THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

Absolute Configuration Determination of Side Chains of Basic Amino Acid Residues Using Water-Soluble Porphyrins-Based Exciton Chirality Method

Shingo Hattori, Kengo Akagawa, Kazuaki Kudo, and Kazuyuki Ishii

J. Phys. Chem. B, **Just Accepted Manuscript** • DOI: 10.1021/acs.jpcb.6b07925 • Publication Date (Web): 14 Sep 2016

ACS Publications

The Journal of Physical Chemistry B is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Absolute Configuration Determination of Side Chains of Basic Amino Acid Residues Using Water-Soluble Porphyrins-Based Exciton Chirality Method

Shingo Hattori, Kengo Akagawa, Kazuaki Kudo and Kazuyuki Ishii*

Institute of Industrial Science, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8505, Japan

*(K. I.) E-mail: k-ishii@iis.u-tokyo.ac.jp. Telephone: +81-3-5452-6306. Fax: +81-3-5452-6306

Abstract

We demonstrated that the circular dichroism (CD) exciton chirality method based on the supramolecular interactions of *meso*-tetra(4-sulfonatophenyl)porphyrin ($MTPPS_4$, M = Zn or H_2) was applicable for the determination of the absolute configuration between the side chains of two basic amino acid residues of stable monomeric β-hairpin peptides (tryptophan zipper: Trpzip). When $MTPPS_4$ was added to an aqueous solution containing Trpzip, a bisignate CD signal was detected in the Soret band region in addition to a decrease in absorbance. These spectral changes indicated the formation of a supramolecule consisting of Trpzip and $MTPPS_4$ via electrostatic interactions between the positively charged lysine residue of Trpzip and the negatively charged sulfonate group of $MTPPS_4$. On the basis of the Job plots, the supramolecular structure of Trpzip-$ZnTPPS_4$ is ZnP-Tz-ZnP or ZnP-Tz-ZnP-Tz-ZnP, while that of Trpzip-H_2TPPS_4 is $-(-H_2P-Tz-)_n-$ (MP and Tz denote $MTPPS_4$ and Trpzip, respectively). In order to explain the bisignate CD spectra of the supramolecules, a plausible model, i.e., ZnP-Tz-ZnP, was carefully analyzed by the CD exciton chirality method: two orthogonalized electric transition dipole moments of each $MTPPS_4$ and the effects of free rotation of $MTPPS_4$ around the electrostatic bonding axis were considered. The exciton-coupled CD spectral pattern based on $ZnTPPS_4$ reflected the absolute

configuration between the side chains of two lysine residues. Our approach can be used to determine the absolute configuration of side chains of other peptides with two basic amino acid residues in the amino acid sequences.

Introduction

Circular dichroism (CD) is a useful tool to obtain structural information for chiral compounds in solution. In particular, the CD exciton chirality method is an efficient method for determining the absolute configurations of various chiral organic compounds, including natural products.[1-3] In these analyses, when chromophores are introduced into substituents of a chiral compound, the sign of the bisignate CD signal in the absorption band of the chromophores reflects the absolute configuration of the chiral compound. Thus, structural information for chiral compounds can be obtained non-empirically.[1-3]

Porphyrins are versatile chromophores for the exciton chirality method for the following reasons. First, intense extinction coefficients at the B band of porphyrins, which is generally called as the Soret band, enhance the CD intensity based on the exciton chirality. Second, since the Soret band is observed in the visible region, the spectral overlap with small natural chromophores, i.e., benzene derivatives, imidazole, and indole, is generally negligible, which simplifies CD analyses. Third, various functional groups can be introduced at several sites, such as *meso*-positions and *β*-positions, in porphyrin rings. Thus, there are many reports of the exciton chirality

method using porphyrins.[4-37] For example, the group of Nakanishi and Berova has reported the determination of absolute configurations of various cyclic compounds, using 5,10,15,20-tetraphenylporphyrin (H_2TPP) derivatives covalently linked to the corresponding cyclic compounds.[6-11] They also investigated structural changes in DNA using H_2TPP linked covalently.[12-15] Molinski and colleagues determined the stereochemistry of long-chain lipids in liposomes based on H_2TPP linked covalently.[16-17] The group of Nakanishi and Berova has demonstrated the usefulness of metalloporphyrin dimer-based tweezers to determine absolute configurations of various chiral compounds, on the basis of the coordination of the substituents to the central metals.[18-26] Borhan and colleagues improved the porphyrin tweezer system by increasing the Lewis acidity of metals and using rigid linkers between porphyrins.[27-33] Inoue and colleagues investigated chiral amine-coordinated bisporphyrin systems using exciton CD as well as X-ray structural analyses.[34-36] Recently, Takanami and colleagues expanded the application of bisporphyrin systems to the determination of the absolute configuration of chiral monoalcohols.[37] However, in almost all studies, chemical synthetic procedures, e.g., for the linkage between porphyrins and substrates, dimerization of porphyrins, and the introduction of functional groups to substrates, are needed for CD analyses. Furthermore, these may provide a little structural change of

chiral compounds, while the absolute configurations were not influenced. Thus, it is important to analyze the absolute configuration without chemical synthetic efforts.

A promising approach is the formation of supramolecules. For example, *meso*-tetra(4-sulfonatophenyl)porphyrins ($MTPPS_4$), which are water-soluble porphyrins, is known to form a supramolecule with peptides via electrostatic interactions between the anionic sulfonate groups of $MTPPS_4$ and the cationic amino groups of lysine residues (one of basic amino acids).[38-40] The formation of supramolecules is confirmed by exciton-coupled CD in the Soret band region. However, these CD spectra have not been analyzed by the exciton chirality method, and this may be because their supramolecular structures are unknown. If the absolute configuration between side chains of peptides can be determined by the sign of the bisignate CD signal of $MTPPS_4$ linked to the peptide via electrostatic interactions, i.e., one type of supramolecular interactions, the exciton chirality method based on the supramolecular interactions can be implemented without chemical synthetic procedures. In order to establish the exciton chirality method based on electrostatic interactions, a peptide whose structure is well-characterized should be employed for exciton-coupled CD analyses as the initial step.

Herein, we report exciton-coupled CD analyses of the supramolecule consisting of $MTPPS_4$s (M = Zn or H_2) and a stable monomeric β-hairpin peptide, tryptophan zipper (Trpzip), via electrostatic interactions (Figure 1). Trpzip is an appropriate model to demonstrate the exciton chirality method based on the supramolecular interactions, because its structure in solution has been well-characterized by two dimensional NMR, and because it has two cationic protonated lysine residues.[41-43] On the basis of the Job plots, we found the formation of supramolecular oligomers, i.e., $-(-MP-Tz-)_n-$ (MPs and Tz denote $MTPPS_4$s and Trpzip, respectively). Because the chiral exciton coupling between $MTPPS_4$ and its neighboring $MTPPS_4$ affords a bisignate CD signal, the exciton-coupled CD spectra were analyzed by the use of a plausible model, i.e., ZnP-Tz-ZnP. Although only one effective electric transition dipole moment of each porphyrin has been considered in most previous studies, two orthogonalized electric transition dipole moments of each $MTPPS_4$ ($\boldsymbol{\mu}_{ix}$ and $\boldsymbol{\mu}_{iy}$) were considered in the present analyses. Also, the effects of free rotation of $MTPPS_4$ (θ_i) around the bond axis based on electrostatic interactions between the anionic sulfonate group of $MTPPS_4$ and the cationic amino group of the lysine residue were calculated. Based on these analyses, we propose the usefulness of our exciton chirality method based on the supramolecular interaction to determine the absolute configuration of side chains of peptides with two

lysine residues in the amino acid sequences.

