Structural Studies on Single Particles and Biomolecules

Janos Hajdu, *Uppsala University*

Gyula Faigel, *Institute for Solid State Physics and Optics, Budapest*

Keith Hodgson, *Stanford Synchrotron Radiation Laboratory*

Chris Jacobsen, *State University of New York at Stony Brook*

Janos Kirz, *State University of New York at Stony Brook*

Gerd Materlik, *HASYLAB, DESY, Hamburg*

John Miao, *Stanford Synchrotron Radiation Laboratory*

Richard Neutze, *Uppsala University*

Carol V. Robinson, *New Chemistry Laboratory, Oxford University*

David Sayre, *State University of New York at Stony Brook*

Abraham Szöke, *Lawrence Livermore National Laboratory*

Edgar Weckert, *HASYLAB, DESY, Hamburg*

David van der Spoel, *Uppsala University*

MYCOPLASMAS

The smallest creatures capable of self-replication

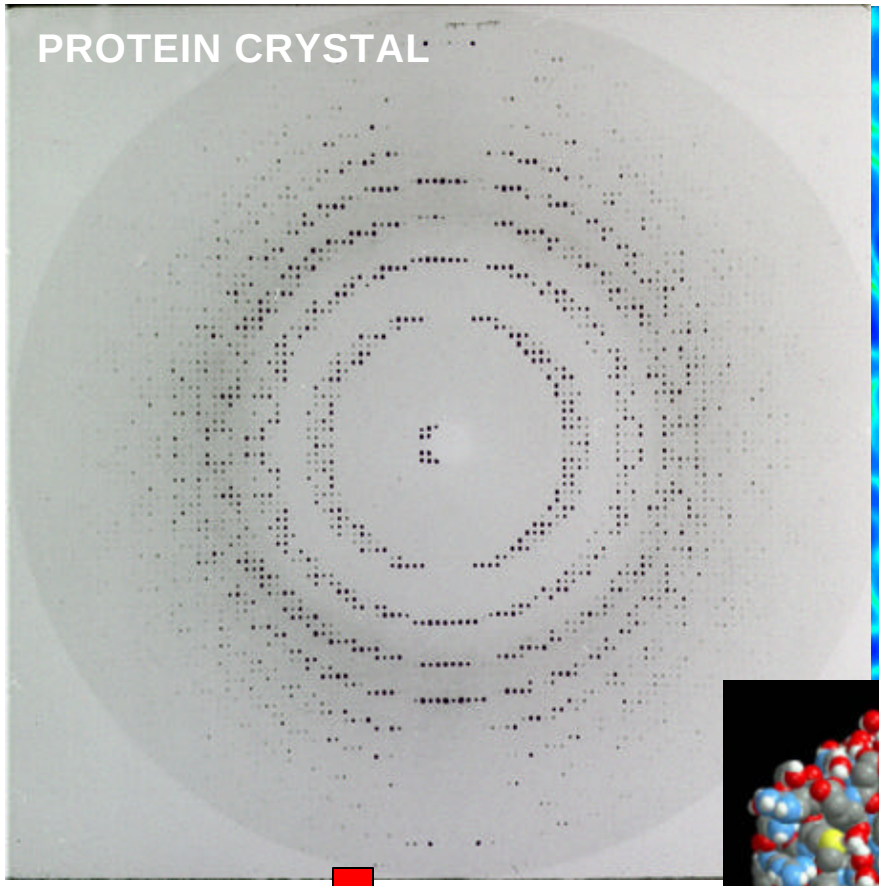
- ~300 nm Ø (cell membrane: 8 nm)
- Solvent content: 60-70%

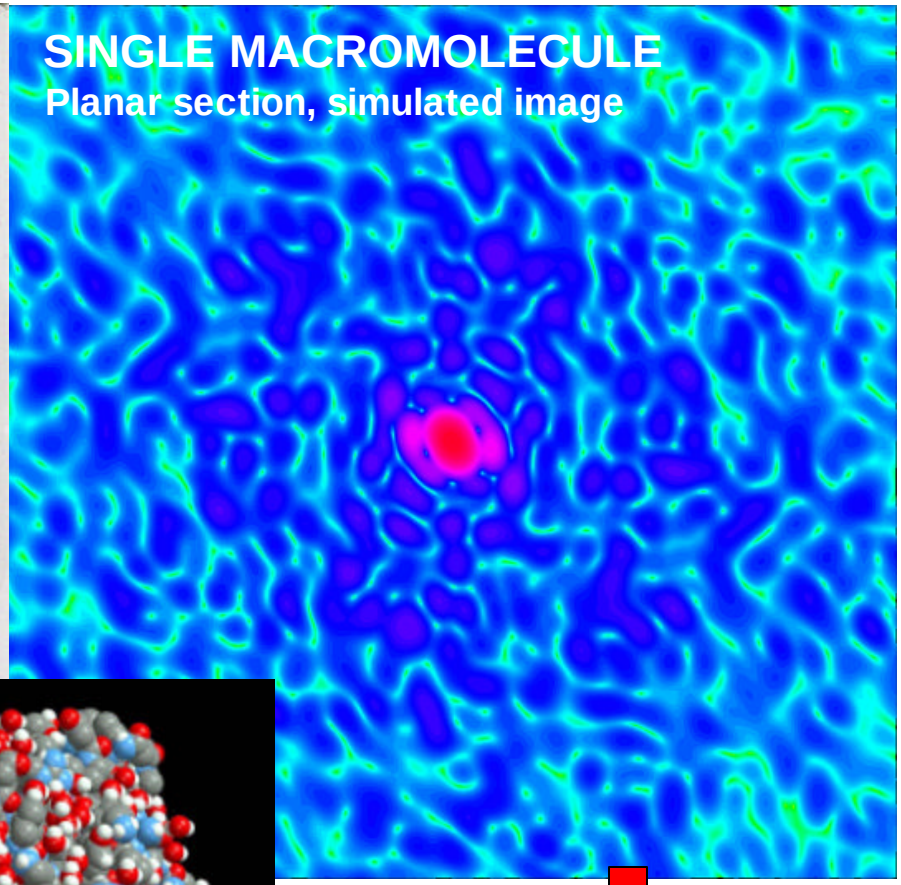
1 DNA (genome size: 600 - 1,300 kbp)
400 ribosomes
10,000 RNA molecules
50,000 protein molecules
400,000,000 water/solute molecules

