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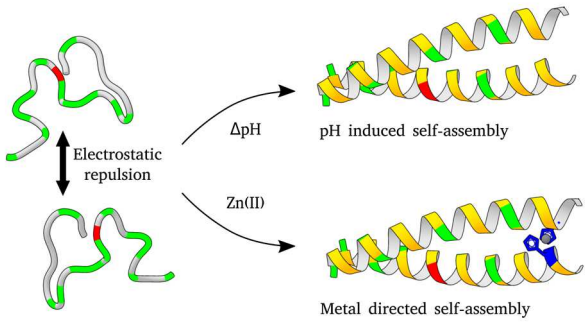
SwitCCh: Metal-site design for controlling the assembly of a coiled-coil homodimer

Jana Aupič, Fabio Lapenta, and Roman Jerala*

Abstract: 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 molecular machines tailored for specific applications. Here we explore metal-binding site design to engineer a peptide-based conformational switch called SwitCCh that assembles into a homodimeric coiled-coil in response to the addition of Zn(II) ions or low pH. 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.

One of the current challenges in the protein design field is the design of proteins that change their conformation in response to environmental cues.[1,2] Coiled-coils (CC), characterized by the heptad repeat $(abcdefg)_n$, are useful scaffolds for engineering conformational flexibility, since the free energy difference between different oligomeric states can be very low.[3] Hitherto, CC assemblies responsive to pH,[4–6] metal ions,[7–13] light,[14] redox conditions,[15] hydrophobic ligands[16] or phosphorylation[17] have been successfully designed. Additionally, CC strand displacement using peptides with different affinities to guide the exchange of CC assemblies has recently been presented.[18] Metal ions are particularly suitable for controlling conformational changes due to their ubiquitous role in various biological systems.[19] Zinc is one of the most prevalent protein binding metals as it is found in more than half of all known human metalloproteins.[20] Due to the high occurrence of Zn(II) binding sites in proteins, the preferred binding residues and coordination geometry are well understood,[21,22] which enabled elucidation of principles for designing metalloproteins.[23] While helical bundles[7] or peptides comprising non-canonical amino acids[8] have been previously made responsive to Zn(II) ions, the design of Zn(II)-dependent CC dimers that could be biologically encoded and therefore used *in-vivo* remained a challenge. Recently, orthogonal sets of CC dimers[24–26] have been utilized to construct complex protein assemblies.[24,27–29] Ligand-mediated assembly of CC dimers would be therefore of considerable interest for various biotechnological applications such as sensing, encapsulation and drug delivery. Here we present the design of a CC dimer-based conformational switch whose assembly is mediated by metal ions as well as by pH (Scheme 1), and is furthermore orthogonal to the existing heterodimeric CC set used for the construction of coiled-coil protein origami (CCPO) cages.[26,30]



Scheme 1. Scheme of the designed conformational switching.

For the design of metal ion chelating site the geometry of Zn(II) binding sites in natural metalloproteins was taken into account. Zinc ions are generally coordinated by 4 residues, most often Cys and His, in a tetrahedral arrangement around the metal center,[22] where the distance between the coordinating atoms and Zn(II) is approximately 2 Å.[21,22] A minimal Zn(II) binding site, on the other hand, comprises two amino acid residues, with His as the prevalent ligand.[31] As target metal sites we chose $ZnCys_4$, a common zinc binding site, and the minimal $ZnHis_2$ motif.[31] Peptides P11SN and P9SN from the orthogonal CC set were chosen as scaffolds for the target $ZnCys_4$ and $ZnHis_2$ metal sites, respectively.[29] To maintain orthogonality to the peptides in the CC set, the introduced mutations were restricted to a small number of interacting residue positions. Introduction of a functional Zn(II) regulatory site into a CC dimer requires a net repulsion between the peptide chains in the absence of metal ions that prevents dimerization. Under neutral pH conditions P9SN and P11SN do not form homodimers, due to electrostatic repulsion between amino acids at *e* and *g* heptad position (Figure 1A). However, in the case of P9SN, low pH values neutralize the negative charge of Glu residues, resulting in the assembly of a CC homodimer (Figure 1B).

