Quick purification of protein complexes for structural proteomics, using grpahene and single walled carbon nanotubes

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In our postgenomic era, understanding of protein–protein interactions by characterizing the structure of the corresponding protein complex is becoming increasingly important. An important problem is that different protein complexes have different degrees of stability over time; many protein complexes have a half-life of only several or several tens of minutes. The typical purification methods for protein complexes, such as tandem affinity purification and multiple chromatographic separations, are time consuming and therefore are only suitable for stable protein complexes. Up till now, a quick and efficient protein complex purification method for 3D structure characterization has not been developed.

My new research-line aims at developing a quick and efficient method that is suitable for structural characterization of unstable protein complexes. In this project, single walled carbon nanotubes (SWNTs) and graphene oxide (GO) are used to 'fish' the target protein complex through affinity interaction by chemically binding affinity pairs on SWNTs and GO and the target protein complex respectively, as shown in Figure 1 A-E.

The protocol will be validated in single particle structure determination by cryo-EM, as shown in Figure 1 F-G. Any captured protein complex on a SWNT can be flash frozen and transferred into a cryo electron microscope (EM) for imaging without removal of the SWNTs, because SWNTs are compatible with EM characterization. The native structure of the protein complex can be kept intact during the whole process without any additional treatment.

We developed a novel method for preparing SWNT(GO)•streptavidin complexes via the biotin–streptavidin recognition.[1,2] Capturing biotinylated DNA, fluorophores, Au nanoparticles (NPs), and DNA/protein complexes on the SWNT (GO)•streptavidin complexes demonstrate their usefulness as a docking matrix, especially in our project, for purification of unstable protein complexes and structural characterization using cryo-electron microscopy.

References

Purification.

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Figures

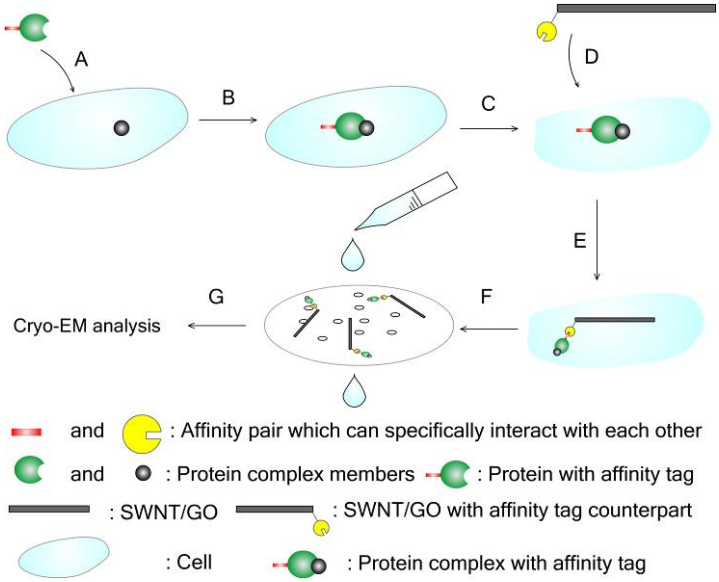

Figure 1 Schematic representation of capturing protein complex using SWNT and GO.