

COMMENTARY

Handy water: Chiral superstructures around peptide β -sheets

Saranya Pullanchery^a and Sylvie Roke^{a,b,c,1}

Water is a key component of biological systems. Traditionally, water is considered as the background against which biology evolves. However, recently, it is becoming apparent that water is very much an essential part of the system. One could even ask to what extent water determines the structure of proteins, membranes, DNA, and the more complex biomachinery that is composed of multiple compounds. To answer this question, it is important to understand how the hydration shell of a biomolecule responds to its structural changes. Chirality (or handedness) is one of the most fundamental aspects of biomolecular structure. Two molecular groups are chiral when they have the same chemical structure but are each other's mirror image. Amino acids in proteins are chiral, and although they can exist as left (L-) and right-handed (D-) mirror images, only L-enantiomers are found in nature. In contrast, sugars and DNA occur only in D-form. Macromolecules constructed from chiral building blocks form either exclusively L- or D-structures. The vast majority of biological reactions in aqueous environments are fine-tuned for their speed and accuracy by using chiral selectivity. Determining the structural properties of water around chiral biomolecules is therefore important to understand the complexity of life itself. However, experimentally measuring the structure of water in contact with a biomolecule or larger molecular assemblies is a challenging task. In PNAS Perets et al. (1) implement an elegant approach that combines interferometric chiral sum frequency generation (SFG) spectroscopy, isotopic exchange experiments, and molecular dynamics (MD) simulations to understand the relationship between peptide β -sheets and their hydrating water. (L-) and (D-) antiparallel peptide β -sheets are demonstrated to impart their chirality to adjacent water molecules, leading to chiral superstructures of water around peptides that extend for approximately five hydration layers.

When two laser pulses of infrared and visible frequencies are spatially and temporally overlapped

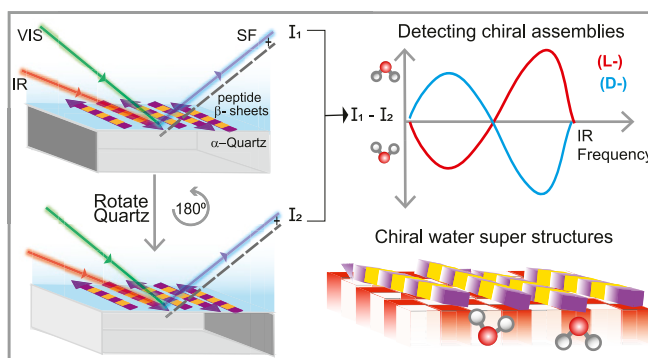


Fig. 1. Revealing chiral water superstructures using interferometric SFG. Hydrated films of antiparallel peptide β -sheet assemblies are formed on the surface of α -quartz. The detected chiral SFG electromagnetic field is added to that of α -quartz, leading to intensities I_1 and I_2 . I_1 and I_2 are measured for two different orientations of the α -quartz crystal. $I_1 - I_2$ provides the direction of the electromagnetic field which reports on the up/down orientation of dipoles. The sketched spectra show mirror-image signals observed for L- vs. D-enantiomers. The illustration at the bottom right depicts the checkerboard-like pattern of water dipole orientations that form the chiral structure, revealed from MD simulations by Perets et al. (1).

on a quartz window that is in contact with an aqueous solution of the strongly amphiphilic peptides, sum frequency photons are generated exclusively from regions lacking centrosymmetry. At the interface between the biomolecules and water, symmetry is necessarily broken. The symmetry requirements of SFG further make it an excellent tool to probe chiral ordering of various biomolecules at interfaces (2). Even though the majority of chiral SFG studies were done with molecules possessing intrinsic chirality, it is in theory possible that a chiral interface orders an achiral molecule into a chiral extended superstructure, which can potentially generate chiral SF photons (3). MD simulations have predicted that the local variations in DNA–water hydrogen bonding interactions combined with the spatial confinement imposed by the

^aLaboratory for Fundamental BioPhotonics (LBP), Institute of Bioengineering (IBI), School of Engineering (STI), École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland; ^bInstitute of Materials Science and Engineering (IMX), School of Engineering (STI), École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland; and ^cLausanne Centre for Ultrafast Science, École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland

Author contributions: S.P. and S.R. wrote the paper.

The authors declare no competing interest.

This open access article is distributed under [Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 \(CC BY-NC-ND\)](https://creativecommons.org/licenses/by-nc-nd/4.0/).

See companion article, "Mirror-image antiparallel β -sheets organize water molecules into superstructures of opposite chirality," [10.1073/pnas.2015567117](https://doi.org/10.1073/pnas.2015567117).

¹To whom correspondence may be addressed. Email: sylvie.roke@epfl.ch.

Published January 8, 2021.

