Intracellular Delivery of Model Proteins Using Fluoro-Crown Ether Phosphate

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Bioactive molecules such as small drugs, peptides, proteins, and RNAs are highly promising therapeutic agents due to their target specificity and biocompatibility[1,2]. Existing delivery strategies, including nanoscale carriers like inorganic nanoparticles, synthetic polymers, and cell-penetrating peptide-based complexes, have addressed these issues by promoting membrane permeability and endosomal escape. However, biosafety concerns and the cargo's physicochemical limitations often constrain these approaches. Leveraging our recent findings on the polar and compact structure of cyclic fluoro-crown ether phosphate (CyclicFP), we propose a novel strategy to enhance the cellular uptake of various cargoes. The mechanism centers on CyclicFP's ability to disrupt the hydrogen-bonded water network at the cell membrane, promoting cargo penetration through the cell membrane.

As a proof of concept, we investigated the cellular uptake of dye-labeled CyclicFPs. CyclicFP-X was internalized into cells even at 4 °C, a condition that inhibits energy-dependent endocytosis, suggesting direct membrane permeation. In contrast, CyclicP-NBD (a nonfluorinated analog) and AcyclicFP displayed negligible internalization under the same conditions. Furthermore, we assessed the uptake of CyclicFP-sfGFP, which successfully entered cells but exhibited fluorescence signals predominantly within endosomes, indicating entrapment. In contrast, the sfGFP control without CyclicFP showed no detectable fluorescence, confirming its inability to cross the membrane.

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