SUPPORTING INFORMATION for：

Highly sensitive MALDI-MS measurement on active ricin: insight from more potential deoxynucleobase-hybrid oligonucleotide substrates

Jiewei Yang,[a] Chenyu Wang,[a,b] Li Luo,[a,c] Zhi Li,[a] Bin Xu,[a] Lei Guo,*[a] and Jianwei Xie[a]

[a.] *State Key Laboratory of Toxicology and Medical Countermeasures, and Laboratory of Toxicant Analysis, Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences, Beijing, 100850, China.*

[b.] *School of Pharmacy, Minzu University, Beijing, 100081, China.*

[c.] *School of Public Health, Hebei Medical University, Shijiazhuang, Hebei Province, 050017, China.*

† Corresponding author: Prof. Dr. Lei Guo, Tel. 86-10-68225893, email: guolei@bmi.ac.cn

Experimental

Chemicals and materials

Acrylamide: bisacrylamide (Acr-Bis, 29:1), ammonium persulfate, Coomassie Brilliant Blue G250, prestained protein ladder (10-180 kDa), and BCA protein concentration determination kit were purchased from Beyotime Biotechnology Co., Ltd. (Beijing, China). Dithiothreitol (DTT), N, N, N', N'-tetramethyl ethylene diamine (TEMED), 2-morpholinoethanesulfonic acid monohydrate (MES), N-(3-dimethylaminopropyl)-N'-ethyl carbodiimide hydrochloride, and tris(hydroxymethyl)aminomethane (Tris) were purchased from Sigma-Aldrich Company (USA). Sodium dodecyl sulfate (SDS) was obtained from VWR International (USA).

The pH meter (Seven Excellence) was purchased from METTLER TOLEDO Instruments Co., Ltd. (Shanghai, China). Biacore T200 was purchased from GE Healthcare Life Sciences Co. (Sweden).

ASF-coupled carboxylate magnetic beads (ASF-MBs) to capture ricin

The suspension of carboxylate MBs (1 mL, 10 mg) was mixed evenly and added into a 2 mL centrifuge tube, the supernatant was then removed by a magnetic separation. An aliquot of 1 mL 100 mM MES (pH 5.0) was added, then was magnetically separated and the supernatant was removed, this procedure was repeated once.

ASF solution of 500 μg (1 g L^{-1} in MES) was added, rotated and mixed at 25°C for 30 min, then 100 μL of the newly prepared EDC solution (10 g L^{-1} in MES) was added and made up with MES to a total volume of 1 mL, rotated and mixed at 25°C for 1 h, and the supernatant was removed by the magnetic separation. An aliquot of 0.1 M Tris-HCl (pH 7.4, 1 mL) was then added to terminate the conjugation. The supernatant was then transferred to 1 mL of 0.1%Tween 20 in PBS (PBST), and then the ASF-MBs were ready for use.

Ricin was mixed with ASF-MBs at a ratio of 1:1-1:10 (w/w), incubated at 37°C for 3.5 h, then the supernatant was magnetically separated and removed. An aliquot of ultra-pure water (200 μL) was added to resuspend the ASF-MBs, rotated and mixed at 25°C for 10 min, the supernatant was then magnetically separated and removed. This procedure was repeated for 5 times. The ricin binding ASF-MBs were then ready for use.

The reaction buffer (30 μL EDTA at 0.5 g L^{-1}, 1 μL DHC at 50 g L^{-1}, 0.4% FA 1 μL, pH 4.2) of 32 μL, 100 μM Rd12 of 15 μL was then added into the ricin binding ASF-MBs, incubated at 37°C for 30 min. The activity of different amount of ricin was then measured by MALDI-MS.

Evaluation of affinity and kinetic constants of ASF-I and ricin binding by SPR

Ricin at 50 μg mL^{-1} was used for covalently coupled onto the channel 2 of CM5 chip, and the resonance unit (RU) of coupling was achieved as 5000 RU, the channel 1 was used as a blank channel. The flow rate was 30 μL/min, the association time was 120 s, and the dissociation time was 3600 s. The loading concentrations of ASF-I were ranged from 0.625 to 10 nM. Multi-cycle kinetic evaluation (MCK) was performed to evaluate the kinetic and binding constants.

