Supporting Information for
"Photocleavable peptide hydrogel arrays for MALDI-TOF analysis of kinase activity"

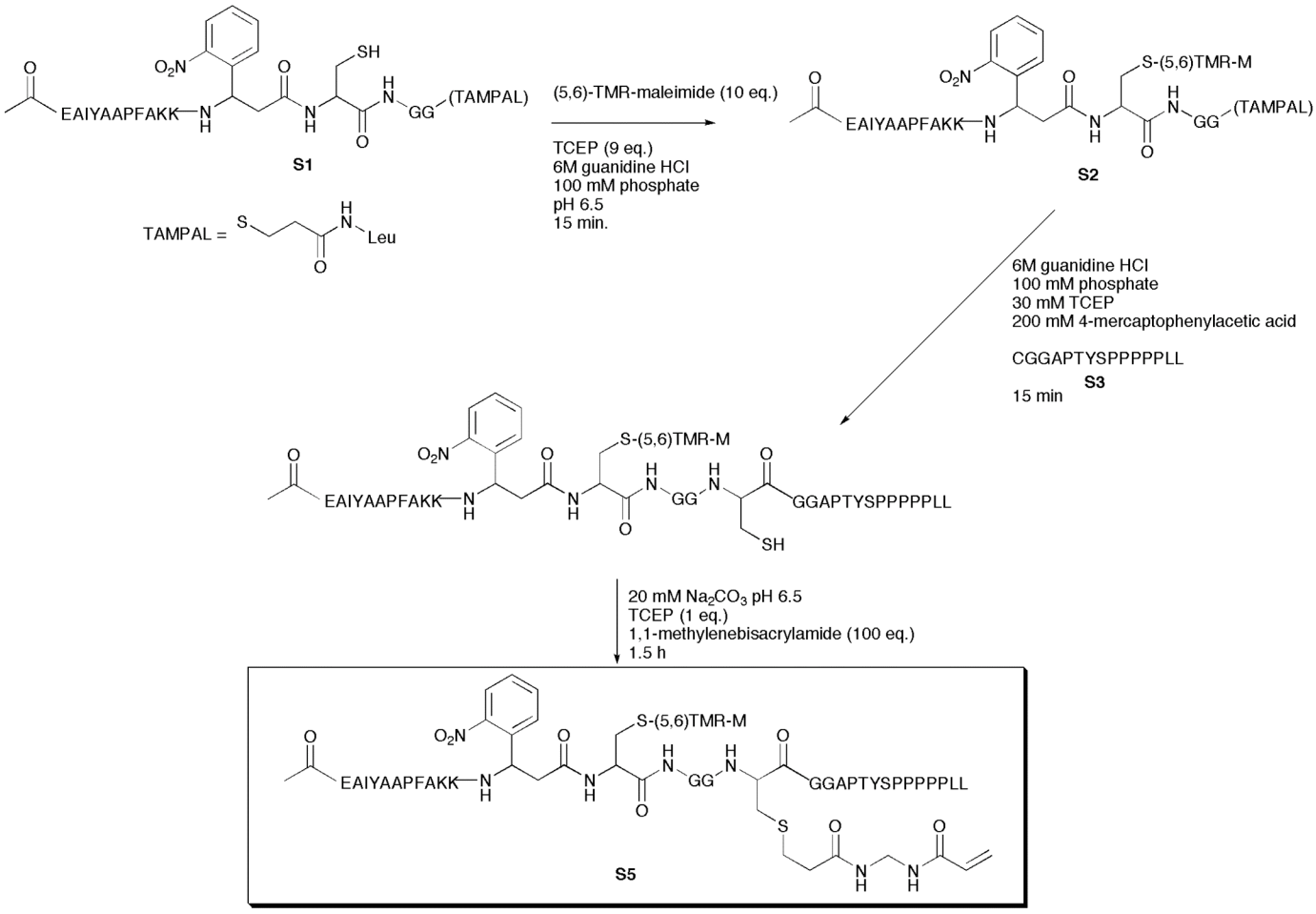
Laurie L. Parker, Shawn B. Brueggemeier, Won Jun Rhee, Ding Wu, Stephen B. H. Kent, Stephen J. Kron and Sean P. Palecek

