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Panel 2

Panel 2 Biotechnology

Research Assessment Exercise (RAE) 2021, self-evaluation

Coordinator: Prof. Per-Åke Nygren
Vice-coordinator: Prof. Per Berglund

Organisation

Organisation schedule

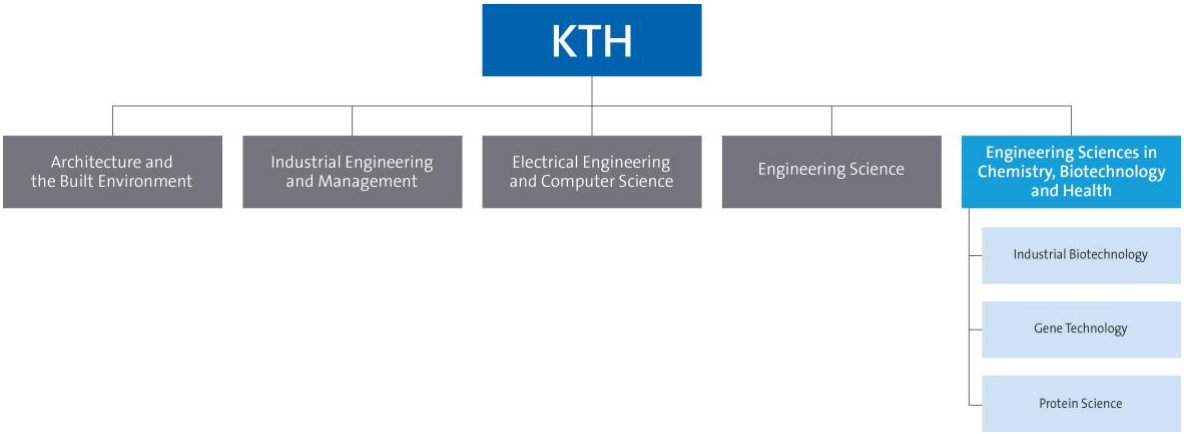


Figure 1: Panel´s position in KTH´s organisation.

Involved units

• School of Engineering Sciences in Chemistry, Biotechnology and Health
• Departments of Gene Technology, Protein Science and Industrial Biotechnology

Department/Division	Head	Deputy Head
Department of Gene Technology *www.kth.se/gte* *No divisions*	Prof. Peter Savolainen *peter.savolainen@scilifelab.se*	Prof. Afshin Ahmadian *afshin.ahmadian@scilifelab.se*
Department of Protein Science *www.kth.se/pro* *Seven divisions*	Prof. Cecilia Williams *cecilia.williams@scilifelab.se*	Prof. Stefan Ståhl *ssta@kth.se*
Division of Systems Biology *www.kth.se/pro/sysbio*	Prof. Mathias Uhlén *mathias.uhlen@scilifelab.se*	N/A
Division of Cellular and Clinical Proteomics *www.kth.se/pro/cellular-proteomics*	Prof. Emma Lundberg *emma.lundberg@scilifelab.se*	N/A
Division of Affinity Proteomics *www.kth.se/pro/affinity-proteomics*	Prof. Peter Nilsson *peter.nilsson@scilifelab.se*	N/A
Division of Nanobiotechnology *www.kth.se/pro/nanobio*	Prof. Aman Russom *aman.russom@scilifelab.se*	N/A
Division of Drug Discovery and Development *www.kth.se/pro/drug-discovery*	Dr. Anders Olsson *anders.olsson@scilifelab.se*	N/A
Division of Protein Technology *www.kth.se/pro/prot-tech*	Prof. Sophia Hober *sophia@kth.se*	N/A
Division of Protein Engineering *www.kth.se/pro/proteineng*	Prof. Per-Åke Nygren *perake@kth.se*	N/A
Department of Industrial Biotechnology *www.kth.se/dib* *No divisions*	Prof. Anthony van Maris *tonvm@kth.se*	Prof. Per Berglund *perbe@kth.se*

CONTENT

Part A: Introduction of panel

1. Description of the research field of the departments included in the research panel

The departments included in **Panel 2 Biotechnology** are Department of Gene Technology, Department of Protein Science and Department of Industrial Biotechnology, all belonging to the KTH School of Engineering Sciences in Chemistry, Biotechnology and Health (CBH School). The overall research field common for all three departments included in Panel 2 is life science/biotechnology, with an emphasis on development and use of technologies for molecular, structural and bioinformatic analyses of *e.g*. nucleic acids, proteins and metabolites in healthy and disease living matter. Several of the research areas addressed involve the generation and handling of large scale proteomic and transcriptomic data. Further, development of biomolecular reagents and materials for research, diagnostic, environmental, industrial and medical applications, as well as cell-based production of a wide range of products, incl. therapeutic proteins, chemicals, viruses and biofuels are central parts.

2. Description of the self-evaluation process for the research panel

The self-evaluation process started already in 2019/20 with "RAE 2020". Initial meetings were held between the coordinator and the respective department heads (incl. deputy heads) to inform about the process. Separate workshops were then arranged by the department heads at which division heads/group leaders were present to discuss the self-evaluation template and how to address the different sections. A first draft version of the Panel 2 report was produced during the spring of 2020, but soon after this the whole RAE 2020 was put on hold when the SARS-CoV-2 pandemic hit. The self-evaluation process was restarted during the winter of 2020/21, now denoted "RAE 2021". In the writing of the self-evaluations, the Department heads (*Sw*. "Prefekt") have been in charge of their respective departmental reports, requesting various contributions from division heads/group leaders. The whole process has been iterative with commenting on draft versions from the senior faculty. The coordinators have served as Links between the central KTH RAE team and the departments, incl. relaying information of the process, distributing various documents and forwarding questions from the departments. Quantitative data on *e.g*. staff situation, bibliometric analyses and financing information (incl. graphs) have been provided by the KTH RAE team. The maximum allowed number of "success stories", or impact cases, (Appendix 1) to be included in the report was set by KTH to a maximum of 12 per panel. A consensus was reached within Panel 2 to split these based on head counts, resulting in 3-6-3 impact cases, respectively, for the three included departments.

3. Identified research panel synergies

Synergies

The three departments have the common overall research goal to contribute to a sustainable future through innovative biotechnological solutions. The approaches taken and specific scientific questions addressed are obviously different depending on the department/division/research group, but many general biotechnological concepts are used by all departments resulting in a good understanding of each other´s work ("speaking the same language") which in turn leads to synergies in respect to *e.g*. valuable exchange of collegial advice, instrument sharing and collaborative projects across the departmental borders. The AdBIOPRO competence centre for advanced bioproduction by continuous processing in which staff from both Dept. of Industrial Biotechnology and Dept. of Protein Science join forces based on complementary competences serves as a good example of synergy within the panel. A further example is the undergraduate level course in Advanced Microbiology and Metagenomics (BB2560) where participating teachers from different departments within the panel have together designed the course plan.

Common challenges

A high dependance on external funding (some of which is tied to a soon retiring faculty), increasing costs for salaries, rent and overheads, difficulties to maintain and replace the instrument

infrastructure, several hinders for expansion of the permanent faculty and a high turn-over of administrative staff are common challenges among all three departments.

Part B: Report for each department

Department of Gene Technology

Self-evaluation
Head of Department: Professor Peter Savolainen

Included divisions:

The department has no divisions

Department of Gene Technology

1. Overall analysis and conclusion; strengths and development areas

a. Limited SWOT-analysis

Overall analysis of 1) Research strengths, 2) Research weaknesses, 3) Organizational strengths, 4) Organizational weaknesses (see SWOT table) and 5) Development areas.

	Strengths	Weaknesses
Research	Bulleted list, in order of magnitude. 1. Broad competence within several key areas of Gene Technology, including development of novel molecular technologies and bioinformatics tools, forensics, plant research, microbiota, and medical and environmental genomics. This creates a fertile environment for multidisciplinary research. 2. Strong competence in technology development propelling key advancements in biological and medical science, and thus sustainability. 3. A strong publication record with high impact publications in a variety of top international journals. This level of high impact publishing has been continuous with a positive trend through the assessment period. 4. Widespread international networks and high local collaboration activity. To meet our requirements for highest level biological and medical competence and sampling, we have sought and established collaborations internationally when required, and locally when applicable. 5. International reputation through high impact and cutting-edge technology development, sought after by many external experts, which has allowed the faculty members to initiate collaborations with key biological and medical expertise.	Bulleted list, in order of magnitude. 1. The department is dependent on cutting edge biological/medical competence, concerning both expertise and samples. To mitigate this, the PIs have built key international collaborations. 2. Sustainability work, especially for environmental research, is carried out by several groups but can be broadened and intensified. 3. Lack of cutting-edge bio-related machine learning expertise.

Organisation	Bulleted list, in order of magnitude. 1. Access to world-class infrastructure, in particular high throughput sequencers and automation robots allowing for high throughput analysis on an internationally competitive scale. 2. The ten research groups have expertise in different academic sub-disciplines but are related enough to form a fertile environment for collaboration between groups, leading to numerous projects in which groups combine synergistically to address research questions of the highest scientific relevance. 3. Strong external funding, including in international competition (e.g. EU-funding) and industrial funding. 4. Geographically united department, with all members able to collaborate and interact on a daily basis, and an open and friendly atmosphere, resulting in frequent scientific discussions and multidisciplinary collaborations. 5. Competent young faculty: three new faculty recruited since last RAE obtained government/EU starting grants.	Bulleted list, in order of magnitude. 1. Gender balance at the department is not entirely equal (40% females and 60% males), but this is improving on the PI level with recent recruitments. 2. Faculty members are dependent on external funding, as the salaries are not fully covered by the university. 3. Limited department activity in the form of formal sessions for scientific discussions.

5) Development areas

We consider the following two development areas to be most important:

- Recruitment of cutting-edge machine learning expertise, both faculty and students. This is needed to advance our research in a time when already very large datasets are growing and the development of AI and machine learning opens up new possibilities.

- Increased internal funding for faculty. Faculty members are dependent on external funding, as the salaries are not entirely covered by the university. This organizational structure creates stress (bad work environment) and takes energy from research.

b. Summary statement on contributions of department on impact, infrastructure and sustainable development

Impact

The Department of Gene Technology [Link] contributes with significant impact through a very strong publication record, with high impact publications in a variety of top international journals. This impactful research contributes to society by development of important tools for medical research and environmental monitoring, by medically important biological findings, by development of sustainable forest and food production, by innovations and patent applications, and by considerable outreach, especially concerning dog history and evolution to a curious general public.

Infrastructure
The department contributes with the numerous molecular and computational methods that have been developed, that have opened up new analysis methods in many scientific fields, and by hosting two national research facilities, most importantly National Genomics Infrastructure (NGI) [Link]. We also contribute to the scientific community by our international and local networking and collaboration activities.

Sustainable development
Most of our research contributes to sustainable development, since it relates to improvement of human health, and to environmental issues, conservation, sustainable forest and food production, and forensics.

2. Research profile

a. General information of the department

The department of Gene Technology is one of nine departments at the School of Engineering Sciences in Chemistry, Biotechnology and Health (CBH), which is one of the five schools at KTH. The department of Gene Technology consists of ten research groups (one of which started in December 2020, and therefore not included in this assessment), and also hosts two national infrastructure platforms at Science for Life Laboratory (SciLifeLab) [Link]; the National Genomics Infrastructure (NGI) [Link] and Clinical Genomics [Link]. Head of department (Prefekt) is Professor Peter Savolainen. The number of faculty is nine and total number of employed, incl. Postdocs and PhD students, is 55. The ten research groups are:

Genomics, PI Professor Joakim Lundeberg
The laboratory of Lundeberg focuses on molecular technology development coupled with computational tools and applications in life sciences. The current research focus relates to spatially resolved genomics studies *in situ*. Applying this strategy to study gene expression has been demonstrated to work remarkably well and allows visualizing and quantifying the transcriptome in regular histological tissue sections, *i.e.* tissue domains can be matched to precise gene expression patterns. Furthermore, data driven methods can be applied to unsupervised discovery of transcriptomic patterns in space that yield information about cell-types, microenvironments or tissue architecture that allows for novel avenues of research in life sciences.

Experimental Genomics, PI Professor Afshin Ahmadian
The research of the Experimental Genomics group focuses on molecular technology development covering the fields of genomics and DNA-assisted proteomics. The group has pioneered the droplet barcoding technology, a method that today is a routine assay in many labs. The technology is used, for example, to haplotype-resolve cancer genomes with single molecule resolution, and to target the central dogma of single cells and characterize surface proteins of single exosomes in heterogeneous cancer samples.

Evolutionary Biology and Forensics, PI Professor Peter Savolainen
Based on large-scale DNA sequencing and method development, this group studies history and evolution of animals, and develops analysis methods for forensic DNA investigations. The group is especially excellent in the field of dog history and evolution, where it has been at the research front the last 20 years.

Statistical Biotechnology, PI Professor Lukas Käll
The group of Statistical Biotechnology develops machine learning-based methods for analysis of modern molecular biology data. It primarily focuses on shotgun proteomics data and implements methods for the assignment of statistical confidence of findings, for clustering of mass spectra and for the facilitation of quantitative analysis from such experiments. The group also works with the pathway-based integration of omics-data.

Environmental Genomics, PI Assoc. Professor Anders Andersson
The group develops molecular and bioinformatics methods for studying complex microbial communities with omics approaches, and studies the function and evolution of microbiomes of various environments, with main focus on marine ecosystems (in particular the Baltic Sea).

Expression Bioinformatics, PI Assoc. Professor Olof Emanuelsson
The group of Expression Bioinformatics performs bioinformatics tool development and analysis with focus on human and plant transcriptomics, in particular transcriptome reconstruction and allele-specific expression from sequencing data.

Regulatory Genomics, PI Assoc. Professor Pelin Sahlén
The Sahlén laboratory focuses on the non-coding part of the human genome and its role in noncommunicable diseases, such as heart disease, diabetes and atopic dermatitis. It develops both experimental and computational tools to chart regulatory networks, to find functional non-coding variants and connect them to the genes they affect. Thus, the aim is to understand the functional impact of non-coding variants in health and disease, to enable its therapeutic use in medicine and the clinic, which is currently lacking.

Applied Genomics, PI Assoc. Professor Patrik Ståhl
The Applied Genomics group has a particular focus on spatially resolved technologies, and have propelled some of the recent advances in the field, in particular in the context of spatial transcriptomics. We have an experimental approach to technology innovation, where we specifically develop new protocols for single-cell and spatial genomics.

Spatial Biology, PI Dr. Stefania Giacomello
The Giacomello laboratory focuses on the study of tissue plasticity from a spatial perspective across systems. This is achieved by developing and applying single-cell and spatial transcriptomics techniques in mammalian and plant organisms.

Molecular Genomics, PI Assistant Professor Anniina Vihervaara
The Molecular Genomics group investigates genome regulation, chromatin architecture and transcriptional mechanisms at a molecular level. We use and develop techniques that track the transcription machinery at a nucleotide-resolution, analysing rate-limiting steps of transcription at genes and enhancers. The mechanistic information gained on genome organization and transcription is applied to physiologically relevant contexts to understand how cells rapidly respond to stress, establish a transcriptional memory, or differentiate into distinct cell types. Assistant Professor Vihervaara`s group joined the department in December 2020, and is therefore not further included in this assessment.

The department also hosts two national infrastructure platforms, the National Genomics Infrastructure (NGI) and Clinical genomics:

National Genomics Infrastructure (NGI), Head of facility Dr. Ellen Sherwood
NGI is a national infrastructure with platform activity in Stockholm and Uppsala [Link]. NGI Stockholm (at SciLifeLab Stockholm) consists of two groups: Genomics Production and Applications Development, and offers state-of-the-art service in the rapidly developing field of massively parallel DNA sequencing. Genomics Production offers sample QC, library preparation and high-throughput sequencing followed by data processing and best practice analysis for a variety of well-established applications (WGS, WES, RNA-seq, ATAC-Seq, etc.). Applications Development has internal developmental programs and collaborates with researchers on applications not supported by Genomics Production. In addition, new computational tools (MultiQC, SAREK, nf-core etc.) are established by the team. KTH is the host for National Genomics Infrastructure and the director is appointed by KTH. Notably, NGI was given the highest rating in a recent evaluation by the Swedish Research Council, and consequently received continued funding as a national research infrastructure for the period 2021-2025.

Please note that NGI is a KTH infrastructure doing a separate RAE 2021 self-evaluation.

Clinical Genomics Facility, Head of facility Dr. Valtteri Wirta
The Clinical Genomics Stockholm facility [Link] provides a dedicated research infrastructure for projects utilising massively parallel/next generation sequencing technologies. All projects are carried out in close collaboration with the Swedish healthcare system. The facility serves as a competence centre assisting the translation of genomics-based tools to routine clinical care. All work is carried out in close collaboration with medical expertise provided by the clinical diagnostic laboratories and patients' managing physicians. Also, the facility aims to improve the capacity of the public health microbiology for national surveillance of infectious diseases and for epidemic preparedness. Karolinska Institute (KI) is the host for the Clinical Genomics facility. The Head of Facility is jointly funded by KI and KTH.

b. Central research questions and themes, knowledge gaps addressed, main research activities activities

The basis for the research at the Department of Gene Technology is application driven method development. Our scientists identify important scientific questions in, for example, medicine or environmental research that cannot be solved with available technology. To solve these problems, they use their skills in technology and method development to establish novel methods, which are subsequently used in applied research.

Thus, in the broad sense, the **knowledge gaps that are identified and addressed** are important scientific questions in medicine, biology and environmental science for which a technical solution is lacking. The **main research activities** are development of novel molecular and computational methods, and subsequent application of these methods to solve the original scientific questions.

The **main research activities** can be broadly categorized into four interconnected categories: **Technology** - development of new molecular concepts and ideas; **Application** - use of molecular tools to provide new biological knowledge in life sciences; **Bioinformatics** - large scale investigations of rich biological data; **Computational Biology** - development of new computational frameworks and models for life science data. Collectively, the department has demonstrated its excellence in all these areas, with publication of highly cited papers in journals like *Science, Nature, Cell, Nature Methods,* and *Nature Biotechnology*.

Given that the ten research groups belong to different sub-disciplines within the gene technology field, a broad palette of research questions and knowledge gaps are addressed at the department, as exemplified for three of the research groups:

Genomics
The need for precise analysis of gene expression in specific parts of tissues has been a major knowledge gap for biological studies and clinical diagnosis. With the development of the spatial transcriptomics technology, this knowledge gap was addressed. The technology is now applied to analysis of a wide range of tissues and to the Human Cell Atlas projects. Further development of technology as well as computational methods are ongoing.

Experimental Genomics
The group works with developing molecular methods within genomics, transcriptomics and DNA-assisted proteomics, and bioinformatics tools tailored to handle the generated data. A knowledge gap that is addressed is the need for cost-effective haplotyping at a genomic scale, for example in cancer diagnostics. A technology was recently developed, that allows for genome-wide haplotyping with single molecule resolution, with unprecedented throughput in a cost-effective manner. Understanding the genetics of cancer necessitates identification of both the variants and the order of these variants in the

two sets of chromosomes. This innovative technology is now applied to haplotype-resolve colon and lung cancer genomes.

Evolutionary Biology and Forensics
A theme of greatest scientific relevance is studies of the origins, evolution and dispersal of the domestic dog. The knowledge gaps that are addressed includes identification of the geographical origin of the dog, which is a vigorously debated question, the routes and dates for the global dispersal of the dogs, and identification of genome evolution coupled to domestication and feralization. These questions are studied by massive DNA sequence analysis of mitochondrial and nuclear genomes, and by phylogeographic analysis, demographics and selection analysis.

Thus, the ten research groups belong to different academic sub-disciplines within the gene technology field. However, they are related enough to create a very fertile environment for multidisciplinary collaboration, where expertise from two or more groups combine synergistically to foster novel scientific ideas and projects of highest scientific relevance. Often, several in-house developed technologies are combined, opening up possibilities for totally novel research. For example, the vast experience in spatially resolved transcriptomics has enabled further studies in plant and environmental research, and studies of genome interactions combines expertise in droplet technology and chromatin profiling. Further examples of collaborations are studies of the role of enhancers in trait evolution in dogs, and computational analyses of the spatial gene expression in Norway spruce cones. The extent of group-to-group collaborations is summarized in Table 1, showing that most research groups have three or more ongoing collaborations within the department.

Table 1. Number of ongoing collaborative projects between the research groups and infrastructure platforms at the department.

	Gen-omics	Exp. Gen.	Evol. Biol.	Stat. Biot.	Env. Gen.	Expr. Bio-inf.	Reg. Gen.	Appl. Gen.	Spat. Biot.	NGI	Clin. Gen.
Gen-omics					1	1		1	3	3	1
Exp. Gen.			3		1		1			1	
Evol. Biol.		3			1		1			1	
Stat. Biot.											1
Env. Gen.	1	1	1						1	1	
Expr. Bioinf.	1										
Reg. Gen.		1	1					1			
Appl. Gen.	1						1			1	
Spat. Biot.	3				1	1					
NGI	3	1	1		1	1		1			
Clin. Gen.	1			1							

c. Contributions to the advancement of the state of the art within the research fields of the department

We here give only a few examples of the large number of major contributions by the department:

RNA-sequencing and, more recently, single-cell RNA-sequencing have seen huge advancements in the past decade. For several years, the missing factor to the transcriptomics puzzle of intact tissues was the spatial component. Our department has led a significant part of the international research development towards a solution for spatially resolved transcriptome analysis. Since 2016 several key papers have been published in high impact journals such as *Science, Nature, Cell, Nature Methods and Nature Communications*, surrounding the theme of the spatial transcriptomics technology developed at the department, as well as applications of the technology. Notably, the spatial transcriptomics method was named "Method of the Year 2020" by the journal *Nature Methods* [Link] and ranked ninth among the "2020 Top Innovations" by the journal *The Scientist* [Link].

Research by the Evolutionary Biology and Forensics group, about Dog origins, evolution and history, has been at the forefront of the field the last 20 years. The group changed the paradigm of the field with an article in *Science* pointing out East Asia instead of Europe or SW Asia as the probable geographical origin of the domestic dog. The group has also pioneered studies of the global dispersal of dogs, to Australia, Oceania, America and Madagascar. It now continues to advance the state-of-the-art with prominent research about the trait evolution connected to the origin and further development of the dog. This research has resulted in 28 articles, 11 of which in high impact (IF>9.4) journals, and totally 4,639 citations.

Metagenomics is today considered a key method for understanding how microorganisms influence human and environmental health. Since a metagenome comprises a soup of DNA fragments from different organisms, a major challenge has been to sort the fragments into genomes of origin (metagenomic binning). The group co-developed the first automatic bioinformatics method for solving this step, presented in *Nature Methods* [Link] and the software has been used to reconstruct microbial genomes from a range of environments in more than 500 studies.

Most genetic variants associated with complex diseases are situated within non-coding regions. With no effective methods for Linking these non-coding regions to the genes they affect, complex disease genomics has lagged behind in terms of clinical and medical translation. The Regulatory Genomics group therefore developed the Capture Hi-C methodology, which combines chromosome conformation capture with targeted sequencing, to generate high-resolution promoter-enhancer maps, Linking non-coding variants with affected genes. The method, presented in an article in *Genome Biology* [Link], is an invaluable tool for evaluating the functional impact of non-coding variants and particularly impactful in complex disease biology.

The Experimental Genomics group has developed a novel Linked-read sequencing technology for whole genome haplotyping (denoted Droplet Barcode Sequencing, DBS), enabling reconstruction of synthetic long reads from short reads into megabase-scale haplotype blocks. This technique does not require complex devices or reagents, implying that any laboratory can adopt the method without investing in instruments and kits. The technology is flexible, enabling phased haplotypes to be obtained from single DNA molecules, and complex metagenomics samples may be studied with single molecule resolution. The single molecule haplotyping approach has made it possible to obtain unprecedented N50 values of 30 megabases, allowing for, e.g., accurate haplotyping and analysis of small and large structural variants in cancer which has not been possible before. The DBS method has been described in articles published in *Nature Communications* (2015) [Link], *Nucleic Acids Research* (2017) [Link] and *Nature Scientific Reports* (2019) [Link].

d. Quality and quantity of contributions to the body of scientific knowledge

Bibliometric statistics show that the Department of Gene technology has a very strong publication record (Figures 1-3). According to the latest "KTH Annual Bibliometric Monitoring (ABM)" [Link], our department had the highest Publication impact (field normalized citations) and Journal impact (journal field normalized citations) among all departments at KTH. Specifically, the Publication impact for 2016-2018 was 2.68, implying that our scientific articles are cited almost three times as often as the average article in Web of Science (168% above field average) and the Journal impact for 2017-2019 was 2.12, implying that the articles were published in journals with citation rates more than two times above the average (112% above field average) (Figure 2). Similarly, 24.6% of the articles were among the 10 percent most cited in its field, and 54.0% were published in the 20 percent most cited journals in its field. The values have been high all through the period 2013-2019, with a clearly positive trend, Publication impact increasing from 1.89 (2013-2015) to 2.68 (2016-2018) and Journal impact increasing from 1.49 (2013-2015) to 2.12 (2017-2019). The fractional publication count (Figure 1) shows an overall steady productivity, and the international co-publication (Figure 3) is above 50% each year.

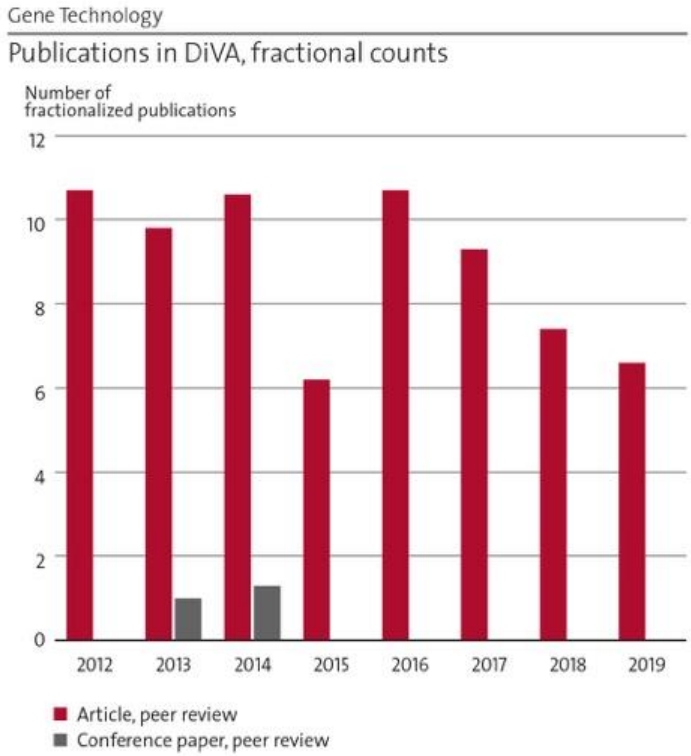


Figure 1. *Number of fractionalized publications 2012-2019.*

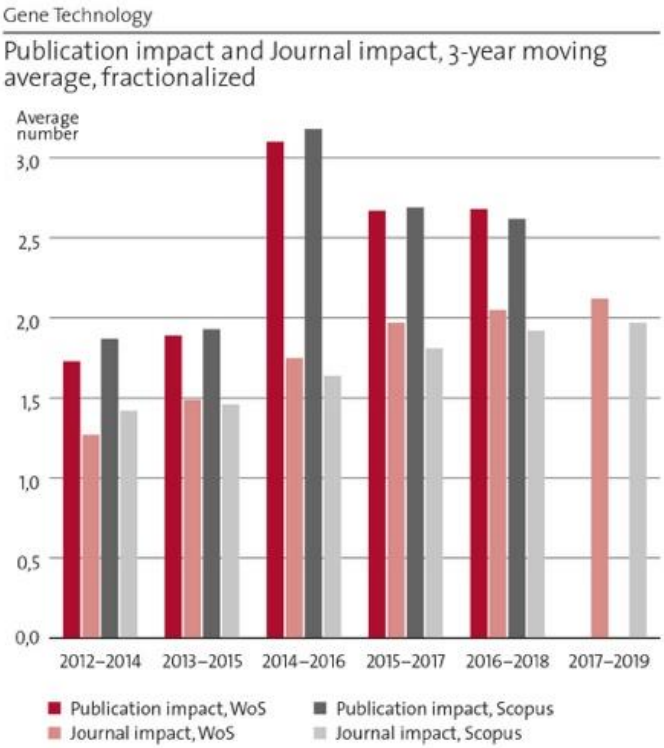


Figure 2. *Publication and journal impact 2012-2019.*

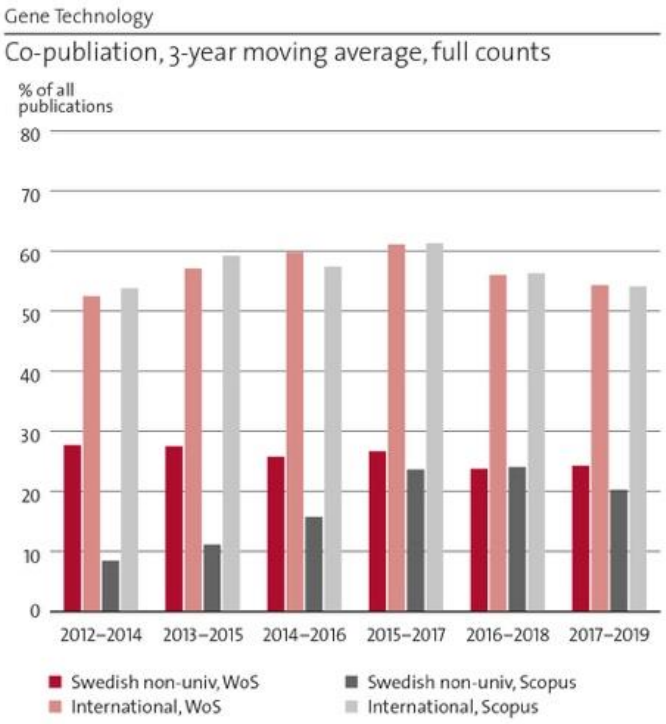


Figure 3. *National and international co-publications 2012-2019.*

The strong publication record and positive trend can be further illustrated by statistics on the actual number of articles published in high impact journals (Impact Factor>9.4, to include PNAS) (Table 2). In the period 2012-2020, totally 53 articles were published in high impact journals, giving an average of 5.9 per year, or 0.65 high impact publications per group leader and year. There is also a notable steady rise in the number of articles, up to around 10 articles yearly (more than one article yearly per group leader) in high impact journals the last three years.

In conclusion, the bibliometrics indicate a high and steadily increasing productivity of excellent research at our department, that attests our focus on application driven method development as an effective strategy for research of highest relevance.

Table 2. Number of publications and high impact publications at the department [Link]

Year	Number of publications	With Impact Factor>9.4
2020	35	13
2019	31	7
2018	30	9
2017	32	5
2016	28	5
2015	25	7
2014	28	5
2013	32	2
2012	35	0
Total	276	53

We want to highlight the following 10 articles, with contribution by all nine group leaders included in the assessment, as examples of excellent research in a broad range of sub-disciplines produced by our department:

Guo-Dong Wang, Weiwei Zhai, He-Chuan Yang, Lu Wang, Li Zhong, Yan-Hu Liu, Ruo-Xi Fan, Ting-Ting Yin, Chun-Ling Zhu, Andrei D Poyarkov, David M Irwin, Marjo K Hytönen, Hannes Lohi, Chung-I Wu, Peter Savolainen, Ya-Ping Zhang. Out of southern East Asia: the natural history of domestic dogs across the world. ***Cell research*** 2016 [Link]. **Number of citations: 226**

Giacomello S, Salmén F, Terebieniec BK, Vickovic S, Navarro JF, Alexeyenko A, Reimegård J, McKee LS, Mannapperuma C, Bulone V, Ståhl PL, Sundström JF, Street NR, Lundeberg J. Spatially resolved transcriptome profiling in model plant species. ***Nature Plants*** 2017 [Link] **Number of citations: 49**

Asp M, Giacomello S, Larsson L, Wu C, Fürth D, Qian X, Wärdell E, Custodio J, Reimegård J, Salmén F, Österholm C, Ståhl PL, Sundström E, Åkesson E, Bergmann O, Bienko M, Månsson-Broberg A, Nilsson M, Sylvén C, Lundeberg J. A Spatiotemporal Organ-Wide Gene Expression and Cell Atlas of the Developing Human Heart. ***Cell*** 2019 [Link] **Number of citations: 84**

Ståhl PL, Salmén F, Vickovic S, Lundmark A, Navarro JF, Magnusson J, Giacomello S, Asp M, Westholm JO, Huss M, Mollbrink A, Linnarsson S, Codeluppi S, Borg Å, Pontén F, Costea PI, Sahlén P, Mulder J, Bergmann O, Lundeberg J, Frisén J. Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. ***Science*** 2016 [Link] **Number of citations: 662**

Vickovic S, Eraslan G, Salmén F, Klughammer J, Stenbeck L, Schapiro D, Äijö T, Bonneau R, Bergenstråhle L, Navarro JF, Gould J, Griffin GK, Borg Å, Ronaghi M, Frisén J, Lundeberg J, Regev A,

Ståhl PL. High-definition spatial transcriptomics for *in situ* tissue profiling. ***Nat Methods***. 2019 [Link] **Number of citations: 161**

Reimegård J, Kundu S, Pendle A, Irish VF, Shaw P, Nakayama N, Sundström JF, Emanuelsson O. Genome-wide identification of physically clustered genes suggests chromatin-level co-regulation in male reproductive development in *Arabidopsis thaliana*. ***Nucleic Acids Res***. 2017 [Link] **Number of citations: 19**

Jakobsson HE, Abrahamsson TR, Jenmalm MC, Harris K, Quince C, Jernberg C, Björkstén B, Engstrand L, Andersson AF (2014) Decreased gut microbiota diversity, delayed Bacteroidetes colonization, and reduced Th1 responses in infants delivered by Caesarean section. ***Gut*** 2014 [Link] Apr;63(4):559-66. **Number of citations: 752**

Sahlén P, Abdullayev I, Ramsköld D, Matskova L, Rilakovic N, Lötstedt B, Albert TJ, Lundeberg J, Sandberg R. Genome-wide mapping of promoter-anchored interactions with close to single-enhancer resolution. ***Genome Biol***. 2015 [Link] **Number of citations: 110**

The, M and Käll, L. Focus on the spectra that matter by clustering of quantification data in shotgun proteomics. ***Nat Commun.*** 2020 [Link] **Number of Citations: 8**

Borgstrom, E., Redin, D., Lundin, S., Berglund, E., Andersson, A.F. and Ahmadian, A. Phasing of single DNA molecules by massively parallel barcoding. ***Nat Commun.*** 2015 [Link] **Number of citations: 27**

e. Engagement in national and international research collaboration within academia and its outcomes

All research groups at our department have very active and substantial international networking and collaborations, which are fundamental for meeting our requirements for highest level biological and medical competence and sampling in applied studies. Space allows only a few of these to be mentioned here:

For the development and application of the spatial transcriptomics method, collaborations offering highest level medical competence and sampling have been instrumental, and have resulted in prominent papers such as Ståhl *et al.*, *Science* 2016; Vickovic *et al., Nature Methods* 2019; Berglund *et al., Nature Comm* 2018 [Link]; Asp *et al., Cell* 2019 and Maniatis *et al.*, *Science* 2019 [Link]. Similarly, for the studies about dog origins, evolution and dispersal, global collections of samples have been necessary for driving research at the absolute research front, which has resulted in numerous high impact publications such as Wang *et al.*, 2016 *Cell Res*. [Link], Wang *et al.,* 2019 *Cell Res*. [Link], and Zhang *et al.,* 2020 *Nat Commun*. [Link]. Further examples are the Environmental Genomics group which has extensive collaborations with marine research groups surrounding the Baltic Sea (Denmark, Germany, Estonia, Finland) in EU-funded research projects (Blueprint and AFISmon) and the Spatial Biology group which has collaborations with NASA Ames Research Center to study the impact of spaceflight on mouse heart and brain.

f. Follow up from previous evaluations

In the RAE from 2012 the panel foresaw that:

"Projects such as the *de novo* sequencing of the economically important species and surveys of the Baltic Sea ... have huge potential and are economically and societally of great importance. The application of high-throughput sequencing in the context of human genetics and the Science for Life Laboratories could make a substantial contribution to the basic understanding of biology which is largely needed these days. In our opinion this is one of areas where the university can generate the impact they seek to have for society".

