Structural Opportunities

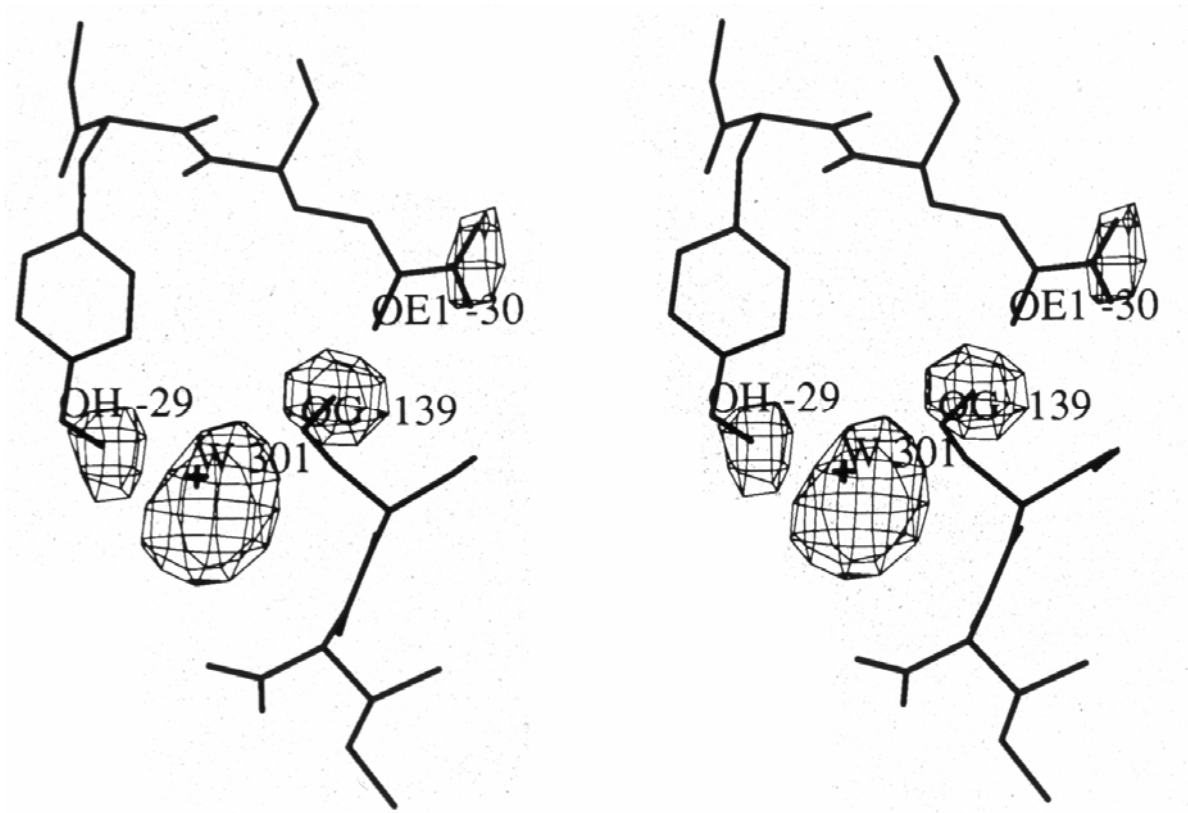
Systems where H's perform function, but.....

Structural details of protein packing are a surprisingly fertile area.

H/D exchange is an underutilized method to study protein dynamics and spatial organization of secondary structure

Hydroxyl orientations are the most valuable probe to assess electrostatic/van der Waals forces in protein packing.

Assignment of charge distributions using X-N synthesis



Kossiakoff, A.A., et. al. Proteins (1992) 12, 223-236.

D_2O-H_2O Solvent difference maps

A powerful and UNBIASED method to locate exchangeable H's.

For protonation states, hydroxyl rotors, H/D exchange, water orientations, deamidation

Easier said than done. Perdeuteration to the rescue

D20 - H20 SOLVENT MAPS

Need: 1 data set D_2O, 1 set H_2O
(H_2O set much more difficult - incoherent scattering)

Success based on:

1) Scattering difference D vs. H (6.7 vs. -3.8)

2) Protein region identical in D_2O and H_2O, therefore in map density = 0

3) All ordered water no further than 4Å from protein surface.

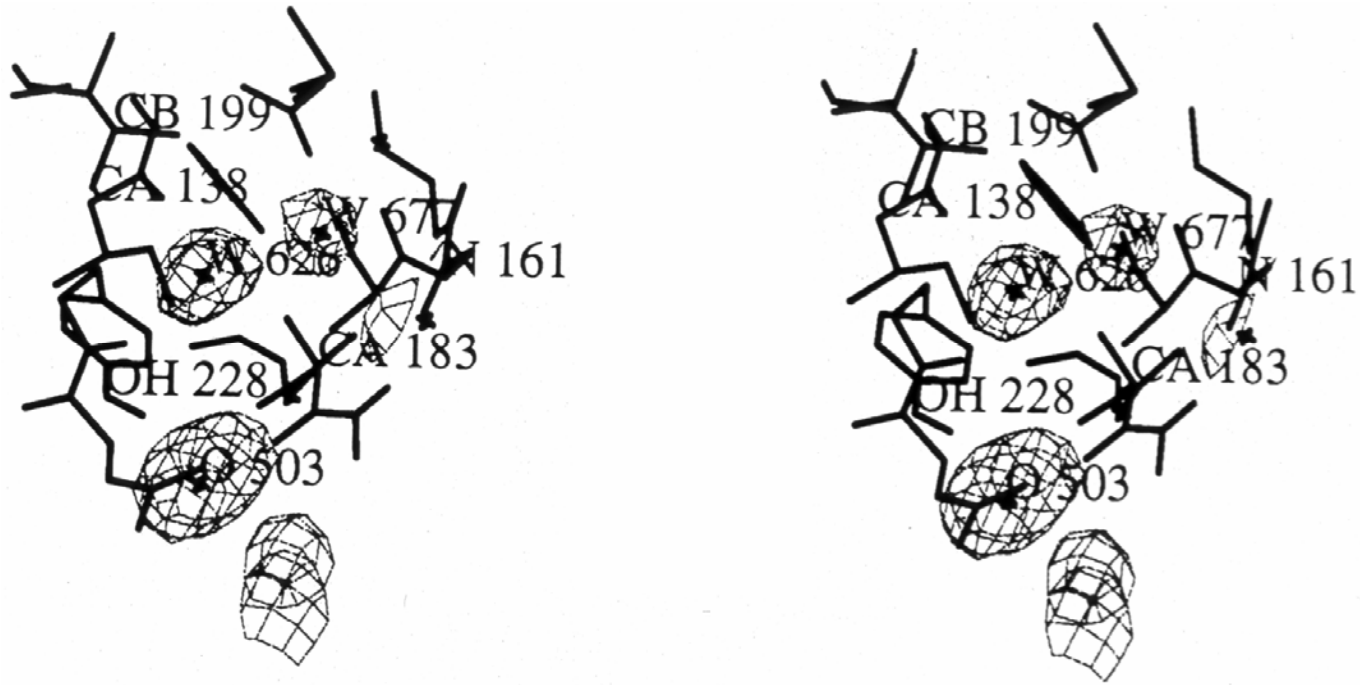
Apply density modification:

a) protein=0 }
bulk=constant } } 60% of cell

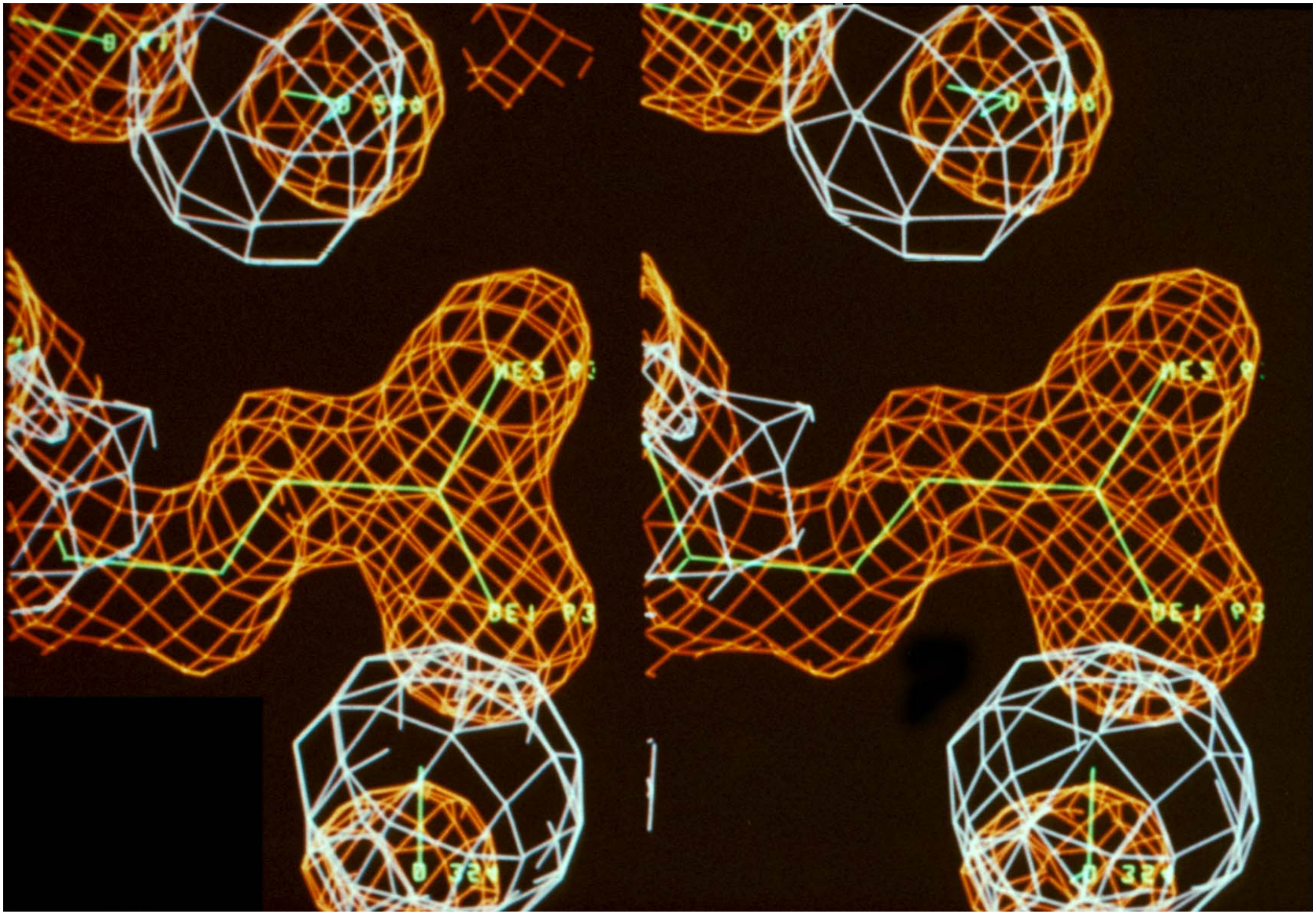
b) no solvent density < bulk average

Strong restraints- lots of feedback

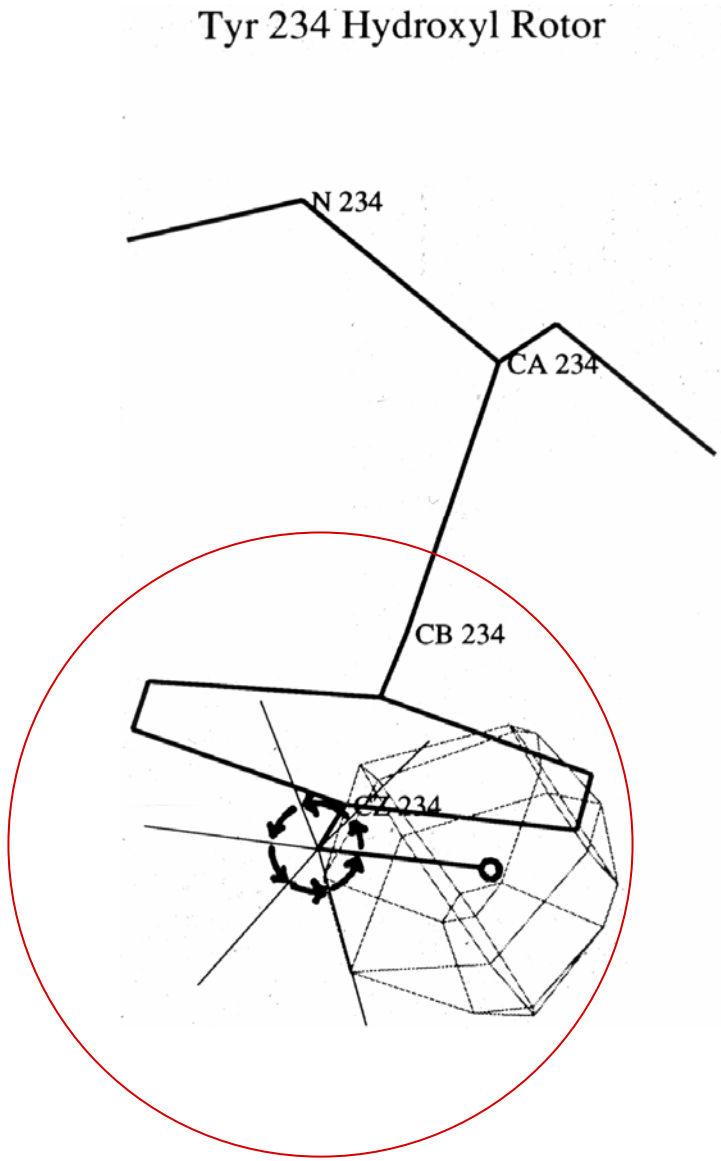
Kossiakoff, A.A., et. al. Proteins (1992) 12, 223-236.



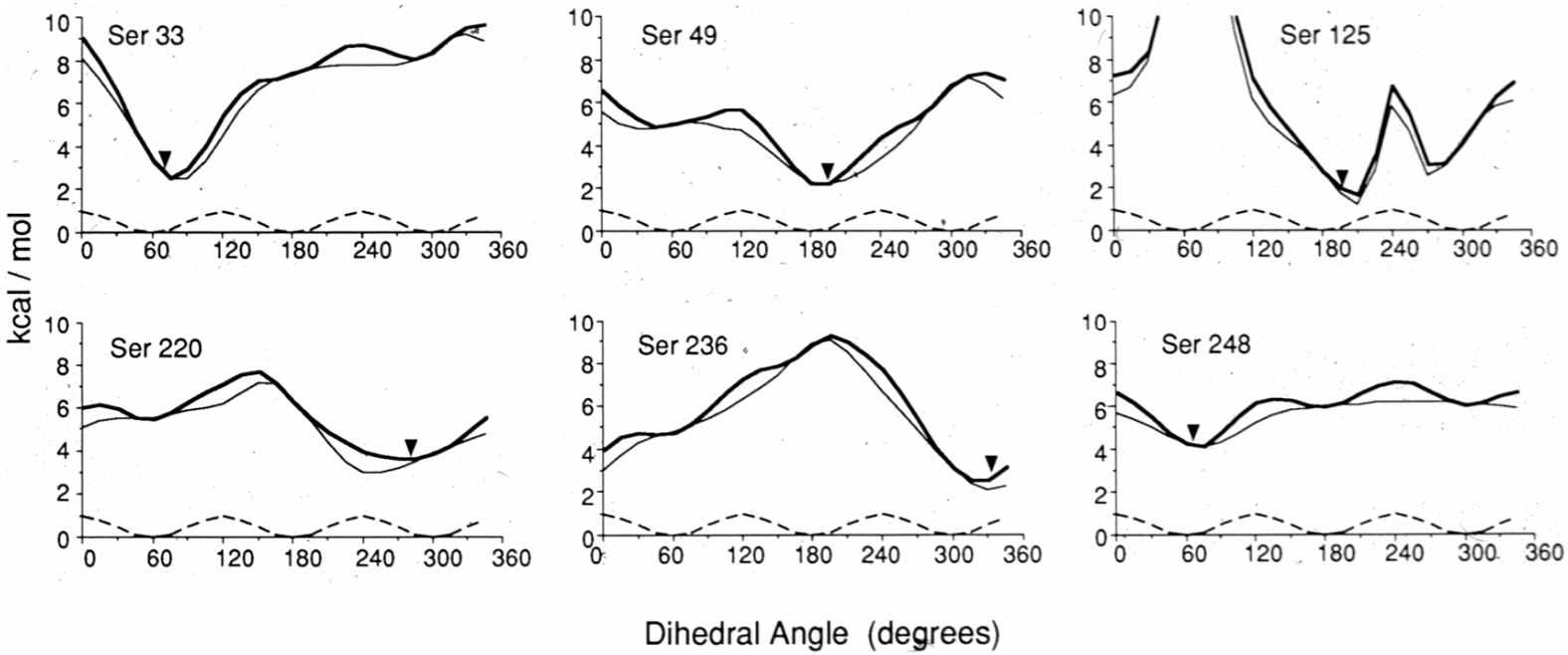
Kossiakoff, A.A., et. al. Proteins (1992) 12, 223-236.

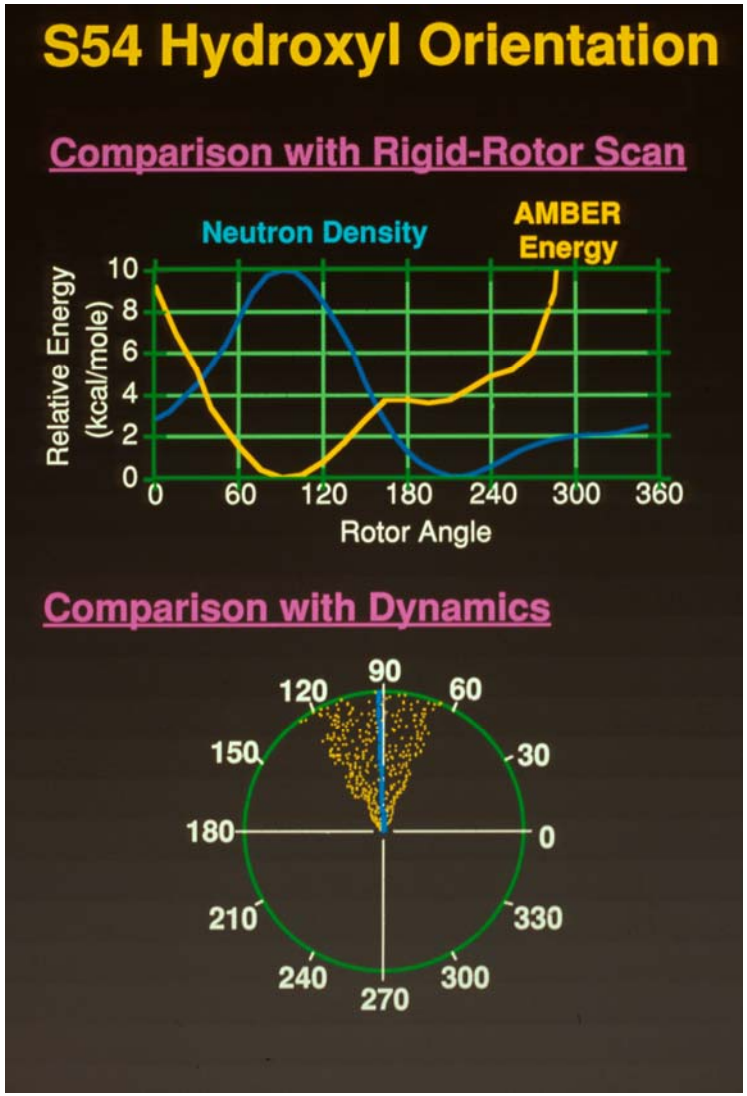


Finer-Moore, J.S. et. al. Proteins (1992) 12, 203-222.

