Conserved Activity of Reassociated Homotetrameric Protein Subunits Released from Mesoporous Silica Nanoparticles

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Supporting Information

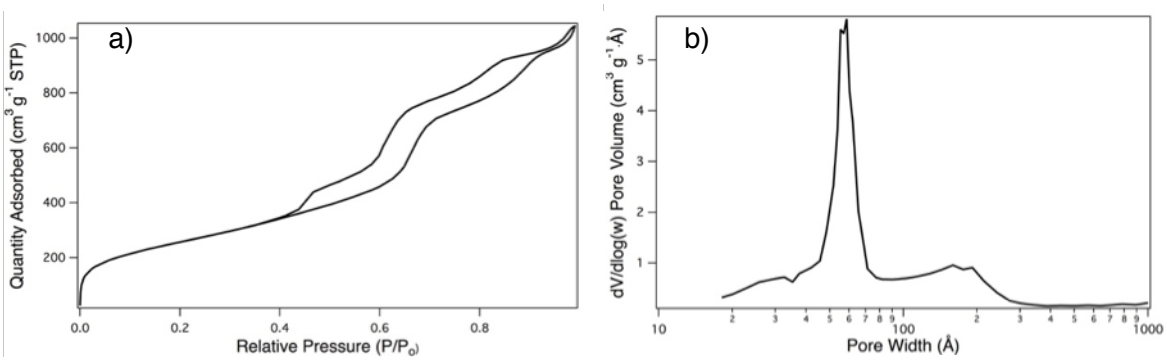

Figure S1. a) Nitrogen sorption isotherm of PEMSN synthesized using mesitylene pore expanding agent using BET calculation, b) BJH pore size distribution of PEMSN showing two pore size distributions centered on 5.8 nm (major peak) and 15.9 nm (minor peak).

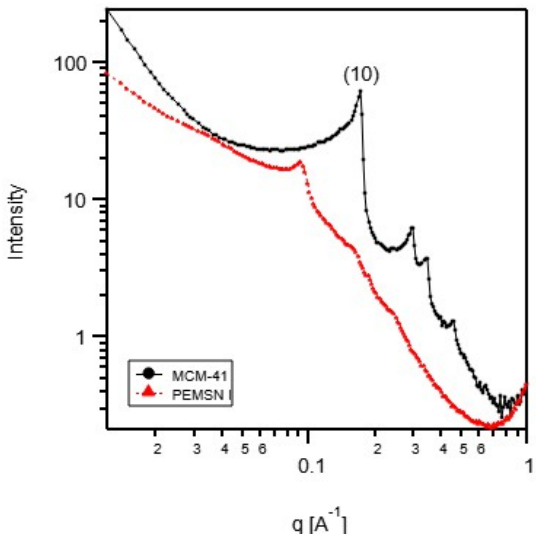

Figure S2. Diffraction peaks present in SAXS data acquired for MCM-41 prepared with (red) and without mesitylene (black).

