Supporting Information for:

Norbornene-functionalised chitosan hydrogels and microgels via an unprecedented photo-initiated self-assembly for potential biomedical applications

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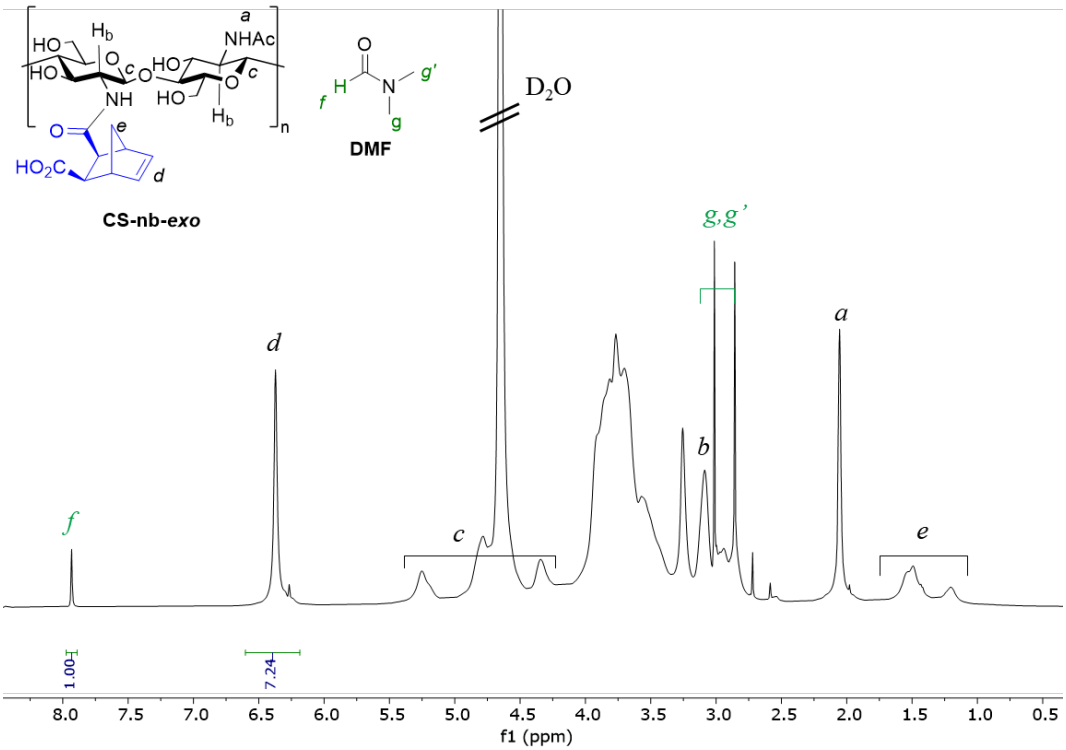

Figure S1. ^{1}H NMR of CS-nb-exo with 1 mM DMF. The degree of functionalisation (DF) was calculated using the integrals corresponding to the protons d and f using Equation S1:

$$DF = \frac{1}{I_f}.C_{DMF}.V_{DMF}\frac{\frac{I_d}{2}}{m_{CS-nb}} \quad \text{(S1)}$$

where:

I_f is the integral of the amide H of DMF (labelled *f* in Figure S1, 1H)

C_{DMF} is the concentration of DMF used to prepare the NMR sample (1 mM)

V_{DMF} is the volume of DMF used to prepare the NMR sample (550 µL)

I_d is the integral of the alkene peaks of norbornene (labelled *d* in Figure S1, 2H)

m_{CS-nb} is the mass of CD-nb dissolved to make up the NMR sample

An example of calculation is given Figure S1 with m_{CS-nb} = 3.20 mg, which gives:

$\text{DF} = 1 \times 1 \times 0.550\frac{\frac{7.241}{2}}{3.20}$ = 0.62 µmol nb/mg CS-nb

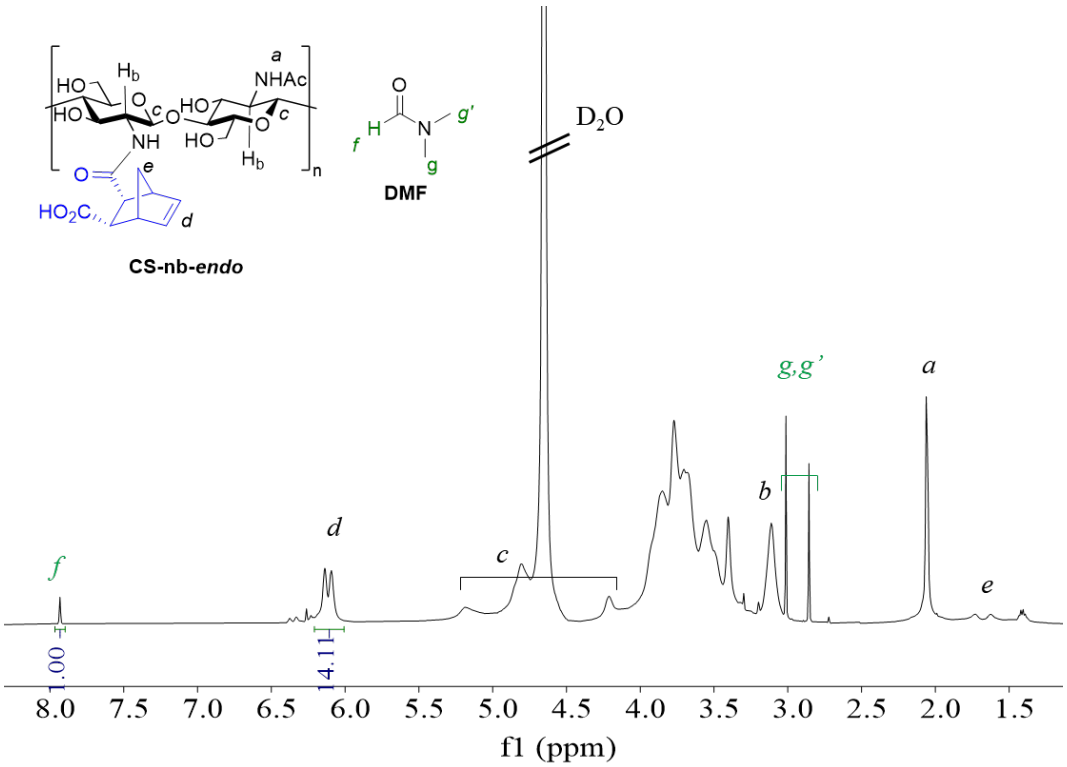

Figure S2. ^{1}H NMR of CS-nb-*endo* with 1 mM DMF. The degree of functionalisation (DF) was calculated using the integrals corresponding to the protons d and f using Equation (S1).

