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CuS@PDA–FA Nanocomposites: A Dual Stimuli-responsive DOX Delivery Vehicle with Ultrahigh Loading level for Synergistic Photothermal-chemotherapies on Breast Cancer

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Electronic Supplementary Information

Supplementary Experimental Section

Preparation of citrate coated CuS and PDA

For CuS preparation, 0.2 mmol $CuCl_2 \cdot 2H_2O$ and 0.136 mmol sodium citrate were added in 100 mL DI water. Then, 20 mL $Na_2S \cdot 9H_2O$ solution (10 mM) was added and stirred for 5 min at room temperature. The mixed solution was heated at 90 °C for 30 min and put rapidly into an ice water bath to cool down. Finally, the citrate-coated CuS nanoparticles were obtained.[1]

For PDA preparation, 300 μL $NH_3 \cdot H_2O$ solution, 4 mL ethanol and 9 mL water were mixed and stirred for 30 min. 1 mL DA solution (50 mg mL^{-1}) was then added into the above mixture. Finally, the solution was stirred at 30 °C for 24 h to obtain PDA nanoparticles.[2]

Calculation of photothermal conversion efficiency

According to previous reports,[3, 4] the important calculation processes are listed below.

$$\theta = \frac{T - T_{surr}}{T_{max} - T_{surr}} \quad (1)$$

$$t = -\tau_s \cdot ln\theta \quad (2)$$

These formulas were applying to the cooling process without laser irradiation. The t is the cooling time, T is the corresponding temperature, T_{max} is the maximum system temperature and T_{surr} is the ambient temperature of surroundings. τ_s is the system time constant which can be obtained by inducing the Equation (2)

$$hS = \frac{m \cdot C_{H_2O}}{\tau_s} \quad (3)$$

$$\eta = \frac{hS(T_{max} - T_{surr}) - Q_{Dis}}{I(1 - 10^{-A_{808}})} \quad (4)$$

m is the mass of CuS, PDA, CuS@PDA or CuS@PDA-FA solution, C_{H_2O} is the specific heat capacity of water. The hS can be obtained by Equation (3). The energy input by pure solvent (Q_{dis})

was measured to be 28 mW. A_{980} is the absorbance of the materials we made at 808 nm. Finally, we can calculate the photothermal conversion efficiency η.

Biocompatibility of CuS and PDA

Firstly, the MCF-7 cells were seeded in 96-well plates and incubated 24 h. Then, the cells were incubated with the fresh medium including citrate-coated CuS, PDA, CuS@PDA (100, 200, 500 μg mL^{-1}). After incubating for 20 h, 10 μL MTT solution (5.0 mg mL^{-1}) was added in each well and the cells were cultured for further 4 h. 100 μL DMSO was added to dissolve the generated formazan crystals. Thereafter, the absorbance was measured at 490 nm by a microplate reader.

Effects of laser irradiation of power density on MCF-7 cells

The effect of laser irradiation of power density on MCF-7 cells was also studied by an MTT assay. We used the 808 nm laser of different power densities (0.6, 0.8, 1.0, 1.5, 2.7 W cm^{-2}) to irradiate cells for 5 min and detected the cell viability by a microplate reader to verify the effect of laser irradiation on cells.

Cellular uptake of CuS@PDA-FA/DOX

Typically, MCF-7 cells were seeded in glass-bottom cell culture dishes and grown for 24 h. The cells were then cultured with CuS@PDA-FA/DOX for 0.5, 1, 2 and 4 h and washed with 10 mM PBS buffer. Subsequently, the cells were fixed with 4% paraformaldehyde. After staining with the DAPI solution for 10 min, the cells were observed by a confocal microscope. The effect of laser irradiation on cellular uptake was also studied. The MCF-7 cells were incubated for 0.5 h with 100 μg mL^{-1} CuS@PDA-FA/DOX in glass-bottom dishes. There were two groups. In Group 1, the cells were irradiated for 5 min by 808 nm laser (0.8 W cm^{-2}). In Group 2, the cells were not irradiated. All

cells were cultured for another 0.5 h and washed by 10 mM PBS buffer. The cells were fixed and stained. Finally, the cells were observed by a confocal microscope.

