Research Assessment Exercise (RAE) 2020

Panel Report

Panel 2: Biotechnology

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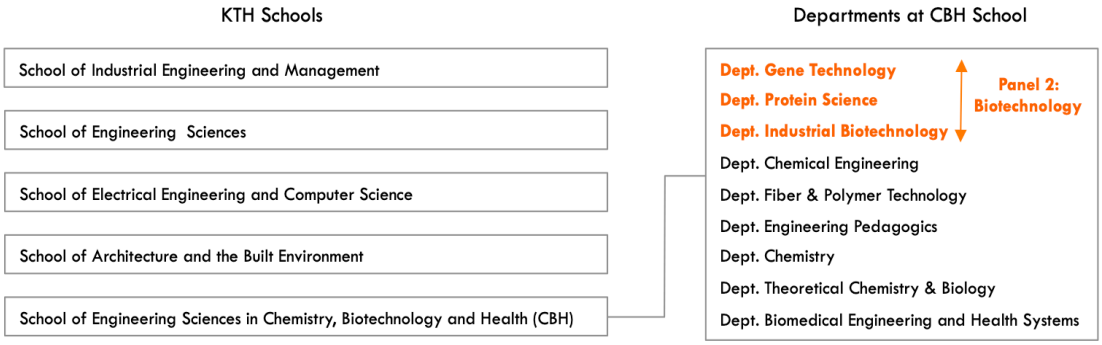
Contents

A) INTRODUCTION

0 a. Description of the research field of the departments included in the panel

The panel no. 2 "Biotechnology" includes the three departments **Gene Technology** (8 faculty), **Protein Science** (19 faculty) and **Industrial Biotechnology** (7 faculty) at the KTH School of Chemistry, Biotechnology and Health (CBH School), KTH.



The overall research field in common for all three departments 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, metabolites in healthy and disease living matter, development of biomolecular reagents and materials for research, diagnostic, environmental, industrial and medical applications, as well as various cell-based production of a wide range of products, incl. therapeutic proteins, chemicals, viruses and biofuels.

0 b. Description of the self-evaluation process for the research panel

The self-evaluation process started with meetings between the coordinator and the resepective 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 the different questions. Department heads have been requesting various contributions (e.g. impact case descriptions and descriptions of research activities) from division heads/group leaders and compiled the final department self-evaluations. The whole process has been iterative with responses and commenting on draft versions from the senior faculty, also including comments from the coordinator. The coordinator wrote a shorter introduction and compiled the three final self-evaluations into a single panel report.

0 c. 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/group, but many general biotechnological concepts are used by all departments resulting in a good understanding of each others work ("speaking the same language") which in turn leads to synergies in respect to e.g. valuable exhange of collegial advise, instrument sharing and collaborative projects across the department borders.

Common challenges

A high dependance on external funding (some of which tied to a soon retiring faculty), increasing costs (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.

RAE 2020 Self-evaluation

Panel 2: Biotechnology

Section 1

Department of Gene Technology

A) REPORT FOR GENE TECHNOLOGY

1. Overall analysis and conclusion; strengths and development areas

a. *Limited SWOT-analysis*

Research

Strengths

- 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.
- Strong competence in technology development propelling key advancements in biological and medical science, and thus sustainability.
- 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.
- 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.
- 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.

Weaknesses

- 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.
- Sustainability work, especially for environmental research, is present among several groups but can be broadened and intensified.
- Lack of cutting-edge machine learning expertise.

Organization

Strengths

- Access to world-class infrastructure, in particular high throughput sequencers and automation robots allowing for high throughput analysis on an internationally competitive scale.
- The nine 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.
- Strong external funding, including in international competition (e.g. EU-funding) and industrial funding.
- 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.
- Competent young faculty: all three new faculty recruited since last RAE obtained government starting grants.

Weaknesses

- 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.
- Faculty members are dependent on external funding, as the salaries are not fully covered by the university.
- Increasing administration, because of decreased administrative support after school merger.
- Rigid and lengthy administration in recruitment. Internationally uncompetitive recruitment procedure (for all types of personnel), in large due to a too lengthy process, based on rigid and slow administrative policies.
- Limited department activity in the form of formal sessions for scientific discussions.

Development areas considered 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.
- Rigid and lengthy administration in recruitment is a problem. International recruitment (for all types of personnel) is hampered due to a too lengthy process, based on rigid and slow administrative policies. This leads to decreased diversity and less influence from other excellent research environments.

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

Impact

The department of Gene Technology 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 has opened up new analysis methods in many scientific fields, and by hosting two national research facilities, most importantly National Genomics Infrastructure (NGI). 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 nine research groups, and also hosts two national infrastructure platforms at SciLifeLab; the National Genomics Infrastructure (NGI) and Clinical Genomics. Head of department (Prefekt) is Professor Peter Savolainen. Number of faculty is eight and total number of employed, Postdocs and PhDs is 48 (excluding NGI and Clinical Genomics). The nine 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 tor 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
Sahlén's lab 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.

Single Cell Technology, PI Assistant Professor Patrik Ståhl
The Single Cell Technology 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.

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. 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.

Clinical Genomics Facility, Head of facility Dr. Valtteri Wirta
The Clinical Genomics Stockholm facility 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 and composition of research team(s)*

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 nine 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 nine 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 collaborative projects between the research groups in the department

	Genom	Exp Genom	Evol Biol	Stat Biot	Env Genom	Expr Bioin	Reg Genom	Single cell	Spat biot	NGI	Clinical
Genom					**1**	**1**		**1**	**3**	**3**	**1**
Exp Genom			**3**		**1**		**1**			**1**	
Evol Biol		**3**			**1**		**1**			**1**	
Stat Biot											**1**
Env Genom	**1**	**1**	**1**						**1**	**1**	
Expr Bioin	**1**										
Reg Genom		**1**	**1**					**1**			
Single cell	**1**						**1**			**1**	
Spat biot	**3**				**1**	**1**					
NGI	**3**	**1**	**1**		**1**	**1**		**1**			
Clinical	**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 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.

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. They 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, ten of which in high impact (IF>9.5) journals, and totally 4,460 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, 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, 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. Our 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), Nucleic Acids Research (2017) and Nature Scientific Reports (2019).

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. According to the latest "Bibliometrics analytics for KTH", our department had the highest Cf (fractionalized field normalized citations) and JCf (fractionalized journal field normalized citations) among the departments of the CBH school. Specifically, the Cf for 2015-2017 was 2.16, implying that our scientific articles are cited more than twice as often as the average article in Web of Science (116% above field average) and the JCf for 2016-2018 was 2.04, implying that the articles were published in journals with twice as high impact than average (104% above field average). Similarly, 22.9% of the articles were among the 10 percent most cited in its field, and 58.5% were published in the 20 percent most cited journals in its field. The values have been high all through the period 2012-2018, with a clearly positive trend, Cf increasing from 1.69 (2012-2014) to 2.16 (2015-2017) and JCf increasing from 1.36 (2012-2014) to 2.04 (2016-2018).

The strong publication record of the department can be further illustrated by statistics from Google scholar, (https://scholar.google.se/citations?user=tM4A8TQAAAAJ), showing a combined yearly number of citations above 5,000 and a combined h-index of 100 for our nine group leaders. The individual h-index for the four professors are 72, 32, 28 and 27, and for the three associate professors 37, 23 and 12, respectively.

Year	Number of Publications	With Impact Factor>9.5
2019	32	8
2018	30	9
2017	32	5
2016	28	5
2015	25	7
2014	28	5
2013	32	2
2012	35	0
Total	**242**	**41**

With 210 articles published 2012-2018, 33 of which in high impact journals, this gives an average of 3.3 peer reviewed articles (0.54 of which in high impact journals) per group leader and year. There is also a notable steady rise in the number of articles in high-impact journals, to almost one article yearly per group leader the last two years. In conclusion, the bibliometrics indicate a high and steady productivity of excellent research at our department.

We want to highlight the following 10 articles, with contribution by all nine group

leaders, 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; 26 (1), 21-33
Number of citations: 157

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 May 8;3:17061. doi: 10.1038/nplants.2017.61.
Number of citations: 29

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 Dec 12;179(7):1647-1660.e19. doi: 10.1016/j.cell.2019.11.025.
Number of citations: 5

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 Jul 1;353(6294):78-82. doi: 10.1126/science.aaf2403.
Number of citations: 338

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 Oct;16(10):987-990. doi: 10.1038/s41592-019-0548-y.
Number of citations: 23

Alneberg J, Bjarnason BS, de Bruijn I, Schirmer M, Quick J, Ijaz UZ, Lahti L, Loman NJ, Andersson AF, Quince C (2014) Binning metagenomic contigs by coverage and composition. ***Nat Methods*** 2014 Nov;11(11):1144-6.
Number of citations: 522

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 Apr;63(4):559-66.
Number of citations: 597

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 Aug 3;16:156
Number of citations: 92

B Zhang, M Pirmoradian, R Zubarev, Käll L, Covariation of peptide abundances accurately

reflects protein concentration differences, ***Molecular & Cellular Proteomics*** 2017, 16 (5), 936-948
Number of citations: 23

Borgstrom, E., Redin, D., Lundin, S., Berglund, E., Andersson, A.F. and Ahmadian, A. (2015) Phasing of single DNA molecules by massively parallel barcoding. ***Nat Commun***, 6, 7173.
Number of citations: 24

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, and only a few of these can be mentioned here:

For all groups working with development and application of methods, collaborations are fundamental for meeting our requirements for highest level biological and medical competence and sampling in applied studies, to result in prominent papers such as Ståhl et al, *Science* 2016; Vickovic et al *Nature Methods* 2019; Berglund et al *Nature Comm* 2018; Asp et al *Cell* 2019 and Maniatis et al Science 2019. Similarly, for our studies about dog origins, evolution and dispersal, global collections of samples have been necessary for our research in the absolute research front, which has resulted in numerous high impact publications such as Wang et al 2016 *Cell Res*, Wang et al 2019 *Cell Res*, and Zhang et al 2020 *Nat Commun*. 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 optimised bioinformatics pipelines with excellent reporting. The department has not obtained a bioinformatics professorship, but has recruited many 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. This is reflected in a relatively low proportion of faculty funding (38%). The main funding of the department is from external sources, obtained in competition (60%). Five of our PI:s currently have governmental funding, from VR or Formas, including all 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 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 Corona epidemics has also implied negative market trends that will affect external funding negatively.

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" "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 for seminar activities, and activities among PhD students and Postdocs such as the "Thursday pub", at SciLifeLab.

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 central JML-work (gender equality, diversity and equal treatment) at the CBH School and keep focus on bullying and harassment, in line with several commentaries in prestigious scientific journals (https://www.sciencemag.org/features/2020/01/academic-bullying-desperate-data-and-

solutions; https://www.nature.com/articles/d41586-018-07532-5; https://www.nature.com/articles/d41586-018-06040-w).

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 eight faculty plus one non-faculty group leader. Furthermore, two more faculty are joining during 2020: one Assistant Professor (SciLifeLab fellow Anniina Vihervaara) who has just been recruited and will start in December, and one position as Professor (50% research and 50% Director for the NGI platform) which is advertised and planned to be filled during this year. With five Professors, three Associate professors and two Assistant professors, there is a good career stage balance. The age of the faculty is relatively low, 38-56 years, mean 50.1 years. With no faculty older than 56 years, there are no retirements planned the next 10 years. The six "original" faculty members were all males. The four new recruitments are three females and one male, giving a faculty of seven males and two females, plus one female 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, four out of the ten group leaders have a non-Swedish background.

Table 2. Overview of research groups

Group	PI	Prof.	Assoc. Prof.	Assist. Prof.	PhDs	Post Docs	PhD Students	Research Eng.
Genomics	Joakim Lundeberg	**1**			**2**	**3**	**10**	**2**
Experimental Genomics	Afshin Ahmadian	**1**				**2**	**1**	**2**
Evol. Biol. and Forensics	Peter Savolainen	**1**				**1**	**1**	
Statistical Biotechnology	Lukas Käll	**1**					**2**	
Environmental Genomics	Anders Andersson		**1**			**2**		
Expression Bioinformatics	Olof Emanulesson		**1**				**1**	
Regulatory Genomics	Pelin Sahlén		**1**			**2**	**2**	
Single Cell Technology	Patrik Ståhl			**1**			**3**	**1**
Spatial Biology	Stefania Giacomello				**1**	**1**	**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 was recruited. At recruitments, equal numbers of the sexes are engaged as experts, to safeguard equal opportunities.

e. *Infrastructure and facilities*

The department is host for the National Genomics Infrastructure (NGI; ngisweden.se) which is an internationally leading infrastructure in genomics and a unique and exceptionally important resource for the Swedish research community. 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. 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 Science for Life Laboratory (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, 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 funding as a national research infrastructure for 2021-2025; 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 optimised 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 spinned 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 from KTH.

NGI has on average invested about 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.

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, Qbit etc). New investments will be sought through grant proposals.

Genome sequencing and genotyping analyses represent a cornerstone in biology and medicine, with a very wide range of applications, and NGI and Clinical Genomics plays a central role in providing these cutting-edge technologies, enabling Swedish research groups and clinical partners to perform world-class research projects and providing world-leading healthcare.

4. Strategies and organization

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 research as set out in "A leading KTH - Development Plan 2018-23" and with the school(s) development plan(s) respectively.*

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. 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 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 imagining and machine learning (Digitilization) to battle society's growing challenges: human disease and global environmental changes.

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.

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.

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, https://www.medbioinfo.se/. 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 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 facts 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 performed at the department 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 Corona epidemics, several ongoing projects have been adjusted to focus on Corona 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 (ISBN 978-91-88727-13-8). 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 exampls 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, 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 Corona 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, the Department of Gene Technology has 60-100% 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.

d. *Impact cases*

We here choose to present five 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.

Spatial Transcriptomics

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 Swedish Research Council and the Knut and Alice Wallenberg Foundation. 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, Salmén et al. Science 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. Nat Commun 2018)*, melanoma *(Thrane et al. Cancer Research 2018)*, ALS *(Vickovic et al. Science 2019)* and developing human heart *(Asp, Giacomello et al. Cell 2019)*. But the focus on delivering a strong technology to the community has been equally important and the department has produced several further key technology developments for a standardized protocol *(Salmén, Ståhl et al. Nat Protocols 2018; Giacomello et al. Nat Plants 2018)* and higher

resolution *(Vickovic et al. Nat Methods 2019)*, and not least key bioinformatics tools, including an automated data processing pipeline *(Navarro et al. Bioinformatics 2017; Wong, Navarro et al. Bioinformatics 2018)* and data visualization software *(Navarro et al. Bioinformatics 2019),* to enable the application of the technology on a broad international scale.

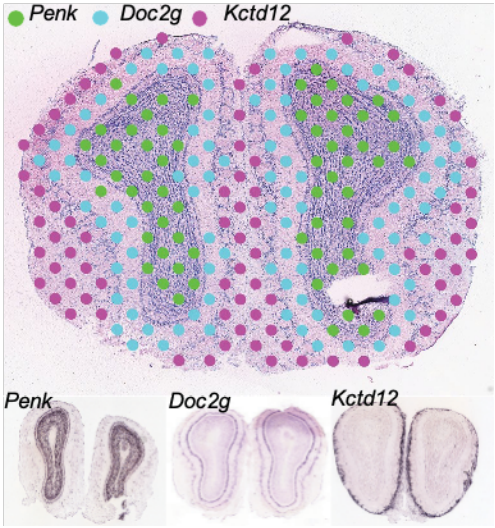


Figure 1: Adopted from Ståhl, Salmén et al. *Science* 2016. The expression pattern of three genes with distinct different spatial expression patterns in mouse brain olfactory tissue. (top) Spatial transcriptomics data is projected on top of a high resolution tissue image. (bottom) Corresponding in situ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

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 startup 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. At the same time, other actors in the field of spatially resolved nucleic acid analysis started emerging. In the past two years the field of spatial nucleic acid analysis, in particular spatial transcriptomics, has become well established and is a leading trend at many genomics conferences around the world. The startup company and the technology was 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.

