SUPPLEMENTARY METHODS

Fabrication of the microfluidic platform

The microfluidic chip was fabricated using two-layer soft lithography. Two different molds were first fabricated by a photolithographic process to create the fluidic channels and the control channels that actuate the values located in the top and bottom layers, respectively, of the PDMS-based chip (1). The fluidic channel mold was made by a three-step photolithographic process. Both round-profile and square-profile channels were created to interface with fully closing regular valves and sieve values, respectively. Upon closure of the valve, round-profile channels close completely while square-profile values leave a small gap near the right-angle corners (see Fig. 2 of reference (2)). In the first photolithographic step, a 45-μm thick negative photoresist (SU8-2025) was spin coated on to a silicon wafer (Silicon Quest, San Jose, USA). After UV exposure and development, a square-profile channel pattern was obtained to generate sieve valve channels with a width of 200 μm and height of 45 μm. In the second step, through the same process, a second layer of 80-μm thick negative photoresist (SU8-2100) was generated on the same wafer to construct the bead columns and circulation chambers with a square-profile channel width of 400 μm and height of 80 μm. In the third step, a 35-μm thick positive photoresist (AZ 100XT PLP) was spin coated on the same wafer to generate the isolation valve round-profile channels with a channel width of 200 μm and a final height of 45 μm (see below). Each alignment was conducted on a Karl Suss aligner (Karl Suss America Inc., Waterbury, VT) to ensure a good match between layers prior to the UV exposure. Once the positive photoresist was developed, the wafer was heated above the glass transition temperature of the positive photoresist. It is through this process that the surface profile of the patterned positive photoresist is transformed into a round-profile channel (for fully closing regular values) while the profile of the negative photoresist remains unchanged (square profile for sieve values). As a concequence, the 35-μm thick positive photoresist square-profile channel results in a 45-μm round-profile channel. The control channel mold was made by fabricating a

30-μm negative photoresist (SU8-2025) pattern on a silicon wafer. In order to achieve reliable performance of each valve, the width of the control channel was set at 250 μm (30 μm height) in sections where the valve modules are located. Before fabricating the device, both the fluidic and control molds were exposed to trimethylchlorosilane (TMSCl) vapor for 2-3 minutes. Well-mixed PDMS (GE, RTV 615 A and B in 5:1 ratio) was poured onto the fluidic mold located in a Petri dish to give a 5 mm-thick fluidic layer. Another portion of PDMS (GE, RTV 615 A and B in 20:1 ratio) was spin-coated onto the control mold (1,800 rpm, 60 s, ramp 15 s) to obtain the control layer. The thick fluidic layer and thin control layer were cured in an 80 °C oven for 25 and 15 minutes, respectively. Instead of the commonly used 10:1 (A:B) ratio, the two ratios above were found to be the best condition for enhancing the adhesion between the two PDMS layers, as the partially solidified A in the control layer (A:B = 20:1) continues to react with excess component B in the fluidic layer (A:B = 5:1) when they are interfaced. After incubation, the thick fluidic layer was peeled off the mold, and holes were punched into the fluidic layer for access of reaction solutions. The fluidic layer was then trimmed, cleaned and aligned onto the thin control layer. After further baking at 80 °C for 60 minutes, the assembled layer was peeled off the control mold, and another set of holes were punched for access to the control channels. These assembled layers were then placed on top of a glass slide that was spin-coated (6,100 rpm, 60 s, ramp 15 s) with PDMS (GE RTV 615 A and B in 20:1 ratio) that had been oven cured for 10 minutes. The device was completed by overnight curing at 80 °C.

