Handle large biomolecular interactions with ease

Protein-Protein interactions: Alpha technologies

Table of contents

When proteins come together Alpha makes things happen

The interactions and binding of proteins are implicated in a large number of biological processes. The need for an efficient, highly sensitive assay to study large protein interactions is increasingly important.

Alpha Technology is a highly flexible, homogeneous, no-wash assay ideal for the measurement of protein interactions and complexes as large as 200 nm in size. A bead-based proximity assay, Alpha Technology offers the possibility to assay many biological targets, including enzymes, receptor-ligand interactions, low-affinity interactions, second messenger levels, DNA, RNA, proteins, peptides, sugars and small molecules.

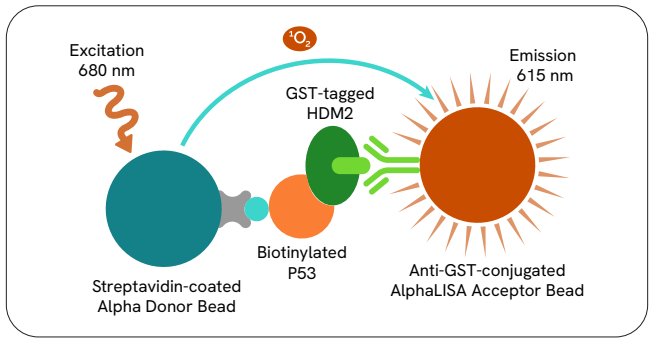
When Alpha Donor and Acceptor beads are brought together, a cascade of chemical reactions is set into motion creating a greatly amplified signal. The highly versatile beads can be coated with various biomolecules enabling detection of unique biological events.

Explore the advantages of Alpha Technology

Choose Alpha Technology for the study of proteins and their interactions using both biochemical and cell-based assays. Alpha Technology offers:

- Ability to detect weak interactions that would be difficult to detect using traditional assays; this allows highly sensitive measurement of a broad range of affinities, from low-affinity interactions to high-affinity interactions (picomolar to low millimolar range)
- Cost-efficiency: use with small amounts of samples and reagents
- Easy miniaturization
- Flexibility – choose custom or off-the-shelf solutions
- No separation steps; homogeneous no-wash assay

- **AlphaLISA**™ – offers the greatest flexibility with a toolbox range of beads to mix and match
- **AlphaScreen**™ – best off-the-shelf solution – ideal for fusion tag detection



Mechanism of action

- The Donor and Acceptor beads can be coated with different molecules depending on the type of reaction or event which needs to be studied
- Interaction between bead-bound molecules and analyte brings Donor and Acceptor beads into close proximity
- The Donor bead is excited with a laser at 680 nm and releases a singlet of oxygen
- The singlet of oxygen can travel up to 200 nm and allows for large interactions to be studied
- The singlet of oxygen excites the Acceptor bead and a light emission is produced (between 520-620 nm) which is proportional to the level of interaction

Take the Alpha challenge today and see how your assay measures up. Visit www.revvity.com

See pages 8 and 9 to learn how your peers are using Alpha Technology to enhance their research.

Case study 1: Protein-peptide small compound screening

Small compound screen for E6/E6AP interaction inhibitors

Assay type

AlphaScreen with recombinant protein and peptide

Revvity products used

- Streptavidin-coated Alpha Donor beads
- GSH or anti-GST AlphaScreen Acceptor beads
- EnVision™ Multilabel Plate Reader

