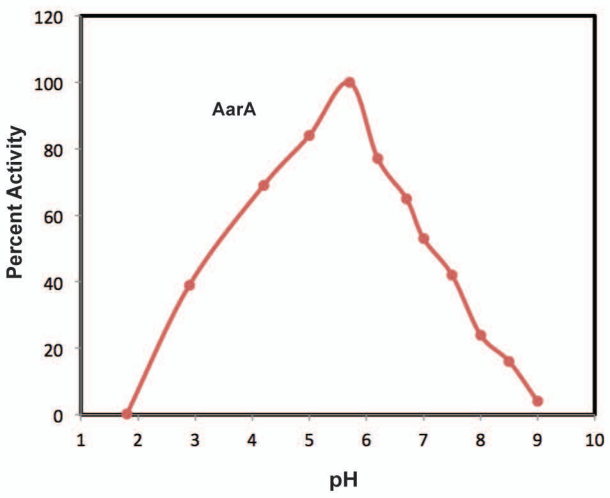
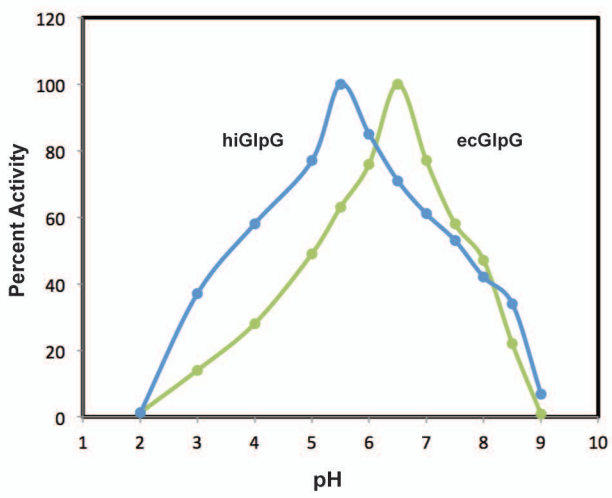
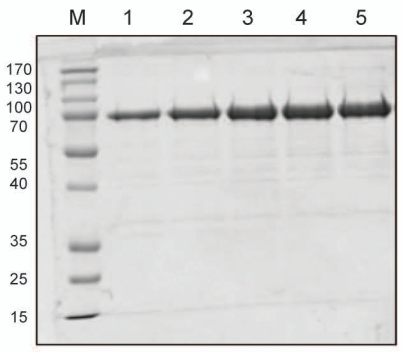
A


AarA
Percent Activity
pH
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10

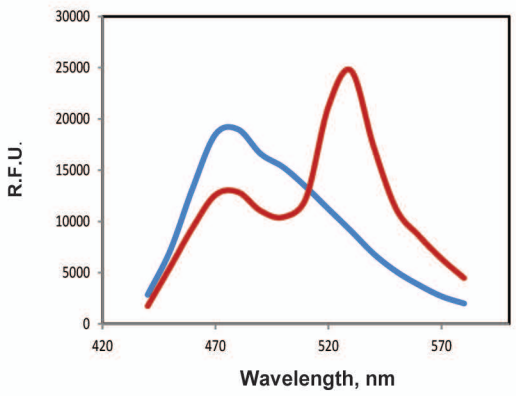
B


hiGlpG
ecGlpG
Percent Activity
pH
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10

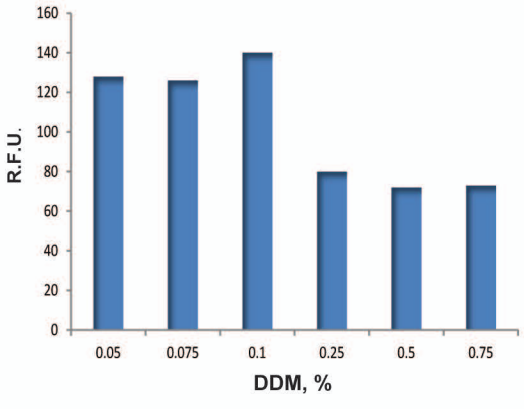
C

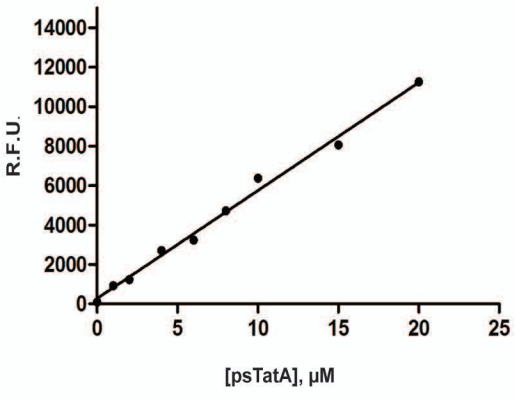

M
1
2
3
4
5
170
130
100
70
55
40
35
25
15

D


R.F.U.
Wavelength, nm
30000
25000
20000
15000
10000
5000
0
420
470
520
570

E


R.F.U.
DDM, %
160
140
120
100
80
60
40
20
0
0.05
0.075
0.1
0.25
0.5
0.75


R.F.U.
[psTatA], μM
14000
12000
10000
8000
6000
4000
2000
0
0
5
10
15
20
25

Supplemental Figure S3. Characterization of psTatA-FRET substrate and activity dependence on pH. (A) AarA activity dependence on pH (red). A. The proteolysis reaction was performed at 37oC for 2 hr in the presence of 0.18 μM of rhomboid protease and 2 μM of full-length psTatA-FRET in 50 mM Tris-HCl, 150 mM NaCl, 20% glycerol, 0.1% DDM and 2 mM DTT reaction buffer, at pH interval spanning from 2 to 9. The pH of the reaction buffer was assessed before and after the addition of all components including substrate to ensure proper buffering capacity. (B) pH dependence on the ability of ecGlpG (blue) and hiGlpG (green) to cleave fluorescein-labelled casein was assessed in a similar way. The highest initial velocity was calculated to determine the percent activity along the pH gradient. Results at pH 4.0, 6.0 and 8.0 were confirmed in Acetate, MES buffer and Tris respectively using both full length psTatA-FRET and non-fluorescently labelled full-length psTatA. (C) SDS-PAGE of purified psTatA-FRET. M – molecular markers (kDa), 1-5 – protein fractions after SEC. (D) Emission spectrum of psTatA-FRET. psTatA-FRET (2 μM) before (red) and after (blue) incubation with the same amount of AarA for 4 hr at 37oC. (E) The dependence of DDM concentration in activity buffer on AarA activity with psTatA-FRET substrate. 2 μM of psTatA- FRET was reacted with 0.18 μM of AarA for 2 hr at 37oC in activity buffer, containing different DDM concentrations. The initial velocities were plotted against the corresponding concentrations of DDM. (F) The linear proportionality between fluorescent signals emitted and the amount of psTatA.