A troubleshooting guide:
Experts share their advice on measuring and analyzing protein interactions

Protein-Protein Interactions

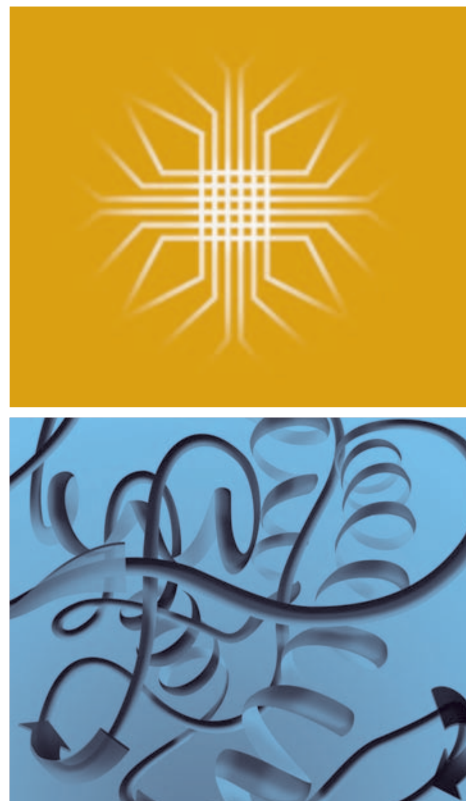
Q&A

Genome Technology

The GenomeWeb Intelligence Network

Protein Interaction Analysis

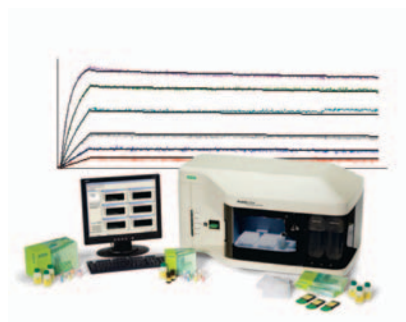
6X6



A Powerful Equation

The ProteOn™ XPR36 protein interaction array system, the ultimate in parallel processing.

Bio-Rad introduces the ProteOn XPR36, a surface plasmon resonance optical biosensor. Take the next step from protein discovery to function with label-free protein-protein interaction analysis. XPR™ technology is a unique 6 x 6 crisscross approach to multiplexing that processes, in parallel, 6 different ligands with 6 different analytes. Perform a complete kinetic analysis, rank a library of antibodies, or test multiple interaction conditions on a single chip, in a single run.



The 6 x 6 interaction array format provides:

- High-throughput parallel sample processing
- Full kinetic data
- Flexible experimental design

Visit us on the Web at **www.bio-rad.com/ad/proteininteraction/**

Visit us on the Web at **discover.bio-rad.com**
Call toll free at 1-800-4BIORAD (1-800-424-6723);
outside the US, contact your local sales office.

BIO-RAD

Table of contents

Letter from the Editor 4

Index of Experts 4

Q1: What are the **benefits of your preferred assay system(s)**? 5

Q2: How do you **eliminate or reduce false positive results**? 8

Q3: How do you **identify structural patterns of multiple interactions**? 9

Q4: What techniques do you use to **reduce non-specific interactions**? 10

Q5: What do you do to **validate suspected protein-protein interactions**? 11

Q6: Which **computational methods do you use** to analyze interactions? 12

Resources 14

Dualsystems Biotech

Looking for novel protein interactions?

We have the solution:

- Screen full-length integral membrane proteins for interaction partners with our DUALmembrane system
- Screen soluble proteins or protein fragments with our optimized two-hybrid system
- Full-length cDNA expression libraries for all screening systems
- Yeast-based recombinant protein expression

All systems available as custom screening services and ready-to-use kits.

www.dualsystems.com

Take advantage of the power of yeast protein interaction screening today!

Letter from the editor

For this latest installment of *Genome Technology*'s technical reference guide series, we've assembled a team of authorities on protein-protein interactions. In this guide, these contributors share their strategies for detecting, characterizing, and analyzing proteins on the move.

