Supplementary Information

Unusual Role of Folate in the Self-Assembly of Heparin-Folate Conjugates into Nanoparticles

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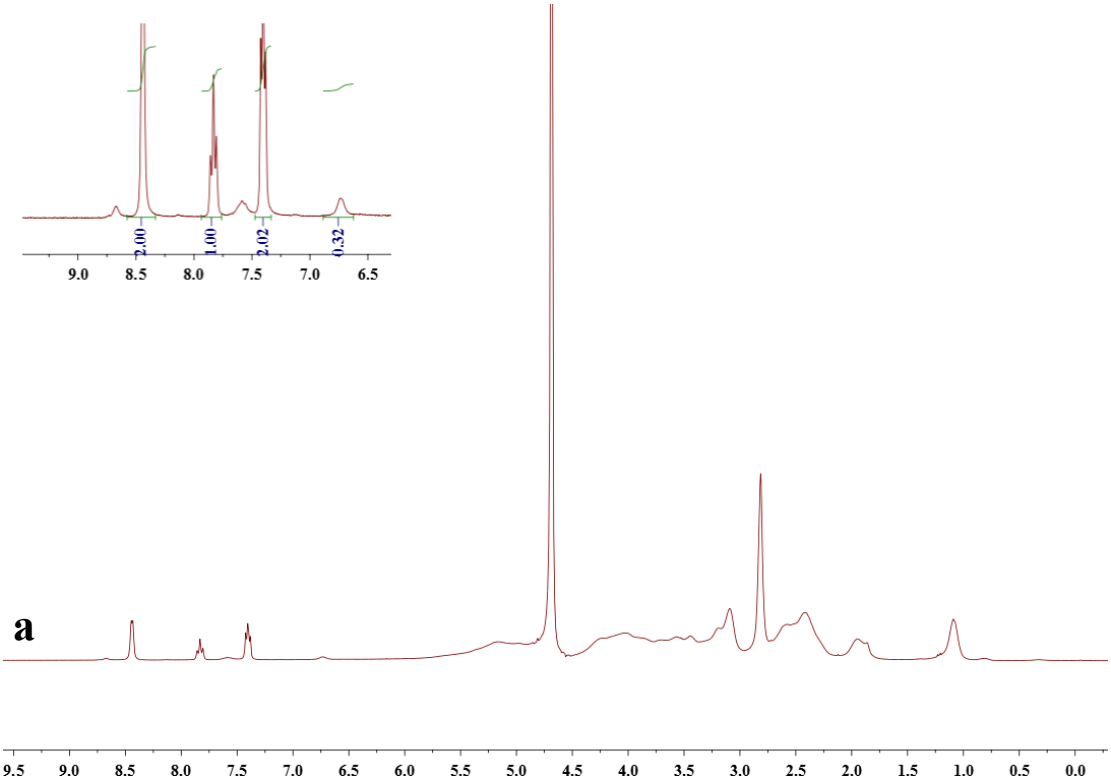
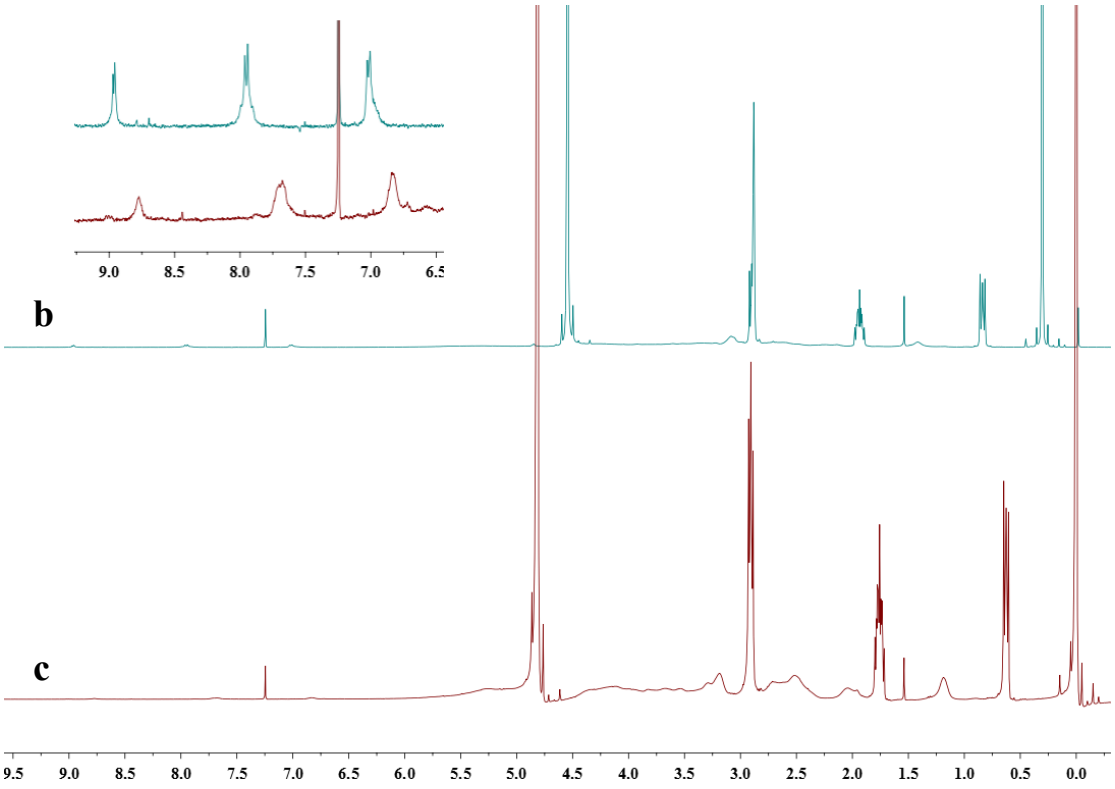
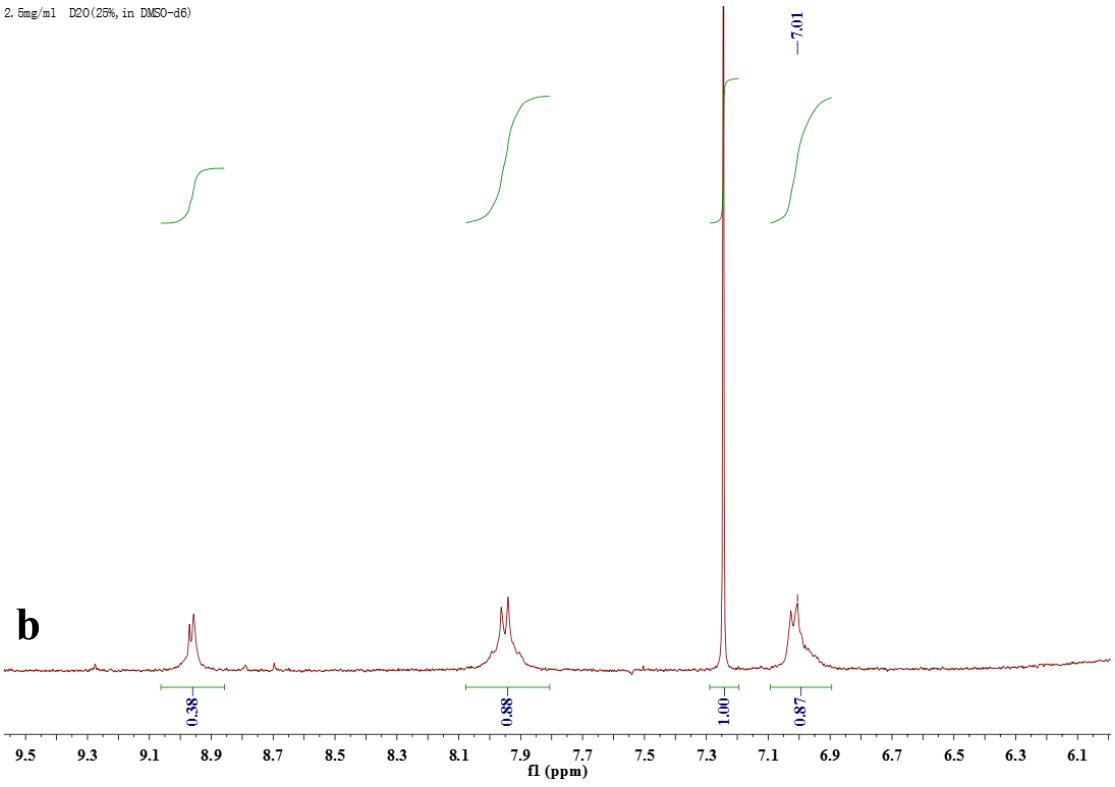