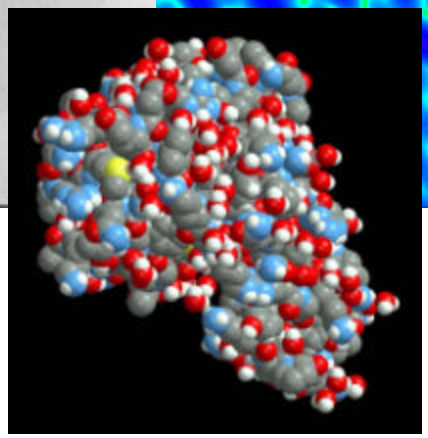
- **Biological samples are highly radiation sensitive**
- **Conventional methods cannot achieve atomic resolution on non- repetitive (or non-reproducible) structures**
- **The limit to damage tolerance is about 200 X-ray photons/Å^2 in crystals (conventional experiments)**
- **The conventional damage barrier can be stretched by very fast imaging**

(Neutze, R., Wouts, R., van der Spoel, D., Weckert, E. Hajdu, J. (2000) *Nature* 406, 752-757)

Scattering by a Crystal and by a Single Molecule







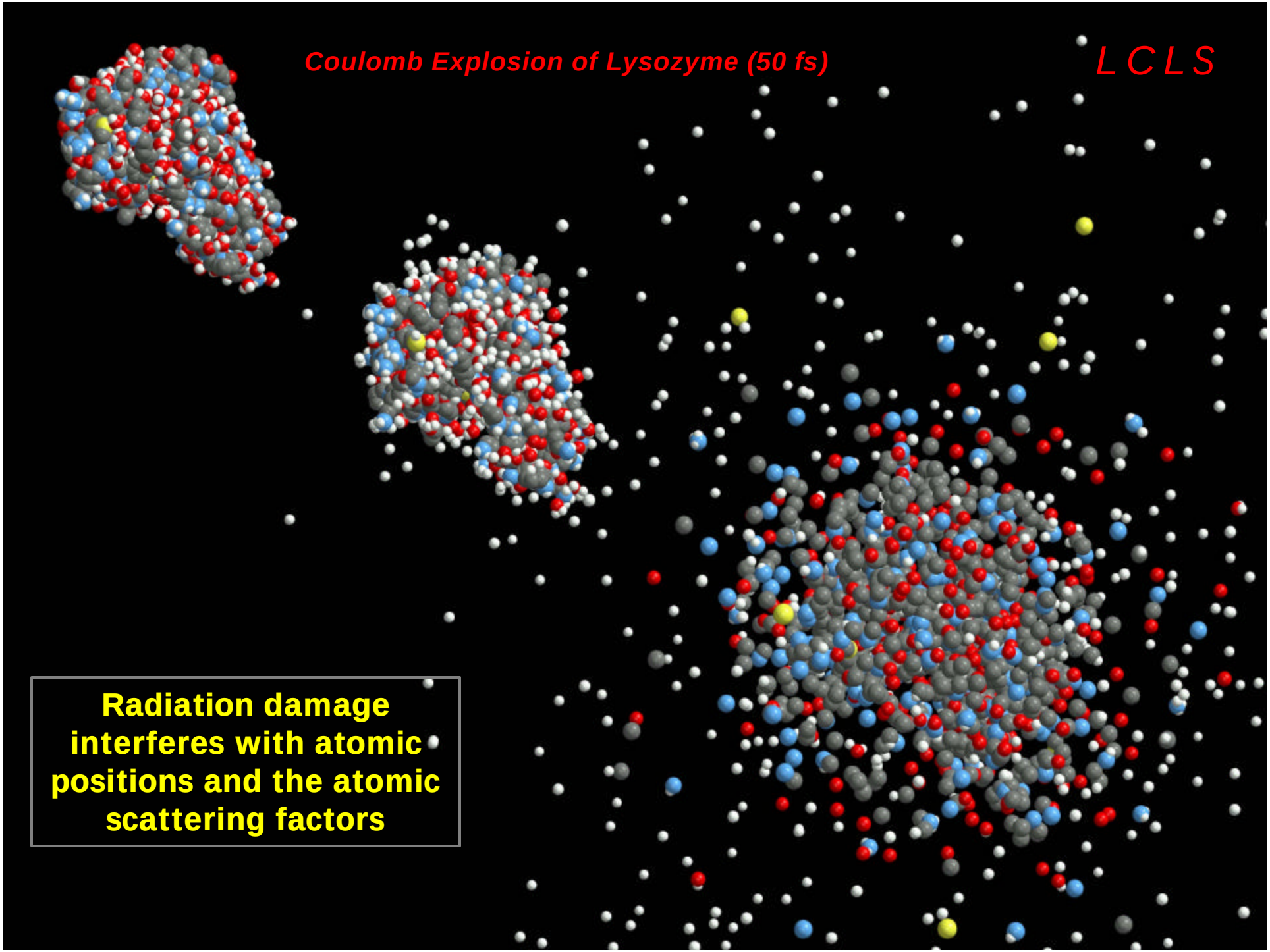
Max. resolution is a function of crystal quality

