## *Supporting Information*

# Selective detection and bio-imaging of $Pd^{2+}$ with novel 'C-CN' bond cleavage of cyano-rhodamine, cyanation with diaminomaleonitrile†

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## CONTENTS

**1. General:**

Unless otherwise mentioned, chemicals and solvents were purchased from Sigma-Aldrich Chemicals Private Limited and were used without further purification. $^{1}$H-NMR and $^{13}$C-NMR are recorded on 400 and 500 MHz instruments rsepectively. For $^{1}$H-NMR and $^{13}$C-NMR spectra, $CDCl_3$ are used as solvents respectively, TMS is used as an internal standard. Chemical shifts are expressed in δ units and $^{1}$H–$^{1}$H in Hz. UV-vis titration experiments were performed on a JASCO UV-V530 spectrophotometer and fluorescence experiment was done using PTI fluorescence spectrophotometer using a fluorescence cell of 10 mm path.

**2. General method of UV-vis and fluorescence titration:**

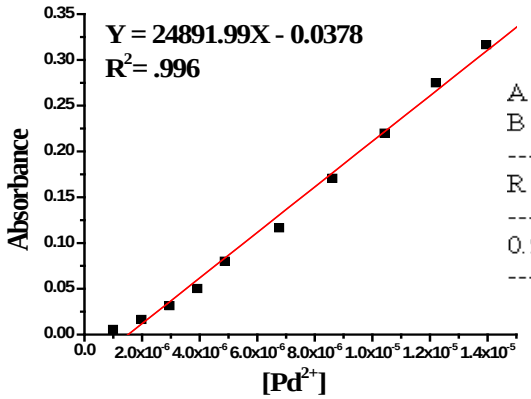
For UV-vis and fluorescence titrations, stock solution of the sensor was prepared (c = 1 x $10^{-5}$ $ML^{-1}$) in MeOH-$H_2O$ (2:8, v/v, 25°C). The solution of the guest cataions using their salts in the order of 2 x $10^{-4}$ $ML^{-1}$ was prepared in water. pH of the solution is adjusted at 7.4 by using 20 mM HEPES buffer. Solutions of various concentrations containing sensor and increasing concentrations of cataions were prepared separately. The spectra of these solutions were recorded by means of UV-vis and fluorescence methods.

**3. Determination of Detection Limit:**

The detection limit DL of **BHI** for $CN^-$ was determined from the following equation. [S1]

DL = K* Sb1/S

Where K = 2 or 3 (we take 3 in this case); Sb1 is the standard deviation of the blank solution; S is the slope of the calibration curve.




| | | | |
|---|---|---|---|
| A | -0.0378 | 0.00609 | |
| B | 24891.99501 | 770.11971 | |
| R | SD | N | P |
| 0.99619 | 0.01034 | 10 | <0.0001 |

**Figure S1:** Absorbance vs. [$Pd^{2+}$] plot with corresponding liner fit analysis data using origin software.

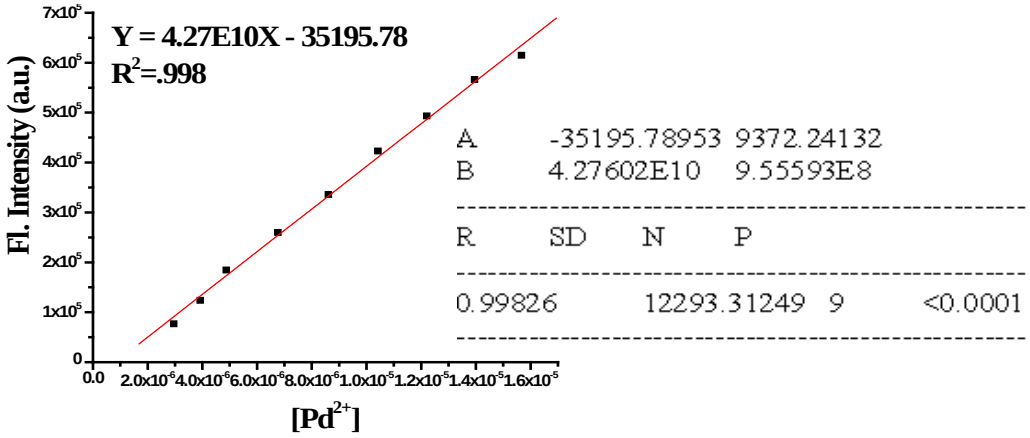



**Figure S2:** Fluorescence intensity vs. [$Pd^{2+}$] plot with corresponding liner fit analysis data using origin software.

Thus using the formula we get the Detection Limit = 0.83 μM (from absorbance data) and 0.57 μM (from fluorescence data) i.e. Rh-CN can detect $Pd^{2+}$ in this minimum concentration.