Contents

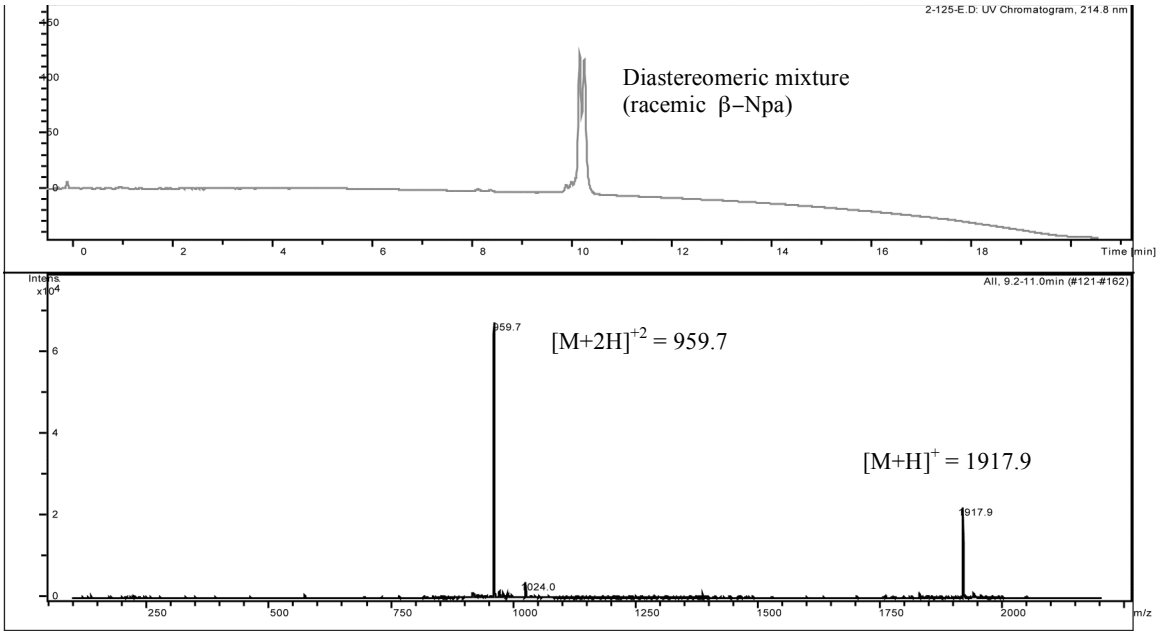
1. Peptide construction and characterization

Peptides were synthesized using Boc SPPS and purified with RP-HPLC as described in the methods section. The reaction scheme for construction of the peptide is given in Scheme S2. The Abltide substrate peptide EAIYAAPFAKK-β–Npa-CGG-COSR (**S1**) was synthesized as a C-terminal TAMPAL thioester, with the photocleavable amino acid between the substrate sequence and the thioester moieties. This thioester peptide was labeled with (5,6)-tetramethylrhodamine maleimide and purified by RP-HPLC (**S2**). The labeled peptide was then conjugated to the c-Abl SH3 ligand peptide CGG*APTYSPPPPP*LL (**S3**) using native chemical ligation to give **S4**. LC-MS data for **S1-5** are given below. UV chromatograms were generated for 214 nm absorbance of peptide bonds, and MS data represents the average mass spectrum across the entire HPLC peak for the peptide.

