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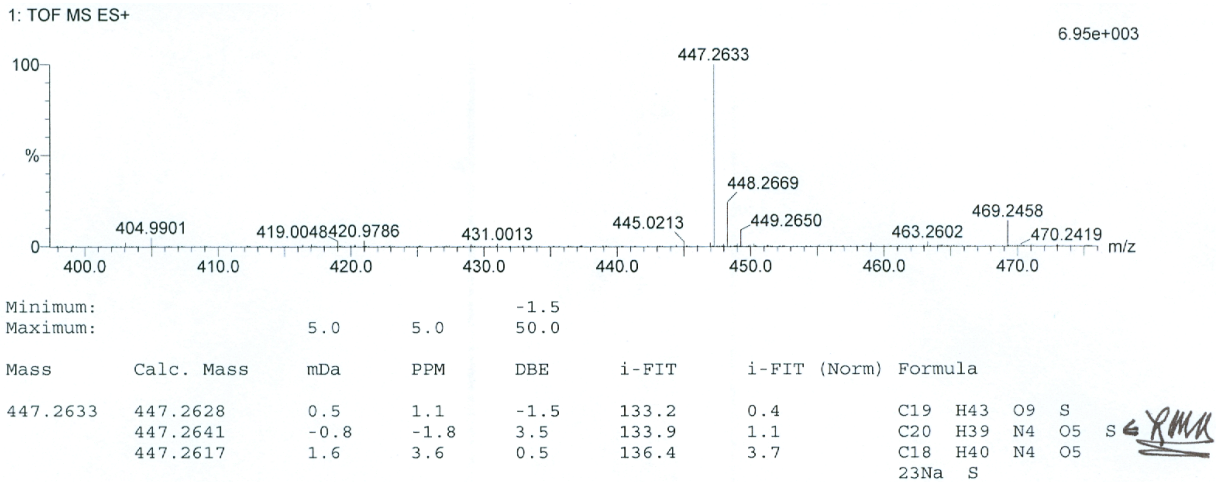
I. Materials and Instrumentation

Biotin, triethyl amine, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), 4, 7, 10-trioxa-1, 13-tridecanediamine, diisopropylethylamine (DIPEA), N, N-dimethylformamide (DMF), and pyrrolidine were purchased from AcrosAdenosine 5'-triphosphate (ATP), glycerol, sodium hydroxide (NaOH), potassium chloride (KCl), magnesium chloride ($MgCl_2$), sodium chloride (NaCl), sodium dodecyl sulfate (SDS), glacial acetic, acetonitrile, staurosporine and SA-Cy5 conjugate were purchased from Fisher. HPLC grade trifluoroacetic acid (TFA) and acetonitrile were obtained from VWR and EM Millipore respectively. N, N-dimethylformamide (DMF) was purchased from Acros Organics. D_2O and CD_3OD were obtained from Cambridge Isotope Labs. Ammonium bicarbonate, β-casein and myelin basic protein were bought from Sigma. PKA, CK2, Abl, CIP, PP1, TC PTP enzymes, sodium fluoride, sodium orthovanadate and Abl peptide were purchased from New England Biolabs. Kemptide and CK2 peptides were bought from Promega. Triton X-100 was purchased from Fluka. HeLa cells were obtained from the National Cell Culture Center (www.nccc.org). Protease inhibitor cocktail was purchased from Calbiochem, and Bovine serum albumin (BSA) was obtained from Santa Cruz Biotechnology. Immobilion P^{sq} PVDF membrane was purchased from Millipore. Sypro Ruby and Pro-Q diamond stain was obtained from Invitrogen. 2-D gel starter kit, ReadyStrip IPG strip (pH 3-10), Ready Gel Tris-HCl Gel were purchased from Bio-Rad.

^{1}H NMR, ^{13}C NMR and ^{31}P NMR were recorded on Varian Mercury (400 MHz) spectrometer. IR spectra were carried out in $CHCl_3$ on FT/IR-460 plus (JASCO Co. Ltd.) spectrometer. High resolution mass spectra (HRMS) were obtained on LCT Premier XT (Waters). HP 8452A Diode array UV-Vis spectrophotometer was used to measure absorbance of ATP-biotin. The purified final product was lyophilized using VirTis BT 3.3 EL Benchtop lyophilizer. A SPD131 DDA ThermoSavant speed vac was used to evaporate solvents *in vacuo*. Quantitative mass spectrometric analysis was performed on MALDI-TOF MS (Bruker Ultraflex). The absorbance values for Bradford assay were measured with a fluorimeter (GENios Plus Tecan). The SDS-PAGE apparatus was purchased from BioRad (Protean III) and a mini-gel setup was used. Western blotting was carried out using the Mini-Transblot Electrophoretic Transfer Cell apparatus from BioRad. Protein IEF cell (Bio-Rad) was used for 2-D gel analysis. The peptides were analyzed using Waters 1525 binary HPLC pump and Waters 2998 photodiode array detector. Reverse phase C-18 column (YMC America, INC 250×4.6 mm, 4μm, 8 nm) was used to separate peptide mixtures. The separated proteins were visualized using a Typhoon 9210 scanner (Amersham Biosciences).

II. Compound Characterization



Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
447.2633	447.2628	0.5	1.1	-1.5	133.2	0.4	C19 H43 O9 S
	447.2641	-0.8	-1.8	3.5	133.9	1.1	C20 H39 N4 O5 S
	447.2617	1.6	3.6	0.5	136.4	3.7	C18 H40 N4 O5 23Na S

Figure S1: HRMS of biotin-PEG amine (**9**).

