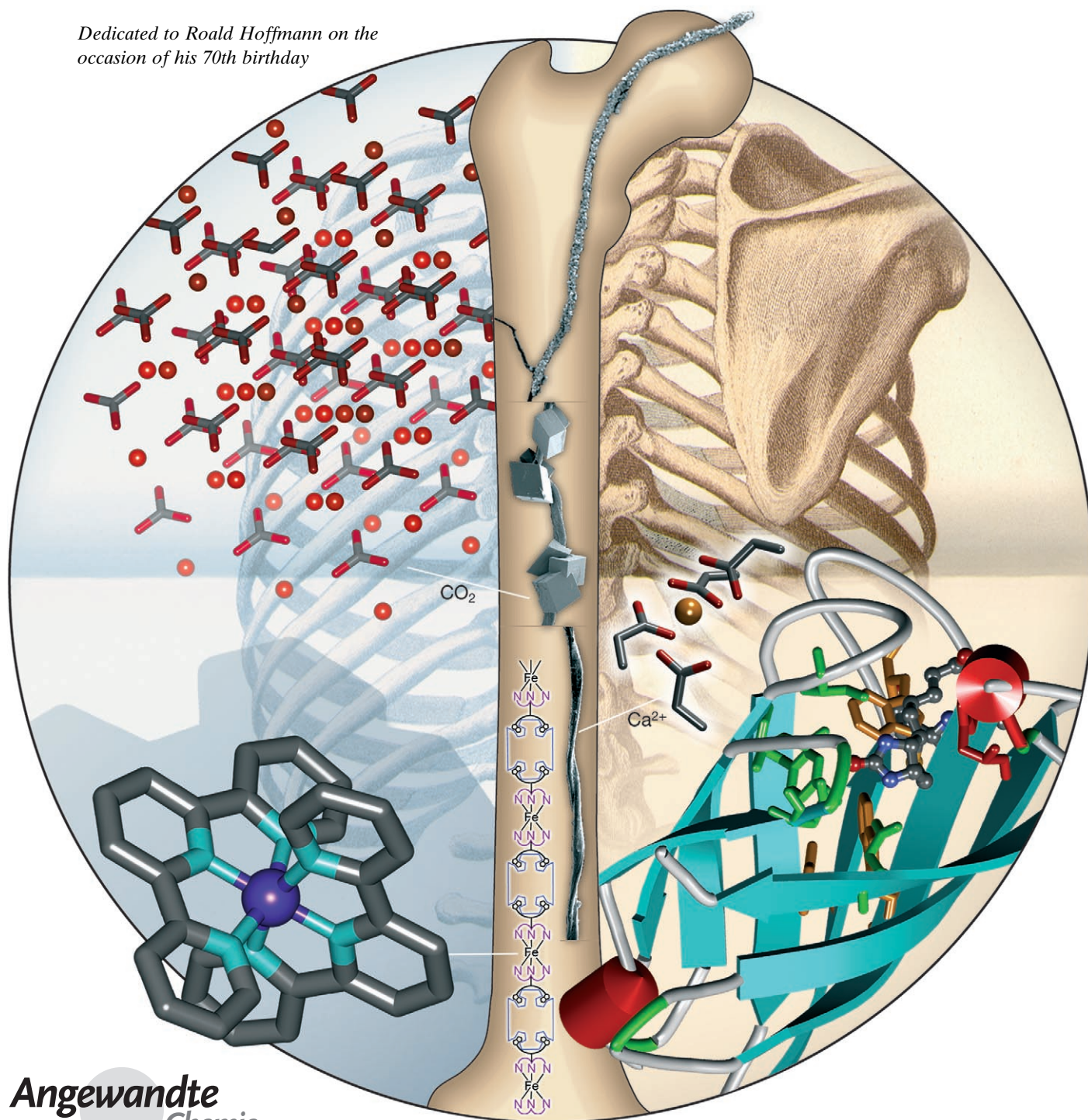


Hierarchical Self-Assembly of One-Dimensional Streptavidin Bundles as a Collagen Mimetic for the Biomineralization of Calcite**

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Dedicated to Roald Hoffmann on the occasion of his 70th birthday



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Over the past two decades, major effort has been dedicated to the metal-directed self-assembly of discrete one-, two-, or three-dimensional frameworks. In addition to their appealing architecture, these coordination polymers (also coined metal–organic frameworks) possess cavities that can accommodate various guest molecules. Such guest–host complexes often display unusual properties such as chemical reactivity, energy or electron transfer, second harmonic generation materials, high-capacity gas storage, catalysis, etc.^[1–6]

