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1 Introduction

The ProNova VINN Excellence Centre for Protein Technology started its operations in April, 2007, as a joint industry-academia collaboration initiated by VINNOVA, involving the Royal Institute of Technology (KTH) as the hosting academic partner and companies in the Swedish life science sector as industrial partners. Through collaboration with the Swedish Human Protein Atlas (HPA) program, the ProNova Centre has a unique access to the wealth of antibody reagents produced in the HPA project, which gives a unique advantage for pioneering exploration of the field of global protein analysis, including for example use of different platforms for multiplexed disease-specific biomarker discovery and serum analysis.

The Centre is now entering stage three (of four), corresponding to the three-year period April 2012 - March 2015. At present, there are 12 industrial partners in the Centre, representing differently sized companies from various niches within the life science field. The organization of the Centre involves a Board and a Centre Management team that includes the Centre Director, the Vice Centre Director, the Program Director and three Program Area Directors. An International Scientific Advisory Board (ISAB) is in place to support the Board in scientific matters, incl. project evaluations. The new and vitalized research program for stage three is organized into three Program Areas, each headed by a Program Area Director: Affinity Tools and Protein Engineering, Array Technologies and Microfluidics. Each Program Area involves a number of academy-industry collaborative projects led by one of the eleven ProNova project leaders from KTH. In addition to KTH, part of the academic research is performed at the Science for Life Laboratory (SciLifeLab) Stockholm, located in Solna.

The present Operational Plan makes the ProNova VINN Excellence Centre well prepared for an exciting third stage of the path towards its visionary goals to be an internationally competitive centre for multi-disciplinary research in protein technology, including functioning as a preferred meeting place for industry and academia for the performance of mutual projects leading to enabling technologies, products and services for protein analysis and proteomics studies.
2 Centre Objectives and Long-term Strategic Plan

2.1 Vision
The long-term vision of the ProNova VINN Excellence Centre for Protein Technology is to further strengthen its present position as an internationally competitive centre for multi-disciplinary research in protein technology, including functioning as a preferred meeting place for industry and academia for the performance of mutual projects leading to enabling technologies and products for protein analysis and proteomics studies.

2.2 Mission
The mission of the Centre is to work with applications and topics reflecting the needs of the Centre parties and to exploit the large and unique ProNova reagent resource. The Centre will strive to operate as an innovative national and international competence resource in the field, to recruit and keep top scientists as faculty, and to attract highly talented and motivated research students and post-doctoral fellows for education and training within the Centre. An important part of the Centre mission is to function as a network and resource for collaborative projects within protein technology for the Swedish life science industry sector. Communication with the industry and commercial exploitation of research results are therefore prioritized issues of the Centre.

2.3 Results in a 2 years and 3 years perspective
2.3.1 Specific results in the Centre - commercial and scientific

Commercial results. The research program during Stage 2 (April 2009-March 2012) was organized into four Program Areas, each containing up to five subprojects. The academic and industrial Partners actively participating in these respective areas have had both the responsibility and opportunity to identify results of potentially commercial value. During Stage 2, two specific scientific results were identified as having commercial exploitation potential, and were also subjects of patent application filings:

• A method for site-specific covalent antibody conjugation using photoactivable and labeled antibody binding proteins; Inventors: Sophia Hober, KTH; Anna Konrad, KTH; Amelie Eriksson Karlström, KTH; Assignee of patent application: GE Healthcare Bio-Sciences AB

• A discovery of elevated levels of the human protein Fibulin as a serum biomarker related to kidney disorders; Inventors: Peter Nilsson, KTH; Jochen Schwenk, KTH; Mathias Uhlén, KTH; Assignee of patent application: Atlas Antibodies AB

The subject matter of these two patent applications clearly demonstrates both the industrial attraction to generated results and the width of the research performed in the Centre, including both methodology aspects and detailed biomolecule questions. In a two- to three-year perspective, the Centre is expected to produce new industrially implementable results in this broad way, reflecting the diverse needs within protein technology of the Centre partners having their prime interests in either methodology for protein analysis and handling/modification, protein biomarker discovery or engineered proteins with desired properties, respectively.

Scientific results. In addition to the commercial results described above, other projects also generated valuable scientific insights and results that have the potential to become subjects of or lead to, directly or indirectly, future Centre-derived patent application filings. For example,
an instrument for generation and high-throughput analysis of water-in-oil microdroplets was built within ProNova, which is to be used during Stage 3 in conjunction with enzyme engineering projects. Further, proteins with novel binding specificities have been and are continuously developed, that have a potential value for biotech applications. Two platforms for use in protein library technologies, based on bacterial surface display technology, have been developed and are ready for use in protein engineering and epitope mapping investigations, respectively, with the potential to lead to novel protein products and high quality antibody reagents.

In a two- to three-years perspective, the Centre expects a continued high production of scientific results, of central importance for both the visibility and branding of the Centre as well as for the contribution to the scientific community.

### 2.3.2 Specific results for the partners (from results from the Centre) - commercial and scientific

The discovery of a plasma biomarker (Fibulin) has the potential to have direct implications for the clinical testing process of new drug candidates. Efficient and sensitive monitoring of toxicity problems associated with a particular compound early in the drug development process could contribute to an increased success rate during expensive late phase trials in patients. This is of central importance for AstraZeneca AB, being a pharmaceutical company. Also, for Atlas Antibodies AB, the formal assignee on the patent application, the discovery may open up for the opportunity to develop a commercial test for measurement of this biomarker.

The antibody labeling technology developed in the Centre addresses a central issue for the many users of labeled antibodies in the world. Efficient and user-friendly means for site-specific and controlled antibody labeling with reporting groups such as biotin, fluorophores, or nucleic acids are expected to be highly appreciated by experimental scientists in the world as well as being implemented in the partner companies’ activities.

### 2.3.3 Implemented results

The two examples given above (section 2.3.2) are the commercial success stories so far, taken into account the filing of patent applications. Due to the long product development phases characterizing the biotechnology field, concrete success parameters such as revenue figures, etc. are not in place at this stage.

Concerning implementation of results in general, several methodological advances made during Stage 2 have resulted in robust platform technologies, from now ready to be exploited in specific applications during coming stages of the Centre.

### 2.4 Goals (in a 5 years perspective)

#### 2.4.1 General Goals (in relation to the VINN Excellence criteria)

The general goals can be summarized as follows:
• To gather and organize a resource of competence to perform needs-motivated research for
the generation of new products, processes and services for the promotion of a sustained
Swedish economy growth.
• To promote an intense, effective and productive interaction climate between the involved
parties leading to new innovative solutions.
• To promote a continued value-building of ideas resulting from the Centre activities.
• To work with a focus on building long-term value for involved parties and Sweden
involving the creation and sustaining of international excellence.

2.4.2 Specific Goals (for this Centre)

Industrial/societal and scientific goals and connected milestones

• To generate new knowledge and results in the field of protein technology, that make a
significant contribution to the enterprises of the Centre industry parties, to the knowledge base
in the academic world and to the society in general.

• The specific Centre goals within the field of protein technology encompass methodology
development for the analysis and engineering of proteins, as well as the use of such platforms
for specific projects in the interest of the Centre parties, as exemplified by the discovery of
novel disease-related biomarkers, development of novel proteins with desired biological
properties, development of methodology for protein labeling, development of innovative
means for constructing and using protein or antibody arrays.

• The specific goals of the Centre are connected with milestones in the form of:
  • Production of scientific publications, reports and conference contributions
  • Producing new Master’s theses, licentiate theses, and doctoral theses based on the
    Centre research
  • Patent applications and granted patents
  • Long-lasting and valuable academy-industry networks
  • Products and services available to the society

Centre performance goals and connected milestones

• To provide an organization and environment promoting the overall Centre goals in terms of a
positive attitude of the personnel conducting well-planned collaborative projects of high
scientific quality in an efficient manner, whilst securing any potential intellectual property
rights emerging from the results.

• The specific performance goals of the Centre are connected with milestones in the form of:
  • The production and partner signing of a high quality Centre Main Agreement and a
    carefully designed Operational Plan
  • The performance of well-attended activities for networking and information
    spreading, e.g. Annual Centre Days, Program Area meetings and Board meetings
  • The ability to recruit and retain top-level students, academic staff members, Board
    members and International Scientific Advisory Board members
  • The ability to attract industry parties to become active members of the Centre
2.5 Strategies

2.5.1 Strategy for implementation of results

The Centre strategy to promote the implementation of results is linked to efforts to ensure that potentially commercially exploitable results emerging from the collaborative research projects are recognized and subject of immaterial right protection considerations. These efforts involve several measures, e.g.:

- The use of a Centre Main Agreement, signed by all parties and including regulations concerning e.g. disclosure of results, confidentiality issues and partner rights to results.
- The implementation of a Centre document denoted “IPR guidelines”, serving as an additional source of information concerning how to proceed in practice when new research results have been produced.
- Program Area meetings, at which results obtained in all the collaborative subprojects of a program area are presented and discussed.
- During Stage 3 the Program Area directors will have a responsibility to establish and maintain a good dialogue with the industrial partners, including keeping record of and follow-up on results from the program area of potential commercial value.

2.5.2 International strategies

Activities in the field of protein technology, and the associated application areas in e.g. medicine, biotechnology and diagnostics are taking place worldwide. To be able to competitively and successfully act on this arena, the Centre personnel and parties need to have intense international contacts and continuously monitor the scientific forefront, incl. trends and innovations made in the field.

The PIs of the Centre have extensive international networks, through larger consortia such as integrated EU-projects, or bi- or multilateral collaborations, including other academic groups and/or industries (see Table 12 for a complete listing of currently funded research projects). These contacts increase the international scientific awareness and serve an important input to the relevance and quality of the Centre projects. To increase the international profile, the Centre is positive to recruitment of international students and postdocs, e.g. through EU programs such as Erasmus Mundus and Marie Curie, and the Centre Management will provide support to individuals applying for such programs. The Centre results are spread through traditional international channels, incl. peer-reviewed scientific journals, conference contributions and through research visits. The International Scientific Advisory Board (see Section 4.1) also contributes to the international influence on the Centre activities.

The Board and management of ProNova is in principle open to involve also the Centre itself (as the legal entity), in national or international collaborations. Although the Centre Main Agreement, carefully prepared to regulate confidentiality and IPR ownership issues between Centre parties makes it complex to intimately engage non-Centre bodies (e.g. other centra) into the ProNova Centre project work, the Centre Board is prepared to consider the engagement of the Centre in an agreement-regulated collaboration if justified by the situation.

2.5.3 Strategy for the Centre beyond Stage 4

The ProNova Centre environment and its organization of the research program into Program Areas strive to create strong and long-lived networks between academy and the involved
industrial partners. After the VINNOVA-supported stages of the Centre, it is expected that Centre fostered academy-industry constellations of larger or smaller sizes continue to collaborate on specific topics in protein technology field. The opportunities also exist that these constellations may in turn be part of larger and financially supported research clusters in different European or global collaborations.
3 Centre Partners

3.1 Industrial Partners

The ProNova VINN Excellence Centre for Protein Technology will in the start of Stage 3 have twelve industry partners representing different company sizes and fields of the life science sector, ranging from global companies to small enterprises, and from drug development to instrument and reagent supply. Brief partner company descriptions can be found in the below.

| ProNova partner since 2009 | Contact info: Caroline Ekblad (caroline.ekblad@affibody.se), Affibody AB, Gunnar Asplunds Allé 24, SE-171 63 Solna, Sweden  
Org. No.: 556665-6913  
Affibody AB is a Swedish biotech company founded in 1998 and situated in Stockholm with ca. 20 employees. The business is focused on using protein engineering to develop next generation biologicals for therapy, diagnostic imaging, and other applications based on its unique proprietary technology platforms: Affibody® molecules and the in vivo half-life extension technology Albumod™.  
Centre contribution:  
Active participation in Program Areas 1 and 2  
Total FTE contribution: 20% |
|---|---|
| ProNova partner since 2007 | Contact info: Hugh Salter (Hugh.Salter@astrazeneca.com), AstraZeneca Translational Science Center, AstraZeneca AB, SE-151 85 Södertälje, Sweden  
Org. No.: 556011-7482  
AstraZeneca AB is one of the world's leading pharmaceutical companies, with over 67,000 employees. The corporate office is located in London, UK, and major R&D sites are found in Sweden, the UK and the US. The company focuses on six therapy areas: cancer, cardiovascular, gastrointestinal, infection, neuroscience and respiratory & inflammation.  
Centre contribution:  
Active participation in Program Area 2  
Total FTE contribution: 10% |
| ProNova partner since 2007 | Contact info: Henrik Wernérus (henrik.wernerus@atlasantibodies.com), Atlas Antibodies AB, AlbaNova University Centre, SE-06 91 Stockholm, Sweden  
Org. No.: 556682-8082  
Atlas Antibodies AB was founded in 2006 by researchers at the Royal Institute of Technology (KTH) in Stockholm and the Rudbeck Laboratory, Uppsala University in Uppsala, Sweden. Atlas Antibodies has ca. 15 employees and is located in Stockholm and its business areas include research reagents and biomarker discovery. Atlas Antibodies distributes products worldwide.  
Centre contribution:  
Active participation in Program Area 2 |
| ProNova partner since 2007 | Contact info: Cristina Glad (cristina.glad@bioinvent.com), BioInvent International AB, SE-223 70 Lund, Sweden  
Org. No.: 556537-7263  
**BioInvent International AB** is a listed (BINV) research-based pharmaceutical company located in Lund, Sweden, with ca. 90 employees. The company focuses on developing antibody drugs, where a commercialization of the candidates will preferably take place in cooperation with established pharmaceutical companies. BioInvent International AB has been an active Partner in the ProNova centre for five years.  
**Centre contribution:**  
Active participation in Program Area 1  
Total FTE contribution: 25% + antibody aliquot contribution |
| ProNova partner since 2007 | Contact info: Åke Danielsson (ake.danielsson@ge.com), GE Healthcare Bio-Sciences AB, Box 605, SE-751 25 Uppsala, Sweden  
Org. No.: 556108-1919  
**GE Healthcare Bio-Sciences AB**, headquartered in the United Kingdom, is a $17 billion unit of General Electric Company (NYSE: GE). GE Healthcare employs more than 46,000 people worldwide, of which 2,000 in Sweden. GE Healthcare has a broad range of products and services within medical imaging, medical diagnostics, patient monitoring systems, drug discovery, and biopharmaceutical manufacturing technologies. In 2006, GE Healthcare AB acquired the Swedish biotech instrument company Biacore AB, a global supplier of systems for protein interaction analysis, an area of increasing importance for scientists in the academic, pharmaceutical, biotechnology and diagnostic markets.  
**Centre contribution:**  
Active participation in Program Area 1  
Total FTE contribution: 15% |
| ProNova partner since 2007 | Contact info: Mats Inganäs (mats.inganas@gyros.com), Gyros AB, Uppsala Science Park, SE-751 83 Uppsala, Sweden  
Org. No.: 556672-5429  
**Gyros AB**, founded in 2000, has ca. 60 employees and its headquarters in Uppsala, Sweden. The company business is based on miniaturization and integration of laboratory applications through its proprietary microfluidics platform, the Gyrolab compact disc (CD) microlaboratory.  
**Centre contribution:**  
Active participation in Program Areas 1 and 2  
Total FTE contribution: 20% |
| | Contact info: Niklas Ahlborg (niklas@mabtech.com), Mabtech AB, Box 1233, SE-131 28 Nacka Strand, Sweden  
Org. No.: 556276-8225  
**Mabtech AB** is a privately owned biotech company founded in 1986, with... |
### Appendix

