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Controlling Self-Assembly of a Peptide-Based Material via Metal-Ion Induced Registry Shift

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Supporting Information

ABSTRACT: Peptide **TZ1C2** can populate two distinct orientations: a staggered (out-of-register) fibril and an aligned (in-register) coiled-coil trimer. The coordination of two cadmium ions induces a registry shift that results in a reversible transition between these structural forms. This process recapitulates the self-assembly mechanism of native protein fibrils in which a ligand binding event gates a reversible conformational transition between alternate forms of a folded peptide structure.

Sequence-specific biomolecules, i.e., proteins and nucleic acids, confer significant advantages as substrates for the construction of structurally complex supramolecular materials.[1−3] Foremost, the sequence−structure correlations that have been elucidated from physical analysis of native biomolecular structures provide a context for the rational design of novel materials.[4−6] However, self-assembly of most designed peptide-based materials occurs commensurately with protein folding. In contrast, native protein assemblies undergo reversible self-association due to subtle shifts in conformation that are propagated hierarchically. We describe herein a mechanism to control supramolecular assembly of a synthetic peptide-based material through registry selection. This process recapitulates the native mechanism of fibril assembly in which a ligand binding event gates a reversible conformational transition between alternate forms of a folded peptide structure.

Peptide **TZ1C2**, a 41-residue sequence comprising 6 heptad repeats (Figure 1), was derived from modification of a previously reported trimeric coiled-coil **TZ1**.[7] Sequence variants of peptide **TZ1** have been shown to self-assemble into high aspect-ratio helical fibrils, in which lateral registration between adjacent helical protomers is enforced through Coulombic interactions between charged residues at the *e*- and *g*-positions of the heptad repeats. Peptide **TZ1C2** differs from **TZ1** in that two cysteine residues were introduced in place of isoleucines at core *a*-positions within the second and fifth heptad sequences. The sulfhydryl groups of the cysteine residues have the latent capacity to serve as ligands for the coordination of metal ions.[8] However, in order to create effective binding sites, the cysteine residues should be oriented proximally across the helical interface, which requires an in-register alignment of helices within a trimeric coiled-coil structure.

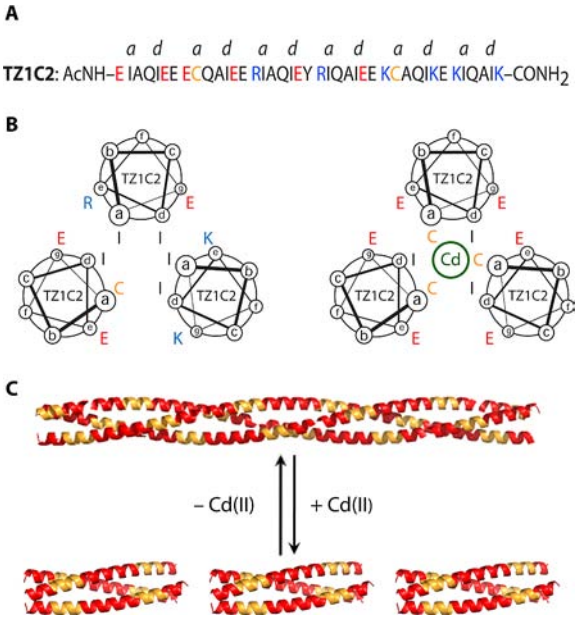

Figure 1. (A) Amino acid sequence of peptide **TZ1C2**. (B) Helical wheel representation of core layer packing within the second heptad of **TZ1C2** in the staggered (left) and aligned (right) orientations. (C) Schematic representation of the registry shift that results from Cd(II) coordination to the cysteine residues of **TZ1C2** (yellow: Cys-containing heptads and red: non-Cys-containing heptads).

Peptide **TZ1C2** can populate two distinct orientations within this structural context. The preferred orientation of helices depends on the presence of a metal ion, such as Cd(II), that can form a stable complex with the thiolate ligands of **TZ1C2**. Metal ion coordination may provide sufficient thermodynamic driving force to overcome electrostatic repulsion between residues at the *e*/*g*-positions and drive realignment of the structure (Figure 1). In the absence of metal coordination,

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electrostatic interactions and steric complementation[9] at core positions should favor the staggered alignment.[7] Thus, the balance of intermolecular forces can be manipulated through changes in environmental conditions to shift the helices reversibly from a staggered (out-of-register)[7,10,11] to aligned (in-register) orientation with a concomitant transition from a fibril to a discrete helical bundle.

