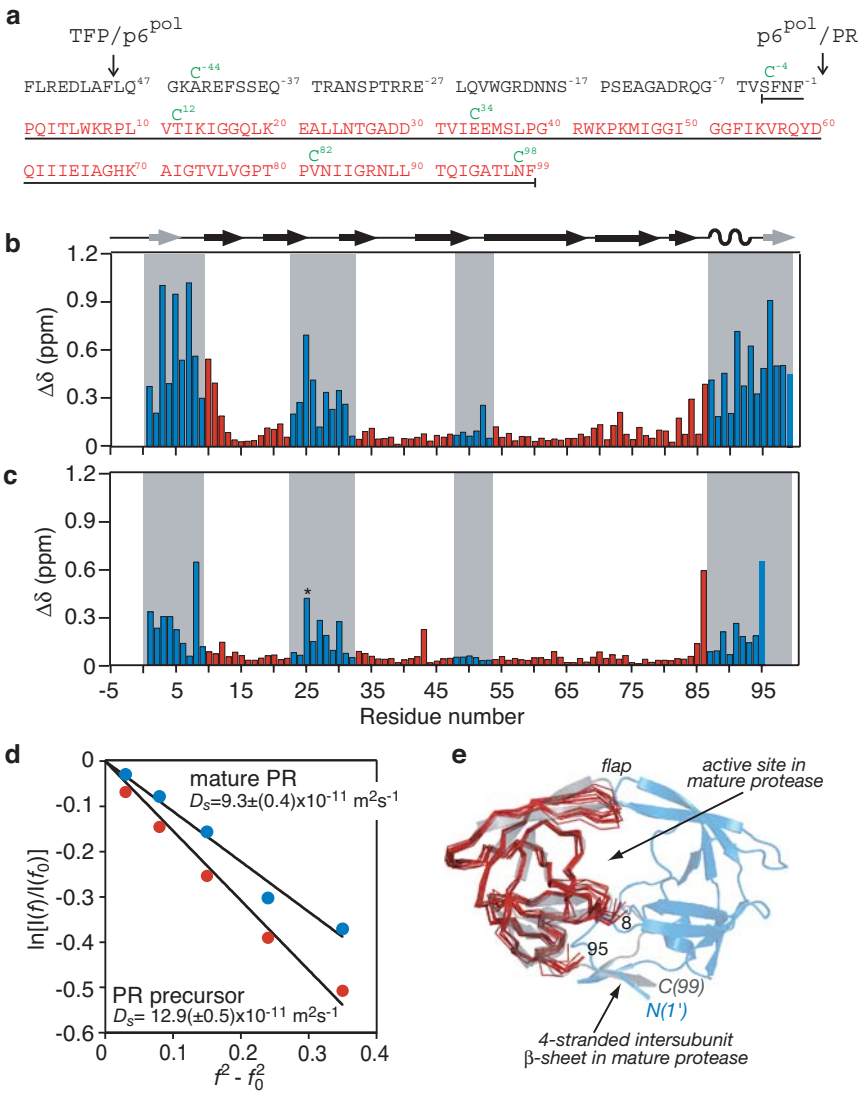

Figure S1 Monomeric structure of the $^{SFNF}PR_{D25N}$ protease mini-precursor. a, Amino acid sequence of the full-length transframe region(TFR)-protease precursor within the Gag-Pol polyprotein of HIV-1. The TFR (in black) comprises two domains, the conserved transframe octapeptide (TFP) followed by the 48 residue p6pol. The protease cleavage sites (arrows) and the substituted (A-44C, T12C, E21C, V82C, N98C) or inserted (-4C) mutation sites for conjugation with the paramagnetic spin-label are indicated in green. The 99 amino acid sequence of the mature D25N protease is shown in red. Underlined in black is the $^{SFNF}PR_{D25N}$ mini-precursor used extensively in the current work. **b,c** Normalized chemical shift difference Δδ (given by $[\Delta\delta^2_{HN} + \Delta\delta^2_N/25 + \Delta\delta^2_{C\alpha}/4]^{1/2}$) relative to **b** the mature PR_{D25N} dimer and **c** the PR_{D25}(1-95) monomer.[5] Residues that are buried at the dimer interface in mature protease are colored in blue and delineated by the grey shaded areas; the asterisk in **c** indicates the site of the D25N mutation; and a schematic of the secondary structure elements in the mature protease is shown above panel **b**. **d**, Translational diffusion measurements for mature PR_{D25N} dimer and the $^{SFNF}PR_{D25N}$ mini-precursor at a subunit concentration of 0.4 mM by pulse field gradient NMR (f and f_0 are the fractional gradient strengths, and $I(f)$ and $I(f_0)$ are the corresponding NMR signal intensities, where the subscript 0 refers to the reference spectrum). The ratio of the two D_s values (0.72±0.04) is fully consistent with the expected value of 0.75 for a $D_s^{dimer}/D_s^{monomer}$ ratio (Full methods, ref. 28), placing an upper limit of about 10% for the population of dimeric species. **e,** Cα traces (red) of the 10 lowest energy structures for the $^{SFNF}PR_{D25N}$ monomer (residues 8-95) computed from chemical shifts using CS-Rosetta superimposed on one subunits (grey ribbon) of the mature protease dimer (main text, ref. 23). The other subunit of the mature protease dimer is shown in light blue.

