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Supporting Information

Multi-functional DNA-conjugated nanohydrogels for aptamer-directed breast cancer cell targeting

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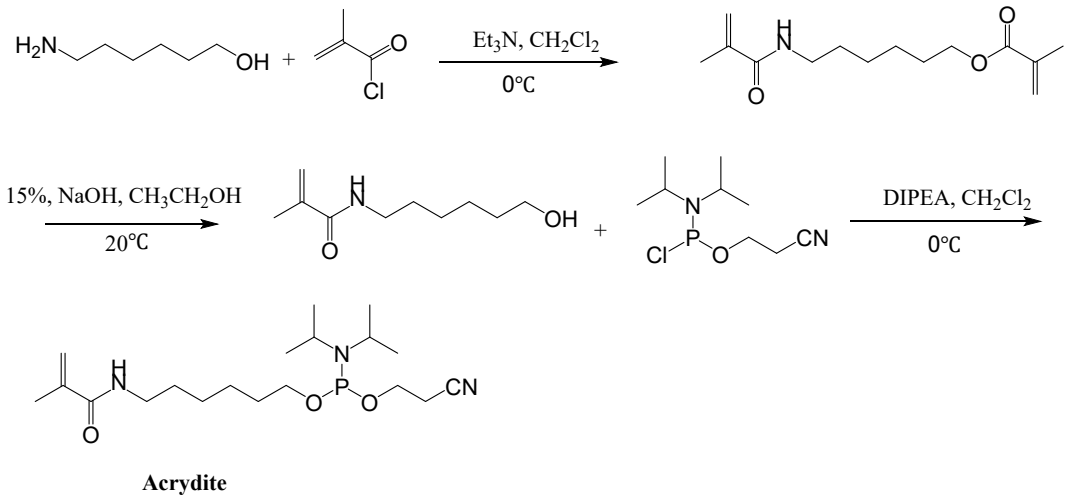
1. Synthesis of acrydite and Acrydite-DNA

The acrydite was synthesized according to previous report (Scheme S1) [1]. 6-amino-1-hexanol (0.5 g, 4.3 mmol) was cooled at 0 °C in dichloromethane, Triehylamine (1.2 mL, 8.6 mmol) was added to the solution, and methacryloyl choride (1.35 g, 12.7 mmol) was added dropwise, the reaction solution was then stirred at 0 °C for 2 hours. After concentrated of the mixture, the mixture was poured into 5 mL ethanol and 15 % sodium hydroxide (2 mL) for 1 h. After evaporation of all solvents, the residue was added dichloromethane (10 mL) and washed with sodium hydrogen carbonate solution (10 mL, 5 mL×2) and sodium chloride (10 mL, 5 mL×2). The resulting material was chromatographed on a silica gel using ethyl acetate to afford 6-hydroxyhexyl methacrylamide (0.64 g, 80 % yield). A solution 6-hydroxyhexyl methacrylamide (0.50 g, 2.70 mmol) in anhydrous CH_2Cl_2 (10 mL) was added slowly N,N'-Diisopropylethylamine (DIPEA) (0.98 g, 7.50 mmol). Then, 2-cyanoethyl diisopropyl chlorophosphoramidite (0.87 mL, 3.25 mmol) was added dropwise to the reaction solution and the mixture was stirred at 0 °C for 2 h. After removing the solvent, the residue was added dichloromethane (10 mL) and washed with sodium hydrogen carbonate solution (10 mL, 5 mL×2) and sodium chloride (10 mL, 5 mL×2) and dried with $MgSO_4$. The residue was chromatographed on a silica gel using a 40:60:3 ethyl acetate-hexane-triethylamine and dried to afford acrydite (0.62 g, 60 % yield) as colorless oil. The final product was coupled with DNA to obtain acrydite-DNA by DNA synthesizer. Acrydite: 1H NMR (400 MHz, CDCl3): δ 5.85 (s, 1H), 5.67 (s, 1H), 5.30 (s. 1H), 3.80-3.75 (m, 2H), 3.70-3.50 (m, 4H), 3.35-3.25 (m, 2H), 2.65 (t, 2H), 1.95 (s, 3H), 1.68-1.52 (m, 4H) 1.47-1.30 (m, 4H) 1.22-1.15 (m, 12H)； ESI-MS, (m/z)：384.9 $[M-H]^-$, Molecular weight of Acrydite-DNA was determined by Sangon Biotech, Shanghai, China (Fig. S1).

