SUPPORTING INFORMATION

Fig. S1. DSC thermograms of dimeric PR (dark red), PR_{D29N} (dark yellow) and PR_{D25N} (orange) in the presence of each of the set of eight inhibitors (28 μM) described in the text. For comparison, DSC scans of the unliganded proteins are also shown. For experimental conditions and data handling, see Materials and Methods and ref. 17. Data shown have been baseline corrected and normalized to the appropriate protease dimer concentration as described. The curves for PR and PR_{D25N} in the absence of inhibitors and in the presence of RPB or DRV are from ref. 17. For PR with DRV, ATV, SQV and APV, biphasic transitions were observed, as discussed in detail for DRV[17]. Results of deconvolution of these overlapping curves (by use of the Origin software provided by MicroCal) are shown as dashed lines. Only the major transitions (colored dashed lines) with the higher T_m are shown in Fig. 2.

Fig. S2. Michaelis-Menten kinetic curve for the hydrolysis of H-Pro-Thr-Glu-Phe-[*p*-NO_2-Phe]-Arg-Leu-OH by 0.13 nM pepsin in 50 mM sodium formate buffer, pH 3, at 28 ºC. Values of K_m and V_{max} were determined from a nonlinear least-squares fit to the data: $K_m = 93 \pm 16$ μM and $V_{max} = 2.08 \pm 0.12$ μM/s. The line is a theoretical curve based on these values.

Fig. S3. Logarithmic correlation between published values of ligand dissociation constants (K_L) for PR/inhibitor complexes (x-axis; see refs. in Table 1) and calculated values (present work) based on T_m shifts (y-axis).

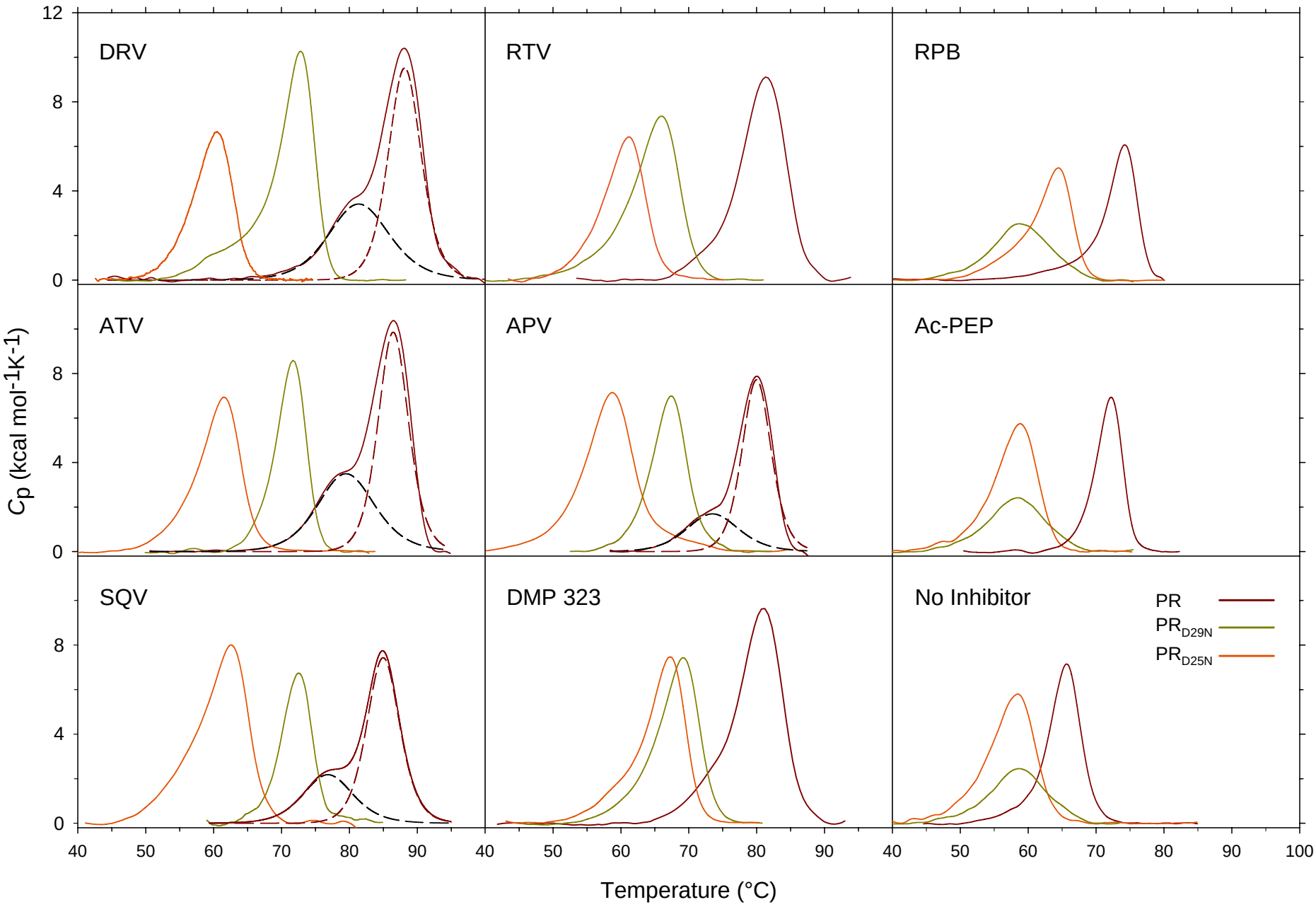
DRV
RTV
RPB
ATV
APV
Ac-PEP
SQV
DMP 323
No Inhibitor
PR
PR$_{D29N}$
PR$_{D25N}$
C_p (kcal mol^{-1}K^{-1})
Temperature (°C)
0
4
8
12
40
50
60
70
80
90
100