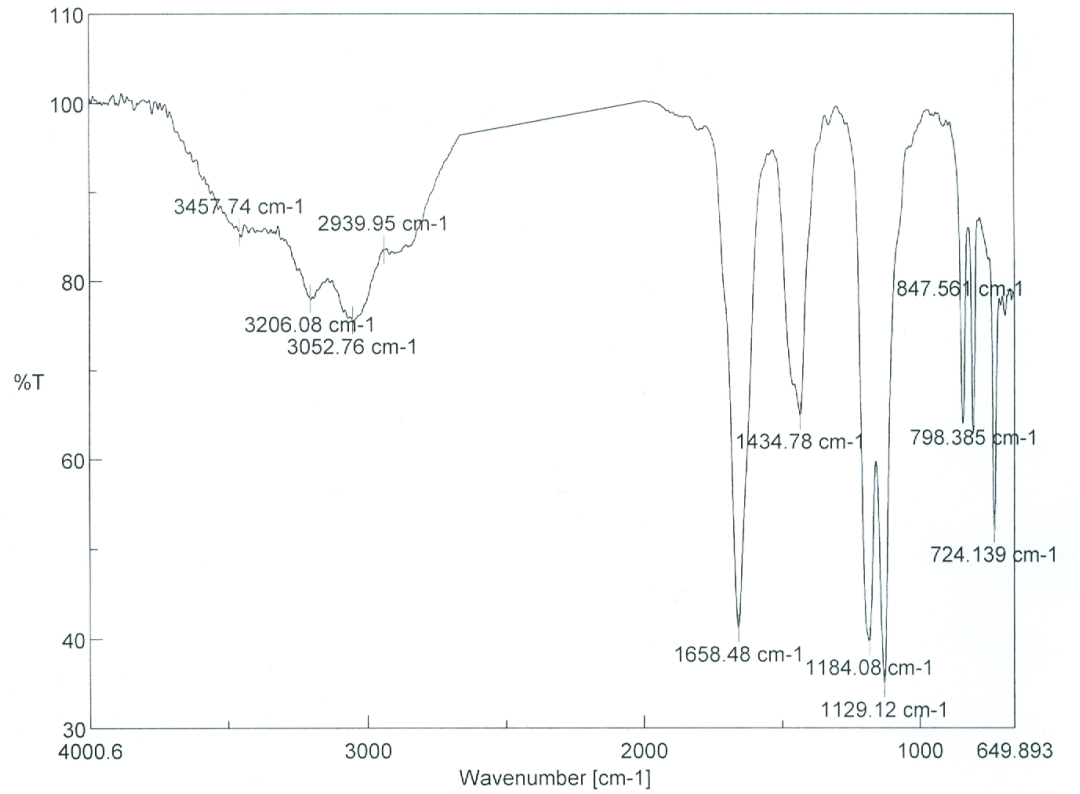


Figure S2: The IR spectrum (4000-650 cm^{-1}) of biotin-PEG amine (**9**) (neat).

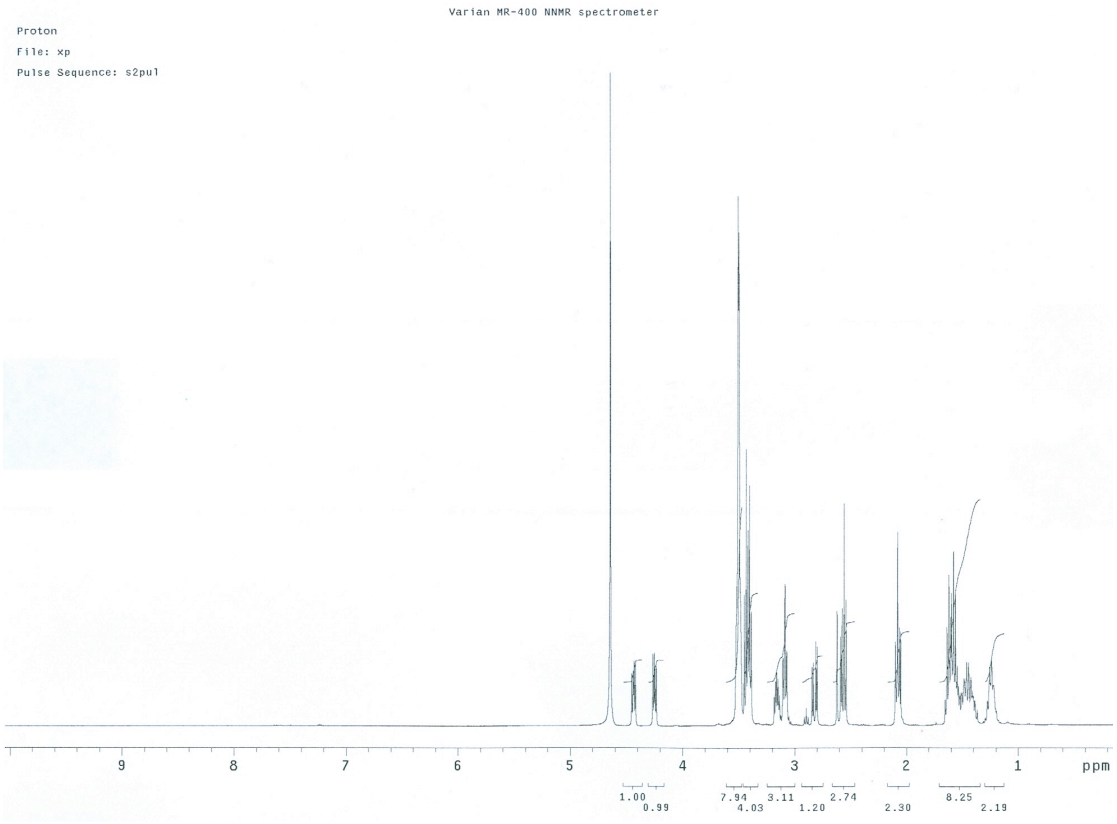


Figure S3: ^{1}H-NMR of biotin-PEG amine (**9**) recorded in D_2O solvent.

