Supporting Information

Combining Microfluidic Networks and Peptide Arrays for Multi-Enzyme Assays

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Procedures

Synthesis of peptide substrates for kinase and phophatase assays. Peptide substrates of kinases and phosphatases used in this work were designed based on previously reported optimal substrate sequences.[1] All peptide substrates were prepared on Fmoc-Rink amide MBHA resin (AnaSpec. Inc., San Jose, CA) using an ABI 430A peptide synthesizer and Fmoc solidphase peptide synthesis methods.

Fabrication of PDMS with microchannels. Poly (dimethylsiloxane) (PDMS) was made from 184 Silicone Elastomer Kit (Dow Corning Sylgard Brand, 10:1 base: curing agent ratio). Channels of rectangular cross section (500 μm wide by 50 μm tall by 1.8 cm long) were fabricated using rapid prototyping in PDMS.[2] Non-crosslinked monomers and short oligomers of dimethylsiloxane were extracted by soaking the PDMS stamp in pentane for 1-2 days, then in toluene for 1 day, in ethyl acetate for 1 day, and finally in acetone for 1 day.[3] The PDMS stamp was then rinsed with ethanol and placed in an oven at 80^{o}C for two days. Holes are punched to make the inlets and outlets of the channels in the PDMS using a 19 Ga needle. The interior surfaces of all PDMS channels and Teflon

tubings were blocked with PBS (pH =7) containing 10 mg mL^{-1} bovine serum albumin (BSA) for 1 hour. The channels were emptied and rinsed with PBS prior to use.

Preparation of self-assembled monolayers (SAMs). Self-assembled monolayers of alkanethiolates on gold were prepared using previously reported procedures.[4] Briefly, gold substrates (10 nm Ti and 50 nm Au on microscope cover glass) were immersed for 8 hours in an ethanolic solution containing a symmetric disulfide presenting tri (ethylene glycol) groups and an asymmetric disulfide presenting one maleimide group and one tri (ethylene glycol) group at a molar ratio of 1: 4. The total concentration of disulfides was 1 mM. The monolayers were then rinsed with ethanol and dried under N_2 flow.

Using μFNs to prepare peptide arrays and perform kinase assays on SAMs. A PDMS stamp with microchannels was sealed to the SAMs on gold substrate and clamped between 2 pieces of 1/4" thick polycarbonate using binder clips. Aqueous solutions were introduced to the channels using syringes (Hamilton Company, Reno, Nevada) attached to the channel by 27 gauge Teflon tubings. To prepare the peptide arrays, separate solutions containing 0.2 mM different peptide substrates in 10 mM Tris-HCl buffer (pH 7.0) were filled into the channels manually (0.5 μL for each channel and 1.5 μL for its inlet and outlet). These solutions were incubated with the maleimide-terminated SAMs in the channels for 30 minutes at room temperature. The channels were emptied, removed from the SAMs and washed with PBS. The SAMs were rinsed with distilled water and dried under N_2 flow. The PDMS stamp was then reapplied on top of the SAMs in a perpendicular direction so that each channel intersected the parallel lines of peptides immobilized previously. Buffer solutions containing different enzymes[5] were introduced respectively into the channels at 150 nL min^{-1} for 1 hour at room temperature using PHD

2000 syringe pumps (Harvard Apparatus, Holliston, Massachusetts). Again the channels were emptied and PDMS was removed from the SAMs. The SAMs were rinsed with water and dried prior to MS analysis.

Mass spectrometry. SAMs on gold substrate were treated with an acetone solution containing 2, 4, 6-trihydroxyacetophenone (THAP, 10 mg mL^{-1}), dried and loaded to a Voyager DE-PRO Biospectrometry mass spectrometer (Applied Biosystems, Framingham, MA). A 337 nm nitrogen laser was used as a desorption / ionization source with accelerating voltage at 20 kV and extraction delay time at 100 ns. All the spectra were acquired using reflector mode for positive ions. 300 laser shots were applied for each spectrum obtained by moving the laser beam to several different locations within a crossing section of the orthogonal channels. For quantitation, the completion of each enzymatic reaction was calculated by measuring the relative intensities (peak heights) of the substrate and product ions ($M+H^+$) (extent of reaction = $I_{product}$ / ($I_{substrate}$ + $I_{product}$).

Preparation of human K 562 cell extracts for kinase and phosphatase assays. Human K 562 cells (American Type Culture Collection CCL-243) were grown in Iscove's modified Dulbecco's medium supplemented with 10% fetal bovine serum (FBS) under 5% CO_2 at 37° C. Approximately 10^7 cells in 10 mL culture medium were washed twice with ice-cold PBS and then lysed in 1 mL buffer containing 50 mM Tris-HCl (pH 7.4), 100 mM NaCl, 1mM EDTA, 1% Triton-100, 10% glycerol, 1mM NaF, 1mM NaV_3O_4, 1mM PMSF, 1.5 μg mL^{-1} chymotrypsin, 0.8 μg mL^{-1} thermolysin, 1 mg mL^{-1} papain, 1.5 μg mL^{-1} pronase, 1.5 μg mL^{-1} pancreatic extract and 0.2 8 μg mL^{-1} trypsin. After incubation on ice for 15 minutes, the lysis mixture was centrifuged and extract supernatant was transferred to fresh tubes and used immediately. For testing the

inhibition of Gleevec on intracellular Abl kinase activity, cells were grown in culture medium containing 50 μM Gleevec for 2 hours at 37 °C prior to cell lysis. For testing the inhibition of peroxide vanadate (pV) on intracellular phosphatases, cells were grown in culture medium containing 10 μM pV for 15 minutes at 37 °C prior to cell lysis. For evaluating the inhibition of calynulin A on intracellular phosphatases, cells were grown in culture medium containing 20 nM calynulin A for 1 hour at 37 °C before they were lysed. In kinase and phosphatase assays on μFN-defined peptide arrays, 6 μL cell extracts was diluted 4-fold with assay buffer containing 50mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 1 mM DTT ,100 μg mL^{-1} BSA supplemented with 0.2 mM ATP and the mixture was flowed through a microchannel for 2 hours at room temperature as described previously.

References:

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(5) Kinase concentrations used in Figure 2A and 2B are as following: : Abl 1 U μL^{-1}, c-Src 0.1 U μL^{-1}, CK I 1 U μL^{-1} , Erk-1 75mU μL^{-1}, PKA 60 U μL^{-1} and PKC (catalytic domain) 0.2 mU μL^{-1}. Kinases were purchased from Calbiochem and New England Biolabs Inc.