Tunable nanogels by host-guest interaction with carboxylate pillar[5]arene for controlled encapsulation and release of doxorubicin

Peng Wei, [a,b] *Gauri Gangapurwala,* [a,b] *David Pretzel,* [a,b] *Limin Wang,* [a,b] *Stephanie Schubert,* [b,c] *Johannes C. Brendel,* [a,b] *Ulrich S. Schubert* *, [a,b]

[a] Laboratory of Organic and Macromolecular Chemistry (IOMC), Friedrich Schiller University Jena, Humboldtstraße 10, 07743 Jena, Germany

[b] Jena Center for Soft Matter (JCSM), Friedrich-Schiller-University, Philosophenweg 7, 07743 Jena, Germany

[c] Institute of Pharmacy and Biopharmacy, Department of Pharmaceutical Technology, Friedrich Schiller University Jena, Lessingstrasse 8, 07743 Jena, Germany

*Correspondence to: U. S. Schubert (E-mail: ulrich.schubert@uni-jena.de)

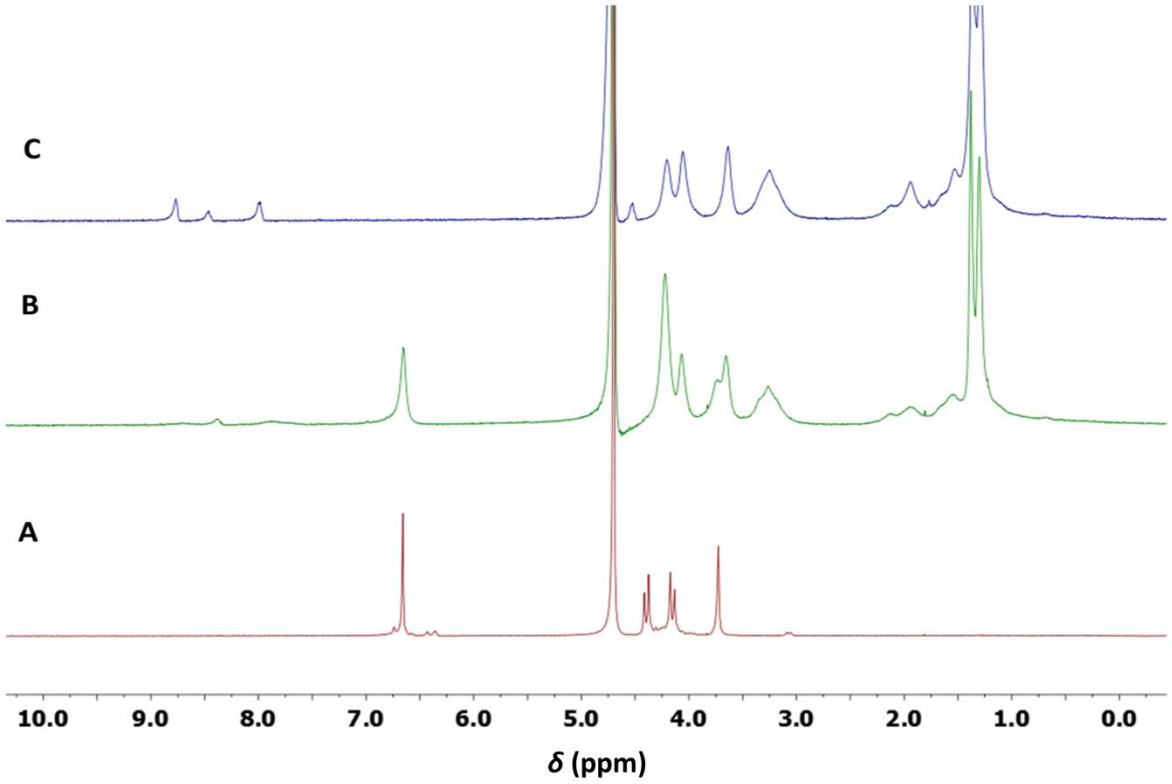

Figure S1 ^{1}H-NMR (D_2O, 300 MHz) spectra of A) **H1** (3.5 mg mL^{-1}), B) **NG** (5 mg mL^{-1}) + 1 eq. **H1** and C) **NG** (5 mg mL^{-1}).

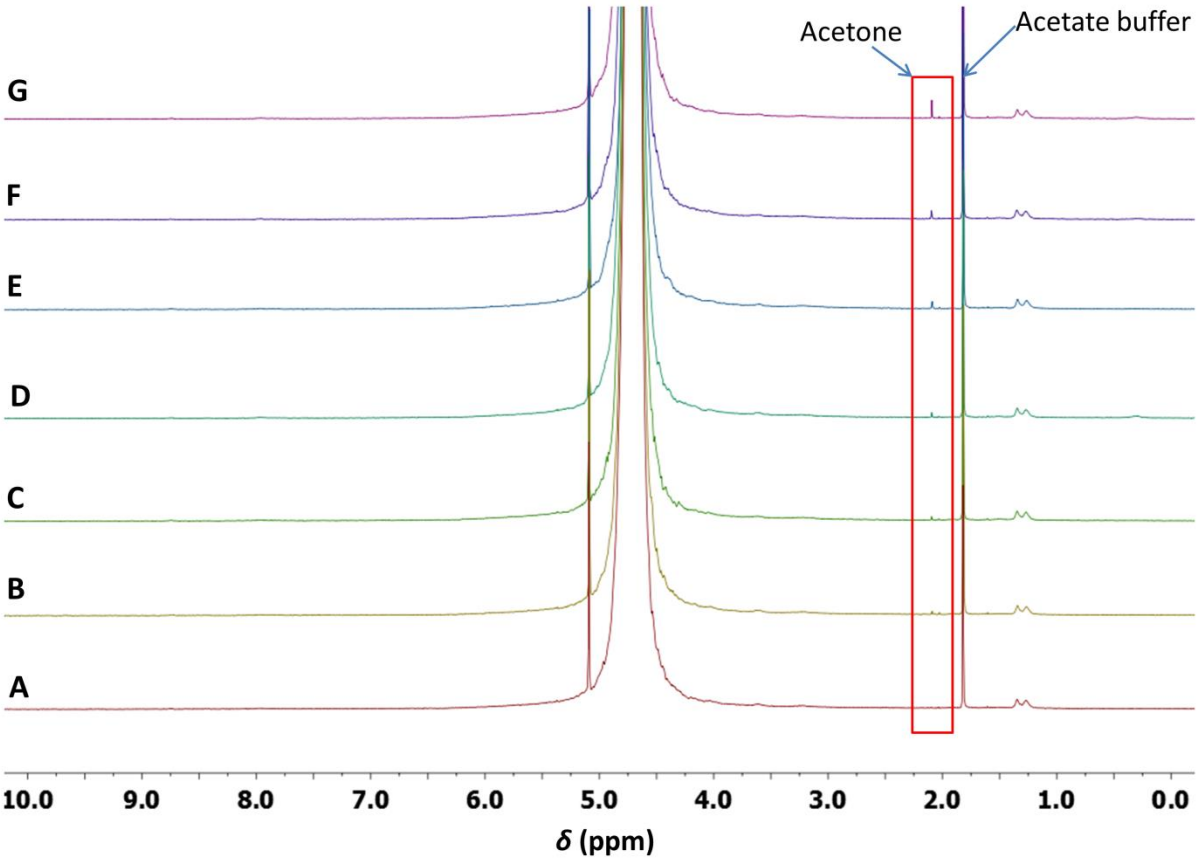

Figure S2 ^{1}H-NMR (D_2O, 300 MHz) spectra of **NG** (5 mg mL^{-1}) at pH = 5.1 after A) 0 h, B) 24 h, C) 48 h, D) 72 h, E) 96 h, F) 168 h and G) 264 h.

