

What is needed to make single particle EM reach its
true potential?

PDB40 Symposium
Cold Spring Harbor Laboratory
29th October 2011

Richard Henderson
MRC-LMB, Cambridge

Cold Spring Harbor Symposium XXXVI 1971



Henderson

Zwick



Rossmann

North



Baldwin

Harrison

214 643752

MAY 13 1967

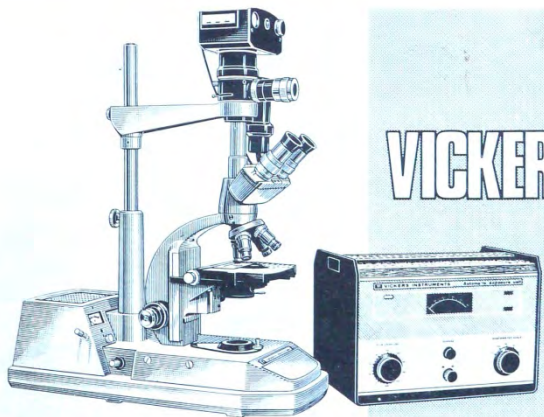
NATURE

A Weekly Journal of Science

Vol. 214, No. 5089

SATURDAY, MAY 13, 1967

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Three-dimensional Structure of Tosyl- α -chymotrypsin

by

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P. B. SIGLER
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Cambridge, England

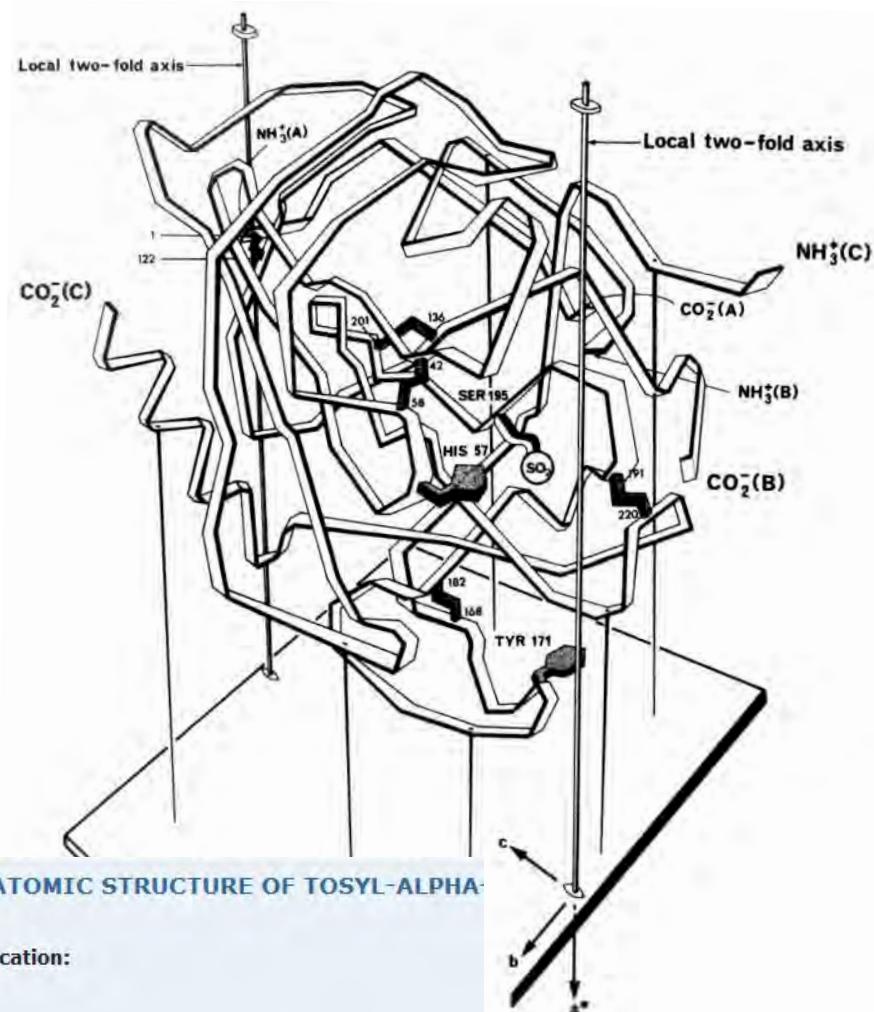
A model is proposed for the structure of an inhibited derivative of an enzyme which hydrolyses proteins. It is based on a map of the electron density distribution at 2 Å resolution and interpreted in terms of a previously reported sequence of 241 amino-acids. The map has been derived from a Fourier synthesis of 24,500 terms and represents two crystallographically independent molecules, each of molecular weight 25,000, which are nearly identical in their tertiary structure. The enzyme is composed almost entirely of extended polypeptide chains. A chemical marker unambiguously defines the position of the active centre. The position and orientation of the residues known to be important in catalysis are clearly seen. The mechanism by which the inactive precursor is converted into an active enzyme is revealed.

An X-ray diffraction study of crystalline α -chymotrypsin—a bovine protease containing 241 amino-acid residues—has been in progress since 1960 (refs. 1–3). We are now able to report the preparation of an electron density map at a resolution of 2 Å which, taken in conjunction with the amino-acid sequence determined by Hartley^{4,5} and Keil and Sorm^{6,7}, reveals in detail the conformation of the enzyme molecule in the crystal.

X-ray Diffraction Measurements

The crystals were grown from ammonium sulphate solutions at pH 4.2 as previously described². The unit cell (space group $P2_1$, $a = 49.1$ Å, $b = 67.4$ Å, $c = 65.9$ Å, $\beta = 101.8^\circ$) contains four molecules of molecular weight 25,000 (refs. 9 and 2). Although the enzyme normally functions as a monomer, the crystallographic asymmetric

use of a single, moderately heavy atom to give accurate phase information at high resolution. Second, using tosyl- α -chymotrypsin as parent, a simpler and more constant pattern of binding of PMA to the protein was achieved. Finally, we anticipated that the interpretation of the map would be aided considerably by the unambiguous identification of the tosylated serine 195, which was likely to be more clearly visualized in the structure of the tosylated enzyme.



2CHA



THE STRUCTURE OF CRYSTALLINE ALPHA-CHYMOTRYPSIN, V. THE ATOMIC STRUCTURE OF TOSYL-ALPHA-

Authors: Birktoft, J.J. , Blow, D.M. 

Release: 1977-01-18

Classification:

Date:

Experiment: X-RAY DIFFRACTION with resolution of 2.00 Å

Compound: 3 Polymers [[Display Full Polymer Details](#) | [Display for All Results](#)]
1 Ligand [[Display Full Ligand Details](#) | [Display for All Results](#)]

Citation: Structure of crystalline α -chymotrypsin. V. The atomic structure of tosyl- α -chymotrypsin at 2 Å resolution.

4CHA



STRUCTURE OF ALPHA- α -CHYMOTRYPSIN REFINED AT 1.68 ANGSTROMS RESOLUTION

Authors: Tsukada, H. , Blow, D.M. 

Release: 1985-04-01

Classification:

Date:

Experiment: X-RAY DIFFRACTION with resolution of 1.68 Å

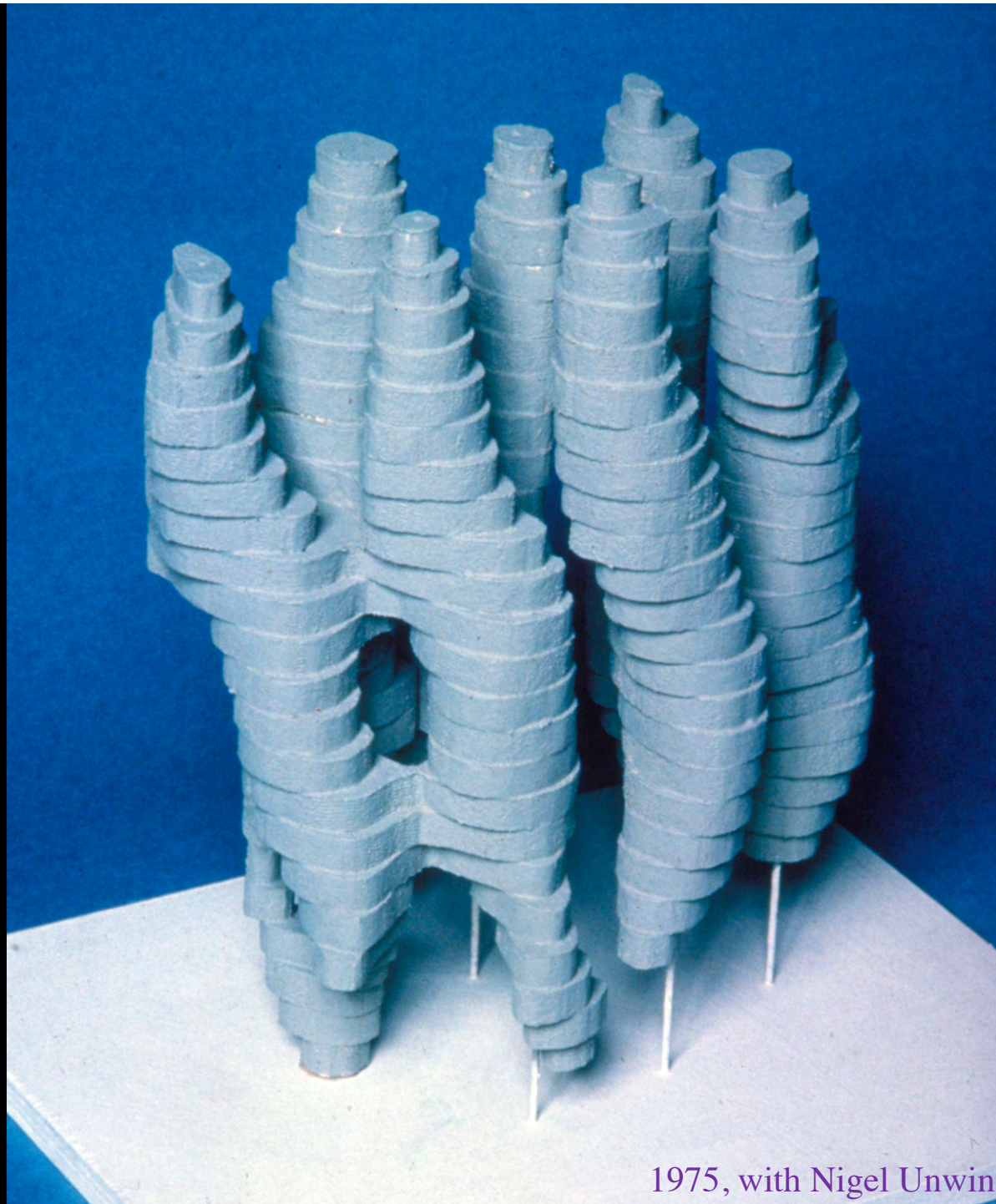
Compound: 3 Polymers [[Display Full Polymer Details](#) | [Display for All Results](#)]

Citation: Structure of alpha-chymotrypsin refined at 1.68 Å resolution. (1985) J.Mol.Biol. **184**: 703-711 [[Display Full Abstract](#) | [Display for All Results](#)]