In the 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, 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.

Droplet Barcode Sequencing

High-throughput sequencing platforms mainly produce 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 whole 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. We applied our bead-based droplet barcoding assay to bacterial 16S sequencing (Borgström et al, Nat Commun. 2015. doi: 10.1038/ncomms8173). 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 we further developed our method and presented Droplet Barcode Sequencing (DBS), 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, Nucleic Acids Res. 2017. doi: 10.1093/nar/gkx436). While phasing of HLA-A was a targeted approach, we sought to haplotype-resolve the entirety of genetic variation and thus utilizing genomics for medical purposes, in particular cancer. To achieve this goal, we adapted the DBS to a library preparation technique for high throughput barcoding of short reads where millions of random barcodes were used to reconstruct megabase-scale phase blocks (Redin et al, Sci Rep. 2019. doi: 10.1038/s41598-019-54446-x). Today we are applying the method to colon cancer and aim to detect structural variants. As DBS is free from complex libraries of barcoded beads, we have easily adapted it to target surface proteins on single cells and single exosomes in lung cancer and immune system, in projects run with researchers at Karolinska Institutet.

Targeted Chromosome Conformation Capture (Capture Hi-C)

Chromosome Conformation Capture (Hi-C) is a powerful approach to identify regions in the genome that are proximal to each other within the nucleus but distal in the genome. Hi-C is instrumental in mapping the overall structure of genomes during different cellular, developmental or pathological states, but lacks the resolution to inform on individual regulatory interactions such as those between promoters and enhancers. To address this gap, we developed the capture Hi-C technology which combines Hi-C with targeted sequencing to produce high-resolution (around 600bp) genome interaction networks. We

started method development around 2011 and established collaborations with industry (Roche Nimblegen Inc.) to obtain access to custom-made product sets required during method development. The method was described in 2015 in Genome Biology and is highly cited in the field. We also worked with KTH Innovation Holding and obtained patenting funds. The method is now patented in Europe and the US, and there is ample interest from industry for the patent use.

Complex diseases such as heart disease and type 2 diabetes have a strong genetic component but almost all variants are non-coding. This poses a problem since, if mutations are in regulating regions, e.g., enhancers, the affected gene can mostly not be directly assigned for causality or risk modulation. Capture Hi-C addresses this problem by connecting such regulatory variants to the genes they regulate, providing potential gene targets for further investigation. We successfully applied Capture Hi-C on atherosclerosis and inflammatory skin disease and found dozens of novel genes and functions for further functional work. In particular, we discovered a targetable gene that plays a major role in endothelial dysfunction in the cardiovascular disease. We also revealed several clinically relevant genes for atopic dermatitis and psoriasis, which could be used for disease management and are currently being assessed for their clinical use. Capture Hi-C is therefore a powerful method that will enable functional understanding of the non-coding parts of the genome and will spearhead the long-awaited use of genomic information in complex disease management, prevention and treatment.

A comprehensive catalogue of Baltic Sea bacterioplankton genomes

Bacterioplankton are key drivers of biogeochemical cycles and important components of marine and freshwater food webs. Since most of them are difficult to culture, learning about their physiology and ecosystem functions has been challenging. With metagenomics, microbial ecosystems can be analysed by shotgun sequencing, but a challenge has remained in sorting the resulting DNA sequences into genomes of origin. In 2014, the Environmental Genomics group developed a new bioinformatics method (the software CONCOCT) together with a group in the UK that clusters metagenomic genome fragments into draft genomes (Alneberg, 2015; https://pubmed.ncbi.nlm.nih.gov/25218180). The software has been used in >500 publications and we started using it to explore the microbial world of the Baltic Sea. In 2015 we applied it on time-series metagenomics data from the Baltic Sea and reconstructed 83 metagenome-assembled genomes (and coined the now widely used term: MAGs)(Hugerth, 2015: https://pubmed.ncbi.nlm.nih.gov/26667648). Comparing these genomes with metagenomic data from around the globe we could show that a global brackish microbiome exists with bacteria that are genetically distinct from their freshwater and marine relatives. More recently (Alneberg, 2020: pmidid coming soon) we expanded the analysis to 124 samples covering the various regions and depths of the Baltic and reconstructed nearly 2000 genomes corresponding to 350 species, most of which have not been found before. In this study we, for the first time, showed that an organism's ecological niche can be predicted directly from its encoded functional genes using machine-learning. The catalogue of genomes provides an important resource for deepening the understanding of aquatic microbiomes, and has already been used in follow-up studies, e.g. in a study published in PNAS (https://pubmed.ncbi.nlm.nih.gov/30322929) investigating how vitamin synthesis (an important ecosystem service provided by microbes) is distributed among microbial

species in the sea.

Semi-supervised machine learning for the analysis of mass spectrometry-based proteomics data

Protein tandem mass spectrometry provides a high-throughput method for detecting and quantifying thousands of proteins in any complex biological sample. The technology thus enables investigation of a huge range of biological questions, from fundamental molecular biology to characterization of disease states in humans and model organisms. Recent, rapid growth in the field of proteomics has been driven in large part by technological advances in tandem mass spectrometry.

Percolator is a machine learning post-processing algorithm that assists in the interpretation of this type of data, using a semi-supervised machine learning approach to significantly increase the proportion of observed spectra that can be mapped to their corresponding generating peptide sequences. The original Percolator paper, from 2007, has been cited 1200 times, including 1072 citations since 2012 (Google Scholar). Several additional papers from us as well as other groups describe the Percolator, this site lists the most prominent, https://scholar.google.se/citations?user=GxPtO0gAAAAJ .While the citation statistics are impressive, they under-count the use of the tool, because it is frequently used as part of commercial products and not cited separately. From the list of software that uses Percolator (https://bit.ly/2Vrpz0X), it is worth pointing out Mascot Server from Matrix Science Ltd, and Proteome Discoverer from Thermo Scientific. Percolator is used in many high-profile mass spectrometry projects, including in the analysis pipelines for both of the published draft maps of the human proteome. Our choice of a liberal, commercialization-friendly open-source software license, as well as our continued maintenance, has made our tools some of the most widely distributed in proteomics.

Percolator, separate correct from incorrect peptide-spectrum matches (PSMs), by combining multiple PSM features into a single, interpretable quality measure. Its primary innovation was to use semi-supervised learning to improve the ability of the learning system to generalize (Figure 1). Percolator avoids the need for manual curation by training a classifier to distinguish between real PSMs (called “target PSMs”) and decoy PSMs that involve shuffled or reversed peptide sequences. The task is semi-supervised because the decoy PSMs have labels (“incorrect”) but the target PSMs are an unlabelled mixture of correct and incorrect PSMs. Percolator uses an iterative semi-supervised machine learning algorithm in which the inner loop is an SVM classifier. The method is fast because Percolator makes use of an optimization algorithm specific to linear SVMs. A cross-validation scheme is employed to allow the target PSMs to also be used for statistical confidence estimation.

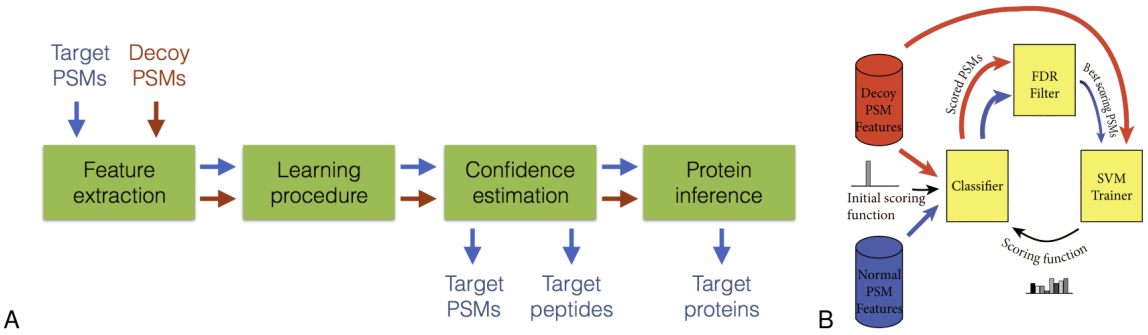


Figure 1: **Overview of the Percolator algorithm.** (A) Components of the software. Output PSMs, peptides and proteins are associated with q-values (a measure of false discovery rate) and posterior error probabilities. (B) The core learning algorithm. The input is a set of feature vectors, labelled as targets and decoys. Targets and decoys are ranked according by a selected feature, and target PSMs with 1% false discovery rate (FDR) are identified. An SVM is trained to discriminate between the selected targets and the full set of decoys. The SVM induces a new ranking, and the procedure iterates until the ranking stops changing.

Since the initial description of Percolator, several competing algorithms have been published. 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. 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." Percolator has been broadly adopted and adapted by the computational mass spectrometry community.

e. *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 five impact cases, 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 above. Similarly, the bioinformatics methods developed for analysing 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 Corona 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 Corona 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.

7. Other

a. *Specifics that the department wishes to mention and describe*

RAE 2020 Self-evaluation

Panel 2: Biotechnology

Section 2

Department of Protein Science

A) REPORT FOR PROTEIN SCIENCE

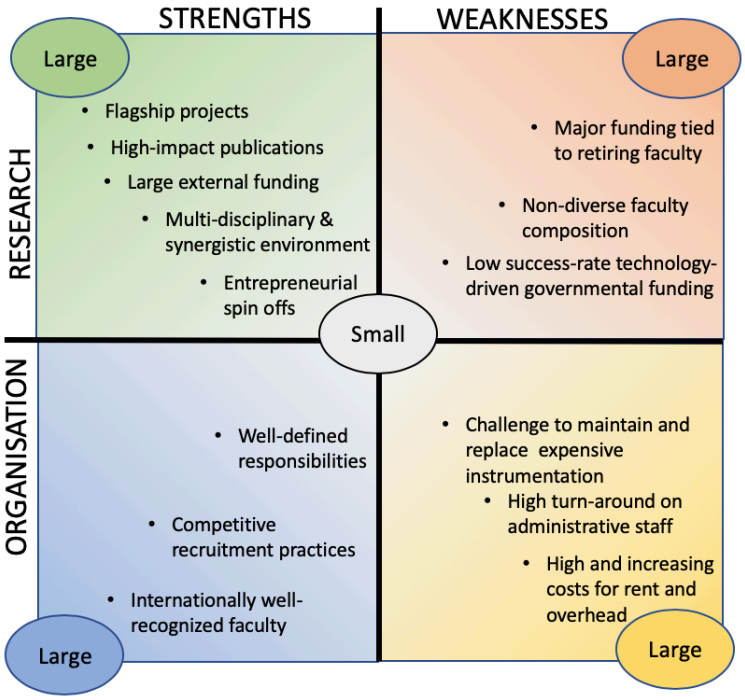
1. Overall analysis and conclusion; strengths and development areas

a. *Limited SWOT-analysis*

The Department of Protein Science (PRO) has assessed strengths and weaknesses, as related to Research and Organization, respectively. This limited SW(OT) analysis is summarized in the figure below, along with the estimated magnitude of each issue. The higher up and farther from the center of the graph an issue is placed, the stronger/weaker is it considered.

We consider our strong publication record (high number of publications, high-impact journals, high citation rates), our flagship projects, and large external funding to be clear **strengths** relating to our research. Our multidisciplinary, skilled, and synergistic competence (protein engineering, transcriptomics, proteomics, microbiomics, bioinformatics, system biology, cell biology, molecular biology, animal models, nanobiotechnology, medical research, clinical competence) is also a strong feature, which our analysis suggests could be further leveraged in terms of more synergy. The many companies associated with faculty at the department is a strength, both because it leverages impact and because it increases opportunities for collaborative networks with industry. On the organizational side, we include our internationally recognized faculty as strengths (evidenced by prestigious memberships such as the Royal Swedish Academy of Science (KVA) and US National Academy of Engineering (NAE), awards such as ERC, Wallenberg fellows, and attracting NIH and EU funding) and the practices that enables us to recruit very competitive SciLifeLab fellows. Our strong connection to the life science industry can also be considered an organizational strength, exemplified by two multi-partner competence centers in protein/cell technology managed by the department.

Our main **weaknesses** relate to dependence on a few highly successful faculty members who drive and fund large flagship projects. If these faculty retire or leave, our department will be strongly impacted, including financially. Some weaknesses are also identified in a



lower success rate in retaining national funding for technology-driven research than we think is achievable, and in a lack of diversity in our faculty composition. For example, we have no female faculty at the Assistant and Associate Professor level, and we also have few faculties from other backgrounds (other universities, ethnic backgrounds, and certain research areas). On the organizational side, we suffer from a high turn-around of administrative personnel, we lack a secure plan for maintaining and replacing expensive instrumentation, and we have unsustainable increases in rent and other indirect/administrative costs.

Among these identified strengths and weaknesses, we have selected five **development areas** as very important for the future of the department:

1. Seize emerging 'data-driven science' opportunities in order to expand our flagship projects
2. Fully leverage our multi-disciplinary competence, synergies, and networks to design new flagship(s) project and attract new sources of funding
3. Build on strong faculty recruitment to diversify faculty composition
4. Improve success rate in national technology-driven grant applications and work to diversify funding
5. Maintain and expand advanced instrumentation and infrastructure

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

Impact: The Department of Protein Science has contributed to significant impact through a large number of high-level publications, including several Science and Nature-family publications, and through our well-publicized flagship projects (see impact cases 1 and 5). Our internationally recognized faculty, research including environmentally friendly breakthroughs (see impact case 4), entrepreneurial efforts (see impact case 3 and 5), and outreach (see impact case 2), and recently our effort into Covid-19 tests, have all received significant international attention and impacted and moved forward the state-of-the art of the research fields, health care, or society significantly.

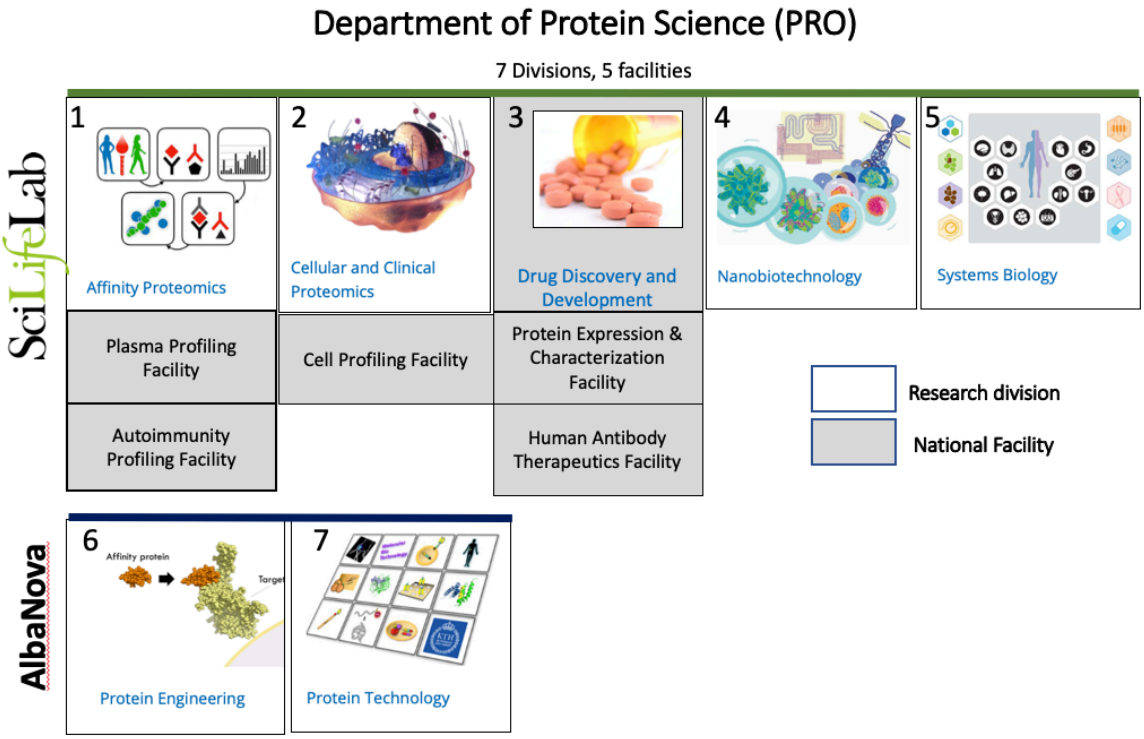
Infrastructure: Our Department has contributed with cutting-edge technologies and infrastructure through own methodological developments, creating the major Human Protein Atlas (proteinatlas.se) open access resource, managing academia-industry centers, and hosting five national facilities. Further, we 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.

