# **Small** Micro

## Supporting Information

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Strong and Elastic Membranes via Hydrogen Bonding Directed Self-Assembly of Atomically Precise Nanoclusters

Anirban Som, Alessandra Griffo, Indranath Chakraborty, Hendrik Hähl, Biswajit Mondal, Amrita Chakraborty, Karin Jacobs, Päivi Laaksonen, Olli Ikkala,\* Thalappil Pradeep,\* and Nonappa\*

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#### 1.0 Materials and methods

All chemicals were commercially available and were used without further purification. Silver nitrate (AgNO<sub>3</sub>, 99%), *p*-mercaptobenzoic acid (*p*MBA, 97%) and sodium borohydride (NaBH<sub>4</sub>, 99.99%) were purchased from Sigma-Aldrich. Also, dimethylformamide (DMF, AR grade), dimethysulfoxide (DMSO, AR grade), toluene (AR grade), methanol (AR grade), citric acid and cesium hydroxide (CsOH) were procured from Sigma-Aldrich. Milli-Q water was used throughout the experiment.

#### 2.0 Synthesis of Na<sub>4</sub>Ag<sub>44</sub>-pMBA<sub>30</sub> nanoclusters

Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> clusters were synthesized using a reported procedure.<sup>27,28</sup> To a stirred solution of AgNO<sub>3</sub> (28 mg) in a mixture of DMSO and water (4:7 volume ratios) under constant stirring, 173 mg of solid *p*MBA was added, and stirring was continued. Subsequently, a 50% aqueous solution of CsOH was added dropwise till the cloudy nature of thiolates became clear and a greenish yellow color appeared. To the reaction mixture, a solution of NaBH<sub>4</sub> (283 mg in 9 mL water) was added dropwise. Upon adding the NaBH<sub>4</sub>, the color of the reaction mixture changed to deep brown, and after 1 h it turned deep red, confirming cluster formation. This crude cluster was precipitated by adding DMF, and the mixture was centrifuged at 5000 rpm. After removing the centrifugate, the cluster was dispersed in citric acid containing DMF solution. The dispersion cluster was then reprecipitated by adding excess toluene and centrifuged at 5000 rpm. The acidification step was repeated one more time. After this, the precipitate was re-dissolved in DMF to get the purified cluster.

#### 3.0 Self-assembly of Na<sub>4</sub>Ag<sub>44</sub>-pMBA<sub>30</sub>

#### 3.1 Microscale colloidal crystals

To precipitate microscale colloidal crystal colloidal crystal sheets, 20  $\mu$ L of the as synthesized dispersion in Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> in DMF was added to 200  $\mu$ L of the solvent (methanol, ethanol, propanol, 1-butanol, pentanol, or acetone). The resulted precipitate was then used to prepare the SEM and TEM samples.

#### 3.2. Preparation of Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> monolayer membranes

To prepare large area monolayer membranes, the purified clusters were first precipitated by adding toluene to 2.0 mL of as synthesized Na<sub>4</sub>Ag<sub>44</sub>-pMBA<sub>30</sub> dispersion in DMF. The solvent was decanted and the precipitate was re-dispersed in 2.0 mL of 1-pentanol. The Na<sub>4</sub>Ag<sub>44</sub>-pMBA<sub>30</sub> dispersion in 1-pentanol was slowly dropped over the surface of milli-Q water in a

beaker using pipette until the entire water surface was covered. The Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> thin membrane which was formed at the water-air interface was subjected to characterization. It is important to mention here that an immediate transfer is better for the stability of the membrane.

#### 4.0 Instrumentation

UV-visible absorption spectra were recorded using a Perkin-Elmer Lambda 25 spectrophotometer in the range 200-1100 nm. Raman spectra were recorded using a Witec Alpha300 S confocal Raman spectrometer with 633 nm (He-Ne) laser using a Zeiss 20x objective. Raman imaging was performed using a Nikon 100x objective using a piezoelectric scanner. Data were processed by Witec Project Plus software package. FT-IR spectral measurements were performed using a Nicolet 380 instrument equipped with an ATR cell. Transmission electron microscopy (TEM) and transmission electron microscopy (TEM) were performed at an accelerating voltage of 300 kV on JEOL 3200FSC. Image acquisition was done by a Gatan 794 multiscan CCD camera. Data were processed by Gatan Digital Micrograph software. Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> monolayer membrane floating on water was transferred on 300 mesh carbon coated copper grid and holey carbon grids and dried before TEM analysis. The TEM images were processed using Gatan Digital micrograph software. For IFFT, an area of interest in the given image was selected and transformed using available in-built functions in Gatan Digital Micrograph.

