

Genetically encoding light-responsive protein-polymers

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Enabling researchers and clinicians to control and manipulate biological agents in space and time is a defining challenge in biology, bioengineering, and medicine. In this context, light is a unique external regulatory element because it can be precisely controlled in location, timing and amplitude. An ideal methodology for encoding and fine-tuning light-responsive behavior in proteins should permit the exact positioning of multiple, small, reversibly photo-switchable groups in the primary sequence of the protein.

Of particular interest to us are two families of unstructured proteins-based biomaterials (PBBs), Elastin and Resilin Like Polypeptides (ELPs and RLPs), which are stimuli responsive, biocompatible biomolecules, that undergo a reversible soluble to insoluble, phase transition in a lower and upper critical transition temperature (LCST/UCST) respectively. Our hypothesis is that by incorporating photoswitchable unnatural amino acids (uAAs) in these PBB sequences, in a site-specific manner, we will also be able to genetically encode a light-based phase transition, enabling us to create polymer based photoswitches.

We have recently described the efficient and accurate genetic incorporation of several azobenzene derivatives as uAAs in proteins and PBPs, allowing for isothermal, reversible, light-mediated soluble-to-insoluble phase transition in these PBPs, with up to a 12 °C difference in the transition temperature upon cis-to-trans. These PBPs can also be designed as self-assembling amphiphilic diblocks, allowing for light-responsive nanostructures.

In similar methods, we evolved another orthogonal aminoacyl tRNA synthetase capable of efficient incorporation of a uAA that is based on a less studied photoswitch- arylazopyrazole (AAP), which exhibits superior properties for bi-directional photo-control. The incorporation of the AAP-uAA into ELPs yielded proteins capable of an isothermal, reversible, and robust light-mediated phase transition, which occurred faster (after only 1 min of light irradiation) and demonstrated a larger transition temperature difference (up to a 45 °C difference in the ELP transition temperature upon a cis-to-trans AAP isomerization) than similar azobenzene-containing ELPs.

We envision that proteins and PBBs with such tailored and tunable light-responsive behaviors could then be manipulated by light in both space and time to sequester or release biomolecules on demand, elucidate complex cellular behaviors, and to use as scaffolds for sophisticated extra- and intra-cellular factories.