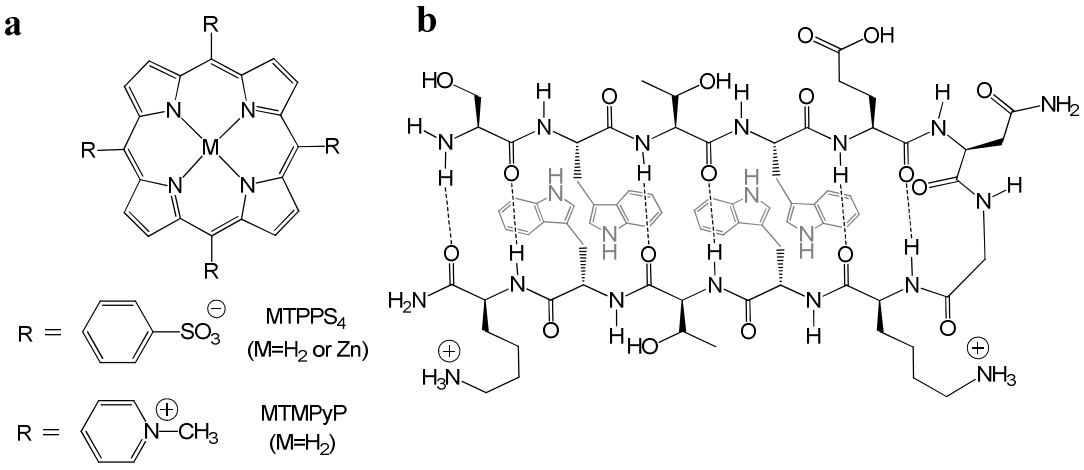


Figure 1. Molecular structures of water-soluble porphyrins (a) and Trpzip (b). The amino acid sequence of Trpzip is SerTrpThrTrpGluAsnGlyLysTrpThrTrpLys-NH_2.

Experimental

Meso-tetra(4-sulfonatophenyl)porphine (H_2TPPS_4, Figure 1a) and *meso*-tetra(*N*-methyl-4-pyridyl)porphine (H_2TMPyP, Figure 1a) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). H_2TPPS_4 was purified using methanol as a solvent to remove salts. $ZnTPPS_4$ was synthesized following previously described methods.[44]

Tryptophan zipper (Trpzip, Figure 1b) was synthesized by standard

9-fluorenylmethyl-oxycarbonyl (Fmoc) solid-phase synthesis procedures in which Fmoc-amino acids and Fmoc-NH-SAL (Rink amide resin) were used.[45-47] The crude product was purified by high-performance liquid chromatography using Shimadzu CLASS-VP system with Senshu Pak PEGASIL C4 SP100 column (25 cm) and guard column (1 cm) to obtain Trpzip. MALDI-TOF-MS measurements were performed on a Bruker BIFLEX III mass spectrometer. **Trpzip:** MALDI-TOF *m*/*e*: 1607 ($M+H^+$).

Electronic absorption and CD spectra were measured using a JASCO V-570 spectrometer and a JASCO J-725 spectrodichrometer, respectively, using 1-mm path length optical quartz cuvettes.

Structural optimizations were performed using Density Functional Theory (DFT: Gaussian 03) with a B3LYP/6-31G* basis set.

Results and interpretations

Electronic absorption and CD spectra of Trpzip and $ZnTPPS_4$ in aqueous solution are shown in Figures 2a–d. The electronic absorption spectrum of Trpzip shows B_b and L_a-L_b bands of indole rings at 220 nm and 280–290 nm, respectively.[48] These absorption bands can be explained by configuration interactions between HOMO → LUMO, HOMO → LUMO+1, HOMO-1 → LUMO, and HOMO-1 → LUMO+1 transitions.[49] The L_b and B_b bands are mainly attributed to a linear combination of HOMO → LUMO+1 and HOMO-1 → LUMO transitions, but the L_a band is mainly attributed to a single HOMO → LUMO transition. In the CD spectrum of Trpzip, exciton-coupled negative/positive signals are observed in the B_b band region, which can be explained by the twisted configurations between tryptophans, i.e., Trp2-Trp11, Trp4-Trp9, and Trp2-Trp9.[50] The electronic absorption spectrum of $ZnTPPS_4$ shows intense Soret and weak Q absorption bands at 422 and 550–600 nm (556 and 596 nm), respectively. These spectroscopic features can be explained by the Gouterman's four-orbital model,[51] in which four frontier orbitals of porphyrins, e.g., HOMO, HOMO-1, LUMO, and LUMO+1, are considered. The Soret band consists of B_x and B_y bands, whose electric transition dipole moments are orthogonalized. In the case of metal porphyrins with D_{4h} symmetry, the B_x and B_y bands are exactly degenerated, and therefore, the spectral

splitting cannot be observed in the Soret band.[4]

The aqueous solution of $ZnTPPS_4$ was added to the aqueous solution of Trpzip in order to investigate the formation of supramolecules. The electronic absorption and CD spectra of the supramolecule in aqueous solution are shown in Figures 2c and 2d, respectively. A bisignate CD signal in the Soret band region was observed in addition to a decrease in the absorbance of the Soret band, when the aqueous solution of $ZnTPPS_4$ was added to the aqueous solution of Trpzip. These results indicate the formation of a Trpzip-based supramolecule (Trpzip-$ZnTPPS_4$). A bisignate CD signal consists of two separated transitions, which show positive and negative CD signals, respectively, and therefore, for one transition or degenerate transitions in a chiral compound, an integral type CD spectral pattern should be seen in contrast to impossibility for a positive/negative CD spectral pattern. On the other hand, the bisignate CD signal can be seen, when chromophores are twisted: this is called exciton-coupled CD.[1-3] Thus, the bisignate CD signal is reasonably attributed to the exciton-coupled CD, which indicates that several twisted $ZnTPPS_4$s exist in Trpzip-$ZnTPPS_4$. In order to determine the concentration ratio of Trpzip-$ZnTPPS_4$, a Job plot of exciton-coupled CD of Trpzip-$ZnTPPS_4$ was employed (Figure 2e). The maximum value of the mole fraction of $ZnTPPS_4$ ($X_{ZnTPPS4}$) was 0.62, which indicated that the concentration ratio of Trpzip and

$ZnTPPS_4$ in the supramolecule was 2:3 or 1:2. The association constant of Trpzip-$ZnTPPS_4$ for the 1:2 supramolecule was roughly evaluated as 10^5 M^{-1}, which is similar to the previous reports describing that supramolecules consisting of peptides and $MTPPS_4$s were formed via electrostatic interactions.[38-40]

