Electronic Supplementary Material (ESI) for Faraday Discussions.
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High-throughput Enzyme Nanopatterning

Xiangyu Liu[a,b], Mohit Kumar[b], Annalisa Calo'[a,b], Edoardo Albisetti[a, b, d], Xiaouri Zheng[a, b], Kylie B. Manning[c], Elisabeth Elacqua[c], Marcus Weck[c], Rein V. Ulijn[b], Elisa Riedo[*a, b]

[a]Tandon School of Engineering, New York University, Brooklyn, NY, USA
[b]Advanced Science Research Center (ASRC), CUNY Graduate Center, New York, NY, USA
[c]Department of Chemistry, New York University, New York, NY, USA
[d]Dipartimento di Fisica, Politecnico di Milano, Milano, Italy

*e-mail: elisa.riedo@nyu.edu

1. Thermolysin size characterization

Below is the dynamic light scattering result of the enzyme solution. It shows that the Thermolysin molecules have an average hydrodynamic diameter of about 5.75 nm comparable to the height difference before and after incubation in enzyme solution (Figure 3), which indicates a monolayer of Thermolysin immobilized inside the pattern.

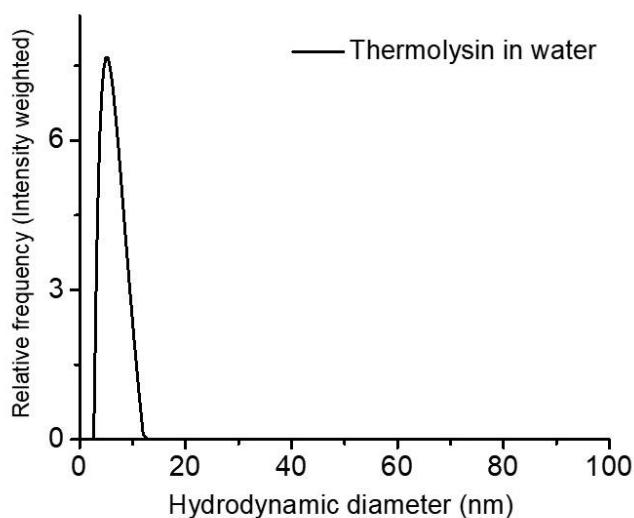

Fig. S1 Dynamic light scattering (DLS) result of thermolysin enzymes showing its hydrodynamic diameter around 5.75 nm.

