

First-principles ECD calculation using RISM-SCF-cSED

First-principles computation of electronic circular dichroism spectra of solvated molecules using RISM-SCF-cSED

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We propose a practical first-principles protocol for computing electronic circular dichroism (ECD) spectra of chiral molecules in aqueous solution by combining time-dependent density functional theory (TDDFT) with the reference interaction site model self-consistent field with constrained spatial electron density (RISM-SCF-cSED), and, when necessary, molecular dynamics (MD)-based conformational sampling. After demonstrating the approach for D-lactic acid in water as a benchmark system with limited conformational variability, we apply a similar workflow to a solvated biomolecule, the terminally capped alanine tetramer (Ac-Ala₄-NMe). The achievements of this study is that incorporating explicit solutewater hydrogen-bonding structure through RISM-SCF-cSED enables quantitative reproduction of the ECD spectrum of the rigid α -helical state. These results establish RISM-SCF-cSED/TDDFT combined as a general and computationally efficient platform for predicting ECD spectra of solvated molecules.

I. INTRODUCTION

Circular dichroism (CD) spectroscopy measures the differential absorption of left- and right-circularly polarized light, which is commonly reported as the differential molar extinction coefficient $\Delta\epsilon(\lambda)$ (or equivalently as molar ellipticity $[\theta](\lambda)$). It is an indispensable technique for elucidating the structure and conformation of chiral molecules, including biomacromolecules such as proteins and nucleic acids¹⁻³. In particular, the electronic circular dichroism (ECD) in far-UV serves as a sensitive probe of the secondary structure of polypeptides and proteins, enabling the identification and quantification of motifs such as right-handed α -helices and β -strands from the characteristic sign pattern and band positions of the spectrum⁴⁻⁷.

From a theoretical viewpoint, the ECD spectrum is characterized by both the excitation energies (band positions) and the signed intensities of the bands. The latter is governed by the rotational strength of each electronic transition, while the corresponding UV-Vis absorption intensity is described by the oscillator strength. Theoretical calculations have played an essential role in interpreting the experimental ECD spectra⁸⁻¹⁰. Because the rotational strength is calculated from the scalar product of the electric and magnetic transition dipole moments, the treatment of electronic excitation is required¹¹⁻¹⁴. While highly sophisticated computational methods such as SAC-CI¹⁵⁻²⁴ or CAS-SCF/PT2²⁵⁻²⁸ have been employed, time-dependent density functional theory (TDDFT) is also widely utilized to predict the absolute configuration of chiral organic compounds and natural products²⁹⁻³², as well as to analyze induced ECD in protein-ligand interactions^{33,34}. In addition, two challenges must be considered to quantitatively calculate the ECD of, especially, biomacromolecules such as polypeptides existing in real systems. One is an adequate representation of conformational fluctuations. For instance, even minor alterations in chromophore orientation have been demonstrated to modify the intensity and even the sign of the bands^{35,36}. In particular, small polypeptides are too short to fold; they take a broader range of conformation. The other is an accurate description of the solvent effects on electronic structure. The polarizable continuum model (PCM) is the standard tool for incorporating bulk solvation, approximating the solvent by its dielectric constant ϵ ^{37,38}. This method can accurately calculate excitation energies and band shapes, particularly when specific solute-solvent interactions are not dominant^{39,40}. Furthermore, combining approaches incorporating a small number of explicit solvent molecules into PCM³², and the quantum mechanics/molecular mechanics (QM/MM) method employing molecular dynamics (MD) simulations is also effective^{41,42}. However, its application

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often entails substantial computational costs. In aqueous solutions, it is readily conceivable that local hydrogen bonding influences transition energies and rotational strength, necessitating computational models that incorporate such a specific solute-solvent interaction.

A promising route is offered by hybrid integral-equation/quantum-chemical approaches, in which the solute wavefunction is self-consistently coupled to the correlation functions of the solvent. The reference interaction site model self-consistent field with constrained spatial electron density (RISM-SCF-cSED) belongs to this family, providing a computationally efficient description of local solvent structure around the solute molecule treated with quantum chemistry^{43–45}. In this study, after the demonstration for a relatively small organic molecule in solution, D-lactic acid in water which has minimal structural changes, the ECD spectrum of the terminally capped alanine tetramer (Ac-Ala₄-NMe) in solution, for which experimental data are available³², was calculated using the RISM-SCF-cSED method. Recent developments and applications of RISM-SCF-cSED demonstrate that it can account for diverse solution phenomena — including reaction thermodynamics, NMR observables^{46–50}, hyper Raman spectra⁵¹ and excited-state properties such as solvatochromism — by directly feeding the solvent-induced potential into the electronic-structure calculation⁴⁵. It should be emphasized that the ECD calculation has not been employed within the RISM-SCF-cSED framework to date, which is a key contribution of the present work. Another contribution is to consider the conformational fluctuation of the solute molecule's geometry, which is often fixed in many quantum chemical computations. Because the original RISM and 3D-RISM are also employed at the fixed geometry, the conformational effect is introduced via MD-based ensemble averaging^{52–54}. The method combining RISM-SCF-cSED averaging provides a unified platform for predicting ECD spectra.

II. THEORETICAL METHODS

We used the RISM-SCF-cSED⁴⁴ method to explicitly treat the solvent distribution around the solute electronic structure. Since the details can be found elsewhere⁴⁵, we briefly describe the method for combinations with excited state calculations. In the RISM^{55,56}, an integral equation theory (IET) of molecular liquids, the solvent structure is described using total and direct correlation function $h_{\alpha s}(r)$, $c_{\alpha s}(r)$. The main equations are the following RISM and closure relation (e.g.

HNC closure);

$$h_{\alpha s}(r) = \sum_{\gamma} \omega_{\alpha\gamma} * c_{\gamma t} * \{\omega_{ts} + \rho_t h_{ts}\}(r), \quad (1)$$

$$h_{\alpha s}(r) = \exp\{-\beta u_{\alpha s}(r) + h_{\alpha s}(r) - c_{\alpha s}(r)\} - 1, \quad (2)$$

where $\beta = 1/k_B T$ and ρ_t is the thermodynamic beta and number density of solvent site t , respectively. Please refer to the literature for details on the intramolecular correlation function ($\omega_{\alpha\gamma}$) and solute-solvent interaction function ($u_{\alpha s}$). In this theory, the solvent structure is obtained as the radial distribution function (RDF) $g_{\alpha s}(r)$ in the following relation;

$$g_{\alpha s}(r) = h_{\alpha s}(r) + 1. \quad (3)$$

To combine with solute QM calculations, the solvent-induced potential V is added to the Fock operator using the SEDD (spatial electron density distribution) scheme⁵⁷⁻⁶¹;

