

SUPPLEMENTAL MATERIAL

The apo-structure of the low-molecular-weight protein tyrosine phosphatase A (MptpA) from *Mycobacterium tuberculosis* allows for better target-specific drug development*

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*Running title: *Structure and function of apo-MptpA by NMR*

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Figure S1

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      1      10      20      30      40      50      60
GIDPFTLKMSSPRERRPA SQAPRLSRRPP PAHQTSRSSPP DTTAPTGSGLL SNRFVNDNGII VDDTTASGTNN

      70      80      90      100     110     120
CPPPPRAAARR RASSPGESPQQ LVIFDLDGTLL TDSARGIVSSS FRHALNHIGAA PVPEGDLATHH

     130     140     150     160     170     180
IVGPPMHETLL RAMGLGESAEE EAIVAYRADYY SARGWAMNSLL FDGIGPLLADD LRTAGVRLAVV

     190     200     210     220     230     240
ATSKAEPTARR RILRHFGIEQQ HFEVIAGASTT DSGRGSKVDVV LAHALAQLRPP LPERLVMVGDD

     250     260     270     280     290
RSHDVDGAAAA HGIDTVVVGWW GYGRADFIDKK TSTTVVTHAAA TIDELREALGG V

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Figure S1. PtkA (Rv2232, M1-V291) amino acid sequence construct. Additional residues G(-7) - K(0) originate from **subcloning**.

Figure S2

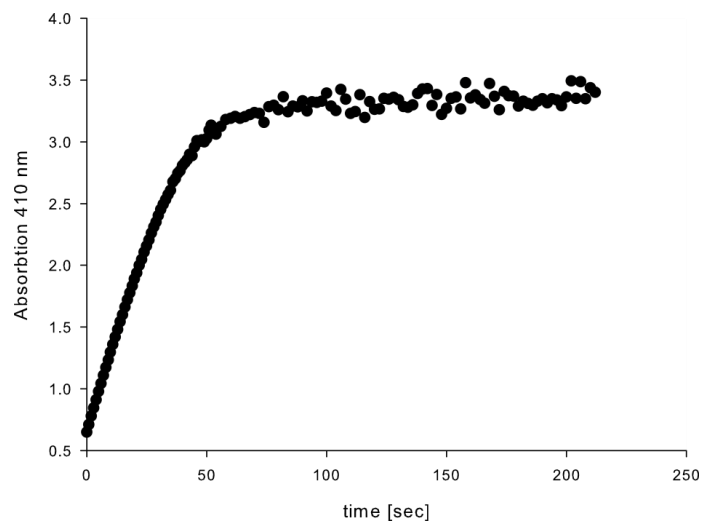


Figure S2. Dephosphorylation reaction of para-Nitrophenylphosphate (25 mM) catalyzed by wild type MptpA (20 μ M in 25 mM Tris-HCl (pH 7.0), 50 mM NaCl, 2 mM EDTA) monitored by the absorption of p-nitrophenol at 410 nm (Varian, Cary50Bio) at room temperature (25 $^{\circ}$ C).

Table S1. Order parameter S^2 , (^1H) - ^{15}N heteronuclear NOE, ^{15}N - T_1 and ^{15}N - T_2 values for MptpA (1.0 mM in 150 mM NaCl, 50 mM HEPES (pH 7.0), 10 mM DTT) at T=298 K using a 600 MHz spectrometer with cryogenic probe. The T_1 longitudinal ^{15}N relaxation rates were determined from spectra with different delays of 10, 100, 400, 600, 800, 1000, 1200 and 1500 ms. T_2 transverse ^{15}N relaxation rates were determined using the following delays 35.2, 70.4, 105.6, 140.8, 176.0, 211.2, 246.4 and 281.6 ms. The (^1H) - ^{15}N hetNOEs were obtained from the ratio of peak intensities ($I^{\text{NOE}}/I^{\text{no NOE}}$) with and without the saturation of amide protons.

