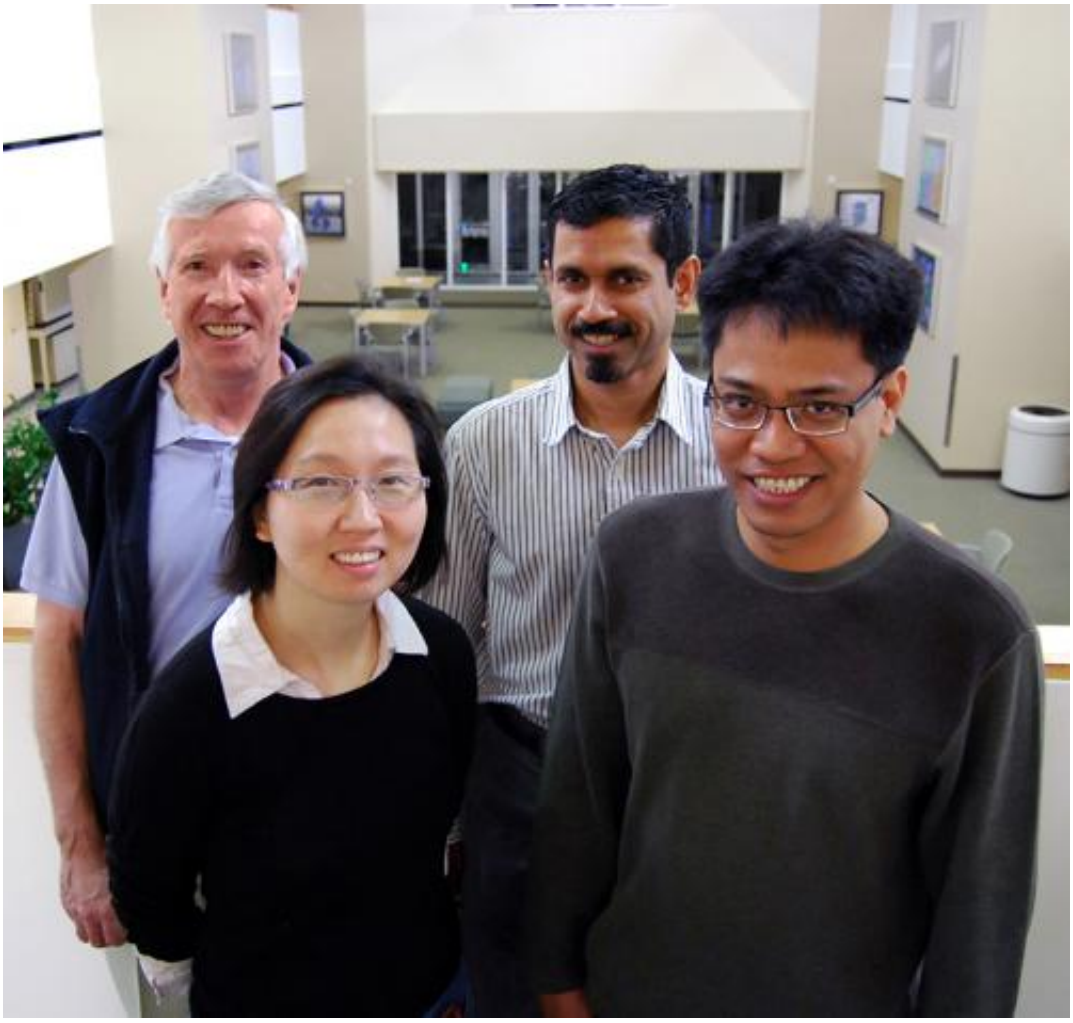
Scientists find new source of versatility so 'floppy' proteins can get things done

June 19 2013



The Scripps Research Institute's Peter Wright (left back), Ashok Deniz (right back), Josephine Ferreon (front left) and Allan Chris Ferreon are authors of the new *Nature* paper. Credit: The Scripps Research Institute.

Many proteins work like Swiss Army knives, fitting multiple functions into their elaborately folded structures. A bit mysteriously, some proteins manage to multitask even with structures that are unfolded and floppy—"intrinsically disordered." In this week's issue of *Nature*, scientists at The Scripps Research Institute (TSRI) report their discovery of an important trick that a well-known intrinsically disordered protein (IDP) uses to expand and control its functionality.

