PICKING THE POCKETS OF PROTEIN–PROTEIN INTERACTIONS

Steve Buckingham

Controlling protein–protein interactions offers a rich vein for the discovery of new drugs, but researchers have shied away from this potentially lucrative area because of the numerous technical difficulties involved. Now, all this is set to change.

As the genomic era gives way to the proteomic era, biologists are asking less about how genes encode proteins and more about how proteins interact with each other. Proteins often use complex networks of interactions to produce a sophisticated signalling network that is capable of well-tuned and highly adaptive responses to environmental stimuli, such as in programmed cell death. Using proteomics, we can now investigate interactions between proteins on an unprecedented scale, even to the point of examining all of the proteins that are expressed in a given cell or tissue. What is particularly exciting is that revealing information about protein–protein interactions could provide the targets for a generation of new drugs.

Monitoring protein chatter

Protein–protein interactions occur in a number of different ways. Antibody–antigen binding is a well described protein–protein interaction. Some proteins act as enzymes that alter the structure of other proteins. For example, the γ-secretase enzyme complex cleaves the amyloid precursor protein into two fragments, and errors in this process are thought to have a central role in the development of Alzheimer's disease. Protein–protein interactions also control the localization of proteins, their substrate-processing activity, and even their tagging for destruction or recycling.

Protein–protein interactions also have a role in signalling between cells. Most cells respond to cues that control their production of enzymes, their metabolic activity, or their growth. For instance, the arrival of a growth factor molecule at a cell membrane causes a pairing (dimerization) of receptor proteins which then sets in motion a signalling cascade within the cell that leads to the appropriate set of responses.

Protein–protein interactions as therapy

A key feature of protein–protein interactions is their variety. Proteins interact in complicated ways because their shapes are so vastly complex. Amino-acid side chains that stick out from the body of the molecule create pits or bumps of different shapes and sizes. Proteins exploit this structural diversity to the fullest, producing binding pockets and recognition sites with varying degrees of specificity and subtlety of interaction. It is this versatility of protein–protein interactions that makes them such a tempting prospect to exploit in the search for new drug targets. Most current drugs target the important binding site of a protein, typically affecting its entire spectrum of operation. This new generation of drugs can act as competitive antagonists, but can also make much more subtle alterations through allosteric inhibition, by only disrupting the way in which a protein interacts with other specific proteins (FIG. 1).

The therapeutic applications of protein–protein interactions are potentially wideranging. Researchers hope to find ways of targeting specific interactions between HIV genes and their targets, overcoming the many limitations (such as viral resistance) that are associated with current treatments. The growth of tumours requires the development of new blood vessels. This process, known as angiogenesis, depends on the interaction of two proteins — metalloproteinase 2 (MMP2) and $\alpha_v\beta_3$, a receptor of the integrin family (see later).

Interrupting the conversation

But versatility comes at a price — ironically, the subtlety of protein–protein interactions presents serious obstacles to research. For one thing, there is a large range of concentrations over which proteins might interact. Affinities

