Turn-on Fluorescent Dopamine Sensing Based on *in Situ* Formation of Visible Light Emitting Polydopamine Nanoparticles

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Ⓢ *Supporting Information*

ABSTRACT: Dopamine is the principle biomarker for diseases such as schizophrenia, Huntington's, and Parkinson's, and the need is urgent for rapid and sensitive detection methods for diagnosis and monitoring of such diseases. In this Article, we report a turn-on fluorescent method for rapid dopamine sensing which is based on monitoring the intrinsic fluorescence of *in situ* synthesized polydopamine nanoparticles. The assay uses only a common base and an acid, NaOH and HCl to initiate and stop the polymerization reaction, respectively, which makes the assay extremely simple and low cost. First, we studied the *in situ* optical properties of polydopamine nanoparticles, for the first time, which formed under different alkaline conditions in order to determine optimum experimental parameters. Then, under optimized conditions we demonstrated high sensitivity (40 nM) and excellent selectivity of the assay. With its good analytical figures of merit, the described method is very promising for detection of dopamine related diseases.
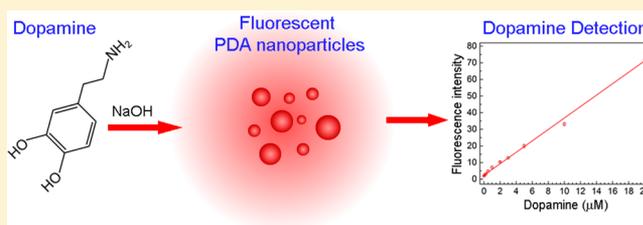
Dopamine (DA), a catecholamine neurotransmitter, regulates many biological processes in central nervous, hormonal, and cardiovascular systems.[1,2] Abnormal DA concentrations in biological fluids (e.g., urine, blood plasma, and extracellular fluid of the central nervous system) can be indicator of several diseases such as schizophrenia and Huntington's and Parkinson's diseases.[3−5] In this regard, sensitive and selective measurement of DA levels is important for diagnosis of these diseases and monitoring of patients.[6] Common DA detection methods utilize electrochemical analysis,[7−9] chromatography coupled with spectroscopy[10,11] (e.g., high-pressure liquid chromatography (HPLC)-fluorescence and gas chromatography/mass spectrometry (GC/MS)) and fluorescent[12−16] or colorimetric probes[1,17] (e.g., organic dyes, quantum dots, and gold nanoparticles). These methods, however, have some limitations. For instance, interference from uric acid (UA) and ascorbic acid (AA) largely limits selectivity of electrochemical methods. Chromatographic methods on the other hand, are time-consuming, labor intensive, and expensive with complicated procedures. Similarly, synthesis of fluorescent or colorimetric probes for DA detection involves complicated and time-consuming procedures.

A straightforward, cost-effective and rapid alternative for DA detection is measuring the optical absorption of oxidation product of dopamine under alkaline conditions.[18−22] These assays use only a common base (e.g., NaOH) or other oxidants (e.g., enzymes) as a reagent, and DA concentration is determined by simply measuring the optical absorption of the resulting brownish oxidation product. Unfortunately, the method demonstrates a poor sensitivity around a few micromolar. In these oxidation studies, the product is assumed as a quinone derivative of dopamine.[18] However, recent studies demonstrated that under alkaline conditions the quinone product is unstable and rapidly polymerized by covalent attachment and aggregation.[23−25] The resulting polymer is commonly referred to as polydopamine (PDA), which is structurally very similar to the natural eumelanin polymers.[26] Although, the fluorescence property of eumelanins is known for more than 40 years and there is ongoing research to understand the interesting optical properties of these materials;[27−29] the fluorescence of PDA is largely unexplored. To our knowledge, only very recently Zhang et al.[30] reported that purified PDA nanoparticles are fluorescent and can be used for cellular imaging.

