Supplementary Information

Recombinant production of influenza hemagglutinin and HIV-1 GP120 antigenic peptides using a cleavable self-aggregating tag

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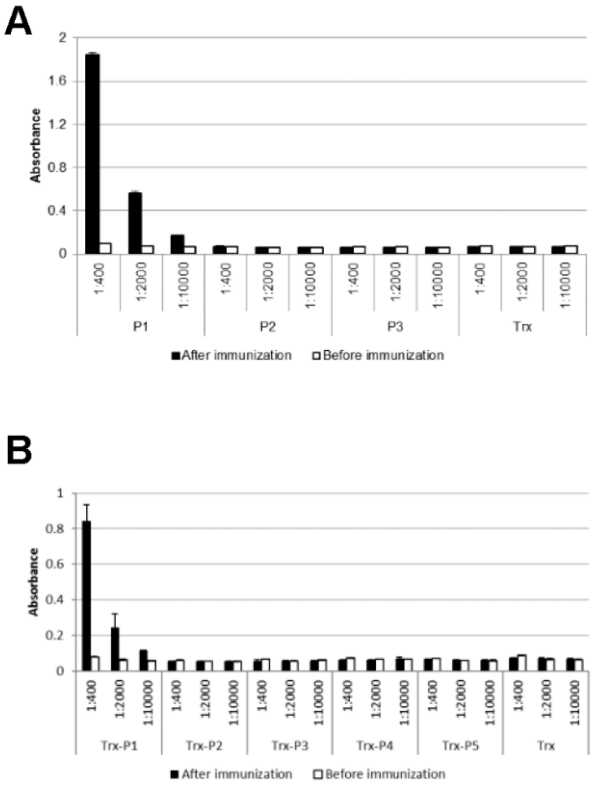
Peptide	Residue number	Position[a]	Sequence	GRAVY[b]
2009 A (H1N1) influenza virus hemagglutinin peptides				
P1	70	423-492	EKRIENLNKKVDDGFLDIWTYNAELLVLLENERTLDYHDSNVKNLYEKVRSQLKNNAKEIGNGCFEFYHK	-0.899
P2	37	387-423	LKSTQNAIDEITNKVNSVIEKMNTQFTAVGKEFNHLE	-0.562
P3	39	22-60	IGYHANNSTDTVDTVLEKNVTVTHSVNLLEDKHNGKLCK	-0.574
P4	32	308-339	PFQNIHPITIGKCPKYVKSTKLRLATGLRNIP	-0.281
P5	65	61-125	LRGVAPLHLGKCNIAGWILGNPECESLSTASSWSYIVETPSSDNGTCYPGDFIDYEELREQLSSV	-0.192
HIV-1 (HXB2) gp120 peptides				
G9	72	207-278	KVSFEPIPIHYCAPAGFAILKCNNKTFNGTGPCTNVSTVQCTHGIRPVVSTQLLLNGSLAEEEVVIRSVNFT	0.197
G31	48	431-478	SNNESEIFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTKAKRRVVQR	-1.102

Supplementary Table S1. Peptide sequences. [a] amino acid positions of the peptides on the respective intact viral envelope protein.[b] GRAVY: Grand average of hydropathicity, The GRAVY value for a peptide or protein is calculated as the sum of hydropathy values of all the amino acids, divided by the number of residues in the sequence.GRAVY value indicates the hydropathy of proteins: a positiveGRAVY score means the protein is hydrophobic anda negative score hydrophilic[1].