Every process in a cell is affected by interactions between proteins, which inform everything from the shape of an organelle to the function of a ribosome. Just as we tailor our own conversations depending on setting, proteins exhibit many different modes of interaction. Long-term interactions result in protein complexes, while briefer protein liaisons may lead to a range of possible chemical modifications. Protein interactions are also behind the phenomenon of signal transduction, by which a message can be relayed across the cellular landscape.

Because they are so integral to physiological function, protein-protein interactions are germane to many lines of research, both basic and clinical. Protein interaction data may yield important information about the molecular basis of disease, and many researchers are making moves to use such information in devising new therapeutics.

Parsing out the ways in which proteins meet is a pretty good starting point for really understanding the molecular conversations that fuel a biological process. That's not to say it's easy. The study of protein interactions has historically involved expertise in biology, biochemistry, and biophysics. So although it's beyond the scope of this guide to present all of the possible tactics used in protein studies today, we did attempt to formulate questions that could yield valuable advice on detecting and analyzing interactions, regardless of your discipline.

Keep this guide nearby if you are interested in picking up a few tricks to spot and monitor protein interactions in your own system of interest. The experts below cover everything from the relative merits of different assays to keeping non-specific interactions to a minimum. Also, be sure to check out the resource guide, which contains reading and resources recommended by our contributors.

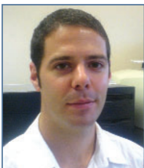
— Jennifer Crebs

Index of experts

All of the expert contributors listed below are based in academic institutions and have no ties to vendors featured in this guide.

Edward Evans

University of Oxford



John James

University of Oxford

Ulf Landegren

Uppsala University

Stephen Michnick

University of Montreal

Moritz Rossner

Max Planck Institute of Experimental Medicine

Igor Stagljar

University of Toronto

What are the benefits of your preferred assay system(s)?

Surface plasmon resonance (SPR) technology was first commercially implemented for analyzing protein-protein interactions by Biacore. Biacore experiments, in our case utilizing a Biacore 3000, involve passing a soluble protein or analyte (*e.g.* a ligand) over an immobilized binding candidate (*e.g.* a receptor) inside a very small flow-cell, controlled by clever microfluidics. Because SPR, induced by the phenomenon of total internal reflection at the base of the flow cell, is sensitive to refractive index changes within the flow cell, changes in the SPR signal can be used to monitor binding-induced changes in protein accumulation within the flow cell in real time.

Historically, there had been many ways to detect protein-protein interactions, such as radioimmunoassay and ELISA. These approaches did not allow binding to be followed in real time and so were confined to the analysis of high-affinity interactions that could tolerate a wash step, such as antibody or hormone interactions. Such methods were thus unsuited for the analysis of very weak interactions, such as those expected to occur at the cell surface (t_{fi}<1s), as the interactions would "fall apart" in the course of washing.

The Biacore was a major advance because it allowed, for the first time, protein interactions to be observed without a wash step. Although not explicitly designed to study very weak interactions, PA van der Merwe and colleagues showed that, using the Biacore, it was possible to observe such interactions by comparing the amount of binding in the test flow cell (with test protein immobilized) versus a control flow-cell (in which a negative control protein is immobilized). A second advantage is that the Biacore collects data rapidly enough to do kinetic analyses of very fast interactions. The third key advantage is that relatively small amounts of protein are required for analysis (40-100 ml is all that's required to get a minimum dataset). This is important because, in order to see very weak interactions, the analyte needs to be at very high concentrations, which might not be achievable if large volumes of protein were required. The BIAcore now represents the state of the art for studying weak interactions.

— Edward Evans

Bioluminescence Resonance Energy Transfer (BRET) relies on the same physical principles as its fluorescent counterpart, FRET, where a donor fluorophore in close enough proximity, typically <10nm, to an appropriate acceptor fluorophore can transfer energy through a non-radiative process. BRET uses *Renilla* luciferase as the donor, a protein that oxidizes its substrate (coelenterazine) and concomitantly emits light in the 400-500nm wavelength range, which is in contrast to FRET where the donor is excited by an external light source. By genetically linking luciferase and an acceptor, invariably a green fluorescent protein (GFP) variant to proteins of interest, it is possible to identify protein interactions and potentially assign stoichiometries to them.