Here, we explored the intrinsic emission properties of PDA nanoparticles which were synthesized by oxidizing DA under different conditions and demonstrated that it can be used as turn-on type fluorescent reporter of DA existence. The introduced method is based on monitoring the visible light emission of *in situ* synthesized PDA nanoparticles, and it is capable to detect very low DA concentrations (detection limit is 40 nM). Note that the reached detection limit is 20−750-fold better than the methods that measure the absorption of oxidization solution of DA (see the Supporting Information,

Table S1). Initially, we studied the fluorescence property of PDA synthesized under different basic conditions. We observed that the emission spectrum of PDA is very dependent on time, base type, and its concentration. Then, under optimized conditions, we performed DA sensing experiments. Finally we showed that the method is very selective to DA, and no interference from AA or UA is observed.

■ MATERIALS AND METHODS

Materials. Dopamine, ascorbic acid, glucose, urea, sucrose, aspartic acid, alanine, glycine, lysine, and hydrochloric acid (37%) were purchased from Sigma-Aldrich (U.S); sodium hydroxide was purchased from Merck (Germany). All chemicals were used as purchased.

Oxidization of Dopamine. A volume of 1.8 mL of freshly prepared dopamine (100 μM) solution in phosphate buffered saline (PBS) (2 mM, pH 7.4) was oxidized by dropwise addition of 200 μL of Tris base (final concentration in the solution is 10 mM) or NaOH (final concentration in the solutions are 0.5 mM, 10 mM, and 20 mM) stock solutions. Time dependent evaluation of UV−vis or fluorescence spectra of the solutions were *in situ* recorded using UV−vis absorption (Cary 5000, Varian) and fluorescence (Eclipse, Varian) spectrophotometers, respectively.

Characterization of Polydopamine Nanoparticles. A volume of 5.4 mL of dopamine solutions (100 μM) in PBS was polymerized using 0.6 mL of NaOH (final concentration in the solution is 20 mM) for 3 h. Then the particles were purified using membrane dialysis. A volume of 2 μL of purified particles dripped on a transmission electron microscopy (TEM) grid, and morphology of the particles were investigated using a TEM (Tecnai G2 F30, FEI). For dynamic light scattering (DLS) measurements, 100 μM of dopamine was polymerized in PBS for 3 h. Then particles were filtered with a 1 μm syringe filter, and their size distribution was measured with Zetasizer Nanoseries (Malvern Instruments).

Stopping the Oxidization Reaction. In order to stop the polymerization reaction, excess acid can be added to the polymerization solution. For instance, 2 mL of dopamine oxidization solution in which the final concentrations of dopamine and NaOH are 100 μM and 20 mM, respectively, is stopped using 42 μL of 1 M HCl.

Sensitivity and Selectivity Experiments. Freshly prepared dopamine stock solutions were diluted in PBS to give final volume of 1.8 mL and concentrations ranging from 0.1 to 20 μM. Polymerization reactions were initiated using 200 μL of NaOH (final concentration is 20 mM). After 30 min, reactions were stopped using 42 μL of 1 M HCl and solutions were further incubated for 10 min. Then, fluorescence spectra of solutions were recorded (excitation wavelength is 370 nm). For selectivity experiments, concentration of all interfering chemicals (ascorbic acid, uric acid, urea, glucose, sucrose, aspartic acid, lysine, alanine, and glycine) was 0.1 mM and other parameters were the same with the sensitivity experiments. All experiments were performed in triplicate.

Absorption Based Assay. The samples were prepared using the same experimental parameters with the fluorescence assay. Absorption of DA oxidization solutions (DA concentrations were between 0.5 and 20 μM) at 360 nm were recorded using a UV−vis absorption (Cary 100, Varian) spectrophotometer.