**ProNova partner since 2007**

<table>
<thead>
<tr>
<th>Company</th>
<th>Contact info</th>
<th>Org. No.:</th>
<th>Centre contribution:</th>
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| Mabtech          | around 40 employees. Mabtech has become a world leader in immunoassays such as ELISpot, Fluorospot and other cellular assays to be used in research on infections, cancer, allergy, autoimmune disease and vaccine development. The head office and research laboratory are located in Stockholm, with local offices in Australia, France, Germany and USA. | N/A                | Active participation in Program Areas 1 and 2  
Total FTE contribution: 20% |
| **ProNova partner since 2009** | **Contact info:** Torben V. Borchert (tvb@novozymes.com), Novozymes A/S, Brudelysvej 26 (1U1.23), 2880 Bagsvaerd, Denmark  
**Org. No.:** N/A  
**Novozymes A/S.** Novozymes, with its 5000 employees, is a world-leading biotechnology company with state-of-the-art expertise in microbiology, biotechnology and gene technology used to develop or refine enzymes and microorganisms that meet the customers' needs and to produce them safely in large quantities.  
**Centre contribution:**  
Active participation in Program Area 3  
Total FTE contribution: 30% | **OLINK BIOSCIENCE**  
**Org. No.:** 556663-6998  
**Olink AB** is a privately held company founded in 2004, with ca. 20 employees, and which develops innovative diagnostic tools and technologies to the life science research and diagnostics community. The company is based at Uppsala Science Park in Sweden.  
**Centre contribution:**  
Active participation in Program Area 1  
Total FTE contribution: 10% | **Phadia**  
**Org. No.:** 556041-3204  
**ThermoFisher Scientific (Phadia AB),** with its headquarters in Uppsala, Sweden has ca. 1500 employees whereof 450 in the headquarter and has been the world leader in its field for more than 25 years in developing, manufacturing and marketing complete blood test systems to support the clinical diagnosis and monitoring of allergy, asthma and autoimmune diseases. ThermoFisher Scientific (Phadia AB) has been an active Partner in the ProNova centre for five years.  
**Centre contribution:**  
Active participation in Program Areas 1, 2 and 3  
Total FTE contribution: 60% |

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ProNova VINN Excellence Centre for Protein Technology
Appendix 1 to Main Agreement: Operational Plan, Stage 3 - Signing version

ProNova partner since 2011

Contact info: Sarah Fredriksson (sarah.fredriksson@genovis.com), Genovis AB, Box 790, SE-22007, Lund, Sweden
Org. No.: 556574-5345
Genovis AB, is based in Lund, Sweden and has ca. 10 employees. The main business field of interest is the development of nanostructures for applications within preclinical research.
Centre contribution:
Active participation in Program Area 1
Total FTE contribution: 10%

ProNova partner during 2007-2009 (Stage 1)

Contact info: Patrik Strömberg (Patrik.Stromberg@sobi.com), Swedish Orphan Biovitrum AB, SE-11276 Stockholm, Sweden
Org. No.: 556038-9321
Swedish Orphan Biovitrum AB (SOBI), with headquarters in Stockholm, Sweden, is a biopharmaceutical company with about 500 employees. The key therapeutic areas of the company are hematological diseases, autoimmune diseases, hereditary metabolic disorders and therapeutic oncology.
Centre contribution:
Active participation in Program Area 2
Total FTE contribution: 15%

The annual industrial partner membership fee for Year 6 will remain unchanged at 20 kSEK, but the Centre plans to increase the membership fees for Year 7 and Year 8.

To become Active Partner of a Centre research project, the industrial partner needs to devote own resources to the projects, corresponding to at least 0.1 FTE (1 industrial FTE = 1,472 kSEK).

3.2 Academic and other Research Performing Partners

KTH Royal Institute of Technology. The Royal Institute of Technology (Kungliga Tekniska Högskolan), is responsible for one-third of Sweden’s capacity for engineering studies and technical research at post-secondary level. KTH is a public university, mainly funded by government grants. It was founded in 1827 and is the largest of Sweden’s universities of technology, and has over 15,000 undergraduate students, more than 1,400 active postgraduate students and a staff of 4,300 people. KTH has appointed a Vice President for Research, Prof. Björn Birgisson, who is also responsible for various issues related to the different centra at KTH.

KTH School of Biotechnology. The KTH School of Biotechnology, with its Dean Prof. Stefan Ståhl, is the host for the ProNova Centre. Eleven project leaders at the School of Biotechnology, representing different complementing competences within protein technology (listed below), are involved in the Stage 3 of the ProNova VINN Excellence Centre:

- Prof. Mathias Uhlén (proteomics, automation and bioinformatics)
- Prof. Sophia Hober (protein expression and engineering)
• Prof. Per-Åke Nygren (protein engineering and combinatorial methods)
• Prof. Stefan Ståhl (medical imaging and protein expression)
• Prof. Helene Andersson Svahn (microfluidics and nanobiotechnology)
• Prof. Peter Nilsson (microarrays and proteomics)
• Prof. Amelie Eriksson Karlström (protein chemistry and peptide synthesis)
• Assoc. Prof. Afshin Ahmadian (microarrays and genetic analysis)
• Assoc. Prof. Jochen Schwenk (suspension arrays and plasma analysis)
• Assist. Prof. John Löfblom (protein engineering and combinatorial methods)
• Dr. Johan Rockberg (flow cytometry and immunotechnology)

KTH Holding AB. KTH Holding AB, a fully owned subsidiary to KTH, is involved in the VINN Excellence Centre as a holding company for Intellectual Property Rights (IPR) transferred from individual KTH researchers to the Centre partner KTH (Holding AB), as a result of their work in Centre projects. With the purpose to regulate the conditions in relation to a transfer of IPR from individual researchers (working under the "Teachers exemption rule"; Sw. "Lärarundantaget") to KTH Holding AB, being a signing Party of the main Agreement, an Agreement has been established, signed by KTH Holding AB as one Party and each of the individual KTH researchers as the other Party.
4 Centre Management and Organization

4.1 Centre Leadership and Management Structure

The Centre organization, including decision making and reporting lines, can be seen in the schematic chart below.

![Centre Organization Diagram]

Centre Organization. A schematic chart describing the different core and support units of the ProNova VINN Excellence Centre for Protein Technology and their reporting lines.

Briefly, the organization is based on the General Meeting as the superior assembly to decide on fundamental Centre issues, incl. the election of a Centre Board. The Centre Board then acts on delegation from the General Meeting to run the Centre. The Centre Board has support from an International Scientific Advisory Board in decisions of scientific matter. A Centre Management Team, running the day-to-day activities of the Centre and reporting to the Centre Board, is composed of the Centre Director, the Vice Centre Director, the Program Director and the Program Area Directors. The Centre Management Team receives administrative support from economists and human resource functions present at the KTH School of Biotechnology. The collaborative academy-industry research activities are arranged in different Program Areas, each containing a number of scientifically related subprojects. A Program Area Director, to whom the different subproject leaders, responsible for the different subprojects, are reporting, manages each Program Area.

The current Centre Director is Prof. Amelie Eriksson Karlström, the Vice Centre Director is Prof. Per-Åke Nygren, and the Program Director is Prof. Mathias Uhlén, all affiliated with
the School of Biotechnology, KTH. A more detailed description of the functional units of the organization is found in the following.

The Centre Board

The Centre Board is composed of members primarily from industry, but also from other organizations. The Centre Board will be formally elected at a General Meeting to be held soon after the official start of Stage 3 of the Centre. The presently suggested members of the future Centre Board are the following:

- **Prof. Björn O. Nilsson (Chairman)**
  President of the Royal Swedish Academy for Engineering Sciences (IVA)

- **Dr. Maris Hartmanis (Vice Chairman)**
  Chief Executive Officer of Medivir AB

- **Dr. Cristina Glad**
  Executive Vice President of BioInvent International AB

- **Prof. Maria Anvret**
  Senior Advisor, Research and External Relations at the University of Gothenburg, The Sahlgrenska Academy

- **Dr. Åke Danielsson**
  Staff Scientist at GE Healthcare Bio-Sciences AB

- **Lisa Ericsson**
  Chief Executive Officer of KTH Holding AB

- **Prof. Björn Birgisson,**
  Vice President for Research, KTH

- **Dr. Marianne Hansson**
  Chief Executive Officer of Atlas Antibodies AB

- **Dr. Hugh Salter**
  Head of AstraZeneca Translation Science Center

The KTH representative (appointed by the President of KTH), Prof. Björn Birgisson, is a new member of the Centre Board for Stage 3. Dr. Marianne Hansson and Dr. Hugh Salter are also proposed as new members of the Centre Board for Stage 3, whereas the other suggested Board members were members of the Centre Board already during Stage 2.

An observatory role in the Centre Board is held by:

- **Margareta Danielsson**
  VINNOVA

To each Board meeting the VINNOVA observer will be invited. The whole or parts of the Centre Management Team will be called in to give an update of the activities and results of the Centre.

In accordance with section 5.2.7 in the Main Agreement, the Board has established written rules for their work:
Rules of Procedure of the Centre Board:

- The Board should consist of at least five members
- Board meetings should be held three to four times per calendar year
- A convening notice should be sent by e-mail to the members of the Board at least seven days prior to the Board meeting
- Minutes will be taken at each meeting and archived
- The Board has a quorum of 50% of its members
- The University’s President may appoint a member of the Board
- Election of Board members shall be done at a General Meeting with all parties present (representative, deputy or proxy)
- Deputies (if appointed) are allowed to be present at Board meetings

In accordance with section 5.2.8 in the Main Agreement, the Board has established written instructions indicating the division of labor between the Centre Board and the Centre Director as well as other entities:

The Board is responsible for:

- Approving the budgets
- Reviewing the outcome of the finances after each Stage
- Project management, including approval of new projects, review of the financial and scientific status of ongoing projects, and premature termination of ongoing projects
- Strategic decisions, concerning e.g. research directions, recruitment and alliances
- Information to Parties concerning operations and annual budgets
- Agreement issues, e.g. related to changes in Party composition, FTE involvement in projects and IPR issues

The Centre Director is responsible for implementing the Centre Board decisions in the Centre activities. The specific duties of the Centre Director are the following:

- To be the official contact person of the Centre towards the industrial partners
- To have regular meetings with the Program Area Directors to jointly oversee the progress of the different projects
- To work with the human resources and economy staff at KTH for the administration of the Centre
- To be responsible for that the progress of the Centre (scientific and financial) is reported at each Board meeting
- To gather information from the Program Area Directors and the industrial partners to be able to report to VINNOVA about the finances and activities of the Centre when required
- To communicate information about the Centre activities to all parties involved through regular newsletters and the Centre web page
• To arrange General Meetings and an Annual Centre Day for the academic and industrial partners
• To manage the project generation process, including arranging meetings, collecting ideas and feedback from both the project leaders and the industrial partners, and presenting a research program proposal to the Centre Board

The duties of the Vice Centre Director are the following:

• To be responsible for the Centre Agreement process before entering a new stage as well as legal issues during an ongoing stage
• To coordinate IPR-related issues in the Centre
• To assist the Centre Director in the day-to-day activities

Program Director
The duties of the Program Director are the following:

• To give strategic input on the Centre research program
• To be the contact person of the Centre towards the International Scientific Advisory Board
• To be responsible for contact and collaborations between the Centre and the Human Protein Atlas (HPA) program
• To be responsible for contact and interactions between the Centre and the Science for Life Laboratory

Centre Management Team
The Centre Management Team is composed of the Centre Director, the Vice Centre Director, the Program Director and the three Program Area Directors. The role of the Centre Management Team is to jointly deal with different aspects of the daily operations and project management. The Centre Management Team has regular meetings to discuss the progress and operations of the Centre.

Project Leaders and Program Area Directors
The Centre Project Leaders are the research leaders actively involved in the Centre research projects. The Centre research is organized in Program Areas, each headed by a Program Area Director, and encompassing two or more related subprojects, which are headed by Project Leaders.

The duties of the Program Area Directors are the following:

• To have regular contacts with the Project leaders in a Program Area.
• To arrange regular Program Area meetings with both academic personnel and the involved industrial partners
• To regularly report the finances and the scientific progress of the subprojects of the Program Area to the Centre Director and also, when requested, to the Centre Board.
• To present the status of the Program Area at Partner Meetings and the Annual Centre Day.
• To have regular contacts with the industrial partners involved in a Program Area.
• To monitor the progress of the research projects in the Program Area and to keep record of potentially patentable inventions.

The duties of the **Project Leaders** are the following:

• To prepare a Project Description form including goals, work packages, and milestones of their project
• To have regular contact with the involved industrial partners and coordinate the activities of the project
• To report the financial and scientific status of the project to the Program Area Director and the Centre Director when required
• To present the status of the project at the Program Area Meetings, the Partner Meetings and the Annual Centre Day

**The International Scientific Advisory Board (ISAB)**

The ISAB is composed of international experts in the fields of protein analysis, proteomics and molecular biotechnology. The current members of the ISAB are the following:

- **Prof. Dolores Cahill**  
  University College of Dublin, Ireland
- **Dr. Thomas Joos**  
  Natural and Medical Sciences Institute, University of Tubingen, Germany
- **Prof. Peter Roepstorff**  
  University of Southern Denmark, Denmark
- **Dr. John McCafferty**  
  University of Cambridge, UK
- **Dr. Fridtjof Lund-Johansen**  
  University of Oslo, Norway
- **Dr. Mike Taussig**  
  The Babraham Institute, Cambridge, UK

In Stage 3, the ISAB will provide the Centre Board with external input into various issues of the projects. The first International Scientific Advisory Board meeting is planned to be held in conjunction with the Annual Centre Day in the fall, 2012, to discuss and give advice on the newly launched Stage 3 research program. The second ISAB evaluation will be performed during the following year, with the main purpose of reviewing the research program to give input to the Centre Board in their decision regarding extension of the project for a third year. Prior to the meetings, the ISAB will be provided with reports of the ongoing Centre projects, and during the meeting, the ISAB will have the opportunity to discuss the projects in-depth.
with the respective project leaders and the Centre Management Team. Comments on the scientific aspects of the projects as well as strategic advice and suggestions on future directions will be given to the Centre Board by the ISAB in a written report after the meeting. To avoid potential conflict of interest, the ISAB members will be asked to declare any collaboration or co-publication with the Centre researchers in connection with preparation of the ISAB reports. The Centre Management is open to invite other internationally recognized scientists with complementary expertise to the ISAB, as the research program evolves during Stage 3.

### 4.2 Forms of Collaboration within the Centre

The main form of collaboration between Centre parties will be through the formation of Program Area groups and Project groups. A Project group consists of one or several academic groups (a Centre project leader with associated research students and technicians) and representatives from those industrial partners who have chosen to be actively involved in the Project. A Program Area group consists of all the Project groups belonging to the same Program Area. Such collaborations will be performed according to a jointly developed Project description for the project in question. Thus, related Projects together form a Program Area, which will have meetings at which the results are presented and discussed in a larger group of people. Typically, the collaborations also include the visiting and use of each other’s facilities and spending shorter or longer periods in the different environments.

### 4.3 Plan for Equality of Opportunity

Three out of the eleven project leaders of the Centre, including the Centre Director, are women. The Centre is a good opportunity for them to be role models for younger female researchers.

During Stage 2, as many as eleven out of thirteen PhD students were female, indicating the Centre may need to increase the efforts to recruit more male PhD students in Stage 3 to gain an equal gender distribution.

As the biotechnology field historically has attracted both genders relatively equally, we foresee that the recruitment base for future appointments within the Centre (new research students, postdocs) will provide a beneficial situation to reach an equal opportunity goal.

### 4.4 The Centre in the University Organization. Interaction with the University Infrastructure

The Centre is organized as a separate financial unit at the KTH School of Biotechnology, to facilitate transparency regarding economy, steering, annual reports etc.

Within the KTH organization, the Centre operates in close contact with the Vice President for Research, Prof. Björn Birgisson, who has been appointed by the President of KTH for this special assignment.

The KTH unit KTH Holding AB is engaged in the Centre as the body handling the KTH researcher’s rights to inventions as a result of the Centre research.
4.5 Centre Communication Strategy and Plan

4.5.1 Communication strategies

The communication strategy of the Centre is to be as active as possible in spreading information about the activities and results of the Centre, both internally and externally, without disclosing sensitive information that may be the subject of future IP protection. Regular internal communication is important to keep all parties informed about the Centre activities and promote the industrial engagement in the projects. External communication is important to attract new industrial partners, and recruit top-class scientists to all levels of positions in the Centre (e.g. PhD students, postdocs, senior researchers), and may also be important to gain more funding to the Centre research. In the Centre budget, a total of 500 kSEK/year has been allocated for costs related to communication and learning activities.

