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ARTICLE

Novel Fluorescent Organic Nanoparticles as Label-free Biosensor for Dopamine in Serum

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Novel fluorescent organic nanoparticles (FONs) composed of an arbitrarily selected organic dye (C2) and an amphiphilic triblock copolymer (Pluronic F127) have been designed and constructed. The as-prepared C2-F127 FONs display uniform morphology with diameter around 300 nm and strong orange fluorescence with emission maximum at 561 nm. When C2-F127 FONs are utilized as fluorescent probe for detecting dopamine, a significant fluorescence quenching can be observed. Such fluorescence quenching is attributed to the formation of polydopamine coated on the surfaces of the FONs, which leads to photo-induced charge transfer between the organic dye molecules and the formed polydopamine. Moreover, C2-F127 FONs are highly selective to dopamine over other biomaterials, such as glucose, uric acid, ascorbic acid, epinephrine and L-DOPA, including in a competitive environment. As C2-F127 FONs demonstrate good dispersion and high stability in biological media, they are further utilized as a label-free biosensor for detecting dopamine in 10% serum, and satisfied sensitivity and selectivity are achieved.

Introduction

Fluorescent sensors have been extensively explored in various applications on chemistry, biology, and environment, owing to their unique advantages, such as low-cost, operational simplicity, reliable, reproducible, and real-time detection.[1-3] The performance of fluorescent sensors is determined to a great extent by the physicochemical properties of the fluorescent probes.[4] Hence, the development of fluorescent probes has attracted considerable research interests over the past few decades. Fluorescent sensory materials based on fluorescent inorganic nanoparticles (FINs),[5] organic dyes,[6] and fluorescent proteins,[7] have been developed as fluorescent probes. However, most of them are exposed to certain disadvantages, such as high biological toxicity, low biocompatibility, or high cost.[8-10] Alternatively, fluorescent organic nanoparticles (FONs) have attracted great attention due to their biocompatible property, structure designability, surface chemistry tailorability and biodegradation potential.[11-14] A general strategy for fabrication of FONs is to encapsulate the hydrophobic fluorescent dyes by amphiphilic polymers which can be self-assembled into nanoparticles in aqueous media.[11] In such FONs, the hydrophilic polymers on the surface of FONs bring high biocompatibility and stability in biological media while the hydrophobic fluorescent dyes are encapsulated in the nanoparticles. However, most of fluorescent materials are suffered from aggregation-caused quenching (ACQ)[15,16] upon formation of FONs. Consequently, highly luminescent FONs based on conventional organic dyes are still pursued.

As a significant neurotransmitter, dopamine (DA, 3,4-dihydroxyphenyl ethylamine) plays an important role in renal, cardiovascular, central nervous, and hormonal systems.[17] It may act as a biomarker for various central nervous system-related diseases, such as Parkinsonism disease, schizophrenia, and anorexia.[18,19] Therefore, detection and quantification of DA is becoming increasingly important in clinical medical practice. Electrochemical methods with simple instrument and fast response time have been a particular choice for DA detection.[20,21] Unfortunately, the coexisting compounds, such as uric acid (UA) or ascorbic acid (AA) in the biological samples can cause great interference due to the close oxidation potential in comparison to that of DA.[20,22-24] In addition, the accumulation of the oxidation product of AA or UA on electrode surface may lead to the electrode fouling with poor selectivity and reproducibility.[23]

In the present study, Pluronic F127 (polyethylene oxide (PEO) – polypropylene oxide (PPO) – PEO triblock copolymer) is utilized as an amphiphilic polymer to encapsulate a hydrophobic fluorescent dye C2 to form C2-F127 FONs. The as-prepared C2-F127 FONs with a strong orange fluorescence were characterized by transmission electron microscopy (TEM), dynamic light scattering (DLS), Fourier transform infrared spectroscopy (FTIR), ultraviolet-visible (UV-vis) and photoluminescence (PL) measurements. The resulting C2-F127 FONs are highly sensitive and selective to DA over other biomaterials due to efficient fluorescence quenching. Their

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biosensor application for DA detection in 10% serum is further investigated.