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Initially, the $ZnCys_4$ regulatory site was designed into the P11SN peptide by placing two Cys residues at *g* and *a* positions of the third heptad repeat, since this arrangement of residues matched best the structure of $ZnCys_4$ sites observed in protein crystal structures (Figure S1). Experimental analysis revealed that the designed peptide folded under acidic conditions but was however completely unresponsive to Zn(II) ions (Figure S1, Table S1), therefore the design had to be modified.

Analysis of Cα-Cα distances between coordinating His residues in protein crystal structures containing the $ZnHis_2$ metal site,[31] identified the geometric arrangement of residues at *e* or *g* heptad positions in the P9SN peptide as the most suitable for the introduction of chelating His residues (Figure 1C, 1D). The mutation was inserted at *g* position of the C-terminal heptad, since CC termini exhibit greater backbone flexibility,[32] allowing the His residues to adopt a favorable coordination geometry. Additionally, Lys8 residue at the second heptad *g* position was mutated into a Glu, in order to decrease the electrostatic repulsion between the central heptads and tune the stability of the dimer at neutral pH. Since only two *e-g* positions were mutated, the resulting peptide, named SwitCCh (Figure 1E), was expected to retain the pH responsiveness of its parent peptide P9SN. Comparison of the SwitCCh dimer model structures with helices in either parallel or antiparallel orientation suggested the former as energetically more favourable[33] (Figure S2). The design was further validated by a 30 ns molecular dynamics (MD) simulation of the CC-Zn(II) complex. The complex remained stable with an average Zn-N distance of 2.2±0.1 Å (Figure 1F, 1G). During MD simulation, the structural agreement of the designed binding site with respect to $ZnHis_2$ sites observed in protein crystal structures increased, with the average RMSD decreasing from 2.8±0.2Å for the initial model to 1.9±0.3 Å (Figure 1H).

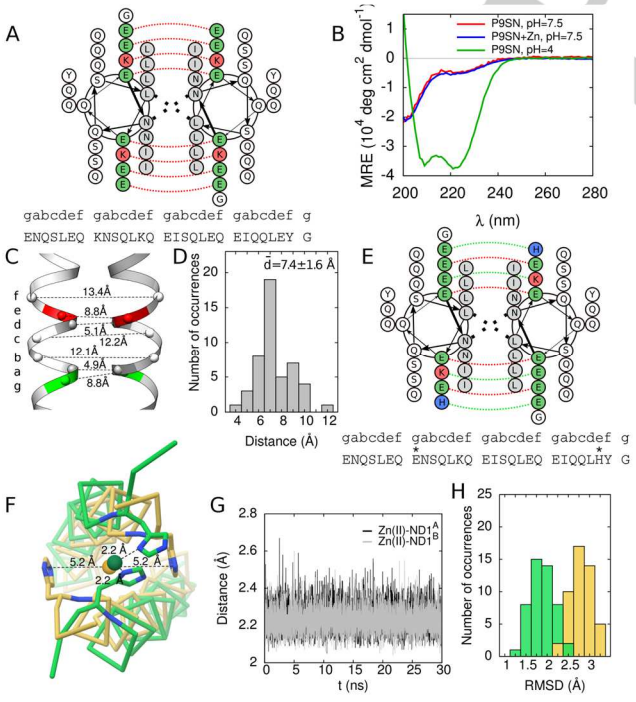

Figure 1. Rational re-design of an orthogonal CC peptide for conformational switching. (A) Helical wheel representation and sequence of the CC peptide P9SN serving as the basis for the design. (B) Circular dichroism (CD) spectra of P9SN peptide under different solution conditions. (C) Cα-Cα distances between matching heptad repeat positions in the P9SN homodimer model. (D) Cα-Cα distances between Zn(II) coordinating residues observed in crystal structures of proteins containing the $ZnHis_2$ site. (E) Helical wheel representation and sequence of the designed SwitCCh peptide. The modifications of the original sequence are marked with an asterisk. (F) Close-up of the Zn(II) binding site after the 30 ns MD simulation (green), superimposed on the initial ISAMBARD-generated model (yellow). (G) Distance between the Zn(II) ion and ND1 His atoms during MD simulation. (H) Distribution of the binding site RMSDs before (yellow) and after (green) MD simulation with respect to $ZnHis_2$ sites found in protein crystal structures. RMSD was calculated over His residues.

