The image above is a simple cartoon, not drawn to scale, of the differential array platform. The silicon wafer array, with micromachined pyramidal wells for holding the receptor derivatized beads, resides within a flow cell attached to the stereoscope stand. Fluid containing the experimental solutions is added to the top of the array and pushed around and through the bead matrix, and out the bottom of the pyramidal wells holding the beads. A charge-coupled device is fixed to the stereoscope that captures 12-bit RGB images and sends them to Image Pro on the computer for further analysis. Red, green, and blue (RGB) pixel density is extrapolated from the images by drawing an area of interest (AOI) for each bead in the image. The AOI is drawn to cover nearly the entire bead. The extrapolated data is processed using Microsoft Excel and principal component
analysis is completed with Statistica. For a more detailed report see reference 11 and the Instrumentation section of this Supporting Information.

Experimental Details

**Materials.** Chemicals for the synthesis of library 1 were purchased from Novabiochem and used without further purification. All solvents used were obtained from dry stills. The proteins used in the assay were obtained from Sigma-Aldrich and ICN and were used without further purification. Buffer components were of reagent grade. The components and fabrication of the micromachined bead array analysis system have been previously reported.\textsuperscript{11, 12}

**Instrumentation.** 29 beads derivatized with receptor 1 and six N-acylated blank beads were placed into individually addressable etched cavities within a 7 x 5 array on a silicon wafer microchip. The loaded array was placed into a flow cell as described previously.\textsuperscript{11, 12} The flow cell was positioned on an Olympus SZX 12 stereoscope that allowed for bottom illumination of the beads in the array using a General Electric Quartzline lamp as the illumination source. Image capture was performed with a 12-bit DVC 1312C (DVC, Austin, TX) charge-coupled device (CCD) mounted on the stereoscope and interfaced with Image Pro Plus 4.0 software (Media Cybernetics). Introduction of indicator, analyte, buffer, and wash solutions into the flow cell was conducted using an Amersham Pharmacia Biotech AKTA Fast Protein Liquid Chromatograph (FPLC) controlled by Unicorn 3.0 Software.
**Assay Conditions.** Each assay was performed at room temperature under continuous flow conditions. All protein and indicator solutions were buffered with HEPES (25 mM) at pH = 7.40. At a flow rate of 0.25 mL/min, 5 mL of each protein (0.355 µM) sample was loaded into the flow cell. To ensure complete removal of non-specifically bound proteins, a three minute HEPES (25.0 mM, pH = 7.4) wash was employed at 1.0 mL/min. Following this rinse, bromopyrogallol red (3.0 µM) was immediately injected at 1.0 mL/min. Indicator-uptake was monitored by capturing 12-bit images every 2 seconds for a total of 215 images. To repeat the experiment, the array was regenerated by washing for 13.5 minutes with 0.15 M NaOH at 1.5 mL/min, for 22 minutes with 0.30 M HCl at 3.0 mL/min, and finally for 4.5 minutes with 0.40 M NaOH at 1.5 mL/min. A 2 minute buffer rinse at 2.0 mL/min followed to rinse any excess base from the array.

**Data Collection and Processing.** Array images were analyzed by drawing an area of interest (AOI) around each bead and evaluating the average red, green, and blue pixel densities within this AOI. For simplification, only the green channel intensity values were utilized for further analysis. The green channel intensity, $I_G$, was converted to an effective green absorbance, $A_G$, using Beer’s Law ($A_G = -\log \left( \frac{I_G}{I_B} \right)$), where $I_B$ was the average green pixel intensity of a blank N-acylated bead. The N-acylated blank beads remained colorless throughout the assay indicating little to no dye uptake.

**Library Synthesis.** Used the Novasyn TG amino resin purchased from Novabiochem with a loading value of 0.26 mmol/g. Synthesis was completed via the route shown in Scheme 1. Used all common amino acids except cysteine. Each was
Fmoc protected and side chains were appropriately protected with $t$-butyl, Pbf, trityl, or Boc.
Synthesis.