The circular dichroism (CD) spectrum of **TZ1C2** (100 μM) in TAPS buffer was consistent with the presence of an α-helical conformation throughout the pH range from 6 to 9 (Figure 2).

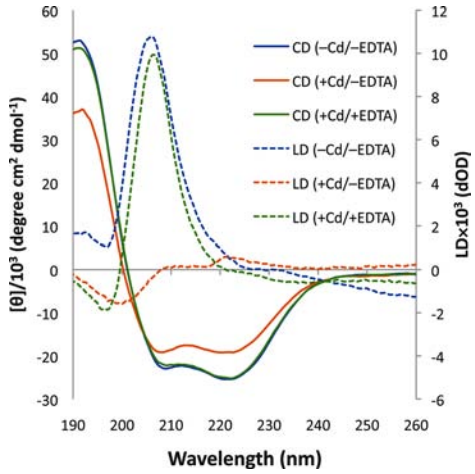

Figure 2. CD and flow LD spectra of peptide **TZ1C2** (100 μM) in TAPS buffer (10 mM, pH 8.5) and NaCl (100 mM).

Flow linear dichroism (LD) of an aqueous solution of **TZ1C2** (100 μM) in TAPS buffer (10 mM, pH 8.5) displays a strong positive signal at 206 nm under a Couette flow of 3000 rpm (Figure 2). The observed spectroscopic response indicated the presence of extended helical assemblies of **TZ1C2** that are stable to flow alignment. The flow LD data suggested a conformational arrangement of **TZ1C2** in which the amide bonds, and consequently, the α-helices are oriented parallel to the flow direction. Similar behavior has been observed in the flow LD spectra of synthetic coiled-coil fibers and tropomyosin under flow alignment.[12] TEM analysis of assemblies derived from **TZ1C2** confirmed the flow LD analysis. High aspect ratio fibrils are observed in negatively stained specimens of **TZ1C2** at concentrations $\geq$100 μM (Figure 3). The combined data support the hypothesis that **TZ1C2** self-assembles into α-helical fibrils in which the peptides adopt a parallel, out-of-register orientation as observed previously for other **TZ1** peptide derivatives.[7]

The scheme for disassembly of the **TZ1C2** fibrils requires the presence of a metal ion to induce a registry shift that orients the cysteine residues within the same layer of the triple helical coiled-coil (Figure 1). Previous investigations have demonstrated that the chalcophilic cadmium(II) ion can bind to structurally related coiled-coil trimers in which cysteines have been substituted into core *a*- or *d*-positions within the heptad repeats.[13−15] Cysteine residues were placed at the *a*-positions of the second and fifth heptad repeats of **TZ1C2**. Prior research had indicated a preference for Cd(II) complex formation at the trigonal binding sites at the *a*-positions of coiled-coil trimers.[16] In addition, cadmium has the advantage that complexation can be monitored using ^{113}Cd NMR spectroscopy[17] and ^{111m}Cd perturbed angular correlation (PAC) spectroscopy,[18] which are

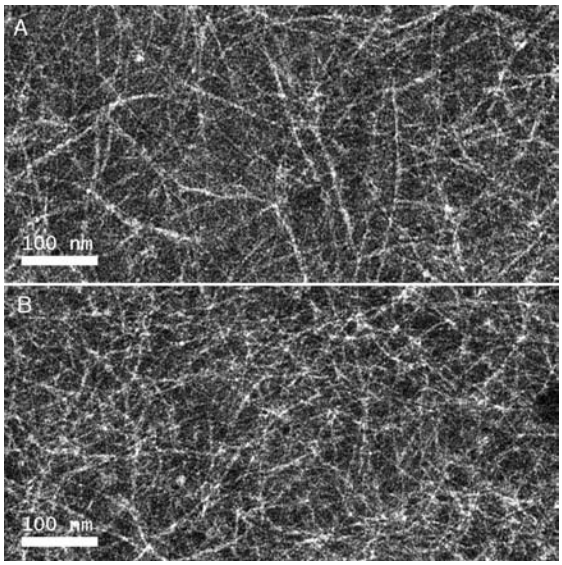

Figure 3. Negative-stain TEM images of **TZ1C2** fibrils (500 μM in TAPS buffer (10 mM, pH 8.5)) and NaCl (100 mM) before Cd(II) addition (A) and after Cd(II) addition followed treatment with excess EDTA (B).

sensitive tools for analysis of ligation environment and coordination geometry of metal ion-binding sites in proteins.[19]

Aqueous solutions of peptide **TZ1C2** (100 μM) were treated with 2 equiv of cadmium(II) nitrate (67 μM) in TAPS buffer (10 mM, pH 8.5, 100 mM NaCl). Each peptide contains two cysteine residues that contribute one-third of a cadmium(II) binding site within the trimeric coiled-coil structure. CD titration experiments suggested that this concentration of cadmium should be sufficient to saturate the two trivalent binding sites created within the trimeric coiled-coil assembly (see Supporting Information, SI). CD spectropolarimetry of Cd(II)-containing solutions of peptide **TZ1C2** indicated that the α-helical conformation was retained in the complex, although the net helicity was reduced with respect to the corresponding value in the absence of cadmium ion (Figure 2).

