

Supporting Information

Sequence-specific response of collagen mimetic peptides to osmotic pressure

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TABLE OF CONTENTS

Estimation of the water content in hexagonally packed triple helices	S3
Figure S1. Amino acid sequence of human collagen I	S4
Figure S2. Experimental setups for the <i>in situ</i> control of the relative humidity.....	S6
Figure S3. Setup for the molecular dynamics simulations.....	S7
Table S1. Comparison of (PPG) ₁₀ structural parameters obtained from XRD and MD at dry conditions	S8
Figure S4. Validation of the lattice plane assignment.	S9
Figure S5. Osmotic pressure response of (POG) ₁₀ and (OOG) ₁₀	S10
Figure S6. Comparison of the lateral packing arrangement of (POG) ₁₀ in the dehydrated and fully hydrated state.	S11
Figure S7. Osmotic pressure effects on the interchain hydrogen bond.	S12
Figure S8. Osmotic pressure response of (PPG) ₄ -ARGSDG-(PPG) ₄	S13
References	S14

Estimation of the water content in hexagonally packed triple helices

To estimate the hydration level in the XRD experiments, it is necessary to know the molecular volume of one tripeptide repeat unit \bar{V}_t and that of the water molecules \bar{V}_w around the triple helices. In the following, we describe the general procedure used to estimate the number of water molecules per amino acid (N_w/N_{AA}) for hexagonally packed CMPs, using $(PPG)_n$ as an example. The same procedure can be used to estimate N_w/N_{AA} for $(POG)_n$ and $(OOG)_n$.

The molar volume of a PPG tripeptide unit in its triple helical state is $169.4 \text{ cm}^3 \text{ mol}^{-1}$,¹ which corresponds to $\bar{V}_t = 281.3 \text{ \AA}^3$. Assuming that PPG is a cylinder with an effective radius r_{eff} , and considering that $(PPG)_n$ adopts a 7/2 structure, the diameter of the helix can be written as

$$r_{eff} = \sqrt{\frac{V}{\pi h}}$$

where V is the volume of the cylinder and h its height. For a 7/2 structure, 7 triplets correspond to a height of $\sim 20 \text{ \AA}$. With $V = 7\bar{V}_t$, the effective diameter equals $2r_{eff} = 1.12 \text{ nm}$.

\bar{V}_w was estimated from crystallographic data (PDB 1a3j²) as

$$\bar{V}_w = \frac{(V_{cell} - n_h \cdot n_t \cdot \bar{V}_t)}{n_w}$$

where n_h is the number of triple helices and n_t is the number of tripeptide units per helix. V_{cell} is the volume of the unit cell and n_w is the number of water molecules in the unit cell. For example, considering a unit cell of the structure PDB 1a3j² with $n_h = 4$, $n_t = 7$ and $n_w = 160$, \bar{V}_w is estimated to be $\sim 42 \text{ \AA}^3$, which is significantly larger than for bulk liquid water ($\sim 30 \text{ \AA}^3$). In the same PDB entry, the total number of amino acids in the unit cell is 84 (i.e. $3 \cdot n_h \cdot n_t$). This ultimately yields $N_w/N_{AA} = 1.9$. Similarly, $N_w/N_{AA} = 2.0$ for another crystal structure of $(PPG)_{10}$ (PDB 1k6f³). Also for $(POG)_{11}$ (PDB 1v6q⁴), 42 water molecules were found for 7 tripeptide units, which again yields $N_w/N_{AA} = 2.0$.

In the next step, we use \bar{V}_w and r_{eff} to assess the hydration level (N_w/N_{AA}) of the CMPs in a hexagonal arrangement. In the geometry shown in Figure 1, the red triangular element contains 0.5 helices. As the volume of a helix is $V = \pi h r_{eff}^2$, and considering again a 7/2 structure, N_w/N_{AA} can be calculated as

$$\frac{N_w}{N_{AA}} = \bar{h} \frac{\left(\frac{\sqrt{3}}{2} a^2 - \pi r_{eff}^2 \right)}{\bar{V}_w}$$

where \bar{h} is the height of the triple helix divided by the number of amino acid contained therein (for a 7/2 structure, \bar{h} is $20/(7 \cdot 3) \text{ \AA}/AA$). For $a = 1.28 \text{ nm}$, i.e. the center-to-center distance at the highest experimentally obtained $R.H.$, $N_w/N_{AA} \approx 1.1$. At $N_w/N_{AA} = 0.8$ and $N_w/N_{AA} = 0.4$, the center-to-center distance is 1.23 nm and 1.15 nm . This corresponds to an intermediate $R.H.$ of $\sim 60 \%$ and very dry conditions, respectively. It must be noted that the considered volumetric relationships may be different at low hydration levels, as the partial molar volume of water may change significantly in this hydration range. Therefore, the reported calculations are not exact. They are only used here as a tool to roughly estimate the water content in hexagonally packed helices.