residue	S^2		hetNOE		T_2		T_1	
	SD		SD		[ms]	SD	[ms]	SD
G0	-	-	-0.7982	0.0005	503.6	43.30	945.3	140.0
M1	0.2821	0.0196	-0.3627	0.0055	231.9	10.20	780.9	81.8
S2	0.2380	0.0115	-0.2568	0.0137	277.0	17.70	800.7	92.7
D3	0.6141	0.1067	0.0868	0.0198	114.7	3.52	791.0	80.9
P4	-	-	-	-	-	-	-	-
L5	0.7762	0.0059	0.6192	0.0170	69.3	1.09	798.7	14.4
H6	0.7836	0.0011	0.7939	0.0226	81.7	2.40	834.0	14.4
V7	0.8132	0.0019	0.7943	0.0256	74.6	2.87	824.8	23.9
T8			0.8196	0.0134	86.4	2.31	785.4	18.3
F9	0.8208	0.0018	0.8161	0.0269	75.2	1.72	841.3	32.6
V10	0.7886	0.0019	0.8094	0.0199	81.2	1.86	786.1	30.0
C 11	-	-	0.8213	0.0185	-	-	830.8	29.7
T12	-	-	-	-	-	-	-	-
G13	-	-	-	-	-	-	-	-
N14	0.8612	0.0055	0.8226	0.0309	69.5	3.42	774.2	19.9
I15	-	-	-	-	-	-	-	-
C16	-	-	0.8759	0.0472	-	-	786.5	104.0
R17	-	-	-	-	-	-	-	-
S18	-	-	-	-	-	-	-	-
P19	-	-	-	-	-	-	-	-
M20	0.7947	0.0122	0.7702	0.0173	72.4	2.78	872.2	21.8
A21	0.7932	0.0337	0.8071	0.0195	69.8	3.33	856.8	27.8
E22	0.8726	0.0008	0.8340	0.0172	65.2	2.03	848.2	35.7
K23	0.8224	0.0005	0.8122	0.0210	72.9	3.39	846.2	32.5
M24	0.8508	0.0029	0.7908	0.0189	70.9	1.64	847.4	37.3
F25	-	-	-	-	-	-	-	-
A26	0.8255	0.0011	0.8166	0.0141	70.9	1.69	853.0	26.9
Q27	0.8362	0.0014	0.8214	0.0135	68.9	1.96	866.6	33.7
Q28	0.8225	0.0028	0.8419	0.0184	73.4	1.64	807.4	29.3
L29	-	-	-	-	-	-	-	-
R30	0.9230	0.1067	0.7881	0.0108	70.5	1.28	789.7	16.0
H31	0.8432	0.0037	0.8395	0.0126	70.6	1.28	781.2	32.4
R32	0.7867	0.0031	0.7769	0.0154	79.3	1.88	882.8	45.6
G33	0.8868	0.0034	0.7784	0.0152	65.0	1.64	840.4	51.2
L34	0.8237	0.0033	0.8004	0.0145	71.8	1.55	848.8	37.0
G35	0.9093	0.0058	0.7580	0.0163	92.0	1.87	798.9	38.0
D36	-	-	-	-	81.8	1.84	809.5	70.6

