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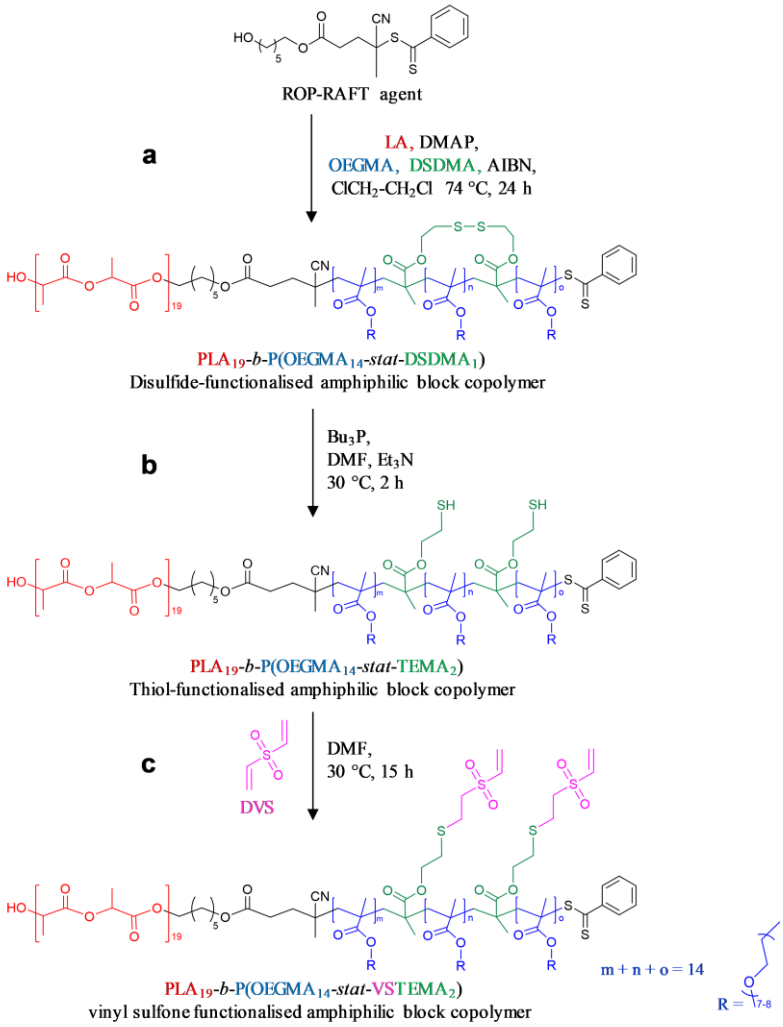
Supporting Information

Functionalization of polymeric nanoparticles with targeting VNAR ligands using vinyl sulfone conjugation

Adam Leach, Marie Finnegan, Mariana S. Machado, Laura Ferguson, John Steven, Peter Smyth, Andrew Porter, Caroline Barelle, Efrosyni Themistou and Christopher J. Scott



Scheme S1. Preparation of vinyl sulfone-functionalized amphiphilic PLA_{19}-*b*-P($OEGMA_{14}$-*stat*-$VSTEMA_2$) diblock copolymers. (a) Synthesis of disulfide-functionalized branched amphiphilic PLA_{19}-*b*-P($OEGMA_{14}$-*stat*-$DSDMA_1$) diblock copolymer from a dual ROP-RAFT agent by simultaneous ROP of LA and RAFT statistical copolymerization of OEGMA and DSDMA. *Polymerization conditions*: $[LA]_0$: $[OEGMA]_0$: $[DSDMA]_0$: $[ROP\text{-}RAFT\ agent]_0$ relative molar ratios 20 : 15 : 1 : 1 and 55% w/w solids at 74 ºC for 24 h. Indicative intramolecular cycle presented for the disulfide bond although both intramolecular cycles and intermolecular branches are expected to be obtained by this reaction. (b) Formation of a thiol-functionalized linear amphiphilic PLA_{19}-*b*-P($OEGMA_{14}$-*stat*-$TEMA_2$) diblock copolymer with thiol groups prepared by reductive cleavage of the disulfide bonds in the methacrylic block of the disulfide-functionalized amphiphilic diblock copolymer. *Reaction conditions*: Bu_3P in DMF at 30 ºC for 2 h with relative molar ratios of $[Bu_3P]_0$: $[Et_3N]_0$: $[disulfide\ bond]_0$ of 3.0 : 2.1 : 1. (c) Synthesis of vinyl sulfone-functionalized linear amphiphilic PLA_{19}-*b*-P($OEGMA_{14}$-*stat*-$VSTEMA_2$) diblock copolymer by conjugation of divinyl sulfone to the thiol-functionalized amphiphilic diblock copolymer. *Reaction conditions*: DMF, 30 ºC, 15 h, excess DVS with relative molar ratio $[DVS]_0$: $[thiol]_0$ of 15 : 1.