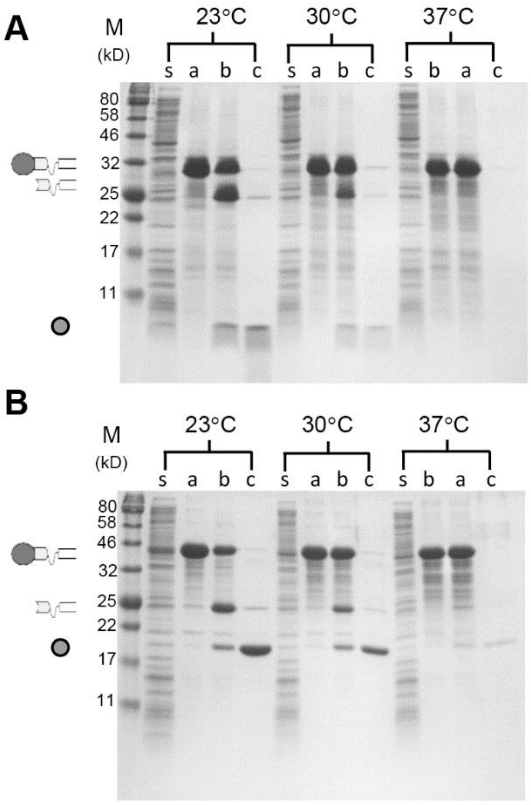
Primer name	Nucleotide sequence[a]	Description
Trx-For	5'-AGTTACATATGAGCGATAAAATTATTC-3'(*Nde*I)	Used to amplify the *trx*A gene, with the introduction of *Bgl* II and *Spe* I sites in the 3'- terminus, and insert between the *Nde* I and *Spe* I sites of pET30a-LipA-I-ELK[L. Xing, MCF, 2011].
Trx-Rev	5'-TCACGACTAGTGCATCTCCCGTGATGCACATTCGCATGATATCTGAACCAGATCTCGCCAGGTTAGCGTCGAGGAAC-3' (*Spe* I, *Eco*R V, *Bgl* II)	
G31-GS linker-For	5'-ATCGTGGATCCGGTTCAGGTTCAGGTTCTAGATCTTACAGCAACAACGAGTCTGA-3'(*Bam*H I, *Bgl* II)	Use to amplify the G31 gene, with the introduction of GS linker sequence in the 5'- terminus, and insert between the *Bgl* II and *Eco*RV sites of pET30a-Trx-I-ELK.
G31-Rev	5'-AGTTCGATATCACGTTGAACAACACGGCGC-3' (*Eco*R V)	

Supplementary Table S2. Oligonucleotides used for the construction of expression vectors.[a]The underlined nucleotides indicate restriction sites in the brackets.



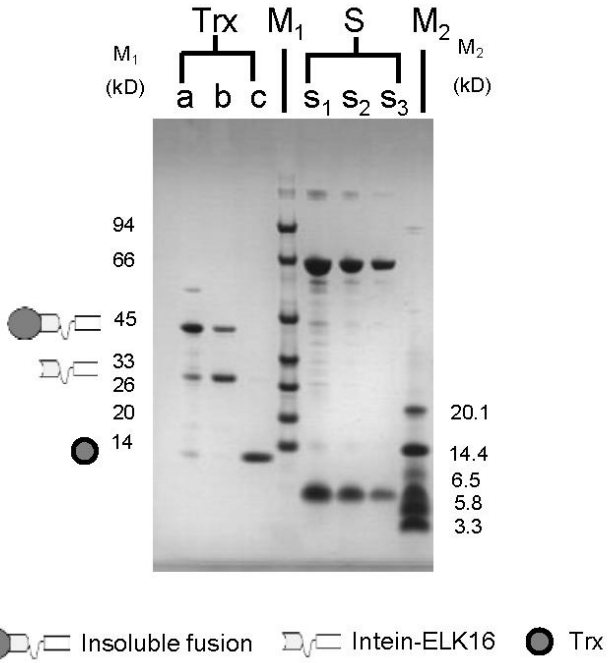
Supplementary Figure S1. Antigenic activities of P1–P5 with or without the Trx tagto mouse antisera

Binding activities of HA peptides P1–P3 (**A**) and P1–P5 with the N-terminal Trx tag (**B**) against the mouse antisera before and after immunization. Figure S1(B) was reproduced from reference 24[2].



