Title: Mechanistic insights of evaporation-induced actuation in supramolecular crystals

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Abstract:

Water-responsive materials undergo reversible shape changes upon varying humidity levels. These mechanically robust, yet flexible structures can exert significant forces and hold promise as efficient actuators for energy harvesting, adaptive materials, and soft robotics. Here we demonstrate that energy transfer during evaporation-induced actuation of nanoporous tripeptide crystals results from strengthening of water H-bonding that drives the contraction of the pores. The seamless integration of mobile and structurally bound water inside these pores with a supramolecular network which contains readily deformable aromatic domains, translates dehydration-induced mechanical stresses through the crystal lattice, suggesting a general mechanism of efficient water-responsive actuation. The observed strengthening of water bonding complements accepted understanding of capillary force induced reversible contraction for this class of materials. These minimalistic peptide crystals are much simpler in composition compared to natural water-responsive materials, and the insights provided here can be applied more generally for the design of high-energy molecular actuators.

Main Text:

Water-responsive (WR) materials that exhibit dramatic structural responses to relative humidity (RH) changes open up opportunities to harvest and utilize evaporation energy¹⁻³, and can be used as high-efficiency RH-driven actuators for applications such as soft robotics and wearable devices^{4,5}. WR materials that have been described to date include both naturally derived and fully synthetic structures. For instance, a π -stacked carbon nitride polymer demonstrates extremely fast (50 ms) WR deformation⁴; zinc-based metal-organic frameworks (MOFs) have recently been developed for water sensing and as thermal imaging probes⁶; and carbon nanotube^{7,8} and graphene⁹-based materials show remarkably high WR energy densities.

In nature, WR behaviors are crucial for life-critical biological functions such as the opening and closing of pine cones¹⁰ or propelling of wheat awns into the ground¹¹. Several of these natural WR materials have been applied outside of their biological context and demonstrated powerful actuations with energy densities ranging from 8 kJ m⁻³ to 21.3 MJ m⁻³¹², which has enabled various applications, including macroscopic machines that are driven by evaporation energy¹. While nature continues to provide inspiration for the development of WR materials, the fundamental actuation mechanisms are not yet understood across the molecular to macroscopic length scales, thus hampering rational design and improvement of these materials.

The best-performing examples share a number of characteristics: they consist of both rigid nanocrystalline regions that are inter-dispersed with less-ordered amorphous (reconfigurable) regions; they typically contain repetitive sequences of hydrophilic and hydrophobic segments that strongly interact through supramolecular interactions; and they consist of hierarchical and porous architectures with high mechanical strength and toughness^{13,14}. Notably, the multi-scale,

hierarchical organization and amphiphilic aspects have been effectively exploited in synthetically derived WR actuators^{3,9}.

At the most fundamental level, a material's water-responsiveness is an energy conversion process that involves absorption and desorption of water molecules which is driven by the chemical potential difference between water bound to the material and that in the surrounding atmosphere. H-bonding and water-surface interactions, as well as reversible, non-covalent interactions within the material's structure are believed to be essential to their energy conversion processes in order to translate molecular interactions to material level deformations^{15,16}.

Designed supramolecular materials¹⁷, including those based on proteins and peptides^{18,19}, have been used as minimalistic mimics of biological materials to develop structures with dynamic porosity²⁰⁻²³, hierarchical organization^{24,25}, coexistence of ordered and disordered regions through H-bonding patterns^{26,27}, and dynamic water absorption and desorption capability^{22,28,29}. Thus, we hypothesized that supramolecular peptide materials could provide ideal systems to unveil the fundamental mechanisms, and specifically the role of porosity and supramolecular connectivity of nanoscale domains in water-responsiveness. Peptide-based materials have dramatically reduced molecular complexity and their sequence-dependent supramolecular interactions can be probed using spectroscopy and diffraction.³⁰

Preparation and Macroscale Water-responsiveness of Tripeptide Crystals

We selected four tripeptide sequences composed of dyads of aromatic residues^{31,32} – tyrosyl-phenylalanine (YF), complemented with amino acids histidine (H) or aspartic acid (D) to favor interactions with water; namely HYF (His-Tyr-Phe), YFH (Tyr-Phe-His), DYF (Asp-Tyr-Phe-NH₂), and YFD (Tyr-Phe-Asp-NH₂), (**Fig. 1a**)³². [²⁷: DYF and YFD were amidated at the carboxyl-terminus (C-terminus) to enhance their aqueous aggregation propensity]. HYF, DYF,

and YFD form needle-like crystals in a buffered solution within minutes (**Fig. 1b**, Methods, Extended Data Figure 1, and Supplementary Figure 1). By contrast, YFH gives rise to amorphous aggregates.