Supplementary Figures

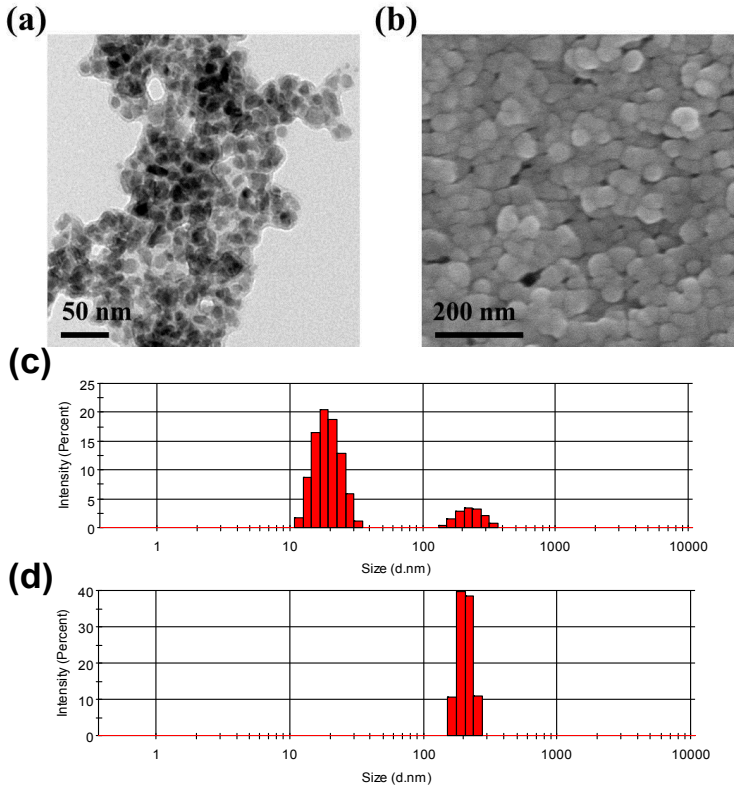

Fig. S1 (a) TEM image of CuS@PDA-FA ,(b) SEM image of CuS@PDA-FA, (c) Dynamic light scattering analyses of CuS@PDA(c) and CuS@PDA-FA(d).

