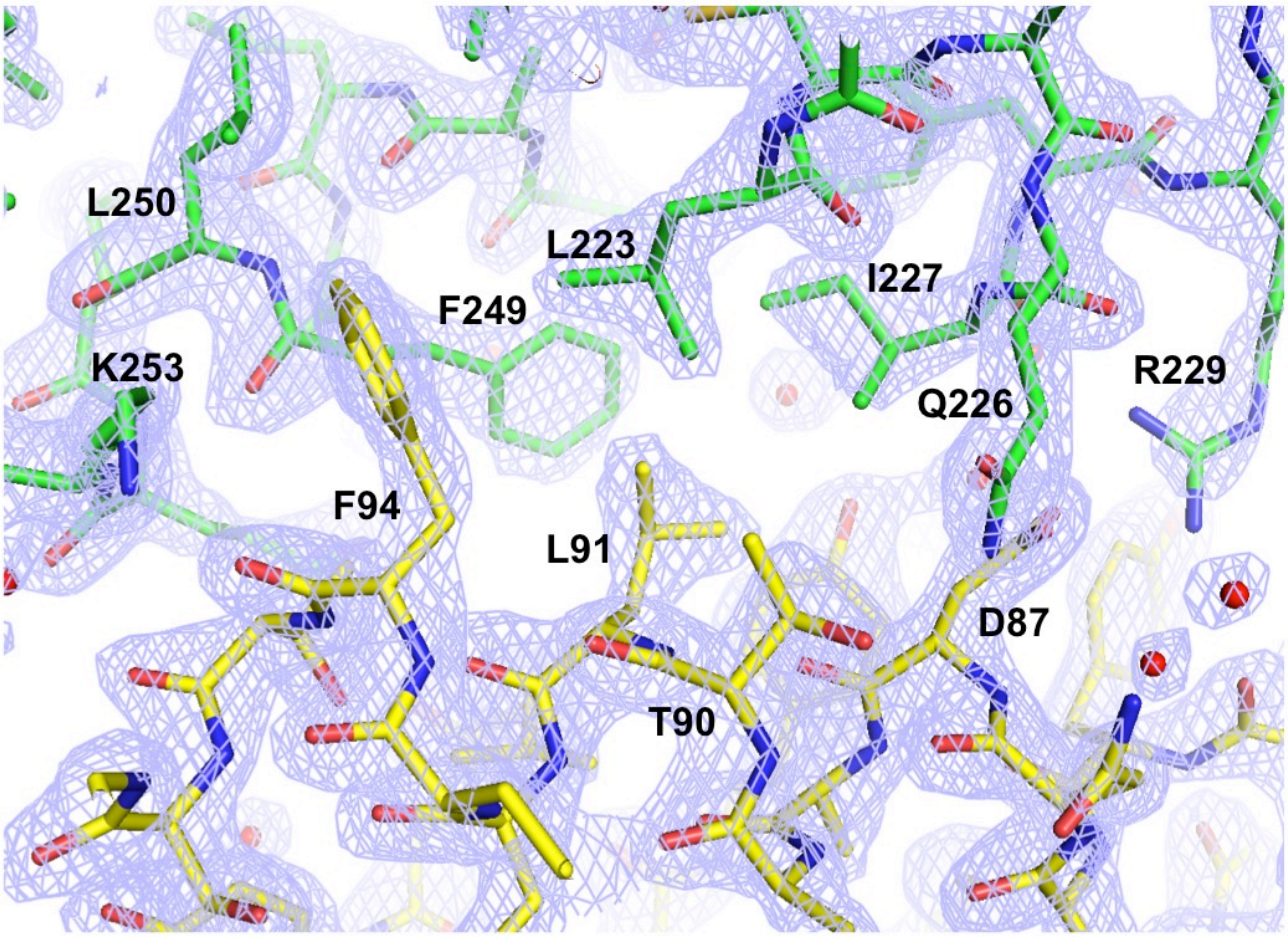

Fig. S1. Electron density for the complex. The 2Fo-Fc map, calculated at 2.30 Å resolution with phases from the final model, is shown as a blue mesh, contoured at 1.8 sigma. Carbon bonds for λ Exo and Redβ CTD are shown in yellow and green, respectively (bonds for nitrogen and oxygen are shown in blue and red, respectively). Key residues of each protein at the interface are labeled.

