

Supporting information

Multi-stage, charge conversional, stimuli-responsive nanogels for therapeutic protein delivery

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1. Cleavage of citraconic amide

1.1. ^1H NMR spectra

20 mg of NGCA was dispersed in 5 ml of acetate buffer solution at pH 5.0 for 8 h incubation. After lyophilization, the residue was dissolved in D_2O for proton NMR measurement.

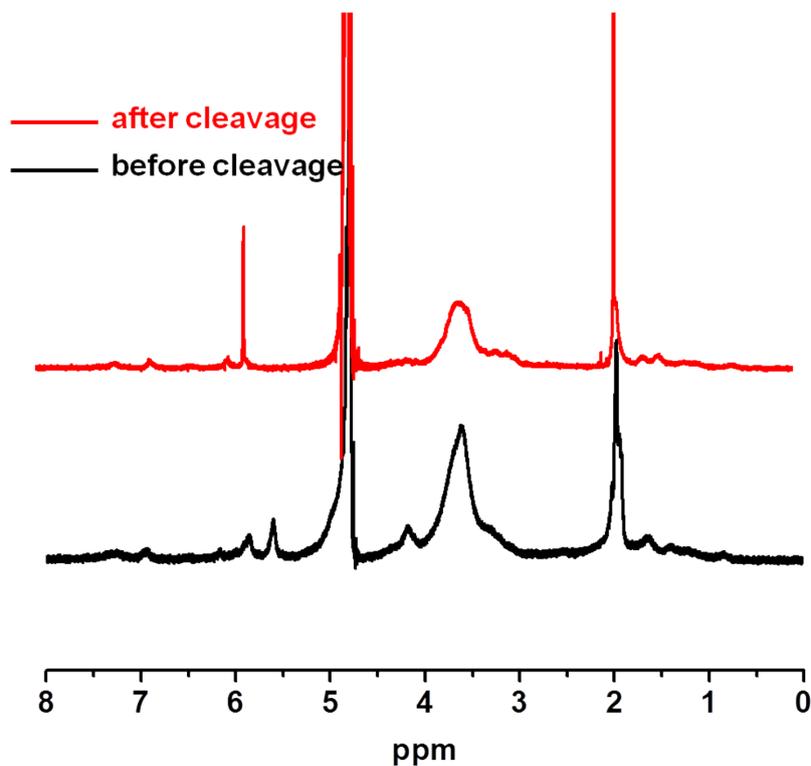


Figure S1. ^1H NMR spectra of NGCA before (black) and after (red) treatment at pH 5.

1.2. Ninhydrin assay

10 mg NGCA was incubated in 10 ml of PBS solution at pH 7.4 or acetate buffer solution at pH 5.0 at 37 °C. At pre-determined time points, the amount of primary amine was determined by Ninhydrin assay [1]. 90% exposed primary amine was determined by the fluorescence of NGCA after being incubated in HCl aqueous solution (pH 3) overnight.

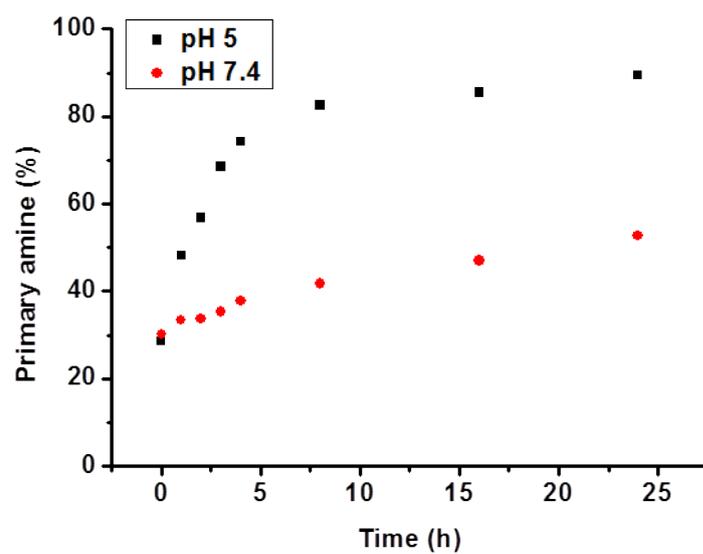


Figure S2. The citraconic amide degradation at pH 7.4 and 5.