#### 4.1 Sample Preparation for AFM

Quantifoil R1.2/1.3 (Electron Microscopy Sciences) grids were plasma cleaned for 10 s in a Solarus plasma cleaner (Gatan, Pleasantown, CA, USA). Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> monolayer membrane was transferred and dried in air and was used for AFM measurements.

#### 4.2 AFM Imaging

Topographical images were recorded by AFM (Bruker Multimode 8) in Quantitative Nanomechanical mapping mode (QNM) in air. In this mode, the probe oscillates much below the sonance frequency of the cantilever recording fast force-distance curves on the sample resulting in high resolution force volume maps. Evaluating these maps, therefore, allows also for a precise determination of the true sample surface. The images were scanned using silicon (Scanasyst, tip radius 2 nm, Bruker) and silicon nitride (MLCT-F, tip radius 20 nm, Bruker) cantilevers, with respectively a resonance frequency of 70 kHz and 125 kHz and a force constant of  $0.4 \text{ N m}^{-1}$  and  $0.6 \text{ N m}^{-1}$ . The scanned image sizes were from 1.5 to 10  $\mu$ m<sup>2</sup>.

#### 4.3 AFM indentation

The AFM indentation experiments were performed with a Bruker Multimode 8.0 Instrument at room temperature using Scanasyst cantilevers (spring constant 0.4 N/m). For each cantilever, the spring constant was calibrated by thermal methods. The Scanasyst probe had soft triangular cantilevers and pyramidal tips (with pyramid angle of 35° and a maximum nominal curvature radius of 12 nm). The actual tip diameter was estimated by tip qualification with Nanoscope software and reported a value of 23.0 nm. The tip qualification measurement was performed with an unused tip. he tip qualification was not performed for every probe used since this procedure might lead to small tip damages. Yet, indentation measurements with probes with and without known tip diameter were compared and no significant difference in the determined E values was found. In the force curve measurements, the approaching and retracting velocity was kept at 500 nm/s, scan rate at 1.25 Hz and the ramp size at 250 nm. Tens of experiments were carried out on samples having been first checked by TEM analysis. The TEM analysis permitted to discard grids not having a uniform coverage or showing bilayers prior to the force indentation  $F(\delta)$  experiments. For each sample, after having recorded the image, AFM indentation measurements were performed at the center of the suspended membrane in two ways: keeping the indentation force constant or increasing it until the rupture of the membrane. The data were processed using a homemade MATLAB script for baseline and contact point correction and extraction of the elastic modulus by the fitting of the approaching curve. The relative humidity was maintained below 50% and the temperature was maintained between 22 -23 °C.

#### 4.4 Raman Spectroscopy

For SERS studies, a WITec GmbH confocal Raman microscope was used. It was equipped with a frequency doubled Nd: YAG diode laser (532 nm) for Raman excitation of the sample. This instrument was equipped with an Olympus BX41 optical microscope (inverted microscope with a  $100 \times 1.4$  NA air objective lens) and a thermoelectrically cooled charge-coupled detector (ANDOR CCD Detection System) with a  $1024 \times 1024$ -pixel format, operating at -80 °C. The initial signals were calibrated using the 520 cm<sup>-1</sup> vibrational mode of silicon. A total of 600 grooves/mm grating was used for collecting SERS spectra. Acquisition time was 0.5 s for all measurements.

#### 4.5 Sample preparation for SERS studies

The AmpB was diluted to the desired concentration using milliQ water. The film of Ag44 was collected on thoroughly cleaned regular glass coverslips. These were held vertically on kim

wipe tissue paper to soak all the excess solvents. Then a drop of 3  $\mu$ L AmpB was added on top of the film. It was left at room temperature in dark to get dried.

#### 4.6 Preparation of AgNP films

Synthesizing pMBA-functionalized AgNP and making a thin film out of that itself is a synthetic challenge. Therefore, we made Ag44 film as usual and kept it overnight at 50 °C. TEM image confirmed that the AgNCs were converted to AgNP while maintaining the morphology of the film. This gave the closest comparable system to our AgNC film. Amp B of desired concentration was then dropcasted on it in as stated above.



