SUPPLEMENTARY MATERIAL

Table S1. Heavy atom derivatives used at 2.5 Å resolution

	Gold*	Mercury*	Platinum*
R_{symm} (%)	9.3	13.5	9.6
R_{ano} (%)	10.2	14.0	10.2
R_{nat} (%)	15.9	25.9	10.2
R_C	0.83	0.97	0.81
Phasing power**	1.7 (1.1)	2.0 (1.2)	0.4 (0.3)
Heavy atom sites	1	4	1

*Gold = Potassium dicyanoaurate(I), $KAu(CN)_2$, Mercury = Thimerosal,

(Ethyl(2-mercaptobenzoate-S)mercury sodium salt, $C_9H_9HgNaO_2S$),

Platinum = Potassium tetrachloroplatinate(II), K_2PtCl_4

**Number in parentheses is for centric zone.

$R_{symm}=\Sigma|<F^+>-<F^->| \,/\, 0.5\Sigma|<F^+>+<F^->|$

$R_{ano}=\Sigma\Sigma|<F^+(j)>-<F^->| + |<F^-(i)>-<F^+>| \,/\, 0.5\Sigma|<F^+>+<F^->|$

$$R_{nat}=\Sigma\,|\,F_{PH}-F_P\,|\,\,/\,\Sigma\,|\,F_P\,|$$

$$R_C=\Sigma\,|\,|\,\,F_{PH}\pm F_P\,|-|\,F_H\,|\,|\,\,/\,\Sigma\,|\,F_{PH}\pm F_P\,|$$

Table S2. Crystallographic data and refinement statistics at 2.5 Å resolution

Crystallographic Data	
Space group	$P4_1$
Unit cell dimensions	
$a = b$ (Å)	66.7
c (Å)	76.6
Resolution range (Å)*	2.28 (20.0 – 2.50)
Measured reflections**	34,166
Unique reflections*	13,201 (10,802)
Completeness (%)	92.2
R_{symm} (%)	9.7
Refinement	
R_{work} (%)	21.0
	19.6 (>2σ)
	18.9 (>3σ)

*Number in parentheses is used for refinement.

**The intensity data were collected on a FAST area-detector diffractometer (Enrauf-Nonius) on a GX21 Elliot X-ray generator operated at 45 kV, 95 mA.

Table S3. Hydrogen bonds between FAD and the protein molecules

FAD	Human		Rat		RNR	
Atom	Atom	Distance (Å)	Atom	Distance (Å)	Atom	Distance (Å)
Isoalloxazine						
N1	T181OG1	3.3	-----	---	-----	---
O2	K110N	3.0	K110N	3.1	K81N	3.1
N3	V108O	2.7	V108O	2.8	L79O	2.7
O4	T184OG1	2.8	T184OG1	2.9	T149OG1	2.6
N5	T184OG1	3.2	T184OG1	3.0	T149OG1	3.3
Ribityl						
O4*	Y93OH	2.8	Y93OH	2.5	Y64OH	2.8
Diphosphate						
O1P	R91NE	2.9	R91NE	3.0	R62OH	2.7
	M126N	2.9	M126N	2.8	M97N	2.9
O2P	S127OG	2.6	S127OG	3.0	T98OG1	2.4
	S127N	2.8	S127N	2.9	T98N	3.0
AO1	R91NH2	3.3	R91NH2	3.3	-----	---

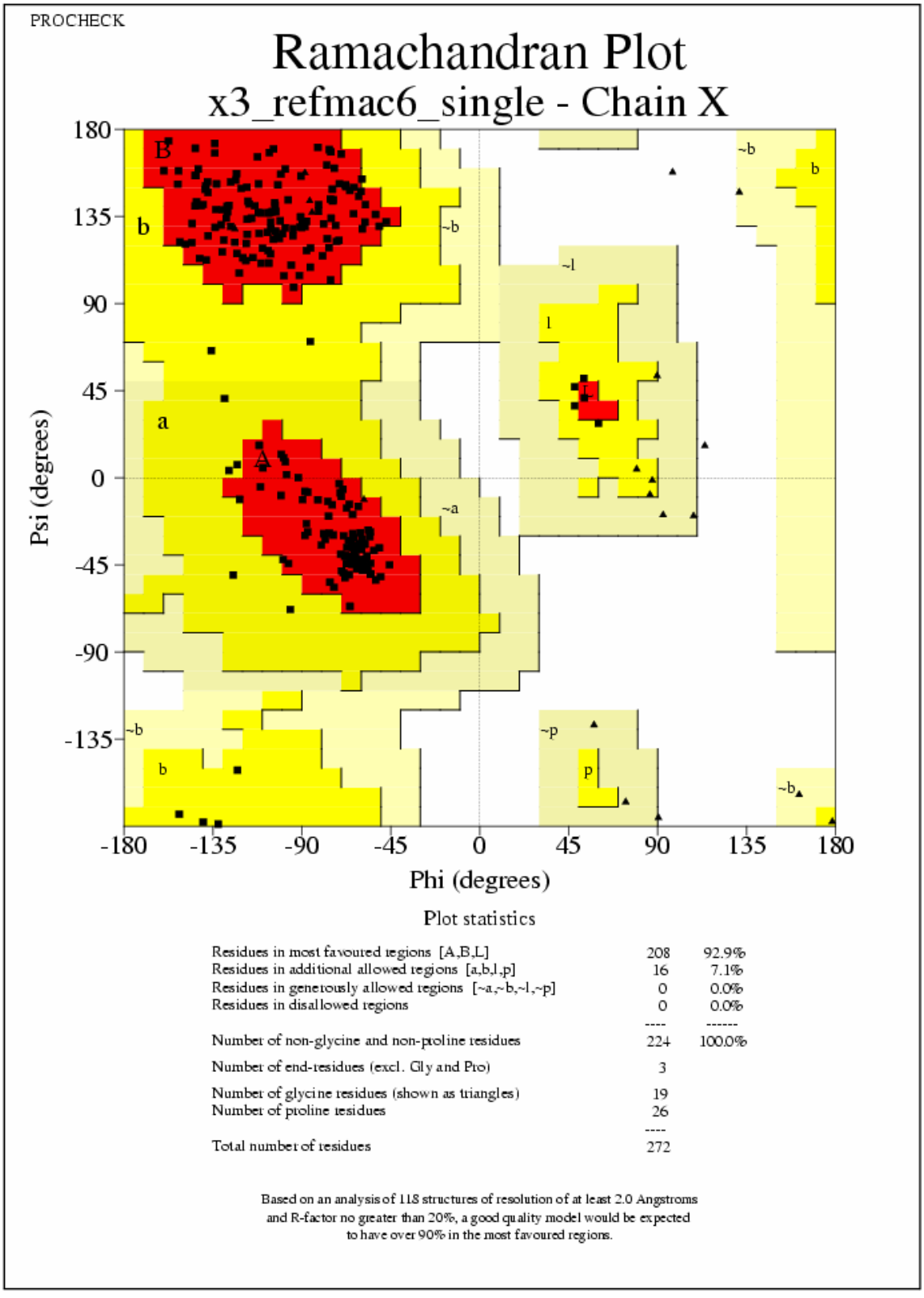
	K125N	3.0	K125N	2.9	L96N	3.0
AO2	R91NE	3.2	R91NE	3.0	-----	---
	R91NH2	3.2	-----	--- -	-----	---
Adenine						
AN6	F113O	2.8	F113O	3.0	F84O	3.0

Table 4S. Positional deviations between human and rat b5R

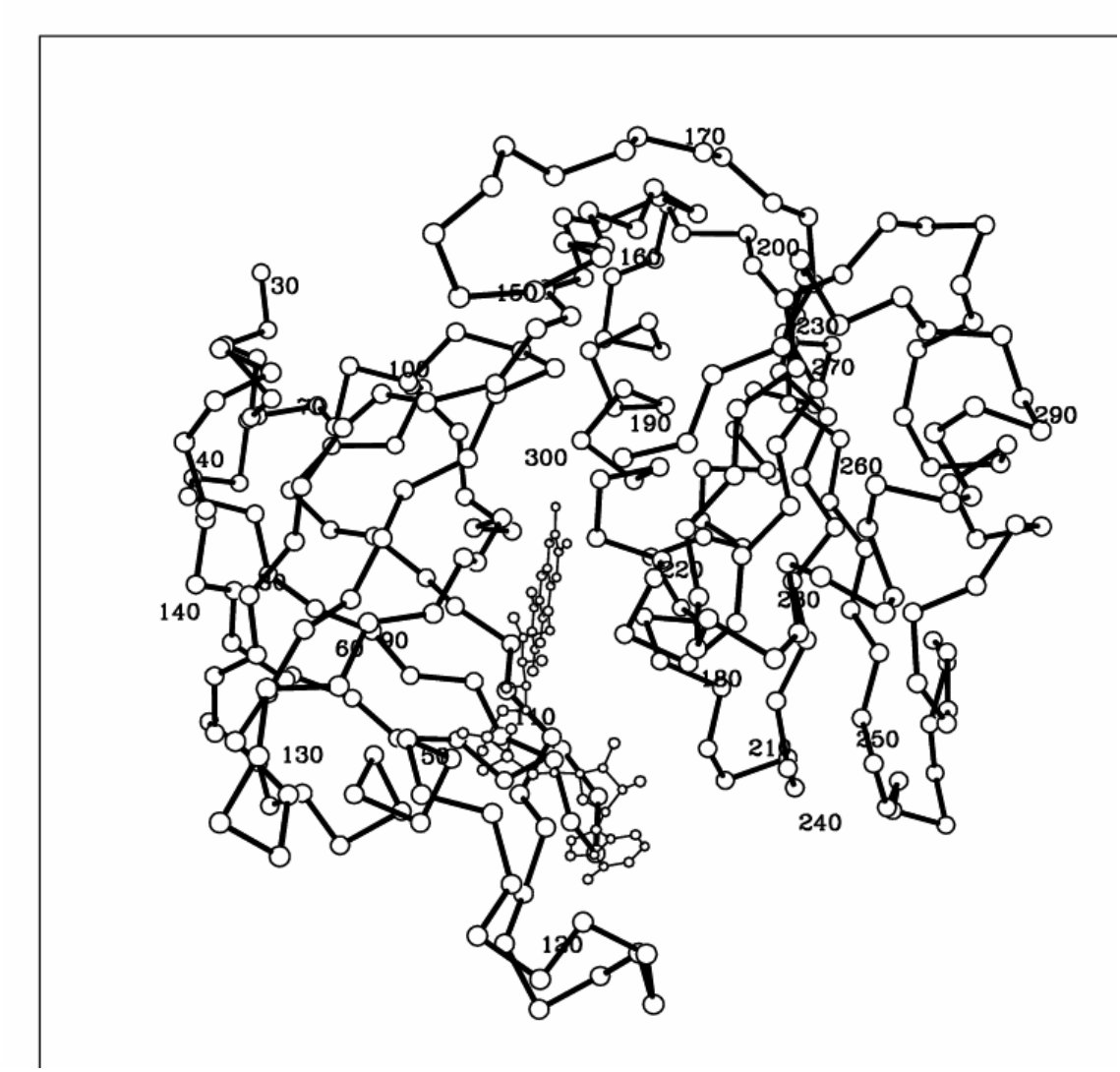
	Positional deviation (Å)		
Atom	rms	average	maximum
All*	1.4 (1.2)	0.9 (0.9)	14.3 (7.6)
Main chain*	0.9 (0.9)	0.8 (0.7)	5.7 (3.0)
Side chain*	1.8 (1.6)	1.2 (1.2)	14.3 (7.5)
CA only*	1.0 (0.9)	0.8 (0.7)	5.7 (3.1)

*Number in parentheses is for without residue 265 to 268.

