Supplementary Material (ESI) for Nanoscale

Kinetic investigation of bioresponsive nanoparticle assembly as a function of ligand design

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Supplementary Information

Experimental Section

Peptide Synthesis. The peptides were synthesised on Rink Amide MBHA resin (Novabiochem, UK) using standard solid phase Fmoc strategy and an HBTU/HOBt protocol. Each amino acid was coupled in two cycles of 30 min each using a 10-fold excess of amino acid and a reagent mix amino acid/HBTU/HOBt/DIPEA of 1/1/1/2. All peptide synthesis reagents were obtained from AGTC Bioproducts (UK), except for Fmoc-ε-Ahx-OH, Fmoc-Tyr(PO(OBzl)-OH)-OH (Novabiochem, UK), and Fmoc-O2Oc-O2Oc-OH (IRIS Biotech, Germany). The peptides were lyophilised, purified by HPLC and identified by mass spectrometry.

Nanoparticle Functionalisation. 20 nm citrate-stabilised gold NPs (BBInternational, UK) were functionalised with monoclonal mouse anti-phosphotyrosine antibody PT-66 (Sigma-Aldrich, UK) according to the protocol published by Kumar *et al*. (*Nat Protoc*, 2008, **3**, 314). The peptide-coated 20 nm citrate-stabilized gold NPs were prepared as follows: 1.5 nmol of peptide in aqueous solution (2 mM stock concentration) was added to 1 mL of 1 nM colloidal gold dispersion stabilised with 0.1% w/v BSA in non-stick eppendorf tubes (Alpha Labs, UK). The mixture was vortexed and an overnight incubation at 4 °C was maintained for the place exchange reaction between the peptides and the citrate molecules on the NP surface. Excess peptide was removed by centrifugation at 13,500g for 20 min and by washing of the pellet twice in assay buffer (20 mM $MgCl_2 \cdot 6H_2O$, 70 μM EDTA, 0.1% w/v BSA in 35 mM HEPES buffer, pH 7.5), before resuspension of NPs in assay buffer to obtain a final concentration of approximately 7 nM.

Surface Plasmon Resonance (SPR) Measurements. All SPR measurements were performed at 25 °C on the Reichert SR7000DC dual channel instrument (Reichert Inc, NY, USA).

For the kinetic titration experiments, SPR chips containing mixed self-assembled monolayers (SAMs) of 10% poly(ethylene glycol)$_6$-COOH and 90% poly(ethylene glycol)$_3$-OH (Reichert Inc) were modified with the anti-phosphotyrosine antibody in the sample channel and a monoclonal mouse anti-phosphoserine control antibody (Abcam, UK) in the reference channel using EDC/NHS chemistry. Both antibodies were immobilised to a comparable surface packing density. The chip surface was blocked with citrate-stabilised gold NPs suspended in 3% w/v BSA in 35 mM HEPES buffer pH 7.5 to minimize non-specific binding of the NPs. A blank solution containing the assay buffer followed by five increasing concentrations of peptide-functionalized gold NPs were then sequentially injected at 100 μL min^{-1} flow rate for each of the six peptide types (**see Table S1**) maintaining the association and dissociation phases of 60 s and 180 s, respectively, for each injection. After subtracting the reference channel signals, the association phase data were fitted to a Langmuir isotherm model and normalised according to their respective B_{max} values (**Fig. S1**). Here, B_{max} corresponds to the SPR signal value obtained at the receptor saturation level.

The SAM packing density of the individual peptide designs was determined using unmodified gold chips (Reichert Inc). The chips were cleaned for 20 min in Piranha solution, followed by thorough washing with ultrapure water and acetone in a sonicator. Directly before use, the chips were treated for 15 min in an UV-Ozone plasma (Bioforce, UK). Next, the gold surface was equilibrated with 10 mM citrate buffer pH 6.0 to mimic NP surface conditions. Peptides were then injected at a concentration of 2 μM in 10 mM citrate buffer pH 6.0 at 2 μL/min flow rate until saturation levels were achieved (approximately 12 h). The peptide density was then quantified and normalised according to the resonance signal (1 uRIU ~ 1 pg/mm2) and specific molecular weight of the peptides (**Fig. S2**). All measurements were performed in duplicate.

