# Elasticity of Mesoporous Nanocapsules Regulates Cellular Uptake, Blood Circulation, and Intratumoral Distribution

Jun Tao[†a], Wenhui Shi[†a], Kun Chen[b], Wei Lu[a], Aaron James Elbourne[c], Lei Bao[d], Lixing Weng[*a,] Xudong Zheng[e], Xiaodan Su[a], Zhaogang Teng[*a], Lianhui Wang[*a]

[a]*State Key Laboratory of Organic Electronics and Information Displays, School of Materials Science and Engineering, Nanjing University of Posts and Telecommunications (NJUPT), Nanjing 210023, P.R. China*
[b]*Guangdong Key Laboratory of New Drug Screening, School of Pharmaceutical Sciences, Southern Medical University, Guangzhou 510515, Guangdong, P. R. China.*
[c]*School of Science, RMIT University, Melbourne, VIC 3000, Australia.*
[d]*School of Engineering, RMIT University, Melbourne, VIC 3000, Australia.*
[e]*School of Environmental & Safety Engineering, Changzhou University, Changzhou 213164, P.R. China.*
**Corresponding author: lxweng@njupt.edu.cn (Lixing Weng), iamzgteng@njupt.edu.cn (Zhaogang Teng), iamlhwang@njupt.edu.cn (Lianhui Wang).*
[†] *These authors contributed equally to this work*

## Experimental

### Materials and methods.

**Materials.** N,N-dimethylformamide (DMF) and Tetraethyl orthosilicate (TEOS) were purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), Bis[3-(triethoxysilyl)propyl] tetrasulfide (TETS), and N-hydroxysulfosuccinimide (NHS) were purchased from Sigma-Aldrich (St. Louis, USA). 1,4-Dioxane was purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). Cetyl trimethyl ammonium bromide (CTAB, 99%) and absolute ethanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sodium hydroxide (NaOH) was purchased from Guangdong Guanghua Sci-Tech Co., Ltd. (Guangdong, China). Concentrated ammonia aqueous solution ($NH_3 \cdot H_2O$, 25~28 wt%) and concentrated hydrochloric acid (HCl, 36~38 wt%) were purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Hyaluronic acid (HA, MW≈100~200 kDa) was purchased from Bloomage Freda Biopharm Co., Ltd. (Shanghai, China). $NH_2$-maleimide and Cy5.5-maleimide were purchased from Xi'an Ruixi Biological Technology Co. Ltd. (Xi'an, China). Phosphate buffered solution (PBS), Dulbecco's modified Eagle's medium (DMEM), and fetal bovine serum (FBS) were purchased from Nanjing Keygen Biotech Co. Ltd. (Nanjing, China). MCF-7 cells were acquired from the American Type Culture Collection (ATCC, Manassas, VA). Deionized water with a resistivity of 18 MΩ·cm was used for all experiments.

**Synthesis of mesoporous organosilica nanoparticles (MONs).** MONs were synthesized by the hydrolysis and condensation of silane (TETS and TEOS) in an alkaline environment. First, 0.16 g of CTAB was dissolved in 30 ml of ethanol, 75 ml of $H_2O$, and 1 ml of $NH_3 \cdot H_2O$ and stirred at 35 °C for 1 h to produce a clarified solution. Then, a mixture of TEOS (0.25 ml) and TETS (0.1 ml) was added to the solution and stirred for another 24 h. The white suspension was collected by centrifugation, washed with ethanol three times, and redispersed in ethanol for future use.

**Modification of hyaluronic acid (HA).** For the modification of HA, $NH_2$-maleimide ($NH_2$-Mal) was grafted onto the surface of MONs via a click reaction. Briefly, 10 mg of MONs and 0.19 g of triphenylphosphine were dispersed in 1.2 ml of water, 4.4 ml of dioxane, and 80 μl of concentrated hydrochloric acid and stirred under nitrogen gas at 40 °C for 2 h. Then, the sulfhydryl-rich MONs were collected and washed with water and redispersed in 1.2 ml of water, 0.12 ml of DMF, and 4 mg of $NH_2$-Mal, and stirred for another 12 h. Subsequently, MONs-$NH_2$ were collected and washed with water three times. For the activation of the carboxyl group in HA, 1 ml of HA (10 mg·ml$^{-1}$) was dispersed in 0.5 ml of EDC (10 mg·ml$^{-1}$) and 0.5 ml of NHS (10 mg·ml$^{-1}$), and then shaken for 3 h. Then, the as-made MONs-$NH_2$ was added to the activated HA solution for 12 h. The MONs-HA was collected by centrifugation, and the residual CTAB in MONs-HA was removed by extraction in ethanol (30 ml) at 30 °C for 3 h. After repeating three times, surfactant-free MONs-HA was obtained.

