Electronic Supplementary Information (ESI)

Unique fluorescent response of hexaphenylsilole to methyl parathion hydrolase: a new signal generating system for the enzyme label

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Figures and tables

UV-vis response of MPH in the presence of MP

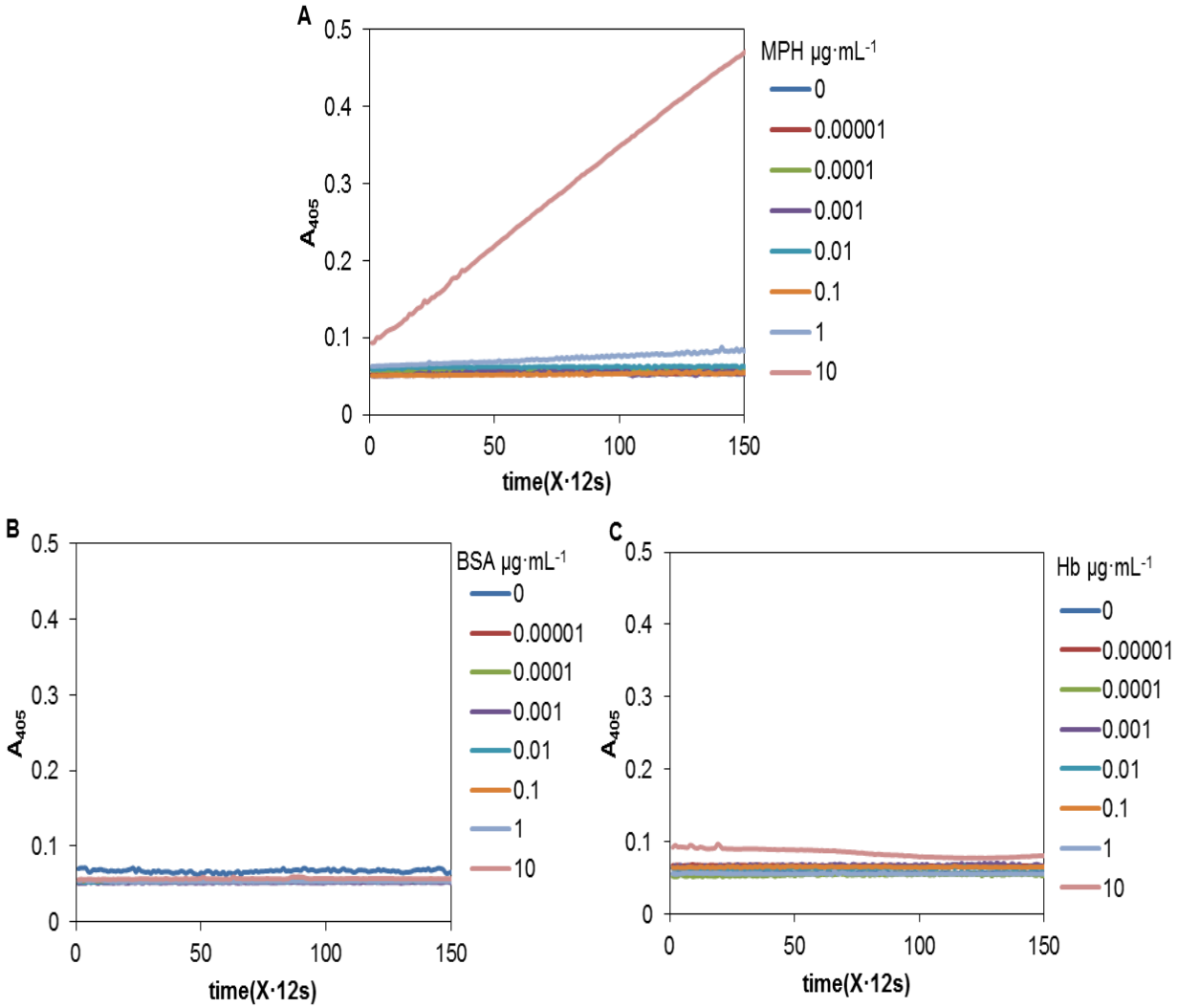

Figure S1. Time-dependence UV absorption of pre-incubated MP(250μM) upon addition of different concentrations (0-10μg·mL^{-1}) of pre-incubated proteins (A) MPH, (B) BSA and (C) Hb in Tris (50mM pH8.0) under UV lamp at 405nm at room temperature.