Microfluidic control interface

The pneumatic control setup consists of 4 sets of eight-channel (eight-valve) solenoid-regulated manifolds controlled through a BOB3 breakout integrated circuit controller board (Fluidigm, San Francisco). Compressed air passed through a gas purifier (Hammond Drierit, Xenia, OH) provides pressure (30 psi) to the manifolds. A total of 25 control lines from the

device are individually connected to the corresponding channels on the solenoid manifolds with stainless steel tubes (23 Gauge, New England Small Tube Corp, Litchfield, NH) using Tygon microbore tubing (Cole-Parmer East, Bunker Court, CA) (Supplementary Fig. 2). When a channel on the manifold is activated, air enters the control line connected to the specific channel, providing pressure to close valves in the microfluidic device. The computer control interface incorporates a digital I/O card (AT-DIO-32HS National Instruments, Austin, TX) that digitally controls the switching of manifolds through the BOB3 breakout controller board, and the LabVIEW software program that allows manual control of individual valves and automation of the pumping.

Beta camera spatial calibration and configuration

The raw images of the position sensitive avalanche photodiode (PSAPD) have inherent spatial distortions. A radioactive printout with a known spatial pattern was used as a calibration tool to image these distortions which were then corrected using an inverse mapping method to arrive at the final spatially corrected image. PSAPDs are intrinsically designed for detection of visible light. Therefore, the top surface of the PSAPD was optically passivated with a 9 μm layer of Mylar coated with 3.3 μm of aluminum to allow operation under room light. For each experiment we also used a sacrificial 3 μm Mylar layer to protect the passivation layer and the PSAPD detector.

Beta camera region of interest (ROI) determination

The spatial distribution of the emitted ^{32}P source is a function of the kinetic energy of the emitted particles, the geometry of the volume containing the isotope (i.e. the microfluidic chamber), and the spatial resolution of the beta camera. The spatial distribution of ^{32}P for our system can be estimated from the experimental results of the ivkra system. From the beta camera image of the lower substrate-capture columns the spatial distribution of the source

signal was measured and a line profile was then drawn across the signal to calculate the full-width tenth-maximum (FWTM). A region of interest (ROI) was then drawn with a width equal to the FWTM of each lower column source in order to count a substantial portion of the emitted signal. Identically sized ROIs were used to measure the radioactivity in the calibration experiments as well as to measure all experimental results from the lower columns.

The geometry of the upper circulation columns required drawing larger ROIs to enclose and measure the emitted signal. The ROIs for the upper chambers of each assay unit were of equal size to ensure that the loading of the radioactive ^{32}P into each of the upper chambers was approximately equal.

No spillover corrections were applied to the measurements.

Reagents for *in vitro* kinase radio assays

All assay reagents were purchased from Fisher Scientific (Pittsburgh, PA) unless otherwise specified. For the microfluidic assay, 0.1% n-dodecyl-beta-D-maltoside (DDM) was added to all buffers to prevent bead clumping (indicated by /DDM in the buffer name). 0.1% DDM had no detectable effect on BCR-ABL kinase activity (data not shown). Wash buffer: 50 mM Tris-HCl (pH 7.5), 150 mM NaCl; Wash/TX buffer: wash buffer + 0.05% Triton X-100; Blocking buffer: 1% bovine serum albumin (Sigma-Aldrich, St. Louis) in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% Triton X-100; Kinase wash buffer: 20 mM Tris-HCl (pH 7.5), 15 mM $MgCl_2$, 5 mM β-glycerophosphate, 1 mM EGTA, 0.2 mM Na_3VO_4, 0.2 mM DTT); Kinase assay buffer: kinase wash buffer plus 0.4 μM protein kinase A inhibitor, 4 μM protein kinase C inhibitor, 4 μM calmidazolium, plus cold ATP and [γ-^{32}P]ATP as indicated below; Termination buffer: 75 mM H_3PO_4; Bind & Wash Buffer: 10mM Tris-HCl (pH 7.5), 1mM EDTA, 2.0 M NaCl.