The major benefit of this approach is that protein-protein interactions can be monitored *in situ*, where the donor- and acceptor-tagged proteins are expressed as a "BRET pair" in an appropriate cell line, often HEK-293T. This is most lucidly seen for membrane-confined proteins, where there is a real paucity of techniques that can probe protein interactions without extracting the target from its

What are the benefits of your preferred assay system(s)?

native environment, which is likely to cause significant artifacts for highly hydrophobic proteins. BRET also offers several advantages over FRET. Principally, luminescent detection of light emission gives an exceptional signal/noise ratio for data, which allows protein-protein interactions to be detected at more physiological expression levels. The absence of an external light source to excite donor fluorophore also obviates problems of photobleaching, unwanted excitation of the acceptor fluorophore, and other optical effects.

— John James

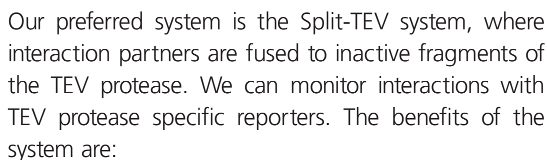
We are using a method we have developed ourselves and published recently to investigate interactions between proteins, directly in genetically unmodified cells and tissues (Söderberg et al., 2006). This method does not depend on overexpression of fusion proteins, but observes natural proteins detected in fixed tissues via antibodies.

— Ulf Landegren

Protein-fragment complementation assays (PCAs) have been variously called "split-protein" or, in the case of fluorescent protein PCAs, renamed biomolecular fluorescence complementation (BiFC), though described method is based on original work of Ghosh et al., 2000. We use the acronym PCA to describe all assays based on protein engineered fragment complementation assays for detecting protein-protein interactions because it is general and follows historically from the literature. PSAs:

1) allow for direct detection of protein-protein interactions *in vivo* and *in vitro* in any cell type
2) allow detection of protein-protein interactions in appropriate subcellular compartments or organelles
3) allow detection of interactions that are specifically induced in response to developmental, nutritional, environmental, or hormone-induced signals
4) allow monitoring of kinetic and equilibrium aspects of protein assembly in cells
5) allow screening for novel protein-protein interactions in any cell type
6) is not a single assay, but a series of assays; thus, an assay can be chosen because it works in a specific cell type that is appropriate for studying that class of interaction
7) are inexpensive, requiring no specialized reagents beyond those necessary for a particular assay and off-the-shelf materials and technology
8) can be automated, allowing high-throughput screening
9) are designed at the level of the molecular structure of the enzymes used; because of this, there is additional flexibility in designing the probe fragments to control the sensitivity and stringencies of the assays
10) can be based on enzymes whose activity can be determined by multiple assay strategies, including by survival-selection or production of a fluorescent product
11) that are based on fluorescent proteins or proteins that bind to fluorescent ligands (*e.g.* DHFR) can be used to determine locations of protein complexes unambiguously.

— Stephen Michnick



Our preferred system is the Split-TEV system, where interaction partners are fused to inactive fragments of the TEV protease. We can monitor interactions with TEV protease specific reporters. The benefits of the system are:

1) Flexibility: We can use fluorescent or luminescent reporter proteins that either can be directly activated by proteolytic cleavage or that are indirectly activated as reporter genes activated by a protease-dependent transcription factor. Practically, any other reporter gene may be used if preferred. We have already also used resistance-conferring reporter to monitor survival as readout.
2) Sensitivity: By using rapamycin-induced interactions of FRB and FKBP at the membrane of living cells, we can detect significant activation with 0.001 nM rapamycin added.
3) Full-length proteins: We can monitor interactions also of full-length membrane proteins in living cells, but the TEV-tags have to be placed properly.
4) Applicability to living cells: The interaction is measured within living cells. So far, we have successfully tested a variety of heterologous cell lines (*e.g.* COS1, Hela, PC12, NIH3T3, CHO) and primary cells (neurons, astrocytes, ES cells).

