Dr. Richard Henderson

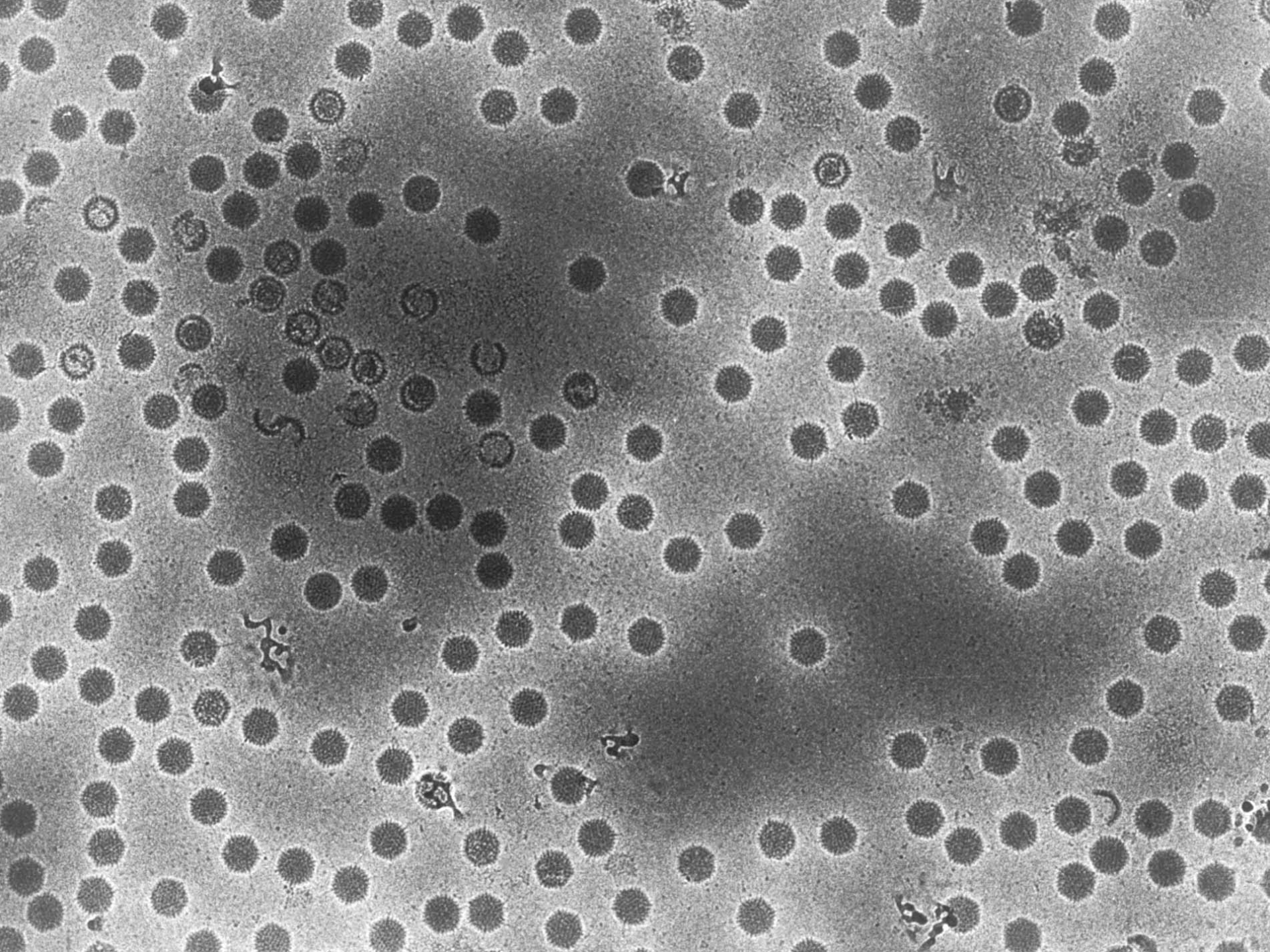
MRC Laboratory of Molecular Biology

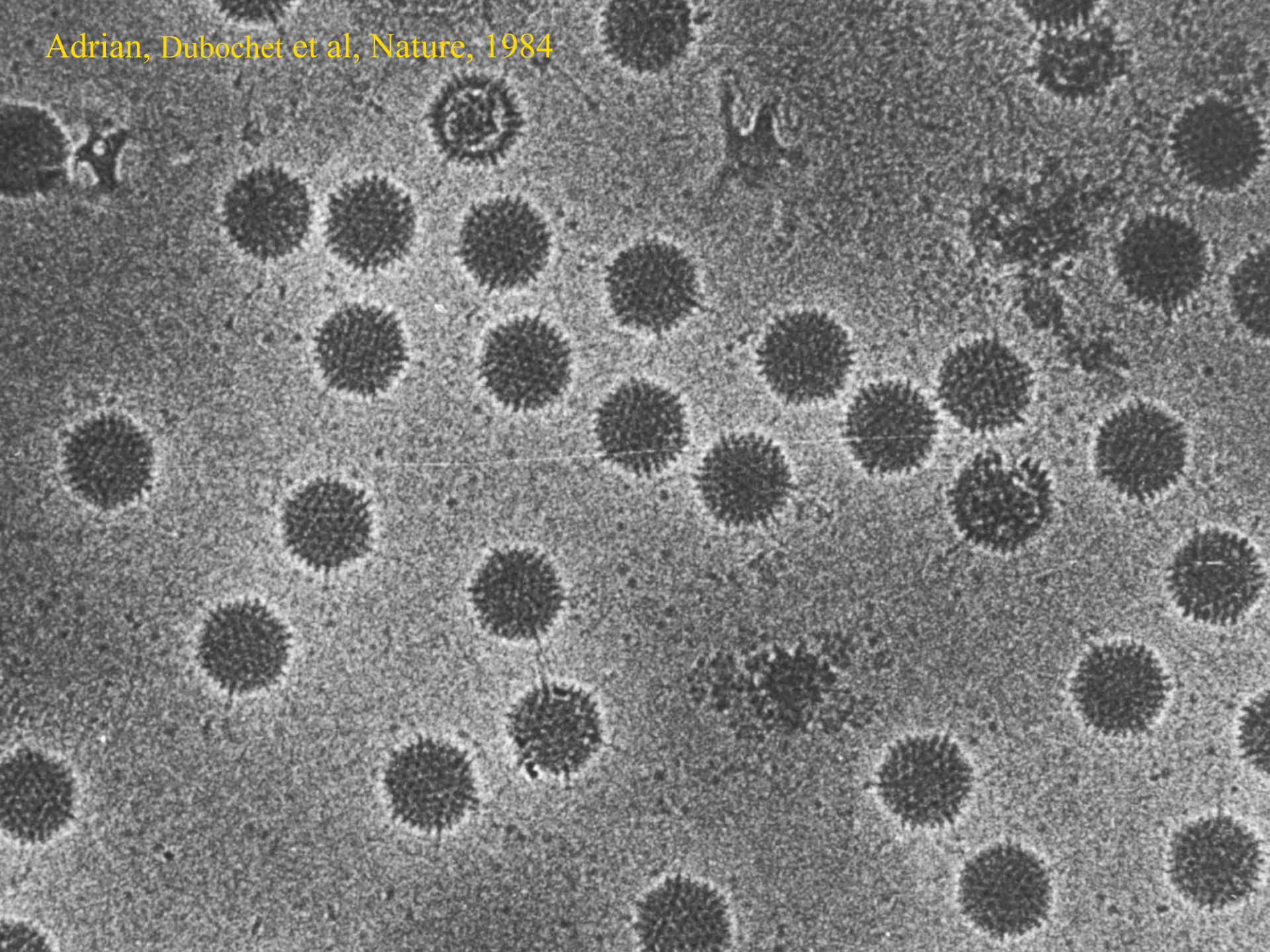
Hills Road, Cambridge CB2 2QH, UK

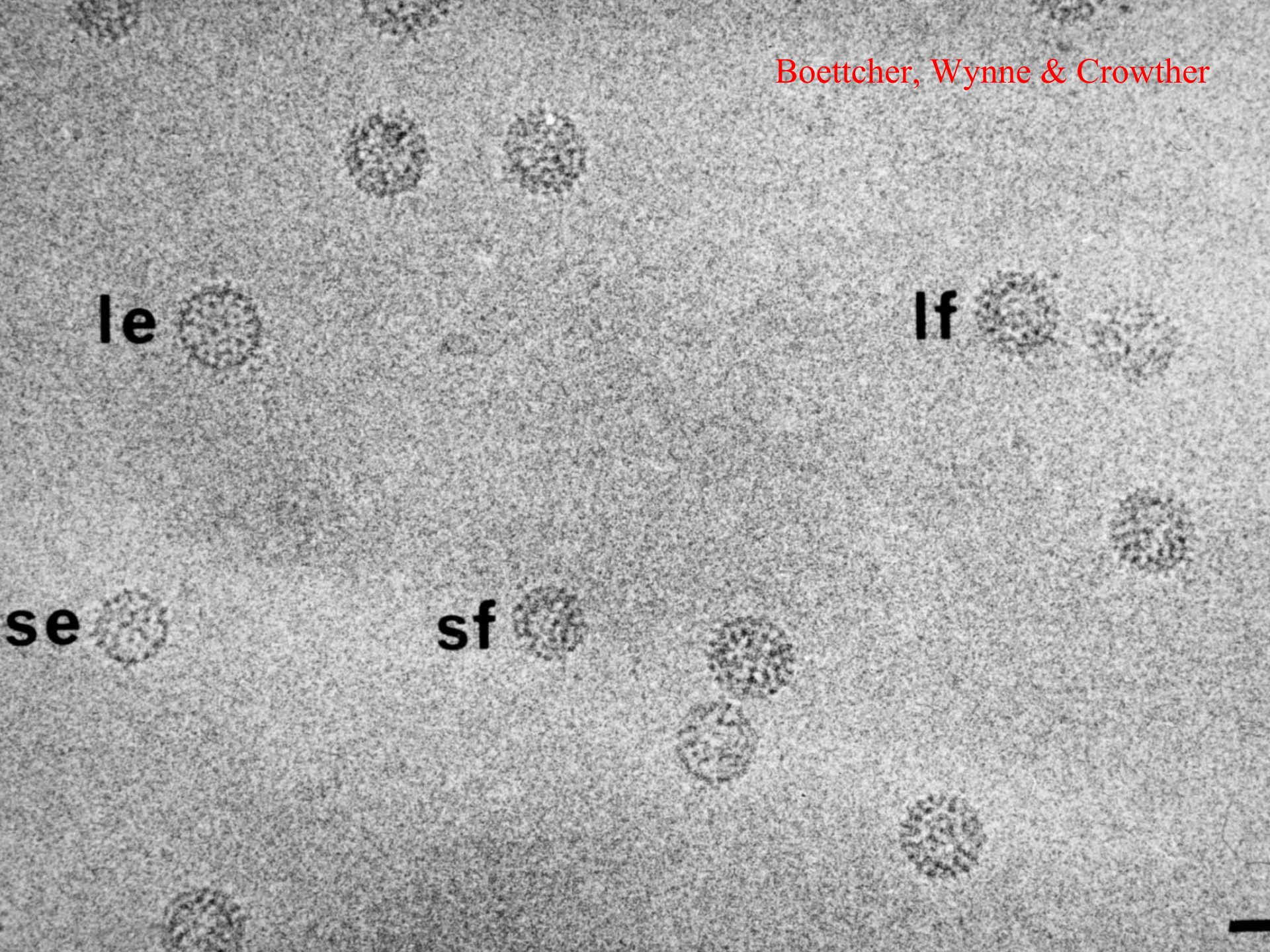
Tel: +44 – (0) 1223-402215

Fax: +44 – (0) 1223-249565

Web: http://www.mrc-lmb.cam.ac.uk

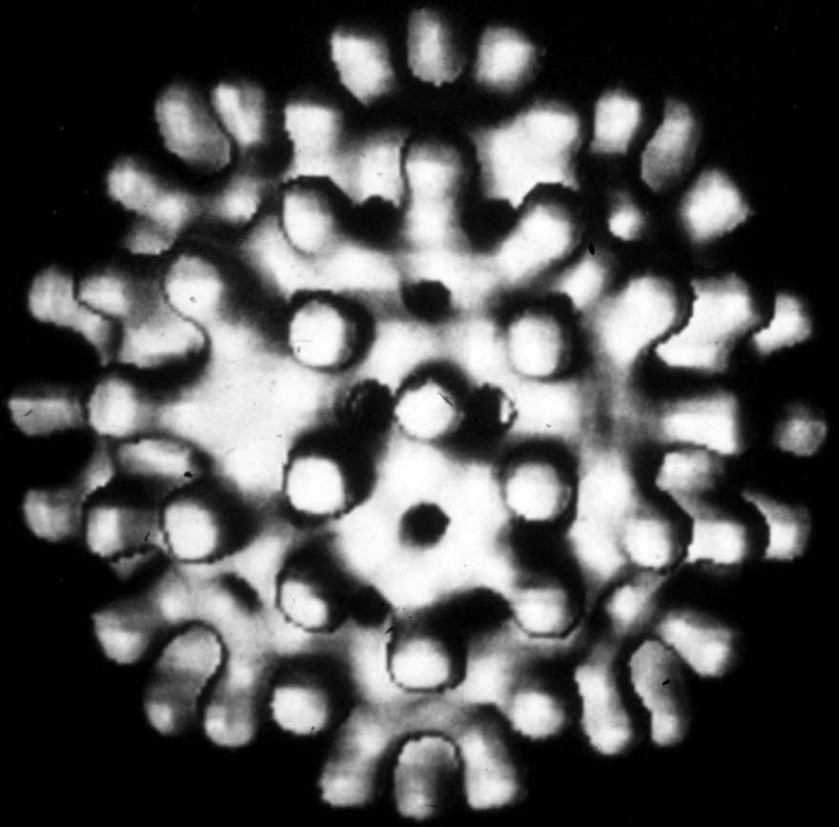


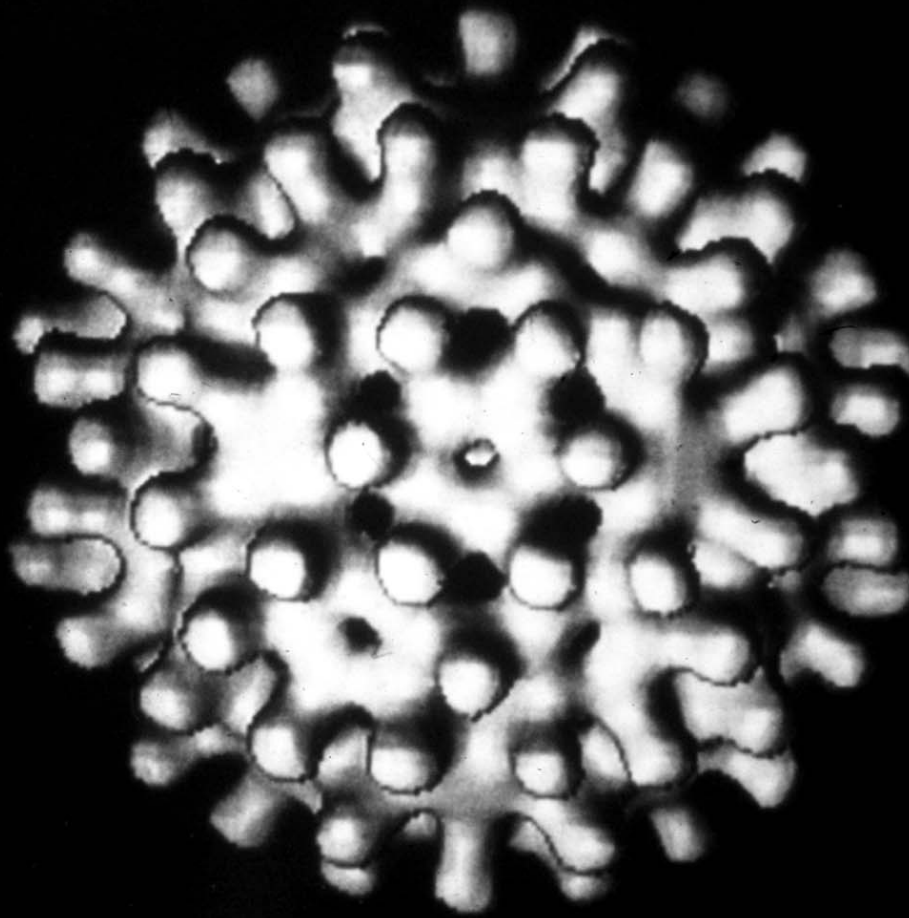

Adrian, Dubochet et al, Nature, 1984

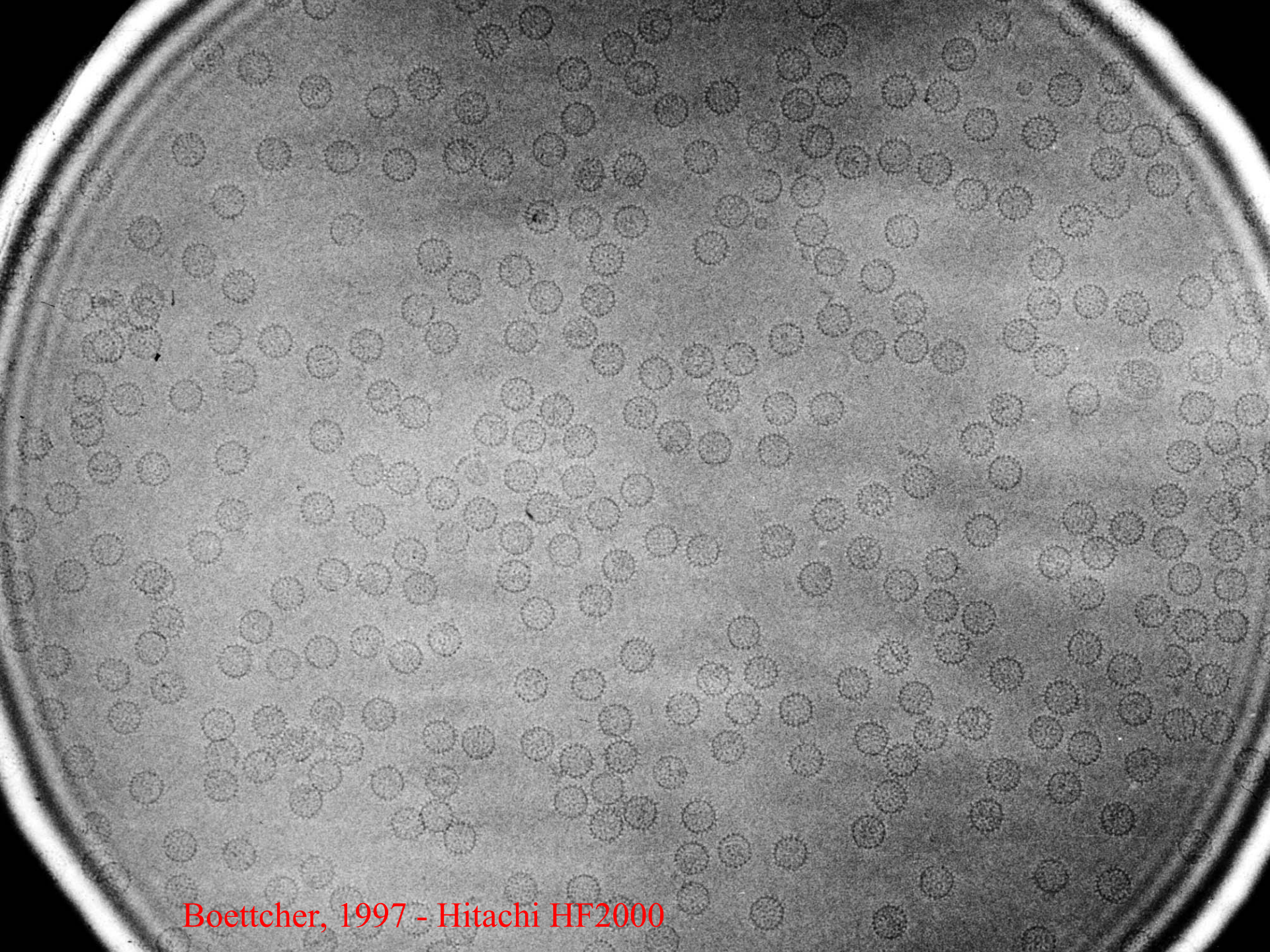