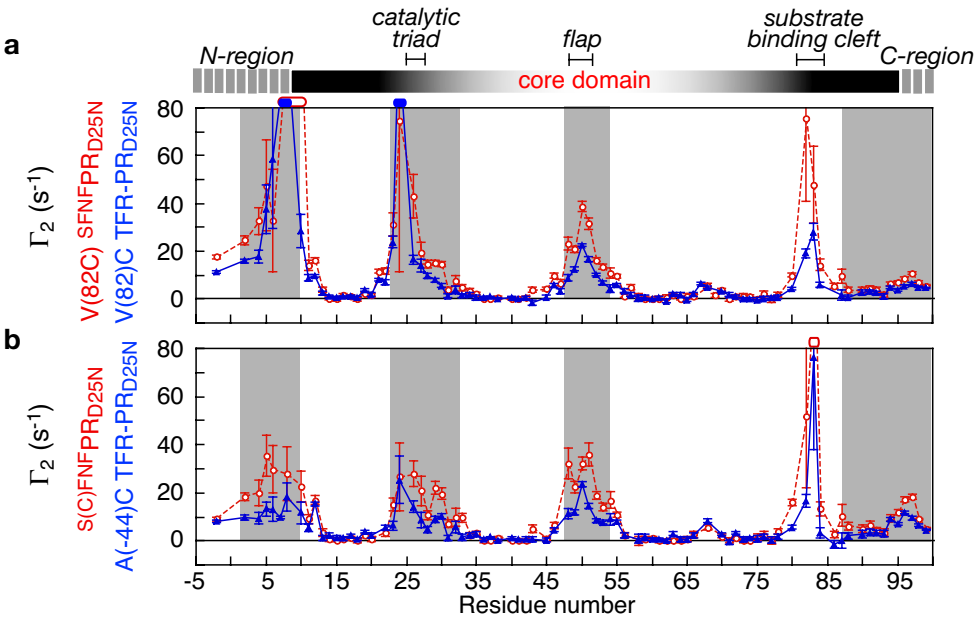

Figure S2. Comparison of intermolecular PREs profiles observed for full length TFR-PR$_{D25N}$ and the mini-precursor (^{SFNF}PR$_{D25N}$ or $^{S(C)FNF}$PR$_{D25N}$). a, Intermolecular PRE profiles from V82C spin-labeled full length TFR-PR$_{D25N}$ (blue triangles) and ^{SFNF}PR$_{D25N}$ (red open circles) at natural isotopic abundance to U-[^{2}H/^{13}C/^{15}N]-^{SFNF}PR$_{D25N}$. **b,** Intermolecular PRE profiles from A(-44)C spin-labeled full length TFR-PR$_{D25N}$ (blue triangles) and from N-terminal spin-labeled $^{S(C)FNF}$PR$_{D25N}$ (red open circles) to U-[^{2}H/^{13}C/^{15}N]-^{SFNF}PR$_{D25N}$. In each case the samples comprise a 1:1 mixture of spin-labeled and isotope labeled components, each at a concentration of ~0.2 mM. In (**a**) the PRE profiles observed from V82C spin-labeled full-length TFR-PR$_{D25N}$ and ^{SFNF}PR$_{D25N}$ are very similar indicating that the same interface for transient dimerization (in the form of encounter complexes) is employed. Likewise in (**b**) the PRE profiles observed from A(-44)C spin-labeled, full length TFR-PR$_{D25N}$ and from N-terminal spin-labeled $^{S(C)FNF}$PR$_{D25N}$ are very similar indicating that the N-terminal residues of TFR-PR$_{D25N}$, including the TFP-p6^{Pol} cleavage site located between residues -48 and -49 (see main text Fig. 1a, and Supplementary Fig. S1a), can insert themselves into the active site of the partner ^{SFNF}PR$_{D25N}$ mini-precursor in the encounter complex ensemble. The slightly lower intensities of the PRE profiles observed for full length TFR-PR$_{D25N}$ relative to the ^{SFNF}PR$_{D25N}$ mini-precursor reflect a decrease in the population of transient TFR-PR$_{D25N}$/^{SFNF}PR$_{D25N}$ dimeric species relative to that of the ^{SFNF}PRD$_{25N}$/^{SFNF}PR$_{D25N}$ transient dimer species. This is consistent with previous NMR data that showed that the protease inhibitor DMP323 was more effective at promoting dimerization of ^{SFNF}PR$_{D25N}$ than of TFR-PR$_{D25N}$ (ref. 5, main text). Residues broadened beyond detection are denoted by open bars. Error bars represent 1 s.d.

