Intrinsic site-selectivity of ubiquitin dimer formation

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Table S1. Values of k ($M^{-1}s^{-1}$) for the reaction of $Ubiquitin^{K\rightarrow C(NTB)}$ and $Ubiquitin^{C77}$ or AlaAlaCys

$Ubiquitin^{K\rightarrow C(NTB)}$	$Ubiquitin^{C77}$ [a]	AlaAlaCys [b]
K6C	17 ± 1	15 ± 2
K11C	90 ± 9	56 ± 4
K27C	3 ± 2	2.4 ± 0.6
K29C	6.9 ± 0.9	4.5 ± 1.0
K33C	10.0 ± 0.3	2.4 ± 0.6
K48C	58 ± 5	16 ± 2
K63C	182 ± 20	122 ± 4

[a] From data in Figure 2A and eq 3.
[b] From data in Figure S1 and eq 3.

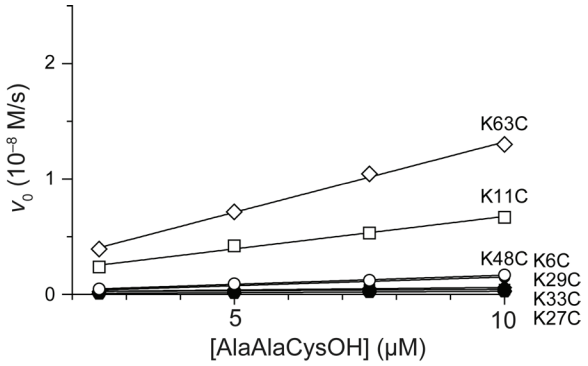


Figure S1. Graph of the rates of formation of ubiquitin dimers between AlaAlaCys (10 μM) and varying concentrations of a $Ubiquitin^{K\rightarrow C(NTB)}$ variant (0–10 μM). Data are for the release of 5-thio-2-nitrobenzoate during a reaction analogous to that shown in Figure 1B.