$$[F_{\text{gas}} + V\Xi^{-1}\Sigma]C = SC\varepsilon, \quad (4)$$

$$V_k = \sum_s \rho_s q_s \int d\mathbf{r} h_{\alpha s}(|\mathbf{r} - \mathbf{R}_{\alpha}|) \int d\mathbf{r}' \frac{f_k(\mathbf{r}')}{|\mathbf{r} - \mathbf{r}'|}, \quad (5)$$

where S , C , ε are the overlap integral, orbital coefficients and orbital energies, respectively. In Eq. (5), ρ_s and q_s are the solvent site density and charge, respectively. $f_k(\mathbf{r})$ is the auxiliary basis set dealing with the spatial distribution of the solute charge. For a precise definition of Ξ , Σ and $f_k(\mathbf{r})$, the reader is referred to Sec. III.B of Ref. 45. In Eq. (5), the correlation function $h_{\alpha s}$ is included, bridging between the QM and IET calculations. Note that $u_{\alpha s}(r)$ in Eq. (2) depends on the density matrix $\mathbf{P}$; $u_{\alpha s} = u_{\alpha s}(r; \mathbf{P})$, where

$$P_{\mu\nu} = 2 \sum_i C_{i\mu}^* C_{i\nu}. \quad (6)$$

These formulas are solved self-consistently in terms of V and $\mathbf{P}$. In order to avoid negative atomic site density, the cSED scheme⁴⁴ was introduced by the following Lagrangian using parameters m and μ ,

$$\Lambda_{\text{cSED}} = \Lambda_{\text{SEDD}} + \frac{2}{m} \mu C^{(m)}[\mathbf{d}], \quad (7)$$

where $C^{(m)}[\mathbf{d}]$ is the penalty function and the reader is referred to Sec. III.C of Ref. 45 for its detailed definition.

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Once a ground state (GS) wavefunction is obtained from the RISM-SCF-cSED procedure, the excited state (ES) properties can be calculated within the TDDFT framework. The excitation energies Ω and the corresponding eigenvectors $(\mathbf{X}, \mathbf{Y})$ are obtained by solving the Casida equation,

$$\begin{pmatrix} \mathbf{A} & \mathbf{B} \\ \mathbf{B} & \mathbf{A} \end{pmatrix} \begin{pmatrix} \mathbf{X} \\ \mathbf{Y} \end{pmatrix} = \Omega \begin{pmatrix} 1 & 0 \\ 0 & -1 \end{pmatrix} \begin{pmatrix} \mathbf{X} \\ \mathbf{Y} \end{pmatrix}, \quad (8)$$

where the orbital energy differences and two-electron integrals in $(\mathbf{X}, \mathbf{Y})$ are augmented by the solvent-induced potential obtained from the RISM-SCF-cSED scheme. In the present RISM-SCF-cSED/TDDFT scheme, the solvation effect on electronic excitations is incorporated through the solvent-induced one-electron potential V obtained self-consistently for the ground state (Eqs. (4)-(7)). The Casida matrices are constructed from the solvent-refined Kohn-Sham orbitals and orbital energies in the presence of V ; consequently, the electron-hole coupling terms (including the two-electron-integral contributions) are evaluated with these solvent-perturbed orbitals, i.e., solvation affects them implicitly via orbital relaxation induced by V rather than via an additional solvent-dependent two-electron operator. Because ECD is an absorption (vertical excitation) property, we adopt a frozen-solvent approximation in the excited-state calculation: the solvent distribution/correlation functions determined for the ground state are kept fixed, and no further solvent relaxation with the excited-state density is considered. The ECD spectra are then simulated from the calculated excitation energies and rotational strengths. The latter is defined for the n -th ES as

$$R_{0n} = \text{Im}(\boldsymbol{\mu}_{0n} \cdot \mathbf{m}_{0n}), \quad (9)$$

where $\boldsymbol{\mu}_{0n}$ and $\mathbf{m}_{0n}$ are the electric and magnetic transition dipole moments between the GS and n -th ES, respectively. They are represented in the TDDFT scheme using the components of the eigenvectors in Eq. (8) ($X_{ja,n}$ and $Y_{ja,n}$) as

$$\boldsymbol{\mu}_{0n} = \sum_{ja} \langle j | \mathbf{r} | a \rangle (X_{ja,n} + Y_{ja,n}), \quad (10)$$

$$\mathbf{m}_{0n} = \sum_{ja} \left\langle j \left| \frac{i}{2} \mathbf{r} \times \boldsymbol{\nabla} \right| a \right\rangle (X_{ja,n} - Y_{ja,n}), \quad (11)$$

where the bracket and indices j and a are the conventional notation. Through this scheme, it becomes possible to estimate the ECD spectrum, which is derived from the solvent-refined wavefunctions and excitation energies. In the present work, we implemented the RISM-SCF-cSED/TDDFT scheme into the Gaussian 16 program package⁶² and all the electronic structure calculations were

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performed using this code. In practice, the present RISM-SCF-cSED/TDDFT calculation is carried out in two steps. First, for a given solute geometry, the coupled RISM and SCF equations (Eqs. (1)-(7)) are iterated until self-consistency is achieved between the solute density matrix P and the solvent-induced potential V . After obtaining the converged ground-state wavefunction and solvent-refined orbital energies, the Casida equation (Eq. (8)) is solved using these quantities to compute the excited states and the ECD spectrum.

III. COMPUTATIONAL DETAILS

A. Shared settings for RISM-SCF-cSED/TDDFT calculations

The RISM equation was solved by using the hyper-netted chain (HNC) closure at 298.15 K. The Lennard-Jones parameters for the solute and solvent molecules are listed in the Supplementary material (SM, Sec. 1.A-B). The bulk solvent site density was set to $\rho_t = 3.336 \times 10^{-2} \text{ \AA}^{-3}$, corresponding to a mass density of $0.99796 \text{ g cm}^{-3}$ for liquid water. The solvent was represented by a rigid three-site water model with $r_{\text{OH}} = 0.9572 \text{ \AA}$ and $\angle\text{HOH} = 104.52^\circ$ (see Sec. 1.B in SM). In the present work, the solvent susceptibility entering the RISM-SCF-cSED calculation was generated from the standard one-dimensional RISM/HNC treatment of the adopted three-site water model at the specified (T and ρ_t) state point. Accordingly, unlike continuum models, no macroscopic dielectric constant was supplied as an external parameter, and no dielectric-consistent RISM (DRISM) correction was applied to force the experimental dielectric constant of water. We adopted this setup to keep the first ECD implementation fully consistent with the established RISM-SCF-cSED protocol and to focus on the role of explicit local solute-water structure. Detailed numerical settings of RISM-SCF-cSED including convergence and grid point information are shown in Sec. 1.C in SM.

