

Non-toxic dimeric peptides derived from the Bothropstoxin-I are potent SARS-CoV-2 and Papain-Like Protease inhibitors

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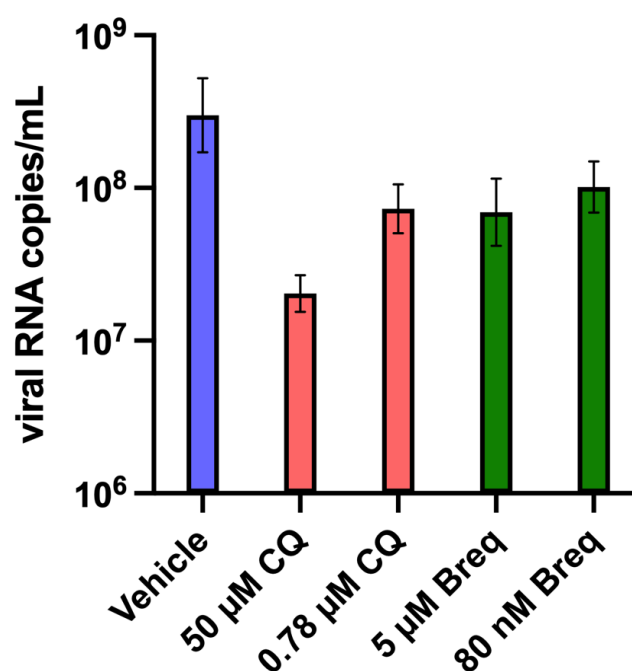


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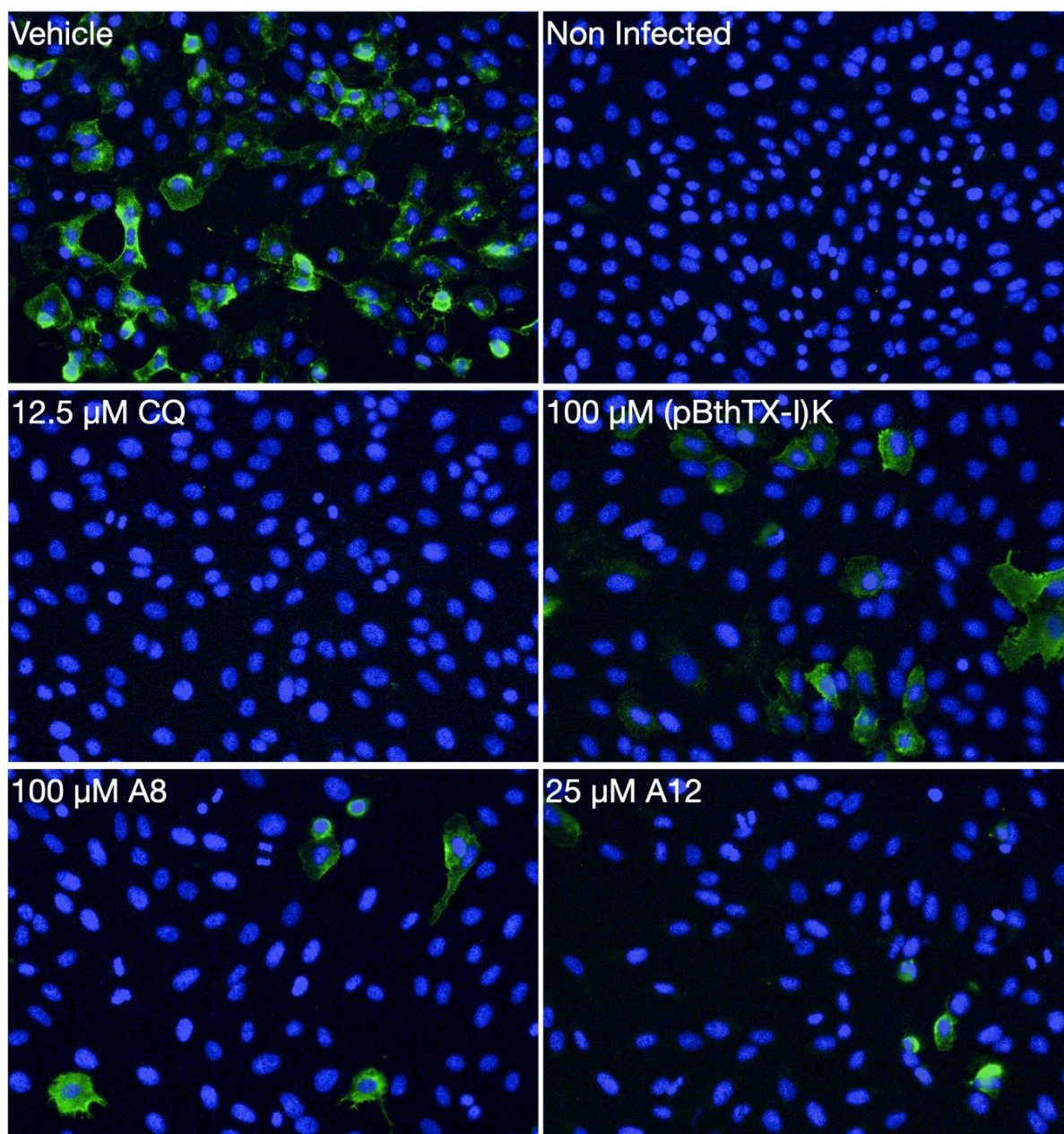