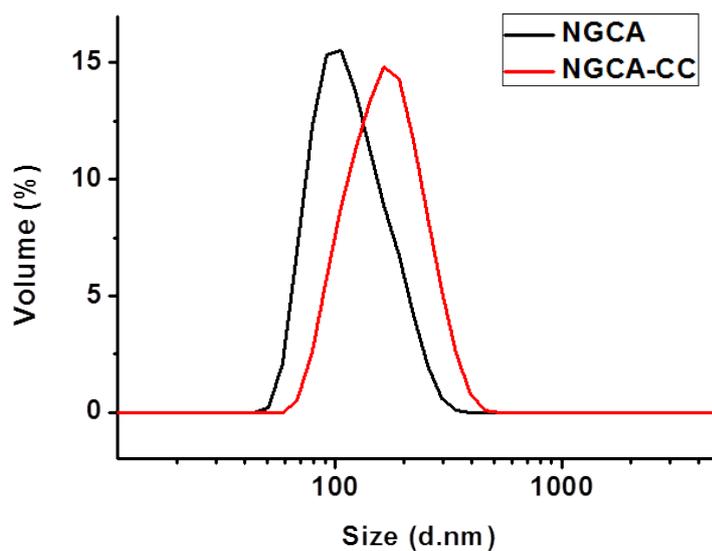


Figure S3. Size distribution of blank nanogel (NGCA) and protein loaded nanogel (NGCA-CC).

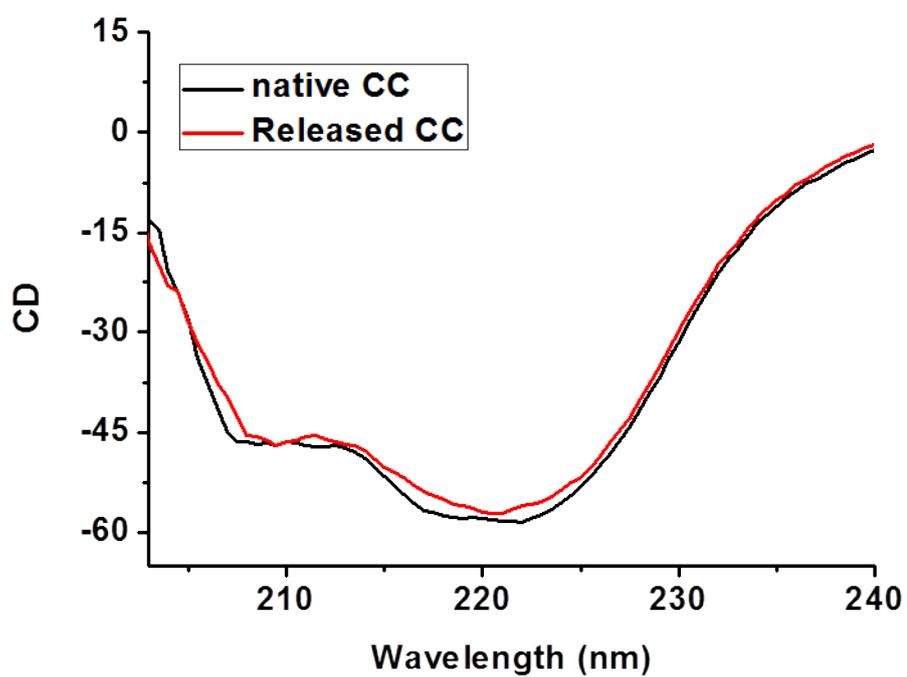


Figure S4. CD spectra of native CC and released CC at a concentration of 500 $\mu\text{g/ml}$.

