UNLOCKING INTRACELLULAR THERAPEUTIC TARGETS THROUGH NOVEL NANOSTRUCTURED BIOMATERIALS

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Nucleic acid cargoes offer unmatched diversity in gene regulatory potential and therapeutics, and understanding of nucleic acid functionality continues to expand rapidly and dramatically through seminal discoveries including RNA interference approaches and gene editing technologies. In nature, the basis for gene regulation is ultimately encoded by the exquisite specificity with which cells are able to control both the location and accessibility of nucleic acid constructs to govern their activation states. My research program seeks to understand and control gene activation using synthetic constructs through nature-inspired approaches to control and quantify cell binding interactions and stability in polymer and peptide nanocarriers. The basis of our approaches is the design of stimuli-responsive polymers and peptides whose interactions with nucleic acids and cells can be controlled dynamically by specific intracellular or external triggers. We exploit our ability to control nucleic acid binding/release and cellular processing to gain new mechanistic insights over nucleic acid delivery, leading to design advances including histone-inspired DNA targeting, light-responsive gene silencing, and collagen turnover-stimulated gene expression. This talk will highlight two ways we have used nature-inspired peptides to control gene transfer in regenerative medicine.

Our approaches are exemplified by our work in histone-targeted nanocarrier design. Histones have received great interest as potential gene carriers for several decades due to their seminal role in chromatin packaging and gene transfer, yet therapeutic efforts with histones have lacked both a well-controlled materials approach and a deeper knowledge of cellular processing mechanisms. Hence, histone-based carriers have failed to reach clinical efficacy. We have capitalized on newly recognized and highly pivotal roles for histone tails in native gene regulatory control to develop a gene transfer method that utilizes native, histone-based processing pathways *via* incorporation of post-translationally modified (PTM) histone tails within controllably-assembled DNA vehicles (polyplexes). Our efforts proved that polyplexes displaying PTM-modified histone tails promote nuclear accumulation, DNA release, transcription, and enhanced transfection. Moreover, our group has combined detailed nanostructure engineering with sophisticated cellular imaging to identify novel aspects in the cell biology framework regulating polyplex transport to the nucleus.

We have also focused on novel mechanisms to exploit nature's ability to harness extracellular matrix (ECM) proteins such as collagens to sequester and control delivery of bioactive nanostructures. Our specific approaches have capitalized on a class of peptides known as collagen-mimetic peptides, or CMPs, that have been recognized for their unique affinity for native collagen, which can be tailored through alterations in CMP amino acid sequence and molecular weight. CMPs incorporate themselves into the natural collagen triple helical structure via strand invasion, in a reversible process previously that has been used to modify extracted collagen *in vitro* and exclusively target remodeling collagen *in vivo.* In our studies, we employed a proline-rich CMP designed to act not only as an adjustable tether to regulate collagen-polyplex affinity, but also as an adhesive/endocytic ligand for polyplexes. The use of a collagen scaffold afforded our system structural support and innate bioactivity to encourage cellular ingrowth and proliferation, whereas altering the extent of the modification of our vector provided additional tunability to allow tailorable release for prolonged time periods. This CMP-based approach also consistently and fully maintained polyplex activity in the presence of serum for at least a week, whereas most bolus and substrate-mediated gene delivery approaches report rapid reductions within hours or a few days, and the level of transgene expression directly correlated with MMP-concentrations and the extent of collagen remodeling, demonstrating "on demand" release. The ability to tailor release over extended periods via physical attachments, combined with the ability to provide cell-trigger release and collagen-mediated uptake, make this approach very attractive for many applications in regenerative medicine.