Max. resolution does not depend on sample quality

LCLS* - *a "never seen regime", for which only predictions and simulations exist

The *LCLS* Beam Interacts with the Matter Through Scattering and Absorption:

- **(1) Photoelectric effect (~90%) followed by Auger emission, shake-up excitations, and interactions between decay channels**
- **(2) Elastic scattering (~7-10%)**
- **(3) Inelastic scattering (~3%)**


Coulomb Explosion of Lysozyme (50 fs)
LCLS
Radiation damage interferes with atomic positions and the atomic scattering factors

XMD interfaced with GROMACS (van der Spoel et al.)

Heating conserving momentum

Bond break through Morse potential

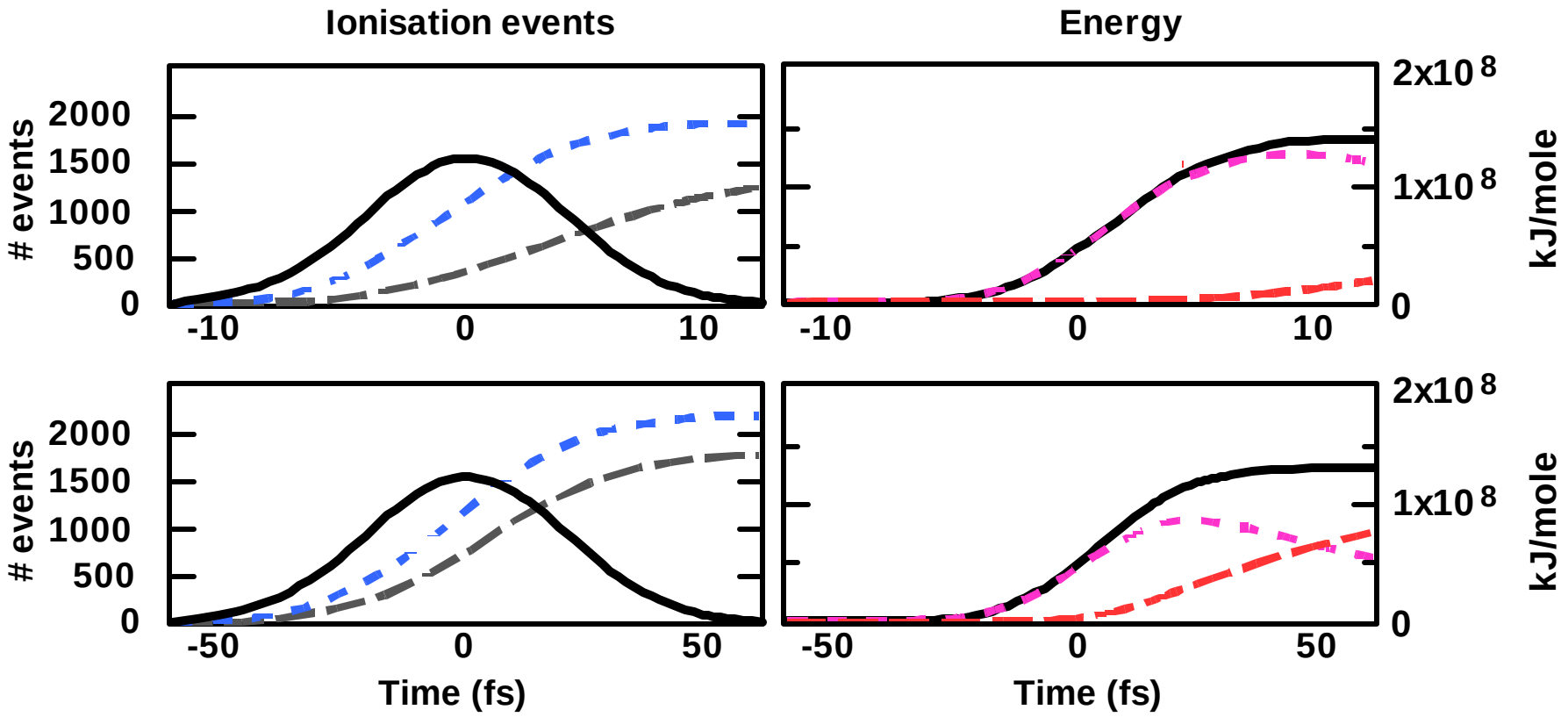
Ionisation primary and secondary effects

Ionisation dynamics calculate changes in the elastic, inelastic and photoelectric cross-sections for each atom during exposure

Inventory kept on all electrons in the sample

(Neutze, R., Wouts, R., van der Spoel, D., Weckert, E. Hajdu, J. (2000) *Nature* 406, 752-757)

