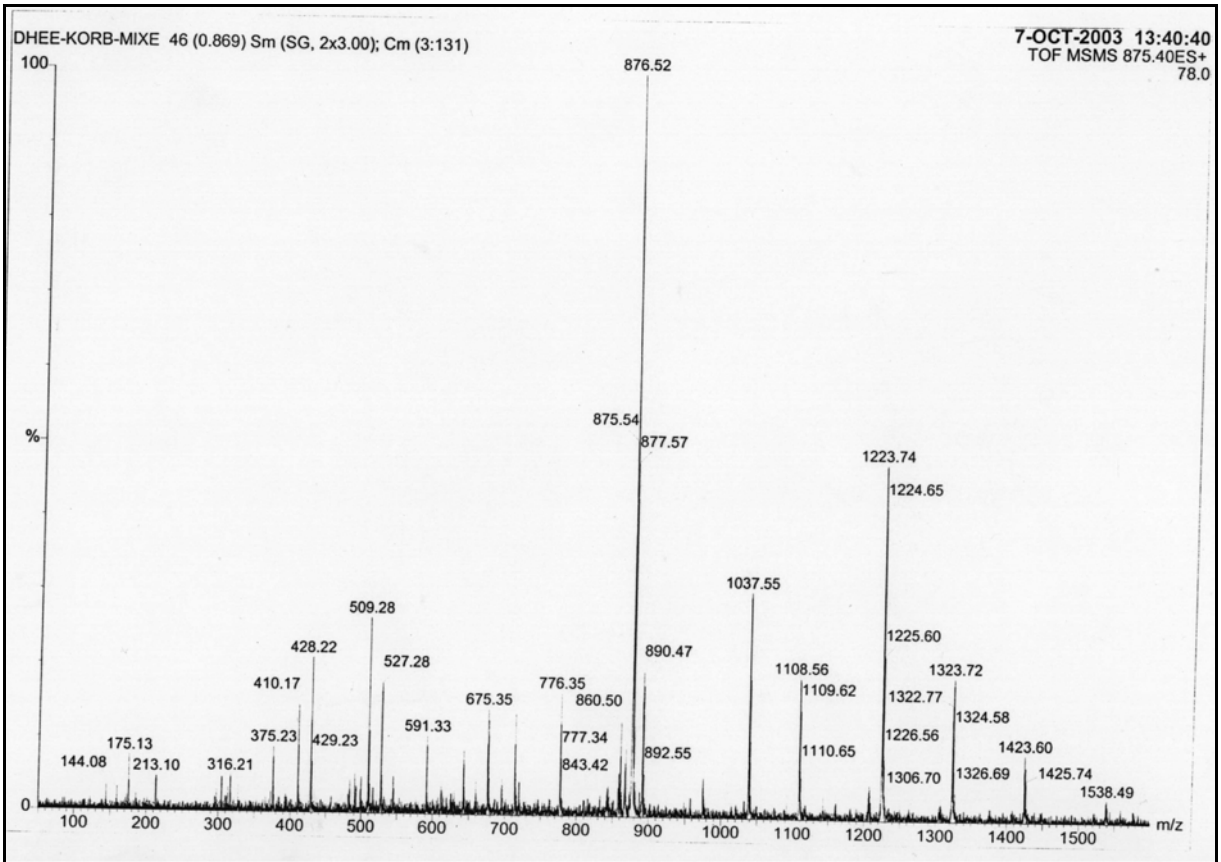
9. Appendix

I. Mass spectrometric proteolytic peptide map from the dissolved crystal after tryptic digest. Peptide sequence, DPNTVDAFNGQTDAER was identified corresponding to amino acid residues 256-271 from KorB.



