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Supplementary Information for

Fluorescence probe for the selective detection of creatinine in aqueous buffer applicable to human blood serum

Siddhartha Pal,[a] Somenath Lohar,[a] Manjira Mukerjee,[a] Pabitra Chattopadhyay,*[a] and Koushik Dhara*[b]

[a] Prof. P. Chattopadhyay, S. Pal, S. Lohar, M. Mukherjee

Department of Chemistry, The University of Burdwan, Golapbag, Burdwan 713104, West Bengal, India.

E-mail: pabitracc@yahoo.com (P.C.)

[b]Dr. K. Dhara

Department of Chemistry, Sambhu Nath College, Labpur, Birbhum 731303, West Bengal, India.

E-mail: dharachem@gmail.com (K.D.)

Materials and methods:

1. Materials and physical methods: All chemicals and reagents were purchased from Sigma and used without further purification. Solvents used for spectroscopic experiments were purified and dried by standard procedures. Fluorescence and absorption spectra were performed using a HITACHI F-4600 Fluorescence Spectrophotometer and Shimadzu 2450 UVSpectrophotometer respectively. Electron spray ionization (ESI) mass spectra were recorded on a Thermo Scientific Exactive Plus Mass Spectrometer. NMR spectra of organic compounds were obtained on a Bruker Advance DPX 400.

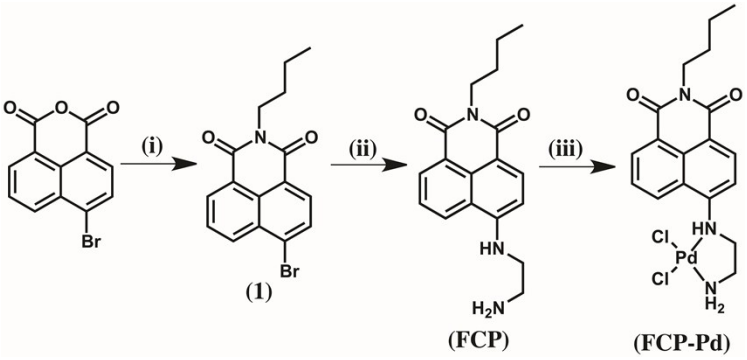
2. General method of fluorescence analyses and absorption studies: FCP-Pd was dissolved in acetonitrile to obtain stock solutions for fluorimetric and absorption titration studies. Desired volume of acetonitrile stock was taken to dilute in 10 mM PBS buffer (0.5 % CH_3CN) of pH 7.2 at 37 °C to reach the final concentration (10 μM) of probe. The fluorescence spectra of FCP-Pd were recorded at an excitation wavelength of 440 nm.

3. HPLC analyses: Reversed-phase HPLC analysis was performed using Agilent 1200 Infinity series with C18 column. HPLC experiments were carried out with an increasing ratio of buffer B (0.1% CH_3COOH in acetonitrile) to buffer A (0.1% CH_3COOH in H_2O). All samples were evaluated by increasing the amount of buffer B from 20 to 90% over 20 min.

4. Collection of real samples for analyses: To establish the application of our newly designed probe, FCP-Pd, to biological samples, a number of different real blood samples were analyzed using the present method.4 ml of blood samples were collected from healthy individuals cooperated by M/S. S. Banerjee Diagnostic Centre (Baburbag, Burdwan, West Bengal, India). Blood samples were collected by percutaneous puncture using a 5 ml sterile syringe attached to a 20 gauge needle and blood samples were centrifuged at 2000 rpm prior to analysis. The supernatant was used and were stored at 4 °C immediately. Serum samples were used as denatured serum after the heat treatment at 90 °C for 5 mints to minimize the unwanted protein probe interactions.[1,2]

LIVE SUBJECT STATEMENT: All experiments were performed in compliance with the relevant laws and institutional guidelines, and also the institutional committee(s) have approved the experiments. The consent was obtained by us for performing the experiments with human subjects.

