Researchers develop method to identify fleetingly ordered structures from intrinsically disordered protein

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(PhysOrg.com) -- A team of scientists from The Scripps Research Institute and the University of California, San Diego (UCSD) have developed a novel technique to observe previously unknown details of how folded structures are formed from an intrinsically disordered protein. The insights could help scientists to better understand the mechanism of plaque formation in neurodegenerative disorders such as Parkinson's and Alzheimer's diseases.

The results of the study, which has broad implications for the field, were recently published in an advanced, online issue of the journal Nature Methods.

The new technique allows previously unheard-of rapid detection—in less than 0.001 seconds—of transiently folded single-molecule structures from a class of often-amorphous molecules known as "intrinsically disordered proteins." The method also permits new types of observations of short-lived protein complexes.

"This exciting new technique allowed us to visualize multiple short-lived folded states," said Scripps Research Associate Professor Ashok Deniz, Ph.D., who led the study with UCSD Professor Alex Groisman, Ph.D., and Yann Gambin, Ph.D., of Scripps Research. "Further, better understanding of complexity during folding may offer more ways to regulate this interesting class of proteins."

The specific protein examined in the study was a-synuclein, which is highly concentrated in neural tissue. The protein has been implicated in Parkinson's and Alzheimer's diseases, as it is found in high concentrations in aggregates from the brains of patients with these conditions.

Mixing It Up

Unlike typical proteins in the cell, intrinsically disordered proteins such as a-synuclein do not adopt a stable globular form in isolation. Rather, intrinsically disordered proteins are like a messy, unfolded string of yarn, whereas typical globular proteins more closely resemble yarn neatly knit into complicated and functional shapes like that of a glove.

Studying intrinsically disordered proteins has been a challenge. Known techniques to determine protein structures are often designed for ordered proteins, and detection of transient shapes in structurally heterogenous proteins such as a-synuclein has been difficult. To remedy this situation, the Deniz and Groisman labs set out to devise advanced technologies to shed light on this novel class of proteins.

The new experimental technique described in the *Nature Methods* paper successfully combines and improves upon two established experimental methods: single-molecule Förster Resonance Energy Transfer (smFRET) and microfludic mixing.

smFRET detection allows for the observation of very small distances (in the range of one billionth of a meter), and can reveal changes in the molecular structure in real time. The method works by transfer of energy between single fluorescent dye molecules used as tags on a protein. One dye (donor) absorbs light and can emit red-shifted fluorescent light, whereas the other dye (acceptor) can receive the energy from the donor and emit even more red-shifted light. The relative amount of light

emitted by the two dyes depends on the distance between them, and hence can be used as a molecular ruler to measure distances in proteins. Microfluidic mixing in high-speed laminar flows has been used previously to rapidly initiate protein-folding reactions, but most observations have been made on a bulk rather than single-molecule level.

Detecting Protein Folding in a Chip

The key innovation of the research was to combine rapid mixing in a high-speed flow with single-molecule detection in a slow flow by abruptly decelerating the flow between mixing and detection regions.

The protein started in an aqueous solution and was mixed with a substance known as sodium dodecyl sulfate (SDS), which is normally used to unfold proteins but also facilitates the folding of a-synuclein due to its special interactions with amphipathic environments. This combination of rapid mixing and detection enabled the discovery of short-lived protein states previously invisible to researchers.

The microfluidic mixing itself was performed inside a small chip housing several hollow channels (or tunnels). The main channel is like a freeway upon which the protein travels. This channel connects at junction points to other inlets (on-ramps to the freeway) or outlets (off-ramps from the freeway).

The two inlets were used to funnel buffer and SDS into the central stream, effectively focusing the central protein stream into a narrow, fast-moving lane, and allowing a rapid switch into a solution containing SDS. Further along the channel at another junction, two outlets forced most of the "traffic" to exit. As a result, the speed of the remaining central part of the stream, or central lane, abruptly decreased.

Protein molecules in this slower, focused stream of protein were then

detected by smFRET. The rapid slowing was a critical new element in the method, providing just enough time for scientists to examine individual slower-moving proteins as they passed by the detector. A movie of the proteins' changing shapes could be recorded over time.

At the inlet (on-ramp), a-synuclein was introduced to negatively charged SDS, and a-synuclein began to fold. Combined with the rapid mixing, the fluorescence from the dye tags—which had been placed far apart on a-synuclein—revealed previously unknown details of transiently folded structures of a-synuclein, observed in the sub-millisecond timeframe.

Dynamic Conditions

Prior to this work, the equilibrium state of a-synuclein in the SDS-containing solution was known to be an extended helix (like the coil of a phone cord) called the F state. This ordered structure exists in the presence of the negatively charged biological membrane or SDS.

"So the question was: 'Do we go directly from the disordered protein to that F state?'" said Deniz. "And the answer from our experiments was, 'No.' We visit an intermediate structure, which has a similar FRET efficiency to what was previously observed to be a helix-kink-helix (I state), like a coil with a kink that bends the coil into a U-shape instead of a straight coil. Surprisingly, even this initial transition is complex, and provides us views of how the protein shape changes soon after binding to its partner molecules. What this means is that, as conditions in the cell are dynamic, these new states might give us many more points of regulation of a-synuclein."

Next in the lab's research, Deniz plans to examine questions including: "Do different a-synuclein structures aggregate differently, and how do they couple to function?" "What triggers the aggregation?" "What exactly are the roles of aggregates?" and "What kinds of structures will

be detected for a-synuclein interacting with other protein, lipid, and small-molecule partners?"

In addition, Deniz believes the developed microfluidic method will improve scientists' understanding of complexity in many other biological and health-related molecules.

More information: www.nature.com/nmeth/journal/v …/abs/nmeth.1568.html

Provided by The Scripps Research Institute

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