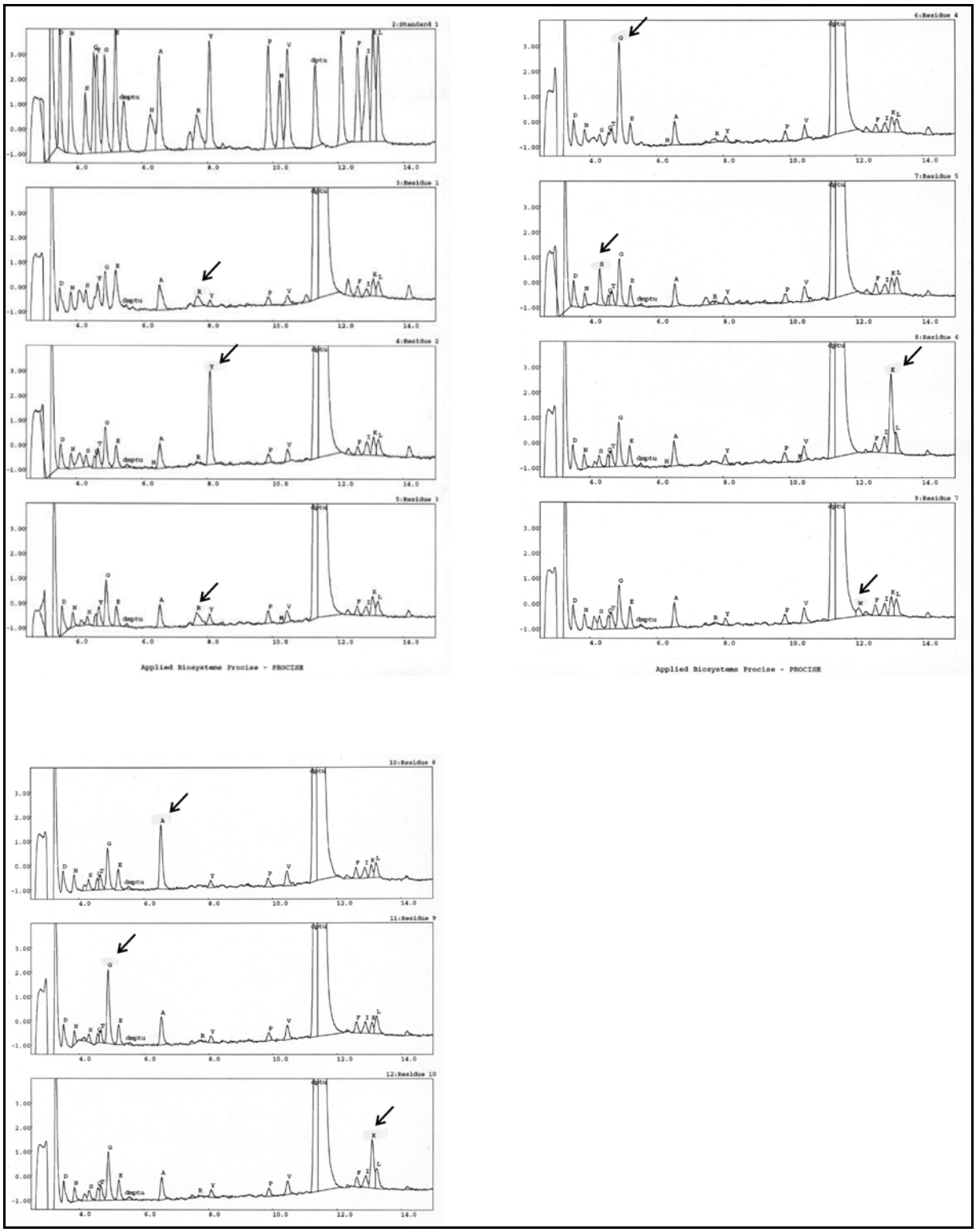
Printed: Tue Oct 07 13:43:54 2003

Average Mass = 1749.7687, Monoisotopic Mass = 1748.7601
Residues 1-16
N-Terminus = H, C-Terminus = OH
Fragment ions: Monoisotopic/Average (1750) m/z ratios with 1 positive charge(s).

a	88.04	185.09	299.14	400.18	499.25	614.28	685.32	832.38	946.43	1003.45	1131.51	1232.55	1347.58
b	116.03	213.09	327.13	428.18	527.25	642.27	713.31	860.38	974.42	1031.44	1159.50	1260.55	1375.58
c''	133.06	230.11	344.16	445.20	544.27	659.30	730.34	877.41	991.45	1048.47	1176.53	1277.58	1392.60
i	88.04	70.07	87.06	74.06	72.08	88.04	44.05	120.08	87.06	30.03	101.07	74.06	88.04
	1	2	3	4	5	6	7	8	9	10	11	12	13
	Asp	Pro	Asn	Thr	Val	Asp	Ala	Phe	Asn	Gly	Gln	Thr	Asp
	16	15	14	13	12	11	10	9	8	7	6	5	4
x	-	1660.72	1563.67	1449.62	1348.58	1249.51	1134.48	1063.44	916.38	802.33	745.31	617.25	516.21
y''	-	1634.74	1537.69	1423.65	1322.60	1223.53	1108.50	1037.47	890.40	776.35	719.33	591.27	490.23
z	-	1617.71	1520.66	1406.62	1305.57	1206.50	1091.48	1020.44	873.37	759.33	702.31	574.25	473.20

a	1418.62	1547.66	-
b	1446.61	1575.66	-
c''	1463.64	1592.68	-
i	44.05	102.06	129.11
	14	15	16
	Ala	Glu	Arg
	3	2	1
x	401.18	330.14	201.10
y''	375.20	304.16	175.12
z	358.17	287.14	158.09

II. N-terminal sequencing results from the dissolved crystal (amino acid residues 117-126, RYRGSKWAGK were identified)



III. Summary of KorB-O—O_B contacts
(Distances in Å)

Residue (contact)	**Protein Molecule**	**Overlapping DNA stands**			
		E/I	**F/J**	**H/K**	**G/L**
Lys171 NZ-O1P (THY3)	A	3.01/2.42			
	B		2.43/2.98		
	C			2.59/2.82	
	D				2.40/3.03
Gly172 N-O2P (BRU2)	A	2.72/2.93			
	B		2.63/2.93		
	C			2.74/3.08	
	D				2.81/2.68
Ser181 (THY12)	A N-O2P OG-O2P		2.70/3.22 3.02/2.53		
	B N-O2P OG-O2P	3.08/2.94 2.54/2.98			
	C N-O2P OG-O2P				2.68/3.34 2.83/2.62
	D N-O2P OG-O2P			3.08/2.97 2.43/3.06	
Thr186 OG1-O2P (THY3)	A	2.68/2.82			
	B		2.96/2.76		
	C			2.66/2.77	
	D				2.73/2.67
Val210 N-O2P (GUA10)	A		3.03/2.81		
	B	3.10/2.84			
	C				2.87/3.01
	D			2.96/2.88	
Thr211 N4 (CYT11) – OG1	A		3.26/2.76		
	B	2.84/3.03			
	C				2.79/2.86
	D			3.50/2.73	
Lys221 NZ-O1P (THY4)	A	3.40/3.10			
	B		3.85/4.24		
	C			3.43/2.90	
	D				2.56/3.00

Arg240 NH1-O6 (GUA10)	A NH1-O6 NH2-N7		3.41/2.89 3.50/2.83		
	B NH1-O6 NH2-N7	3.48/2.84 3.28/2.70			
	C NH1-O6 NH2-N7				3.59/2.68 3.28/2.65
	D NH1-O6 NH2-N7			3.22/2.89 3.15/2.82	
Arg247 (THY5)	A NH1-O2P NH2-O1P	3.75/3.04 3.68/2.73			
	B NH1-O2P NH2-O1P		3.93/2.98 3.88/2.66		
	C NH1-O2P NH2-O1P			3.42/3.05 3.94/2.51	
	D NH1-O2P NH2-O1P				3.46/3.71 2.86/3.36

