Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

A versatile method for the selective core-crosslinking of hyaluronic acid-based nanogels via ketone-hydrazide chemistry: from chemical characterization to *in vivo* biodistribution

*Francielle P. Garcia, Marlène Rippe, Mychelle V. P. Companhoni, Talitha F. Stefanello, Benoit Louage, Simon Van Herck, Lucie Sancey, Jean-Luc Coll, Bruno G. De Geest, Celso V. Nakamura, Rachel Auzély-Velty**

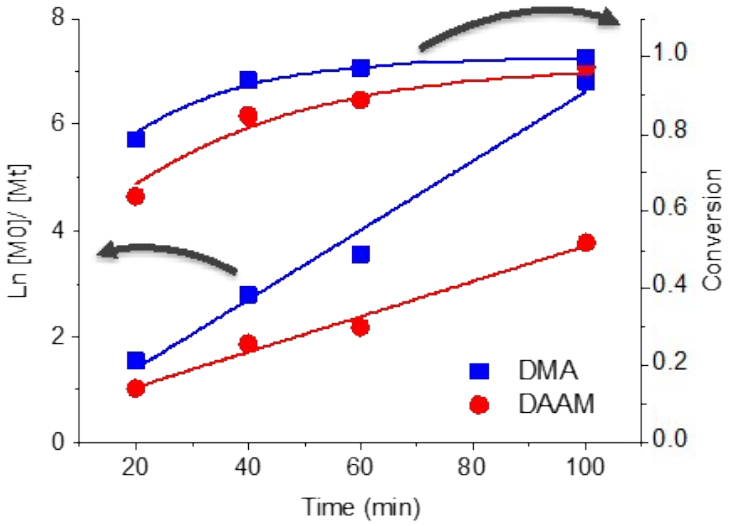


Figure S1. (A) Kinetic curves of copolymerization of DAAM and DMA. Reaction conditions: [DAAM]/[DMA]/[PABTC]/[AIBN] = 1.43//0.71/0.021/4.35×10^{-3} in dioxane at 75 °C.

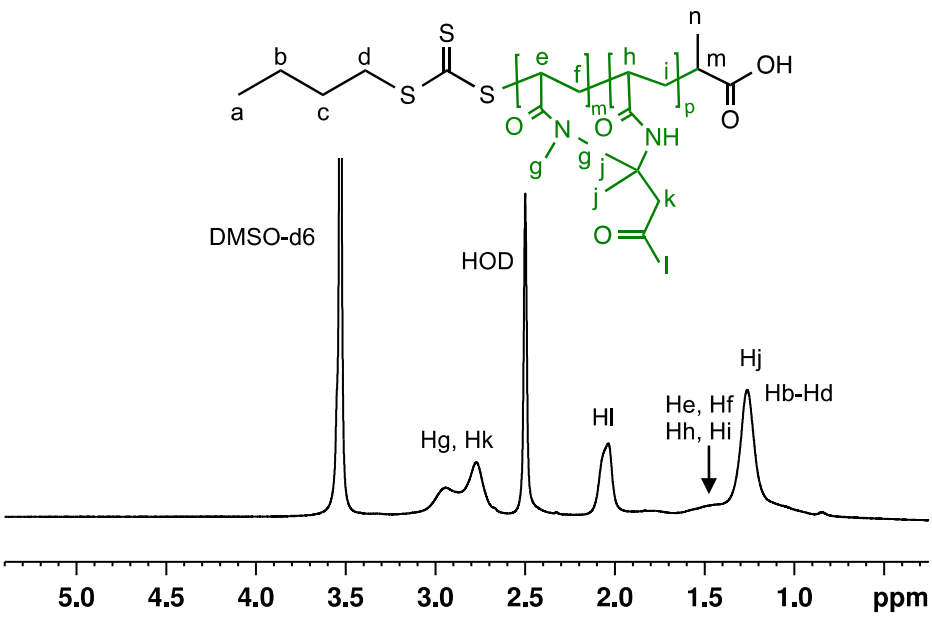


Figure S2. ^{1}H NMR spectrum (400 MHz, 6 mg/mL in DMSO-d6, 10 °C) of the copolymer poly(DAAM-co-DMA).