The flow LD spectrum of **TZ1C2** was significantly reduced in the presence of cadmium(II). The strong positive signal at 206 nm disappeared and was replaced with a weak negative signal at 200 nm (Figure 2). The spectroscopic behavior was consistent with disassembly of **TZ1C2** from extended fibrillar structures into smaller species that could not be aligned under Couette flow. Similarly, TEM analysis did not detect the formation of fibrils in aqueous solutions of **TZ1C2** in the presence of cadmium(II) (see SI). The disassembly was readily reversed in the presence of excess EDTA (200 μM), which can tightly bind to Cd(II) ion.[20] The addition of EDTA restored the original flow LD signal (Figure 2) and induced the formation of fibrils that were observed in the TEM analysis (Figure 3).

The oligomeric state of **TZ1C2** in solution was investigated in the presence and absence of Cd(II) ion by sedimentation velocity analytical ultracentrifugation.[21] The effect of Cd(II) ion was further examined as a function of **TZ1C2** concentration. When **TZ1C2** was measured under low loading concentration (160 μM) in the presence of Cd(II), a slightly heterogeneous sedimentation distribution with a weight average *s*-value of 1.68S was observed. Further analysis by genetic algorithm Monte Carlo[22] methods indicated the presence of a small

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amount of monomeric **TZ1C2** species, as well as a major trimeric species (42% of absorption), and a small amount of a larger oligomer consistent with a hexameric species. Under higher loading concentration (480 μM), the weight average *s*-value shifted slightly to 1.89S, and the relative amount of trimer increased to 62% of absorption at the expense of the other species. This observation is consistent with a reversible mass action effect. In the absence of Cd(II), the sedimentation distributions changed drastically, and 38% of the absorbance corresponded to a monomeric species at low loading concentration (140 μM), while the remaining absorbance sedimented as a heterogeneous mixture with *s*-values ranging between 3 and 7S, corresponding to highly anisotropic aggregates ranging between 0.4 and 1 million Da in size (Figure 4). The latter species are consistent with the fibrils

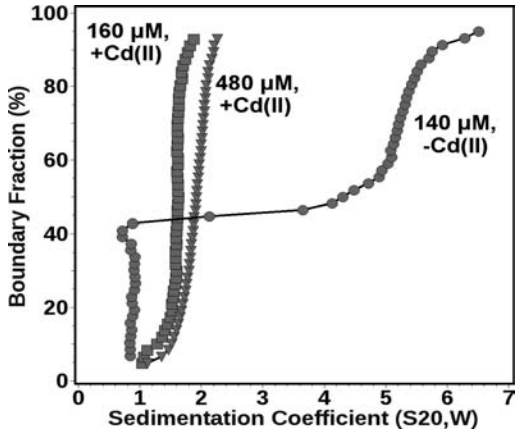

Figure 4. Diffusion-corrected integral sedimentation coefficient distributions obtained from a van Holde-Weischet[23] analysis. Shown are results for **TZ1C2** in the presence of excess Cd(II) at 160 μM (squares) and 480 μM (triangles) and in the absence of Cd(II) at 140 μM (circles) loading concentration.

observed in the TEM images of **TZ1C2**. From these data it can be concluded that Cd(II) facilitates the formation of reversibly self-associating trimer, while in the absence of Cd(II), **TZ1C2** is partially monomeric and partially forms much larger, irreversible aggregates. In addition, nondenaturing nano-electrospray ionization mass spectrometry confirmed the identity of the **TZ1C2** trimer as the dicadmium adduct (Figure S7).

The structural role of the Cd(II) ion in the **TZ1C2** assemblies was interrogated using a combination of ^{113}Cd NMR and ^{111m}Cd PAC spectroscopies (Figure 5) performed on isotopically enriched specimens. A single resonance was observed at 619 ppm in the ^{113}Cd NMR spectrum of an aqueous solution of peptide **TZ1C2** that had been treated with 2 equiv of $^{113}CdCl_2$. The chemical shift was consistent with nearly identical CdS_3X coordination environments for the two structurally similar metal ion binding sites in **TZ1C2**. The ^{113}Cd NMR spectroscopic data were similar to that reported for Cd-(*S*-cysteinyl)$_3$ complexes of structurally analogous coiled-coil trimers within the TRI series.[13,19] These spectroscopic results were interpreted in terms of a dynamic equilibrium on the NMR time-scale for a Cd(II) complex that involved three cysteinyl thiolate groups and a reversibly bound water molecule (X).

