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Focusing on shared subpockets - new developments in fragment based drug discovery

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Abstract

Introduction—Protein–protein interactions (PPIs) are important targets for understanding fundamental biology and for the development of therapeutic agents. Based on different physicochemical properties, numerous pieces of software (e.g PocketQuery, Anchor and FTMap) have been reported to find pockets on protein surfaces and have applications in facilitating the design and discovery of small molecular weight compounds which bind to these pockets.

Areas covered—The authors discuss a pocket-centric method of analyzing protein-protein interaction interfaces, which prioritize their pockets for small molecule drug discovery and the importance of multicomponent reaction (MCR) chemistry as starting points for undruggable targets. The authors also provide their perspectives on the field

Expert opinion—Only the tight interplay of efficient computational methods capable of screening a large chemical space and fast synthetic chemistry will lead to progress in the rational design of PPI antagonists in the future. Early drug discovery platforms will also benefit from efficient rapid feedback loops from early clinical research back to molecular design and the medicinal chemistry bench.

Keywords

ANCHOR; chemical space; multicomponent reaction; POCKETQUERY; Protein–protein interactions; Pocket cluster; Virtual screening

1. Introduction

The complex formation between a receptor and its ligand is determined by a simple balance between enthalpy and entropy ($G = H - TS$). However, the underlying physicochemical

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processes responsible for this equilibrium are poorly understood. These processes are multidimensional and involve different interaction energies such as charge-charge, hydrogen bonding, van der Waals, halogen bondings, (de)solvations, and tight receptor bound waters, conformational changes and dynamics in the two components (e.g. induced fit). With the exception of free energy perturbation methods that have shown progress in modeling and ranking compounds that are close in structure to a known co-crystal [1]. None of the current computational approaches reflects this complex reaction pathway and there is no method to accurately calculate its change in Gibbs free energy or affinity a priori. In fact, currently used ligand docking and energy calculation programs are particular unreliable in ranking series of unrelated compounds, predicting binding poses and energies [1–6]. Not surprisingly, still today, a key challenge in computational drug discovery is the discrepancy between computational predictions and experimentally determined interactions [7]. Thus, most structure based drug discovery methods including fragment based drug discovery concentrate on the analysis of ligand bound receptor complexes to increase affinity. In this small review, we discuss a method of analyzing protein-protein interaction interfaces that prioritizes a feedback loop between pockets for small molecule drug discovery and precision chemistry to develop useful starting points for undruggable targets.

2. The importance of pocket shape and size and clusters thereof

Pockets are indents on the surface of proteins of variable size and are present permanently or temporarily. Often they are formed to accommodate a substrate for an enzymatic transformation or a cofactor for a specific biochemical transformation or they are formed during the recognition process in protein-protein interactions. Pockets in protein targets are of utmost importance to the biochemical mechanism and therefore are often viable starting points for structure-based drug discovery. Pockets can accommodate small molecules and effectively shield the protein surface from surrounding water. Protein pockets are the targets of medicinal chemists. They can be found by manual analysis of Protein Data Bank (PDB) structures but automatic analysis of PPI interfaces can often be more reliable and faster especially considering the relatively large number of PPIs in the PDB. Based on different physicochemical methods, numerous software have been documented to find pockets on protein surfaces and have found applications in the design and discovery of small molecular weight compounds binding to these pockets [8–10].

Analysis of the interface of protein-protein interactions can give a valuable starting point for the design of small molecule antagonists [11]. The diverse nature of these interfaces, however, makes a rule based approach difficult. For instance, small and structurally defined pockets and very large flat and rather featureless interfaces are known (Fig 1). POCKETQUERY (<http://pocketquery.csb.pitt.edu>) is a recently introduced interface analysis server for exploring the properties of protein-protein interaction (PPI) interfaces with a focus on the discovery of promising starting points for small-molecule design [10]. It calculates the change in solvent accessible surface area (SASA) upon binding for each side-chain and estimates the associated binding free energy of each residue and proposes ANCHOR points [12–14]. Interestingly enough the POCKETQUERY found clusters can be exported to the web server ANCHORQUERY or ZINCPHARMER thus comprising a fully-fledged pharmacophore-based drug discovery workflow (Fig 4). POCKETQUERY is different from

other computational pocket analysis methods as it focuses attention on clusters of key interacting residues in a PPI co-crystal. Using a 'druggability' score that includes free energy estimates of hot spots and the size of the pockets available on each cluster, this server estimate the likelihood that the chemical mimicry of a given cluster would result in a small-molecule inhibitor of an interaction [15–17].