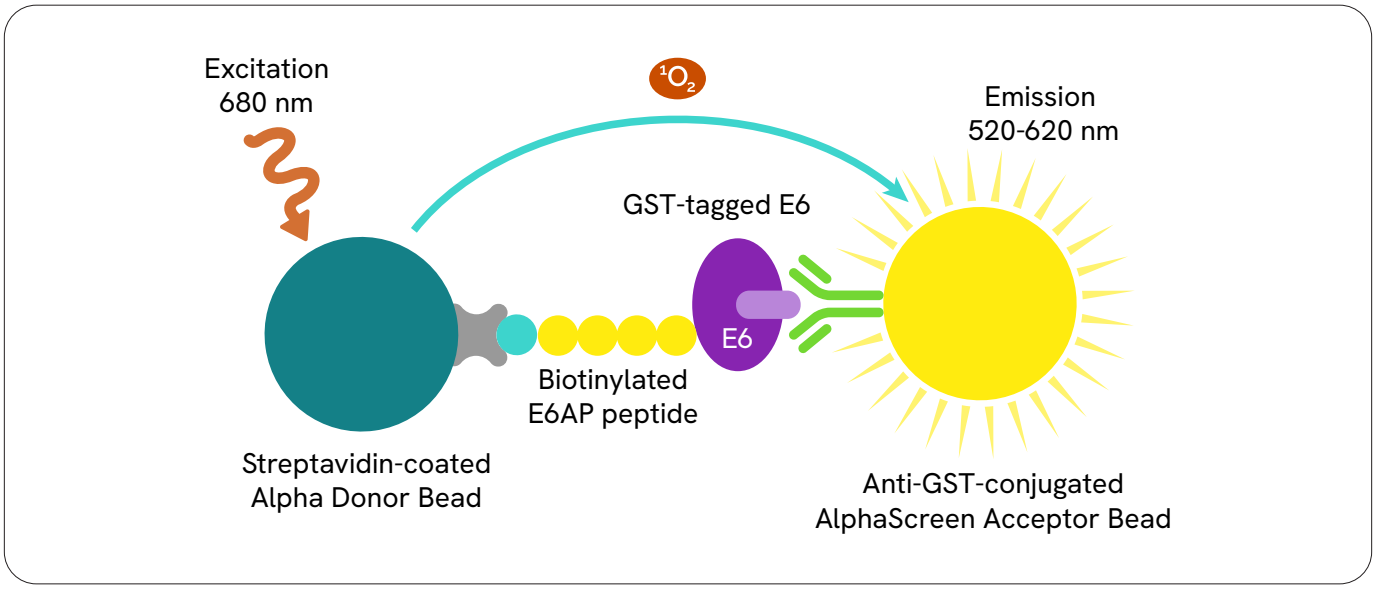
Results

E6 is an oncogene encoded by the human papilloma virus that has been shown to be involved in the development of cervical cancer through binding to the E6-associated protein E6AP, an inactivator of p53.

An AlphaScreen assay was developed to monitor the binding between E6 and the minimal E6-binding domain of the E6AP protein. The assay was validated with a pilot screen using 3000 compounds and enabled the identification of potential inhibitors of the E6/E6AP interaction.

Reference

Sehr, P., Pawlita, M. & Lewis, J. Evaluation of different glutathione S-transferase-tagged protein captures for screening E6/E6AP interaction inhibitors using AlphaScreen. J Biomol Screen 12 (4), 560-567 (2007).



Interaction: Virus-encoded oncogene E6 protein with its cellular target, E6AP.

Chemical Biology Core Facility, EMBL German Cancer Research Center

Case study 2: Measurement of endogenous/exogenous protein binding

Peptide-binding assays to MHC Class I and II

Assay type

AlphaScreen with recombinant proteins and peptide

Revvity products used

- Streptavidin-coated Alpha Donor beads
- AlphaScreen Acceptor beads
- EnVision Multilabel Plate Reader

