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Leucine zipper motif inspiration: a two-dimensional Leucine Velcro-like array in peptide coordination polymers generates hydrophobicity

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Here, we show that the well-known hydrophobic leucine (Leu) zipper motif (also known as the coiled-coil or Leu scissors motif), typically found in proteins, can be used as a source of inspiration in coordination polymers built from Leu-containing dipeptides or tripeptides. We demonstrate that this motif can be extended to form Velcro-like layers of Leu, and that the hydrophobicity of these layers is transferred to coordination polymers, thereby enabling development of a new type of hydrophobic materials.

Researchers seeking to develop new materials and coatings with specific functionalities are continuously inspired by nature.[1] For example, the next generation of adhesives and superhydrophobic surfaces are based on the surface structures of gecko foot-hairs and lotus leaves, respectively.[2] The chemistry found in nature is also an important source of inspiration. For example, adhesive proteins in mussels have been adapted to develop underwater adhesives and coatings,[3] and many supramolecular motifs found in peptides are currently being mimicked in labs to create new biomaterials, such as hydrogels,[4] and new supramolecular nanomaterials.[5]
Among these supramolecular motifs, the leucine (Leu) zipper is a common protein-protein interaction domain consisting of two opposite amphipathic α helices that dimerize in parallel to form a coiled-coil that binds with DNA.[6] The amino acid residues in the hydrophobic side of these amphipathic helices are responsible for the dimerization. In general, these helices consist in a short-range of repetition of seven amino acids, in which the hydrophobic amino acids are located spaced every four and then three residues apart of this sequence. The hydrophobic amino acids of one left-handed helix and one right-handed helix pack together, creating a hydrophobic Leu zipper motif that drives their dimerization. Over the past few years, this supramolecular hydrophobic motif has been successfully used in the assembly of new functional materials. For example, other groups have produced hydrogels and unilamellar spheres using proteins and artificial peptides that incorporate a Leu zipper.[7]

Herein we show the possibility to create hydrophobic synthetic materials being inspired by the hydrophobic nature of the Leu zipper. For this, we have exploited the periodicity of coordination polymers,[8] where it is possible to obtain materials in which the peptide (or amino acid sequence) is repeated along the space by linking this specific peptide to the metal ions. To date, several studies have shown that short peptides can be used to create crystalline coordination polymers. Among them, most of their designs have been focused on obtaining porous materials.[9] For example, Fujita *et al.* have reported the synthesis of artificial protein-like nanocavities by linking a pyridil-modified Gly-Pro-Pro tripeptide with Ag(I) ions.[9b] These nanocavities were effectively used to recognize anions, organic chiral molecules and bio-oligomers. Rosseinsky *et al.* have constructed a two-dimensional Zn(II)-based coordination polymer using the dipeptide Gly-Ala.[9c] This coordination polymer showed adaptable porosity, in which the flexibility of the peptide was shown to be crucial in changing the pore conformation. More recently, the same authors have synthesized other Zn(II)-based coordination polymers using Gly-Thr and Gly-Ser dipeptides.[9d] In these latter studies, it was first proved the importance of the side chains of the residues of these peptides to control the final properties of these porous materials. They found that the hydroxyl groups of the side chains of the Thr and Ser residues and their possibility to form hydrogen bonds with the coordination structure and/or guest solvent molecules determine the rigidity or flexibility of the resulting porous materials.

In the present study, we show that as occurs in nature the side chains of the residues of peptides incorporated in crystalline coordination polymers can self-assemble forming new bioinspired supramolecular arrangements that can impart new functionalities to the resulting materials.

Our crystal engineering strategy[10] begins with the selection of the dipeptide Asp-Leu because it contains two terminal carboxylate groups that can coordinate to metal ions and extend the metal-peptide structure, plus one hydrophobic Leu isobutyl side chain, which is necessary for producing the Leu zipper-like motif (Fig. 1a).