5. Synthesis of FCP-Pd (Scheme S1): Compound 1 and FCP were synthesized using reported procedures.[3] A solution of 0.095 g $Pd(CH_3CN)_2Cl_2$ (0.37 mmol) in acetonitrile (20 ml) is added drop wise to a 0.156 g FCP (0.50 mmol) already dissolved in CH_2Cl_2 (20 ml). The solution mixture was then heated to 50 °C for few minutes and allowed to stir for another 2 h. The reaction mixture was filtered and the filtrate was taken for next few days. The precipitated compound was washed with cold dichloromethane and then dried *in vacuo* to perform the characterization (calculated yield 37%).^{1}H NMR (400 MHz, CD_3CN):δ 0.89-0.93 (t, 3H), 1.28-1.37 (m, 2H), 1.56-1.60 (t, 2H), 3.57-3.72 (m, 4H), 4.00-4.03 (t, 2H), 6.86-6.91 (m, 1H), 7.68-7.76 (m, 1H), 7.82 (s, 1H), 8.26-8.32 (m, 1H), 8.43-8.48 (m, 1H), 8.62-8.66 (t,1H) [Fig. S1]. ^{13}C NMR (100 MHz, d_6-DMSO):δ 13.8, 19.9, 29.9, 42.5, 43.3, 104.1, 108.3, 120.3, 121.9, 124.5, 128.6, 129.4, 130.8, 134.2, 150.4, 163.1, 163.9 [Fig. S2]. HRMS (m/z) found 528.0501 Calculated for $[M+CH_3CN]^{\cdot+}$ 528.0311 [Fig. S3].



Scheme S1 Synthetic routes to obtain FCP-Pd. (i) n-butylamine, glacial acetic acid, reflux 6 h; (ii) *ethylenediamine*, DMF: *N,N-dimethylformamide*, reflux 5 h; (iii) $Pd(CH_3CN)_2Cl_2$, CH_3CN-DCM.

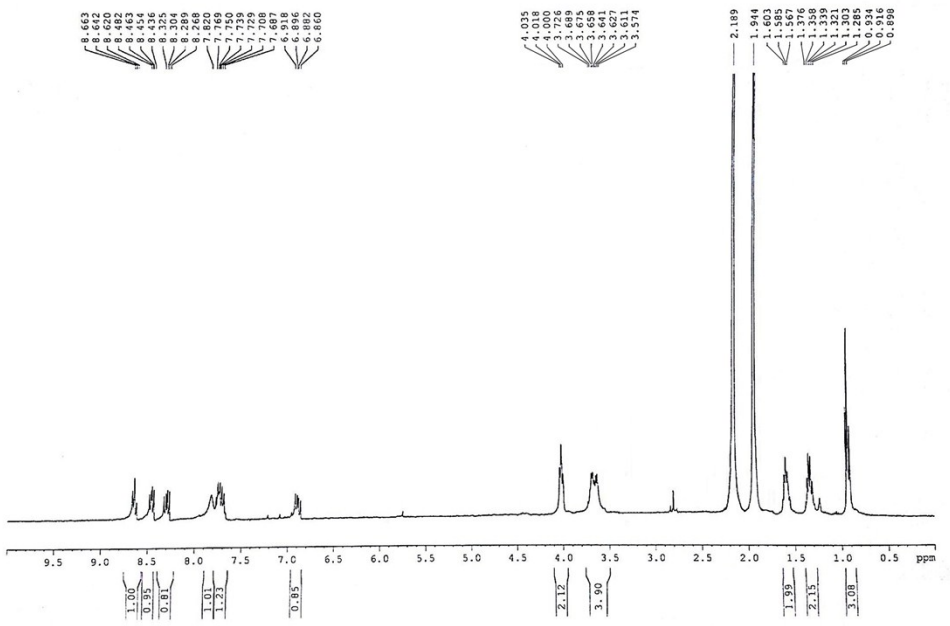


Fig. S1 ^{1}H NMR data of FCP-Pd in CD_3CN.