Table S1. UV response of pre-incubated MP (250μM) upon addition of different concentrations (0-10μg·mL^{-1}) of pre-incubated MPH, BSA and Hb in Tris (50mM pH8.0)

Proteins (μg • mL^{-1})	A_{405}		
	MPH	BSA	Hb
0	0.053	0.053	0.053
10^{-5}	0.053	0.055	0.067
10^{-4}	0.059	0.053	0.054
10^{-3}	0.057	0.052	0.068
10^{-2}	0.063	0.055	0.063
10^{-1}	0.055	0.056	0.065
1	0.085	0.053	0.055
10	0.472	0.057	0.081

pH dependence of A_2HPS fluorescent intensity

Table S2. pH dependence of A_2HPS fluorescent intensity

pH	1	2	3	Average
2.02	7.096	7.068	6.999	7.054
2.98	6.893	7.062	7.092	7.016
4.07	6.948	7.050	6.897	6.965
5.05	8.222	8.421	8.874	8.506
6.07	82.068	82.267	81.510	81.948
7.05	102.883	104.156	104.790	103.943
8.02	113.365	116.156	116.686	115.402
9.05	114.394	114.671	116.607	115.224
10.04	115.680	116.877	116.800	116.452
11.00	118.601	118.617	118.529	118.582

Measured under Ex=370nm, Em=490nm at room temperature.

Fluorescent response of A_2HPS to MPH at a low pH condition

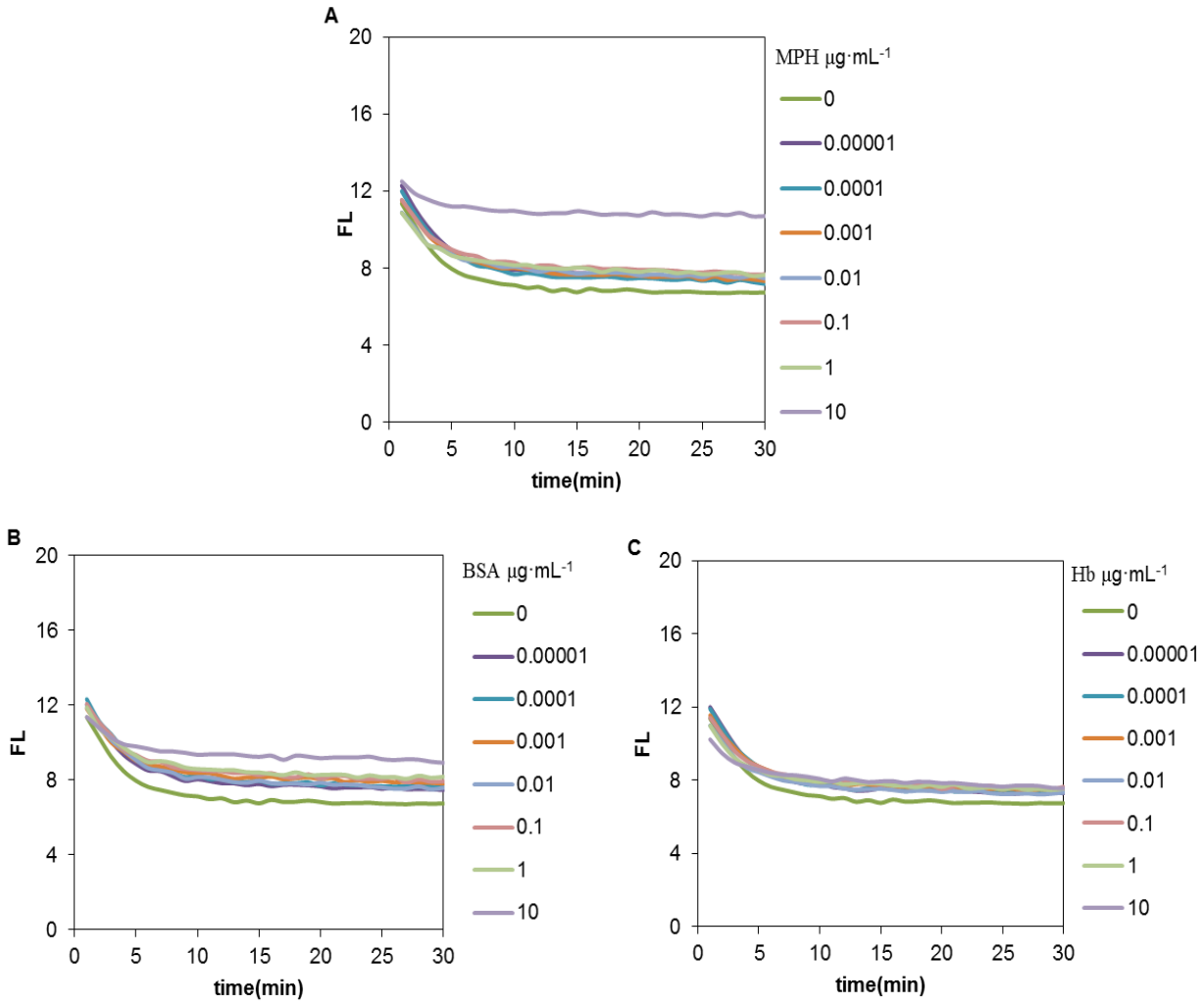

Figure S2. Time-dependence fluorescence intensity of A_2HPS probe (28.8μM) upon addition of different concentrations (0-10μg·mL^{-1}) of (A) MPH, (B) BSA and (C) Hb in Tris (50mM pH4.0) at room temperature.