Sustainable development: Nearly all research performed at the department is focused on improving health and healthcare, or by researching novel environmentally friendly fuels and materials.

2. Research profile

a. *General information of the department*

Dept. of Protein Science (PRO, Head: Cecilia Williams, ≈200 employees, 19 faculties)
PRO is one of nine Departments within the School of Engineering Sciences of Chemistry, Biotechnology and Health (CBH) at KTH. The department is divided into seven divisions, split between two geographical locations (five divisions are located at SciLifeLab and two at AlbaNova, as illustrated in the figure). The department hosts 5 National Facilities, all located at SciLifeLab. The department also manage a multi-partner competence centers in protein/cell technology (until 2024), and is part of several governmentally funded research environments (VR).



1. Division of Affinity Proteomics (Head: Peter Nilsson, 20 employees)
This division includes research groups led by two professors (Peter Nilsson and Jochen Schwenk), the Plasma Profiling Facility headed by researcher Claudia Fredolini and the Autoimmunity Profiling facility headed by researcher Roland Sjöberg. The research in the division is based the development and application of multiplexed immunoassays technologies for the detection and characterization of proteins and antibodies. 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 focussed on proteomic profiling in neurogenerative and inflammatory disease, while the Schwenk Lab researches in the context of cancer and metabolic disorders. The labs share their exquisite infrastructures with their respective facilities to make these accessible for the research community to primarily study proteins in the circulation or other body fluids. While mostly using reagents used in the research are derived from the Human Protein Atlas and associated initiatives, several additional commercial systems have been established.

2. Division of Cellular and Clinical Proteomics (Head: Emma Lundberg, 30 employees)
This division includes three faculty-led research groups (Emma Lundberg, Cecilia Williams, Jacob Odeberg), reasearcher-led groups (Burcu Ayoglu, Lynn Butler), and a National Cell Profiling facility (headed by researcher Charlotte Stadler). The research of this division uses large-scale technologies applied on patient material or model systems to understand basic molecular mechanisms underlying health and diseases. In the interface between bioimaging, proteomics and artificial intelligence the Lundbergs 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. The Lundberg group is also the creators of the Cell Atlas of the Human Protein Atlas. The Williams group investigates topics such as the roles of hormones and high-fat diet for colorectal cancer and the gut microbiome, using omics, mechanistic, and functional studies, including in vivo animal studies, and clinical samples. The Odeberg group works closely with the Butler group and investigates cardiovascular disease with a focus on biomarkers and the role of endotheliumusing clinical samples, omics and functional studies using in vitro models. The Ayoglu group has a systems immunology focus and applies high-throughput and spatially-resolved omics techniques for measurement and imaging of molecules such as RNA and proteins in single cells to resolve immune cell diversity, variation and developmental trajectories in health and disease. The Cell Profiling facility at SciLifeLab provides national access to advanced technology for spatial proteomics to the research community.

3. Division of Drug Discovery and Development (Head: Anders Olsson, 10 employees)
This division is purely made up of National Facilities, and consists of the Human antibody therapeutics (HAT) facility lead by researcher Helena Persson and Protein Expression and Characterisation (PEC) facility, led by researcher Anders Olsson. Both facilities are part of the Drug Discovery and Development (DDD) platform at SciLifelab. The mission is to deliver well-characterized protein targets and therapeutic antibody candidates to the drug development projects driven by the platform. This division provides academic access to its competence and instrumentation to serve the platforms national mission to develop academic discoveries into innovations. Research performed within the division is primarily focused on method and technology development to further improve its capabilities to fulfil its mission.

4. Division of Nanobiotechnology (Head: Aman Russom, 20 employees)
This division includes two faculty-led research groups (Aman Russom, Håkan Jönsson), and one adjunct industrial researcher. Building on state-of-the-art micro- and nanotechnology, the research of this division is focused on microfluidics for different biological applications. This includes development of various microfluidics tools for clinical applications, such as high-throughput droplet microfluidics, including sorting of cells, and production of 3D microtissue for screening and precision medicine applications. Further, different microfluidic based point-of-care devices for cellular and molecular diagnostics, with focus on sample preparation and integration with nucleic acid analysis, are developed.

5. Division of Systems Biology (Head: Mathias Uhlén, 40 employees)
This division includes four faculty-led groups (Mathias Uhlén, Paul Hudson, Adil Mardinglou, Saeed Shoaie) and three researcher-led groups (Fredrik Edfors, Cristina Al-Khalili Szigyarto and Linn Fagerberg) focusing on systems biology-related research spanning from photosynthetic bacteria, microbial research, human biology, systems medicine, metabolic and network modelling, protein science, antibody engineering and precision medicine. The topics range from basic research in 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. Most of their expertise is on photosynthetic cyanobacteria, though litho-autotrophic, H2-consuming bacteria is also studied. 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 complex diseases including obesity, non-alcoholic fatty liver disease (NAFLD), type 2 diabetes (T2D) and certain types of cancers. The Uhlén lab heads the Human Protein Atlas program and is responsible for data management and visualization of the data in the program. The group is also active in the field of data-driven life science. The Al-Khalili lab is involved in research on neuromuscular disorders and the database Antibodypedia. The Fagerberg lab is responsible for the integration of multi-omics data, related to the precision medicine efforts and the Human Protein Atlas program. A major focus of the effort is to pursue systems biology approaches related to large-scale data. The Edfors lab is focusing on targeted proteomics involving "in-house" developed methods for absolute quantification of proteins in blood and tissues. The group is involved in several collaborative programs both with industry and academia. Finally, the Shoaie lab is headed by a newly appointed SciLifeLab fellow who is setting up a new research group starting in the summer of 2020. The main focus of this group is microbiome research and the relationship between the microbiome profile and various clinical parameters.

6. Division of Protein Enginering (Head: Per-Åke Nygren, 25 employees)
This division includes five research groups (Per-Åke Nygren, Amelie Karlsström Eriksson, Stefan Ståhl, Torbjörn Gräslund, John Löfblom). The research field of protein engineering encompasses a large number of activities. A focus is on using rational and directed evolution principles for development of small and robust non-immunoglobulin affinity proteins as well as antibodies (incl. bi-specifics). Using various techniques, incl. gene fusion, chemical conjugation, sortase-mediated coupling and photochemistry, developed affinity proteins are in many instances tailor-made for specific applications. Several projects are addressing cancer, incl. different 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-lifes and in vivo mode-of-actions are also addressed. Large efforts are also made in the field of neurodegenerative diseases, incl. Alzheimer´s disease and Frontotemperal dementia (FTD), based on therapeutic use of affinity proteins. Method development is continuosly addressed, incl. new or refined concepts for selection of desired clones from large libraries. Also, biotechnological uses of protein engineering are addressed, incl. novel concepts for mild affinity chromatography using innovative ligands developed by protein engineering. Recently, efforts have been initiated to integrate the affinity protein technology in multi-domain scaffolds to facilitate structural determinations of different proteins via cryo-EM.

7. Division of Protein Technology (Head: Sophia Hober, 35 employees)
This division includes three research groups (Sophia Hober, My Hedhammar, Johan Rockberg), as well as an Albanova-located section of the Protein Atlas Project (section led by researcher Hanna Tegel). The department pursues research in different areas related to protein production and purification. Furthermore, there is a large focus on development of novel proteins and protein-based materials for biomedical applications. A large pipeline for protein production and purification is set up, both for production in mammalian cells and bacteria. After purification, the proteins are used for different purposes including phenotypic assays and development of binders. To improve the production efficiency, cell line engineering after OMICS analyses is applied. Systems for improved secretion of AAV

virus to allow for a higher productivity aiming for gene therapy are developed. Also in focus is the development of protein-based binders for biotechnology and medical applications. Small bispecific binders with capable of binding to both serum albumin (for half-life extension) and and a therapeutic target are developed. Furthermore, a calcium dependent scaffold is developed which can be tailor-made for use in affinity chromatography purifications to different proteins of interest and enable mild elution conditions for e.g. protein-based therapeutics. Furthermore, engineering of bi-specific antibodies is performed. Within the department, recombinant spider silk proteins are functionalized at either genetic or protein level, to achieve bioactive materials. The main focus here is to utilise the bioactive silk formats to mimic the *in vivo* environment and promote cells to form functional tissue for both clinical applications (e.g. transplantations) and models systems (e.g. drug testing).

b. *Central research questions and themes, knowledge gaps addressed, main research activities and composition of research team(s)*

The Department of Protein Science performs research and education primarily in the area of technology-driven health-related research. The research includes development and use of novel technology platforms, proteomics, transcriptomics, microbiomics, nanobiotechnology, protein engineering, imaging, bioinformatics, and system biology. Such technologies are applied to medically relevant research topics such as cancer, Alzheimer's, cardiovascular diseases, and Covid-19. Other sustainable themes, such as creating bacteria producing fuels, are also pursued. The research has generated several so-called flagship projects, of which the Human Protein Atlas (impact case 1) and combinatorial affinity protein engineering (affibodies, ADAPTS, impact case 5) are two examples, along with numerous fruitful international collaborations, centre formations with academia and industry, and spin-off entrepreneurial companies. The below table summarizes central research themes of the department, and highlights existing or potential synergies between the divisions, and related activities of each division is described in further detail below.

Central Research Themes: *Division:*	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Molecular Method Developments (display technologies, directed evolution, mammalian cell production systems, affinity-based assays, antibody validations, image analysis, microfluidics)	x	x	x	x	x	x	x
Human Health (Basic biology, Cell biology, Health, Diagnostics, Therapy, Cancer, Neurodegenerative disorders, Cardiovascular diseases)	x	x		x	x	x	x
Biomedical Applications (protein engineering, spider silk, nanodevices, medical imaging tracers, biomarker discovery/assays)	x	x		x	x	x	x
Environment (fuel production, waste water)		x			x		x

Activities with the division of Affinity Proteomics (1) is devoted to **affinity-based biomarker discovery** and validations. This is explored within **neuroproteomics** and various **autoimmune disorders** and enabled through the development and utilization of different types of protein array formats using protein fragments and antibodies from the Human Protein Atlas. Several interdisciplinary research projects are also centered around **multiplexed immunoassays** for exploring the **plasma proteome**, and to map the differences and variations of proteins between individuals, health and disease states. Affinity assays are developed and applied across different disease areas such as **cancer**

(e.g. prostate, breast, pancreas), **thrombosis**, **diabetes**, and **wellness**.

Within the division of Cellular and Clinical Proteomics (2) several **basic cell biology** research questions are pursued, such as proteome-wide **subcellular localization** (impact case 2), the **cell cycle** proteome, the role of **non-coding RNAs**, and **molecular mechanisms** underlying **hormone signalling** and cells functions. The research is in large part focused on human health, including **endothelial** cells in **cardiovascular** disease, **biomarkers,** understanding how hormone signalling and **diet** impacts the **gut microbiome** and **colorectal cancer**, **sex differences** in the **immune system**, and **exosome** signalling in the **microenvironment** of cancer.

At the division of Drug Discovery and Development (3), the main objective is to deliver to projects in the Drug Discovery and Development platform. Research is therefore focused on method and technology development to further this capability, such as establishing a **high-throughput pipeline** for expression, purification and **characterisation of antibody candidates** at small and medium scale; developing a panel of **analytical methods** to assess developability of antibody candidates based on their biophysical properties including non-specific interactions and aggregation propensity; using DOE approaches and **microscale bioreactors** to optimize **transient expression** protocols in mammalian cells; systematic evaluation of signal sequences to **optimize expression** in **mammalian** cells; generation of novel **scFv** and **Fab libraries**; designing new approaches for **affinity maturation** of antibodies; establishing and applying methods for **hit finding** using DNA-encoded libraries; and expanding the expression/purification/characterisation tool box to address **difficult-to-express proteins** and to tailor proteins for **drug development purposes**.

The research at division of Nanobiotechnology (4) focuses on **microfluidic tools** for manipulating cells and molecules at the micro- and nano-scale, and developing **point-of-care devices** for cellular and molecular diagnostics. Examples include microfluidics-based methods that uses blood as “liquid biopsy” for clinical **cancer diagnostics**; isolation of microorganisms from whole blood for **sepsis diagnostics**, and development of **3D microtissues** as model for **organ-on-chip** applications.

The division of Systems Biology (5) is behind the international effort of **Human Protein Atlas** (impact case 1). This was started in 2003 with the aim to **map all human proteins** in cells, tissues and organs using integration of various omics technologies, including antibody-based imaging, mass spectrometry-based proteomics, transcriptomics and systems biology. The database has now more than 300,000 visitors per month and it is one of the most visited biological databases in the world. The program was recently selected by the EU infrastructure ELIXIR as a core database resource due to its fundamental importance for a wider life science research community. **Production of biofuels from sunlight** (impact case 4) involves one of the most important tasks for a future sustainable society to be able to provide renewable energy without the use of fossil fuel. The goal is a production system where bacteria fix CO_2 and convert it directly to fuel. Here, photosynthetic cyanobacteria are used that can produce energy directly from sunlight, and litho-autotrophic bacteria that use energy derived from hydrogen. **Precision medicine** (impact case 6) is another important field of activities for the division. The dawn of many new omics tools for analysing clinical samples such as genomics, proteomics, transcriptomics, and metabolomics has opened up new possibilities to study both health and disease with high throughput along with high analytical precision and clinical accuracy. We have used the resources created within the **Human Secretome** Project and the Human Protein Atlas program combined with the infrastructure built up in the Science for Life Laboratory to initiate one of the world’s most comprehensive “personal omics profile” program. **Drug development** (impact case 5) is also an important part of the activities in

the division to take the basic research into applications of societal importance. The research has to a large extent been medically oriented, but recently also research aimed for environmental applications have been initiated. 16 start-up companies have their origin at the division and at present (April 2020), seven human clinical trials are being pursued by these start-up companies.

The research field of **protein engineering** is addressed both at the division of Protein Engineering (6) and Division of Protein Technology (7). A focus is on using rational and directed evolution principles for development of small and robust **non-immunoglobulin affinity proteins** as well as **antibodies** (incl. bi-specific antibodies). Using various techniques (incl. gene fusion, chemical conjugation, sortase-mediated coupling and photochemistry) affinity proteins are developed and tailor-made for specific applications. Several projects are addressing **cancer**, and explores different 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 made on therapeutic use of affinity proteins for **neurodegenerative diseases**, such as Alzheimer´s disease and Frontotemporal dementia (FTD). Method development is continuously addressed, including on new or refined concepts for selection of desired clones from large libraries. Improvements for biotechnological uses of protein engineering are also addressed, such as developing novel concepts for mild affinity chromatography using innovative ligands developed by protein engineering. Recently, efforts have been initiated to integrate the affinity protein technology in multi-domain scaffolds to facilitate structural determinations of different proteins via cryo-EM. Impact of this area is further described in impact case 5.

