JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Communication
pubs.acs.org/JACS

Supramolecular Metal-Coordination Polymers, Nets, and Frameworks from Synthetic Coiled-Coil Peptides

Nathan A. Tavenor, Matthew J. Murnin, and W. Seth Horne*

Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, United States

Supporting Information

ABSTRACT: Metal coordination and peptide-directed self-assembly are two proven methods for creating defined supramolecular architectures. Here, we report a new class of crystalline materials based on coiled-coil peptides bearing unnatural metal-chelating terpyridine moieties. High-resolution structural characterization of lattices formed in the presence of Cu^{2+} reveals a general assembly mechanism. Subtle sequence variation in the modular synthetic ligand dictates assembly morphology.

The power of metal coordination to drive molecular assembly has motivated 2 decades of research on metal organic frameworks (MOFs).[1] In that time, the structural modularity of simple organic ligands has been utilized to produce diverse crystalline materials with tunable structures and properties. The functional versatility resulting from this level of control has led to MOF applications in areas such as catalysis, gas capture, and sensing.[2]

Peptides are another flexible starting point for creating ordered supramolecular assemblies.[3] Careful design of amino acid sequence can yield complex architectures held together via specific contacts made possible by the folded structure of the underlying building blocks.[4] MOFs and peptide-based materials both rely on a modular building unit and the marshalling of predictable noncovalent forces to drive assembly. Fertile ground exists at the intersection between these areas, where metal coordination and peptide folding work in concert to create ordered supramolecular materials.

Metals enjoy a rich history in protein design,[5] and recent efforts have expanded their application to form larger biomolecular architectures.[6] Pioneering work has shown that expressed proteins bearing metal-coordinating side chains[7] or incorporating such groups by binding a cognate small-molecule ligand[8] can form highly ordered lattices. Here, folding is a key factor in dictating supramolecular morphology, and maturing design rules in these systems are approaching those for small organic ligands in precision and sophistication.[9] Metal coordination has also been used for the supramolecular organization of shorter synthetic peptides that form defined quaternary structures (collagen[10] and α-helical coiled-coil[11]). These systems are notable for coupling peptide-directed association and metal binding as orthogonal assembly forces[12] in a fully synthetic ligand. Coiled coils are intriguing in this context, as defined folds can be specified in as few as 20 residues, stability and stoichiometry are readily tunable, and the scaffolds are highly amenable to modification.[13] The above features have led to the widespread use of coiled coils in peptide-based materials.[14]

An important consideration in peptide-based coordination framework design is selection of metal-binding group(s). Canonical amino acids have been used to great effect, but artificial moieties are a powerful alternative.[6] Although widely employed in small-molecule supramolecular systems,[15] 2,2′:6′,2″-terpyridine has found limited application in protein-based materials.[6,8,11a]

The structural versatility of coiled-coil folding and terpyridine metal binding motivated us to explore systems combining both. Our central hypothesis was that peptide self-assembly and terpyridine-directed metal coordination could work in concert to create ordered supramolecular architectures. We further reasoned that subtle sequence changes that alter coiled-coil symmetry and valency with respect to metal-binding groups could control assembly structure. Here, we report the fruits of efforts to test this hypothesis. The results obtained suggest a general approach to highly ordered, structurally tunable supramolecular metal-peptide materials.

After a series of exploratory studies (see Supporting Information (SI) for details), we arrived at peptides **1**−**3** as a starting point (Figure 1). These sequences are based on reported de novo coiled coils of similar length and sequence composition but different oligomerization state (dimer, trimer, or tetramer).[16] Each bears one or two unnatural terpyridine-functionalized residues (Tpy, **X**) at sites solvent-exposed in the folded state. Peptides **1**−**3** were synthesized by microwave-assisted solid-phase methods, and the Tpy residues introduced through on-resin modification of an orthogonally protected diaminobutanoic acid derivative (Scheme S1). Circular dichroism scans and thermal melts suggest that **1**−**3** all fold and assemble to form stable coiled-coil quaternary structures in dilute aqueous solution (Figure S3). High-resolution structural characterization, detailed below, confirmed the expected oligomerization state in each case (Figure 1D).

