Supporting Information

Thiol Ligand-Mediated Exfoliation of Bulk Sulfur to Nanosheets and Nanodots: Application in Antibacterial Activity

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1. Ligand synthesis:

1.1 Characterization of ligands

For the characterization of the ligands, a Bruker spectrometer operating at 400 MHz was employed for the acquisition of NMR spectra, with TMS (tetramethyl silane) serving as an internal standard. The chemical shift values in the NMR data were reported in "δ" parts per million (ppm). The multiplicity of each NMR peak was indicated as follows: "s" for singlet, "d" for doublet, "t" for triplet, "q" for quartet, "br" for broad, and "m" for multiplet. High-resolution mass spectra were obtained using the XEVO G2-XS QTof instrument.

1.2 Synthesis of thiol-ligands:

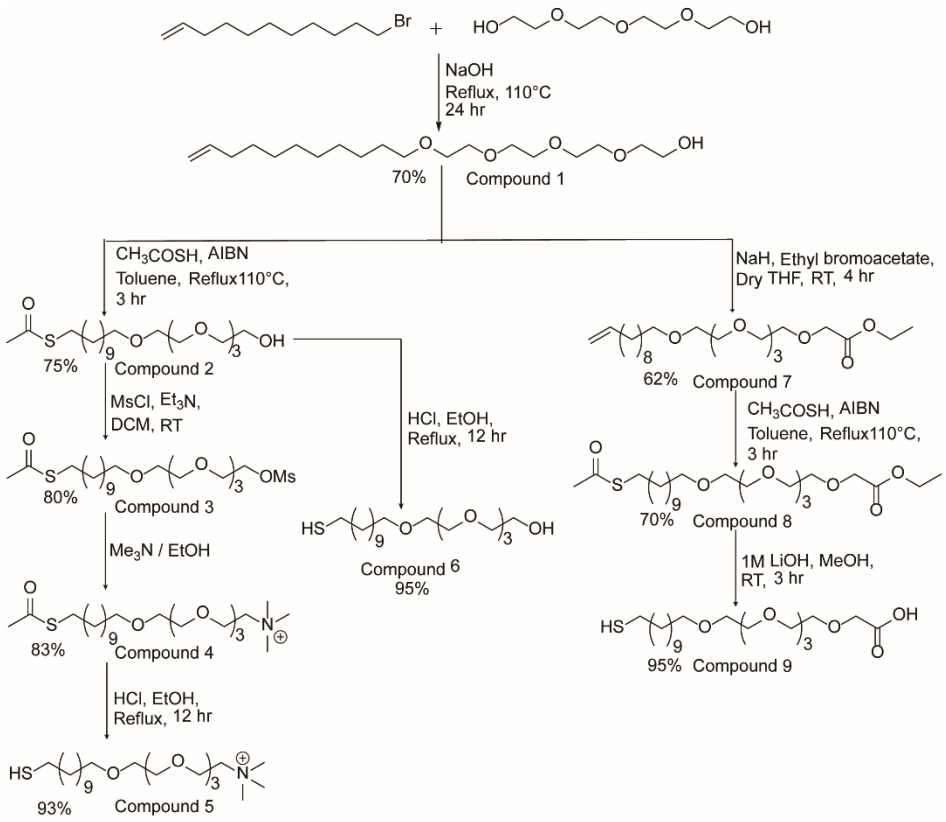

Figure S1. Scheme for synthesis of thiolated ligands.

Synthesis of compound 1:

A mixture comprising 21.4 mmol of sodium hydroxide and 106.5 mmol of tetraethyleneglycol was subjected to stirring at 100°C under an argon atmosphere for approximately 30 minutes. Subsequently, 11-bromoundec-1-ene (21.4 mmol) was added gradually. Following a 24-hour reaction period, the reaction mixture was cooled to room temperature, and the product was extracted via hexane washes, repeated six times. All the hexane portions were combined and concentrated in a rotary evaporator, giving yellow oil containing a mixture of mono-alkylated and di-alkylated products. The monoalkylated product was further purified through column chromatography, affording a yield of 70%.

Synthesis of compound 2:

In a two-necked round bottom flask (RB) under an argon atmosphere, Compound 1 (5.77 mmol) was placed, and then 15 ml of dry toluene was added. Subsequently, Azobisisobutyronitrile (AIBN) (0.3 equivalent) was added, followed by the addition of thiol acetic acid (11.44 mmol). The resulting mixture was refluxed at 110°C for a duration of 3 hours. Following this reflux period, the reaction mixture was dissolved in ethyl acetate and subjected to two washes with a saturated sodium bicarbonate solution. The organic layer was then dried using anhydrous sodium sulfate and concentrated using a rotary evaporator. The crude reaction mixture underwent purification via column chromatography, leading to the isolation of the purified product, which was characterized by ^{1}H-NMR, yielding a 75% yield.

Synthesis of compound 3:

In a round bottom flask (RB), Compound 2 (4.4 mmol) was taken and then dissolved in 20 ml of dichloromethane (DCM) at ice-cold temperatures. To this reaction mixture, triethylamine (8.80 mmol) was introduced at 0°C and stirred for 15 minutes. Subsequently, methane sulfonyl chloride (6.6 mmol) was added dropwise. The reaction mixture was stirred at room temperature overnight. Upon completion of the reaction, DCM was evaporated, and the resulting crude mixture was re-dissolved in ethyl acetate. It underwent two washes with a dilute HCl solution (0.1 M) and three washes with a saturated sodium bicarbonate solution. The organic layer was then subjected to drying using anhydrous sodium sulfate, followed by vacuum concentration and purification via column chromatography. Confirmation of the product's identity was achieved through ^{1}H-NMR analysis, yielding an 80% yield.

Synthesis of compound 4:

Compound 3 (3.65 mmol) was first dissolved in 15 ml of ethanol (EtOH), and 7.3 mmol of trimethylamine (33% in EtOH) was added. The reaction mixture was stirred under a nitrogen atmosphere at room temperature, until the starting material disappeared, as monitored by TLC (thin-layer chromatography). If necessary, additional trimethylamine was added after TLC monitoring at regular intervals. The resulting product was purified by trituration with hexane: ether mixture (1:1) and placed in the refrigerator overnight. The precipitated product was collected and characterized by ^{1}H-NMR, with a typical yield of approximately 83%.

Synthesis of compound 5 (positive thiol ligand):

Compound 4 (3.736 mmol) was initially dissolved in 20 ml of ethanol (EtOH). Subsequently, 1 ml of hydrochloric acid (HCl) was added, and the mixture was refluxed for a duration of 12 hours. Following this reflux period, the solvent was removed by evaporation, and the resulting product underwent purification through trituration, a process that was repeated three times using hexane: diethyl ether mixture (1:1). During each trituration step, the precipitated product was collected, and its identity was confirmed through ^{1}H-NMR and HRMS analysis. The overall yield of the compound was 93%.

Synthesis compound 6 (neutral thiol ligand):

Compound 2 (2.36 mmol) was initially dissolved in ethanol (EtOH), and then 1 ml of hydrochloric acid (HCl) was introduced. The mixture was subsequently refluxed for a period of 12 hours. Upon completion of the reaction, the reaction mixture underwent washing with a brine solution. The concentrated organic layer was then subjected to column chromatography. The resulting product was confirmed through ^{1}H-NMR and HRMS analysis, and it yielded a 95% yield.

Synthesis of compound 7 :

Sodium hydride (6.92 mmol) was placed in a round-bottom flask (RB) and maintained at 0°C under an argon atmosphere. Subsequently, 10 ml of dry tetrahydrofuran (THF) was added. Compound 1 (5.77 mmol) was placed under an argon atmosphere and dissolved in dry THF in a separate RB. This solution of Compound 1 was then added to the RB containing sodium hydride in THF. After 15 minutes, ethylbromoacetate was slowly introduced into the reaction mixture, which was kept at a low temperature under an argon atmosphere. The mixture was stirred at room temperature for 4 hours. After completion of the reaction, the reaction was quenched by adding a few drops of water, and the resulting crude mixture was concentrated

using a rotary evaporator. Subsequently, the crude mixture was dissolved in ethyl acetate and subjected to washing with a brine solution. The concentrated organic layer was then purified through column chromatography. The identity of the purified product was confirmed via ^{1}H-NMR analysis, and it yielded a 62% yield.

Synthesis of compound 8:

The procedure resembles that of compound 2. The resulting product was validated through ^{1}H NMR analysis, yielding a 70% yield.

Synthesis of compound 9 (negative thiol ligand):

Compound 8 (1.96 mmol) was initially dissolved in 15 ml of methanol (MeOH). Subsequently, 4 ml of 1 M lithium hydroxide solution was added to the solution and stirred at room temperature for 3 hours. The resulting solution was then cooled to 0°C. To adjust the pH to approximately 2, 1 M HCl was added. The solvent was subsequently evaporated, and the residue was re-dissolved in ethyl acetate. This solution underwent three washes with brine. The concentrated organic layer was then subjected to purification through column chromatography. The purified product was confirmed via ^{1}H-NMR and HRMS analysis.

Compound 5 (positive thiol ligand) (R= -CH$_3$): ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.984 (br, 2H, -CH_2-N^+-), 3.679-3.589 (14H, -O-CH_2-CH_2-O-), 3.468-3.434 (t, 2H, -CH_2-**CH_2**-O-), 3.375 (s, 9H, -N^+ (CH_3)$_3$), 2.775 (s, 3H, CH_3-SO_3-), 2.712- 2.506 (m, 2H, -CH_2-SH), 1.615-1.562 (m, 4H, -CH_2-), 1.283 (m, 14H, -CH_2).

Compound 6 (neutral thiol ligand) ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.771-3.609 (m, 16H, -O-CH_2-CH_2-O-), 3.492-3.458 (t, 2H, - CH_2-**CH_2**-O-), 2.719-2.517 (m, 2H, -**CH_2**-SH), 1.625-1.588 (m, 4H, -CH_2-), 1.289 (m, 14H, -CH_2).

