ProNova VINN Excellence Centre
for Protein Technology

Project status reports
November 26th, 2012
Research Program Stage 3

1. Affinity Tools and Protein Engineering
   - Affibody
   - BioInvent
   - GE Healthcare
   - Genovis
   - Gyros
   - Mabtech
   - Olink
   - ThermoFisher Scientific (Phadia)

2. Array Technologies
   - Affibody
   - AstraZeneca
   - Atlas Antibodies
   - Gyros
   - Mabtech
   - SOBI
   - ThermoFisher Scientific (Phadia)

3. Microfluidics
   - Novozymes
   - ThermoFisher Scientific (Phadia)
## Project resources and spendings (after 25% of project period)

### Affinity Tools & Protein Engineering

| 1A. Development of an *E. coli* display system | 320 kSEK |
| 1B. Exploitation of immunoglobulin-binding domains for antibody labeling | 640 kSEK |
| 1C. Antibody labeling for preclinical *in vivo* imaging applications | 640 kSEK |
| 1D. Detection systems based on split-protein complementation | 640 kSEK |
| **Total: 1659 kSEK (28%)** | **4480 kSEK (2 years)** |

### Array Technologies

| 2A. Antibody characterization and purification | 640 kSEK |
| 2B. Antigen microarrays and autoimmunity repertoires | 640 kSEK |
| 2C. Advancing antibody bead arrays for biomarker discovery | 640 kSEK |
| 2D. Immunosequencing (iSeq) for highly multiplex protein analysis | 320 kSEK |
| **Total: 164 kSEK (26%)** | **4480 kSEK (2 years)** |

### Microfluidics

| 3A. Droplet microfluidics | 480 kSEK |
| 3B. Lateral flow microarray assays | 480 kSEK |
| **Total: 204 kSEK (11%)** | **1920 kSEK (2 years)** |

**Report period: April 2012 – September 2012**

**Total: 5440 kSEK/year**
Program Area 1: Affinity Tools and Protein Engineering

Program Area Director: John Löfblom

1A. Development of an *E. coli* display system
Personnel (KTH): John Löfblom (PI), Stefan Ståhl (co-PI), Filippa Fleetwood (PhD student), Ken Andersson (PhD student)
Industrial partner: Affibody AB

1B. Exploitation of immunoglobulin-binding domains for antibody labeling
Personnel (KTH): Sophia Hober (PI), Sara Kanje (PhD student)
Industrial partners: BioInvent International AB, Gyros AB, Mabtech AB and Olink AB

1C. Antibody labeling for preclinical *in vivo* imaging applications
Personnel (KTH): Amelie Eriksson Karlström (PI), Kristina Westerlund (PostDoc), Anna Perols (PhD student)
Industrial partners: BioInvent International AB and Genovis AB

1D. Detection systems based on split-protein complementation
Personnel (KTH): Per-Åke Nygren (PI), Amrita Singh-Blom (PostDoc), Feifan Yu (PhD student)
Industrial partners: GE Healthcare Bio-Sciences AB and ThermoFisher Scientific (Phadia AB)
1A. Development of an *E. coli* display system

**Project aims:** The subproject is focused on development of a new *E. coli*-based protein/peptide library display system for epitope mapping and combinatorial protein engineering.

**Current status:**

WP1: Construction of an expression cassette for library applications.
- Putative OmpT-sites mutated in model affinity protein (Affibody molecule) and normalization tag (ABP)
- Mutated Affibody molecule and ABP displayed in a functional manner on the outer membrane (verified by FACS)
- New unique OmpT-site (+His-tag) introduced (sequence ver. ongoing)

WP2: Evaluation of different promoter systems and strains.
- Three different inducible promoters for expression in *E. coli* have been obtained

**Dissemination of results/IPR considerations:**
- No patentable results identified yet.
1B. Exploitation of immunoglobulin-binding domains for antibody labeling

**Project aims:** To create a Fab binding protein with crosslinking abilities for antibody labelling

**Current status:**

- New double mutants
  - K31A_K28A
  - K31W_K28A
  - Q32A_K28A
  - Q32W_K28A
  - K28A_D40A
  - K31A_D40A
  - Q32A_D40A
  - N35A_D40A
  - N35W_D40A -> N35W_D40T

- Ongoing:
  - Synthesis of double mutants with BPA at
    - T11
    - E19
    - G38
    - T11_E19
    - Biotin on L50

**Dissemination of results/IPR considerations:**

- Discussions regarding patentability ongoing
**1C. Antibody labeling for preclinical *in vivo* imaging applications**

**Project aims:** to explore the ProNova technology for site-specific labeling of antibodies, based on immunoglobulin-binding domains in combination with photoactivatable probes, for preclinical *in vivo* imaging by e.g. SPECT, PET, MRI or optical imaging.