Materials and methods

Reagents and instruments

Pluronic F127 (M_n = 10000) and DA were purchased from Energy Chemical and Aladdin Company, respectively. The other reagents were analytical grade. All chemicals in this study were obtained from the manufactures without purification unless specifically noted. The serum samples were kindly provided by Shanghai General Hospital and stored at 4 °C. The buffer involved in this work was Tris-HCl buffer. The pH measurement was carried out on a Mettler-Toledo Delta FiveGo pH meter. Deionized (DI) water (MillQ, 18.2 MΩ) was used throughout the study.

UV-vis spectra were obtained from UV-2500 spectrometer (Shimadzu, Japan). PL measurements were conducted on an RF5301 spectrometer (Shimadzu, Japan). The fluorescence measurements were performed in a 1 mm quartz cuvette. The solution volume for each measurement was 100 μL. TEM images were obtained from JEM-2100F electron microscope (JEOL, Japan). FTIR spectroscopy was obtained from IRAffinity spectrometer (Shimadzu, Japan). Zeta potential and DLS measurements were performed on Zetasizer Nano ZS analyzer (Malvern, UK). The photoluminescence quantum yield was measured by QM40 Fluorescence Spectrometer (PTI, USA).

Preparation of C2-F127 FONs

The synthesis of organic dye C2 has been reported elsewhere.[25] To fabricate the FONs, 50 mg F127 was dissolved in 20 mL DI water and then mixed with 20 mg C2 in approximately 20 mL THF. After a few minutes, THF was removed by a vacuum rotary evaporator. To remove the excess organic dyes, the resulting mixture after high speed centrifugation was washed by DI water for 3 times. Then a stable orange solution of C2-F127 FONs in water were obtained and stored at 4 °C before further characterizations and applications.

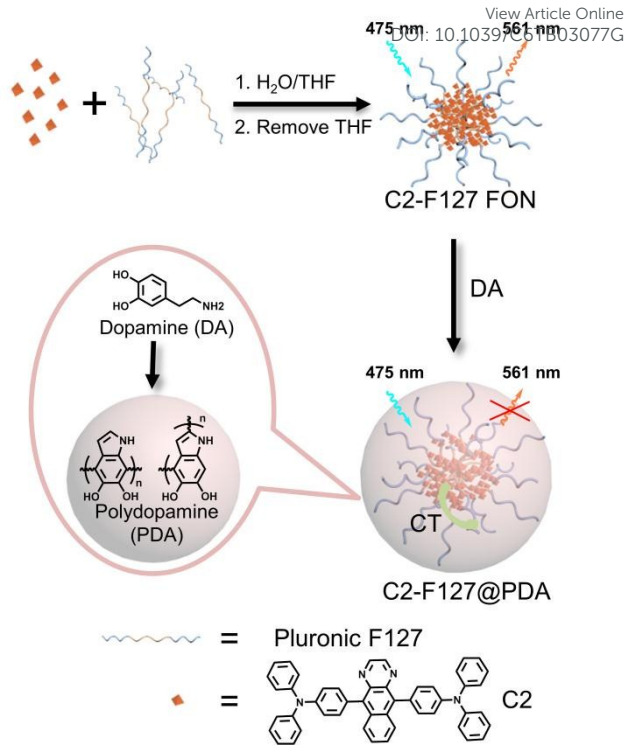
Preparation of biosensor

In accordance with conventional experiments, various amounts of DA were added into the aqueous solution of the synthesized C2-F127 FONs. Then the mixture was incubated for 90 minutes to detect the fluorescence intensity changes. All the experiments were performed in buffer solution. In real samples, the experiments were conducted in 10% diluted serum similarly as the one in 100 μL of Tris-HCl buffer (10mM, pH = 9.5). The concentration of C2-F127 FONs was around 50 μg/mL in the experiments of optimizing sensing conditions, while 10 μg/mL for sensing DA in Tris-HCl buffer and 10% serum.

Results and discussion

Synthesis and characterization of C2-F127 FONs

The synthesis of the triblock copolymer Pluronic F127 (PEO-PPO-PEO) based FONs is depicted in Scheme 1. It has been well studied that amphiphilic block copolymers are tended to form nanoparticles in proper solvent. Herein, the PPO block is hydrophobic while the PEO blocks are hydrophilic. After removing the organic solvent THF by a rotary evaporator, organic nanoparticles with PEO-rich surface can be formed. To endow the organic nanoparticles with fluorescence, one of our previously reported hydrophobic dyes was arbitrarily selected and initially mixed with F127 before self-assembly.[25] Numerous organic dye molecules were then encapsulated in F127 based nanoparticles because of the strong interactions between the hydrophobic segments of F127 and the aromatic rings in C2, while the hydrophilic segments of F127 covered the surface to create a water-soluble periphery. The as-prepared C2-F127 FONs were further characterized by TEM



Scheme 1 Schematic illustrations of the preparation of C2-F127 FONs and the mechanism of DA sensing.