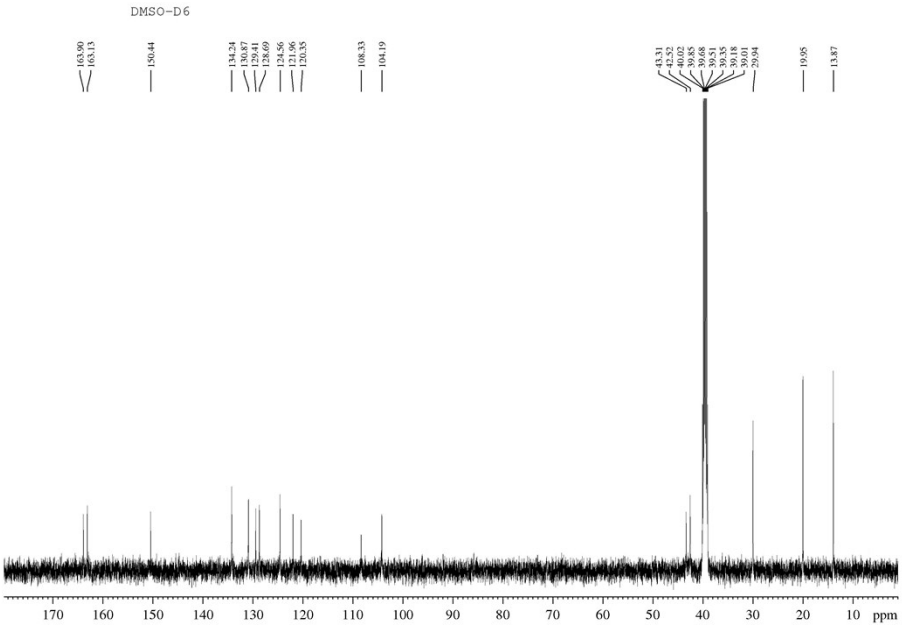


Fig. S2 ^{13}C NMR data of FCP-Pd in d_6-DMSO.