3. Precision chemistry and ANCHOR-based virtual screening as a determinant for success

The chemical space useful for drug discovery is subject of an ongoing debate. Within the huge chemical space of synthesizable small organic compounds, there is only a small fraction of potentially active compounds of interest for further investigation to become drugs. The development of new synthetic scaffolds is the most important challenge for identifying new lead structures during the drug discovery process. A key observation, however, is that many general chemical backbones are accessible by so-called multicomponent reactions (MCRs) [21, 22].

MCRs are organic chemical reactions of three or more starting materials in one pot to yield a complex product. This is in sharp contrast to classical organic reaction which are one component or two component reactions. In order to yield a complex product via classical 1- or 2-component reaction a sequential multistep synthesis is necessary. Thus MCR provides a huge advantage in convergence, time and effort to yield a complex product.

A recent analysis of ligand receptor interaction revealed that small molecules in average are not leveraging enough polar contacts to the receptor [23]. Undergoing polar contacts has advantages for selectivity, solubility and drug-like properties and potency while keeping molecular weight low. Thus an important goal of medicinal chemistry aims is to maximize polar contacts to better resemble the interaction patterns that natural molecules present [24]. However the practitioner well knows that it will almost always be harder to match polar contacts than to gain affinity through the addition of hydrophobic substituents. This can be explained by the tight distance and angle dependency of the polar contacts and on the other hand the unavailability of suitable chemical building blocks to accomplish an optimal polar contact between ligand and receptor. A fast and straightforward way to optimize polar ligand receptor contacts which can solve the above dilemma is multicomponent reaction (MCR) chemistry [21, 22]. MCR is an alternative strategy in different drug discovery stages including lead discovery and pre-clinical process development. The advantages of MCRs, superior atom economy, simple procedures, the one-pot character, and increasing number of derivatives allow the resource and cost effective, fast, and convergent synthesis of diverse compound libraries, and highly improve the efficiency to explore the chemical space with limited synthetic effort [25]. The LEGO type architecture of MCR chemistry allows for the fast and easy construction of superior ligand-receptor contacts based on the building block approach (Fig 2). Other properties of compound libraries that should enhance the chances of binding are 3D features such as 3D shape (non flatness) and number of chiral centers. Yet, the analysis of chemotypes in the receptor shape and current screening libraries reveals a discrepancy. Namely, while receptor shapes of protein-protein interactions are often larger

and have more 3D features than typical pharma targets such as kinases or proteases, current screening libraries are often rich in flat heterocycles and do not cover large surface areas due to their shape and size restrictions [26]. Natural products are a large and diverse class of compounds that can overcome problems of current synthetic libraries. However, they have drawbacks of their own such as difficult accessibility, complex multi-step synthetic access for optimizations and often poor ADME properties. Novel synthetic chemistry that can overcome these limitations is the rich world of MCR scaffolds (Fig 3) [21, 22]. Therefore modern screening library design should incorporate ideas of structural systems pharmacology of post genomic targets [27].

Identifying or predicting potential druggable sites and developing novel chemistry are only one aspect of the problem. A more technical limitation is our capabilities to screen the large and diverse chemical space entailed by MCR scaffolds. To solve this problem, we developed a web-based virtual screening platform called ANCHORQUERY (<http://anchorquery.csb.pitt.edu/>) [30]. ANCHORQUERY is a specialized interactive pharmacophore-based search engine whose aim is to facilitate the design of MCR-based small molecule (ant)-agonists of protein–protein interactions. ANCHORQUERY is unparalleled in its focus to design amino acid side chains analogs to bury at protein–protein interfaces as essential building block of small molecules.