To test the WR behavior of these crystals in a macroscale experiment, a polyimide film was coated with a crystals/adhesive composite – see Materials for details of composite and film construction (**Fig. 1c**, Supplementary Figure 2-4, and Supplementary Note 1). Upon periodically alternating RH, we found that polyimide films with HYF and, to a lesser extent DYF, can reversibly bend, while YFD responds only weakly (**Fig. 1c-e** and Supplementary Video 1 and 2). Based on maximum curvatures of polyimide films, the WR actuation energy density of these HYF, DYF, and YFD crystals is estimated to be $\sim 88 \text{ kJ m}^{-3}$, $\sim 18 \text{ kJ m}^{-3}$, and $\sim 5 \text{ kJ m}^{-3}$, respectively, demonstrating clearly that peptide sequence strongly influences WR behavior (see Supplementary Note 2 and Supplementary Video 8).

We established that tripeptide crystals exhibit water-containing pores of varying size, and further exhibit variable supramolecular organization of peptide components within the crystal lattice. HYF's crystal structure was determined using single-crystal X-Ray diffraction (SC-XRD) (see Supplementary Table 1 and Methods) with the crystal structures of DYF and YFD reported previously²⁷. The crystal structure of HYF was found to be rhombohedral, with lattice parameters $a = b = 41.202(5) \text{ \AA}$, $c = 4.951(6) \text{ \AA}$, $\alpha = \beta = 90^\circ$, and $\gamma = 120^\circ$ (**Fig. 1f**, Supplementary Video 3, Supplementary Note 3, and Supplementary Table 1). We note that HYF crystals form parallel and triangular-shaped pores (28.3% of the unit cell volume) that contain water molecules with differing degrees of bonding and mobility (**Fig. 1, f and h**). In the xy-plane, the network is stabilized by H-bonding between the tyrosine hydroxyl and the C-terminus and between water molecules and both the amino-terminus (N-terminus) and the C-terminus

around the aqueous pores (**Fig. 1h**). Tripeptide molecules further interact through H-bonding, aromatic stacking, and electrostatic interactions in the z-direction (**Fig. 1g**). The areas between the pores show clusters of phenylalanine side chains which are readily deformable through aromatic slippage.

In contrast to HYF, previously reported DYF and YFD are both monoclinic systems (Extended Data Figure 2, Supplementary Table 1²⁷, and Supplementary Video 4 and 5). Tripeptide conformations and water inclusion within these crystals are substantially different. Specifically, within YFD, the N-terminus forms an intramolecular salt bridge with the aspartic acid side chain and the phenol/phenyl pair of YF form an intramolecular π -stack. This leads to less intermolecular connectivity and thus reduced water-responsiveness of YFD crystals. Beyond differences in intermolecular connectivity within the crystals, chemical structure and geometry of the aqueous pores also varies, which results in differential water-occupancy (**Fig. 1f** and Extended Data Figure 2). Compared to DYF, the ability of HYF to accommodate both ordered and disordered water molecules in pores that are interspersed with deformable aromatic regions (**Fig. 1h**)²³ correlates to a more significant macroscale response to changes in RH.

Water-responsiveness of Micron-scale Tripeptide Crystals

A customized atomic force microscope (AFM) that allows for rapid adjustment of local RH while monitoring the topographical change was used to quantify shape changes (**Fig. 2, a-b**). When the local RH is alternated between 10% and 90%, a HYF crystal of $\sim 2\ \mu\text{m}$ in width shows rapid actuation (1.2 s to expand upon hydration and 2.9 s to contract upon dehydration, **Fig. 2, b-c**). These response times are much faster compared to those of the macroscopic HYF/polyimide films (8.0 s for hydration and 109 s for dehydration, **Fig. 1, d-e**). This size dependence can be explained by faster water diffusion in smaller structures and the adhesive barrier for the