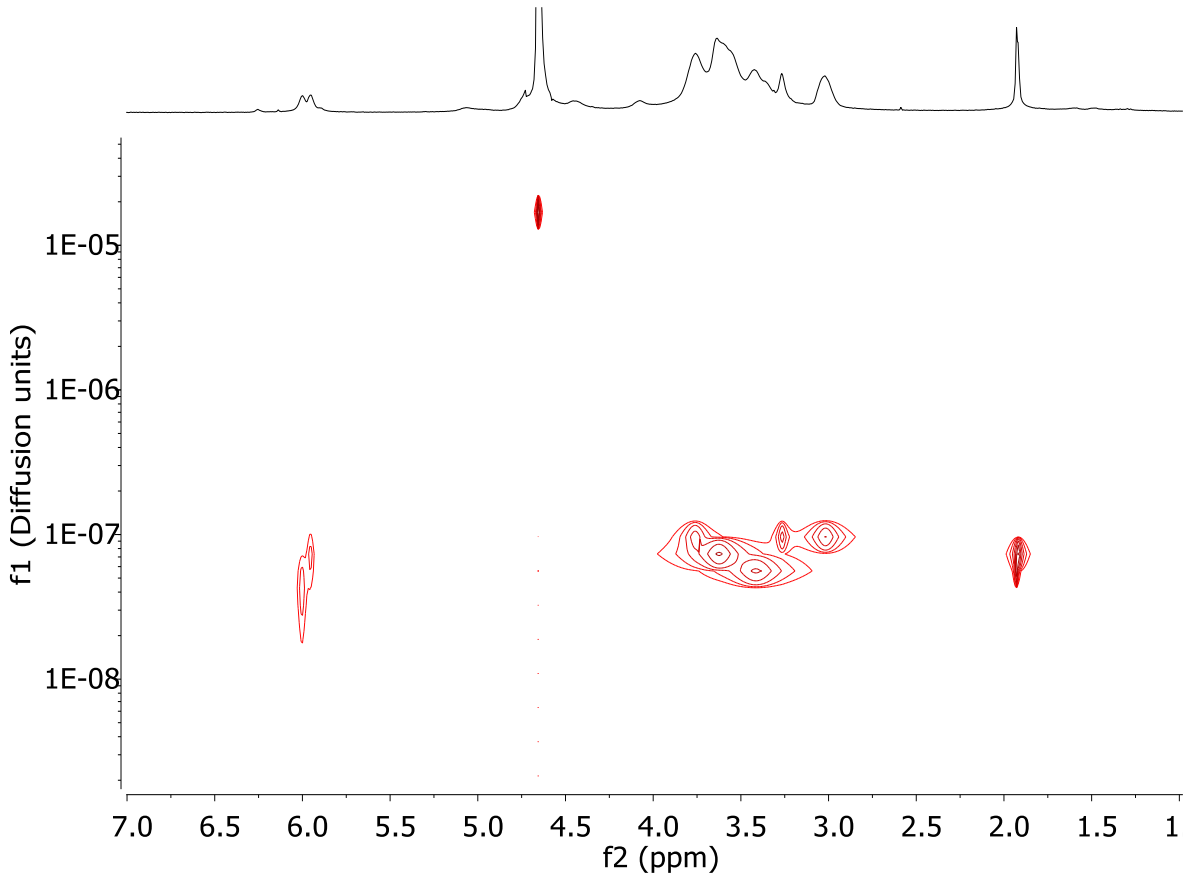

Figure S3. DOSY NMR of CS-nb-*endo*.

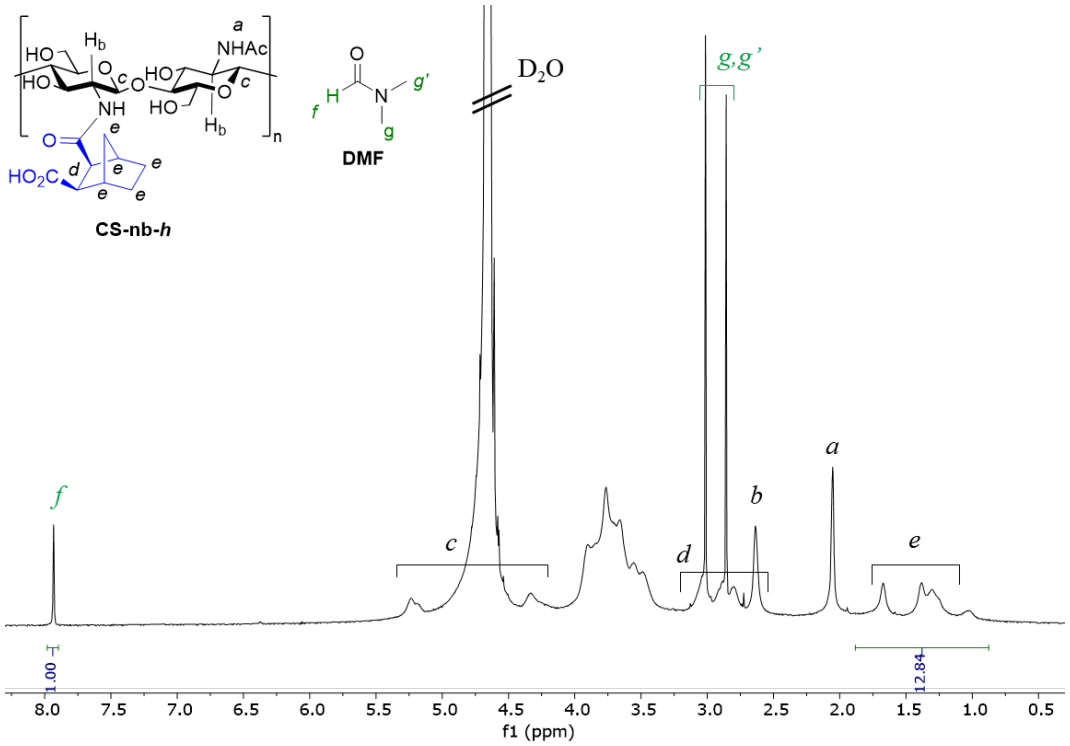

Figure S4. ^{1}H NMR of CS-nb-h with 1 mM DMF. The degree of functionalisation (DF) was calculated using the integrals corresponding to the protons d and f using Equation (7-1).