— Moritz Rossner

In my lab, we primarily use yeast-based genetic screening assays to identify and characterize protein-protein interactions (PPIs). These assays include the well-known Yeast Two-Hybrid (YTH) system, originally invented by Stan Fields in 1989, and the split-ubiquitin Membrane Yeast Two-Hybrid assay (or MYTH) developed in our lab in 1998.

Yeast-based assays have several advantages when compared to conventional biochemical assays: since all interactions are detected within growing, intact cells, the likelihood of introducing artifacts due to the destruction of cellular compartments by cell lysis and harsh extraction conditions are diminished. Furthermore, both direct and indirect PPIs can be detected. In addition, as no washing or purification steps are involved, there is greater opportunity to detect weak or transient interactions. Yeast-based assays also offer flexibility, as demonstrated by the great number of variations on the original YTH system, which allow, for example, the identification of protein complexes (so-called yeast three-hybrid systems), modification-dependent interactions (*e.g.* phosphorylation or acetylation dependent), protein-RNA interactions, protein-DNA interactions, small molecule-protein interactions or the identification of compounds which alter a PPI.

The major goal of our research is to identify proteins associated with numerous yeast and human integral membrane proteins using the MYTH assay on a systematic scale to provide comprehensive membrane PPI maps. Due to their pivotal role in many cellular processes, their direct link to human diseases and their extracellular accessibility to drugs, the identification of proteins associated with integral membrane proteins is desirable. However, due to their complex chemical properties, membrane proteins are difficult to purify for in vitro assays and ill-suited for identification of their interacting partners using the current *in vivo* assays. Because *(continued on p.13)*

How do you eliminate or reduce false positive results?

SPR as we implement it, using a Biacore 3000, is not ideally suited to screening large numbers of candidate ligands for a protein, because only three candidates can be tested simultaneously. Other Biacore machines recently released can be used for this application. However, SPR is ideal for testing proposed interactions because false positive results are extremely rare, provided precautions are taken to exclude non-specific interactions. The purity of the injected analyte and the immobilized material is, of course, also important. Finally, care must be taken that the method of immobilization used for the binding partners will not also capture the analyte independently of its protein-protein interactions. To give a trivial example, which we have seen all too often with new users, if a mouse Fc-fusion construct is immobilized via an antibody against all Fc isotypes, you cannot then test for antibody binding, because it too will be captured by the immobilized secondary antibody.

— Edward Evans

Because a BRET experiment involves the expression of potential interacting proteins as a BRET pair, it is not well suited to a high-throughput approach to analyzing protein-protein interactions. As long as care is taken to minimize and account for the effects of non-specific interactions, false positive results should be rare.

— John James

At present our method is not used in high-throughput, and we can use appropriate controls to confirm that we do not have nonspecific signals.

— Ulf Landegren

1) PCAs are designed at the level of the molecular structure of the enzymes used; because of this, there is additional flexibility in designing the probe fragments to control the sensitivity and stringencies of the assays.
2) Non-interacting proteins. A PCA response should not be observed if non-interacting proteins are used as PCA partners; nor should overexpression of a non-interacting protein alone compete for the known interaction.
3) Partner protein interface mutations. A point or deletion mutation of a partner that is known to disrupt an interaction should also prevent a PCA response.
4) Competition. A PCA response should be diminished by the simultaneous overexpression of one or other of the interacting proteins that is not fused to a complementary PCA fragment.
5) Fragment swapping. An observed interaction between two proteins should occur regardless of which interacting proteins is attached to which PCA fragment.
6) Induction or inhibition of interactions in response to developmental, nutritional, environmental, or hormone-induced signals linked to the interaction can validate a novel interaction as being biologically relevant to a specific cellular process.

— Stephen Michnick

Since we always measure with a kinetically uncoupled reporter (min range for the direct and h range for the indirect reporters), we first reduce the time between transfection and analysis. Another option with our system is to reduce, in transient tranfections, the amount of

(continued on p.13)

How do you identify structural patterns of multiple reactions?