Boettcher, Wynne & Crowther
le
lf
se
sf

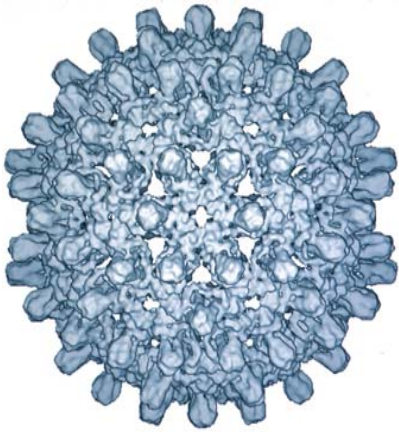
T=3

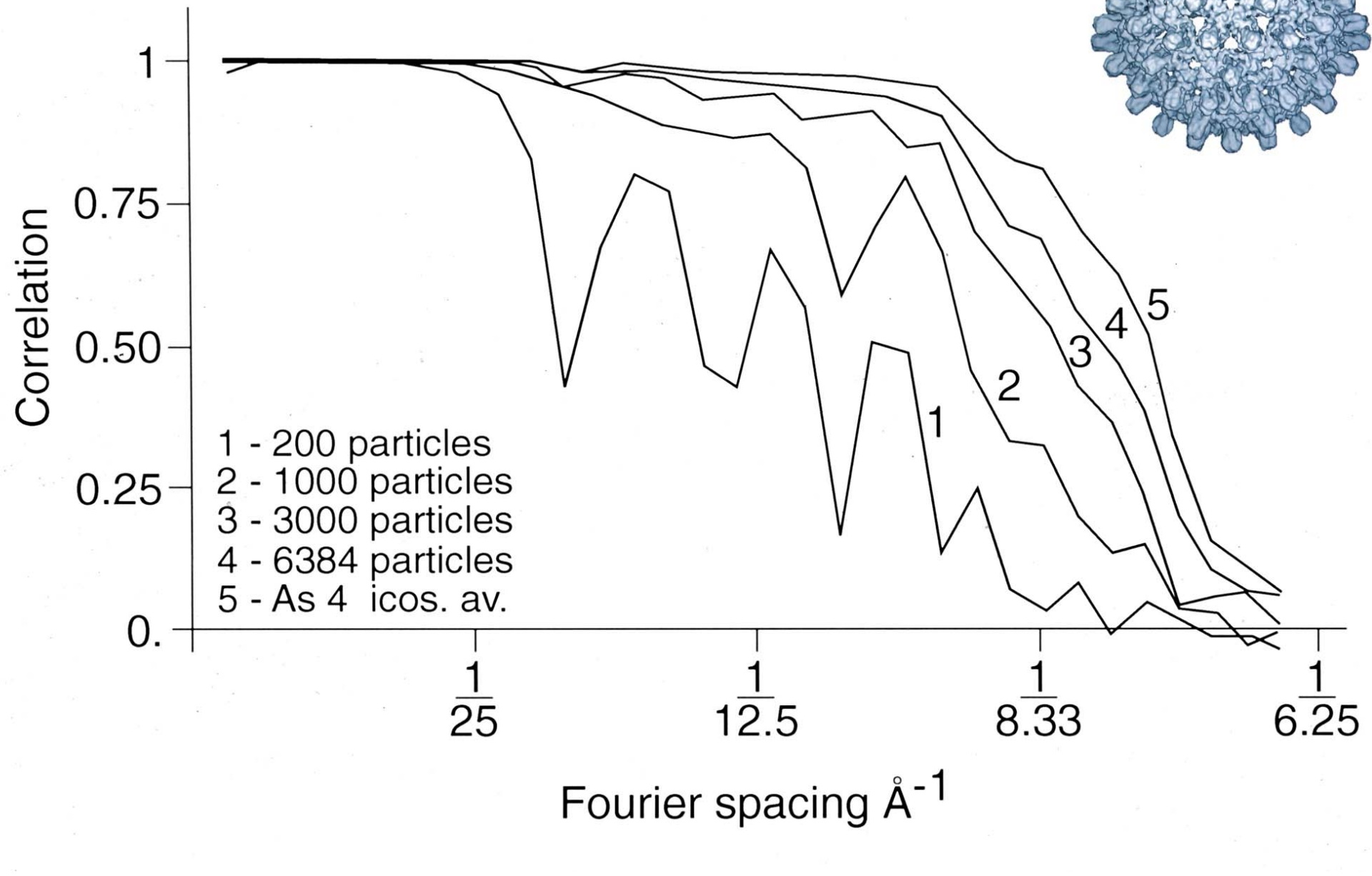
T=4

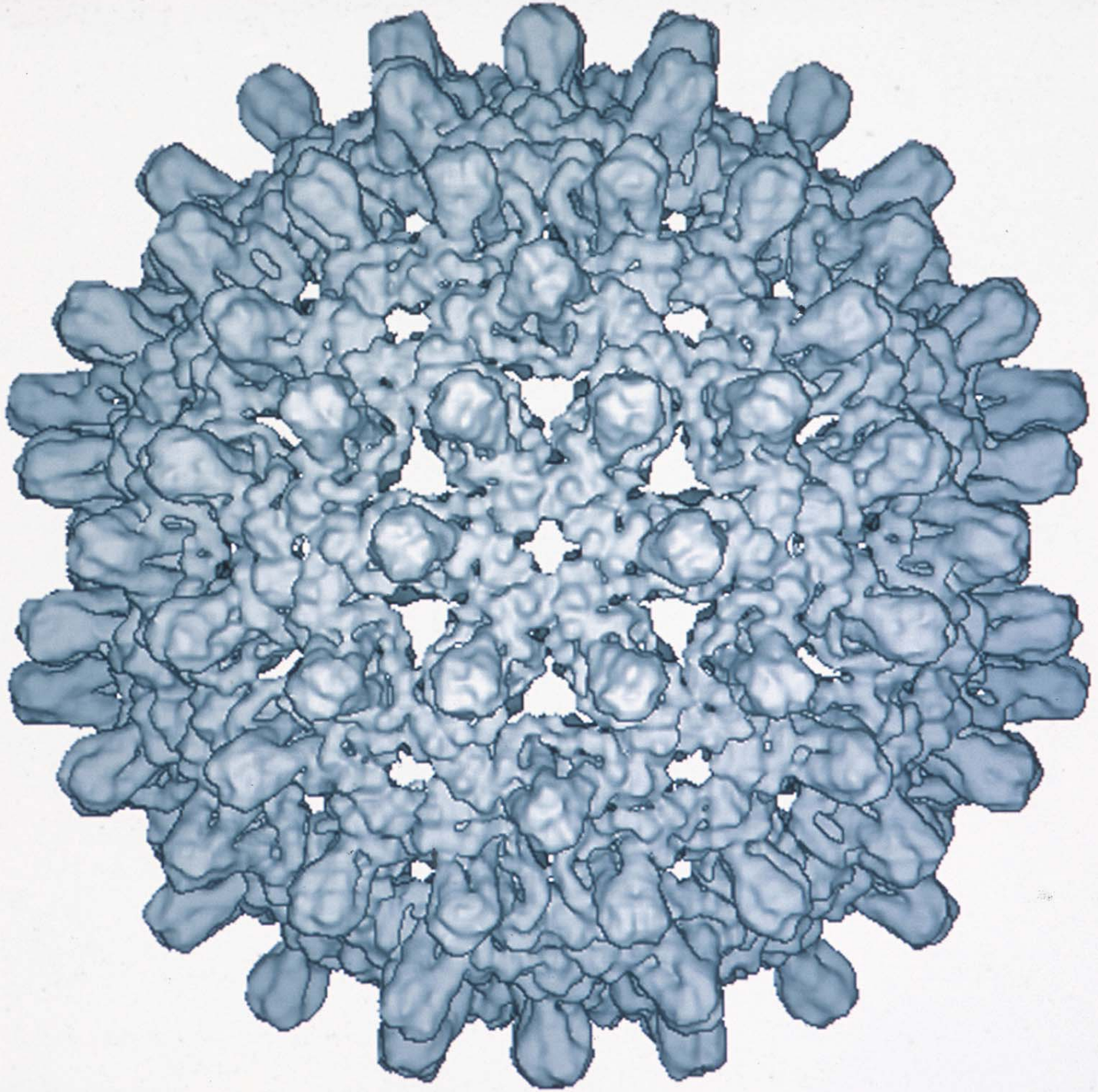




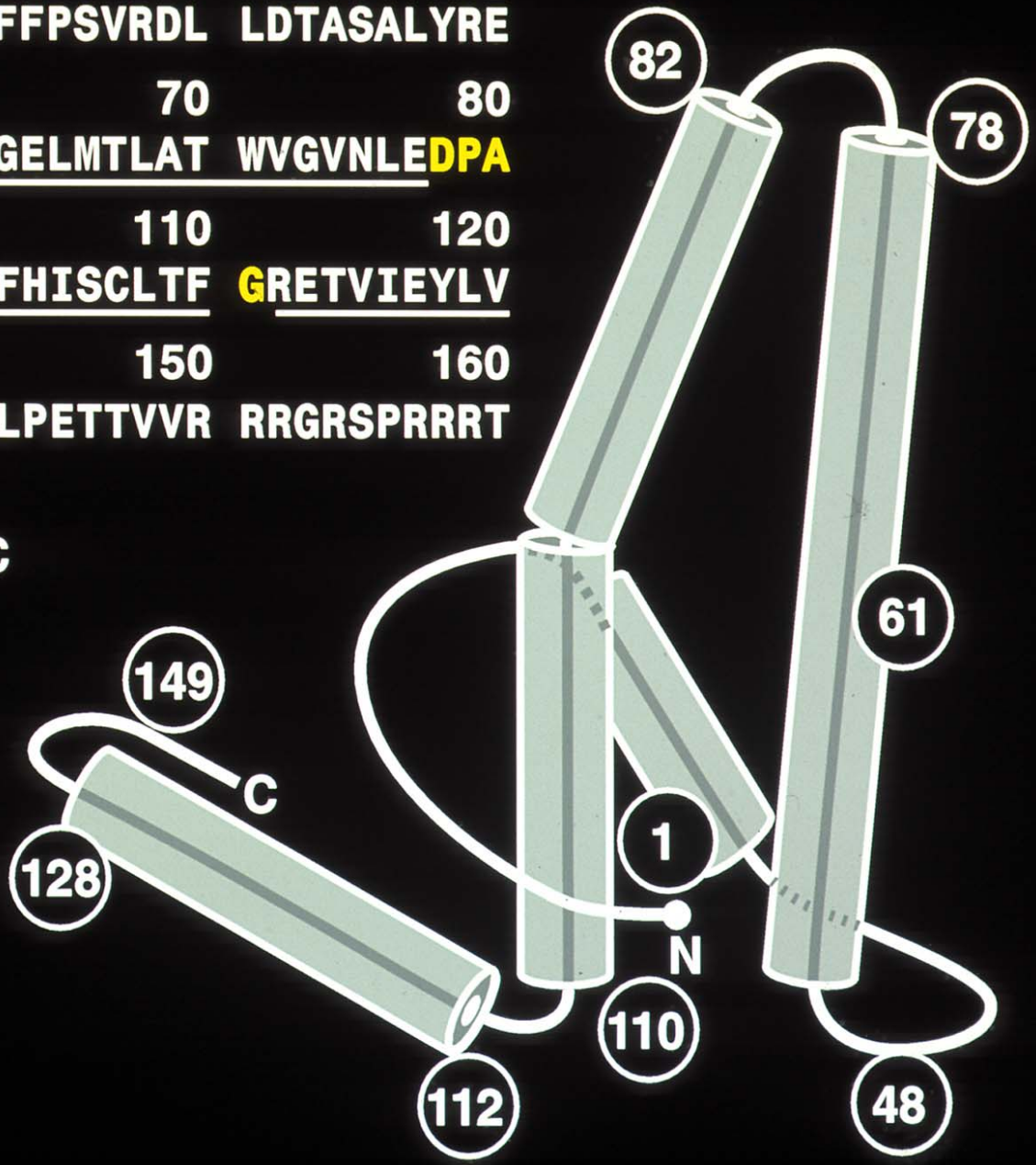

Boettcher, 1997 - Hitachi HF2000




Correlation
1
0.75
0.50
0.25
0.
1 - 200 particles
2 - 1000 particles
3 - 3000 particles
4 - 6384 particles
5 - As 4 icos. av.
1
2
3
4
5
1/25
1/12.5
1/8.33
1/6.25
Fourier spacing Å-1

