# Supporting information

# Stimuli-directed selective detection of Cu$^{2+}$ and Cr$_2$O$_7^{2-}$ ions using pH-responsive chitosan-polyaminoamide fluorescent microgel in aqueous media.

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**EXPERIMENTAL SECTION:**

**Materials:** 1, 4-Diaminobutane (99%) purchased from Sigma, water-soluble low molecular weight chitosan (extrapure 90% DA) purchased from SRL. DABCO purchased from CDH. Deuterium oxide (D$_2$O, 99.9 atom % D), N, N'-Methylene (bis)acrylamide (MBA) 3x cryst purchased from SRL. HPLC grade water purchased from SRL. Tetrahydrofuran (THF) was purchased from SRL and N – isopropyl acryl amide (NIPAM) was purchased from Sigma Aldrich. Sodium hydroxide pellets and glacial acetic acid were purchased from CDH. Potassium dichromate (AR), Cobalt (II) nitrate hexahydrate, potassium (I) chloride, and Cadmium (II) nitrate tetrahydrate were purchased from CDH. Sodium arsenate (AR), sodium cyanide, sodium azide

(AR), sodium hydrogen carbonate, sodium iodate, and sodium fluoride were purchased from CDH. Sodium sulphate was purchased from Fisher Scientific. Sodium chloride was purchased from SRL. Barium (II) nitrate and calcium (II) chloride were purchased from CDH. Magnesium sulphate was purchased from SRL. Lead (II) chloride, nickel (II) chloride, zinc (II) chloride, ferrous (II) chloride, copper (II) chloride, iron (III) chloride hexahydrate, chromium (III) chloride hexahydrate (AR) were purchased from CDH. For all the experiments, HPLC-grade water was used.

**Synthesis of NANO-PAMAM:**

Via an aza-Michael addition reaction between N, N'-methylene-bisacrylamide ($A_2$ monomer) and 1, 4 -diaminobutane ($B_4$ monomer), polyaminoamide (NANO-PAMAM) was synthesized. The unique method for the synthesis of NANO-PAMAM was described in the following way: In 2.5 ml of water, N, N'- Methylene(bis)acrylamide (MBA, 0.250 g, 1.62 mmol) was added at room temperature, the resulting suspension was stirred for 10 min. In order to start the polymerisation reaction, 1,4- diaminobutane was added to the aqueous suspension of MBA maintaining the molar ratio for MBA to di-amine (2:1). The aqueous suspension was clear after 20 min of stirring. After 90 min from the addition of diamine. The aqueous solution of N – isopropyl acrylamide (NIPAM 0.906 g, 8.1 mmol, excess) was added after 90 min from the addition of diamine, and at room temperature, the solution was stirred for more than 48 hours. In tetrahydrofuran (THF), the resulting solution was precipitated and purified. To remove unreacted excess NIPAM, the precipitated product was washed 3 times with THF.

To prepare the aqueous solution of the product with a concentration of 10 mg/ml, the product was then dried and immediately solubilized in water.

**Synthesis of NANO-PAMAM-CHT:**

NANO-PAMAM-CHT was synthesized through the aza-Michael addition reaction between NANO-PAMAM and chitosan. The reaction was optimized by varying the weight ratio of NANO-PAMAM to chitosan (1:1, 1:1.5, and 1:2). At the weight ratio of (NANO-PAMAM: CHT = 1:2), we can get around 100% conversion from NANO-PAMAM to NANO-PAMAM-CHT and the method of synthesis is as follows: 0.02 g of NANO-PAMAM was dissolved in water and 0.04 g of chitosan was dissolved in 0.5 ml water followed by the addition of 24.44 mg DABCO (1,4-diazabicyclooctane). Two solutions were mixed and stirred for 48 hours at room temperature. The solution was then precipitated in acetone and the precipitated product was dried at room temperature. The purified product was stored in the refrigerator.

**Table S1:** Optimization table for the synthesis of NANO-PAMAM-CHT.

| NANO-PAMAM: chitosan (weight ratio) | Base | Temperature | Time (hours) | Solvent | Conversion |
|---|---|---|---|---|---|
| 1:1 | DABCO | Room temp. | 48 | Water | 36% |
| 1:1.5 | DABCO | Room temp. | 48 | Water | 75% |
| 1:2 | DABCO | Room temp. | 48 | Water | 100% |

**Nuclear Magnetic Resonance (NMR) Spectroscopy–** A Bruker 400 $MH_Z$ spectrometer was used for $^1$H-NMR analysis. For $^1$HNMR analysis, $D_2O$ was used as an NMR solvent.

**FT-IR Spectroscopy-** On a Perkin Elmer spectrum 400 FT-IR spectrophotometer, Fourier transform infrared (FTIR) spectra of NANO-PAMAM and NANO-PAMAM-CHT were recorded and the collection was from 500 to 4000 $cm^{-1}$ for 64 scans at 4 $cm^{-1}$.

**Size Exclusion Chromatography (SEC) –**To understand the average molecular weight and the architecture of NANO-PAMAM-CHT, size exclusion chromatography (SEC) was used. The system consists of Shimadzu i-series plus integrated HPLC attached with triple detector - multi-angle light scattering detector (Wyatt DAWN HELEOS LS II), Viscoster, and Refractive index detector (Wyatt Optilab T-rEX). The SEC eluent was water (containing 0.01% $NaN_{3)}$, the flow rate was 0.75 mL/min and the temperature of the column was 35 ˚C. ASTRA 7.3.0 software (Wyatt Technological Corporation) was used for data collection and processing.

**Ground state interaction by UV-vis spectrophotometer:**

Ground state interaction between NANO-PAMAM-CHT and $Cr_2O_7^{2-}$ was confirmed by a UV-vis spectrophotometer. Normally, we prepared the aqueous solution of NANO-PAMAM-CHT by dissolving NANO-PAMAM-CHT in water with a concentration of 0.25 mg/ml, and the prepared aqueous solution was poured into the quartz cuvette with a path length of 1 cm and width of 1 cm. A red shift (~20 nm) of the absorption spectra for (NANO-PAMAM-CHT) in the presence of $Cr_2O_7^{2-}$ was observed at 370 nm with respect to absorption spectra for pure $Cr_2O_7^{2-}$ solution, which was not observed in the presence of $Cu^{2+}$ ion.

**Hydrodynamic Radius ($R_h$) determination:**

For the dynamic light scattering (DLS) study, we prepared the aqueous solution of NANO-PAMAM-CHT by dissolving NANO-PAMAM-CHT in water with a concentration of 0.5 mg/ml, and the prepared aqueous solution was poured into the quartz cuvette (4 winded) with path length

1 cm and width of 1 cm. To understand the average hydrodynamic diameter of NANO-PAMAM-CHT, a Litesizer 500 particle analyzer from Anton Paar was used. Size and the particle size distributions were calculated by using the CONTIN plot.

**Atomic force microscopy (AFM)** – Atomic force microscope (AFM) topography imaging of NANO-PAMAM-CHT was taken from Agilent Technologies 5500 and the analysis was done in noncontact mode. At a resonance frequency of 289 kHz, silicon cantilever probes were used with a spring constant of 42 N/m. In order to study the topography of NANO-PAMAM-CHT nanoparticles, an aqueous solution of NANO-PAMAM-CHT was prepared with a concentration of 1 mg/mL. To observe the surface morphology of NANO-PAMAM-CHT, an aqueous solution was coated on a silicon wafer and dried on the hot plate at 60°C.

**Transmission Electron Microscopy (TEM):**

By using JEOL TEM with an accelerating potential of 200 kV, transmittance electron microscope (TEM) images were analyzed.

**Fluorescence spectrophotometer:**

In order to obtain the fluorescence excitation and emission spectrum of NANO-PAMAM-CHT in the presence and absence of different analytes with slit 2, a fluoro max-4P spectrofluorometer (Horiba jobin Yvon) was used. A four-winded quartz cuvette was used for these measurements.

**pH meter:**

pH meter with the model OAKTON PC 2700 was used to prepare pH solutions from 3 to 11.

**Lifetime measurement:**

Lifetime measurement of the NANO-PAMAM-CHT was done in the presence and absence of the analyte with the help of time-correlated single photon counting (TCSPC) technique and the excitation at 375 nm by using a diode laser (excitation source). A spectrophotometer from Edinburgh instrument (model: lifeSpec II, U.K) was used for the fluorescence decay study. To collect the signal, a detector from Hamamatsu MCP PMT (3809U) was used. Lifetime measurement was carried out by using four winded quartz cuvettes with a path length of 1cm. Finally, fluorescence decay emissions were measured at the magic angle of 54.7°C and F 900 decay analysis software was used for the data collection.

