Strong Out-of-Plane Vibrations and Ultrasensitive Detection of Dopamine-like Neurotransmitters

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ABSTRACT: The detection of monoamine neurotransmitters has become a vital research subject due to their high correlations with nervous system diseases, but insufficient detection precisions have obstructed diagnosis of some related diseases. Here, we focus on four monoamine neurotransmitters, dopamine, norepinephrine, epinephrine, and serotonin, to conduct their rapid and ultrasensitive detection. We find that the low-frequency (<200 cm⁻¹) Raman vibrations of these molecules show some sharp peaks, and their intensities are significantly stronger than those of the high-frequency side. Theoretical calculations identify these peaks to be from strong out-of-plane vibrations of the C–C single bonds at the joint point of the ring-like molecule and its side chain. Using our surface enhanced low-frequency Raman scattering substrates, we show that the detection limit of dopamine as an example can reach 10 nM in artificial cerebrospinal fluid. This work provides a useful way for ultrasensitive and rapid detection of some neurotransmitters.

Monamine neurotransmitters, such as dopamine (DA), norepinephrine (NE), epinephrine (EE), and serotonin (5-hydroxytryptamine; 5HT), play a crucial role in memory, emotional, cognitive, and motor functions.1–3 Abnormal levels of these molecules in cerebrospinal fluid (CSF) are associated with various nervous system diseases such as depression, schizophrenia, and Parkinson’s and Alzheimer’s diseases.4–6 Therefore, the concentrations of monoamine neurotransmitters in CSF are considered to be important diagnostic markers for some nervous diseases.7 So far, some methods have been used to detect these monoamine neurotransmitters including electrochemistry,8 fluorescence,9 and chromatography.10 However, their intrinsic drawbacks limit wide clinical applications of these methods. For instance, the electrochemical neurotransmitter sensor may confuse molecular sorts with structures similar to the target molecule.8 The fluorescent sensor needs a fluorescence dye label and complex experimental techniques.9 The chromatographic method has a limited spatial resolution and is time-consuming.10 Low-intensity high-frequency Raman bands (>200 cm⁻¹) of some monoamine neurotransmitters have been acquired in previous studies, and few have been applied in disease detection.11 Our recent research shows that the low-frequency Raman spectrum of histamine molecules in the wavenumber range of <200 cm⁻¹ is significantly stronger than the high-frequency spectrum and can be used in a high-precision probe of histamine concentration.11

In this work, we carefully examine the low-frequency Raman spectra of four kinds of monoamine neurotransmitters, DA, NE, EE, and SHT, and find them to have strong intensities and high specificities. Similar results occur when they exist in solution environments such as CSF. Based on the density functional theory (DFT) calculations, we prove that the strong low-frequency Raman vibrations originate from strong out-of-plane vibrations of the carbon–carbon single bonds at the joint point of the ring-like molecule and its side chain. Previously, DA cannot be identified effectively in the widely used electrochemical method.8,9 As an example, we currently conducted practical detection of DA molecules in artificial CSF with the help of the surface-enhanced low-frequency Raman scattering (SELFRS) substrates. The obtained results show that the detected concentration can reach 10 nM (signal–noise ratio > 3), which is in the range of 0.5–25 nM in human CSF.8 Such a high detection limit can easily distinguish DA from other structurally similar neurotransmitters such as NE. Combined with surface enhanced Raman scattering technology, we realize the ultrasensitive, rapid, and high-selectivity detection of dopamine.

The high- and low-frequency Raman spectra of DA, NE, EE, and SHT powders are shown in Figure 1a. We can see that the low-frequency signals are significantly stronger than the high-frequency ones. The two strongest peaks in the low- and high-frequency ranges are highlighted in red and blue. Histamine, as
an important bioactive small molecule, has also been reported to have similar phenomena (Figure S1 in the Supporting Information).11 All of these molecules consist of a ringed group and a carbon side chain, so the strong low-frequency Raman spectra should be related to similar molecular structures (Figure S2 in the Supporting Information). It is known that histamine has two nitrogen atoms in the imidazole ring and one nitrogen atom in the side chain. Such a molecular structure makes high electron density distributions concentrated on three areas, two being on the C atoms of the ring and one being on the side chain, and only torsions of the C−C bonds in the side chain can dramatically cause electron density changes of the three areas to produce a great molecular polarizability modification.11 This is the reason that causes the observed strong low-frequency Raman peaks in histamine. For DA, NE, EE, and 5HT, they have a similar ringed structure, but some oxygen atoms (two for DA, NE, and EE, and one for 5HT) bond to the C ring to form the C−O bonds (Figure 1a). This possibly makes high electron density areas concentrated on these oxygen atoms, away from the ring group. As a result, maximal low-frequency Raman polarizability modification would occur due to the difference vibration of the C−C single bond at the joint point of the ring-like molecule with its side chain. Such structural modifications may significantly affect the frequencies and intensities of the low-frequency modes so that the vibration modes are of the specificity in the low-frequency Raman spectrum. Our careful DFT calculations confirm that the strongest low-frequency peaks of the four neurotransmitters stem from out-of-plane vibrations of the carbon−carbon single bonds at the joint point of the ring-like molecule and its side chain (red bars in Figure 1a and Tables S1−S4 in the Supporting Information). A large change of the electron density distribution intuitively shows this conclusion (Video 1). Changes of the Raman polarizability densities for the strongest low- and high-frequency modes are presented in red and blue zones in Figure 1b,12 respectively. Compared to the high-frequency stretching vibrations (Video 2), such C−C out-of-plane vibrations cause obvious large variations of the Raman polarizability densities. The hydrochlorides of the four neurotransmitters also exhibit the same characteristics, and the strongest peaks occur in the low-frequency region (Figure S3 in the Supporting Information). Due to the specificities of the four molecules, their Raman spectra are different in peak positions (Figure S4 in the Supporting Information), and so, they can easily be distinguished by low-frequency Raman scattering.

