Versatility from Protein Disorder

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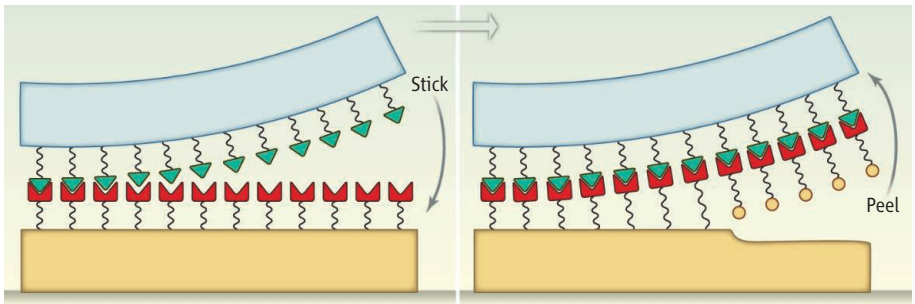
Science **337**, 1460 (2012);
DOI: 10.1126/science.1228775



Stick it and rip it. In a new, high-resolution method for nanofabrication, Liao *et al*. show that rubber stamps (top; blue) with siloxyl groups (green) on their surfaces react covalently with hydroxyl-terminated (red) self-assembled monolayers (SAMs) on gold (bottom; gold) at points of physical contact. Peeling the stamp away initiates fracture near the surface layer of the gold, yielding SAMs with precisely patterned geometries.

in the unprinted areas. Past attempts to minimize these effects achieved some limited success through the use of molecular inks with large molecular weights (*9*). Liao *et al*. take a completely different approach, with even better results. Instead of using stamps to print molecules onto bare surfaces in the usual way, they exploit chemically functionalized stamps to remove molecules from preformed, unpatterned SAMs. Here, upon physical contact, covalent bonds form between the stamp and reactive groups exposed on the surface of the SAM. Peeling the stamp away "mechanically desorbs" molecules from the SAM in regions defined by contact with the stamp. SAMs patterned in this way can then serve as molecular templates for etching the underlying substrate or for guiding the deposition of other materials (*2*–*4*). The most important practical aspect of this technique, termed chemical lift-off lithography, is that its resolution in patterning SAMs exceeds that of previous soft lithographic techniques.

Optimized surface chemistries are critical to the successful operation of the process. Liao *et al*. find that Si-OH groups on PDMS and hydroxyl-terminated SAMs on gold rapidly and covalently react to form strong Si-O-SAM linkages that remain intact as the stamp is removed. Here, mechanical fracture occurs within a near-surface region of the gold, such that both the SAM and, roughly, a monolayer of gold atoms peel off of the substrate (see the figure). These steps of contact-induced chemistry followed by nanoscale fracture can occur in minutes, over areas limited only by the sizes of the stamp and substrate, and with efficiencies of SAM removal that approach ~80%. Furthermore, the edges of the patterns can be extremely sharp and well defined, with roughness at the level of only a few nanometers. As a result, patterns with dimensions in the nanometer regime are possible. Liao *et al*. demonstrate 40-nm features, apparently limited only by the sizes of the relief features on the stamps.

The capabilities demonstrated by Liao *et al*., especially with such an extremely simple printing method, offer powerful modes of use in research, particularly when overlay registration is not required. Further development of the technique to eliminate such constraints will require engineering innovation. Fundamental extension of the resolution will demand improved understanding of the underlying mechanisms and, in particular, the relative roles of the molecular chemistry of the SAMs, the materials science of the substrate and stamp, and the physics of nanoscale fracture. Such topics represent appealing opportunities for interdisciplinary work, with strong potential for impact in nanoscience and nanotechnology alike.

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10.1126/science.1228493

STRUCTURAL BIOLOGY

Versatility from Protein Disorder

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Synergy between disordered regions and structured domains increases the functional versatility of proteins and their interaction networks.

Many protein functions can be attributed to segments (domains) that fold independently and adopt specific three-dimensional structures (*1*). Intrinsically disordered regions (*2*, *3*) are unstructured segments whose amino acid compositions prevent autonomous folding. Some eukaryotic proteins are either fully disordered (intrinsically disordered proteins) or structured, but most have both types of regions (see the figure). The notion that disordered regions are largely passive is being actively challenged by the idea that they perform diverse functions, and that synergy between structured and disordered regions expands the functional repertoires of proteins.

Understanding how disordered regions mediate function requires accurate physical descriptors of sequence-disorder relationships. Recent studies based on a combination of polymer theory, computer simulations, and biophysical experiments have revealed some coarse-grain conformational properties of disordered regions. Sequences enriched in polar amino acids and deficient in hydrophobic residues form compact, globular conformations (*4*, *5*). As the net charge per residue within disordered regions increases, they undergo continuous transitions to loosely packed ellipsoidal random coils (*6*, *7*).