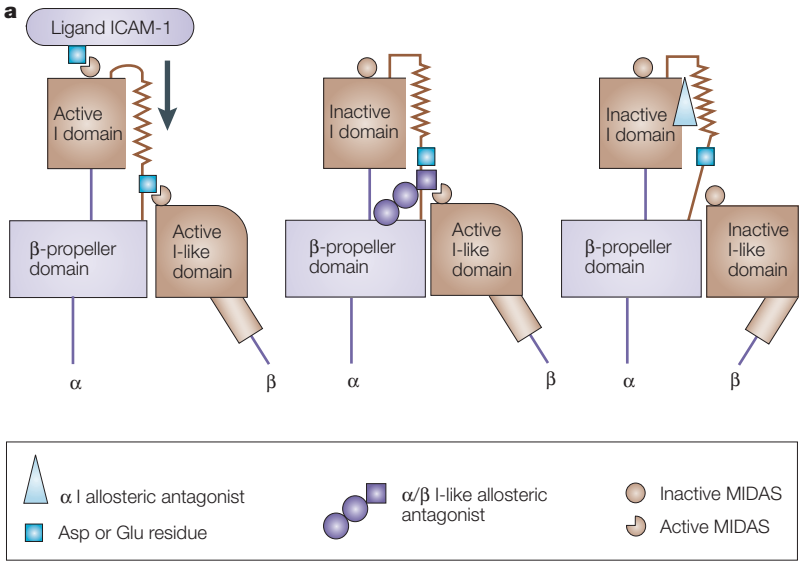

Figure 1 | **Model of allosteric inhibition of LFA1 by small molecules.** The α- and β- chains of LFA1 (αL and CD18, respectively) interact with each other at the headpiece domains. In the active conformation of LFA1, ICAM1 binds to the I-domain, which is held in the active conformation through interactions between the α7 helix with the I-like domain. α/β I-like domain antagonists bind at the junction between the α- and β-chains, interrupting the α7/I-like domain interaction. I-domain antagonists bind in a hydrophobic groove next to the α7 helix, which also interrupts the α7/I-like domain interaction. Modified from Arkin, M. R. & Wells, J. A. Small-molecule inhibitors of protein–protein interactions: progressing towards the dream. *Nature Rev. Drug Discov*. **3**, 301–317 (2004) © Macmillan Magazines Ltd.

between proteins can vary depending on the specific cellular function being studied and the precise nature of the immediate chemical environment, especially the pH and concentration of calcium ions. Even if you find something that inhibits a protein–protein interaction, it can be difficult to determine how specific its effect is, or even which of the proteins is being targeted, as there are many molecular routes to the same phenomenon.

Another problem has to do with size. Large or medium-sized peptides have often been used to modulate protein–protein interactions, and plenty of antibodies and other proteins are available to do this. But to be of therapeutic use, molecules must be small enough to get to the site of target-protein interaction, which is often hidden away inside cells.

But how can such small molecules have an effect at the large protein–protein interaction site? The interacting surfaces of the proteins are many times larger than a small molecule. To make matters worse, X-ray structures of protein–protein pairs often do not reveal the deep pockets that mark some binding sites. As if that were not enough, proteins are also remarkably flexible structures, being in constant motion between different conformational states with similar energies, and important fluctuations in the binding area would not show up in X-ray crystal structures anyway.

Thankfully, an inhibitory molecule does not have to cover the entire binding area. Binding pockets have 'hot spots' — small areas of bumps and holes that largely determine binding (FIG. 2). The problem is how to find them.

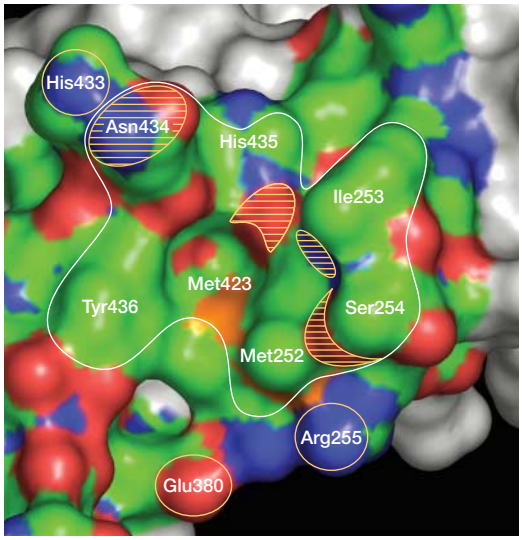

Figure 2 | **Hot spots at protein–protein interaction sites.** Although protein–protein interactions occur over a large surface area, X-ray crystallography and site-directed mutagenesis have shown that many protein–protein interfaces contain compact, centralized regions of residues — 'hot spots' — that are crucial for the interaction. Many proteins function by binding to multiple partners, and these proteins tend to use the same hot spot, which adapts to present the same residues in different structural contexts. Here, the Fc domain of immunoglobulin has been co-crystallized with three protein ligands and one phage-optimized peptide, and these X-ray structures indicate a common hot spot for the four ligands. The binding site is coloured by atom type and the consensus binding site is outlined. The central hydrophobic site is outlined in white, hydrogen-bonding interactions are shown by hashed lines and salt bridges are outlined in yellow. Modified from Arkin, M. R. & Wells, J. A. Small-molecule inhibitors of protein–protein interactions: progressing towards the dream. *Nature Rev. Drug Discov*. **3**, 301–317 (2004) © Macmillan Magazines Ltd.