Compound 9 (negative thiol ligand) ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.021 (s, 2H,-O-CH_2-CO-), 3.674-3.594 (m, 16H, -O-CH_2-CH_2-O-), 3.477-3.444 (t, 2H, -CH_2-**CH_2**-O-), 2.721-2.685 (m, 2H, -**CH_2**-SH), 1.707-1.293 (m, 18H, -CH_2-).

1.3 Synthesis of non-thiol ligands:

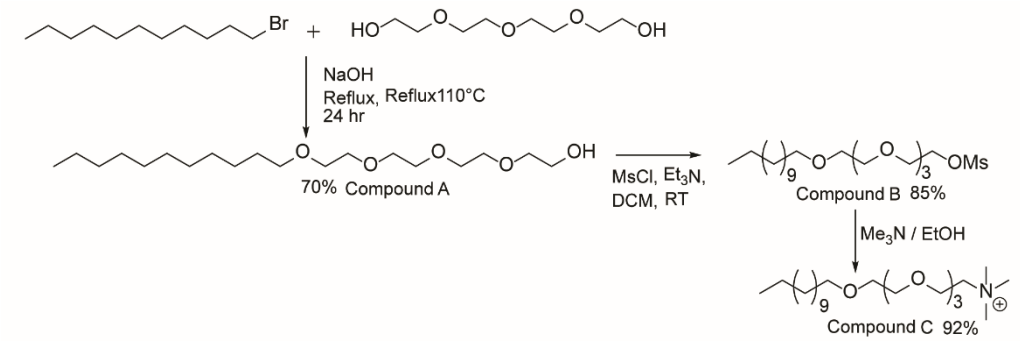

Figure S2. Scheme for synthesis of non-thiolated ligands.

Synthesis of compound A (neutral non-thiol ligand):

At 100°C in an argon environment, a mixture consisting of tetraethylene glycol (127.6 mmol) and sodium hydroxide (12.76 mmol) was stirred for around 30 minutes. Then 11-bromoundecane (12.76 mmol) was slowly added. After 24 hours, the reaction mixture was cooled to room temperature. The crude product was extracted from the reaction mixture using hexane repeatedly for six times. All the hexane portions were combined and concentrated in rotary evaporator, giving yellow oil containing a mixture of mono-alkylated and di-alkylated products. The mono-alkylated product was purified by column chromatography with a yield of 70%. The purified product was characterized by ^{1}H-NMR.

Synthesis of compound B:

The neutral non-thiol ligand was dissolved in 20ml of DCM in an RB maintained at 0ºC. Then triethylamine (2.87 mmol) was added to the reaction mixture and stirred for 15 minutes. Thereafter, methane sulfonyl chloride (2.87 mmol) was added slowly to the reaction mixture and stirred at room temperature overnight. After the completion of the reaction, the DCM was evaporated and the crude mixture was dissolved in ethyl acetate, followed by two washes with dilute HCl (0.1M) and saturated sodium bicarbonate solution. The organic layer was dried with anhydrous sodium sulfate, vacuum-concentrated, and purified using column chromatography. The product was validated by ^{1}H-NMR with an 85% yield.

Synthesis of compound C (positive non-thiol ligand):

Compound B (1.429 mmol) was dissolved in 10 mL of DCM, followed by addition of 2.858 mmol of trimethylamine (33% in EtOH). The reaction was allowed to stir at room temperature in an argon environment until the starting material disappeared, as measured by TLC. If necessary, trimethylamine was added at regular intervals based on the amount of starting

material left. The product is purified using the trituration process in Hexane and diethylether (1:1) and stored in the refrigerator overnight. With a 92% yield, the precipitated product was collected and analysed by ^{1}H-NMR.

Compound A (neutral non-thiol ligand) ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.0 (br, 2H, O-CH_2-**CH_2**-N^+), 3.942-3.930 (br, 2H, O-**CH_2**-CH_2-N+), 3.691-3.681 (br, 2H, -CH_2-**CH_2**-O-), 3.636-3.573 (m, 12H, -O-CH_2-CH_2-O-), 3.449 (s, 9H, -N^+ $(CH_3)_3$), 2.768 (s,3H, -OMs), 1.599-1.489 (m, 2H, -CH_2-CH_2-O-), 1.273 (s, 16H, -CH_2), 0.910-0.878 (t, 3H,-CH_3).

Compound C (positive non-thiol ligand): ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.745-3.578 (m, 16H, -O-**CH_2-CH_2**-O-), 3.471-3.437 (t, 2H, -CH_2-**CH_2**-O-), 1.599-1.548 (m, 2H, -**CH_2**-CH_2-O-), 1.264 (br, 16H, -CH_2-), 0.901-0.868 (t, 3H, -CH_3).

2. SEM sample preparation of MRSA:

For the preparation of SEM samples, MRSA was cultured in LB media, harvested during the mid-log phase through centrifugation at 5000 rpm for 6 minutes, and the OD was adjusted to 0.1. Subsequently, the bacterial pellet was resuspended in PBS and treated with BASNs with the corresponding MBC value for 2 hr. After the incubation, the bacterial suspension underwent another round of centrifugation at 5000 rpm for 5 minutes. Following this step, the bacteria were fixed using a 3% glutaraldehyde solution for an additional hour. Post-glutaraldehyde fixation, the samples were dehydrated using an ethanol gradient and then drop-casted onto a silicon wafer. Prior to imaging with SEM, the samples were sputtered with a layer of gold.

3. TEM sample preparation for bacterial imaging:

MRSA bacteria were collected during their mid-logarithmic growth phase, characterized by an OD of 0.1 at 620 nm. These bacterial cultures were then subjected to thorough triple washes with PBS buffer. Following this, the bacterial cells were resuspended in deionized (DI) water. The bacterial suspensions were subsequently treated with BASNs at concentrations corresponding to their MBC. This treatment was carried out for a duration of 1 hour at a temperature of 37°C. After the incubation period, any unbound material was effectively removed through centrifugation. For TEM sample preparation, 10 µL of the treated bacterial solution was carefully drop-casted onto carbon-coated copper grids that had been previously subjected to UV treatment. This deposition was allowed to sit undisturbed for a duration of 10 minutes. Afterward, any excess solution was gently blotted away using blotting paper. To

enhance contrast and facilitate TEM imaging, negative staining by uranyl acetate (0.5%) was done.

4. Cell viability assay:

The Cytotoxicity was evaluated by subjecting HeLa cells to an MTT assay to access the effects of BASNs. The experimental process commenced by seeding the HeLa cells onto 96-well plates at 15,000 cells/mL density. In each well, 100 µL of Dulbecco's Modified Eagle Medium (DMEM) was dispensed, and the cells were allowed to undergo overnight incubation to establish their initial conditions. Then exfoliated BASNs with varying concentration was added. Subsequently, the cells were subjected to an extended incubation period of 24 hours. Subsequently, cell viability was assessed using MTT dye (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). After a 4-hour incubation period with the dye, viable cells converted the dye into an insoluble formazan. The formazan crystals were then dissolved in 100 µL of DMSO, and the relative cell viability was determined by measuring the absorbance at 570 nm.

Additional Figures

5. [1]HNMR Spectra of compound 5 (positive thiol ligand)

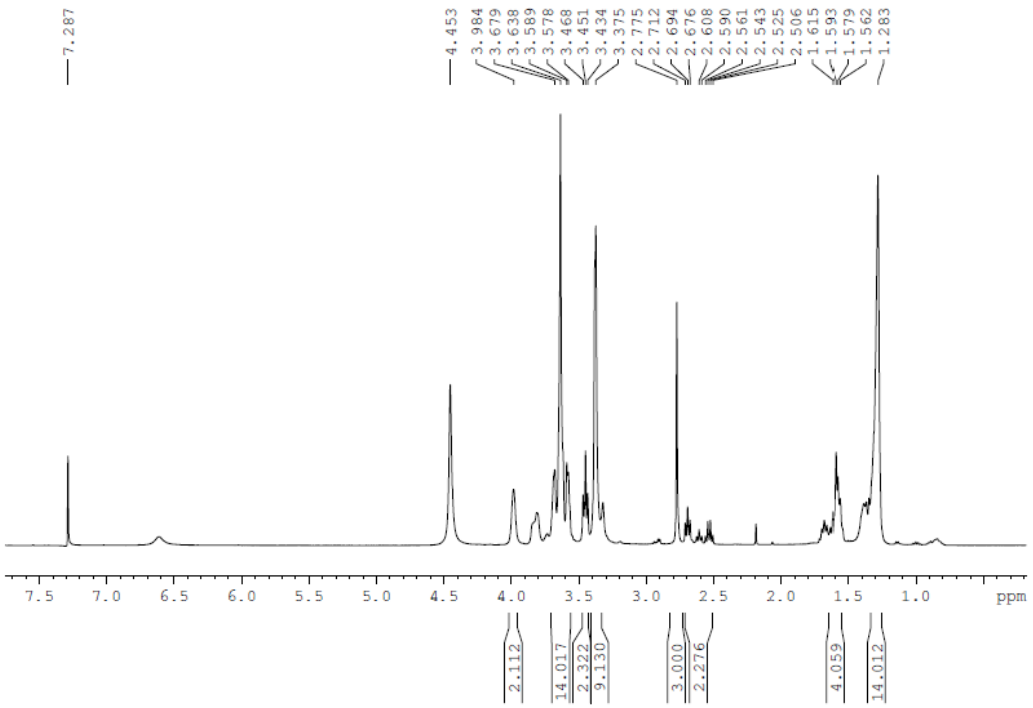

Figure S3. [1]H NMR spectra of positive thiol ligand.