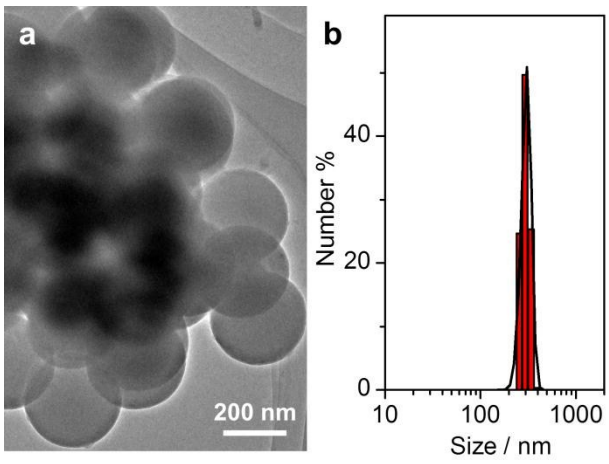


Fig. 1 (a) TEM image and (b) size distribution of C2-F127 FONs.

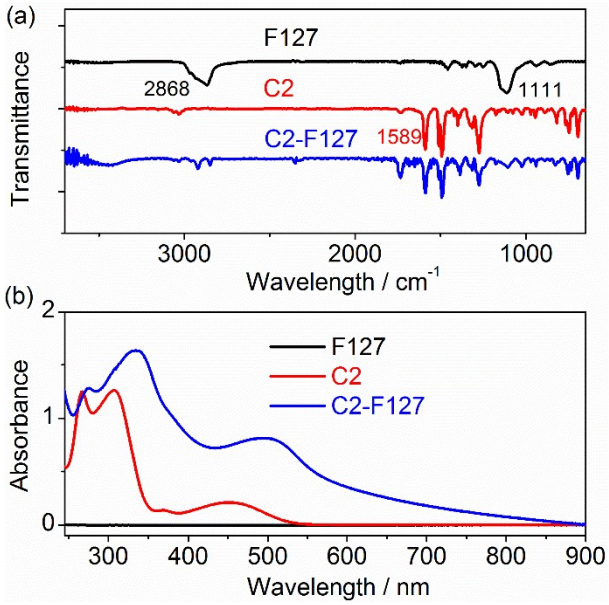


Fig. 2 (a) FTIR and (b) UV-vis absorption spectra of F127, C2, and C2-F127 FONs.

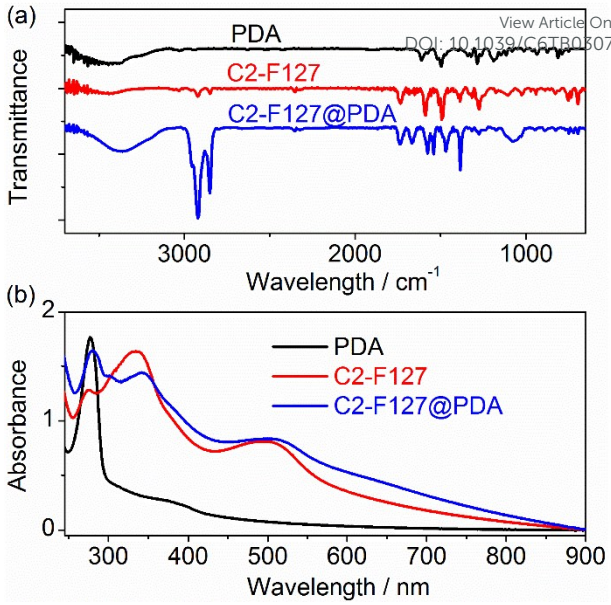


Fig. 3 (a) FTIR and (b) UV-vis absorption spectra of PDA, C2-F127 FONs, and C2-F127@PDA.

and DLS measurements. As illustrated in Fig. 1a, a typical TEM image displays that the average diameter is around 300 nm. The DLS measurement reveals relatively uniform morphology of C2-F127 FONs in DI water (Fig.1b). The size distribution is 310 ± 30 nm, which is slightly different from those measured by TEM and probable caused by the shrinkage and accumulation of micelle during the drying process. Moreover, the PL properties of the as-prepared C2-F127 FONs in aqueous solution were investigated. As shown in Fig. S1, it exhibits an intense emission with PL maximum at 561 nm. The PL quantum yield of the FONs in aqueous solution was measured to be 10.4% by a Fluorescence Spectrometer.