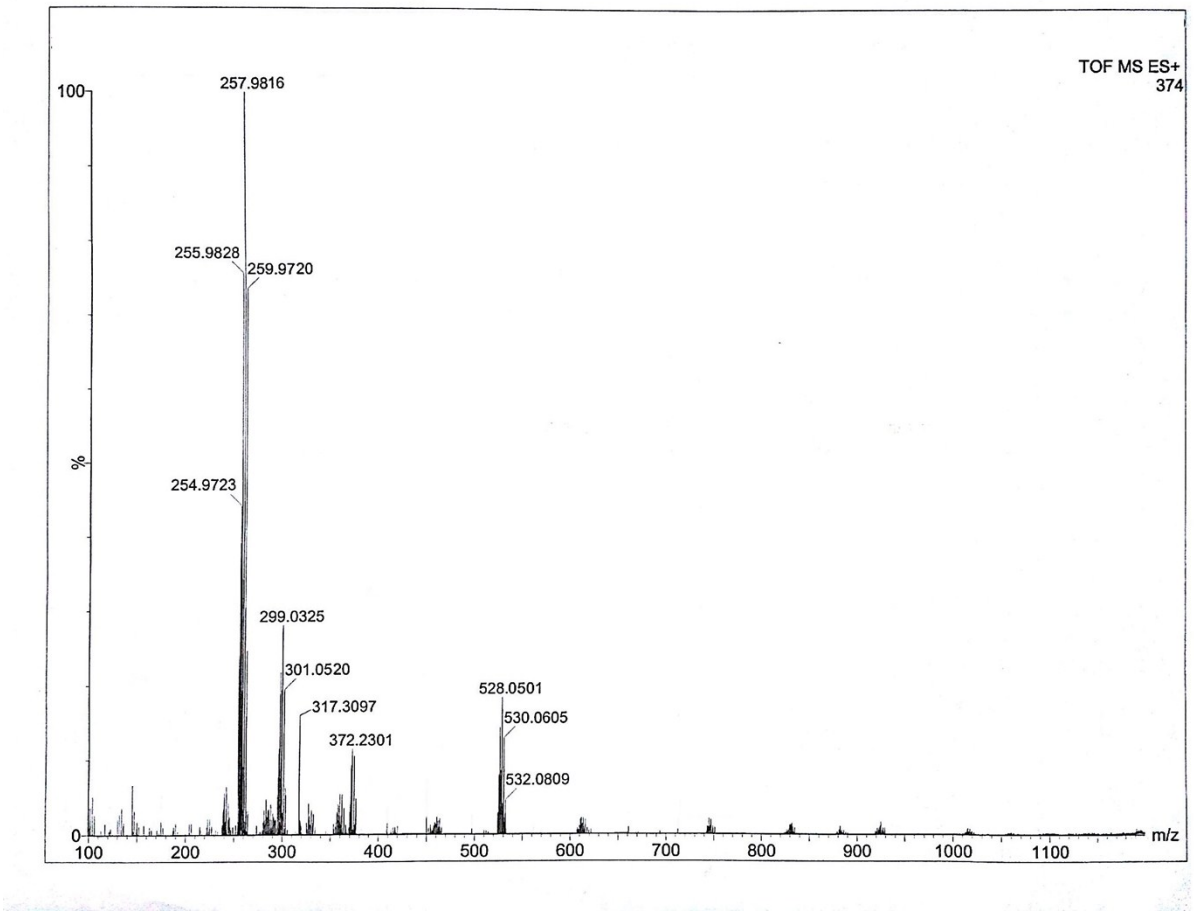


Fig. S3 HRMS data of FCP-Pd.

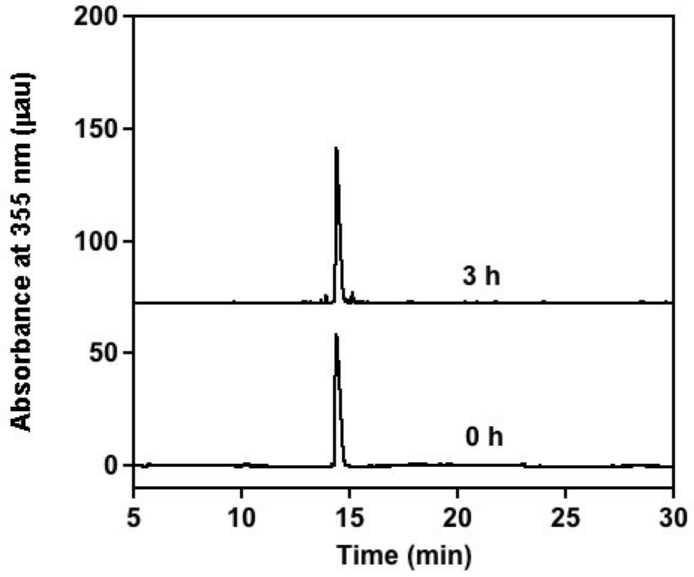


Fig. S4 Reversed-phase HPLC analyses of the stability of the probe, FCP-Pd, was incubated for 3 h in the reaction buffer [10 mM PBS, 0.5 % CH_3CN] of pH 7.2 at 37 °C.

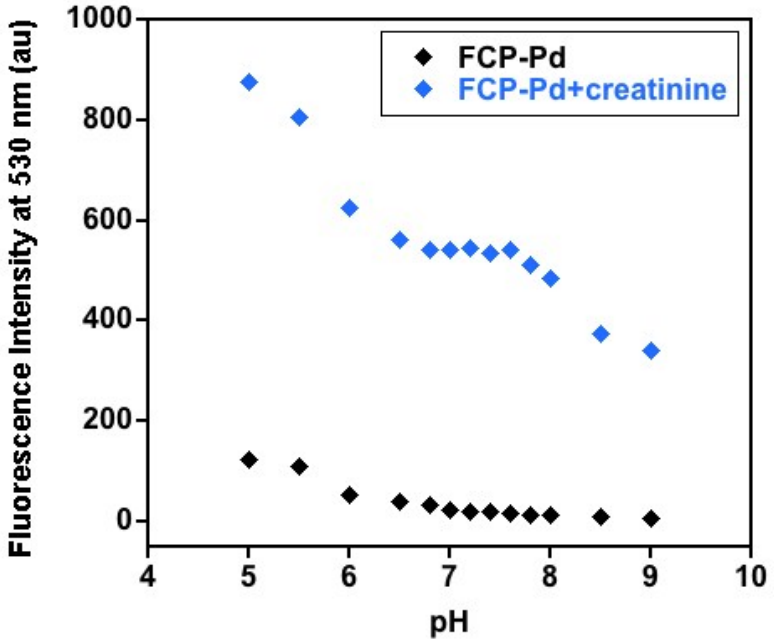


Fig. S5 Fluorescence responses at 530 nm of FCP-Pd (10 μM) to creatinine (300 μM) incubated for 30 min in reaction buffer of various pH at 37 °C (ex: 440 nm).

