Supplemental data

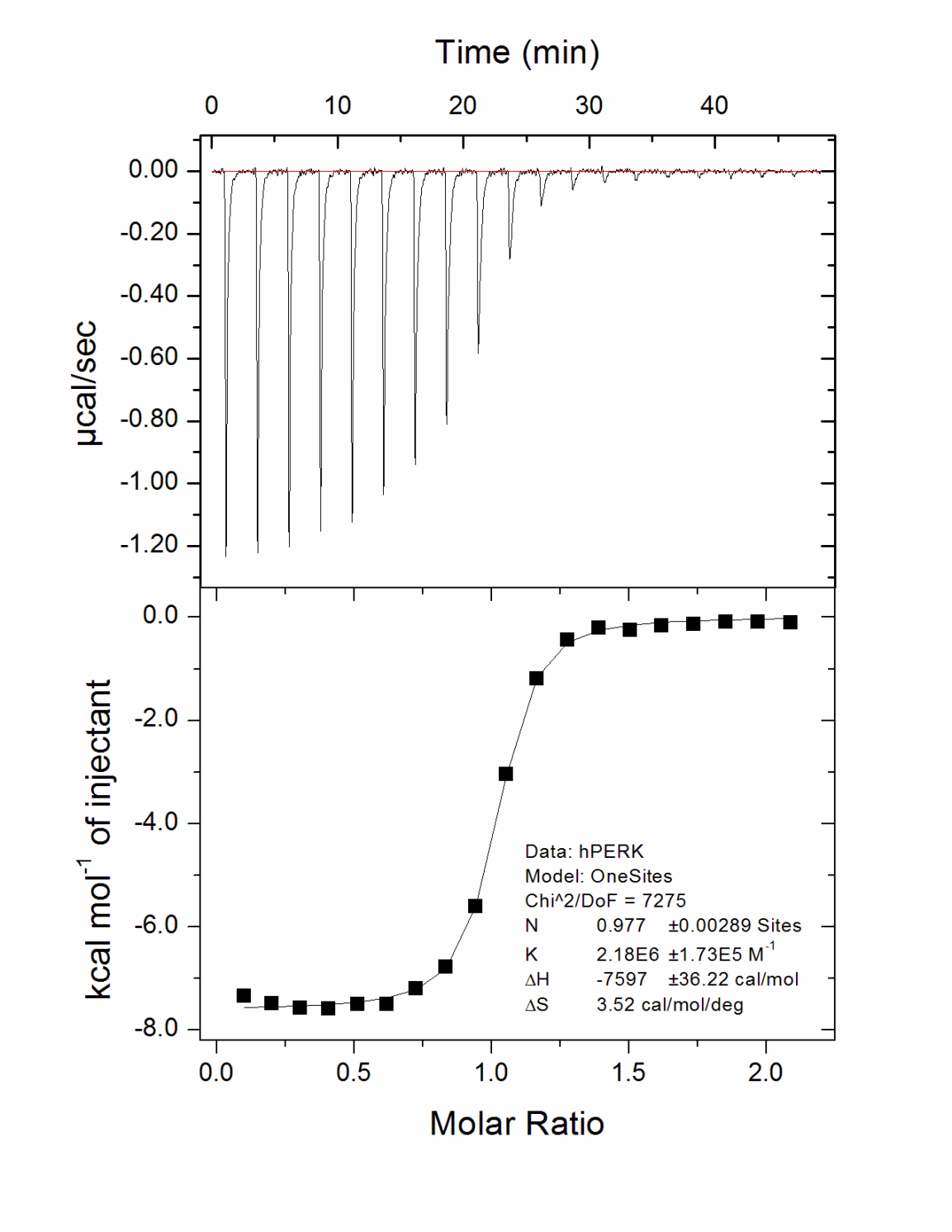

Fig. S1a

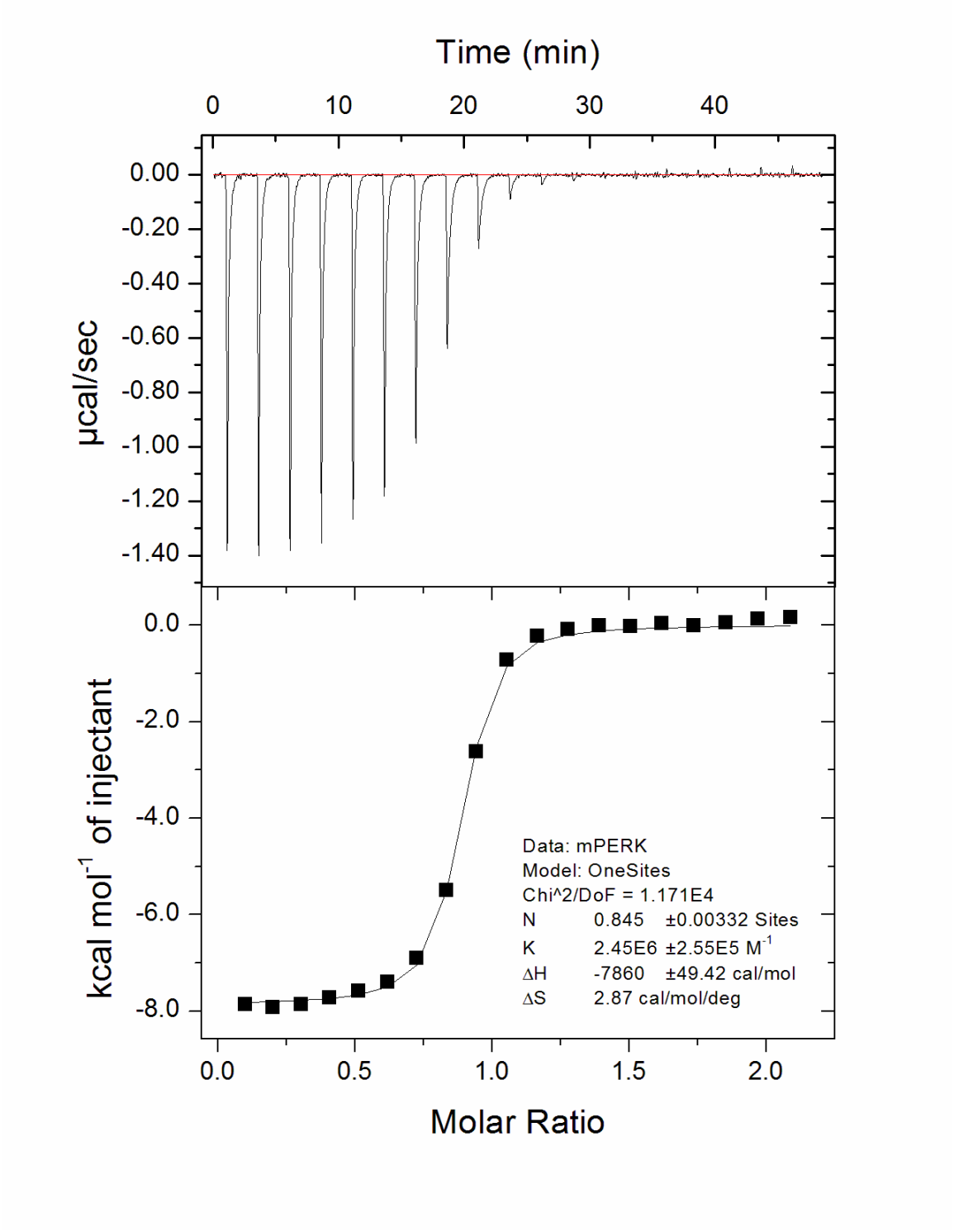
Time (min)
0
10
20
30
40
0.00
-0.20
-0.40
-0.60
-0.80
-1.00
-1.20
-1.40
µcal/sec
0.0
-2.0
-4.0
-6.0
-8.0
kcal mol-1 of injectant
Data: mPERK
Model: OneSites
Chi^2/DoF = 1.171E4
N 0.845 ±0.00332 Sites
K 2.45E6 ±2.55E5 M-1
ΔH -7860 ±49.42 cal/mol
ΔS 2.87 cal/mol/deg
0.0
0.5
1.0
1.5
2.0
Molar Ratio