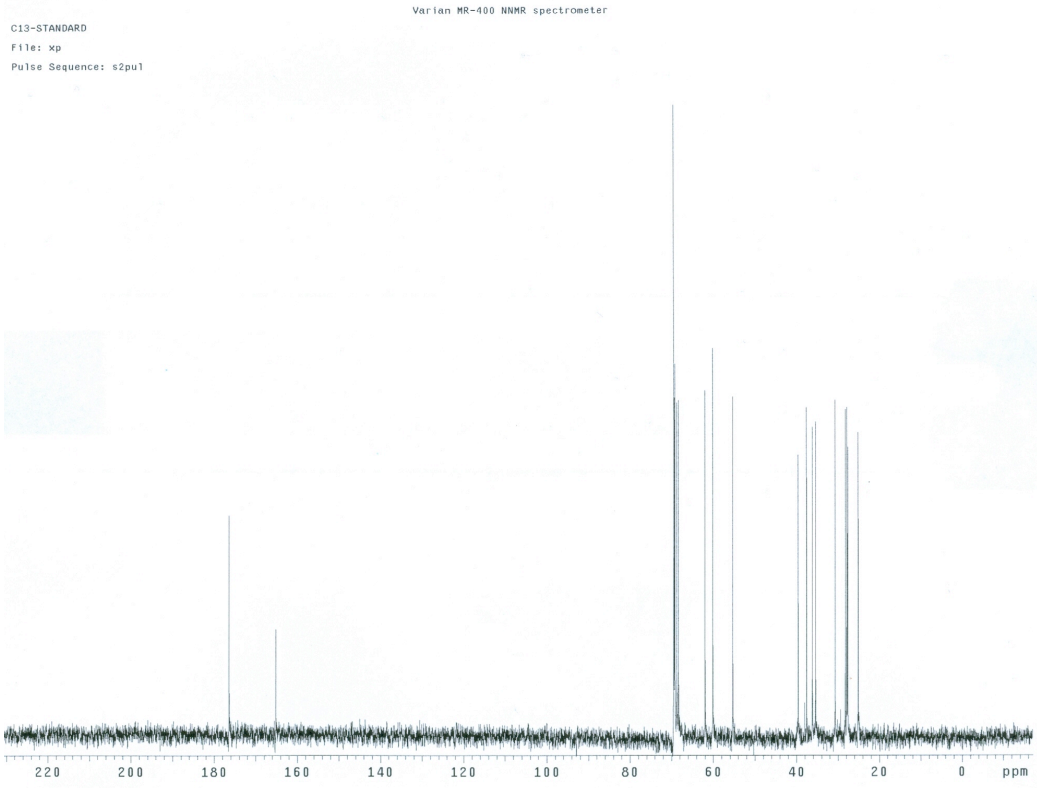
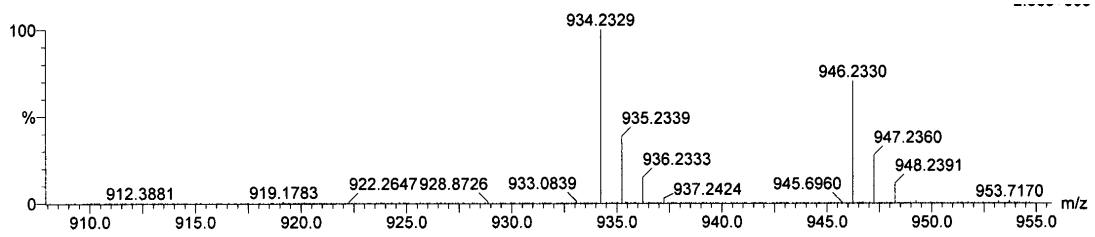


Figure S4: ^{13}C-NMR of biotin-PEG amine (**9**) recorded in D_2O solvent.



Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
934.2329	934.2312	1.7	1.8	8.5	140.0	0.6	C28 H52 N9 O17 23Na P3 S
	934.2337	-0.8	-0.9	11.5	140.2	0.8	C30 H51 N9 O17 P3 S
	934.2353	-2.4	-2.6	12.5	142.9	3.6	C33 H52 N7 O15 23Na P3 S

Figure S5: HRMS of ATP-biotin (**2**).

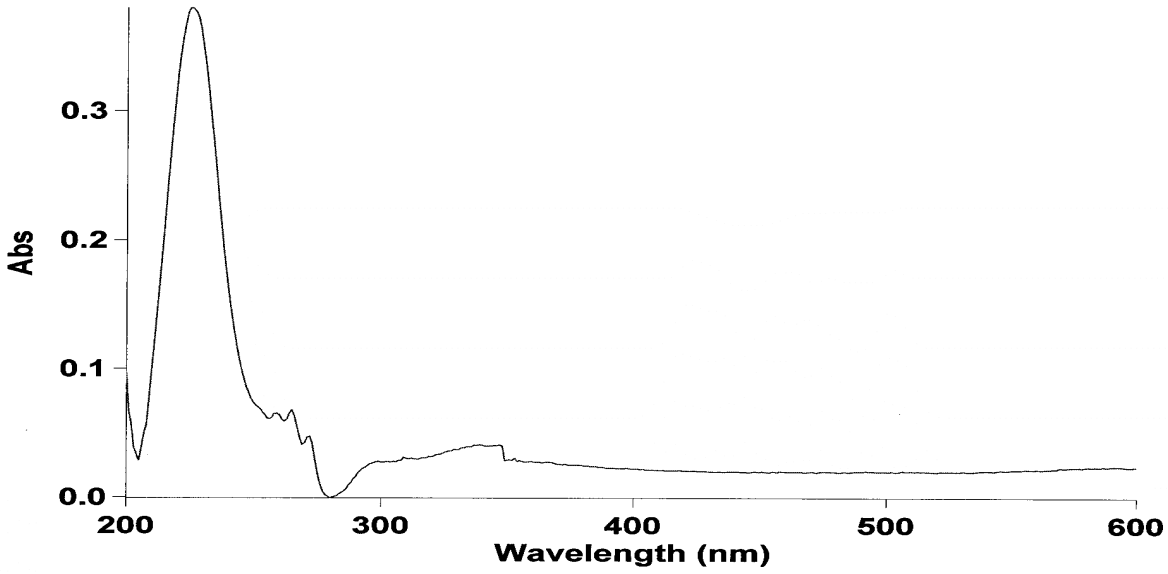


Figure S6: The UV absorbance spectrum (200-600 nm) of ATP-biotin (**2**) in methanol.