The secondary structure of the SwitCCh peptide under different solution conditions was studied by circular dichroism (CD). The CD spectrum of the SwitCCh peptide at pH 7.5 corresponded to the random coil state (Figure 2A). After introducing Zn(II) into the solution two minima at 208 and 222 nm appeared in the CD spectrum, indicating the formation of a CC structure (Figure 2A). An excess of Zn(II) was needed to complete the transition to the CC state (Figure 2B). K_D for binding of Zn(II) to the SwitCCh CC homodimer in 1:1 stoichiometry was 400±10 µM (Figure 2B inset). The amount of helical secondary structure at final Zn(II) concentration was estimated at 64%. As expected, the CD spectrum of the peptide under the acidic conditions confirmed CC formation (Figure 2A) like in the case of P9SN. The helical content of P9SN at pH 4 (99%) was higher in comparison to SwitCCh peptide (84%). This is most likely due to the decrease in the number of Glu-Glu interactions in the re-design. Monitoring the CD spectrum as a function of pH revealed SwitCCh has almost a digital response; the transition occurred within a narrow pH range, spanning approximately 1 pH unit, with a transition midpoint around pH 5 (Figure 2C). Thermal denaturation scans showed a 30 °C increase in the melting temperature, T_m, of SwitCCh upon addition of Zn(II) or decrease in pH (Figure 2D).

Size exclusion chromatography coupled to multi angle light scattering (SEC-MALS) measurements of the SwitCCh peptide gave a molecular weight consistent with the monomeric state at neutral pH (Figure 2E), while in the presence of Zn(II) (Figure 2F) or at pH 4 (Figure 2G) the measured mass corresponded to a dimer. Dissociation constants of the CC assembly in different solution conditions were quantitatively examined using dilution isothermal titration calorimetry (ITC) experiments (Figure S3). Our data indicated the SwitCCh CC assembly is stabilized more strongly by low pH than by Zn(II) (K_D 2 µM and 10 µM, respectively). This is consistent with CD measurements, where SwitCCh exhibited higher helical content under acidic condition than in the presence of Zn(II) and a slightly higher T_m. The lower helical content and thermodynamic stability of SwitCCh-Zn(II) complex is most likely due to remaining unfavorable electrostatic interactions between Glu residues. No heat effects were observed at pH 7.5 in the absence of Zn(II).

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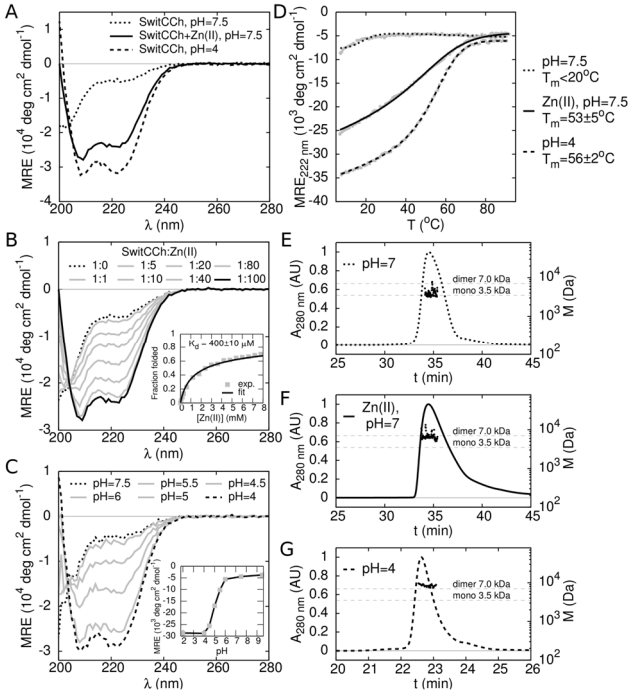

Figure 2. Secondary structure, stability and oligomeric state of the SwitCCh peptide in response to Zn(II) ions and pH. (A) CD spectra of SwitCCh under different solution conditions. The CD spectrum in the presence of Zn(II) ions corresponds to SwitCCh:Zn(II) molar ration of 1:100. (B) Effect of increasing Zn(II) concentration on SwitCCh CD spectrum; (inset) Fraction of the SwitCCh in the folded state as a function of Zn(II) concentration. (C) Effect of pH on SwitCCh CD spectrum; (inset) MRE at 222 nm as a function of pH. (D) Temperature denaturation profiles for SwitCCh under different solution conditions. Dots mark experimental data, while lines represent model-fit. SwitCCh:Zn(II) molar ratio is as in panel A. (E, F, G) SEC-MALS chromatographs for SwitCCh under different solution conditions. Black dots represent molecular weight, while dashed grey lines mark the masses expected for the monomeric and dimeric state.