Table S1 Sequences of the synthesized DNA.

Name	DNA sequences
Acrydite-DNA	5’-Acrydite-d (AAA AAA AAA AAA AAA AAA AA)-3’
MUC1-aptamer	5’-GCA GTT GAT CCT TTG GAT ACC CTG GTT TTT TTT TTT TTT TTT TTT TT-3’
T-DNA	5’-TTT TTT TTT TTT TTT TTT TT-3’



Scheme S1 Synthetic route of acrydite

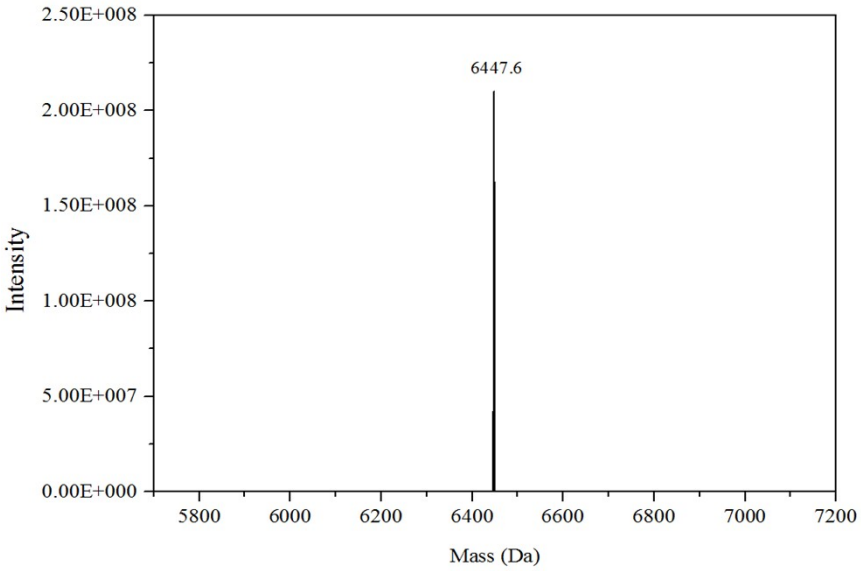


Fig. S1 Mass spectrum of acrydite-DNA

2. Swelling behaviour of DNA-conjugated nanohydrogels

Swelling tests were conducted with freeze-dried DNA-conjugated nanohydrogels by immersing it in ultrapure water.[2] The concentration of gel-solution was 1 mg/mL. At predetermined time intervals, the sample was centrifuged followed by removing the supernatant. the precipitate was wiped with a filter paper to remove the surface water, and then weighted. The swelling ratio (SR) was calculated by the following equation:

$$SR=(W_t-W_0)/W_0$$

where W_0 is the initial weight of dried nanohydrogels, W_t is the weight of nanohydrogel at time t after immersing in ultrapure water. Three samples were tested for each group.