Since then our department has performed very successful research in all these areas, specifically sequencing of the Norway spruce genome, surveys of microbiomes in the Baltic Sea, and studies of spatial gene expression in human tissues.

The RAE from 2012 recommended to do:

"Further reinforcement both of the side of maintaining platforms state-of-the-art and investment into development of forward-looking activities, such as investment into advanced bioinformatics, *e.g.* development of a bioinformatics professorship in pathways and network analysis".

The National Genomics Infrastructure (NGI), hosted by our department, has continued to provide DNA sequencing service using state-of-the art instrumentation, and has established highly optimized bioinformatics pipelines with excellent reporting. Notably, several of the methods used by NGI have been developed in collaboration with research groups at the department. The department has now also recruited a professor, Tuuli Lappalainen, who is expert in human genetics and functional genomics. She will join the department in May 2021, and will act as Director for NGI [Link]. The department has also recruited several postdocs with good bioinformatics and computational skills.

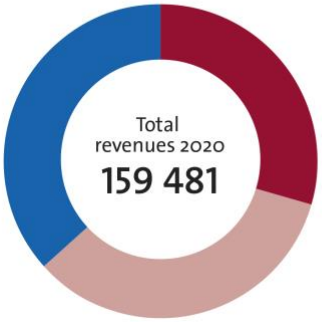
3. Viability

a. Funding; internal and external

The principal activity for the faculty at the Department of Gene Technology is research, with only around 15% of time spent for bachelor and master level teaching, which is reflected in a relatively low proportion of basic funding for teaching. The main funding of the department is from external sources obtained in competition, "Research grants" (Figure 4). Five of our PI:s currently have governmental funding, from The Swedish Research Council (VR) or Formas, including three group leaders recruited since last RAE who obtained career starting grants. There is also substantial funding in several EU projects. The largest proportion of funding comes from private foundations, especially from Knut and Alice Wallenberg foundation (KAW), Erling Persson Family Foundation, and Swedish Cancer Society. The largest income, "Other revenues", concerns mainly activities in the two national infrastructure platforms, the National Genomics Infrastructure (NGI) and Clinical genomics, and does not relate directly to research at the department. The large external funding reflects very successful previous work and our strong publication record. Considering the positive trend for our already strong publication record, continued funding opportunities seem good. Still, the successful external funding has a backside in that the faculty relies on continued external funding to keep their activities, including salary for Faculty members since salaries are not fully covered by the university. The Covid-19 pandemic has also implied negative market trends that will affect external funding negatively.

Gene Technology

Sources of research income (2012, 2016, 2020)



Research income, kSEK	2012	2016	2020
Government grants for research and doctoral studies		25 390	28 756
Research grants		53 976	41 643
Contract research funding		0	668
Other revenues		5 0498	88 410
Financial income		334	4
Total revenues	128 592	147 930	159 481

Figure 4. *Comparison of research incomes 2016 and 2020.*

b. Academic culture

The academic culture at the department is characterized by collegiality, and an open and friendly atmosphere. The group leaders have their offices in the same part of the department, so they meet daily in the corridor or at lunch or coffee breaks. This results in frequent opportunity for scientific discussions, and it is easy to get help for solving big or small scientific problems also outside of collaborations, which is a help to move projects forward. All this helps to build collaborations among the different research groups. This has resulted in numerous collaborations among the groups where different expertise can be combined in multidisciplinary studies.

Also among Postdocs and PhD students, there is a helpful attitude and sharing of knowledge among students in the different research groups. Thus, students from different groups help each other and discuss scientific questions. The focal point for these meetings is the lunchroom/coffee machine.

In a more organized form, scientific questions are discussed at two weekly seminar series about "DNA/RNA science" and "gene technology", where the daily experiments and the latest literature is discussed. However, participation by group leaders is normally low, and other formal sessions for group leaders and students to discuss science are missing, which is thus a point for improvement. There are also opportunities at SciLifeLab for seminar activities, and for activities among PhD students and Postdocs such as the "Thursday pub".

In order to keep a nice working atmosphere, the department is actively involved in discussions about academic culture at KTH. Faculty members are part of the JML-work (gender equality, diversity and equal treatment) at the CBH School and SciLifeLab, and keep focus on bullying and harassment, in line with several commentaries in prestigious scientific journals [Link], [Link], [Link].

c. Current faculty situation

The department has been in a growth phase the last few years, from six faculty members five years ago to today's nine faculty plus one non-faculty group leader (Figure 5, Table 3). Furthermore, one more faculty member (professor and new director for the NGI platform, Tuuli Lappalainen) and one non-faculty group leader (ERC starting grantee, Ian Hoffecker) are joining during 2021. With five Professors, four Associate professors and one Assistant professor, there is a good career stage balance. The age of the faculty is relatively low, 38-57 years, mean 47.8 years. With no faculty older than 57 years, there are no retirements planned the next 10 years. The six "original" faculty members were all males. The four new faculty recruitments are three females and one male, giving a faculty of seven males and three females, plus one male and one female non-faculty group leader. Thus, a more even gender balance is evolving. At recruitments, equal numbers of the sexes are engaged as experts, to safeguard non-biased evaluations. Notably, six of the twelve group leaders have a non-Swedish background. Except the faculty, the department has a good gender balance, consistent with the biotechnology field in general.

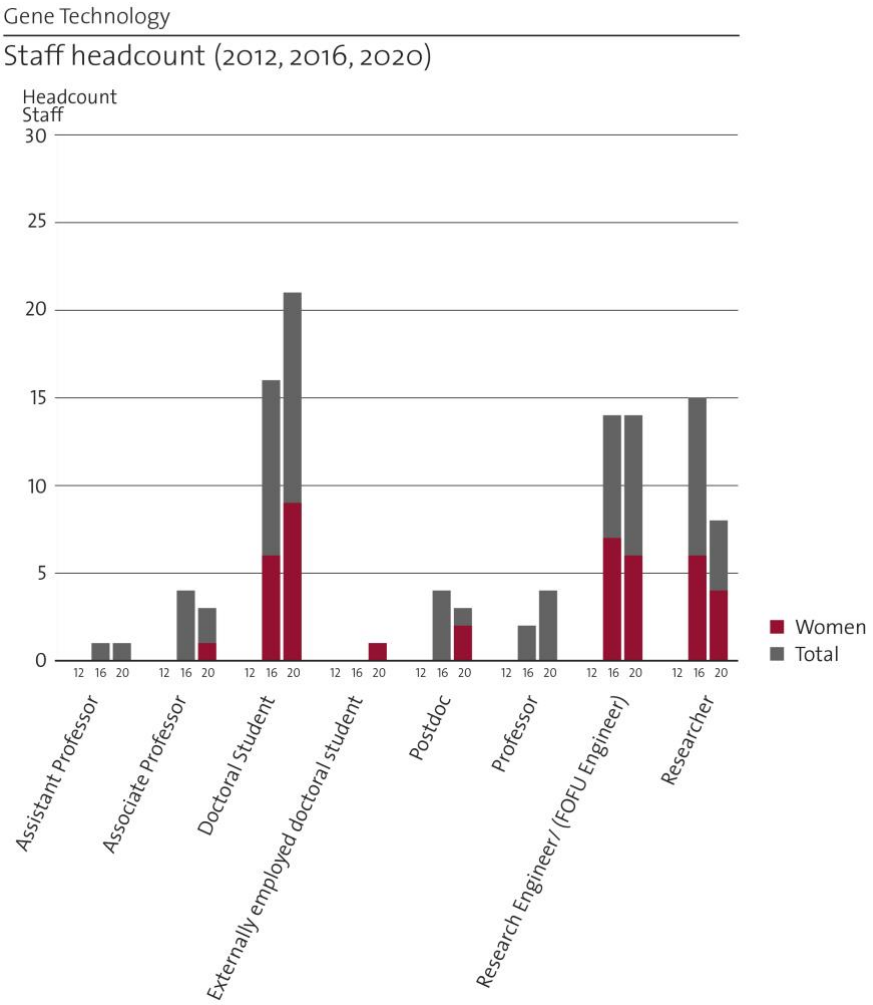


Figure 5. *Staff headcounts (2012), 2016 and 2020.*

Table 3. Overview of current research groups.

Group	PI	Prof	Assoc. Prof	Assist. Prof	PhDs	Post Docs	PhD Stud.	Res. Eng.
Genomics	Joakim Lundeberg	1			2	3	10	2
Experimental Genomics	Afshin Ahmadian	1				2	1	1
Evol. Biol. and Forensics	Peter Savolainen	1			1		1	
Statistical Biotechnology	Lukas Käll	1					2	1
Environmental Genomics	Anders Andersson		1			2		
Expression Bioinformatics	Olof Emanuelsson		1				1	
Regulatory Genomics	Pelin Sahlén		1			2	2	
Applied Genomics	Patrik Ståhl		1				4	
Spatial Biology	Stefania Giacomello				1	1	2	2
Molecular Genomics	Anniina Vihervaara			1				

d. Recruitment strategies

Our department is building its scientific success on technique and method development that is applied to important scientific problems within Life science. This strategy has been built on recruitment of a mix of expertise in both molecular biology and bioinformatics, at the faculty as well as PhD/Postdoc level. In later years, given the increasing data amounts in the field, skills in bioinformatics, programming and machine learning have become increasingly important for our research projects. With still growing data amounts we see both a need for, and a potential in, increased application of machine learning in many projects. We therefore see a need to recruit more scientists and students with good skills in machine learning and computational biology. We also aim to increase our efforts in environmental research and plant genetics, which is why researcher Stefania Giacomello was recruited. At recruitments, equal numbers of the sexes are engaged as experts, to safeguard equal opportunities.

e. Infrastructure and facilities

The Department of Gene Technology is host for the National Genomics Infrastructure (NGI) [Link] which is an internationally leading infrastructure in genomics and a unique and exceptionally important resource for the Swedish research community (please note that NGI is also a KTH infrastructure doing a separate RAE 2021 self-evaluation). NGI has more than 20 years of experience in providing large-scale genotyping (Uppsala) and DNA sequencing as a service using state-of-the art instrumentation from low to high throughput needs (MiSeq, NovaSeq, Sequel (Stockholm, Uppsala)). Significant efforts are made at NGI to evaluate emerging technologies and develop novel methods in

the rapidly changing field of genomics, to a large extent in collaboration with researchers at the Department of Gene Technology. This allows NGI to offer a unique combination of extensive consultative support (planning and project design meetings) and executive support (sequencing/ genotyping and pipeline analysis) for high-throughput DNA sequencing and genotyping to academic and industrial users in Sweden. The NGI facilities are placed at SciLifeLab in Stockholm and Uppsala and funded by the Swedish Research Council (VR), the member universities (KTH, UU, KI, SU), SciLifeLab and the Knut and Alice Wallenberg Foundation. As of 2019 the total NGI staff compromises >70 FTEs (15 of which employed by the Department of Gene Technology), including heads of facilities, project coordinators, staff for laboratory and informatics, IT and databases.

In 2019, NGI received the highest rating by the Swedish Research Council, and continued funding as a national research infrastructure for 2021-2025. During 2019, 57,501 samples were sequenced, generating 711 tera base pairs, and 50,123 samples were genotyped, generating 3.5 billion genotypes; 1,145 projects were performed, and 217 of these had PIs that were first time users of NGI. NGI is partner in a number of EU grants including EATRIS-plus, EASI-Genomics, PRECODE. NGI is also a major contributor to the nf-core project, which has established use of highly optimized bioinformatics pipelines with excellent reporting and validated releases to ensure reproducibility, a key for NGS core facilities. The turnover for NGI during 2019 was 208 MSEK.

In 2014, an infrastructure was spun out from NGI to specifically establish clinical implementation of genomics at SciLifeLab, the Clinical Genomics Facility. Sequencing instruments are shared with NGI. Today the facility consists of >30 FTEs and the facility provides >120 WGS analyses per month and >6,000 WGS samples since 2014. The turnaround is 5-14 days for clinical samples. The focus is on custom developed informatics tools adapted to clinical routine. Infrastructure grants from Karolinska Institute has continuously secured needed investments. The facility is hosted by Karolinska Institutet but has employed staff (including the Head of facility) from KTH.

NGI has on average invested around 10 MSEK per year into new instruments. Funding for these instruments has been granted by SciLifeLab, Swedish Scientific Council, Knut and Alice Wallenberg Foundation and host universities and has secured NGI's role as provider of a world-leading genomics infrastructure. The ambition is to continue the level of investments into genomics.

As mentioned above, many activities at NGI originally stem from applications on the research side of the Department of Gene Technology. Massive parallel sequencing as well as sequence capture are two families of technologies which were applied on the research side before being transferred into infrastructure activities at NGI. A more recent example is the integration of Spatial Transcriptomics as a service at NGI, and there are currently seven ongoing collaborations between researchers at the department and NGI (See Table 1 above) based on development of novel methods.

In addition to NGI, the Department of Gene Technology has established a broad infrastructure for tissue handling (culture, cryosectioning, microtome, Chromium), imaging (brightfield and fluorescent microscopes and scanners), robotics for molecular work (Agilent, MBS) and analytical instruments (PCR, QPCR, Bioanalyzer, Qbi, *etc.)*. New investments will be sought through grant proposals.

4. Strategies and organisation

a. Goals for development 5–10 years ahead

The research at Gene Technology is at the research front in several of its sub-disciplines, with a very strong publication record. This success is based on our expertise in method development and on very active international networking and collaboration with other strong research group in multidisciplinary constellations.

We aim to continue in this tradition, and aspire to be a world-leading department specialized in technology development within molecular and computational biology, characterized by

interdisciplinary collaborations and applications in medicine, environmental science and genome evolution.

Gene technology is to a large degree driven by massive data amounts and we foresee that continued technology development will lead to even larger data amounts. With the development of stronger machine learning-based analyses there will be opportunities to further drive the research front. A major strategy is therefore to recruit computational competence and to work with a high degree of integration of experimental activities, bioinformatics analysis and computational biology.

b. Congruence with university-level goals for “A leading KTH" as set out in KTH's "Development Plan 2018-23” (page 5) [Link].

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KTH is Sweden's largest technical university and one of Europe's leading technical and engineering universities. KTH has always demonstrated strong leadership in pushing technologies forward for the benefit of mankind. Basic and applied research are performed side-by-side at KTH and interdisciplinary research is conducted in parallel with research in specific fields. This approach encourages versatile solutions and the innovative climate at KTH creates many opportunities to realize great ideas. As a result of the first RAE 2008, six research focus areas have been created at KTH that work as platforms for multidisciplinary research; Digitalization, Energy, Industrial Transformation, Life Science Technology, Materials and Transport. In addition to scientific excellence, these areas build on KTH's strong tradition of addressing future social challenges. The department is very active in these platforms with, for example, group leader Peter Savolainen acting as Director for the Life Science Technology platform.

The ongoing projects at the Department of Gene Technology are well in line with the overall strategic plan of KTH, since they build on interdisciplinary expertise using innovative molecular approaches (Life Science Technology) and advanced computational frameworks for imaging and machine learning (Digitilization) to battle society's growing challenges: human disease and global environmental changes.

As described under section 4d "Strategies for high quality", below, our research is "based on application driven method development, on curiosity-driven science and academic freedom, and on collaboration with other excellent scientists". Our department has a large academic impact, NGI is an important piece of national infrastructure for molecular life sciences, we work extensively on visibility in the wider society and the department has an increasing awareness of societal impact. Our research is based on interdisciplinary collaborations within the department as well as with national and international expertise. At the school level, there is research cooperation with the Department of Protein science, but there are opportunities for broadening cooperation to other departments at the school within, e.g., plant genomics.

c. Leadership structure and collegial structure

The department has a flat organization, with a Head of Department (Prefekt) and the nine PI:s (group leaders). The group leaders meet at four formal meetings per year. However, most of the interaction happens on a daily basis in corridors and the lunch room. Important decisions are made collectively involving all group leaders.

The department also organizes much of the research collectively. Basic chemicals and laboratory supply and consumables are funded and purchased collectively. The Postdocs and PhD students from all research groups have their lab benches in a single lab, and share the same office landscapes. Consequently, there is a helpful attitude and sharing of knowledge among students in the different research groups. Typically, inexperienced students can turn to experienced students in any other research group for help.

The group leaders have their offices in the same part of the department, so they meet daily in the corridor or at lunch or coffee breaks. This results in frequent opportunity for scientific discussions, and for quick help with scientific or administrative questions. All this helps to build collaboration among the different research groups. This has resulted in numerous collaborations among the groups which have gained from synergistic combinations of the diverse expertise at the department. The Covid-19 pandemic has limited physical interaction, staff working remotely as far as possible. However, the good collegial structure relies on the pre-Covid situation, and possibilities for more frequent face-to-face meetings will hopefully soon be possible.

d. Strategies for high quality

The successful research at this department has been based on application driven method development, on curiosity-driven science and academic freedom, and on collaboration with other excellent scientists. Our scientists identify important scientific questions in, for example, medicine or environmental research that need new technological methodologies to be solved. In the application of these methods we turn to the owners of the actual scientific question, for example medical scientists near the clinic. The very large amount of national and international collaborations indicate that we have been an attractive collaboration partner.

The group leaders are generally ambitious but humble and there is an open and friendly atmosphere at the department. Therefore, there is a constant scientific discussion going on where it is easy to get help for solving big or small scientific problems. Help is often close at hand to move projects forward. We have a mix of expertise in different interconnected subjects. It seems that the department has gathered a critical mass of scientific knowledge which is a fertile environment for novel scientific ideas.

The gene technology field is presently evolving very fast. An important factor for success is therefore the ability to pick up the latest methods and use them for new scientific questions. Or department has been at the research front in several of our research areas, succeeding to move with the general development. Finally, a factor for the success is probably simply that we have ambitious researchers that strive to publish in the best journals. This ambition for high impact publications also works as a quality assurance strategy.

A challenge as well as development opportunity is the growing data amounts in the field. The technique development will in the near future be driven by handling and analysis of massive data amounts. Maintaining and updating our computational competence in key areas such as machine learning and AI will therefore be of major importance for the department, and recruitment of students with this competence will be a key factor for continued success.

For optimal dissemination of our work we need to continue our collaborations with the end-users of our technology. We will continue to use public data repositories and open code repositories and to publish articles as open access, and increase our efforts with highlighting our results through press releases.

In conclusion, our strategy for high quality relies on recruitment of ambitious but humble scientists and a collaborative and friendly working atmosphere, collaborations with excellent scientists nationally and internationally, method development aimed at solving important societal needs, keeping up with technique development, and focus on publishing in high impact journals.

5. Interaction between research and teaching

a. Interaction between research and teaching at all three levels (BSc, MSc, PhD) of education

There is a high degree of interaction between research and teaching at the department of Gene Technology. In common for all three levels of teaching is that the competence at the department is both

broad and deep. This is because the teachers represent different sub-disciplines, giving a broad combined competence within the gene technology-field, while they are all active scientists close to the research front, with deep knowledge in their respective sub-disciplines. Thus, the broad competence of the teachers ensures that the biotechnology program at CBH has teachers with competence across all important aspects of the biotechnology field, while their high scientific standard in their respective fields implies that they can teach the state-of-the-art and include material that are not yet included in the course books.

There are numerous examples of research influencing the courses and increasing the quality of teaching:

At the bachelor level, the course in Genetics (BB1070) includes the latest findings about human evolution and its medical consequences and the course in Gene Technology (BB1190) includes the latest advances in forensics, based on expertise from the Evolutionary Biology and Forensics group.

At the master level, Molecular Biomedicine (BB2290) covers the use of genome and epigenome information in medicine and the latest genomic technologies used for prevention, clinical and treatment purposes. Bioinformatics (BB2441) covers fundamental methods and computational tools for analysing biomolecules, some of which have been developed by researchers at the department. Advanced microbiology and metagenomics (BB2560) covers how microbes affect the health of humans and impact the environment, and teaches omics-based methods (including some developed at the department) for studying microbes and microbiomes. The course Analysis of data from high-throughput molecular biology experiments (BB2490/BB2491) was given 2011-2019 and include a part where the students worked in real research projects (from the Environmental Genomics and Expression Bioinformatics groups and from invited colleagues) with actual data and research questions for which the research groups wanted answers. Furthermore, our research on the latest leading technologies, such as single cell analysis and spatial transcriptomics, is incorporated into the courses Applied Gene Technology and Large Scale Data Analysis (BB2255 and CB2040). Notably, the course Applied Gene Technology spans from cutting-edge molecular techniques to their related bioinformatics aspects, a design which is possible because of the interdisciplinary research that is currently ongoing at our department. Importantly, most of the bachelor and master course related above were initiated and designed by the teachers from Gene technology. The teachers from the Gene technology department have therefore had considerable impact on the biotechnology bachelor and master programs.

At the doctoral level, the high standard of the scientists at our department ensures that the PhD students are offered very proficient supervision for their research projects, and that they work in projects that are at or near the research front. There are many high impact articles published at the department and PhD students are mainly first and second authors on these. This shows that the doctoral students work in relevant and highly competitive PhD projects.'

The department plays a key role in the VR-funded research school on medical bioinformatics [Link]. Particularly, we are responsible for the course Algorithmic Bioinformatics. The department also offers four doctoral level seminar courses in DNA/RNA science (FCB3081-FCB3084), as well as four doctoral level seminar courses in gene technology (FCB3071-FCB3074). These courses form a backbone for PhD students at the department, as well as students at other departments and universities, who need to follow the latest trends in the gene technology field. Researchers at the department are also engaged in organising and teaching at SciLifelab courses for PhD students and other researchers from around the country. For example, the course Introduction to Metagenomics and Single Cell analysis (run 2014-2016) was co-organised by the Environmental Genomics group.

6. Impact and engagement in society

a. Relevance of research to society at large

Gene technology is of the greatest relevance to the society at large. The use of gene technology in general society is growing yearly, with biomedical and clinical applications at its core. A key example from the Gene technology department is the Clinical Genomics platform [Link] which performs DNA analysis on patient samples for the Karolinska Hospital and other hospitals in Sweden on a daily basis.

Biomedical and clinical applications is one of the major outcomes of our research. A prominent example is data generated with the spatial transcriptomics technology to create maps of gene activity in human organs, which in turn can be leveraged not only by other research institutions, but by pharmaceutical companies and in the long run public authorities in the health care sector. The impact of the spatial transcriptomics technology in particular is exemplified by its spin-off into a startup company which was recently acquired by one of the leading and most quickly developing technology companies in the field of nucleic acid research, 10X Genomics.

Another example is software from Statistical Biotechnology which is currently a cornerstone of the mass spectrometry-based Proteomics community, particularly the software percolator which is the almost universal method of choice for reporting the performance difference between wet-lab procedures. Percolator has also become an integral part of many commercial and open-source software. Furthermore, a software for accurately quantifying proteins (Quandenser) has recently been adopted by the clinical genomics community, as a method to effectively narrow down which SNPs that may have consequences for a patient's proteotype. Hence, patients may soon be treated based on information from this software.

Also, the technologies developed by the Experimental Genomics group are applied to clinical samples and thus of interest to the society. The core technology, the Droplet Barcode Sequencing (DBS) method, is used to haplotype genomes of colon cancer patients with the ultimate goal of accurately finding all small and large structural variants, which was previously, if not impossible, very difficult. Further development of DBS (to DBS-Pro) has led to investigating surface proteins on millions of single exosomes in lung cancer patients that have undergone therapy. The DBS-Pro technology is also applied for analyzing genes, transcripts and proteins of single cells in the immune system.

In addition to the biomedical and clinical applications, the research at our department has applications that are relevant and useful to a large number of other sectors in society:

The research about the history and evolution of the domestic dog and related canids is of great interest for the general public, for dog organizations and breeders, for public authorities, and for veterinary as well as human medicine. The dog has had a unique position in the human society during the last 10,000 years, as the first domesticated animal and the only one accompanying humans to every continent in ancient times. Today, the dog is an important part of the society, especially through its tight emotional bonds to humans, but also through its many duties, in guarding, hunting, herding, rescuing, tracking, and as aid for the disabled. Consequently, there is a huge interest for information about the history, evolution, behaviour and phenotype of the dog from the general public. This interest is mirrored by the large number of interviews given to the world press by group leader Peter Savolainen and the demand for public lectures and popular science articles, related below. It is notable that this research has rewritten history books and Wikipedia pages. The research also explains the genetics behind medical disorders, inbreeding and behaviour, knowledge which is of greatest importance for kennel clubs and breeders and which gives basic medical knowledge for veterinary and human medicine and for dog breeding. The research is also of importance for wolf and dingo conservation.

The gene technology department was pioneers in forensic analysis of DNA from animal hairs, and further method development of hair analysis is ongoing. This improvement of forensic methods is of interest for the police and the judicial system and for the safety of all citizens.

All projects of the Spatial Biology group are of interest to society. A collaboration with the Expression Bioinformatics groups and with the Sundström group at SLU can potentially affect the breeding of forest tree plants and contribute to improved forest tree seed production, of great interest to the Swedish forest industry. A study of how spaceflight alters the gene expression of mouse heart and brain, in collaboration with NASA, aims not only to advance spaceflight conditions for astronauts but also to translate the findings into modern medicine to promote human health on Earth. Moreover, the final goal of studies of the development of wheat spikes and the host-pathogen spatial gene expression interactions is to improve the overall production and resistance of agricultural plant species to benefit the world population.

The Environmental Genomics group's mapping of the Baltic Sea plankton biome contributes to better ecosystem models which are of importance for proper management of the sea. In collaboration with SMHI the group is also setting up sequencing-based monitoring of marine plankton, which will lead to faster and more accurate monitoring of plankton (including toxin-producing algae) in the waters surrounding Sweden.

Importantly, because of the Covid-19 pandemic, several ongoing projects have been adjusted to focus on Covid-19 related research and several new projects have been initiated. For example, several projects study the effects and potential treatment of COVID-19 in the lung, based on the spatial transcriptomics technology, and a project for monitoring Coronavirus counts in wastewater has been initiated.

b. Research dissemination beyond academia

The department works actively to disseminate the research results beyond academia through press releases, public lectures and popular science articles, and strives to be available to the news media. We also strive to publish all articles as Open access, which is an immense help to the non-scientist public to find and access articles they are interested in. Likewise, whenever possible, data is deposited in public data repositories.

Group leader Peter Savolainen has been interviewed about the research on dog history and evolution more than 60 times by World press, TV and radio, e.g., National Geographic, Discovery Channel, New York Times, Le Monde, El Pais, AP and BBC, and in TV and radio documentaries, and the work has been cited by several thousand newspaper and magazine articles and web pages. This dissemination to the society has been actively promoted through totally 10 press releases. Peter Savolainen has been engaged in the conservation of the Australian dingo, in letters to government and state Ministers for environment. He has also written four popular science articles and has given more than 40 popular science lectures, to the general public, school classes and kennel clubs.

Group leader Anders Andersson has lectured several times about “Our microbial planet” at “KVA’s inspiration day for teachers” where high-school teachers from all over Sweden learn about novel research, and at high-schools in Stockholm and at the “Senior-akademin”. He has produced a policy paper on the potential of meta-omics in marine monitoring and a report to the government agency Havs- och vattenmyndigheten on environmental monitoring of marine waters using metabarcoding [Link]. Group leader Stefania Giacomello has participated in a Swedish podcast series titled “Have we gone to Mars yet?” to disseminate her joint effort with NASA. Group leader Patrik Ståhl has written more than 1,000 technology review articles for the nationally leading magazine Life Science Sweden.

Innovation and seeking intellectual property rights are key components within the research at the department of Gene Technology. For example, we have applied for patents for biological findings as well as technical innovations, from plants to spatially resolved technologies. In the funded collaborations with industry we have noted an overall increased interest in IP protection and more than five patent applications were filed during 2018/2019. A specific example is group leader Patrik Ståhl who is author of more than 10 patents and patent applications and co-founder of two startup companies, with technologies based on his research. The latest of these, based on the spatial transcriptomics technology, was recently acquired by one of the leading companies in the field of nucleic acid research products, 10X Genomics. Another example is Pelin Sahlén who holds the patent for the Capture Hi-C application [Link], which has great potentials to propel the clinical translation of non-coding variants, which is particularly impactful in complex disease genomics.

The Statistical Biotechnology group strives to make all their source code available in open code repositories, such as GitHub and Sourceforge under open software licenses. This is based on a believe that the procedures for interpreting biological data should be kept transparent. This is not only a question of returning value for the received funds, but important for letting the scientific community ensure the integrity of reported results. A positive side effect is that open-source software is adopted into other labs and companies, and is also cited more frequently than proprietary software.

c. Sustainability and the United Nations' Sustainable Development Goals (SDG)

The research at the department is connected to several of the United Nations' Sustainable Development Goals (SDGs) since most of the molecular and bioinformatics methods developed at the department are applied in biomedical or environmental research.

Currently, the majority of application projects are related to ***SDG3 - Good Health and Well-being*** as they focus on human health: on understanding how the human body responds to stressors as well as causes and mechanisms of diseases. This leads to the ability to cure and diagnose illness such as heart failure, cancer, Alzheimer's, rheumatoid arthritis, and filariasis. In response to the Covid-19 pandemic, several new health related projects have been initiated. Several other projects are aimed at tackling environmental issues. For example, the metagenomics survey of the Baltic Sea has generated data that is now used to increase the understanding of crucial ecological processes driven by microbes, such as biogeochemical cycles and vitamin production, thus relating to ***SDG14: Life Below Water***. Moreover, the project on the Swedish Biodiversity Data Infrastructure (SBDI) will be an important instrument for monitoring biodiversity changes in response to global change in terrestrial and aquatic ecosystems, hence working towards ***SDG14*** as well as ***SDG15: Life on Land***. Also, the work about the genetics and genomics of wolves and dingoes, of importance for conservation and management issues, as well as the studies on Norway spruce reproductive processes, important for reforestation issues and sustainable forestry, are related to ***SDG15***. Furthermore, the studies of various plant species using genomics and spatial expression analysis tools are aimed at sustainable food production, targeting ***SDG2: Zero Hunger***. Finally, several projects concern development of improved forensic methods, which is of interest for the police and the judicial system as well as for the safety of all citizens, hence relating to ***SDG16: Peace and Justice Strong Institutions.***

Overall, since most of our projects are applied in biomedical or environmental research, the Department of Gene Technology has 80-100% research dedicated to sustainable development according to the United Nations' SDG:s. Thus, almost all our research is already related to sustainable development. Concerning actual sustainable development (human development while sustaining the natural systems), researcher Stefania Giacomello was recruited in 2018 to broaden the work on sustainable development into plant spatial biology. She has a Formas Future Research Leaders grant for reforestation related research, and funding from BASF for research on sustainable food production.

In conclusion, the majority of the research at the department relates to the United Nations' SDG:s, especially to ***SDG3 - Good Health and Well-being***, but also to several other of the goals. ***SDG3*** will continue to be of greatest relevance, but the department strives to expand its research about ecology and about food and forest production.

d. Structure for increased impact

The previous scientific success of the department is heavily based on application-driven method development and academic freedom. As exemplified by the three impact cases presented below, our researchers identify important scientific questions that need novel technological methods to be solved, and the methods are then applied to real life problems within, e.g., medicine and environment. Thus, it is our scientists' curiosity and their engagement in societal issues and the humanities, like medicine, environment, evolution and history, in combination with their excellent technological know-how, that drives the research. Through this process, our research has already had considerable impact on society, and it will be the basis for our future research.

Importantly, several of our most important projects are now coming into the phase where the practical application of the method is executed in, for example, medical studies. This is especially the case for the Spatial Transcriptomics and Capture Hi-C technologies described in the impact cases presented below. Similarly, the bioinformatics methods developed for analyzing bacterioplankton genomes are now applied to ecological studies of the Baltic Sea. Therefore, within the next few years, an increased impact on society is to be expected for the already ongoing projects.

Through collaboration between research groups, the technology developed at the department is now also exploited for addressing additional societal needs. For example, spatial transcriptomics, besides being vastly applied to medical questions, is employed to understand plant development and plant infection processes, with long-term impact on forest ecology and industry and on food production. Another recent project has a Space Biology focus, investigating the impact of spaceflight microgravity on mouse heart in collaboration with NASA. This will impact not only space flight programs but also our understanding of how extreme environments affect human health and how medicine can be pushed forward in order to respond to such changes.

Several projects are also changing to a more applied focus. For example, the research about dog evolution is focusing increasingly on the behavioural and morphological difference between dogs and wolves and among different types of dogs. This implies that the research, in addition to being of the greatest interest to a curious general public and to dog owners and breeders, is now becoming increasingly important as a basis for veterinary and human medicine.

Because of the Covid-19 pandemic, several of our projects are also adjusted to battle this disease, for example by improved lung diagnostics and monitoring of infection levels in the society, demonstrating our scientists' engagement and the impact of our research on this societal need.

The successful research at the department of Gene technology is based on curiosity driven research. It is to a large degree the scientists' engagement in societal issues that identifies the research questions, resulting in the great impact achieved by our research in the society. With the engagement of KTH in sustainability, with current very urgent societal challenges like Climate change and the Covid-19 pandemic, and with the United Nations' Sustainable Development Goals, there is an increased awareness of societal issues and of our ability as scientists to address them. This implies that our research will in future become even more focused on impact and engagement in society."

e. Impact cases

We here choose to present three impact cases which are all examples of the major strength of our research: application driven method development. Our scientists have identified important scientific questions in

medicine, environment or basic research that cannot be solved with the available technology. They have therefore developed novel methods that are subsequently used in applied research (see Panel 2 Impact cases 1-3 in Appendix 1).

