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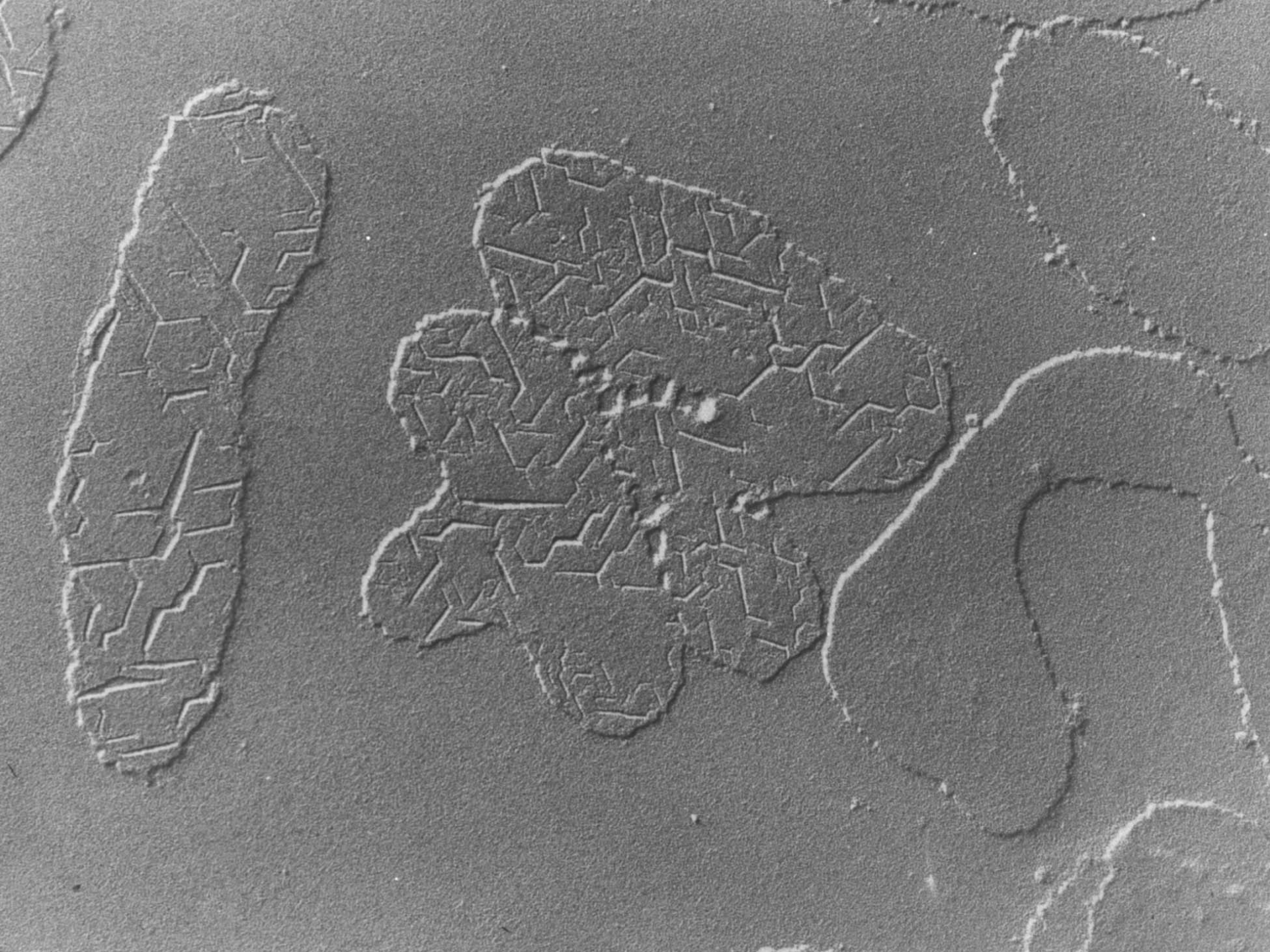
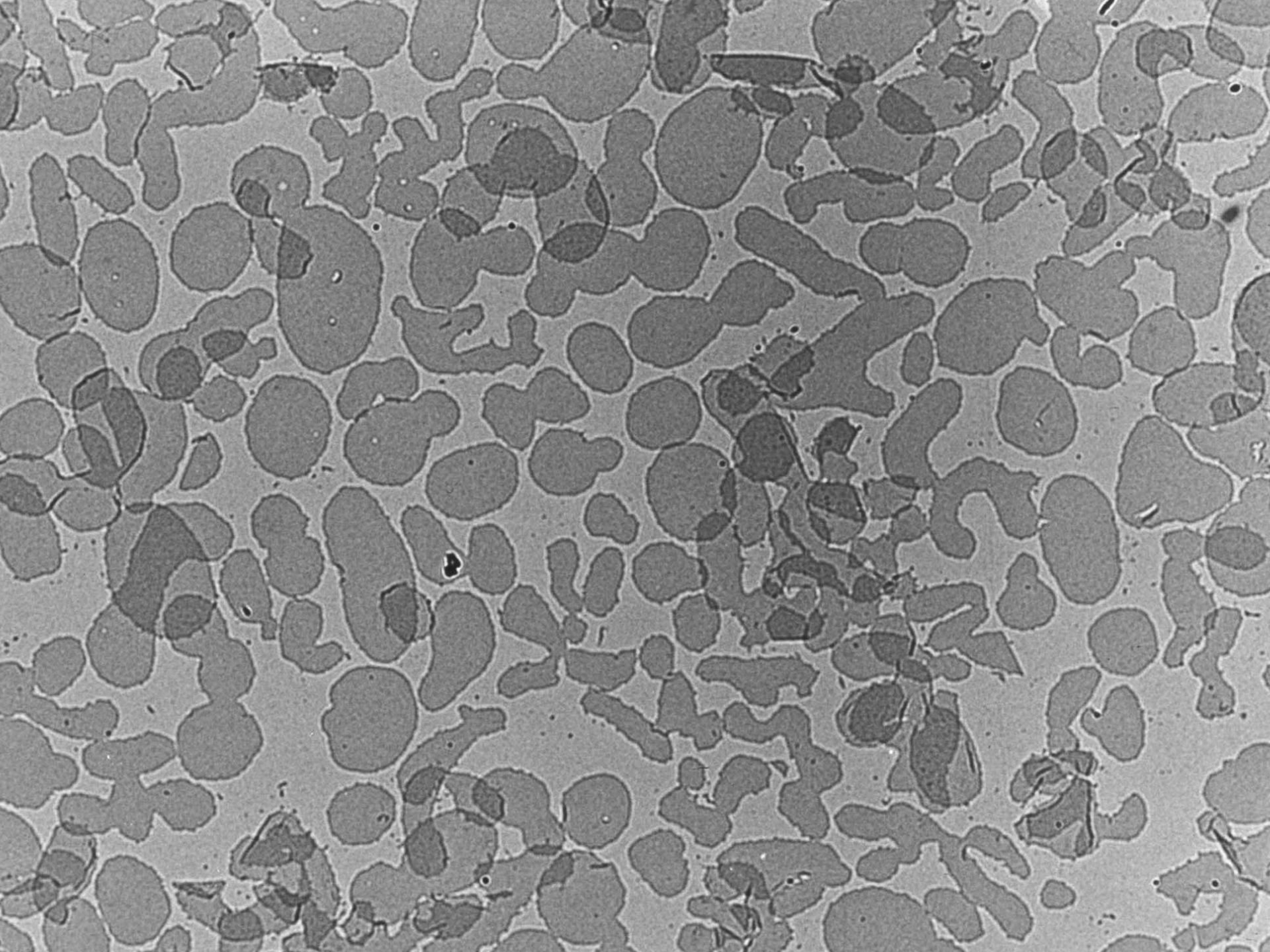
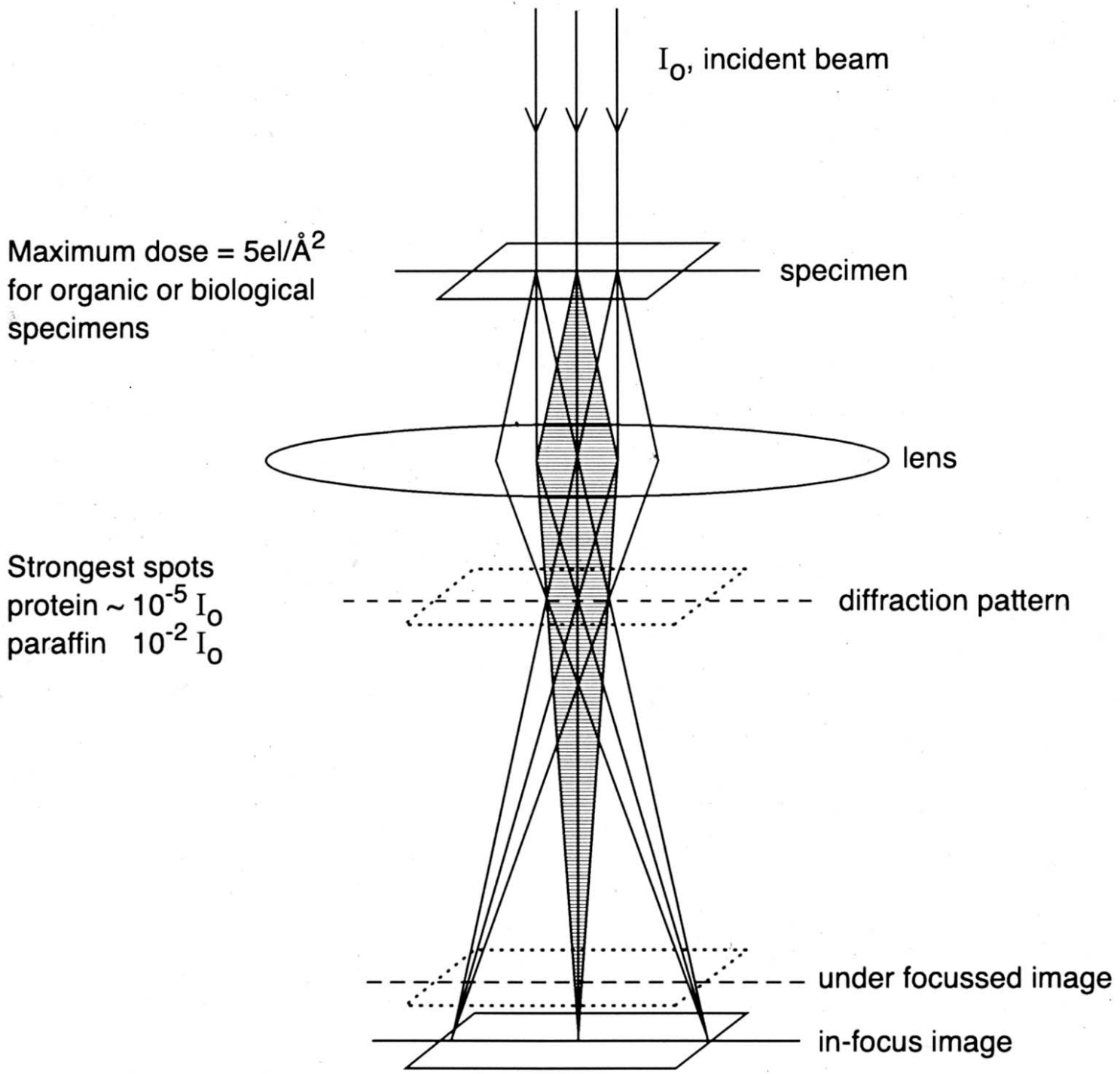
Electron Crystallography