6. HRMS spectra of compound 5 (positive thiol ligand)

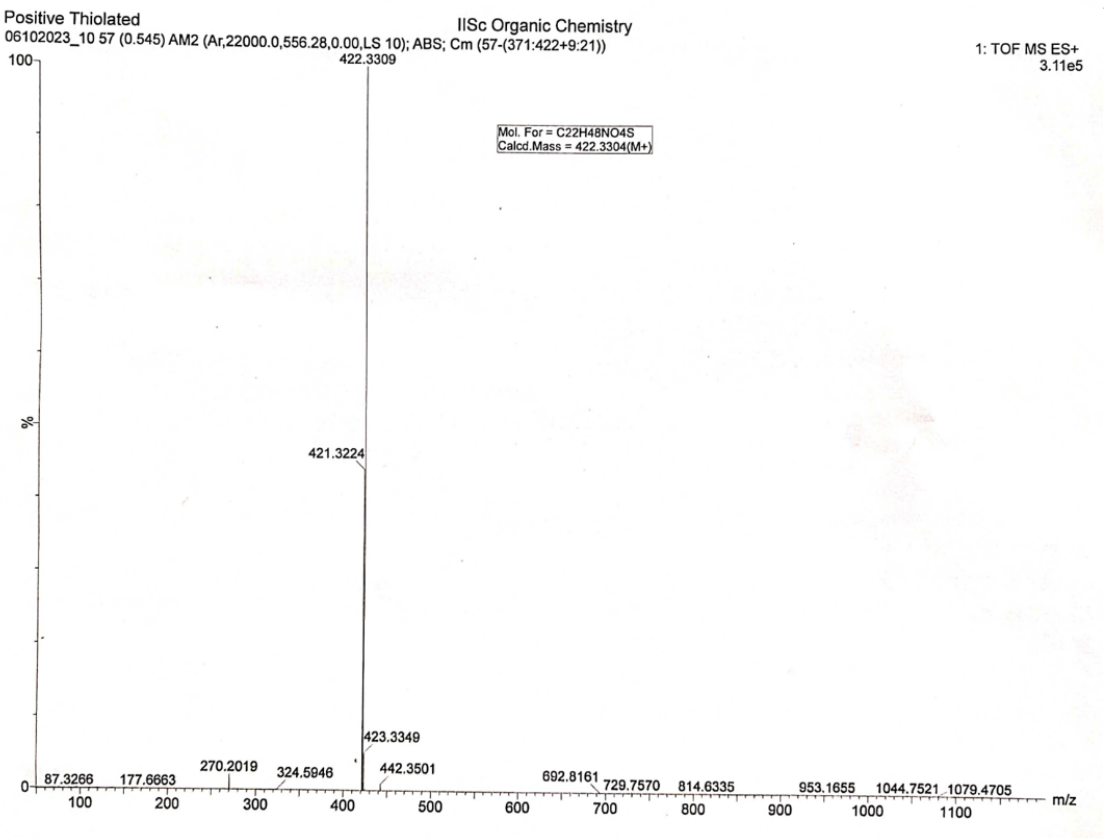

Figure S4. HRMS spectra of positive thiol ligand.

7. [1]HNMR Spectra of compound 6 (neutral thiol ligand)

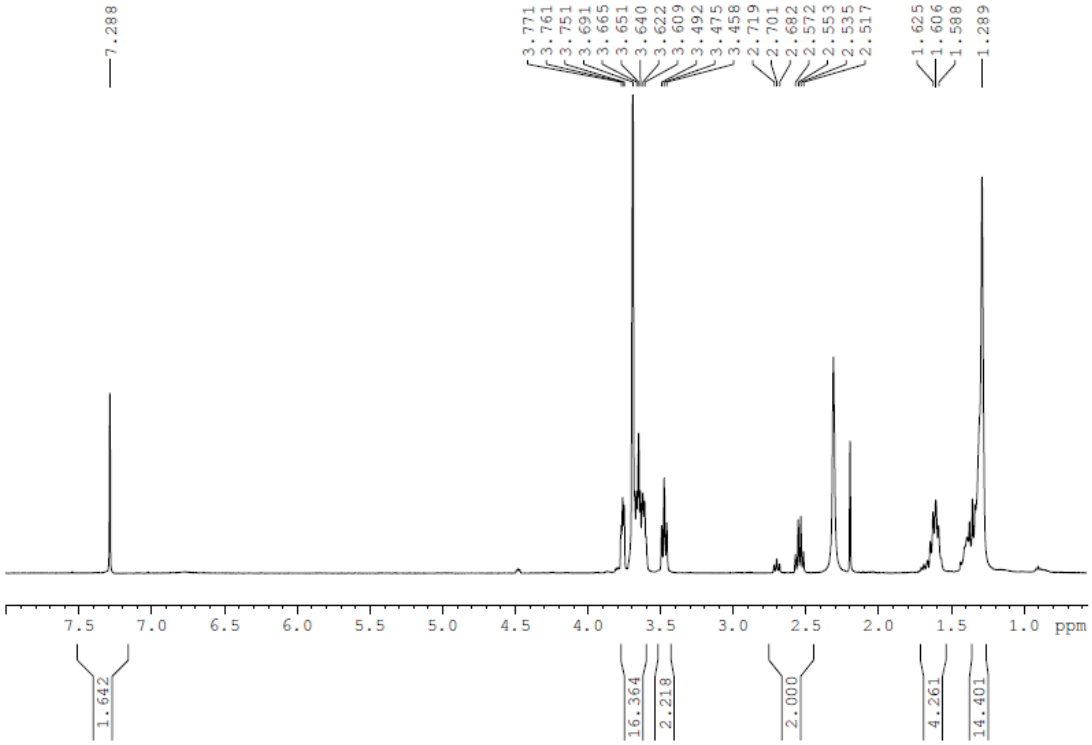

Figure S5. [1]H NMR spectra of neutral thiol ligand.

8. HRMS spectra of compound 6 (neutral thiol ligand)

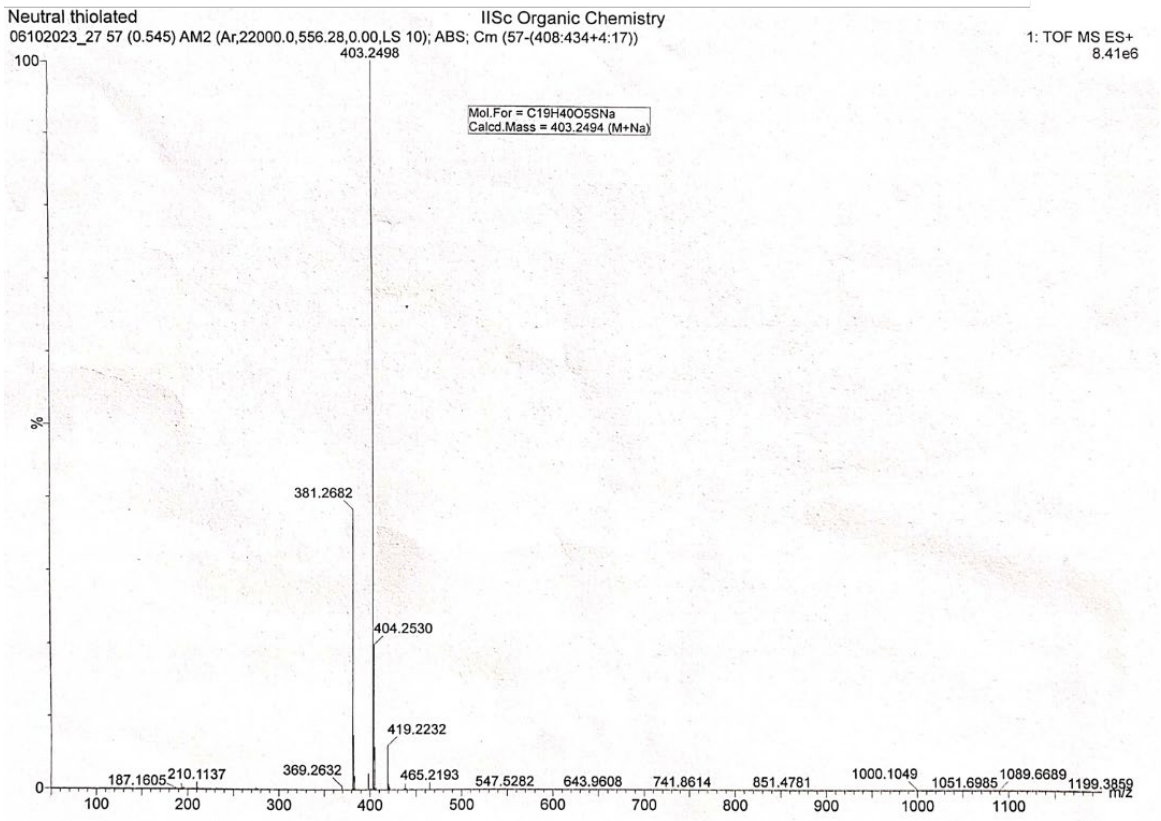

Figure S6. HRMS spectra of neutral thiol ligand.