Fig. S1b

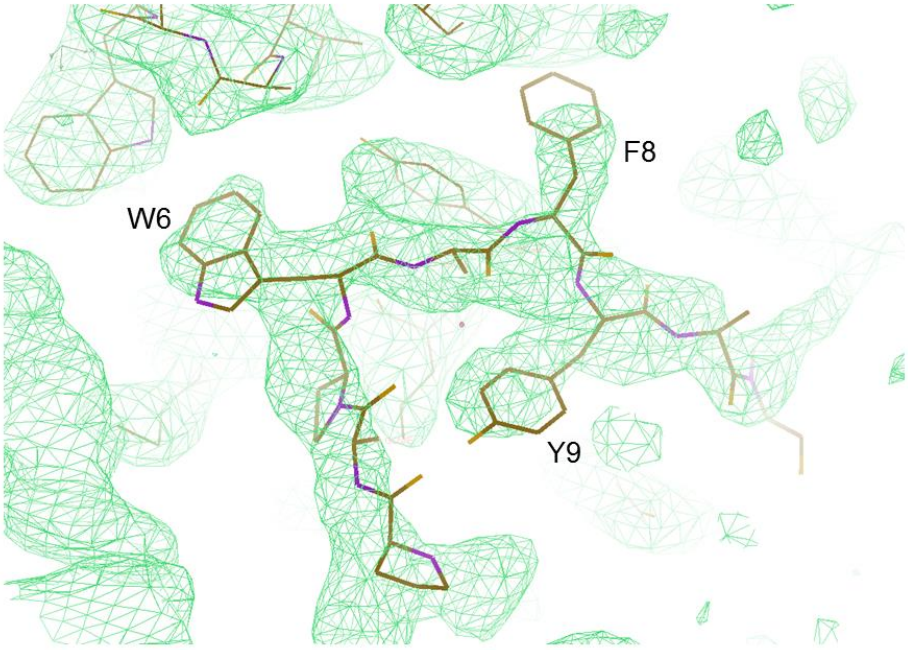

Fig. S2a

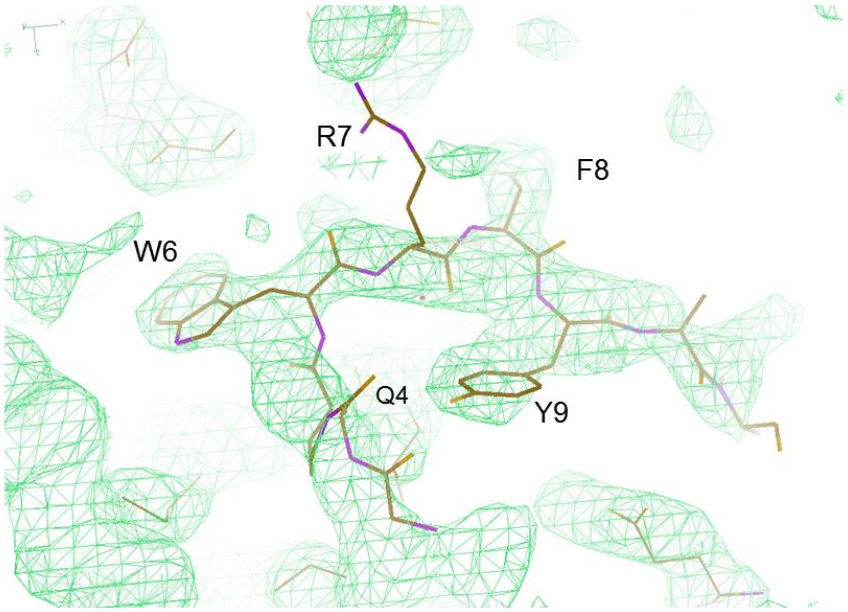