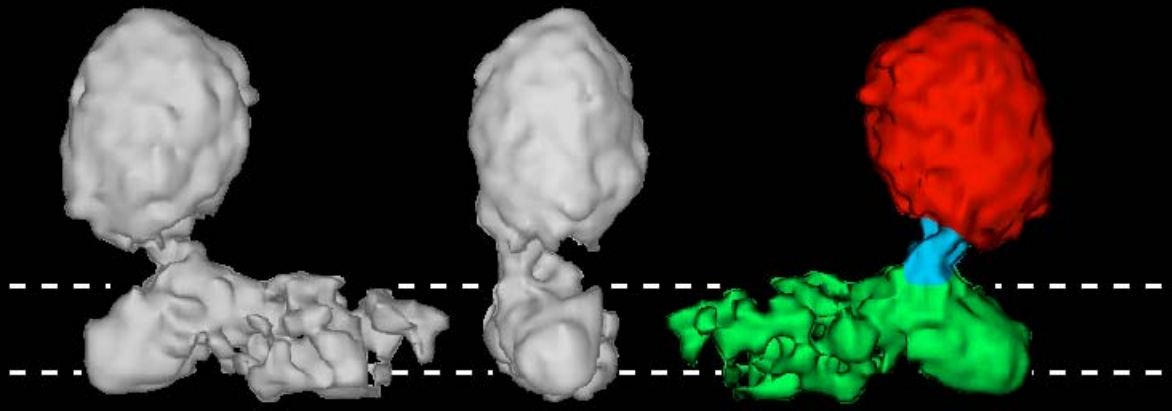


10	20	30	40
MDIDPYKEFG	ATVELLSFLP	SDFFPSVRDL	LDTASALYRE
50	60	70	80
ALESPEHCSP	HHTALRQAIL	CWGELMTLAT	WVGVNLEDPA
90	100	110	120
SRDLVVSYVN	TNMGLKFRQL	LWFHISCLTF	GRETVIEYLV
130	140	150	160
SFGVWIRTPP	AYRPPNAPIL	STLPETTVVR	RRGRSPRRRT
170	180		
PSPRRRRSQS	PRRRRSQSRE	SQC	



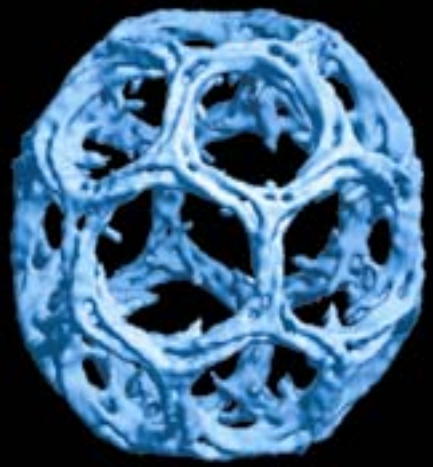
Bovine Complex I at 22 Å (symmetry C1)

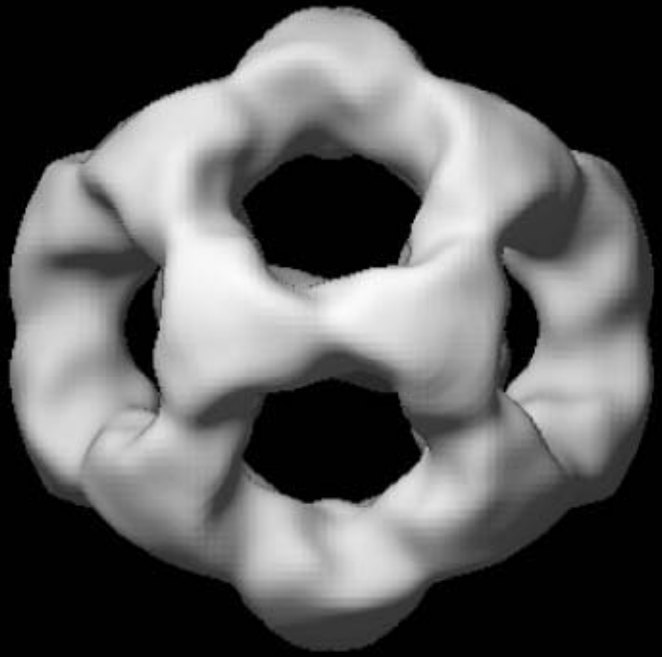
Grigorieff (1998) J.Mol.Biol. 277, 1033-1046



