How stabilizing disordered proteins may lead to the next generation of medical applications

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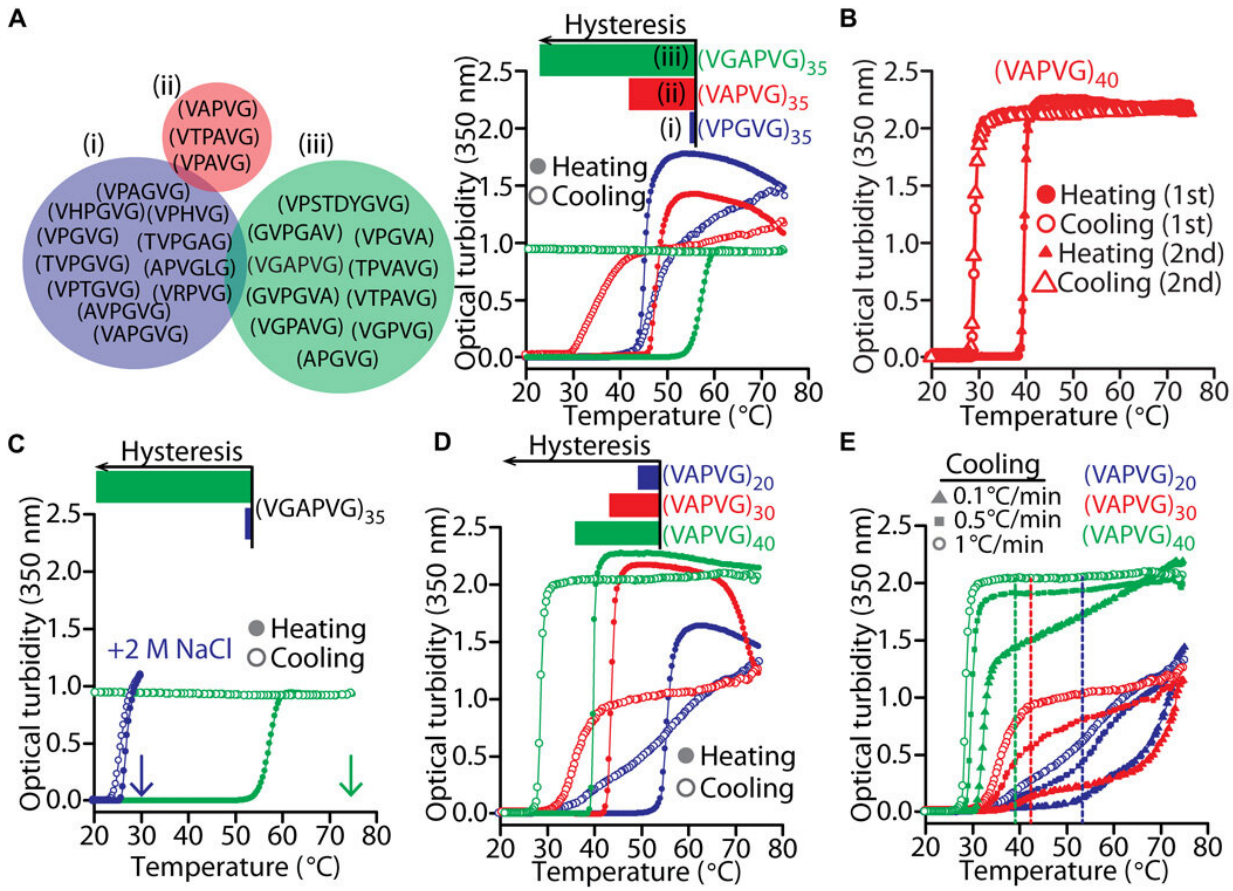

LCST IDPPs exhibit a wide range of hysteretic phase behaviors. (A) Analysis of the reversible phase behavior of LCST IDPPs in our library revealed three groups of repeat motifs, wherein motifs in each group encode one of three types of phase behavior characterized by differences in the degree of thermal hysteresis seen on cooling below the cloud point temperature, ranging from (i) negligible (~0°C) and (ii) moderate (10° to 30°C) to (iii) large, environmentally sensitive hysteresis. Here, we show temperature-dependent optical turbidity over

a full cycle of heating and cooling pass the Tcp for three representative IDPPs that exhibit the full range of observed hysteretic behaviors. As a guide to the eye, each panel includes a legend with a qualitative indicator of the degree of hysteresis for each repeat motif. (B) IDPPs made of (VAPVG) repeats exhibit highly reproducible degrees of thermal hysteresis over multiple cycles of phase separation. (C) Extension of data in (A) examining the phase behavior of (VGAPVG)35 to show its large, environmentally sensitive hysteresis, as it shows (in separate experiments) large or negligible thermal hysteresis depending on the maximum temperature (shown by arrows) reached during the heating part of the cycle. (D) Hysteretic phase behavior of IDPPs with an increasing number of (VAPVG) repeats. (E) Analysis of IDPPs in (D) but varying the cooling rate (from 1° to 0.1°C/min). To improve data visualization, the corresponding Tcp on heating are shown as vertical dashed lines. All optical turbidity measurements were performed at a fixed concentration of 50 μM in PBS, with heating and cooling at 1°C/min, unless otherwise stated. Credit: *Science Advances* (2019). DOI: 10.1126/sciadv.aax5177