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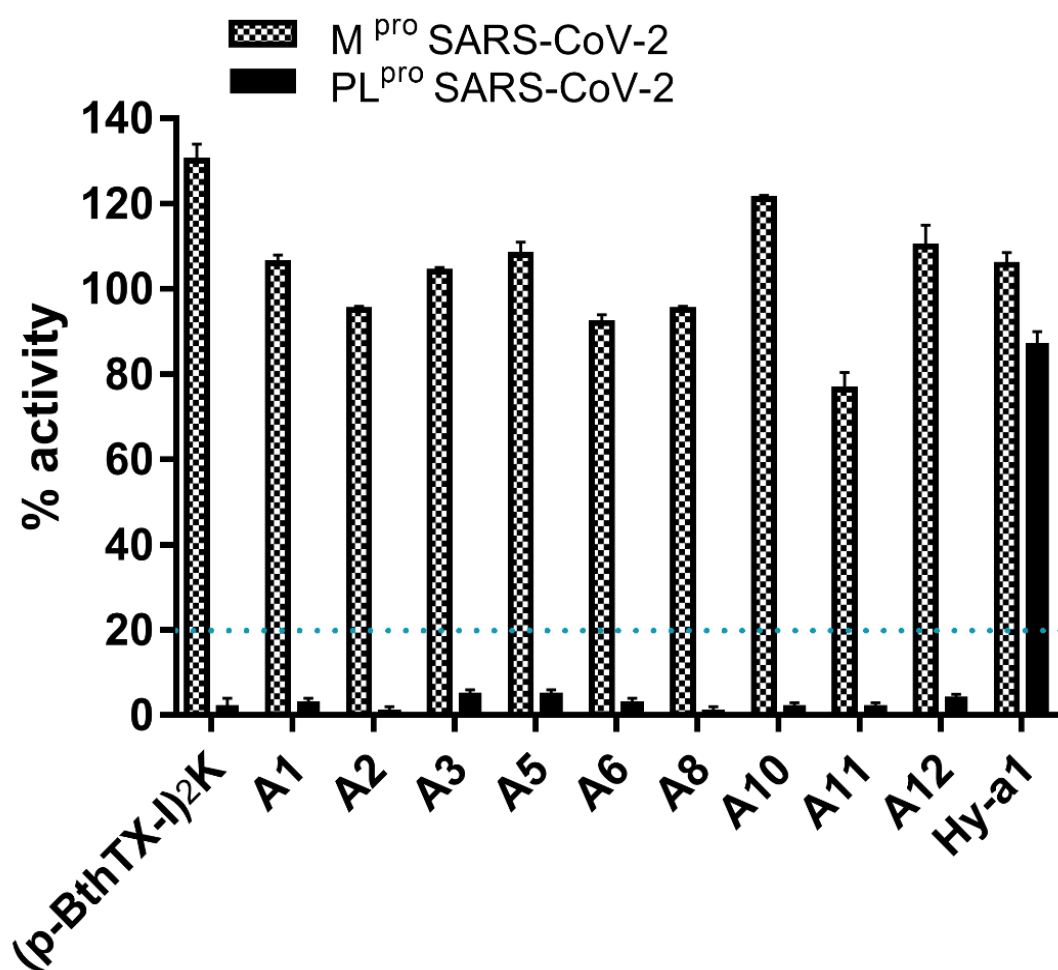
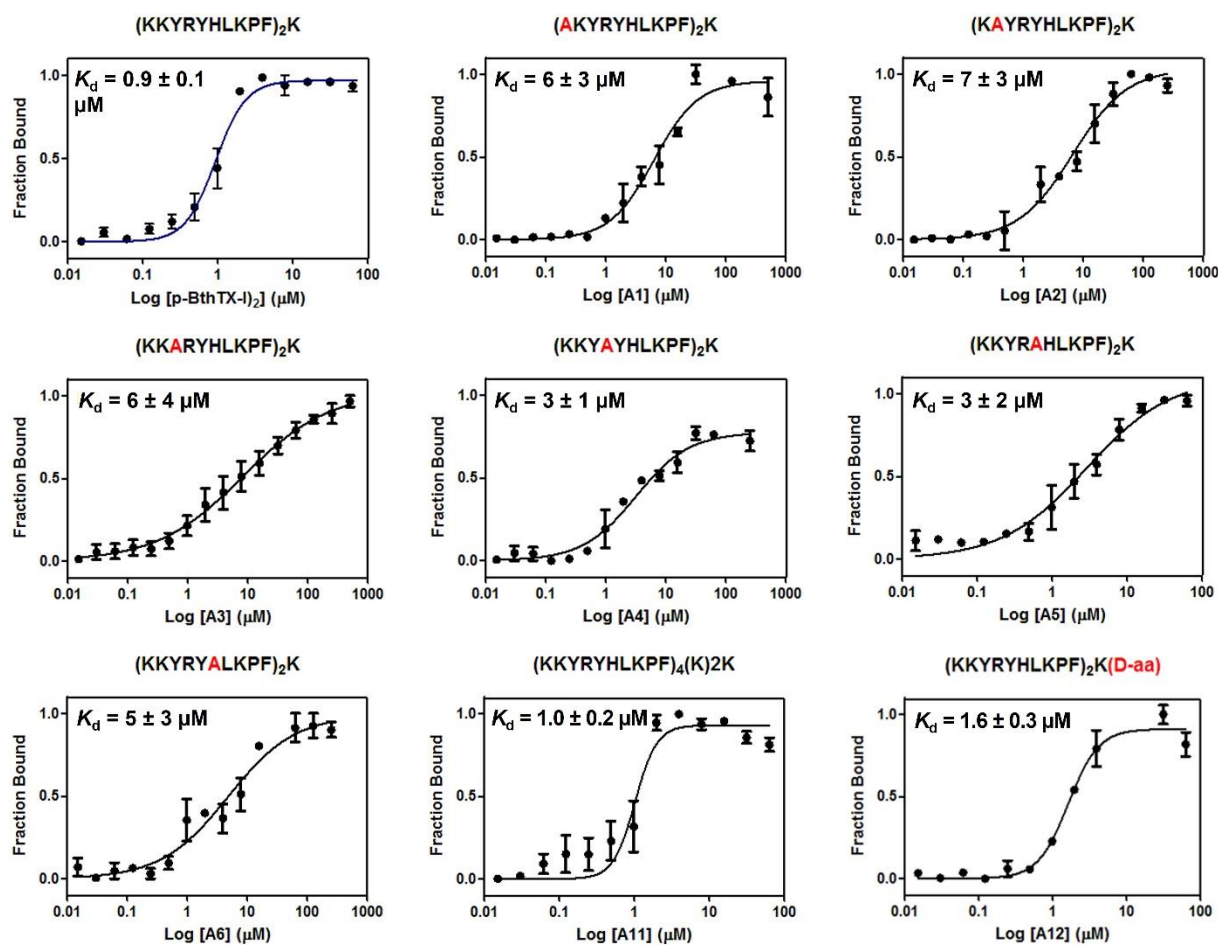


