Supplementary Methods and Results

The Cys-AL (CGGAPTYSPPPPPLL) and Cys-AT (CEAIYAAPFAKKK) peptides were tethered to the surface *in trans* through sequential Michael additions via the crosslinker bisacrylamide. The kinase reaction and chemiluminiscent detection were performed as described in Materials and Methods. Cys-AT at 1 mM showed a moderate phosphorylation signal while Cys-AL at the same concentration was not phosphorylated (Fig. S1). Combining 1 mM Cys-AT with 1 mM Cys-AL increased the signal roughly two fold while combining 0.25 mM Cys-AT with 0.25 mM Cys-AL resulted in a signal that was still stronger than 1 mM Cys-AT alone.

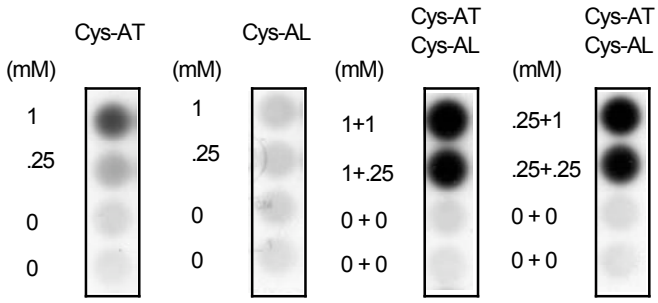


Fig. S1. Enhanced phosphorylation of Cys-AT with Cys-AL. Cys-AT and Cys-AL alone and combination of Cys-AT and Cys-AL in the indicated concentration were immobilized to hydrogel surface, followed by the kinase assay with 25 µg of K562 cell lysate.

To quantitate peptide immobilization, we synthesized Cys-AT with biocytin replacing the terminal lysine (Cys-AT-Biocytin, CEAIYAAPFAKKB). The density of peptide immobilized on the surface is low, in the picomole range, thus it cannot be measured with typical detection methods. Briefly, after the plate was activated, either 50 µl SR415 or bisacrylamide were added at 25 mM. The Cys-AL and Cys-AT-Biocytin peptides were added to the hydrogel plate in 1:1 ratio at 0.5, 0.05, or 0 mM. The plate was again washed and then blocked overnight with 10% BSA in 50 mM TBS, pH 7.5 at 4°C. The FluoReporter kit (Invitrogen, Carlsbad, CA, U.S.A.) was then used to determine the amount of biocytin bound as a direct

