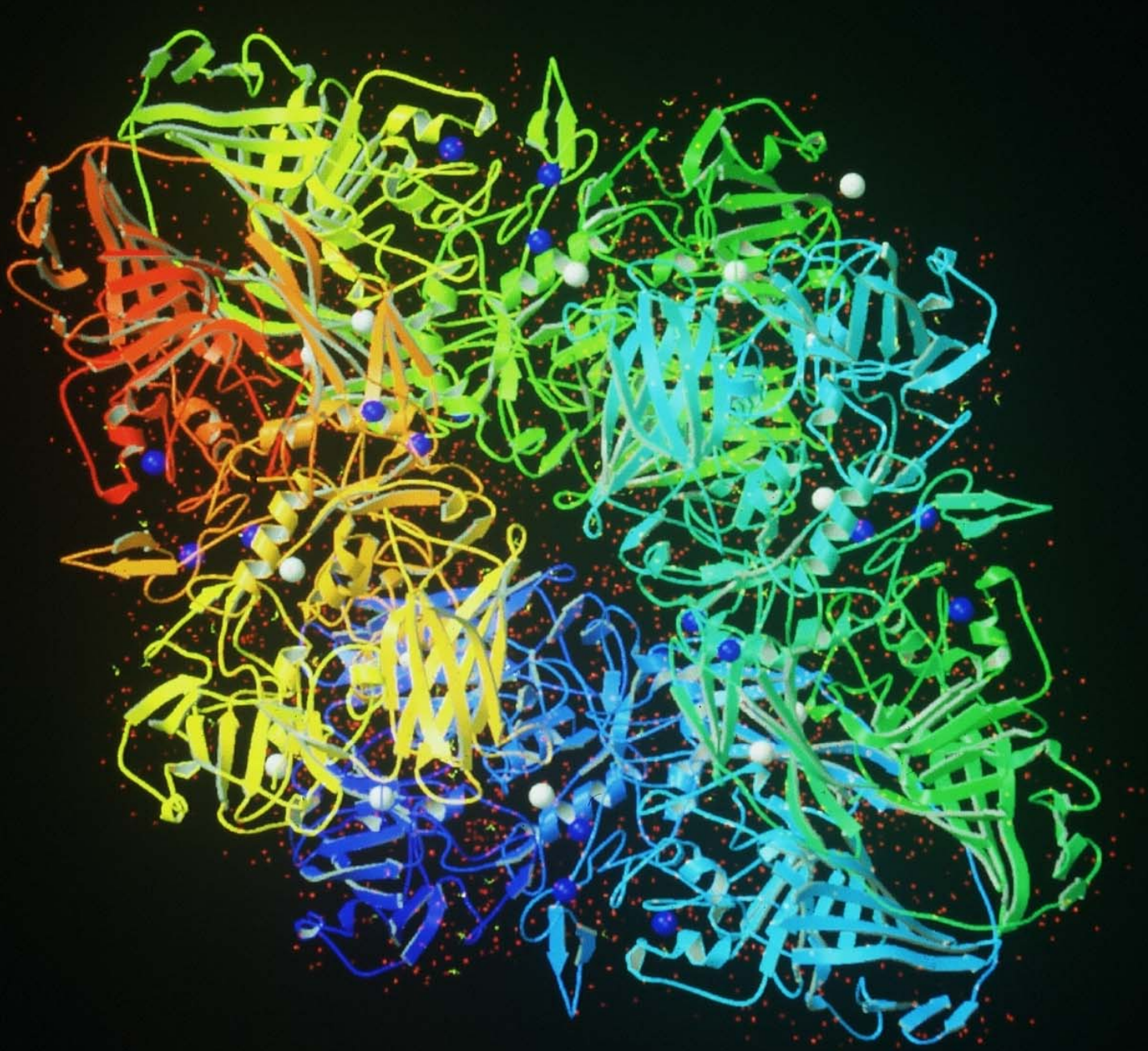
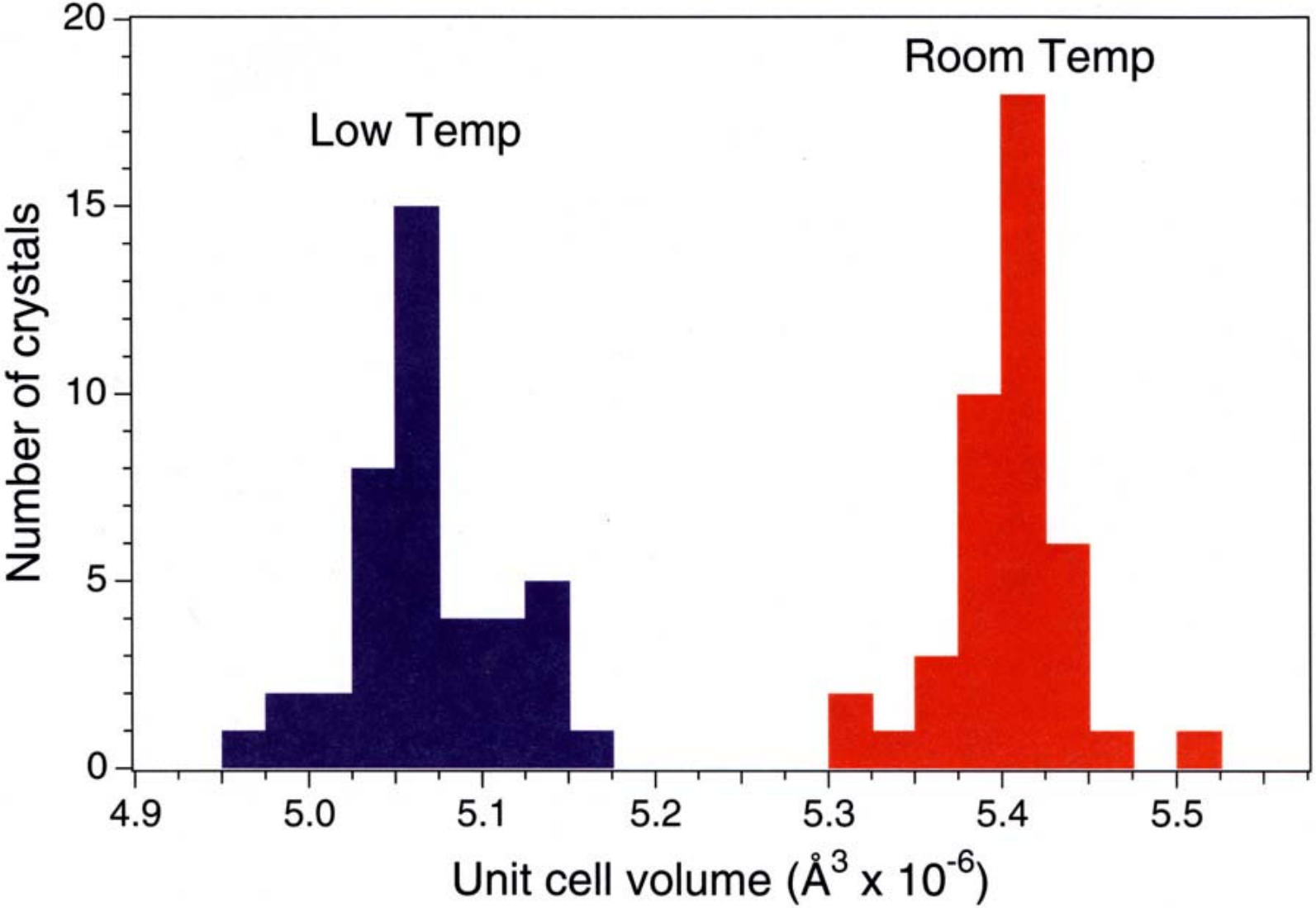
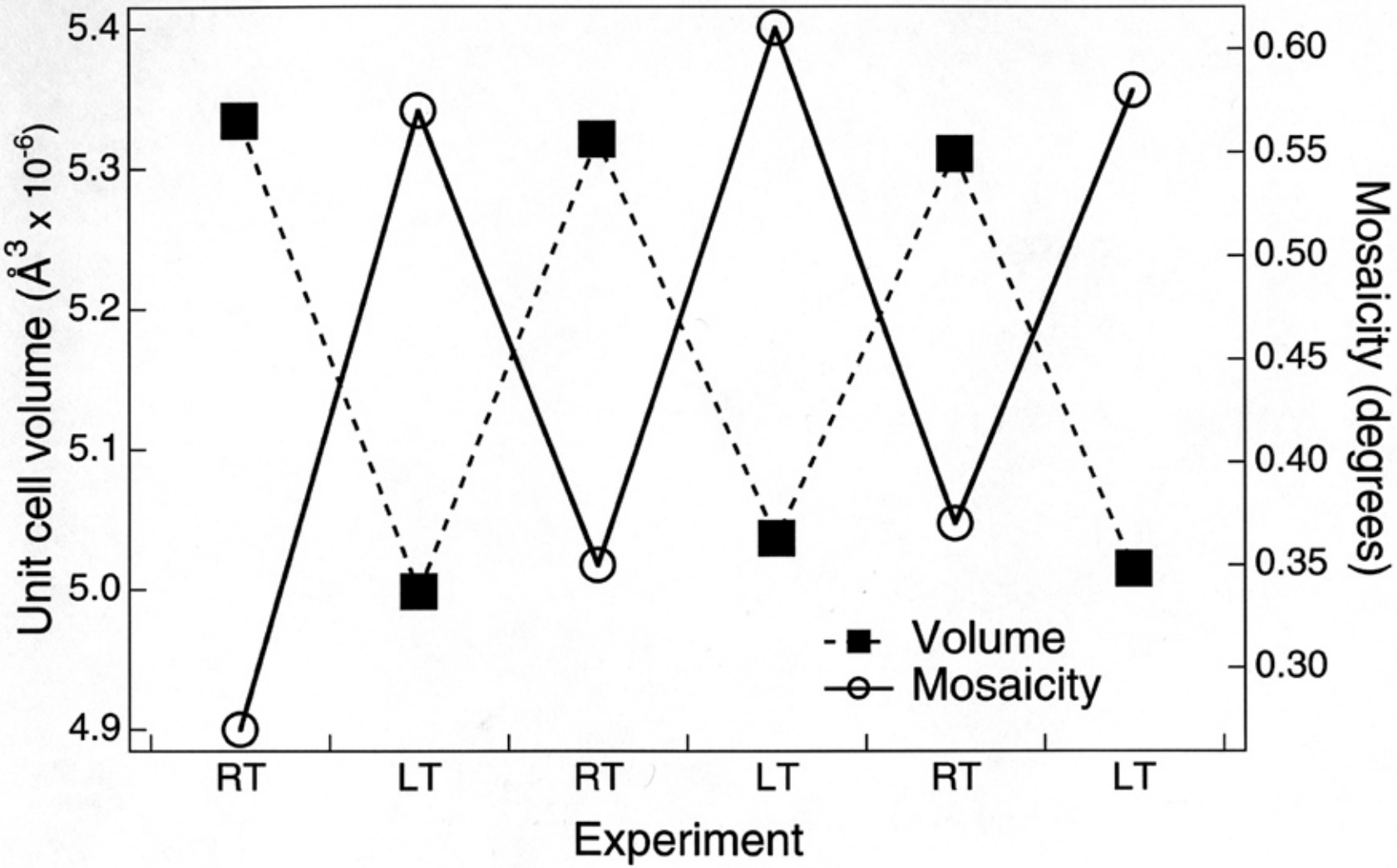
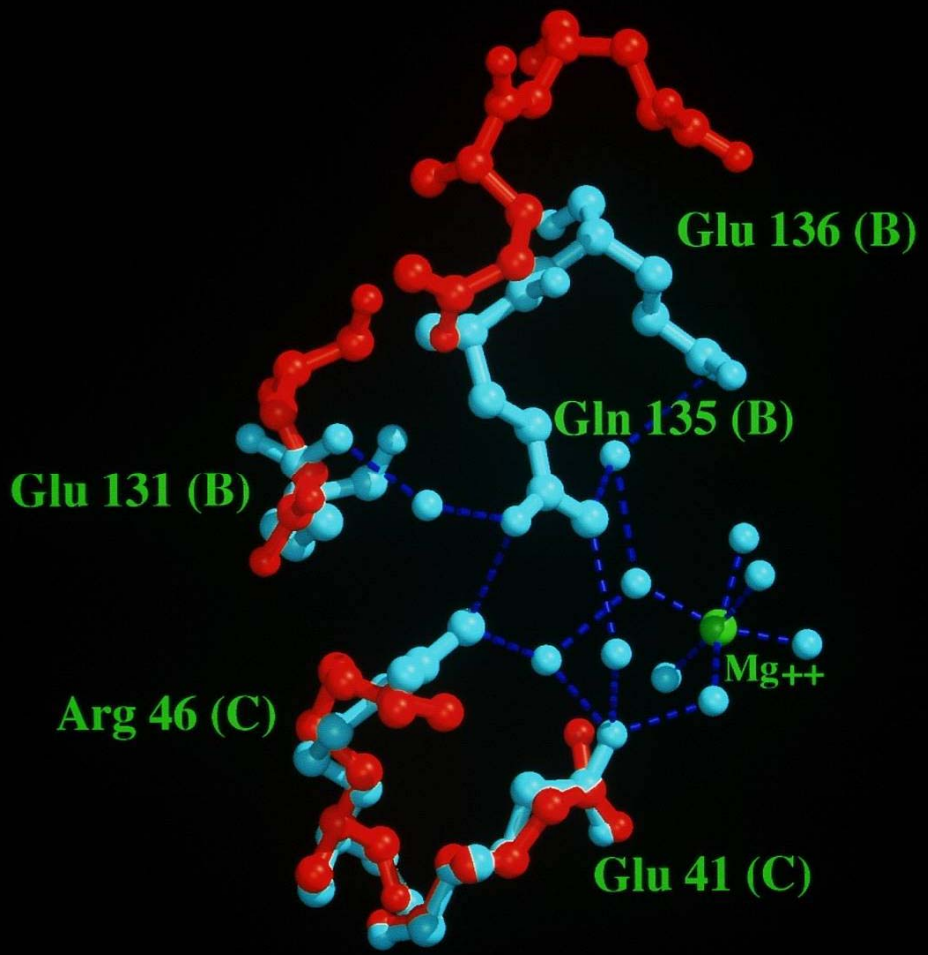
The effects of flash-freezing on the structure of β-galactosidase

