

## Protein–Protein Interactions as Targets for Novel Therapeutics

a report by

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The lack of novel, validated targets continues to severely limit the emergence of new therapeutics. Much of the effort to address this need is directed towards the screening of compounds on target proteins that belong to classes of molecules known to have yielded druggable substances in the past. Membrane-bound receptors, ion channels and enzymes such as kinases or proteases are thus widely explored as specific tools for the identification of small-molecule drugs. G-protein-coupled receptors, for instance, constitute nearly 40% of currently used targets of the most commonly used drugs, such as beta-blockers, beta-agonists and dopamine or serotonin antagonists.<sup>1</sup> Altogether, however, such receptors, kinases and proteases probably do not constitute even 10% of the 20,000 genes identified in the human genome. Many of the other encoded proteins have no obvious binding or enzymatic activity, as deduced from structural homology, and have thus not yet been considered as potential tools for drug discovery.

### Protein–Protein Interactions as Targets for Drug Discovery

To further expand the number of targets, several groups have considered inhibiting interactions between two or more proteins. While long considered unlikely to be successful, a number of recent studies now confirm the feasibility of this approach, and several small-molecule inhibitors of protein–protein interactions (PPIs) are in clinical trials.<sup>2–5</sup> This progress results from better validation of PPI data and corresponding validation as drug targets, as well as from improved knowledge of the structural requirements of small-molecule inhibitors of PPIs based on better understanding of the PPI interfaces.<sup>2,5</sup> Stringent quality control applied to a combination of complementary novel procedures to the study of PPIs has allowed a number of groups to define one-to-one interactions precisely. These procedures range from iterative two-hybrid-in-yeast to co-immuno-precipitation or co-affinity-chromatography to surface plasmon resonance analysis.<sup>4</sup>

### Target Validation

Several approaches have been developed to validate PPIs that could serve as targets for the development of novel therapeutics. Mono-specific antibodies can be raised against one of the interacting partners, or small interfering RNA can be prepared to block the synthesis of one of the protein partners: each of these types of reagents can thus be evaluated

for its effect on the pathway in which the proteins are active.

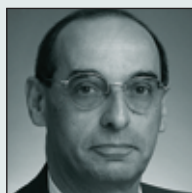
### Protein–Protein Interfaces and Conceptual Approaches for Inhibitors

In contrast to what is generally stated, the PPI interfaces between two proteins are not necessarily large and flat: the study of crystallised antigen antibody complexes had already shown the relative importance of a few interacting residues from each protein. The bulk of the binding energy could thus reside in a relatively small area that could be occupied by a small-molecule inhibitor; a number of ‘epitope’-containing residues or small compounds had indeed been shown to be capable of inhibiting the antigen–antibody reaction. Such energetic ‘hotspots’, which often contain aromatic residues, have been elegantly discussed by Arkin and Wells,<sup>2</sup> who were among the pioneers of their discovery.<sup>6</sup> The same group also confirmed the adaptability of the PPI interface upon interaction with small-molecule binders, thus enhancing the probability of a good fit. Finally, the advent of fragment-based screening – in which a low-affinity binder is combined with a second, complementary low-affinity binder – has led to the synthesis of hybrid high-affinity inhibitors through ‘tethering’ or ‘assembly’ of two compounds.<sup>7,8</sup> *Figure 1* shows four examples of small-molecule inhibitors of PPIs. All four are rather bulky structures and are rich in phenyl rings. The remainder of this short review summarises some of the most relevant reports that have appeared in recent years describing small-molecule inhibitors of PPIs. I will restrict myself to studies in which compounds have been developed as the basis for novel therapeutics. In order to organise this discussion, I have listed in *Table 1* a number of areas in which such analyses have been conducted.

### Ligand Receptors

The obvious place to look for inhibitors of PPIs is, of course, where natural compounds are known to bind specifically and with high affinity – namely receptors. The interleukin (IL)-1 receptor is a good example of such a protein. When mapping the binding site of a small-molecule inhibitor of this cytokine, Arkin<sup>6</sup> discovered that the compound was actually binding in a so-called ‘hot spot’ where binding also occurs with the IL-1 receptor, thus demonstrating a direct interference between the small-molecule inhibitor and the PPI between natural ligand and receptor. The same group has further expanded their observations to other examples.<sup>2</sup>

While most binding of natural ligands indeed occurs in well identified binding sites, a number of studies have now revealed a resulting displacement of binding of associated proteins, mostly through allotropic ‘adaptive’ changes in the receptor, but also through direct disruption of interactions. A good example is the glucocorticoid receptor, which binds immunosuppressive and anti-inflammatory hormones produced by the adrenal glands; for this purpose it requires effective interaction with a chaperone protein, Hsp90. This interaction, effectively inhibited by drugs



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**Table 1: Recent Examples of Selected Protein–Protein Interactions Disrupted by Small-molecule Inhibitors**