4.5.2 Internal communication - activities and resources

**Target groups**
- Industrial and academic Partners of the Centre as well as VINNOVA

**Objectives**
- To spread information of the activities and results from the Centre research activities, incl.
  - The present research program
  - New project proposals
  - Project-related information (results, protocols, reports)
  - Centre-related publications from the Centre partners
  - Position opportunities
- To spread information of the meeting activities of the Centre, incl.
  - Invitations to Centre meetings (i.e. Partner Meetings, Annual Centre Days, etc)
  - Presentation handouts and protocols from the Centre meetings

**Goal**
- To satisfy the expectations and needs from the Centre Partners related to information access.

**Strategy**
- An internally accessible (password-protected) Centre webpage for downloading newsletters, protocols, scientific updates, project progress reports, meeting handouts etc.
- Distribution of Newsletters from the Centre (pdf-documents sent by E-mail)
- Program Area Meetings
- Partner Meetings
- Annual Centre Day

4.5.3 External communication - activities and resources

**Target groups**
- National and international scientists
- The public/society in general
- Partner customers and their financial contacts
- Media (newspapers, TV/radio, scientific journals)

**Objective**
- To inform broadly about Centre activities and results
- To attract additional resources to the Centre
Goal

• To fulfill an important information spreading duty
• To create an awareness of the Centre
• To build a network
• To increase the possibility of constructive contacts with external parties (ideas, criticism, collaborations, licensing)
• To increase the resources for Centre activities
• To engage in EU projects (optional)
• To facilitate recruitment of suitable students and researchers

Strategy

• Publicly accessible web page (not password-protected) for information to the society about:
  - Centre objectives
  - Centre Partners (academic and industrial)
  - Research Program (without disclosing patentable project ideas and results)
  - Publications from the Centre
  - Position opportunities
• Oral and poster presentations of Centre projects and results at national and international meetings, workshops and conferences.

4.6 Learning activities for Centre progress incl. resources

In the Centre budget, a total of 500 kSEK/year has been allocated for costs related to communication and learning activities. The learning activities will include:

• Meetings with the International Scientific Advisory Board (ISAB). These meetings involve presentations of the ongoing projects followed by discussions with the members of the ISAB to collect their input and possible criticism of the projects and the Centre research strategy.

• The Annual Centre Day. An Annual ProNova Centre Day is arranged each year with focus on the results of the Centre as well as to promote center and social networking. The meeting is subject to "invitation only" and each industrial partner can send up to five representatives to the whole day (or part of the day).

• Participation of PhD students in courses. The PhD students of the Centre participate in courses to both deepen and broaden their knowledge in matters of relevance for the Centre research activities.

• Participation of project leaders in courses. The Centre management is positive to the participation of project leaders in activities leading to better project management and understanding of the industrial working conditions.

• Research student seminars. In addition to Centre meetings (project meetings, Annual Centre Day), several opportunities exist for all research students of the Centre to meet and present and discuss results and experiences with other research students at various seminars.

• Participation in scientific meetings and conferences. Members of the Centre are encouraged to participate in national and international workshops and conferences to collect new input and to spread the results of the Centre projects (after securing any IPR possibilities). To promote the participation in international meetings, ProNova travel grants
are awarded up to a gross sum of 250 kSEK/year are awarded to ProNova younger academic personnel who apply (subject of assessment of relevance).

• Participation in activities organized by VINNOVA. Members of the Centre participate in activities organized/initiated by VINNOVA, such as the Annual VINNOVA Centre Days organized by VINNOVA for the Centre Directors.

• Visits to international research groups. PhD students and other members of the Centre are encouraged to make short-term visits to external leading international research groups, to learn new techniques that are relevant to the Centre activities. The Centre will reserve financial resources to support such visits, which can be applied for to the Centre Director.
5 Research Program

5.1 Centre Research Profile (SWOT analysis)

The profile of the Centre research is "Protein technology", with an emphasis on high throughput (affinity) biotechnology, involving development of new methodologies, technologies, systems and principles enabling target molecule detection and quantification in various formats, including development, modification and use of reagents capable of selective interaction with given target molecules. A primary source of such reagents originate from the close interaction between the Centre and The Swedish Human Protein Atlas (HPA) project (www.proteinatlas.org), which generates numerous highly validated polyclonal rabbit antibody reagents towards human target proteins. These antibodies are provided to the Centre for use in the Centre research projects by contributions from KTH and the partner Atlas Antibodies AB. The unique opportunity that the Centre has in terms of being in the international forefront of multiplex analyses of human proteins (via the HPA reagent bank) is a driver for value- and knowledge-creating projects of interest for the partner companies. In addition, projects devoted to the development of new technologies for protein analysis and protein library production and screening/selection applications are central to the research program.

The research program for Stage 3 spans three Program Areas: (1) Affinity Tools and Protein Engineering, (2) Array Technologies and (3) Microfluidics, with 10 projects in total. Below is shown a Strengths/Weaknesses/Opportunities/Threats (SWOT) analysis of the Centre research profile and associated operations:

<table>
<thead>
<tr>
<th>STRENGTHS</th>
<th>WEAKNESSES</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Expertise and internationally engaged project leaders</td>
<td>• Too broad research program</td>
</tr>
<tr>
<td>• Motivated students/staff</td>
<td>• - lack of focus/critical mass</td>
</tr>
<tr>
<td>• A large and engaged industry network</td>
<td>• Project leaders heavily committed also in other projects</td>
</tr>
<tr>
<td>• Board members with vast experience from Swedish life science industry and protein technology</td>
<td></td>
</tr>
<tr>
<td>• ISAB members with internationally recognized expertise in proteomics and protein technology</td>
<td></td>
</tr>
<tr>
<td>• A broad research program</td>
<td></td>
</tr>
<tr>
<td>• Unique antibody/antigen resources</td>
<td></td>
</tr>
<tr>
<td>• Working Main Agreement (IPR) in place</td>
<td></td>
</tr>
<tr>
<td>• Routines are up and running</td>
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<table>
<thead>
<tr>
<th>OPPORTUNITIES</th>
<th>THREATS</th>
</tr>
</thead>
<tbody>
<tr>
<td>• &quot;First-to-discover&quot; biomarkers/autoantigens</td>
<td>• Loss of active industrial engagement</td>
</tr>
<tr>
<td>• Productive cross-talks between partners</td>
<td>• Competition from outside the Centre</td>
</tr>
<tr>
<td>• Innovative projects leading to new IPR production</td>
<td>• Administrative fatigue (reporting etc.)</td>
</tr>
<tr>
<td></td>
<td>• Freedom-to-operate-problems for industrial parties exploiting Centre innovations</td>
</tr>
<tr>
<td></td>
<td>• Scientific challenges (proposed projects/principles don`t work)</td>
</tr>
</tbody>
</table>
5.2 In a 5-years Perspective (years 6-10)

Looking forward, the ProNova Centre is presently starting its Stage 3 (Q2 2012 - Q1 2015), corresponding to operational years 6-8, to be followed by a Stage 4 (Q2 2015 - Q1 2017), corresponding to the last two years of the 10-year Centre period with support from VINNOVA. In these next five years the research of the Centre will have a continued emphasis on needs-driven research, but will gradually shift its focus from early research and technology development towards application, implementation and technology transfer of the methods and platforms developed and discoveries made in the Centre.

One of the criteria for upcoming post-operation evaluation of success or not for the ProNova Centre will be whether or not the Centre has provided results that already have or will be possible for ProNova’s partners to implement into products or services of public or industrial interest. This fact should be reflected in the long-term strategy concerning prioritization between candidate projects to be addressed by the Centre (see figure below).

**ProNova research program development options.** Schematic illustration showing options for the ProNova Centre concerning the balance in the research program in Stages 3 and 4 concerning the number of projects and program areas and their different potentials to generate results which are implementable in a near future by the Centre Partners (closeness to applicability).

For the Stage 3, some Stage 2 subprojects have been terminated and the Stage 2 subprojects to be continued have been re-evaluated for industrial interest and potential of implementation. Further, new projects will be started, and at this stage of the Centre period (i.e. approximately mid-term), some such candidate projects are still of relatively low "implementation maturity", and thus instead bringing new and innovative ideas of high future potential into the research program. One option for the continued research program development (towards Stage 4) will be to consolidate within some Program Areas, involving shifting the focus from support of a relatively large number of smaller topics, to an further increased support of a few topics showing promising implementation opportunities (Route A in the figure). In parallel, other Program Areas may still be worthwhile to continue via support to a larger number of less...
commercially implementation-mature subprojects, however showing great future potential by other measures, such as providing innovative methodologies or information of value for in-house projects run by the ProNova partners (Route B in the figure). The balance between these two options, or other opportunities, will be subject of future ProNova Centre Board considerations. One important aspect here is that the ProNova Centre should not be directly involved in product development operations.

The present activities of the ProNova Centre are to a significant extent related to the use of antibody reagents derived from the Human Protein Atlas (HPA) project (www.proteinatlas.org), via contributions from both Atlas Antibodies AB and KTH. At the end of Stage 2, no less than 18,850 antibodies (and two aliquots of each) are available for ProNova projects. Even if this collection already is huge, it is expected to continue to grow substantially during the coming years as the HPA project moves towards completion. Approaching a situation with a complete collection of antibodies towards the human proteome constituents may influence the future scientific questions addressed by the projects in the research program. As auxiliary platform technologies for high throughput multiplexed biological analyses develop, e.g. microarrays, mass spectrometry, microdroplet technologies, and other novel principles addressed in ongoing ProNova projects, the content with which these platforms are supposed to be filled will presumably become a bottleneck for most groups or consortia. Here, the ProNova antibody collection will provide the Centre with a unique reagent bank that potentially could be used in combination with new groundbreaking platforms to ask pan-proteome questions.

During Stage 2, parts of the Centre research staff moved to the Science for Life Laboratory (SciLifeLab) in Stockholm, which is a new research centre dedicated to high-throughput molecular bioscience and translational medicine. During Stages 3 and 4, the Centre plans to further strengthen the links and increase its interactions with SciLifeLab, which is expected to create beneficial effects for both parties. For the ProNova Centre, the close connection of the Centre to SciLifeLab will bring access to the new infrastructure of SciLifeLab, including state-of-the-art instrumentation for DNA sequencing, mass spectrometry and confocal microscopy, and it will provide new opportunities for research collaborations. For SciLifeLab, the connection to the ProNova Centre will give access to the network of Swedish life science industries and bring an increased focus on scientific questions of relevance for the industry, which is expected to leverage the investments made in the new research centre.

5.3 Stage 3 (Year 6-8)

5.3.1 Research Program

The research program of Stage 3 is organized in three different Program Areas, each headed by an assigned Program Area Director and involving a number of related projects. The industrial relevance of the program has been secured through the Stage 3 planning phase and is manifested through active participation in at least one Program Area and project by all twelve Stage 3 industrial partners. In total, the research program involves eleven project leaders from KTH and twelve industrial partners. As advised by VINNOVA, some of the Centre resources are left open for the start of new projects within Stage 3, to provide some flexibility into the Centre activities.

An overview of the Program Areas and the different projects is given below.
Program Area 1: Affinity Tools and Protein Engineering

Program Area Director: John Löfblom

This program area is focused on the development of new molecular reagents for use in protein detection and involves both protein engineering efforts to generate and improve the properties of affinity reagents as well as the development of display technologies for selection of new variants from protein libraries.

Projects:

1A: Development of an *E. coli* display system
Project leader: John Löfblom
Co-project leader: Stefan Ståhl
Industrial partner: Affibody AB

1B: Exploitation of immunoglobulin-binding domains for antibody labeling
Project leader: Sophia Hober
Industrial partners: BioInvent International AB, Gyros AB, Mabtech AB and Olink AB

1C: Antibody labeling for preclinical *in vivo* imaging applications
Project leader: Amelie Eriksson Karlström
Industrial partners: BioInvent International AB and Genovis AB

1D: Detection systems based on split-protein complementation
Project leader: Per-Åke Nygren
Industrial partners: Affibody AB, GE Healthcare Bio-Sciences AB and ThermoFisher Scientific (Phadia AB)

Program Area 2: Array Technologies

Program Area Director: Peter Nilsson

This program area is focused on technology development for the characterization, purification and use of antibodies for protein profiling and biomarker discovery. Different array technologies (planar and suspension bead) are used as enabling tools in the projects.

Projects:

2A: Antibody characterization and purification
Project leader: Johan Rockberg
Co-project leader: Mathias Uhlén
Industrial partners: Affibody AB, Atlas Antibodies AB, Gyros AB and Swedish Orphan Biovitrum AB.

2B: Antigen microarrays for profiling autoimmunity repertoires
Project leader: Peter Nilsson
Industrial partners: Affibody AB and ThermoFisher Scientific (Phadia AB)

2C: Advancing antibody bead arrays for biomarker discovery
Project leader: Jochen Schwenk
Industrial partners: Affibody AB, AstraZeneca AB, Atlas Antibodies AB, Gyros AB and Mabtech AB

2D: Immunosequencing (iSeq) for highly multiplex protein analysis  
Project leader: Afshin Ahmadian  
Industrial partner: Atlas Antibodies AB

Program Area 3: Microfluidics

Program Area Director: Helene Andersson Svahn

This program area is focused on microfluidic technologies for protein analysis. One of the projects explores the use of droplet microfluidics for high-throughput enzyme screening and in the other project diagnostic assays based on lateral flow microarrays are developed.

Projects:

3A: Evolution and screening of enzymes for industrial conditions  
Project leader: Helene Andersson Svahn  
Industrial partner: Novozymes A/S

3B: Lateral flow microarray assays  
Project leader: Helene Andersson Svahn  
Industrial partner: ThermoFisher Scientific (Phadia AB)

5.3.2 Specific Projects

See Section 10 for detailed Project Descriptions.

5.4 Gender perspective in the research programme

The research program has no bias towards scientific questions related to a particular gender (e.g. diseases like breast cancer and prostate cancer are considered to be of equal importance). The Centre Board and Management will keep a continuous awareness of this important issue. The fact that both men and women are well represented in the Centre, will provide a good basis for non-gender biased Centre operations.
6 Plan for evaluation in relation to the general and specific Centre goals

Part of the financial resources will be dedicated to evaluation of the Centre results and progress.

6.1 Self-evaluation including indicators

The Centre self-evaluation is a critical process to provide an effective and well-functioning organization, with a high level of enthusiasm and inspirational spirit. The Centre will strive to operate in a climate that is open to dialogues about these issues and to provide channels for constructive criticism and suggestions for improvements if needed.

The progress of the Centre (as outlined below) will continuously be monitored and summarized to provide a basis for internal discussions as well as for reporting to VINNOVA when requested:

- number of publications (incl. co-publications between academia and industry)
- number of theses (PhD/Licentiate/Master)
- number of poster contributions
- number of invitations to meetings (oral presentation, keynote addresses)
- number of patent applications and granted patents
- level of multiple-project engagement by industrial partners
- level of industrial utilization of Centre results
- mobility of Centre staff between the academic site and the industry
- level of new (Centre-induced) collaborations between industrial Centre Parties
- level of employee satisfaction with the work climate (typically assessed in surveys and/or interviews)

6.2 Systematic measurement of results

The results generated (i.e. as described above: publications, theses, meeting presentations, patent applications, etc.) will be continuously monitored for each project, each Program Area, and summarized for the entire Centre. For each Board Meeting (approximately four times per year), the Centre Director will gather this information and present it to the Board to give an overview of the progress of the Centre projects.

6.3 Systematic evaluation of technical and scientific outcome

In addition to the self-evaluation, the technical and scientific outcome of the Centre will be evaluated by meetings and other interactions with the International Scientific Advisory Board, incl. a mid-term review process of the projects after approximately two years. On these occasions, status reports of the ongoing projects will be presented to the ISAB both orally and in writing, and the ISAB will be asked to review the projects and leave a written statement with their thoughts on the scientific relevance/background, project work package content, performance and advice for the continued work.

6.4 Evaluation of the industry-university collaboration

The degree of industrial engagement is critical for the success of the Centre and different relevant indicators (i.e. as described above: the level of industrial engagement in the different projects, industry/academia co-publications, industrial utilization/implementation of Centre results, and the mobility of Centre staff between academia and industry) will therefore be
continuously monitored and recorded. This information will be presented to the Board by the Centre Director at the Board Meetings.

