Reaching for high-hanging fruit in drug discovery at protein–protein interfaces

小组：　　冯施雨　柳叶茂
报告人：　　冯施雨
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Reaching for high-hanging fruit in drug discovery at protein–protein interfaces

James A. Wells[1,2] & Christopher L. McClendon[3]

Targeting the interfaces between proteins has huge therapeutic potential, but discovering small-molecule drugs that disrupt protein–protein interactions is an enormous challenge. Several recent success stories, however, indicate that protein–protein interfaces might be more tractable than has been thought. These studies discovered small molecules that bind with drug-like potencies to 'hotspots' on the contact surfaces involved in protein–protein interactions. Remarkably, these small molecules bind deeper within the contact surface of the target protein, and bind with much higher efficiencies, than do the contact atoms of the natural protein partner. Some of these small molecules are now making their way through clinical trials, so this high-hanging fruit might not be far out of reach.

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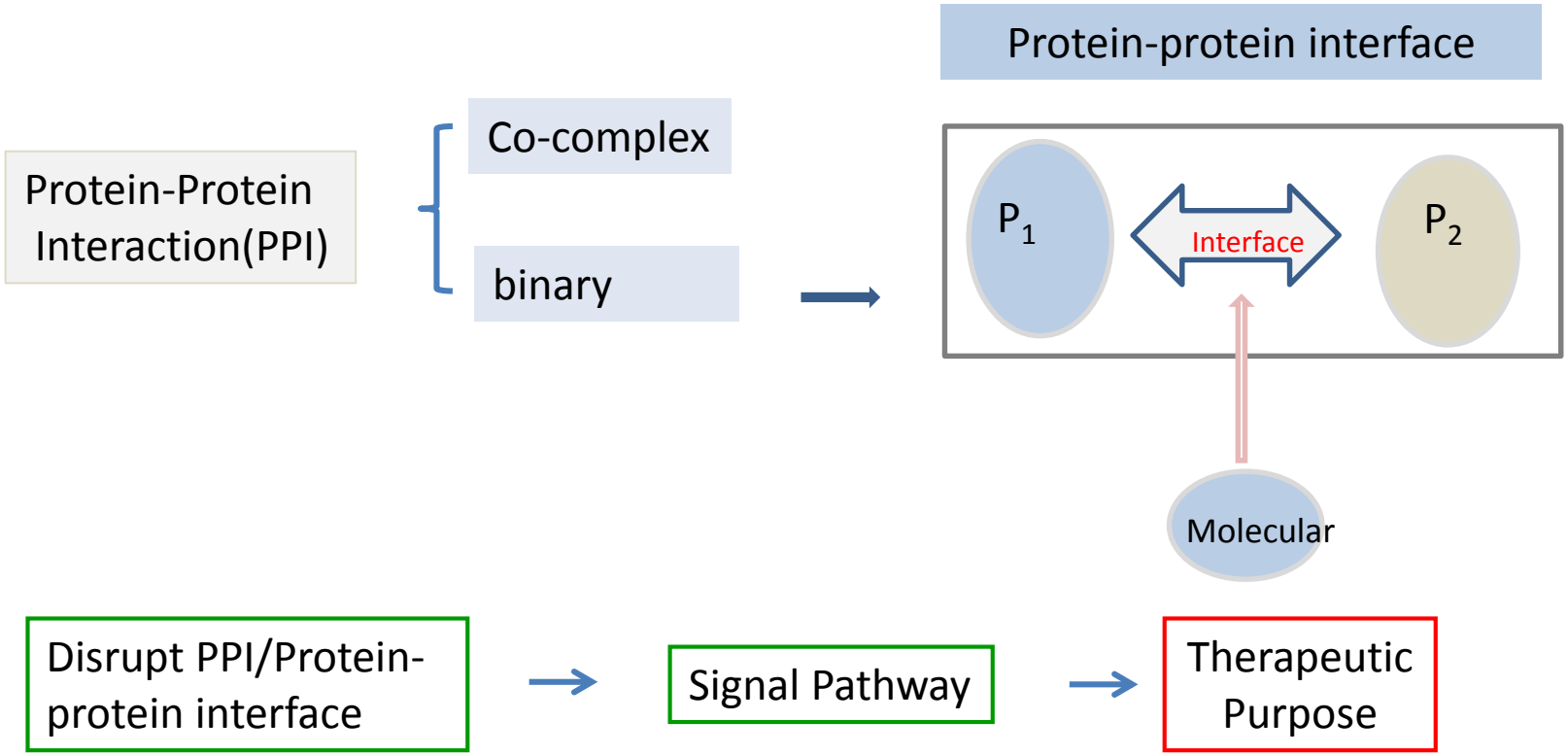
Introduction

Targeting the interfaces between proteins has huge therapeutic potential, but discovering small-molecule drugs that disrupt protein–protein interactions is an enormous challenge.



