Supporting Information

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Fig. S1. Surface-rendered 3D reconstructions of the R-peptide (*Left*), the mature (*Center*), and the L651A mutant Env (*Right*) viruses at full volume (mesh) and at two thresholds of increased density (magenta). Top, side, and bottom views are shown. (Scale bar, 50 Å.)

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162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179
144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161
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54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71
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Fig. S2. Map projections and class averages of the 3D reconstruction of the native R-peptide Env. Map projections (even-numbered images) are shown with the corresponding class averages (odd-numbered images) (*Right*).

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Fig. S3. Map projections and class averages of the 3D reconstruction of the activated intermediate (isomerization-arrested stage; IAS) form of the R-peptide Env. Map projections (even-numbered images) are shown with the corresponding class averages (odd-numbered images) (*Right*).

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54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	7
18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	3

Fig. S4. Map projections and class averages of the 3D reconstruction of the R-peptide Env mutant L651A. Map projections (even-numbered images) are shown with the corresponding class averages (odd-numbered images) (*Right*).



Fig. S5. Asymmetric triangles showing the distribution of images in the classes in the Env reconstructions of the R-peptide Env (*A*), the IAS form of the R-peptide Env (*B*), and the R-peptide L651A Env mutant (*C*), as displayed by EMAN (http://blake.bcm.edu/emanwiki/EMAN/). The grayscale shows the number of images per class. The number of particles included in the datasets and the resolution achieved (Å) are shown below each triangle.



Fig. S6. Fourier shell correlation plots for final maps of the R-peptide Env, the IAS R-peptide Env, and the R-peptide L651A Env mutant. The resolution of the final reconstruction was determined using a 0.5 cutoff of the Fourier shell correlation between the "odd" and "even" image reconstructions. The resolutions were estimated to 21, 22, and 22 Å, respectively.

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