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Supporting Information

From Proof-of-Concept Material to PEGylated, Modularly Targeted Ultrasound-Responsive Mesoporous Silica Nanoparticles.

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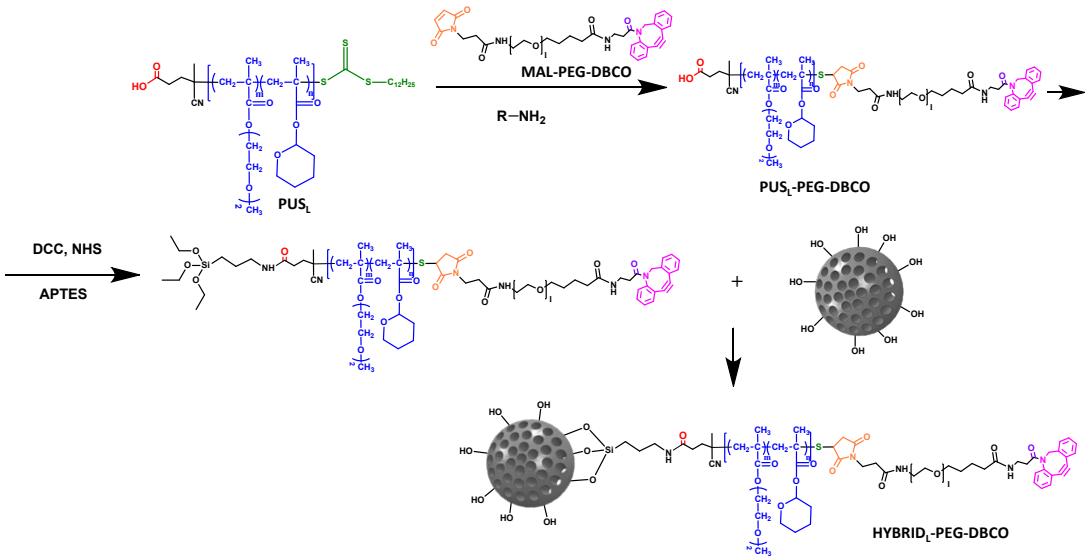
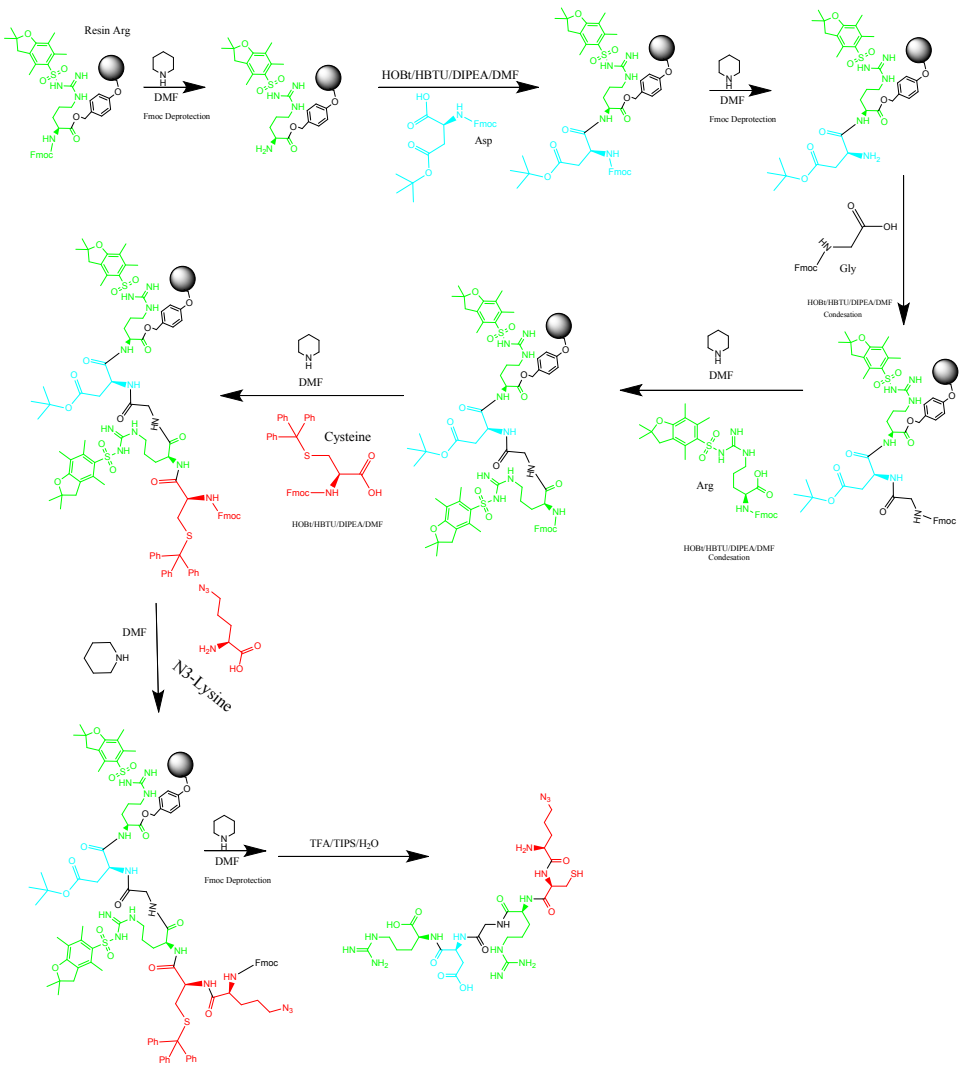
Table S1. Amount of different reagents employed to obtain PUS samples with different molecular weights.