Other setups are in the following way. Unless otherwise stated, TDDFT calculations were performed at the B3LYP/6-311++G(d,p) level. In addition, for the rigid α -helical Ac-Ala₄-NMe benchmark, we also tested other exchange-correlation functionals (PBE0, M06-2X, CAM-B3LYP, and ω B97X-D) with the same 6-311++G(d,p) basis set to assess the functional dependence of the calculated ECD spectrum; the results are summarized in Fig. S2 and Table S2 of the Supplementary Material. After the RISM-SCF-cSED/TDDFT calculations, the obtained transitions were convoluted using a Gaussian function with a full-width at half-maximum (FWHM) of 0.7 eV, whereas

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0.35 eV was used for the UV-Vis spectra. When we computed the ensemble-averaged spectrum, it was obtained by direct averaging of the individual snapshot spectra. In figures showing ensemble data, we plot all individual snapshot spectra together with their arithmetic mean rather than a shaded uncertainty band, because the spectral spread is strongly wavelength-dependent and can be non-Gaussian or even sign-changing; this representation is intended to show the full configurational variation without implying a formal confidence interval. Because the *NPT* MD trajectory already provides a Boltzmann-distributed ensemble under the employed force field, no additional reweighting was applied. The calculated spectra were plotted using the Multiwfn program^{63,64}, and the experimental spectra were extracted using WebPlotDigitizer⁶⁵. The digitized experimental ECD curves were used on a relative vertical scale. Accordingly, the comparisons below focus on the sign pattern, band positions, and normalized intensity ratios rather than on absolute CD magnitudes. All the MD simulations were performed with the Amber 25 package⁶⁶.

B. D-lactic acid in aqueous solution

To demonstrate the effect of incorporating conformational fluctuations into the spectral simulation, we chose D-lactic acid (DLA) as a simple chiral model system. Because its conformational ensemble in solution is expected to be influenced primarily by rotation about the C^{*}–C(=O) bond, DLA provides a convenient minimal example for assessing how such torsional motion affects the calculated ECD spectrum. Force-field parameters for the neutral DLA solute were generated with GAFF2⁶⁷ and AM1-BCC partial charges^{68,69}. The solute was solvated in a periodic TIP3P⁷⁰ water box with a 12 Å buffer, resulting in 12 solute atoms and 828 water molecules (2496 atoms in total). After solvent relaxation followed by an unrestrained minimization, the system was heated from 0 to 300 K for 50 ps under *NVT* with harmonic restraints (5 kcal mol^{−1} Å^{−2}) on solute heavy atoms. Subsequently, *NPT* equilibration was carried out at 300 K and 1 atm with weakened restraints (2 kcal mol^{−1} Å^{−2}) for 200 ps and then without restraints for 500 ps. Production MD was performed in the *NPT* ensemble for 3.6 ns. A 2 fs time step was used with SHAKE constraints on bonds involving hydrogen atoms⁷¹. Long-range electrostatic interactions were treated with the particle-mesh Ewald method⁷², and a 10 Å cutoff was applied to nonbonded interactions. Temperature was controlled by a Langevin thermostat ($\gamma = 2$ ps^{−1}), and pressure was controlled isotropically (relaxation time 2 ps). Coordinates were saved every 10 ps during the production MD. For the ECD calculations, 50 solute snapshots were selected from the production trajectory

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at approximately uniform intervals (ca. 70 ps between analyzed snapshots; i.e., not every saved frame). Water molecules were removed from these snapshots, and the resulting solute geometries were used as input for the RISM-SCF-cSED/TDDFT calculations, in which the 10 lowest-lying excited states were computed. In contrast to Ac-Ala₄-NMe discussed below, we did not introduce a separate fixed-geometry benchmark for D-lactic acid, because the purpose here was to assess ensemble-averaging effects in a small chiral solute without an experimentally isolated rigid reference state.

C. Ac-Ala₄-NMe in aqueous solution

The experimental curve used for comparison is the difference ECD spectrum for Ac-HAAAH-NH₂ reported in Fig. S6a of Ref. 32. It was obtained by subtracting the additive-free spectrum from the spectrum measured under the His/Co²⁺-stabilized condition. Because the experimental peptide contains His residues and differs from Ac-Ala₄-NMe in sequence, terminal capping, and metal-coordination environment, this curve is not an absolute experimental ECD spectrum of Ac-Ala₄-NMe. Following the interpretation in Ref. 32, we use it only as an experimentally extracted proxy for the α -helical backbone contribution. Therefore, the comparison is restricted to the normalized line shape, sign pattern, and band positions, rather than absolute CD intensity.

Ac-Ala₄-NMe in aqueous solution takes an extended structure to form more hydrogen bonds to the environmental water molecules, but experimentally, the α -helical conformation was observed upon insertion of His residues and addition of Co²⁺ ion³², implying that the conformation is stabilized by strong metal coordination. In the experimental setup of Ref. 32, His residues are introduced into the peptide sequence to provide metal-binding sites, and Co²⁺ added to the solution forms coordination bonds with these His side chains. This His-Co²⁺ coordination is known to stabilize the peptide backbone into an α -helical preference in this system. In Ref. 32, the experimental curve used here as the α -helical reference was not a raw spectrum measured for the stabilized sample alone. Rather, it was obtained by subtracting the additive-free spectrum from the spectrum recorded under the His/Co²⁺-stabilized condition, and was used as an approximate experimental proxy for the α -helical contribution. Therefore, in the present fixed-geometry benchmark, the calculated spectrum of the idealized α -helical Ac-Ala₄-NMe structure is compared directly with this experimentally extracted difference spectrum; no difference operation was applied on the theoretical side. In other words, the α -helix is likely to be more rigid than that of a typical peptide helix,

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and conformational fluctuation is virtually suppressed. Therefore, we fixed all the Ramachandran dihedrals at $(\phi, \psi) = (-57.0, -47.0)$, which is one of the ideal α -helical parameters⁷³, and other degrees of freedom were then relaxed using the standard DFT (B3LYP) and the 6-31G basis set. The electronic structures of the GS and 30 lowest-lying ESs were calculated with the B3LYP functional referring to previous studies^{32,41}. For comparison, ES calculations were performed in the gas phase and with the integral-equation formalism of PCM (IEF-PCM, $\epsilon = 78.3553$). As there are many states in the low-lying region of the isolated α -helix, the number of states in the gas phase calculation was increased to 50. Accordingly, the α -helix calculations provide a fixed-geometry benchmark to compare the solvent models (gas phase, IEF-PCM, and RISM-SCF-cSED) against the experimentally extracted α -helical contribution. Note that this comparison is not intended to represent the full conformational ensemble of the additive-free peptide.