Table S3. Fluorescence intensity of 28.8μM A_2HPS probe upon addition of different concentrations (0-10μg·mL^{-1}) of MPH, BSA and Hb in Tris (50mM pH4.0)

Proteins (μg • mL^{-1})	FL		
	MPH	BSA	Hb
0	6.751	6.751	6.751
10^{-5}	7.437	7.472	7.294
10^{-4}	7.198	7.603	7.355
10^{-3}	7.351	7.814	7.436
10^{-2}	7.483	7.591	7.331
10^{-1}	7.691	7.939	7.639
1	7.665	8.190	7.533
10	10.720	8.933	7.560

The data was recorded at 30^{th} min. Ex=370nm, Em=490nm.

Table S4. I/I_0 of 28.8μM A_2HPS-probe upon addition of different concentrations (0-10μg·mL^{-1}) of MPH, BSA and Hb in Tris (50mM pH4.0)

Protein (μg • mL^{-1})	I/I_0		
	MPH	BSA	Hb
10^{-5}	1.102	1.107	1.081
10^{-4}	1.066	1.126	1.090
10^{-3}	1.089	1.158	1.101
10^{-2}	1.108	1.125	1.086
10^{-1}	1.139	1.176	1.132
1	1.135	1.213	1.116
10	1.588	1.323	1.120

The data was recorded at 30th min. Ex=370nm, Em=490nm.