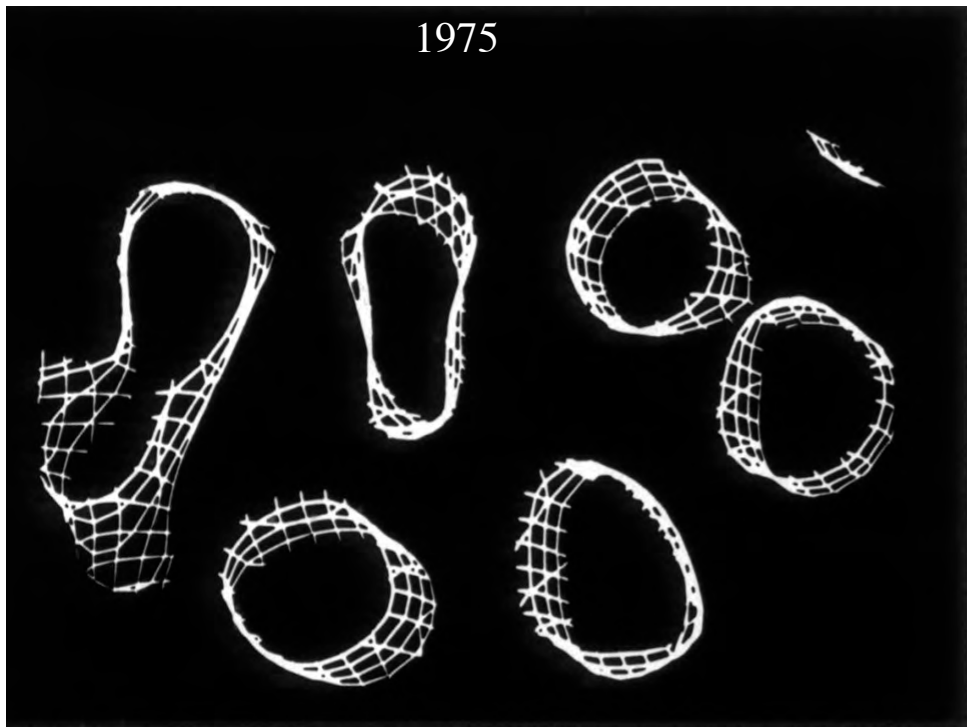


Walther Stoeckenius: *Halobacterium salinarum*

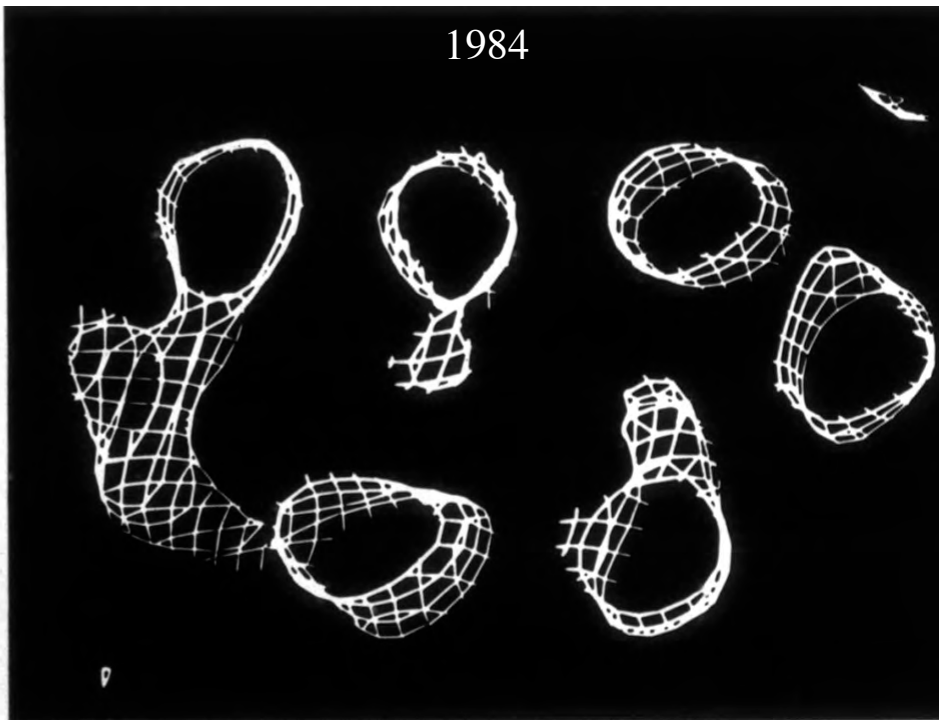


1975, with Nigel Unwin

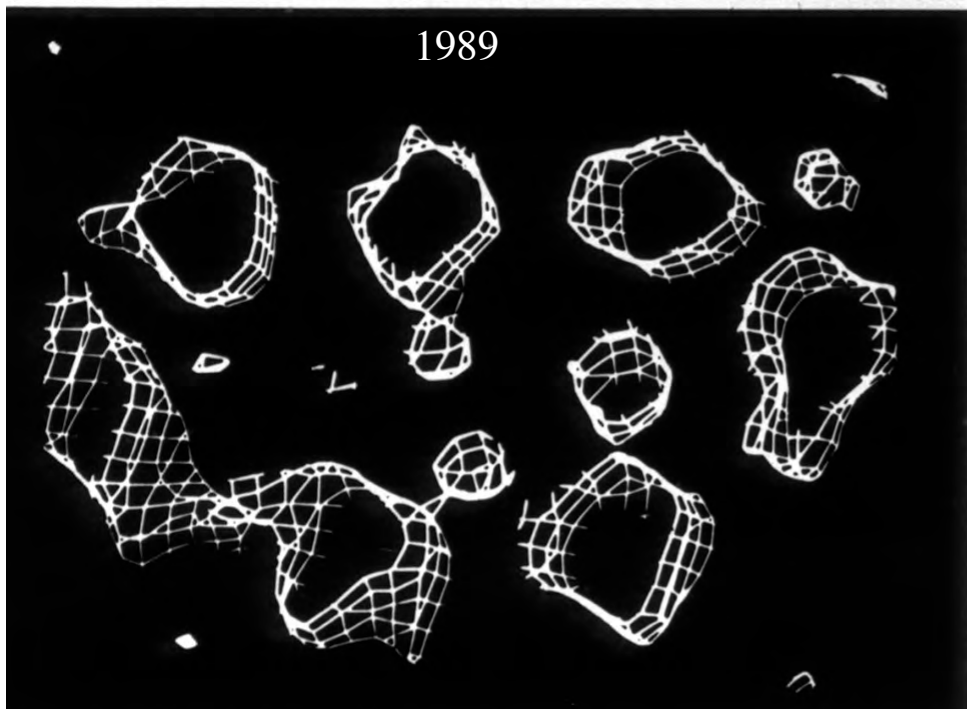
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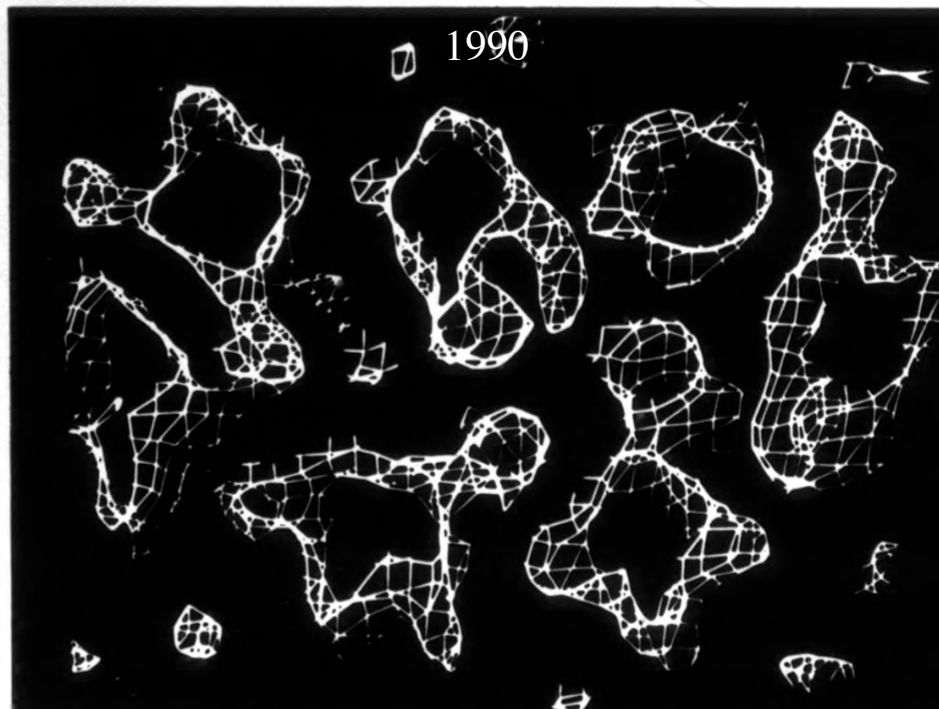
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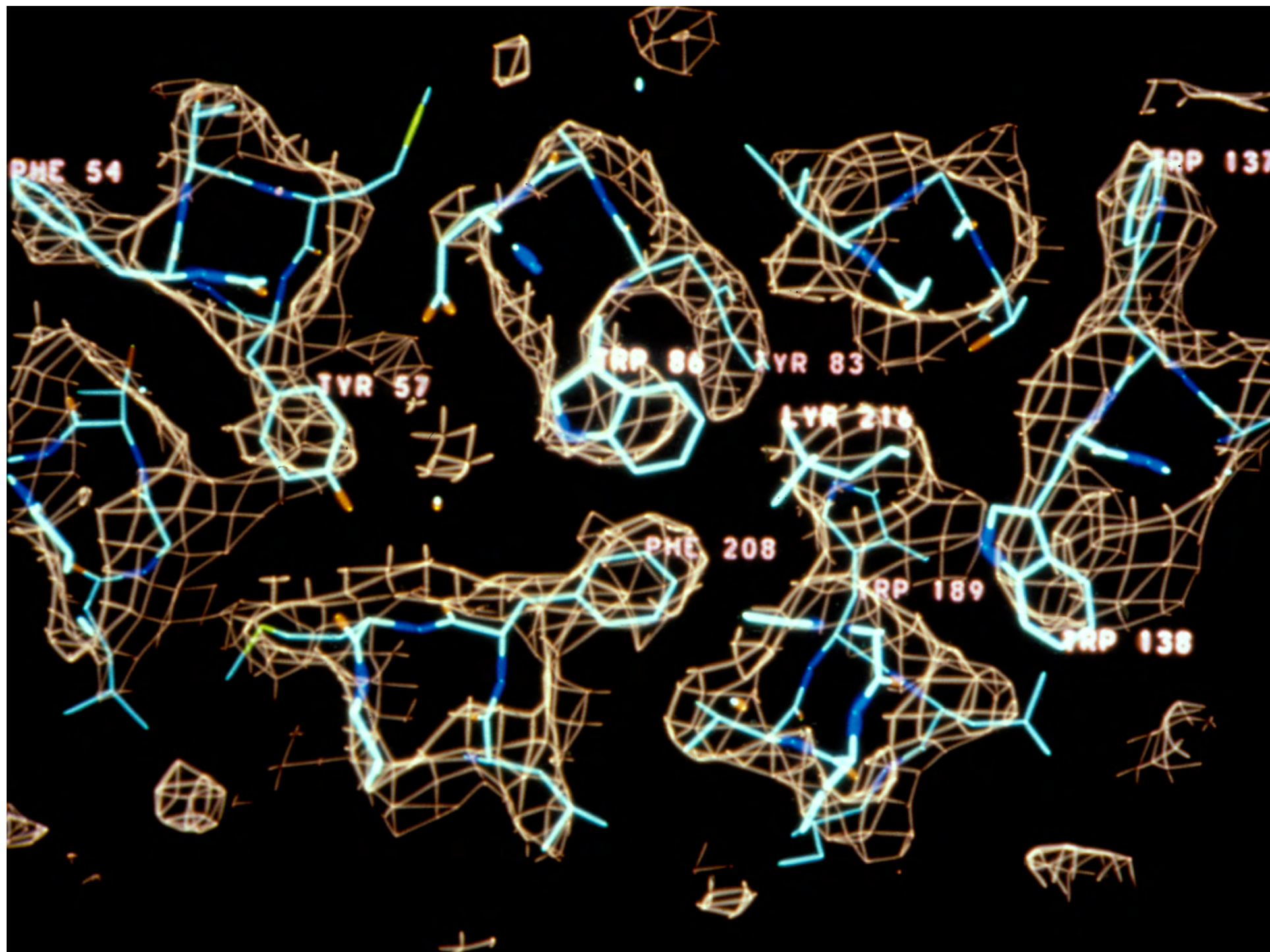


