Thermoelectric conductors is the absence of a band gap in the former. Electrons can strongly interact with holes in gapless graphene (6), and this process changes the “sign” of the velocity renormalization correction compared with the case of electron-electron interaction.

Strong electron-hole interactions may cause the electronic liquid in graphene to become highly viscous (10). The mutual viscous friction forces electrons and holes to move together, so that the effective charge contributing to the low-frequency optical response of the electron liquid is diminished. In theory, this effect should inhibit plasmons but enable another type of collective excitation—energy waves or “demons” (11)—to exist at small \( \omega \) and \( q \) (see the figure). The thermal photocurrent mapping technique devised by Lundeberg et al. (2, 4) appears to be particularly promising for detection of these elusive modes.

Lundeberg et al. point out that their method of determining the nonlocal complex conductivity \( \sigma'(q, \omega) + i \sigma''(q, \omega) \) is applicable to other quantum materials, including low-dimensional conductors, superconductors, and Weyl semimetals. A technical precondition for such experiments is the ability to use nano-optical imaging at cryogenic temperatures, which recently became available (12). Plasmonic imaging in graphene at liquid helium temperature are also highly desirable because scattering by phonons in this regime will be reduced, whereas the observables associated with many-body physics are expected to be enhanced. We anticipate that future studies will address yet another unresolved issue pertaining to the analysis of the linewidth of plasmonic modes in graphene that is determined by the ratio \( \sigma'(q, \omega)/\sigma''(q, \omega) \). Plasmonic images, including those reported in (2–4), prompt us to reimagine the sheer scope of unresolved problems that can be tackled with this innovative experimental approach.

How do miniproteins fold?

A high-throughput study yields libraries of miniproteins that help to explain how proteins are stabilized

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How does the amino acid sequence of a protein chain determine and maintain its three-dimensional folded state? Answering this question—a key aspect of the protein-folding problem (1)—would help to explain how multiple noncovalent interactions conspire to assemble and stabilize complicated biomolecular structures; to predict protein structure and function from sequence for proteins that cannot be characterized experimentally; and to design new protein structures that do not exist in nature (2).

On page 168 of this issue, Rocklin et al. use parallel protein design on a massive scale to create thousands of miniprotein variants and to determine what sequences specify and stabilize these structures (3). The work opens up considerable possibilities for protein folding and design.

Miniproteins are polypeptides shorter than 40 to 50 residues with stable tertiary structures (folds) that contain a limited number of secondary structure elements, such as \( \alpha \) helices and \( \beta \) strands. By contrast, larger proteins have hundreds of amino acids that are often arranged in complex three-dimensional structures. Thus, miniproteins simplify the protein-folding problem and potentially allow in-depth examinations of sequence-structure-stability relationships without complications from larger protein contexts. Unfortunately, only a few miniproteins that are stable without covalent cross-links or stabilizing metal ions are currently available for such studies (4).

In their study, Rocklin et al. combine high-throughput DNA synthesis and cloning (5, 6) with methods for selecting stably folded proteins (7–9). They implement the latter by expressing libraries of miniproteins on the surface of yeast; tagging the displayed proteins with a fluorescent dye; and discriminating between stable and unstable folds through their ability to resist or succumb to protease treatment, respectively (see the figure). Proteins that survive are rescued by fluorescence-activated cell sorting and then identified by deep sequencing. However, the team’s experiments go beyond a yes/no measure of protein resilience, providing a semi-quantitative measure of stability.

To demonstrate the approach, the authors first apply their method to many variants of a small number of known miniproteins. With the method established, they turn their attention to four classes of de novo miniproteins, which they design computationally using Rosetta (10): \( \alpha\alpha\alpha \), \( \beta\alpha\beta \), \( \alpha\beta\alpha \), and \( \beta\beta\alpha\beta \) folds, where each Greek letter represents an \( \alpha \) helix or a \( \beta \) strand in the peptide string.