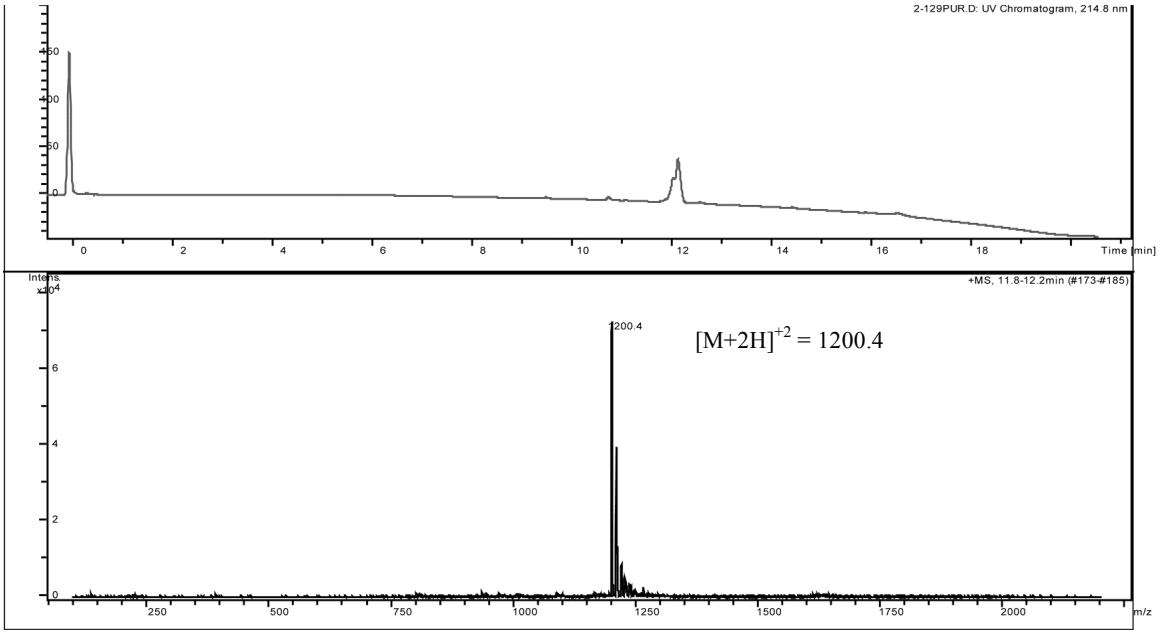
Scheme S1.



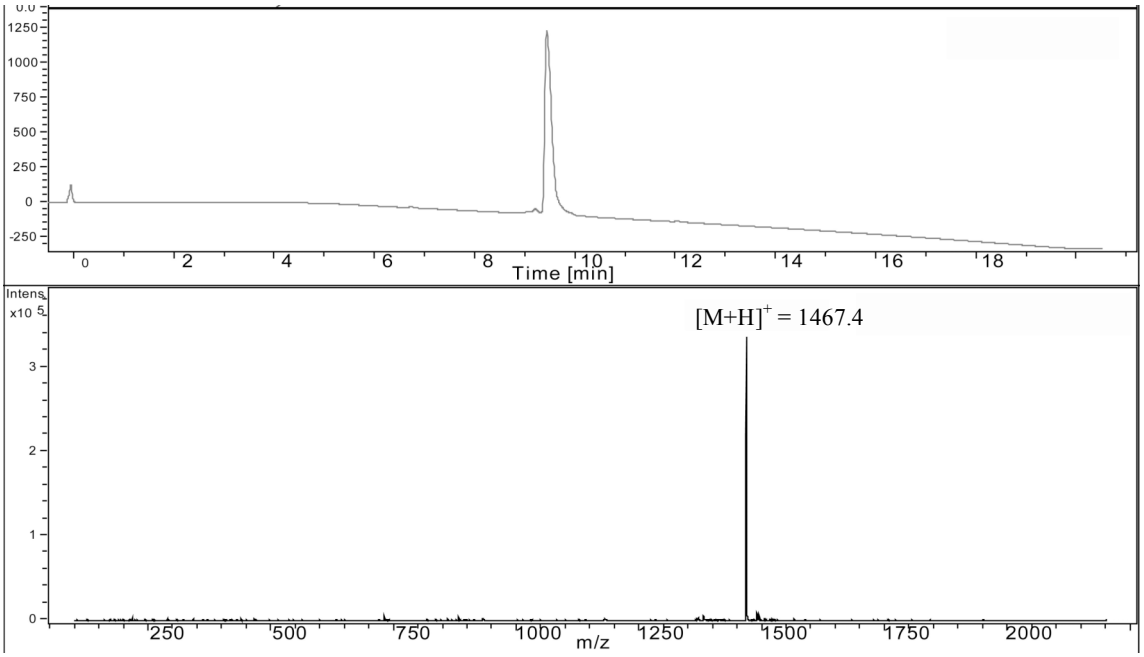
S1.