1. Spatial Transcriptomics
2. Developing molecular tools for translating genomic information for clinical use
3. Interpreting biological data with Artificial Intelligence

Department of Protein Science

Self-evaluation
Head of Department: Professor Cecilia Williams

Included divisions:

Division of Systems Biology
Division of Cellular & Clinical Proteomics
Division of Affinity Proteomics
Division of Nanobiotechnology
Division of Drug Discovery & Development
Division of Protein Technology
Division of Protein Engineering

Department of Protein Science

1. Overall analysis and conclusions; strengths and development areas

a. Limited SWOT-analysis

Overall analysis of 1) Research strengths, 2) Research weaknesses, 3) Organizational strengths, 4) Organizational weaknesses and 5) Development areas.

	Strengths	Weaknesses
Research	Bulleted list, in order of magnitude. 1. Flagship projects 2. High-impact 3. Large external funding 4. Multidisciplinary and synergistic environments, including with private sector	Bulleted list, in order of magnitude. 1. Major funding tied to retiring faculty 2. Low diversity among faculty 3. Low success-rate in certain governmental funding panels for technology development (VR-NT)
Organisation	Bulleted list, in order of magnitude. 1. Internationally recognized faculty 2. SciLifeLab location and national facilities 3. Competitive recruitment practices 4. Well defined responsibilities	Bulleted list, in order of magnitude. 1. Increasing costs for rent and overhead 2. High turnaround of administrative staff 3. Maintaining and replacing expensive instrumentation

***Figure 1**. The Department of Protein Science (PRO) has assessed strengths and weaknesses related to Research and Organization, respectively.*

1) Research strengths: Relating to our research, we consider our our flagship projects, strong publication record (high number of publications, high-impact journals, high citation rates), and large external funding to be clear strengths. Our multidisciplinary, skilled, and synergistic competence (including protein engineering, nanobiotechnology, omics technologies, bioinformatics, system biology, cell biology, molecular biology, medical research, clinical competence) is also a strong feature. Our strong relations with both public and private sector, exemplified by several current or recent multi-partner competence centers being managed by the Department (such as VINNOVA-funded centers: CellNova [Link] and AAVNova; Novo Nordisk Foundation-funded KTH CHO Cell factory group, Center for Biosustainability; Wallenberg Centre for Protein Research (WCPR) [Link], and KTH Center for Applied Precision Medicine KCAP). Also, the many start-up companies associated with our faculties (including Affibody AB, Atlas Antibodies AB, Atlas Therapeutics AB, ScandiBio Therapeutics AB, Abclon, Amylonix AB and more) are strengths and indicative of our impact on society. This leverages impact and also increases opportunities for collaborative networks with industry and healthcare.

2) Research weakness: We have a strong concentration of external funding tied to one highly successful faculty member. This level of funding may be difficult to maintain once he retires or leaves, and thus constitute a risk or weakness during a transition period. Also, having heavily externally funded research as we do may risk a trade off with curiosity-driven research (in relation to industry demands) and/or premiering shorter and safer project (that can be published before the next funding cycle). Some weaknesses are also identified in a lower success rate in obtaining certain national funding for technology-driven research (VR-NT) than we think should be achievable. We also believe we would benefit from a higher diversity in our future faculty composition. For example, few faculties have a non-Swedish background (around 4 out of 20) or are non-KTH graduates (5 out of 20). Notably, we have no

female faculty at the Assistant and Associate Professor level, and no female faculty at all with diverse background (non-Swedish), signaling that we need to work on our gender and diversity strategies.

3) Organizational strength: Our internationally recognized faculty is a clear strength. This is evidenced by prestigious memberships in the Royal Swedish Academy of Science (KVA), Royal Swedish Academy of Engineering Sciences (IVA), and US National Academy of Engineering (NAE), awards such as from European Research Council (ERC), Knut and Alice Wallenberg foundation (KAW), including Wallenberg Academy Fellows, US National Institute of Health (NIH), and Swedish Research Council funding. Our recruitment practices that enable us to attract highly competitive faculty, including our location at the highly renowned campus SciLifeLab and the process to recruit SciLifeLab fellows in international competition, adds to this strength and helps us maintain a high level of our faculty. We also lead several national facilities for advanced technologies, located at SciLifeLab. This attests to our expertise in molecular technologies and our high-quality method development practicies. It also makes the technologies accessible to us and others. Further, we have well-defined responsibilities for maintaining instruments, protocols, and scientific exchange (seminar series, retreats) at the Department, providing a foundation for research of high quality.

4) Organizational weakness: On the organizational side, we are concerned by unsustainable increases in rent and other indirect or administrative costs. We suffer from a high turn-around of administrative personnel, which frequently leads to our faculty having to spend extensive time and attention to administrative tasks, and also risks miscommunication and errors (e.g., in financial reports to grants agencies). We do not have a fully secure plan for maintaining and replacing expensive instrumentation.

5) Development areas: Among these identified strengths and weaknesses, we have selected five development areas as very important for the future of the Department. Several of these (1-4) constitute a strategy to compensate for potential loss of funding and flag ship projects associated with retiring faculty.

1. **Expand our present flagship projects,** transferring knowledge and seizing emerging ‘data-driven life science’ opportunities.
2. **Attract new sources of funding,** increase synergies and networks to fully leverage our multi-disciplinary competence and enable design of new flagship project(s).
3. **Diversify faculty composition,** build on our strong SciLifeLab fellow recruitment strategy to attract internationally recognized candidates, including females.
4. **Improve success rate** in national technology-driven grant applications. For example, encourage participation in grant review study sections, encourage a high rate of applications, and provide guidance when needed.
5. Develop a plan to maintaining and expanding advanced instrumentation and **infrastructure**

b. Summary statement on contributions of departments on impact, infrastructure and sustainable development

Impact: The Department of Protein Science has contributed to significant impact, including through a large number of high-level publications in *Science* and *Nature*-family journals. Our faculty has impacted society and sustainable development through our flagship health projects (see *impact case 4*), outreach (see *impact case 5*), entrepreneurial efforts (see *impact cases 6 and 8*) and environmentally friendly breakthroughs (see *impact case 7*). During the year of the pandemic (from March 2020) the Department has made an outstanding contribution to society, contributing to both healthcare, testing, pandemic surveillance, research and expertise related to Covid-19 (see *impact case 9*). Further, by

creating the major open access resources, and by managing academia-industry centers, we contribute to society with knowledge, data, and collaborations. We contribute to an active and creative scientific environment through our ideas, competences, skills, data for data-driven research, networks of national and international collaborations, and taking part in numerous meetings, seminars, discussions, and conferences. Our activities have received significant national and international attention and impacted and moved forward the state-of-the art of the research fields, health care, or society significantly. Several faculties from the Department have also been active communicating with the surrounding society, governmental agencies, government, the regional healthcare (Region Stockholm), politicians, and media (national television, newspapers, radio).

Infrastructure: Our Department was instrumental in the establishment of SciLifeLab (2010), with the aim to provide state-of-the art technologies at a national level. We have contributed to infrastructures through our cutting-edge technologies, methodological developments, and expertise. Resources generated within our Human Protein Atlas (HPA) project [Link] has enabled facilities at SciLifeLab to offer them to researchers nationally. As a result, we have over time contributed significantly to the national facilities located at SciLifeLab. Today, we host five national facilities. The more than 50 000 antibodies generated within HPA are used in the facility for ***Cell Profiling***, which provides immunofluorescence staining of cells at a subcellular level, and for ***Translational Plasma Profiling,*** which uses multiplexed immunoassays to surveil proteins in human bodily fluids. The more than 42 000 human protein fragments generated within HPA are used for custom-designed protein and peptide arrays in the facility for ***Autoimmunity and Serology Profiling.*** This facility provides serological analysis of autoantibodies in serum or plasma, as well as assists with antibody validation. Further, our protein engineering expertise underlies two facilities within the Drug Discovery Development platform: ***Human Antibody Therapeutics*** which produces human antibodies from in-house constructed phage libraries, and ***Protein Expression and Characterization*** which produces recombinant proteins from bacterial and mammalian expression systems. The facilities are highly important to us. We are at a technological university, and we are a technology-driven department. The field develops rapidly, and we depend on novel technology, related instruments, competences, and networks. This is the base for our activities. Hosting these facilities help us sustain the technologies and expand our method development, it also provides access to high-level expertise and a variety of high-quality techniques for our health/environment-focused research, also for those of us not experienced in each specific technology. Our facilities and infrastructure activities expand our networks both nationally and internationally. Together, this helps us maintain the high quality of our research and attract external funding.

Sustainable development: Nearly 100% of the research performed at the Department is related to sustainable development. By focusing on improving health or environment we impact sustainable development in many areas. As described in more detail in *impact cases 4-9*, our research contributes to better understanding of disease development and their treatments, addressing UN ***SDG3 Good health and wellbeing.*** Our research has also led to phase I-III clinical trials (e.g., for liver disease), development of affibodies for cancer imaging, biomarkers for neurodegenerative diseases, testing for Covid-19 disease, antibodies and wastewater-based Covid-19 community surveillance. Other work is directed towards development of point-of-care tests for diagnostics, including detecting critical neonatal illnesses to reduce the mortality rate in newborns, especially in low-income countries. This work is specifically addressing ***SDG 3.2: To end preventable deaths of newborns and reduce neonatal mortality***, and ***SDG 3.8: Access to safe, effective, quality and affordable essential medicines and vaccines for all***. Further, through genetic manipulation of bacteria, work is ongoing to create novel environmentally friendly fuels which contributes to ***SDG 7: Affordable and clean energy***.

RAE2021 exercise: The Department wishes to receive constructive feedback relating to whether we are developing in the right direction and tips on how to improve further. For example, which aspects

are most worthwhile to focus on? Can or should we collaborate more within the Department? Are there suitable funding mechanisms we should strive for as a group rather than individuals? If so, how can we increase synergies between individual faculties and divisions and combine forces? We also would like feedback on how to optimally advance and support our faculty and staff.

2. Research profile

a. General information of the department

Dept. of Protein Science [Link] (PRO, Head: Cecilia Williams, ≈180 employees, 20 faculties).

PRO is one of nine Departments within the School of Engineering Sciences of Chemistry, Biotechnology and Health (CBH) at KTH. Main activities at PRO are related to molecular life sciences and cancer, both of which are two strategic research areas at KTH (Vision 2027). The Department is divided into seven divisions and located at two geographically separated campuses: Five divisions are located at SciLifeLab and two at AlbaNova University Center [Link] (see Figure 2). PRO hosts five National Facilities, all located at SciLifeLab (see figure). PRO also manages multi-partner competence centers related to protein/cell technology and is part of several governmentally funded research environments (Swedish Research Council, VR).

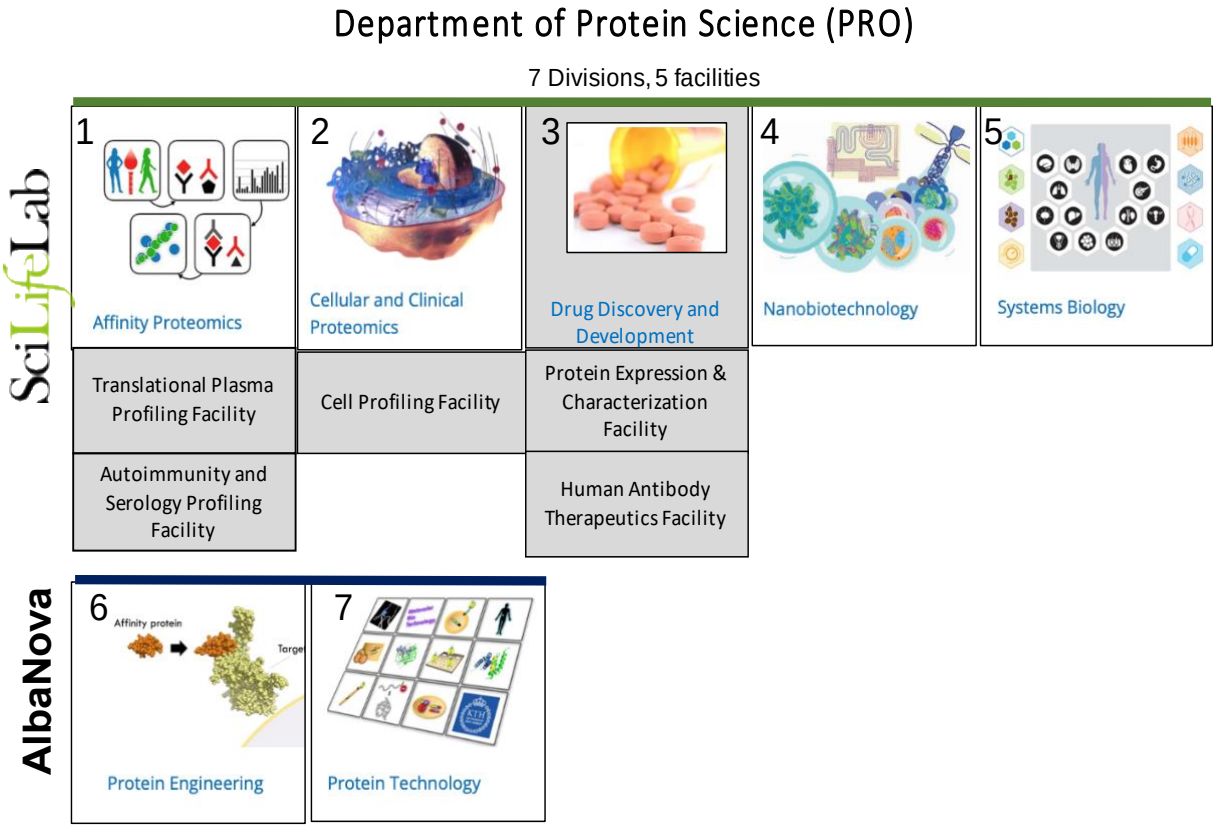


Figure 2. *The organization within the Department of Protein Science into seven divisions and located in two different locations, AlbaNova University Ccenter and SciLifeLab. Grey shade indicates National Facilities.*

b. Central research questions and themes, knowledge gaps addressed, main research activities

Central research themes and main activities: PRO researches questions fundamental to technology-driven health-related research. Central themes that several of the different divisions are oriented around include *protein engineering* and related method developments (display technologies, directed evolution, mammalian cell production systems, affinity-based assays, spider silk engineering, antibody

validations, microfluidics) for *biomedical applications* (medical imaging tracers, biomarker discovery/assays, diagnostics, therapeutics, Covid-19 testing and surveillance). This is applied to *basic research* within *human health* (including within basic biology, cell biology, cancer development, neurodegenerative disorders, cardiovascular diseases). Environmental research (fuel production, wastewater) is also performed, but with fewer faculties involved. The research has generated several so-called flagship projects, of which the Human Protein Atlas (see *impact case 4*) and combinatorial affinity protein engineering including affibodies and ADAPTS (see *impact case 8*) are examples. The research has generated numerous fruitful international collaborations, center formations with academia and industry, and spin-off entrepreneurial companies. Our research profile is cross-disciplinary, and includes protein engineering, genomics/transcriptomics, proteomics, microbiomics, cell biology, molecular biology, mechanistic molecular biology research, imaging analysis, bioinformatics, system biology, cancer research, medical research, animal models, nanobiotechnology, and clinical competence. Central activities and compositions of each division are described in further detail below.

1. Division of Affinity Proteomics (Head: Peter Nilsson, ~20 employees)
This division includes research groups led by two professors (Peter Nilsson and Jochen Schwenk), and two National facilities (the Translational Plasma Profiling Facility headed by researcher Claudia Fredolini and the Autoimmunity and Serology Profiling Facility headed by researcher Ronald Sjöberg). The research in this division is based on *development and application of multiplexed immunoassays* technologies for detection and *characterization of proteins and antibodies primarily in the circulation or other bodily fluids*. One common theme of the research groups is the large-scale and high-throughput search and validation of protein biomarkers to assess health and disease states. The Nilsson lab focuses on proteomic profiling in *neurogenerative and inflammatory disease*, while the Schwenk lab researches markers of *cancer and metabolic disorders*. Their labs share their exquisite infrastructures, and resources from the Human Protein Atlas, with their respective facilities to make these accessible for the research community. The division has also, together with division of Protein Technology, developed serological methods for the analysis of SARS-CoV-2 antibodies in blood (applied in more than 130,000 clinical samples by March 2021).

2. Division of Cellular and Clinical Proteomics (Head: Emma Lundberg, ~30 employees)
This division includes three research groups led by professors (Emma Lundberg, Cecilia Williams, Jacob Odeberg), and a National Cell Profiling facility (headed by researcher Charlotte Stadler). This facility provides national access to advanced technology for spatial proteomics. The research of this division uses large-scale technologies applied to analyze cells, clinical material, or animal model systems to understand *basic molecular mechanisms underlying health and diseases*. In the interface between bioimaging, proteomics, and artificial intelligence the Lundberg group aims to define the *spatiotemporal subcellular organization of the human proteome*, with the goal to understand how variations in protein expression patterns contribute to cellular function and disease. Prof. Lundberg is also the creator and director of the Cell Atlas of the Human Protein Atlas. The Williams group studies *cancer*, specifically the *role of hormones* in colorectal cancer development, and corresponding interaction with the *gut microbiome*, using mouse models and clinical samples, omics, mechanistic, and functional studies. The Odeberg group investigates *cardiovascular disease* with a focus on biomarkers and the role of the endothelium, using clinical samples, in vitro models, omics and functional studies.

3. Division of Drug Discovery and Development (Head: Anders Olsson, ~10 employees)
This division consists of two national facilities: Human Antibody Therapeutics (HAT, led by researcher Helena Persson) and Protein Expression and Characterization (PEC, led by researcher Anders Olsson). Both facilities are part of the Drug Discovery and Development (DDD) platform at SciLifeLab. The platforms national mission is to develop academic discoveries into innovations. The two facilities deliver *well-characterized protein targets and therapeutic antibody candidates to drug development* projects driven by the platform and provide academic access to competence and instrumentation.

Research performed within this division is primarily focused on *method and technology development*, in order to further improve its capabilities.

4. Division of Nanobiotechnology (Head: Aman Russom, ~20 employees)
This division includes two faculty-led research groups (Prof. Aman Russom, Assoc. Prof. Håkan Jönsson), and one adjunct industrial researcher. Building on state-of-the-art micro and nanotechnology, this develops and applies *microfluidics for biological and medical applications*. This includes tools for high-throughput droplet microfluidics, sorting of cells, and production of *3D microtissue for screening and precision medicine applications*. Further, microfluidic-based *point-of-care devices* for cellular and molecular diagnostics, including nucleic acid analysis, of various conditions, are developed.

5. Division of Systems Biology (Head: Mathias Uhlén, ~40 employees)
This division includes four faculty-led groups (Prof. Mathias Uhlén, Assoc. Profs. Paul Hudson, and Adil Mardinoglu, and Assist. Prof. Saeed Shoaie) who focus on systems biology-related research. This spans from photosynthetic bacteria, microbial research, human biology, systems medicine, metabolic and network modelling, protein science, and precision medicine. The topics range from basic research into human and microbial biology to more applied research, including clinical applications in *cancer, infectious diseases, cardiovascular diseases, autoimmune diseases* and *neurobiology*. The Hudson lab pursues applied and fundamental research in the field of metabolism of autotrophic (*CO2-fixing) bacteria*, photosynthetic cyanobacteria, and litho-autotrophic, H2-consuming bacteria. The Mardinoglu lab develops GEnome-scale Metabolic models (GEMs) for human cells/tissues and employ these comprehensive models in the analysis of the omics data obtained from subjects with diseases including *obesity, non-alcoholic fatty liver disease*, *type 2 diabetes* and certain types of cancers. The Shoaie lab is headed by our newly appointed SciLifeLab fellow who set up a new research group in 2020. The main focus of his group is the *microbiome* and its relation to clinical parameters. The Uhlén lab heads the *Human Protein Atlas* flagship program [Link] and is responsible for its data management and visualization. Subgroups led by researchers include the Al-Khalili lab (*neuromuscular disorders* and the database Antibodypedia) [Link], the Fagerberg lab (integration of multi-omics data, related to the *precision medicine* efforts and the Human Protein Atlas program), and the Edfors lab (*targeted proteomics* for absolute quantification of proteins in blood and tissues).

6. Division of Protein Engineering (Head: Per-Åke Nygren, ~25 employees)
This division includes five research groups led by professors (Per-Åke Nygren, Amelie Eriksson Karlström, Stefan Ståhl, Torbjörn Gräslund, John Löfblom). With a protein engineering focus, they develop antibodies, including bi-specific, and small and robust non-immunoglobulin affinity proteins using *rational and directed evolution principles*. The goal is to develop *tailor-made affinity proteins* for specific applications. This involves applying various techniques, including gene fusion, chemical conjugation, sortase-mediated coupling and photochemistry. Several projects address *cancer*, including concepts for *in vivo* imaging, delivery of drugs, signal blocking, exosome research, and pro-drug activation via the use of engineered affinity proteins. General concepts for delivery of biologics across the blood brain barrier, regulation of their circulation half-lives and *in vivo mode-of-actions* are also addressed. Large efforts are directed towards therapeutic use of affinity proteins in *neurodegenerative diseases,* including Alzheimer´s disease and Frontotemporal dementia. Also, biotechnological uses of protein engineering are addressed, such as novel concepts for mild affinity chromatography using innovative ligands developed by protein engineering. Recently, efforts have been initiated to integrate affinity protein technology with multi-domain scaffolds to facilitate structural determinations of proteins via *cryo-EM*.

7. Division of Protein Technology (Head: Sophia Hober, ~35 employees)
This division includes three research groups led by professors (Sophia Hober, My Hedhammar, Johan Rockberg), as well as an AlbaNova-located section of the Human Protein Atlas project (led by researcher Hanna Tegel). This division pursues research related to protein production and purification, including *development of novel proteins and protein-based materials for biomedical applications*. The

division has set up a large *pipeline for protein production* using mammalian cells or bacteria. Engineering of cell lines based on omics analyses are applied to improve the *production quality and yield*. The proteins are to be used as therapeutic products, research tools in phenotypic assays, or as targets for generation of novel binders. Together with the Division of affinity proteomics, this division has taken a lead in the development of serological tests for CoVID-19 and served the Swedish community by analyzing clinical samples (>130,000 tests). Another focus is the development of protein-based binders for biotechnology and medical applications. Small *bispecific binders* capable to bind both serum albumin (for half-life extension) and a therapeutic target are developed. Furthermore, a calcium-dependent scaffold tailor-designed for usage in affinity chromatography purifications is developed. This can be used to enable mild elution conditions for e.g., protein-based therapeutics. Moreover, the division has established accredited lab environments for work with viral systems and has a strong focus *improved secretion of adeno-associated virus* which could allow a higher productivity of *gene therapy* agents are developed (Rockberg). The focus is on both capsid engineering and cell engineering for bioproduction of gene therapy, to improve targeting, viral titer and viral quality. Also, within the division, *recombinant spider silk proteins* are functionalized at genetic or protein level, to achieve bioactive materials (Hedhammar). The main focus is to utilize bioactive silk to mimic the *in vivo* environment and promote cells to form functional tissue for clinical applications (e.g., transplantations) or model systems (e.g., drug testing).

Knowledge gaps addressed: The Department's research activities address biological, medical or environmental knowledge gaps, focusing especially on technological limitations. From our technology-oriented aspect, we identify knowledge gaps where we can contribute. Close and interactive collaborative work with clinical researchers, hospitals, or industry, ensures a high relevance of researched questions. Below are examples of knowledge gaps we are actively aiming to address.

Basic cell biology. Humans have around 23 000 different basic proteins, and numerous more variants. We lack information of the function of the majority of these. Knowledge of each protein's precise location is a prerequisite to begin to understand its function. ***We do not, however, know where all proteins are located, in which tissues, cells and subcellular location***. This is addressed in our flagship project the Human Protein Atlas (HPA, *impact case 4*) which used large-scale production of antibodies to all proteins along with high-throughput analysis to address tissue and cell locations of the human proteome. Building on this, the *Cell Atlas* (*impact case 5*) set out to determine corresponding subcellular localizations, using large-scale immunofluorescence and image processing. Expansions of this project has focused on understanding and characterizing proteins specifically involved in the cell cycle.

Diseases. **Several major diseases lack efficacious treatments because we don't understand the molecular mechanism(s) behind the disease.** As part of HPA the Pathology Atlas aims to close major knowledge gaps relating to proteins altered (expression, location) in **cancer**. Other research teams at PRO studies specific proteins involved in cancer or cancer development, applying omics, bioinformatics, protein engineering, molecular, functional and animal studies. This includes studying unknown roles of non-coding RNAs, and how hormones impact cancer development. Other research is dedicated to understanding **cardiovascular diseases**, which includes the HPA *Blood Atlas*, and efforts to identify corresponding biomarkers (see below). Also, the brain and **neurological diseases** is a focus that several groups approach. From defining the *Brain Atlas*, to biomarkers and therapeutics (see below)

Biomarkers: Biomarkers in plasma or serum, can be used for screening, for diagnostics, or to determine treatment progress. Biomarkers in tissue sections can be used for diagnostics, to determine treatment (precision medicine) and/or prognosis. However, **a number of diseases cannot be screened or diagnosed, because there are no known biomarkers (yet).** The human blood contains numerous proteins secreted by tissues, and antibodies produced by the immune system, which are reflective of the disease. However, the **knowledge of which proteins are secreted into the**

blood, which disease they may signal, or which autoimmune antibodies are circulated, is poor. One reason is difficulties in performing large-scale high-quality analysis of the proteome, in sufficiently large cohorts. This gap is addressed by several groups at PRO. Some work on defining which proteins are present in blood (*Blood Atlas*) and can be secreted into the blood (the *Human Secretome Project*) [Link], others develop methods for affinity-based biomarker discovery, and investigates cohorts and perform validation of proposed biomarkers. Examples include efforts to identify serum biomarkers for **autoimmune disorders, neurodegenerative diseases, cancer, thrombosis, diabetes**, as well as general **wellness-illness**.

Point-of-care devices. Once a biomarker has been defined, for it to have an impact on healthcare, a user-friendly application is required. If less complicated laboratory equipment is needed for the analysis, the dissemination will be quicker and the impact higher. Developing diagnostic micro- and nano-scale point-of-care devices has potential to significantly improve health care. Such application can significantly speed up diagnostics and be especially suitable for low-income countries that may lack advanced labs. Activities at the Department include developing microfluidics-based methods for "liquid biopsy", for example testing blood for cancer diagnostics, nasopharyngeal samples or saliva for SARS-CoV-2 detection, and isolation of microorganisms from whole blood for sepsis diagnostics. Other directions include development of 3D microtissues for "organ-on-chip" applications.

Precision medicine. The arrival of omics tools for large-scale analysis of genes and proteins such as genomics, proteomics, transcriptomics, and metabolomics, opened up new possibilities to study both health and disease in a high-throughput manner. This led to a paradigm shift in the outlook of adapting therapies to each individual. By using new and more targets that are specific for each subtype of disease or condition, and for each patient, treatment can be tailor made. Enabling precision medicine is an important field of activities for PRO (see e.g., *impact case 4*). Resources created within HPA and the *Human Secretome Project* have been combined with the infrastructure at SciLifeLab to initiate one of the world's most comprehensive "personal omics profile" program. Many individual projects in several divisions are also focused on identifying new drug targets and related biomarkers.

Therapeutics: ***Drug development*** has a very direct societal importance and is of high relevance for precision medicine. Our Department strives to contribute to this in several areas (some examples are provided in impact case 8). Antibody therapeutics, for example, have enormous potential to treat, prevent or improve previously uncurable diseases. Antibodies or related binders (e.g., affibodies) can, in theory, be directed to any protein or peptide, and can also be combined with therapeutics for targeted delivery such as chemotherapeutic agents to specific cells or organs, and thereby reduce adverse effects in the patient. Binders can also be used for imaging and diagnostics, for example detecting cancer metastases. However, there are unknowns in ***how to optimally select or design binders that are specific, stable and do not raise adverse effects in vivo***. Groups at PRO use rational and directed evolution principles (incl. development of new methodologies) to develop small and robust non-immunoglobulin affinity proteins and bi-specific antibodies tailor-made for specific applications (including cancer and neurodegenerative diseases). Also, diverse antibodies and proteins are **needed as research tools** and to develop therapeutics, and there is a high need to produce these. To meet this need, PRO develops high-throughput pipelines for expression, purification and characterization of antibody candidates at small and medium scale. This includes development of a panel of analytical methods to assess antibody candidates based on their biophysical properties, systematic evaluation of signal sequences for optimized expression in mammalian cells; generation of novel scFv and Fab phage libraries; design of new approaches for affinity maturation of antibodies; establishment of methods for hit-finding using DNA-encoded libraries; addressing difficult-to-express proteins, and to tailor proteins for drug development purposes. ***Limiting factors and bottlenecks for production of proteins in mammalian cells*** are identified, and ***cell engineering is used*** to improve the yield and quality of ***biologicals***. This includes improving the host cells or modifying the expression strategy.

Novel materials for medical applications: There are high needs to develop ***new materials*** for various medical applications. A breakthrough approach based on recombinantly engineered spider silk is used at our Department. Via genetic functionalization of soluble monomeric silk fiber constituents and/or site-selective conjugations to already formed fibers, bio-compatible materials can be made. These fibers can promote cell growth and differentiation *in vivo* and are suitable as **materials for medical applications**. The main focus is to utilize the bioactive silk formats to mimic the *in vivo* environment and promote the formation of functional tissues that can be used for transplantations or as model systems for example for drug testing (*impact case 6*).

Microbiome: There is increasing evidence that the bacterial composition (microbiome) within our bodies, such as in the human gut or oral cavity, impacts our health and predisposition for different diseases. However, there are ***large knowledge gaps, including what the compositions of the different microbiomes are, how they change and why, and how specific bacterial species influence clinical parameters and vice versa***. These questions are addressed by several research groups at PRO. From how to best handle this type of big data (methods for optimal genomic and bioinformatic analysis, system biology approaches), to studies of relationship between microbiomes the host and parameters such as diet, obesity, inflammation, hormones, gender or cancer, and how specific bacterial strains correlate with clinical parameters.

Green biofuels: Another aspect of bacteria is their usage in production. Other groups at PRO are investigating their ability to **produce biofuels from sunlight and carbon dioxide** (see *impact case 7*). Finding new efficient sources to provide renewable energy without the use of fossil fuel is one of the most important tasks for a future sustainable society. A system where bacteria fix CO_2 and convert it directly to fuel, photosynthetic cyanobacteria that can produce energy directly from sunlight, or litho-autotrophic bacteria that use energy derived from hydrogen are venues that are investigated here.

c. Contributions to the advancement of the state of the art within the research fields of the department

KTH has led the massive effort to create the Human Protein Atlas (HPA) and a multitude of related atlases. This effort has resulted in more than 600 scientific publications by consortium members, including many in high-impact journals, such as *Nature* and *Science*. The resource is publicly available for the wider life science research community. Due to its fundamental importance, this KTH-based map of human biology has been selected by the EU-based organization ELIXIR [Link] as a ***core database resource***. The atlas is one of the most visited biological databases in the world, with more than 150,000 visits by researchers from numerous countries each month. The project has also generated an enormous resource of antibodies which are useful for technology development, research of human biology and disease, and development of precision medicine (*impact case 4*). This had led to generation of a *Cell Atlas* characterizing subcellular localizations and, for example, the changing proteome of the cell cycle (*impact case 5),* and a *Pathology Atlas* characterizing human cancers. PRO has made numerous contributions to understand cancer and its development, including specific biomarkers, molecular mechanisms, and how cancers are impacted by sex and hormones. Biomarkers for cardiovascular diseases, neurological diseases, autoimmune disorders, and wellness have also been proposed and evaluated in clinical cohorts. Related point-of-care prototypes have been developed and tested (for example for diagnosis of sepsis, cancer, and COVID-19). Small scaffold-based affinity proteins, affibodies, and ADAPTs, have been developed to targets of relevance (e.g., Alzheimer's disease and cancer), and corresponding pre-clinical proof-of-principle therapy studies have been performed. Similarly, improved delivery of chemotherapeutic agents to tumors via affinity principles have been achieved (pre-clinically) and a number of medical imaging concepts have been described of which one is in late-stage clinical development for HER2-based breast cancer diagnostics. A platform for production of adeno-associated virus (AAV) for gene therapy has been designed, and methods for production of functionalized spider silk materials for biomedical applications have been developed. Significant improvements in the design and production of proteins or antibodies for therapeutics, including achieving increased circulation times, improved mammalian cell production and purification,

have brought the state-of-the-art forward. Toolboxes have been developed to express difficult-to-express proteins, to generate high-quality combinatorial protein libraries for selection purposes, and to validate antibodies. Methods for imaging analysis, including machine learning, have been explored in multiple projects. Lately, the Department has contributed to speed up SARS-CoV-2 testing, COVID-19 serology, and exploring pandemic wastewater surveillance. Further, several genetically modified cyanobacteria strains, capable of converting light and CO2 into the biofuel butanol, have been developed, along with novel tools for cell modeling, genetic engineering and cell analysis. Altogether, PRO has significantly expanded the horizons of what is achievable.

d. Quality and quantity of contributions to the body of scientific knowledge

The bibliometrics for our Department, as provided for 2012-2019 by the KTH-internal publication record system DiVA, show that we are very productive. We have in total published 586 peer-reviewed articles over this time period, with a total *fractionalized publication record of 219.6*. This is considered very high. The yearly record is relatively stable over recent years, with a peak year 2016 (Figure 3), and overall a slight upwards trajectory. Not only do we publish a large number of studies, but we are also highly cited: We have a fractionalized ***publication impact around 2.0*** *(Web of Science, WoS, 2012-2017,* Figure 4*). Fractionalized* average citations are above 11, more than 13% of fractionalized publications are top-10%-cited articles, and few are uncited (9.3% on average). The journals we published in are of high quality, the *fractionalized journal impact* is up to 1.41 (2016-2019, Fig. 4). Furthermore, the journal impact has also increased over the years (from 1.22 to 1.37-1.41, per WoS, Figure 4). Our share in top-20% journals is high and increasing (from 43% 2013-2015 to 50.7% 2017-2019, Scopus). Over 96% of our full count of published articles are included in WoS).