**4. Time dependent absorbance and fluorescence spectra of Rh-CN in addition with $PdCl_2$.**

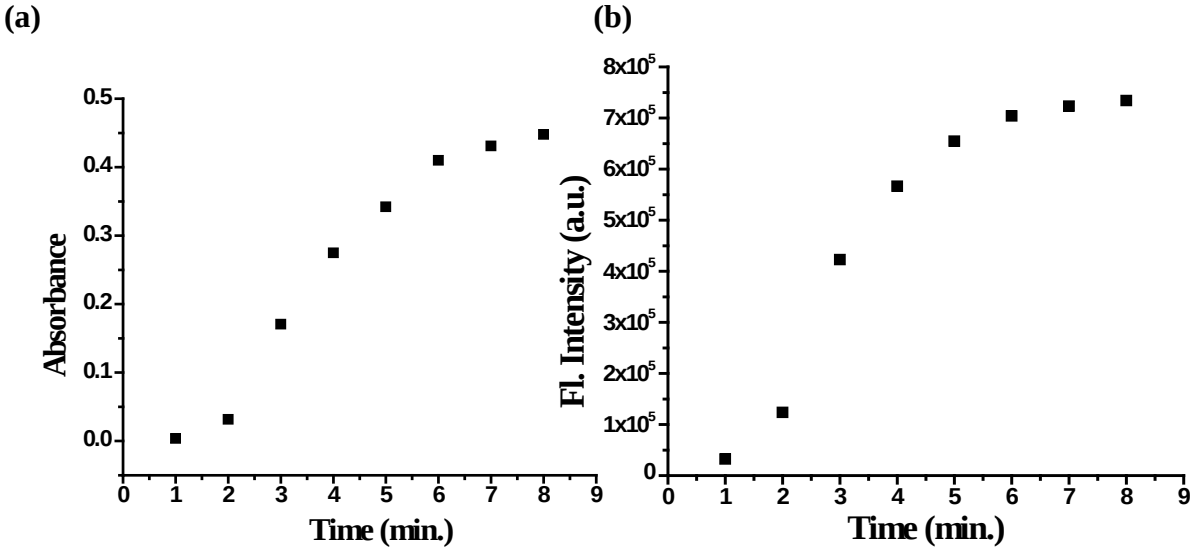



**Figure S3:** Time dependent absorbance (a) and Fluorescence intensity (b) of Rh-CN with addition of 4 equiv. $PdCl_2$ at a time.

## 5. Determination of fluorescence quantum yield:

Here, the quantum yield φ was measured by using the following equation,

$\varphi_x = \varphi_s ( F_x / F_s)( A_s / A_x)(n_x^2/ n_s^2)$

Where,

X & S indicate the unknown and standard solution respectively, φ = quantum yield,

F = area under the emission curve, A = absorbance at the excitation wave length,

n = index of refraction of the solvent. Here φ measurements were performed using anthracene in ethanol as standard [φ = 0.27] (error ~ 10%).

The quantum yield of Rh-CN itself is 0.005 is remarkably change into 0.442 (around 88 fold enhancements) after addition of $PdCl_2$.

## 6. Methods for the preparation:

**Synthesis of cyano-rhodamine (Rh-CN):** Rh-CN was synthesized in one step new reaction from rhodamine 6G. A solution of rhodamine 6G hydrochloride (1 g, 2.26 mmol) and diaminomaleonitrile (250 mg, 2.3 mmol) in EtOH (10 mL) was refluxed for 12hr in presence of 2.5ml of triethylamine. After checking the TLC plot (a less polar UV active but non-fluorescence spot is appeared) the solvent is evaporated in vacuum. Then the reddish colored gummy compound is purified through column chromatography using DCM as a solvent. The pure light pink colored compound is then recrystallized from $CHCl_3$-MeOH (1:1) (437.5mg, 35% yield). The single crystal of the compound is also grown in the mixed solvent $CHCl_3$-MeOH (1:1) with slow vaporization technique (figure S6 and figure S7)

**$^1$H NMR ($CDCl_3$, 400 MHz):** δ (ppm): 8.173 (d, 1H, *J* = 7.88), 7.559 (S, 1H), 7.364 (d, 2H, *J* = 5.6 Hz), 6.708 (s, 2H), 6.284 (s, 2H), 3.780 (q, 2H, *J* = 7.06 Hz), 3.448 (s, 2H), 3.187 (d, 4H, J = 6.48 Hz), 1.967 (s, 3H), 1.280 (q, 6H, J = 8.46 Hz), 0.976 (t, 3H, *J* = 7.1 Hz).