In a physiological pH environment, the four neurotransmitters mainly exist in the form of monocations, and the N atom in the tail chain attracts an additional proton (Figure S2 in the Supporting Information).13−16 Due to their peculiar molecular structure, the conformations of these DA-like molecules with a ringed group and carbon side chain are very sensitive to the surrounding environment, and main manifestations are the changes of the dihedral angles of the C−C bonds in the carbon side chain, which has a great influence on the low-energy vibrations.11,17,18 Since molecular conformations determine biological activity, we carefully examined the Raman spectra of the four neurotransmitters in aqueous solution with pH of 7.4. The full Raman spectra of the four molecules are exhibited in Figure S5 in the Supporting Information, and the characteristic vibration regions in the wavenumber ranges of 50−250 and 500−1000 cm−1 are shown

![Figure 1](https://example.com/figure1.png)

**Figure 1.** (a) Experimental (black lines) and simulated (green lines) Raman spectra of DA, NE, EE, and 5HT powders. The insets show their molecular structures. The two strongest peaks in low- and high-frequency sides are highlighted in red and blue bars, respectively. (b) Changes of the Raman polarizability densities associated with the two strongest peaks. The left column is for the low-frequency peaks, and the right column is for the high-frequency peaks. The polarizability density is normalized, and the absolute isovalue is set to 0.15 with the positive signal in blue and the negative one in red.
in Figure 2a. We can see that the low-frequency peaks have far stronger intensities than the high-frequency ones, but they occur in a form of the broad band, and the most probable peak positions blue-shift more than 10 cm$^{-1}$ compared to those of their solid powders.

To understand the origin of the broad band, we consider the solvent-induced modification of the dihedral angles $\theta$ and $\varphi$ in the side chain under the coexistence of several different conformations. The ring group and the first carbon atom in the side chain form plane 1 (yellow), the carbon chain (C–C–C) forms plane 2 (blue), and the carbon chain and nitrogen atom (C–C–N) form plane 3 (red). $\theta$ is the angle between plane 1 and plane 2, and $\varphi$ is the angle between plane 2 and plane 3. Figure 2b depicts the structures of the minimum energy states, in which the dihedral angles $\theta$ and $\varphi$ are marked in light blue and pink. The most possible conformations must be some stable structures with the lowest energies, and thus, we theoretically calculated the energies of all possible conformations with various $\theta$ and $\varphi$ to ensure a full description of all low-energy conformers. Using the DFT B3LYP method, we scan for the conformational energy landscape as functions of the dihedral angles $\theta$ and $\varphi$. The computational details have been described in our previous work. The 3D presentations of the energy landscapes are shown in Figure 2c for the four neurotransmitters in water at pH = 7.4 (a representation of energy, $E$, as functions of the dihedral angles $\theta$ and $\varphi$ of the four neurotransmitters). The ratios of three minimum energy conformations of the four neurotransmitters are marked with yellow, green, and blue numbers.

Figure 2. (a) Experimental Raman spectra acquired from DA, NE, EE, and 5HT molecular solutions (0.1 M) (black line). The four solutions are prepared using 0.1 M hydrochloric acid at pH = 7.4. Yellow, green, and blue columns illustrate the calculated Raman peaks of three minimum energy conformations. Simulated Raman spectra are obtained according to the ratio of each minimum energy conformation (red lines). (b) The structures of the minimum energy states and their dihedral angles $\theta$ and $\varphi$ (marked in light blue and pink). (c) The landscapes of the four neurotransmitter structures in water at pH = 7.4 (a representation of energy, $E$, as functions of the dihedral angles $\theta$ and $\varphi$ of the four neurotransmitters). The ratios of three minimum energy conformations of the four neurotransmitters are marked with yellow, green, and blue numbers.
SHT in their mixture systems. However, this can be met using our currently presented Raman method because of the specificity of the low-frequency mode positions (Figure S4 in the Supporting Information). This makes accurate molecular discrimination possible by the Raman spectroscopy for the four neurotransmitters.