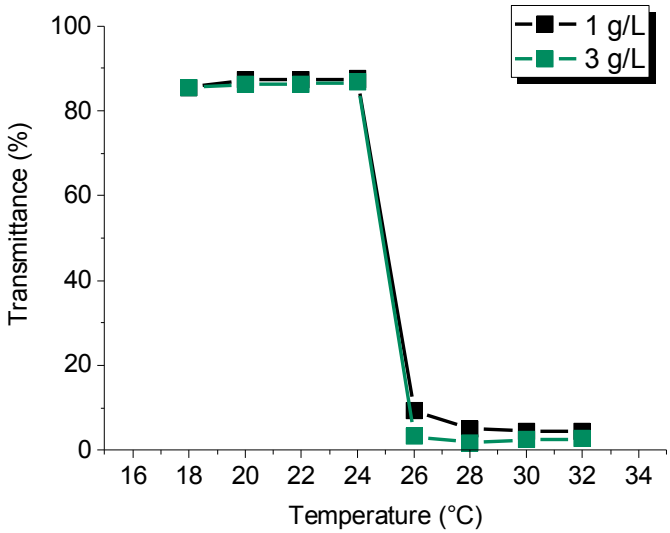


Figure S3. Phase transition of poly(DAAM-co-DMA) in PBS at 1 and 3 g/L as measured by UV/Vis spectroscopy at 500 nm. The cloud point temperature was found to be 24 °C for the two concentrations tested.

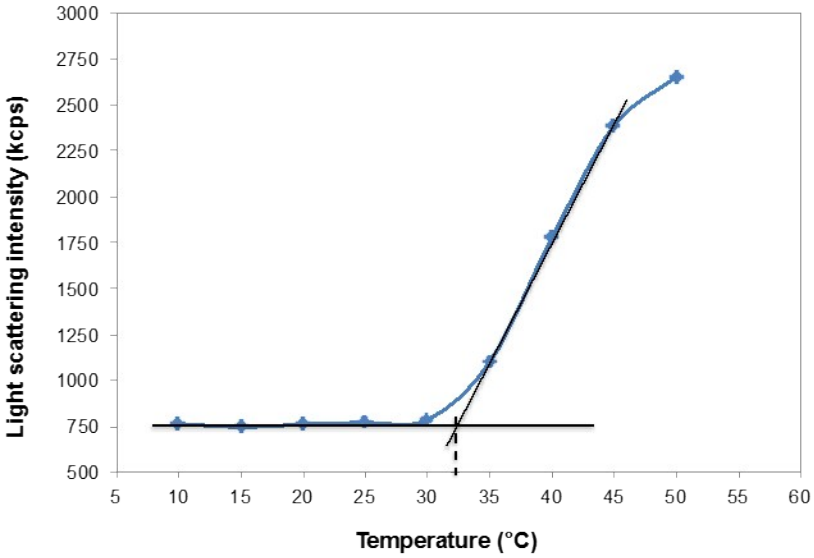


Figure S4. Light scattering intensity of a solution of HA-m-poly(DAAM-co-DMA) in PBS at 0.5 g/L as a function of temperature. The critical aggregation temperature was found to be 32 °C.

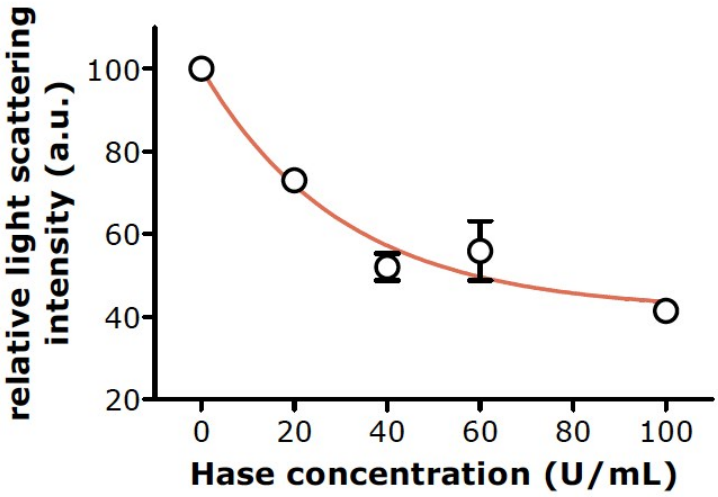
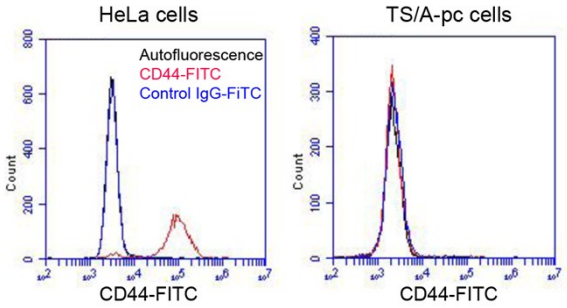


Figure S5. Relative light scattering intensity of suspensions of nanogels (crosslinked with an IDH to ketone molar ratios of 0.5) measured at 37 °C 1 h after addition of HAase at different concentrations.



