SUPPORTING MATERIAL

Pressure and Temperature Effects on the Activity and Structure of the Catalytic Domain of Human MT1-MMP

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Pressure effect on the rate constant

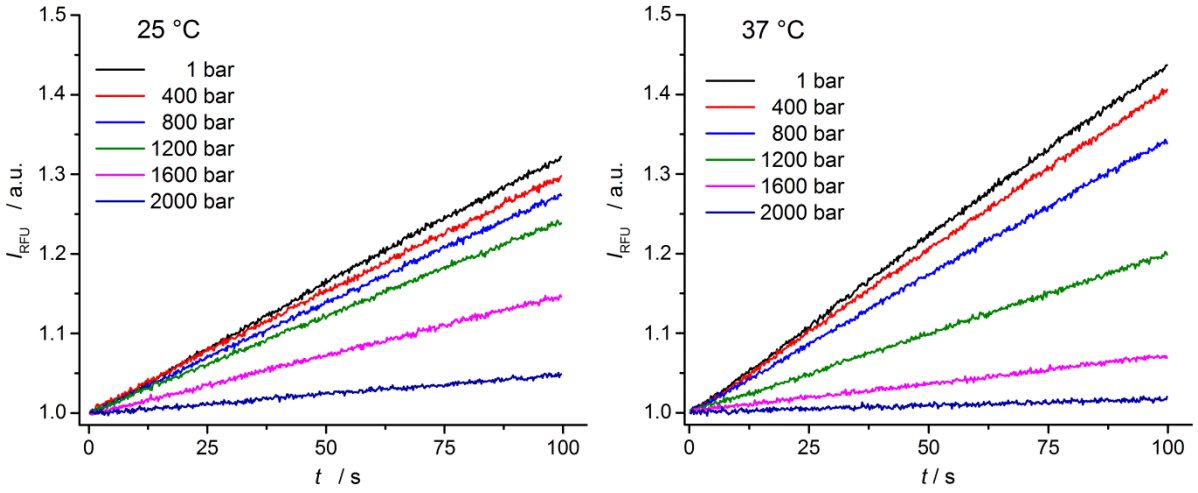

Fig. S 1 Pressure-dependent activity measurements for the hydrolysis of a collagen-like peptide by the catalytic domain of MT1-MMP. The signal detected corresponds to the relative fluorescence intensity as a function of time at 25 °C (left) and 37 °C (right). Reaction slopes exhibited nearly linear behavior during the course of the reaction, implying saturating enzyme concentration with substrate.

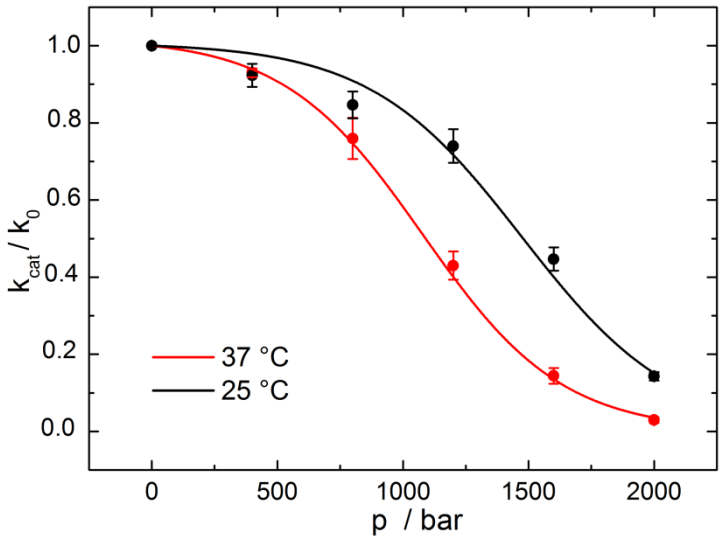

Fig. S 2 Pressure dependence of the enzymatic activity of MT1-MMP at 25 °C (298 K) and 37 °C (310 K). k_{cat}/k_0 corresponds to the ratio between the rate constant at pressure p and the rate constant at atmospheric pressure (1 bar).

