

Hypoxia-responsive drug-drug conjugated nanoparticles for breast cancer synergistic therapy

Ruilong Zhang,^{a,b,⊥} Yan Li,^{b,⊥} Miao Zhang,^{a,b} Qunwei Tang^{*,a} and Xin Zhang^{*,b}

^a Institute of Materials Science and Engineering, Ocean University of China, Qingdao, Shandong Province, 266100, PR China. E-mail: tangqunwei@ouc.edu.cn

^b National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing, 100190, PR China. E-mail: xzhang@ipe.ac.cn

[⊥] R. L. Zhang and Y. Li contributed equally to this work.

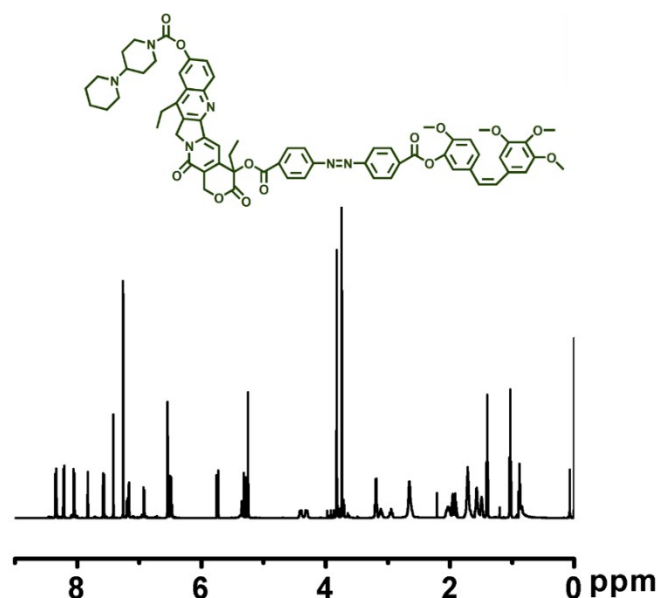


Fig. S1 ¹H NMR spectra of IR-AZO-CA4 (600 MHz, CDCl₃).

¹H NMR spectroscopy (600 MHz, CDCl₃ δ ppm) was carried out to characterize the IR-AZO-CA4: 0.087 (-C-CH₂-CH₃, t, 3H), 1.04 (Py-CH₂-CH₃, t, 3H), 1.40 (Pip-N-CH₂-CH₂-CH₂-CH₂-CH₂, t, 6H), 1.48 (Pip-CH₂-CH₂-CH₂-CH₂-N-C=O, t, 4H), 1.56 (-C(OH)-CH₂-CH₃, m, 2H), 1.72 (Pip-N-CH₂-CH₂-CH₂-CH₂-CH₂-N-, t, 4H), 2.02 (-CH₂-N(-C=O)-CH₂-, m, 4H), 2.65 (Py-CH₂-CH₃, m, 2H), 3.18 (Pip-(CH₂))

C(H)-CH₂, t, 1H), 3.75 (Ph-O-CH₃, s, 6H), 3.82 (Ph-O-CH₃, s, 6H), 5.29 (-N-CH₂, m, 2H), 5.72 (Py-CH₂-O-C(=O)-, m, 2H), 6.48 (Ph-CH=CH-Ph, m, 2H), 6.54 (CH₃O- Ph-H, s, 2H), 6.91 (CH₃O- Ph-H, d, 1H), 7.15 (O=C-O-CH₃O-Ph-H, d, 1H), 7.18 (Ph-H), dd, 1H), 7.22 (Py-C(H)=C(O)-, s, 1H), 7.42 (Py-(N)C=C(H)-, s, 1H), 7.58 (Py-C(H)=C(H)-C(O)=, d, 1H), 7.83 (Py-C(H)=C(H)-C(O)=, d, 1H), 8.04 Ph-H, m, 4H), 8.20 (IR-Ph-H, m, 2H), 8.34 (CA4-Ph-H, m, 2H).