However, the ^{111m}Cd PAC spectroscopic data appear to refute a similar scenario for the cadmium(II) complex of

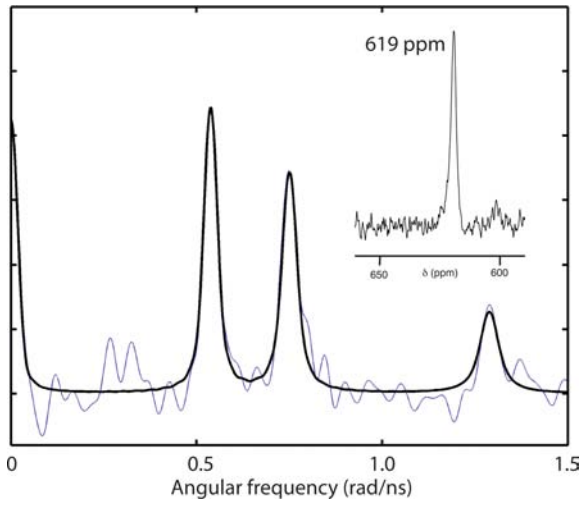

Figure 5. Fourier transformed ^{111m}Cd PAC spectroscopic data (blue: experimental data; black: fit) for the Cd(II) complex of **TZ1C2**. Inset: ^{113}Cd NMR spectrum of the Cd(II) complex of **TZ1C2** trimer.

TZ1C2. The ^{111m}Cd PAC spectrum of fully complexed **TZ1C2** gives a single very well-defined nuclear quadrupole interaction (NQI), indicating that all Cd(II) ions are found in practically the same coordination geometry, i.e., the two Cd(II) binding sites are highly similar (Figure 5). The NQI, and thus the local structure, is different from those observed for Cd(II) complexes of the TRI peptide family. Both the frequency (ω_0 ~0.399 rad/ns) and the asymmetry parameter (η ~0.61) are relatively high for the **TZ1C2** complex. The high-asymmetry parameter indicates the absence of axial symmetry, i.e., idealized trigonal planar CdS_3, and tetrahedral CdS_3X structures can be ruled out. The combined spectroscopic data suggest the presence of a single type of Cd(II) binding site, most likely of the CdS_3X type, in which the X-ligand is not in dynamic exchange on the time scales observable by the spectrocopic techniques. The deviation from an axially symmetric coordination environment may arise from structural distortions due to electrostatic repulsion between similarly charged *e*/*g*-residues at the Cd(II) coordination sites within the in-register coiled-coil trimer (Figure 1).

These data demonstrate that metal ion coordination can reversibly control chain registry and, consequently, the assembly state of a designed nanomaterial. A similar phenomenon has been observed adventitiously for β-sheet assemblies,[24,25] in which pH-dependent changes in strand registry can trigger reversible transitions between fibril and nanotube structures.[24] In addition, experimental evidence suggests that a registry shift between helices in an antiparallel coiled-coil dimer within the cytoskeletal motor protein dynein may underlie the mechanism of locomotion.[26] Thus, control of chain alignment represents an attractive strategy for the design of dynamically reconfigurable nanoscale materials.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental methods of peptide synthesis and characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Hauser, C. A.; Zhang, S. *Chem. Soc. Rev.* **2010**, *39*, 2780−90.

(2) Woolfson, D. N.; Mahmoud, Z. N. *Chem. Soc. Rev.* **2010**, *39*, 3464−79.

(3) Tørring, T.; Voigt, N. V.; Nangreave, J.; Yan, H.; Gothelf, K. V. *Chem. Soc. Rev.* **2011**, *40*, 5636−46.

(4) King, N. P.; Sheffler, W.; Sawaya, M. R.; Vollmar, B. S.; Sumida, J. P.; André, I.; Gonen, T.; Yeates, T. O.; Baker, D. *Science* **2012**, *336*, 1171−4.

(5) Lanci, C. J.; MacDermaid, C. M.; Kang, S. G.; Acharya, R.; North, B.; Yang, X.; Qiu, X. J.; DeGrado, W. F.; Saven, J. G. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 7304−9.

