Supplementary Material (ESI) for Chemical Communications

Single-step kinase inhibitor screening using a peptide-modified gold nanoparticle platform

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SUPPLEMENTARY INFORMATION

Experimental Section

Materials. 20 nm citrate-stabilized gold NPs and light microscopy silver enhancement kit were purchased from BBInternational, UK. Kinases v-Src and v-Abl were bought from SignalChem, Canada and Calbiochem, USA, respectively. Monoclonal mouse anti-phosphotyrosine antibody, adenosine 5'-triphosphate disodium salt hydrate (ATP), bovine serum albumin and enzyme inhibitor staurosporine were purchased from Sigma-Aldrich, UK. Peptide substrate Ac-IpYGEFKKKC was synthesized by Genscript, USA. Monoclonal mouse anti-phosphoserine antibody was procured from Abcam, UK. All other reagents used were of analytical grade. Buffers were prepared using standard protocols and used at 1× dilution unless and otherwise stated. Milli-Q water of resistivity ≈ 18 MΩ cm was used throughout the experiments.

Peptide Substrates. Peptides Ac-IYGEFKKKC and Ac-IYAAPKKGGGGC were synthesized in house by standard solid phase Fmoc synthesis on a MBHA Rink Amide resin (Novabiochem, UK) using the HBTU/HOBt protocol with ten fold excess of amino acids. All other reagents were obtained from AGTC Bioproducts (UK). The peptides were lyophilized and their identity and purity were determined by mass spectroscopy and reverse-phase HPLC.

Peptide-functionalized Gold NPs. Peptide gold NPs were prepared as described previously (12). Briefly, approx. 1.4 nmols of peptide was added per 1 mL of 1 nM gold NP suspension containing 0.1% w/v BSA in non-stick eppendorf tubes (Alpha Labs, UK). BSA was included to stabilize the NP suspension against aggregation during the peptide immobilization step and to minimize any non-specific adsorption. The mixture was incubated overnight at 4 ^{o}C and then washed twice by centrifugation at 13,500g for 20 min to remove excess peptide that did not chemisorb on the gold surface. The NPs were finally resuspended in assay buffer (20 mM $MgCl_2.6H_2O$, 70 μM EDTA, 0.1% w/v BSA in 35 mM HEPES buffer, pH 7.5).

Solution-phase Assays. 50 μL reaction mixtures containing peptide-conjugated gold NPs (28 μL of 4 nM in assay buffer), kinase (16.25 μL of varying concentrations in assay buffer), ATP (0.75 μL of 10 mM in 10 mM Tris buffer, pH 8) and antibody (5 μL neat in PBS) were incubated at 37 ^{o}C for different periods of time. Control experiments were performed by replacing the enzyme/antibody solution with an equal volume of assay buffer. In the enzyme inhibitor experiments, varying concentrations of inhibitor were included in the 50 μL reaction mixtures containing 20 nM v-Src / 2.3 nM v-Abl and 10 μM ATP (final concentrations). The DMSO concentration was adjusted to 2% v/v. The extent of NP agglutination was measured by UV-Visible absorption spectroscopy (NanoDrop ND1000, UK) over 400 – 750 nm wavelength range. The results were quantified as the ratio of peak absorbance at 528 nm at any time ($A_{t=t}$) and at time zero ($A_{t=0}$). All experiments were performed in duplicate.

Dot Blot Assays. Antibodies diluted in PBS were immobilized on a nitrocellulose membrane (0.45 μm pore size, Sigma-Aldrich, UK) using 0.4 μL antibody solution per spot. The membrane was blocked with 3% w/v BSA in HEPES buffer for 1 h. Per every 15 spots or so, approx. 1.5 mL of reaction mixture consisting of peptide-functionalized gold NPs (1 mL of 1 nM in assay buffer), kinase (0.5 mL of varying concentrations in assay buffer) and ATP (5 μL of 10 mM in 10 mM Tris buffer, pH 8) were allowed to react for 2 h at 37 ^{o}C and then incubated on the membrane at room temperature for 30 min. The membrane was washed with surplus deionized water to remove all free ions from the system and silver enhanced for 11 min following the kit instructions. Kinetic experiments were performed by directly incubating 1 nM of chemically pre-phosphorylated peptide-functionalized gold NPs for different time lengths at room temperature followed by 8 min of silver enhancement. Control experiments without enzyme were performed by replacing the enzyme with an equal amount of assay buffer. In kinase inhibition experiments, different amounts of inhibitor were included in the reaction mixtures such that the final v-Src / v-Abl, ATP and DMSO concentrations were 1 nM / 0.55 nM, 5 μM and 1% v/v, respectively. The spots were imaged using a Dyversity photoimager (Syngene, Cambridge, UK). The spot darkness was quantified by the optical densitometry analysis technique as described elsewhere (16). Briefly, the average opacity I_s of each spot was measured using the luminosity histogram tool in Photoshop (version 7.0.1) and normalized by the opacity of the background I_b. The results were expressed as an optical darkness ratio, $ODR = I_b - I_s / I_b$. All experiments were performed at least in duplicate.

