Switching of macromolecular ligand display by thermoresponsive polymers mediates endocytosis of multi-conjugate nanoparticles.

Edward J Sayers[1], Johannes P Magnusson,[2] Paul R Moody,[1] Francesca Mastrotto,[2,3] Claudia Conte[2], Chiara Brazzale,[3] Paola Borri,[4] Paolo Caliceti,[3] Peter Watson,[4] Giuseppe Mantovani,[2] Jonathan Aylott,[2] Stefano Salmaso,[3] Arwyn T Jones[1] and Cameron Alexander[2].

[1]Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, Wales.

[2]School of Pharmacy, University of Nottingham, University Park, Nottingham, UK.

[3]Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy.

[4]School of Biosciences, Cardiff University, Cardiff, Wales.

Supporting Information

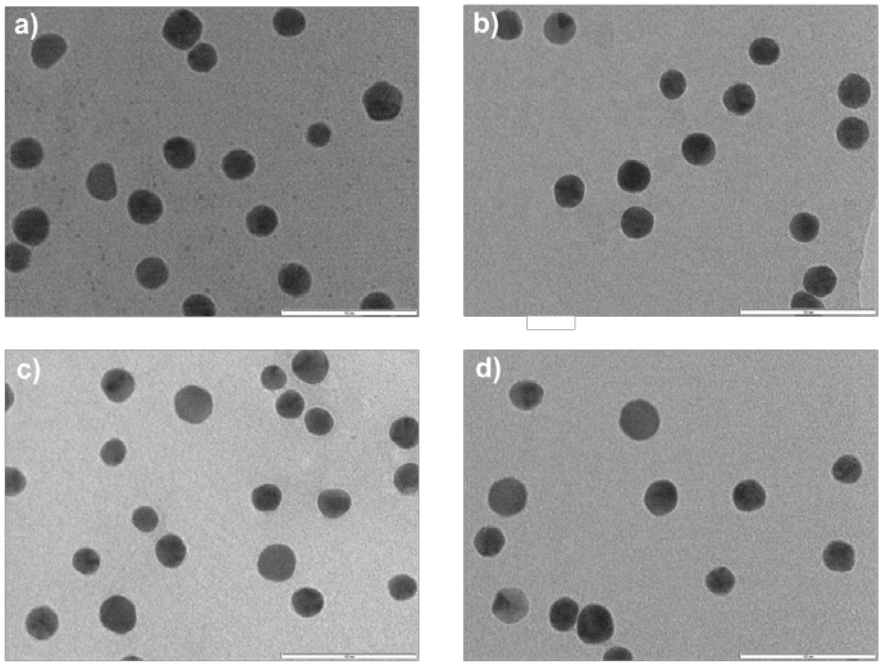

Figure S1 –TEM analysis of functionalized gold nanoparticles; a) Tf-AuNP-1, b) Tf-AuNP-2, c) pPEGMA-AuNP-1, d) pPEGMA-AuNP-3. Scale bar = 100 nm

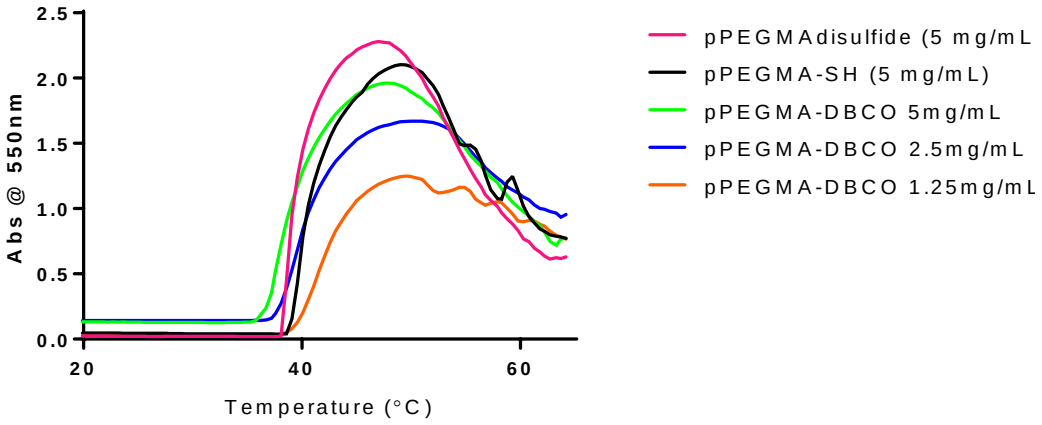

Figure S2 – Cloud point analysis of thermoresponsive polymers before reduction and after derivatization with DBCO. Transition onset: pPEGMA disulfide – 38.2 °C, pPEGMA-SH – 38.5 °C, pPEGMA – DBCO (5 mg/mL) = 35.8 °C, pPEGMA – DBCO (2.5 mg/mL) = 37.2 °C, pPEGMA – DBCO (1.25 mg/mL) = 38.9 °C.