IV. RESULTS AND DISCUSSION

A. D-lactic acid in aqueous solution

DLA is a compact chiral hydroxy acid that provides a useful test case for assessing how conformational fluctuations and hydration jointly shape the ECD response in aqueous solution^{74,75}. In water, its carboxyl and hydroxyl groups are strongly coupled to hydration through hydrogen bonding, while the intrinsic flexibility of the solute is governed by only a few torsional degrees of freedom. In particular, the relative arrangement between the chiral center and the carboxyl chromophore can be characterized primarily by the torsion about the $C^*-C(=O)$ bond, with the hydroxyl torsion further modulating the local hydrogen-bonding motif. Because this low-dimensional conformational variability coexists with pronounced solvent interactions, DLA may serve as an instructive test case to examine how thermally accessible structural fluctuations and hydration effects are reflected in the ECD response.

The aqueous-phase ECD spectrum of lactic acid has been characterized in multiple wavelength windows. First, Bocková *et al.* reported UV-ECD spectra of both D- and L-lactic acid (together with related hydroxycarboxylic acids) in aqueous solution over 170–280 nm using synchrotron-radiation circular dichroism (SRCD)⁷⁴. The spectra of the two enantiomers are quasi-perfect mirror images, which provides an experimental justification for comparing the D-enantiomer spectrum with the sign-inverted L-enantiomer spectrum. In this wavelength range, the spectrum is

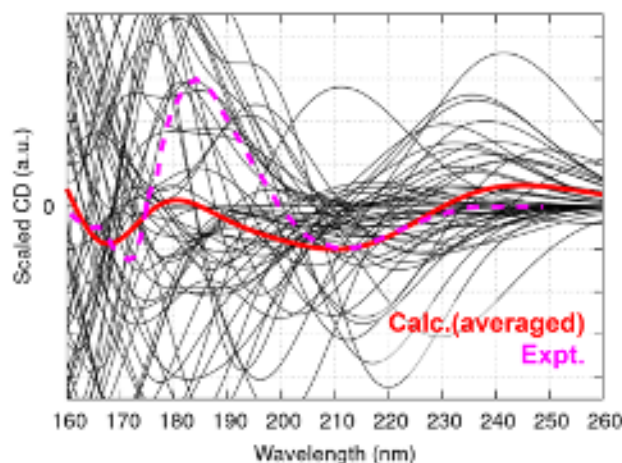


FIG. 1. The calculated ECD spectra of DLA in water by the RISM-SCF-cSED method. The black lines are the spectrum at each sampled structure, and the red is their simple ensemble average. The dashed magenta line is the D-enantiomeric corresponding experimental result digitized from Ref. 75 for L-enantiomer and sign inverted to represent the D-enantiomer.

dominated by a broad band with an extremum near 210 nm, assigned to the $n\text{-}\pi^*$ transition of the carboxyl chromophore. The reported solutions had pH 2–3.1, where lactic acid is predominantly present in the undissociated (protonated) form.

Second, Fukuyama *et al.* extended the aqueous-phase ECD spectrum of lactic acid down to 160 nm by measurements from 250 to 160 nm at 25 °C using a synchrotron-radiation ECD instrument, and additionally cross-checked the 250–190 nm region with a conventional CD spectrometer⁷⁵. The L-lactic-acid spectrum exhibits two positive bands around 211 and 172 nm and one negative band around 184 nm. Their sample solutions were adjusted to pH 5.1–5.4 using NaOH, and thus the experimental protonation state may differ from the neutral form considered in the present MD/RISM-based workflow; we therefore treat the comparison primarily as a benchmark for the overall sign pattern and band positions in water across a broad wavelength window.

Figure 1 compares the ensemble-averaged ECD spectrum obtained here for DLA with the sign-inverted experimental spectrum⁷⁵. A key point emerging from Table I is that the present protocol reproduces the positions of the dominant extrema in water within a few nanometers ($\lambda_{\min} \approx 210$ nm and $\lambda_{\max} \approx 181\text{--}184$ nm), whereas the relative intensity between the far-UV positive band and the ~ 210 nm minimum is not captured quantitatively. Specifically, after normalization by the

TABLE I. Maximum and minimum wavelength and the corresponding ECD intensity ratio in Figure 1.

	$\lambda_{\max}$ (nm)	$\lambda_{\min}$ (nm)	Intensity ratio ^a
Calc.	181	210	1/0.15
Expt. ⁷⁵	184	211	1/2.98

^a Intensity ratios were calculated from the absolute values of the vertical axis, normalized with respect to the negative peak around 210 nm.

~210 nm minimum, the calculated intensity of the positive band near ~181 nm is 0.15, while the experimental counterpart near ~184 nm is 2.98. In other words, although the sign pattern and band positions are qualitatively reproduced, the present calculation substantially underestimates the relative contribution of the short-wavelength positive feature (around 180–190 nm) compared to the $n\text{-}\pi^*$ -dominated band around 210 nm. This discrepancy is also consistent with the large configuration-to-configuration variability visible below ~200 nm (black curves in Fig. 1), suggesting that the ensemble-averaged signal in this region is particularly sensitive to conformational/hydration fluctuations and may require more extensive sampling and/or refined modeling to achieve quantitative agreement.

In the 200–240 nm window, the calculated profile reproduces the experimentally observed sign and band position of the dominant feature around 210 nm, consistent with the assignment of this band to the $n\text{-}\pi^*$ transition of the carboxyl chromophore. In contrast, the shorter-wavelength region (roughly 170–195 nm) exhibits a noticeably larger discrepancy in spectral shape, most prominently in the relative intensity of the positive band centered near ~185 nm in the experimental spectrum (for the D enantiomer). This behavior is consistent with the fact that the far-UV region involves higher-energy transitions that are more difficult to assign and more sensitive to both solute conformations and local hydration patterns. Fukuyama *et al.* emphasized that the calculated spectra of lactic acid are greatly influenced by the dihedral angles of the COO^- and OH groups, and that individual hydrated structures can yield substantially different peak positions and intensities. Their analysis also indicates that the ~185 nm region is not governed by a single simple contribution, but can be influenced by multiple transitions (including contributions beyond the dominant $n\text{-}\pi^*$ character), making this region intrinsically more demanding for theory. Accordingly, the present underestimation of the 180–190 nm intensity relative to the ~210 nm band can be interpreted as a practical manifestation of this sensitivity: the averaged spectrum in this range is affected by

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the balance among multiple high-energy transitions and therefore becomes more susceptible to incomplete configurational sampling and methodological approximations.

A second important consideration is the coupling between torsional fluctuations and hydration motifs. Fukuyama *et al.* demonstrated that the ECD peak positions and intensities are markedly affected by the dihedral angles of the COO^- and OH groups in lactic acid, and that the OH torsion can strongly modulate the ECD spectrum for geometries where the OH oxygen is proximal to an oxygen of the COO^- group. They further concluded, from comparison with L-alanine, that a network with relatively loose hydrogen bonding around the COO^- and OH groups is responsible for the flexible conformations of hydroxy acids and their complicated side-chain dependence relative to amino acids. In the present MD/RISM workflow, the configuration-to-configuration variability is indeed larger in the 170–195 nm region than in the $n\text{-}\pi^*$ -dominated 200–240 nm region. This suggests that rare but spectroscopically important torsion/hydration motifs may contribute disproportionately to the far-UV band, motivating additional sampling and/or enhanced-sampling strategies if quantitative agreement in this region is sought.