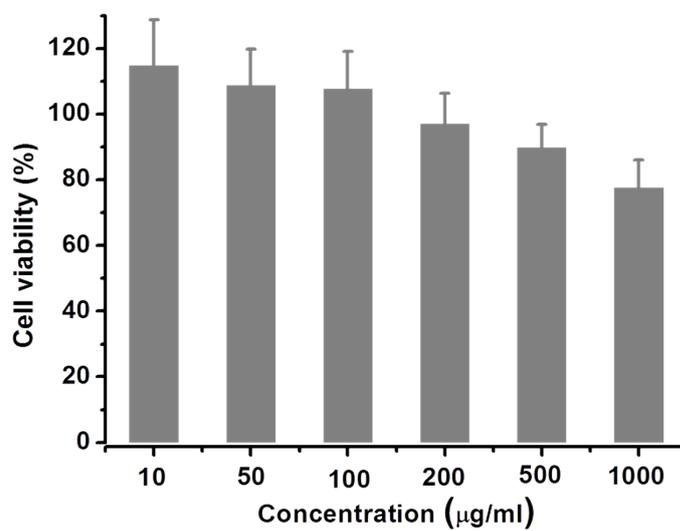


Figure S5. Cell viability of MCF-7 cells after the treatment of different concentrations of NGCA for 48 h. The cell viability of untreated control was set to 100%.

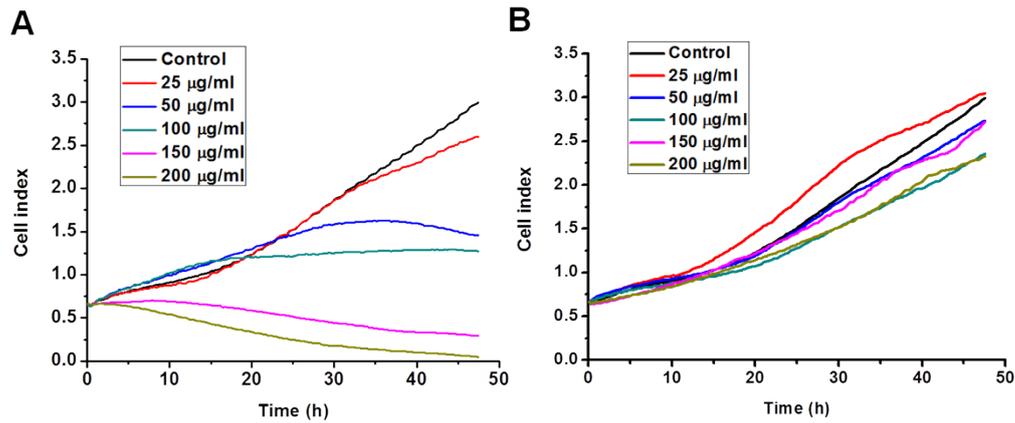


Figure S6. Cytotoxicity profiles of MCF-7 cells determined by an xCelligence RTCA device. Cell proliferation over time under the treatment of (A) NGCA-CC and (B) CC at different concentrations of CC.