Meeting the challenge

Dale Boger at the Scripps Research Institute, San Diego, is one chemist who is tackling the challenge head on. Boger thinks that the problem is largely in the mind. "It's a question of perception," he says. "There aren't so many examples of these small molecules to start from, so people get the general idea that it must be difficult to do."

In fact, small molecules of this kind have already been used to disrupt protein–protein interactions. The drug colchicine, for example, has long been used by biologists to disrupt the interaction between α- and β-tubulins in microtubules, which allow cell division — another crucial process in tumour formation.

If you want to find small molecules that interfere with protein–protein interactions, the best place to start is examining peptidomimetics — short, synthesized peptide fragments that mimic the most common peptide motifs, such as an α-helix or β-sheet. Often this peptidomimetic can be a simple α-helix that will tuck itself into a binding pocket and prevent protein–protein interactions. From these starting points, Boger's group used a technique called solution-phase combinatorial chemistry to generate a library of some 40,000 variants of these peptidomimetics.

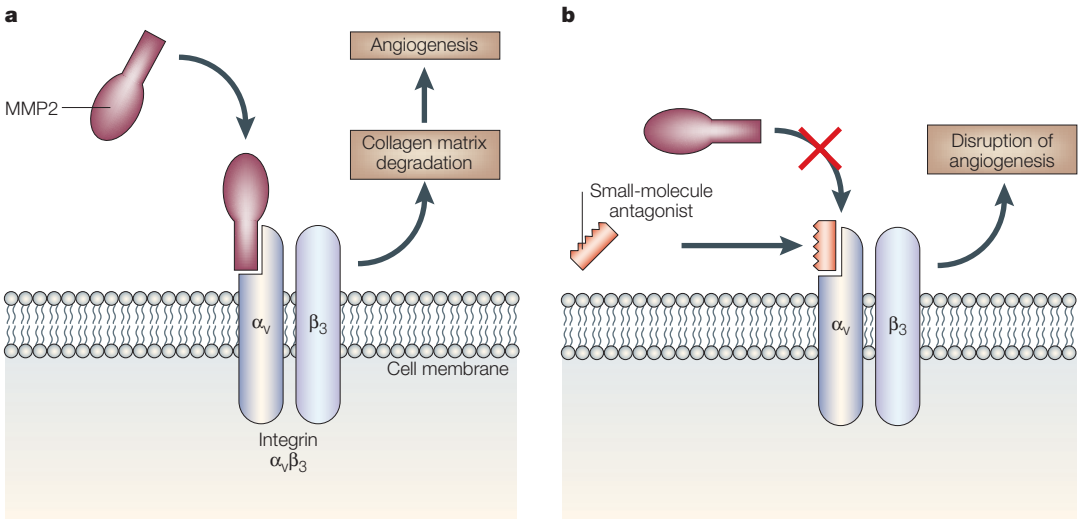

Figure 3 | **Protein–protein interactions and angiogenesis.** The growth of tumours depends on the development of new blood vessels, a process that is known as angiogenesis. **a** | The interaction of two proteins, metalloproteinase 2 (MMP2) and $\alpha_v\beta_3$ (a membrane-based receptor of the integrin family), is crucial to this process. Angiogenesis begins when MMP2 interacts with the α_v subunit of $\alpha_v\beta_3$, which then degrades the collagen matrix that surrounds cells, making room for new blood vessels to proliferate. **b** | Small-molecule inhibitors that block the ability of MMP2 to interact with $\alpha_v\beta_3$ would disrupt angiogenesis, and provide a powerful novel therapy for cancer.