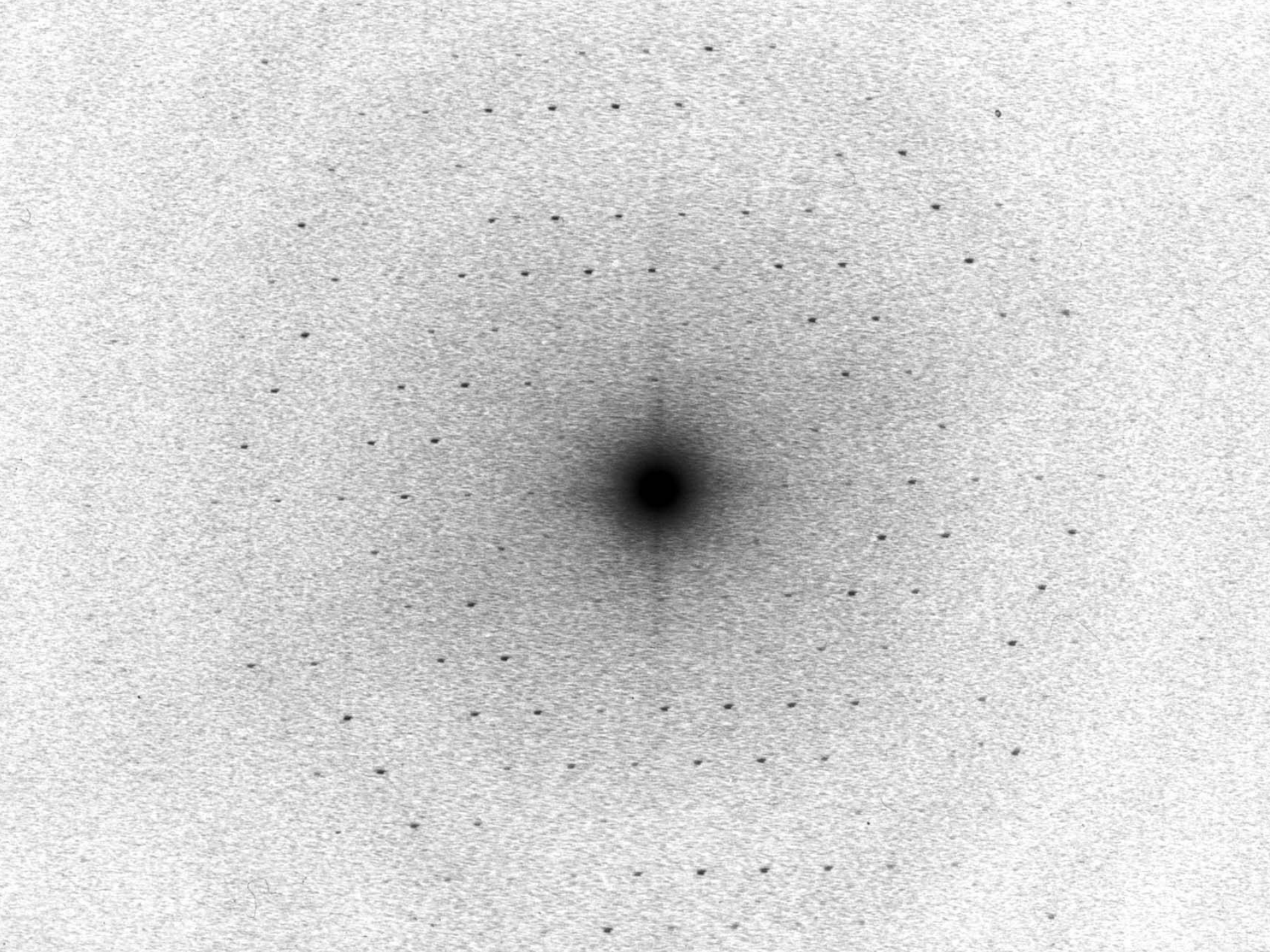
- Our current approach
- Problems and pitfalls
- Lessons from 2D for other cryoEM