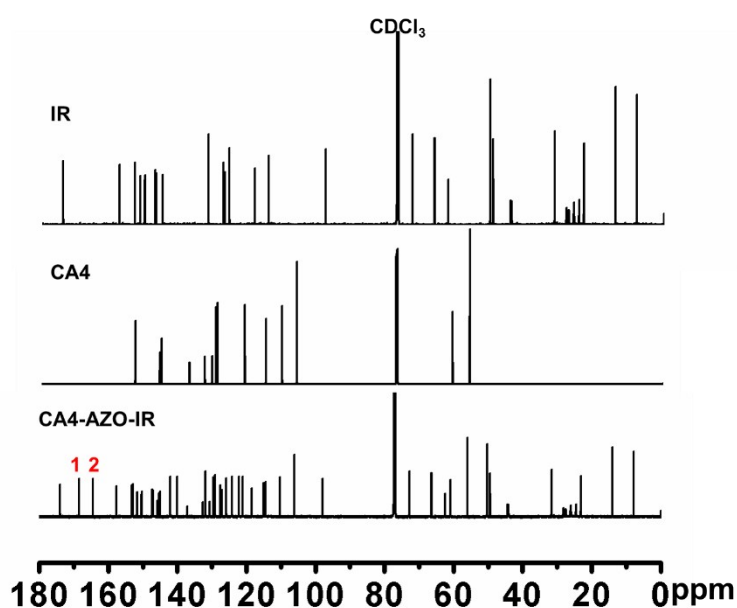


Fig. S2 ¹³C NMR spectra of IR-AZO-CA4 (600 MHz, CDCl₃)

Two new peak appears at 168.3 (1) and 164.4 (2) ppm corresponding to the -COO- group in the ¹³C NMR. ¹³C NMR δ (ppm): 174.1, 168.3, 164.4, 157.6, 152.8, 153.2, 151.6, 150.4, 150.2, 147.2, 146.9, 145.8, 145.2, 145.1, 142.2, 140.1, 137.2, 132.7, 131.8, 130.9, 129.5, 129.1, 127.4, 126.9, 125.8, 124.2, 122.1, 121.1, 118.4, 115.1, 114.4, 110.3, 106.2, 97.9, 72.8, 66.3, 62.4, 60.8, 55.9, 50.2, 49.4, 44.2, 31.6, 28.2, 26.1, 24.5, 23.1, 13.9, 7.9.

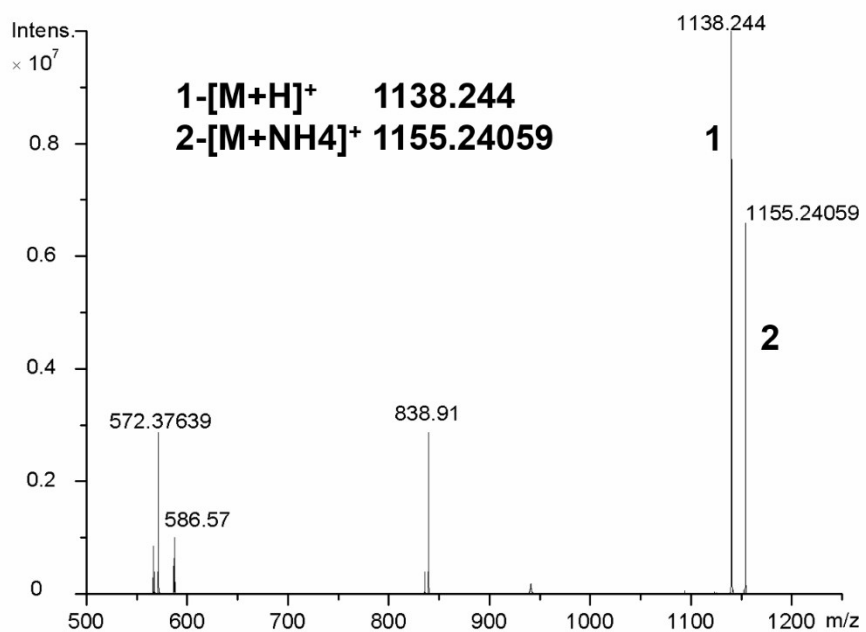


Fig. S3 High resolution mass spectroscopy to determine the exact mass and the corresponding molecular formula of the IR-AZO-CA4.