A37	0.9015	0.1765	0.7560	0.0145	93.4	1.94	862.9	33.0
V38	0.8305	0.0023	0.7837	0.0131	75.6	1.39	817.5	28.4
R39	0.7755	0.0019	0.8266	0.0244	83.1	3.97	842.6	19.9
V40	0.8431	0.0010	0.7745	0.0170	70.5	2.54	807.8	24.3
T41	0.8102	0.0016	0.8444	0.0201	76.5	3.19	844.7	64.2
S42	0.8238	0.0030	0.8600	0.0181	66.4	1.75	795.6	34.1
A43	0.8113	0.0019	0.7869	0.0138	78.3	1.78	790.8	33.0
G44	0.7919	0.0034	0.7569	0.0248	66.8	3.02	827.5	36.2
T45	0.8491	0.0009	0.8114	0.0217	70.6	2.72	803.1	29.7
G46	0.8208	0.0022	0.7690	0.0190	78.4	1.71	789.0	35.4
N47	0.8272	0.0045	0.7559	0.0221	72.9	2.64	780.6	72.2
W48	0.8722	0.0004	0.8079	0.0218	76.8	4.49	708.8	37.2
H49	0.9195	0.0028	0.8002	0.0221	66.3	2.43	771.7	51.8
V50	0.7547	0.0044	0.6988	0.0113	82.0	1.73	832.2	40.6
G51	0.8303	0.0002	0.7778	0.0214	73.8	1.93	814.0	20.7
S52	0.8368	0.0039	0.8303	0.0147	75.3	0.98	785.5	46.0
C53	0.7182	0.0025	0.7971	0.0130	81.0	1.88	886.6	65.6
A54	-	-	-	-	-	-	-	-
D55	0.8054	0.0013	0.8691	0.0262	77.0	1.92	830.7	37.1
E56			0.8166	0.0197	83.9	1.19	744.2	49.4
R57	0.8314	0.0005	0.8018	0.0203	76.3	2.42	787.7	28.4
A58	0.7675	0.0009	0.8253	0.0169	83.4	2.93	841.6	36.2
A59	-	-	-	-	79.6	1.49	-	-
G60	0.7993	0.0082	0.8269	0.0160	61.0	1.72	852.6	24.2
V61	0.8568	0.0084	0.7834	0.0128	64.7	1.48	767.2	13.5
L62	0.7535	0.0045	0.7658	0.0171	83.9	1.50	827.9	40.5
R63	0.8048	0.0021	0.7854	0.0144	77.5	1.30	796.3	23.8
L62	-	-	0.8008	0.0122	-	-	-	-
H65	0.7600	0.0015	0.7836	0.0143	83.2	2.09	889.7	39.8
G66	0.8384	0.0044	0.7978	0.0151	56.4	1.54	842.1	23.5
Y67	0.7826	0.0021	0.8282	0.0141	75.5	1.87	869.7	34.6
P68	-	-	-	-	-	-	-	-
T69	0.9001	0.0071	0.7956	0.0170	93.1	2.20	848.1	39.6
D70	-	-	-	-	-	-	-	-
H71	-	-	0.7964	0.0131	-	-	-	-
R72	0.8155	0.0378	0.7892	0.0132	67.4	0.98	863.5	27.3
A73	0.7949	0.0014	0.8250	0.0178	75.8	1.45	857.5	27.3
A74	0.7998	0.0828	0.8196	0.0189	71.1	1.00	836.6	44.3
Q75	0.8367	0.0175	0.8027	0.0192	56.6	3.96	795.5	18.7
V76	0.8813	0.0025	0.7986	0.0211	63.9	1.72	821.2	16.8
G77	0.7262	0.0037	0.7675	0.0259	88.3	3.12	846.1	55.1
T78	0.8453	0.0025	0.7976	0.0195	74.2	1.17	815.5	58.1
E79	0.8732	0.0058	0.7886	0.0145	70.6	1.30	780.1	68.9
H80	0.7601	0.0033	0.7778	0.0124	81.7	1.50	861.9	37.0
L81	0.8573	0.0014	0.8126	0.0197	72.6	1.50	785.9	24.5
A82	0.8159	0.0018	0.7711	0.0113	75.7	1.24	824.5	37.2
A83	0.8226	0.0016	0.8182	0.0112	70.6	1.15	868.2	21.2

