Supplementary Information for

Protease inhibition mechanism of camelid-like synthetic human antibodies

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Table of Content

Figure S1:

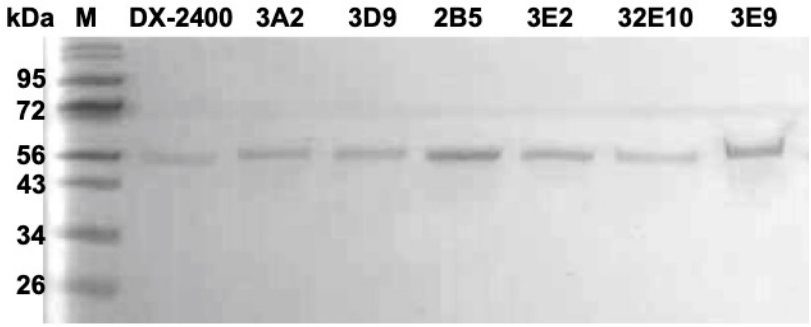

Figure S1. SDS-PAGE of purified Fabs. Fabs were expressed in periplasmic space of *E. coli* BL21 cells and purified by using Ni-NTA chromatography. Purified Fabs were analyzed by SDS-PAGE at non-reducing condition.

Figure S2:

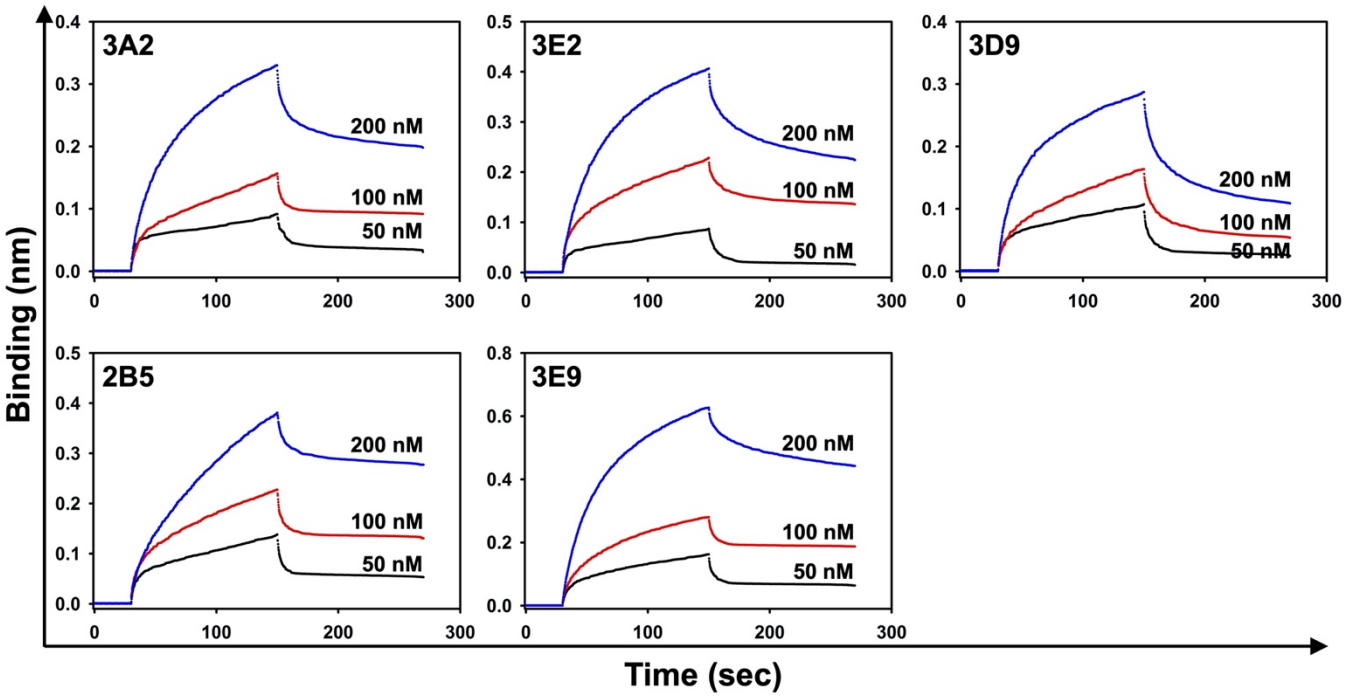

Figure S2. Binding kinetics of Fabs on cdMMP-14. Association constants (k_{on}) and dissociation constants (k_{off}) were determined by using biolayer interferometry (BLI) with biotinylated cdMMP-14 immobilized on streptavidin sensor, and values of binding affinity K_D were calculated.