This is what SPR is most suited to doing, *i.e.* allowing you to precisely characterize the binding properties of a particular protein interaction. With proteins of known structure, a series of point mutations can then be made in one of the partners in order to map the binding surface using Biacore-based binding assays. The obvious caveat here is that each mutant must fold correctly in order to be informative. Many researchers make alanine mutants on the surface of a protein to look for the binding site, the so-called "alanine scanning" approach. However, this amounts only to removal of the side chain, and therefore identifies only those residues contributing free energy to binding. Since not all residues contribute binding energy, it follows that this will give an incomplete map of the binding surface.

> "We recommend an initial screen of surface residues from the known structure." *— Edward Evans*

We therefore recommend an initial screen of surface residues identified from the known structure using what we call "drastic" mutations. This involves changing the charge and increasing the size of a residue. In practice it usually means mutating everything to arginine except histidine, lysine, and arginine itself, which are all changed to glutamic acid. Such changes are likely to completely disrupt binding if the residue is involved in the binding interface in any way and should give a clear delineation of the interacting surface.

If a mistake is made and a structurally important residue is mutated, this will completely destroy expression of the protein. This is very important because if the protein expresses well it means that a surface residue has been targeted and it eliminates the chance of false negatives, i.e. the false conclusion that a residue is important for binding when it is in fact required for folding. Alanine mutations can later be used to determine the source of binding energy.

— Edward Evans

Although structural information is invaluable in making sure the BRET assay is implemented correctly, it does not rely on explicit knowledge of protein structure to analyze potential interactions. Proteins are expressed as a pair, therefore only allowing one potential interaction to be investigated at one time. Where structures suggest a pairwise interaction, however, it is simply a case of expressing the two targets as donor- and acceptor-tagged pairs in a suitable cell line and performing the BRET assay.

— John James

Our methods, termed P-LISA for proximity ligation *in situ* assay, gives staining patterns in microscopic specimens. We have shown that the method permits detection of sets of three interacting proteins.

— Ulf Landegren

Variation of polypeptide linker length between interacting proteins and reporter protein fragments can be used to evaluate whether an interaction is likely direct or indirect.

— Stephen Michnick

What techniques do you use to reduce non-specific interactions?

The key to avoiding non-specific interactions is to ensure that high-quality soluble protein is used as the analyte. It is therefore important to have a very good expression system so that you have enough protein to purify properly. We favor mammalian expression systems that fold proteins well and purify our proteins via an affinity tag followed by gel-filtration (at least). The analyte should be as close to the native form as possible and be entirely free of aggregates. Where possible, we use protein straight after size exclusion gel-filtration on an HPLC system for binding analysis using the Biacore, as even very small amounts of aggregate may affect measured binding properties and these can build up even after overnight storage at 4℃ or following a freeze-thaw cycle. Of course, the analyte protein could be naturally prone to non-specific interactions, so it is also important to have a good control immobilized for comparison.

— Edward Evans

Non-specific interactions are an inevitable consequence of protein diffusion, which is greatly enhanced when proteins are confined to the crowded environment of the plasma membrane. Confirming that the expression levels of fluorophore-tagged molecules are within a physiological range, *e.g.* by FACS, can minimize unwanted interactions as well as by ensuring that the protein is correctly localized within the cell, usually by microscopy. We have found that with our BRET experiments, all membrane proteins give significant but non-specific energy transfer at the cell surface. In order to account for this, proteins of known stoichiometry are used to distinguish these random interactions from specific protein-protein association. By varying the relative amount of acceptor- and donor-tagged proteins it is further possible to gain quantitative information about the interaction, where independence from these changes in the acceptor/donor ratio is the hallmark of random interactions. Expression of proteins that are known not to interact as a BRET pair can also help to identify what the profile of this type of interaction would look like in comparison to the unknown interaction.

— John James

Controls are important to set a reliable background. The background is usually a bit higher for interactions of two membrane proteins compared to a membrane and one soluble protein.