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The Asp-Leu peptide was reacted with $Zn(NO_3)_2·6H_2O$ in a mixture of ethanol and water at 85 °C for 24 hours to produce the coordination polymer of formula [Zn(Asp-Leu)] (**1**) (see ESI† for experimental details). Close examination of the crystal structure of **1** reveals the formation of coordination layers, each one built up from pure left-handed helices running along the *b* axis that are inter-coordinated along the *a* axis (Fig. 1b,c). These helices, which result from the pathway defined by the linkage between Zn(II) ions and Asp-Leu linkers through their carboxylate groups, are composed of one *"-Zn-Asp-Leu-Zn-Asp-Leu-Zn-"* sequence per turn, such that two Leu isobutyl side chains are pointing up and down the layers per turn (Fig. 1b,c). In these helices, Zn(II) ions adopt a square-pyramidal geometry (Addison parameter[11] $\tau = 0.36$) coordinated to two carboxylate-O atoms of two different Asp-Leu linkers (also confirmed by infrared spectroscopy; ESI† Fig. S1 and S7a). The remaining coordination positions are occupied by a carbonyl-O atom, which underpins the internal linkages in the helices, and by one amino-N atom and one carboxylate-O atom, which are responsible for linking the consecutive helices along the a axis (Fig. 1c).

In the aforementioned configuration, the isobutyl side chains of a helix of one coordination layer, plus those of the helix of a consecutive layer, together form 1D inter-digitated Leu motifs (hereafter called Leu_{inter} motif) running along the *b* axis (Fig. 1d). The distances between neighbouring Leu isobutyl chains (from here onwards, using the –CH carbon of the isobutyl group as the reference carbon atom for simplicity) in this Leu_{inter} motif are 6.325(39) Å, 6.075(25) Å and 5.440(4) Å. In addition, the proximity of Leu_{inter} motifs along the *a* axis involves Van der Waals interactions between Leu isobutyl side chains that extend from one to two dimensions (Fig. 1d, right). The distances between the isobutyl side chains of two consecutive Leu_{inter} motifs are 6.070(1) Å, 6.075(25) Å and 6.325(39) Å (Fig. 1d, right). The aforementioned interactions define hydrophobic Velcro-like Leu layers extending along the *ab* plane. Interestingly, although the helices that form **1** are not the typical α helices found in nature, the average distances between the isobutyl chains involved in the infinite Velcro-like Leu assembly (d = 5.977 Å) are comparable with those observed in the twisted ladder Leu zipper motif present in different proteins (e.g. basic leucine zipper domain (bZIP) class transcription factors, and tropomyosin, having protein data bank codes 2zta, d = 6.298 Å; 2e42, d = 6.290 Å; 2e43, d = 6.119 Å; 2h7h, d = 6.226 Å; and 2g9j, d = 5.748 Å; Fig. 1e).