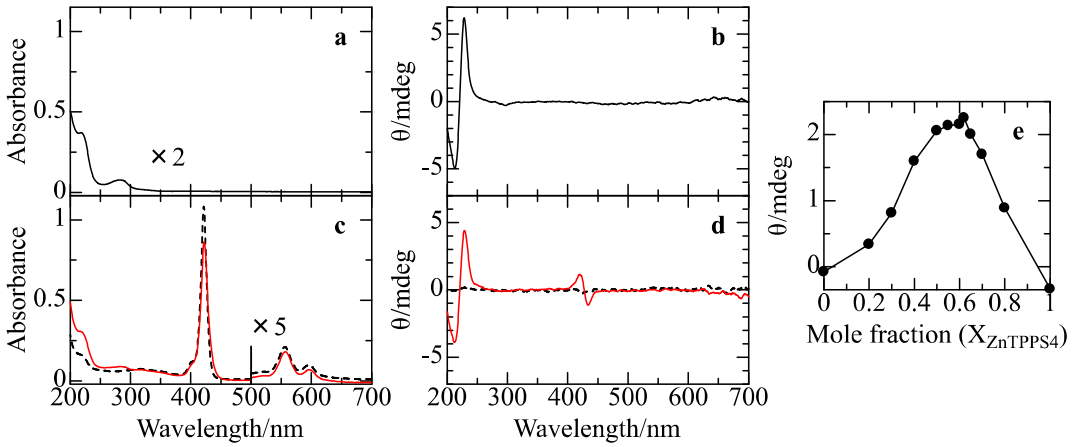


Figure 2. Typical electronic absorption and CD spectra (c, d: red) and a Job plot of CD (e) of Trpzip-$ZnTPPS_4$ in aqueous solution, as well as those of Trpzip (a, b) and $ZnTPPS_4$ (c, d: broken) in aqueous solution ([Trpzip] = 19 μM, [$ZnTPPS_4$] = 31 μM). In (e), the intensities are defined as the difference between a positive peak at 420 nm and a negative peak at 434 nm ([Trpzip] + [$ZnTPPS_4$] = 50 μM, $X_{ZnTPPS4}$ = [$ZnTPPS_4$]/([Trpzip] + [$ZnTPPS_4$])).

The electronic absorption and CD spectra of Trpzip and H_2TPPS_4 in aqueous solution are shown in Figures 3a–d. The electronic absorption spectrum of H_2TPPS_4 showed intense Soret and weak Q bands at 413 and 510–640 nm (516, 553, 581, and 635 nm), respectively. In comparison with $ZnTPPS_4$, Q bands of H_2TPPS_4 are great in number, and this can be explained by a change in the symmetry of the π-conjugated system, i.e., $ZnTPPS_4$ (D_{4h}) → H_2TPPS_4 (D_{2h}). Thus, the B_x and B_y transitions of H_2TPPS_4 are quasi-degenerate.

When the aqueous solution of H_2TPPS_4 was added to the aqueous solution of Trpzip, the electronic absorption and CD spectra are shown in Figures 3c and 3d, respectively. Similarly to the $ZnTPPS_4$ system, in the H_2TPPS_4 system, exciton-coupled positive/negative CD signals in the Soret band region were observed as well as a decrease in the absorbance of the Soret band. This indicates the formation of a Trpzip-based supramolecule containing several twisted H_2TPPS_4s (Trpzip-H_2TPPS_4). The Job plot showed that the maximum value of mole fraction of H_2TPPS_4 ($X_{H2TPPS4}$) was approximately 0.5, which indicated the concentration ratio of Trpzip and H_2TPPS_4 was 1:1 (Figure 3e).

On the other hand, distinguishable CD signals and a change in the absorbance of the

Soret band were not observed after the addition of H_2TMPyP. This indicates that the negatively charged sulfonate groups play an important role in the formation of the Trpzip-based supramolecules.[38-40]

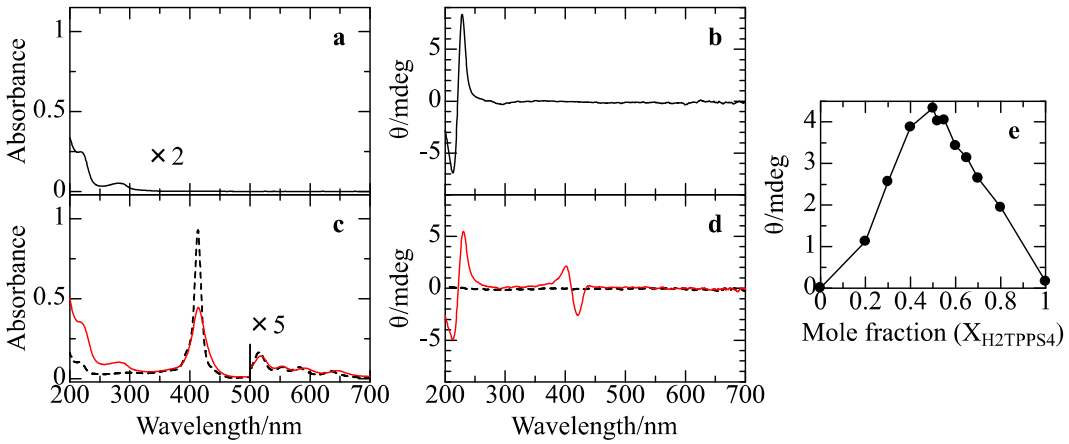


Figure 3. Typical electronic absorption and CD spectra (c, d: red) and a Job plot of CD (e) of Trpzip-H_2TPPS$_4$ in aqueous solution, as well as those of Trpzip (a, b) and H_2TPPS$_4$ (c, d: broken) in aqueous solution ([Trpzip] = 25 μM, [H_2TPPS$_4$] = 25 μM). In (e), the intensities are defined as the difference between a positive peak at 403 nm and a negative peak at 422 nm ([Trpzip] + [H_2TPPS$_4$] = 50 μM, $X_{H2TPPS4}$ = [H_2TPPS$_4$]/([Trpzip] + [H_2TPPS$_4$])).

Discussion

Based on the CD and electronic absorption spectra, Trpzip formed a supramolecule with $MTPPS_4$s via electrostatic interactions.[38-40] Based on the comparison between $MTPPS_4$s and H_2TMPyP, four anionic deprotonated sulfonate groups in $MTPPS_4$s should contribute to the electrostatic interaction with Trpzip. In Trpzip, two cationic protonated lysine residues (Lys8 and Lys12) are plausible counterparts towards the anionic deprotonated sulfonate groups, since the basicity of H_2N- in the terminal amide groups is negligible, and since the terminal amino group of a serine residue (Ser1) forms the hydrogen bonding with a carbonyl group. Because of four anionic deprotonated sulfonate groups in $MTPPS_4$s and two cationic protonated lysine residues in Trpzip, $MTPPS_4$s (MPs, M = H_2 or Zn) and Trpzips (Tzs) can form oligomers, which is represented as -(-MP-Tz-)$_n$- (n: the degree of oligomerization). On the basis of the Job plot, the supramolecular structure of Trpzip-$ZnTPPS_4$ is ZnP-Tz-ZnP or ZnP-Tz-ZnP-Tz-ZnP, while that of Trpzip-H_2TPPS_4 is -(-H_2P-Tz-)$_n$-. The exciton-coupled CD spectra originate from the twisted $MTPPS_4$ chromophores, whose twisted configurations should correlate with the chiral configuration between Lys8 and Lys12 in Trpzip. Furthermore, even in the oligomer, -(-MP-Tz-)$_n$-, the chiral exciton interaction between $MTPPS_4$ and its neighboring $MTPPS_4$ should mainly contribute to

the CD intensity, because the exciton interaction is inversely proportional to the cube of the distance between the $MTPPS_4$ chromophores. Thus, a supramolecule which contains one Trpzip and two $ZnTPPS_4$, ZnP-Tz-ZnP, is considered as a plausible model for explaining the exciton-coupled CD spectra of Trpzip-$MTPPS_4$.