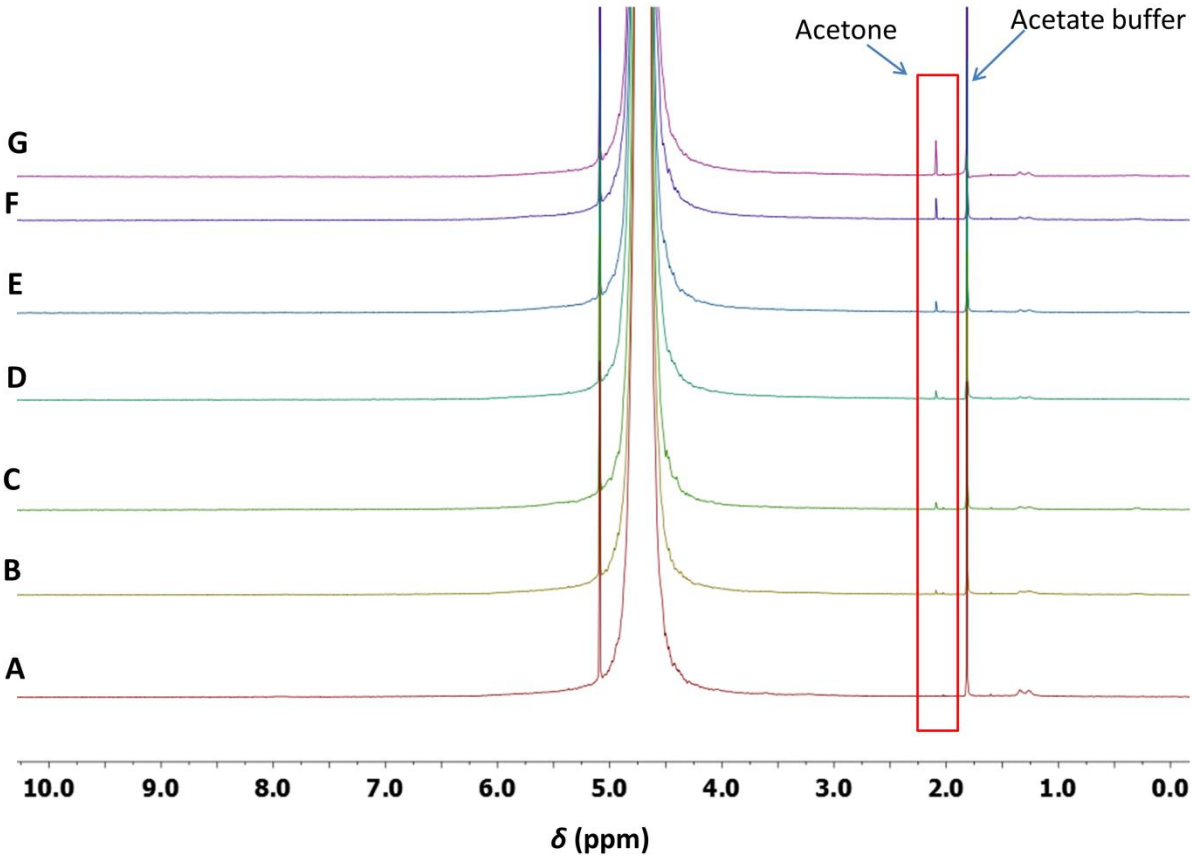

Figure S3 ^{1}H-NMR (D_2O, 300 MHz) spectra of **NG** (5 mg mL^{-1}) + 0.05 eq. **H1** at pH = 5.1 after A) 0 h, B) 24 h, C) 48 h, D) 72 h, E) 96 h, F) 168 h and G) 264 h.