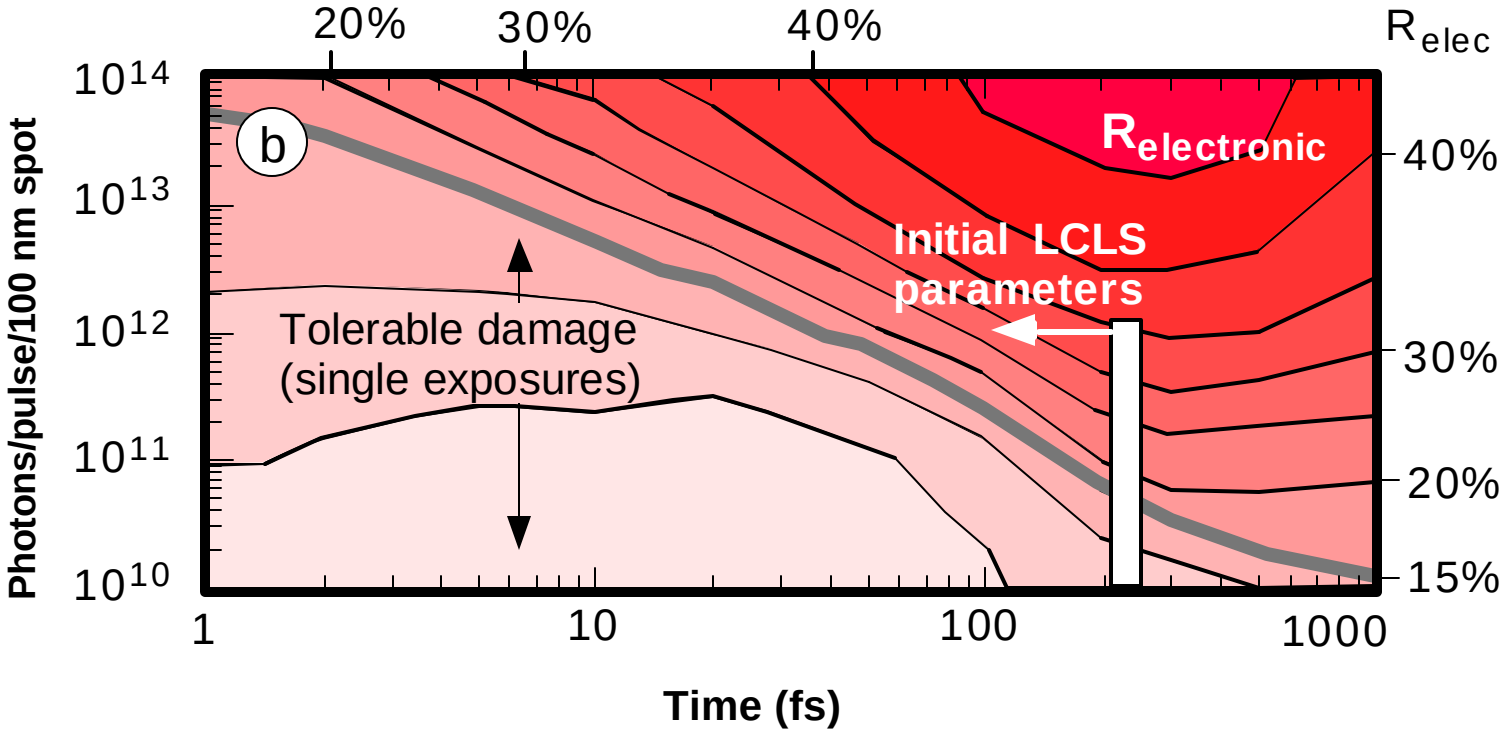
$3x10^{12}$ photons/100 nm diameter spot ($3.8x10^{6}$ photons/Å^2, 12 keV)



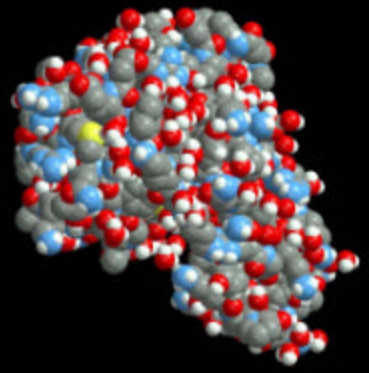
Ionisation and subsequent sample explosion causes diffraction intensities to change

Agreement factor:

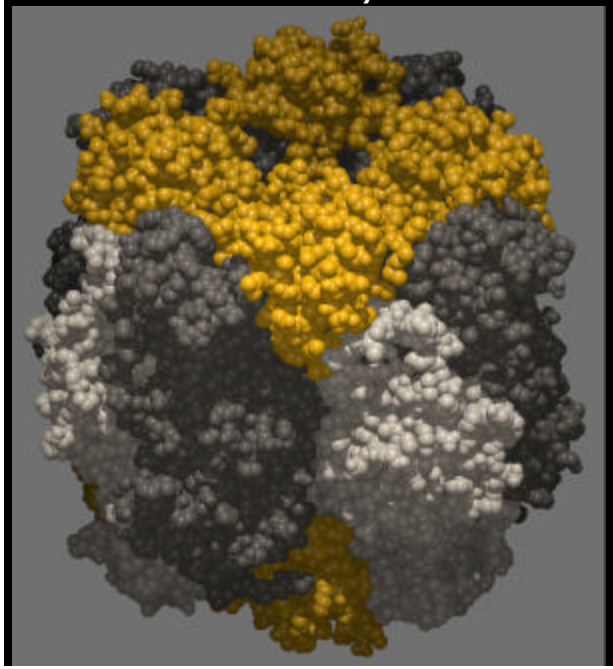
$$R = \frac{\left| \sqrt{I(t)} - \sqrt{I_o} \right|}{\sqrt{I_o}}$$