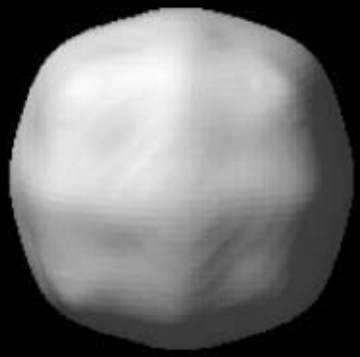
Clathrin at 22 Å (symmetry D6)

Smith,Grigorieff,Pearse (1998) EMBO J. 17, 4943-5953



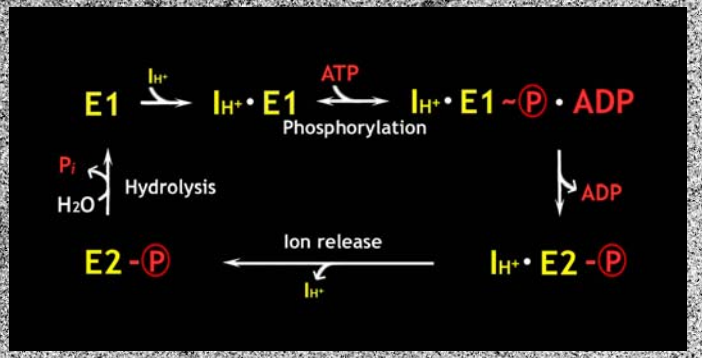

PDH

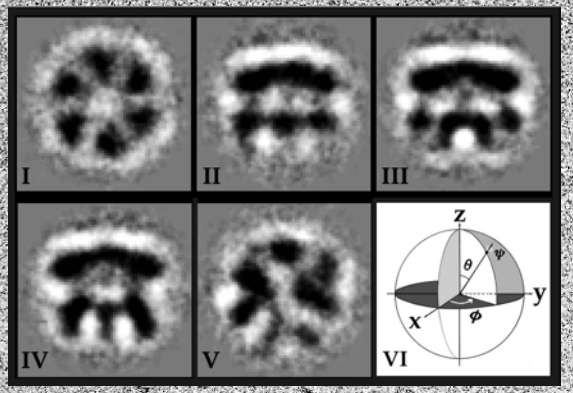

H+-ATPase

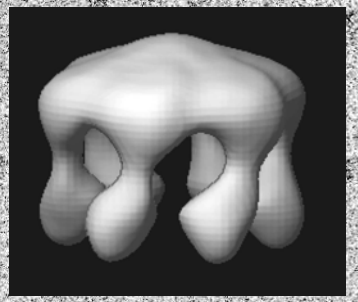

apoferritin

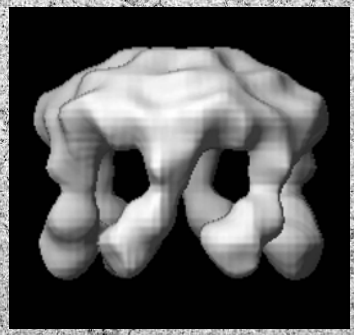

β-galactosidase

Rhee,Scarborough & Henderson, EMBO J., 2002

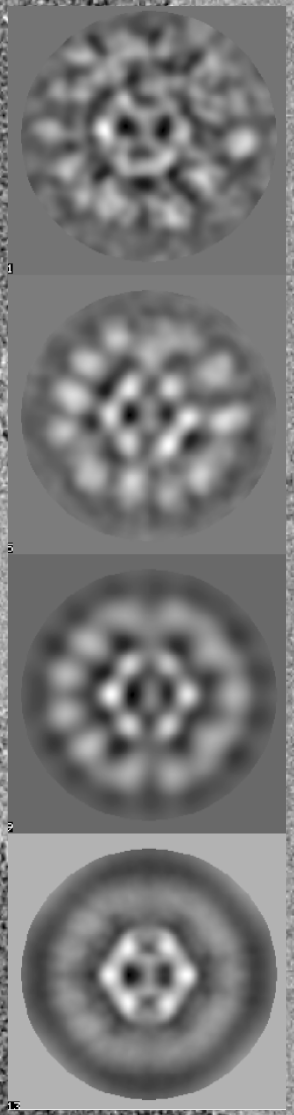




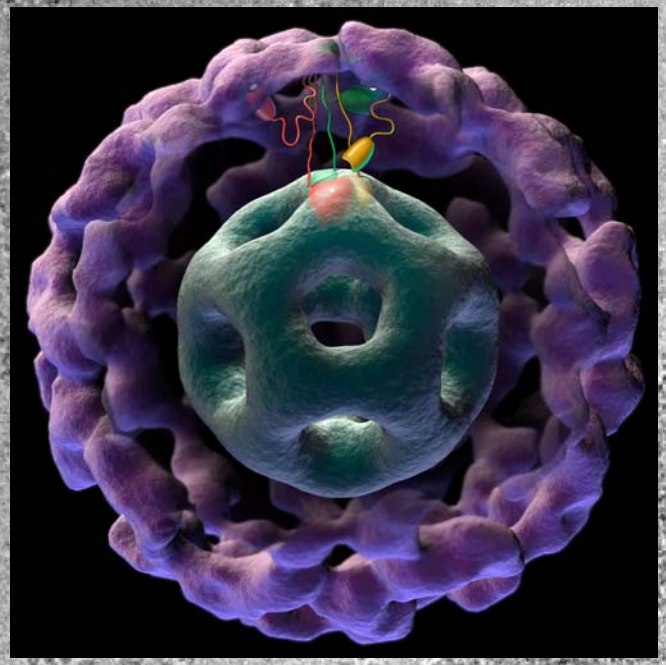




Milne et al EMBO J. 21: 5587-559 (2002)

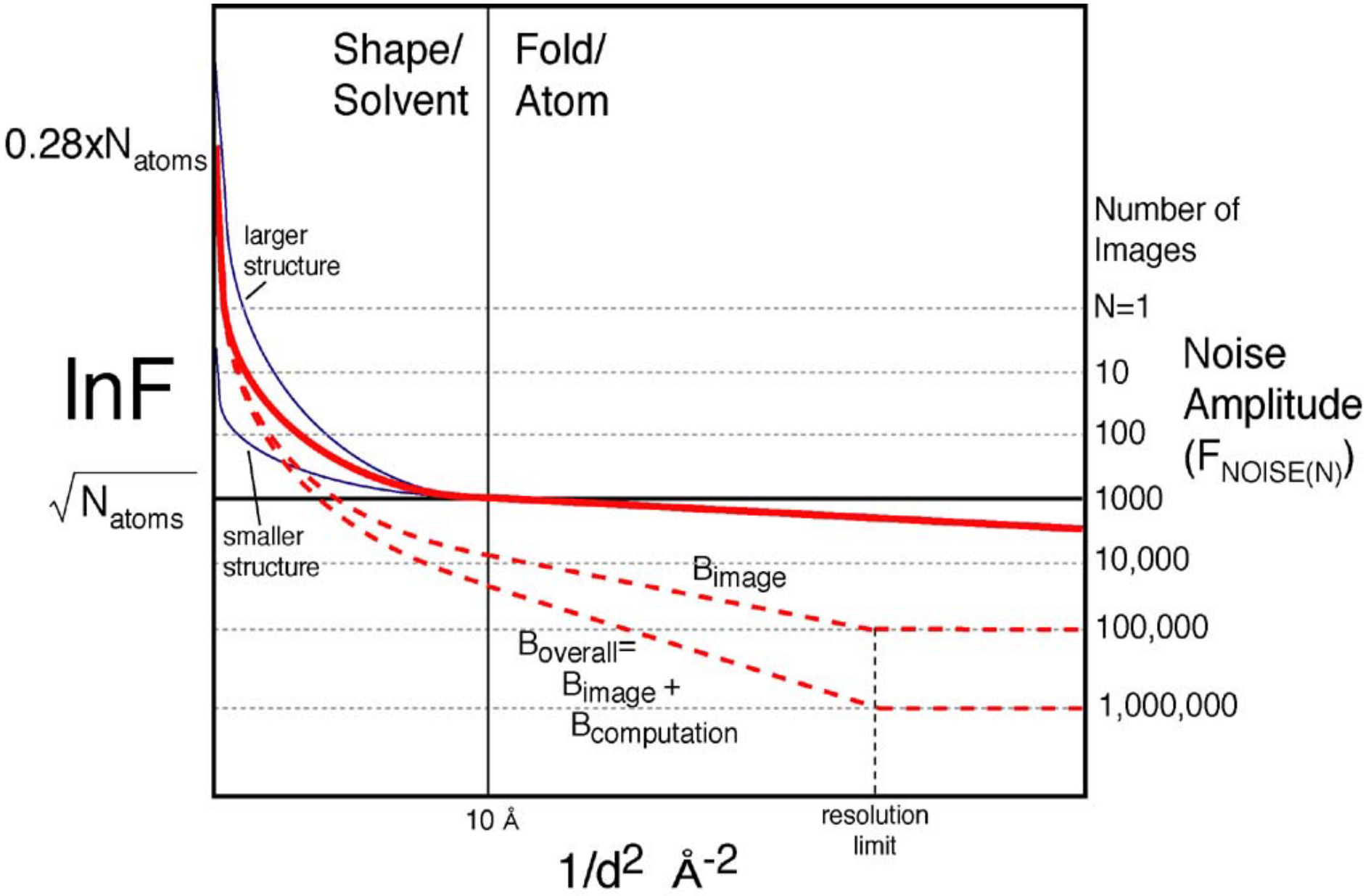


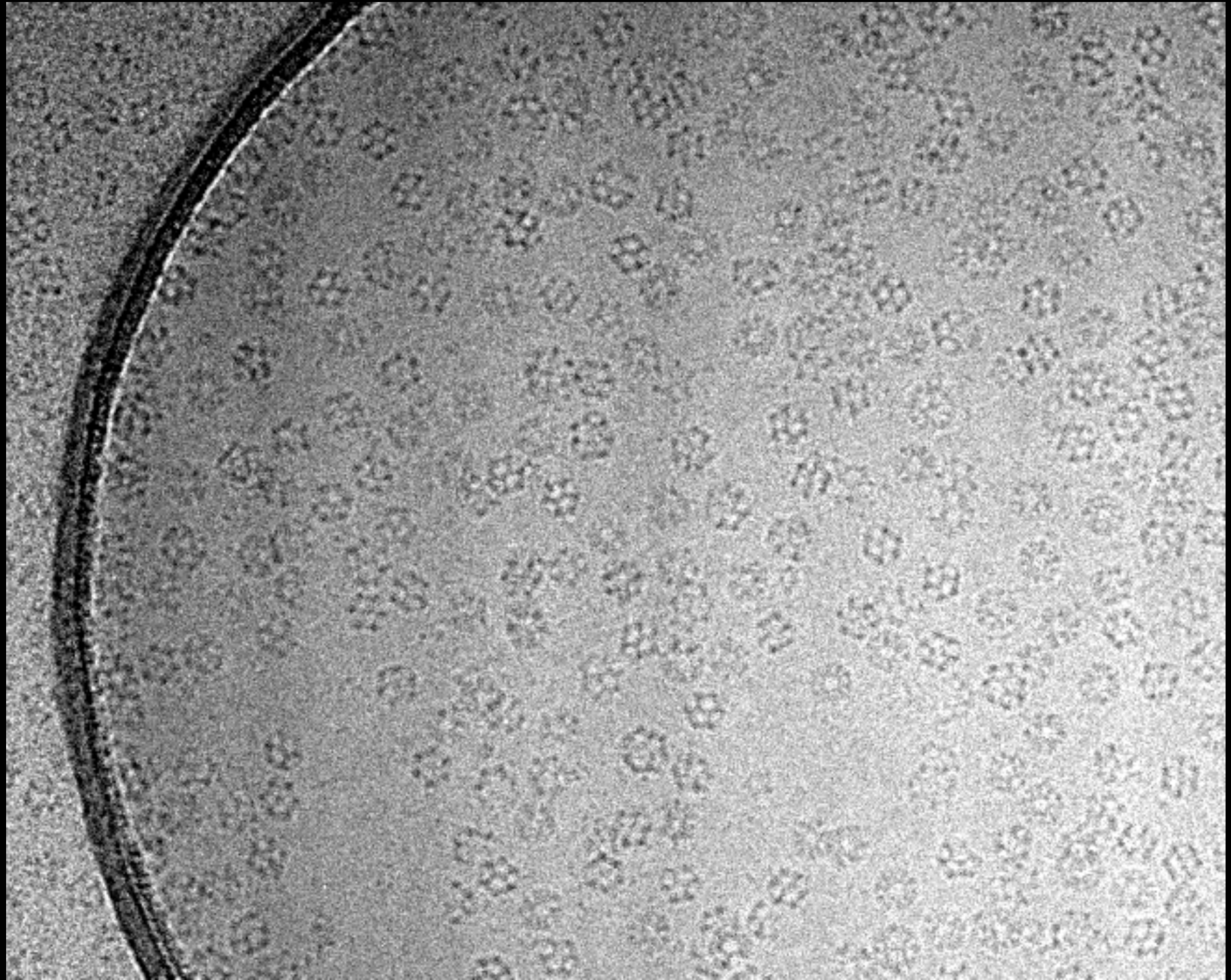
QuickTime™ and a
SGI decompressor
are needed to see this picture.