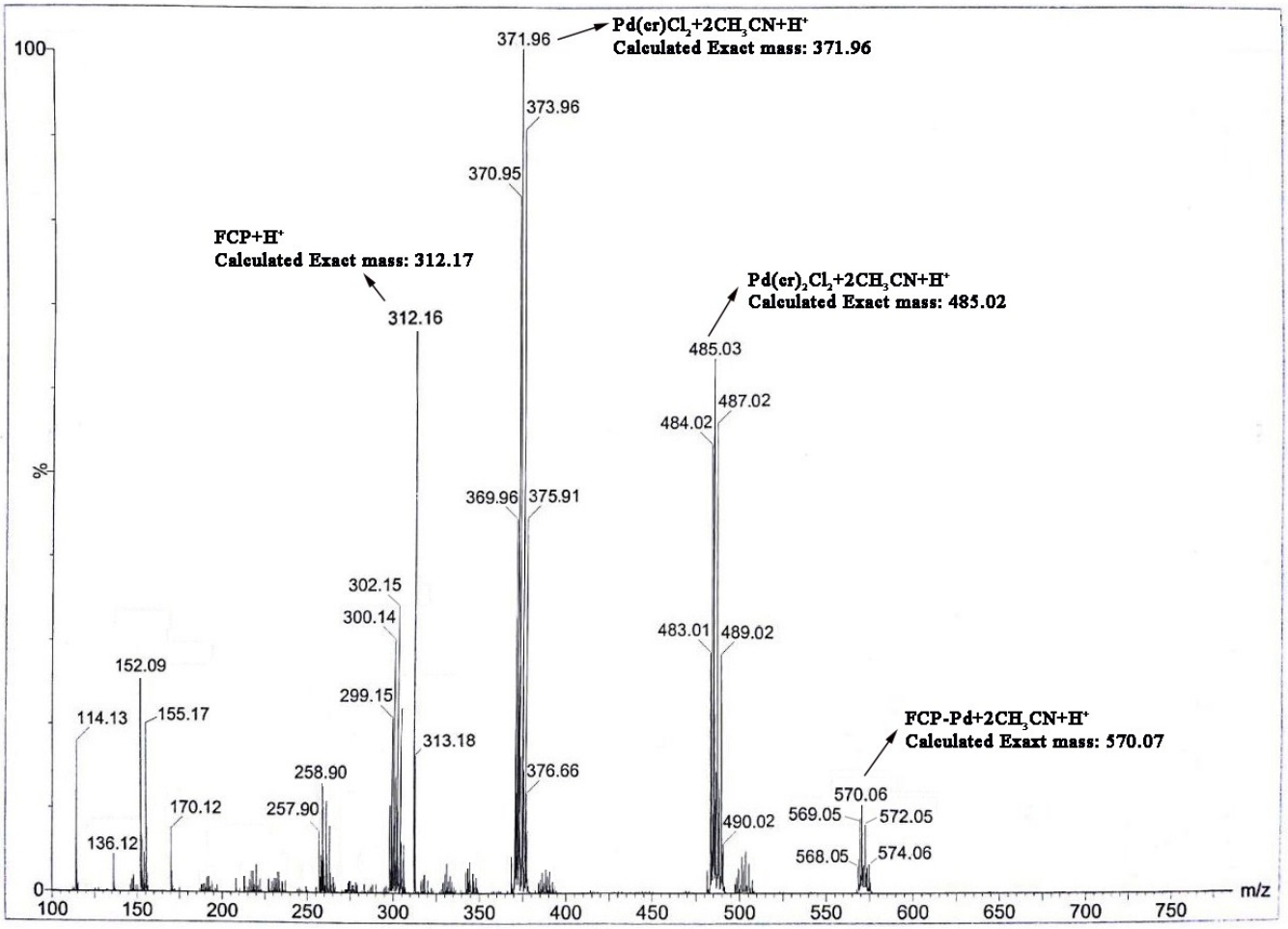


Fig. S6 ESI-MS spectrometry analysis of the reaction mixture of FCP-Pd and creatinine, confirms the presence of $Pd(cr)_2Cl_2$ complex and the formation of free ligand FCP.

