Chemists bring mixed folded proteins to life

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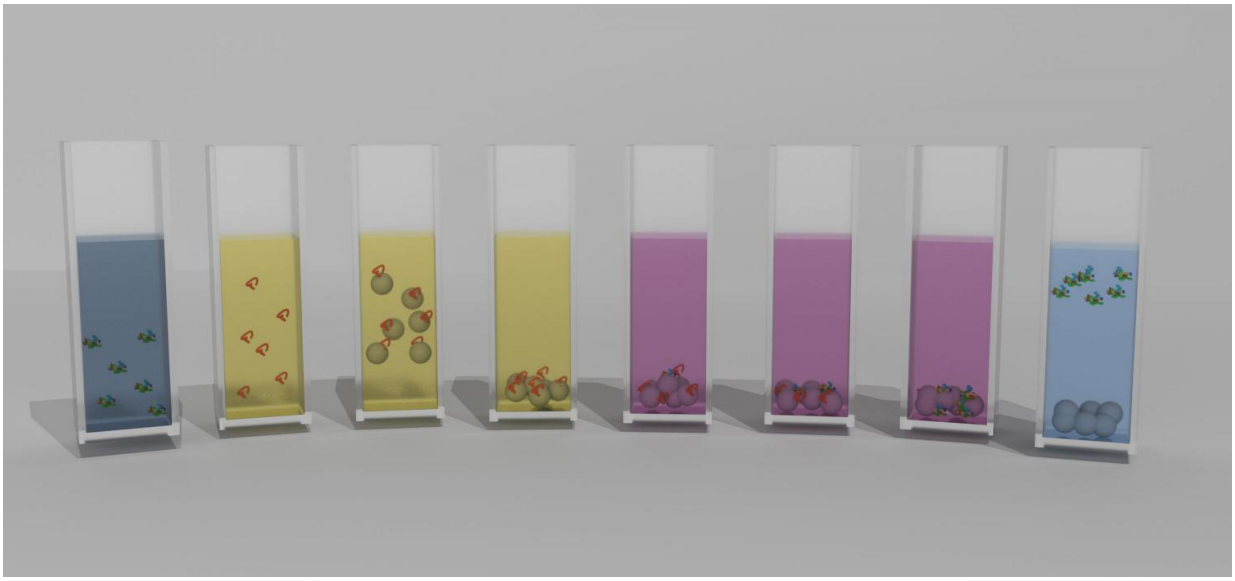

The alumina nanoparticle-assisted enzyme refolding process is shown. Credit: ITMO University

Scientists from ITMO University in Saint Petersburg and Hebrew University in Jerusalem have found a way to recover a protein structure after its chemical denaturation. The method is based on electrostatic interaction between folded, or denatured, proteins and alumina, which unwrap them. The authors highlight the versatility of the method, which works for both specific molecules and multiprotein systems—no previous technique has been able to recover mixtures of enzymes before. This can simplify and cheapen the production of drug proteins for Alzheimer's and Parkinson's treatment. The study appeared in *Scientific*

Reports.

Proteins, especially accelerators of chemical reactions, are the basis of the pharmaceutical and food industries. Meanwhile, 80 percent of these substances are lost during synthesis. Influenced by unfavorable factors like strong acids, alkalis or heating, proteins denature, losing their native shape and any chemical activity. Thus, the industry seeks a universal method for recovering protein structure, which could make the production cheaper and more effective. To manufacture enzyme-based drugs and foods on an industrial scale, it is especially important to find a way to recover mixtures of proteins, since renaturating each particular type of enzyme separately is costly and inefficient.

Russian chemists in cooperation with foreign colleagues have proposed a solution to this issue with a process that gives a second life to proteins, returning their molecules to the original form after denaturation.

In the new research, the chemists unfolded molecules of three enzymes: carbonic anhydrase, phosphatase and peroxidase. Denatured by a strong alkaline, the proteins were mixed with nanoparticles of alumina in water. Due to electrostatic interaction, the enzymes attracted the nanoparticles and engaged them in forming a supramolecular complex with physical rather than chemical bonds.

This shell of nanoparticles protected protein molecules from aggregation, enabling the scientists to easily extract them from the aggressive media. Washed from denaturing substances, the enzymes restored their structure by themselves. "Constant exposure of denaturing agents and the tendency of curling macromolecules to aggregation are major obstacles for recovering proteins. When removing these factors, we were able to regenerate our objects," says Katerina Volodina, a second-year graduate student at ITMO University.

Changing pH, the scientists separated nanoparticles from proteins showing that the substances involved in the experiment can be repeatedly used.

The authors applied their method to a mixture of two enzymes: carbonic anhydrase and phosphatase (CAB and AcP). For these proteins, the portion of renaturated molecules was more than half, an unprecedented result. "Renaturating of multiprotein mixtures has never been done before. But my colleagues and I believe that further research in this area is in the great interest of pharmaceutical companies right now. Theoretically, our method can simplify and cheapen the manufacture of drugs for Alzheimer's or Parkinson's therapy. Many of these medicines are made of proteins," notes Katerina Volodina.

Besides its versatility and high performance, the technology proposed by ITMO University's chemists is also fast and low cost. The scientists are going to refine the approach mostly to renaturation of proteins in complex mixtures.

More information: Katerina V. Volodina et al, Alumina nanoparticle-assisted enzyme refolding: A versatile methodology for proteins renaturation, *Scientific Reports* (2017). DOI: 10.1038/s41598-017-01436-6

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