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Highly Sensitive Detection of the Interaction Occurring Between Phage Displayed Peptides and their Target using AlphaScreen™

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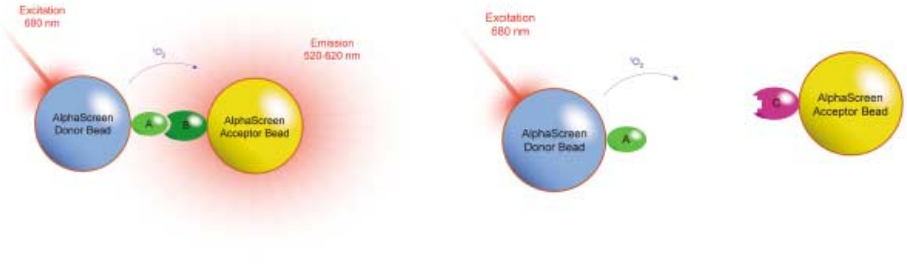
1 Abstract

Phage display is growing in popularity as a screening tool in functional genomics and proteomics. The throughput of this technology is however limited by the selections strategies available to date. Standard selection procedures are mainly based on ELISA and include the use of either antigens coated directly or indirectly (ex. streptavidin) on plastic surfaces, or biotinylated antigens captured by streptavidin-coated paramagnetic beads. Automation of these procedures is difficult since they require numerous separation steps. Furthermore, a high rate of false positives is often observed as a result of the high amounts of both target immobilized on solid support and phage particles required to produce a signal when a relatively low affinity interaction prevails between these binding partners. AlphaScreen is a homogenous non-radioactive HTS platform featuring assay geometry benefits allowing one to detect both high and low affinity interactions with high sensitivity. The AlphaScreen Phage display assay involves the capture of phage particles by Acceptor beads conjugated with anti-M13 antibodies whereas a biotinylated-target is captured by Streptavidin-Donor beads. When phages expressing specific peptides interact with the target of interest, both Acceptor and Donor beads are brought into proximity and the AlphaScreen signal is produced. AlphaScreen was used to 1) titer phage particles from bacterial lysate, 2) measure the interaction between peptides (expressed as g3p fusions) to specific targets, and 3) measure the interaction between soluble polypeptides (non g3p-fusion) to specific targets. In each case, AlphaScreen was effective at measuring specific interactions with high sensitivity.

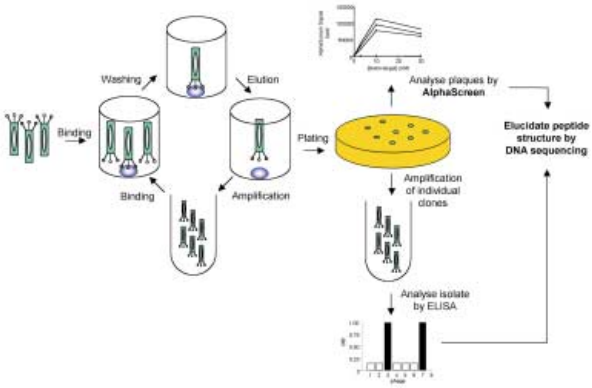
2 Principles of AlphaScreen

AlphaScreen is a bead-based non-radioactive Amplified Luminescent Proximity Homogeneous Assay. When biological interactions bring the Donor and Acceptor beads into close proximity, reactive oxygen generated by irradiation of the Donor beads initiates a luminescence/fluorescence cascade in the Acceptor beads. This process leads to a highly amplified signal with light output in the 520-620 nm range.

When the Acceptor and Donor beads are not in proximity, the reactive oxygen decays and only a very low background signal is generated.



3 Where Does AlphaScreen Fit?



Standard phage selection procedures are mainly based on ELISA and include the use of either antigens coated directly or indirectly (ex. streptavidin) on plastic surfaces, or biotinylated antigens captured by streptavidin-coated paramagnetic beads. In contrast to ELISA, AlphaScreen assays are easy to automate and allow one to measure high and low affinity interactions prevailing between low concentrations of binding partners. AlphaScreen is therefore a means of optimizing the throughput following the bio-panning process. The sensitivity of AlphaScreen allows one to take phage particles from a colony (plaque) and measure their interaction with a specific target.

