Project name:
Yeast surface display of Protein A mimics: Directed evolution, characterization and application

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Project overview
Affinity purification requires the availability of binding reagents with both the necessary molecular recognition properties (affinity, association rate, specificity, reversibility) and industrial processing properties (robustness to immobilization and multiple rounds of purification and regeneration). Protein A meets this need for monoclonal antibody purification, however falls short in requiring acidic elution conditions that partially denature and consequently cause the aggregation of many antibodies.
Advances in molecular modelling *in silico*, genetic engineering and biological and chemical combinatorial methods, combined with high-throughput screening (HTS) technologies provided important means for generating and selecting new biologically active molecules, tailored to specific biotechnological needs. These molecules that often display enhanced robustness, resistance, stability and cost efficient production as compared to their natural templates include engineered antibody-like molecules such as affibodies (Nord et al., 2001, Fernandez, 2004), novel antibody-mimic domains based on resistant protein scaffolds (Vaughan and Sollazzo, 2001, Holt et al., 2003, Lipovsek et al., 2007) small peptides (Fassina et al, 2001; Liu et al., 2007), and triazine-based synthetic affinity ligands that mimic the interaction of natural specific receptors with their complementary proteins (Lowe et al., 2001).
The main goal of this sub-project is to develop single fibronectin Ig domains with binding affinity mimicking that of Protein A by a yeast-surface display combinatorial approach. The particular aim is to develop new purification methodologies, with emphasis on milder elution conditions to avoid antibody aggregation. The stability and binding properties of the Protein A mimics will be engineered by both directed evolution and rational design. The Protein A mimics will be thoroughly characterized and used as alternative ligands for the affinity purification of antibodies from different species, classes and subclasses. The thermal stability, stability to caustic treatment, and binding properties will be engineered by directed evolution, and then thoroughly experimentally characterized.
These binders will also be used as secondary reagents for the development of standard immunoassays such as ELISAs, and immunofluorescence methods.

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Faculty involved:
K. Dane Wittrup (MIT), Angela Taipa (IST)

PhD student(s) involved:
MIT: Margaret Pawlowski, Ben Hackel. IST: TBA

Expected deliverables:
At the completion of task 1 it is expectable that complex libraries of different sizes displaying Fn3 variants (Protein A mimics) will have been designed and partially synthesized. At the completion of task 2 it is expectable that libraries of different sizes will have been characterized and optimized for the selection of medium-to high affinity binders that mimic the interaction of Protein A with immunoglobulins from different types and species. By a dual screening approach is expected that leads of Protein A mimics (3-5) with high affinity to IgG and improved elution/stability profiles have been identified for further development and application.
Potentially the greatest impact of this task / work will be the isolation of affinity purification ligands that do not require denaturing conditions for elution. Soluble selected lead ligands will be produced and affinity purified in high quantities, and available for further bioprocessing studies and immuno applications. At the completion of this task, it is expected that ligands for the major species and subclasses of IgG will have been identified and thoroughly characterized. At the end of this task, it is expected that an efficient process for the one-step purification and/or resolution of IgG from different species and subclasses by affinity chromatography with a cost-effective, durable and stable lead-adsorbent will be well established an optimized.

Results:
We have constructed the fibronectin library and isolated initial lead binders against rabbit, goat, and human IgGs. These will serve as leads for further evolution.

Timeline (through August 08):
The IST student will come to MIT this summer to commence a year in residence in the Wittrup lab on the project.