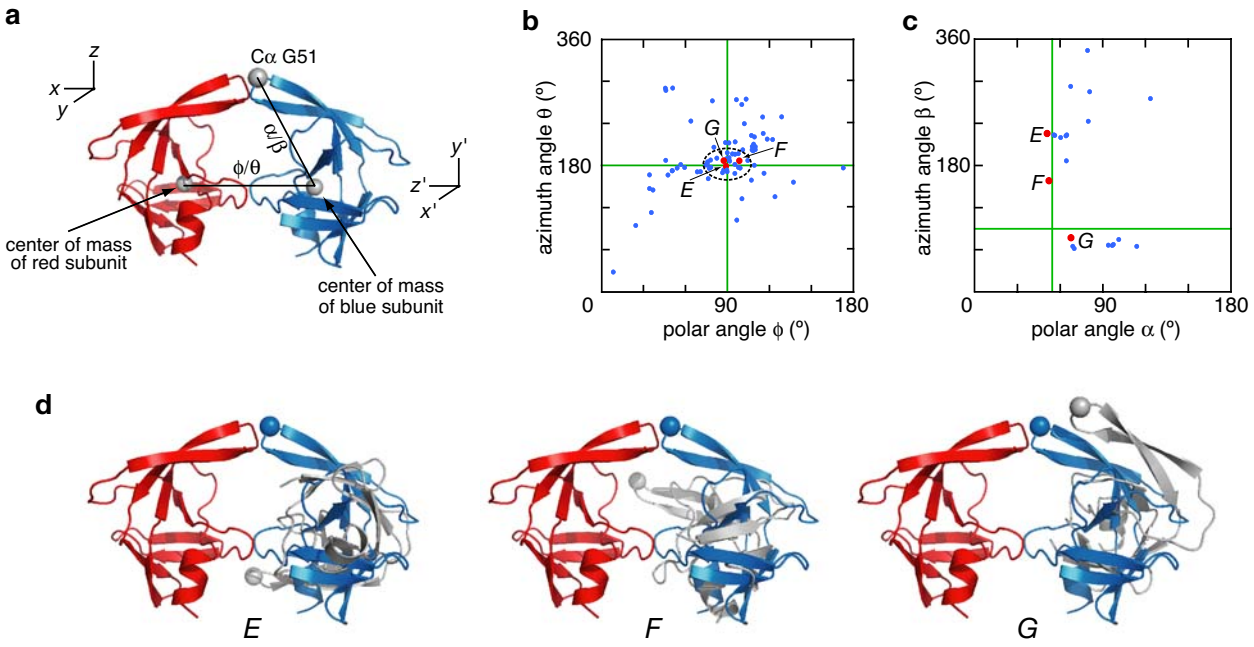

Figure S3. Relative orientation of subunits in the $^{SFNF}PR_{D25N}$ mini-precursor encounter complex ensembles. a, Definition of the two polar coordinate systems. The first (polar angle ϕ and azimuth angle θ) describes the orientation of the vector joining the center of masses of the two subunits (shown as grey spheres) to an external axis system with the *z* axis corresponding to the C_2 symmetry axis of the mature dimer. The second (polar angle α and azimuth angle β) describes the orientation of a vector joining the center of mass of the second subunit to an arbitrarily chosen atom of the same subunit (Cα atom of Gly51) relative to an axis system with the *z′* axis given by the vector joining the center of masses of the two subunits (with the red subunit corresponding to the fixed reference subunit). **b,** ϕ,θ spherical angles for the encounter complexes observed in the N_e = 4 ensembles calculated at a population of 5% heterodimer (20 x 4 structures). The ϕ,θ spherical angles for the mature HIV-1 protease dimer are located at the green cross-hairs. The dashed circle delineates the encounter complexes with ϕ,θ angles close to that of the mature dimer. The structures labeled *E*, *F* and *G* (red dots) correspond to the structures with the closest ϕ,θ angles, the lowest DRMS and the lowest atomic rms displacement, respectively, relative to the mature dimer. **c,** α,β spherical angles for the encounter complexes lying within the dashed circle shown in **b.