

Spotlight

Ensembles of synthetic polymers mimic biological fluids

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Recently a report by Ruan *et al.* in *Nature* described how relatively simple random heteropolymers can replicate the properties of biological fluids. These polymers capture the segmental-level interactions between proteins and could enhance folding of membrane proteins, improve stability, and enable DNA sequestration in a chemistry specific manner.

The complexity of biological fluids represents a significant challenge to understanding how biomolecules such as proteins interact with their environment. In contrast to the molecular-level precision of individual proteins, the composition of biological fluids cannot be molecularly defined and varies as a function of time and stimulus. As a result, proteins are expected to have evolved to exist in the context of this complex molecular environment. A recent report by Ruan *et al.* in *Nature* has explored how the complexity of interactions present in biological fluids can be recapitulated using simple random heteropolymers [1].

Traditionally, protein function has been understood in the context of the specific sequence of amino acids and the overall 3D structure of the molecule. To first understand the potential physicochemical environment present in biological fluids, Ruan *et al.* mined the UniprotKB database [2] to extract information on the apparent hydrophobicity and sequential arrangement of amino acids in known globular and membrane proteins in the context of 50

residue segments. The use of these two physicochemical descriptors allowed for projection of the complexity of protein sequence into two dimensions using principle component analysis. However, the diversity of sequence that can be obtained by considering even the basic set of 20 common amino acids, setting aside the potential for non-canonical residues and/or the effects of post-translational modifications, represents a chemical space that is too vast to be experimentally tractable.

Building upon knowledge from previous efforts [3–6], the team decreased the scale of the potential chemical space by considering sequence effects in the context of only four categories: charged, neutral and hydrophilic, hydrophobic, and very

hydrophobic. They then generated libraries of random heteropolymer ensembles by combining varying amounts of 3-sulfopropyl methacrylate potassium salt (SPMA) as the charged monomer, oligo(ethylene glycol) methacrylate (OEGMA) as the neutral and hydrophilic monomer, and then methyl methacrylate (MMA) and 2-ethylhexyl methacrylate (EHMA) as the hydrophobic and very hydrophobic monomers, respectively (Figure 1A). Importantly, they used control over both monomer composition and reactivity ratios to obtain ensembles of polymers with similar composition but different levels of blockiness [5]. Figure 1B,C demonstrates how the chemical nature of an example random heteropolymer ensemble, with each chain having the same composition and size for the individual blocks, traverses the principle component space