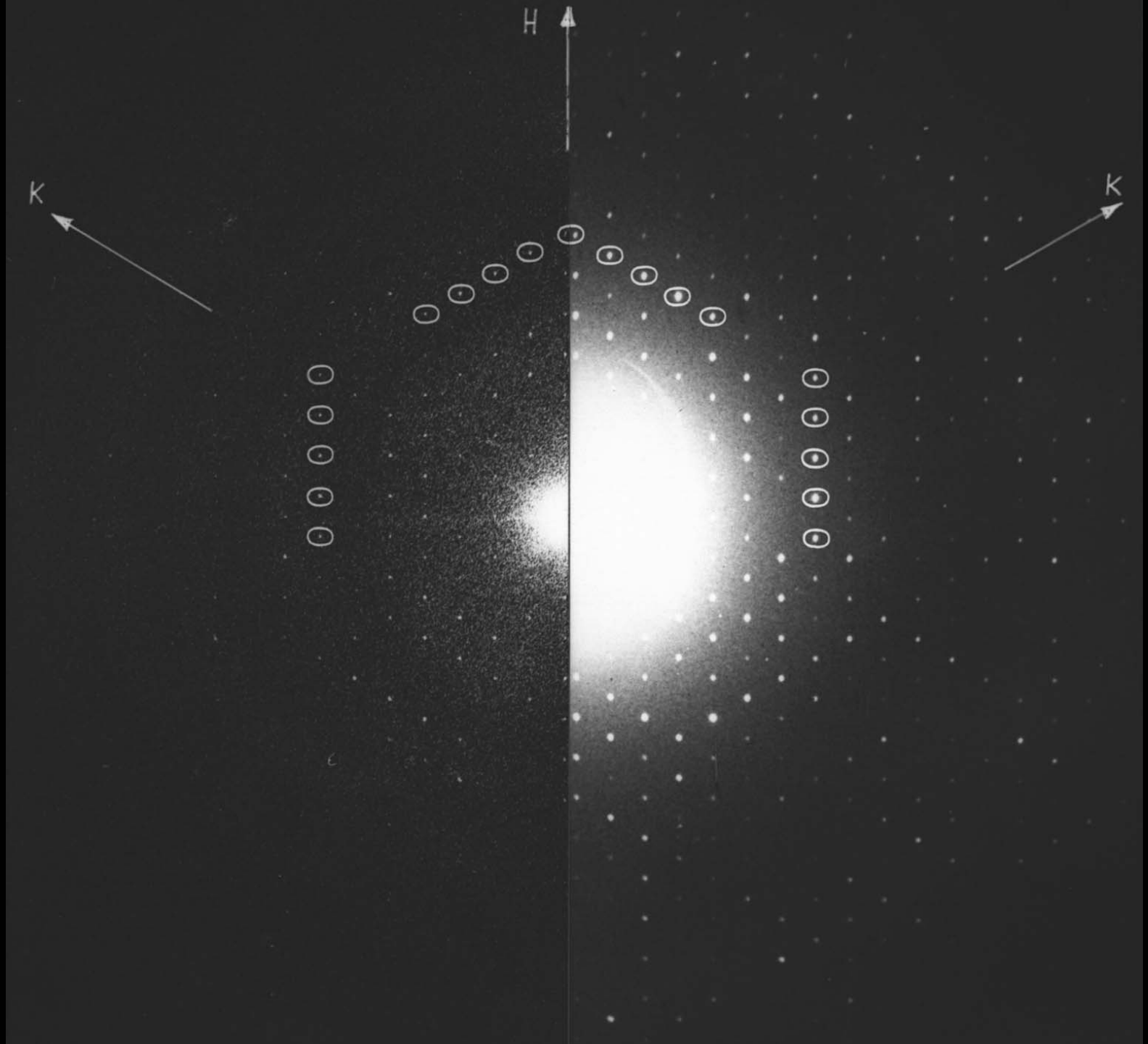
Halobacterium salinarum

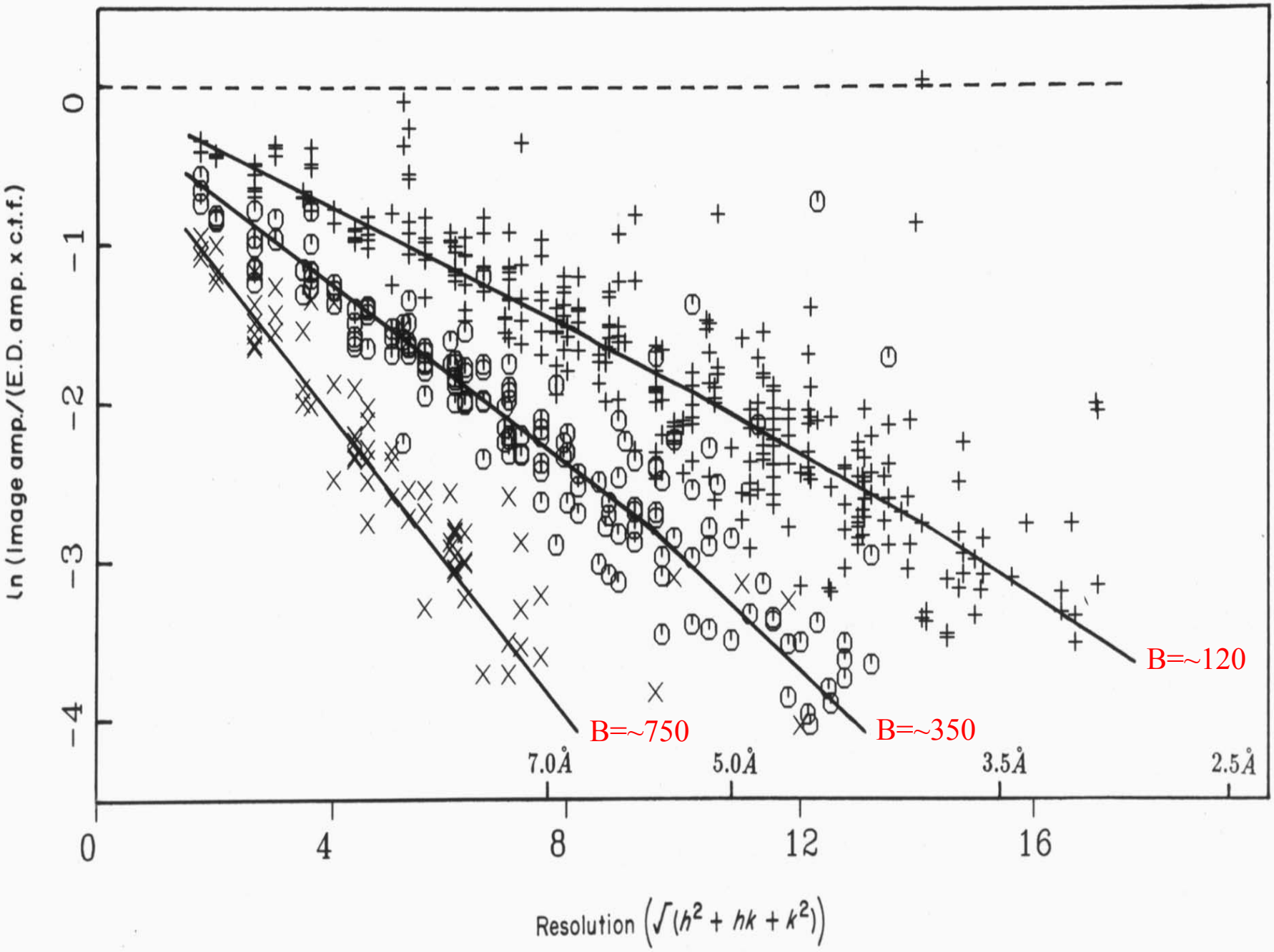


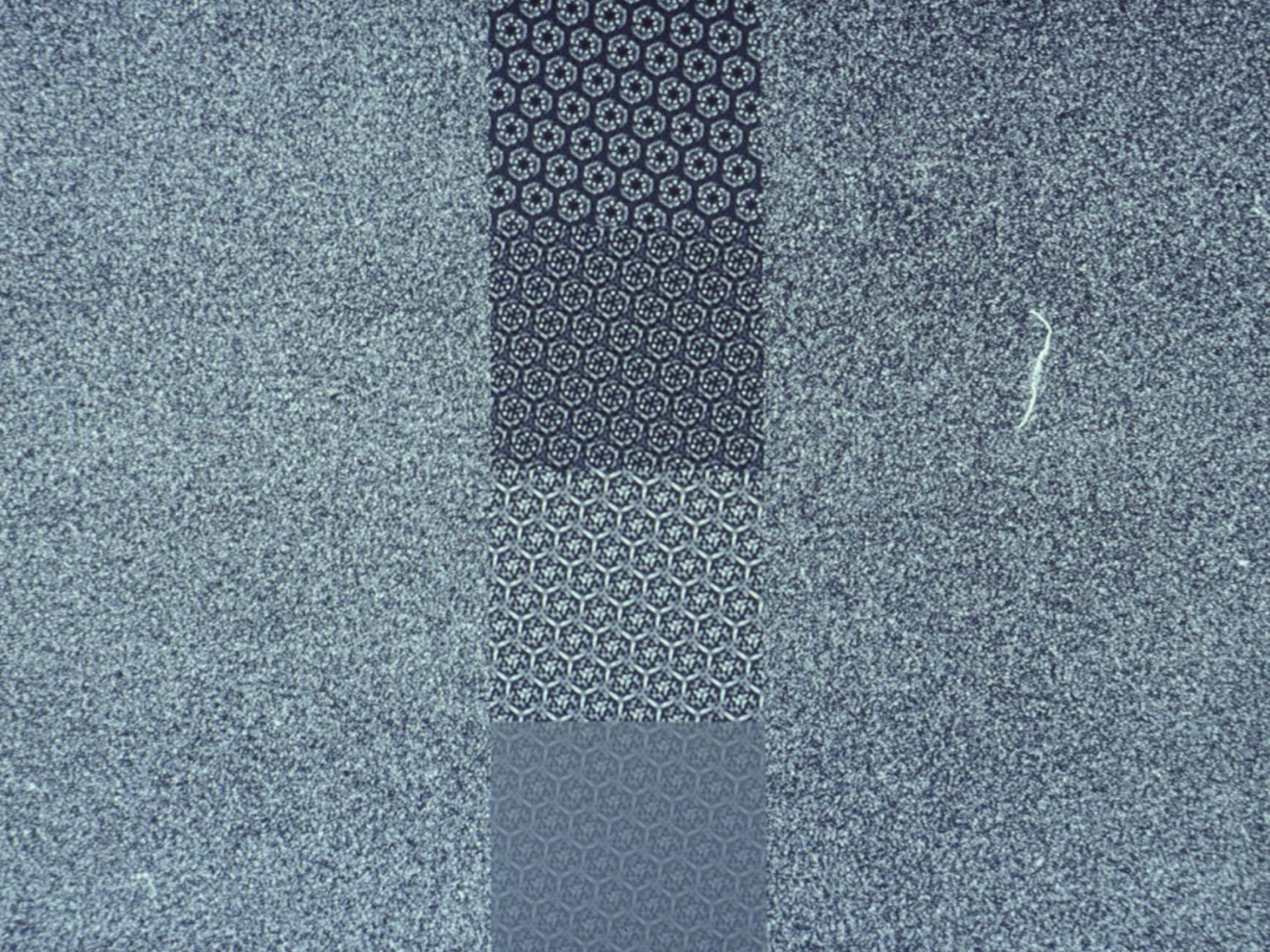


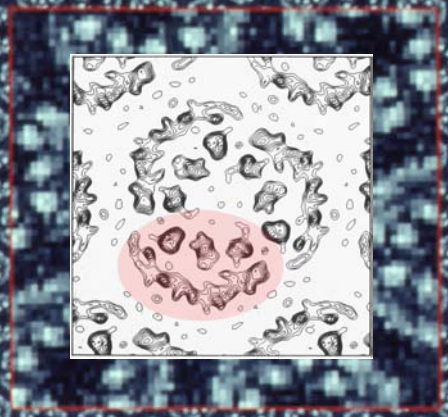

I_o, incident beam
Maximum dose = 5el/Å^2
for organic or biological
specimens
specimen
lens
Strongest spots
protein ~ 10^{-5} I_o
paraffin 10^{-2} I_o
diffraction pattern
under focussed image
in-focus image

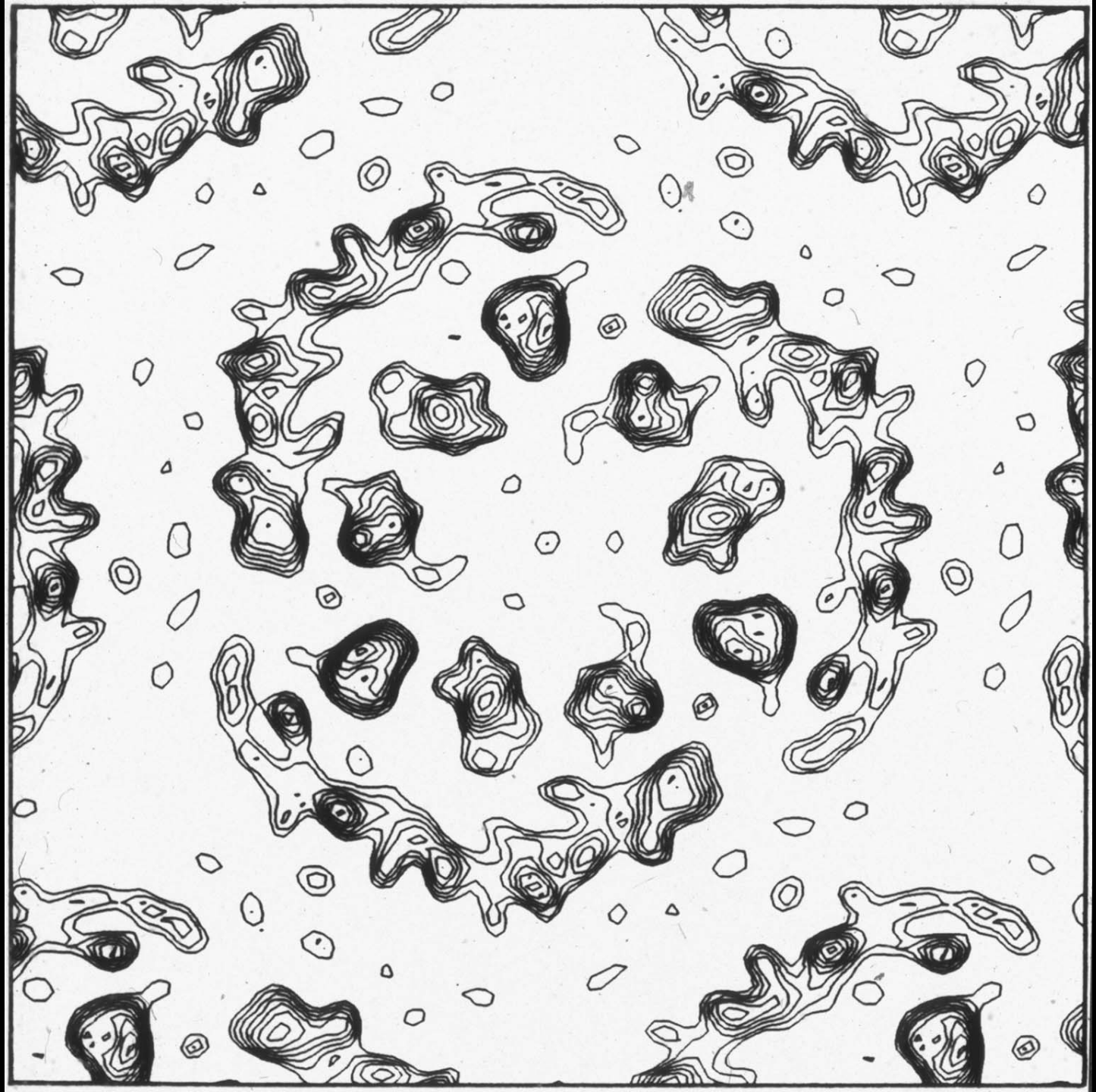



H
K
K


ln (Image amp./(E.D. amp. x c.t.f.))
0
−1
−2
−3
−4
7.0Å
5.0Å
3.5Å
2.5Å
0
4
8
12
16
Resolution $\left(\sqrt{(h^2 + hk + k^2)}\right)$
B=~120
B=~350
B=~750