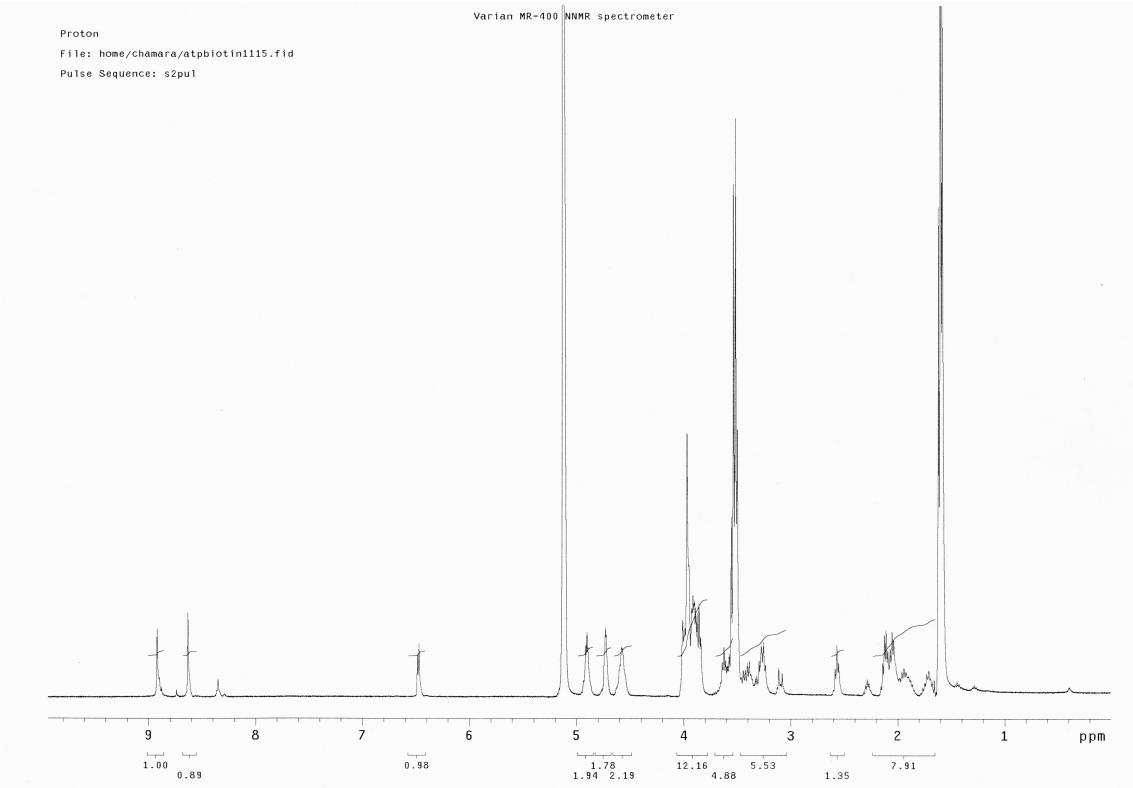


Figure S7: ^{1}H-NMR of ATP-biotin (**2**) recorded in D_2O solvent.

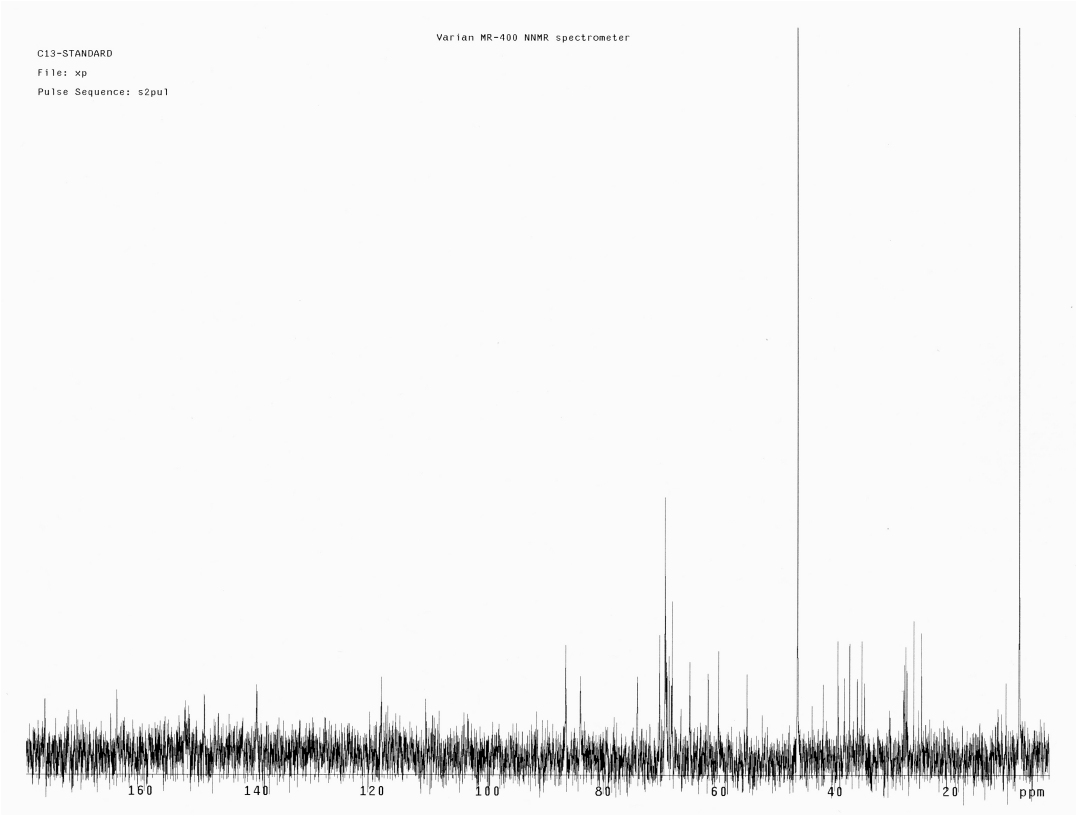


Figure S8: ^{13}C-NMR of ATP-biotin (**2**) recorded in D_2O solvent.