Differences between protein–protein interactions and protein–small-molecule interactions

contact surfaces	protein–protein interactions	protein–small-molecule interactions
area	approx1,500–3,000 Å2	approx300–1,000 Å2
shape	generally flat	Present grooves and pockets

Challenges：

Unlike the classic proteins for which small-molecule drugs have been designed , protein–protein interactions do not have natural small-molecule partners. Thus, efforts to discover drugs that bind to a protein–protein interface do not have the luxury of starting from a small natural substrate or ligand.

I. Most contact surfaces in protein–protein interfaces also involve amino-acid residues that are not contiguous in the polymer chain.

II. High-throughput screening (HTS) does not routinely identify com-pounds that disrupt protein–protein interfaces.

III. Biopharmaceuticals such as monoclonal antibodies and polypeptide hormones almost always bind to protein–protein interaction surfaces, there are few approved small-molecule drugs that do so.

Several lines of evidence provide hope

for finding small molecules that target protein–protein interfaces.

I. Although PPI interfaces are large mutational studies to find ‘hotspots’

II. Proteins involved in protein–protein interactions can be ‘promiscuous’, binding to several targets using the same hotspot region. Structural studies show that these promiscuous contact surfaces are adaptable, allowing one protein to engage a range of structur-ally diverse partners.

III. Moreover, peptides selected for binding to one of the partners in a protein–protein pair (by using phage display) often compete with the natural protein partner for binding to the hotspot.

Thus, there seem to be many chemical solutions for tight binding, and large contact surfaces can be engaged by more-compact structures.

Protein-protein Interfaces——"hotspots"