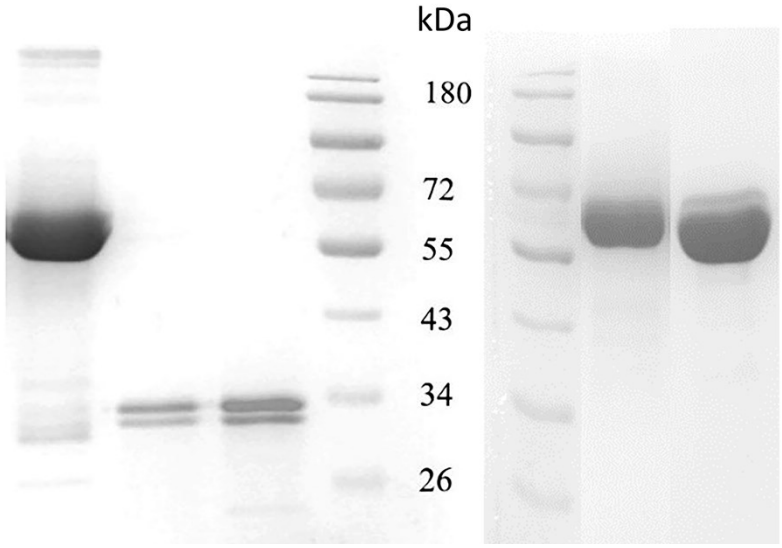

Fig. S1 SDS-PAGE of the self-prepared biotoxins at milligram yield. Aliquots of 12 µL ricin (5 g L^{-1}), RTA (1 g L^{-1}), RTB (1 g L^{-1}), AAG (2 g L^{-1}), and abrin (2 g L^{-1}) were loaded, respectively. The protein ladder was loaded at 3 µL (unit: kDa).

Ricin was obtained from the crude extract of castor beans of *Ricinus communis* L. by affinity chromatography and followed size exclusion chromatography. RTA and RTB were obtained by denaturing the holotoxin ricin by DTT and then separated by affinity chromatography. Due to glycosylation heterogeneity, RTA appeared as a double band of 30 kDa and 32 kDa[1]. Abrin and AAG were obtained from the crude extract of the seeds of *Abrus precatorius* L. by affinity chromatography.

