Table S1. Catalytic properties (at 25 ºC, pH 7.5) of MMP-12 variants with mild substitutions and activities nearly intact.

MMP-12 variant [a]	*Location of mutation*	*General Substrate FS-6*	**fEln-100**		
		k_{cat}/K_m, $M^{-1}s^{-1}$	k_{cat}/K_m, $M^{-1}s^{-1}$	K_m, μM	k_{cat}, 10^{-2} s^{-1}
MMP-12	-	133,800 ± 6000	10,690 ± 430	1.06 ± 0.04	1.06 ± 0.04
Y132S	hA	158,900 ± 4800	9710 ± 390	0.86 ± 0.04	0.83 ± 0.03
S142E	hA	144,600 ± 4300	9130 ± 370	1.15 ± 0.04	1.05 ± 0.04
V144A	hA	141,700 ± 4300	9030 ± 360	1.18 ± 0.05	1.07 ± 0.04
K148T	sII	146,700 ± 4400	10,200 ± 410	1.06 ± 0.04	1.08 ± 0.04
N153Y	II-III loop	132,600 ± 4000	10,850 ± 430	1.04 ± 0.04	1.13 ± 0.05
V162S	sIII	157,800 ± 4700	10,380 ± 420	1.29 ± 0.04	1.34 ± 0.05
A164V	sIII	148,800 ± 4500	10,410 ± 420	1.10 ± 0.04	1.15 ± 0.05
G166R	sIII	140,100 ± 4200	9830 ± 390	0.97 ± 0.04	0.95 ± 0.04
D200E	V-B loop	144,800 ± 4300	9470 ± 380	0.88± 0.03	0.84 ± 0.03
S207V	V-B loop	143,300 ± 4300	9440 ± 380	0.97 ± 0.04	0.92 ± 0.04
I255R	hC	136,000 ± 4100	9770 ± 390	0.99 ± 0.04	0.97 ± 0.04
I255V	hC	138,400 ± 4200	9980 ± 400	1.08 ± 0.04	1.08 ± 0.04

[a] Each mutation is found at the equivalent position in a less elastolytic homologue such as MMP-3, 8, 10, 11, 26, 27, or MT-MMP.