9. [1]HNMR Spectra of compound 9 (negative thiol ligand)

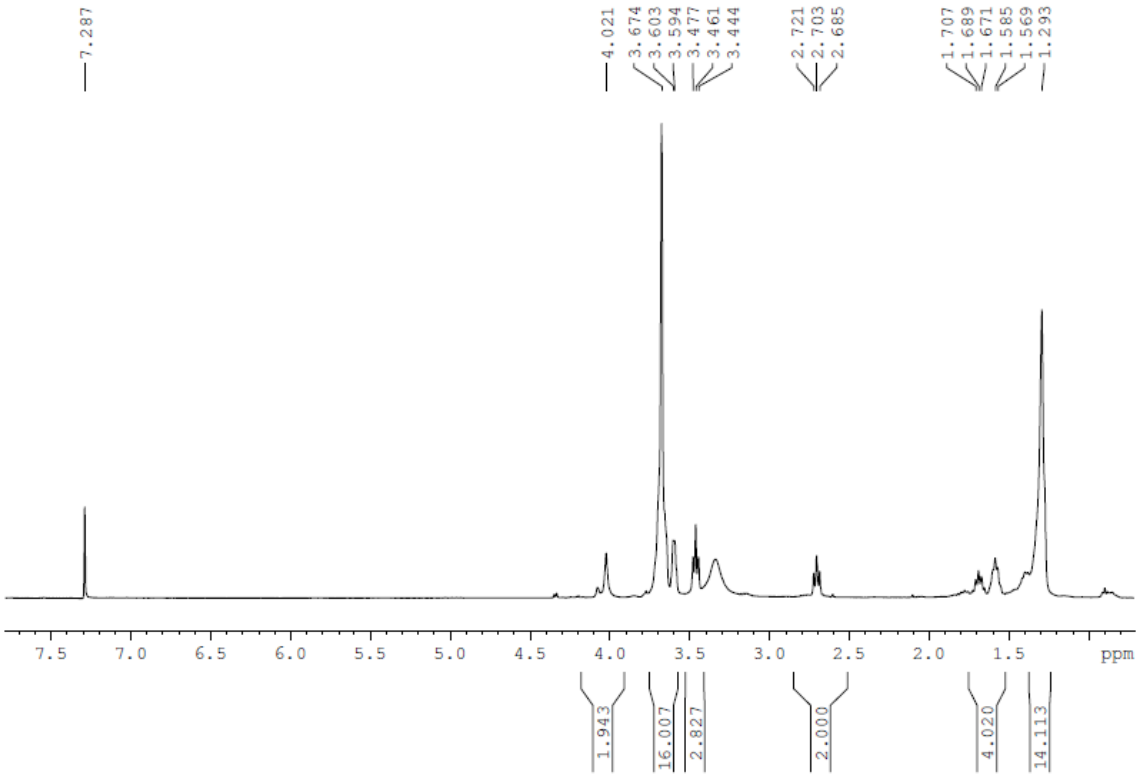

Figure S7. [1]H NMR spectra of negative thiol ligand.

10. HRMS spectra of compound 9 (negative thiol ligand).

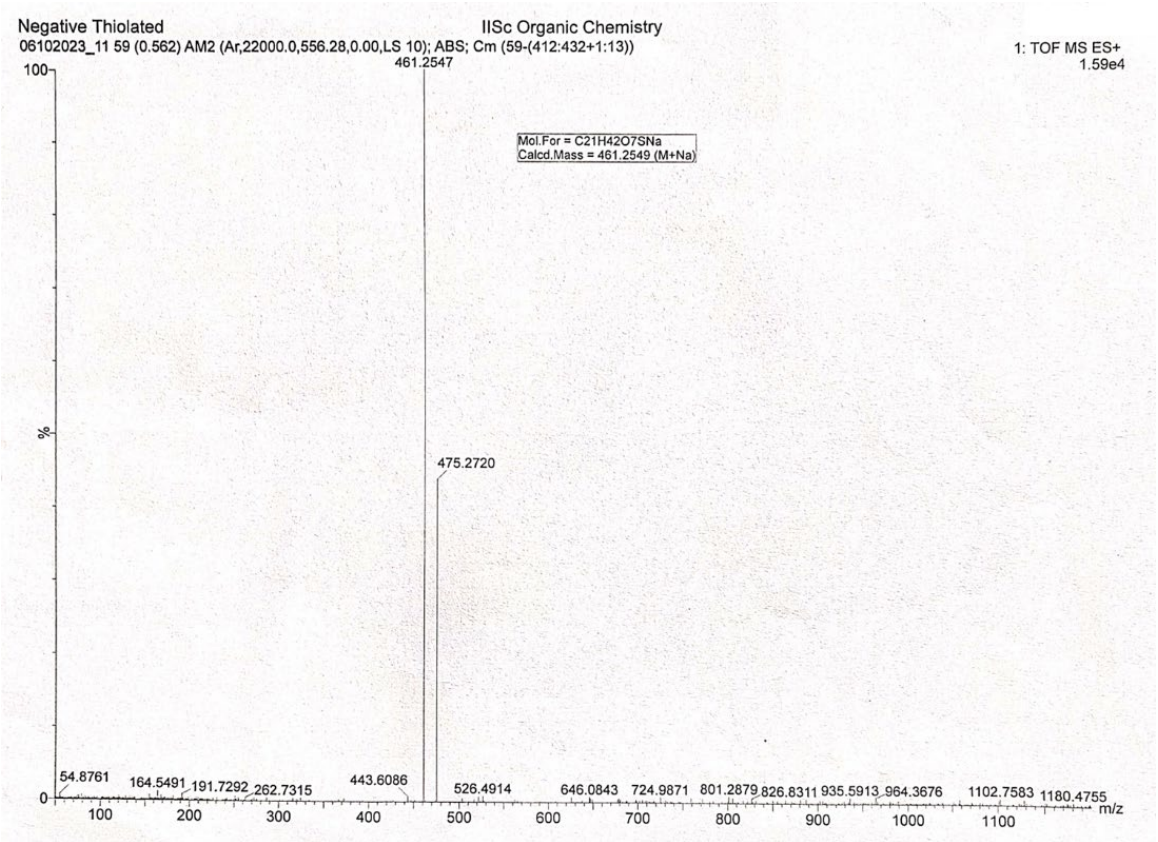

Figure S8. HRMS spectra of negative thiol ligand.

11. [1]HNMR Spectra of compound A (neutral non-thiol ligand).

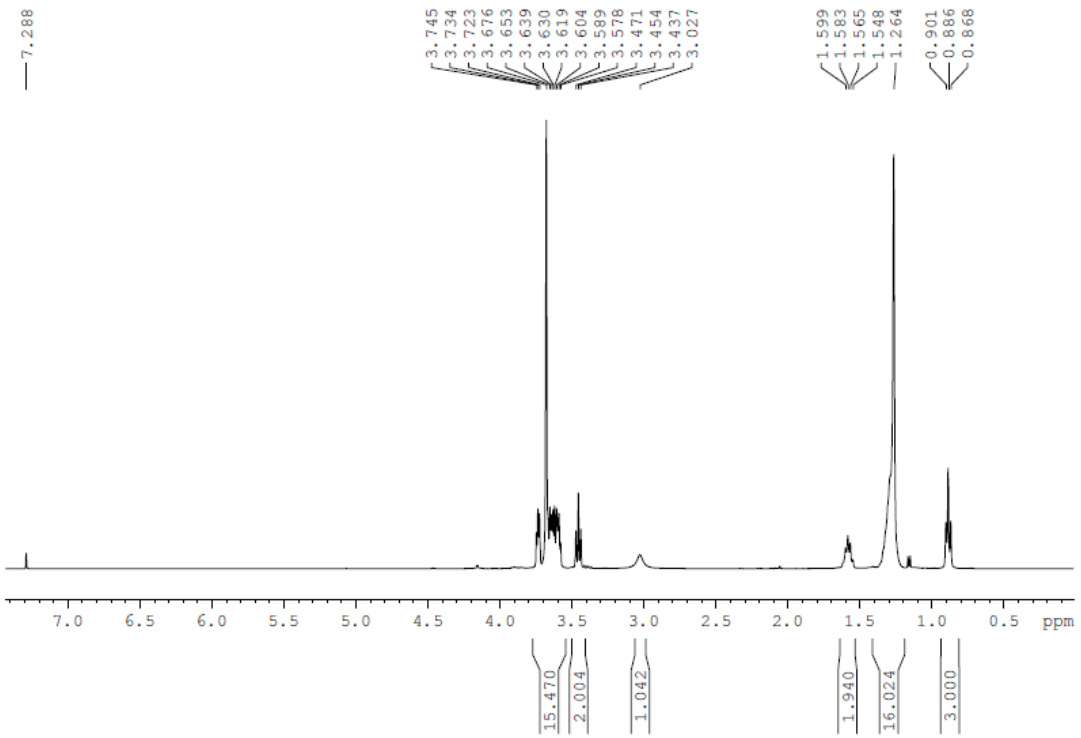

Figure S9. [1]H NMR spectra of neutral non-thiol ligand.

12. HRMS spectra of A (neutral non-thiol ligand).

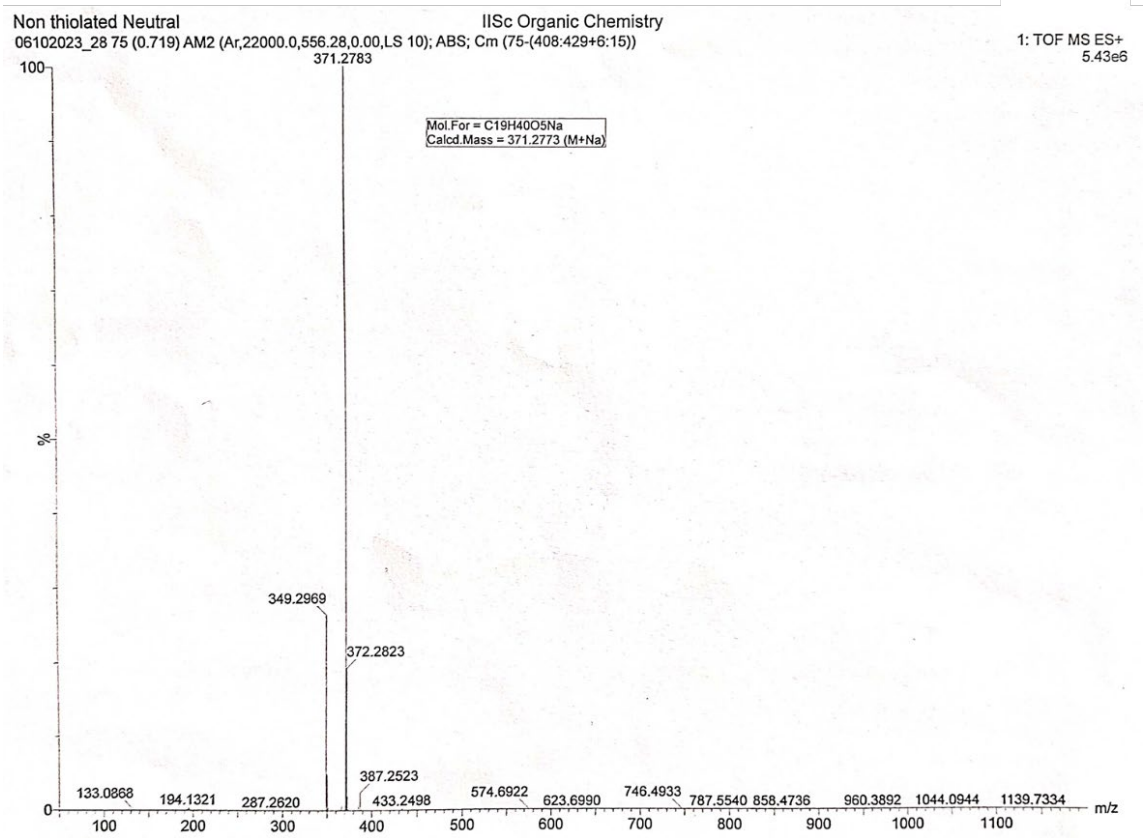

Figure S10. HRMS spectra of neutral non-thiol ligand.

