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Supplementary information

Functional Magnetic Nanoparticles for Protein Delivery Applications: Understanding Protein-Nanoparticle interactions

Rajat Sharma, Daniel Ungar, Edward Dyson, Stephen Rimmer and Victor Chechik

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Fig. S7.1.2. Effect of IONP diameter on the heat-triggered TRF release: Western blot analysis (**i-ii**) of TRF release in the presence of 10 mg/ml RNaseB after incubation for 1 h at the indicated temperature using 7 nm (**i**) and 11 nm IONPs (**ii**) coated with 19 kDa PNIPMAM.

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Fig. S10.2.2. Western blot analysis following 10 mg/ml BSA treatment of 0.5 mg 19 kDa PNIPMAM @ 16 nm IONPs incubated with 1µg IgG at pH 7.4 for 30 and 60 min at 37 °C and 45 °C.

Fig. S11.1.1. Reactivity of the anti-human TRF antibody against TRF orthologs found in various sera: fetal bovine serum (FBS), goat serum and pig serum were screened with anti-human TRF primary and HRP conjugated goat anti rabbit secondary antibodies for TRF detection. 10 µL of the 10 % FBS, pig serum and goat serum are loaded with various amounts of pure TRF.

Fig. S11.1.2. Specificity of the IgG antigens present in various serums (10 %) to the goat anti bovine IgG antibody: fetal bovine serum (FBS), goat serum and pig serum. 10 µL of the 10 % FBS, pig serum and goat serum are loaded with various amounts of pure bovine IgG.

Fig. S11.2.1. Western blot analysis following 10 % goat serum treatment of 0.5 mg 19 kDa PNIPMAM @ 16 nm IONPs incubated with 1µg IgG at pH 7.4 for 30 and 60 min at 37 °C and 45 °C, respectively.

Fig. S11.2.2. Magnetic heating triggered IgG release from PNIPMAM @ IONPs at 37 °C: Western blot analysis following 10 % goat serum treatment of 0.5 mg 19 kDa PNIPMAM @ 16 nm IONPs incubated with 1µg IgG at pH 7.4 for for 0, 10, 20 and 30 min at 37 °C with and without magnetic heating (10 sec on / 30 sec off).

Fig. S12.1. Western blot analysis of TRF (25 ng) under reducing conditions following incubation at 45 °C for at different times and centrifugation. Denaturated protein would be removed during centrifugation; no change in band intensity suggests lack of denaturation.

Fig. S12.2. Western blot analysis of IgG (50 ng) under reducing conditions following incubation at 45 °C for at different times and centrifugation. Under these conditions, IgG (160 kDa) separates to 2 bands: heavy (50 kDa) and light (25 kDa). Denaturated protein would be removed during centrifugation; no change in band intensity suggests lack of denaturation.

Table S13.1. Conditions to make different w/v (%) SDS-PAGE gels.

S1. PNIPMAM to NDA-PNIPMAM: MALDI-MS

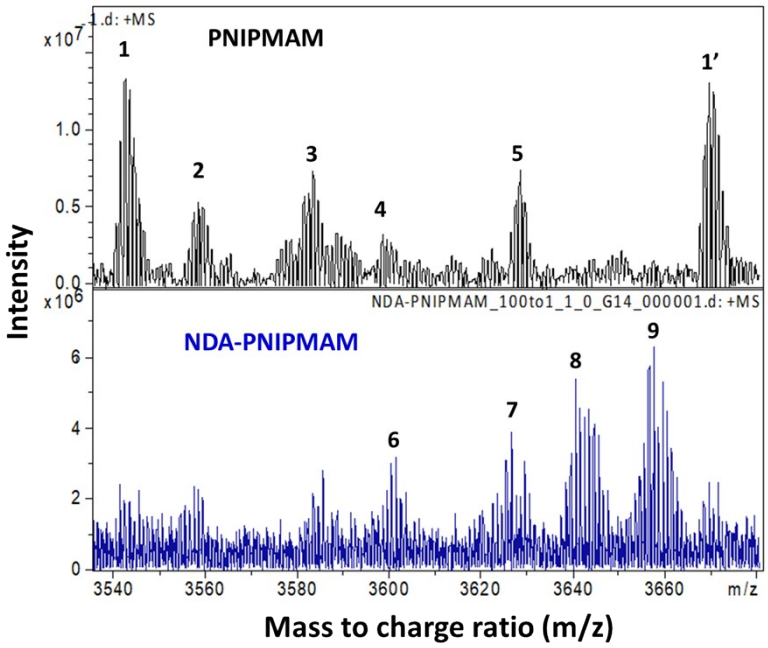


Fig. S1.1. MALDI-MS spectrum of PNIPMAM showing a set of 4 peaks replaced by a set of 4 new peaks in NDA-PNIPMAM confirmed the successful end group functionalization of PNIPMAM with NDA.

S2. Standard curve of 6-nitrodopamine (NDA)

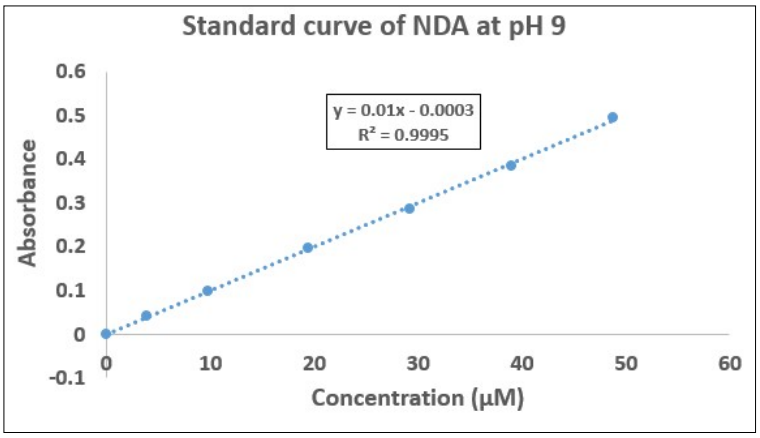
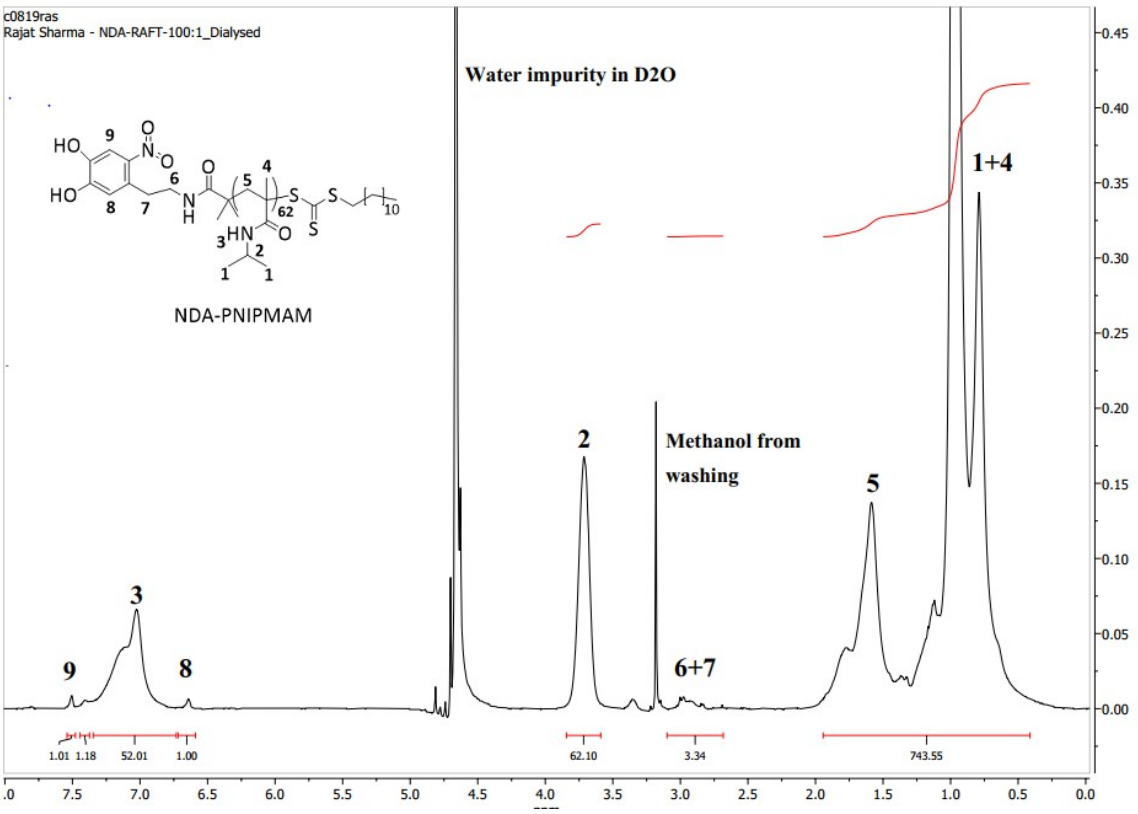


