Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Probing the kinetics of quantum dot-based proteolytic sensors

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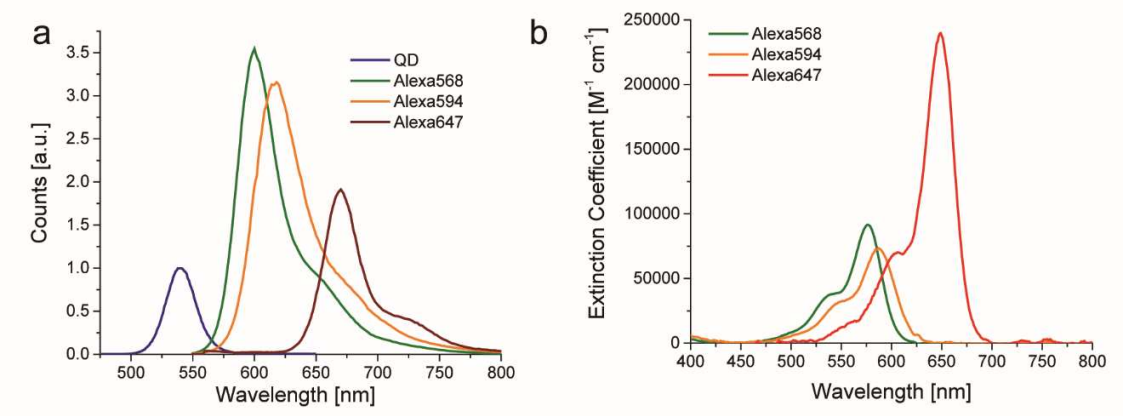

Fig. S1 a) Emission spectra of the components of the enzyme sensors. Areas have been normalized to the QY of each component. b) Extinction coefficient of the acceptor components of the enzyme sensors

Table S1 Spectroscopic properties of the QDs and fluorophores utilized in this work

	λ_{abs} [nm]	ε [M^{-1} cm^{-1}]	λ_{Em} [nm]	QY
QD	523*	185,000	540	0.22
Alexa568	576	91,300	600	0.69
Alexa594	588	73,000	617	0.66
Alexa647	649	239,000	670	0.33

*Corresponds to the first absorption band QY = quantum yield

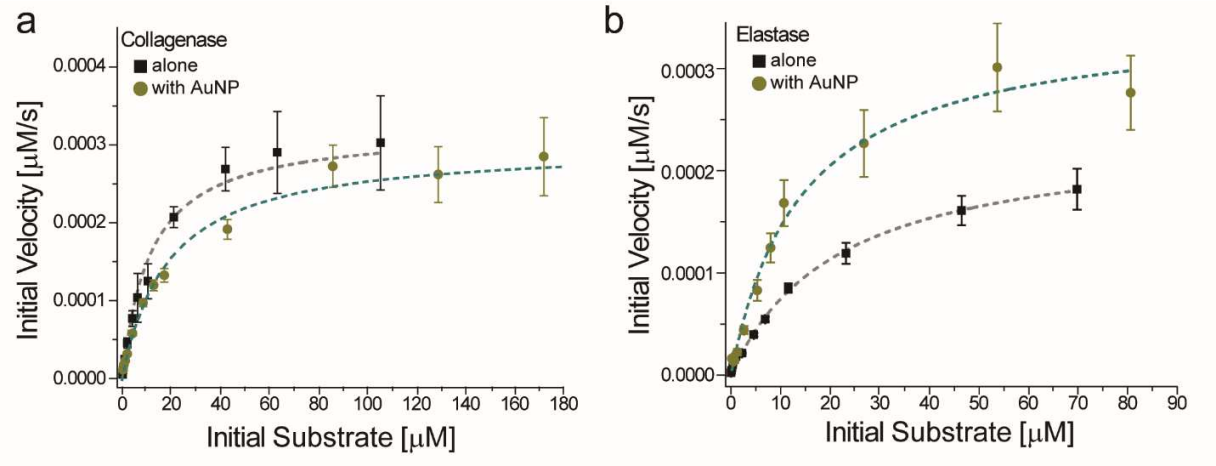

Fig. S2 Classical MM graph of enzymes and substrates in 1× PBS with 10 mM Ca^{+2}. Black squares are alone in solution, grey line MM fit. Gold circles consists of the same condition with the addition of 3.1 nM of AuNPs, cyan line MM fit to this experiment. a.) Collagenase [1.064 nM]. b.) Elastase [0.193 nM]