PUS sample	CDTPA (mg)	ABCVA (mg)	MEO_2MA (mL)	THPMA (mL)
PUS_S	15	4	1.8	0.2
PUS_M	8	3	1.8	0.2
PUS_L	5	2	1.8	0.2



Scheme S1. Synthetic procedure employed to obtain the PEGylated, DBCO-modified ultrasound-responsive material, $HYBRID_L$-PEG-DBCO.



Scheme S2. Synthesis scheme employed to obtain RGD-N_3 by standard solid-phase techniques using Fmoc-coupling chemistry.

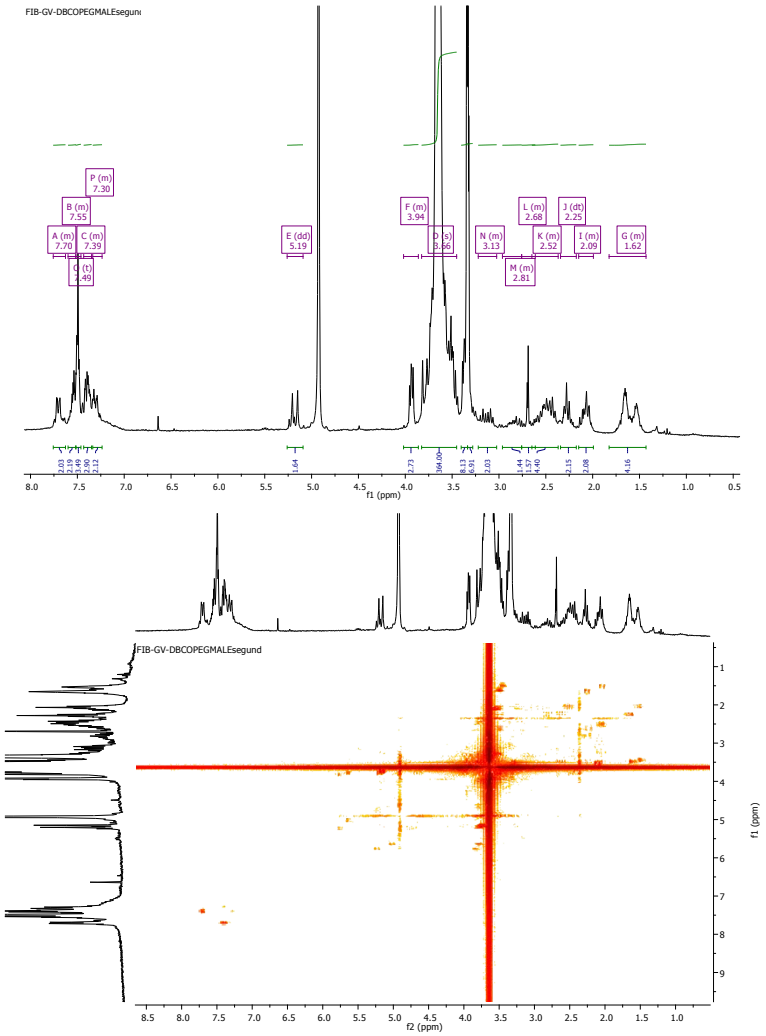


Figure S1. ^{1}H NMR spectrum and ^{1}H NMR COSY of MAL-PEG-DBCO in MeOD.
^{1}H NMR (250 MHz, MeOD) δ 7.69 (ddd, *J* = 14.0, 10.2, 3.8 Hz,2H, 2xCHAr, DBCO), 7.60 – 7.51 (m, 2H, 2xCHAr, DBCO), 7.49 (m, 4H, 2xCH, maleimide, 2xCHAr, DBCO), 7.39 (ddd, *J* = 8.9, 3.7, 2.2 Hz, 2H, 2xCHAr, DBCO), 7.30 (dt, *J* = 8.7, 5.1 Hz, 2H, , 2xCHAr, DBCO), 5.20 (s, *J* = 8.6 Hz, 1H, CH_2, DBCO), 5.15 (s, 1H, CH_2, DBCO), 4.02 – 3.86 (m, 