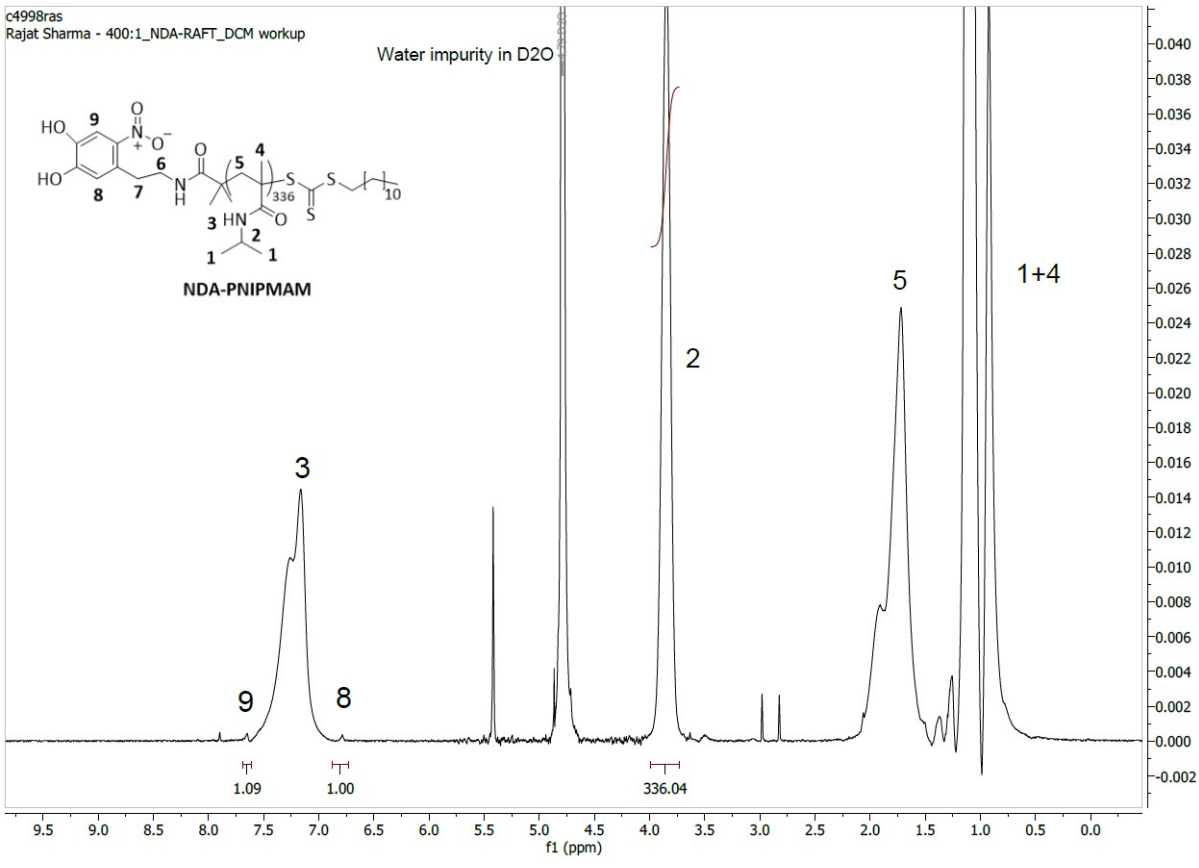
Fig. S2.1. Standard curve of NDA at pH 9 used to calculate the molar extiction coefficient of NDA = 9600 $mol^{-1}cm^{-1}$.

S3. ^{1}H-NMR of NDA-PNIPMAM

4.7 kDa



19 kDa



86 kDa

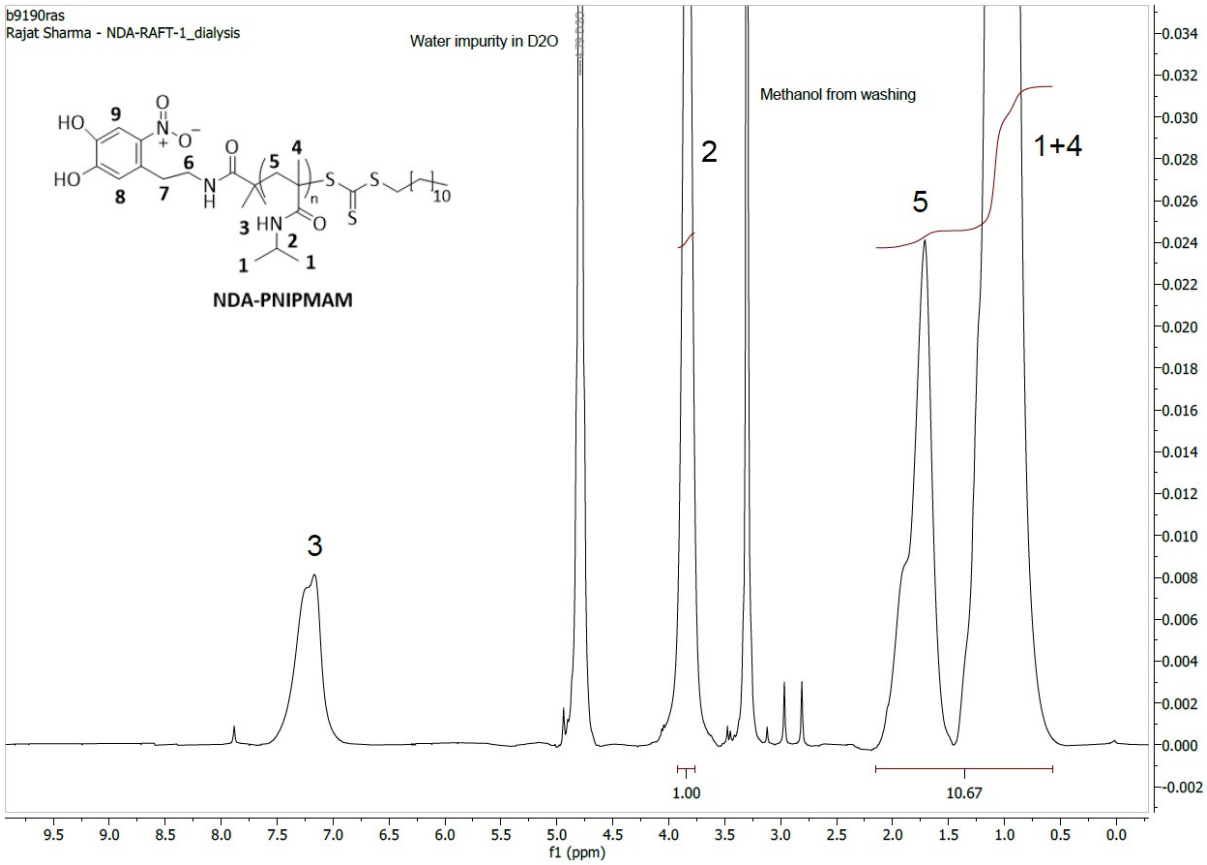


Fig. S3.1. ^{1}H-NMR of NDA-PNIPMAM in D_2O.