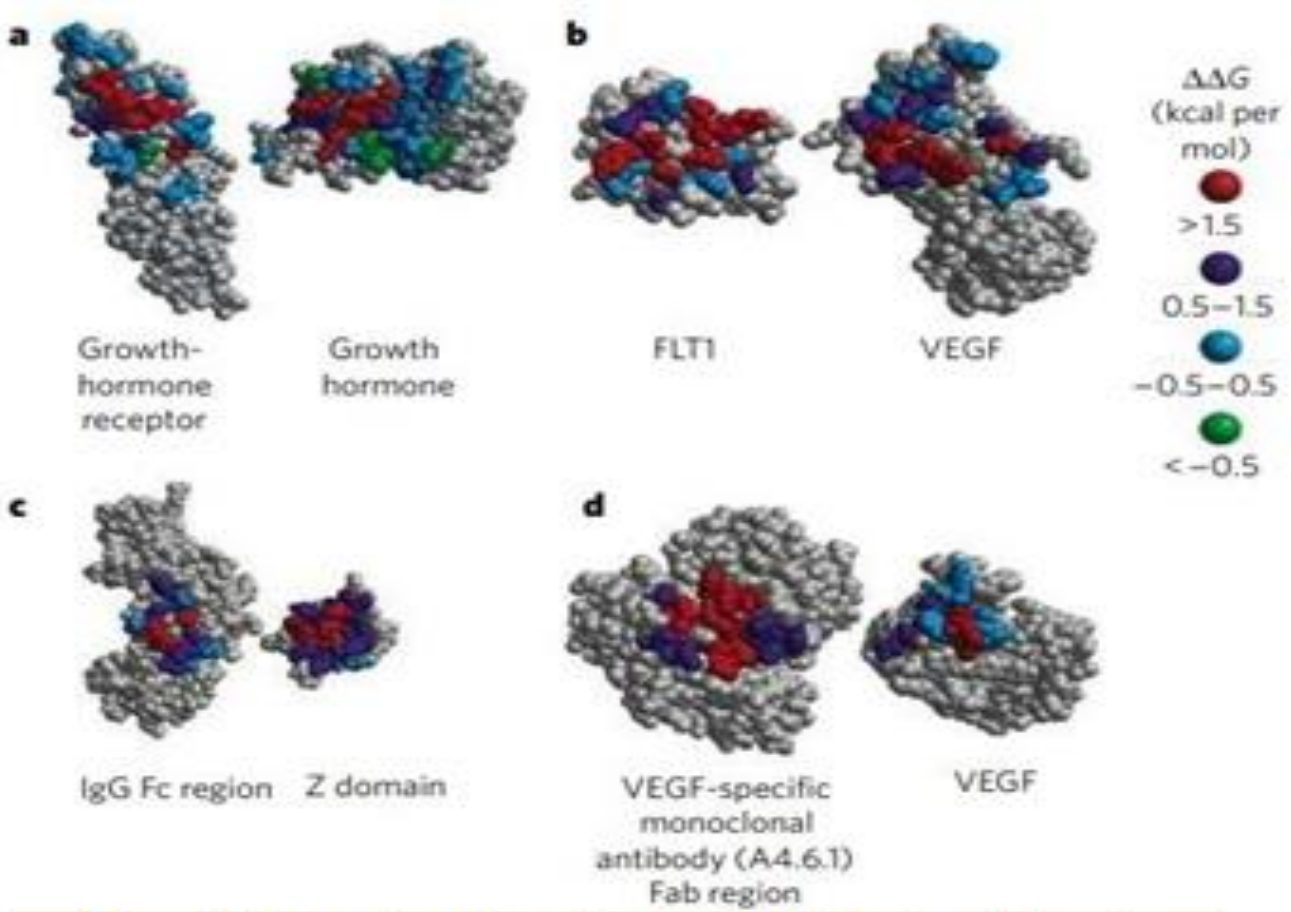


Figure 1 | Examples of protein–protein interface hotspots.

Alanine-scanning mutational analysis (replacing each amino acid, in turn, with alanine) was carried out on the contact surfaces of four pairs of interacting proteins.

Assemble/Design Molecule

These molecules were assembled in a fragment-based approach guided by X-ray structures and medicinal chemistry, and inspired by the previous drug-discovery efforts of Jefferson Tilley and co-workers at F. Hoffmann-La Roche.

- fragment-binding data
- structures of compounds bound to IL-2
- medicinal chemistry
- structure–activity relationships (SAR)

More recently, another class of small molecule that targets TNF was discovered, by using fragment screening.

Six examples of PPIs and the small molecules

Table 1 | Comparison of protein and small-molecule binding partners

Ligand	Molecular mass (Da)	PDB identity of complex	Affinity (μM)*	Ligand efficiency (kcal per mol per non-hydrogen atom)†	References
IL-2					
IL-2 receptor α-chain	24,790	1Z92	0.0105	0.11	33
SP4206	663	1PY2	0.06	0.21	31, 34
BCL-X_L					
BAD-derived peptide (amino acids 100–126)	3,110	2BZW	0.0006	0.16	89
ABT-737	813	2YXJ	0.0006	0.23	47
HDM2					
p53-derived peptide (amino acids 15–29)	1,808	1YCR	0.6	0.12	51
Nutlin-3	581	1RV1‡	0.09	0.24	53
Benzodiazepinedione	566	1T4E	0.067	0.31	54, 55
HPV E2					
E1	24,630	1TUE	0.06	0.14	62
Compound 23	684	1R6N‡	0.006	0.28	60, 61
ZipA					
FtsZ-derived peptide (amino acids 367–383)	2,024	1F47	21.6	0.13	63
Compound 1	425	1Y2F	12	0.23	67
TNF					
Subunit protein	17,381	1TNF	ND	ND	90
SP304	548	2AZ5	13	0.17	70