— Moritz Rossner

Non-specific interactions are less of a problem in yeast-based systems than in biochemical assays, as there are no purification steps involved. However, non-specific interactions can occasionally occur, for example if two proteins that are normally located in separate cellular compartments are co-expressed in the yeast cell. These proteins may interact, although under normal circumstances such an interaction would never be observed since the two proteins would not co-localize in the same compartment.

Non-specific interactions can be controlled either by increasing the stringency of selection (for example by using the triple reporter strains), or by lowering the expression levels of both a bait and prey protein, or, finally, by carrying out additional confirmation assays after the screen.

— Igor Stagljar

What do you do to validate suspected protein-protein interactions?

The Biacore is itself the ideal validation method — it allows detailed characterization of individual interactions. When high-throughput instruments are more widely used (*e.g.* Biacore FlexChip), interactions identified by screening one analyte against hundreds of candidates would then be individually validated on a more conventional instrument such as the Biacore 3000 (or the updated T100).

— Edward Evans

Where BRET analysis shows an interaction between proteins and there is structural information available that suggests a potential binding interface, mutagenesis provides a means to directly test whether this interface is responsible for the observed binding. By using mutations that should have a drastic effect on the interaction, a decrease in BRET values compared to the wild type is strong evidence that this region is important. Where detailed structural information in not available, chimeric proteins can be used to narrow down the region of the molecule that is responsible for the observed interaction. Performing the BRET analysis on homologous proteins can also shed light on whether a suspected interaction is likely to have been conserved through evolution and hence may have a functional consequence.

— John James

Induction or inhibition of interactions as measured by PCA in response to a pathway-specific stimulus acts as a first-pass validation that a novel interaction is biologically relevant to a specific cellular process.

— Stephen Michnick

So far, we have been using mainly well characterized pairs, but I am sure that for novel interactions the sequential application of at least two non-complementary methods is essential.

This means that you should not verify a FRET result with a BRET assay and not a Split-TEV results with a Spli-Luci assay. If possible, a Split-Enzyme assay should be complemented with a biochemical approach (*e.g.* CoIP) if sensitivity is not limiting.

— Moritz Rossner

The common approach is to verify a particular PPI by co-immunoprecipitating the two proteins from human cell extracts. Furthermore, we are using a modified Bioluminescence Resonance Energy Transfer (BRET) assay developed in the Bouvier lab that monitors in real time the interactions between an integral membrane bait protein and its interactor in living human cells.

Alternatively, co-localization of the two putative interaction partners can be used to infer if the two proteins might interact in their native environment.

If a putative interaction can be confirmed by any of the above-mentioned methods, the next step is functional assays. Such assays include loss-of-function screens (*e.g.* knockout mice or RNAi-mediated gene suppression in cell lines or organisms), gain-of-function (overexpression in cell lines or transgenic mice) studies, or *in vitro* enzymatic assays.

— Igor Stagljar

Which computational methods do you use to analyze interactions?

Initial analysis of sensorgrams (the traces produced during SPR) can be done using the Biaevaluation software provided with Biacore systems. This allows comparison with controls, subtraction of background signal, and other simple manipulations of the data for comparison between experiments. This software can also be used to analyze carefully collected kinetic data for individual interactions. For rapid estimation of affinities, equilibrium binding experiments can be analyzed in any mathematical software with a curve fitting function. Following subtraction of background response (from the control flow cell), binding response is plotted against the concentration of analyte injected and fitted to a hyperbolic curve as dictated by the binding model (in most cases, for monomeric proteins we use a simple 1:1 Langmuir model). Analysis of changes in affinity with temperature by curve fitting to the non-linear van't Hoff equation can also be used to obtain thermodynamic constants for an interaction.