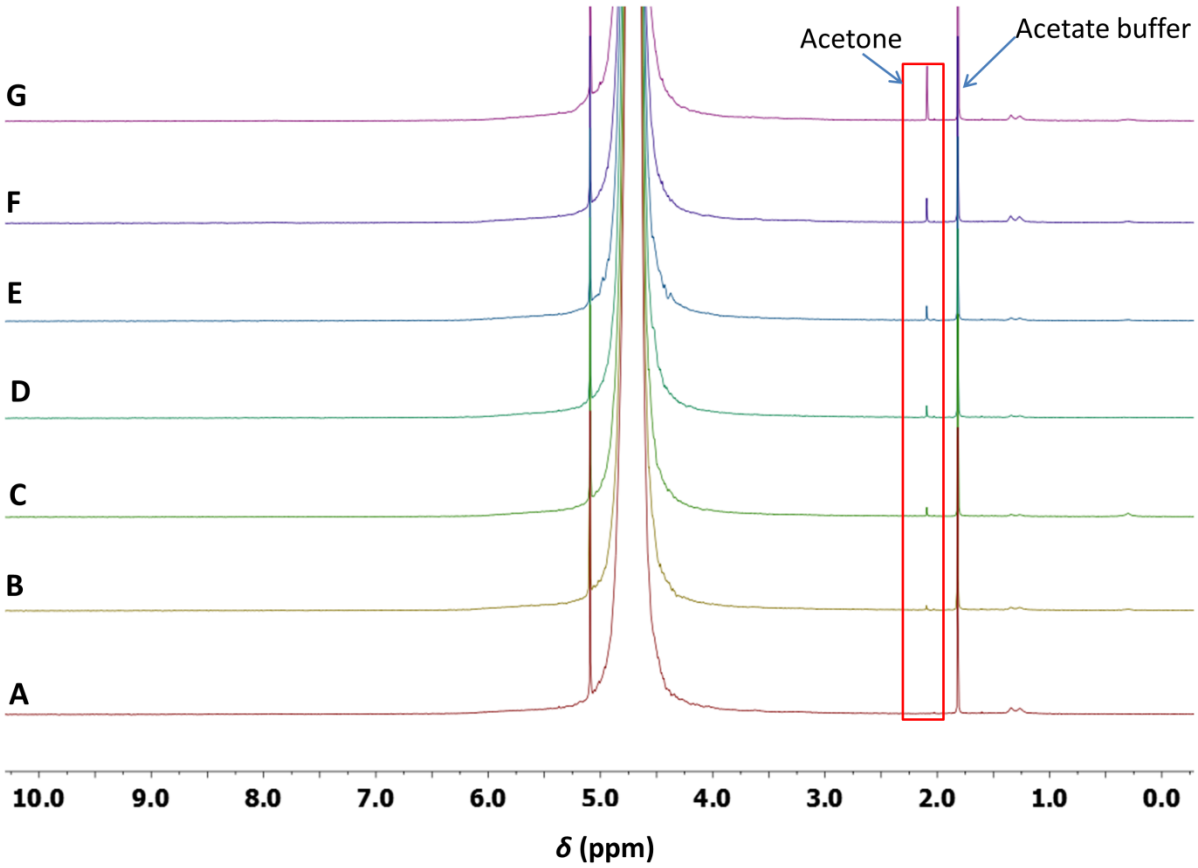

Figure S4 ^{1}H-NMR (D_2O, 300 MHz) spectra of **NG** (5 mg mL^{-1}) + 0.1 eq. **H1** at pH = 5.1 after A) 0 h, B) 24 h, C) 48 h, D) 72 h, E) 96 h, F) 168 h and G) 264 h.

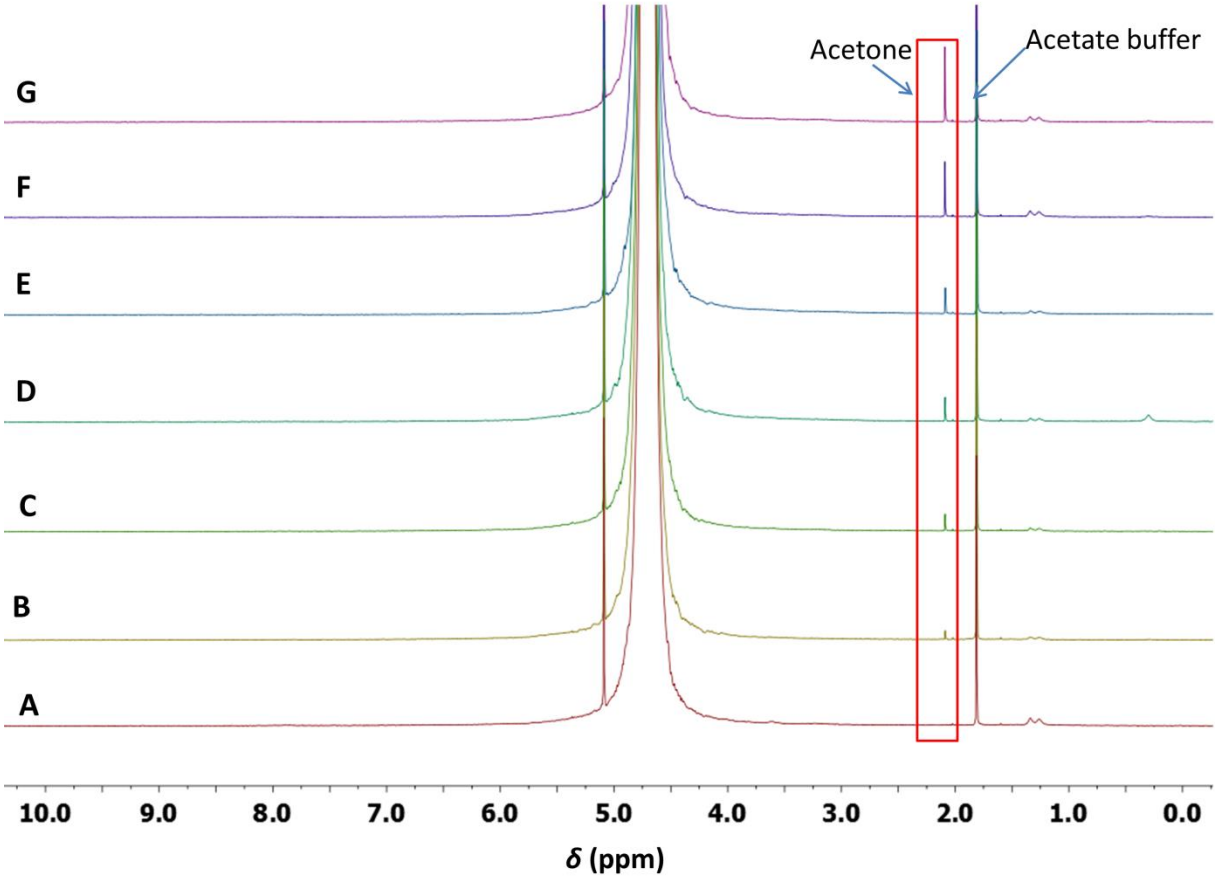

Figure S5 ^{1}H-NMR (D_2O, 300 MHz) spectra of **NG** (5 mg mL^{-1}) + 0.2 eq. **H1** at pH = 5.1 after A) 0 h, B) 24 h, C) 48 h, D) 72 h, E) 96 h, F) 168 h and G) 264 h.