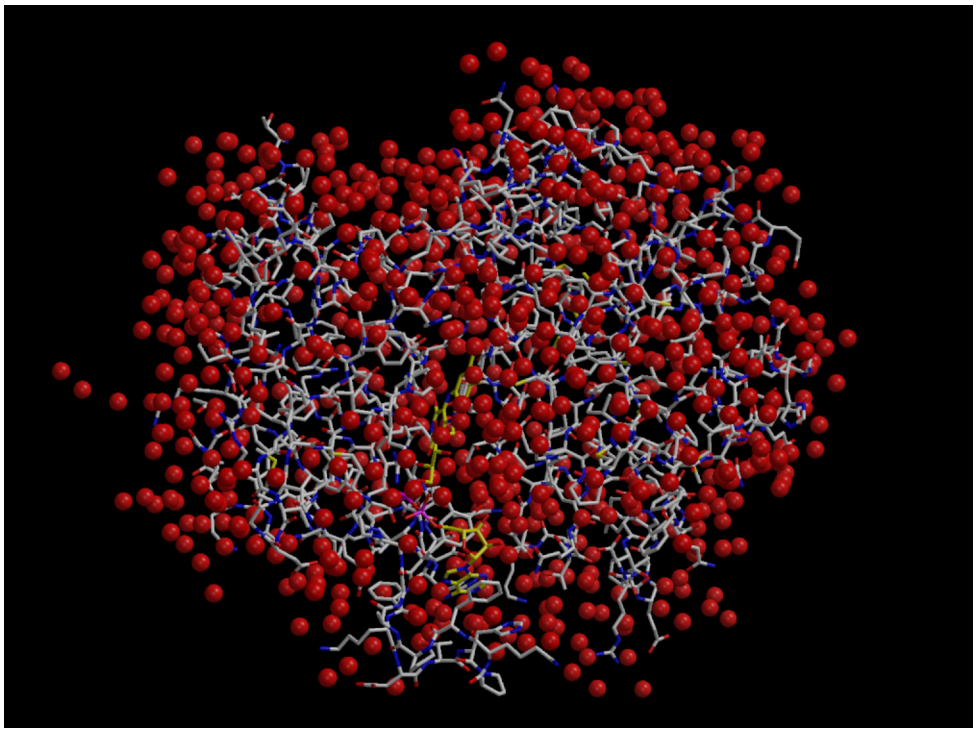
(**Figure S1**) A Ramachandran plot of the final model of human b5R.



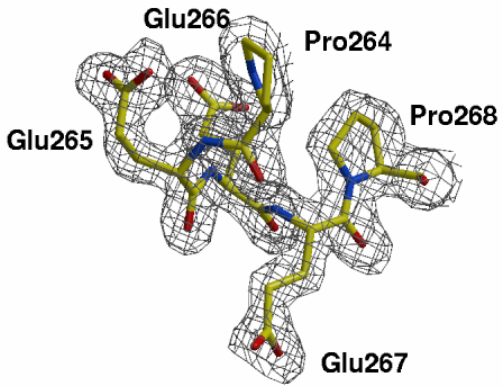
(**Figure S2**) The Cα-chain trace of human b5R in the same perspective as Figure 1 in the text.



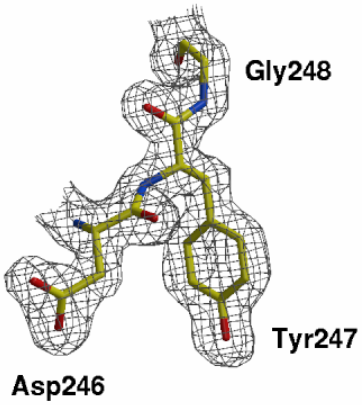
(**Figure S3**) A human NADH-cytochrome b_5 reductase molecule with water molecules (red spheres).



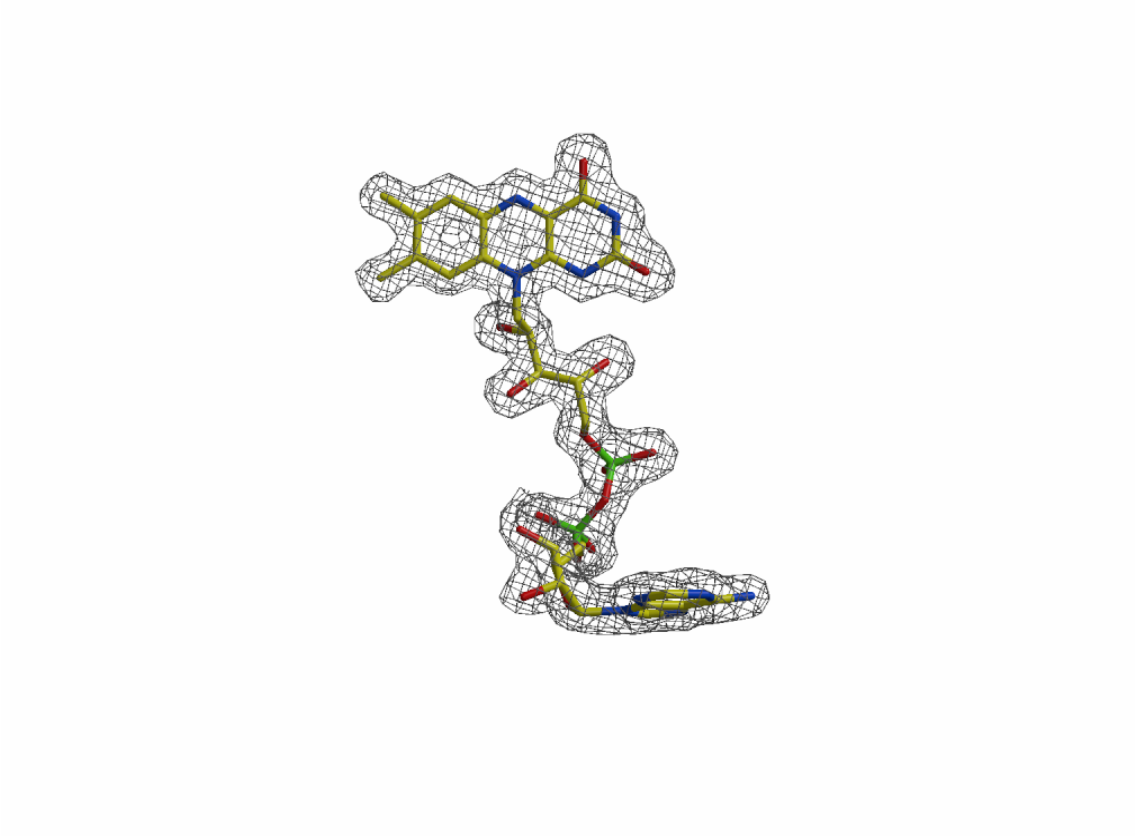
(**Figure S4**) The final 2Fo-Fc electron density contoured at 0.8 σ with the final b5R model. The conformation of the region from Glu265 to Pro268 is different from that of rat b5R as described in the section, 3.2 Comparison with Rat b5R.



(**Figure S5**) Tyr247, which has main-chain torsion angles, (φ, ψ) = (−122.5, −151.0), is superposed on the final 2Fo-Fc map. The torsion angles are unusual, but the model fits well to the electron density map.



(**Figure S6**) The FAD model superposed on the final 2Fo-Fc map. The view perpendicular to the isoalloxazine ring plane.



(**Figure S7**) A schematic drawing of the hydrogen-bond system around FAD of human b5R, excluding water molecules. The hydrogen bonds in the figures of human b5R, rat b5R, and corn nitrate reductase (RNR) are summarized in Table S3.

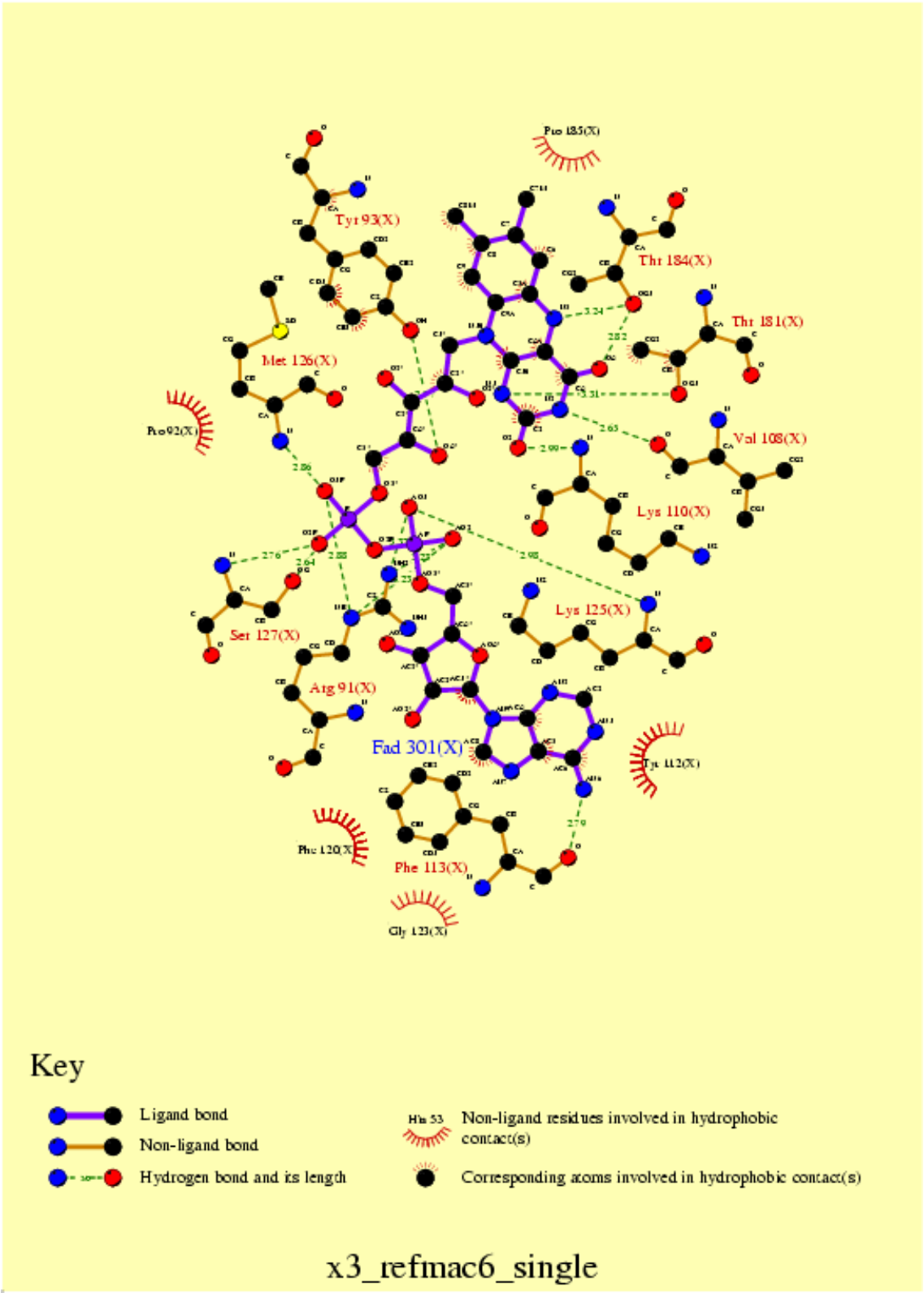
(**Figure S8**) A schematic drawing of the hydrogen-bond system around FAD of rat b5R, excluding water molecules.

(**Figure S9**) A schematic drawing of the hydrogen-bond system around FAD of corn nitrate reductase (RNR), excluding water molecules.