— Edward Evans

The detected light emission from the donor and acceptor molecules must all be corrected for background signals and especially for the presence of donor emission in the acceptor channel, which can be very significant. These values can then be used to derive the ratio between acceptor and donor emission, which corresponds to the BRET value. When attempting to derive stoichiometric data from a BRET analysis, any dependence of the BRET values on the acceptor/donor ratio must be fitted to a prescribed hyperbolic equation using a non-linear fitting algorithm that is found in most graphing software. Analysis of structural information about the protein of interest can be invaluable in determining the most appropriate terminus of the protein to fuse the BRET fluorophores to, as well as giving an approximate hydrodynamic diameter of the protein that may affect the maximal level of energy transfer observed.

— John James

Broadly-based Bayesian inference tools to link interactions to gene regulatory and biochemical networks.

— Stephen Michnick

Interaction databases such as DIP, BIND, or GRID are important tools to quickly check whether a particular interaction has been identified before. They are also helpful when you have to collect data on putative new interactors.

On a more complex level, support vector machines (SVMs) have witnessed increased application in the past years, especially when processing interaction data from high-throughput screens. SVMs can be used to attach a confidence level to a particular interaction based on information that includes the Gene Ontology annotations of biological process, molecular function, protein localization, transcriptional regulation, and essentiality of the genes encoding the proteins. All these parameters can help one to judge whether the identified PPIs are real or artifactual.

Lastly, we use the Osprey and the NAViGaTOR software packages developed in the Tyers and Jurisica lab for visualizing and analyzing PPI networks in 2D and 3D format.

— Igor Stagljar

Q1: What are the benefits of your preferred assay system(s)? (continued from p.7)

the MYTH assay works in intact yeast cells and because it's the only technology thus far reported to work as a screening system to find protein interactors of membrane proteins, the system has a great perspective in proteomics research. For example, MYTH provides an opportunity for therapeutic development by identifying novel drug targets for the diagnosis and treatment of many human diseases.

— Igor Stagljar

Q2: How do you reduce false positives? (continued from p.8)

DNA, either of the reporter or one interaction partner. Stable expression of at least one partner is another option and increased the stimulus-dependency of a GPCR b-arrestin interaction assay several-fold in our hands.

— Moritz Rossner

In yeast-based assays, false positive interactions are best reduced by using careful controls. In the so-called "bait-dependency test," putative interactors isolated in a screen are re-assayed against the "bait" protein used in the screen and a set of carefully chosen controls. Only those "prey" proteins which interact with the original bait but not with any of the controls is considered a true interactor and is pursued for further analyses. If this assay is performed with care, most false positives can be excluded early on.

Other strategies include checking the identity of isolated putative interactors against a reference database of commonly identified false positives (for example, the Golemis lab keeps a valuable list of false positives identified in YTH screens) or using computer-based algorithms to filter out false positives which spuriously interact with many baits.

One of the most effective strategies implemented in the past years has been the generation of YTH strains harboring multiple reporter genes. Compared to first generation strains, these "triple reporter strains" greatly reduce the number of false positives in a typical MYTH screen because activation of all three reporters is required.

— Igor Stagljar

If you want to increase sensitivity and avoid microarray artefacts...

Talk to Tecan

HS Pro microarray hybridization stations with ABS™

The HS 400™ Pro and HS 4800™ Pro are the perfect solutions for superior microarray processing. Fully automated – from pre-hybridization to slide drying – the instruments maximise the sensitivity and reproducibility you expect from your microarray results.

Only Tecan's HS Pro systems have ABS technology (patent pending) to prevent hybridization artefacts by excluding bubbles from the process. A variety of low volume hybridization chambers will even enable processing 2 independent arrays on 1 slide, on a throughput of up to 48 slides in a run.

The HS Pro systems are part of the Tecan Microarray Suite, a range of instruments and solutions from microarray processing and scanning to data analysis. They are easy to use, require less maintenance, and offer the flexibility to process different microarray applications. HS Pro systems are just one example of how Tecan is in touch with what you need most.

To find out more about HS Pro or any of our products, please talk to Tecan on the number below.

www.tecan.com

Liquid Handling & Robotics | Detection | Sample Management | Components | Services & Consumables

Switzerland +41 44 922 89 22 USA +1 919 361 5200 Other +43 62 46 89 33

List of resources

Our panel of experts referred to a number of publications and online tools that may be able to help you get a handle on interacting proteins.