S4. TEM of NDA-PNIPMAM-coated IONPs

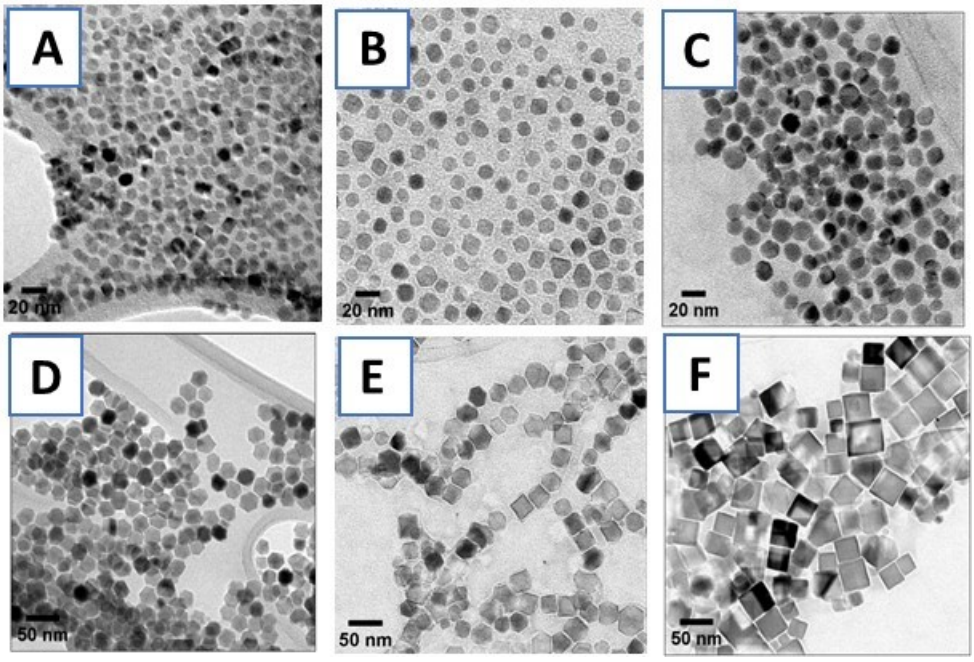


Fig. S4.1. TEM images of IONPs coated with 19 kDa PNIPMAM: A) 7.3 ± 1.4 nm, B) 11.0 ± 2.0 nm, C) 15.4 ± 2.1 nm, D) 19.1 ± 2.3 nm nano-octahedrals, E) 27.4 ± 3.6 nm and F) 33.4 ± 4.9 nm nanocubes (± denotes standard deviation, n ≥ 100).

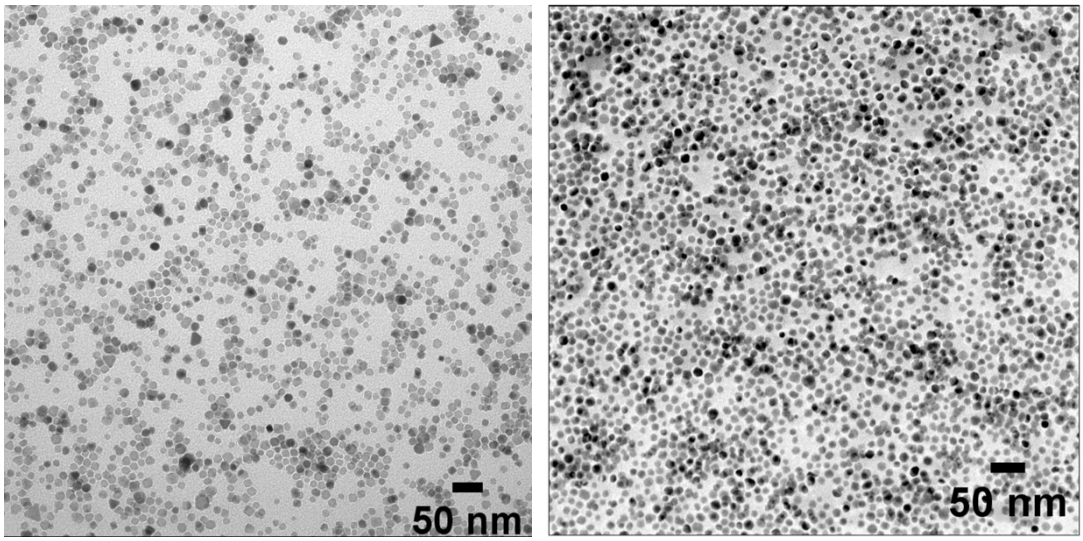


Fig. S4.2. TEM images of 16 nm IONPs coated with 19 kDa PNIPMAM after storage in physiological buffer for 1 week (left) and 6 months (right).

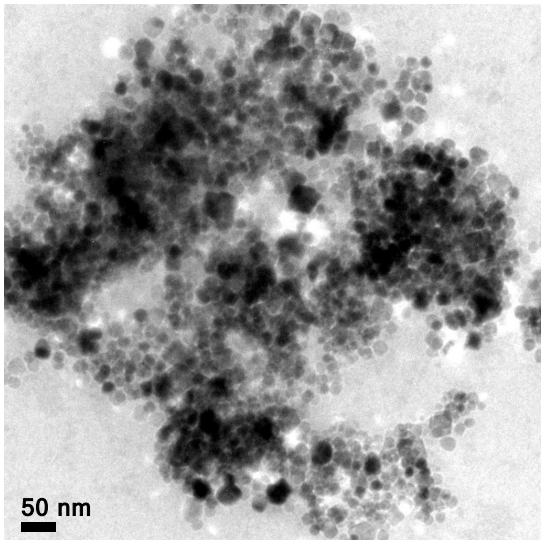


Fig. S4.3. TEM images of 16 nm IONPs coated with 19 kDa PNIPMAM loaded with TRF protein and stored in physiological buffer for 1 week.