The dimeric coiled coil formed by peptide **1** bears four Tpy residues; two were intended to direct formation of a linear coordination polymer and the remaining two promote ordering into a larger lattice (see SI for details). We subjected **1** to crystallization trials by hanging drop vapor diffusion in the presence of transition metals known to form stable bis-terpyridine complexes in aqueous solution (Zn^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, and Cu^{2+}).[17] Crystals were readily obtained only with Cu^{2+}; this was surprising, as it has the lowest propensity among

Received: January 19, 2017
Published: February 5, 2017

DOI: 10.1021/jacs.7b00651
J. Am. Chem. Soc. 2017, 139, 2212−2215

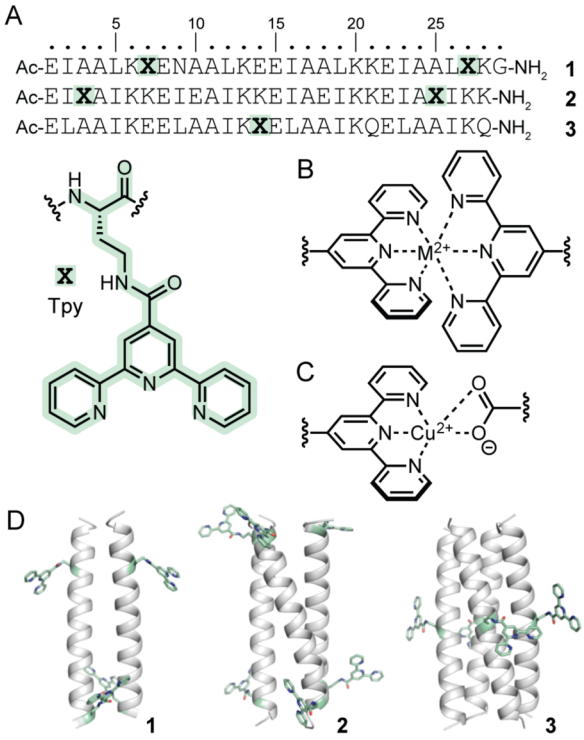

Figure 1. (A) Sequences of peptides **1**−**3** and structure of the Tpy residue (**X**). (B, C) Complex between two terpyridines and a divalent transition metal (B) and between terpyridine, Cu^{2+}, and a carboxylate (C). (D) Coiled-coil quaternary structures formed by **1**−**3**.

the series to form the desired Tpy−M^{2+}−Tpy motif and often favors ternary complexes.[18] Optimization of crystallization conditions yielded diffraction quality single crystals of peptide **1** (crystal form *a*), and the structure was solved to 2.2 Å resolution (Figure 2, PDB ID 5U59).

Supporting our central hypothesis, the noncovalent interactions that make up the crystal lattice consist entirely of (1) coiled-coil hydrophobic interfaces and (2) interchain contacts involving Tpy residues and Cu^{2+} ions. The asymmetric unit is made up of a single α-helix, and the dimeric coiled-coil is created by a crystallographic 2-fold symmetry axis. The dimer (Figure 2A) is virtually identical to that of a variant lacking the Tpy residues.[16b] The remaining two crystallographically independent contacts between chains involve proximal Tpy residues (Figure 2B,C); however, neither showed the simultaneous coordination of Cu^{2+} by two Tpy side chains (Figure 1B). Instead, a carboxylate from citrate in the crystallization buffer replaced one of the terpyridine moieties (Figure 1C). Such terpyridine−Cu^{2+}−carboxylate complexes are known and have been shown to be stable in aqueous solution.[19]

The above result led us to reason that other carboxylate ligands besides citrate may be able to bridge Cu^{2+}-bound Tpy residues. To test this idea, we refocused optimization efforts with an aim to obtain crystals of peptide **1** replacing citrate with a different carboxylate linker. These experiments yielded a new crystal form (*b*) from a buffer containing terephthalate, and the structure of this crystal was solved to 3.2 Å resolution (Figure 3, PDB ID 5U5A).

Like crystal form *a*, the asymmetric unit of crystal form *b* consists of a single α-helix, and the coiled-coil dimer is created by a crystallographic symmetry axis. The peptide folds are

