Packing together. The crystallization of colloidal particles and biomacromolecules is intrinsically related to the interactions between the particles, which can be controlled by their charge (graph at left). Repulsive spheres can easily be crystallized by restricting their volume. Proteins and binary mixtures of oppositely charged particles can be crystallized by precise adjustment of the interactions into a weakly attractive regime. However, if the interactions between the particles are strongly attractive, rapid precipitation of amorphous aggregates occurs. The micrograph images are of colloidal crystals assembled by restricting the free volume of repulsive latex spheres (left), crystals of the protein lysozyme obtained under slightly attractive interactions (center), and a nanoparticle crystal assembled under controlled electrostatic attraction (right).

Interestingly, the idea that the key to crystallization is achieving a precise balance among weak attractive interactions has been actively explored in the field of protein crystallization for more than a decade. Proteins are large, complex molecules of nonuniform shape and charge, which have been shown to crystallize only under conditions of slightly attractive interactions when both positively and negatively charged groups are present on their surfaces (7). The intricate fundamentals of the attractive electrostatic interactions between nanoparticles in a crystal are still not understood in depth. It seems that the concepts developed for proteins may now provide a roadmap for nanoparticle crystallization.

Future research in nanoparticle assembly may bring closer the areas of biomacromolecule and nanoparticle crystallization. Could a similar charge-balancing approach be applied to binary mixtures of proteins, or mixtures of proteins and nanoparticles? A large variety of nanoparticles of special shape and properties have been synthesized in the past few years, but little is yet known about their self-assembly. New “zwitterionic” particles, having patches of negative and positive charges on their surfaces, could soon be synthesized and crystallized by adjustment of the interactions in a manner similar to the crystallization of proteins. Thus, nanoparticle crystallization and assembly may not only yield new nanomaterials, but could also provide insights into how to control colloidal forces on the nanoscale.

**References**

8. www.sciencemag.org on November 17, 2017

---

**Hitting the Hot Spots of Cell Signaling Cascades**

**John Joseph Grubb Tesmer**

Transient protein–protein interactions are hallmarks of intracellular signaling cascades triggered by heterotrimeric guanine nucleotide-binding proteins, or G proteins (1). Hundreds of cell surface receptors for hormones and other extracellular factors activate G proteins, thereby regulating nearly all aspects of cell physiology. These receptors are the targets of a large fraction of the pharmaceutical drugs being used today. Thus, small molecules that negatively or positively modulate the protein–protein interactions of G proteins could likewise be powerful therapeutic agents (2, 3). However, drugs that target protein–protein interfaces are harder to develop than those that target the active sites of enzymes, which are often found in deep, well-defined pockets on the protein surface. Many of the protein–protein interfaces found in signaling cascades are comparatively flat and expansive. They also tend to be adaptable, meaning that a signaling protein can use the same surface to bind to a structurally diverse set of targets (1, 2, 4). This renders it difficult to find a drug that can turn one particular signaling pathway on or off without affecting the others.

One way to overcome at least some of these hurdles is to identify compounds that target the so-called “hot spot” of the protein–protein interface (5). In many transient protein–protein interactions, a majority of the binding energy is contributed by only a few amino acid residues within the interface. These “hot” amino acids tend to cluster together in a relatively small, central region surrounded by a ring of less energetically important and more water-accessible residues (6–8). Thus, small molecules can disrupt a comparatively large protein–protein interface by binding to the hot spot or, alternatively, to an allosteric site that alters its conformation (2).