Next, the selectivity of the SwitCCh peptide for metal-ion binding was investigated (Figure 3A). Among the investigated metals, only Cu(II) induced CC formation (86% helical content). Thermal stability of SwitCCh in the presence of Cu(II) was slightly lower than in the presence of Zn(II) with T_m=44±4 °C (Figure 3B). The K_D for binding of Cu(II) was 64±2 µM per binding site, where the best fit was obtained for Cu(II):CC dimer binding ratio of 4:1 (Figure 3C). The K_D of the SwitCCh homodimer in the presence of Cu(II) was 1 µM (Figure 3D). The observed lack of discrimination between Zn(II) and Cu(II) might be due to greater coordinational flexibility of Cu(II) in comparison to other metal ions, which allows Cu(II) to bind in various coordination geometries.[34] However, the observed difference in the stoichiometry of binding indicates the mechanisms by which Zn(II) and Cu(II) induce self-assembly of the SwitCCh peptide differ. Additionally, at higher temperatures and concentrations Cu(II) often led to aggregation of the SwitCCh peptide (Figure S4). For this reason, and due to it being less appropriate for application in biological systems, we did not proceed with Cu(II) in further experiments.

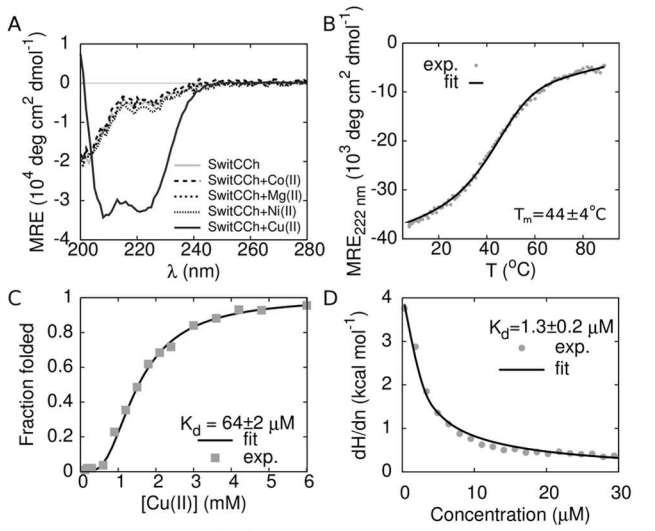

Figure 3. Effect of selected divalent metal ions on the secondary structure of the SwitCCh peptide. (A) CD spectra of SwitCCh at neutral pH and in the presence of selected metal ions. (B) Temperature denaturation profile for SwitCCh in the presence of Cu(II). (C) Fraction of the peptide in the folded state as a function of Cu(II) concentration. (D) Heat effects during micro calorimetric titration of the SwitCCh-Cu(II) complex into matching buffer.

The orientation of individual peptide chains in the CC assembly was assessed by examining concentration dependence of the T_m. For these experiments, we used a variant of the SwitCCh peptide with an additional Cys residue at the C terminus, which allowed covalent linking of the two peptide chains via disulfide bond (Table S1). T_m of the disulfide-linked SwitCCh assembly was not affected by its concentration, corroborating the parallel chain orientation[25,35] (Figure 4A, Figure S5). In contrast, T_m of SwitCCh in the absence of crosslinking strongly depended on its concentration (Figure 4A, Figure S5).