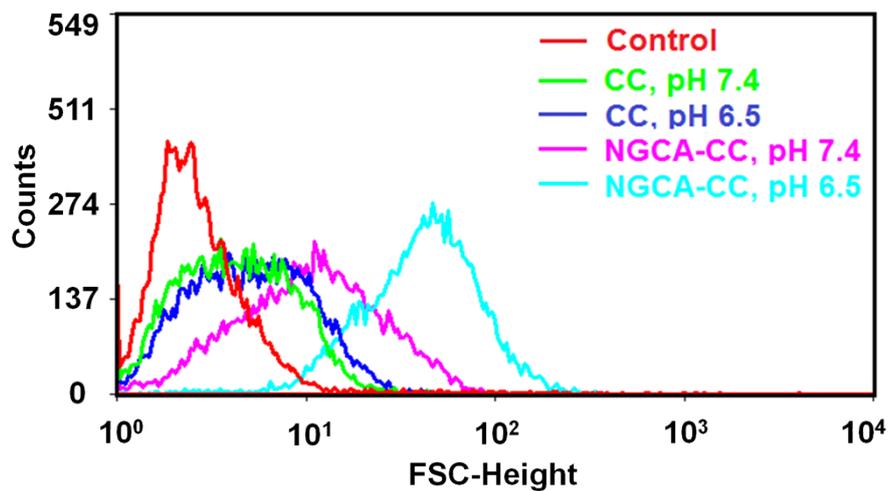


Figure S7. Flow cytometry profiles of MCF-7 cells treated with free CC or NGCA-CC for 1 h at pH 7.4 and 6.5.

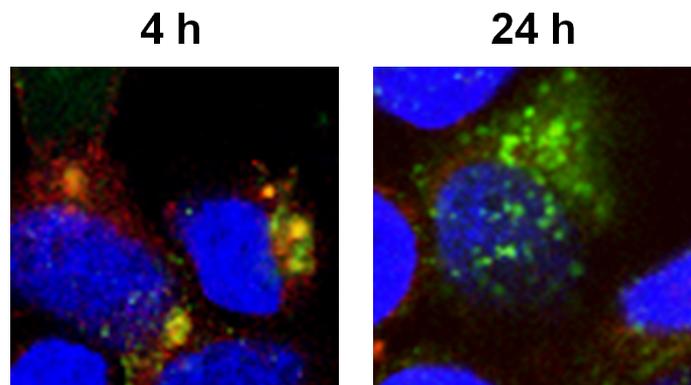


Figure S8. CLSM images of MCF-7 cells after 4 h and 24 h treatment with NGCA-CC.

