Supporting Information for

Spatial Mapping of Protein Adsorption on Mesoporous Silica Nanoparticles by Stochastic Optical Reconstruction Microscopy (STORM)

Alden M. Clemments,[a] Pablo Botella,[b] and Christopher C. Landry[a]

[a]Department of Chemistry, University of Vermont
82 University Place, Burlington, VT 05405, U.S.A.
[b]Instituto de Tecnología Química, (Universitat Politècnica de València-Consejo Superior de Investigaciones Cientificas)
Avenida de los Naranjos s/n, 46022 Valencia, Spain

Table of Contents

1. Synthesis and Characterization of MSNs

Materials

All chemicals were purchased from Sigma Aldrich unless otherwise noted. Alexa Fluor 647 was purchased from Thermo Scientific and used according to the manufacturer's protocol. All proteins were purchased from Athen Research & Technologies.

Synthetic Procedures

Synthesis of 830 nm Mesoporous Silica. Mesoporous silica of 830 nm was synthesized according to a previously published protocol[1]. Ethanol (100 %, 138 g, mol) was combined with Milli-Q water (162 g, mol) in an Erlenmeyer flask equipped with a magnetic stir bar. Ammonium hydroxide (28.95 wt %, 11.6 mL) was then added and the solution was briefly mixed. Cetyltrimethylammonium bromide (CTAB, 0.280 g, mol) was then added and stirred until all of the CTAB had dissolved. After 5 min., tetraethylorthosilicate (TEOS, 1.388 mL, mol) was added and the reaction proceeded at r.t. for 2 h. Following, the material was isolated via centrifugation (15,000 *g*, 10 min). The material was then thoroughly washed with a mixture of water and ethanol before drying under vacuum for 24 h. Removal of the surfactant was achieved by calcining the material at 550^{o}C for 6 h.

Synthesis of Large Pore Mesoporous Silica. Pore-expansion of the parent material was carried out according to a previously published protocol[2]. The calcined, small pore material was suspended in ammonium hydroxide (1 M, 40 mg particles/mL) in a Telfon bottle and briefly sonicate using a Branson 2510 sonicator operating at 40 kHz. The Telfon-sealed bottle was then placed in a 100^{o}C and the reaction was allowed to proceed for 30 min. After removing from the oven, the material was isolated via centrifugation and repeatedly washed with ethanol before drying under vacuum for 24 h.

Characterization of Nanoparticles

Nanoparticle morphology and size were studied by transmission electron microscopy (TEM) using a JEOL 1400 microscope operating at 80 kV. Samples were dispersed in ethanol, transferred to carbon-coated copper grids, and then immediately imaged. Nitrogen gas physisorption isotherms were measured in a Micromeritics Flowsorb apparatus. Surface area calculations were carried out using the BET method, pore size distributions were calculated using to the KJS adjustment of the BJH method.[3]

2. Protein Labeling and Adsorption

Protein Labeling and Quantification

Proteins were used as received from Athens Research and Technologies. Proteins were reconstituted in PBS (10 mM, pH 7.4) at a concentration of 1 mg/mL. An aliquot containing 200 μg of protein was then

transferred to a new eppendorf and NHS-terminated Alexa Fluor 647 (20 µg, 10 mg/mL DMSO) was added to this solution. The reaction was allowed to proceed in the dark for 2 h at r.t. Separation of 647-conjugated protein and free dye was performed using a Sephadex G-25 column (eluent: 10 mM PBS, pH 7.4). Protein solutions were then concentrated using nanostep centrifugation filters (MW cutoff > 4 kDa). Finally, protein concentrations were determined using the Pierce BCA Protein Assay Kit.

Protein Adsorption

Calcined particles were suspended in PBS (10 mM, pH 7.4) at a concentration of 1 mg/50 µL. The particle suspension was then sonicated to disperse any aggregates. An aliquot containing 1 mg of particles was then transferred to a new eppendorf and diluted to a final concentration of (1 mg/0.5 mL) with PBS containing 647-labeled protein (100 µg protein). The adsorption proceeded for 1 h before isolating the particle-protein complexes via centrifugation (1 min, 14,800 rpm). The protein-adsorbed samples were then resuspended in PBS (1 mL), sonicated, and centrifuged. This process was repeated three times in order to remove any loosely bound protein.