■ RESULTS AND DISCUSSION

Under alkaline conditions, DA is spontaneously oxidized to its quinone derivative and then autopolymerized to form PDA (Scheme 1).[23,24] Polymerization of DA solution (1 mM) in

Scheme 1. Schematic Representation of Fluorescent PDA Nanoparticle Formation
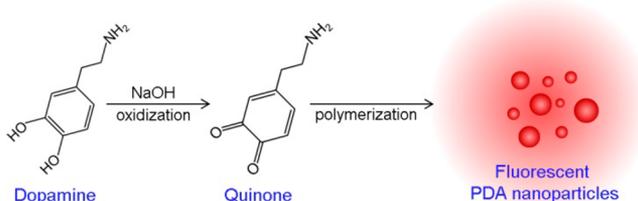
PBS (pH 7.4) was *in situ* investigated using UV−vis spectroscopy for 3 h (see the Supporting Information, Figure S1). After NaOH addition (final concentration is 20 mM), a peak around 350 nm appeared which indicates the quinone formation[24] and its intensity gradually increased for 3 h. The broad peak around 470 nm shows the intramolecular cyclization of the quinone derivative[24] which is an intermediate product in the polymerization of DA (see the Supporting Information, inset of Figure S1).[23] After 10 min, this broad peak disappeared and absorption at all wavelengths between 400 and 700 nm increased gradually, which shows the formation of PDA.[24] In order to characterize the PDA nanoparticles formed after 3 h of oxidization reaction, we polymerized 100 μM DA using 20 mM NaOH. Particle size distribution of PDA nanoparticles is given in Figure 1, which
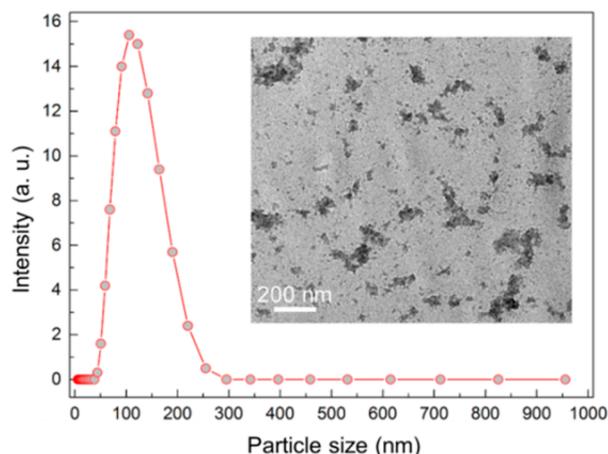

Figure 1. Particle size distribution of PDA nanoparticles. The graph shows the particle size distribution of PDA nanoparticles which were prepared after oxidization of 100 μM DA solution for 3 h in the presence of 20 mM NaOH. Inset shows the TEM images of the purified DA oxidization solution showing the polydisperse and irregularly shaped PDA nanoparticles.

shows the broad size distribution of particles with diameters ranging from a few nanometers to hundreds of nanometers. Figure 1 inset shows the TEM image of 3 h oxidized PDA particles. PDA nanoparticles were irregular in shape and have broad particle size distribution which is in accordance with DLS results.

The resulting PDA nanoparticles are intrinsically fluorescent (will be discussed in detail below). Initially, we explored the effect of different oxidation conditions on the fluorescence of oxidation product of DA (i.e., PDA nanoparticles) in order to determine optimum experimental conditions for DA sensing. We compared two bases that are commonly applied in DA oxidization;[18,23] Tris base and NaOH at the same pH and molarity. We measured the *in situ* fluorescence of 100 μM DA which is oxidized under different basic conditions for 1 h and compared the fluorescence intensities at 510 nm at different time intervals. The results are presented in Figure 2 and Figure
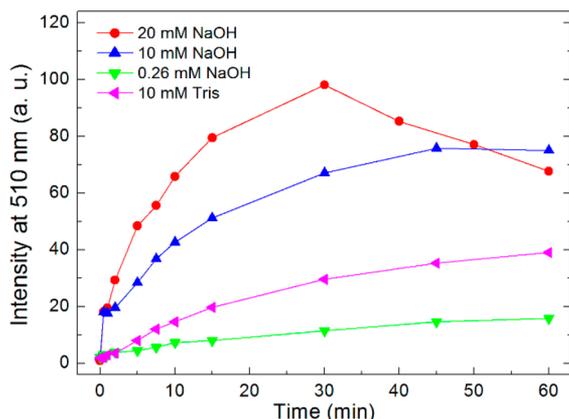

Figure 2. Time dependent fluorescence intensity at 510 nm of 100 μM DA solutions, which were oxidized at different basic conditions.