**$^{13}$C NMR ($CDCl_3$, 125 MHz**): δ (ppm): 166.01, 162.14, 157.86, 137.01, 134.56, 131.22, 128.36, 115.36, 114.85, 113.81, 56.67, 55.85, 55.50, 47.00, 43.22, 40.85, 26.81, 16.01, 14.50, 12.07.

**MS (ESI MS):** (m/z, %): 470.23 [(Rh-CN+$H^+$)$^+$, 100 %].

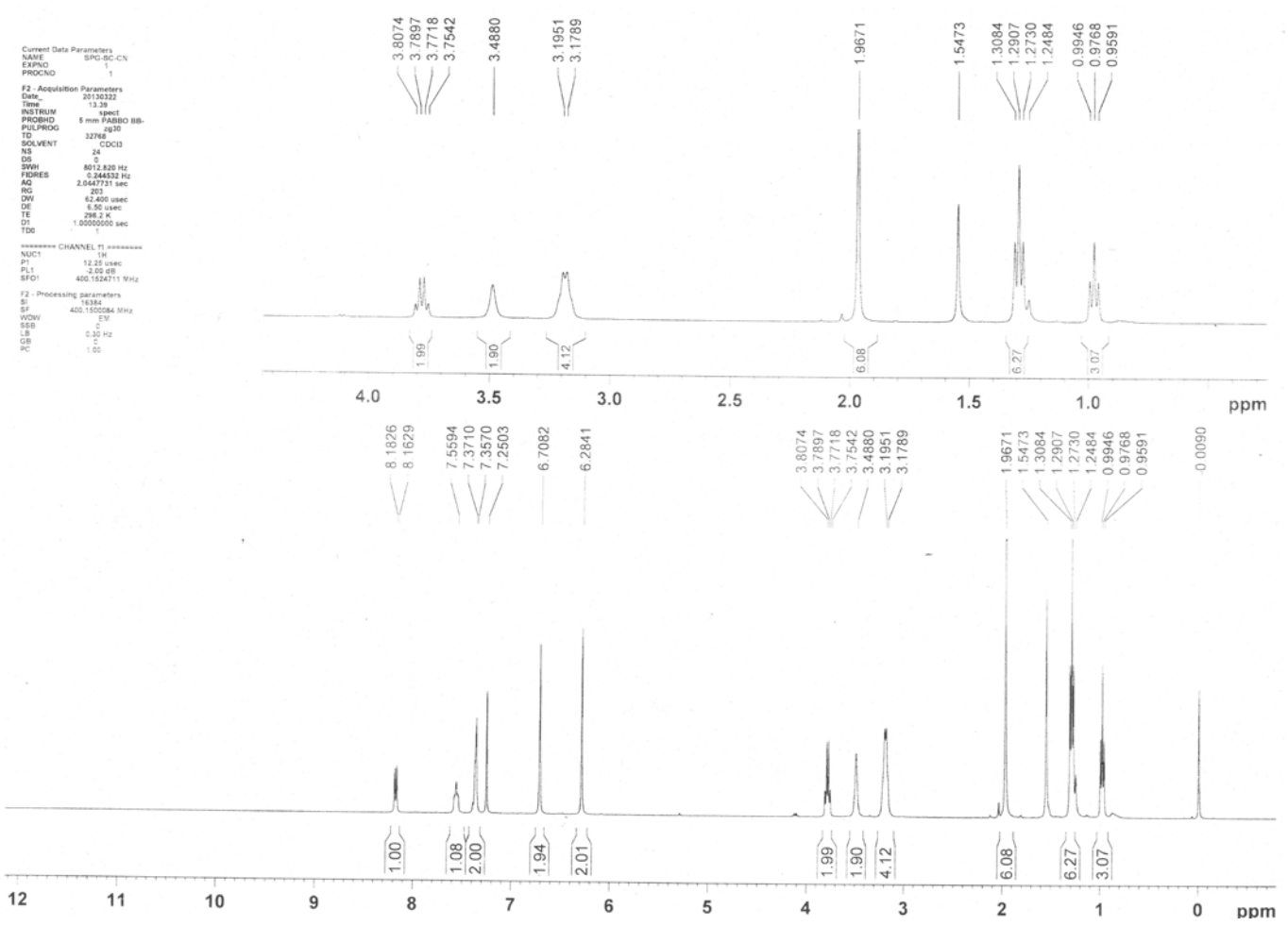
**Synthesis of Rhodamine 6G from Rh-CN with the action of $Pd^{2+}$:** Rh-CN is mixed with one equivalent of $PdCl_2$ in methanolic water at rt for 10 min to give a bright reddish pink color solution. On removing the solvent, a blackish solid product was obtained which was used for NMR and MASS after purified from column chromatography (DCM as a solvent).