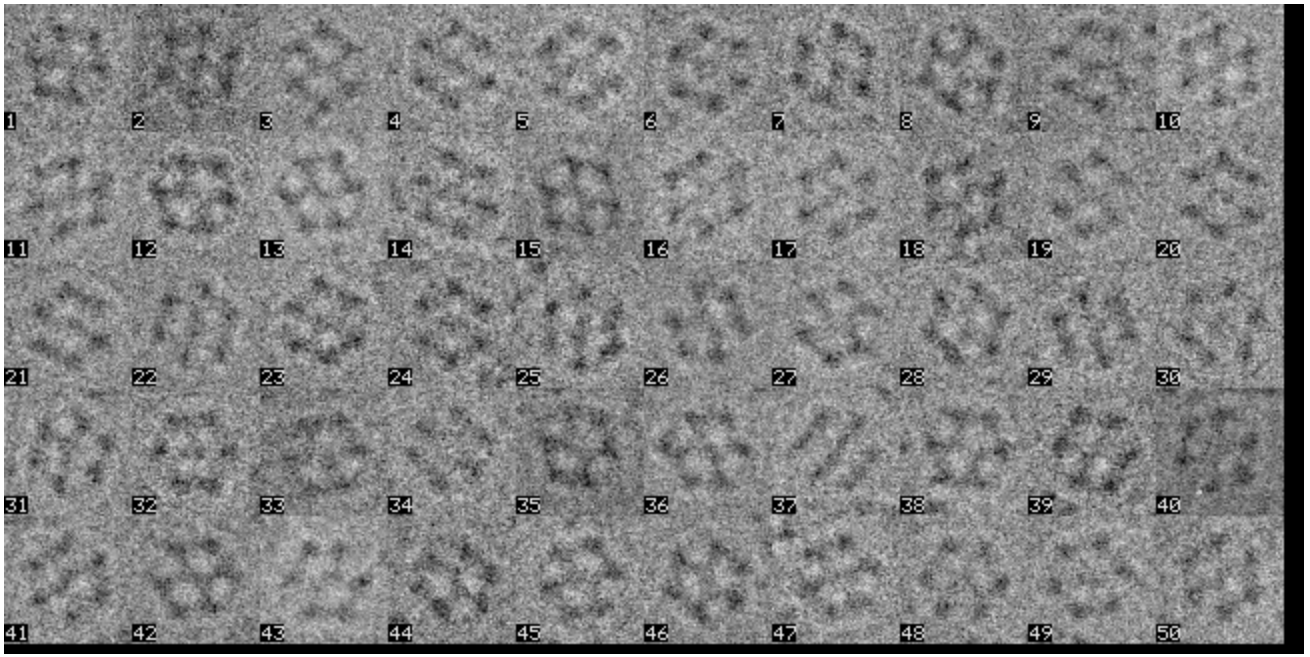
Single particle approaches (Peter Rosenthal)

- Use of tilted pairs (absolute hand, parameter optimisation)
- Sharpening and signal-to-noise weighting

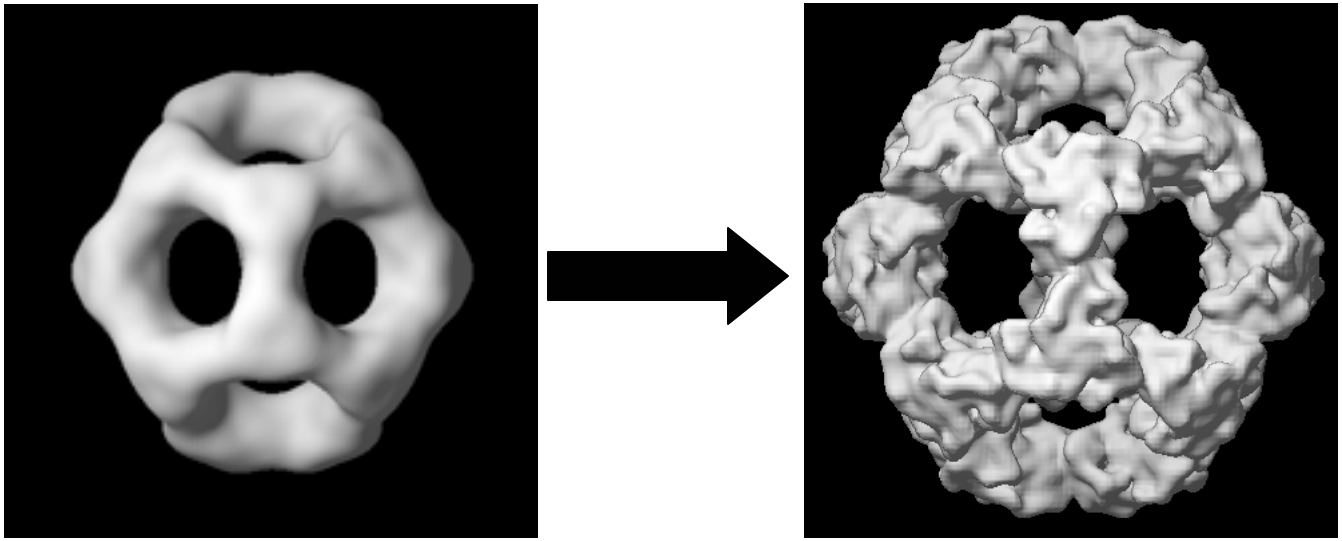

Shape/
Solvent
Fold/
Atom
0.28xN atoms
larger
structure
lnF
√N atoms
smaller
structure
Number of
Images
N=1
10
100
1000
10,000
100,000
1,000,000
Noise
Amplitude
(F NOISE(N))
B image
B overall=
B image +
B computation
10 Å
resolution
limit
1/d2 Å-2