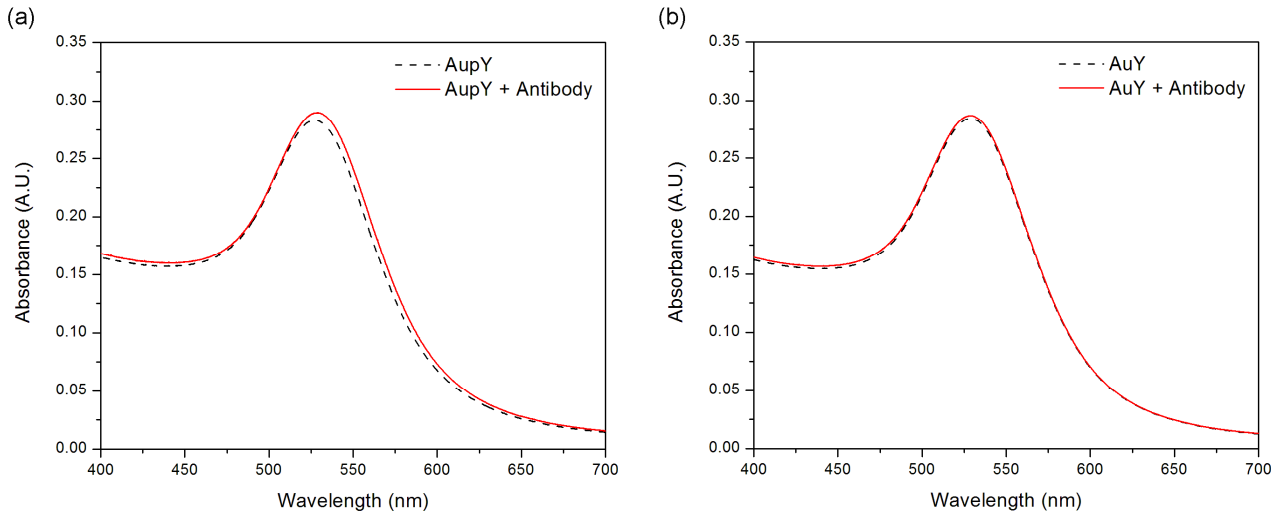

Figure SI-1. The accessibility of the phosphopeptide for antibody binding investigated using UV-Visible spectroscopy. A small 10 nM quantity of anti-phosphotyrosine antibodies was added to a suspension of gold NPs that were conjugated to either (**a**) chemically pre-phosphorylated (AupY) or (**b**) non-phosphorylated peptides (AuY). A spectral peak red-shift of 2 nm only in the case of (a) indicated a specific binding between the antibodies and phosphopeptides. The UV-Vis spectra were collected in 1 cm path-length PMMA cuvettes using a Unicam UV-500 UV-Vis spectrophotometer.