**$^{13}$C NMR ($CDCl_3$, 125 MHz**): δ (ppm): 166.02, 162.14, 157.87, 145.47, 143.26, 140.81, 137.47, 134.61, 130.45, 121.81, 121.05, 105.11, 104.05, 55.87, 47.01, 30.15, 16.02, 14.51, 12.09.

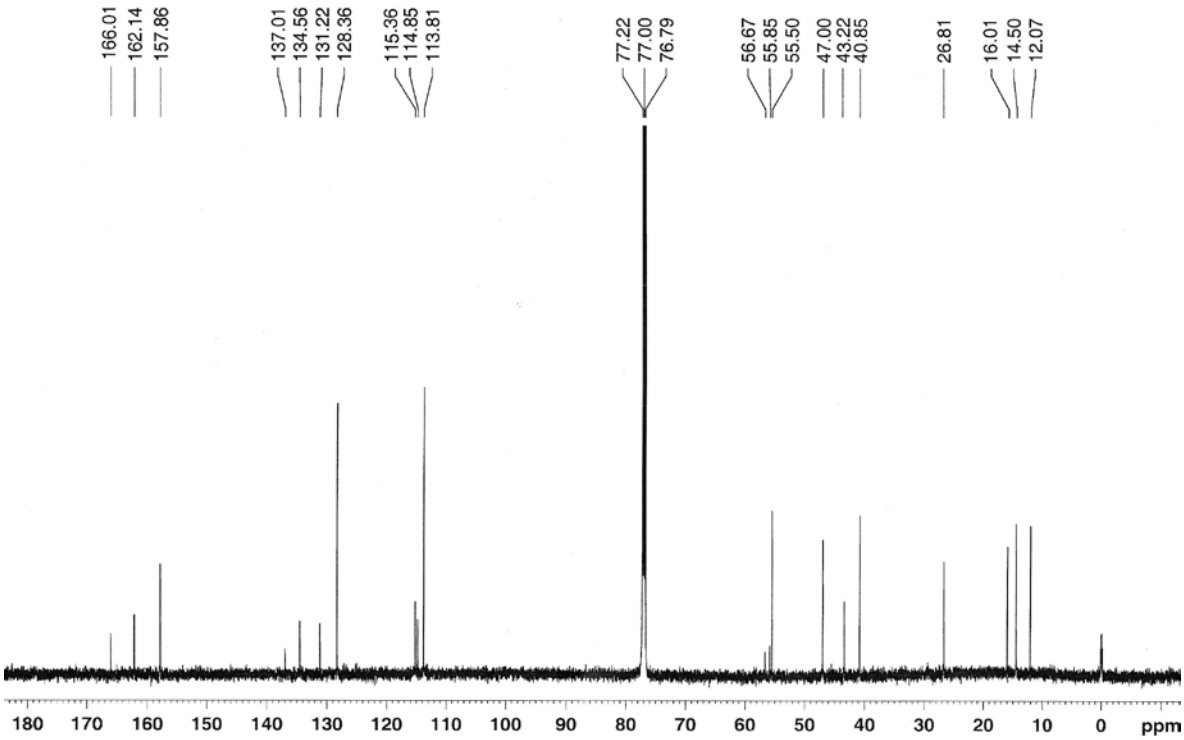
**MS (ESI MS):** (m/z, %): 443.22 [(Rh+$H^+$)$^+$, 100 %].