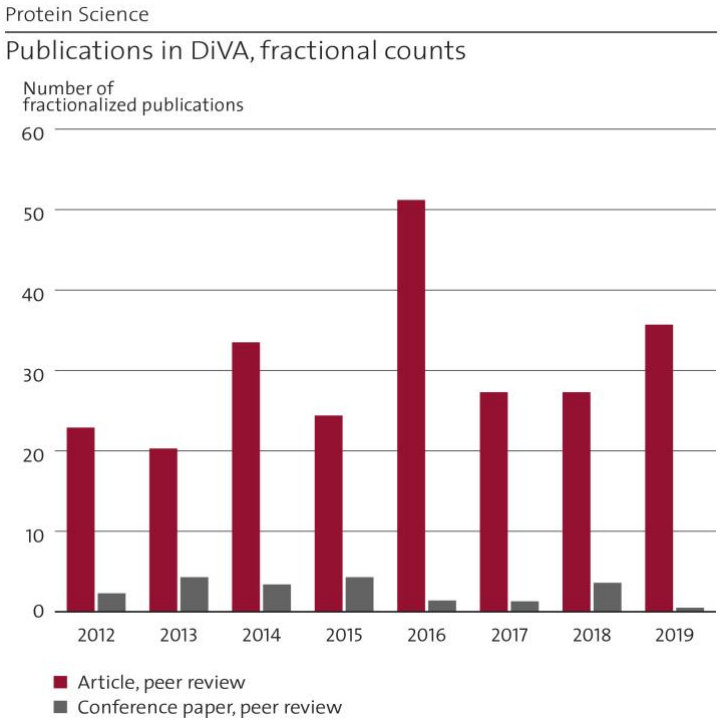


Figure 3. *Fractional counts of publications 2012-2020.*

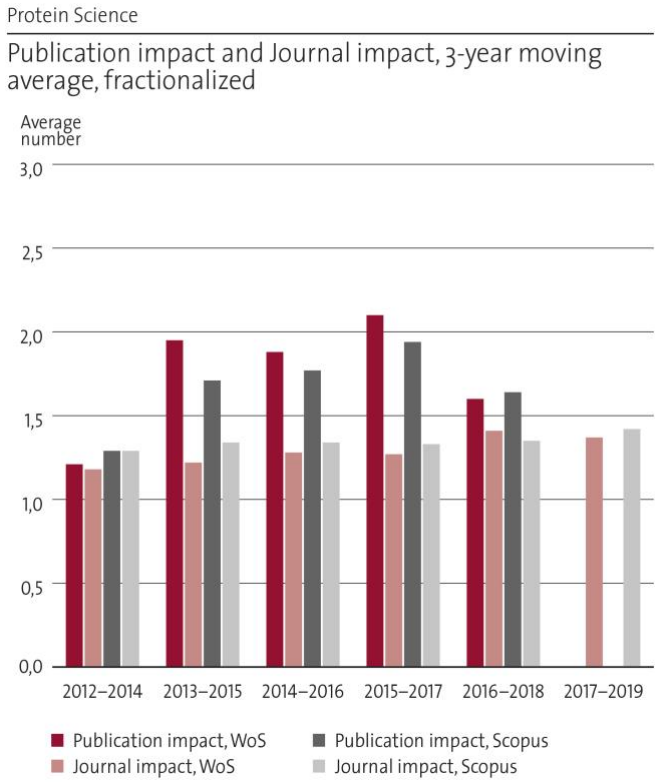


Figure 4. *Publication and journal impact 2012-2019.*

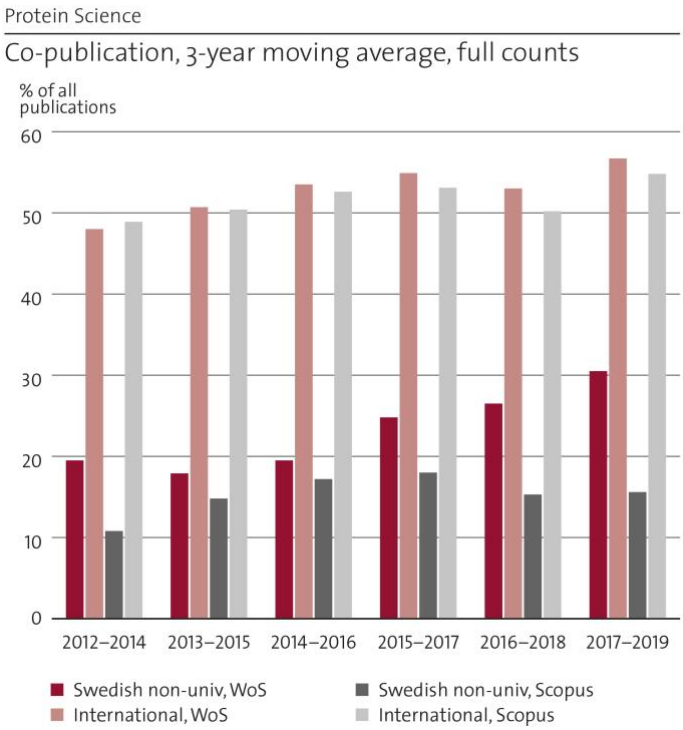


Figure 5. *National and international co-publications 2012-2019.*

It is noteworthy that PRO holds one of the most cited scientists in Sweden (Uhlén). Moreover, over half of our publications are co-published with international collaborators, with a slight year-to-year increase. We have also produced 52 doctoral theses during 2013-2019. Overall, our contribution to scientific knowledge puts us among the top departments at KTH and is in line with the ***most prominent research institutions in the world***.

Publications that the Department wants to highlight.

We have published multiple articles in the high impact journals *Nature*, *Science* and *JAMA* since the last RAE evaluation 2012. This list of ten articles exemplifies some of the breath of the research more recently performed at PRO. (Bold fold indicates authors from the Department, # indicates corresponding author, JIF indicates its recent journal impact factor, citations according to Scopus):

1. **Mahdessian D, Cesnik AJ, Gnann C, Danielsson F, Stenström L, Arif M, Zhang C, Le T, Johansson F, Shutten R, Bäckström A, Axelsson U, Thul P,** Cho NH, Carja O, **Uhlén M, Mardinoglu A, Stadler C,** Lindskog C, **Ayoglu B,** Leonetti MD, Pontén F, **Sullivan DP, Lundberg E#**. (2021) Spatiotemporal dissection of the cell cycle with single-cell proteogenomics. *Nature* 590:649-654 [Link] (JIF>42)
2. Havervall S, Rosell A, Phillipson M, Mangsbo SM, **Nilsson P, Hober S**, Thålin C. (2021) Research Letter: Symptoms and Functional Impairment Assessed 8 Months After Mild COVID-19 Among Health Care Workers. *JAMA* e215612 [Link] (JIF>45)
3. Bruzelius M, **Iglesias MJ**, **Hong MG**, **Sanchez-Rivera L,** Gyorgy B, Souto JC, Frånberg M, Fredolini C, Strawbridge RJ, Holmström M, Hamsten A, **Uhlén M,** Silveira A, Soria JM, Smadja DM, Butler LM, **Schwenk JM**, Morange PE, Trégouët DA, **Odeberg J#.** (2016) PDGFB, a new candidate plasma biomarker for venous thromboembolism: results from the VEREMA affinity proteomics study. *Blood* 128:e59-e66. [Link] (JIF>16) 16 citations.
4. Subramanian K, Neill DR, Malak HA, Spelmink L, Khandaker S, Dalla Libera Marchiori G, Dearing E, Kirby A, Yang M, Achour A, **Nilvebrant J, Nygren P-Å,** Plant L, Kadioglu A, Henriques-Normark B. (2019) Pneumolysin binds to the mannose receptor C type 1 (MRC-1) leading to anti-inflammatory responses and enhanced pneumococcal survival. *Nat Microbiol.* 4:62-70. [Link] (JIF>15) 29 citations.
5. A micro-dispenser for long-term storage and controlled release of liquids. **Kazemzadeh A,** Eriksson A, Madou M, **Russom A#**. (2019) *Nat. Commun*. 10:189. [Link] (JIF>13) 9 citations.
6. Andersson S, Sundberg M, Pristovsek N, **Ibrahim A**, Jonsson P, Katona B, Clausson CM, Zieba A, Ramström M, Söderberg O, **Williams C#**, Asplund A. (2017) Insufficient antibody validation challenges oestrogen receptor beta research. *Nat. Commun*. 8:15840 [Link] (JIF>13) 93 citations.
7. **Yao L, Shabestary K, Björk SM, Asplund-Samuelsson J, Joensson HN, Jahn M, Hudson EP#**. (2020) Pooled CRISPRi screening of the cyanobacterium Synechocystis sp PCC 6803 for enhanced industrial phenotypes. *Nat. Commun*. 11:1666 [Link] (JIF >13) 22 citations.
8. Eisenhut P, **Mebrahtu A, Moradi Barzadd M, Thalén N,** Klanert G, Weinguny M, Sandegren A, Su C, Hatton D, Borth N, **Rockberg J#**. (2020) Systematic use of synthetic 5'-UTR RNA structures to tune protein translation improves yield and quality of complex proteins in mammalian cell factories. *Nucleic Acids Res*. 48(20):e119 [Link] (JIF>11)
9. **Nilebäck L, Widhe M, Seijsing J,** Bysell H, Sharma PK, **Hedhammar M.** (2019) Bioactive Silk Coatings Reduce the Adhesion of *Staphylococcus aureus* while Supporting Growth of Osteoblast-like Cells. *ACS Appl Mater Interfaces* 11:24999-25007 [Link] (JIF>8.5) 10 citations.

10. **Ståhl S#, Gräslund T, Eriksson Karlström A**, Frejd FY, **Nygren P-Å, Löfblom J**. (2017) Feature review: Affibody Molecules in Biotechnological and Medical Applications. *Trends Biotechnol*. 35:691-712 [Link] (JIF>14) 104 citations.

e. Engagement in national and international research collaboration within academia and its outcomes

National academic collaborations (selected examples)	**Value & outcome**
Profs. Vladimir Tolmachev/Anna Orlova (UU)	Provides expertise in tracer-development for medical imaging, and performs mice studies in imaging and targeted drug development (> 50 joint publications)
Prof. Jan Linnros (KTH), Dr Apurba Dev (UU), Prof. Rolf Lewensohn (KI), Dr Kristina Viktorsson (KI).	Multi-disciplinary collaboration established with focus on method development for the analysis of extracellular vesicles in lung cancer.
Profs. Caroline Graff, Lars-Olov Wahlund (KI)	A consortium Swedish FTD initiative, in frontotemporal dementia has been established [Link], conducting multidisciplinary FTD research
Profs. Adnane Achour (KI), Birgitta Henriques Normark (KI), Anders Håkansson (LU).	Provides expertise in structural biology and bacterial infections research. Co-drivers of several projects.
MD Johan Hartman (Karolinska University Hospital)	Provides pathological and clinical expertise within breast and colorectal cancer, clinical samples (joint grants and publications).
Prof. Lars Engstrand (KI)	Provides expertise in microbiome research, registry epidemiology, and SARS-CoV-2 testing (joint publications).
Prof. Per Hall (KI)	Provides expertise on breast cancer, joint HMT grant and collaboration on analysis of circulating biomarkers.
International academic collaborations (selected examples)	**Value & outcome**
Prof. Thomas Wisniewski, New York University	Provides expertise in Alzheimer's disease, performs studies in transgenic mice (joint publications]
DTU Denmark, Prof. Lars Nielsen (UCSD), Prof. Nathan Lewis (BOKU Vienna), Prof Nicole Borth	Provides expertise in CHO cell engineering and systems biology
Abclon, Seoul South Korea	Bispecific antibodies, *in vivo* experiments
Stanford, California	Developmental atlas, grants
Prof Jonna Frasor, University of Illinois at Chicago	Provides expertise and reagents for hormone signaling and relation inflammation, joint publications
Assoc. Prof. Maria Bondesson, Indiana University	Provides assays for endocrine disruptors, zebrafish assays, joint publications
Weiliang Xia, Shanghai Jiao Tong University	Provides expertise in exosomal research, joint publications.
Prof. Thomas Sakmar, Rockefeller University	Provides expertise in protein interactions studies of membrane receptors
Tromsö Hospital, Norway	Clinical samples, grants

f. Follow up from previous evaluations

The key **general** recommendations from RAE 2012 to our **School** (then *School of Biotechnology*) and relevant central **KTH** guidance were to:

1) Recruit truly international tenure-track assistant professors educated abroad in order to maintain intellectual diversity; Use the school's top international reputation to attract absolute prime international researchers to KTH, take advantage of this unique position in its succession planning, KTH could be become the "CERN" of Biotechnology in Europe.

Response: PRO has recruited three international tenure-track assistant professors as SciLifeLab fellows.

2) Work actively in achieving a more equal gender balance.
   Response: Our Department is working along the CBH school developmental plans towards increasing female faculty/teachers. We aim to raise awareness on all levels. The Head of Department has taken part in a KTH-wide leadership program with focus on gender (GOFL) and all Division Heads have been offered education in gender balance and equal treatment. We have specifically worked to expand female faculty, recruiting two female faculties (My Hedhammar 2014, Cecilia Williams 2015) and actively worked to retain current faculty (Emma Lundberg, 2020).
3) Even though the School is very successful, its leadership should consider restructuring its research areas (UoAs) and Divisions in response to the dynamic scientific developments and funding situation. The same goes for certain individual professors and researchers that the panel found misplaced in the current organization.
   Response: A major re-organization in 2018 led to the creations of a new School (CBH), and our new Department (PRO).
4) The leadership of the School must also consider improving the cooperation and synergy between the various divisions. It was felt that there today was a risk for duplication of efforts and fragmentations of the research and infrastructural investments.
   Response: The five national facilities that PRO hosts at SciLifeLab has reduced fragmentation of major infrastructure investments. Our Department's aim to improve synergies is still work-in-progress.
5) On a more specific note, the School needs to think more strategic with respect to its access to prime competence in Structural Biology.
   Response: We have covered needed expertise through recruitments (*e.g*. postdoc with expertise in X-ray crystallography), and there is some relevant expertise available within the CBH School (e.g., crystallography: Christina Divne, Dept. Industrial Biotechnology; Cryo-EM: Carsten Mim and Hans Hebert, Div. Structural Biotechnology, Dept. Biomedical Engineering and Health Systems).

The key **specific** recommendations from RAE 2012 for our **Department** (then *Medical Biotechnology* and *Proteomics*) were to:

Recommendation: Reinforce maintaining of platforms state-of-the-art

Response: We have developed our platforms and expanded significantly, including in the set-up of 5 national facilities.

Recommendation: Investment into forward-looking activities, such as investment into advanced bioinformatics, e.g., development of a bioinformatics professorship in pathways and network analysis, or recruit a professor in bioinformatics.

Response: We have recruited considerable bioinformatic competence (including faculty Adil Mardinoglu, 2015; Saeed Shoaie, 2020).

Recommendation: Regarding the HPA program: for the plasma/serum studies, we should invest in having two affinity reagents for each protein, and in a strict clinical validation strategy together with statisticians to go from discovery to clinical applications.

Response: This has been set up on a wide scale.

Recommendation: International mobility should be more encouraged. Both from the incoming and outgoing side.

Response: Several faculties have been recruited after international mobility (Paul Hudson, USA, 2014; Adil Mardinoglu, UK, 2015; Cecilia Williams, USA, 2015; Saeed Shoaie, UK, 2020). One of our faculties has been Visiting Assoc. Prof. at Stanford School of Medicine (USA) for 2.5 years

(Emma Lundberg, 2018-2020), with support from KTHs program for sabbaticals. Further, our PhDs increasingly go for a postdoc fellowship (e.g., to Stanford).

Recommendation: The division of Nanobiotechnology has a limited number of senior persons, which should be addressed with new strategic hires.

Response: During RAE 2012, this Divison only had one faculty, Professor Helene Andersson Svahn. Since then, the Division lost this highly successful professor (who left for industry) but has promoted its group leader to associate and then full Professor (Aman Russom). Furthermore, a new assistant, now associate, professor (Håkan Jönsson) was hired. There has also been a rearrangement of the departmental organizational structure since then. The Department's divisions now, in general, are smaller, with most having only 2-3 faculties per division. Nevertheless, although this division with its two faculties now has as many senior faculties as other divisions, its topic is somewhat more separate, and we believe this division is still in need of reaching a larger critical mass. We are considering further strategic hires, alternatively, other ways of organizing this Division in order to increase synergies.

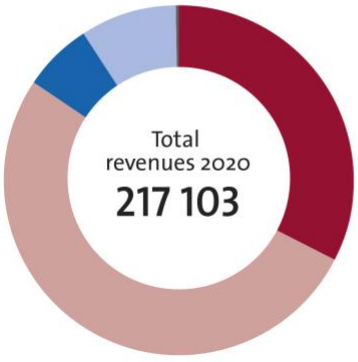
3. Viability

a. Funding; internal and external

Our Department is research-intensive. Governmental funding (faculty base funding through KTH, including so called Strategic SFO Funds) provides only a third of our budget (32.6%, 2020) (Figure 6). Furthermore, this funding appears to be decreasing (from 38% in 2016). We hope this may increase slightly with the recent governmental proposal to increase the basic funding, but we fear this will be offset by increasing overheads and rental costs. Our main funding is external (research grants: 58%).

Protein Science

Sources of research income (2012, 2016, 2020)



Research income, kSEK	2012	2016	2020
Government grants for research and doctoral studies	0	68 625	70 943
Research grants	0	101 993	112 916
Contract research funding	0	731	13 861
Other revenues	0	7 500	19 371
Financial income	0	226	12
Total revenues	0	179 075	217 103

Figure 6. *Comparison of research incomes (2012), 2016 and 2020*

Private foundations represent a majority of this, especially KAW (Knut and Alice Wallenberg foundation), Erling-Persson family foundation, Schörling foundation, Tussilago foundation (Lichtenstein), Novo Nordisk Foundation (Denmark), the Swedish Cancer Society, Heart and Lung foundation, and the Brain foundation. The Governmental funding agencies, such as the Swedish Research Council (VR), VINNOVA, and FORMAS, are also very important for PRO. The external funding is obtained in competition. While this ensures a high quality of the projects, it is also usually short term (1-5 years). This impacts how we plan our research, tilting it towards safer and shorter-term projects that can generate publications before next grant round. In conclusion, our Department is more dependent on external funding than most departments at KTH, and especially on private foundations.

b. Academic culture

Our departmental culture values support and sharing. We often share instruments and to make this work well, each instrument has a responsible person. This person teaches users how to handle the instrument, organizes lists for bookings, and arranges for services and repairs. Similarly, different labs or divisions commonly share know-how or skills with each other, at all levels, from students to professors. Each lab has regular lab meetings to help each other out and offer advice, and these interactions expand on common weekly or bi-weekly journal clubs, and on our regular division meetings. At each site, morning meetings are held a few times per semester to discuss common issues between divisions (instruments, practicalities, or research related) at so called BIO-fika (AlbaNova) or KTH-fika (SciLifeLab) meeting ("fika" means coffee break). Many of our faculties also takes part in committee work for the school (CBH) or central KTH and are active in the global academic citizenship (peer-review, academic editors, grant review for funding bodies, and other).

We consider PRO to have a creative and positive atmosphere, that we value. A positive atmosphere stimulates creativity and collaborations. We value both curiosity-driven and applied research. For creativity to thrive, research cannot only be viewed as excellent and highly competitive, but also requires a measure of “play” and that the individual researcher feels secure to try out new ideas. We try to encourage a friendly atmosphere, that senior faculty sets a good example and help younger faculties with advice, offer collaboration, and other assistance. Professors usually have a non-official type of mentoring to younger tenure-track or aspiring researchers.

Several seminar series function as meeting points between researchers at all levels, both locally at AlbaNova and at SciLifeLab (including its wider community). Both sites offer topic-oriented seminars or events, PhD and postdoc cross-sectional presentation series, and numerous seminars with international invited speakers. Organizing committees are responsible for arranging most of these events and are usually changed yearly. Both sites welcome all staff. Further to this, PRO (in non-pandemic times) organizes regular off-site retreats, often together with the neighboring Department of Gene Technology. These are occasions where PhD-students and postdocs present their research, with plenty of time for discussion and possibilities to identify synergies. This is combined with team-building activities, dinner, and over-night stays. This has proven to be an important factor for our supporting and positive academic culture.

c. Current faculty situation

PRO has around 180-200 employees (2017-2019), including PhD students. We have 20 faculty (assistant professors, associate professors, full professors) of which six are women (although one is on long-term leave of absence) (Figure 7). Faculty thus makes up about 10% of the Department’s employees. Compared to most departments, this is a low proportion. The majority of employees (2019) are researchers (28%), research engineers/engineers/technicians (25%), and PhD students (24%). Postdocs make up a smaller fraction (7%). The many researchers and engineers are, in part, explained by the National facilities hosted by PRO (Translational Plasma Profiling, Autoimmunity and Serology Profiling, Cell Profiling, Human Antibody Therapeutics, and Protein Expression and Characterization) and the Human Protein Atlas flagship project. These types of entities require a larger fraction of positions for routine work, which are not as suitable for doctoral projects or academic careers.

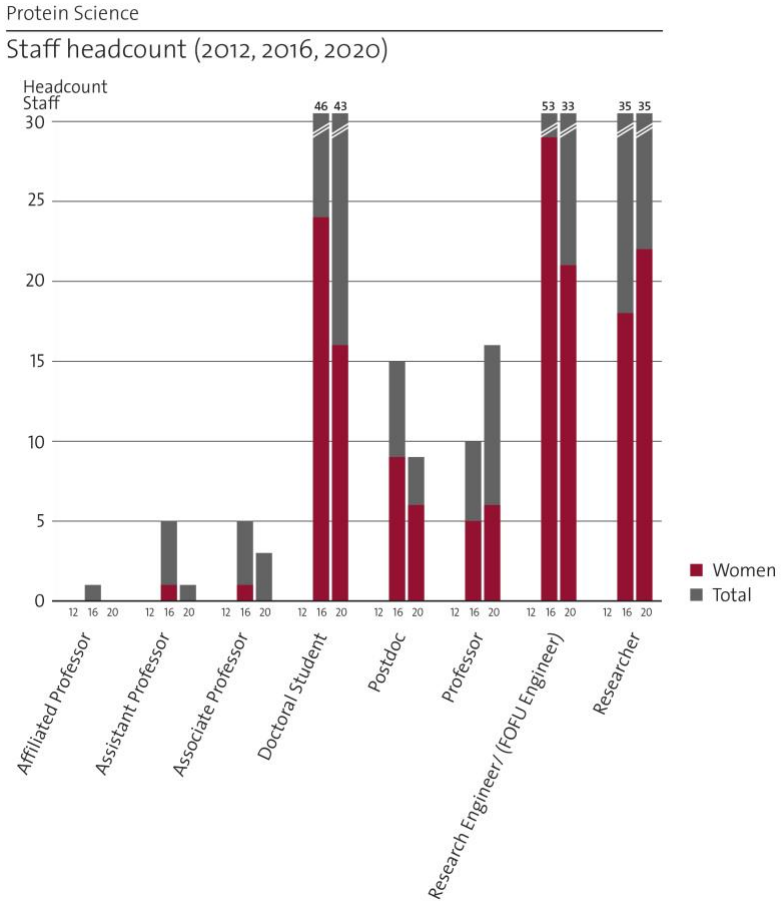


Figure 7. *Staff headcounts (2012), 2016 and 2020*

Composition of research team: As evident from the description above, PRO's 20 faculties, along with around 150 researchers, research engineers, doctoral students, and postdocs apply various aspects of biotechnology to drive national facilities and a wide repertoire of research projects focused on health, basic biology, and environment. This requires staff with diverse backgrounds and expertise in specific technologies, tools, and subjects. Several academic sub-disciplines are present. We also benefit from numerous multi-disciplinarily collaborations, where SciLifeLab function as an important hub for networking and information exchange.

We have one professor approaching retirement age (which is currently 68 but will increase to 69). It will be 8-10 years until the next faculties in line 'have to' retire. However, if desired by the individual, retirement can be taken from age 55. This means that nearly half (9 out of 20) of our faculty could choose retirement in five years' time.

PRO at a whole is balanced with respect to gender (nearly 50/50). However, there is a clear gender gap depending on position. Most women are hired at non-career-oriented positions (researchers/research engineers/technicians). Although a majority of master students in our field are women (approx. 60%), our PhD-students are now more often men (almost 2 out of 3). At the faculty level, it is even more unbalanced, with only 30% (6/20) women. Among active faculty (one of our female faculties is on long-term leave), 25% are women. We especially lack female representation among our younger faculty, as all our associate (3) and assistant (1) professors are men. Thus, unless we recruit new faculty, our gender balance will not improve.

We also have few faculties with a non-Swedish (around 4 out of 20) or non-KTH (5 out of 20) background.

d. Recruitment strategies

Our faculty recruitment process is an integrated part of the CBH school recruitment cycle. Our Department can nominate areas or topics that fits our strategy for required competences. We nominate topics based on synergy we envision, or competences we are missing, in areas/divisions where we have a budget proposal for how to provide as start-up funding. School-wide nominations are then discussed and ranked at the School's leader group level (Heads of Departments, Head of School, Head of Administration). Once approved, a wide search is initiated, and ads posted in *Science*, *Nature* Jobs, LinkedIn, and other venues. In addition, since a part of PRO is located at SciLifeLab, we can utilize the SciLifeLab Fellows program to attract and recruit tenure-track assistant professors. This is a career program aiming at strengthening Swedish research in Molecular Biosciences and the research environment at SciLifeLab. The four host universities have used parts of their governmental strategic funding (SFO) to recruit a number of young research leaders. At KTH, the topics for these faculty positions can be suggested by the five different Schools, which are then prioritized by the KTH SciLifeLab committee. Priority is based on strategic relevance of the subjects for the development of SciLifeLab and considers coordination with the other host universities. This is also discussed in the SciLifeLab Strategic Council and involves the SciLifeLab management and representatives of the other host universities. By providing the new recruitments with a substantial financial start-up package, it has been possible to attract excellent, internationally competitive candidates for the positions. Three KTH SciLifeLab Fellows, in the subjects of Microbial Bioenergy Production (Paul Hudson), Systems Biology (Adil Mardinoglu), and Bioinformatics (Saeed Shoae), have been placed at PRO. These recruitments have already proven successful through extraordinary scientific output.

Non-faculty recruits include those with complementing expertise, such as Anders Olsson with significant industrial experience of drug development at AstraZeneca as Head of Protein Expression and Characterization facility and Head of Division at PRO. Several other heads of national facilities have been recruited similarly (incl. Helena Persson Lotsholm from Lund University, Claudia Fredolini from George Mason University, USA). Others have complemented research groups with specific expertise at different levels (e.g., researchers with animal in vivo expertise; post doc with crystallography expertise). At the doctoral student level, several international recruits have been made through the Marie Curie Networks (at least 8), and many other through each research group in open competition. At all levels, we aim to complement the Department with skills and backgrounds that can create synergies.

e. Infrastructure and facilities

Our faculties were instrumental in the establishment of SciLifeLab. SciLifeLab aims to provide state-of-the art technologies at a national level as well as to create synergies and creative environments for break-through life science. In relation to technologies, we have contributed with cutting-edge technologies, methodological developments and expertise. We have also provided resources generated within our HPA project. The more than 50 000 polyclonal antibodies generated within HPA are used in the facility for *Cell Profiling*, which provides immunofluorescence staining of cells at a subcellular level, and *Translational Plasma Profiling,* which uses multiplexed immunoassays to surveil proteins in human body fluids. The more than 42 000 recombinant human protein fragments generated within HPA (for use as antigens) are also used for custom-designed protein arrays applied in the facility for *Autoimmunity and Serology Profiling*. This facility provides analysis of autoantibodies in body fluids, as well as antibody validation. Further, our protein engineering expertise underlies two national facilities within the Drug Discovery Development platform: *Human Antibody Therapeutics* which produces human antibodies from in-house constructed phage libraries, and *Protein Expression and Characterization* which provides recombinant proteins from bacterial and mammalian expression systems. As a result, we now host 5 national facilities. While these national facilities are important to Sweden as they provide our technologies, expertise, and resources to the whole country, they are also highly important to us. We are a technological university, and we are a technology-driven department. The field develops rapidly, and we depend on novel technology, and related instruments, competences

and networks are the base for activities. Hosting these national facilities help us sustain the technologies and expand our method development, it also provides access to high-level expertise and a variety of high-quality techniques for our health/environment-focused research, also for our younger researchers. Furthermore, the synergies and creative environments that SciLifeLab generates, and that we are part of, are incredibly important for our research. These activities expand our knowledge, networks both nationally and internationally, enables collaborations, inspiration for novel ideas and boosts creativity. All this helps us maintain a high quality in our research and attract external funding.

4. Strategies and organization

a. Goals for development 5–10 years ahead

Our ambition for the future is to maintain, but also expand, the strong position we have acquired. Our Department's success in attracting external funding is viewed as a strength, but also a risk. Considering that a disproportional part of this funding is tied to one or a few individuals, our funding situation may change drastically when these faculty retire or depart. In case unexpected changes to funding or faculty employment occur, this large proportion of permanent staff on external funding is a significant financial risk. We will need strategies and time to manage this. We also need to plan for transfer of knowledge, especially within our flagship projects which were initiated by faculty that are likely to retire in the coming years. Further, we foresee opportunities for chartering new territory, including taking charge in a massive KAW-funded effort into data-driven life sciences [Link]. Another aspect is that we note that grant applications for governmental funding within the field of Biotechnology (reviewed by the VR-NT study section), which is an area that we are strong in, has an unpredictably low success rate. Our research appears to not fit into VR-NTs funding priorities, or the applications are not written in a form that its panels value. Our overall conclusion is that PRO should plan for transfer of knowledge, aim to diversify our external funding, including striving to increase the success rate in VR-NT funding but also find new avenues for funding. We also need to look over how we can plan for our general smaller-scale infrastructure at the Department, including the aggregated service costs for maintaining the instrument park in good functional shape which is demanding (e.g., for biosensors, flow sorter, HPLC), replacement or new common machines and other. Based on our own analysis of our current performance, strengths and weaknesses, we have identified four development areas as very important for the future of the Department: **Expand our flagship projects,** for example by developing in new directions, seizing emerging 'data-driven science' opportunities**, attract new sources of funding,** by increase synergies and networks to fully leverage our multi-disciplinary competence and enable design of new flagship(s) project, **improve success rate** in national technology-driven grant applications, and to maintain and expand instrumentation and **infrastructure.** Our strategies to reach these goals are described here in short:

1. Expand our flagship projects including **transfer of knowledge**: We have, in general, systems set up to organize transfer of knowledge. At the smaller scale, for example, the Department has a clear definition of responsible persons for all methods, instruments and reagents, including a process to hand over responsibilities when positions end. There is also an existing informal mentorship between leading PIs and younger faculty and researchers, who can grow to take over different subprojects. However, for our large flagship projects, this also requires capacity to write proposals and fund exceptionally large and costly projects. Transfer of such tasks, including expansion of large flagship projects, is however not routine tasks that we have a set up process for. Nevertheless, we have initiated work to seize emerging 'data-driven life science' (DDLS) opportunities, and we have placed a faculty strategically in the steering group of the KAW-funded DDLS effort. PRO will aim to recruit one of the assistant professorships that is part of this effort in the coming couple of years and has prepared to create such a position. We are already at the forefront of KTH in both research and entrepreneurial activities (patent, spin offs), and we aim to continue this.

2. Attract new sources of funding. If we can increase synergies to fully leverage our multi-disciplinary competence, we believe we have the capacity to design of new flagship project(s). Building on the above, the KAW DDLS will be an important source of funding. We also apply for *e.g*. VINNOVA funding for larger projects, we see opportunities for increased EU funding, and we will also aim to identify new sources.

3. Improve success rate in national technology-driven grant applications: We believe we have the capacity to be more successful in these funding mechanisms than we currently are. We may need to encourage our faculties to submit more proposals, work on the structure of grant applications that are submitted or offering more support to applicants (focused grant writing workshops, internal review system). We may also need to work on being active strategically and take part in the peer review process, to shape the focus of what is being funded.

4. Maintain and expand advanced instrumentation and infrastructure: As stated above, our infrastructure is extraordinarily important to us. We need to assure the infrastructures development, that they stay ahead of the state-of-the art, and that we maintain key personnel. We will need to make a plan for applications for infrastructure funding, for budgeting and purchases of new equipment. We also need to develop a career management plan for staff at our National facilities. Currently, as they are not faculty, they do not have well-defined career stages.

b. Congruence with university-level goals for research as set out in “A leading KTH ” as set out in KTH´s “Development Plan 2018-23” (page 5) [Link].

Our research is very much in line with two of the prioritized areas of KTH. As described throughout this text, PRO has application-oriented research that is to a large extent curiosity-driven basic research, with numerous interdisciplinary collaborations. We focus on areas prioritized by KTH, including Life Science Technology (sustainable development) and advanced computational frameworks for imagining and machine learning (digitalization) to battle society's growing challenges: human disease and global environmental changes. The general goals for KTH and at the CBH School level also fit well with our Department: our faculty is active in education that reflect the top-level research conducted (see below), our applied research stems from fundamental research and multidisciplinary collaborations (see above), we have top-level infrastructures, and our research and education have an impact on society (see *impact cases*). Further, we strive to develop professional leadership, and include digitalization, sustainable development, internationalization and gender equality in our activities.

c. Leadership structure and collegial structure

Our department is led by a Head of Department (Cecilia Williams), and sorted into seven divisions each led by a Division Head. Each division has a number of research groups led by Principal Investigators (PIs), which are our faculty members and, in some cases, researchers. The PIs, to a large extent, form the research milieus. The two divisions that are located at AlbaNova, Protein Engineering (Nygren) and Protein Technology (Hober), have chosen to have their research groups intermingled, while at SciLifeLab the research groups are more spread out and for practical reasons defined by the localization (house and floor) of each group or division. Two divisions that are co-localized at SciLifeLab, Affinity Proteomics and Cellular & Clinical Proteomics share lab spaces with Systems biology. A leadership group assists the Head of Department with strategy, work environment issues, educations, and other taks. The leadership group includes our deputy Head (Stefan Ståhl), our seven Division Heads (Sophia Hober, Per-Åke Nygren, Aman Russom, Peter Nilsson, Emma Lundberg, Mathias Uhlén, Anders Olsson) and our vice School Head (Amelie Eriksson Karlström) and meet regularly 4 to 5 times a year.

d. Strategies for high quality

The collegial structure at both sites aims for a collaborative atmosphere, with significant sharing of instrumentation and resources, and with a possibility for everyone to take part in the research

discussions. In this manner everyone's competence is viewed as a resource that can maximize the quality of our research. Both senior and junior faculty/researchers and colleagues are involved in supervision, in creating and maintaining policy documents relating to supervision, instruments, lab responsibilities, calling for regular floor meetings, cleaning and organization days, seminars, and journal clubs (where also publishing, journal quality and impact, review processes, are discussed). We encourage both curiosity-driven research and applied, problem-solving research. With active supervision and a clear structure for maintaining top working conditions (including policy documents and tasks divisions, responsible persons for instrumentation, processes, and reagents) we ensure the highest standard of our activities. Further, since the majority of our funding is from external sources and obtained in competition, there is a continuous strive to publish the results in high-impact journals, which helps increase the chances to maintain the level of external funding. We also aim to recruit top talents from the forefront of research in the order to get influx of valuable competence. Our faculties are key to achieving our goals, and in terms of our faculty composition, strategic new hires can assist in maintaining a rapid and current development. We are well suited for an upcoming focus on big data, but we need to combine this and create synergies with focused basic and translational research. We anticipate we may need to recruit at least three assistant or associate professors in the next 5-10 years. Faculty funding and relevant teaching assignments will be critical for such renewals. If the SFO funding will be continued, this may give us an opportunity to recruit SciLifeLab fellow(s) and attract excellent young new faculty. Considering our lack of younger female faculty, we will take steps to ascertain that female candidates apply. We will aim to identify suitable candidates and design the positions accordingly and encourage applicants by reaching out to them directly. We will also pay attention to the selection of expert reviewers and aim to select unbiased experts that will give all candidates a fair ranking. We are well aware that a balance of genders and other backgrounds among faculty, students and staff, is important from several perspectives. Recruitment from as large pool as possible increases the opportunity to recruit highly merited persons. Having many perspectives presented when formulating research topics and interpreting the data expands the chances for high-quality research. Also, it is critically important that students know that there are equal opportunities and that gender, nationality, or a diverse background should not hinder aspirations. Finally, it is simply not acceptable if we are biased in who we hire.