Fig. S2b

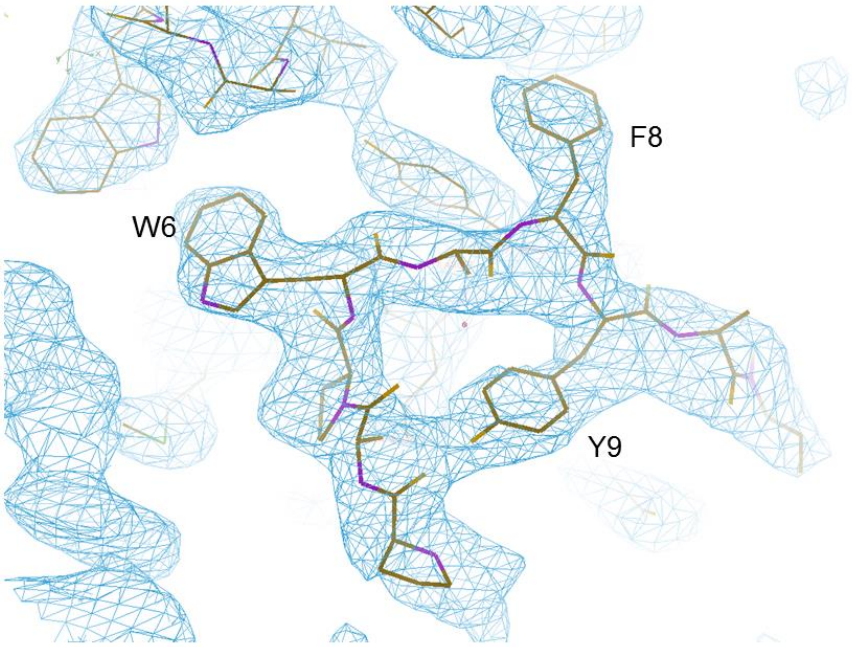

Fig. S2c

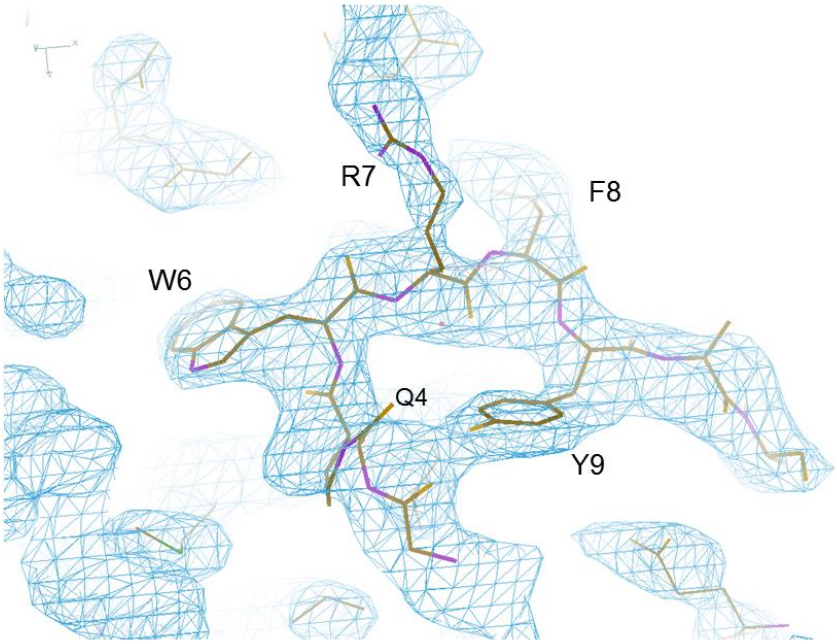

Fig. S2d