"We've found what is probably a general mechanism by which IDPs modulate their activities," said TSRI Professor Peter E. Wright, who is Cecil H. and Ida M. Green Investigator in Biomedical Research and member of TSRI's Skaggs Institute for Chemical Biology. Wright was a senior investigator for the study, along with TSRI Associate Professor Ashok A. Deniz.

The study focused on an IDP known as the adenovirus "early region 1A oncoprotein" (E1A). An adenovirus starts producing copies of E1A shortly after it infects a cell. E1A proteins interact with a variety of key cellular molecules to quickly subvert the cell's replication machinery for the benefit of the virus.

Links to Disease

E1A is worth studying not just because it facilitates adenovirus infections, but also because it's a prime example of an IDP. Such proteins frequently play outsized roles in cells, as crucial "molecular hubs" within very large protein-interaction networks. IDPs also include proteins that are linked to major diseases, including the tumor suppressor protein p53, the alpha synuclein protein of Parkinson's disease, and the amyloid beta and tau proteins of Alzheimer's disease.

The simple, flexible structures of IDPs are often promiscuously "sticky," which in principle explains why they would have multiple molecular

partners. But IDPs don't connect willy-nilly with other proteins, and scientists have wondered how they regulate their diverse interactions.

Wright's laboratory and others have been studying these interactions using a technique called nuclear magnetic resonance (NMR) spectroscopy. However, E1A's intrinsic stickiness means that it tends to aggregate at NMR-friendly concentrations, rendering this method of analysis problematic. (Most proteins, by folding up into complex shapes, effectively cloak their stickier bits.)

A Sensitive Technique

For the new study, Wright and his colleagues turned to Deniz, whose laboratory specializes in the use of sensitive, cutting-edge techniques to study the dynamics of disordered proteins and other biological molecules. One of these techniques, a quantum optics method known as single-molecule FRET, uses a tiny fluorescent beacon system to register distances between selected parts of a protein. In effect, this allows investigators to monitor in real time the shape-changes of E1A—characterized by Wright's laboratory in earlier work—which mark its rapid couplings and uncouplings with other proteins.

"The technique is sensitive enough that we can use it at extremely low protein concentrations, even focusing on single E1A proteins to avoid the loss of information that comes from the usual averaging of results over multiple proteins," Deniz said.

Postdoctoral fellows Allan Chris M. Ferreon and Josephine C. Ferreon, in the Deniz and Wright laboratories, respectively, used the single-molecule FRET method to detail the strengths ("affinities") with which E1A binds to two of its most important protein partners. By mapping how these binding affinities change under different conditions, they were able to obtain key insights into how E1A manages its multiple

interactions.

Achieving Complexity

First, like many folded proteins, E1A turns out to employ a basic regulatory mechanism called allostery: when one protein partner binds at one part of the E1A structure, it changes the ability of the other major binding site on E1A to bind other partners.

For most proteins that use allostery, this change makes partner-binding at the other site more likely ("positive cooperativity"). For a minority, it makes partner-binding at the other site less likely ("negative cooperativity"). But E1A turns out to have the capacity for either positive cooperativity or negative cooperativity between its two major binding regions—depending on whether a third part of the protein is occupied. "Allostery itself is a mechanism for modulating a protein's functions, and here we show that E1A takes it to another level by modulating allostery—modulating the modulation, in effect," said Josephine Ferreon.

The finding helps explain how E1A generates and manages its functional complexity—a complexity that for viral proteins seems particularly necessary, considering how tiny viral genomes are in comparison to those of their animal hosts. Moreover, some of E1A's key binding partners in infected cells are themselves hub-type IDPs. "So now you multiply the complexity—and you can see how proteins such as E1A manage to achieve so much so quickly within a cell," said Allan Ferreon.

Wright regards the study as the start of a rewarding line of investigation using sensitive techniques such as single-molecule FRET. "The fact that we can get around the usual technical obstacles relating to IDPs and do these single-molecule experiments really opens up the study of IDP hub interactions," he said.

Deniz concludes, "We're definitely going to be studying more of these hub proteins, and I think we're going to discover other fundamental principles by which they achieve complex layers of biological regulation and function."

More information: "Modulation of allostery by protein intrinsic disorder" doi:10.1038/nature12294

Provided by The Scripps Research Institute