Switch-on fluorescent strategy based on N and S co-doped graphene quantum dots (N-S/GQDs) for monitoring pyrophosphate ions in synovial fluid of arthritis patients

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Abstract

This work reports a facile and effective approach for preparing graphene quantum dots co-doped with nitrogen and sulfur (N-S/GQDs). The proposed method comprised pyrolysis of citric acid with glutathione and dialysis treatment. The fabricated N-S/GQDs exhibited blue luminescence and a high fluorescence quantum yield of 36.3%. N-S/GQDs can be sensitively quenched by Fe^{3+} , which was absorbed on the edge of N-S/GQDs through the coordination interaction between Fe^{3+} and N, S elements. The subsequent introduction of pyrophosphate ion (PPi) resulted in the recovery of the fluorescence of Fe^{3+} -quenched N-S/GQDs. The stronger coordination reactivity between Fe^{3+} and PPi induced changes in fluorescence. PPi concentration was facilely quantified through the recovered amplitudes of the fluorescence intensities of N-S/GQDs. Under the optimized experimental condition, increased fluorescence intensity showed a linear relationship to PPi concentration (1 μM to 1000 μM) in serum samples. Additionally, the proposed method featured sensitive and selectivity for the detection of PPi in synovial fluid, suggesting the potential application for simple and accurate clinical diagnosis of arthritic diseases.

Keywords: Nitrogen and sulfur co-doped graphene quantum dots; Switch-on fluorescence; Pyrophosphate ion (PPi); Fe^{3+} ; synovial fluid

1. Introduction

Pyrophosphate ion ($\text{P}_2\text{O}_7^{4-}$, PPI) is an important biological metabolite because it participates in several biochemical reactions, such as ATP hydrolysis, DNA polymerization, and other metabolic processes [1,2]. Studies revealed that PPI level is related to the pathological processes of arthritis; therefore, qualitative and quantitative analyses of PPI can be used for differential diagnosis of diseases such as chondrocalcinosis or calcium pyrophosphate dihydrate (CPPD) crystal deposition [3]. High levels of PPI are present in the synovial fluid of patients suffering from CPPD and chondrocalcinosis [4]. Recent European League Against Rheumatism (EULAR) recommendation indicated that CPPD should be applied to the umbrella term of diseases, including acute calcium pyrophosphate (CPP) crystal arthritis, osteoarthritis with CPPD, and calcium pyrophosphate deposition (CPPD) may present different clinical phenotypes [5,6]. CPPD crystal sedimentation causes swelling of joints, such as knee, wrist, shoulder, and hip and calcium deposition in cartilago articularis, meniscus, synovial, and articular tissues [7]. Thus, development of a facile, selective, and sensitive strategy for monitoring PPI levels in synovial fluid is significant in clinical diagnosis and therapy of CPPD and other arthritic diseases.

Synovial fluid contains abundant and complex components, such water, mucinous proteins, hyaluronic acid, and saccharide-rich molecules.

During inflammation of joints, the chemical composition and cellular constituent of synovial fluid change. Determining these changes requires the selective and sensitive monitoring of clinically important species, such as PPI. Various technologies have been developed for PPI analysis, including fluorescent biosensors [8,9], colorimetry [10], and tandem mass spectrometry [11]; nevertheless, simple and specific methods for PPI monitoring must be developed to sustain the high demand for facile clinical diagnosis and sustainable arthritis therapy [12].

Switch-type sensing strategies, including fluorescent routine and colorimetric methods, have been widely used for special target analytes because of their sensitivity, specificity, visualization ability, and convenience [13–18]. Studies suggested that carbon nanomaterials can be used as a fluorescent probe for switch-on monitoring of ions and small molecules [19,20], especially for graphene quantum dots (GQDs) [21,22]. Various fluorescent methods provide optimal PPI sensing performance [12, 23], but their synthesis procedures are complicated; moreover, the selectivity of these methods for PPI in complex synovial fluid remains inadequate [9, 24].