Finally, the protonation state is another factor that can influence both intensities and band positions, especially because the dominant chromophore is the carboxyl group. The SRCD measurements⁷⁴ were performed at pH 2–3.1, where lactic acid is predominantly protonated, whereas Fukuyama *et al.* adjusted their solutions to pH 5.1–5.4 using NaOH⁷⁵. Because the present MD sampling was performed for the neutral (protonated) form, the comparison to the spectrum⁷⁵ should be interpreted cautiously, particularly for relative intensities in the short-wavelength region. A fully quantitative comparison across experiments conducted at different pH values would require explicit consideration of acid–base equilibrium, e.g., by performing MD/RISM sampling for both protonated and deprotonated forms and combining their spectra with appropriate weights at the experimental pH. Within the present revision, we restrict ourselves to 50 snapshots for the neutral form, and we leave such protonation-state averaging and further increase of the sample size for the far-UV convergence as important future tasks.

Overall, the present calculation with 50 snapshots captures the dominant feature around 210 nm and reproduces the qualitative sign pattern in water across a broad wavelength window, supporting the applicability of the MD+RISM-SCF-cSED strategy to a small, low-dimensional conformational system. At the same time, the remaining discrepancy in the far-UV region around 180–190 nm—most clearly seen in the intensity ratio summarized in Table I—highlights a practical limitation: higher-energy bands are more sensitive to conformational/hydration fluctua-

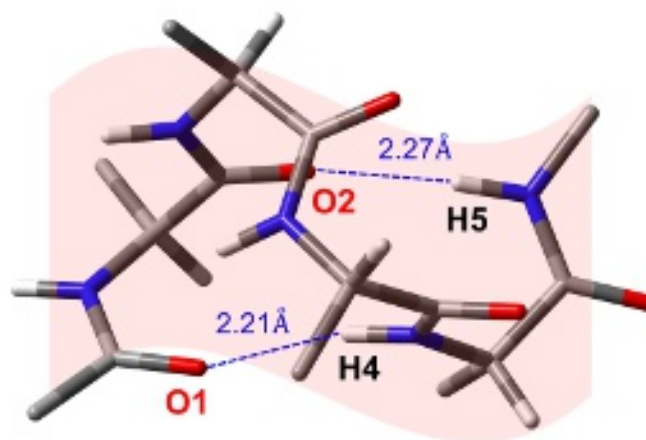


FIG. 2. The α -helical structure of Ac-Ala₄-NMe. For simplicity, H atoms of the methyl groups are omitted. The numbering of O and H atoms and intramolecular hydrogen-bond distances (in Å) are also shown.

tions and protonation state, and may demand more extensive sampling and/or refined electronic-structure/solvation treatment for quantitative agreement.

B. Ac-Ala₄-NMe in aqueous solution

We examined the solvent effect on the ECD spectrum at the α -helix structure, which is shown in Figure 2. In this conformation, the oxygen atoms O1 and O2 form intramolecular hydrogen bonds with the H4 and H5 amide hydrogens of the residues three positions ahead, at distances of 2.21 and 2.27 Å, respectively.

The ECD and UV-Vis spectra computed using the RISM-SCF-cSED method, together with the experimentally extracted α -helical difference spectrum from Ref. 32, are shown in Figure 3. For comparison, the spectra calculated in the gas phase (an isolated peptide) and with the IEF-PCM are also included. All three calculations (RISM-SCF-cSED, IEF-PCM, and gas phase) were performed as single-point TDDFT evaluations on the identical solute geometry; therefore, the differences in the spectra arise solely from the different solvent treatments. The RISM-SCF-cSED spectrum gives the closest agreement with the normalized experimental difference spectrum among the tested models, particularly in the sign pattern and the positions of the positive and negative bands commonly associated with the π - π^* (~ 190 nm) and n - π^* (~ 220 nm) regions. Table II summarizes the peak wavelengths and the ECD intensity (height) ratios, normalized with respect to the positive band. Because the experimental curve is an extracted difference spectrum

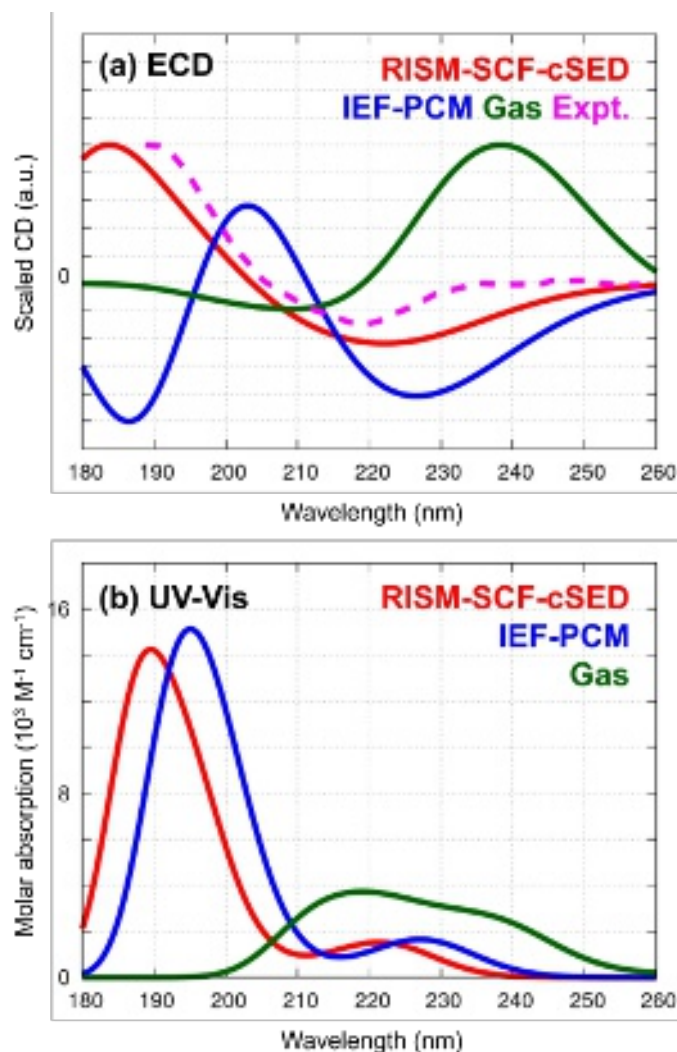


FIG. 3. (a) Comparison of calculated ECD spectra of α -helical Ac-Ala₄-NMe in water by RISM-SCF-cSED (red line), IEF-PCM (blue line), and in the gas phase (dark-green line) with the experimentally extracted α -helical difference spectrum from Ref. 32, Fig.S6 (dashed magenta line). The experimental curve corresponds to the spectrum recorded under the His/Co²⁺-stabilized condition minus the additive-free spectrum (for the details, see the main text). (b) Calculated UV-Vis spectra by the corresponding methods.