## 7. $^1$H NMR, $^{13}$C NMR and ESI MS spectra of Rh-CN and product with reaction of $PdCl_2$:

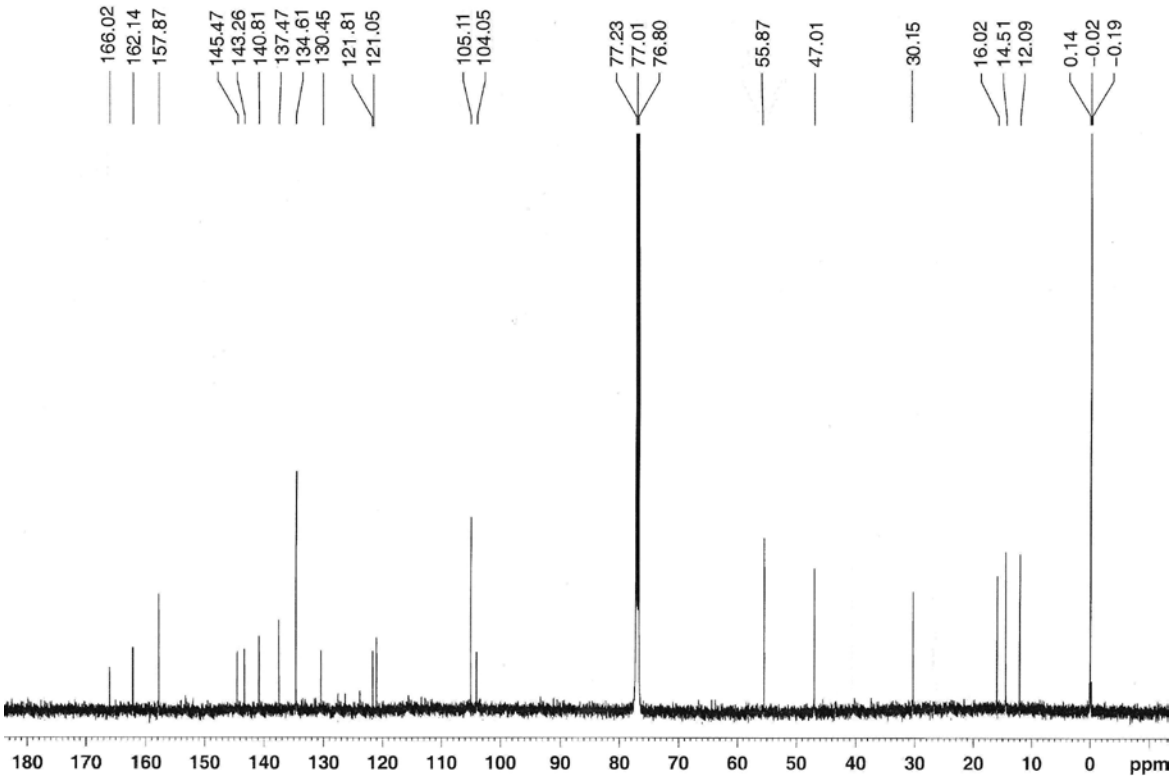
### $^1$H NMR of Receptor i.e. Rh-CN (in $CDCl_3$):