2. Enzyme activity

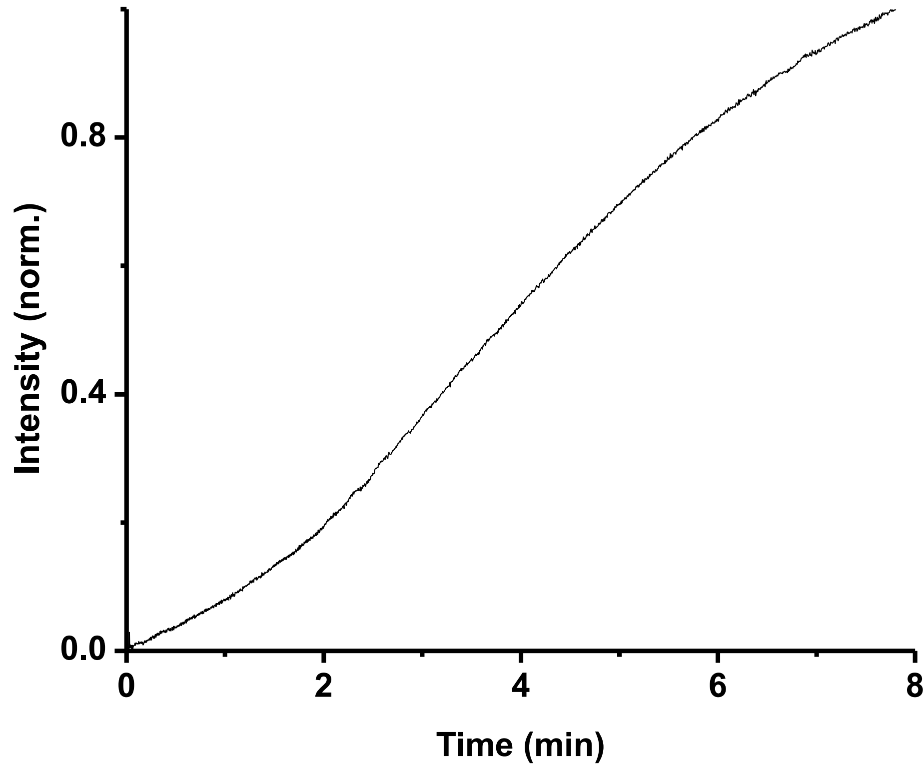

Fig. S2 FRET peptide-based assay confirms the peptidase activity of surface immobilized thermolysin. λ_{ex}= 340 nm, λ_{em}= 520 nm.

The FRET peptide substrate, E(EDANS)-GT ↓ LGK-(DABCYL), was purchased from China Peptides and dissolved in DMSO to a final concentration of 0.5mM. The downward arrow in the sequence represents the position for enzyme cleavage. Fluorescence emission spectra were measured on a Jasco FP-6500 spectrofluorimeter with light measured orthogonally to the excitation light, at a scanning speed of 500 nm min^{-1}. 1 μl of the FRET solution were added to 1 ml of water in a 10 mm quartz cuvette. Then the patterned surface with enzyme immobilization was inserted into the cuvette and fluorescence intensity was monitored at 520 nm over time.

3. Characterization of Alexa-Thermolysin conjugates

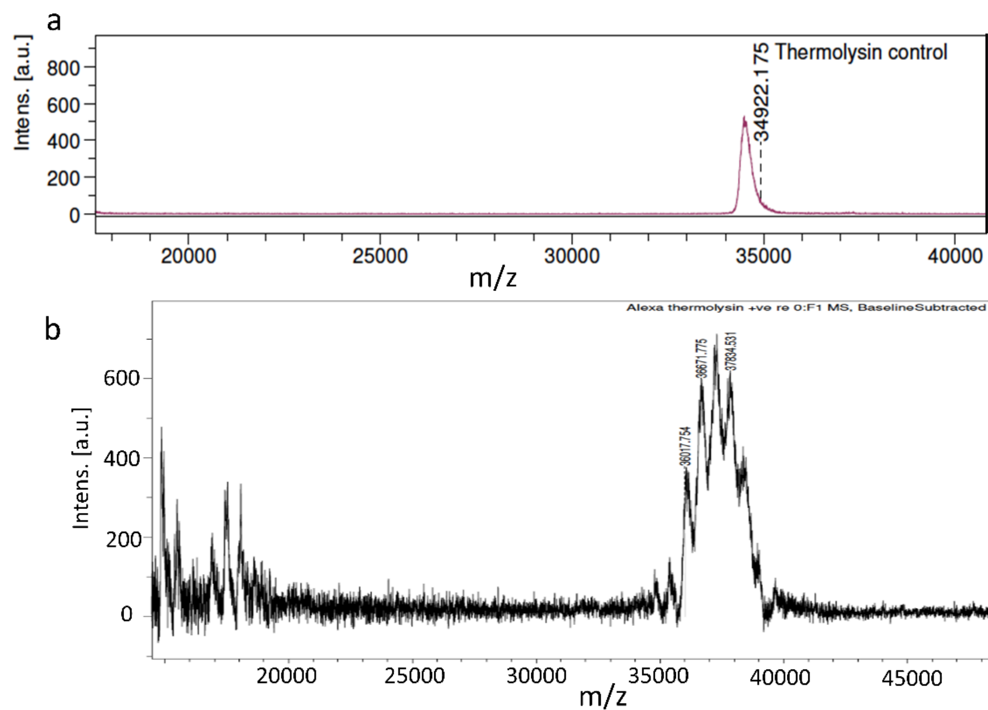

Figure S3. Comparative Mass-Spectrometry (MALDI) analysis of the Thermolysin before (a) and after (b) Alexa-488 conjugation. As evident, the Alexa-Thermolysin shows higher mass compared to pristine thermolysin, confirming successful conjugation. Multiple MS peaks indicate multiple Alexa-488 molecules attached to the enzyme.