Cell line	Autofluo	CD44-FITC
HeLa	3 437 ± 46	108 486 ± 68
TS/A-pc	2 334 ± 56	2 483 ± 203

Figure S6. CD44-expression in HeLa and TS/A-pc cells, observed by FACS. Autofluorescence (black), isotypic control (blue) and CD44-FITC labeled cells (red) were measured for both cell lines. Results indicated the mean of fluorescence intensity ± coefficient of variation observed on 10,000 cells, for autofluorescence and CD44-FITC.

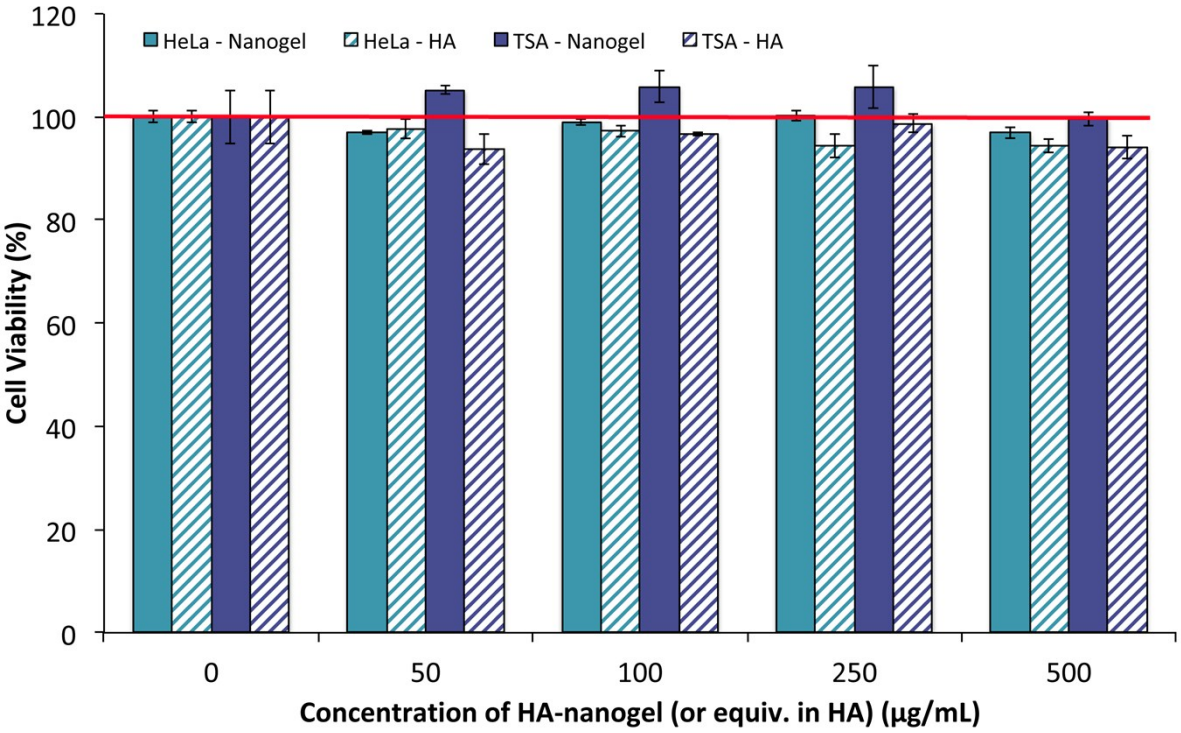


Figure S7. Cell viability of HeLa and TSA cells, 72 h after addition of HA nanogels (crosslinked with an IDH to ketone molar ratios of 0.5) or equivalent dose of initial HA (from 12.5 to 125 µg/mL), at different concentrations. The cell viability was assessed using MTS assay (n=3/condition).