The degree of functionalisation (DF in µmol of nb/mg of CS-nb) was calculated by ^{1}H NMR by dissolving 2-to-5 mg of CS-nb in D_2O containing 1 mM of anhydrous DMF as:

$$DF = \frac{1}{I_f}.C_{DMF}.V_{DMF}\frac{\frac{I_e}{6}}{m_{CS-nb}} \quad \text{(S2)}$$

where:

I_f is the integral of the amide H of DMF (labelled *f* in Figure 3-5, 1H)

C_{DMF} is the concentration of DMF used to prepare the NMR sample (1 mM)

V_{DMF} is the volume of DMF used to prepare the NMR sample (550 µL)

I_e is the integral of the alkane peaks of norbornene ring, excluding those part of the succinic ring (labelled *e* in Figure 3-5, 6H)

m_{CS-nb} is the mass of CD-nb dissolved to make up the NMR sample

An example of calculation is given Figure S3 with m_{CS-nb} = 2.10 mg, which gives:

$$\text{DF} = 1 \times 1 \times 0.550\frac{\frac{12.84}{6}}{2.10} = 0.56 \text{ µmol nb/mg CS-nb}$$

Table S1. Summary of reaction conditions needed for hydrogel formation.

Entry	Reaction conditions				CS derivative (2 w:v%)			
	Crosslinker	UV	IRG	2% AcOH	**Native**	**nb-*endo***	**nb-*exo***	**nb-*h***
(a)	✓	✓	✓	✓	Liq.	Gel	Gel	Liq.
(b)	✓	✓	-	✓	Liq.	Liq.	Liq.	Liq.
(c)	-	✓	✓	✓	Liq.	Gel	Gel	Liq.
(d)	-	-	✓	✓	Liq.	Liq.	Liq.	Liq.
(e)	-	✓	-	✓	Liq.	Liq.	Liq.	Liq.
(f)	-	✓	✓	-	Liq.	Liq.	Liq.	Liq.

Table S2. Summary of the acid screening performed for hydrogel formation.

	Acetic acid			**Citric acid**			**HCl**		
Concentration (M)	**0.8**	**0.35**	**0.08**	**0.8**	**0.35**	**0.08**	**0.8**	**0.35**	**0.08**
CS-nb-*endo*	Gel	Gel	Gel	Gel	Gel	Gel	Gel	Gel	Gel
CS-nb-*exo*	Gel	Gel	Gel	Gel	Gel	Gel	Gel	Gel	Gel

Figure S5. Digital photographs of CS-nb-*exo* hydrogel (left) and microgel (right).

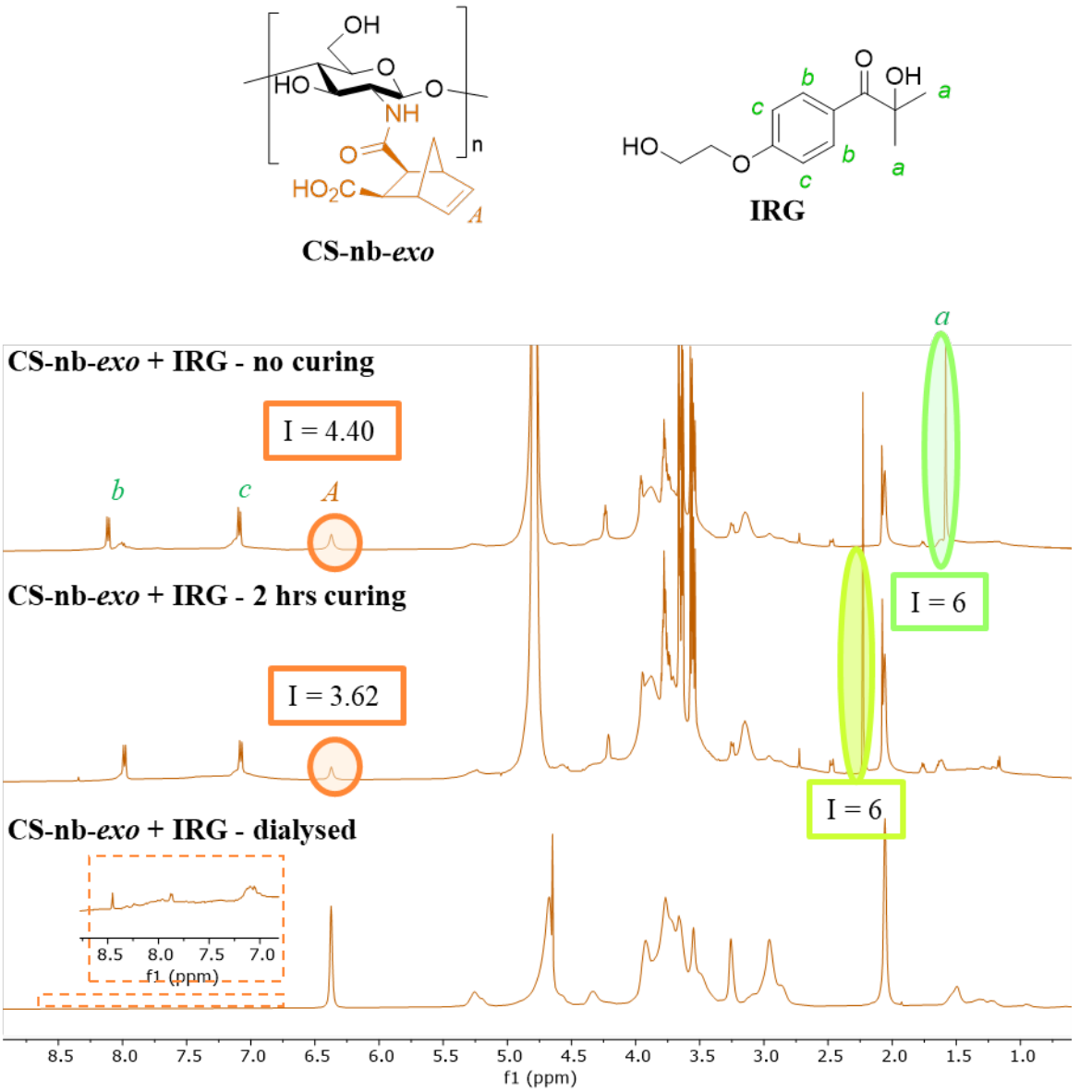

Figure S6. *In situ* ^{1}H NMR studies of CS-nb-exo coupling with IRG before (top, green) and after (middle, red) UV exposure, and presat of the purified resulting polymer (bottom, blue).