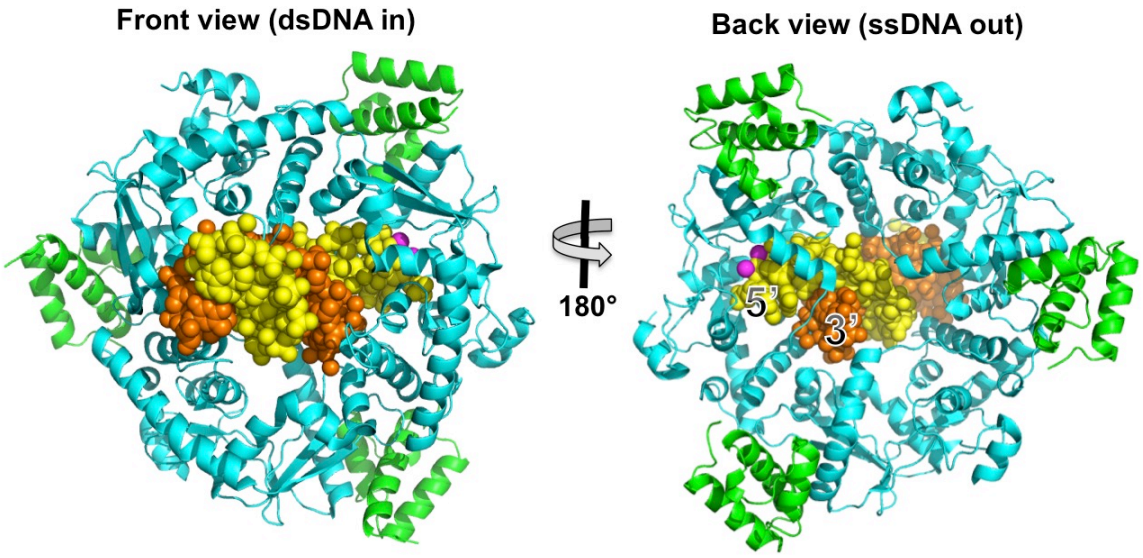

Fig. S2. Positions of the three Redβ C-terminal domains relative to the DNA substrate on the λ Exo trimer. The three Redβ CTDs from the structure determined here were superimposed onto the structure of the λ Exo trimer in complex with DNA (PDB code 3SM4, 39). The DNA is shown with the 5'-strand that is cleaved in yellow and the 3'-strand that will form the overhang in orange (labeled at right). The two-active site Mg^{2+} ions are shown in magenta. The view on the left shows the front of the λ Exo trimer, the side at which the dsDNA substrate would enter. The view on the right shows the back, the side from which the nascent 3'-overhang would emerge.