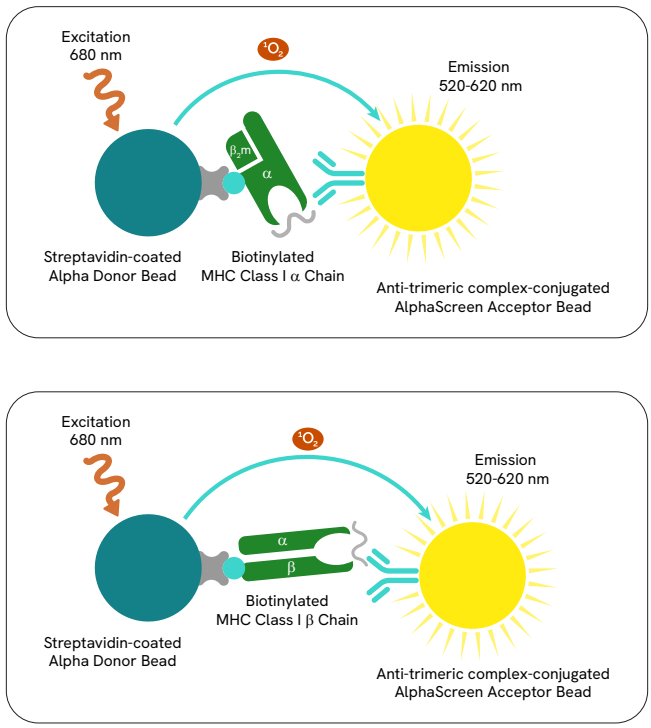
Results

MHC Class I and II molecules specifically bind to peptides derived from endogenous or exogenous proteins, respectively, presenting them at the cell surface for recognition by circulating T cells, thereby eliciting an immune response. In these two studies, the authors developed an AlphaScreen assay to detect and measure affinities for peptide binding to MHC Class I and II making use of recombinant biotinylated HLA subunits and conformation-specific antibodies recognizing the trimeric protein complexes. Only through the binding of specific peptides will an AlphaScreen signal be generated.

References

Justesen, S., Harndahl, M., Lamberth, K., Nielsen, L.L., Roder, G. & Buus, S. Functional recombinant MHC class II molecules and highthroughput peptide-binding assays. Immunome Res 5:2 (2009).
Harndahl, M., Justesen, S., Lamberth, K., Roder, G., Nielsen, M. & Buus, S. Peptide binding to HLA class I molecules: homogenous, highthroughput screening and affinity assays. J Biomol Screen 14 (2), 173-180 (2009).

Laboratory of Experimental Immunology, University of Copenhagen



Interaction: Peptide bridging two proteins.

Case study 3: High throughput screening of protein interactions

High throughput screening of Hsp90/ cochaperone HOP interaction inhibitors

Assay type

AlphaScreen with recombinant protein and peptide

Revvity products used

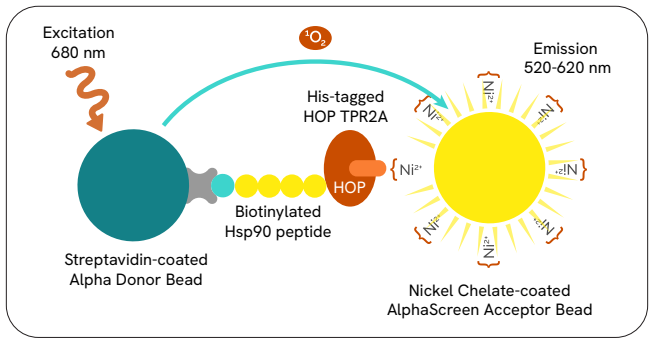
- Streptavidin-coated Alpha Donor beads
- AlphaScreen Acceptor beads (nickel chelate-conjugated)
- EnVision Multilabel Plate Reader

Results

The anticancer drug target Hsp90, through its interaction with a number of cochaperones, enables the folding and maturation of a battery of oncogenic signaling proteins. An AlphaScreen assay was used to measure the interaction between the 20-mer C-terminal peptide of Hsp90 and the tetricopeptide repeat (TPR) domain of Hsp90/Hsp70-organizing protein HOP. Following automation optimization of the assay into a 1536-well format, the assay was screened against an NIH Genomics Center library of 76,134 compounds to uncover potential Hsp90-HOP inhibitors.

Reference

Yi, F., Zhu, P., Southall, N., Inglese, J., Austin, C.P., Zheng, W. & Regan, L. An AlphaScreen-based high-throughput screen to identify inhibitors of Hsp90-cochaperone interaction. J Biomol Screen 14, 273-281 (2009).