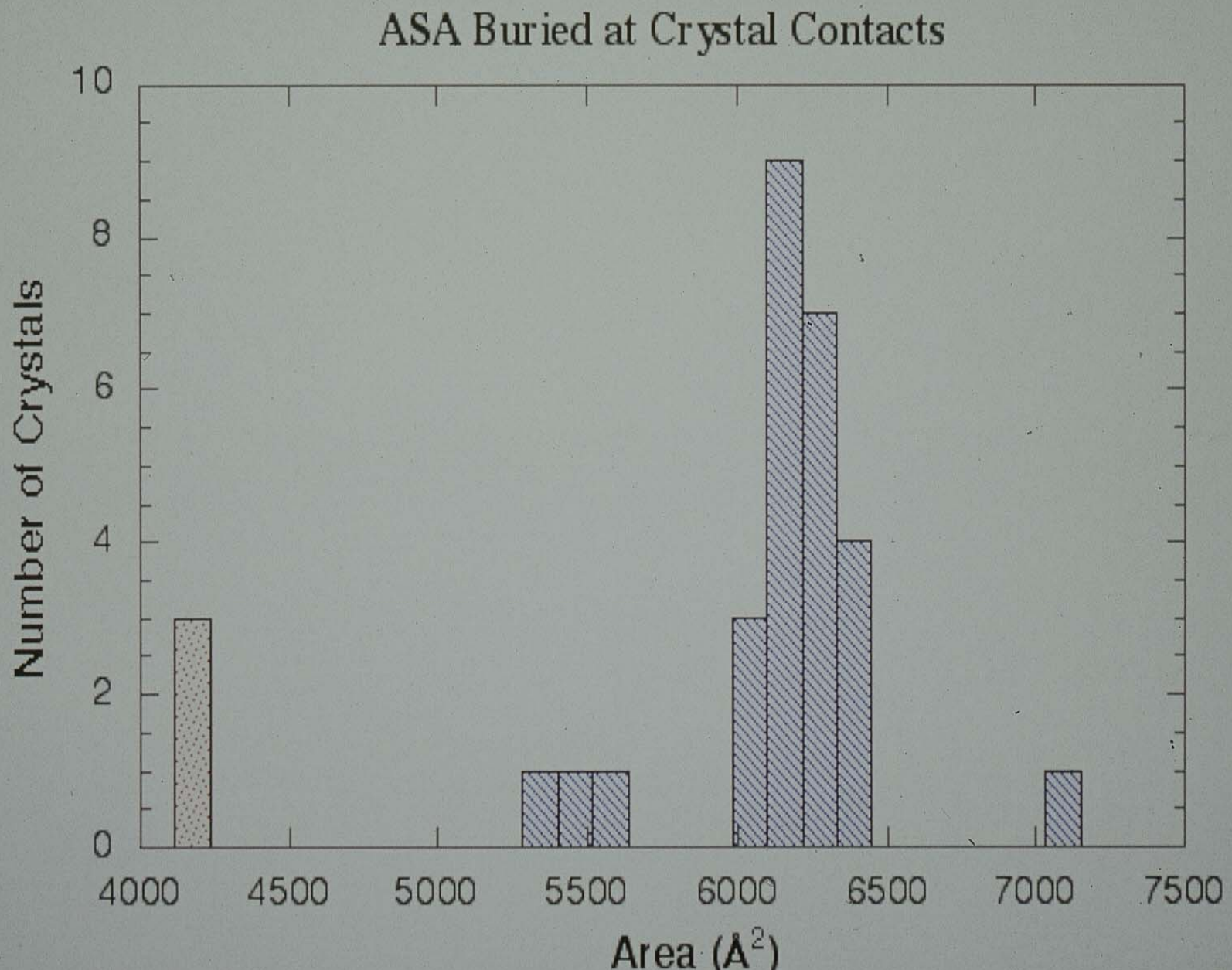
Doug Juers

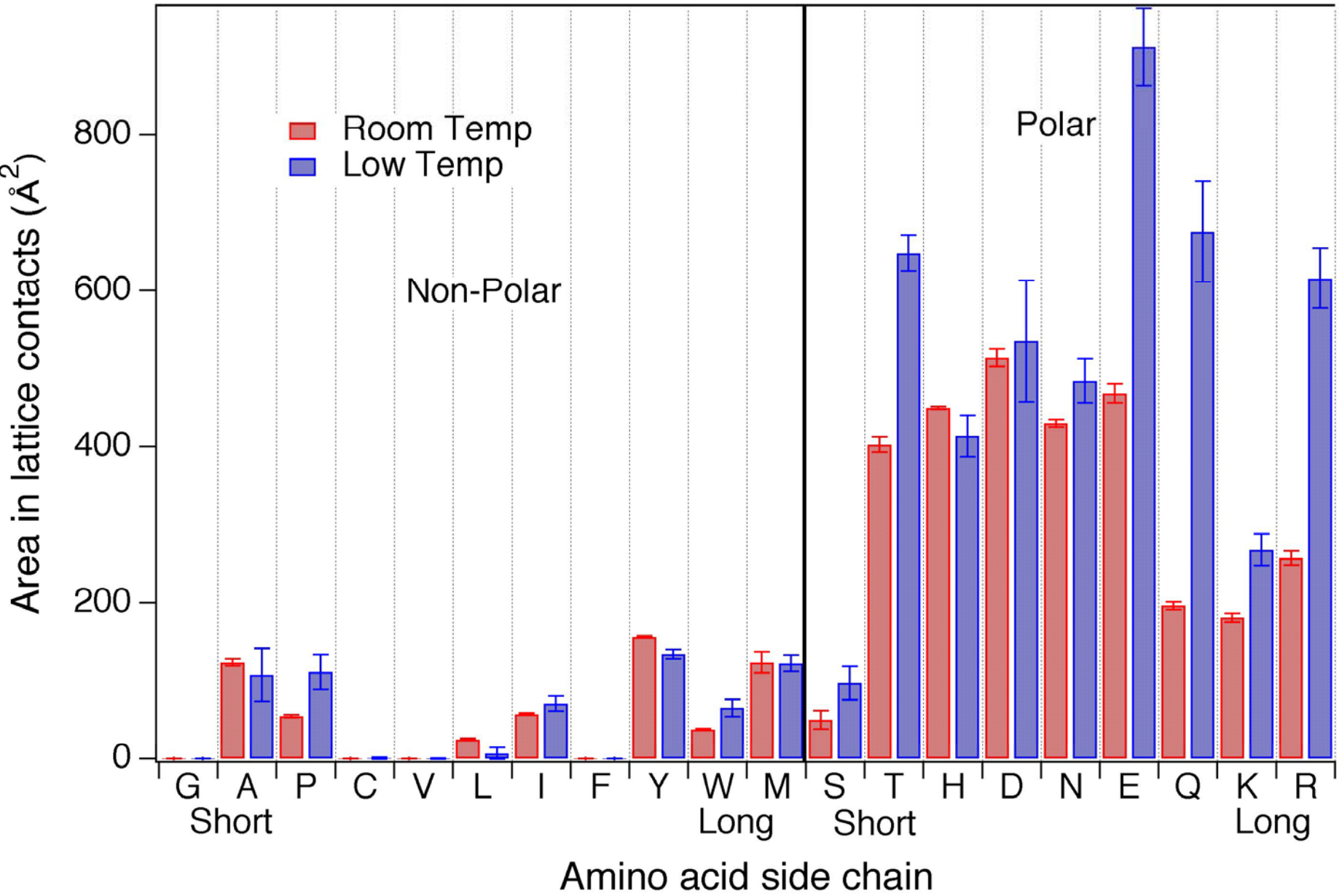


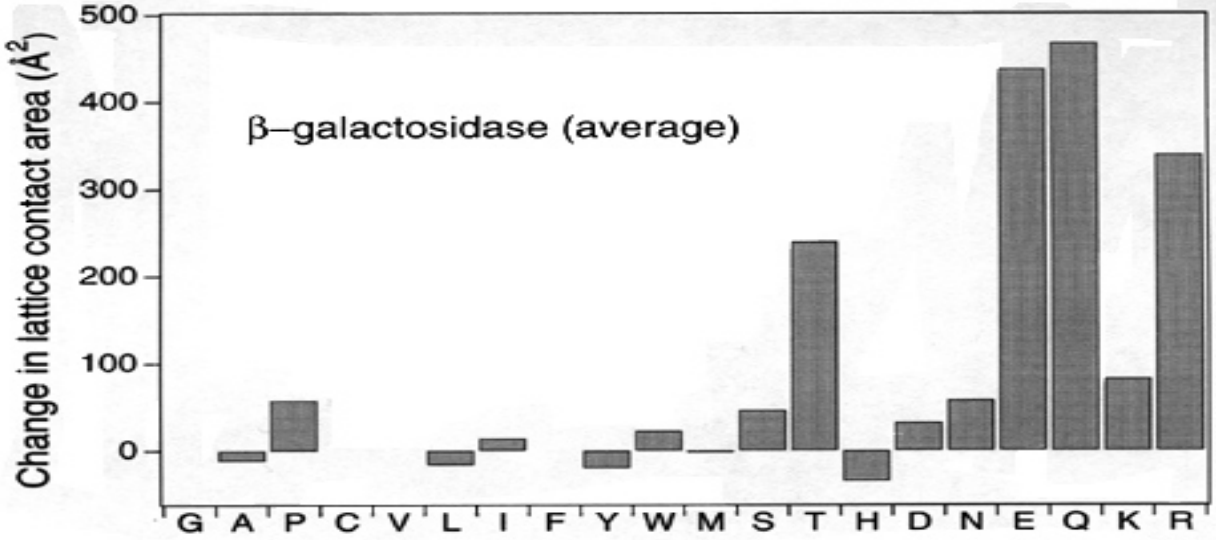

Low Temp
Room Temp
Number of crystals
0
5
10
15
20
4.9
5.0
5.1
5.2
5.3
5.4
5.5
Unit cell volume (Å3 x 10-6)

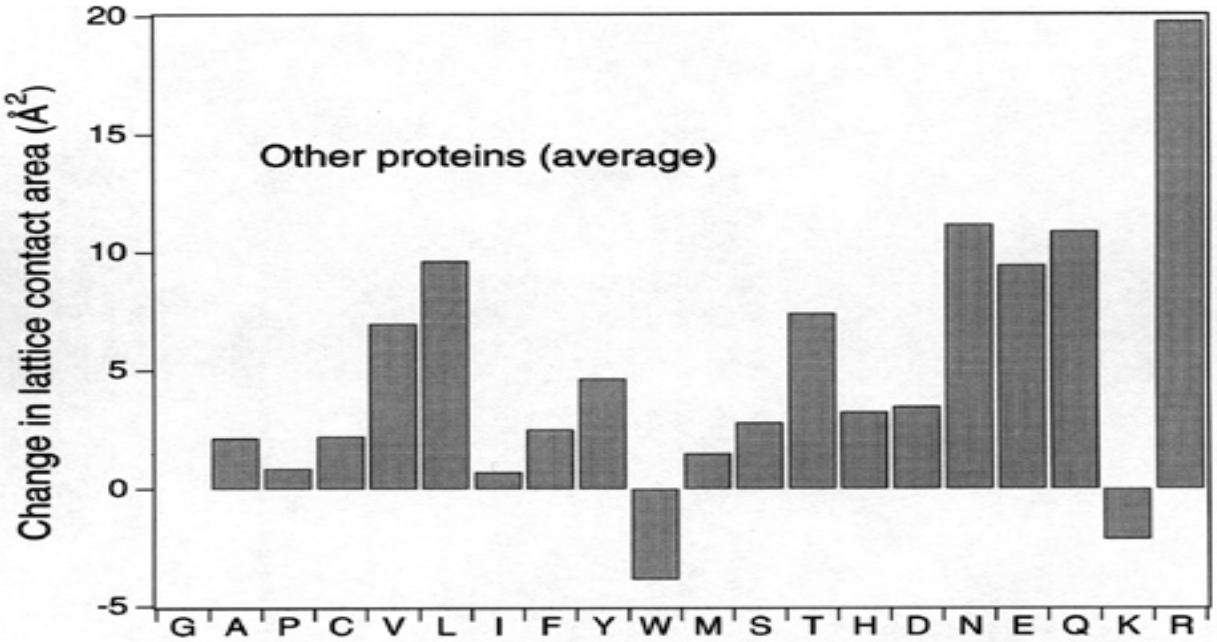

Unit cell volume (Å^3 x 10^{-6})
5.4
5.3
5.2
5.1
5.0
4.9
Mosaicity (degrees)
0.60
0.55
0.50
0.45
0.40
0.35
0.30
Volume
Mosaicity
RT
LT
RT
LT
RT
LT
Experiment

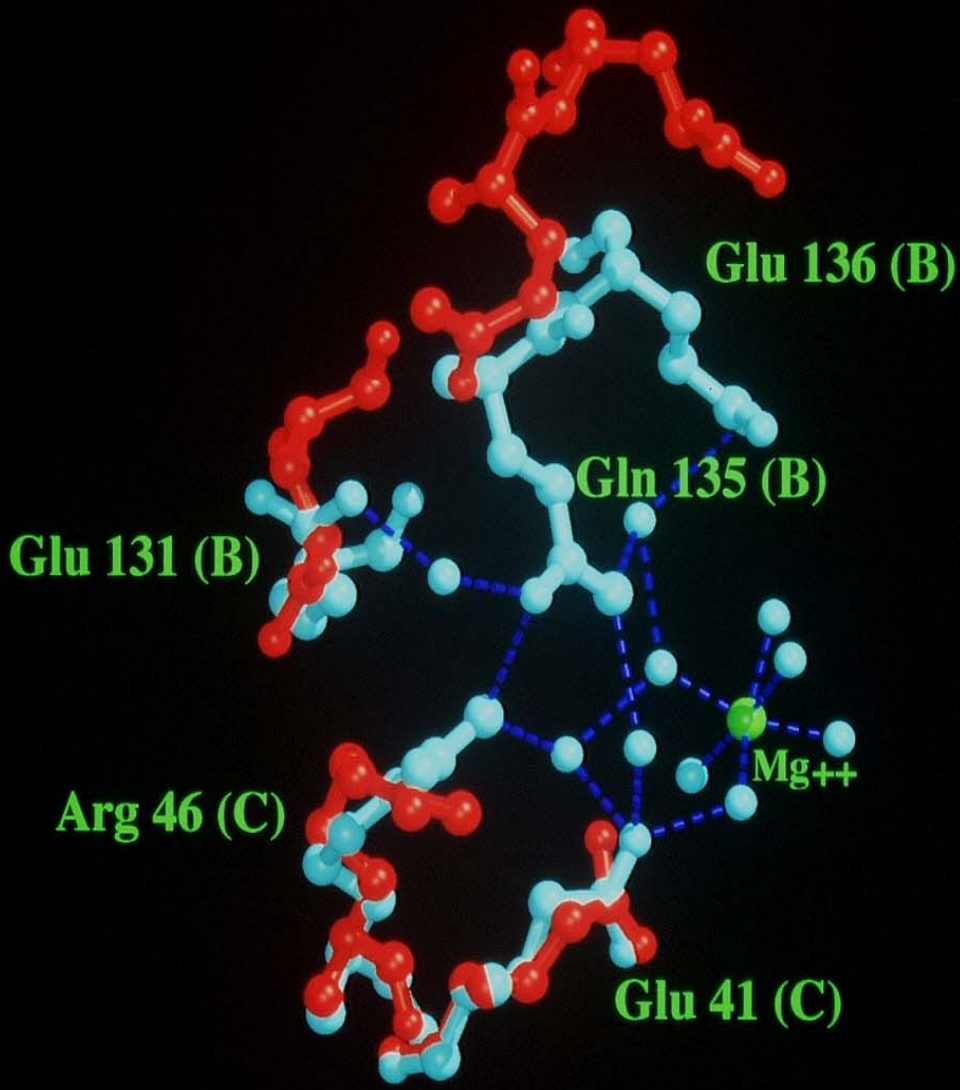

Glu 136 (B)
Gln 135 (B)
Glu 131 (B)
Mg++
Arg 46 (C)
Glu 41 (C)