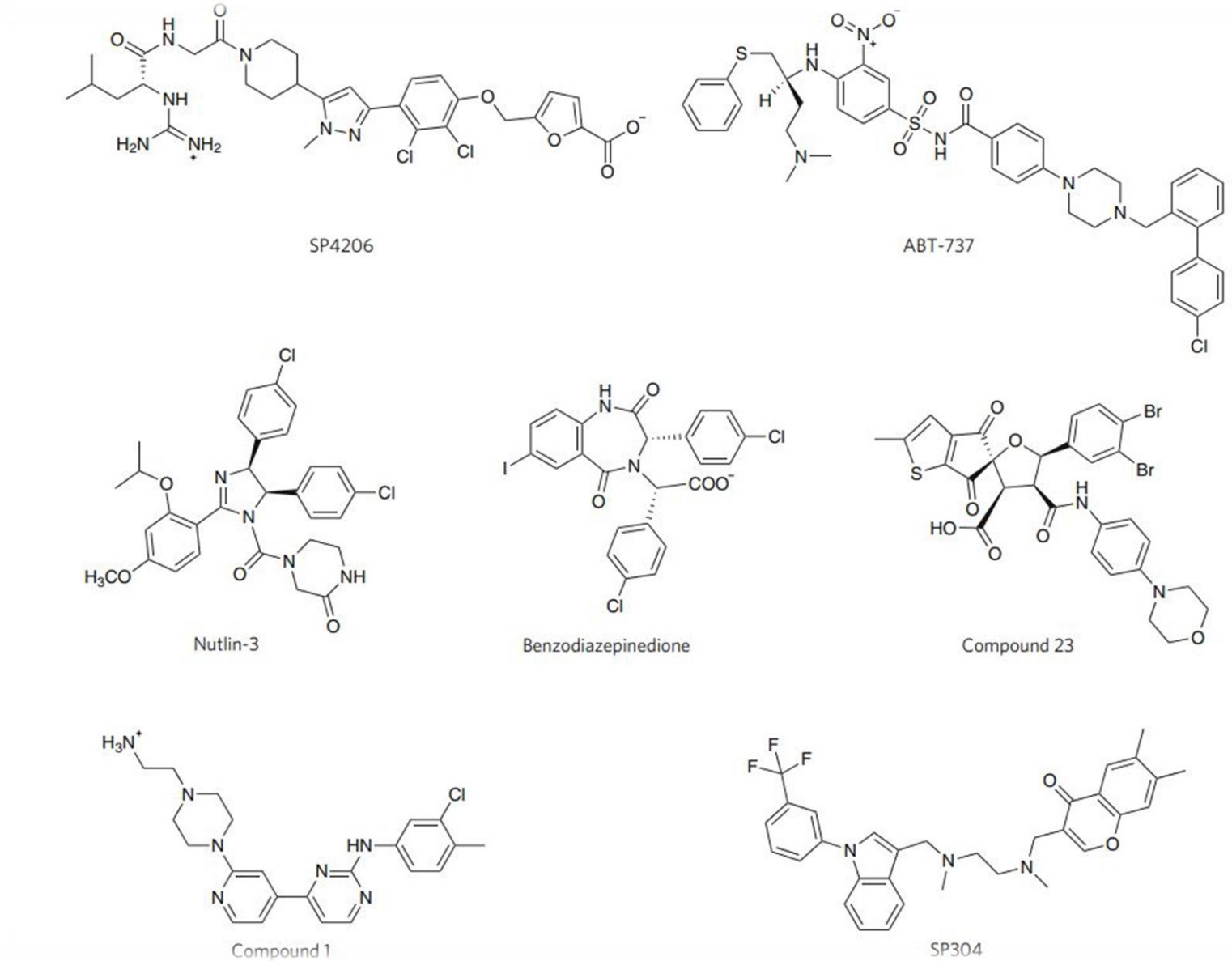
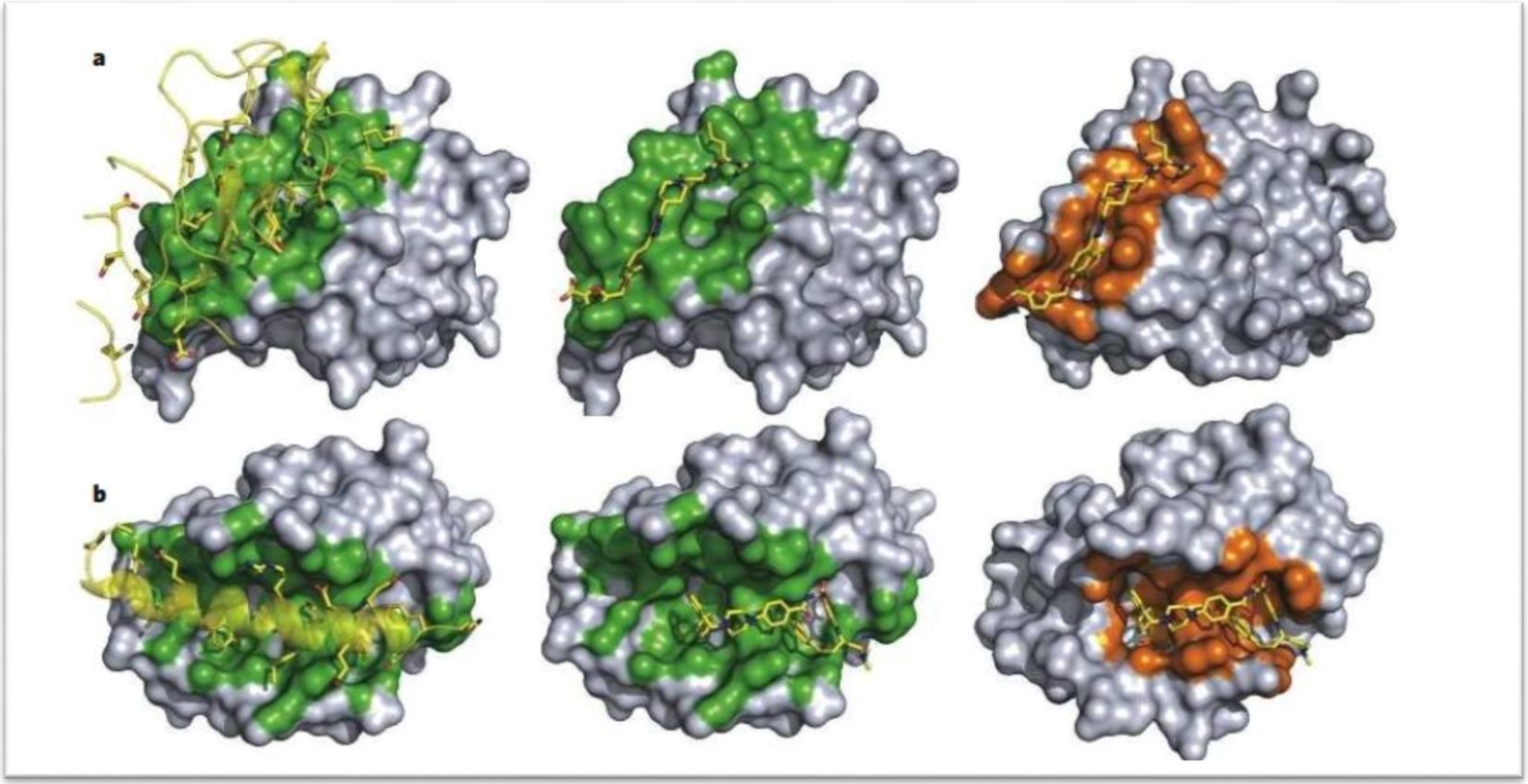