This work proposes a technically simple yet sensitive and selective strategy for fluorescent monitoring of PPI in the synovial fluid of patients with arthritis. Citric acid was pyrolyzed in the presence of reduced glutathione (GSH) to prepare N and S co-doped graphene quantum dots

(N-S/GQDs). The possible assembly of Fe^{3+} on the surface of N-S/GQDs and the electron transfer effect can quench the fluorescence of N-S/GQDs [25,26]. Phosphate ions exhibit high affinity for metal ions through coordination [24], especially binding of multiposphates and Fe^{3+} through Fe-O-P bonds [25, 27]. We designed and developed a N-S/GQDs-based switch mediated by the Fe^{3+} quenching effect and triggered by the interaction of PPI with Fe^{3+} to reproduce fluorescence. Scheme 1 illustrates the switch-on mechanism of N-S/GQDs with Fe^{3+} for PPI assay in synovial fluids of arthritis patients. The current sensing technique exhibits potential for clinical diagnosis of arthritis.

2. Experimental

2.1. Reagents and Apparatus

Citric Acid (CA) were purchased from Tokyo chemical industry Co.,Ltd. (Japan). Rhodamine 6G, Cadmium chloride (CdCl_2), Nickelous sulfate (NiSO_4), and cobalt sulfate (CoSO_4) were purchased from aladdin industrial corporation (China). Glutathione (GSH), Adenosine triphosphate (ATP) and adenosine diphosphate (ADP) were purchased from Sigma-Aldrich Chemical Co. (USA). All other reagents of analytical reagent grade were purchased from Sinopharm Chemical Reagent Co., Ltd. (China), and were used without further purification. Ultrapure water obtained from a Millipore water purification system was used in all experiments.

Ultraviolet-visiblelight (UV-vis) absorption spectra were recorded in 1-cm path-length quartz cuvettes on UV-2250 in UV-vis spectrometer in the range of 200-600nm. Fluorescence spectra were collected using a Cary Eclipse FL12020M009 (Agilent Technologies, USA). The samples were excited at 346 nm and the fluorescence emission ranged from 350 to 550 nm, in steps of 5 nm. All UV-vis absorption and fluorescence measurements were performed at room temperature under ambient conditions. Fourier Transform Infrared Spectroscopy (FT-IR) was collected using a NICOLET iS50 Infrared Spectroscopy. (Thermo Fisher Scientific, USA). Atomic force microscopy (AFM) and transmission electron microscopy (TEM) images were obtained on a Bruker Multimode 8 SPM system in tapping mode and a JEOL JEM-1400 transmission electron microscope, respectively. XPS was performed on a Thermo ESCALAB 250XI.

2.2. Synthesis of N-S/GQDs

The N-S/GQDs was prepared according to previously described methods with special modifications [25]. Briefly, 2.0 g of Citric Acid (CA) was mixed with 0.6 g of Glutathione (GSH) in 5 mL beaker and heated to 200 °C using a heating mantle. About 5 min later, the mixture of CA and GSH was liquated. Subsequently, the color of the liquid was changed from colorless to pale yellow, and then brown in 15 min. The liquid was transferred into a beaker containing 100 mL deionized water with

continuous stirring. And then, the pH of the sample solution was modified to be pH 5 with NaOH. Herein, dialysis was chosen as the purification method for N-S/GQDs due to the characteristic of environmental friendliness, timeliness and cost-efficiency. The sample solution with pH 5 was dialysis with molecular weight cut-off of 2000 for 8 h, and then the purified N-S/GQDs stock solution with the concentration defined as 20 mg•mL⁻¹ (the 2.0 g mass of citric acid was divided by the volume of 100mL) was stored in darkness at 4 °C before use.

