Supporting Information

Assessment of DNA Complexation onto Polyelectrolyte-coated Magnetic Silica Nanoparticles

Ana B. Dávila-Ibáñez,[1] Niklaas J. Buurma,[2]* Verónica Salgueiriño,[1]*

[1]*Departamento de Física Aplicada, Universidade de Vigo, 36310, Vigo (Spain)*
[2]*Physical Organic Chemistry Centre, School of Chemistry, University of Cardiff, Cardiff (UK)*

* Buurma@Cardiff.ac.uk; * vsalgue@uvigo.es

Experimental

Chemicals. Iron pentacarbonyl ($Fe(CO)_5$), octyl ether, oleic acid, poly(5)oxyethylene-4-nonylphenyl-ether (NP-5), poly (sodium 4-styrenesulfonate) (PSS) (Mw ≈ 70,000), poly (diallyldimethylammonium chloride) (PDADMAC) (Mw < 200,000), ammonium hydroxide solution (NH_4OH (28-30% NH_3)) and sodium chloride were supplied by Sigma-Aldrich. Chloroform was supplied by Merck. Calf thymus (CT DNA) was supplied by Sigma-Aldrich and fish sperm (FS DNA) DNA was from Acros. All chemicals were used as received.

Synthesis of Fe_3O_4 nanocrystals. 6.6 ± 0.7 nm Fe_3O_4 nanocrystals were synthesized as follows;[1] 0.4 mL of $Fe(CO)_5$ (3.04 mmol) were injected into a mixture containing 20 mL of octyl ether and 1.72 mL of oleic acid (5.42 mmol) at 100 °C (previously deoxygenated using N_2 flow). The resulting mixture was slowly heated up to 280-290 ºC and left to reflux for 2h, now under open-air atmosphere. A change of color from yellow to black was observed. After that, the solution was cooled to room temperature under continuous magnetic stirring and later treated with an excess of ethanol. The nanoparticles formed were washed and separated by centrifugation at least three times, and finally dispersed in 20 mL of hexane with oleic acid (20 μL).

Silica Coating. For incorporation in silica, Fe_3O_4 nanocrystals were first transferred to cyclohexane. For a typical reverse microemulsion synthesis,[2] 2.6 mL of NP-5 was

dispersed in 18 mL of cyclohexane and stirred for 15 min (850 rpm). Subsequently, 100 mL of Fe_3O_4 nanocrystals were added, after which different volumes (20-50 mL, depending on the final silica shell thickness) of TEOS (dispersed in 2 mL of cyclohexane) and 580 mL of ammonium hydroxide solution were added. Between the additions, the reaction mixture was stirred for 15 min. After the last step, the mixture was stirred for 1 min and stored at room temperature for 48 h. The silica-coated magnetite nanoparticles were washed twice adding 10 mL of ethanol to the reaction mixture, were centrifuged and finally redispersed in water.

Surface modification (precursor multilayer polyelectrolyte film). One layer of polyelectrolyte or a three-layer polyelectrolyte film (PE_3) was deposited by the adsorption of PDADMAC in the first case, or alternating PDADMAC, PSS and PDADMAC onto the silica-coated magnetite nanoparticles as indicated elsewhere,[3] in such a way that the subsequent layers of deposited polymer have opposite charge, hence layers are predominantly adsorbing through electrostatic interactions. This one or these three layers of polyelectrolytes produce a smoother, more uniform and positively charged surface onto the nanoparticles, compared to the initial negatively charged silica surface without any modification.

DNA Complexation. Since the properties of the magnetoplexes prepared by adding DNA to the nanoparticles differ from those prepared by adding nanoparticles to the DNA, we have performed both procedures; adding aliquots of stock solutions of DNA (with a concentration of 0.072 g/L = 0.1108 mM in terms of base pairs, prepared two days in advance, in water) to 2 mL of a solution of the PE_1- or PE_3-functionalized silica-coated magnetite nanoparticles (using different concentrations of nanoparticles) and, vice versa, by adding aliquots (typically 20 to 60 μL) of the positively charged nanoparticles to 2 mL of 0.0277 mM DNA (FS or CT DNA, concentration in terms of base pairs) solutions, prepared diluting from the stock solutions. Samples were mechanically stirred throughout the complexation experiments.