Assuming a pressure-induced displacement of a conformational equilibrium between two conformational states, A ⇄ B, of the enzyme with different activity, data were fitted with the integral form of the equation for the pressure-dependence of the equilibrium constant, K_{eq} (1,2):

$$K_{eq} = \frac{[A]}{[B]} = e^{(p_{1/2}-p)\Delta V°/RT}$$

Since k_{cat}/k_0 can be assumed to be proportional to the fraction of MT1-MMP in the most active conformation of the enzyme, x_A, we can write:

$$x_A = \frac{[A]}{[A]+[B]} = \frac{1}{1+e^{-(p-p_{1/2})\Delta V°/RT}}$$

The values of the pressure-induced standard molar volume change, $\Delta V°$, and the "half pressure" of the conversion, $p_{1/2}$, at which [A] = [B], amounts to $\Delta V°$ = -93 ± 6 mL mol^{-1} and $p_{1/2}$ = 1080 ± 25 bar (r^2 = 0.9998) at 37 °C and $\Delta V°$ = -85 ± 10 mL mol^{-1} and $p_{1/2}$ = 1475 ± 60 bar (r^2 = 0.997) at 25 °C, respectively.

Temperature and pressure effect on secondary structure

Figures S 3 and S 4 display the temperature-dependent changes of the FSD and 2nd derivative spectra of the amide I' band of MT1-MMP. The FSD spectrum of the native protein (Fig. A, blue lines) exhibits nine peaks corresponding to nine underlying secondary structure elements, approved by the 2nd derivative spectra (Table 4 in the main article for assignment). Therefore, nine Gaussian shaped bands were fitted to the temperature dependent data revealing information about changes in secondary structure elements while temperature is increased.