6.5 Preparation for the VINNOVA evaluation

The Centre Management will prepare all reports and documents requested by VINNOVA in connection to future evaluations of the Centre.
7 Actions taken on recommendations given in the Evaluation Report of Stage 2

7.1 Recommendations to the Centre and 7.2 Actions taken

The following recommendations were received from the international expert panels after the VINNOVA evaluation of Stage 2 of the ProNova Centre, with meetings on November 14-15, 2011, and on March 7, 2012:

**Long-term Vision, Mission and Strategy**

**Recommendation #1:**
That the Centre increases its focus on long-term strategies for enabling transfer of fundamental research results, including methodologies, tools and platforms, to innovations that are taken up by industry.

Centre’s response:
The transfer of Centre research results to innovations taken up by industry is, and will continue to be, of central importance for the Centre. The current strategy used by the Centre to promote such transfer involves several modules, incl. items related to formal matters or working principles:

- A research program (see section 5.3.1 Research Program and sections 9. Program Area Descriptions and 10. Project Descriptions) developed jointly by industry and academia, to ensure an industrial "end-user" interest in the Centre activities and potential results.
- A carefully designed Centre Main Agreement, signed by all Parties in the Centre, ensuring formal protection of the potential commercial values of the Centre results via strict "file-before-publish" principles concerning potentially exploitable Centre results and well defined regulations concerning ownership of new IPR.
- A policy document/manual denoted "IPR Guidelines", serving as a practical guide for how to initiate and proceed with potentially valuable results emerging from the Centre activities.
- Regular meetings to communicate Centre results (Annual Centre Day, Program Area meetings, Site visits)

The Centre is convinced that the actions already taken as described above will result in the generation of exploitable results at an increased frequency as the projects and methodology platforms in the Centre mature. However, to be prepared for and facilitating the handling of new IPR cases during Stage 3, the Centre will encourage the Program Directors to, with the industry representatives, specifically identify and keep an updated list of potentially patentable inventions (see the part Project Leaders and Program Area Directors in section 4.1 Centre Leadership and Management Structure).

**Scientific Quality and Productivity**

**Recommendation #2:**
That the Centre accelerates the employment of a wider range of biochemical and biophysical techniques for quantitative characterization of antibodies and other affinity proteins.
particularly interaction and stability analyses including development of relevant expertise in the Centre or via collaborations including with the existing KTH facilities.

Centre’s response:
The Centre is open to include new methods that are relevant for biomolecular characterization and will for example take advantage of the close contact with SciLifeLab Stockholm and make use of the instrumentation available at this site during Stage 3 (see section 5.2 In a 5-years Perspective (years 6-10) for a description of the planned increased interaction with SciLifeLab).

Recommendation #3:
That the Centre submits as part of the operational plan to the Board and VINNOVA, explicit strategies and actions for increasing the international profile and collaborations of the Centre and that the full, relevant, scientific publication record of the PIs and senior scientists be listed. The evaluation guidelines suggest a method of noting publications that are not funded by the Centre.

Centre’s response:
The Centre Board and Management have so far not prioritized the engagement of the Centre in national or international collaborations. However, if a situation appears that could motivate an agreement-regulated collaboration with an external centre or consortium, the Centre Board is willing to re-consider this assessment if justified by the situation (see section 2.5.2 International strategies).
To better visualize the full international and national engagement of the PIs, full scientific publication records of the PIs and senior scientists will be listed in future reports, where projects not funded by the Centre will be clearly marked, as proposed by the Panel.

Centre Partners

Recommendation #4:
That the annual partner cash contribution be significantly increased in Stage 3, particularly for large companies.

Recommendation #5:
That the cash and in kind contributions of new companies, particularly foreign companies, joining in Stage 3 should reflect the earlier, substantial investments (in cash and in kind) by other partners, the University and VINNOVA during Stages 1 and 2.

Recommendation #6:
That the annual partner in kind contribution associated with personnel time be increased in Stage 3.

Centre’s response:
The Centre Board considers the present strategy, which involves a relatively low annual membership fee in combination with an active engagement in the Centre projects via own work by the industrial partners, to be a success factor for the Centre. Nevertheless, in response
to the recommendations by VINNOVA, the Centre Board and Management will work on the implementation of a new strategy, with increased cash contributions from the industrial partners during Years 7 and 8 of Stage 3 (see section 3.1 Industrial Partners).

The cash contribution for Year 6 will not be changed, and any companies not agreeing with the new conditions will have the option to leave the Centre before the start of Year 7 or Year 8, respectively, giving three months’ notice of termination, without any financial consequences (see Main Agreement; Section 16 – Premature Resignation).

Regarding new industrial partners, the only new company joining the Centre with the start of Stage 3, is Swedish Orphan Biovitrum (SOBI). Since Biovitrum/SOBI was a member of the Centre during Stage 1 (2007-2009), and the company does not get the rights to any results produced in the Centre during Stage 2, the Board does not consider it reasonable to demand an extra entry fee from SOBI for joining the Centre in Stage 3.

It is anticipated that as the research program matures, there will be more focus on testing and evaluation of the new technologies developed in the Centre, which will lead to increased activity in the companies. The present budget for Stage 3 should be seen in the light of the criticism put forward by the evaluation panels regarding companies not fulfilling their budgeted contributions during Stage 2, and it thus reflects a minimal commitment level agreed upon by the industrial partners. However, the Centre will have a strong focus on increasing the industrial activities, to make it likely that a higher-than-budgeted level of the in kind contributions associated with personnel time is reached during Stage 3.

Recommendation #7:
That a method of establishing the value of in kind contributions associated with transfer of biological materials (from the Centre to the partners and vice versa) and with analytical services be developed by individuals who are arms-length to all associated with the Centre.

Centre’s response:
During Stage 3, the Centre will put more attention on the valuation of the biological materials (e.g. patient sample cohorts) provided by the Centre parties, to be able to report the true value of the in kind contributions and give a better account of the industrial partners’ commitment. Concerning the value of the HPA antibodies obtained from KTH and Atlas Antibodies AB, the Centre will perform an independent valuation of the antibodies for the financial report of Stage 3. However, the enclosed budget for Stage 3 is based on a preliminary 50% discount of the current list price for the antibody aliquots (see section 8. Financing Plan and Budget for Stage 3).

Organisation and Management of the Centre

Recommendation #8:
That a representative of the senior management of KTH be a member of the Board.

Recommendation #9:
That members of the Board be predominately representative of the set of organizations that have a commitment of cash or in kind to the Centre.

Recommendation #10:
That a representative of the smaller privately held partner companies be a member of the Board.

Centre’s response:
The senior management of KTH has appointed Prof. Björn Birgisson, Vice President for Research, as the KTH representative for Stage 3.

The proposed Centre Board for Stage 3 is expanded to encompass nine members, compared to the seven members during Stage 2, to comply with the recommendations by the evaluation panels without losing the competence provided by the earlier members of the Centre Board. The proposed Centre Board for Stage 3 is composed of four members affiliated with Centre partner companies (two large companies and two SMEs), one member affiliated with KTH Holding AB, one representative of KTH, and three members affiliated with organizations (industry or academy) that are not part of the Centre (see part on The Centre Board in section 4.1 Centre Leadership and Management Structure).

Recommendation #11:
That a new Management Team position be created to focus on building and maintaining relationships with industry with a view to increasing partner cash and in kind contributions and possibly growing the partner complement.

Centre’s response:
It is of high priority for everyone involved in the Centre that the research projects are successful and lead to results that are both of high scientific value and of high industrial relevance. The new Management Team, including the Director, Vice Director, Program Director and Program Area Directors, will during Stage 3 work jointly on strengthening the connections to the industrial partners (see section 4.1 Centre Leadership and Management Structure). As a consequence, the Centre does not see the need for having a specific position in the Management Team dedicated to working with industry relations, and instead wants to include the entire Management Team in the contacts with the partner companies. By including the whole team, the Centre can work on improving the relationships with the industry on different levels: from engaging in research-related discussions with the personnel directly involved in the Centre research projects, to financial and strategic discussions performed on a management level.

Regarding the joining of new partner companies, the Centre is positive to include new parties that complement the existing industrial partners, and will make use of various communication channels to increase the visibility of the Centre and attract new companies (see section 4.5 Centre Communication Strategy and Plan). As evident from the joining of the industrial partner Genovis AB during Stage 2, which was accomplished without difficulties, the Centre already has practices in place to handle the accession of a new partner during a running stage.

The ISAB

Recommendation #12:
That the ISAB be reconstituted with arms-length people.
Recommendation #13:  
That the ISAB meets annually during Stage.

Centre’s response:  
The Centre acknowledges that collaborations with the members of the ISAB leading to co-publication should be avoided or at least be kept to a minimum, but, on the other hand that close connections and exchange of ideas with the ISAB should be promoted. To find a balance in this respect the Centre will investigate any potential conflict of interest in connection with each ISAB assignment (see part on The International Scientific Advisory Board (ISAB) in section 4.1 Centre Leadership and Management Structure).

According to the conditions of “VINNOVA:s Allmänna villkor för VINN Excellence Center”, the Centre is to establish a scientific advisory board with international expertise, for support in scientific questions. However, there is no guidance in this document regarding the meeting frequency of the International Scientific Advisory Board. The Centre has planned for two meetings with ISAB during Stage 3, but may invite the whole or parts of the ISAB to additional meetings to provide input on the research program, if it is considered valuable by the Centre Management and Board.

Training Personnel of High Competence

Recommendation #14:  
That the Centre implements a plan for the recruitment and selection of international students and researchers.

Centre’s response:  
To promote recruitment of international students and researchers, all vacant positions in the ProNova Centre will be announced on both the official KTH website, as well at the ProNova website. In addition, the Centre PIs will make use of their extensive personal international networks to find the best possible candidates for available positions. The Centre encourages and will promote participation in European programs, e.g. Erasmus Mundus and Marie Curie (see section 2.5.2 International strategies).
8 Financing Plan and Budget for Stage 3

Template for calculation of in kind contributions in the Centre budget:

Industry:
- 1 FTE = 1 472 kSEK/year
  
  (800 SEK/hr x 1 840 hr/year)

Academia:
- Senior researcher (Prof./Assoc. Prof.): 1 500 kSEK/year
- Senior researcher (PhD/Postdoc): 960 kSEK/year
- PhD student: 800 kSEK/year
- Administrative personnel: 600 kSEK/year

  (including salary, social costs, indirect costs, consumables, etc.)

In kind contribution of HPA antibodies:
The value is calculated from an estimated annual contribution of 2 x 2 500 antibody aliquots.
The list price (April, 2012) for an antibody aliquot is € 315, which equals approx. 2 800 SEK.
The estimated value of the antibodies is based on a preliminary 50% discount, i.e. 1,400
SEK/aliquot.
The annual contribution, each from KTH and Atlas Antibodies AB, is: 2 500 x 2 800 x 0.50 =
3 500 000 SEK.

Overhead costs:
The budgeted overhead costs in Table 9 Overall Expenditures are calculated as a 51% addition to the salary expenses. The overhead costs include:

  - Rent (14%)
  - University (KTH) overhead (27%)
  - School of Biotechnology overhead (10%)

The budgeted material and running costs are calculated as 10% relative to the salary expenses.
Note that according to a decision by VINNOVA, the allowed indirect costs may not be higher than a 35% addition to other costs (excluding equipment) for the part of the budget covered by VINNOVA funds.

The annual VINNOVA contribution to the budget of 7 MSEK is for each year divided into the following cost items:

- Salaries: 4260 kSEK
- Material and running costs: 425 kSEK
- Communication and learning activities: 500 kSEK
- Overhead costs: 1815 kSEK

The budgeted overhead costs for the VINNOVA contribution thus correspond to an addition of 35% relative to the other costs, as approved by VINNOVA: \((4260 + 425 + 500) \times 0.35 = 1815\) kSEK.

However, the actual overhead costs at KTH correspond to an addition of 51% relative to the salary expenses: \(4260 \times 0.51 = 2173\) kSEK.

The difference, \(2173 - 1815 = 358\) kSEK, is covered by the cash contribution from KTH.

The remaining cash contributions from KTH and the industrial partners are used to cover the other budgeted costs for salaries, material/running costs, and overhead costs, that are shown in Table 9: Overall Expenditures.
Table T8: Overall resources available (cash and in kind)

This table should present the overall resources available (cash as well as in-kind) for center activities, one row for each financial source.

<table>
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<th>Affiliation</th>
<th>Year 6 Budget (kSEK)</th>
<th>Year 6 Outcome (kSEK)</th>
<th>Year 7 Budget (kSEK)</th>
<th>Year 7 Outcome (kSEK)</th>
<th>Year 8 Budget (kSEK)</th>
<th>Year 8 Outcome (kSEK)</th>
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### Table 9: Overall Expenditures

List all expenses for the centre at an aggregated level.

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**Affinity AB:**
- **Internal staff:** 294
- **AstraZeneca AB:**
- **Internal staff:** 147
Table 10: Research Personnel

List all personnel working in the centre. Preferably group them in order to use the information in other parts of the report.

Only indicate personnel over 5 % FTE

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation (financing source)</th>
<th>University degrees</th>
<th>Category title, status / position</th>
<th>Degree of activity in the center</th>
<th>Budget</th>
<th>Outcome</th>
<th>Degree of activity in the center</th>
<th>Budget</th>
<th>Outcome</th>
<th>Degree of activity in the center</th>
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<td>Genovis AB:</td>
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Summary Industry Personnel: 3605

Year 6

Atlas Antibodies AB: 368
BioInvent International AB: 221
GE Healthcare Bio-Sciences AB: 147
Genovis AB: 147
Gyro AB: 294
Mabtech AB: 294
Novozymes A/S: 442
Olink Bioscience AB: 147
Swedish Orphan Biovitrum AB: 221
ThermoFisher Scientific (Phadia AB): 883
Summary Industry Personnel: 3605
Table 11: Project expenditures

Include all contributions that supports the Centre activities

Follow up that resources have been used for learning activities and communication (5% of VINNOVA funding), list of projects and financial size

<table>
<thead>
<tr>
<th>Programme Areas</th>
<th>Year 6 Budget (kSEK)</th>
<th>Year 6 Outcome (kSEK)</th>
<th>Year 7 Budget (kSEK)</th>
<th>Year 7 Outcome (kSEK)</th>
<th>Year 8 Budget (kSEK)</th>
<th>Year 8 Outcome (kSEK)</th>
<th>Summary Stage 3 Outcome (kSEK)</th>
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<td>Management and administration</td>
<td>Cash: 7 740</td>
<td>14 735</td>
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<td>8 865</td>
<td>16 215</td>
<td>17 080</td>
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<td>5 400</td>
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<td>communication, etc.</td>
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<td>Antibodies to be used in the Centre projects</td>
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<td>1. Affinity Tools and Protein Engineering</td>
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<td>4 405</td>
<td>6 720</td>
<td>6 975</td>
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<td></td>
<td>2 240</td>
<td>2 240</td>
<td>4 485</td>
<td>6 720</td>
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<td>2. Array Technologies</td>
<td>2 240</td>
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<td>4 485</td>
<td>6 720</td>
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<td>3. Microfluidics</td>
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</table>

Summary Stage 3: 8 865 kSEK
Table 12: Related Research Grants

List grants granted, applied for and under preparation - project title, total amount applied for, duration of project, funding source, date of application and any comment you might have.

Only indicate grants that are bigger than € 70 000 and explicitly strengthens the center activities without directly financing it.