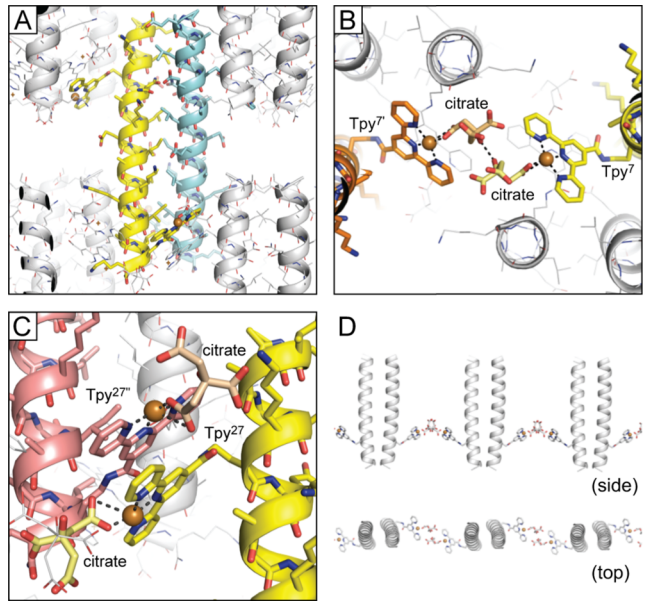

Figure 2. Crystal structure of peptide **1** (crystal form *a*). (A) Dimeric coiled-coil fold. (B) Interface between coiled coils in which two Tpy−Cu^{2+}−citrate units are connected via hydrogen bonding between citrates. (C) Interface where two Tpy−Cu^{2+}−citrate units are stacked via the Tpy hydrophobic surface. (D) Supramolecular polymer embedded in the crystal lattice.

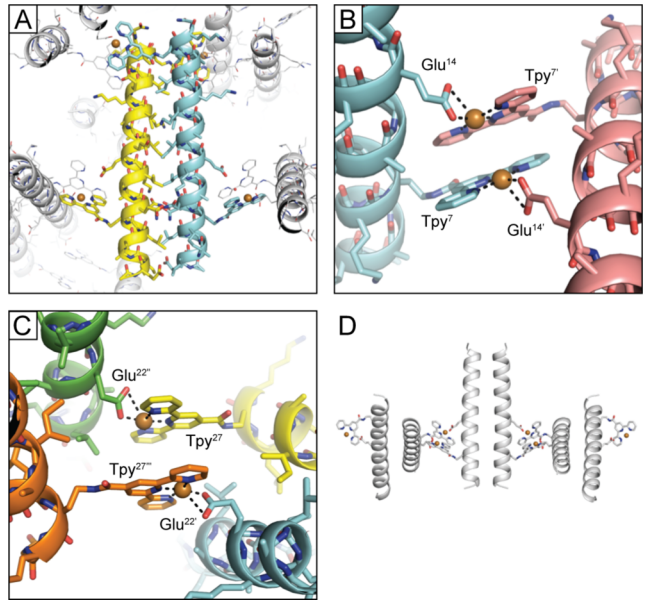

Figure 3. Crystal structure of peptide **1** (crystal form *b*). (A) Dimeric coiled-coil fold. (B, C) Interfaces between coiled coils in which neighboring chains are connected by Tpy−Cu^{2+}−Glu linkages. (D) Coordination polymer embedded in the lattice.

virtually identical in the two crystals, and the contacts that make up the lattice are composed entirely of coiled-coil hydrophobic interfaces and Cu^{2+}-bound Tpy residues in both. Despite these similarities, the lattices differ fundamentally in the packing between coiled-coil units. In crystal form *b*, every carboxylate participating in a Tpy−Cu^{2+}−carboxylate motif comes from a Glu side chain on a neighboring peptide (Figure 3B,C). From its absence in the lattice, we hypothesize the terephthalate may facilitate crystallization by acting as a stabilizer and leaving

DOI: 10.1021/jacs.7b00651
J. Am. Chem. Soc. 2017, 139, 2212−2215

group for bound Cu^{2+} at the edge of the growing crystal. Every coiled coil in crystal form *b* is connected to every other chain through Tpy−Cu^{2+}−Glu coordination and embedded in the lattice is an extended supramolecular polymer consisting of alternating peptide−peptide and metal-coordination interfaces (Figure 3D).

The serendipitous finding that Tpy−Cu^{2+}−Glu coordination could create ordered supramolecular metal−peptide assemblies led us to test the generality of this observation. Thus, we designed sequences **2** and **3** guided by results for **1**. Judicious harnessing of coiled-coil symmetry and Tpy placement yielded crystalline assemblies with alternate morphologies. The coiled coil formed by peptide **2** displays six Tpy units near the termini of a trimeric scaffold and was intended to form an extended 3-dimensional framework. The coiled-coil formed by peptide **3** bears four Tpy residues at the midpoint of a tetrameric junction and was intended to form tetragonal sheets. We attempted to predict and engineer the Glu residues interacting with the terpyridine moieties in each case to varying degrees of success (see SI for details).