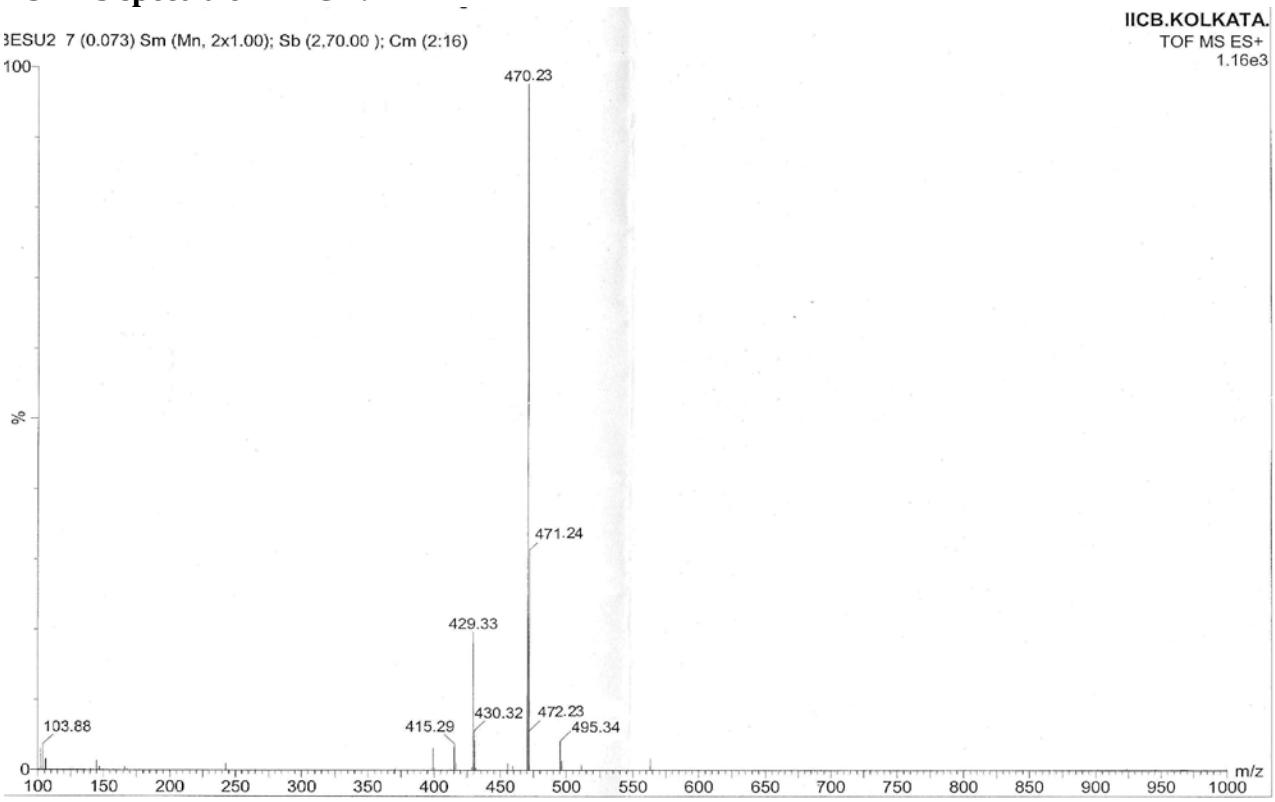

**$^{13}$C NMR of Rh-CN (in $CDCl_3$):**



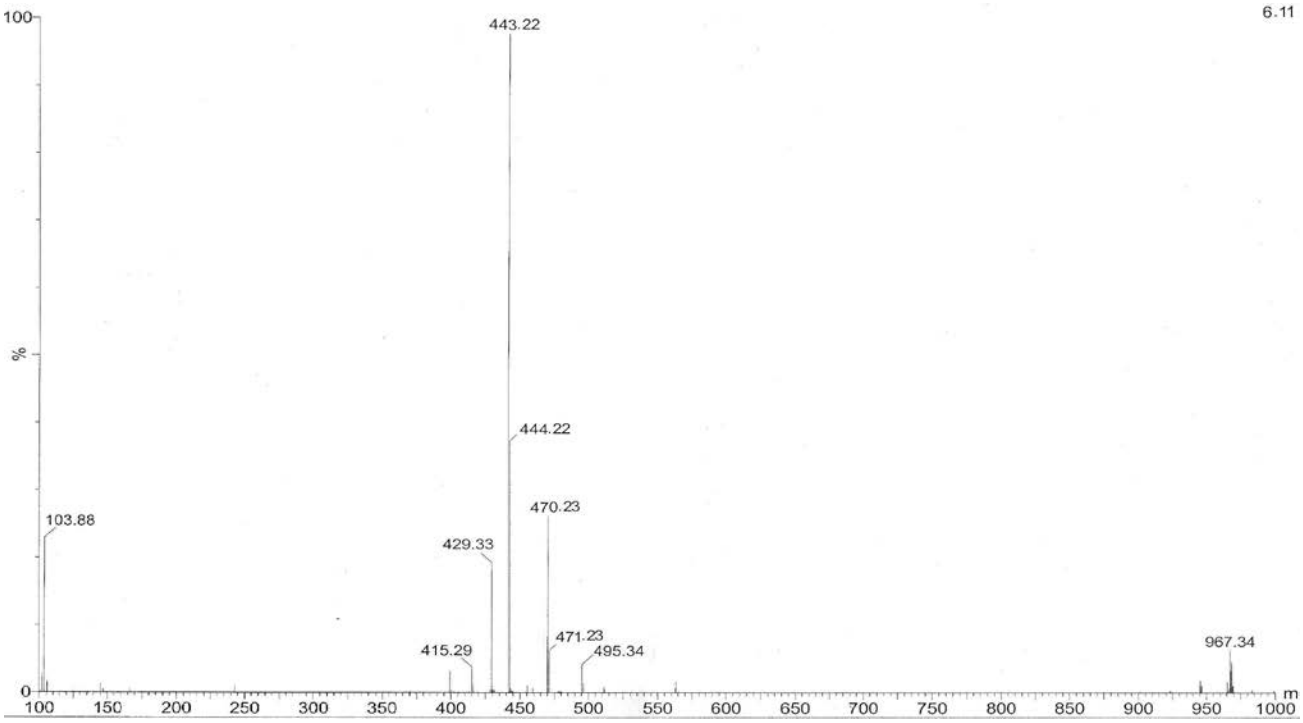

**$^{13}$C NMR of Rh-CN + $PdCl_2$ (in $CDCl_3$):**



**ESI MS spectra of Rh-CN:**




**ESI MS spectra of Rh-CN + $PdCl_2$:**



**8. Absorbance and fluorescence spectral data data:**

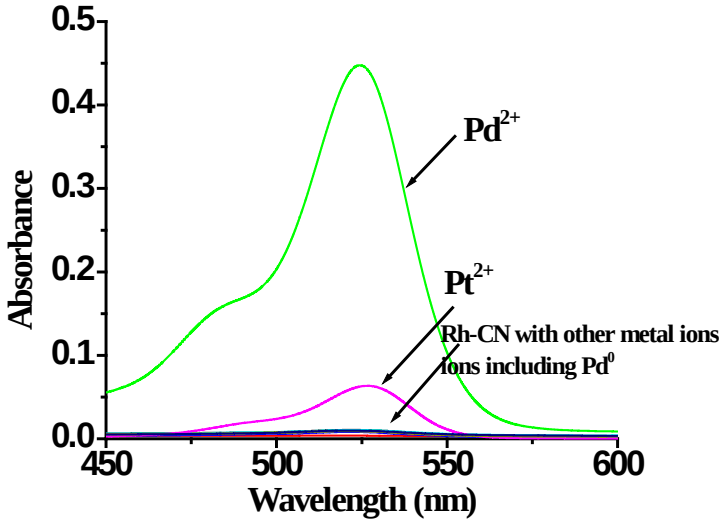



**Figure S4:** UV-vis absorption titration spectra of receptor (c = 1 x $10^{-5}$ M) in presence 6 equivalent of all the tested ions (except $Pd^{2+}$: 4 equiv.) in MeOH-$H_2O$ (2:8, v/v, at pH =7.4, 20 mM HEPES buffer).