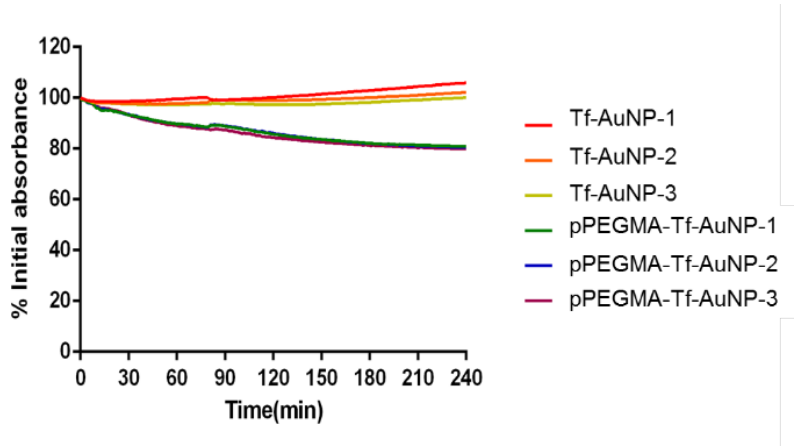

Figure S3 – Colloidal stability of nanoparticles in DMEM media.

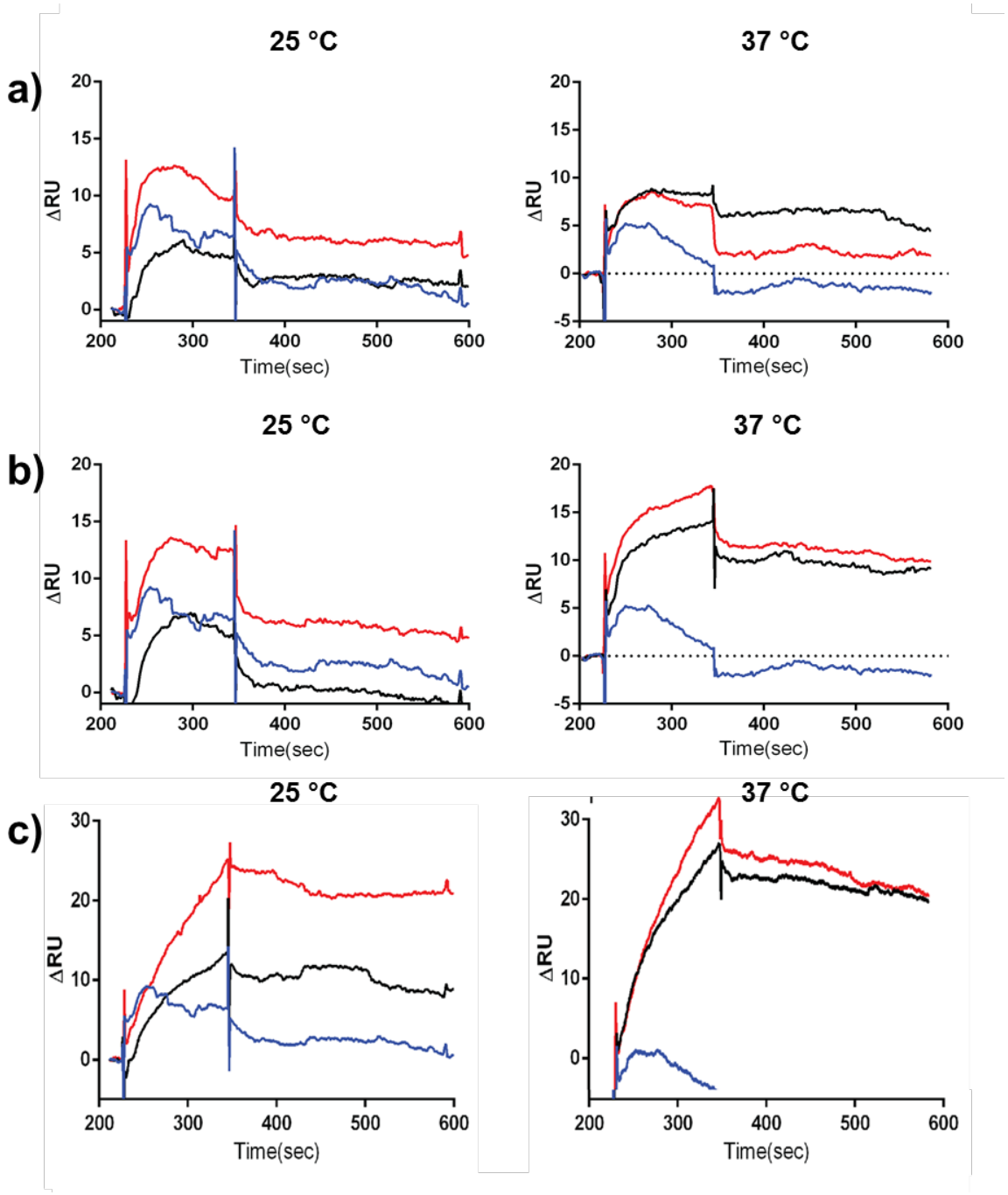

Figure S4: SPR affinity of nanoparticles batches towards transferrin receptor at 25 °C and 37 °C. In a) pMPC-AuNP (blue trace), Tf-AuNP- 1 (black trace) and pPEGMA-Tf-AuNP-1 (red trace); b) pMPC-AuNP (blue trace), Tf-AuNP- 2 (black trace) and pPEGMA-Tf-AuNP-2 (red trace); c) pMPC-AuNP (blue trace), Tf-AuNP- 3 (black trace) and pPEGMA-Tf-AuNP-3 (red trace).

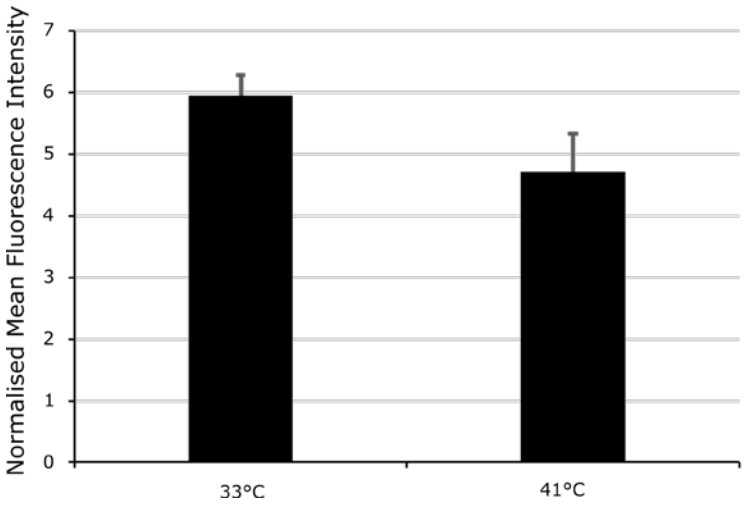

Figure S5 – Uptake of Tf-Alexa488 at 33°C and 41°C in HeLa cells.

