Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information

Optimized polydopamine coating and DNA conjugation onto gold nanorods for single nanoparticle bioaffinity measurements

Marwa M. Azab[a,b], Rédha Cherif [c], Aryanne L. Finnie[a], Maha M. Abou El-Alamin[b], Maha A. Sultan[b] and Alastair W. Wark*[a]

[a] *Centre for Molecular Nanometrology, Technology and Innovation Centre, Dept. of Pure & Applied Chemistry, University of Strathclyde, 99 George St., Glasgow, G1 1RD, UK.*

[b] *Analytical Chemistry Department, Faculty of Pharmacy, Helwan University, 11795, Cairo, Egypt.*

[c] *Université Pierre et Marie Curie, 4 Place Jussieu, 75252 Cedex 05, France.*

Supporting Data

Figs. S1-S12 and Table S1 featuring additional data on polydopamine (PDA) growth characterization including on different sized NRs plus DNA attachment and NR-DNA conjugate characterization.

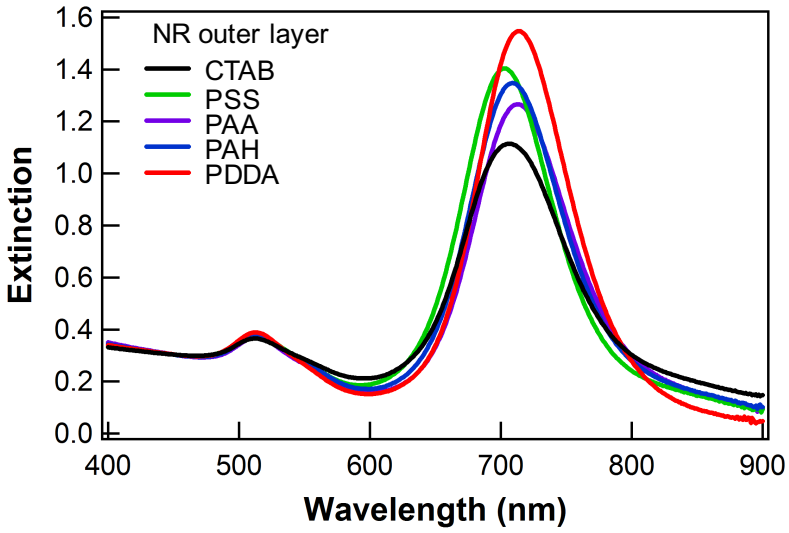

Fig. S1 Extinction spectra of NR stock solutions prior to PDA coating described in Figs. 1-3. This includes NRs suspended in 1 mM CTAB or with PSS, PAH, PDDA or PAA as the outermost layer. The polyelectrolyte wrapped samples were washed in water 3x prior to PDA layer formation. All spectra have been normalized to the same intensity at 450 nm for comparison purposes.

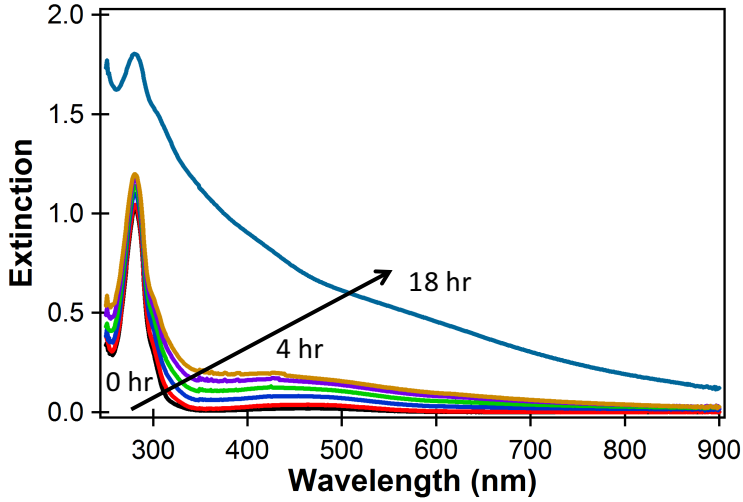

Fig. S2 UV-vis spectra monitoring PDA formation in the absence of nanoparticles. A dopamine hydrochloride concentration of 0.3 mM in Tris pH 8.5 buffer was used with spectra shown at 0, 1, 2, 3, 4 hours and overnight (18 hours).