The next task is to choose your protein–protein interactions. Boger focused on some carefully selected interactions which are not only steps in fundamental cellular pathways but might also be the targets for therapies. When he turned his attention to protein–protein interactions involved in angiogenesis, Boger struck gold.

As mentioned before, tumours depend on angiogenesis to survive. If their blood supply can be kept in check, tumour growth can be controlled. Angiogenesis begins with interaction of the integrin ($\alpha_v\beta_3$) and MMP2 (FIG. 3). This degrades the collagen matrix surrounding the cell, making room for the new budding blood vessel.

Boger built a library of compounds using $\alpha_v\beta_3$ molecules immobilized on a solid substrate, and has identified a compound that blocks interactions between the two proteins. It works by interfering with the site that controls the localization of the target protein MMP2 within the cell. "The really exciting thing is that we can inhibit an enzyme's function by targeting an enzyme's location site without affecting its catalytic site," says Boger. This raises the possibility of finding drugs that affect an enzyme in one tissue without affecting the same enzyme in another tissue. The long sought-after 'magic bullet' might not be so very far away after all.

Going to pieces

An alternative approach to combinatorial chemistry is to screen a number of small organic compounds, called fragments, find the ones that bind to your protein and

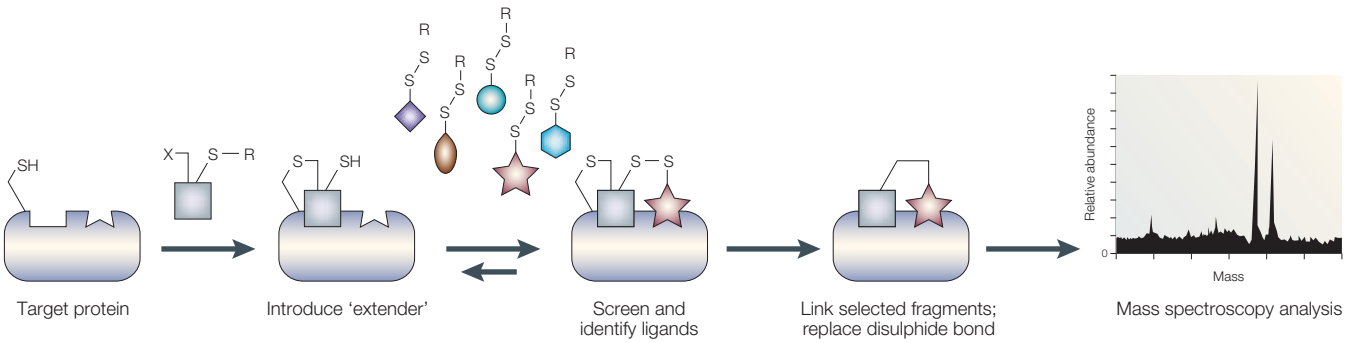

Figure 4 | **The tethering approach.** One approach to finding inhibitors of protein–protein interactions is to screen small organic compounds, called fragments, find those that bind to your protein and then 'stitch together' the fragments to find the most potent molecule. In an extended form of the approach, a target protein is engineered to contain a cysteine mutation (SH) near an interaction site. The protein is then probed with test fragments that contain disulphide (S–S) bonds. When a fragment meets the target area it becomes chemically bonded to the cysteine and can then be identified using mass spectrometry. The main advantage of this approach is that it starts with an enriched source of lead compounds, which are themselves good starting points because they are less hydrophobic and are therefore likely to be good building blocks for drugs.

then start stringing the fragments together to find the most potent molecule. The fragments can be initially screened using techniques such as nuclear magnetic resonance (NMR), or by an ingenious technique called 'tethering' (FIG. 4).

