Synthesis and Assembly of Nanoscale Biological-Material Hybrids and Their Use for Selective Cellular Delivery

by

Shyam Sundhar Bale

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Examining Committee:

Prof. Jonathan S. Dordick, Thesis Adviser

Prof. Ravi S. Kane, Thesis Adviser

Prof. Steven Cramer, Member

Prof. George Plopper, Member

Rensselaer Polytechnic Institute
Troy, New York

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ABSTRACT

Developments in recent years have illustrated the impact of nanoscale materials as unique supports for biomolecules for applications ranging from biocatalysis to cellular delivery, and biosensing. This thesis is focused on understanding the interactions between nanoscale materials and proteins, and exploiting these interactions for the synthesis of hybrid nanomaterials, protein-mediated assembly, and cellular delivery.

We first demonstrated the use of conjugates of proteins with carbon nanotubes to form protein-nanotube-nanomaterial conjugates under mild conditions. The formation of nanoparticles was influenced by the glycan content of the protein used. This technique may be useful in forming functional nanocomposites that combine biotic function with abiotic structural features. Another advantage of proteins is that they present a rich variety of functional groups that may be used as orthogonal reactive handles for further nanotube functionalization.

Next, the cytoskeletal protein tubulin was conjugated with multi-walled carbon nanotubes (MWNTs) to achieve the protein-driven assembly of MWNTs and to transport MWNTs under conditions that mimic the intracellular transport of a living cell. In one approach, cut-MWNTs were complexed with tubulin to form nanotube-based conjugate assemblies in a concentration dependent manner to form linear bundles (lower concentration) and petal like conformations (higher concentrations). Furthermore, the immobilized tubulin retained its ability to polymerize into microtubular structures that encapsulated the nanotubes when free tubulin was added. The polymerized structures inherited the functionality of tubulin specifically, motility in a gliding assay when using immobilized kinesin molecules. In another approach, microtubules (polymerized tubulin) were tested for their ability to transport MWNTs attached as “cargo” to their lattices. Specifically, streptavidin functionalized cut-MWNTs were attached to biotinylated microtubules and the assembly was driven by kinesin immobilized on engineered surfaces. Furthermore, when other nanoparticles were attached to the nanotube (i.e. silver) and subsequently to the motile microtubule, the complex hybrid structures were also glided by the immobilized kinesin.

In a final study, nanoparticle-mediated protein delivery was used to target cellular machinery. Specifically, we used silica nanoparticles to deliver “active” proteins to the

cytoplasm of mammalian cells – human breast cancer cells (MCF-7) and rat neural progenitor stem cells (NSC). The delivery of proteins such as ribonuclease A and anti-p-AKT antibodies, that can influence cell functioning only when present in the cell cytoplasm, resulted in apoptosis. We also demonstrated the ability to deliver proteins to specific sub-cellular locations. To that end, a GFP complementation system with GFP_{1-10} and GFP_{11} was adapted to act as a sensor for protein delivery, and to aid in visualizing protein localization at the target site. Introduction of proteins to cells is a powerful alternative to the introduction of genetic material, such as DNA and siRNA – commonly used modes to influence cell function. Nanoparticle-mediated protein delivery may be particularly useful for loss/gain of function studies that target a specific posttranslationally modified form of a protein (e.g., various kinases involved in signaling pathways, glycosylated proteins, etc.)