3D Specimen

Different 2D Projected Images

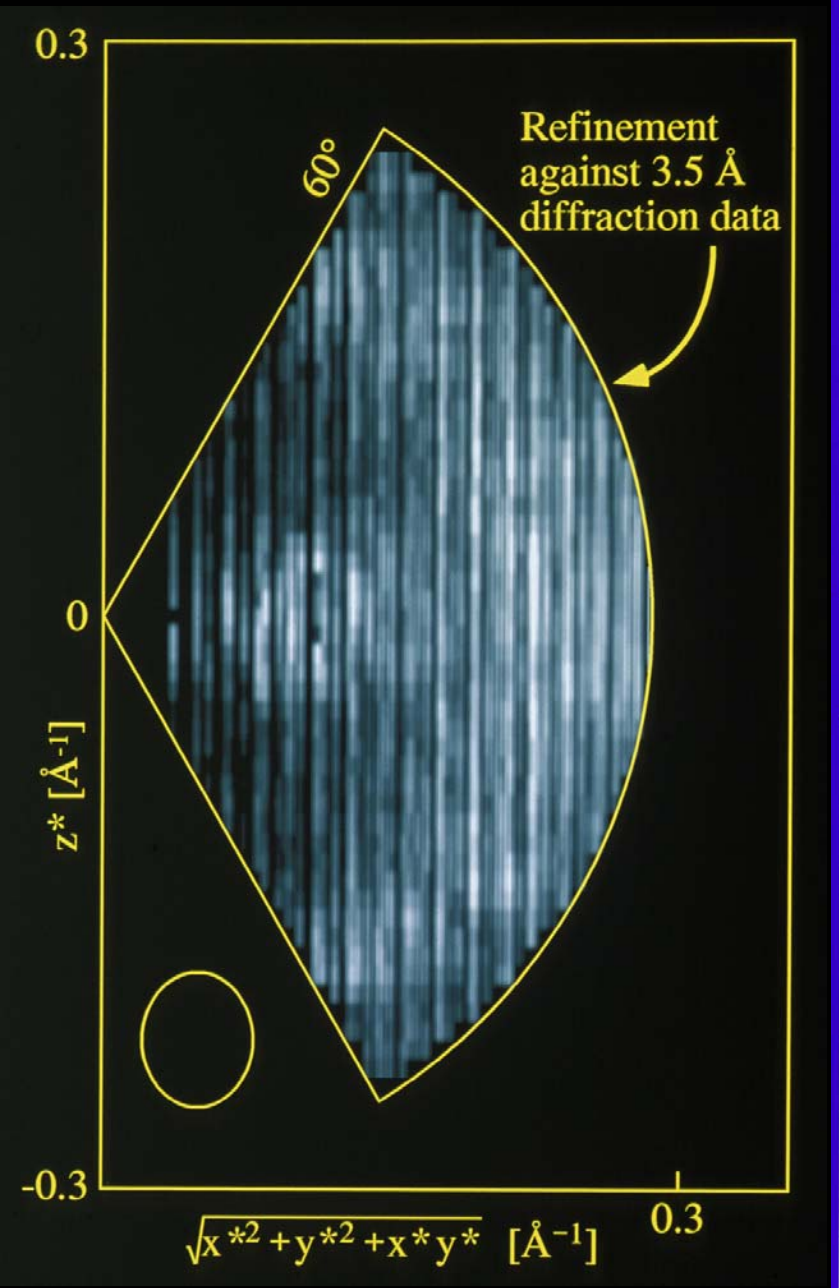
2D Fourier transforms

are

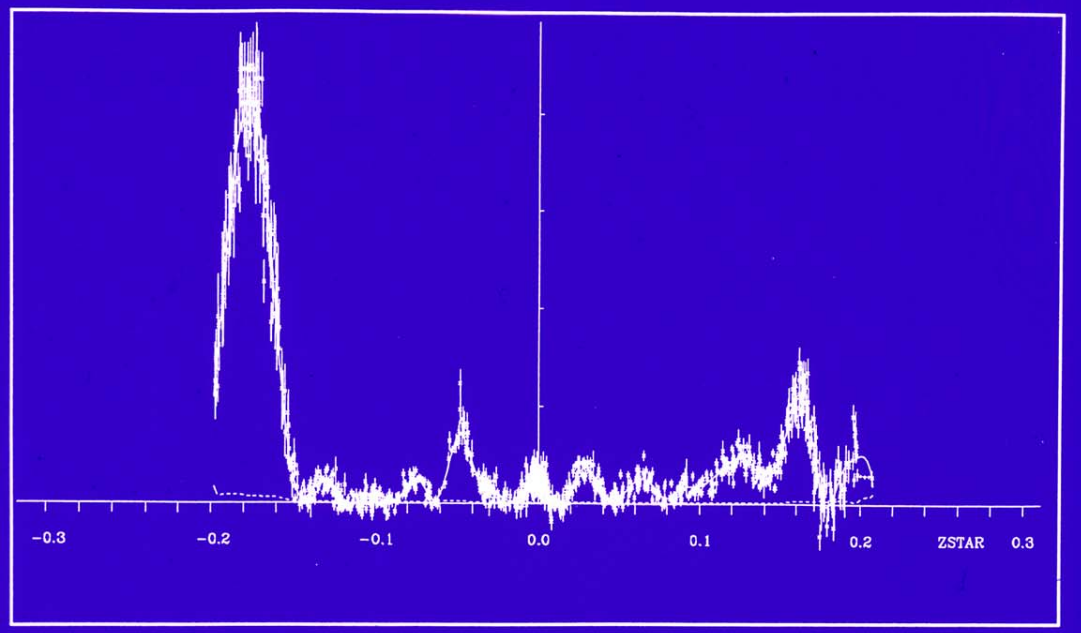
Sections of 3D Fourier Transform

FOURIER | INVERSION

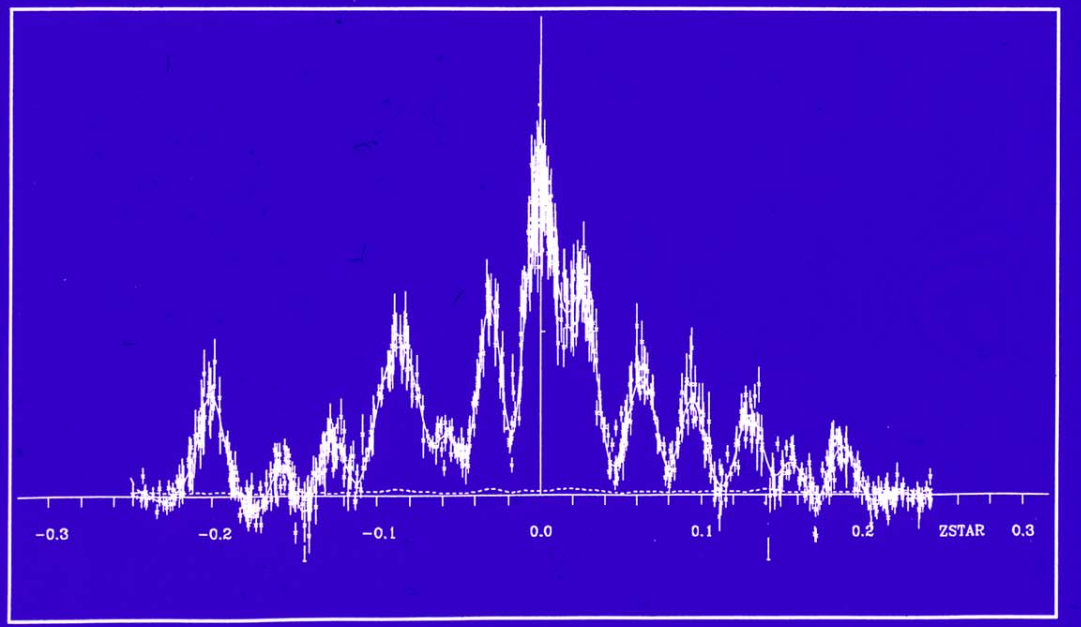
3D Map

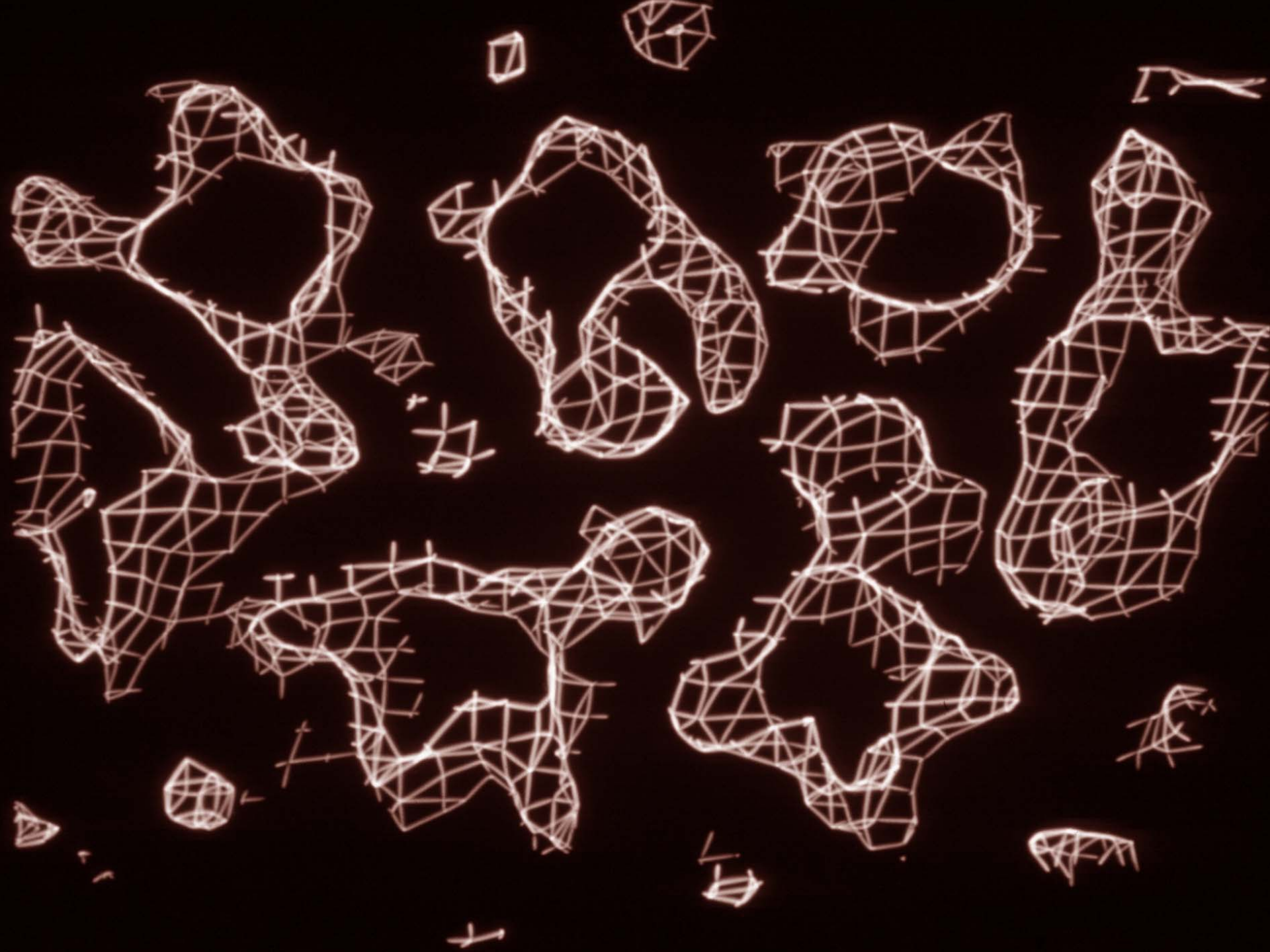

0.3
0
-0.3
z* [Å-1]
0.3
√x*2 +y*2 +x*y* [Å-1]
60°
Refinement
against 3.5 Å
diffraction data

