In contrast to short helical peptides, constrained peptides, and foldamers, the design and fabrication of crystalline 3D frameworks from the β-sheet peptides are rare because of their high self-aggregation propensity to form 1D architectures. Herein, we demonstrate the formation of a 3D porous honeycomb framework through the silver coordination of a minimal β-sheet forming a peptide having terminal metal coordinated 4- and 3-pyridyl ligands.

The development of artificial, self-organized three-dimensional (3D) framework structures from biomolecular building blocks such as proteins/peptides has evolved for a broad range of applications in the fields of molecular recognition, separation, catalysis, materials and biomedical sciences. As metal ions are ubiquitous in nature, the concept of metal-coordinated self-assembly was introduced to simplify the design rule of 3D porous frameworks based on multiple hydrogen-bonds and hydrophobic interactions. Initially, ultra-short peptides lacking defined secondary structures were employed for metal coordination and were shown to produce exciting adaptable, and tunable porous crystalline frameworks. With the advent of chemical tools for sequence engineering of the defined structure, there has been rapid progress in the development of helical conformation-based metal-coordinated frameworks using longer peptides such as collagen and coiled-coil peptides, short peptides (Pro-rich), and foldamers.

On the other hand, β-sheets are major structural motifs observed in natural proteins and their interactions play crucial roles in protein dimerization and oligomerization, protein–protein interaction, and peptide/protein aggregation. Comprehending intermolecular inter-action of β-sheets is not only important for protein folding, but also holds significant relevance in the realm of proteinopathies and therapeutics. For instance, amyloidosis, one of the greatest health threats of the 21st century, is characterized by the accumulation of amyloid fibrils caused by the uncontrolled aggregation of β-amyloid (Aβ) peptides through cross-β structure formation. Moreover, β-sheets also serve as a valuable resource for the development of peptide-based materials for bionanotechnological applications. Concurrently, in living systems, β-barrels, formed by the 3D cylindrical self-assembly of β-sheets, are one of the two pure protein tertiary structures found in binding proteins, pores, and enzymes in many variations. While the fabrication of 1D filamentous or fibrous stochastic assemblies from β-sheets is relatively common, the generation of artificial crystalline 3D porous frameworks is highly challenging.

In this context, metal-coordinated self-assembly of β-sheets could reveal an important development in the goal to achieve 3D porous architectures. Suitable natural amino acid-based metal coordinating side chains or artificial metal binding ligands can be installed into the peptide strand to participate in the metal coordination and drive the polymerization process to form crystalline 3D supramolecular networks. Recently, Sawada et al. judiciously utilized metal-coordinated self-assembly to artificially construct a steric zipper arrangement of β-sheets, which is a common hydrophobic packing in amyloid fibrils. Nowick et al. strategically incorporated N-methylated amino acids in one of the β-strands of an artificial macrocyclic β-sheet and demonstrated that 3D crystalline frameworks can be fabricated from the metal-directed self-assembly of macrocyclic β-sheets. Furthermore, Fujita et al. presented the construction of artificial β-barrel structures having large cylindrical pores through metal-driven
assembly of octapeptides comprising β-strands and loop-forming sequences. However, the existing literature offers only limited evidence and understanding on the metal-directed self-assembly of minimal β-sheet forming peptides into diverse complex structures, such as 3D crystalline porous honeycomb frameworks.

Herein, we report the design, synthesis and metal-directed higher order self-assembly of two minimal tripeptides having different coordinating sites into 3D crystalline porous honeycomb frameworks with well-defined porous channels and supramolecular metallogels with impressive mechanical properties.

In order to understand the metal-driven construction of complex 3D architectures, two short peptides P1 and P2, as depicted in Scheme 1, were designed and synthesized. Both 3- and 4-pyridyl metal binding ligands were used to examine the influence of coordinating sites on the metal-driven folding and assembly of these short peptides. All the peptides were synthesized using a solution phase fragment condensation strategy, purified through reverse phase HPLC, and characterized by mass spectrometry and nuclear magnetic resonance (NMR).

