Design of a coiled-coil protein origami with controllable self-assembly

SpT-05-03

J. Aupič[I], F. Lapenta[I], Ž. Strmšek[I], R. Jerala[I,II]

[I]National Institute of Chemistry, Ljubljana, Slovenia, [II]EN-FIST Centre of Excellence, Ljubljana, Slovenia

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. Coiled-coil dimers are present in many natural proteins and have been used to construct synthetic protein nanostructures, such as coiled-coil protein origami (CCPO) cages. Design of coiled-coils with dynamic self-assembly, mediated by a selected environmental signal, would enable the construction of CCPO cages for encapsulation, targeted delivery or controlling the protein activity of packaged cargo. Here, we utilized metal-binding site design to engineer an orthogonal set of Zn(II)-responsive coiled-coil heterodimers. Circular dichroism (CD) 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) ions. Additionally, the designed peptides also acted as pH switches, since low pH prevented coordination of Zn(II) and led to coiled-coil disassembly. The designed set was employed for the construction of several single-chain triangular folds. CD and SAXS analysis demonstrated the designs' dynamic Zn(II)-dependent folding behaviour, indicating the developed coiled-coil set could be used for controlling the assembly and disassembly process of larger CCPO cages and other coiled-coil based nanostructures. Furthermore, we showed that by selecting the building blocks with the appropriate thermodynamic stability, the temperature range where Zn(II) ions exhibit the greatest effect on the equilibrium between the folded and unfolded state could be tuned according to the desired application.