Crystals of **3** were grown in the presence of Cu^{2+}, and the structure solved to 2.1 Å resolution (Figure 4, PDB ID 5U5B).

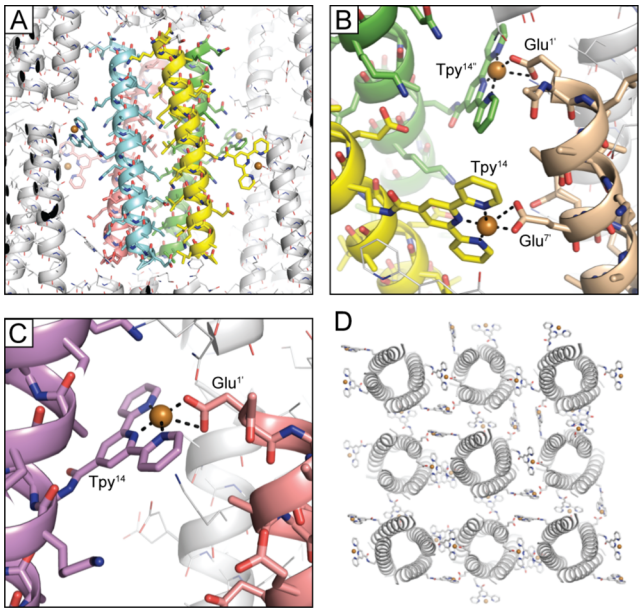

Figure 4. Crystal structure of peptide **3**. (A) Tetrameric coiled-coil fold. (B, C) Interfaces between coiled coils in which neighboring chains are connected by Tpy−Cu^{2+}−Glu linkages. (D) Two-dimensional net embedded in the lattice.

The asymmetric unit consists of six chains: four in the expected parallel tetrameric coiled coil, and the other two creating a second tetramer via a 2-fold symmetry axis. The single Tpy residue in every chain engages in a Tpy−Cu^{2+}−Glu interface with a neighboring tetramer in the lattice (Figure 4B,C). The result is the expected infinitely propagating two-dimensional net (Figure 4D). The arrangement of tetramers in the net leads to layers that stack in a knobs-into-holes fashion to form the third dimension of the lattice (Figure S4).

Crystals of trimeric coiled-coil peptide **2** were grown in the presence of Cu^{2+}, and the structure was solved to 2.4 Å resolution (Figure 5, PDB ID 5U5C). The asymmetric unit consists of the expected trimeric fold (Figure 5A), and the coiled coils are held together in the lattice entirely by Tpy side

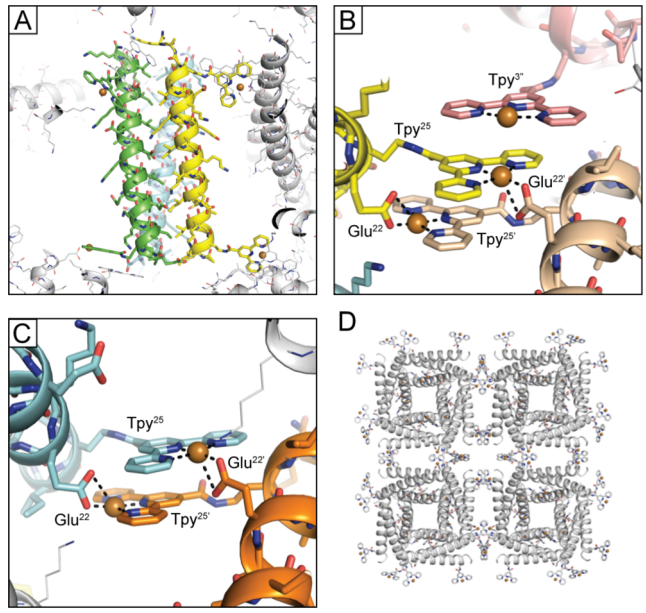

Figure 5. Crystal structure of peptide **2**. (A) Trimeric coiled-coil fold. (B, C) Interfaces between coiled coils in which neighboring chains are connected by Tpy−Cu^{2+}−Glu linkages. (D) Extended framework that makes up the lattice.

chains. Among four crystallographically independent interfaces, the same Tpy−Cu^{2+}−Glu coordination motif seen for peptides **1** and **3** (Figure 5B,C) is found at most sites, alongside stacking of the aromatic systems without Glu coordination (Figure 5B). These contacts generate an extended framework (Figure 5D).