(3) Novasyn TG amino resin 2 (1.2 g, 0.31 mmol) was added to a solid-phase reaction vessel (50 mL reaction vessel with a frit for evacuating reaction solutions and wash solutions) and allowed to swell for 30 min. A solution of Fmoc-Lys(Boc)-OH (0.292 g, 0.62 mmol), 1-hydroxybenzotriazole (HOBt) (0.084 g, 0.62 mmol), benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) (0.322 g, 0.62 mmol), and diisopropylethylamine (DIEA) (0.216 mL, 1.24 mmol) was prepared in DMF (5 mL). This was mixed at room temperature for 4 h. The reaction solution was evacuated and the resin was rinsed with DMF, methanol, methylene chloride (DCM), and hexanes. DMF (5 mL) was added to the resin along with acetic anhydride (0.1 g, 1 mmol) and dimethylaminopyridine (DMAP) (1 mg). This mixed for 30 minutes to cap all unreacted amines. Again the resin was washed as before, and then 20% piperidine in DMF (5 mL) was added to deprotect the Fmoc protecting groups. This mixed for 5 min. Again 20% piperidine in DMF (10 mL) was added and mixed for 5 min. The resin was washed twice with DMF, DCM, methanol, and hexanes. The resin was then dried on the hi-vac.

(4) 3 was initially swelled in THF/DCM (1:1, 5 mL) for 30 min. A solution of 4-nitrophenylchloroformate (0.312 g, 1.55 mmol) in THF/DCM (1:1, 5 mL) and DIEA (0.27 mL, 1.55 mmol) was added to the resin. This mixed for one hour and the solution
was evacuated. The resin was rinsed several times with 1:1 THF/DCM, and then dried on the hi-vac.

(5) 4 was added to a solution of 1,3,5-tris-aminomethyl-2,4,6-triethyl benzene (0.62 g, 2.5 mmol) in THF/DCM (6 mL, 1:1) and DMF (4 mL). This mixed overnight and was evacuated. The resin was then washed with DMF followed by 5% butylamine in DMF until the solution remained colorless. The resin was then washed twice with methanol, DCM, and hexanes. The resin was dried on the hi-vac.

(7) 5 was swollen in dry DMF (5 mL) for 30 min. A solution of 6 (1.45 g, 2.2 mmol), PyBOP (1.14 g, 2.2 mmol), HOBt (0.30 g, 2.2 mmol), and DIEA (0.76 mL, 4.4 mmol) was prepared at 0 ° C in DMF (5 mL) as the solution tends to warm upon addition of DIEA. After the solution cooled to room temperature it was added to the swollen resin. It was spun for 4 h. and then the solution was evacuated. A Kaiser test was performed and was negative. The resin was washed with methanol, DCM, DMF, and hexanes. The Fmoc groups were removed with 20% piperidine in DMF (10 mL). The resin was washed twice with methanol, DCM, DMF, and hexanes. A Kaiser test was performed and was positive. The resin was dried on the hi-vac.

(8) The library was synthesized using standard split-and-pool combinatorial chemistry. Resin 7 was split into 19 equal portions. One of the 19 amino acids (0.47 M), HOBt (0.47 M), DIEA (0.47 M), and PyBOP (0.47 M) solutions were added to each of the resin portions and mixed overnight in DMF. The reaction solution was evacuated and the resin
washed with methanol, DCM, DMF, and hexanes. DMF (5 mL) was then added to the resin along with acetic anhydride (0.1 g, 1 mmol) and DIEA (0.47 M). Following evacuation and rinses, 20% piperidine in DMF (5 mL) was added and mixed for 10 minutes. This was evacuated and the resin was rinsed with methanol, DCM, DMF, and hexanes. All 19 portions of the resin were mixed together once again and then split again into 19 equal portions. The synthetic split-and-pool procedure was performed until three amino acids were added to both arms of the receptor. The acid-labile protecting groups on the amino acid side chains were removed using a TFA/water/1,2-ethanediol/triisopropylsilane (94:2.5:2.5:1, 12 mL) solution. The resin was washed twice with DCM, methanol, and hexanes, thoroughly dried on the hi-vac, and subsequently used in the array.