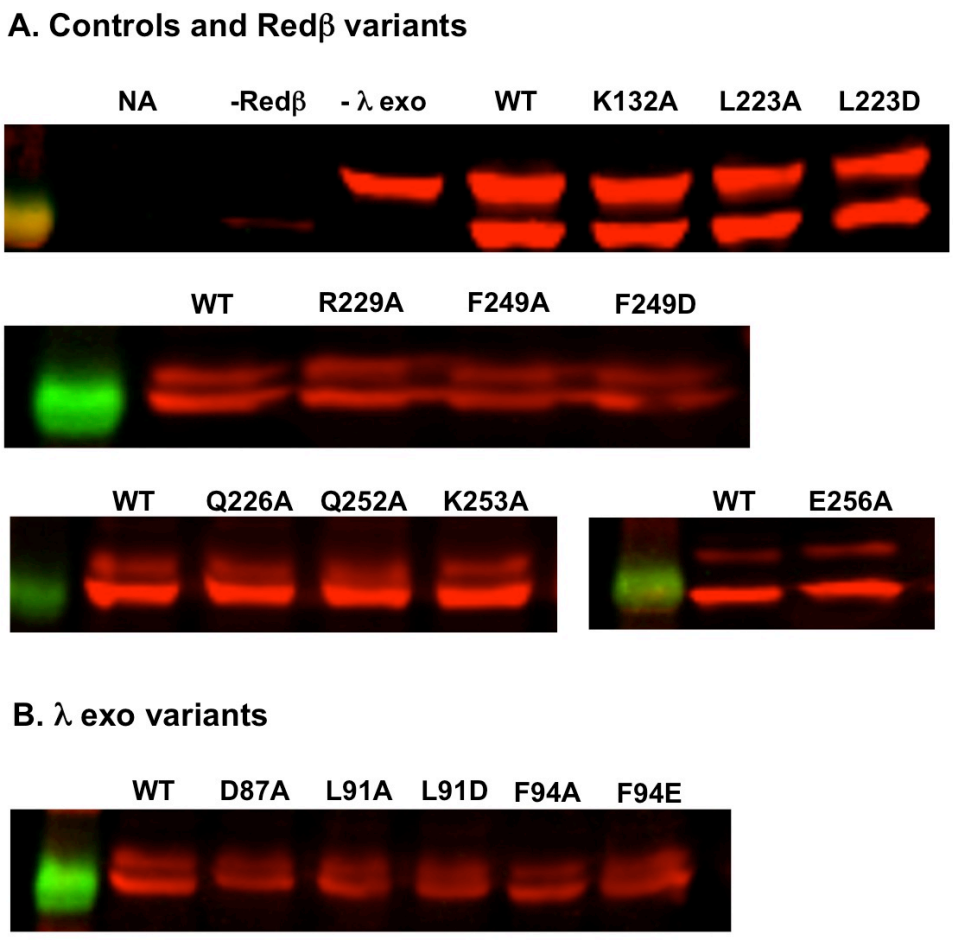

Fig. S3. Western blot analysis of λ Exo and Redβ protein expression in the cells used for *in vivo* ssDNA recombination assays. Protein expression was induced in GB2005 cells using procedures identical to that used for ssDNA recombination experiments, except that the culture volume was 50 ml instead of 1.2 ml. Cell lysates were prepared by sonication, and the soluble portion of the lysate after centrifugation was run on a 13.5 % SDS-PAGE gel, blotted onto nitrocellulose, incubated with a polyclonal antibodies raised against both proteins, λ exo and Redβ. The very top panel shows controls for no arabinose induction (NA), and the Redβ and λ Exo deletions. The left two lanes of each gel show a molecular weight marker (25 kDa, green band) followed by a sample of the WT proteins. Notice that the expression levels of both proteins for all of the protein variants are similar to WT.

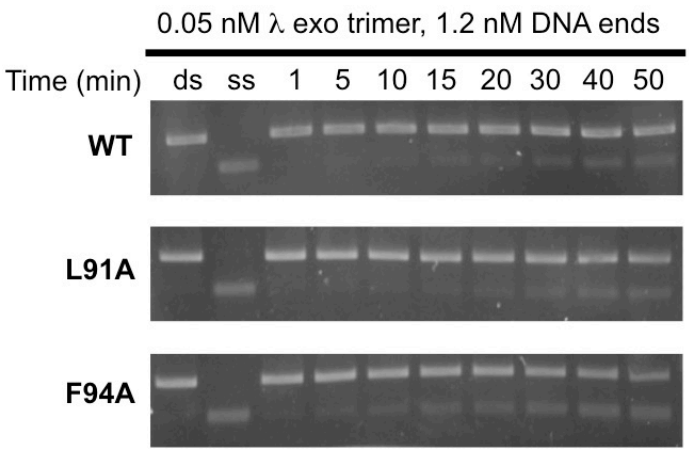

Fig. S4. The L91A and F94A variants of λ Exo that are defective for binding Redβ and for dsDNA recombination are fully active as exonuclease enzymes. The 2 kB linear pUC19 DNA substrate was incubated with a limiting amount of λ Exo trimer for the indicated timepoints, electrophoresed on a 0.8% agarose gel, and stained with SYBR Gold, which stains both ds and ssDNA. The appearance of a band for ssDNA product, due to processive cleavage of the opposing strand, is seen to similar extents for all three proteins, indicating that the mutations do not affect nuclease activity. The first two lanes on each gel show controls for the dsDNA substrate and ssDNA product.

