Evaluation of
ProNova VINN Excellence Centre for Protein Technology
A VINN Excellence Centre
at
KTH

1. Introduction
On 7 November 2014, the Chair of the Centre Board, Björn O. Nilsson, board members, the Centre Director, Per-Åke Nygren, colleagues of the ProNova VINN Excellence Centre, PhD students, external partners, and university representatives had a formal interview with three members of the evaluation team (Mary O’Kane (Chair) and Alison McKay as generalists and Kristiina Takkinen as specialist). The evaluation team also included Sabeth Verpoorte as the remote specialist evaluator. At interview Mats Jarekrans, Margareta Danielsson and Thomas Eriksson were also present on behalf of VINNOVA. We thank all members of the Centre and the VINNOVA teams for their efforts in providing information for the evaluation via the self-evaluation report and the meeting with the evaluation team.

This evaluation is particularly focused on the output from the Centre in the form of scientific, societal and industrial results and the impact of this output.

ProNova operates using a model that differs somewhat from that used by most of the other VINN Excellence Centres and from the model implied by the VINN Excellence Evaluation Guidelines. In ProNova partner companies (large and small) pay a common (relatively low) cash contribution but contribute in-kind resources differentially, depending on the level to which they wish to engage with particular Centre projects. While the industry partners are consulted extensively at the beginning of each Stage as to what projects they would like the Centre to engage in, the Board takes the final decision on exactly what will constitute the projects for that Stage. The Centre then aims to (and does) deliver top-level research in carrying out these projects so that industry partners can pick up top-level know-how through participating in the projects. However, the Centre does not use any specific industrial/economic targets as Centre key performance indicators. Rather the Centre has agreed with VINNOVA that it be judged primarily on its scientific impact, confident that the partner companies will make good use of what they learn and that, long term, this will lead to good industrial impacts.

It was established at interview that this ProNova operating model had been agreed with VINNOVA when the Centre was established and that, in retrospect, there clearly had been misunderstandings at previous evaluations as the relevant evaluation teams had not been made aware that this particular operating model applied to this Centre.

Recommendation to VINNOVA
That VINNOVA revise the evaluation guidelines to indicate that VINN Excellence Centres should be evaluated against the agreed (with VINNOVA) success criteria in each Centre’s Operational Plan for that Stage.

2. Long-term Vision, Mission and Strategy
The Vision, Mission and Strategy for the Centre are appropriate.
3. How the Centre addressed the recommendations of the previous Review
The Centre has addressed the recommendations of the previous evaluation reasonably given the misunderstanding about its operating model, referred to above.

4. Centre Partners
The Centre has a good range of industrial partners, each of which pays a cash membership fee to participate in the Centre. The participation of at least one non-academic partner in each Stage 3 project is a strength of the Centre. In response to Recommendation 7 from the Stage 2 evaluation, the Centre has established a way of calculating the in-kind value of antibody reagents.

Processes for needs identification and project selection are open and transparent within the Centre.

5. Scientific Quality and Productivity
Research area, competence profile, people, facilities, critical size, and processes for ideas generation
With the Centre in its 8th year of operation, the research area of protein technology remains extremely relevant for the medical and life sciences. Proteins come in an enormous variety of forms having a wide range of functions, from metabolism to immune response, DNA replication to structural and mechanical function. Much remains to be learned about the role of proteins in cellular processes, particularly those related to disease pathophysiology. Being able to detect and analyse proteins is thus essential for the further understanding of these processes.

There are three program areas related to affinity tools and protein engineering, array technologies, and microfluidics. They form a complementary program requiring researchers who have a skill set ranging from biotechnology through biochemistry to engineering. The competence profile of the Centre includes 38 researchers whose combined expertise covers all program areas. The 13 academic project leaders are all recognized experts in their individual fields, and are active in terms of both written output and training young researchers. The facilities at the KTH and SciLifeLab appear to be excellent. The connection of ProNova with the Human Protein Atlas, which offers the Centre access to a huge set of greater than 21,900 antibodies for screening human proteins, offers researchers a unique and powerful tool.

As to the Centre’s size, there are 12 projects, described as “small” in the report, running in parallel, and divided up fairly evenly over the three programs. 38 researchers means an average of about 3 scientists per project – which more or less confirms the description “small”. Given that there are 10 participating company partners, 12 is a good number of projects, with the possibility for companies to select and participate in their projects of choice. More projects for the same number of companies and academic researchers is not recommended, in the view of the evaluators, as this would dilute efforts in all projects and risk far fewer significant results. The companies are significantly different in their expertise and the products they represent, and there is industrial interest and active participation in most of the academic projects running. The Centre appears to have achieved a critical size to pursue most of the work proposed by the program area leaders.
As described in the Plans for Development section of the Stage 3 evaluation report (pp, 28-31), it may be time to consolidate efforts into a smaller number of larger projects that build on results of more advanced Stage 3 projects. The idea of reorganizing the projects into two new program areas (Next Generation Diagnostic and Global Views on Autoimmunity, and Anti-Drug Response and Allergy) is a good one, as it takes existing projects and recasts them into new fields. In this way, company interests in the Centre will be maintained and the mission of the Centre to impact products, services or standards in companies and society as a whole will achieve greater unity and a more concrete form.

