Supplementary Information:

Measurement of Protein Tyrosine Phosphatase Activity in Single Cells by Capillary Electrophoresis

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MATERIALS AND METHODS

Materials. N-α-(9-fluorenylmethyloxycarbonyl) protected amino acids, 2-(6-Chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU), N-hydroxybenzotriazole (HOBt), 1,3-diisopropylcarbodiimide (DIC), and TentaGel Rink (TGR) resin were obtained from EMD Novabiochem. 6-Carboxyfluorescein (6-FAM) was obtained from Anaspec and recombinant phosphatases were purchased from Millipore. Bovine serum albumin (BSA) was from Calbiochem, Dulbecco's Modified Eagle's Medium (DMEM) from Cellgro, penicillin/streptomycin (PS) and 0.25% Trypsin from Gibco, and fetal bovine serum (FBS) from Atlanta Biologicals. Sylgard 184 polydimethylsiloxane (PDMS) was purchased from Dow Corning. All other chemicals were purchased from either Sigma or Fisher.

Peptide Synthesis and Characterization. Peptide synthesis was accomplished by coupling 5 equivalents (eq) of N-α-Fmoc protected amino acid in N,N-dimethylformamide (DMF) with HCTU (5 eq) and N-methylmorpholine (10 eq) for 20 min. Unreacted amines were then acetylated using 1:1 acetic anhydride in DMF. N-terminal deprotection was achieved using two 15 min incubations with 30% (v/v) piperidine in DMF. Coupling of 6-carboxyfluorescein (3 eq) to the peptide N-terminus was performed manually using DIC (3 eq) and HOBt (3 eq) in DMF for 24 h, followed by two 15 min incubations with 30% (v/v) piperidine in DMF. Cleavage from TGR resin and side-chain deprotection was accomplished by incubating in 95% TFA, 2.5% triisopropylsilane, 2.5% water for 3 h at room temperature. TFA was evaporated under a stream of N_2 gas, followed by peptide precipitation with ice-cold diethyl ether. Peptides were allowed to dry overnight then dissolved in water for purification by C18 reversed-phase HPLC, followed by lyophilization and storage at -20 ^{0}C. Peptide molecular weight was confirmed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry

(MALDI-TOF-MS) using an AB4800 (Applied Biosystems, Carlsbad, CA) and α-cyano-4-hydroxycinnamic acid (CHCA) matrix.

Capillary Electrophoresis. Capillaries were conditioned before use with successive rinses of 0.1 M NaOH (12 h), water (1 h), 0.1 M HCl (6 h), and water (12 h). Laser-induced fluorescence (excitation: 488 nm, detection: 532 nm) was used for detection of fluorescent peptides. On the commercial system, samples were loaded into the capillary by applying positive pressure (0.5 psi for 5 s) to the capillary inlet followed by separation at 8 kV (267 V/cm) with a negative outlet. On the customized system, electrokinetic sample loading (79 V/cm for 5 s, negative outlet) was followed by separation at 10 kV (263 V/cm). Data were acquired with software written in Labview (National Instruments, Austin, TX) or with 32 Karat software (Beckman Coulter, Brea, CA) and analyzed using Matlab (The Mathworks, Natick, MA) and Origin (OriginLab, Northampton, MA) software.

Recombinant Phosphatase Activity Assay. PTP was diluted (11.1 pg/ μL for PTPN1, 2.8 pg/ μL for PTPN2) in 90 μL of reaction buffer (60 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 150 mM sodium chloride, 0.17 mM dithiothreitol, 0.83 (v/v)% glycerol, 0.017 (w/v) % BSA, 0.002% Brij-35, pH 7.2), followed by addition of pTS13 in water to final peptide concentrations of 780 nM or 280 nM for reactions with PTPN1 or PTPN2, respectively, and the reactions were allowed to proceed at 21 °C for 10 min. Reactions were terminated by addition of an equal volume of 200 μM HCl and samples were stored at -20 °C prior to analysis. CE of each sample was performed and the percent dephosphorylated peptide was calculated as the area of the dephosphorylated peptide peak divided by the total area of all peptide peaks on the electropherogram.

Determination of Kinetic Constants. The Michaelis constant, K_M, and maximum reaction velocity, V_{max}, were determined for the interaction of recombinant PTPN1 and PTPN2 with pTS13 by measuring initial reaction rates over a range of pTS13 concentrations. Recombinant PTPN1 (3.3-6.7 pg/ μL) or PTPN2 (0.67-1.7 pg/ μL) in reaction buffer was incubated with pTS13 (110-1390 nM) as described above. The amount of product formed was measured by CE at multiple time points for each substrate concentration and initial rates were plotted versus substrate concentration. Nonlinear regression of the data using the Michaelis-Menten equation was performed using Origin software and kinetic constants were calculated.

Statistical Analysis. The distribution of the difference between the mean %pTS13 remaining in each treatment group and the control group was calculated using bootstrapping.[22] 10,000 bootstrap replicates were sampled (with replacement) from each group and the means of each bootstrap replicate were calculated. The distribution of the mean differences was estimated by the distribution of the differences between the appropriate pairs of bootstrap replicates. 95% confidence intervals for each difference were estimated using the bias-corrected and accelerated (BCa) method. The p-value for testing the null hypothesis of no difference in mean between each treatment group and the control group was estimated by dividing the number of bootstrap differences 0 or less by the total number of bootstrap replicates (i.e. 10,000).