Electronic Supplementary Material (ESI) for Analytical Methods.
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Supporting Information

Development of a RPLC-UV Method for Monitoring Uncleaved HIV-1 Envelope Glycoprotein

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$$\%\ uncleaved\ gp140 = \frac{Peak\ area\ _{uncleaved\ gp140}}{Peak\ area\ _{all\ peaks\ (gp120\ +\ uncleaved\ gp140\ +\ gp41)}}\ x\ 100$$

Equation S1 Calculation to determine the percentage of uncleaved gp140 is shown and can be achieved using automatic peak integrations via the LC software for routine analysis and reporting. Peak areas can also be normalized against molecular weight (weighted molar impurity).

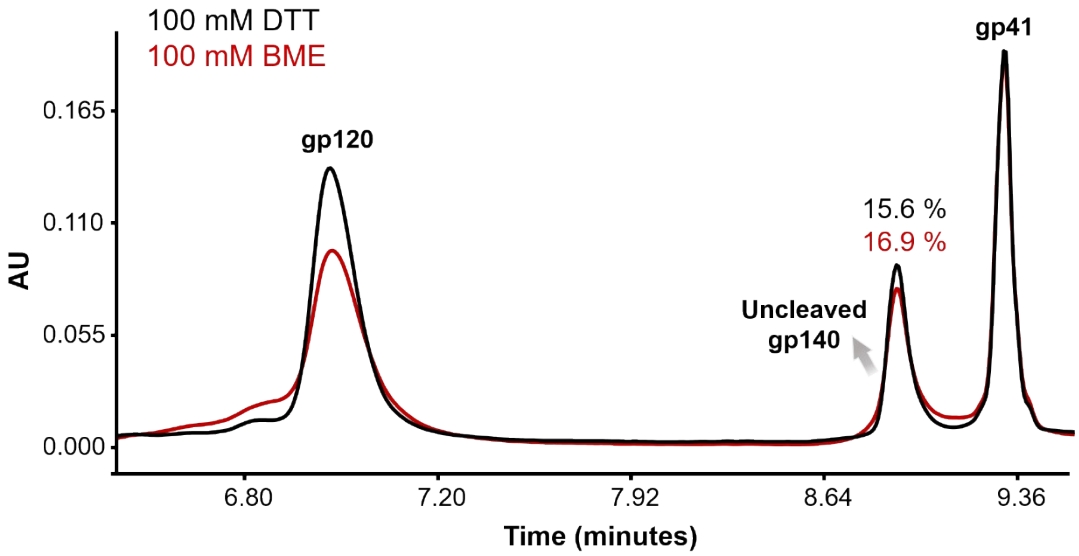


Fig. S1 Normalized overlays of the RPLC-UV profiles of Env incubated at 90°C for 5 min with 100 mM DTT (black trace) or 100 mM BME (red trace).

Table S1 Different concentrations of DTT (50 mM to 400 mM) were incubated with Env trimer developmental material for 5 min at 90°C to evaluate its effect on the % gp140 results.