13. [1]HNMR Spectra of compound C (positive non-thiol ligand).

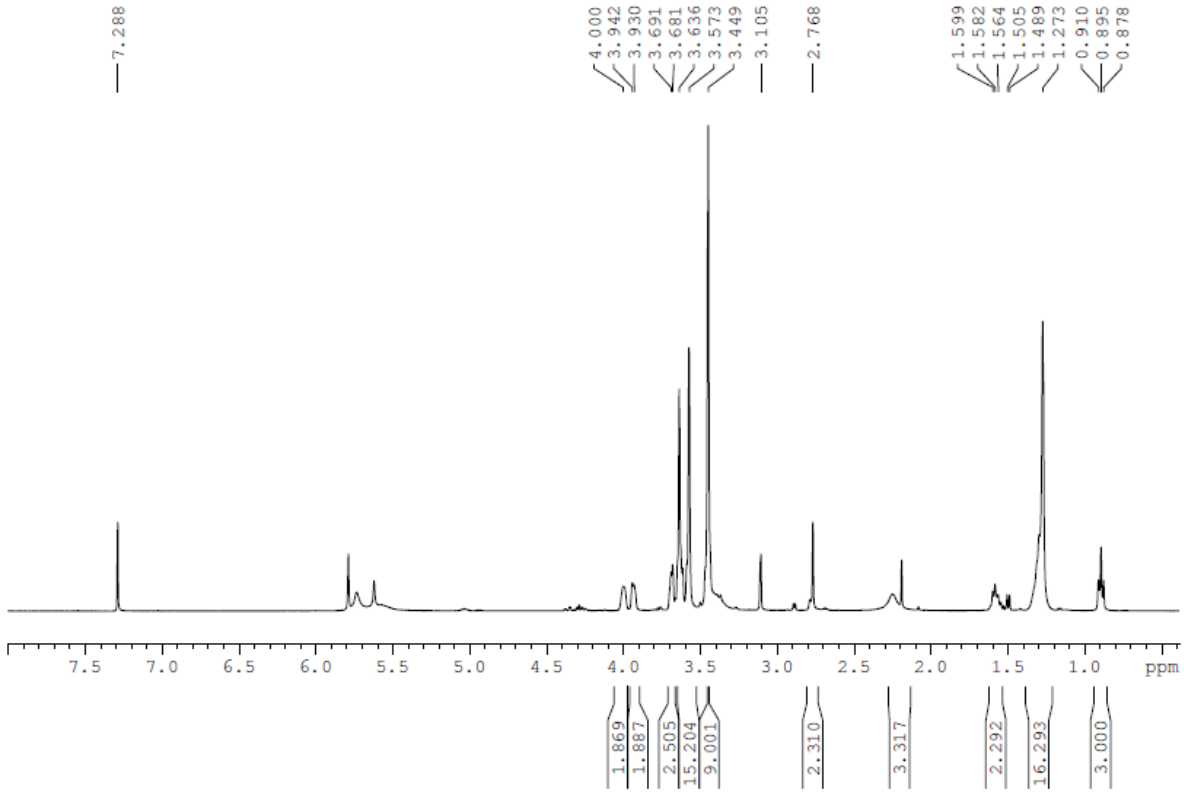

Figure S11. [1]H NMR spectra of positive non-thiol ligand.

14. HRMS Spectra of compound C (positive non-thiol ligand).

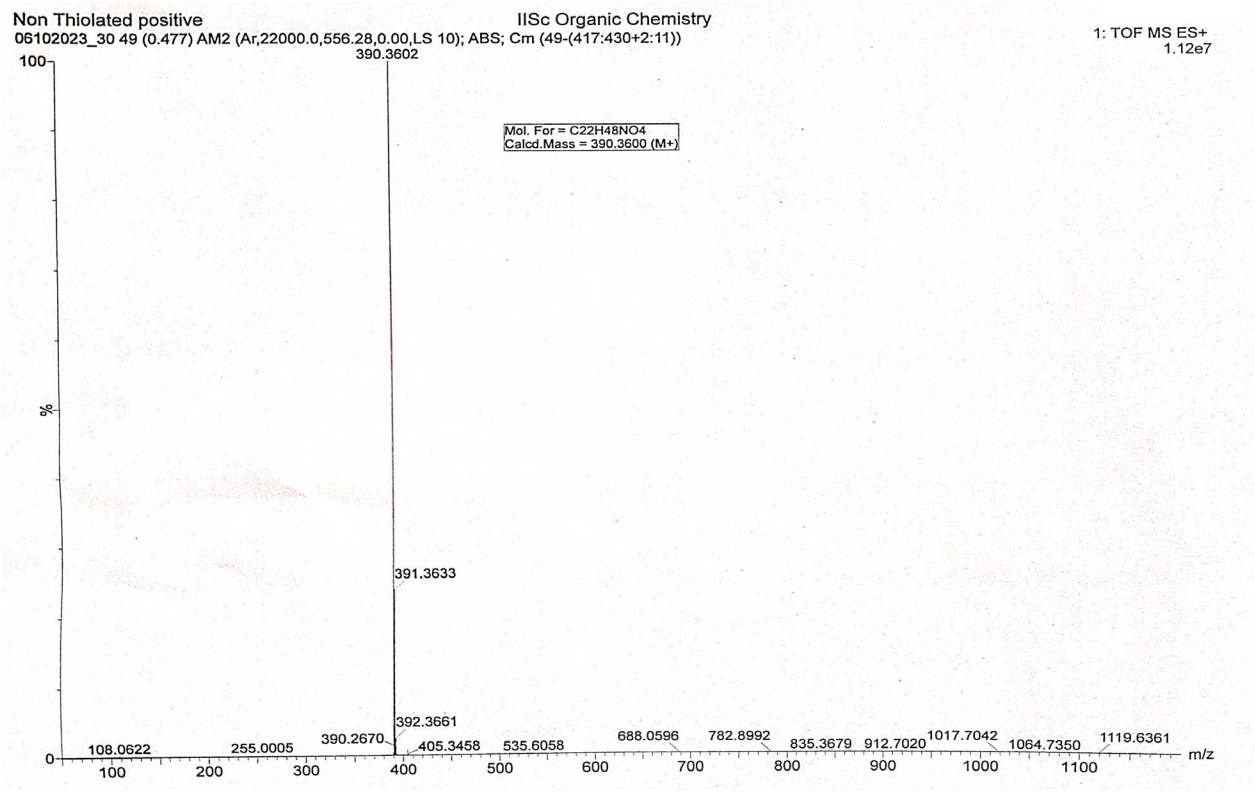

Figure S12. HRMS spectra of positive non-thiol ligand.