**Current status:**

**WP1:** Optimization of antibody photoconjugation yield
- different benzophenone-labeled Z domain variants synthesized and tested
- Z32BPA shows high photocoupling efficiency to human IgG1

**WP2:** Labeling via functionalized nanoparticles
- PEG-coated superparamagnetic iron oxide nanoparticles (11 nm) from Genovis AB
- Z5BPA-thiol coupled to nanoparticle
- UV conjugation to antibodies

**Stage 2 project:** Development of conjugation chemistries for DNA labeling of antibodies
- DNA labeling of antibodies is performed via the ZSBPA domain conjugated to a peptide nucleic acid (PNA) tag
- Proof-of-concept: immuno-PCR

**Dissemination of results/IPR considerations:**
- No patentable results identified. The results of the optimized photoconjugation yield will be summarized in a short publication/letter. DNA labeling via PNA tag; manuscript in preparation.
1D. Detection systems based on split-protein complementation

**Project aims:** To investigate split-protein complementation principles for use in different detection systems in biotechnology.

**Current status:**
- No spontaneous complementation
- Complementation is depending on fused/linked affinity system

**WP1:** Homogenous detection system based on Ab-Z\textsubscript{MBP}-mCherry conjugates
- Different fusions between a Z photocoupling probe and subfragments of mCherry cloned and produced
- Photoconjugation of detection antibodies performed
- Target detection tests ongoing
- Linker lengths to be further varied

**WP2:** Monitoring of fusion protein cleavage via detection of split GFP reconstitution
- GFP 1-10 expression construct designed and received from DNA 2.0
- pGEX-6P-1 vector (GE’s GST system) has been genetically modified to contain the GFP 11 element (sequence to be verified)
- Gene for first model target protein T7 RNA polymerase (99 kDa) successfully PCR amplified from *E. coli* BL21 chromosome
- Cleavage/complementation tests to be performed (w/o target protein)

**Dissemination of results/IPR considerations:**
- No patentable results identified yet.
Program Area 2: Array Technologies

Program Area Director: Peter Nilsson

2A. Antibody characterization and purification
Personnel (KTH): Johan Rockberg (PI), Mathias Uhlén (co-PI), Anna-Luisa Volk (PhD student)
Industrial partners: Affibody AB, Atlas Antibodies AB, Gyros AB and SOBI AB

2B. Antigen microarrays and autoimmunity repertoires
Personnel (KTH): Peter Nilsson (PI), Julie Bachmann (PostDoc), Ronald Sjöberg (PhD student/Res. Engineer), Anna Häggmark (PhD student), Maja Neiman (PhD student)
Industrial partners: Affibody AB and ThermoFisher Scientific (Phadia AB)

2C. Advancing antibody bead arrays for biomarker discovery
Personnel (KTH): Jochen Schwenk (PI), Mun-Gwan Hong (PostDoc), Sanna Byström (PhD student), Elin Birgersson (Res. Engineer)
Industrial partners: Affibody AB, AstraZeneca AB, Atlas Antibodies AB, Gyros AB and Mabtech AB.

2D. Immunosequencing (iSeq) for highly multiplex protein analysis
Personnel (KTH): Afshin Ahmadian (PI), Mahya Dezfooli (PhD student)
Industrial partner: Atlas Antibodies AB
2A. Antibody characterization and purification

**Project aims:** 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

**Current status:**

![Data Set 4: Woll_D02](image1)

**WP1: Epitope mapping of structural epitopes**
- Three target antigens decided (EGFR, HER2 and SOBI target)
- Domain and multidomain fragments designed (around 25-30)
- All fragments amplified, cloned and sequenced
- First structures just tested in *S.carnosus* (picture) and the 4 domains of HER2 express successfully
- Next to test binding of affibody molecules and reference reagents

![WP2: Epitope mapping using high-density peptide planar arrays for generation of antibody-pairs](image2)

- Test using smaller set of proteins using old array trying reaction conditions for array mappings
- Initial choice of antigens by industry partners
- Next order designed arrays for analysis of chosen proteins

**Dissemination of results/IPR considerations:**
- No patentable results identified yet.
2B. Antigen microarrays and autoimmunity repertoires

**Project aims:** Explore the potential of antigen microarrays as a tool to generate autoimmunity signatures in body fluids and new knowledge about the autoimmunity repertoires in various types of diseases as well as in non-disease individuals.