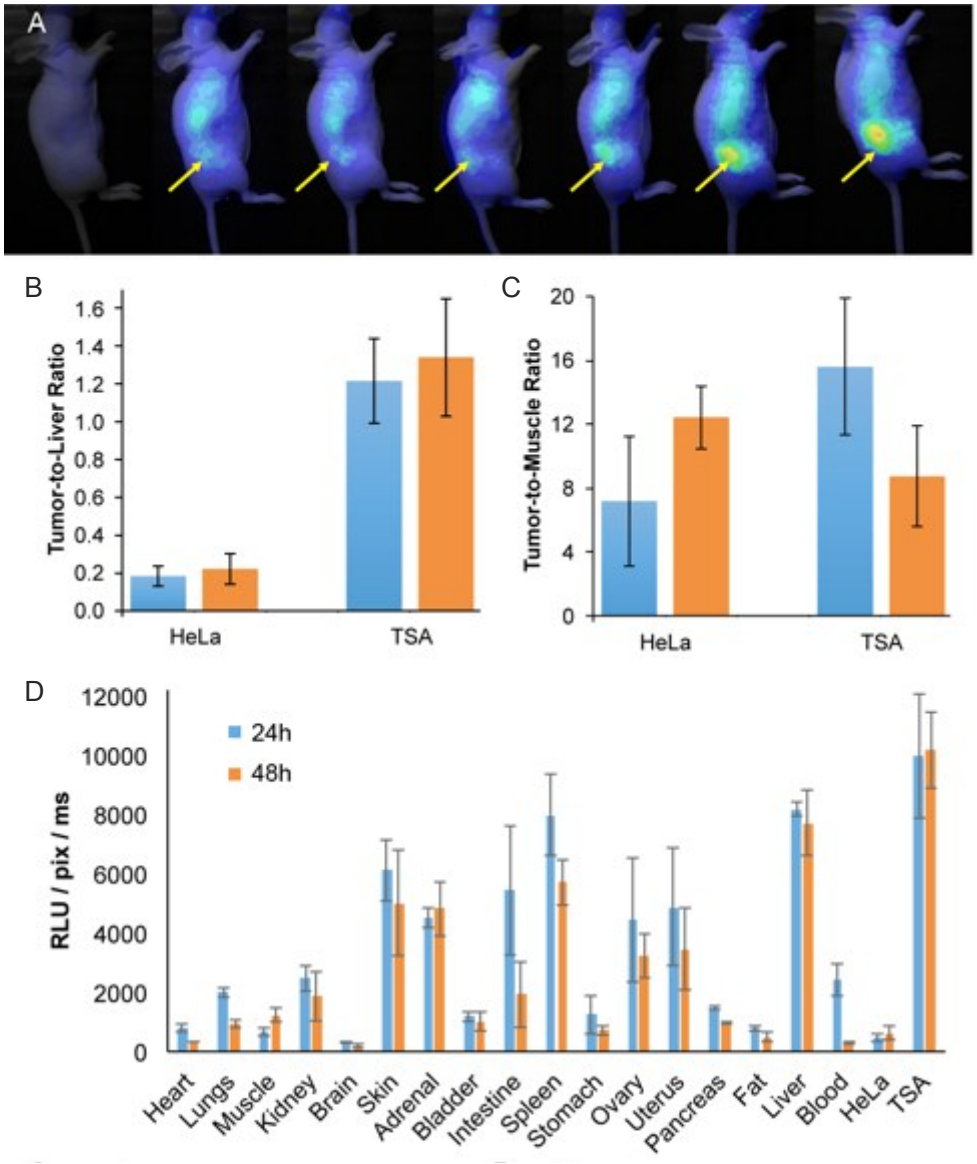


Figure S8. (A) *In vivo* near-infrared fluorescence (NIRF) images of the time dependent biodistribution of Cy5.5-labeled HA in TS/A-pc and HeLa tumor-bearing mice (n = 3/tumor type). The tumor was engrafted subcutaneously on the right flank of the mice. The fluorescence was measured at the following time elapse after administration: 0 min, 30 min, 1 h, 2 h 30, 5 h, 24 h, and 48 h. (B) Fluorescence intensity of the tumor-to-liver and, (C) tumor-to-muscle ratios from exised organs, sampled at 24 h (blue) and 48 h (orange) post-injection for (B) and (C). The results are expressed as the mean ± SD (n=3). (D) Quantification of the *ex-vivo* biodistribution of Cy5.5-labeled HA in mice, 24 h and 48 h after administration. ROIs were defined on the extracted organs to semi-quantify the amount of photons detected per pixel. The results in each organ are expressed as the mean ± SD (n=6).