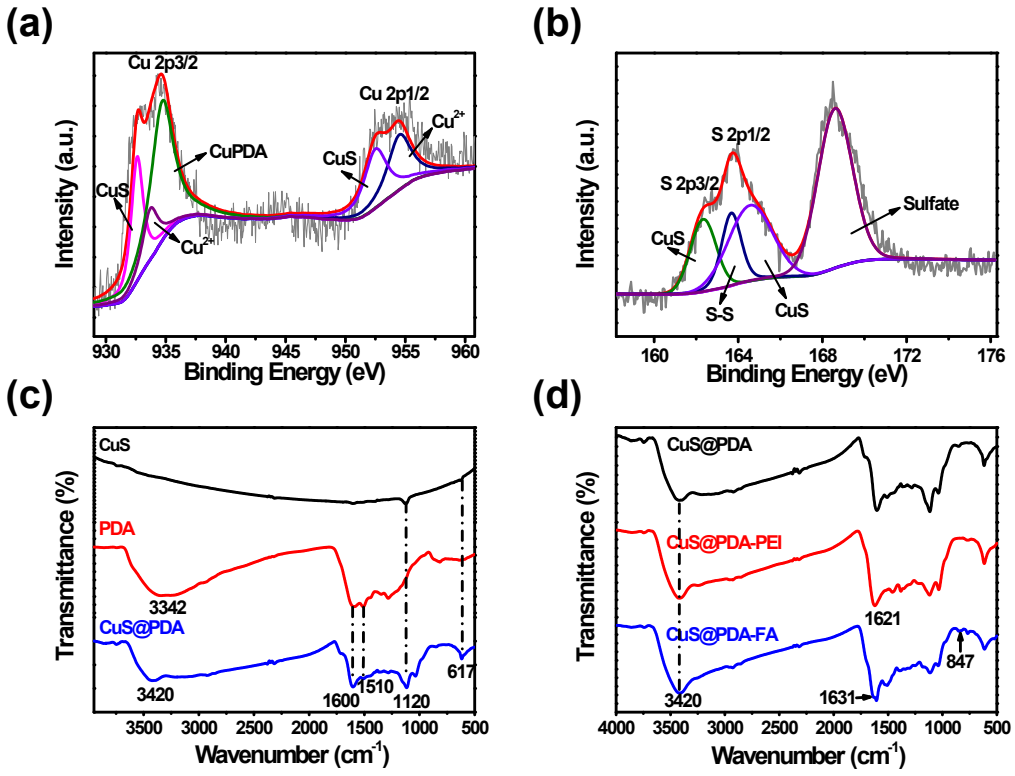

Fig. S2 XPS scans of CuS@PDA: (a) Cu 2p spectrum and (b) S 2p spectrum; FTIR spectra of (c) CuS, PDA, CuS@PDA and (d) CuS@PDA, CuS@PDA-PEI, and CuS@PDA-FA.

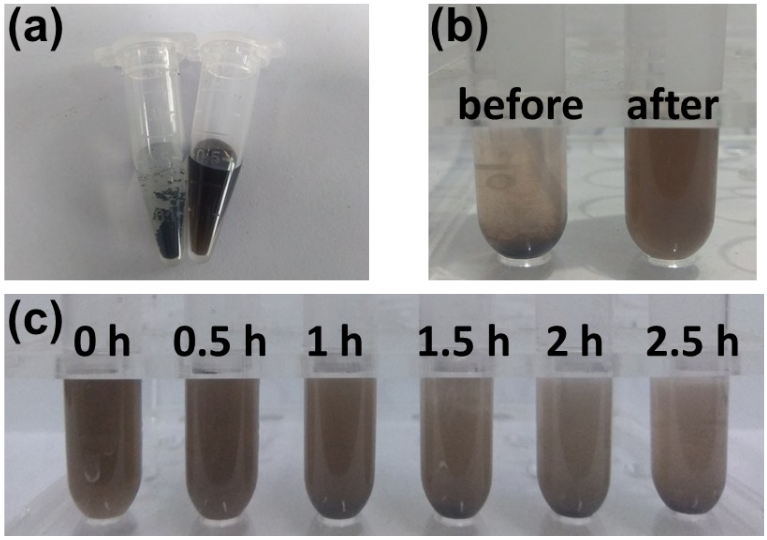

Fig. S3 (a) Photograph of CuS (left) and CuS@PDA (right) under the 808 nm laser irradiation (1.5 W cm^{-2})for 10 min, (b) Photos of the CuS@PDA-FA/DOX dispersion before/after sonication, (c) Photos of the CuS@PDA-FA/DOX dispersion standing at different times.