3. STORM Preparation and Imaging

Slides and Buffer preparation

In order to reduce non-specific adsorption of 647-labeled protein, all imaging slides were thoroughly cleaned in potassium hydroxide (1 M) and piranha solution (3:1, H_2SO_4: H_2O_2) following to a previously published protocol[4]. Imaging buffer was prepared fresh before each experiment and was prepared according to a previously published recipe.

Particle-Protein Imaging

After preparing the glass slides and buffer, protein-adsorbed particles were suspended in 100 µL of PBS. After sonicating the sample, the sample was aspirated onto the glass imaging slide. After allowing the particles to settle on the glass slide (2-3 min) the excess PBS was removed. Imaging buffer (1.5 mL) was then very slowly added to the dish and the sample was immediately imaged on a Nikon Eclipse Ti-E microscope. The composition of the buffer used for these experiments consisted of Tris buffer (1 mL, 50 mM Tris-HCl (pH 8.0), 10 mM NaCl, 10% glucose), an oxygen scavenging system (0.5mg mL^{-1} glucose oxidase, 40 µg mL^{-1} catalase), and 2-mercaptoethanol (MEA, final concentration 100 mM). Fresh imaging buffer was prepared prior to each experiment. For all samples, a minimum of 20,000 frames was collected to generate the final STORM image. A minimum peak height value of 300 was used for all samples.

4. Sphere Fitting and Analysis

Sphere Fitting

The sphere of best fit was found by minimizing the function[5]:

$$J = \sum_{i=1}^{I}((x_i - x_c)^2 + (y_i - y_c)^2 + (z_i - z_c)^2 - {r_c}^2)^2$$

I = number of data points

(x_i, y_i, z_i) = ith measurement of the origin

(x_c, y_c, z_c) = center of sphere

r_c = radius of sphere

$$\begin{bmatrix} x_c \\ y_c \\ z_c \end{bmatrix} = \frac{1}{2}\begin{bmatrix} \sum_i x_i\,(x_i - \bar{x}) & \sum_i x_i\,(y_i - \bar{y}) & \sum_i x_i\,(z_i - \bar{z}) \\ \sum_i y_i\,(x_i - \bar{x}) & \sum_i y_i\,(y_i - \bar{y}) & \sum_i y_i\,(z_i - \bar{z}) \\ \sum_i z_i\,(x_i - \bar{x}) & \sum_i z_i\,(y_i - \bar{y}) & \sum_i z_i\,(z_i - \bar{z}) \end{bmatrix}^{-1} \begin{bmatrix} \sum_i ({x_i}^2 + {y_i}^2 + {z_i}^2)(x_i - \bar{x}) \\ \sum_i ({x_i}^2 + {y_i}^2 + {z_i}^2)(y_i - \bar{y}) \\ \sum_i ({x_i}^2 + {y_i}^2 + {z_i}^2)(z_i - \bar{z}) \end{bmatrix}$$

$$r_c = \sqrt{\frac{1}{I}\sum_{i=1}^{I}((x_i - x_c)^2 + (y_i - y_c)^2 + (z_i - z_c)^2)}$$

Distance Calculations

Once the center of the sphere had been determined (x_c, y_c, z_c), the distance from each data point to the center was determined according to the following calculation:

$$d = \sqrt{(x_i - x_c)^2 + (y_i - y_c)^2 + (z_i - z_c)^2}$$

d = distance from origin to data point (nm)