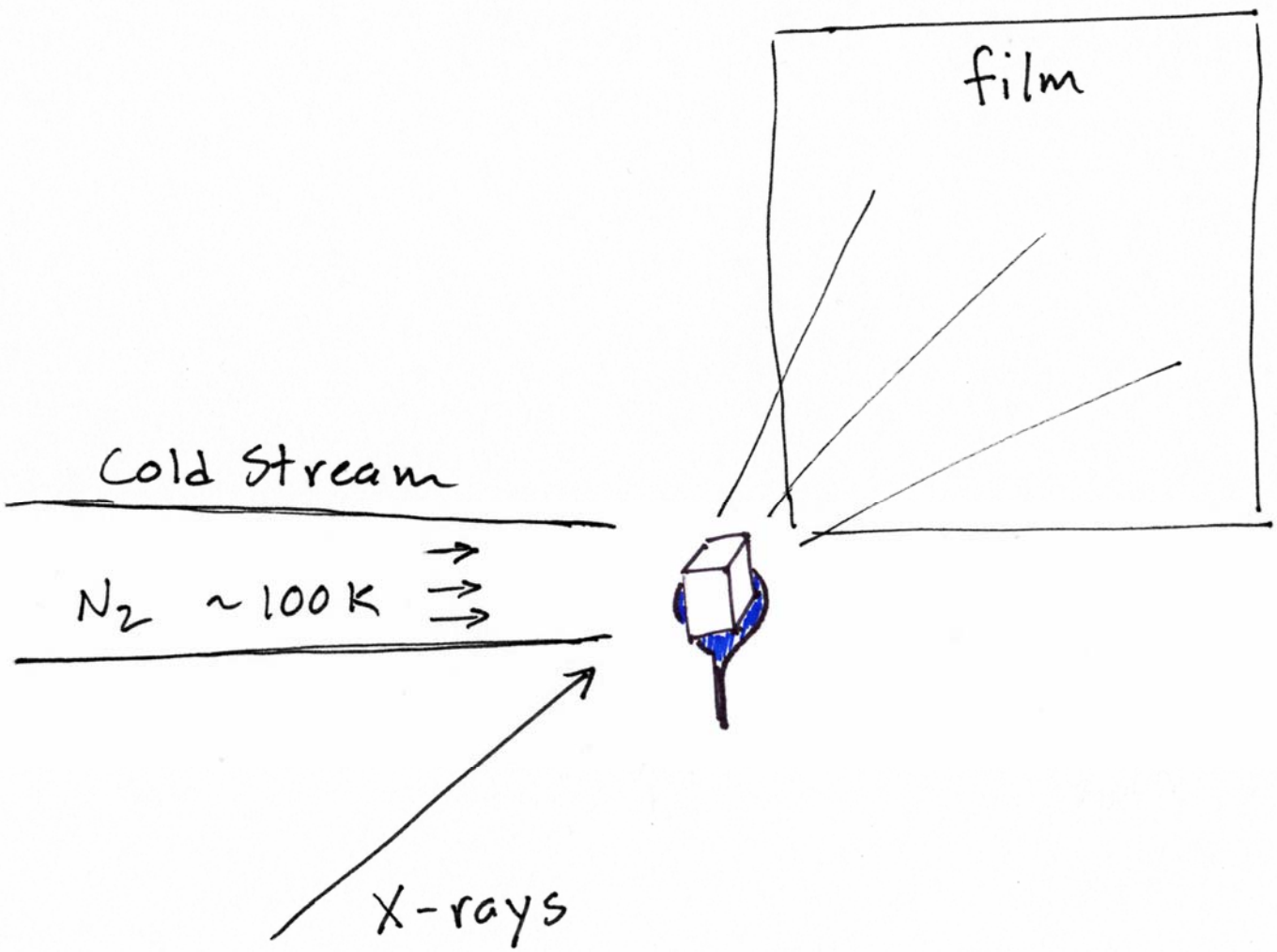

ASA Buried at Crystal Contacts
Number of Crystals
0
2
4
6
8
10
4000
4500
5000
5500
6000
6500
7000
7500
Area (Å^2)


Area in lattice contacts (Å^2)
800
600
400
200
0
Room Temp
Low Temp
Non-Polar
Polar
G
A
P
C
V
L
I
F
Y
W
M
S
T
H
D
N
E
Q
K
R
Short
Long
Short
Long
Amino acid side chain

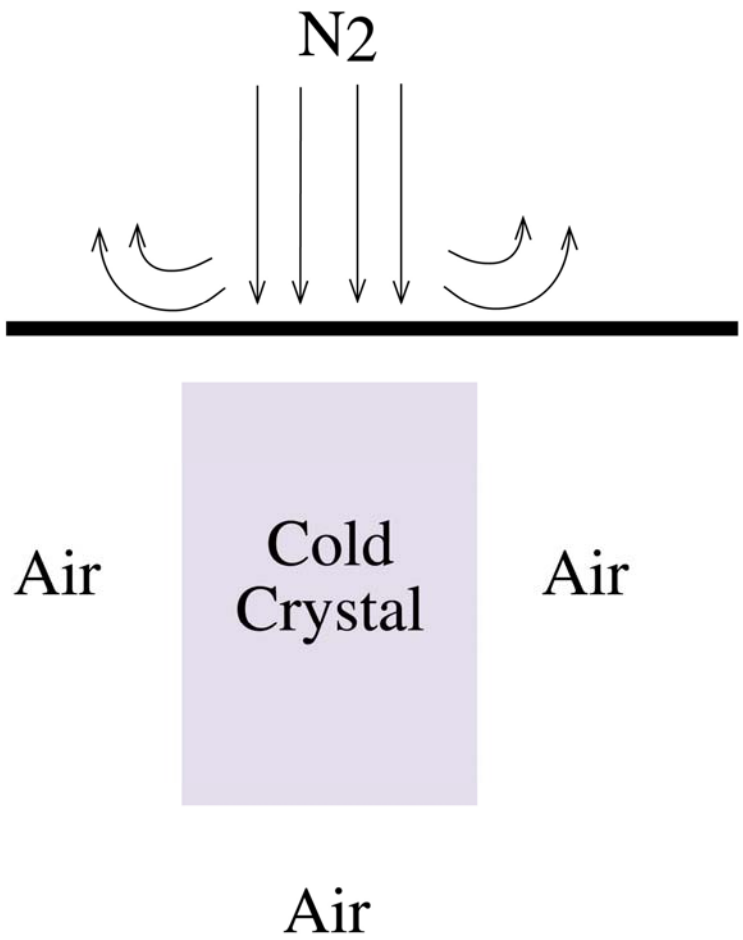

β–galactosidase (average)
Change in lattice contact area (Å²)
500
400
300
200
100
0
G A P C V L I F Y W M S T H D N E Q K R


Other proteins (average)
Change in lattice contact area (Å²)
20
15
10
5
0
-5
G A P C V L I F Y W M S T H D N E Q K R


Glu 136 (B)
Gln 135 (B)
Glu 131 (B)
Mg++
Arg 46 (C)
Glu 41 (C)