2.3. Switch-on fluorescent sensing for PPI

30 μ L of N-S/GQDs was mixed with an amount of Fe³⁺, and then, variable concentrations of pyrophosphate ion (PPI) were incubated with above solution for 30 min at 37 °C and the volume of mixture was adjusted to 300 μ L with Tris-HCl buffer (10 mM, pH 7.0). The final concentration of N-S/GQDs and Fe³⁺ was controlled to be 2 mg•mL⁻¹ and 500 μ M, respectively. Subsequently, the fluorescence intensity of the sensing solution was measured with excitation at 346 nm.

2.4. Analysis of PPI in human synovial fluid

Aliquots of 100-fold diluted human synovial fluid (30 μ L) were mixed 30 μ L of N-S/GQDs and 500 μ M Fe³⁺ for 30 min at 37°C, and then the fluorescent intensity was recorded. The concentration of PPI in synovial fluid was seen as C_{Sample}. Subsequently, the synovial fluid spiked with constant concentration of PPI. The adding standard samples were

treated same as above. The concentrations of standard additions were seen as C_{SS} . Each C_{Sample} and C_{SS} was tested in triplicate.

2.5. MTT testing

Briefly, 180 μ L of HL-60 cell lines which account for about 6000 per wells was added to 96 well plates, and then mixed with 20 μ L different concentration of N-S/GQDs and N-S/GQDs containing 500 μ M Fe^{3+} at 37°C in the environment of 5% CO_2 . After incubated for 24 h, 20 μ L 5mg $\cdot mL^{-1}$ MTT was added into each well in the plate and incubated for 4 h in 37°C and 5% CO_2 . After centrifuging at 4600 rpm for 8min, remove the supernatant from every well. 100 μ L DMSO was added to each well to extract and solubilize the formed azan crystal in 20 min. Finally, the absorption of the plate was tested at 570 nm wavelength. Each compound and control was operated in triplicate. The percentage of proliferation was calculated by the following formula: Proliferation (%) = $[(OD_{sample} - OD_{control}) / OD_{control}] * 100$.

3. Results and discussion

3.1 Characterization of N-S/GQDs

N-S/GQDs were fabricated through pyrolysis of citric acid in the presence of GSH at 200 °C; this temperature is lower than previously reported pyrolysis temperature [25]. The morphology of N-S/GQDs was investigated. The TEM image shows that the as-prepared N-S/GQDs exhibited uniform size, with an average diameter of 3 nm, and were well

monodispersed (Fig. 1A). The AFM results indicated that topographic heights of N-S/GQDs were about 1.5 nm (Fig. S1), suggesting that the prepared N-S/GQDs contain about two graphene layers. In addition, XPS measurement was performed to probe the composition of N-S/GQDs. Fig. 1B shows that N, S, C, and O were present in the XPS survey spectrum of N-S/GQDs; hence, N and S were successfully co-doped into the as-prepared GQDs. The atomic percentages of N, S, C, and O were found to be 7.93%, 2.16%, 59.32%, and 30.57%, respectively. The main peak at 399.8 eV of the high-resolution N1s spectrum in Fig. S2A indicates that N was doped into the prepared N-S/GQDs through the formation of N-C bonds [26, 28]. The high-resolution S2p spectrum of N-S/GQDs in Fig. S2B shows two peaks at 164.3 and 163.2 eV; these peaks are consistent with the -C-S-C- covalent bond of thiophene-like S in the prepared quantum dots [29]. This result confirms the doping effect of S in N-S/GQDs. Moreover, the C1s spectrum (Fig. S2C) revealed the presence of C=C/C-C (284.5 eV) in the aromatic ring, as well as C-N/C-S (285.8 eV) and C=O (288.2 eV), thereby confirming the doping effect of N and S in the synthesized N-S/GQDs through facile pyrolysis. Furthermore, the main peak of the C1s spectrum at a low binding energy (284.5 eV) demonstrated the reduction of GQDs and proved that doping of N and S mainly occurred during pyrolysis of citric acid in the presence of GSH [28]. The chemical components of N-S/GQDs confirmed by XPS results

were in accordance with the FT-IR results (Fig. S3).