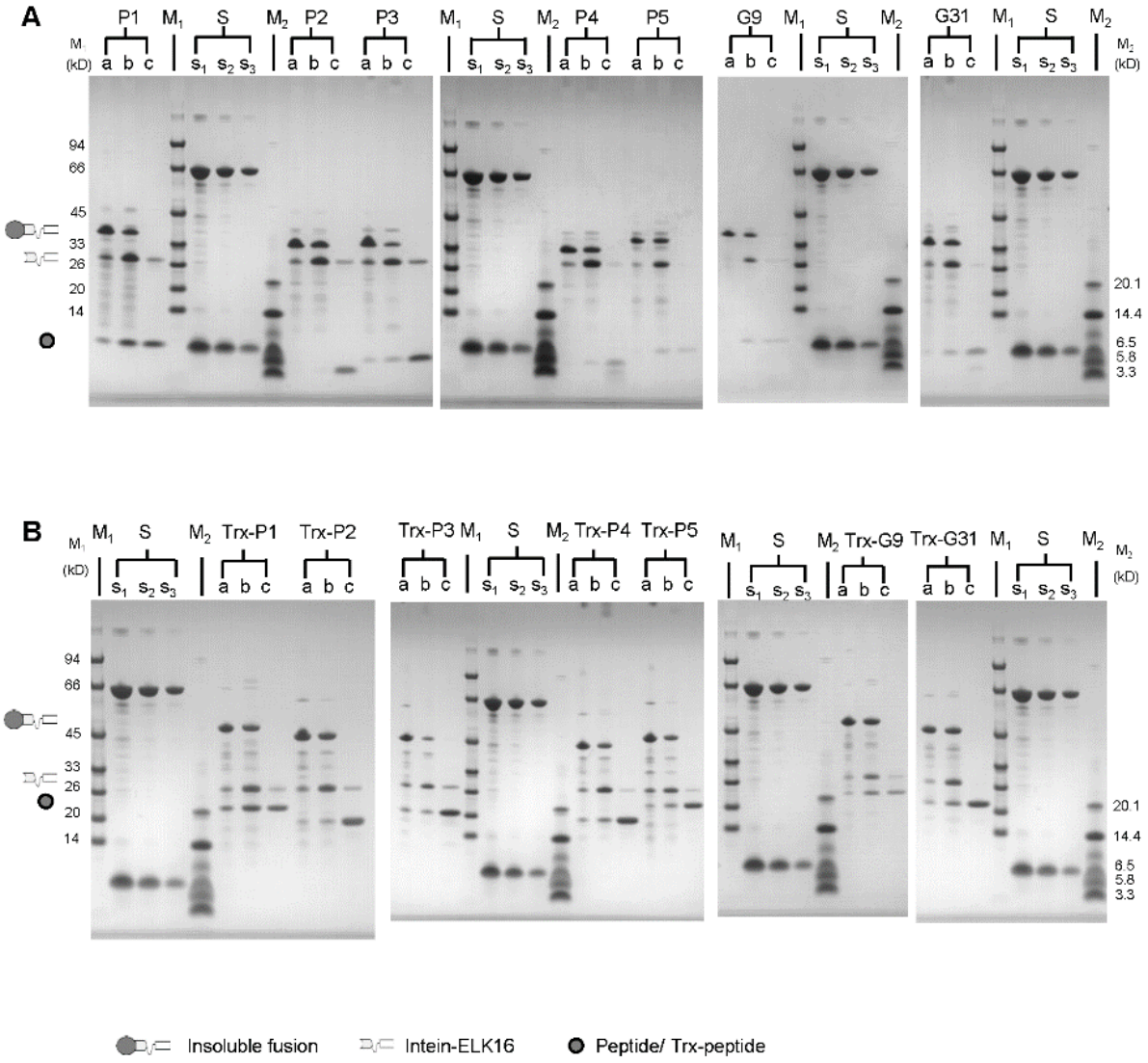
Supplementary Figure S2. Expression temperature optimization.

Expression and purification of the HIV-1 (HXB2) envelope glycoprotein GP120 peptides G31 without (**A**) and with (**B**) Trx tag at three different expression temperatures, as detected by SDS-PAGE. Lane s, soluble fraction of the cell lysate; Lane a, insoluble fraction of the cell lysate; lanes b and c, insoluble and soluble fractions of the cleaved fusion protein respectively.



Supplementary Figure S3. The full-length gel of the gel in Figure 1.

SDS-PAGE results of Trx. Lane a, insoluble fraction of the cell lysate; lanes b and c, insoluble and soluble fractions of the cleaved fusion protein respectively; lanes s1, s2 and s3, quantification standards consisting of bovine serum albumin (BSA, 66.5 kDa) at 3, 1.5 and 0.75 μg/lane and aprotinin (6.5 kDa) at 1.5, 0.75 and 0.3 μg/lane respectively. The molecular weights of the protein standards M1 and M2 are indicated on the left and right sides respectively.



Supplementary Figure S4. The full-length gel of the gel in Figure 2.

Expression and purification of the 2009 A (H1N1) influenza hemagglutinin (HA) peptides P1–P5 and HIV-1 (HXB2) envelope glycoprotein GP120 peptides G9, G31 without (A) and with (B) Trx tag, as detected by SDS-PAGE. Lane a, insoluble fraction of the cell lysate; lanes b and c, insoluble and soluble fractions of the cleaved fusion protein respectively. The loading volume of the protein samples was 1:2:4 for lanes a, b and c respectively. Lanes s1, s2 and s3, quantification standards consisting of bovine serum albumin (BSA, 66.5 kDa) at 3, 1.5 and 0.75 μg/lane and aprotinin (6.5 kDa) at 1.5, 0.75 and 0.3 μg/lane respectively. The molecular weights of the protein standards M1 and M2 are indicated on the left and right sides respectively.

References

1. Kyte, J. & Doolittle, R. F. A simple method for displaying the hydropathic character of a protein. *J Mol Biol***157**, 105-132 (1982).
2. Xu, W., Han, L. & Lin, Z. Screening of random peptide library of hemagglutinin from pandemic 2009 A(H1N1) influenza virus reveals unexpected antigenically important regions. *PloS one***6**, 1-11 (2011).