Optimise phage display workflows with dip-in kinetic analysis

Kris Ver Donck, Filip Delport | FOx BIOSYSTEMS NV, Agoralaan Abis, 3590 Diepenbeek

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Breaking limits in bioanalysis

Abstract

Phage display in conjunction with biopanning is a frequently used strategy in the selection process for single domain antibodies and other binding proteins with specificity to a target antigen. Here we present a workflow optimization by monitoring biopanning and performing early selection using kinetic characterization direct on phage libraries and phage clones. From clone selection with off-rate ranking to real-time kinetics characterization providing k_{on} and k_{off} data on normalized clones, more insight and efficiency as compared to endpoint techniques such as ELISA.

Figure 1 – Binding measurement method using dip-in sensors

Fiber optic surface plasmon resonance (FO-SPR) dip-in probes in 96 well plates on WHITE FOx. Probes functionalized with the target protein were used to selectively bind peptides and proteins expressed on M13 phage coat proteins.

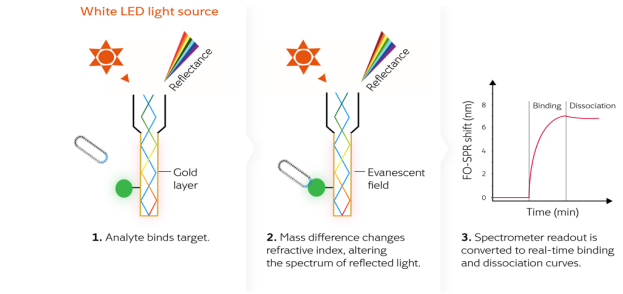


Figure 2 - Monitoring panning for polyclonal section guidance

(A) M13 phage libraries expressing lactoferrin binding peptides are grown and captured on lactoferrin-functionalized sensor probes. (B-C) clones eluted at pH 2.5, 2.0 and 1.5 to be used in subsequent culture cycles. The selection process is monitored with FO-SPR. Real-time binding sensorgrams of phage binding for the three subsequent biopanning rounds are shown.

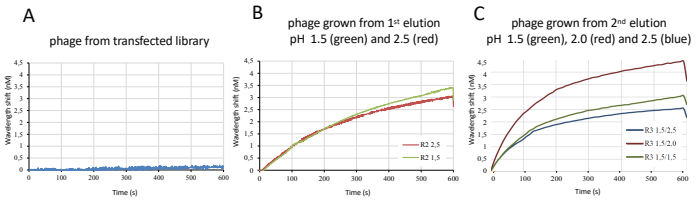
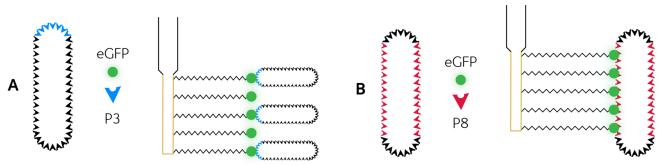


Figure 3 – Biopanning for eGFP binding phage

M13 phage libraries expressing peptides on either p3 or p8 viral protein are grown, captured on streptavidin-biotin-eGFP coated microtiterplates and eluted to be used in subsequent culture cycles for 3 rounds of panning.



Methods

As a model phage display system, libraries of filamentous M13 phage were used, displaying peptides with selective affinity for a target protein, either at the N-terminus of the p3 protein (5 sites at end of the viral filament) or p8 protein (up to 2700 sites along the viral filament). Phage were incubated with *Escherichia coli* at 37 °C for 30 min. Infected cells were grown overnight in NZY-tetracycline-medium for phage amplification.

BIOPANNING: 1) Lactoferrin: libraries of M13 phage displaying peptides with selective affinity for lactoferrin were used. Solid phase affinity selected phage were eluted for 5 minutes in 10 mM glycine, pH 2.5, 2.0 and 1.5 successively. Phage grown from elution were tested for binding to lactoferrin-immobilized FO-SPR sensor probes. 2) eGFP: libraries of M13 phage displaying peptides with selective affinity for enhanced green fluorescent protein (eGFP) were subjected to three rounds of solid phase selection in streptavidin-coated microtiter plates using biotinylated eGFP. Single clone phage were isolated, tested for binding to eGFP comparing ELISA and FO-SPR and identified using DNA sequencing.

FO-SPR BINDING CHARACTERIZATION: Streptavidin probes were used on WHITE FOx to capture biotinylated eGFP 10 µg/mL as a selective binding layer. Phage binding (at pH 6.0) and phage dissociation steps were performed for 15 minutes each. Probes were washed for 60s in 10 mM glycine pH 2 for cleaning.

