Electronic Supplementary Material (ESI) for Nanoscale.

-Supporting Information-

Direct Silica Coating of Drug Crystals for Ultra-high Loading

Neng Wang,‡ Weiwei Zhou,‡ Miao Yan, Mengmeng Zhang, Hong Wang, and Hongyu Chen**

Institute of Advanced Synthesis, School of Chemistry and Molecular Engineering, Jiangsu National Synergetic Innovation Center for Advanced Materials, Nanjing Tech University, Nanjing, 211816, P. R. China.

Email: ias_hwang@njtech.edu.cn, iashychen@njtech.edu.cn

Experimental Section

Materials

All chemical reagents were used without further purification. Pentyltriethoxysilane (PTES), 4-mercaptobenzoic acid (4-MBA, 98%), benzoic acid (BA, 99.5%) and sodium dodecyl sulfate (SDS, 98.5%) were purchased from Sigma Aldrich. (3-mercaptopropyl)triethoxysilane (MPTES, 98%) was purchased from energy chemical. Trimethoxysilylpropanethiol (MPTMS, 95%) was purchased from Alfa Aesar. Perylene (98%), paclitaxel (99%), carvedilol (98%), irinotecan (98%), itraconazole (98%), $NH_3 \cdot H_2O$ (AR grade, 25-28% w/w), tetraethyl orthosilicate (TEOS) and tetramethylene (THF, 99.8%), *N,N'*-dimethylformamide (DMF, 99.8%), dimethyl sulfoxide (DMSO, 98%) and all other chemicals were purchased from Aladdin. Deionized water (resistance > 18.2 MΩ/cm) was used in all reactions. Copper specimen grids (300 mesh) with formvar/carbon support film were purchased from Beijing Zhongjingkeyi Technology Co.

Characterization

TEM images were collected from a HT7700 Transmission Electron Microscopy (Japan) operated at 100 kV and a Talos L120C (USA) model operated at 120 kV. Florescence spectra were measured from a PerkinElmer LS55 (USA) and collected from sample solutions in a cuvette (pathlength = 1.00 cm) on a steady-state spectrofluorometer with the excitation wavelength of 335 nm. UV-vis spectra were collected on a Lambd 750 UV-vis spectrophotometer (USA). Thermogravimetric analysis (TGA) were measured from a Mettler Toledo TGA2 (Switzerland) and carried out under a flow of O_2 with a temperature ramp of 10 ^oC/min from 30 ^oC temperature to 600 ^oC.