(6) Brodin, J.; Ambroggio, X.; Tang, C.; Parent, K.; Baker, T.; Tezcan, F. A. *Nat. Chem.* **2012**, *4*, 375−382.

(7) (a) Zimenkov, Y.; Dublin, S. N.; Ni, R.; Tu, R. S.; Breedveld, V.; Apkarian, R. P.; Conticello, V. P. *J. Am. Chem. Soc.* **2006**, *128*, 6770−1. (b) Dublin, S. N.; Conticello, V. P. *J. Am. Chem. Soc.* **2008**, *130*, 49−51.

(8) Peacock, A. F. A.; Iranzo, O.; Pecoraro, V. L. *Dalton Trans.* **2009**, 2271−80.

(9) Schnarr, N. A.; Kennan, A. J. *J. Am. Chem. Soc.* **2002**, *124*, 9779−83.

(10) Potekhin, S. A.; Melnik, T. N.; Popov, V.; Lanina, N. F.; Vazina, A. A.; Rigler, P.; Verdini, A. S.; Corradin, G.; Kajava, A. V. *Chem. Biol.* **2001**, *8*, 1025−32.

(11) Papapostolou, D.; Smith, A. M.; Atkins, E. D.; Oliver, S. J.; Ryadnov, M. G.; Serpell, L. C.; Woolfson, D. N. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 10853−8.

(12) Bulheller, B. M.; Rodger, A.; Hicks, M. R.; Dafforn, T. R.; Serpell, L. C.; Marshall, K. E.; Bromley, E. H.; King, P. J.; Channon, K. J.; Woolfson, D. N.; Hirst, J. D. *J. Am. Chem. Soc.* **2009**, *131*, 13305−14.

(13) Matzapetakis, M.; Farrer, B. T.; Weng, T. C.; Hemmingsen, L.; Penner-Hahn, J. E.; Pecoraro, V. L. *J. Am. Chem. Soc.* **2002**, *124*, 8042−54.

(14) Li, X.; Suzuki, K.; Kanaori, K.; Tajima, K.; Kashiwada, A.; Hiroaki, H.; Kohda, D.; Tanaka, T. *Protein Sci.* **2000**, *9*, 1327−33.

(15) Kharenko, O. A.; Ogawa, M. Y. *J. Inorg. Biochem.* **2004**, *98*, 1971−4.

(16) Matzapetakis, M.; Pecoraro, V. L. *J. Am. Chem. Soc.* **2005**, *127*, 18229−33.

(17) Hemmingsen, L.; Olsen, L.; Antony, J.; Sauer, S. P. *J. Biol. Inorg. Chem.* **2004**, *9*, 591−9.

(18) Hemmingsen, L.; Sas, K. N.; Danielsen, E. *Chem. Rev.* **2004**, *104*, 4027−62.

(19) Iranzo, O.; Jakusch, T.; Lee, K. H.; Hemmingsen, L.; Pecoraro, V. L. *Chemistry* **2009**, *15*, 3761−72.

(20) Andersen, O. *Environ. Health Perspect.* **1984**, *54*, 249−66.

(21) Demeler, B. *Current Protocols in Protein Science*; Wiley: Hoboken, NJ, 2010; unit 7.13, Chapter 7.

(22) (a) Brookes, E.; Demeler, B. *GECCO '07 Proceedings of the 9th annual conference on Genetic and evolutionary computation*; ACM: New York, p 361−368. (b) Demeler, B.; Brookes, E. *Colloid Polym. Sci.* **2008**, *286*, 129−137.

(23) Demeler, B.; van Holde, K. E. *Anal. Biochem.* **2004**, *335*, 279−288.

(24) (a) Liang, Y.; Pingali, S. V.; Jogalekar, A. S.; Snyder, J. P.; Thiyagarajan, P.; Lynn, D. G. *Biochemistry* **2008**, *47*, 10018−26. (b) Mehta, A. K.; Lu, K.; Childers, W. S.; Liang, Y.; Dublin, S.; Dong, J.; Snyder, J. P.; Skanthakumar, S.; Thiyagerajan, P.; Lynn, D. G. *J. Am. Chem. Soc.* **2008**, *130*, 9829−35.

(25) Petkova, A. T.; Buntkowsky, G.; Dyda, F.; Leapman, R. D.; Yau, W. M.; Tycko, R. *J. Mol. Biol.* **2004**, *335*, 247−60.

(26) Kon, T.; Imamula, K.; Roberts, A. J.; Ohkura, R.; Knight, P. J.; Gibbons, I. R.; Burgess, S. A.; Sutoh, K. *Nat. Struct. Mol. Biol.* **2009**, *16*, 325−33.