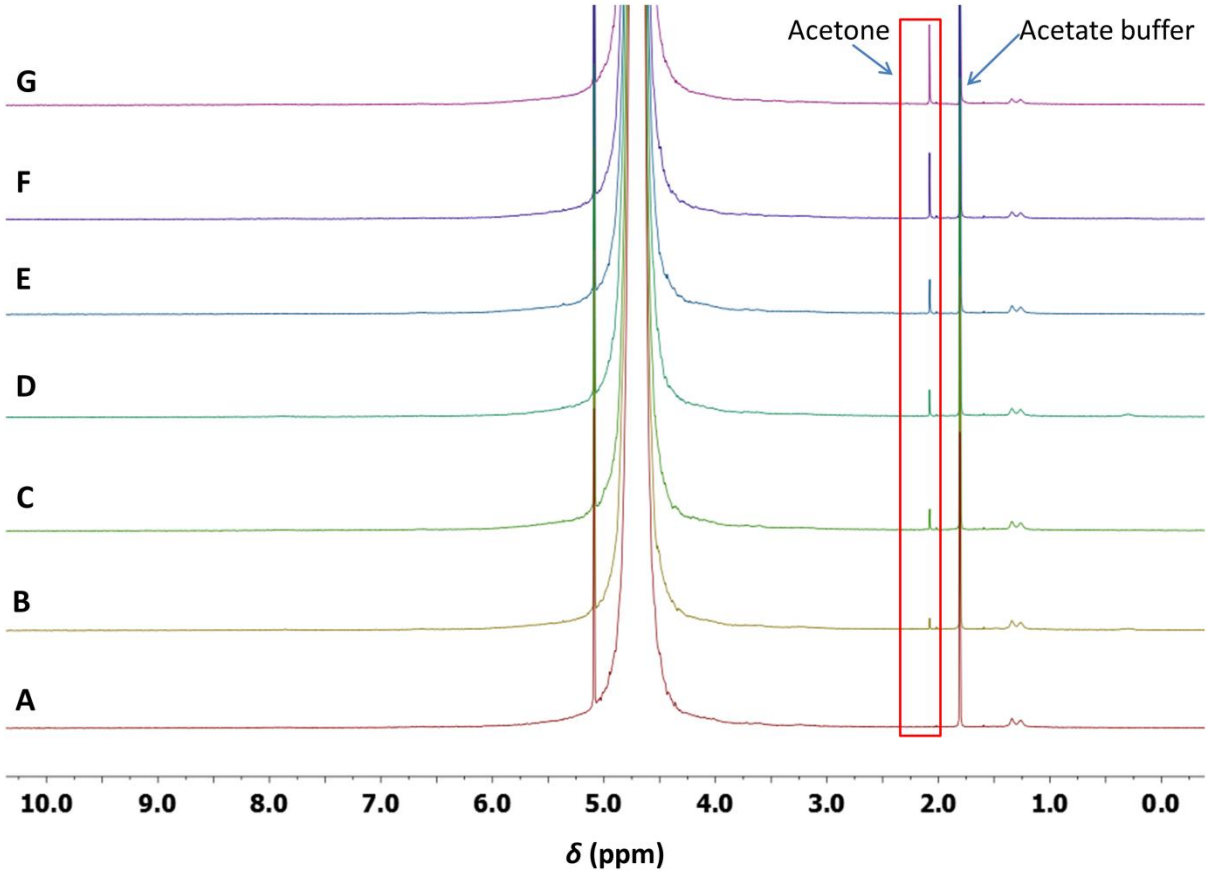

Figure S6 ^{1}H-NMR (D_2O, 300 MHz) spectra of **NG** (5 mg mL^{-1}) + 0.5 eq. **H1** at pH = 5.1 after A) 0 h, B) 24 h, C) 48 h, D) 72 h, E) 96 h, F) 168 h and G) 264 h.

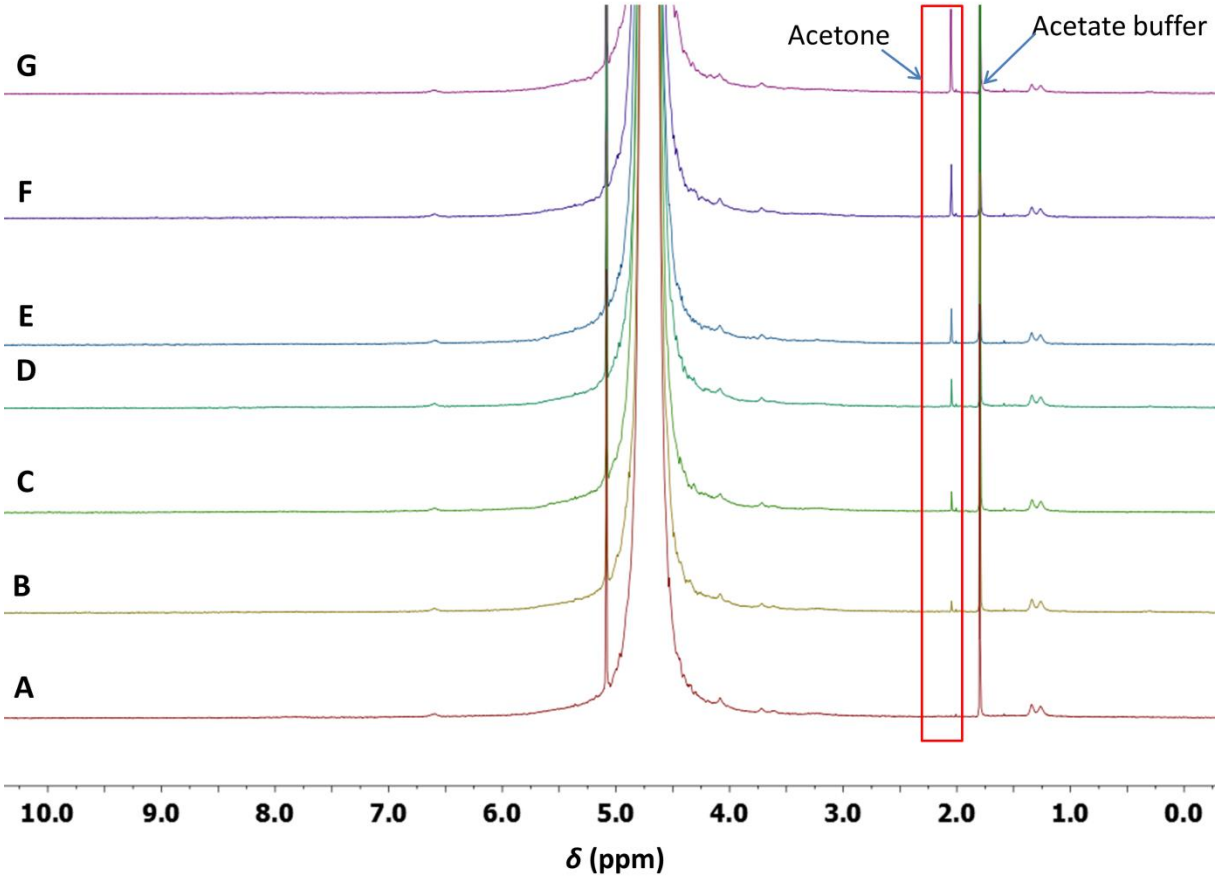

Figure S7 ^{1}H-NMR (D_2O, 300 MHz) spectra of **NG** (5 mg mL^{-1}) + 1 eq. **H1** at pH = 5.1 after A) 0 h, B) 24 h, C) 48 h, D) 72 h, E) 96 h, F) 168 h and G) 264 h.