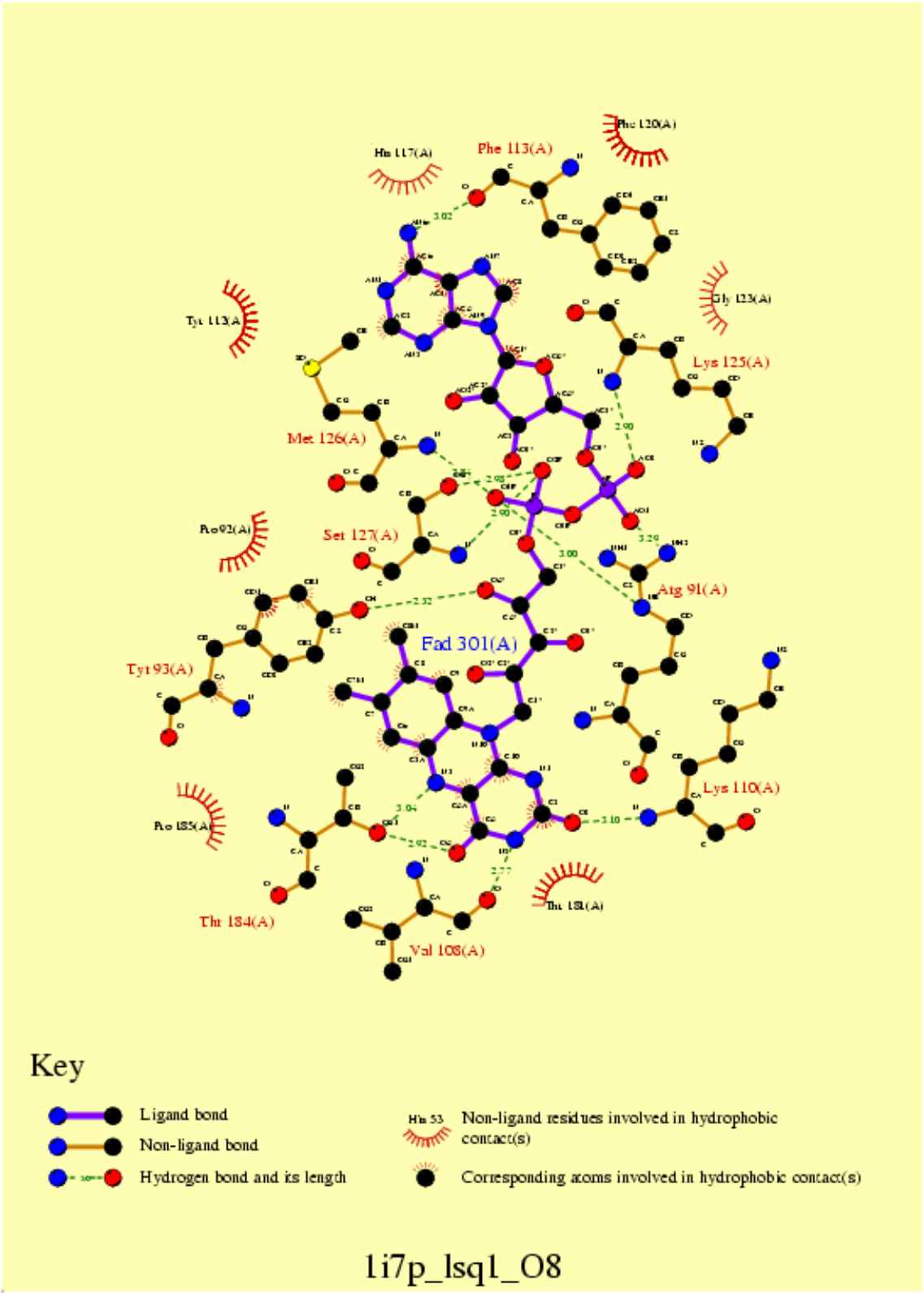
(**Figure S10**) A schematic drawing of the structure from residue 263 to 269 in human b5R, whose hydrogen-bond system is different from that in rat b5R as seen in the following Figure S11.

(**Figure S11**) A schematic drawing of the structure from residue 263 to 269 in rat b5R. The difference of the structures is described in the text.

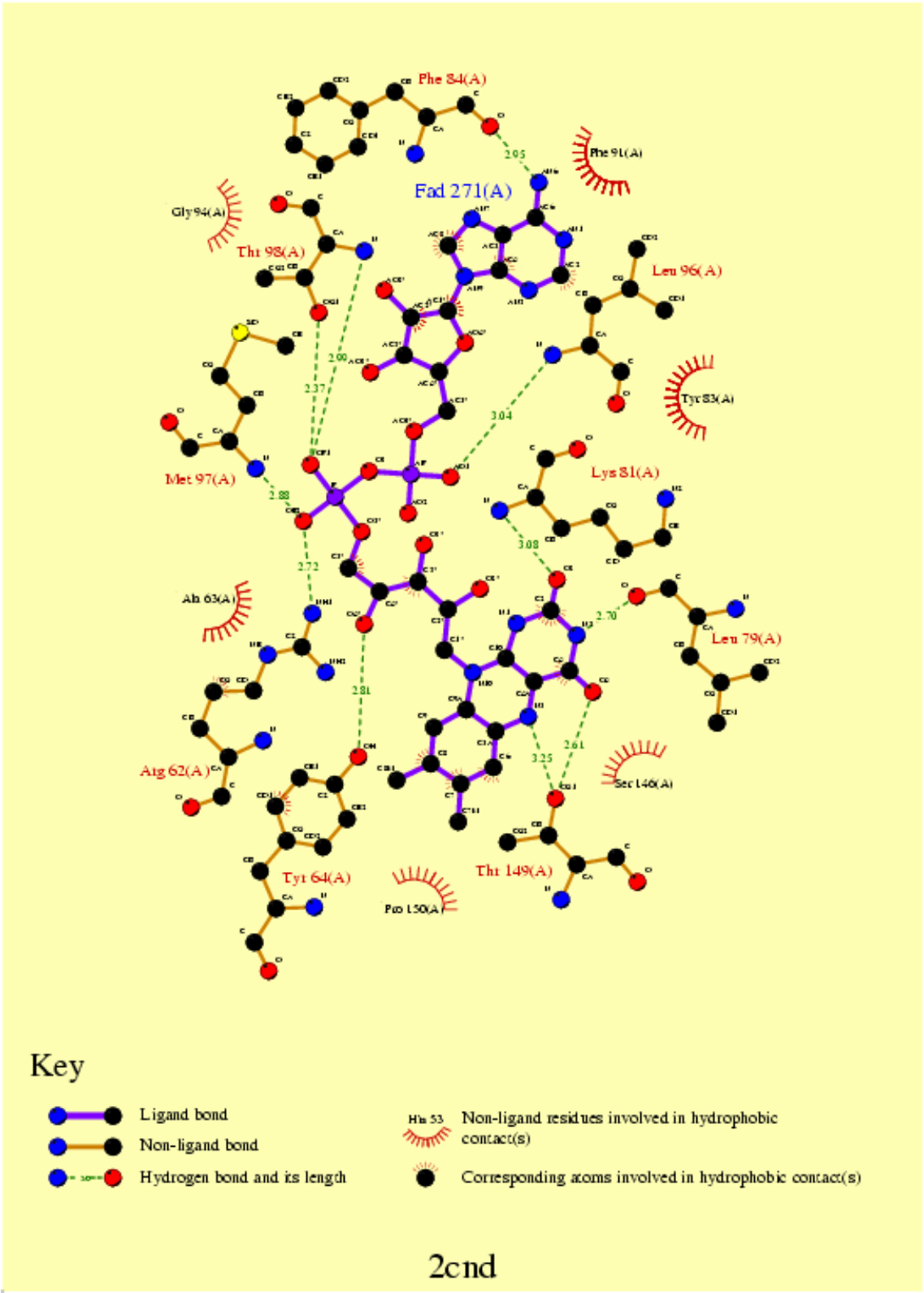
(Figure S7)


Pro 185(X)
Tyr 93(X)
Thr 184(X)
Thr 181(X)
Met 126(X)
Pro 92(X)
Val 108(X)
Lys 110(X)
Lys 125(X)
Ser 127(X)
Arg 91(X)
Fad 301(X)
Tyr 112(X)
Phe 120(X)
Phe 113(X)
Gly 123(X)
Key
Ligand bond
Non-ligand bond
Hydrogen bond and its length
His 53
Non-ligand residues involved in hydrophobic contact(s)
Corresponding atoms involved in hydrophobic contact(s)
x3_refmac6_single

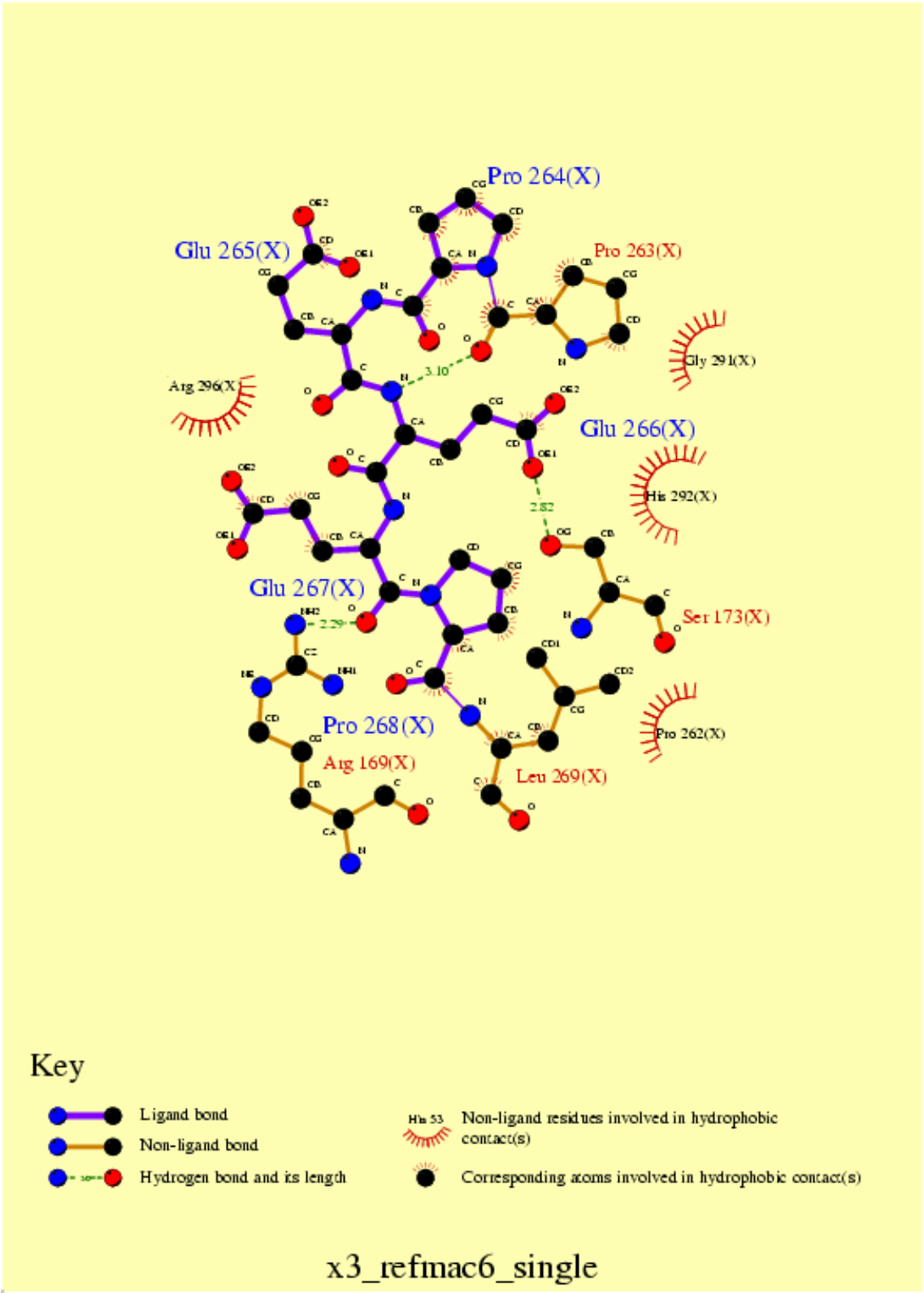
(Figure S8)


Phe 113(A)
Tyr 113(A)
Gly 123(A)
Lys 125(A)
Met 126(A)
Pro 92(A)
Ser 127(A)
Arg 91(A)
Fad 301(A)
Tyr 93(A)
Lys 110(A)
Pro 185(A)
Thr 184(A)
Thr 181(A)
Val 108(A)
Key
Ligand bond
Non-ligand bond
Hydrogen bond and its length
His 53
Non-ligand residues involved in hydrophobic contact(s)
Corresponding atoms involved in hydrophobic contact(s)
1i7p_lsq1_O8