crystals/adhesive composites, complying with the classic Fick's law of normal diffusion. During repeated actuations, HYF crystals displayed a height change of ~8% (**Fig. 2, b and d**). The micron-scale tripeptide crystal structures have asymmetric morphology, thereby resulting in a non-uniform WR strain in different dimensions. Considering the size changes, we estimate that HYF's volume increases ~24.6% upon hydration (HYF) (**Fig. 2d** and Supplementary Figures 5), while DYF and YFD increase by ~13.9% and ~3.7%, respectively (**Fig. 2f** and Extended Data Figures 3 and 4), which is consistent with the trends observed in the polyimide film deformation experiment (**Fig. 1c**). The micron-scale HYF crystal's actuation energy density ($\sim 6.5 \text{ MJ m}^{-3}$, see calculation details in Methods and Supplementary Note 4) compares well with reported values for natural materials^{1, 33} and is higher than that observed in the macroscopic HYF/polyimide films due to the energy dissipation of the adhesive used in these films.

Mass changes as a result of water absorption and desorption during hydration/dehydration cycles were investigated using Dynamic Vapor Sorption (DVS), giving 148.2% for HYF (**Fig. 2, e and f**), 41.8% for DYF, and 11.3% for YFD (**Fig. 2f**, Extended Data Figures 3 and 4, and Supplementary Note 5). These results show substantially more water uptake than the volume expansions which were determined by AFM suggest. These differences are related to water adsorption at the next hierarchical level, *i.e.*, on crystal surfaces and interfaces between aggregated crystallites, which are also visible by SEM (Supplementary Figure 1) and evidenced by the hysteresis loop of water sorption isotherms analyzed using the Young-Nelson (YN) model (**Fig. 2e**, Extended Data Figure 3, d-e, i-j, Supplementary Figure 6, and Supplementary Note 6)^{34,35}.

To understand how changes in the RH affect the peptide scaffold, powder X-ray diffraction (PXRD) was performed within a T/%RH environment-controlled chamber to observe the water

induced lattice deformation (see Methods). For HYF crystals, since the stacking distance d is relatively short (**Fig. 1g**), the majority of diffracting planes of 2θ from 5° to 40° are parallel to the z -direction (**Fig. 1g** and Methods). During the dehydration processes, the diffraction peaks from polycrystalline HYF samples show a general shift to higher angles (**Fig. 2, g-h**, Supplementary Figure 7, and Supplementary Note 7), which is consistent with shrinkage of the lattice as the size of water pores is reduced. When RH is further decreased to $\sim 20\%$, major peaks lose their intensity and broaden considerably, which suggests a sudden phase change of HYF crystals. This abrupt change at $\sim 20\%$ RH also correlates to the size and mass changes observed in our AFM and DVS experiments (**Fig. 2, b-f**). In contrast, DYF crystals' diffraction pattern shows only minor shifts (Supplementary Figure 8), and YFD permanently loses its original crystalline structure (Supplementary Figure 9 and 10). Both HYF and DYF crystals fully regain their original diffraction patterns upon rehydration (Supplementary Figure 7 and 8).

The lattice parameter changes of HYF and DYF were calculated from a Pawley refinement of the individual PXRD diffraction patterns at each RH (**Fig. 2i**, Extended Data Figure 4, and Supplementary Figure 11). The refined cell parameters confirm that the HYF crystal's unit cell volume reduces by $\sim 7.5\%$ as %RH decreased from 90% to 10%. However, the AFM measurements show more than a 20% change in volume (**Fig. 2d**), which indicates that the tripeptide's polycrystalline structures and the non-uniformly orientated crystallites further amplify its lattice deformation (**Fig. 1f** and Supplementary Figure 1).

Molecular Level Water-responsiveness of Tripeptide Crystals

The reversible phase transitions for HYF and DYF were further investigated using Molecular Dynamics (MD) simulations (see **Fig. 3, a-e**, Methods, Extended Data Figure 5 and 6, and Supplementary Note 8). The simulated changes in pore dimensions agree with observed changes

in lattice parameters (a and b) from the Pawley refinement, which shows a dramatic shrinking and disordering of HYF pore structures starting from $\sim 20\%$ - 30% relative hydration (R. Hyd.) as water leaves (**Fig. 3, b and d**, and Supplementary Video 6). Upon rehydration, the pore regains its original structure, owing to the HYF crystals' reversible intermolecular interactions. In contrast, DYF shows no appreciable deformation of its pore structure (**Fig. 3, c and e** and Supplementary Video 7).