15. Yield of the material after exfoliation and after centrifugation

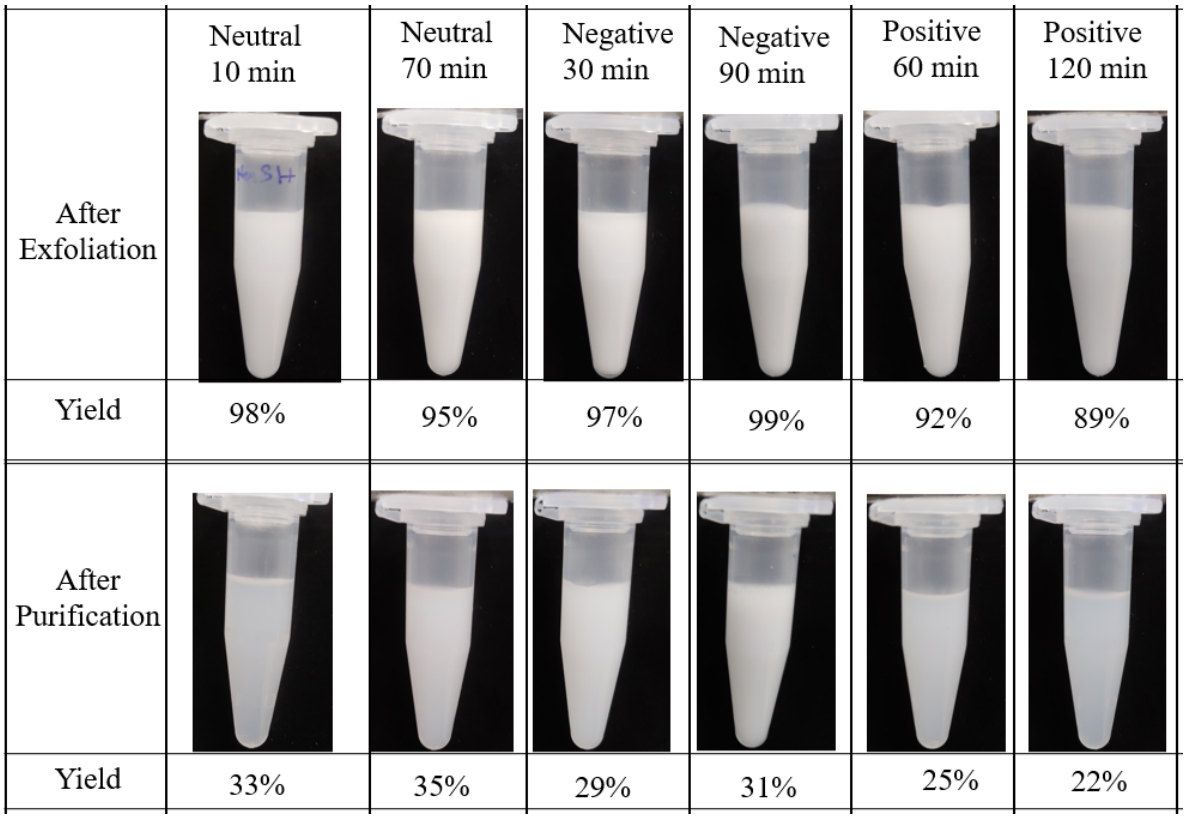

Figure S13. The image showing the exfoliated substances alongside their yield post-exfoliation and subsequent centrifugation.

16. Height distribution of nanosheets and nanodots

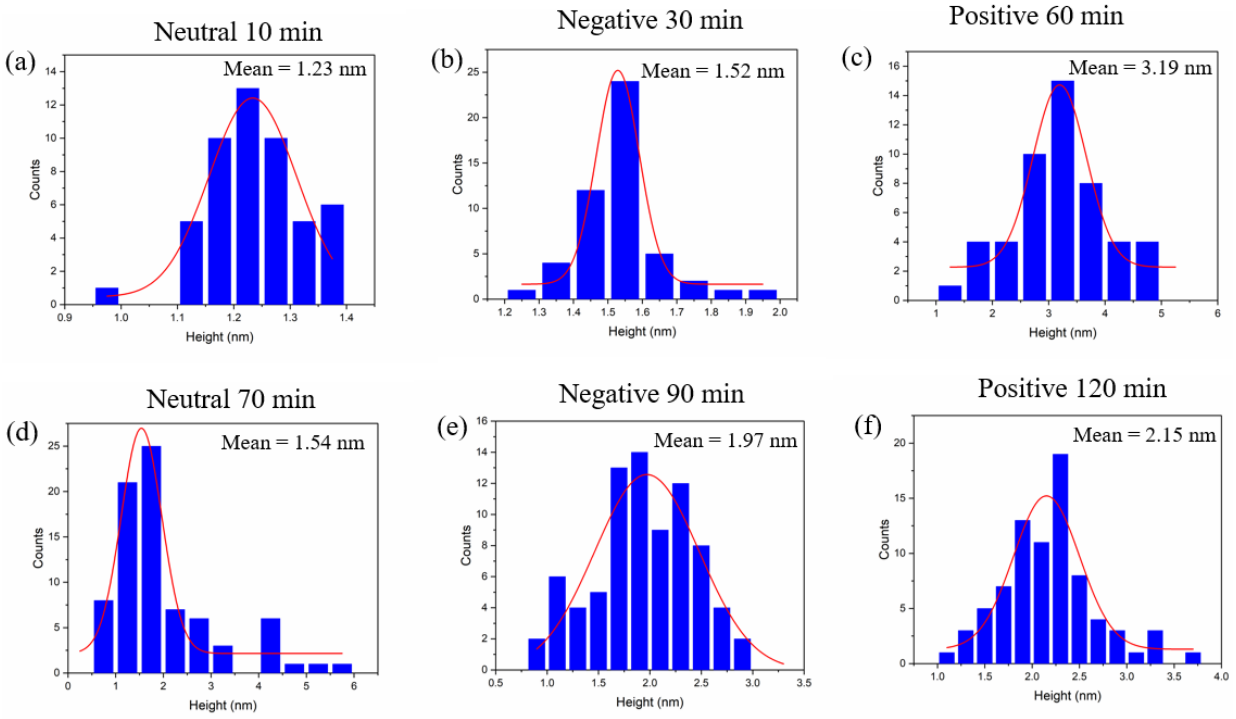

Figure S14. Thickness distribution of the exfoliated material measured from AFM image. (a) Neutral nanosheets, (b) negative nanosheets, (c) positive nanosheets, (d) neutral nanodots, (e) negative nanodots, (f) positive nanodots.

17. Neutral thiol ligand mediated exfoliation of sulfur

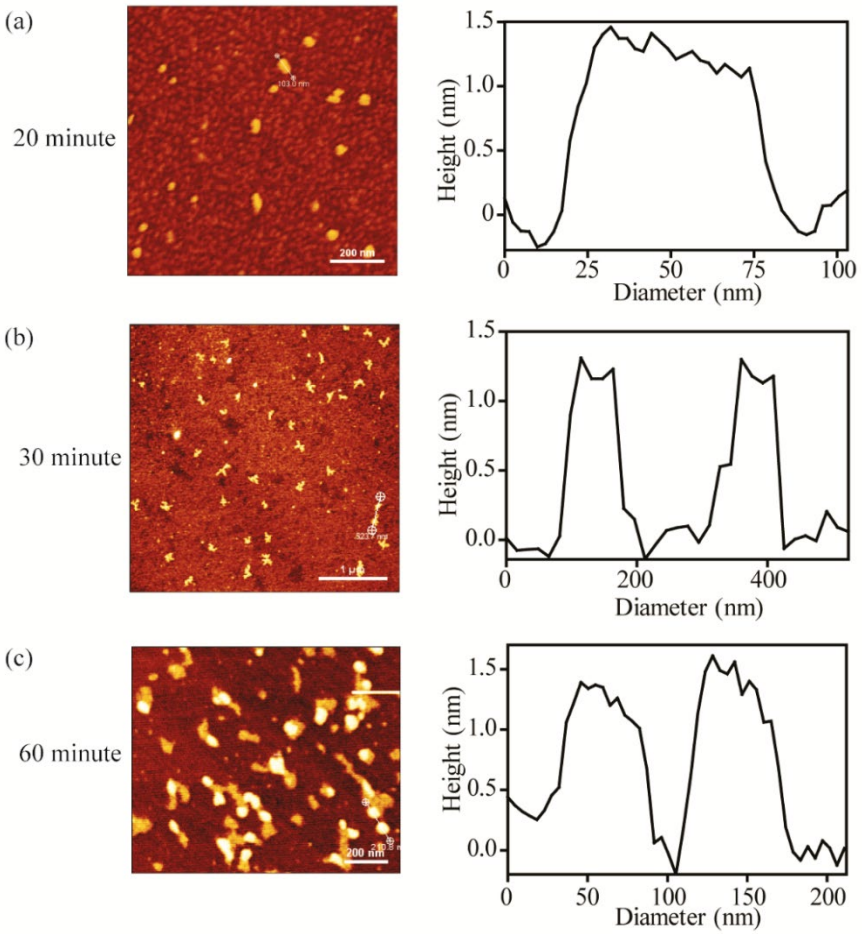

Fig. S15. Neutral thiol mediated exfoliation of bulk sulfur; (a) at the end of 20 minutes, (b) at the end of 30 minutes, (c) at the end of 60 minutes. The AFM image suggests that at the end of 60 minutes nanodots begins to form.

18. DLS measurement of the exfoliated nanostructures.

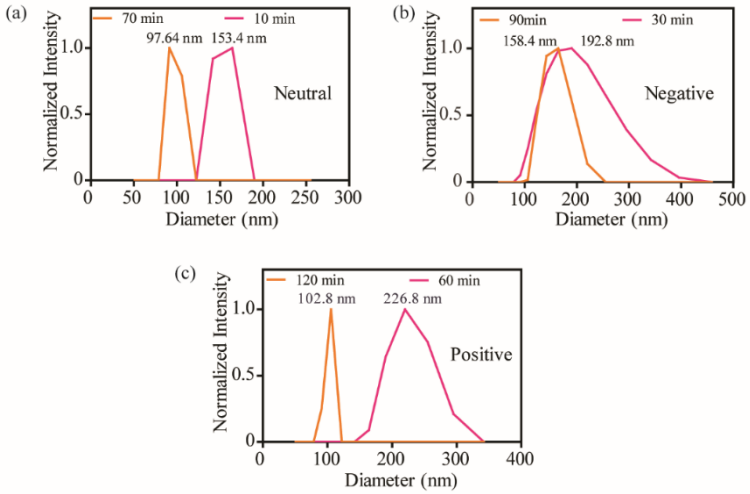

Fig. S16. (a) DLS measurement of neutral thiol ligand mediated exfoliated sulfur at the end of 10 min and 70 min. (b) DLS measurement of negative thiol ligand mediated exfoliated sulfur at the end of 30 min and 90 min. (c) DLS measurement of positive thiol ligand mediated exfoliated sulfur at the end of 60 min and 120 min.