Quench effect of MP and PNP on fluorescence of A_2HPS

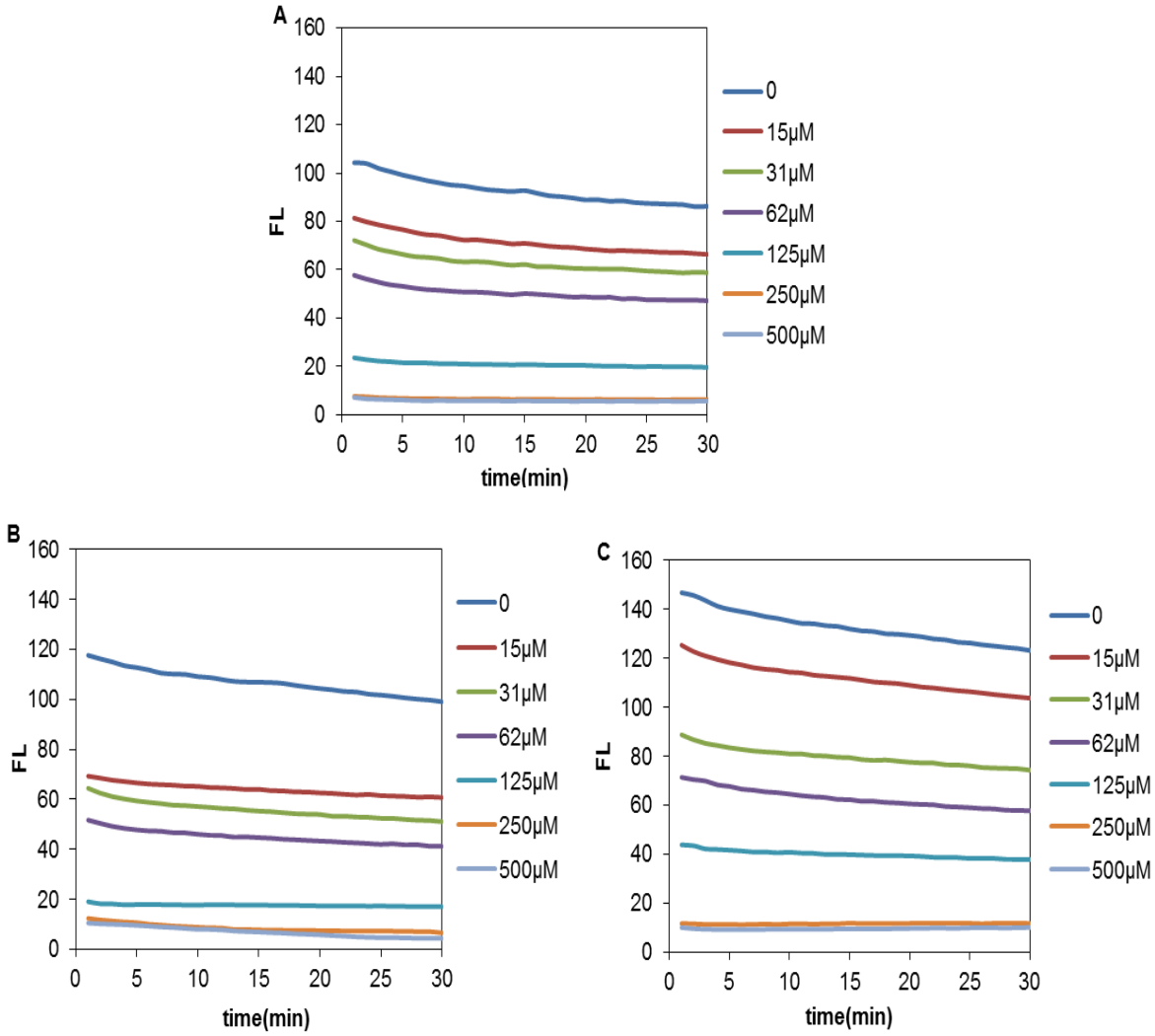

Figure S3. Time-dependence fluorescence intensity of A_2HPS in the presence of different concentrations of MP (0-500μM) in Tris (50mM pH8.0). (A) 14.4μM A_2HPS, (B) 28.8μM A_2HPS and (C) 72μM A_2HPS at room temperature. Ex=370nm, Em=490nm.