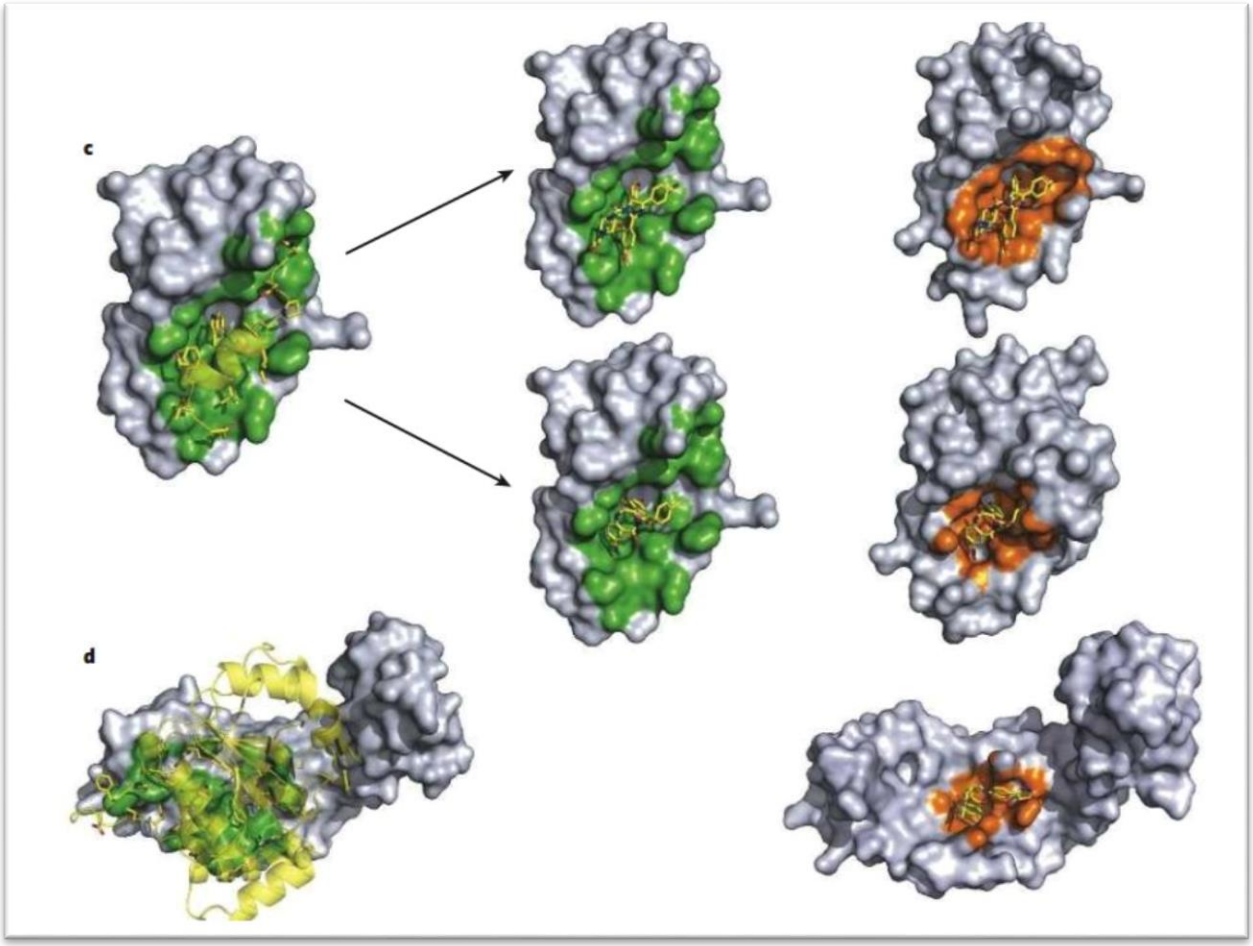