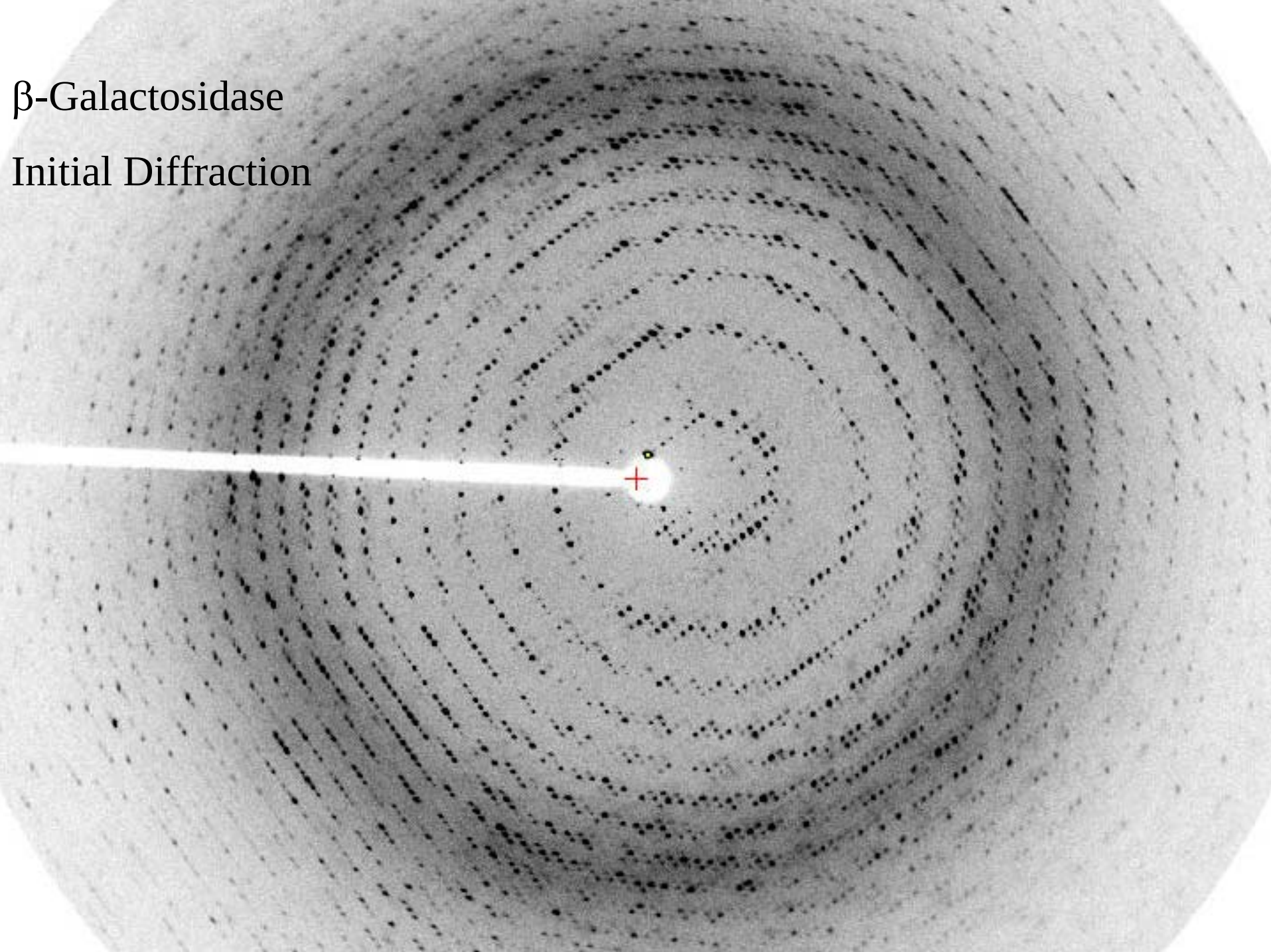

film
Cold Stream
N_2 ~100 K
X-rays

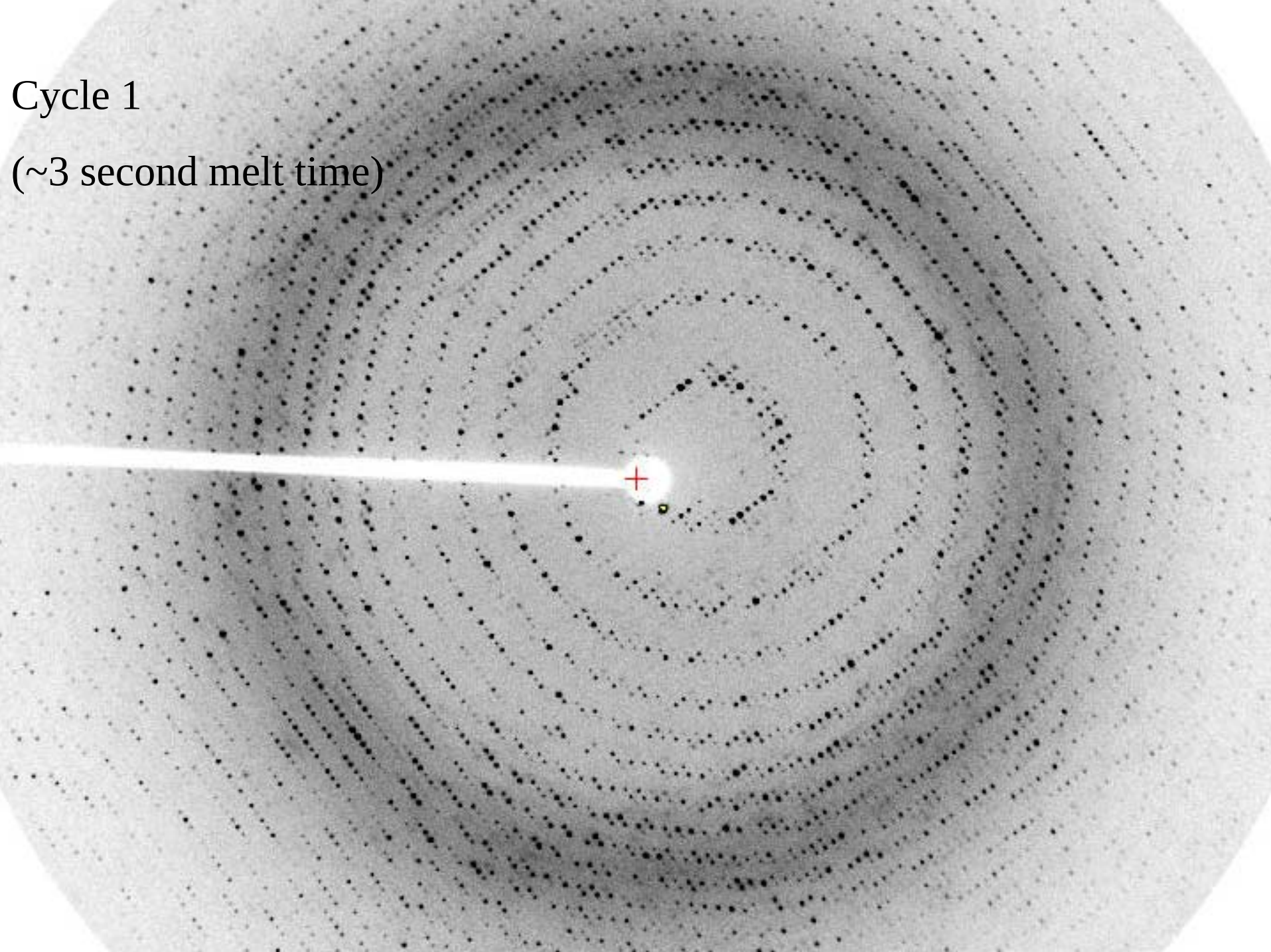
Blocked Cold Stream

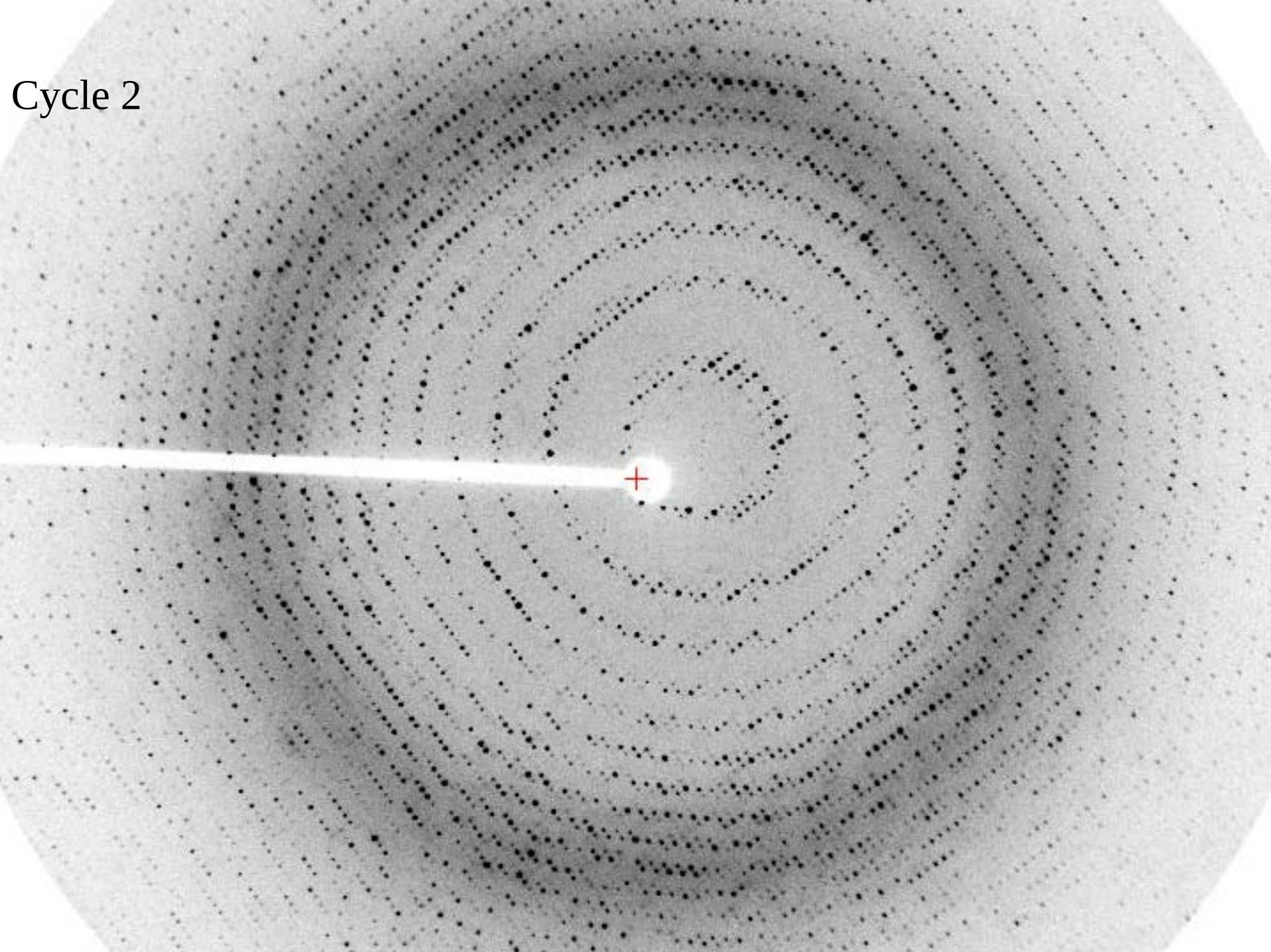


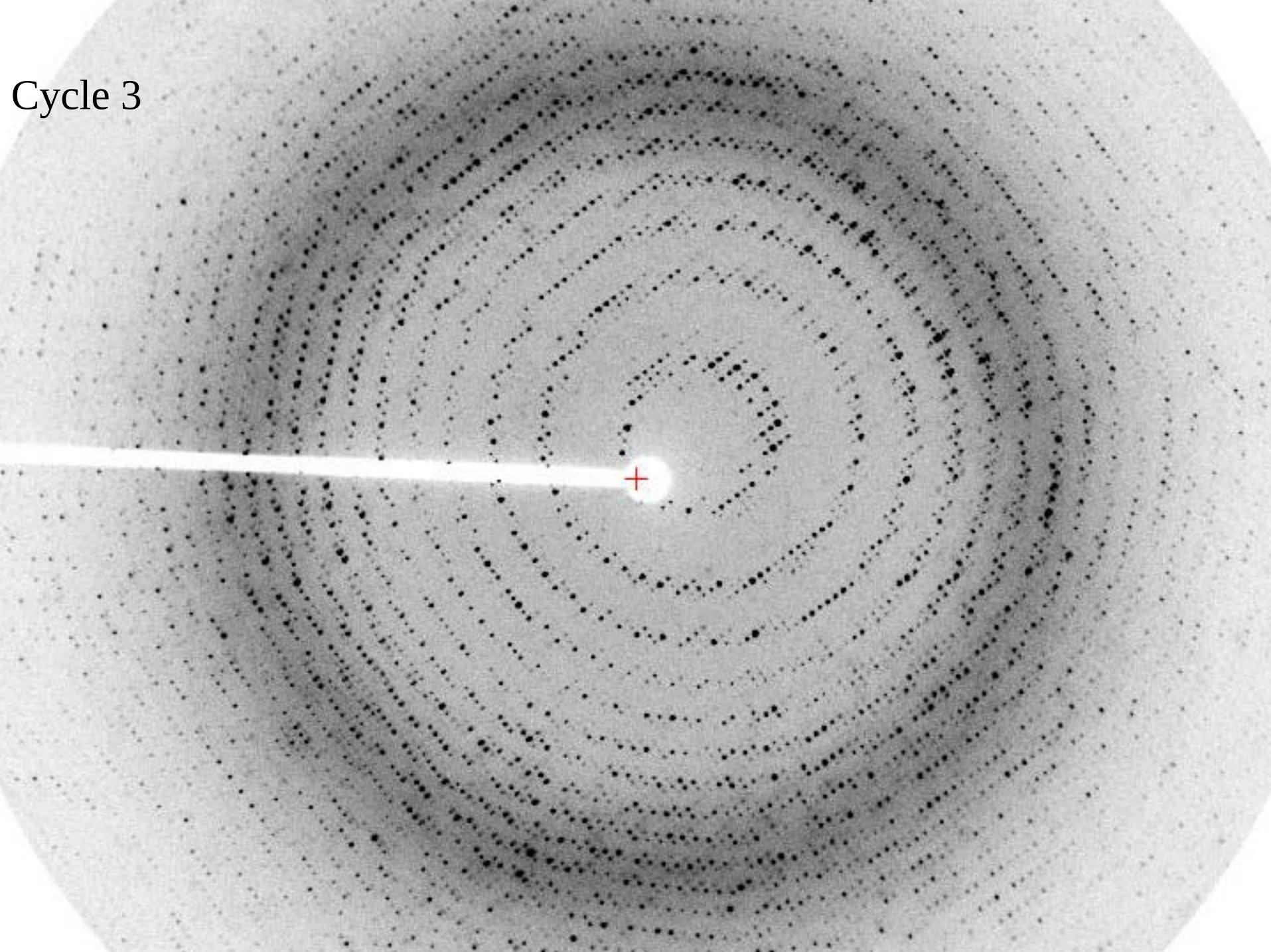
β-Galactosidase

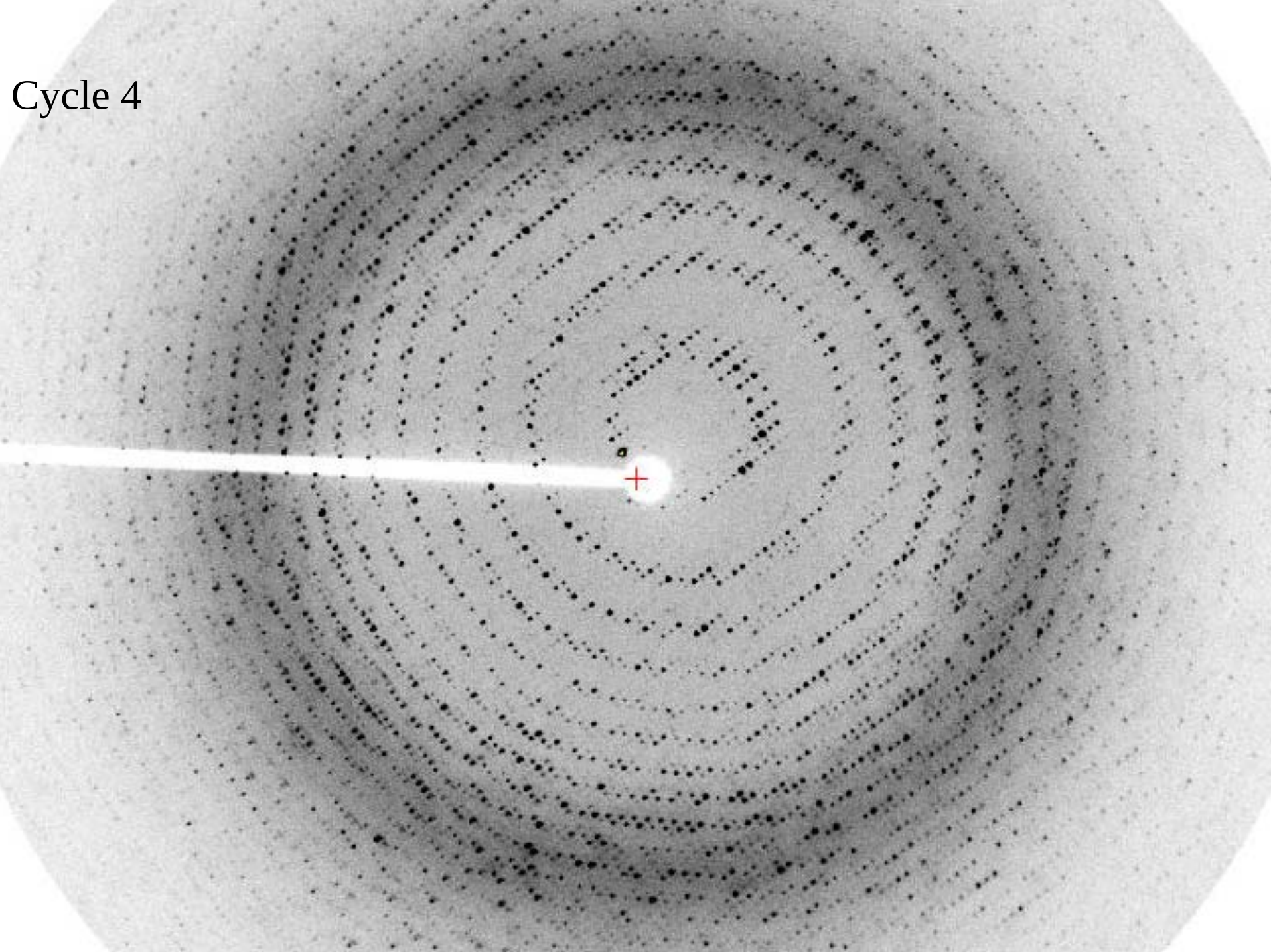
Initial Diffraction

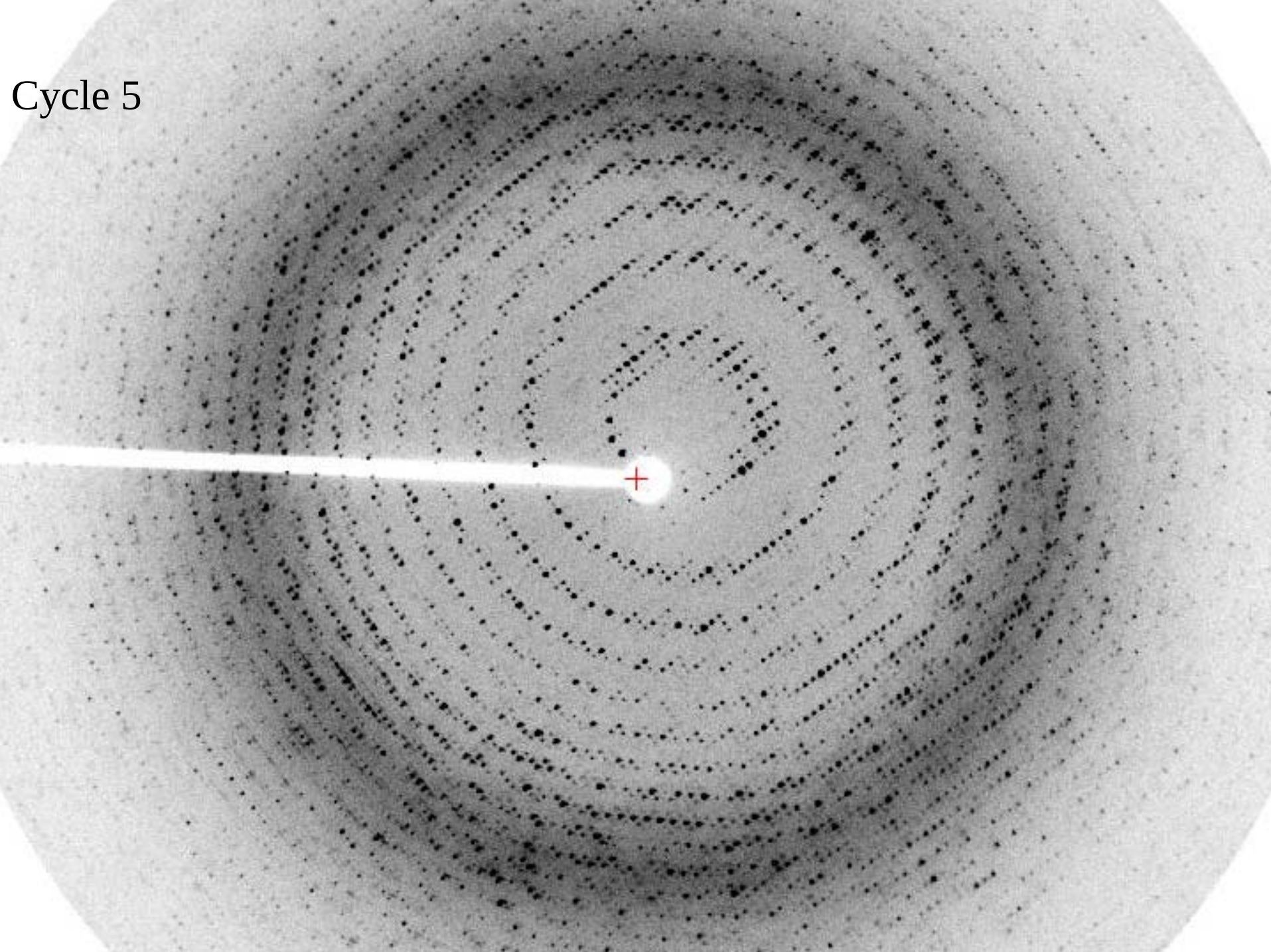


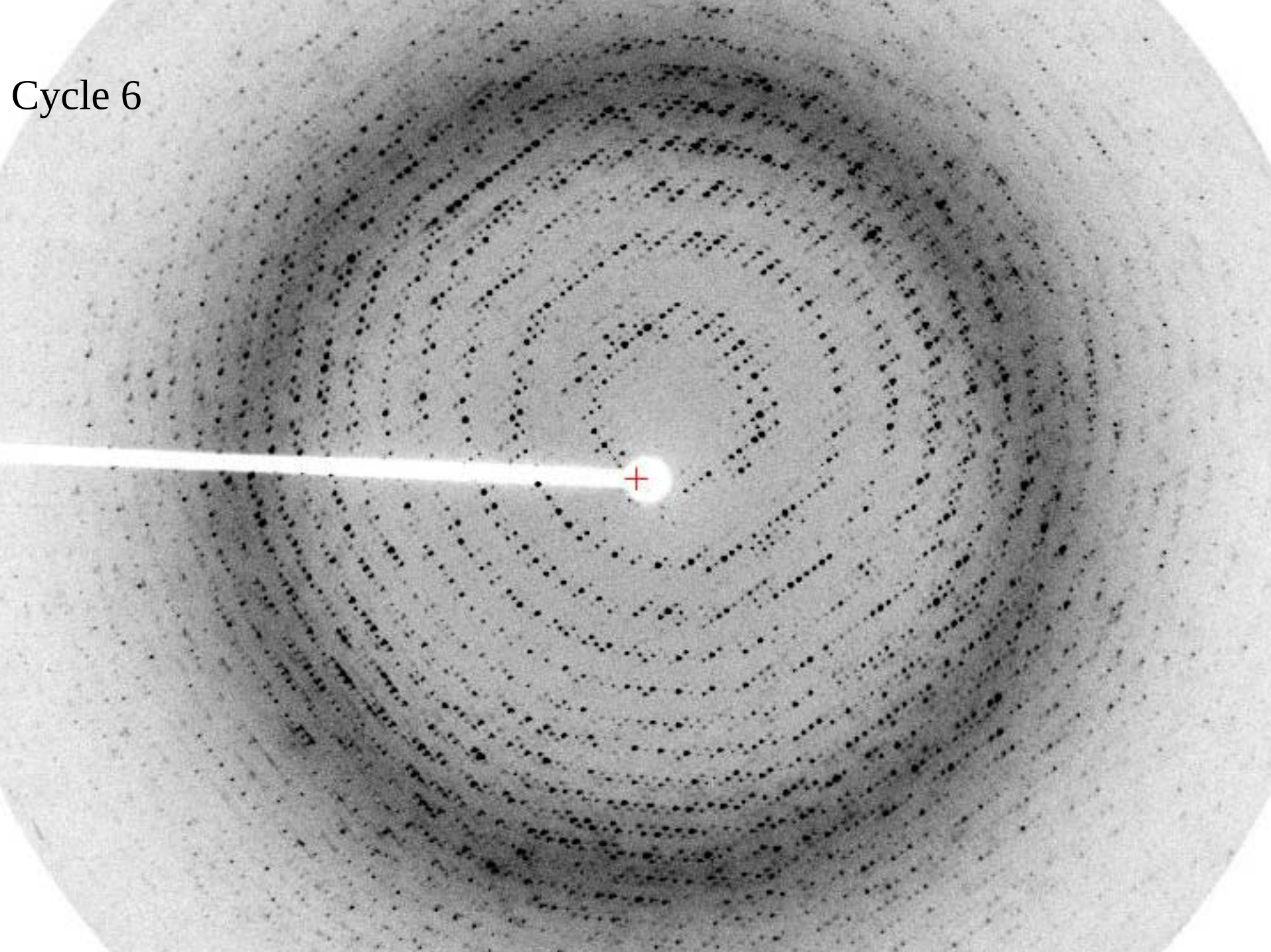

Cycle 1
(~3 second melt time)

