CHIN. PHYS. LETT. Vol. 37, No. 8 (2020) 087504 **Express Letter**

Unexpectedly Strong Diamagnetism of Self-Assembled Aromatic Peptides

Haijun Yang(杨海军)[1,2], Zixin Wang(王子鑫)[3], Liuhua Mu(木留华)[2], Yongshun Song(宋永顺)[4], Jun Hu(胡钧)[1], Feng Zhang(张峰)[3,5*], and Haiping Fang(方海平)[4,1*]

[1]*Shanghai Synchrotron Radiation Facility, Zhangjiang Laboratory (SSRF, ZJLab), Shanghai Advanced Research Institute, Chinese Academy of Sciences, Shanghai 201204, China*
[2]*Division of Interfacial Water, CAS Key Laboratory of Interfacial Physics and Technology, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China*
[3]*State Key Laboratory of Respiratory Disease, Guangzhou Institute of Oral Disease, Stomatology Hospital, Department of Biomedical Engineering, School of Basic Medical Sciences, Guangzhou Medical University, Guangzhou 511436, China*
[4]*School of Science, East China University of Science and Technology, Shanghai 200237, China*
[5]*Biomedical Nanocenter, School of Life Science, Inner Mongolia Agricultural University, Hohhot 010011, China*

(Received 28 June 2020; accepted 5 July 2020; published online 9 July 2020)

There is a considerable amount of work that shows the biomagnetism of organic components without ferromagnetic components at the molecular level, but it is of great challenge to cover the giant gap of biomagnetism between their experimental and theoretical results. Here we show that the diamagnetism of aromatic peptides is greatly enhanced for about 11 times by self-assembling, reaching two orders of magnitude higher than the mass susceptibility of pure water. The self-assembly of aromatic rings in the peptide molecules plays the key role in such a strong diamagnetism.

PACS: 75.90.+w, 87.90.+y, 87.14.ef DOI: 10.1088/0256-307X/37/8/087504

Biomagnetism has attracted tremendous interests in the past few decades.[1] Magnetic fields originating from the heart, brain, skeletal muscles, and isolated nerve and muscle preparations have been detected.[2,3] High static magnetic fields make cells change their orientation, proliferation, microtubule and mitotic spindle orientation, and their DNA and cell cycle.[4] However, not all these organisms and cells contain enough ferromagnetic components. Further, it was found recently that self-assembled aromatic peptide nanotubes could align in strong magnetic fields[5,6] although these nanotubes were believed to be non-ferromagnetic. There have been efforts trying to understand the biomagnetism of organic components without ferromagnetic components. For example, early in 1970s, Worcester and Pauling attributed the diamagnetic property of proteins to aromatic rings and peptide bonds, and estimated the magnitude of diamagnetic susceptibility of these structures.[7,8] Tendler and Lee argued that the alignment of an aromatic peptide nanotube in magnetic fields was mainly originated from the ordered structure of aromatic rings in the peptide nanotubes, given that the aromatic rings have a large diamagnetic anisotropy.[5,6] However, all their experimental measurements and calculations on the diamagnetic susceptibility are based on the dehydrated samples, partly because the existence of water makes the experimental measurement very difficult. For real biological systems, peptides are usually assembled in aqueous solution, and to the best of our knowledge, there is still no report on the magnetism of the assembled peptides. On the other hand, it was found that non-dehydrated DNA shows a paramagnetic upturn in low temperature.[9]

In this work, we designed an ingenious experiment to measure the magnetism of assembled aromatic peptides. The mass susceptibility of aromatic peptides with the sequence of AYFFF is greatly enhanced for about 11 times by self-assembling, reaching two orders of magnitude higher than the mass susceptibility of water. The finding is helpful for understanding of magnetic properties of organic components and will benefit practical applications including peptide assembly, medicine, physiology, and psychology.