By using the following equation, we have calculated the weight-average lifetime –

$$<\tau> = \sum_{i=1}^{N} (C_i \tau_i)$$

$$C_i = B_i / \sum_{i=1}^{N} B_i$$

Where, $\tau_i$ = The fluorescence decay time

$\quad\quad$ $B_i$ = The pre-exponential factor

**Quenching efficiency:**

% quenching was calculated by using the following equation-

$$\% \text{ quenching} = \frac{I_0 - I}{I_0} \times 100$$

Where $I_0$ is the fluorescence intensity of NANO-PAMAM-CHT in the absence of a quencher, and $I$ is the fluorescence intensity of NANO-PAMAM-CHT in the presence of a quencher.[1]
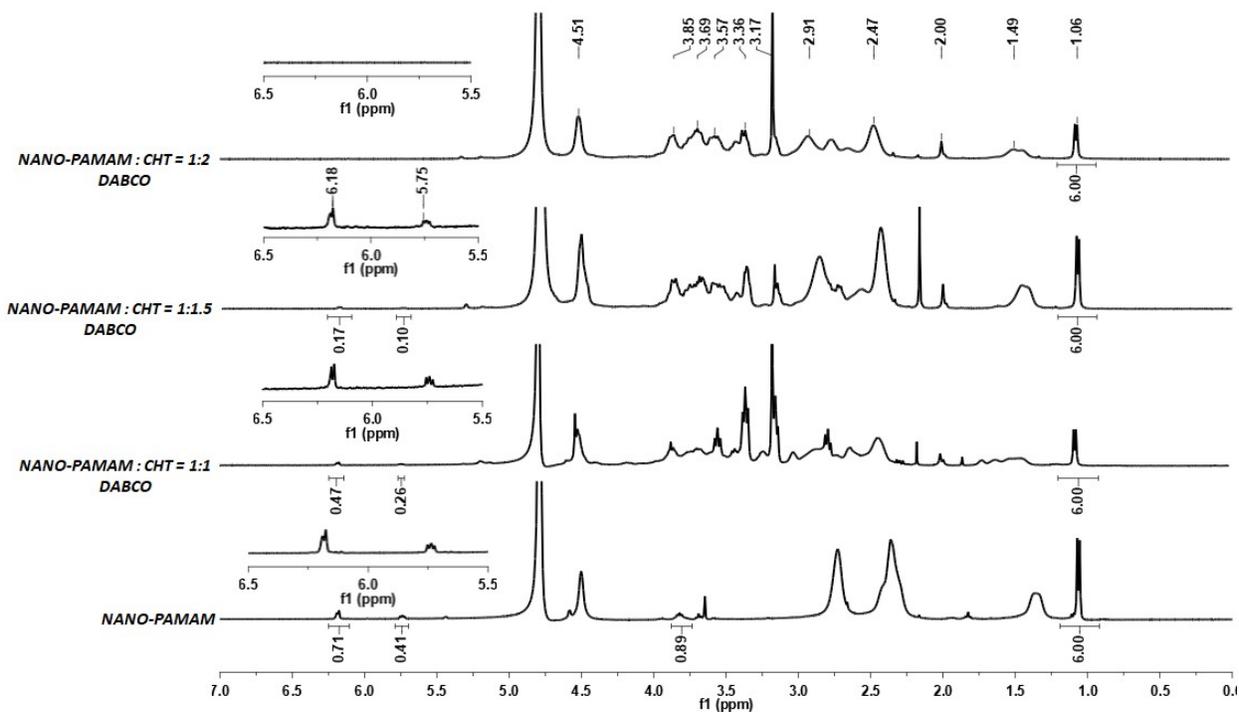
**Figure S1:** $^1$H-NMR analysis of NANO-PAMAM and NANO-PAMAM-CHT with the variation of weight ratio of NANO-PAMAM to chitosan in presence of base, DABCO.
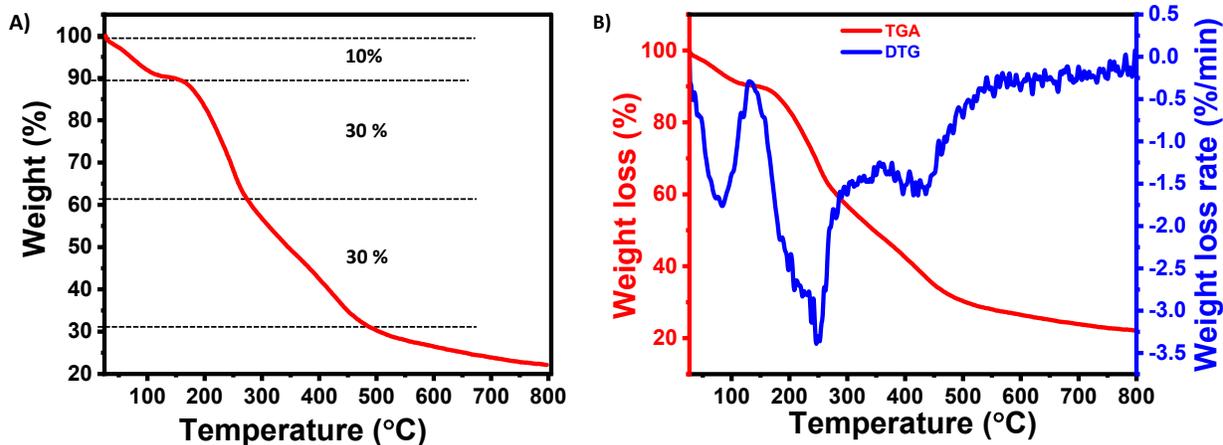


**Figure S2:** A) Thermo gravimetric analysis (TGA) of NANO-PAMAM-CHT, B) TG-DTG thermogram of NANO-PAMAM-CHT.
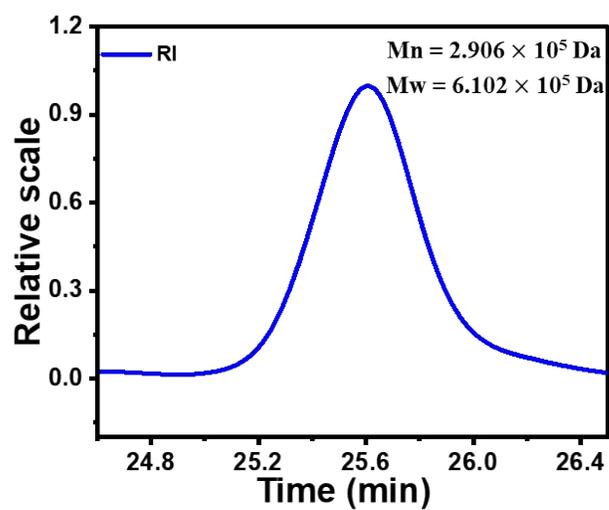
**Figure S3:** A) SEC chromatogram of the microgel, NANO-PAMAM-CHT.
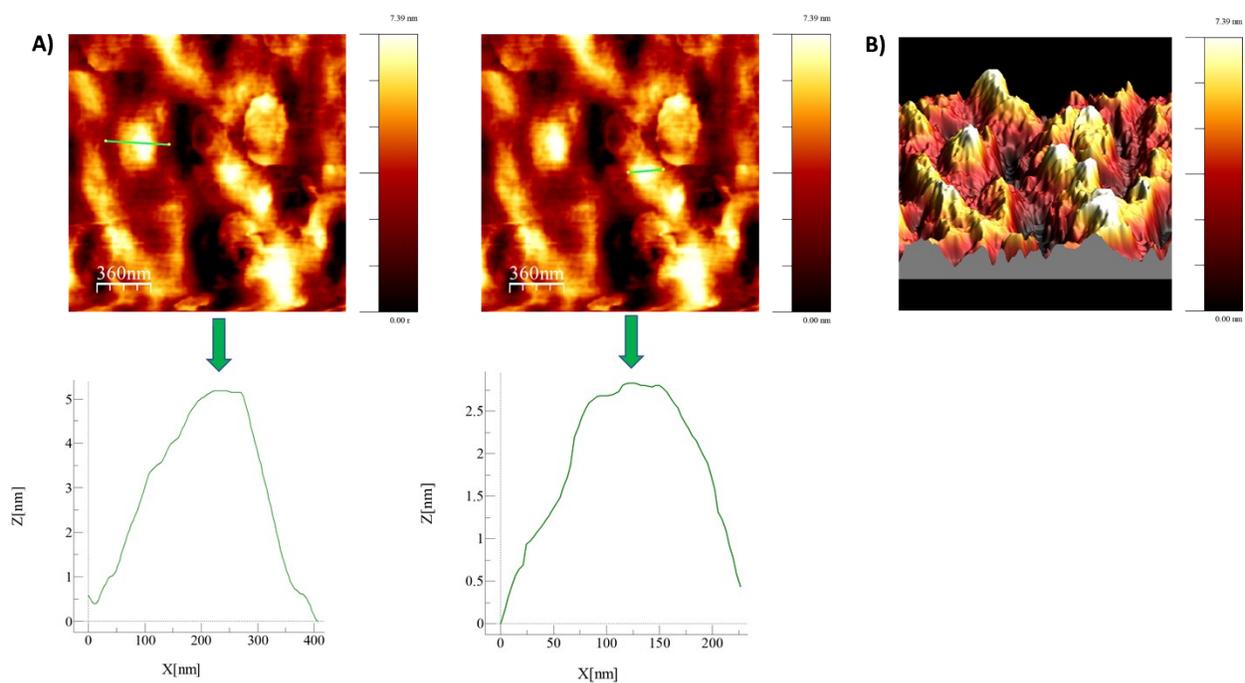