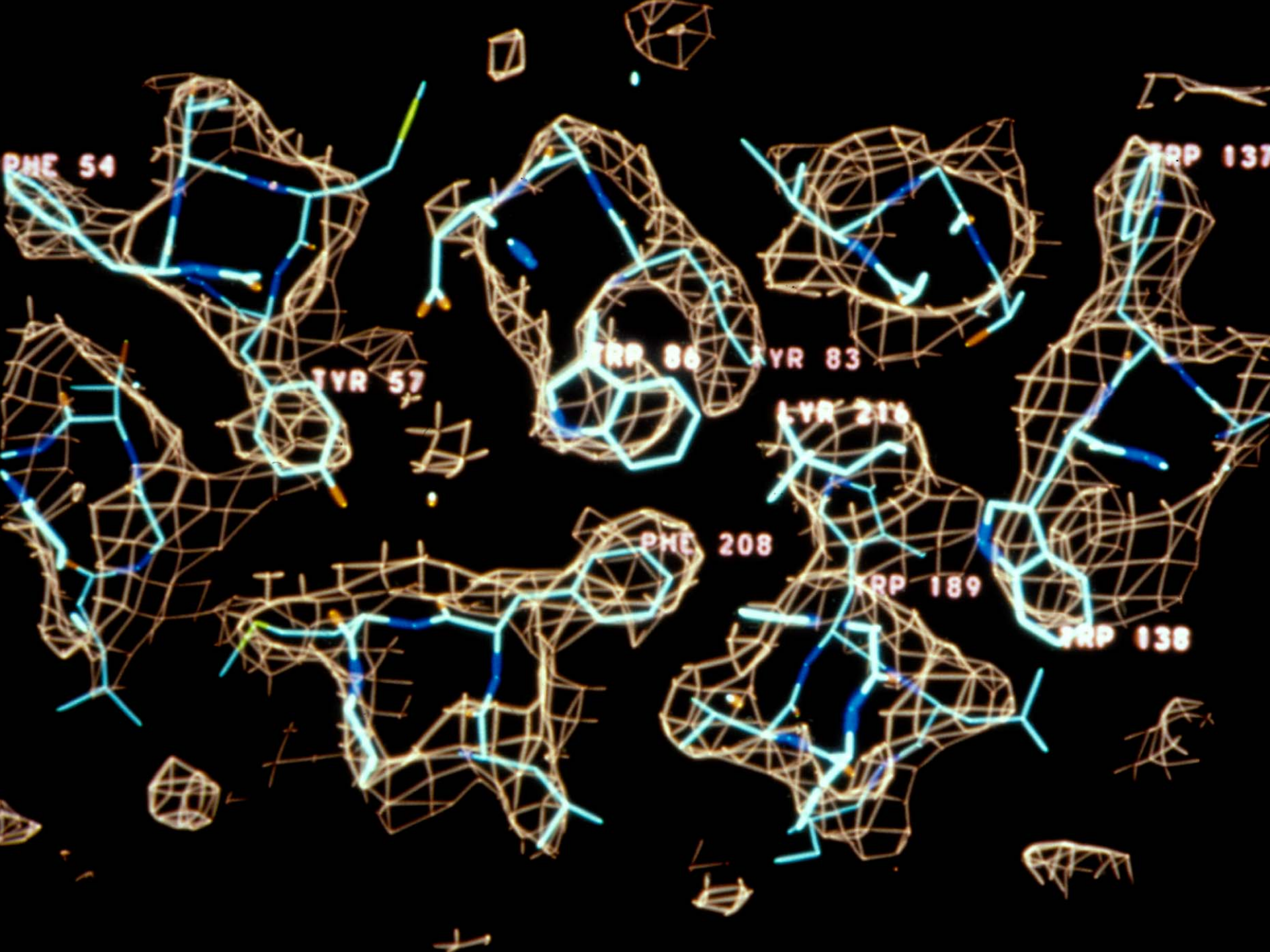
1,6

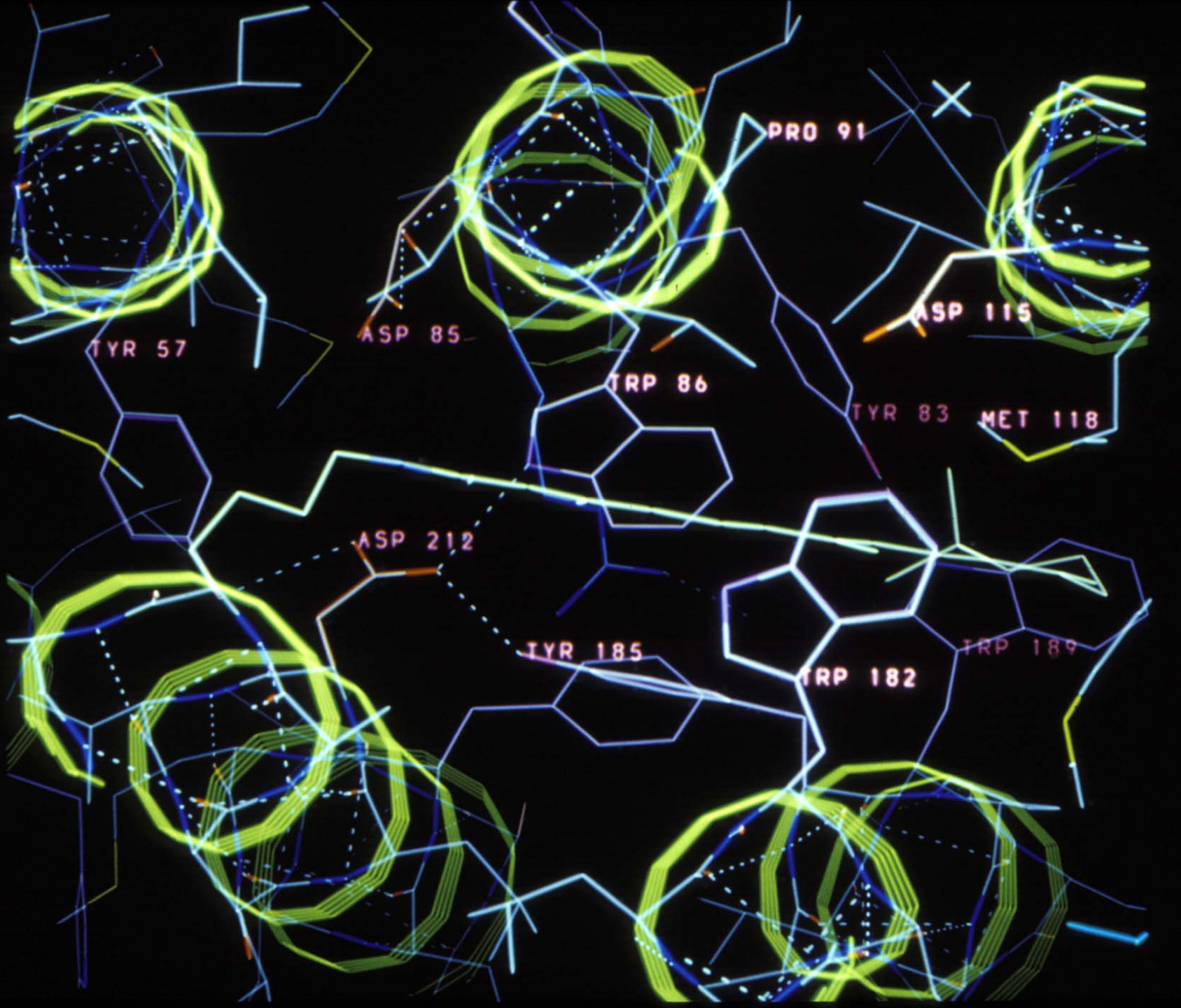

-0.3
-0.2
-0.1
0.0
0.1
0.2
ZSTAR
0.3

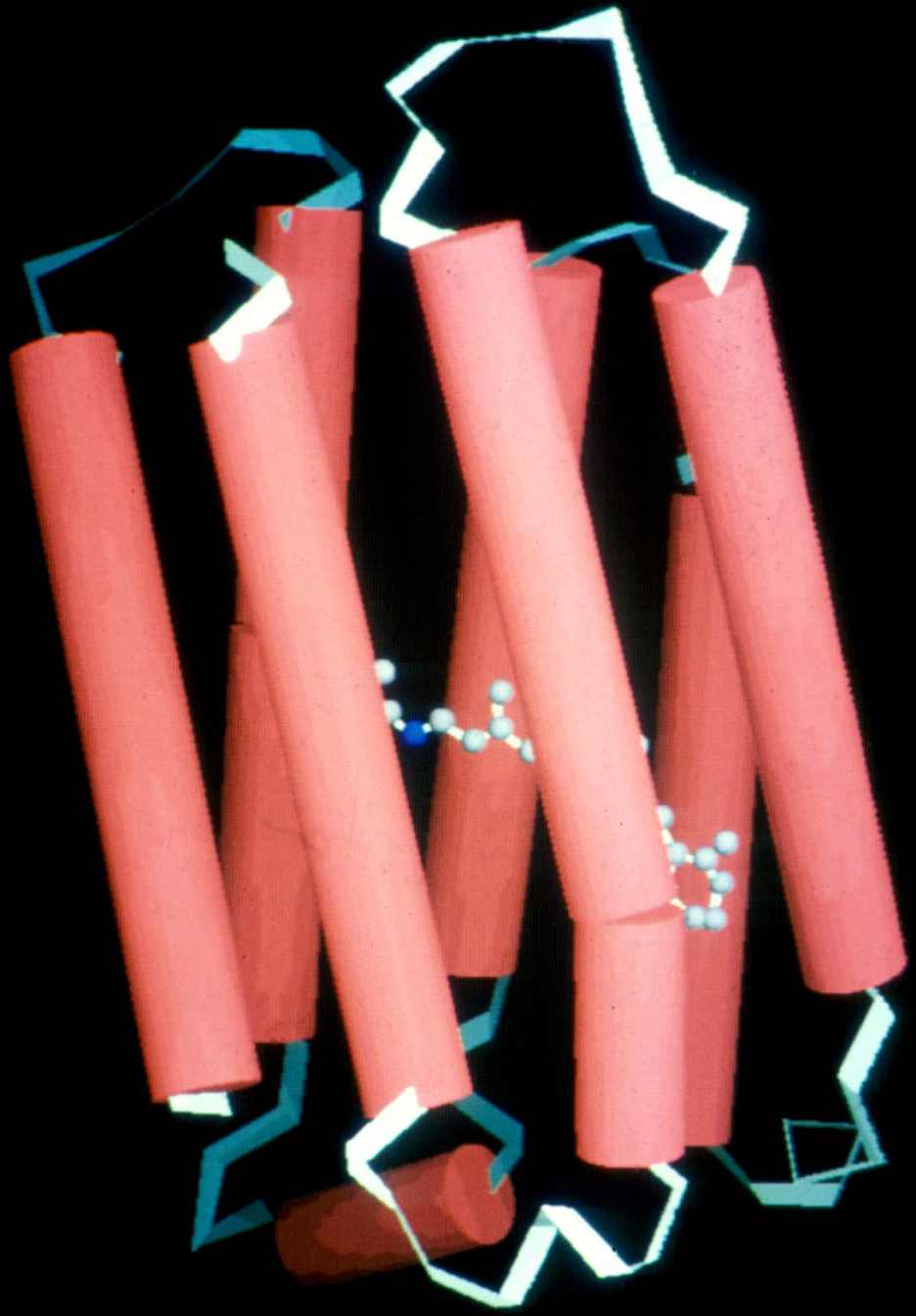
2,8


-0.3
-0.2
-0.1
0.0
0.1
0.2
ZSTAR
0.3

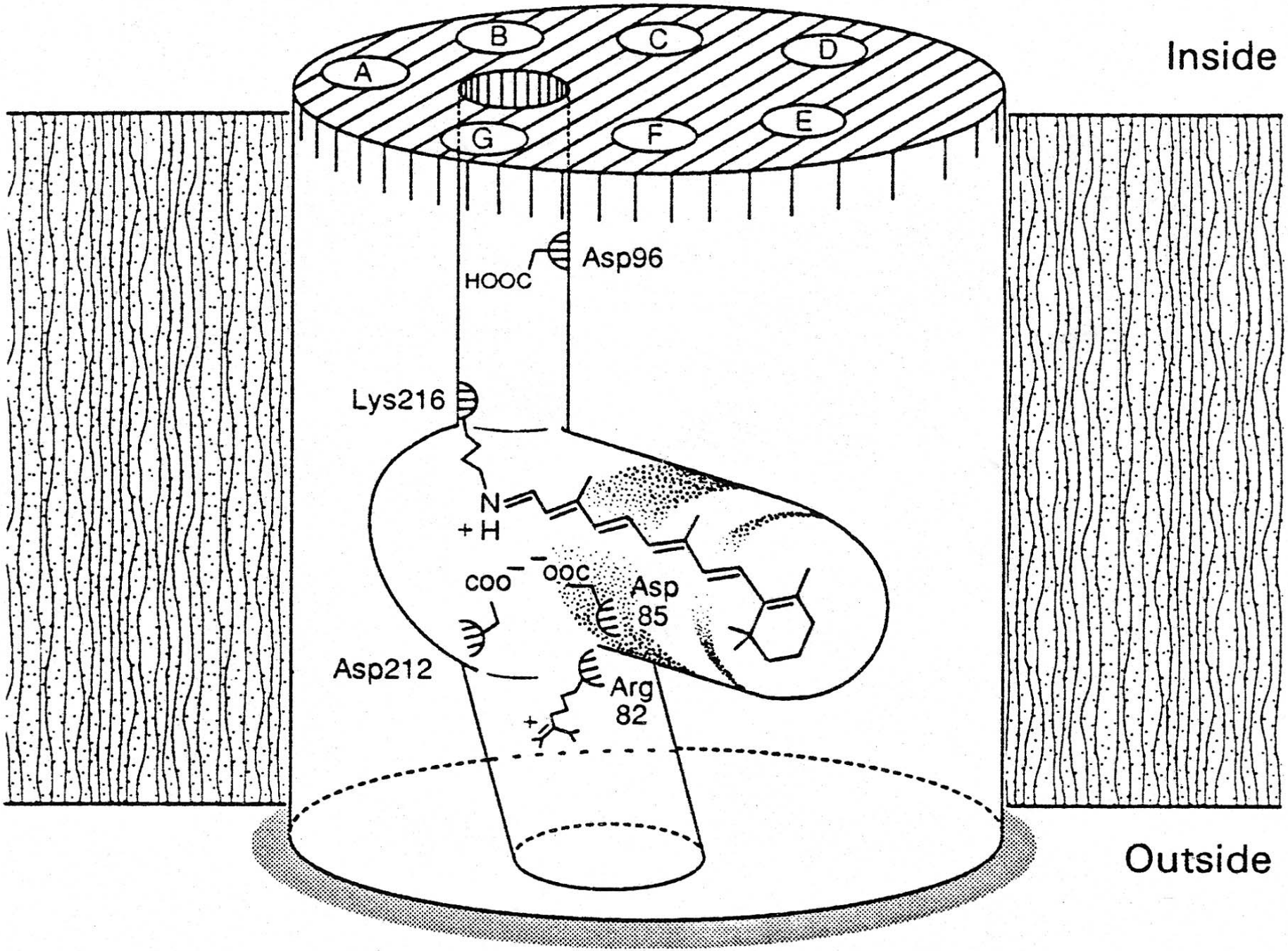


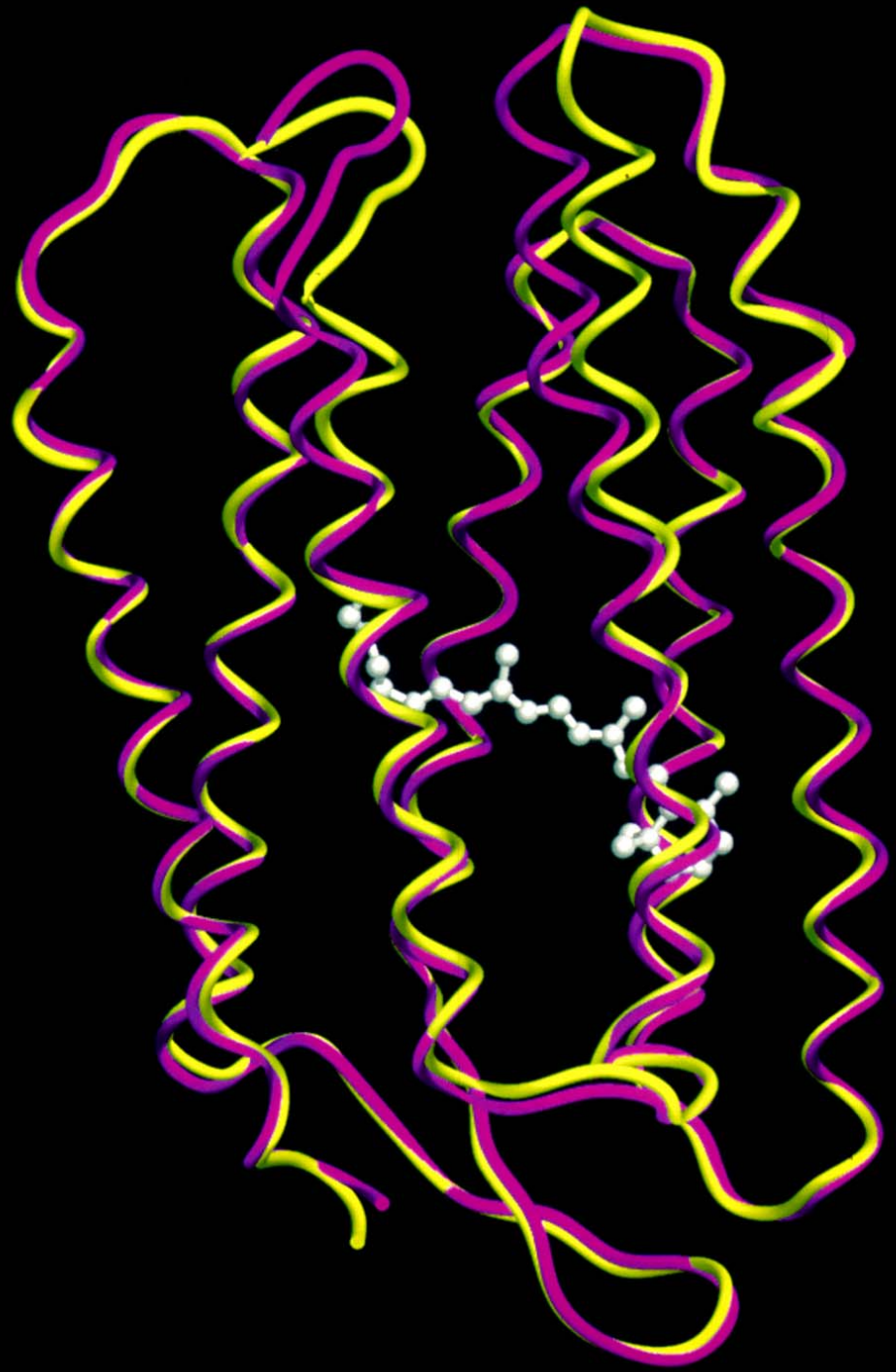

PHE 54
TYR 57
TRP 86
TYR 83
TYR 214
PHE 208
TRP 189
TRP 138
TRP 137


PRO 91
TYR 57
ASP 85
ASP 115
TRP 86
TYR 83
MET 118
ASP 212
TYR 185
TRP 189
TRP 182



Bacteriorhodopsin


A
B
C
D
E
F
G
Inside
Outside
Asp96
HOOC
Lys216
N
+H
COO⁻
⁻OOC
Asp
85
Asp212
Arg
82
+



3D structures from 2D crystals

Bacteriorhodopsin	3.5 Å	*
Bacteriorhodopsin	3.0 Å	*
DOC bacteriorhodopsin	6.0 Å	
Bacteriorhodopsin p22121	6.5 Å	
Porin PhoE	6.0 Å	
Plant LHC-II	3.4 Å	*
Rhodopsin frog p2	6.5 Å	
Tubulin dimer	3.7 Å	*
Aquaporin-1	3.8 Å	*
Aquaporin	4.0 Å	*
Halorhodopsin	5.0 Å	
Glutathione transferase	6.0 Å	
SecYEG complex	8.0 Å	
Plant photosystem II RC	8.0 Å	
Neurospora H+-ATPase	8.0 Å	
Gap junction channel	7.5 Å	
NhaA Na/H antiporter	7.0 Å	
Glycerol channel GlpF	6.9 Å	
Oxalic acid transporter OxlT	6.0 Å	
EmrE multidrug transporter	7.0 Å	

Problems & pitfalls

- Space group determination
- Twinning
- Comparison to X-ray structures

The 17* Two-Sided Plane Groups Allowed For Biological Molecules

Unit cell	Two-sided plane groups	Corresponding 3-dimensional space group	Projection symmetry
oblique	p1	P1	p1
	p21	P2(*c*-axis unique)	p2
rectangular	p12	P2(*b*-axis unique)	pm
	$p12_1$	$P2_1$	pg
	c12	C2	cm
	p222	P222	pmm
	$p222_1$	$P222_1$	pmg
	$p22_12_1$	$P22_12_1$	pgg
	c222	C222	cmm
square	p4	P4	p4
	p422	P422	p4m
	$p42_12$	$P42_12$	p4g
hexagonal	p3	P3	p3
	p312	P312	p3m1
	p321	P321	p31m
	p6	P6	p6
	p622	P622	p6m

The two-sided plane group nomenclature was first proposed by W.T. Holser (Z.krystallogr. **110**, 266-28, (1958)). His nomenclature has the following main rules.

(a) Cell type is indicated first by small letter, *p* or *c* (primitive or centred)

(b) The axis perpendicular to the plane is always chosen as the *z*-axis.

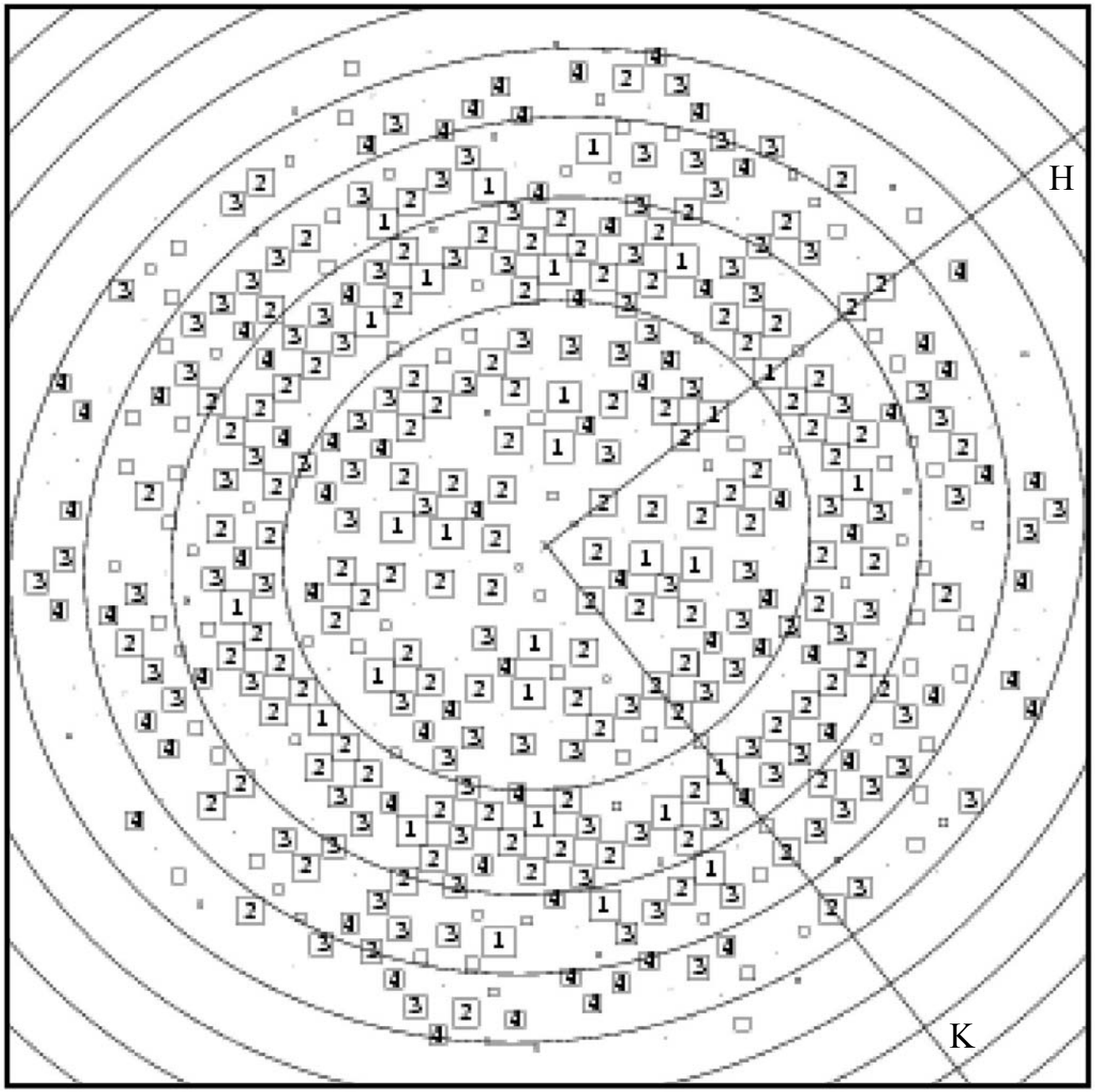
(c) The symmetry along this axis is always described by the first symbol following the cell type.

e.g. p3 $p42_12$

* Note that there 80 two-sided plane groups in total, of which there are only 17 which do not contain inversion centres or mirror or glide planes.

```
C#################### ALLSPACE ################################################
C  Table of phase comparisons to be made