The successful hybrid of organic dye C2 in the FONs was verified by FTIR and UV-vis absorption spectroscopies. Fig. 2a displays the FTIR spectra of F127, C2, and C2-F127 FONs. For F127, two intense and sharp peaks at 1111 and 2868 cm^{-1} are attributed to the stretching vibration of C-O and C-H bond, respectively.[14] A rather weak band peaked at 3500 cm^{-1} is assigned to the stretching vibration of the terminal hydroxyl groups in F127.[26] For organic dye C2, a moderate band at 1589 cm^{-1}, assigning to the stretching vibration of the aromatic skeleton, can be observed.[25] Upon the formation of C2-F127 FONs, both characteristic vibration bands of C2 and F127 are found in the FTIR spectrum, which indicates the successful combination of C2 in F127 in the synthesized FONs. Moreover, the synthesized nanoparticles were also characterized by UV-vis spectroscopy. Fig. 2b demonstrates the absorption spectra of F127, C2, and C2-F127 FONs in solutions. Organic dye C2 in dichloromethane solution exhibits two distinct absorption bands. The absorption band in the visible region originates from the π–π* electron transition of the conjugated backbone, while the other one in the ultraviolet region is assigned to the intramolecular charge transfer from the electron-donating triphenylamine unit to the central electron-withdrawing benzoquinozaline unit. When C2 is hybridized with F127 formed nanoparticles, significant bathochromic shifts can be observed for both absorption bands, which probably results from the aggregation and intermolecular interactions. Therefore, both FTIR and UV-vis absorption spectra confirm the successful synthesis of C2-F127 FONs.

The colloidal stability was investigated by measuring the zeta potential which was calculated from the streaming potential using the Smoluchowski relationship:[27]

$$\zeta = \frac{\Delta E}{\Delta P}\frac{\eta\,\lambda}{\varepsilon\varepsilon_0}$$

where ζ, η, λ, and $\varepsilon\varepsilon_0$ are the zeta potential, the solution viscosity, the solution conductivity, and the dielectric permittivity of water. $\Delta E/\Delta P$ is the streaming potential, namely the slope of the potential difference versus pressure difference curve.[27] The zeta potential of F127 and C2-F127 FONs in Tris-HCl buffer (10 mM, pH = 9.5) were determined to be -29.8 and -26.4 mV, respectively, which suggests relatively stable properties of the FONs.[28] Moreover, the photo-stability of the FONs was further evaluated by measuring the fluorescence intensity at 561 nm over a period of 10000 s. It can be found that the fluorescence intensity keeps almost constant upon excitation at 475 nm (Fig. S1).

Mechanism of DA sensing

The as-prepared C2-F127 FONs in aqueous solution exhibit an intense emission with maximum at 561 nm. Upon addition of excess DA, an efficient fluorescence quenching can be observed for the FONs. Such fluorescence quenching is attributed to the photo-induced charge transfer (PCT) between the organic dye molecules and the already formed polydopamine (PDA).[29] The formation of C2-F127@PDA and the sensing strategy of the biosensor are illustrated in Scheme 1. It is well known that DA tends to self-polymerize into PDA in alkaline environment.[30,31] During polymerization, PDA will spontaneously form a conformal and continuous coating layer