(x_i, y_i, z_i) = x, y, z coordinates of individual data point

(x_c, y_c, z_c) = center of sphere

5. Figures

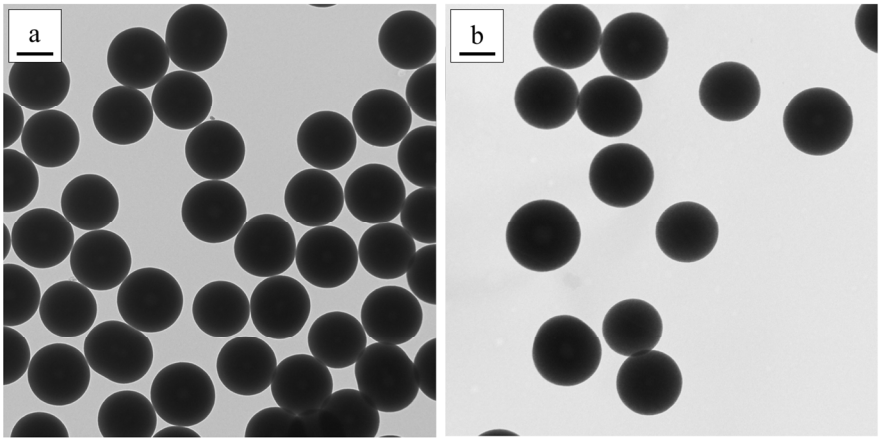

Figure S1. TEM micrographs of small pore (**a**) and large pore (**b**) mesoporous silica. Scale bar : 500 nm.

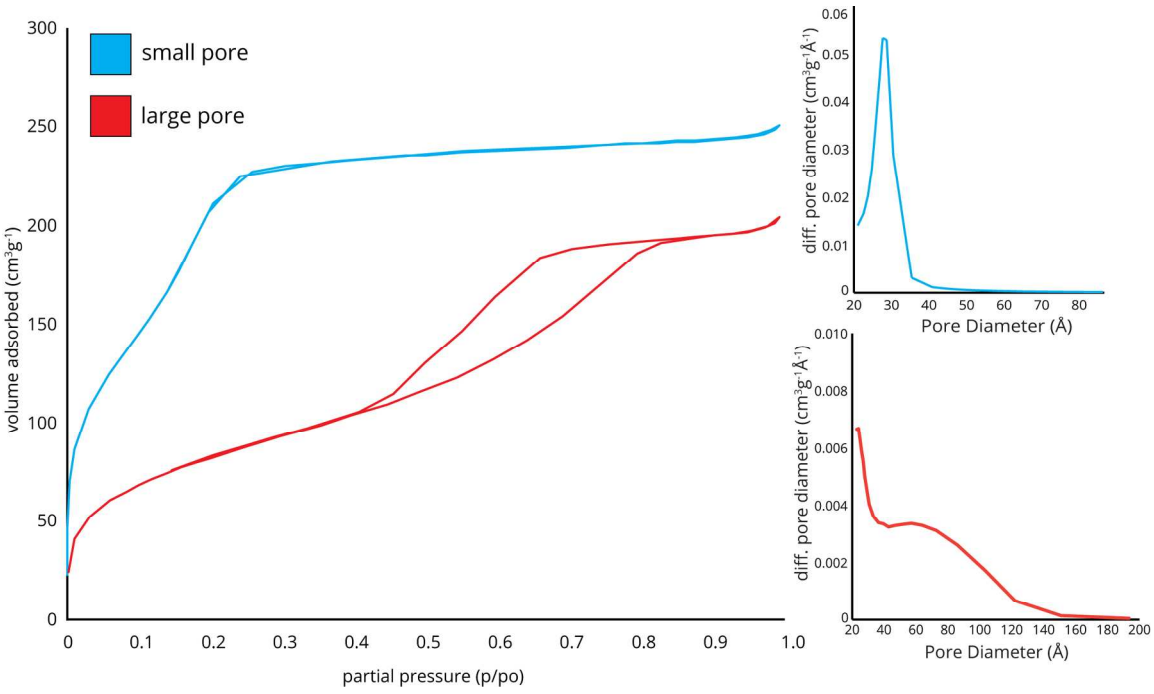

Figure S2. N_2 physisorption isotherms and pore distributions of small and large pore mesoporous silica.