HeLa cells were pulsed with 10nM Tf-A488 for 2hrs in serum free medium containing 0.1% BSA before washing and imaging. Uptake represents >100 cells from 10 individual images, error bars represent inter-image standard deviation.

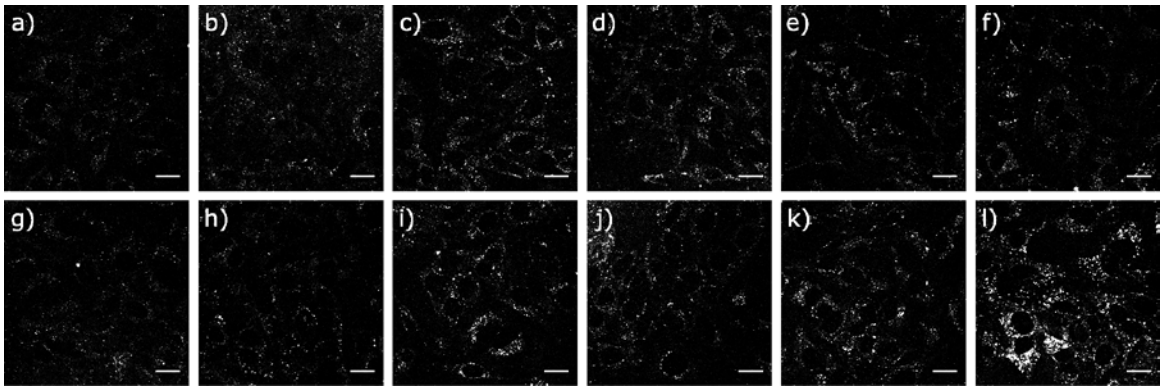

Figure S6: Non false-colored images from Figure 3 to show effect of temperature-on the endocytosis of AuNPs and conjugates in HeLa cells evaluated by confocal microscopy. Images (a-c) and (g-i) show Tf-AuNP-1, -2, -3 at 33 °C and 41 °C, respectively, while images (d-f) and (j-l) show corresponding polymer-coated particles pPEGMA-Tf-AuNP-1, -2, -3 across the same temperature ranges.