UV-vis and fluorescence techniques were used to investigate the optical properties of N-S/GQDs. As shown in Fig. 1C, N-S/GQDs exhibited two absorption peaks centered at 215 and 330 nm, which correspond to the π - π^* transition of C=C and n- π^* transition of C=O, respectively [29,30]. Under the 346 nm excitation, the N-S/GQDs solution exhibited strong purple-blue fluorescence centered at 418 nm. Additionally, the fluorescence quantum yield (QY) of the prepared N-S/GQDs was about 36.3% with rhodamine 6G as the calculated reference standard. N-S/GQDs prepared through pyrolysis of citric acid in the presence of GSH exhibited properties similar to previously reported GQDs [29]. The high QY of the prepared N-S/GQDs was due to the doping effect of heteroatoms and the special defining features, which regulate the fluorescence property [31]. Furthermore, the maximum emission wavelength red shifted to about 2 nm with excitation from 330 nm to 380 nm, illustrating the nearly excitation-independent fluorescent property (Fig. 1D). As previously reported [25, 32], photoabsorption and photoluminescence peaks can red shift with increasing particle size; thus, a small red shift range suggests a uniform particle size distribution [33]. Therefore, we can presume that the prepared N-S/GQDs possess a homogeneous particle size, which agree well with the TEM results. The stability of N-S/GQDs was also investigated through long-term

experiment (Fig. S4). The fluorescence response of N-S/GQDs can maintain 93% of the original intensity at the end of a 90-day period when stored at 4 °C, indicating the excellent stability of N-S/GQDs.

3.2 Mechanism for the sensing strategy of PPI by using N-S/GQDs combined with Fe^{3+}

As shown in Fig. 2A, the addition of Fe^{3+} into the dispersion of the N-S/GQDs led to an observable quenching intensity. However, the TEM images (Fig. S5) revealed that the introduction of Fe^{3+} did not cause a considerable aggregation of N-S/GQDs, which differed from the Ni^{2+} -treated carbon quantum dots [34]. These results revealed that N-S/GQDs can absorb Fe^{3+} on their edges through the interaction of Fe^{3+} with N and S, which is caused by C-N and C-S groups in N-S/GQDs and the strong coordination affinity of Fe^{3+} [26, 29]. Fe^{3+} sensitively quenches the fluorescence of N-S/GQDs through electron transfer facilitated by the interaction between N-S/GQDs and Fe^{3+} [25, 26, 29]. As depicted in Fig. 2A, the addition of PPI into the N-S/GQD solution containing Fe^{3+} allowed the fluorescence to substantially recover from quenching. In this state, PPI can protect N-S/GQDs from interacting with Fe^{3+} ; this interaction is stronger than the interaction of Fe^{3+} with C-N and C-S groups in N-S/GQDs. With the formation of the Fe^{3+} -PPI complex, the protected N-S/GQDs simultaneously switched on the remarkable fluorescence; thus, the recovered fluorescent intensity can be used to

quantify the concentration of PPI.

The UV-vis spectra of different testing processes were determined to verify the sensing mechanism. As shown in Fig. 2B, the specific absorption peaks of N-S/GQDs remained constant whether PPI was present or not, suggesting that PPI alone did not affect the optical property of N-S/GQDs. With the addition of PPI, the characteristic absorption peak of Fe^{3+} at 293 nm blue shifted to 260 nm, suggesting that the coordination reaction between Fe^{3+} and PPI formed a strong Fe^{3+} -PPI complex. Interestingly, the absorption band of Fe^{3+} almost disappeared with the addition of Fe^{3+} into N-S/GQDs alone. The absorption band of N-S/GQDs was also enhanced and red shifted from 338 nm to 341 nm. The changeable absorption behavior of N-S/GQDs and Fe^{3+} could be due to the adsorption and electron transfer between the dots and Fe^{3+} [26, 29]. With further addition of PPI, the new absorption band centered at 285 nm appeared. Compared with the absorption curve of Fe^{3+} -PPI system, the appearance of the new band confirmed the formation of the Fe^{3+} -PPI complex. Additionally, the absorption band centered at 340 nm and ascribed to the $n\text{-}\pi^*$ transition of C=O of N-S/GQDs remained but exhibited reduced intensity. This change in absorption suggested that PPI can detach Fe^{3+} from the N-S/GQDs through the strong coordination interaction between PPI and Fe^{3+} and the formation of a stable Fe^{3+} -PPI complex. The UV-vis spectra confirmed