**Current status:**

**WP1:** Establish a standardized analytical framework.
- Automated experimental procedures established for sample handling on planar microarrays
- Procedure for bead-based verification phase established
- An advance LIMS and data warehouse has been partly developed for data analysis and visualization

**WP2:** Establish procedures for profiling of other body fluids.
- Both CSF, cerebrospinal fluid, and BAL, bronchoalveolar lavage are now being explored for autoimmunity signatures within the areas of multiple sclerosis and sarcoidosis

**WP3:** Develop a citrullination assay.
- Initial citrullination experiments have been performed, without any success

**WP4:** Explore multiplex profiling and affibody molecules as detection reagents
- This will be initiated 2013

**Dissemination of results/IPR considerations:**
- Results from autoimmunity profiling reveal massive heterogeneity among individuals
2C. Advancing antibody bead arrays for biomarker discovery

**Project aims:** Improve translation versatility and performance of bead-based protein profiling

**Current status:**

**WP1 – Advancing throughput for screening**
- 384-plex and 384-well assays established
- Co-profiling efforts ongoing, manuscript on CSF analysis to be submitted
- Segmented arrays in development

**WP2 - Advancing assays for screening**
- First test on emulsions performed, more to be done
- Implementation of Affibody molecules is ongoing

**WP3 - Verification assays for discoveries**
- First protocol for screening of antibody pairs in use
- Epitope mapped monoclonal antibodies to Fibulin-1 in preparation

**WP4 – Verifying technologies for new targets**
- Platform integration to be initiated

**WP 5 – Biomarker discovery and verification**
- Three DILI studies in data analysis (Acetaminophen, Momenta, Ethiopia)
- COPD study to be initiated

**Dissemination of results/IPR considerations:**
- Protein profiling using 10,000 antibodies in cancer and cardiovascular diseases
2D. Immunosequencing (iSeq) for highly multiplex protein analysis

**Project aims:** The main focus of the project is on a novel principle for simultaneous detection and quantification of protein abundances in complex samples, where a combination between immunorecognition (DNA-labeled antibodies) and massively parallel DNA sequencing is applied. The challenges to label antibodies with DNA, which is of general interest for the growing field of DNA-assisted proteomics, are addressed.

**Current status:**

**WP1: Immunosequencing enabled by DNA-antibody carrying magnetic particles.**
- An automated protocol is established for solid-phase conjugation
- Five antibodies, coupled to barcode probes on magnetic particles, were tested on PrEST arrays in a 3+2 manner (3 positives and 2 negative controls)
- Resulting amplicons of the barcoded probes were massively parallel sequenced.
- The 3 positive controls gave between 18000-49000 reads while the negative controls resulted in only 300-500 reads

**WP2: Conjugation in droplets.**
- This project is not initiated yet

**WP3: Antibody labeling.**
- A fully automated protocol for labeling scarce amounts of antibody is established
- Successful labeling with different fluorophores and biotin is done
- Stability of the labeled antibodies is under investigation

**Dissemination of results/IPR considerations:**
- A manuscript describing fully automated biotinylation and fluorescence labeling of antibodies is prepared. A second manuscript describing iSeq is under preparation.
Program Area 3: Microfluidics

Program Area Director: Helene Andersson Svahn

3A. Droplet microfluidics
Personnel (KTH): Helene Andersson Svahn (PI), Håkan Jönsson (Co-PI/PostDoc), Staffan Sjöström (PhD student), Yunpeng Bai (PostDoc)
Industrial partner: Novozymes A/S

3B. Lateral flow microarray assays
Personnel (KTH): Helene Andersson Svahn (PI), Jesper Gantelius (Co-PI/PostDoc), Thiru Raja Chinivasamy (PostDoc)
Industrial partner: ThermoFisher Scientific (Phadia AB)
3A. Droplet microfluidics

**Project aims:** To select functionally improved enzyme variants from a bacterial library by droplet microfluidics based high throughput screening

**Current status:**

**WP1:** Enzymatic activity based droplet sorting of amylase library
- Picoinjection and droplet sorting functional enabling workflow for screening at non-native conditions
- High throughput droplet based sorting of mock library shows high enrichment of amylase producing bacterial strain

**WP2:** Controlled retrieval/output of single clones and downstream droplet PCR
- Droplets interfaced with FACS through solidification for controlled output of encapsulated clones
- Emulsion intact following standard thermocycling (improved oil-surfactant formulation)

**Dissemination of results/IPR considerations:**
- Discussions ongoing with partner company
3A. Droplet microfluidics

**Project aims:** To select functionally improved enzyme variants from a bacterial library by droplet microfluidics based high throughput screening

**Current status:**

Injection of sub-picoliters in 1000 droplets per second

Analysis and selection of 2000 droplets per second
3B. Lateral flow microarray assays

Project aims: Improve sensitivity and detection options for LFM

Current status:

- In-house made dual-labelled GNP (anti-IgG, anti-IgE, Neutravidin and enzymes HRP, ALP)
- Employed with LFM in Rheumatoid Arthritis (RA), allergy and antibody array serum analysis

Next step: novel NPs

- In-house made dual-labelled GNP (an@-IgG, an@-IgE, Neutravidin and enzymes HRP, ALP)
- Employed with LFM in Rheumatoid Arthritis (RA), allergy and antibody array serum analysis

Dissemination of results/IPR considerations:

- A manuscript showing LFM in RA-analysis is planned to be submitted this year
- There could be IPR considerations for the method and novel GNPs.