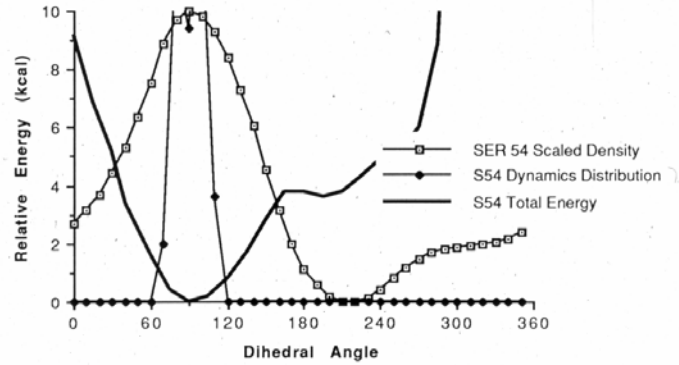


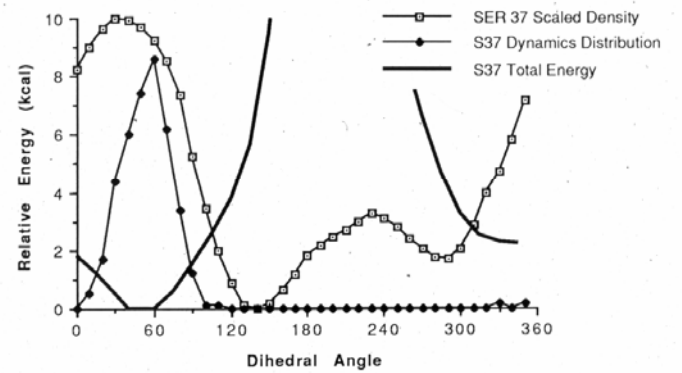
Kossiakoff, A.A., et. al. Proc. Natl. Acad. Sci. USA (1990) 87, 4468-4472.

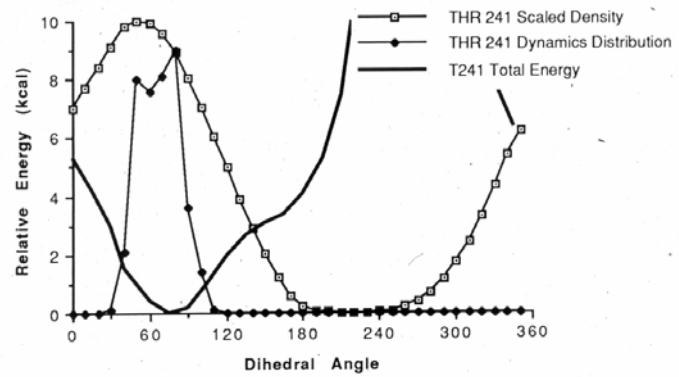

Ser 33
Ser 49
Ser 125
Ser 220
Ser 236
Ser 248
kcal / mol
Dihedral Angle (degrees)
0
60
120
180
240
300
360
2
4
6
8
10

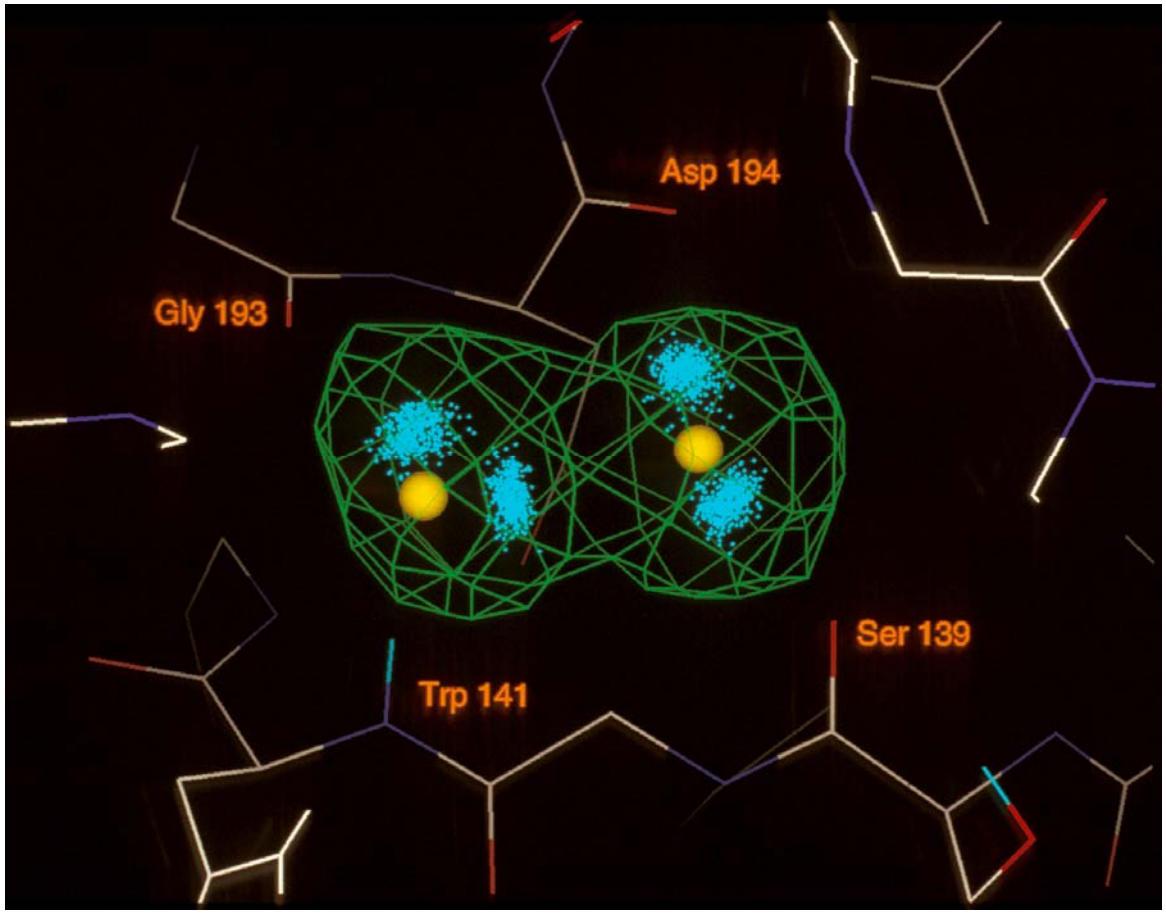


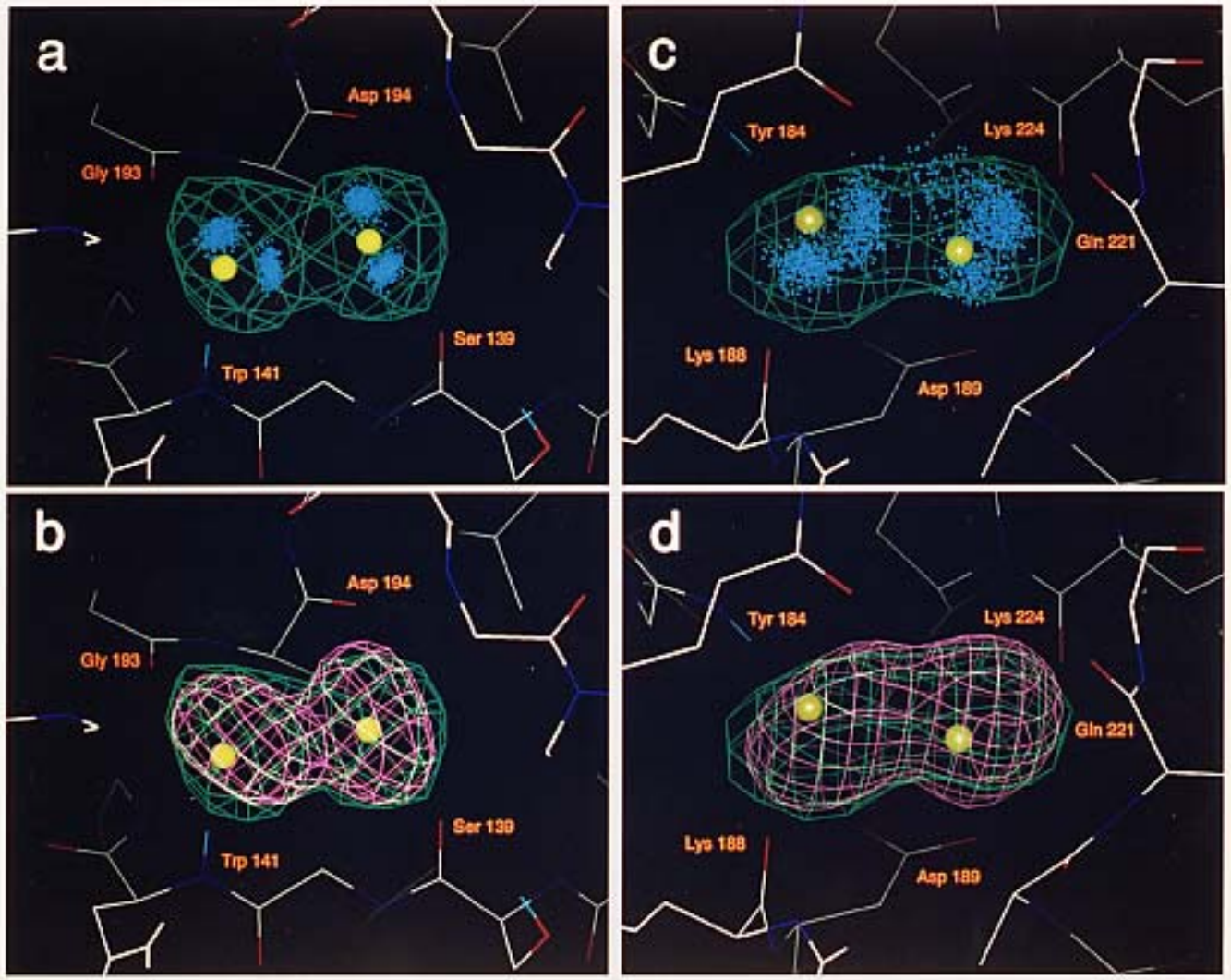
McDowell, R. S. and Kossiakoff, A.A. “A comparison of neutron diffraction and molecular dynamics structures: Hydroxyl group and water molecule orientations in trypsin. J. Mol. Biol. (1995) 250, 553-570.