Characterization. TEM measurements were performed on a Philips CM12 instrument operating at an acceleration voltage of 120 kV. Samples for TEM were prepared by placing a drop of the dispersions on a Cu grid letting the liquid evaporate at room temperature. To study their magnetic properties using a superconducting quantum interference device (SQUID magnetometry) the magnetic nanoparticles (before and after being coated with silica) were precipitated and the dried samples were measured independently. Laser Doppler electrophoresis (Zetamaster 2000, Malvern Instruments

Ltd.) was used to measure electrophoretic mobilities. UV-visible spectra were recorded over the range 200-600 nm using a JASCO V630 UV-visible spectrophotometer with a Peltier thermostating unit and a JASCO V650 UV-visible spectrophotometer with an AutoPeltier thermostating unit with 6-cell changer PAC-743R. The UV-visible spectroscopy titrations of DNA solutions using the magnetic nanoparticles were performed in a 1 cm pathlength cuvette. Isothermal titration calorimetry experiments were carried out at 25 ºC on a high-precision VP-ITC microcalorimeter (MicroCal, LLC Northampton, MA). In the calorimetry experiments, we measured directly the heat effects, and hence the enthalpy, associated with DNA-PE binding. All solutions were degassed for at least 7 minutes before the titrations. ITC experiments were carried out in deionized water or in the presence of 100 mM NaCl. In nanoparticle dilution experiments in the absence of added salt, a 0.739 mM (for sample A) a 0.616 mM, or a 0.308 mM (for sample C) solution was injected into deionized water. The nanoparticle dilution experiment in the presence of 100 mM NaCl involved the dilution of a 0.585 mM solution (sample C) into a 100 mM NaCl solution. For all ITC experiments, nanoparticle concentrations are reported in terms of positive charges as estimated based on an idealized scenario assuming that precipitation in the UV-visible experiments is caused solely by charge neutralization. For DNA-binding experiments in the absence of added salt, solutions of nanoparticles (0.739 mM for sample A or 0.308 mM for sample C) were titrated into a 0.0277 mM (in terms of base pairs) solution of FS DNA. The DNA-binding experiment in the presence of 100 mM NaCl involved the titration of a 0.585 mM nanoparticles solution (sample C) into a 0.0277 mM solution of FS DNA. For experiments in the absence of added NaCl, successive injections (5 or 15 mL) were performed at intervals of 300 s (for sample A) or 400 s (for sample C). For experiments in the presence of 100 mM NaCl, injections of 20 mL at 600 s intervals were carried out. The instrument was operated in high-gain mode, applying a reference power of 10 mcal s^{-1} while stirring the sample cell contents at 416 rpm (in the absence of added salt) or 307 rpm (in the presence of added salt). All nanoparticle samples containing 100 mM NaCl were vortexed immediately before use.

Sample	Diameter (nm)	PE_n	ζ (mV)
A	25.9±2.7	1	49.2±0.7
B	28.6±2.5	1	49.6±1.5
C	30.0±2.9	3	39.2±0.6
D	31.8±2.4	1	58.6±1.4
E	38.0±2.0	3	41.3±1.2

Table S1. Summary of the silica-coated magnetite nanoparticles formulations used in this study. Diameter and ζ-potential were measured before and after the polymer deposition, respectively.