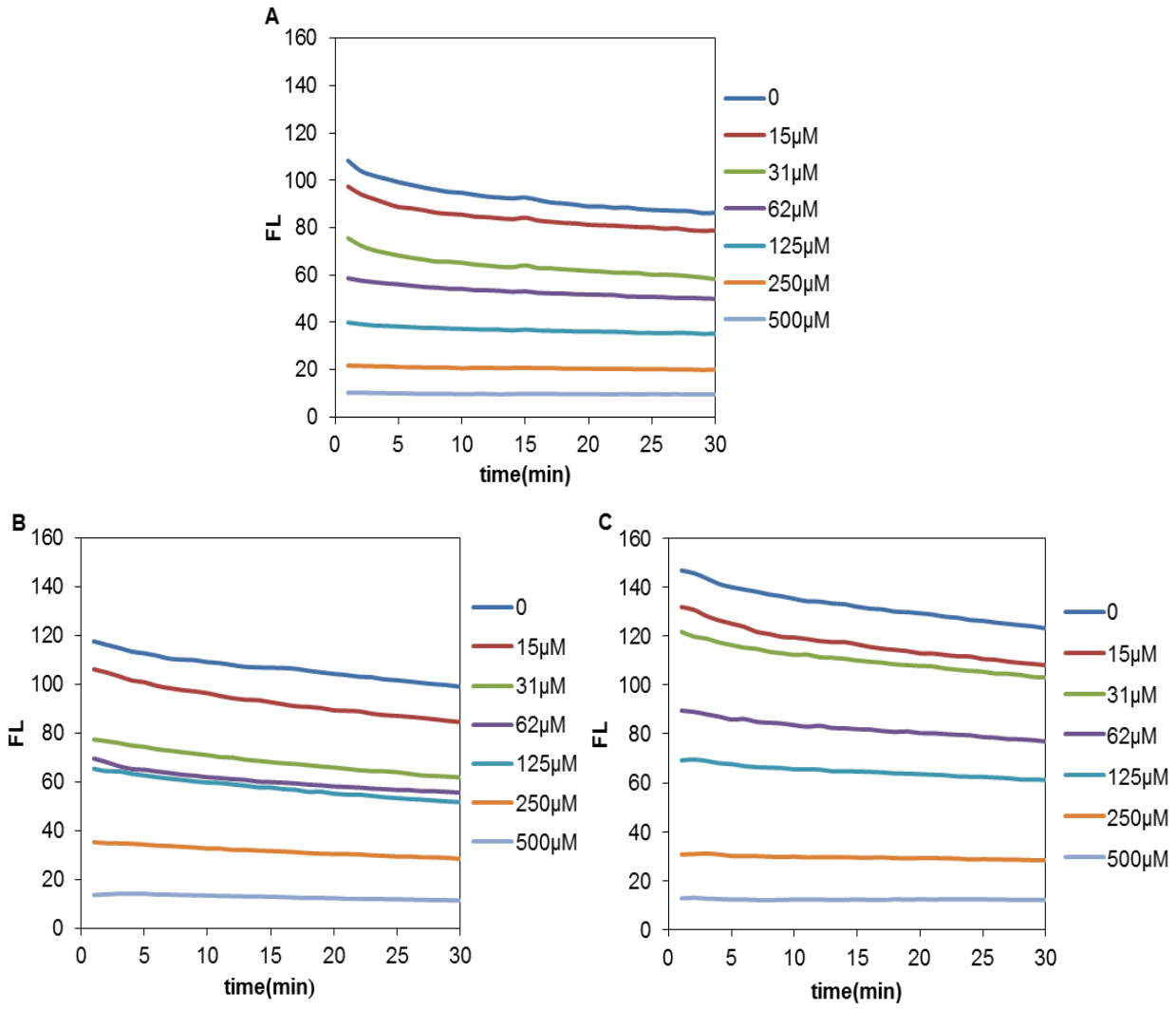

Figure S4. Time-dependence fluorescence intensity of A_2HPS in the presence of different concentrations of PNP (0-500μM) in Tris (50mM pH8.0). (A) 14.4μM A_2HPS, (B) 28.8μM A_2HPS and (C) 72μM A_2HPS at room temperature. Ex=370nm, Em=490nm.

Table S5. Fluorescent intensity of quench effect of MP and PNP

MP/PNP（μM）	FL					
	14.4μM A_2HPS		28.8μM A_2HPS		72μM A_2HPS	
	MP	PNP	MP	PNP	MP	PNP
0	86.264	86.264	99.059	99.059	123.118	123.118
15	66.393	78.729	60.744	84.580	103.664	108.087
31	58.830	58.274	51.087	61.950	74.283	103.084
62	47.231	49.956	41.246	55.687	57.715	76.943
125	19.689	35.226	17.062	51.801	37.727	61.233
250	6.367	20.091	6.625	28.575	11.660	28.401
500	5.721	9.595	4.440	11.563	10.011	12.267

The data was recorded at 30^{th} min. Ex=370nm, Em=490nm.

Table S6. I/I_0 of quench effect of MP and PNP

MP/PNP（μM）	I/I_0					
	14.4μM A_2HPS		28.8μM A_2HPS		72μM A_2HPS	
	MP	PNP	MP	PNP	MP	PNP
15	0.770	0.912	0.613	0.854	0.842	0.878
31	0.682	0.676	0.516	0.625	0.604	0.838
62	0.548	0.579	0.416	0.562	0.469	0.625
125	0.228	0.408	0.172	0.523	0.306	0.497
250	0.074	0.233	0.067	0.288	0.095	0.231
500	0.066	0.111	0.045	0.117	0.081	0.100