Figure S1-S9

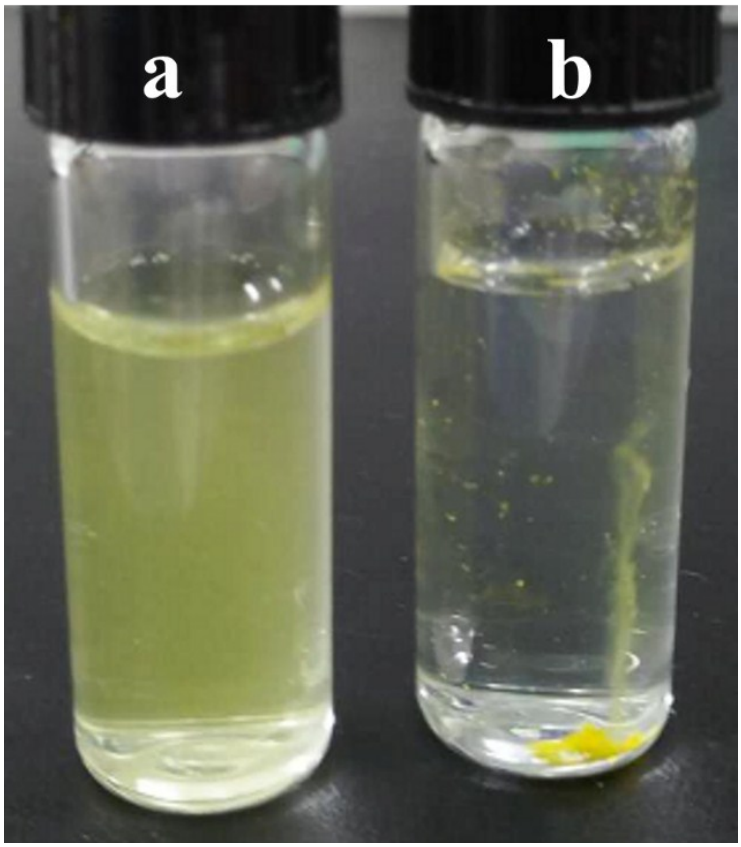

Figure S1. The photographs of (a) perylene crystals@silica, and (b) perylene crystals were incubated for one month, respectively.

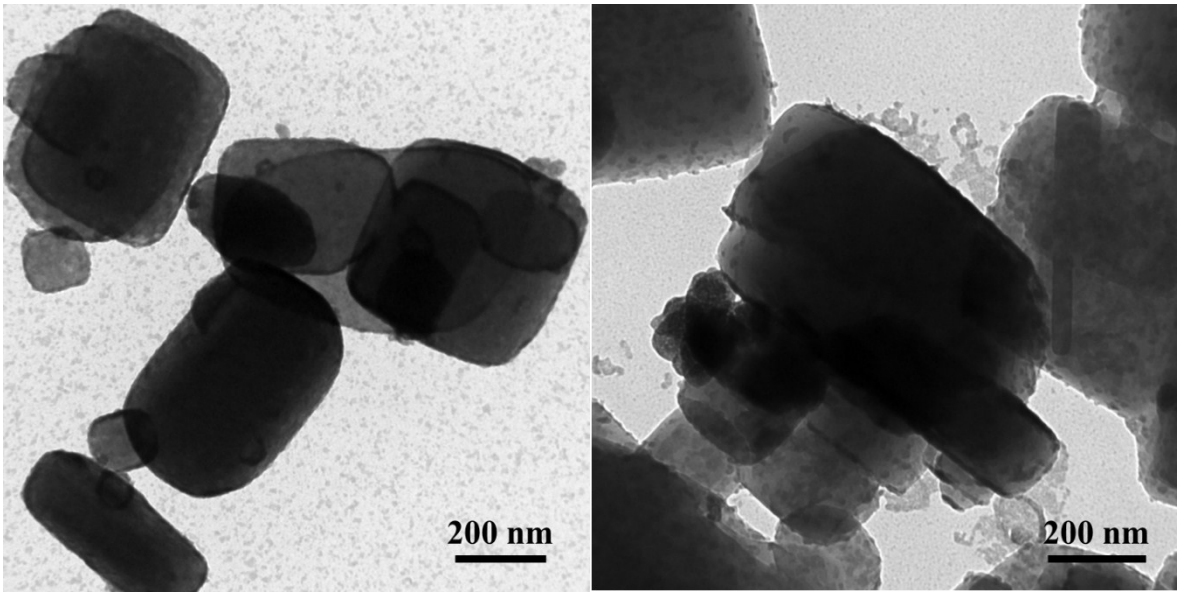

Figure S2. TEM images of the synthesized perylene@silica when benzoic acid was used as the ligand.

4-MBA as the ligand: Low concentration of 4-MBA (0.2 mM) would give few molecules that can be absorbed on the surface of organic crystals, and thus the crystals are easily aggregated and difficult to be encapsulated with the silica shells (Figure S3a). When the concentration of 4-MBA is high to 2 mM, the ligands tend to self-assembly into crystals, which can also be encapsulated with the silica shells (Figure S3b).