Figure S3: Single concentration inhibition screening of peptide analogs (10 μ M) against M^{pro} and PL^{pro} from SARS-COV-2.

A



B

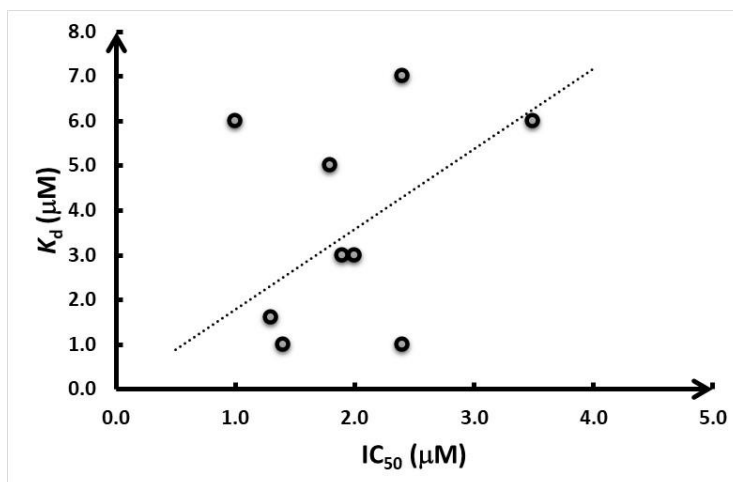


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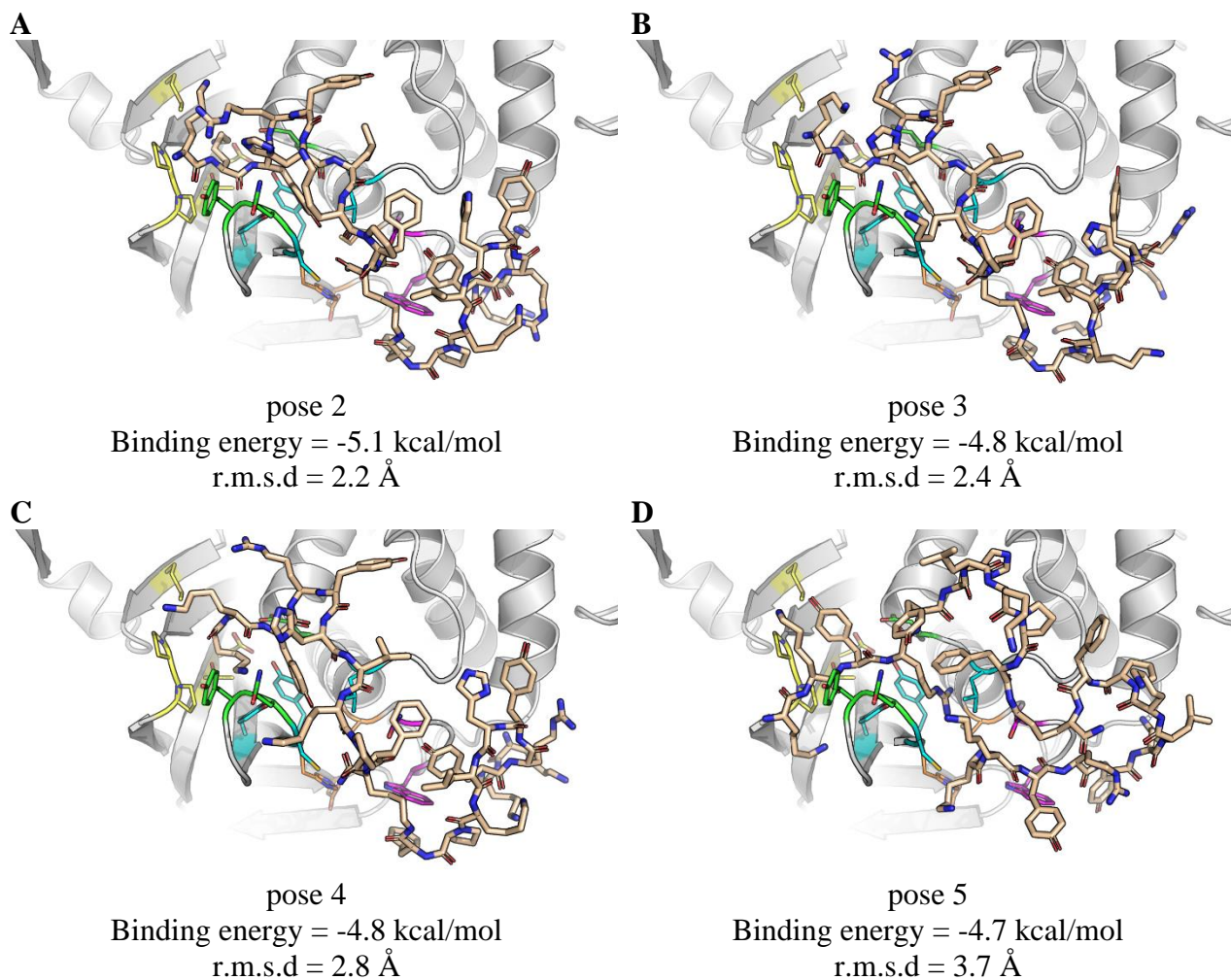
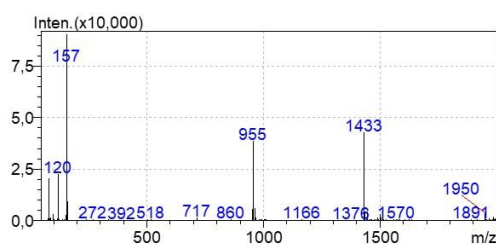
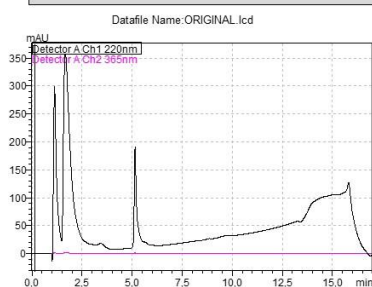