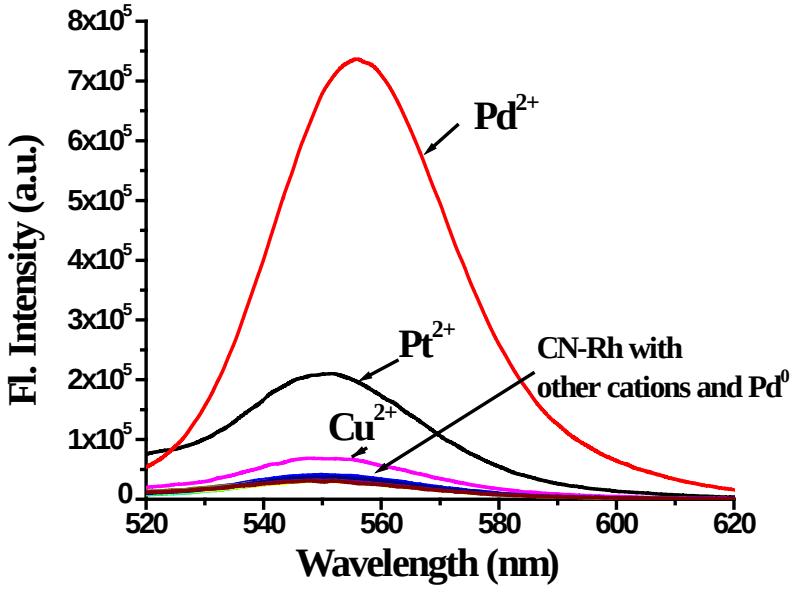



**Figure S5:** Fluorescence titration spectra of receptor (c = 1 x $10^{-5}$ M) in presence 6 equivalent of all the tested ions (except $Pd^{2+}$: 4 equiv.) in MeOH-$H_2O$ (2:8, v/v, at pH =7.4, 20 mM HEPES buffer).

**9. Details of cell culture and fluorescence microscopy:**

HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and penicillin-streptomycin (0.5 U/ml of penicillin and 0.5 μg/ml streptomycin) on cover slip in 35 mm dishes at 37$^{o}$C in an atmosphere of air with 5% $CO_2$ and constant humidity. The cells were initially incubated with the addition of 50 μM of $Pd^{2+}$ in the growth medium for 45 minutes. After washing three times with phosphate buffered saline (PBS) fresh growth medium containing 50 μM of the receptor (Rh-CN) was added and the cells were further incubated for 45 minutes. Following the incubation the cells were washed three times with PBS and the imaging was carried out using Zeiss AxioObserver Fluorescence Microscope equipped with an Apotome apparatus.

**10. <u>X-ray Crystallography</u>**

Single crystal suitable for X-ray analysis was performed on Bruker APEX II Duo CCD area-detector diffractometer using Mo Kα radiation (λ = 0.71073 Å). Data collection was performed using the *APEX2* software, whereas the cell refinement and data reduction were performed under the *SAINT* software. The crystal structure was solved by direct method and refined against $F^2$ by full-matrix least-squares refinement using *SHELXTL* package. The non-hydrogen atoms were refined anisotropically, whereby the N-bound and C-bound hydrogen atoms were located in difference fourier maps (N–H = 0.83(2) - 0.86(2) Å) and positioned geometrically (C–H = 0.95 - 0.99 Å), respectively. The final refinement converged well. Absorption correction was applied to the final crystal data by using the *SADABS* software. Crystallographic data for compound Rh-CN (fs237) has been deposited with the Cambridge Crystallographic Data Center No. CCDC934861