5. Interaction between research and teaching

a. Interaction between research and teaching at all three levels (BSc, MSc, PhD) of education

There is a high degree of interaction between research and teaching at the Department. Our teachers represent different sub-disciplines and are able to give a broad combined competence within affinity proteomics, protein engineering, biomarkers, therapeutics and precision medicine, nanobiotechnology, systems biology, cell biology, biomedicine, novel biomaterials, microbiome and a large spectrum of basic cell biology and clinical biotechnical applications. Since all are active researchers close to the research front, their deep knowledge ensures that PRO contributes to the teaching within the Biotechnology program KTH. There are numerous examples of research influencing the courses.

At the bachelor level, our faculties teach *Introduction to Biotechnology* (7.5 European Credit Transfer ECTS, BB1010, Johan Rockberg), *Gene technology* (7.5 ECTS, BB1190, Paul Hudson), *Microbiology* (6 ECTS, BB1030, Johan Nilvebrant), *Eucaryotic cell biology* (7.5 EDU, BB1160, Torbjörn Gräslund), *Analysis of biomolecules* (6 ECTS, BB1200, John Löfblom) and *Purification of biomolecules* (6 ECTS, BB1210, My Hedhammar).

At the master's level our faculty teaches in three master programs: Medical Biotechnology, Medical Biotechnology, Industrial and Environmental Biotechnology, and Molecular Techniques in Life Science. The latter represents a joint educational effort between KTH, Karolinska Institutet, and Stockholm University, with teaching that reflects the research focus of SciLifeLab. Our faculties teach *Downstream processing of biological products* (7.5 ECTS, CB2010, Sophia Hober), *Proteomics* (7,5

ECTS, CB2080, Jochen Schwenk), *Immunology* (7.5 ECTS, BB2446, Jacob Odeberg) and *Clinical applications of biotechnology* (7.5/6 ECTS, CB2020/CB2021, Cecilia Williams).

We also lead several project courses, where student take part in active research. These are sorted into degree projects in the first and second cycles: Degree Project in Biotechnology (First Cycle, 15 ECTS, BB101X, BB102X, BB103X, BB104X, Cristina Al-Khalili Szigyarto), Project in molecular life science (Second Cycle, 7,5 ECTS, CB2050, Håkan Jönsson), and Degree Project in Biotechnology (Second Cycle, 30 ECTS, BB201X, Torbjörn Gräslund)

The topics of the courses are designed based on what a graduate in respective program is required or expected to know when entering the workplace (whether in industry, governmental agencies, or academia). The faculties develop the courses and shaped them around the research front where they are active. This ensures an up to date, near the research front level of the course. The medical/environmental focus is in line with our research. The education and the courses are gradually updated to reflect the performed research (including new methods, new technology, new applications). Old courses are discontinued, and new courses are started, to reflect changing societal needs. We strive to maintain the principle that every teacher should be active in research, and that every faculty should be active in teaching.

PRO further contributes to the quality of education through faculties or researchers being responsible persons for undergraduate education (vice GA, Torbjörn Gräslund) and for two of the master programs (Medical Biotechnology and Industrial and Environmental Biotechnology, through Program Director, PA, Cristina Al-Khalili) at the CBH school.

At the doctoral level, faculty and researchers are active as supervisors to PhD students within their research projects at or near the research front. The high standard of the researchers at our department ensures that there is a high scientific level of the projects, and many high impact articles are published at PRO with PhD students as first and second authors. Our department also offers doctoral level seminar courses in proteomics and omics (FCB3010, FCB3011, FCB3012, FCB3013), protein engineering (BB3430, BB3431, BB3432, BB3433), protein science (BB3021, BB3022, BB3023, BB3024), and molecular biotechnology (BB3061, BB3062, BB3063, BB3064) which are all directly connected to the research being performed. These courses form a backbone for PhD students at the department, as well as students at other departments and universities, who need to follow the latest trends in the protein technology/life science field. Researchers at the department are also engaged in organizing and teaching other courses, including in relation to the SciLifeLab national facilities and technologies, for PhD students and researchers from around the country.

6. Impact and engagement in society

a. Relevance of research to society at large

A major focus of our research is to improve public health and to invent solutions related to a more sustainable world. This includes providing a large number of fundamental tools (databases over the human molecular landscape) useful for other researchers and research-intense pharma companies, as a base for research efforts and hypothesis building. These efforts have the potential to provide immense long-term impact on human health. This public data is accessible also for an interested general audience, through a pedagogical web-interface at the portal www.proteinatlas.org [Link]. and educational videos. Several concepts and reagents emerging from the department have already been implemented into practical use in the society (HER2 *in vivo* imaging, other affibody/ADAPT-based reagents, treatment of NAFLD, Pyrosequencing). This has, for example, resulted in 16 start-up companies which have their origin at the Department and at present seven human clinical trials which are being pursued by these start-up companies. Further descriptions are provided in our impact cases (see Section 6e).

b. Research dissemination beyond academia

The science we produce are disseminated beyond academia through several approaches. One is through our competence centers, that include multiple collaborations with industry and the healthcare sector, enabling the research to be directly spread into those avenues.

Another is through interactions with politicians and governmental agencies. Faculties take part in joint collaborations between, for example, KTH and Region Stockholm (Health care and regional planning). As a striking example, the Department was imperative for the creation of SciLifeLab: Through interactions with Ministers of Education and Research, faculties have provided inspirations and input for its conceptualization and planning, as well as active advice to the budget set for research (forskningspropositionen). In addition, the COVID-19 pandemic has highlighted how our institution can contribute to society: Several faculties assisted the Public Health Agency of Sweden (Folkhälsomyndigheten), interacted with ministers of the government, took part in advisory expert committees relating to vaccines, and assisted with large-scale testing (supported by large private foundation KAW and funds from the Stockholm county), thereby significantly helping to disseminate research and assist with the response (See *impact case 9*)

Faculties also actively disseminate science to both governmental agencies, industry, and the public through associations such as the Royal Swedish Academy of Engineering Sciences (IVA), Royal Swedish Academy of Science (KVA).

Further, research is disseminated to the general populations through media and popular presentations and articles. Also, a highly publicized effort to spread novel data of human proteins together with a general introduction to cell biology through a popular on-line gaming platform (see *impact case 5*), has had a large impact. Research originating from PRO is quite visible in both Swedish and international media, and highly so during the COVID-19 pandemic (*impact case 9*). Other efforts include participation at the Museum of Technological Sciences (Tekniska museet), Museum of the Nobel Prize, digital exhibitions, displays and other. Our Department also regularly hosts visits from high-school students (gymnasium) , s well as their teachers, for recruitment to KTH bachelor education. This includes presenting our research, sharing information about our programs and courses, supplying advice, sometimes offering lab projects, several times a year. To capture wider talent, improve diversity, and inspire more young students to a career within research, a yearly student-driven research project called "iGEM Stockholm" [Link] is hosted by the Department (by Johan Rockberg). Student groups in the KTH-initiated program competes in a global competition (*International Genetically Engineered Machine* iGEM) originating from University of Massachusetts Institute of Technology MIT in Boston 2003. Since its start in 2015 'iGEM Stockholm' has trained over 110 students in the field of synthetic biology. Stockholm teams have been nominated to several iGEM awards and won gold, silver, and overall winner. Students from KTH, KI, SU, Berghs school of communication, Konstfack University of Arts, Crafts and Design and others, have initiated, funded and executed research ideas of their own. Team members include students within chemical engineering, biotechnology, electrical engineering, medical doctors, bioentrepreneurs, toxicology, design, programming, conceptual design, and marketing. Output from this activity includes research reports, posters, new scientific tools, prototypes, scientific debates, children´s books, board games, computer games, and numerous TV and newspaper interviews in Swedish and global press.

c. Sustainability and the United Nations' Sustainable Development Goals (SDG)

Good health and well-being are critical for a sustainable society, and our research activities relate strongly to this (addressing SDG 3 Good health and wellbeing). Nearly all (100%) research performed at the department is focused on improving health or environment, and we thus impact sustainable development in many areas. We expect that our research will lead to improved prevention, diagnosis and treatment of several conditions, including cancer and neurodegenerative diseases. We have contributed to several clinical trials based on research from our flagship projects focused on wellness and diseases (see *impact cases 4 and 8*), through outreach (see *impact case 5*), and entrepreneurial

efforts (see *impact cases 6 and 8*), which help improve sustainability. Our research contributes to better understanding of disease development and their treatments, has led to phase I-III clinical trials (e.g., for liver disease), development of affibodies for cancer imaging, and biomarkers for neurodegenerative diseases. During the pandemic (from March 2020) PRO has made an outstanding contribution to society's sustainability, contributing with activities, instruments, and research for healthcare (including testing), wastewater-based community surveillance, as well as dedicated science related to Covid-19 (see *impact case 9*). Other work is directed towards development of point-of-care tests for diagnostics, including detecting critical neonatal illnesses to reduce the mortality rate in newborns, especially in low-income countries (addressing SDG GOAL 3.2 to end preventable deaths of newborns and reduce neonatal mortality, and SDG GOAL 3.8 access to safe, effective, quality and affordable essential medicines and vaccines for all). Further, through genetic engineering of bacteria, work is ongoing to create novel environmentally friendly fuels (contribute to SDG GOAL 7, affordable and clean energy, see *impact case 7*). For over 10 years, our Department has co-hosted the Novo Nordisk Center for Biosustainability, where 'cellular factories' are designed to replace petrochemical raw material and produce for example chemicals, plastic, biomaterials and pharmaceuticals. PRO has contributed with methods for genome editing, sequencings of microbes, high-throughput screenings (droplet microfluidics), biosensors (with reporter signaling), and more. We also collaborate with the private sector (e.g., AstraZeneca, AlfaLaval, Vironova) to help improve yield and quality of pharmaceuticals and improve their production sustainability.

We also contribute through our teaching, where we have incorporated the 17 SDG into the curriculum of most of our courses. All of our active teachers are also engaged in research relating to either health or environment. In our courses, we further strive to have teacher teams, in order to reduce vulnerabilities to the course (if a teacher is unavailable, sick, or leaves). Our faculties and researchers with responsibility for educational quality (vGA, PA) has taken specific courses focusing on gender and equality. Our teachers of basic courses include topics such as basic behavioral sciences. Most courses are closely Linked to and integrated with the research environment, ensuring up-to-date topics in the curriculum and that students can perform their thesis project in our labs (or at another university, private sector, or governmental agency of their choice). The educational programs are designed to offer continuous and progressing training of abilities, skills and values. This includes collaboration skills, critical thinking, presentation techniques, sustainability, and research ethics, throughout the students' education.

d. Structure for increased impact

As exemplified by our impact cases above, our research has considerable impact on society. This is to a large extent based on the relevance of our research combined with a pro-active outreach to society. The relevance is assured by our faculties' interests, curiosity, and engagement with society coupled with their technological and molecular biology expertise. The pro-active outreached, exemplified by interactions with governmental actors, politicians, healthcare leaders, schools and media, also stems from an interest and engagement with society which drive this development. This is exemplified by the recent COVID-19 pandemic (see *impact case 9*). Our faculties immediately stepped up and interacted with leaders in the region, country and funding bodies, and improved and helped out with COVID-19 testing, wastewater-based surveillance, and initiated of numerous research project to better understand the disease (such as identifying underlying biological mechanism for why some are more severely affected). This demonstrates that our Department is engaged in our society and how this contributes to impact.

The successful research at PRO is to a large extent based on curiosity-driven research. Curiosity identifies the needs and research questions, helps engage collaborations and stake holders, and in the end generates impact. Importantly, several of our flagship projects are now in the phase where even further impact can be expected, for example as a result of clinical studies and applications in the health care settings. Further, with the urgent societal challenges relating to climate change and the pandemic

and with KTH's explicit focus on sustainability and the United Nations' Sustainable Development Goals, there is an ever-increasing awareness of societal issues. Our Department's research can help to address this, and we expect that this will only increase our engagement in society in the future.

In order to maintain and increase our impact, it is critical that we have a structure that values and encourages curiosity-driven research, engagement with society, and ensures that our faculties and researchers can work at optimal conditions. The latter includes both physical conditions (access to state-of-the-art instruments, labs) and non-physical conditions, such as having enough time dedicated to research, support in achieving funding, limitations of administrative tasks, and that we have an including, open, and supporting atmosphere. Discussions on best practices and experiences between faculty, sharing contacts and information (*e.g.* funding agencies, governmental agencies, media) and training in for example communication (including with media) can be important aspects of this.

e. Impact cases

We provide six examples where we believe we have made a significant impact (see Panel 2 Impact cases 4-9 in Appendix 1):

4. Building an international core resource to explore human biology and disease
5. Engaging a wider community in the classification of images
6. BioSilk – recombinant silk for biomedical applications
7. Direct conversion of CO2 to biofuels with bacteria
8. Case study: Drug development and human clinical trials
9. Confronting the COVID-19 pandemic

Department of Industrial Biotechnology

Self-evaluation
Head of Department: Professor Antonius van Maris

Included divisions:
The department has no divisions

Department of Industrial Biotechnology

1. Overall analysis and conclusions; strengths and development areas

a. Limited SWOT-analysis

Overall analysis of 1) Research strengths, 2) Research weaknesses, 3) Organizational strengths, 4) Organizational weaknesses (see SWOT table) and 5) Development areas.

	Strengths	Weaknesses
Research	1. A joint ambition to perform excellent research on fundamental, application inspired topics with broad relevance for sustainability development of industry and society. 2. Strong Links between the research and teaching activities within the department at the 1st, 2nd and 3rd cycle. 3. With foreseen retirements, a redevelopment plan was initiated that in a 5-6 year period will result in recruitment of 5 new faculty members with diversity in career stage. With support at KTH and school level this will result in anchoring of central topics with at least two faculty members and realignment to strategically relevant topics that are uncovered by faculty (e.g. environmental biotechnology and mammalian cell-based bioprocessing). 4. During this transition, the department has an increasing number of publications, whilst maintaining impact and representation in top 20% journals, which is a good sign, given that the long-term impact of the staff renewal still has to come.	1. DIB should increase the level of external funding through both large initiatives as well as personal grants should increase to improve research quality, quantity and impact. In turn, this should increase the average number of temporary scientific personnel (PhD students and post-docs) per faculty and thereby the research output and critical mass. 2. Increasing PhD student salaries and rental cost, combined with high overhead contrast the (much) slower increasing funding through science councils and basic funding. This disqualifies certain sources of funding that do not cover the costs and decreases the relative scientific quantity from the remaining grants. 3. Although the improved work environment and communication has already resulted in many good scientific discussions amongst the different experts within the department, internal collaboration can improve further with the aim to result in more co-publications and joint grants. 4. Long-term funding for medium-cost infrastructure is increasingly challenging. Specifically that infrastructure that is too big for common personal grants from research councils, but small for dedicated grants. Options to apply for specific yearly strategic KTH funding are no longer available for this.

	Strengths	Weaknesses
Organisation	1. A positive work environment with a flat organisation, collegiality, communication and transparency, making it possible to share and use a wide array of research infrastructure, including a pilot plant with 600 L bioreactor, not only within the department, but also within the school. 2. A well-balanced staff with role models of both genders. This remains a point of attention, since with the relatively low total number of staff numbers, decisions by (external) recruitment boards have a large impact on this. 3. In the RAE2012 recommendation were made to improve internal KTH collaboration and to support collaborative efforts, and additionally to strengthen structural biology activities. Presently, structural biology activities within DIB are strengthened through a collaborative PhD student with the Chemistry department as well as strategic funding at the school level. 4. Another RAE2012 recommendation concerned exchange of experience and enlargement of critical mass. The department of Industrial Biotechnology currently enjoys collaboration with other scientists that also work on biocatalysis, metabolic engineering and environmental biotechnology in other departments of KTH. Two recruitments in process.	1. Faculty funding. Increased levels of permanent faculty funding (currently ±42% of salary & overhead) would be beneficial for external recruitment and the competitive position of KTH. Additionally, this would improve work enjoyment and development of long-term research vision by faculty, which is a requirement for excellent research. 2. The recruitment process for new faculty is extremely slow. This mostly seems to be caused by too many steps and a lack of urgency on centralized procedures. Additionally, external candidates are poorly informed about the status of ongoing procedures. Excellent candidates, especially the sought after excellent female ones, drop out of the procedures. This is something KTH cannot afford in combination with the lack of a competitive offers. 3. A very high turnover in administrative staff for finances, HR, legal support has resulted in lack of knowledge retention, loss of time and efficacy for researchers and inability to go beyond the basic first support needs over the past 3.5 years. Together with these functions mostly being centralized, this leaves researchers feeling unsupported and wondering what they get in return for the high overhead costs.

5) Development areas

- Continuation of the ongoing renewal of both the faculty and strategic topics within the department, including currently ongoing recruitments for a faculty position on biocatalysis for green chemistry and one for mammalian-cell-based bioprocessing and a faculty position on environmental biotechnology.

- Increased external funding through activity of individual researchers, coaching by experienced staff and increased familiarity of the department head with the unspoken inner-workings of setting up large consortia in Sweden.

- We hope that boundary conditions required for scientific performance, including improved and local administrative support, quicker recruitment procedures and improved financial aspects, such as faculty funding, lower overhead and constrained rent increases, will dramatically improve during the coming years.

Ensuing from the above the quantity and quality of the scientific output of the department is expected to increase.

b. Summary statement on contributions of departments on impact, infrastructure and sustainable development

Impact

The main impact of the department has been on the following topics with further details described in the specific impact cases:

- Yeast strains and underlying knowledge from proof-of-principle to full-scale production of fuels and chemicals (*impact case 10*).
- Fundamental insight into structure and function of membrane proteins through Integrative structural biology for advancement of industrial & medical biotechnology.
- Proof-of-principle on site removal of pharmaceutical residues at municipal waste-water treatment facilities for a cleaner Baltic sea (*impact case 11*).
- Advancing the field of biocatalysis through a large international network of small & medium sized enterprises mixed with leading research groups in the field (*impact case 10*).
- Knowledge hub for industry and academia on advanced bioproduction of pharmaceuticals through high cell density mammalian-cell-based processes (*impact case 12*).

Infrastructure

Aside from appropriate research infrastructure needed for the strategic topics of the departments (described under section 3.e), the department has a pilot facility for research, teaching and contract research to scale up bioprocesses up to 600L cultivation volume, including down-stream processing equipment.

Sustainable development

The fundamental, application-inspired research of DIB on production of fuels (UN #7), chemicals and materials from sustainable agriculture and forestry resources or by use of more benign reaction conditions contribute to a carbon neutral society and minimizing further climate change (UN #12&13).

Removal of pharmaceuticals and excess nutrients from municipal waste water improves quality of life in surface waters (UN #14), which in turn ensures availability of suitable drinking water (UN #6) and improves the health of the Baltic sea. Other environmental biotechnology solutions more specifically target developing countries, including projects with the Swedish Development Agency SIDA.

Process-intensification and continuous bioprocessing for mammalian-cell-based bioproduction directly contributes to responsible production (UN #12). Through cost reduction and improved product quality this research also improves health- and well-being (UN #3) and cheaper therapies for rare diseases provided by biopharmaceuticals also reduces inequality (UN #10).

2. Research profile

a. General information of the department

The Department of Industrial Biotechnology (DIB) [Link] provides societally relevant biotechnological options for sustainable production of pharmaceuticals, (fine) chemicals, materials and fuels, as well as contributing to clean water and environment. DIB has seven faculty positions and an additional four independent researchers. Together with temporary and support staff DIB numbers around 40 people. To maximize the benefits from increased collaboration and communication DIB currently has a flat organization without division structure. We are based at floor 1 and floor 2 of the AlbaNova University Centre.

DIB is undergoing a process of renewal that aims to both improve the scientific quality and impact, as well as the ability to acquire external funding, whilst maintaining excellent contributions to the education programs of KTH. Four out of seven faculty members present in 2016 left KTH, have retired, or will retire during the coming two years (Humble 2018; Larsson in 2019; Veide in 2021 and Nyrén in ±2022). As part of this process, a new head of department has been recruited externally (van Maris 2016) and 4 additional new faculty members with diversity career stages have been or will be recruited, thereby either anchoring existing topics amongst the faculty or open new strategic opportunities.

b. Central research questions and themes, knowledge gaps addressed, main research activities

The research of DIB is divided in the following five themes:

Mammalian-cell-based bioprocess technology focusses on the bioproduction of therapeutic biologics using mammalian/human cells. This team advances the state-of-the-art of the field through: (i) Continuous perfusion culture at very high cell density for intensified production of therapeutic biopharmaceuticals using established mammalian/human cells such as CHO and HEK293 cells. (ii) Mathematical modelling of these processes in view of process optimization and control. (iii) Collaborations with industrial partners in many projects and areas as well as active involvement in cross-disciplinary projects with teams experts in purification process, system identification and automatic control, artificial intelligence, cell and vector engineering, omics, nanotechnology, instrumentation, bioproduction of human primary cells, bioproduction of human stem cells and analytical chemistry. The group also applies its expertise in mammalian cells bioprocessing to solve severe technological bottlenecks in the newer field of production of viral vectors for gene therapy.

Biocatalysis and enzymology. Enzyme technology is one of the corner stones of Industrial Biotechnology. The research in this area involves both fundamental and applied enzymology and focusses on applied enzyme catalysis in combination with enzyme engineering for the sustainable synthesis of functional bio-based polymers and fine chemicals. The state-of-the-art of the field is developed through: Molecular modeling-guided rational design of enzyme specificity, development of novel multicatalytic one-pot reaction cascades, engineering and molecular-level understanding of transaminase stability and, fundamental lipase/acyltransferase applications for the development of powerful chemo-enzymatic routes to polymers and materials. Important in-house interaction on a daily basis with DIB experts on molecular biology (Assoc. Prof. Ezcurra) and structural biology (Prof. Divne) is naturally important but also the collaboration with other KTH departments (*e.g.* fibre & polymer technology, chemistry/glycoscience and chemistry/physical chemistry). Furthermore, the theme has a strong and rewarding tradition to participate in external research networks (see *impact case 10*).

Integrative Structural Biology focusses on fundamental understanding of structure-function relationships of enzymes of importance for metabolic reactions relevant to health and environmental objectives. Specifically, ongoing projects concern enzymes that play key roles in: virulence and pathogenicity of notorious plant pathogens; neurological disorders and cancer; synthesis of glycoconjugates as part of protein glycosylation; degradation of valuable plant polysaccharides (e.g. cellulose and hemicellulose) and lignin components for production of value-added sugar-based

compounds and improved bioprocessing of biomass. Two of the projects are performed in close collaboration with other researchers at CBH, namely Assoc. Prof. Ines Ezcurra (DIB) on the project concerning new therapeutics for plant disease (Ezcurra is an expert in plant molecular biology/biotechnology) and Assoc. Prof. Magnus Johnson (CHE) on the influence of lipid and protein composition on membrane characteristics.

Environmental Biotechnology focusses on the removal of pharmaceutical residues from waste water streams and valorization of residue streams. This team has shown how pharmaceuticals are distributed throughout the Baltic Sea catchment area and how on-site technology at municipal waste-water treatment facilities can mitigate this in the future. Additionally, novel concepts for water treatment using plant-based coagulant proteins and magnetic nanoparticles are investigated in collaborations with third world countries through the Swedish Development Agency SIDA. Concepts are developed to valorize residue streams, such as food waste, through biotechnology. Recruitment of an external senior faculty member would improve profiling of the existing environmental biotechnological know-how and increase the focus on excellent research on entirely novel concepts.

Microbial Bioprocess technology focusses on metabolic engineering and process development for production of fuels, chemicals and proteins. This team advances the state-of-the-art of the field through: (i) Chassis engineering and proof-of-concept with established industrial work horses such as *S. cerevisiae* and *E. coli*, (ii) pioneering research with aerobic- and anaerobic thermophiles with foreseen advantages over current processes and (iii) collaboration with academic and industrial partners for process development in up to 600L bioreactors. Aside from internal complementarity, this theme benefits from collaborations with DIB experts in biocatalysis and enzymology, structural biology, environmental biotechnology, as well as external collaborations on electrochemical biology, chemical engineering and genomics.

c. Contributions to the advancement of the state of the art within the research fields of the department

Mammalian-cell-based bioprocess technology

- New methods for process development of continuous perfusion process at very high density of mammalian/human cells.
- Novel tools for process modelling of mammalian cell culture, aiming at simulation, prediction and model-based control.
- Application of bioprocessing expertise to the new field of Advanced Therapy Medicinal Products (ATMP) such as viral vector for gene therapy and primary human cells to develop new methods enabling scale up.

Biocatalysis and enzymology

- Advancing the knowledge of transaminase enzymology and rational design of enzyme specificity.
- Enzyme stability engineering for industrial process conditions and molecular-level understanding of mechanisms of enzyme inactivation.
- Applied and fundamental lipase/acyltransferase enzymology and engineering towards selective processes for bio-based polymers and resins

Integrative Structural Biology

- Seminal work on key enzymatic processes in crystalline cellulose degradation.
- Important contribution to the recent paradigm shift in lignocellulose degradation by elucidating the structure-function relationship of novel oxidative enzymes.

- Novel fundamental understanding of glycan biosynthesis.
- Structure-mechanistic foundation for biosynthesis of plant pathogen phyto-pathogenic exopolysaccharides.

Environmental Biotechnology

- Analysis and demonstration of the spread of pharmaceutical residues from different catchment areas throughout the Baltic Sea region with a specific focus on the modelling of environmental concentrations of carbamazepine in this region.
- Water purification by bio-functionalized nanoparticles with plant coagulant protein enabling regeneration and reusage
- Demonstration and analysis of different techniques for on-site removal of pharmaceutical residues from municipal waste water and specific Baltic Sea catchment areas, resulting in a handbook for policymakers and users that is available at http://waterchain.eu/best-practices/

Microbial Bioprocess Technology

- Development and proof-of-principle for application of surface-displayed tyrosinase and melanization of *E. coli* for removal of pharmaceutical residues and electro-biochemical diagnostics.
- Advanced the knowledge of national and international companies (SME and non-SME) through contract research, collaborations and knowledge exchange based on bioprocess engineering know-how and pilot plant.
- Novel metabolic- and evolutionary engineering concepts for production of bioethanol, mono- and dicarboxylic acids in yeast, *Clostridium thermocellum* and *E. coli*.

d. Quality and quantity of contributions to the body of scientific knowledge

The number of (fractional) publications published by DIB is showing an increasing trend (Figure 1), which is a positive sign in the middle of a rejuvenation/transition of the faculty. The quality of journals we publish in is above average for the field with a close to 100% coverage in Web of Science, with a representative share of publications in top 20% journals. The impact of our publications follows the field average for those journals with a slight positive trend (Figure 2). We aim for this to increase in line with the faculty renewal process and realignment of research topics. Continuous attention is paid to educate staff and junior scientists in awareness of open access, predatory publishing and predatory conferences. Realization of increased external funding, which is the main ambition for the coming period, will also increase the quantity of the research output through the increase of the critical mass. A similar trend was observed for 2020.

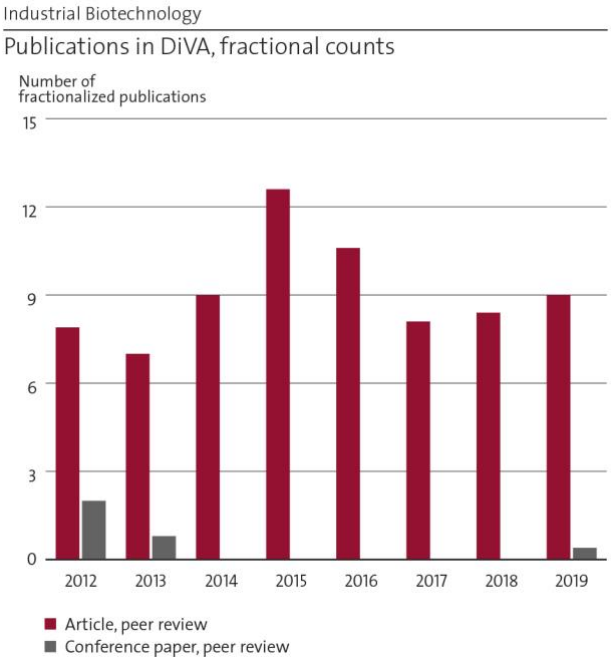


Figure 1. *Number of fractionalized publications 2012-2019.*

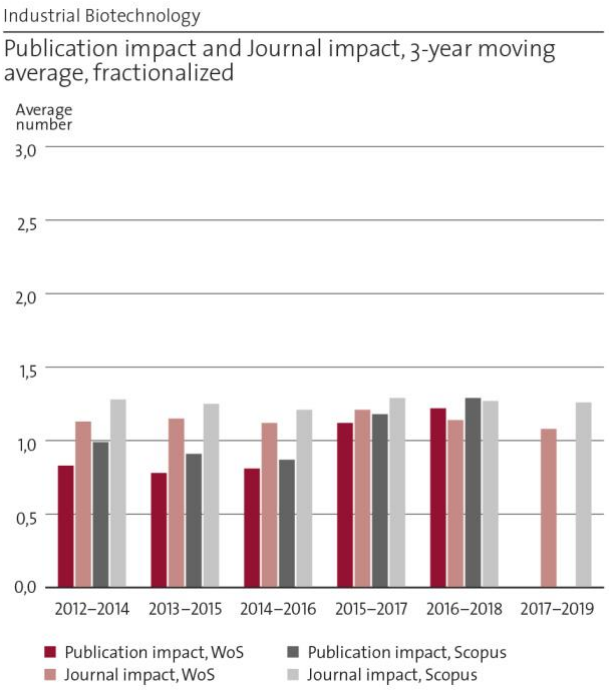


Figure 2. *Publication and journal impact 2012-2019.*

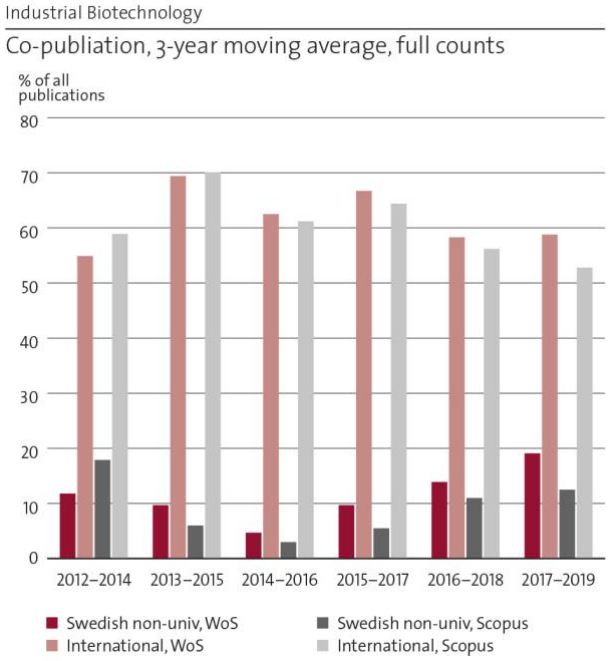


Figure 3. *National and international co-publications 2012-2019.*

Highlights from the publications of DIB are:

Björlenius *et al.* (2018). Pharmaceutical residues are widespread in Baltic Sea coastal and offshore waters - Screening for pharmaceuticals and modelling of environmental concentrations of carbamazepine. *Sci. Total Env.*, 633:1496-1509 [Link].

Clincke *et al.* (2013). Very high density of CHO cells in perfusion by ATF or TFF in WAVE bioreactor. Part I. Effect of the cell density on the process. *Biotechnology Progress* 29:754 [Link].

Dalecka *et al.* (2020). Constructive use of filamentous fungi to remove pharmaceutical substances from wastewater. *J. Water Process Eng.*, 33 [Link].

Finnveden *et al.* (2019). Mono-substitution of symmetric diesters: Selectivity of *Mycobacterium smegmatis* Acyltransferase variants. *Catal. Sci. Technol.*, 9, 4920–4927 [Link].

Guo, F., & Berglund, P. (2017). Transaminase Biocatalysis: Optimization and Application. *Green Chem.*, 19, 333 – 360 [Link].

Gustavsson *et al.* (2016). Biocatalysis on the surface of *Escherichia coli*: melanin pigmentation of the cell exterior. *Scientific Rep.*, 6, 36117 [Link].

Marques *et al.* (2018). Combined engineering of disaccharide transport and phosphorolysis for enhanced ATP yield from sucrose fermentation in *Saccharomyces cerevisiae*. *Met. Eng.*, 45, 121-133 [Link].

Schwarz, H. *et al.* (2020). Small-scale bioreactor supports high density HEK293 cell perfusion culture for the production of recombinant Erythropoietin. *J. Biotechnology* 309: 44-52 [Link].

Tan *et al.* (2015). Structural basis for cellobiose dehydrogenase action during oxidative cellulose degradation. *Nature Communications*, 6, 7542 [Link].

VanArsdale *et al.* (2020). A Coculture Based Tyrosine-Tyrosinase Electrochemical Gene Circuit for Connecting Cellular Communication with Electronic Networks. *ACS Synthetic Biology*, 9, 1117-1128 [Link].

e. Engagement in national and international research collaboration within academia and its outcomes

The national Vinnova Competence Centre for Advanced BioProduction by Continuous Processing, AdBIOPRO, provides novel technology for manufacturing of therapeutic biologics with industrial focus [Link]. AdBIOPRO is driven by the complementary expertise of five teams at KTH, Lund University and the Karolinska Cell Therapy Centre (KCC) at Karolinska University Hospital together with seven industrial partners, Sobi, Cobra Biologics, BioInvent, GE Healthcare, Valneva, Lab-on-a-Bead, and CellProtect Nordic Pharmaceutical. The Centre budget is 86 MSEK over 5 years and is renewable for a new period of 5 years (over 5 years: cash funding of 37.5 MSEK from Vinnova, industrial partners and KTH, and 48.5 MSEK as in kind). This centre combines world-leading academic, industrial and medical experts and is central to further development of this field at KTH, Stockholm, Sweden and beyond.