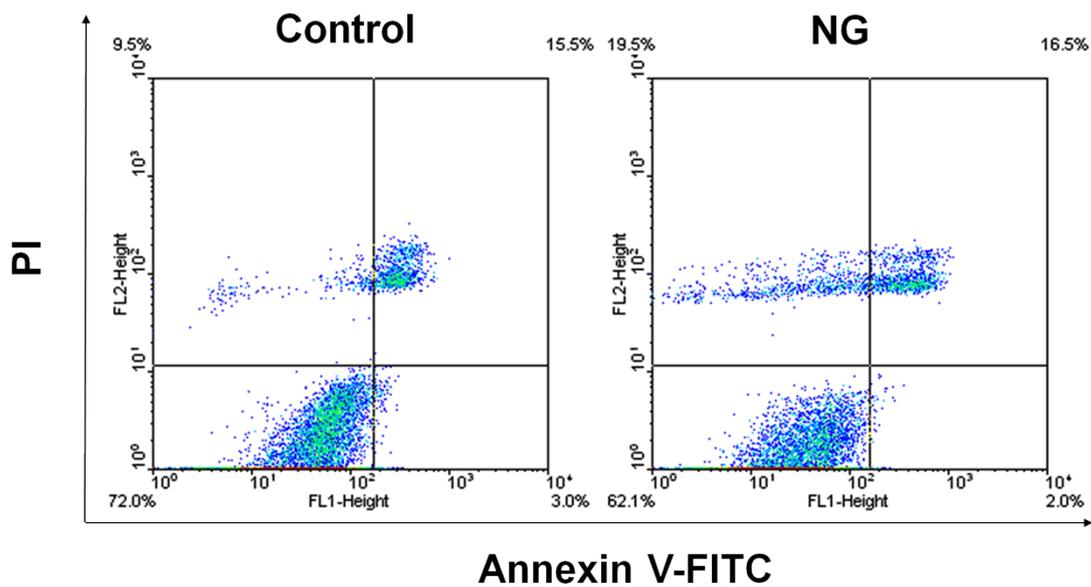
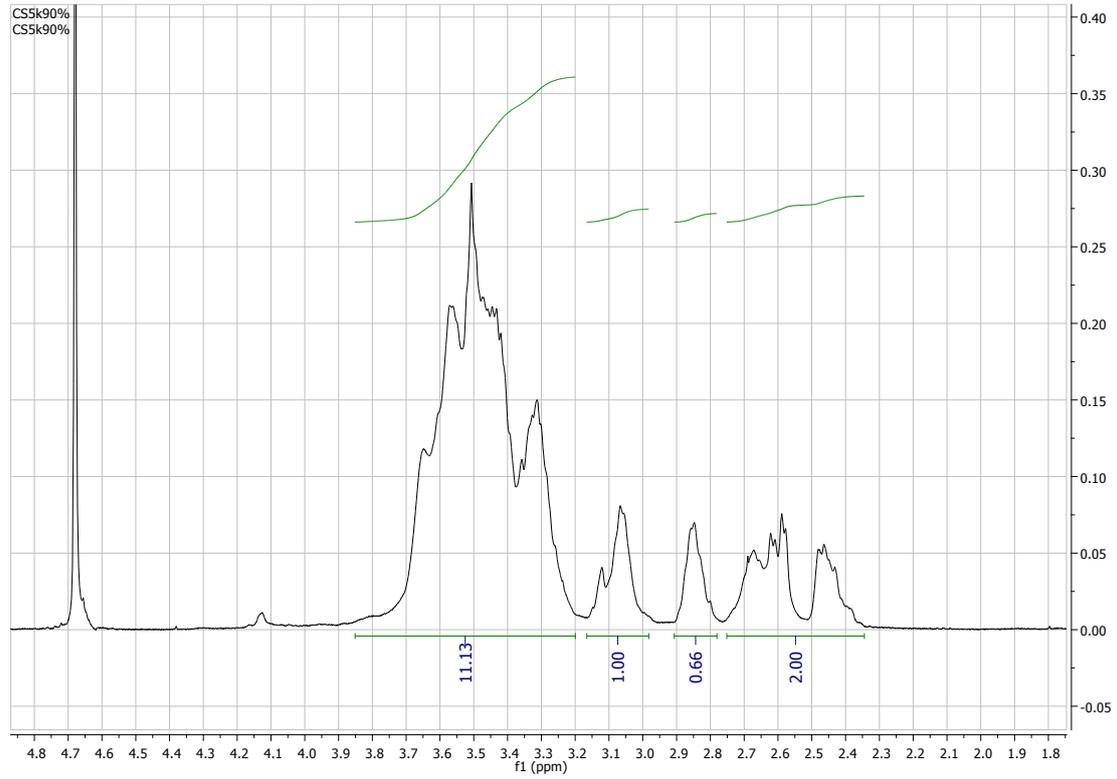


Figure S9. Contour diagram of Annexin V-FITC/PI flow cytometry of MCF-7 cells after 48 h treatment of NGCA-CC at a concentration of 500 $\mu\text{g/ml}$.