IV. Summary of KorB-O—O_B contacts mediated by water molecules (Distances in Å)

Residue (contact)		Protein Molecule	Overlapping DNA stands			
			E/I	F/J	H/K	G/L
Lys171N	W63*	A	2.86 2.40/2.64			
-2BRU O1P	W222*	B		3.13 2.16/2.51		
	W136*	C			2.89 2.40/2.22	
	W60*	D				3.02 3.06/2.20
Pro182 O	W6	A	2.94 2.92/2.43			
3THY O2P	W35	B		2.81 2.71/3.31		
	W14	C			2.87 2.44/3.48	
	W13	D				2.86 2.51/3.00
Thr186OG1	W6	A	3.38 2.92/2.43			
3THY O2P	W35	B		3.53 2.71/3.31		
	W14	C			3.39 2.44/3.48	
	W13	D				3.27 2.51/3.00
Arg208N	W46	A		2.72 2.93/2.73		
9Cyt/GuaO2P	W78	B	2.97 2.68/2.81			
	W145	C				2.83 2.69/3.00
	W74	D			2.80 2.63/2.72	
Asp209	W46	A N- 9Cyt/GuaO2P		3.05 2.93/2.73		
	W131	OD1-10GuaO2P		3.29 2.85/2.83		
	W22*	OD2-9Cyt/GuaO2P		2.89 3.09/2.18		
	W78	B N-9Cyt/GuaO2P	3.25 2.68/2.81			
	W12	OD1-10GuaO2P	3.33 3.23/2.43			
	W32	OD2-9Cyt/GuaO2P	2.71 3.27/2.44			
	W145	C N -9Cyt/GuaO2P				3.16 2.69/3.00
	W18	OD1-10GuaO2P				3.07 2.93/2.67
	W41*	OD2-9Cyt/GuaO2P				2.65 2.06/3.20
	W74	D N –9Cyt/GuaO2P			3.20 2.63/2.72	
	W1	OD1-10GuaO2P			3.33 3.22/2.38	
	W108	OD2-9Cyt/GuaO2P			2.83 3.65/2.44	
Thr211	W131	A N & OG1 -10GuaO2P		3.15 & 2.71 2.85/2.83		
	W12	B N & OG1 -10GuaO2P	3.07&2.56 3.27/2.43			
	W18	C N & OG1 -10GuaO2P				2.99 & 2.56 2.93/2.67
	W1	D N & OG1 -10GuaO2P			3.05 & 2.64 3.14/2.38	

Glu215OE2	W86	A -7GuaO6	3.42 2.87/2.94			
	W119	-6AdeN7	3.08 3.38/3.25			
	W236	B -7GuaO6		3.49 3.12/2.56		
	W40	-6AdeN7		2.87 3.41/3.82		
	W45	C -6AdeN7			3.03 3.80/3.08	
	W65	-7GuaO6			3.37 3.45/2.04	
	W114	D -7GuaO6				3.37 2.74/3.06
	W156	-6AdeN7				2.87 3.25/3.74
Thr218	W143	A OG1-5ThyO2P	2.70 3.36/3.29			
	W159	OG1-4ThyO2P	3.22 2.45/2.65			
	W26	B OG1-5ThyO2P		2.75 3.19/3.37		
	W181	OG1-4ThyO2P		2.68 2.85/2.58		
	W144	C OG1-5ThyO2P			2.79 3.12/3.36	
		----no 2nd water				
	W121	D OG1-4ThyO2P				2.81 2.77/2.59
	W147	OG1-5ThyO2P				2.83 2.67/3.02
Ile238 O	W124*	A		2.92 3.34/2.36		
9Cyt/GuaO2P						
	W210	B	2.67 3.65/2.47			
	W248	C				2.95 2.89/3.00
	W74	D			4.11 2.63/2.72	
Arg240N	W112	A -O2P		2.89 2.79/3.01		
8Cyt	W25	B -O2P	3.06 2.72/3.01			
	W28	C -O2P				2.89 2.77/2.95
	W194	D -O2P			2.83 2.86/3.00	
Ala183N	W21	A		3.07		
		13AdeN7		2.93/3.56		
	W5	B	3.20			
		13AdeN7	2.77/3.33			
	W3	C				3.10
		13AdeN7				2.91/3.35
	W8	D			3.17	
		13AdeN7			3.24/2.67	
Gly241N	W167*	A		2.69 3.50/2.24		
8CytO2P	W43*	B	2.87 3.43/2.08			
	W169*	C				2.99 2.18/2.90
	W263*	D			2.80 3.17/1.99	

*** Water molecules with half occupancy**

V. Van der Waals interactions

(Distances in Å)