$\alpha 1$ GPMGPSGPRGLOGPOGAOGPQGFQGPOGEOGEOGASGPMGPRGPOGPOGK
 $\alpha 1$ GPMGPSGPRGLOGPOGAOGPQGFQGPOGEOGEOGASGPMGPRGPOGPOG
 $\alpha 2$ GPMGLMGPARGPOGAAAGAOGPQGFQGPAGEOGEOGQTGPAGARGPAGPO

$\alpha 1$ NGDDGEAGKPGROGERGPOGPQGARGLOGTAGLOGMKGHRGFSGLDGAKG
 $\alpha 1$ KNGDDGEAGKPGROGERGPOGPQGARGLOGTAGLOGMKGHRGFSGLDGAKG
 $\alpha 2$ GKAGEDGHOGKPGROGERGVVGPQGARGFOGTOGLOGFKGIRGHNGLDGL

$\alpha 1$ DAGPAGPKGEOGSOGENGAOGQMGPARGLOGERGROGAOGPAGARGNDGAT
 $\alpha 1$ GDAGPAGPKGEOGSOGENGAOGQMGPARGLOGERGROGAOGPAGARGNDGGA
 $\alpha 2$ KGQOGAPGVKGEOGAOGENGTOGTTGARGLOGERGRVGAOGPAGARSDG

$\alpha 1$ GAAGPOGPTGPAGPOGFOGAVGAKGEAGPQGPRGSEGPQGVRRGEOGPOGP
 $\alpha 1$ TGAAGPOGPTGPAGPOGFOGAVGAKGEAGPQGPRGSEGPQGVRRGEOGPOG
 $\alpha 2$ SVGPVGPAGPIGSAGPOGFOGAOGPKGEIGAVGNAGPAGPAGPRGEVGLO

$\alpha 1$ AGAAGPAGNOGADGQOGAKGANGAOGIAGAOGFOGARGPSGPQGPGGPOG
 $\alpha 1$ PAGAAGPAGNOGADGQOGAKGANGAOGIAGAOGFOGARGPSGPQGPGGPO
 $\alpha 2$ GLSGPVGPOGNPGANGLTGAKGAAGLOGVAGAOGLOGPRGIGOPVGAAGA

$\alpha 1$ PKGNSGEOGAOGSKGDTGAKGEOGPVGVQGPOGPAGEEGKRGARGEOGPT
 $\alpha 1$ GPKGNSGEOGAOGSKGDTGAKGEOGPVGVQGPOGPAGEEGKRGARGEOGP
 $\alpha 2$ TGARGLVGEOGPAGSKGESGNKGEOGSAGPQGPOGPSGEEGKRGPNGEAG

$\alpha 1$ GLOPOGERGGOGSRGFOGADGVAGPKGPAGERGSOGPAGGPKGSOGEAGR
 $\alpha 1$ TGLOGPOGERGGOGSRGFOGADGVAGPKGPAGERGSOGPAGGPKGSOGEAG
 $\alpha 2$ SAGPPGPOGLRGSOGSRGLOGADGRAGVMGPPGSRGASGPAGVRGPNGDA

$\alpha 1$ OGEAGLOGAKGLTGSOGSOGPDGKTGPOGPAGQDGROGPPGPOGARGQAG
 $\alpha 1$ ROEAGLOGAKGLTGSOGSOGPDGKTGPOGPAGQDGROGPPGPOGARGQA
 $\alpha 2$ GROEOGLMGPRGLOGSOGNIGPAGKEGPVGLOGIDGROGPIGPAGARGE

$\alpha 1$ VMGFOGPKGAAGEOGKAGERGVOGPOGAVGPAGKDGEAGAOGPOGPAGPA
 $\alpha 1$ GVMGFOGPKGAAGEOGKAGERGVOGPOGAVGPAGKDGEAGAOGPOGPAGP
 $\alpha 2$ PGNIGFOGPKGPTGDOGKNGDKGHAGLAGARGAOGPDGNNGAOGPOGPQ

$\alpha 1$ GERGEQGPAGSOGFQGLOGPAGPOGEEAGKOGEQGVODLGAOGPSGARGE
 $\alpha 1$ AGERGEQGPAGSOGFQGLOGPAGPOGEEAGKOGEQGVODLGAOGPSGARG
 $\alpha 2$ VQGGKGEQGPPPGPOGFQGLOGPSGPAGEVGKOGERGLHGEFGLOGPAGPR