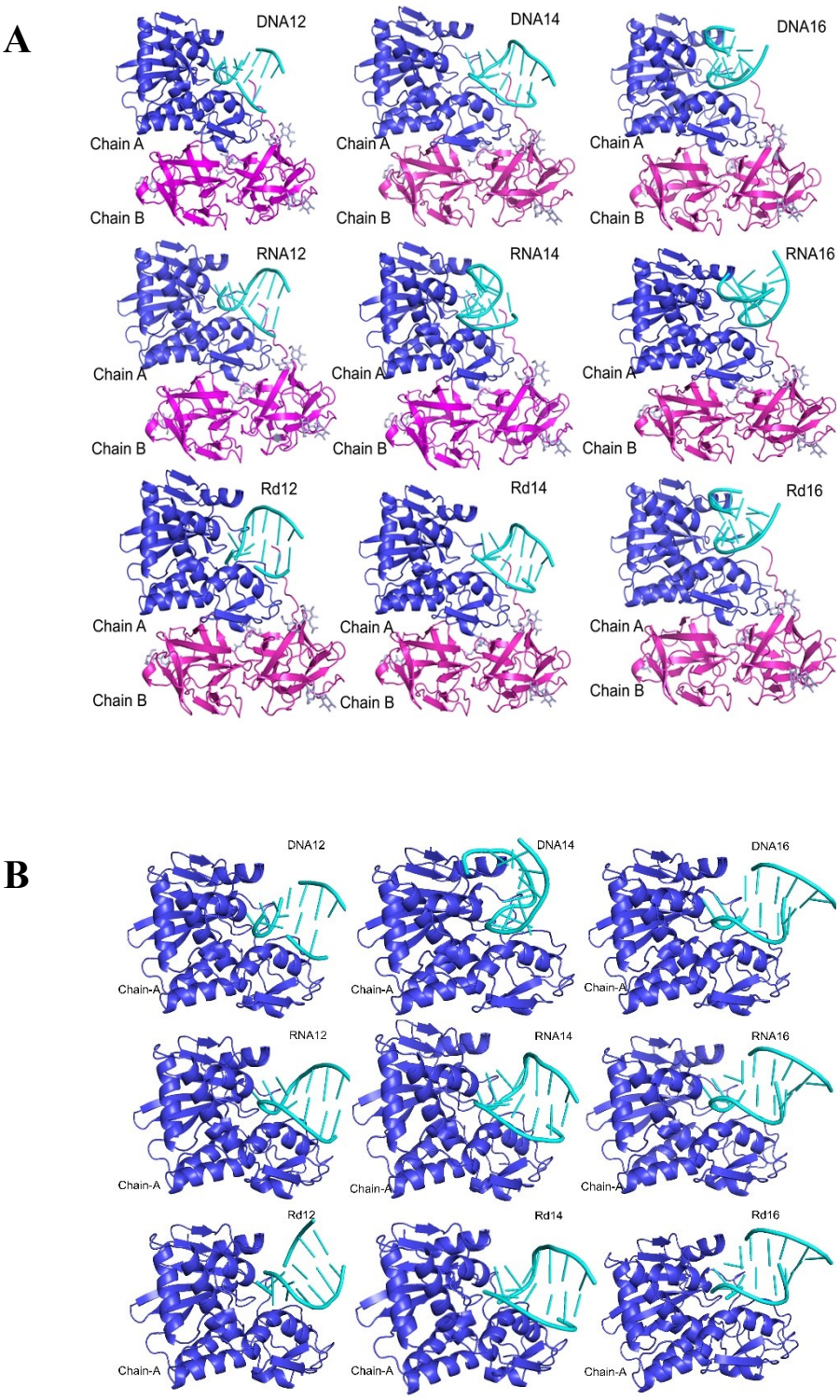

Fig. S2 The molecular docking simulation of ricin (A) and RTA (B) with all nine oligo(deoxy)nucleotide substrates. The grey colour in ricin B chain resembles the glycosylation group.

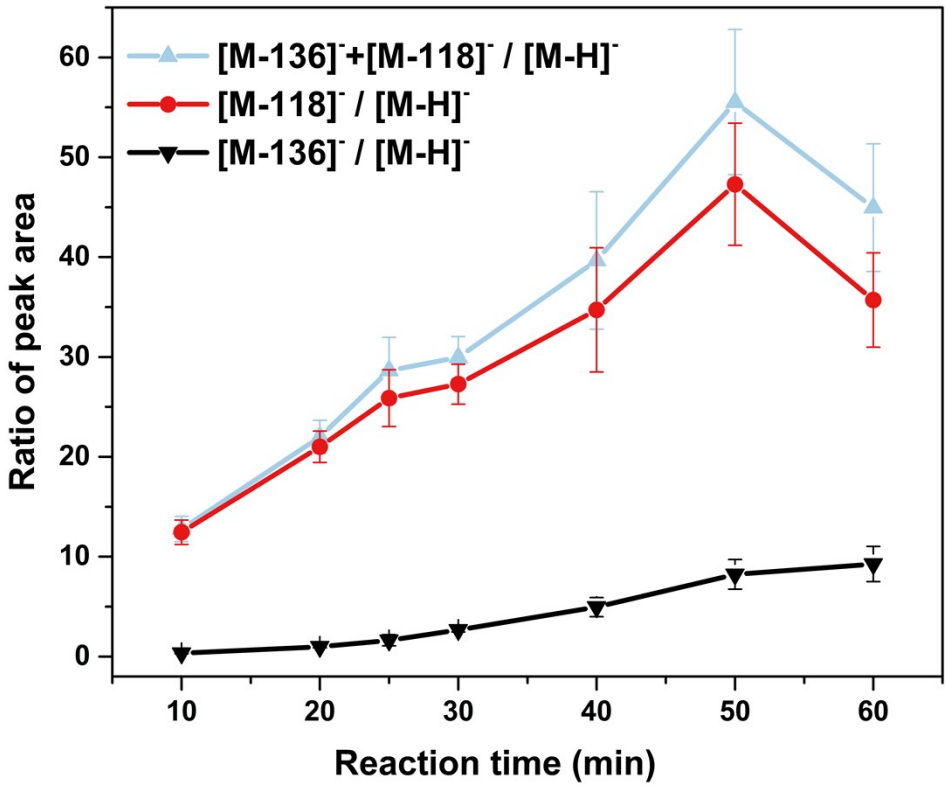

Fig. S3 The concurrence of different forms of products during the reaction process. Conditions: 30 μM Rd12, 0.5 μg mL^{-1} ricin, 55°C.