To understand the unambiguous conformations of peptide ligand P1, we performed crystallization under different conditions. Unfortunately, we were unsuccessful in obtaining single crystals of peptide P1, and thus 2D NMR spectroscopy was employed to comprehend the conformational preferences. The analysis of the ROESY spectrum (DMSO-d6 at 298 K) of P1 showed very weak NH ↔ NH ROEs; however, the existence of strong C=H ↔ NH ROEs between i and i + 1 residues supported the extended conformation (Fig. S1–S3, ESI†). Folding and assembly of P1 upon coordination to Ag+ was investigated after growing the single crystals of the P1–AgBF4 complex. Fourier-transform infrared (FTIR) analysis was performed of the isolated metal–peptide crystals to understand the secondary structure of the ligand in a metal complex, which showed a sharp amide I band at 1638 cm⁻¹ demonstrating the presence of predominant β-sheet conformation (Fig. S4, ESI†). Furthermore, a single crystal of P1–AgBF4 complex suitable for diffraction was subjected to an X-ray diffraction study. Diffraction data were collected for 0.90 Å resolution and refined in the R32 space group. Atomic level structural analysis revealed that in the asymmetric unit, two nearly-identical P1 monomer subunits (P1A and P1B) were connected in a head-to-head fashion through linear 4py–Ag–4py coordination and crystallized with one BF4⁻ counterion (Fig. 1a). In the individual P1 unit, the two aromatic side chains of the Phe residues are arranged in the same face relative to the peptide backbone, while the Leu side chain and pyridyl termini are displayed on the opposite side of the peptide backbone (Fig. 1a). The torsion angles around the Leu2 residue appeared to play a pivotal role in dictating the overall structural feature of the peptide backbone. The allowed torsion angles of the Leu2 residue were found to be localized within the extended β-sheet region of the Ramachandran plot, with ϕ2 and ψ2 values of −152° and 158° for P1A and −129° and 149° for P1B, respectively (Table S1, ESI†). Individual β strands further interacted with the adjacent strands that are oriented in an antiparallel fashion thereby forming a supramolecular antiparallel β-sheet structure. Fig. 1b displays the consensus pattern of hydrogen bonding (average N⋯O distances of 2.85 Å in strand P1A and 2.83 Å in strand P1B) between strands in each of the antiparallel sheets illustrating that their organization allows for pairing of nearly all intermolecular backbone hydrogen-bonds. Further stabilization of the β-sheet conformation is achieved through aromatic–aromatic interactions between the phenyl ring of the Phe3 residue of one strand and the 4py motif of the next strand (center-center distance = 4.56 and 5.73 Å for P1A and P1B, respectively). This in turn forces the 3py motif adjacent to Phe3 to reside in a bending position as compared to the remaining backbone of the strand. In this arrangement of strands, aromatic residues (Phe1, Phe3) appear on one face of the dimeric β-sheet, while the Leu2 side chain appears on the other face. Further assembly of these sheets along both faces can form a dry interface and stabilize through tight interactions in the self-complementary association of the hydrophobic side chains like that of steric zippers.

More intriguingly, the NH and CO groups of the Phe1 residue of each β-strand form intermolecular hydrogen bonds with the NH and CO groups of the Phe1 residue in the adjacent
strand N5–O7 = 2.91 Å and N11–O3 = 2.88 Å (Fig. 1c). This, coupled with the strong π–π stacking interaction between the 4-pyridyl group (centroid–centroid distance 3.84 Å) and metal coordination (4Py–Ag–4Py), results in the formation of a triangular structure with a central channel (Fig. 1c). Moreover, within the triangular structure, each silver ion is further coordinated with the C-terminal pyridine of another two β-strands to form a tetrahedral complex (Fig. 1d).