**Synthesis of MONs-HA with wide range of Young's moduli.** MONs-HA with different Young's moduli were synthesized via an interior preferential etching approach. Typically, 2 mg of MONs-HA was dispersed in 1 ml of sodium hydroxide at different concentrations (0, 20, 50, 100 and 200 mM) for 15 min. Then five $MONs_{0\text{-}200}$-HA, denoted as $MONs_0$-HA, $MONs_{20}$-HA, $MONs_{50}$-HA, $MONs_{100}$-HA, $MONs_{200}$-HA, with different Young's moduli, were collected by centrifugation and washed with water three times.

**Fluorescent modification.** Briefly, 60 mg of $MONs_{0\text{-}200}$-HA was dispersed in 12 ml of water, 0.19 mg of Cy5.5-mal, and 1.2 ml of DMF for 12 h. $MONs_{0\text{-}200}$-HA-Cy5.5 was obtained by centrifugation and washed with water. The Uv-vis spectra were measured and shown in Fig. S4.

***In vitro* cytotoxicity assessment.** Briefly, MCF-7 cells were seeded in 96-well plates at a density of 1 × 10$^4$ cells per well for 12 h. Then, $MONs_{0\text{-}200}$-HA/DMEM suspensions at different concentrations (0~200 μg·ml$^{-1}$) were added to each well and cocultured for another 24 h. Then, the culture solution was replaced by 10 μl of CCK-8 and 90 μl of DMEM for 4 h. The cell viability was calculated by the following formula:

$$\text{Viability}\ (\%) = \frac{\text{the absorbance of the material group at 490 nm}}{\text{the absorbance of the control at 490 nm}} \times 100\%$$

***In vitro* cellular uptake.** MCF-7 cells were seeded in 6-well plates at a density of 1 × 10$^6$ cells per well for 12 h. Then, 50 μl of $MONs_{0\text{-}200}$-HA-Cy5.5/DMEM suspension (equal in particle numbers) was added to each well for 3 h. Subsequently, the cells were digested, washed with PBS three times, and redispersed in 500 μl of PBS. Cellular uptake was measured by flow cytometry. In addition, the cells incubated with $MONs_{0\text{-}200}$-HA-Cy5.5 were fixed with paraformaldehyde, stained with DAPI and analyzed by confocal laser scanning

microscopy (CLSM). Relative cellular uptake (RCU) was calculated by the following formula:

$$RCU = \frac{\text{the uptake efficiencies of MONs - HA}}{\text{Control}}$$

***In vivo* blood circulation.** The same amount of $MONs_{0\text{-}200}$-HA was injected into healthy BALB/c mice through the tail vein. Afterward, blood (0.5 ml) was collected from the eyeball vein at different time points. The Si element in the blood was measured by inductively coupled plasma (ICP). All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Southern Medical University and approved by the Animal Ethics Committee of Southern Medical University.

***In vivo* distribution.** The 4T1 tumor-bearing mice were randomly divided into five groups (n = 6) and i.v. injected with the same number of $MONs_{0\text{-}200}$-HA-Cy5.5. The in vivo distribution of $MONs_{0\text{-}200}$-HA-Cy5.5 at different time points (1, 4, 8, and 12 h) was observed by fluorescence imaging.

**Characterization.** Scanning electron microscopy (SEM, Hitachi S4800, Tokyo, Japan) and transmission electron microscopy (TEM, Hitachi HT7700, Tokyo, Japan) were used to characterize the morphologies of MONs-HA. Hydrodynamic size and zeta potential were measured by a dynamic light scatterer. Elemental mapping images were obtained by an FEI Talos F200X electron microscope. Atomic force microscope (AFM) measurements were conducted using a Cypher ES Atomic Force Microscope (Oxford Instrument, Asylum Research, Santa Barbara, CA, USA) at room temperature (25 °C). Images were collected using amplitude-modulated-AFM (AM-AFM). Young's modulus of MONs-HA was measured using contact mode. A Tap300Al-G cantilever (Budgetsensors) with a nominal spring constant (ks) of 40 nN·nm-1·sp was used for each measurement. Each cantilever was calibrated using the thermal spectrum method, while the lever sensitivity was determined using force spectroscopy. The spring constant was resolved via the inverse optical lever sensitivity (InVOLS). Fourier transform infrared (FT-IR) spectra was measured by a NEXUS870 FT-IR spectrometer (Nicolet Instruments Inc. Madison, WI, USA).