PDA growth kinetics study on NR@PSS with longitudinal LSPR λmax at ~700 nm:

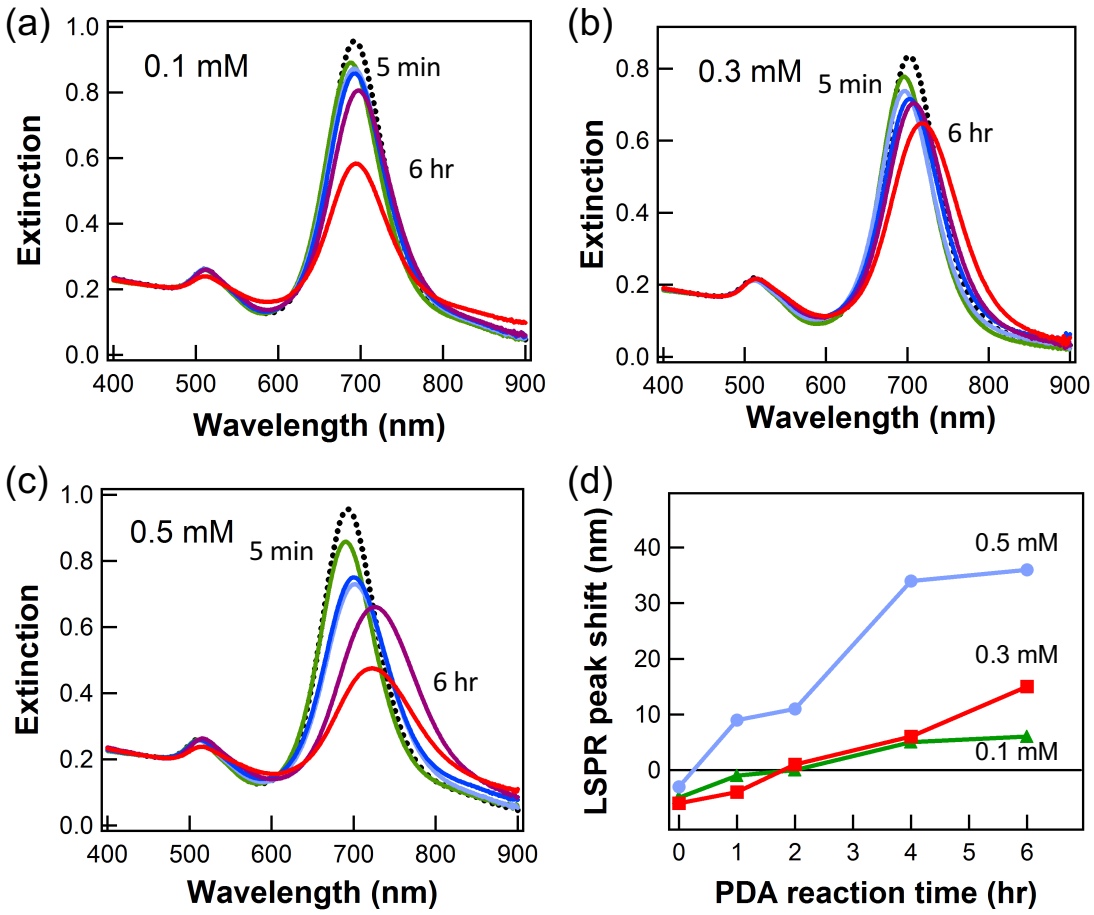

Fig. S3 Monitoring PDA growth on NR@PSS with an initial LSPR λ_{max} at ~700 nm, where all the reaction parameters are the same except for the concentration of dopamine hydrochloride, which was (a) 0.1 mM, (b) 0.3 mM and (c) 0.5 mM. A plot of the LSPR peak shift is shown in (d). Each measurement was acquired with a separate reaction aliquot with times of 5 min, 1, 2, 4 and 6 hr prior to centrifugation and suspending in Tris buffer. The black dotted line represents the PSS@NR's prior to PDA coating. All spectra have been normalized to the same intensity at 450 nm for comparison purposes.