D84	-	-	-	-	-	-	-	-
L85	0.8237	0.0002	0.7837	0.0126	77.0	1.46	792.3	13.8
L86	0.8790	0.0009	0.8333	0.0224	66.6	3.44	782.6	34.4
V87	0.7711	0.0546	0.8171	0.0200	82.5	2.77	829.9	35.5
A88	-	-	0.8040	0.0203	83.3	2.51	784.6	33.6
L89	0.8231	0.0011	0.7974	0.0290	80.6	4.94	776.9	31.1
D90	-	-	0.8444	0.0474	-	-	899.4	106.0
R91	-	-	0.7804	0.0219	68.6	3.09	807.7	55.9
N92	-	-	-	-	70.7	2.33	759.7	23.1
H93	-	-	-	-	-	-	-	-
A94	0.8060	0.0010	0.8110	0.0151	80.8	6.18	809.0	43.5
R95	0.8070	0.0007	0.7844	0.0139	73.4	2.32	873.9	35.5
L96	0.8419	0.0006	0.8108	0.0110	74.6	1.91	783.3	16.6
L97	0.7950	0.0010	0.7677	0.0173	78.6	3.01	832.1	21.6
R98	0.8344	0.0204	0.8064	0.0143	73.2	1.04	819.0	17.9
Q99	0.9586	0.0048	0.7883	0.0114	77.1	0.79	799.7	10.0
L100	0.7757	0.0002	0.8037	0.0135	84.2	1.94	820.1	17.1
G101	0.9033	0.0091	0.7979	0.0145	90.6	2.14	835.4	27.6
V102	0.7969	0.0055	0.8437	0.0156	72.4	1.01	915.1	28.1
E103	0.7584	0.0015	0.7723	0.0156	84.4	1.76	852.8	27.6
A104	0.8773	0.0006	0.7790	0.0131	71.5	2.22	754.4	26.6
A105	-	-	-	-	72.8	2.58	795.2	51.5
R106	0.8169	0.0026	0.7683	0.0136	77.2	1.65	812.4	30.2
V107	0.7958	0.0017	0.7797	0.0111	80.0	1.25	816.3	19.7
R108	0.8294	0.0024	0.8076	0.0158	66.7	1.68	795.5	18.1
M109	0.7498	0.0016	0.7678	0.0199	84.3	3.27	839.6	56.3
L110	0.8626	0.0043	0.7988	0.0229	70.7	1.23	800.0	59.5
R111	0.8086	0.0049	0.8368	0.0301	76.9	2.33	801.8	54.0
S112	0.8729	0.0016	0.8448	0.0184	72.2	2.30	765.7	40.5
F113	0.8916	0.0011	0.8277	0.0175	65.4	2.24	819.2	40.2
D114	-	-	-	-	-	-	-	-
P115	-	-	-	-	-	-	-	-
R116	0.8310	0.0017	0.6816	0.0159	75.1	2.39	777.2	36.5
S117	-	-	0.6598	0.0197	-	-	809.4	66.5
G118	0.6562	0.0456	0.5947	0.0202	114.6	5.92	703.1	82.2
T119	-	-	-	-	-	-	-	-
H120	-	-	0.5808	0.0437	-	-	-	-
A121	-	-	0.5166	0.0334	-	-	801.2	69.9
L122	0.8158	0.1071	0.6017	0.0135	84.2	1.59	751.1	48.7
D123	0.7616	0.0129	0.7085	0.0142	82.7	1.22	853.5	37.3
V124	0.6619	0.0044	0.4950	0.0185	96.6	3.04	875.3	54.0
E125	-	-	0.8059	0.0200	81.8	2.46	798.7	20.0
D126	0.8619	0.0064	0.8005	0.0284	69.6	3.72	790.1	36.6
P127	-	-	-	-	-	-	-	-
Y128	0.7784	0.0007	0.7398	0.0166	79.9	3.01	838.9	29.2
Y129	0.8379	0.0236	0.8269	0.0162	71.8	1.16	802.3	34.5
G130	0.7402	0.0498	0.7602	0.0128	75.6	2.33	906.3	44.3