The mass and molecular formula of IR-AZO-CA4 were determined by HR-MS (positive, Bruker, USA) m/z 1138.24 $[M + H]^+$, 1155.24 $[M + NH_4]^+$; calcd for $C_{65}H_{64}N_6O_{13}$: 1137.24, found: 1138.24.

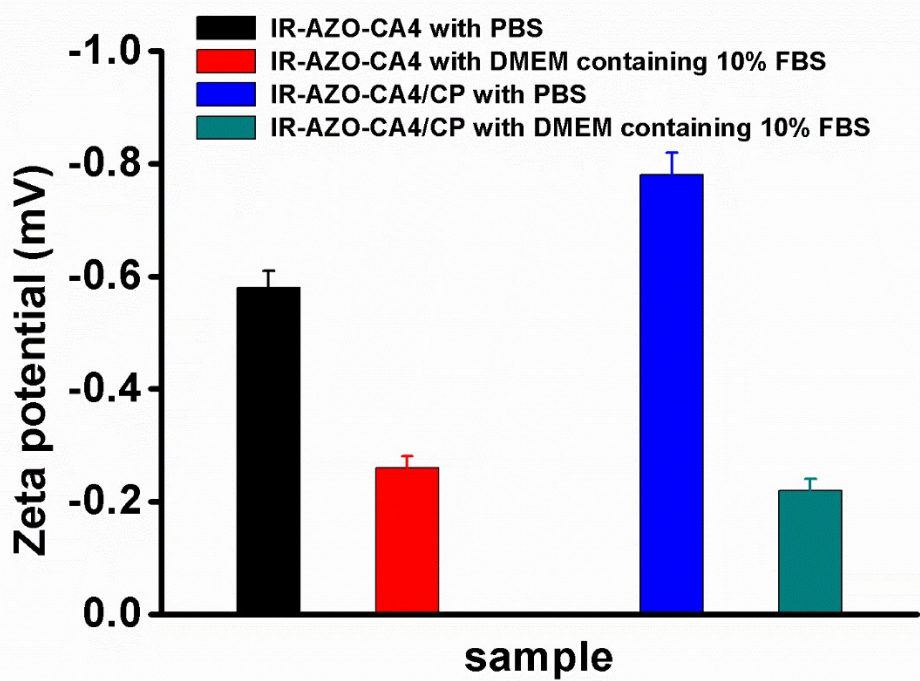


Fig. S4 Zeta potential for the nanoparticles before and after mixed with DMEM containing FBS