Engineering of recombinant spider silk is used to generate **novel materials for medical applications**. Via genetic functionalization of soluble monomeric silk fiber constituents and/or site-selective conjugations to already formed fibers, bio-compatible materials suitable for promoting cell growth and differentiation are produced. The main focus here is to utilise the bioactive silk formats to mimic the *in vivo* environment to promote the formation of functional tissues for both clinical applications (e.g. transplantations) and for use as models systems in e.g. drug testing (Impact case 3).

Within the field of **cell engineering for production of biologicals** efforts are performed to identify limiting factors and bottlenecks in mammalian cells affecting the yield and quality of produced proteins and when possible use genetic engineering to either improve the host cells or modify the expression strategy, or both, to improve the production.

Composition of research team: The research profile of the department is dependent on cross-disciplinary expertise, including protein engineering, genomics/transcriptomics, proteomics, microbiomics, cell biology, molecular biology, mechanistic molecular biology research, imaging analysis, bioinformatics, system biology, medical research, animal models, nanobiotechnology, and clinical competence. Further, to have diverse backgrounds represented, including gender among faculty and team level, is important both from the perspective of assuring that the most merited persons are recruited from as large base as possible (men and women are e.g. equally educated within biotechnology, with women having slightly higher grades/merits) and to have as many perspectives as possible present when formulating research topics and interpreting the data. We have several academic sub-disciplines present and benefit from multi-disciplinarily to a large extent. However, our analysis has concluded that additional competence in e.g. immunology would strengthen the current research profile, and contribute significantly to research related to several important research themes, including cancer, Alzheimer's, and

the Protein Atlas project.

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

Contributions to the advancement of the state of the art within the research fields of the divisions of the department	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Method development: In house-developed method for increased circulation times of protein drugs, based on engineered albumin-binding domain; Development of HTP pipeline for production and purification of proteins in mammalian cells; Development of systems-biology toolbox allowing for construction of tuned mammalian host cells for specific proteins that are difficult to express; Establishment of methods for generation of high-quality combinatorial protein libraries for selection purposes; Development of pipeline for antibody validation; Ligands for downstream processing in mAb production; Large-scale confocal immunofluorescence, Imaging analysis, Machine learning, G coupled protein receptors molecular interactions.	x	x	x	x	x	x	x
Biomedical applications: In house-developed small scaffold proteins Affibodies and ADAPTs, incl. pre-clinical proof-of-principle for Alzheimer´s disease; Generation of medical imaging concepts in late-stage clinical development for HER2-based breast cancer diagnostics; Profiling biomarkers of neurodegenerative disorders; Platform for production of adeno-associated virus (AAV) for gene therapy; Development of methods for production of functionalized spider silk materials for biomedical applications; Sars-CoV-2 tests	x	x		x	x	x	x
Human health: Human Protein Atlas production of open access resource and datasets, Pathology Atlas; Cell Atlas; Improved chemotherapy agent, Characterization of sex differences and hormonal impact in colorectal cancer and microbiomes; Cardiovascular diseases biomarkers, clinical trials.	x	x	x	x	x	x	x
Environment: fuel production, Sars-CoV-2 waste water		x			x		x

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

The bibliometrics for our department, as provided by the KTH-internal system DIVA 2012-2018, shows that we have published 535 peer-reviewed articles, and 86 other articles, and that more than 95% are included in the World of Science (WoS). Over half of the publications are co-published with international collaborators. We have also produced 18 book chapters, 36 peer-reviewed conference papers, 5 licentiate and 46 doctoral theses. This approximates to an average production of 4 peer-reviewed scientific articles per faculty and year (535 articles, 19 faculties, 7 years). We consider this all as evidence of a high productivity of research.

Further, our departments work is highly visible. The scientific articles are cited 62% above the field's average, and published in journals with 38% higher impact than average (fractionalized citations average Cf =1.62 and average journal Cf = 1.38). Moreover, this is on a steady upward trajectory. Scrutinizing the data per year, we note that visibility and impact has increased each year (citations nearly doubled from 2012 (Cf 1.12) to 2018 (Cf 2.06); journal impact increased from 1.28 to 1.47). Nearly all published articles have received citations (uncited share is only 0.1) and 12.4% are among the top-10% most highly cited articles in the field. Further, the h-index (Google

Scholar) range between 18-119 (median 32) for our Professors and between 16-34 (median 21) for our Associate Professors. It is noteworthy that one of the members of the department (Uhlén) is one of the most cited scientists in Sweden in all research fields (h=119) and has the highest h-index ever for a researcher at KTH (according to eleventh edition of Google Scholar Science, September 2019).

Publications that the department wants to highlight:

Human Health:

- M Uhlén *et al*. Tissue-based map of the human proteome. **Science** 347: 1260419 (2015) *Presents the tissue localization of >90% of all human proteins. >4100 citations* JIF>40
- PJ Thul *et al.* A subcellular map of the human proteome. **Science** 356 (6340), eaal3321 (2017 *>600 citations* JIF>40
- W Ouyang *et al.* Analysis of the Human Protein Atlas Image Classification competition. **Nature methods** 16 (12), 1254-1261 (2019) JIF>28
- M Uhlén *et al.* (2017) "A pathology atlas of the human cancer transcriptome" *Science* 357(6352) (2017) *>700 citations,* JIF>40
- M Uhlén *et al.* "A genome-wide transcriptomic analysis of protein-coding genes in human blood cells" **Science** 366 (6472): eann9198 (2019), JIF>40
- Ibrahim A, *et al*. Colitis-induced colorectal cancer and intestinal epithelial estrogen receptor beta impact gut microbiota diversity. **Int J Cancer**. (2019) 144(12):3086-3098. PMID: 30515752
- E. Sjöstedt et al "An atlas of the human, pig and mouse brain" **Science** 367 eaay5947 (2020), JIF>40

Biomedical applications:

- Seijsing *et al.* An engineered affibody molecule with pH-dependent binding to FcRn mediates extended circulatory half-life of a fusion protein. **Proc. Natl. Acad. Sci. USA**, 111: 17110-5 (2014). *The presented results have led to ongoing phase I clinical trials for autoimmune disease.* JIF>9
- M Bruzelius *et al.* PDGFB, a new candidate plasma biomarker for venous thromboembolism: results from the VEREMA affinity proteomics study. **Blood**. 128(23):e59-e66 PMID: 27742707 (2016) JIF>16
- Ståhl et al., "Affibody molecules in biotechnological and medical application" **Trends Biotechnol**. 35: 691-712 (2017). *Highly cited (>100 citations) review summarizing > 500 affibody-related publications.*
- Boutajangout et al., "Affibody-mediated sequestration of amyloid ß demonstrates preventive efficacy in a transgenic Alzheimer's disease mouse model" **Frontiers**. **Ag. Neuroscience** 11, doi: 10.3389 (2019). "*Describes how an affibody targeting monomeric Aß prevents the development of AD symptoms in mice*.

Method development:

- Lundqvist M *et al*. Solid-phase cloning for high-throughput assembly of single and multiple DNA parts. **Nucleic Acids Res**. (2015) 43(7):e49. PMID: 25618848
- Honarvar H, *et al*. Feasibility of Affibody Molecule-Based PNA-Mediated Radionuclide Pretargeting of Malignant Tumors. **Theranostics**. 2016 6(1):93-103. PMID: 26722376
- S Andersson *et al*. Insufficient antibody validation challenges oestrogen

receptor beta research. 8:15840 **Nature Comm**. (2017) *Key antibody validation study (among top 5% cited publications, >100 citations*) JIF >11

- Sullivan D *et al*. Deep learning is combined with massive-scale citizen science to improve large-scale image classification **Nat Biotech** (2018) 36(9):820-828. JIF>35
- Kanje S *et al*. Protein Engineering Allows for Mild Affinity-based Elution of Therapeutic Antibodies. **J Mol Biol**. (2018) 430(18 Pt B):3427-3438. PMID: 29886013
- Johansson U *et al*. Assembly of functionalized silk together with cells to obtain proliferative 3D cultures integrated in a network of ECM-like microfibers. **Sci Rep.** (2019) 9(1):6291. PMID: 31000733

Environment:

- Yao L, Shabestary K, Björk SM, Asplund-Samuelsson J, Joensson HN, Jahn M, Hudson EP. Pooled CRISPRi screening of the cyanobacterium Synechocystis sp PCC 6803 for enhanced industrial phenotypes. **Nat Commun**. 2020 11(1):1666. PMID: 32245970

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

National academic collaborations	**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).	KAW/Erling Persson project w. Amelie Eriksson Karlström. 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, [https://frontallobsdemens.se] 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 run at Div. of Protein engineering.
Assoc. Prof. and MD Johan Hartman (KI and pathologist at Södersjukhuset)	Region Stockholm HMT project. Provides pathological and clinical expertise within breast and colorectal cancer, clinical samples.(joint publications)
Lars Engstrand (KI), Nele Brusselaers (KI)	Provides expertise in microbiome research.
International academic collaborations	**Value & outcome**
Prof. Thomas Wisniewski, New York University	Provides expertise in Alzheimer's disease, performs studies in transgenic mice (2 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
Tromsö Hospital, Norway	Clinical samples, grants

f. *Follow up from previous evaluations*

The key recommendation from RAE 2012 was to recruit a professor in bioinformatics. We have since recruited an Associated Professor (Adil Mardinoglu, 2015) and an Assistant

Professor (Saeed Shoaie, April 2020) in bioinformatics.
In regards to the on-going monitoring (2019) evaluations, we are also working in-line with the CBH school developmental plans towards increasing female faculty/teachers, supporting individuals developments, to fairly distribute workloads (committee work et.c.), developing teaching merits, leadership and equal opportunities, research ethics, publications strategies, and increasing synergies between groups and divisions.

3. Viability

a. *Funding; internal and external*

Our department is research-intensive. The university provides about a third (32%, 2018-2019) as faculty funding, and this funding appears to be decreasing (was slightly less in 2019 compared to 2018; and less in 2017 compared to 2016 in the equivalent previous divisions). Our main funding is external (58%, 2018-2019) and obtained in competition. Private foundations represent a vast majority with KAW (Knut and Alice Wallenberg foundation), Stefan Persson (H&M), Schörling, the Cancer foundation, the Brain foundation and other. Governmental funding (Swedish research council VR, FORMAS, VINNOVA) is also substantial, but not as large as could be expected. Additional smaller contributions (3%) are generated through external assignments "uppdrag", often private companies or hospitals, or 'other' funding (5%).

A critical factor we observe is our Department's dependence on external funding. Considering that a disproportional large part of this funding is tied to a few individuals (which may retire or depart) and that we have many permanently employed researchers and engineers, we are at a significant financial risk.

Further, we note that grant applications for governmental funding within the field of *Biotechnology* (VR-NT), an area that we are strong in, has an unpredictable low success rate. Our department's research appears to not fit in VRs funding priorities, or the applications are not written in a form that its panel values. Our department should aim to diversify its external funding, strengthening individual faculties with less funding, and overall aim to increase success rate in VR-funding.

b. *Academic culture*

The Department organizes regular off-site retreats, together with the neighbouring 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 synergy effects. We consider this a key factor for our academic culture. Further, a creative and positive atmosphere exists within the divisions, which we consider a factor that stimulates collaborations. Several seminar series further function as meeting points between researchers at all levels, and the divisions located at SciLifeLab takes part in this community, with topic-oriented 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. A noted limitation is a lack of time, especially at the faculty side, to attend relevant meetings.

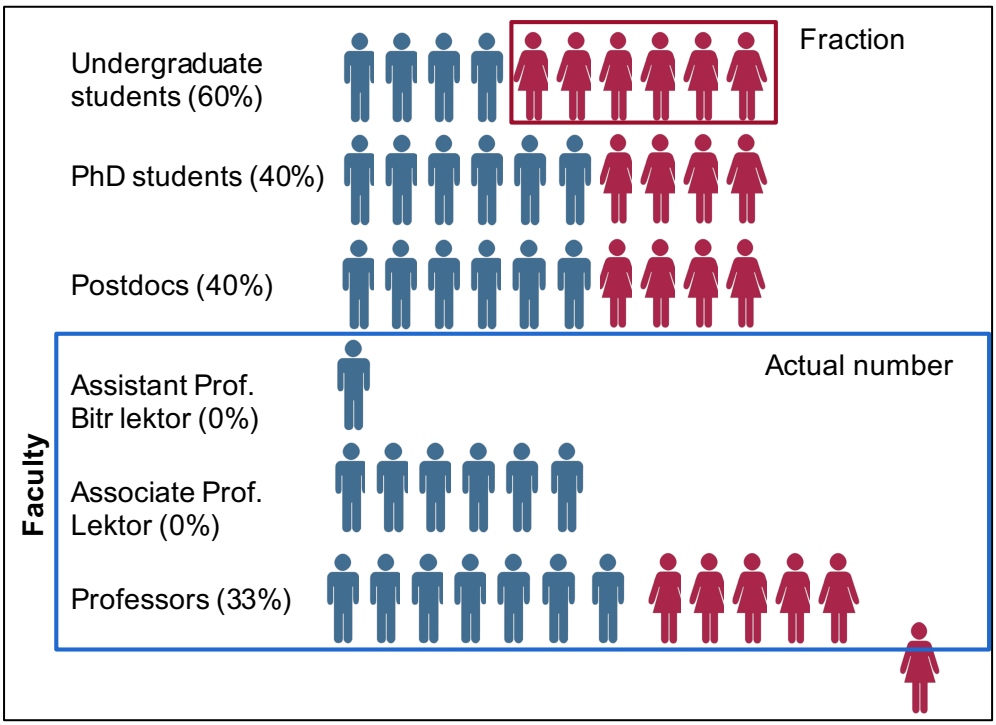
c. *Current faculty situation*

The faculty (19 positions, but one is on permanent leave of absence) makes up less than 10% of the Department's employees. We consider this a low proportion. According to 2019 figures, a third (30%) of the staff are PhD students and 7% are postdocs. The majority of our employees are research engineers/engineers/technicians (38%) or researchers (>25%). This pattern (large fraction of researchers/engineers and low fraction of faculty) is primarily explained by the Human Protein Atlas project and that several of SciLifeLab's national facilities (Plasma Profiling, Autoimmunity Profiling, Cell Profiling, and parts of Drug Discovery and Development) are part of the department. These projects and facilities include a larger fraction of routine positions that require a lower educational level than research-intensive projects.

Age: Three professors will be 60 or older this year, and an additional three will be so within five years, and would be expected to retire in the foreseeable future (guidelines are 67 years, but retirement can be taken from 55 years of age).

Gender: The department at a whole is balanced with respect to gender (women 52%, men 48%) but most women are hired at non-career-oriented positions (researchers/research engineers/technicians: 59-65% women). Although a slight majority of master students in our field are women (approx. 60%), our PhD-students and postdocs are more often men (63% and 60%, respectively). We have five female professors (38%) but all our Associate (5) and Assistant (1) Professors are men. We thus miss female representation among our younger faculty (Figure).