Figure 2 Examples of small molecules that inhibit protein–protein interactions.



a, IL-2 bound to its natural protein partner IL-2Ralpha (left), and IL-2 bound to the small molecule SP4206 (right).

b, Bcl-XL bound to a peptide derived from one of its natural protein partners, BAD, and Bcl-XL bound to the small molecule ABT-737.



c, HDM2 bound to a peptide derived from its natural protein partner p53, and HDM2 bound to the small molecule Nutlin-2 (upper) or a benzodiazepinedione (lower).
d, HPV-18 E2 bound to HPV-18 E1, and HPV-11 E2 bound to the small molecule compound 18. The centre panel is not shown, because HPV-18 and HPV-11 are related but not identical.

TNF disruptors

- The cytokine tumour-necrosis factor (TNF)(肿瘤坏死因子) is a key factor in inflammatory responses and is therefore an important drug target. Biological therapeutics that target TNF have been approved for treating arthritis. Not surprisingly, there is considerable interest in developing small molecules or peptides that can disrupt the interaction between TNF and its receptors, TNFR1 and TNFR2.

- For example, small (13-residue) TNFR1-derived peptides that bind to TNF with moderate affinity (Kd approximately 5 microM) have been found, and photoactive small molecules that inhibit the TNF–TNFR1 interaction by labelling a site near where the receptor binds have also been discovered.