for a His-containing peptide rather than an absolute spectrum of Ac-Ala₄-NMe, the comparison should be interpreted as a relative line-shape benchmark. Note that the RISM-SCF-cSED and experimental difference spectra are normalized to the positive band near 190 nm, whereas the IEF-PCM spectrum is normalized to the negative band near 188 nm and the gas-phase spectrum to the positive band near 240 nm. For this relatively rigid α -helical structure, we also examined the dependence of the calculated ECD spectrum on the choice of density functional and basis set; the

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TABLE II. Maximum and minimum wavelength and the corresponding ECD intensity ratio in Figure 3(a).

	$\lambda_{\max}$ (nm)	$\lambda_{\min}$ (nm)	Intensity ratio
Calc.(RISM-SCF-cSED)	184	222	1/0.44
Expt. ³²	189	219	1/0.30

TABLE III. Maximum wavelength λ and molar absorption coefficient ϵ in Figure 3(b).

	λ (nm)	ϵ ($10^3 \text{ M}^{-1} \text{ cm}^{-1}$)
RISM-SCF-cSED	190	14.2
	221	1.5
IEF-PCM	195	15.1
	227	1.7

results are summarized in Figures S1 and S2 and Tables S1 and S2. In the IEF-PCM calculation, the negative rotational strength in the $n\text{-}\pi^*$ region is successfully reproduced, but both positive and negative peaks appear in the $\pi\text{-}\pi^*$ region, resulting in an artificial "−+" split pattern. To further examine the difference between the solvent models, we consider the UV-Vis properties, i.e., the excitation energies (Figure 3 (b)). Both models show the strong (~ 190 nm) and weak (~ 220 nm) absorptions, with their wavelengths at the absorption maxima and the corresponding molar absorptivities shown in Table III. Compared with IEF-PCM, the RISM-SCF-cSED results are blue-shifted by about 5-6 nm, while the overall spectral profile remains virtually the same apart from this parallel shift. Unfortunately, the experimental UV-Vis was not available. It is likely that the red shift of the strong peak in IEF-PCM partially contributes to the artificial split pattern observed in its ECD spectrum. On the other hand, the gas-phase spectrum shows a significant deviation, with the characteristic absorption around 190 nm absent. The analysis of the TDDFT results shows that each of these three spectra consists of multiple transitions among numerous molecular orbitals, making it difficult to characterize the absorption spectra. Note that, due to the helical structure, it is not possible to define molecular planes or π -orbitals clearly. While there are many small transitions around 220 nm in the gas phase, the relative transition energies are considerably higher due to solvation. In this system, the α -helical structure in the ground state possesses a significant dipole moment, 18.6 Debye, which is further enhanced to 24.4 Debye (IEF-PCM) or 27.2 Debye (RISM-SCF-cSED). The ground state is likely more stabilized than the

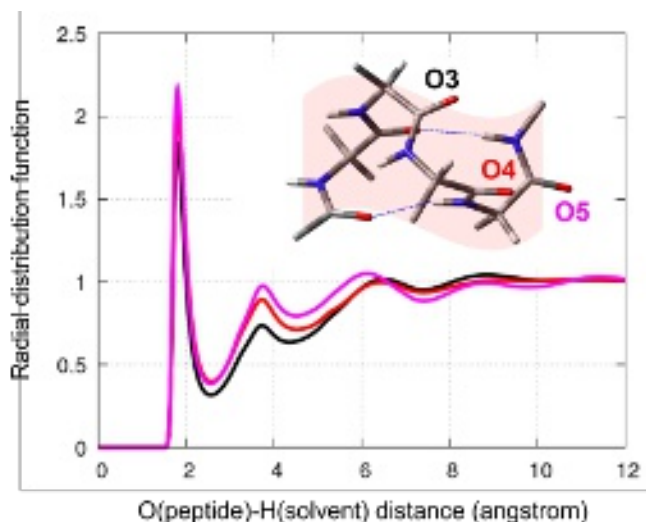


FIG. 4. RDFs between the O atoms in Ac-Ala₄-NMe (solute peptide) and the H atoms in water (solvent). The numbering of O atoms are also shown.

excited states, and the energy gap widens in aqueous environments. Similarly, the observed ECD spectra cannot be assigned to a single transition, and the analysis of the ECD spectrum in the π - π^* region obtained from the two solvent models is more complex, as it requires consideration of not only the oscillator strengths in the UV but also the magnetic dipole contribution. Consequently, the solvation effect is crucial for accurately computing UV-Vis and ECD calculations; its absence renders these calculations insufficient. Note that the same structure was employed all over the computations, meaning that the differences are attributed only to the differences in the electronic structure.

Typical RDFs calculated by RISM-SCF-cSED are plotted in Figure 4. These oxygen atoms (O3, O4 and O5) are not involved in the intramolecular hydrogen bondings (see Figure 2), which are exposed to the aqueous environment. Sharp RDF peaks were observed at distances slightly shorter than 2 Å around each oxygen atom, indicating the formation of hydrogen bonds with the solvent water. While these carbonyl oxygens of the peptide are responsible for both the n - π^* and π - π^* transitions, the corresponding molecular orbitals are not localized at a specific bond, instead spread over the molecule. As mentioned above, the occupied π -orbitals are likely to be more stabilized by the solvation through the hydrogen bonding with the environment, increasing the excitation energies.

V. CONCLUSION

In the present work, we extended the RISM-SCF-cSED method to compute ECD spectra of solvated molecules within a TDDFT framework. As an initial demonstration, we examined D-lactic acid in aqueous solution, a compact chiral hydroxy acid with low-dimensional conformational variability. The ensemble-averaged spectrum obtained from 50 MD snapshots followed by RISM-SCF-cSED/TDDFT calculations reproduced the dominant aqueous feature around 210 nm and the qualitative sign pattern in the 170–240 nm region, although discrepancies in the far-UV intensity indicate the need for broader sampling and explicit treatment of protonation-state effects for fully quantitative agreement.

For the α -helix structure, considered to form under the condition with His residues and Co^{2+} , the ECD spectrum computed with the RISM-SCF-cSED shows good agreement with the experimentally extracted α -helical difference spectrum. The UV-Vis absorptions computed by TDDFT using this method and IEF-PCM remarkably resemble each other, suggesting a similarity between the respective orbitals altered by the solvation. Overall, the present results demonstrate that RISM-SCF-cSED/TDDFT is a useful framework for treating solvent effects in ECD calculations.