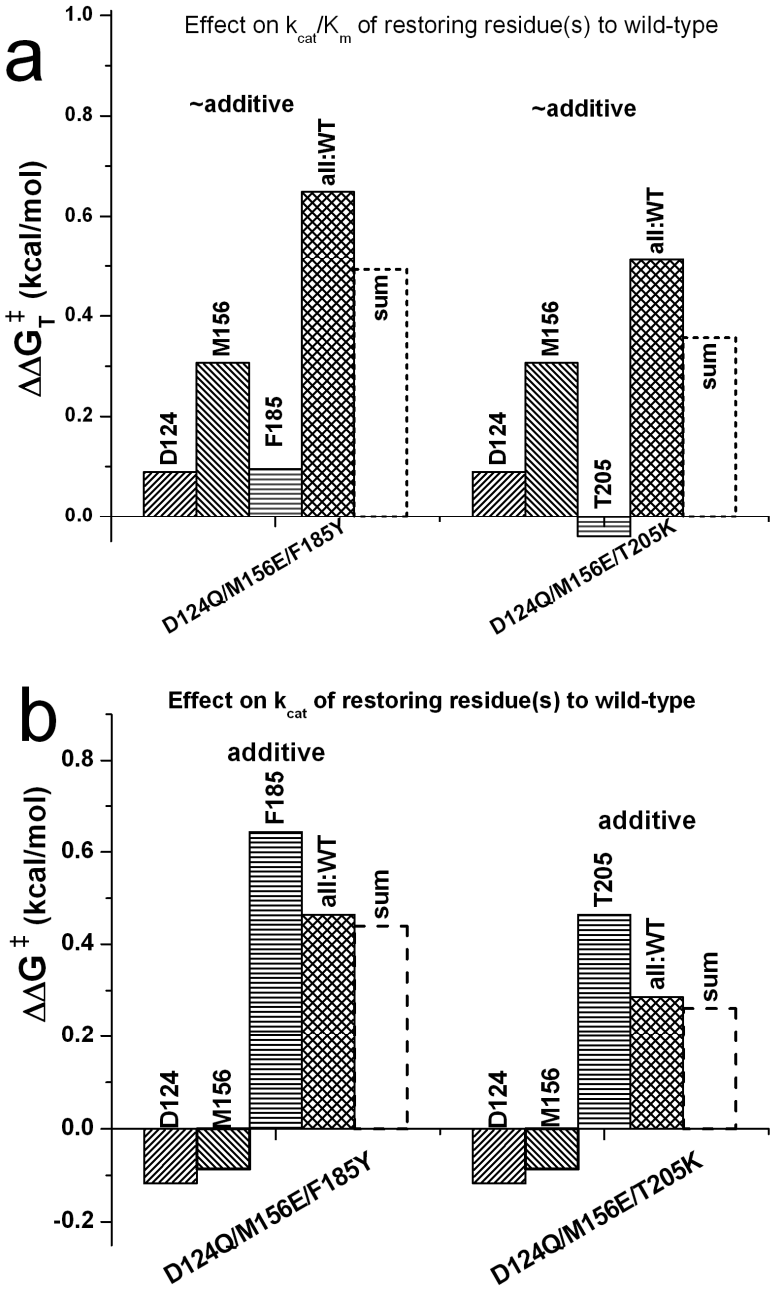

Fig. S1. "Double" mutant cycle analysis of effects on (a) k_{cat}/K_m and (b) k_{cat} for triple mutations that include a point mutation on either extreme of the active site cleft: F185Y on the unprimed side or T205K on the primed side. The data are plotted as described in the legend of Fig. 4. $\Delta\Delta G^{\ddagger}_T$ is calculated using eq. 1 and $\Delta\Delta G^{\ddagger}$ using eq. 3.