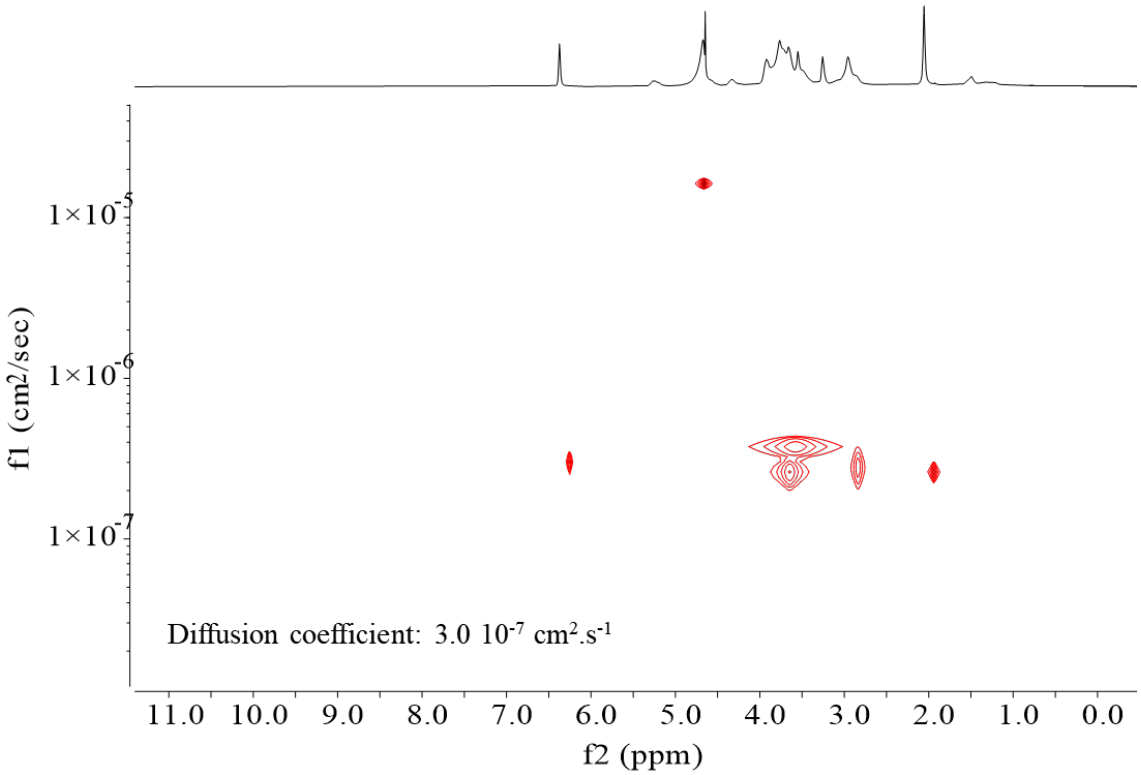

Figure S7. DOSY NMR of CS-nb-*exo* reacted with IRG under UV-B for 2 hrs after dialysis

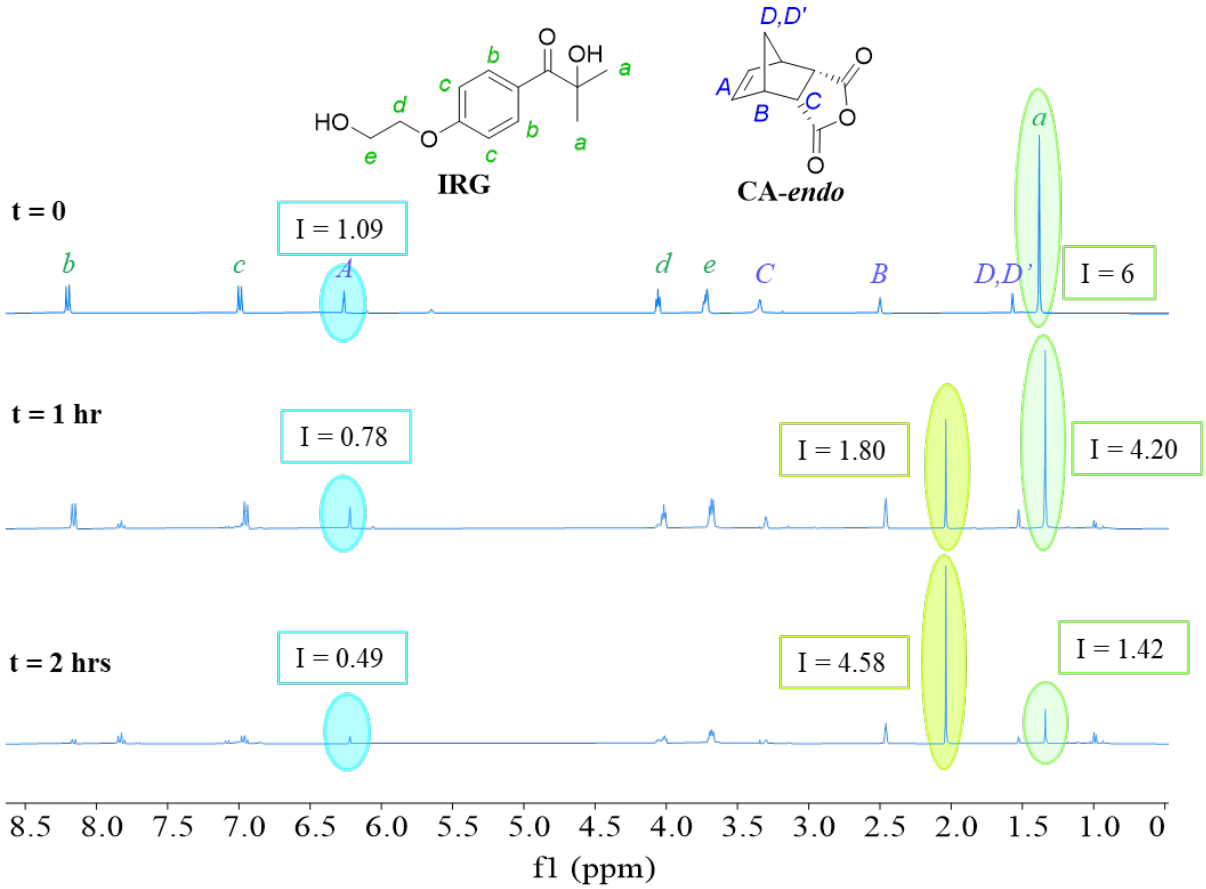

Figure S8. *In situ* ^{1}H NMR kinetics studies of the reactivity of CA-*endo* with IRG in 2% AcOD-d_4/D_2O.