Interaction: Hsp90 with its cochaperone HOP.

Dept. Molecular Biophysics & Biochemistry, Yale University NIH Chemical Genomics Center

Case study 4: Simultaneous measurement of protein interactions and phosphorylation

MAP kinase ERK2 dissociation from MAP2K MEK1 upon phosphorylation

Assay type

AlphaScreen and AlphaLISA in a single well with recombinant proteins

Revvity products used

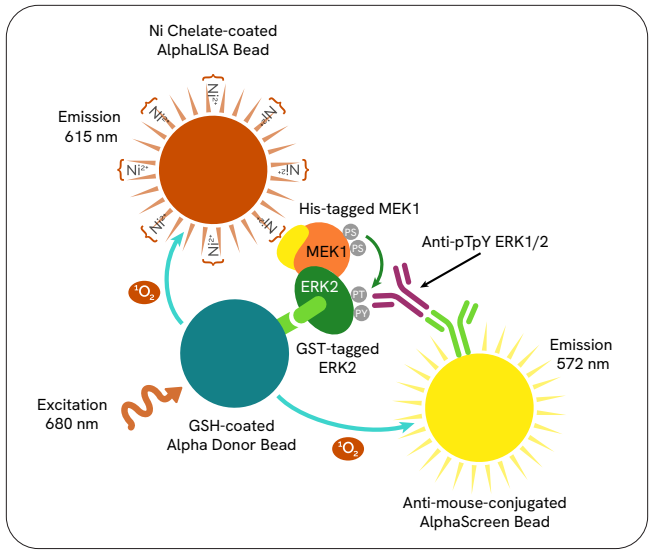
- Alpha Donor beads (GSH-conjugated)
- AlphaScreen Acceptor beads (Anti-mouse IgG)
- AlphaLISA Acceptor beads (nickel chelate-conjugated)
- EnVision Multilabel Plate Reader

Results

This assay fuses the versatility of AlphaScreen and AlphaLISA platforms. In its unphosphorylated state, GST-ERK2 binds His-MEK1. In the presence of ATP, MEK1 phosphorylates ERK2, causing both proteins to dissociate. Phosphorylation-interaction patterns generated are characteristic for each enzymesubstrate pair and were used to compare mechanisms of action and selectivity of phosphatases as well as small-molecule kinase inhibitors, allowing to discriminate between an ATP-competitor and an allosteric modulator.

Reference

Arcand, M., Roby, P., Bossé, R., Lipari, F., Padrós, J., Beaudet, L., Marcil, A. & Dahan, S. Single-well monitoring of protein-protein interaction and phosphorylation-dephosphorylation events. Biochemistry 49 (15), 3213-3215 (2010).



Interaction: Simultaneous monitoring of enzyme activity and protein-protein interaction.

Bio-discovery, Revvity

Alpha Toolbox. Create your own possibilities.

When you need to develop an assay to measure complex biological interactions, reach for the Alpha Toolbox. It gives you a wide range of beads coated with commonly used protein tags and binding motifs, allowing you to build an assay to measure virtually any biological interaction. If you don't see the binding protein you need, we provide unconjugated beads so you can coat them yourself or we can custom coat them for you.

The Alpha Technology Toolbox can be used to detect:

- Proteins, peptides or oligonucleotides that are doubly labeled with the tag and biotin
- Protein-protein, protein-peptide, protein-DNA and/or peptide-DNA interactions where the binding partners are biotinylated and tagged, respectively

Alpha technology toolbox beads for protein-protein interaction studies

Alpha donor beads
Anti-FLAG†
Anti-mouse IgG†
Anti-rabbit IgG†
Glutathione
Nickel Chelate
Protein A†
Strep-Tactin†
Streptavidin
Unconjugated