**Figure S4:** A) AFM micrograms with corresponding height profile graph of NANO-PAMAM-CHT. B) 3D AFM microgram of NANO-PAMAM-CHT
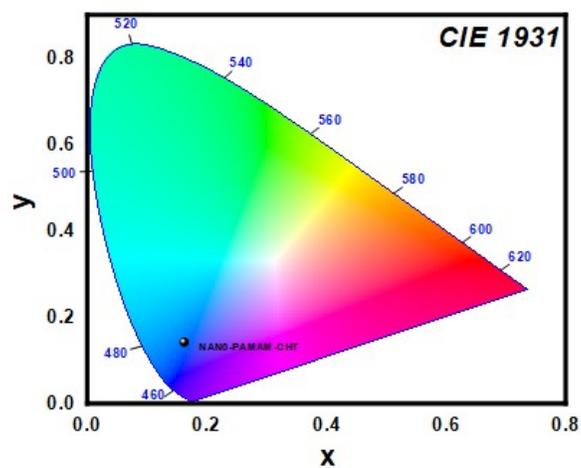
**Figure S5**:  CIE 1931 chromaticity diagram for NANO-PAMAM-CHT.
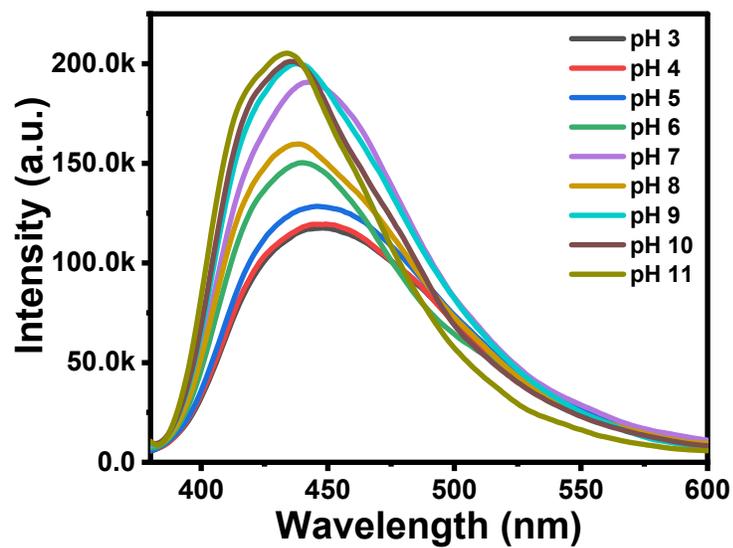


