Electronic Supplementary Material (ESI) for Analyst

A novel protease activity assay method based on an engineered autoinhibited protein using an enzyme-linked immunoassay

Hyun Kyung Yoon[a] and Tae Hyeon Yoo[a,b] *

[a]Department of Molecular Science and Technology, [b]Department of Applied Chemistry and Biological Engineering, Ajou University, Suwon, 443-749, Korea

* Correspondence should be addressed to T.H.Y.
Tel: +82-31-219-3543
Fax: +82-31-219-1610
E-mail: taehyeonyoo@ajou.ac.kr

Supporting Information

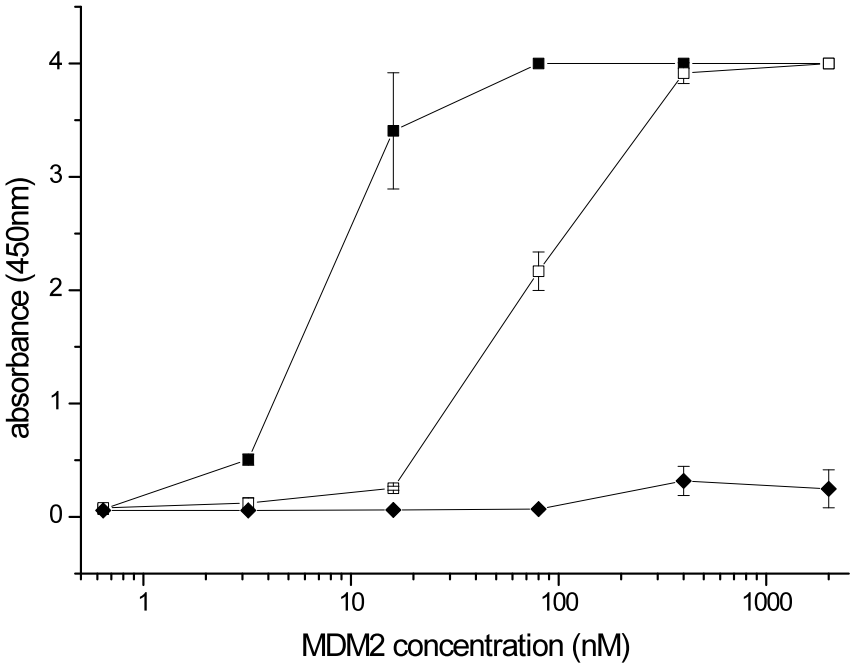

Fig. S1. Comparison of the binding affinity of GST-P27L (■), GST-PMI-N8A (□), and blank (◆) to MDM2. GST-P27L, GST-PMI-N8A, or blank (coating buffer) was coated on 96-well plates, and the binding of MDM2 was evaluated by anti-His_6-HRP conjugate.

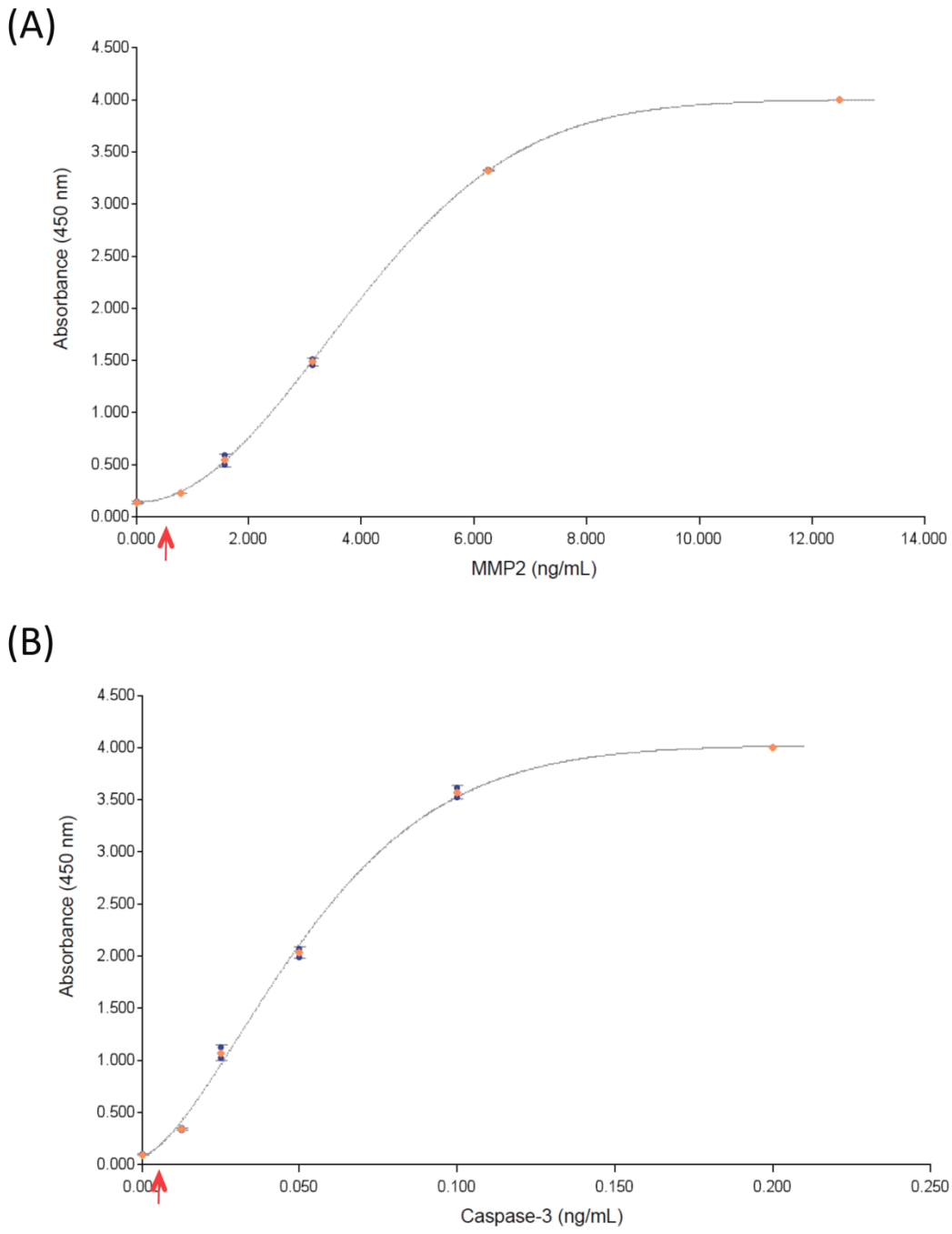

Fig. S2. Standard curves of MMP2 (A) and caspase-3 (B). The data of Fig. 2D at 2 h for MMP2 and Fig. 2E for caspase-3 were fitted with the nonlinear equation of Absorbance = (A-D)/(1+(Concentration of protease/C)^B)^E+D using Gen5 (v. 2.00) (BioTek); A, B, C, D, and E are parameters obtained by fitting the ELISA signals to the nonlinear equation. The red arrows indicate the limit of detection (0.5 ng/mL for MMP2 and 3 pg/mL for caspase-3) determined as the concentration of protease that produces an absorbance value equivalent to the average negative value plus 3 × standard deviation using the standard curve.