Computational Design of a light and Ca^{2+} switchable Miniprotein

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The design of metalloproteins has emerged as a valuable approach to customize their properties and broaden their functional capabilities by incorporating metal cofactors. Recent studies have shed light on the recognition of metal-ligand coordination geometries in proteins, highlighting their essential roles in biological reactions. In this study, we employed the Rosetta design structural modeling toolbox and sequence optimization techniques to achieve a motif-swapped dimer configuration of the indicator protein. We focused on predicting stable binding sites for calcium ions, which were further validated through molecular dynamics simulations. Our goal was to create a photo-switchable calcium indicator by combining an EF-hand motif with spiropyran, ensuring optimal metal binding. Using a screening and optimization process tailored specifically for calcium ion binding, we identified the CaBS.F candidate with a phenylalanine (Phe) sequence. Experimental characterization revealed its selective binding to Ca^{2+} ions, distinguishing it from Zn^{2+} and Mg^{2+}. The NMR structure of the designed hybrid closely matched the design model, as evidenced by similar root-mean-square deviations (RMSDs) across all atoms in the binding site.As computational and experimental techniques continue to advance, expanding our knowledge of metallopeptides structures and functions, there is a growing interest in de novo design and redesign of these peptides. The aim is to surpass the inherent diversity found in nature. Photoswitchable metallopeptides, which combine metal ions or complexes with photoresponsive properties, hold promise for applications in fields such as optoelectronics and biosensing. In summary, this study highlights the use of design strategies and molecular simulations to create a photo-switchable calcium indicator by incorporating an EF-hand motif and spiropyran. The optimized design demonstrated selective binding to calcium ions and showcased the potential for advancing the field of metallopeptide design beyond the constraints of natural diversity, with implications for optoelectronics and biosensing applications.