Supporting information

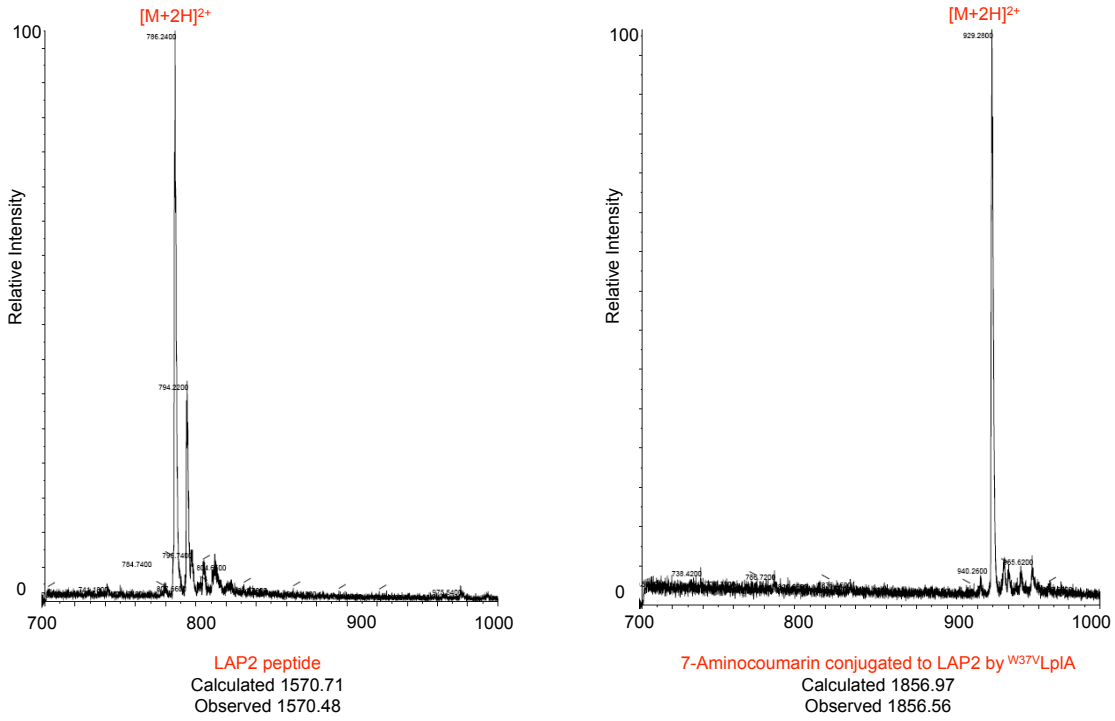

Figure S1. ESI mass spectra of the 7-aminocoumarin-LAP conjugate and the LAP starting material (starred peaks in Figure 2C).

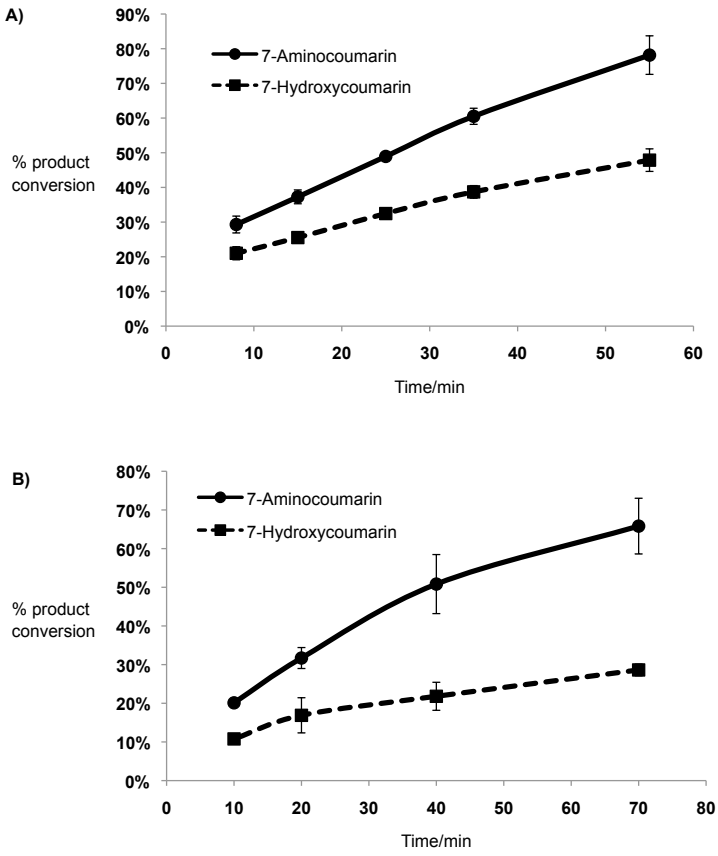

Figure S2. Comparison of 7-aminocoumarin and 7-hydroxycoumarin ligation kinetics by ^{W37V}LplA. Either 500 μM of each probe was used (top), or 100 μM of each probe (bottom). Product conversions were measured using HPLC. Measurements were performed in triplicate. Error bars, ± 1 s.d.

7-Aminocoumarin and 7-hydroxycoumarin pH profiles (Figure 2B)

Fluorescence emission was recorded for 150 μM solutions, using a TECAN Safire Microplate Reader and a plastic transparent-bottomed 384-well plate (Greiner). pH 3-10 buffers were prepared as the table below. The exact pH values of buffer were measured, and slightly adjusted by using <2mL of 1M HCl or NaOH solution, prior to use.

pH	1M acetic acid solution	1 M sodium acetate solution	
pH=3	98.2 mL	1.8 mL	
pH=4	84.7 mL	15.3 mL	
pH=5	35.7 mL	64.3 mL	
pH=6	5.2 mL	94.8 mL	
pH	1M disodium hydrogen phosphate solution	1M HCl solution	1M NaOH solution
pH=7	75.6 mL	24.4 mL	--
pH=8	95.5 mL	4.4 mL	--
pH=9	95.5 mL	4.5 mL	--
pH=10	96.6 mL	--	3.4 mL