Fast Screening for Expression, Biophysical-properties and Affinity (FASEBA)

Summary

With FASEBA one can obtain information on expression levels, biophysical properties and affinities of a large number of proteins at early stage without protein purification.

What are the problems

Lengthy process and uncertain outcome of antibody/protein engineering due to

(Purification and) characterization of a large number of candidates, many of which

may have poor expression

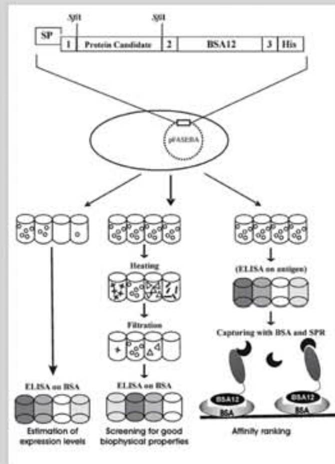
may form aggregates and have low stability

may not have desired affinity

➔ Lack of an efficient approach for screening candidates at early stage

Our approach

1. Construct an antibody/protein library fused to a **protein anchor**
2. Obtain cell lysates containing candidate protein-anchor protein (BSA12) fusions
3. Expression level: Specifically and completely capture individual fusion proteins and determine the amount of fusion proteins captured
4. Biophysical property: Treat samples under denaturation condition, remove formed aggregates and compare percentage of fusion proteins removed
5. Affinity: Inject antigen to captured candidate protein-anchor protein fusions, and analyze binding profiles by surface plasmon resonance



Requirements to the protein anchor and BSA12

Extreme affinity to its base	K_D = 4 pM
Readiness of interruption of such interaction	100% removal of binding by pH 2 solution
Resilient base	BSA can resist many rounds of surface regeneration
Stability	$T_m = 70\ ^{o}C$
Small in size	13 kDa

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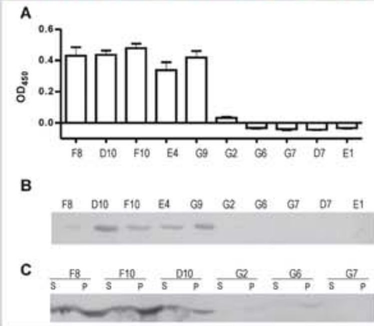
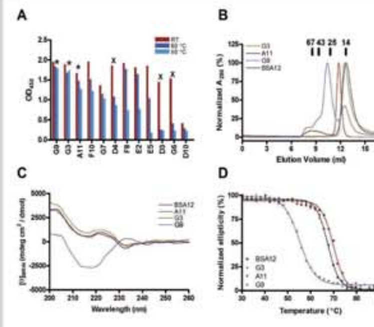
Estimation of expression levels



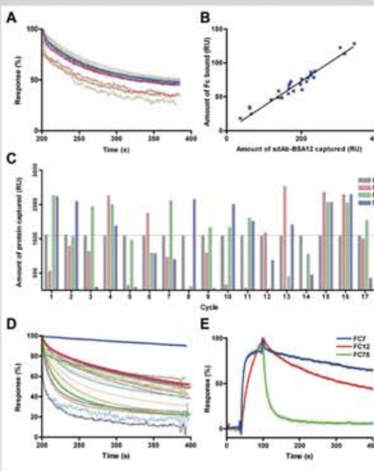
Readings of ELISA on BSA (A) is a good indicator of the expression levels of both candidate protein -anchor protein fusions (B) and candidate proteins themselves (C). In addition, expression levels can be quantified while measuring affinities of the candidate proteins by injecting the fusion proteins onto BSA surface (data not shown).

Screening of biophysical properties



Two of the three human single domain antibodies (sdAbs) predicted to have good biophysical properties selected by FASEBA (* in A) exist mainly as monomer (B), have typical circular dichroism profiles of stable sdAbs (C) and have high thermostabilities (D), whereas the yields of all three proteins predicted to have bad biophysical proteins (x in A) are too poor to be purified for analysis.

Ranking of dissociation rates



Good data reproducibility (A and B) is the prerequisite of affinity ranking. Resilient BSA surface, seen as practically identical level of protein captured through 17 rounds of the experiment (C, dashed line), provides a solid basis for automated affinity estimation. Measurement of binding profiles of 48 sdAbs generated an order of their dissociation rates (D), which is the same as the order of their affinities (E). Accurate affinities can be determined using a SPR instrument allowing injection of multiple concentrations of the antigen.

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