Career stage balance: We have 13 professors (one is on permanent leave) and six Associate Professors, but only one Assistant Professor (newly recruited in 2020). We consider this quite unbalanced (Figure). In order to achieve a sound balance, and replacing upcoming retirements, we will need to prepare for recruitment of at least three assistant or associate professors within the next five years. Faculty funding and relevant teaching assignments will be critical for such renewals. Further, we will take steps to ascertain that female candidates apply and are considered.



d. *Recruitment strategies*

Faculty recruitment is part of the CBH school recruitment cycle. The department nominates topics where we need competences and/or find a strategic need. Two thirds of our Department are located at SciLifeLab and recruits tenure-track faculties through the SciLifeLab Fellows program. This is a career program aiming at strengthening Swedish research in Molecular Biosciences and the research environment at the Science for Life Laboratory. The four host universities have used parts of their SFO budgets to recruit a number of young research leaders to SciLifeLab. At KTH, the topics for these new faculty positions are selected in a process where the KTH schools are invited to suggest suitable subjects, which are prioritized by the KTH SciLifeLab committee based on the strategic relevance of the subjects for the development of SciLifeLab, and coordination with the other host universities by discussions in the SciLifeLab Strategic Council, involving both SciLifeLab management and representatives of the host universities. By providing the new recruitments with a substantial financial start package, it has been possible to attract excellent internationally competitive candidates for the positions. Three of the KTH SciLifeLab Fellows recruited so far, in the subjects of Microbial Bioenergy Production, Systems Biology, and Bioinformatics, are placed at the Department of Protein Science.
Postdocs, researchers, and PhD candidates are recruited by the respective research groups, and approved by the Head of department and/or Head of School.

e. *Infrastructure and facilities*

It is challenging to find funding for infrastructure and expensive instrumentation since the research councils do not have dedicated calls for infrastructure. Further, the aggregated service costs for maintaining the instrument park in good functional shape are demanding (e.g. for biosensors, flow sorter, HPLC).

4. Strategies and organization

a. *Goals for development 5–10 years ahead*

In 5-10 years, in addition to faculty renewal as stated above, a major aim will be to maintain the very strong position we have in the international forefront within several research areas. A challenge will be to replace the activity of top level highly successful professors that will reach retirement. Strategic new hires can assist in maintaining a rapid and current development. We are especially well suited for an upcoming focus on big data.

b. *Congruence with university-level goals for research as set out in "A leading KTH - Development Plan 2018-23" and with the school(s) development plan(s) respectively.*

The general goals for KTH and CBH very well fits with the goals of our department (PRO)

- the education should reflect the top-level research conducted
- the applied research should emanate from fundamental research and multidisciplinary collaborations
- digitalization, sustainable development, internationalization and equality should be obvious keywords
- top-level infrastructures and professional leadership are key
- the research and education should have an impact on society

c. *Leadership structure and collegial structure*

The research is typically led by Principle Investigators (PIs), which are our faculty members and, in some cases, researchers. These are sorted into divisions, led by a division head. It is to a large extent the PIs that forms the research milieus. The two divisions 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 for practical reasons defined by the localization (house and floor) of each group or division. At both sites a collaborative atmosphere is dominating, with significant sharing of instrumentation and resources, and with a possibility for everyone to take part in the research discussions. In this manner the competences of everyone is used as a strategy to maximize creativity.

d. *Strategies for high quality*

Since the majority of the funding is from external sources and obtained in competition, there is a continuous strive to publish the results in high-impact journals, which in itself increases the chances to maintain the level of external funding. At the same time, we will aim to recruit top talents from international groups in the forefront of research in the order to get influx of valuable competence.

5. Interaction between research and teaching

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

The research areas are closely connected to the research education (PhD-level) and thus defined by areas where external funding can be obtained. The medical/environmental focus is likely to be maintained, as the continuous development of technology platforms needed for our research. The education and thus also the courses should gradually be updated to reflect the performed research (including new methods, new technology). We strive to maintain the principle that every teacher should be a researcher.

The department (main PI: J. Rockberg) has been hosting (supplying advice, reagents and lab space) several local iGEM teams during the evaluation period. iGEM stands for *International Genetically Engineered Machine (igem.org)* and is the premier international competition in synthetic biology which has been around since 2003 when it was started as a course at the world-renowned University of Massachusetts Institute of Technology, MIT, in Boston. The Stockholm teams, consisting of undergraduate students in life science have in general been very successful in the competition (several gold and silver medals).

6. Impact and engagement in society

a. *Relevance of research to society at large*

The focus of the research is to improve public health and to come up with solutions related to a more sustainable world. This includes providing a large number of fundamental tools (databases over the human body molecular landscape) useful for other researchers (incl. research-intense pharma companies) to base their research efforts and hypothesis driving on. The long-term impact on human health of these efforts has the potential to be immense. Public data accessible for an interested general audience, through a pedagogical web-interface (see proteinatlas.org). Several concepts and reagents emerging from the department have already been implemented into practical use in the society (HER2 imaging, other affibody/ADAPT-based reagents, NAFLD, Pyro).

b. *Research dissemination beyond academia*

Through our competence centers and multiple collaborations with industry and the healthcare sector, research is disseminated widely outside the academia. Several faculties are also active within funding agencies (e.g. Vetenskapsrådet and Cancerfonden), in steering boards, and through visits. The Department is also quite visible in Swedish and international media, as well as within e.g. on-line gaming platforms (see Impact case 2). Faculties also have regular interactions with schools and their students within Stockholm and Sweden.

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

The 17 UN Sustainable Development Goals has been incorporated well into the courses the faculties of the Department are teaching. Further, our research activities relate strongly to Goal nr. 3: Good health and well-being, and in some respect to several other (environmental, education, gender equality)

d. *Impact cases*

Impact case 1: The Human Protein Atlas

Impact Case 2: Engaging a wider community in the classification of images

Impact Case 3: BioSilk – recombinant silk for biomedical applications

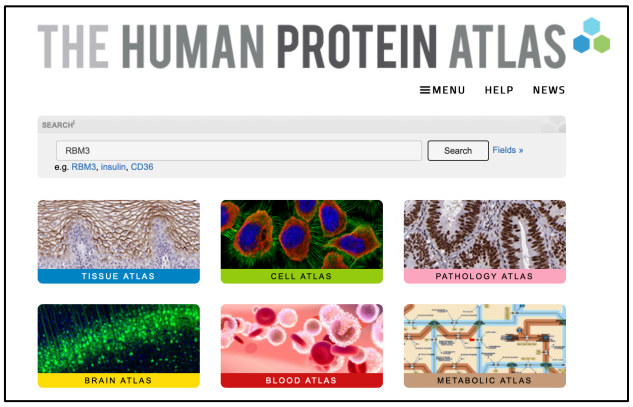
Impact Case 4: Direct conversion of CO_2 to biofuels with bacteria

Impact case 5: Drug development and human clinical trials

Impact case 6: Precision medicine

Impact case 1: The Human Protein Atlas

The Human Protein Atlas (HPA) program is an international effort started in 2003 with the aim to map all human proteins in cells, tissues and organs using integration of various omics technologies, including antibody-based imaging, mass spectrometry-based proteomics, transcriptomics and systems biology. The main funding to the HPA consortium has been provided by the Knut and Alice Wallenberg Foundation (Sweden), and the mapping has been focused on cells and tissues of human origin.



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 current version HPA program consists of six separate parts (www.proteinatlas.org), each focusing on a particular aspect of the genome-wide analysis of the human proteins, including the Tissue Atlas showing the distribution of the proteins across all major cells, tissues and organs in the human body (2015), the Cell Atlas showing the subcellular localization of proteins in single cells (2017) and the Pathology Atlas showing the impact of protein levels on survival of cancer patients (2017). In the last year, three new additions have been launched; the Blood Atlas showing the proteins across the major immune cells, including also data regarding the number and concentrations of human blood proteins (2019), the Brain Atlas showing the proteins located to different regions of the human brain as well as the brains of pig and mouse (2020) and the Metabolic Atlas with a model of the metabolic enzymes in humans and their location to different tissues (2020).

The database has more than 300,000 visitors per month and it is one of the most visited biological databases in the world. More than five peer-reviewed publications are published in average by external groups every day that has used the antibodies generated in the program and the HPA program has thus so far contributed to several thousands of external publications in the field of human biology and disease. Furthermore, the program was recently selected by the organization ELIXIR as a core database resource due to its fundamental importance for a wider life science research community. In addition, the program has led to eight start-up companies from the KTH group and led to drug candidates which are now in four human clinical trials (March 2020).

In addition, we have spent considerable efforts to validate the antibodies used in the program. 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 (Uhlen) as chair. A new concept for enhanced validation of antibodies has been launched and more than 10,000 of the antibodies used in the Protein Atlas effort are now validated using the strategy proposed by the IWGAV.

Key publications:

1. Uhlén *et al.* (2015) "Tissue-based map of the human proteome" ***Science*** 347: 1260419
2. Uhlén *et al.* (2016)" A proposal for antibody validation" ***Nature Methods,*** 13: 823-7
3. Uhlén *et al.* (2017) "A pathology atlas of cancer transcriptome" ***Science*** 357 (6352): eaan2507
4. Thul *et al.* (2017) "A subcellular map of the human proteome" ***Science*** 356 (6340): eaal3321
5. Uhlén *et al.* (2019) "The human secretome" ***Science Signaling*** 12(609)
6. Uhlén *et al.* (2019) "A genome-wide transcriptomic analysis of protein-coding genes in human blood cells" ***Science*** 366 (6472): eann9198
7. Sjöstedt *et al.* (2020) "An atlas of the human, pig and mouse brain" ***Science*** 367 eaay5947

Impact Case 2: Engaging a wider community in the classification of images

Pattern recognition and classification of images are key challenges throughout the life sciences. We are continuously working with crowed-sourced and machine learning solutions for such problems related to the images in the HPA Cell Atlas.

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 massively-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. During 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, and 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 (2).

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, I organized the Human Protein Atlas Image classification challenge hosted by Kaggle (owned by Google). Over 2000 teams participated with deep learning models for pattern recognition in microscope images, significantly improving the state-of-the-art. We are now at the point where we can quantify spatial patterns for integration with other multi-omics data (3). Through such crowed 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.

Key publications:

1. Sullivan D *et al*. Deep learning is combined with massive-scale citizen science to improve large-scale image classification*.* ***Nat Biotechnol***. 2018 Oct;36(9):820-828
2. Ouyang W *et al.* Analysis of the Human Protein Atlas Image Classification competition. ***Nat Methods***. 2019 Dec;16(12):1254-1261.
3. Peplow M. Citizen Science lures gamers into Sweden's Human Protein Atlas. *Nature Biotechnol.* 2016 May 6;34(5):452-3. *News article highlighting the significance of Project Discovery.*

Impact Case 3: BioSilk – recombinant silk for biomedical applications

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 has from this established a scalable process for reproducible production of defined and highly pure spider silk proteins.

Moreover, methods for functionalisation 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 functionalisation 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 behaviour 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.

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 utilising BioSilk, a silk protein functionalised 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. BioSilk is since 2019 available as a commercial product, produced by Spiber Technologies AB and distributed by BioLamina AB. Together with researchers with competence in specific medical fields, methods for 3D culture of cells in BioSilk is currently being developed for several applications within regenerative medicine including nerve conduits, wound healing, cancer models and diabetes treatment.

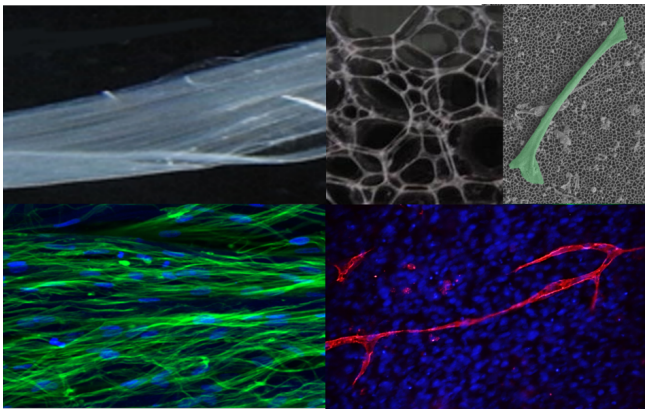


Fig. 1. Upper row: Recombinant silk in the format of fibers, 3D network and nanowire. Lower row: Cells integrated in 3D BioSilk and their spontaneous formation of a microvessel.

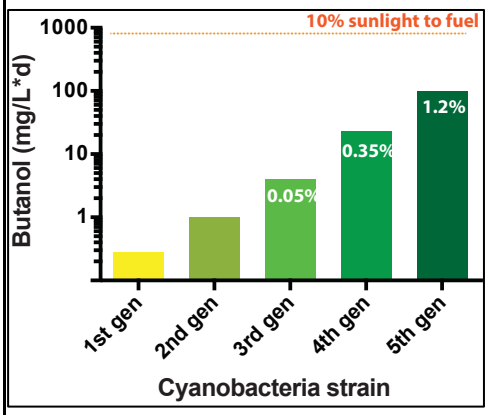
Key publications:

1. Hedhammar *et al.* Structural Properties of Recombinant No
2. nrepetitive and Repetitive Parts of Major Ampullate Spidroin 1 from Euprosthenops australis: Implications for Fiber Formation. ***Biochemistry***. *2008;47(11):3407-17.*
3. Jansson R *et al.* Recombinant spider silk genetically functionalized with affinity domains. ***Biomacromolecules.*** *2014 12;15(5):1696-706.*
4. Widhe M *et al.* A fibronectin mimetic motif improves integrin mediated cell biding to recombinant spider silk matrices**.** ***Biomaterials****. 2016;74:256-66.*
5. Gustafsson L *et al.* Structuring of Functional Spider Silk Wires, Coatings, and Sheets by Self-Assembly on Superhydrophobic Pillar Surfaces. ***Adv Mater****. 2018;30(3).*
6. Johansson U *et al.* Assembly of functionalized silk together with cells to obtain proliferative 3D cultures integrated in a network of ECM-like microfibers. ***Sci Rep****. 2019 9(1):6291*

Impact Case 4: Direct conversion of CO_2 to biofuels with bacteria

One of the most important tasks for a future sustainable society is to be able to provide renewable energy without the use of fossil fuel. An interesting solution is to use solar energy combined with biologically solutions, such as gene editing and metabolic engineering. In the KTH group, new types of biofuel production system are being developed, where bacteria fix CO_2 and convert it directly to fuel. Here, photosynthetic cyanobacteria are used that can produce energy directly from sunlight, and litho-autotrophic bacteria that use energy derived from hydrogen. Both of these substrates can be obtained cheaply and sustainably. The system is thus promising to reduce biofuel costs, but extensive metabolic engineering is needed to create strains that can convert CO_2 to fuels with high efficiency.

In one area of research, the KTH group is exploring how metabolic flux is controlled in these strains, particularly within the CO_2-fixing Calvin cycle. We use advanced systems biology techniques to quantify cellular metabolites and enzymes under various environmental conditions; these data are then fed into mathematical models of metabolism. Systems biology can provide insight to guide genetic engineering. For example, the most in-depth analysis of the cyanobacteria proteome was recently published, where over 1200 proteins were quantified. This data showed that carbon fixation is likely regulated post-translationally, so that simple overexpression of enzymes is not the most effective way to increase CO_2 fixation or biofuel production rates. These results have led us to also explore allosteric regulation in the Calvin cycle. From metabolomics data, we created "design rules," for novel synthetic metabolic pathways, to ensure that these pathways contain high-abundance metabolites, so that biofuel production will have a high driving force.