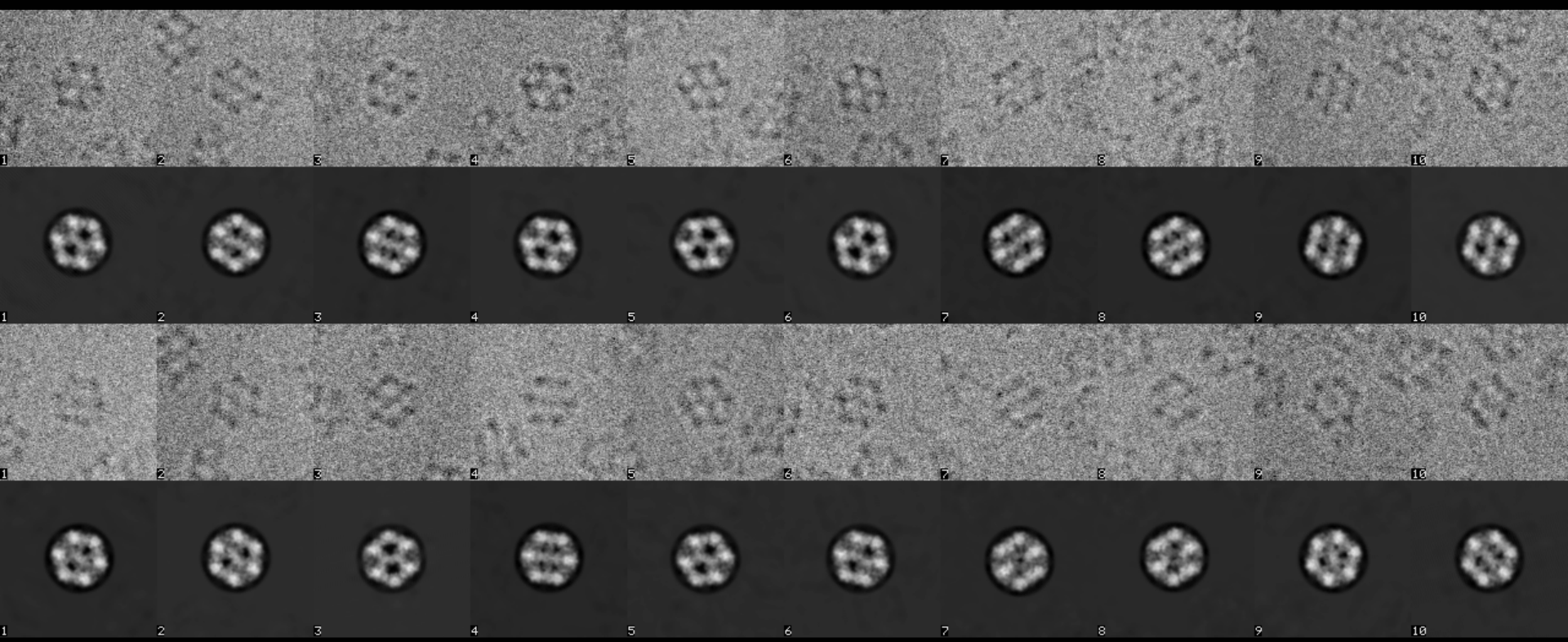


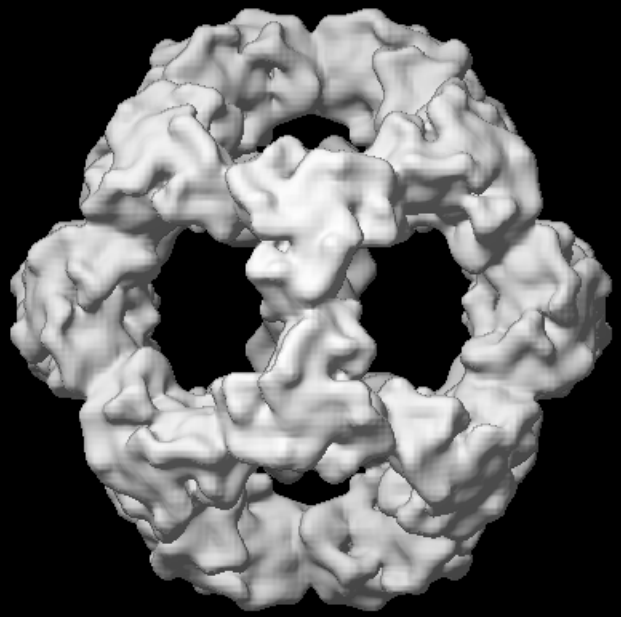
PARTICLE IMAGES

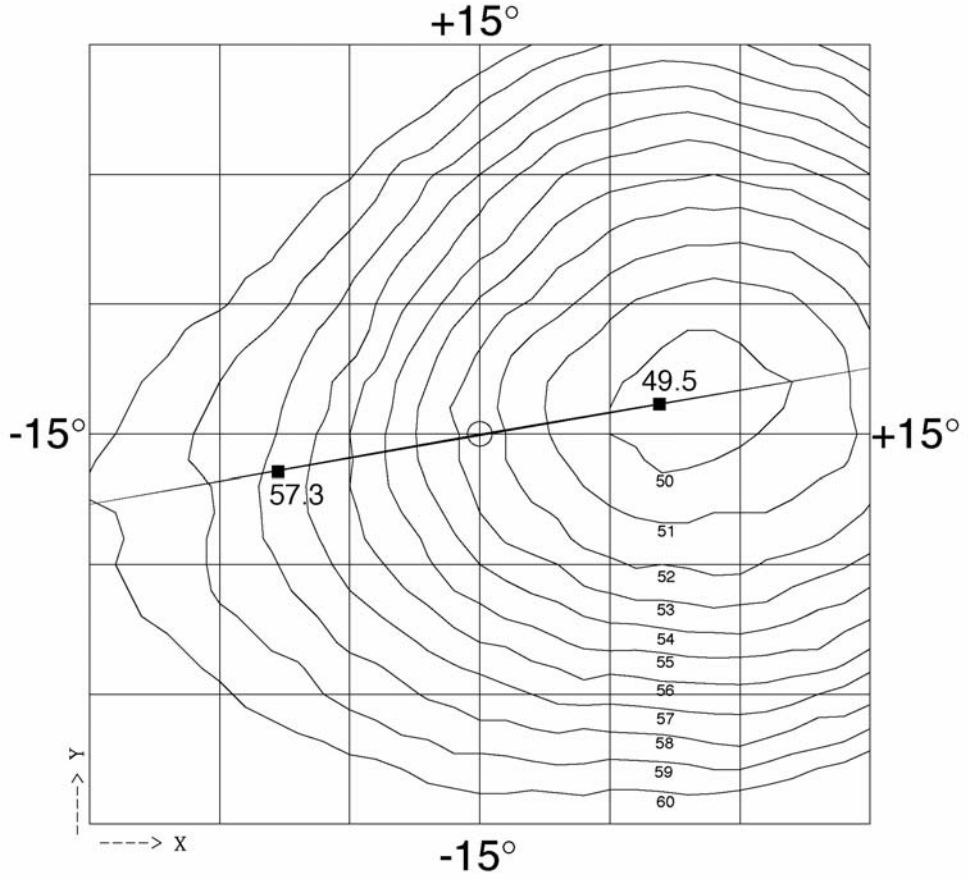


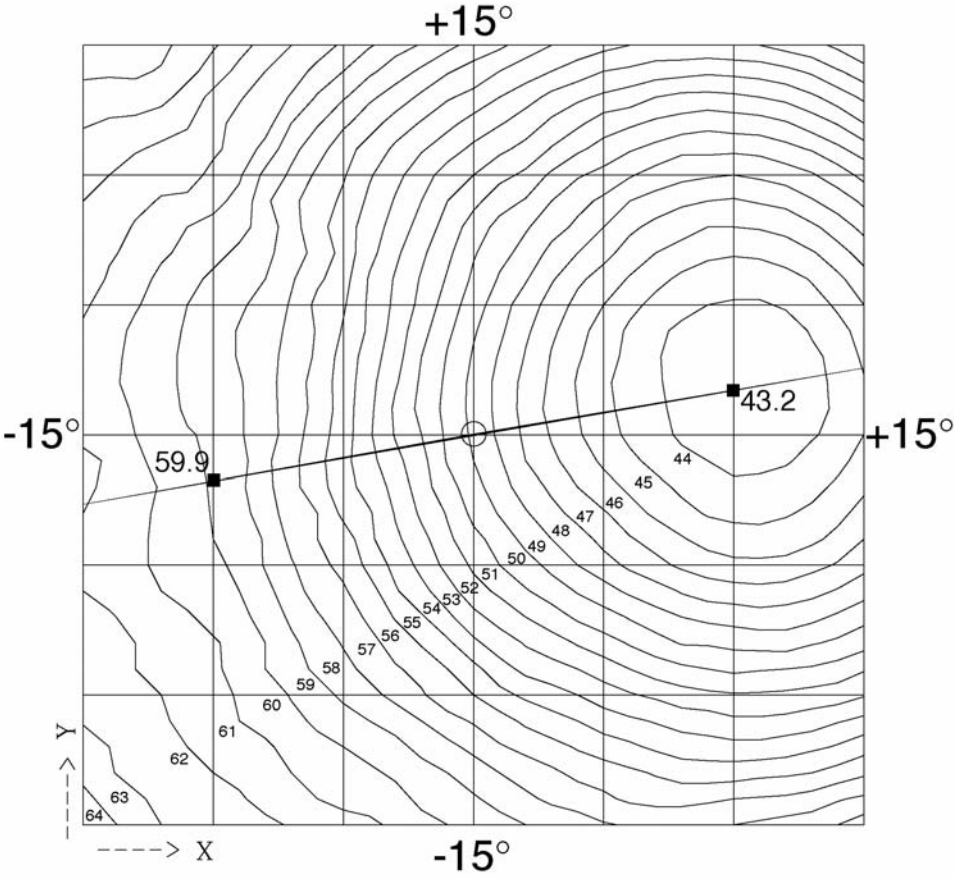
STARTING MODEL

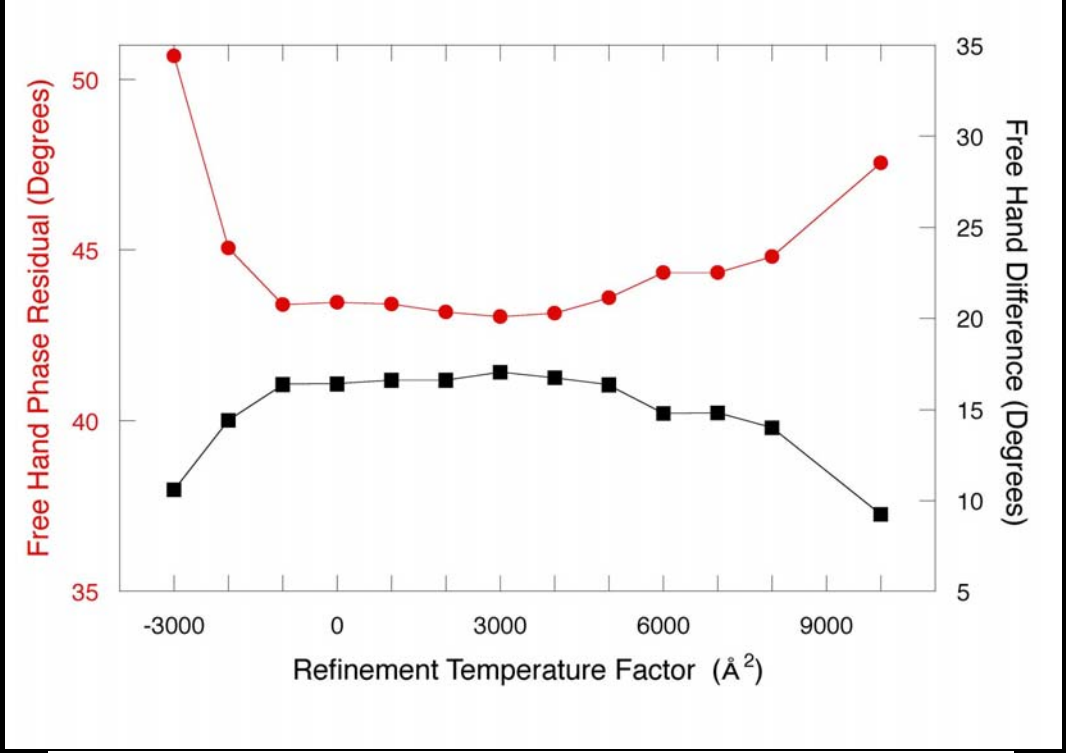


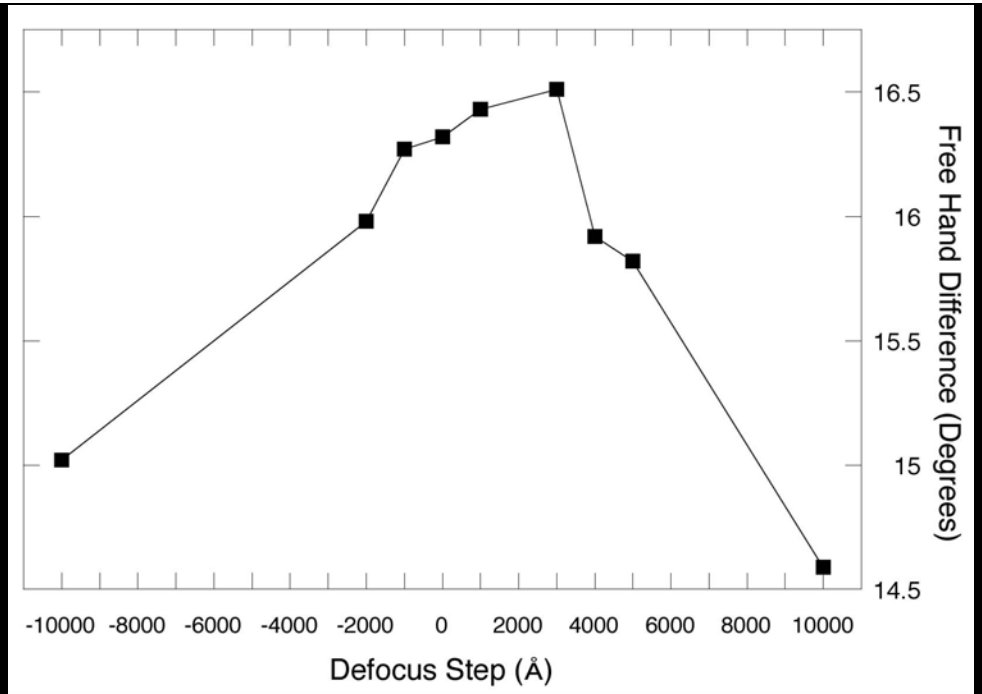

1 2 3 4 5 6 7 8 9 10
1 2 3 4 5 6 7 8 9 10
1 2 3 4 5 6 7 8 9 10
1 2 3 4 5 6 7 8 9 10

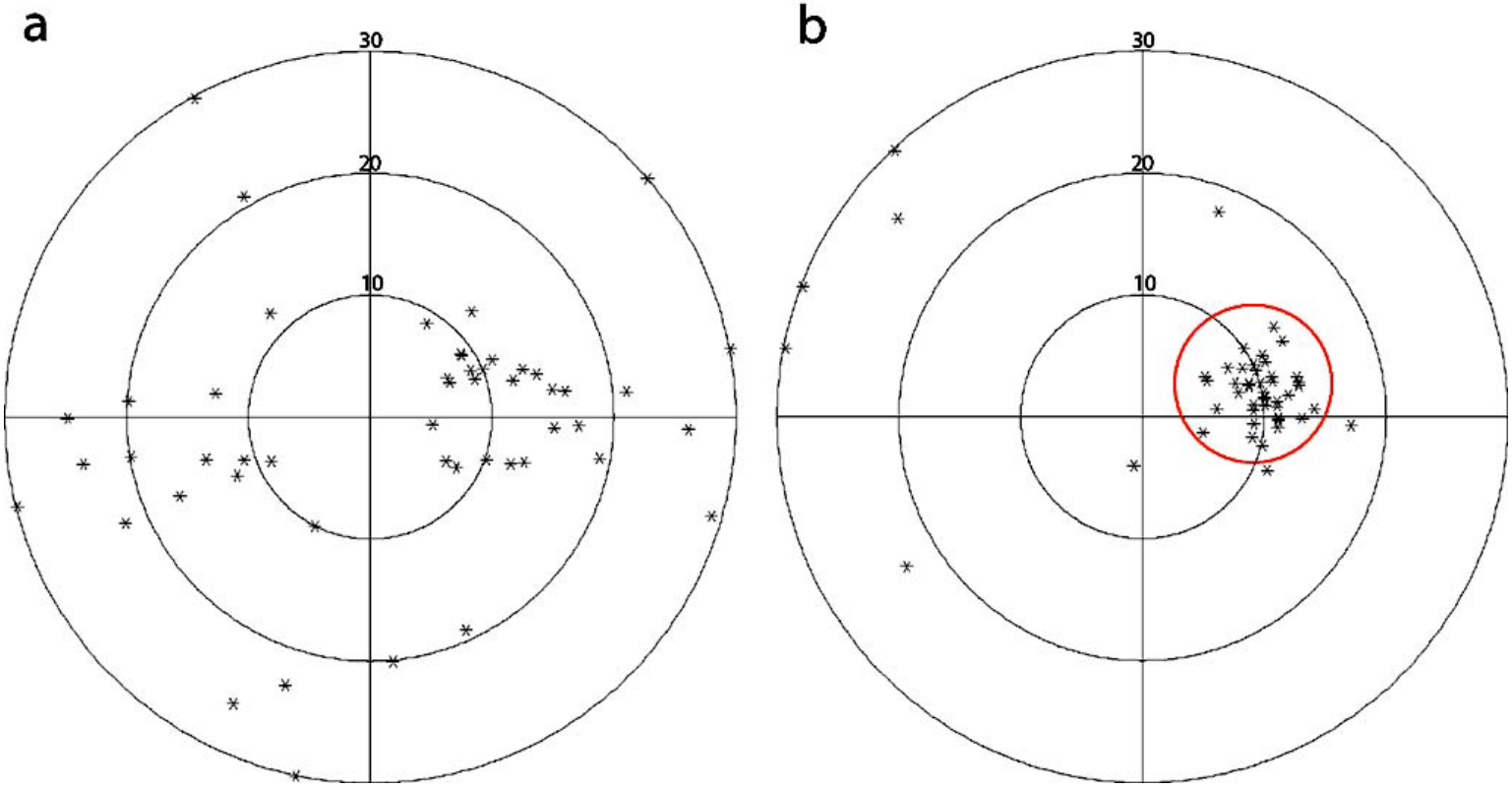



+15°
-15°
+15°
-15°
49.5
57.3
50
51
52
53
54
55
56
57
58
59
60
Y <----
----> X


+15°
-15°
+15°
-15°
43.2
59.9
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
Y <----
----> X