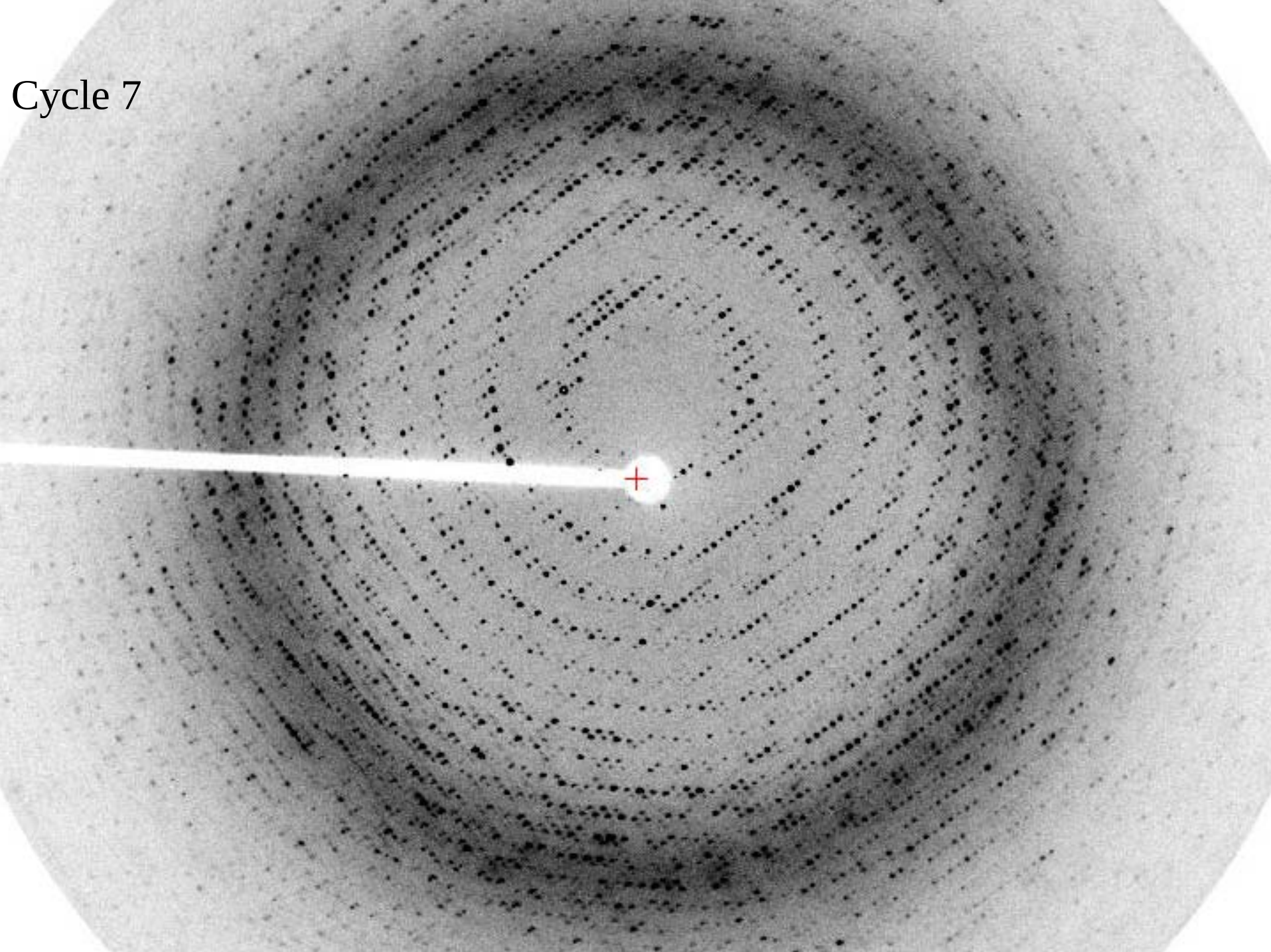

Cycle 2

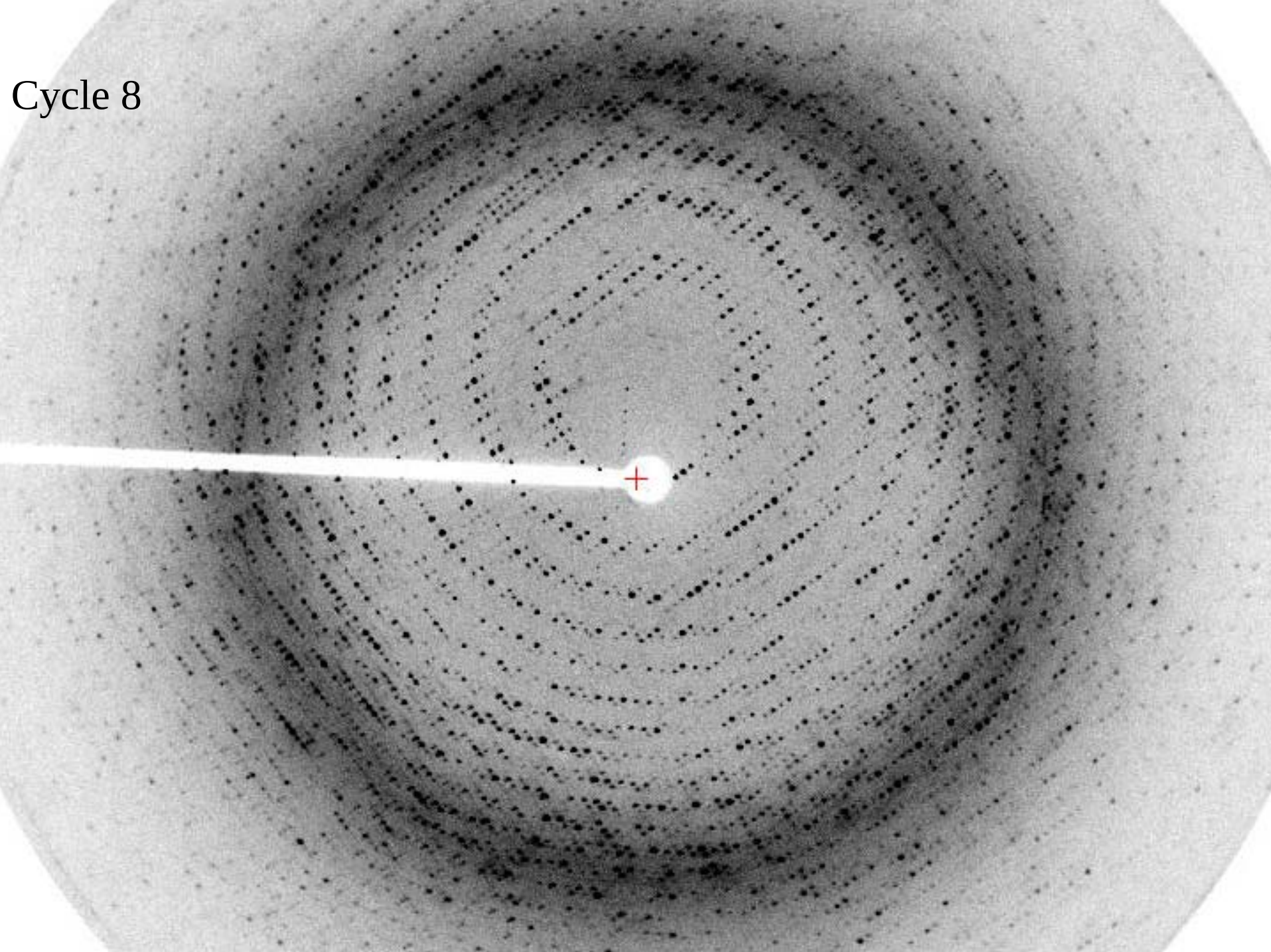

Cycle 3

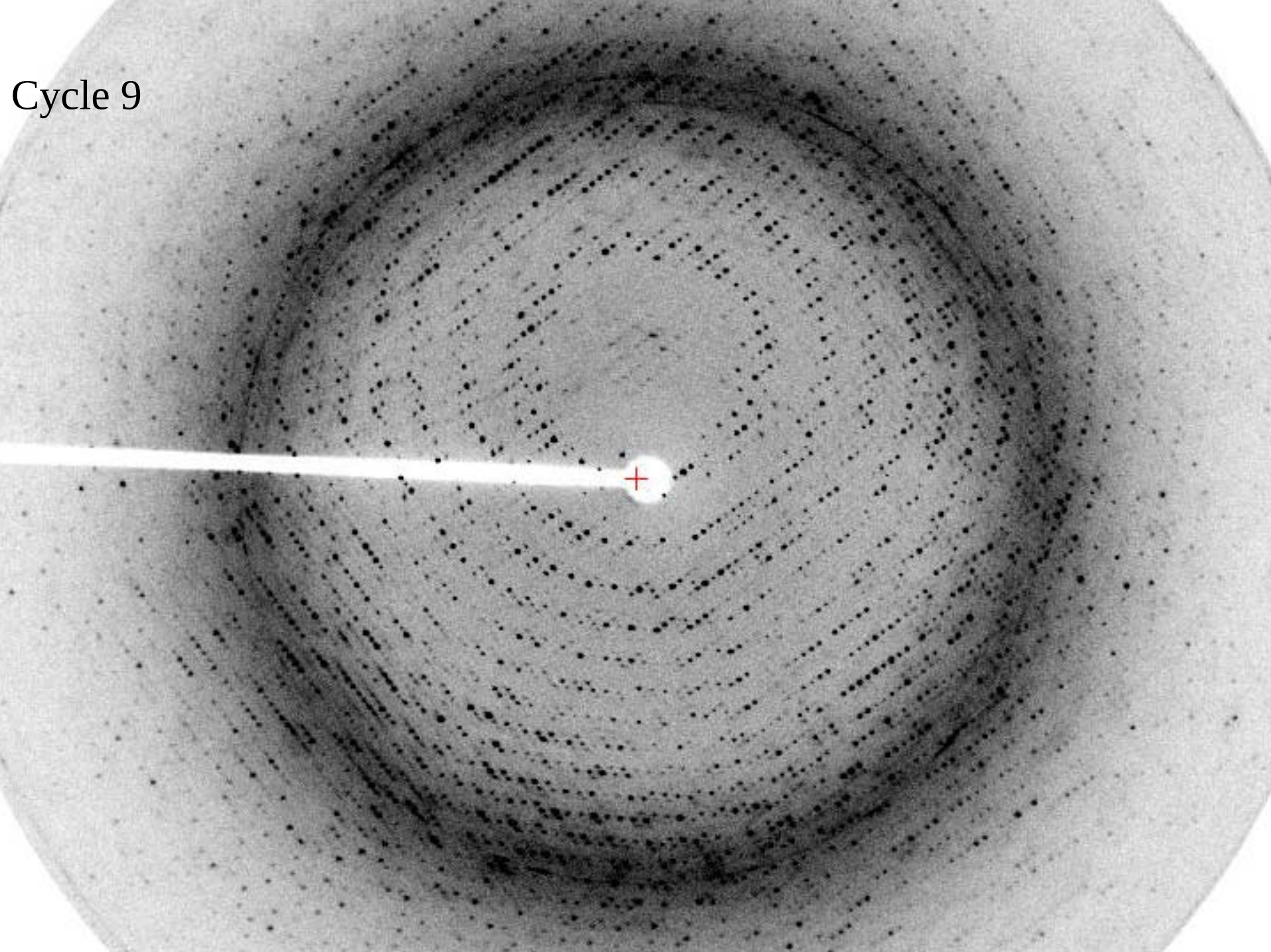

Cycle 4

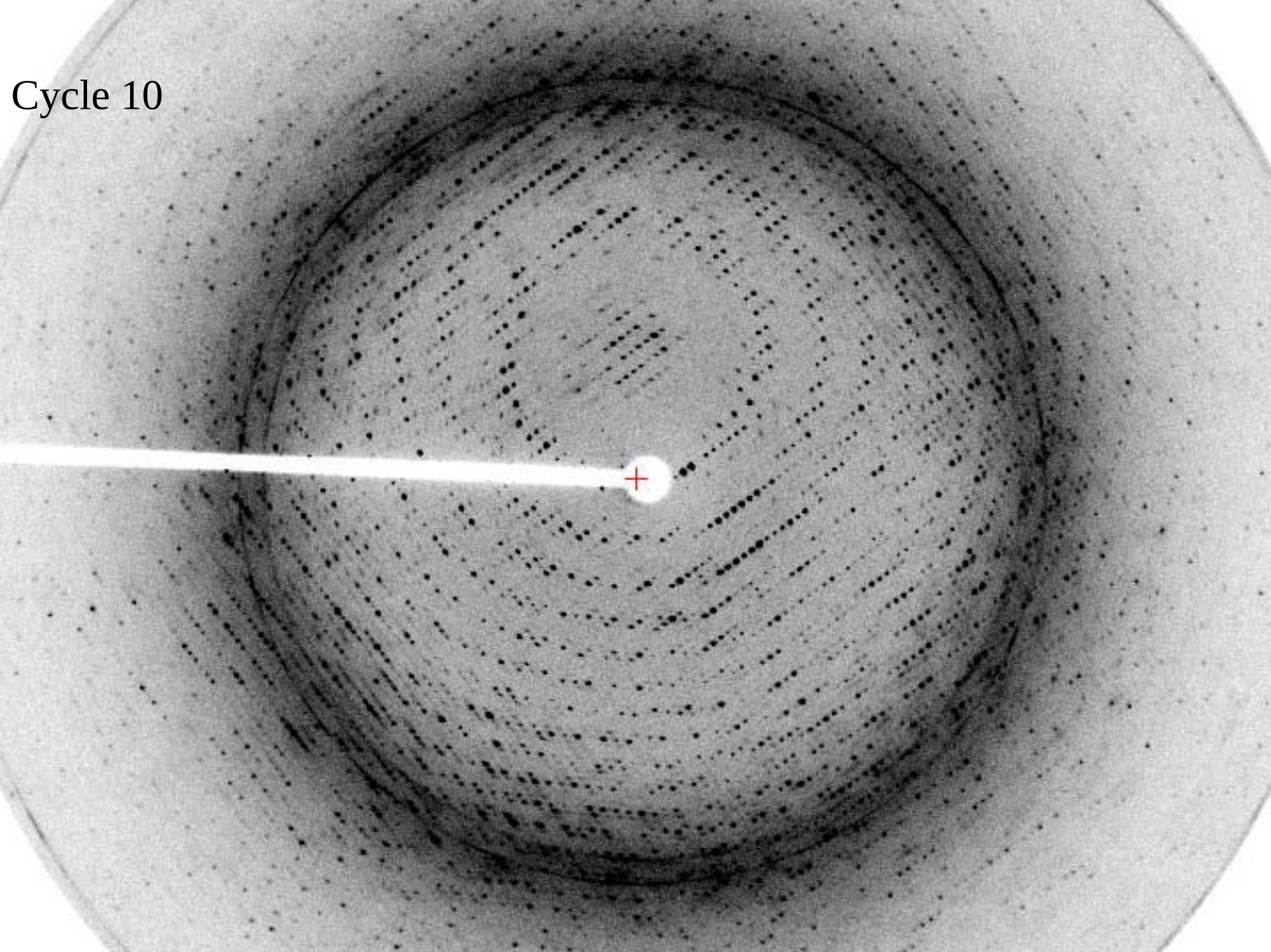

Cycle 5

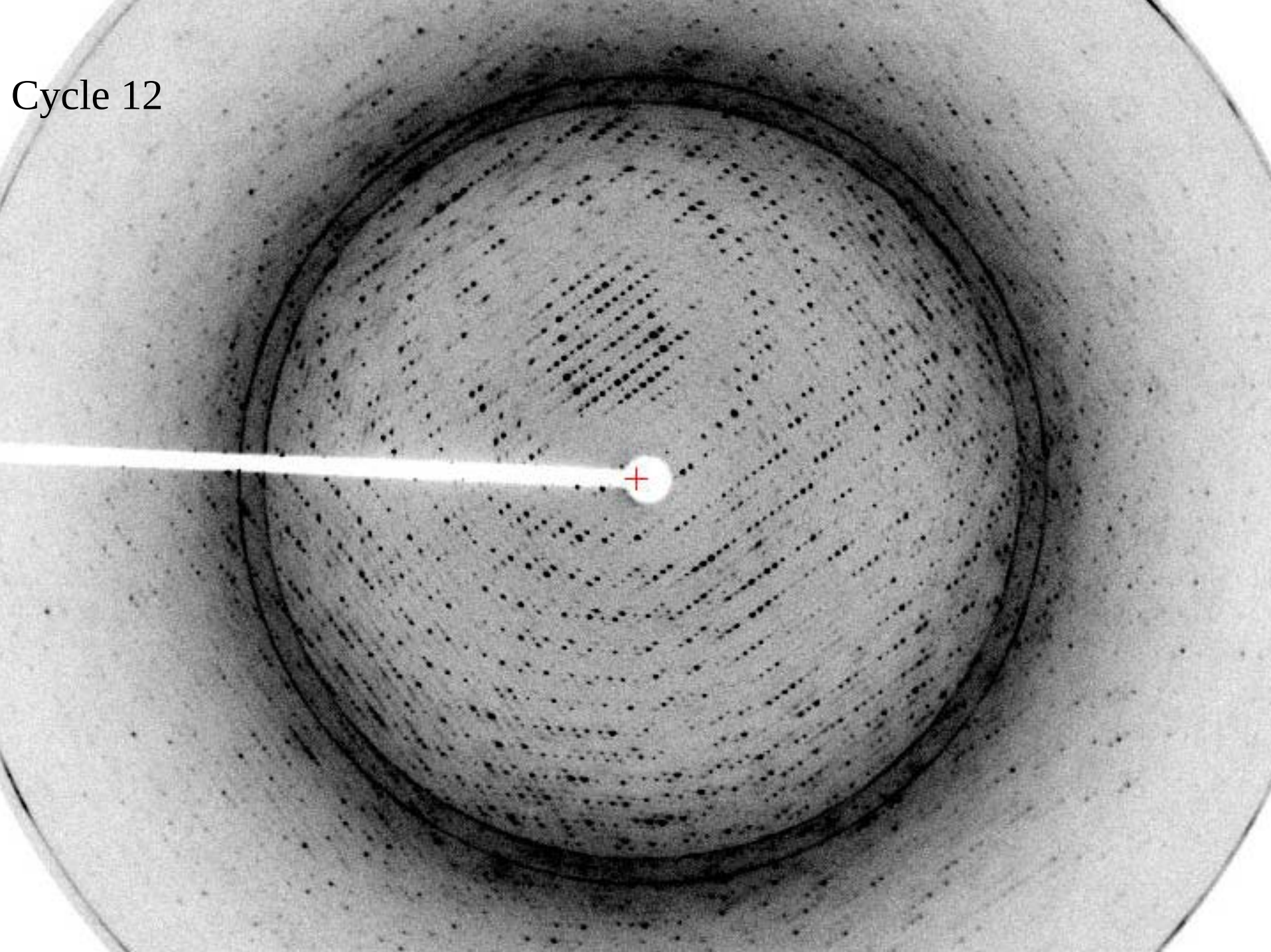

Cycle 6

