Chiral Phonons in Biomolecules: Spanning from Molecules to Biomaterials

*Minkyu Kim and Vladimir V. Tsukruk**

School of Materials Science and Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA

* Corresponding Author E-mail: vladimir@mse.gatech.edu

Total words: 1,450

1. What is chirality in molecules and in natural materials

The word "chiral" which was introduced by Lord Kelvin in the end of 19 century[1] stems from the Greek word "*χέρι* (kheir)" that means "hand" where left and right hand are not superimposable each other.[2] Chirality indicates asymmetric property in such a manner that a structure and its mirror image are non-superimposable. Chirality is essential for individual organic molecules, their crystals, and biomaterials. The complexity of life is originated from the chiral monomers that compose biological polymers.[3] In nature, chiral biomolecules are ubiquitous in biopolymers across multi-length scales, including amino acids (AAs), peptides, proteins, DNA/RNA and different secondary structures (**Fig 1a**).[4] The chirality of these biomolecules provides biological specificity in various cellular processes such as molecular recognition, immune reaction, and cellular membrane trafficking.[5] For instance, humans can only break down *D*-sugars but not *L*-sugars since *L*-sugars cannot bond to the enzymes.[6]

In modern technologies, chiral biomolecules play a crucial role in pharmaceutical industry, health monitoring, biosensing, and enantiomers separation.[4,5,7] For instance, the drugs that possess the same chemical formula but are enantiomerically different show the different efficiency and side effects in clinical studies.[7] In order to control these phenomena, accurate and precise identification of chiral properties of biomolecules is vital. Since chiral molecules possess an optical activity, optical tools such as circular dichroism (CD) are widely exploited for their characterization in UV and visible wave-range for not only to detect the chirality but also handedness of chiral molecules and biomaterials in very minute quantities (**Fig. 1a**).[8] For this reason, CD has frequently been utilized to glance at chirality and handedness of target practical materials such as cellulose and chitin nanocrystals in fruits, beetles, and exoskeletons.[9,10] These chiral biomaterials can offer vivid structural colors because of matched helical pitch length with visible light wavelength. However,

overlapping of electronic and vibrational resonances and excessive absorption mask fine details of the origin of chiral light polarization appearance.