Crude <sup>I</sup> cluster in DMF

NC membranes on water surface

NC dispersion when added to 2/75 (v/v) 1-pentanol/water



Figure S1. Synthesis of Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> nanoclusters and membrane formation.



**Figure S2.** Absorption spectra and mass spectrometric characterization of Na<sub>4</sub>Ag<sub>44</sub>-pMBA<sub>30</sub>. a) UV-Vis spectra of Na<sub>4</sub>Ag<sub>44</sub>-pMBA<sub>30</sub> nanoclusters in solution. b and c) ESI-Mass spectra of Na<sub>4</sub>Ag<sub>44</sub>-pMBA<sub>30</sub>. d) Shows the top view of L3 pMBA ligand bundles and interlayer H-bonding (note: L2 bundles are not shown for clarity).



**Figure S3.** TEM images of 2D crystals obtained when Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> dispersion in DMF was dispersed in various solvents. a and b) TEM images of 2D nanosheets obtaine upon adding a solution of Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> in DMF to methanol. c-e) HR-TEM of 2D nanosheets in methanol showing Moire patterns due to overlapping layers. f) Ethanol. g) 1-Propanol. h) 1-Butanol and i) Acetone.



**Figure** S4. HR-TEM of Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> monolayer membranes. a and b) HR-TEM of the selected area of monoayer membrane deposited on 300 mesh gold grid with ultrathin carbon support membrane. Insets showing the FFTs of the respective images indicating long range hexagonal array of Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> monolayer membranes. c) The steps invovived in IFFT.



**Figure S5.** Raman spectra and optical images of Na<sub>4</sub>Ag<sub>44</sub>-pMBA<sub>30</sub> monolayer membrane and the dropcasted Na<sub>4</sub>Ag<sub>44</sub>-pMBA<sub>30</sub> dispersion. a) Raman spectra showing a comparison between Ag<sub>44</sub>ML and the dropcasted Ag<sub>44</sub> solution. b) Comparision of different spectral region reveal the presence of monolayer and overlapping layers with bright peaks in Raman imaging (c). d) Optical image of a portion of the membrane obtained using interfacial assembly. e) FT-IR spectra of pMBA. f) FT-IR spectra of Na<sub>4</sub>Ag<sub>44</sub>-pMBA<sub>30</sub> membrane.



**Figure S6.** TEM micrographs of Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> monolayer membranes. a) TEM image of Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> monolayer membrane stretched over a a gold grid with holey carbon support membrane. b) TEM image of a Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> monolayer membranes in a single hole. c) TEM image of holey grid after sputter coating with platinum showing the holes are empty as confirmed by high magnification image in d.



**Figure S7.** TEM micrographs of Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> membranes deposited on holey grid after sputter coating with platinum.



**Figure S8.** Real height images of Na<sub>4</sub>Ag<sub>44</sub>-pMBA<sub>30</sub> membrane suspended on holey carbon grid. a and b) Scan sizes 2.1  $\mu$ m, c and d) Scan sizes 3.5  $\mu$ m.



**Figure S9.** Real height images of Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> membranes deposited on silicon wafers.



**Figure S10.** AFM image of the holey carbon support without the Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> monolayer membrane deposited. a and b) 3D view of the AFM image and height profile, respectively, showing an estimated rim height is 20 nm.



**Figure S12.** AFM height images before and after indentation of Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> monolayer membranes. a-c) AFM height images recorded at different scan sizes for Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> monolayer membrane suspended on the TEM grid with holey carbon substrate. d-e) AFM images after indentation upon one or more closely different spots.



**Figure S13.** Overlap of multiple Force – indentation curves. Overlap of  $11 F(\delta)$  approaching curves indenting at the center of a Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> monolayer membrane suspended on the TEM grid with holey carbon support.



**Figure S14. SERS measurements.** a) Raman spectra of *p*MBA. b) The reproducibility of SERS spectra of Amp B deposited on multiple Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> membranes. c) TEM image of thermally treated Na<sub>4</sub>Ag<sub>44</sub>-*p*MBA<sub>30</sub> membrane resulted in larger AgNPs. d) Raman spectra of AgNP, Amp B and Amp B deposited over AgNP film showing no detectable changes in SERS spectra.