Apoptosis and Cell-cycle Pathway Proteins	Ligand Receptors
Bak-BH3/Bcl2 <sup>12</sup>	TNFalpha trimer <sup>8</sup>
Bak-BH3/Bclxl <sup>11</sup>	Co-activator/Oestrogen-alphaR <sup>19</sup>
Bak-BCl2 <sup>13</sup>	HSP90/GR <sup>9</sup>
Calcineurin/NFAT <sup>20</sup>	IL2/IL2R <sup>2,7</sup>
CDK2/CyclinA <sup>21</sup>	
LFA1/ICAM-1 <sup>22,23</sup>	<b>Virus/Host-cell Proteins</b>
MDM2/p53 <sup>24</sup>	HCV-NS5B/CyclophilinB <sup>17</sup>
Myc/Max <sup>25</sup>	HIV-gag/CyclophilinA <sup>14</sup>
Tcf4/beta-catenin <sup>26</sup>	HIV-1/CCR5 <sup>15</sup>
	HIV/Tat <sup>16</sup>

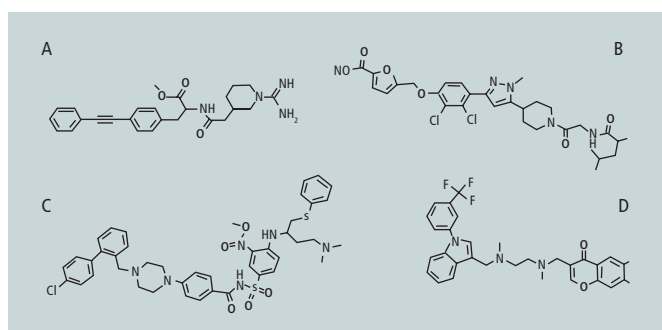
such as geldanamycin or cisplatin, has now been mapped using a variety of structural mutants.<sup>9</sup>

### Apoptosis and Cell-cycle Proteins

Considerable efforts have also gone in to identifying inhibitors of PPIs involving BH3 domain-certain protein and their Bcl2 or Bclx partners, which are active in apoptosis of various cell types. A number of convincing studies has now been published.<sup>10–12</sup> At least one study describes an inhibitor of Bcl-2 family proteins to induce regression of solid tumours in animal studies.<sup>13</sup> This compound, which is potent at subnanomolar concentrations, is poorly soluble and has a molecular weight of 813 Daltons, but was nevertheless considered suitable for clinical development. As with several other inhibitors of PPI, it is rich in phenyl groups (see *Figure 1*).<sup>5</sup>

### Virus/Host Proteins

One area in which considerable effort has been made to identify inhibitors of PPI is in host pathogen interactions, and more particularly virus/host protein interactions. The initial difficulty in identifying anti-HIV compounds has led a number of groups to try to block interactions between HIV and host proteins, including CD4, cyclophilin A (CsA), tat and gag.<sup>14–16</sup> Many of these initial reports were not followed up by more definitive studies. One of these compounds – maraviroc, which inhibits the interaction between HIV and its CCR5 co-receptor – is now in phase

**Figure 1: Examples of Structures of Small-molecule Inhibitors of Protein–Protein Interaction Targets**

(A) IL-2 antagonist Ro26-4550 (IC<sub>50</sub> 3–6μm), discovered serendipitously through medicinal chemistry.<sup>27</sup> (B) IL-2 antagonist (IC<sub>50</sub> 60nm), discovered through the fragment-based tethering approach.<sup>7</sup> (C) ABT-737 (IC<sub>50</sub> ~0.1nm), an inhibitor of Bcl-2, Bcl-XL and Bcl-w discovered using SAR by NMR.<sup>13</sup> (D) TNFα antagonist (IC<sub>50</sub> ~10μm), discovered through a fragment-based approach.<sup>8</sup>

Source: permission from Whitty and Kumaravel, 2006.<sup>5</sup>

II clinical studies based on the observation that it strongly reduces plasma viral load in HIV-infected people orally treated with the compound.<sup>15</sup> Similarly, the well-known immunosuppressive agent CsA was also initially described for its potential ability to slow down HIV infection by interfering in the interaction between the viral gag protein and cyclophilin A, one of the host-cell receptors for CsA.<sup>14</sup> More recently, the cyclophilin B-mediated effect of CsA on the NS5B polymerase of hepatitis C virus<sup>17</sup> led to currently ongoing clinical studies.<sup>18</sup>

### Conclusion

While progress is not being made nearly as quickly as was expected after the first successes, there is nevertheless a growing body of evidence that suggests that PPIs are not necessarily as intractable as first concluded using traditional screening approaches. Using novel approaches such as fragment combination, tethering and screening libraries developed using molecular scaffolds more complex than those found in the more usual high-throughput screening (HTS) collections of compounds a number of inhibitors have indeed been discovered that are now progressing towards clinical development. ■

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