Collectively, we have shown here that designed coiled-coil peptides bearing terpyridine side chains can assemble to form highly ordered supramolecular architectures. The assemblies are held together by two orthogonal forces: peptide quaternary structure and metal coordination based on Tpy−Cu^{2+}−Glu motifs. Subtle variations in peptide sequence that alter coiled-coil stoichiometry and/or placement of metal-binding residues give rise to drastically different assembly morphologies: 1-dimensional coordination polymers, two-dimensional nets, and three-dimensional frameworks. Although the current work falls short of robust design rules for established systems based on small molecules and expressed proteins, we anticipate that the structures reported are the tip of the iceberg of possibilities through systematic variation in the highly modular synthetic peptide ligand. The prospect of exploiting this modularity to introduce new functionality (e.g., catalytic, photophysical, electronic) suggests wide ranging potential applications from this new family of materials. An important open question in such future work is whether these assemblies can be created outside the context of a crystal.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.7b00651.

Supplementary figures, text, and methods (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*horne@pitt.edu

DOI: 10.1021/jacs.7b00651
J. Am. Chem. Soc. 2017, 139, 2212−2215

ORCID

W. Seth Horne: 0000-0003-2927-1739

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the National Science Foundation (DMR1149067). Synchrotron experiments were carried out at Southeast Regional Collaborative Access Team (SER-CAT) beamline 22-ID at the Advanced Photon Source, Argonne National Laboratory. Supporting institutions may be found at www.ser-cat.org/members.html. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-Eng-38.

■ REFERENCES

(1) (a) Kondo, M.; Yoshitomi, T.; Matsuzaka, H.; Kitagawa, S.; Seki, K. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1725. (b) Li, H.; Eddaoudi, M.; Groy, T. L.; Yaghi, O. M. *J. Am. Chem. Soc.* **1998**, *120*, 8571.

(2) (a) Seo, J. S.; Whang, D.; Lee, H.; Jun, S. I.; Oh, J.; Jeon, Y. J.; Kim, K. *Nature* **2000**, *404*, 982. (b) Rosi, N. L.; Eckert, J.; Eddaoudi, M.; Vodak, D. T.; Kim, J.; O'Keeffe, M.; Yaghi, O. M. *Science* **2003**, *300*, 1127. (c) Ma, L.; Falkowski, J. M.; Abney, C.; Lin, W. *Nat. Chem.* **2010**, *2*, 838. (d) Lee, C. Y.; Farha, O. K.; Hong, B. J.; Sarjeant, A. A.; Nguyen, S. T.; Hupp, J. T. *J. Am. Chem. Soc.* **2011**, *133*, 15858. (e) Bloch, E. D.; Queen, W. L.; Krishna, R.; Zadrozny, J. M.; Brown, C. M.; Long, J. R. *Science* **2012**, *335*, 1606.

(3) (a) De Santis, E.; Ryadnov, M. G. *Chem. Soc. Rev.* **2015**, *44*, 8288. (b) Yeates, T. O.; Liu, Y.; Laniado, J. *Curr. Opin. Struct. Biol.* **2016**, *39*, 134.

(4) (a) Padilla, J. E.; Colovos, C.; Yeates, T. O. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 2217. (b) Ryadnov, M. G.; Woolfson, D. N. *Nat. Mater.* **2003**, *2*, 329. (c) Sinclair, J. C.; Davies, K. M.; Venien-Bryan, C.; Noble, M. E. M. *Nat. Nanotechnol.* **2011**, *6*, 558. (d) Fletcher, J. M.; Harniman, R. L.; Barnes, F. R.; Boyle, A. L.; Collins, A.; Mantell, J.; Sharp, T. H.; Antognozzi, M.; Booth, P. J.; Linden, N.; Miles, M. J.; Sessions, R. B.; Verkade, P.; Woolfson, D. N. *Science* **2013**, *340*, 595.

(5) Peacock, A. F. A. *Curr. Opin. Chem. Biol.* **2013**, *17*, 934.

(6) Zou, R.; Wang, Q.; Wu, J.; Wu, J.; Schmuck, C.; Tian, H. *Chem. Soc. Rev.* **2015**, *44*, 5200.