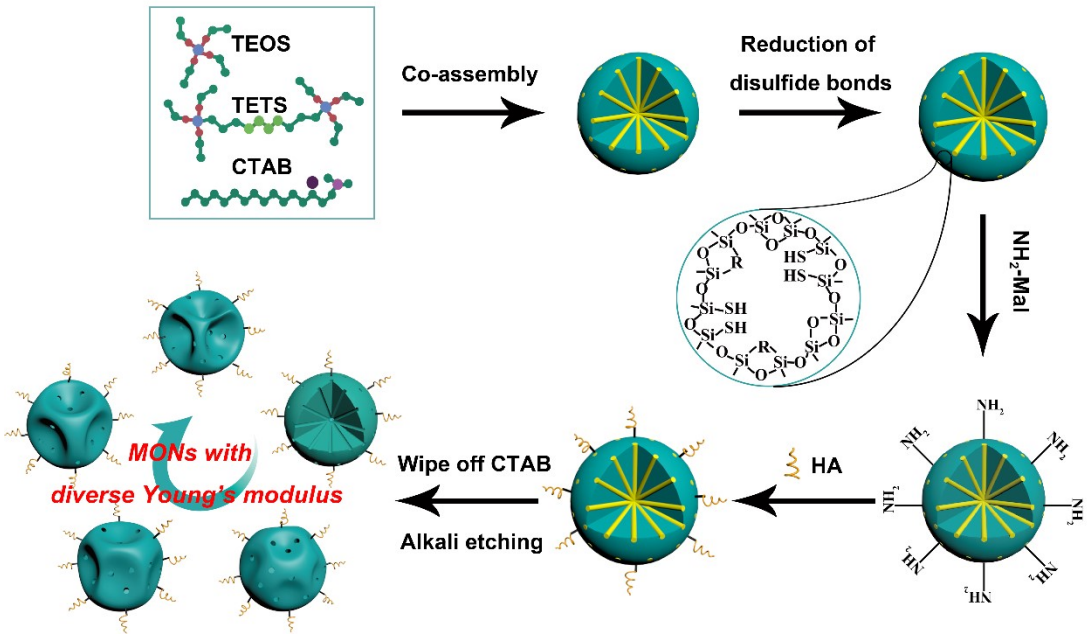

**Fig. S1.** Schematic illustration of the synthesis of deformable $MONs_{0\text{-}200}$-HA

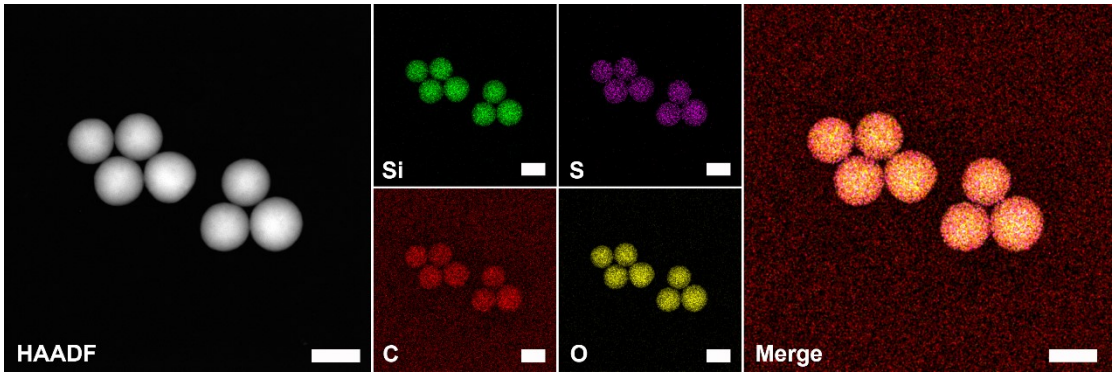


**Fig. S2.** High-angle annular dark-field scanning TEM image, elemental mapping images of Si, S, C, O, and merge image.

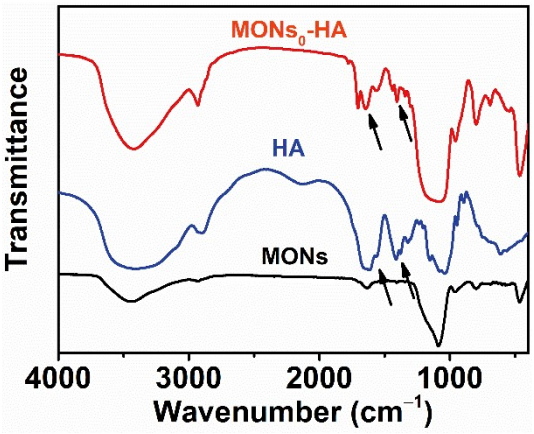


**Fig. S3.** FT-IR spectra of MONs, HA and $MONs_0$-HA.

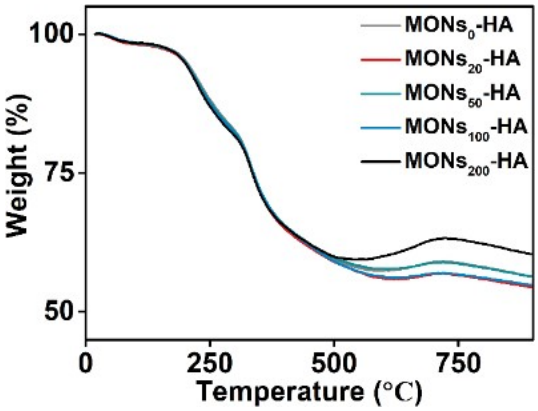