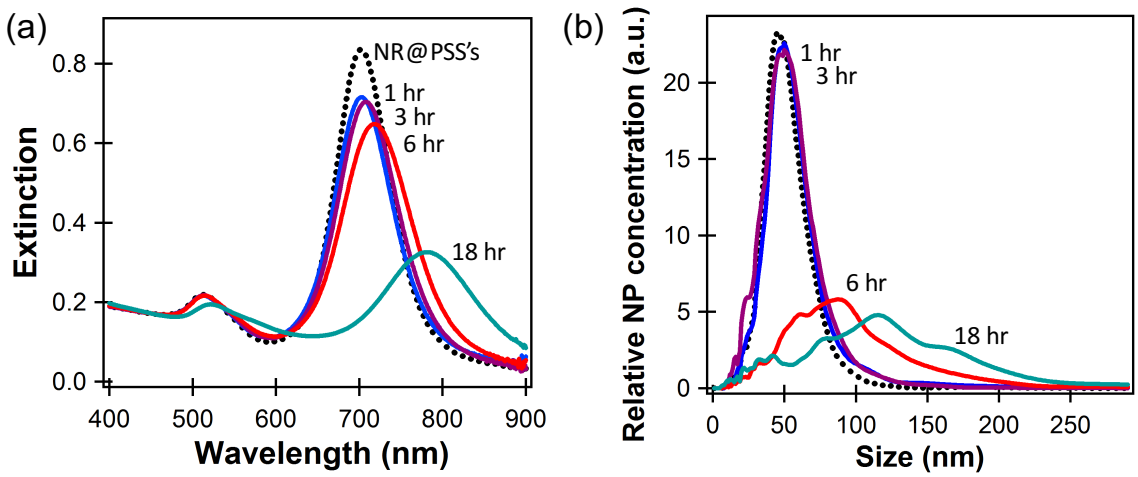

Fig. S4 Comparison of (a) extinction spectra and (b) nanoparticle tracking analysis of PDA layers grown on PSS-coated NR's as described in figure 2(a) in the main article. Samples obtained for reaction mixing times (prior to centrifugation and resuspension) of 1, 3, 6 hours and overnight are shown alongside the starting PSS-NR's data. The significantly broader and damped profiles shown in (b) for 6 hours and overnight did not show a significant decrease in particle density when comparing videos at different particle dilutions. Thus, the increase in average size and size distribution is affiliated with thicker PDA layer growth rather than NR aggregation. The initial starting concentrations of stock NR's and dopamine were the same for all the measurements with care taken to minimise NR losses during the sample clean-up.

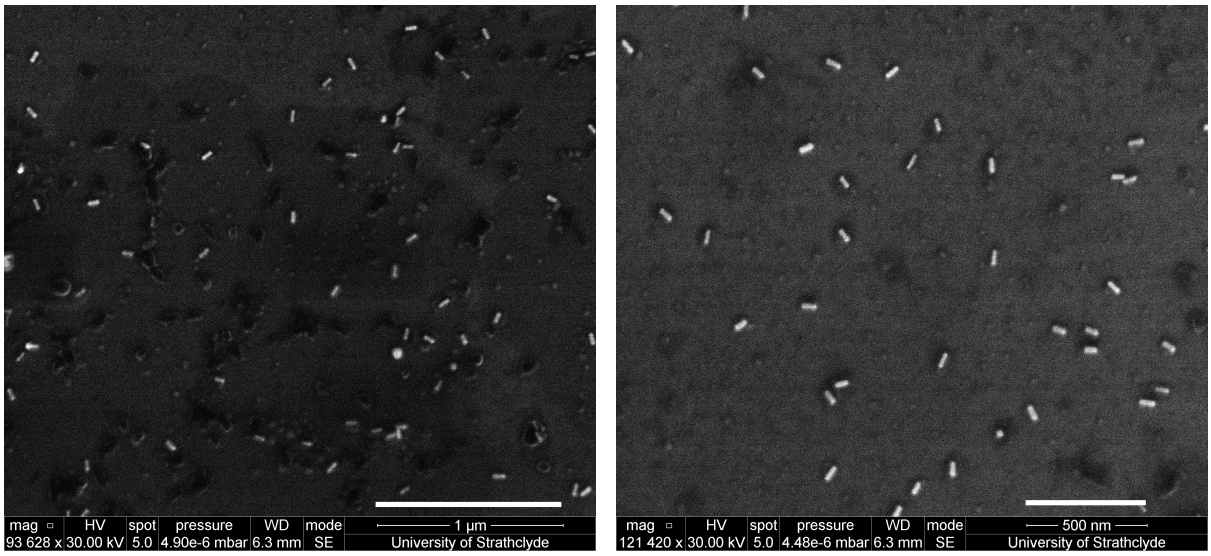

Fig. S5 Representative SEM images of NR@PSS@PDA's prepared with 6 hours of polymer growth time. The samples were prepared by exposing a polyelectrolyte (PDDA) coated Si wafer to the colloid solution for a few minutes before rinsing with water to prevent drying induced aggregates forming. This provides additional evidence that the NR's remain individually dispersed.