Additionally, the reversibility of Zn(II)-induced structuralization was investigated (Figure 4B). Adding an equimolar amount of EDTA, a strong chelator of Zn(II), to the solution of SwitCCh-Zn(II) complex, caused the minima at 208 and 222 nm to disappear from the CD spectra, indicating a return to the random coil state (Figure S6). Introducing additional Zn(II) into the same solution led to helicity returning, rather remarkably, to the original value observed before the addition of EDTA. The switching between the monomeric and CC state could be performed without significant losses for three full cycles, after which the amplitude of the response decreased, yet clearly remained observable. This gradual loss of reversibility was likely due to the accumulation of the metal ion chelator in solution upon serial addition of the same amount of each reagent, as the experiments were performed without removal of metal ions and EDTA or increasing amounts of added reagents.

Finally, the orthogonality of the SwitCCh peptide to the previously published orthogonal CC peptide set most frequently used in designed protein cage structures (CCPOs)[26,29] was examined (Figure 4C). At pH 7.5 the SwitCCh peptide did not interact with any of the peptides comprising the set (Figure 4D). Addition of Zn(II) to the peptide solutions did not interfere with the

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orthogonality of the CC set, while the SwitCCh peptide formed only homodimers and did not interact with any of the set members (Figure 4E). At pH 4 the orthogonality of the set is disrupted, since it is strongly based on electrostatic complementarity. Therefore orthogonality of the SwitCCh peptide under acidic conditions is not relevant.

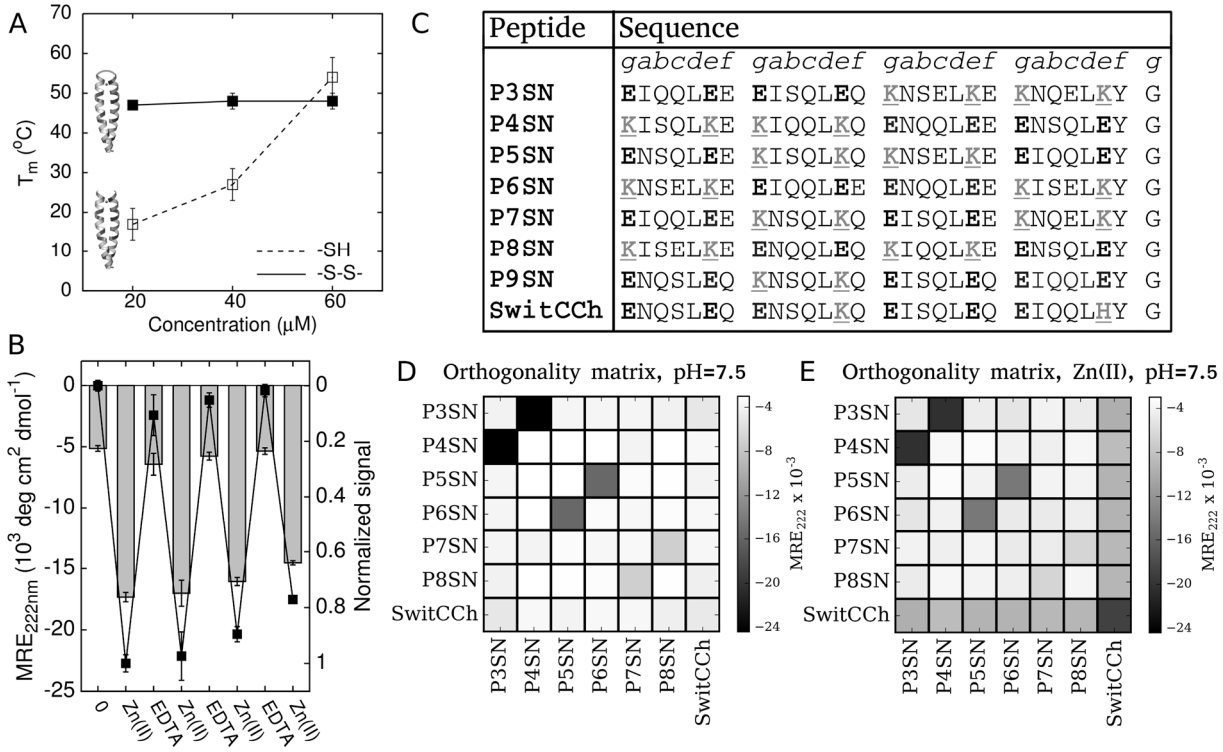

Figure 4. Reversibility and orthogonality properties of the SwitCCh peptide. (A) Effect of peptide concentration on the melting temperature of the covalently (filled squares) and non-covalently (hollow squares) linked assembly in the presence of Zn(II). (B) CD signal at 222 nm after consecutive additions of Zn(II) and EDTA to solution of the SwitCCh peptide. Bars depict MRE_{222nm}, while squares correspond to normalized signal. (C) Amino acid sequence of the negatively supercharged orthogonal CC set (P3SN-P8SN), P9SN and the designed SwitCCh peptide. (D, E) Orthogonality matrix under physiological pH conditions and in the presence of Zn(II).