3.1. Example: Mdm2-p53

Mdm2-p53 is a PPI which is structured only upon complex formation but otherwise partially disordered. Multiple cocrystal structures between mdm2 and p53, related peptide, peptoids, stapled peptides and small molecules are published and can serve as a valuable source of structural analysis (Table 1). For apo-mdm2 no x-ray structure has been reported despite intensive trials of crystallization world-wide. The NMR structure however is reported and shows no preformed pocket anyhow similar to the p53-mdm2 complex [31]. The mdm2 structure however reveals a small indent which upon complex formation will result in a deep, structured and extended pocket with several subpockets accommodating the archetypical hot spot F19W23L26 of an amphipathic α -helix of p53 (Fig 5). It can be hypothesized that in the complex forming trajectory the first contact is made by a weak interaction of W23 poking into the minor mdm2 indent and in an ordered, cooperative, higher-order complex formation, fashion the binding cleft is step-wise opening by the countermovement of the α 1' and α 2' helix of mdm2. In fact molecular dynamic studies are proposing such a trajectory and might be used as a model for the cooperative complex formation [32].

Analysis of the p53 mdm2 interface clearly reveals a hot spot consisting of 3 sub pockets filled by the three p53 peptide derived amino acid side chains F¹⁹W²³L²⁶. The tryptophan is most deeply buried by POCKET QUERY and thus consists the ANCHOR and is in fact a good starting point for the discovery of mimicking small molecules [39, 40]. Additionally W²³ forms a hydrogen bond to the mdm2-L⁵⁴ thus introducing selectivity. Feeding the POCKETQUERY derived pocket cluster into ANCHORQUERY reveals hits based on multiple chemical scaffolds after resynthesis and screening of the virtual hits [41, 42]. All hits are based on precision MCR chemistry and can be synthesized and tested in a fast

manner. Several scaffolds are now optimized to low nM cellular active compounds. Multiple ANCHORQUERY derived hits have been characterized by co-crystallisation with mdm2. Interestingly the alignment of the W23 ANCHOR with the ANCHOR part of the small molecules is almost perfect (Fig 5).

4. Conclusion

Protein–protein interactions (PPIs) are important biologically targets for the development of chemical investigations and therapeutic agents but in the same time the design of compounds that are capable of interfering with protein–protein interactions is difficult. Many methods based on purely computational approaches to discover small molecule for PPI are highly underdeveloped. ANCHOR and POCKETQUERY are web-based tools whose aim is to facilitate the analysis of protein-protein interfaces with regard to its suitability for small molecule drug design, whereas ANCHORQUERY is a specialized interactive pharmacophore search technology. The ultimate goal of these open access technologies to enable the rational design and synthesis of new chemical probes based on interactive structure based virtual screening of large libraries of synthesizable compounds.

5. Expert Opinion

Currently the complex formation of a protein-protein interaction with the dynamic trajectory and its energy landscape is very poorly understood. Even less understood is the rational process towards drug modulating PPIs. The importance of pockets and clusters of pockets addressable by molecular entities of different flavors, including small molecules, stapled peptides or macrocycles is emerging as a key principle towards next generation therapeutics of postgenomic targets currently viewed as undruggable. With all the uncertainties of binding energy prediction and dissection into different components such as charge-charge interaction, hydrophobic interactions, hydrogen bonding, pi stacking interaction, halogen bonding and sulfur contacts just to name a few, with the poor understanding of structural water in pockets, is it nevertheless possible to learn from structural biology and use the knowledge for rational structure based drug design? Current methods based on purely computational approaches to discover small molecule for PPI are highly underdeveloped and are not expected to result in conceptual break through [43, 44]. Rather the combination of novel experimental with computational approaches will lead to a gain of knowledge leading to a deeper understanding of receptor ligand interactions and novel drugs for so-called intractable targets. Prioritization of amino acid clusters in PPI interfaces adjusted to different types of chemistries (small molecules, macrocycles) followed by anchor focused virtual screening of large and chemically accessible libraries is one such promising approach, e.g. using the herein described combination of the software POCKETQUERY, ANCHORQUERY, and precision MCR chemistry. A focus on single residue subpockets embedded in a pocket cluster resulting from interacting peptide epitopes can provide valuable starting points for the design and development of chemical modulators. Many PPIs will be very difficult to target with classical small molecules. Novel libraries of PPI specific compound collections will be needed to effectively target such PPIs. For other targets new chemistries in between small molecules and proteins will be required including stapled peptides or macrocycles and further future chemical developments will be needed in this

area. The full potential of post genomic targets with its wealth of biology and potential for human health will only be fully uncovered by stepping novel trails of biophysical and chemical methods [45]. Early drug discovery platform will tremendously benefit from efficient, rapid feedback loops from early clinical research back to molecular design and the medicinal chemistry bench.

