

## SYSTEMS BIOLOGY

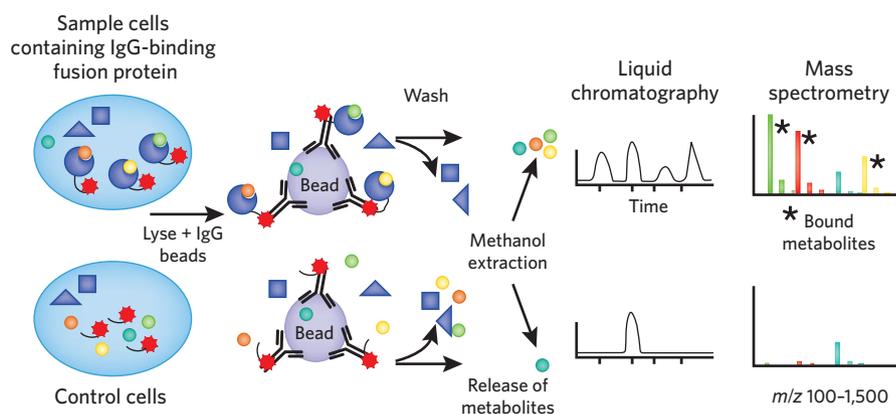
# Metabolites do social networking

To find out which metabolites bind to which proteins, one does not need to start with a hypothesis: it is now easiest just to do the experiments. As it turns out, some metabolites are quite promiscuous, at least in yeast.

Douglas B Kell

The first step in any study of systems or network biology is to work out what interacts with what to produce a qualitative map of the network<sup>1</sup>. Particularly for the networks involved in small-molecule metabolism, these maps or models are relatively commonplace, but although many linkages are well known, we certainly do not know all the interactions, and the problem is combinatorial. In principle, any metabolite could interact with any protein, and to test every small molecule (say, 1,000) with every enzyme (say, 2,000) serially at 10 concentrations would require an unfeasible number of experiments. When one takes into account that molecules can influence each other's binding and must therefore be tested together, the problem becomes even less tractable, as the number of combinations of just three metabolites from a set of 1,000 is ~100 million. Thus, a simple, generic screen for assessing small molecule–protein binding in high-throughput parallel assays is necessary to help complete network maps that include metabolites. This is what Li *et al.* have now provided in yeast<sup>2</sup>.

The search for ligands that bind to proteins is not confined to natural ligands, and much of modern pharmacology is concerned with finding (semi-) synthetic small molecules that bind to macromolecular targets (successful drugs are often in fact related structurally to natural metabolites<sup>3</sup>). A particularly useful and generic method for detecting such binding is mass spectrometry, as the mass of a small molecule in a well-designed experiment is usually sufficient to differentiate it from other molecules, and scientists can thus test and discriminate many hundreds of small molecules in parallel. This mass spectrometry approach is well established for candidate pharmaceuticals, which can indeed be tested several hundred at a time<sup>4</sup>, and, in general, the more hydrophobic a molecule is, the more promiscuous it tends to be<sup>5,6</sup>. A related approach<sup>7</sup> detects substrates for enzymes with unknown activities through the incubation of the protein candidate with a rich metabolite cocktail and determining which metabolite masses



**Figure 1** | Assessing the binding of yeast metabolites to a protein of interest. Proteins fused to an IgG-binding domain (top) or the IgG-binding domain alone (bottom) were expressed in yeast. The proteins along with interacting metabolites were isolated with IgG-loaded magnetic beads, washed and extracted in methanol. The molecules were detected and identified using LC-MS, and metabolites bound by the protein of interest (\*) were detected in the fusion sample (top) but not in the IgG-binding domain alone sample (bottom).

have been changed as a result of a reaction catalyzed by the enzyme.

Li *et al.*<sup>2</sup> expressed yeast proteins of the ergosterol biosynthesis pathway or kinases fused to an immunoglobulin G (IgG)-binding domain. The proteins, along with interacting metabolites, were isolated with IgG-loaded magnetic beads, which were subsequently washed and extracted in methanol, releasing the associated metabolites (Fig. 1). These molecules were detected and identified using LC-MS. A control using cells with no recombinant protein allowed metabolites that bound nonspecifically to the beads to be subtracted. Because they metabolize reasonably hydrophobic molecules that might therefore be moderately promiscuous, enzymes in the ergosterol biosynthesis pathway were Li *et al.*'s initial focus. They discovered that lanosterol is particularly promiscuous, as was pentaporphyrin 1, a molecule not previously associated with this pathway. In addition, ergosterol bound to several protein kinases unrelated to its biosynthetic pathway, often with 1:1 stoichiometry—a totally unexpected result. Particularly striking was the finding that ergosterol is important for the activity of a highly conserved yeast kinase, Ypk1, a homolog

of the mammalian Akt protein kinase family, which has been implicated in a variety of diseases. Armed with evidence for these novel interactions, they determined the binding constants for ergosterol–protein kinases directly *in vitro*, and although tightness of binding is not itself a criterion for specificity, the numbers, in the low micromolar range, imply that the binding was unlikely to be adventitious.

The special significance of this paper is that the original binding between metabolites and proteins takes place *in vivo*, and the authors have found some novel and unexpected interactions. It remains unclear how tightly the small molecules must bind a protein to survive the washing steps, and the functional significance of these interactions also remains to be determined. But the knowledge that a particular molecule binds to a protein *in vivo* means that enzymologists can assess the functional consequences of these interactions directly and focus on a very small but prioritized subset of the potential small molecule–protein combinations. Overall, it is clear both that small molecules can interact with many more proteins than one might have expected<sup>8</sup> and that this is typically necessary for them to have substantial effects in biochemical networks<sup>9,10</sup>. More generally,

it is deceptively easy to assume, once one has identified a function or a set of interactions for a particular protein, that this is its only function or set of interactions. Data-driven approaches, where one seeks novel or unknown interactions in an unbiased way, as here, are the easiest way out of this trap.

Improving our understanding of small molecule–protein interactions is critical for developing more accurate systems biology models, and the kinds of data produced by the Li *et al.* approach<sup>2</sup> are vital. Extending the method to the study of other organisms is a

very obvious next step, not least to metabolic network modeling and drug discovery in humans, for which these novel interactions will have substantial significance. No hypothesis is necessary<sup>11</sup>.

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#### Competing financial interests

The author declares no competing financial interests.