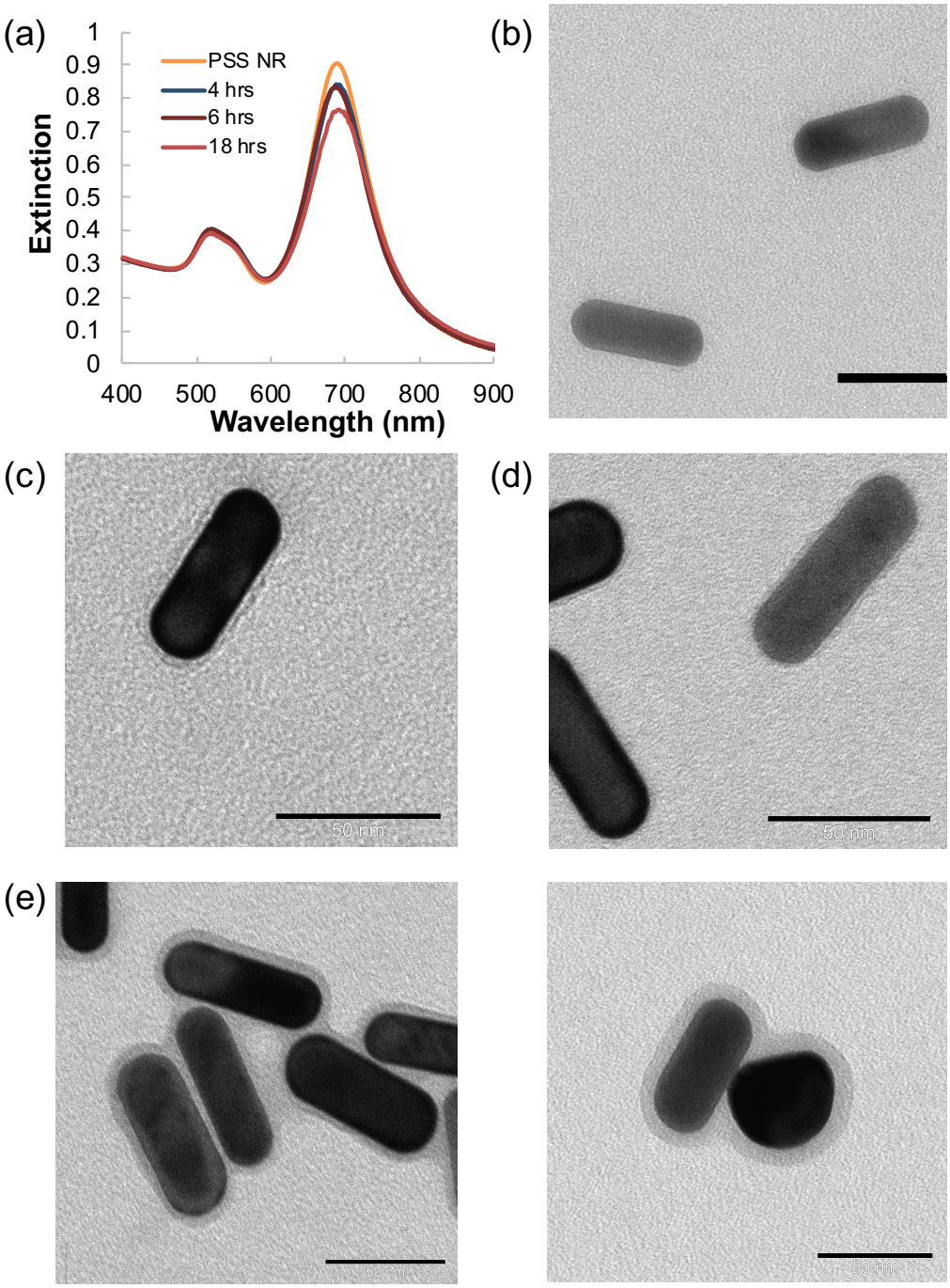

Fig. S6 (a) Extinction spectra and TEM images of (b) NR@PSS, (c – e) NR@PSS@PDA prepared using a dopamine concentration of 0.3 mM dopamine with reaction times of (c) 4 hrs, (d) 6 hrs and (e) 18 hrs. Scale bars = 50 nm. These were prepared using a different NR stock solution from that used elsewhere in this work at a similar LSPR.