Figure S3:

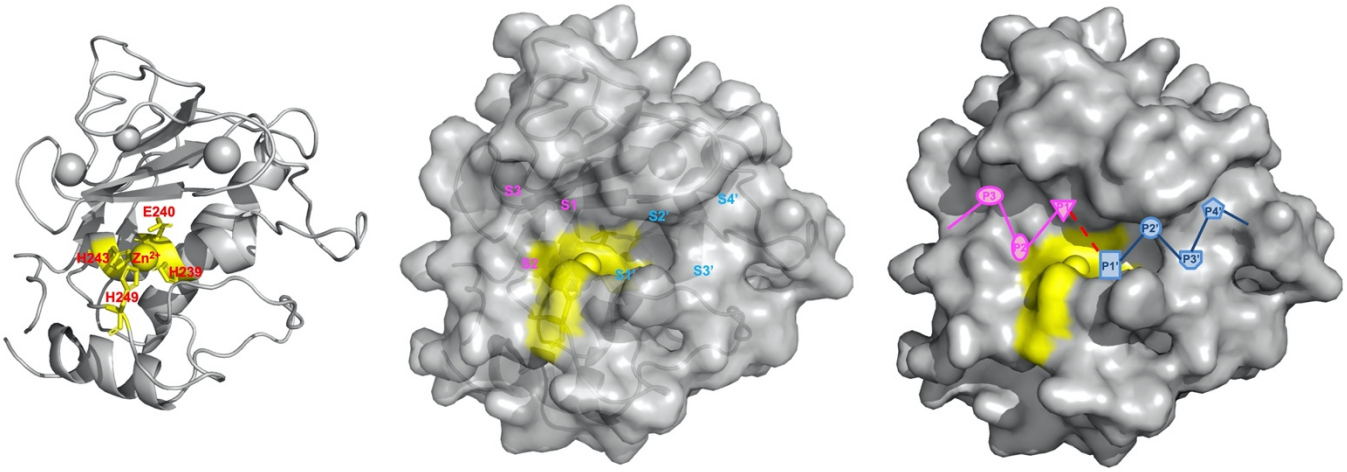

Figure S3. Active cleft of MMP-14. (Left) Catalytic zinc and its coordinating residues form the active site of MMP-14. **(Middle)** Located at either side of the catalytic zinc, the prime subsites (in pink) and the non-prime subsites (in blue) sculpt a cleft-like structure on the surface of cdMMP-14. **(Right)** A conceptual polypeptide substrate is accommodated within the cleft structure, promoting hydrolysis of the scissile bond between its P1 and P1' positions.

Figure S4:

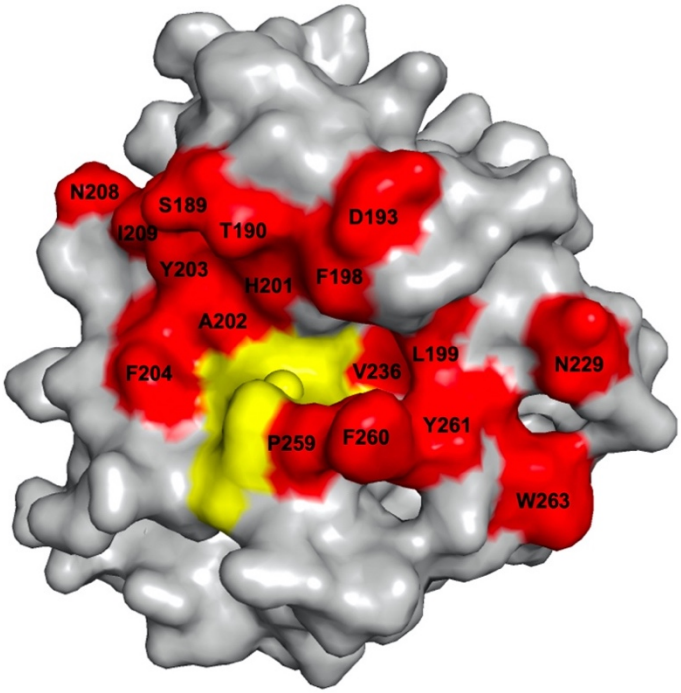

Figure S4. nTIMP-2 binding surface on cdMMP-14. The residues interacting with nTIMP-2 are in red. Catalytic zinc and its coordinating histidines (His239, His243 and His249) are in yellow. PDB=1BQQ.