C    - not comparable
C    1 directly identical
C    H differ by 180*H       JSIMPLE  = number to compare directly
C    K differ by 180*K       JSCREW   = number to compare + 180 * M
C    HK differ by 180*(H+K)     where M = H*JH180 + K*JK180
C
C
C  SPACEGROUP  H=-h +h -h +k +k -k -k +h -h +k -k -h +h -h +h  JSIMPLE
C         H=                    -k +k -k +k   JSCREW
C refin
C  prog # symb  K=+k -k -k +h -h +h -h -h +h +h +h -h +k -k     JH180
C         K=            -k +k -k +k               JK180
C
C  1   1  p1   - - - - - - - - - - - - - - - - 0 0 - -
C  2   2  p2   - - 1 - - - - - - - - - - - - - 1 0 - -
C  3   3b p12   1 - - - - - - - - - - - - - - - 1 0 - -
C  4   "a  "   - 1 - - - - - - - - - - - - - - 1 0 - -
C  5   4b p121  K - - - - - - - - - - - - - - - 0 1 - 180
C  6   "a  "   - H - - - - - - - - - - - - - - 0 1 180 -
C  7   5b c12   1 - - - - - - - - - - - - - - - 1 0 - -
C  8   "a  "   - 1 - - - - - - - - - - - - - - 1 0 - -
C  9   6  p222  1 1 1 - - - - - - - - - - - - - 3 0 - -
C 10   7b p2221  H H 1 - - - - - - - - - - - - - 1 2 180 -
C 11   "a  "   K K 1 - - - - - - - - - - - - - 1 2 - 180
C 12   8  p22121 HK HK 1 - - - - - - - - - - - - - 1 2 180 180
C 13   9  c222  1 1 1 - - - - - - - - - - - - - 3 0 - -
C 14   10 p4    - - 1 - 1 1 - - - - - - - - - - 3 0 - -
C 15   11 p422  1 1 1 1 1 1 1 - - - - - - - - - 7 0 - -
C 16   12 p4212  HK HK 1 1 HK HK 1 - - - - - - - - - 3 4 180 180
C 17   13 p3    - - - - - - - - - - 1 - 1 - - - 2 0 - -
C 18   14 p312  - - - - - - - 1 - 1 1 - 1 - - 1  5 0 - -
C 19   15 p321  - - - 1 - - - 1 - 1 - 1 - 1 -  5 0 - -
C 20   16 p6    - - 1 - - - - - - - 1 1 1 1 - -  5 0 - -
C 21   17 p622  - - 1 1 - - 1 1 1 1 1 1 1 1 1  11 0 - -
C
C  Notes:-
C    1. Compare all possible pairs of phases each with error E.
C    2. Error comparing 2 different reflections is  1.414 * E.
C    3. Error comparing reflections to its Friedel is  2.0 * E.
C    4. So Friedel comparisons should have less weight ????
C    5. Note that the convention in the two dimensional spacegroups is that
C         the first in-plane symmetry axis is the a-axis. Therefore,
C         when ALLSPACE indicates that the spacegroup has the in-plane
C         axis along b, it is better to switch the indexing to ensure
C         compatibility with the normal convention as programmed in
C         ORIGTILT, rather than using the REVHK option in ORIGTILT,
C         which will switch the indexing on input.
```