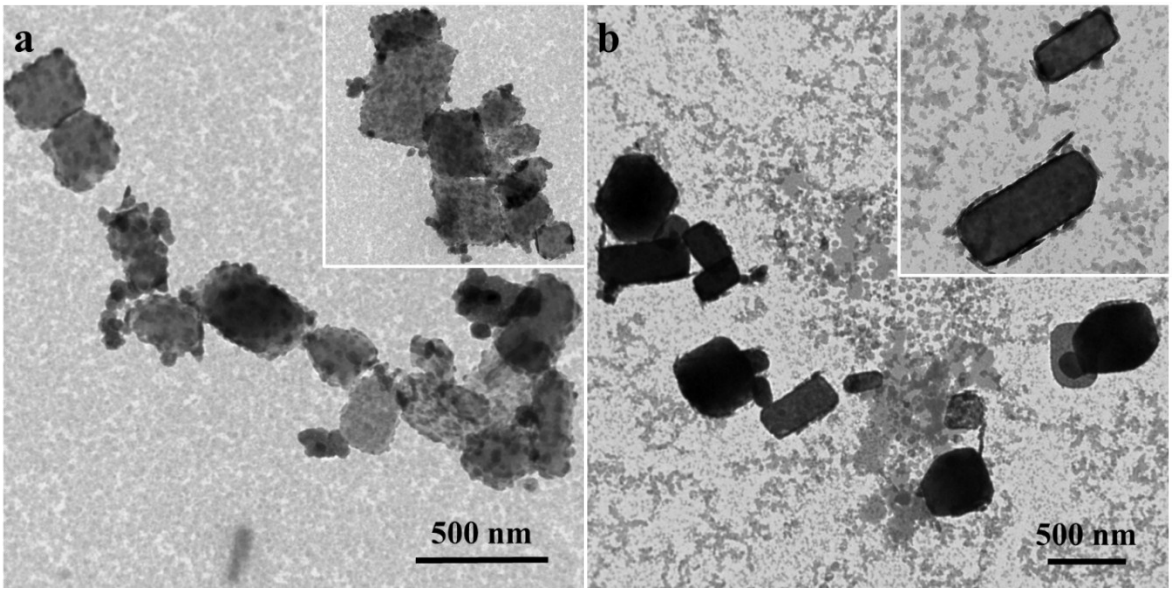

Figure S3. TEM images of the perylene@silica synthesized with 4-MBA as the ligand: (a) 0.2 mM, and (b) 2 mM.

Sequence of adding ligands: When the ligand PTES was added after the crystal formation, partial aggregation of crystals occurred (Figure S4). The PTES first hydrolyses into amphiphilic molecule, and then covers the crystal surface. However, such hydrolysis process is too slow to prevent the aggregation of crystals. As a result, the crystal is not completely covered or the silica shell was not uniform.