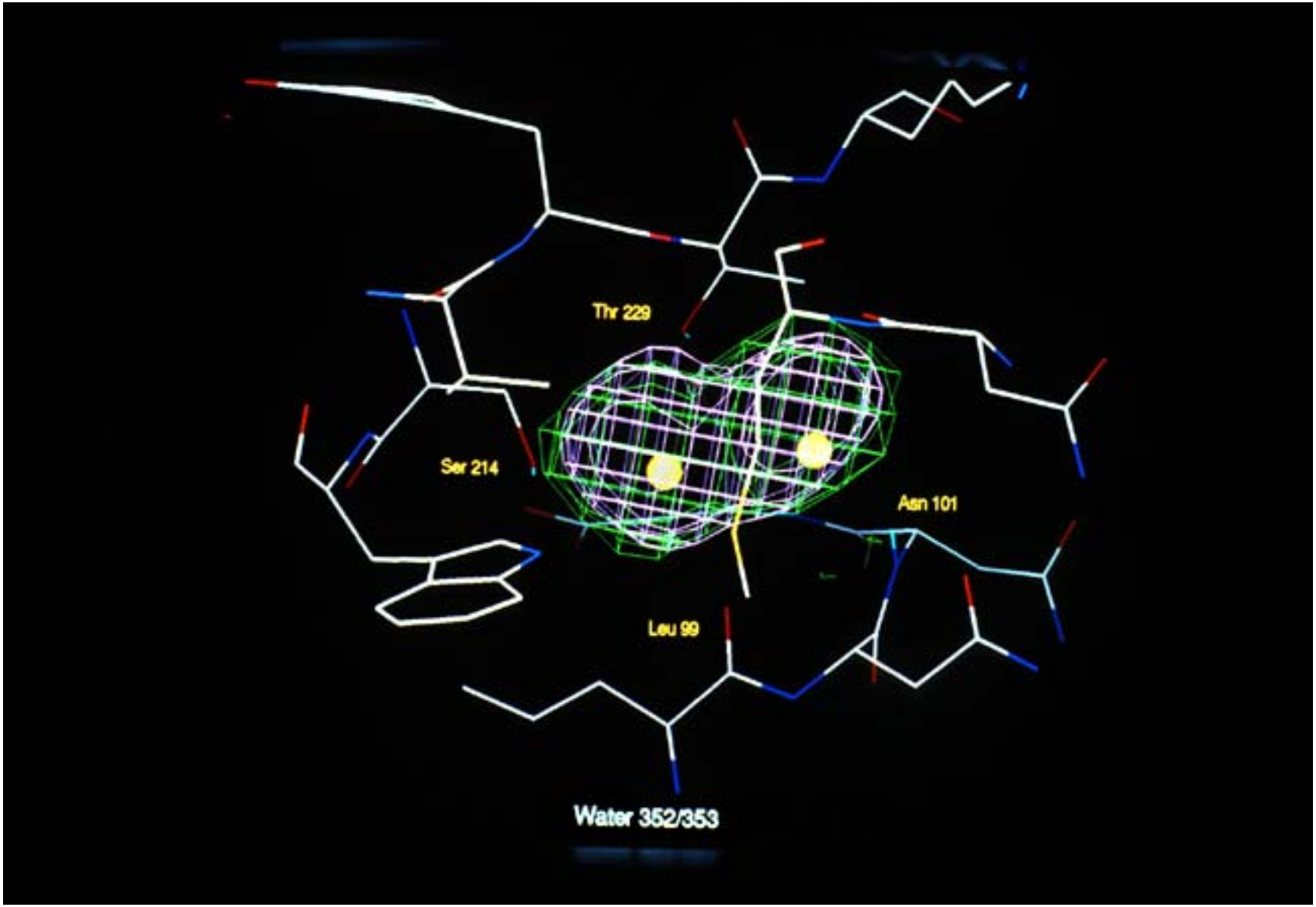

Relative Energy (kcal)
0
2
4
6
8
10
0
60
120
180
240
300
360
Dihedral Angle
SER 54 Scaled Density
S54 Dynamics Distribution
S54 Total Energy


Relative Energy (kcal)
0
2
4
6
8
10
0
60
120
180
240
300
360
Dihedral Angle
SER 37 Scaled Density
S37 Dynamics Distribution
S37 Total Energy

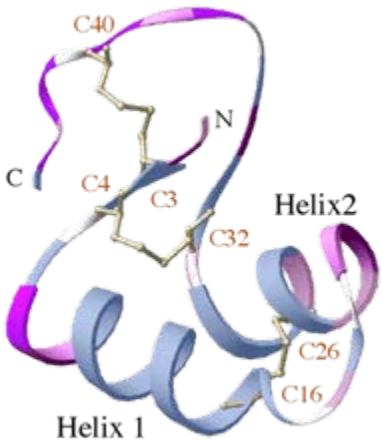

Relative Energy (kcal)
0
2
4
6
8
10
0
60
120
180
240
300
360
Dihedral Angle
THR 241 Scaled Density
THR 241 Dynamics Distribution
T241 Total Energy


Asp 194
Gly 193
Ser 139
Trp 141


a
Asp 194
Gly 193
Ser 139
Trp 141
c
Tyr 184
Lys 224
Gln 221
Lys 188
Asp 189
b
Asp 194
Gly 193
Ser 139
Trp 141
d
Tyr 184
Lys 224
Gln 221
Lys 188
Asp 189