Table S2 Kinetic parameters of MM determinations of proteolytic enzymes

Enzyme	Collagenase	Elastase
V_{max} [μM s^{-1}]	$3.2\times10^{-4} \pm 0.2\times10^{-4}$	$2.4\times10^{-4} \pm 0.1\times10^{-4}$
K_m [μM]	12 ± 1	22 ± 2
K_{cat} [s^{-1}]	0.30 ± 0.02	1.20 ± 0.02
K_{cat}/K_m [mM^{-1} s^{-1}]	25 ± 3	55 ± 5

With 3.1 nM of AuNPs coated with CL-4 ligands in solution

Enzyme	Collagenase	Elastase
V_{max} [μM s^{-1}]	$3.0\times10^{-4} \pm 0.1\times10^{-4}$	$3.5\times10^{-4} \pm 0.3\times10^{-4}$
K_m [μM]	18 ± 1	14 ± 2
K_{cat} [s^{-1}]	0.28 ± 0.02	1.80 ± 0.05
K_{cat}/K_m [mM^{-1} s^{-1}]	15 ± 3	129 ± 8

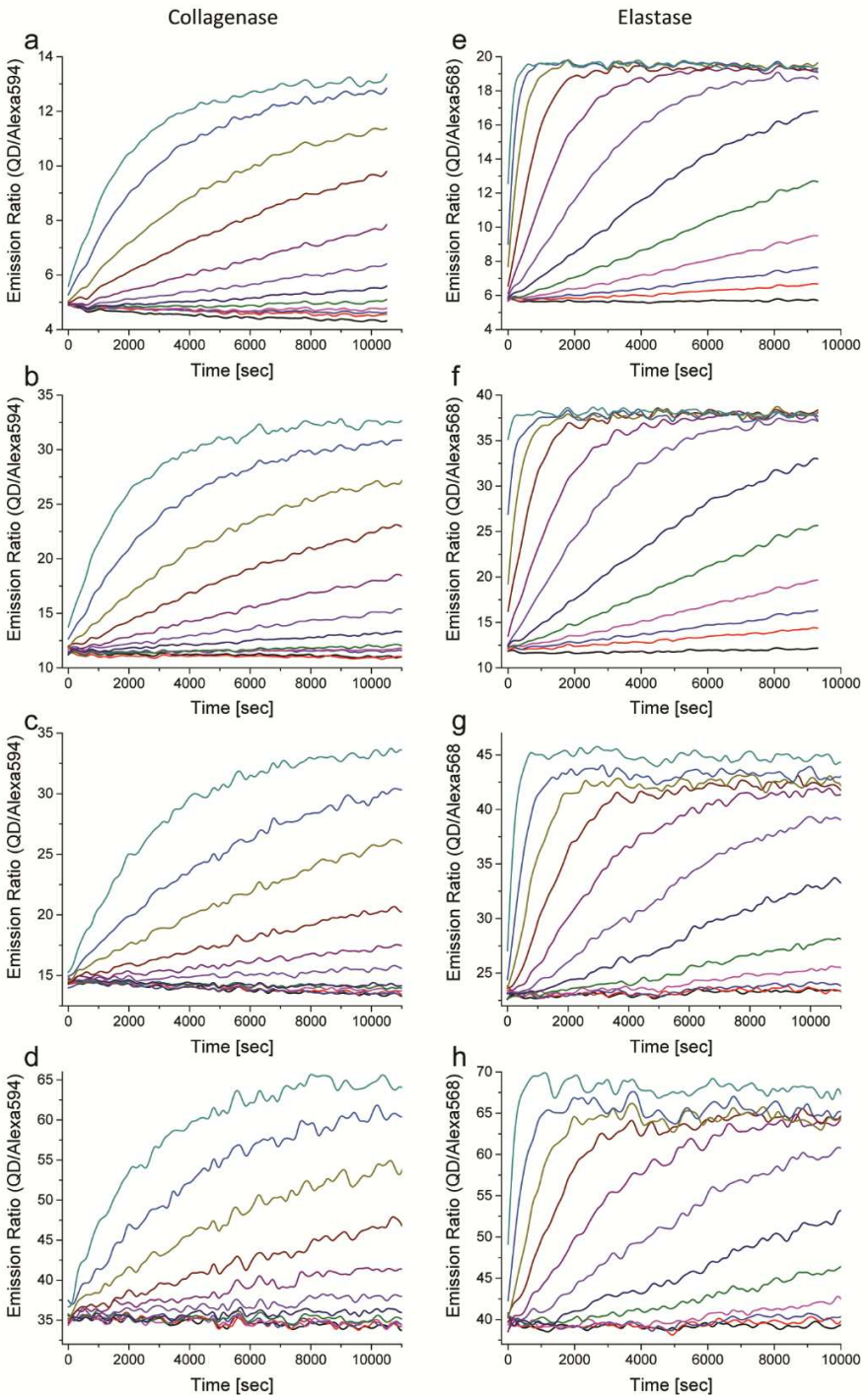

Fig. S3 Progress curves of QD conjugated with labeled substrate. Collagenase (a-d): Left Column from top down: 14.5P, 11P, 10P, 6P. Collagenase enzyme 2-fold dilutions of concentration from 946 nM to 0.9 nM including a zero enzyme control. Elastase (e-h): Right column from top down: 12 pep, 10 pep, 7 pep, 5.5 pep. Elastase enzyme 2-fold dilutions of concentration from 576 nM to 0.6 nM including a zero enzyme control