Off-chip *in vitro* kinase radio assays

Protein A/G agarose beads (40-160 μm; Pierce, Rockford, IL) from 40 μl of slurry were pre-washed twice with 200 μl wash/TX buffer. Washed beads were resuspended with 400 μl of 10 μg/ml anti-c-ABL antibody (OP20, Millipore, Temecula, CA) in wash/TX buffer. The beads were coated with antibody overnight at 4 °C with gentle rocking, followed by three washes with 400 μl blocking buffer. The antibody-coated beads were next used to capture kinase from 400 μl (20 μg/μl, or 4×10^7 cell equivalents) of cell lysate during an 8 h incubation at 4 °C with gentle rocking, followed by two washes with 500 μl wash buffer and two with 500 μl kinase wash buffer. After washing, the beads were resuspended in 40 μl kinase wash buffer and warmed to 25 °C. 40 μl kinase assay buffer (containing 200 μM cold ATP and 17 nM [γ-^{32}P]ATP (3 Ci/μMole; PerkinElmer, Waltham)) and 80 μl of 200 μM Abltide-biotin conjugate peptide substrate (Millipore) were added to the resuspended beads and the kinase reaction proceeded for 15 min at 25 °C. The reaction was terminated with 120 μl 75 mM H_3PO_4 and incubation on ice for 15 min. 9 μl of each reaction was spotted to SAM2 Biotin Capture Membrane squares followed by washing and quantitation by scintillation counting per the instructions of the manufacturer (Promega, Madison, WI). The reaction solution was used to determine the specific activity of hot ATP.

Microfluidic *in vitro* kinase radio assays

Protein G-conjugated polystyrene beads (6.7 μm; Spherotech Inc., Lake Forest, CA) were washed twice and resuspend in 50 μl wash/TX/DDM buffer. All microfluidic chip columns were pre-washed with wash/TX/DDM buffer. The pre-washed Protein G beads were loaded into the microfluidic chip filling 4/5th of the upper column (80 nL). Three incubation steps with circulation were performed sequentially. For antibody coating, pre-washed beads were incubated with 20 μg/ml anti-c-ABL antibody in wash/TX/DDM buffer for 30 min at room

temperature (RT). For kinase capture, either undiluted (20 μg/μl) or lysis buffer diluted cell lysate was incubated with the antibody-coated beads for 30 min at RT. During the bead re-trapping and washing steps, the microfluidic chip was cooled by placing it on ice. For the kinase reaction, a mixture of 2.5 parts kinase wash/DDM buffer, 2.5 parts kinase assay/DDM buffer (containing 40 μM cold ATP, or 1.5 μM for drug inhibition experiments (3-5)), 1 part [γ-32P]ATP stock (5 μCi/μL, 3 Ci/μMole, 1.7 μM), 6 parts Abltide-Biotin peptide substrate (50 μM, or 200 μM for linearity experiments) was incubated with the kinase coated beads for 15 min at RT (concentrations listed are prior to fresh mixing) (Supplementary Fig. 1). In each step, the new solvent was loaded into the circulation chamber (370 nL) while the beads were trapped in the upper column. The beads were then released and mixed to homogeneity using the circulation pumping protocol with flow reversal every 80 sec to prevent bead clumping (Supplementary Video 1). Between the first and second incubations the beads and circulation chamber were washed with blocking/DDM buffer, and between the second and third incubations they were washed with kinase wash/DDM buffer. The beads were washed by flowing the wash solvent through the loosely packed trapped beads. Equal loading of the radiolabeled ATP was verified by collecting a 1 min image using the beta charged particle camera (Fig. 2b). During the last incubation, the lower chamber was washed with Bind & Wash/DDM buffer and loaded with streptavidin-coated polystyrene beads (6.7 μm; Spherotech Inc.) that had been prewashed and resuspended in Bind & Wash/DDM buffer. The kinase reaction was terminated and the substrate was captured on the streptavidin beads by using a 75 mM H_3PO_4 solution to push the substrate containing liquid through the lower column using a flow reversal protocol. The streptavidin beads were washed with 75 mM H_3PO_4, and the chip was quantitatively imaged using the beta camera (20 min acquisition).

Prior to developing the bead trap and release methods, we attempted to use chromatography-style methods of passing reagent-loaded solvents through packed beads for

the bead coating and related steps. However, in our hands this resulted in inhomogeneous and inefficient results. The likely explanation is sub-optimal chromatography conditions such as the bead diameter being only approximately 10-fold smaller than the channel width and the use of low pressures (25-30 psi) that are the maximum compatible with integrated PDMS microfluidic chip designs.

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