The Integrative Structural Biology team is actively performing research at a large number of national and international infrastructure facilities and platforms including BioMAX at MAXIV, Cryo-EM Sweden, SciLifeLab (Stockholm University), 3D-EM (Karolinska Institute), Protein Science Facility (Karolinska Institute), Diamond Light Source (UK), SOLEIL Synchrotron (France), BESSY II Synchrotron (Germany), DESY PETRA III Synchrotron (Germany) and European Synchrotron Radiation Facility ESRF Grenoble (France). Furthermore, the team is in a close collaboration with Assoc. Prof. Magnus Johnson at the Dept. of Chemistry (CBH) regarding membrane studies using the novel technology nano-IR microscopy, where CBH at KTH is the first in Sweden, and one of a few globally, to own this type of new infrastructure. The team is also closely collaborating with Assoc. Prof. Ines Ezcurra who is an expert in plant molecular biology.

The Formas Bioraf project was a national collaboration network between three different research groups at KTH, the Research Institutes of Sweden (RISE) and the University of Borås with support and strong interest from an industrial panel including Ragn-Sells, AKZO Nobel and Lantmännen. The total budget was 24 MSEK over a period of 5 years. Aside from 5 cross-disciplinary trained PhD students and 25 peer reviewed publications two major outcomes of this project are: the demonstration of the use of biocatalytic esterifications to valorize residue streams of forestry, and (ii) membrane bioreactor-based valorization of food residues with VFAs as platform intermediate. For both topics, follow up projects are being pursued together with the industrial partners.

Several scientists at DIB have been and are engaged in various successful ITN applications (innovative training networks, “European Marie Curie”-type of networks) in recent years. Many of the ITN project partners are companies (both SMEs and large enterprises). Examples of ITNs at DIB are STACCATO (Dr. Chotteau, 2019-2022), Biocascades (Prof. Berglund, 2015-2018), REFINE (Dr. Martinell, 2012-2016) and INTERfaces (Prof. Berglund, 2020-2023). Each participation has secured funding for 1-2 KTH PhD students. These and other large European networks are and have been of immense importance for future continued research funding for DIB in the form of various spin-off projects and they constitute excellent PhD education platforms providing the next generation of European PhD graduates in industrial biotechnology.

f. Follow up from previous evaluations

The only direct comments for DIB was:

"Cooperation within KTH is not optimal. Here an optimization would bring much benefit with respect to exchange of experience and enlargement of the critical mass."

Within DIB cooperation has already improved, benefitting from joint seminars and better communication. Also, cooperation with other departments within KTH has been intensified through joint supervision of PhD students with the departments of Chemistry (with financial CBH support), Chemical Engineering, Protein science and Intelligent Systems (EECS). Where needed, new faculty recruitment has been used to strengthen the critical mass on strategically important topics.

Additionally, the department head has regular discussions with faculty in other departments that work on related topics in Metabolic Engineering, Environmental Biotechnology and Biocatalysis.

Another comment was made at the school level, but is also relevant for DIB:

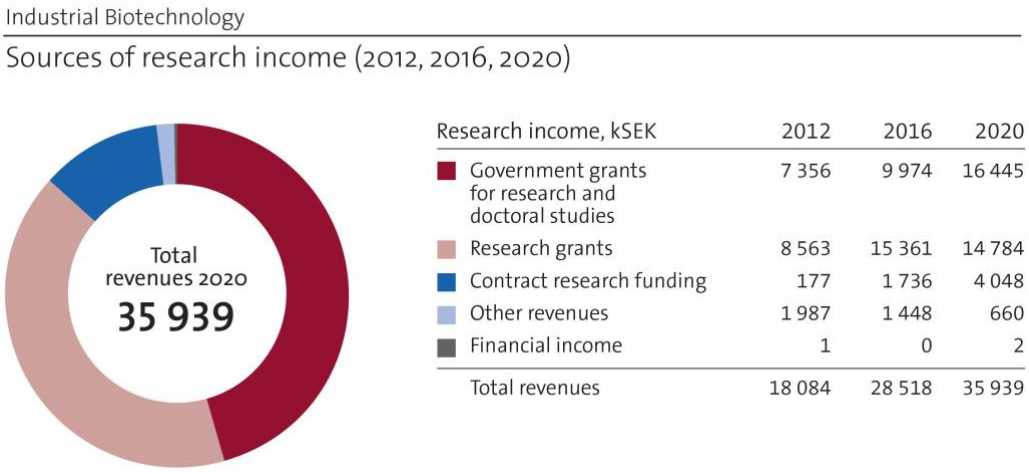
"On a more specific note the School needs to think more strategic with respect to its access to prime competence in Structural Biology."

This has resulted in allocation of school merger funds to a joint PhD student between Prof. Divne's Integrative Structural Biology group and the department of Chemistry. Additionally, this group has received strategic resources to increase its scientific impact and growth potential.

3. Viability

a. Funding; internal and external

The research funding of the department over the past seven years (2014-2020) has on average been 34.5 MSEK per year with a small increasing trend. The sources for this research income were on average: 38% from basic KTH funding, 50% external funding from research grants, 8% from contract research and 4% other sources. The three years chosen in the centrally provided figure 4 below show a somewhat misleading trend, since 2012/2013 were not representative for the current faculty composition of DIB (incoming transfers) and 2016 was a (low) outlier.



Research income, kSEK	2012	2016	2020
Government grants for research and doctoral studies	7 356	9 974	16 445
Research grants	8 563	15 361	14 784
Contract research funding	177	1 736	4 048
Other revenues	1 987	1 448	660
Financial income	1	0	2
Total revenues	18 084	28 518	35 939

Figure 4. *Comparison of research incomes 2012, 2016 and 2020.*

Together with the funding for teaching (which is not in the figure on research funding; >6 MSEK/year), to which DIB makes a very significant contribution in both quantity and quality, this provides a viable basis for the current number of faculty. However, to realize our research ambitions, it is important to achieve an upward trend of the external funding after replacement of retiring faculty members and continued development of existing faculty members. The applied funding agencies Formas and Vinnova are the quantitatively most important funding agencies. DIB aims to increase the external funding by 50%-100% over the coming 5 years. Examples of currently under-represented sources of funding are VR, the Wallenberg foundation and EU funding. Underlying changes in faculty composition and the recent return of a key faculty member (Prof. Berglund) from his central role as vice dean of faculty, which although valuable for DIB and KTH has been detrimental for the development of his scientific group, are expected to have a positive influence. It is worth noting that the

contract research is a crucial contribution to operating and maintaining the pilot facility for (contract) research and education.

b. Academic culture

Over the past four years DIB has dramatically improved the communication between researchers and at the department level. The main aim of this is to stimulate discussion, collaboration, exchange of expertise and sharing of infrastructure and thereby increase both scientific quality and improve the work environment. The importance of this is shared amongst the staff and participation is high. During the last pandemic year, this has helped tremendously in keeping up a good spirit, finding solutions for teaching as well as research and maintaining a responsible work environment apart-together. The following meeting places play an important role in this and all are continued (online) during the pandemic:

- The coffee/lunch table - an often underestimated place to discuss and align
- Bilateral meetings with daily supervisors for PhD students and postdoc and with department head for staff. Goal: Detailed discussion, experimental and strategy.
- Regular meeting with all members working on a specific research theme (weekly to monthly depending on the needs). Goal: Information and knowledge sharing.
- Monthly meeting of the DIB faculty. Goal: Communication, identification of needs and strategic planning. Faculty especially appreciates the efficient translation of information from KTH to CBH to the department.
- DIB wide seminars every two weeks where everyone from starting MSc students to established faculty presents and discuss their work. Goal: Information and exchange of expertise. Additionally, items like bibliometrics, open access, predatory publishing etc. are also discussed during this seminar series.
- Seminar series for PhD students from DIB or other departments. Goal: Knowledge exchange and develop critical thinking about experimental design, data analysis and thereby align with national 3rd cycle education goals.
- Twice per year each individual floor of the department meets (floor 2 together with the division of Glycoscience) to discuss the work environment, safety and detailed operation of the labs. This is routinely combined with an all-hands cleaning day.
- CBH-wide seminars. One per year everyone at CBH has the opportunity to meet, discuss science and exchange knowledge. In addition, all PhD student of the school meet once per year. Two meetings per year that target people with shared interest in different topics (e.g. biofuels, materials etc.) will be added to this.

c. Current faculty situation

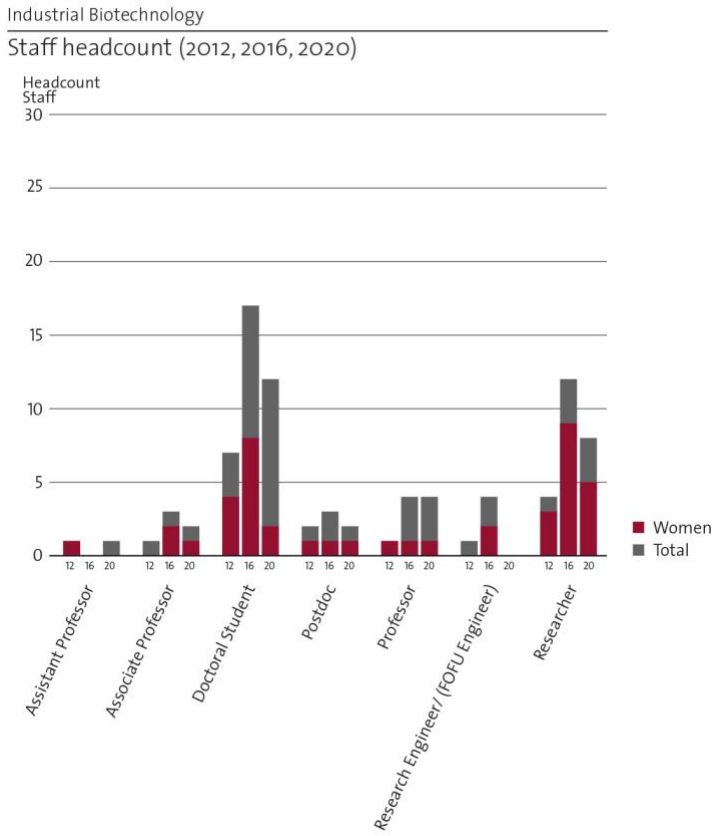


Figure 5. *Staff headcounts 2012, 2016 and 2020.*

The composition of the research team on the five focus topics is as follows:

Mammalian-cell-based bioprocess technology is led by Dr. Chotteau (Rec 2008; Bioprocessing, Automatic Control) with 1 lab manager, 3 post-docs, 6 PhD students and a lab technician.

Biocatalysis and enzymology is currently led by PIs Prof. Berglund (fine chemicals synthesis) and Dr. Martinell (polymer synthesis) and will be supplemented by the asst. prof. in Biocatalysis and Green Chemistry under recruitment.

Integrative Structural Biology is led by Prof. Christina Divne whose team currently includes three PhD students, and one junior researcher.

Environmental Biotechnology is led by Dr. Kuttuva Rajarao with assistance from Prof. van Maris with 5 (exchange) PhD students. For a large part of the evaluated period, this team also included Dr. Björlenius who left at the start of 2019. Prof. van Maris is also a member of the board of the cross-disciplinary KTH Water Centre.

Microbial Bioprocess technology is led by Prof. van Maris (Rec 2016; Biochemical Engineering), Assoc. Prof Veide (ret. 2021; Biochemical Engineering) and Assist. Prof. Gustafsson (Rec. 2019; Industrial Microbiology) with 1 lab manager, 1 post doc and 4 PhD students.

DIB has a well-balanced staff with role models of both genders and spread across career stages. There is no underlying reason for a gender bias on any of our research topics and excellent candidates of all genders are available for all the academic career stages. Although two research themes are currently male-dominant, this fluctuation is caused by the recent graduation of many female PhD students. The outcome of ongoing and foreseen recruitments will have a large impact on the gender balance in view of the relatively low total number of faculty numbers. The management of DIB can only influence this with the recruitment strategy and by making sure DIB provides an optimal work environment. Elements that are out of direct control of DIB are actual shown interests by candidates, decision by the centralized recruitment board and the slow centralized processes and uncompetitive conditions that cause excellent candidates of both genders, but especially excellent female candidates, to accept positions elsewhere.

The faculty renewal process that included the recruitment of Prof. van Maris and Dr. Gustafsson, is continuing with faculty recruitment on strategic topics, including currently ongoing recruitments for a faculty position on biocatalysis for green chemistry and one for mammalian-cell-based bioprocessing and a future faculty position on environmental biotechnology. Upon completion of the current faculty development plan, a group of four retirement estimated between 2031-2033 will be the next natural opportunity for strategic realignment.

Three independent researchers play a central role in the current operation of DIB and their well-being and performance gets the same attention of that of the rest of the faculty. Simultaneously KTH does not offer this group of researchers a clear career perspective. Within DIB no new independent researcher positions are created. Through retirement and/or creation of new faculty positions where strategically relevant, the number of independent researchers is foreseen to decrease.

d. Recruitment strategies

All recruitments at KTH are advertised through the central KTH webpage for vacancies. Depending on the level of the recruitment, the following communication actions are used to stimulate excellent candidates of both genders to apply:

- Distribution according to standard KTH channels. When suitable candidates are present amongst the students of the department these are also encouraged to apply.
- The extensive LinkedIn network of the faculty members.
- Advertisement in *Nature* Jobs for faculty positions and key post-docs.
- E-mail to distribution list of group- and department leaders of international high quality groups.
- E-mail to ‘point out’ vacancy to selected high-potential candidates of both genders.
- To safeguard equal opportunities, the standard recruitment procedures are secured by the human resource administration in collaboration with the faculty of DIB.

e. Infrastructure and facilities

Important research infrastructure of DIB includes:

- Extensive set of bioreactors for quantitative physiology and process development at 0.5-10 L scale under aerobic and anaerobic conditions.
- A pilot plant with bioreactors from 20-600 L scale including downstream processing equipment for (dis)continuous biomass separation, homogenization, liquid-liquid separation, chromatography and filtration.
- Clean rooms, including bioreactors for high cell density cultivation of various mammalian cell lines (including human cell lines).

- Infrastructure for enzymology, organic synthesis and structural biology, including gas chromatography, organic solvent-based HPLC, fume hoods equipped with Ar gas lines.
- Mobile, container-based pilot facilities for evaluation of on-site environmental biotechnological removal of pharmaceuticals from waste-water streams.

KTH, The CBH School and DIB together have made or are making various investments in the maintenance and upgrading of the pilot plant, bioreactors and acquisition of a highly accurately mass-spec for analysis of gas-streams leaving the bioreactors. It is foreseen that other big investments will either be made through applications for specific grants or future strategic resources from KTH. Investments in small equipment can routinely be made from project budgets or through the shared departmental overhead. The main challenge is to replace or expand the infrastructure that falls in between these categories, but is essential for current operation, such as analytical infrastructure, or future competitiveness, such as liquid handling robots for automated work flows and high throughput screening.

4. Strategies and organization

a. Goals for development 5–10 years ahead

Vision: Advancing the sustainability development of industry and society with critical mass on five thriving fundamental, application-inspired research themes (section 2b) each anchored in the organization by one or more faculty with increased quality and quantity of both external funding and research output, whilst maintaining an excellent contribution to the core educational programs of KTH.

The mission to achieve this started with the appointment of a new head of department and professor in Biochemical Engineering in 2016, continued with the recruitment of an assistant professor in Industrial Microbiology in 2019 and the currently ongoing recruitment of an assistant professor in biocatalytic processes for green chemistry. These positions form an integral part of the faculty development within CBH. Two other important developments within the existing faculty will influence the research environment with DIB, with expected positive impact on the scientific quality and –impact. This includes the return of Prof. Per Berglund (mid 2019) from his tenure as vice dean of faculty of KTH and the promotion of Prof. Christina Divne to full professor and additional support for her research group.

Education, training and research in the field of mammalian-cell-based bioprocessing is highly relevant for both the international large industry, as well as start-ups, in the greater Stockholm area and is currently not represented amongst the faculty of KTH. This field has previously been identified and supported as strategically important by Vinnova and KTH. Over the past years, Dr. Veronique Chotteau has proven her still growing potential as very talented independent researcher, who runs an almost completely externally funded (*e.g.* Vinnova Centre of Excellence AdBioPro) research group. Given the strategic value of a faculty position in mammalian-cell-based bioprocessing this topic has been prioritized by CBH for faculty recruitment and is currently at the interview stage.

Another highly relevant topic of research and education within DIB is the use of biotechnological processes for clean water- and removal of environmental pollution created by human activity and, where possible, turn these into valuable resources. However, also this topic is not anchored in a faculty position. Reallocation of basic funding gives the option to change this by opening the recruitment of a visionary and scientific faculty member at associate- or full professor level. With the proposed recruitments and developments, the faculty of DIB will maintain a good career stage- and gender balance as well as a solid future-proof research portfolio with a good connection to the education at 1st, 2nd and 3rd cycle.

b. Congruence with university-level goals for research as set out in “A leading KTH" as set out in KTH´s "Development Plan 2018-23” (page 5) [Link].

The ambitions of DIB naturally align with the ambitions of KTH and the CBH school. Our curiosity-driven applied basic research provides sustainable biotechnological solutions for Energy, Environment and Materials, which are also 3 out of the 4 focus areas of research within CBH. We collaborate with industry, international academia and we have increased our collaborative efforts within KTH in the recent years. The joint DIB research infrastructure is used for teaching at the 1st, 2nd and 3rd cycle levels as well as for our research.

Another shared passion of DIB is to Link societally relevant research to teaching and training of highly-skilled engineers and scientists driven by a desire to change society in a prosperous and sustainable direction. This is shown by the large and appreciated contribution of all DIB faculty to the teaching of KTH, as well as by Prof. Divne as director and Assoc. Prof. Ines Ezcurra as program director Biotechnology within 3rd cycle education at CBH.

c. Leadership structure and collegial structure

The leadership structure of DIB is flat with the department head (and vice head of department) as direct line manager for the other 10 faculty and independent researchers. The faculty and independent researchers in turn directly supervise their own post-docs, PhD students and where applicable lab managers. This is a purposely chosen and regularly discussed structure to optimize opportunities for communication and collaboration. The leadership team of the department of 10 faculty and researchers meets once a month to discuss and communicate news from the school board, discuss the work environment, science and strategic future development. Faculty working together within the 5 research themes (section 2b) organize additional regular in-depth group discussions. DIB also actively contributes to collegial activities at school and central level, such as Prof. Berglund’s appointment as KTH vice dean from 2010-2019 and membership of Dr. Ezcurra and Gustafsson of the CBH school assembly.

d. Strategies for high quality

During the ongoing process of faculty renewal, the recruitment of excellent candidates for the faculty positions, as well as strategic selection of the research fields, is of the greatest importance. Achievement of critical mass both in faculty per topic as well as increasing the number of temporary personnel per faculty member is important to consolidate research quality and quantity. The balance between the education of PhD students and the generally higher scientific output of post-docs is actively considered. (Inter)national collaboration is an important pillar underlying the current successes of DIB and will remain such in the future. Newly recruited and existing staff is coached towards these goals. In both formal (personal development dialogues and salary meetings) as well as informal meetings quality and quantity of scientific output as well as the balance between applied and fundamental science is regularly discussed. Publication strategy, including open access and predatory publishing has been a returning topic during the DIB seminar series. Additionally, KTH and especially CBH with support from DIB stimulate open access publication by providing additional funds for this.

5. Interaction between research and teaching

a. Interaction between research and teaching at all three levels (BSc, MSc, PhD) of education

Research and teaching at all three cycles is strongly integrated within the culture of DIB. All faculty members thoroughly enjoy teaching and view the combination of research and teaching as an essential part of being a faculty member at a university. Additionally, many of the topics of expertise of the DIB staff are also important for the students participating in the KTH education programs, their future employers and society. Therefore, strong Links exist between the research and teaching activities within the department at the 1st, 2nd and 3rd cycles:

- Examples of our research are included in our many continuously updated 1st cycle courses where that is conducive to achieve the main learning goals of the courses.
- Already in the 1st cycle, students are welcome into our labs during for instance the cultivation technology lab course or one of the BSc end projects.
- At the 2nd cycle, the courses of the MSc Industrial and Environmental Biotechnology provide even more opportunity for the students to be exposed to and/or take part in our research during case studies and lab courses in for instance Biocatalysis, Environmental Bioprocess Technology or the Bioprocess Design course that is fully integrated within the department.
- Teachers at DIB supervise many MSc end projects that are either performed fully embedded within the research lines of the department or at a company with DIB supervisors.
- During the times the BSc and MSc students are within the DIB labs, they share the same coffee and lunch room, which decreases the distance and facilitates communication (e.g. career advice etc.) in later years. This is of course negatively affected by Covid-19.
- PhD students are an essential part of the research and teaching community at DIB, whilst being immersed in their own tailor-made 3rd cycle education projects of which participation in education activities is an important part.
- PhD students are educated in critically reviewing both each other's own research as well as publications by external scientists during a seminar series dedicated to the DIB PhD students.
- The Director of 3rd cycle education at the CBH school, Christina Divne, is a faculty member of DIB.

Assistance of PhD students during various 1st and 2nd cycle teaching activities provides a crucial opportunity for the PhD students to gain experience in teaching. All PhD students that participate in these teaching activities also receive didactical education themselves.

6. Impact and engagement in society

a. Relevance of research to society at large

A common theme and strength in the research of DIB is a drive to develop knowledge and concepts that contribute to achieving and improving sustainable production of chemicals, fuels and pharmaceuticals. DIB also develops concepts for cleaner water and environment. This drive towards sustainable production is itself highly relevant to society at large (See 6c). As indicated throughout this document, this strongly benefits from collaboration with many different industrial and academic partners (see for instance section 2e and the impact cases in section 6e below). For instance the KTH Water Centre, of which van Maris is a board member, creates a network of research groups across KTH together with local government, non-governmental organizations and many large- and small companies.

Although DIB actively disseminates societally relevant and interesting research results to a broad audience (see 6b), this can be done more frequently and by more of the scientists. There are also opportunities to increase the direct valorization of research through patent applications and starting spin-off companies with the help of KTH innovation. Successful examples of intellectual property valorization are the sale and/or licensing of for instance the surface display of tyrosinase (Larsson) and the pentose-fermenting yeast strains (van Maris).

The global impact of improved sustainable production benefits all societal groups. Although improved equality is not the main focus of the research of DIB, there are specific research examples that contribute to this: (i) Improved efficacy and lower cost of production of biopharmaceuticals can lower the cost of drugs for rare diseases and thereby reduce inequality. (ii) development of environmental biotechnological solutions for (waste) water treatment that are suitable for operation in developing

countries. (iii) decreased dependence on fossil oil decreases the dependence on a scarce and geographically unequally distributed resource.

b. Research dissemination beyond academia

Outside academia DIB disseminates research to the following actors:

- Industry. Many collaborations with industry are described throughout this document, including scientific collaboration, co-funding, licensing of intellectual property etc.
- Local governments and governmental organizations. An example of this is the publication of a handbook for policymakers and users for removal of pharmaceuticals and other xenobiotics from (waste) water [Link].
- Students. Trained PhD- and MSc-students are the most important type of valorization by a university. Already during their studies, many students perform their MSc end project at companies or institutions. After their formal training, many students that pursued their education at DIB spread relevant knowledge to society, providing feedback to DIB and contribute to the teaching and research. Results from the research are often incorporated into the (under)graduate education.
- Children, high-school students and teachers. DIB regularly received visits from school classes. Prof. Berglund served on the board of the Science House 2018-2019, a joint KTH-Stockholm University science maker space for school children visited by 80 000 children and their teachers annually. Van Maris regularly gave lectures to groups of high-school students and teachers, but still has language limitations in Sweden.
- General public. DIB utilizes multiple platforms to reach out to the general audience: (i) Online, such as for example through the blog of the ITN Biocascades project, where the PhD students communicated and discussed popular science, (ii) Articles in newspapers, such as for instance the results of the Centre of excellence AdBIOPRO that have appeared in Framtidens Forskning, NyTeknik, Dagens Nyheter, Aftonbladet, Life-time, and in Veckans Affärer. (iii) Workshops, such as organized by AdBIOPRO in Sept 2019 with attendees from a broad audience interested in medical products or a lecture by van Maris at a workshop for the general public organised by the Swedish Water House (SIWI).

This broad range of activities is mostly the result of the personal interest of individual researchers and further improvement of the dissemination beyond academia will be an important topic amongst the faculty of DIB for the coming years, including training of newly recruited faculty.

c. Sustainability and the United Nations' Sustainable Development Goals (SDG)

80-100% of the DIB research is related to sustainable development. Sustainable production (UN #12) of chemicals, fuels or pharmaceuticals is underlying most research lines within DIB, thereby mitigating for instance climate change (UN #13) and improving life on land (UN #15). This is partially also reflected in the sustainability bibliometric analysis of DIB publications. Strangely enough UN#12 itself is not reflected in the bibliometric analysis of KTH, which most likely reflects that this analysis is currently looking for a too narrow window of terms. Additionally, increased efficacy and decreased cost of pharmaceutical production, can boost health for a larger group of people (UN #3) and reduce inequality (UN #10). In the evaluation period, the department has run the Formas Bioraf project to increase utilization of residual stream, acquired an individual Formas grant for the same topic and included sustainability in all new recruitment, to maintain the integration of sustainability in DIB research. Other research lines address removal of pharmaceuticals and excess nutrients from municipal waste water which improves quality of life in surface waters (UN #14), and in turn ensures availability

of suitable drinking water (UN#6) and improves the health of the Baltic sea. Other environmental biotechnology solutions more specifically target developing countries, including projects with the Swedish Development Agency SIDA.

d. Structure for increased impact

To increase impact and to anchor strategically important and societally relevant research topics, KTH, the CBH school and DIB decided to use strategic resources for recruitment of three new faculty members, which included appointment of an externally-recruited head of department (van Maris; 2017). Research impact is a topic that is discussed during the yearly development dialogues between the scientists and the head of department. Special attention will be paid to the engagement of newly recruited faculty members in these activities. Discussion on best practices and experience between the faculty of DIB will be used to increase societal engagement through non-scientific dissemination of the research results.

e. Impact cases

We provide three examples where we believe we have made a significant impact (see Panel 2 Impact cases 10-12 in Appendix 1):

10. Industrial Biotechnology for sustainable fuels and chemicals
11. Environmental Biotechnology for Water Treatment: The Baltic as a case study
12. Very high density continuous culture for biologics production

Appendix 1: Impact cases

Impact case 1: Spatial Transcriptomics

Department of Gene Technology

Summary of the impact

Spatial Transcriptomics was developed at KTH, Department of Gene Technology, between 2010 and 2016, in collaboration with researchers at Karolinska Institute. It was collectively named *Method of the Year 2020* by Nature Methods, and *2020 Top 10 innovations* by *The Scientist*. The technology has been commercialized by 10x Genomics and is available to scientists on a global scale in a reagent-kit format. The technology is being used by researchers and clinicians across many medical disciplines including on samples from human cancer, brain and heart tissue. It is also applied in impactful international projects, e.g., a NASA project to analyse samples from space flights. In industry the technology is widespread, and the department has ongoing collaborations with both 10x Genomics and AstraZeneca. The impact is also visible through numerous recent publications in high impact journals including *Science*, *Cell*, and *Nature Methods*.

Underpinning research

In 2010 the department started the development of a technology aimed at capturing nucleic acids, in particular messenger RNAs, from tissue samples, with maintained positional information. The concept in itself was very simple and is based on arraying DNA-primers, containing nucleic acid barcodes and an RNA-capture sequence, into spatially distinct positions on a microscopic glass slide. A tissue section is then put on top of the slide, and permeabilized, allowing capture of the RNA contents onto the barcode-containing primers and subsequent library preparation and sequencing. If successful, the technology would enable researchers around the world to leave the world of bulk RNA-seq of tissues, and instead enter the world of spatially resolved RNA-seq. At the start, the laws of nature, and in particular diffusion, were seemingly against the small research team designated with the task to develop the technology. However, with a tradition of pushing the research front in genomics technology development, the department invested what limited resources were available at the time. After a couple of small grants in 2011 and 2012, the research team was able to expand and come up with a first proof of concept, and in 2013 and 2014 the team was able to attract large grants from the Knut and Alice Wallenberg Foundation, the Swedish Foundation for Strategic Research and the Swedish Research Council. This allowed the department to recruit and expand its operations with focus on Spatial Transcriptomics. In 2016, the group published a landmark paper in *Science* (Ståhl *et al.* 2016), for the first time describing full transcriptome capture with maintained spatial resolution from mouse brain and human breast cancer specimens. With the technology at hand, the department has been able to carry out several high impact studies on key biological specimens, such as prostate cancer (Berglund *et al.* 2018), melanoma (Ji *et al.*, 2020), ALS (Maniatis *et al.,* 2019) and developing human heart (Asp 2019). But the focus on delivering a strong technology to the community has been equally important. The department has produced several further key technology developments for a standardized protocol (Salmén *et al.,* 2018; Giacomello *et al.,* 2018) and higher resolution (Vickovic *et al.,* 2019), and not least key bioinformatics

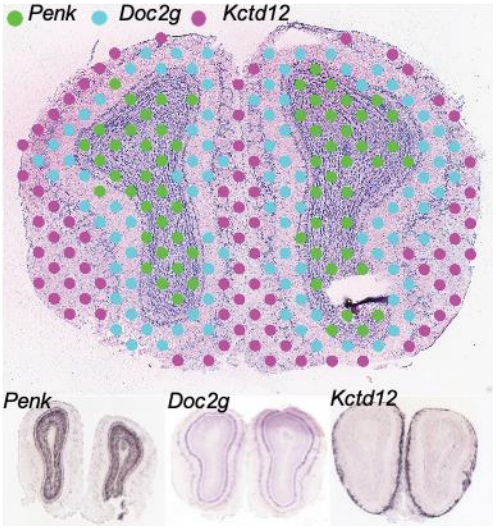


***Figure 1**: Adopted from Ståhl, Salmén et al. Science 2016. The expression pattern of three genes with distinctly different spatial expression patterns in mouse brain olfactory tissue. (top) Spatial transcriptomics data is projected on top of a high resolution tissue image. (bottom) Corresponding in situ hybridization staining patterns of the same genes in three separate tissue sections*

tools, including an automated data processing pipeline and data visualization software to enable the application of the technology on a broad international scale.

The dissemination of the Spatial Transcriptomics technology into the research community is well characterized by the above developments, coupled with numerous conference presentations and seminars surrounding the technology. However, to truly spread the technology and allow it to impact the broader society, it was decided that the technology should be commercialized through a start-up company, also entitled Spatial Transcriptomics. The company was founded based upon several patent applications describing the core technology. It soon became clear that the demand for using the technology outside of the traditional research community was large, and in particular pharmaceutical companies had an interest in leveraging the power of the technology to better understand the impact of their drugs in different tissue specimens. Even NASA is now using Spatial Transcriptomics. The start-up company and the technology were acquired in 2018 by one of the fastest growing companies in the life science research field, 10X Genomics. Since the acquisition, the Spatial Transcriptomics technology has been launched in a kit format and is today available to any actor within the field of life science across the globe. It has been featured in many high impact publications across several clinical fields. In 2020, spatially resolved transcriptomics was collectively named Method of the Year 2020 by *Nature Methods*, and also named in the list of top 10 innovations of 2020 by *The Scientist*.

In future, the technology has the potential to be included in a clinical diagnostic setting. Today, pathologists look at tissue sections with the help of one or a few histological stains, and make a human assessment on the contents. On the same tissue section, the Spatial Transcriptomics technology would allow the same pathologist to get an unbiased view of all gene expression events. By incorporating data from not one or a few, but all genes at once, bioinformatics algorithms allow for the generation of gene expression maps covering all parts of the tissue, and allow for comparisons to earlier known gene expression patterns, for instance from different cell types or disease states. In the end, building on knowledge from comparing thousands of samples, this will allow the pathologist to be presented with an unbiased assessment of the contents of a tissue, allowing for even more informed decisions in the further treatment of patients.

Research publications (citations according to Web of Science):

- Ståhl *et al.* (2016) Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. *Science*. [Link], 406 citations.
- Berglund *et al.* (2018) Spatial maps of prostate cancer transcriptomes reveal an unexplored landscape of heterogeneity. *Nat Commun*. [Link], 71 citations.
- Ji *et al.* (2020) Multimodal Analysis of Composition and Spatial Architecture in Human Squamous Cell Carcinoma. *Cell 2020*. [Link], 22 citations.
- Maniatis *et al.* (2019) Spatiotemporal dynamics of molecular pathology in amyotrophic lateral sclerosis. *Science*. [Link], 66 citations.
- Asp *et al.* (2019) A Spatiotemporal Organ-Wide Gene Expression and Cell Atlas of the Developing Human Heart. *Cell*. [Link], 46 citations.
- Salmén *et al.* (2018) Barcoded solid-phase RNA capture for Spatial Transcriptomics profiling in mammalian tissue sections. *Nat Protocols*. [Link], 33 citations.
- Giacomello *et al.* (2018) Spatially resolved transcriptome profiling in model plant species. *Nat Plants*. [Link], 24 citations.
- Vickovic *et al.* (2019) High-definition spatial transcriptomics for in situ tissue profiling. *Nat Methods*. [Link], 91 citations.

Sources to corroborate the impact

Forbes: 10x Genomics buys spatial transcriptomics in bet on genetic tools [Link].
Nature: Method of the year 2020 [Link].
AstraZeneca: Our technologies/multi-omics [Link].
The Scientist: 2020 Top ten innovations [Link].

Impact case 2: Developing molecular tools for translating genomic information for clinical use

Department of Gene Technology

Summary of the impact

The price for sequencing a whole genome has in the last decade dropped drastically and, accordingly, the use of massively parallel sequencing platforms has increased. However, translating the genomic information into clinical settings has been lagging behind. One reason is that the complex haplotype structure of the human genome is lost in genome-wide sequencing. Another reason is that the vast number of variants are not located in protein coding regions which hampers their functional characterization based on sequencing data only. To address these challenges the Experimental Genomics and the Regulatory Genomics groups have developed molecular tools to resolve the haplotypes and to connect the non-coding mutations to the genes that they affect.