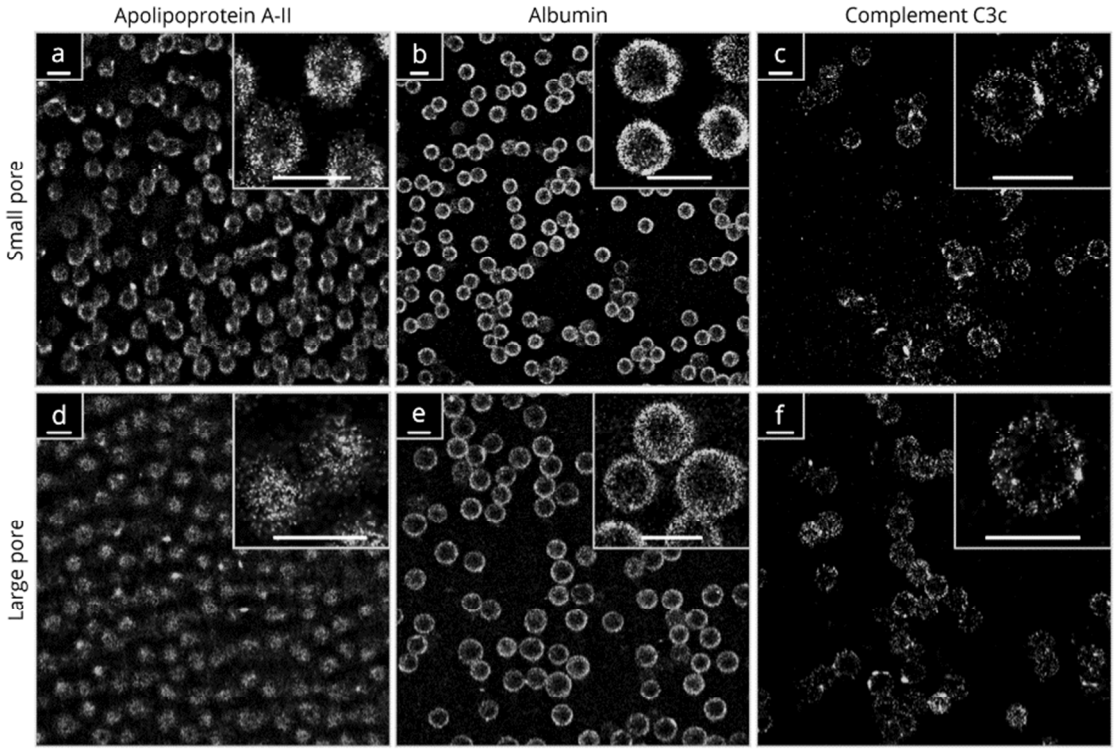

Figure S3. Protein adsorption on small and large pore mesoporous silica observed by STORM. Adsorption of apolipoprotein A-II (**a, d**) , albumin (**b, e**), and complement C3c (**c, f**) on small pore (**a, b, c**) and large pore (**d, e, f**) mesoporous silica particles. Scale bars, 1000 nm

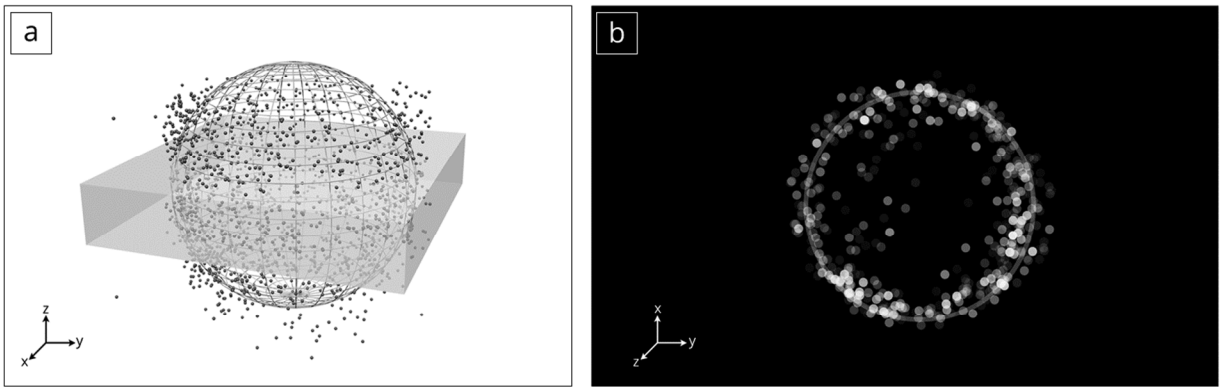

Figure S4. Sphere fitting model and analysis on selected spheres. (**a**) Following acquisition of STORM data, a least squares sphere fitting algorithm was applied to all data points associated with a selected particle, and the sphere of best fit was determined. Once the particle origin was calculated, all molecules within 100 nm of the particle center (along the z-axis) were extracted from the data set. (**b**) To highlight the depth by which a particular protein penetrated into the MSN, the data points were flattened and plotted as a function of their fluorescence intensity.