In summary, through rational metal-site design a two-state metal ion-regulated conformational CC dimer switch has been constructed. Engineering metal binding sites represents an additional layer of design that can be included into the selected scaffold with minimal sequence perturbation. The small number of required mutations showcases the fine interplay between hydrophobic and electrostatic interactions in CC dimers. More generally, our results demonstrate SwitCCh peptide could be used as a versatile tool in polymer and biomimetic chemistry, e.g. for biosensing or controlling protein activity, as a model system for studying biologically relevant molecular mechanisms, or for regulating the assembly of protein nanostructures or bio-inspired materials.

Experimental section

Experimental details are provided as part of supporting information.

Acknowledgements

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Keywords: protein design • coiled-coil • metalloproteins• self-assembly• conformational switching

[1] V. Stein, K. Alexandrov, *Trends Biotechnol.* **2015**, *33*, 101–110.
[2] X. I. Ambroggio, B. Kuhlman, *Curr. Opin. Struct. Biol.* **2006**, *16*, 525–530.
[3] A. R. Thomson, C. W. Wood, A. J. Burton, G. J. Bartlett, R. B. Sessions, R. L. Brady, D. N. Woolfson, *Science* **2014**, *346*, 485–488.
[4] R. Lizatović, O. Aurelius, O. Stenström, T. Drakenberg, M. Akke, D. T. Logan, I. André, *Structure* **2016**, *24*, 946–955.