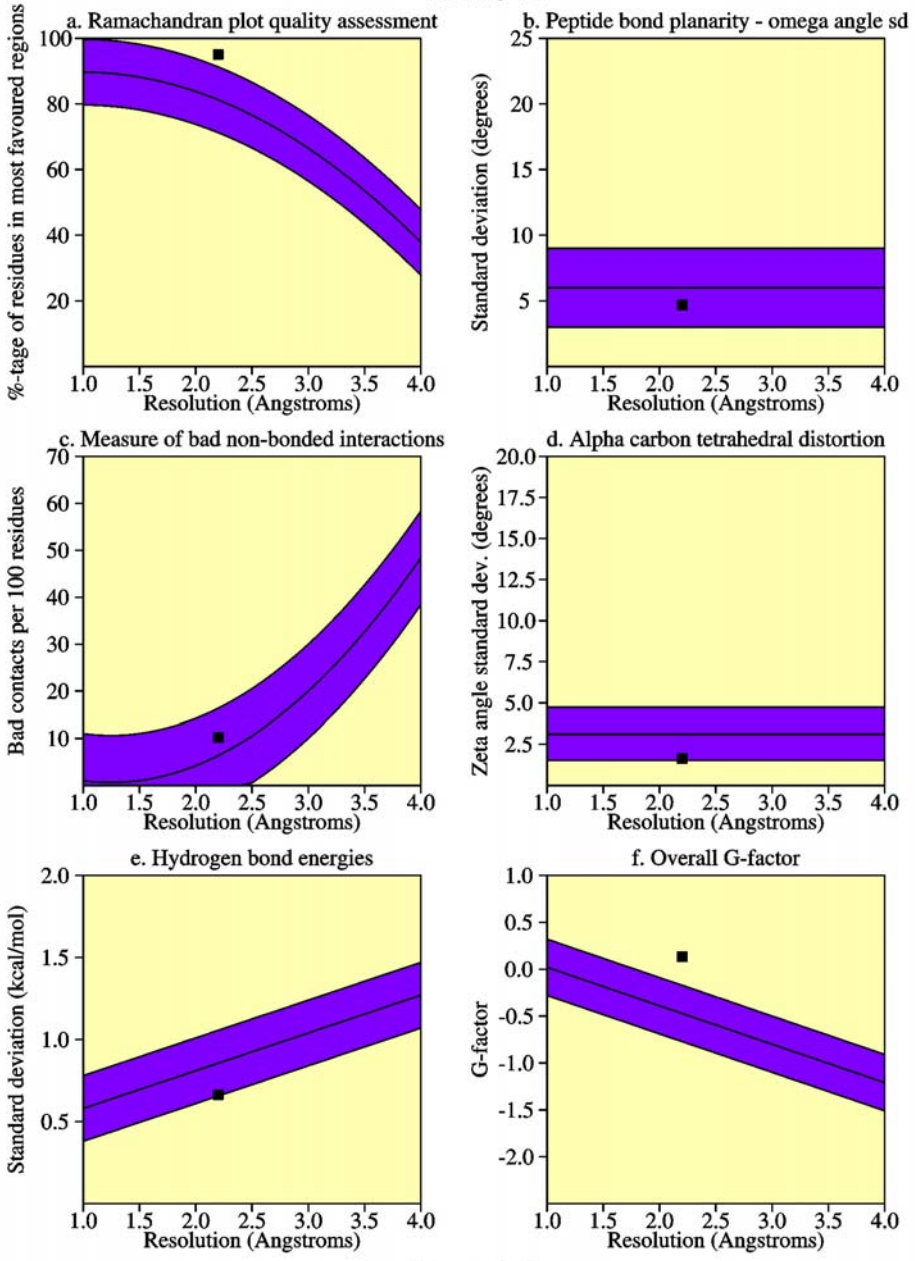
Residue (contact)	**Protein Molecule**	**Overlapping DNA stands**			
		E/I	**F/J**	**H/K**	**G/L**
Ser181OG -C5M 12Thy	A		3.69/4.57		
	B	3.64/4.49			
	C				3.85/4.71
	D			4.73/4.07	
Pro182CB -C5M 3Thy	A	3.84/3.53			
	B		3.28/4.03		
	C			3.06/4.29	
	D				3.69/3.54
Ala183CB	A -C5M 4Thy -C5M 12Thy	3.83/4.25	3.47/3.62		
	B -C5M 4Thy -C5M 12Thy	3.45/3.66	3.81/4.38		
	C -C5M 4Thy -C5M 12Thy			3.95/4.34	3.55/3.78
	D -C5M 4Thy -C5M 12Thy			3.65/3.69	3.91/4.36
Thr186OG1 C5M 3Thy	A	3.49/3.87			
	B		3.52/4.06		
	C			4.02/3.62	
	D				4.55/3.39
Thr186CB C5M 4Thy	A	3.60/4.13			
	B		3.43/4.30		
	C			3.81/3.94	
	D				3.76/4.46
Gln187OE1 C5M 12Thy	A		3.91/3.51		
	B	3.94/3.58			
	C				3.80/3.66
	D			3.89/3.65	

VI. PROHECK summary of 1R71 (KorB-O—O_B complex)

PROCHECK

Main-chain parameters

1R71

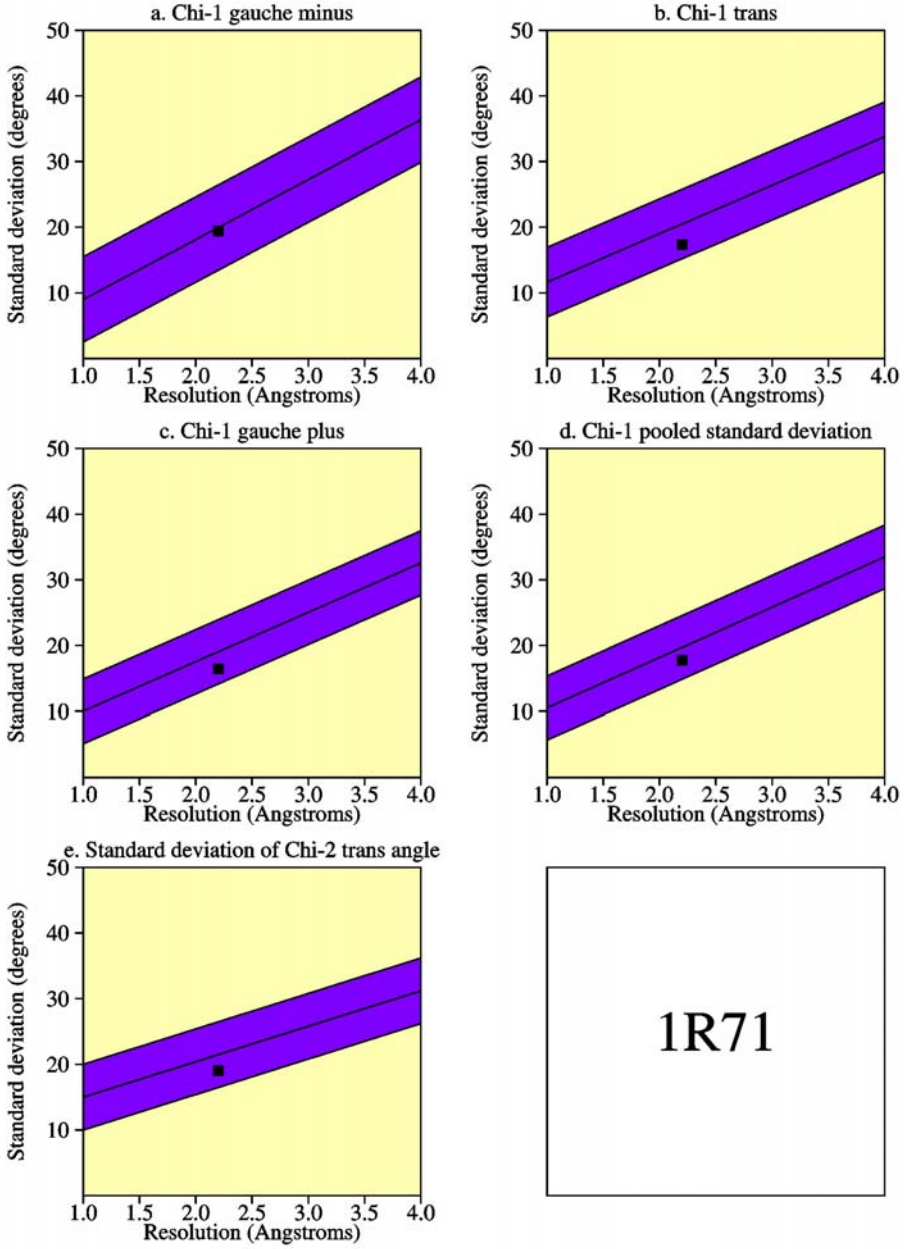


Plot statistics

Stereochemical parameter	No. of data pts	Parameter value	Comparison values Typical value	Band width	No. of band widths from mean	
a. %-tage residues in A, B, L	409	95.1	81.3	10.0	1.4	BETTER
b. Omega angle st dev	450	4.7	6.0	3.0	-0.4	Inside
c. Bad contacts / 100 residues	48	10.2	6.4	10.0	0.4	Inside
d. Zeta angle st dev	433	1.6	3.1	1.6	-0.9	Inside
e. H-bond energy st dev	323	0.7	0.9	0.2	-1.0	Inside
f. Overall G-factor	469	0.1	-0.5	0.3	2.0	BETTER