that PPI can considerably change the optical properties of N-S/GQDs in the presence of Fe^{3+} , which agrees with the fluorescence recovery results of N-S/GQDs in Fig. 2A and the monodispersed morphology dots in Fig. 1A.

The time-resolved fluorescence decay of N-S/GQDs at different conditions was discussed to clearly understand the sensing mechanism of PPI. As shown in Fig.S6, the average lifespan of N-S/GQDs is 7.7 ns, which decreased to 6.1 ns after reacting with Fe^{3+} . The reduced lifespan revealed that the quenching mechanism was due to the ultrafast electron-transfer process between N-S/GQDs and Fe^{3+} [35]. However, the lifespan of N-S/GQDs increased to 7.5 ns with further addition of PPI, which indicated that PPI can disassociate Fe^{3+} from N-S/GQDs through strong interactions.

3.3 Optimization of sensing conditions

Fluorescence recovery as a function of time was monitored to investigate the kinetic behavior of PPI upon reacting with N-S/GQDs containing Fe^{3+} . In this study, several temperatures with different reaction times were surveyed to investigate the reaction behavior, as shown in Figs. 3A to 3F. With increasing reaction temperatures, the required time for the recovered fluorescence to plateau was gradually shortened, as shown in Fig. 3G. The recovered fluorescence intensities usually reach the maximum values in different reaction temperatures. When immobilization

time was controlled at 30 min, the recovered fluorescence intensity increased, then decreased, and eventually reached the strongest fluorescence intensity at 37 °C (Fig. 3G). Thus, 37 °C and 30 min were selected as the optimal condition for PPI sensing through N-S/GQDs containing Fe^{3+} .

In the proposed sensing strategy, the coordination interaction between PPI and metal ions was introduced to switch on the quenched N-S/GQDs by using metal ions. Thus, finding a suitable metal ion with the highest N-S/GQD-quenching efficiency is important. The C-N and C-S bonds in the structure of N-S/GQDs can serve as ligands for variable metal cations; thus, the quenching effect of different cations (500 μM) was compared in Fig. 4A. Fe^{3+} can almost thoroughly quench the fluorescence intensity of N-S/GQDs, whereas the N-S/GQD-quenching efficiency of other common metal cations is negligible. As a result, Fe^{3+} was selected in this work for PPI sensing.

The quenching process of N-S/GQDs by Fe^{3+} was investigated. Fig. 4B shows that the fluorescence of N-S/GQDs gradually decreased with increasing amounts of Fe^{3+} . Fluorescence intensity reached the minimum platform stage when the concentration of Fe^{3+} reached 500 μM , as shown in the inset of Fig. 2A. As a result, 500 μM Fe^{3+} was used as the effective condition for PPI quantification.

3.4 Sensing performance of PPI based on N-S/GQDs

The selectivity of the proposed method toward different kinds of species that can possibly interfere with the detection of PPI was investigated. As illustrated in Fig. 5, separate addition of anions did not change the fluorescence obviously, whereas the fluorescence for PPI testing was significantly increased. These results suggest that common anions did not interrupt PPI sensing in the current method. Furthermore, the interference of similar species, such as ATP and ADP, on the PPI assay is considered negligible, which was indicated by the low fluorescence changes upon ATP or ADP addition.