SUPPLEMENTARY MATERIAL

The supplementary material (SI.pdf) contains additional information and data on the computational parameters (S1), and other results of the Ac-Ala₄-NMe calculation (S2).

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AUTHOR DECLARATIONS

Conflict of interest

The authors have no conflicts to disclose.

Author contributions

Yuji Takabayashi: Data curation (lead); Formal analysis (lead); Investigation (lead); Visualization (lead); Writing - original draft (lead). Kosuke Imamura: Software (lead); Supervision (equal). Daisuke Yokogawa: Supervision (equal). Hirofumi Sato: Conceptualization (lead); Funding acquisition (lead); Project administration (lead); Supervision (equal); Writing - review & editing (lead).

DATA AVAILABILITY STATEMENT

The deposited files include the optimized α -helical Ac-Ala₄-NMe structure and the analyzed D-lactic-acid snapshot coordinates.

REFERENCES

- ¹W. C. Johnson Jr., "Circular Dichroism and Its Empirical Application to Biopolymers," in Methods of Biochemical Analysis (John Wiley & Sons, Ltd, 1985) pp. 61–163.
- ²G. D. Fasman, ed., Circular Dichroism and the Conformational Analysis of Biomolecules, 1st ed. (Springer New York, NY, 1996).
- ³N. Berova, K. Nakanishi, and R. W. Woody, eds., Circular Dichroism: Principles and Applications, 2nd ed. (Wiley-VCH, New York, 2000).
- ⁴N. J. Greenfield, *Anal. Biochem.* **235**, 1 (1996).
- ⁵I. Warnke and F. Furche, *WIREs Comput. Mol. Sci.* **2**, 150 (2012).
- ⁶D. M. Rogers, S. B. Jasim, N. T. Dyer, F. Auvray, M. Réfrégiers, and J. D. Hirst, *Chem* **5**, 2751 (2019).
- ⁷A. Micsonai, É. Bulyáki, and J. Kardos, "BeStSel: From Secondary Structure Analysis to Protein Fold Prediction by Circular Dichroism Spectroscopy," in

- Structural Genomics: General Applications, edited by Y. W. Chen and C.-P. B. Yiu (Springer US, New York, NY, 2021) pp. 175–189.
- ⁸M. Pecul and K. Ruud, in *Response Theory and Molecular Properties (A Tribute to Jan Lindenberg and Poul Jørgensen)*, Adv. Quantum Chem., Vol. 50, edited by H. Jensen (Academic Press, 2005) pp. 185–212.
- ⁹J. Autschbach, *Chirality* **21**, E116 (2009).
- ¹⁰M. Srebro-Hooper and J. Autschbach, *Annu. Rev. Phys. Chem.* **68**, 399 (2017).
- ¹¹L. Rosenfeld, *Zeits. f. Physik* **52**, 161 (1929).
- ¹²E. U. Condon, *Rev. Mod. Phys.* **9**, 432 (1937).
- ¹³T. D. Bouman, B. Voigt, and A. E. Hansen, *J. Am. Chem. Soc.* **101**, 550 (1979).
- ¹⁴T. Yamazaki, Y. Aoki, and A. Imamura, *Chem. Phys. Lett.* **295**, 431 (1998).
- ¹⁵S. Bureekaew, J. Hasegawa, and H. Nakatsuji, *Chem. Phys. Lett.* **425**, 367 (2006).
- ¹⁶Y. Honda, A. Kurihara, M. Hada, and H. Nakatsuji, *J. Comput. Chem.* **29**, 612 (2008).
- ¹⁷J. Seino, Y. Honda, M. Hada, and H. Nakatsuji, *J. Phys. Chem. A* **110**, 10053 (2006).
- ¹⁸T. Miyahara, J. Hasegawa, and H. Nakatsuji, *Bull. Chem. Soc. Jpn.* **82**, 1215 (2009).
- ¹⁹Y. Honda, A. Kurihara, Y. Kenmochi, and M. Hada, *Molecules* **15**, 2357 (2010).
- ²⁰T. Miyahara, H. Nakatsuji, and H. Sugiyama, *J. Phys. Chem. A* **117**, 42 (2013).
- ²¹T. Miyahara and H. Nakatsuji, *J. Phys. Chem. A* **117**, 14065 (2013).
- ²²T. Miyahara, H. Nakatsuji, and T. Wada, *J. Phys. Chem. A* **118**, 2931 (2014).
- ²³T. Miyahara and H. Nakatsuji, *J. Phys. Chem. A* **119**, 8269 (2015).
- ²⁴T. Miyahara, H. Nakatsuji, and H. Sugiyama, *J. Phys. Chem. A* **120**, 9008 (2016).
- ²⁵M. Schreiber, R. Vahrenhorst, V. Buss, and M. P. Fülcher, *Chirality* **13**, 571 (2001).
- ²⁶A. Gilbert and J. Hirst, *J. Mol. Struct. THEOCHEM* **675**, 53 (2004).
- ²⁷Z. Li, D. Robinson, and J. D. Hirst, *Faraday Discuss.* **177**, 329 (2015).
- ²⁸Z. Li and J. D. Hirst, *Chem. Sci.* **8**, 4318 (2017).
- ²⁹F. Furche, R. Ahlrichs, C. Wachsmann, E. Weber, A. Sobanski, F. Vögtle, and S. Grimme, *J. Am. Chem. Soc.* **122**, 1717 (2000).
- ³⁰K. M. Specht, J. Nam, D. M. Ho, N. Berova, R. K. Kondru, D. N. Beratan, P. Wipf, R. A. Pascal, and D. Kahne, *J. Am. Chem. Soc.* **123**, 8961 (2001).
- ³¹J. Autschbach, T. Ziegler, S. J. A. van Gisbergen, and E. J. Baerends, *J. Chem. Phys.* **116**, 6930 (2002).
- ³²J. Kaminský, J. Kubelka, and P. Bouř, *J. Phys. Chem. A* **115**, 1734 (2011).