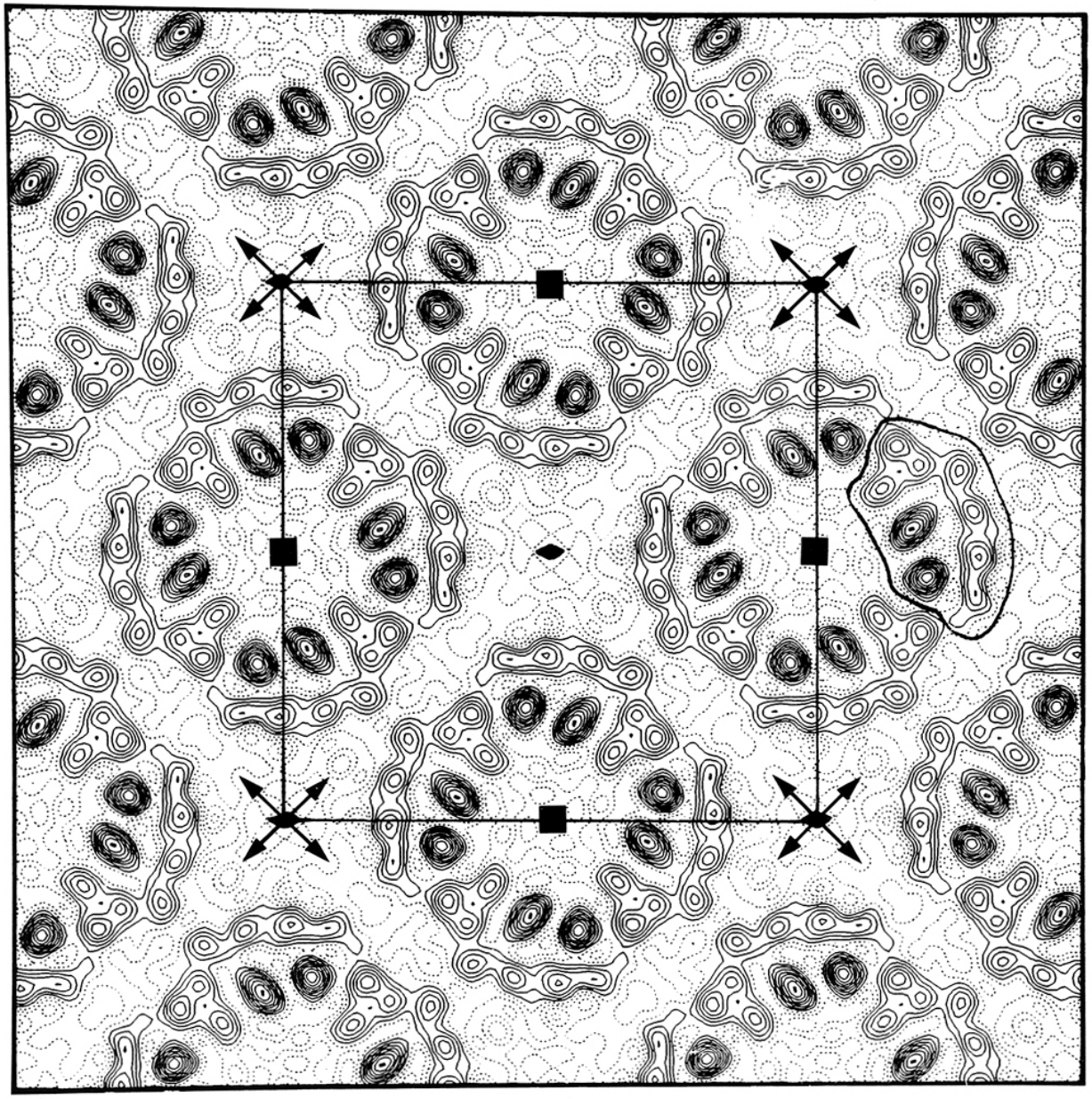
Halorhodopsin CTFg000 defocus=5650,7050,15, 19.2.94, CTF refinement

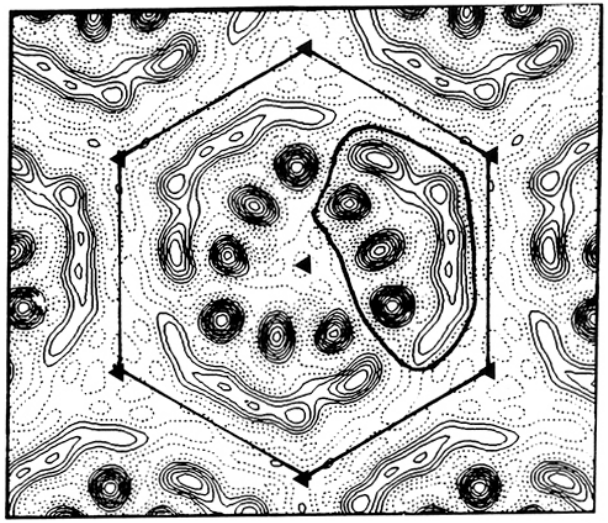


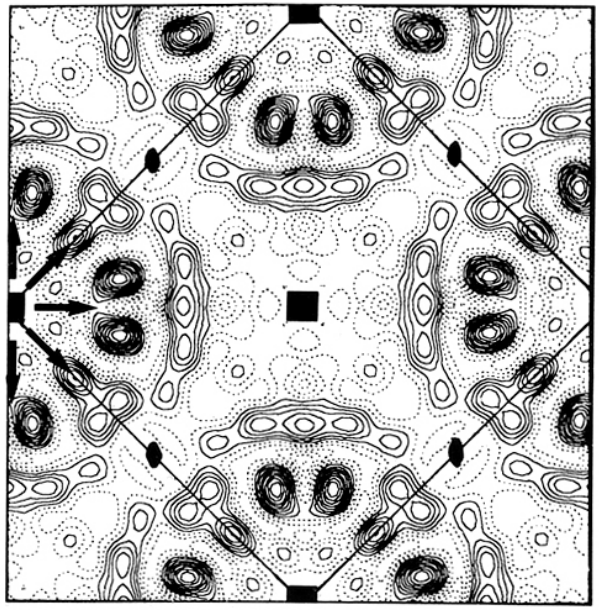
Halorhodopsin film 2464s

SPACEGROUP	Phase resid(No) v.other spots (90 random)	Phase resid(No) v.theoretical (45 random)	OX	OY	Target residual based on statistics taking Friedel weight into account
1 p1	34.4 432	25.9 432			
2 p2	42.8* 216	21.4 432	130.1	-21.6	51.7
3b p12_b	77.7 167	11.3 14	-49.5	12.0	35.2
3a p12_a	80.1 169	30.1 18	-180.0	-21.8	35.4
4b p121_b	29.3* 167	10.1 14	-139.7	120.0	35.2
4a p121_a	21.4* 169	9.3 18	138.0	68.1	35.4
5b c12_b	77.7 167	11.3 14	-49.5	12.0	35.2
5a c12_a	80.1 169	30.1 18	-180.0	-21.8	35.4
6 p222	64.9 552	21.5 432	-49.7	158.3	41.2
7b p2221b	59.6 552	37.7 432	-139.5	-111.9	41.2
7a p2221a	63.0 552	37.8 432	-139.7	68.2	41.2
8 p22121	32.3* 552	21.5 432	130.2	158.2	41.2
9 c222	64.9 552	21.5 432	-49.7	158.3	41.2
10 p4	30.8* 528	21.5 432	130.1	-21.7	41.5
11 p422	57.2 1173	21.5 432	130.1	158.1	37.6
12 p4212	28.2* 1173	21.6 432	130.1	158.1	37.6

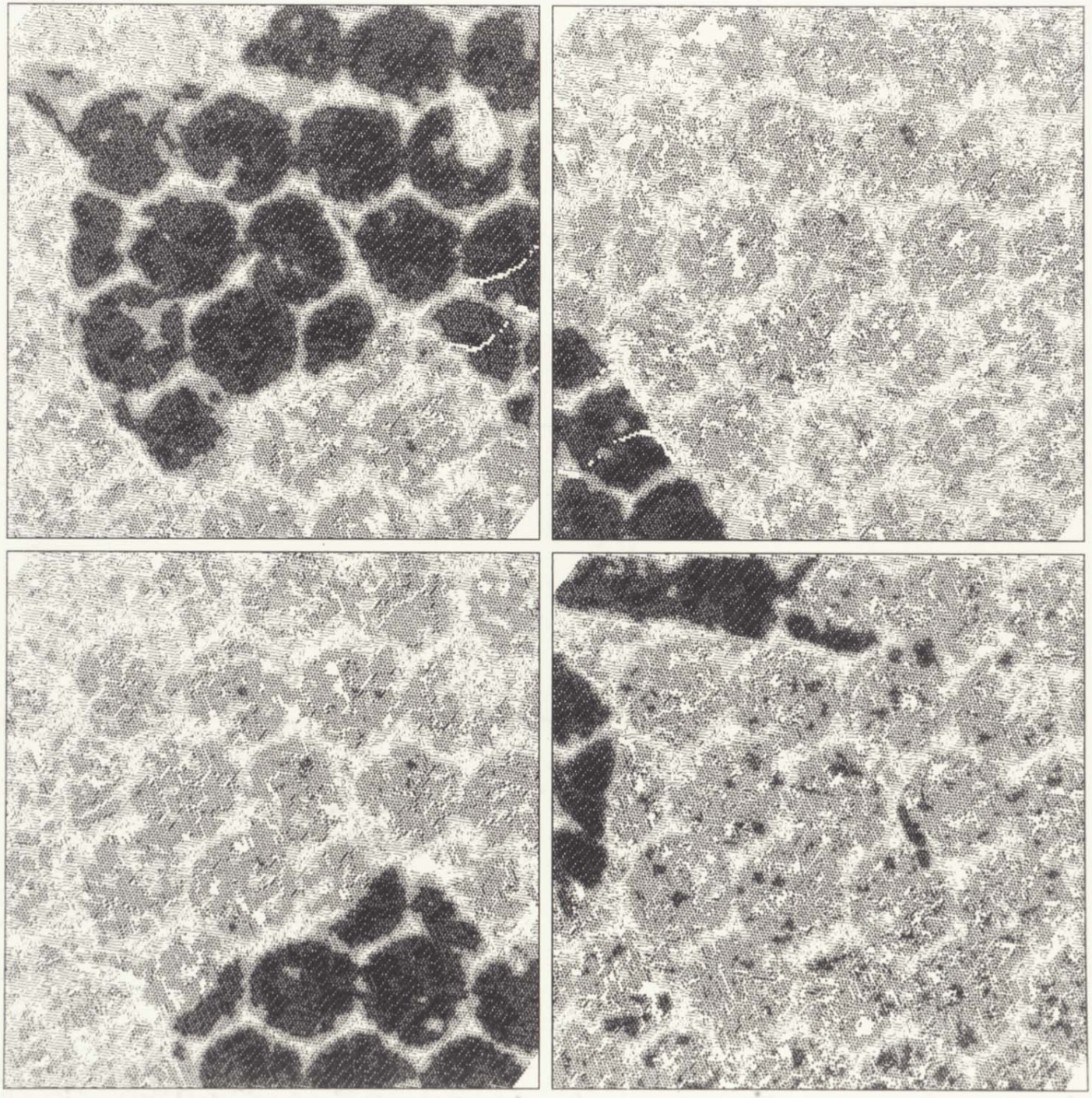
*= acceptable
!= should be considered
`= possibility