S5. DLS data for IONPs

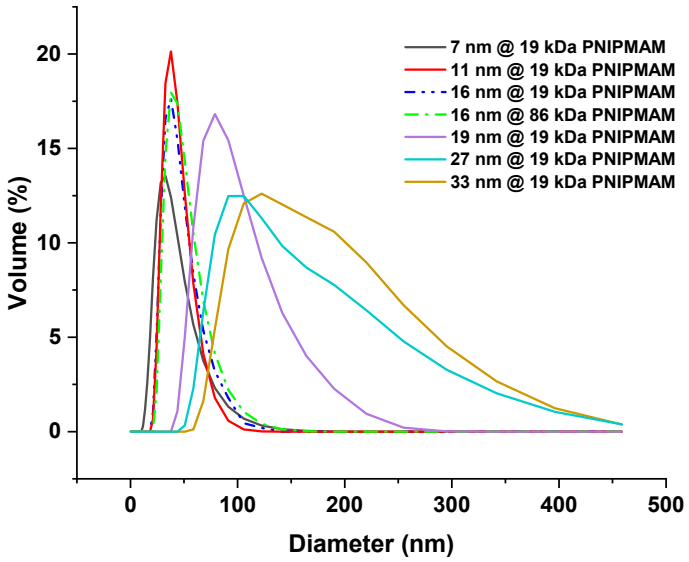


Fig. S5.1. DLS data for PNIPMAM @ IONPs.

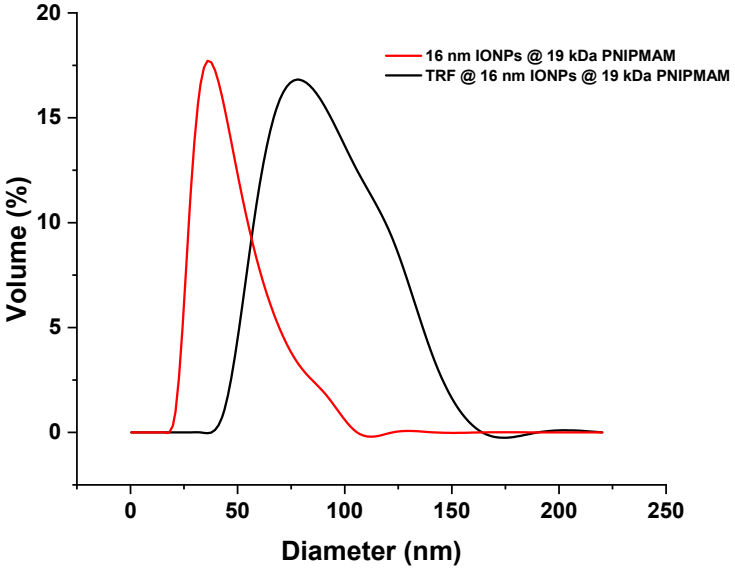


Fig. S5.2. DLS data for 19 kDa PNIPMAM @ 16 nm IONPs before and after protein encapsulation (TRF).

S6. Magnetic heating data for citric acid coated IONPs

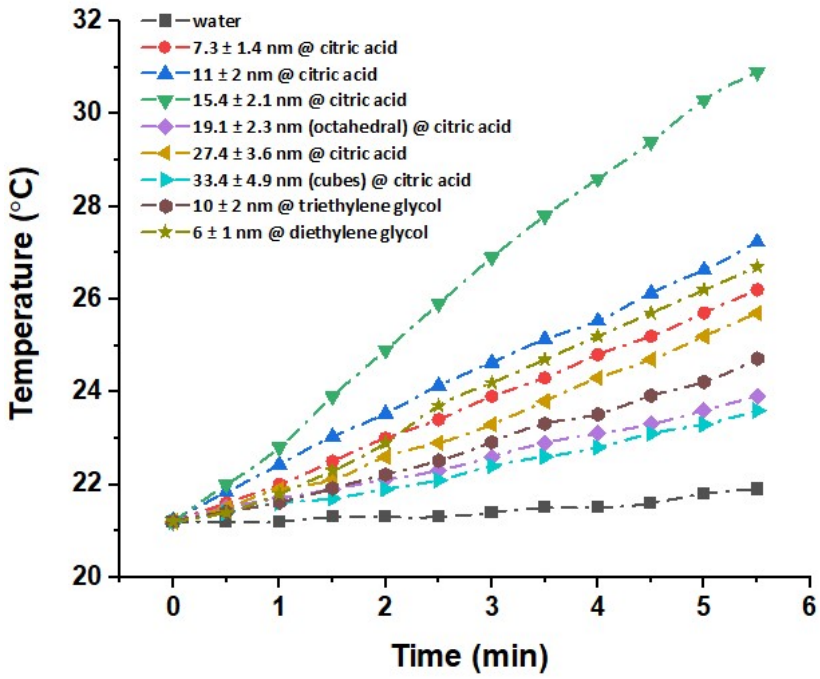


Fig. S6.1. Heating curve analysis of different size and shaped water dispersible IONPs. Effect of different ligands on NPs surface on their magnetic heating behaviour is also reported here (polyols and citric acid). Measurement conditions: 0.45 ml of 10 mg/ml [Fe], AMF strength of 28.7 mT and frequency of 102.4 kHz).

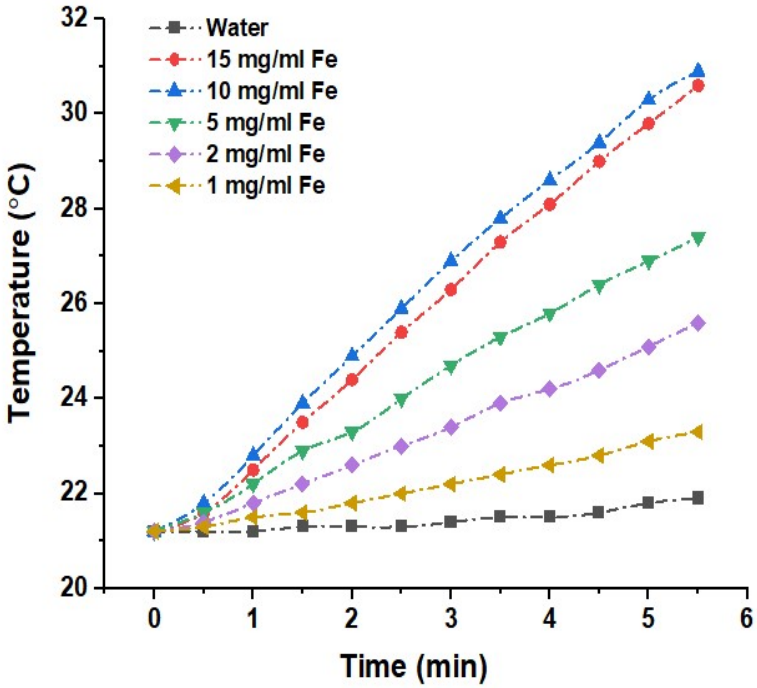


Fig. S6.2. Concentration dependence on the magnetic heating of IONPs: Different concentrations of 16 nm citric acid @ IONPs, AMF strength of 28.7 mT and frequency of 102.4 kHz.

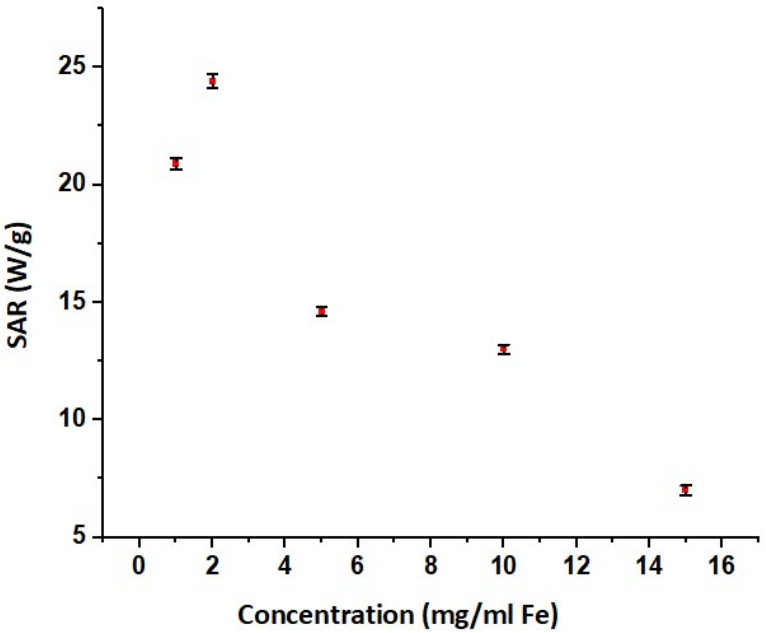


Fig. S6.3. Concentration dependence on the SAR values of 16 nm citric acid @ IONPs (AMF strength = 28.7 mT and frequency = 102.4 kHz). (n = 3, error bars denote standard error).