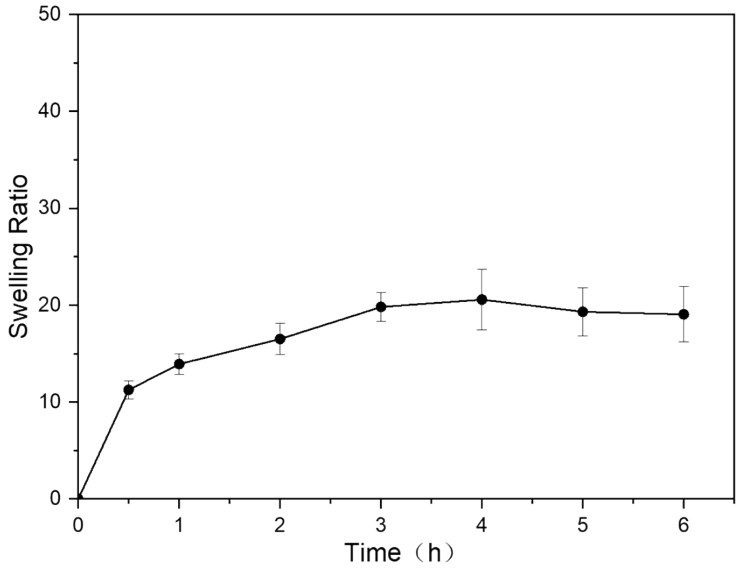


Fig. S2 Swelling behavior of DNA-conjugated nanohydrogels in water.

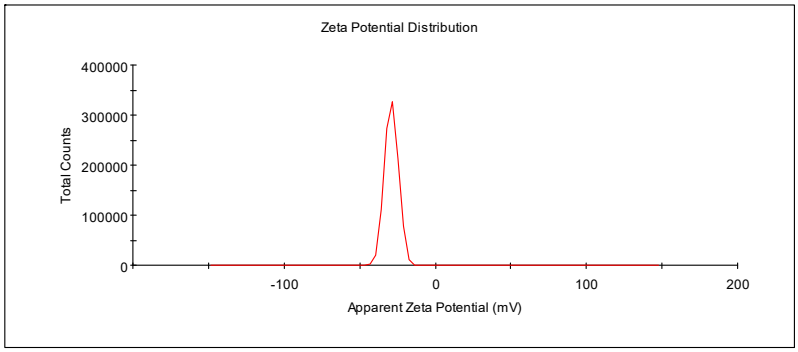


Fig. S3 *Z*-potential chart of DNA-conjugated nanohydrogels.

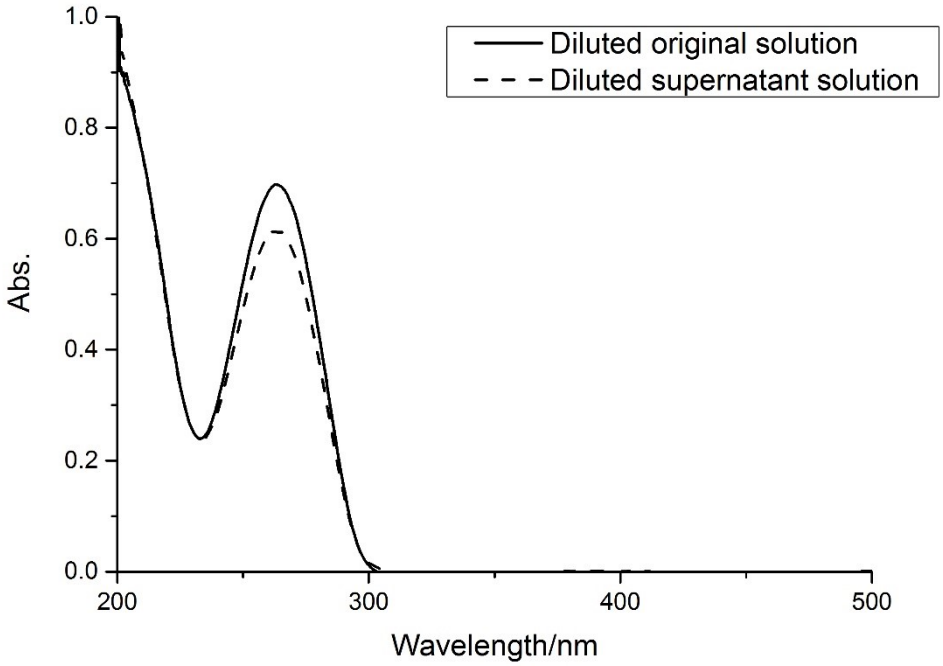


Fig. S4 UV-visible spectra of diluted original MUC-1 aptamer solution (solid line) and diluted supernatant solution (dashed line) containing unbound MUC-1 aptamers.