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[5] C. Minelli, J. X. Liew, M. Muthu, H. Andresen, *Soft Matter* **2013**, *9*, 5119–5124.
[6] K. Pagel, S. C. Wagner, K. Samedov, H. Von Berlepsch, C. Böttcher, B. Koksch, *J. Am. Chem. Soc.* **2006**, *128*, 2196–2197.
[7] K. Suzuki, H. Hiroaki, D. Kohda, H. Nakamura, T. Tanaka, *J. Am. Chem. Soc.* **1998**, *120*, 13008–13015.
[8] W. D. Kohn, C. M. Kay, B. D. Sykes, R. S. Hodges, *J. Am. Chem. Soc.* **1998**, *120*, 1124–1132.
[9] K. Pagel, T. Seri, H. von Berlepsch, J. Griebel, R. Kirmse, C. Böttcher, B. Koksch, *ChemBioChem* **2008**, *9*, 531–536.
[10] X. I. Ambroggio, B. Kuhlman, *J. Am. Chem. Soc.* **2006**, *128*, 1154–1161.
[11] M. R. Berwick, D. J. Lewis, A. W. Jones, R. A. Parslow, T. R. Dafforn, H. J. Cooper, J. Wilkie, Z. Pikramenou, M. M. Britton, A. F. A. Peacock, *J. Am. Chem. Soc.* **2014**, *136*, 1166–1169.
[12] G. R. Dieckmann, D. K. MoRorie, D. L. Tierney, L. M. Utschig, C. P. Singer, T. V. O'Halloran, J. E. Penner-Hahn, W. F. DeGrado, V. L. Pecoraro, *J. Am. Chem. Soc.* **1997**, *119*, 6195–6196.
[13] M. L. Zastrow, V. L. Pecoraro, *Coord. Chem. Rev.* **2013**, *257*, 2565–2588.
[14] A. M. Ali, M. W. Forbes, G. A. Woolley, *ChemBioChem* **2015**, *16*, 1757–1763.
[15] G. P. Dado, S. H. Gellman, *J. Am. Chem. Soc.* **1993**, *115*, 12609–12610.
[16] L. Gonzalez, J. J. Plecs, T. Alber, *Nat. Struct. Biol.* **1996**, *3*, 510–515.
[17] L. Szilák, J. Moitra, D. Krylov, C. Vinson, *Nat. Struct. Mol. Biol.* **1997**, *4*, 112–114.
[18] K. Gröger, G. Gavins, O. Seitz, *Angew. Chemie - Int. Ed.* **2017**, *56*, 14217–14221.
[19] A. Galdes, B. L. Vallee in *Metal Ions in Biological Systems: Volume 15: Zinc and Its Role in Biology and Nutrition* (Ed.: H. Sigel), CRC Press, New York, 1983, pp. 1-54.
[20] A. Azia, R. Levy, R. Unger, M. Edelman, V. Sobolev, *Proteins Struct. Funct. Bioinforma.* **2015**, *83*, 931–939.
[21] D. S. Auld, *BioMetals* **2001**, *14*, 271–313.
[22] I. L. Alberts, K. Nadassy, S. J. Wodak, *Protein Sci.* **1998**, *7*, 1700–1716.
[23] S. L. Guffy, B. S. Der, B. Kuhlman, *Protein Eng. Des. Sel.* **2016**, *29*, 327–338.
[24] L. S. R. Small, M. Bruning, A. R. Thomson, A. L. Boyle, R. B. Davies, P. M. G. Curmi, N. R. Forde, H. Linke, D. N. Woolfson, E. H. C. Bromley, *ACS Synth. Biol.* **2017**, *6*, 1096–1102.
[25] K. E. Thompson, C. J. Bashor, W. A. Lim, A. E. Keating, *ACS Synth. Biol.* **2012**, *1*, 118–129.
[26] H. Gradišar, R. Jerala, *J. Pept. Sci.* **2011**, *17*, 100–106.
[27] W. M. Park, M. Bedewy, K. K. Berggren, A. E. Keating, *Sci. Rep.* **2017**, *7*.
[28] H. Gradišar, S. Božič, T. Doles, D. Vengust, I. Hafner-Bratkovič, A. Mertelj, B. Webb, A. Šali, S. Klavžar, R. Jerala, *Nat. Chem. Biol.* **2013**, *9*, 362–6.
[29] A. Ljubetič, F. Lapenta, H. Gradišar, I. Drobnak, J. Aupič, Ž. Strmšek, D. Lainšček, I. Hafner-Bratkovič, A. Majerle, N. Krivec, et al., *Nat. Biotechnol.* **2017**, *35*, 1094–1101.
[30] I. Drobnak, H. Gradišar, A. Ljubetič, E. Merljak, R. Jerala, *J. Am. Chem. Soc.* **2017**, *139*, 8229–8236.
[31] C. Andreini, G. Cavallaro, S. Lorenzini, A. Rosato, *Nucleic Acids Res.* **2012**, *41*, D312–D319.
[32] J. M. Fletcher, G. J. Bartlett, A. L. Boyle, J. J. Danon, L. E. Rush, A. N. Lupas, D. N. Woolfson, *ACS Chem. Biol.* **2017**, *12*, 528–538.
[33] C. W. Wood, J. W. Heal, A. R. Thomson, G. J. Bartlett, A. Ibarra, R. L. Brady, R. B. Sessions, D. N. Woolfson, *Bioinformatics* **2017**, *33*, 3043–3050.
[34] M. M. Harding, *Acta Crystallogr. Sect. D Biol. Crystallogr.* **2001**, *57*, 401–411.
[35] E. K. O'Shea, R. Rutkowski, P. S. Kim, *Science* **1989**, *243*, 538–542.

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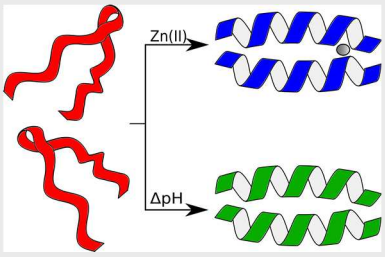
Entry for the Table of Contents
Layout 1:

COMMUNICATION

Zinc control to major coil: Metal site engineering is exploited to design a peptide that self-assembles into a CC dimer in response to Zn(II) ions or low pH. The monomer-dimer conformational switching is reversible and can be performed for several cycles, highlighting the peptide's potential for development of synthetic molecular machines.



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