Effect of polyelectrolyte outer layer on subsequent PDA growth:

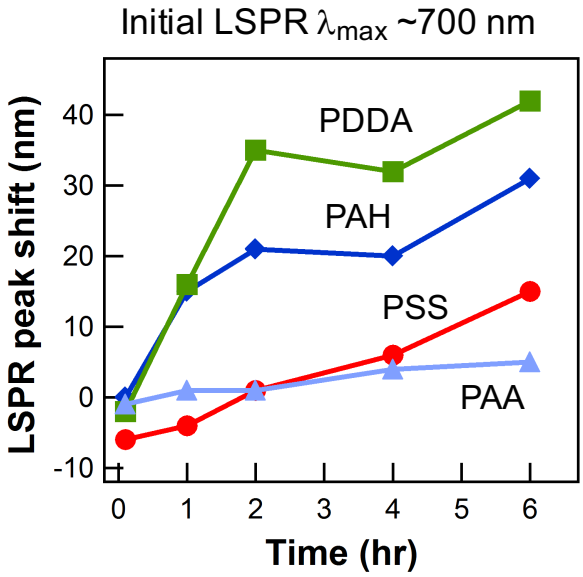

Fig. S7 Comparison of longitudinal LSPR λ_{max} peak shifts for PDA layer growth at different reaction times on NR stocks with different outer polyelectrolyte layers as described in Fig. 2 in the main article. The concentration of dopamine HCl was fixed at 0.3 mM and the NR stocks fixed at 0.27 nM for each experiment with the data points acquired after centrifugation and resuspending the NR's in water.

PDA growth kinetics study on NR@PSS with longitudinal SPR λmax at ~770 nm:

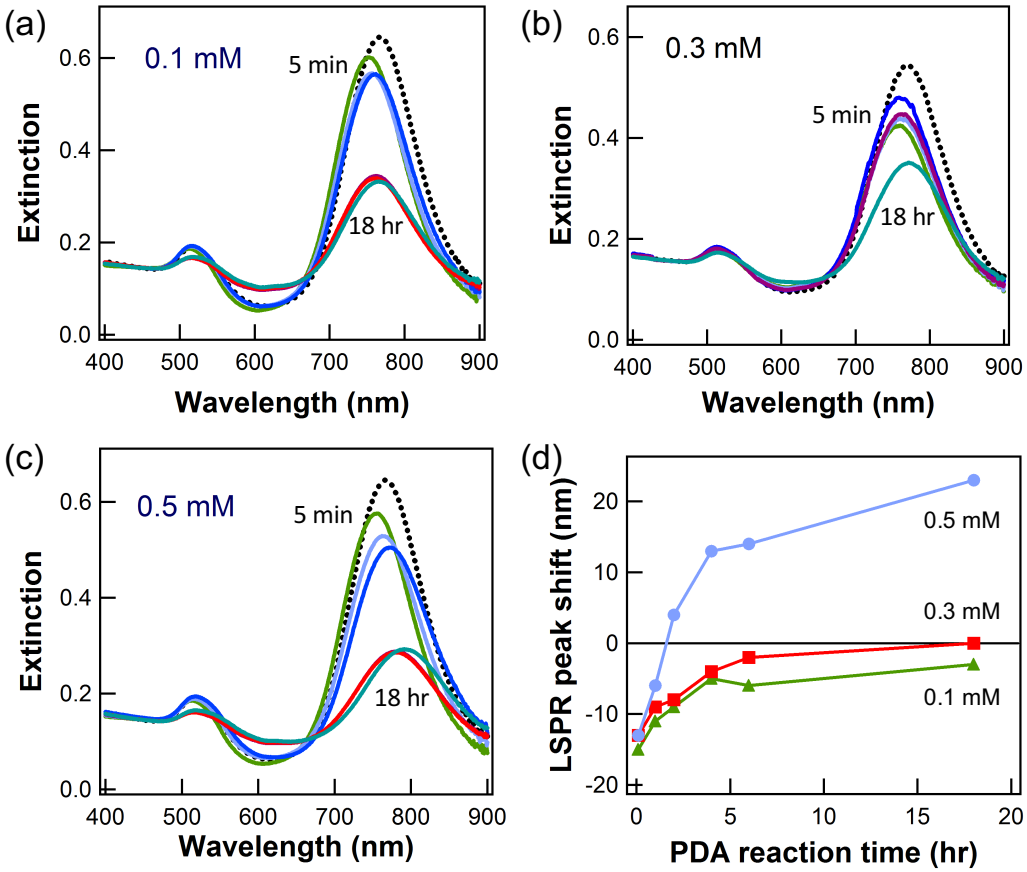

Fig. S8 Monitoring PDA growth on NR@PSS with an initial LSPR λ_{max} at ~770 nm, where all the reaction parameters are the same except for the concentration of dopamine hydrochloride, which was (a) 0.1 mM, (b) 0.3 mM and (c) 0.5 mM. A plot of the LSPR peak shift is shown in (d). Each measurement was acquired with a separate reaction aliquot with times of 5 min, 1, 2, 4, 6, and 18 hr prior to centrifugation and suspending in water. The black dotted line represents the NR@PSS colloid prior to PDA coating. All spectra have been normalized to the same intensity at 450 nm for comparison purposes.

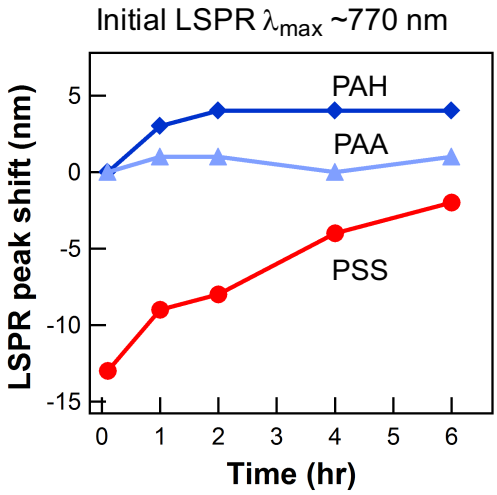

Fig. S9 Comparison of longitudinal LSPR peak shifts for PDA layer growth on NR@PSS's with an initial LSPR λ_{max} at ~770 nm. The same layer combinations were used as described in Fig. S8 above with the concentration of dopamine and NR stocks fixed at 0.3 mM and 0.22 nM respectively. Each data point was acquired after centrifugation and resuspending the NR's in water.

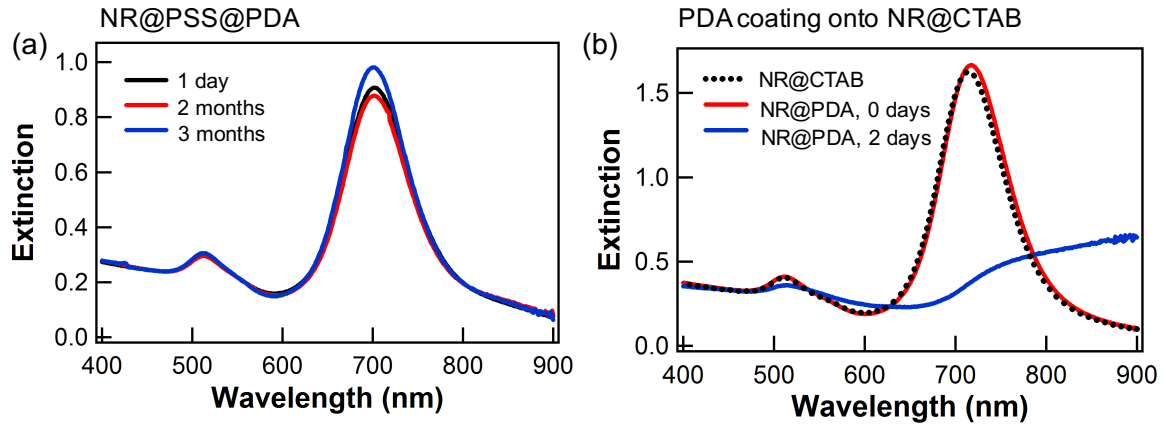

Fig. S10 Extinction measurements demonstrating typical colloidal stability of (a) NR@PSS@PDA and (b) NR@PDA. In (b), 0.3 mM dopamine HCl was mixed for 30 min with the NR stock resuspended in 1 mM CTAB. This was a relatively rare example of where centrifugation/resuspension in water could be performed with no significant aggregation, but longer-term stability is much poorer than NR@PSS@PDA. All spectra have been normalized to the same intensity at 450 nm for comparison purposes.