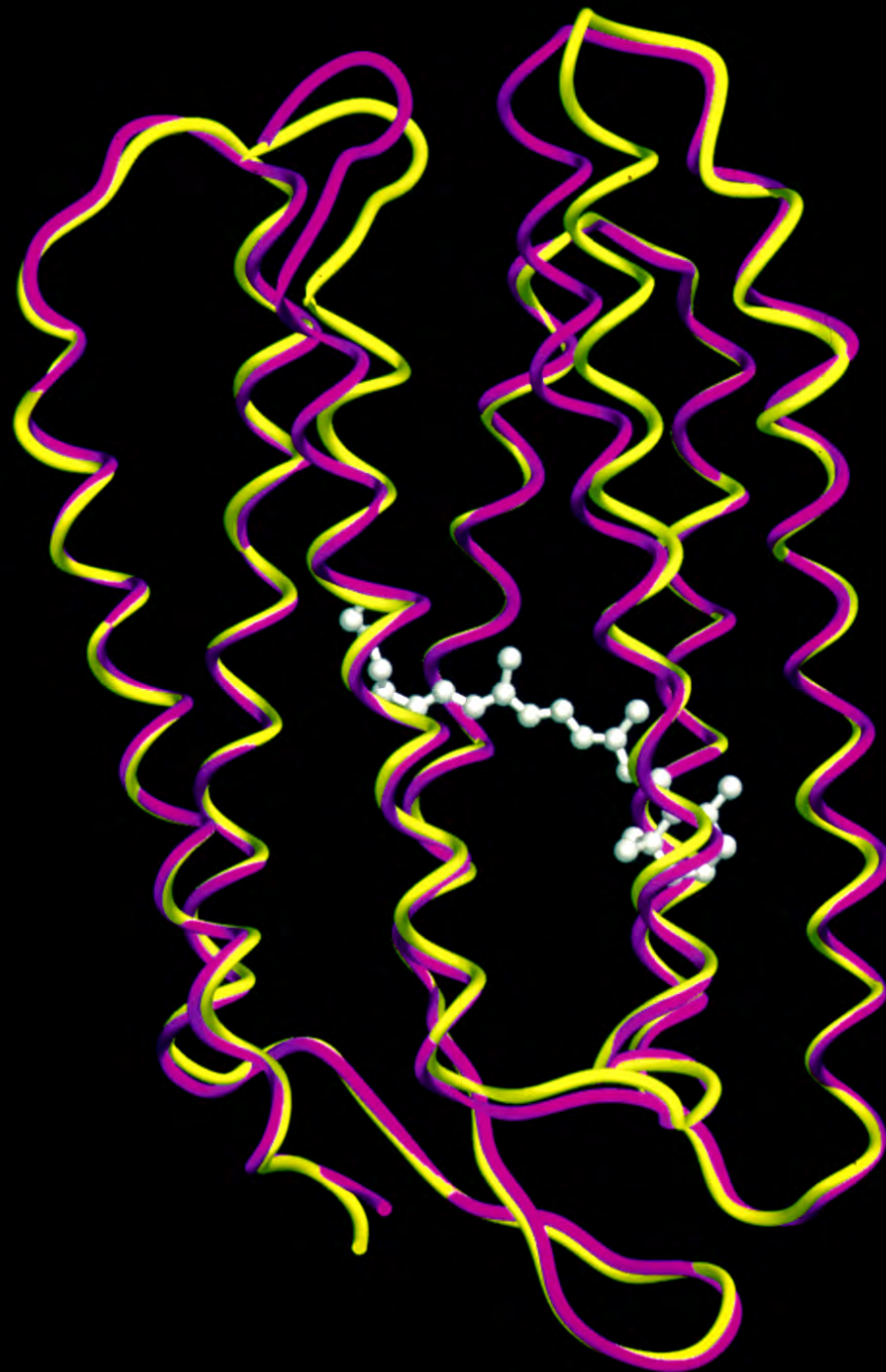
1989



1990







1brd
2brd
1fbb
1fbk

3D structures from 2D crystals (* = atomic model)

Bacteriorhodopsin	3.5, 3.0 Å	*
DOC bacteriorhodopsin	6.0 Å	
Bacteriorhodopsin p22 ₁ 2 ₁	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-0	1.8 Å	**
Aquaporin-1	3.8 Å	*
Aquaporin-4	2.8 Å	*
Halorhodopsin	5.0 Å	
Glutathione transferase	3.5 Å	*
Prostaglandin E2 synthetase	3.6 Å	*
SecYEG complex	8.0 Å	
Plant photosystem II RC	8.0 Å	
Neurospora H ⁺ -ATPase	8.0 Å	
Gap junction channel	7.5, 6.0 Å	
NhaA Na ⁺ /H ⁺ antiporter	7.0 Å	
Glycerol channel GlpF	6.9 Å	
Oxalic acid transporter OxIT	6.0 Å	
EmrE multidrug transporter	7.0 Å	

Some early single particle cryoEM structures from Cambridge

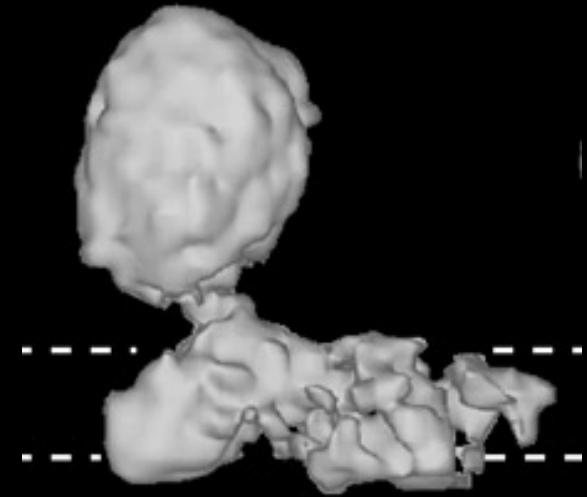
B



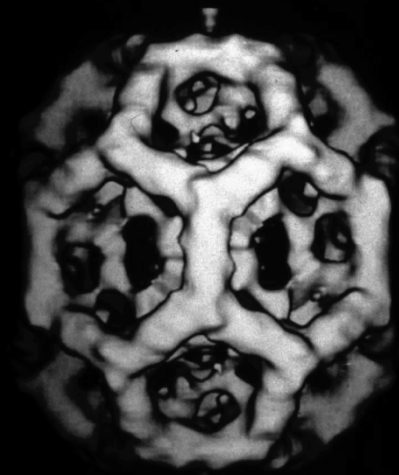
F-type ATPase 0.6 MDa



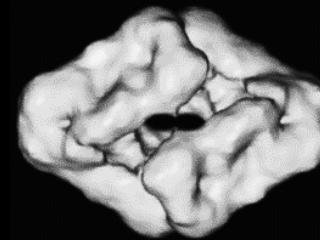
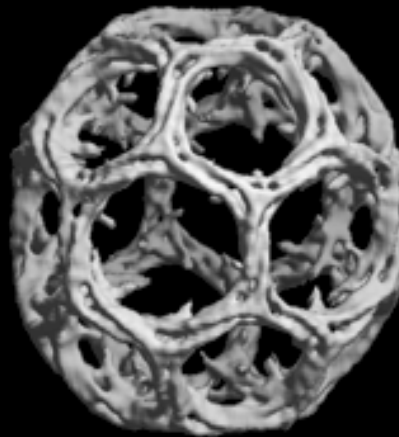
P-type H⁺-ATPase 0.6 MDa



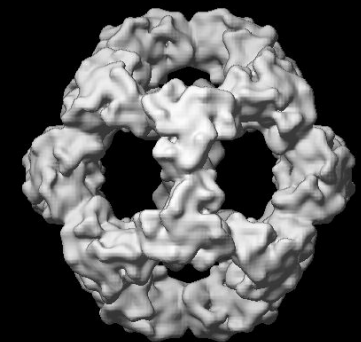
Complex I 0.9 MDa



Clathrin cage 20 MdD



betaGal
0.45 MDa



PDH 1.6 MDa

Potential of cryoEM

- (a) How do we know how well we should be able to do?
- (b) How do we know we are right – validation?
- (c) How do we improve?

- Relate theory to experiment
- Tilt pair analysis to assess orientation determination
- Calculate control map with high resolution noise substituted
- Better detectors
- Minimise specimen movement & image blurring

Human Rotavirus DLP Zhang et al & Grigorieff
3.8 Å, B-factor 450Å² (2008) PNAS **105**, 1867-72.