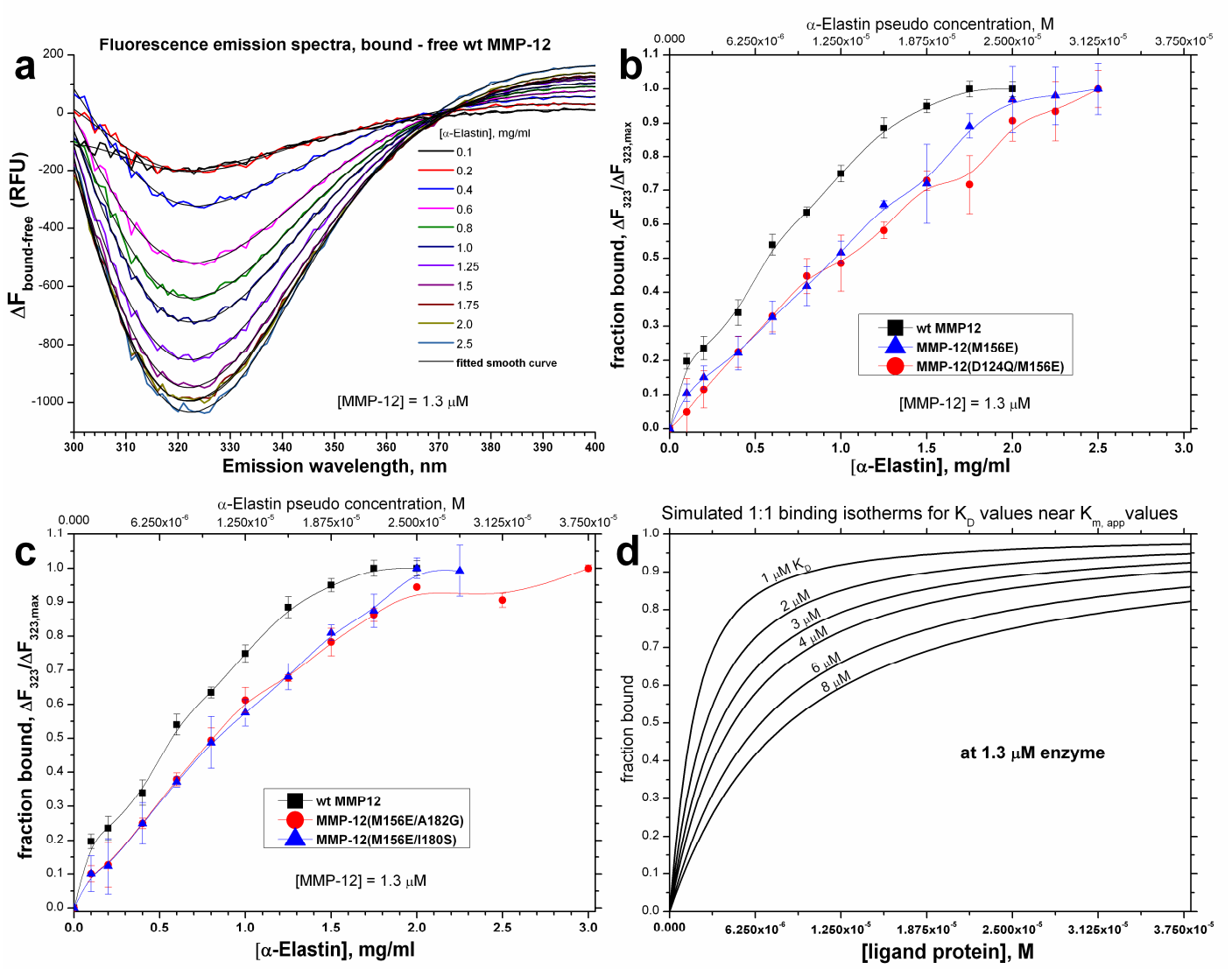

Fig. S2. Comparison of affinity for α-elastin of MMP-12 variants that have apparent K_m increased by more than two-fold. (a) Intrinsic tryptophan fluorescence changes of 1.3 μM MMP-12 variants were monitored as a function of increasing α-elastin concentration which quenched the fluorescence emission of the MMP, which is maximal at 323 +/1 nm. Elastin lacks any tryptophan. (b,c) The titrations with α-elastin were conducted in triplicate for wt MMP-12 and the four mutants having K_m increased > two-fold, each of which contain the M156E lesion. The fluorescence emission changes were normalized to a scale of 0 to 1 using the maximum fluorescence change at the plateau reached from 2 to 3 mg/ml. α-elastin (*1*) is a continuum of species migrating with apparent mobility on SDS-PAGE ranging from ~10 kDa to > 150 kDa. By oversimplifying this heterogeneity to an average MW of ~80 kDa, the α-elastin concentration was converted to the pseudo, apparent molar concentration labeled atop (b, c) for comparison with (d). (d) For comparison, simulated binding isotherms for simple single-site binding are plotted for K_D values approximately spanning the range of fitted K_m values.