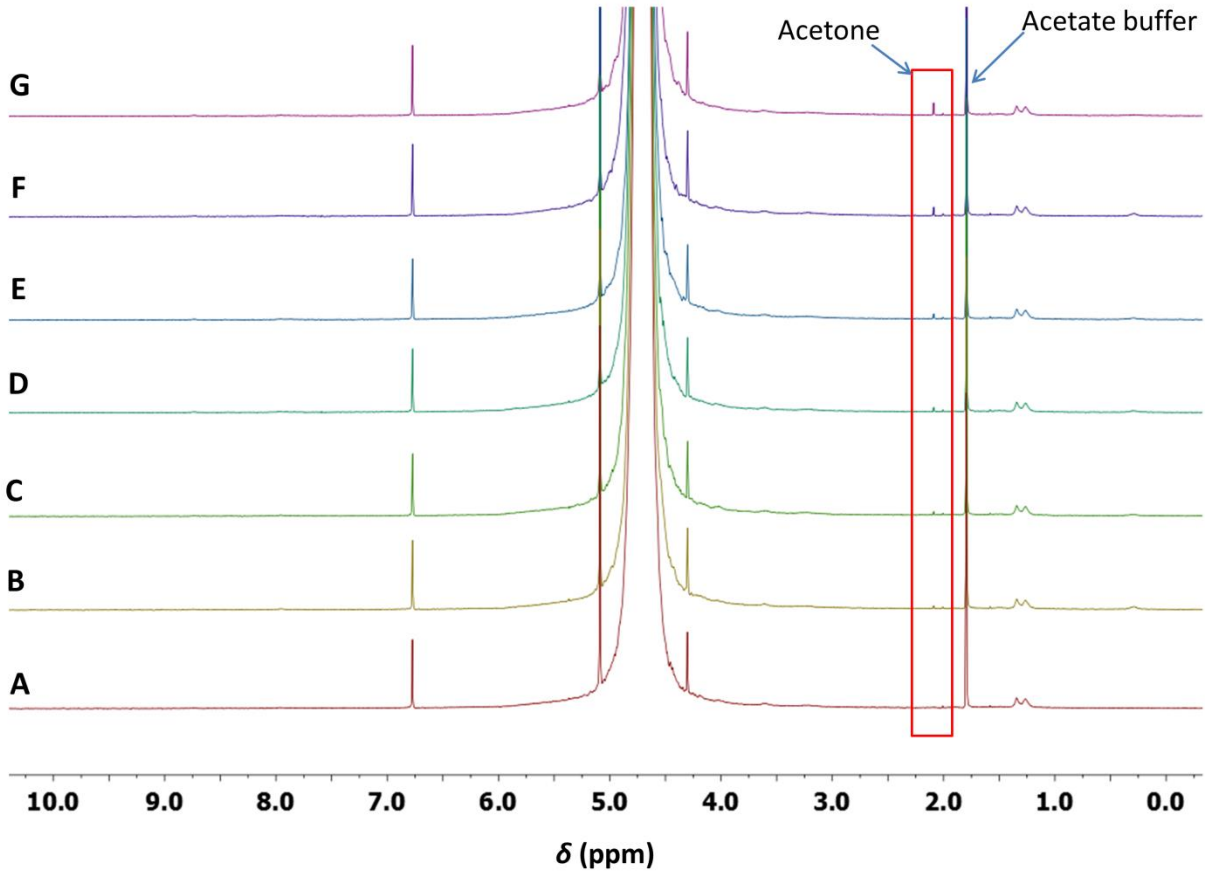

Figure S8 ^{1}H-NMR (D_2O, 300 MHz) spectra of **NG** (5 mg mL^{-1}) + 5 eq. **H2** at pH = 5.1 after A) 0 h, B) 24 h, C) 48 h, D) 72 h, E) 96 h, F) 168 h and G) 264 h.

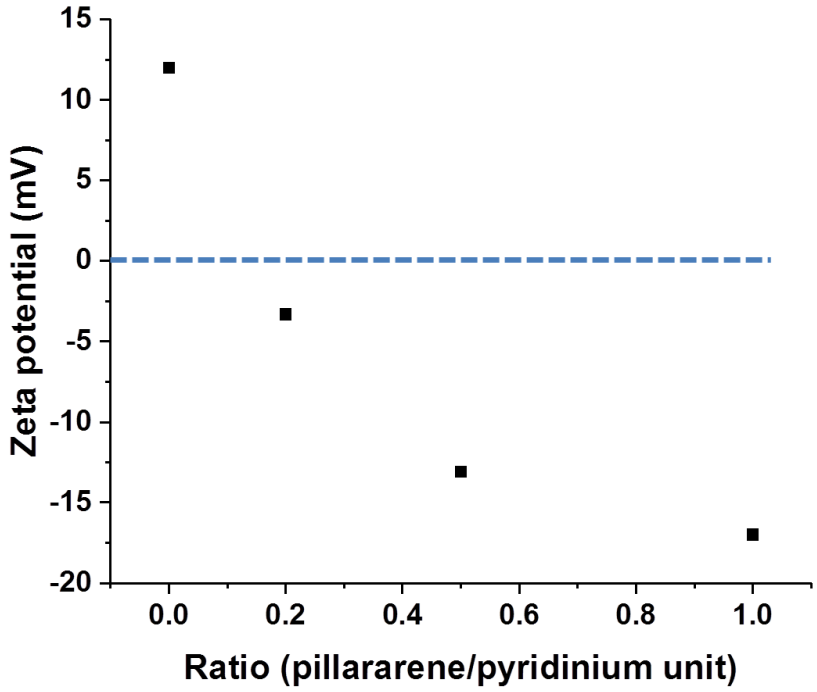

Figure S9 Zeta potential of the respective nanogels at different ratios of **H1** to pyridinium unit of the nanogel; all measurements were performed in phosphate buffer (pH 5.1, 10 mM).

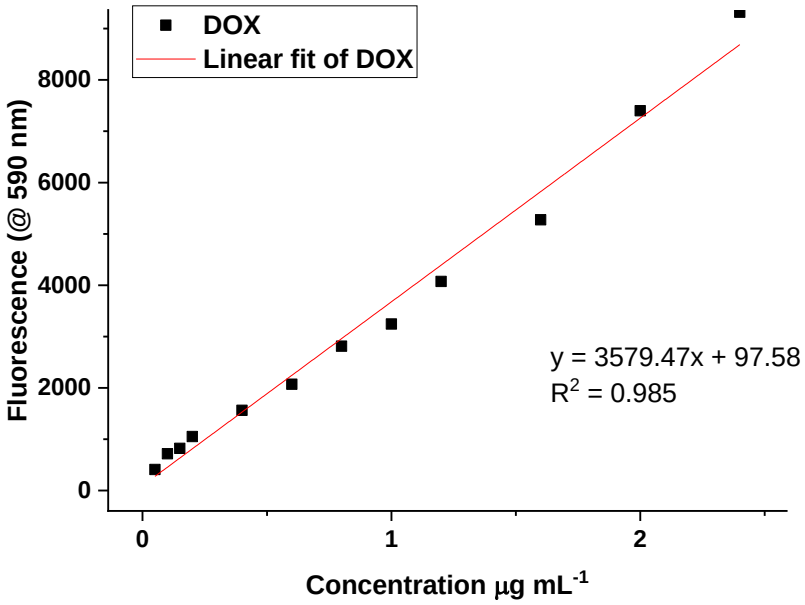

Figure S10 Calibration curve of doxorubicin hydrochloride in DMSO.

