Predicting Self-Assembly of Globular Protein-Polymer Bioconjugates

Aaron Huang

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Abstract

Globular proteins offer powerful solutions for addressing challenges in the fields of medicine, industry, defense, and energy. Enzymes perform reactions with high efficiency and specificity, allowing for minimal generation of undesired side products even while exhibiting rapid turnover—traits difficult to replicate in synthetic catalysts. These targets make proteins attractive tools for immobilization to form functional catalysts and sensors. Nevertheless, there are many challenges in creating these advanced materials. The activity of the protein must be retained, and control over the structure of the material is desirable. Protein-polymer block copolymers offer an attractive solution to these issues. These materials have been shown to self-assemble into ordered nanodomains while retaining protein activity. However, the phase behavior of these materials is not fully understood due to the complex nature of anisotropic interactions between the proteins.

Within this thesis, a method for creating highly-active thin-film catalysts from myoglobin-PNIPAM bioconjugates is established by flow-coating these materials onto solid supports and then cross-linking them with glutaraldehyde. These catalysts exhibit considerable stability and perform reactions 5-10 times more efficiently than catalysts formed using other common immobilization techniques. However, the self-assembly and structural control of this catalyst was observed to be poor, and it was hypothesized that the poor self-assembly relative to mCherry and EGFP systems could be a consequence of the protein shape. In order to probe the effect of protein shape on self-assembly, a panel of mCherry bioconjugates with differing conjugation sites was studied using small-angle x-ray scattering. The self-assembly behavior of these conjugation site variants was observed to be robust with only minor differences in phase boundaries and observed phases resulting from the changes in conjugation site. However, observed changes in the domain spacing signaled that modifications to conjugation site offer control over protein orientation within the domains.

Based on studies showing that polymer chemistry in bioconjugates has a significant effect on self-assembly, an attempt to quantify these protein-polymer interactions was made using contrast-variation small-angle neutron scattering on mCherry and polymer blends. This technique allows for decomposition of the scattering intensity into its component parts corresponding to correlations between the 3 different pairs of the 2 species in the blends. From this analysis, it was determined that the best ordering bioconjugates have primarily repulsive interactions that can be described using a depletion layer model. Lastly, the effect of protein properties was screened using a large library of bioconjugates made from 11 different proteins. The primary observed trend was that order increases as molecular weight increases, but a narrow region around 28-30 kDa was observed where bioconjugate ordering was significantly enhanced and additional nanostructures were accessible.

Thesis supervisor: Bradley D. Olsen
Title: Paul M. Cook Career Development Professor
Massachusetts Institute of Technology