Figure S5:

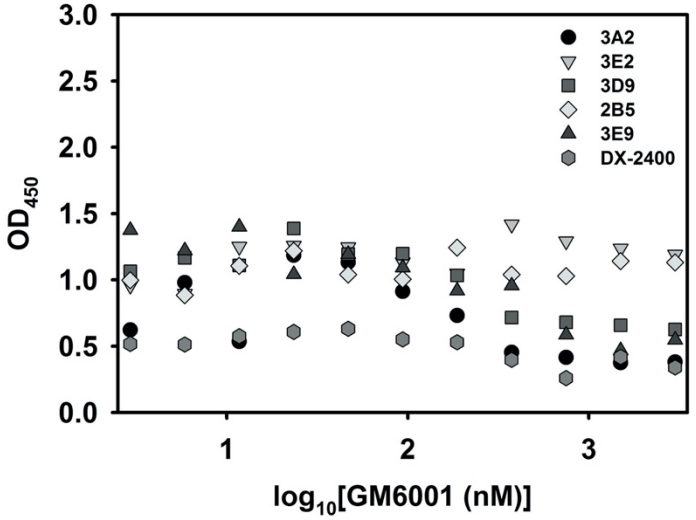

Figure S5. Competitive ELISA with GM6001. Immobilized cdMMP-14 was incubated with mixtures of 200 nM of Fabs (3A2, 3D9, and DX-2400) or 1 μM of Fabs (3E2, 2B5, and 3E9) and 3 nM - 3 μM GM6001. Signals were developed with anti-Fab-HRP.

Figure S6:

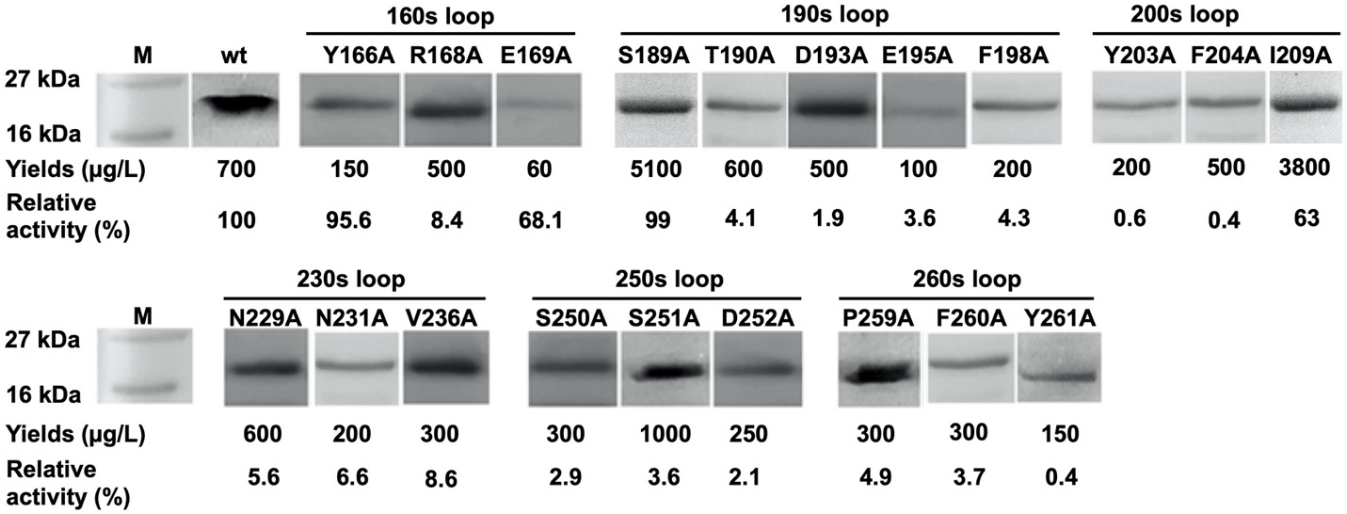

Figure S6. Production of cdMMP14 alanine point mutants. SDS-PAGE of produced cdMMP-14 mutants. Each cdMMP-14 mutants were cloned, periplasmically expressed and purified. Yields and enzymatic activities were measured to compare with wt cdMMP-14.

Figure S7:

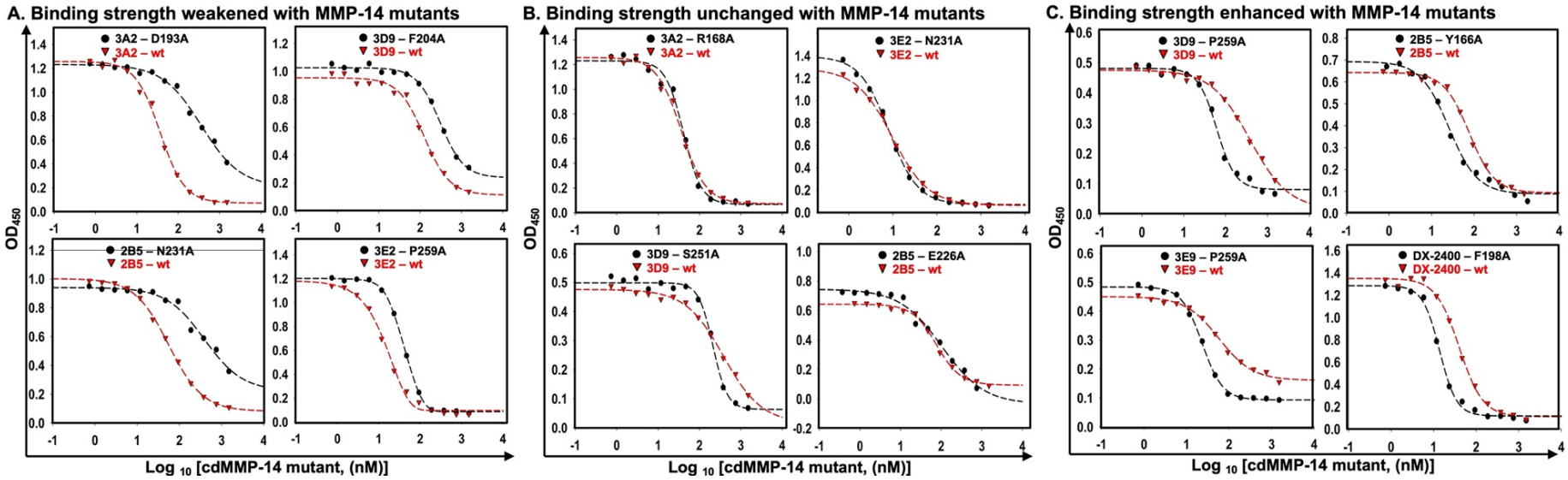

Figure S7. Additional exemplary results of competitive ELISA. Binding strength **(A)** weakened, **(B)** unchanged or **(C)** enhanced with MMP-14 mutants.

Figure S8:

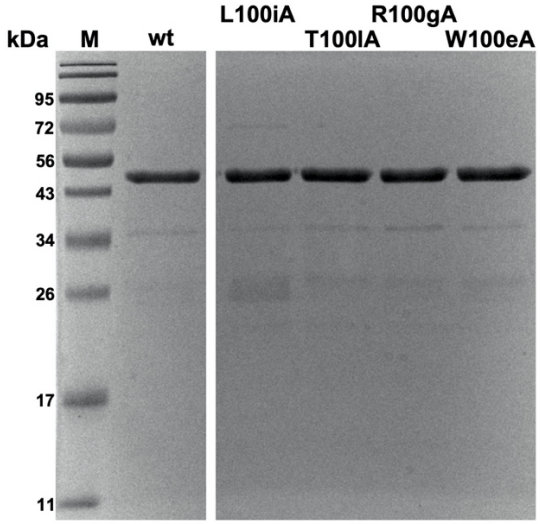

Figure S8. Production of Fab 3A2 mutants. Fabs were periplasmically expressed and purified Fabs were analyzed by SDS-PAGE at non-reducing condition.

Figure S9:

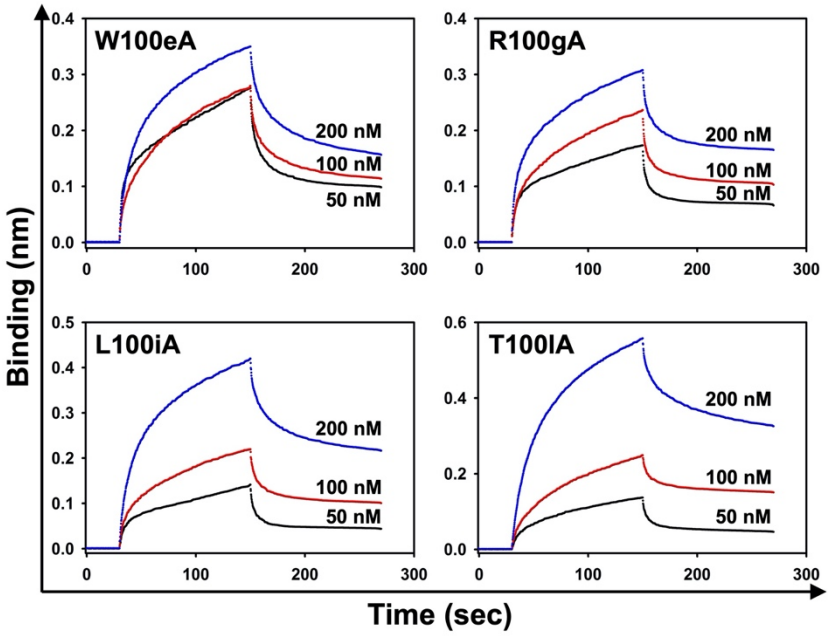

Figure S9. Biolayer interferometry results of Fab 3A2 mutants. Biotinylated cdMMP-14 was immobilized on streptavidin sensor and binding kinetics of Fabs, i.e. k_{on} and k_{off} were measured to determine affinity K_D.