Fluorescent response of A_2HPS to MPH in the presence of MP

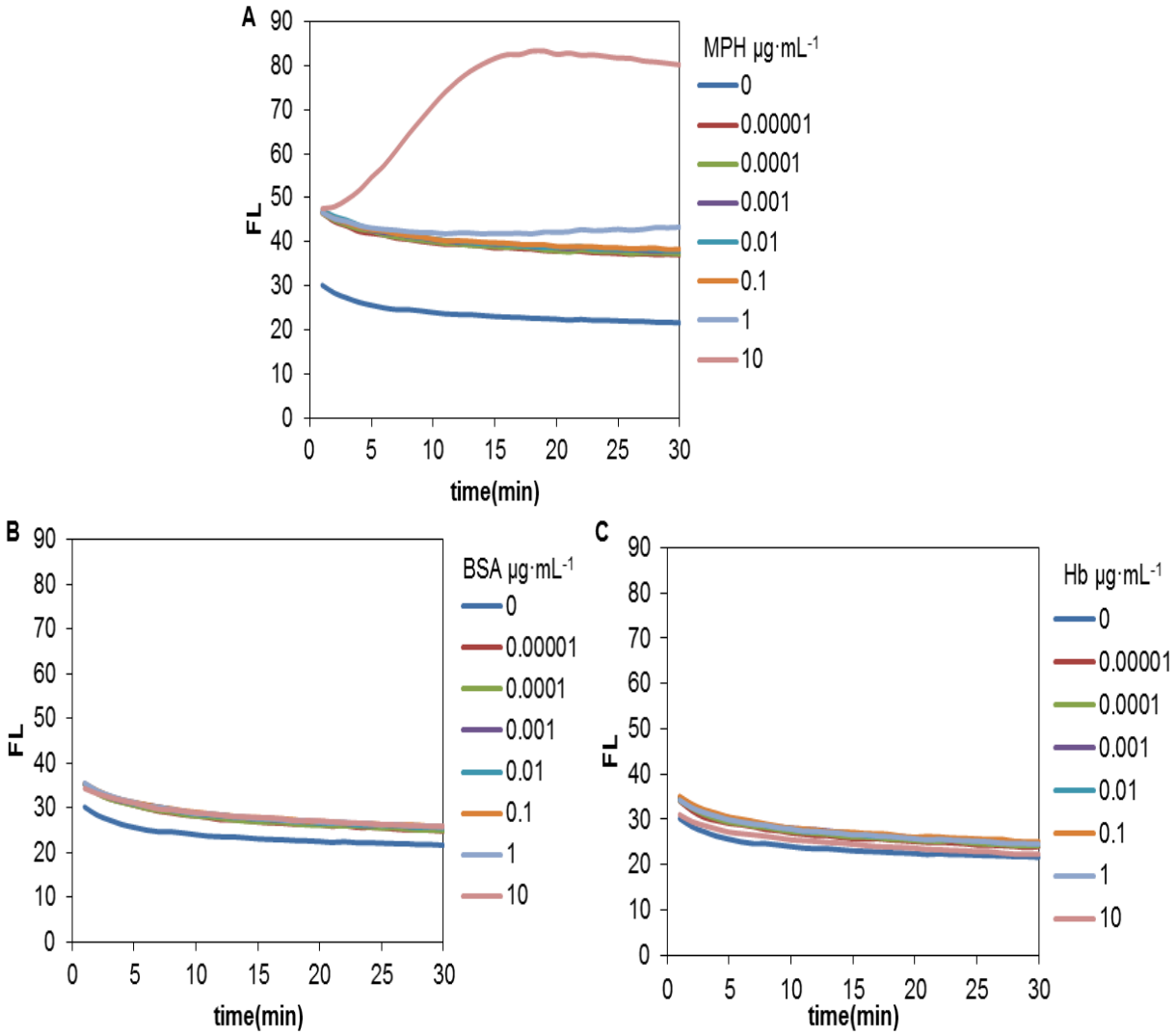

Figure S5. Time-dependence fluorescence intensity of pre-incubated MP-A_2HPS upon addition of different concentrations (0-10μg·mL^{-1}) of pre-incubated proteins (A) MPH, (B) BSA and (C) Hb in Tris (50mM pH8.0) at room temperature. Ex=370nm, Em=490nm.

Table S7. FL intensity of pre-incubated MP-A_2HPS upon addition of different concentrations (0-10μg·mL^{-1}) of pre-incubated MPH, BSA and Hb in Tris (50mM pH8.0)

Protein (μg • mL^{-1})	FL		
	MPH	BSA	Hb
0	21.588	21.588	21.588
10^{-5}	37.044	24.755	23.962
10^{-4}	37.271	24.863	24.227
10^{-3}	37.958	25.654	24.778
10^{-2}	37.979	25.440	24.528
10^{-1}	38.360	25.785	25.057
1	43.343	25.750	24.543
10	80.225	25.810	22.265

The data was recorded at 30^{th} min. Ex=370nm, Em=490nm.

Table S8. I/I_0 of pre-incubated MP-A_2HPS upon addition of different concentrations (0-10μg·mL^{-1}) of pre-incubated MPH, BSA and Hb in Tris (50mM pH8.0)

Protein (μg • mL^{-1})	I/I_0		
	MPH	BSA	Hb
10^{-5}	1.715	1.146	1.109
10^{-4}	1.726	1.151	1.122
10^{-3}	1.758	1.188	1.148
10^{-2}	1.758	1.179	1.136
10^{-1}	1.776	1.194	1.161
1	2.007	1.192	1.136
10	3.714	1.195	1.031