In parallel to these developments, surfaces and cavities spanned by protein higher-order aggregates have been exploited for molecular encapsulation and for templating nanoparticle synthesis. Typical examples include protein fibrils,^[7,8] ferritin,^[9,10] S-layers,^[11–13] antibodies,^[14] peptide amphiphiles,^[15] capsids,^[16–19] leucine zippers,^[20] etc.^[21–26]

Controlled protein aggregation is a critical process in many areas, ranging from biomineralization^[27] to neurodegenerative diseases.^[28] In contrast with molecular tectonics, however,^[5,29] the de novo design of higher-order architectures by using proteins as building blocks (e.g. protein tectonics) remains challenging.^[21–24,30–34]

In the spirit of coordination polymers, we reasoned that one may exploit the versatility of transition-metal connectors in conjunction with proteins bearing tethered ligands to create discrete one-, two-, or three-dimensional metal–organic protein frameworks (MOPF; Figure 1). For this proof-of-principle study, we used a linear Fe(terpy)₂ moiety (terpy = terpyridine) bearing four biotin anchors, [Fe(Biot₂-terpy)₂]²⁺ complex (the connector), in conjunction with streptavidin (hereafter referred to as Sav, the linker) to afford a one-dimensional MOPF. We show that in the presence of calcium ions and CO₂-rich vapors, these MOPF aggregates form bundles that template the biomineralization of calcite.

Based on our previous experience with the compatibility between organometallic moieties and proteins, we selected a noncovalent approach based on the biotin–streptavidin technology to assemble MOPFs.^[35–41] Streptavidin is a slightly acidic (isoelectric point, pI = 6.3) homotetrameric protein that bears four biotin binding sites.^[42,43] For this proof-of-principle study, we selected a preorganized bis-biotinylated terpyridine ligand Biot₂-terpy that binds to two *cis*-related sites in streptavidin.^[44–47] A ferrous ion was chosen as terpyridine binds in a cooperative fashion ($K_1 = 1.26 \times 10^7 \text{ M}^{-1}$, $K_2 = 6.31 \times 10^{13} \text{ M}^{-1}$),^[48] thus ensuring the exclusive

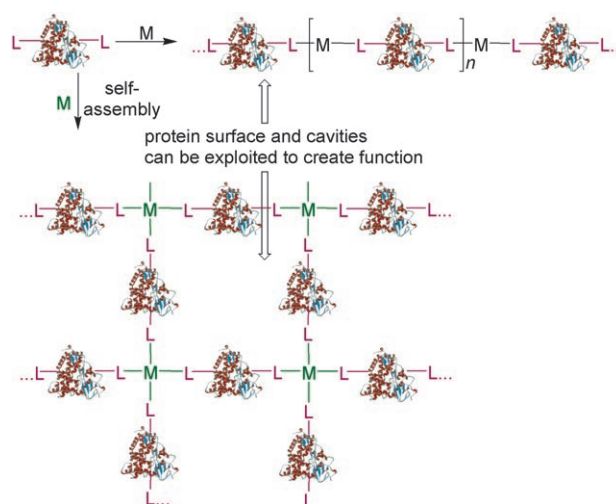


Figure 1. Extending the concept of coordination polymers to MOPFs. The nature of the metal M and the denticity of the ligand L determines the topology of the self-assembled MOPF.

formation of a linear connector bearing four biotin anchors (Figure 2). Experimental details for the synthesis and the characterization of the [Fe(Biot₂-terpy)₂]²⁺ complex are described in the Supporting Information.

Despite the presence of many potentially coordinating functionalities on the streptavidin surface, the selected metal + ligand + protein triad ensures the exclusive formation of a one-dimensional noncovalent MOPF upon addition of streptavidin to afford ([Fe(Biot₂-terpy)₂]²⁺⊂Sav)_∞. The characteristic [Fe(terpy)₂]²⁺ complex absorption band ($\epsilon_{557 \text{ nm}} = 9237 \text{ cm}^{-1} \text{ M}^{-1}$) allows one to assess the integrity of the linear connector throughout the hierarchical self-assembly process. The biotin-binding event between the [Fe(Biot₂-terpy)₂]²⁺ ion and Sav can be conveniently monitored with the disappearance of an induced CD signal, $\lambda_{\text{max}} = 505 \text{ nm}$, caused by the displacement of 2-(4'-hydroxyazobenzene)benzoic acid (HABA, a hydrophobic dye weakly bound within the biotin-binding pocket) by the [Fe(Biot₂-terpy)₂]²⁺ ion (see the Supporting Information).^[37,49]