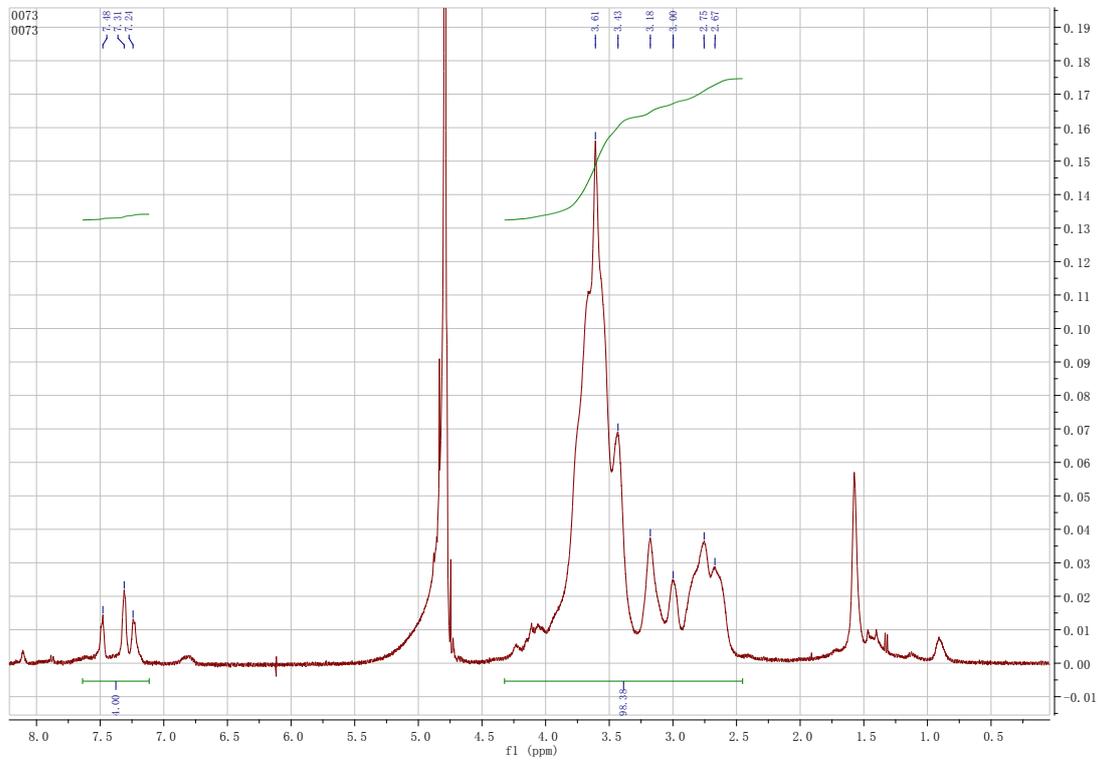
[1] E. W. Yemm, E. C. Cocking and R. E. Ricketts, *Analyst*, 1955, **80**, 209-214.

NMR spectra

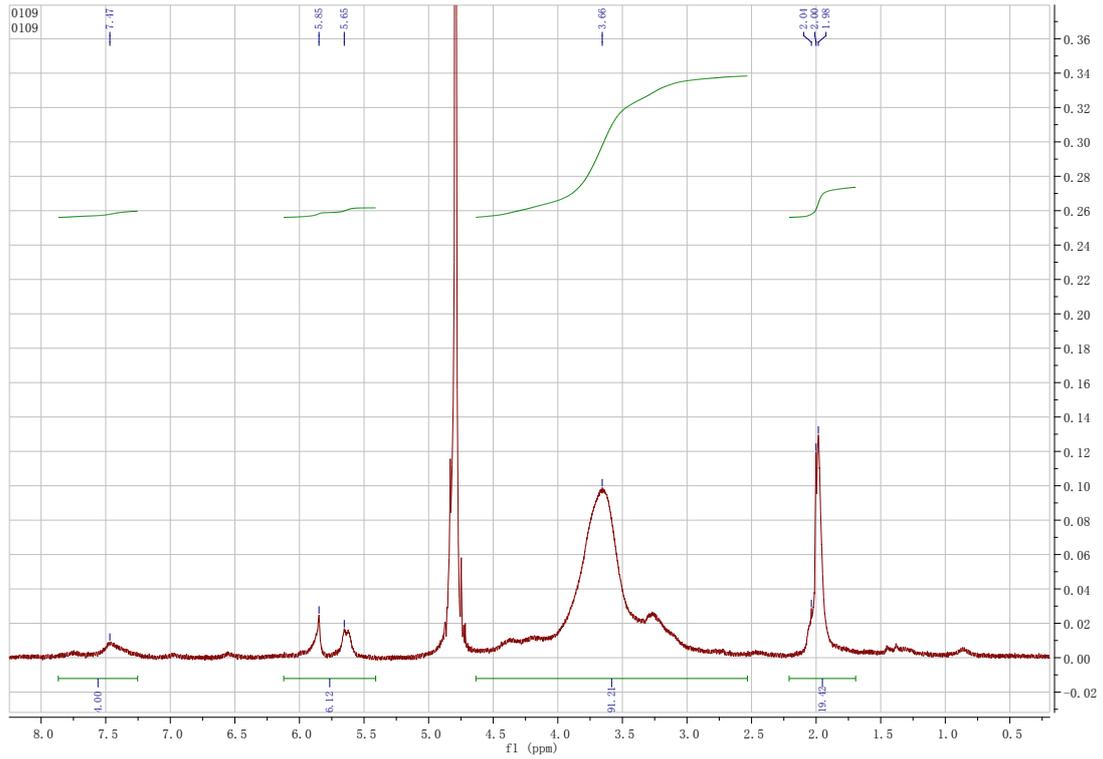
dPG-90%NH₂ (dPGA)