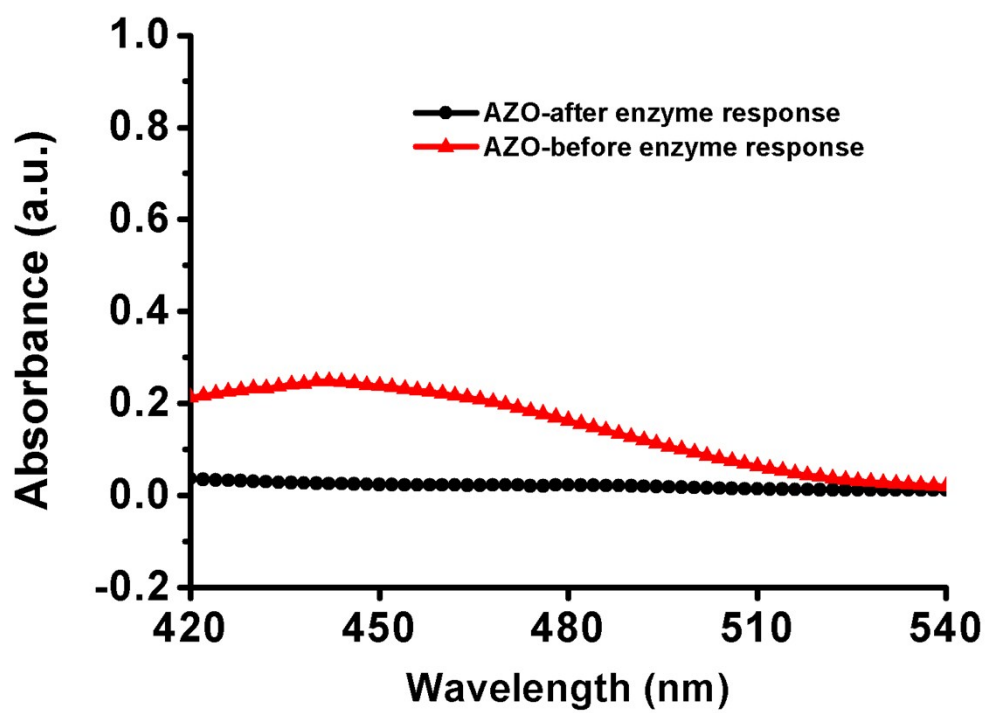


Fig. S5 UV/vis absorption spectrum of IR-AZO-CA4 molecule (red line) and cleavage AZO bond of IR-AZO-CA4 molecule (black line) in DMSO.

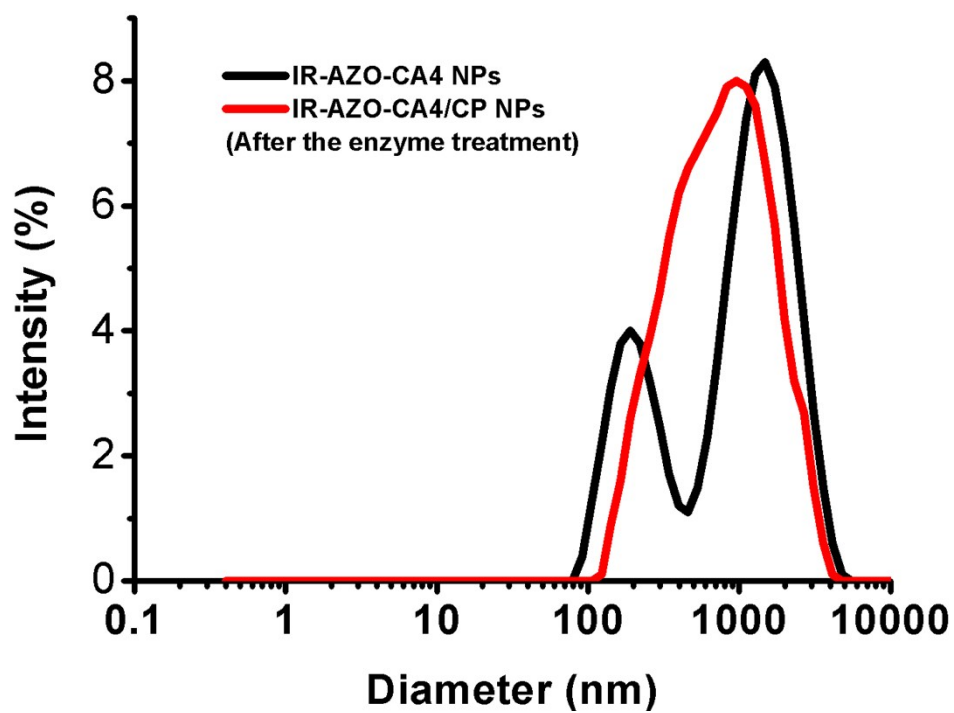


Fig. S6 The size distribution of IR-AZO-CA4 and IR-AZO-CA4/CP after the enzyme treatment.