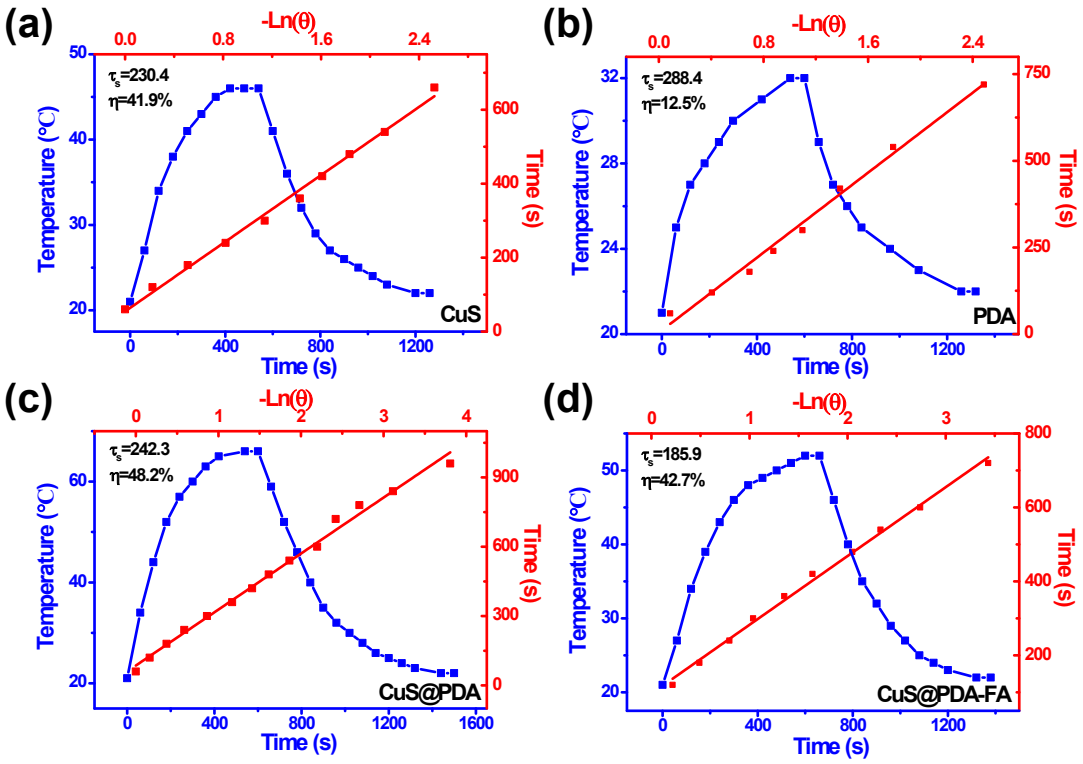

Fig. S4 Steady-state heating curve of (a)CuS, (b)PDA, (c)CuS@PDA and (d)CuS@PDA-FA

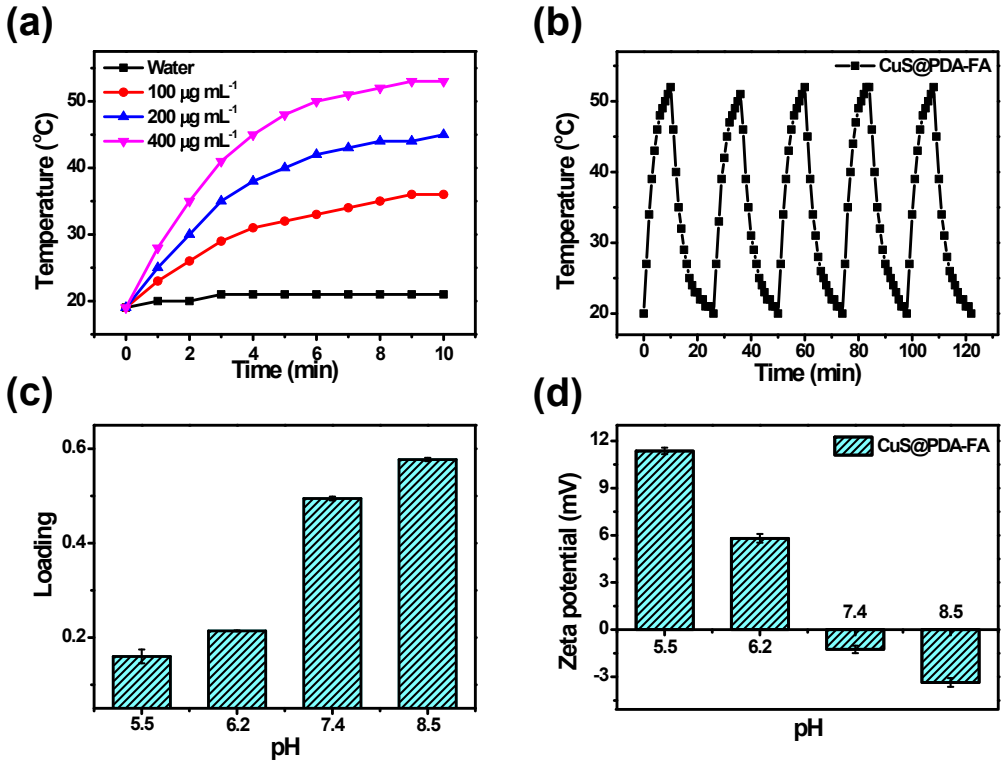

Fig. S5 (a) The photothermal profles of DI water and CuS@PDA-FA NPs solution with different concentrations under 808 nm laser irradiation (1.5 W cm^{-2}). (b) Photothermal stability study of CuS@PDA NPs. (c) DOX loading efficiency on CuS@PDA-FA in 10 mM PBS buffer with various pH values. (d) Zeta potential of CuS@PDA-FA in PBS buffer with different pH values.