Under the optimized experimental conditions, the fluorescence recovery of N-S/GQDs containing 500 μM Fe^{3+} with different PPI concentrations was measured. As shown in Fig. 6A, the fluorescence intensity of N-S/GQDs was gradually restored when the concentration of PPI added to the sensing system was increased from 0 μM to 1000 μM . Fig. 6B presents the relationship between the recovered fluorescence value (ΔF) and the concentration of PPI. In the range of 1–1000 μM , the linear equation is given by $\Delta F = 0.4818 X + 2.491$ ($R^2 = 0.9937$) ($\Delta F = F_{\text{ppi}=x\mu\text{M}} - F_{\text{ppi}=0\mu\text{M}}$, while $F_{\text{ppi}=x\mu\text{M}}$ refers to the fluorescent intensity of N-S/GQDs containing 500 μM Fe^{3+} after reacting with different concentrations of PPI, $F_{\text{ppi}=0\mu\text{M}}$ refers to the fluorescent intensity of N-S/GQDs containing 500 μM Fe^{3+} without PPI), where X is the concentration of PPI (μM). Based on the calculations of $3\sigma/S$, the limit of

detection was obtained at 0.81 μM .

The possible practical application of the proposed fluorescence sensor for clinical PPI sensing was tested. Diluted fetal calf serum was used to simulate a biological environment to investigate whether the complex system can interrupt the selected sensing of PPI or not. As shown in Fig. 6C, the fluorescence intensities of the recovered GQDs gradually increased with increasing PPI concentration increased. The increase in ΔF also exhibited a good linear relationship within 1–1000 μM with the calibration plot of $\Delta F = 0.3303X + 34.42$ ($R^2 = 0.9948$) ($\Delta F = F_{\text{ppi}=x\mu\text{M}} - F_{\text{ppi}=0\mu\text{M}}$, while $F_{\text{ppi}=x\mu\text{M}}$ refers to the fluorescent intensity of N-S/GQDs containing 500 μM Fe^{3+} after reacting with different concentrations of PPI, $F_{\text{ppi}=0\mu\text{M}}$ refers to the fluorescent intensity of N-S/GQDs containing 500 μM Fe^{3+} without PPI), where X is the concentration of PPI in the diluted serum. The results illustrate that the current method can be used for simple and accurate measurement of PPI in practical biological samples.

3.5 Determination of PPI in synovial fluid

PPI sensing in human synovial fluid was performed to investigate the validity of the proposed method. The fluorescence intensity before and after the addition of diluted synovial fluid in N-S/GQDs system containing 500 μM Fe^{3+} was measured. PPI concentration was calculated from the corresponding measured value by using the linear equation $\Delta F =$

$0.4818X + 2.491$ ($R^2 = 0.9937$). Moreover, the recovery of the standard PPI addition was tested through the same process. As shown in Table 1, the detected PPI levels in synovial fluids of arthritis patients fluctuated from 1.10 mM to 4.12 mM. The recoveries of addition of 100 μ M PPI of the proposed assay of PPI in synovial fluids were also satisfactory, illustrating the high precision of the developed fluorescence strategy. The detection of PPI levels in real synovial fluids of arthritis patients demonstrates that the proposed fluorescence assay is a promising PPI-monitoring strategy for laboratory medicine.

3.6 MTT assay

The excellent optical performance encouraged us to investigate the cell cytotoxicity of N-S/GQDs and N-S/GQDs with Fe^{3+} using a MTT assay [32]. To determine the toxicity, the viabilities of HL-60 cell lines treated with N-S/GQDs and N-S/GQDs containing Fe^{3+} were compared (Fig. S7). The viability of the cells remained higher than 85% despite their incubation with high concentrations N-S/GQDs and N-S/GQDs containing Fe^{3+} for 24 h. Furthermore, the cell viability of N-S/GQDs containing Fe^{3+} was higher than that of single N-S/GQDs, suggesting the low toxicity of the fabricated N-S/GQDs containing Fe^{3+} for PPI testing.