Environmental-controlled Fourier Transform Infrared Spectroscopy (FTIR) further revealed dehydration-induced changes in intermolecular bonding interactions within the peptide structures. We found that, during dehydration, HYF crystals' amide I band shifts to a lower frequency, and the amide II band shifts to a higher frequency (**Fig. 3f** and Supplementary Figure 12), indicating a strengthening in H-bonding interactions between peptides upon dehydration^{36,37}. This observation is consistent with the decrease in the H-bond distances that were determined by the PXRD refinement and MD simulations of the pore. As the RH drops below 20% , the H-bond lengths decrease and the overall dimensions of the pore contract (**Fig. 3d**). However, there were no observable changes in the amide I and amide II bands for DYF and YFD crystals (**Fig. 3g**, Supplementary Figure 13 and 14), suggesting less elastic energy is transferred to DYF and YFD structures than that to HYF. Once again, this is consistent with the PXRD and MD simulations that show no effective change in the dimensions of the pore as a consequence of the decrease in RH. (**Fig. 3e**).

Dehydration-induced H-bonds Strengthening

To further understand the water-peptide interactions at the pore surface during HYF's WR expansion and contraction, we investigated the H-bond interactions between primary intrinsic water and the exposed H-bonding sites that form the surface of the aqueous pores using MD

simulation (**Fig. 4, a-f**, Supplementary Figure 15 and 16, Methods, and Supplementary Note 8).

As the hydration level is decreased from 100% to 10%, paradoxically, the occupancy of the H-bonding sites dramatically increases (**Fig. 4, c and e**, Extended Data Figure 5, and Supplementary Figure 17), indicating that less water is able to exchange with pore surface H-bonding donors and acceptors and the proportional interaction energy between the water and peptide consequently increases (**Fig. 4d**, Methods, and Supplementary Note 8). We note that while water's cohesive energy generally will include multiple effects^{38,39}, the dominant role that H-bonding has in this process leads us to use the term H-bonding energy as a useful proxy for this study. After a ~10% increase in the H-bonding occupancy with the initial reduction in R. Hyd. from 100% to 90%, the H-bonding occupancy remains stable until ~30% R. Hyd., and finally shows a significant increase at the lowest hydration levels (**Fig. 4c**). This is particularly evident in the case of the terminal phenylalanine carboxylate group (OT, **Fig. 4, b-d**) and the backbone nitrogen of histidine (N, **Fig. 4, b-d**) in which a 20% increase in the occupation level is observed. This ability of water to bond more strongly to the nanopore surface as the hydration level decreases suggests that they are able to transfer the pressure and work done by mobilized water. This ductile structure allows the system to undergo multiple deformation cycles without compromising the internal structure of the material. This observation is confirmed by the difference between the chemical potential inside and outside the pore observed in the MD simulations. As the simulation equilibrates, water redistributes between the inside and the outside of the pore dictated by the difference in the chemical potential. This is evidenced by a comparison of the average number of water molecules in the cross-section of the pore over the simulation frames (**Fig. 4f**). Comparing 10% R. Hyd. to 100% R. Hyd., the overall quantity of water molecules in the system is reduced by 90%, but the average quantity of water in the pore is

reduced by only around 50% as the disordered water molecules are removed from the pore and only those forming the first solvation shell of the peptide network remain, leading to a contraction of the pore (**Fig. 3d**).

This understanding is consistent with the lower WR behavior observed for the DYF crystals, where the pore size is significantly smaller, and the majority of the H-bonding sites exist between the DYF monomers with only the aspartate side chain and the aspartate backbone nitrogen interacting with water molecules (**Fig. 4, g and h**). In contrast to HYF crystals, the occupancy of these H-bonding sites decrease from ~30% at 100% R. Hyd. to ~15% at 10% R. Hyd. (**Fig. 4, i-k**, Extended Data Figure 6, and Supplementary Figure 18). The weaker interactions between the residues at the pore surface and water molecules result in a reduction in the ability to translate energy into structures' elastic energy, which is seen through the consistent reduction in the average number of water molecules in the cross-section of the pore as the humidity decreases (**Fig. 4l**). Our observations suggest that the H-bonding network in the aqueous nanopores between water and the peptide pore surface is crucial to water-responsiveness. Further evidence was provided by exchanging H₂O with D₂O which dramatically reduced HYF's WR behaviors - shifts in amide I and II bands were not observed in FTIR (Supplementary Figure 19) and macroscopic experiments on polyimide films showed significant reduced actuation (Supplementary Figure 20).