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Article highlights

- Pockets in protein targets are important to the biochemical mechanism.
- Analysis of the interface of protein-protein interactions can give a valuable starting point for the design of small molecule antagonists
- The development of new synthetic scaffolds is the most important challenge for identifying new lead structures during the drug discovery process.
- MCR is an alternative strategy in different drug discovery stages including lead discovery and pre-clinical process development
- ANCHORQUERY is a specialized interactive pharmacophore-based search engine whose aim is to facilitate the design of MCR-based small molecule (ant)-agonists of protein–protein interactions.

This box summarizes key points contained in the article.

Fig 1.

Open book presentation exemplifying the diversity of PPI classes. A) An α -helix mediated PPI exemplified by the receptor protein mdm2 (surface) and the p53 α -helix (cartoon), comprising a buried interface of 722 Å² (cyan surface) and representing an important anticancer drug target (PDB ID 1YCR) [18]. B) The proprotein convertase subtilisin/kexin type 9 (PCSK9, surface) and a low-density lipoprotein receptors (LDLRs, cartoon) interaction burying 515 Å². The LDLR is held together by multiple small contributions of hydrogen bonds and hydrophobic interactions (PDB ID 3BPS) [19], but otherwise the

interface is rather featureless for small molecule intervention, C) The interacting surface of Il17 (surface) and Il17 receptor (cartoon) is very large, burying $\sim 2400 \text{ \AA}^2$ solvent accessible surface area and not showing well-defined deep cavities of classical drug targets (PDB ID 4HSA) [20]. Interfaces calculated with PISA, rendering with PYMOL.

Building Blocks

Scaffolds

Fig 2. Precision MCR chemistry based on simple, diverse and commercially available building blocks. Hundreds of scaffolds can be assembled from their building blocks in one or very few synthetic steps.

Fig 3. Shape Diversity and 3Dness of MCR scaffolds. Left: 3D crystal structures of compounds build by precision MCR chemistry. Two molecules in the second row feature unusual pyramidal amide nitrogen atoms. Right: Principal moment of inertia (PMI) analysis of random MCR libraries of 4 MCR scaffolds compared with random ZINC compounds carves out a considerable higher 3D character of MCR libraries [28, 29].

Fig 4.

Small Molecule PPI Antagonist Discovery Workflow: Analysis of the interface using POCKETQUERY, transfer of the target clusters into ANCHORQUERY, refinement of the pharmacophore, virtual screening of virtual MCR library of several millions of compounds, screening of virtual hits. After a few cycles of refinement, best virtual hits can be synthesized by following the provided on-line MCR synthesis protocols.

Fig 5.

Above: The mdm2-pocket is induced by its ligands. Left is the NMR structure of mdm2 (PDB ID 1Z1M), right the p53-peptide mdm2 structure (PDB ID 1YCR) focusing on the hot spot ANCHOR region F¹⁹W²³L²⁶ (sticks). Below: Four novel scaffolds based on precision MCR chemistry have been discovered and co-crystallized with mdm2. In each box the PDB identifier is given; the small molecule indole ANCHOR and the 1YCR-derived W²³ anchor (red line) is aligned.

Table 1

Selection of antagonists of p53-mdm2 PPI characterized by crystal structures.

PDB ID	ligand	Ref
1YCR	p53 derived peptide 	[18]
3W69	Small molecule: dihydroimidazothiazole 	[33]
4OBA	Small molecule: morpholinone 	[34]
4JSC	Small molecule: pyrrolidine (RG7388) 	[35]
3LBK	Small molecule: imidazole 	[36]
3TPX	D-peptide 	[37]
3V3B	Stapled peptide 	[38]