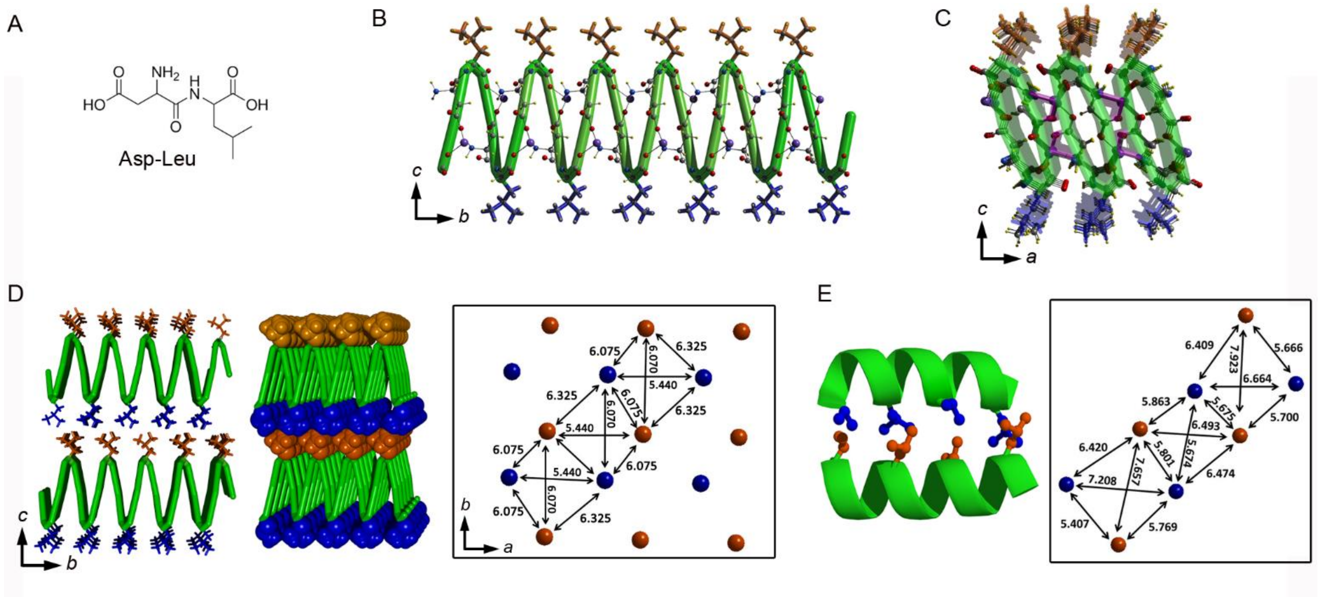

Fig. 1 a) Chemical structure of Asp-Leu; b-c) Helices of the sequence "*-Zn-Asp-Leu-Zn-Asp-Leu-Zn-*" run along the *b* axis and are interconnected, creating 2D-layers that extend throughout the *ab* plane. In this arrangement, the leucine residues point upwards and downwards relative to the sheets. The violet bond in c) represent the linking of the helices along the *a* axis; d) The Leu_{inter} motif is running along the *b* axis (left). The Velcro-like Leu motif (middle) arises from association of Leu_{inter} motifs along the *a* axis. On the right, representation of the –CH atoms of the Leu isobutyl side chains within the Velcro-like Leu motifs in **1** and their corresponding distances in Å; e) Representation of the typical Leu zipper motif in the bZIP yeast transcriptional activator CGN4 (protein data bank code 2zta). On the right, view of the –CH atoms of the Leu isobutyl side chains within the characteristic ladder-type Leu zipper motif in CGN4 and their corresponding distances in Å. In b-e, the Leu isobutyl chains pointing upwards and downwards are colored in orange and blue, respectively.

Compound **1** can therefore be seen as a repetition of coordination layers linked by Leu Velcro. Since **1** grows as two-dimensional plate-like crystals, it is feasible to imagine a strong correlation between the macroscopic shape of the crystals and the growth of the coordination layers. We confirmed this hypothesis by indexing the plate face of a single-crystal of **1**, which was identified as the [*hk0*] direction (Fig. 2a). In this way, the coordination layers that propagate along the *ab* plane are aligned parallel to the flat surfaces of these platy crystals, meaning that the Leu Velcro connects the layers along the direction perpendicular to this surface. Thus, we reasoned that the two flat surfaces of each platy crystal must be composed of half of this Velcro-like Leu motif, conferring them with strong hydrophobicity (Fig. 2a). To confirm our hypothesis, we performed water contact-angle measurements on crystals of **1** hand-packed on a glass surface. The contact angle (Θc) was 138° ± 1, a value which is characteristic of a hydrophobic solid. To maximize the (001)-oriented assembly of these crystals, **1** was also packed on a plastic surface by drop-casting a dispersion of pre-sonicated (for 10 min) crystals in water (Fig. 2c). In this case, the Θc was 134° ± 1. Finally, we characterized the wetting properties of **1** shaped as a disk pellet, which was formed by pressing the product as a dry crystalline powder under a pressure of 2 tons for 5 min (Fig. 2d). As expected, this pellet which showed a roughness factor r (defined as the ratio of the actual area of the rough surface to its projected area on the horizontal plane) of 1.10 ± 0.03, was also found to be hydrophobic, with a Θc of 114° ± 1.1 (Fig. 2e). We also quantified the contact angle hysteresis (CAH), the value of which was 32°. This relatively large CAH value was attributed to the surface roughness of the disk pellet, which may be responsible for the contact-line pinning.

To further determine the importance of the Leu Velcro to the resulting hydrophobicity of **1** and to prove that not all Leu-containing peptides would produce hydrophobic materials, we created the new coordination polymer [Zn(Leu-Asp)] (**2**), which is very similar in composition to the former but lacks the Leu Velcro. To this end, we switched to the "inverted" dipeptide Leu-Asp as the linker while maintaining Zn(II) as the metal ion (Fig. 2f). Reaction of Leu-Asp and $Zn(CH_3COO)_2{\cdot}2H_2O$ using similar conditions as for the synthesis of **1**, afforded **2** (ESI†).