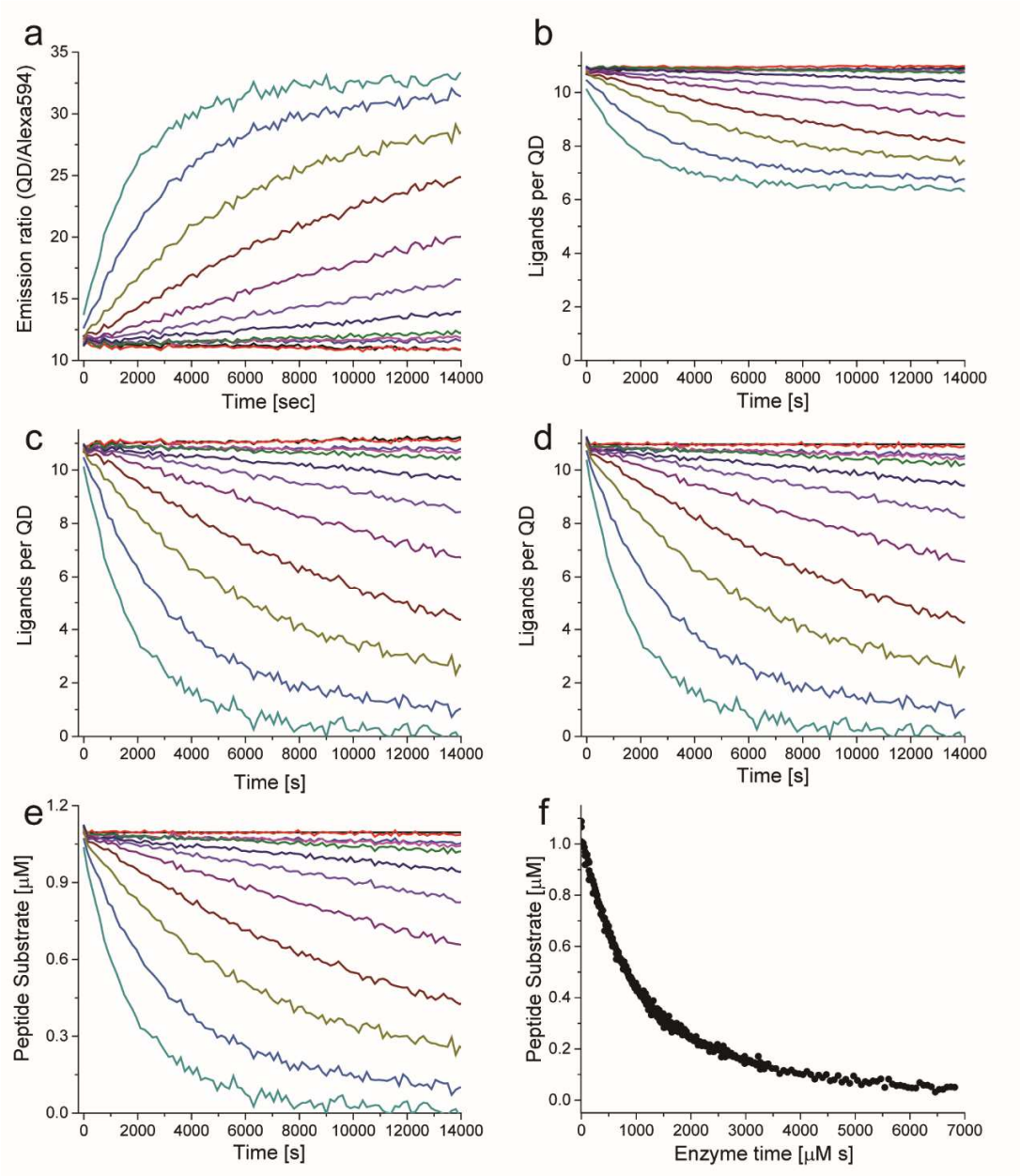

Fig. S4 Step-by-step transformations of the 11P/QD Collagenase data set. a) Raw emission ratio curves. b) Using the calibration curves to determine the initial amount of P/QD. c) Using the pre-digested calibration curves to find the end-point of the reaction. d) Baseline correction for instrumental drift. e) Multiplication by QD concentration to obtain peptide substrate consumption progress curves. f) Enzyme time peptide substrate consumption progress curves