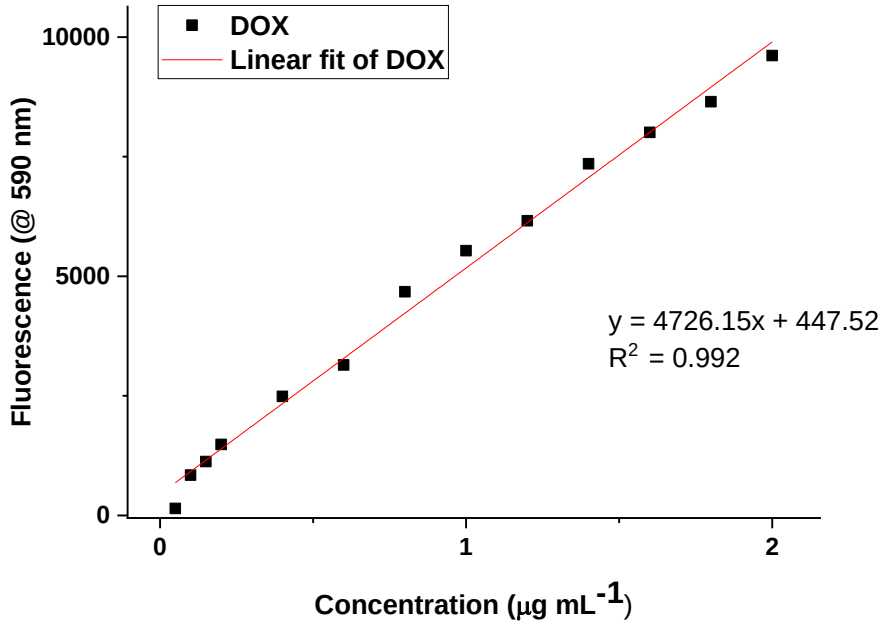

Figure S11 Calibration curve of doxorubicin hydrochloride in phosphate buffer (10 mM, pH = 7.4).

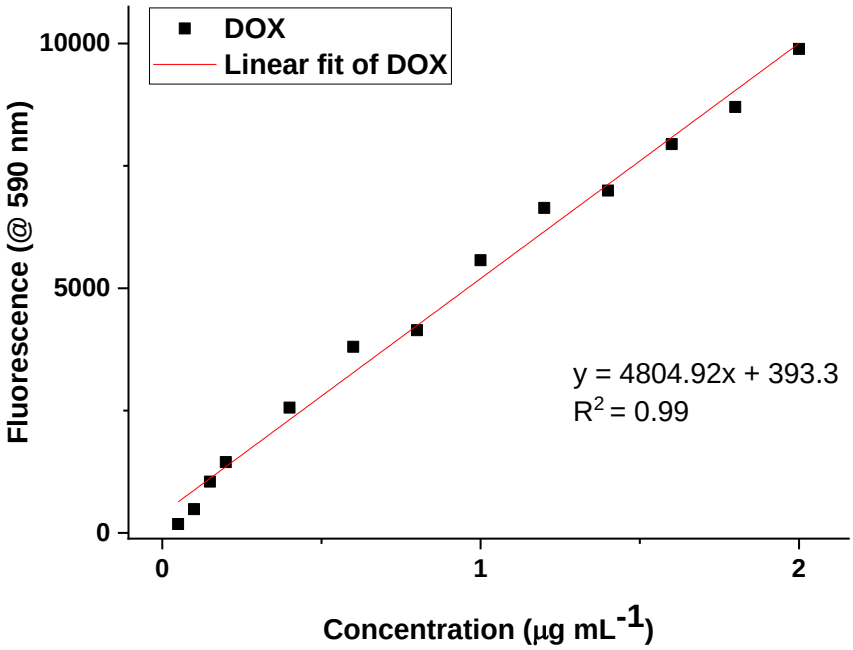

Figure S12 Calibration curve of doxorubicin hydrochloride in acetate buffer (10 mM, pH = 5.1).

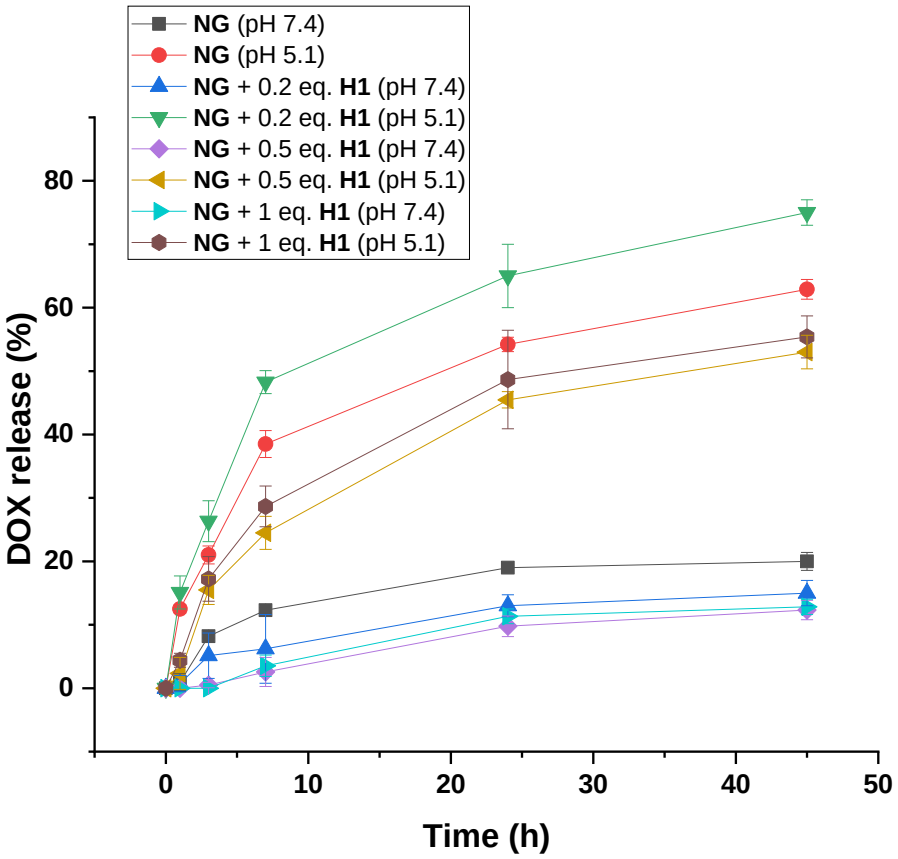

Figure S13 Release of DOX from nanogels with varying amounts of **H1** at pH values of 7.4 and 5.1.