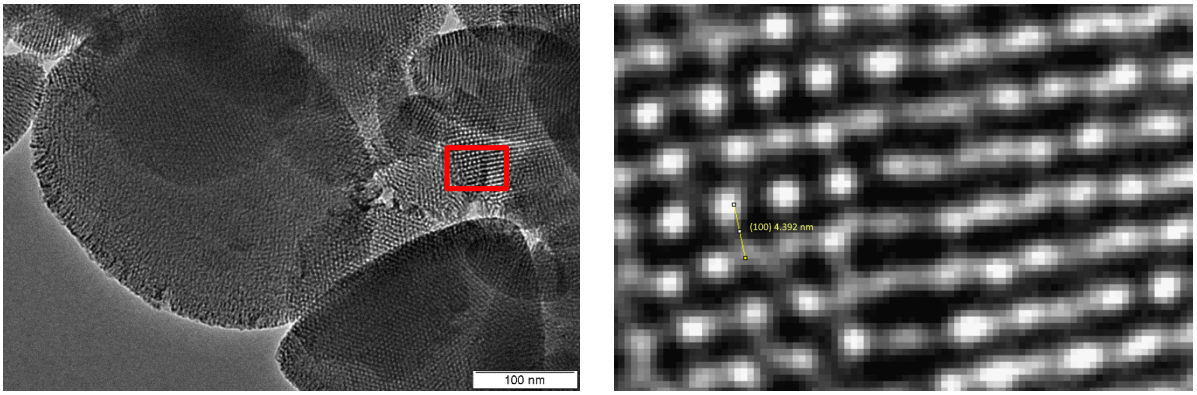

Figure S3. TEM images of the PEMSN. The right image is a magnification of the inset depicted on the box outlined in the image on the left. $d_{(100)}$ was measured by *ImageJ* as 4.4 nm.[6]

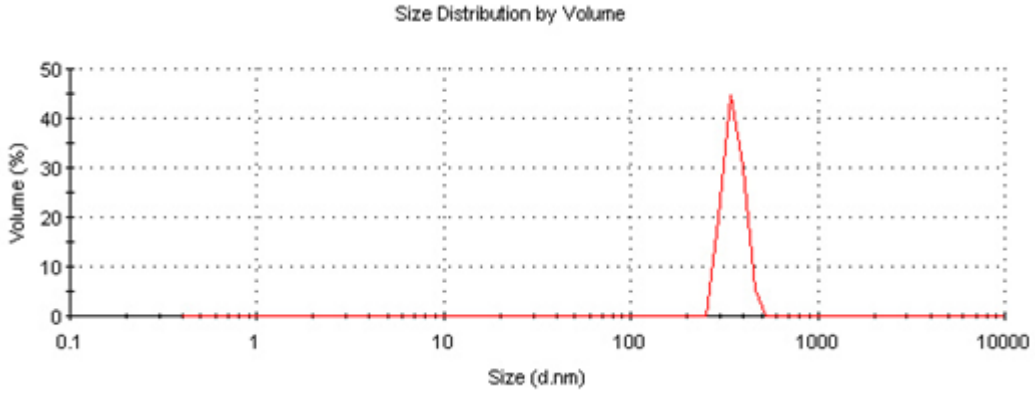

Figure S4. Dynamic light scattering measurement of particle size distribution of PEMSN suspended in 10 mM PBS pH 7.4 showing an average size of 300 nm.

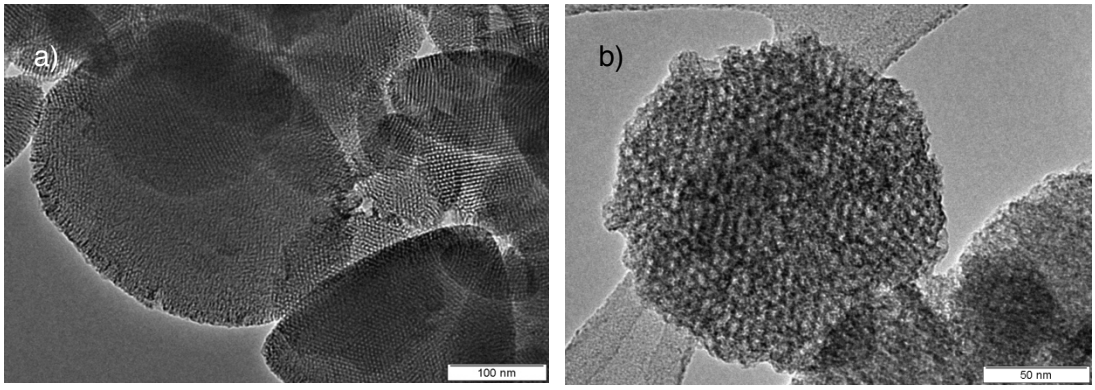

Figure S5. TEM images of PEMSN before (a) and after (b) and *Con A* loading.