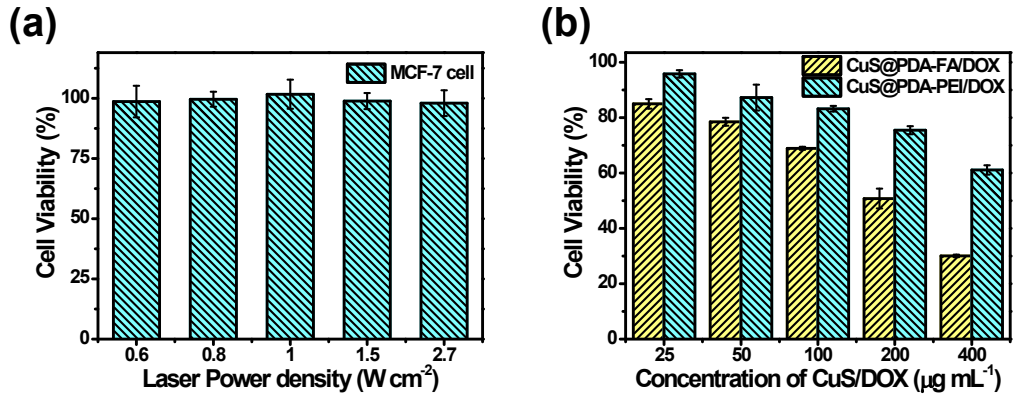

Fig. S6 (a) Cell viability of MCF-7 cells incubated with different power densities of laser irradiation. (b) Cell viability of MCF-7 cells incubated with CuS@PDA-FA/DOX and CuS@PDA-FA/DOX nanocomposites at various concentrations.