Publications

Ghosh, I; Hamilton, A. D.; Regan, L.
Leucine Zipper Assisted Protein Reassembly: Application to the Green Fluorescent Protein
J. Am. Chem. Soc., 2000, 122, 5658-5659.

Söderberg O, Gullberg M, Jarvius M, Ridderstråle K, Leuchowius KJ, Jarvius J, et al.
Direct observation of individual endogenous protein complexes *in situ* by proximity ligation.
Nat Methods. 2006 Dec;3(12):995-1000.

Van der Merwe PA, Brown MH, Davis SJ, Barclay AN.
Affinity and kinetic analysis of the interaction of the cell adhesion molecules rat CD2 and CD48.
EMBO J. 1993 Dec 15;12(13):4945-54.

Websites

BIND
http://bond.unleashedinformatics.com/Action

Bouvier Lab BRET page
http://www.mapageweb.umontreal.ca/bouvier/bret/index.html

DIP
http://dip.doe-mbi.ucla.edu/

Dualsystems Biotech
www.dualsystems.com

Golemis Lab False Positive List
http://www.fccc.edu/research/labs/golemis/InteractionTrapInWork.html

GRID
http://www.thebiogrid.org/index.php

NAViGaTOR
http://ophid.utoronto.ca/navigator

Osprey
http://biodata.mshri.on.ca/osprey/servlet/Index

Sunrise Science Products

Looking for Yeast Media?

- *S. cerevisiae* and *S. pombe* formulations
- Amino acid dropout mixtures
- Dropout and yeast nitrogen base
- Sugars, supplements and agar
- Custom mixtures at no extra charge

High-Quality Affordable Ready-to-Ship

www.sunrisescience.com • info@sunrisescience.com

Proteomics Europe

4-5 September 2007 Amsterdam, The Netherlands

Keynote Speakers Already Confirmed

Ruedi Aebersold
Professor, Systems Biology, Institute of Biotechnology, ETH-Zürich and Faculty of Natural Sciences, University of Zürich

Dolores Cahill
Professor,Translational Science, Conway Institute, University College Dublin

Check ProteomicsEurope.com for updates

Welcome to the inaugural Proteomics Europe conference and exhibition to be held at the Hilton Amsterdam. We are assembling an excellent list of influential speakers for the agenda and will have a large exhibition alongside.

The conference is co-located with the European Biomarkers Summit. Registered delegates will also have access to this meeting ensuring a very cost-effective trip

Call for Papers - Become Part of the Event!

Oral presentation Submission Deadline:
25 February 2007

Poster Submission Deadline:
8 July 2007

If you would like to submit a proposal for an oral or poster presentation at this meeting, please visit ProteomicsEurope.com for details.

Possible Agenda Topics:

- Protein Arrays and their Applications
- Protein Expression
- High Throughput Proteomics
- Bioinformatics
- Disease-Related Proteomics

Exhibition Details

Our exhibition currently features the following exhibitors:

Digilab BioVisioN | Fusion Antibodies | Genomic Solutions | GenTel® BioSciences | Invitrogen | Metanomics Health | PerkinElmer | Proteome Sciences | Qiagen

Please contact us as soon as possible if you would like to receive information about exhibition & sponsorship opportunities:

Paul Raggett, Exhibition Manager
tel: +44 (0)1787 315117
email: paul.raggett@selectbiosciences.com

Corporate Sponsors

ProteomicsEurope.com

SELECT BIOSCIENCES
Delivering the Difference

GE Healthcare

Biacore

Success is based on knowledge, not chance

A system for success

Making the right decision at the right time is crucial to your success, from basic research through drug discovery and development, to manufacturing and QC.

So wherever you work, the highest quality, information-rich interaction data from a Biacore™ system enables you to make each critical decision with confidence – because every Biacore system is supported by more than 15 years of knowledge and experience.

Select the perfect solution for your application – from the company that continues to set the standard for label-free interaction analysis.

www.biacore.com

imagination at work