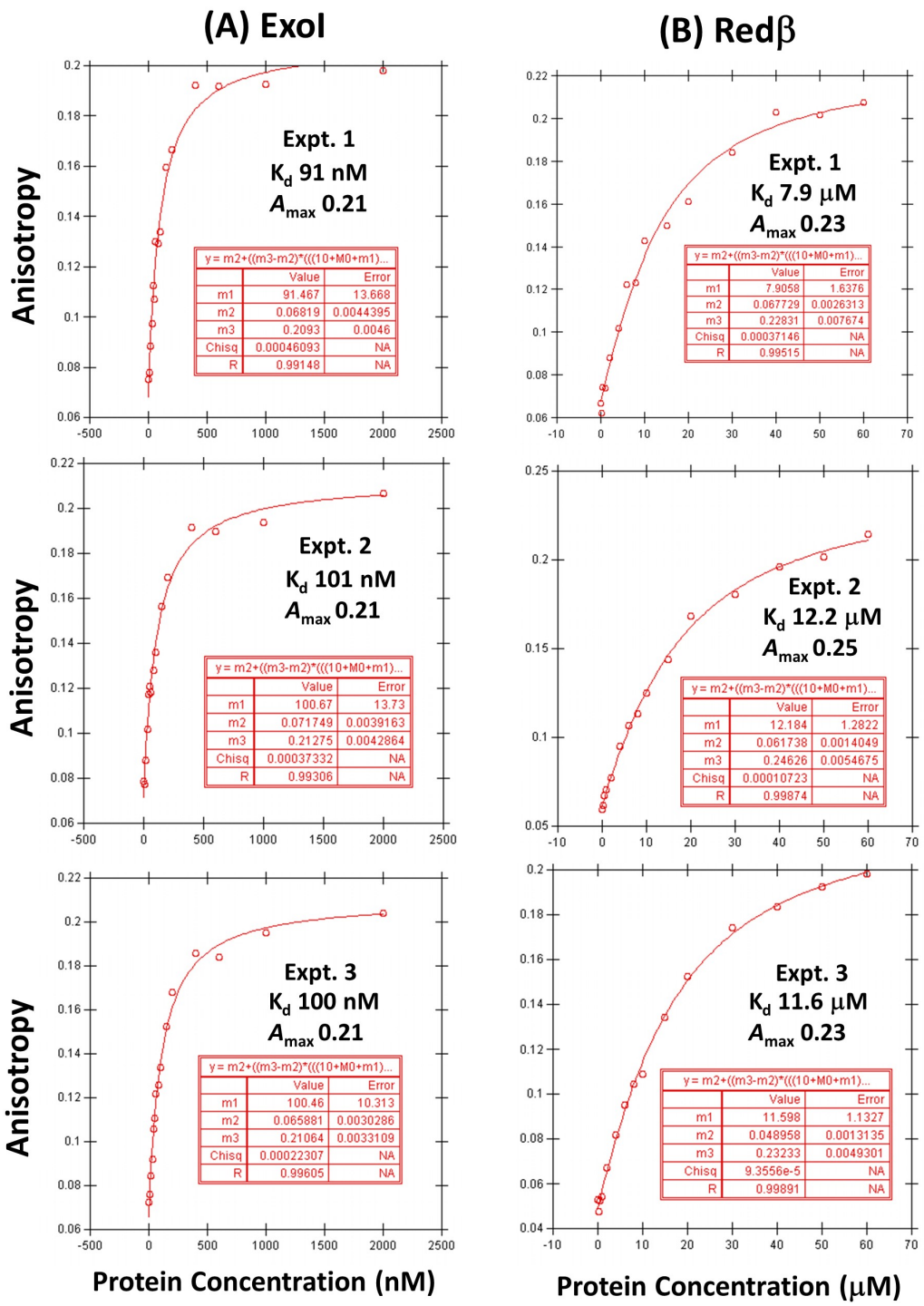

Fig. S5. Fluorescence anisotropy titrations of ExoI (A) and Redβ (B) into the FAM-labeled SSB-Ct peptide. The plots show fits of the anisotropy data to a 1:1 binding model in KaleidaGraph as described in Materials and Methods, where m1 is the fitted K_d, m2 is the minimum anisotropy (A_{min}), and m3 is the maximum anisotropy (A_{max}). Three separate individual experiments are shown for each protein, yielding apparent dissociation constants of 98 ± 5 nM for ExoI, and 10.6 ± 2.3 μM for Redβ.