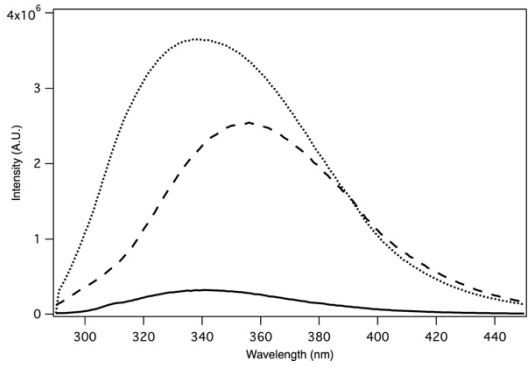

Figure S6. Fluorescence measurements at an excitation wavelength of 280 nm of native *Con A* (dotted line), dissociated *Con A* (dashed line), and re-associated *Con A* (solid line). Dissociated protein shows a red shift from 337 nm to 352 nm. Reassociated *Con A* after diffusing out of the mesopores of MSN showing a blue shift from 352 nm back to the 337 nm characteristic of the native *Con A*. Fluorescence intensity of the re-associated *Con A* is very low because this sample was diluted until the urea concentration was negligible such that the protein concentration was 0.7 µM. Measurements were taken on samples that had not been exposed to pepsin.

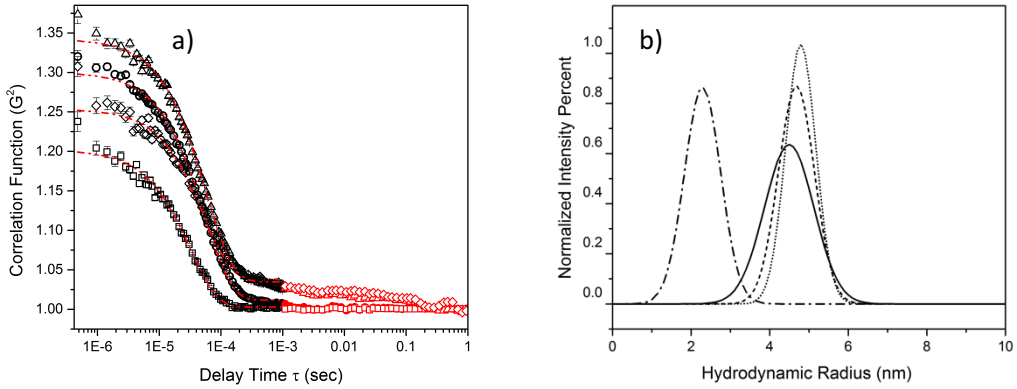

Figure S7. *Con A* size measurements from dynamic light scattering in respective buffers. The figures are a) correlation functions for native *Con A* (circles), dissociated *Con A* (squares), reassociated *Con A* post pepsin digestion (diamonds), and reassociated *Con A* without exposure to pepsin (triangles) and b) dynamic light scattering size measurements for *Con A* showing dissociation and reassociation to approximate size of native protein. The solid line represents data for native *Con A*, the alternating dots and dashes line for dissociated *Con A*, the dashed line for reassociated *Con A*, and the fine dotted line for reassociated *Con A* post pepsin digestion. For similarly sized particles the y-intercept, initial ratio of scattering intensity, is proportional to the concentration of analytes, though not quantitatively, a decrease is shown for the post pepsin digest sample.