**Figure S6**:  Fluorescence emission spectra of NANO-PAMAM-CHT at different pH.
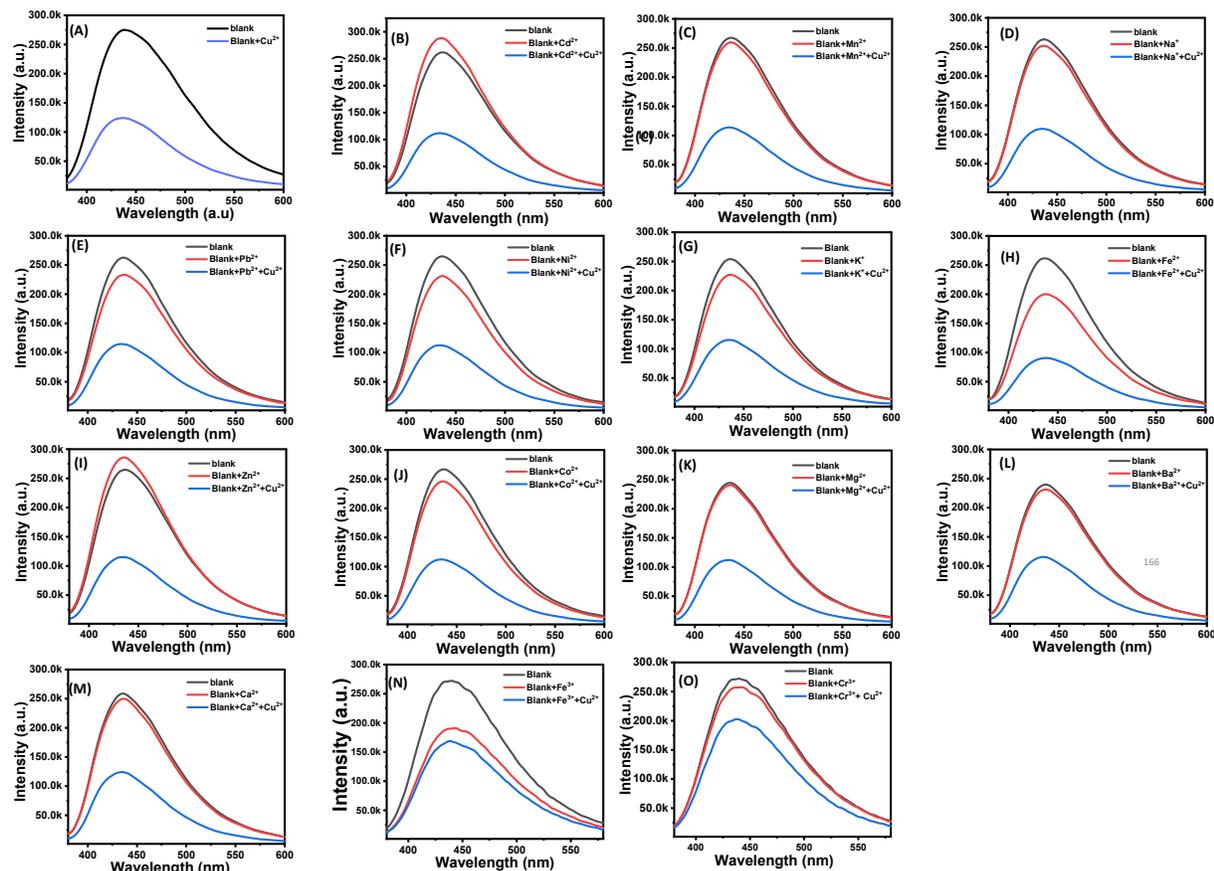
**Figure S7:** A) $Cu^{2+}$ ion sensing by NANO-PAMAM-CHT in absence of other interfering cation and $Cu^{2+}$ ion sensing by NANO-PAMAM-CHT in presence of other interfering cations B) $Cd^{2+}$ ion C) $Mn^{2+}$, D) $Na^+$, E) $Pb^{2+}$, F) $Ni^{2+}$, G) $K^+$, H) $Fe^{2+}$, I) $Zn^{2+}$, J) $Co^{2+}$, K) $Mg^{2+}$, L) $Ba^{2+}$, M) $Ca^{2+}$, N) $Fe^{3+}$, O) $Cr^{3+}$ in aqueous solution.
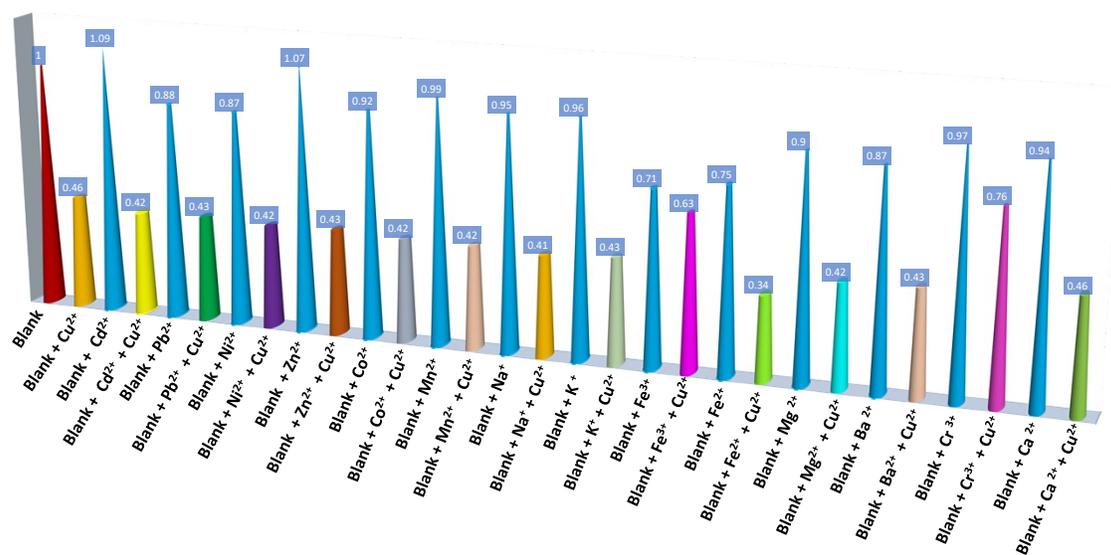
**Figure S8:** $Cu^{2+}$ ion sensing by NANO-PAMAM-CHT in the presence of various competing metal ions in aqueous solution.
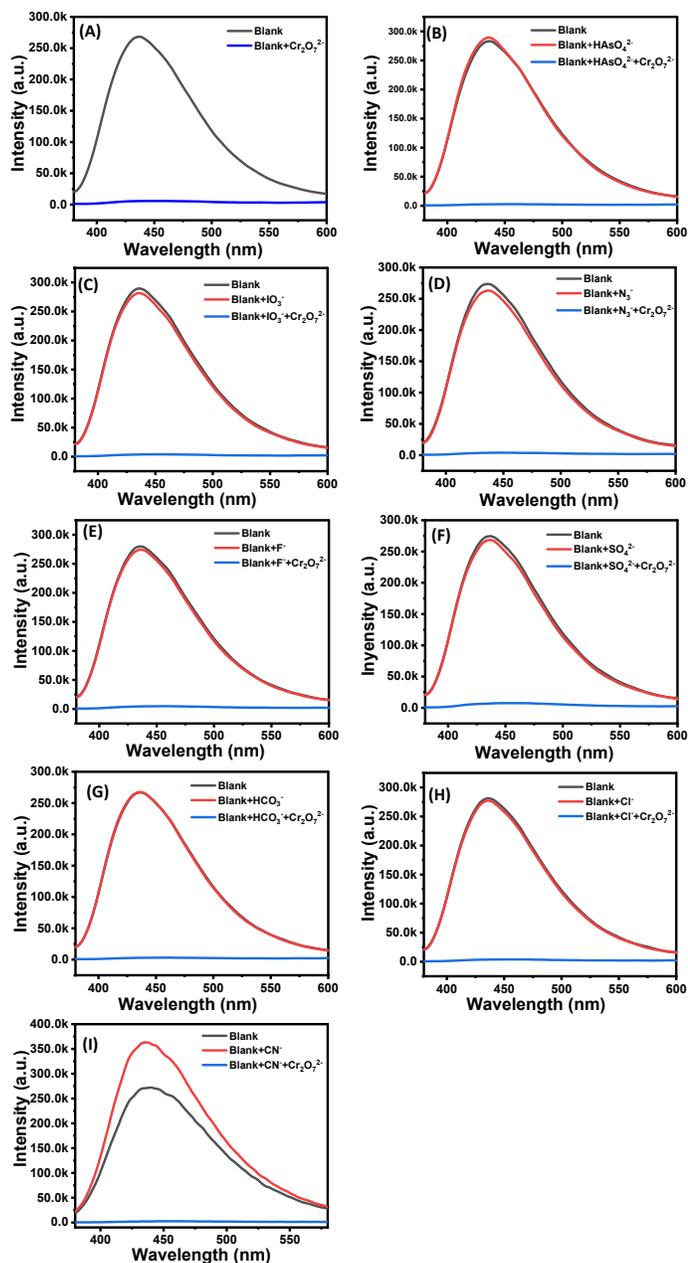
**Figure S9:** A) $Cr_2O_7^{2-}$ ion sensing by NANO-PAMAM-CHT in absence of other interfering anion in aqueous solution and $Cr_2O_7^{2-}$ ion sensing by NANO-PAMAM-CHT in presence of other interfering anions B) $HAsO_4^{2-}$ , C) $IO_3^-$ , D) $N_3^-$ , E) $F^-$, F) $SO_4^{2-}$ , G) $HCO_3^-$ and H) $Cl^-$, I) $CN^-$ in aqueous solution.
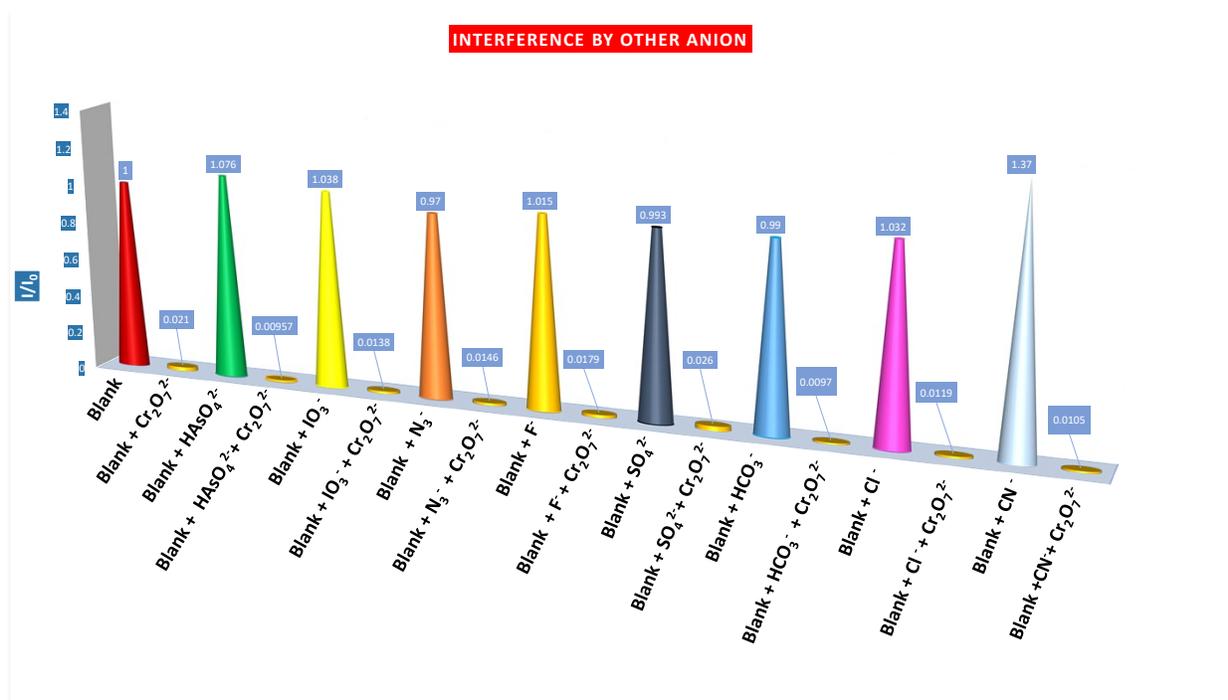
**Figure S10:** $Cr_2O_7^{2-}$ ion sensing by NANO-PAMAM-CHT in presence of various competing anions in aqueous solution.
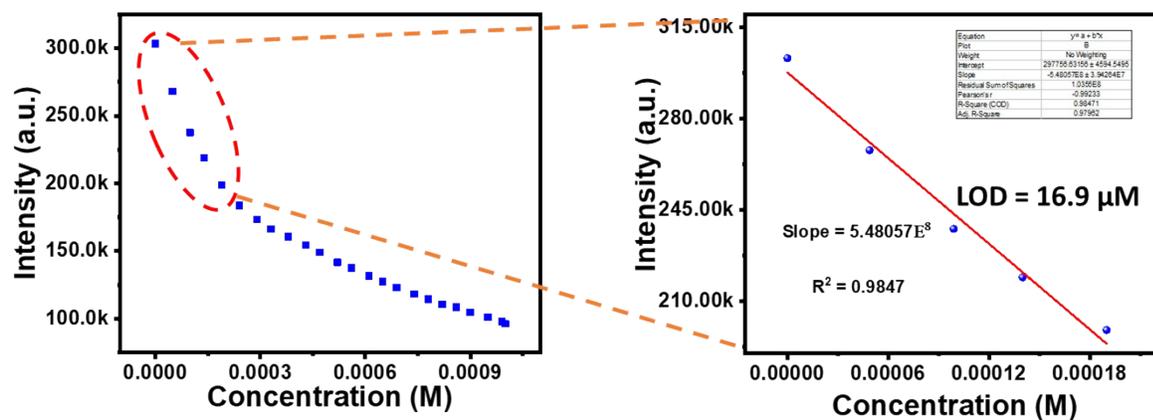