Conclusion

An economical, simple, and efficient method for the preparation of N-S/GQDs with high QY was developed. The introduction of GSH in the

fabrication of N-S/GQDs served as the source of N and S. Moreover, the abundance of C-N and C-S groups on the surface of N-S/GQDs allowed the coordination between Fe^{3+} and N-S/GQDs, resulting in sharp fluorescence quenching. The fluorescence recovery of N-S/GQDs was used to quantitatively evaluate PPI concentration because the formation of complexes between Fe^{3+} and PPI was facilitated by the addition of PPI. Furthermore, the proposed method can be applied to accurately detect PPI concentration in synovial fluid of arthritis patients.

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Figures and Figure captions

Scheme 1. Schematic illustration of switch-on fluorescent strategy for PPI sensing based on N-S/GQDs containing Fe^{3+} .

Figure 1.(A) TEM image of prepared N-S/GQDs, (B) XPS survey and (C) UV-vis absorption (red), fluorescence excitation (black) and emission(blue) spectra of N-S/GQDs, (D) The emission curves of N-S/GQDs with the different excitation wavelengths. Insert of (C) shows the fluorescence image of N-S/GQDs.

Figure 2. (A) Fluorescence spectra of pure N-S/GQDs, N-S/GQDs containing Fe³⁺, and N-S/GQDs containing Fe³⁺ with different concentration PPi. (B) UV-vis spectra of pure N-S/GQDs, N-S/GQDs containing Fe³⁺, N-S/GQDs containing Fe³⁺ with PPi, solution containing Fe³⁺ and PPi, pure Fe³⁺, pure PPi, N-S/GQDs and PPi.

Figure 3. The effect of temperature to the recovered fluorescence intensity of the addition of PPI to N-S/GQDs containing Fe^{3+} as a function of time (from A to F) , A: 25°C; B:30°C; C:37°C; D:40°C; E:45°C; F:50°C. (G) The changeable fluorescence intensity of N-S/GQDs as a function of temperature.

Figure 4. The quenching effect of different metal ions with the concentration of 500 μM (A) and different concentrations of Fe^{3+} (B) to N-S/GQDs. Inset of B shows the trend of the fluorescence intensity of N-S/GQDs as a function of Fe^{3+} .

Figure 5. The recovered fluorescence intensity of N-S/GQDs containing $500 \mu\text{M Fe}^{3+}$ after reacting with PPI ($500\mu\text{M}$), ATP ($500\mu\text{M}$) and ADP ($500\mu\text{M}$), and other interfering common anions ($1000 \mu\text{M}$).

Figure 6. The recovered fluorescence intensities ($\Delta F = F_{\text{PPI}=\text{x}\mu\text{M}} - F_{\text{PPI}=0\mu\text{M}}$) (A, C) and calibration curves (B, D) of N-S/GQDs containing 500 μM Fe^{3+} after reacting with different concentrations of PPI in Tris-HCl (A, B) or 10% diluted serum with Tris-HCl (C, D).

Table 1. The sensing results and the recovery of PPI in diluted synovial fluid of arthritis patients

Samples	C _{Sample} ^a (μM)	C _{added} (μM)	C _{SS} ^a (μM)	Recovery (%)
1	41.2±0.68	100	140.4±1.09	99.1
2	38.7±0.36		129.6±0.34	90.9
3	31.2±0.30		130.7±2.54	99.5
4	36.3±1.82		137.6±1.82	101.4
5	11.0±0.29		110.2±0.97	99.1
6	38.0 ±2.54		145.1±1.94	107.1
7	39.5±0.86		132.6±1.38	93.1
8	38.2±0.70		151.8±1.31	113.6
a: the data stood the average value of three independent testing				