Each strand of the tetrahedral complex forms intermolecular H-bonds with adjacent β-strands to produce dimeric β-sheet structures. The overall packing results in a hexameric assembly of β-sheets and produces a channel-like structure (internal diameter of 6.32 Å) along the c-axis (Fig. 1e). In contrast to the general affinity of β-sheets to form 1D supramolecular polymers, the current 3D assembly of artificial β-sheets into well-defined supramolecular oligomers is rare and comparable to the formation of artificial β-barrel structures (Fig. 1e and f). In the organization of the barrel-like hexamer, aromatic residues (Phe2 and 4-Py) are extended towards the cavity and create a hydrophobic core inside the hexamer. Both metal coordination and aromatic–aromatic interactions play a significant role in stabilizing the overall structure of the framework. Due to suitable volume and perfect fitting into the void space, the counter anions of the Ag salt, BF4−, are encapsulated inside the pore and thereby block the channels. In the higher order packing, utilizing metal coordination and hydrophobic stacking, each dimeric β-sheet participates in two other kinds of pore formation along with the one discussed above. The opposite end of the β-sheet that did not participate in the hexameric pore formation coordinated in two different ways. The 3Py motif interacts in a head-to-tail fashion with the adjacent β-sheet through 3Py–Ag–3Py coordination and organizes in a helical structural arrangement (Fig. S6, ESIT). Three β-sheet units complete a helical turn and five such units fabricate the internal cavity of the helical structure. The overall packing extends six Leu2 residues towards the cavity and thus produces a hydrophobic core of diameter 8.49 Å (Fig. S6, ESIT). This end of the β-sheet further interacts with another adjacent β-sheet through both pyridyl motifs in a 3Py–Ag–4Py and 4Py–Ag–3Py fashion and fabricates another helical structural arrangement (Fig. S7, ESIT). The top view of the helical structure displays the formation of a nearly circular cavity with an average diameter of 3.99 Å (Fig. S7, ESIT). The supramolecular arrangement situates amide groups at the inner edge of the pore, enhancing accessibility for hydrophilic guests. Excitingly, the higher order packing of the [AgBF4]3 complex along the c-axis has resulted in a 3D porous honeycomb-like framework comprising three distinct pores, each distinguished by its unique size and nature (Fig. 2). The voids for the structure as mapped by the contact surface (probe radius 1.2 Å) indicating a void volume of 19,984 Å3, which is ~42% of the unit cell volume (calculated by using mercury). An attempt to study the gas adsorption capability of the framework under vacuum showed an insignificant amount of N2 or CO2 uptake (Fig. S8, ESIT). However, peptide-based frameworks comprising flexible ligands could exhibit different classes of porosity, like static porosity, dynamic porosity, and cooperative porosity with diverse nature of stability suitable for biotechnological applications such as catalysis, selective encapsulation, and tissue engineering.

From the above observation it is clear that the coordination mode of the 4-pyridyl nitrogen plays a significant role in the higher-order structure formation of the [(AgBF4)P1]n complex. Furthermore, to understand this phenomenon in more detail, we have synthesized the same sequence replacing only the N-terminal 4-pyridine by 3-py (P2). Like P1, P2 has a latent property to form an extended structure, which was further supported by 2D-NMR spectroscopy. The absence of NH ↔ NH ROEs and the presence of strong CnH ↔ NH ROEs in the ROESY spectrum provides evidence for the extended type of conformation (Fig. S10, ESIT). Interestingly, when an ethanolic solution of P2 (250 µL of 30 mM of P2 in EtOH) was added to 250 µL of 30 mM of an aqueous solution of AgBF4 in a glass vial and kept for a few minutes after sonication, formation of a stable gel was observed. This was confirmed by the vial inversion method (Fig. 3a). The mechanical properties of the metallogel were evaluated by rheological measurements. Dynamic frequency sweep (at 0.1% strain over 0.1–100 Hz) revealed a linear viscoelastic region. The greater storage modulus (G’) compared to the loss modulus (G”) across a wide range of frequencies confirmed its viscoelastic nature (Fig. 3b). In addition, a strain sweep experiment at a constant frequency of 1 Hz demonstrated that as the strain increased, the gel ruptured, and at 17% strain, complete gel rupture occurred (Fig. 3c). To comprehend the morphological features of the metallogel, transmission electron microscopy (TEM) was employed (Fig. 3d). The results revealed that the metallogel is composed of entanglements of fibrillar networks. FTIR analysis of the dried metallogel exhibited an amide I band at 1637 cm−1 indicating β-sheet-mediated aggregation in the gel state (Fig. S11, ESIT). Moreover, we performed X-ray powder diffraction of lyophilized gel samples, which showed broad diffraction peaks at 4.2 and ~10.5° that are consistent with a cross-β structure pattern of the amyloid fibril (Fig. S12, ESIT).
Intriguingly, upon exposure to ambient light, the transparent metallogel changed to a light brown color indicating the formation of silver nanoparticles (Fig. 3e inset), which was further supported by TEM micrographs (Fig. 3e and Fig. S13, ESI†). In summary, we have showcased the successful development of a crystalline 3D-porous honeycomb framework from the metal-driven folding and higher order assembly of a minimal β-sheet-forming peptide. Using two different metal binding sites at the two termini, it was possible to direct the metal-coordinated self-assembly process of β-sheets into a β-barrel like architecture. Furthermore, we have also demonstrated how slight alteration of the ligand induces a shift in the metal-driven self-assembly process, transitioning it from a 3D crystalline framework to a supramolecular fibrillar network. This observation holds significant importance in unravelling the design principles of this new class of systems and providing guidance for the metal-driven self-assembly of β-sheets into 3D frameworks.

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Conflicts of interest

There are no conflicts to declare.

Notes and references


