Supporting Information

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A Peptide Photoaffinity Probe Specific for the Active Conformation of the Abl Tyrosine Kinase

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SUPPLEMENTAL FIGURES

Figure S1. Sensitivity of Abl detection with ABL-Z probe. Purified WT Abl was added in the indicated amounts to cleared HEK293T cell lysate (2 mg/mL protein) and treated with ABL-Z probe. Following irradiation, reactions were treated with avidin beads to precipitate the probe and associated Abl. Avidin bead samples (top) and lysates prior to avidin treatment (bottom) were immunoblotted to detect Abl. Amounts of Abl in the range of 0.3 – 3 ng (2.4 – 24 fmol) are detectable.

Figure S2. Inhibition of Abl by STI571 and dasatinib. WT Abl from the lysate shown in Figure 3 in the main text was isolated by FLAG affinity purification and subjected to radiolabel kinase assay using ABL tide as the substrate (peptide) in the presence or absence of dasatinib or STI571 (10 µM).
**EXPERIMENTAL PROCEDURES**

**Peptide synthesis.** Protected amino acids and resin for solid phase peptide synthesis were ordered from Anaspec or Advanced Chemtech, DMF was from American Bioanalytical, and DCM was from Sigma. Peptides and photoaffinity labels were synthesized on Wang resin preloaded with lysine or ε-lysylbiotin using standard Fmoc chemistry in DMF on a Thuramed Tetras automated peptide synthesizer. Peptides were cleaved from the resin in 85% TFA/5% water/5% phenol/5% trisopropylsilane and precipitated with cold ether. Precipitated peptide was air dried, suspended in 50% acetonitrile/H2O, lyophilized to dryness and then purified by C18 reverse-phase HPLC in acetonitrile/water/0.1%TFA. Peptide concentrations were determined by measuring the absorbance at 280 nm. Purified peptides were characterized by MALDI-TOF mass spectrometry (Voyager DE PRO): calculated m/z for ABL-Z ([M+H]+) 2029.4, found 2029.6; calculated m/z for ABLtide ([M+H]+) 1530.8, found 1531.2; calculated m/z for scrambled ABLtide ([M+H]+)1530.8, found 1531.25. For ABL-Z we also observed a peak in the MALDI-TOF mass spectrum corresponding to the expected photolysis product with the phenyl azide converted to the corresponding aniline (calculated m/z 2003.4, found 2003.7), a likely consequence of MALDI irradiation. We further confirmed the identity of ABL-Z by ESI-MS: calculated m/z for ABL-Z ([M+2H]+) 1015.2, found 1015.0.

**Kinase expression.** Full-length, C-terminally His6-tagged WT mouse Abl protein was expressed in baculovirus-infected Hi5 insect cells and purified using Ni-nitrilotriacetic acid resin as previously described.[1] Insect cell produced Abl was used for all experiments using WT Abl exclusively.

For expression in mammalian cells, the coding sequence for full length mouse Abl (isoform IV encoding the N-terminally myristylated form) was amplified by PCR using the following primers: 5’-GTCAGGATCCACCATTGGGCCAGCAGCCTG-3’ (forward) and 5’-CTGAGCGGCGCCACCTCCGGACAATGTCGC-3’ (reverse). The PCR product was subcloned into the BamHI and NotI sites of a modified pcDNA3 vector engineered to encode C-terminal His6 and FLAG epitope tags. The GPP (G2A/P242E/P249E) mutant was generated by the QuikChange method (Stratagene) and confirmed by sequencing. WT and mutant Abl were prepared by overexpression in human embryonic kidney HEK293T cells followed by FLAG affinity purification. Plasmids were transiently transfected using Lipofectamine2000 (Invitrogen) into HEK293T cells in 6 cm plates. 48 hours following transfection, each plate was lysed in lysis buffer (500 µL, 1% Triton X100, 20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na3VO4, 1 mM DTT, 1 mM PMSF, 10 µg/mL leupeptin, 2 µg/mL pepstatin A, and 10 µg/mL aprotinin). Cleared lysates were tumbled with M2 FLAG affinity resin (Sigma, 15 µL per plate) for 2 hr at 4°C. Beads were washed twice with lysis buffer and then twice with wash buffer (50 mM HEPES, pH 7.4, 100 mM NaCl, 5 mM β-glycerophosphate, 0.1 mM Na3VO4, 0.01% Igepal CA630, 10% glycerol) and then protein was eluted with FLAG peptide (0.5 mg/mL, 20 µL per starting plate) in wash buffer. To prepare phosphorylated Abl, prior to elution beads were equilibrated to λPP buffer (20 mM Tris, pH 7.5, 100 mM NaCl, 5 mM DTT, 0.1 mM EDTA, 2 mM MnCl2, 0.05% Triton X100) then treated with λPP (400 U, New England Biolabs) in λPP buffer (100 µL) for 30 min at room temperature. Beads were then washed twice with wash buffer and eluted as described above. Abl protein was quantified by scanning a Coomassie-stained polyacrylamide gel with a LI-COR Odyssey imager using BSA as a standard.
The Gateway entry vector pDONR221 harboring the coding sequence for full length human Src was purchased from Life Technologies. The Src coding sequence was recombined using LR clonase (Life Technologies) as directed by the manufacturer into the vector pDEST27, a mammalian expression vector producing N-terminal GST fusion proteins. To produce Src kinase, HEK293T cells were transiently transfected with pDEST27-Src and cell lysates prepared as described above for expression of Abl. Cleared lysates were tumbled with Glutathione Sepharose 4B beads (GE Healthcare, 15 µL per plate) for 2 hr at 4°C. Beads were washed as described above for Abl purification, and then protein was eluted with reduced glutathione (two volumes, 6 mg/mL in wash buffer).

