Protein interactions *à la carte*

This special issue brings together an array of novel methodologies to study protein-protein interactions. For the study of both interaction networks and individual protein complexes, the diversity of approaches is as impressive as the individual developments.

To conclude a 'methods-rich' year, welcome to this special issue on protein-protein interactions! Remarkably, most of the items on this theme that you will find in these pages are not commissioned reviews but primary research papers. The only reason we were able to assemble such a collection of high-quality papers on protein-protein interactions is the tremendous interest that this field of research is presently enjoying and the recognition that once again, methodological developments will be needed to move the field forward. The diverse array of approaches presented here is a testimony to the activity of different branches of a healthy field using multiple lenses to observe a complex problem.

Only a few years ago, it may have been inaccurate to talk about the 'field' of protein interactions, but this type of study has now evolved from an ancillary analysis to an area of research. The description of the yeast two-hybrid assay in the early 1990s led to a revolution in the study of protein-protein interactions. For the first time it was possible to screen hundreds, even thousands, of protein pairs for interactions. In the wake of the two-hybrid revolution, complementary methods were developed such as protein microarrays, and more physiological setups like purification of protein complexes from cells followed by identification of their components by sensitive analytical methods—in particular mass spectroscopy. This panoply of tools helped bring interaction studies to the proteome scale, and with the publication of landmark reports on large interaction networks in yeast and human cells over the past few years, interactomics was born.

The yeast two-hybrid assay had one effect that may not have been appreciated at the time. It served as the trigger for a division of the protein community between researchers striving to elucidate huge interactomes and those focusing on individual proteins or pathways. Arguably, the main reason for this schism was the high rate of false positives and false negatives that affected early high-throughput assays. Because of this limitation, results were received by many with skepticism, even open criticism. Subsequent developments have alleviated the problem, but it is fair to say that specificity and sensitivity remain an issue for all high-throughput protein-protein interaction screening methods. Nevertheless, the resulting interactome maps provide an unprecedented perspective on biological phenomena—one that will be needed to understand cell biology.

The complexity of biological function relies on protein interactions. Most proteins exert their function not alone, but paired with another protein or as a part of larger complexes whose modularity helps generate functional diversity from a limited number of original components. After completion of the inventory of parts thanks to genomic annotation and proteomics efforts, the next challenge will be to understand interactions at the proteome-wide level.

Thus, high-throughput screens clearly are the wave of the future, but no single approach will suffice. Rather, a combination will be crucial to circumvent the limitations of each screening system, and expanding the diversity of high-throughput screens by adapting more methods to large-scale and multiplex format will be key. Improvements to existing methods, especially in terms of specificity and sensitivity, are still required in addition to more efficient ways to validate the hits they generate—either experimentally, or by implementing confidence ratings based on experimental and contextual parameters.

Regardless of the success of these improvements, we certainly have not seen the last of studies on individual protein complexes. In fact, these approaches are essential to understand molecular mechanisms, and simply to answer questions that most screens are ill-equipped to address—such as taking into account temporal regulation of protein expression, protein movements between cell compartments or to tackle membrane proteins.

The body of knowledge accumulated from studies of single proteins is also a tremendous resource to complete and validate information obtained from high-throughput screens. One of the challenges of interactomics is to integrate this knowledge with new large-scale explorations—bridging the schism. Several databases have embarked on the enormous task of capturing all protein-protein interaction information by manually curating the literature, but a large proportion of the reports lack details that would be required to make the information useful in the context of network analysis. To facilitate the capture of information, a document proposing a list of 'minimal information required for reporting a molecular interaction experiment' (MIMIx) has been drafted, which compromises between the user's need for all relevant details and the burden on the scientist reporting the information. This document is currently up for public comment while being considered for publication at *Nature Biotechnology*. Readers who eventually will find themselves on the reporting or data-mining side are encouraged to evaluate the document and provide feedback to *Nature Biotechnology* editors (http://www.nature.com/nbt/consult/index.html).

But if you are hungry for new methods, what does this issue have to offer? In terms of improvements to existing high-throughput screens, two papers propose complementary enhancements to existing approaches. On page 1013, the group of Angela Bauch and Giulio Superti-Furga present new tags for tandem affinity purification (TAP), the workhorse of two recent genome-wide interactome studies in yeast. The new tags provide higher yields and better specificity, opening the door to TAP-tag purification and mass spectrometry analysis in mammalian cells, where so far the procedure has performed poorly.