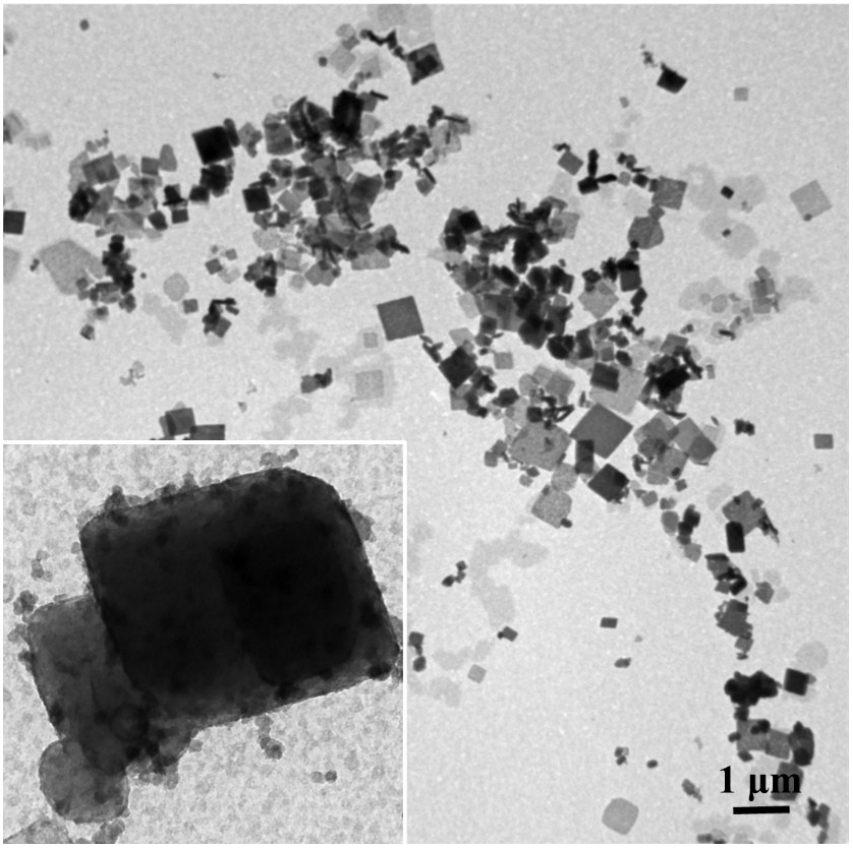

Figure S4. TEM images of perylene@silica if the ligand PTES was added after the crystal crystallization.

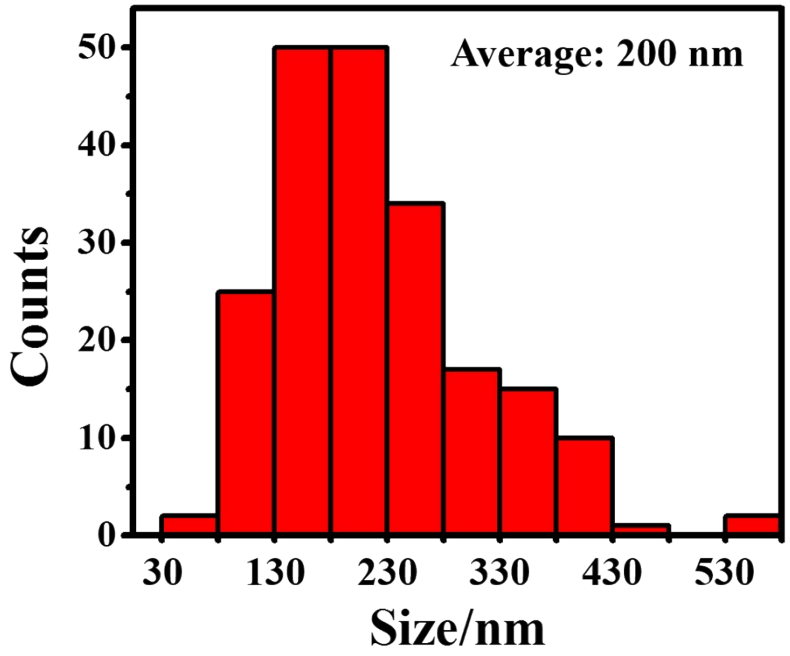

Figure S5. The size distribution of the perylene@silica nanoparticles.

Calculation of loading efficiency. The calculation of loading efficiency as following:[1]

$$\text{Loading content} = \frac{\text{the mass of perylene encapsulated in silica}}{\text{the mass of perylene@silica}}$$

$$\text{Encapsulation efficiency} = \frac{\text{the mass of perylene encapsulated in silica}}{\text{the mass of original perylene for encapsulation}}$$

Loading content was calibrated by the UV-vis absorbance. The as-synthesized perylene@silica (500 mL) was dried, and then dispersed in a DMF solution (6 mL). The mixture was vortexed for 1 h to completely dissolve the crystals. The mass of perylene in silica was further quantized by the UV-vis absorbance spectra. After that, the solution was centrifuged at 6000 rpm for 15 min. The core-free silica shells were collected at the bottom of eppendorf tube, and washed with DMF 5 times. Finally, the silica shells were dried in the oven at 80 ˚C, and then weighed, getting the mass of silica shell.