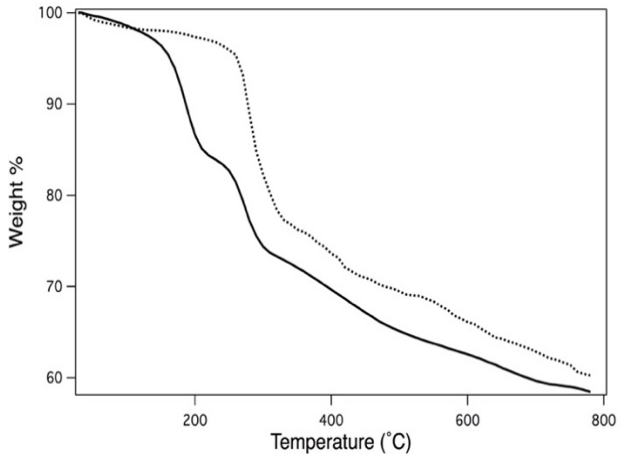

Figure S8. Thermogravimetric analysis (TGA) of buffer loaded (dotted line) indicating a 39.8 wt% loss and buffer with *Con A* loaded (solid line) PEMSN indicating a 42 wt% loss by 800 ˚C. The weight percent difference between the buffer loaded blank final mass and the protein loaded PEMSN final mass was taken to determine how many milligrams of protein were loaded per gram (22 mg/g PEMSN). The weight loss due to silanol condensation is accounted for in the blank. From this and the molecular weight of *Con A* (54,000 g/mol), a loading in micromoles protein per gram PEMSN can be determined (0.407 μmol/g PEMSN). This was then compared to the original concentration to which the particles were exposed to determine what percentage was loaded (50.9%).

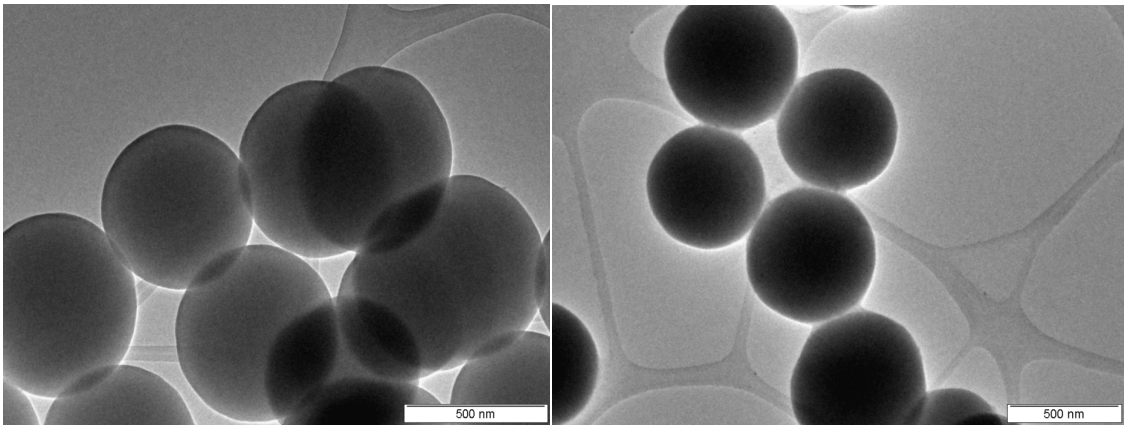

Figure S9. TEM images of nonporous silica nanoparticles (Stöber process)