DNA minor grooves should result in heterogeneous hydration (4), implying the existence of chiral water structures. Although conceptually straightforward, experimental verification of this idea has only been achieved very recently. McDermott et al. reported chiral water superstructures around DNA duplexes immobilized on a silica surface (5). The SFG spectra measured in the O–H stretch frequency region indicated the presence of chiral water structures, templated by the DNA double helix. The presence of chiral water structures was also detected from water confined inside aquaporin-inspired artificial channels embedded in supported lipid bilayers (6). The water inside the channels exhibits enhanced dipole ordering and uses the supramolecular chirality of the channels as a template. In 2019, Perets et al. suggested the presence of chiral water structures next to antiparallel β -sheets formed by the model peptide LK₇ β at the quartz interface (7). However, unambiguous demonstration requires a more rigorous experiment: showing that inversion of the peptide's chirality also inverts the chirality of the water.

The LK₇ β peptides form chiral β -sheet assemblies, whereas the individual strands do not. β -sheets from L-/D-peptides also form interfacial mirror-image assemblies. Thus, while the amide I vibrational mode of the individual strands is SFG-inactive, it is active for chiral β -sheet assemblies (7). Showing that L- and D-peptides have inverted chiral structures requires the measurement of the SF electromagnetic field components, which have a direction that inverts for D or L structures. The SFG intensity alone does not reveal this information. To distinguish between the electromagnetic field components, Perets et al. (1) have cleverly used interferometric SFG (a.k.a. phase-resolved or heterodyne-detected SFG). Positive or negative electromagnetic field directions directly correlate with molecular orientation, and it can be determined if vibrational modes are oriented in an upward or downward direction with respect to the interface. The field directions can be determined by interfering the peptide/water interfacial electromagnetic field with that of an underlying quartz window, which has a known response that depends on the azimuthal angle. Measuring at two different azimuthal angles allows the retrieval of the molecular orientation (8) (Fig. 1). It was also confirmed that mirror-image β -sheet assemblies of LK₇ β exhibit opposite orientations in both C–H and N–H modes, as they should since they are each other's chiral enantiomer (9). With this advancement a further question about the chirality of biomolecules and the hydration shell can now be answered: Do left- or right-handed chiral water superstructures exist around peptides?

In this work, Perets et al. (1) answer this question experimentally and also investigate the length scale over which the superstructures extend, and the interactions that lead to it. Using interferometric SFG in combination with ¹⁸O substitution they reveal that the vibrational O–H stretch band of water contains two O–H stretch modes of water that are separated by 200 cm⁻¹, in combination with two N–H stretch modes from the peptide backbone. When the chiral handedness of the peptide assembly is reversed, the water modes undergo a concomitant flip in sign. Thus, switching from L- to D-peptides, not only the interferometric response of the amide modes reverts in orientation but also the water molecules that are directly in contact with it. The dipolar orientation of water molecules in the chiral superstructure is therefore determined by the chiral handedness of the underlying surface. Note that it is not possible to deduce whether the chiral superstructure adopts the same or the reverse chirality as the peptide.

Perets et al. (1) also provide further insight into the origin and nature of the handedness of water using MD simulations. For a simulated peptide composed of five amino acids there is an

asymmetry in the number of N–H groups exposed to water on the exterior strands. This asymmetry leads to a lack of reflection plane on the β -sheet assemblies themselves, which is responsible for their chirality. It can be expected that the water directly interacting with these groups follows this asymmetric pattern too. Indeed, it is observed that the alternating hydrophobic and hydrophilic regions on the LK₇ β peptide assembly order the water molecules in a checkerboard-like pattern of up/down orientations. This ordering pattern comprises a chiral superstructure and extends up to approximately five hydration layers around the peptide.

In PNAS Perets et al. implement an elegant approach that combines interferometric chiral sum frequency generation (SFG) spectroscopy, isotopic exchange experiments, and molecular dynamics (MD) simulations to understand the relationship between peptide β -sheets and their hydrating water. (L-) and (D-) antiparallel peptide β -sheets are demonstrated to impart their chirality to adjacent water molecules, leading to chiral superstructures of water around peptides that extend for approximately five hydration layers.

The MD simulations also reveal that water dipoles within the first hydration shell from the backbone of the peptide assembly have significantly different vibrational frequencies, depending on what the dipole orientation is. Therefore, by comparison, the two separate water modes observed in the measured interferometric SFG spectra likely originate from water molecules with different hydrogen bonding strengths. Taking the simulations one step further, the interferometric chiral SFG response of water surrounding L- and D-LK₇ β assemblies was computed. The calculated SFG spectrum matches one of the experimentally observed water peaks, whereas the lower-frequency peak that is observed in the experiment is absent. Interestingly, the calculated SFG spectrum also revealed a higher-frequency water mode that was outside the range of experimental observation.