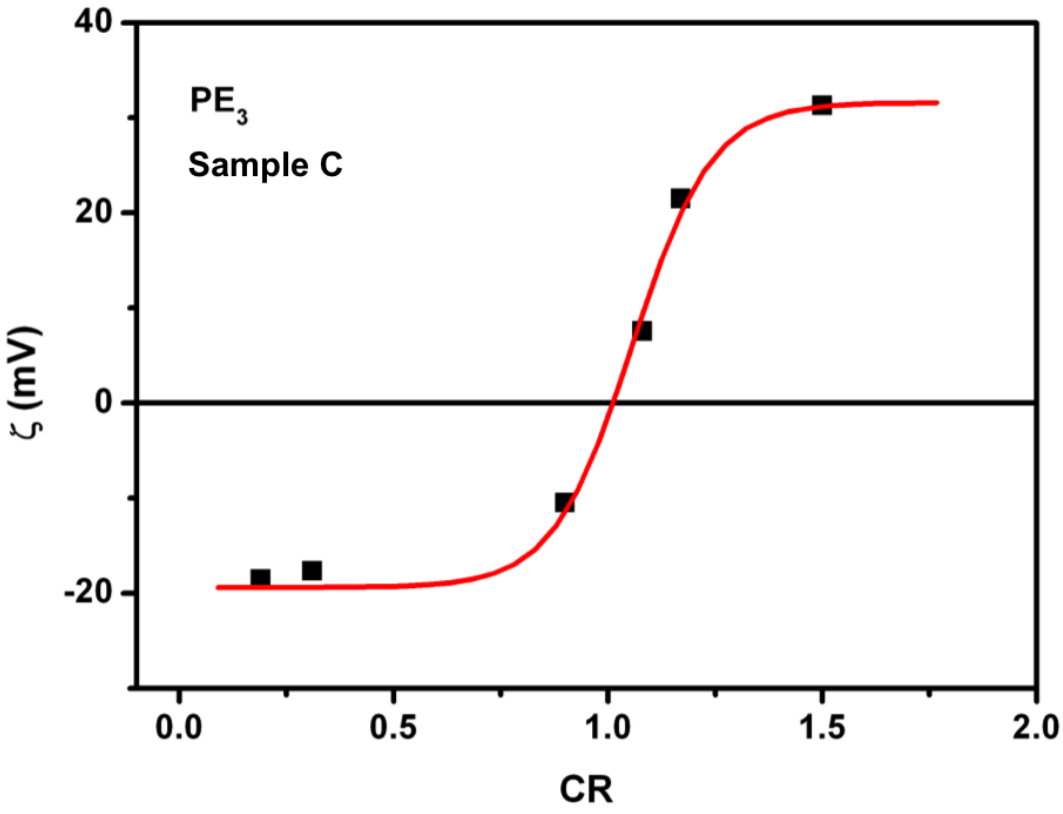

Figure S1. ζ-potential values obtained upon mixing independently different volumes of the magnetic nanoparticles solution (sample C) with a fixed volume of a 0.0037 mM CT DNA solution in order to vary the charge ratio (CR).

