FINAL PUBLISHABLE SUMMARY REPORT

Project No: 220701

ExIV – Expanding the Potential of in vitro Compartmentalised Screening and Selection Approaches

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Research training.

The PURE system (Protein Synthesis Using Recombinant Elements) was adapted for selection of binding proteins using SNAP-tag display was adapted for SNAP-tag display of antibody fragments in selections for protein binders to lysozyme (as a model target protein) . The SNAP-tag technology had been developed in the Cambridge group and consists of the *in vitro* creation of a covalent genotype-phenotype linkage between DNA and expressed protein in microdroplets that ensure that the very protein encoded by a linear DNA fragment is covalently coupled to it. Recent work of the Hollfelder group had shown that the protein expression efficiency in droplets in limited and may significantly impede progress in directed evolution in droplets. Therefore I probed whether these issues could be addressed in a different formulation, adding DNAK, GroEL and protein disulfide isomerases (PDIs). These measures have been shown to improve the expression of antibodies, for which selections have to be carried out under oxidative conditions. To this end I have performed model selections that yielded antibodies with μM binding affinity. Controls suggested that successful selections were only possible using the newly developed protocol: In the presence of 3.5 mM of glutathione, the addition of PDI improved recovery by >30-fold. Western blotting showed that antibody binding is not compromised under these conditions.

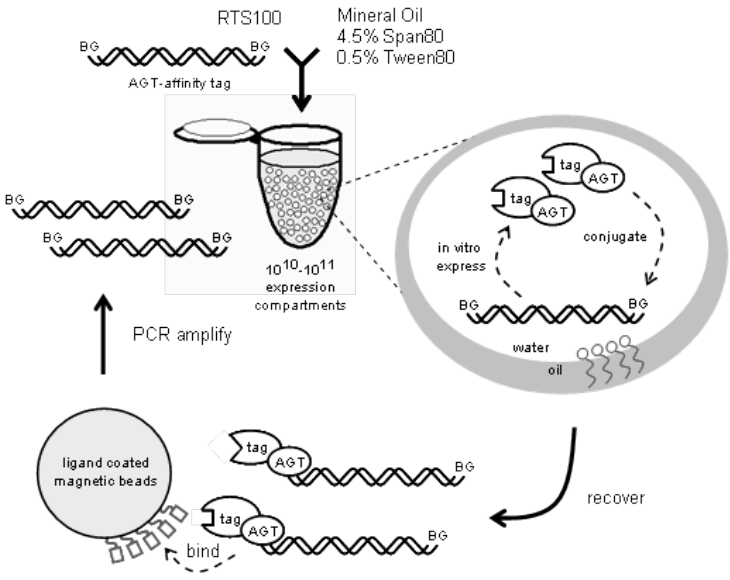

Figure. A covalent genotype phenotype linkage. A linear DNA template coding for a fusion protein comprised of AGT and the protein to be selected is labeled with benzyl guanine (BG), a substrate of AGT. Templates are then compartmentalized in water-in-oil emulsion droplets, so that on average, less than one DNA template is present per droplet. In the presence of a coupled *in vitro* transcription-translation system, AGT-fusion proteins are expressed and react covalently and irreversibly with BG. Following recovery, fusions carrying the target protein are enriched on a solid phase immobilized ligands that bind the target protein, and the coding DNAs amplified by PCR.

Building in this procedure I wanted to conduct selections with carbohydrate binding domains (CBDs) and started to clone a selection of these proteins. A number of these proteins did not show good expression in *in vitro* expression systems, neither in PURE, nor in commercial Roche or Invitrogen kits. Towards the end of the project I have established that visible enrichment can be observed in model selections with one CBD. Proper selections could not be performed in the timeframe of the project, because of the many practical obstacles encountered. However, other members of the host group will take up this project in the future. The – by now robust – protocol to perform selections from protein libraries with disulfide bonds will help to achieve the goal of actual selection of CBDs. I have set up assays for catalytic selections that will allow screening of a catalytically competent protein after evolving the binding capabilities of the CBD.

Transfer of knowledge.
All transfer of knowledge objectives have all been addressed. On the experimental level I have introduced several new "tricks" into the Hollfelder group that have helped to make the protein expression in emulsion microdroplets more efficient. At the same time I have learned to use the droplet-based directed evolution system developed in Cambridge and have experienced its power, which will be an excellent tool to take back to Japan.
I have attended training events and seminars that deal with microdroplets in microfludics (part of a weekly interdisciplinary series of events) and have learned about other ongoing projects in the Hollfelder groups in groups seminars and personal discussions.
I have interacted with researchers in two EU networks coordinated by Dr Hollfelder, ProSA and ENEFP. The use of the PURE system was initially not common in these networks and I hope that I have been useful for implementing this system more widely in European groups.

Dissemination
At network meetings organised by Dr. Hollfelder who is coordinator of two relevant networks, ProSA and ENEFP, I have been able to present my work to an international audience, received useful feedback and have been able to get in touch with many European researchers, junior and senior. In preparation for these presentations, Dr Hollfelder worked with me on my presentations skills and helped me to maximise the impact of my presentations.
Target groups to use this technology are researchers in biotech companies and academic groups that are interested in protein engineering, eg of generating protein therapeutics based on antibody or related scaffolds or, more generally binding proteins or domains for a wide range of applications.