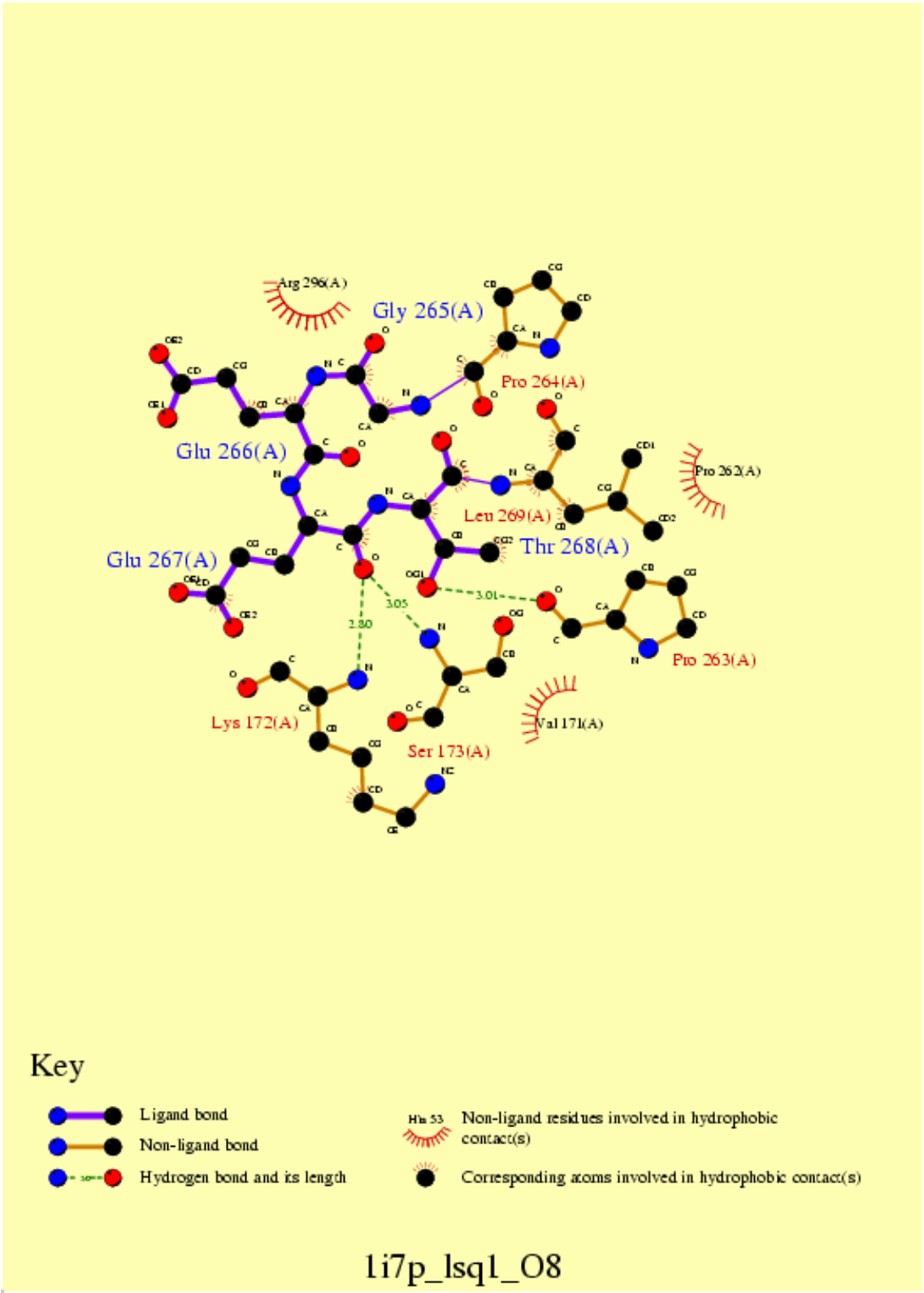
(Figure S9)


Phe 84(A)
Phe 91(A)
Fad 271(A)
Gly 94(A)
Thr 98(A)
Leu 96(A)
Tyr 83(A)
Met 97(A)
Lys 81(A)
Leu 79(A)
Ala 63(A)
Arg 62(A)
Ser 146(A)
Tyr 64(A)
Thr 149(A)
Pro 150(A)
Key
Ligand bond
Non-ligand bond
Hydrogen bond and its length
His 53
Non-ligand residues involved in hydrophobic contact(s)
Corresponding atoms involved in hydrophobic contact(s)
2cnd

(Figure S10)


Pro 264(X)
Glu 265(X)
Pro 263(X)
Gly 291(X)
Arg 296(X)
3.10
Glu 266(X)
His 292(X)
2.82
Glu 267(X)
2.29
Ser 173(X)
Pro 268(X)
Pro 262(X)
Arg 169(X)
Leu 269(X)
Key
Ligand bond
Non-ligand bond
Hydrogen bond and its length
His 53
Non-ligand residues involved in hydrophobic contact(s)
Corresponding atoms involved in hydrophobic contact(s)
x3_refmac6_single

(Figure S11)

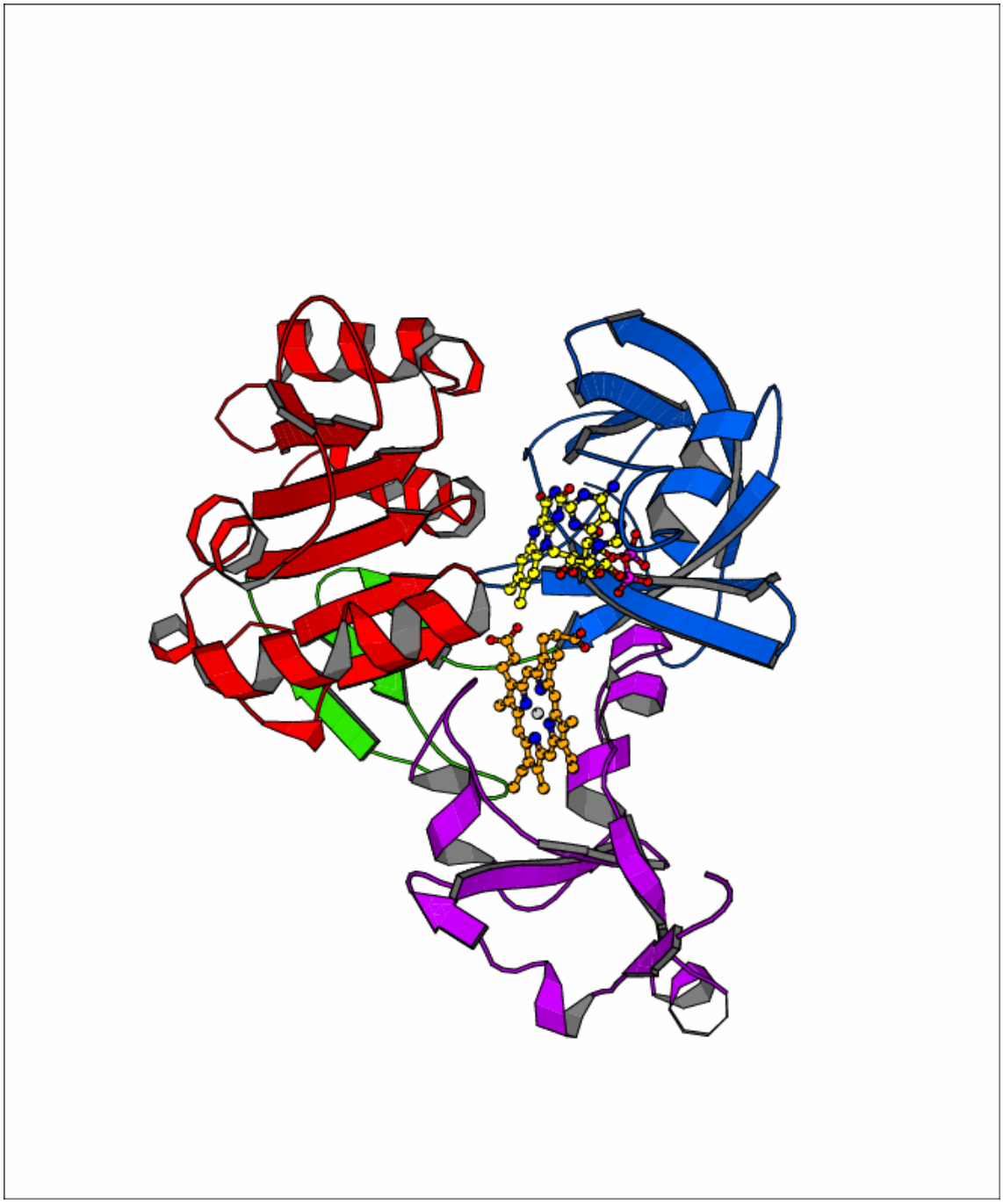

Arg 296(A)
Gly 265(A)
Pro 264(A)
Glu 266(A)
Pro 262(A)
Leu 269(A)
Thr 268(A)
Glu 267(A)
3.01
3.05
2.80
Pro 263(A)
Lys 172(A)
Val 171(A)
Ser 173(A)
Key
Ligand bond
Non-ligand bond
Hydrogen bond and its length
His 53
Non-ligand residues involved in hydrophobic contact(s)
Corresponding atoms involved in hydrophobic contact(s)
1i7p_lsq1_O8

(**Figure S12**) A model of b5/b5R complex molecule with the same orientation of isoalloxazine ring and heme plane as those of flavocytochrome b_2. The b5 molecule (in magenta) is in collision with the loops in the FAD domain (in blue) of b5R.

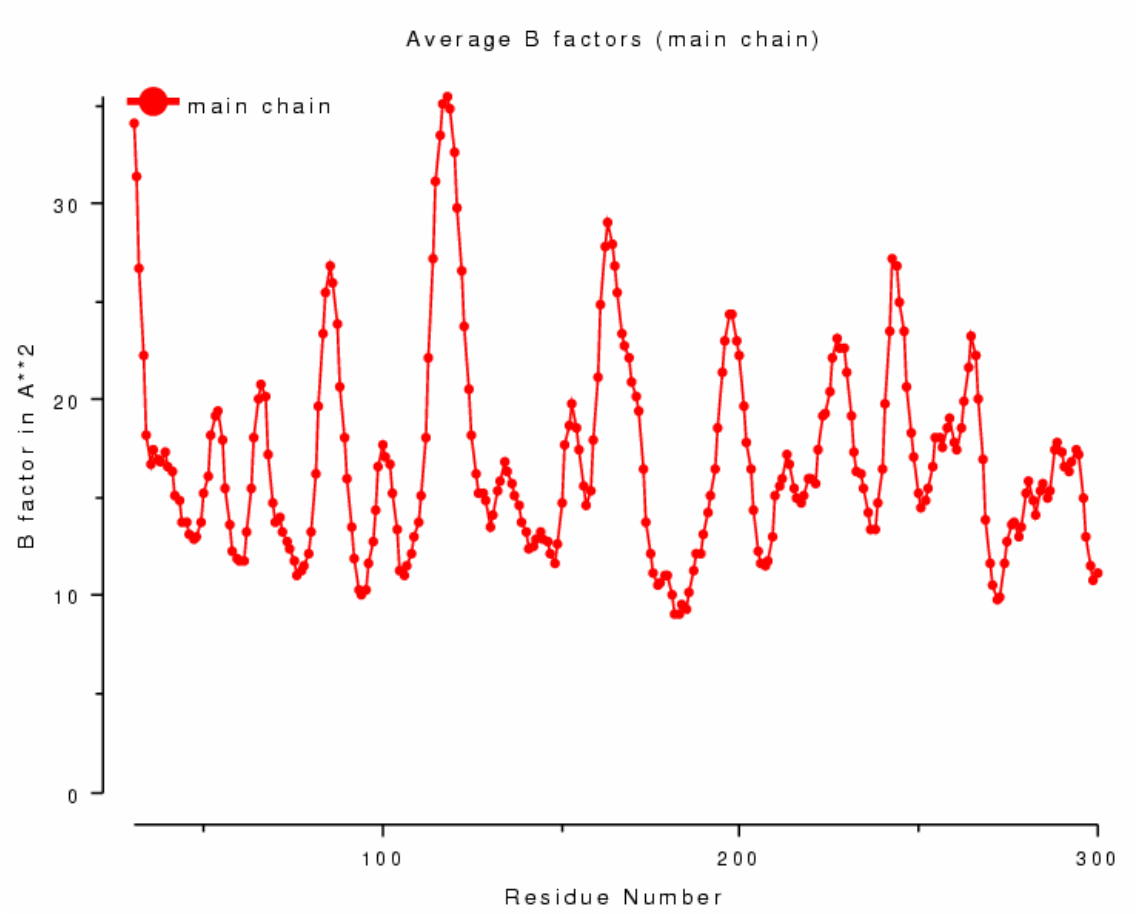
(**Figure S13**) The most probable model of b5/b5R complex, which is same as the model of b5/RNR complex molecule by Lu et al.. The differences between the models are described in the text.