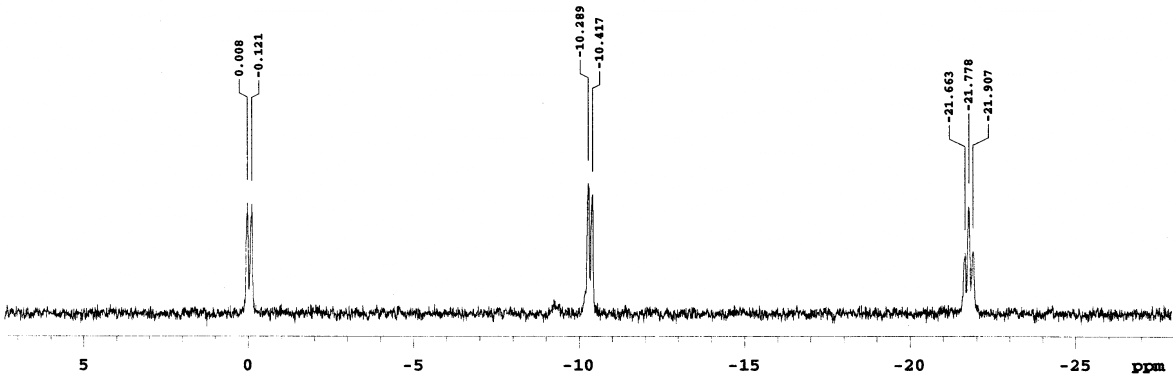


Figure S9: ^{31}P-NMR of ATP-biotin (**2**) recorded in D_2O solvent.

III. Quantitative MALDI-TOF MS analysis

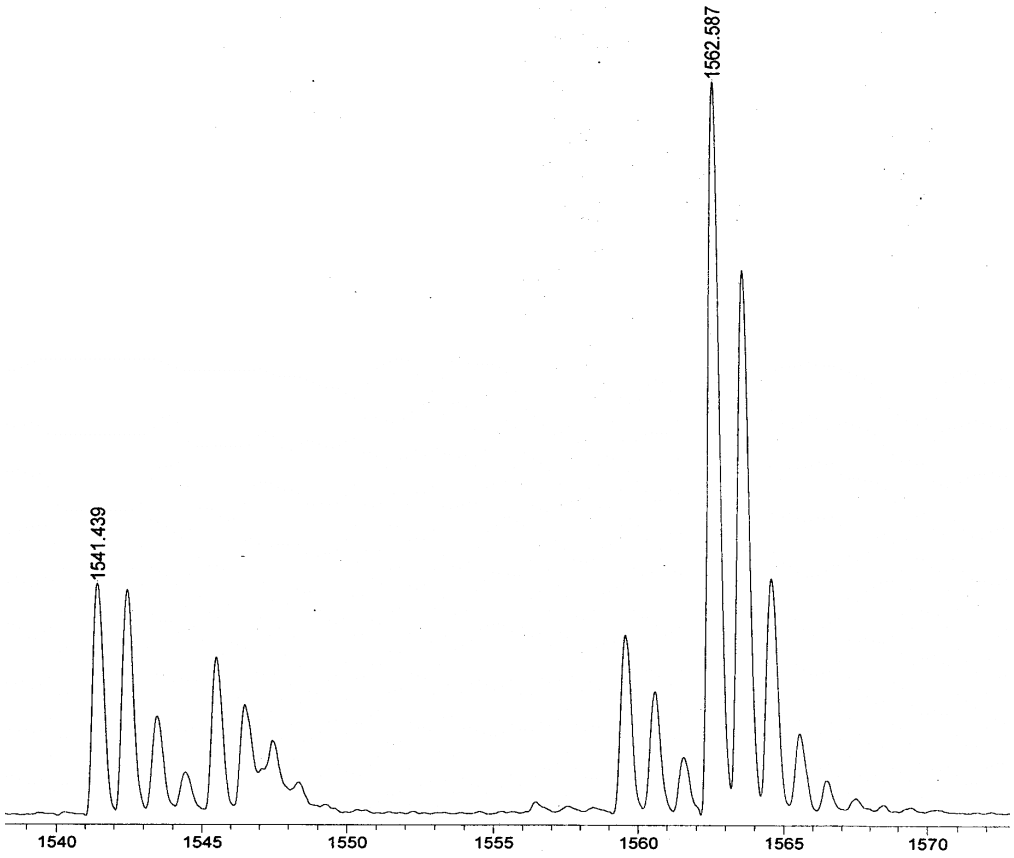


Figure S10: Quantitative MALDI analysis of biotinylation with CK2 kinase, CK2 peptide substrate (RRREEE**T**EEE), and either ATP (**1**) or ATP-biotin (**2).** The peak at 1541.439 m/z corresponds to hepta-methylated phosphopeptide after reaction with ATP-biotin and acidic cleavage of phosphoramide bond, while the peak at 1562.587m/z corresponds to the deuterated hepta-methylated phosphopeptide after phosphorylation with ATP. Percent conversion of this single trial is 53%.

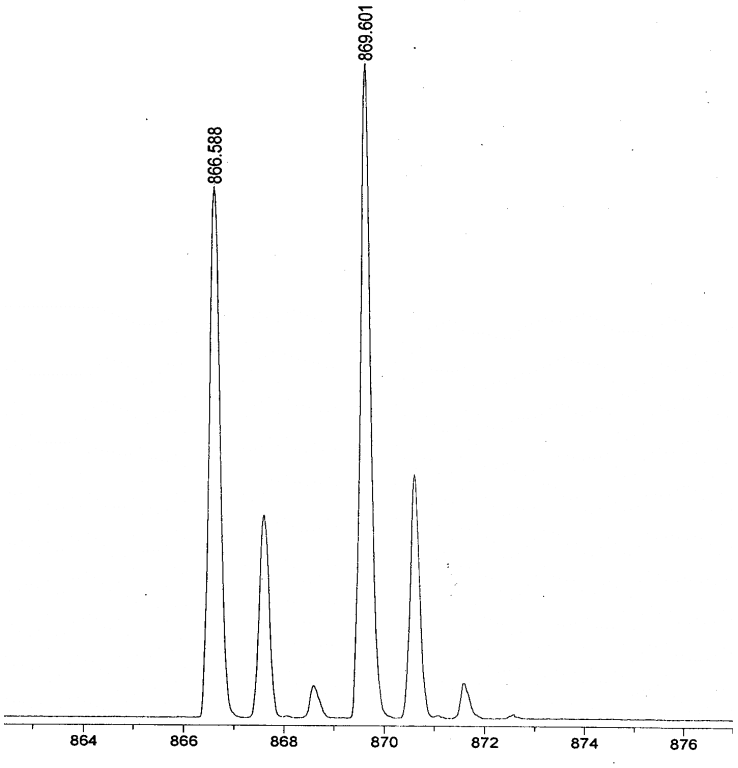


Figure S11: Quantitative MALDI analysis of biotinylation with PKA kinase, PKA peptide substrate Keptide (LRRA**S**LG), and either ATP (**1**) or ATP-biotin (**2).** The peak at 866.588 m/z corresponds to mono-methylated phosphopeptide after reaction with ATP-biotin and acidic cleavage of phosphoramide bond, while the peak at 869.601 m/z corresponds to the deuterated mono-phosphopeptide after phosphorylation with ATP. Percent conversion of this single trial is 82%.