19. Neutral non-thiol ligand mediated exfoliation of sulfur

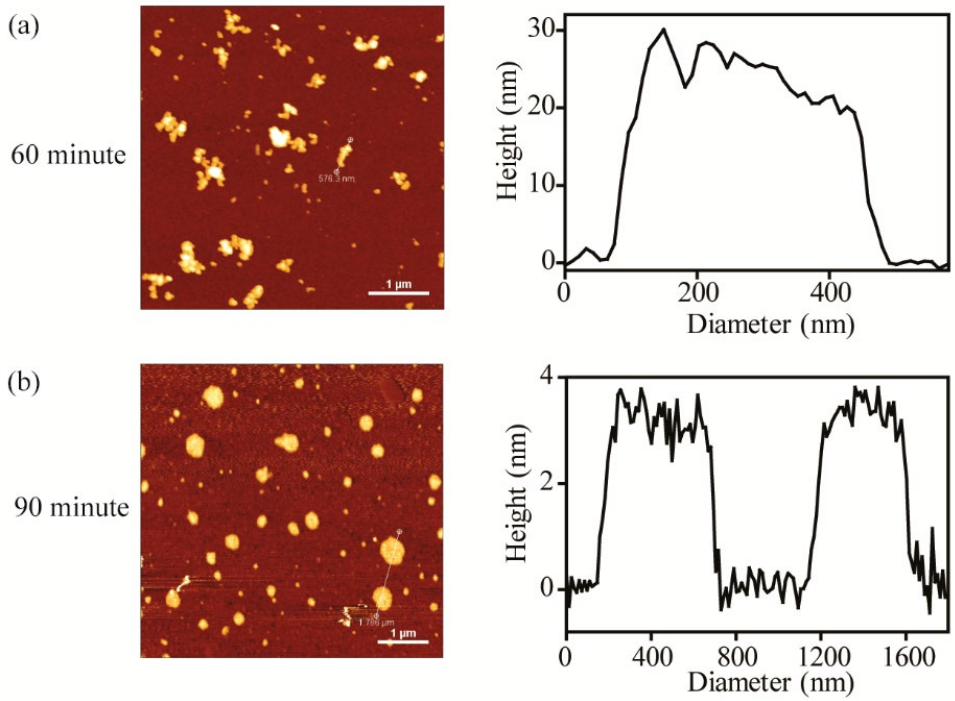

Fig. S17. Neutral non-thiol mediated exfoliation of bulk sulfur; (a) at the end of 60 minutes, (b) at the end of 90 minutes. AFM image suggests that it took 90 minutes of sonication layered nanosheet to form.

20. Positive non-thiol ligand mediated exfoliation of sulfur

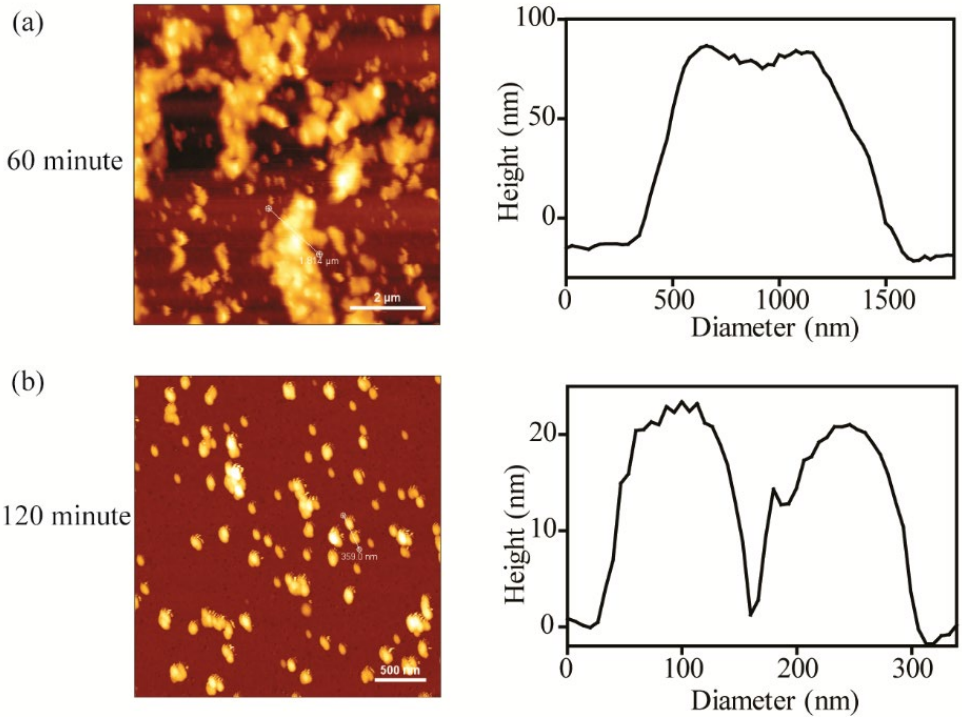

Fig. S18. Positive non-thiol ligand mediated exfoliation of bulk sulfur; (a) at the end of 60 minutes, (b) at the end of 90 minutes. AFM image suggests that non-thiol positive ligand have less efficacy to exfoliate bulk sulfur.

21. Fluorescence emission spectra of exfoliated NSs and NDs

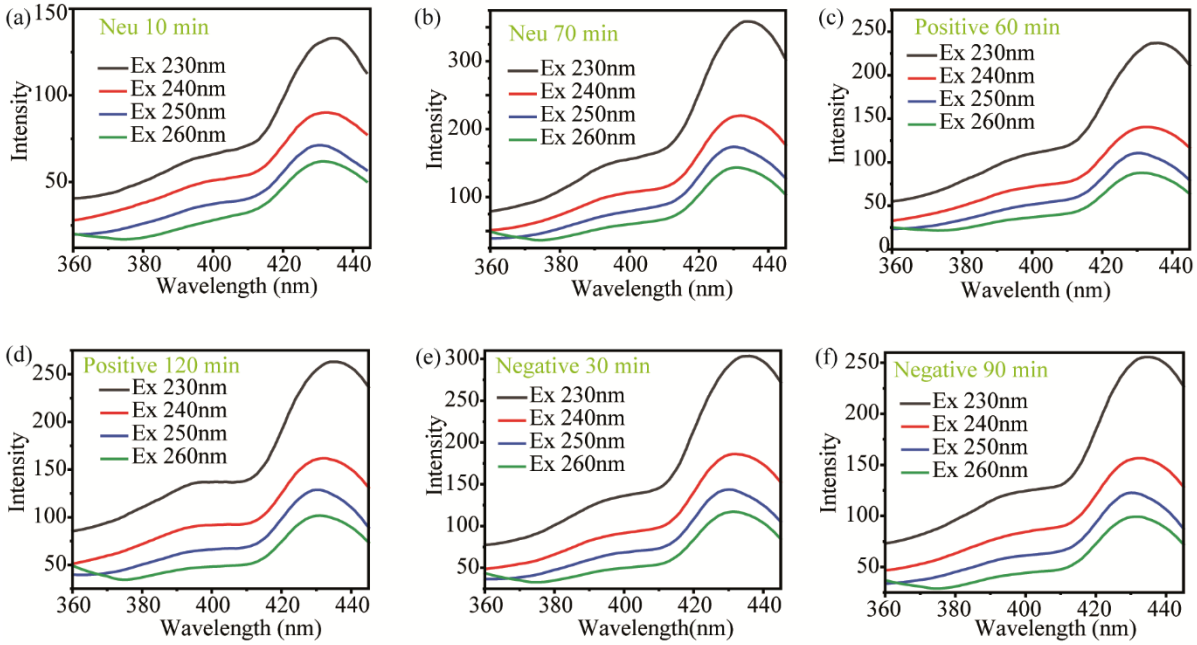

Fig. S19. Fluorescence emission spectra (a) neutral NSs, (b) neu NDs, (c) positive NSs, (d) positive NDs, (e) negative NSs, (f) negative NDs. Fluorescence emission spectra suggests excitation independent emission.

22. Raman spectra of exfoliated NDs

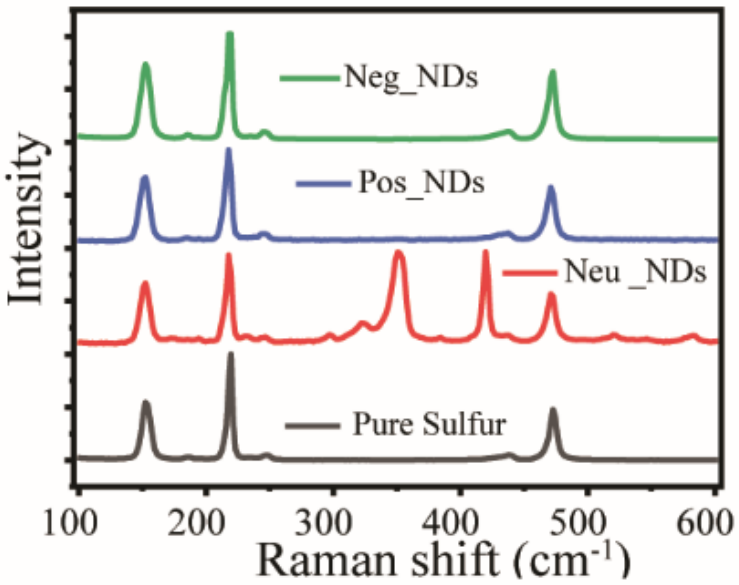

Fig. S20. Raman spectra of exfoliated nanosheets.