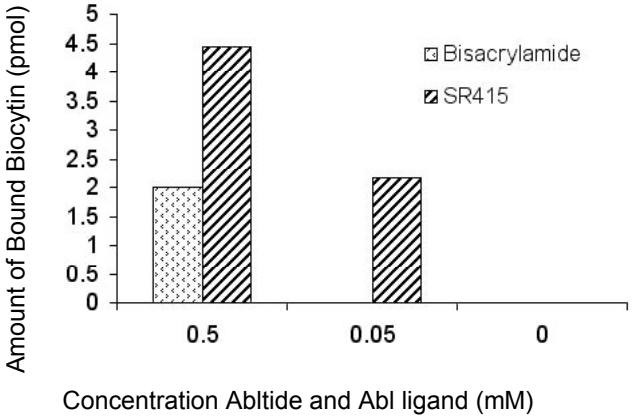


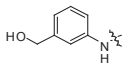
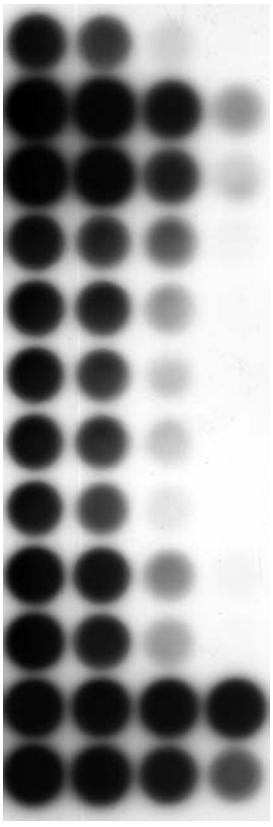
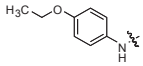
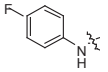
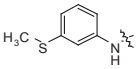
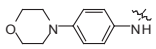
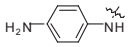
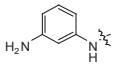
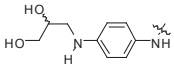
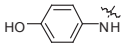
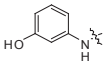
Fig. S2. Comparison of the binding capacity of Cys-Abltide with Biocytin at C-terminus via different crosslinkers. The peptide was immobilized to the hydrogel plate as described in Materials and Methods. Cys-AL and Cys-AT-Biocytin peptides were attached at 0.5, 0.05, or 0 mM to the hydrogel plate via biscarylamide or SR415. The FluoReporter kit was used to determine the amount of Biocytin present on the surface.

measure of tethered Cys-AT. First, 50 µl per well 1X PBS was added to each well. Next, 50 µl per well 2X Biotective Green reagent was added. After a five minute incubation at room temperature, the fluorescence was read with a Bio-Rad Molecular Imager FX using excitation at 488 nm and emission at 532 nm. A standard curve was set up to evaluate biocytin levels. The FluoReporter kit showed SR415 significantly increased the amount of Cys-AT-Biocytin on the hydrogel surface compared to bisacrylamide (Fig. S2).

A screen of all 27 pyridopyrimidine compounds from the focused library is shown in Fig. S3.

We also demonstrated that the assay could be used to compare the effects of weaker small-molecule inhibitors on Bcr-Abl kinase activity. Here, K562 cell extracts were treated with imatinib (5 µM) and the 80 inhibitors from the BioMol ScreenWell™ Kinase Inhibitor Library (200 µM each). None of the compounds in the ScreenWell library are potent inhibitors of c-Abl or Bcr-Abl, although PP1 and PP2 are known Src family kinase inhibitors. In the presence of imatinib the majority of compounds appeared to have no further inhibitory effect on Bcr-Abl activity; however, several compounds diminished Cys-AT phosphorylation significantly. In particular, the inhibitors staurosporine (compound number 6), AG1478 (#18), damnacanthal (#22), PP1 (#24), and PP2 (#52) each appeared to decrease Bcr-Abl activity markedly compared to imatinib (5 µM) alone (Fig. S4 and Table.S1).

Screen of 27 candidate Abl/Bcr-Abl inhibitors for apparent IC_{50} values

No	Inhibitor	R Group	1 10 10^2 10^3 (nM)	IC_{50} (nM)
1	PD166326			~20
2	PD173958			~500
3	PD173956			~300
4	PD173955			~100
5	PD173952			~35
6	DV1-10			~20
7	DV2-43			~20
8	DV2-281			~15
9	DV2-47			~45
10	DV2-89			~40
Without compound				
IM				

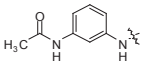
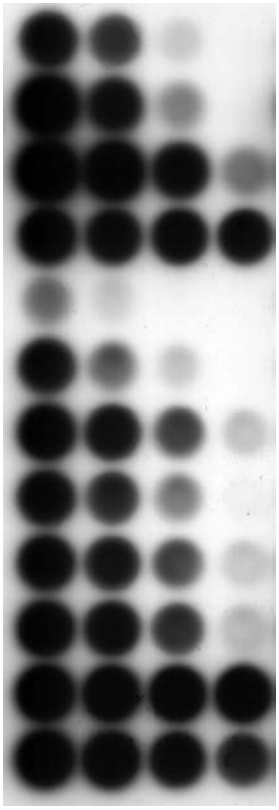
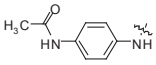
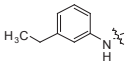
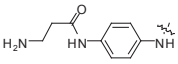
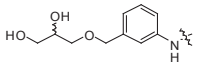
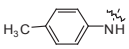
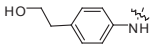
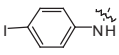
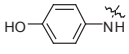
11	DV-M016			~20
12	DV-M017			~55
13	DV2-87			~1000
14	DV2-103			>1000
15	DV2-273			<1
16	DV2-271			~8
17	DV3-081			~150
18	DV3-096			~40
19	DV2-37			~150
20	DV2-53			~300
Without compound				
IM				