**Figure S11:** LOD plot for $Cu^{2+}$ ion at pH 7.
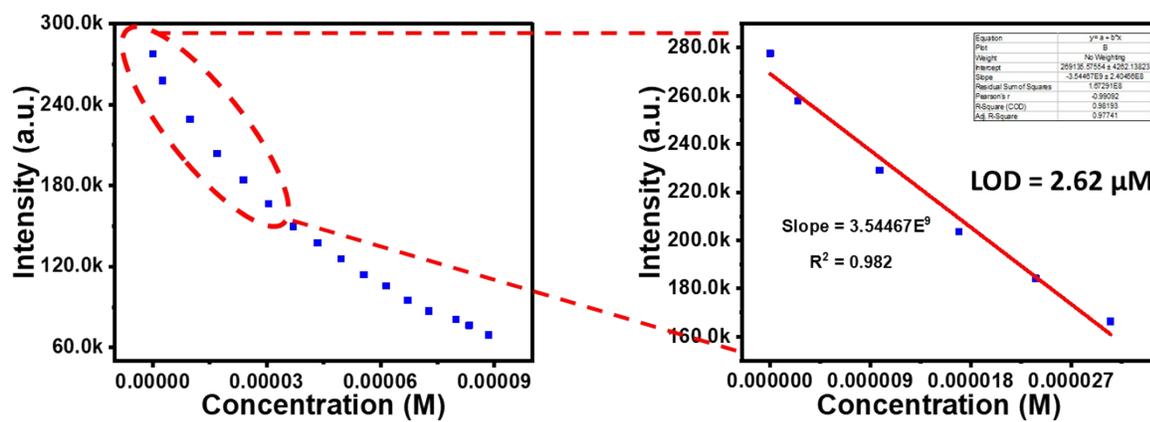
**Figure S12:** LOD plot for $Cr_2O_7^{2-}$ ion at pH 7.

**Table S2:** Comparison table for NANO-PAMAM-CHT in the presence of $Cu^{2+}$ with the other fluorescence probe reported in literature.

| SL. NO | Compounds | Analytes | Medium | Limit of detection (LOD) | Ref. |
|---|---|---|---|---|---|
| 1. | Chitosan-BODIPY | $Cu^{2+}$ | Water | 4.75 µM | [2] |
| 2. | Coumarine based AIEE probe | $Cu^{2+}$ | Acetonitrile/water mixture (1:9 v/v) | 28.0 µM | [3] |
| 3. | N-doped carbon dot | $Cu^{2+}$ | Water | 45.87 µM | [4] |
| 4. | N-doped carbon dot | $Cu^{2+}$ | Water | 10.0 µM | [5] |
| 5. | Carbon dot-based pH sensor | $Cu^{2+}$ | pH solution (At pH 4) | 40.0 µM | [6] |
| 6. | Spherical nanocellulose | $Cu^{2+}$ | Water | 16.22 µM | [7] |
| 7. | C=N based PAMAM polymer dot | $Cu^{2+}$ | Water | 7.46 µM | [8] |
| 8. | NANO-PAMAM-CHT | $Cu^{2+}$ | Water | 16.9 µM | This work |

**Table S3:** Comparison table for NANO-PAMAM-CHT in the presence of $Cr_2O_7^{2-}$ with the other fluorescence probe reported in literature.