2H, CH_2-N, maleimide), 3.66 (s, broad, 364 H, 90x(CH_2-CH_2-O), PEG), 3.22 – 3.02 (m, 2H, CH_2-NHCO), 2.96 – 2.76 (m, 2H, CH_2-NHCO), 2.65 – 2.37 (m, 4H, 2xCH_2-CONH), 2.26 (dd, *J* = 13.0, 5.8 Hz, 2H, CH_2, maleimide chain), 2.08 (dd, *J* = 12.5, 5.4 Hz, 2H, CH_2, maleimide chain), , 1.82 – 1.57 (m, 2H, CH_2, maleimide chain), 1.58 – 1.46 (m, 2H, CH_2, maleimide chain).

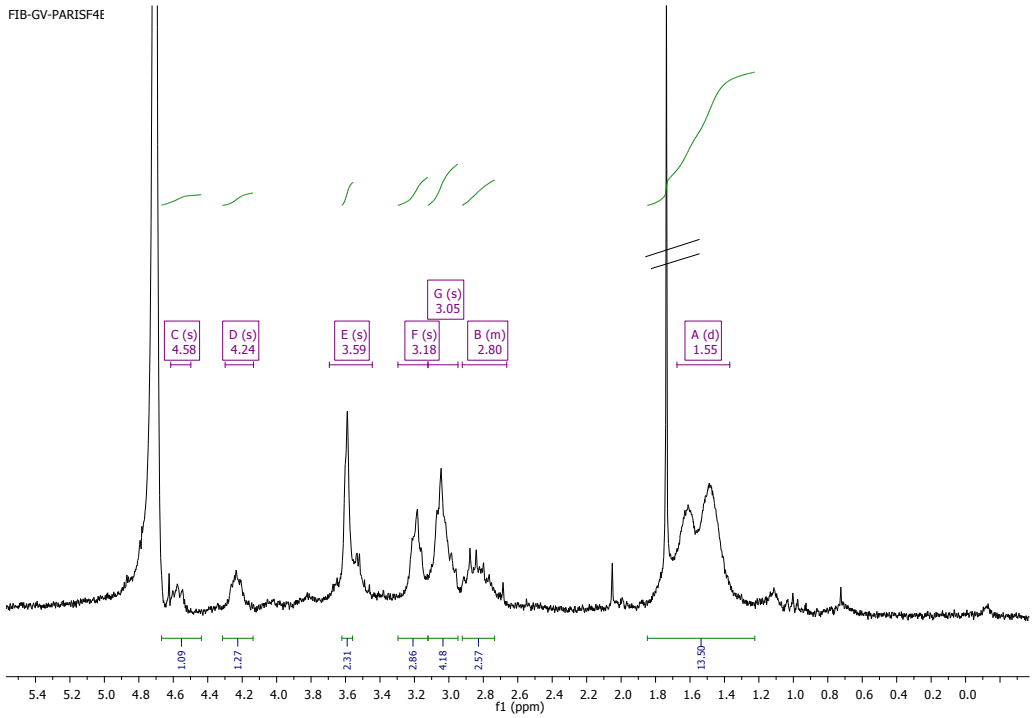


Figure S2. ^{1}H NMR spectrum of RGD-N_3 in D_2O.
^{1}H NMR (250 MHz, D_2O) δ 4.58 (1H, CH, Cys); 4.35 – 4.10 (2H, 2xCH, Arg), 3.60 (s, broad, 2H, CH_2, Gly), 3.33 – 3.13 (m, 3H, CH_2, Cys, CH Lys-N_3), 2.85 (s, broad, 4H, 2xCH_2, Arg), 2.83 – 2.64 (m, 2H, CH_2, Asp), 1.60 (s, broad, 6H, 2xCH_2, 2xArg, CH_2, Lys-N_3), 1.50 (s, broad, 8H, 2xCH_2, Lys-N_3, 2xCH_2, 2xArg).
MALDI/TOF/TOF= 713.080 m/z [M^+-SH] (100%);