covering the surfaces of C2-F127 FONs via covalent and noncovalent bond, especially with bidentate catechol-mediated H-bonding.[32,33] The TEM image of C2-F127@PDA reveals that C2-F127 FONs are covered by the formed polydopamine films (Fig. S2). However, due to the strong intermolecular interactions among the polydopamine molecules, the C2-F127 FONs covered with PDA films aggregate seriously. The formation of C2-F127@PDA were verified by FTIR and UV-vis absorption spectroscopies. The samples of PDA and C2-F127@PDA were prepared in Tris-HCl buffer (10 mM, pH = 9.5). The restulting mixture were centrifugated and washed by DI water for several times. As shown in Fig. 3a, PDA displays a broad vibration band around 3419 cm^{-1}, which is assigned to the stretching vibrations of O-H and N-H bonds.[34] When DA is self-polymerized and coated on C2-F127 FONs, both characteristic vibration bands of PDA and C2-F127 can be found in the FTIR spectrum. Similarly, the aqueous solution of PDA exhibits an absorption band at 278 nm, corresponding to the π-π* electron transition of the conjugated backbone. Upon coating the surface of C2-F127 FONs with a PDA layer, apart from the absorption peak of PDA, two absorption bands of organic dye C2 can be also observed. Therefore, both FTIR and UV-vis absorption spectra reveal the successful coverage of PDA on the surfaces of C2-F127 FONs. Furthermore, to prove the PDA formation around the particles, the fluorescence response of C2-F127 FONs upon addition of DA and PDA were investigated. The addition of DA (100 μM) was incubated in deionized water (*ca.* pH = 7) for 90 minutes in this control experiment where the polymerization rate of DA was too slow and the formation of PDA was negligible. No obvious fluorescence quenching could be found when either DA or PDA was added into the FONs in aqueous solution. This is obviously due to the large distance between C2-F127 FONs and free DA/PDA which disables the photo-induced charge transfer between the organic dye molecules and DA/PDA.[29] Therefore, the explanation of the fluorescence quenching from free DA molecules and/or PDA chains/particles can be excluded. The formed PDA layers coated on the FONs act as electron acceptor and hence quench the emission of the FONs,

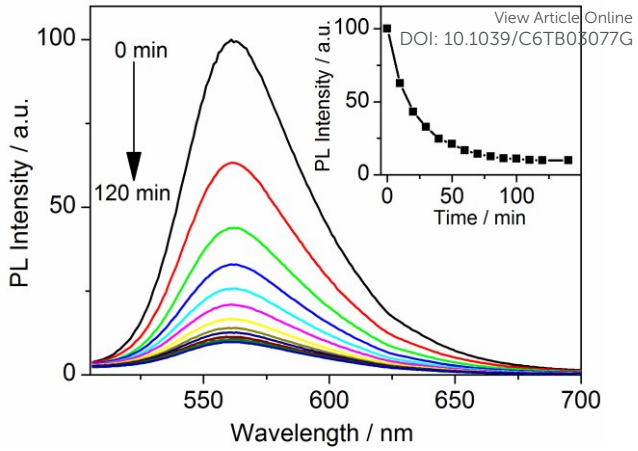


Fig. 5 PL spectra of C2-F127 FONs (*ca.* 50 μg/mL) in Tris-HCl buffer recorded after addition of 500 μM DA with different time (excited at 475 nm). The quenching kinetics is shown as inset.

similar to gold nanoparticles,[35,36] graphene oxide,[6] and other 2D nanosheet.[37,38]

Optimization of sensing conditions

To achieve the highest sensitivity for C2-F127 FONs based biosensor, several sensing parameters were firstly optimized, including the pH values of the buffer solution and the analyte addition time. Fig. 4 displays the PL profiles of C2-F127 FONs (*ca.* 50 μg/mL) upon addition of DA in Tris-HCl buffer with pH value ranging from 6.5 to 10.0. Initially, C2-F127 FONs exhibit an intense emission with PL maximum at 561 nm. The pH value of the buffer has little effect on either the PL intensity or the PL maximum of C2-F127 probe, suggesting the stable PL property of the FONs. However, upon adding 500 μM DA to the solution of C2-F127 FONs in Tris-HCl buffers, the emission intensity dramatically decreases. When the pH value increases from 6.5 to 10.0, C2-F127 FONs based biosensor shows enhanced sensitivity to DA. Previous studies have demonstrated that DA is much easier to self-polymerize to PDA in alkaline media than in acid solution.[30,31] Hence the highest quenching efficiency at the pH about 8.5-10 is