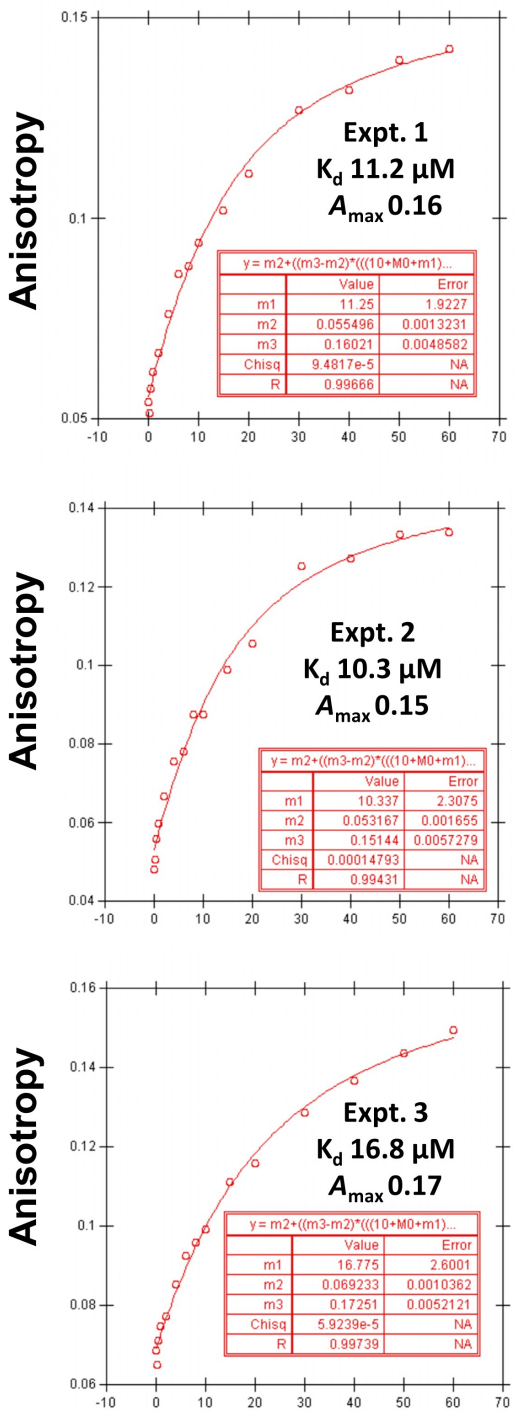

Fig. S6. Fluorescence anisotropy titrations of the λ Exo – Redβ complex into the FAM-labeled SSB-Ct peptide. The plots show fits of the anisotropy data to a 1:1 binding model in KaleidaGraph as described in Materials and Methods, with parameters defined as in Figure S5. Three separate individual experiments are shown, yielding an apparent dissociation constant of 12.8 ± 3.5 µM.