23. Cysteine assisted exfoliation of bulk sulfur

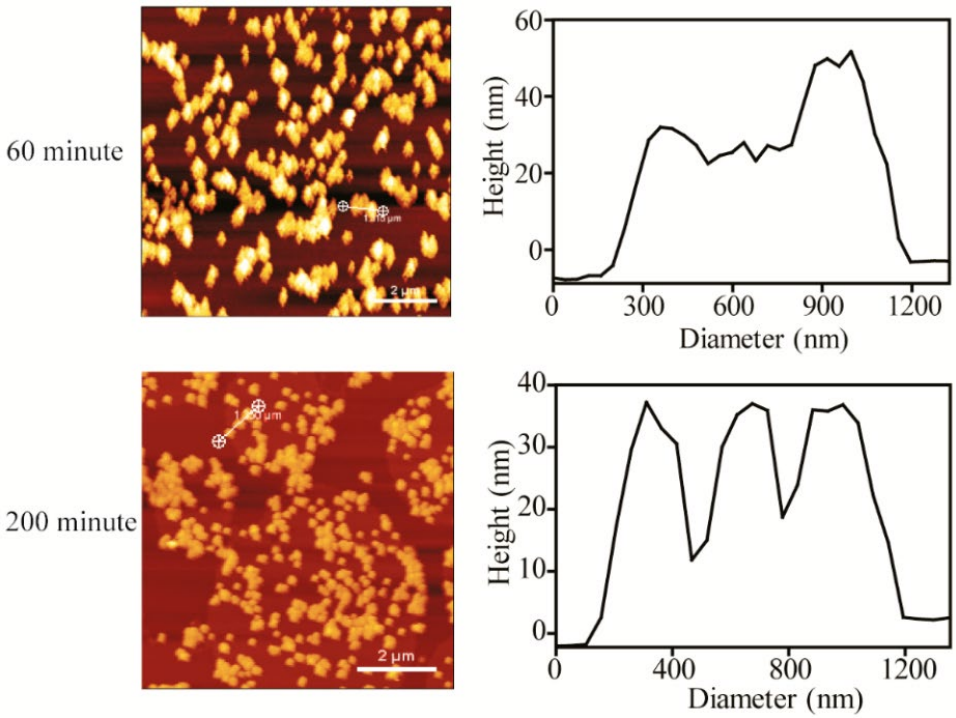

Fig. S21. Cysteine mediated exfoliation of bulk sulfur; (a) at the end of 60 minutes, (b) at the end of 200 minutes.

24. 3-Mercaptopropionic acid assisted exfoliation of bulk sulfur

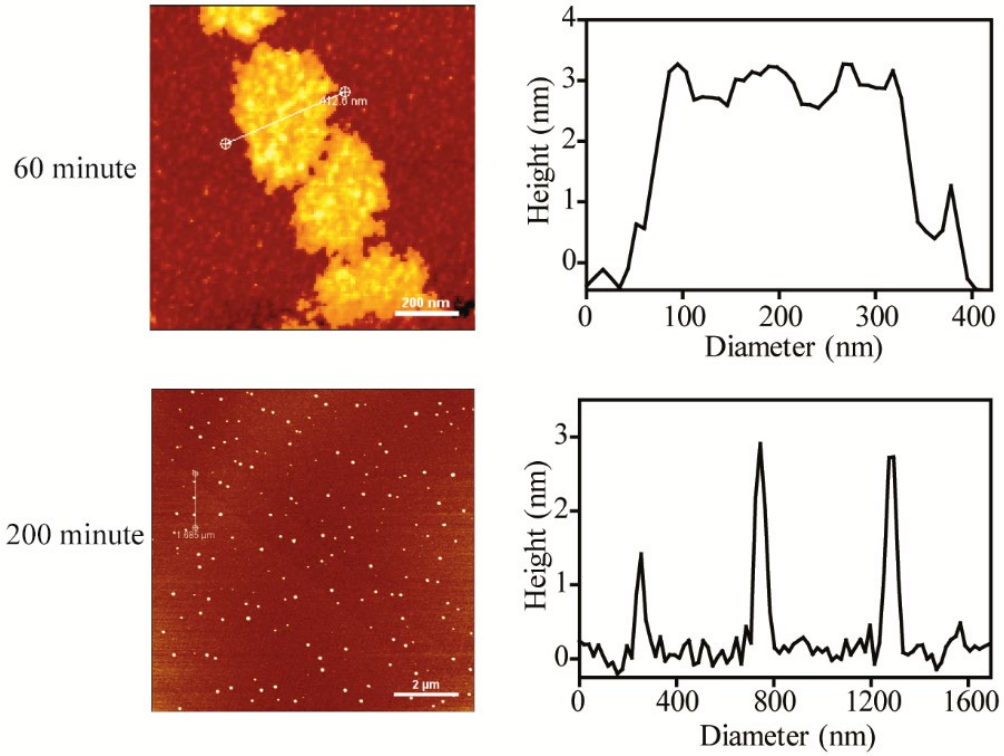

Fig. S22. 3-Mercaptopropionic acid mediated exfoliation of bulk sulfur; (a) at the end of 60 minutes, (b) at the end of 200 minutes.

25. BA assisted exfoliation of bulk sulfur to NDs

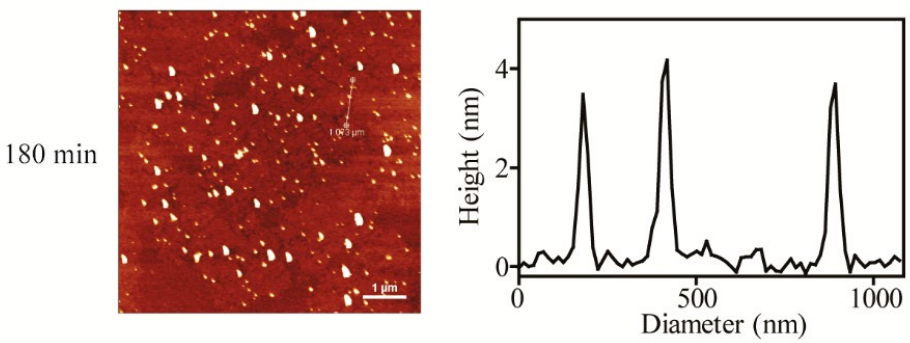

Fig. S23. 4-mercaptophenylboronic assisted exfoliation of bulk sulfur to nanodots after 180 minutes of probe sonication.

26. Characterization of BASNs

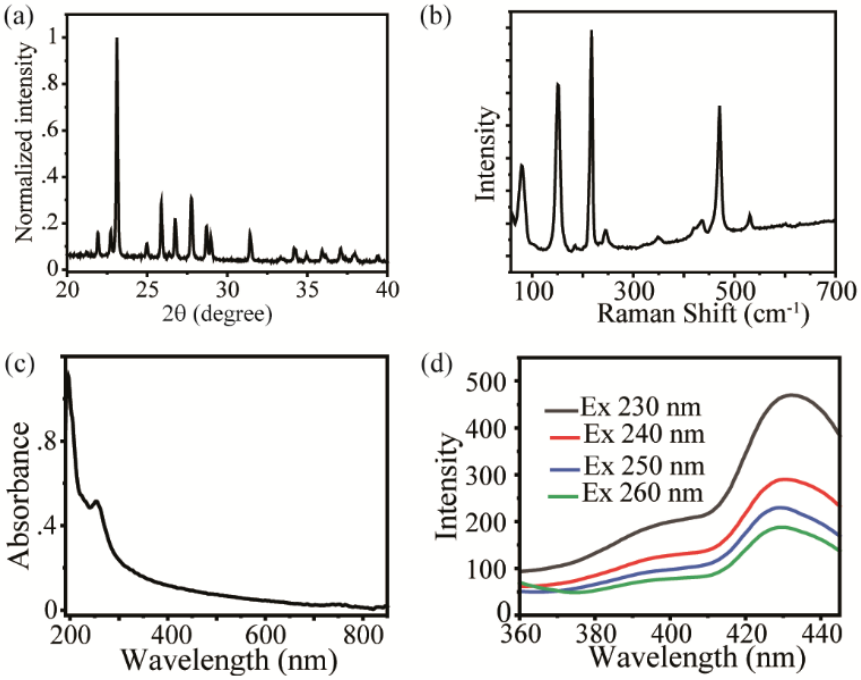

Fig. S24. (a) XRD of BASNs, (b) Raman spectra of BASNs, (c) UV-Vis spectra of BASNs, (d) Fluorescence emission spectra of BASNs.

27. Antibacterial activity of BSA (Bovine Serum Albumin) exfoliated nanosheet

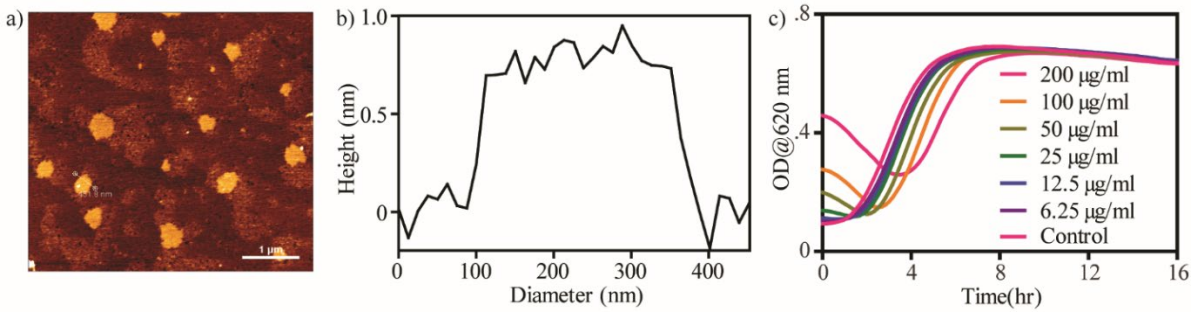

Fig. S25. (a) Exfoliation of bulk sulfur to nanosheets mediated by BSA after 120 minutes. (b) Height profile of the exfoliated nanosheet. (c) Bacterial growth kinetics of MRSA bacteria in the presence of BSA stabilized sulfur nanosheets.

28. Evaluation of cytotoxicity of BASNs against *HeLa* cell lines

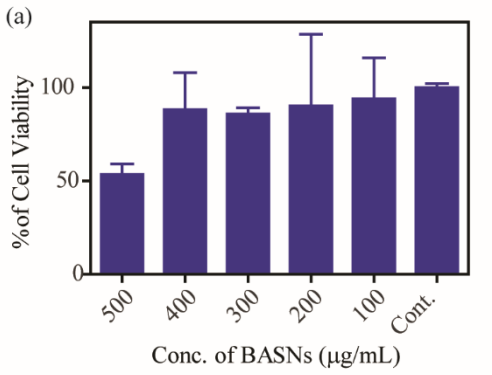

Fig. S26. (a) Cellular toxicity of BASNs against *HeLa* cell lines.