Protein Nanocapsules for Therapeutic Applications

Yi Tang, UCLA

Proteins are the engines of life that perform essential functions inside cells, such as enzyme catalysis, signal transduction, gene regulation and maintaining a fine balance between cell survival and programmed death. Many diseases arise from the alterations in the functions of intracellular proteins. Therefore, a general method of delivering active forms of proteins to specific cells and organs in living organisms is an important goal in many medical applications, including cancer therapy, vaccination, regenerative medicine, treating loss-of-function genetic diseases and imaging. From a therapeutic perspective, protein-based approaches may be safer than gene therapy because no random or permanent genetic changes are involved, but only transient actions of proteins are needed.

The main barriers of delivering target proteins to the intracellular space result from intrinsic properties of most proteins, including the large sizes, varying surface charges and fragile tertiary structures. When administered into serum, native proteins can suffer from serum instability and can be rapidly degraded or inactivated. Most native proteins are also membrane impermeable due to electrostatic repulsions. Therefore like drug, DNA and siRNA delivery systems, appropriate delivery vehicles for escorting proteins to the cytosol are highly important. In addition to the obstacles mentioned above, the vehicle often needs to help the protein cargo in endolysosomal escape. Importantly, to successfully reach the various desired subcellular compartments, such as the cytosol, the mitochondria and the nucleus, the delivery vehicle must be able to escape the endosomal pathway to avoid being trafficked through endomembrane compartments and being subject to clearance and degradation under harsh lysosomal conditions. To date, the most commonly used approach for intracellular protein delivery is the genetic fusion of the target protein to protein transduction domains (PTDs) or cell-penetrating peptides (CPPs). Despite many practical advantages of the protein transduction technology, the main concern of this method is the inefficient escape from the endosome to the cytosol, leading to CPP-tagged cargoes sequestered in intracellular vesicles.

In the past decade, nanocarrier-based intracellular protein delivery approaches have generated considerable interest and have been shown to be promising strategies. These nanosized carriers include lipid-containing colloidal systems such as liposomes and solid lipid nanoparticles, polymeric nanocarriers, inorganic nanoparticles/nanotubes and protein-based carriers. Target protein cargoes can be loaded into various nanocarriers using different strategies, including direct conjugation *via* either chemical or genetic modifications, physical adsorption and covalent/noncovalent encapsulation. One of the key functions of the nanocarriers is to serve as shields to protect proteins from premature degradation and various denaturing interactions with the biological environment. They can increase the stealth of the delivered protein by concealing antigenic and immunogenic epitopes and attenuating receptor-mediated uptake by the reticuloendothelial system (RES). The high surface area to volume ratio of nanocarriers also leads to improved pharmacokinetics and biodistribution of payload. Another crucial feature of the nanocarrier-based delivery system is the increased flexibility of tailoring the chemical and physical properties of the vehicle through controlled synthesis, assembly and facile biocompatible chemical modifications. Key particle properties such as size, surface charge and displayed ligands can be customized to facilitate cell

penetration and endolysosomal escape, as well as to optimize stability, targeting specificity and cargo release kinetics.

Here we present a new nanoscale approach to cytosolic protein delivery using reverse encapsulation of protein cargo in a degradable polymeric layer. The polymer shell can serve as a protective layer that shields the protein from proteases and denaturants; as well as presenting a positively charged vehicle for cellular internalization. Our lab recently used this strategy to synthesize biodegradable nanocapsules that can be used to deliver a variety of protein targets. The single-protein nanocapsules were prepared through interfacial polymerization around the native proteins with monomers and biodegradable crosslinkers. The deposit of monomers and crosslinkers is facilitated by electrostatic interactions and is therefore noncovalent in nature. Degradation of the crosslinker upon entry into the cell leads to disassembly of the nanocapsule layer and release of the protein cargo. Nanocapsules formed using this method are uniform in size with overall positive surface charge, and are internalized by different cells through endocytosis. We demonstrate the utility of the nanocapsule approach in triggering programmed cell death and artificial control of gene expression in various human cell lines.