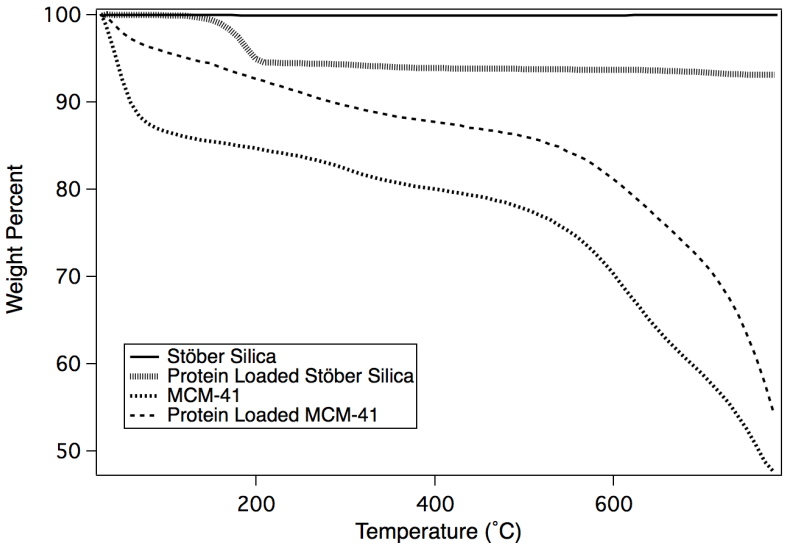

Figure S10. Thermogravimetric analysis (TGA) of nonporous silica (Stöber silica), *Con A* loading on nonporous silica nanoparticles, MCM-41 type mesoporous silica, and *Con A* loading onto MCM-41. The weight loss before 200 ˚C is attributed to water loss/ silanol condensation and accounted for in the blanks. Calculations were done as described in Figure S9. These data show a loading of 6% onto the nonporous silica and 27.5% onto the MCM-41.

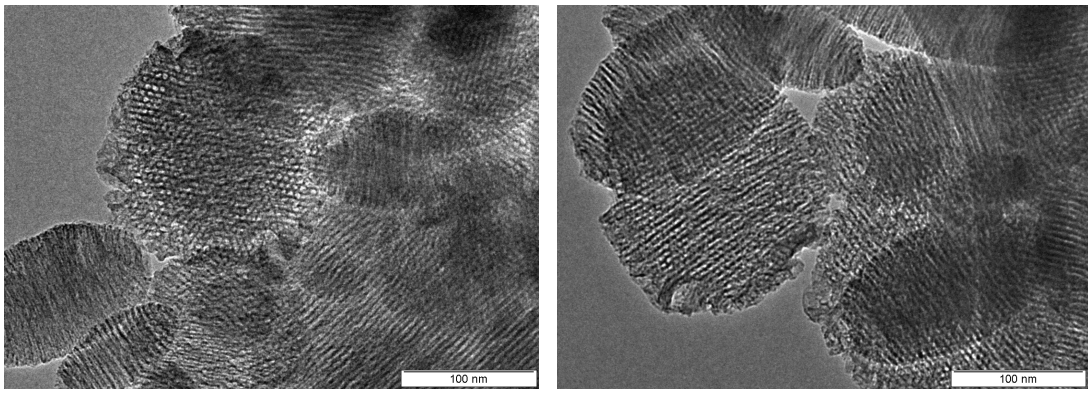

Figure S11. TEM images of PEMSN without protein loading stained with uranyl acetate.

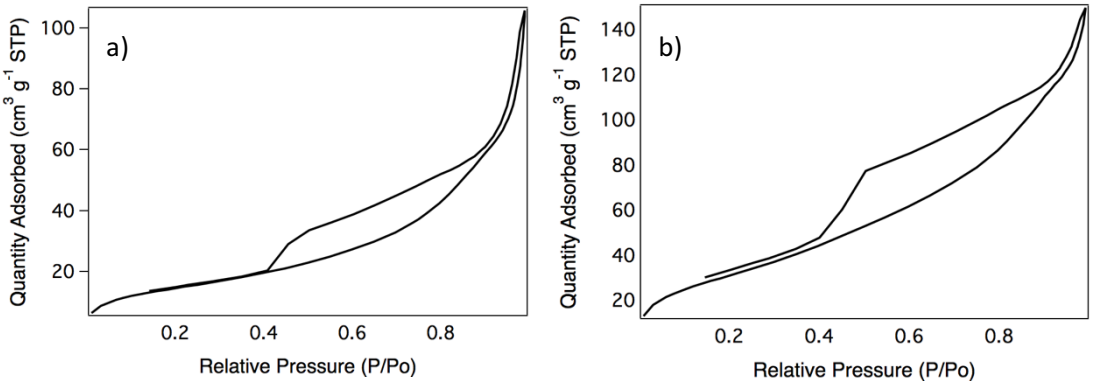

Figure S12. BET of a) PEMSN after loading *Con A* subunits and b) pore expanded, *Con A* loaded PEMSN after a pepsin digestion. The pore volume (0.14 cm^3 g^{-1} to 0.2 cm^3 g^{-1}) and surface area (53.7 m^2 g^{-1} to 118 m^2 g^{-1}) are relatively unchanged between the two, which indicated *Con A* within the PEMSN pores were not digested by pepsin.

