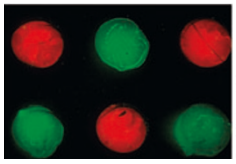
MELTING THE MOLTEN GLOBULE

Although much research has gone into studying protein folding, the difficulties of studying a potentially heterogeneous ensemble of pathways by bulk solution measurements has made it difficult to draw firm conclusions about the pathways involved. Single-molecule measurements offer an approach for watching an individual protein molecule fold; however, previous studies have lacked the resolution to observe folding intermediates. In a recent issue of *Science*, Marqusee, Bustamante and colleagues used single-molecule-force-measuring optical tweezers to observe an individual ribonuclease H (RNase H) protein fold. When the molecule was stretched, either of two distinct transitions occurred: one at a lower force that indicated that the molecule being stretched was not being pulled from the fully folded state, and one at a higher force that corresponded with full unfolding of the protein. During relaxation, the protein refolded through the same partially folded intermediate. A kinetic analysis of the unfolding of the intermediate revealed that the intermediate was a compact but soft and mechanically compliant state, quite distinct from the native, fully folded state. Combined with the relatively low force required to unfold the intermediate, the kinetic analysis suggests that the intermediate has a molten globule structure with only weakly formed tertiary contacts. These results provide the first evidence that the molten globule state is a necessary and on-pathway intermediate in a protein folding pathway. Studies on additional proteins will be necessary to determine if a molten globule intermediate is a general requirement for protein folding. (*Science* **309**, 2057–2060, 2005) *JK*

Membrane printing made easy

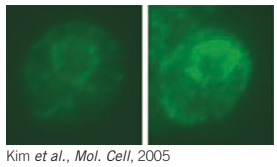


Dr. Michael Mayer

Biological membranes maintain barriers between cells and organelles, mediate transport of cellular materials and nucleate signaling cascades. Because membranes contain a complex mixture of lipids, proteins and small molecules, understanding membrane structure and function under physiological condhitions remains a challenging problem. The desire to analyze membrane properties in parallel has been aided by methods for the synthesis of spatially arrayed supported bilayers. For example, microcontact printing allows the stamping of biomolecules on to surfaces, much in the same way that rubber stamps transfer ink to paper. Majd and Mayer now report the reproducible printing of supported lipid bilayer arrays using a patterned stamp made of an agarose gel, which was 'inked' with lipids. The agarose stamp was used to print 100 replicates of a lipid array without additional inking. Medium- to high-density bilayer arrays, including a range of lipid types and composition, were readily prepared and demonstrated membrane fluidity and stability comparable to standard bilayers. Demonstrating the utility of the arrays, the authors reproduced known properties of annexin V, which preferentially binds to membranes with elevated levels of negatively charged lipids in a calcium-dependent manner. In addition, they showed that membrane fluidity was perturbed by cholesterol and by nimesulide, a nonsteroidal anti-inflammatory drug. The current approach offers a straightforward way to synthesize supported lipid arrays, which should prove useful for future studies of membrane biochemistry (*Angew. Chem. Int. Ed.*, published online 27 September 2005, doi:10.1002.anie.200502189). *TLS*

Research highlights written by Greg Watt, Mirella Bucci, Joanne Kotz and Terry L. Sheppard

Sweet kiss of death



Kim *et al.*, *Mol. Cell*, 2005

Protein folding and glycosylation in the endoplasmic reticulum (ER) is essential for proteins that either reside in the ER or are trafficked through the secretory pathway. Proteins that fail to fold properly, within biochemically defined time limits, are precisely targeted for degradation after dislocation from the ER to the proteasomes. How the ER distinguishes conformational variants of the same molecule and what signals are required for recognition, retention and dislocation have remained unclear. In the recognition step alone, there are at least two distinct ER-associated degradation (ERAD) pathways, depending on the protein substrate. ERAD-C and ERAD-L substrates are distinguished by the location of their misfolded domain (cytosolic or lumenal) and their different biochemical requirements for folding. These domains can include both protein and carbohydrate components. Three research teams now conclude that this is indeed the case and that one protein can recognize both components within ERAD substrates in *Saccharomyces cerevisiae*. Each group implicates the ER-resident protein Yos9p in binding to N-linked glycans on an ERAD-L substrate and in subsequent proteasomal degradation of the substrate. Szathmary *et al.* show that this interaction requires a specific glycan moiety, although, curiously, it is not one that is specific to misfolded proteins. Bhamidipati *et al.* help resolve this conundrum by finding that Yos9p could bind substrates independently of the carbohydrate. Kim *et al.* suggest that, depending on the particular substrate, Yos9p can act together or upstream of Mnl1p, another ERAD substrate receptor. Together, these studies point to a role for Yos9p both in recognizing misfolded proteins through glycan and unfolded protein determinants and in linking the recognition and dislocation steps of ERAD. (*Mol. Cell* **19**, 741–751, 753–764, 765–775, 2005) *MB*

Bacterial glycan builders

Campylobacter jejuni bacteria are known biosynthesize N-linked glycoproteins, in which the glycans are thought to be important for host cell invasion. Glycoprotein assembly in this bacterium is analogous to the process in eukaryotic cells whereby oligosaccharide transfer from a complex lipid-linked donor to an asparagine group on a target protein is mediated by an oligosaccharyltransferase. In *C. jejuni*, the donor substrate is a lipid-linked heptasaccharide, which is constructed by a team of specific glycosyltransferases. As only four of these enzymes catalyze the six monosaccharide addition steps required for heptasaccharide assembly, precisely how they function is unclear. To address this, two recent papers by Imperiali and coworkers report the *in vitro* chemoenzymatic reconstitution of lipid-linked heptasaccharide biosynthesis in *C. jejuni*. The enzymes responsible for protein glycosylation are located on one gene locus, termed *pgl*. The authors obtained recombinant forms of the four glycosyltransferases (PglA, PglJ, PglH and PglI) that catalyze heptasaccharide biosynthesis. They showed that the first two enzymes (PglA and PglJ) sequentially transferred two *N*-acetyl-galactosamine (GalNAc) residues to a chemically synthesized lipid-linked derivative of bacillosamine, an atypical bacterial sugar, to give a trisaccharide derivative. The next transferase (PglH), unconventionally, catalyzed the addition of three more GalNAc residues to give a linear hexasaccharide. Synthesis of the complete heptasaccharide was achieved by the last enzyme (PglI), which added a branching glucose unit to the hexasaccharide chain. This study shows the precise organization of glycoprotein glycan biosynthesis in *C. jejuni*. (*J. Am. Chem. Soc.*, published online 14 September 2005, doi:10.1021/ja054265v, and *Proc. Natl. Acad. Sci. USA* 102, 14255–14259, 2005) *GW