| SL. NO | Compounds | Analytes | Medium | Limit of detection (LOD) | Ref. |
|---|---|---|---|---|---|
| 1. | Carbon nano-cluster | $Cr_2O_7^{2-}$ | Water | 0.9 µM | [9] |
| 2. | poly-N-phenyl anthranilic acid (PNPA) | $Cr_2O_7^{2-}$ | sulfuric acid solution | 25.0 µM | [10] |
| 3. | P(Fmoc-Glu-OH) | $Cr_2O_7^{2-}$ | Water | 3.33 µM | [11] |
| 4. | Eu (III) coordination polymer | $Cr_2O_7^{2-}$ | Water | 10.07 µM | [12] |
| 5. | Fluorescent coordination polymer [Zn(L)(H2L)]n | $Cr_2O_7^{2-}$ | Water | 11.4 µM | [13] |
| 6. | Zn(II)-based coordination polymer | $Cr_2O_7^{2-}$ | Water | 63.65 µM | [14] |
| 7. | Covalent triazine polymer SMCOP-2 | $Cr_2O_7^{2-}$ | Water | $4.1 \times 10^{-4}$ M | [15] |
| 8. | Isophthalic acid-doped polymer dots | $Cr_2O_7^{2-}$ | Water | 0.03 µM | [16] |
| 9. | NANO-PAMAM-CHT | $Cr_2O_7^{2-}$ | Water | 2.62 µM | This work |
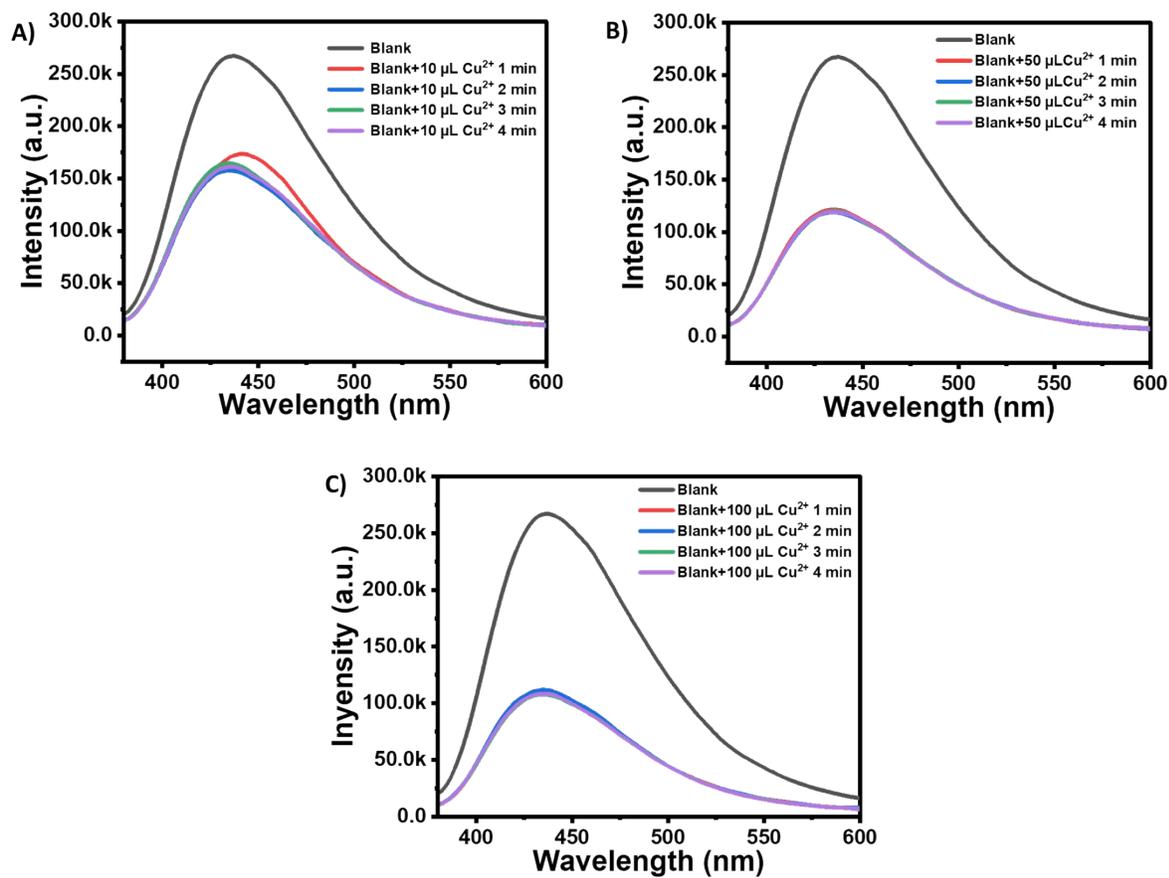
**Figure S13:** Emission spectra for the kinetic study of NANO-PAMAM-CHT in the presence of Cu²⁺ ion (10 mM) after addition of A) 10 µL b) 50 µL C) 100 µL.
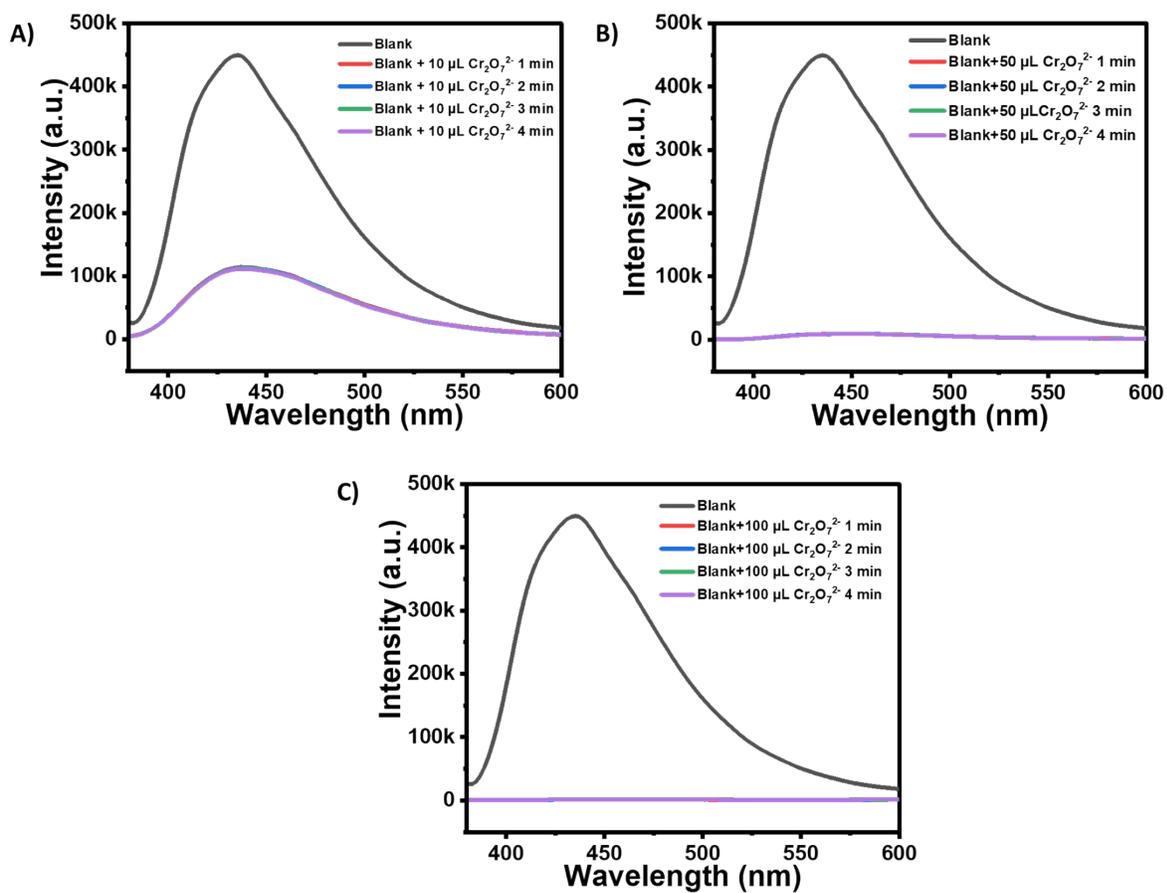
**Figure S14:** Emission spectra for the kinetic study of NANO-PAMAM-CHT in the presence of $Cr_2O_7^{2-}$ ion (10 mM) after addition of A) 10 µL B) 50 µL C) 100 µL.
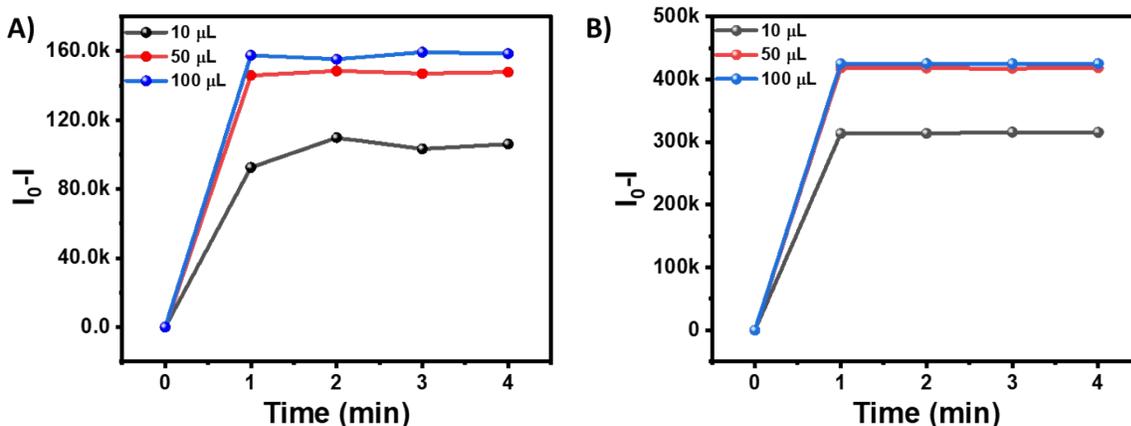
**Figure S15:** A) Kinetic study of NANO-PAMAM-CHT in the presence of $Cu^{2+}$ ion in aqueous solution. B) Kinetic study of NANO-PAMAM-CHT in the presence of $Cr_2O_7^{2-}$ ion in aqueous solution.
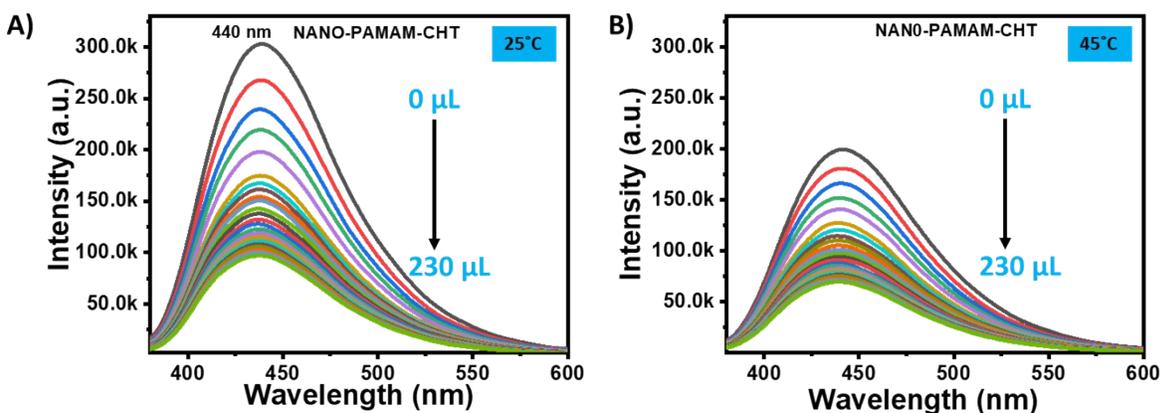