S2 (Supporting Information). At the same pH value (9.6), Tris is more effective than NaOH in terms of generating fluorescent PDA nanoparticles. On the other hand, at the same molarity (10 mM), NaOH is almost 2 times more effective than Tris base. Also, we explored fluorescence of the polymerization solution at different NaOH concentrations, and we selected 20 mM as optimal for the sensing experiments. Therefore, we used 20 mM NaOH to oxidize DA in the rest of the study.

The fluorescence spectra of synthesized PDA nanoparticles after 3 h of oxidation, which were prepared using 100 μM DA, is given in Figure 3a. We observed that fluorescence behavior of the resulting PDA nanoparticles is very similar to eumelanin polymers in which the fluorescence spectrum is excitation wavelength dependent and can be tuned in the whole visible spectra.[28] With the increasing excitation wavelength, the emission maximum of the PDA nanoparticle solution shifts to the longer wavelengths (Figure 3b). Also, emission intensity is excitation wavelength dependent; it initially enhances with increasing wavelengths and then started to decrease gradually. The maximum emission intensities were recorded between the excitation wavelengths of 350 and 400 nm. Therefore, we selected 370 nm as the excitation wavelength for DA sensing experiments.

Figure 3c shows the fluorescence spectra of 100 μM DA solution recorded at different time intervals up to 6 h. After NaOH addition, two emission peaks immediately formed at around 470 and 530 nm, respectively. In the first 30 min, intensities of the both peaks gradually increased. Interestingly, after 30 min the intensity of the peak around 530 nm started to decrease and it completely disappeared in 2 h. The peak intensity around 470 nm, on the other hand, decreased slightly between 30 and 60 min but after this point it started to increase again. After 6 h, only an intense peak around 470 nm was
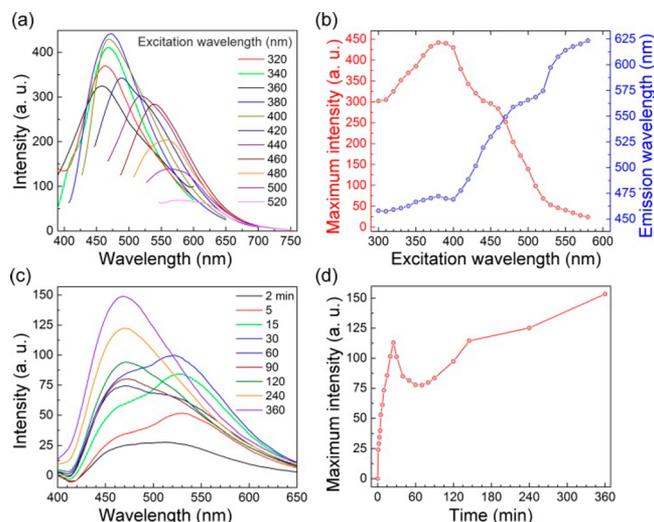

Figure 3. Emission properties of PDA nanoparticles: (a) excitation wavelength dependent fluorescence spectra of PDA nanoparticles, (b) change of the fluorescence intensity maxima and wavelength of PDA nanoparticle solution as a function of excitation wavelength, (c) time dependent fluorescence spectra of 100 μM DA solution oxidized using 20 mM NaOH, and (d) fluorescence intensity maximum change of the same solution over time.

observed. The peak around 530 nm may correspond to an intermediate product in the polymerization reaction, which is formed and consumed in 1 h. We believe that *in situ* fluorescence measurements of the DA oxidation solution can give information about the structure of PDA, which is still not completely identified;[31] however, it is beyond the scope of this study. Figure 3d shows the emission intensity maxima of the same solution during polymerization. In the first minutes of polymerization, the fluorescence intensity sharply increased, after about 30 min it started to decrease, and after 90 min it began to raise again. Accordingly, we selected 30 min as the incubation time for sensing experiments.