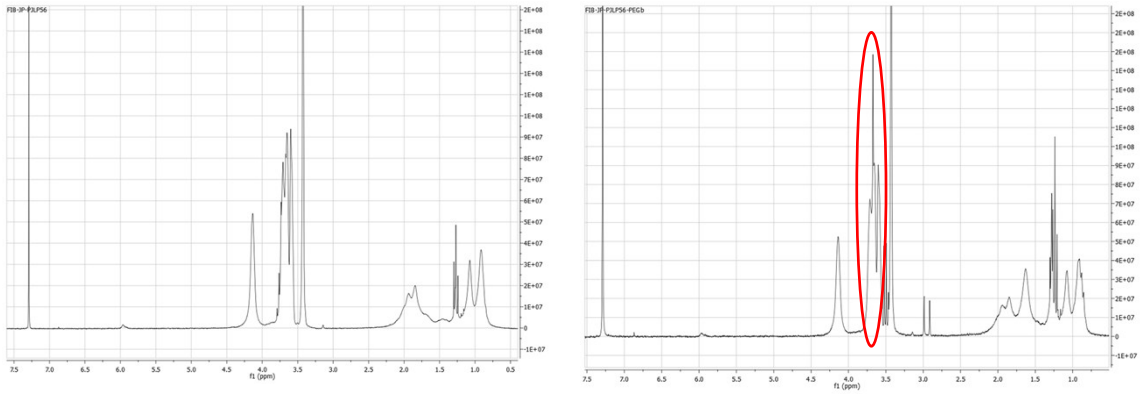


Figure S3. ^{1}H NMR spectra of PUS_L (left) and PUS_L-PEG (right) in $CDCl_3$.

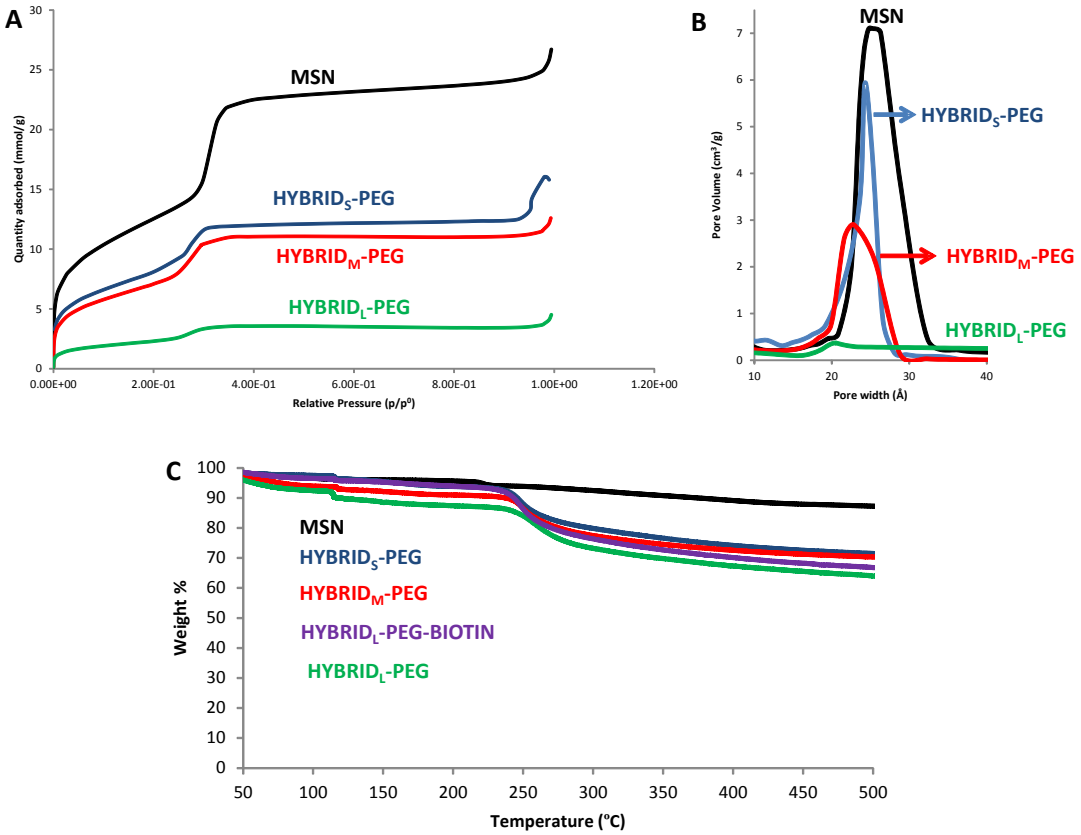


Figure S4. Characterization of the obtained materials by N_2 adsorption/desorption: adsorption isotherms (A), pore size distribution (B) and by thermogravimetric analysis (C).