Thr 229
Ser 214
Asn 101
Leu 99
Water 352/353

Crambin at high resolution

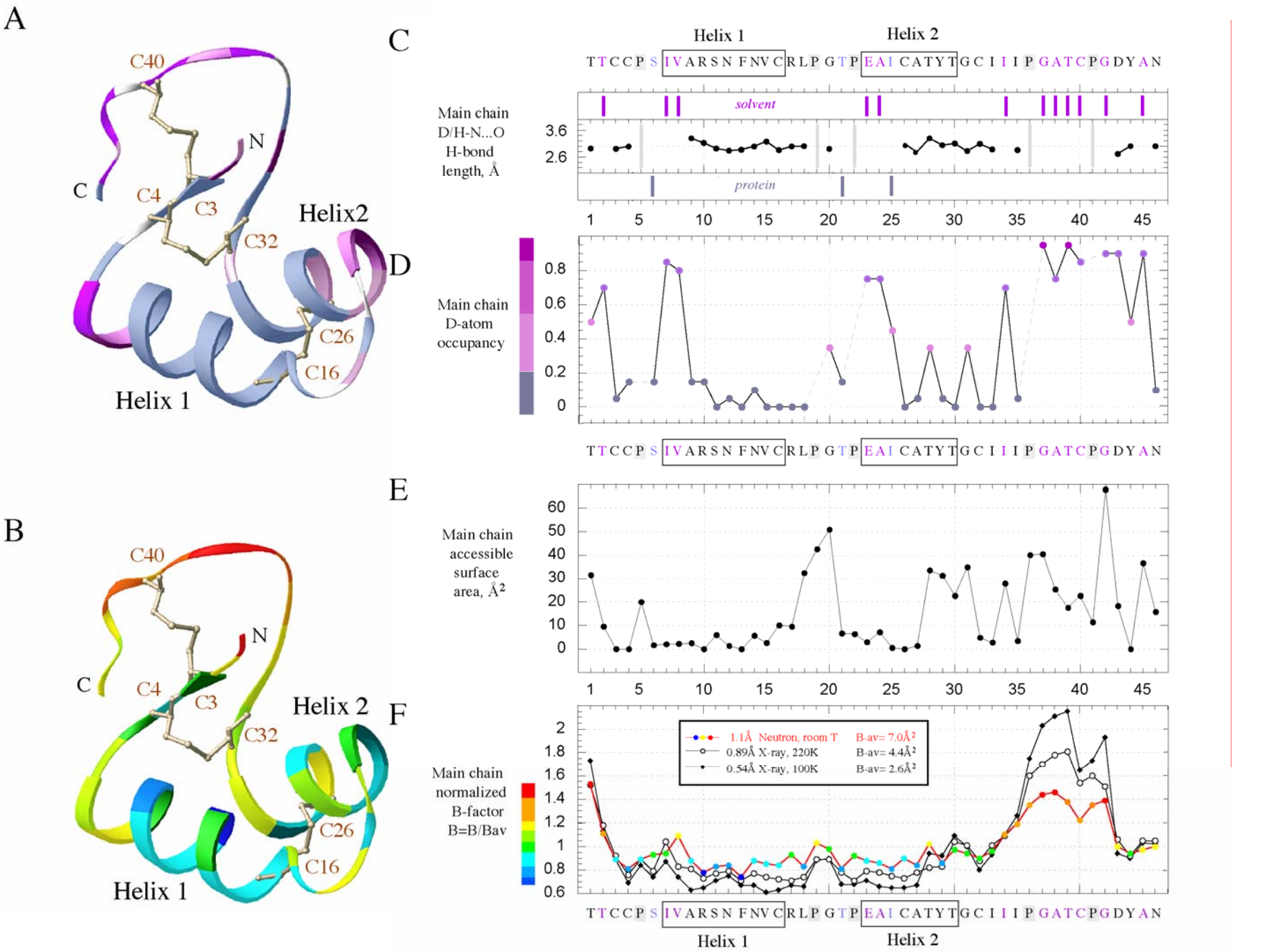


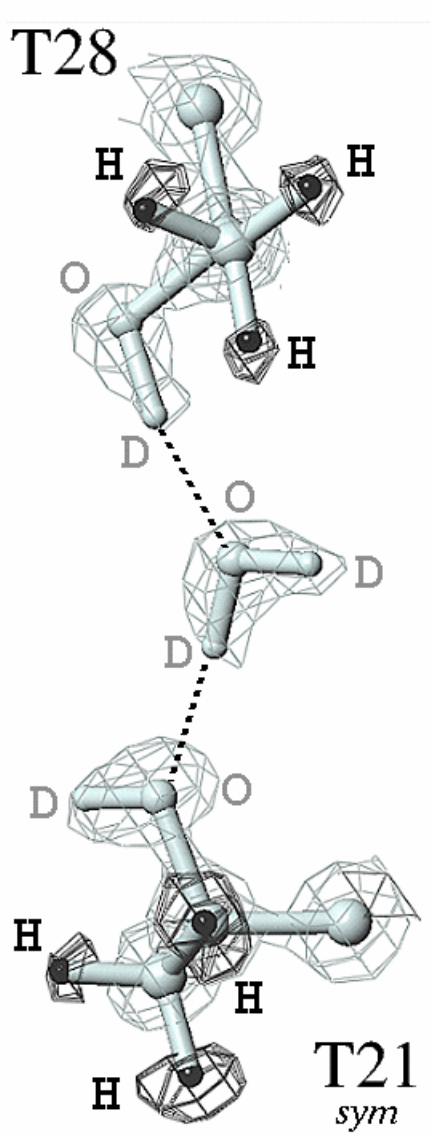
Data from M. Teeter

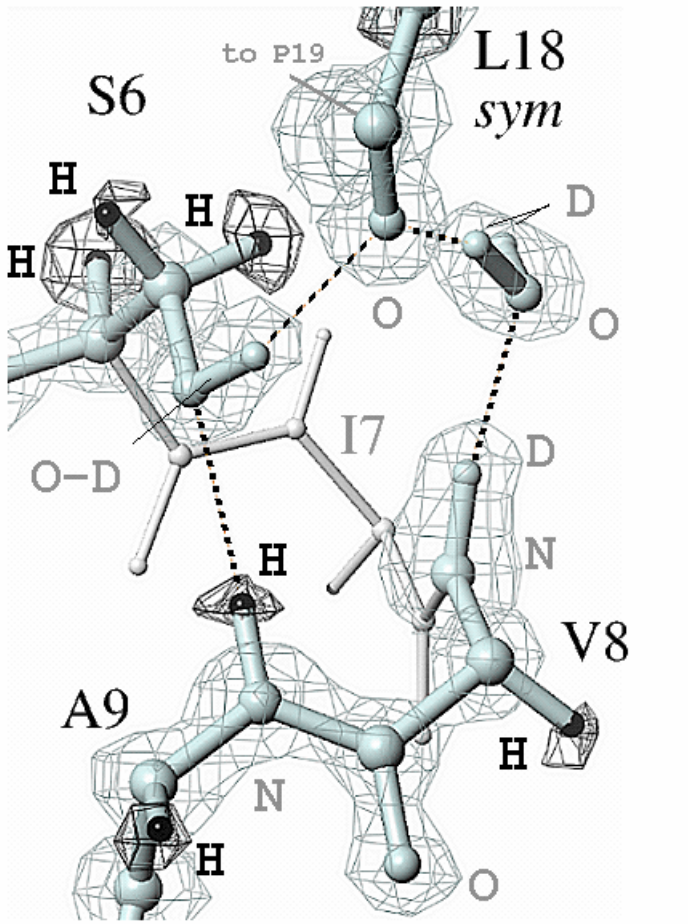
Table 1. Crystal data and refinement statistics

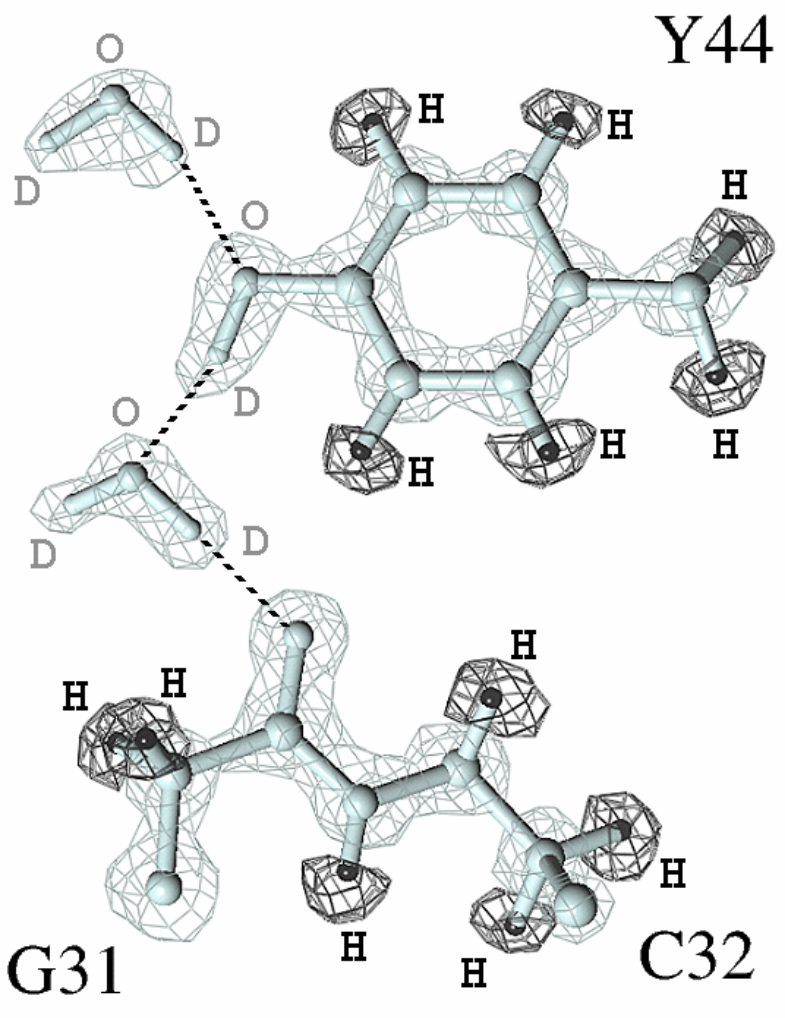
Space group	P 2_1	**Unit cell parameters**	a=41.01 b=18.69 c=22.64 β=90.63^o
Resolution,	25-1.1		
Unique reflections	11,256	**Completeness, %**	82.9
R factor, %	15.6	**R-free, %**	21.2
No.of a.a. residues in refinement	46	**No.of solvent molecules in refinement:**	29OD_2+37O=66
Protein non-hydrogen atoms	337	**Protein H/D**	322
Rmsd bonds ( )	0.02	**Rmsd angles (^{o})**	2.4
Average B-factor, ^{2}	10.9		

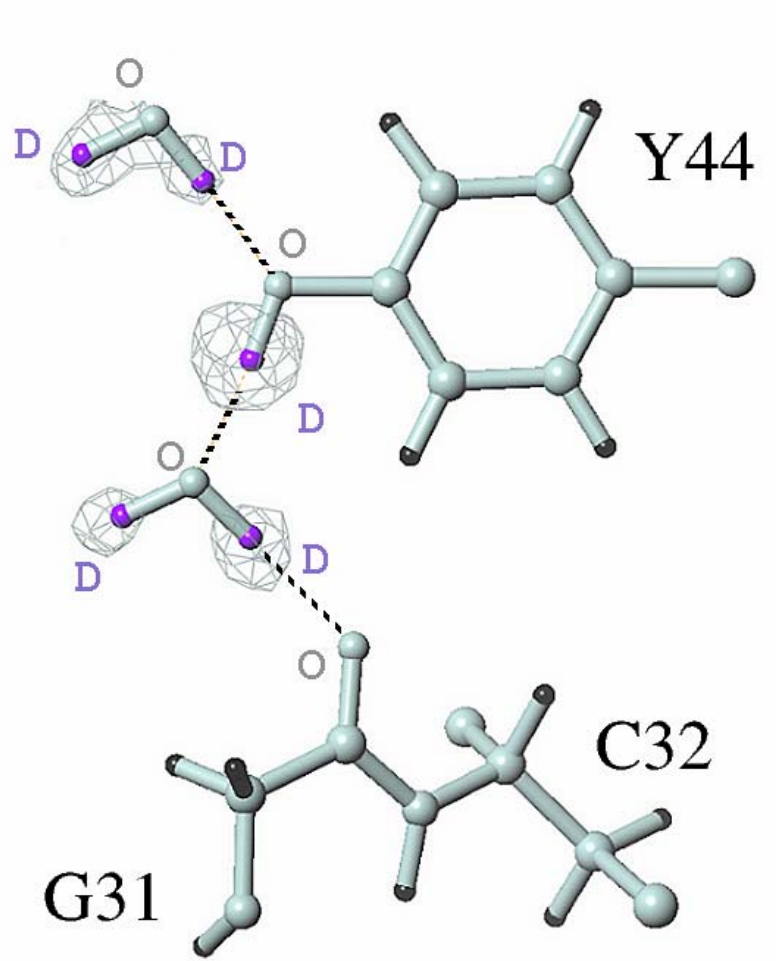
[b] Restrained anisotropic refinement was performed in Refmac5. The D and H atoms were included in the refinement.