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Estimation of loading content. Taking a crystal cube with an average size of $(200\ nm)^3$ and 5 nm thick silica shell, and considering their respective density of 1.35 g/mL and 1.91 g/mL. Thus,

Mass of perylene = $200^3\ nm^3 \times 1.35\ g/mL = 1.08 \times 10^{-14}\ g$;

Mass of silica = $(205^3 - 200^3)\ nm^3 \times 1.91\ g/mL = 1.17 \times 10^{-15}\ g$;

Loading content = $1.08 \times 10^{-14}\ g \div (1.08 \times 10^{-14}\ g + 1.17 \times 10^{-15}\ g) = 90\%$.

Encapsulation efficiency. To obtain the encapsulation efficiency, the perylene@silica nanoparticles were centrifuged from the solution, and dispersed into DMF to dissolve the perylene, which amount was obtained by measuring UV-vis absorbance to be 72% of the total amount used (Fig. S6d).

In contrast, for micelle system (Fig. S6a), the loading amount of the drugs depends on the solubilizing capacity of the hydrophobic core. For mesoporous nanoparticles (Fig. S6b), the loading amount relies on its affinity with the carrier surface. In such two cases, the maximum loading content is achieved when the solubility equilibrium or adsorption equilibrium is respectively reached. As a result, there is still a large amount of "free" drug molecules in the solution. For hollow structures, the drug is generally loaded by post-diffusion. In most cases, the drug can only enter the cavity in the form of a single molecule, and the encapsulation efficiency depends on the volume of the cavity. Maximum load is achieved when the drug concentration between the inside and outside of the cavity is the same. However, the volume occupied by the cavity is only a small fraction of the drug solution. The volume ratio of the cavity to the solution is the encapsulation efficiency. Thus, the low encapsulation efficiency can be obtained (Fig. S6c).[2]