From here, using the above model, the CD spectrum of Trpzip-$MTPPS_4$ was comprehensively analyzed in order to establish the exciton chirality method based on electrostatic interactions. Although it is difficult to obtain the optimum structure of whole ZnP-Tz-ZnP with flexible structures using DFT calculations, the following five procedures were used for structural evaluations based on the absolute configurations between $MTPPS_4$s in ZnP-Tz-ZnP. (1) The plausible structure of Trpzip consisting of both a rigid anti-parallel β-hairpin moiety and flexible residues was obtained from previous studies, in which the structure of Trpzip in solution was experimentally proposed based on the NMR spectrum (Protein Data Bank code: 1LE1),[41] and this structure was supported by CD analyses for tryptophans.[50] (2) The electrostatic interactions between the cationic protonated lysine residues of Trpzip and the anionic deprotonated sulfonate groups of $MTPPS_4$ were evaluated theoretically using the DFT calculation of the optimum structure of a model supramolecule, i.e., benzenesulfonate-methylammonium. (3) The optimum structure of $MTPPS_4$ was

theoretically evaluated using the DFT calculation, and the result was consistent with a previous X-ray structural analysis.[56] (4) CD spectra were calculated using the CD exciton chirality method with plausible structures of whole ZnP-Tz-ZnP, which were obtained by considering the rotations along the bond axis based on electrostatic interactions between the anionic deprotonated sulfonate group and the cationic protonated amino group, in addition to the procedures (1)–(3). (5) By comparison with the observed CD spectra, the absolute structural information of Trpzip-$MTPPS_4$ was extracted.

Initially, the structural optimization using DFT calculations was performed for benzenesulfonate-methylammonium, which corresponds to the supramolecular bond. In the optimum structure, the anionic benzenesulfonate was almost linearly connected to the cationic methylammonium moieties, as shown in Figure 4a. This is reasonably explained by the formation of a triangle pole consisting of three positively charged protons and three negatively charged oxygens. Thus, plausible structures of whole ZnP-Tz-ZnP were proposed based on the procedures (1)–(3), as shown in Figure 4b. Here, the rotations along the bond axis were considered as a function of the rotation angle (θ_i: i = 1, 2).

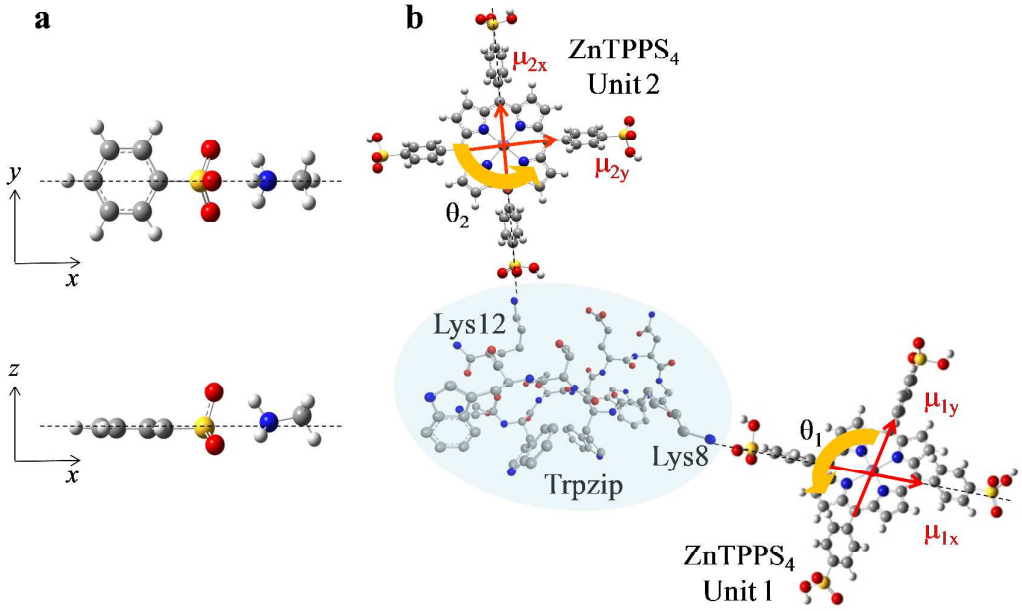


Figure 4. Optimum structures of benzenesulfonate-methylammonium (a) and the proposed structure of ZnP-Tz-ZnP (b). In (a), the upper and lower panels show the structures of benzenesulfonate-methylammonium in the *x*-*y* and *x*-*z* planes, respectively. In (b), the structure of Trpzip was obtained from PDB code 1LE1, while the structure of $ZnTPPS_4$ was obtained using the DFT calculation (B3LYP/6-31G*).

In order to calculate the CD spectra, i.e., effects of CD on electronic transitions, the excited-state energies and the rotational strengths were evaluated for electronic transitions. In almost all previous studies of the exciton-coupled CD based on

porphyrins, only one electric transition dipole moment approximately along the bond axis between the targeted chiral molecule and the porphyrin was considered for determining absolute configurations.[6-11] However, two transitions ($\boldsymbol{\mu}_x$ and $\boldsymbol{\mu}_y$), whose electric transition dipole moments are degenerate and orthogonal to each other, exist in the Soret band region. Further, the off-diagonal matrix elements should be considered not only for the chiral exciton interaction between $\boldsymbol{\mu}_{1x}$ and $\boldsymbol{\mu}_{2x}$ but also for those between $\boldsymbol{\mu}_{1x}$ and $\boldsymbol{\mu}_{2y}$, $\boldsymbol{\mu}_{1y}$ and $\boldsymbol{\mu}_{2x}$, and $\boldsymbol{\mu}_{1y}$ and $\boldsymbol{\mu}_{2y}$, where $\boldsymbol{\mu}_{iy}$ (i = 1, 2) denotes the $\boldsymbol{\mu}_y$ of unit i.[52-54] Thus, the determinant of ZnP-Tz-ZnP should be evaluated for calculating four excited-state energies, $E_n(\theta_1, \theta_2)$ (n = 1–4), and four eigenfunctions, $\Psi_n(\theta_1, \theta_2)$ (n = 1–4), which is represented by the linear combination of basis function $\phi_{ij}(\theta_i)$, which describes unit i (i = 1, 2) in the excited state j ($j = x, y$), as follows:

$$\begin{array}{c} \phi_{1x}(\theta_1) \quad \phi_{1y}(\theta_1) \quad \phi_{2x}(\theta_2) \quad \phi_{2y}(\theta_2) \\ \begin{vmatrix} \sigma_0 - E(\theta_1,\theta_2) & 0 & V_{1x2x}(\theta_1,\theta_2) & V_{1x2y}(\theta_1,\theta_2) \\ 0 & \sigma_0 - E(\theta_1,\theta_2) & V_{1y2x}(\theta_1,\theta_2) & V_{1y2y}(\theta_1,\theta_2) \\ V_{1x2x}(\theta_1,\theta_2) & V_{1y2x}(\theta_1,\theta_2) & \sigma_0 - E(\theta_1,\theta_2) & 0 \\ V_{1x2y}(\theta_1,\theta_2) & V_{1y2y}(\theta_1,\theta_2) & 0 & \sigma_0 - E(\theta_1,\theta_2) \end{vmatrix} = 0 \end{array} \quad (1)$$

$$\Psi_n(\theta_1,\theta_2) = \sum_{j=x,y} \sum_{i=1}^{2} c_{nij}(\theta_i)\phi_{ij}(\theta_i) \quad (2)$$