**Scientific output and impact of scientific results**

Program Area 1, “Affinity tools and protein engineering”, contains five projects. In the project 1A (PIs Prof Ståhl and Dr Löfblom) an E.coli bacterial display system with an optimised expression vector has been developed based on earlier published and patented innovation of a German group. The patent is expiring around 2016 and after that this E.coli display system, which is now ready for construction of large libraries of biobinders, can be used by the ProNova industrial partners for the development of biopharmaceuticals. Affibody AB is the industrial partner providing know-how for library constructions and biobinder selections.

In the projects 1B (PI Prof Hober), 1C and 1E (PI Prof Eriksson Karlström) and 1D (PI Prof Nygren) the small Ig-binding domains of protein G have been engineered to achieve site-specific labelling of the Fc or Fab regions of an antibody molecule e.g. for immunoassay, immobilization and in vivo imaging applications. The advantage of this ProNova-invented technique is the capability to synthesize the labelling peptides domains chemically with desired functional groups. In the project 1B a variant of the C2 domain with two mutations and incorporated photo activable amino acid p-benzophenylalanine to two different positions, specific labels for human and mouse Fab fragments have been produced. Project 1C has further developed the ProNova method for site-specific labelling of antibodies for in vivo imaging applications. Variants of protein Z domain have been engineered to enhance the labelling efficiency of human and mouse IgG1, commonly used in therapy and diagnostics. A further approach includes conjugation of these site-specific labelled antibody molecules with optimized linkers to magnetic nanoparticles for molecular imaging applications. In the project 1D a homogeneous, one-step immunoassay for antigens has been established. The ProNova technique for site-specific covalent labelling of antibodies is further exploited to label antibodies (binding nearby epitopes) with sub-fragments of the reporter enzyme beta-lactamase. Applicability of this “mix-and-measure” assay is demonstrated for the model target HER2. In the project 1E (started in April 2013) the aim is to evaluate antibody labelling with 18F for site-specific photo conjugation to antibodies especially for PET imaging applications. The results of all these antibody labelling projects are scientifically interesting and could impact the product development of the industrial partners as stated e.g. by GE Healthcare Bio-Sciences, Genovis AB, Mabtech AB and AstraZeneca AB.

Program Area 2, “Array technologies”, contains three projects. In the project 2A, “Antibody characterization and purification” (PI Dr Rockberg), tools to characterize the nature of the antibody binding linear or conformational epitopes have been established using peptide arrays or Staphylococcal display. The characterization of the binding properties is important for the validation of best performing antibodies e.g. for immunoassays. The impact of this project is clearly summarized by the industrial partners Affibody and SOBI AB exploiting the developed technique to map the
conformational binding site of a therapeutic Affibody providing understanding of the binding mechanism of this drug candidate important for the further development phase.

In the project 2B, “Antigen microarrays and autoimmunity repertoires” (PI Prof Nilsson), the main aim is to exploit extensive peptide or protein domain arrays representing the human proteome for identification of autoimmune targets. This project exploits the unique large collection of human Protein Epitope Signature Tags (PrESTs) available to ProNova through the Human Protein Atlas project. The power of this technology was convincingly demonstrated by identification of new multiple sclerosis (MS) disease associated autoantigen candidates. Of the candidates anoctamin (ANO2) protein, also expressed in the brain, was revealed as a prominent MS target. This finding is highly interesting scientifically and moreover has an important impact providing tools for the development of more precise diagnostics and treatment of the MS disease.

In the project 2C, “Advancing antibody bead arrays for biomarker discovery” (PI Assoc Prof Schwenk), sensitive dual antibody based immunoassays are developed and optimized for validation and clinical assay set ups of protein biomarkers. The assay optimization has been done for CDH5 and FABP1 proteins identified during Stages 2 and 3 as biomarkers of liver failure in a single binder screening assay. The identification of the liver failure biomarkers is one of the key results of the ProNova programme. If further validation, that is currently going on in the IMI project SAFE-T, verifies that these proteins are new and improved safety biomarkers of liver toxicity, the impact for enhanced drug development process can be exceptionally high.

In the project 2D, “Immunosequencing (iSeq) for highly multiplex protein analysis” (PI Assoc.Prof Ahmadian), a miniaturized, multiplexed bead-assisted assay based on immunorecognition of the biomarker with DNA-labelled antibodies combined with high-throughput sequencing is under development. The developed multiplexed DNA-barcoding approach for labelling of valuable antibodies at nanogram scale is highly advantageous. The industrial partner Atlas Antibodies AB is providing the antibodies for the project.