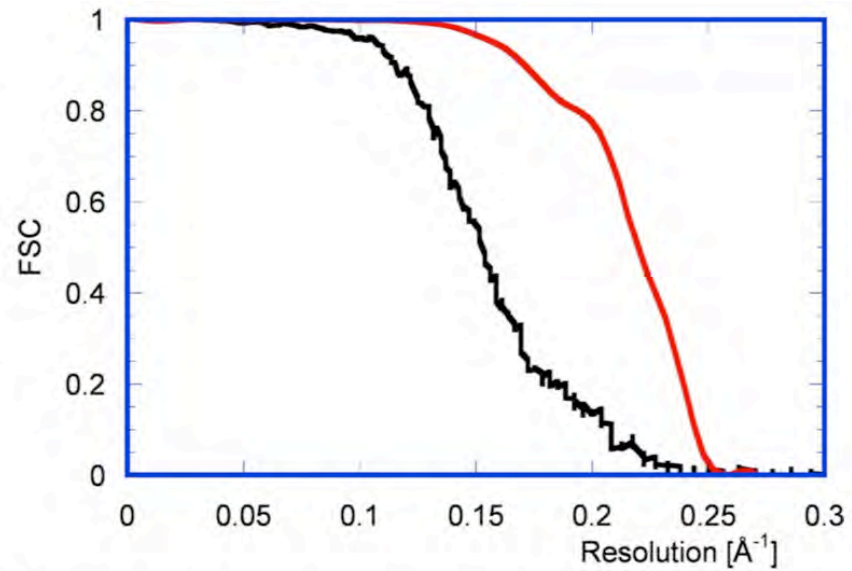
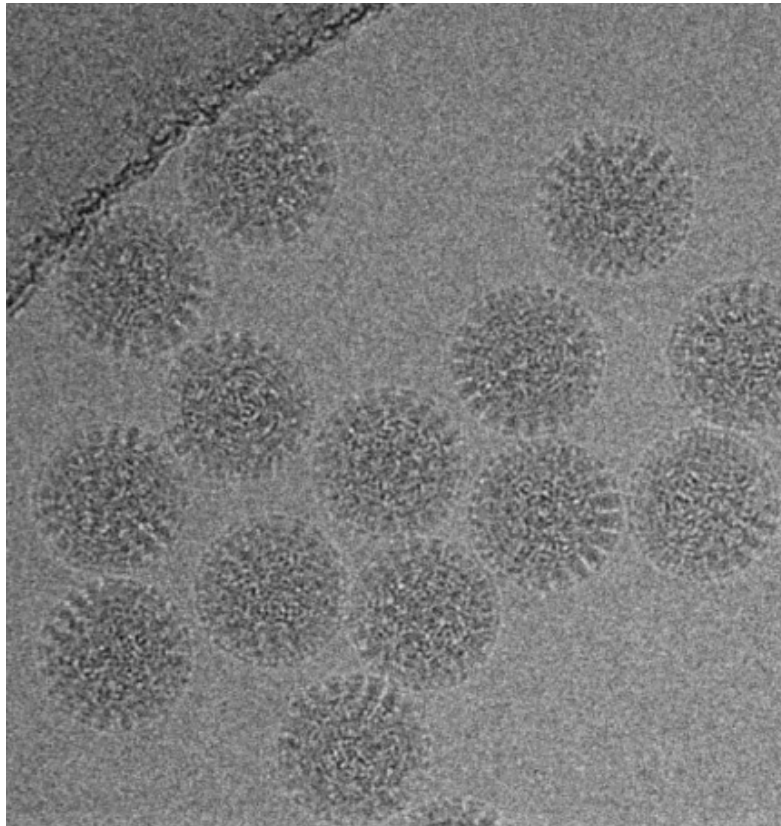
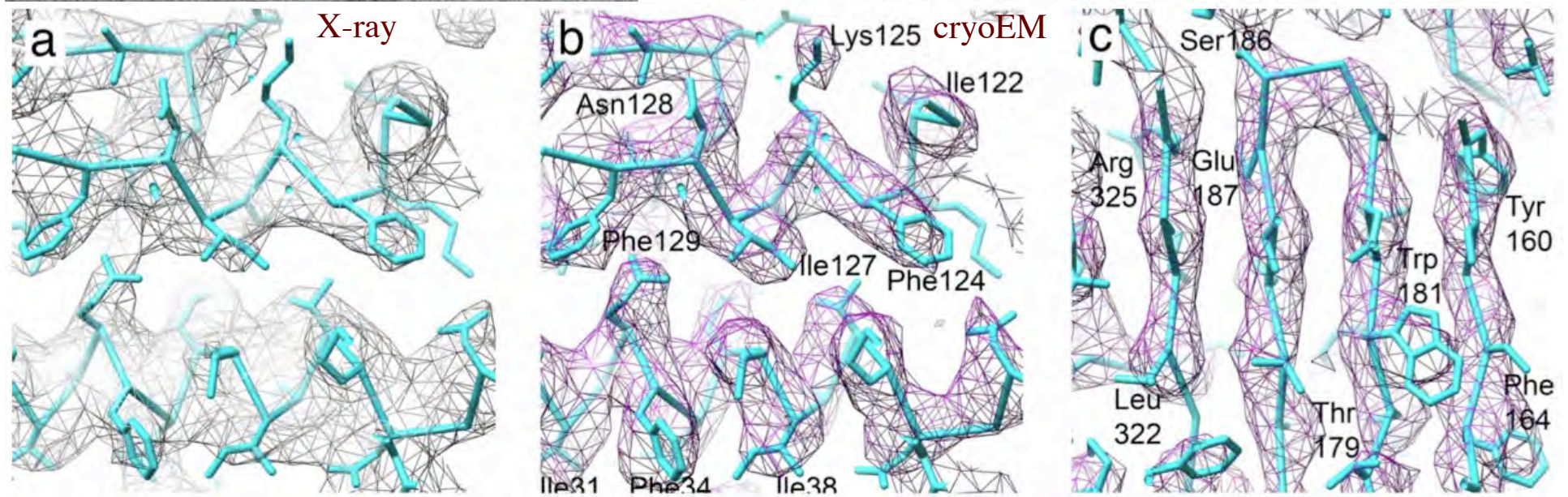
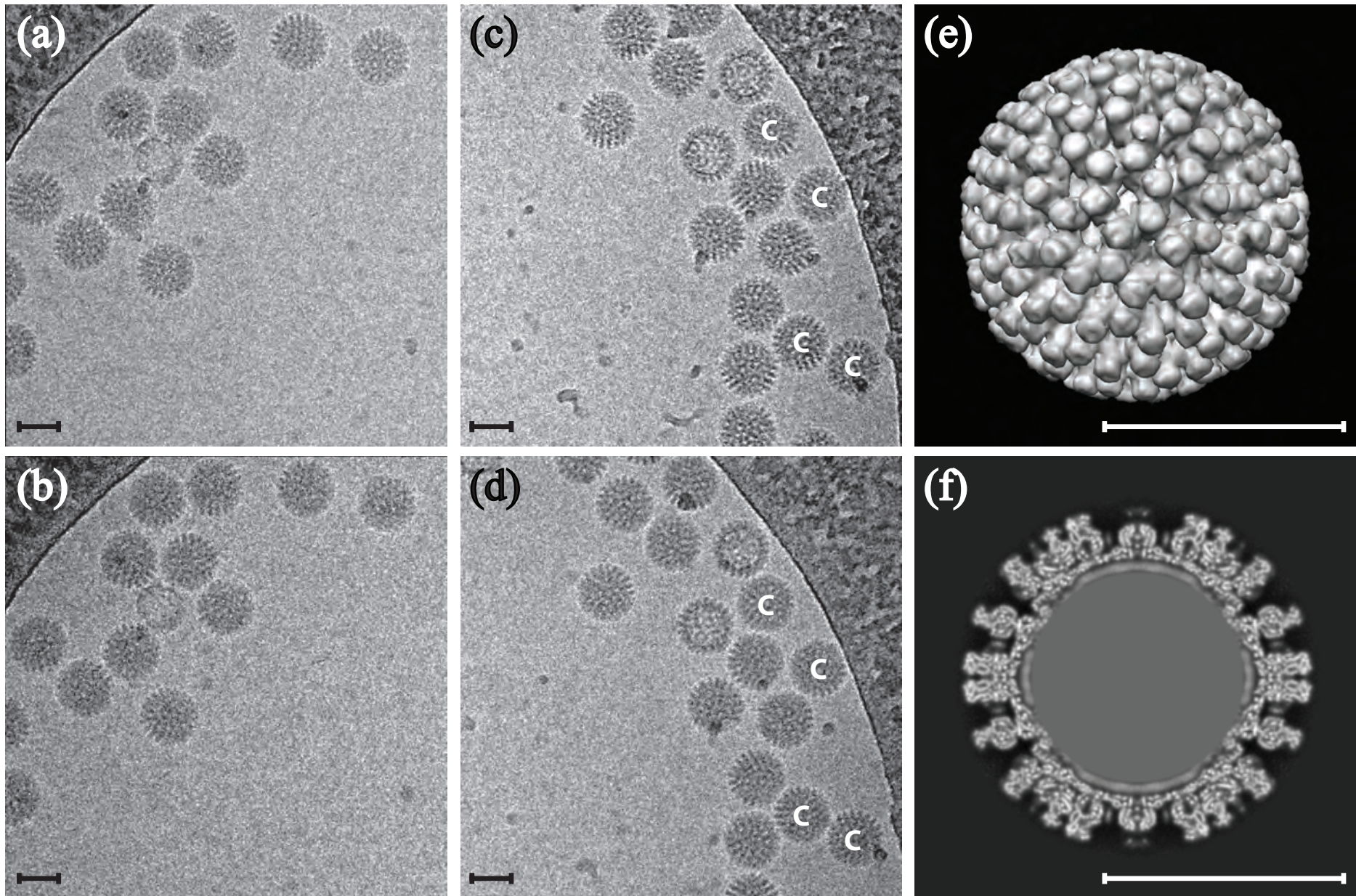


Fig. 4. FSC curves before (black) and after (red) 13-fold nonicosahedral averaging. The black curve suggests a resolution of 5.1 Å (0.143 threshold value), and the red curve indicates a resolution of 4.1 Å.