S7. Represented blots for the TRF loading/release with different competitor proteins

S7.1. Effect of the polymer chain length on the TRF release

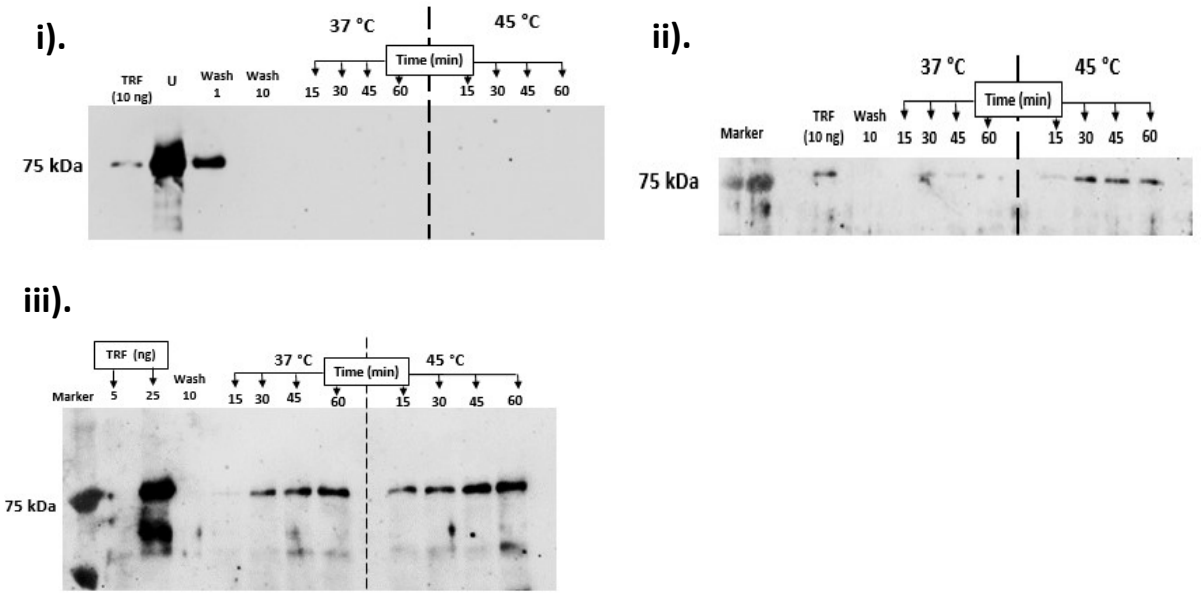


Fig. S7.1.1. Effect of **i-iii)** polymer chain length on heat-triggered TRF release: Western blot analysis (**i-ii**) of TRF release in the presence of 10 mg/ml RNaseB after incubation for 1 h at the indicated temperature using 16 nm IONPs and 4.7 kDa (**i**), 19 kDa (**ii**), and 86 kDa (**v**) polymer.

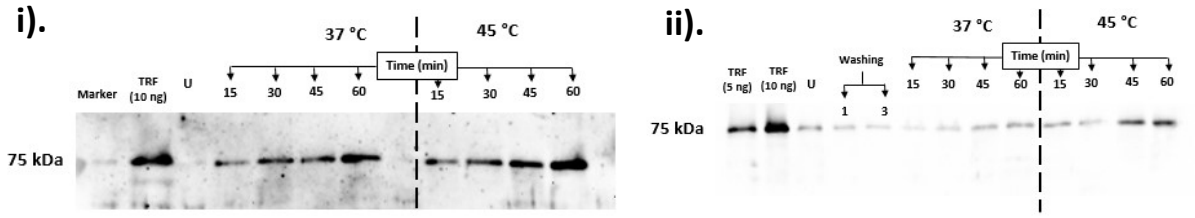


Fig. S7.1.2. Effect of IONP diameter on the heat-triggered TRF release: Western blot analysis (**i-ii**) of TRF release in the presence of 10 mg/ml RNaseB after incubation for 1 h at the indicated temperature using 7 nm (**i**) and 11 nm IONPs (**ii**) coated with 19 kDa PNIPMAM.

S7.2. Glycosylated proteins – RNaseB, OVL and IgG

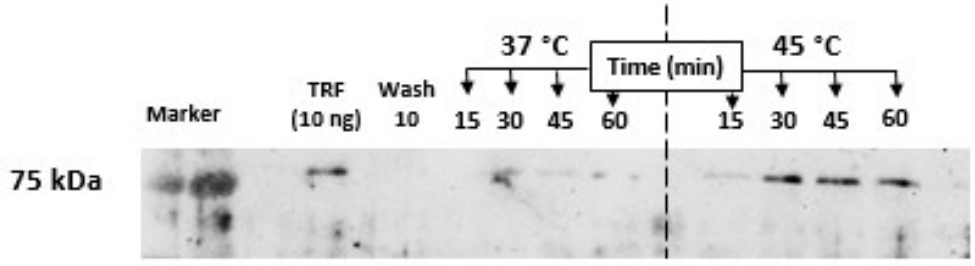


Fig. S7.2.1. Western blot analysis following 10 mg/ml RNaseB treatment of 0.5 mg 19 kDa PNIPMAM @ 11 nm IONPs incubated with 1µg TRF at pH 7.4 for 15, 30, 45 and 60 min at 37 °C and 45 °C.

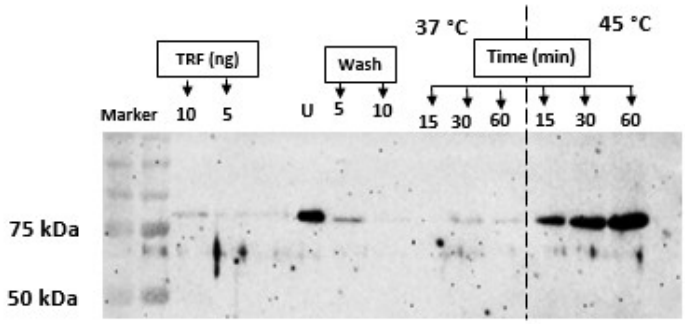


Fig. S7.2.2. Western blot analysis following 10 mg/ml OVL treatment of 0.5 mg 19 kDa PNIPMAM @ 16 nm IONPs incubated with 1µg TRF at pH 7.4 for 15, 30 and 60 min at 37 °C and 45 °C.