The methods developed by Experimental and Regulatory Genomics are today used, in collaborative efforts with researchers at Karolinska Institute, to investigate several types of cancers, complex diseases such as cardiovascular and inflammatory disease, and immune diseases. The Capture Hi-C method developed at the Regulatory Genomics Lab is patented in Europe and the US, and licensed for use by a company in the UK. It was used to discover 23 clinically targetable genes for the management and treatment of atopic dermatitis and psoriasis. This result was disseminated through a press release and picked up by several newspapers and online news agencies. The Droplet Barcode Sequencing (DBS) technology developed at Experimental Genomics Lab is paving the way for affordable haplotype-resolved sequencing of whole genomes in clinical settings, and is open source to achieve maximal societal use. Both Capture Hi-C and the DBS technology are presently evaluated for use by NGI at SciLifeLab, to offer novel and affordable genomics tools to the research community in Sweden.

Underpinning research

High-throughput sequencing platforms produce mainly short-read data, resulting in a loss of phasing information for the vast majority of the genetic variants analyzed. For most clinical applications, it is vital to know which variant alleles are connected to each individual DNA molecule. In 2012, the Experimental Genomics group started to develop a technology to address this issue. This was done through a new approach for massively parallel barcoding and phasing of single DNA molecules, working within droplets for generating a primer library with millions of uniquely barcoded beads. When compartmentalized with single DNA molecules, the beads were used to amplify and tag any target sequences of interest, enabling coupling of the biological information from multiple loci (Borgström *et al.,* 2015). Although the method was unique, use of beads as a means to barcode DNA molecules or the content of single cells was expensive and laborious. The company 10X Genomics that published their method the year after also used beads (gel-beads) and their technology is very expensive. To address this issue, the method was further developed into Droplet Barcode Sequencing (DBS), which is a novel approach for creating Linked-read sequencing libraries by uniquely barcoding the information within single DNA molecules in emulsion droplets, without the aid of specialty reagents or microfluidic devices. Barcode generation and template amplification was performed simultaneously in a single enzymatic reaction, greatly simplifying the workflow and minimizing assay costs compared to alternative approaches. The method was first applied to phase multiple loci targeting all exons of the highly variable Human Leukocyte Antigen A (HLA-A) gene (Redin *et al.,* 2017) and was later further developed to haplotype-resolve the entirety of genetic variations in the human genome, in megabase-scale phase blocks (Redin *et al.,* 2019). Today, the method is applied for detecting structural variants in colon cancer. As DBS is free from complex libraries of barcoded beads, it was easily adapted to target surface proteins on single exosomes and on single cells in lung cancer and immune diseases, in collaborations with researchers at Karolinska Institute. The method is also applied to investigate chromatin accessibility in single cells. Accessible chromatin or open regions of the genome are primary positions for regulatory elements and this type of epigenetic modification has recently gained interest for locating epigenetic changes that lead to, e.g., cell differentiation and disease development. In

particular, analysis of single cells has been in focus to increase the resolution, but single cell investigations have, to this date, been limited to analysis of only a few thousand cells. However, by exploiting the DBS technique, data on accessible chromatin was recently generated for one million single cells in a single reaction, opening a new chapter in locating disease causing epigenetic changes.

The expression of genes is tightly regulated in cells. Promoters that are located at the start of the genes receive and process information from the cellular space to decide when and how much the gene will be expressed. However, there are also other sequences, called enhancers, that take part in this decision process. Since enhancers carry specific epigenetic marks, they are fairly straightforward to locate. However, unlike promoters, enhancers can be located very distal to the gene that they regulate, and the nearest gene is often not regulated by the enhancer. Therefore, it is not a trivial task to determine which gene(s) the enhancer regulates. Here, the structure of the genome plays a fundamental role: two DNA regions can be several kilobases away in the genome but very close to each other in the three-dimensional space of the nucleus, because of the folding of the genome. It was to exploit this genome folding information, to pair the enhancers to the genes they regulate, that the Capture Hi-C methodology was developed. Capture Hi-C combines chromosome conformation capture with targeted sequencing to find interactions between promoters and enhancers. The method produces the highest resolution regulatory interaction maps to date. Method development was started around 2011 and collaborations with industry (Roche Nimblegen Inc.) was established to obtain access to custom-made product sets required during method development. The method was described in 2015 in Genome Biology (Sahlén *et al.,* 2015) and is highly cited in the field. With assistance from KTH Innovation Holding, funds were obtained for patenting.

Inflammatory skin conditions such as atopic dermatitis and psoriasis have a strong genetic component but almost all the associated variants are non-coding. This poses a problem since, if mutations are in regulating regions, e.g., enhancers, the affected gene cannot be directly assigned for risk modulation. Capture Hi-C addresses this problem, providing potential gene targets for further investigation. To discover genes involved in the onset and progress of inflammatory skin pathologies, Capture Hi-C was combined with transcriptomic and epigenomic datasets. The results were published in the most-cited journal within the allergy/immunology field (Sahlén *et al.,* 2020). The research group has applied Capture Hi-C also on cardiovascular (Åkerborg *et al.,* 2019), liver (Cavalli *et al.,* 2020) and immune (Pradhananga *et al.,* 2020) disease contexts.

Research publications (citations according to Web of Science):

- Borgström *et al.* (2015) Phasing of single DNA molecules by massively parallel barcoding. *Nat Commun*. [Link], 15 citations.
- Redin *et al.* (2017.) Droplet Barcode Sequencing for targeted Linked-read haplotyping of single DNA molecules. *Nucleic Acids Res*. [Link], 7 citations.
- Redin *et al.* (2019) High throughput barcoding method for genome-scale phasing. *Sci Rep*. [Link], 1 citation.
- Sahlén *et al.* (2015) Genome-wide mapping of promoter-anchored interactions with close to single-enhancer resolution. *Gen Biol.* [Link], 69 citations.
- Sahlén *et al.* (2020) Chromatin interactions in differentiating keratinocytes reveal novel atopic dermatitis- and psoriasis-associated genes. *JACI*. [Link], 0 citations.
- KTH Press release: Study identifies more genes that are likely behind psoriasis and eczema [Link].
- Åkerborg *et al.* (2019) High-Resolution Regulatory Maps Connect Vascular Risk Variants to Disease-Related Pathways. *Circulation*. [Link], 4 citations.
- Cavalli *et al.* (2020) A Multi-Omics Approach to Liver Diseases: Integration of Single Nuclei Transcriptomics with Proteomics and HiCap Bulk Data in Human Liver. *Omics*. [Link], 2 citations.
- Pradhananga *et al.* (2020) Promoter anchored interaction landscape of THP-1 macrophages captures early immune response processes. *Cell Immun*. [Link], 1 citation.

Impact case 3: Interpreting biological data with Artificial Intelligence

Department of Gene Technology

Summary of the impact

While much of the methods-development within modern molecular biology experimentation has led to a standardization of experimental protocols, it has also introduced new challenges. The terabytes of data from DNA sequencing, RNAseq, or proteomics analysis in any standard medical, environmental or biological experiment has moved the focus from generating data to interpreting the results. Manual interpretation of data is not seen as accurate enough and it is in any case not able to scale to newer instrumentation. Hence, much hope is directed towards artificial intelligence (AI) for interpreting data. Here we will give two examples of how this type of AI-based methods development at the Department of Gene Technology has led to societal impact in the areas of metagenomics and proteomics data analysis.

The program CONCOCT, co-developed by the Environmental Genomics group, is a program for reconstructing microbial genomes from metagenomics data. It has more than 750 citations and has been applied in studies on all kinds of microbiomes of relevance to human health and the environment. The Environmental Genomics group has applied CONCOCT for large-scale reconstruction of plankton genomes from the Baltic Sea (Hugerth *et al.,* 2015, Alneberg *et al.,* 2020). The mapping of the Baltic Sea plankton biome contributes to better ecosystem models which are of importance for proper management of the sea. In collaboration with SMHI, the group is setting up sequencing-based monitoring of marine plankton, which will lead to faster and more accurate monitoring of plankton (including toxin-producing algae) in the waters surrounding Sweden. CONCOCT has also been applied by the National Bioinformatics Infrastructure (NBIS) for their metagenomics workflow, and thus supports many Swedish scientists in their work.

Percolator, developed by Prof. Lukas Käll, is a cornerstone of the analysis of mass spectrometry data for protein analysis and is currently seen as the reporting standard for proteomics data. From the list of software that uses Percolator [Link], it is worth pointing out some of the commercial actors, such as Mascot Server from Matrix Science Ltd, and Proteome Discoverer from Thermo Scientific. Percolator has been downloaded from Github 5755 times as of January 2020. Prior to 2009 it was hosted on Sourceforge, where it was downloaded 3085 times. Conda Percolator has been downloaded ~4000 times since it became available in December 2019. The seminal paper (Käll *et al.,* 2007), has been cited more than 1450 times (1045 WoS), whereof the vast majority (950 citations) are since 2016 (Google Scholar). Notably, all these figures are gross underestimates of the user base because most users obtain Percolator as a part of other software packages and Percolator is not always cited separately.

Underpinning research

Complex communities of microbes - microbiomes - play important roles for human health and for the environment. Insights into the metabolism and functional potential of individual microbial species can be gained from their genomes, but since most microbes are hard to isolate, sequencing their genomes is challenging. Metagenomics - shotgun sequencing of DNA from a microbial community - circumvents the need for cultivation, but here a challenge has been to decipher which DNA fragments are derived from the same species. The program CONCOCT, co-developed by the Environmental Genomics group (Alneberg *et al.,* 2014), solves this problem and automatically bins metagenomic contigs into species level clusters. The software uses an unsupervised learning algorithm (Gaussian Mixture Model) to cluster contigs based on their sequence composition and abundance distribution patterns.

Percolator is the dominant software for analyzing spectrum identifications produced by tandem mass spectrometry for proteomics. The Percolator software solves the problem of how to tell correct from incorrect identifications of peptides in experiments. The program takes as input database search results produced by any one of a variety of search tools and then applies a semi-supervised machine learning

approach to rank the identified spectra based on an assessment of the statistical significance of the peptide-spectrum matches. Percolator was originally developed by Lukas Käll, during his postdoc period at the University of Washington, and the software is still actively maintained and applied to novel applications (The *et al.* 2016).

Research publications (citations according to Web of Science):

- Alneberg *et al.* (2014) Binning metagenomic contigs by coverage and composition. *Nat Methods* [Link], 469 citations.
- Hugerth *et al.* (2015) Metagenome-assembled genomes uncover a global brackish microbiome. *Genome Biol.* [Link], 83 citations.
- Alneberg *et al.* (2020) Ecosystem-wide metagenomic binning enables prediction of ecological niches from genomes. *Commun Biol.* [Link], 3 citations.
- Käll *et al.* (2007) Semi-supervised learning for peptide identification from shotgun proteomics datasets. *Nat Methods* [Link], 1045 citations.
- The *et al.* (2016) Fast and accurate protein false discovery rates on large-scale proteomics data sets with Percolator 3.0. *Journal of the American Society for Mass Spectrometry* [Link], 79 citations.

Sources to corroborate the impact

CONCOCT is used in the metagenomics workflow of the National Bioinformatics Infrastructure (NBIS) [Link]. The work of implementing DNA sequencing for plankton monitoring has so far resulted in a report for the Swedish Agency for Marine and Water Management (Karlson *et al.*, 2018), and a follow up report is under way.

Since the initial description of Percolator, several competing algorithms have been published, demonstrating the establishment of the idea of using semi-supervised machine learning to improve the scoring of spectral matches. For example, the widely used PeptideProphet algorithm was modified in 2008 to use the semi-supervised target-decoy approach pioneered by Percolator. An independent research group published a comparison of three different search engines in combination with five post-processing methods (Tu *et al.,* 2015). The primary conclusion was that “combinations involving Percolator achieved markedly more peptide and protein identifications at the same FDR level than the other 12 combinations for all data sets.” Several additional papers from us, as well as other groups, describe novel applications of Percolator. For instance, two independent groups have adopted Percolator to re-score spectrum matches by comparing observed spectra with spectra predicted by the fragment-ion peak intensity predictors MS2PIP and Prosit, fully independent of traditional database search engines.

Karlson *et al.* (2018) Miljöövervakning av växtplankton i Kattegatt och Östersjön med rDNA-barcoding och mikroskopi. Havs- och vattenmyndighetens rapport 2018:22. ISBN 978-91-88727-13-8 [Link].

Tu *et al.* (2015). Optimization of search engines and postprocessing approaches to maximize peptide and protein identification for high-resolution mass data. *Journal of proteome research*, 14(11), 4662-4673 [Link].

Impact case 4: Building an international core resource to explore human biology and disease

Department of Protein Science

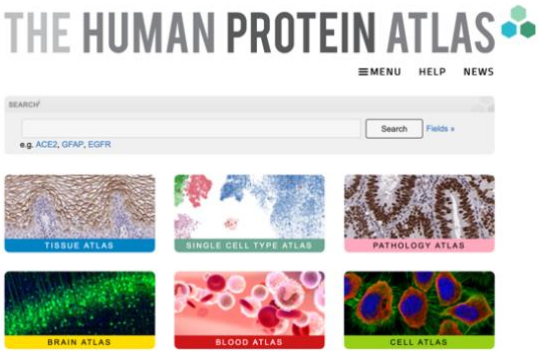
Summary of the impact

KTH has led the international effort to create a Human Protein Atlas (HPA), which started in 2003 with the aim to map all human proteins in cells, tissues and organs using integration of various technology platforms. The KTH-based map of human biology now consists of more than 15 million on-line pages with "in-house" generated data, including 10 million annotated bioimages generated by the consortium. All the data in the knowledge resource is open access to allow scientists both in academia and industry to freely explore all our compiled data on the human proteome. The resource has led more than 600 publications by the consortium members, including many in high-impact journals, such as Nature and Science. The program has been selected by the EU-based organization ELIXIR as a core database resource due to its fundamental importance for a wider life science research community. Using this resource, we have initiate one of the world's most comprehensive analysis of health and disease using proteome profiling. We have also carried out extensive translation efforts, including the generation of multiple patent applications and the start of eight "spin-out" companies.

The impact of the research has been:

- to allow more than 150,000 researchers from more than 150 countries to visit the knowledge resource every month making the atlas one of the most visited biological databases in the world.
- to provide a resource of antibodies and information for research around the world, leading to citations from more than 10 publications every day on average by external groups.
- to provide a resource and technology base for clinical research leading to more than 200 per-reviewed publications by the consortium-members in the field of precision medicine.
- to generate translation research with eight biotech companies from the program conducting at present five human clinical trials

Underpinning research

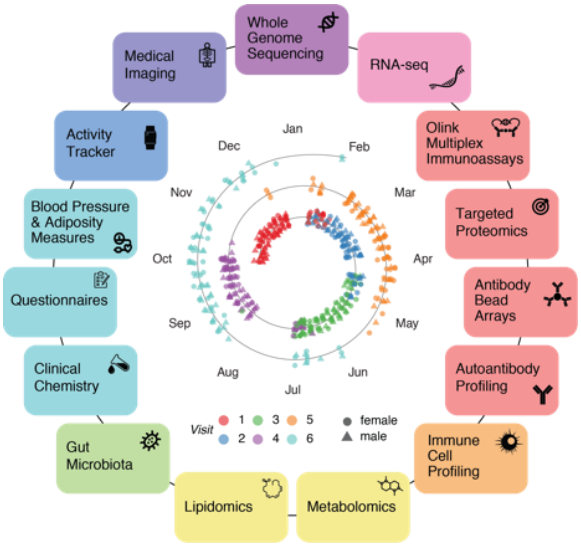


The program consists of 20 research groups mainly at KTH (Science for Life Laboratory), but also research groups at AlbaNova University Center (KTH), Chalmers (Göteborg), Karolinska institutet, Uppsala university, Lund university, Umeå university and several international collaborators, including research groups in Denmark, Germany, France, UK, USA, South Korea, Japan, China and India. The main funding has come from several grants to Prof. Mathias Uhlén extended over almost 20 years from the Knut and Alice Wallenberg Foundation (Sweden). The current version of the HPA program consists of six separate parts (www.proteinatlas.org) and this has recently been described in detail in a Digital Booklet published in November 2020 by *Science*/AAAS [Link]. In this booklet, 35 milestones for the project are described in detail. In addition, a set of 18 YouTube videos have been produced to communicate to the general public.

The database has now more than 300,000 visits per month from 150,000 researchers and it is thus one of the most visited biological databases in the world. The HPA program has thus so far contributed to several thousands of external publications in the field of human biology and disease. An International Working Group for Antibody Validation (IWGAV) has been formed with representatives from several major academic institutions (such as NIH, Stanford, Harvard and EMBL) with KTH (Uhlén) as chair.

In addition, the program has led to eight start-up companies from the KTH group (Atlas Antibodies AB, Atlas Therapeutics AB, Atlas Intressenter AB, Abclon (South Korea), ScandiBio Therapeutics AB, ScandiEdge Therapeutics AB, A05 Diagnostics AB and ProteomEdge AB) with pharmaceutical candidates which are now in five human clinical trials (March 2021).

We have also used the resource created within the Human Protein Atlas Project and the infrastructure built up in the SciLifeLab to initiate one of the world's most comprehensive "personal omics profile" program. All collected samples have been analyzed on a set of omics platforms in a world-unique manner (see figure). The results support an individual-based definition of health and show that comprehensive protein profiling in a longitudinal manner is a path forward for precision medicine. Significant achievements have also been obtained in this context within the field of biomarker discovery. More than 200 publications have been published with authors from the KTH group in the field of biomarker discovery and precision medicine, and some of these discoveries are now pursued in clinical settings as part of the start-up companies.



Research publications (citations according to Scopus):

- Uhlén *et al.* (2015) Tissue-based map of the human proteome. *Science* [Link], 4350 citations
- Uhlén *et al.* (2016) A proposal for antibody validation. *Nature Methods* [Link] 231 citations
- Uhlén *et al.* (2017) A pathology atlas of cancer transcriptome. *Science* [Link] 899 citations
- Thul *et al.* (2017) A subcellular map of the human proteome. *Science* [Link] 703 citations
- Uhlén *et al.* (2019) A genome-wide transcriptomic analysis of protein-coding genes in human blood cells. *Science* [Link] 52 citations
- Sjöstedt *et al.* (2020) An atlas of the human, pig and mouse brain. *Science* [Link] 2 citations
- Mahdessian *et al.* (2021) Spatio-temporal dissection of the cell cycle with single cell proteogenomics. *Nature* [Link]
- Zong *et al.* (2021) Next-generation plasma profiling to monitor health and disease. *Nature Communications* [Link]

Sources to corroborate the impact

Selected by the EU-based organization ELIXIR as a core database resource due to its fundamental importance for a wider life science research community [Link].

More than 200 per-reviewed publications only in the last five years (2016-2020) leading to more than 8000 citations last year (see Google Scholar: Mathias Uhlén)

More than 20 patent applications have been filed with results from the program.

Eight biotech companies have started as a result of the program leading to more than 100 MSEK in export income (last year).

The publication in *Science* in 2015 (Uhlén *et al.*, 2015), is the most cited of all scientific publications from Sweden in the last six years. More than 6,000 citations according to Google Scholar [Link].

Digital Booklet published in *Science* in 2020 outlining 35 milestones achieved by the project [Link].

Educational YouTube videos have been produced using "in-house" 3D image technology aimed at students (educational) and the general public [Link].

Impact case 5: Engaging a wider community in the classification of images

Department of Protein Science

Summary of the impact

Pattern recognition and classification of images are key challenges throughout the life sciences. At KTH we are continuously working with crowd-sourced and machine learning solutions for such problems related to the images in the Human Protein Atlas (HPA) images.

The impact of the research has been:

- Engagement of over 300,000 gamers worldwide in Citizen Science effort. This effort is often described as a milestone in citizen science and can be considered a massive public educational effort, since the game provided participants with insights into modern Life Science and cell biology.
- Engagement of over 2,500 teams of computational scientists worldwide in image classification challenges, establishing the HPA images as a benchmark dataset.
- Several high-impact publications.

Underpinning research

The work outlined in this impact case has mainly been performed by Prof. Emma Lundberg and her research group at the CBH school at KTH.

First, we teamed up with the Icelandic gaming company CCP games and a Swiss start-up MMOS to perform citizen science in a novel way. The idea was to make use of the massive amount of time spent on computer games. In contrast to previous gamified citizen science efforts, we thought it would be more efficient to inject the scientific task into an existing computer game. We developed “Project Discovery”, a scientific mini-game within the massive multiplayer online Science-fiction game EVE Online. Here players could aid the HPA by recognizing protein expression patterns in the massive number of microscopic images, integrated into the EVE game visuals, mechanics and narrative. The game was launched in 2016 and this was the first time ever a scientific project has been integrated into an existing computer game. In one year, 320,000 people have played the game and spent over 70 working years classifying 33 million images. Altogether, the approach turned out highly effective, and the gamers provided classification with a higher accuracy than any published machine learning model. This aided the expansion of the number of location classes annotated in HPA.

Second, we used deep learning to build an automated Localization Cellular Annotation Tool (Loc-CAT). This tool classifies proteins into 29 subcellular localization patterns and is the first published model that can manage multi-label issue (half of all proteins are localized to multiple compartments in the cell) and performs robustly across different cell types. This model was compared side-by-side with the citizen science and performed at par (better at common classes, worse at rare classes). We further demonstrated that the performance of the model could be significantly improved by augmenting it with a pseudo-gamer model. (1) Project Discovery received a lot of media attention and has been described as trailblazing the field of Citizen Science.

To build even better models for image classification (ideally better and more reproducible than humans), we turned to convolutional neural nets. In order to establish the HPA Cell Atlas as a benchmark dataset and to effectively survey a multitude of potential DNN solutions, we organized the Human Protein Atlas Image classification challenge hosted by Kaggle (owned by Google). Over 2 000 teams participated with deep learning models for pattern recognition in microscope images, significantly improving the state-of-the-art, as published in a paper collaboratively written with the

top-ranking teams (2). We are now at the point where we can quantify spatial patterns for integration with other multi-omics data (3,4).

To enable studies of single cell proteome spatial heterogeneity we recently organized another challenge "Human Protein Atlas – Single Cell Classification" again hosted by Kaggle. Here the aim is to make use of weak image-level labels to provide precise single cell labels. The challenge is ongoing and will end in May 2021. *Nature Methods* has again expressed interest in publishing the results as an analysis article.

In summary, through such crowd-sourced approaches, we have been able to develop novel superior models for image classification, raise awareness of the Human Protein Atlas, and establish our images as a benchmark dataset in the field of computer vision. Cell Biology is transforming to a data-driven discipline, and we expect that these studies and the developed computational models will underpin this transformation at the CBH School as well as in the field of cell mapping.

Research publications (citations according to Scopus):

- Sullivan *et al.* (2018) Deep learning is combined with massive-scale citizen science to improve large-scale image classification. *Nature Biotechnology* [Link] 44 citations.
- Ouyang *et al.* (2019) Analysis of the Human Protein Atlas Image Classification competition. *Nature Methods* [Link] 10 citations.
- Stenström *et al.* (2020) Mapping the nucleolar proteome reveals a spatiotemporal organization related to intrinsic protein disorder. *Mol Syst Biol.* [Link] JIF: 9.0 4 citations.
- Qin *et al.* Mapping Cell Structure across scales by fusing protein images and interactions. [Link] (bioRxiv, in revision for *Nature*)

Sources to corroborate the impact

HPA challenge #1 Human Protein Atlas image classification [Link]

HPA challenge #2 Human Protein Atlas - Single cell classification [Link].

Landhuis E. Deep learning takes on tumors. *Nature*, 21 April 2020 [Link].

Peplow M. Citizen Science lures gamers into Sweden's Human Protein Atlas. *Nature Biotechnol.* 2016 [Link]. News article highlighting the significance of Project Discovery.

The Economist: An atlas of where proteins are found in cells, 10 Dec 2016 [Link].

The Wall Street Journal: Videogamers are recruited to fight tuberculosis and other ills, 3 May 2016 [Link].

Wired UK: How thousands of gamers are helping to decode the human body, 28 Apr 2016 [Link].

PC World: How EVE Online players are solving real-world science problems: Meet Project Discovery, 15 Apr 2016 [Link].

Impact case 6: BioSilk – recombinant silk for biomedical applications

Department of Protein Science

Summary of the impact

Methods for recombinant production of spider silk proteins with various functionalizations have been developed in Prof. My Hedhammar's research group. These spider silk proteins have the unique ability to assemble into silk-like materials with both favorable mechanical properties and specific bioactivity. Methods for formulation of bioactive silk of defined microstructure have been developed. These can be used in several biomedical applications, including 3D cell culture.

Underpinning research

At the department, extensive knowledge and experience of recombinant protein production is gathered. This has contributed to the development of a process that allows production of even the very aggregation prone spider silk proteins in non-denatured form. Thereby, the spider silk proteins maintain their unique ability to self-assemble into silk-like materials under benign aqueous conditions. The spin-off company Spiber Technologies AB [Link] has from this established a scalable process for reproducible production of defined and highly pure spider silk proteins.

Methods for functionalization of silk with peptides and domains with preserved bioactivity in the silk format could thereby be developed. Examples of bioactive moieties successfully incorporated into silk materials are affinity domains, cell adhesion motifs, growth factors and antibacterial enzymes. The functionalization can be done either at genetic or protein level, which thus expands the variety of the silk toolbox. The usage of bioactive silk is currently further explored for various applications in both national and international collaborations.

Extensive investigation of the behavior of various silk protein modules at interfaces had led to the understanding of how silk proteins assemble, which enabled development of methods for processing into various silk formats such as fibers, films, coatings and 3D-foams. By usage of microsystems, it is also possible to produce defined silk structures in the nano- and micro-size range.

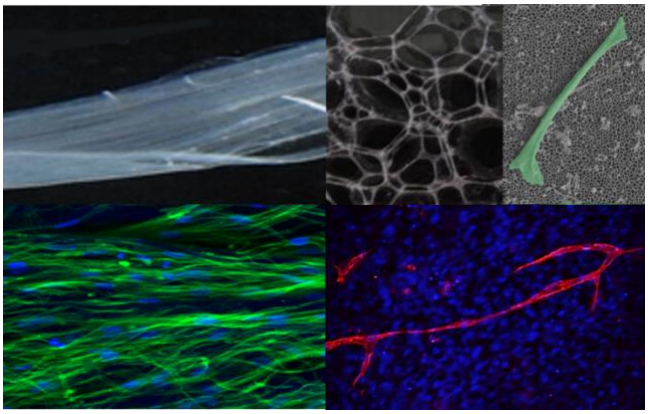


Fig. 1. *Upper row: Recombinant silk in the format of fibers, 3D network and nanowire. Lower row: Cells integrated in 3D BioSilk and their spontaneous formation of a microvessel.*

Thanks to the discovery that assembly of recombinantly produced silk proteins can occur at very mild conditions (air-water interface at room temperature) a method for simultaneous integration of mammalian cells could be developed. By utilizing BioSilk, a silk protein functionalized with a cell binding motif, and the process for 3D formulation, a method for 3D culture of cells in an environment resembling the natural extracellular matrix could be obtained.

Together with researchers with competence in specific medical fields, methods for 3D culture of cells in BioSilk are currently being developed for several applications within regenerative medicine including nerve conduits, wound healing, cancer models and diabetes treatment.

Research publications (citations according to Scopus):

- Hedhammar *et al.* (2008) Structural Properties of Recombinant Nonrepetitive and Repetitive Parts of Major Ampullate Spidroin 1 from Euprosthenops australis: Implications for Fiber Formation. *Biochemistry* [Link] 110 citations.

- Jansson *et al.* (2014) Recombinant spider silk genetically functionalized with affinity domains. *Biomacromolecules* [Link] 42 citations.
- Widhe *et al.* (2016) A fibronectin mimetic motif improves integrin mediated cell biding to recombinant spider silk matrices. *Biomaterials* [Link] 47 citations.
- Gustafsson *et al.* (2018) Structuring of Functional Spider Silk Wires, Coatings, and Sheets by Self-Assembly on Superhydrophobic Pillar Surfaces. *Adv. Mater.* [Link] JIF: 27 21 citations.
- Johansson *et al.* (2019) Assembly of functionalized silk together with cells to obtain proliferative 3D cultures integrated in a network of ECM-like microfibers. *Sci Rep.* [Link] 8 citations.

Sources to corroborate the impact

The company Spiber Technologies AB was founded in 2008 around the silk technology [Link].

BioSilk is since 2019 available as a commercial product, produced by Spiber Technologies AB and distributed by BioLamina AB [Link].

BioSilk is used as a substrate for differentiation of stem cells in 3D within the EU project STACCATO [Link].

BioSilk is used as support in the development of advanced tissue models, within the EU project GUTVIBRATIONS [Link].

Impact case 7: Direct conversion of CO_2 to biofuels with bacteria

Department of Protein Science

Summary of the Impact

Background. One of the most important tasks for a future sustainable society is to be able to provide energy and industrial chemicals without the use of fossil fuels. An interesting solution is to use solar energy combined with biological tools, such as gene editing and metabolic engineering. In the KTH group of Paul Hudson, these new types of production system are being developed, where engineered bacteria fix CO_2 and use it to synthesize any desired compound. One example is photosynthetic cyanobacteria that can produce liquid fuels or chemicals directly from sunlight, another is litho-autotrophic bacteria that use energy derived from hydrogen. The systems aim to reduce emissions in several sectors of the chemical industry.

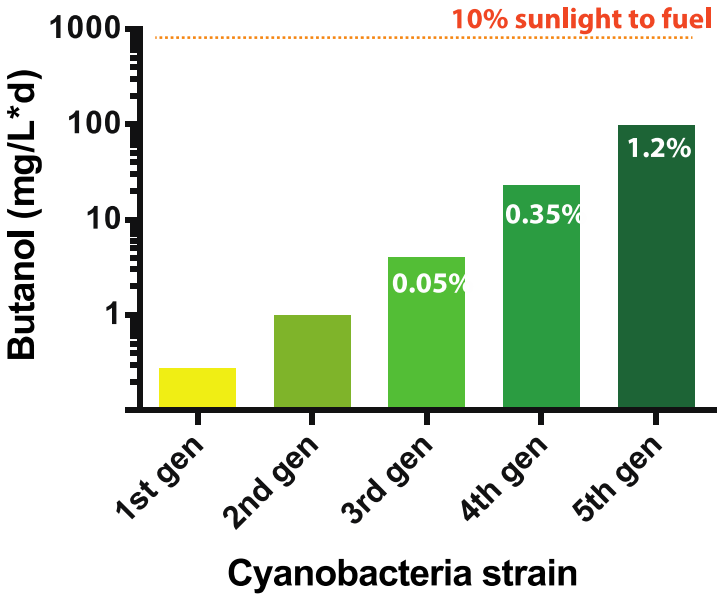
Impact. The group has created several GMO cyanobacteria strains that convert light and CO2 into the biofuel butanol. In designing and constructing these strains, novel tools for cell modeling, genetic engineering and cell analysis were developed. The impact has been primarily through technical reports and articles, public interviews or lectures on the potential of biological solutions to climate change, and workshops to train students on biological engineering techniques. One collaboration with Photanol, a Dutch SME, will lead to a small pilot-scale cultivation of the KTH GMO cyanobacteria (H2020 RIA project GA 760994). Current embodiments are not efficient enough to produce compounds at economic competitiveness; a ten-fold increase in efficiency is required. Research is ongoing to increase rates and efficiency of these strains.

Underpinning research

The KTH group is exploring how metabolism is controlled in CO_2 fixing bacteria, with focus on the primary CO_2-fixing reactions, the Calvin cycle. Two approaches are pursued: i) to increase the CO_2 fixation rate, and ii) to increase the fraction of fixed CO_2 that is converted to the desired compound.

A systems biology approach provides insight to guide genetic engineering. For example, an in-depth analysis of the cyanobacteria proteome showed that carbon fixation is likely regulated post-translationally, results that have led to mapping of allosteric regulation of Calvin cycle enzymes. We expect that discoveries on cyanobacterial Calvin cycle will also have relevance for C3 crop plant engineering, as the chloroplast shares a common ancestor with cyanobacteria. All generated systems biology data and software for metabolic modeling is publicly available.

The KTH group pioneered the use of CRISPR/Cas gene editing and CRISPR-interference in cyanobacteria. These tools allowed us to rapidly test genetic engineering strategies to increase productivity. For example, we created several cyanobacteria strains to produce the biofuel n-butanol, a potential gasoline replacement that can also be upgraded to jet fuel. Through a model-guided genetic engineering campaign, we have gradually increased light-to-fuel conversion efficiency (see Figure). Our current strains convert approximately 1% of incoming light energy into butanol with carbon partitioning to fuel >20%. Using Life-Cycle analysis of a hypothetical cyanobacteria butanol



"farm," in Sweden, the KTH group calculated that an increase in conversion efficiency to 10%, near the theoretical maximum, would make cyanobacteria biobutanol more sustainable than 2nd generation bioethanol.

To further increase the rate and efficiency, the KTH group is pursuing high-throughput screening of genetic libraries and transferring successful genetic interventions into novel strains that are suitable for industrial use.

Research publications (citations according to Scopus):

- Jahn *et al.* (2018) Growth of Cyanobacteria Is Constrained by the Abundance of Light and Carbon Assimilation Proteins. *Cell Reports* [Link] (JIF: 8.1) 27 citations.
- Asplund-Samuelsson *et al.* (2018) Thermodynamic analysis of computed pathways integrated into the metabolic networks of *E. coli* and *Synechocystis* reveals contrasting expansion potential. *Metabolic Engineering* [Link] (JIF: 7.3) 13 citations.
- Shabestary *et al.* (2018) Targeted repression of essential genes to arrest growth and increase carbon partitioning and biofuel titers in cyanobacteria. *ACS Synthetic Biology* [Link] (JIF: 7.2) 31 citations.
- Yao *et al.* (2020) Pooled CRISPRi screening of the cyanobacterium *Synechocystis* sp. PCC 6803 for enhanced industrial phenotypes. *Nature Communications* [Link] (JIF: 13.1) 22 citations.
- Nilsson *et al.* (2020) Environmental impacts and limitations of third-generation biobutanol: Life cycle assessment of n-butanol produced by genetically engineered cyanobacteria. *Journal of Industrial Ecology* [Link] (JIF: 6.5) 6 citations.

Sources to corroborate the impact

Public outreach

Print

- Can solar energy fuel the world? [Link] Hudson EP, Uhlen, M. (2016) in *Environmental Reality: Rethinking the Options – His Majesty King Carl XVI Gustaf of Sweden's 12th Royal Colloquium*.
- Så kan solbränsle ersätta fossil energi [Link]. Article in magazine *Forskning och Framsteg* (2020). Highlights our work (Swedish).
- Bränsle från gröna cellfabriker [Link]. Article by Ph.D. student Jan Karlsen printed in *Skärgårdsredaren* (2017 Nr 4, Swedish).

Video

- Framtidens Energi ("Energy of the Future"; subtitled in English) [Link]. Episode 8 in video series Jakten på Kraften produced by Vattenfall (2019) [Link].
- Genome Editing to Feed, Fuel, and Cure [Link]. Lecture by Paul Hudson at symposium hosted by the Royal Swedish Academy of Engineering Science (IVA). (2018).
- Using CRISPRi for fundamental and applied biology in cyanobacteria [Link]. Recorded video by Paul Hudson (19 min, 2020). The 2 minute version [Link].