AlphaScreen acceptor beads*
Protein A
Unconjugated

AlphaLISA acceptor beads	
Anti-c-myc	Anti-rabbit IgG (Fc-specific)
Anti-chicken IgY†	Anti-rat IgG (Fc-specific)
Anti-DIG	Anti-sheep IgG†
Anti-FITC†	Anti-V5†
Anti-FLAG	Glutathione (GSH) GNA†
Anti-GFP†	Nickel Chelate
Anti-goat IgG (Fc-specific)	Protein A
Anti-GST	Protein G
Anti-His†	Protein L†
Anti-human IgG (Fc-specific)	Raw-NH2†
Anti-M13 Phage†	*Strep*-Tactin†
Anti-MBP†	Streptavidin†
Anti-mouse IgG (Fc-specific)	Unconjugated
Anti-mouse IgM†	

AlphaScreen fusion tag detection kits**
c-Myc
DIG
FITC
FLAG
GST
HA
Histidine (Nickel Chelate)
Biotinylated-GST
Biotinylated-HIS

Custom coatings and conjugations are available.
*AlphaScreen is another Acceptor bead type, which emits at 520-620 nm.
**Kits include both a Donor and Acceptor bead.
†Coming soon.

Want us to design an assay for you?
Learn how we can customize one to your specific requirements.

Detection and automation. Performance that measures up.

More and more researchers are discovering the tremendous advantages of our exclusive Alpha Technology. And as the only provider of integrated, validated Alpha detection and automation solutions, we can enhance every stage of your critical protein-protein experiments. With an offering this comprehensive, why go anywhere else?

State-of-the-art detection

Multilabel plate readers

Only Revvity plate readers have been validated for Alpha Technology to deliver optimal results time after time. Available for a wide range of applications and budgets.

Higher quality performance

Revvity microplates

When you start with a higher quality microplate, you'll end up with optimal results. Choose from AlphaPlate™, OptiPlate™, CulturPlate™ or ProxiPlate™.

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Seamless reliability

Integration of JANUS with the EnVision multilabel plate reader

Prepare your assays with JANUS, and select the EnSpire or EnVision plate reader for more efficient sample preparation and reproducible results.