Fluorescence intensity of MP quenched A_2HPS at different temperature

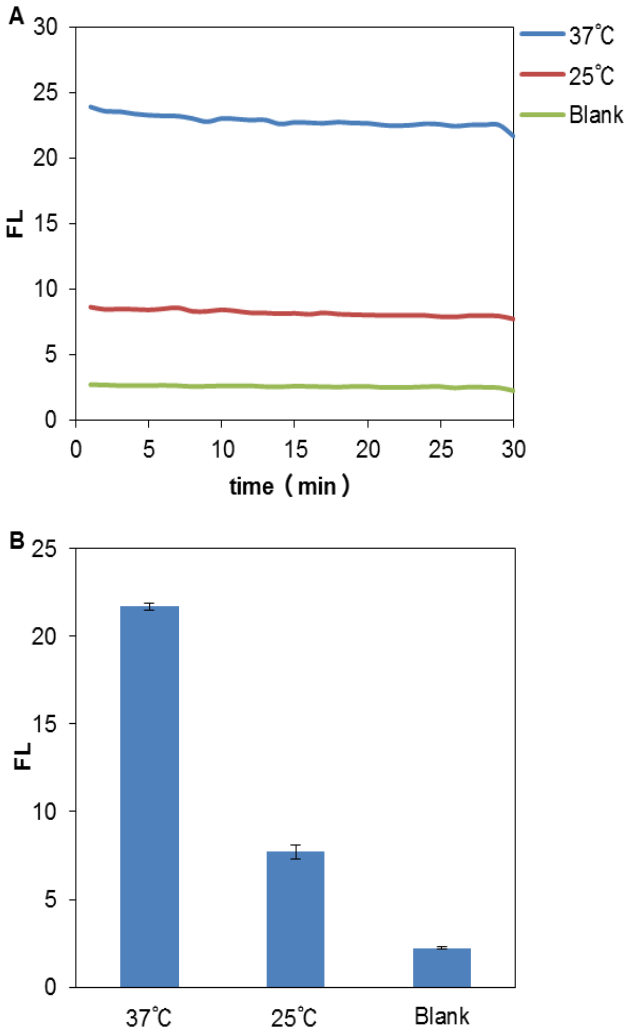

Figure S6. (A) Spectra of time-dependence of fluorescence intensity of 28.8μM A_2HPS in the presence of 250μM MP quenched in Tris (50mM pH8.0) at different temperature. (B) Bar graph of the fluorescence intensity recorded at 30th min. E=370nm, Em=490nm.

The kinetics of the MPH catalysis of the turnover of MP

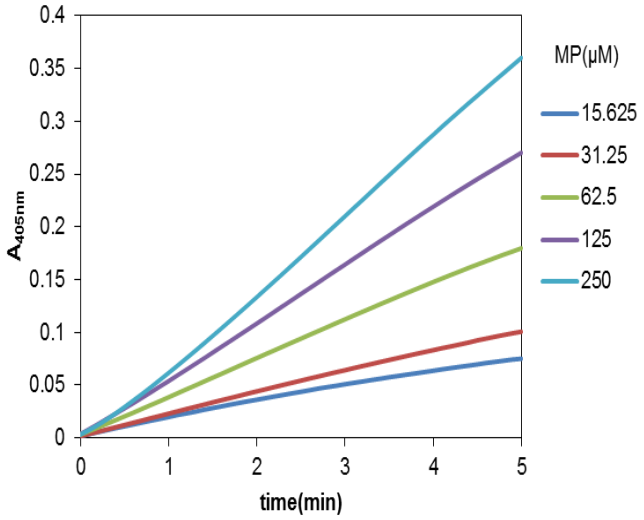

Figure S7. Progress curves of a set of enzyme-catalyzed reactions with different starting concentrations of MP (15-250μM). The A_{405nm} was recorded continuously for 5 min.

Table S9 The value of ΔA_{405nm} measured from the above experiment

MP (μM)	15.625	31.25	62.5	125	250
ΔA_{405nm} (5min)	0.0735	0.0991	0.1770	0.2670	0.3570

Table S10 The A_{405nm} of PNP of different concentrations (50 mM pH8.0 Tris buffer)

PNP（μM）	1	2	3	mean
blank	0.079	0.077	0.076	0.077
5	0.108	0.103	0.101	0.104
10	0.144	0.146	0.142	0.144
20	0.219	0.225	0.216	0.220
40	0.363	0.378	0.367	0.369
80	0.644	0.636	0.639	0.640
100	0.789	0.8	0.791	0.793

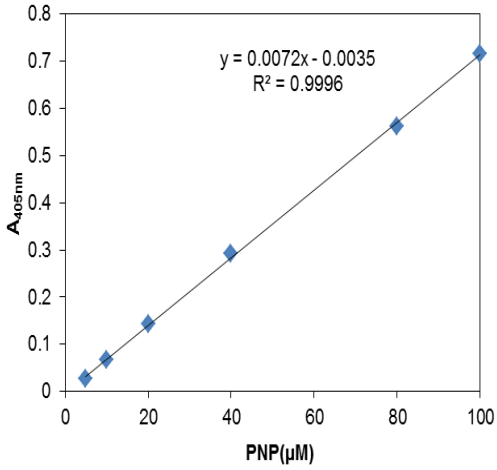

Figure S8. The spectrophotometric working plot for the concentration of PNP.

