

P013 Development of a solution based assay for screening selective anchor disruptors of AKAP-PKA interactions
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AlphaScreen (amplified luminescence proximity homogenous assay) is a bead based screening technology used to study biomolecular interactions in a medium to high throughput environment. The biomolecules to be analysed are separately and specifically immobilized on two different bead types; acceptor and donor beads. A complex of these two molecules will then bring the respective beads in proximity leading to an energy transfer from the acceptor bead to the donor bead, producing a luminescent/fluorescent signal (the AlphaScreen signal).

We are currently using this technology to study different AKAP-PKA interactions, with the aim of characterizing the potency of different high affinity peptides to function as anchoring disruptors, or compete with AKAP binding for the regulatory subunit of PKA (RI/RII). The RII specific AKAP95 and the dual-specific AKAP149 have been selected for this purpose.

The prototypic anchoring disruptor Ht31 efficiently competed AKAP95 and AKAP149 binding to RII, and AKAP149 binding to RI. RIAD, a RI specific anchoring disruptor was one order of magnitude more efficient than Ht31 in competing the AKAP149 interaction with RI, and this peptide was also verified by this method to be highly RI specific.