PROCHECK

Side-chain parameters
1R71



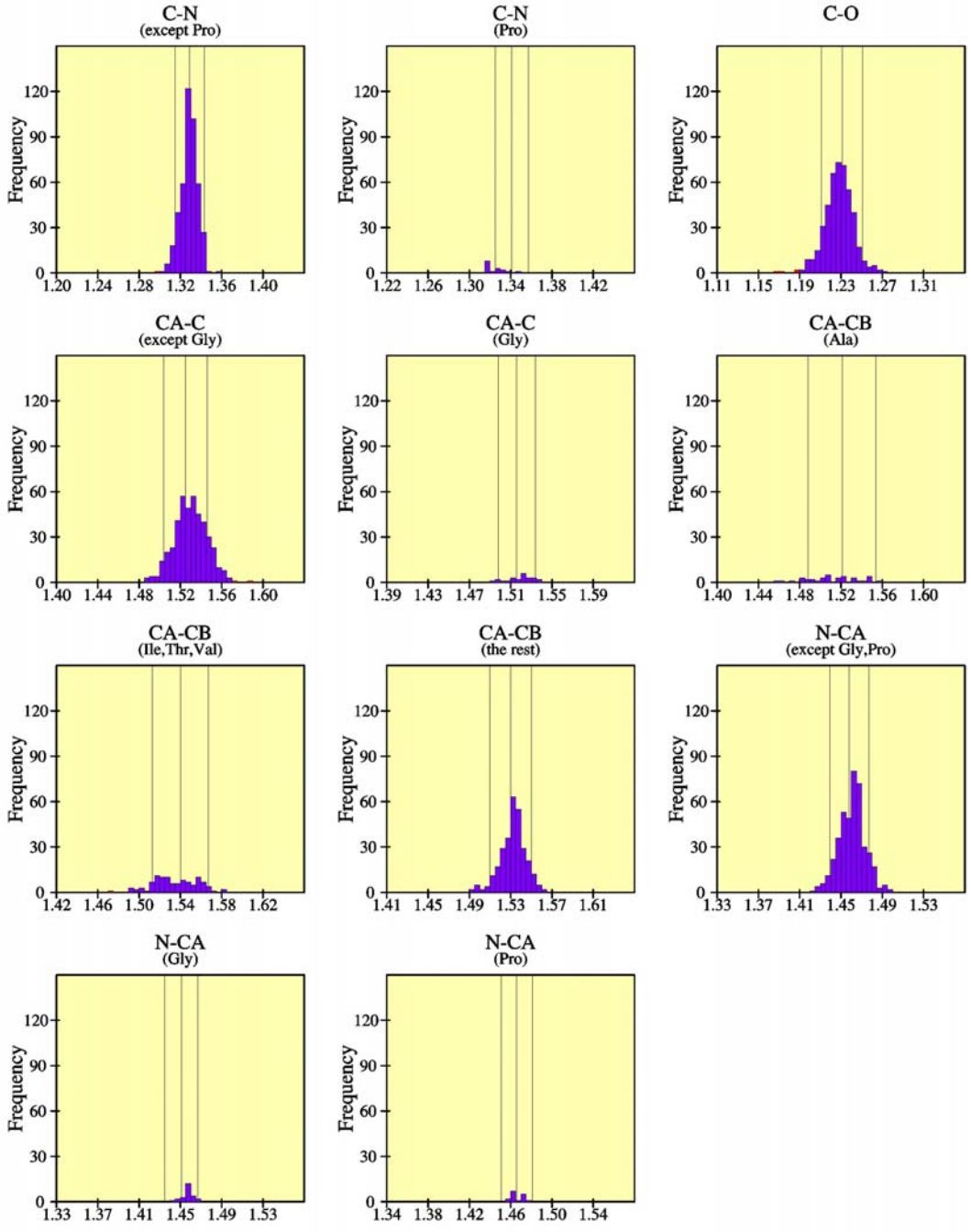
Plot statistics

Stereochemical parameter	No. of data pts	Parameter value	Comparison values Typical value	Band width	No. of band widths from mean	
a. Chi-1 gauche minus st dev	33	19.4	19.9	6.5	-0.1	Inside
b. Chi-1 trans st dev	119	17.4	20.5	5.3	-0.6	Inside
c. Chi-1 gauche plus st dev	229	16.4	19.0	4.9	-0.5	Inside
d. Chi-1 pooled st dev	381	17.7	19.7	4.8	-0.4	Inside
e. Chi-2 trans st dev	146	19.0	21.5	5.0	-0.5	Inside