D131	0.8016	0.0016	0.7634	0.0157	78.6	3.35	805.0	39.5
H132	-	-	-	-	-	-	-	-
S133	0.8355	0.0037	0.7727	0.0143	69.0	1.32	839.8	75.4
D134	0.8290	0.0859	0.7817	0.0135	63.7	1.90	875.8	52.6
F135	0.8743	0.0058	0.8137	0.0188	68.6	2.38	838.9	63.9
E136	-	-	-	-	71.1	1.58	853.1	21.0
E137	0.9083	0.0098	0.8026	0.0126	76.8	1.11	809.7	22.2
V138	0.8064	0.0014	0.7793	0.0147	73.6	1.25	870.8	23.8
F139	0.7976	0.0042	0.8335	0.0172	60.5	1.79	884.1	34.4
A140			0.8655	0.0113	68.7	1.85	820.4	19.0
V141	0.8286	0.0003	0.8018	0.0155	69.6	3.07	868.3	44.9
I142	-	-	-	-	72.9	1.38	889.3	34.5
E143	0.8272	0.0016	0.8020	0.0132	71.0	3.55	856.2	22.4
S144	0.7928	0.0024	0.7830	0.0126	74.0	1.15	890.8	36.0
A145	0.8877	0.0037	0.8341	0.0154	53.5	1.74	771.7	23.1
L146	0.7665	0.0523	0.7581	0.0173	73.3	1.19	894.5	39.2
P147	-	-	-	-	-	-	-	-
G148	0.7925	0.0020	0.7732	0.0169	71.2	3.46	891.8	28.3
L149	0.7747	0.0032	0.8200	0.0175	68.5	2.10	869.4	21.5
H150	0.7922	0.0174	0.8354	0.0168	72.0	1.33	870.8	13.9
D151	0.8391	0.0143	0.8211	0.0127	68.3	1.17	865.5	18.5
W152	-	-	-	-	-	-	-	-
V153	-	-	0.7794	0.0139	83.8	2.44	-	-
D154	0.8629	0.0424	-	-	66.9	2.07	853.2	44.0
E155	0.8371	0.0007	0.7951	0.0142	68.9	1.51	860.7	22.3
R156	0.7960	0.0025	0.8165	0.0154	75.3	1.51	875.9	35.3
L157	-	-	-	-	-	-	-	-
A158	0.8616	0.0070	0.6982	0.0127	85.7	2.82	790.7	18.0
R159	0.7010	0.0077	0.5097	0.0110	102.4	1.52	756.2	41.4
N160	0.4080	0.0065	0.3012	0.0145	172.8	3.49	683.8	96.5
G161	0.1832	0.0037	-0.2631	0.0051	332.9	12.30	813.5	88.6
P162	-	-	-	-	-	-	-	-
S163	0.1195	0.0084	-1.0553	0.0019	525.3	18.10	1125.0	31.0

