# Project Status Reports

**October 12, 2010**

## Research Programme of Stage 2

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<th>Program</th>
<th>Programme Director</th>
<th>Project Leaders</th>
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<td>1. Affinity Tools</td>
<td>Sophia Hober</td>
<td>Amelie E. Karlström, Per-Åke Nygren, Afshin Ahmadian and Sophia Hober</td>
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<td>2. Epitope Technologies</td>
<td>Stefan Ståhl</td>
<td>Mathias Uhlén and Stefan Ståhl</td>
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<td>3. Plasma Analysis for Biomarker Discovery</td>
<td>Peter Nilsson</td>
<td>Jochen Schwenk, Lisa Berglund and Peter Nilsson</td>
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<td>4. Microfluidics and Nanobiotechnology</td>
<td>Helene Andersson Svahn</td>
<td>Helene Andersson Svahn</td>
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![Budgets for Programme Areas 1-4](image)
Programme Area Director: Prof. Sophia Hober

Personnel (KTH):
- 1A: Sophia Hober (PI) and Anna Konrad (PhD student)
- 1B: Amelie Eriksson Karlström (PI) and Peter Järver (PostDoc)
- 1C: Afshin Ahmadian (PI) and Mahya Dezfouli (PhD student)
- 1D: Per-Åke Nygren (PI) and Feifan Yu (PhD student)

Industrial partners: Atlas Antibodies AB, Mabtech AB, Olink AB, GE Healthcare Bio-Sciences AB, Affibody AB, Gyros AB, BioInvent International AB, Ortho-Clinical Diagnostics Uppsala AB.

Funds: 2 100 kSEK/year for two years
- 1A: 600 kSEK/year
- 1B: 600 kSEK/year
- 1C: 300 kSEK/year
- 1D: 600 kSEK/year

Total spending: (Apr 2009 – Jun 2010) (15 months; 62.5% of project duration)

<table>
<thead>
<tr>
<th>Category</th>
<th>Amount (SEK)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salaries</td>
<td>1 353 400</td>
</tr>
<tr>
<td>Rent</td>
<td>146 700</td>
</tr>
<tr>
<td>Overhead</td>
<td>434 800</td>
</tr>
<tr>
<td>Reagents</td>
<td>224 000</td>
</tr>
<tr>
<td>Travel</td>
<td>24 300</td>
</tr>
<tr>
<td>Other costs</td>
<td>200</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2 182 400</strong></td>
</tr>
</tbody>
</table>

52% of 4 200 kSEK

Subprojects:

1A. Exploitation of immunoglobulin-binding domains for antibody labelling (PI: Sophia Hober)

1B. Development of conjugation chemistries for DNA labelling of antibodies (PI: Amelie Eriksson Karlström)

1C. DNA-based barcode probes for multiplexed immunoassays (PI: Afshin Ahmadian)

1D. Development of immunoglobulin-binding domains with new specificities (PI: Per-Åke Nygren)

Status:
- Two new biotinylated variants of the Z domain for improved binding of streptavidin have been synthesized; evaluation in progress.
- Site-directed mutagenesis of the C2 domain to delete the Fc-binding capacity in favor of Fab-binding, work in progress. Eight different mutants have been constructed, produced and purified; evaluation in progress.
- Antibody-DNA conjugation via PNA (Peptide Nucleic Acid) tags for selective DNA hybridization, work in progress. PNA has successfully been chemically synthesized and conjugated to Z5BPA.
- Random antibody-DNA conjugation has been performed by a commercial kit based on hydrazone chemistry (Solulink). Optimization of washes in order to decrease the background signal has been successful.
- Site-directed mutagenesis of the Z domain to improve the binding to the mouse IgG1 antibody subtype. Improved binding to mIgG1 shown for ZF5R and ZF5I mutants. The ZF5I mutant was synthesized with BPA in position 24 and successfully UV-conjugated to mIgG1 and mIgG2. Evaluation of ZF5I as a ligand for affinity purification of antibodies from mouse hybridoma supernatants, work in progress.
Programme Area Director: Prof. Stefan Ståhl

Personnel (KTH):

2A: Stefan Ståhl (PI), John Löfblom (PostDoc), Nina Kronqvist (PhD student) and Magdalena Malm (PhD student)

2B: Mathias Uhlén (PI), Johan Rockberg (PostDoc) and Barbara Hjelm (PhD student)

2C: Mathias Uhlén (PI), Johan Rockberg (PostDoc) and Barbara Hjelm (PhD student)


Funds: 1 200 kSEK (+ 147 kSEK)/year for two years

2A: 600 kSEK/year
2B: 300 kSEK/year
2C: 300 kSEK/year

Total spendings: (Apr 2009 – Jun 2010) (15 months; 62.5 % of project duration)

Salaries: 1 414 000
Rent: 156 600
Overhead: 445 600
Reagents: 253 800
Travel: 32 400
Other costs: 300
Total: 2 302 700 SEK

Status:

• A new protocol for subcloning of randomized library DNA to the modified vector and transformation to bacteria has been established in collaboration with Affibody AB. The new protocol in combination with the new vector is currently being evaluated.

• Evaluation of bead-based selection of staphylococcal cells displaying recombinant proteins on the surface. If successful, bead-based selection will provide a complement to FACS for isolation of positive cells from cell libraries. The method would be useful both for selection of new affinity proteins as well as for epitope mapping purposes. Bead-based selection is readily automated and such possibilities will be investigated. A combination approach using first bead-based selection followed by FACS is currently being planned.