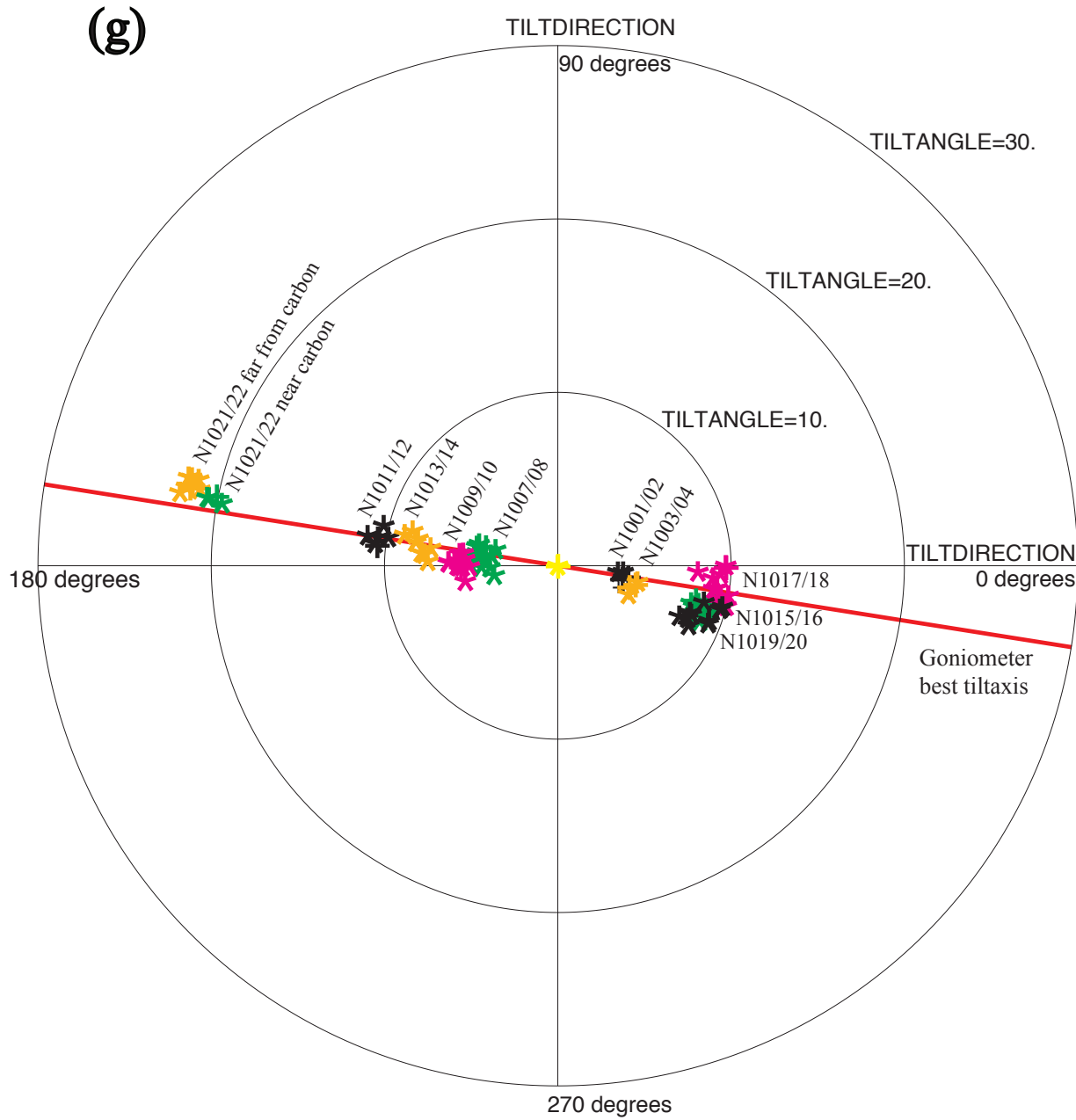


Rotavirus tilt pair images: James Chen & Niko Grigorieff, Brandeis



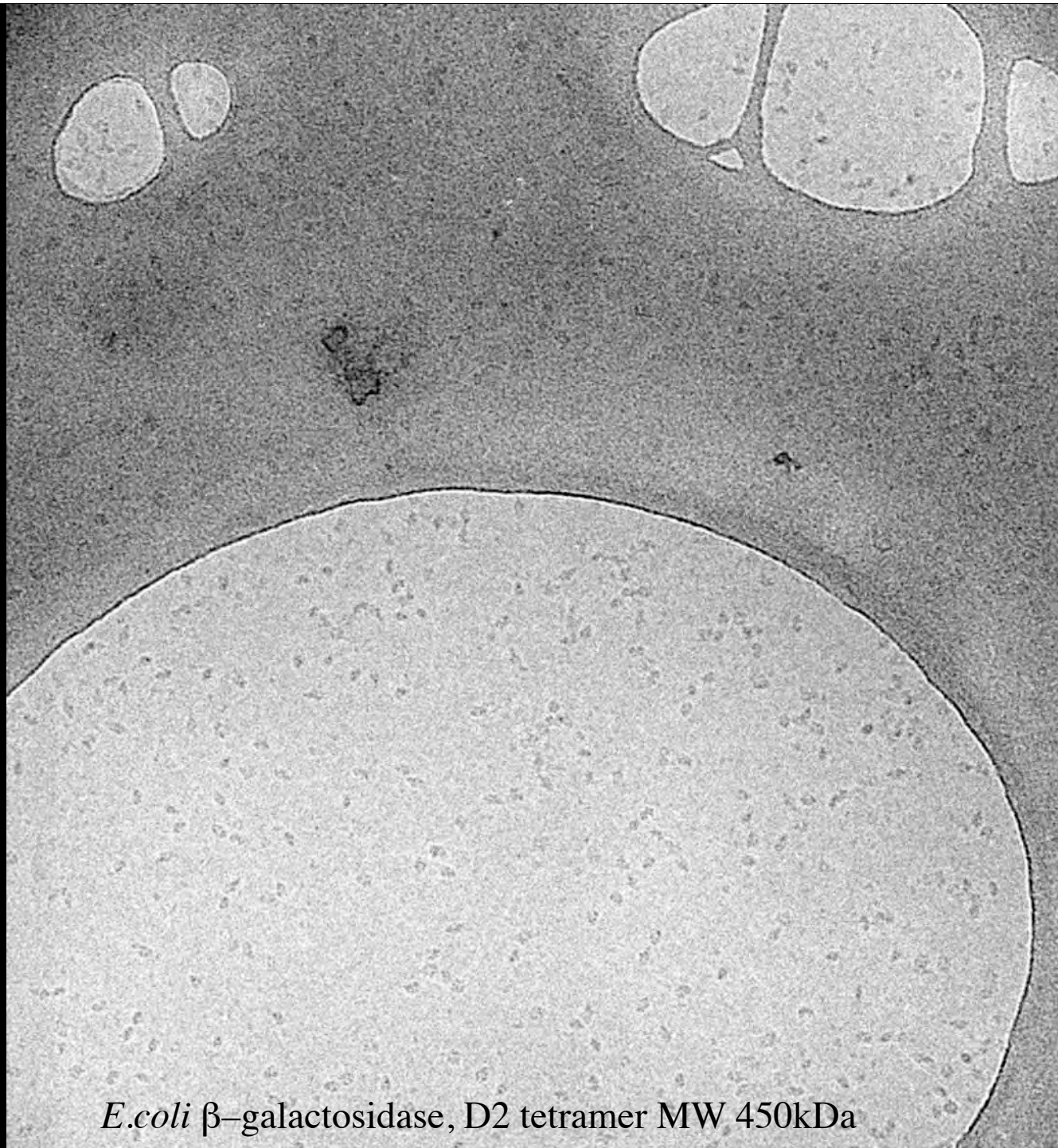
500 Å

Rotavirus: 10 tilt pairs, Chen & Grigorieff



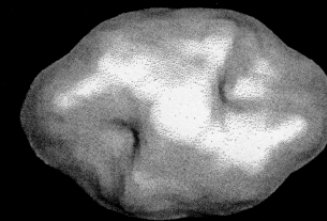
Film pair	<TANG> (sd)	Nom. TANG
N1001/2	+3.83 (± 0.20)	+5.0
N1003/4	+4.50 (± 0.21)	+5.0
N1007/8	-4.24 (± 0.39)	-5.0
N1009/10	-5.67 (± 0.33)	-5.0
N1011/12	-10.4 (± 0.44)	-10.0
N1013/14	-8.07 (± 0.63)	-10.0
N1015/16	+8.67 (± 0.45)	+10.0
N1017/18	+9.34 (± 0.53)	+10.0
N1019/20	+8.83 (± 0.81)	+10.0
N1021/22	-21.14 (± 0.95)	-20.0