<table>
<thead>
<tr>
<th>Project Title</th>
<th>Status</th>
<th>Total amount applied for</th>
<th>Duration of project</th>
<th>Funding source</th>
<th>Date of application</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Development of microwell platform</td>
<td>Granted / Applied /</td>
<td>750 kSEK</td>
<td>2009</td>
<td>Industry</td>
<td>2009</td>
<td>Helene Andersson Svahn (yearly grant)</td>
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<td>Faculty support</td>
<td>Granted</td>
<td>1 MSEK</td>
<td>2012</td>
<td>KTH</td>
<td>Helene Andersson Svahn</td>
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<td>Multidisciplinary BIO</td>
<td>Granted</td>
<td>2.86 MSEK</td>
<td>2009-2012</td>
<td>SSF</td>
<td>2008</td>
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<td>Novo Nordisk Foundation Center for Biosustainability</td>
<td>Granted</td>
<td>8 MSEK</td>
<td>2012-2015</td>
<td>NovoNordisk-fonden</td>
<td>2008</td>
<td>Helene Andersson Svahn</td>
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<td>Droplet Microfluidics for enzyme and biomarker screening</td>
<td>Granted</td>
<td>3.95 MSEK</td>
<td>2012-2015</td>
<td>VR</td>
<td>2011</td>
<td>Helene Andersson Svahn</td>
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<td>Next generation sample preparation, high throughput PCR and analytical</td>
<td>Granted</td>
<td>5 MSEK</td>
<td>2012-2016</td>
<td>Formas</td>
<td>2011</td>
<td>Helene Andersson Svahn</td>
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<td>Slaphtylococcal surface display - a powerful protein-engineering platform</td>
<td>Granted</td>
<td>2.7 MSEK</td>
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<td>VR</td>
<td>2009-04-15</td>
<td>Stefan Ståhl, John Löfblom</td>
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<td>A trispecific biotherapeutic agent, targeting the HER2 and HER3 receptors</td>
<td>Granted</td>
<td>1.8 MSEK</td>
<td>2011-2013</td>
<td>Cancerfonden</td>
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<td>Stefan Ståhl</td>
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<td>Development and preclinical validation.</td>
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<tr>
<td>Sel-tagged proteiner som nya biomarkörer vid PET-avbildning</td>
<td>Granted</td>
<td>4 MSEK</td>
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<td>SSF</td>
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<td>A Human Protein Atlas</td>
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<td>KAW</td>
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<td>translational research</td>
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<td>CAGEKID: Cancer genomics of the kidney</td>
<td>Granted</td>
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<td>EU-FP7</td>
<td>Mathias Uhlén, Fredrik Pontén, et al</td>
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<td>PRIMES: Protein Interaction Machines in Oncogenic EGF signaling</td>
<td>Granted</td>
<td>5.918 MSEK</td>
<td>2011-2016</td>
<td>EU-FP7</td>
<td>Mathias Uhlén, Cristina Al-Khalli Szigyarto, et al</td>
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</table>
Table 12: Related Research Grants

List grants granted, applied for and under preparation - project title, total amount applied for, duration of project, funding source, date of application and any comment you might have. Only indicate grants that are bigger than € 70 000 and explicitly strengthens the center activities without directly financing it.

<table>
<thead>
<tr>
<th>Project Title</th>
<th>Status</th>
<th>Total amount applied for</th>
<th>Duration of project</th>
<th>Funding source</th>
<th>Date of application</th>
<th>Comments</th>
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<tr>
<td>Proteome biology of cardiovascular disease</td>
<td>Granted</td>
<td>8.278 MSEK</td>
<td>2010-2014</td>
<td>NIH</td>
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<td>Mathias Uhlén, Jacob Odeberg et al</td>
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<tr>
<td>Droplet microfluidics and functionalized nanowires for detection of circulating tumour cells</td>
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<td>KAW</td>
<td>2011</td>
<td>Amelie Eriksson Karlström, Helene Andersson Svaeh, Afshin Ahmadian and Joakim Lundeberg</td>
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<td>Affibody molecules 2.0</td>
<td>Granted</td>
<td>2 MSEK</td>
<td>2010-2012</td>
<td>VR</td>
<td>2009</td>
<td>Per-Åke Nygren</td>
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<td>IDRE: Multiplex disease marker diagnostics based on the ISET platform</td>
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<td>2009-2014</td>
<td>VINNOVA</td>
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<td>Sophia Hober, Thomas Laurell, et al</td>
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<td>Uppsala-Umeå comprehensive cancer consortium</td>
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<td>2009</td>
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<td>Biobank Profiling</td>
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<td>SciLifeLab</td>
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<td>Jochen Schwenken</td>
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<td>Protein Array Technology</td>
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<td>SciLifeLab</td>
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PROGRAM AREA 1:  
AFFINITY TOOLS AND PROTEIN ENGINEERING

1. Summary

This program area is focused on the development of new molecular reagents for use in protein detection and involves both protein engineering efforts to generate and improve the properties of affinity reagents as well as the development of display technologies for selection of new variants from combinatorial protein libraries.

2. Period

Stage 3: 2012-04-01--2015-03-31

3. Academic personnel involved

Dr. John Löfblom (Program Area Director)  
Prof. Stefan Ståhl  
Prof. Sophia Hober  
Prof. Per-Åke Nygren  
Prof. Amelie Eriksson Karlström  
Dr. Kristina Westerlund  
M. Sc. Filippa Fleetwood  
M. Sc. Sara Kanje  
M. Sc. Anna Perols  
M. Sc. Feifan Yu

4. Industry partners involved (incl. contribution to the Program Area)

Affibody AB (0.1 FTE)  
BioInvent International AB (0.15 FTE)  
Gyros AB (0.1 FTE)  
Mabtech AB (0.1 FTE)  
Olink AB (0.1 FTE)  
Genovis AB (0.1 FTE)  
GE Healthcare Bio-Sciences AB (0.1 FTE)  
ThermoFisher Scientific (Phadia AB) (0.1 FTE)

5. List of projects

1A: Development of an *E. coli* display system  
Project leader: John Löfblom
Co-project leader: Stefan Ståhl

1B: Exploitation of immunoglobulin-binding domains for antibody labeling
Project leader: Sophia Hober

1C: Antibody labeling for preclinical *in vivo* imaging applications
Project leader: Amelie Eriksson Karlström

1D: Detection systems based on split-protein complementation
Project leader: Per-Åke Nygren

### 6. Total Program Area budget (kSEK)

<table>
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<th>Year 4</th>
<th>Year 5</th>
<th>Total Stage 3</th>
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### 7. Other information

- 

### 8. Background IPR/material declaration

**Background Information:**
None

**Background Results:**
None
PROGRAM AREA 2: ARRAY TECHNOLOGIES

1. Summary

This program area is focused on technology development for the characterization, purification and use of antibodies for protein profiling and biomarker discovery. Different array technologies (planar and suspension bead) are used as enabling tools in the projects.

2. Period

Stage 3: 2012-04-01--2015-03-31

3. Academic personnel involved

Prof. Peter Nilsson (Program Area Director)
Prof. Mathias Uhlén
Assoc. Prof. Jochen Shwenk
Assoc. Prof. Afshin Ahmadian
Dr. Johan Rockberg
Dr. Julie Bachmann
Dr. Mun-Gwan Hong
M. Sc. Anna Häggmark
M. Sc. Maja Neiman
M. Sc. Ronald Sjöberg
M. Sc. Sanna Byström
M. Sc. Mahya Dezfouli
M. Sc. Elin Birgersson

4. Industry partners involved (incl. contribution to the Program Area)

Affibody AB (0.1 FTE)
AstraZeneca AB (0.1 FTE)
Atlas Antibodies AB (0.25 FTE)
Gyros AB (0.1 FTE)
Mabtech AB (0.1 FTE)
Swedish Orphan Biovitrum (0.15 FTE)
ThermoFisher Scientific (Phadia AB) (0.2 FTE)

5. List of projects

2A: Antibody characterization and purification
ProNova Stage 3
PROGRAM AREA DESCRIPTION

Project leader: Johan Rockberg
Co-project leader: Mathias Uhlén

2B: Antigen microarrays for profiling autoimmunity repertoires
Project leader: Peter Nilsson

2C: Advancing antibody bead arrays for biomarker discovery
Project leader: Jochen Schwenk

2D: Immunosequencing (iSeq) for highly multiplex protein analysis
Project leader: Afshin Ahmadian

6. Total Program Area budget (kSEK)

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7. Other information

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8. Background IPR/material declaration

Background Information:
None

Background Results:
(1) "Biomarker of renal impairment" (EP11173081.8).
Approval to listing obtained from Atlas Antibodies AB.
1. Summary

This program area is focused on microfluidic technologies for protein analysis. One of the projects explores the use of droplet microfluidics for high-throughput enzyme screening and in the other project diagnostic assays based on lateral flow microarrays are developed.

2. Period

Stage 3: 2012-04-01--2015-03-31

3. Academic personnel involved

Prof. Helene Andersson Svahn (Program Area Director)
Dr. Håkan Jönsson
Dr. Jesper Gantelius
Dr. Thiru Raja
M. Sc. Staffan Sjöström

4. Industry partners involved (incl. contribution to the Program Area)

Novozymes A/S (0.3 FTE)
ThermoFisher Scientific (Phadia AB) (0.3 FTE)

5. List of projects

3A: Evolution and screening of enzymes for industrial conditions
Project leader: Helene Andersson Svahn

3B: Lateral flow microarray assays
Project leader: Helene Andersson Svahn

6. Total Program Area budget (kSEK)

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7. Other information

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8. Background IPR/material declaration

Background Information:
None

Background Results:
None
PROJECT DESCRIPTION

1. Program area and project designation/title

Program Area: 1. Affinity Tools and Protein Engineering
Project: A. Development of an E. coli display system

2. Project duration

Period 1: 2012-04-01--2014-03-31 (Centre Years 6 and 7)
Period 2 (conditional): 2014-04-01--2015-03-31 (Centre Year 8), if a continuation is approved by the Centre Board

3. Project resources:

KTH / ProNova resources:

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<td>Stefan Ståhl</td>
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<td>Filippa Fleetwood</td>
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Template for calculation of in kind contributions:
Industry: 1 FTE = 1 472 kSEK/year (800 SEK/hr x 1840 hr/year)
Academia: Senior researcher (Prof./Assoc. Prof.): 1 500 kSEK/year, Senior researcher (PhD/Postdoc): 960 kSEK/year, PhD student: 800 kSEK/year, Administrative personnel: 600 kSEK/year

Summary of resources:

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- Page 51 of 93 -
4. Project summary, incl. goals

The subproject is focused on development of a new E. coli-based display system for epitope mapping and combinatorial protein engineering. The aim of the project is to construct an E. coli display vector based on an AIDA-I expression system for future library applications. The new technology is aimed to be a complement to the in-house developed staphylococcal display platform for epitope mapping and combinatorial protein engineering. The AIDA-I technology is a promising system for E. coli display and potential advantages include: i) high transformation frequency -> large libraries, ii) convenient single-strain transformation schemes (as compared to the staphylococcal system requiring E. coli as passage strain), iv) high surface expression level, v) high tolerance for large passenger proteins, and vi) straightforward production of soluble proteins aimed for detailed characterization through a simple strain-shift.

The aim is to construct a new surface display vector for E. coli and investigate its potential for library applications.

5. Project work packages

Work package 1: Construction of an expression cassette for library applications.

This work package includes construction of an expression cassette for display of protein and peptide libraries on E. coli, including:

Subcloning of a gene encoding an affinity protein (e.g. an Affibody molecule) in fusion to the AIDA-I gene for surface expression on E. coli. Functional surface expression will be investigated using flow cytometry. Affibody AB will provide relevant affinity proteins.

Subcloning of a gene encoding a reporter protein (e.g. an albumin-binding protein) in between the AIDA-I gene and the Affibody molecule. The reporter protein will function both as a spacer and as a reporter tag for monitoring of the surface expression level and normalization during FACS. Functional surface expression will be monitored using flow cytometry.

Subcloning of a gene encoding a His-tag and an OmpT protease recognition site in between the ABP and the Affibody molecule. The OmpT recognition site will enable secretion of His-tagged affinity proteins in OmpT-positive E. coli strains. The same plasmid can thereby be used for surface anchoring in OmpT negative strains and secretion of soluble affinity protein in OmpT positive strains. This is of significant convenience since other systems typically require subcloning, expression and purification of proteins aimed for characterization. Cleavage efficiency will be investigated using flow cytometry, IMAC, SDS-PAGE and mass spectrometry.

Work package 2: Evaluation of different promoter systems and strains.

Three different inducible promoters (T7 promoter, arabinose promoter and rhamnose promoter) will be investigated in terms of expression regulation, surface expression level and growth bias. A panel of different OmpT positive and OmpT negative strains will be investigated in terms of mainly expression level but also viability during FACS.

In this work package we will optimize subcloning and transformation protocols for construction of large libraries using the new display vector. Affibody AB will provide know-how and protocols. The work package also includes investigation of enrichment efficiency using a model affinity protein library displayed on E. coli and FACS for isolation of binders. Affibody AB will provide affinity proteins for the proof-of-principle library.

6. Milestones and deliverables

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7. Project-specific considerations related to ProNova main agreement

7.1 Relation to Results in earlier ProNova projects (Stages 1 and 2), not yet subject of patent application filing.

The subproject is related to former subproject 2A of Stage 2.

7.2 Relation to protected Results in earlier ProNova projects (Stages 1 and 2).

NA

7.3 Relation to own existing IP (property of either academic staff or involved industrial partners).

-
7.4 Other considerations.
PROJECT DESCRIPTION

1. Program area and project designation/title

Program Area: 1. Affinity Tools and Protein Engineering
Project: B. Exploitation of immunoglobulin-binding domains for antibody labelling

2. Project duration

Period 1: 2012-04-01--2014-03-31 (Centre Years 6 and 7)
Period 2 (conditional): 2014-04-01--2015-03-31 (Centre Year 8), if a continuation is approved by the Centre Board

3. Project resources:

KTH / ProNova resources:

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Industry partner resources:

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Summary of resources:

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4. Project summary, incl. goals

Our successful results from earlier stages of the ProNova project show that site-specific covalent labeling of antibodies using an IgG (Fc)-binding domain containing photoactivable probes is both efficient and specific. In this coming stage of ProNova, we propose to further develop this method by broadening the usefulness of the targeted labeling to include more IgG species and sub groups, as well as IgG fragments. Also, the possibility to attach more than one IgG-binding group (labeling) to one antibody will be explored.

We will mainly focus our efforts to a protein domain denoted C2, a Streptococcal protein G-derived protein natively displaying both Fc and Fab (CH1) binding ability. Here, using protein engineering we will investigate if the Fc binding activity could be destroyed, to result in a strictly Fab binding probe. Mutagenesis has already been made in order to define the most important amino acids for the affinity of the C2 domain to Fc. Furthermore possible amino acid changes able to destroy the Fc affinity have been evaluated. This information together with an earlier developed and optimized method for chemical synthesis of C2 will be used for incorporation of the photoactivable probe BPA. The possibilities of insertion of the BPA-molecule will be defined by exploration of the published structure of the C2-Fab-complex. Thereafter, synthesis of the novel C2-domain with BPA in different positions will take place. The effectiveness of the different positions for covalent linkage will be thoroughly evaluated. Also, if higher affinity than offered by the inherent binding site is needed, a small DNA-library will be designed and created. This will give us the possibility to select protein molecules with higher affinity to increase the effectiveness of the conjugation. This novel molecule will, together with the earlier developed Z-variant, be explored and optimized for different applications, both for single labeling and together as dual labeling/solid phase coupling.

5. Project work packages

Work package 1: Elimination of Fc-binding

In this work package we will focus on the production, purification and characterization of the mutated C2 variants with decreased affinity to Fc. Thereafter a combination of the most promising mutants will be made to achieve a molecule with no affinity to Fc but retained Fab-binding.

WP1.1 Directed mutagenesis to delete the Fc affinity

WP1.2 Production and purification of constructed mutants

WP1.3 Analysis of structure and function of the mutants

Work package 2: SPPS and incorporation of BPA

Next step will be to, by solid phase protein synthesis, introduce the photoactivable probe BPA on different positions in the C2 domain. These will be characterized in order to reveal the most efficient way to covalently link C2 to Fab by photoactivation. This step will benefit from the earlier developed method for synthesis of C2.

WP2.1 Synthesis and purification of the earlier designed mutants with BPA at different positions

WP2.2 Analyses of the crosslinking ability of the produced proteins
Work package 3: Labelling

In order to use the covalently linked molecule for labelling or directed attachment, different functional groups will be incorporated in the domain by protein synthesis. Various positions for these functional groups will be evaluated. The C2 domains will also be labelled with groups that give means for detection.