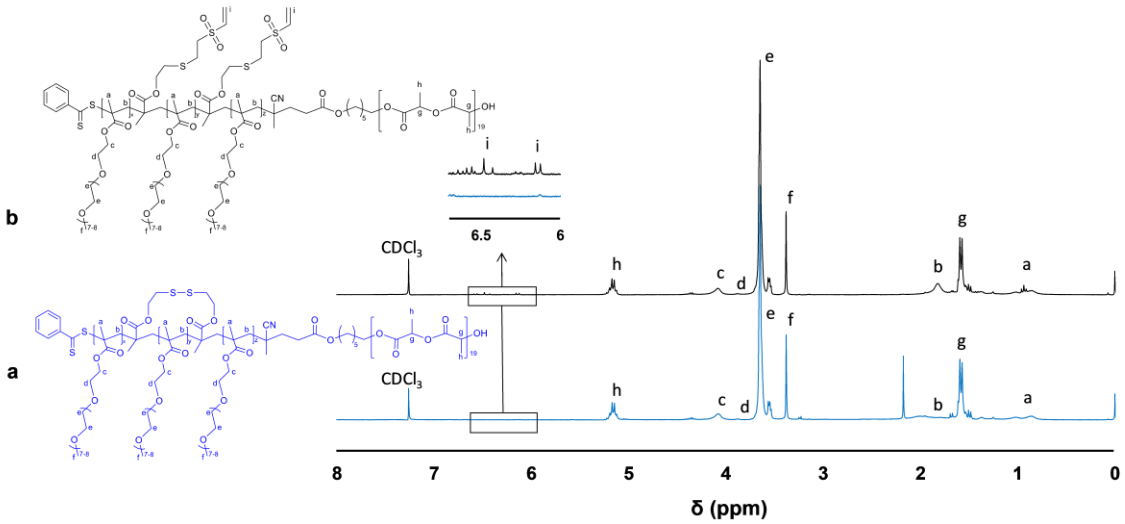


Figure S1. ^{1}H NMR ($CDCl_3$) spectra of (a) disulfide-functionalized branched amphiphilic PLA_{19}-*b*-P($OEGMA_{14}$-*stat*-$DSDMA_1$) and (b) vinyl-sulfone functionalized linear amphiphilic PLA_{19}-*b*-P($OEGMA_{14}$-*stat*-$VSTEMA_2$) diblock copolymers.