Figure S3

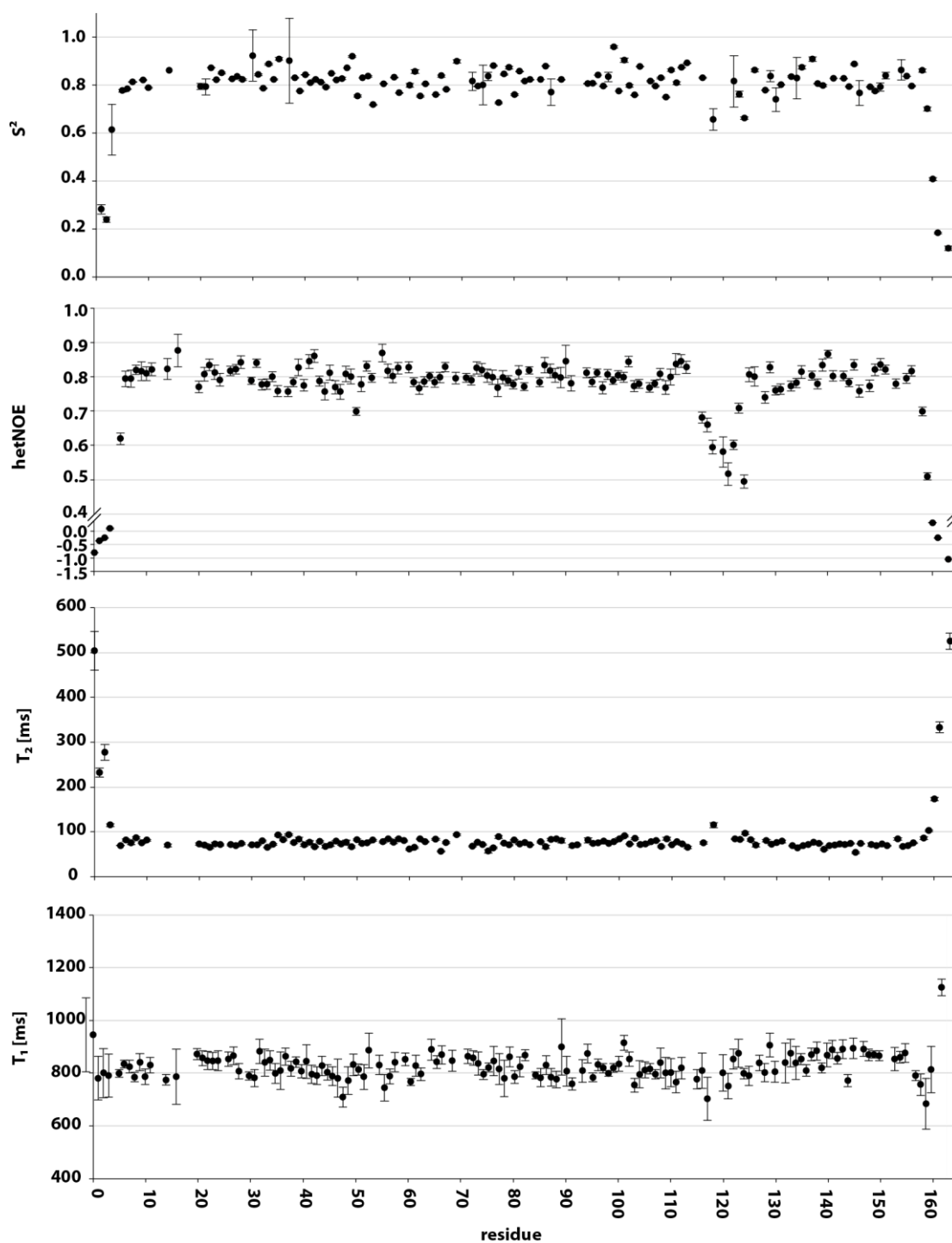


Figure S3. Heteronuclear relaxation data and S^2 order parameter of MptpA

Figure S4 and S5

In the phosphorylation assay for MptpA both enzymes (MptpA : PtkA, ratio 4:1) were incubated overnight at 4°C in the reaction buffer (300 mM NaCl, 50 mM Tris-HCl (pH 8.0), 10 mM β -Mercaptoethanol, 10 mM MgCl_2) containing 10 mM ATP. Phosphorylation was monitored by ^{31}P NMR spectroscopy (Fig. S4) and MALDI-MS (Fig. S5). 1D ^{31}P NMR spectra with ^1H inverse gated decoupling were recorded at a 300 MHz spectrometer. The spectrometer was locked on D_2O . Figure S4, A shows the expected lack of autophosphorylation activity of MptpA, while the autophosphorylation activity of PtkA can be verified due to ATP hydrolysis (ADP corresponding resonances at -5.78 ppm and -10.04 ppm) as well as with detection of a new peak resonating at -1.05 ppm (Fig. S4, B). The 1D ^{31}P spectrum of the phosphorylation reaction mixture containing MptpA, PtkA and ATP (Figure S4, C) shows one additional peak at 3.92 ppm.