[b] R factor = Σ | |F(obs)| - |F(calc)| |/Σ|F(obs)|, R-free is the same calculated with 5% data withheld from refinement

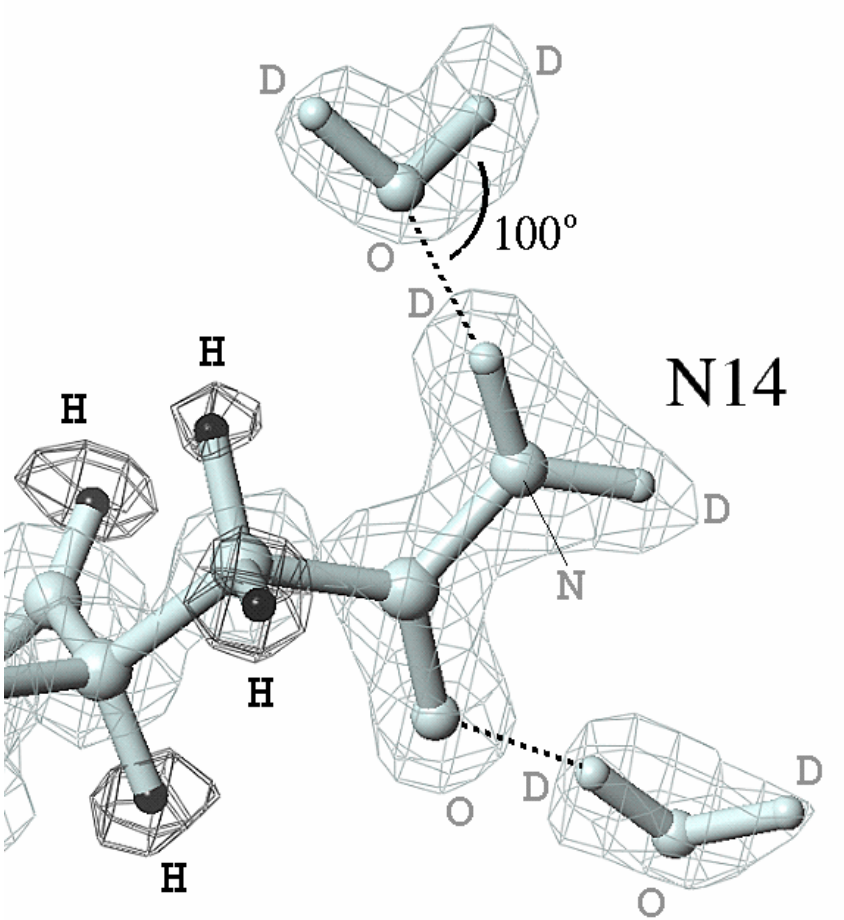

A
C40
N
C
C4
C3
Helix2
C32
D
C26
C16
Helix 1
B
C40
N
C
C4
C3
Helix 2
C32
C26
C16
Helix 1
C
Helix 1
Helix 2
TTCCPSIVARSNFNVCRLPGTPEAICATYTGCIIIPGATCPGDYAN
Main chain D/H-N...O H-bond length, Å
solvent
protein
3.6
2.6
1 5 10 15 20 25 30 35 40 45
Main chain D-atom occupancy
0.8
0.6
0.4
0.2
0
TTCCPSIVARSNFNVCRLPGTPEAICATYTGCIIIPGATCPGDYAN
E
Main chain accessible surface area, Å²
60
50
40
30
20
10
0
1 5 10 15 20 25 30 35 40 45
F
Main chain normalized B-factor B=B/Bav
2
1.8
1.6
1.4
1.2
1
0.8
0.6
1.1Å Neutron, room T B-av= 7.0Å²
0.89Å X-ray, 220K B-av= 4.4Å²
0.54Å X-ray, 100K B-av= 2.6Å²
TTCCPSIVARSNFNVCRLPGTPEAICATYTGCIIIPGATCPGDYAN
Helix 1
Helix 2