2-125-E.D: UV Chromatogram, 214.8 nm
Diastereomeric mixture
(racemic β–Npa)
Time [min]
All, 9.2-11.0min (#121-#162)
959.7
[M+2H]+2 = 959.7
[M+H]+ = 1917.9
1917.9
1024.0
m/z

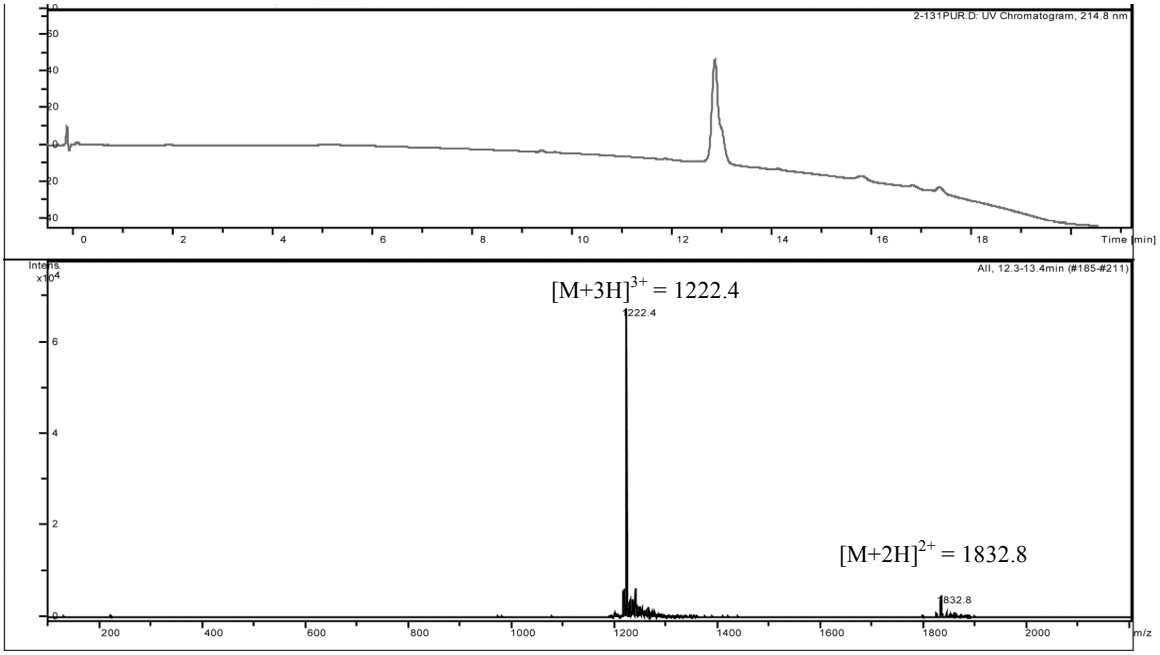
S2.