Upon standing at room temperature, the dilute purple solution containing the metal + ligand + protein triad slowly becomes turbid, suggesting the formation of polymeric material {[Fe(Biot₂-terpy)₂]²⁺⊂Sav)_∞}. In the presence of calcium ions (0.05 M), threads appear within minutes and can be visualized by scanning electron microscopy (SEM, Figure 3a,b). We speculate that the presence of aspartate and glutamate side chains (48 in total for the Sav tetramer)^[50] on streptavidin's surface favors the chelation of calcium, eventually resulting in the cross-linking of MOPF to form bundles with a micrometer diameter and a millimeter length (Figure 3a,b). Calcium-induced bundle formation of polyacrylate fibers on carboxylate-terminated SAMs was reported by Tremel and co-workers.^[51]

In the presence of carbon dioxide vapors, the MOPF bundles template the nucleation, the growth, and the assembling of calcite microcrystals (Figure 3). The biomineralization experiments were carried out by using the diffusion method in a closed dessicator.^[52,53] A solution of ammonium

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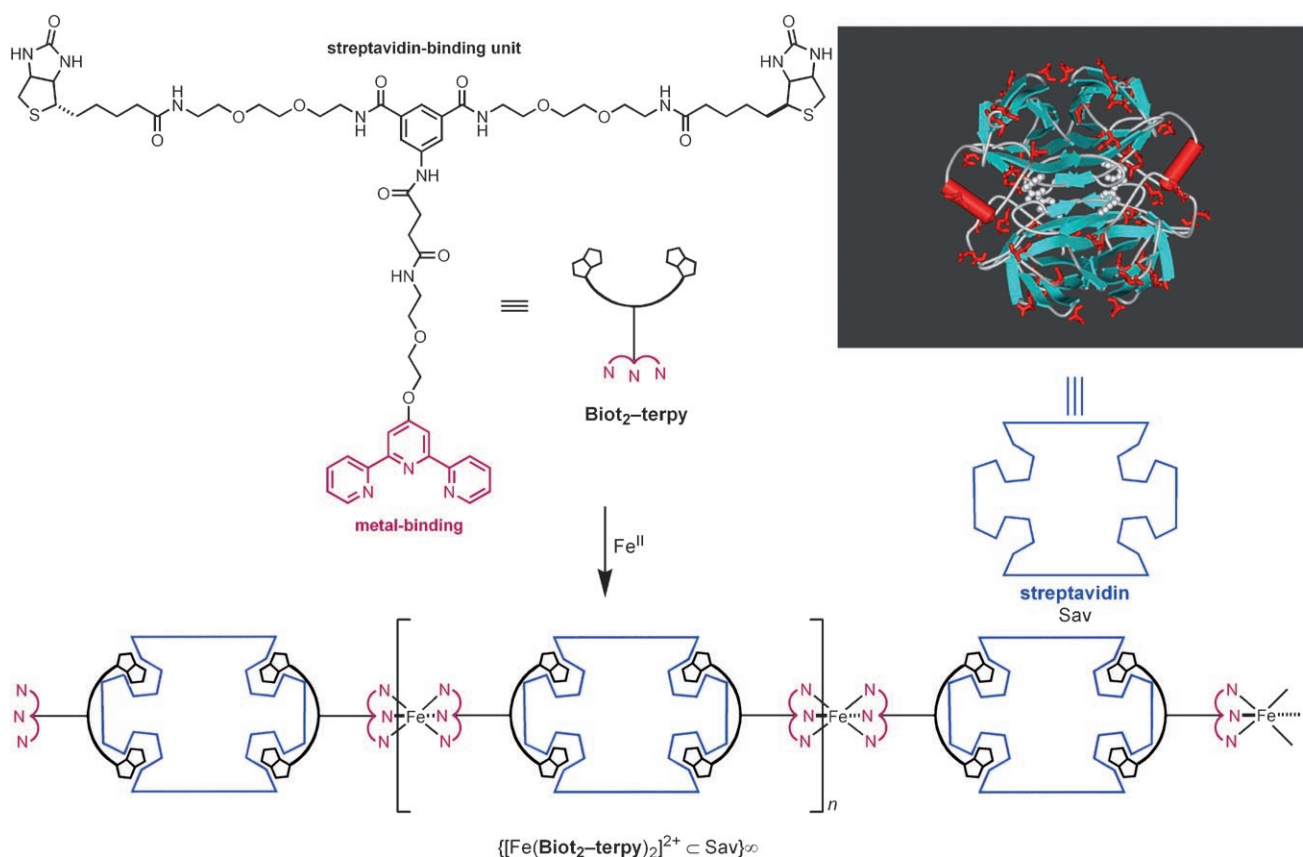