Biomedical engineers from Duke University have demonstrated that they can create stable materials from engineered disordered proteins by altering the environmental triggers that cause them to undergo phase transitions.

This discovery shines a light on previously unexplored behaviors of disordered proteins and allows researchers to create novel materials for applications in drug delivery, tissue engineering, regenerative medicine and biotechnology.

The research appeared online on Oct. 18 in *Science Advances*.

Proteins function by folding into 3-D shapes that interact with different biomolecular structures. Researchers previously believed that proteins needed to fold into a specific fixed shape in order to function, but in the

last two decades, engineers seeking to create novel materials for biomedical applications have turned their attention to intrinsically disordered proteins, called IDPs, which dynamically shift among a wide array of structures.

IDPs are especially useful for biomedical purposes because they can undergo phase transitions — changing from a liquid to a gel, for example, or a soluble to an insoluble state, and back again — in response to environmental triggers, like changes in temperature. This ability has made IDPs a go-to tool for long-term drug delivery, as IDPs can be injected in liquid form into the body and then solidify into a gel depot that slowly releases medication.

But while their flexible structure makes IDPs useful in a variety of applications, researchers previously thought that this flexibility limited the stability of the resulting materials.

In their recent paper, Ashutosh Chilkoti, the chair of Duke Biomedical Engineering, and Felipe Garcia Quiroz, a Ph.D. graduate of the Chilkoti Lab who is a postdoctoral fellow at Rockefeller University, demonstrate that they can precisely tune the stability of IDP-based materials by controlling how quickly IDPs associate and dissociate in response to environmental cues.

"Unlike well-folded proteins, conventional IDPs have a hard time shielding different parts of their structures from each other," Quiroz said. "So as IDPs become more abundant in a solution they begin to collide and clash frequently, with some of their exposed structures weakly sticking together and rapidly breaking apart."

If the rate of association and dissociation is equal, the IDP is in equilibrium and it doesn't undergo any behavior change. But if something in the environment changes, such as temperature, then

segments of the IDPs stick together for longer periods of time, and they break apart with less frequency, resulting in a phase transition from a soluble to an insoluble state that can be harnessed to build materials.

Upon removing the environmental stimulus, however, conventional IDPs go back to exhibiting very weak associations, and the previously assembled materials fall apart.

In their new work, Chilkoti and Quiroz created materials using newly-designed IDPs that change phase at different temperatures, and demonstrated that upon phase separation, these IDPs are knocked out of their usual equilibrium behavior. This triggers a process known as hysteresis, in which IDPs will stick together even if the environmental trigger of the initial phase transition is removed.

"What is exciting about our new work is that we've shown that we can dial the degree of hysteresis to identify designs in which these proteins will stick together readily, and once those associations emerge, it becomes very difficult to break them," Quiroz said. "IDPs are typically thought as being weakly sticky, but we now show that it's possible to design super sticky IDPs, which become very stable building blocks."

"That super stickiness only emerges after we apply an environmental trigger, so they otherwise behave as regular IDPs and we don't have to worry about their stickiness as we handle them," Quiroz said. "From a materials perspective, many of our favorite materials are those that are easy to prepare, but can rapidly mature to a state that is highly stable and difficult to disrupt. Cement is a great example of this."

By showing that they could make a highly stable material out of IDPs, Quiroz said, they could build on earlier work with IDPs in fields like regenerative medicine. For example, in their liquid form, IDPs can flow into a wound cavity, adopt its shape and then phase into a gel to provide

structural support and recruit key cells for tissue repair.

Because current IDP-based materials lack stability, their effect is short-lived as they erode fairly quickly, but this new approach could make IDPs a good source of new materials for wound-healing.

"IDPs have had a set of known characteristics, and we have been working within that range of characteristics to explore potential biomedical applications for the last two decades," Quiroz said. "But now we essentially have new tools to play with, and that allows us to be more creative. Our discovery adds complexity to what we are able to do with IDP-based materials for applications spanning materials science and biology, which is exciting."

More information: Felipe Garcia Quiroz et al. Intrinsically disordered proteins access a range of hysteretic phase separation behaviors, *Science Advances* (2019). DOI: 10.1126/sciadv.aax5177

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