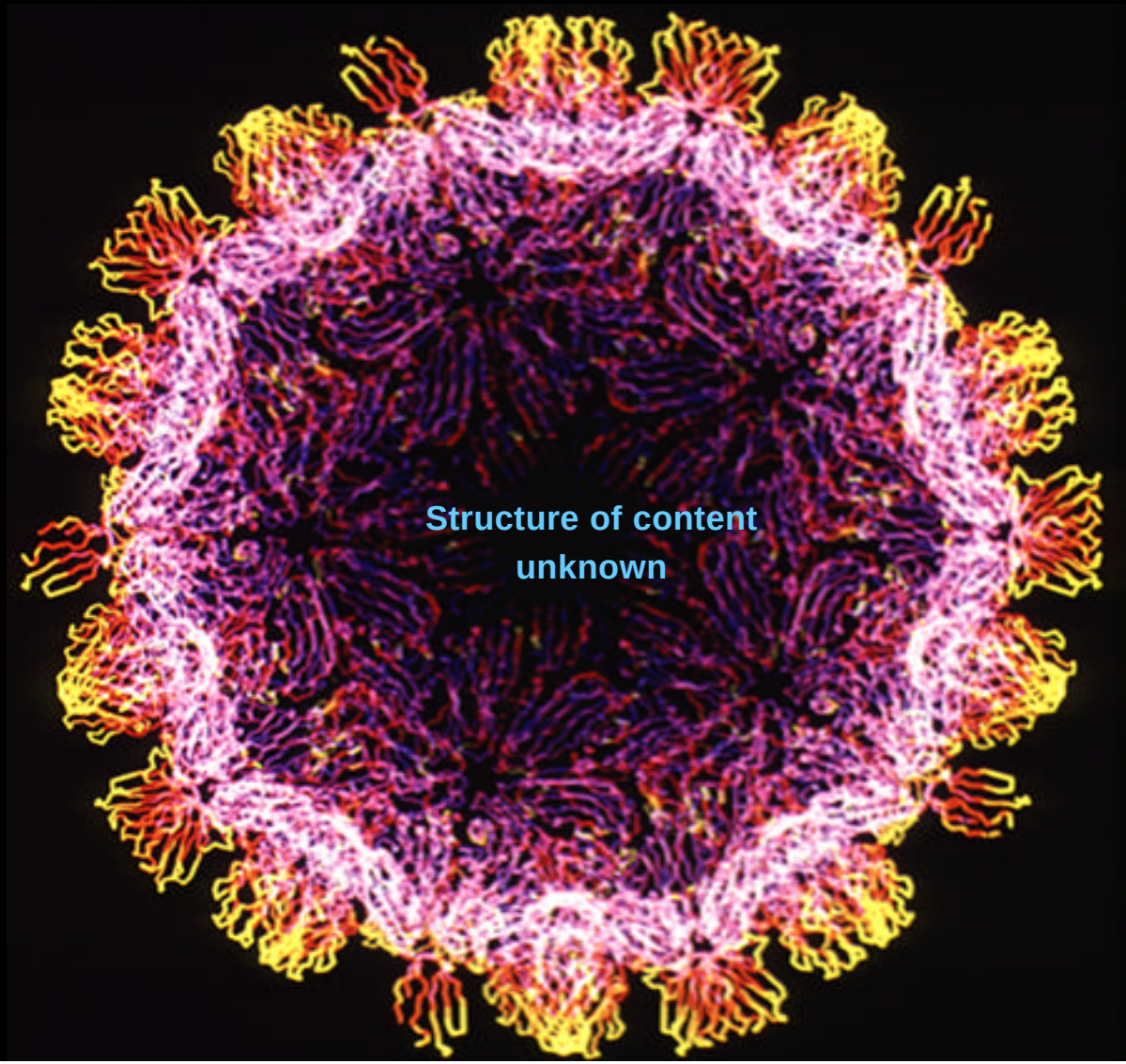
LYSOZYME 19,806 Da



RUBISCO 562,000 Da



HRV ~3,000,000 Da



Calculated Limits of Resolution with $R_{electronic}$ = 15 %

Pulse duration (FWHM)	10 fs	50 fs	100 fs	230 fs
Photons/pulse (100 nm spot) (R = 15%)	$5x10^{12}$	$8x10^{11}$	$3x10^{11}$	$5x10^{10}$
Single lysozyme molecule MW: 19,806	26 Å	30 Å	>30 Å	>30 Å
3x3x3 cluster of lysozymes Total MW: 535,000	<2.0 Å	3.0 Å	6.5 Å	12 Å
Single RUBISCO molecule MW: 562,000	2.6 Å	4.0 Å	20 Å	30 Å
Single viral capsid (TBSV) MW: ~3,000,000	<2.0 Å	<2.0 Å	<2.0 Å	2.4 Å

Single virus particles look very promising

- **Single viral particles - structure of the viral genome**
- **Nanoclusters**
- **Structural kinetics on nanometer-sized samples**
- **Nanocrystals**
- **Two dimensional crystalline arrays**
- **X-ray diffraction tomography of whole cells**
- **X-ray scattering from intact cells**