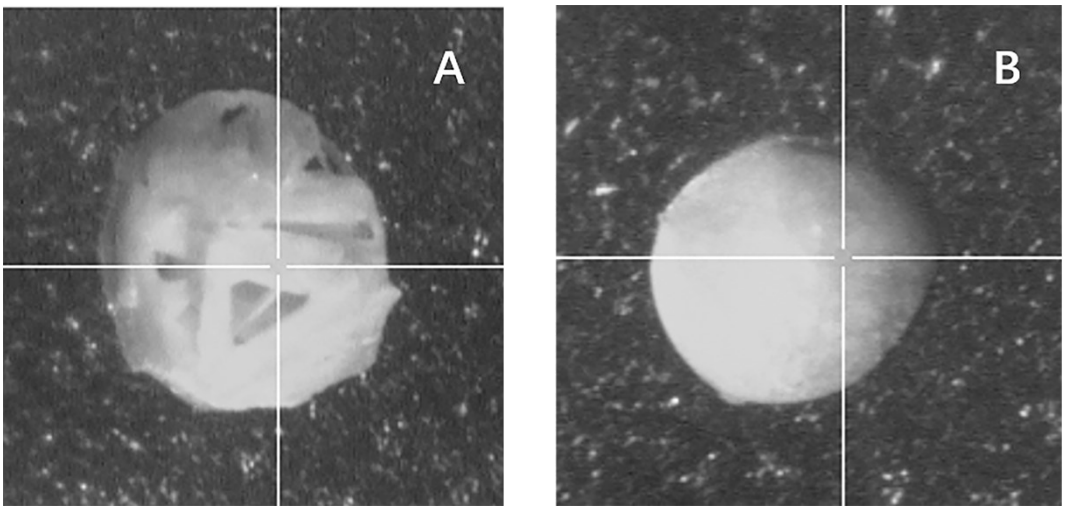

Fig. S4 Effect of the termination of ammonia water on crystallization.

A. before ammonia water added; B. with ammonia water termination.

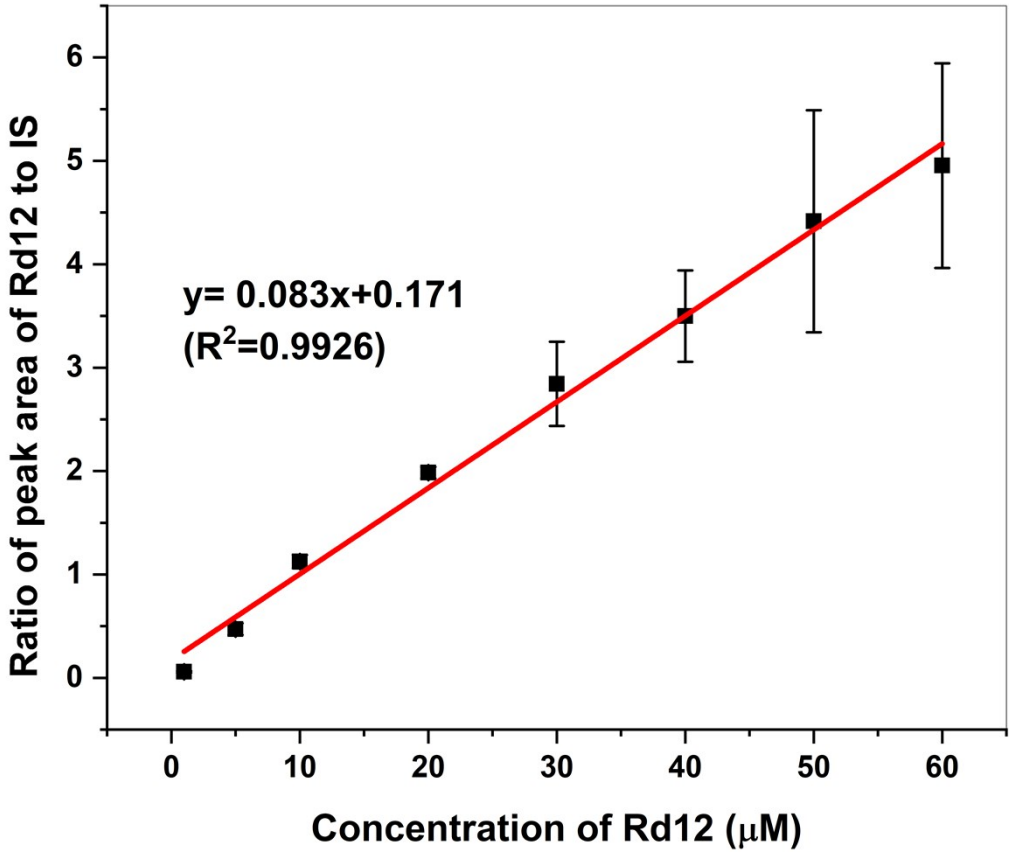

Fig. S5 Linear range of Rd12 in MALDI-MS when using the internal standard of one oligonucleotide of 10 nt (IS10b, GCGCGGGCGC).