Table S2 Drug loading capacity and efficiency of Dox in DNA-conjugated nanohydrogels.

Sample code	DNA-conjugated nanohydrogels (mg)	Dox (mg)	DLC (wt%)	DLE (wt%)
1	10	2	15.8	95.1
2	10	4	26.85	91.8
3	10	6	35.76	88.4
4	10	8	39.7	82.3
5	10	10	42.9	75.5

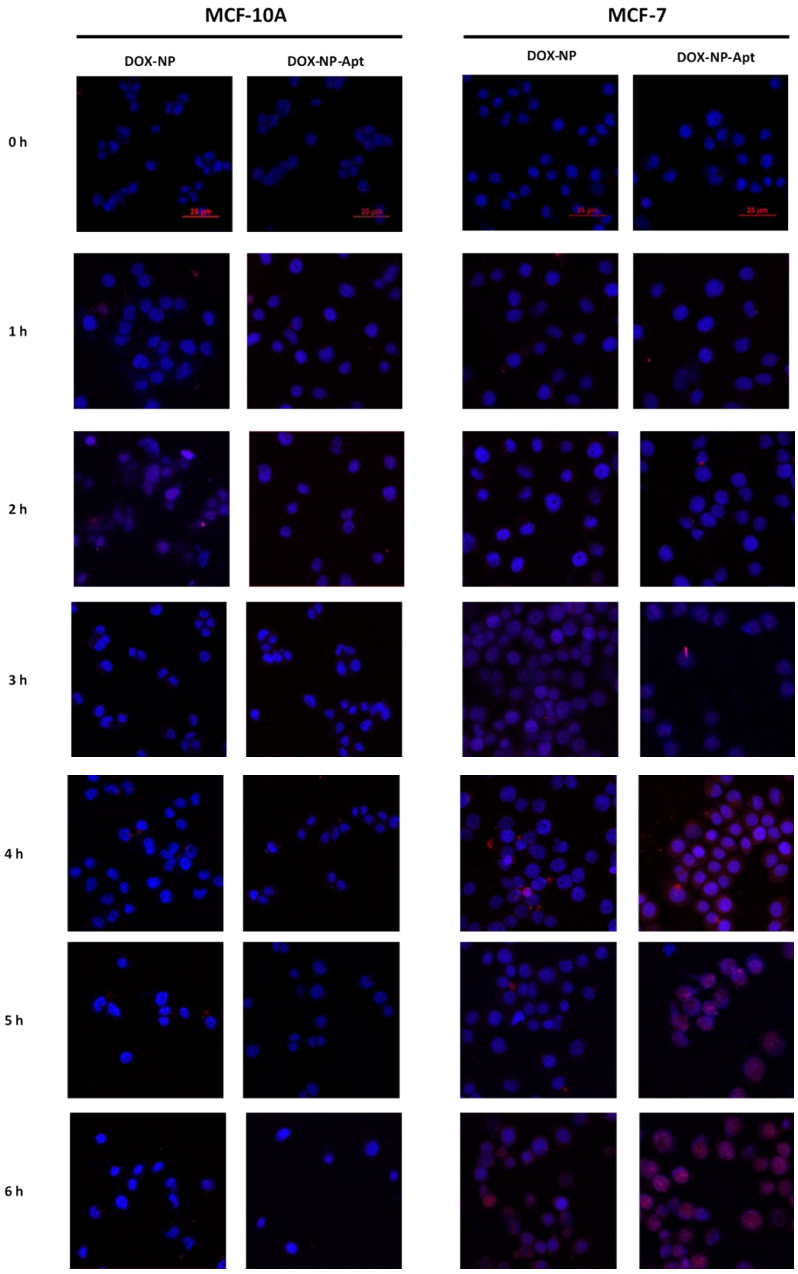


Fig. S5 Representative confocal images of the uptake of MCF-7 and MCF-10A cells treated with Dox-loaded nanohydrogel particles (Dox-NP) or aptamer-functionalized Dox-NP (Dox-NP-Apt) at different times. MCF-7 and MCF-10A cells were treated with Dox-NP or Dox-NP-Apt. The photographs were analyzed at 600× magnification.