The Program Leader of the Microfluidics program, Prof Andersson-Svahn, is well respected in the microfluidics community, as supported by the substantial number of invitations she has received for oral presentations both in Sweden and abroad over the past three years, at international conferences and institutes. Her particular strength in the past few years is the application of microfluidic approaches to clinically relevant problems which require high-throughput information generation. She has successfully established microdroplet technology in her lab for directed enzyme evolution, as well as high-density protein arrays on paper for allergen screening. Novozymes, the industry partner in this former work, comments on the fact that the company has direct access to training in this approach via ProNova, and sees promise for future developments in the company. ThermoFisher (formerly Phadia) is a large contributor of lateral flow assays to the market, and therefore has been involved in the allergen test strip development. Both developments are at the forefront of microfluidics research in terms of applications development. At the same time, the Andersson-Svahn group also carries out technology developments to realize these applications. The pico injector, designed to inject pL amounts of reagents into microdroplets containing single cells or other biological entities, is an example of this. Her group, consisting of researchers with different backgrounds, is thus very versatile and inventive. Prof Andersson-Svahn and her coworkers have published in high-impact journals over the period 2012 to 2014. In fact, of the Centre's
publications in 2012, her paper in Angewandte Chemie has garnered the most citations (29 – an impressive number in less than two years).

**International comparators with other Centres and Collaborations**
The Affinity Tools and Protein Engineering and Array Technologies programmes in the ProNova Centre are scientifically excellent and in the forefront when compared to other international groups. The ProNova Centre has unique access to the largest validated antibody resource against human proteome as well to the protein epitope signature tags (PrESTs) of whole human proteome generated in the Human Protein Atlas project. This unique position regarding human proteome specific reagents is efficiently exploited throughout the ProNova programme.

The Microfluidics program in the ProNova Centre, though perhaps somewhat small, is excellent and compares well with other international groups. The program has found an important niche, namely protein and (single) cell analysis. To the best of this reviewer’s knowledge, the inclusion of microfluidics as a program in a larger protein technology centre is quite unique.

**Overall conclusion - scientific quality and productivity**
The Affinity Tools and Protein Engineering and Array Technologies programmes are scientifically excellent and productive as measured by the publications in this highly competitive scientific field. The Microfluidics Program program has demonstrated an excellent level of scientific quality and productivity. Prof Andersson Svahn’s publications have outperformed those of her colleagues in terms of citations. She has become well known for her work in the area of microdroplets applied to biochemical and clinical problems.

**6. Output and Impact - output from and impact of the Centre in the form of societal and industrial results with particular focus on impact on Centre partners**
The Centre’s scientific output is recognised as being excellent and of high academic impact. Its impact on industry is more diffuse, and so difficult to quantify directly. However, at interview the Centre Director presented an “impact landscape” that illustrated the different kinds of impact coming from the Centre and the industrial partners described the high value they associate with knowledge and know-how that is coming from the Centre. In addition, two company representatives presented very strong examples of how the Centre’s work impacted their product development processes. These included results being used in the following ways: as the basis of proof-of-concept projects within companies; in the discovery and development of biomarkers; and as integral parts of the company’s innovation processes.

**7. Organisation and Management of the Centre**
The governance and management of the Centre appear sound. The evaluation team notes the Centre intends to rethink the operation and composition of its International Scientific Advisory Board for Stage 4.

The management finances tables appear complete. The notes associated with the finance tables were very useful for the evaluation. The Centre was allocated 21.75 MSEK from VINNOVA for Stage 3; there has been a small underspend on this because Stage 3 is not due for completion until March 2015. KTH is expected to contribute in excess of 26 MSEK and the industrial partners 1.24 MSEK in cash and in excess of 20 MSEK in kind.
8. Training Personnel of High Competence
The PhD students we met at the interview valued their membership in the Centre and noted that their engagement with industry partners through projects improves their appreciation of potential applications of their work. The students found their interactions with the wider industry group in Centre-run events useful and identified more opportunities for work experience, e.g. a week in a company doing lab work which a small number of students already receive, as an improvement opportunity.

Recommendation: That the Centre explores the provision of work experience opportunities for PhD students in partner companies.

9. Long-term development during stage 4 and beyond
The evaluation team notes the Centre’s clear thinking about the issues to face for Stage 4. The Centre will work during Stage 4 to find a way to preserve valuable aspects of the Centre beyond Stage 4, noting that direct continuation at a lower level is unlikely because of the modest industry contributions. Bilateral projects are a possibility however.

Recommendation to Strengthen the Centre
In summary, our recommendation to the Centre is:
Recommendation: That the Centre explores the provision of work experience opportunities for PhD students in partner companies.

Recommendation to VINNOVA
That VINNOVA revise the evaluation guidelines to indicate that VINN Excellence Centres should be evaluated against the agreed (with VINNOVA) success criteria in each Centre’s Operational Plan for that Stage.

Conclusion
ProNova is performing well. The evaluation team recommends continued funding.

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