$\alpha 1$ RGFOGERGVQGPOGPAGPRGANGAOGNDGAKGDAGAOGAOGSOGAOGLOG
 $\alpha 1$ ERGFOGERGVQGPOGPAGPRGANGAOGNDGAKGDAGAOGAOGSOGAOGLO
 $\alpha 2$ GERGPPGESGAAGPTGPIGSRGSPSGPOGPDGNKGEPEGVVGAVGTAGPSGP

$\alpha 1$ MOGERGAAGLOGPKGDRGDAGPKGADGSPGKDGVRGLTGPIGPOGPAGAO
 $\alpha 1$ GMOGERGAAGLOGPKGDRGDAGPKGADGSPGKDGVRGLTGPIGPOGPAGA
 $\alpha 2$ SGLOGERGAAGIOGGKGEKGEPEGLRGEIGNPGRDGARGAOGAVGAOGPAG

$\alpha 1$ GDKGESGSPGAGPTGARGAOGDRGEPGPOGPAGFAGPOGADGQOGAKGE
 $\alpha 1$ OGDKGESGSPGAGPTGARGAOGDRGEPGPOGPAGFAGPOGADGQOGAKG
 $\alpha 2$ ATGDRGEAGAAGPAGPAGPRGSPGERGEVGPAGPNGFAGPAGAAGQOGAK

 $\alpha 1$ OGDAGAKGDAGPOGPAGPAGPOGPIGNVGAOGAKGARGSAPOGATGFOG
 $\alpha 1$ EODAGAKGDAGPOGPAGPAGPOGPIGNVGAOGAKGARGSAPOGATGFO
 $\alpha 2$ GERGAKGPKGENGVVGPPTGPVGAAGPAGPNPPGPPPAGSRGDGGPPGMTGF

 $\alpha 1$ AAGRVGPOGPSGNAGPOGPOGPAGKEGGKGRGETGPAGROGEVVGPOGPO
 $\alpha 1$ GAAGRVGPOGPSGNAGPOGPOGPAGKEGGKGRGETGPAGROGEVVGPOGP
 $\alpha 2$ OGAAGRTGPOGPSGISSGPOGPOGPAGKEGLRGPRGDQGPVGRTGEVGAVG

 $\alpha 1$ GPAGEKGSOGADGPAGAOGTPGPQGIAGQRGVVGLOGQRGERGFOGLOGP
 $\alpha 1$ OGPAGEKGSOGADGPAGAOGTPGPQGIAGQRGVVGLOGQRGERGFOGLOOG
 $\alpha 2$ PPGFAGEKGPSGEAGTAGPOGTPGPQGLLGAOGILGLOGSRGERGLOGVA

 $\alpha 1$ SGEOGKQGPSGASGERGPOGPMGPOGLAGPOGESGREGAOGAEGSOGRDG
 $\alpha 1$ PSGEOGKQGPSGASGERGPOGPMGPOGLAGPOGESGREGAOGAEGSOGRD
 $\alpha 2$ GAVGEOGPLGIAGPOGARGPOGAVGSOGVNGAOGAAGRDNOGNDGPOGR

 $\alpha 1$ SOGAKGDRGETGPAGPOGAOGAOGAPGPVGPAGKSGDRGETGPAGPTGPV
 $\alpha 1$ GSOGAKGDRGETGPAGPOGAOGAOGAPGPVGPAGKSGDRGETGPAGPTGP
 $\alpha 2$ DGQOGHKGERGYOGNIGPVGAAGAPGPHGPVGPAGKHGDRGETGPSPGVG

 $\alpha 1$ GPVGARGPAGPQGPRGDKGETGEQDRGIKGRGFSGLQGPOGPOGSOGE
 $\alpha 1$ VGPVGARGPAGPQGPRGDKGETGEQDRGIKGRGFSGLQGPOGPOGSOG
 $\alpha 2$ PAGAVGPRGSPGQGIKGRGDKGETGEQDRGIKGRGFSGLQGLPGLKGHNGLQGLPGIAGHH

 $\alpha 1$ QGSPGASGPAGPRGPOGSAGAOGKDGLNGLOGPIGOOGPRGRTGDAGPVG
 $\alpha 1$ EQGSPGASGPAGPRGPOGSAGAOGKDGLNGLOGPIGOOGPRGRTGDAGPV
 $\alpha 2$ GDQAPGSVGPAGPRGPAGPSGPAGKDGRTGHPGTVPAGIRGPQGHQGP

 $\alpha 1$ POGPOGPOGPOGPP
 $\alpha 1$ GPOGPOGPOGPOGPP
 $\alpha 2$ AGPOGPOGPOGPPGVS