DTT (mM)	% gp140
50	15.5
100	15.3
200	15.1
400	15.3

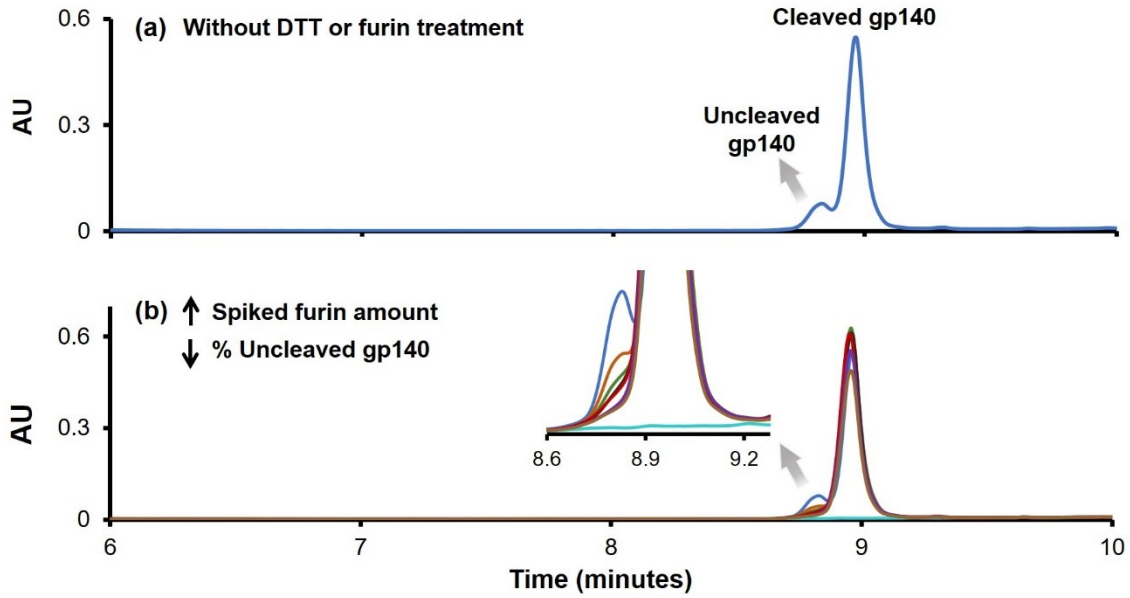


Fig. S2 UV traces of purified Env glycoprotein under non-reduced conditions: (a.) without furin treatment and (b) spiked with varying amounts of furin and incubated for 6 hours at room temperature. 0.5 mg/mL of purified Env sample was incubated with 0 µL (blue), 0.2 units (orange), 0.4 units (green), 1 unit (black), 2 units (red), 10 units (purple), and 20 units (brown) of furin. The teal trace was a negative control of 10 units of furin without Env.

Table S2 Intermediate precision of the RPLC-UV assay over two days: Percent uncleaved gp140 reported for an Env trimer developmental lot using different LC systems on different days.

Sample (n)	LC System A - Day 1 % gp140	LC System A – Day 2 % gp140	LC System B - Day 1 % gp140
1	14.7	15.4	15.2
2	14.5	15.3	14.6
3	14.6	15.4	14.7
4	14.6	15.4	14.7
5	14.7	15.3	14.8
6	14.6	15.3	14.5
AVG (n = 18): 14.9%			
%CV: 2.40%			

Table S3 Intermediate precision of the RPLC-UV assay over a 9-month period: Percent uncleaved gp140 reported for an Env trimer developmental lot that was used as a system suitability control across different analytical runs.

Sample (n)	% gp140								
	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Run 9
1	16.1	16.2	15.4	15.9	16.4	16.5	16.0	15.6	15.9
2	15.9	16.4	15.9	15.6	16.1	16.6	16.0	15.6	16.0
3	N/A	N/A	N/A	15.8	N/A	16.8	16.1	15.6	16.0
4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	15.7	N/A
5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	15.7	N/A
AVG (n = 25): 16.0% %CV: 2.17%									