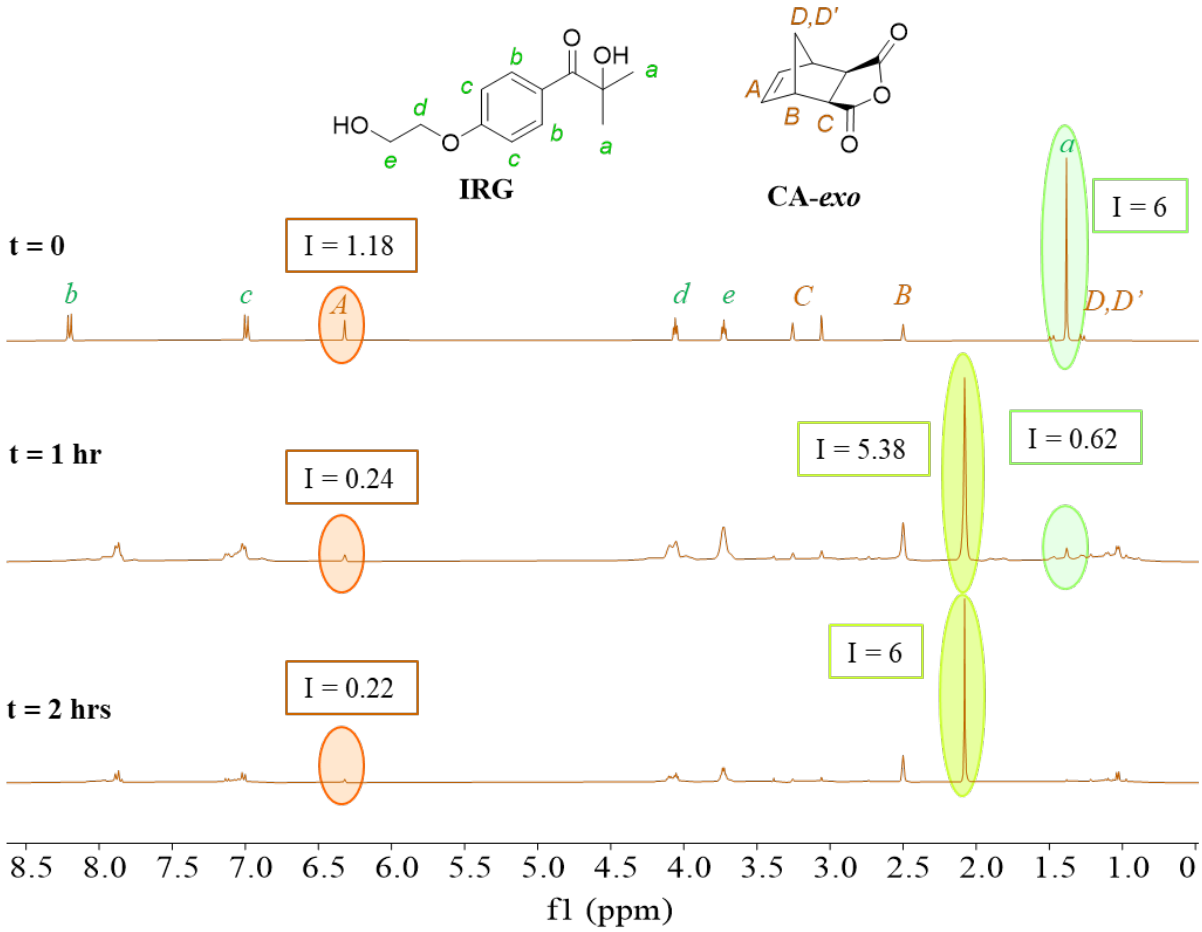

Figure S9. *In situ* ^{1}H NMR kinetics studies of the reactivity of CA-*exo* with IRG in 2% AcOD-d_4/D_2O.

