

## ABSTRACT

*De novo* protein design represents an exciting opportunity to explore the conformational space unsampled by nature and develop novel protein folds and functionality. One of the current challenges in the protein design field is the design of proteins that change their conformation in response to environmental cues. Conformational change of proteins in response to chemical or physical signals is the underlying principle of many regulatory and transport mechanisms in biological systems. The ability to design proteins whose conformational state can be precisely and reversibly controlled would facilitate the development of smart bio-inspired materials or molecular machines tailored for specific applications. We explored metal-binding site design to engineer peptide-based conformational switches that assemble into a dimeric coiled-coil in response to the addition of Zn(II) ions. Coiled-coil dimers are present in many natural proteins and have been used to construct synthetic protein nanostructures. Firstly, we designed a peptide called SwitCCh that formed a parallel homodimeric coiled-coil in the presence of Zn(II) or low pH. The addition of Zn(II) promoted formation of a parallel homodimer with an increase in thermal stability by more than 30 °C. The peptide could be reversibly cycled between the coiled-coil and random conformation. Furthermore, the SwitCCh peptide was orthogonal to the previously developed coiled-coil dimer set, indicating it could be used for regulated self-assembly of coiled-coil based nanostructures and materials. We further advanced our work by utilizing metal-binding site design to render a previously designed orthogonal set of coiled-coil heterodimers Zn(II)-responsive. Circular dichroism spectroscopy and size exclusion chromatography coupled to multi-angle light scattering confirmed the designed peptides assembled into coiled-coil heterodimers only in the presence of Zn(II). Additionally, designed peptides also acted as pH switches, since low pH prevented coordination of Zn(II) and lead to disassembling of coiled-coils. Our results showed the incorporation of a metal binding site not only preserved orthogonality, but that it is also a viable strategy for increasing the size of orthogonal sets. The designed Zn(II)-responsive coiled-coils were used for the construction of a triangular fold, whose assembly and disassembly was under the control of Zn(II) ions, demonstrating the designed set could facilitate the development of coiled-coil protein cages with easily controllable folding and unfolding.