The time dependent fluorescence spectrum of the DA oxidization solution can be a source of error in the analytical measurements. To overcome this problem and obtain stable emission characteristic over time, we added excess HCl to DA solutions after a certain time of oxidization and stopped the polymerization reaction by making the solution acidic. Figure 4a shows the fluorescence spectra of a DA oxidation solution (100 μM) before and after HCl addition. In the first 15 min, emission intensity increases gradually as expected. After HCl addition, the fluorescence spectrum of the solution shifted to shorter wavelengths and becomes very stable over time. In Figure 4b, the emission intensity at 510 nm was plotted as a function of time. After HCl addition intensity at 510 nm increased slightly and then it was almost constant for at least 45 min.

In order to evaluate the performance of the assay, we tested different concentrations of DA between 0.1 and 20 μM with the assay. First, oxidation of dopamine solutions at different concentrations in PBS was initiated using NaOH (20 mM) and after 30 min excess HCl was added to stop the reaction. Then, fluorescence spectra of the DA solutions were recorded (Figure 5a). As expected with the increasing DA concentration, fluorescence of the solution becomes brighter. Figure 5b shows the fluorescence intensities at 510 nm as a function of DA concentration. We observed that the response of the assay is
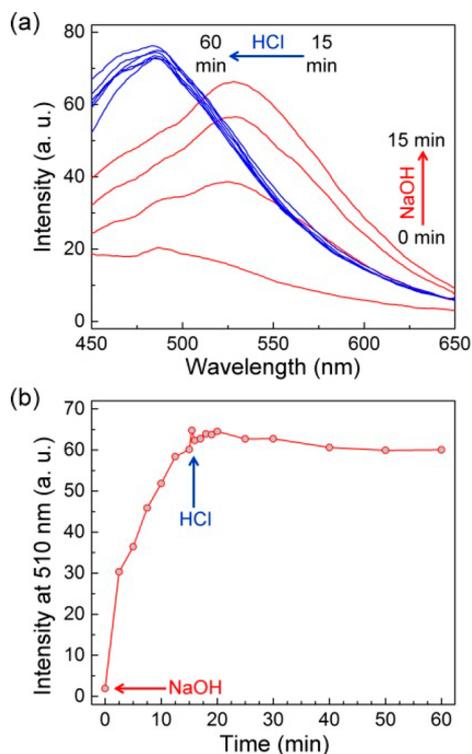
Figure 4. Stopping the polymerization reaction of DA using HCl: (a) fluorescence spectra of 100 μM DA, which is oxidized using 20 mM NaOH and after 15 min excess HCl was added; (b) fluorescence intensity at 510 nm change with time before and after HCl addition.
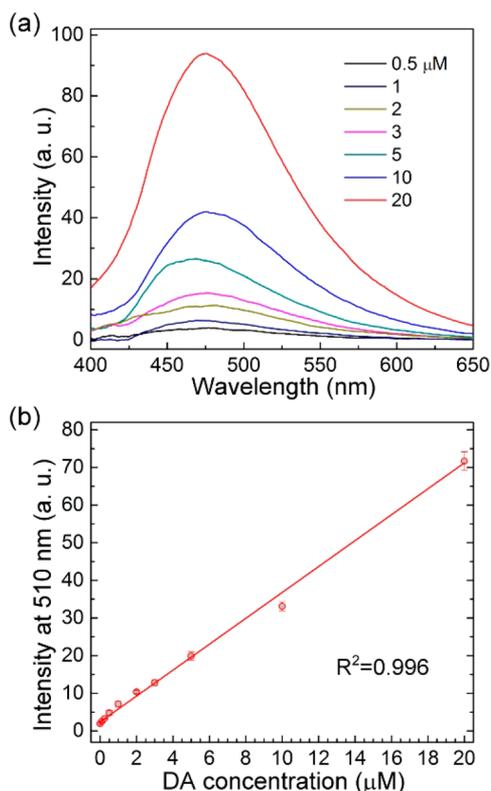

