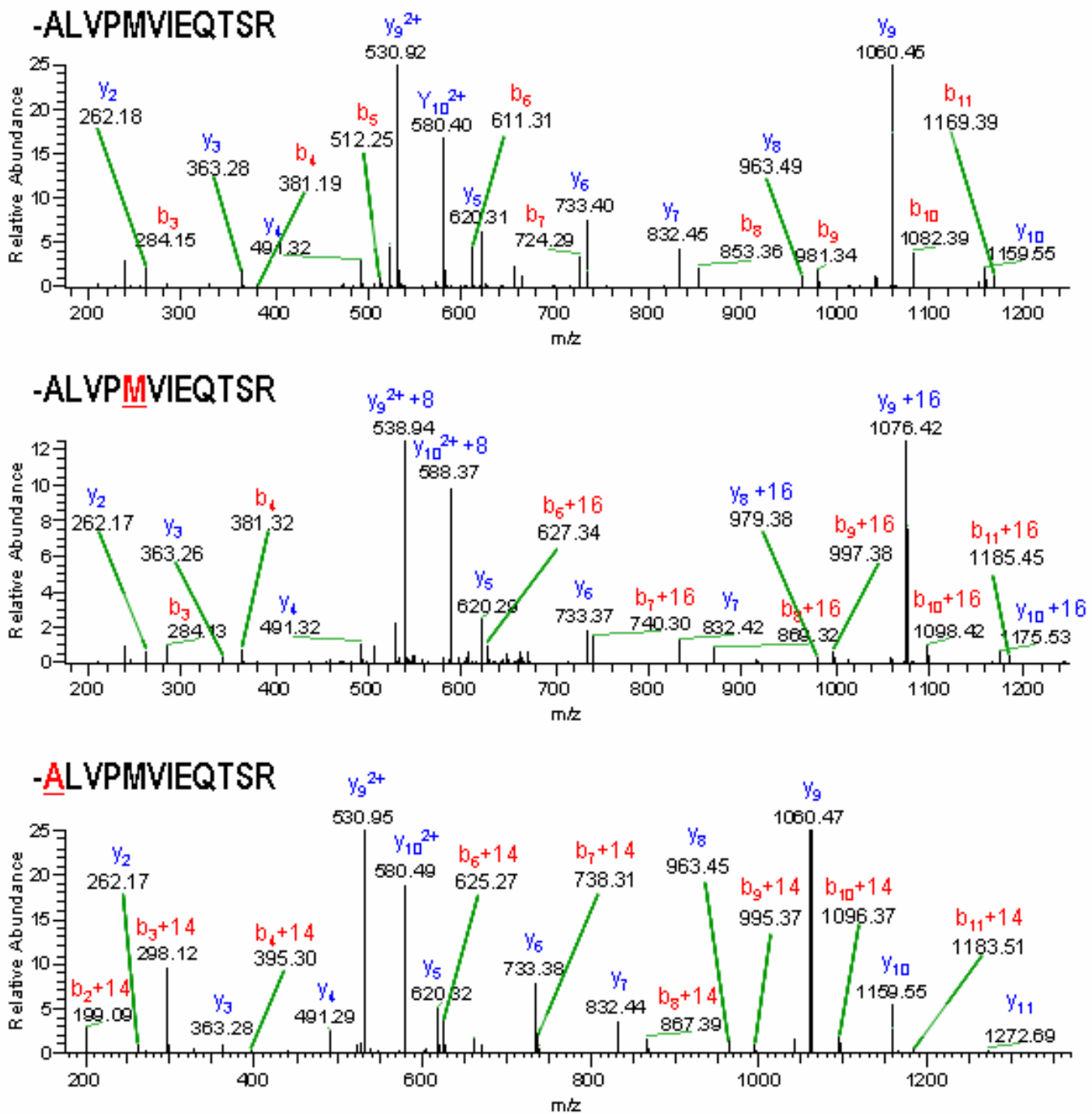


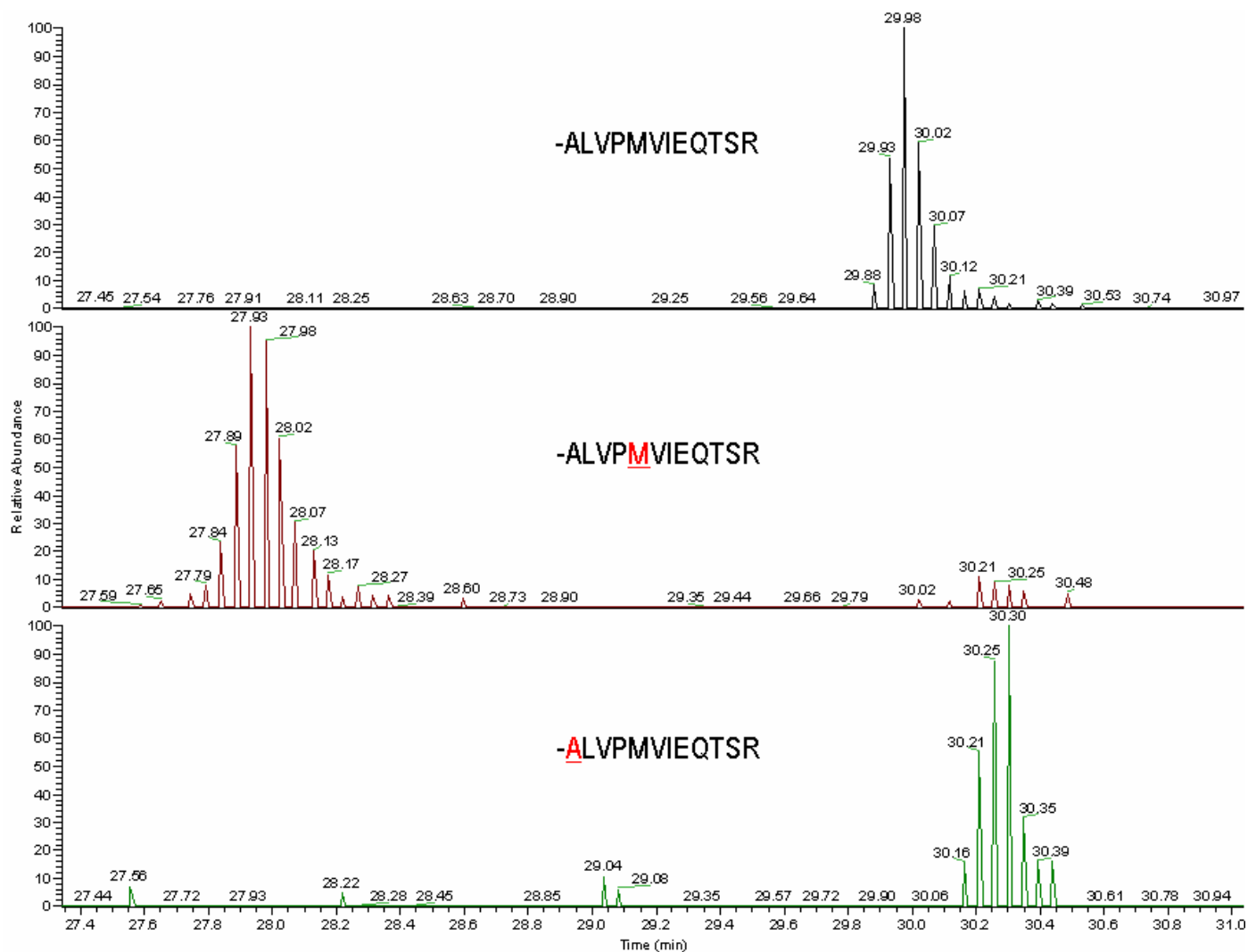
# Supporting Information

## **The ClpP N-terminus coordinates substrate access with protease active site reactivity**

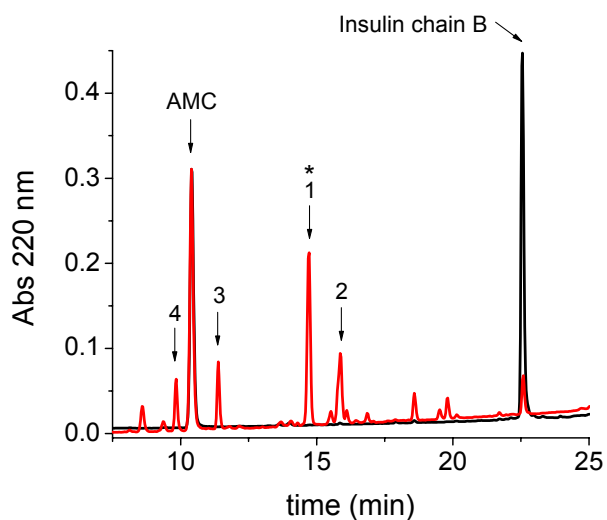
Laura D. Jennings,<sup>‡</sup> Jen Bohon,<sup>§</sup> Mark R. Chance,<sup>§</sup> and Stuart Licht\*,<sup>‡</sup>