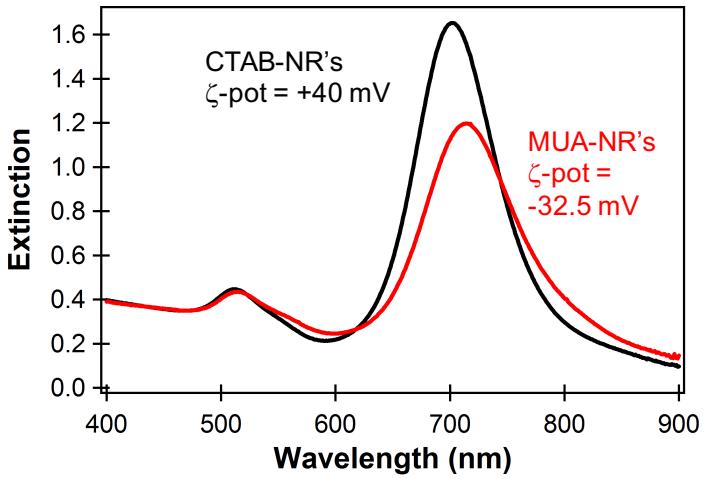

Fig. S11 Extinction spectra of NR stock (in 1 mM CTAB) and following MUA functionalization after two centrifuge/resuspend cycles in 0.01 M borate buffer to remove excess MUA. Zeta-potential (ζ-pot) values are also given for both colloids. All spectra have been normalized to the same intensity at 450 nm for comparison purposes.

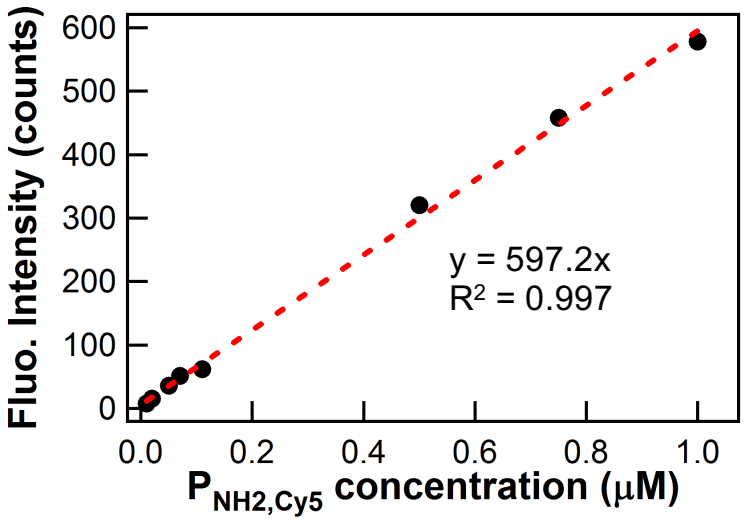

Fig. S12 Calibration plot monitoring bulk fluorescence at different concentrations of ssDNA sequence $P_{NH2,Cy5}$. The fluorimeter instrument settings were selected in order to establish a linear response across this concentration range. In order to ensure repeatability from day-to-day, a standard solution was used to normalize the signal acquisition.

Sample	Zeta-Potential (mV)			
	Before PDA	30 mins growth	4 hrs growth	End
NR@PSS@PDA	–28 (± 2.4)	–40 (± 4.5)	–41.7 (± 3.2)	–38 (± 1.8) (18 hrs)
NR@PSS@PAH@PDA	+30.4 (± 5.2)	–33.60 (± 1.6)	–38.7 (± 3.3)	–18.5 (± 4.7) (6 hrs)
NR@PSS@PDDA@PDA	+28.1 (± 2.1)	–41.1 (± 2.9)	–32.4 (± 3.1)	–37.1 (± 3.9) (6 hrs)
NR@PSS@PAH@PAA@PDA	–37.7 (± 3.5)	–40.8 (± 1.0)	–40.4 (± 2.0)	–25.6 (± 7.6) (18 hrs)

Table S1 Representative zeta-potential values measured on various positive and negative charged polymer coated NR substrates. The PDA reaction was stopped after various reaction times and the NR suspension centrifuged and resuspended in water prior to measurement.