(Figure S12)

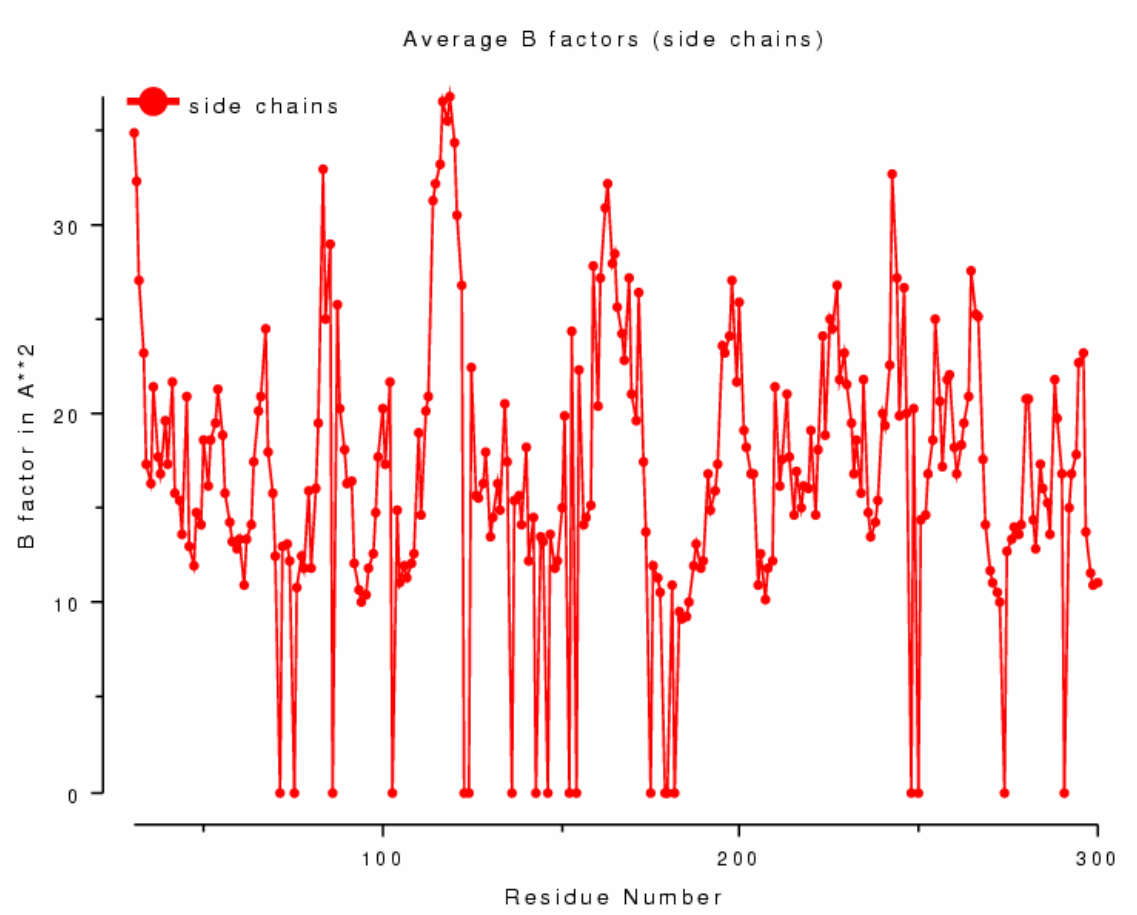
(Figure S13)



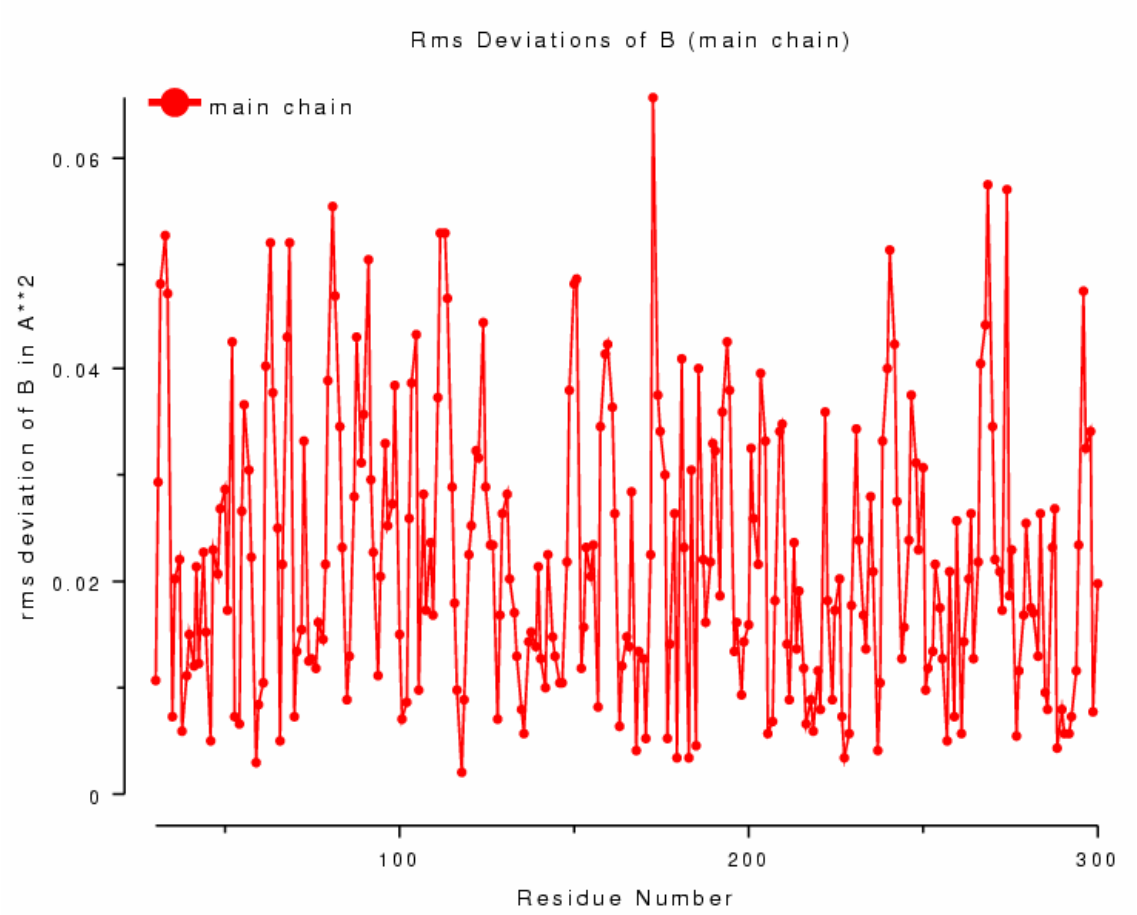
(**Figure S14**) Average B values *versus* residue numbers for main chain residues.



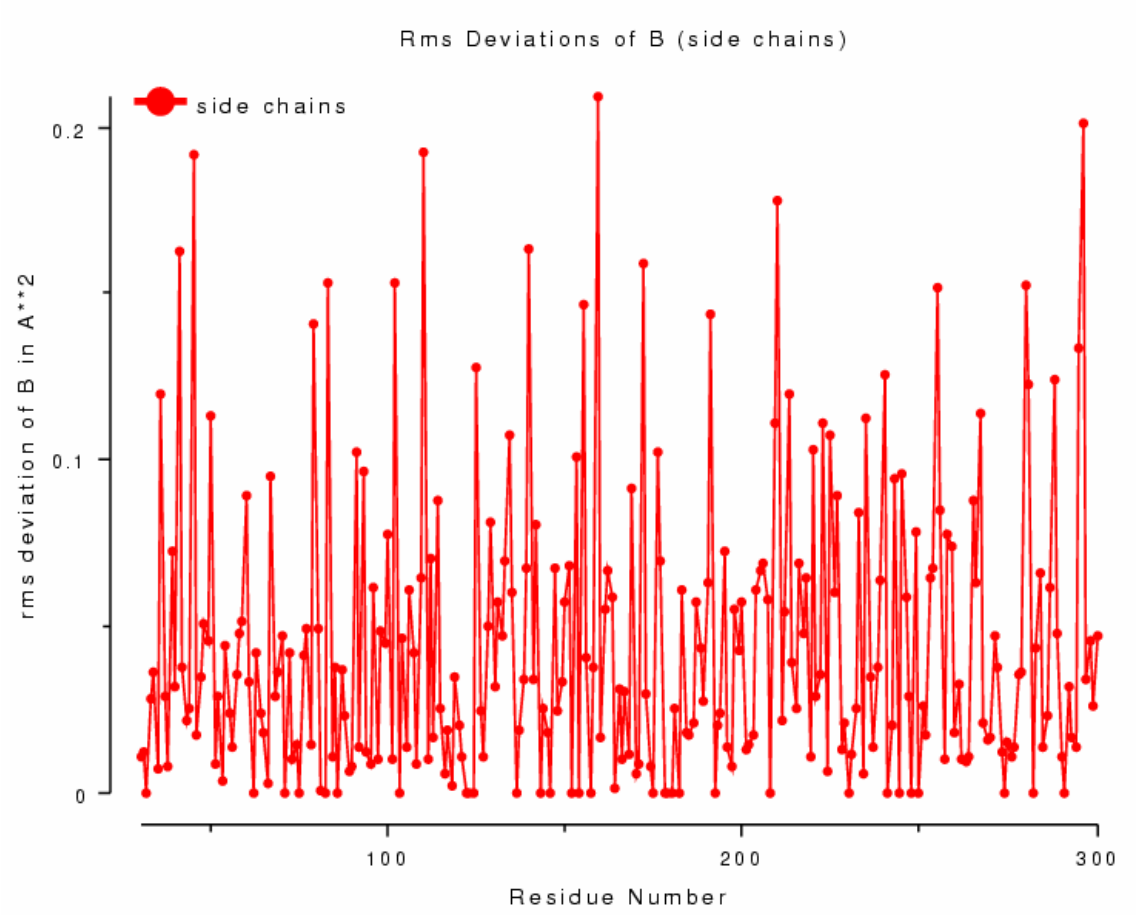
(**Figure S15**) Average B values *versus* residue numbers for side chain residues.



(**Figure S16**) Average rms B *versus* residue numbers for main chain residues.



(**Figure S17**) Average rms B *versus* residue numbers for side chain residues.