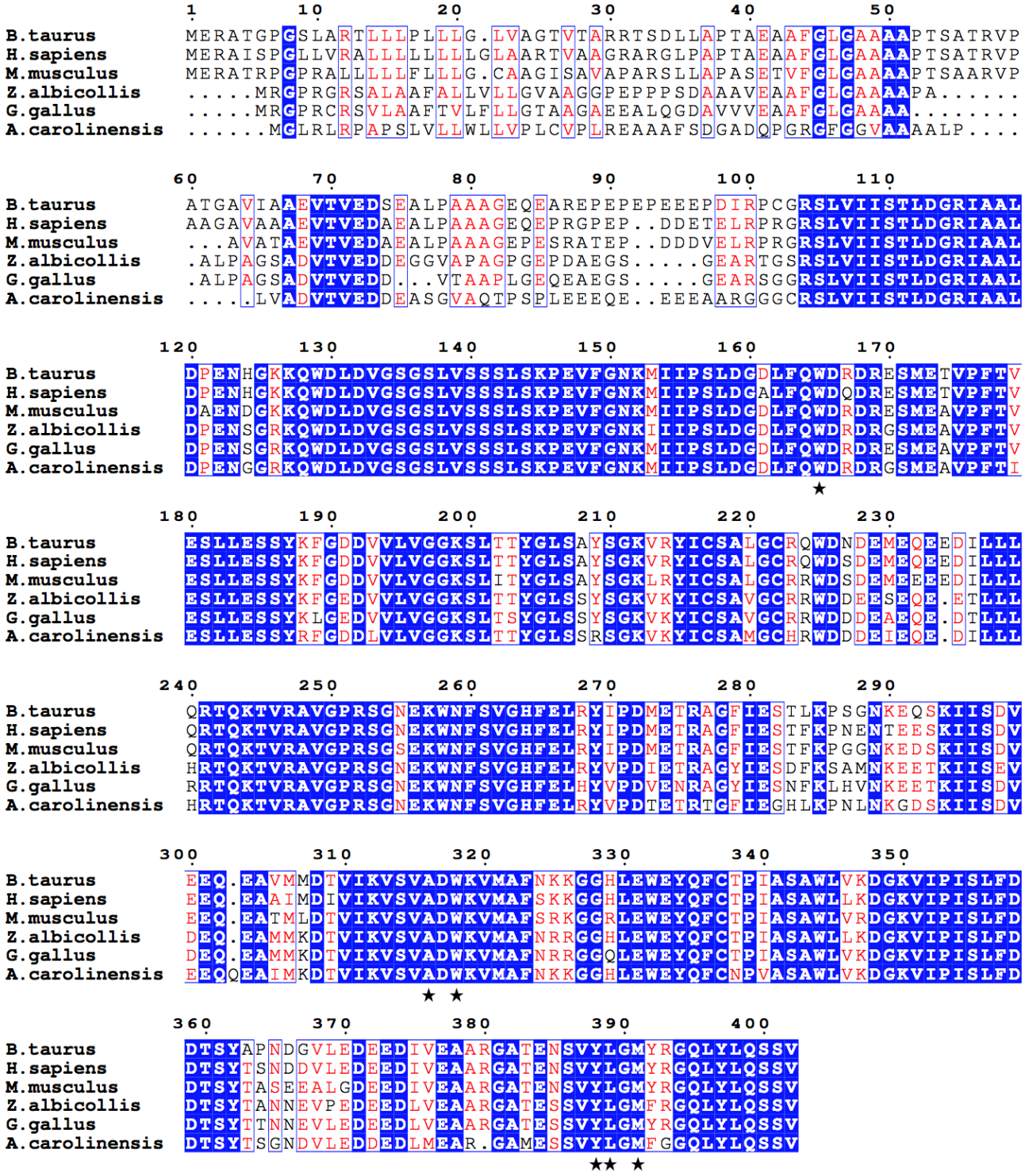
B.taurus
H.sapiens
M.musculus
Z.albicollis
G.gallus
A.carolinensis