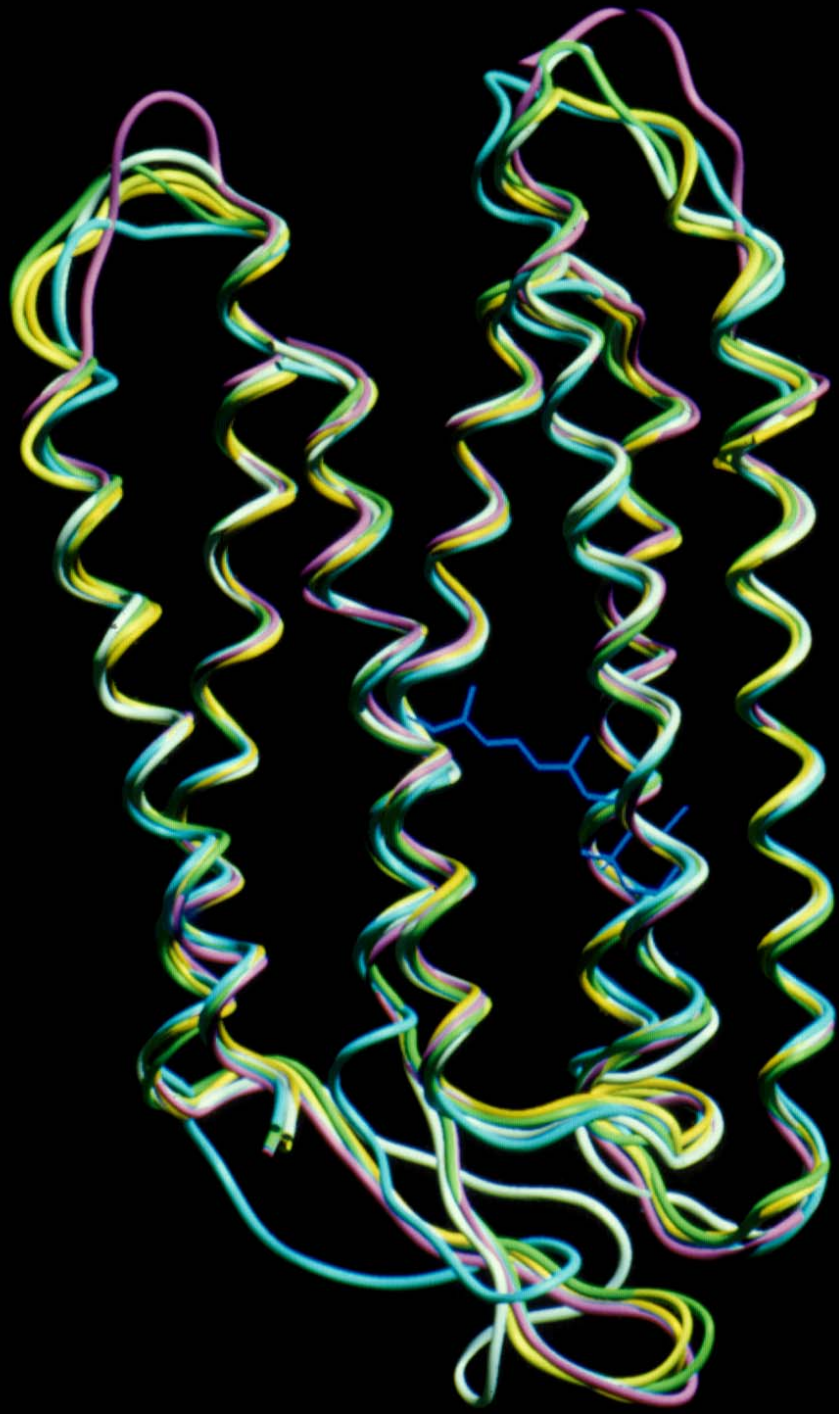






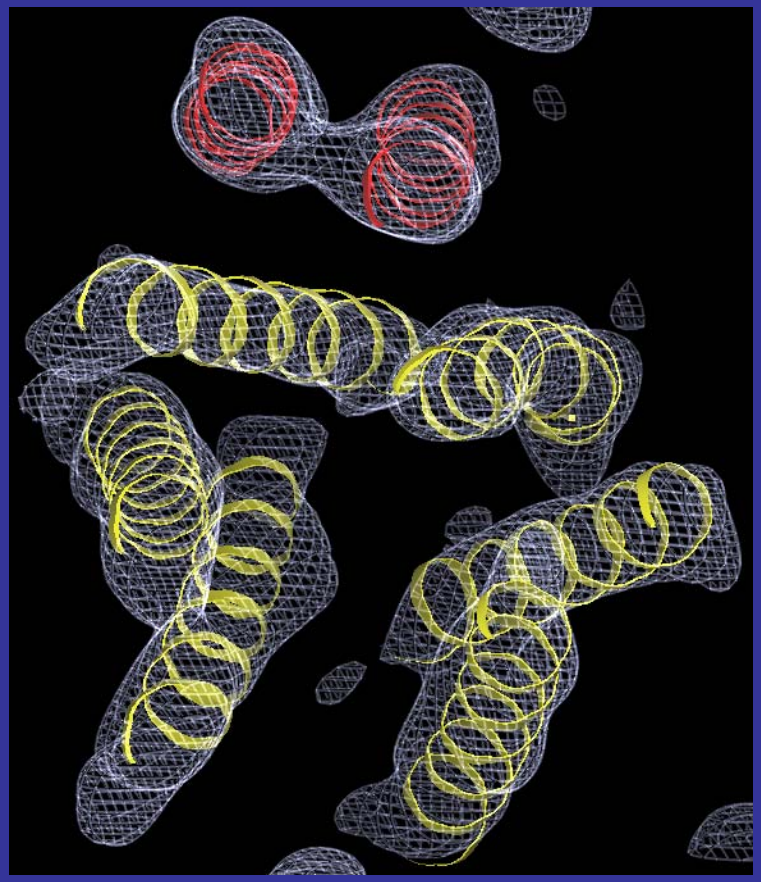
ccorvtk080.cor



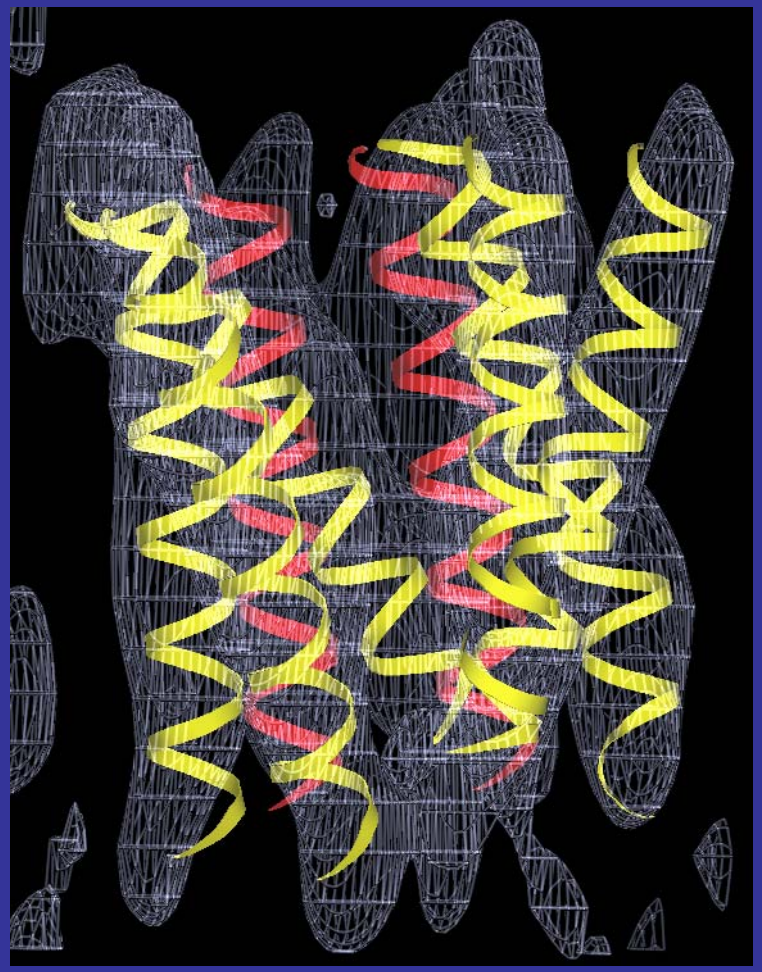


The multidrug transporter EmrE is an asymmetric homodimer

Tate & Ubarretxena-Belandia, EMBO J. (2003) 22, 6175



Top view



Side view

Key considerations

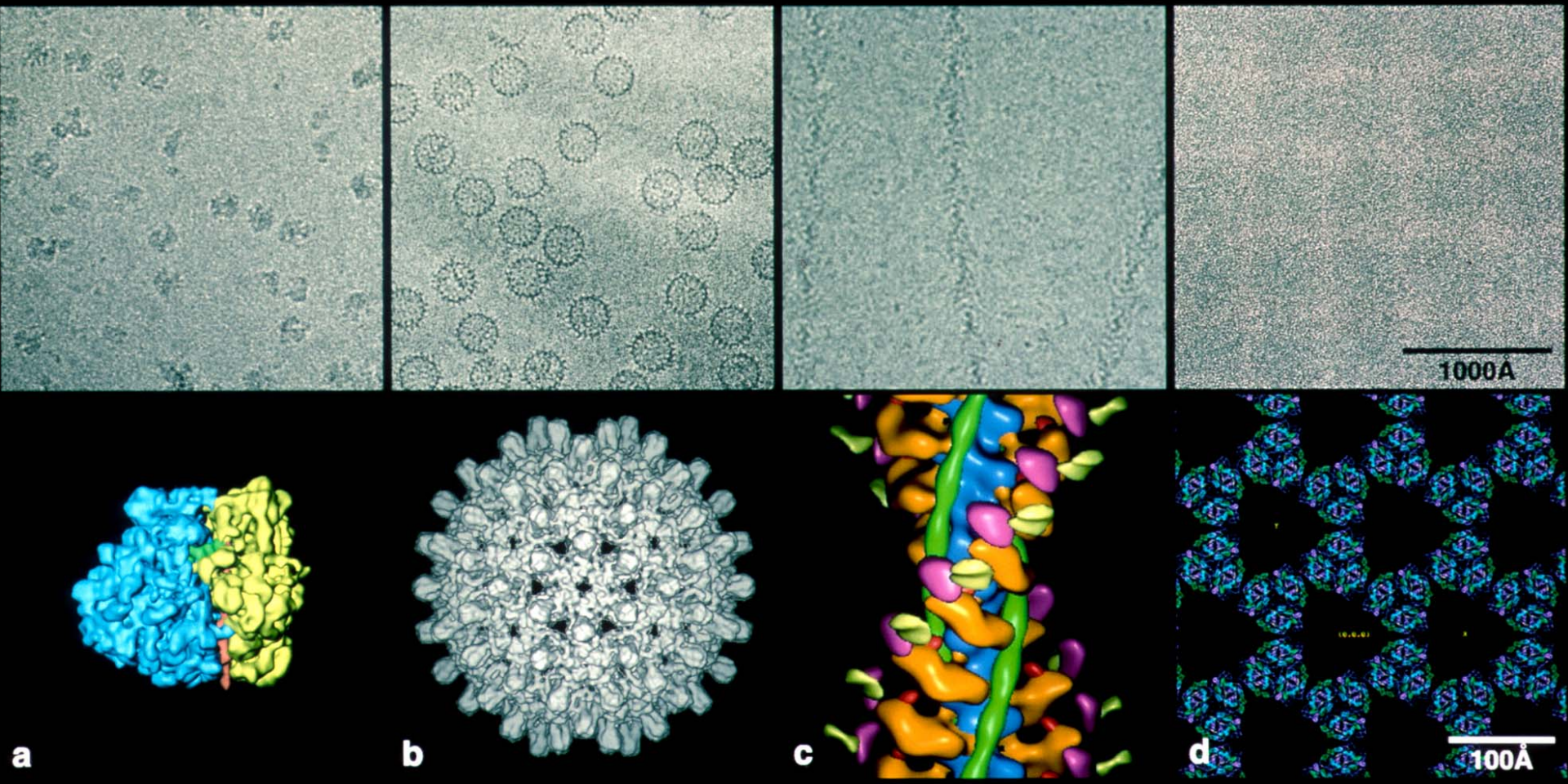
- Tilt-transfer-function (TTF, TTBOX, etc)
- Creating reference area using MAKETRAN
- Size of reference box (optimize)

Future problems to be solved

- Better images
- Flatness of 2D crystals
- Treatment as single molecules/unit cells

Lessons from 2D not yet general

- Beam tilt
- Differential magnification



Some state-of-the-art cryo-EM structures - see Baker & Henderson, Int Tables for Cryst, Vol F