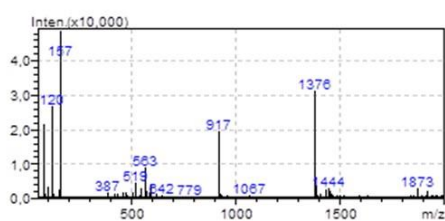
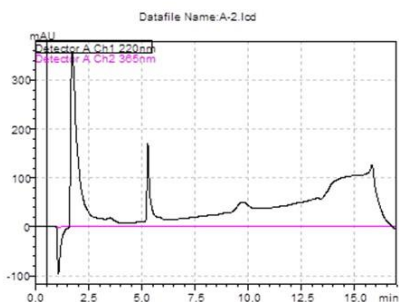
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(pBthTX-I)2K



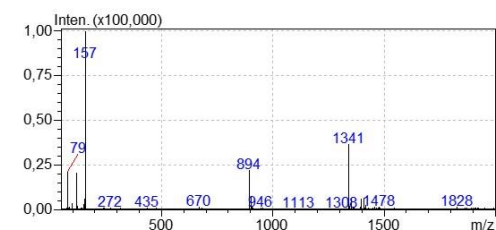
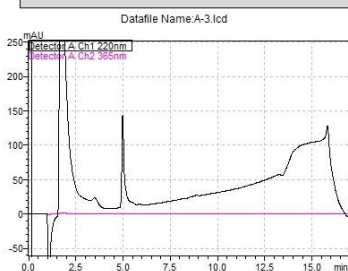
$M+1H^+ = 2866$
 $M+2H^+ = 1433$
 $M+3H^+ = 955$

A2



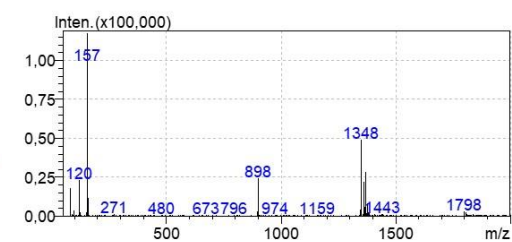
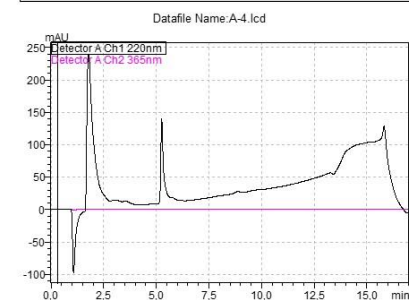
$M+1H^+ = 2752$
 $M+2H^+ = 1376$
 $M+3H^+ = 917$

A3



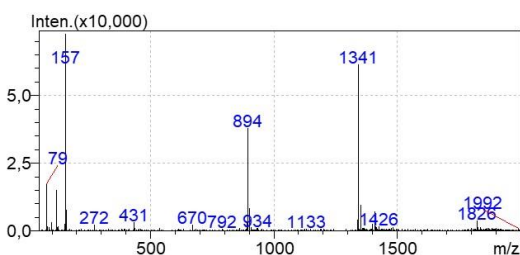
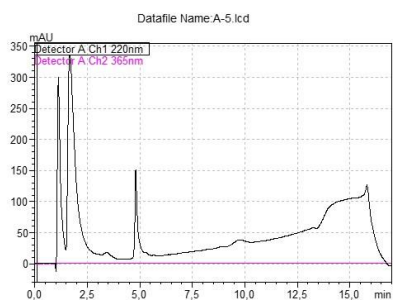
$M+1H^+ = 2682$
 $M+2H^+ = 1341$
 $M+3H^+ = 894$