“...Rocklin et al. have taken high-throughput, data-driven protein design, selection, and optimization to new heights...”

To cover swaths of sequence space, the team generate diverse libraries with minimal sequence identity between members.

They then use iterative rounds of protease selection and stability scoring, testing different hypotheses, and introducing tweaks to the design methods and protocols at each stage. The value of these tweaks is apparent from the improved success rate—the proportion of stable proteins in the starting library—which reaches 87% for one target. However, both the initial and final design success rates depend critically on the fold being targeted, with the \( \alpha\alpha\alpha \) fold proving easiest and the \( \alpha\beta\alpha \) fold most difficult to optimize.

Through sequence analyses of many thousands of these new and also existing miniprotein folds from other studies, the authors highlight several key sequence and structural features. First, a long-established basic tenet of protein folding and design shines through: the importance of burying nonpolar surfaces. This is not surprising, but Rocklin et al. quantify the effect, showing that stable variants require more than 30 Å² for each residue of buried hydrocarbon.

Second, of the initial computational designs, those containing peptide fragments geometrically similar to ones known from the

References

Protein design cycle

Rocklin et al. use an iterative design cycle to create stable miniproteins. After initially designing miniprotein folds using computational tools, they express them and test their stability, followed by further optimization cycles.

1 Miniprotein structures are designed computationally using a fragment-based approach in Rosetta.

2 Libraries of amino acid sequences are generated to best fit these structures. Those sequences are synthesized via high-throughput DNA synthesis and cloning.

3 The resulting proteins are expressed on the surface of yeast.

4 Stable variants are selected based on resistance to treatment with protease.

5 The sequences of stable variants are analyzed to determine sequence–stability relationships, which are fed back into the design cycle.

Protein Data Bank of protein structures fared better in the selection process than those with more geometrically distant matches; i.e., the former gave more stable sequences. This could be a consequence of using Rosetta to achieve the design frameworks, given that it is a fragment-based design approach. In the future, it will be interesting to see how starting points from parametric and other design approaches perform (11–13).

Third, one relationship not included or tweaked during the iterative process—it simply emerges from the analysis—is the importance of having charged side chains at the termini of the α helices that oppose the terminal partial charges of the helices. This concurs with studies of model peptides that form freestanding α helices in solution, where helix formation is attributed to local capping effects (14).

Despite the impressive and expansive nature of the study, there are gaps to fill and more steps to take. Although the authors have characterized many sequences for the target designs by circular dichroism spectroscopy, size-exclusion chromatography, and thermal and chemical denaturation, and have verified a small number of structures by nuclear magnetic resonance spectroscopy, more high-resolution structural details would be welcome—for instance, from x-ray crystallography. Such structures would allow sequence-structure-stability relationships to be rationalized in terms of specific noncovalent interactions that likely underlie them. For example, the study points to stabilizing roles for aromatic residues at surface-exposed sites of α helices and β strands in miniproteins, which hint at noncovalent interactions particular to this class of side chain.

In an unrelated but pertinent study, Baker et al. recently designed, characterized, and interrogated another monomeric miniprotein, PPα. This miniprotein has a compact structure comprising a polyproline II helix and an α helix that are connected by an intervening loop (15). A key determinant of PPα’s stability comes from intimate CH–π interactions between tyrosine residues of the α helix and proline residues of the buttressing polyproline II helix. Studying the role and interplay of these and other noncovalent interactions will be critical for feeding back into and improving computational design methods.

In their study, Rocklin et al. have taken high-throughput, data-driven protein design, selection, and optimization to new heights, bringing us closer to solving aspects of the protein-folding problem. A combination of high-throughput studies of the sequence-structure-stability relationships described by Rocklin et al. and drilled-down, fully quantitative examinations of the noncovalent interactions within (mini)proteins will bring us even closer to solving this long-standing problem. In turn, this will facilitate better engineering of natural and de novo proteins.

REFERENCES AND NOTES


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