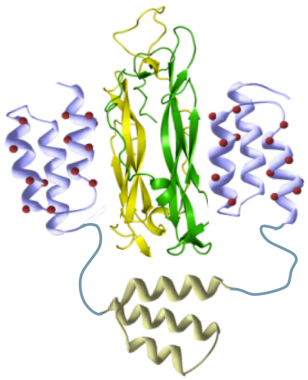
We have also pioneered synthetic biology in these strains, in particular CRISPR/Cas gene editing and variants in cyanobacteria. This tool allowed us to rapidly test genetic engineering strategies to increase biofuel production. For example, we have created several cyanobacteria strains to produce the biofuel n-butanol, which is a potential gasoline replacement, and can also be upgraded to jet fuel. Through a series of model-guided genetic engineering, we have gradually increased the productivity (see Figure). Our current strains can convert approximately 1% of incoming light energy into butanol. We are now testing strategies to de-regulate central carbon metabolism, in order to increase efficiency up to 10%, which is near the theoretical maximum. We are also working to increase stability of these strains, so that they produce biofuel over a period of months. If successful, this biological solution to cyanobacteria-produced butanol would make this renewable biofuel economically superior to fossil fuel and thus have huge impact on our attempts to move towards a sustainable society

Key publications:

1. Yao, L., Cengic, I., Anfelt, J. & Hudson, E. P. Multiple Gene Repression in Cyanobacteria Using CRISPRi. ***ACS Synthetic Biology* 5**, 207–212 (2016).
2. Jahn, M. *et al.* Growth of Cyanobacteria Is Constrained by the Abundance of Light and Carbon Assimilation Proteins. ***Cell Reports* 25**, 478–486 (2018).
3. Asplund-Samuelsson et al. Thermodynamic analysis of computed pathways integrated into the metabolic networks of E. coli and Synechocystis reveals contrasting expansion potential. ***Metabolic Engineering* 45** 223-236 (2018).
4. Yao et al. Pooled CRISPRi screening of the cyanobacterium *Synechocystis* sp. PCC 6803 for enhanced industrial phenotypes. ***Nature Communications*** *(*2020*)*

Impact case 5: Drug development and human clinical trials

The research groups at the department have for many years been focusing on translational efforts to take the basic research into applications of societal importance. The research has to a large extent been medically oriented, but recently also research aimed for environmental applications have been initiated. Approximately 100 inventions have led to patent applications with inventors from the KTH researchers and more than 20 start-up companies have their origin at the department. At present, nine human clinical trials are being pursued by these start-up companies.



A lot of these efforts are based on the unique scaffold protein, named affibody, developed in the department more than 20 years ago. This molecule has been shown promise as a "next-generation biological drug", this being an alternative to therapeutic antibodies. The first therapeutic affibody molecules have now entered clinical development and more than 500 studies using the affibody-concept have been published, many from external groups. In the last years, applications for medical imaging has been described including researchers from the department, for example for imaging of breast cancer patients, which is in late-stage clinical evaluation. In addition, another scaffold molecule has been developed in the department for increased half-life of biopharmaceuticals using an albumin-binding domain (ABD). As an example, affibody molecules specific for IL-17, a well-known driver of psoriasis, have been formatted into a small dimeric "IL-17 trap" with sub-picomolar affinity and fused to ABD for prolonged long plasma half-life (see figure). The molecule is now in phase II clinical development in patients with moderate-to-severe psoriasis and more than 200 patients have been treated with excellent clinical effect and no reported adverse effect. Another example is an affibody molecule binding the neonatal Fc receptor (FcRn) in a pH-dependent manner, allowing for endosomal recycling. The concept has advanced into clinical evaluation for the purpose of treating autoimmune diseases by depletion of IgG. A novel scaffold protein was recently devised, the ADAPT, based on the structure of ABD. A HER2-specific ADAPT was recently evaluated in a first medical imaging trial in 20 breast cancer patients, with indeed impressive results, and therapeutic application are being considered.

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

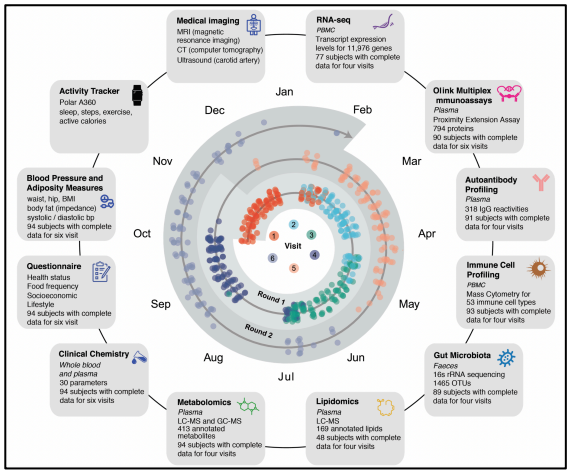
Start-up company	Year	Description
Abclon	2010	Therapeutic antibodies (phase 1 clinical trial, cancer)
Atlas Therapeutics	2011	Merged with Alligator Bioscience, cancer therapeutics
Antibodypedia	2013	Portal for validated antibodies
ScandiBio Therapeutics	2017	Three clinical trials initiated (Fat liver, Alzheimer's and Parkinson's)
Amylonix	2018	Drugs for Alzheimer disease (in preclinical testing)
ProteomEdge	2019	Precision medicine
MindForce Game Lab	2019	Science and gaming

Key publications:

1. Ståhl *et al.* (2017) "Affibody molecules in biotechnological and medical applications" ***Trends Biotechnol***. 35: 691.
2. Seijsing *et al.* (2014) "An engineered affibody molecule with pH-dependent binding to FcRn mediates extended circulatory half-life of a fusion protein" ***PNAS*** 111, 17110.
3. Seijsing *et al.* (2018) "In vivo depletion of serum IgG by an affibody molecule binding the neonatal Fc receptor" ***Sci. Reports*** 8, 5141.

4. Mardinoglu *et al.* (2014) “Genome-scale metabolic modelling of hepatocytes reveals serine deficiency in patients with non-alcoholic fatty liver disease” ***Nature Communications*** 5, 3083.

Impact case 6: Precision medicine



The human blood proteins are important as a target for diagnostics and therapy. At KTH, we have launched one of the world´s largest analysis of the human blood proteins named the Human Secretome Project funded by the Erling Persson Foundation, the Wallenberg Foundation and the governmental agency VINNOVA in collaboration with the pharmaceutical company AstraZeneca, The Novo Nordisk Foundation Center for Biosustainability in Denmark and several biotech companies in Sweden and abroad. The aim of the program is the create a world unique resource of all human secreted blood proteins using a high-throughput mammalian cell factory. This resource can then subsequently be used to study human biology as well as a valuable asset for development of new diagnostics and potential pharmaceutical drugs. So far 3000 full-length genes have been generated using synthetic biology and out of these approximately 1600 have been produced and purified using an “in-house” developed down-stream processing scheme. This unique resource has been used for phenotypic screens by AstraZeneca and is also used for development of both antibody-based and mass spectrometry-based protein assays both by the KTH groups and the industrial partners.

An important aspect of precision medicine is to probe and define the differences in molecular profiles among healthy and diseased individuals. The dawn of many new omics tools for analyzing clinical samples such as genomics, proteomics, transcriptomics and metabolomics has opened up new possibilities to study both health and disease with high throughput along with high analytical precision and clinical accuracy. We have used the resource created within the Human Secretome Project and the infrastructure built up in the Science for Life Lab to initiate one of the world’s most comprehensive “personal omics profile” program, called the Swedish SciLifeLab SCAPIS Wellness Profiling (S3WP) program, based on the thorough two-year longitudinal analysis of individuals between 50 and 65 years old. All collected samples are analyzed on a comprehensive set of omics platforms (se figure) including plasma proteome analysis based on proximity extension assay, autoimmunity profiling, immune cell profiling based on mass cytometry, gut microbiota analyses and plasma metabolite profiling. 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 towards precision medicine. This with focus on the analysis of plasma and serum, enabled through the development of technologies for high-throughput, highly multiplexed and both untargeted as well targeted autoantibody and protein profiling. By utilizing the unique and very large resource of affinity reagents created within the Human Protein Atlas as well as within The Human Secretome, have both antibody and mass spectrometry-based approaches, and combinations thereof, been developed. More than 100 publications have been generated with authors from the KTH group, providing important insights how to move towards blood- and proteomics-based precision medicine.

Key publications:

1. Ayoglu *et al.* (2016) “Anoctamin 2 identified as an autoimmune target in multiple sclerosis” ***Proc Natl Acad Sci USA***
2. Neiman *et al.* (2019) “Individual and stable autoantibody repertoires in healthy individuals” ***Autoimmunity***
3. Uhlén *et al.* (2019) “The human secretome” ***Science Signaling***

e. *Structure for increased impact*

1. Identification of important/relevant scientific questions

Keeping a high awareness of the scientific landscape/forefront through seminars, journal clubs, internal discussions, networking, conferences. Inspire ourselves to keep a creative/curious working athmosphere.

2. Publishing strategy

Strive to publish results in high impact journals (increased visibility/impact).

Acting professionally, creating high quality data

3. Entrepenuership

Inspire PhD students to take courses in entrepreneurship and patenting issues.

Inspire to a mindset continously asking if results could have a commericial application/value

4. Dissemination of results to the society in large.

Publish news on the KTH website.

Spread data via popular science channels (open lectures, student visits, etc.)

Participate in the public debate if relevant.

7. Other

a. *Specifics that the department wishes to mention and describe*

RAE 2020 Self-evaluation

Panel 2: Biotechnology

Section 3

Department of Industrial Biotechnology

A) REPORT FOR INDUSTRIAL BIOTECHNOLOGY

1. Overall analysis and conclusion; strengths and development areas

a. *Limited SWOT-analysis*

Strengths in research

- A joint ambition to perform excellent research on fundamental, application inspired topics with broad relevance for sustainability development of industry and society.
- Strong links between the research and teaching activities within the department at the 1st, 2nd and 3rd cycle.
- 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. This started with recruitment of a new department head with a good network in international industry and academia in 2016. With support at KTH and school level this will also 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) .
- 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.

Strengths in organisation

- A positive work environment with a flat organisation, collegiality, communication and transparancy, 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.
- 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.
- 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 strenghtened through a collaborative PhD student with the Chemisty department as well as strategic funding at the school level.
- Another RAE2012 recommendation concerned exchange of experience and enlargement of critical mass. The department of Industrial Biotechnology currently enjoy collaboration with other scientists that also work on biocatalysis, metabolic engineering and environmental biotechnology in other departments of KTH.

Weakness in research

- The main attention point to improve research quality, quantity and impact is too increase the level of external funding through both large initiatives as well as personal grants. 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.
- Increasing PhD student salaries and rental cost, combined with high overhead are in contrast to the slower increasing funding from the science councils and low basic funding from KTH (and the Swedish government). This disqualifies certain sources of funding that do not cover the costs and decreases the relative scientific quantity from the remaining grants.
- 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.
- 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.
- Mammalian-cell-based bioprocessing is a topic of high strategic relevance internationally as well as for KTH, Stockholm and Sweden. Although Dr. Veronique Chotteau runs a highly successful research group, e.g. leading the national Vinnova initiative AdBioPro, this topic is currently not anchored by a faculty position.

Weakness in organisation

- The low level of permanent faculty funding (±42% of salary & overhead) is prohibitive for external recruitment and the competitive position of KTH. Additionally, having to continuously meet short-term needs, is prohibitive for work enjoyment and for the development of the long-term research vision by individual scientist that is required for excellent research.
- 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 centralised 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.
- A very high turnover in administrative staff for finances, HR, legal support has resulted in lack of knowledge retention, lost of time and afficacy for reseachers and inability to go beyond the basic first support needs over the past 2.5 years. Together with the high level of centralisation leaves the researchers feeling unsupported and wondering what they get in return for the high overhead costs.

Development areas

- Continuation of the ongoing renewal of the faculty and strategic topics within the department, including a faculty position on 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 should increase.

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

Impact

- Yeast strains and underlying knowledge from proof-of-principle to full-scale production of fuels and chemicals.
- Fundamental insight into structure and function of membrane proteins through Integrated 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.
- Advancing the field of biocatalysis through a large international network of small & medium sized enterprises mixed with leading research groups in the field.
- Knowledge hub for industry and academia on advanced bioproduction of pharmaceuticals through high cell density mammalian-cell-based processes.

Infrastructure

- 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) provides societally relevant biotechnological options for sustainable production of pharmaceuticals, chemicals, materials and fuels, as well as contributing to clean water and environment. DIB has 7 faculty positions and an additional 4 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 Alba Nova 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 CBH. 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 ±2021). 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 and composition of research team(s)

The research of DIB is divided in the following 5 themes:

Mammalian-cell-based bioprocess technology focusses on the bioproduction of therapeutic biologics using mammalian/human cells. Dr. Chotteau (Rec 2008; Bioprocessing, Automatic Control) leads this theme with 1 lab manager, 3 post-docs, 6 PhD students and a lab technician. 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 new 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. The complementary competence profiles of the existing PIs Prof. Berglund (fine chemicals synthesis) and Dr. Martinell (polymer synthesis) will be supplemented by the asst. prof. in Biocatalysis and Green Chemistry under recruitment. 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).

Integrative Structural Biology focusses fundamental understanding of structure-function relationships of enzymes of importance for metabolic reactions relevant to health and environmental objectives. This theme is led by Prof. Christina Divne whose team currently includes three doctoral students, and two junior researchers of which one is a visiting researcher. 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 valorisation of residue streams. This theme is led by Dr.Kuttuva Rajarao (Scientist) 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. 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 valorise 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. Prof. van Maris is also a member of the board of the cross-disciplinary KTH Water Centre.

Microbial Bioprocess technology focusses on metabolic engineering and process development for production of fuels, chemicals and proteins. This theme is led by Prof. van Maris (Rec 2016; Biochemical Engineering), Assoc. Prof Veide (ret. 2021; Biochemical Engineering) and Assis. Prof. Gustafsson (Rec. 2019; Industrial Microbiology) with 1 lab manager and 4 PhD students. 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.

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.

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

Integrated 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 phytophatogenic exopolysaccharides.

Environmental Biotechnology

- Analysis and demonstration of the spread of pharmacautical 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 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 biethanol, mono- and dicarboxylic acids in yeast and *E. coli*.

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

The number of publication published by DIB is showing an increasing trend, 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 journal. We aim for this to increase in line with the faculty renewal process and realignment of research topics. Continuous attention is paid to educate of staff and junior scientist in awareness of open access, predatory publishing and predatory conferences. Realisation of increased external funding, which is the main ambition for the coming period, will also increase the quantity and through critical mass, also the quantity of the research output. 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.

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

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

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

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

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

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.

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

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

VanArsdale et al. (2020). A Coculture Based Tyrosine-Tyrosinase Electrochemical Gene Circuit for Connecting Cellular Communication with Electronic Networks. ACS Synthetic Biology, doi: 10.1021/acssynbio.9b00469.

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. 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.

Structural Biology, internal collaboration, national infrastructure (Chrstina). 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 Instiutet), Protein Science Facility (Karolinska Institutet), Diamond Light Source (UK), SOLEIL Synchrotron (France), BESSY II Synchrotron (Germany), DESY PETRA III Synchrotron (Germany), European Synchrotron Radiation Facility ESRF Grenoble (France). Furthermore, the team is in a close collaboration with Asso. Prof. Magnus Johnson at the Dept. of Chemistry (CBH) regarding membrane studies using the novel technology nano-IR microscopy, where CBH is the first in Sweden, and one of few globally, to own this type of new equipment. The group 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 3 different research group at KTH, the Research Institute 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 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 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 department 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 Integrated Structural Biology group and the department of Chemistry. Additionally this group has received strategic resources to increase it's scientific impact and growth potential.

3. Viability

a. Funding; internal and external

The research funding of the department over the past 6 years (2014-2019) has on average been 34 MSEK per year with 37% from basic KTH funding KTH, 52% external funding from research grants, 7% from contract research and 5% other sources (Table 1). The years 2012-2013 are excluded from this analysis in view of a significant change due to transfer of faculty to DIB.

Table 1. Yearly research funding of Department of Industrial Biotechnology in MSEK/year.

	2014-2015	2016-2017	2018-2019	Average
Basic funding KTH	12.8	10.2	14.6	12.5
External funding	23.0	16.3	14.1	17.8
Contract research	1.9	1.6	3.5	2.3
Other	1.1	2.3	1.6	1.7
Total	38.9	30.4	33.8	34.4

Together with the funding for teaching (which is not in the table; >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 invert the downward trend of the external funding, which partially reflects the transition from retiring to new faculty members, but also a decrease in acquisition by other 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 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 3 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 these meetings is shared amongst the staff and attendance is high. This includes the following meeting places:

- 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 montly 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 level 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, item like bibliometry, 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 meeting 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

The faculty of DIB, which for the purpose of this evaluation includes the independent researchers, is well-balanced with role models of both genders as well as career stages (Table 2). However, as can be seen from the table, 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 centralised recruitment board and the slow centralised processes and uncompetitive conditions that cause excellent candidates of both genders to accept positions elsewhere. Upon completion of the current faculty development plan, a group of 4 retirement estimated between 2031-2033 will be the next natural opportunity for strategic realignment.