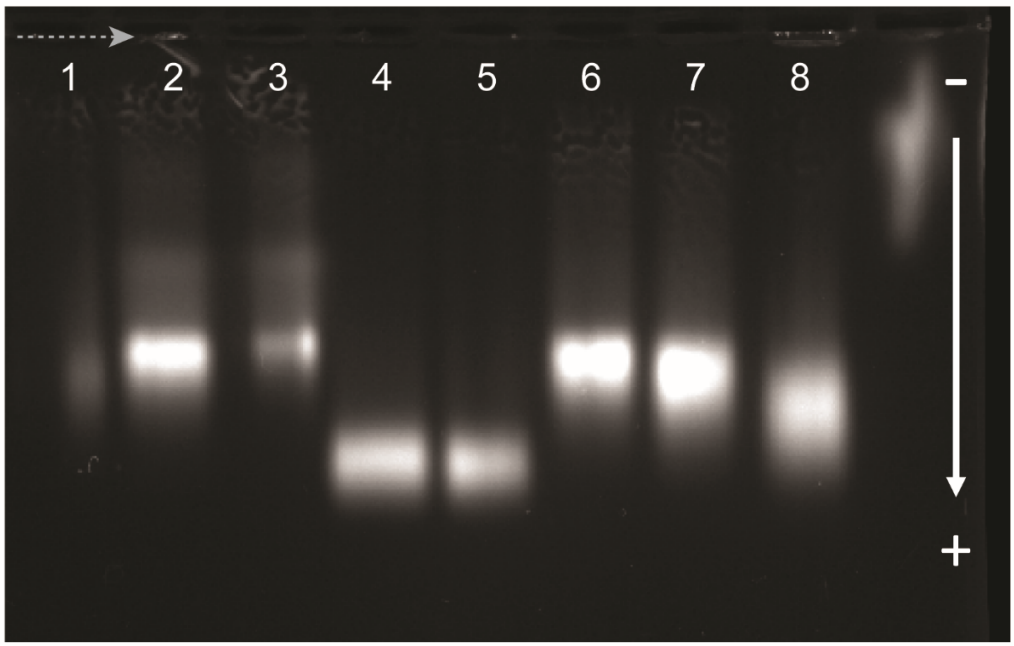

Fig. S5 Electrophoresis of 540 nm QDs with increasing varying amounts of labeled peptide substrate, 1% agarose gels, detected by QD and Alexa dye fluorescence. Dotted arrow marks the origin. Lane 1-3 collagenase substrate peptide = 5.5 pep/QD, 11 pep/QD L, 16 pep/QD respectively. Lane 4-5: QD alone. Lane 6-8 elastase substrate peptide = 17 pep/QD, 11 pep/QD L, 5.5 pep/QD respectively