(**Figure S18**) Rfactors *versus* resolution

TABLE TITLE:

Cycle 6. Rfactor analysis, F distribution v resln

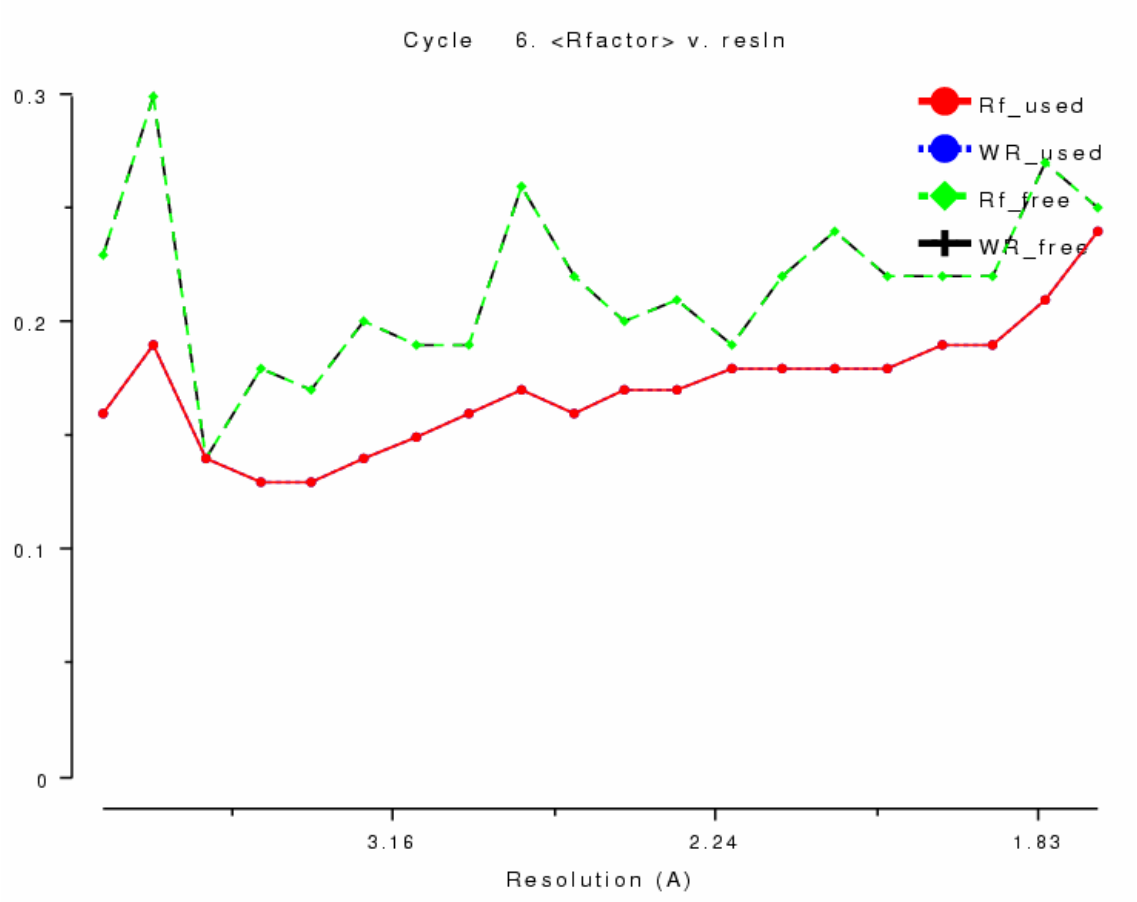
GRAPH TITLE:

Cycle 6. <Rfactor> v. resln

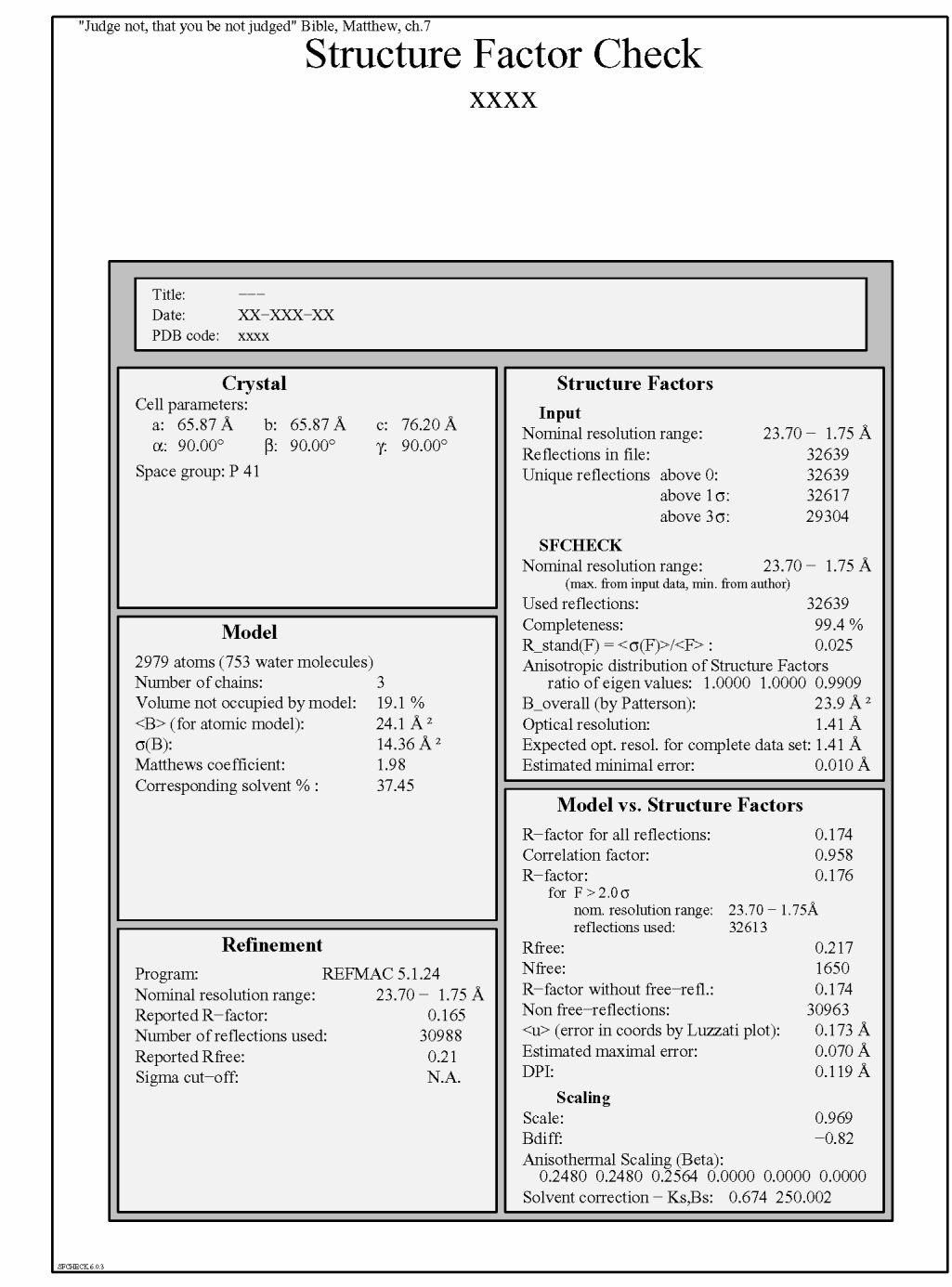
COLUMN TITLES:

M(4SSQ/LL) : M(4SSQ/LL)
Rf_used : Rf_used
WR_used : WR_used
Rf_free : Rf_free
WR_free : WR_free

M(4SSQ/LL)	Rf_used	WR_used	Rf_free	WR_free
0.01	0.16	0.16	0.23	0.23
0.03	0.19	0.19	0.30	0.30
0.04	0.14	0.14	0.14	0.14
0.06	0.13	0.13	0.18	0.18
0.07	0.13	0.13	0.17	0.17
0.09	0.14	0.14	0.20	0.20
0.11	0.15	0.15	0.19	0.19
0.12	0.16	0.16	0.19	0.19
0.14	0.17	0.17	0.26	0.26
0.16	0.16	0.16	0.22	0.22
0.17	0.17	0.17	0.20	0.20
0.19	0.17	0.17	0.21	0.21
0.20	0.18	0.18	0.19	0.19
0.22	0.18	0.18	0.22	0.22
0.24	0.18	0.18	0.24	0.24
0.25	0.18	0.18	0.22	0.22
0.27	0.19	0.19	0.22	0.22
0.29	0.19	0.19	0.22	0.22
0.30	0.21	0.21	0.27	0.27
0.32	0.24	0.24	0.25	0.25


Cycle 6. <Rfactor> v. resln
0.3
0.2
0.1
0
Rf_used
WR_used
Rf_free
WR_free
3.16
2.24
1.83
Resolution (A)

(**Figure S19**) Structure Factor Analysis



"Judge not, that you be not judged" Bible, Matthew, ch.7

Structure Factor Check

XXXX

Title: ---
Date: XX-XXX-XX
PDB code: xxxx

Crystal

Cell parameters:
a: 65.87 Å b: 65.87 Å c: 76.20 Å
α: 90.00° β: 90.00° γ: 90.00°
Space group: P 41

Model

2979 atoms (753 water molecules)

Number of chains:	3
Volume not occupied by model:	19.1 %
<B> (for atomic model):	24.1 Å²
σ(B):	14.36 Å²
Matthews coefficient:	1.98
Corresponding solvent % :	37.45

Refinement

Program:	REFMAC 5.1.24
Nominal resolution range:	23.70 – 1.75 Å
Reported R-factor:	0.165
Number of reflections used:	30988
Reported Rfree:	0.21
Sigma cut-off:	N.A.

Structure Factors

Input

Nominal resolution range:		23.70 – 1.75 Å
Reflections in file:		32639
Unique reflections	above 0:	32639
	above 1σ:	32617
	above 3σ:	29304

SFCHECK

Nominal resolution range: 23.70 – 1.75 Å
(max. from input data, min. from author)

Used reflections:	32639
Completeness:	99.4 %
R_stand(F) = <σ(F)>/<F> :	0.025

Anisotropic distribution of Structure Factors
ratio of eigen values: 1.0000 1.0000 0.9909

B_overall (by Patterson):	23.9 Å²
Optical resolution:	1.41 Å
Expected opt. resol. for complete data set:	1.41 Å
Estimated minimal error:	0.010 Å

Model vs. Structure Factors

R-factor for all reflections:	0.174
Correlation factor:	0.958
R-factor:	0.176

for F > 2.0 σ
nom. resolution range: 23.70 – 1.75Å
reflections used: 32613

Rfree:	0.217
Nfree:	1650
R-factor without free-refl.:	0.174
Non free-reflections:	30963
<u> (error in coords by Luzzati plot):	0.173 Å
Estimated maximal error:	0.070 Å
DPI:	0.119 Å

Scaling

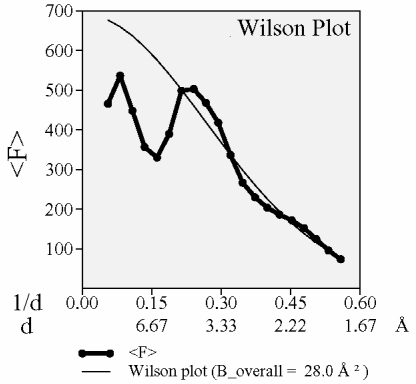
Scale:	0.969
Bdiff:	-0.82

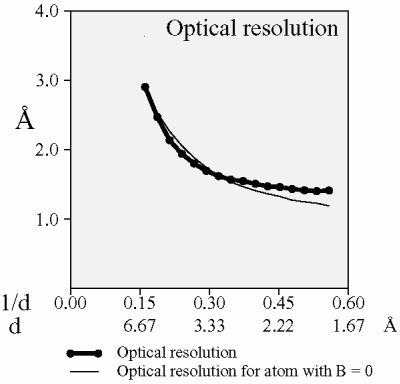
Anisothermal Scaling (Beta):
0.2480 0.2480 0.2564 0.0000 0.0000 0.0000
Solvent correction – Ks,Bs: 0.674 250.002