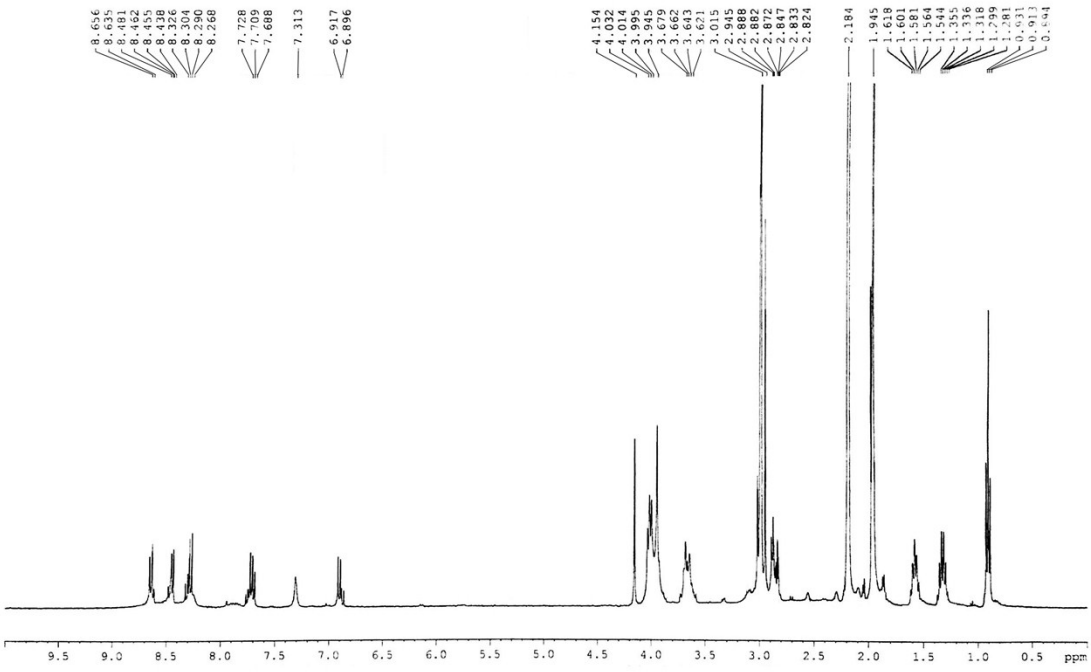


Fig. S7 ^{1}H NMR study of reaction mixture of FCP-Pd and creatinine.

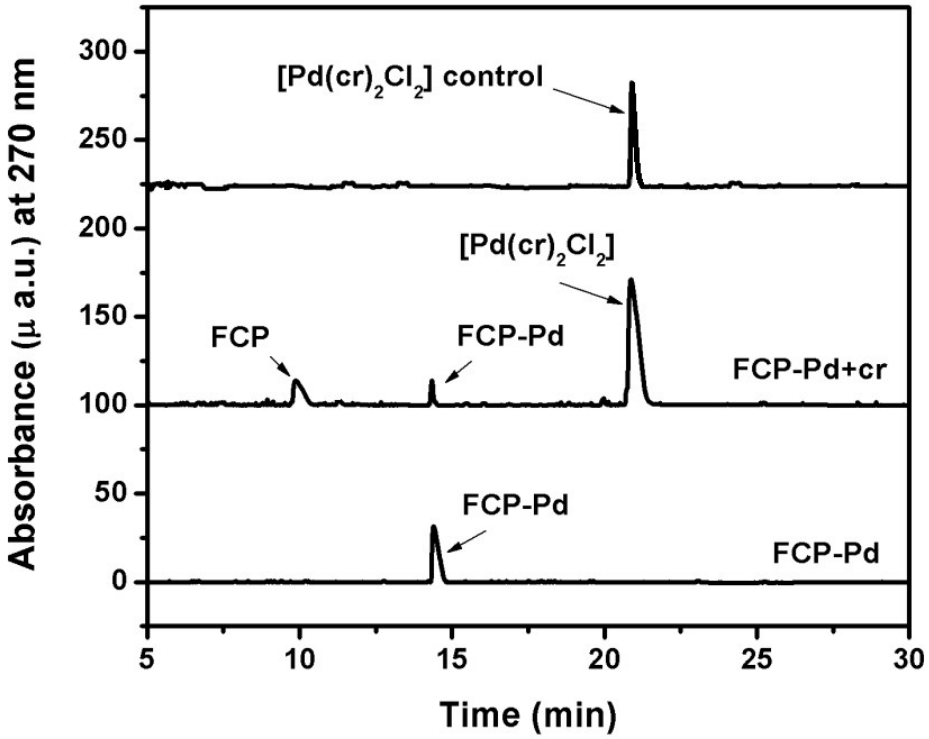


Fig. S8 Reversed-phase HPLC analyses of the reaction progress of FCP-Pd with creatinine in reaction buffer of pH 7.2 at 37 °C (characteristic absorbance band of $Pd(cr)_2Cl_2$ at 270 nm).