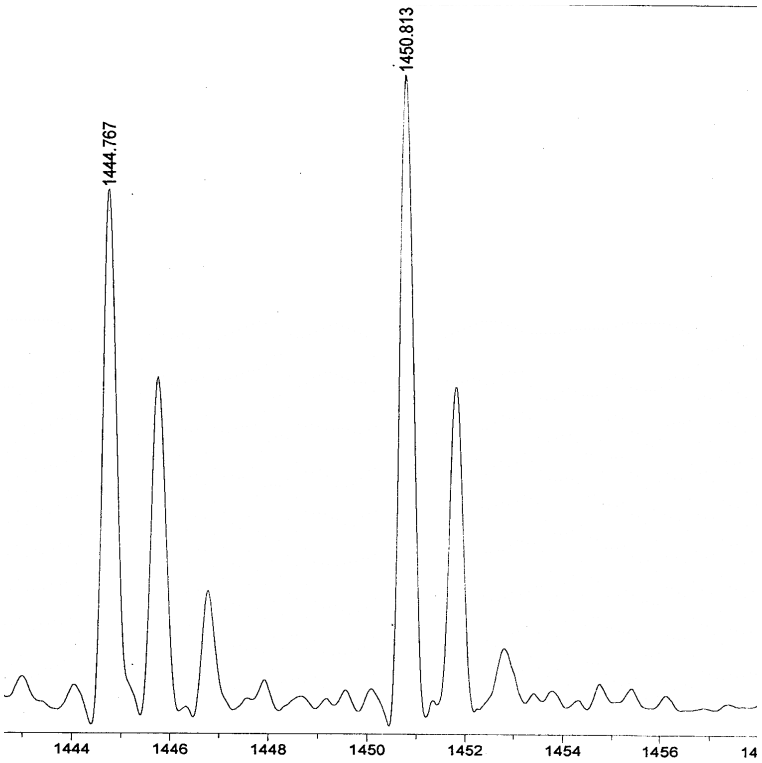
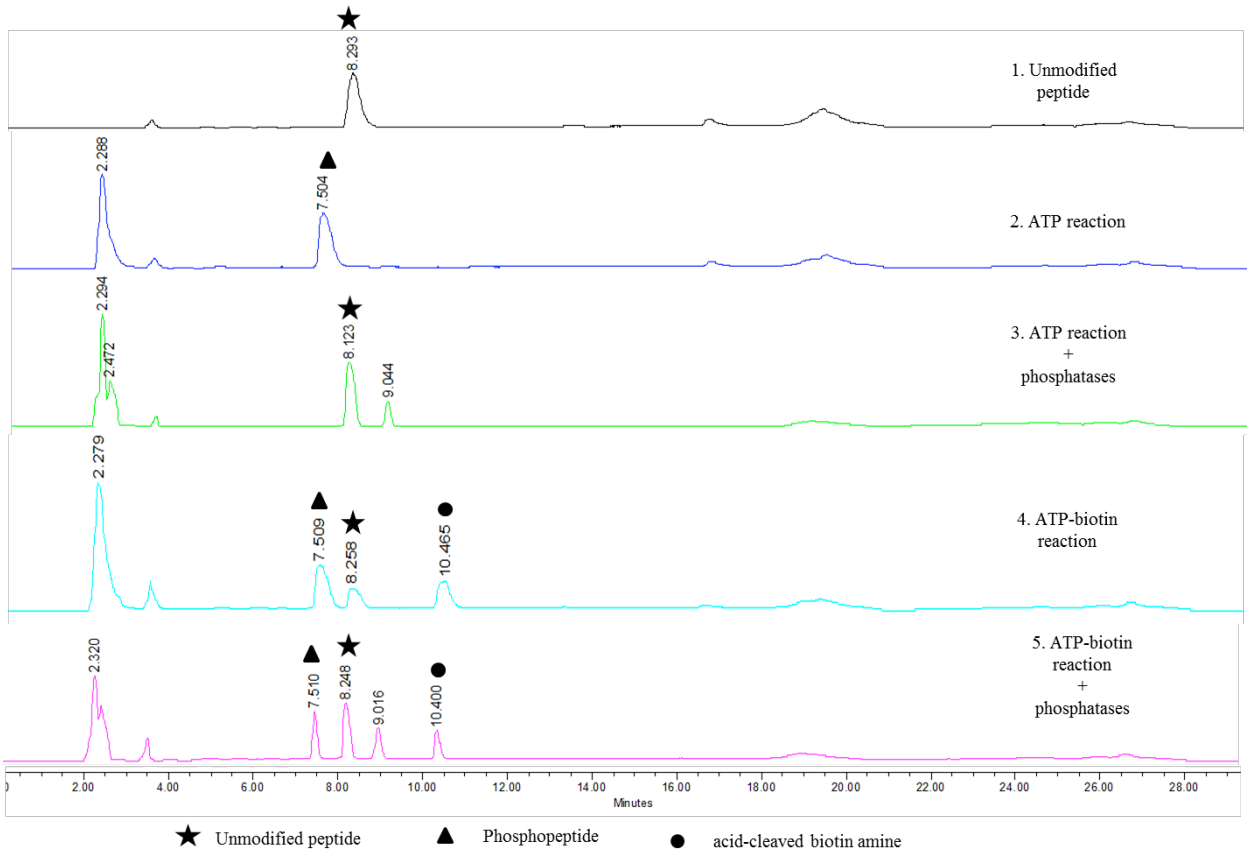