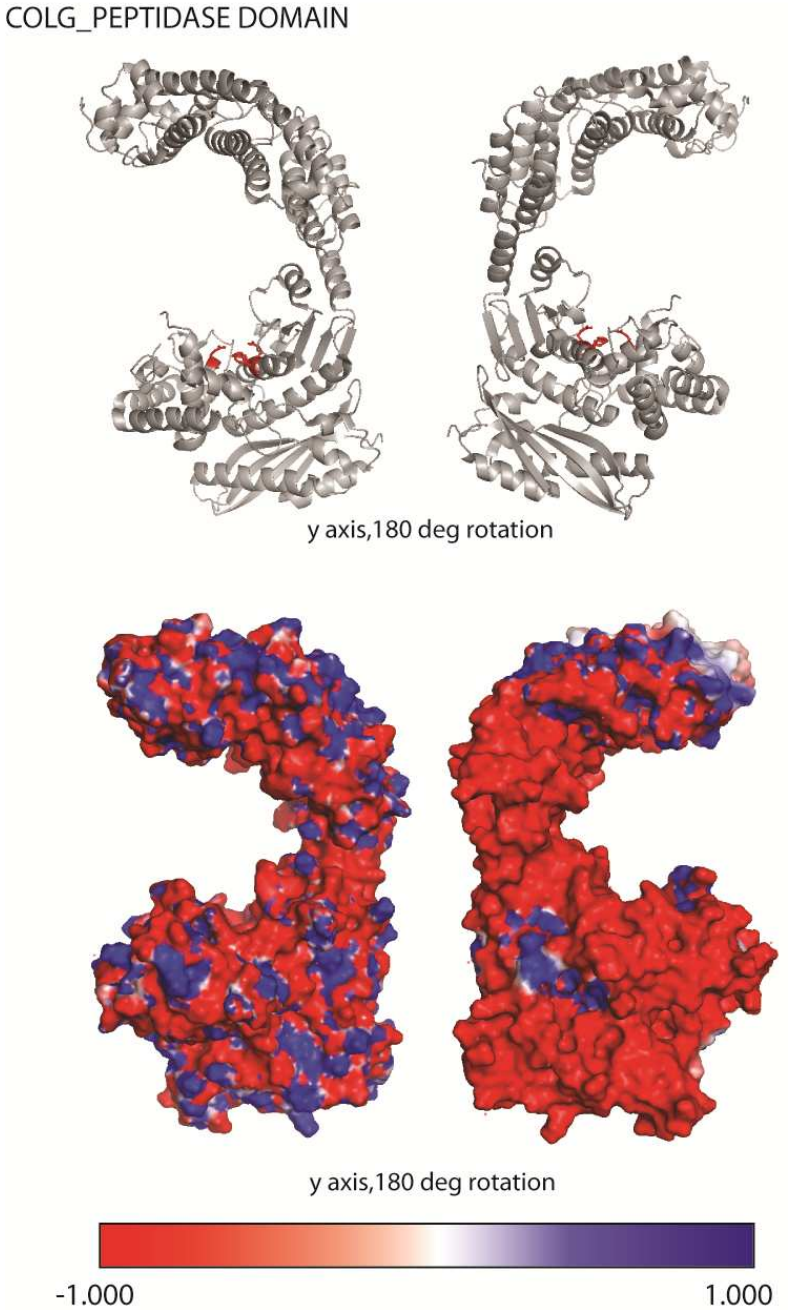

Fig. S6 Collagenase, peptidase domain active site and surface charge distribution. Top, cartoon representation of the peptidase domain of ColG Collagenase, PDB 2Y3U. Red residues are Zn coordinating and involved in collagen binding. Bottom, surface charge representation colored by electrostatic potential, red indicates localization of negative residues, blue indicates a higher presence of positive residues