Scientific references

Reference	Donor bead	Acceptor bead
Al-Mawsawi, L.Q., Christ, F., Dayam, R., Debyser, Z. & Neamati, N. Inhibitory profile of a LEDGF/p75 peptide against HIV-1 integrase: insight into integrase-DNA complex formation and catalysis. FEBS Lett 582, 1425-1430 (2008).	Anti-FLAG	Ni-NTA
Arcand, M., Roby, P., Bossé, R., Lipari, F., Padrós, J., Beaudet, L., Marcil, A. & Dahan, S. Single-well monitoring of protein-protein interaction and phosphorylation-dephosphorylation events. Biochemistry 49 (15), 3213-3215 (2010).	Glutathione	Ni-NTA: Anti-mouse
Baima, E.T. et al. Novel insights into the cellular mechanisms of the anti-inflammatory effects of NF-kappaB essential modulator binding domain peptides. J Biol Chem 285, 13498-13506 (2010).	Glutathione	Anti-FLAG
Bartholomeeusen, K. et al. Differential interaction of HIV-1 integrase and JPO2 with the C terminus of LEDGF/p75. J Mol Biol 372, 407-421 (2007).	Streptavidin; Anti-FLAG	Ni-NTA; Anti-FLAG
Becker, M.N., Todd, T.M. & Moyer, R.W. An Amsacta moorei entomopoxvirus ortholog of the poly(A) polymerase small subunit exhibits methyltransferase activity and is non-essential for virus growth. Virology 375, 624-636 (2008).	Streptavidin	Anti-FLAG
Campbell, L.A. et al. Decreased recognition of SUMO-sensitive target genes following modification of SF-1 (NR5A1). Mol Cell Biol 28, 7476-7486 (2008).	Streptavidin	Ni-NTA
Courtney, H.S., Zhang, Y., Frank, M.W. & Rock, C.O. Serum opacity factor, a streptococcal virulence factor that binds to apolipoproteins A-I and A-II and disrupts high density lipoprotein structure. J Biol Chem 281, 5515-5521 (2006).	Streptavidin	Ni-NTA
De Rijck, J. et al. Overexpression of the lens epithelium-derived growth factor/p75 integrase binding domain inhibits human immunodeficiency virus replication. J Virol 80, 11498-11509 (2006).	Streptavidin	Ni-NTA
Fujii, N. et al. An antagonist of dishevelled protein-protein interaction suppresses beta-catenin-dependent tumor cell growth. Cancer Res 67, 573-579 (2007).	Streptavidin	Anti-GST
Gesellchen, F., Prinz, A., Zimmermann, B. & Herberg, F.W. Quantification of cAMP antagonist action *in vitro* and in living cells. Eur J Cell Biol 85, 663-672 (2006).	Streptavidin	Anti-GST
Greenhalgh, C.J. et al. SOCS2 negatively regulates growth hormone action *in vitro* and *in vivo*. J Clin Invest 115, 397-406 (2005).	Streptavidin	Ni-NTA
Haas, T. et al. The DNA sugar backbone 2′ deoxyribose determines toll-like receptor 9 activation. Immunity 28, 315-323 (2008).*	Streptavidin	Protein A
Hamm, S. et al. Alternating 2′-O-ribose methylation is a universal approach for generating non-stimulatory siRNA by acting as TLR7 antagonist. Immunobiology 215, 559-569 (2010).*	Streptavidin	Protein A
Harndahl, M., Justesen, S., Lamberth, K., Roder, G., Nielsen, M. & Buus, S. Peptide binding to HLA class I molecules: homogenous, high-throughput screening and affinity assays. J Biomol Screen 14 (2),173-180 (2009).	Streptavidin	Anti-trimeric
Hombrouck, A. et al. Virus evolution reveals an exclusive role for LEDGF/p75 in chromosomal tethering of HIV. PLoS Pathog 3, e47 (2007).	Anti-MBP	Ni-NTA
Hornung, V. et al. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. Nature 458, 514-518 (2009).*	Streptavidin	Ni-NTA
Hou, Y. et al. Screening for antiviral inhibitors of the HIV integrase-LEDGF/p75 interaction using the AlphaScreen luminescent proximity assay. J Biomol Screen 13, 406-414 (2008).	Glutathione	Ni-NTA
Jerga, A. & Rock, C.O. Acyl-Acyl carrier protein regulates transcription of fatty acid biosynthetic genes via the FabT repressor in Streptococcus pneumoniae. J Biol Chem 284, 15364-15368 (2009).	Streptavidin	Ni-NTA
Jerga, A., Miller, D.J., White, S.W. & Rock, C.O. Molecular determinants for interfacial binding and conformational change in a soluble diacylglycerol kinase. J Biol Chem 284, 7246-7254 (2009).**	Streptavidin	Ni-NTA
Justesen, S., Harndahl, M., Lamberth, K., Nielsen, L.L. & Buus, S. Functional recombinant MHC class II molecules and high-throughput peptide-binding assays. Immunome Res 5, 2 (2009).	Streptavidin	Custom
Kadkhodayan, S. et al. Evaluation of assay technologies for the identification of protein-peptide interaction antagonists. Assay Drug Dev Technol 5, 501-513 (2007).	Streptavidin	Ni-NTA
Kitamura, N., Kaminuma, O., Kitamura, F. & Miyatake, S. Characterization of binding activity between nuclear factor of activated T cells and calcineurin by amplified luminescent proximity homogeneous assay. J Immunol Methods 312, 105-110 (2006).	Streptavidin	Ni-NTA
Kota, S., Coito, C., Mousseau, G., Lavergne, J. & Strosberg, A.D. Peptide inhibitors of hepatitis C virus core oligomerization and virus production. J Gen Virol 90, 1319-1328 (2009).	Glutathione	Anti-FLAG

All interactions are protein-peptide/protein-protein unless otherwise indicated.
*Protein-nucleic acid
**Protein-lipid or protein-sugar