Table S1. The different concentrations of peptide-functionalised particles injected during the SPR study for the various peptides used.

	pY-1	**pY-2**	**pY-3**	**pY-4**	**pY-5**	**pY-6**
Injection 1	3.95 pM	3.15 pM	3.15 pM	3.25 pM	2.69 pM	2.99 pM
Injection 2	19.8 pM	15.76 pM	15.76 pM	16.2 pM	13.4 pM	14.9 pM
Injection 3	98.8 pM	78.8 pM	78.8 pM	81.2 pM	67.2 pM	74.8 pM
Injection 4	0.46 nM	0.39 nM	0.39 nM	0.41 nM	0.34 nM	0.35 nM
Injection 5	2.47 nM	1.97 nM	1.97 nM	2.03 nM	1.68 nM	1.87 nM

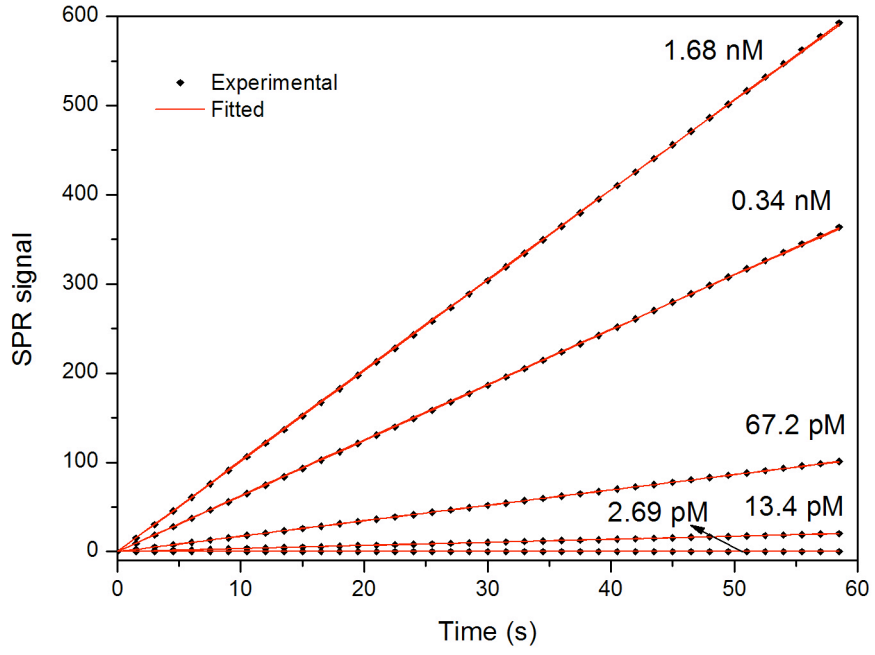

Figure S1. Derivation of the apparent on-rate constants by data-fitting to a Langmuir isotherm model, exemplified for pY-5. The SPR association phase data obtained for the five different concentrations of pY-5-functionalized NPs were fitted using the Langmuir isotherm model for a B_{max} value of 7920 to obtain an apparent k_{on} value of $2.39 \cdot 10^6 \pm 0.02 \cdot 10^6$ M^{-1} s^{-1}.