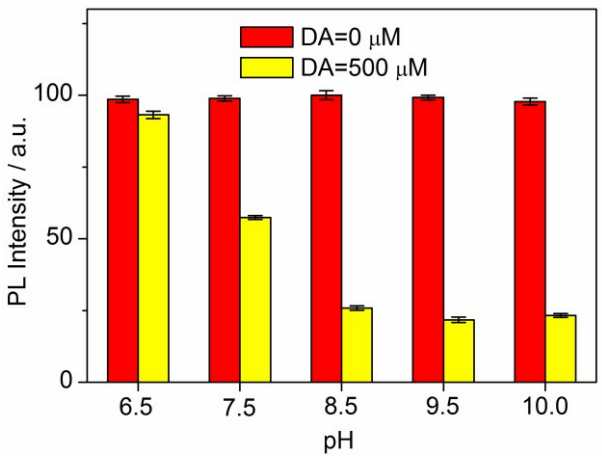


Fig. 4 Fluorescence emission response profiles of C2-F127 FONs (*ca.* 50 μg/mL) upon addition of DA in Tris-HCl buffers with different pH values. Error bars are estimated from three replicate measurements.

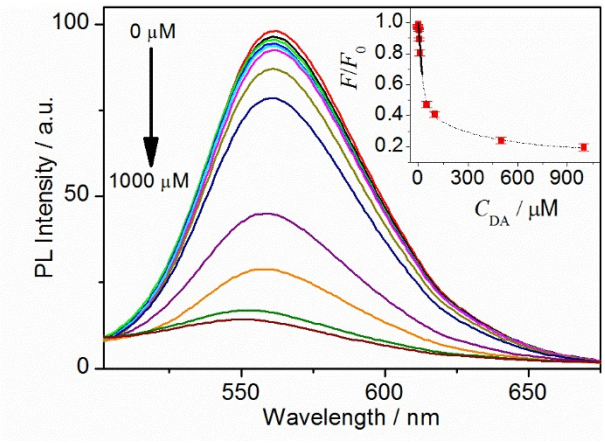


Fig. 6 PL spectra of C2-F127 FONs (*ca.* 10 μg/mL) upon addition of DA with different concentrations. Inset illustrates the calibration curve of DA detected with FONs.

attributed to the PDA films adsorbed on the surface of C2-F127 FONs. Therefore, the sensing properties of C2-F127 FONs for DA are investigated in Tris-HCl buffer with a pH value of 9.5.

The sensitivity of C2-F127 based biosensor is also affected by the addition time of DA analyte. Fig. 5 shows the PL spectra of C2-F127 FONs (*ca.* 50 μg/mL) in Tris-HCl buffer recorded after addition of 500 μM DA at different time. It can be found that upon addition of DA analyte, the fluorescence intensity quickly decreases in the initial minutes and decreasing speed slows down after 30 minutes. The inset of Fig. 5 shows the quenching kinetics which reaches to equilibrium in around 90 minutes. These results suggest that the self-polymerization of DA is almost terminated after 90 minutes, meanwhile the maximum fluorescence quenching is achieved. Therefore, the PL spectra of C2-F127@PDA were recorded 90 minutes after the addition of DA.

Analytical performance

C2-F127 FONs can be used as a biosensor for DA detection due to the efficient fluorescence quenching effect. Hence the PL spectra of C2-F127 FONs (*ca.* 10 μg/mL) upon addition of different concentrations of DA were recorded under the optimized conditions. As shown in Fig. 6, the fluorescence intensity of C2- F127 FONs decreases gradually with the DA concentration increasing. The calibration plots exhibit good linear relationship between the fluorescence intensity of the C2-F127 FONs and the DA concentration in range of 0.1 to 10 μM. The limit of detection (LoD) and the limit of quantification (LoQ) are recalculated to be 35 and 100 nM, respectively, according to the method described by Armbruster and Pry[39] (for details, see the supporting information). The linear regression equation is F/F_0 = 0.9666 - 0.01521C_{DA} with a correlation coefficient (R^2) of 0.995.