Figure S4

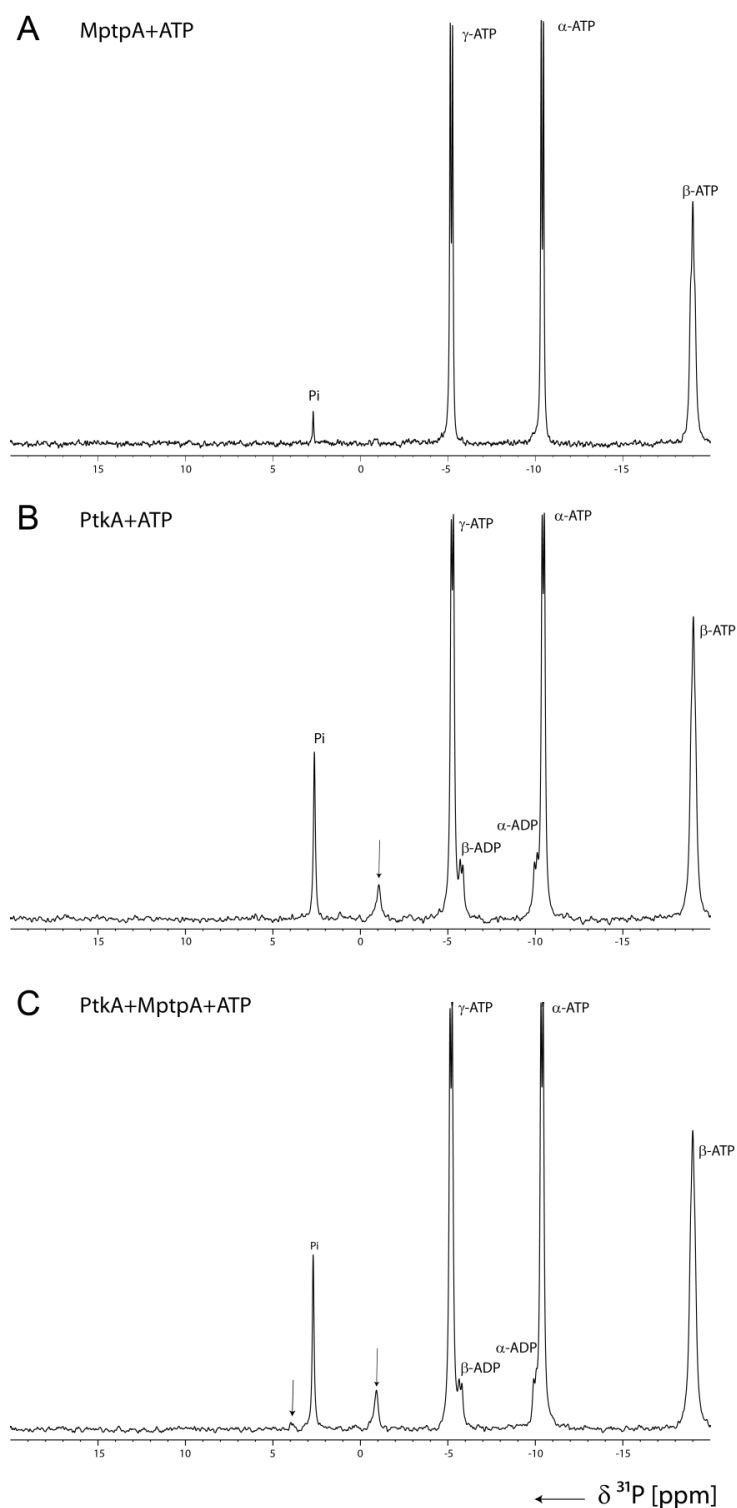


Figure S4. ^{31}P NMR spectroscopy of phosphorylation reaction. A. Autophosphorylation assay of MptpA (200 μM MptpA, 10 mM ATP) B. Autophosphorylation assay of PtkA (50 μM PtkA, 10 mM ATP) C. Incubation, showing phosphorylation of MptpA by PtkA (200 μM MptpA, 50 μM PtkA, 10 μM ATP). Resonances corresponding to phosphorylated tyrosine residues in PtkA and MptpA are indicated by arrows. All experiments were recorded with 3072 number of scans (TD = 4096, AQ = 0.24 s).