Figure S1. Amino acid sequence of human collagen I ($\alpha 1$ chain: Swissprot P02452; $\alpha 2$ chain: Swissprot P08123), highlighting the staggered arrangement of the $\alpha 1$ and $\alpha 2$ chains.⁵ The prodomains and telopeptides are not shown. The overlap regions⁶ are shaded in grey. The hydroxyproline (O) posttranslational modification is assigned according to a published X-ray structure of rat collagen I (PDB 3hr2).⁷ The PPG and POG tripeptide units are highlighted. The OOG tripeptide unit located in the fifth overlap region contains 3R-hydroxy-2S-proline in the Xaa position⁸ so that this OOG unit is not identical to the OOG tripeptide unit studied here. Red sequences have been predicted to be responsive to changes in osmotic pressure.⁹ The sequence GARGSD ($\alpha 2$ chain) is inserted into the host-guest peptide used in this study.

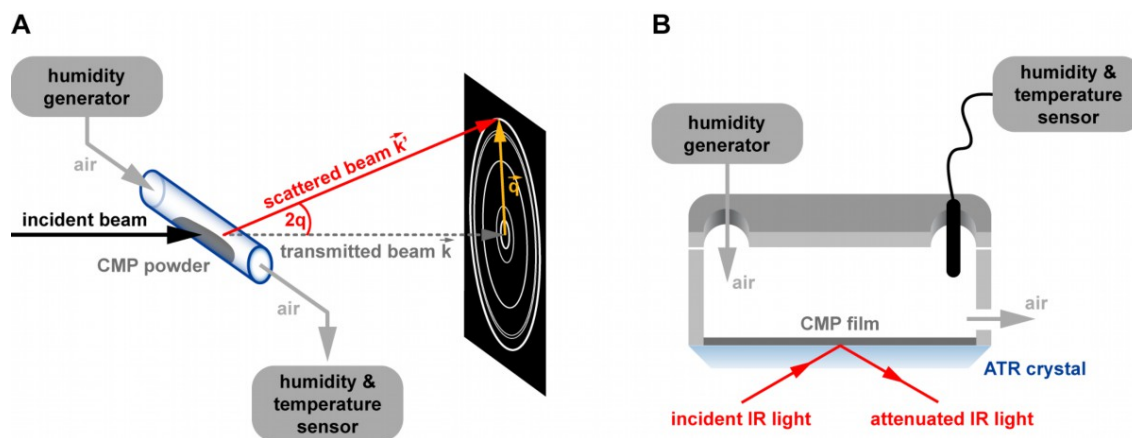


Figure S2. Experimental setups for the *in situ* control of the relative humidity. A) Humidity control during X-ray scattering experiments. The lyophilized CMP powder was placed inside a glass capillary. Air of controlled relative humidity ($R.H.$), provided by a humidity generator, was flushed through the capillary. The $R.H.$ values reported throughout the manuscript were obtained from a humidity and temperature measurement at the exit of the capillary. B) FTIR in a humidity-controlled environment. Dried CMP films were prepared on the ATR crystal from aqueous CMP solutions. The ATR crystal was subsequently placed into a chamber and air of controlled $R.H.$ was flown through the chamber. The chamber was equipped with a humidity and temperature sensor.