Figure 5. Sensitivity of the dopamine assay. (a) Fluorescence response of the assay against different concentrations of DA. (b) Assay response as a function of DA concentration which indicates the good linearity of the sensor in the studied region.

highly linear ($R^2 = 0.996$) in the studied concentration region. Relative standard deviations (RSD) of three separate experiments in Figure 5b are between 0.2% and 2.4% indicating the good reproducibility of the sensor response. Using this calibration line, we calculated limit of detection (LOD) and limit of qualification (LOQ) values of the assay as 40 nM and 120 nM, respectively. The achieved LOD and LOQ values of our fluorescence based assay is much better than absorption based methods (see the Supporting Information, Table S1) which typically have LOD and LOQ values above the miromolar level. In addition, to directly compare the performance of our fluorescence assay with the absorption based method, we measured the absorption of DA solutions which were oxidized using the same conditions with fluorescence experiments. We observed that for the concentrations above 1 μM there is a statistically significant difference in the absorption value between blank and DA solutions (see the Supporting Information, Figure S3). For the concentrations below 1 μM, the absorption assay could not detect the presence of DA, which is also in accordance with the previous reports.[18−22]

Lastly, we tested the selectivity of the assay using possible interfering chemicals such as AA, UA, amino acids, and sugars. The concentration of interfering substances is 100 μM. None of the tested chemicals produced a fluorescence signal even at this high concentration (Figure 6). To further demonstrate the
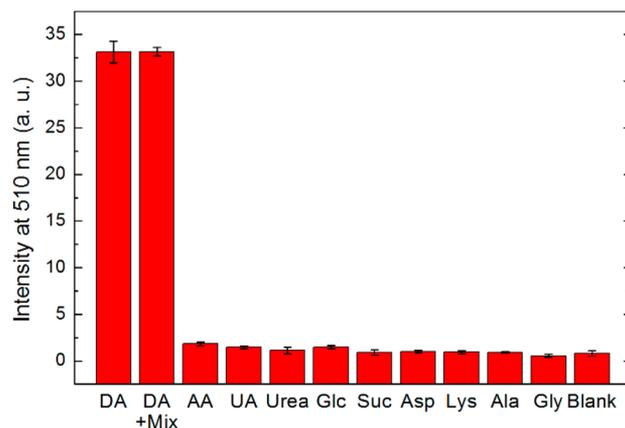

Figure 6. Selectivity of the dopamine assay. DA concentration is 10 μM and concentration of other substances is 100 μM. Fluorescence response was only observed for DA indicating the excellent selectivity of the assay (DA, dopamine; AA, ascorbic acid; UA, uric acid; Glc, glucose; Suc, sucrose; Asp, aspartic acid; Lys, lysine; Ala, alanine; Gly, glycine; Mix, mixture of all interfering chemicals).

selectivity of the method, we measured the fluorescence of 10 μM DA oxidization solution in the presence of mixture of all of interfering chemicals that used in this study (concentration of interfering chemicals was 100 μM). Presence of these chemicals in the DA assay has no effect on the fluorescence response (Figure 6). The observed excellent selectivity over DA of our assay is due to its specific response against PDA nanoparticle formation.

■ CONCLUSION

In conclusion, we developed a facile assay for detection of DA, which is based on measuring the fluorescence signal of the oxidation product of the DA. In order to determine optimum conditions for DA detection, we initiated the polymerization of DA at different oxidation conditions and recorded time

dependent emission spectra of these solutions. Accordingly, we achieved a DA detection limit of as low as 40 nM. In addition, we did not observe any interference from AA and UA and as well as many amino acids and sugars. Compared with other DA detection methods, our assay is extremely simple and low cost, which make the method very promising for detection of DA deficiency related diseases.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Table comparing performance of the assay with previous assays, time dependent UV−vis spectra of dopamine oxidation solution, *in situ* fluorescence spectra of dopamine solutions which were oxidized at different basic conditions, and a graph showing the sensitivity of the absorption based assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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