Table 2 Faculty composition (including independent researchers) based on career stage and gender, including foreseen promotions and planned recruitments.

	Current Situation			5 years from now		
	Women	Men	Recruit	Women	Men	Recruit
Professor	1	3		1	2	1
Associate Professor	1	1		1	1	1
Assistant Professor		1	1			1
Researcher	3	1		2	1	
Total	5	6	1	4	4	3

Four 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. 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. Suitable candidates are

present amongst the students of the department these are also encouraged to apply.
- The extensive Linkedin network of the faculty.
- Advertisement in Nature Jobs for faculty positions and key post-docs.
- E-mail to distribution list of group- and department leaders of 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 safeguard 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 teaching programmes 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 (a.o. 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.

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 - Development Plan 2018-23” and with the school(s) development plan(s) respectively.

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 as well as increased our collaborative efforts within KTH in the recent years. The joint DIB research infrastructure is used for teaching at the undergraduate and post graduate level 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 of 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 11 faculty and researchers meets once a month to discuss the 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 of education from 2010-2019.

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 cycle:

- 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, lab courses in for instance Biocatalysis, Environmental Bioprocesstechnology or a 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 facilitiates communication (e.g. career advice etc.) in later years.
- PhD students are an essential part of the research and teaching community at DIB, whilst being immersed in their own tailor made 3rd cycling education projects of which participation in educaiton activities is an important part.
- PhD students are educated in critically reviewing each other's research and publications by external scientist 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 6d below). For instance also 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 organisation 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 organisations. An example of this is the publication a handbook for policymakers and users for removal of pharmacauticals and other xenobiotics from (waste) water (waterchain.eu/best-practices).
- Students. Trained PhD- and MSc-students are the most important type of valorization by a univeristy. Already during their studies, many students perform their MSc end project at companies or institutions. After their formal training, many students that pusued 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 has language limitations in Sweden.
- General public. DIB utilises 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

from their (http://www.biocascades.eu/what-is-genetic-engineering/). (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 also reflected in the sustainability bibliometric analysis with coverage of many DIB publications. Strangely enough UN#12 itself is not reflected in the bibliometric analysis of KTH, which most likely reflects that this analysis is looking 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 utilisation 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 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.

d. *Impact cases*

Impact Case 1: Very high density continuous culture for biologics production

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 production based on mammalian cells in a majority of biological drugs nowadays. The legacy is still fed-batch process (i.e. batch process fed with time). However this is rapidly evolving with the adoption of novel technologies to meet new challenges and competitiveness. In this perspective, 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. This production intensification is now considered as the ultimate goal for some companies e.g. Sanofi, Novartis, and such process intensification is applied by most large Pharma companies. The drivers behind this are several: 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.

Chotteau's lab was the first group to publish extremely HCDP of antibody producing CHO cells in Clincke *et al*. 2013. This work in collaboration with GE Healthcare resulted in patent US20140011270A1. These 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 M.S., Konstantinov K.B. and Cooney C. 2015. The future of industrial bioprocessing: batch or continuous? *Biotechnol Bioeng* 112:648. The article Clincke *et al.* 2013 is the most cited of all papers published in Biotechnology Progress during 2011-2019. This work has positioned the group as world-leader in mammalian cell-based perfusion processes.

Following this success several projects of HCDP were then carried out in collaboration with different industrial partners as listed in Table 1. 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. In 2016, Prof. Mathias Uhlén, KTH, together with AstraZeneca launched the Wallenberg

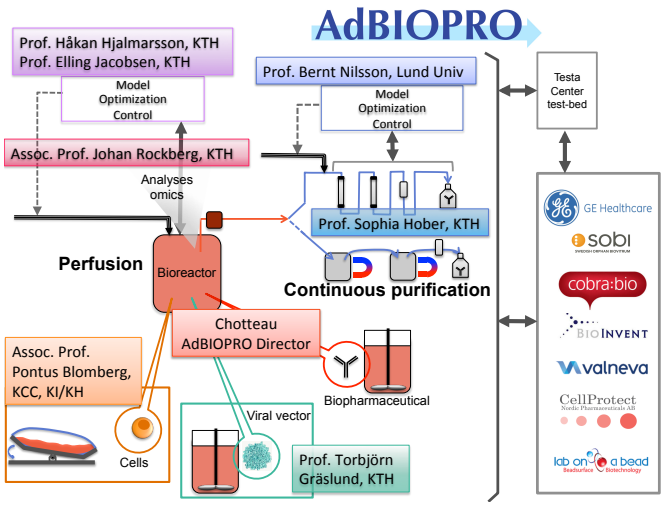


Figure 1: AdBIOPRO overview, showing the research areas of the Centre and the partners from academia, public sector and industry

Centre for Protein Research, where Chotteau's group was responsible for HCDP using the human cell line HEK293 to produce proteins. The group successfully developed a new small-scale perfusion system, suitable for HEK293 cells despite their higher shear stress sensitivity (Schwarz et al. 2020). Furthermore, in collaboration with Assoc. Prof. Johan Rockberg, cell characterisation by omics revealed mechanisms taking place in HCDP of CHO and HEK293 cells. Thanks to this leading position in HCDP, Chotteau became Director of AdBIOPRO Competence Centre for Advanced Bioproduction by Continuous Process (Figure 1), 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 characterisation, with focus on biopharmaceutical manufacturing, adeno-associated virus and cell therapy. Furthermore, Chotteau is leading or involved in other projects related to perfusion processes (see Table). Chotteau is 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 */Main applicant – Funding or Sponsors [Period]* / Collaborators
Competence Centre for Advanced BioProduction, AdBIOPRO */Chotteau - 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); GE Healthcare – SE; Sobi – SE; Cobra Biologics – UK, SE; Valneva – SE; BioInvent – SE; Lab-on-a-Bead – SE
Competence Centre Wallenberg Centre for Protein Research/*Prof. M. Uhlén, KTH - AstraZeneca & Wallenberg Foundation [2016-2018]*/ KTH (3 departments), AstraZeneca – UK
iConsensus, Integrated control and sensing platform for biopharmaceutical cultivation process high-throughput development and production */Chotteau - EU-IMI H2020 and biopharma partners [2018-2022]* / 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; Synthon – NL; UCB – BE; Presens – DE; m2P lab – DE; Ipratech – BE; Kantisto – NL; Iprasense – FR; Micronit – NL; PaiaBio – DE; Ramcon - DK
Staccato, European Industrial Doctorate for enhancing upstream biopharmaceutical manufacturing process development through single cell analysis */Dr. C. Clark, NIBRT - EU-EID H2020 (Marie Sklodowska Curie) [2019-2022]* / Dr. C. Clark (Bioinformatics, NIBRT, Ireland); Prof. N. Barron (Omics, Univ College Dublin, Ireland); Dr. J. Bones (MS, NIBRT, Ireland); Prof. M. Hedhammar (Biomaterial, KTH)
SmartFD, Smart feed design */Chotteau - Vinnova - PiiA Process Industrial IT and Automation [2017-2019]* / Prof. Francisco Vilaplana (Glycoscience, KTH), GE Healthcare – SE, Cobra Biologics – UK, SE
DL2, Data-Limited Learning of Complex Dynamical Systems */Assoc. Prof. D. Broman, KTH - KTH Digital Futures [2019-2024]*/ KTH (3 departments)
AAVNova, AAV production for gene therapy */Assoc. Prof. J. Rockberg, KTH - Vinnova and AstraZeneca [2019-2022]* / Assoc. Prof. J. Rockberg (Cell Engin., KTH); AstraZeneca – UK; Vironova - Sweden
Centre for Advanced Medical Product, CAMP */Dr. Jukka Lausmaa, RISE – Vinnova [2018-2023]* / Assoc. Prof. P. Blomberg (GMP production of ATMP, KCC - Karolinska Hospital)

Clincke M.F., ..., Chotteau V. (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

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

Impact case 2: Environmental Biotechnology for Water Treatment: The Baltic as a case study

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.eu/best-practices). 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 (Fig. 1). One of the pharmaceuticals investigated, the anti-epileptic drug carbamazepine, was widespread in coastal and offshore seawaters (present in 37 of 43 samples). In order 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.

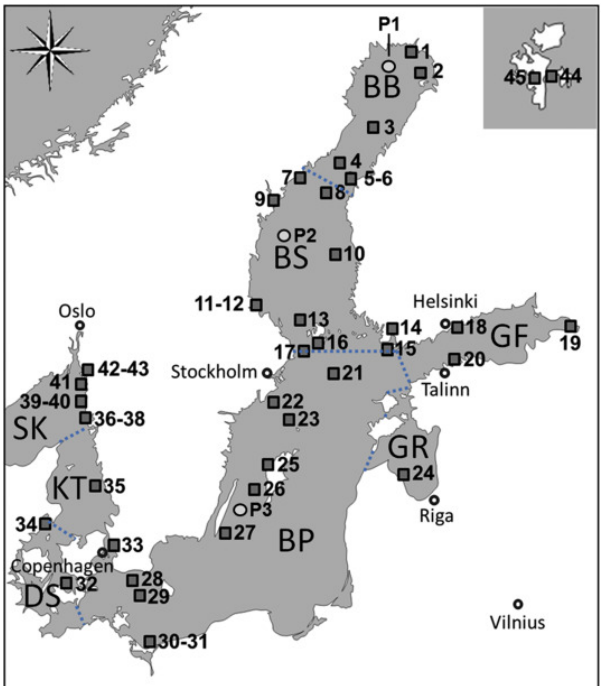


Figure 1 Baltic Sea with sampling points and main sub-basins: BB = Bothnian Bay, BS = Bothnian Sea, GF = Gulf of Finland, GR = Gulf of Riga, BP = Baltic Proper, DS = Danish Straits, KT = Kattegat and SK = Skagerrak. Control samples (44 and 45) were taken at Svalbard.

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). Supported by scale-down experiments in the lab, on-site pilot container-based pilot plants (Fig. 2) 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 online available at waterchain.eu/best-practices.

Figure 2. Mobile pilot plant for removal of pharmaceutical residues from effluent of waste-water treatment facilities.

DIB Environmental Biotechnology 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.

Björlenius, B. (2018). Pharmaceuticals – improved removal from municipal wastewater and their occurrence in the Baltic Sea. Doctoral dissertation, KTH Royal Institute of Technology, Sweden.

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 Environ., 633:1496-1509. doi: 10.1016/j.scitotenv.2018.03.276

Impact case 3: Biocatalysis at the interface of industry and academia

The Biocatalysis research at KTH is picked up by companies and is spread through active networking for more than 40 years. In recent years the impact is manifested in a successful international conference series, commercialization of the developed sustainable transaminase technology and many co-publications with companies.

The industrial and academic impact of the research of the Biocatalysis group is illustrated by its extensive network, which has been built through participation in FP7, H2020, Vinnova and Formas projects over the recent years. Figure 1 shows the major collaborators and networks since the previous RAE in 2012. Important examples constitute the ITNs *Biocascades* 2015-2018 (P. Berglund), *IRENE* 2009-2012 (M. Martinell), *REFINE* 2012-2016 (M. Martinell) and *INTERfaces* 2020-2023 (P. Berglund). These projects have resulted in five PhDs graduating from KTH. Important networking has also been taken place by the active participation in the Cost Actions CM0701 *CASCAT - Cascade Chemo-Enzymatic Processes: New Synergies between Chemistry and Biochemistry* 2008-2012 and CM1303: *Systems Biocatalysis* 2013-2017 where one important feature has been the short scientific missions aimed for exchange of people. All these networking have resulted in 33 co-authored papers with international and national academic and industrial collaborators 2012-2020.

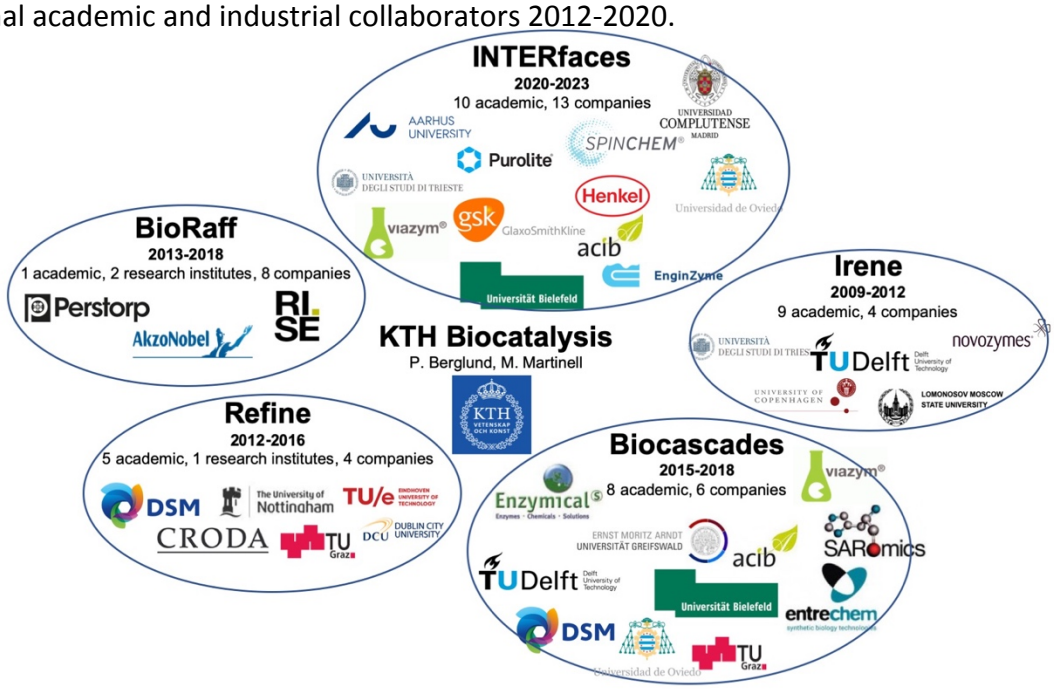


Figure 1. KTH Biocatalysis major networking 2012-2020.

The Biocatalysis group at KTH originates from the beginning of the 1980s when Prof Karl Hult from Biochemistry joined forces with Prof Torbjörn Norin from Organic chemistry. This cross-disciplinary research has since then fostered more than 40 graduated doctoral students now active in companies and academia all over the world, and has put KTH as one of the well-known top universities on the European Biocatalysis scene through participation in numerous international research projects and networks over the years.

The start-up of the company EnginZyme AB (www.enginzyme.com) around 2014 is an important evidence for the impact of the KTH Biocatalysis research. The CEO and founder

of EnginZyme, Dr Karim Engelmark Cassimjee is a PhD graduate from the KTH Biocatalysis group in 2012 (P. Berglund supervisor). The pioneering work, which today is the major technology platform of the company, was published in two papers during Dr Cassimjee's PhD studies at KTH and is based on a general immobilization methodology through a His-tag of the enzymes (Cassimjee *et al*. 2008 and 2011).

Transaminase chemistry is today a worldwide versatile technology for the sustainable synthesis of chiral amines. At KTH, transaminases started to be explored in the national Vinnova-funded consortium BIO-AMINES 2008-2011 (P. Berglund and G. Larsson) involving KTH and the companies AstraZeneca, Cambrex Karlskoga AB and SARomics Biostructures AB. The project was a kick-start for transaminase chemistry at KTH, partly funded 4 PhD students and resulted in 7 joint published peer-reviewed papers.