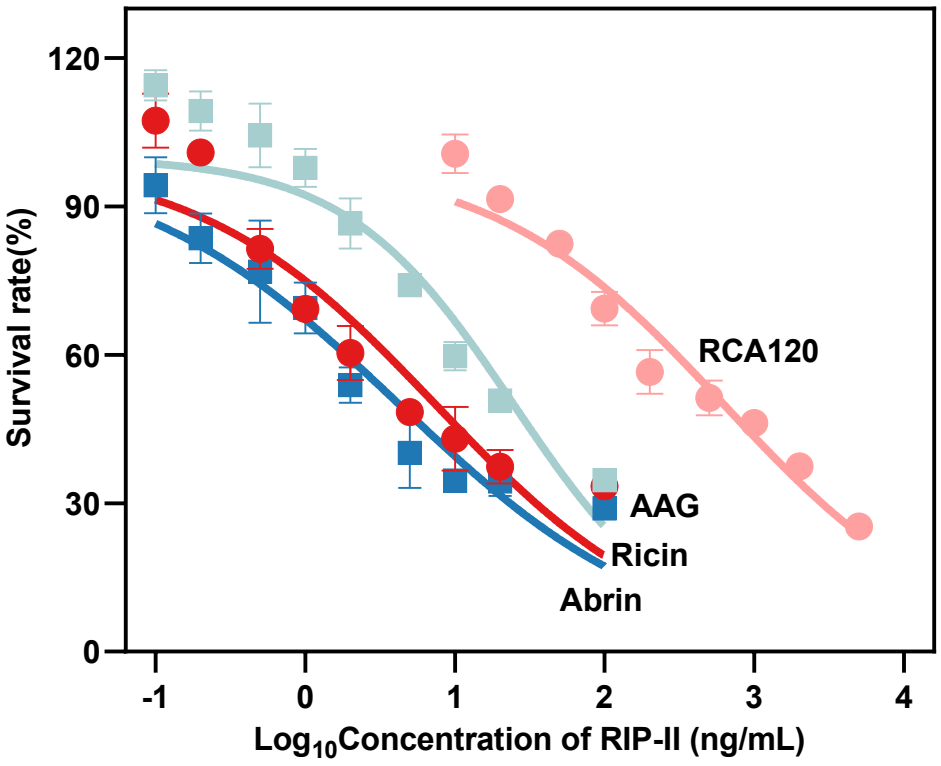

Fig. S6 Cytotoxicity test using HeLa cells with RIP-IIs at 37°C of 24 h was shown as an *in vitro* comparison.