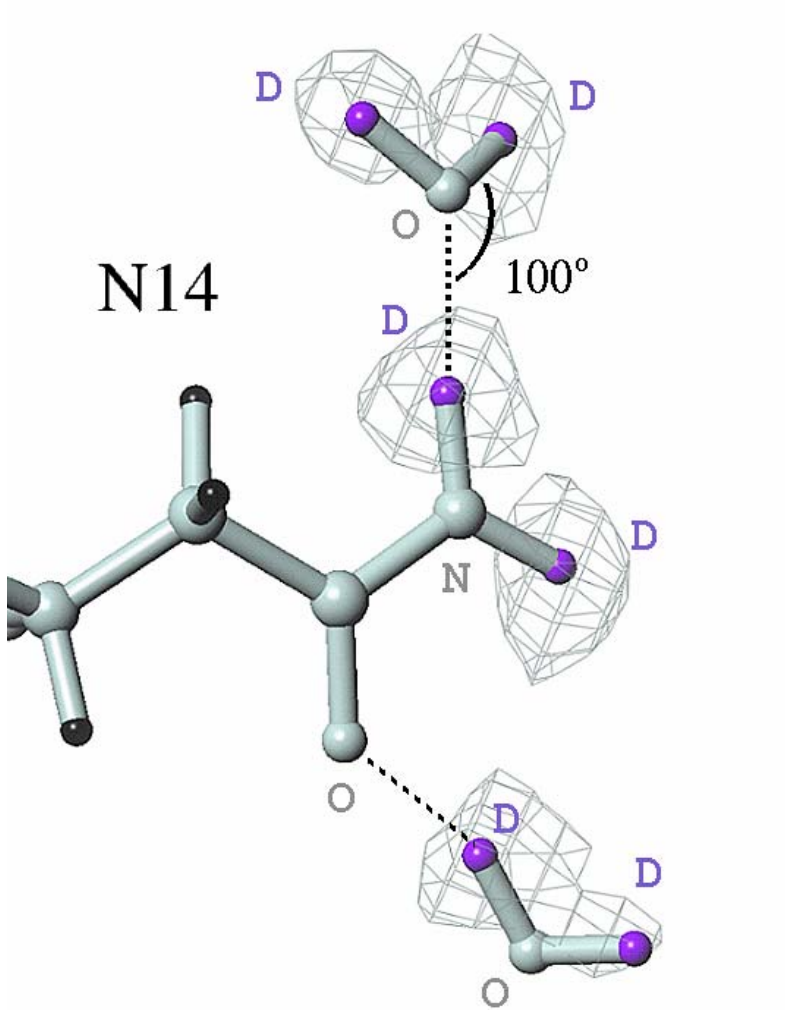

T28
H
H
O
H
D
O
D
D
D
O
H
H
H
T21
sym

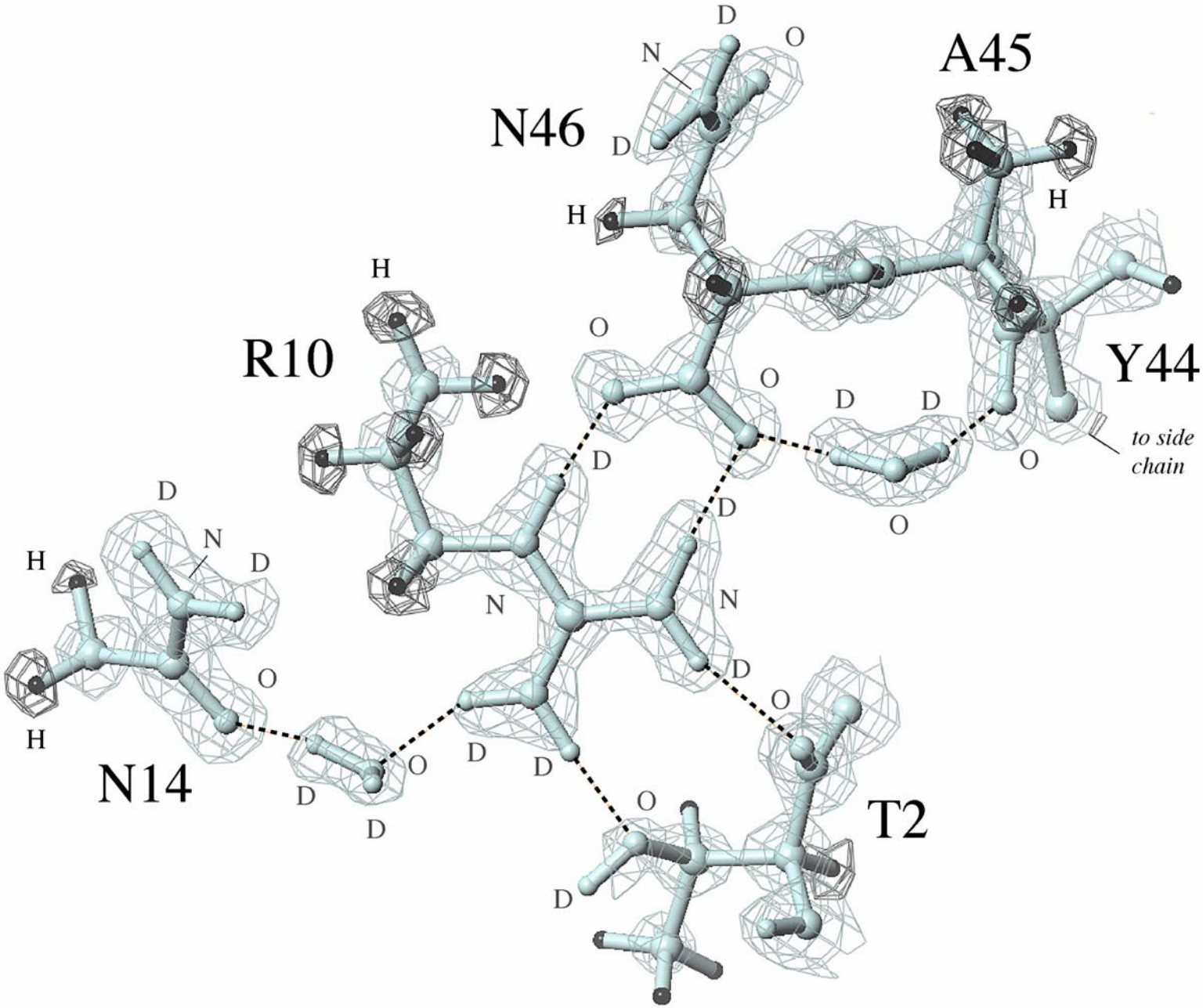

to P19
L18
sym
S6
H
H
H
D
O
O
I7
O–D
D
H
N
V8
A9
H
N
H
O

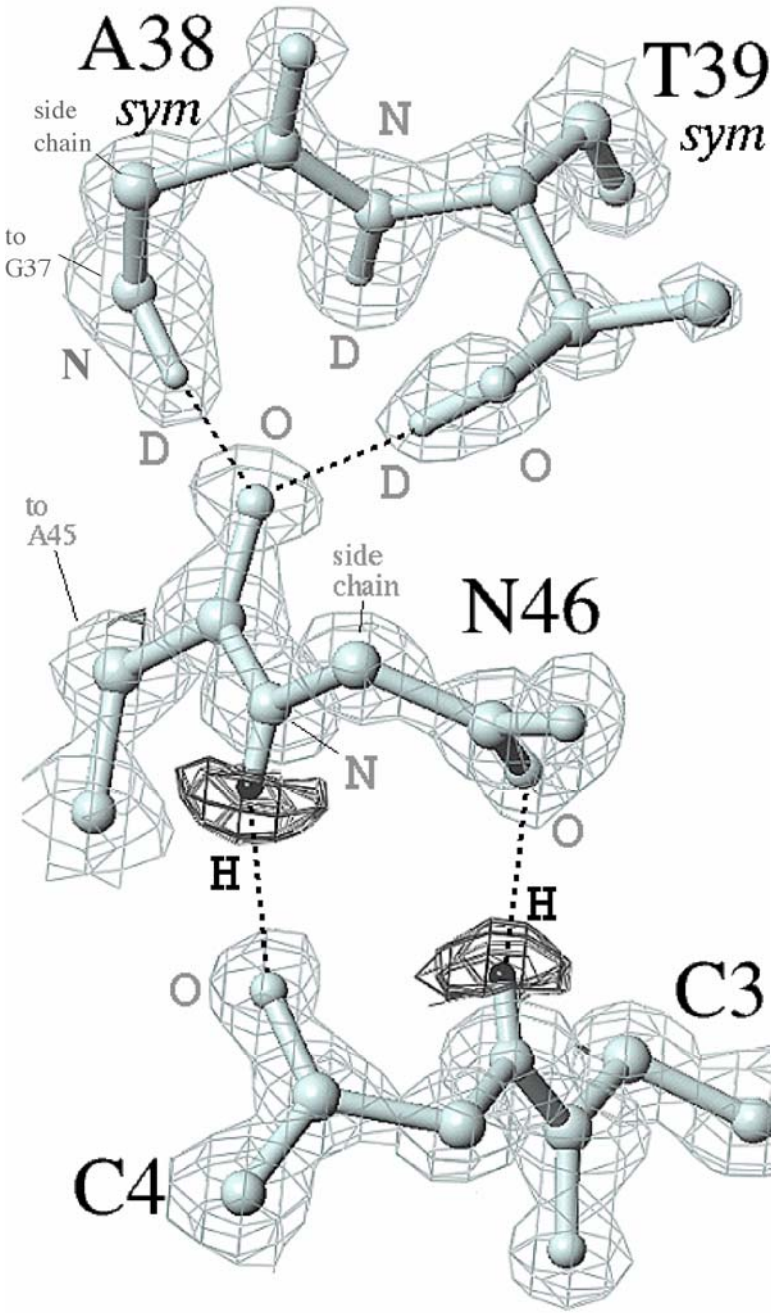

Y44
O
D
D
O
D
O
D
D
H
H
H
H
H
H
H
H
H
H
G31
C32


O
D
D
Y44
O
D
O
D
D
O
C32
G31

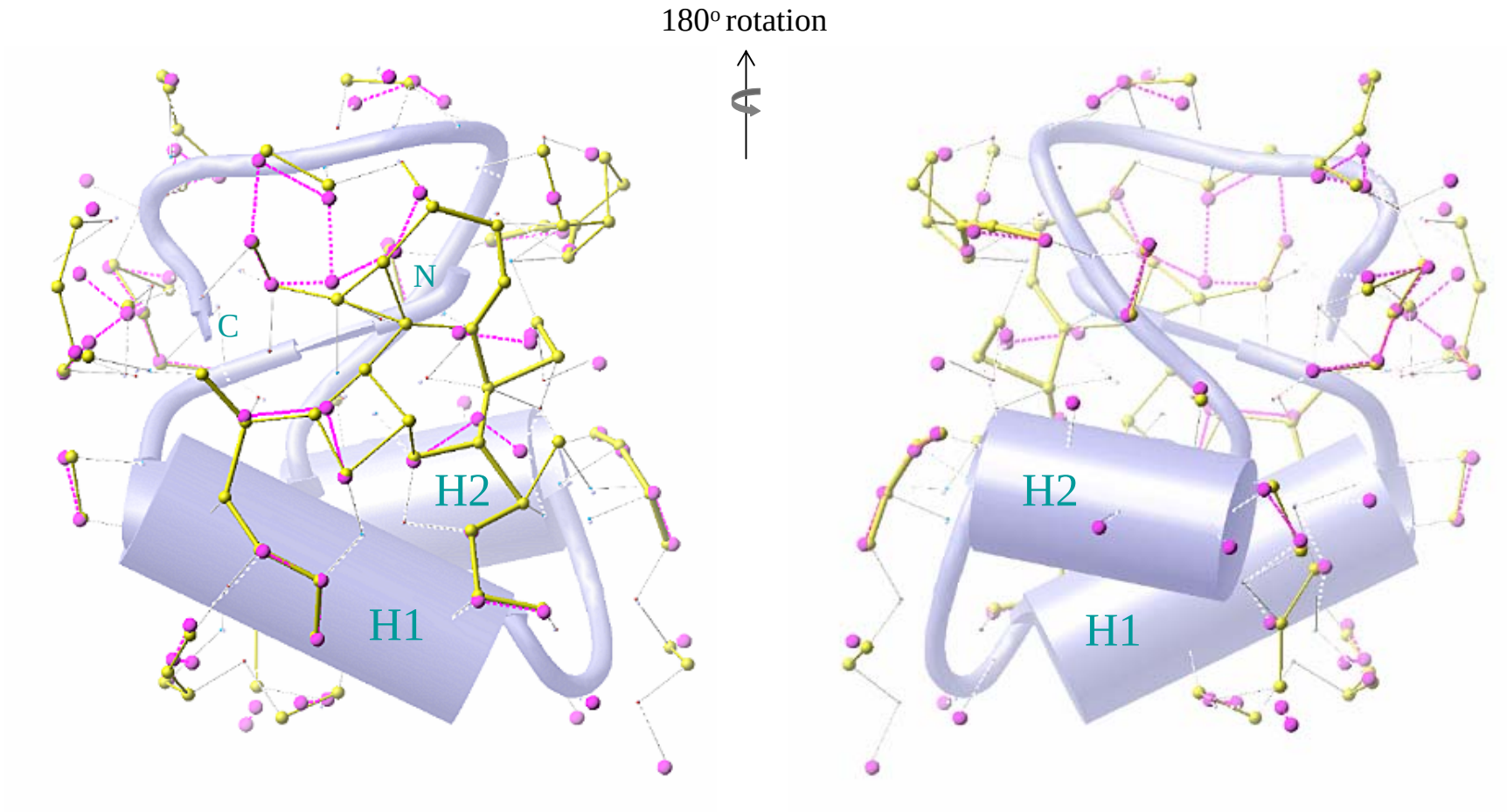

D
D
O
100°
D
N14
H
H
D
N
H
D
D
O
H
O


D
D
O
100°
N14
D
D
N
D
O
D
D
O

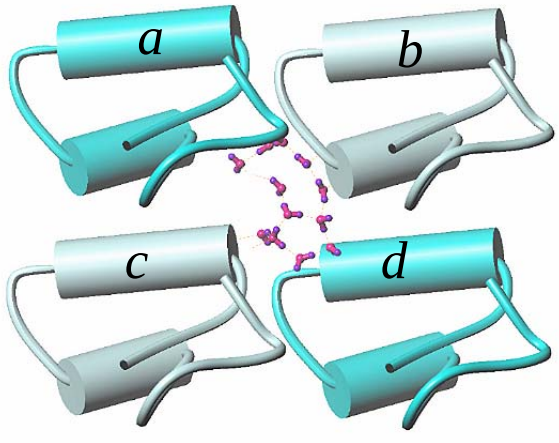

N46
A45
R10
Y44
N14
T2
to side chain

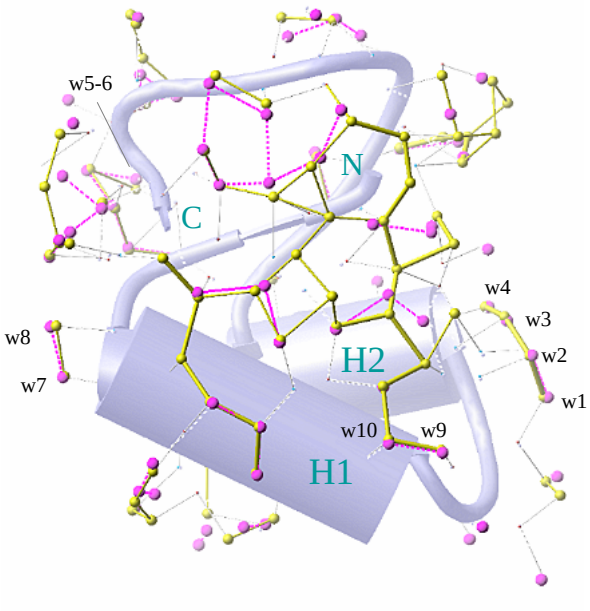

A38
sym
side
chain
T39
sym
N
to
G37
N
D
D
O
D
O
to
A45
side
chain
N46
N
O
H
H
O
C3
C4