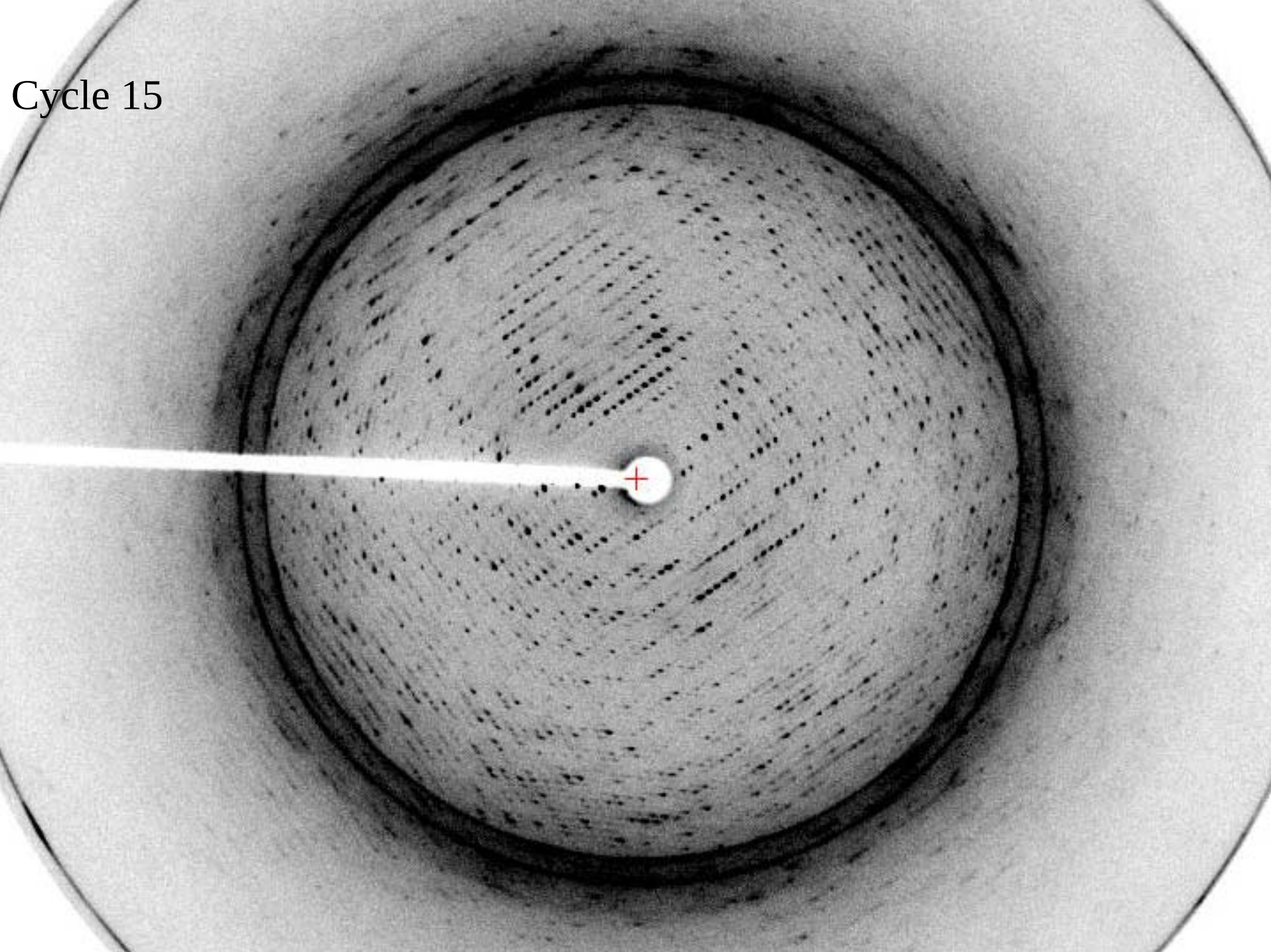

Cycle 7

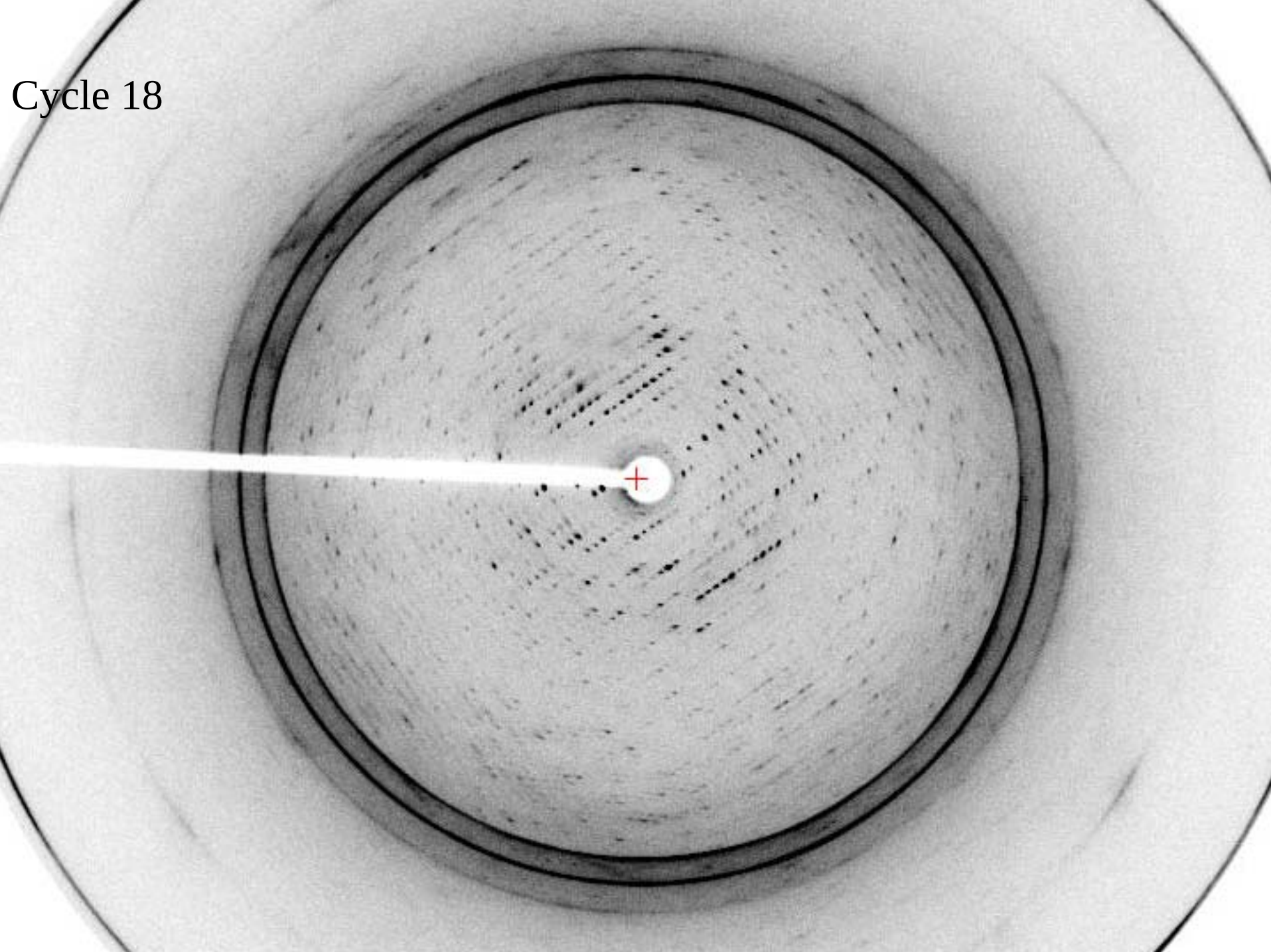

Cycle 8

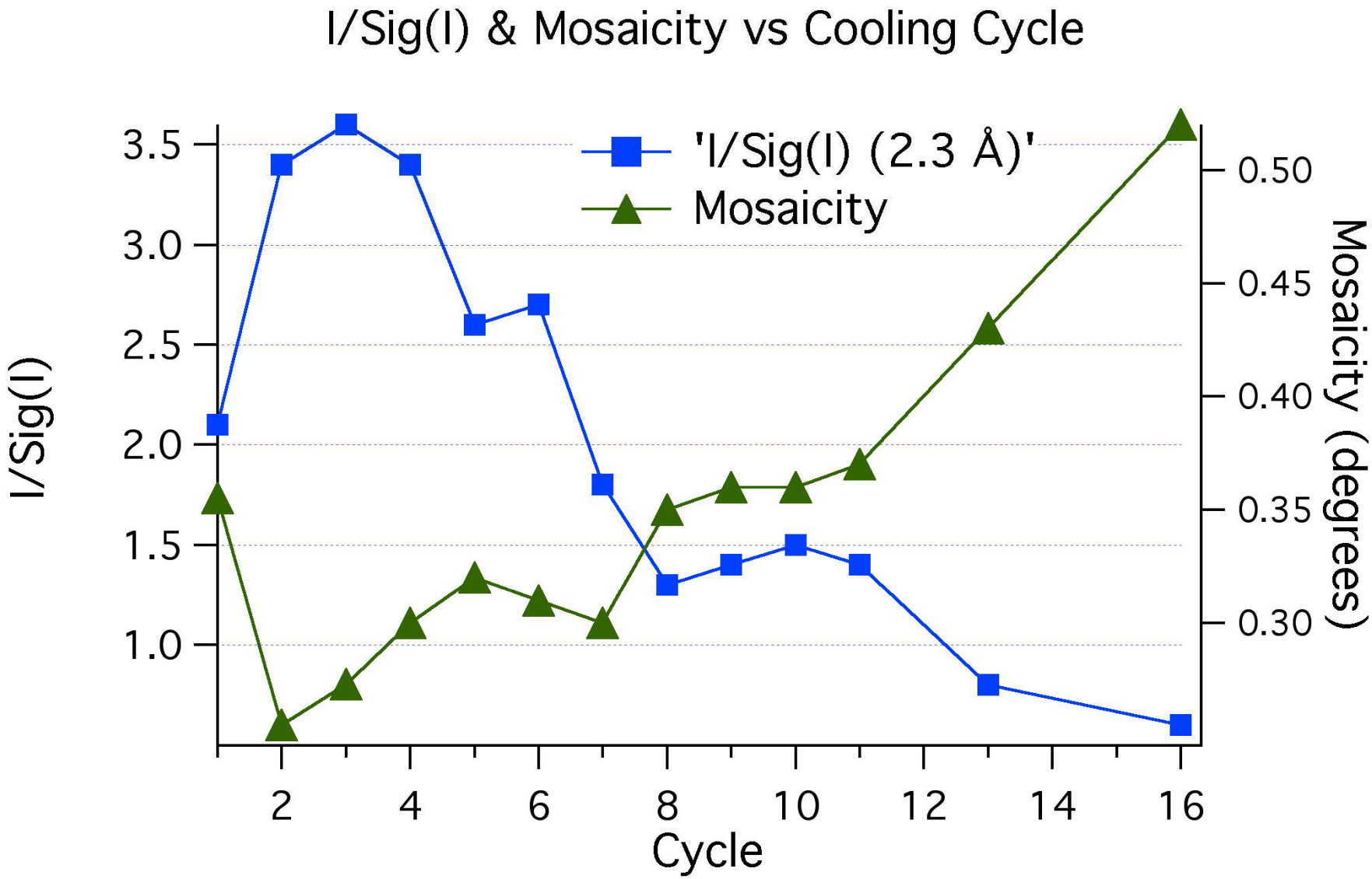

Cycle 9


Cycle 10

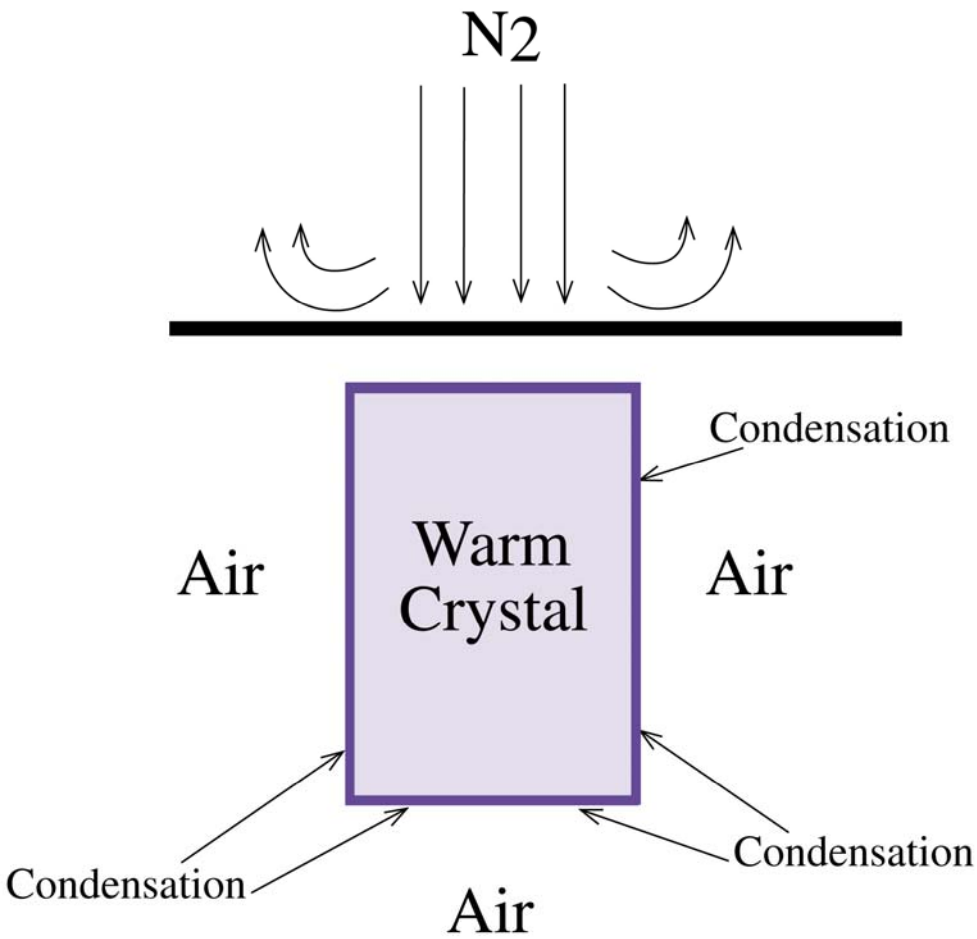

Cycle 12

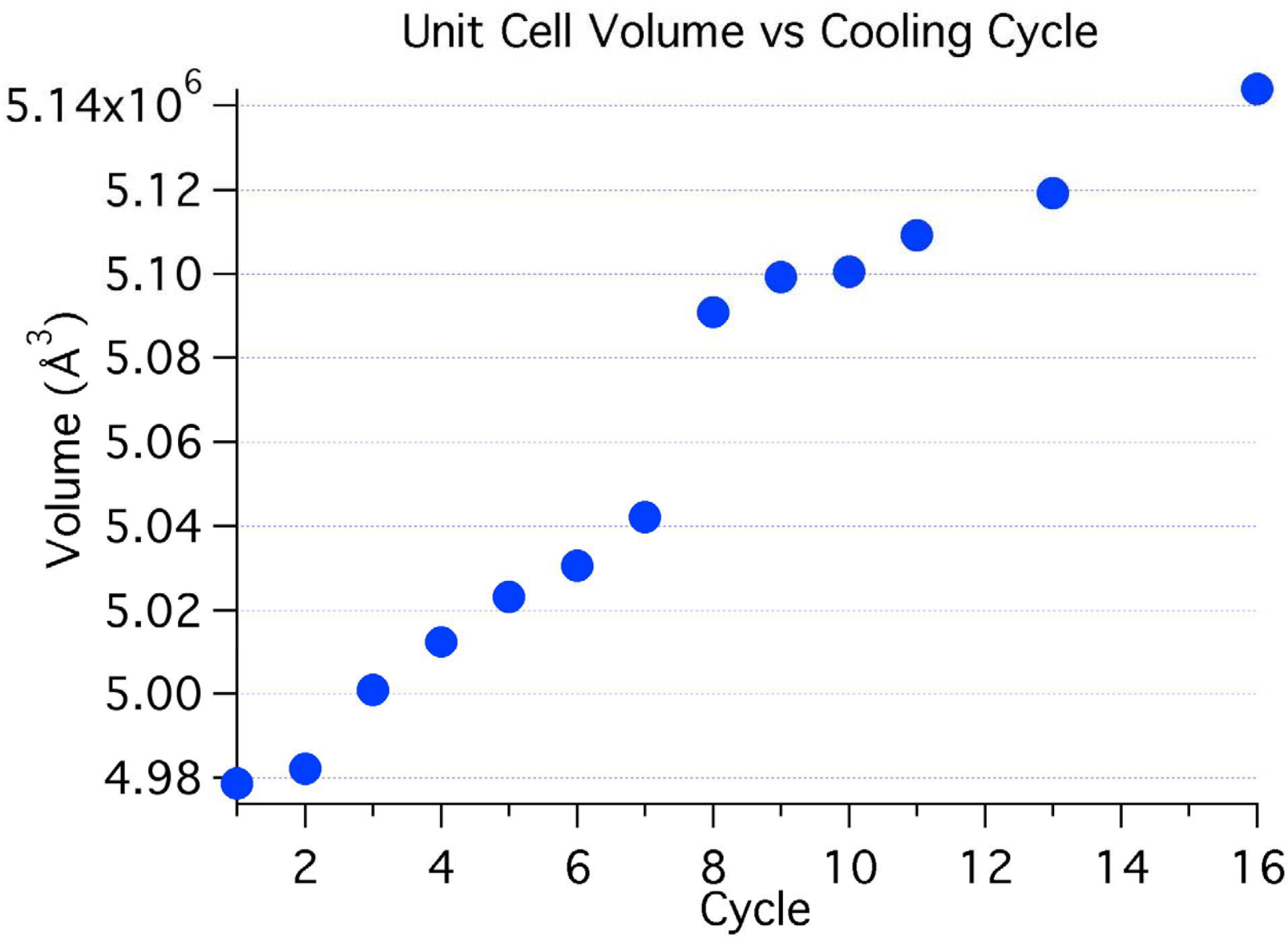

Cycle 15