SFCHECK 6.0.3

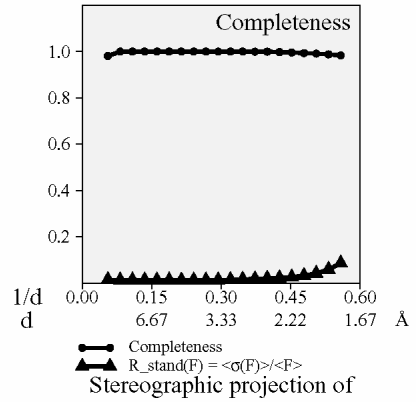
Structure Factor Check

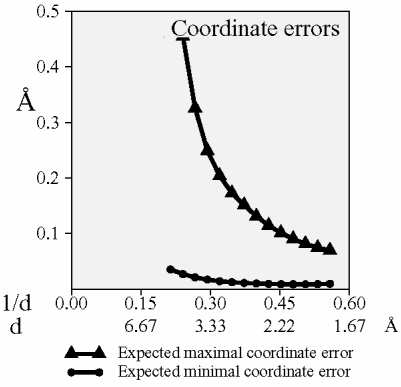
xxxx



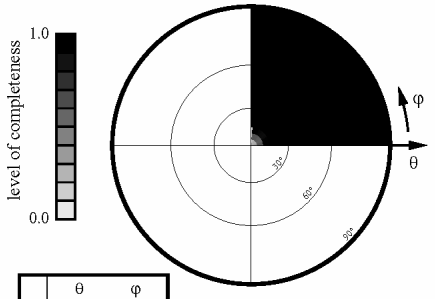


Wilson plot (B_overall = 28.0 Å ²)



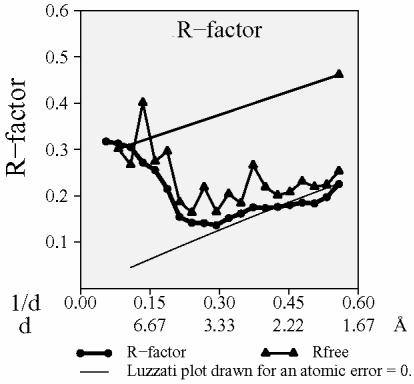


Stereographic projection of
the averaged radial completeness



	θ	φ
h	90.00	0.00
k	90.00	90.00
l	0.00	0.00

polar coordinates of the
crystallographical axes

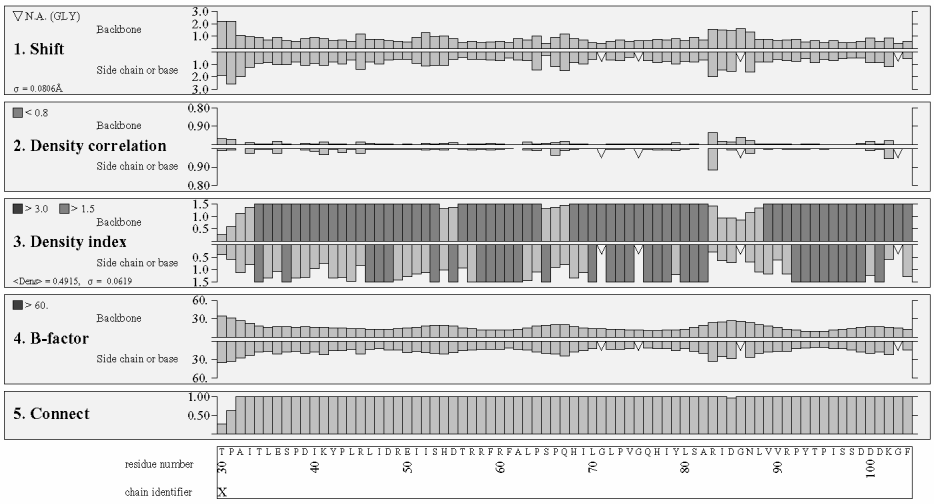


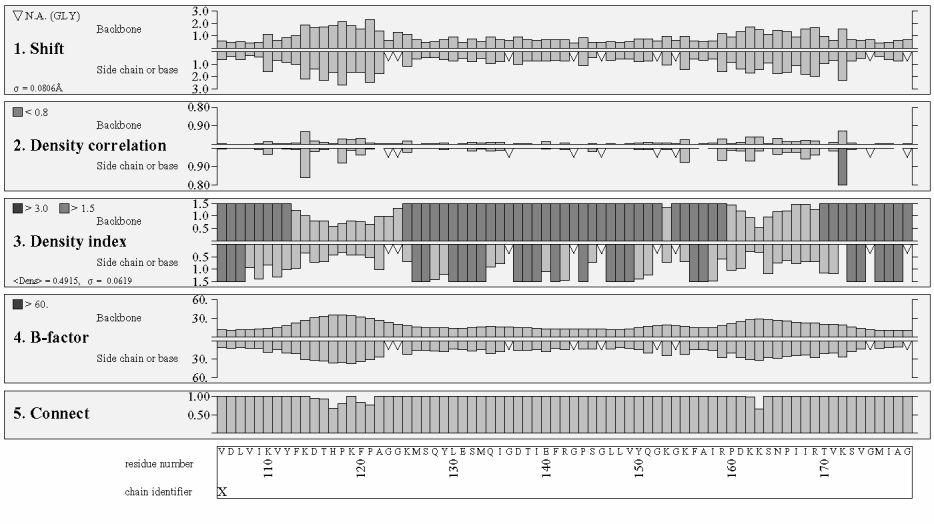
SFCHECK 6.0.3

Structure Factor Check

xxxx

Local estimation



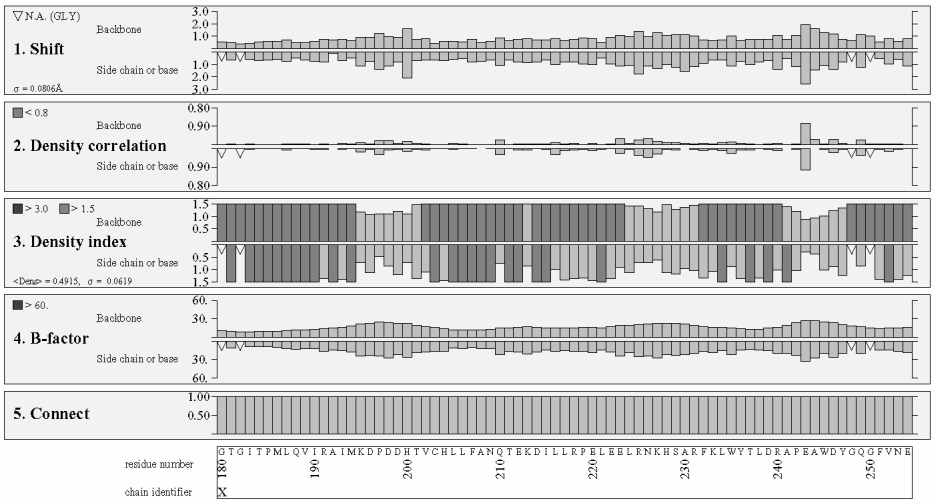


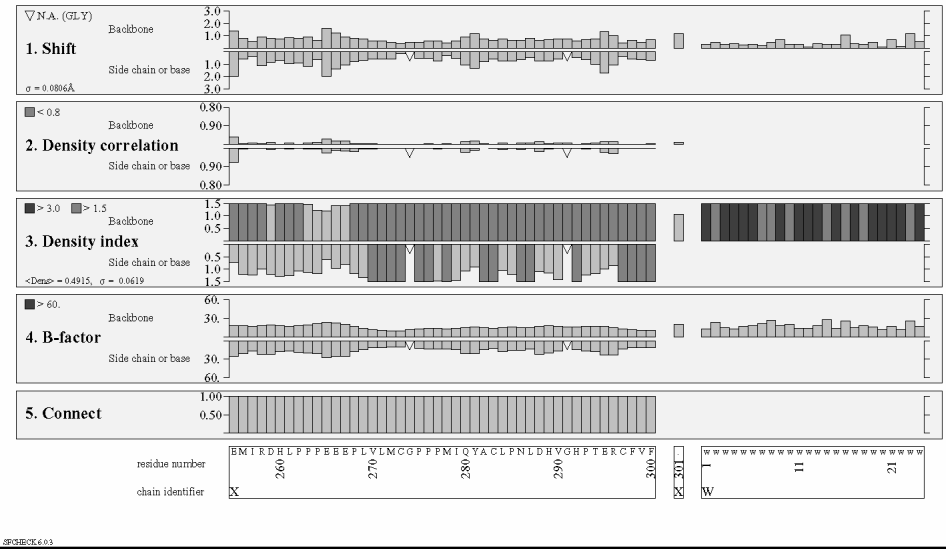
SFCHECK 6.0.3

Structure Factor Check

xxxx

Local estimation (2)

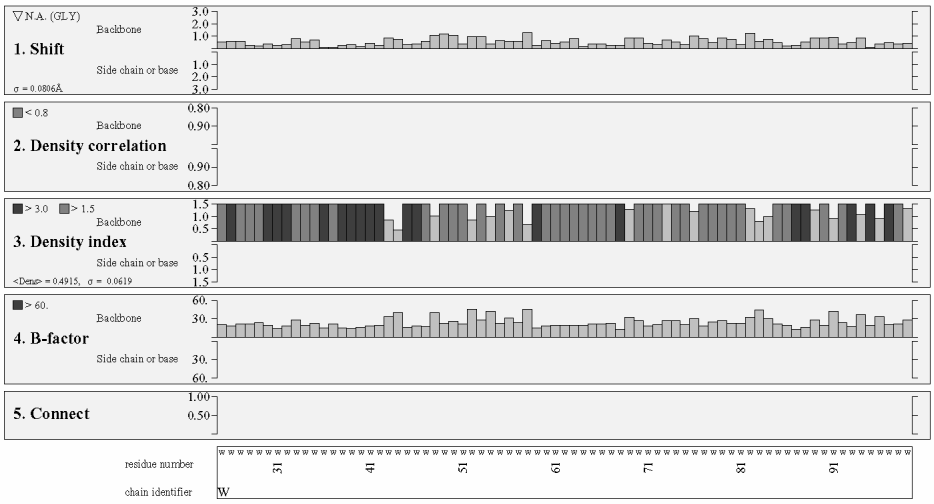


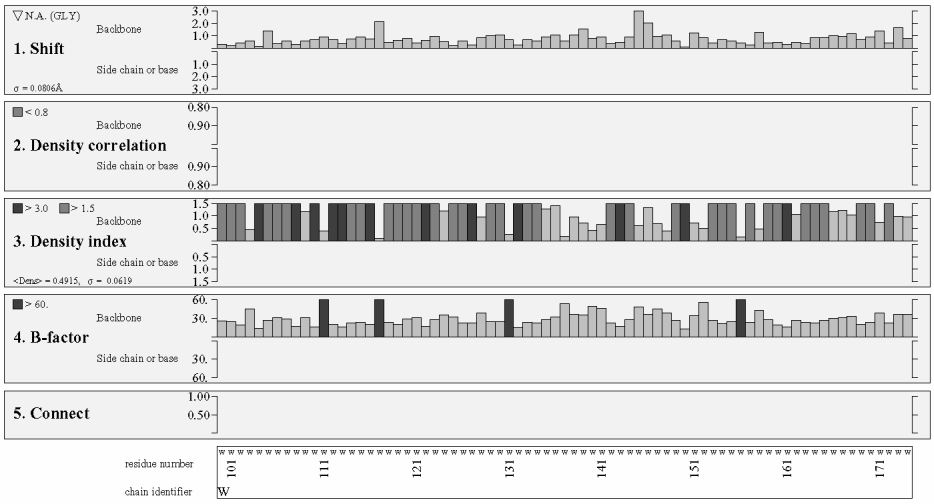


Structure Factor Check

xxxx

Local estimation (3)