Perets et al. (1) bring into focus the intertwining of peptide and water structure. The results emphasize the need to go beyond simplistic models that treat water as the background medium, if we are to understand biological processes at the molecular level. Additionally, the direct verification and observation of chiral water superstructures next to proteins will open up a number of exciting opportunities as chiral recognition is fundamental across biochemical processes in living systems. Most of the ligands and drug molecules interact with their protein target in a chiral-selective manner. Understanding the role of chiral hydration shells in these interactions will require the integration of experimental and computational studies in the static and dynamic regimes. Besides interferometric SFG, other nonlinear optical approaches could be used in combination, to address even better the emerging complexity of aqueous systems. Sum frequency and second harmonic scattering are examples of techniques that can probe integrative *in vitro* systems, such as studying hydration of liposomes (10) with and without proteins (11). Along with hydration shell structure, recent theoretical and experimental advances have provided insights into the hydration dynamics

of biomolecules (12, 13). More recently, second harmonic imaging was used to infer spatiotemporal hydration dynamics of in vitro freestanding membrane-pore systems (14, 15) as well as in living cells (16). Water molecules inside a peptide ion channel have been shown to exhibit enhanced dipolar ordering and these dipoles reorient along the interfacial field lines of the transported ions (14). One could now ask how the chiral water structures are important in such dynamic events. Studying the spatiotemporal dynamics of water in and around various complex

biomachineries is an exciting task to be achieved in coming years. A continuously evolving set of nonlinear spectroscopic and imaging tools, along with more accurate in vitro model systems and better simulation toolboxes, will help to further unravel the intriguing role of water in biomolecular interactions.

Acknowledgments

We thank the Julia Jacobi Foundation and the Swiss National Science Foundation (Grant 200021-182606-1).

- 1 E. A. Perets *et al.*, Mirror-image antiparallel β -sheets organize water molecules into superstructures of opposite chirality. *Proc. Natl. Acad. Sci. U.S.A.*, 10.1073/pnas.2015567117 (2020).
- 2 E. C. Y. Yan, L. Fu, Z. Wang, W. Liu, Biological macromolecules at interfaces probed by chiral vibrational sum frequency generation spectroscopy. *Chem. Rev.* **114**, 8471–8498 (2014).
- 3 G. J. Simpson, Molecular origins of the remarkable chiral sensitivity of second-order nonlinear optics. *ChemPhysChem* **5**, 1301–1310 (2004).
- 4 E. Duboué-Dijon, A. C. Fogarty, J. T. Hynes, D. Laage, Dynamical disorder in the DNA hydration shell. *J. Am. Chem. Soc.* **138**, 7610–7620 (2016).
- 5 M. L. McDermott, H. Vansalous, S. A. Corcelli, P. B. Petersen, DNA's chiral spine of hydration. *ACS Cent. Sci.* **3**, 708–714 (2017).
- 6 I. Kocsis *et al.*, Oriented chiral water wires in artificial transmembrane channels. *Sci. Adv.* **4**, eaao5603 (2018).
- 7 E. A. Perets, E. C. Y. Yan, Chiral water superstructures around antiparallel β -sheets observed by chiral vibrational sum frequency generation spectroscopy. *J. Phys. Chem. Lett.* **10**, 3395–3401 (2019).
- 8 X.-H. Hu, F. Wei, H. Wang, H.-F. Wang, α -quartz crystal as absolute intensity and phase standard in sum-frequency generation vibrational spectroscopy. *J. Phys. Chem. C* **123**, 15071–15086 (2019).
- 9 X.-H. Hu *et al.*, N-H chirality in folded peptide LK γ β is governed by the C α -H chirality. *J. Phys. Chem. Lett.* **11**, 1282–1290 (2020).
- 10 N. Smolentsev, C. Lütgebaucks, H. I. Okur, A. G. F. de Beer, S. Roke, Intermolecular headgroup interaction and hydration as driving forces for lipid transmembrane asymmetry. *J. Am. Chem. Soc.* **138**, 4053–4060 (2016).
- 11 J. Dedic, S. Rocha, H. I. Okur, P. Wittung-Stafshede, S. Roke, Membrane-protein-hydration interaction of α -synuclein with anionic vesicles probed via angle-resolved second-harmonic scattering. *J. Phys. Chem. B* **123**, 1044–1049 (2019).
- 12 M.-C. Bellissent-Funel *et al.*, Water determines the structure and dynamics of proteins. *Chem. Rev.* **116**, 7673–7697 (2016).
- 13 D. Laage, T. Elsaesser, J. T. Hynes, Water dynamics in the hydration shells of biomolecules. *Chem. Rev.* **117**, 10694–10725 (2017).
- 14 O. B. Tarun, M. Y. Eremchev, A. Radenovic, S. Roke, Spatiotemporal imaging of water in operating voltage-gated ion channels reveals the slow motion of interfacial ions. *Nano Lett.* **19**, 7608–7613 (2019).
- 15 O. B. Tarun, C. Hanneschläger, P. Pohl, S. Roke, Label-free and charge-sensitive dynamic imaging of lipid membrane hydration on millisecond time scales. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 4081–4086 (2018).
- 16 M. E. P. Didier, O. B. Tarun, P. Jourdain, P. Magistretti, S. Roke, Membrane water for probing neuronal membrane potentials and ionic fluxes at the single cell level. *Nat. Commun.* **9**, 5287 (2018).