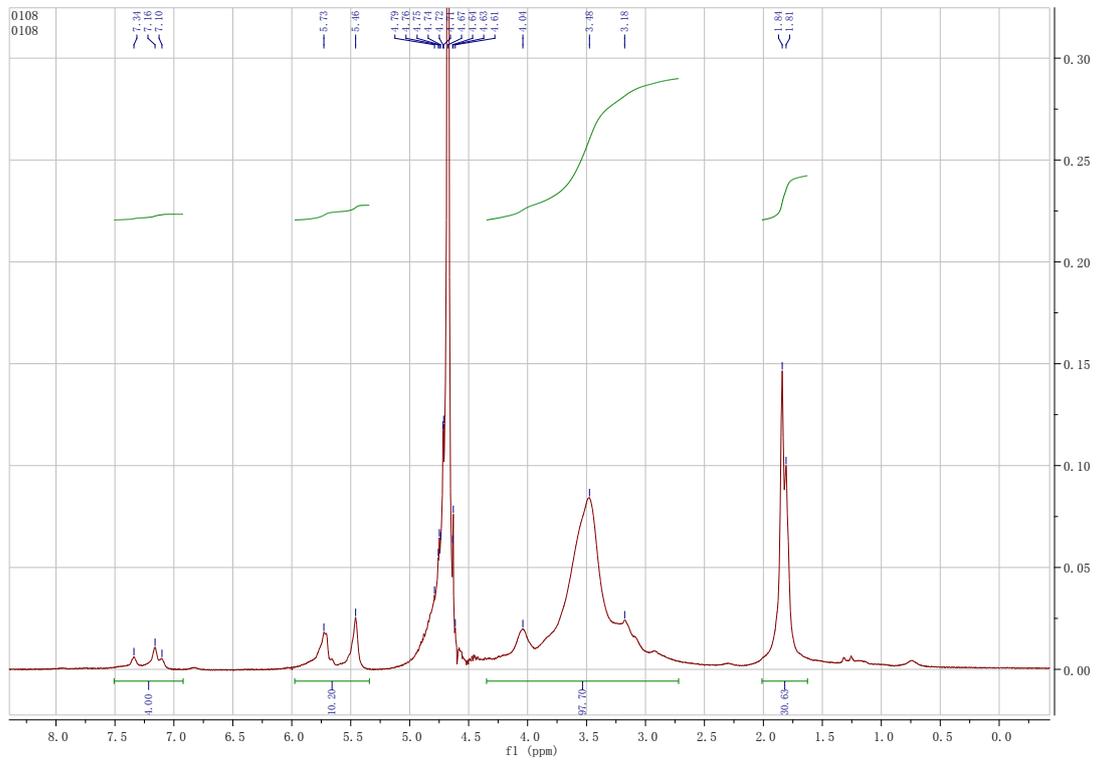
dPGA-5%FPBA



dPGA-5%FPBA-35%CA



dPGA-5%FPBA-50%CA



dPGA-5%FPBA-65%CA

