CHEMICAL BIOLOGY

More charges against aggregation

Michele Vendruscolo and Christopher M. Dobson

Protein aggregation causes problems for biotechnology and leads to many fatal human diseases. But a grasp of the physical principles involved enables 'superproteins' to be designed that have exceptional solubilities.

Proteins evolved under the stringent conditions imposed by the cellular environment. This means that, out of the vast number of possible amino-acid sequences in proteins, only a tiny fraction actually occur in nature. The advent of protein engineering[1] provides an opportunity to create proteins that have sequences that have never been found in living organisms, and that could have properties unparalleled in their natural counterparts. Reporting in the *Journal of the American Chemical Society*, Liu and colleagues[2] show that it is possible to design functional proteins with very high electrostatic charge that turn out to be far more soluble in water than their naturally occurring analogues.

Although a detailed knowledge of the biological factors that influence the behaviour of proteins is crucial to an in-depth understanding of their fundamental nature, considerable insights can also be gained by examining their physical and chemical properties. This idea has been reinforced by the discovery[3] that such properties are closely linked to the tendency of proteins to aggregate into non-functional polymeric structures, often known as amyloid assemblies. These structures are best known as the basis for the plaques that form in Alzheimer's disease, and they, or their precursors, can be highly toxic[3]. The need to avoid aggregation has limited amino-acid sequences to those that yield proteins with a relatively narrow range of specific physical attributes — such as hydrophobicity and net electrostatic charge.

Yet natural proteins have overcome many of these limitations to exhibit a wide range of solubilities. Such versatility enables them to work in diverse environments — from non-polar lipid membranes to the acid-bath of the stomach — and suggests that altering their solubility even more radically could result in artificial, 'high-performance' proteins. Moreover, if enzymes can be made to function in inorganic solvents other than water[4], and in organic solvents other than the lipids that make up biological membranes[5], new catalytic roles might emerge. This type of research has great potential for the chemical industry, and already allows carefully engineered proteins to be self-assembled into technologically valuable materials in the laboratory[6].

It follows, too, that a promising therapeutic strategy[7] to combat protein-deposition disorders such as Alzheimer's disease is to produce slightly more soluble versions of the proteins whose aggregation is the root cause of the problem. Such modified proteins will reduce the tendency of their natural counterparts to aggregate, while remaining compatible with their cellular environment[8]. This strategy might be aided by the actions of molecular 'chaperones' that protect the mutated proteins and promote their safe interactions with their environment[9].

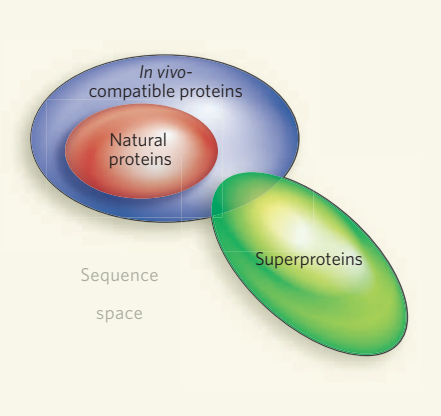

Figure 1 | An exploration chart of protein amino-acid sequences. The number of possible amino-acid sequences that can make proteins of the size found in living systems is enormous (larger than 10^{400}), and can be represented as the 'sequence space'. The number of naturally occurring proteins is much smaller (probably fewer than 10^{12}); such proteins have broadly similar physical properties, and form a cluster (red oval) in sequence-space. They are surrounded (purple region) by sequences that have not been found in nature, but whose properties might not differ much from those of natural proteins. 'Superproteins' are now being engineered that ignore at least some biologically imposed restrictions on amino-acid sequence, and these occupy regions of sequence space (green oval) unexplored by natural proteins. Such molecules can be designed to have unusual properties, such as Liu and colleagues' highly charged proteins[2], which resist aggregation.

With all of this in mind, Liu and colleagues[2] set out to increase the solubility of proteins dramatically. They began by modifying green fluorescent protein (GFP), which is widely used as an optical reporter for monitoring cellular processes. Using protein engineering, the authors produced GFP variants with a net charge ranging from –30 to +46; for comparison, the net charge of most natural proteins is in the range of –10 to +10. By clever design, these supercharged versions of GFP not only maintained their structural stability *in vitro*, but also remained soluble when exposed to conditions that normally cause proteins to aggregate — such as heating to high temperatures, or treatment with a chemical additive that causes the protein to denature. Liu and colleagues[2] then went on to modify other proteins, and found that the process of supercharging can be achieved without altering the proteins' normal functions.

The authors' results[2] are impressive. It will be fascinating to explore the extent to which such radically altered proteins can avoid unfavourable interactions *in vivo* with other cellular components, interactions that could result in toxicity. Even if such events occur, an armoury of additional design tools is available to modify the proteins further to provide therapeutic compounds.

More generally, the evidence from this study[2] suggests that the ability to use protein engineering and design techniques in new ways to sample largely unexplored amino-acid sequences holds great promise for applications in medicine, biotechnology and even materials science. These opportunities arise from the recognition that it may be possible to overcome the stringent limitations that evolution has imposed on the physical and chemical properties of naturally occurring proteins. ■

Michele Vendruscolo and Christopher M. Dobson are in the Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK.

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Correction

In the News & Views article "Atomic physics: A whiff of antimatter soup" by Clifford M. Surko (*Nature* 449, 153–155; 2007), the first prediction of the positronium atom is ascribed to John Wheeler in 1946. In fact the story, as recounted for example by Helge Kragh (H. Kragh *J. Chem. Educ.* 67, 196–197; 1990), is more complex: Stjepan Mohorovičić proposed a similar 'electrum' atom in 1934, although the antiparticles of his system were not the positrons of quantum theory; and Arthur E. Ruark, in work published in 1945 and at the time unknown to Wheeler, coined the term 'positronium' for "an unstable atom composed of a positron and a negative electron".

In the same News & Views article, it is also stated that a γ-ray laser could be made from a Bose–Einstein condensate of positronium molecules (Ps_2); in fact, positronium atoms (Ps) would be the starting point.