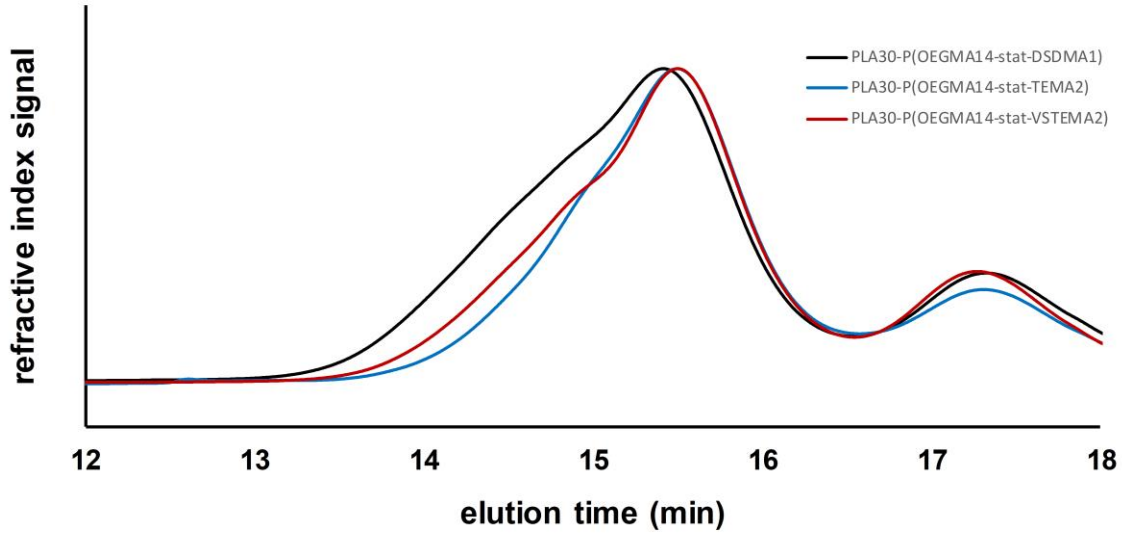


Figure S2. SEC (THF) chromatograms of disulfide-functionalized branched amphiphilic PLA_{19}-*b*-P($OEGMA_{14}$-*stat*-$DSDMA_1$), thiol-functionalized linear amphiphilic PLA_{19}-*b*-P($OEGMA_{14}$-*stat*-$TEMA_2$) and vinyl-sulfone functionalized linear amphiphilic PLA_{19}-*b*-P($OEGMA_{14}$-*stat*-$VSTEMA_2$) diblock copolymers.

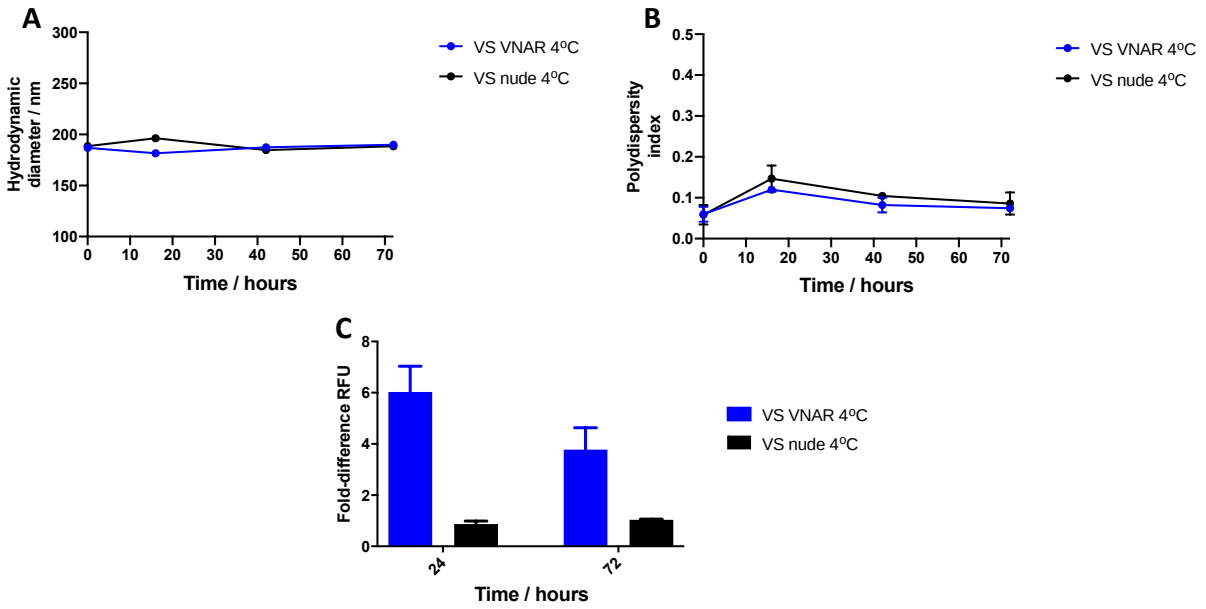


Figure S3. Assessment of VNAR-functionalized (VS VNAR) and vinyl-sulfone functionalized (VS nude) nanoparticle formulations stored in PBS buffer suspension at 4ºC between 0 and 72 h at predetermined intervals: (A) Hydrodynamic diameter by DLS, **(B)** polydispersity index value by DLS and **(C)** binding by fluorescent plate based binding assay, data presented as fold difference fluorescence between DLL4-Fc coated and uncoated wells at 24 and 72 h post VNAR conjugation.