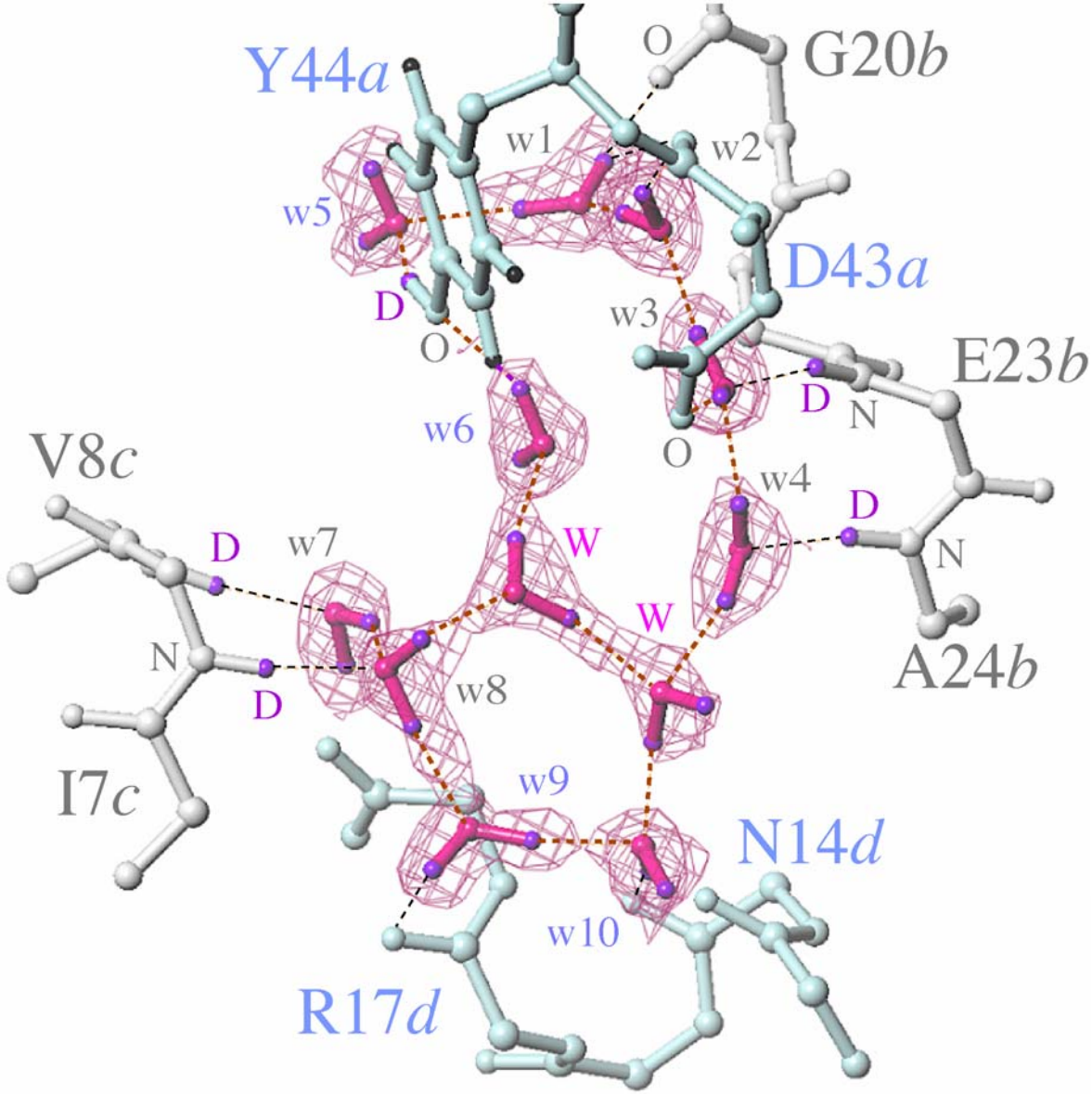
First hydration shell

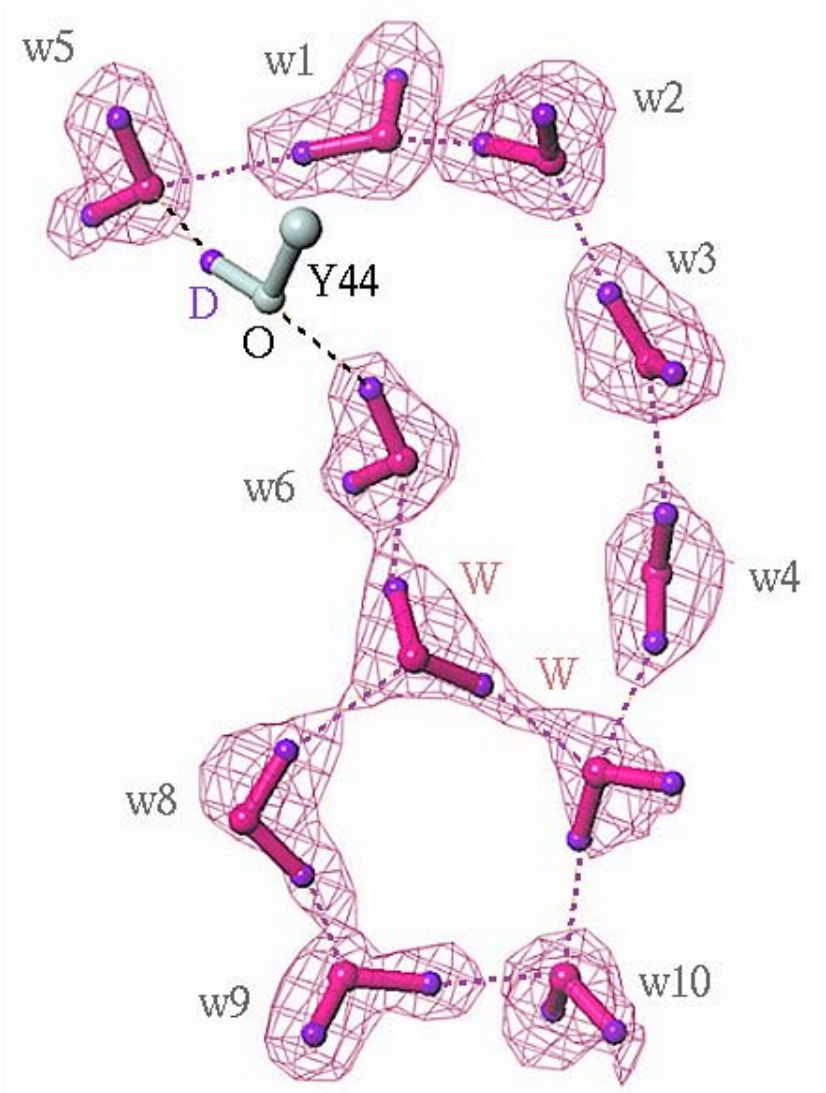


View along crystal axis *a*

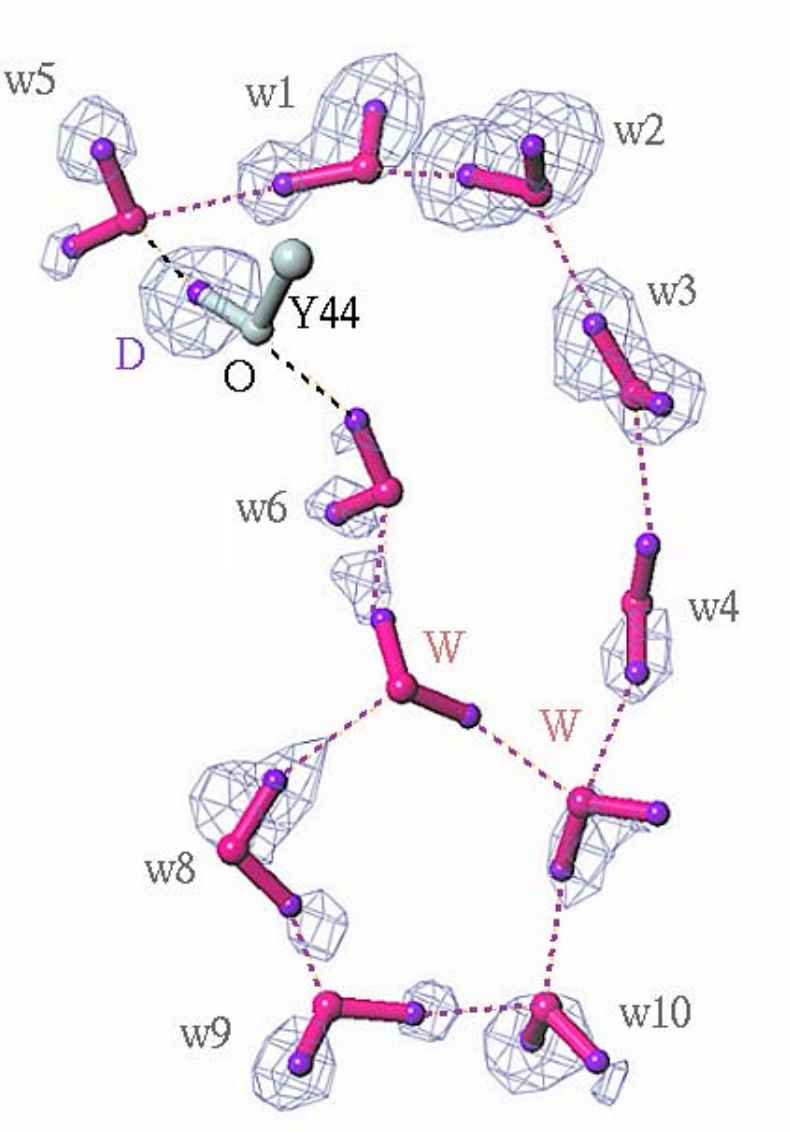




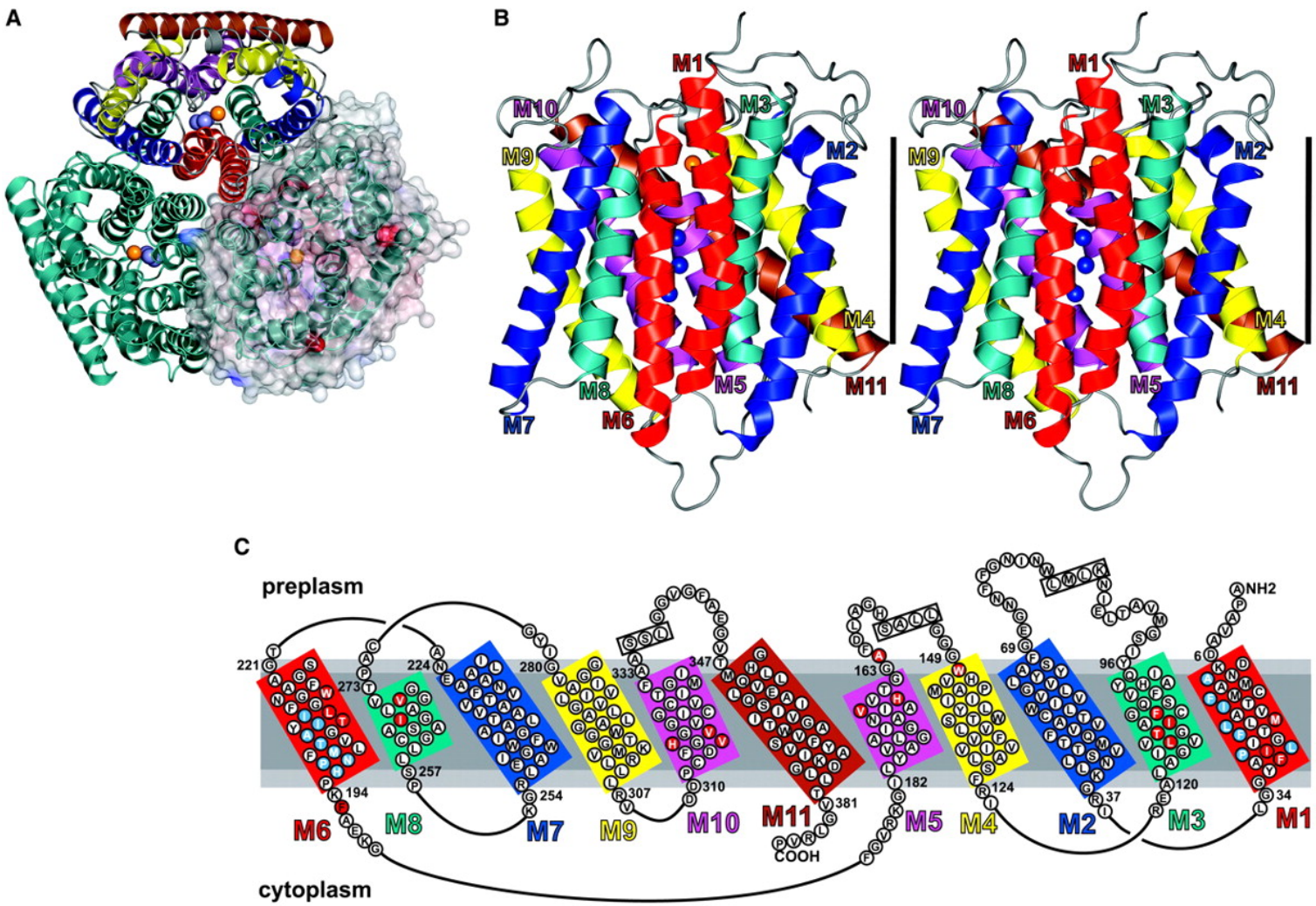




2Fo-Fc at 1.2σ level



Omit Fo-Fc at 2.5σ level



The ammonia channel- Bob Stroud et. al.

The ammonia/ ammonium channel?