Here, σ_0 denotes the Soret band energy of the $ZnTPPS_4$ monomer, which was 2.37×10^5

cm^{-1} according to the electronic absorption spectrum. The off-diagonal matrix elements, $V_{ijkl}(\theta_i, \theta_k)$ (i = 1, 2, $i \neq k$ = 1, 2, $j = x, y$, $l = x, y$), which are functions of rotation angles, i.e., θ_i and θ_k, are expressed by a point-dipole approximation as follows.

$$V_{ijkl}(\theta_i, \theta_k) = \frac{\boldsymbol{\mu}_{ij}(\theta_i) \bullet \boldsymbol{\mu}_{kl}(\theta_k)}{|\mathbf{r}_{ik}|^3} - 3\frac{(\boldsymbol{\mu}_{ij}(\theta_i) \bullet \mathbf{r}_{ik})(\boldsymbol{\mu}_{kl}(\theta_i) \bullet \mathbf{r}_{ik})}{|\mathbf{r}_{ik}|^5} \quad (3)$$

Here, $\mathbf{r_{ik}}$ is a distance vector between units i and k, and was 34 Å based on the proposed structure of ZnP-Tz-ZnP. $\boldsymbol{\mu}_{\mathbf{ij}}$ was evaluated to be 7.3 Debye by fitting from the observed absorption spectrum.[55,57] Here, we defined the bond axis as parallel to $\boldsymbol{\mu}_{\mathbf{ix}}$ and perpendicular to $\boldsymbol{\mu}_{\mathbf{iy}}$. In these calculations, θ_i (i = 1, 2) was changed point by point at 30 degree steps, and thus, 144 structures were considered. Thus, four excited-state energies, $E_n(\theta_1, \theta_2)$ (n = 1–4), and four eigenfunctions, $\Psi_n(\theta_1, \theta_2)$ (n = 1–4), were calculated for each of the 144 structures. The rotational strength for each transition was calculated using $\Psi_n(\theta_1, \theta_2)$, as follows.

$$R_n = \pi\sigma_0 \left(\sum_{j=x,y} \sum_{i=1}^{2} c_{nij}(\theta_i) \boldsymbol{\mu}_{ij}(\theta_i) \right) \bullet \left(\sum_{j=x,y} \sum_{i=1}^{2} c_{nij}(\theta_i) \mathbf{r}_i \times \boldsymbol{\mu}_{ij}(\theta_i) \right) \quad (4)$$

For each of the 144 structures of ZnP-Tz-ZnP, a CD spectrum consisting of four transitions was calculated using their excited-state energies and rotational strengths. Figure 5 shows the 144 CD spectra, in which a Gaussian line shape function was

employed for each transition. We found that both positive/negative and negative/positive signs were obtained depending on θ_i. For example, a positive/negative spectral pattern was calculated when $\theta_1 = \theta_2 = 0°$ (Figure 6a, red line). In contrast, a negative/positive spectral pattern was obtained when $\theta_1 = 0°$ and $\theta_2 = 90°$ (Figure 6a, blue line). This indicates that $\boldsymbol{\mu}_{iy}$ dependent on θ_i affects the sign of CD. A positive/negative spectral pattern was calculated by averaging 144 CD spectra (Figure 6b), which indicates that the positive/negative spectral pattern is dominant. The positive/negative spectral pattern calculated for the average of the 144 CD spectra successfully reproduced the observed CD spectrum (Figure 6c). Based on the linear connection between the anionic deprotonated sulfonate group and the cationic protonated amino group, we concluded that the exciton-coupled CD spectral pattern based on $ZnTPPS_4$ reflected the absolute configuration between the side chains of two lysine residues, i.e., the bond directions between a nitrogen atom of the amino group and a neighboring carbon atom. Although some flexibility of the side chains of lysine residues should be also considered, the observed absolute configuration corresponds to the average structure in solution.

It is noteworthy that the CD spectral pattern is dependent on θ_i when $\boldsymbol{\mu}_{iy}$ is considered. With reference to most previous studies,[6-11] a CD spectrum was also calculated using

the general exciton chirality method with only $\boldsymbol{\mu}_{ix}$, which is independent of θ_i (Figure 6a, black line). In the CD spectrum calculated using only $\boldsymbol{\mu}_{ix}$, a positive/negative spectral pattern is seen, which is identical to the average of the calculated 144 CD spectra and the observed CD spectra. This similarity indicates that the general exciton CD method using only $\boldsymbol{\mu}_{ix}$ reflects the dominant spectral pattern at least in this system. On the other hand, when $\boldsymbol{\mu}_{iy}$ is considered, the CD spectral pattern is strongly dependent on θ_i, which indicates that the general exciton chirality method using only $\boldsymbol{\mu}_{ix}$ is not always correct, and that it is important to employ the exciton chirality method using both $\boldsymbol{\mu}_{ix}$ and $\boldsymbol{\mu}_{iy}$ in order to evaluate the absolute configuration when $MTPPS_4$ cannot be freely rotated. Further, the CD magnitude calculated using only $\boldsymbol{\mu}_{ix}$ ($|\Delta\varepsilon_{max}| = 37.6$, $|g_{CD}| = 3\times10^{-4}$ at 427 nm) was 4-fold compared with the magnitude of the average of 144 CD spectra that were calculated using both $\boldsymbol{\mu}_{ix}$ and $\boldsymbol{\mu}_{iy}$ ($|\Delta\varepsilon_{max}| = 9.9$, $|g_{CD}| = 1\times10^{-4}$ at 427 nm). This is because 144 CD spectra contain positive/negative and negative/positive spectral patterns. Although it is tough to quantitatively discuss the observed CD magnitude because of the coexistence of Trpzip-$ZnTPPS_4$ and a small amount of bare $ZnTPPS_4$, which prevents from determining the exact ε and $\Delta\varepsilon$ values for Trpzip-$ZnTPPS_4$, the magnitude of observed CD spectrum ($|g_{CD}| = 1\times10^{-4}$ at 434 nm), was comparable to that of the average of calculated CD spectrum. Thus, our calculation results indicate that

both the calculations using μ_{ix} and μ_{iy} and the averaging processes for various configurations are needed to quantitatively evaluate the CD magnitude.