Figure S5

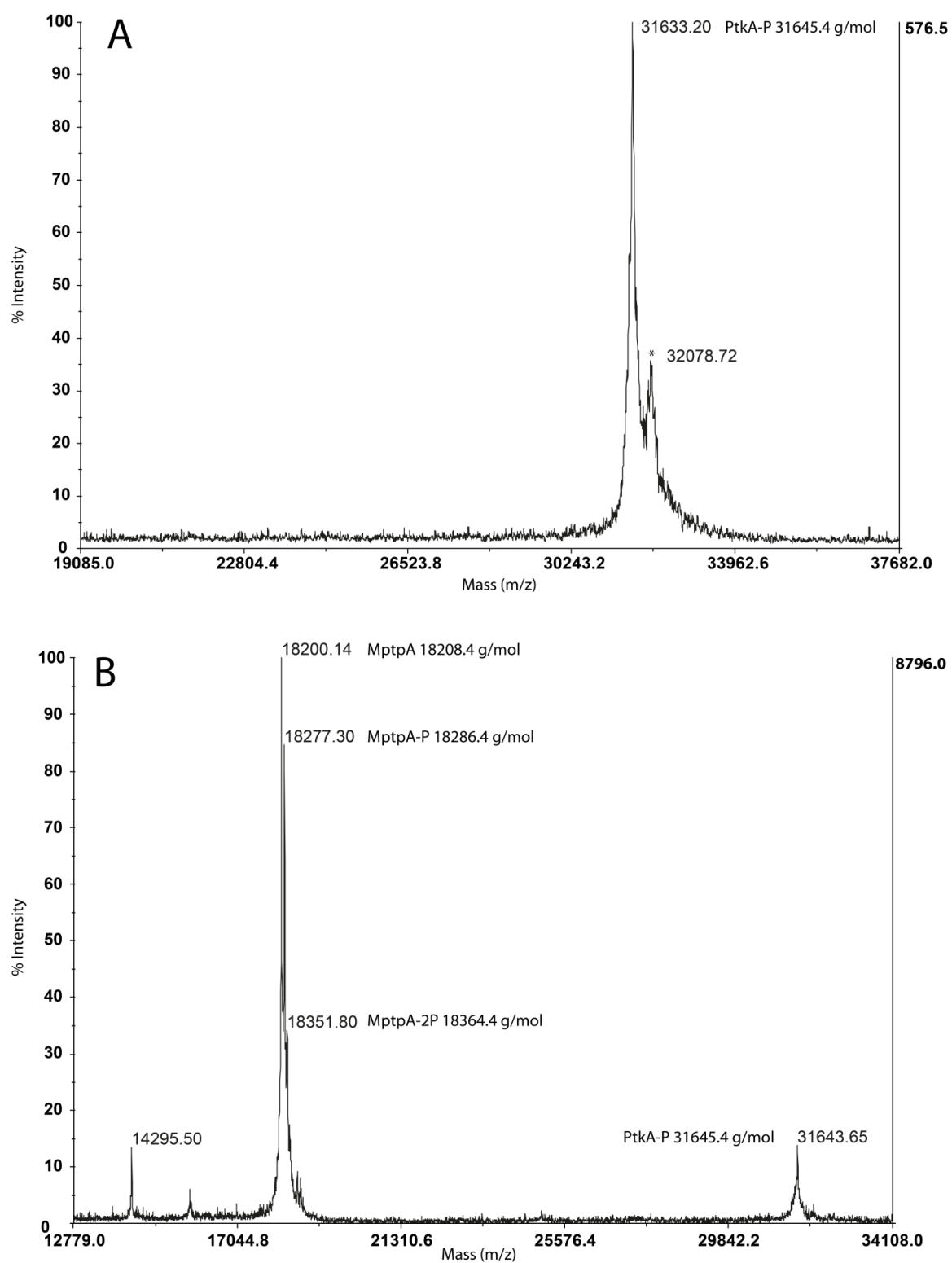


Figure S5. MALDI-MS annotated with experimental and theoretical molecular mass A. **Autophosphorylation assay** of PtkA (50 μ M) incubated with 10 mM ATP (* unassigned signal) B. Phosphorylation of MptpA (200 μ M) catalyzed by PtkA (50 μ M) in presence of 10 mM ATP.