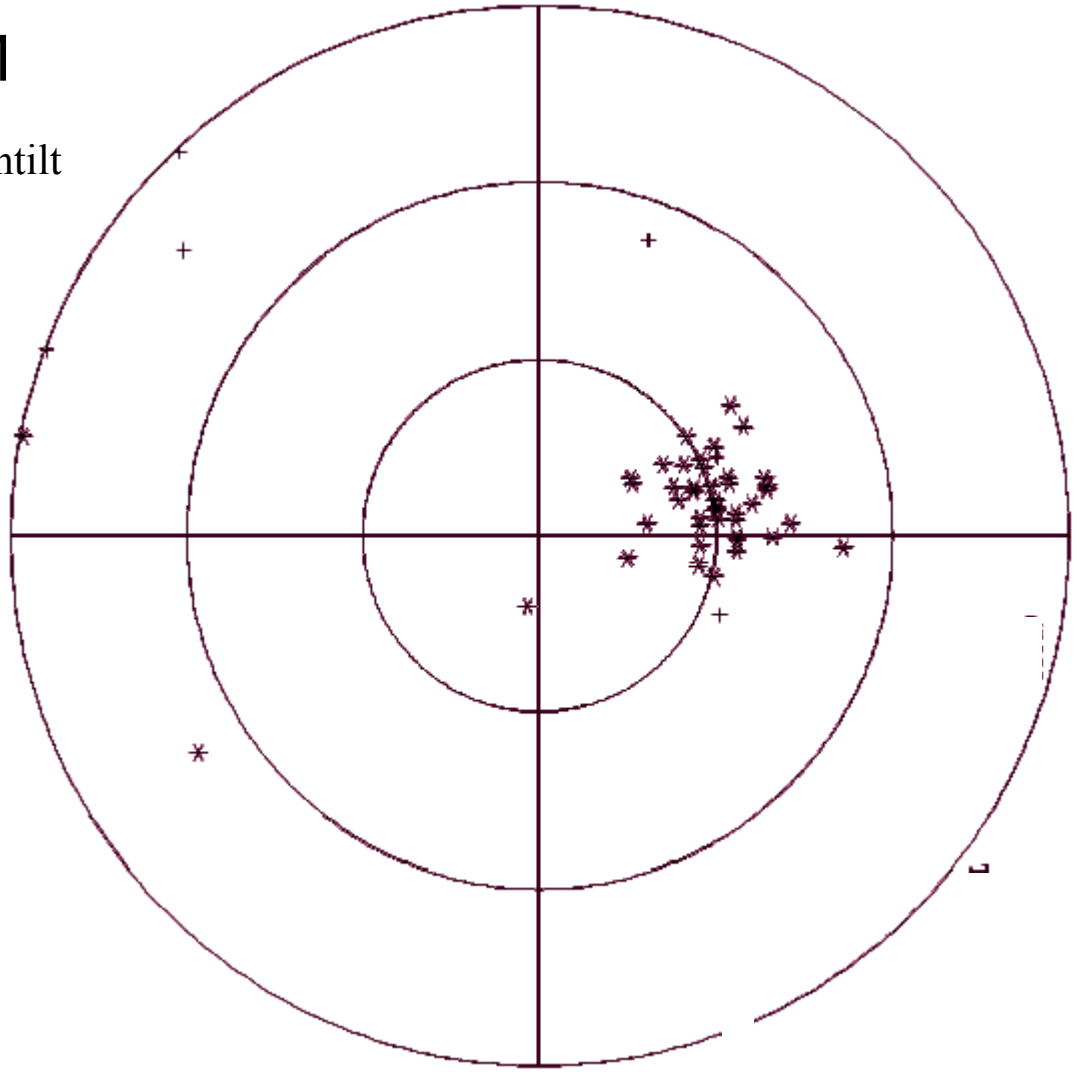

Free Hand Phase Residual (Degrees)
50
45
40
35
Free Hand Difference (Degrees)
35
30
25
20
15
10
5
-3000
0
3000
6000
9000
Refinement Temperature Factor (Å^2)


16.5
16
15.5
15
14.5
Free Hand Difference (Degrees)
-10000
-8000
-6000
-4000
-2000
0
2000
4000
6000
8000
10000
Defocus Step (Å)


a
30
20
10
b
30
20
10

TILT AXIS FOR EACH PARTICLE PAIR AFTER OPTIMIZATION

CALCULATED FROM $(\psi,\theta,\varphi)_{tilt,}$ and $(\psi,\theta,\varphi)_{untilt}$



For two independent half sets of data

Cross-correlation = Ctest

$Ctest = \Sigma(S + N1)(S + N2) / \Sigma(S^2 + 2SN + N^2)$

$= S^2 / (S^2 + N^2)$

where S = signal and N = N1 = N2 = noise in half dataset

Comparing the full set of data to a perfect reference set

Cross-correlation = Cref

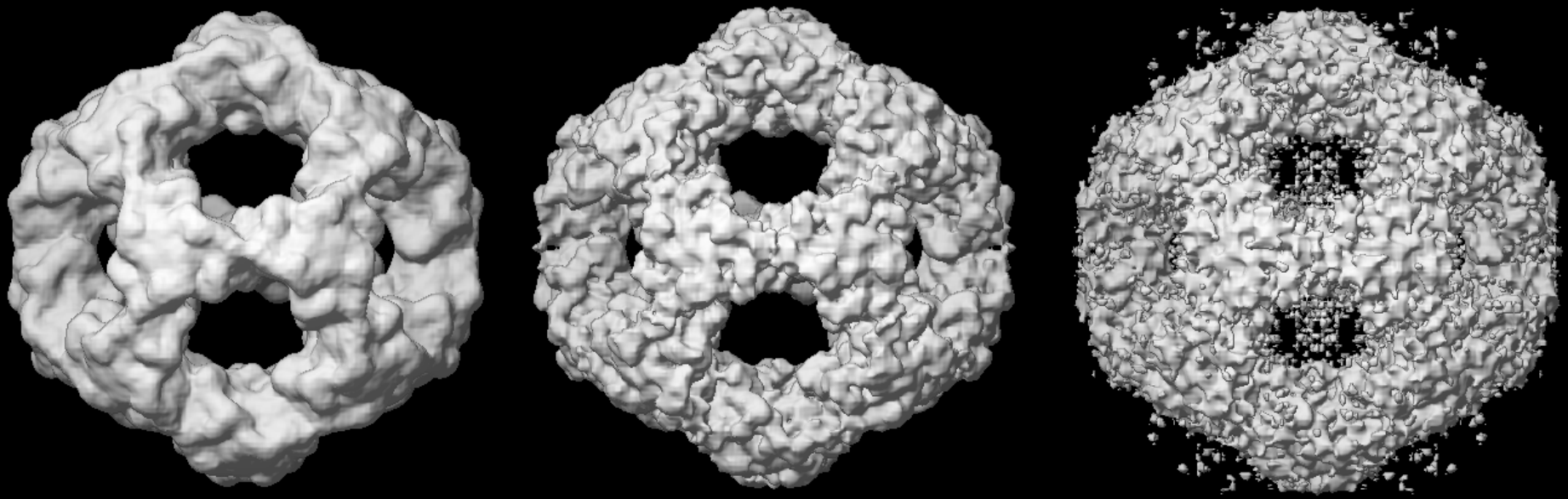
$Cref = S^2 / (\sqrt{S^2} \cdot \sqrt{(S^2 + N^2/2)})$