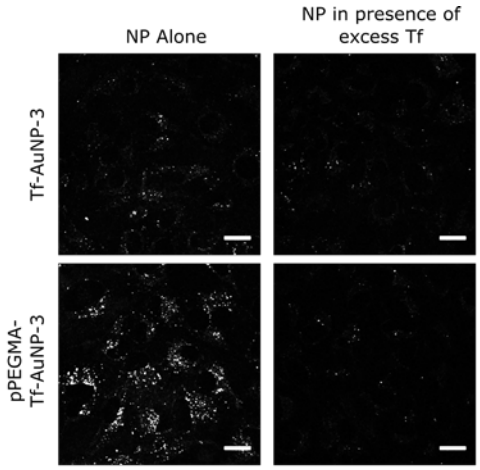

Figure S7: Non false-colored images from Figure 5 to show competition of Tf-AuNP-3 and pPEGMA-Tf-AuNP-3 with excess Tf. HeLa cells were incubated with either Tf-AuNP-3 or pPEGMA-Tf-AuNP-3 in the presence or absence of 625 nM Tf for 2 h in serum free medium containing 0.1% BSA at 41°C before washing and imaging.

Table S1 –Sizes of nanoparticles by light scattering analysis. The most abundant diameter given (i.e. size at peak height in the number distribution ± 3 nm), with most significant sub-population in brackets.

Nanoparticle	Diameter/ nm, 33 °C	Diameter/ nm, 37 °C	Diameter/ nm, 41 °C
Tf-AuNP-1	48	51	342 (60)
Tf-AuNP-2	50	50	60
Tf-AuNP-3	49	50, (80)	530 (80)
pPEGMA-Tf-AuNP-1	55	58, (91)	460 (80)
pPEGMA-Tf-AuNP-2	57	61, (104)	700 (70)
pPEGMA-Tf-AuNP-3	59	56	63 (450)

Table S2 – Zeta potentials of nanoparticle

Nanoparticle	ζ / mV, 33 °C	ζ / mV, 37 °C	ζ / mV, 41 °C
Tf-AuNP-1	-8.6 ± 1.7	-15.2 ± 4.3	-13.1 ± 1.7
Tf-AuNP-2	-7.8 ± 0.4	-7.0 ± 1.4	-18 ± 2.4
Tf-AuNP-3	-6.0 ± 0.3	-17.6 ± 1.2	-18.5 ± 0.1
pPEGMA-Tf-AuNP-1	-6.1 ± 0.2	-13.8 ± 3.2	-19 ± 0.4
pPEGMA-Tf-AuNP-2	-5.9 ± 0.3	-18.0 ± 3.8	-17.4 ± 0.8
pPEGMA-Tf-AuNP-3	-7.9 ± 1.5	-9.0 ± 1.5	-11.3 ± 0.5

Table S3

Polymer	mM thiol [a]	mM thiol (theor) [b]
pPEGMAdisulfide	0.0069	2.39
pPEGMA-SH	2.3	1.64
pPEGMA-DBCO	0.061	0.98

Ellman's assay for disulfide, reduced and modified polymer solutions. a) Free thiol concentration measured by Ellman's assay. b) Theoretical concentration of free thiol in solution

if polymers were fully reduced and non-modified. Polymer concentrations were calculated from M_n GPC.

Table S4. Transferrin AuNP - conjugation. Tf degree of conjugation and particle recovery.

ample	Conditions	TF grafting (no. TF/AuNP)	Concentration (AuNP/mL)	% AuNP recovery
Tf-AuNP-1	2 eq. Tf/AuNP	0.7	1.33×10^{14}	99.5%
Tf-AuNP-2	4 eq. Tf/AuNP	1.3	1.23×10^{14}	92.1%
Tf-AuNP-3	8 eq. Tf/AuNP	3.0	1.27×10^{14}	94.9%