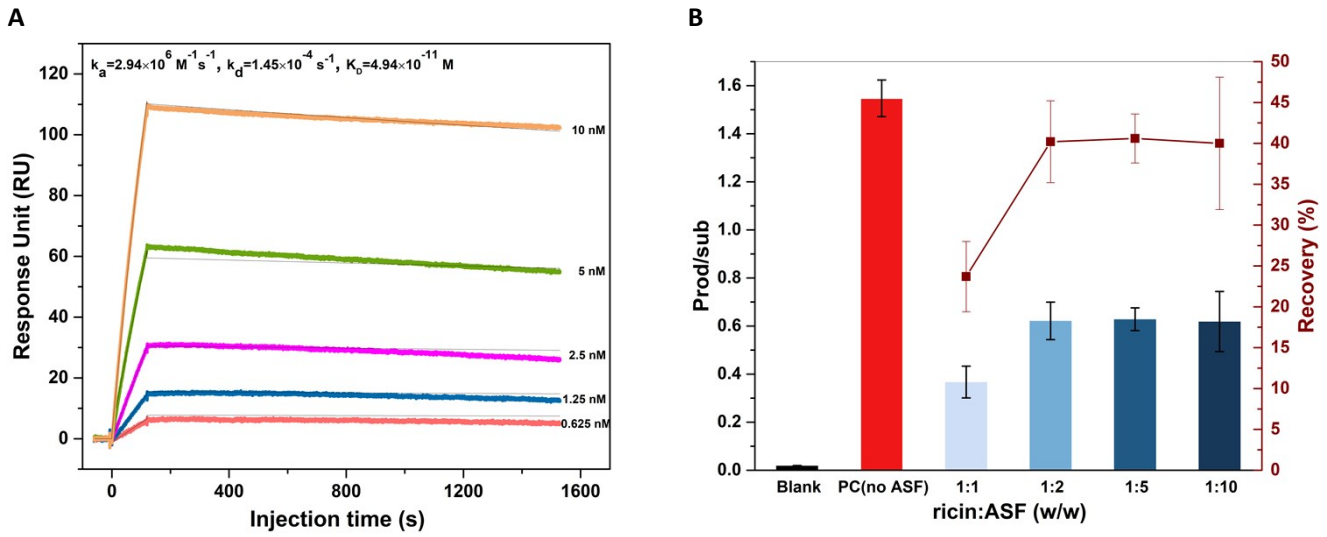

Fig. S7 Affinity and kinetic constants of ASF-I and ricin binding by SPR (A); and the ASF-MBs capture capability toward ricin at a different ratio between ricin and ASF-I (w/w).

Left vertical axis: Prod/sub; right vertical axis: recovery. The loading concentration of ricin was 1 μg mL^{-1}, and the recovery, *i.e.*, the ASF-MBs capture efficiency was calculated as 40.6% (B).

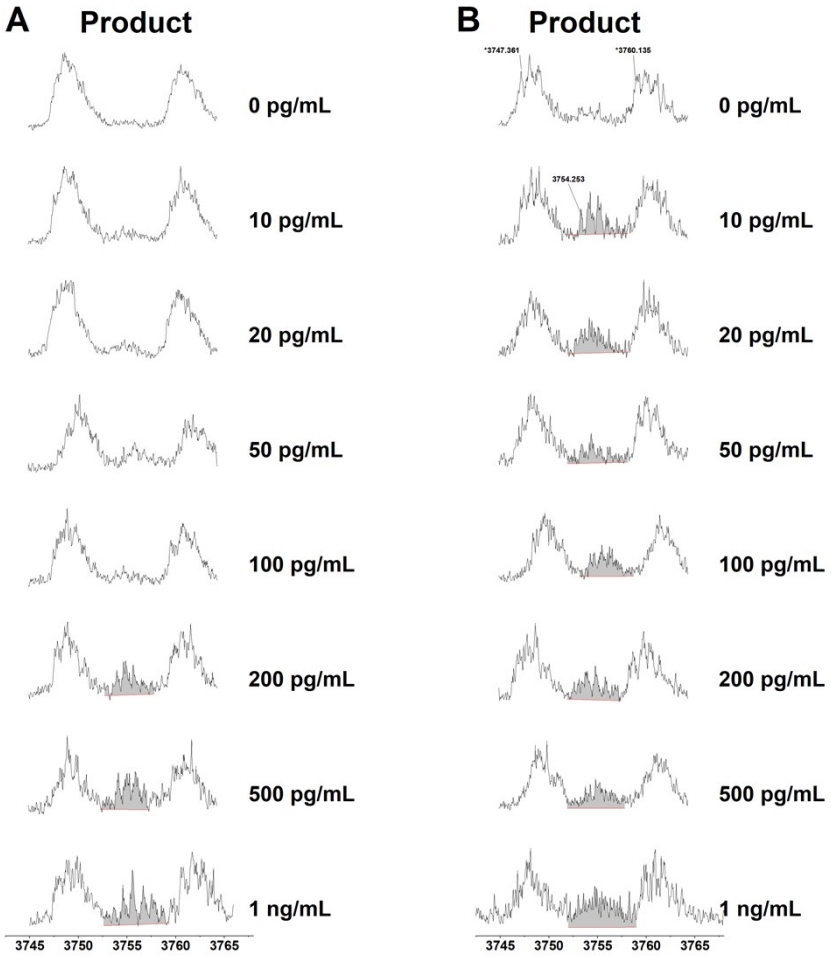

Fig. S8 The LOD (1 ng mL^{-1}) and lower concentrations in the MALDI-MS spectra.
Conditions: 37°C, 10 min(A), 30 min(B), the peaks marked* represent the inherent peaks coexisted in the spectra but without any interference.

Reference

1. M. Pasetto, E. Barison, M. Castagna, P. Della Cristina, C. Anselmi and M. Colombatti, *J. Biol. Chem.*, 2012, **287**, 7367-7373.