Fig.1

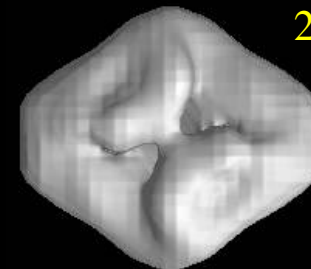


E.coli β -galactosidase, D2 tetramer MW 450kDa

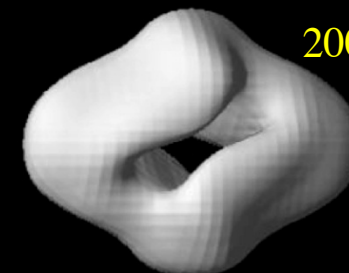
1997



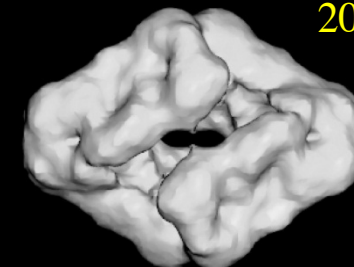
2002



2005



2011



Shaoxia Chen: LMB Cambridge

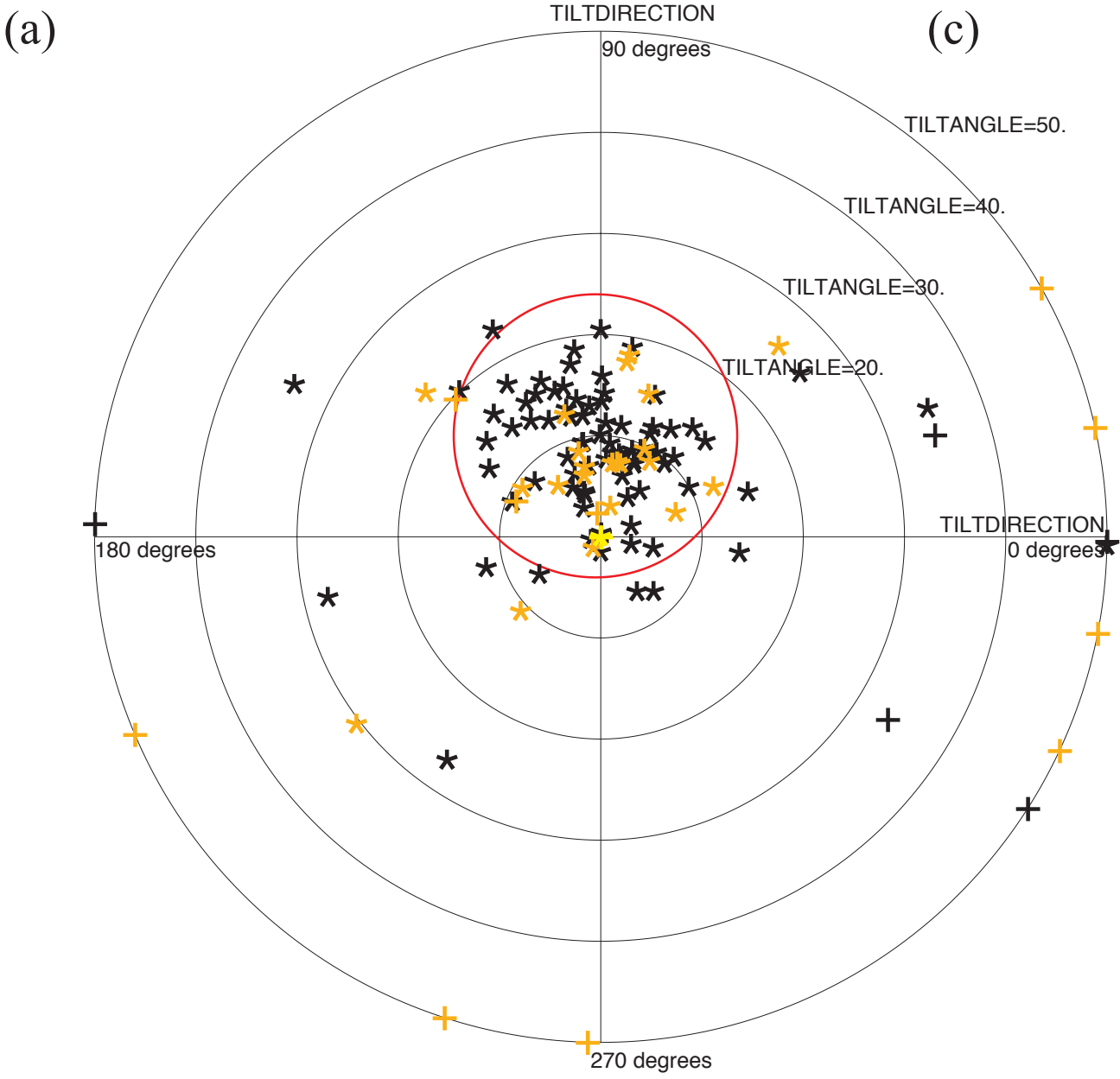
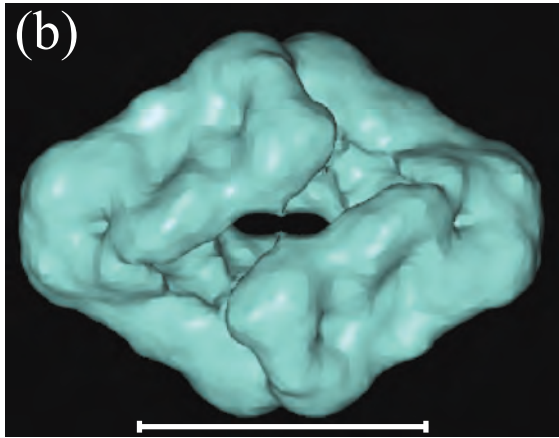
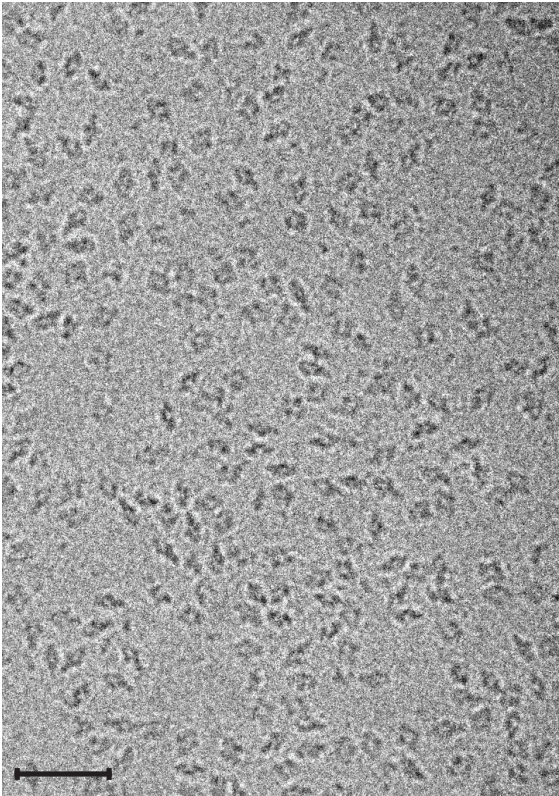


Fig.2

Table 2 - Overview of tilt pair statistics

Specimen	Symmetry	Particle size	Molecular Weight	Number of tilt pairs	Number of particles	Successful alignment (%)	Mean/maximum angular error (degs)	
Rotavirus DLP	I2	700 Å	50 <u>MDa</u>	10	95	100/100	0.25	1.0
CAV	I2	255 Å	2.7 <u>MDa</u>	1	45	62/82	2.5	3.5
FAS	D3	260x220 Å	2.6 <u>MDa</u>	2	44	59/95	4.0	6.0
70S ribosomes	C1	270x260 Å	2.6 <u>MDa</u>	12	220	45/75	4.0	5.0
PDH-E2CD	I1	280 Å	1.6 <u>MDa</u>	1	50	62/94	3.0	4.0
<u>Thermus V-ATPase</u>	C1	250x140 Å	0.6 <u>MDa</u>	1	50	54/80	10.0	16.0
Bovine F-ATPase	C1	250x140 Å	0.6 <u>MDa</u>	1	29	52/79	20.0	25.0
<u>DNA-PKcs</u>	C1	150x120 Å	0.47 <u>MDa</u>	14	108	44/81	15.0	17.0
<u>β-galactosidase</u>	D2	<u>180x130x95 Å</u>	0.45 <u>MDa</u>	2	119	74/91	10.0	14.0