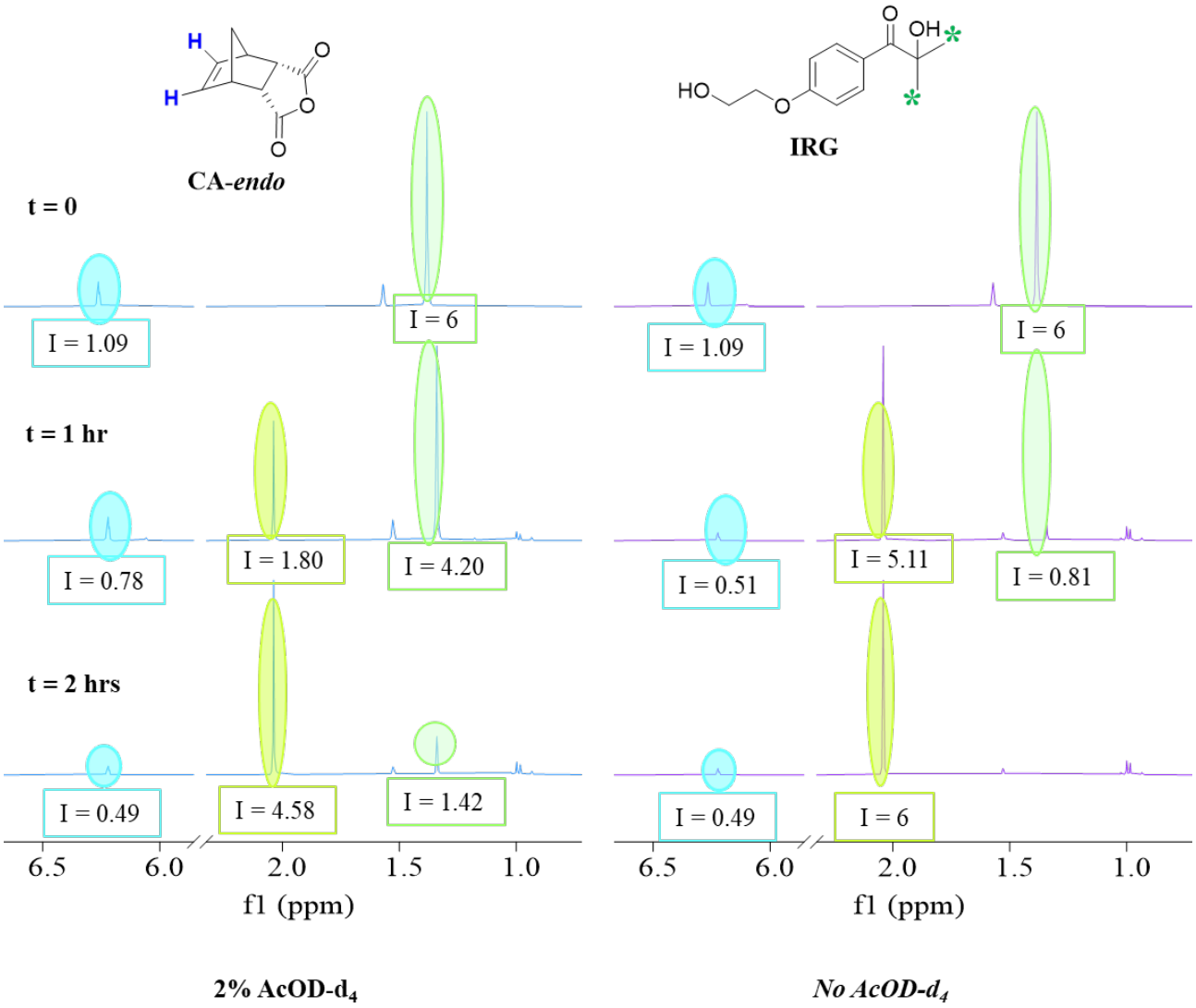

Figure S10. *In situ* ^{1}H NMR kinetics studies of the reactivity of CA-*endo* with IRG in 2% AcOD-d_4/D_2O (left) and in pure D_2O (right).

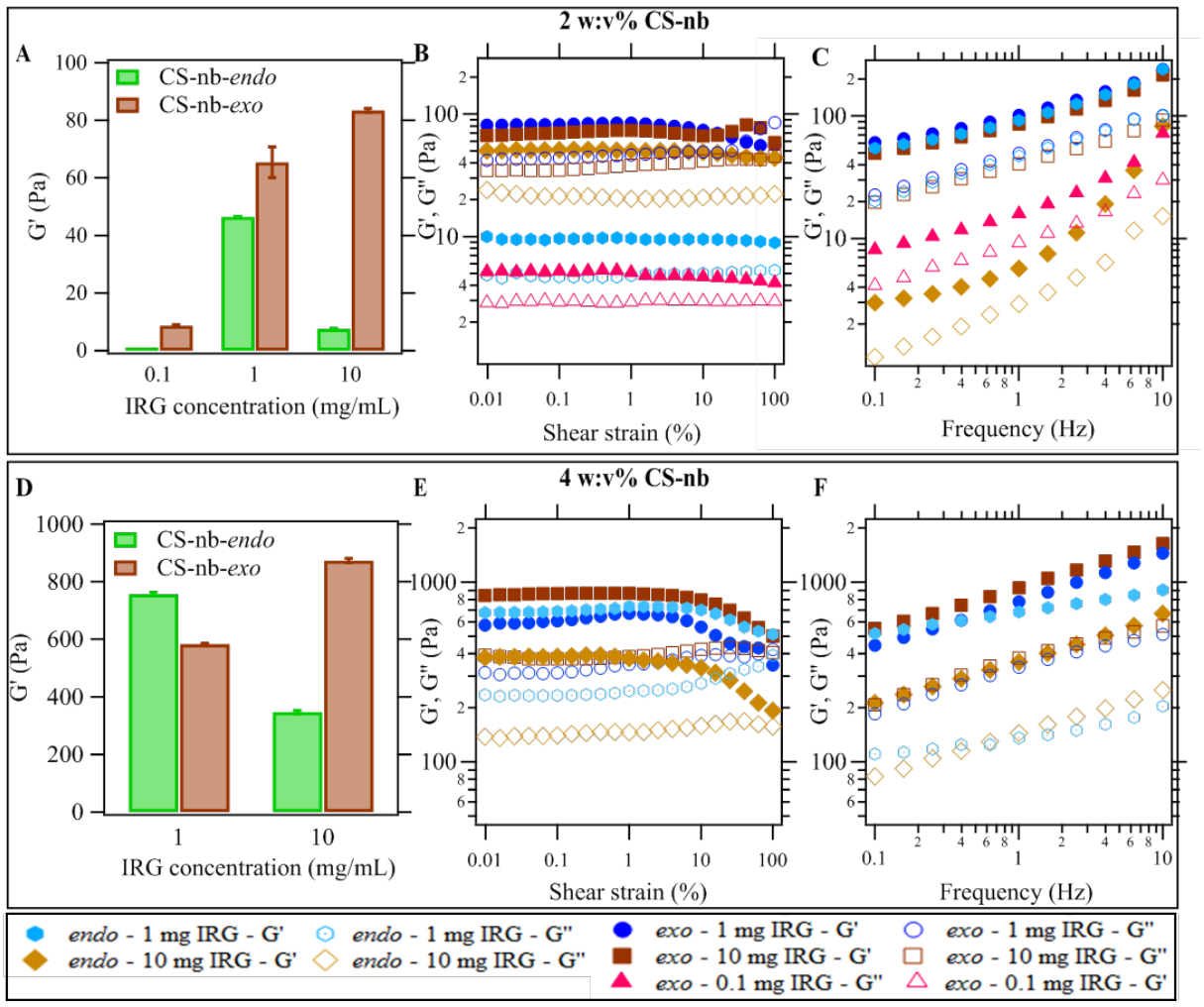

Figure S11. Rheology measurements of CS-nb-*endo* and -*exo* hydrogels: averaged G' obtained by amplitude sweep of the -endo and the -exo hydrogels with varying IRG concentrations at CS concentration of 2 (A) or 4% (D). Amplitude sweep (B, E) and frequency sweep (C, F) of CS hydrogels respectively at 2 or 4 w:v% with varying IRG concentrations.

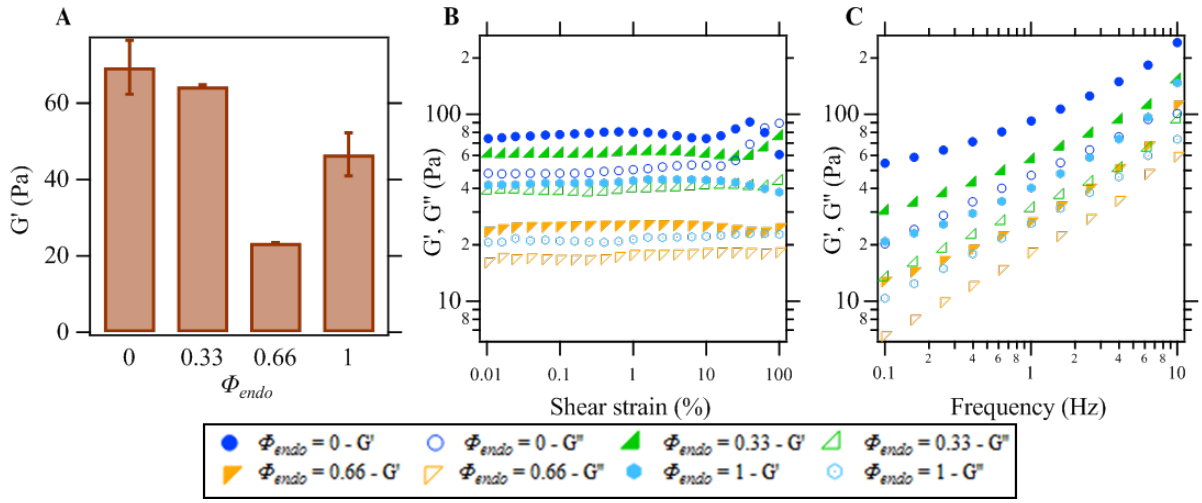

Figure S12. Mixed -*endo*/-*exo* hydrogel rheological properties when varying the ratio of CS-nb-*endo* ϕ_{endo} from 0 to 1: A) average G', B) amplitude sweep and C) frequency sweep.