**Figure S16:** A) Fluorescence emission spectra of NANO-PAMAM-CHT with gradual addition of $Cu^{2+}$ ion (10 mM) at 25°C in aqueous solution. B) Fluorescence emission spectra of NANO-PAMAM-CHT with the gradual addition of $Cu^{2+}$ ion (10 mM) at 45°C in aqueous solution.
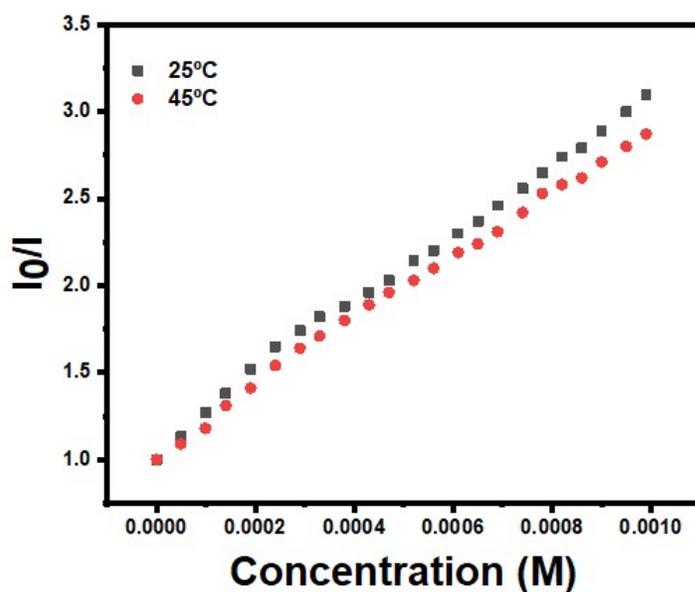
**Figure S17:** Intensity ratio vs concentration plot for $Cu^{2+}$ ion sensing by NANO-PAMAM-CHT at 25° C and at 45° C in aqueous solution.
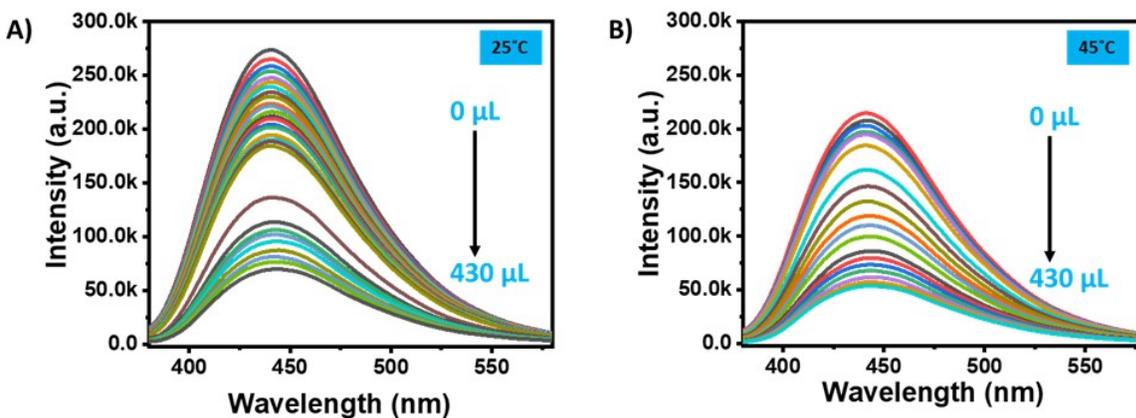


**Figure S18:** A) Fluorescence emission spectra of NANO-PAMAM-CHT with gradual addition of $Cr_2O_7^{2-}$ ion (500 µM) at 25°C in aqueous solution. B) Fluorescence emission spectra of NANO-PAMAM-CHT with the gradual addition of $Cr_2O_7^{2-}$ ion (500 µM) at 45°C in aqueous solution.
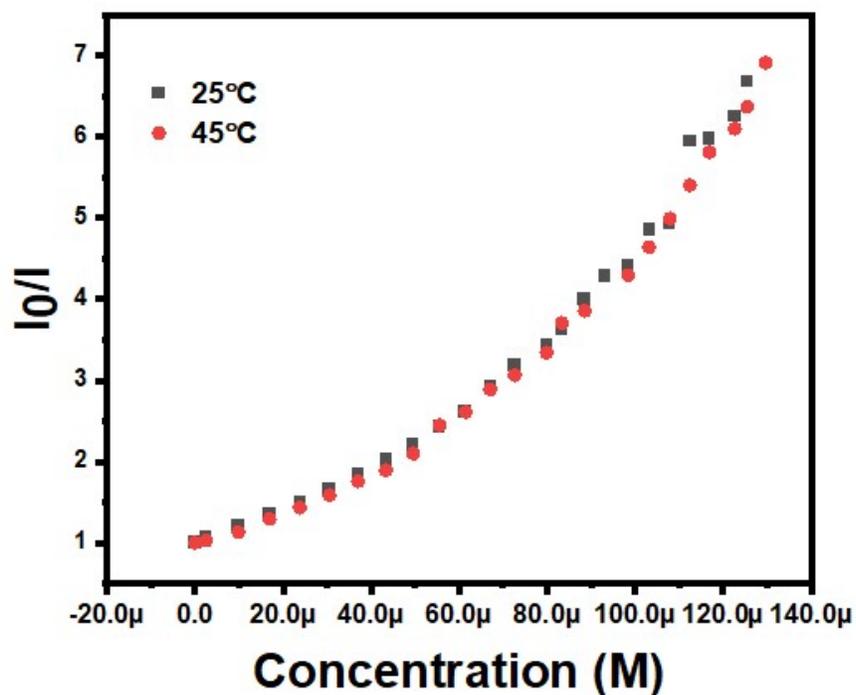
**Figure S19:** Intensity ratio vs concentration plot for $Cr_2O_7^{2-}$ ion sensing by NANO-PAMAM-CHT

at 25° C and at 45° C in aqueous solution.
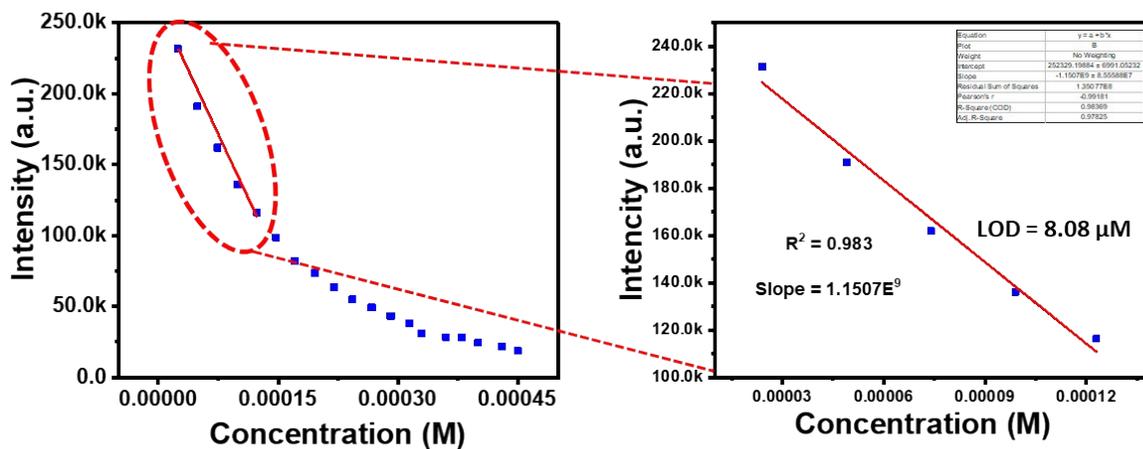