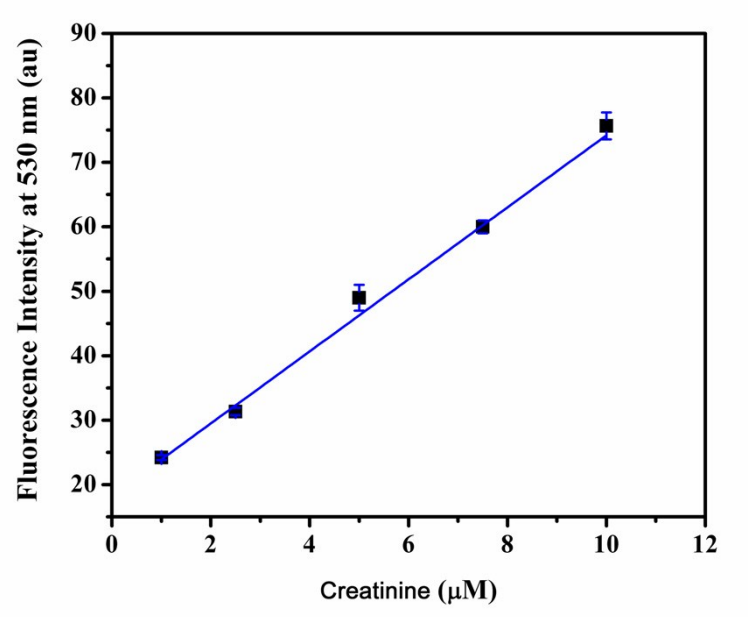


Fig. S9 Plot for the determination of limit of detection (LOD) for creatinine.

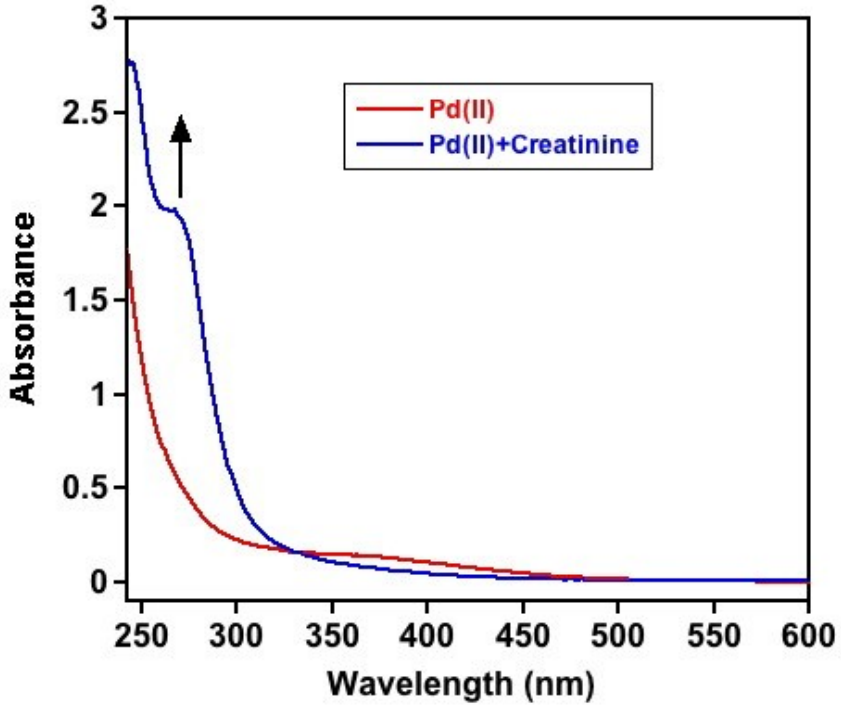


Fig. S10 UV-vis absorption spectra of $PdCl_2$ and $PdCl_2$ with creatinine (1:1) incubated at 37 °C in reaction buffer.