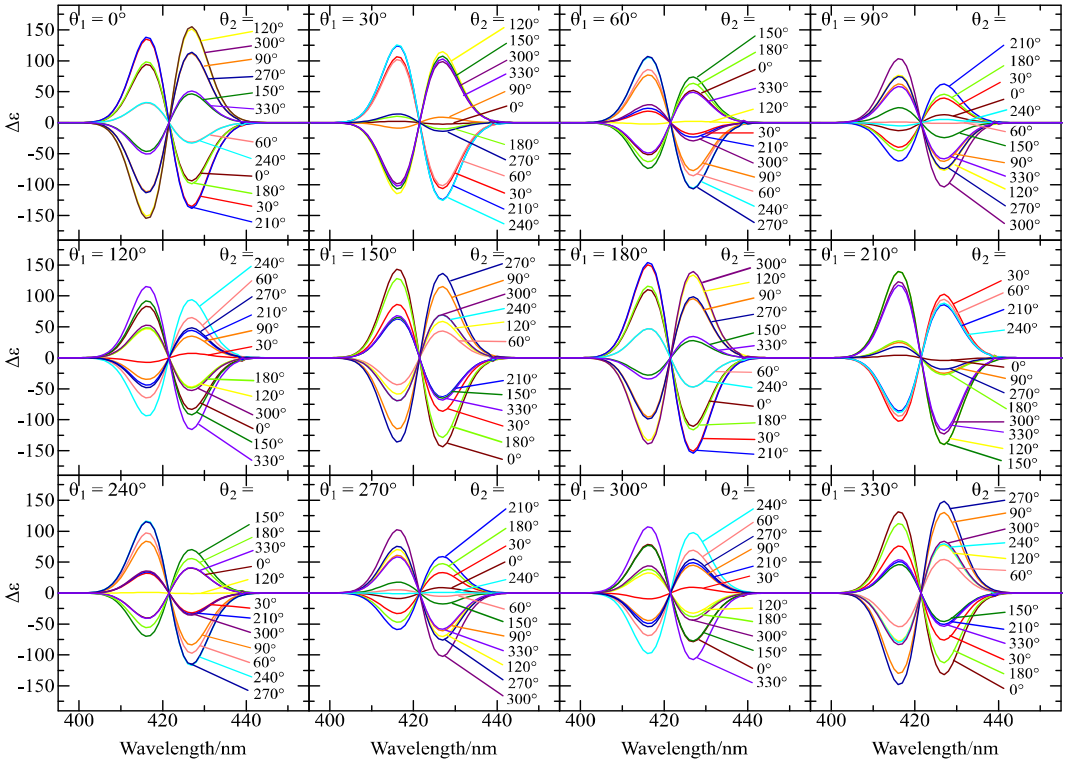


Figure 5. 144 calculated CD spectra of ZnP-Tz-ZnP (brown: θ_2 = 0°, red: θ_2 = 30°, pink: θ_2 = 60°, orange: θ_2 = 90°, yellow: θ_2 = 120°, dark green: θ_2 = 150°, yellowish green: θ_2 = 180°, blue: θ_2 = 210°, sky blue: θ_2 = 240°, navy blue: θ_2 = 270°, purple: θ_2 = 300°, bluish purple: θ_2 = 330°).

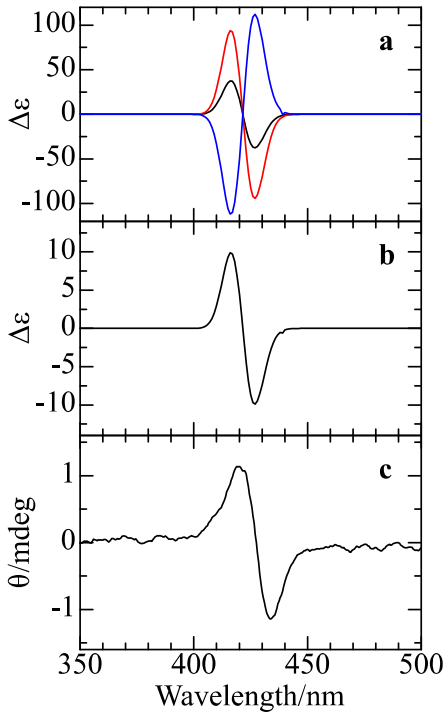


Figure 6. Calculated CD spectrum (a) of ZnP-Tz-ZnP (red: $\theta_1 = \theta_2 = 0°$, blue: $\theta_1 = 0°$ and $\theta_2 = 90°$), the average of the calculated 144 CD spectrum of ZnP-Tz-ZnP (b), and the observed CD spectrum of Trpzip-ZnTPPS$_4$ (c). In (a), red line and blue line show the CD spectra calculated using both $\boldsymbol{\mu}_{ix}$ and $\boldsymbol{\mu}_{iy}$, while black line shows the CD spectrum calculated using only $\boldsymbol{\mu}_{ix}$.

Conclusions

In this study, we investigated supramolecules consisting of Trpzip and $MTPPS_4$. A plausible model, i.e., ZnP-Tz-ZnP, was carefully analyzed by the CD exciton chirality method, and the absolute configuration between side chains of two lysine residues in Trpzip was evaluated. Our approach, the CD exciton chirality method based on the supramolecular interactions, will be useful for absolute configuration determination of side chains of other peptides with two basic amino acid residues in the amino acid sequences.

Acknowledgements

This work was supported by a Grants-in-Aid for Scientific Research (Category B No. 16H04128) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References and Notes

1. Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy: Exciton Coupling in Organic Stereochemistry;* University Science Books: Mill Valley, CA, 1983.
2. Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy: Exciton Coupling in Organic Stereochemistry;* Tokyo Kagaku Dojin: Tokyo, JPN, 1982.
3. Harada, N.; Nakanishi, K. The Exciton Chirality Method and Its Application to Configurational and Conformational Studies of Natural Products. *Acc. Chem. Res.* **1972**, *5*, 257-263.
4. Huang, X.; Nakanishi, K.; Berova, N. Porphyrins and Metalloporphyrins: Versatile Circular Dichroic Reporter Groups for Structural Studies. *Chirality* **2000**, *12*, 237-255.
5. Berova, N.; Pescitelli, G.; Petrovic, A. G.; Proni, G. Probing Molecular Chirality by

CD-Sensitive Dimeric Metalloporphyrin Hosts. *Chem. Commun.* **2009**, 5958-5980.

6. Matile, S.; Berova, N.; Nakanishi, K.; Novkova, S.; Philipova, I.; Blagoev, B. Porphyrins: Powerful Chromophores for Structural Studies by Exciton-Coupled Circular Dichroism. *J. Am. Chem. Soc.* **1995**, *117*, 7021-7022.
7. Matile, S.; Berova, N.; Nakanishi, K.; Fleischhauer, J.; Woody, R. W. Structural Studies by Exciton Coupled Circular Dichroism over a Large Distance: Porphyrin Derivatives of Steroids, Dimeric Steroids, and Brevetoxin $B^{\perp}$. *J. Am. Chem. Soc.* **1996**, *118*, 5198-5206.
8. Matile, S.; Berova, N.; Nakanishi, K. Exciton Coupled Circular Dichroic Studies of Self-Assembled Brevetoxin-Porphyrin Conjugates in Lipid Bilayers and Polar Solvents. *Chem. Biol.* **1996**, *3*, 379-392.
9. Rickman, B. H.; Matile, S.; Nakanishi, K.; Berova, N. A Two-Step Chemical / Chiroptical Method for Determining Absolute Configurations of α-Hydroxy Acids. *Tetrahedron* **1998**, *54*, 5041-5064.
10. Jiang, H.; Huang, X.; Nakanishi, K.; Berova, N. Nanogram Scale Absolute Configurational Assignment of Ceramides by Circular Dichroism. *Tetrahedron Lett.* **1999**, *40*, 7645-7649.
11. Tanaka, K.; Itagaki, Y.; Satake, M.; Naoki, H.; Yasumoto, T.; Nakanishi, K.; Berova,

N. Three Challenges toward the Assignment of Absolute Configuration of Gymnocin-B. *J. Am. Chem. Soc.* **2005**, *127*, 9561-9570.