** The red dots correspond to the points labeled *E*, *F* and *G* in **b**. The α,β spherical angles for the mature HIV-1 protease dimer are located at the green cross-hairs. **d,** Ribbon diagrams of the encounter complexes corresponding to the points labeled *E*, *F* and *G* in **b** and **c**. The isotopically-labeled and spin-labeled subunits in the encounter complexes are shown in red and grey, respectively, and the blue subunit corresponds to the orientation relative to the red subunit seen in the mature dimer. The position of the Cα atom of Gly51 at the tip of the flap of the grey and blue subunits is shown as a sphere.

Table S1 Cα atomic rms displacement, DRMS and spherical angles for the $^{SFNF}PR_{D25N}$ encounter complexes labeled *E*, *F* and *G* in Fig. S3 (as well as in Fig. 2d).

Structure	Cα RMS[1] (Å)	DRMS[2] (Å)	ϕ (°)	θ (°)	α (°)	β (°)
E	16.7	3.8	89.0	179.0	50.8	225.7
F	10.4	2.6	98.5	186.8	51.9	158.5
G	10.1	4.7	87.4	186.9	67.6	77.1
Mature dimer			90.0	180.0	54.6	90.0

[1]The Cα atomic rms displacement relates to the rms displacement between the spin-labeled subunit in the precursor encounter complex (grey subunit in Fig. S3d) and its position in the mature protease (blue subunit in Fig. S3d).
[2]DRMS is given by (Full methods, ref. 40):

$$DRMS = \frac{1}{N}\sum_{i,j} | d_{i,j}^{precursor} - d_{i,j}^{mature} |$$

where N is the number of distinct residue pairs (i, j), and $d_{i,j}^{precursor}$ and $d_{i,j}^{mature}$ are the distance matrices in a calculated precursor encounter complex structure and the mature HIV-1 protease dimer structure, respectively.