In this work, we take DA as an example to show the serviceability of the low-frequency Raman spectrum with the interference of the other three neurotransmitters. A 10 mM aqueous solution of each neurotransmitter was adjusted with dilute hydrochloric acid to pH = 7.4. The dopamine solution was respectively mixed with the other three solutions in varying proportions. A 50 μL aliquot of each mixture was dripped onto a clean silicon wafer and dried at 60 °C. The corresponding low-frequency Raman spectra were measured and shown in Figure 3a−c. We can see that the DA−NE-mixed sample shows three strong peaks at 90 cm\(^{-1}\) from DA and at 81 and 100 cm\(^{-1}\) from NE. The intensities of the three peaks vary with their relative concentration proportions. The peak-to-peak intervals are also large enough, indicating that the origins of these peaks are distinguishable in situ (Figure 3a).

For EE, it has four weak peaks at 72, 79, 87, and 112 cm\(^{-1}\), whereas the DA−EE-mixed sample could show an additional strong peak at 90 cm\(^{-1}\) (Figure 3b). Hence, we can recognize DA molecules when the concentration ratio of DA relative to EE rises. For the DA−5HT-mixed sample, the three peaks at 82, 96, and 115 cm\(^{-1}\) are from 5HT, and the 90 cm\(^{-1}\) peak is from DA. They can be clearly distinguished in all the samples with different concentration proportions of DA:5HT (Figure 3c). It is known that normal concentrations of DA, NE, EE, and 5HT in human CSF are in the ranges of 0.5−25, 0.8−5.3, 0.1−1.6, and 0.5−5.1 nM, respectively. To further check the serviceability of the low-frequency Raman detection, we also prepared equal proportions of basal solution mixed

Figure 3. Low-frequency Raman spectra of the dried DA−NE (a), DA−EE (b), and DA−SHT (c) mixtures.

Figure 4. (a) Low-frequency Raman spectra of the dried DA mixtures with NE, EE, and 5HT. Here, 0, 2, 4, 6, 8, 10 mM DA standard concentration solutions are simulated to show the DA release process. (b,c) The peak fittings of the low-frequency Raman spectra of the dried 6 and 10 mM DA mixtures with NE, EE, and SHT. (d) The integral intensity of the 90 cm\(^{-1}\) peak versus DA concentration in NE−EE−SHT-mixed samples.
together with 5.3 mM of NE, 1.6 mM of EE, and 5.1 mM of 5HT, based on their normal concentration ranges in human CSF. The mixed basal solution was then mixed with 0, 2, 4, 6, 8, and 10 mM DA standard concentration solutions, respectively, and adjusted to have pH ≈ 7.4 with hydrochloric acid. Figure 4a shows the corresponding low-frequency Raman spectra. We can see that the characteristic peak of DA at 90 cm⁻¹ strengthens with increasing DA concentration and becomes the strongest one in all the detected peaks when the DA concentration reaches 6 mM. To see the influence of the Raman peaks from NE/EE/5HT on the basal intensity at 90 cm⁻¹ from DA, we carried out the Lorentz division and fitting of these Raman spectra. Two typical results from the NE–EE–5HT mixture samples with 6 and 10 mM DA concentrations are presented in Figure 4b,c, respectively. It can be seen that the Raman spectra of the two mixture samples can all be divided into eight peaks in which the 79 and 87 cm⁻¹ peaks are from EE, 81 cm⁻¹ is from NE or 5HT, 96 and 120 cm⁻¹ are from 5HT, 100 and 132 cm⁻¹ are from NE, and 90 cm⁻¹ is from DA. These Raman peaks of the three neurotransmitters, NE, EE, and 5HT, have an influence on the baseline at 90 cm⁻¹ from DA, but it is weak compared to the DA signal. The 90 cm⁻¹ characteristic peak is always the strongest in all the detected peaks. In Figure 4d, we give the dependence of the integral intensity of the 90 cm⁻¹ peak on DA concentration. A good linear relationship can be achieved, indicating that DA can effectively be distinguished from the four neurotransmitters by multipeak-fitting. So, it is reasonable to infer that NE, EE, and 5HT hardly affect the identification of DA by the low-frequency Raman spectra at normal CSF level.