WP3.1 Synthesis and purification of the earlier designed mutants with molecules that can be used for protein labeling and detection.

WP3.2 Analyses of the produced molecules

Work package 4: Improvement of affinity

In order to increase the affinity of the novel C2 domain to Fab, a small genetic library will be produced. A 3D-structure for the proteins (Fab and C2) in complex is available which allows for selection of positions for the introduction of randomized positions. The library will be used for selection, either with phage display or by using a cell display system based on Staphylococci, of variants with higher affinity to Fab.

WP4.1 Design of a DNA-library based on data from the structurally determined affinity surfaces.

WP4.2 Production and transformation of the library

WP4.3 Selection of binders with increased affinity to Fab

WP4.4 Evaluation of the acquired binders

WP4.5 SPPS of the selected binder with incorporated BPA groups

Work package 5: Validation

The novel variants of the protein domains will be functionally evaluated in different assays, such as ELISA, confocal microscopy, FACS etc.

WP5.1 Validation of the first generation of C2 molecules with deleted Fc affinity

WP5.2 Validation of molecules with BPA in different positions

WP5.3 Validation of different labeling groups

WP5.4 Validation of C2 molecules with increased Fab affinity

6. Milestones and deliverables

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7. Project-specific considerations related to ProNova main agreement

7.1 Relation to Results in earlier ProNova projects (Stages 1 and 2), not yet subject of patent application filing.

This project is a continuation of subproject 1A in Programme Area 1 from ProNova Stage 2.

7.2 Relation to protected Results in earlier ProNova projects (Stages 1 and 2).

This subproject is in part a continuation of a methodology developed during ProNova stage 2. The IP rights to the methodology were protected by a patent application filed by GE Healthcare Bio-Sciences on September 5, 2011.

7.3 Relation to own existing IP (property of either academic staff or involved industrial partners).

- 

7.4 Other considerations.

-
PROJECT DESCRIPTION

1. Program area and project designation/title

Program Area: 1. Protein Engineering and Affinity Tools
Project: C. Antibody labeling for preclinical in vivo imaging applications

2. Project duration

Period 1: 2012-04-01--2014-03-31 (Centre Years 6 and 7)
Period 2 (conditional): 2014-04-01--2015-03-31 (Centre Year 8), if a continuation is approved by the Centre Board

3. Project resources:

KTH / ProNova resources:

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Academia: Senior researcher (Prof./Assoc. Prof.): 1 500 kSEK/year, Senior researcher (PhD/Postdoc): 960 kSEK/year, PhD student: 800 kSEK/year, Administrative personnel: 600 kSEK/year

Summary of resources:

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4. Project summary, incl. goals

The goal of the subproject is to explore the technology earlier developed in the ProNova Centre for site-specific labeling of antibodies, based on immunoglobulin-binding domains in combination with photoactivable probes, for in vivo applications. In contrast to standard bioconjugation techniques, this labeling technology is not dependent on the number and distribution of surface-exposed functional groups, which may vary significantly between individual antibodies. Instead, the immunoglobulin-binding domain carrying the reporter label is attached to a defined position of the antibody and the efficiency of the reaction is expected to be similar for all antibodies of the same subtype. It can therefore be anticipated that the strategy will be a convenient way to evaluate and compare different antibodies, since the effect that the labeling may have on the properties of the antibody (e.g. denaturation or loss of binding activity) will be comparable within a panel of different antibodies. In the project, the photoconjugation strategy will be explored for covalent coupling of antibodies to radionuclides, magnetic nanoparticles and near-IR fluorescent probes, for preclinical imaging by e.g. SPECT, PET, MRI or optical imaging.

5. Project work packages

Work package 1: Optimization of antibody photoconjugation yield

During Stages 1 and 2 of the Centre, different positions in the immunoglobulin-binding Z domain have been used for incorporation of the photoactivable probe, benzophenone (BP). The photoactivable probe has been introduced either as part of an amino acid (benzophenylalanine, BPA) or conjugated to the side chain of a cysteine residue (via a maleimido-benzophenone reagent) (see figure below).

![Chemical drawings showing the side chains of a) benzoxyphenylalanine, b) cysteine conjugated with benzophenone-4-maleimide, and c) lysine coupled to 4-benzoylbenzoic acid.](image)

It is possible that the different lengths and flexibility of the linker between the benzophenone moiety and the peptide backbone can affect the antibody binding and conjugation efficiency. In order to optimize the efficiency of the photoconjugation reaction for covalent coupling to the Fc part of human IgG1, four different variants will be synthesized and tested. The construct giving the highest antibody labeling yield will then be used in subsequent work packages. The positions
tested will be Phe5 and Gln32, which will be replaced with either benzoylphenylalanine or lysine coupled to 4-benzoylbenzoic acid (to mimic the longer linker achieved by conjugating benzophone-4-maleimide to cysteine) (see figure above).

WP1.1 Synthesis of four BP-labeled Z domain variants
WP1.2 Evaluation of binding affinity and photoconjugation efficiency

Work package 2: Labeling via functionalized nanoparticles

Magnetic nanoparticles fabricated by e.g. iron oxide can be visualized by magnetic resonance imaging (MRI). In this project, the immunoglobulin-binding domains will be covalently coupled to functionalized magnetic nanoparticles, followed by site-specific UV-conjugation to an antibody. The performance of the photoconjugated antibody-nanoparticles, in terms of stability and functionality, will be compared with conjugates produced by standard bioconjugation techniques. The antibody-nanoparticles will be evaluated in vitro using various platforms such as biosensor analysis, Luminex immunoassays, flow cytometry and other cell-based assays, before investigation in vivo. The biodistribution and in vivo half-life of the antibody-nanoparticle conjugates will be assessed in normal mice. Promising conjugates can be further investigated by studying tumor targeting in immunocompromised mice with xenografted tumors. In addition to the inherent properties conferred by the nanoparticle material (e.g. magnetism), the nanoparticles can be functionalized with suitable reporter groups (e.g. fluorophores or PET nuclides) required for the intended application.

WP2.1 Establishment of a protocol for antibody conjugation to nanoparticles
WP2.2 In vitro evaluation of stability and binding activity
WP2.3 Studies of the biodistribution in normal mice
WP2.4 Tumor targeting in xenografted mice

Work package 3: Fluorescent labeling using near infrared dyes

For in vivo fluorescence imaging, problems with tissue autofluorescence and high background can be circumvented by using near infrared (NIR) dyes. In the project, different NIR dyes (e.g. the XenoFluor 750 dye, or the DyLight 755 dye) will be coupled to the Z domain and evaluated for photoconjugation to the antibody. Since the photoconjugation reaction involves UV irradiation of the protein there is a risk of photodecomposition of the fluorophore. The photobleaching effect on different fluorophores will be investigated and promising NIR dyes will be selected for further studies. The properties of the selected antibody-NIR conjugates will be investigated by different in vitro methods and by in vivo optical imaging, analogous to the conjugates produced in WP2.

WP3.1 Evaluation of antibody conjugation to NIR dyes
WP3.2 In vitro evaluation of stability and binding activity
WP3.3 Studies of the biodistribution in normal mice
WP3.4 Tumor targeting in xenografted mice

Work package 4: Radioactive labeling via a radionuclide chelator-protein conjugate
The immunoglobulin-binding domain will be synthesized with a radionuclide chelator moiety, which can be labeled with a radionuclide, before or after UV-conjugation to the antibody. An advantage of labeling the immunoglobulin-binding protein before conjugation to the antibody is that this small protein is significantly more stable than many antibodies to the harsh reaction conditions that may be required for efficient radiolabeling. As a first proof-of-concept, the DOTA chelator will be introduced in position 58 of the Z domain. The protein will be labeled with indium-111 and photoconjugated to antibodies, followed by evaluation of the radioconjugate stability and binding specificity in vitro, followed by in vivo studies by SPECT to assess the biodistribution and tumor targeting properties in animal models.

WP4.1 Evaluation of antibody conjugation to indium-111 using DOTA chelator

WP4.2 In vitro evaluation of stability and binding activity

WP4.3 Studies of the biodistribution in normal mice

WP4.4 Tumor targeting in xenografted mice

Work package 5: Labeling effects on antibody effector functions

When using an immunoglobulin-binding domain for covalent labeling of the antibody, the antibody interaction sites with the neonatal receptor (FcRn), located in the Fc region, may be blocked by the bound immunoglobulin-binding domain, which in turn is expected to reduce the conjugate’s half-life in circulation and possibly enhance the contrast for imaging applications. On the other hand, for therapeutic applications the blocking of the antibody effector sites may reduce the therapeutic efficiency of the antibody. To study this effect, the antibody conjugates produced in WP2, WP3 and WP4 will be evaluated for binding to Fc receptors using flow cytometry and further tested for ADCC and CDC activity using in vitro models.

WP5 Investigation of ADCC, CDC and FcR binding for the labeled conjugates

### Milestones and deliverables

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- Page 62 of 93 -
7. Project-specific considerations related to ProNova main agreement

7.1 Relation to Results in earlier ProNova projects (Stages 1 and 2), not yet subject of patent application filing.

This project is in part based on results from subprojects in Programme Area 1: Affinity Tools during Stage 2.

7.2 Relation to protected Results in earlier ProNova projects (Stages 1 and 2).

This subproject is in part a continuation of a methodology developed during ProNova stage 1. The IP rights to the methodology are protected by a patent application filed with the Swedish Patent and Registration Office on September 6th, 2010, by GE Healthcare Bio-Sciences: Hober, S., Eriksson Karlström, A. & Konrad, A. "Method for labeling of compounds".

7.3 Relation to own existing IP (property of either academic staff or involved industrial partners).

- 

7.4 Other considerations.

-
PROJECT DESCRIPTION

1. Program area and project designation/title

Program Area: 1. Affinity Tools and Protein Engineering  
Project: D. Detection systems based on split-protein complementation

2. Project duration

Period 1: 2012-04-01--2014-03-31 (Centre Years 6 and 7)  
Period 2 (conditional): 2014-04-01--2015-03-31 (Centre Year 8), if a continuation is approved by the Centre Board

3. Project resources:

KTH / ProNova resources:

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Template for calculation of in kind contributions: Industry: 1 FTE = 1 472 kSEK/year (800 SEK/hr x 1840 hr/year); Academia: Senior researcher (Prof./Assoc. Prof.): 1 500 kSEK/year; Senior researcher (PhD/Postdoc): 960 kSEK/year; PhD student: 800 kSEK/year; Administrative personnel: 600 kSEK/year

Summary of resources:

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4. Project summary, incl. goals

The development of principles for selective detection of a desired target structure in samples of complex composition remains a challenge. Such methods should ideally be capable of combining quick read-outs and easy-to-use handling with high selectivity. One already demonstrated hallmark principle towards this goal relies on a combined use of two (or more) separate affinity probes, both of which are required to bind the target structure for the generation of a signal, such as in “sandwich” type ELISA assays and proximity ligation assays, both typically utilizing pairs of non-competing antibodies for recognition of separate epitopes on a target structure. A different principle, based on so called split-protein technology, is also frequently used to study protein-protein interactions. This principle is based on a physical splitting of a given reporter protein into two fragments, each fragment being inactive when produced and studied on its own. However, when brought together the two fragments combine and reconstitute the active reporter (complementation).

In the proposed project, a panel of novel principles for target molecule detection will be investigated, all based on split-protein technology:

1) Antibody and split-fluorescent protein-based target detection (WP 1-4)

The aim of the project is to investigate a principle for selective and sensitive detection of target molecules, in which three components are used in combination:

1) A pair of antibodies recognizing different epitopes on the same target molecule; 2) Site-specific conjugation of antibodies via photoactivable immunoglobulin binding protein domains; 3) A fluorescent protein, split into fragments not spontaneously associating unless brought together by other means.

In the format to be investigated, two antibodies will be separately and covalently conjugated to two different probe fusion proteins, each consisting of two moieties: a) an immunoglobulin binding protein (typically the staphylococcal protein A derived and Fc binding Z domain or variants thereof), containing an introduced unique cysteine residue to which a photoactivable maleimide benzophenone group can be selectively conjugated; b) one of two fragments of a suitable fluorescent protein, such as mCherry (Fig. 1A). Hence, any favorite non-cloned, non-recombinant antibody can be converted into a half-probe of the kind useful in the assay.

The mCherry (Fig. 1B) is a red fluorescent protein characterized by being monomeric, devoid of cysteines and capable of being split into two fragments that can associate into a fluorescent protein when brought together. In an assay situation, the two antibody-probe-mCherry half reagents would be used in combination to detect a target protein via red fluorescence, appearing only when a target protein is present. The signal would be proportional to the number of
reconstituted mCherry reporters, in turn reflecting the number of target molecules present (acc. to law of mass action).

The proposed system is similar to the proximity ligation assay, but instead of a nucleic acid-dependent read out, the present system is based on fluorescence. In contrast to FRET, based on a relatively complex read out of the crosstalk between two recruited fluorophores coupled to the two antibodies, the present principle will generate a simpler read out (dark or red).

The antibody labeling technology using photoactivatable immunoglobulin binding domains was developed in ProNova during stages 1 and 2.

2) Monitoring fusion protein cleavage via split-protein fluorescent protein detection (WP 5-7)

The aim of the project is to investigate a principle for real-time monitoring of processes in which a recombinant fusion protein is site-specifically cleaved by an added protease.

The principle is based on that the recombinant fusion protein to be cleaved typically contains four moieties: 1) An gene fusion affinity tag used for recovery of the fusion protein; 2) a linker composed of a particular amino acid sequence corresponding to a beta strand normally present in a variant of Green Fluorescent Protein (GFP 11 element of "superfolder" GFP); 3) A peptide sequence serving as substrate for an added protease and 4) a target protein of interest to be purified (Fig. 2A).

In solution, free GFP 11 peptide moieties can spontaneously associate and complement large GFP moieties denoted GFP 1-10, consisting of all other amino acids of the GFP reporter. Such complementation results in an active GFP protein visible via its fluorescence. The present assay will depend on that a GFP 11 peptide, when present between to flanking proteins is not capable of complementing GFP 1-10 proteins present in the same sample. Thus, only when the fusion protein is cleaved by the added protease, the GFP 11 peptide is presented in a manner that allows it to complement the large GFP 1-10 docking partner (Fig 2B).

If this holds true, the assay has the potential to be useful in quantitative monitoring, in real time, the cleavage process of recombinant fusion proteins.

3) Single-step immunoglobulin quantification (WP 8-10)

The aim of the project is to investigate a potentially very simple principle for quantification of immunoglobulins in a sample, such as in crude sera or plasma, in eluates after chromatographic purification of mAbs or polyclonal antibodies or after surface immobilization. The principle is based on the construction and use of two "half-reagents", each composed of a fragment of an immunoglobulin binding protein domain (e.g. C2 from streptococcal protein G, Fig. 3) linked to one of two fragments of the red fluorescein protein reporter mCherry (see Project 1).

The assay principle is based on the assumption that the complementation of the C2 halves will be promoted by, or even better dependent on, the presence of the affinity partner for a
reconstituted C2 domain in the sample, i.e. immunoglobulins (Fc). A stabilization of reconstituted C2 domains will be accompanied by a reconstitution also of the thereto fused mCherry reporter halves, resulting in red fluorescence (Fig. 3).

Previous studies of the 56-residue C2 domain has shown that the domain can be split into two fragments, res. 1-40 and res. 41-56, respectively, that can reconstitute to form a native-like structure. The affinity (Kd) between these two fragments is weak, in the order of 10-100 μM. Mutations in the 1-40 fragment have been found that either stabilize or destabilize the wild type C2 domain, suggesting that the affinity between the two moieties probably can be tuned to suit the assay principle described here. The mCherry parts are described to be essentially non-capable of spontaneous self-complementation.

5. Project work packages

Approx. Year 1

Work package 1:
- Preparation of Histag6-ZF5I+Q32C domain-linker-mCherry1-159 and Histag6-ZF5I+Q32C domain-linker-mCherry160-237 gene fusion constructs in expression plasmids, incl. short (S), medium (M) and long (L) linker region variants.
- Preparation of full-length Histag6-mCherry1-237 expression plasmid construct.