Fig S3A

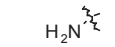
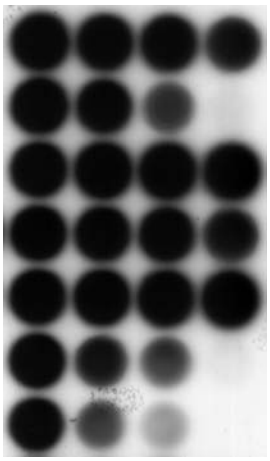
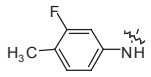
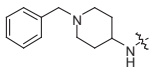
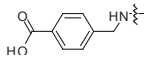
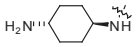
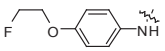
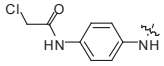
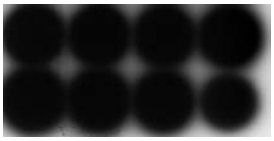
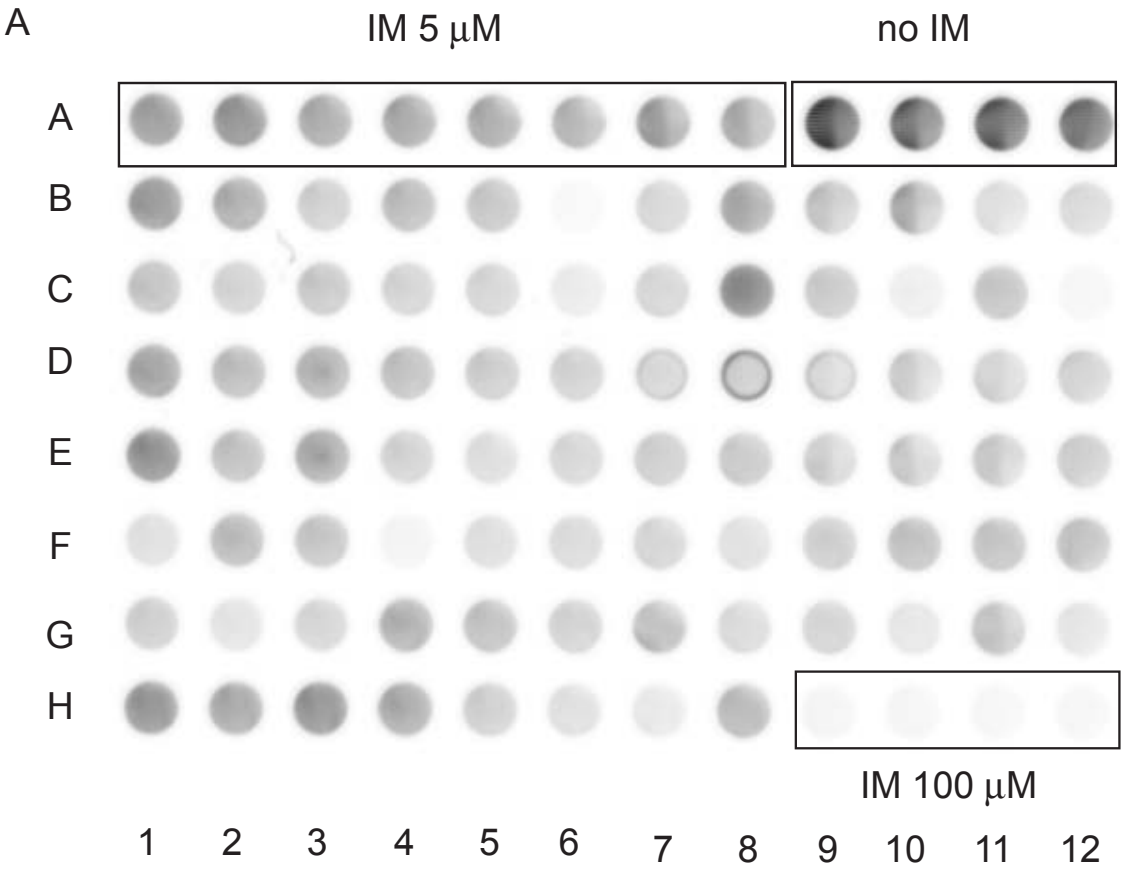
No	Inhibitor	R Group	1 10 10^2 10^3 (nM)	IC_{50} (nM)
21	DV2-287			>1000
22	PD180970			10-100
23	DV2-167			>1000
24	DV2-171			>1000
25	DV2-155			>1000
26	DV5-31			~200
27	DV2-289			~20
Without compound				
IM				