Detection and analysis of monoamine neurotransmitter concentrations in CSF are integral to the diagnosis and study of psychiatric disorders. It is obvious that the low-frequency Raman scattering provides the potential to detect multiple neurotransmitters simultaneously. To test its effectiveness, we use a kind of surface enhanced low-frequency Raman scattering (SELFRS) substrate to show the detection precision. We still choose DA as a target neurotransmitter to test the possibility. Various pathologic states are associated with disturbed DA concentrations in CSF, which can induce depression, schizophrenia, and Alzheimer’s and Parkinson’s diseases and so on. For example, abnormal rising of the DA concentration in CSF is connected with schizophrenic disease, and the synthesis difficulty of DA relates with depression and Parkinson’s diseases.

The designed SELFRS substrate consists of Au@SiO₂ nanoparticles (NPs) and an omniphobic surface. Au@SiO₂ NPs provide “hot spots” to enhance the Raman signals and an omniphobic surface to concentrate the test solution. Preparation details of the SELFRS substrates are similar to those described in our previous work. A typical clinical detection process of DA in CSF includes the following steps (Figure S6 in the Supporting Information). First, CSF is uniformly mixed with Au@SiO₂ NPs in a volume ratio of 1:3, and the mixed solution is adjusted to pH = 7.4 with phosphate buffer for simulating the body fluid environment. Second, 40 µL of the mixed liquid is dripped onto an omniphobic surface and evaporated at 60 °C to form a small-size aggregate. Finally, the low-frequency Raman spectra of the aggregate are measured, and a diagnosis is made.

The current Raman spectra were acquired on a T64000 Raman system with a micro-Raman backscattering geometry at normal laser incidence (514.5 nm). The power illuminating the sample was ~3 mW. The acquisition time of each Raman spectrum was 30 s. All the measurements were conducted at room temperature. The low- and high-frequency Raman spectra of the phosphate buffer solution with DA (pH = 7.4) dried on the SELFRS substrate are shown in the left side of Figure 5a. We can see that two strongest peaks marked with red and green vertical dotted lines appear at 90 and 751 cm⁻¹ (757 cm⁻¹ in solid powder). With decreasing DA concentration, the intensity of the peak at 751 cm⁻¹ falls sharply and can no longer be distinguished from the noise signal when the concentration is lower than 10⁻⁷ M. For the peak at 90 cm⁻¹, it can still be identified in the 10⁻⁹ M sample. The relationships between intensities and concentrations of the two peaks are shown in Figure 5b (red/green lines). Such a DA concentration dependence in the 10⁻⁵ to 10⁻⁹ M range almost
covers the dynamic ranges of dopamine in normal human CSF, serum, and urine (red line). To confirm the practical feasibility of the SELFRS method in CSF, we repeated the above experiments using artificial CSF (ACSF), and the corresponding SELFRS results are depicted on the right side of Figure S6. The strongest low-frequency Raman peak at 90 cm\(^{-1}\) is marked by a blue dotted line, and the dependence of its intensity on DA concentration is shown in Figure 5b (blue line). We can see that the detection limit of this method in ACSF is 10\(^{-8}\) M, reaching the concentration of DA in normal human CSF. To compare the advantages of the SELFRS method with currently existing ones, we list the detection limit and testing time of dopamine concentration adopted by currently existing methods in Table S5 in the Supporting Information. Obviously, the SELFRS method has sufficient detection accuracy and short testing time, suitable for wide clinical applications. The above results provide the feasibility of a DA concentration challenge test in body fluids using our current SELFRS method.

Not only monoamine neurotransmitters but also some other amino acids consist of a ringed group and carbon side chain such as phenylalanine, tryptophan, and histidine. They are essential amino acids for humans and indispensable nutrients for our body. As important building blocks of proteins, their deficiencies or abundances will lead to a variety of diseases including stunting, depression, schizophrenia, Parkinson’s reaction, and so on. Ultrasensitive detection of these amino acids in our body will guide our nutritional balance. The SELFRS method reported in this work provides a direct, rapid, and ultrasensitive way to detect amino acids or even some structures of proteins.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpcl.2c00737.

Molecular structures of DA, NE, EE, and 5HT, experimental and calculated Raman spectra, normal mode descriptions based on potential energy distribution, typical detection process of SELFRS method, and comparison of the detection limit and testing time of dopamine concentration adopted by currently existing methods (PDF)

Variation of the electron density distribution caused by the strong out-of-plane vibrations of the C–C single bonds of DA at the joint point of the ring-like molecule and its side chain: low-frequency 83 cm\(^{-1}\) mode (MP4)

Variation of the electron density distribution caused by the stretching vibrations of C–O and C–C bonds of DA at the ring group: high-frequency 757 cm\(^{-1}\) mode (MP4)

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Notes
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