Crowning Proteins: Modulating the Protein Surface Properties Using Crown Ethers

A research team led by Academician Andrew H.-J. Wang, a Distinguished Research Fellow at the Institute of Biological Chemistry has developed a new tool for the non-covalent modification of protein surfaces via small, cyclic organic molecules called crown ethers. The team included Drs. Cheng-Chung Lee, and Manuel Maestre-Reyna, both postdoctoral research fellows at the Institute of Biological Chemistry; and Dr. Kai-Cheng Hsu and Dr. Jinn-Moon Yang, from the Institute of Bioinformatics and Systems Biology, National Chiao Tung University. The results of the research were published online in the prestigious journal *Angewandte Chemie*, which specializes in important discoveries in applied chemistry, on October 5.

As protein function is directly linked to protein surface properties, structural information is key in the development of the majority of protein applications in the biomedical, biotechnological, and chemical fields. Due to the complexity of protein molecules, it is very difficult to predict and rationally modulate the protein structure, which results in most protein structures being experimentally determined.

Most protein structures are solved by a method termed protein X-ray crystallography, for which protein crystals need to be grown. Since crystals rely on regular surface-surface interactions to grow, protein X-ray crystallography is often itself hindered by the very feature it tries to clarify, the protein surface. If the protein surface cannot form regular contacts, and this is very difficult to know beforehand without a protein structure, the protein will not crystallize, and no protein structure can be obtained. In order to break this vicious circle, Dr. Wang and his team used crown ethers, which they proved were capable of attaching to protein surfaces. Furthermore, they showed that protein-crown ether complexes presented a surface which was better suited for crystallization. Crown ethers can interact both with positive charges on the protein surface, and with themselves. In this way, they neutralize the former repulsive forces, and yield new attractive forces via the latter. Since crown ethers bound selectively to the protein surface, these new attractive forces are displayed in regular patterns, which, as mentioned above, are highly conducive to successful crystallization.

Showing how, and where, crown ethers interact with proteins may open the door to many new applications. For example, most modern drugs act by binding a target protein in

the human body, yielding a protein-drug complex with new properties, which the protein alone lacks. Currently, there is a single crown ether-based drug, and its mechanism of action is unclear. Dr. Wang's research team was able to propose a definite mode of action for crown ether-based drugs. They showed that, under some circumstances, crown ethers can profoundly change the overall protein structure, resulting in the aforementioned new properties.

The discovery of crown ether-based protein surface modulation will also be very useful for the chemical industry. Enzymes are extremely efficient and specific chemical reactors, but most of them will only work in water, or water-like solvents. On the other hand, most reactions in a chemical plant take place in organic media. Several strategies have been developed to bridge this gap, including the use of proteins from organisms which live in extreme conditions, which are characterized by being particularly tough and stable. Other strategies are based on coating the protein with small molecules, including crown ethers, which protect them from the adverse conditions. Even though there are some applications, such as crown ether-cytochrome complexes, the mechanism by which crown ethers protect enzymes was not well understood. Thanks to Dr. Wang's research, there is now a rational basis to crown ether-mediated enzyme protection, and therefore a foundation on which to develop more efficient enzyme protecting molecules.

The first co-authors of the study were Dr. Cheng-Chung Lee and Dr. Manuel Maestre-Reyna, who both working in Dr. Wang's lab. The research leading to the current publication was supported by the Core Facilities for Protein Structural Analysis (CFPSA), the National Center for High-Performance Computing (NCHC), and the National Applied Research Laboratories (NARLabs) of Taiwan. Structural data was obtained at the National Synchrotron Radiation Research Center (NSRRC), Taiwan, and SPring-8, Japan.

The complete article entitled: "Crowning proteins: modulating the protein surface properties using crown ethers" can be found at the *Angewandte Chemie* journal website at: http://onlinelibrary.wiley.com/doi/10.1002/ange.201405664/pdf

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