Fig S3B

The screen was performed using the standard assay conditions: 25 μl 0.05 mM Cys-AT and Cys-AL were bound to a hydrogel plate with SR415 as the crosslinker. During the kinase reaction, 0.005 U c-Abl were treated with a titration of each compound (1, 10, 100 or 1000 nM) for 1 h at 30°C with 10 μM ATP, where without compound is DMSO only and IM is imatinib mesylate. Detection was done with chemiluminiscence. The x-ray film was scanned and the density of each spot was quantified by Quantity one software. The relative density of each spot was obtained by normalized to the untreated control and apparent IC_{50} calculated as the concentration of the compounds corresponding to a 50% decrease in relative density.

Fig S4. Model screen with BioMol Screen-Well^{TM} Kinase Inhibitor Library. (A) Kinase assays were with K562 cell lysates with 200 μM each compound from the Library (B1 to H8) in the presence of 5 μM IM. (B) The relative fluorescence reading is calculated by: (fluorescence reading of the compound)/ (mean fluorescence at 5 μM IM) *100. The solid line is the mean of all the compounds and the dashed lines represent one standard deviation (SD).



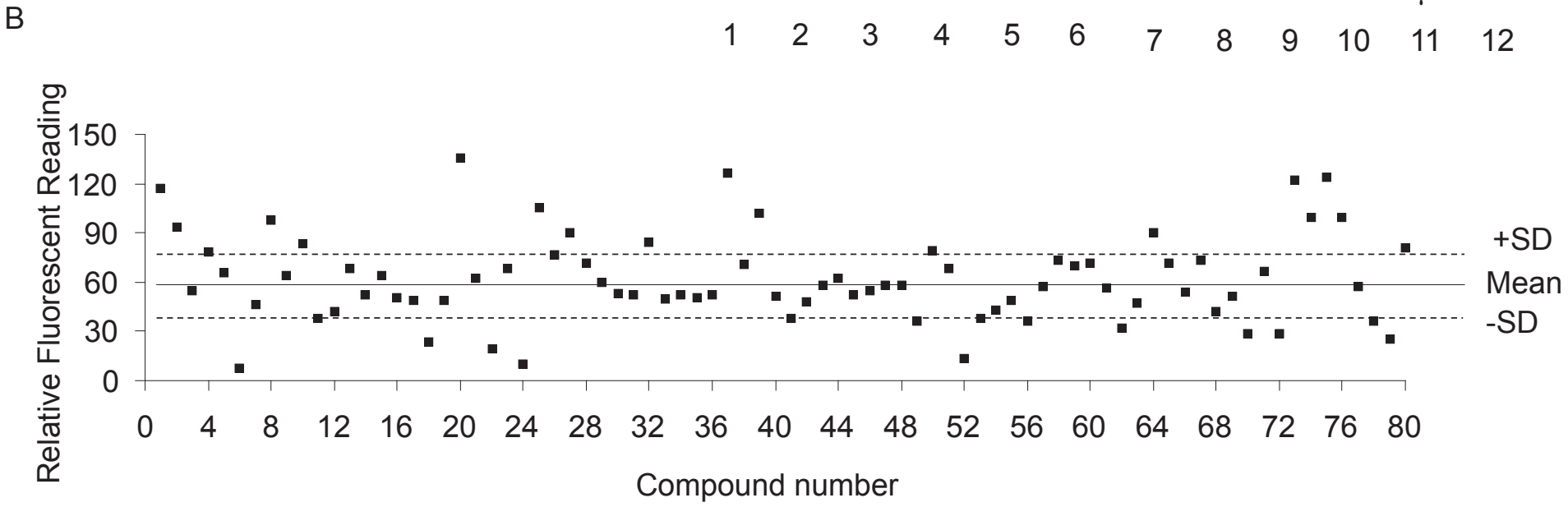


Table S1 The BioMol ScreenWell^{TM} compounds name and plate location

Number	Plate Location	Inhibitor Name
1	B1	PD-98059
2	B2	U-0126