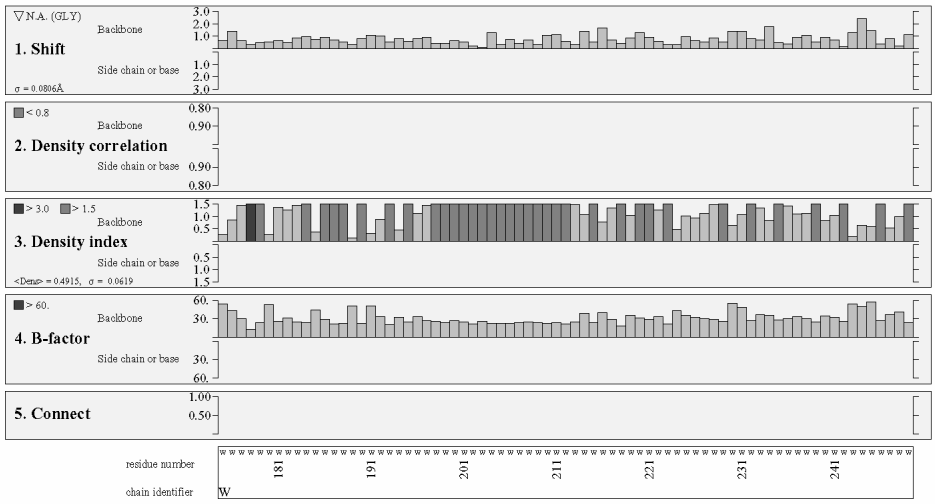


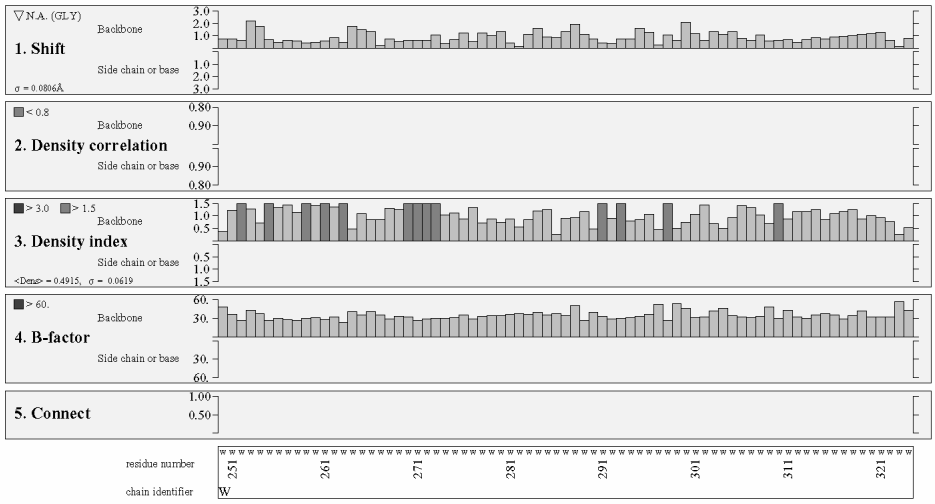
SFCHECK 6.0/3

Structure Factor Check

xxxx

Local estimation (4)



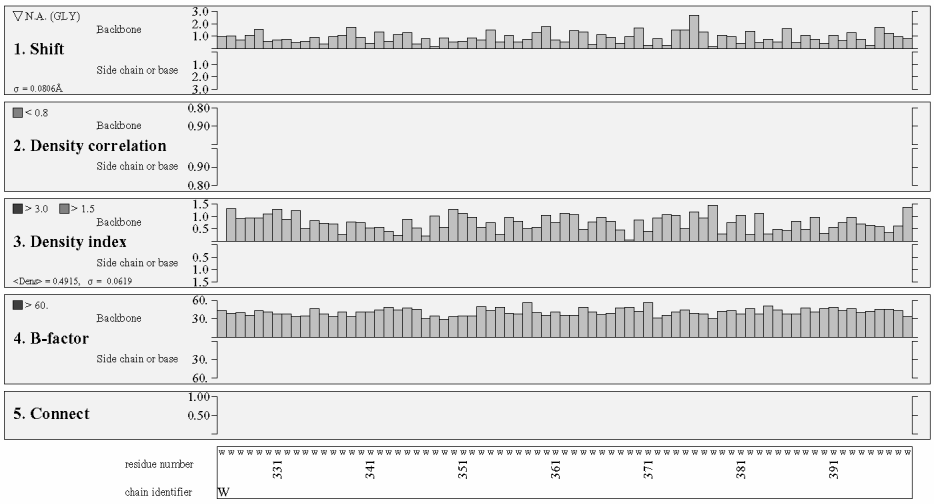


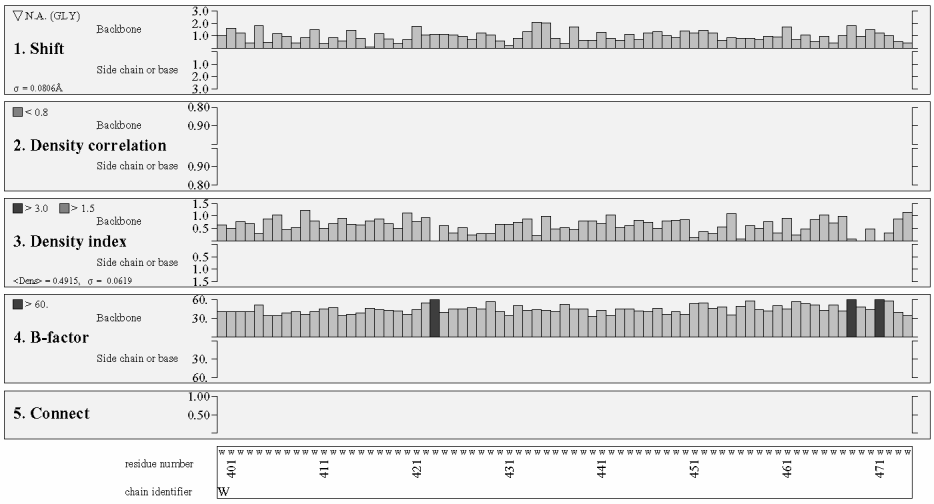
SPCHECK 6.0.3

Structure Factor Check

xxxx

Local estimation (5)



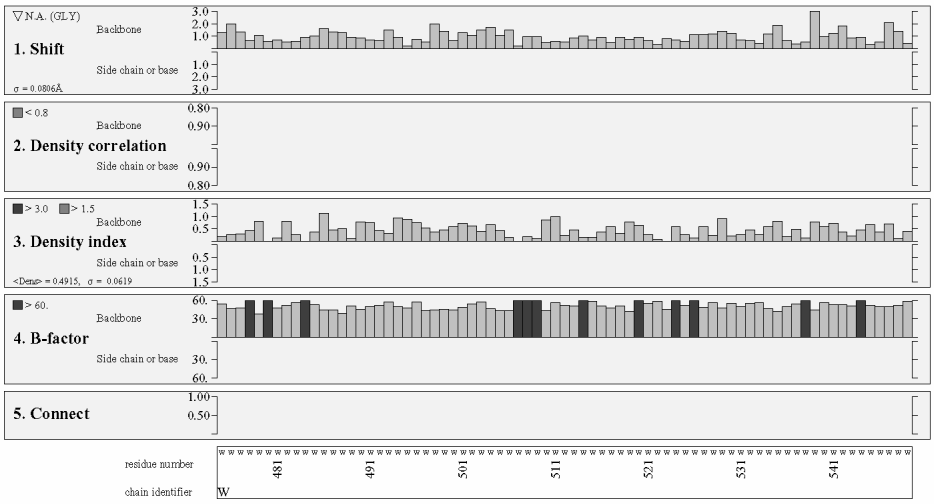


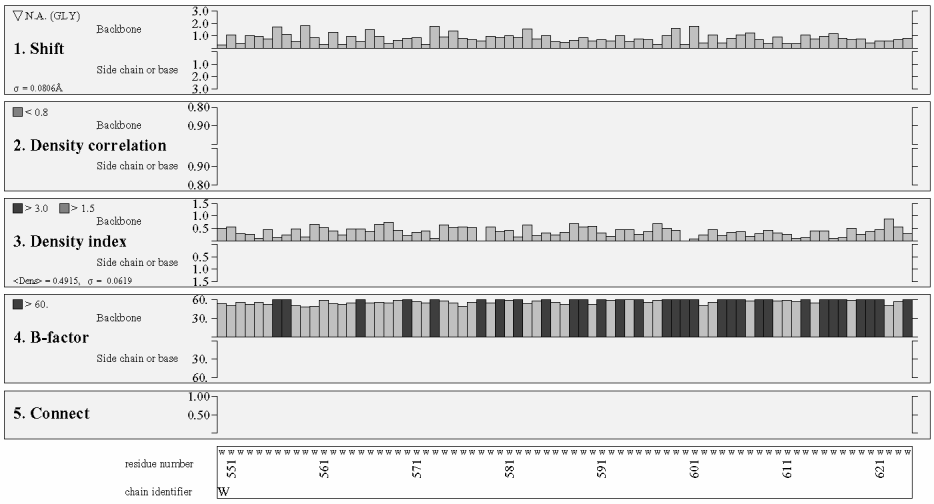
SFCHECK 6.0/3

Structure Factor Check

xxxx

Local estimation (6)



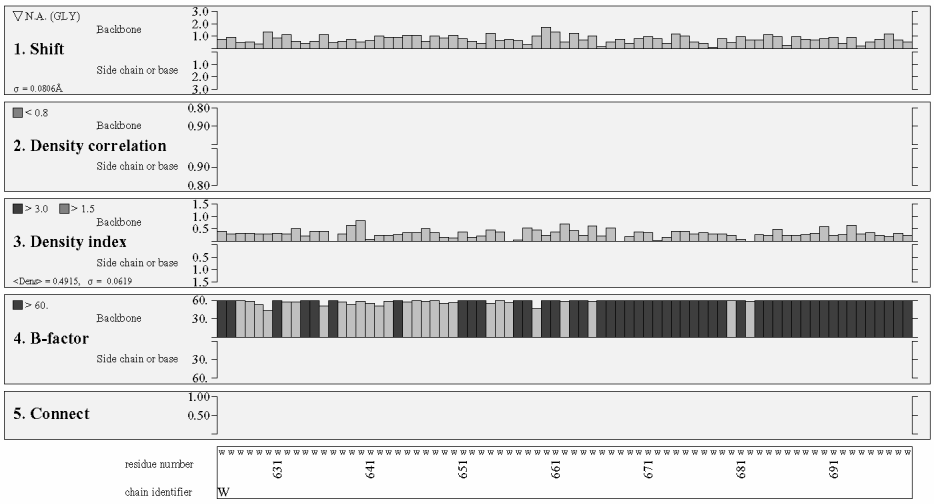


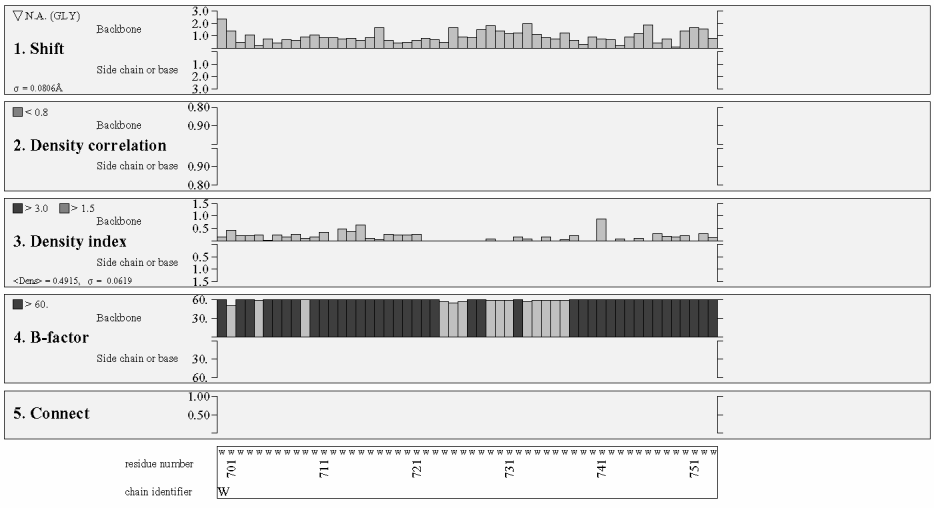
SFCHECK 6.0.3

Structure Factor Check

xxxx

Local estimation (7)





SFCHECK 6.0.3