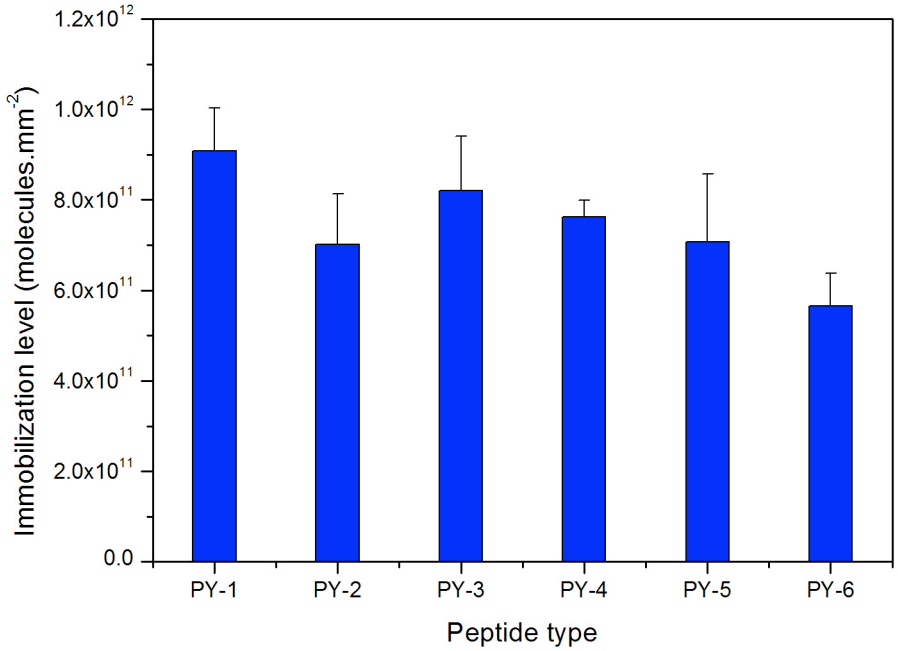

Figure S2. Determination of the peptide-specific SAM packing density on a gold surface. SPR chips with plain gold surface were cleaned and pre-equilibrated with 10 mM citrate buffer pH 6. Peptides were individually injected in a concentration of 2 μM in the same buffer for 12 h at a flow rate of 2 μL/min. The resonance signals at SAM saturation levels were then converted into mass (1 μRIU ~ 1 pg/mm^2) and further normalised to the molecular weight of the specific peptide. Columns represent arithmetic means of duplicate measurements.

Solution-Phase NP Aggregation. Reaction mixtures containing equimolar concentrations of peptide-conjugated gold NPs (7 nM in assay buffer having 0.1% w/v BSA) and antibody-modified gold NPs (7 nM in assay buffer having 0.1% w/v BSA) were incubated at either room temperature or 37 ^{o}C. The NP aggregation progress was monitored by UV-Vis spectrophotometry (NanoDrop ND1000, UK) measured over the 400–750 nm wavelength range. The extent of aggregation at any time was quantified by calculating the A/D ratio as described previously (Laromaine et al., *JACS*, 2007, **129**, 4156). The kinetic rate constants were then derived by fitting the data to a reaction model developed by Gupta et al. (*Small*, 2010, epub ahead of print) also described briefly below. Experiments were performed at least in duplicate.

Fitting equation: $$\frac{A}{D} = \left(\frac{k_{on}\, n}{2}\right) t + c$$

Here, k_{on} is the apparent forward reaction rate constant, n is initial peptide- and antibody-NP concentration, t is the time of aggregation, $c = 1/2n$ is an arbitrary constant of integration and the A/D (aggregated/dispersed area) ratio is an optical parameter determined by UV-Vis spectroscopy that allows us to estimate the relative concentrations of the aggregated versus dispersed NPs at any time. The dispersion region D is calculated by integrating the area under the plasmon resonance peak spanning from 490 to 526 nm and aggregated region A is the area integrated from 541 to 750 nm. Since the above rate equation is valid only in the early stages of reaction when the aggregated NP complexes are in the dimeric form, fitting of the A/D data collected in the first couple of minutes allowed us to calculate the initial slopes of the curves and hence, the k_{on} values.