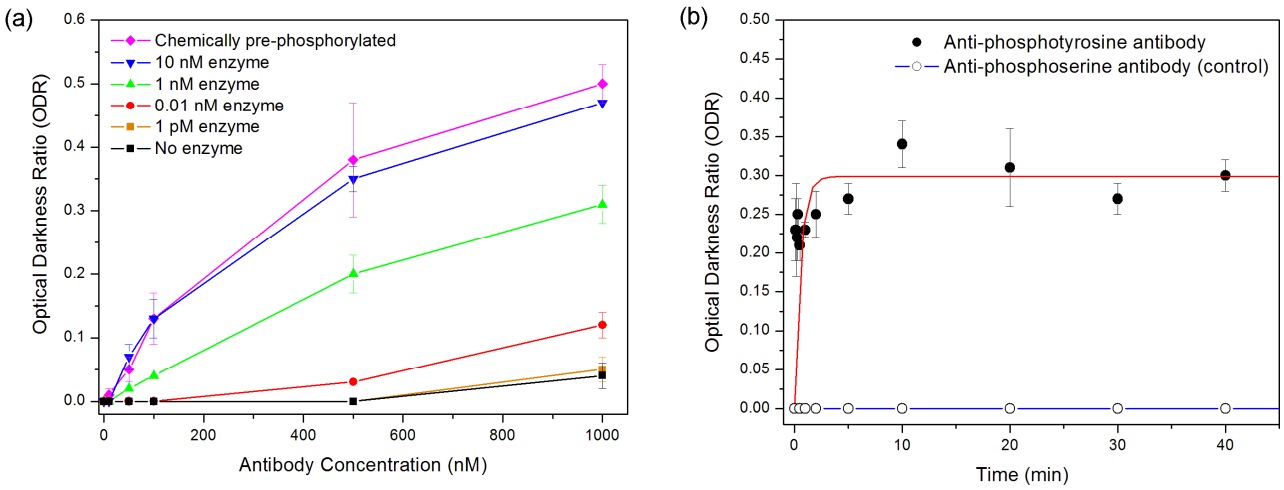

Figure SI-2. The colorimetric response of dot blot assays as a function of the experimental parameters. (**a**) The effect of enzyme and antibody concentrations on assay signal illustrated a monotonic rise in ODR with increasing concentrations of each species. The upper and lower limits of enzyme detection were found to be 10 nM and 10 pM kinase, respectively, as determined by comparing their performance with experiments using chemically pre-phosphorylated peptides or zero enzyme samples. (**b**) Varying the gold NP incubation time revealed that the signal saturation occurred within the first few minutes from the start of the incubation process. Repeating the experiment with a control antibody resulted in no signal over time suggesting the high specificity of the assay.

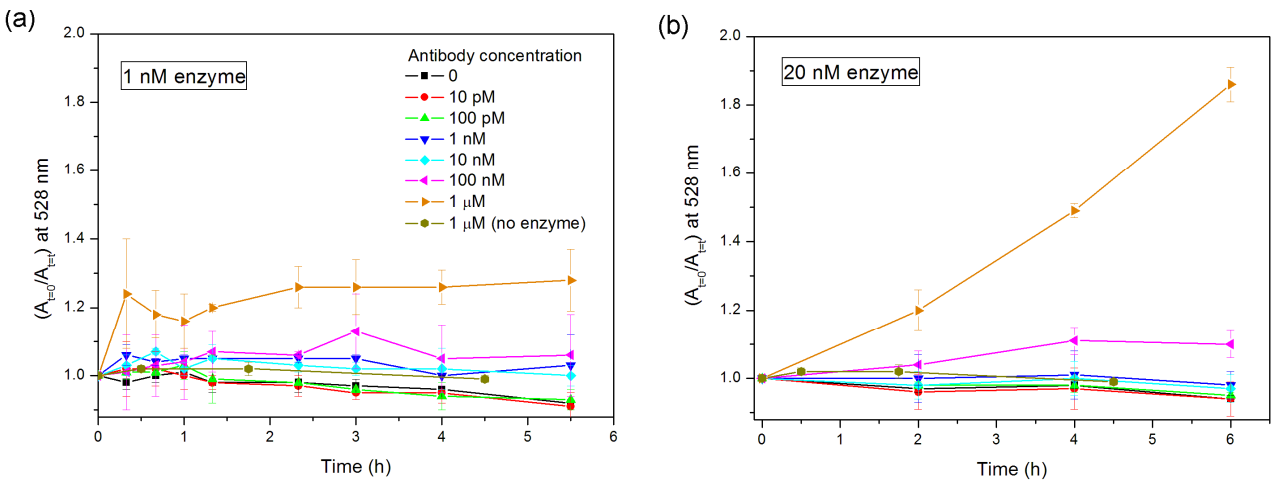

Figure SI-3. Optimization of the experimental conditions in terms of antibody and enzyme concentration with respect to the response of solution-phase assays. (**a**) The aggregation of enzymatically-phosphorylated NPs was seen to occur with antibody concentrations between 0.1-1 μM with an optimal response at 1 μM. At all other antibody concentrations or in the absence of enzyme, the assay signal was measured to be zero. (**b**) The assay response increased 1.5 times when the enzyme concentration in the reaction mixture was increased twenty fold but the optimal antibody concentration for maximum NP aggregation remained at 1 μM.