A high selectivity for the target analyte is an important requirement for a biosensor concerning their further practical applications. Therefore, the selectivity of DA over other biomaterials was carried out by comparing the fluorescence intensity of C2-F127 FONs (*ca.* 10 μg/mL) upon the addition of DA, uric acid (UA), ascorbic acid (AA), glucose, epinephrine (EP), L-DOPA, and some common metal ions, such as Zn^{2+} and Ca^{2+}. As shown in Fig. 7a, the fluorescence intensity is slightly affected by the above control substances. Moreover, various common substances in practical samples perhaps interfere with the sensitivity of the target analyte. Subsequently, a competitive experiment of DA detection with existence of the above substance was also operated to discuss the specificity of our biosensor. As shown in Fig. 7b, the fluorescence intensity variation caused by the presence of interfere substance is less than 10%, indicating the existing interference does not interrupt the DA detection and this biosensor can be further used for real sample analysis. Furthermore, the reproducibility of the biosensor is also a key factor in the practical application. In order to test the reproducibility of biosensor, two different concentrations of DA, 1 μM and 10 μM, were tested by six independently prepared biosensors. The value of relative standard deviation (RSD) was determined to be 3.9% and 7.1%

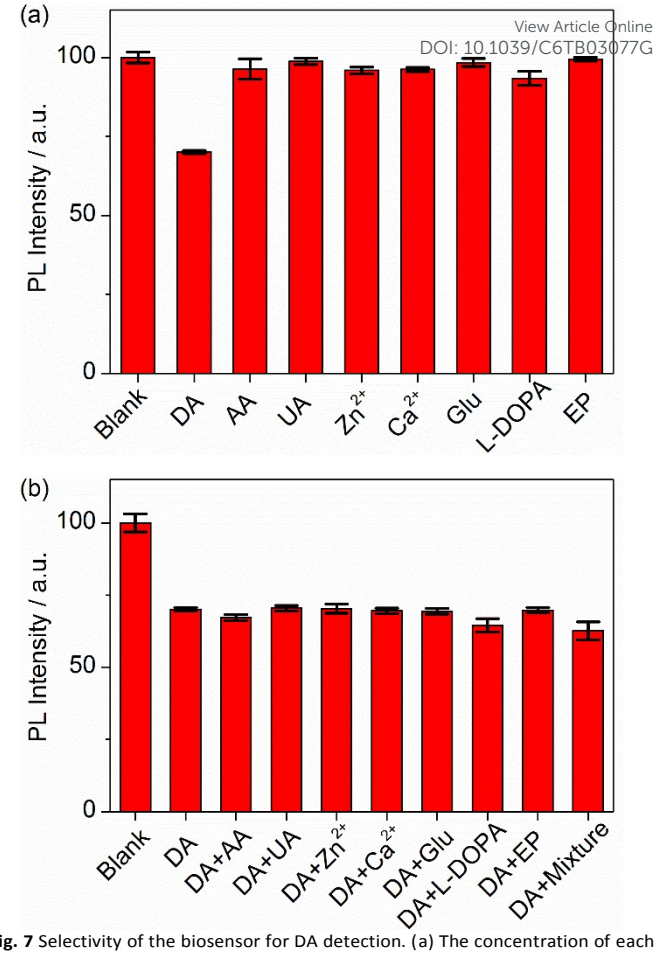


Fig. 7 Selectivity of the biosensor for DA detection. (a) The concentration of each analyte is 10 μM. (b) The concentrations of other interfering substances are 10 μM (containing 10 μM DA). The concentration of C2-F127 FONs is 10 μg/mL and Error bars are estimated from three replicate measurements.

respectively, which illustrate the acceptable precision and reproducibility for the biosensor.

Application analysis in 10% serum samples

In order to evaluate the feasibility of the sensing system in complex biological samples, the assay of DA was conducted in 10% serum samples, a real complex media containing a variety of proteins and other contaminants. As shown in Fig. 8a, upon the addition of DA, the fluorescence intensity of the as-prepared FONs (*ca.* 10 μg/mL) gradually decreases. When the DA concentration is lower than 10 μM, a linear fitting can be obtained for F/F_0 as a function of C_{DA} (Fig. 8a and 8b). Therefore, this turn-off biosensor displays satisfied sensitivity and selectivity for the detection of DA in 10% serum. Moreover, the calibration curves of the fluorescence to various concentrations of DA in Tris-HCl buffer and 10% serum are plotted and shown in Fig. 8b. It can be found that the sensitivity of this biosensor for DA in 10% serum is slightly lower than that in Tris-HCl buffer. This is probably owing to the interference of the biomacromolecules in the serum which act as coating substrates during the DA self-polymerization processes and thus slightly consume the DA in the serum samples.[22]