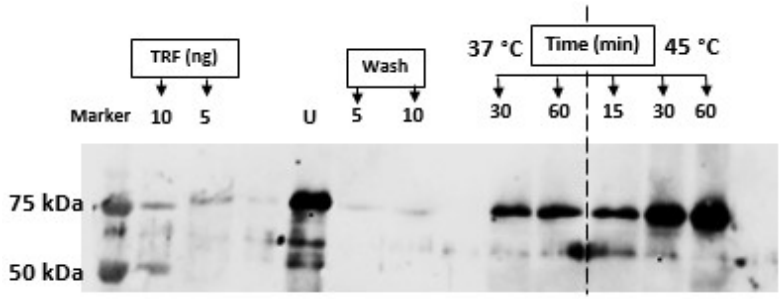


Fig. S7.2.3. Western blot analysis following 10 mg/ml IgG treatment of 0.5 mg 19 kDa PNIPMAM @ 16 nm IONPs incubated with 1µg TRF at pH 7.4 for 15, 30 and 60 min at 37 °C and 45 °C. For washings, OVL (10 mg/ml) was used as a competitor (9 washes) followed by buffer (20 mM HEPES, 100 mM NaCl, pH 7.4) washes (3).

S7.3. Non-glycosylated proteins- RNaseA and BSA

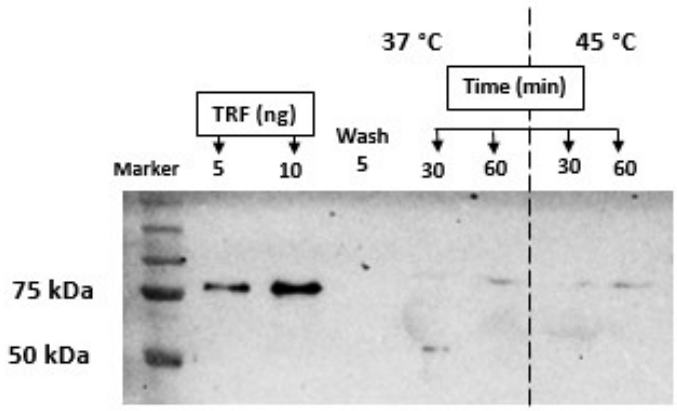


Fig. S7.3.1. Western blot analysis following 10 mg/ml RNaseA treatment of 0.5 mg 19 kDa PNIPMAM @ 16 nm IONPs incubated with 1µg TRF at pH 7.4 for 30 and 60 min at 37 °C and 45 °C.

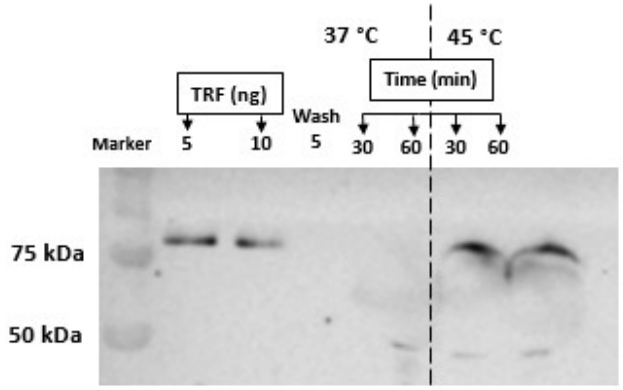


Fig. S7.3.2. Western blot analysis following 10 mg/ml BSA treatment of 0.5 mg 19 kDa PNIPMAM @ 16 nm IONPs incubated with 1µg TRF at pH 7.4 for 30 and 60 min at 37 °C and 45 °C.

S8. Sugars as competitor

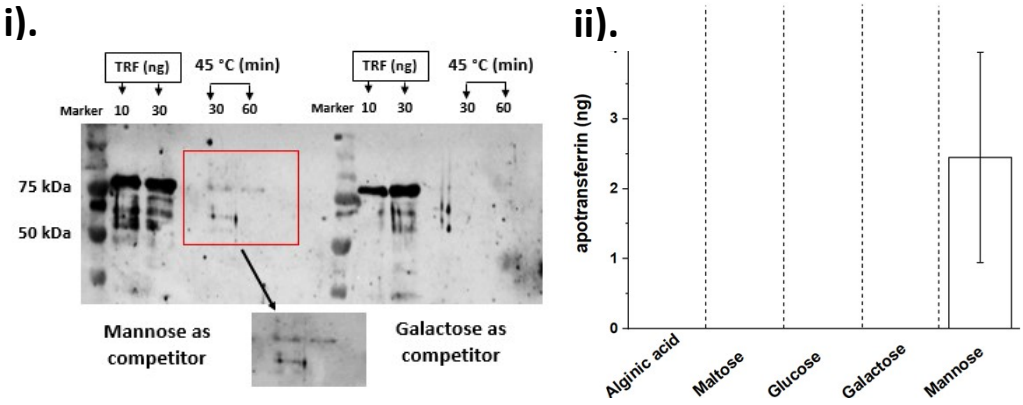


Fig. S8.1. Studying saccharides-NPs interactions: i) Western blot analysis for the triggered TRF release from PNIPMAM @ IONPs in a temperature-dependent manner with mannose and galactose and ii) quantification of the TRF release with various sugars as competitor.

S9. Standard curve used to quantify GFP

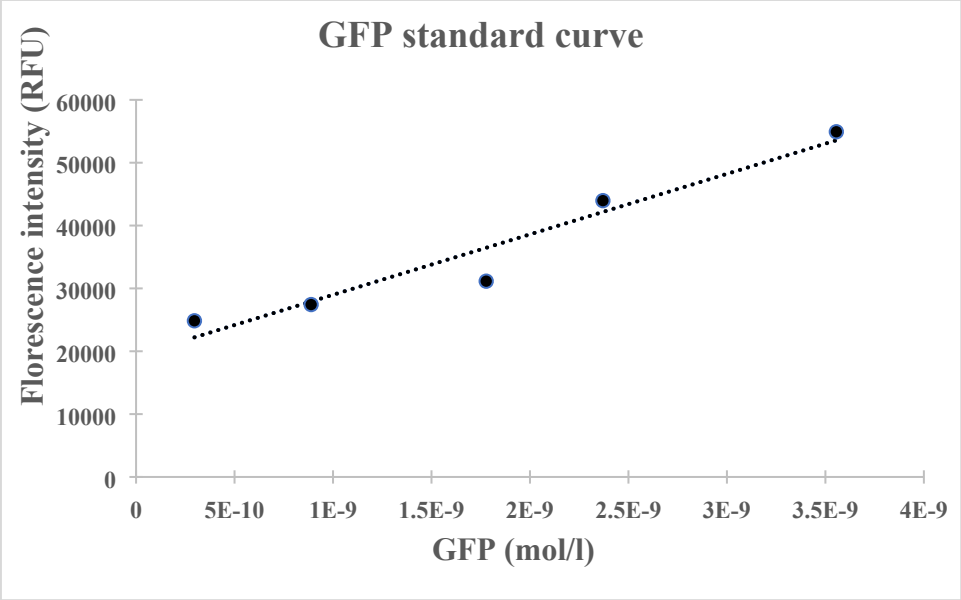


Fig. S9.1. Standard GFP curve used for the analysis of GFP loading/release samples.