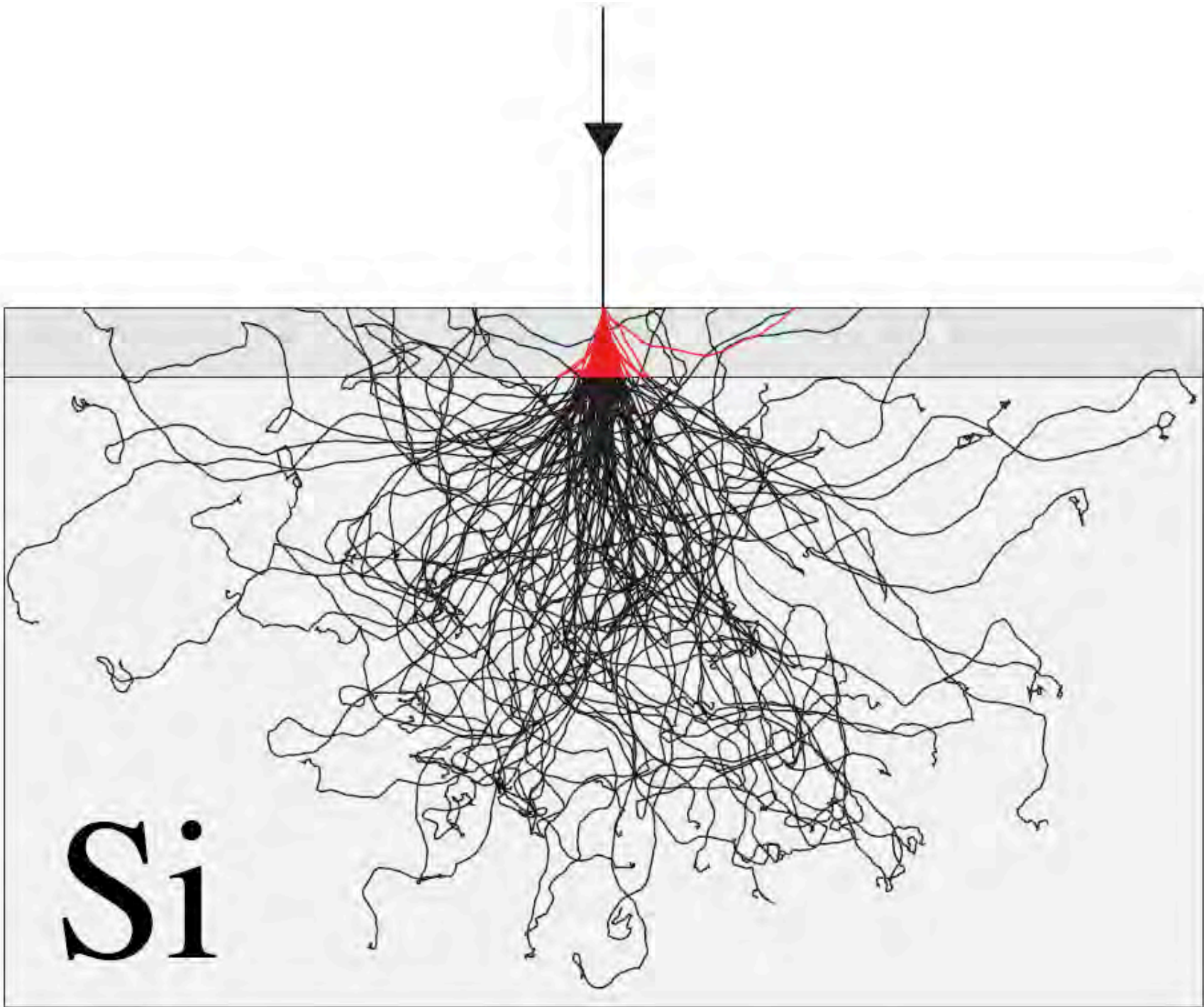
Table 3 – Effect of angular accuracy on $B_{\text{computation}}$ and map resolution

Particle size (diameter / MW)	Angular orientation error (degrees)				
	0.5	2.0	5.0	10.0	20.0
150 Å / 500 kDa	3	40	250	1000	4000
250 Å / 3MDa	7	110	700	2800	11000
700 Å / 50 MDa	55	900	5600	>20000	>80000

Angular error is translated into an apparent B-factor due to computational blurring of the 3D map, using the formula $B = (\Delta\theta \cdot D)^2 / 2200$, where $\Delta\theta$ is the orientation error in degrees and D is the particle diameter in Å. In previously published work, B-factors of $B=1000$ have given 8.7 Å resolution maps¹⁹, 750 gave 7.0 Å resolution³ and $B=240$ gave 3.3 Å⁹, though other factors including the number of particles in the dataset are also important. This suggests that a two to three-fold improvement in orientation accuracy would allow structures of around 500 kDa to reach near-atomic (~4 Å) resolution, without too many particles being required. The box shadings (white, pale grey, dark grey) represent the likelihood of obtaining high (3-5 Å), medium (6-10 Å) or low (below 12 Å) resolution maps with the given error in orientation angles.

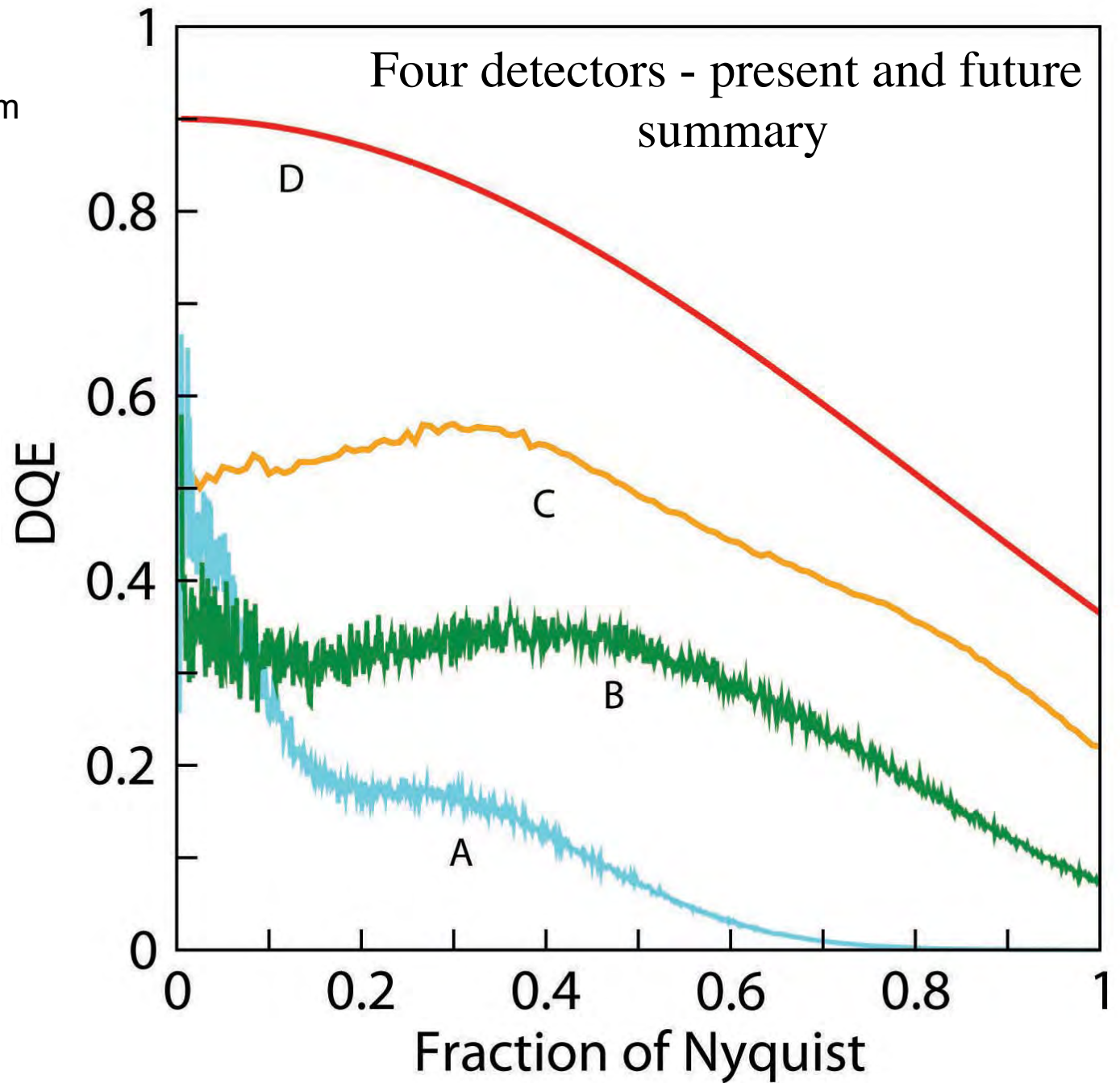
How to improve: solve two remaining problems

- beam-induced movement [evidence from many observations: visible blurring, asymmetric Thon rings, large B-factors, model systems like paraffin, vertical movements observed in tilted 2D crystals, rotavirus ice film doming, liquid helium (Wright et al, 2007) effects larger]
- orientational accuracy [information in images must be increased]



- A Ultrascan 4000 15 μ m
- B SO-163 film 7 μ m
- C Backthinned CMOS
- D Electron counting

D represents the performance of the proposed new electron counting detector



Acknowledgements

- PDH Peter Rosenthal
- CAV Tony Crowther
- Rotavirus James Chen, Niko Grigorieff
- 70S Ribosome Lori Passmore
- DPKase Phoebe Stewart
- FAS Luciano Ciccarelli
- F-type ATPase John Rubinstein
- V-type ATPase Wilson Laue, John Rubinstein
- betaGal Shaoxia Chen
- Detectors Greg McMullan, Wasi Faruqi