Outlook

We propose that the crucial role of an integrated H-bonding network at the material/water interface which is connected with deformable regions, as we observed in HYF crystals could also be relevant to other naturally occurring and synthetic materials. Clearly, the maximum energy density of a WR material is primarily determined by the total amount of absorbed/desorbed water

molecules and their chemical potential during hydration/dehydration cycles. To efficiently convert the chemical potential to mechanical energy, the H-bond network should be strong enough to transfer pressure and volume changes induced by water absorption and desorption to surrounding supramolecular structures - which themselves should be readily deformable - as we observed in HYF crystals. The hierarchical organizations can provide high mechanical stiffness (Supplementary Table 2) and ductility to effectively store energy elastically or transfer it to external loads. These findings provide insights of nature's powerful WR behaviors, and could serve as design criteria for synthetic approaches to develop high energy WR actuators.

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Figure Legends

Fig. 1. Tripeptide crystals exhibit water-responsiveness. (a) Chemical structures of HYF, DYF, and YFD. (b) Tripeptides crystallized in phosphate buffer (PB) at pH 8, scanning electron microscope images (scale bar, 2 μm) of HYF, DYF, and YFD crystals. (c) Crystal-coated polyimide films reversibly curve and straighten in response to RH changes (scale bar, 2 mm). (d, e) WR actuation behavior of HYF coated polyimide film in response to alternating humidity and its hydration and dehydration speed (e). (f) HYF exhibits a rhombohedral crystal structure with intrinsic trigonal aqueous pores, boundaries are colored blue. Edge length of ~ 15.2 Å, pore volume is 2062 Å³, corresponding to 28.3% of the unit cell volume. (g) Cross-section view of (f) shows that tripeptides are arranged through H-bonding and aromatic stacking in the z-direction, and the distance d between layers in hydrated state is $4.951(6)$ Å. Blue lines indicate tripeptide-tripeptide H-bonds. (h) Top view shows tripeptide molecules are interacting through H-bonding and structured water molecules in the xy-plane. Red lines indicate water-water and water-tripeptide H-bonds. Blue lines indicate tripeptide-tripeptide H-bonds. Error bars represents standard deviations calculated from three measurements.

Fig. 2. WR properties of micron-scale tripeptide crystals. (a, b) AFM topography measurement shows the expansion of HYF crystals. The dynamic height change in (b) was monitored while local RH was rapidly alternated between 10% and 90. (c) HYF's hydration and dehydration speed. (d) Volume and height vs. %RH of HYF. (e) DVS water sorption isotherms of HYF crystals. Mass change vs. RH curve show hysteresis between 20% RH and 40% RH during hydration and dehydration cycles. (f) % volume and mass change of HYF, DYF and YFD between 10% and 90% RH. The volume change in (d) is estimated from the height, width, and

length changes of individual crystals. (g) PXRD showing diffraction pattern changes upon rehydration. Zoom-in of $(7\bar{5}0)$ plane diffraction indicating shift towards higher 2θ values (h). (i) % lattice parameter changes of HYF and DYF between 10% and 90% RH from Rietveld refinement of the PXRD data. Error bars represent standard deviations calculated from three measurements.

Fig. 3. Reversible WR deformation of tripeptide crystals at molecular level. (a) MD simulated crystal structure on an aqueous pore structure of HYF crystals at 90% RH. (b, c) Comparisons of the HYF (b) and DYF (c) lattice parameter changes a/b derived from the Pawley refinement with MD simulated pore width in response to RH (R. Hyd.) changes. (d, e) Representation of the change in the internal pore volume for HYF (d) and DYF (e). The change in the area for each cross section was determined through the decrease in the average pore width. (f, g) FTIR spectra of HYF (f) and DYF (g) shows that HYF's amide I and amide II bands shifting during dehydration process, whereas DYF remain unchanged. Error bars represent standard deviations calculated from three measurements.

Fig. 4. Dehydration induced H-bonds strengthening in HYF. (a, e, g, k) Top views of MD simulated HYF and DYF showing water molecules occupancy. (b, h) Tripeptide residues of HYF and DYF that form H bonding with water. HYF's symmetric nature of the pore results in three repeats of the same residues forming the circumference of the pore (a), with each residue in the tripeptide structure offering an H-bonding potential with water (e). H-bonding occurrence (c, i) and energy (d, j) of HYF and DYF side chains vs % RH. (f, l) Average number of water molecules in the cross-section of the pore over the simulation frames vs. R. Hyd. Error bars represent standard deviations calculated from three measurements.