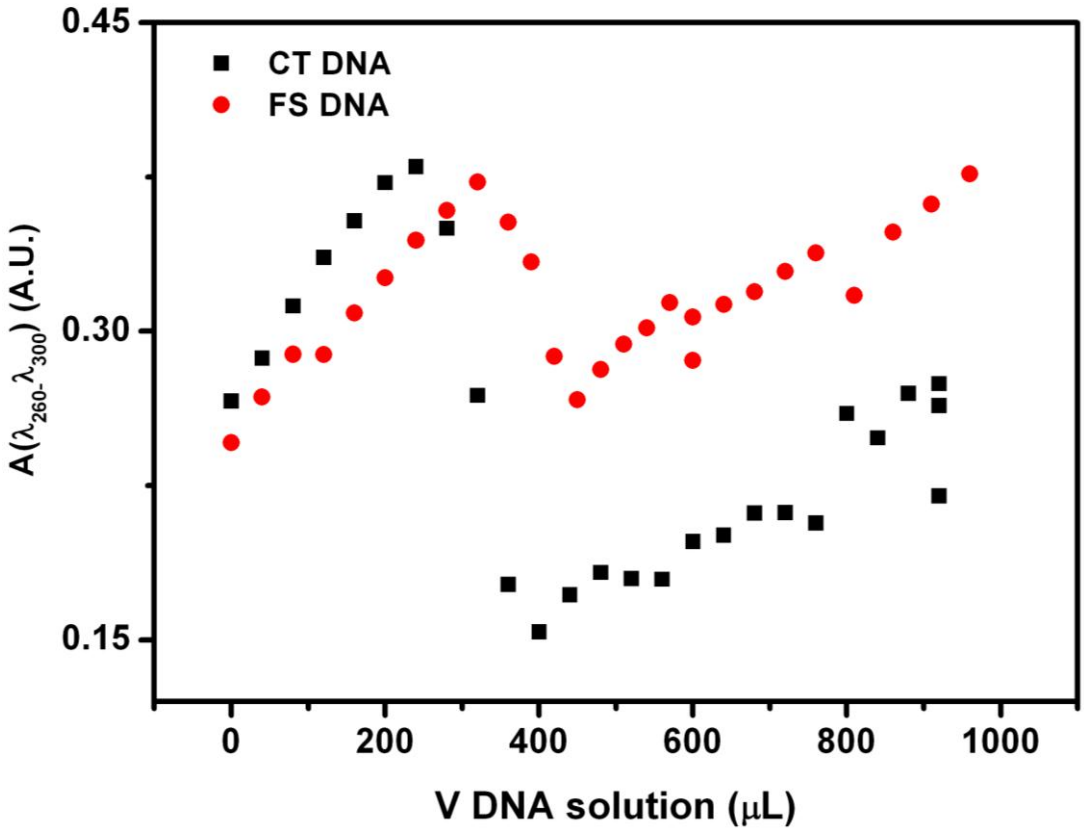

Figure S2. Absorption band intensity upon adding aliquots of 0.1108 mM DNA solutions (CT (black squares) and FS (red dots)) to a fixed amount of magnetic nanoparticles (samples B and A, respectively) (reverse titration).

Quantification of nanoparticle concentration

If we assume that the sudden drop in absorbance corresponds to full binding of all of the FS DNA in the initial 2 mL of solution, then the added volume of nanoparticles indicates that the nanoparticle concentration in terms of positive charges in the stock solution is 0.168 mM. An alternative approach considers the plot in Figure 3a (black open symbols) in terms of the change in absorbance to quantify how much DNA has been removed from the solution ($[FS\ DNA]_i$ = 0.0277 mM, $[FS\ DNA]_f$ = 0.0167 mM). Assuming that the removed DNA forms part of the precipitated magnetoplexes and that the remaining DNA is free at the inflection point on the curve ($V_{NPs} \approx 660$ μL), the final concentration of nanoparticles in terms of moles of positive charges would be 0.082 mM in this titration.

Description of the ITC Experiments

Interaction of (bio)molecules, i.e. going from the free to the bound state, causes changes in the Gibbs energy of the system (ΔG), representing underlying changes in enthalpy (ΔH - heat associated with the interactions that take place) and entropy (ΔS), according to the well-known equation $\Delta G=\Delta H-T\Delta S$. An isothermal titration calorimeter consists of two essentially identical cells; the reference cell (for experiments involving aqueous solutions typically containing water) and the sample cell; usually containing host molecules (for example biomacromolecules such as DNA, enzymes, cyclodextrins, etc.) into which a ligand (defined as a compound that binds to the host and corresponds in our case to the polyelectrolyte-functionalized nanoparticles) is titrated at a fixed temperature. Both binding partners (DNA and the positively charged nanoparticles) need to be in the same solvent or solvent mixture to avoid any background heat effects that arise from dilution and/or mixing of unequal solvents or buffers. The heat effects in the sample cell relative to the reference cell are detected by semiconductor thermopiles and the instrument applies thermal power (µcal/s) in order to actively compensate for the ligand-induced heat effects in the sample cell and thus brings the two cells into thermal equilibrium. The raw data are presented as a series of peaks measured as power (measured in µcal/s) versus time and the integration of these peaks and correction to a per mole basis yields the molar heat effect corresponding to every ligand addition and thus to the overall binding isotherm.[4,5] In order to get meaningful data, it is necessary to carry out the titrations in the range of concentrations where significant binding takes place. In this regard, it is necessary to design an experiment by selecting appropriately the following parameters for to the present study: DNA concentration in the calorimeter cell, ligand (polyelectrolyte-primed nanoparticles) concentration in the syringe, total volume of the ligand solution to be injected, volume of each injection, time between injections and experimental temperature. Because it is not possible to accurately determine the concentration of the magnetic nanoparticles in solution, we chose to estimate our “ligand concentration” using the alternative approach already introduced, expressing the concentration of magnetic nanoparticles in terms of available positive charges, using the data from the previous UV-visible experiments.

