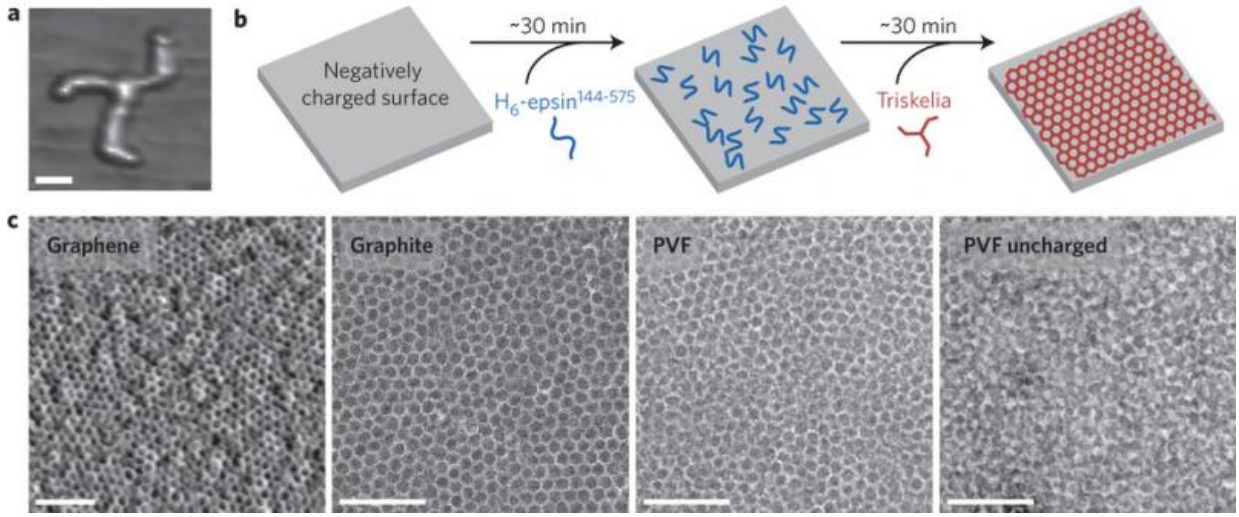
Clathrin as a biotech substrate: Immobilization and functionalization

September 22 2015, by Heather Zeiger



Assembly of clathrin lattices on different surfaces. Credit: (c) 2015 *Nature Nanotechnology*, DOI: 10.1038/NNANO.2015.206

(Phys.org)—The base-pairing properties of DNA, combined with our abilities to create synthetic DNA in the laboratory have led to advances in nanoscale architecture and molecular device designs. Less research has been done with proteins, even though proteins, like DNA, are made of individual subunits whose unique chemical properties can be exploited to functionalize protein sheets or immobilize the proteins on a surface. Certain proteins have desirable properties for molecular devices.

A group of researchers from Hannover Medical School, University College London, Georg August University, and the Centre for Nanoscale Microscopy and Molecular Physiology of the Brain in Germany have shown that clathrin, a lattice-forming protein complex used for vesicle transport in eukaryotic cells, can be immobilized onto a variety of surfaces and functionalized with nanoparticles and enzymes. Furthermore, the clathrin lattice can be stored and re-activated without losing its functionality, making it a practical substrate for molecular devices. Their work appears in *Nature Nanotechnology*.

Clathrin is employed in vesicle transport across membranes in eukaryotic cells. It forms a lattice structure that can be either a two-dimensional sheet or a three-dimensional cage. Clathrin is comprised of a three-legged protein complex, known as a triskelion. The triskelia self-assemble into lattices which encloses a membrane into a polyhedral cage. The triskelion has heavy chains and light chains. A lattice can be made of triskelia that are both heavy and light chains or just heavy chains. In this study, the light chains are functionalized with nanoparticles or enzymes.

Dannhauser, et al. found that two-dimensional clathrin lattices will form on several types of surfaces. They immobilized clathrin using a portion of an adaptor protein, H_6-epsin. In the body, clathrin attaches to membranes through adapter proteins, so for purposes of immobilization on a surface, Dannhauser, et al. tested whether the same mechanism can apply to a variety of surfaces in the laboratory setting. They produced immobilized clathrin lattices on graphene, polymers, glass, and metals.

The surface-lattice interaction can be controlled using NaSCN. NaSCN is known to impede three-dimensional clathrin assembly, so they used it to disassemble the two-dimensional, surface-bound lattice. After treating with 0.05 M NaSCN, the lattice became disordered. Removal of the NaSCN showed some of the lattice features remained and treating with

more triskelia caused the lattice to re-form. Higher concentrations of NaSCN were used to remove the lattice completely. However, the H_6-epsin linker remained intact even at higher concentrations of NaSCN, showing that the linker is highly robust while the lattice can be easily removed.

Unfortunately the immobilized clathrin lattice is only stable for tens of minutes, which is impractical for use as a device. Therefore, Dannhauser, et al. tested various crosslinking strategies. They found that 4-azido-2,3,5,6-tetraoroenzo acid succinimidyl ester (ATFB) to be a good candidate for crosslinking. It covalently links clathrin to H_6-epsin. Additionally, the lattice can be dehydrated by first crosslinking with glutaraldehyde and then using uranyl acetate. AFM studies show that lattice activity can be restored upon rehydration. Crossliniking combined with dehydration allowed them to store the lattices for months at a time.

Finally, the clathrin lattice was functionalized with gold nanoparticles and with a co-enzyme called auxilin via incorporating modified light chains to a lattice consisting of heavy chains. Imaging studies confirmed functionalization of both the nanoparticles and the enzyme. Auxilin is used in living cells together with the enzyme Hsc70 to remove clathrin lattices from membranes. Preliminary studies showed that auxilin seems to maintain its enzymatic activity by the way it disassembled the immobilized clathrin lattice. While additional studies are needed, this experiment demonstrates that the lattice assembly can be functionalized with diverse particle types.

This research looks at how clathrin can be used for molecular devices and nano assembly. Dannhauser, et al. demonstrate its practicality by immobilizing the lattice on various surfaces, increasing its lifetime through crosslinking and dehydration, and functionalizing it with an inorganic nanoparticle and an enzyme.

More information: "Durable protein lattices of clathrin that can be functionalized with nanoparticles and active biomolecules" *Nature Nanotechnology*, DOI: 10.1038/NNANO.2015.206

Abstract

Biological molecules that self-assemble and interact with other molecules are attractive building blocks for engineering biological devices. DNA has been widely used for the creation of nanomaterials, but the use of proteins remains largely unexplored. Here, we show that clathrin can form homogeneous and extended two-dimensional lattices on a variety of substrates, including glass, metal, carbon and plastic. Clathrin is a three-legged protein complex with unique self-assembling properties and is relevant in the formation of membrane transport vesicles in eukaryotic cells. We used a fragment of the adaptor protein epsin to immobilize clathrin lattices on the substrates. The lattices span multiple square millimetres with a regular periodicity of 30 nm and can be functionalized via modified subunits of clathrin with either inorganic nanoparticles or active enzymes. The lattices can be stored for months after crosslinking and stabilization with uranyl acetate. They could be dehydrated and rehydrated without loss of function, offering potential applications in sensing and as biosynthetic reactors.