First-principles ECD calculation using RISM-SCF-cSED

- ³³S. Ionescu, I. Matei, C. Tablet, and M. Hillebrand, *Phys. Chem. Chem. Phys.* **15**, 11604 (2013).
- ³⁴C. Ota, T. Konishi, S. Tanaka, and K. Takano, *Chem. Phys. Chem.* **25**, e202300593 (2024).
- ³⁵A. Kumar, S. E. Toal, D. DiGuseppi, R. Schweitzer-Stenner, and B. M. Wong, *J. Phys. Chem. B* **124**, 2579 (2020).
- ³⁶M. Monti, M. Stener, and M. Aschi, *J. Comput. Chem.* **43**, 2023 (2022).
- ³⁷J. Tomasi, B. Mennucci, and R. Cammi, *Chem. Rev.* **105**, 2999 (2005).
- ³⁸B. Mennucci, *WIREs Comput. Mol. Sci.* **2**, 386 (2012).
- ³⁹B. Mennucci, J. Tomasi, R. Cammi, J. R. Cheeseman, M. J. Frisch, F. J. Devlin, S. Gabriel, and P. J. Stephens, *J. Phys. Chem. A* **106**, 6102 (2002).
- ⁴⁰M. Pecul, D. Marchesan, K. Ruud, and S. Coriani, *J. Chem. Phys.* **122**, 024106 (2004).
- ⁴¹J. Šebek, Z. Kejík, and P. Bouř, *J. Phys. Chem. A* **110**, 4702 (2006).
- ⁴²J. Jiang, D. Abramavicius, B. M. Bulheller, J. D. Hirst, and S. Mukamel, *J. Phys. Chem. B* **114**, 8270 (2010).
- ⁴³H. Sato, *Phys. Chem. Chem. Phys.* **15**, 7450 (2013).
- ⁴⁴D. Yokogawa, *Bull. Chem. Soc. Jpn.* **91**, 1540 (2018).
- ⁴⁵K. Imamura, D. Yokogawa, and H. Sato, *J. Chem. Phys.* **160**, 050901 (2024).
- ⁴⁶T. Yamazaki, H. Sato, and F. Hirata, *Chem. Phys. Lett.* **325**, 668 (2000).
- ⁴⁷T. Yamazaki, H. Sato, and F. Hirata, *J. Chem. Phys.* **115**, 8949 (2001).
- ⁴⁸K. Imamura, T. Yamazaki, D. Yokogawa, M. Higashi, and H. Sato, *J. Chem. Phys.* **152**, 194102 (2020).
- ⁴⁹K. Imamura, D. Yokogawa, M. Higashi, and H. Sato, *J. Chem. Phys.* **157**, 204105 (2022).
- ⁵⁰K. Imamura, M. Higashi, Y. Kobayashi, H. Kageyama, and H. Sato, *J. Phys. Chem. B* **126**, 3090 (2022).
- ⁵¹K. Suda, K. Yagi, and D. Yokogawa, *J. Phys. Chem. Lett.* **16**, 8666 (2025).
- ⁵²T. Miyata and F. Hirata, *J. Comput. Chem.* **29**, 871 (2008).
- ⁵³N. Yoshida and T. Yamaguchi, *J. Mol. Liq.* **385**, 122418 (2023).
- ⁵⁴N. Chiangraeng, H. Nakano, P. Nimmanpipug, and N. Yoshida, *J. Mol. Liq.* **386**, 122503 (2023).
- ⁵⁵D. Chandler and H. C. Andersen, *J. Chem. Phys.* **57**, 1930 (1972).
- ⁵⁶F. Hirata and P. J. Rossky, *Chem. Phys. Lett.* **83**, 329 (1981).
- ⁵⁷S. Ten-no, F. Hirata, and S. Kato, *Chem. Phys. Lett.* **214**, 391 (1993).
- ⁵⁸S. Ten-no, F. Hirata, and S. Kato, *J. Chem. Phys.* **100**, 7443 (1994).
- ⁵⁹H. Sato, F. Hirata, and S. Kato, *J. Chem. Phys.* **105**, 1546 (1996).

- ⁶⁰D. Yokogawa, H. Sato, and S. Sakaki, *J. Chem. Phys.* **126**, 244504 (2007).
- ⁶¹D. Yokogawa, H. Sato, and S. Sakaki, *J. Chem. Phys.* **131**, 214504 (2009).
- ⁶²M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, “Gaussian 16 Revision C.02,” (2016), Gaussian Inc. Wallingford CT.
- ⁶³T. Lu and F. Chen, *J. Comput. Chem.* **33**, 580 (2012).
- ⁶⁴T. Lu, *J. Chem. Phys.* **161**, 082503 (2024).
- ⁶⁵A. Rohatgi, “WebPlotDigitizer, Version 5.2. (<https://automeris.io>),” (2024).
- ⁶⁶D. A. Case, H. M. Aktulga, K. Belfon, I. Y. Ben-Shalom, J. T. Berryman, S. R. Brozell, F. S. Carvahol, D. S. Cerutti, T. E. Cheatham, III, G. A. Cisneros, V. W. D. Cruzeiro, T. A. Darden, N. Forouzeshe, M. Ghazimirsaeed, G. Giambasu, T. Giese, M. K. Gilson, H. Gohlke, A. W. Goetz, J. Harris, Z. Huang, S. Izadi, S. A. Izmailov, K. Kasavajhala, M. C. Kaymak, I. Kolossvary, A. Kovalenko, T. Kurtzman, T. S. Lee, P. Li, Z. Li, C. Lin, J. Liu, T. Luchko, R. Luo, M. Machado, M. Manathunga, K. M. Merz, Y. Miao, O. Mikhailovskii, G. Monard, H. Nguyen, K. A. O’Hearn, A. Onufriev, F. Pan, S. Pantano, A. Rahnamoun, D. R. Roe, A. Roitberg, C. Sagui, S. Schott-Verdugo, A. Shajan, J. Shen, C. L. Simmerling, N. R. Skrynnikov, J. Smith, J. Swails, R. C. Walker, J. Wang, J. Wang, X. Wu, Y. Wu, Y. Xiong, Y. Xue, D. M. York, C. Zhao, Q. Zhu, and P. A. Kollman, *Amber 2025* (2025), University of California, San Francisco.
- ⁶⁷J. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman, and D. A. Case, *J. Comput. Chem.* **25**, 1157 (2004).
- ⁶⁸A. Jakalian, B. L. Bush, D. B. Jack, and C. I. Bayly, *J. Comput. Chem.* **21**, 132 (2000).
- ⁶⁹A. Jakalian, D. B. Jack, and C. I. Bayly, *J. Comput. Chem.* **23**, 1623 (2002).

First-principles ECD calculation using RISM-SCF-cSED

- ⁷⁰W. L. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey, and M. L. Klein, J. Chem. Phys. **79**, 926 (1983).
- ⁷¹J.-P. Ryckaert, G. Ciccotti, and H. J. Berendsen, J. Comput. Phys. **23**, 327 (1977).
- ⁷²T. Darden, D. York, and L. Pedersen, J. Chem. Phys. **98**, 10089 (1993).
- ⁷³S. Arnott and S. Dover, J. Mol. Biol. **30**, 209 (1967).
- ⁷⁴J. Bocková, N. C. Jones, U. J. Meierhenrich, S. V. Hoffmann, and C. Meinert, Commun. Chem. **4**, 86 (2021).
- ⁷⁵T. Fukuyama, K. Matsuo, and K. Gekko, Chirality **23**, E52 (2011).