ESAB (European Section of Applied Biocatalysis of the EFB) is active on the European arena to promote the Biocatalysis field. Its' Scientific Committee (P. Berglund member) supports scientific conferences. The need of a transaminase conference as a driver was identified by the ESAB SciComm in 2012 and the first conference was arranged at KTH in 2013, *1st International Symposium on Transaminase Biocatalysis* (chairs: P. Berglund and John Woodley, DTU). The successful first event with 120 participants in Stockholm was followed by Transam2.0 in Greifswald 2015 (chair: Prof Uwe Bornscheuer) with a slightly broadened scope but with kept numbering, Amine Biocat 3.0 in Manchester in 2017 (chair: Prof Nick Turner), Amine Biocatalysis 4.0 (chair: Prof Bernhard Hauer) in Stuttgart 2020 and the fifth version in Groningen 2022 (chair: Prof Gerrit Poelarends). The series has attracted great interest from industry with >30% of the participants and presenters coming from companies.

The collaboration with Prof Armando Córdova at Mid Sweden university on the cascade synthesis of the pepper fruit compound capsaicin, involving transaminase biocatalysis in a multi-catalytic one-pot process (Anderson *et al.* 2014), also led to a patent on multi-catalytic transformation of alcohols into amines (Córdova *et al.* 2015). This patent was taken over by the company Organofuel Sweden AB in 2016, which is now offering capsaicin on the market (www.organofuelsweden.com). Thus, biocatalysis technology developed at KTH can not only lead to novel industrial processes but can also provide consumer products and thereby fulfill the goal to increase the contribution of industrial biotechnology for a sustainable society.

Anderson, M., Afewerki, S., Berglund, P., & Córdova, A. (2014). Total synthesis of capsaicin analogues from lignin-derived compounds by combined heterogeneous metal, organocatalytic and enzymatic cascades in one pot. *Adv. Synth. Catal*., *356*, 2113-2118.

Cassimjee, K. E., Kourist, R., Lindberg, D., Larsen, M. W., Thanh, N. H. Widersten, M., Bornscheuer, U. T. & Berglund, P. One-step enzyme extraction and immobilization for Biocatalysis applications. (2011) *Biotechnol. J*., *6*, 463-469.

Cassimjee, K. E., Trummer, M., Branneby, C. & Berglund, P. (2008). Silica-Immobilized His_6-tagged enzyme: Alanine racemase in hydrophobic solvent. *Biotechnol. Bioeng*., *99*, 712-716.

Córdova, A., Berglund, P., Anderson, M., & Afewerki, S. (2015). Patent: Efficient synthesis of amines and amides from alcohols and aldehydes by using cascade catalysis. US20170174618 A1, CA2943677A1, CN106458854A EP3122715A1, WO2015144902A1

Impact Case 4: Integrative structural biology for sustainable development

Structural biology is a cornerstone of modern biotechnology. Recent advances in techniques like cryo-EM allow new and previously unseen details of life to be elucidated at high resolution, and functional implications to be drawn that pave the way for engineering of new and altered protein characteristics, drug design and optimized enzyme-based bioprocesses.

Our primary scientific aim is to advance the collective understanding of fundamental biological processes with implications for life that have high probability of future development of applications that promote sustainable development. The main focus is on health and efficient use of global natural resources with emphasis on biological systems and processes involving carbohydrates, glycans and glycoconjugates.

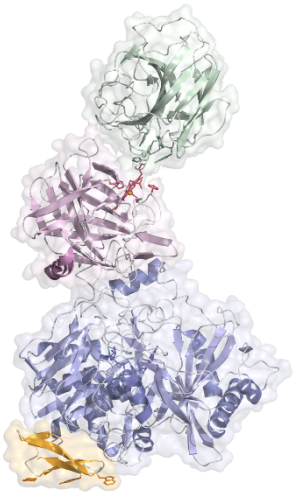


Fig.1. Fungal oxidative CDH-LPMO complex. (Tan et al. Nature Communications, 2015).

Illustrative examples include our seminal work on the structure of the key hydrolytic enzyme cellobiohydrolase I, CBHI (Divne et al. 1994), and later, on oxidative cellulose degradation (Tan et al. 2015) of recalcitrant crystalline cellulose. The work on CBHI allowed rationalization of the enzyme's function and mode of action (Divne et al. 1996), and paved the way for applications and patents related to plant-biomass treatment for biofuel applications (Genencore International Inc.). The more recent work on the oxidative (CDH-LPMO) system by us (Tan et al. 2015; Fig 1.), and other research groups, overhauled the classical "hydrolytic only" model to establish and consolidate the currently prevailing "hydrolytic-oxidative" model. Fundamental and applied research on biomass utilization has obvious relevance for sustainable development by means of efficient use of natural resources, production of value-added products, fine chemicals and next generation biofuels. However, our scope also includes research that can benefit human health.

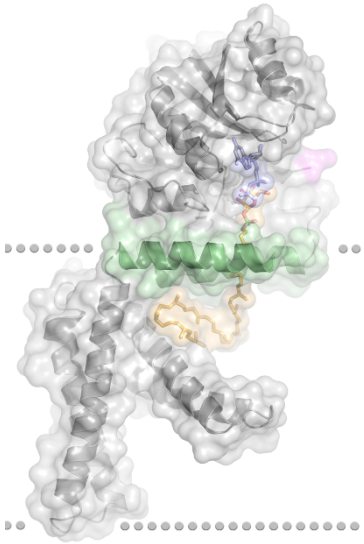


Fig.2. Structure of DPMS (Gandini et al. Nature Communications, 2017).

Despite membrane proteins making up 60% of the (current) druggable proteome, little is known about their structure and function. Polysaccharide- and glycoconjugate-synthesizing membrane enzymes have a fundamental role in a wide range of diseases including cancer, but also in applied research. As an example, we recently published the first structure of the transmembrane enzyme dolichylphosphate mannose synthase (Fig.2.), which catalyzes the glycolipid carrier required for protein glycosylation (Gandini et al. 2017). The structural data allowed rationalization of several human mutations leading to severe forms of congenital disorders of glycosylation.

The future potential of integrative structural biology is illustrated by several new projects started in 2019 with high relevance for human and plant health. Examples include mammalian sphingolipid-synthesizing enzymes with relevance for a wide range of neurological disorders and cancer, plant pathogen enzymes that synthesize glycoconjugates required for virulence and pathogenicity, and bacterial lipoprotein transport protein complexes, also with relevance to plant disease. The strategic relevance for KTH is also illustrated by financial support for an internal CBH collaboration using the first nano-IR microscope available in Sweden (Assoc. Prof. Magnus Johnson) to study protein and lipid composition of membranes with an emphasis on signaling membrane microdomains).

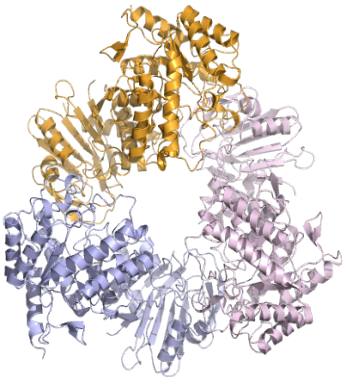


Fig.3. Exo-β-mannosidase from the hindgut of the wood-feeding termite Reticulitermes flavipes determined by a hybrid X-ray and cryo-EM approach (to be published).

Despite considerable advancements regarding cellulose degradation, significantly less progress has been made towards efficient use of other plant cell-wall components such as hemicelluloses and lignin. We recently determined the structure of a 200 kDa large hemicellulose-degrading enzyme (Fig.3) using a hybrid X-ray and cryo-EM approach. This illustrates the advantage of taking an integrative methodological approach, in this case, characterization of novel enzymes with potential for improved biomass utilization.

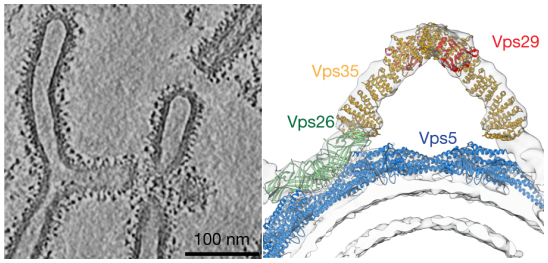


Fig.4. Left, a cryo-ET tomogram section of membrane tubules with a 15-nm thick coat of the retromer complex; and right, high-resolution 3D-image reconstruction of the complex (Kovtun et al. Nature 561:561, 2018).

An exciting recent development in the field of integrative structural biology concerns high-resolution cryo-electron tomography (cryo-ET) with cryo-FIB milling (cryo focused ion beam), a technique that allows visualization of cellular structures and macromolecules *in situ* of a cell at high resolution (Fig. 4). As an expansion of our integrative structural biology approach, cryo-ET/FIB would be particularly interesting for studying protein and ganglioside complexes at the membrane interface of neuronal cells.

Gandini, R., Reichenbach, T., Tan, T. C., & Divne, C. (2017). Structural basis for dolichylphosphate mannose biosynthesis. *Nature Communications*, *8,* 120. [6 citations]

Tan, T. C., Kracher, D., Gandini, R., Sygmund, C., Kittl, R., Haltrich, D., Hällberg, B. M., Ludwig, R. & Divne, C. (2015). Structural basis for cellobiose dehydrogenase action during oxidative cellulose degradation. *Nature Communications*, *6,* 7542. [92 citations]

Seminal paper: Divne. C., Ståhlberg, J., Reinikainen, T., Ruohonen, L., Pettersson, G., Knowles, J. K., Teeri. T. T., & Jones, T. A. (1994). The three-dimensional crystal strcuture of the catalytic core of cellobiohydrolase I from *Trichoderma reesei*. *Science, 265*, 524–528. [504 citations]

Seminal paper: Divne. C., Ståhlberg, J., Teeri. T. T., & Jones, T. A. (1996). High-resolution crystal structures reveal how a cellulose chain is bound in the 50 A long tunnel of cellobiohydrolase I from *Trichoderma reesei*. *Journal of Molecular Biology, 275*, 309–325. [324 citations]

Funding agencies: The Swedish Research Council VR, Formas, The Swedish Childhood Cancer Fund, Oscar and Lili Lamm's Foundation, KTH

Impact Case 5: Yeast engineering for sustainable production of fuels and chemicals

Engineered yeasts produce sustainable chemicals and fuels and thereby decrease the release of greenhouse gasses and the reliance on oil. The last decade yeast-based processes for amongst others lactic acid, succinate, the platform-chemical farnesene and lignocellulosic ethanol started. Research of Ton van Maris and his colleagues played a central role in these advances in yeast metabolic engineering.

Proof-of-principle work on pentose fermenting yeasts by van Maris and his colleagues overcame a major hurdle that long prevented realisation 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). 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 (Fig. 1). 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). 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.

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

The success of yeasts in industrial biotechnology is also exemplified by heterologous products such as lactic acid, farnesene and succinic acid that greatly benefit from the robustness of this microorganism. Pioneering research in this field was performed by engineering *Saccharomyces cerevisiae* strains that no longer produce ethanol in close collaboration with the UK/USA-based company Tate & Lyle. Combining metabolic engineering and laboratory evolution resulted in a pyruvate hyper-accumulating yeast strain, which can alternatively be converted to lactic acid. Subsequent engineering of pyruvate carboxylation, oxaloacetate reduction and functional expression of heterologous dicarboxylic acid transport provided the basis for production of malate or

succinate. These findings either directly (patents) or indirectly (scientific publications) contributed to the realization of industrial yeast-based processes for production of these carboxylic acids.

Introduction and optimization of heterologous product formation pathways has now become mainstream in industry. Consequently, the academic frontier has moved too and van Maris' activities shifted to development of novel concepts to broaden the scope and further improve the efficacy of industrial biotechnology. These concepts include: (i) Increased free-energy conservation in central metabolism, such as for instance engineering of transport and phosphoroclastic of split disaccharide metabolism. (ii) Improving supply of central precursors that in turn improve formation of a broad spectrum of products, such as illustrated by externsive work on cytosolic acetyl-CoA supply (van Rossum et al. 2016) with direct relevance to for instance production of farnesene as an important platform molecule for biofuel, flavours, fragrances and cosmetic ingredients. (iii) improving robustness of yeast through novel laboratory-evolution strategies, such as on-off laboratory evolution for constitutive acetic acid tolerance with high relevance for performance in lignocellulosic hydrolysates.

In 2016 van Maris transitioned from Delft University of Technology to KTH Royal Institute of Technology. Supported by KTH and the Swedish Science Council VR, his yeast research will focus on design, test and build of new anaerobic pathways for microbial products that hitherto can only be made aerobically. Microbial metabolic pathways for anaerobic production of a chemical provide by definition the most efficient stoichiometric biotechnological option when thermo-dynamically feasible. Based on proof-of-principle anaerobic production with yeast (Fig. 2), general pathway-design principles will be derived that can be applied to economically efficient and sustainable production of chemicals and thereby increasing the contribution of industrial biotechnology to a sustainable society.

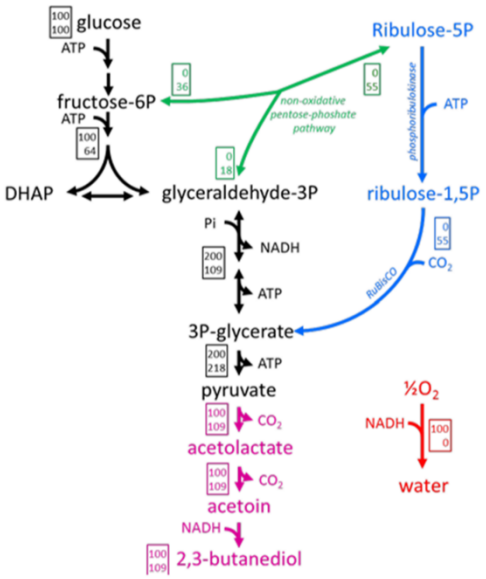


Fig.2. Schematic representation of the increased yield and elimination of oxygen consumption by optimal (bottom flux) versus current state-of-the-art pathway (top flux) for 2,3-butanediol production.

Guadalupe Medina, V. Almering, M.J.H., van Maris, A.J.A., Pronk, J.T. (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., 76, 190-195.

Guadalupe-Medina, V., Wisselink, H.W., Luttik, M.A.H., de Hulster, E., Daran, J.M., Pronk, J.T., van Maris, A.J.A. (2013). Carbon dioxide fixation by Calvin-Cycle enzymes improves ethanol yield in yeast. Biotechnol. Biofuels, 6, 125.

Papapetridis, I., Goudriaan, M., Vázquez Vitali, M. Keijzer, N.A., van der Broek, M., van Maris, A.J.A., Pronk, J.T. (2018). Optimizing anaerobic growth rate and fermentation kinetics in *Saccharomyces cerevisiae* strains expressing Calvin-cycle enzymes for improved ethanol yield. Biotechnol. Biofuels, 11, 17.

van Rossum, H.M., Kozak, B.U., Pronk, J.T., van Maris, A.J.A. (2016). Engineering cytosolic acetyl-coenzyme A supply in *Saccharomyces cerevisiae*: Pathway stoichiometry, free-energy conservation and redox-cofactor balancing. Met. Eng., 36, 99-115.

Wisselink, H.W., Toirkens, M.J., Wu, Q., Pronk, J.T., van Maris, A.J.A. (2009). Novel evolutionary engineering approach for accelerated utilization of glucose, xylose and arabinose mixtures by engineered *Saccharomyces cerevisiae* strains. Appl. Environ. Microbiol., 75, 907-914.

Funding agencies: The Kluyver Centre for Genomics of Industrial Fermentation, Dutch Research Council NWO, BE-Basic Foundation, DSM and the Swedish Research Council VR.

e. *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.

7. Other

a. *Specifics that the department wishes to mention and describe*