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Hot spot-based design of small-molecule inhibitors for protein–protein interactions

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ABSTRACT

Protein–protein interactions (PPIs) are important targets for the development of chemical probes and therapeutic agents. From the initial discovery of the existence of hot spots at PPI interfaces, it has been proposed that hot spots might provide the key for developing small-molecule PPI inhibitors. However, there has been no review on the ways in which the knowledge of hot spots can be used to achieve inhibitor design, nor critical examination of successful examples. This Digest discusses the characteristics of hot spots and the identification of druggable hot spot pockets. An analysis of four examples of hot spot-based design reveals the importance of this strategy in discovering potent and selective PPI inhibitors. A general procedure for hot spot-based design of PPI inhibitors is outlined.

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Protein–protein interactions (PPIs) play a pivotal role in most biological processes. The interface between two proteins typically has an area of 1500–3000 Å^2 with approximately 750–1500 Å^2 of surface area buried in each protein.[1–3] The formation of a protein–protein complex is largely driven by hydrophobic effects,[4] which occur between the nonpolar regions of protein residues through van der Waals contacts. Electrostatic complementarity of the interacting protein surfaces between two proteins promotes the formation and lifetime of the complex. For some interfaces hydrogen bonding and electrostatic interaction play a major role in steering one protein to dock onto the binding site of the second protein.

Characteristics of hot spots and hot regions: The residues on the protein–protein interface do not contribute equally to PPIs. A small subset of residues contribute to the majority of the binding free energy; they are called hot spots.[5] A hot spot is defined as a residue which substitution by an alanine leads to a significant decrease in the free energy of binding ($\Delta\Delta G_{binding} > 1.5$ kcal/mol).[5] The experiment that involves individually mutating interface residues to alanine, eliminating side-chain atoms beyond C_β, and then measuring the effect of individual side chain on binding affinity is called alanine scanning. A survey of alanine scanning data indicated that the amino acid composition of hot spots was enriched in tryptophan (W), arginine (R), and tyrosine (Y).[6] This trend of residue enrichment was also reproduced by a different surface analysis approach using clustered interface families.[7] Energetic hot spots from alanine scanning correlate with structurally conserved residues.[8] The number of the structurally conserved residues, in particular the energetic hot spots, increases with the expansion of the interacting surface area. Typically, hot spot density on the protein–protein interface composes 10% of the binding site residues.[9]

The free energy of binding between two proteins is not a simple summation of the contribution from individual hot spots. Hot spots tend to occur in clusters. Within the cluster, the tightly packed hot spots are in contact with each other and form a network of conserved interactions called hot regions.[10] One example of hot regions in a protein–protein interface is shown in Figure 1. The contributions of hot spots within one hot region are cooperative to stabilize PPIs. Hot regions are networked and contribute dominantly to the stability of PPIs. The energetic contributions between two hot regions can be additive[11] or cooperative.[12]

The protruding hot region of one protein packs against the concave hot region of the other protein.[4,13] Figure 2 illustrates a typical arrangement of hot spot and hot region. Residues 1–4 in Figure 2 constitute the top hot region for the interactions between proteins A and B while residues 5–8 form the bottom hot region. For the top hot region residues 1 and 3 make a protruding hot region, and residues 2 and 4 create a concave hot region. The projecting hot spot, residue 1 in Figure 2, makes a direct contact with hot spot 2 in the concave hot spot pocket. Residue 3 organizes the orientation of projecting hot spot 1, and 4 supports the structure of the hot spot pocket. Not only the alanine mutations of hot spots 1 and 2 but also

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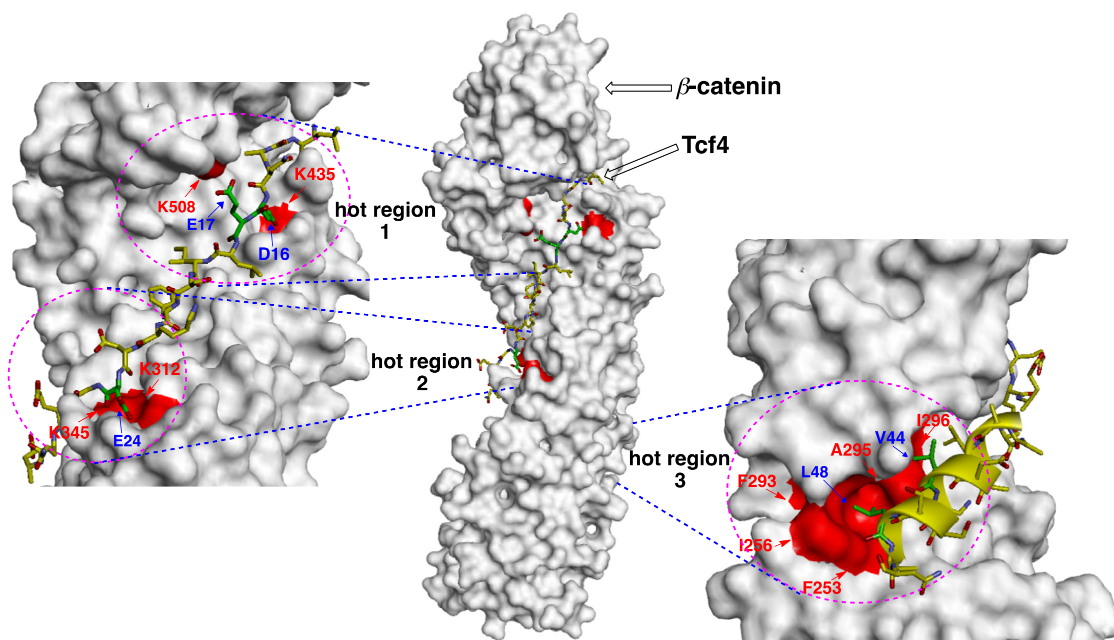