PROCHECK

Main-chain bond lengths

1R71



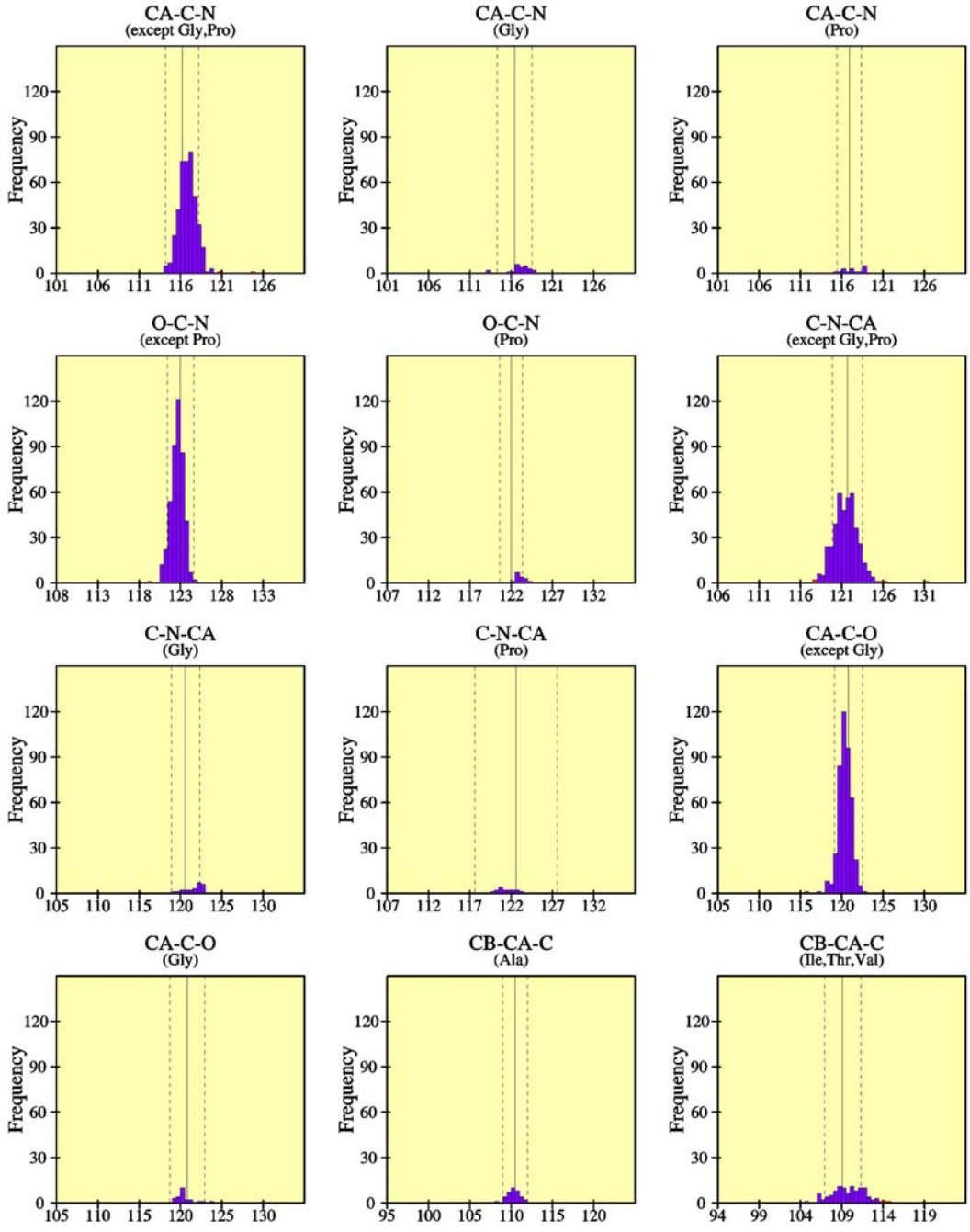
Black bars > 2.0 st. devs. from mean.

Solid and dashed lines represent the mean and standard deviation values as per Engh & Huber small-molecule data.

PROCHECK

Main-chain bond angles

1R71



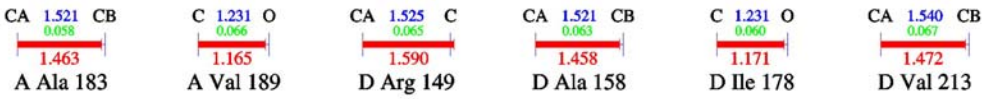
Black bars > 2.0 st. devs. from mean.

Solid and dashed lines represent the mean and standard deviation values as per Engh & Huber small-molecule data.

PROCHECK

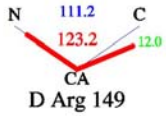
Distorted geometry
1R71

Main-chain bond lengths



Bonds differing by > 0.05A from small-molecule values. Values shown: "ideal", difference, actual

Main-chain bond angles



Bond angles differing by > 10.0 degrees from small-molec values. Values shown: "ideal", actual, diff.

Planar groups

Sidechains with RMS dist. from planarity > 0.03A for rings, or > 0.02A otherwise. Value shown is RMS dist.

VII. PROMOTIF summary of 1R71 (KorB-O—O_B complex)

1R71_summary_01.ps

Summary: 1R71

TRANSCRIPTION/DNA MOL_ID: 1; MOL_ID: 1;

Sequence:

EADQVIENLQRNELTPREIADFIGRELAKGKKKGDIAKEIGKSPAFITQHVTLLDLPEKIADAFNTGRVRDVTVVNELVT
AFKKRPEEVEAWLDDDTQEITRGTVKLLREFLDEYNEADQVIENLQRNELTPREIADFIGRELAKGKKKGDIAKEIGKSP
AFITQHVTLLDLPEKIADAFNTGRVRDVTVVNELVTAFKKRPEEVEAWLDDDTQEITRGTVKLLREFLDEADQVIENLQR
NELTPREIADFIGRELAKGKKKGDIAKEIGKSPAFITQHVTLLDLPEKIADAFNTGRVRDVTVVNELVTAFKKRPEEVEA
WLDDDTQEITRGTVKLLREFLDNEADQVIENLQRNELTPREIADFIGRELAKGKKKGDIAKEIGKSPAFITQHVTLLDLP
EKIADAFNTGRVRDVTVVNELVTAFKKRPEEVEAWLDDDTQEITRGTVKLLREFLDE

Number of residues is: 457 Number of chains is: 4

Number of strands is: 0 Number of alpha helices is: 32 Number of 3,10 helices is: 4

Percentage of strand is: 0.0 Percentage of alpha helix is: 68.5 Percentage of 3,10 helix is: 1.8

Secondary Structure Summary:

HTHTHHGHTHHTHHTHTHTHGHTHHTHHTHTHTHGHTHHTHHTHTHTHGHTHHTH

HELICES

Location	Type
C 224 -C 232	H
C 240 -C 249	H
D 139 -D 147	H
D 154 -D 166	H
D 171 -D 177	H
D 182 -D 189	H
D 190 -D 192	3
D 196 -D 203	H
D 210 -D 222	H
D 224 -D 232	H
D 240 -D 251	H

