Supporting information

A promiscuous glutathione transferase transformed into a selective thiolester hydrolase

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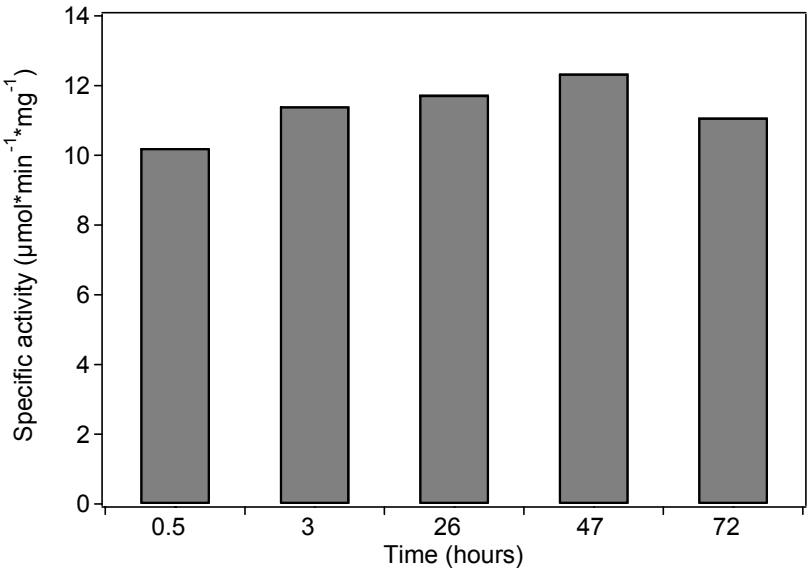
The stability of A216H as measured by the specific activity (μmol*min^{-1}*mg^{-1}) towards CDNB.

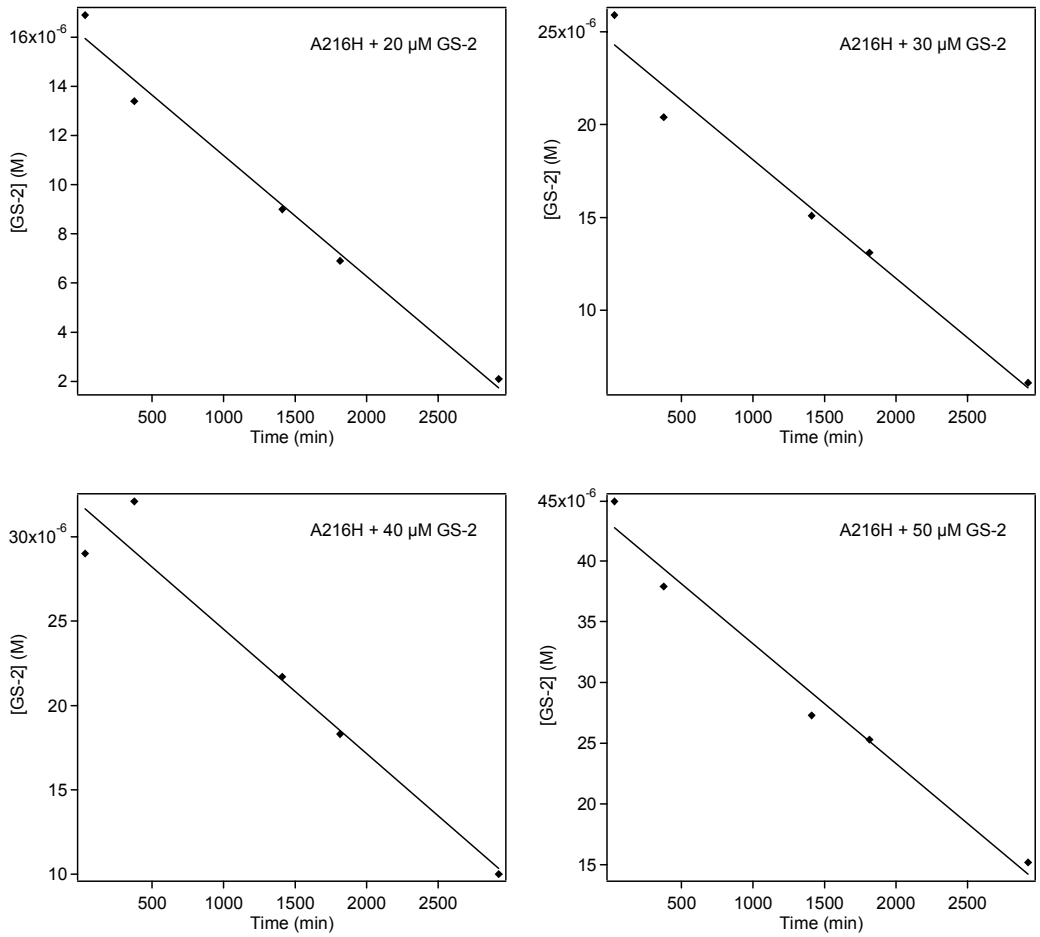


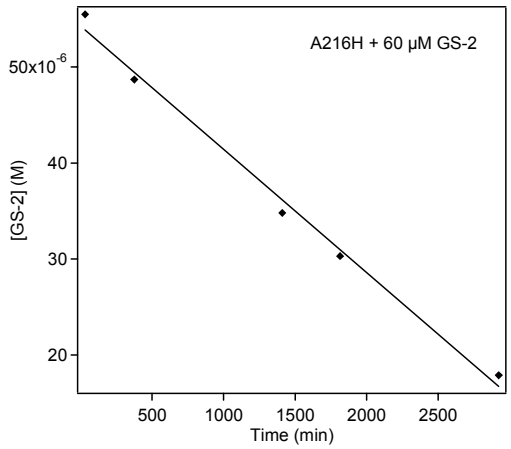
Acetonitrile gradients used for the experiments analyzed by HPLC.