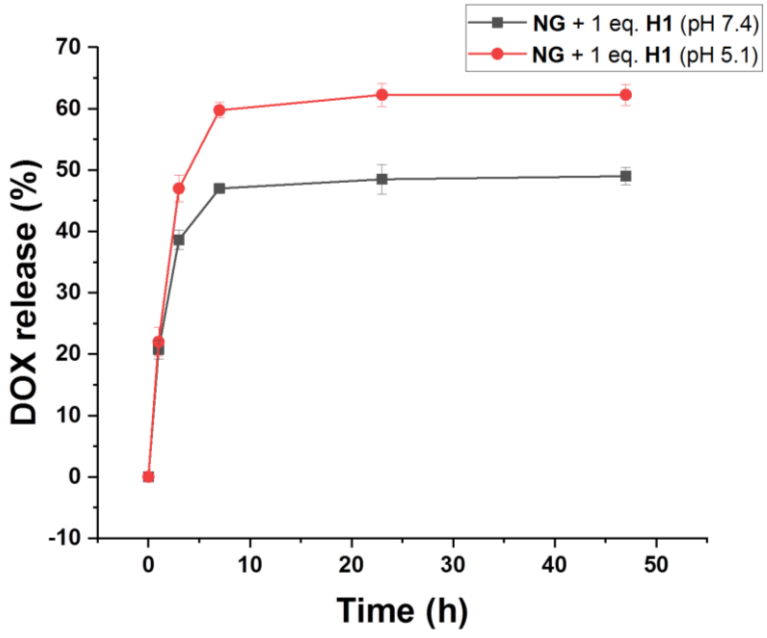

Figure S14 Release of DOX from nanogels (DLC = 16 wt%) with 1 eq. **H1** at pH values of 7.4 and 5.1.

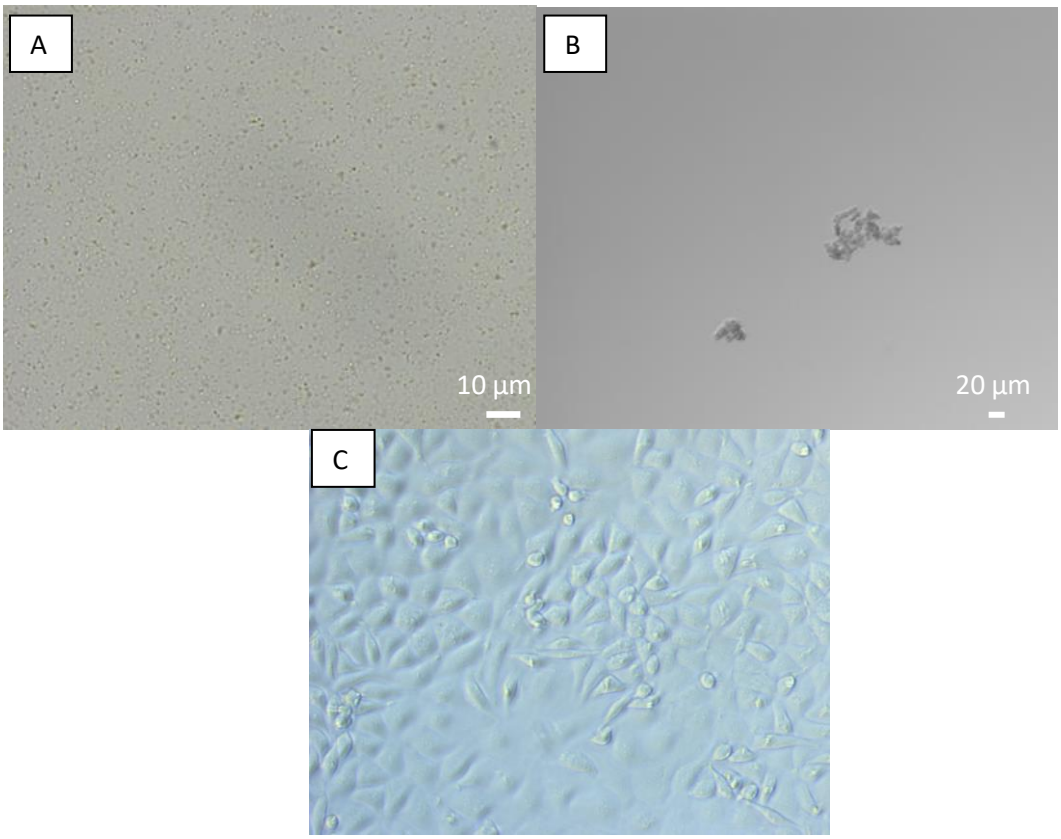

Figure S15: Microscope images of the **NG** + 1eq. **H1** in DMEM with serum (A) and DMEM without serum (B) at concentrations of 1mg mL^{-1} after incubation for 2 h at 37 °C. In the presence of L929 cells **NG** + 1eq. **H1** was stable in DMEM without serum for up to 48 hours at 37 °C (C). The agglomerates in (B) originate from the bottom of the plate and no aggregates from the sample.

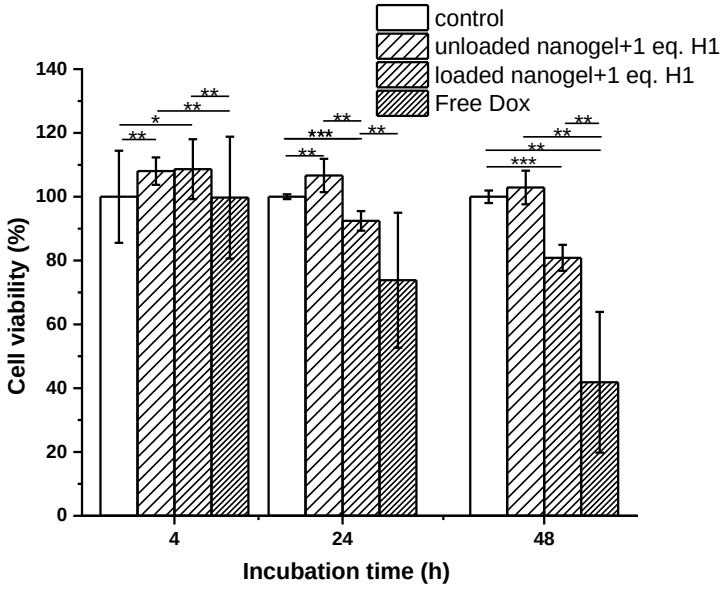

Figure S16: Cell viability of L929 mouse fibroblasts after incubation with unloaded nanogels (500 μg mL^{-1}, DOX loaded nanogels (500 μg polymer and 7.3 μg DOX mL^{-1}) and free DOX (7.3 μg DOX mL^{-1}) for 4, 24, and 48 h respectively. Data represent mean values ± SD of 6-replicates per measured sample (Mann-Whitney test, *p≤0.5;**p≤0.01;***p≤0.001).

The graph shows a clear effect of free DOX on the cell viability. DOX loaded nanogels also show cytotoxic effect but less than free DOX. This can be due to the difference in the uptake mechanism of the free DOX and DOX loaded nanogel in the cells.