A4



$M+1H^+ = 2696$
 $M+2H^+ = 1348$
 $M+3H^+ = 898$

A5



$M+1H^+ = 2682$
 $M+2H^+ = 1341$
 $M+3H^+ = 894$

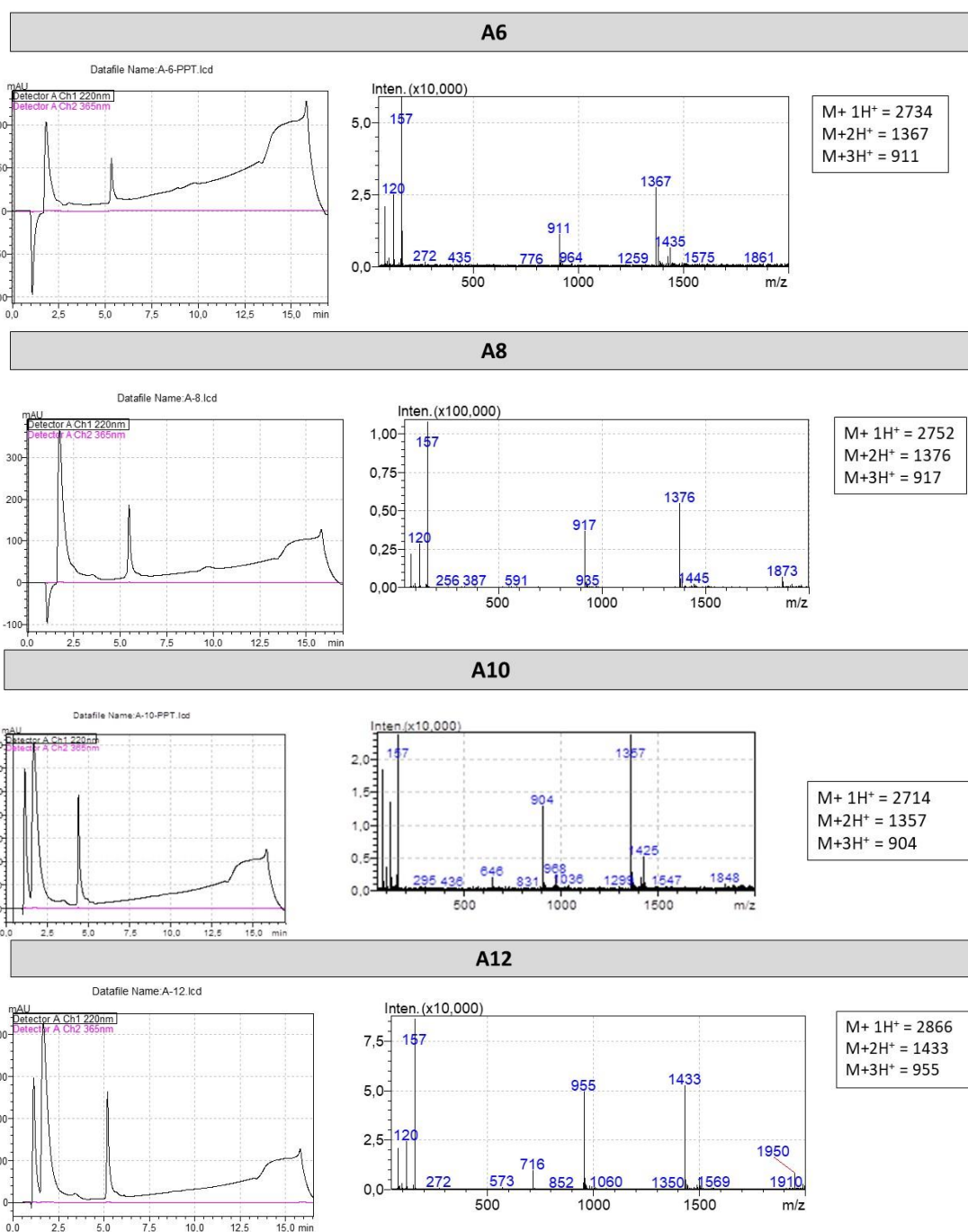


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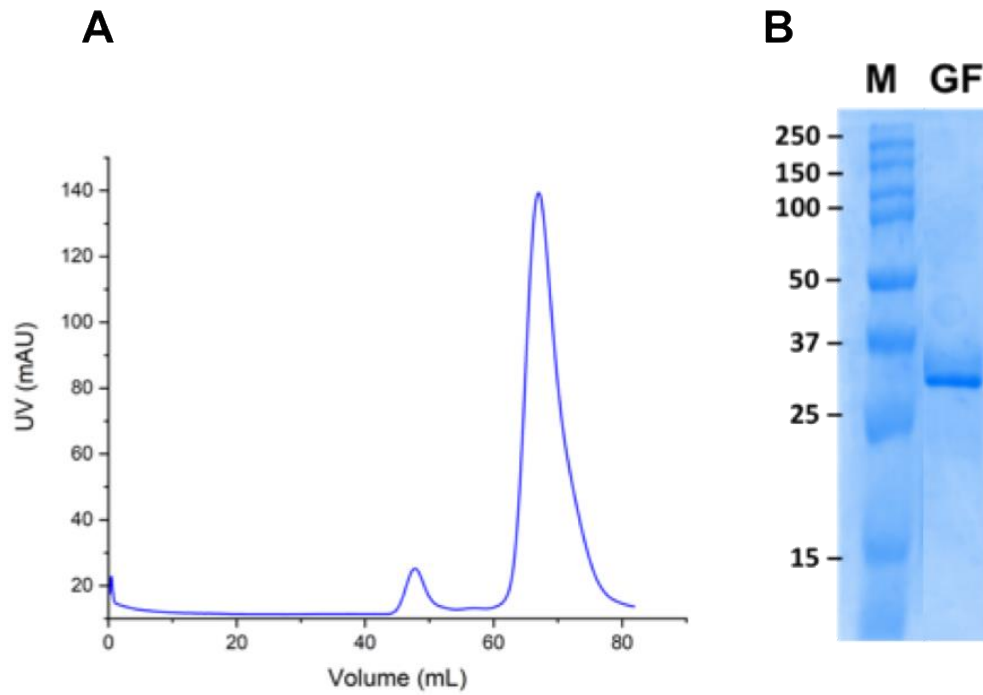