1. Spraying Techniques

Sample selection and injection

Nanodroplets, Cryogenic Temperatures, High Vacuum

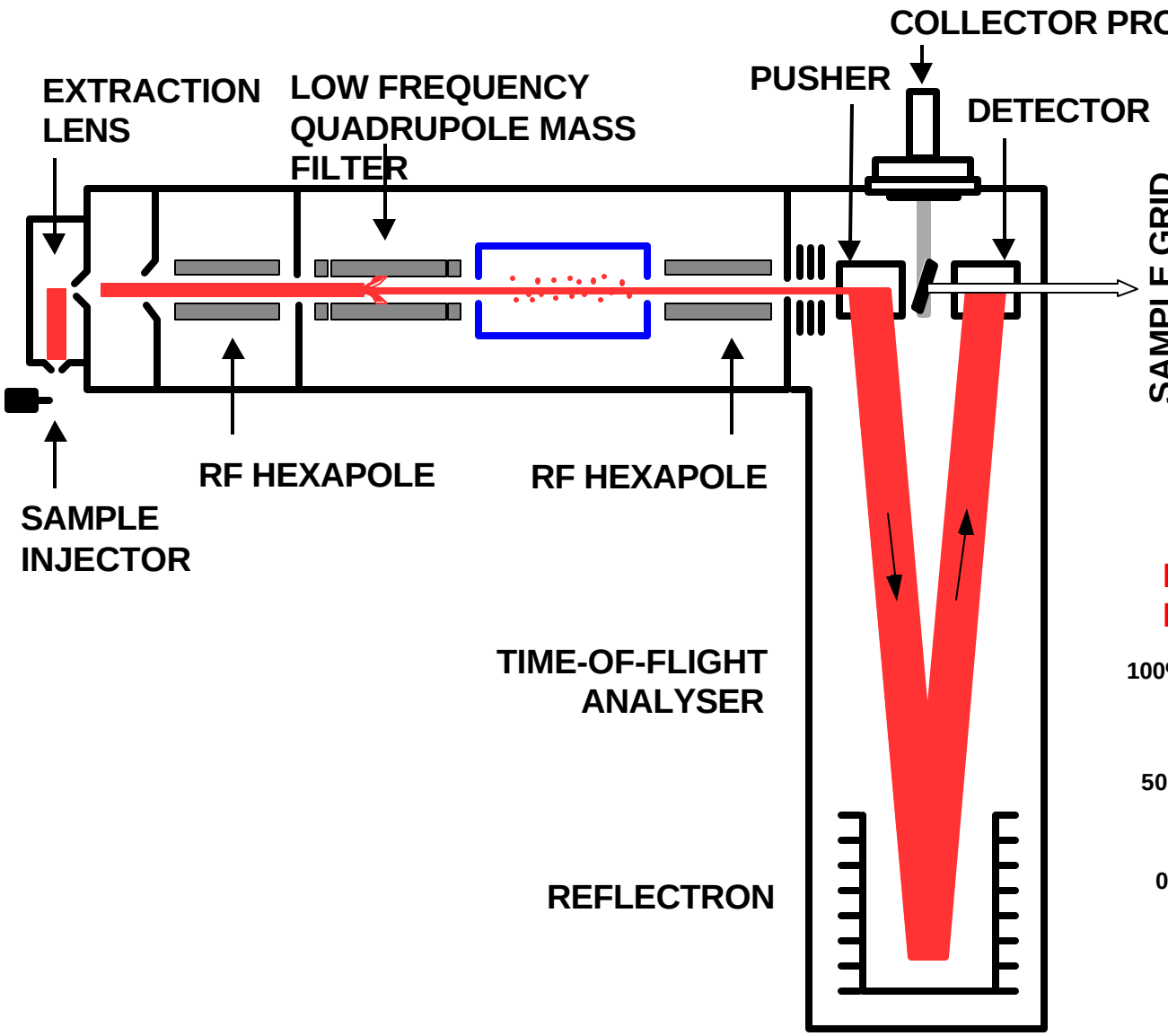
- **Native proteins,**
- **Viruses,**
- **Nanoclusters,**
- **Nanocrystals,**
- **Cell organelles,**
- **Intact cells**