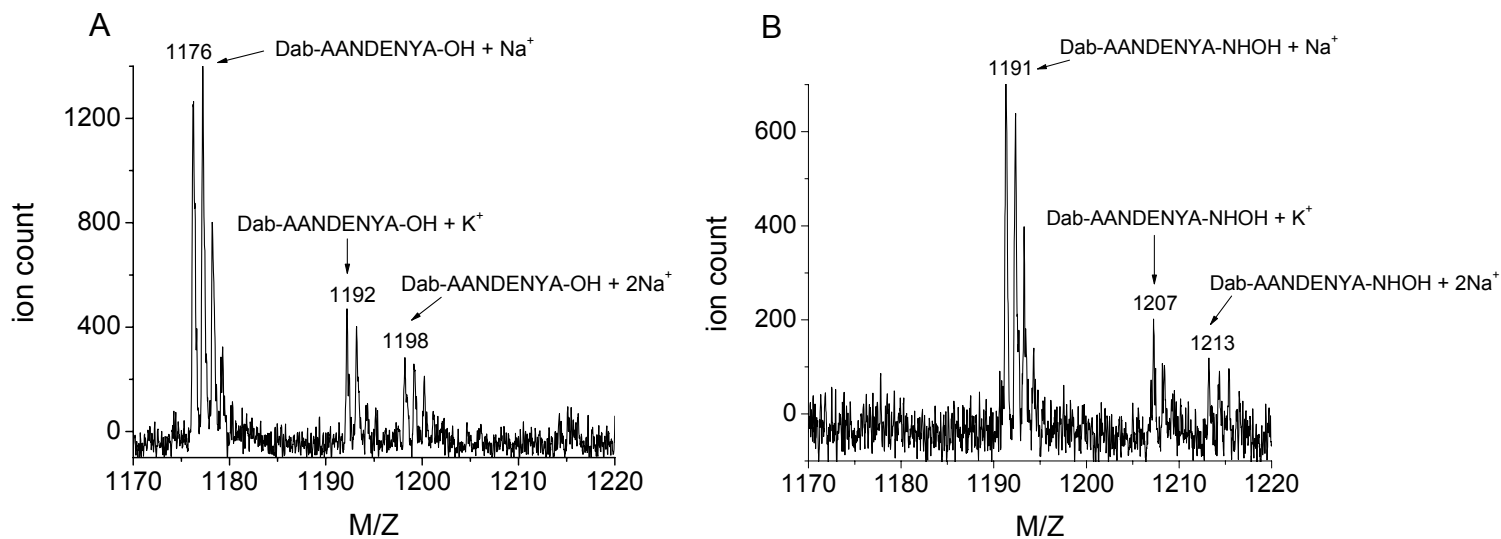
**Figure S1.** Synchrotron footprinting tandem MS spectra of unmodified (top), modified methionine 5 (middle) and modified alanine 1 (bottom) ClpP N-terminal (1-12) peptides. Observed y- and b-ions are labeled.



**Figure S2.** Synchrotron footprinting chromatographic traces for the unmodified (top), modified methionine 5 (middle) and modified alanine 1 (bottom) peptides.



**Figure S3.** HPLC trace of insulin chain B degradation. Shown in black is degradation of 50  $\mu$ M insulin chain B with 1  $\mu$ M ClpP<sub>14</sub>, 2  $\mu$ M ClpA<sub>6</sub>, and 2 mM ATP $\gamma$ S at t=0. Shown in red is t=10 minutes. The most abundant product is labeled with an asterisk. (All data shown in Figure 2 are with the most abundant product.)



**Figure S4.** MALDI analysis of ssrA-dabysl products. A) MALDI spectrum of carboxylic acid product peak. B) MALDI spectrum of hydroxamic acid product peak.

## Supporting Text

The mechanism in Figure 8 includes a step in which the acyl-enzyme intermediate is partitioned between a catalytically active conformation that can hydrolyze the intermediate rapidly and an inactive conformation that cannot catalyze rapid acyl-enzyme intermediate hydrolysis and is assumed to be stable (i.e., re-activation is assumed to be negligible). This mechanism accounts for the observation of a rapid kinetic phase, where most of the enzyme is actively turning over, and the transition to a slow kinetic phase, where most of the enzyme is in the inactive form. Here, a simplified quantitative model is used to illustrate how the kinetic partitioning between the conformations can predict the amplitude of the rapid phase (i.e., the average number of turnovers before the enzyme transitions into the inactive conformation).

The probability  $p$  that the enzyme proceeds through the active conformation rather than entering the inactive conformation can be expressed as:

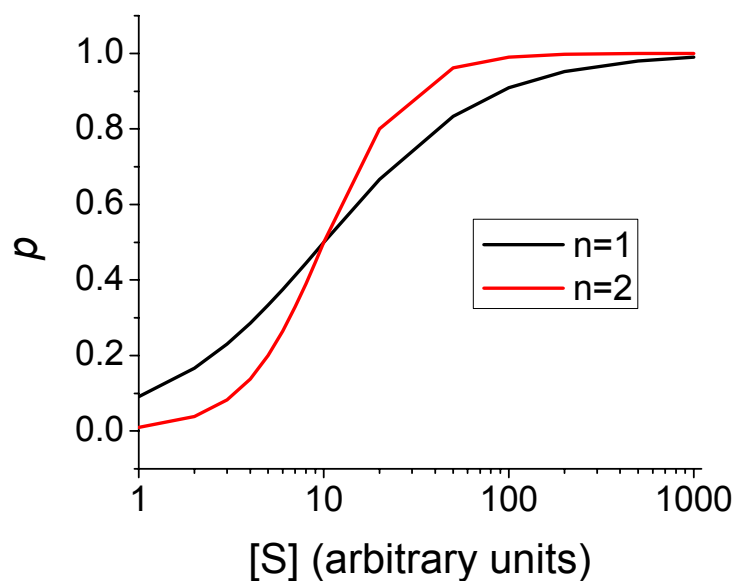
$$p = \frac{k_{\text{act}}[S]^n}{k_{\text{act}}[S]^n + k_{\text{inact}}},$$

where  $k_{\text{act}}$  is the rate constant for transition into the active conformation,  $k_{\text{inact}}$  is the rate constant for transition into the inactive conformation,  $[S]$  is the concentration of substrate peptide, and  $n$  is the effective kinetic order of substrate peptide binding. This expression allows the dependence of  $p$  on  $[S]$  to be determined for arbitrary values of  $k_{\text{act}}$ ,  $k_{\text{inact}}$ , and  $n$  (Fig. S3). With increasing  $[S]$ , the probability of productive turnover increases, approaching unity in the limit of  $[S] \rightarrow \infty$ . The increase of  $p$  with increasing  $[S]$  becomes steeper when  $n > 1$ .

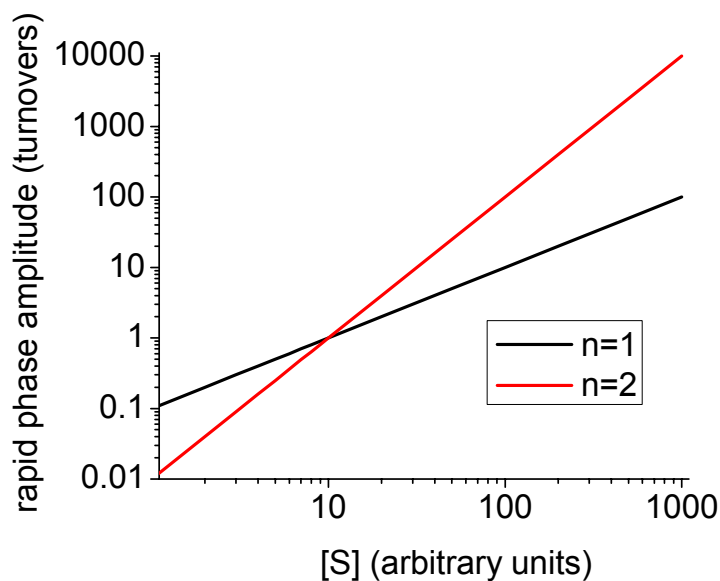
The number of turnovers before transition into the inactive conformation will be described by a geometric distribution<sup>1</sup> with expected value  $(1-q)/q$ , where  $q=1-p$ . This expected value gives the amplitude of the rapid phase. A plot of the dependence of this quantity on  $[S]$  for  $n=1$  and  $n=2$  shows that the rapid phase amplitude is proportional to  $[S]^n$  (Fig. S4). Experimentally, the rapid phase appears to exhibit a steep dependence on  $[S]$ ; this observation is consistent with substrate and/or N-terminal peptide binding at multiple sites being required for efficient partitioning into the active state. In practice, an absolute requirement for multiple binding events is likely to be an oversimplification, but a similar analysis holds for MWC cooperativity (in that case,  $n$  is analogous to the Hill coefficient, and can have non-integer values). Such cooperativity might also be observed if acylation of the active site serine drives the enzyme into its activated conformation.

## Reference

1. Feller, W. "An Introduction to Probability Theory and its Applications," vol. 1, 3<sup>rd</sup> ed., Wiley, New York (1968), pp. 164-166.



**Figure S5.** Dependence of  $p$  (probability of partitioning to productive turnover) on  $[S]$  (substrate concentration).  $k_{\text{act}}$  is set at 1, and  $k_{\text{inact}}$  is set at 0.1 (arbitrary units) for the purposes of illustration.



**Figure S6.** Dependence of rapid phase amplitude (average number of turnovers in the rapid phase) on  $[S]$  (substrate concentration).  $k_{\text{act}}$  is set at 1, and  $k_{\text{inact}}$  is set at 0.1 (arbitrary units) for the purposes of illustration. The rapid phase amplitude is proportional to  $[S]^n$ , where  $n$  is the number of substrate molecules bound in the active form of the enzyme.