Figure S1

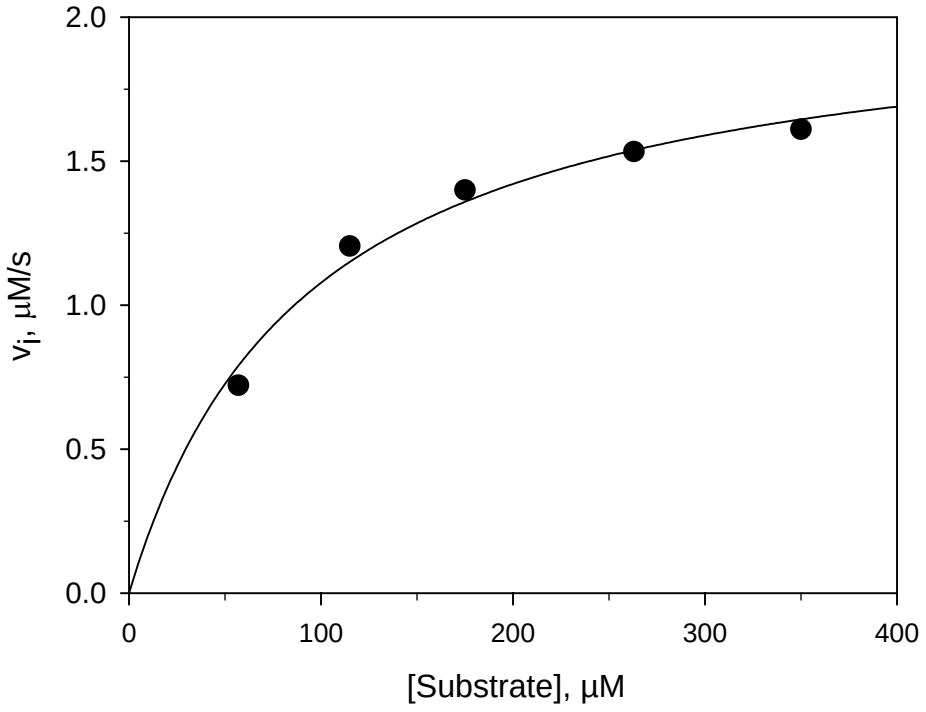

Figure S2

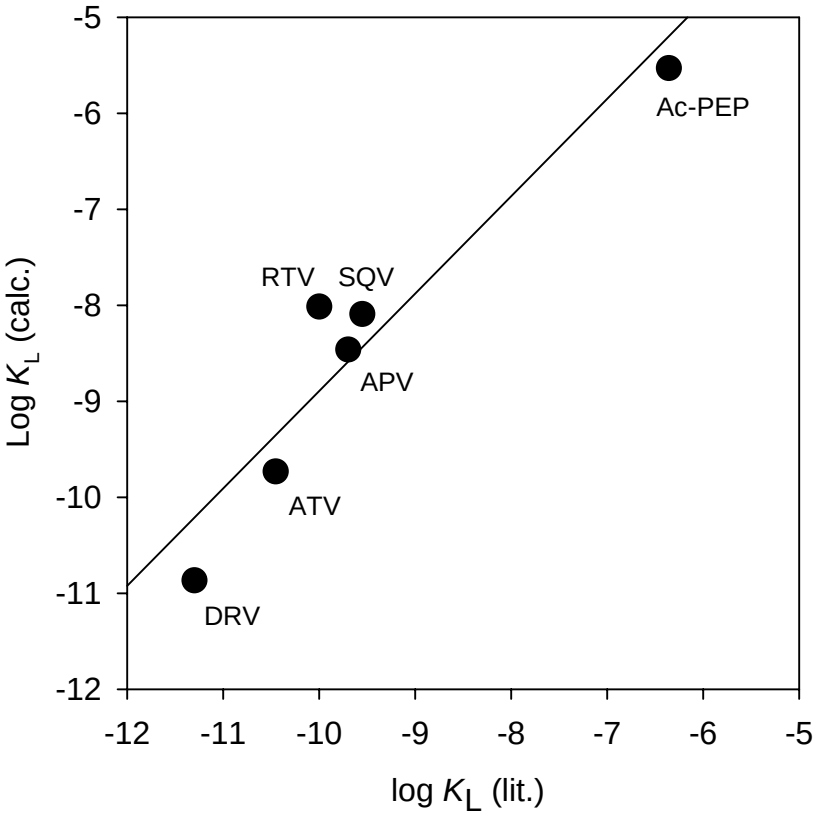
-5
-6
-7
-8
-9
-10
-11
-12
Log K_L (calc.)
-12
-11
-10
-9
-8
-7
-6
-5
log K_L (lit.)
Ac-PEP
RTV
SQV
APV
ATV
DRV

Figure S3