3	B3	SB-203580
4	B4	H-7
5	B5	H-9
6	B6	Staurosporine
7	B7	AG-494
8	B8	AG-825
9	B9	Lavendustin A
10	B10	RG-14620
11	B11	Tyrphostin 23
12	B12	Tyrphostin 25
13	C1	Tyrphostin 46
14	C2	Tyrphostin 47
15	C3	Tyrphostin 51
16	C4	Tyrphostin 1
17	C5	Tyrphostin AG 1288
18	C6	Tyrphostin AG 1478
19	C7	Tyrphostin AG 1295
20	C8	Tyrphostin 9
21	C9	HNMPA
22	C10	Damnacanthal
23	C11	Piceatannol
24	C12	PP1
25	D1	AG-490
26	D2	AG-126
27	D3	AG-370
28	D4	AG-879
29	D5	LY 294002
30	D6	Wortmannin
31	D7	GF 109203X
32	D8	Hypericin
33	D9	Ro 31-8220
34	D10	Sphingosine
35	D11	H-89
36	D12	H-8
37	E1	HA-1004
38	E2	HA-1077
39	E3	HDBA
40	E4	KN-62

Number	Plate Location	Inhibitor Name
41	E5	KN-93
42	E6	ML-7
43	E7	ML-9
44	E8	2-Aminopurine
45	E9	N9-Isopropyl-olomoucine
46	E10	Olomoucine
47	E11	iso-Olomoucine
48	E12	Roscovitine
49	F1	5-Iodotubercidin
50	F2	LFM-A13
51	F3	SB-202190
52	F4	PP2
53	F5	ZM 336372
54	F6	SU 4312
55	F7	AG-1296
56	F8	GW 5074
57	F9	Palmitoyl-DL-carnitine Cl
58	F10	Rottlerin
59	F11	Genistein
60	F12	Daidzein
61	G1	Erbstatin analog
62	G2	Quercetin dihydrate
63	G3	SU1498
64	G4	ZM 449829
65	G5	BAY 11-7082
66	G6	DRB
67	G7	HBDDE
68	G8	SP 600125
69	G9	Indirubin
70	G10	Indirubin-3'-monooxime
71	G11	Y-27632
72	G12	Kenpaullone
73	H1	Terreic acid
74	H2	Triciribine
75	H3	BML-257
76	H4	SC-514
77	H5	BML-259
78	H6	Apigenin
79	H7	BML-265 (Erlotinib analog)
80	H8	Rapamycin