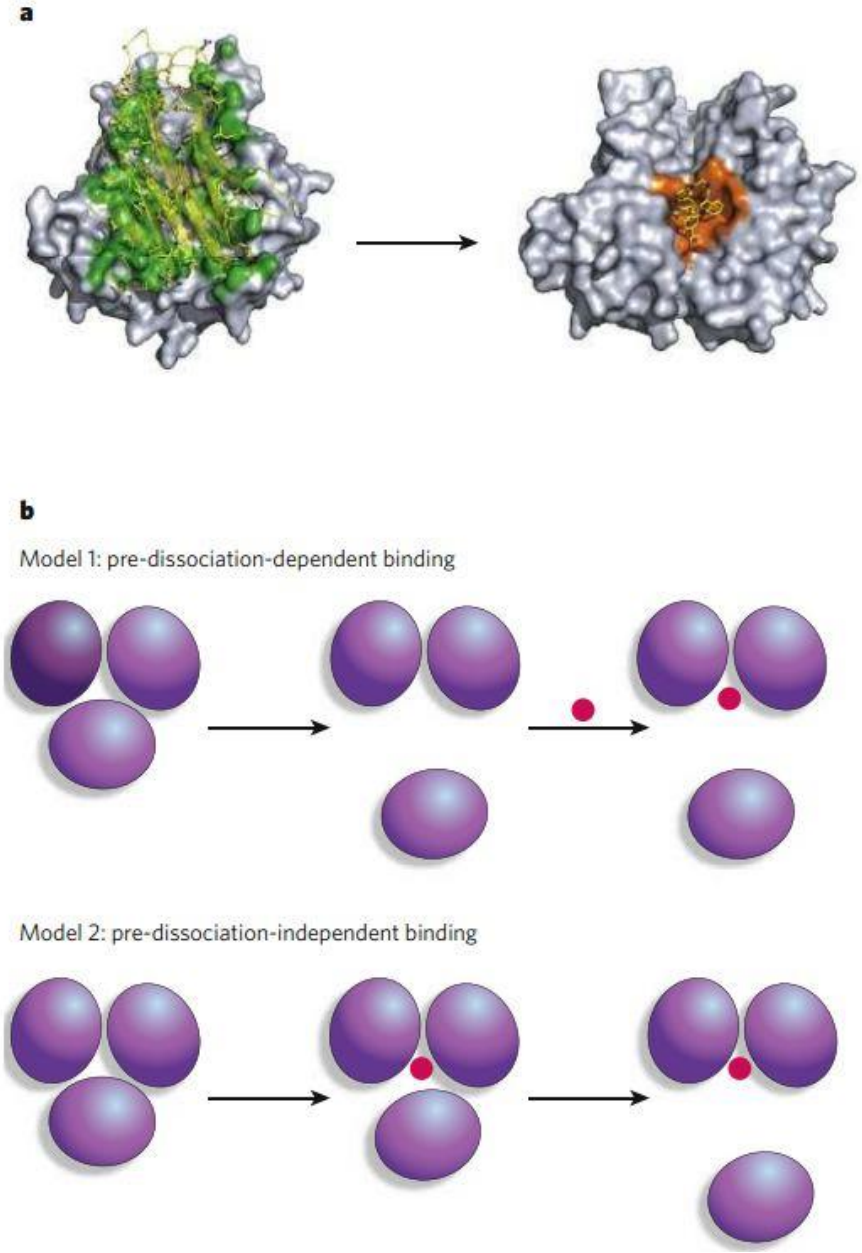


Figure 4 | Disruption of TNF by a small molecule.

a, The structure of TNF, which is composed of three monomers, is shown on the left. The structure of the TNF dimer in complex with the small molecule SP304 is shown on the right

b, There are two models for how small molecules could block the formation of TNF trimers:
In model 1, one of the monomers of TNF must completely dissociate before the small molecule can bind.
In model 2, the small molecule can intercalate into the TNF complex and associate, which facilitates dissociation of a monomer. SP304 accelerates the rate of monomer dissociation (by more than 600-fold), which supports model 2.

Myths about disrupting protein–protein interfaces

- **Protein–protein contact surfaces**

One myth is that the large and flat contact surfaces seen in structures of protein complexes are rigid and do not present cavities for small molecules to bind.

- **Screening for protein–protein interface inhibitors**

Another myth is that screening does not work for protein–protein inter-faces.

- **Affinity of protein–protein interactions**

A further myth is that native protein complexes have a higher affinity than protein–small-molecule complexes and cannot be competed away.

- **Size of small molecules that disrupt protein–protein interactions**

Another myth is that small molecules that target protein–protein interfaces are too large to be drugs.

Affinity of protein–protein interactions

Table 2 | Ligand efficiencies of other small molecules that inhibit protein–protein interactions

Target	Compound	PDB identity of complex	Affinity (μM)	Ligand efficiency (kcal per mol per non-hydrogen atom)	References
Bcl-X_L	Compound 31	1YSI	0.036	0.27	49
HPV E2	Compound 18	1R6N	0.04	0.25	60, 61
ZipA	Compound 3	1Y2G	83.1	0.22	67
Clostridium botulinum neurotoxin B	Doxorubicin	1I1E	9.4	0.18	91
β-Catenin	PNU-74654	–	0.45	0.36	92
ARF1–ARNO complex	LM11	–	49.7	0.22	93
Dishevelled	FJ9	–	29	0.23	94
Rac	NSC23766	–	50	0.19	95
CD4 D1	J2	–	100	0.22	96
HIV gp120	NBD-556	–	47	0.26	97
EIF4E	4EGI-1	–	25	0.22	98
CD80	Compound 9	–	0.28	0.37	99