Figure 2. Structure of the bis-biotinylated (black) terpyridine (violet) Biot₂-terpy, which, upon reaction with Fe^{II}, forms a linear tetrabiocinylated connector, the $[\text{Fe}(\text{Biot}_2\text{-terpy})_2]^{2+}$ complex. In the presence of streptavidin (right insert emphasizing the 48 acidic side chains and the stick representation shown in red),^[50] linear coordination polymers, $[[\text{Fe}(\text{Biot}_2\text{-terpy})_2]^{2+} \subset \text{Sav}]_\infty$, are formed.

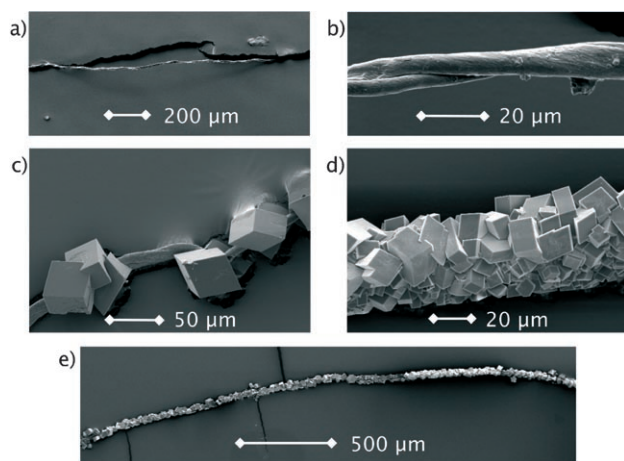


Figure 3. Scanning electron micrographs (gold-coated): a) MOPF bundles obtained from $[[\text{Fe}(\text{Biot}_2\text{-terpy})_2]^{2+} \subset \text{Sav}]_\infty$ solutions ($8 \times 10^{-7} \text{ M}$) containing CaCl_2 (0.05 M) after 5 min at room temperature and b) the close-up view. c–e) Biomineralized $[[\text{Fe}(\text{Biot}_2\text{-terpy})_2]^{2+} \subset \text{Sav}]_\infty$ bundles: c) $8 \times 10^{-6} \text{ M}$, $[\text{CaCl}_2] = 0.01 \text{ M}$, 12 h; d, e) $8 \times 10^{-6} \text{ M}$, $[\text{CaCl}_2] = 0.05 \text{ M}$, 12 h.

carbonate served as a CO_2 source that diffused in CaCl_2 solutions ($c = 0.01\text{--}0.05 \text{ M}$) containing the MOPF building blocks ($c = 8 \times 10^{-6} \text{ M}$). Manipulations were carried out in a

laminar flow hood in a clean room by using six-well plates, and each experiment was performed in triplicate. The CO_2 atmosphere was maintained over 12 h at room temperature after which each sample was scrutinized under a polarized-light optical microscope (see the Supporting Information). Control experiments revealed that the presence of calcite crystal wires only occurred in the wells containing both the $[\text{Fe}(\text{Biot}_2\text{-terpy})_2]^{2+}$ complex and Sav building blocks.

Calcite microcrystals have been grown on a wide variety of templates, including Kevlar,^[54–56] nylon,^[57] self-assembled monolayers, proteins, etc.^[51,58–68] Reminiscent of collagen processing,^[69] the approach delineated herein represents an example of hierarchical self-assembly in which each stage involves building blocks of increasing size such that the resulting supramolecular structure spans over six orders of magnitude, from nanometer-sized covalent building blocks to millimeter calcite wires.^[70]

We have demonstrated that streptavidin (the linker) combined with a linear tetrabiocinylated connector, the $[\text{Fe}(\text{Biot}_2\text{-terpy})_2]^{2+}$ complex, spontaneously self-assemble into a one-dimensional metal–organic protein framework. In the presence of calcium ions, these MOPFs form bundles that serve as a template for the biomineralization of calcite. This hierarchical self-assembly process relies on noncovalent interactions that are typical of both coordination polymers (dative M–L bonds) and of higher-order protein aggregates

(protein–protein interactions) to yield millimeter-sized one-dimensional biomineralized protein matrices.

Current efforts in the group include a) the characterization of the hierarchical self-assembly process by using light-scattering experiments, b) the generation of two- and three-dimensional MOPFs by using polytopic connectors, and c) the exploitation of these frameworks for templating the growth of various nanoparticles.

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