G-TURNS

Location	Seq	Type
D 232 - 234	DDD	INV

HELICES

Location	Type
A 141 -A 148	H
A 154 -A 166	H
A 171 -A 178	H
A 182 -A 189	H
A 190 -A 192	3
A 196 -A 203	H
A 210 -A 222	H
A 224 -A 232	H
A 240 -A 251	H
B 139 -B 148	H
B 154 -B 166	H
B 171 -B 177	H
B 182 -B 189	H
B 190 -B 192	3
B 196 -B 203	H
B 210 -B 222	H
B 224 -B 232	H
B 240 -B 250	H
C 141 -C 148	H
C 154 -C 166	H
C 171 -C 177	H
C 182 -C 189	H
C 190 -C 192	3
C 196 -C 203	H
C 210 -C 222	H

B-TURNS

Location	Seq	Type
A 205 - 208	GRVR	VIII
A 233 - 236	DDTQ	I
B 205 - 208	GRVR	VIII
B 233 - 236	DDTQ	I
C 205 - 208	GRVR	VIII
C 233 - 236	DDTQ	I
D 147 - 150	LQRN	IV
D 205 - 208	GRVR	VIII
D 233 - 236	DDTQ	I

G-TURNS

Location	Seq	Type
B 250 - 252	LDE	INV
C 249 - 251	FLD	INV

1R71_helix_tab01.ps

Helices: 1R71

Helix Number	Start Residue	End Residue	Helix Type	No. of Residues	Sequence
1	A 141	A 148	α	8	DQVIENLQ
2	A 154	A 166	α	13	PREIADFIGRELA
3	A 171	A 178	α	8	KGDIAKEI
4	A 182	A 189	α	8	PAFITQHV
5	A 190	A 192	3_{10}	3	TLL
6	A 196	A 203	α	8	EKIADAFN
7	A 210	A 222	α	13	VTVVNELVTAFKK
8	A 224	A 232	α	9	PEEVEAWLD
9	A 240	A 251	α	12	RGTVKLLREFLD
10	B 139	B 148	α	10	EADQVIENLQ
11	B 154	B 166	α	13	PREIADFIGRELA
12	B 171	B 177	α	7	KGDIAKE
13	B 182	B 189	α	8	PAFITQHV
14	B 190	B 192	3_{10}	3	TLL
15	B 196	B 203	α	8	EKIADAFN
16	B 210	B 222	α	13	VTVVNELVTAFKK
17	B 224	B 232	α	9	PEEVEAWLD
18	B 240	B 250	α	11	RGTVKLLREFL
19	C 141	C 148	α	8	DQVIENLQ
20	C 154	C 166	α	13	PREIADFIGRELA
21	C 171	C 177	α	7	KGDIAKE
22	C 182	C 189	α	8	PAFITQHV
23	C 190	C 192	3_{10}	3	TLL
24	C 196	C 203	α	8	EKIADAFN
25	C 210	C 222	α	13	VTVVNELVTAFKK
26	C 224	C 232	α	9	PEEVEAWLD
27	C 240	C 249	α	10	RGTVKLLREF
28	D 139	D 147	α	9	EADQVIENL
29	D 154	D 166	α	13	PREIADFIGRELA
30	D 171	D 177	α	7	KGDIAKE

1R71_helix_tab02.ps

Helices: 1R71

Helix Number	Start Residue	End Residue	Helix Type	No. of Residues	Sequence
31	D 182	D 189	α	8	PAFITQHV
32	D 190	D 192	3_{10}	3	TLL
33	D 196	D 203	α	8	EKIADAFN
34	D 210	D 222	α	13	VTVVNELVTAFKK
35	D 224	D 232	α	9	PEEVEAWLD
36	D 240	D 251	α	12	RGTVKLLREFLD

1R71_helix_geom01.ps

Helix Geometry: 1R71

Helix Number	Length (A)	Unit Rise (A)	Residues per turn	Pitch (A)	Deviation from ideal (degrees)
1	12.22	1.47	3.63	5.34	12.4
2	19.97	1.50	3.60	5.39	4.9
3	12.52	1.54	3.66	5.65	12.4
4	12.81	1.54	3.65	5.62	13.4
5	-	-	-	-	-
6	12.08	1.45	3.69	5.35	13.6
7	20.23	1.54	3.51	5.42	5.6
8	14.00	1.49	3.61	5.37	8.6
9	18.85	1.51	3.70	5.60	9.5
10	15.48	1.50	3.59	5.39	6.1
11	20.01	1.50	3.64	5.45	5.6
12	10.95	1.50	3.59	5.38	10.2
13	12.78	1.54	3.63	5.58	13.5
14	-	-	-	-	-
15	12.00	1.44	3.70	5.32	14.2
16	20.20	1.54	3.53	5.43	4.8
17	14.05	1.49	3.60	5.38	10.4
18	16.67	1.47	3.66	5.38	7.5
19	12.75	1.52	3.72	5.64	12.3
20	20.12	1.50	3.61	5.41	4.8
21	10.93	1.49	3.63	5.40	9.4
22	12.71	1.52	3.65	5.55	13.1
23	-	-	-	-	-
24	12.21	1.48	3.70	5.46	14.7
25	20.27	1.55	3.52	5.45	5.6
26	14.39	1.53	3.67	5.62	10.5
27	15.36	1.48	3.62	5.37	3.5
28	13.54	1.42	3.66	5.20	9.2
29	20.12	1.50	3.61	5.43	5.9
30	10.90	1.48	3.63	5.39	9.2

1R71_helix_geom02.ps

Helix Geometry: 1R71

Helix Number	Length (A)	Unit Rise (A)	Residues per turn	Pitch (A)	Deviation from ideal (degrees)
31	12.78	1.53	3.62	5.55	13.3
32	-	-	-	-	-
33	11.95	1.44	3.70	5.33	13.6
34	20.31	1.55	3.53	5.47	6.1
35	14.15	1.51	3.64	5.49	10.4
36	18.62	1.49	3.68	5.49	8.9

VIII. SFCHECK summary

Structure Factor Check
1R71

Title: CRYSTAL STRUCTURE OF THE DNA BINDING DOMAIN OF KORB IN COMPLEX WITH THE OPERATOR DNA
Date: 17–OCT–03
PDB code: 1R71

Crystal

Cell parameters:

a: 110.44 Å	b: 110.44 Å	c: 160.53 Å
α: 90.00°	β: 90.00°	γ: 120.00°

Space group: P 32 2 1

Model

6769 atoms (347 water molecules)

Number of chains:	**13**
Volume not occupied by model:	57.9 %
<B> (for atomic model):	44.2 $Å^2$
σ(B):	19.75 $Å^2$
Matthews coefficient:	2.98
Corresponding solvent % :	58.43

Refinement

Program:	REFMAC 5.1.24
Nominal resolution range:	30.00 – 2.20 Å
Reported R–factor:	0.195
Number of reflections used:	54758
Reported Rfree:	0.25
Sigma cut–off:	N.A.