S10. IgG release with different competitors

S10.1. Glycosylated proteins – RNaseB, OVL and TRF

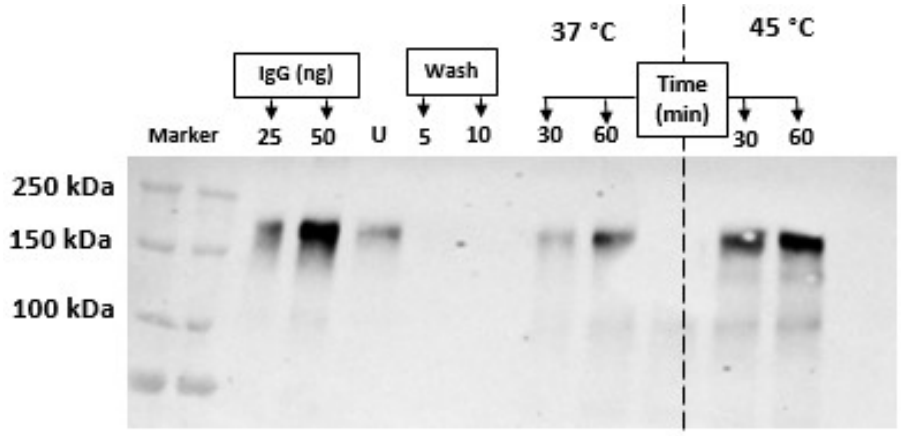


Fig. S10.1.1. Western blot analysis following 10 mg/ml RNaseB treatment of 0.5 mg 19 kDa PNIPMAM @ 16 nm IONPs incubated with 1µg IgG at pH 7.4 for 30 and 60 min at 37 °C and 45 °C.

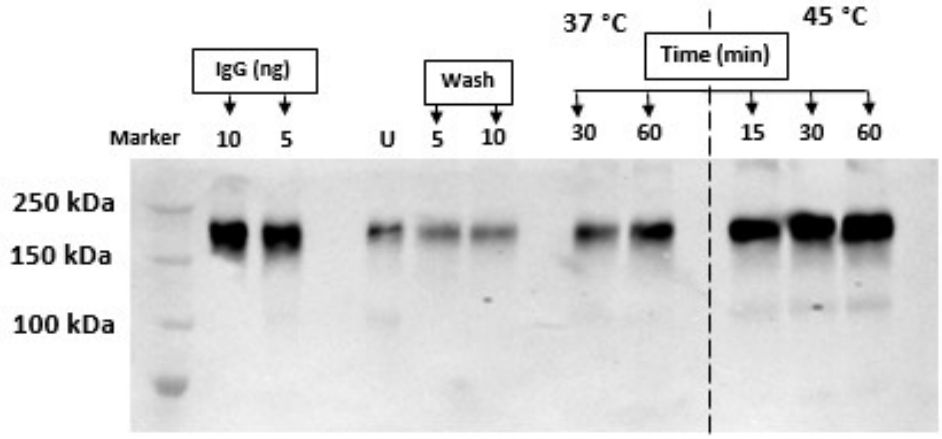


Fig. S10.1.2. Western blot analysis following 10 mg/ml OVL treatment of 0.5 mg 19 kDa PNIPMAM @ 16 nm IONPs incubated with 1µg IgG at pH 7.4 for 30 and 60 min at 37 °C and 45 °C.

S10.2. Non-glycosylated proteins – RNaseA and BSA

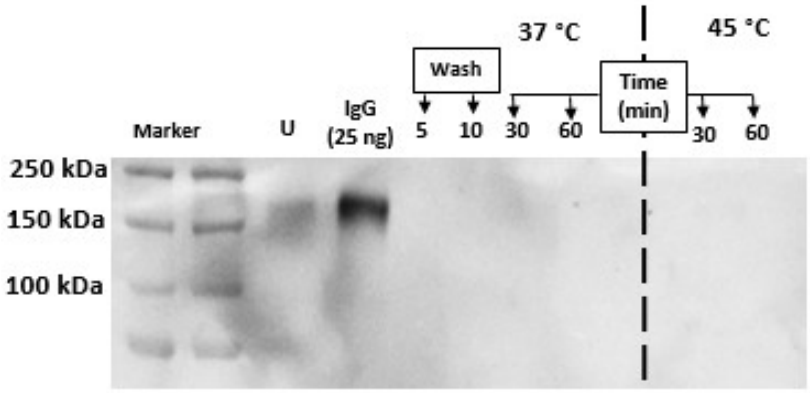


Fig. S10.2.1. Western blot analysis following 10 mg/ml RNaseA treatment of 0.5 mg 19 kDa PNIPMAM @ 16 nm IONPs incubated with 1µg IgG at pH 7.4 for 30 and 60 min at 45 °C.

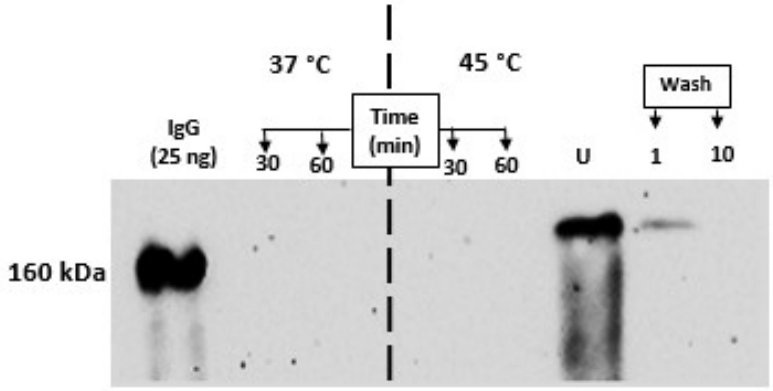


Fig. S10.2.2. Western blot analysis following 10 mg/ml BSA treatment of 0.5 mg 19 kDa PNIPMAM @ 16 nm IONPs incubated with 1µg IgG at pH 7.4 for 30 and 60 min at 37 °C and 45 °C.

S11. Serum as a competitor

S11.1. Reactivity of the orthologs of the guest proteins (TRF and IgG) present in various serums (bovine, goat and pig serum)

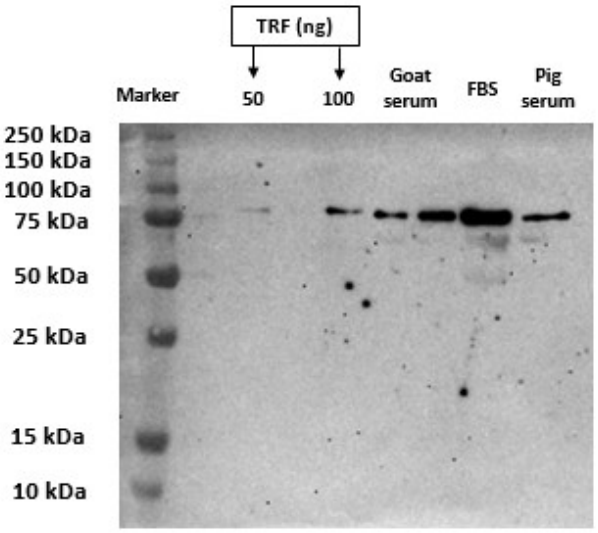


Fig. S11.1.1. Reactivity of the anti-human TRF antibody against TRF orthologs found in various sera: fetal bovine serum (FBS), goat serum and pig serum were screened with anti-human TRF primary and HRP conjugated goat anti rabbit secondary antibodies for TRF detection. 10 µL of the 10 % FBS, pig serum and goat serum are loaded with various amounts of pure TRF.