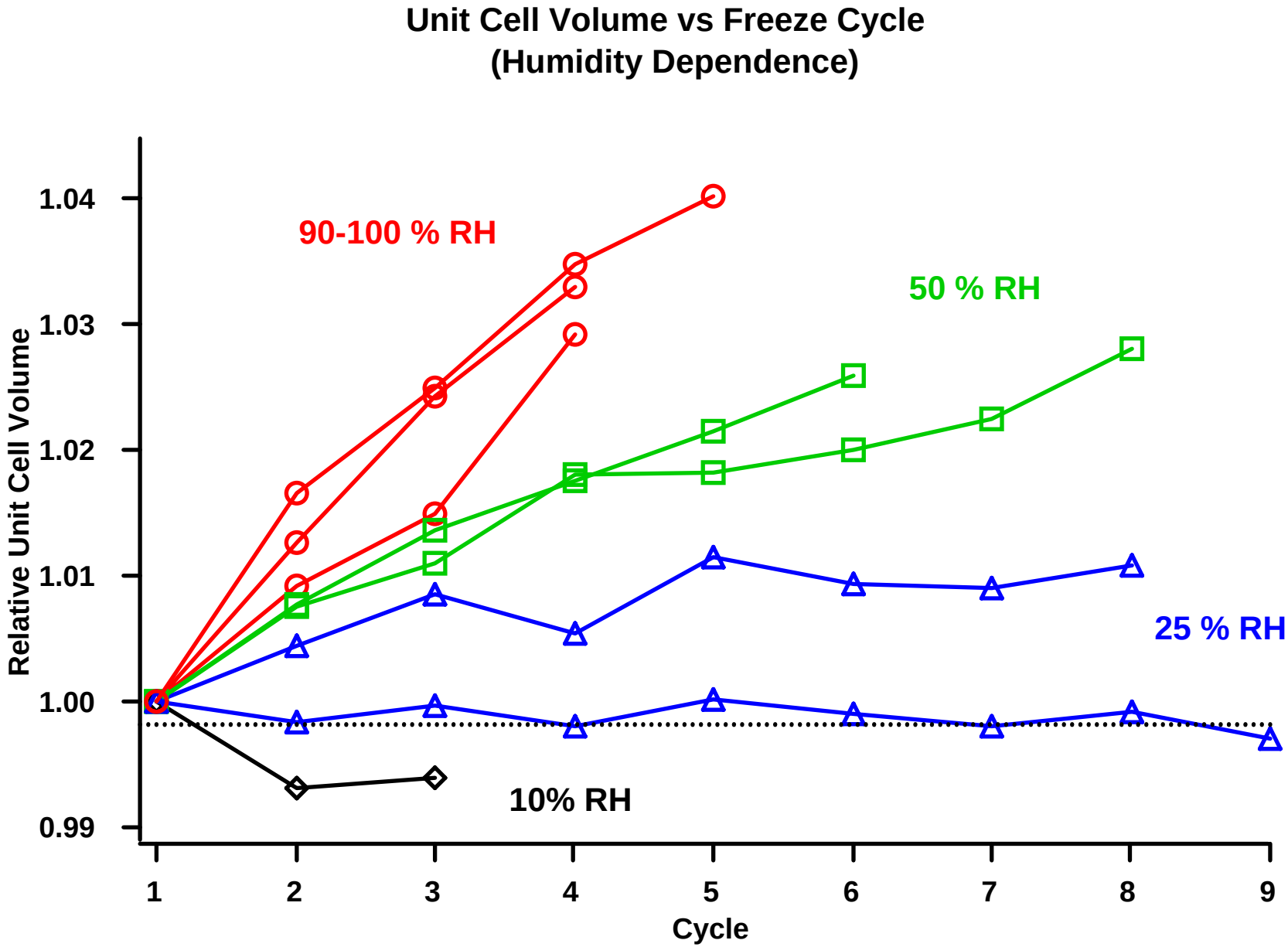

Cycle 18

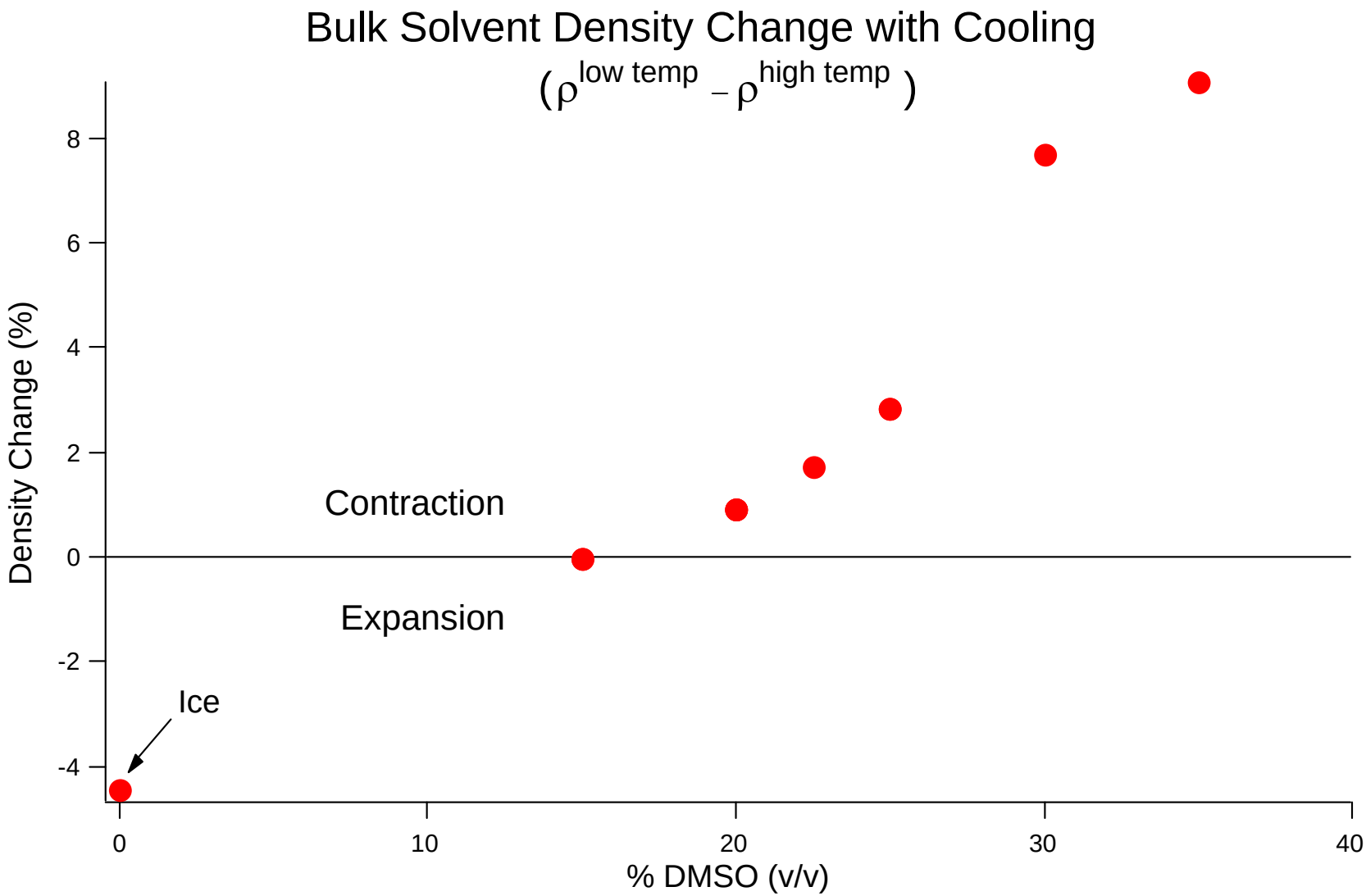

I/Sig(I) & Mosaicity vs Cooling Cycle
'I/Sig(I) (2.3 Å)'
Mosaicity
I/Sig(I)
Mosaicity (degrees)
Cycle
3.5
3.0
2.5
2.0
1.5
1.0
0.50
0.45
0.40
0.35
0.30
2
4
6
8
10
12
14
16