The growing list of features attributed to disordered regions suggests that they act as molecular rheostats to support a continuum of conformational states and transitions. These features enable disordered regions to mediate highly specific interactions with multiple binding partners (*2*, *3*). Conformational fluctuations of these regions can control the exposure of short linear motifs (*8*) that interact with protein domains, thereby regulating protein interactions. Posttranslational modifications within or near these linear motifs may modulate conformation and affinities, thus increasing the functional capabilities of disordered regions. Examples include the tails

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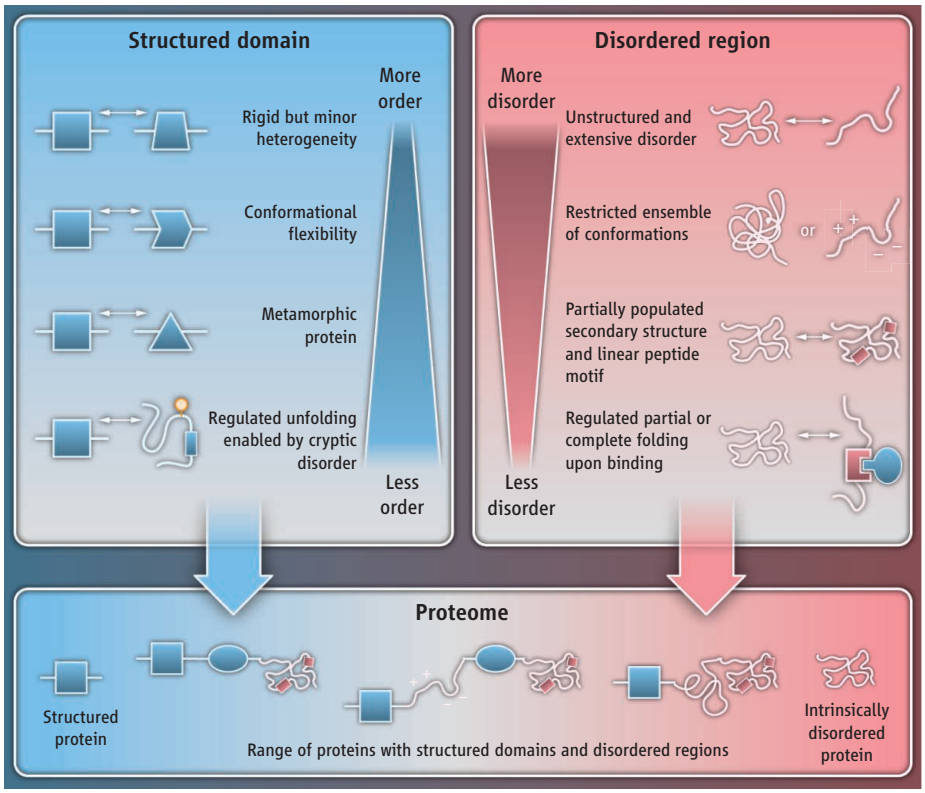

Determinants of protein function. Structured domains and disordered regions are fundamental units of protein function. Most proteins in eukaryotic proteomes contain both types of regions. The bar thickness shown for each type of region indicates a continuum and extent of conformational heterogeneity.

of histone proteins, several receptor kinases, and proteins that control the cell division cycle (*2*, *3*, *9*). A disordered region can also display multiple motifs, giving rise to multivalent interactions that drive the formation of micrometer-sized assemblies as recently reported for actin regulatory proteins (*10*). These assemblies may afford higher-order spatial organization and increase the local concentrations of proteins, and thereby regulate diverse cellular processes. Further, it was recently observed that the number and density of linear motifs within disordered regions can be regulated posttranscriptionally by alternative splicing of messenger RNA. Tissue-specific splicing events appear to alter the disordered regions that harbor binding motifs while leaving the structured regions intact (*11*). This can lead to rewiring of molecular interaction networks and new functional consequences. For example, a nearby structured region, such as a kinase domain, may become capable of acting on (phosphorylating) different proteins (new substrates) that bind to the spliced, disordered segment containing the linear motif. Given the critical roles mediated by such regions, the abundance of proteins with disordered regions is highly regulated to prevent nonfunctional promiscuous interactions that can cause disease (*12*).

Upon binding to an interaction partner, disordered regions can either undergo disorder-to-order transitions or preserve their disordered state by forming "fuzzy complexes" (*13*, *14*). Specific regions within structured domains (or folded regions of a bound disordered region) undergo regulated unfolding. This form of cryptic disorder (*15*) is an example of emergent behavior as it can generate new disordered states by coupling a binding event or posttranslational modification of structured domains to an order-disorder transition. Although current computational methods can readily identify disordered regions, uncovering regions of cryptic disorder encoded within structured domains remains a challenge. In addition, disordered regions also have regulatory roles that include an ability to induce local unfolding within adjacent structured domains and facilitate allosteric communication between structured domains.

How do disordered regions evolve? Because they do not require a defined structure for their function, disordered regions may be more tolerant of mutations than structured domains. This might confer an advantage if a new motif emerges through convergent evolution. Although evolutionarily conserved motifs can be identified through sequence analysis, the discovery of new motifs that are not conserved but are still functional represents a challenge for the future. If sequences of disordered regions change rapidly, do such mutations increase the risk of disease? Analysis of diverse cancer-associated mutations shows a dominant clustering within structured regions of proteins, suggesting that disordered regions provide robustness to mutation-induced effects on phenotype (*16*). Continued studies of sequence variations in normal and diseased cells are necessary to fully understand the contributions of disordered regions to fitness and disease.

Although in vitro experiments and molecular simulations provide important insights, disordered regions need to be studied in biologically relevant contexts to understand how complex functions emerge through the synergy between structured domains and disordered regions. Controlling protein function by modulating the degree of disorder through sequence design should reveal how natural variations in disordered regions affect the emergence of new phenotypes. These challenging issues are inspiring the development of new interdisciplinary approaches, from studies of single molecules and protein ensembles in cells, to deciphering the behavior of disordered proteins within entire biological systems. Complementing computational approaches, functional studies, and systems-level analyses of disorder with biophysical investigations will yield important insights regarding sequence-disorder-function relationships.

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Acknowledgments: We thank the MRC (M.M.B.), NIH (R.W.K. and R.V.P.), American Lebanese Syrian Associated Charities (R.W.K.), and NSF (R.V.P.) for financial support.

10.1126/science.1228775