• Construction of an epitope library consisting of fragments of 50 different protein targets, work in progress. A modified target list of 71 proteins defined in collaboration with the industrial partners has been established. PCR amplification, fragmentation, and cloning from full-length cDNA clones has been undertaken for 68 different fragments. A library in S.carnosus with a library size of approx. 5 x 10^6 members is ready for surface expression evaluation. A protocol for optimized library construction has been published (Rockberg et al, Curr. Protoc. Immunol., 2010).

• Construction of epitope libraries for 12 proteins with known 3-D structure, work in progress. A pilot study with a human tryptophanyl-tRNA synthetase (WARS) library for epitope mapping of a polyclonal and a monoclonal antibody has been published (Hjelm et al, New Biotechnology, 2009). The project is proceeding according to plan with the construction of the libraries for the 12 different proteins.
Programme Area Director: Assoc. Prof. Peter Nilsson

Personnel (KTH):

3A: Peter Nilsson (PI) and Mårten Sundberg (research engineer)
3B: Jochen Schwenk (PI), Ulrika Igel (PhD student), Maja Neiman (PhD student) and Anna Häggmark (PhD student)
3C: Jochen Schwenk (PI), Ulrika Igel (PhD student), Maja Neiman (PhD student) and Anna Häggmark (PhD student)
3D: Peter Nilsson (PI), Burcu Ayoglu (PhD student), Ronald Sjöberg (research engineer) and Anna Häggmark (PhD student)
3E: Lisa Berglund (PI) and Linn Fagerberg (PhD student)


Funds:

2 100 kSEK (+ 294 kSEK)/year for two years

3A: 600 kSEK/year
3B: 300 kSEK/year
3C: 600 kSEK/year
3D: 300 kSEK/year
3E: 300 kSEK/year

Total spendings: (Apr 2009 – Jun 2010) (15 months; 62.5 % of project duration)

Salaries: 995 300
Rent: 120 700
Overhead: 328 100
Reagents: 141 900
Travel: 24 500
Other: 17 400
Total: 1 627 900 SEK

39 % 4 200 kSEK

Subprojects:

3A: Development of antibody arrays for parallel analysis of thousands of protein targets (PI: Peter Nilsson)
3B: Development of high-throughput analysis of serum/plasma based on suspension bead arrays (PI: Jochen Schwenk)
3C: Analysis of disease sample cohorts (PI: Jochen Schwenk)
3D: Development of large PrEST-arrays for analysis of autoimmunity (PI: Peter Nilsson)
3E: Bioinformatic analysis of the human secretome (PI: Lisa Berglund)

Status:

• A new microarray instrument from ArrayJet has been installed in September, 2010, and a new beta-site scanner from CapitalBio has been installed in October, 2010.
• The massive pilot study of antigen-based plasma profiling with 96 MS plasma samples on 11,520 antigens (210 microarray slides) has been completed and analyzed. The first validation phase will be done on Luminex beads with 384 selected antigens. Experiments of antigen-based profiling with CSF from the MS patients have been initiated. A validation cohort for the kidney toxicology findings has been received and is analyzed (at KTH and AstraZeneca). The initial findings are supported.
• For sample preparation, heat has been evaluated in terms of the impact on protein profiles and heating after sample labeling is preferred due to lower variance. Additives such as SDS have been evaluated, and while SDS in assay buffer does result in clogged assay buffer, washing with low concentrations of SDS appears promising. A protocol for Affibody molecule-based depletion of IgG and albumin is now available and being used to prepare serum and plasma samples for Western blot analysis. Only small amounts of sample is consumed and up to 48 samples can be depleted in parallel.
• Protocols for validation by Western blot and immunoprecipitation are now available. Protocols for MS-based analysis and sandwich immunoassays currently under development.
• Analysis of all the 20,734 human genes in the Ensembl database for the presence of signal peptide, transmembrane regions and ER-retention signals using different prediction methods. 15% of the genes (3,211 genes) predicted to encode a secreted protein. Comparison to lists of secreted proteins in literature.
### Programme Area 4. Microfluidics and Nanobiotechnology

**Programme Area Director:** Prof. Helene Andersson Svahn  
**Personnel (KTH):**  
4A: Helene Andersson Svahn (PI) and Håkan Jönsson (PhD student)  
4B: Helene Andersson Svahn (PI) and Jesper Gantelius (PostDoc)  
**Industrial partners:** Phadia AB, Novozymes A/S, GE Healthcare Bio-Sciences AB.  
**Funds:** 600 kSEK (+ 147 kSEK)/year for two years  
4A: 300 kSEK/year  
4B: 300 kSEK/year  
**Total spendings:** (Apr 2009 – Jun 2010) (15 months; 62.5 % of project duration)  
- **Salaries:** 515 700  
- **Rent:** 59 700  
- **Overhead:** 169 200  
- **Reagents:** 48 300  
- **Total:** 792 900 SEK

### Status:

- We have implemented dual channel fluorescence detection and a method for patterning injectable electrodes on the droplet platform.  
- We have successfully performed PCR reactions in microfluidic droplets and developed passive sorting at rates of >10000 droplets/second.  
- We have detected enzyme secreted from encapsulated bacteria inside the droplet by collecting the fluorescence from a fluorogenic substrate processed by the enzyme.  
- The lateral flow microarray setup is currently further evaluated in collaboration with Phadia AB, for antibody microarrays (IgE and Fab-fragment-based), autoantigen peptides for analysis of Rheumatoid Arthritis, and Protein Eptope Signature Tags (PrESTs). A PrEST lateral flow microarray could hold the potential of rapid and inexpensive quality control testing of purified IgGs generated from PrESTs.

### Subprojects:

- **4A:** Development of “in-house” microdroplet fluidics (PI: Helene Andersson Svahn)  
- **4B:** Development of lateral flow microarray platforms for on-site diagnosis (PI: Helene Andersson Svahn)