(7) (a) Salgado, E. N.; Radford, R. J.; Tezcan, F. A. *Acc. Chem. Res.* **2010**, *43*, 661. (b) Brodin, J. D.; Ambroggio, X. I.; Tang, C.; Parent, K. N.; Baker, T. S.; Tezcan, F. A. *Nat. Chem.* **2012**, *4*, 375.

(8) Burazerovic, S.; Gradinaru, J.; Pierron, J.; Ward, T. R. *Angew. Chem., Int. Ed.* **2007**, *46*, 5510.

(9) Sontz, P. A.; Bailey, J. B.; Ahn, S.; Tezcan, F. A. *J. Am. Chem. Soc.* **2015**, *137*, 11598.

(10) Przybyla, D. E.; Chmielewski, J. *J. Am. Chem. Soc.* **2008**, *130*, 12610.

(11) (a) Vandermeulen, G. W. M.; Tziatzios, C.; Schubert, D.; Andres, P. R.; Alexeev, A.; Schubert, U. S.; Klok, H.-A. *Aust. J. Chem.* **2004**, *57*, 33. (b) Dublin, S. N.; Conticello, V. P. *J. Am. Chem. Soc.* **2008**, *130*, 49. (c) Tangbunsuk, S.; Whittell, G. R.; Ryadnov, M. G.; Vandermeulen, G. W. M.; Woolfson, D. N.; Manners, I. *Chem. - Eur. J.* **2012**, *18*, 2524. (d) Nepal, M.; Sheedlo, M. J.; Das, C.; Chmielewski, J. *J. Am. Chem. Soc.* **2016**, *138*, 11051.

(12) Wei, P.; Yan, X.; Huang, F. *Chem. Soc. Rev.* **2015**, *44*, 815.

(13) Parry, D. A.; Fraser, R. D.; Squire, J. M. *J. Struct. Biol.* **2008**, *163*, 258.

(14) (a) 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. (b) Stevens, M. M.; Flynn, N. T.; Wang, C.; Tirrell, D. A.; Langer, R. *Adv. Mater.* **2004**, *16*, 915. (c) Zhou, M.; Bentley, D.; Ghosh, I. *J. Am. Chem. Soc.* **2004**, *126*, 734. (d) Raman, S.; Machaidze, G.; Lustig, A.; Aebi, U.; Burkhard, P. *Nanomedicine* **2006**, *2*, 95. (e) Ryadnov, M. G. *Angew. Chem., Int. Ed.* **2007**, *46*, 969. (f) 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. (g) Staples, J. K.; Oshaben, K. M.; Horne, W. S. *Chem. Sci.* **2012**, *3*, 3387.

(15) (a) Hofmeier, H.; Schubert, U. S. *Chem. Soc. Rev.* **2004**, *33*, 373. (b) Constable, E. C. *Chem. Soc. Rev.* **2007**, *36*, 246.

(16) (a) Zaccai, N. R.; Chi, B.; Thomson, A. R.; Boyle, A. L.; Bartlett, G. J.; Bruning, M.; Linden, N.; Sessions, R. B.; Booth, P. J.; Brady, R. L.; Woolfson, D. N. *Nat. Chem. Biol.* **2011**, *7*, 935. (b) Fletcher, J. M.; Boyle, A. L.; Bruning, M.; Bartlett, G. J.; Vincent, T. L.; Zaccai, N. R.; Armstrong, C. T.; Bromley, E. H. C.; Booth, P. J.; Brady, R. L.; Thomson, A. R.; Woolfson, D. N. *ACS Synth. Biol.* **2012**, *1*, 240.

(17) (a) Holyer, R. H.; Hubbard, C. D.; Kettle, S. F. A.; Wilkins, R. G. *Inorg. Chem.* **1966**, *5*, 622. (b) Calì, R.; Rizzarelli, E.; Sammartano, S.; Siracusa, G. *Transition Met. Chem.* **1979**, *4*, 328. (c) van der Gucht, J.; Besseling, N. A. M.; van Leeuwen, H. P. *J. Phys. Chem. B* **2004**, *108*, 2531.

(18) Sigel, H. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 394.

(19) (a) Wang, P.; Moorefield, C. N.; Panzer, M.; Newkome, G. R. *Chem. Commun.* **2005**, 4405. (b) Zhou, W.; Wang, X.; Hu, M.; Guo, Z. *J. Inorg. Biochem.* **2013**, *121*, 114.

DOI: 10.1021/jacs.7b00651
J. Am. Chem. Soc. 2017, 139, 2212−2215