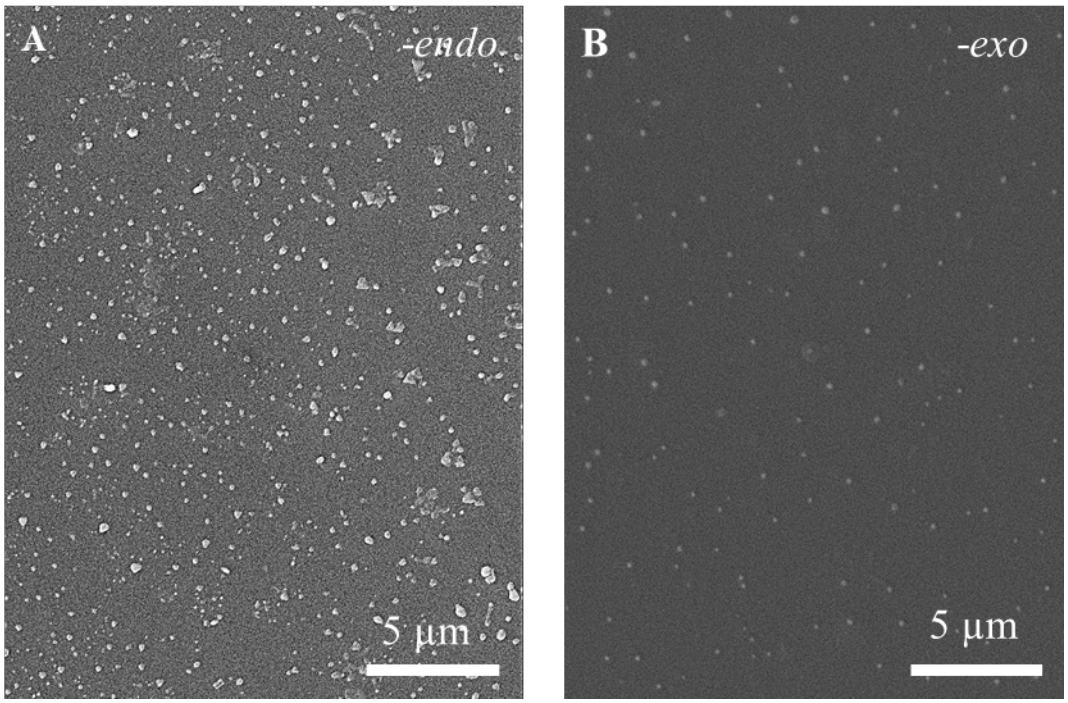

Figure S13. SEM images of CS-nb-*endo* (A) and -*exo* (B) microgels.