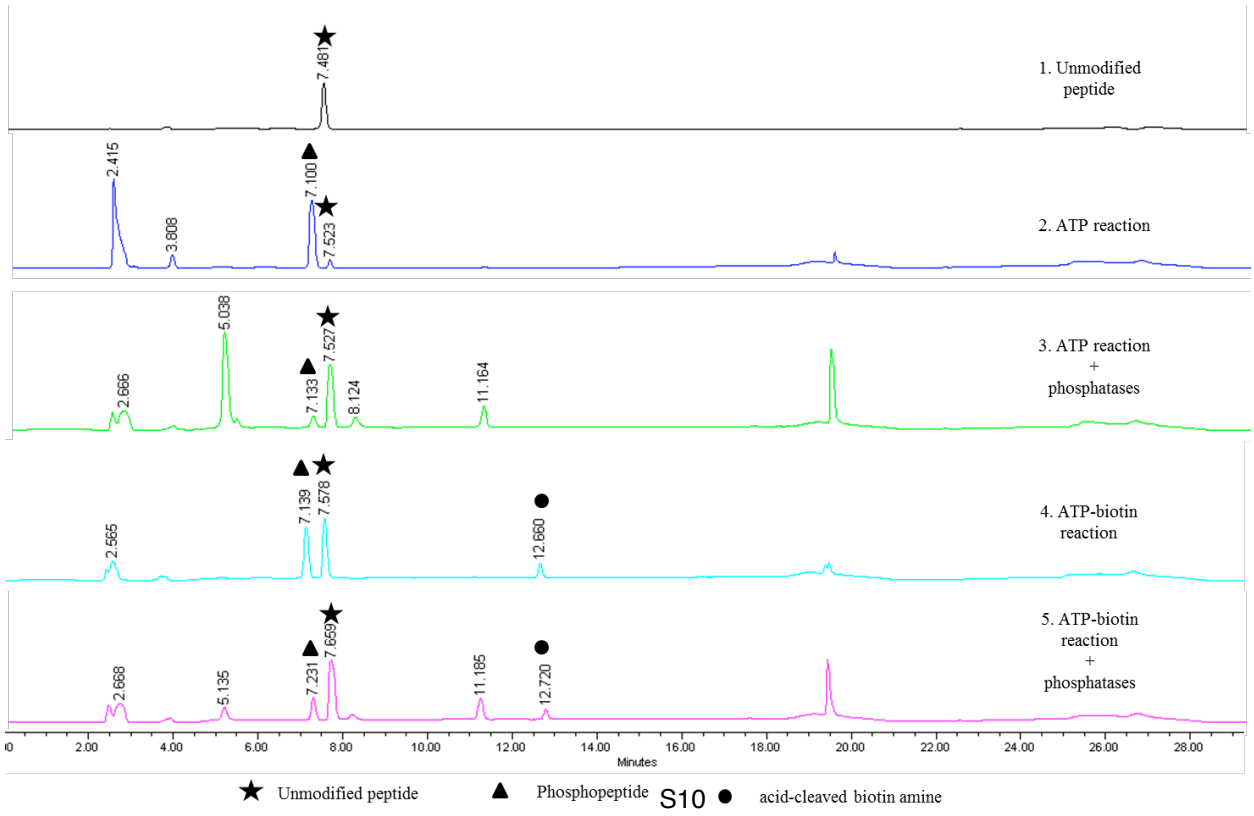
Figure S12: Quantitative MALDI analysis of biotinylation with Abl kinase, Abl peptide substrate (EAI**Y**AAPFAKKK), and either ATP (**1**) or ATP-biotin (**2).** The peak at 1444.767 m/z corresponds to di-methylated phosphopeptide after reaction with ATP-biotin and acidic cleavage of phosphoramide bond, while the peak at 1450.813 m/z corresponds to the deuterated di-phosphopeptide after phosphorylation with ATP. Percent conversion of this single trial is 81%.

IV. Complete HPLC traces of Figure 2

(A) HPLC trace for reactions performed using PKA and PP1.



(B) HPLC trace for reactions performed using CK2 and CIP.



(C) HPLC traces for reactions performed using Abl and TCPTP.

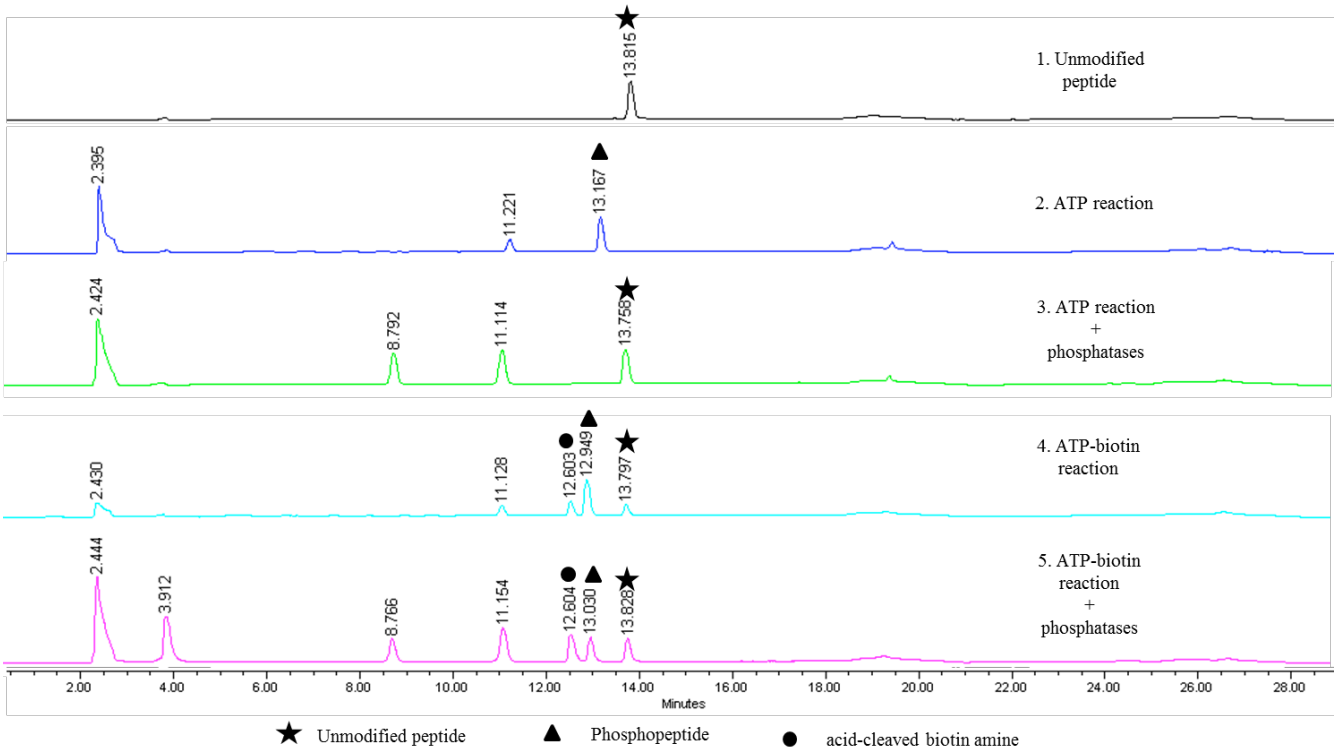
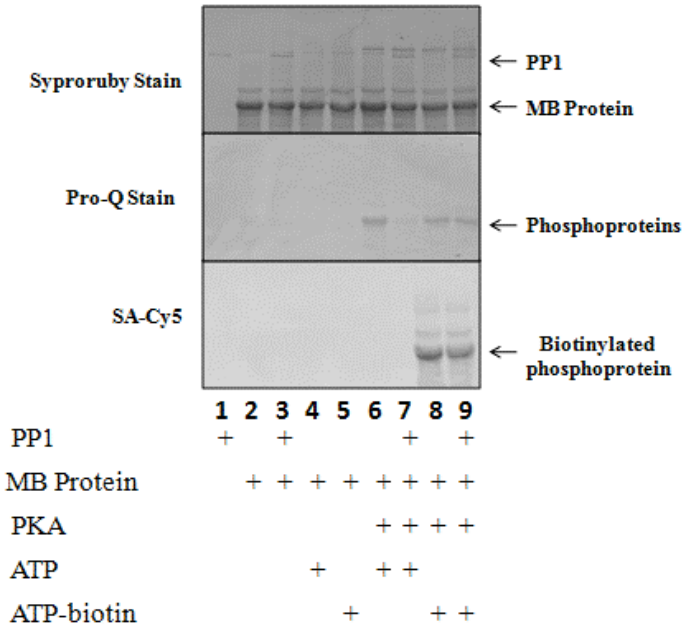