The AYFFF aromatic peptide powders were dispersed into pure water (Milli-Q, 18.2 MΩ), namely as the *dispersed state*, where the peptide concentration keeps at 1.0 mg/mL. Then the peptide dispersions were stored still at 20℃ for 16 hours, namely as the *assembled state*. Figure 1(b) displays the typi-

Supported by the National Natural Science Foundation of China (Grant Nos. U1632135, 11974366, U1932123, 51763019 and U1832125), the Key Research Program of Chinese Academy of Sciences (Grant No. QYZDJ-SSW-SLH053), and the Fundamental Research Funds for the Central Universities.
*Corresponding authors. Email: fanghaiping@sinap.ac.cn; fengzhang1978@hotmail.com
© 2020 Chinese Physical Society and IOP Publishing Ltd

cal self-assembled AYFFF peptide microfibers, which are longer than 10 μm with the width of 300 nm and the height of ∼150 nm. For comparison, the AYFFF peptide powders were dissolved in an organic solvent dimethyl sulfoxide (DMSO), in which there is only a few or even negligible amount of assembled peptides. This state is namely as the *dissolved state*.

We used a high-precision electronic balance (XPR205, METTLER TOLEDO, Switzerland) sensitive to 0.01 mg to measure the weight of specimen with or without a fixed magnetic field. We took 1.0 mL peptide aqueous suspensions of 1.0 mg/mL to fill a plastic tube, diluted with 1.0 mL pure water, shaken well and placed onto a support on the tray of the electronic balance. The magnetic field was achieved by putting an NdFeB rare-earth permanent magnet on a fixed position on the upper cover of the balance, as shown in Fig. 1(a). The readings of the balance with and without the magnet are noted as w_2 and w_1, respectively. Then $(w_2 - w_1)$ is the magnetic force exerting on the specimen. Here, for simplicity, we used the unit of mg (equivalently 9.8×10^{-6} N) as the metric for the magnetic force shown in the readings of the balance. The specimen includes the plastic tube, the tube filled with water/DMSO, the tube filled with water/DMSO and the dissolved peptide, the tube filled with water and the dispersed peptide, and the tube filled with water and the assembled peptide. For each specimen, four samples were prepared and the corresponding forces were measured. We note that the good repeatability of this magnetic force measurement system has been demonstrated by measuring the magnetic force of the plastic tube, in which the standard deviation from the four samples is less than 1%.