Figure S7: SARS-CoV-2 PL^{pro} purification. **A)** Chromatogram of Size Exclusion Chromatography step; **B)** SDS-PAGE gel of PL^{pro}: M – molecular weight marker; GF – fraction eluted at Size Exclusion Chromatography step.

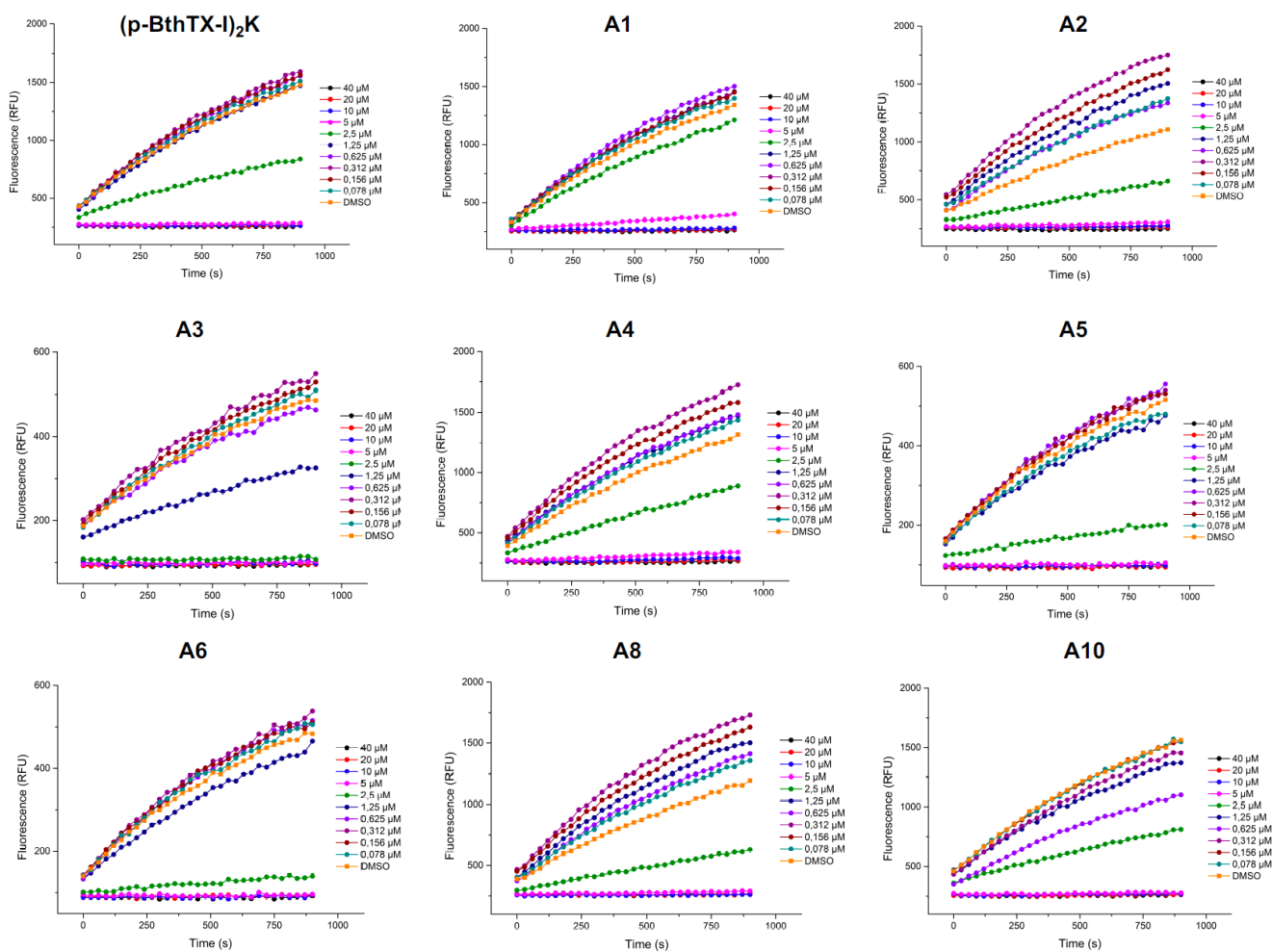


Figure S8. Representative time-dependent traces of the substrate fluorescence at different inhibitor concentrations.

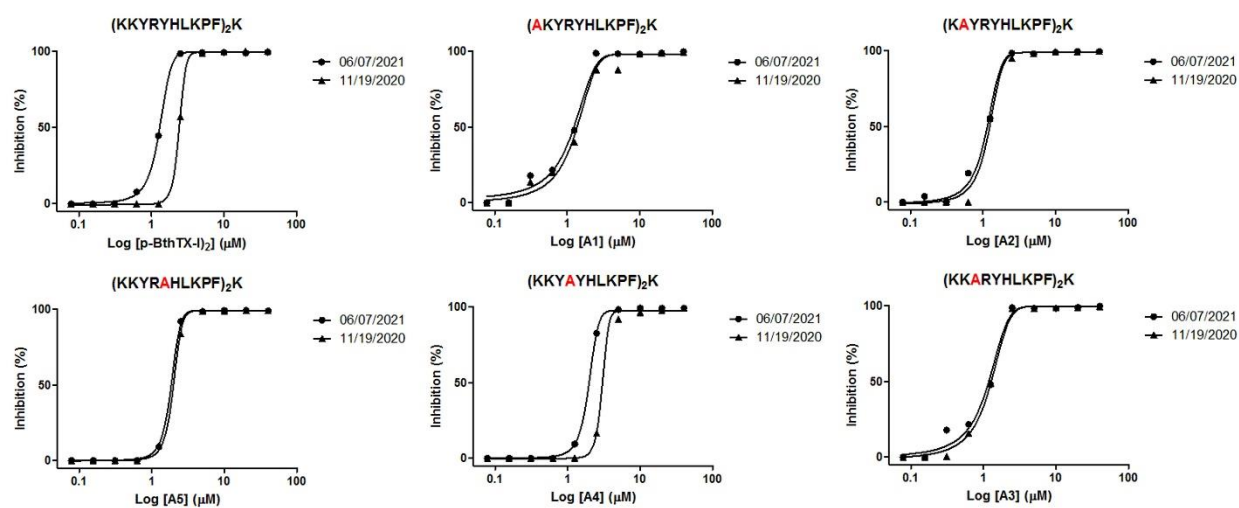


Figure S9. IC₅₀ plots of representative peptides collected in November/2020 and June/2021.

Table S1: Inhibitory activity of (pBthTX-I)₂K and analogs against SARS-CoV-2 and PL^{Pro} at 100 μ M and 10 μ M, respectively. The substituted Ala residue on the sequence of each peptide is indicated as red.