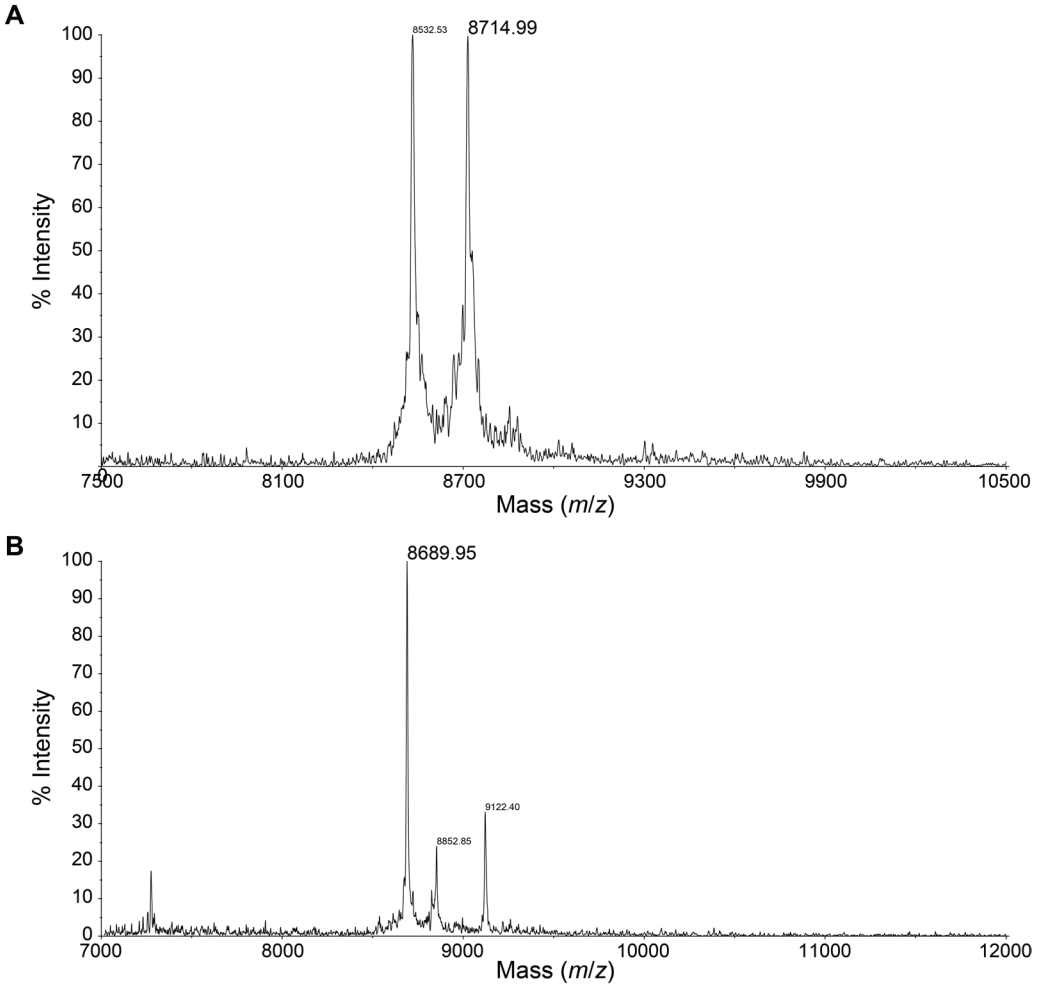


Figure S2. Characterization of monomeric ubiquitin variants by MALDI–TOF mass spectrometry. (A) A Ubiquitin$^{K\rightarrow C(NTB)}$ variant, K48C ubiquitin treated with DTNB (expected *m*/*z* 8715). The disulfide bond is labile to cleavage during analysis by MALDI–TOF mass spectrometry. (B) Ubiquitin^{77C} (expected *m*/*z* 8687).