4 Methods

Phage-displayed peptides (competition assay)

The reagents were added in white opaque 384-well microplates in the following order:

- 5 µl competitor or assay buffer
- 5 µl Purified phage (5x106 pfu/well final) (incubate 30 min. at RT)
- 10 µl of a solution composed of Biotin-target (10 nM final) and Donor beads (50 µg/ml final)
- 5 µl Acceptor beads (20 µg/ml final)

Plates were incubated for 60 min. at RT and read

Soluble Fab's (competition assay)

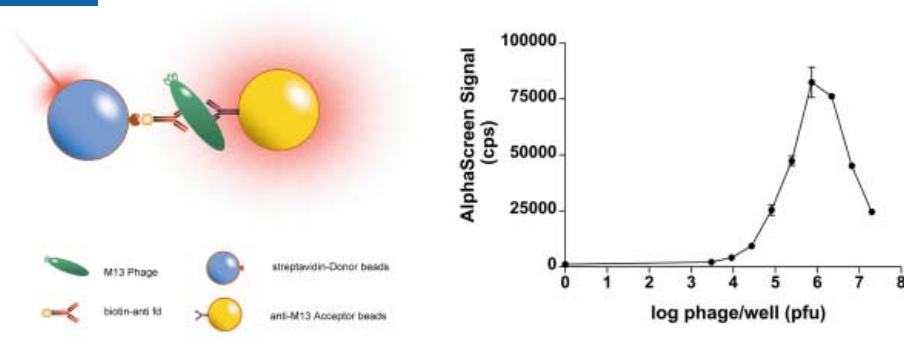
The reagents were added in white opaque 384-well microplates in the following order:

- 5 µl Protein A-Acceptor beads (20 µg/ml final)
- 5 µl anti-k or anti-l IgG (3 nM final) (incubate 30 min. at RT)
- 5 µl soluble Fab
- 5 µl competitor or assay buffer (incubate 30 min. at RT)
- 5 µl of a solution composed of Donor beads (20 µg/ml final) and biotin-target (100 nM final)

Plates were incubated for 60 min. at RT and read

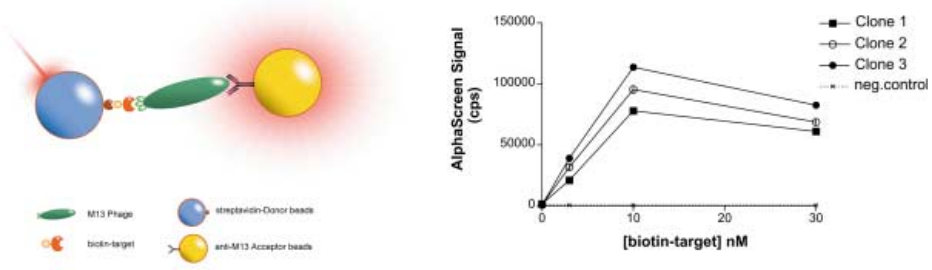
Assay buffer: 25 mM Hepes pH 7.4, 100 mM NaCl and 0.1% BSA.

5 Detection of Phage Particles in Solution



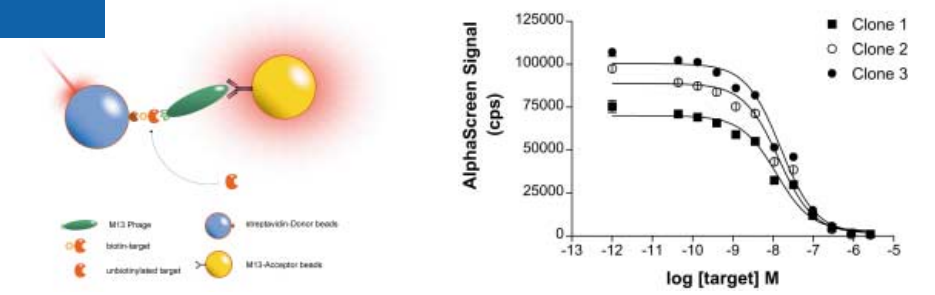
Left Panel: Phages in solutions are simultaneously captured by anti-M13 Acceptor beads and biotin-anti fd IgGs bound to Streptavidin-Donor beads. Both Acceptor and Donor beads are then brought into proximity which allows the AlphaScreen signal generation. ***Right panel:*** Increasing concentrations of phages in solutions lead to a proportional increase of the AlphaScreen signal. Above 10^6 phages/well, both anti-M13 and biotin-anti fd antibodies become saturated and a progressive signal decrease is observed. A significant signal is detectable with as little as 10^4 phages/well.