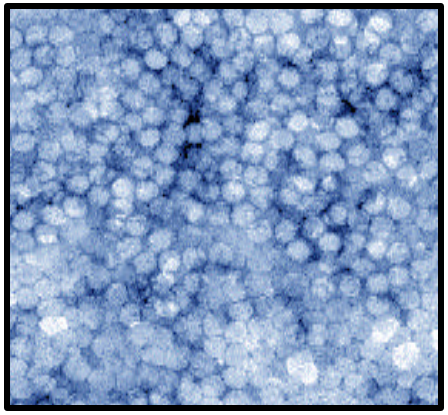
2. Sample embedded in vitreous ice

Goniostat, Cryogenic Temperatures, High Vacuum

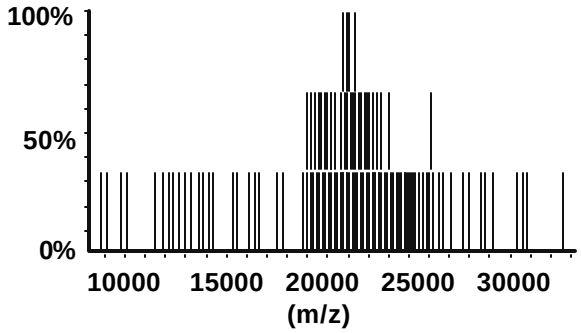
- **Intact cells, cell organelles**

Prototype High Mass Q-TOF Mass Spectrometer



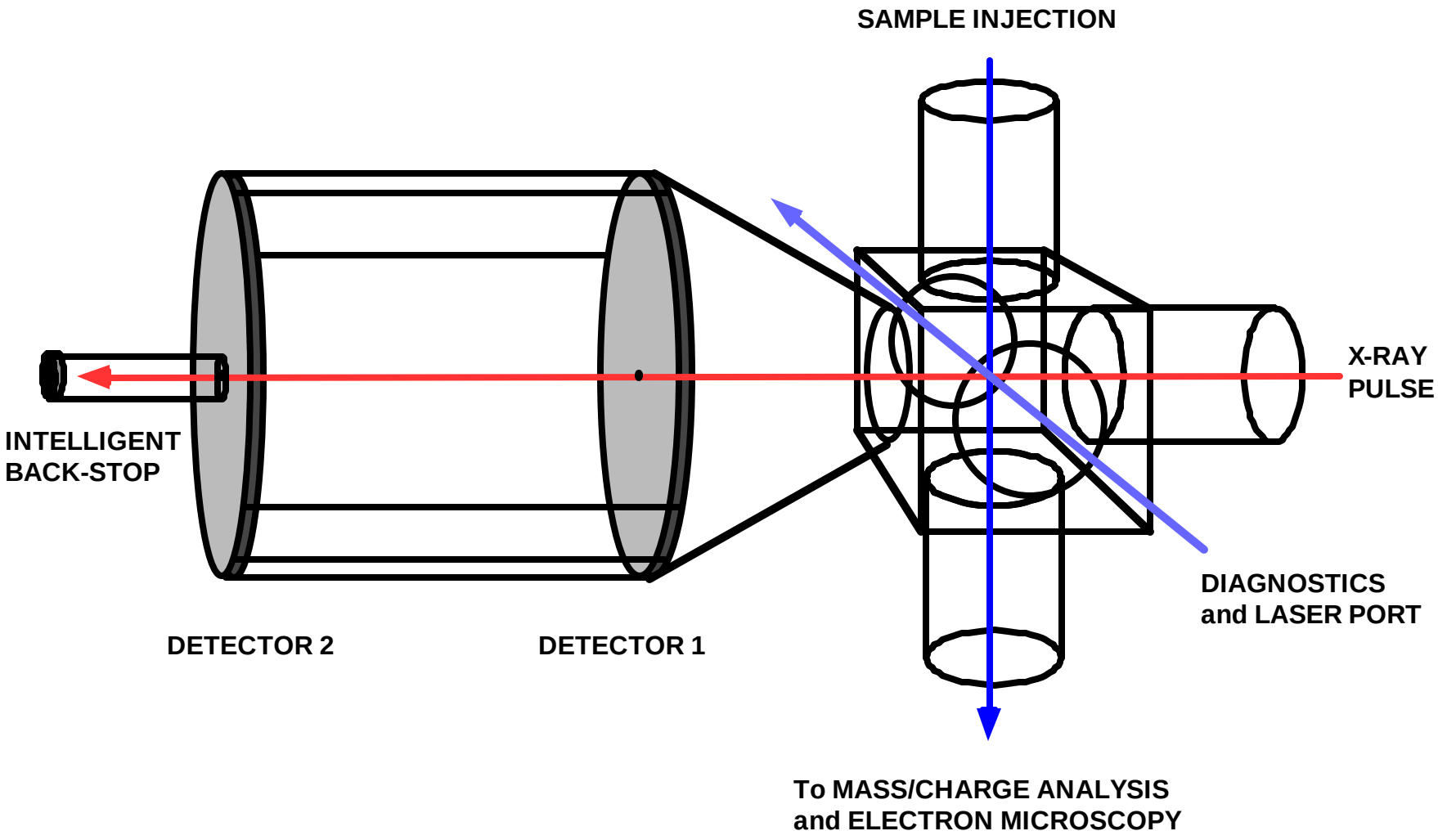


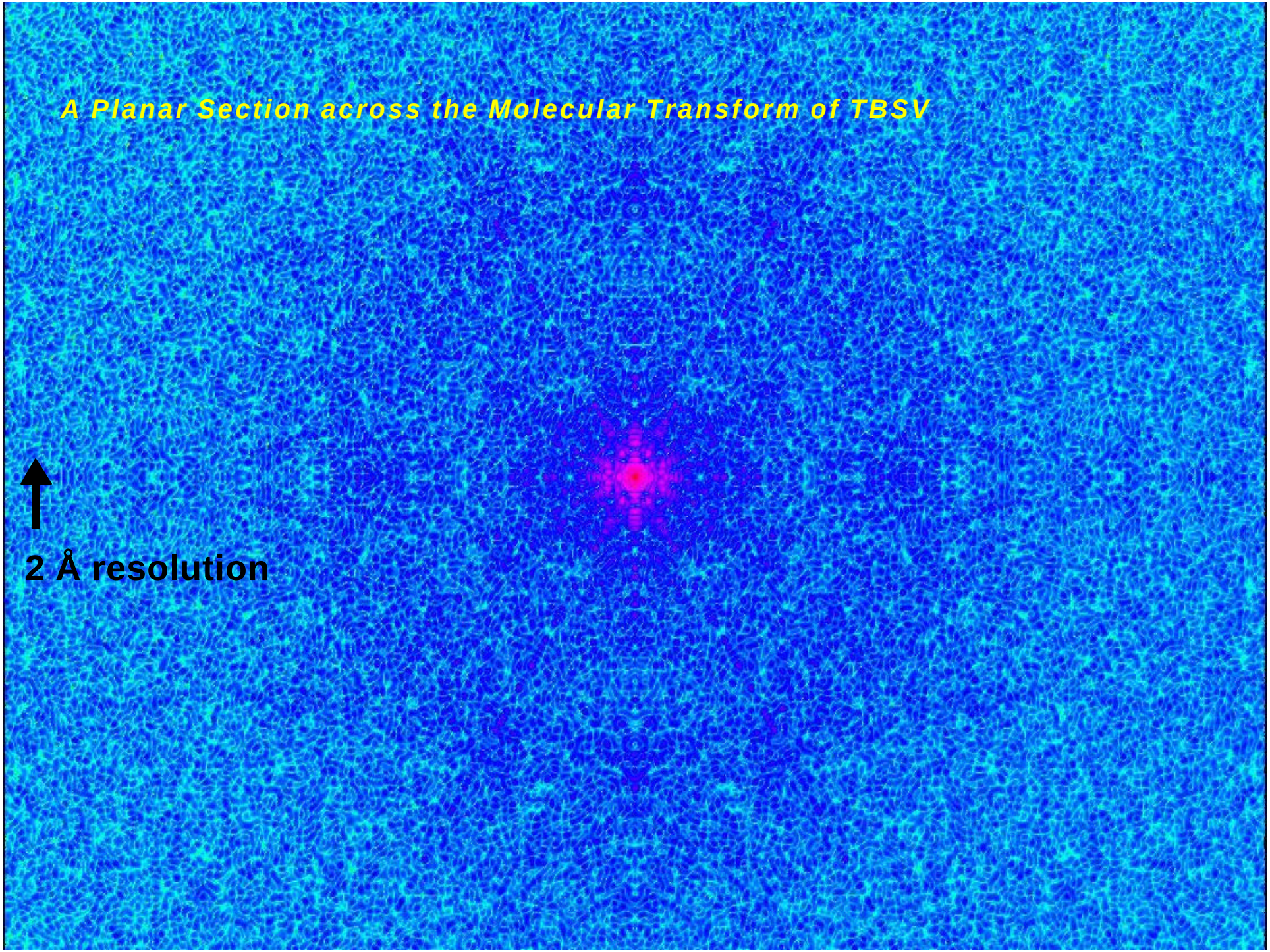
FIRST MASS MEASUREMENT:
MS2 virus: 2,484,700 Da