Figure 1. Crystal structure of β-catenin in complex with T-cell factor (Tcf) shows three hot regions (PDB IDs, 1G3J and 2GL7). Hot region 1 includes K435 and K508 of β-catenin and D16 and E17 of Tcf4. Hot region 2 includes K312 and K345 of β-catenin and E24 and E29 of Tcf4. Hot region 3 includes F253, I256, F293, A295, and I296 of β-catenin and V44 and L48 of Tcf4.

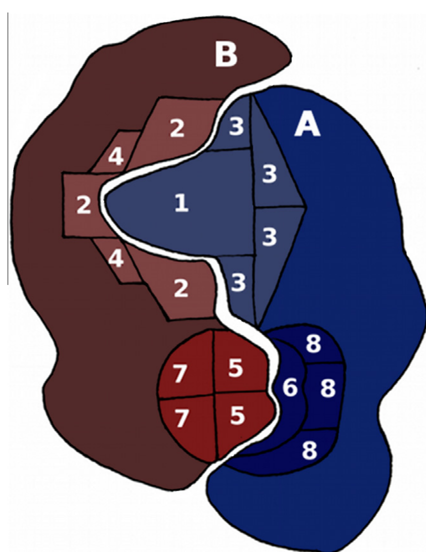

Figure 2. Illustration of hot spots and hot regions in the protein–protein interface (adapted from Golden et al.[13]). The top hot region has a projecting hot spot, **1**, from protein A. This projecting residue binds to a complementary surface pocket of protein B, which is lined by residues that are labeled **2**. The residues on protein A that help to orient projecting hot spot **1** are labeled **3**. The residues on protein B that help to form the concave hot region are labeled **4**. The bottom hot region has two projecting hot spots from protein B that are labeled **5**. The concave surface pocket residue of protein A is labeled **6**. The residues in protein B that support the projecting hot spot are labeled **7**. The residues in protein A that support the formation of the concave hot region are labeled **8**.

the mutations of residues 3 and 4 would greatly affect the free energy of binding between proteins A and B. Therefore, residues 1–4 are all called energetic hot spots in the alanine scanning experiments. The projecting hot spots, 1 and 5 in Figure 2, are also named anchor residues if the change of their solvent accessible surface areas (SASA) upon binding is >0.5 Å^2. [14,15]

The concave hot regions are usually pre-organized in the unbound state prior to protein complexation,[14,16] as demonstrated in Figure 3. The existence of such ready-made recognition motifs implies that the binding pathway can avoid kinetically costly structural rearrangement at the core of the binding interface, allowing for a relatively smooth recognition process. Once the protruding hot region is docked to the concave hot region, an induced fit process further contributes to the formation of the final high-affinity complex.

Alanine scanning experiments to unravel hot spots are relatively time-consuming and labor-intensive. In some cases, the results of the alanine scanning experiments could be inconclusive. For example, the alanine mutation of residues that participate in forming concave hot regions likely gives rise to nonadditive $\Delta\Delta G_{\text{binding}}$ values. The alanine mutations could affect the free energy of binding by a mechanism unrelated to the PPIs at the interface, for example, by destabilizing the unbound state of the protein or altering its conformation. Therefore, hot spots identified by alanine scanning experiments could be false positives in the sense that they do not reflect energetically important binding interactions with the partner protein. In addition, alanine scans could miss a binding hot spot that mostly involves interaction of backbone rather than side-chain atoms. Computational methods have been developed to predict hot spots. These methods are complementary to the alanine scanning experiments and provide valuable insights into the nature of protein–protein complexation.[17] Some computational methods calculate the changes of free energy of binding upon mutation using calibrated free energy functions, such as Robetta[18] and FOLDEF.[19] A second group of computational methods incorporate molecular dynamics simulations in computational alanine scanning.[20] The third group covers knowledge-based methods that learn the relationship between hot spots and various residue features from training data, and then predict new hot spots.[21] Also, hybrid approaches, which integrate the strengths of the machine learning and energy-based methods, have been developed and applied to predict protein hot spots.[22]

Solvation also plays an important role in protein–protein association. Hot spots are often surrounded by energetically less important residues that shape like an O-ring to occlude bulk water molecules from the hot spot.[6] The affinity of a hot region depends not only on the energetically critical hot spots located near the

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