This product also shows 2D layers that run along the *ac* plane but which exhibit a honeycomb-**hcb** topology. In these layers, each Zn(II) ion exhibits a trigonal bipyramid geometry ($\tau = 0.62$) coordinated to two carboxylate-O atoms and one carbonyl-O atom, occupying the equatorial positions, and to one carboxylate-O atom and one amino-N atom, which occupy the axial positions (ESI†, Fig. S7b). In this configuration, the Leu residues are located almost parallel to the honeycomb two-dimensional framework (Fig. 2g), thus preventing the formation of either $\mathrm{Leu}_{\mathrm{inter}}$ or Velcro-like Leu motifs between layers. In this case, the packing between neighbouring zig-zag honeycomb layers is achieved by several H-bond interactions established between the NH, NH_2 and carboxylate groups of the Leu-Asp peptide along the *b* axis (ESI†, Fig. S8 and Table S5).

Water contact-angle measurements on crystals of **2** hand-packed on a glass surface or shaped into a disk pellet (r = 1.06 ± 0.01) revealed a Θc of nearly 0° in both cases (Fig. 2h-i), confirming the hydrophilic character of this product and, by extension, the importance of the Leu Velcro-motif for conferring hydrophobicity to **1**.

Finally, to generalize our crystal-engineering strategy, we returned to the Leu-rich Asp N-terminus peptide family, from which we selected the tripeptide Asp-Leu-Leu as a new ditopic linker (Fig. 3a). Reaction of Asp-Leu-Leu and $Zn(NO_3)_2{\cdot}6H_2O$ in pure water at 85 °C for 48 hours yielded [Zn(Asp-Leu-Leu)(H_2O)] (**3**), which also incorporates the Leu Velcro motif (ESI†).

In **3**, the geometry around the Zn(II) ions can be described as a trigonal bipyramid ($\tau = 0.73$), in which two carboxylate-O atoms and the amino-N atom occupy the equatorial positions, and one carbonyl-O atom and the coordinated water molecule occupy the axial positions (ESI†, Fig. S7c). Like **1** and **2**, **3** also forms coordination layers that extend along the *ab* plane. In each of these layers the Leu isobutyl side chains point upwards and coordinated water molecules point downwards (Fig. 3b), or *vice versa*, thereby creating H-bonded (between the coordinated water molecules) dimeric-layers with upwards/downwards-pointing Leu residues (Fig. 3c, ESI†, Table S5). This configuration resembles the single layers of **1**, and thus, also generates the $\mathrm{Leu}_{\mathrm{inter}}$ and Velcro-like Leu motifs.

In **3**, the distances of the Leu isobutyl chains in the $\mathrm{Leu}_{\mathrm{inter}}$ motif are 6.027(78) Å and 6.200(5) Å, whereas the distances between the isobutyl side chains of two consecutive $\mathrm{Leu}_{\mathrm{inter}}$ motifs along the *a* axis in the Leu Velcro are 6.027(78) Å, 6.200(5) Å, 6.528(82) Å and 8.700(5) Å (ESI†, Fig. S9). Owing to this similarity, **3** exhibits similar hydrophobicity to that of **1**. Indeed, water contact-angle measurements revealed a Θc of