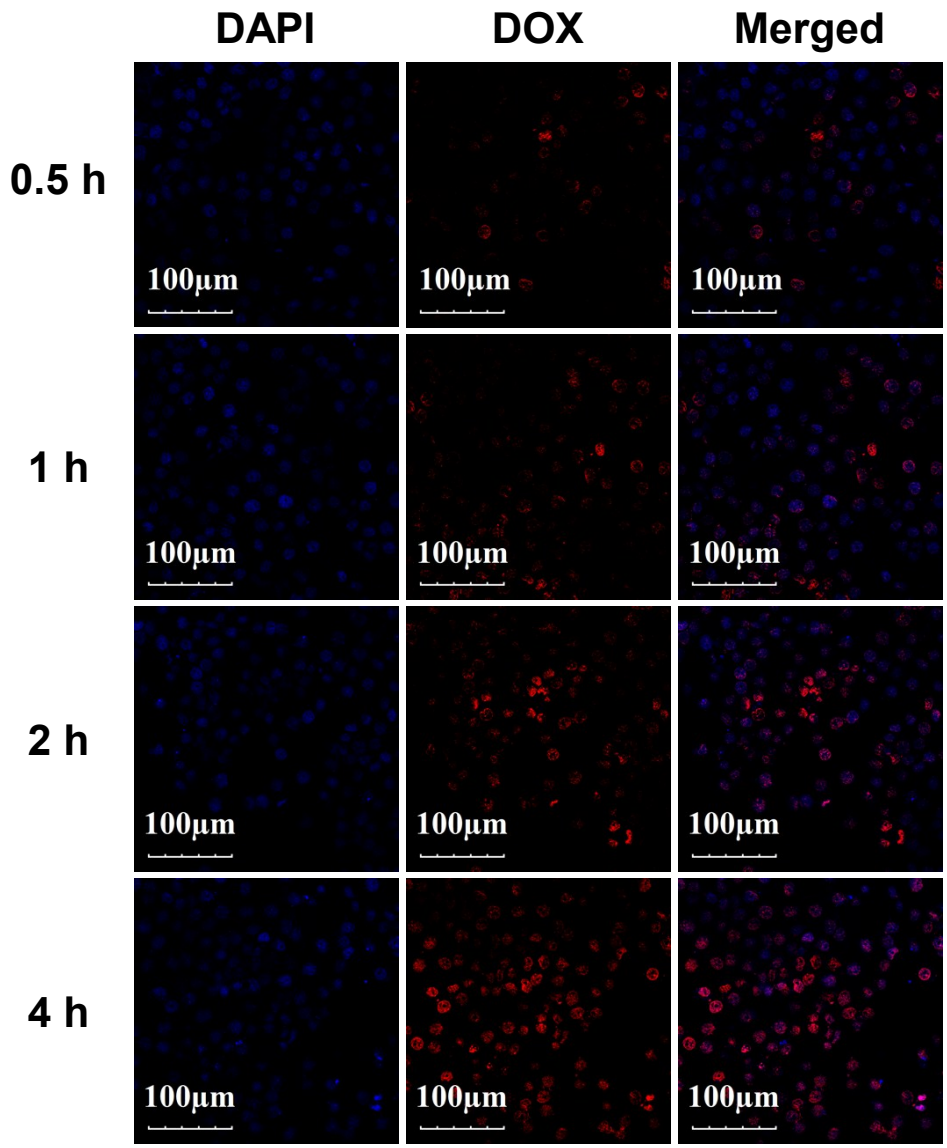

Fig. S7 CLSM images of MCF-7 cells incubated with CuS@PDA-FA/DOX nanocomposites for 0.5, 1, 2 and 4 h, respectively.

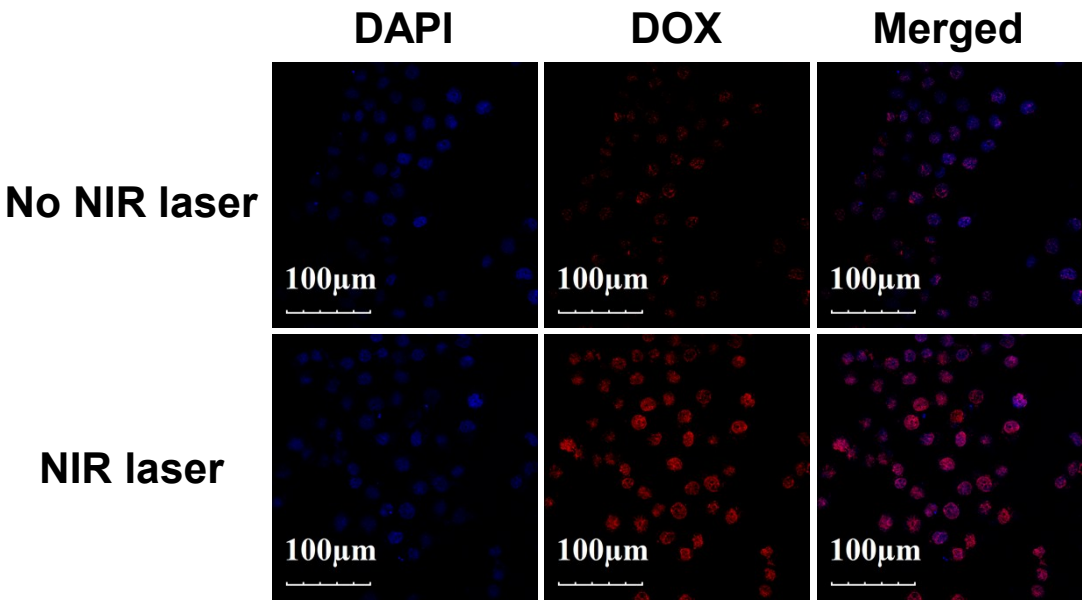

Fig. S8 CLSM images of MCF-7 cells incubated with CuS@PDA-FA/DOX nanocomposites for 1 h without or with NIR irradiation (0.8 W cm^{-2}, 5 min).

Table S1 Summary of DOX loading level of various materials

Materials	Loading mechanism	Loading level (mg mg^{-1})	Reference
CuS@copolymer	electrostatic interaction, hydrogen bonding	0.153	5
MoS_2@BSA	electrostatic interaction, the 2D nature of MoS_2 and the high surface area of flower-like structure	0.34	6
CuS–PAA	electrostatic interaction	0.21	7
Ultrasmall Pd nanosheets	Pd–N coordination bonding	0.053	8
PB@mSiO_2-PEG	electrostatic interaction	1.31	9
CDPGM	π–π stacking and hydrogen-bonding interaction	0.40	2
HA capped MSNs	the surface-to-volume ratio and strong electrostatic interaction	0.14	10
CuS/GO	electrostatic interaction	1.75	11
CuS@PDA-FA	electrostatic interaction, π–π stacking and large specific surface area	4.2	this work

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