12. Balaz, M.; Holmes, A. E.; Benedetti, M.; Rodriguez, P. C.; Berova, N.; Nakanishi, K.; Proni, G. Synthesis and Circular Dichroism of Tetraarylporphyrin-Oligonucleotide Conjugates. *J. Am. Chem. Soc.* **2005**, *127*, 4172-4173.
13. Balaz, M.; Napoli, M. D.; Holmes, A. E.; Mammana, A.; Nakanishi, K.; Berova, N.; Purrello, R. A Cationic Zinc Porphyrin as a Chiroptical Probe for Z-DNA. *Angew. Chem. Int. Ed.,* **2005**, *44*, 4006-4009.
14. Balaz, M.; Li, B. C.; Steinkruger, J. D.; Ellestad, G. A.; Nakanishi, K.; Berova, N. Porphyrins Conjugated to DNA as CD Reporters of the Salt-Induced B to Z-DNA Transition. *Org. Biomol. Chem.* **2006**, *4*, 1865-1867.
15. Mammana, A.; Pescitelli, G.; Asakawa, T.; Jockusch, S.; Petrovic, A. G.; Monaco, R. R.; Purrello, R.; Turro, N. J.; Nakanishi, K.; Ellestad, G. A., et al. Role of Environmental Factors on the Structure and Spectroscopic Response of 5'-DNA-Porphyrin Conjugates Caused by Changes in the Porphyrin-Porphyrin Interactions. *Chem. Eur. J.* **2009**, *15*, 11853-11866.
16. MacMillan, J. B.; Molinski, T. F. Long-Range Stereo-Relay: Relative and Absolute

Configuration of 1,*n*-Glycols from Circular Dichroism of Liposomal Porphyrin Esters. *J. Am. Chem. Soc.* **2004**, *126*, 9944-9945.

17. MacMillan, J. B.; Linington, R. G.; Andersen, R. J.; Molinski, T. F. Stereochemical Assignment in Acyclic Lipids Across Long Distance by Circular Dichroism: Absolute Stereochemistry of the Aglycone of Caminoside A. *Angew. Chem. Int. Ed.* **2004**, *43*, 5946-5951.
18. Huang, X.; Rickman, B. H.; Borhan, B.; Berova, N.; Nakanishi, K. Zinc Porphyrin Tweezer in Host-Guest Complexation: Determination of Absolute Configurations of Diamines, Amino Acids, and Amino Alcohols by Circular Dichroism. *J. Am. Chem. Soc.* **1998**, *120*, 6185-6186.
19. Huang, X.; Borhan, B.; Rickman, B. H.; Nakanishi, K.; Berova, N. Zinc Porphyrin Tweezer in Host-Guest Complexation: Determination of Absolute Configurations of Primary Monoamines by Circular Dichroism. *Chem. Eur. J.* **2000**, *6*, 216-224.
20. Kurtán, T.; Nesnas, N.; Li, Y.-Q.; Huang, X.; Nakanishi, K.; Berova, N. Chiral Recognition by CD-Sensitive Dimeric Zinc Porphyrin Host. 1. Chiraoptical Protocol for Absolute Configurational Assignments of Monoalcohols and Primary Monoamines. *J. Am. Chem. Soc.* **2001**, *123*, 5962-5973.
21. Huang, X.; Fujioka, N.; Pescitelli, G.; Koehn, F. E.; Williamson, R. T.; Nakanishi,

K.; Berova, N. Absolute Configurational Assignments of Secondary Amines by CD-Sensitive Dimeric Zinc Porphyrin Host. *J. Am. Chem. Soc.* **2002**, *124*, 10320-10335.

22. Solladié-Cavallo, A.; Marsol, C.; Pescitelli, G.; Bari, L. D.; Salvadori, P.; Huang, X.; Fujioka, N.; Berova, N.; Cao, X.; Freedman, T. B., et al. (*R*)-(+)- and (*S*)-1-(9-Phenanthryl)ethylamine: Assignment of Absolute Configuration by CD Tweezer and VCD Methods, and Difficulties Encountered with the CD Exciton Chirality Method. *Eur. J. Org. Chem.* **2002**, 1788-1796.

23. Proni, G.; Pescitelli, G.; Huang, X.; Quraishi, N. Q.; Nakanishi, K.; Berova, N. Configurational Assignment of α-Chiral Carboxylic Acids by Complexation to Dimeric Zn-Porphyrin: Host-Guest Structure, Chiral Recognition and Circular Dichroism. *Chem. Commun.* **2002**, 1590-1591.

24. Proni, G.; Pescitelli, G.; Huang, X.; Nakanishi, K.; Berova, N. Magnesium Tetraarylporphyrin Tweezer: A CD-Sensitive Host for Absolute Configurational Assignments of α-Chiral Carboxylic Acids. *J. Am. Chem. Soc.* **2003**, *125*, 12914-12927.

25. Chen. Y.; Petrovic, A. G.; Roje, M.; Pescitelli, G.; Kayser, M. M.; Yang, Y.; Berova, N.; Proni, G. CD-Sensitive Zn-Porphyrin Tweezer Host-Guest Complexes, Part 2:

Cis- and *Trans*-3-Hydroxy-4-aryl/alkyl-β-Lactams. A Case Study. *Chirality* **2010**, *22*, 140-152.

26. Petrovic, A. G.; Vantomme, G.; Nergrón-abril, Y. L.; Lubian, E.; Saielli, G.; Menegazzo, I.; Cordero, R.; Proni, G.; Nakanishi, K.; Carofiglio, T., et al. Bulky Melamine-Based Zn-Porphyrin Tweezer as a CD Probe of Molecular Chirality. *Chirality* **2011**, *23*, 808-819.
27. Li, X.; Tanasova, M.; Vasileiou, C.; Borhan, B. Fluorinated Porphyrin Tweezer: A Powerful Reporter of Absolute Configuration for *Erythro* and *Threo* Diols, Amino Alcohols, and Diamines. *J. Am. Chem. Soc.* **2008**, *130*, 1885-1893.
28. Li, X.; Borhan, B. Prompt Determination of Absolute Configuration for Epoxy Alcohols via Exciton Chirality Protocol. *J. Am. Chem. Soc.* **2008**, *130*, 16126-16127.
29. Li, X.; Burrell, C. E.; Staples, R. J.; Borhan, B. Absolute Configuration for 1,n-Glycols: A Nonempirical Approach to Long-Range Stereochemical Determination. *J. Am. Chem. Soc.* **2012**, *134*, 9026-9029.
30. Tanasova, M.; Borhan, B. Conformational Preference in Bis(porphyrin) Tweezer Complexes: A Versatile Chirality Sensor for α-Chiral Carboxylic Acids. *Eur. J. Org. Chem.* **2012**, 3261-3269.

31. Tanasova, M.; Anyika, M.; Borhan, B. Sensing Remote Chirality: Stereochemical Determination of β-,γ-, and δ-Chiral Carboxylic Acids. *Angew. Chem. Int. Ed.* **2015**, *54*, 4274-4278.

32. Tanasova, M.; Vasileiou, C.; Olumolade, O. O.; Borhan, B. Enhancement of Exciton Coupled Circular Dichroism with Sterically Encumbered Bis-Porphyrin Tweezers. *Chirality* **2009**, *21*, 374-382.

33. Anyika, M.; Gholami, H.; Ashtekar, K. D.; Acho, R.; Borhan, B. Point-to-Axial Chirality Transfer–A New Probe for "Sensing" the Absolute Configurations of Monoamines. *J. Am. Chem. Soc.* **2014**, *136*, 550-553.