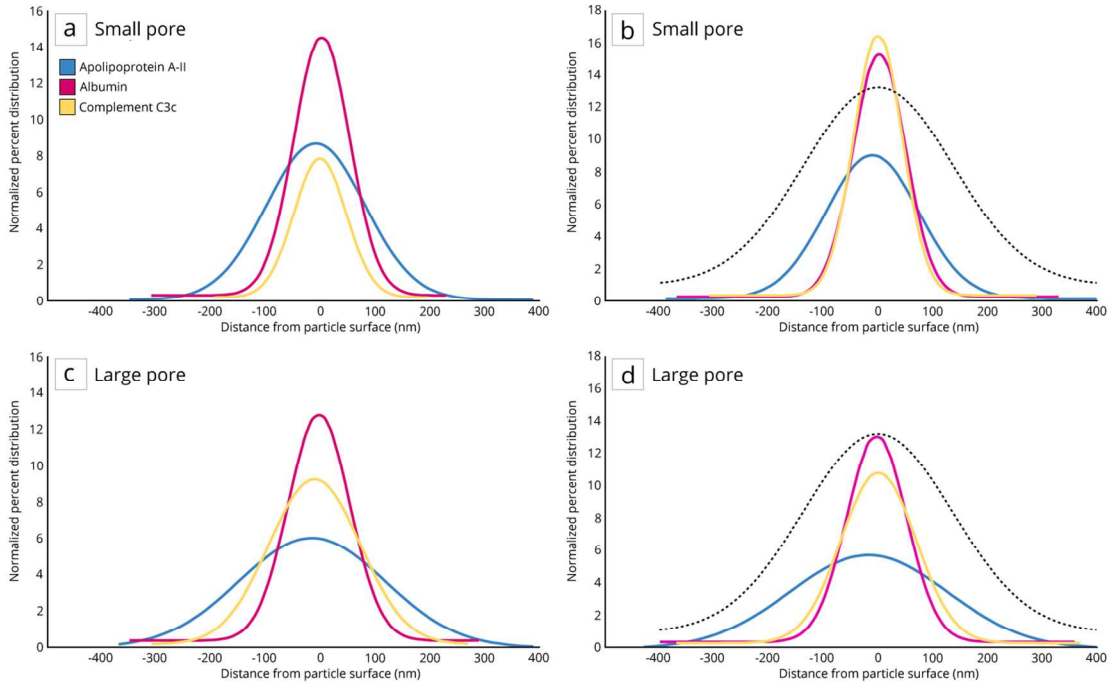

Figure S5. Distribution of distances on the specific particles shown in **Figure 2** in the manuscript: (**a**) small pore; (**c**) large pore. The dashed line represents theoretical distribution in a hollow sphere. (**b, d**) Distribution of distances averaged over 10 particles.

6. References

(1) Nooney, R. I.; Thirunavukkarasu, D.; Chen, Y.; Josephs, R.; Ostafin, A. E. *Chem Mater* **2002**, *14*, 4721.

(2) Sayari, A.; Yang, Y.; Kruk, M.; Jaroniec, M. *J Phys Chem B* **1999**, *103*, 3651.

(3) Kruk, M.; Jaroniec, M.; Sayari, A. *Langmuir* **1997**, *13*, 6267.

(4) Chandradoss, S. D.; Haagsma, A. C.; Lee, Y. K.; Hwang, J.-H.; Nam, J.-M.; Joo, C. *J Vis Exp : JoVE* **2014**, 50549.

(5) Jennings, A. L.; Black, J.; Allen, C. *Shock & Vibration* **2013**, *20*, 503.