Work package 2:
- Recombinant production, purification and maleimide benzophenone conjugation of Histag6-ZF5I+Q32C domain-linker-mCherry1-159 and Histag6-ZF5I+Q32C domain-linker-mCherry160-237 fusion proteins.
- Production and purification of full-length mCherry protein (reference protein for fluorescence measurements)

Work package 3:
- Labeling of anti-target antibody reagents with maleimide benzophenone conjugated Histag6-ZF5I+Q32C domain-linker-mCherry1-159 and Histag6-ZF5I+Q32C domain-linker-mCherry160-237 fusion proteins.

Work package 4:
- Target protein detection trials.

Approx. Year 2

Work package 5:
- Preparation of Histag6-C2domain1-40-linker-mCherry1-159 and Histag6-C2 domain160-237 gene fusion constructs in expression plasmids, incl. short (S), medium (M) and
long (L) linker region variants.

**Work package 6:**
- Recombinant production and purification of \(\text{His}_6 \cdot \text{C2domain}_{1-40} \cdot \text{linker} \cdot \text{mCherry}_{1-159}\) and \(\text{His}_6 \cdot \text{C2domain}_{41-56} \cdot \text{linker} \cdot \text{mCherry}_{160-237}\) gene fusion constructs.
- Biosensor interaction studies (between immobilized Fc to either halves and both).

**Work package 7:**
- Target protein (IgG Fc) detection trials.

**Approx. Year 3**

**Work package 8:**
- Preparation of \(\text{GST} \cdot \text{Superfolder GFP}_{11\text{fragment}} \cdot \text{substrate linker} \cdot \text{multicloning linker} \cdot \text{(and Target protein)}\) expression plasmid construct.
- Preparation of \((\text{GST}) \cdot \text{Superfolder GFP}_{1-10\text{fragment}}\) expression plasmid construct.

**Work package 9:**
- Production and purification of \(\text{GST} \cdot \text{Superfolder GFP}_{11\text{fragment}} \cdot \text{substrate linker} \cdot \text{Target protein}\)
- Production and purification of \((\text{GST}) \cdot \text{Superfolder GFP}_{1-10\text{fragment}}\) protein

**Work package 10:**
- Tests of monitoring \(\text{GST} \cdot \text{Superfolder GFP}_{11\text{fragment}} \cdot \text{substrate linker} \cdot \text{Target protein fusion}\) protein cleavage via Superfolder GFP complementation

**Note:** All three systems described above may be subject of one or additional cycles of engineering/optimization to result in moieties with suitable properties, such as modification of fragment-fragment interaction affinities, substitution of the ZF51+Q32C for a different Z variant, modification of linker lengths, adjustment of Superfolder GFP\(_{11\text{fragment}}\) peptide presentation or affinity for the Superfolder GFP\(_{1-10\text{fragment}}\) or adjusting the protease substrate sequence, etc.

### 6. Milestones and deliverables

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7. Project-specific considerations related to ProNova main agreement

7.1 Relation to Results in earlier ProNova projects (Stages 1 and 2), not yet subject of patent application filing.

The Z domain variant denoted \( Z_{\text{F51} + Q_{32C}} \) and mentioned in subproject 1 above was developed as part of Program Area 1 in ProNova Stage 2. The domain showing higher-than-wild type affinity for mouse IgG1 and showing good photocoupling coupling yields to the same has not yet been discussed in terms of patent application or not.

7.2 Relation to protected Results in earlier ProNova projects (Stages 1 and 2).

The Z domain-based photocoupling technology as such, discussed in subproject 1 above, was subject of patent application filing during ProNova Stage 2. GE Healthcare Bio-Sciences AB was assigned as owner to the IPR.

7.3 Relation to own existing IP (property of either academic staff or involved industrial partners).

GST gene fusion system vectors and reagents, mentioned as examples in subproject 2 above, are sold by GE Healthcare Bio-Sciences AB.

7.4 Other considerations.
PROJECT DESCRIPTION

1. Program area and project designation/title

Program Area: 2. Array Technologies
Project: A. Antibody characterization and purification

2. Project duration

Period 1: 2012-04-01--2014-03-31 (Centre Years 6 and 7)
Period 2 (conditional): 2014-04-01--2015-03-31 (Centre Year 8), if a continuation is approved by the Centre Board

3. Project resources:

KTH / ProNova resources:

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Industry partner resources:

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Template for calculation of in kind contributions:
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Academia: Senior researcher (Prof./Assoc. Prof.): 1 500 kSEK/year, Senior researcher (PhD/Postdoc): 960 kSEK/year, PhD student: 800 kSEK/year, Administrative personnel: 600 kSEK/year

Summary of resources:

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4. Project summary, incl. goals

Project goal
To develop epitope-mapping methods suitable both for mapping of binders to linear and structural epitopes and utilize epitope information to generate antibody pairs for sandwich-based assays.

Project description
The project is split into two work packages: A) Epitope mapping of structural epitopes and B) Epitope mapping using high density planar arrays for generation of antibody-pairs.

A) Epitope mapping of structural epitopes
Several types of binding molecules including Affibody molecules and antibodies occasionally require their target protein to present a folded structure for binding to take place. Determination of epitopes for such binders would benefit of using folded protein domains rather than shorter peptides typically used in other mapping experiments.
In this project we would like to develop a platform for epitope mapping of structural epitopes based on *S. carnosus* cell surface display.
The project involves the mapping of Affibody molecules and/or antibodies to selected targets. In the following four target suggestions are made based on communication with the partner companies and interest from KTH (these might be subject to change):

1. Her2 (positive control with 3-D structure of target-binder complex known)
2. EGFR (binder available)
3. Target selected by SOBI (binder available)
4. Target selected by Affibody AB (binder available)

For each target, the subproject consists of two parts:
1. Expression of individual folded domains on *S. carnosus* followed by binding assay (FACS) to establish folding and binding.
2. Generate alanine-mutated variants in selected positions with oligonucleotide-directed mutagenesis to explore residues of importance for binding.

The domains and libraries are analyzed using Fluorescent Activated Cell Sorting using fluorescently labeled binders, either directly or via a secondary reagent and the clones to which a decreased binding affinity is observed are subjected to genotyping by DNA sequencing.

B) Epitope mapping using high-density peptide planar arrays for generation of antibody-pairs
We would like to explore the possibility to use high-density arrays for epitope mapping of antibodies and subsequently use this information to generate antibody-pairs suitable for sandwich applications. Based on photo-lithographic in situ synthesis techniques we are able to synthesize 12-20-mer peptides and we expect more than 2 million peptides to be included on the array. The arrays will be available through two independent established collaborations with Claus Schaffer (Schafer-N A/S Denmark) and/or Nimblegen/Roche (Germany). Dedicated slides for epitope mapping of ten polyclonal antibodies chosen together with the partner companies will be used as evaluation set for the development of a general method where binders first are
mapped and the epitope information later used for affinity purification of epitope specific binders. The slide design will also include various amino acid scanning techniques to allow precise mapping of the contribution of each amino acid in the epitope of the antibodies for a deeper understanding of the interaction.

5. Project work packages

A) Epitope mapping of structural epitopes

Work package A1.1-1.2: In-silico design of protein fragments
Structural analysis and design of protein fragments and domains. This is including simulations and literature studies of suitable domains/fragments to be chosen for expression. This process is done in close collaboration with the industrial partners utilizing their expertise on the targets. This also includes choice and acquiring of relevant positive controls to later be able to verify a native folding state of the target protein domains when displayed on the cell surface.

Work packages A2.1-2.3: Generation of domain /protein fragment structured clones
PCR, cloning and sequencing of domain and protein fragment constructs in *E. coli* and transformation into *S. carnosus*.

Work package A3: Binding analysis of domains / protein structures
FACS experiments with clones to identify expression level, ability to express a folded structure using positive controls and evaluation of binder ability to recognize the different protein fragments and structures.

Work package A4. Mutagenesis of domains / structures
Generation of numerous and sequentially distributed alanine mutants of domains clones earlier shown reactivity towards investigated binders. The knowledge of surface exposed residues is considered in the design. Analysis is done using FACS of clones followed by genotyping by sequencing.

Work package A5. Data analysis
This includes the compilation of data and projection of the epitopes onto 3D-structures of the target proteins.

Detailed Work package goals:

1. Rationally design several protein regions, which are believed to generate, folded structures based on computer simulations and knowledge from 3D structures and literature.
2. Design a model system to validate folded structures on *S. carnosus* for the four targets.
3. Design primers, amplify, clone into pSCEM2 vector and sequence verify constructs in *E. coli*.
4. Demonstrate ability to express at least some domains on *S. carnosus* using Albumin Binding Protein (ABP)
5. Demonstrate ability to functionally express at least some folded structures on the cell surface
6. Demonstrate ability to bind the structures expressed using the binders available
7. Design dedicated mutagenesis oligos based on positive binding detected and produce *S. carnosus* variants with these variations.
8. Perform epitope mapping experiments using the mutagenesis collection of cells and identify the amino acids involved in the binding event

**B) Epitope mapping using high-density peptide planar arrays for generation of antibody-pairs**

**Work package B1: In-silico design of protein arrays**
Based on the ten antigen chosen together by the partner companies several peptide designs are generated to cover and represent the antigens different ways. Strategies will be developed together with the partners to achieve a design suitable for detailed epitope mapping using synthetic peptides of different length

**Work package B2: Optimization of reaction conditions**
Here various concentrations, temperature, dilutions, scanning time and other conditions are evaluated for the epitope mapping of a reference pAb.

**Work package B3: Epitope mapping of ten antibodies in multiplex**
Here the ability to map pooled antibodies and the effects on the quality of the epitope mapping thereof are investigated.

**Work package B4. Data analysis and epitope extraction**
This includes the compilation of the epitope mapping data from the peptide binding experiments to identify epitopes. These epitopes are projected onto 3D-structures of the target proteins to identify suitable combinations of antibody-pairs. Results from the different amino acid scanning techniques to precise determine the contribution of each amino acid in the epitope to the binder is analyzed and used to generate position specific scoring matrices and sequence logos using bioinformatics tools.

**Work package B5. Affinity purification to generate epitope specific antibody-pairs**
Based on the epitope mapping peptides corresponding to individual peptides are synthesized as biotinylated 15-20mers. The peptides are coupled to columns and used for generation of epitope specific antibodies.

**Work package B6. Validation of epitope specific antibodies**
The epitope specific antibodies are validated using several assays including, western blot, Luminex specificity mapping and as suitable antibody pairs using techniques agreed upon with collaboration partners, including sandwich Elisa.
6. Milestones and deliverables

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- Design of domain fragments
- Design of model system
- PCR of fragments
- Cloning of fragments
- Transformation into S. camosus
- Evaluation of surface expression
- Evaluation of domain fold
- Evaluation of domain fold
- Design of alanine mutations
- FACS of mutated clones
- Sequencing of clones
- Epitope analysis using 3D structure
- Array design
- Optimization of conditions
- Multiplex analysis
- Data analysis & epitope extraction
- Affinity purifications of binders
- Validation of binders

7. Project-specific considerations related to ProNova main agreement

7.1 Relation to Results in earlier ProNova projects (Stages 1 and 2), not yet subject of patent application filing.

- 

7.2 Relation to protected Results in earlier ProNova projects (Stages 1 and 2).

- 

7.3 Relation to own existing IP (property of either academic staff or involved industrial partners).

- 

7.4 Other considerations.

-
PROJECT DESCRIPTION

1. Program area and project designation/title

Program Area: 2. Array Technologies
Project: B. Antigen microarrays and autoimmunity repertoires

2. Project duration

Period 1: 2012-04-01--2014-03-31 (Centre Years 6 and 7)
Period 2 (conditional): 2014-04-01--2015-03-31 (Centre Year 8),
if a continuation is approved by the Centre Board

3. Project resources:

KTH / ProNova resources:

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Industry partner resources:

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Template for calculation of in kind contributions:

Industry: 1 FTE = 1 472 kSEK/year (800 SEK/hr x 1840 hr/year)
Academia: Senior researcher (Prof./Assoc. Prof.): 1 500 kSEK/year, Senior researcher (PhD/Postdoc): 960 kSEK/year, PhD student: 800 kSEK/year, Administrative personnel: 600 kSEK/year

Summary of resources:

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- Page 75 of 93 -
4. Project summary, incl. goals

The selectivity of all antibodies produced within the Human Protein Atlas is verified on antigen microarrays. More than 30,000 antibodies have so far been validated on these microarrays where 384 different antigens are spotted on each batch. A new batch is produced approximately every second week and thereby has a unique resource of arrayed antigens been generated.

The antigen microarrays have been shown to also have large potential as a tool for the discovery of new autoimmunity targets. Serum, plasma and CSF have so far been used in pilot studies with promising results where large numbers of possible new targets have been identified, but with large variance between individuals. The full potential still remains to be explored and the aim is to generate new knowledge about the autoimmunity repertoires in several types of diseases as well as in non-diseased individuals.

The possibility to extend the range of different types of autoantibodies to analyze will be explored. That would mean to include not only IgG in general, but also the subtypes of IgG as well as IgM, IgA and IgE. It would here also be important to evaluate different types of detection reagents and to do develop procedures which could enable multiplexed detection of these autoantibodies.

**Partner contribution** to the project will be mainly technical, methodological and experimental input on various assay development, multiplexed detections and data analysis, as well as specific reagents for these activities. Partners will also contribute with well-characterized sample collections.

5. Project work packages

**Work package 1: Establish a standardized analytical framework.**

This will include visualizations and statistical methods for initial selection of targets of potential interest. There is a need to increase the understanding of the potential quantitative value of the antigen array based screening data and to compare that with data that has been put into a binary context, where all targets are defined as either having human antibodies binding to them or not, based on various relatively subjective criteria. The aim is also to create a data warehouse-like structure where data from various antigen profiling projects are collected which would enable broader analysis of larger data sets.

This WP will be performed with engagement from ThermoFisher Scientific who will be active here and contribute with experience and now how from similar types of data sets which they have generated internally and they will also be able to utilize the resulting outcome.

**Work package 2: Establish procedures for profiling of other body fluids.**

The main sample types that we aim to be able to utilize as alternatives and complement to plasma and serum are CSF, cerebrospinal fluid, and BAL, bronchoalveolar lavage. CSF will enable us to extend the initial plasma profiling of the autoimmunity repertoire within multiple sclerosis and other coming neurodegenerative diseases by correlating the plasma generated profiles with CSF based profiles from the same individuals. Autoimmunity profiles for BAL will be compared
with plasma from the same individuals within the context of sarcoidosis. ThermoFisher will be engaged in the optimizations of the technical procedures with the new sample types.

**Work package 3: Develop a citrullination assay.**

Within rheumathoid arthritis citrullinated antigens are well established as autoimmune targets. We aim here to be able to do large array based screenings for new citrullinated autoantigens, which could potentially be very informative and enable increased understanding of rheumatoid arthritis. We will explore the possibilities of enzymatically introducing the citrullination on the antigen and initially that will be done in solution or on beads before spotting the antigens on the arrays. The main aim is to be able to citrullinate the antigens when they are already spotted. This would enable a very broad screening for all different anti-citrullination-based autoantibodies.

**Work package 4: Explore multiplex profiling and affibody molecules as detection reagents.**

We will here utilize the currently existing affibody molecules generated to bind to various immunoglobulins, i.e. IgG, IgA, IgM and IgE. We aim, together with Affibody to establish herein appropriate procedures for the labeling/conjugation of the affibody molecules or utilizing anti-affibody molecules for the detection of the presence of the human autoantibodies. Also antibodies directed to immunoglobulin subgroups will be utilized and potentially combined with the affibody molecules. We will explore possibilities for multiplexed detection of all various types of immunoglobulins. ThermoFischer has long experience and in-house generated know-how that will be utilized to enhance the possibilities to explore the total panel of immunoglobulins.

### 6. Milestones and deliverables

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7. Project-specific considerations related to ProNova main agreement

7.1 Relation to Results in earlier ProNova projects (Stages 1 and 2), not yet subject of patent application filing.

This project is a continuation of a project in Programme Area 3 from ProNova Stage 2. There are no results from that phase which are presently under consideration for any patent application discussions.