**Photoaffinity labeling.** Purified His-tagged Abl (10 µg/mL) was incubated with ABL-Z (5 µM) in reaction buffer (50 µL, 20 mM Tris, pH 7.5, 100 mM NaCl, 10 mM MgCl₂, 100 µM AMP-PNP) in the presence of ABLtide or scrambled peptide competitor at the indicated concentration on ice for 10 min. Samples were UV irradiated (254 nm, 200,000 µJ/cm², 5 sec) in a Stratalinker (Stratagene). 4x SDS-PAGE loading buffer (12.5 µL) was added immediately and samples heated to 95 °C for 2 min. Samples were subjected to SDS-PAGE in duplicate and transferred to a PVDF membrane, which was probed with either streptavidin-HRP (Millipore) or anti-Abl (K-12 rabbit polyclonal antibody from Santa Cruz Biotechnology). Labeling of purified FLAG-tagged Abl (WT and mutant) was done in the same manner except that reduced glutathione (5 mM) was included in the reaction buffer and irradiation was carried out for 1 min.

For labeling reactions done with cell lysates, expression vectors for FLAG-tagged WT or mutant Abl or empty control plasmid were transiently transfected into HEK293T cells as described above. Lysates were prepared as above except that DTT was left out of the lysis buffer. Cleared lysates (150 µL) were preincubated with STI571 (10 µM) or dasatinib (1 µM) for 30 min on ice where indicated, and then treated with probe and irradiated as described above. After labeling, the reaction was quenched by adding β-mercaptoethanol (10 µL). A portion (20 µL) of the reaction was set aside for detection of total Abl in the cell lysate, and the remainder was diluted with lysis buffer to 1 mL and treated with NeutrAvidin agarose resin (Thermo Scientific, 25 µL) for 1 hr at 4 °C with tumbling. Beads were then washed three times with lysis buffer (1 mL) and then boiled 10 min in 1X SDS-PAGE loading buffer (100 µL). Lysate and bead samples (10 µL) were subjected to SDS-PAGE and probed for biotin and Abl as above. For the experiment shown in Figure S1, reactions were carried out in an identical manner except that the indicated amount of Abl kinase purified from insect cells was added to lysates before the addition of probe.

**Peptide kinase assays.** Abl kinase activity was determined by radiolabel kinase assay using the ABLtide substrate. Kinase (18 ng in 30 µL kinase reaction buffer, 20 mM Tris, pH 7.5, 100 mM NaCl, 10 mM MgCl₂, 50 µM ATP with 0.5 µCi/µL [γ-³²P]-ATP) and ABLtide (10 µM) were incubated at 30 °C for 5 min, and portions (20 µL) of each reaction were transferred to a P81 phosphocellulose filters. After extensive washing with 0.85% H₃PO₄, filters were immersed briefly in acetone, air-dried, and analyzed by scintillation counting.
SUPPLEMENTAL REFERENCES