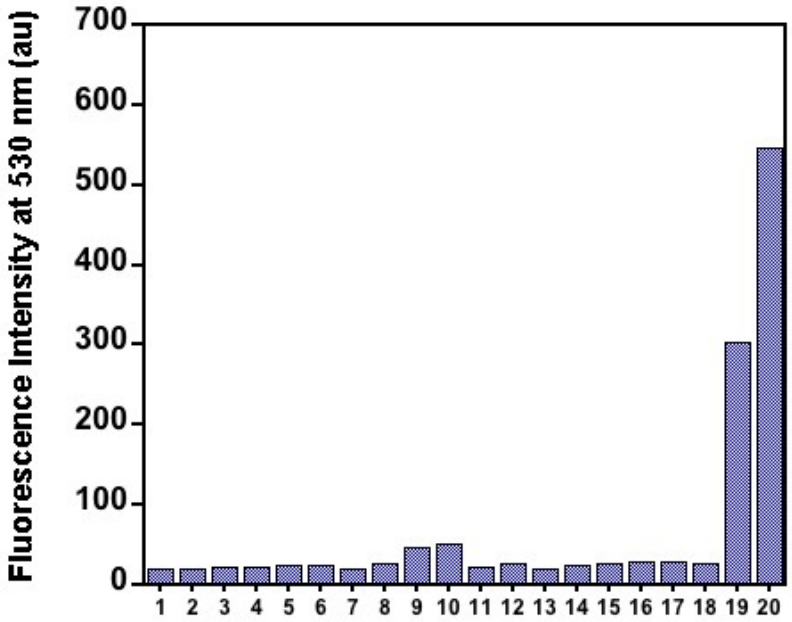


Fig. S11 The fluorescence intensity (at 530 nm) change profile of FCP-Pd (10 μM) in presence of some interfering metal ions and/or anions and various biologically important species incubated for 30 min in reaction buffer of pH 8.0 at 37 □C (ex: 440 nm).(1) None, (2)Ca^{2+}, (3) Mg^{2+}, (4) Fe^{3+}, (5) Zn^{2+}, (6) I^{-}, (7) NO_3^{-}, (8) PO_4^{3-}, (9) histidine, (10) proline, (11) uric acid, (12) urea, (13) thiourea, (4) creatine, (15) glucose, (16) fructose, (17) lactose, (18) cholesterol, (19) 100 μMcreatinine and (20) 300μMcreatinine.

Table S1 Determination of creatinine in different human blood serum samples using FCP-Pd incubated at 37 °C for 30 min and existing colorimetric Jaffe method.

Serum samples	Creatinine estimated by FCP-Pd (μM)	Using existing colorimetric Jaffe method
Sample01	2.50	2.32
Sample02	3.01	2.85
Sample03	3.71	3.58
Sample04	4.32	4.25
Sample05	5.10	5.13
Sample06	89.22 (normal level)	89.29 (normal level)
Sample07	190.53 (injurious kidney condition)	190.68 (injurious kidney condition)

Table S2 Recovery of creatinine by adding standard amount of 30 μM creatinine to different human blood serum samples using two methods

Serum samples	Creatinine estimated by FCP-Pd (μM)		Using existing colorimetric Jaffe method	
	Total creatinine after addition (μM)[a]	Recovery (%)	Total creatinine after addition (μM)[a]	Recovery (%)
Sample01	32.2 ± 0.2	99.00	28.5 ± 0.3	88.18
Sample02	32.6 ± 0.3	98.76	29.1 ± 0.2	88.58
Sample03	33.2 ± 0.2	98.51	30.5 ± 0.4	90.83
Sample04	33.9 ± 0.3	98.77	31.6 ± 0.3	92.26
Sample05	34.8 ± 0.4	99.14	34.4 ± 0.2	97.92
Sample06	114.6 ± 0.3	96.12	115.9 ± 0.3	97.16
Sample07	209.2 ± 0.3	94.86	211.5 ± 0.2	95.84

[a]Average of three replicate experiments

References:

1 R. Wetzel, M. Becker, J. Behlke, H. Billwitz, S. Bohm, B. Ebert, H. Hamann, J. Krumbiegel and G. Lassmann, *Eur. J. Biochem.*1980, **104**, 469-478.

2 V. Raikos, *Food Hydrocolloides* 2010*,* **24**, 259-265.

3 B. Liu and H.Tian, *Chem. Commun.* 2005, 3156-3158.