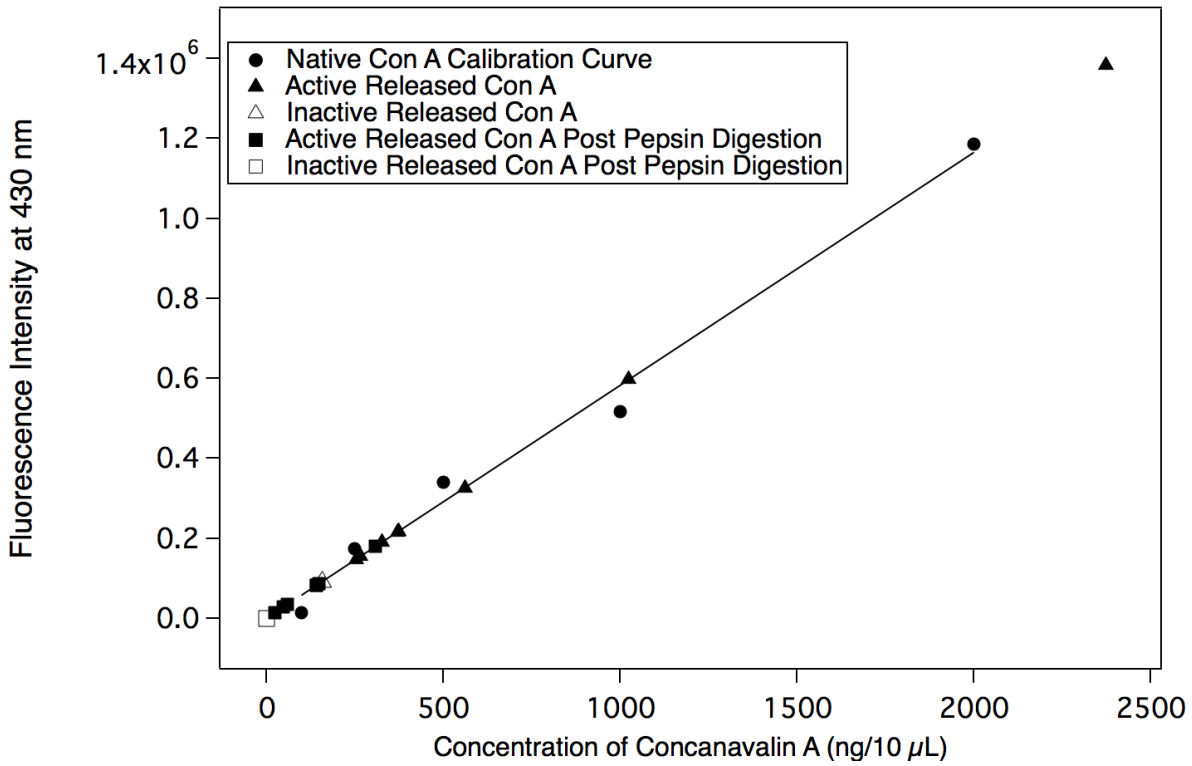

Figure S13. Native *Con A* calibration curve for the *o*-phthalaldehyde fluorescence quantification of released *Con A* activity showing: 3% unbound protein and 97% bound protein for the *Con A* released from PEMSN, and negligible unbound protein and nearly 100% bound protein for the *Con A* released from PEMSN that was exposed to pepsin. One milliliter of *Con A* solution was added to a 2 mL Sephadex G-100 column, the inactive protein was collected over 2 min, and the bound protein was collected in 2 min increments for 20 min where each fraction consisted of 4 mL. The excitation wavelength was 340 nm and emission was monitored at 430 nm.