Software

- Hudson Lab software [Link]. POPPY (metabolic pathway development), and K1 (a kinetic model of the Calvin cycle)

Impact case 8: Case study: Drug development and human clinical trials

Department of Protein Science

Summary of the impact

The research groups at the Department of Protein Science, CBH, KTH, have for many years been focusing on translational efforts to take the basic research into clinical applications of great medical importance. Approximately 100 inventions have led to patent applications with inventors from the KTH group and more than 20 start-up companies have their origin at the department. Taken together, more than ten human clinical trials have been, or are being pursued, by these start-up companies.

Underpinning research

Several of these efforts are based on the unique scaffold protein, named the affibody molecule, developed in the department more than 20 years ago. Affibodies have shown great promise as tracers for medical imaging but recently also as “next-generation" biological drugs, this being an alternative to more traditional therapeutic monoclonal antibodies. The first therapeutic affibody molecules have now entered clinical development, and more than 500 investigations using the affibody-concept have been published, many from external groups.

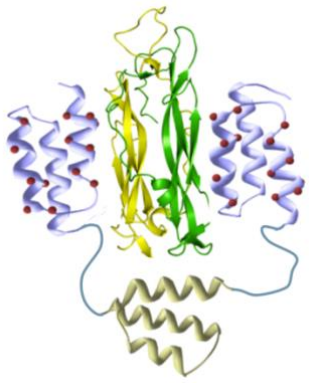


Figure 1. *Two IL-17-binding affibodies (purple) are Linked together via the ABD-domain (beige). The homodimeric IL-17 depicted in green and yellow.*

The small affibody molecules, which are cleared rapidly from the circulation, have been found very suitable for medical imaging applications when conjugated to a suitable radionuclide. A high affinity HER2-specific affibody molecule has been extensively investigated and found safe and efficacious as a medical-imaging agent in humans. Clinical investigations for imaging of breast cancer patients have been performed in Germany, Denmark and Sweden (Uppsala) and is currently in late-stage clinical evaluation. The excellent safety profiles of the affibodies have recently encouraged investigations for biotherapy applications.

The clinically most advanced affibody molecule is currently an engineered IL-17 specific ligand trap, formatted into a small 18 kDa dimeric construct with sub-picomolar affinity (Figure 1) with plasma half-life extended by fusion to an engineered albumin binding domain (ABD). This affibody construct was in clinical evaluations found to be safe, well tolerated, and efficacious in a phase II clinical trial [Link] in more than 200 patients with moderate-to-severe psoriasis, with excellent clinical effect and no reported adverse effect. The same IL-17 ligand trap will now also be clinically evaluated for treatment of psoriatic arthritis and also other autoimmune diseases.

This high-affinity ABD domain, also developed at our department, extends the half-life of fused protein drug candidates to that of serum albumin (ca three weeks in humans), and this technology has been applied to increase the circulation times for biopharmaceuticals from international pharma companies.

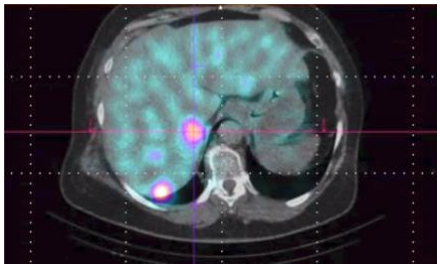


Figure 2. *Two liver metastases are clearly visualized in a breast cancer patient by HER2-mediated SPECT imaging using a ^{99m}Tc-conjugated HER2-specific ADAPT.*

Based on the structure of the ABD, a novel scaffold protein was recently devised, the ADAPT. A HER2-specific ADAPT was recently successfully evaluated in a first medical imaging trial in 20 breast cancer patients [Link], with indeed impressive results (Figure 2). These promising findings will likely encourage future therapeutic applications.

ScandiBio Therapeutics AB [Link] is a biotechnology company founded by researchers from KTH. A platform for AI-based

modelling of biology and medicine has been developed to allow potential treatment of diseases with metabolic dysfunction. The company has developed drug candidates consisting of a combination of several metabolic activators aimed to improve for patients with mitochondrial dysfunction. A large number of human clinical trials have been initiated using one of these drug candidates to treat several diseases with metabolic problems], including COVID-19, Alzheimer´s Disease, Parkinson´s Disease and Non-Alcoholic Fat Liver Disease (NAFLD).

Research publications (citations according to Scopus):

- Seijsing, *et al.* (2014) An engineered affibody molecule with pH-dependent binding to FcRn mediates extended circulatory half-life of a fusion protein. *Proc. Natl. Acad. Sci. USA* [Link] 25 citations. *The presented results have led to ongoing phase I clinical trials for autoimmune disease.*
- Ståhl *et al.* (2017) Featured review: Affibody molecules in biotechnological and medical applications. *Trends Biotechnol.* [Link] (JIF: 14.3) 104 citations.
- Sörensen *et al.* (2016) Measuring HER2-Receptor Expression In Metastatic Breast Cancer Using [(68)Ga]ABY-025 Affibody PET/CT. *Theranostics* [Link] (JIF: 8.7) 103 citations.
- Jonsson *et al.* (2021) Engineering of a femtomolar affinity binding protein to human serum albumin. *Protein Eng. Des. Sel.* [Link] (2008) 126 citations.
- Garousi *et al.* (2015) ADAPT, a novel scaffold-protein based probe for radionuclide imaging of molecular targets that are expressed in disseminated cancers. *Cancer Res.* [Link] (JIF: 9.1) 30 citations.
- Bragina *et al.* (2021) Phase I study of 99mTc-ADAPT6, a scaffold protein-based probe for visualization of HER2 expression in breast cancer. *J. Nucl. Med.* [Link] (JIF: 7.9) 3 citations.
- Altay *et al.* (2021) Combined metabolic activators accelerate recovery in mild-to-moderate COVID-19 *MedRxiv* [Link].

Sources to corroborate the impact

Recently, several start-up companies have been spun out from the department working with drug development and in the following a selected list is presented with year of inauguration.

Start-up company	Year	Description
Abclon (South Korea)	2010	Therapeutic antibodies (clinical trial, cancer). Listed on COSDAQ
Atlas Therapeutics AB	2011	Merged with Alligator Bioscience. Listed on NASDAQ (Stockholm)
ScandiBio Therapeutics AB	2017	Clinical trials initiated (COVID-19, Fat liver, Alzheimer's disease and Parkinson's disease)
Amylonix AB	2018	Drugs for Alzheimer's disease (in preclinical testing)
ProteomEdge AB	2019	Precision medicine. Stratification of patients using protein profiling.
MindForce Game Lab AB	2019	Science and gaming. Clinical trials to be initiated for bipolar disorder.

Impact case 9: Confronting the COVID-19 pandemic

Department of Protein Science

The SARS-CoV-2 and COVID-19 pandemic hit Sweden in March 2020. Testing capabilities, healthcare, and elderly homes were not prepared, and Sweden suffered a health crisis. Limited testing was a major hurdle, which led to difficulties in understanding the spread, and estimate what proportion of the population had been exposed to the virus, including those with mild or even no symptoms. Our Department stepped in forcefully and contributed significantly to confronting these problems.

Summary of the impact

- Large-scale PCR testing has been set-up to aid Swedish health care – more than 600,000 tests performed during 2020 commissioned from the government.
- Provided the Public Health Authority, Region Stockholm Healthcare, Stockholm's Infection Control physician, politicians, and the public with surveillance and early warning of 2nd and 3rd pandemic waves. Immediate impact
- Provided an antibody testing method that within 12 months from initiation of development was applied for analysed over 130,000 samples, assisting the Swedish Public Health Authority and local hospitals in determining antibody levels in populations, hospital staff, workplace. The method is now used as reference by the Public Health Authorities and RISE. Immediate impact
- Assisted as expert in Sweden's Scientific Reference group for Covid-19 vaccines.
- Completed human clinical trials (phase 2 and 3) to treat COVID-19 patients with a drug developed by KTH researcher using AI-based research.
- Supported the research community with COVID19 sample analyses, and initiated research projects to understand the virus and tackle this and future pandemics. Potential future impact.

Underpinning research

Based on a generous grant from the Wallenberg Foundation, a test capacity for COVID-19 was set-up at as a collaboration between research groups at Karolinska Institute (Lars Engstrand) and KTH (Mathias Uhlén). More than 600,000 tests for active infection were carried out in 2020 through a stream-lined PCR-testing platform. In the end of the year, researchers from KTH and KI started a company (A05 Diagnostics) to continue testing outside the university setting. More than 300,000 tests were conducted during the first three months of operation.

Due to the difficulties in measuring the full spread of the pandemic, and with hopes of achieving fast results of infections and ability to predict hospitalizations, a cross-disciplinary collaboration between our faculty with experiences of low-copy number PCR analysis (Cecilia Williams, SciLifeLab) and wastewater expert (Zeynep Cetecioglu, Dept. of Chemical Engineering) set out to sample wastewater and measure SARS-CoV-2 RNA. In March 2020, methods were evaluated, modified, and applied. Weekly measurements were then able to surveil the pandemic and provided early warnings of 2nd (Fall 2020) and 3rd (early Spring 2021) waves.

At the same time, another constellation at PRO with long-term expertise design and high-throughput production of proteins (Sophia Hober, My Hedhammar, Hanna Tegel, Albanova), and of multiplex antibody analyses (Peter Nilsson, SciLifeLab), initiated serological screening. A highly sensitive and specific multiplex analysis of immune response against COVID-19 in plasma samples was developed and applied in a high-throughput format (>130,000 samples). Also, a cell-free high-throughput assay for assessment of SARS-CoV-2 neutralization capability was developed. This assisted the Swedish Public Health Authority and local hospitals in determining antibody levels in populations and hospital staff. To capture the spread of the virus in the general population, serology assays were applied on dried blood collected with home sampling devices (Jochen Schwenk, Claudia Fredolini, SciLifeLab, together with Niclas Roxhed, EESC, KTH). In addition, one of our faculties (Sophia Hober) has acted as expert adviser to the Swedish vaccination reference group and to the Strategic Consultancy Group (SCG) for the AstraZeneca COVID-19 Vaccine development.

A KTH start-up company, ScandiBio Therapeutics, has developed a candidate drug using an AI-based approach for patients with COVID-19. The drug consists of four metabolic activators designed to improve mitochondrial function. A phase 3 clinical study was completed in December 2020 and demonstrated that patients with COVID-19 experienced a significant reduction in recovery time when receiving the drug. The results of the study build on findings from Phase 2 clinical data published in October 2020. The treatment also improved liver health and decreased levels of inflammatory markers. This therapy may become an important complement to help combat the pandemic.

Finally, multiple research projects have been initiated to help speed up testing of nasopharyngeal swab samples, from 1-hour tests (*Centrifugal microfluidic platform to perform loop-mediated isothermal amplification (LAMP) directly from heat-inactivated samples,* Aman Russom) to antigen testing *(Rapid SARS-CoV-2 diagnostics via affinity capture and LC-MS/MS* Per-Åke Nygren, Fredrik Edfors, and others). Others aim to improve treatments (*Spatial single cell mapping of SARS-CoV-2 interacting host proteins for quick and targeted drug repurposing,* by Charlotte Stadler, Emma Lundberg, SciLifeLab), understand its transmission (*Role of host proteins incorporated on SARS-CoV-2 virions in transmission dynamics, pathogenesis and individual immune response*, Claudia Fredolini, Helena Persson, SciLifeLab) to finding markers that predict severe COVID-19 (*Identification of plasma biomarkers for risk stratification of hospitalized Covid-19 patients,* Jacob Odeberg, Lynn Butler, SciLifeLab).

Research publications (citations according to Scopus):

- Rudberg *et al.* (2020) SARS-CoV-2 exposure, symptoms and seroprevalence in healthcare workers in Sweden. *Nature Communications* [Link] (JIF: 13) 25 citations.
- Altay *et al.* (2020) Current Status of COVID-19 Therapies and Drug Repositioning Applications. *iScience* [Link] (JIF: 4.4) 21 citations.
- Jafferali *et al.* (2021) Benchmarking virus concentration methods for quantification of SARS-CoV-2 in raw wastewater. *Science of the Total Environment* [Link] (JIF: 6.5) 10 citations.
- Havervall *et al.* (2021) Symptoms and Functional Impairment Assessed 8 Months After Mild COVID-19 Among Health Care Workers *JAMA* [Link] (JIF:45)
- Dillner *et al.* (2021) High amounts of SARS-CoV-2 precede sickness among asymptomatic healthcare workers. *The Journal of Infectious Diseases* [Link] (JIF: 5.7)
- Dillner *et al.* (2021) Antibodies to SARS-CoV-2 and risk of past or future sick leave. *Scientific Reports* [Link]
- Hassan *et al.* (2021) SARS-CoV-2 infections among personnel providing home care services for older persons in Stockholm, Sweden. *J Internal Medicine* [Link] (JIF: 6.9)
- Roxhed *et al.* (2021) Multianalyte serology in home-sampled blood enables an unbiased assessment of the immune response against SARS-CoV-2. *Nature Communications, accepted* (JIF: 13.1)
- Altay *et al.* (2021) Combined metabolic activators accelerate recovery in mild-to-moderate COVID-19. *MedRxiv* [Link]

Sources to corroborate the impact

- COVID-19 data portal Sweden: SciLifeLab Autoimmunity and Serology profiling facility SARS-CoV-2 antibody test statistics [Link].
- Wastewater surveillance is reported for 6 major areas of Stockholm [Link].
- Antibody testing method as reference method (Public Health Authorities and RISE).
- National media reports (including national television: Aktuellt, SVT-Morgonstudion, SVT-Helgstudion, Vetenskapens värld, TV4-Nyhetsmorgon, TV4 News, National radio: P1 Studio Ett, Vetenskapsradion, and national newpapers and web sites: Dagens Nyheter, Expressen, Aftonbladet, International media *e.g.* Bloomberg)
- Clinical studies have been published (see Altay *et al.*, 2021) and reported on company web page (www.scandibio.com). Information about a start-up diagnostic company, A05 Diagnostics, can be found on the web [Link].

Impact case 10: Industrial Biotechnology for sustainable fuels and chemicals

Department of Industrial Biotechnology

Summary of the impact

Sustainable chemicals and fuels produced by biocatalysis or microorganisms decreases release of greenhouse gas and reliance on oil. The last decade, yeast-based processes for lactic acid, succinate, farnesene, lignocellulosic ethanol, as well as cell-free enzymatic cascade processes for production of fine chemicals started in industry. Research of Prof. Berglund, van Maris and their colleagues made important contributions to these advances.

Underpinning research: microbial production

Proof-of-principle work on pentose fermenting yeasts by van Maris and his colleagues overcame a major hurdle that long prevented realization of yeast-based processes for alcoholic fermentation of lignocellulosic biomass. Combining metabolic engineering and laboratory evolution enabled efficient consumption of the pentose sugars xylose and arabinose forms (Wisselink *et al.,* 2009). Additional original research turned the common fermentation inhibitor acetic acid into an additional carbon source for ethanol production and simultaneously eliminated the production of the major by-product glycerol, thereby increasing the potential industrial ethanol yield on sugar by 6% (Guadalupe Medina et al. 2010). The flexibility of this yeast platform expanded further by expression of the Rubisco enzyme from a CO_2-fixating bacterium, together with a spinach gene enabling elimination of glycerol formation also in the absence of acetic acid (Guadalupe-Medina *et al.,* 2013; Papapetridis *et al.,* 2018). Success of yeasts in industrial biotechnology is also exemplified by heterologous products such as lactic acid, farnesene and succinic acid. Pioneering research on engineering *Saccharomyces cerevisiae* for removal of ethanol production, production of carboxylic acids, increased free-energy conservation, improved pre-cursor supply and increased robustness, either directly (patents) or indirectly (publications) contributed to this.

Sources to corroborate the impact

Figure 1. *Poet-DSM Project Liberty is a cellulosic ethanol plant using engineered yeast, together with many other technological advances, to produce renewable biofuel from corncobs, leaves, husk and stalks. Image: dsm.com*

The underlying technology and patents now are is a central part of the core technology of the DSM yeast concept as for instance used in the Poet-DSM project Liberty (Figure 1). Press releases on these findings and project Liberty have resulted in exposure to a broad audience. Despite technical maturity, project Liberty scaled back from commercial operation to a research and development mission in November 2019, in response to U.S.A. domestic political developments on the Renewable Fuel Standard.

Underpinning research: biocatalysis

Also in the field of biocatalysis, intensive industry-academic collaborations resulted in impact through industrial applications. The extensive network contributing to this, which was built through participation in FP7, H2020, Vinnova and Formas projects since the previous RAE in 2012, is shown in Figure 2, resulting in 33 co-authored papers with international and national academic and industrial collaborators.

Sources to corroborate the impact of the work of the KTH Biocatalysis over the past eight years is also illustrated through:

- Start-up company EnginZyme AB (2014) [Link] with KTH Biocatalysis graduate Dr. Karim Engelmark Cassimjee as CEO and founder. The major technology platform of the company was published in two papers during Dr Cassimjee's PhD studies at KTH (Cassimjee *et al.,* 2008 and 2011).
- Establishment of successful two-yearly series of ESAB-supported International Symposia on Amine Biocatalysis (1st edition in Stockholm in 2013 chairs Berglund & Woodley). The series has attracted great interest from industry with >30% of the participants and presenters.
- Research in collaboration with Prof Córdova on the cascade synthesis of the pepper fruit compound capsaicin (Anderson *et al.,* 2014), led to a patent on multi-catalytic transformation of alcohols into amines (Córdova *et al.,* 2015). The company Organofuel Sweden AB acquired the patent in 2016 and is now offering capsaicin.

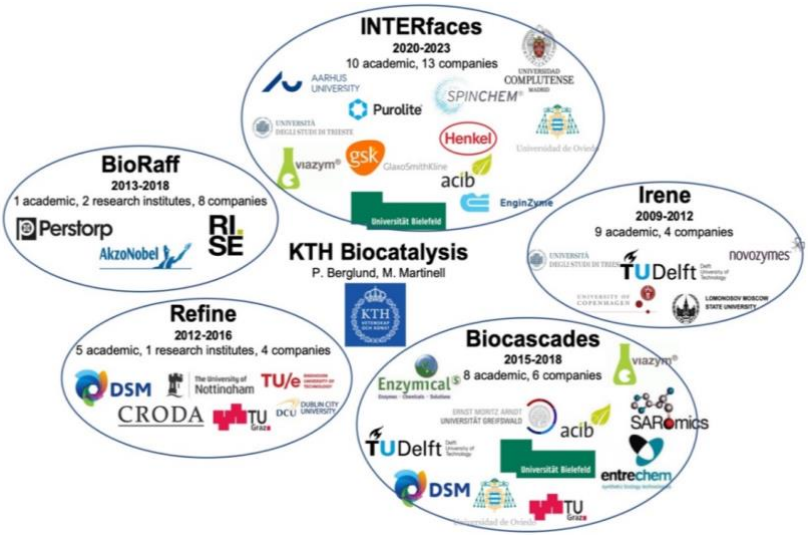


***Figure 2**. KTH Biocatalysis major networking 2012-2020.*

Research publications (citations according to Web of Science)

- Anderson, M *et al.* (2014). Total synthesis of capsaicin analogues from lignin-derived compounds by combined heterogeneous metal, organo¬catalytic and enzymatic cascades in one pot. *Adv. Synth. Catal.* [Link] 21 citations.
- Cassimjee, K. *et al.* (2011) One-step enzyme extraction and immobilization for Biocatalysis applications. *Biotechnol. J.*, [Link] 18 citations.
- Cassimjee, K. *et al.* (2008). Silica-Immobilized His6-tagged enzyme: Alanine racemase in hydrophobic solvent. *Biotechnol. Bioeng.* [Link]. 11 citations.
- Córdova, A *et al.* (2015). Patent: Efficient synthesis of amines and amides from alcohols and aldehydes by using cascade catalysis. US20170174618 A1 [Link], CA2943677A1, CN106458854A EP3122715A1, WO2015144902A1
- Guadalupe Medina, V. *et al.* (2010). Elimination of glycerol production in anaerobic cultures of a *Saccharomyces cerevisiae* strain engineered to use acetic acid as an electron acceptor. *Appl. Environ. Microbiol.* [Link] 99 citations.
- Guadalupe-Medina, V. *et al.* (2013). Carbon dioxide fixation by Calvin-Cycle enzymes improves ethanol yield in yeast. *Biotechnol. Biofuels* [Link] 65 citations.
- Papapetridis, I. *et al.* (2018). Optimizing anaerobic growth rate and fermentation kinetics in *Saccharomyces cerevisiae* strains expressing Calvin-cycle enzymes for improved ethanol yield. *Biotechnol. Biofuels* [Link] 20 citations.
- WisseLink, H.W. *et al.* (2009). Novel evolutionary engineering approach for accelerated utilization of glucose, xylose and arabinose mixtures by engineered *Saccharomyces cerevisiae* strains. *Appl. Environ. Microbiol.* [Link] 177 citations.

Impact case 11: Environmental Biotechnology for Water Treatment: The Baltic as a case study

Department of Industrial Biotechnology

Summary of the impact

Accumulation of pharmaceuticals and other non-natural compounds affects surface- and drinking water quality worldwide. DIB Environmental Biotechnology measured and modelled pharmaceutical residues throughout the Baltic Sea catchment area, investigated the efficacy of different removal methods at Swedish wastewater treatment facilities and published a handbook for policymakers and users: Waterchain: Best practices [Link].

Underpinning research

Human activity made the Baltic Sea one the most polluted seas in the world. Increasing global use of pharmaceutical products is leading to increasing contaminations in surface- and groundwater. In a large nautical sampling campaign, we investigated the environmental concentrations of a selection of 93 pharmaceuticals in 43 locations in the Baltic Sea and Skagerrak (Figure 1). One of the pharmaceuticals investigated, the anti-epileptic drug carbamazepine, was widespread in coastal and offshore seawaters (present in 37 of 43 samples).

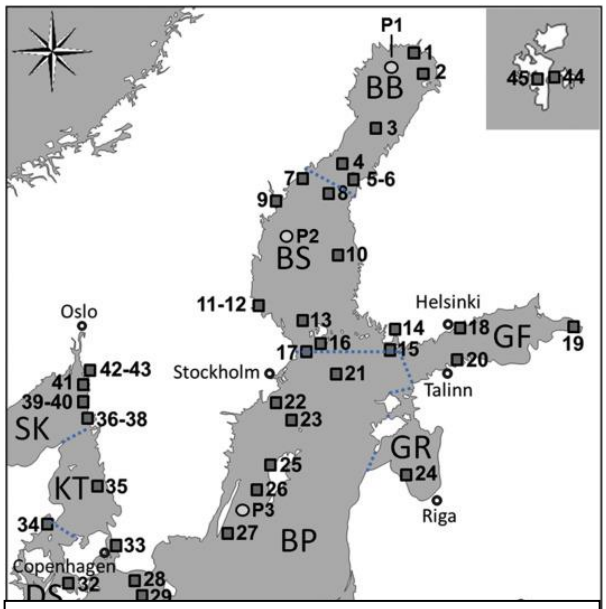


***Figure 1.**. Baltic Sea with sampling points and main sub-basins: BB = Bothnian Bay, BS = Bothnian Sea, GF = Gulf of Finland, GR = Gulf of Riga, BP = Baltic Proper, DS = Danish Straits, KT = Kattegat and SK = Skagerrak. Control samples (44 and 45) were taken at* Svalbard.

To predict concentrations of pharmaceuticals in the sub-basins of the Baltic Sea, a mass balance-based grey box model was set up and the persistent (Björlenius *et al.,* 2018), widely used carbamazepine was the model substance. The model contains hydrological and meteorological characteristics of the sub-basin, removal data from smaller watersheds and wastewater treatment plants, and statistics relating to population, consumption and excretion rate of carbamazepine in humans. Based on an estimated half-life under average Baltic conditions of 3.5 years, the model predicted average environmental concentrations of carbamazepine in sub-basins that were virtually identical to the measured concentrations, amounting to 0.57-3.2 ng/L depending on sub-basin location. With the long turnover time of the Baltic Sea, this illustrates the importance of removing pharmaceuticals as far upstream as possible.

Our research assessed the point source of pollutants and identified possible technologies for a potential reduction in the inflow of nutrient and hazardous substances to the Baltic Sea. Analysis by DIB shows that conventional wastewater treatment plants (WWTPs) only partially remove most of the pharmaceuticals with efficacies ranging between 100% to -60% (deconjugated or released from non-solubilized state) with the variation depending on the compound and technology (Björlenius, 2018).

Sources to corroborate the impact: Supported by scale-down experiments in the lab, on-site pilot container-based pilot plants (Fig. 4) were used to assess the efficacy of different end-of-pipe treatment options, including ozonation, activated carbon, sand filters and/or nano filtration at the sites of the WWTPs of Knivsta, Henriksdal-Stockholm, Kungsängsverket-Uppsala, Kungsängverket-Västerås Nykvarn-Linköping and Käppala. Although nanofiltration achieved 90% removal, significant volumes of retentate would require further treatment. Ozonation with 5-7 g/m3 ozone resulted in 87-95% removal of APIs. The pilot plants with granular and powdered activated carbon (GAC) and (PAC) removed more than 95% of the APIs. Pre-screening of different types and suppliers of activated carbon products was essential in view of the observed broad variation in adsorption capacities. Recirculation of PAC or increased contact times improved the removal of APIs. Analysis of pharmaceutical residues together with analysis of biomarkers showed that both granular-activated-carbon treatment and ozonation could efficiently remove pharmaceutical residues in WWTPs and decrease the biomarker response. To broaden the reach of the obtained result a handbook was published for policymakers and other users, which is available online [Link].

Figure 4. *Mobile pilot plant for removal of pharmaceutical residues from effluent of waste-water treatment facilities.*

DIB Environmental Bio-technology is currently developing and investigating alternative technologies for removal of pharmaceutical residues from conventional wastewater or from more highly concentrated upstream sources such as hospitals or manufacturing sites, including: Melanin producing *E. coli* cells, degradation and removal by filamentous fungi, functionalized recyclable magnetic nanoparticles and development of treatment technologies suitable for sustainable operation in developing countries, such as Rwanda and Bolivia in collaboration with the Swedish development agency SIDA. Additionally, removal of hazardous material is also the focus of the recently started EU Interreg CleanStormWater project, which also focusses on minimizing the release of pollutants into the Baltic Sea.

Research publications (citations according to Web of Science):

- Björlenius, B. (2018). Pharmaceuticals – improved removal from municipal wastewater and their occurrence in the Baltic Sea. Doctoral dissertation, KTH Royal Institute of Technology, Sweden. [Link]
- Björlenius, B. *et al.* (2018). Pharmaceutical residues are widespread in Baltic Sea coastal and offshore waters - Screening for pharmaceuticals and modelling of environmental concentrations of carbamazepine. *Sci Total Environ*. [Link] 36 citations.

Impact case 12: Very high density continuous culture for biologics production

Department of Industrial Biotechnology

Summary of the impact

KTH published a world-record of highest CHO cell density in continuous culture, in 2013. This was the starter of several other projects, including the creation of a high density process of HEK239 cells. Increasing the cell density enables significant process intensification, which supports both optimal economy and better sustainability.

The landscape of the biopharmaceutical industry has evolved from production based on microorganisms towards mammalian cells for biologics, nowadays. The legacy is still fed-batch process (i.e. batch process fed with time). However, to meet new challenges and keep competitiveness, continuous high cell density perfusion culture (HCDP), where medium is steadily added and harvest continuously removed, has recently received high interest. An important enabler for this technology in industry was the achievement of very high cell density, for which our pioneer results of extreme cell densities were an important proof-of-concept. World-wide, HCDP production intensification is now considered as the ultimate goal for some companies *e.g.* Sanofi, Novartis, and applied by most large Pharma companies in various ways. The drivers behind this are: reduction in CAPEX by high productivity in small bioreactors, more robust technology, need of flexibility, compatibility with disposable equipment, potential improved product quality, and support from FDA and EMA.

Underpinning research (citations according to Web of Science)

Chotteau's lab was the first group to publish extremely HCDP of antibody producing CHO cells in:

- Clincke *et al.* 2013. Very High Density of CHO Cells in Perfusion by ATF or TFF in WAVE Bioreactor™ *Biotechnology Prog*. Part I. Effect of the Cell Density on the Process [Link] ; and Part II: Applications for Antibody Production and Cryopreservation [Link]. This work in collaboration with Cytiva (SE) resulted in patent US20140011270A1 [Link].

The article Clincke *et al.,* 2013 is the most cited of all papers published in Biotechnology Progress during 2011-2019 (105 and 63 citations). This work has positioned the group as world-leader in mammalian cell-based perfusion processes.

Since then, HCDP in various aspects has been reported with several collaborators:

- Zhang *et al.* 2015. Very high cell density perfusion of CHO cells anchored in a non-woven matrix-based bioreactor. *J. Biotechnology* [Link] – collaboration CerCell (DN) and Belach (SE). 17 citations.
- Zamani *et al.* 2018. High cell density perfusion culture has a maintained exoproteome and metabolome, *Biotechnology* [Link]– collaboration AstraZeneca (UK), Prof. J. Rockberg (KTH) and Prof. A. Mardinoglu (KTH). 11 citations.
- Gomis-Fons *et al.* 2020 Model-based design and control of a small-scale integrated continuous end-to-end mAb platform. *Biotechnology Prog*. [Link]. Collaboration Prof. B. Nilsson (Lund Univ. - SE), Cobra Biologics and Cytiva. 4 citations.
- Zhang *et al.*2020. Control of IgG glycosylation in CHO cell perfusion cultures by GReBA mathematical model supported by a novel targeted feed, TAFE. *Metabolic Engineering* [Link] - collaboration Prof. H. Hjalmarsson (KTH) and Cytiva. 1 citation (Google Scholar)

Furthermore, the work of HCDP using human HEK293 cells, *e.g:*

- Schwarz *et al.,* 2019. Small-scale bioreactor supports high density HEK293 cell perfusion culture for the production of recombinant Erythropoietin. *Journal of Biotechnology* 309:44-52. [Link] – collaboration AstraZeneca and Prof. J. Rockberg (KTH), is now cited in literature for viral vector production. 5 citations.

Sources to corroborate the impact

The group’s world-record results have been a significant contribution to the field to increase the targeted cell concentrations in industrial perfusion processes, with Chotteau's work cited as proof-of-

concept in opinion papers such as Croughan *et al.,* 2015: "The future of industrial bioprocessing: batch or continuous?" [Link]. Chotteau has been often invited to present the group's results at international conferences attended by the industry, *e.g.* Bioprocess International/KNect365, Bioprocess Summit. She will be one of the four scientists asked to present the first educational webinar of the Integrated Continuous Bioproduction Conference of the ECI Engineering Conference International about HCDP in June 2021 [Link].

Following this success several projects of HCDP were then carried out in collaboration with different industrial partners and groups as listed in Table below. In 2016, Prof. Matthias Uhlén, KTH, together with AstraZeneca launched the Wallenberg Centre for Protein Research, where Chotteau's group was responsible for HCDP using HEK293 cells. Thanks to her leading position in HCDP, Chotteau became Director of AdBIOPRO Competence Centre for Advanced Bioproduction by Continuous Process, funded by Vinnova and industrial partners. Enlarging the group's perspectives, this Centre allies HCDP with continuous purification process, tools of mathematical modelling and omics characterization, for biopharmaceutical, adeno-associated virus and cell therapy. Furthermore, Chotteau is leading or involved in other projects related to perfusion processes (see Table); Coordinator of EU-IMI project iConsensus and partner of EU-ITN project Staccato. Expertise in perfusion process is also applied within mathematical modelling, and for the production of ATMP's, *e.g.* AAV, or cell therapy.

Centres or Projects *Funding or Sponsors [Period]* / Collaborators	**Main applicant**
Competence Centre for Advanced BioProduction, AdBIOPRO [Link] *Vinnova, industrial partners and KTH [2017-2022 (potentially -2027)]* / KTH (6 departments); B. Nilsson (Chem. Eng., Lund Univ); P. Blomberg (Karolinska Centre for Cell Therapy KCC); Cytiva – SE; Sobi – SE; Cobra Biologics – UK; Valneva – SE; BioInvent – SE; MAGic Bioprocessing – SE, XNK Therapeutics – SE	**V. Chotteau**
Competence Centre: Wallenberg Centre for Protein Research [Link] *AstraZeneca & Wallenberg Foundation [2016-2018]*/ KTH (3 departments), AstraZeneca – UK	Prof. M. Uhlén (KTH)
iConsensus, Integrated control and sensing platform for biopharmaceutical cultivation process high-throughput development and production [Link] *EU-IMI and biopharma partners [2018-2022]* / A. Russom (KTH), Åsa Emmer (KTH), E. Jacobsen (KTH) A. Van de Wouver (Mons Univ, BE); B. Hitzmann (Hohenheim Univ, DE); - J. Büchs (Aachen Technical Univ, DE); Sanofi - BE, DE, USA; Bayer – DE; GSK - USA, BE; Pfizer – USA; Rentschler – DE; Byondis – NL; UCB – BE; Presens – DE; m2P lab – DE; Ipratech – BE; Kantisto – NL; Iprasense – FR; Micronit – NL; PaiaBio – DE; Ramcon - DK	**V. Chotteau**
Staccato, European Industrial Doctorate for enhancing upstream biopharmaceutical manufacturing process development through single cell analysis [Link] *EU-EID H2020 (Marie Sklodowska Curie) [2019-2022]* / C. Clark (Bioinformatics, NIBRT, Ireland); N. Barron (Omics, Univ College Dublin, Ireland); J. Bones (MS, NIBRT, Ireland); M. Hedhammar (Biomaterial, KTH)	Dr. C. Clark (NIBRT-Ireland)
SmartFD, Smart feed design [Link] *Vinnova - PiiA Process Industrial IT and Automation [2017-2019]* / F. Vilaplana (Glycoscience, KTH), Cytiva – SE, Cobra Biologics – UK&SE	**V. Chotteau**
DL2, Data-Limited Learning of Complex Dynamical Systems [Link] *KTH Digital Futures [2019-2024]*/ H. Hjalmarsson, S. Chatterjee, D. Broman (KTH - 3 departments)	Assoc. Prof. D. Broman (KTH)
AAVNova, AAV production for gene therapy *Vinnova and AstraZeneca [2019-2022]* / J. Rockberg; AstraZeneca – UK; Vironova – SE; AlfaLaval - SE	Prof. J. Rockberg (KTH)
Centre for Advanced Medical Products, CAMP [Link] *Vinnova [2018-2023]* / P. Blomberg (KCC - Karolinska Hospital); AstraZeneca – SE; MAGic Bioprocessing - SE	Dr. Jukka Lausmaa (RISE)