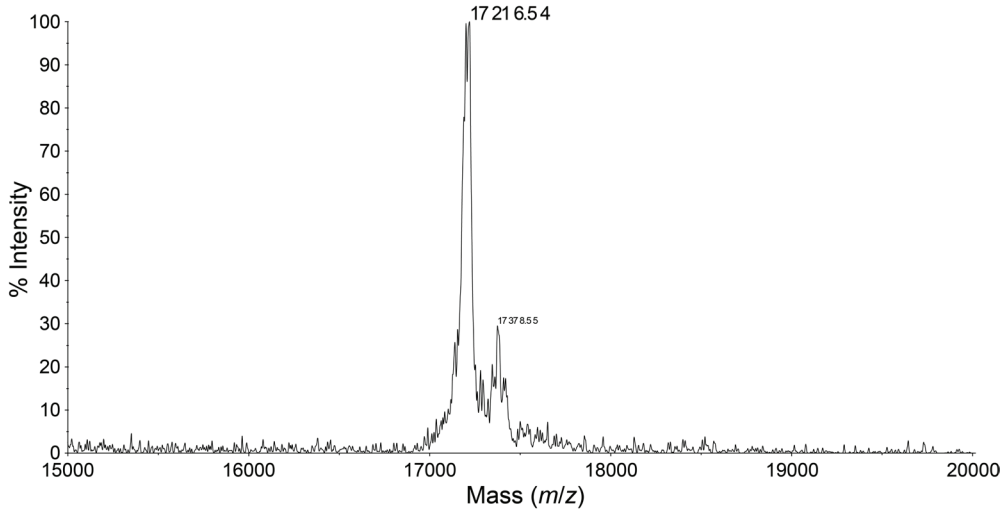
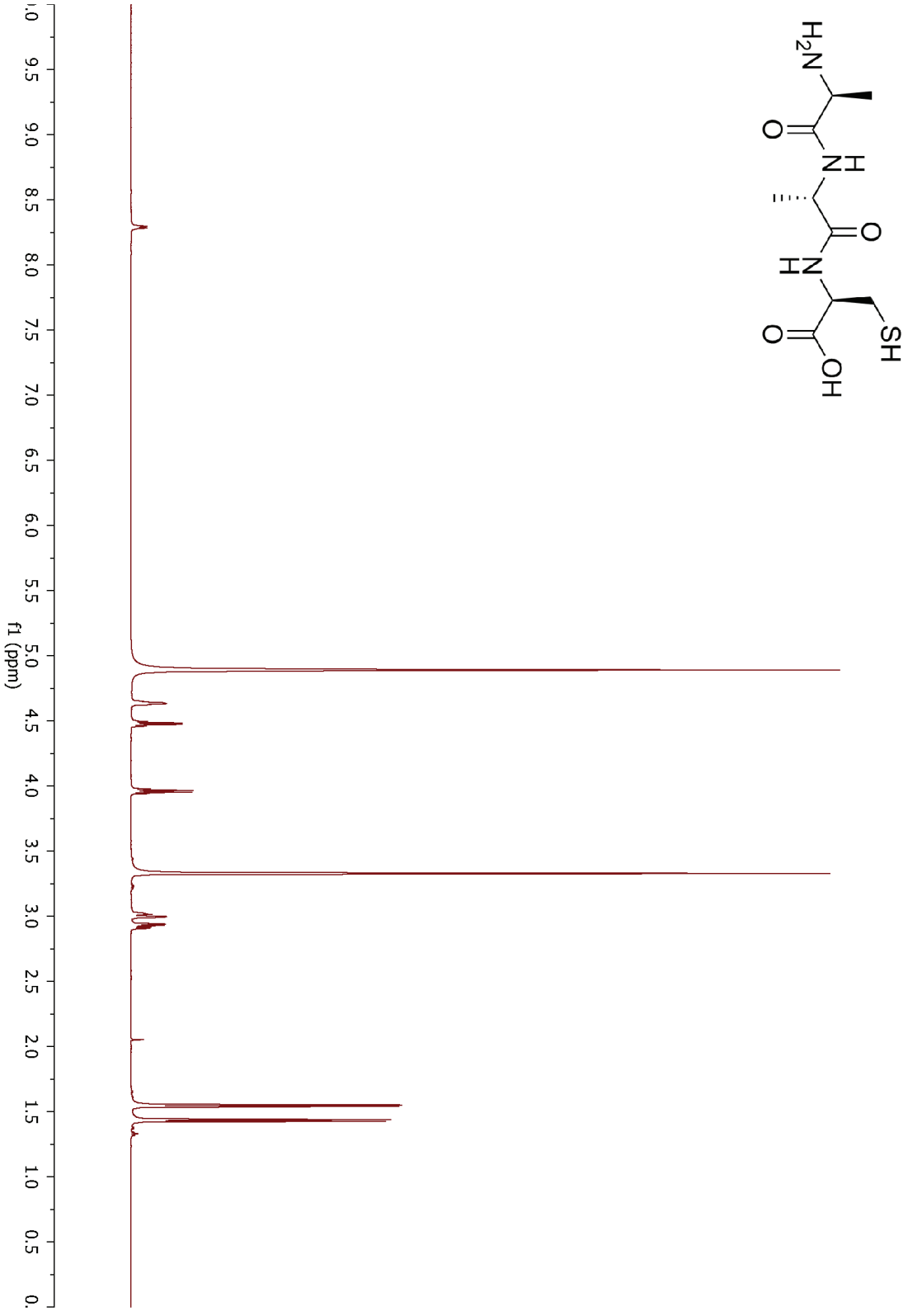


Figure S3. Characterization of a ubiquitin dimer by MALDI–TOF mass spectrometry, Ubiquitin^{77C}–Ubiquitin^{K48C} (expected *m*/*z* 17217).

^{1}H NMR Spectrum (Methanol-d_4) of AlaAlaCys



^{13}C NMR Spectrum (Methanol-d_4) of AlaAlaCys