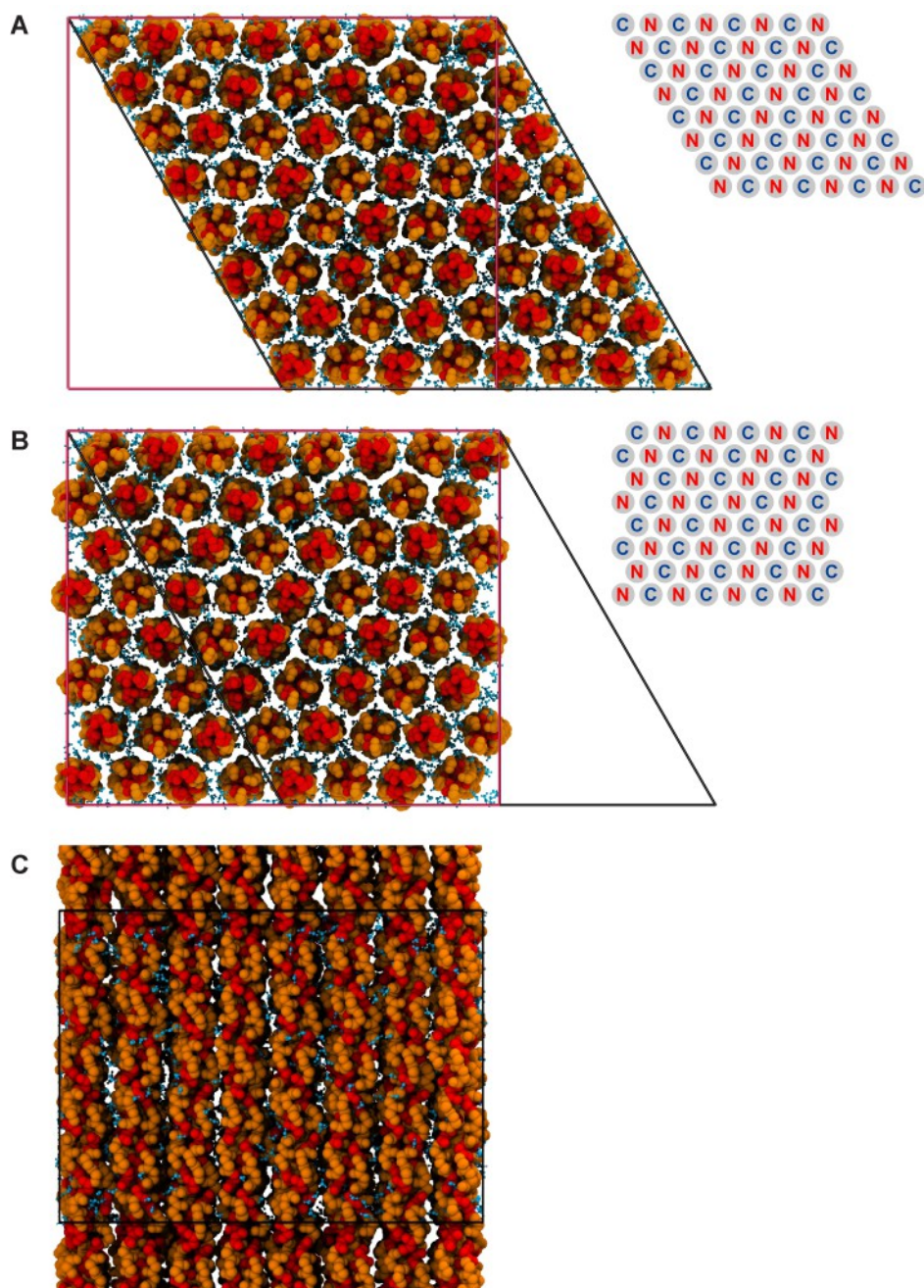


Figure S3. Setup for the molecular dynamics simulations. A) Top view showing the hexagonal organization of PPG-based triple helices under dehydrated conditions ($0.4 N_W/N_{AA}$). The simulations were performed with 8×8 quasi-infinitely-long triple helices in an antiparallel arrangement (see $N \rightarrow C$ orientations in the miniaturized schematic illustration). Each triple helix contains 7 PPG tripeptide units and is connected on both ends with its own periodic image. The simulation box is highlighted as a black trapezoid. The box dimensions were $9.7 \text{ nm} \times 9.7 \text{ nm} \times 6.1 \text{ nm}$ while the angle between the two base vectors was 120 degrees. These parameters were fixed during the entire simulation time. Periodic boundary conditions were applied in all 3 dimensions. B) Simulation box (red rectangle) after “rectangularization”, required for the calculation of the structure factor $S(q)$. C) Side view of the simulation box (black rectangle) showing the quasi-infinite length of the triple helices in the axial direction.

Table S1. Comparison of (PPG)₁₀ structural parameters obtained from XRD and MD at dry conditions (XRD: $R.H. \approx 10\%$; MD: $0.4 N_W/N_{AA}$). For all peaks n , the experimental values of q_{n_XRD} and d_{n_XRD} are given. For the simulated data, q_{n_MD} as well as the Miller indices are provided. The Miller indices (h_{MD} , k_{MD} and l_{MD}) refer to the unit cell containing 8×8 CMPs, as used in the MD simulations. For example, a (0,8,0) plane in the simulations corresponds to a (0,1,0) plane in a standard hexagonal cell.

Peak n	d_{n_XRD} (nm)	q_{n_XRD} (nm ⁻¹)	q_{n_MD} (nm ⁻¹)	h_{MD}	k_{MD}	l_{MD}
1	1.002	6.30	6.03	0	8	0
2	0.704	8.92	8.50	0	8	6
3	0.553	11.36	10.39	16	0	0
4	0.500	12.56	12.01	0	16	0
5	0.484	12.98	12.07	8	12	6
6	0.433	14.50	13.48	16	8	6
7	0.285	22.04	21.41	0	0	21