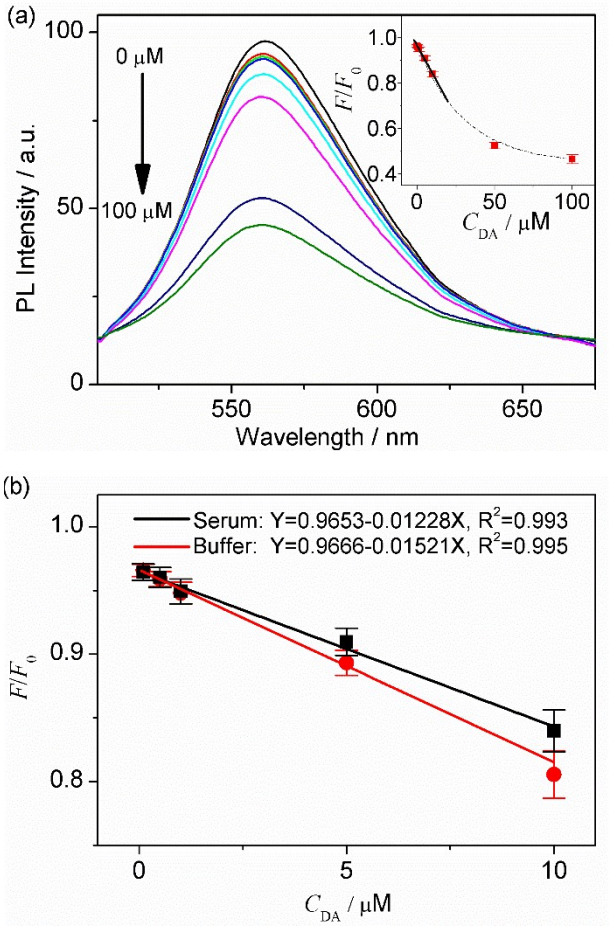


Fig. 8 (a) PL spectra of C2-F127 FONs (*ca.* 10 μg/mL) upon addition of DA with different concentrations in 10% serum. Inset illustrates the calibration curve of DA detected with C2-F127 FONs. (b) Calibration curves of fluorescence to various concentrations of DA in Tris-HCl buffer and 10% serum.

Conclusions

In conclusion, novel FONs based on an arbitrarily selected organic dye C2 and a triblock copolymer Pluronic F127 have been facilely synthesized. The as-prepared C2-F127 FONs are highly sensitive and selective to DA over other biomaterials, such as glucose, UA, AA, EP, and L-DOPA. The recognition of DA is owing to the formation of PDA covering on the surfaces of the FONs, which efficiently quenches the fluorescence of the FONs due to the PCT interactions between the organic dye molecules and the formed PDA. Moreover, C2-F127 FONs are exploited as label-free biosensor for detecting DA in 10% serum and satisfied sensitivity and selectivity are achieved. Therefore, these results suggest that the proposed strategy can be applied to DA detection in practical blood sample, which is of great importance in diagnosis and therapy of central nervous system related diseases.

Acknowledgements

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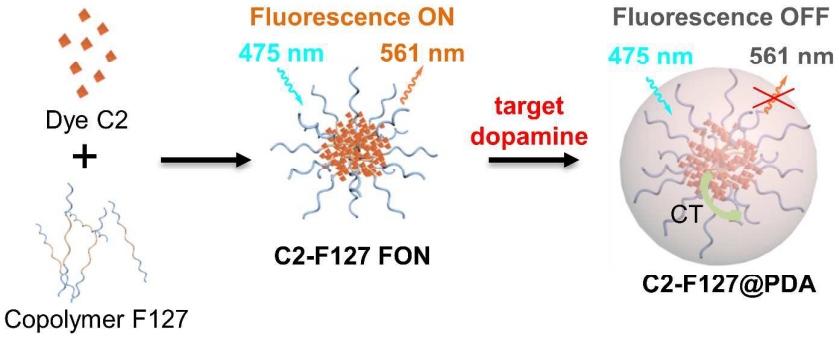

Fluorescent organic nanoparticles composed of organic dyes and triblock copolymers have been constructed and utilized as fluorescent biosensor for dopamine.