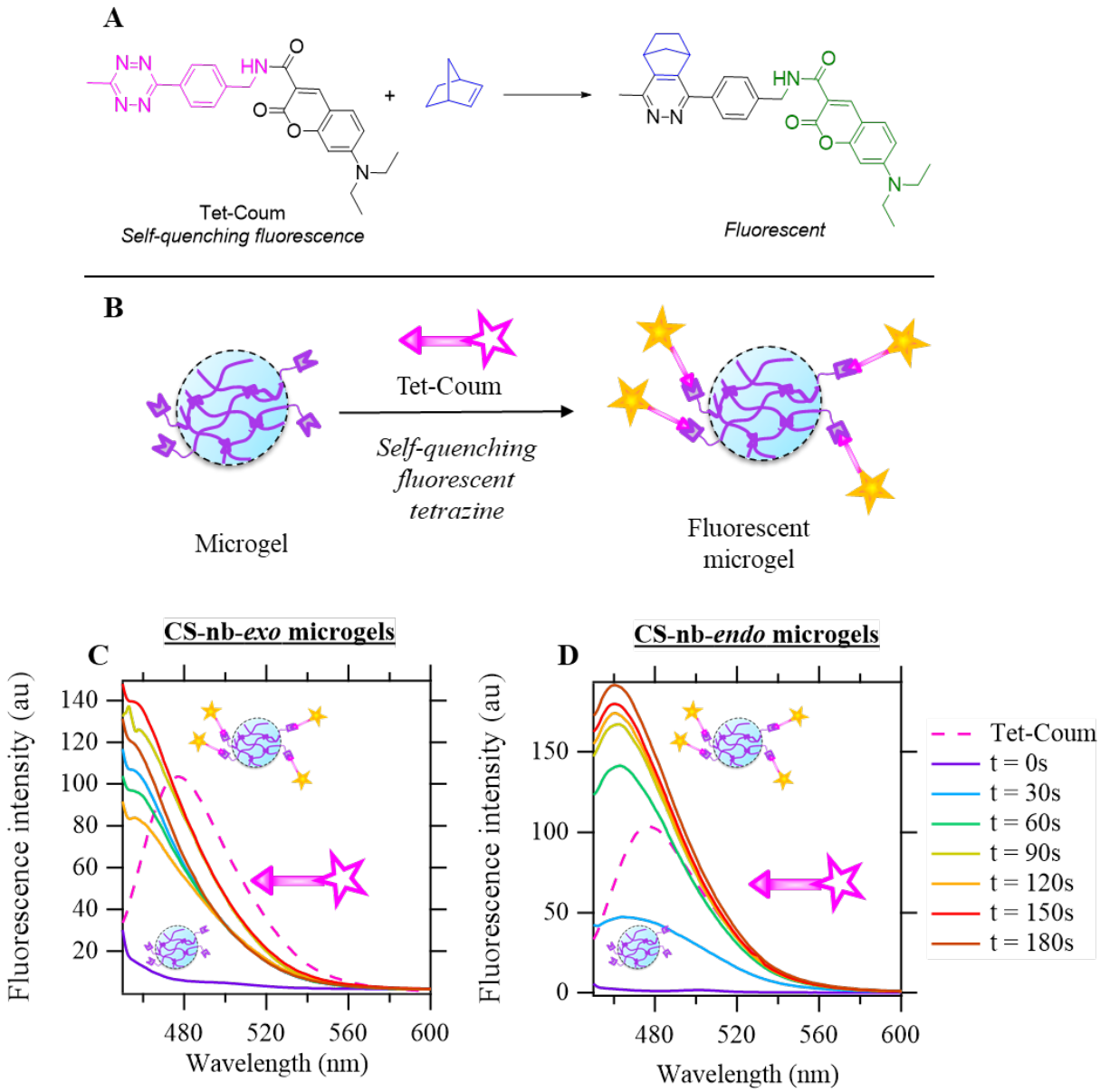

Figure S14. Functionalisation of remaining nb groups on CS-nb microgels with the self-quenching fluorescent probe Tet-Coum: reaction scheme (**A**), microgel functionalisation principle (**B**) and functionalisation of CS-nb-*endo* (**C**) and -*exo* (**D**) microgels measured by fluorescence.

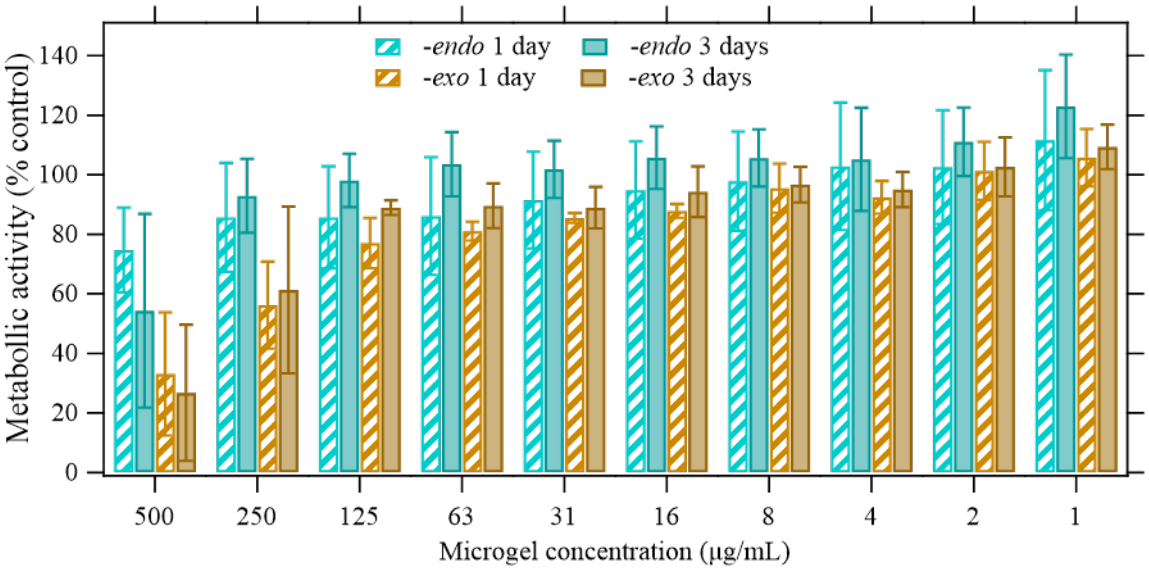

Figure S15. Metabolic activity of HDF cells treated with CS-nb-*endo* and -*exo* microgels after 1 and 3 days.

Cytotoxicity assays

Human dermal fibroblasts (HDF) were cultured in Minimal Essential Medium (MEM) supplemented with GlutaMAX™ , 10% fetal bovine serum (FBS), and antibiotic-antimycotic (AntiAnti). Confluent cultures were detached from the surface using trypsin (Tryp LE Express) before experiments and plated at 5×10^3 cells/well in 96-well plates. The cells were incubated 24 h after plating with microgels at concentrations varied between 500 and 1 µg/mL (4 replicates per condition, i.e., toxicant concentration and time point, all performed in triplicates) for 1 or 2 days. At the required incubation time, the medium was removed and wells were rinsed twice with phosphate buffer saline (PBS). Metabolic activity and cell viability were measured by feeding the cells with FBS-free medium containing 5% Alamar Blue (AB, metabolic activity) and 3 µM Calcein AM (cell viability) for 1 h. Fluorescence was recorded with a CLARIOstar plate reader (AB excitation 515– 555 nm, emission 510–530 nm; Calcein AM excitation 414–483, emission 510–530 nm). All results were background-corrected with a solution of media containing the two dyes and expressed as a percentage of control consisting of cells not exposed to microgels.

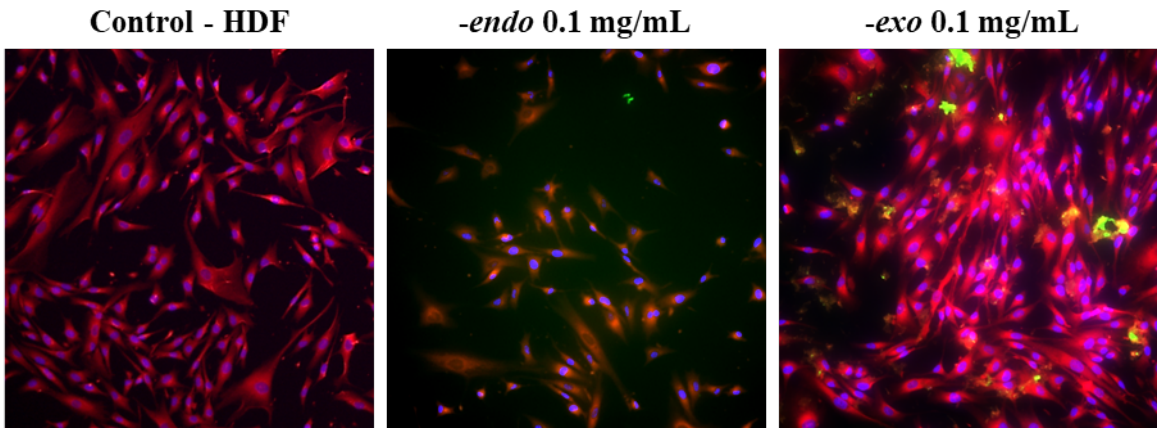

Figure S16. Localisation of Eosin-labelled (green) microgels after 24 hrs incubation. Legend: blue (nuclei, DAPI), red (cell membrane), green (Eosin-labelled microgels). *Exo*-microgels presented aggregation outside the cells.

References

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