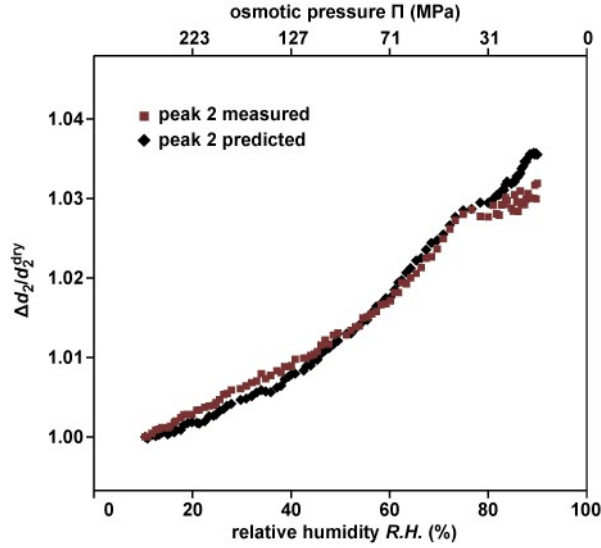


Figure S4. Validation of the lattice plane assignment. Assuming that the lattice planes predicted by MD are correct, the changes in the Bragg distances can be obtained from the experimental data. For example, considering the angular relationships suggested in Table 1, it is predicted from the simulations that peak 2 is related to a lattice plane with an in-plane component (0,8,0) and an axial component (0,0,6). The angle between this lattice plane and the x,y plane, at any relative humidity, is described by the relationship $\alpha^{RH} = \tan^{-1} \left(\frac{21 d_7^{RH}}{6 d_1^{RH}} \right)$, where d_1^{RH} and d_7^{RH} are the experimental values of d_1 and d_7 at any value of $R.H.$. If this assumption is correct, $d_2^{RH} = d_1^{RH} \sin \alpha^{RH}$ at any $R.H.$. The fact that the calculated $\frac{\Delta d_2}{d_2^{dry}} = \frac{d_2^{RH} - d_2^{10\%}}{d_2^{10\%}}$, where $d_2^{10\%}$ refers to the position of d_2 at the lowest experimentally used $R.H.$, falls onto the experimental curve for d_2 confirms that the CMP can be correctly described with the packing of the MD simulations.

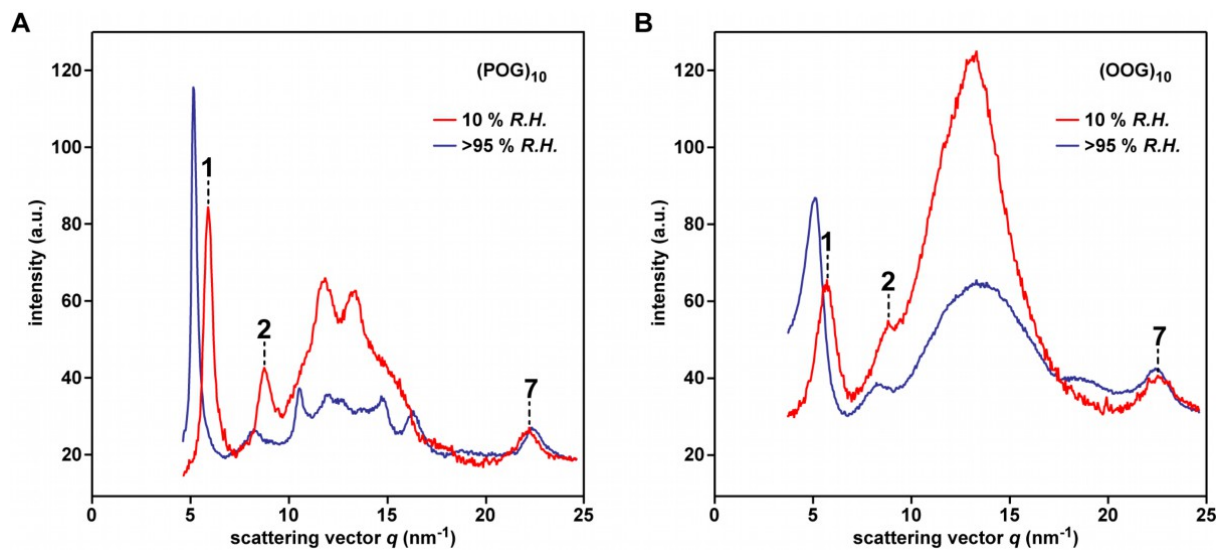


Figure S5. Osmotic pressure response of (POG)₁₀ and (OOG)₁₀. (A) Scattering profile of (POG)₁₀, measured at the lowest (10 %) and highest accessible (>95 %) relative humidity (*R.H.*). (B) Scattering profile of (OOG)₁₀, measured at the lowest (10 %) and highest accessible (>95 %) *R.H.*.

