Using FO-SPR to select for nanobodies in phage display

FOx BIOSYSTEMS
Breaking limits in bioanalysis

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Abstract

Phage display in conjunction with biopanning is a frequently used strategy in the selection process for nanobodies or other expressed binding proteins with specificity to a target antigen. Here we present an FO-SPR based approach that combines both fast kinetic characterization and an efficient selection cycle in a single step. As the assay is based on real-time kinetics monitoring rather than end point characterization such as ELISA, it presents potential for selection and characterization on affinity over avidity.

Figure 1 - Real time selection process monitoring

Fiber optic surface plasmon resonance (FO-SPR) dip-in probes in 96 well plates on White FOx. eGFP (green) funtionalized probes were used to selectively bind peptides expressed on M13 phage coat proteins.

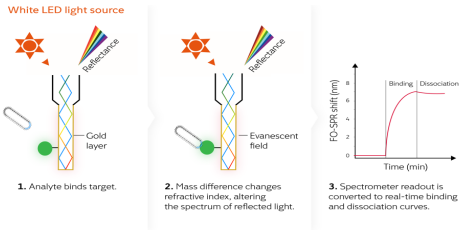


Figure 2 - FO-SPR Panning clone section process

M13 phage libraries expressing anti-eGFP binding peptides are grown, captured on eGFP funtionalised sensor probes and eluted for 3 culture cycles. The selection process is monitored with FO-SPR readout. The 3rd cycle individual clones are cultured for detailed characterization. FO-SPR real time binding sensorgrams of phage binding for the 3 subsequent biopanning rounds are shown (B-C-D).

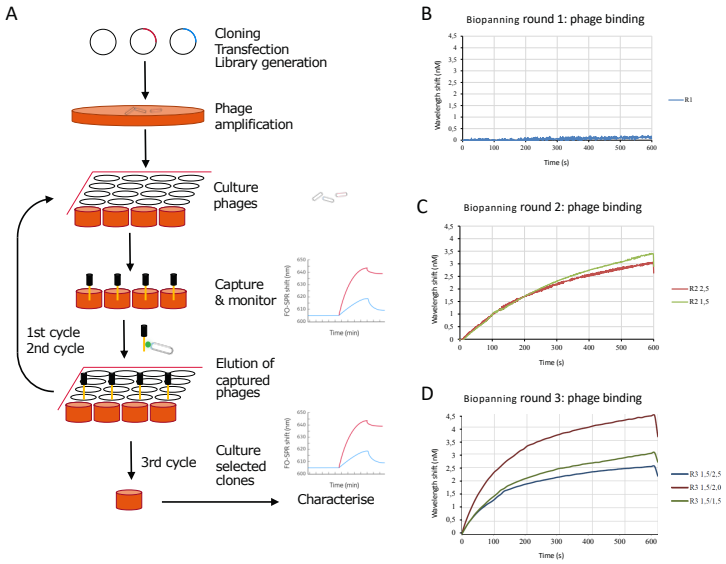
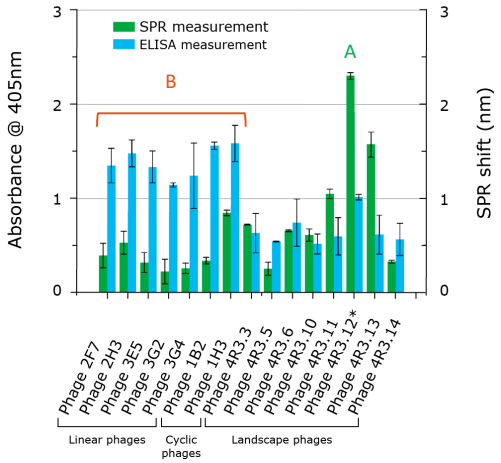


Figure 3 - Comparison to ELISA

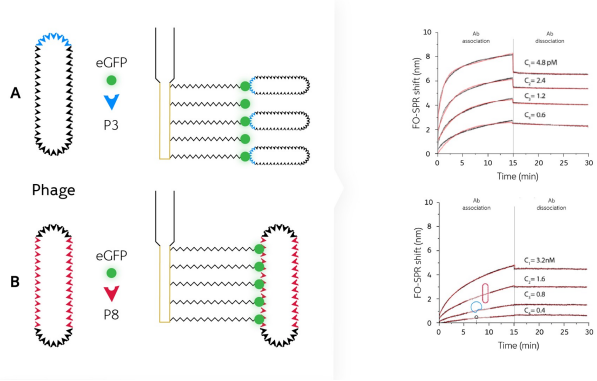
Results differ between ELISA and FO-SPR characterization data: Clones B scoring high on ELISA score low on FO-SPR and present a nanomolar K_d, while clone(s) A score high on FO-SPR, low on ELISA, and present a KD down to picomolar value. ELISA as an endpoint technique prefers avidity of many interactions over the kinetically determined affinity of a single interaction by FO SPR.



phage	k_{on} (s^{-1} M^{-1})	k_{off} (s^{-1})	K_d (M)	
1H3	$1.2 \pm 0.1 \times 10^{8}$	$2.7 \pm 0.1 \times 10^{-3}$	$2.3 \pm 0.3 \times 10^{-11}$	
1F7	$3.8 \pm 0.3 \times 10^{8}$	$4.0 \pm 0.6 \times 10^{-3}$	$1.1 \pm 0.2 \times 10^{-11}$	
1A6	$3.6 \pm 0.2 \times 10^{8}$	$6.0 \pm 0.2 \times 10^{-3}$	$1.6 \pm 0.4 \times 10^{-11}$	
4R3.12	$1.9 \pm 0.1 \times 10^{11}$	$2.2 \pm 0.1 \times 10^{-3}$	$1.2 \pm 0.1 \times 10^{-14}$	B
4R3.11	$2.3 \pm 1.1 \times 10^{8}$	$1.0 \pm 0.1 \times 10^{-3}$	$4.5 \pm 0.9 \times 10^{-12}$	

Figure 4 - Characterization

Clones selected from the 3rd cycle of biopanning were analyzed for kinetic affinity using FO-SPR. Selected low-affinity clones with multiple P8 binding sites presented nanomolar KD values, while high affinity clones with limited number of P3 binding sites presented picomolar KD values.



Methods

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

Three rounds of selection were performed. From the third panning round, single clone phages were isolated, identified (through DNA sequencing), and tested for binding to eGFP using ELISA and FO-SPR technology.

Streptavidin functionalized probes were used to capture biotinylated eGFP 10 µg/mL as selective binding layer. Phage binding (at pH 6.0) and phage dissociation steps were performed for 15 minutes each, in 100 µL buffer. Probes were eluted for 60 s in 10 mM glycine pH 2.

ELISA was performed with streptavidin coated microtiterplates 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-nitrophenylphosphatein in 1 M diethanolamine, 1 mM $MgCl_2$ pH 9.8 was added and monitored at 405 nm for 15 minutes.

Conclusions

This study presents FO-SPR based kinetic analysis of protein binding affinities, where viral particles are used as a whole with either the minor coat protein p3 or the major coat protein p8 expressing peptides selective for eGFP were tested in a biopanning procedure.

This difference in affinities could be partially explained with the orientation of phages, as the high density of p8 coat protein expresses peptides throughout the entire phage surface, allowing for multivalent interactions also resulting in a decreased dissociation. While the P3 phages showed strong binding with less interactions.

The advantage of this method is in the combination of fast panning selection cycles of only 30 minutes combined with the immediate readout of the kinetic profile of the sample. An additional benefit is in the preferential selection of strong binders over multivalent weak binders.

Acknowledgement

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