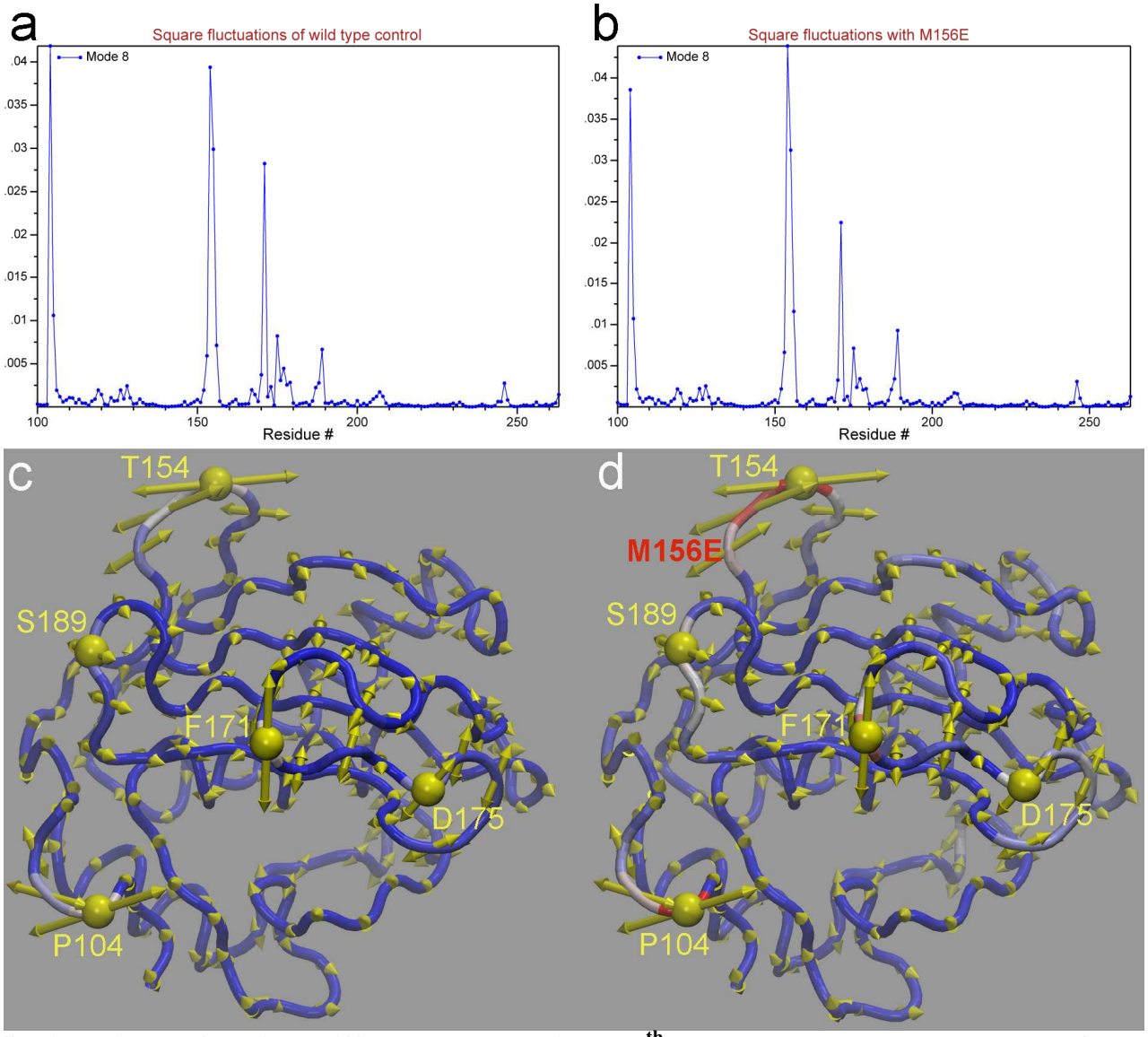

Fig. S3. Amplitude of motions differed detectably in the 8th lowest normal mode, as a result of the M156E mutation of MMP-12. The amplitude of fluctuations is represented on an arbitrary scale both by the the vertical axes of panels *a* and *b* and the length of arrows in panels *c* and *d*. Results are shown for the unmodified, lowest energy NMR structure at left and carrying the M156E substitution at right. Labels in *c* and *d* mark residues with locally high fluctuations that were changed by M156E. In *c* and *d*, red backbone coloration indicates relatively larger fluctuations and white medium-sized fluctuations. The simulations used the anisotropic network model available at an online server (*2*).

Movie S1. Fluctuations of normal mode 8 with M156E. The simulation in the accompanying .mpg file is that of Fig. S3b,d and was generated using the Normal Mode Wizard and Movie Maker plugin of VMD (*3*).

REFERENCES

1. Partridge, S. M., Davis, H. F., and Adair, G. S. (1955) The chemistry of connective tissues. 2. Soluble proteins derived from partial hydrolysis of elastin, *Biochem J 61*, 11-21.
2. Eyal, E., Yang, L.-W., and Bahar, I. (2006) Anisotropic network model: systematic evaluation and a new web interface, *Bioinformatics 22*, 2619-2627.
3. Humphrey, W., Dalke, A., and Schulten, K. (1996) VMD: visual molecular dynamics, *J Mol Graph 14*, 33-38, 27-38.