**Table S1:** X-ray crystallographic data and structure refinement

| Compounds | Compound 1 (CCDC934861) |
|---|---|
| Formula | $C_{29}H_{31}N_3O_3$ |
| Formula Weight | 469.57 |
| Crystal System | Orthorhombic |
| Space Group | *Pbca* |
| T, K | 100 |
| Z | 8 |
| a,Å | 15.2374 (13) |
| b,Å | 14.6371 (12) |
| c,Å | 21.6658 (18) |
| α, β, γ, deg | 90, 90, 90 |
| Volume, $Å^3$ | 4832.2 (7) |
| dcalcd , $g/cm^3$ | 1.291 |
| μ, $mm^{-1}$ | 0.08 |
| independent reflections | 5447 |
| Reflections with $I > 2\sigma(I)$ | 4415 |
| θ range, deg | 1.9–27.3 |
| GOF ($F^2$) | 1.04 |
| *R*int, $R[F^2 > 2\sigma(F^2)]$ | 0.048, 0.043 |
| $wR(F^2)$ | 0.116 |

**Table S2:** Hydrogen-bond geometry (Å, º)

| *D*—H···*A* | *D*—H | H···*A* | *D*···*A* | *D*—H···*A* |
|---|---|---|---|---|
| N3—H1*N*3···N1$^{i}$ | 0.83 (2) | 2.44 (2) | 3.2053 (17) | 153.9 (18) |
| N2—H1*N*2···O2$^{ii}$ | 0.86 (2) | 2.58 (2) | 3.3513 (17) | 150.8 (17) |
| C12—H12*A*···N1$^{iii}$ | 0.95 | 2.61 | 3.5247 (18) | 161 |
| C21—H21*C*···O2$^{ii}$ | 0.98 | 2.59 | 3.348 (2) | 134 |

Symmetry codes: (i) −*x*+1, *y*−1/2, −*z*+1/2; (ii) −*x*, −*y*+1, −*z*+1; (iii) −*x*+1/2, *y*−1/2, *z*.

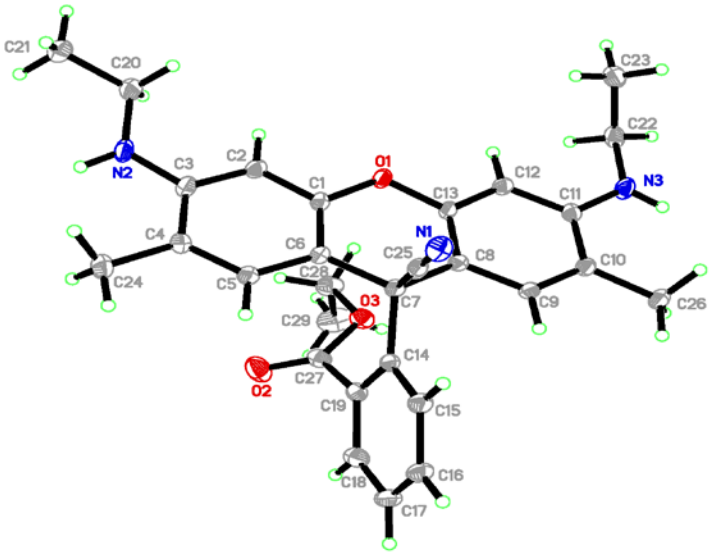



**Figure S6:** The structures of the Rh-CN (CCDC934861), showing 50% probability displacement ellipsoids for non-H atoms and the atom-numbering scheme. Hydrogen atoms are shown as spheres of arbitrary radius.

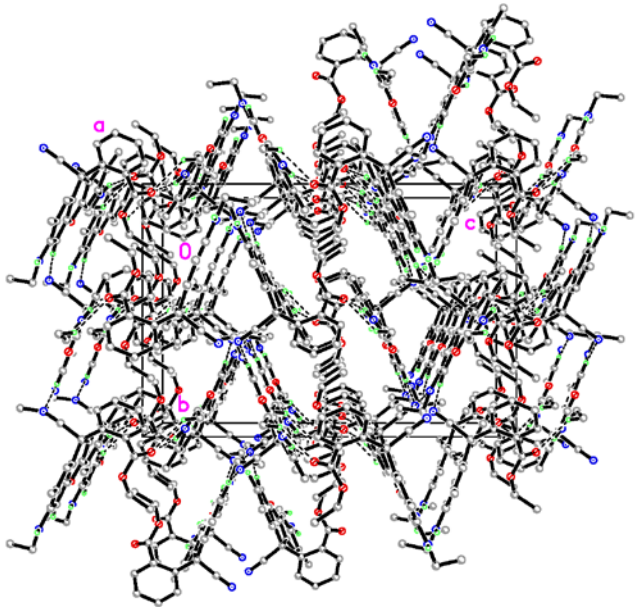



**Figure S7:** The molecules are linked to form a three-dimensional network. H atoms not involved in intermolecular interactions (dashed lines) have been omitted for clarity.

**11. References:**

[S1]. M. Zhu, M. Yuan, X. Liu, J. Xu, J. Lv, C. Huang, H. Liu, Y. Li, S. Wang, D. Zhu, *Org. Lett.*, 2008**, 10**, 1481.