Table S11. Initial velocity as a function of substrate (MP) concentration for MPH enzymatic reaction

S (μM)	V (μM·s^{-1})	1/S (μM^{-1})	1/V ($s \cdot \mu M^{-1}$)
15.625	0.052	0.0040	5.992
31.25	0.104	0.0080	7.985
62.5	0.208	0.0174	11.967
125	0.417	0.0412	21.053
250	0.833	0.0825	28.052

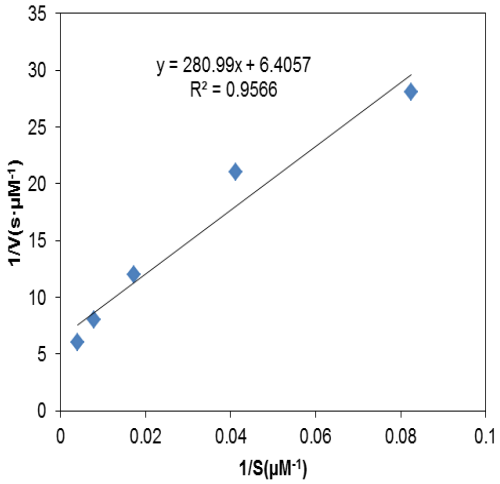

Figure S9. Lineweaver—Burk double-reciprocal plot for selected data from Table S11 within the range of [S] =15.625—250μM

The calculation of enzyme kinetics parameters

According to the Lineweaver - Burk double inverse formula

$$\frac{1}{V} = \frac{\mathrm{Km}}{Vm} \cdot \frac{1}{S} + \frac{1}{Vm}$$

Hence,

$$\mathrm{Vm} = \frac{1}{6.4057} = 0.156\mu\mathrm{M} \cdot \mathrm{s}^{-1} = 9.36 \times 10^{-3} m\mathrm{M} \cdot min^{-1}$$

$$\mathrm{Km} = 280.99\mathrm{Vm} = 280.99 \times 0.156\mu\mathrm{M} \cdot s^{-1} = 43.83\mu\mathrm{M}$$

$$\mathrm{Kcat} = \frac{Vm}{[E]} = \frac{0.156 \times 10^{-6}\mathrm{mol/s} \cdot \mathrm{L} \times 3.5 \times 10^{4}\mathrm{g/mol}}{0.5 \times 10^{-3}\mathrm{g/L}} = 10.92\mathrm{s}^{-1}$$
$$= 655.2 min^{-1}$$

Table S12. Estimates of the kinetic constants Vm, Km and Kcat from Lineweaver - Burk double inverse formula of the data from Table S11

Vm ($mM \cdot min^{-1}$)	Km (mM)	Kcat (min^{-1})
9.36×10^{-3}	0.0438	655.2

Fluorescent response of A_2HPS to low concentration of MPH in the presence of MP

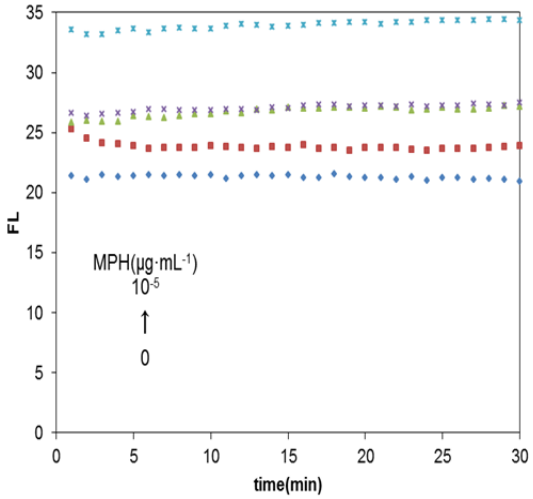

Figure S10. Time-dependence fluorescence intensity of pre-incubated A_2HPS-MP upon addition of different concentrations (0-10^{-5}μg·mL^{-1}) of pre-incubated MPH. A_2HPS-MP was incubated with the MPH for 4h before the fluorescence detection.

Table S13. FL intensity of pre-incubated MP-A_2HPS upon addition of different concentrations (0-10^{-5}μg·mL^{-1}) of pre-incubated MPH in Tris (50mM pH8.0)

μg·mL^{-1}	FL			
	1	2	3	AVE
0	20.331	20.075	24.448	20.951
10^{-8}	23.954	23.784	23.863	23.867
10^{-7}	27.389	29.832	24.280	27.167
10^{-6}	28.404	27.477	27.466	27.782
10^{-5}	35.947	35.670	31.432	34.350

Table S14. I/I_0 of pre-incubated MP-A_2HPS upon addition of different concentrations (0-10μg·mL^{-1}) of pre-incubated MPH in Tris (50mM pH8.0)

MPH(μg·mL^{-1})	I/I_0
10^{-8}	1.139
10^{-7}	1.297
10^{-6}	1.310
10^{-5}	1.639