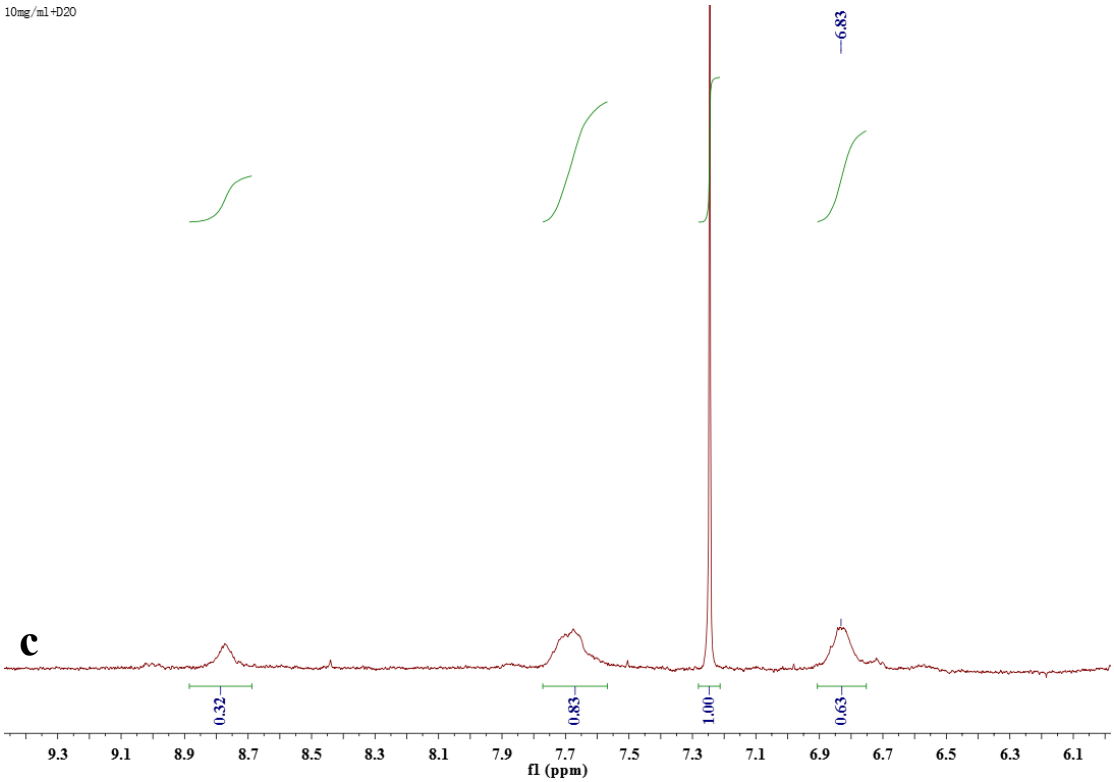
Determining the Ratio between folate on the surface and Inside the Core of HF Nanoparticles by ^{1}H NMR