6 Detection of Phage Displayed Peptide / Biotinylated-Target Interaction



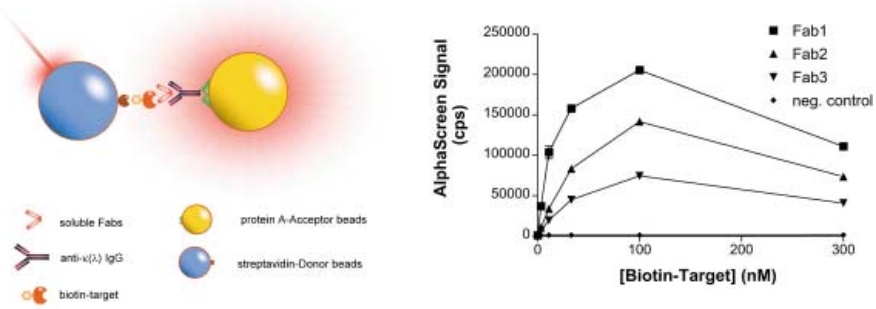
Left panel: Acceptor beads conjugated with anti-M13 antibodies are used to capture phages and the biotin lated-target is captured by Streptavidin-Donor beads. When phages expressing specific peptides interact with the target of interest, both Acceptor and Donor beads are brought into proximity and the AlphaScreen signal is then produced. ***Right Panel:*** Phage clones expressing specific peptides were incubated along with increasing concentrations of biotinylated targets (0-30 nM final). Clones 1, 2 and 3, known to express peptides specific for the biotinylated target, generated a significant signal. The clone expressing a non-specific peptide sequence (neg.control) did not produce any detectable signal.

7 Phage Displayed Peptide: Competition Assay



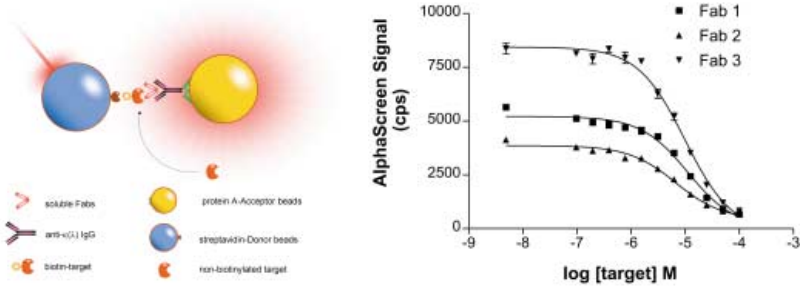
Left Panel: To further assess the specificity of the phage / target interaction, competition assays involving unlabeled target were performed. Acceptor beads conjugated with anti-M13 antibodies are used to capture phages and the biotinylated-target is captured by Streptavidin-Donor beads. When phages expressing specific peptides interact with the target of interest, both Acceptor and Donor beads are brought into proximity and the AlphaScreen signal is then produced. Addition of unlabeled target competes the phage-displayed peptide binding to biotin-target. ***Right Panel:*** Phage clones expressing specific peptides were incubated along with a fixed concentration (10 nM) of biotinylated target and increasing concentrations of unlabeled target (0-3 µM final). The interaction occurring between clones 1, 2 and 3 and the biotinylated target was inhibited in a concentration-dependent manner by the unlabeled target.

8 Soluble Fabs: Detection of Fabs-Biotin-Target Interaction in Bacterial Culture



Left panel: Protein-A coated Acceptor beads are used to capture anti-k or l light chain IgGs and the biotinylated-target is captured by Streptavidin-Donor beads. Soluble Fab's interacting specifically with the target of interest will brought the Acceptor and Donor beads into proximity allowing the AlphaScreen signal to be generated. ***Right Panel:*** Soluble Fab's were incubated along with increasing concentrations of biotinylated targets (0-300 nM final). Fab's 1, 2 and 3, known to interact specifically with the biotinylated target, produced a signal. As expected, the non-specific Fab (negative control) did not produce any significant signal.

9 Soluble Fabs: Competition Assay



Left panel: To further assess the specificity of the soluble Fab / target interaction, competition assays involving unlabeled target were performed. Protein-A coated Acceptor beads are used to capture anti-κ or λ light chain IgGs and the biotinylated-target is captured by Streptavidin-Donor beads. Soluble Fab's interacting specifically with the target of interest will bring the Acceptor and Donor beads into proximity allowing the AlphaScreen signal to be generated. Excess of unlabeled target competes the Fab's binding to biotinylated target. ***Right panel:*** Fab's were incubated along with a fixed concentration (10 nM) of biotinylated target and increasing concentrations of unlabeled target (0-100 µM final). The interaction occurring between Fab's 1, 2 and 3 and the biotinylated target was inhibited in a concentration-dependent manner by the unlabeled target.

10 Conclusions

AlphaScreen was shown to be efficient at:

- Measuring phage titer from bacterial lysates
- Detecting the interaction between peptides expressed as g3p-fusion to specific targets
- Detecting the interaction between soluble polypeptides (ex. Fab's) to specific targets.

Since it allows one to detect specific interactions more rapidly and with high sensitivity, AlphaScreen constitutes a valuable and powerful alternative to classical ELISA for phagedisplay assays.

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