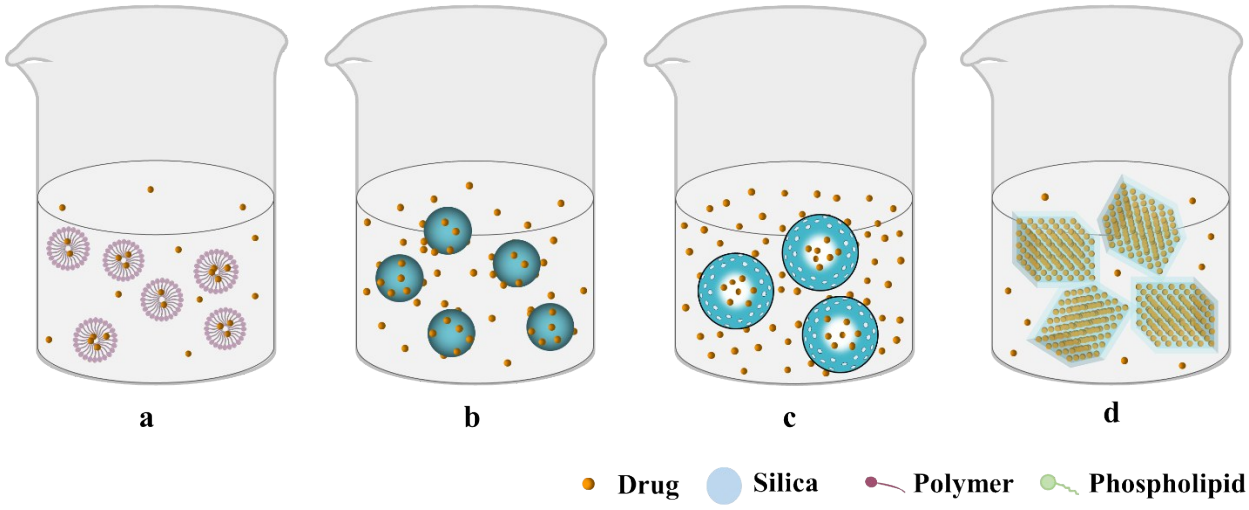

Figure S6. Schematics illustrating the encapsulation efficiency of four loading strategies: drug molecules are (a) encapsulated in the hydrophobic core of micelles, (b) absorbed on the surface of mesopores, and c) enclosed into the cavity of hollow structures; (d) drug crystals are encapsulated in the silica shells.

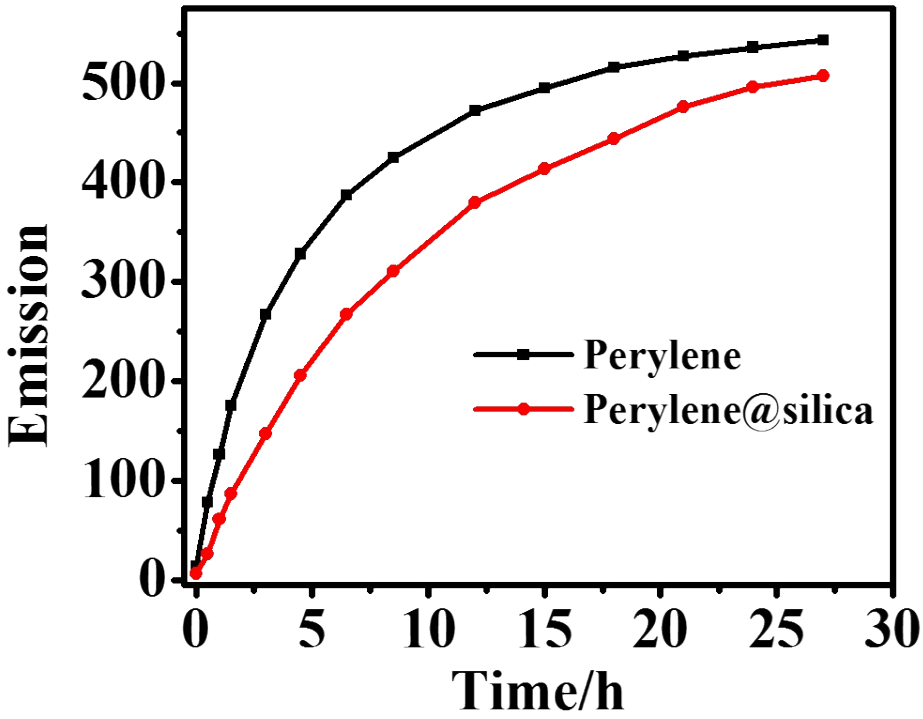

Figure S7. The release curve of perylene crystal (black) and perylene@silica (red) in SDS micelle solution.

Estimation of crystals number. Assuming the concentration of perylene is 0.01 mM and the volume of perylene solution is 1 mL. Thus,

Mass of perylene = 0.01 mM x 1 mL x 252.3 g/mol = 2.523×10^{-6} g;

Assuming the average size of perylene crystal cube is 200 nm. The density of perylene crystal is 1.35 g/mL. Thus,

Number of perylene crystal = $(2.523 \times 10^{-6}$ g ÷ 1.35 g/mL) ÷ $(200 \text{ nm})^3 = 2.3 \times 10^8$