Reference	Donor bead	Acceptor bead
Koury, E.J. et al. Characterization of ligands for thyroid receptor subtypes and their interactions with co-regulators. Steroids 74, 270-276 (2009).	Streptavidin	Anti-GST
Lawrence, H.R. et al. Identification of a disruptor of the MDM2-p53 protein-protein interaction facilitated by high-throughput in silico docking. Bioorg Med Chem Lett 19, 3756-3759 (2009).	Glutathione	Ni-NTA
Mills, N.L., Shelat, A.A. & Guy, R.K. Assay optimization and screening of RNA-protein interactions by AlphaScreen. J Biomol Screen 12, 946-955 (2007).*	Streptavidin	Anti-FITC
Mohamed, M.R. et al. Proteomic screening of variola virus reveals a unique NF-kappaB inhibitor that is highly conserved among pathogenic orthopoxviruses. Proc Natl Acad Sci U.S.A. 106, 9045-9050 (2009).	Ni-NTA	Anti-GST
Moll, D. et al. Biomolecular interaction analysis in functional proteomics. J Neural Transm 113, 1015-1032 (2006).	Streptavidin	Anti-GST
Oikawa, T. et al. Identification of a small-molecule inhibitor of the interaction between Survivin and Smac/DIABLO. Biochem Biophys Res Commun 393, 253-258 (2010).	Streptavidin	Ni-NTA
Penmatsa, H. et al. Compartmentalized cyclic adenosine 3',5'-monophosphate at the plasma membrane clusters PDE3A and cystic fibrosis transmembrane conductance regulator into microdomains. Mol Biol Cell 21, 1097-1110 (2010).	Streptavidin	Anti-FLAG
Pioszak, A.A., Parker, N.R., Suino-Powell, K. & Xu, H.E. Molecular recognition of corticotropin-releasing factor by its G-protein-coupled receptor CRFR1. J Biol Chem 283, 32900-32912 (2008).	Streptavidin	Ni-NTA
Rouleau, N. et al. Highly sensitive assays for SUMOylation and small ubiquitin-like modifier-dependent protein-protein interactions. Anal Biochem 375, 364-366 (2008).	Streptavidin; Glutathione	Ni-NTA; Glutathione
Savkur, R.S. et al. Ligand-dependent coactivation of the human bile acid receptor FXR by the peroxisome proliferator-activated receptor gamma coactivator-1alpha. J Pharmacol Exp Ther 312, 170-178 (2005).	Ni-NTA	Anti-GST
Sehr, P., Pawlita, M. & Lewis, J. Evaluation of different glutathione S-transferase-tagged protein captures for screening E6/E6AP interaction inhibitors using AlphaScreen. J Biomol Screen 12, 560-567 (2007).	Streptavidin	Anti-GST; Glutathione
Shan, L. et al. PCSK9 binds to multiple receptors and can be functionally inhibited by an EGF-A peptide. Biochem Biophys Res Commun 375, 69-73 (2008).	Streptavidin	Ni-NTA
Shi, C. et al. Lectin-like domain of thrombomodulin binds to its specific ligand Lewis Y antigen and neutralizes lipopolysaccharide-induced inflammatory response. Blood 112, 3661-3670 (2008).**	Streptavidin	Protein A
Shih, H.H. et al. CRP is a novel ligand for the oxidized LDL receptor LOX-1. Am J Physiol Heart Circ Physiol 296, H1643-1650 (2009).	Streptavidin	Ni-NTA
Silverman, J. et al. Multivalent avimer proteins evolved by exon shuffling of a family of human receptor domains. Nat Biotechnol 23, 1556-1561 (2005).	Streptavidin	Protein A
Skelton, N.J. et al. Origins of PDZ domain ligand specificity. Structure determination and mutagenesis of the Erbin PDZ domain. J Biol Chem 278, 7645-7654 (2003).	Streptavidin	Anti-GST
Stokka, A.J. et al. Characterization of A-kinase-anchoring disruptors using a solution-based assay. Biochem J 400, 493-499 (2006).	Streptavidin	Anti-GST
Tolbert, W.D. et al. A mechanistic basis for converting a receptor tyrosine kinase agonist to an antagonist. Proc Natl Acad Sci U.S.A. 104, 14592-14597 (2007).	Streptavidin; Ni-NTA	Ni-NTA
Uehara, Y., Mochizuki, M., Matsuno, K., Haino, T. & Asai, A. Novel high-throughput screening system for identifying STAT3-SH2 antagonists. Biochem Biophys Res Commun 380, 627-631 (2009).	Streptavidin	Anti-FITC
Wallace, M., Worrall, E., Pettersson, S., Hupp, T.R. & Ball, K.L. Dual-site regulation of MDM2 E3-ubiquitin ligase activity. Mol Cell 23, 251-263 (2006).	Streptavidin	Protein A
Wang, W. et al. The crystal structures of human steroidogenic factor-1 and liver receptor homologue-1. Proc Natl Acad Sci U.S.A. 102, 7505-7510 (2005).	Streptavidin	Antibody
Wigle, T.J. et al. Screening for inhibitors of low-affinity epigenetic peptide-protein interactions: an AlphaScreen-based assay for antagonists of methyl-lysine binding proteins. J Biomol Screen 15, 62-71 (2010).	Streptavidin	Ni-NTA
Yeh, C. et al. C-terminal repeats of Clostridium difficile toxin A induce production of chemokine and adhesion molecules in endothelial cells and promote migration of leukocytes. Infect Immun 76, 1170-1178 (2008).**	Not Listed	Not Listed
Yi, F. & Regan, L. A novel class of small molecule inhibitors of Hsp90. ACS Chem Biol 3, 645-654 (2008).	Streptavidin	Ni-NTA
Yi, F., Zhu, P., Southall, N., Inglese, J., Austin, C.P., Zheng, W. & Regan, L. An AlphaScreen-based high-throughput screen to identify inhibitors of Hsp90-cochaperone interaction. J Biomol Screen 14, 273-281 (2009).	Streptavidin	Ni-NTA