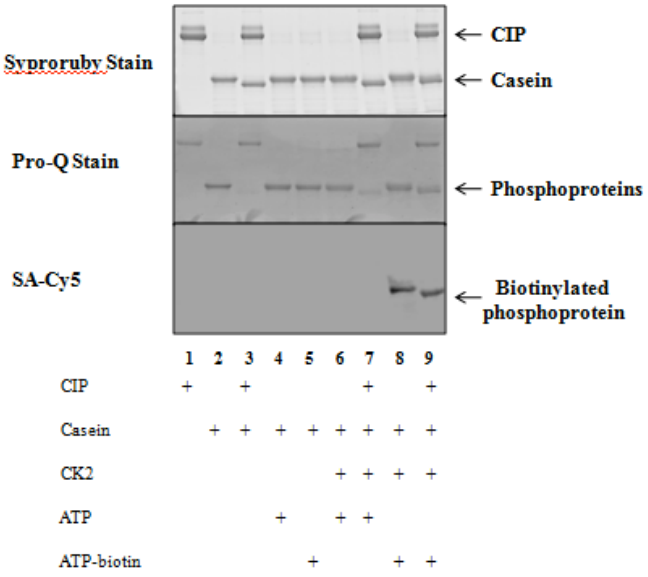
Figure S13: HPLC traces for the peptides (panel 1) after phosphorylation (panel 2), phosphorylation followed by phosphatase treatment (panel 3), biotinylation (panel 4) or biotinylation followed by phosphatase treatment (panel 5). Reactions were performed using (A) PKA, the PKA substrate peptide (LRRASLG), and PP1, (B) CK2, the CK2 peptide (RRREEETEEE), and CIP, or (C) Abl, the Abl substrate peptide (EAIYAAPFAKKK), and TC PTP. The unmodified peptide, phosphopeptide, and biotin amine byproduct of the acid-mediated cleavage of the phosphoramidate bond are shown with symbols. The other peaks observed in the spectra are components from the manufacturer-supplied kinase and/or phosphatase buffer.

V. Complete gel images related to Figure 3

(A) Gel images for reactions performed using PKA and PP1.



(B) Gel images reactions performed using CK2 and CIP.



(C) Gel images for reactions performed using Abl and TC PTP.

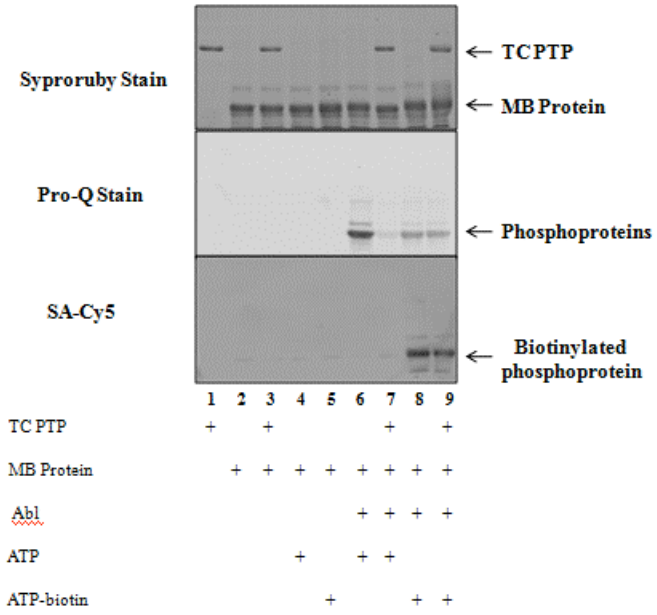


Figure S14: Full gel images for full length protein experiments containing (A) PKA, MBP substrate, and PP1, (B) CK2, β-casein substrate, and CIP, or (C) Abl, MBP substrate, and TC PTP. Lanes 6-9 here are shown in Figure 3 of the manuscript. Several additional control reactions are also shown, with reaction contents indicated under each lane.