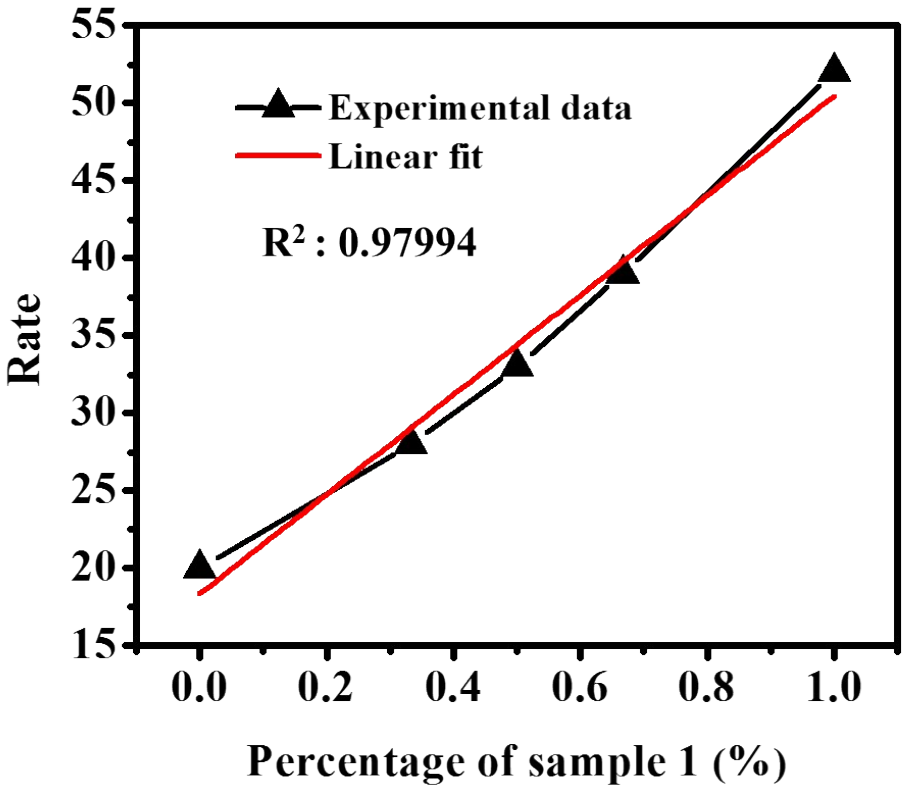

Figure S8. The release rate when perylene releasing from the mixture of perylene@silica (sample 1) and those modified with MPTES (sample M), assuming that the release rate of all the samples is constant rate at initial six hours. The content of sample 1 in the mixture is 0%, 33%, 50%, 67% and 100%, respectively.

The estimate of paclitaxel loading content. The silica to paclitaxel mass ratio can be estimated based on their volume and density. Taking paclitaxel crystal with cylinder shape and an average size of π × 552 × 630 nm^3 and 4 nm thick silica shell (Figure S9), and considering their respective density of 1.39 g/mL and 1.91 g/mL, the loading content is estimated to be 82%.

The specific calculation process is as follows:

Volume of silica = π × 57× 630 × 4 nm^3 + 2 × (π × 592 × 4) nm^3 = 990000 nm^3

Mass of silica = 990000 nm^3 × 1.91 g/mL = 1.89 × 10^{-15} g

Volume of paclitaxel = π × 552 × 630 nm^3 = 5987090 nm^3

Mass of paclitaxel = 5987090 nm^3 × 1.39 g/mL = 8.32 × 10^{-15} g

Loading content = 8.32 × 10^{-15} g ÷ (8.32 × 10^{-15} g + 1.89 × 10^{-15} g) = 82%