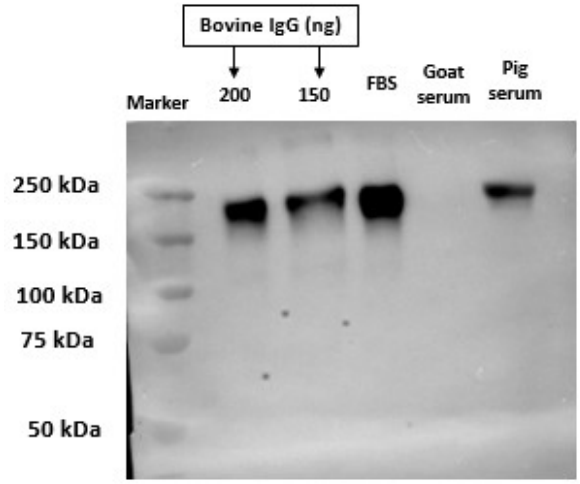


Fig. S11.1.2. Specificity of the IgG antigens present in various serums (10 %) to the goat anti bovine IgG antibody: fetal bovine serum (FBS), goat serum and pig serum. 10 µL of the 10 % FBS, pig serum and goat serum are loaded with various amounts of pure bovine IgG.

S11.2. IgG release with goat serum as a competitor

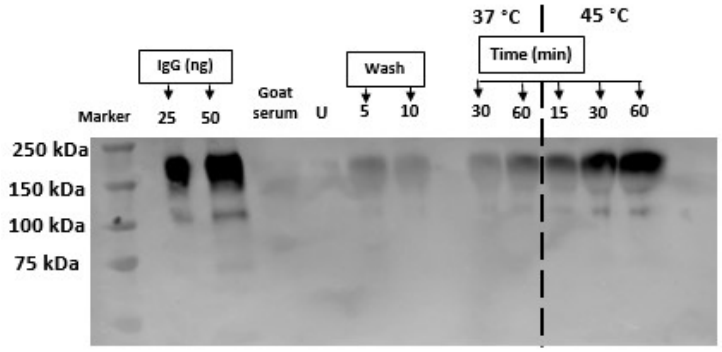


Fig. S11.2.1. Western blot analysis following 10 % goat serum treatment of 0.5 mg 19 kDa PNIPMAM @ 16 nm IONPs incubated with 1µg IgG at pH 7.4 for 30 and 60 min at 37 °C and 45 °C, respectively.

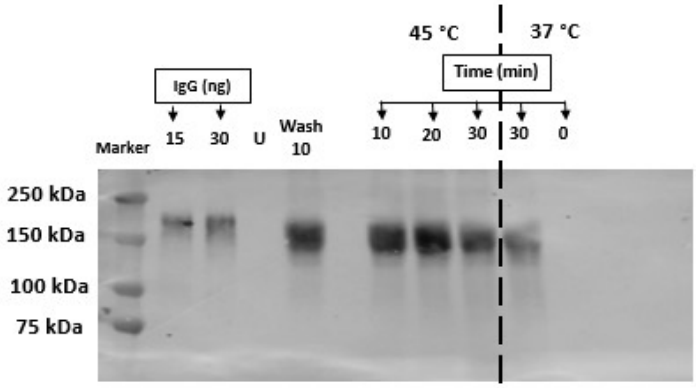
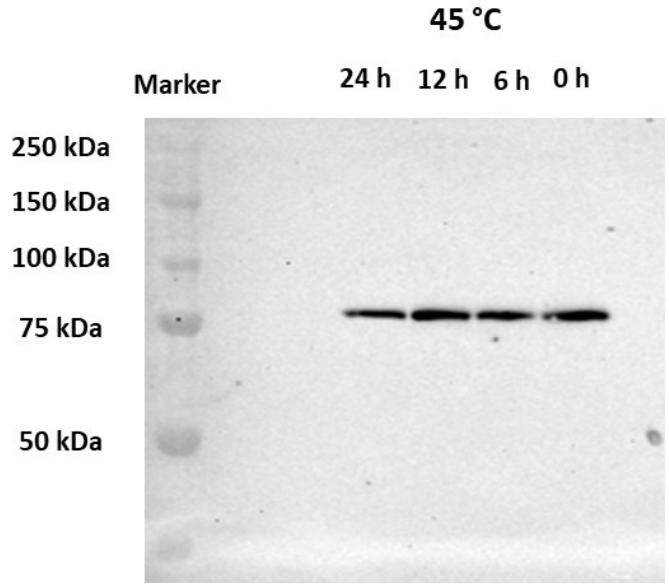


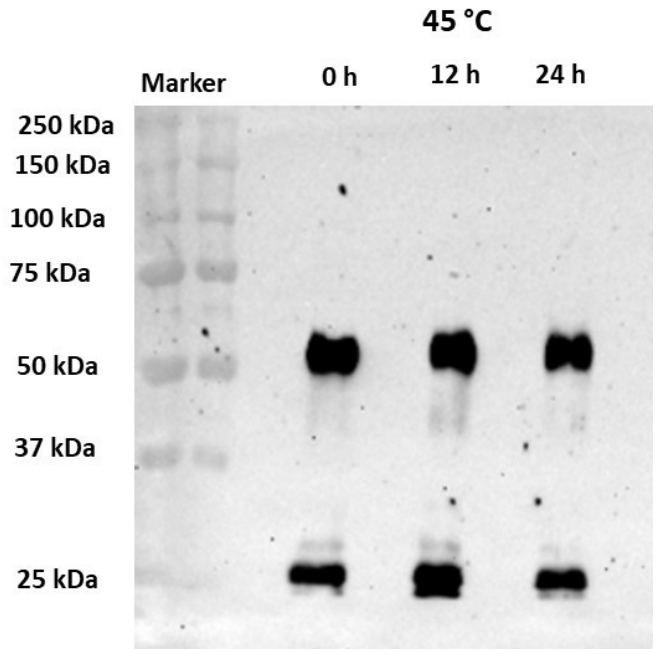
Fig. S11.2.2. Magnetic heating triggered IgG release from PNIPMAM @ IONPs at 37 °C: Western blot analysis following 10 % goat serum treatment of 0.5 mg 19 kDa PNIPMAM @ 16 nm IONPs incubated with 1µg IgG at pH 7.4 for for 0, 10, 20 and 30 min at 37 °C with and without magnetic heating (10 sec on / 30 sec off).

S12. Protein denaturation at 45 °C



Bands	Protein (ng)
0 h	30
6 h	28.7
12 h	29.4
24 h	28.9

Fig. S12.1. Western blot analysis of TRF (25 ng) under reducing conditions following incubation at 45 °C for different times followed by centrifugation. Denaturated protein would be expected to aggregate and therefore be removed during centrifugation; no change in band intensity suggests lack of denaturation.



Bands	Protein (ng)
0 h (top)	50
0 h (bottom)	50
12 h (top)	47.8
12 h (bottom)	49.3
24 h (top)	47.4
24 h (bottom)	48.6

Fig. S12.2. Western blot analysis of IgG (50 ng) under reducing conditions following incubation at 45 °C for different times followed by centrifugation. Under these conditions, IgG (160 kDa) separates to 2 bands: heavy (50 kDa) and light (25 kDa). Denaturated protein would be expected to aggregate and therefore will be removed during centrifugation; no change in band intensity suggests lack of denaturation.

S13. SDS-PAGE gels

Table S13.1. Conditions to make different w/v (%) SDS-PAGE gels.

Percentage	**Separating gel (w/v)**				**Stacking Gel 4 (w/v)**
	7.5 (50-250 kDa)	**10 (30-150 kDa)**	**12 (20-100 kDa)**	**15 (10-75 kDa)**	
H_2O (ml)	6	5.1	4.2	3	2.7
Resolving Buffer (ml)	3	3	3	3	1.125
30 % Acrylamide (ml)	3	3.9	4.8	6	0.6
10% APS (µl)	100	100	100	100	40
TEMED (µl)	20	20	20	20	10