Tethering begins by engineering the target protein with a cysteine mutation near an interaction site. The protein is then probed with test fragments containing disulphide bonds. When a fragment hits the target area, it becomes chemically bonded to the cysteine and can then be identified using mass spectrometry. Whether tethering or NMR is used, the beauty of the fragment discovery approach is that it starts with an enriched source of lead compounds, which are themselves good starting points because they are less hydrophobic and therefore likely to be good building blocks for drugs.

Jim Wells at Sunesis Pharmaceuticals has pioneered the tethered fragment approach to identify hot spots on the interleukin receptor, IL-2. His group were able to determine some of the key conformational changes in the molecule that accompany binding to tethered fragments. They found two subsites, a rigid one and a more mobile one (FIG. 5), of which the latter proved more effective in binding the fragments. This has one important implication for how we should go about discovering new drugs that act on protein–protein interactions — sites are often flexible and therefore one should be wary of designing small molecules purely on the basis of structural analyses.

Targeting protein–protein interactions is clearly more challenging than traditional approaches to the identification of small-molecule inhibitors of protein targets. Nevertheless, researchers are making progress and some of the obstacles are gradually being eroded. Molecules have been identified that allosterically inhibit the function of inducible nitric oxide synthase by binding to the haem cofactor in the protein active site, which disrupts protein dimerization. Recently, small-molecule inhibitors of the MDM2–p53 tumour suppressor protein interaction have generated excitement in the field. MDM2 impairs the ability of p53 to repair potential cancer-causing breakages in genes, and disturbing this interaction could be a novel strategy of cancer therapy. It is hoped that these and other successes will encourage more interest and research in this area, and that what seemed impossible only a few years ago might now become probable.

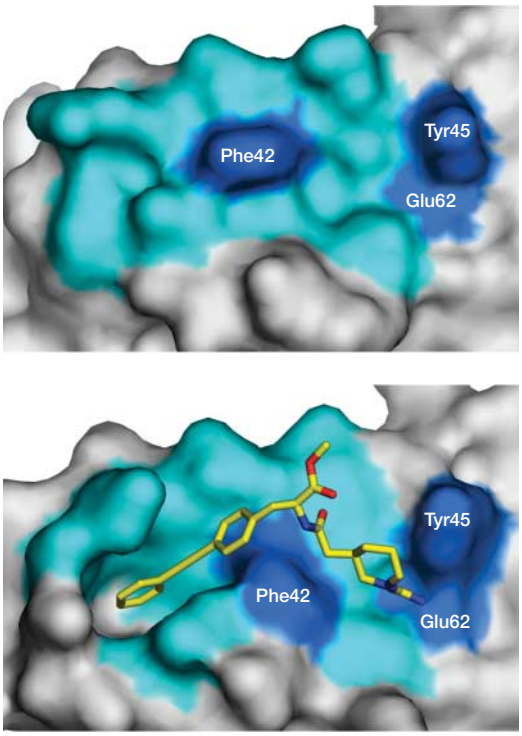

Figure 5 | **Hot spots on the interleukin receptor, IL-2.** **a** | Structure of unliganded IL-2. **b** | Structure of the small-molecule Ro26-4550 bound to IL-2. Hot-spot residues for binding to IL-2 are shown in light blue (moderately important) and dark blue (very important). Ro26-4550 binds at the same hot spot, which seems to be highly complementary to the Ro26-4550 structure. On binding of Ro26-4550 and other small-molecule inhibitors, the IL-2 binding surface undergoes a conformational change in the hydrophobic portion of the site (to the left of the structures), while remaining relatively fixed in the guanidine-binding portion of the site (to the right of the structures). Modified from Arkin, M. R. & Wells, J. A. Small-molecule inhibitors of protein–protein interactions: progressing towards the dream. *Nature Rev. Drug Discov*. **3**, 301–317 (2004) © Macmillan Magazines Ltd.

FURTHER READING

Arkin, M. R. & Wells, J. A. Small-molecule inhibitors of protein–protein i nteractions: progressing towards the dream. *Nature Rev. Drug Discov*. **3**, 301–317 (2004)
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