Fig. S3

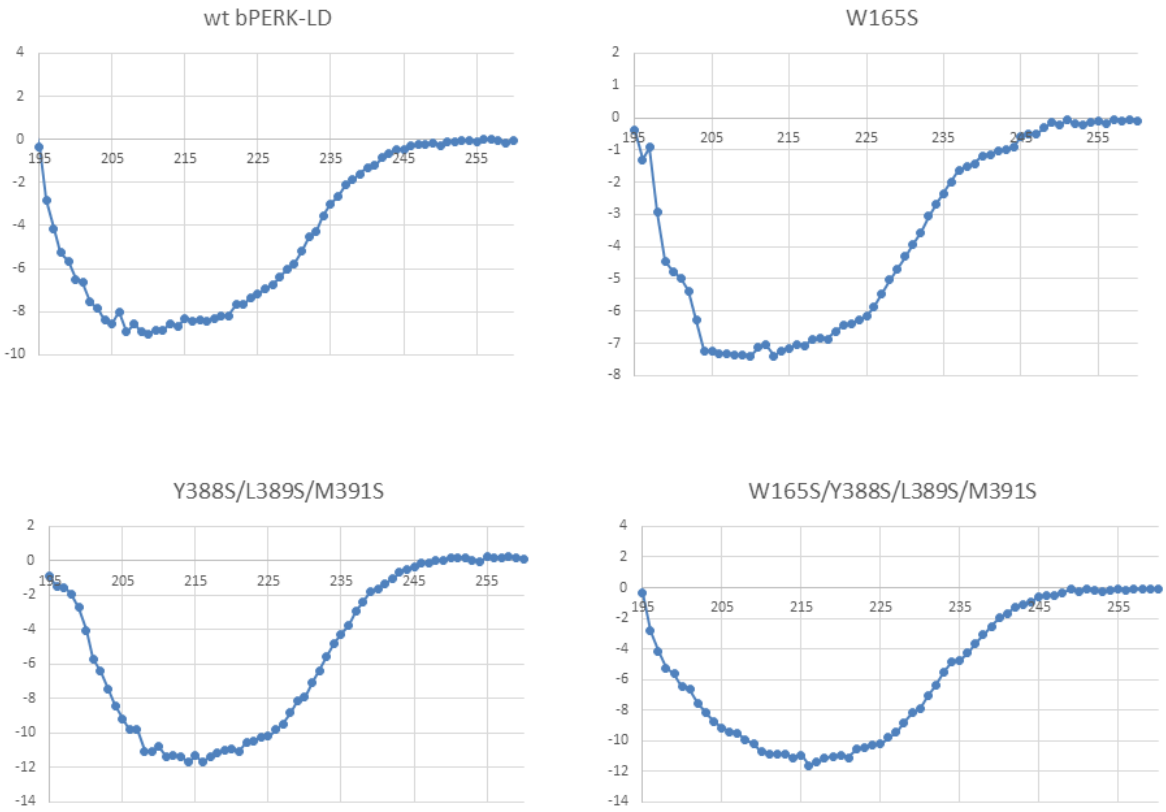

Fig. S4

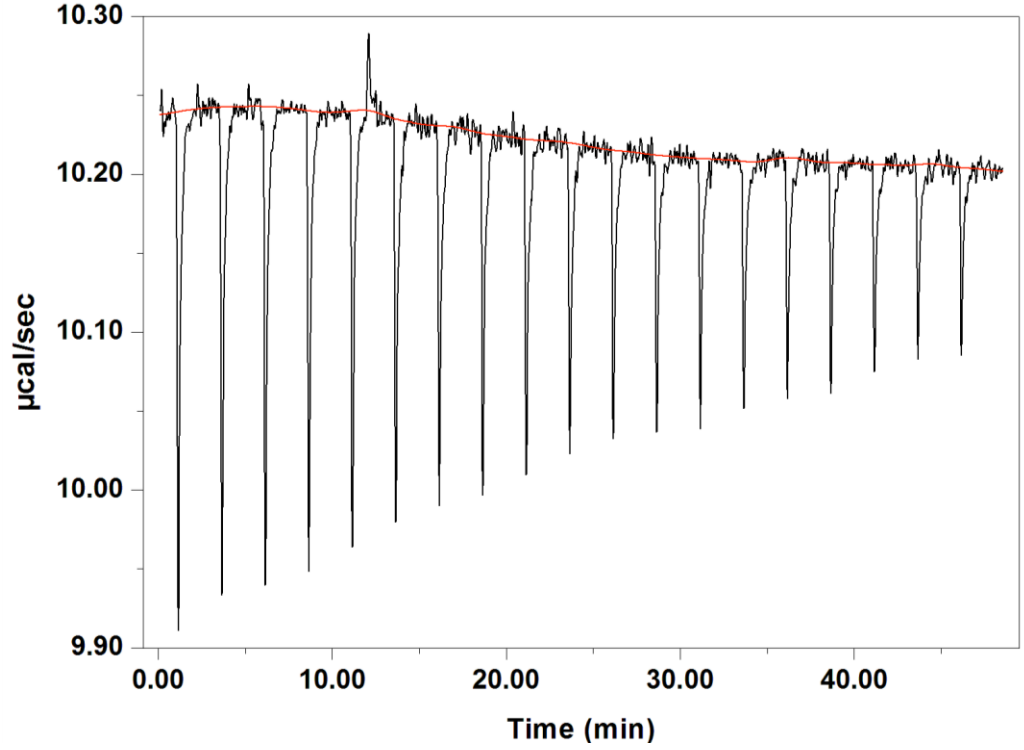

Fig. S5a

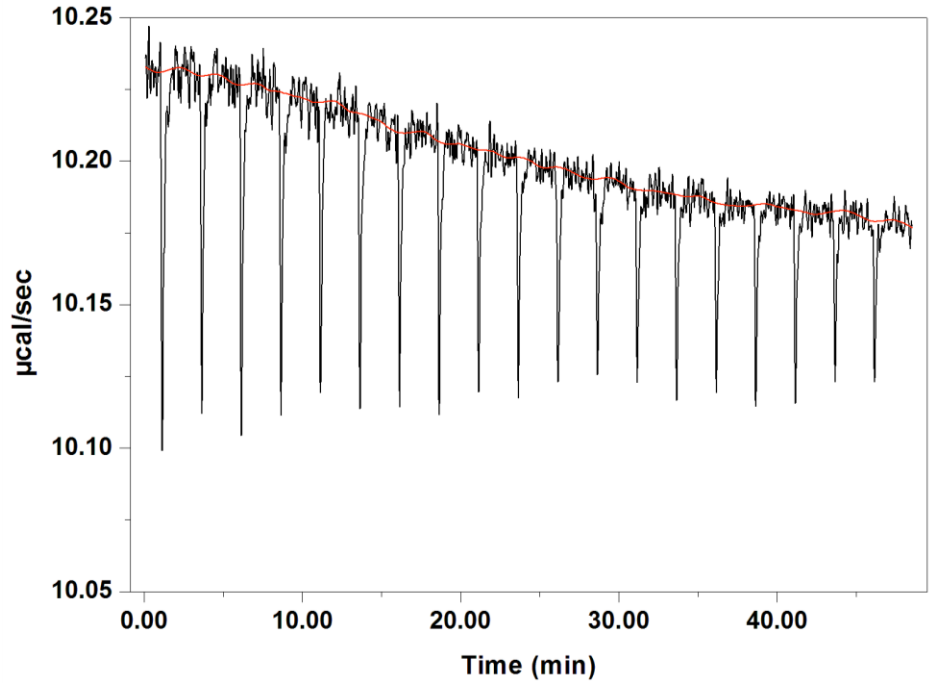

Fig. S5b

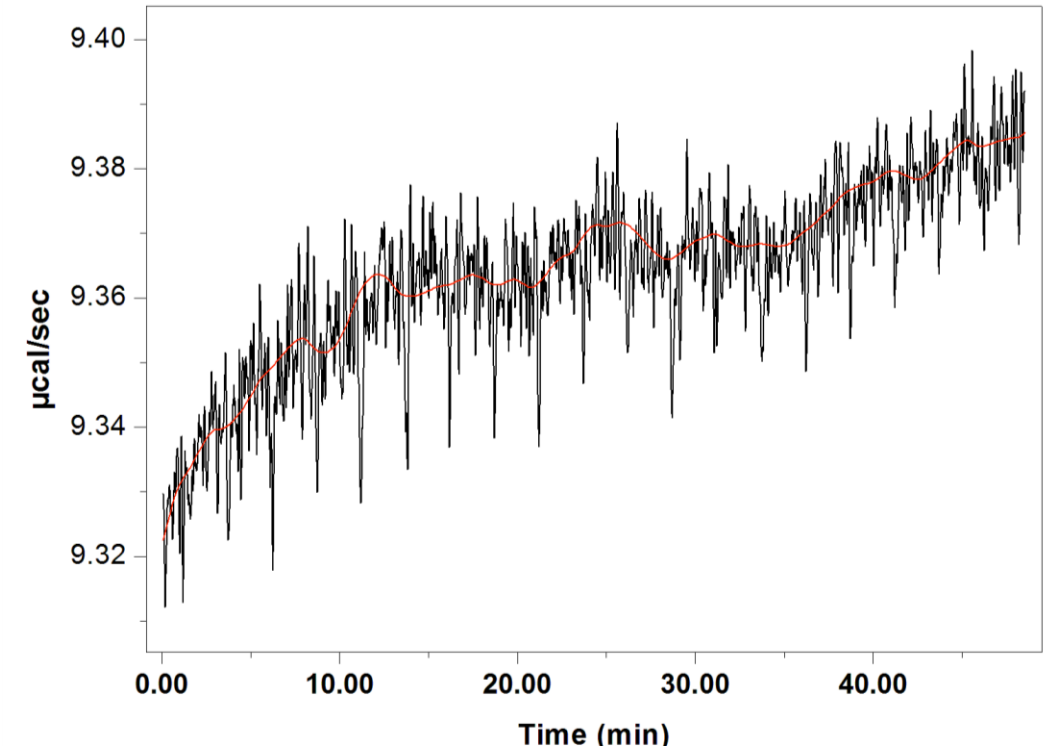

Fig. S5c

Figure legends

Fig. S1. ITC experiments to measure the direct binding between human and mouse PERK luminal domain and the peptide substrate P16 was performed by use of a MicroCal ITC colorimeter (MicroCal, Northampton, MA) at 25 ^{o}C. PERK luminal domain and the peptide were dialyzed against the same buffer (10 mM Tris-HCl pH 7.5, 100 mM NaCl). The reaction cell contained 400 µl of 0.1 mM PERK-LD , and the injection syringe was filled with 140 µl of 1 mM peptide substrate. Each titration experiment was performed by using 20 injections of 2µl with 4 second duration and a 150 second interval between injections. Peptide substrate P16 was injected into the buffer as

control experiments. a) data for human PERK luminal domain b) data for mouse PERK luminal domain.

Fig. S2. a) The omit electron density map in green (weighted 2Fo-Fc map) around the peptide substrate binding site. The peptide substrate was removed from the model for calculating the