Blocked Cold Stream



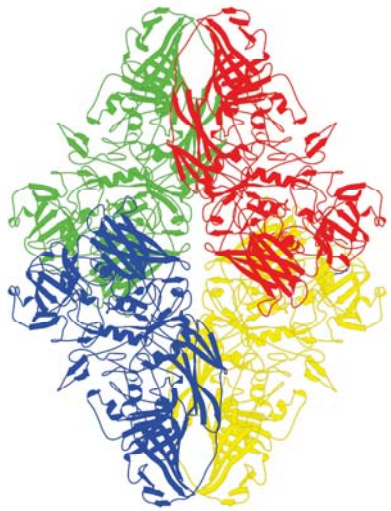

Unit Cell Volume vs Cooling Cycle
5.14x10^6
5.12
5.10
5.08
5.06
5.04
5.02
5.00
4.98
Volume (Å^3)
2
4
6
8
10
12
14
16
Cycle

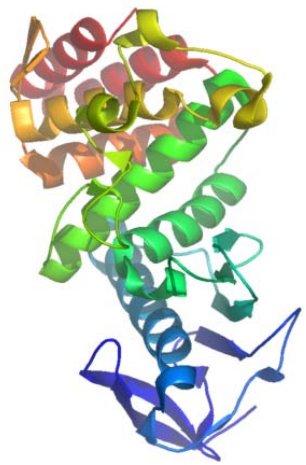

Unit Cell Volume vs Freeze Cycle
(Humidity Dependence)
Relative Unit Cell Volume
1.04
1.03
1.02
1.01
1.00
0.99
90-100 % RH
50 % RH
25 % RH
10% RH
1
2
3
4
5
6
7
8
9
Cycle


Bulk Solvent Density Change with Cooling
(ρ low temp − ρ high temp)
Density Change (%)
8
6
4
2
0
-2
-4
Contraction
Expansion
Ice
0
10
20
30
40
% DMSO (v/v)

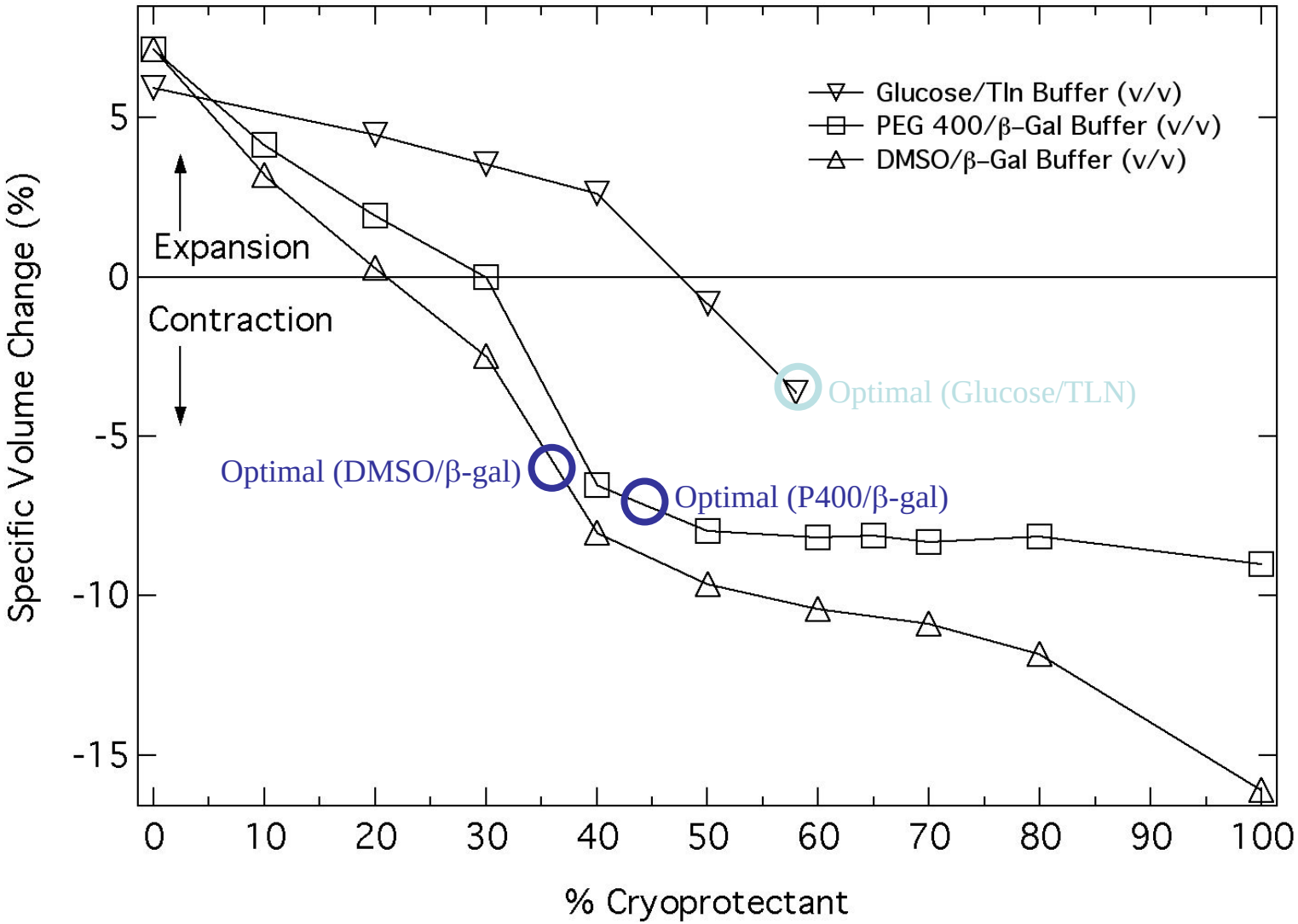
Systems Studied

	β-Galactosidase	Thermolysin
Space Group	$P2_12_12_1$	$P6_422$
Unit Cell	154 x 174 x 204 Å	94 x 131 Å
Fraction Solvent	58 %	49 %
Precipitant	PEG 8000	Water
UC Vol Change	- 5.2 %	- 3.6 %
Cryoprotectant	30 % (v/v) DMSO 40 % (v/v) PEG 400	60 % (w/v) glucose with external oil





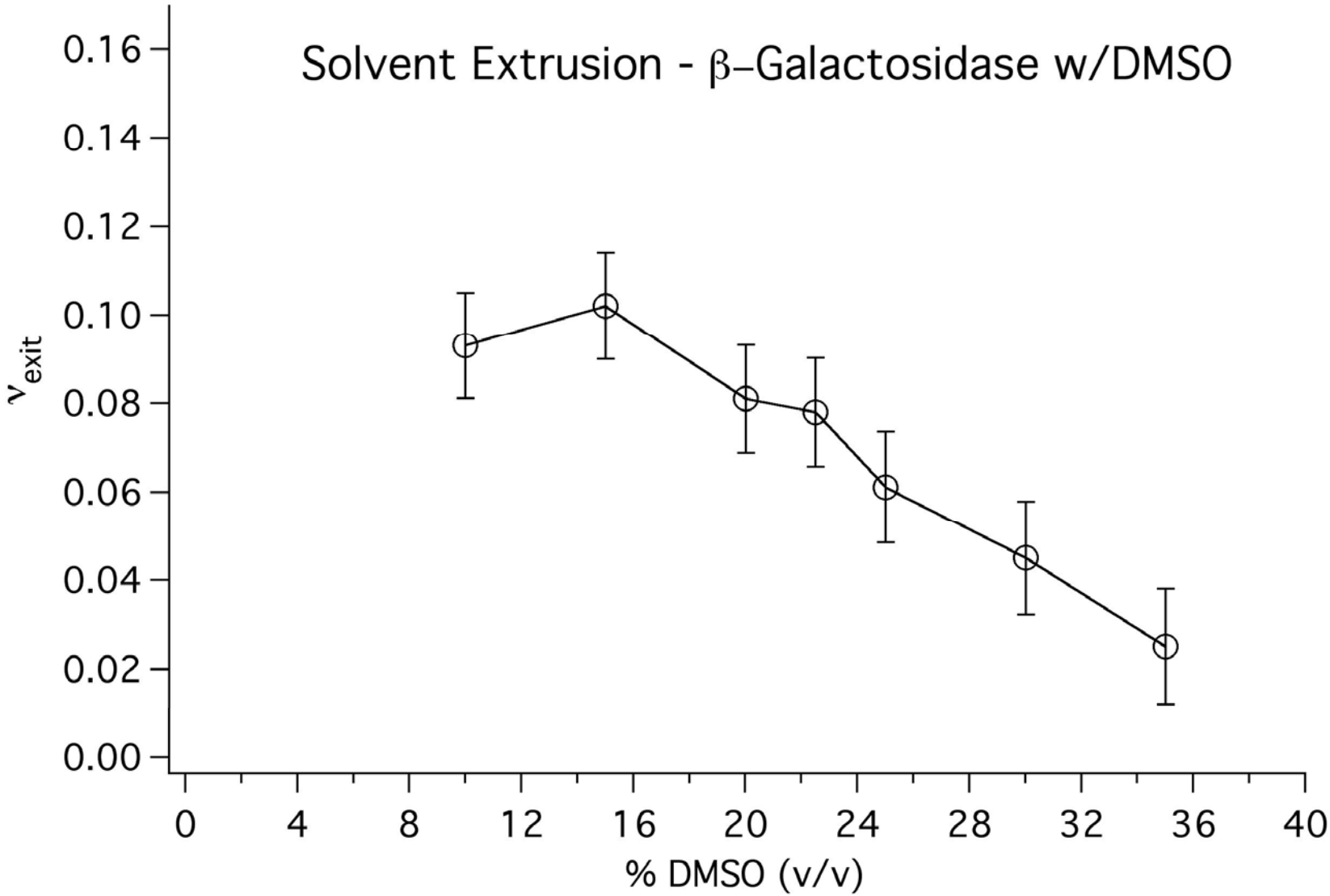
Specific Volume Change of Bulk Solvent with Cooling (RT -> 77 K)

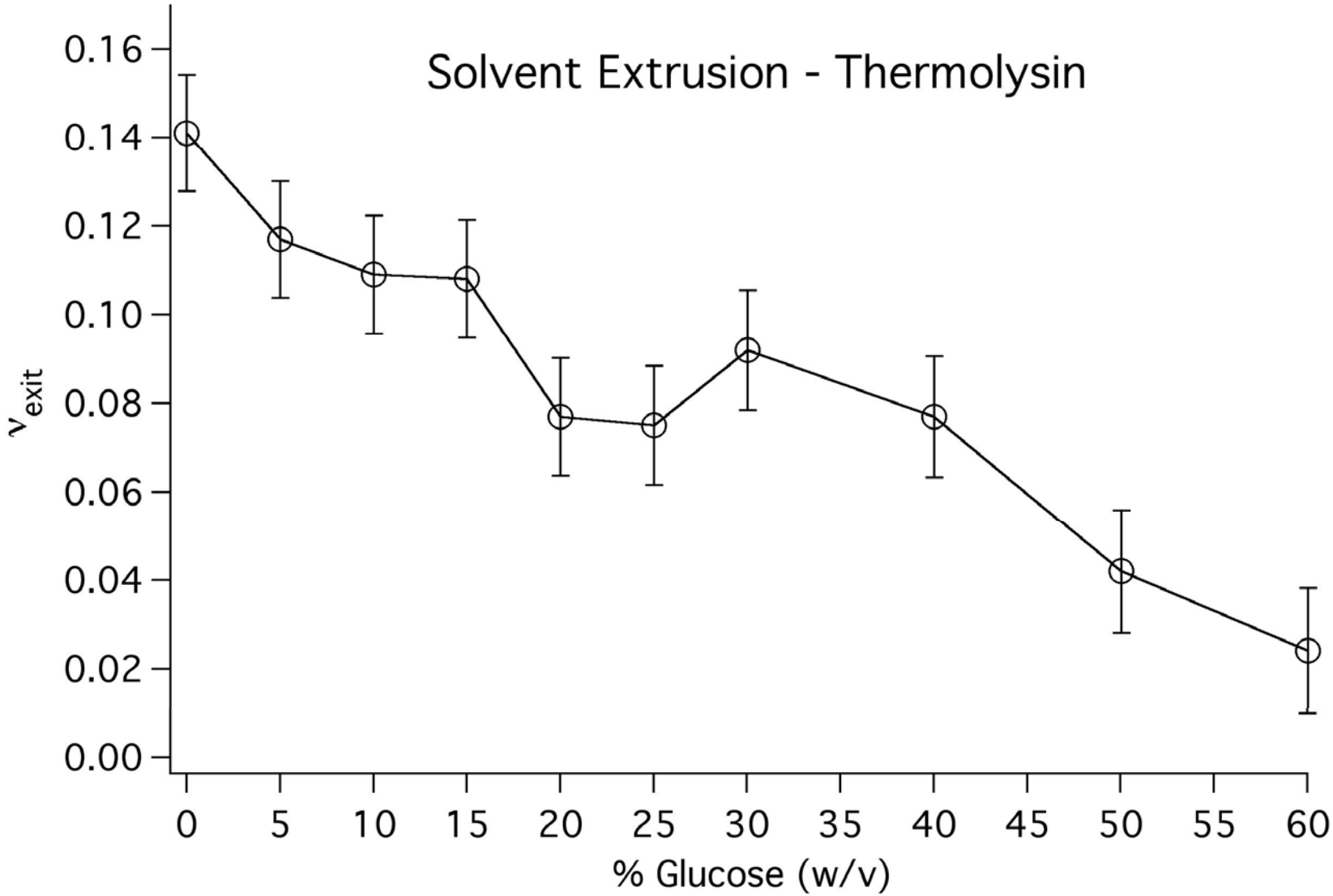


Relation Amongst Parameters - Solvent Extrusion

$$\nu_{exit} = \Delta_{sol} + \frac{(\nu_{prot}\Delta_{prot} - \Delta_{cell})(1 - \Delta_{sol})}{1 - \nu_{prot}}$$

Protein	ν_{exit} (at optimal cryoprotectant)
β-gal / DMSO	0.038 - 0.010
β-gal / PEG 400	0.018 - 0.004
Thermolysin / glucose	0.020


Solvent Extrusion - β–Galactosidase w/DMSO
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
v_{exit}
0
4
8
12
16
20
24
28
32
36
40
% DMSO (v/v)


Solvent Extrusion - Thermolysin
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
v_{exit}
0
5
10
15
20
25
30
35
40
45
50
55
60
% Glucose (w/v)

Overall Summary

1. The optimal cryoprotectant concentration for cryo-cooling appears to be that which allows the bulk solvent contraction to best compensate for the protein and lattice contraction.
2. ‘In situ’ annealing involves, at least in part, tuning of the thermal contraction of the bulk solvent by transporting water into or out of the crystal during the room temperature phase.

Acknowledgment

Doug Juers