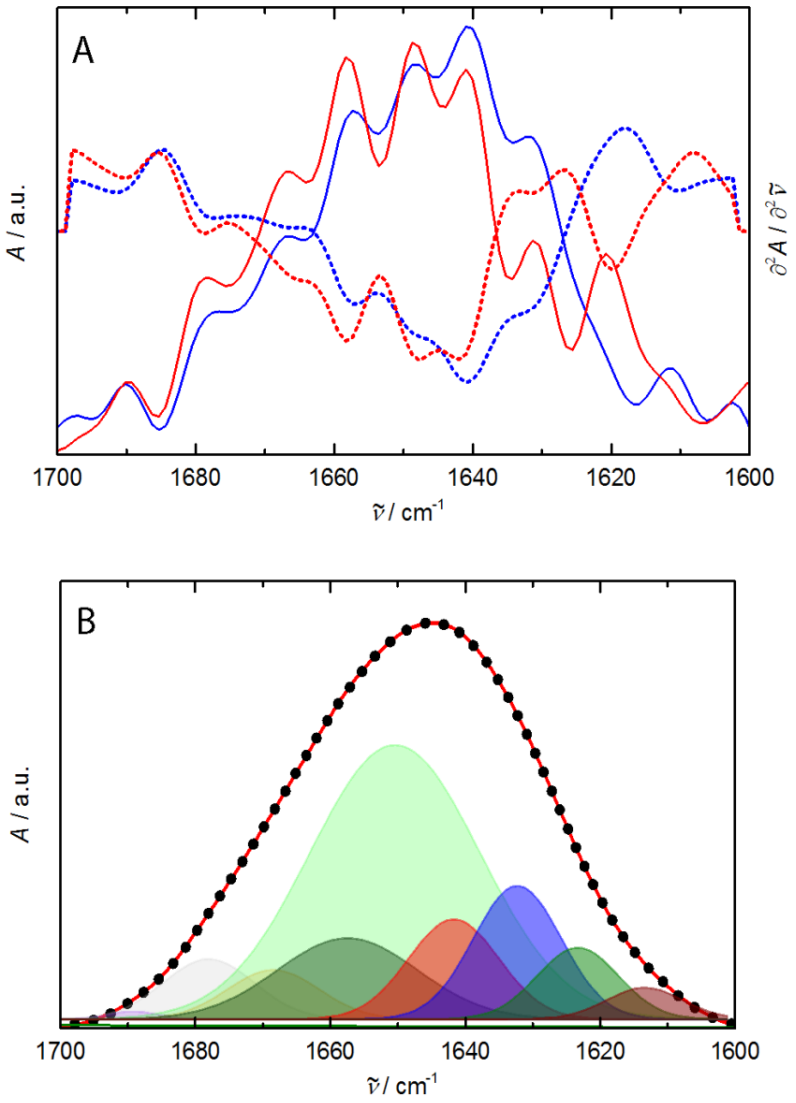

Fig. S 3 (**A**) FSD (solid lines) and 2nd derivative (dashed lines) spectra of the amide I' band of MT1-MMP (20 °C in blue and 90 °C in red). (**B**) Amide I' band of MT1-MMP at 20 °C (black filled circles) and fitted curve (red line) generated by the superposition of the underlying Gaussian shaped subbands.

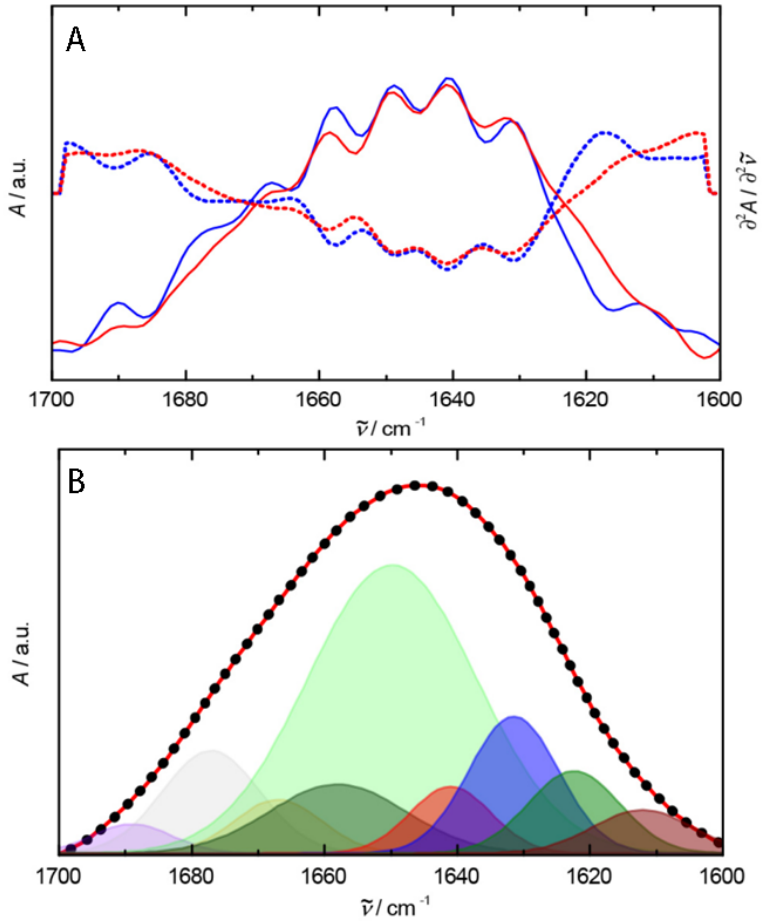

Fig. S 4 (**A**) FSD (solid lines) and 2nd derivative (dashed lines) spectra of the amide I' band of MT1-MMP (pressure of 1 bar in blue and 11 kbar in red). (**B**) Amide I' band of MT1-MMP at 1 bar (black filled circles) and fitted curve (red line) generated by the superposition of the underlying Gaussian-shaped subbands.