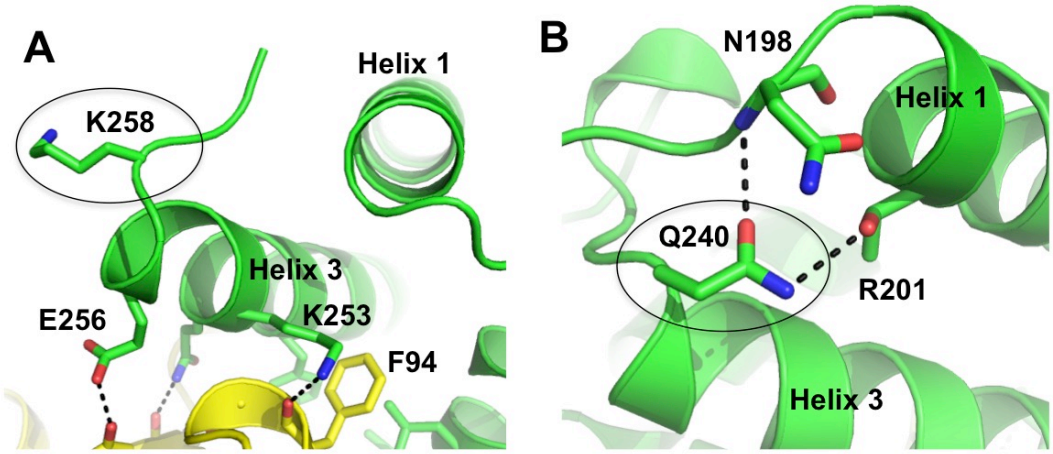

Fig. S7. Interactions of Lys-258 and Gln-240. These two residues were previously implicated as being at the interface with λ Exo (22), but in the structure they are not. (A) Lys-258 is near the very C-terminal end of the CTD, pointing out into solvent. (B) Gln-240 is not near the interface with λ Exo, but could stabilize the folding of the CTD by forming hydrogen bonds that bridge helices 1 and 3. Glu-191, another residue implicated in this study (22) is not shown in these figures, as it is part of the disordered N-terminal region of the CTD (residues 183-194) that is not visible in the structure.

Table S1. Data collection and refinement statistics*.

Data Collection	
Space Group	P3(1)21
Cell Dimensions	
a,b,c, Å	122.5, 122.5, 147.9
α,β,γ, °	90, 90, 120
Resolution, Å	106.1–2.30 (2.34–2.30)
R_{merge}	0.13 (58.0)
$I/\sigma I$	8.6 (1.6)
Completeness, %	99.7 (99.1)
Redundancy	3.9 (2.3)
Refinement	
Resolution, Å	106.1–2.30
No. reflections	54,662
R_{work}/R_{free}	0.195/0.253
No. atoms	
Protein	6889
Ligand/ion	80
Water	604
B factors, Å^2	
Protein	32.4
Ligand/ion	76.5
Water	31.6
rmsd	
Bond lengths, Å	0.009
Bond angles, °	1.27
Ramachandran	
Favored	837 (97.2%)
Allowed	19 (2.2%)
Outlier	5 (0.60%)

*Diffraction data were collected from a single crystal.

Table S2. Atomic interactions at the λ Exo – Redβ CTD interface. The distances between the indicated atoms in the three independently determined interfaces in the crystal structure (chain A of λ Exo with chain D of Redβ CTD, and similarly for B with E and C with F) are given in Å.

Redβ CTD	**λ exo**	**A-D**	**B-E**	**C-F**
Hydrophobic				
Leu-223 CD1	Thr-90 CG2	3.7	3.5	3.7
Phe-294 CZ	Leu-91 CD2	3.9	4.0	4.4
Leu-223 CD1	Leu-91 CD1	3.8	3.5	4.0
Ile-227 CG2	Leu-91 CD2	3.9	4.3	3.8
Ile-227 CD1	Leu-91 CD1	4.1	4.0	4.5
Leu-250 CD2	Phe-94 CZ	3.7	3.8	3.7
Leu-223 CD2	Phe-94 CE2	3.8	3.9	4.2
Lys-253 CD	Phe-94 CZ	3.6	3.7	3.8
Gln-252 CB	Thr-95 CG2	3.9	4.2	3.9
Electrostatic				
Gln-226 NE2	Thr-90 OG1	2.9	2.9	2.9
Gln-226 NE2	Asp-87 OD1	3.0	2.9	2.9
Gln-226 OE1	Thr-90 OG1	3.5	3.4	3.4
Arg-229 NH1	Gln83 OE1	2.5	2.6	2.7
Arg-229 NH2	Asp-87 OD2	2.7	3.1	3.0
Arg-229 NH2	Gln-83 (C=O)	3.0	2.9	2.8
Arg-229 NH2	Gln-83 OE1	3.5	3.3	3.8
Arg-229 NE	Asp87 OD2	3.1	3.3	3.1
Gln-252 NE2	Leu-184 (C=O)	2.9	2.7	2.9
Lys-253 NZ	Phe-94 (C=O)	2.9	2.8	2.9
Glu-256 OE1	Try-186 OH	2.5	3.4	3.7