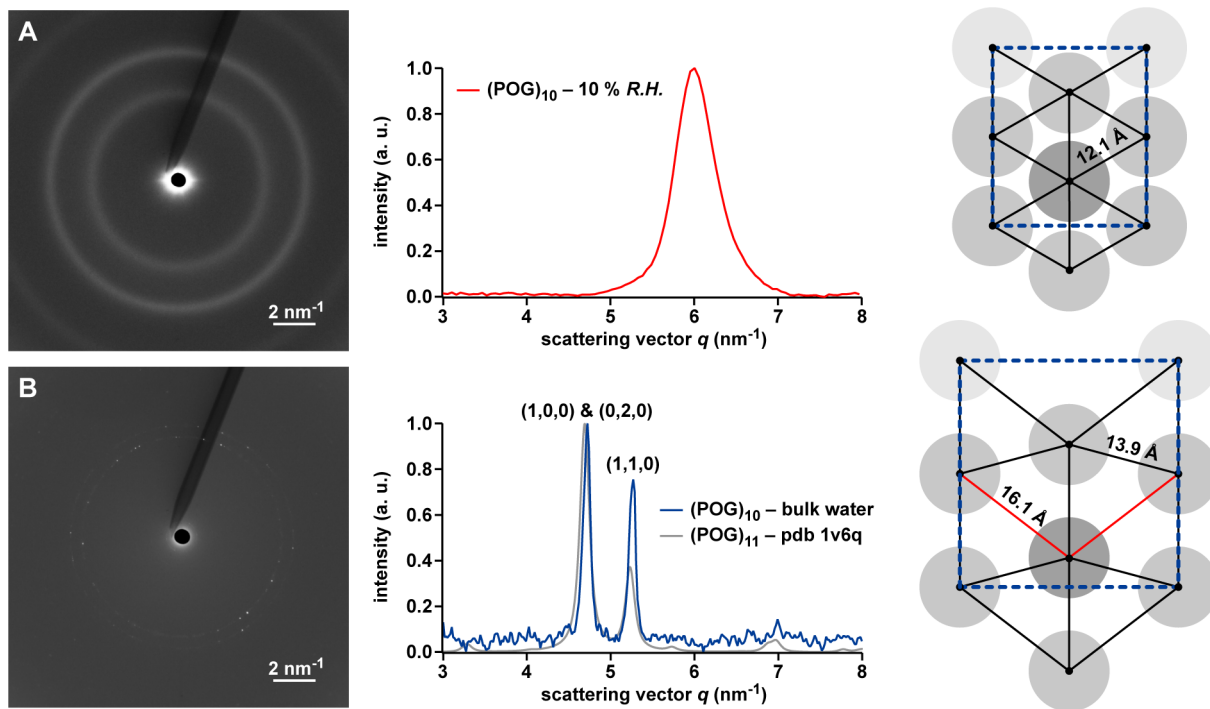


Figure S6. Comparison of the lateral packing arrangement of $(\text{POG})_{10}$ in the dehydrated and fully hydrated state. A) Lateral packing arrangement in dry conditions (10 % *R.H.*). The 2D scatter pattern and the $S(q)$ profile highlight peak 1 that was assigned to the lateral center-to-center distance between triple helices. B) Lateral packing arrangement of triple helices in bulk water (5 mg ml^{-1}). The 2D scatter pattern and the $S(q)$ profile shows the appearance of two new peaks. These peaks are consistent with the diffraction pattern calculated for the rotationally averaged crystal structure of $(\text{POG})_{11}$ (PDB 1v6q⁴). In this configuration, the number of nearest neighbors is four. The distances to the first (nearest) neighbors are shown as black lines, while the second neighbors are highlighted in red.