Although this type of approach has fantastic scalability (virtually all proteins can be tagged), adding tags to proteins may affect their behavior—a problem that interactome studies must address by using different tags at different positions in proteins. More fundamentally, the requirement for protein overexpression can be problematic, even though it does improve sensitivity. In contrast, focusing on endogenous proteins has typically been hampered by the need for good antibodies and the lack of specificity. In the report on page 981, Matthias Selbach and Matthias Mann address the specificity problem by combining RNAi-based 'negative selection' with their previously developed metabolic labeling system to study endogenous protein complexes.

Two other papers report new methods based on protein-fragment complementation, in which interaction between two proteins fused to complementary fragments of a reporter protein results in functional complementation and reporter activity. Although not yet implemented in high-throughput format, these types of techniques allow the observation of protein interaction in living cells, some with subcellular resolution. On page 977, the original developer of the protein-fragment complementation assay, Stephen Michnick, describes an improved assay with better sensitivity and the capacity to measure reversible interactions. As an alternative, Moritz Rossner and colleagues (p. 985) describe the first split-protease assay, which exploits a protease to activate secondary reporters of choice, thus providing greater flexibility in the readout and expanding the possibilities for multiplexing.

An additional perk of complementation assays is that native protein localization can usually be maintained, provided that expression levels are kept low. Explanation of some protein behaviors, however, will require observation of interaction events between native proteins in their natural environment. This, of course, is a challenge of both sensitivity and specificity. But one that was met, at least in fixed cells, by the group of Ulf Landegren who adapted the proximity ligation assay to allow interaction detection *in situ* (p. 995).

Ultimately, single-molecule studies may be necessary to obtain complete descriptions of some protein interactions. Atomic force microscopy (AFM), for example, can quantify interaction forces between individual proteins. In this issue Simon Scheuring's team (p. 1007), describing the first AFM measurements on unsupported patches of membrane, shows how this technique may even help to settle many outstanding questions regarding membrane-protein organization and interactions.

Transmembrane proteins, in fact, constitute a difficult case, for which none of the common techniques of interaction measurement are particularly well adapted. Their membrane dependence complicates many assays, and the associated spatial constraints potentially result in confounding random interactions. The case of the G protein–coupled receptor (GPCR) family discussed on page 1001 illustrates this situation. Based on recent evidence, many researchers now accept that GPCRs exist primarily as functional dimers. However, Simon Davis' group examines the question of receptor dimerization using a more quantitative framework to distinguish true dimerization from random interactions. Their results question the notion that many of these receptors dimerize.

This example illustrates how different methods, owing to inherent biases or limitations, may provide different answers to interaction questions. It is therefore crucial to multiply the approaches, varying the window through which one looks at an interaction, to validate observations and get past the limitations of each technique. With this in mind, we hope you enjoy this array of new methods, and the accompanying News and Views that compare them and put them into perspective.

Call for comments!

Nature Methods is pleased to introduce *methagora*, an online space to debate methodological affairs.

Methodological questions, big and small, are at the core of everyone's research, day after day, technology after technology. Now and again, a methodological question even affects an entire field of research—for example, when divergent methodological approaches provide contradicting results or when the misuse of a method in a specific context risks casting doubt on its reliability in general.

We believe that both seasoned users and neophytes—as well as editors—can learn a lot from constructive discussions about these issues. Sometimes a simple clarification from experts will suffice, other times multiple opinions will have to be heard and weighed for a better understanding of the methodological problems at stake.

To facilitate such discussions, *Nature Methods* is pleased to introduce *methagora*, an online commenting forum dedicated to methodological topics.

Regularly, the editors will seed a debate by posting a topic and inviting comments. Most often, the topic will be directly linked to a paper recently published in the journal, but we will also consider unrelated methodological issues of importance to a particular community of scientists. And then, we call for your participation!

Commenting is simple. Comments submitted online will be posted after a rapid screen by the editors to ensure that they are relevant, appropriate and free of obvious commercial interests. We encourage, but do not require, authors to sign their posted comments, and we request a valid email address only to allow correspondence between the author of the comment and the editors.

Currently *methagora* is hosting a discussion on the most appropriate way of minimizing the reporting of false positives in large-scale RNAi screens. We are seeking opinions from a cross-section of the community of RNAi users in the hope of reaching a consensus of best practices that can be used as reference for users and editors.

The second ongoing debate concerns methods to measure protein-protein interactions in the particular case of G protein–coupled receptors (GPCRs). The results presented by Davis and colleagues in this issue (p. 1001), which call into question the widely accepted notion that all GPCRs exist as functional dimers, invite reflections on the native form of these proteins, but also on the methods used to determine their interactions.

We trust you will be interested in these debates and in forthcoming topics. So please visit the *methagora* site frequently and participate in the discussions. Have your say on the methods at the core of your research!

Methagora can be found at http://blogs.nature.com/nmeth/methagora/