$= \sqrt{(S^2/(S^2 + N^2/2))} = (2 \cdot Ctest / (1 + Ctest))^{1/2}$

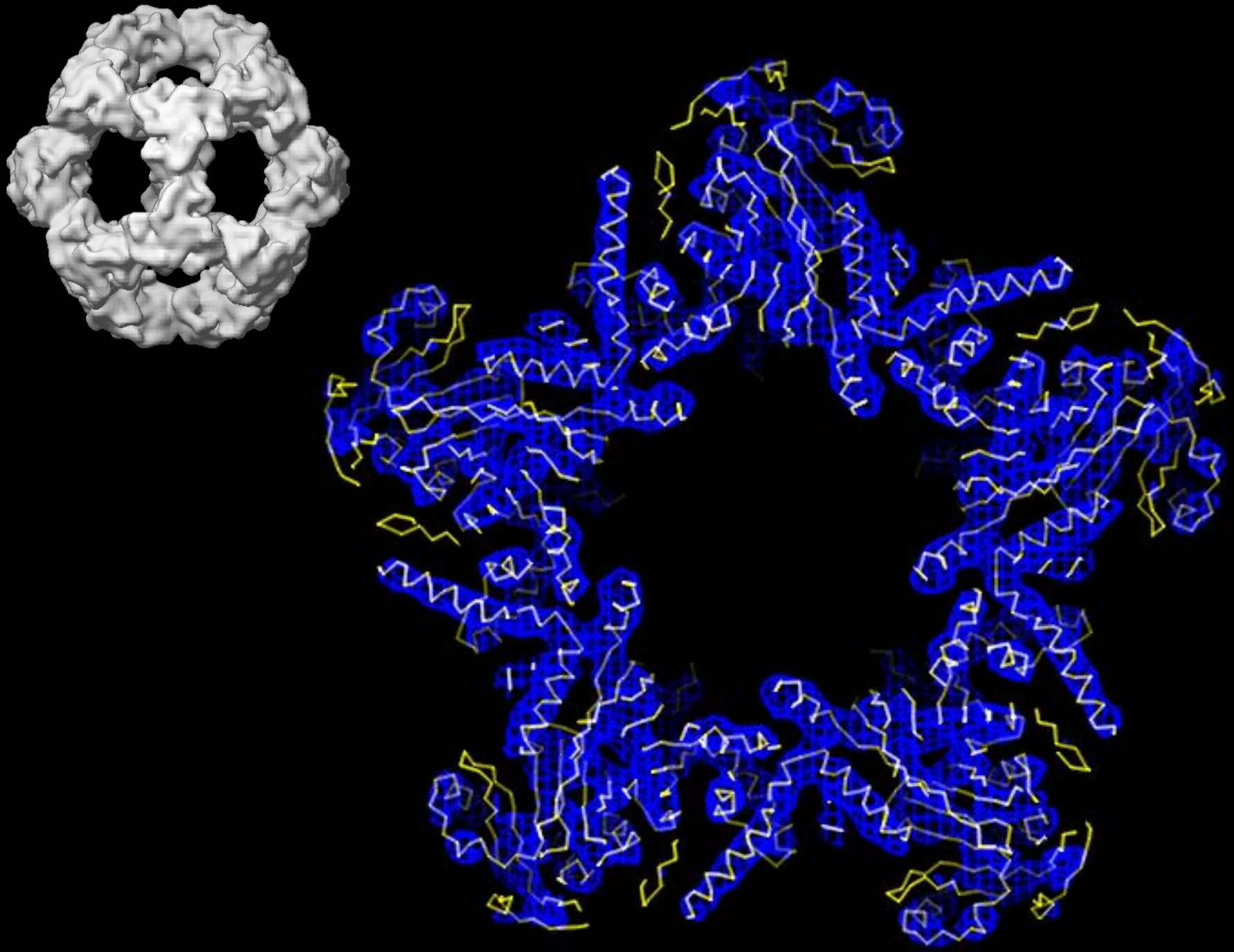
Therefore

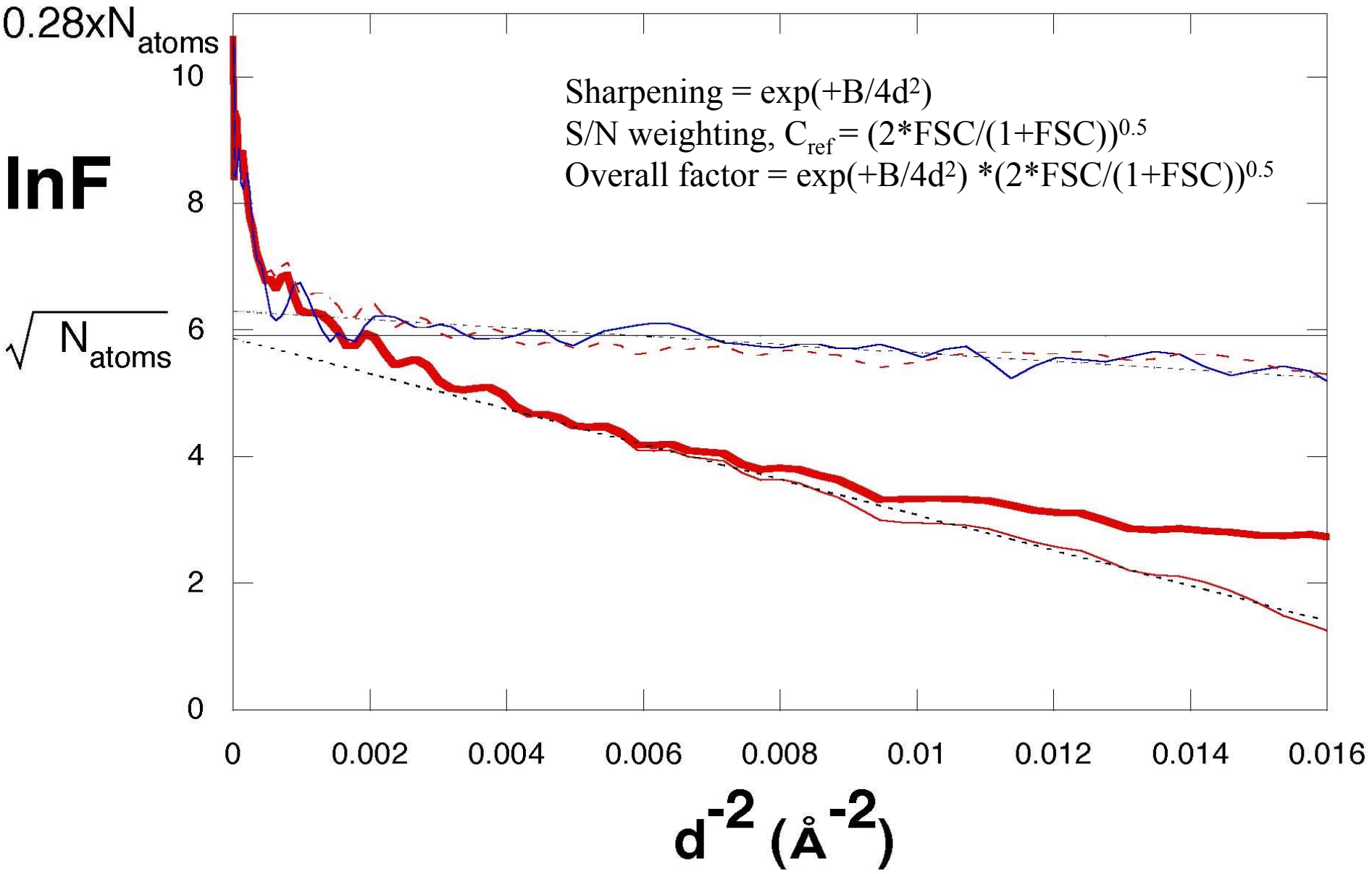
When $S^2 = N^2$ Ctest = 0.500 and Cref = 0.816 = fom

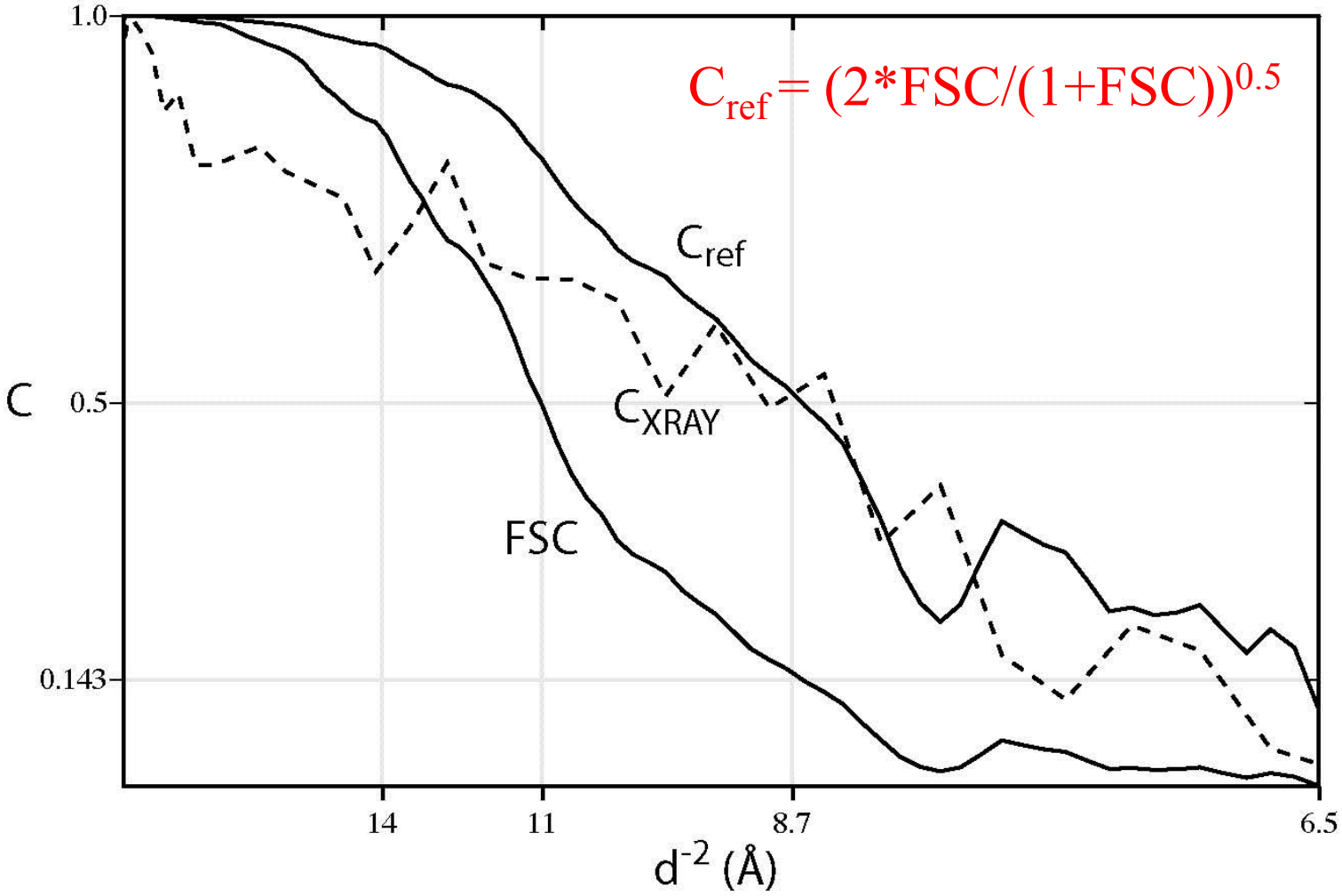
When $6S^2 = N^2$ Ctest = 0.143 and Cref = 0.500 = fom

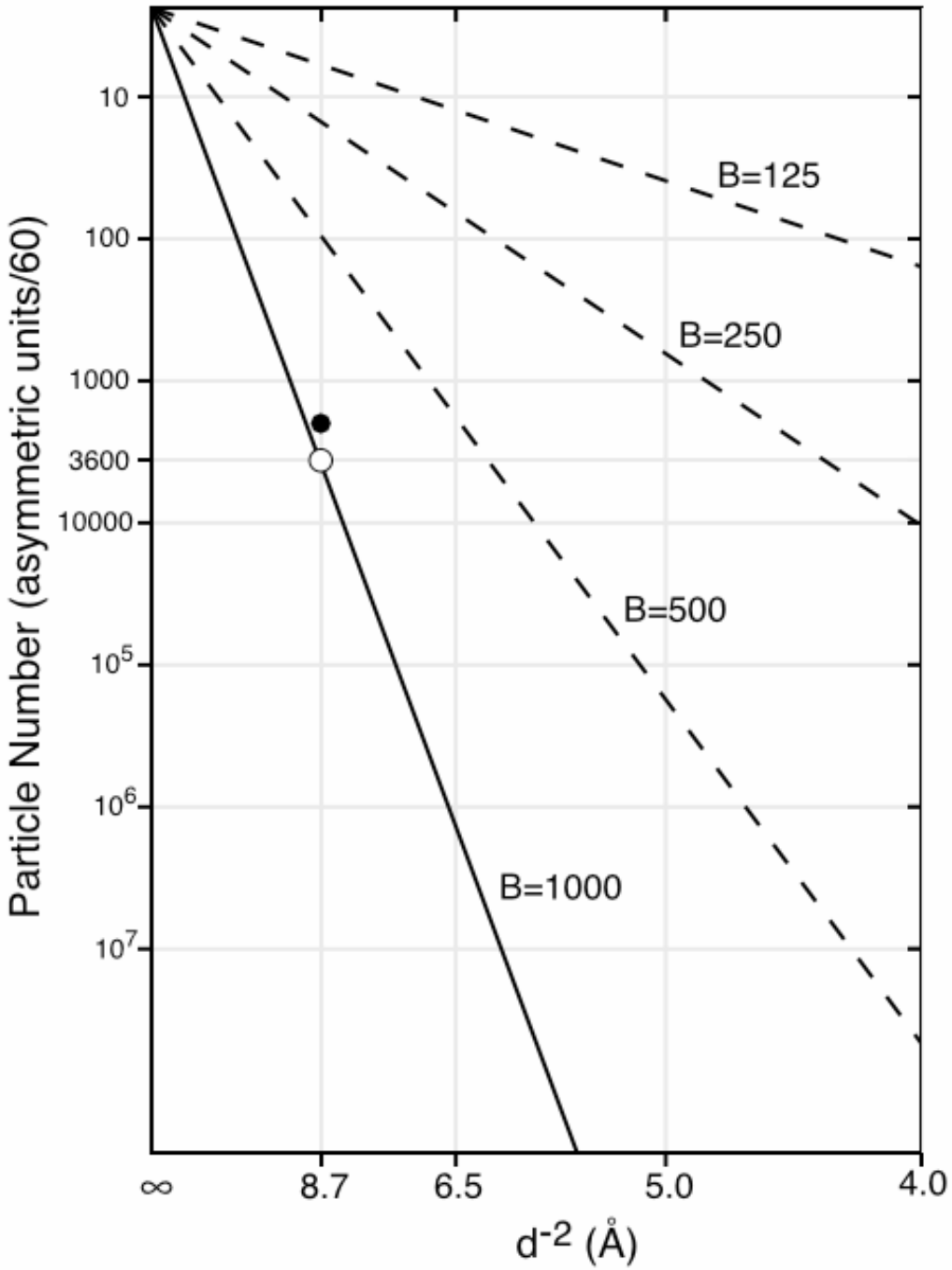


3667 particles




0.28xN atoms
lnF
√ N atoms
Sharpening = exp(+B/4d²)
S/N weighting, C ref = (2*FSC/(1+FSC))^0.5
Overall factor = exp(+B/4d²) *(2*FSC/(1+FSC))^0.5
10
8
6
4
2
0
0
0.002
0.004
0.006
0.008
0.01
0.012
0.014
0.016
d-2 (Å-2)


1.0
0.5
0.143
C
$C_{ref} = (2*FSC/(1+FSC))^{0.5}$
C_{ref}
C_{XRAY}
FSC
14
11
8.7
6.5
d^{-2} (Å)


Particle Number (asymmetric units/60)
10
100
1000
3600
10000
10^5
10^6
10^7
B=125
B=250
B=500
B=1000
∞
8.7
6.5
5.0
4.0
d^{-2} (Å)

Acknowledgements

Adenovirus	Adrian, Dubochet, Lepault & McDowell
Hepatitis B virus cores	Böttcher, Wynne & Crowther
Frealign, Complex I, Clathrin	Grigorieff, Smith & Pearse
H^+-ATPase	Rhee, Scarborough
Pyruvate dehydrogenase	Rosenthal, Milne, Subramaniam, Perham, et al
EM simulator	McMullen