Methods:

Materials and crystal preparation: Tripeptides were purchased from CSBio (purity, > 95%) All solvents were bought from Sigma Aldrich. To obtain peptide micro-crystals the YFD, DYF and HYF tripeptides were dissolved to 20 mM in 0.1 M phosphate buffer at pH 8, vortexed and sonicated. Tripeptides micro-crystals spontaneously formed in the buffered solution within 20 minutes at room temperature. In order to obtain larger crystals for single crystal X-ray diffraction (Extended Data Figure 1), 5 mM of tripeptide solid was dissolved in 0.1 mM PB, heated to 75 °C, left to cool down to room temperature for 72 hrs and kept at 4 °C. All the obtained crystals were needle-shaped. For characterization experiments the crystals were washed twice in fresh PB buffer (pH 8).

Tripeptide crystals/polyimide films: The macroscale actuation behavior of the crystals was demonstrated by gluing the peptide crystals onto polyimide sheets. Briefly, the 8 μm thick polyimide sheet substrate (SPEX) was cut into rectangular pieces of 5 mm length and 3 mm width. The substrates were then cleaned with deionized water and ethanol and allowed to dry for 120 seconds. The polyimide substrates were subsequently placed in a plasma chamber (Fischione M1070 NanoClean) for ionization, thus allowing the surface to hold charge and attract the solution to be deposited more easily. Plasma oxidation was run at 100% power with 75% argon and 25% oxygen gas for 90 seconds. The tripeptide crystals with dimensions ~ 1.5 μm in width and length ranging from ~ 30 μm to 110 μm (Supplementary Figure 2) were mixed with Elmer's glue solution (Elmer's Products), and resultant slurry was applied on the substrate using a micropipette (two layers of 13 μL crystals/adhesive mixture). The films coated with crystals/adhesive slurry were then allowed to dry to 40% RH. Photographs of the curved films

were obtained using a digital camera (Canon EOS Rebel SL1). The curvature of the films was analyzed using Image J software (NIH Image). The energy density was calculated based on the elastic energy of curved films and the volume of peptide crystals(see calculation details in Supplementary Note 1).

WR speed determination: WR speeds of tripeptide crystals in macro and micro scales were determined using a humidity-controlled chamber and an Atomic Force Microscope (AFM) (Multimode 8, Bruker), respectively. RH was rapidly alternated between 10% and 90% by injecting dry lab air and water-saturated air, controlled by a solenoid valve (VK332Y-6G-M5, SMC) programed by LabVIEW (National Instruments). A commercial RH sensor (HIH-4021-003, Honeywell) was placed near samples to continuously monitor local RHs. Tripeptide crystals' macroscale response was recorded using a digital camera. The micron-scale response was measured by monitoring crystals' dynamic height changes using an AFM probe (SCANASYST-AIR) placed on top surfaces of crystals. The time constants τ were calculated by fitting the obtained hydration and dehydration curves to exponential decay /growth functions ⁴⁰:

$$y = Ae^{-x/\tau} \quad (1)$$

$$y = A(1 - e^{-x/\tau}) \quad (2)$$

where A is the actuation amplitude.

Single crystal X-ray structure determination: Single crystal data were acquired on a Bruker SMART APEX II diffractometer equipped with a CCD detector. The X-ray beam generated from a sealed Mo tube was monochromated by a graphite crystal and collimated by a MonoCap collimator. The wavelength of the incident beam is 0.71073 Å ($\lambda_{\text{Mo-K}\alpha}$). The crystal temperature

(100 K) was controlled by an Oxford Cryosystems 700+ Cooler. The HYF, DYF and YFD crystals were mounted on a 0.15 mm MicroMount loop (MiTeGen) for measurement. The 2-dimensional diffraction image data were processed using the APEX2 software (version 2013.12) for data reduction, correction and unit cell refinement. The structure was solved by SHELXT (Sheldrick 2015) and refined with full-matrix least squares by SHELXL (Sheldrick 2017). Non-hydrogen atoms were refined with anisotropic displacement parameters, and hydrogen atoms on carbon atoms and N1 and N3 were placed in idealized positions and refined with riding models, and hydrogen atoms on N4, N5, and O3 were found from DF maps and refined with a restrained distance of N-H 0.86 Å and O-H 0.84 Å. All water molecules are disordered and their occupancies were refined. The selected crystal data were listed in Table S1. The crystallographic information files (CIFs) of HYF were deposited in the Cambridge Crystallographic Data Centre (CCDC, no. 1917273). The crystal structures of DYF and YFD reported previously²⁷ can be found in the CCDC with the numbers of 1539905 (DYF) and 1539906 (YFD).