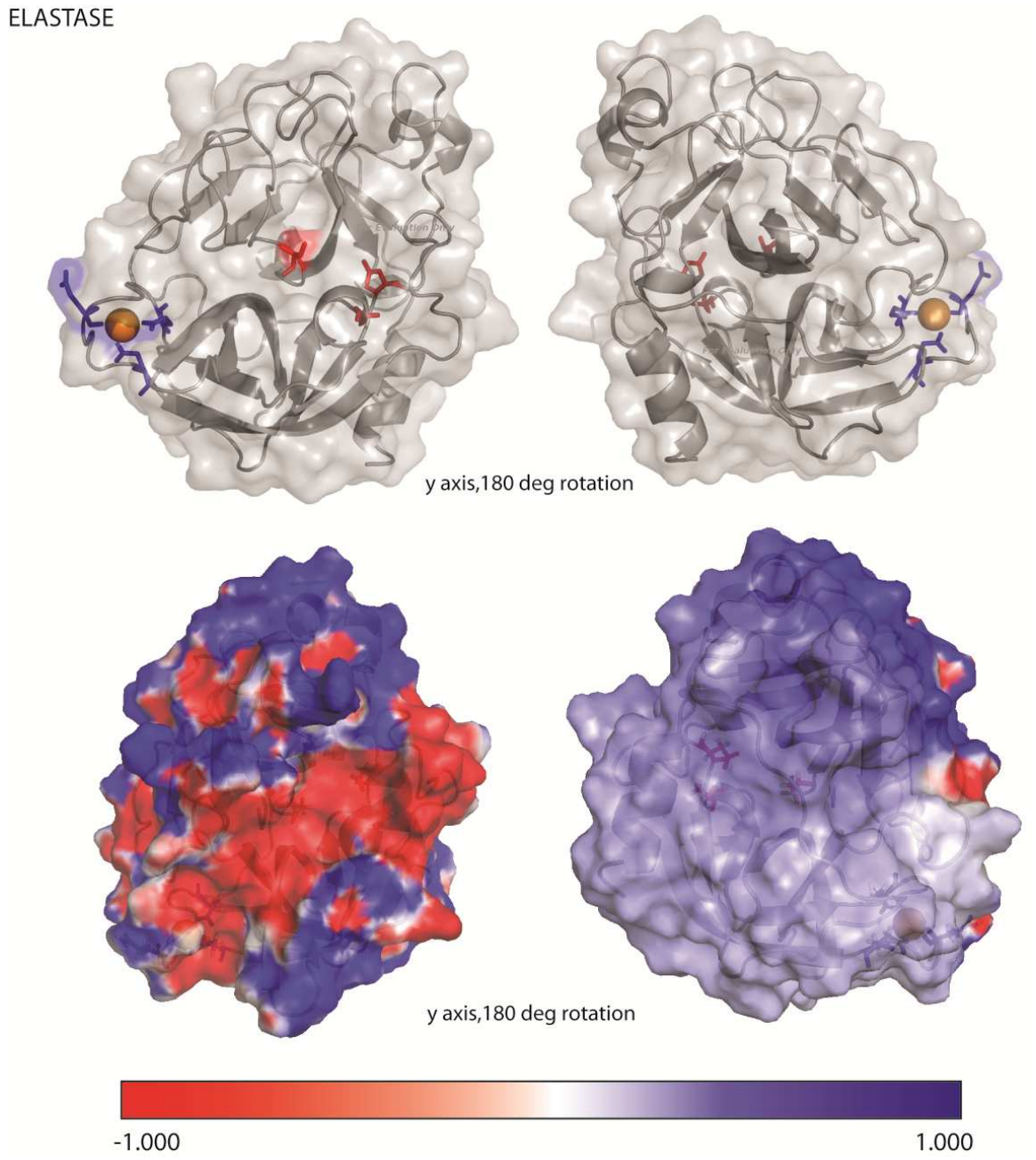

Fig. S7 Elastase, active site and surface charge distribution. Top, cartoon representation of elastase, PDB 1B0E. Active site: Blue residues are calcium coordinating, red residues are involved in charge relay. Bottom, surface charge representation colored by electrostatic potential, red indicates localization of negative residues, blue indicates a higher presence of positive residues