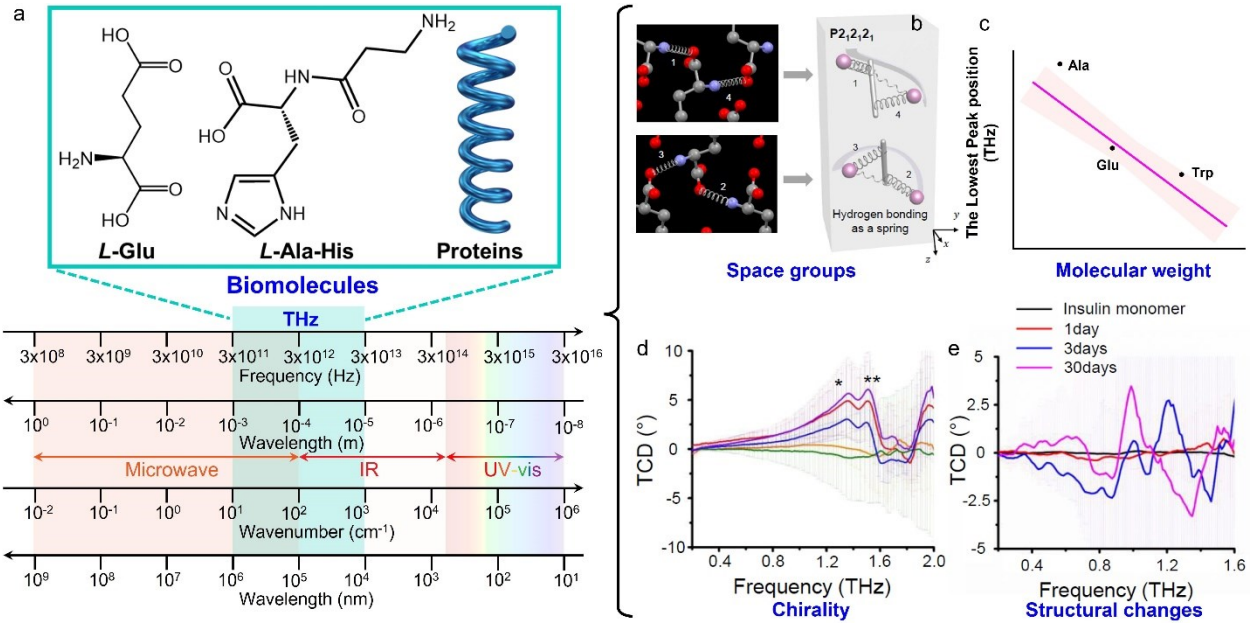

Fig. 1 | Multilevel understanding of chirality of biomolecules and their structures is offered by terahertz time-domain polarimetry in different frequency-wavelength domains in comparison with traditional spectroscopies (a). THz absorption (TA) spectra unveil the information regarding space group (**b**) as well as molecular weight of biomolecules (**c**). THz circular dichroism (TCD) spectra show that degree of chirality in *L*-Ala-His from five different manufactures can be quantitively and qualitatively revealed (**d**); secondary structural changes of the insulin nanofibrils from α-helix to β-sheet can be identified by change in chiral phonon mode in TCD spectra (**e**). Note: **a** and **c** are drawn here and **b, d, e** are adapted from Ref. 12.

2. Unique characteristics of THz spectroscopy

Thus, in attempts to mine more information on chiral state of biomaterials recent attention shifted to terahertz spectroscopy (THz). The related diapasons correspond to: 10^{11}-10^{13} Hz in frequency domain, $3x10^{-3}$ - $3x10^{-5}$ m in large wavelength domain, 3-300 cm^{-1} in wavenumber domain, and $3x10^{4}$ - $3x10^{6}$ nm in nanoscale wavelength domain (**Fig. 1a**).[11,12] This non-trivial THz domain stands alone in realm of traditional domains adopted in different physics communities that explore microwave, infra-red, UV-visible spectroscopies as illustrated in **Fig. 1a**. Overall, TCD spectroscopy is considered as a unique analytical tool for exploring the chiral properties of a various type of biomolecules and related materials, which can find its effective applications in chemical synthesis, structural changes of proteins, and biological diagnosis.[12]

3. The current research provides strong advantages for measuring chiral phonons

Standard THz absorption (TA) measurements display large reliance on sample preparation, which

exacerbates TCD.[12] Apparently, small existence of the opposite enantiomer of AA and impurities can also alter their crystallizations, giving rise to spectral perversion and peak broadening. Since AAs can possess diverse crystalline phases and hydration states, additional XRD measurement is demanded for their careful identifications. Overall, despite some phonon resonances in THz range were measured, chirality identification was difficult in practical establishment of THz circular dichroism (TCD) spectroscopy.[12]

Now, reporting in *Nature Photonics*, Choi and co-workers from USA, Japan, and Brazil have utilized a THz time-domain polarimetry (THz-TDP) with moving XY scanning.[12] This approach renders correct measurements and identification of chiral phonons by obtaining statistically averaged TA and TCD spectra. The TA for each pixel was directly obtained from fast Fourier transform from the electric fields measured in the time-domain along the x and y directions, while TCD spectra for each pixel were calculated using the Stokes equations. When the uniquely developed TA and TCD combination allows accurate information of biomolecules concurrently, including space groups, chirality, structural changes, and molecular weight demonstrated for 20 AAs (**Fig. 1b-e**). Two dominant types of TA spectra can be derived from this method. *Type 1* spectra show the monotonically increasing absorptions with broad peaks, whereas *Type 2* spectra display the multiple sharp peaks. The shape of TA spectra is correlated with space group of AAs, which enables the visual identifications of space group (**Fig. 1b**). Besides, Choi and co-workers discovered that there is negative correlation between molecular weight of AA and the lowest THz resonance peak position in TA spectra (**Fig. 1c**). The TCD spectra of *L*-Ala-His utilized in health supplements from five different manufactures (**Fig. 1d**). All *L*-Ala-His from different company shows the peaks in TCD, indicating that the chirality existence of tested *L*-Ala-His. The intensities ratio between the TA and TCD peaks should be identical for all products or small sample-to sample differences. Large intensity ratio difference can be related with crystalline structural differences in the specific *L*-Ala-His formulations and presence of achiral and chiral impurities.