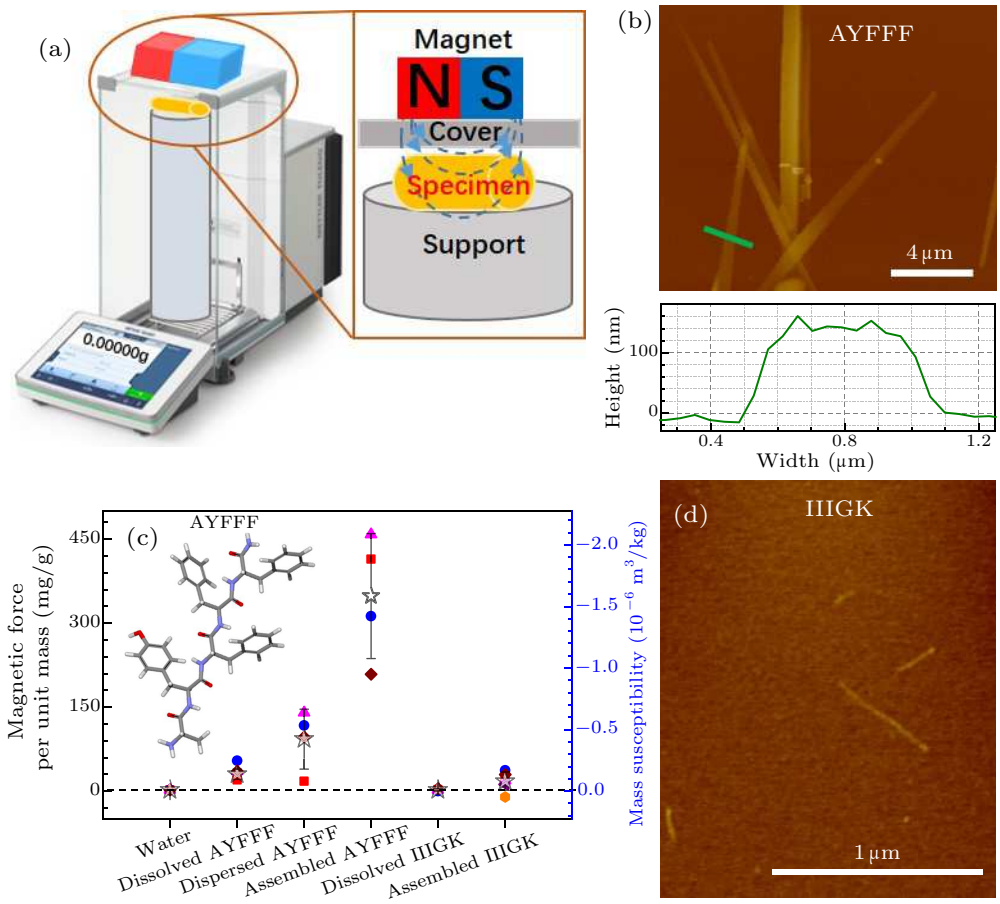

Fig. 1. (a) Experimental setup for measuring the magnetism of peptide. The magnet is sitting on the upper glass cover of the electronic balance (inset). The specimen is put on a support placed in the cover of the electronic balance. (b) AFM images of the self-assembled microfibers of AYFFF aromatic peptides at 20℃ in pure water. The height profile corresponding to the green line marked at the position on the AYFFF microfiber. (c) Average magnetic forces per unit mass of the peptides $\langle w^{\rm p}/m^{\rm p}\rangle$ at dissolved, dispersed, and assembled states, respectively, together with water as a reference $\langle w^{\rm w}/m^{\rm w}\rangle$. All the colorful solid scatters display the experimental data, and the grey hollow stars represent their average values with error bars showing their standard deviations. (d) AFM images of the self-assembled nanofibers of a control nonaromatic peptide IIIGK. Note: both peptides AYFFF and IIIGK are synthesized with the C-terminal amidation in order to increase their biological activities via generating a closer mimic of native proteins.

We measured the magnetic forces of the plastic tube, plastic tube filled with pure water, plastic tubes filled with both the peptides at different states and the pure water of the same amount, which were denoted by w_i^t, $w_i^{\rm tw}$, and $w_i^{\rm twp}$ $(i = 1, 2)$, respectively. Then, the forces contributed by only water and peptide are

$$w_i^{\rm w} = w_i^{\rm tw} - w_i^t, \quad w_i^{\rm p} = w_i^{\rm twp} - w_i^{\rm tw}.$$

The magnetic forces exerting on the water and peptide are

$$w^{\rm w} = w_2^{\rm w} - w_1^{\rm w}, \quad w^{\rm p} = w_2^{\rm p} - w_1^{\rm p}.$$

The magnetic force per unit mass of the water and peptide are $w^{\rm w}/m^{\rm w}$ and $w^{\rm p}/m^{\rm p}$, where $m^{\rm w}$ and $m^{\rm p}$ are the mass of water and peptide, respectively.

Figure 1(c) displays the average magnetic force per unit mass of peptide $\langle w^{\rm p}/m^{\rm p}\rangle$ at different states, together with the average magnetic force per unit mass of water $\langle w^{\rm w}/m^{\rm w}\rangle$ as a reference. It clearly shows that $\langle w^{\rm p}/m^{\rm p}\rangle$ increases in the order of peptides at the dissolved state, at the dispersed state, and at the assembled state. Interestingly, the $\langle w^{\rm p}/m^{\rm p}\rangle$ at the assembled state is about 11 times larger than the value at the dissolved state. This is *surprising* and *unexpected* since the elements of the peptides at all the above-mentioned different states are the same. For the dispersed state, part of the peptides may self-assemble so that the value of $\langle w^{\rm p}/m^{\rm p}\rangle$ is in between the values at dissolved state and at assembled state. We can estimate the mass susceptibility of the self-assembled AYFFF peptides, which reaches $-1.6 \times 10^{-6}\,{\rm m}^3/{\rm kg}$, by comparing its value of $\langle w^{\rm p}/m^{\rm p}\rangle$ of $\sim$350 mg/g to the value of $\langle w^{\rm w}/m^{\rm w}\rangle$ of 2.0 mg/g, considering that the mass susceptibility of pure water is well known as $-9.1 \times 10^{-9}\,{\rm m}^3/{\rm kg}$.