Code	Peptide Sequence	M.W. (Da)	SARS-CoV-2	PL ^{Pro}
			% of inhibition at 100 μ M	% of inhibition at 10 μ M
(pBthTX-I) ₂ K	(KKYRYHLKPF) ₂ K	2868	27	98 \pm 2
A1	(AKYRYHLKPF) ₂ K	2754	4	97 \pm 1
A2	(KAKYRYHLKPF) ₂ K	2754	10	99 \pm 1
A3	(KKARYHLKPF) ₂ K	2684	57	95 \pm 1
A4	(KKYAYHLKPF) ₂ K	2698	10	97 \pm 1
A5	(KKYRAHLKPF) ₂ K	2684	70	95 \pm 1
A6	(KKYRYALKPF) ₂ K	2736	22	97 \pm 1
A8	(KKYRYHLAPF) ₂ K	2754	29	99 \pm 1
A10	(KKYRYHLKPA) ₂ K	2716	7	98 \pm 1
A11	(KKYRYHLKPF) ₄ (K)2K	5848	54	98 \pm 1
A12	(KKYRYHLKPF) ₂ K(d-aa)	2868	69	96 \pm 1
Hy-a1	IFGAILPLALGALKNLIK-NH ₂	1865	59	13 \pm 3

n.d. = not determined