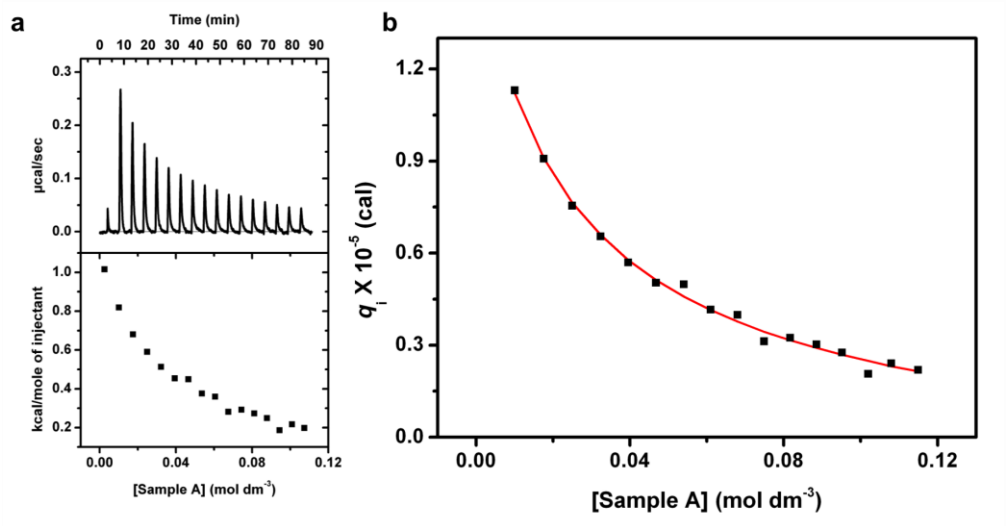

Figure S3. ITC dilution measurements for sample A (a). Raw ITC data from injecting 15 µL aliquots of a 0.739 mM solution of the nanoparticles into water (top). Heat per injection obtained from integrating the heat flow over time (bottom). Fit of a self-aggregation model for molecular ligands to the observed heat effects (b).

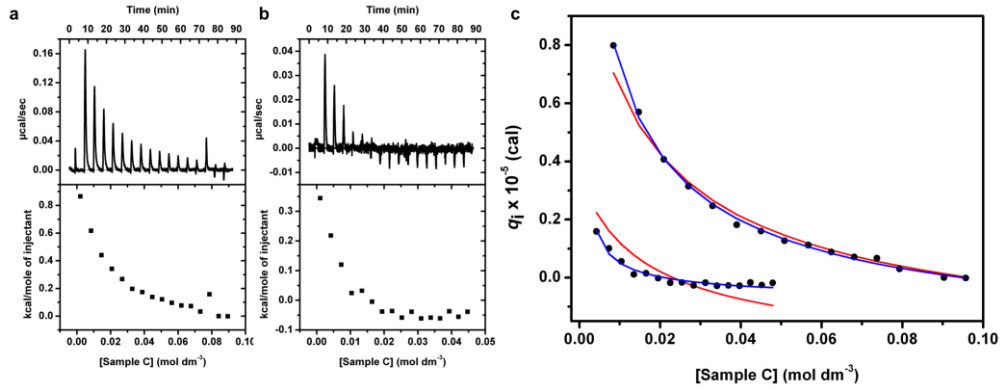

Figure S4. ITC data for sample C dilution. ITC dilution measurements for sample C (a and b (half concentrated)). Raw ITC data from injecting the nanoparticles into water (top) and heat per injection obtained from integrating the heat flow over time (bottom). Heat of dilution fits for the sample C dilutions from ITC experimental data (c).