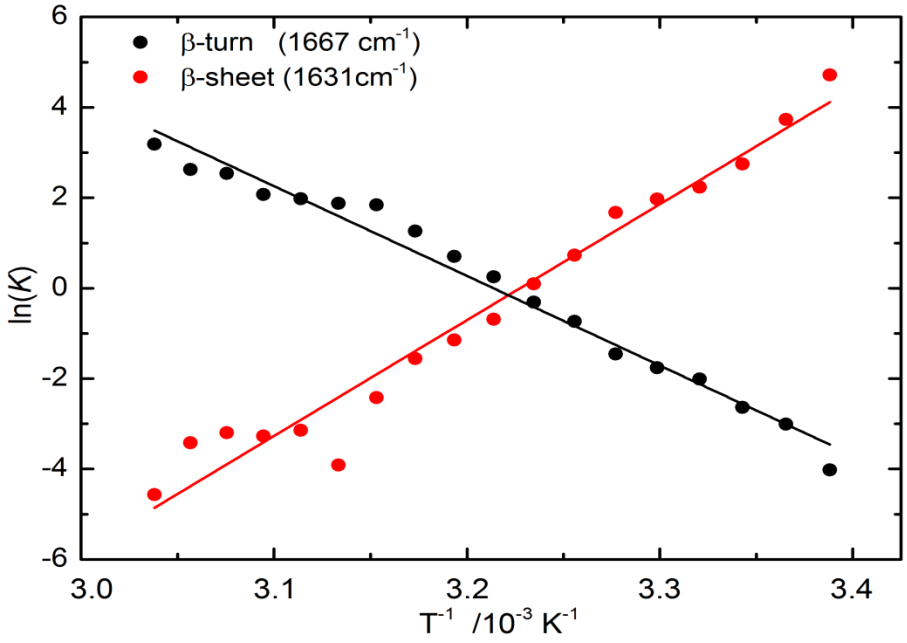

Fig. S 5 Van't Hoff plots constructed from the temperature-dependence of the equilibrium constant for the structural transition at 1667 (β-turn, black) and 1631 cm^{-1} (β-sheet, red). Data were fitted with $\ln K = -\Delta H/RT + \Delta S/R$ where R is the gas constant. The linear correlation coefficients correspond to $r = 0.989$ (β-turn, black) and $r = 0.982$ (β-sheet, red), respectively.

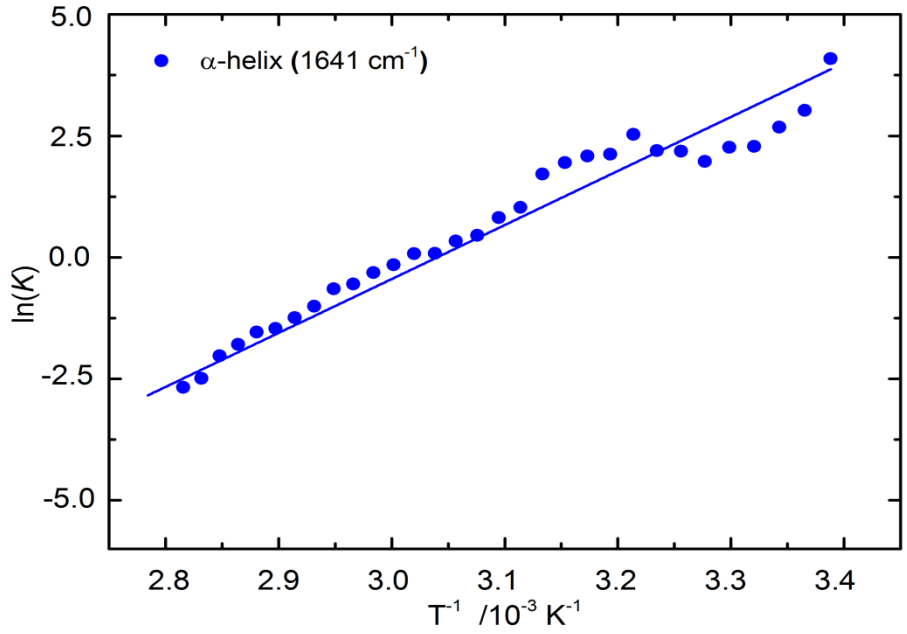

Fig. S 6 Van't Hoff plots constructed from the temperature-dependence of the equilibrium constant for the structural transition at 1641 cm^{-1} (α-helix). The linear correlation coefficient corresponds to $r = 0.978$.

Supporting References

1. Weber G. 1992. Protein interactions. Chapman and Hall, New York.
2. Sineva E. V. and D. R. Davydov. 2010. Cytochrome P450 from Photobacterium profundum SS9. A piezophilic bacterium exhibits a tightened control of water access to the active site. *Biochemistry* 49:10636-10646.