Structure Factors

Input

Nominal resolution range:		27.61 – 2.20 Å
Reflections in file:		57669
Unique reflections	above 0:	57669
	above 1σ:	57020
	above 3σ:	42783

SFCHECK

Nominal resolution range: (max. from input data, min. from author)	27.61 – 2.20 Å
Used reflections:	57669
Completeness:	99.5 %
R_stand(F) = <σ(F)>/<F> :	0.045
Anisotropic distribution of Structure Factors ratio of eigen values:	0.9089 0.9089 1.0000
B_overall (by Patterson):	35.6 $Å^2$
Optical resolution:	1.74 Å
Expected opt. resol. for complete data set:	1.74 Å
Estimated minimal error:	0.031 Å

Model vs. Structure Factors

R–factor for all reflections:	0.234
Correlation factor:	0.929
R–factor:	0.239
for F > 2.0σ	
nom. resolution range:	30.00 – 2.20 Å
reflections used:	57017
Rfree:	0.285
Nfree:	2872
R–factor without free–refl.:	0.236
Non free–reflections:	54145
<u> (error in coords by Luzzati plot):	0.285 Å
Estimated maximal error:	0.148 Å
DPI:	0.261 Å

Scaling

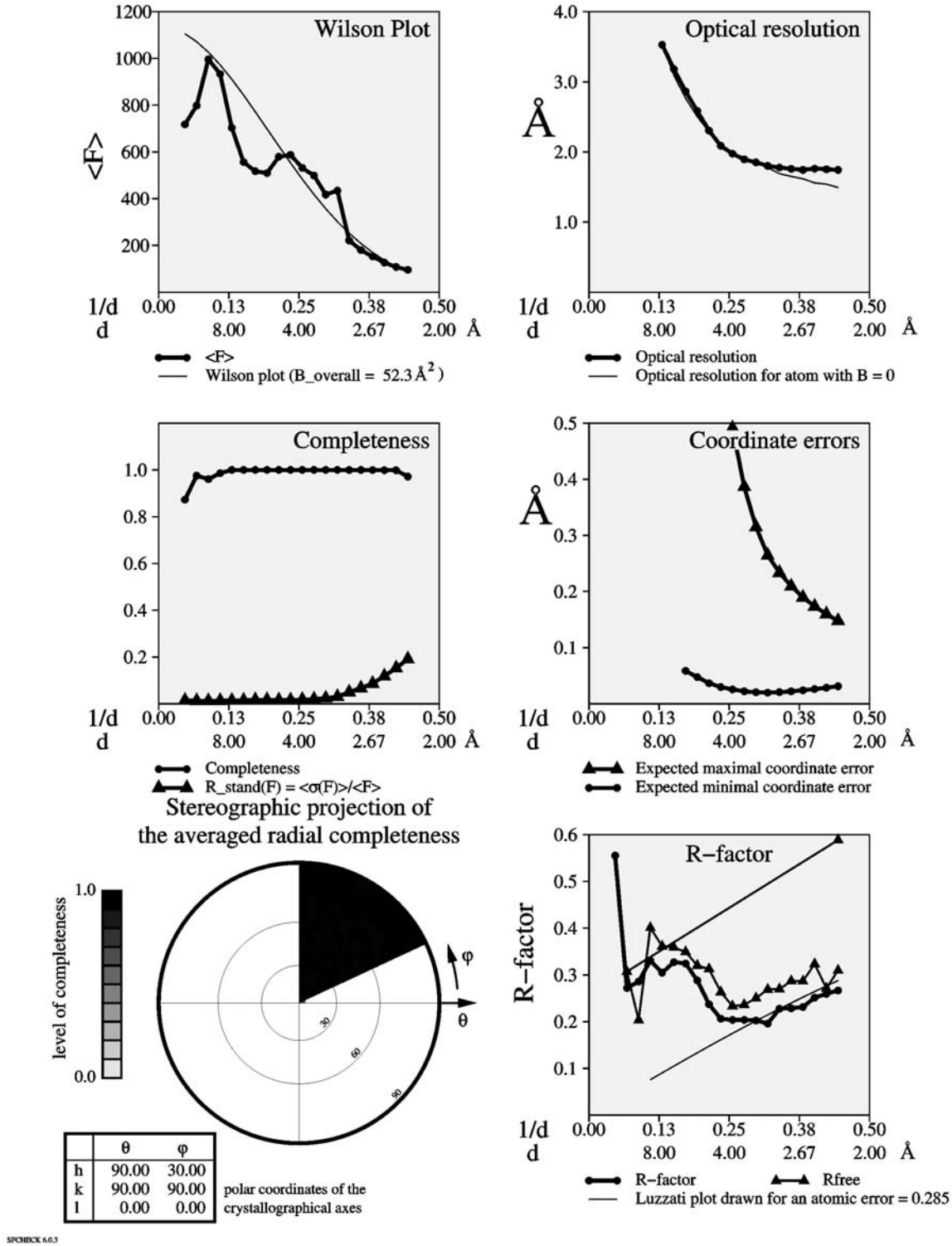
Scale:	0.826
Bdiff:	–5.99

Anisothermal Scaling (Beta):
0.7451 0.7451 0.9883 0.3726 0.0000 0.0000

Solvent correction – Ks,Bs: 0.805 238.647

SFCHECK 6.0.3

Structure Factor Check
1R71



	θ	φ
h	90.00	30.00
k	90.00	90.00
l	0.00	0.00