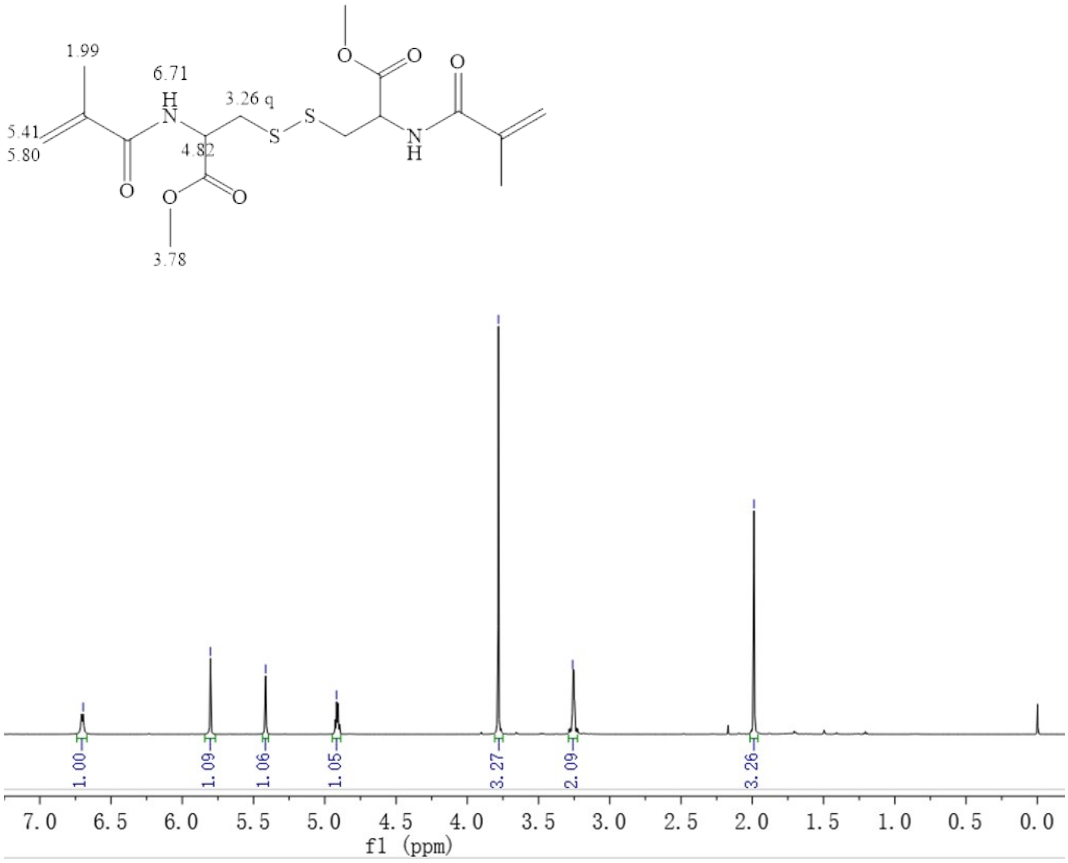


Fig. S6 ^{1}H NMR spectrum of *N,N'*-dimethacryloylcystine dimethyl ester.

References

1. H. Zhao, G. Jiang, J. Weng, Q. Ma, H. Zhang, Y. Ito and M. Liu, *J. Mater. Chem. B*, 2016, **4**, 4648-4651.

2. C. Su, J. Liu, Z. R. Yang, L. Jiang, X. F. Liu and W. Shao, *Int. J. Biol. Macromol.*, 2020, **161**, 1140-1148.