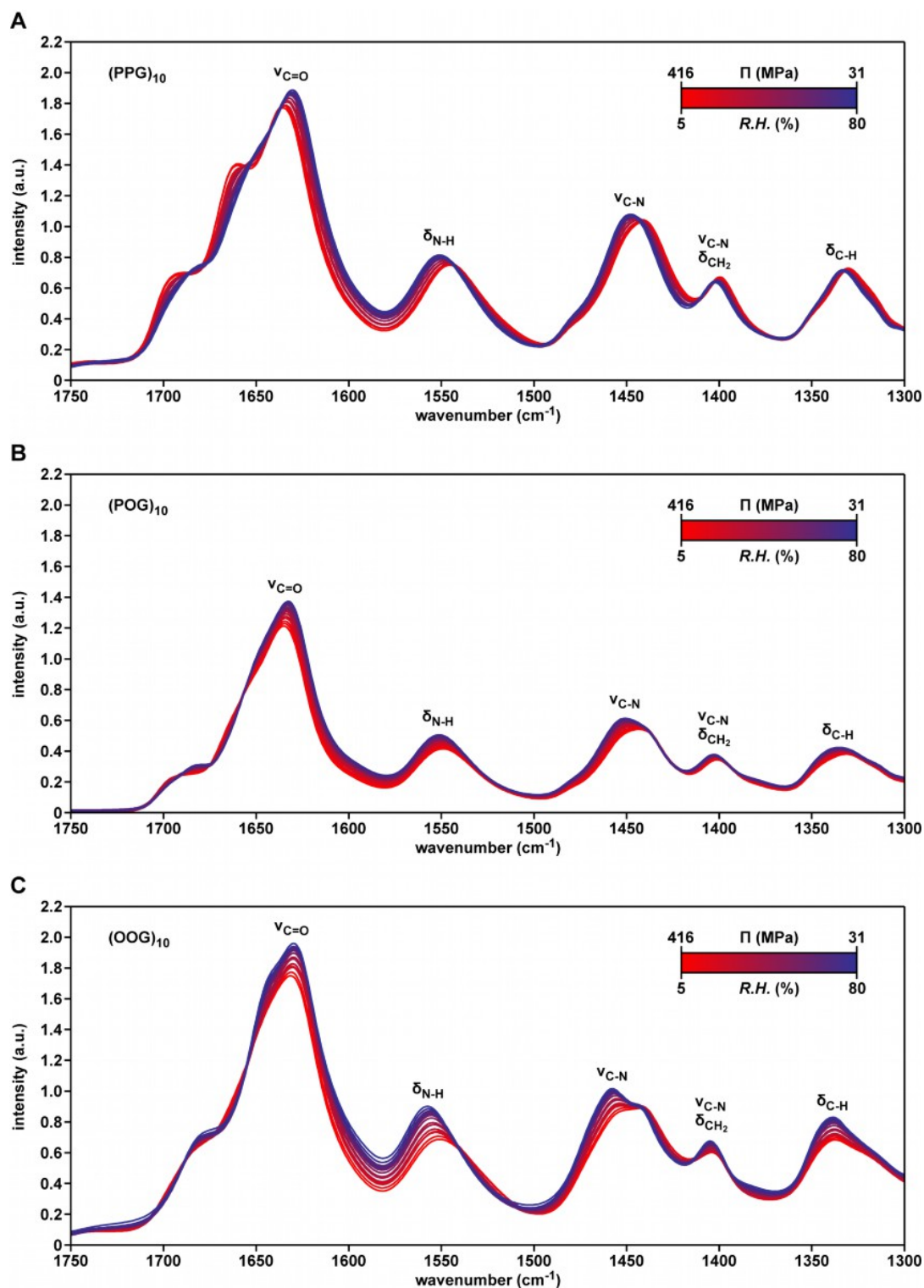


Figure S7. Osmotic pressure effects on the interchain hydrogen bond, monitored by FTIR. A) FTIR spectra of (PPG)₁₀, recorded when increasing the relative humidity (*R.H.*) from 5 % to 80 %. B) FTIR spectra of (POG)₁₀. C) FTIR spectra of (OOG)₁₀. The spectra shown correspond to steps of $\sim 5\%$ *R.H.*.

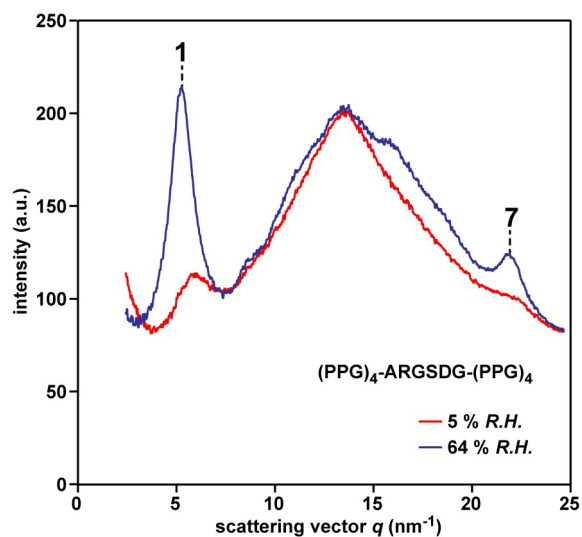


Figure S8. Osmotic pressure response of (PPG)₄-ARGSDG-(PPG)₄. The scattering profiles at the lowest (5 %) and highest (64 %) measured *R.H.* values are shown. Assuming that the PPG host sequence and the region of the guest sequence respond as independent units, one may expect two components under peak 7. As the expected shift is small ($\sim 1 \text{ nm}^{-1}$) compared to the peak width (FWHM $\sim 1 \text{ nm}^{-1}$) it is not possible to resolve these different components.

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