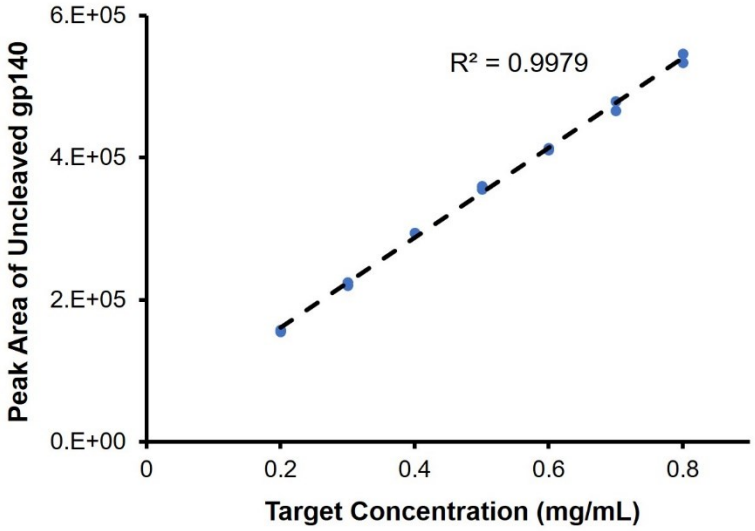
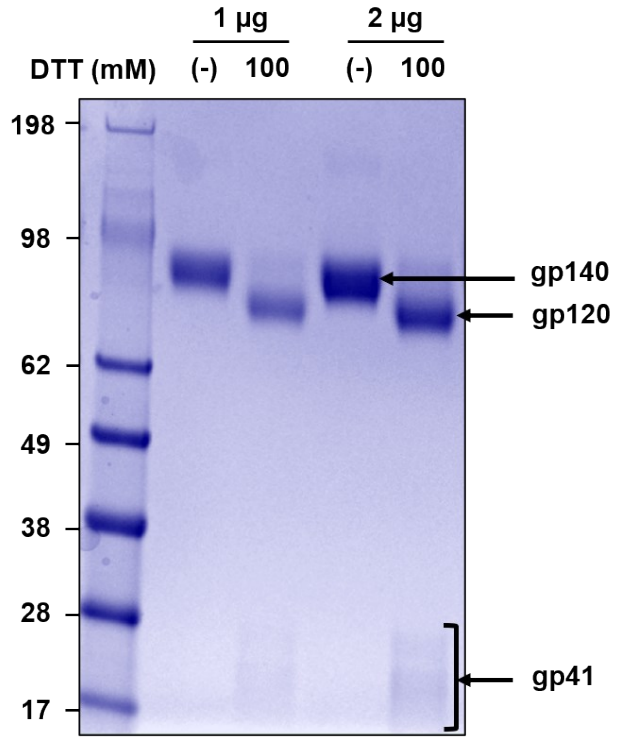


Fig. S3 Uncleaved gp140 peak areas plotted against known sample concentrations.

Table S4 Accuracy assessment: Percent uncleaved gp140 was reported by RPC-UV analysis. The samples marked with asterisks were analyzed three times on three different days with either single or duplicate injections (n = 5).

Expected % Uncleaved gp140	% Average Recovery (n = 2)
2.93	103.5*
4.34	102.5
5.20	104.6
5.76	101.6
8.59	103.3
12.0	102.7
14.3	101.3
19.9	101.7
22.8	101.0
24.2	101.1
25.6	100.4*
AVG: 102%	
%CV: 2.77%	

(a)



(b)

Assay	Loading Amount (µg)	% gp140	% gp120	% gp41
SDS-PAGE	1	17.9	69.5	12.5
	2	15.7	70.1	14.3
RPLC-UV	1	9.4	63.0	27.6

Fig S4. Comparison of SDS-PAGE vs. RPLC-UV analyses. (a) SDS-PAGE analysis of reference lot BG505.DS.SOSIP.664 with and without the presence of 100 mM DTT and incubated at 90°C for 5 min. (b) Comparison of SDS-PAGE and RPLC-UV results for determining the relative percentage of uncleaved gp140.

Experimental protocol for SDS-PAGE analysis: Env trimer sample was mixed with NuPAGE™ LDS sample buffer (4X, Thermo Fisher Scientific) with and without 100 mM DTT and incubated at 90°C for 5 min. Approximately, 1 and 2 µg of Env trimer sample were loaded and separated on a 4 -12% Tris-Glycine gel using 1x MOPS SDS running buffer. SeeBlue™ Plus2 Pre-stained Protein Standard was used as a molecular weight ladder. The protein bands were visualized by staining with GelCode™ Blue Safe Protein Stain (Coomassie-dye reagent). Densitometry measurement was performed using ImageQuant.