ELISA: was performed in streptavidin-coated microtiter plates using biotinylated eGFP for 1.5h at RT, followed by incubation with rabbit anti-fd bacteriophage antibody and alkaline phosphatase conjugated goat anti-rabbit IgG for 1h at RT. After extensive washing with MES, the substrate (p-nitrophenylphosphatedin in 1 M diethanolamine, 1 mM $MgCl_2$, pH 9.8) was added and monitored at 405 nm for 15 minutes.

Figure 4 - Comparison with ELISA

Comparison of FO-SPR with ELISA for M13 clones expressing eGFP-binding peptide with a low number of P3-expressed binding sites (P3), elevated number of P3-expressed binding sites (P3*), and P8-expressed binding sites (P8) to eGFP.

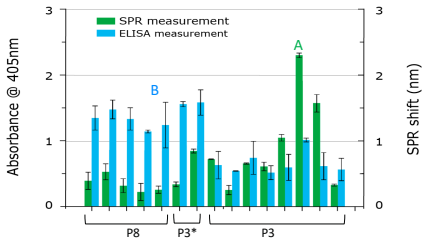
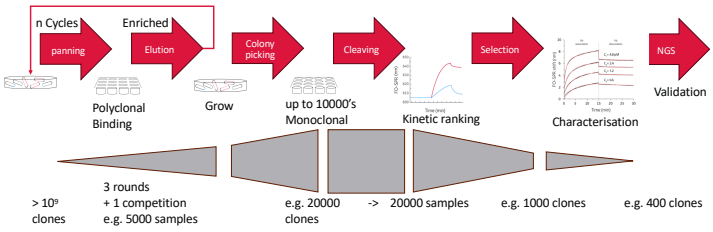


Figure 5 – Workflow optimization

(A) Regular workflow biopanning, clone selection and characterization. ELISA preference for high producers and high avidity samples. Numbers of clones indicated as example; numbers can differ by project.



(B) Efficiency gain by fast in workflow monitoring, and kinetic characterization direct on phage. Validation only on a limited number of selected clones, using the same kinetic analysis method. Numbers of clones indicated as example; numbers can differ by project.

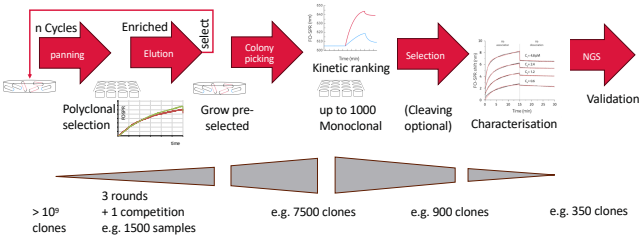
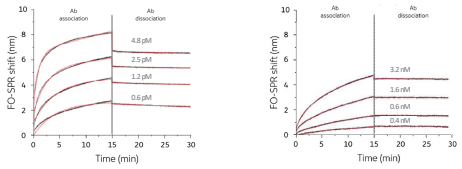


Figure 6 - Characterization

Clones selected from biopanning against eGFP were analyzed for kinetic affinity using dip-in FO-SPR. Selected low-affinity clones presented nanomolar K_d values, while high affinity clones presented picomolar K_d values. Kinetic profiles are shown for the FO-SPR top scoring clone (left) (A in fig 4) with a K_d of $1.2x10^{-14}$ M which scored low on ELISA and an example of the ELISA top scoring clones (right) (B in fig 4) with a K_d of $1.3x10^{-11}$ M which scored low on FO-SPR.



Conclusions

This study presents WHITE FOx dip-in FO-SPR based kinetic analysis of protein binding affinities on whole M13 phage particles, where p3 or p8 coat protein expressing constructs were tested in a biopanning procedure selective for a target protein.

The advantage of this analysis method is i) it can be combined with solid phase selection based biopanning workflows such as magnetic beads or ELISA plates to provide fast selection of polyclonal eluates, and ii) the same method can provide kinetic ranking and detailed kinetic characterization on monoclonal phage directly, reducing number of samples and the need for a cleaving cycle to validate clones. In a confirmation run selected cleaved clones can be analyzed with the same assay protocols on WHITE FOx. No protein isolation or sample purification was required. The label-free kinetic characterization allows for individual determination of k_{on} and k_{off} and their contribution to the K_d for each clone, which is not provided by an endpoint method like ELISA.

Acknowledgement

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