**Figure S20:** LOD plot for $Cr_2O_7^{2-}$ ion at pH 3.
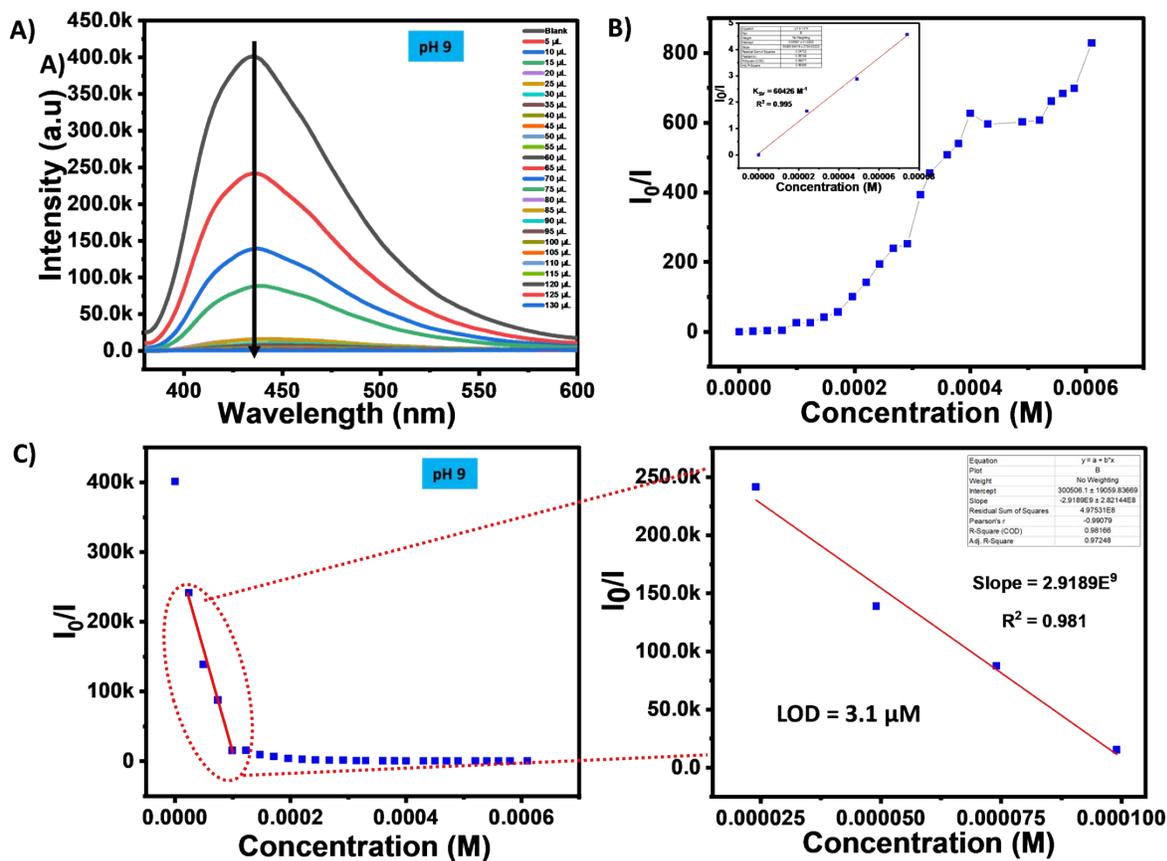
**Figure S21:** A) Fluorescence emission spectra of NANO-PAMAM-CHT at the pH 9 B) Stern-volmer plot. C) LOD plot for $Cr_2O_7^{2-}$ ion at pH 9.
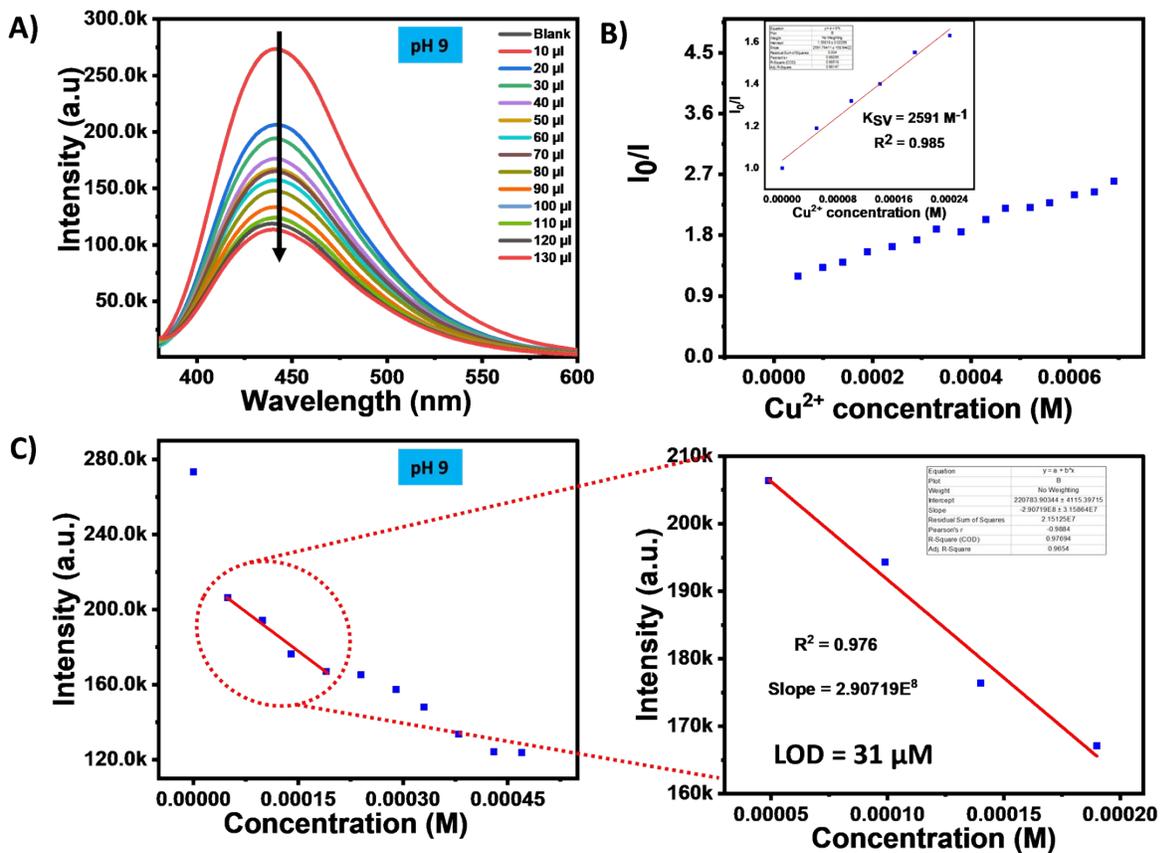
**Figure S22:** A) Fluorescence emission spectra of NANO-PAMAM-CHT at the pH 9 B) Stern-volmer plot. C) LOD plot for $Cu^{2+}$ ion at pH 9.
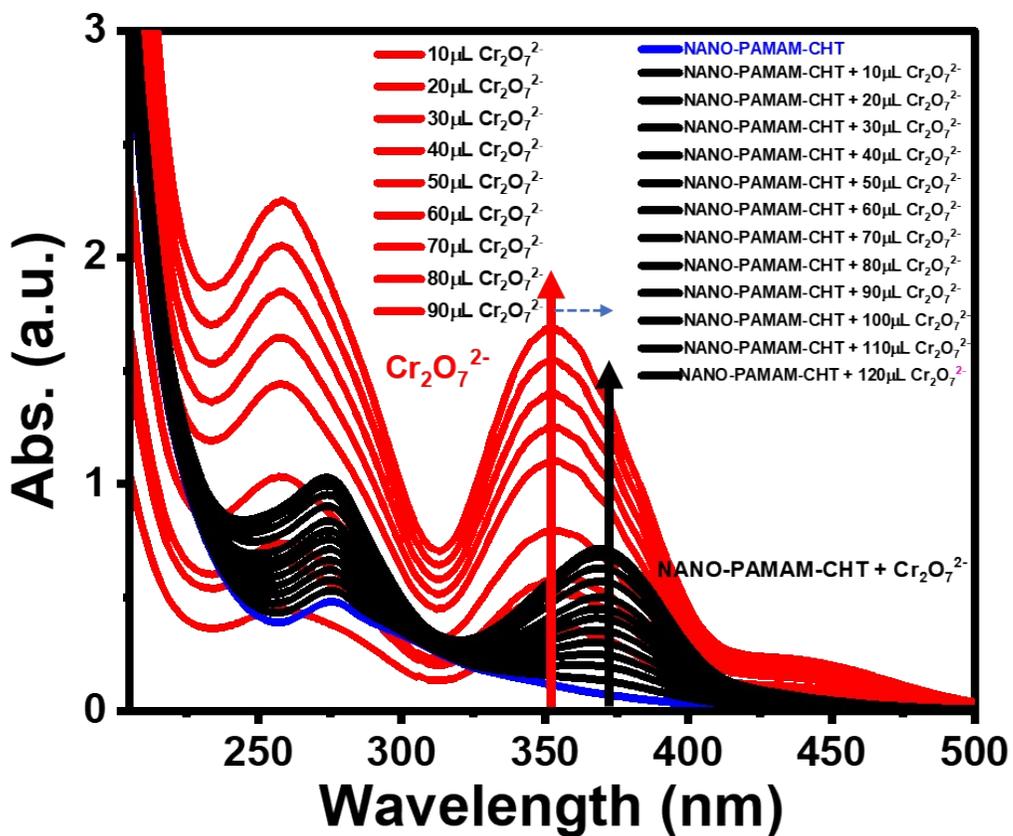
**Figure S23:** Absorption spectra of pure $Cr_2O_7^{2-}$ solution in aqueous solution and NANO-PAMAM-CHT in absence and presence of $Cr_2O_7^{2-}$ ion in aqueous solution (significant red shift was noted compared to pure dichromate solution, ~ 20 nm).

**Table S4:** Fluorescence decay parameters of NANO-PAMAM-CHT in absence and presence of $Cu^{2+}$ ion.

| Sample | $a_1$ | $a_2$ | $a_3$ | $t_1$ | $t_2$ | $t_3$ | $\chi^2$ |
|---|---|---|---|---|---|---|---|
| NANO-PAMAM-CHT | 11.46 | 34.85 | 53.68 | 0.2869 | 1.6395 | 6.1995 | 1.026 |
| NANO-PAMAM-CHT-$Cu^{2+}$ | 37.63 | 62.37 | | 0.7345 | 5.2414 | | 1.033 |

**Table S5:** Fluorescence decay parameters of NANO-PAMAM-CHT in absence and presence of $Cr_2O_7^{2-}$ ion.

| Sample | $a_1$ | $a_2$ | $a_3$ | $t_1$ | $t_2$ | $t_3$ | $\chi^2$ |
|---|---|---|---|---|---|---|---|
| NANO-PAMAM-CHT | 8.59 | 32.62 | 58.79 | 0.1711 | 1.6162 | 5.8399 | 1.010 |
| NANO-PAMAM-CHT-$Cr_2O_7^{2-}$ | 8.41 | 31.68 | 59.92 | 0.1653 | 1.5637 | 5.7363 | 1.068 |

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