D1, amino-terminal variable-region-like domain
EIF4E, eukaryotic translation initiation factor 4E
ARF1, ADP-ribosylation factor 1
ARNO, ARF nucleotide-binding-site opener (also known as PSCD2)
HIV, human immunodeficiency virus
gp120, glycoprotein 120

Size of small molecules that disrupt protein–protein interactions

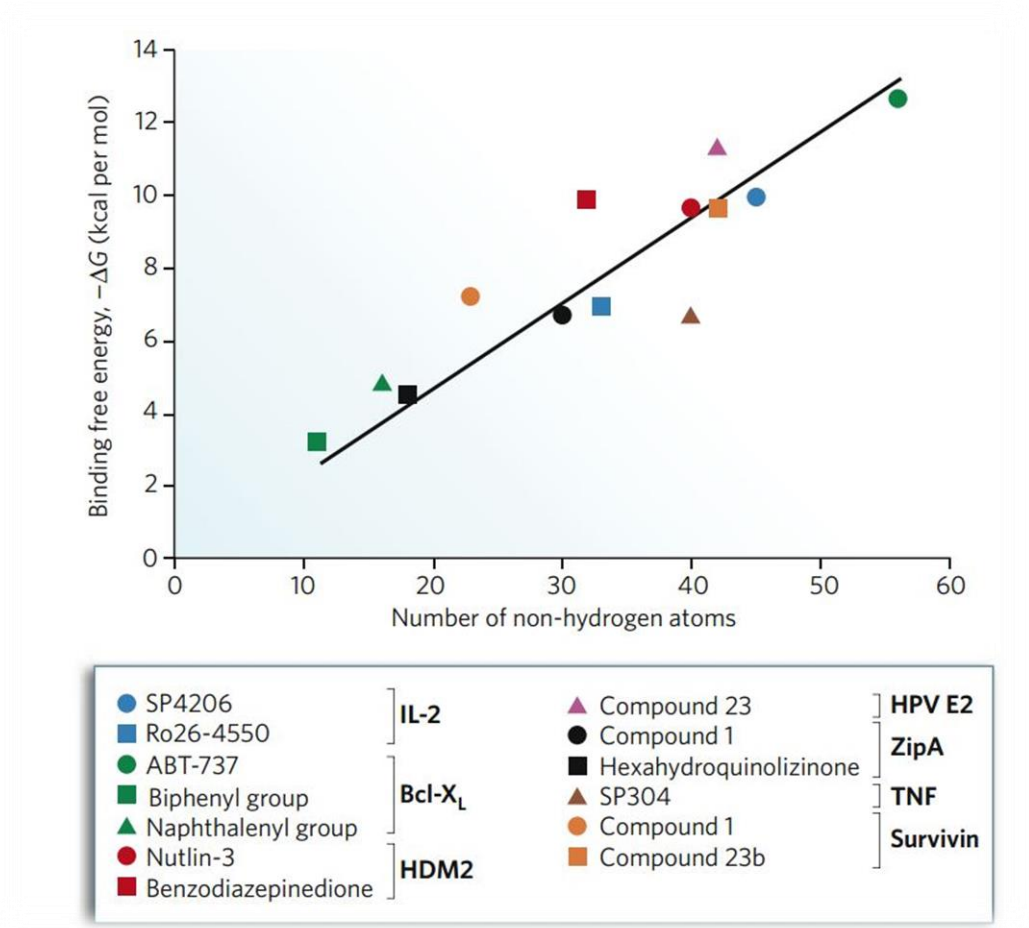


Figure 5 | Relationship between compound potency and size for small molecules that inhibit protein–protein interactions.

Prospects and challenges for drug discovery

In the past five years, there has been remarkable progress in identifying, characterizing and developing small molecules that bind to protein–protein contact surfaces:

⇨ Fragment-screening methods

Improved computational:
'growing' fragments into
higher-affinity small molecules

⇒

more widely adopted:
Cheaper
more sensitive
higher throughput

⇨ In a recent study, high-affinity inhibitors were computationally docked to protein-conformation snapshots obtained from 10-nanosecond molecular-dynamics simulations.

⇨ If we assume that protein–protein interactions have a lower 'ceiling' for ligand efficiency than more traditional targets, then the drug-discovery community will need to improve ADME properties of larger compounds. these compounds are specific for their targets

Clearly, recent efforts have lifted us a rung higher in the quest to reach this class of high-hanging fruit.

THANK YOU FOR YOUR ATTENTION