The observation of such a strong diamagnetism for the peptides at the assembled state is *unexpected*. After careful examination of the molecular structure of AYFFF peptide, we presumed that the aromatic rings may be the origin of the strong diamagnetism since the aromatic ring has a large magnetic susceptibility compared with the other components in the peptides. We then performed measurements with a nonaromatic peptide with the sequence of IIIGK. With the same methods as used for AYFFF peptides, we obtained both the *assembled state* and *dissolved state* of this nonaromatic peptide. The nanofibers with the typical length less than 1 µm and the width of about 10 nm can be seen at the assembled state, as shown in Fig. 1(d). The values of $\langle w^{\rm p}/m^{\rm p}\rangle$ of the nonaromatic IIIGK peptide at the assembled state and at the dissolved state were then measured, respectively, both of which are close to $\langle w^{\rm w}/m^{\rm w}\rangle$, the mass susceptibility of pure water. This result confirms that aromatic rings in peptide molecules play the key role in the unexpected strong diamagnetism of aromatic peptides after self-assembling.

In summary, we have *experimentally* shown that the diamagnetism of peptides, which is generally supposed to be of the same order of magnitude of the diamagnetism of water, is unexpectedly strong at the assembling state, with the mass susceptibility reaching two orders of magnitude higher than the mass susceptibility of pure water. The self-assembly of the aromatic rings in the peptides plays a key role for such a high diamagnetism. We note that the element contents in the dispersed state and in the assembled state are the same. The only difference is that the *assembled state* is obtained by storing the tube with water and peptides at 20℃ for 16 hours. The great increase of the diamagnetism induced by the transformation from the dispersed state to the assembled state further demonstrate the importance of the assembling.

The observation of such a strong diamagnetism for the aromatic peptides at the assembled state is *surprising* and *unexpected*. It provides a challenge to theoretical investigation. We guess that the coupling between the aromatic rings, which can greatly reduce the effect of thermal fluctuation, may be the key. Our findings provide a firm step towards the understanding of the magnetism of biomolecules, as well as applications such as the magnet induced nano-/microfabrication,[10] nuclear magnetic resonance imaging (MRI),[11,12] and a new pathway for brain-computer interface.[13]

References

[1] Crease R 1989 *Science* **245** 1041
[2] Hobbie R K and Roth B J 2007 *Intermediate Physics for Medicine and Biology* (New York: Springer) p 203
[3] Lorant S J S 1977 *Biomagnetism: A Review* pSLAC-PUB-1984
[4] Tao Q, Zhang L, Han X, Chen H, Ji X and Zhang X 2020 *Biophys. J.* **118** 578
[5] A Hill R J, Sedman V L, Allen S, Williams P, Paoli M, Adler-Abramovich L, Gazit E, Eaves L and Tendler S J B 2007 *Adv. Mater.* **19** 4474
[6] Kwon S, Kim B J, Lim H K, Kang K, Yoo S H, Gong J, Yoon E, Lee J, Choi I S, Kim H and Lee H S 2015 *Nat. Commun.* **6** 8747
[7] Pauling L 1979 *Proc. Natl. Acad. Sci. USA* **76** 2293
[8] Worcester D L 1978 *Proc. Natl. Acad. Sci. USA* **75** 5475
[9] Nakamae S, Cazayous M, Sacuto A, Monod P and Bouchiat H 2005 *Phys. Rev. Lett.* **94** 248102
[10] Nanni G, Petroni S, Fragouli D, Amato M, De Vittorio M and Athanassiou A 2012 *Microelectron. Eng.* **98** 607
[11] Gong N J, Dibb R, Bulk M, van der Weerd L and Liu C 2019 *NeuroImage* **191** 176
[12] Jones K M, Pollard A C and Pagel M D 2018 *J. Magn. Reson. Imaging* **47** 11
[13] Abdulkader S N, Atia A and Mostafa M S M 2015 *Egypt. Inf. J.* **16** 213