Micron-scale crystals' WR strain measurement: The WR strain was characterized by using an AFM (Bruker), where local RH was controlled by injecting air with a certain ratio of dry lab air and water-saturated air. After local RH is stabilized, crystals' topologies were obtained using an AFM probe (SCANASYST-AIR, Bruker) under the Peak Force Tapping mode. Crystals' WR strain was analyzed by comparing their height at certain RHs to their initial height at 10% RH.

Stiffness measurement: The stiffness of tripeptide crystals at various RHs was characterized by performing the AFM Nano-indentation using a MESP-V2 probe (Bruker). Prior to measurements, the crystals were washed twice in the phosphate buffer. The spring constant of

the cantilever was calibrated using a thermal tune method, and the average value was 2.5 N/m. Washed crystals were deposited on top of a silicon wafer and left in a dry environment overnight. The RH was adjusted using dry and humid air and continuously monitored by a humidity sensor (HIH-4021-003, Honeywell) placed in the AFM chamber. When RH is stabilized at a certain level, force vs. indentation depth curves were obtained and analyzed by Nanoscope software (Bruker). Young's modulus was calculated by using the Hertz model:

$$(F)^{2/3} = \left(\frac{4}{3} \frac{E}{(1-\nu)^2} \sqrt{R}\right)^{2/3} \delta \quad (3)$$

where F is the indentation force, E is the Young's modulus, ν is the Poisson ratio, R is the tip radius, and δ is the indentation depth.

Micron-scale HYF crystals' energy density estimation: The energy density of the micron-scale HYF crystal is estimated by its elastic energy difference between 10% RH and 90% RH:

$$U_m = \frac{1}{4} (E_{10} + E_{90}) \varepsilon^2 \quad (4)$$

where E_{10} and E_{90} are Young's moduli of HYF crystal at 10% RH and 90% RH, respectively. ε is the WR strain between 10% RH and 90% RH along the thickness direction.

Powder X-ray diffraction analysis: XRPD data was collected on a Bruker D8 Discover diffractometer fitted with an Anton Paar CHC+ variable temperature chamber and variable humidity generator. The temperature was stable and set for 25 °C. The collimated X-rays were generated from a Cu tube ($\lambda_{\text{Cu-K}\alpha} 1.540596 \text{ \AA}$). The samples were prepared by grinding single crystals to fine powders in a mortar. The diffraction patterns for crystallized peptides were obtained for two humidity controlled cycles by 10% step increase/decrease.

Fourier-transformed infrared spectroscopy: Peptide crystals were applied between two 2 mm thick CaF₂ windows and separated by a 27 μ m polytetrafluoroethylene spacer and mounted into a flow cell (Harrick Scientific). The FTIR spectra were acquired in a Bruker Vertex 70 spectrometer with a spectral resolution of 2 cm⁻¹. The humidity sensor was placed in close proximity to the sample and a stream of humid air passed through the flow cell to alter humidity. All spectra were corrected to the phosphate buffer. For peptide crystal analysis we assumed constant temperature throughout the spectra collection to avoid thermal artifacts. For the H₂O/D₂O exchange experiment, HYF crystals were exposed to an environment with saturated D₂O for 24 hours. After the FTIR spectra collection for two hydration and dehydration cycles in D₂O, the same sample was re-exposed to H₂O, and data were collected during another two cycles.

Dynamic vapor sorption: Water vapor sorption isotherms (sorption/desorption, 2 cycles) were measured on an SMS DVS Advantage 1 (Surface Measurement Systems, Middlesex, UK) at a test temperature of 25°C. Cycles were carried out by 10% step RH increase/decrease. Samples of starting mass *ca.* 0.3 mg were added to 10 mm diameter quartz pans. Automatic endpoint determination was used for each incremental isotherm step set to 0.002% dm/dt for all steps, with a minimum time per step of 10 min and maximum time per step of 4 h. A nitrogen carrier gas was used at a total gas flow rate of 200 cm³/min the duration of the experiment. Images were captured at the end of each stage to record any visible changes in the samples. DVS Advantage Advanced Analysis Suite version 7.0.13.1 was used to analyse the data to produce a % mass vs actual % RH plot, and an isotherm plot. The sample mass at the end of the test at 5% RH was used as the zero mass for calculations of % mass change.