On page 443 of this issue, Bonacci et al. (9) use a computer-based “virtual” screen of only 1990 structurally diverse compounds (available from the U.S. National Cancer Institute) to identify molecules that bind to the β and γ subunits of a G protein (Gβγ). G proteins consist of three subunits. In the classic G protein signaling cascade, Gβγ subunits are released as a complex from the α subunit (Go) after activation of an associated receptor at the cell surface. The Gβγ complex can subsequently...
Targeting the Gβγ bull's-eye. Small-molecule compounds that inhibit Gβγ signaling were identified by targeting its “hot spot” in a virtual chemical screen. The structure of Gβγ is depicted as a ribbon diagram superimposed with its molecular surface. The red bull's-eye marks the hot spot that contains residues believed to be important for binding most if not all of the diverse protein targets of Gβγ. The white ring symbols regions that can also participate in protein interfaces but may not be as energetically important or are more solvent accessible (however, regions other than those shown in red or white also interact with Gβγ targets). A peptide that helped define the hot spot of Gβγ is shown as a green ribbon (PDB code 1XHM).

interact with and regulate an array of downstream signaling proteins such as phospholipase C-β, G protein–coupled receptor kinase 2 (GRK2), and phosphatidylinositol 3-kinase. These structurally diverse proteins bind (or are expected to bind) to a surface of Gβγ that overlaps the binding site of Gα (10–14), accounting for the ability of Gα to keep Gβγ from signaling in the absence of extracellular signals (by forming the inactive Gαβγ heterotrimer).

Despite the large surface area of Gβγ that is buried within the Gαβγ complex, it was previously shown that small peptides derived from a phage display library could differentially inhibit the binding of Gβγ to Gα and to its various downstream targets. The peptides bind to a site in Gβγ1 that lies near the center of the surface known to interact with Gα and the effector protein GRK2 (15, 16). Although peptides are not usually good drug candidates, they can still be very useful in defining the functional sites on protein surfaces that can then be targeted by small-molecule drugs (17). Accordingly, Bonacci et al. used the structure of Gβγ bound to one of the phage display peptides to define the hot spot of Gβγ (see the figure), and then used a molecular modeling computer program to dock compounds of known structure into the hot spot. By evaluating several different metrics, the authors narrowed the library to 85 likely compounds, which they then experimentally tested for Gβγ binding. Of these, nine compounds inhibited the binding of phage-display peptides to Gβγ by 50% at high nanomolar to mid-micromolar concentrations. Several of the most promising inhibitors were then shown to be efficacious at modulating Gβγ function in vivo.

This is not the first example of small-molecule screening, or even a virtual one, that has successfully identified a modulator of a protein-protein interface. For example, a similar virtual screen identified a compound that could inhibit the various protein interactions of the small molecular weight G protein Racl, a key regulator of the cytoskeleton and cell proliferation (18). The remarkable aspect of the work reported by Bonacci et al. is that they identified molecules, presumably targeting the same site, that differentially modulate the interactions of Gβγ with its various downstream targets in vitro, in cultured cells, and in an animal model where the physiological effects of one of the compounds was evaluated. Thus, it is possible not only to identify small molecules that directly modulate the function of G proteins, but also to find, with great efficiency, compounds that can turn off one signaling pathway while preserving or even augmenting others. Such compounds could help elucidate the specific pathways regulated by Gβγ under different physiological conditions and may ultimately lead to the development of therapeutic agents for diseases in which Gβγ is expected to play a maladaptive role, such as heart failure (19).

It will be important to accurately define the Gβγ binding sites of the compounds identified by Bonacci et al., as they could reside outside of the intended hot spot, either in an allosteric site or in a region of Gβγ that interacts with only a subset of its effectors. Accordingly, high-resolution crystallographic structures of Gβγ in complex with these inhibitors would provide great insight into the specific regions of Gβγ most important for its various protein interactions and facilitate the design of drugs with higher affinity and selectivity. There is also a strong possibility that higher affinity drugs can be identified by a larger survey of chemical compounds, wherein tens of thousands of molecules are typically screened. Finally, it will be interesting to test whether the compounds identified differentially interact with the many possible species of Gβγ (there are genes for at least five isoforms of Gγ and 12 of Gγ in humans). The hunting season for drugs that target other G protein interfaces is now open.

References
Hitting the Hot Spots of Cell Signaling Cascades
Grubb John Joseph Grubb

Science 312 (5772), 377-378.
DOI: 10.1126/science.1126903

Use of this article is subject to the Terms of Service.