**Fig. S4.** The thermal gravity curves of $MONs_{0\text{-}200}$-HA.

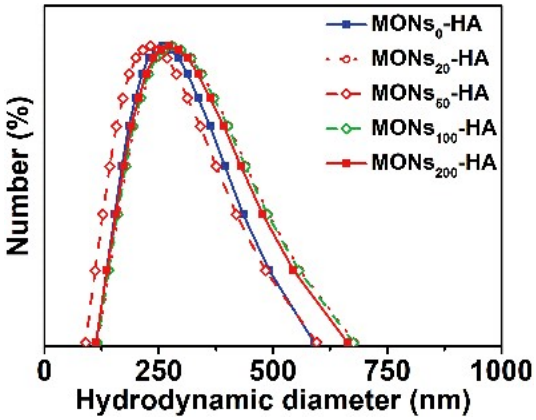


**Fig. S5** The hydrodynamic diameters of $MONs_{0\text{-}200}$-HA in PBS

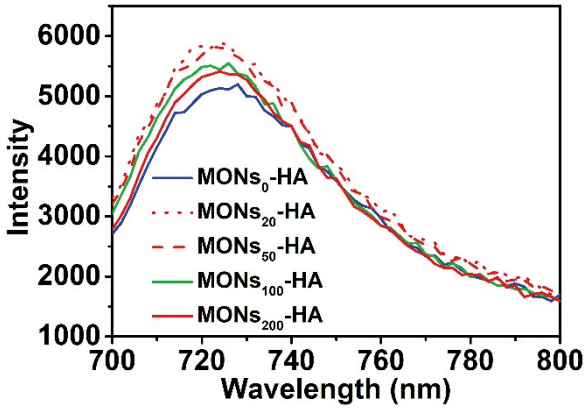


**Fig. S6.** Fluorescence emission spectrum of $MONs_{0\text{-}200}$-HA.

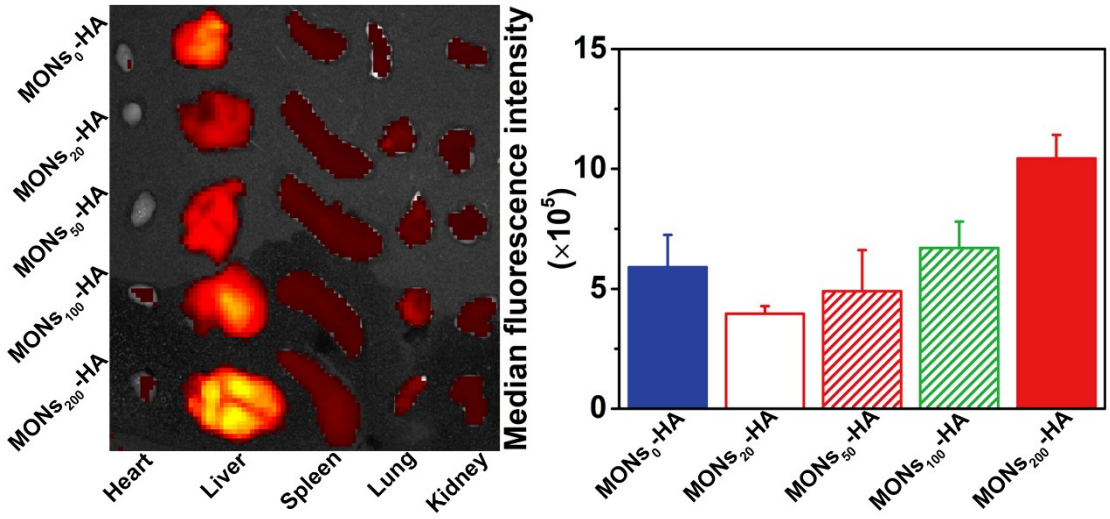


**Fig. S7.** *ex vivo* analysis of major organs (heart, liver, spleen, lung, and kidney) and median fluorescence intensity.