2-129PUR.D: UV Chromatogram, 214.8 nm
Time [min]
+MS, 11.8-12.2min (#173-#185)
1200.4
[M+2H]+2 = 1200.4
m/z

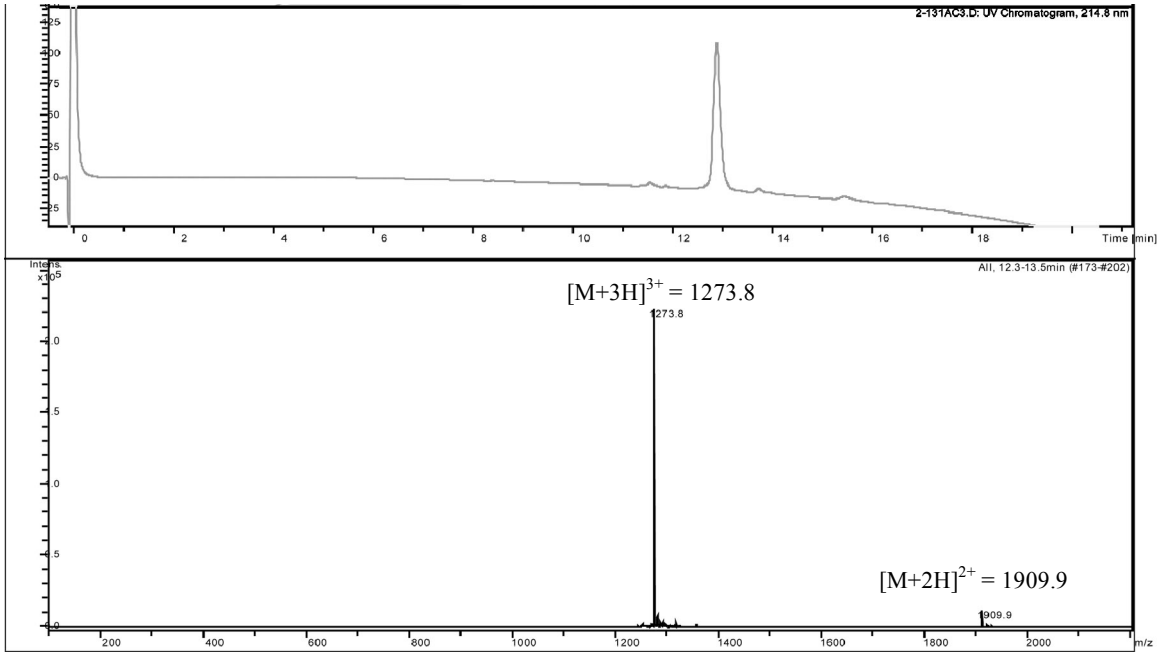
S3.


Time [min]
Intens.
x10 5
[M+H]+ = 1467.4
m/z

S4.


2-131PUR.D: UV Chromatogram, 214.8 nm
Time [min]
All, 12.3-13.4min (#185-#211)
Intens.
x10^4
[M+3H]3+ = 1222.4
1222.4
[M+2H]2+ = 1832.8
1832.8
m/z

S5.



2. Enzyme specific activity determination for these studies

The specific activites of the preparations of enzymes (v-Abl and c-Abl) used in these studies was determined in solution for the particular phosphorylation motif (EAIYAAPFAKK) and kinase buffer conditions, and data semi-quantitation and normalization techniques, in order to have relevant information for normalization of their relative activities on the surface. The kinase reactions were carried out in solution using 50 μM peptide (Abltide substrate sequence CKGEAIYAAPFAKKKG) in the same kinase buffer and ATP concentration as for the surface reactions (see materials and methods in the main meanuscript text) and three concentrations of enzyme. The reaction was quenched after 5 minutes in order to ensure less than 10% activity (necessary for obtaining relevant kinetic information) and analyzed by spotting in triplicate on a MALDI target and analyzing in linear positive and linear negative modes. The % phosphorylated peptide signal observed was normalized as described in the materials and methods. The % phosphorylated peptide signal observed per pmol kinase was defined as the "units" (U) of activity for that kinase. For the enzyme preparations used here, the activity of c-Abl was found to be ~4-5 fold higher than that for v-Abl (Fig. S1). Thus the amount of phosphorylated product observed for the kinase reactions on the slides was normalized to the % product observed per unit of kinase. While this is not an absolutely quantitative measure of specific activities, it is the most relevant way to assess the activities of the kinases for reactions that are analyzed in the same way, as in our experiments.

Fig. S1. Enzyme specific activity measurements for these experiments.