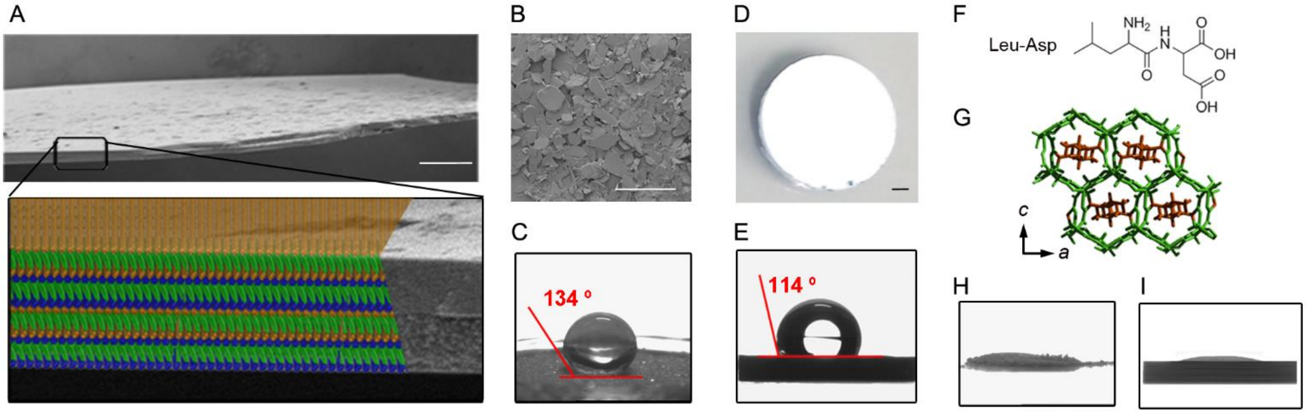

Fig. 2 a) Single-crystal face-indexing reveals that the predominant macroscopic crystal surface comprises Leu isobutyl side chains pointing upwards. The inset is a schematic representation of the disposition of the layers on the macroscopic crystals, the scale bar is 10 µm; b-e) Crystals of **1** were sonicated, and then carefully deposited onto a plastic surface for contact angle measurements on (001)-oriented crystals (b), which revealed a value of 134° (c). When the crystals were shaped into a pressed-pellet disk (d), the contact angle decreased to 114° (e). The scale bars are 200 µm (b) and 1.6 mm (c); f) The chemical structure of the peptide Leu-Asp; g) In **2**, Leu isobutyl side chains run parallel to the 2D-layer along the crystallographic *ac* plane; h-i) Contact-angle image of the hydrophilic compound **2** in the form of hand-packed crystals (h) and pressed-pellet disk (i).

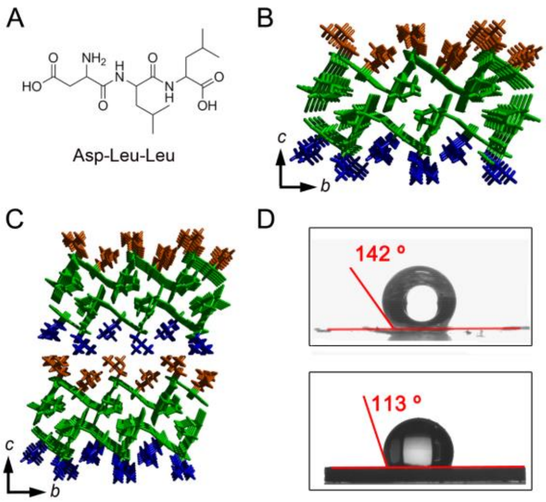

Fig. 3 a) The chemical structure of the Asp-Leu-Leu peptide; b) In **3**, 2D-layers are H-bonded through the coordinated water molecules, yielding dimeric-layers in which the Leu residues point up and down the *c* axis; c) The dimeric-layers generate Velcro-like Leu motifs along the *ab* plane; d) Contact angle image of hand-packed crystals of **3** (top) and of a pressed pellet disk of **3** (bottom). In b-c, the Leu isobutyl chains pointing upwards and downwards are colored in orange and blue, respectively.

142° ± 2 for crystals of **3** hand-packed on a glass surface, and a Ɵc of 113° ± 2 and a CAH of 33.4° for **3** shaped into a disk pellet (r = 1.12 ± 0.01) (Fig. 3d).

Conclusions

In conclusion, we have reported here that linking short Leu-containing peptides to metal ions affords coordination polymers that exhibit a repetitive, Leu-rich sequence, in which their isobutyl groups can assemble intro a novel, strongly hydrophobic, 2D Velcro-like Leu motif. This motif, which can be compared to the 1D Leu-zipper interaction found in proteins, confers the resulting materials with hydrophobicity. We believe that in the future coordination of peptides with metal ions can be a new strategy for finding new ways of assembling peptides, thus opening up new avenues for discovering new supramolecular and bioinspired materials with interesting properties such as hydrophobicity

Notes and references

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