Moreover, the authors monitored the amyloid fibrilization of insulin monomer using the TA and TCD spectra. As known, amyloid fibrillation, a process that soluble proteins misfolds into insoluble fibrils during transformation from α-helix to a β-sheet conformation, is an indication for various health disorders including skin cancer, Alzheimer syndrome, and type II diabetes.[3] In this study, chiral phonon modes of insulin nanofibrils were monitored by TCD (**Fig. 1e**). The TCD peak at 1.2 THz was altered from positive to negative with development of the nanofibers, demonstrating the change of structural change from right-handed in the α-helix to left-handed in the β-sheets, thus offering non-invasive and label-free TCD-based toolbox.

4. Research trend and expectations in chiral biomaterials exploration

We suggest that the experimental approach developed in this research will be further expanded into identification the unique chiral properties of other kinds of advanced materials beyond amino acids considered in this work including other biopolymers, inorganic-organic hybrids, and inorganic nanomaterials for tailored active photonic and photonic applications. For example, circularly polarized light scattering and luminescence is widely explored in visible range for novel

structural photonic natural materials for optical computing, optical communication, camouflaging, chiral sensing, and optical data storages.[4] In addition to biological natural materials mentioned above, intense related research can be extended to chiral inorganic-organic as well as inorganic materials such as chiral metal-organic frameworks,[13] chiral transition metal oxides,[14] and chiral ceramics[15].

Achieving long-range ordered and defect-free organization is critically important task for obtaining high quality optical grade materials with, however, vast attention paid to their properties in visible and near-infra red wave-range (**Fig. 1a**).[16] However, their distinguish scattering, absorption, polarization, and emission properties rarely explored in THz region because of experimental challenges. These properties can be related to interaction of electromagnetic radiation with matter in terms of chemical composition, local chirality, and molecular, nanoscale, and microscale organization. To date, signatures of these relationships in THz frequencies and microwave wavelengths remind largely unknown in diverse research communities in physics, chemistry, biology, and materials.

References

1 Lord, K. *J. Oxford Univ. Junior Sci. Club* **18**, 25 (1894).
2 Pasteur, L. C. R. Hebd. *Seances Acad. Sci.* **26**, 535–538 (1848).
3 Alberts, B. et al. *Molecular Biology of the Cell* 6th edn (Garland Science, 2014).
4 Zhang, L., Wang, T., Shen, Z. & Liu, M. *Adv. Mater.* **28**, 1044-1059 (2016).
5 Nguyen, H.V.-T. *et al. Nat. Chem.* **14**, 85-93 (2022).
6 Ni, B. and Cölfen, H. *SmartMat*, **2**, 17-32 (2021).
7 Brooks, W. H., Guida, W. C. & Daniel, K. G. *Curr. Top. Med. Chem.* **11**, 760–770 (2011).
8 Choi, W.J. *et al. Nat. Mat.* **18**, 820-826 (2019).
9 Kim, M. *et al. Adv. Mater.* **33**, 2103674 (2021).
10 Xiong, R. et al. *Chem. Soc. Rev.* **49**, 983-1031 (2020).
11 Mándi, A. & Kurtán, T. *Nat. Prod. Rep.* **36**, 889-918 (2019).
12 Choi, W.J. et al. *Nat. Photon.* NPHOT-2021-02-00215C (need the doi of current paper) (2022).
13 Sharifzadeh, Z., Berijani, K. & Morsali, A. *Coord. Chem. Rev.* **445**, 214083 (2021).
14 Li, Y. *et al. Adv. Mater.* **32**, 1905585 (2020).
15 Fan, J. & Kotov, N.A. *Adv. Mater.* **32**, 1906738 (2020).
16 Kim, M. *et al. ACS Nano* **15**, 19418-19429 (2021).