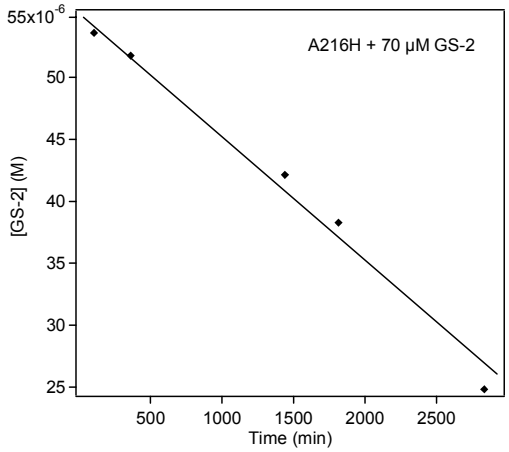
The GS-thiolesters in experiment A to D (with exceptions listed below) were analyzed by the following gradient:
0-2 min: 10% ACN 0.1%TFA, flow = 1 mL/min
2-30 min: 10→66% ACN 0.1%TFA, flow = 1 mL/min
30-31 min: 66→90% ACN 0.1%TFA, flow = 1 mL/min
31-35 min: 90% ACN 0.1%TFA, flow = 3 mL/min
35-35,5 min: 90→10% ACN 0.1%TFA, flow = 3 mL/min
35,5-41 min: 10% ACN 0.1%TFA, flow = 2 mL/min
41-45 min: 10% ACN 0.1%TFA, flow = 1 mL/min

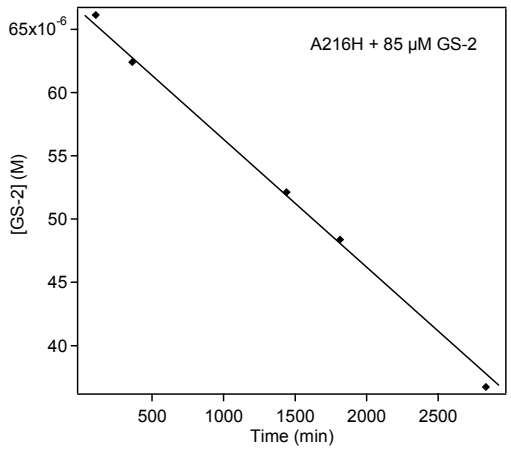
GS-2 in experiment B, C, D and in the saturation kinetic experiment as well as GS-8 in experiment B and GS-12 in experiment C were analyzed by following gradient:
0-2 min: 10% ACN 0.1%TFA, flow = 1 mL/min
2-60 min: 10→66% ACN 0.1%TFA, flow = 1 mL/min
60-61 min: 66→90% ACN 0.1%TFA, flow = 1 mL/min
61-65 min: 90% ACN 0.1%TFA, flow = 3 mL/min
65-65,5 min: 90→10% ACN 0.1%TFA, flow = 3 mL/min
65,5-71 min: 10% ACN 0.1%TFA, flow = 2 mL/min
71-75 min: 10% ACN 0.1%TFA, flow = 1 mL/min

The concentration of GS-2 as a function of time when incubated with A216H. The data is derived from HPLC analysis using relative responses.









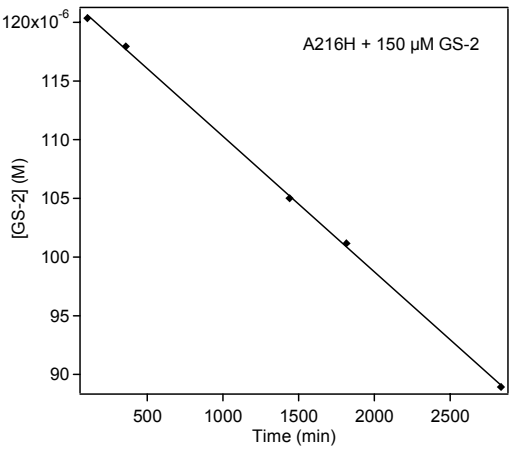


***Table**. Saturation kinetics of A216H-catalyzed hydrolysis of GS-2.*

[GS-2] [μM]	Time points [min]					
4.4	155	345	1950	3165		
10	14	1357	1925	3071		
20	30	375	1410	1815	2925	
22	155	345	1950	3165		
30	14	1357	1925	3071		
30	30	375	1410	1815	2925	
35	20	150	480	1260	1640	3440
40	14	1357	1925	3071		
40	30	375	1410	1815	2925	
45	30	375	1410	1815	2925	
50	14	1357	1925	3071		
50	30	375	1410	1815	2925	
55	30	375	1410	1815	2925	
58	105	360	1440	1815	2835	
60	30	375	1410	1815	2925	
60	105	360	1440	1815	2835	
65	105	360	1440	1815	2835	
70	105	360	1440	1815	2835	
75	105	360	1440	1815	2835	
80	105	360	1440	1815	2835	
85	105	360	1440	1815	2835	
150	105	360	1440	1815	2835	
175	14	1357	1925	3071		