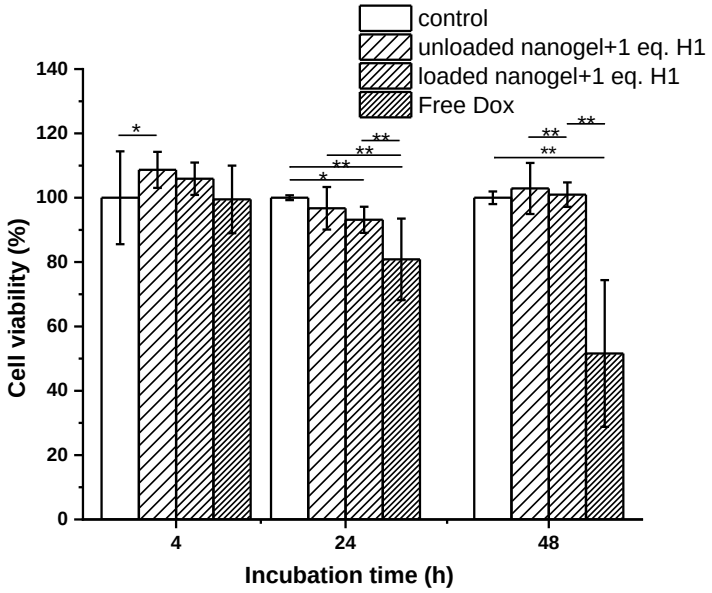

Figure S17: Cell viability of L929 mouse fibroblasts after incubation with unloaded nanogels (100 μg mL^{-1}), DOX loaded nanogels (100 μg polymer and 1.4 μg DOX mL^{-1}) and free DOX (1.4 μg DOX mL^{-1}) for 4, 24, and 48h respectively. Data represent mean values ± SD of 6-fold measured samples (Mann-Whitney test, *p≤0.5;**p≤0.01;***p≤0.001).

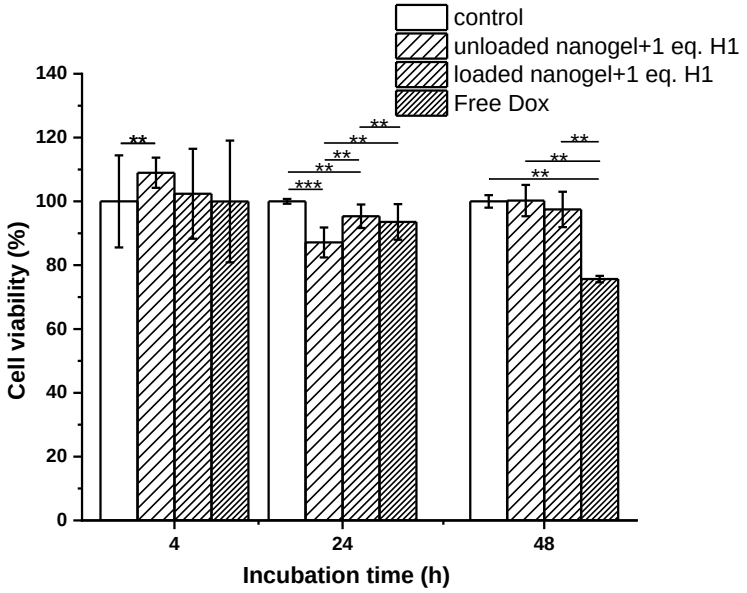

Figure S18: Cell viability of L929 mouse fibroblasts after incubation with unloaded nanogels (50 μg mL^{-1}), DOX loaded nanogels (50 μg polymer and 0.7 μg DOX mL^{-1}) and free DOX (0.7 μg DOX mL^{-1}) for 4, 24, and 48 h respectively. Data represent mean values ± SD of 6-fold measured samples (Mann-Whitney test, *p≤0.5;**p≤0.01;***p≤0.001).

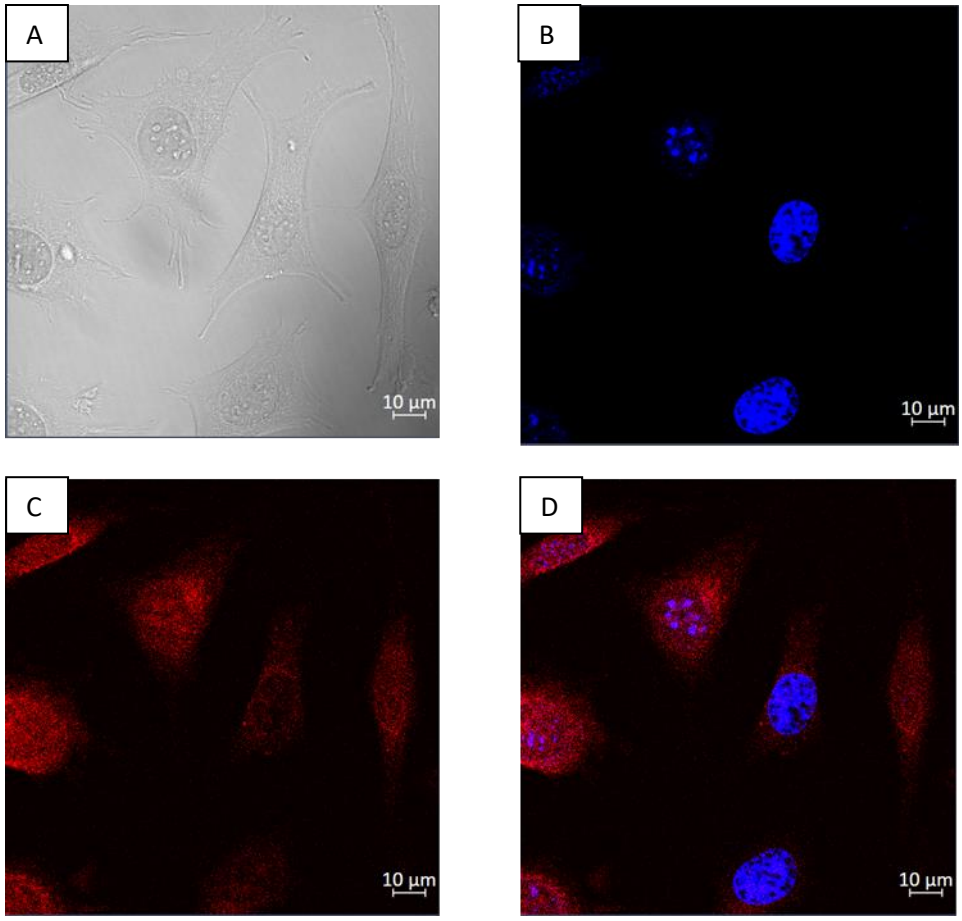

Figure S19: CLSM images of adherent L929 cells after 24 h incubation at 37 °C with free DOX at a concentration of 14.7 µg mL^{-1}. Transmitted light (A) and cell nuclei (B), free DOX in the cells(C) and overlay of all channels (D). The fluorescence signal is correlated with the applied stain specifically for the nuclei. Overlay of all channels proves (D) an intracellular localization of the free DOX.