34. Borovkov, V. V.; Yamamoto, N.; Lintuluoto. J. M.; Tanaka, T.; Inoue, Y. Supramolecular Chirality Induction in Bis(zinc porphyrin) by Amino Acid Derivatives: Rationalization and Applications of the Ligand Bulkiness Effect. *Chirality* **2001**, *13*, 329-335.

35. Lintuluoto, J. M.; Borovkov, V. V.; Inoue, Y. Direct Determination of Absolute Configuration of Monoalcohols by Bis(magnesium porphyrin). *J. Am. Chem. Soc.* **2002**, *124*, 13676-13677.

36. Borovkov, V. V.; Fujii, I.; Muranaka, A.; Hembury, G. A.; Tanaka, T.; Ceulemans, A.; Kobayashi, N.; Inoue, Y. Rationalization of Supramolecular Chirality in a

Bisporphyrin System. *Angew. Chem. Int. Ed.* **2004**, *43*, 5481-5485.

37. Hayashi, S.; Yotsukura, M.; Noji, M.; Takanami, T. Bis(zinc porphyrin) as a CD-Sensitive Bidentate Host Molecule: Direct Determination of Absolute Configuration of Mono-Alcohols. *Chem. Commun.* **2015**, *51*, 11068-11071.
38. Kovaric, B. C.; Kokona, B.; Schwab, A. D.; Twomey, M. A.; Paula, J. C.; Fairman, R. Self-Assembly of Peptide Porphyrin Complexes: Toward the Development of Smart Biomaterials. *J. Am. Chem. Soc.* **2006**, *128*, 4166-4167.
39. Kuciauskas, D.; Caputo, G. A. Self-Assembly of Peptide-Porphyrin Complexes Leads to pH-Dependent Excitonic Coupling. *J. Phys. Chem. B* **2009**, *113*, 14439-14447.
40. Andrade, S. M.; Costa, S. M. B. Spectroscopic Studies on the Interaction of a Water Soluble Porphyrin and Two Drug Carrier Proteins. *Biophys. J.* **2002**, *82*, 1607-1619.
41. Cochran, A. G.; Skelton, N. J.; Starovasnik, M. A. Tryptophan Zippers: Stable, Monomeric β-Hairpins. *Proc. Nat. Acad. Sci. USA* **2001**, *98*, 5578-5583.
42. Wu, L.; McElhency, D.; Takekiyo, T.; Keiderling, T. A. Geometry and Efficacy of Cross-Strand Trp/Trp, Trp/Tyr and Tyr/Tyr Aromatic Interaction in a β-Hairpin Peptide. *Biochemistry* **2010**, *49*, 4705-4714.
43. Markiewicz, B. N.; Oyola, R.; Du, D.; Gai, F. Aggregation Gatekeeper and

Controlled Assembly of Trpzip β-Hairpins. *Biochemistry* **2014**, *53*, 1146-1154.

44. Mori, S.; Ishii, K.; Hirakawa, Y.; Nakamura, R.; Hashimoto, K. In Vivo Participation of Artificial Porphyrins in Electron-Transport Chains: Electrochemical and Spectroscopic Analyses of Microbial Metabolism. *Inorg. Chem.* **2011**, *50*, 2037-2039.
45. Akagawa, K.; Kudo, K. Construction of an All-Carbon Quaternary Stereocenter by the Peptide-Catalyzed Asymmetric Michael Addition of Nitromethane to β-Disubstituted α,β-Unsaturated Aldehydes. *Angew. Chem. Int. Ed.* **2012**, *51*, 12786-12789.
46. Akagawa, K.; Sen, J.; Kudo, K. Peptide-Catalyzed Regio- and Enantioselective Reduction of α,β,γ,δ-Unsaturated Aldehydes. *Angew. Chem. Int. Ed.* **2013**, *52*, 11585-11588.
47. Akagawa, K.; Sakai, N.; Kudo, K. Histidine-Containing Peptide Catalysts Developed by a Facile Library Screening Method. *Angew. Chem. Int. Ed.* **2015**, *54*, 1822-1826.
48. Platt, J. Classification of Spectra of CataCondensed Hydrocarbons. *J. Chem. Phys.* **1949**, *17*, 484-495.
49. Serrano-André, L.; Roos, B. O. Theoretical Study of the Absorption and Emission

Spectra of Indole in the Gas Phase and in a Solvent. *J. Am. Chem. Soc.* **1996**, *118*, 185-195.

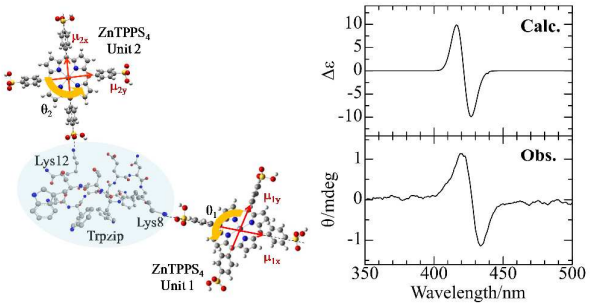
50. Roy, A.; Bour, P.; Keiderling, T. A. TD-DFT Modeling of the Circular Dichroism for a Tryptophan Zipper Peptide with Coupled Aromatic Residues. *Chirality* **2009**, *21*, E163-E171.
51. Gouterman, M. *in Porphyrins;* Dolphin, D., ed.; Academic Press: New York, 1978; Vol. 3, pp 1-165.
52. Kitagawa, Y.; Segawa, H.; Ishii, K. Magneto-Chiral Dichroism of Organic Compounds. *Angew. Chem. Int. Ed.* **2011**, *50*, 9133-9136.
53. Kobayashi, N.; Narita, F.; Ishii, K.; Muranaka, A. Optically Active Oxo(phthalocyaninato)vanadium(IV) with Geometric Asymmetry: Synthesis and Correlation between the Circular Dichroism Sign and Conformation. *Chem. Eur. J.* **2009**, *15*, 10173-10181.
54. Pescitelli, G.; Gabriel, S; Wang, Y.; Fleischhauer, J.; Woody, R. W.; Berova, N. Theoretical Analysis of the Porphyrin–Porphyrin Exciton Interaction in Circular Dichroism Spectra of Dimeric Tetraarylporphyrins *J. Am. Chem. Soc.* **2003**, *125*, 7613-7628.
55. Sultan, N. A. M.; Maiya, B. G.; Swamy, M. J. Thermodynamic Analysis of

Porphyrin Binding to Monordica Charantia (Bitter Grourd) Lectin. *Eur. J. Biochem.* **2004**, *271*, 3274-3282.

56. Fleischer, E. B.; Miller, C. K.; Webb, L. E. Crystal and Molecular Structures of Some Metal Tetraphenylporphyins. *J. Am. Chem. Soc.* **1964**, *86*, 2342-2347.

57. The observed Soret absorption band of $ZnTPPS_4$, which is doubly degenerate, was reproduced by a Gaussian function, by which the magnitudes of electric transition dipole moments ($\boldsymbol{\mu}_{ix}$ and $\boldsymbol{\mu}_{iy}$) were experimentally evaluated.

TOC image


ZnTPPS4
Unit 2
μ2x
μ2y
θ2
Lys12
Trpzip
Lys8
θ1
μ1y
μ1x
ZnTPPS4
Unit 1
Calc.
Obs.
Δε
θ/mdeg
Wavelength/nm
10
5
0
-5
-10
1
0
-1
350
400
450
500