Use of coiled-coil domains to direct the self-assembly of protein-polymer conjugates

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Protein-polymer conjugates have been demonstrated to greatly enhance protein activity and sensitivity in catalytic and biosensing applications. To achieve this enhanced activity, however, it is critical that the conjugates self-assemble into well-defined nanostructures containing regions of densely-packed proteins. While nearly all enzymes and binding proteins can be conjugated to a polymer, few of these conjugates display strong ordering due to the physical properties of the protein being poorly suited for self-assembly. Furthermore, these properties cannot be significantly altered without also affecting the protein's catalytic or binding capabilities. As such, there is a great need for simple and general protein modifications that can promote strong ordering as a conjugate.

In this study, strongly associating coiled-coil domains, supercoiled bundles of alpha helices wrapped around each other, are fused to the protein block of protein-polymer conjugates to promote self-assembly. One of the two constituent alpha helices of a heterodimeric coiled-coil is appended to either the *N*- or *C*-terminus of a binding protein previously demonstrated to exhibit weak ordering. Small-angle X-ray scattering (SAXS) measurements indicate that homogeneous solutions of the conjugates containing a single coiled-coil domain generally self-assemble as weakly as the unmodified binding protein. When two conjugates containing complementary coiled-coil sequences are combined in an equimolar ratio, though, well-defined nanostructures are formed with SAXS peak full width at half maximum (FWHM) values 2- to 4-fold smaller than those for the bare binding protein. The ordering quality of the coiled-coil fusion conjugates is also shown to be highly correlated with biosensing capabilities, and biosensors constructed from the most well-ordered conjugates display sensitivities over an order of magnitude greater than those made from conjugates lacking coiled-coil fusions.