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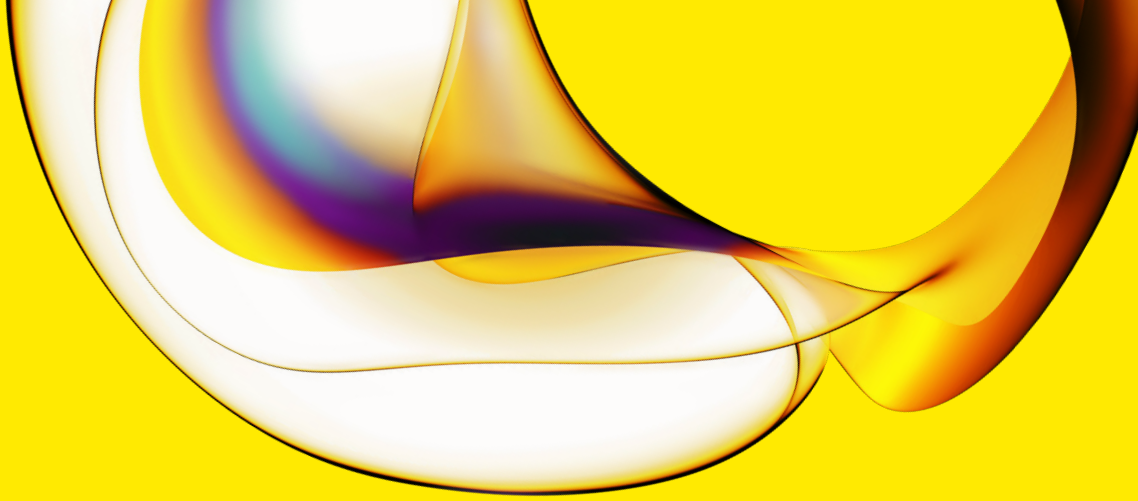

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