Sample	**[NP] (mM charges)**	**K x 10^3 (M^{-1})**	**ΔH x 10^3 (cal mol^{-1})**
Sample A	0.739	3.48	-1.55
Sample C	0.616	42.5	-1.87
Sample C	0.308	361	-1.28
Sample C	0.308 and 0.616 (global analysis)	21.5	-1.60
Titrations were performed using nanoparticles in the injection syringe (both in aqueous solution at 25 ºC)			

Table S2. Thermodynamic parameters describing nanoparticle dilution experiments.

Sample	K (M^{-1})	ΔH (kcal mol^{-1})	n
Sample A (17 inj)	$> 10^9$	-1.8	1.2
Sample A (52 inj)	$> 10^9$	-2.0	1.1
Sample C	$> 10^9$	-1.8	1.8

Titrations were performed using 0.0277 mM FS-DNA in the calorimeter cell and nanoparticles in the injection syringe (with concentrations of 0.739 mM for sample A and 0.308 mM for sample C, both in terms of available positive charges), both in aqueous solution at 25 ºC.

Table S3. Thermodynamic parameters for nanoparticle-DNA binding processes

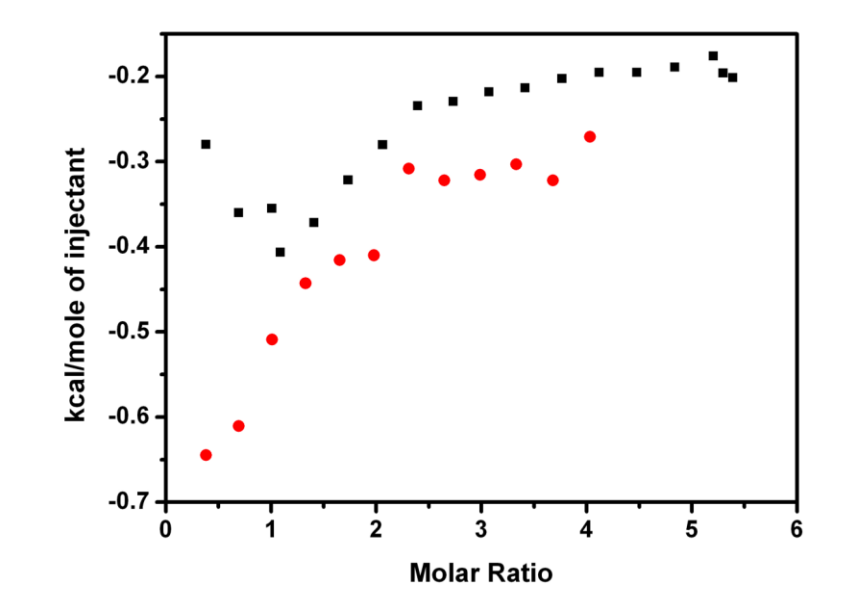

Figure S5. Integrated heat effects for sample C; dilution (red circles) and titration into 0.0277 mM FS DNA (black squares), both in the presence of 100 mM NaCl and at 25ºC.

References

1. T. Hyeon, S. S. Lee, J. Park, Y. Chung, H. B. Na, *J. Am. Chem. Soc.*, 2001, **123**, 12798.
2. R. Koole, M. M. van Schooneveld, J. Hilhorst, C. De Mello Donega, D. C. Hart, A. van Blaaderen, D. Vanmaekelbergh, A. Meijerink, *Chem. Mater.*, 2008, **20**, 2503.
3. F. Caruso, H. Lichtenfeld, M. Giersig, H. Möhwald, *J. Am. Chem. Soc.*, 1998, **120**, 8523.
4. N. J. Buurma, I. Hag, *Methods*, 2007, **42**, 162.
5. T. Wiseman, S. Williston, J. F. Brandts, L.-N. Lin, *Anal. Biochem.*, 1989, **179**, 131.