omit map. After refinement by simulated annealing from 1000K, the map was calculated and contoured at 1.2 rmsd. The residues W6, F8 and Y9 are labeled for the peptide substrate P16. The orientation for the PERK-peptide complex molecule in this panel is similar to that in Fig. 3b. The peptide P16 is shown in thick lines and the PERK luminal domain structure is shown by thinner lines. b) the omit map in green around the peptide substrate binding site. The orientation of this omit map is rotated about 90º along the horizontal axis from that in Fig. S2a. The residues Q4, W6, R7, F8 and Y9 are labeled for the peptide substrate P16. c) the electron density map in blue (weighted 2Fo-Fc map). The orientation of this map is the same as that in Fig. S2a. The map was contoured at 1.2 rmsd. d) the electron density map in blue (weighted 2Fo-Fc map). The orientation of this map is the same as that in Fig. S2b. The electron density of side chains of the solvent-exposed residues Q4 and R7 are not as well defined as those for W6, F8 and Y9.

Fig. S3. The sequence alignment of PERK luminal domain from various species. The residue numbering in the figure is based on bovine PERK sequence. The conserved residues are shown in blue. The residues that may be involved in forming the peptide-binding groove for the misfolded proteins are marked by stars.

Fig. S4. The CD spectrums for bovine PERK luminal domain and its mutants. The PERK luminal domain proteins were concentrated to 2mg/ml in Tris 10mM (pH7.9), NaCl 10mM and subjected

for the measurements. The experiments were carried out by use of a Jasco J-815 CD spectropolarimeter.

Fig. S5. ITC experiments to measure the direct binding between bPERK-LD mutants and the peptide substrate was performed by use of a MicroCal ITC colorimeter (MicroCal, Northampton, MA) at 25 ^{o}C. a: W165S, b: Y388S/L389S/M391S, c: W165S/Y388S/L389S/M391S. bPERK-LD mutants and the peptide were dialyzed against the same buffer (10 mM Tris-HCl pH 7.5, 100 mM NaCl). The reaction cell contained 400 μl of 0.1 mM bPERK-LD mutants, and the injection syringe was filled with 140 μl of 1 mM peptide substrate. Each titration experiment was performed by using 20 injections of 2μl with 4 second duration and a 150 second interval between injections. Peptide substrate P16 was injected into the buffer as control experiments.