HYF molecular dynamics simulations: A single channel from the crystal structure was studied in order to investigate the effect of hydration on the dimensions of the crystal. The crystallographic information file (CIF) obtained from the single-crystal X-ray crystallography experiment was opened in CCDC Mercury 3.10.3 and visualised using the crystal packing function. The packed crystal structure was written to a protein data bank (PDB) file, which was then annotated with CHARMM residue and atom names. This channel was comprised of 10 stacked crystals in the Z axis (corresponding to the cell's 'c' vector), including all HYF monomers in direct contact with the water in the pore and those immediately adjacent to these (in order to help maintain the structural integrity of the pore). This channel was surrounded by 15 Angstroms of water at standard density using the solvate plugin for VMD 1.9.3. This structure was taken to be 100% hydration. A protein structure file (PSF) for the CHARMM36 forcefield was generated using the psfgen plugin for VMD. To change the hydration level, water was removed at random such that only n% of the original water remained, where n was the intended hydration level. The hydration levels simulated were 0%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%. Given that this approach is not strictly equivalent to measuring the relative humidity experimentally, we distinguish in the text and figure captions between the experimental RH and the computational relative hydration (R. Hyd.). Each of these hydration levels was equilibrated in the NVT ensemble at 300 K for 20 ns (with a 2 fs timestep) using NAMD 2.12 and the CHARMM36 forcefield for proteins.⁴ The SETTLE algorithm was used to keep water molecules rigid. Bonds between hydrogen atoms and their bonded heavy atoms were also constrained. The Langevin thermostat was used to maintain the temperature. The central six layers of the crystal were analyzed to see how the inter-layer distance (the average of the backbone C-N distances between layers) and the width of the pore

(the average of the histidine CE - tyrosine CE distances, used as a proxy for cell '*a*' and '*b*' vectors) varied over the course of the simulation. The MDAnalysis package for Python was used to probe these distances.

Hydrogen bond analysis and interpretation: To investigate the hydrogen bonding of the residues closest to the internal surface of the pore, the presence of hydrogen bonds to solvent was investigated for each relative hydration simulation. These bonds were measured using the MDAnalysis library for Python. Hydrogen bonds were defined as a hydrogen bond length of less than 3 Å and a donor-H-acceptor angle of greater than 120° (the default settings). For selected residues, H-bonds were monitored across the 6 central layers for each frame. The average was taken over these six layers across the rotationally symmetric sites in the pore. As simulations were performed in triplicate for each R. Hyd., analysis was performed separately for each trajectory and a mean hydrogen bond formation incidence was obtained for each site, along with a standard deviation. The H-bonding energy is calculated from the occupancy of the site using the Boltzmann factor. That is, we do not rely specifically on the force field interaction terms to extract the H-bonding energy from the potential energy function. Rather, we apply the Boltzmann distribution to determine the potential energy of the H-bond associated with a given probability for the site to be occupied. Note that the potential energy is estimated by proportional probabilities (Boltzmann factor) averaged from a number of frames in the simulation at each states. For this purpose, we assume a proportionality constant of 1. Thus, although the absolute cohesive energy of the water may be proportionally larger or smaller than the reported value, the trends and relative magnitudes of the interactions are correct. Further details of the water occupancy analysis are contained in the Supplementary Information.

Data availability: The data supporting the findings of this study are included in the main text and in the Supplementary Information files, and are also available from corresponding author upon reasonable request. Crystallographic data of HYF can be found in the Cambridge Crystallographic Data Centre (CCDC) with accession number 1917273. The crystal structures of DYF and YFD reported previously²⁷ can be found in the CCDC with accession numbers 1539905 (DYF) and 1539906 (YFD).

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Author contributions: X.C. and R.V.U. conceived and initiated the project. R.P. grew tripeptides crystals. R.P. and S.M. performed FTIR measurements and analyzed the data. T.H. and T.T. performed MD simulations. H.W. and R.P. performed AFM measurements and analyzed the data. A.M. and D.B. conducted PXRD and DVS experiment and data analysis. C.Z. initiated the crystallization of HYF. C.T.H. conducted SC-XRD experiments and solved single crystal structures. T.W. and X.C. characterized crystal morphologies. All authors contributed to data analysis and discussed the results. R.P., T.T., R.V.U., and X.C. wrote the paper. T.T., R.V.U., and X.C. supervised the project.

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612 **Competing interests:** Authors declare no competing interests.