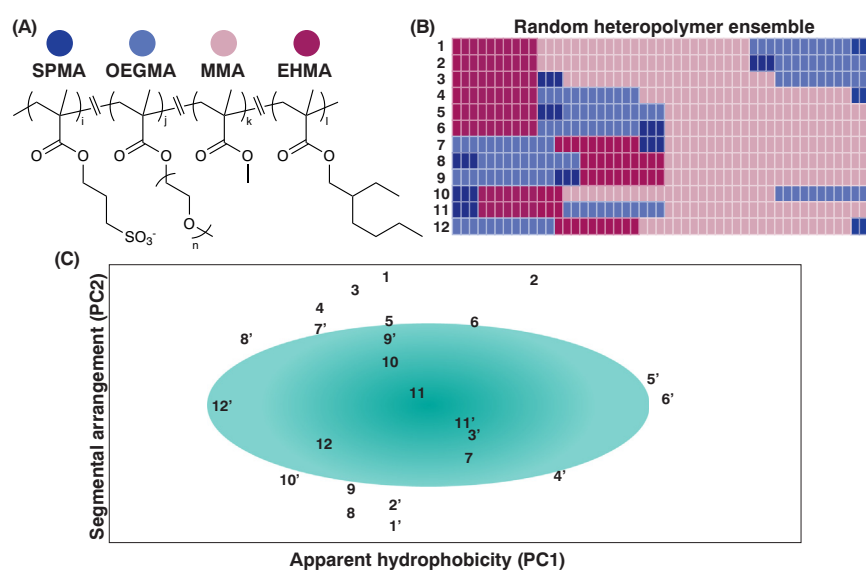


Figure 1. (A) Chemical structure of the four-component random heteropolymer used in this study. Copolymers were composed of 3-sulfopropyl methacrylate potassium salt (SPMA) as the charged monomer, oligo(ethylene glycol) methacrylate (OEGMA) as the neutral and hydrophilic monomer, methyl methacrylate (MMA) as a hydrophobic monomer, and 2-ethylhexyl methacrylate (EHMA) as the very hydrophobic monomers. Degree of hydrophilicity/hydrophobicity is indicated by shades of blue/red. (B) Schematic depiction of sequences for one example random heteropolymer ensemble with fixed composition. (C) The sequences from (B) are projected onto principal component space defined with regards to the apparent hydrophobicity (PC1) and the segmental arrangement (PC2). Sequences indicated with a prime (e.g., 1 versus 1') are the reverse of the numbered sequence shown in (B). Shading indicates the broader region over which polymers from this general ensemble would be expected to fall. Data and figures were adapted with permission from Ruan *et al.* [1].

Trends in Biochemical Sciences

defined by hydrophobicity (PC1) and the segmental arrangement (PC2). This analysis was also performed to determine how the various ensembles match with the chemical characteristics of both globular and membrane proteins.

Having demonstrated the similarity in physicochemical characteristics of their random heteropolymers and the various families of proteins, the authors looked to evaluate the utility of their materials for stabilizing proteins. First, the authors tested whether their random heteropolymers could help to stabilize the post-translational folding of three different membrane proteins generated via cell-free expression. Very excitingly, they observed increases in the yield of folded protein in a manner that correlated directly with the similarity of the hydrophobicity and segmental arrangement of residues in the protein of interest and the ensemble of random heteropolymers used. They then extended this effort to examine the ability of a selected ensemble of heteropolymers to stabilize the complex mixture of proteins present in fetal bovine serum (FBS) against thermal denaturation. Despite the presence of such a complex mixture, they were able to demonstrate both a decrease in sample precipitation and retention of the activity of FBS in cell culture, even after 2.5 hours of heating at 52°C. Importantly, the authors propose that it is the similarity in hydrophobicity and segmental arrangement between the synthesized random heteropolymer ensembles and the various proteins that helps to simulate the range of intermolecular interactions present in biological fluids. This hypothesis would therefore eliminate the need to try and create a precisely defined molecular environment to mimic a biological fluid that is in fact challenging to define and continually fluctuating. In fact, the ensemble of heteropolymers performed

better than equivalent diblock copolymers with the same overall compositions, highlighting the role for a wider range of segmental interactions.

One question might therefore be why a particular range of hydrophobicities and sequence arrangements is so beneficial for the heteropolymer ensemble to be able to interact effectively with proteins. For the family of heteropolymers deemed most similar to that of globular proteins, single-molecule unfolding experiments showed force-extension behavior characteristic of folded, partially folded, and disordered states, a level of structural heterogeneity that is expected to be similar to that of a biological fluid. Simulation results further demonstrated the ability of the polymers to rearrange their structure in the presence of a hydrophobic surface, suggesting the potential for a polymer, for example, to provide on-demand matching of segments for interaction with a protein as it undergoes folding.

Last, the authors explored the potential for their random heteropolymers to undergo liquid–liquid phase separation to mimic the behavior of so-called ‘membraneless-organelles’ by creating new ensembles that matched the hydrophobicity and segmental characteristics of proteins that are known to undergo phase separation [7]. In fact, matching these characteristics was shown to be necessary to achieve phase separation at biologically relevant conditions, as compared with the previously generated heteropolymer ensembles. Interestingly, the hydrophobicity that was necessary to help drive this kind of liquid–liquid phase separation was also observed to affect the ability of membrane proteins to refold after thermal denaturation. The authors also demonstrated the ability to incorporate DNA into these

condensates by incorporating positively charged groups, analogous to many biologically relevant structures.

In summary, the recent work by Ruan *et al.* demonstrated the tremendous potential of random heteropolymer ensembles for use in mimicking biological fluids. Such materials could have tremendous potential in both a scientific context and for applications where, for example, higher thermal stability of an enzyme or a biologic might be needed. Additionally, the focus on ensembles of polymers abrogates the need for residue-level sequence control, a factor that has long been a distinguishing feature between biological and synthetic materials.

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Declaration of interests

No interests are declared.

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