Method 1. By comparing the integration values of folate at 6.6 ppm with pyridine at 7.8 ppm, 0.32 and 1.00 respectively, we estimated the molar ratio of solvated folate in the sample is 0.16[0.32/(1*2)] times than pyridine, then we can calculated the solvated folate of the sample is 0.179mg, the total folate is 1.183mg by the UV analysis, so ratio between folate on the surface of HF nanoparticle to the total folate is 15.1%.


b
c
9.0
8.5
8.0
7.5
7.0
6.5
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0


2.5mg/ml D2O(25%,in DMSO-d6)
7.01
b
0.38
0.88
1.00
0.87
9.5 9.3 9.1 8.9 8.7 8.5 8.3 8.1 7.9 7.7 7.5 7.3 7.1 6.9 6.7 6.5 6.3 6.1
f1 (ppm)



Method 2 we use coaxial insert containing *d*-Chloroform as the internal reference, In both experiments, we set the chloroform peak area to 1. By comparing the integration values of folate in D_2O/*d*-DMSO mixture (D_2O:*d*-DMSO =1:3 v/v, 0.15 mL D_2O, 0.45 mL *d*-DMSO) and D_2O (0.6 mL)with peaks 7.01 and 6.83 ppm, which is 0.87 and 0.63 respectively, because the concentration of folate in D_2O/*d*-DMSO mixture is 2.5mg/mL, While in D_2O is 10 mg/mL, so the total folate should four times than in D_2O/*d*-DMSO mixture, so ratio between folate on the surface of HF nanoparticle to the total folate is 18.1% by compare the integration values, that is 0.63/(0.87*4).

Active Targeting Effects not Compromised

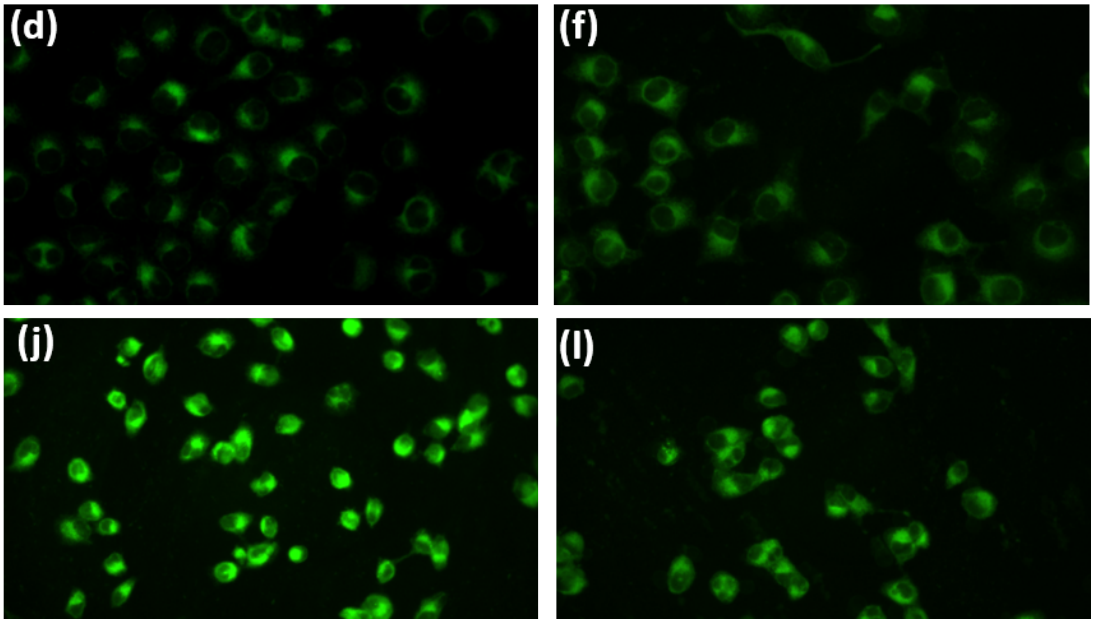


Fig.3 Fluorescent images of KB-3-1 and A549 cells incubated with HF-488 NPs for 4h (d, f, , j, l: Fluorescent images 500×).

Grou p	1	2	3	4	5	Mean Fluorescence
d	25.08	24.37	18.32	21.40	25.32	22.90
f	21.99	21.06	19.21	20.40	22.63	21.06
j	92.40	91.74	94.10	84.92	93.06	91.24
l	41.53	52.35	49.69	42.27	52.80	47.73

Table 1. Cellular uptaken of HF-488 NPs by KB-3-1 (folate receptor overexpressed cell lines) and A549 (folate receptor deficient cell lines), HF uptaken by KB-3-1 is 4 times as much by A549.