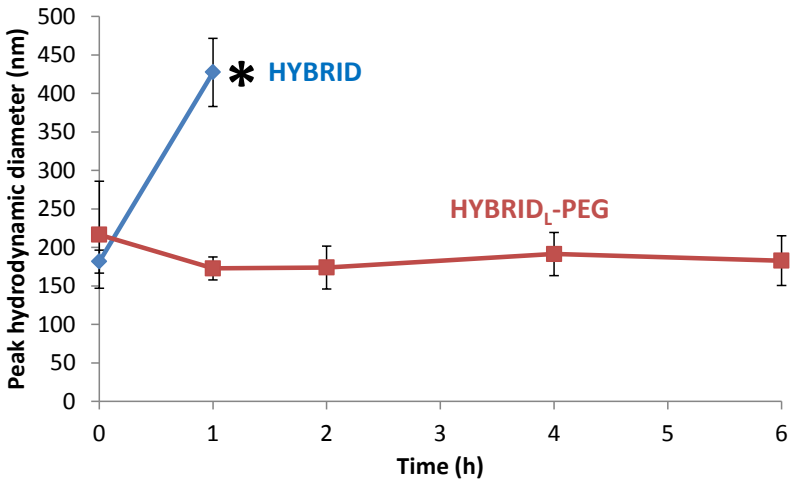


Figure S5. Suspension stability experiments of the prepared nanoparticles performed by DLS at different time points in suspension in 1 mM PBS. * The sample HYBRID was completely aggregated after 1 h, and no valid measurement could be performed afterwards.

Copper-Free Azide–Alkyne Cycloaddition with TAMRA-N_3

To react TAMRA-N_3 with MAL-PEG-DBCO, 2 mg of MAL-PEG-DBCO were dissolved in 300 µL of PBS and 2 µL of the stock TAMRA-N_3 solution were added (1 mg/mL in DMSO). The mixture was stirred at 37 ºC for 1 h. Then, the polymer was purified by G-25 Sephadex column size exclusion chromatography.

For the reaction of TAMRA-N_3 with PUS_L-PEG-DBCO, 10 mg of PUS_L-PEG-DBCO were dissolved in 1 mL of cold deionized (DI) water, and 2 µL of the stock TAMRA-N_3 solution were added. The mixture was stirred at 4 ºC overnight, and the polymer was then precipitated in diethyl ether and centrifuged.

To conjugate TAMRA-N_3 with DBCO-modified hybrid, 3 mg of $HYBRID_L$-PEG-DBCO were dispersed in 500 µL of DI water, and 6 µL of the stock TAMRA-N_3 solution were added. The mixture was stirred at 4 ºC overnight. The material was then collected by centrifugation and thoroughly washed with ethanol and cold water.

TAMRA fluorescence emission was then checked in DI water (Ex: 555 nm; Em: 575 nm) (Figure S2).

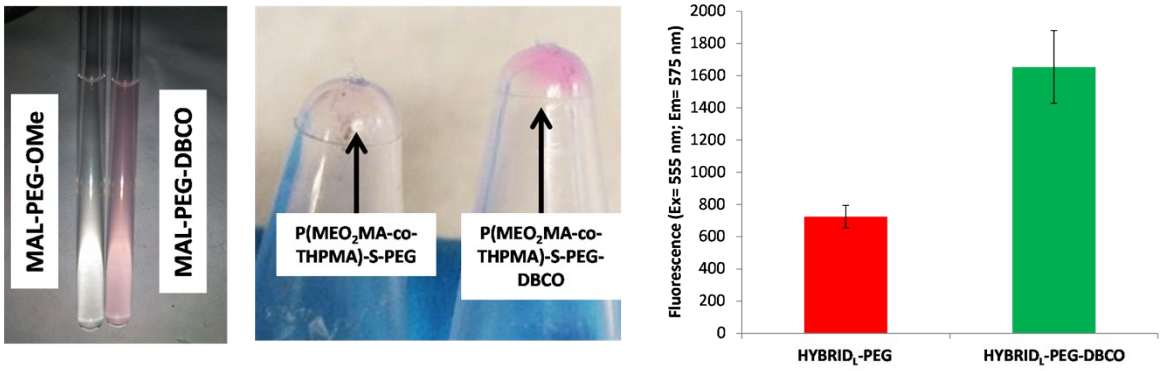


Figure S6. Reaction with TAMRA-N_3 of MAL-PEG-DBCO (left), PUS_L-PEG-DBCO (center) and $HYBRID_L$-PEG-DBCO (right). Control experiments were performed using MAL-PEG-OMe, PUS_L-PEG and $HYBRID_L$-PEG.