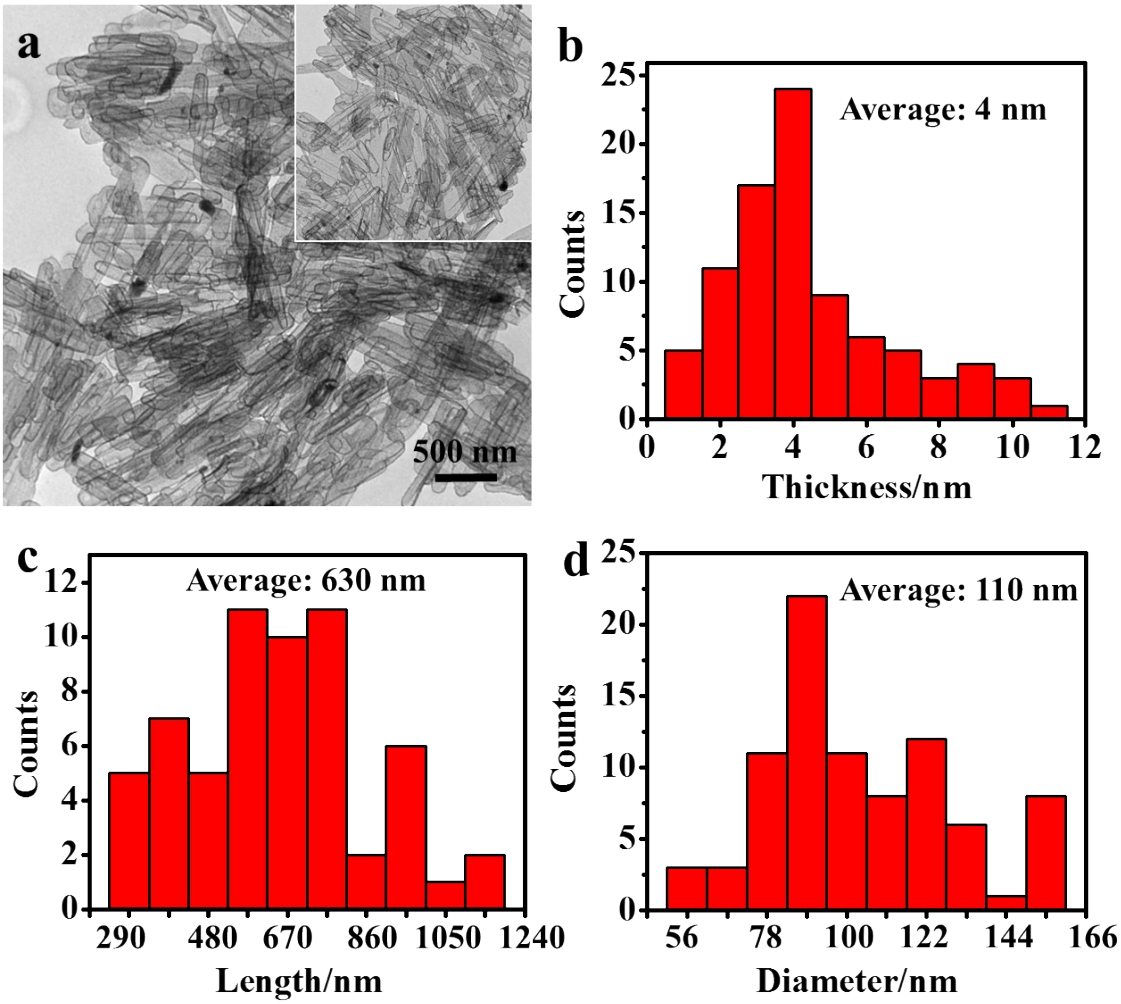

Figure S9. a) TEM images of paclitaxel@silica after dissolving paclitaxel; b) the histogram of silica shell thickness; the histogram of paclitaxel crystal c) length, and d) diameter, respectively.

References

(1) Cunningham, A. J.; Robinson, M.; Banquy, X.; Leblond, J.; Zhu, X. X., Bile Acid-Based Drug Delivery Systems for Enhanced Doxorubicin Encapsulation: Comparing Hydrophobic and Ionic Interactions in Drug Loading and Release. *Mol. Pharm*. **2018**, *15*, 1266-1276.

(2) Wang, N.; Cheng, X.; Li, N.; Wang, H.; Chen, H., Nanocarriers and Their Loading Strategies. *Adv. Healthcare Mater*. **2019**, *8*, 1801002.