7.2 Relation to protected Results in earlier ProNova projects (Stages 1 and 2).

-

7.3 Relation to own existing IP (property of either academic staff or involved industrial partners).

-

7.4 Other considerations.

-
PROJECT DESCRIPTION

1. Program area and project designation/title

Program Area: 2. Array Technologies
Project: C. Advancing antibody bead arrays for biomarker discovery

2. Project duration

Period 1: 2012-04-01--2014-03-31 (Centre Years 6 and 7)
Period 2 (conditional): 2014-04-01--2015-03-31 (Centre Year 8), if a continuation is approved by the Centre Board

3. Project resources:

KTH / ProNova resources:

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Industry partner resources: (proposals below need confirmation from partners)

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Template for calculation of in kind contributions:
Industry: 1 FTE = 1 472 kSEK/year (800 SEK/hr x 1840 hr/year)
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4. Project summary, incl. goals

The goal of this project is to improve the translation versatility and the performance of bead-based protein profiling. This includes increasing the number of samples and sample types (co-profile body fluids cerebrospinal fluid, urine, saliva, and pancreatic juice) per analysis, and focuses the transition from single-binder screening assays to utilizing pairs of antibodies for the detection of protein targets. To further address assay performance, alternative affinity reagents (Affibody molecules) and oil-water emulsions will be evaluated for to enhance the detectability of target. The fourth focus is to evaluate the reagents and assays for using a different platform, while a fifth subject to exercise described developments in a disease-related project remains provisional.

5. Project work packages

Work package 1 – Advancing throughput for screening

We seek to develop a protocol for routine antibody coupling, sample labeling and assays implementation using the 384-well format for to improve sample turnaround time and throughput. We will investigate the possibility to use the bead arrays for body fluid co-profiling (as segmented arrays) and for studying proximal fluids in parallel to serum/plasma tailored assay protocols will be developed. To design and extract the key information from co-profiling efforts of both proximal body fluids alongside plasma, respective data analysis is required (exhale air trapped on a filter). In addition, verification assays for the respective body fluids are needed. We will evaluate epitope-purified and monoclonal antibodies and Affibody molecules as capture reagents in multiplexed assays.

Work package 2 - Advancing assays for screening

We will survey the market for emulsions for protein science and analyze the possibilities to create emulsions using the current assay buffers, and determine the stability and breakability of emulsions, plus heating of samples encapsulated in bead emulsions. In this WP, the performance of Affibody molecules in emulsion assays will be assessed. We aim at combining the developments from WP2 to build dual binder emulsion assays and employ epitope-purified and monoclonal antibodies, as well as Affibody molecules.

Work package 3 - Verification assays for discoveries

Here, the development of dual-binder will be preceded in assays where bead-captured antigens are being detected by low quantities of detection antibodies from the same species. In this WP, the evaluation of anti-rabbit detection reagents with minimal recognition of capture antibodies, assay time, sample concentrations, sample types, assay conditions, buffer components and the most suitable degree of multiplexicity will be evaluated e.g. for Fibulin-1 with Atlas Antibodies AB. We will investigate the use of epitope-purified antibodies and monoclonal antibodies and Affibody molecules to build dual-binder assays.

Work package 4 – Verifying technologies for new targets

A translation of the assay to another platform (Gyros) is anticipated to “disconnect” potential findings from platform bias. The usability of polyclonal, epitope-purified as well as monoclonal
antibodies are evaluated (e.g. for Fibulin-1), which the aim to identify antibody properties suitable for Gyros and other assays. A sandwich assay based on Gyros’ antibody properties is built to quantify Fibulin-1. The gained insight is used to streamline the development of sandwich assays to other, low abundant targets.

Work package 5 – Biomarker discovery and verification

The WP aims at verifying indications from plasma analysis of liver toxicology with the project partner AZ and encompasses the developments from other WPs. Furthermore, the potential of exhaled air with protein content trapped on a filter will be explored as a sample source for biomarker discovery within the respiratory diseases asthma and COPD will be analyzed (AZ Møndal).

6. Milestones and deliverables

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7. Subproject-specific considerations related to ProNova main agreement

7.1 Relation to Results in earlier ProNova projects (Stages 1 and 2), not yet subject of patent application filing.

This project is a continuation of a project in Programme Area 3 from ProNova Stage 2, where results were obtained that are presently considered to be filed as patent application. A potential finding from liver toxicology studies could be extended into the analysis of urine.

7.2 Relation to protected Results in earlier ProNova projects (Stages 1 and 2).

This subproject is in part a continuation of a methodology developed during ProNova stage 2. Results created in the new phase would allow strengthening the association of Fibulin-1 as biomarker for renal impairment (patent application filed), because of the urine analysis protocol.

7.3 Relation to own existing IP (property of either academic staff or involved industrial partners).

Results created in the new phase would allow strengthening the association of Fibulin-1 as biomarker for renal impairment (inventors on filed patent application are part of this subproject).

7.4 Other considerations.

This sub-project is merged from the planned activities for “Biomarker discovery in translational science” and “Improving bead arrays and beyond”. AstraZeneca-Mölndal has expressed the ambition to follow AstraZeneca-Södertälje within the biomarker discovery project area, setting a focus onto respiratory diseases. This will also enable to follow-up fruitful biomarker discovery project within liver toxicology that was initiated during phase 2.
PROJECT DESCRIPTION

1. Program area and project designation/title

Program Area: 2. Array Technologies
Project: D. Immunosequencing (iSeq) for highly multiplex protein analysis

2. Project duration

Period 1: 2012-04-01--2014-03-31 (Centre Years 6 and 7)
Period 2 (conditional): 2014-04-01--2015-03-31 (Centre Year 8), if a continuation is approved by the Centre Board

3. Project resources:

KTH / ProNova resources:

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<td>Afshin Ahmadian</td>
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Template for calculation of in kind contributions:

Industry: 1 FTE = 1 472 kSEK/year (800 SEK/hr x 1840 hr/year)
Academia: Senior researcher (Prof./Assoc. Prof.): 1 500 kSEK/year, Senior researcher (PhD/Postdoc): 960 kSEK/year, PhD student: 800 kSEK/year, Administrative personnel: 600 kSEK/year

Summary of resources:

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4. Project summary, incl. goals

The project aims to continue the development from Stage II of ProNova focusing on a new approach for DNA-assisted antibody-based proteomics. The main focus of the project is on a novel principle for simultaneous detection and quantification of numerous proteins in complex samples, where a combination between immunorecognition (DNA-labeled antibodies) and massively parallel DNA sequencing is applied. In two accompanying work packages, the challenge to label antibodies with DNA in a controlled manner is addressed. These work packages are also of general interest for the growing field of assays involving protein/DNA combination reagents.

5. Project work packages

Work package 1:

Our results from Stage II of ProNova (Program Area 1, Subproject 1C) shows that multiplex protein assays using barcoded DNA probes is possible and that direct DNA conjugation to antibodies is not always necessary as DNA probes and antibodies may simultaneously be coupled to beads, providing a physical link. A mixture of such DNA-antibody carrying beads are let to bind to proteins in a sample of interest (e.g. a reverse phase array) through antibody-antigen interactions, followed by thorough washing. A subsequent in situ PCR of remaining barcoded DNA molecules and sequencing (incl. an integrated counting) of resulting amplicons allows for a reciprocal quantification of the proteins in the sample. However, the relatively large beads (2.8 μm) used hitherto as links between DNA and antibodies may negatively influence the precision of the quantification. Thus, in the coming stage we propose use of considerably smaller particles (10-100 nm) that not only will provide a more accurate quantification of protein abundances but also open up the possibilities to investigate protein-protein interactions. Here, antibodies and barcoded DNA molecules will be attached (covalently or non-covalently) to the surface of nanoparticles and the antibodies will be allowed to bind to proteins on a solid surface. Similar to the previous assay, the proteins will be quantified by first performing in situ PCR using universal primers and then sequencing the amplicons. To detect proteins involved in a protein-protein interaction, antibodies towards the proteins will bring the DNA-carrying nanoparticles close to each other, allowing e.g. proximity ligation assays to be performed. Using this approach, direct probe conjugation to antibodies may be omitted and the fact that hundreds of probes are attached to the nanoparticles leads to a more sensitive detection.

Work package 2:

Single molecule DNA barcoding of antibodies is a challenging task because it is quite difficult to control the reaction conditions and thereby preventing conjugation of more than one DNA molecule. In this proposal we aim to address this issue by utilizing so called emulsion PCR (em-PCR) technology commonly used for clonal amplification in the majority of massively parallel sequencing platforms and adapt it for single molecule conjugation. Here, conditions for carrying out coupling reactions in individual aqueous droplets will be investigated.
Work package 3:

We have previously developed a fully automated protocol for direct DNA labeling of scarce amounts of antibodies on protein A coated magnetic beads. The reactions are carried out on magnet-equipped robotic stations capable of processing 96 antibodies in parallel in less than two hours. To date, two different fluorophores have been used on a few antibodies and successfully tested on antigen microarrays. In stage III of ProNova we aim to investigate the performance of labeled antibodies over time (storage and its effect on labeled proteins). In addition, the protocol will be adapted to conjugate other labeling agents.

6. Milestones and deliverables

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7. Project-specific considerations related to ProNova main agreement

7.1 Relation to Results in earlier ProNova projects (Stages 1 and 2), not yet subject of patent application filing.

- 

7.2 Relation to protected Results in earlier ProNova projects (Stages 1 and 2).

- 

7.3 Relation to own existing IP (property of either academic staff or involved industrial partners).

- 

7.4 Other considerations.

-
PROJECT DESCRIPTION

1. Program area and project designation/title

Program Area: 3. Microfluidics
Project: A. Droplet microfluidics

2. Project duration

Period 1: 2012-04-01--2014-03-31 (Centre Years 6 and 7)
Period 2 (conditional): 2014-04-01--2015-03-31 (Centre Year 8), if a continuation is approved by the Centre Board

3. Project resources:

KTH / ProNova resources:

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Template for calculation of in-kind contributions:

Industry: 1 FTE = 1 472 kSEK/year (800 SEK/hr x 1840 hr/year)
Academia: Senior researcher (Prof./Assoc. Prof.): 1 500 kSEK/year, Senior researcher (PhD/Postdoc): 960 kSEK/year, PhD student: 800 kSEK/year, Administrative personnel: 600 kSEK/year

Summary of resources:

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- Page 87 of 93 -
4. Project summary, incl. goals

In this project we seek to expand the activities in enzyme screening and analysis developed in the 2nd phase of the ProNova center and utilize the droplet microfluidics system developed therein. Building on this foundation we propose to expand the range of enzymes screened to e.g. cellulases, xylanases or proteases as well as the range of non-native industrially relevant conditions under which the enzymes are screened. This will involve the development of enzyme specific assays suitable for the enzymes and conditions of commercial interest. Here, additional modes of detection of enzyme activity other than fluorescence, such as absorbance and fluorescence polarization are proposed.

5. Project work packages

Work package 1: Enzymatic activity based droplet sorting of amylase library
1.1 Droplet sorting of bacterial model system
1.2 Amylase library development
1.3 Droplet screening of bacteria expressed amylase library
1.4 Droplet screening of bacteria expressed amylase library at non-native condition

Work package 2: Controlled retrieval/output of single clones and downstream droplet PCR
2.1 Clonal retrieval of droplets
2.2 Clonal downstream droplet PCR

Work package 3: 2nd enzyme assay screen
3.1 2nd Droplet based enzyme assay
3.2 Development of 2nd enzyme type bacterial library
3.3 Assay validation w. cell expressed enzyme model system
3.4 Droplet based enzyme screening of 2nd enzyme

Work package 4: High temperature and multiple incubation condition screening
4.1 Concurrent single enzyme clone analysis for multiple conditions in a single experiment
4.2 Library analysis at increased temperature

6. Milestones and deliverables

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### 7. Project-specific considerations related to ProNova main agreement

#### 7.1 Relation to Results in earlier ProNova projects (Stages 1 and 2), not yet subject of patent application filing.

- 

#### 7.2 Relation to protected Results in earlier ProNova projects (Stages 1 and 2).

- 

#### 7.3 Relation to own existing IP (property of either academic staff or involved industrial partners).

- 

#### 7.4 Other considerations.

-
PROJECT DESCRIPTION

1. Program area and project designation/title

Program Area: 3. Microfluidics
Project: B. Lateral Flow Microarray Assays

2. Project duration

Period 1: 2012-04-01--2014-03-31 (Centre Years 6 and 7)
Period 2 (conditional): 2014-04-01--2015-03-31 (Centre Year 8), if a continuation is approved by the Centre Board

3. Project resources:

KTH / ProNova resources:

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<tr>
<td>Project Leader</td>
<td>Helene Andersson Svahn</td>
<td>2012-04-01--2015-03-31</td>
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<tr>
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<td>Jesper Gantelius</td>
<td>2012-04-01--2015-03-31</td>
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Industry partner resources:

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Template for calculation of in kind contributions:
Industry: 1 FTE = 1 472 kSEK/year (800 SEK/hr x 1840 hr/year)
Academia: Senior researcher (Prof./Assoc. Prof.): 1 500 kSEK/year, Senior researcher (PhD/Postdoc): 960 kSEK/year, PhD student: 800 kSEK/year, Administrative personnel: 600 kSEK/year

Summary of resources:

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4. Project summary, incl. goals

A great variety of purified allergen components exist, that may be used in specific immunoassays to reveal patient reactivity, which often is associated with allergic symptoms. Several multiplexed approaches for screening individuals or cohorts of patients are currently available, such as planar and suspension bead microarrays, yet there is a lack of assays that offer high performance in terms of sensitivity, specificity, low variability and multiplexing ability while at the same time being cost-efficient, easy to use, and convenient to interpret for end users. In this project, novel approaches for planar microarray analysis in combination with lateral flow devices are developed and characterised within the field of allergy diagnosis, in an attempt to fill this gap.

5. Project work packages

**Work Package 1:** The development of a number of clinically relevant lateral flow protein microarray devices, and evaluation of their performance in autoimmunity and allergy diagnosis as compared with conventional commercial clinical microarray assays.

**Work Package 2:** The development of a rapid lateral flow based assay that through immunochromatographic principles can reveal kinetic characteristics of polyclonal antibody populations towards several antigens simultaneously.

**Work Package 3:** Investigation and implementation of alternative detection systems for lateral flow microarrays, such as enzymatically enhanced colorimetry, Red/Ox electrochemistry and dendrimeric amplification strategies for improved sensitivity.

6. Milestones and deliverables

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<td>Integrated prototypes working in end-user mode tested on larger patient cohorts</td>
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7. Project-specific considerations related to ProNova main agreement

7.1 Relation to Results in earlier ProNova projects (Stages 1 and 2), not yet subject of patent application filing.

-  

7.2 Relation to protected Results in earlier ProNova projects (Stages 1 and 2).

-  

7.3 Relation to own existing IP (property of either academic staff or involved industrial partners).

The project contains technologies that are protected by IPR owned by Jesper Gantelius and Helene Andersson Svahn, KTH, listed as project leader for the subproject, and Phadia AB.

7.4 Other considerations.

-  

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| WP 3.2 | | | | x | Novel detection strategies integrated as alternative in all other lateral flow assays in house and tested on limited patient cohorts. |
| WP 3.3 | | | | x | Successful novel detection strategies published, integrated into in-house assays and applied to larger cohorts. |
Correction

The agreed annual contribution by the industrial partner Affibody AB to projects 2B: “Antigen microarrays and autoimmunity repertoires”, and 2C: “Advancing antibody bead arrays for biomarker discovery”, is 2.5% FTE to each of the projects as also stated in this corrected Operational Plan for ProNova Stage 3 (incl. Budget and Project Plans). However, in the Main Agreement, it is still erroneously stated that the contribution is 5% FTE to each of the projects.

1 This correction of the Operational Plan made by the Centre management is awaiting formal approval by the Centre Board.
PROGRAM AREA: TITLE

1. Summary

2. Period

3. Academic personnel involved

4. Industry partners involved (incl. contribution to the Program Area)

5. List of projects

6. Total Program Area budget (kSEK)

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7. Other information

8. Background IPR/material declaration