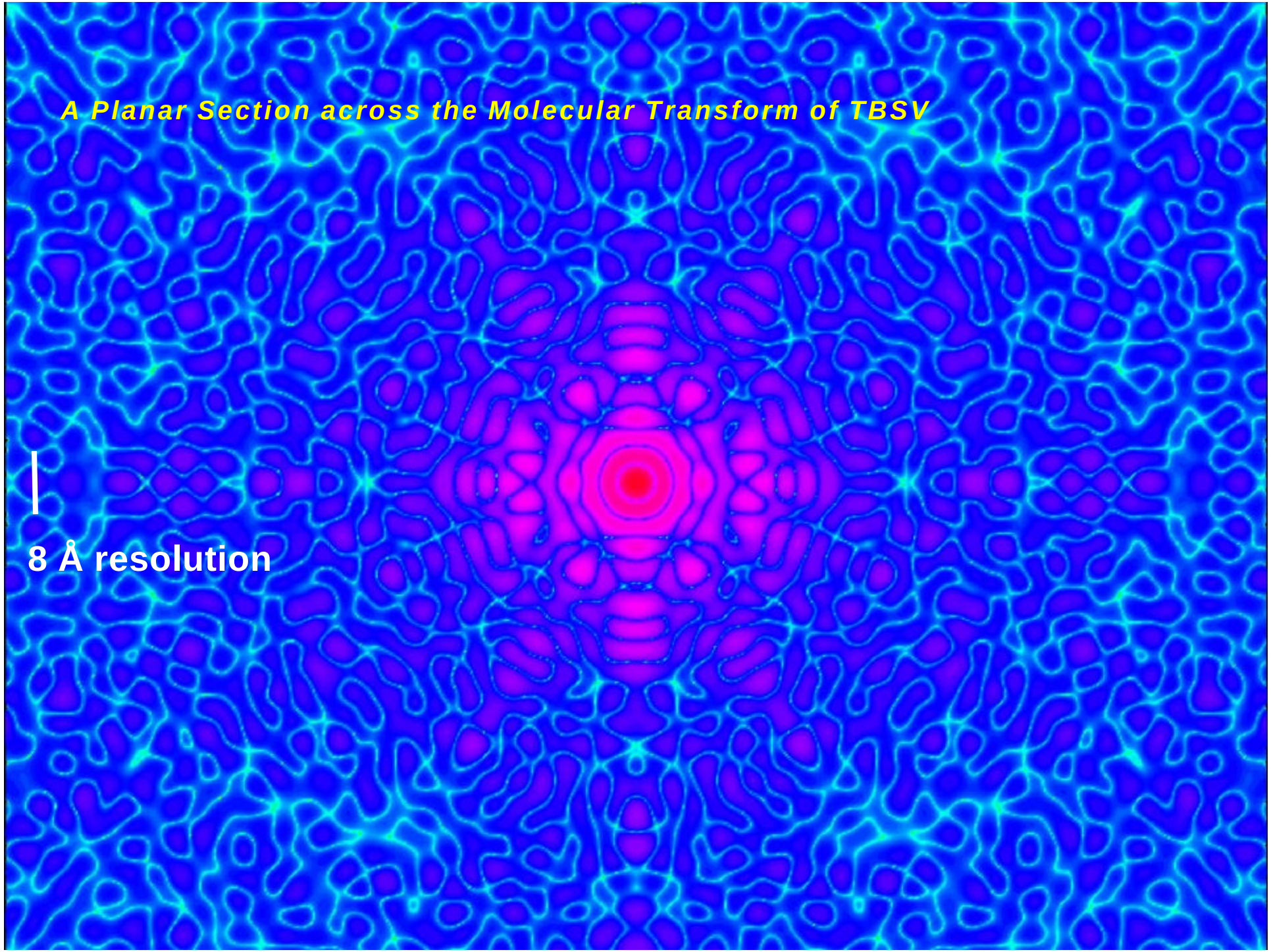


(Tito et al. (2000) *J. Am. Chem. Soc.* 122, 3550-3551)

Interaction Chamber and Detector Arrangement

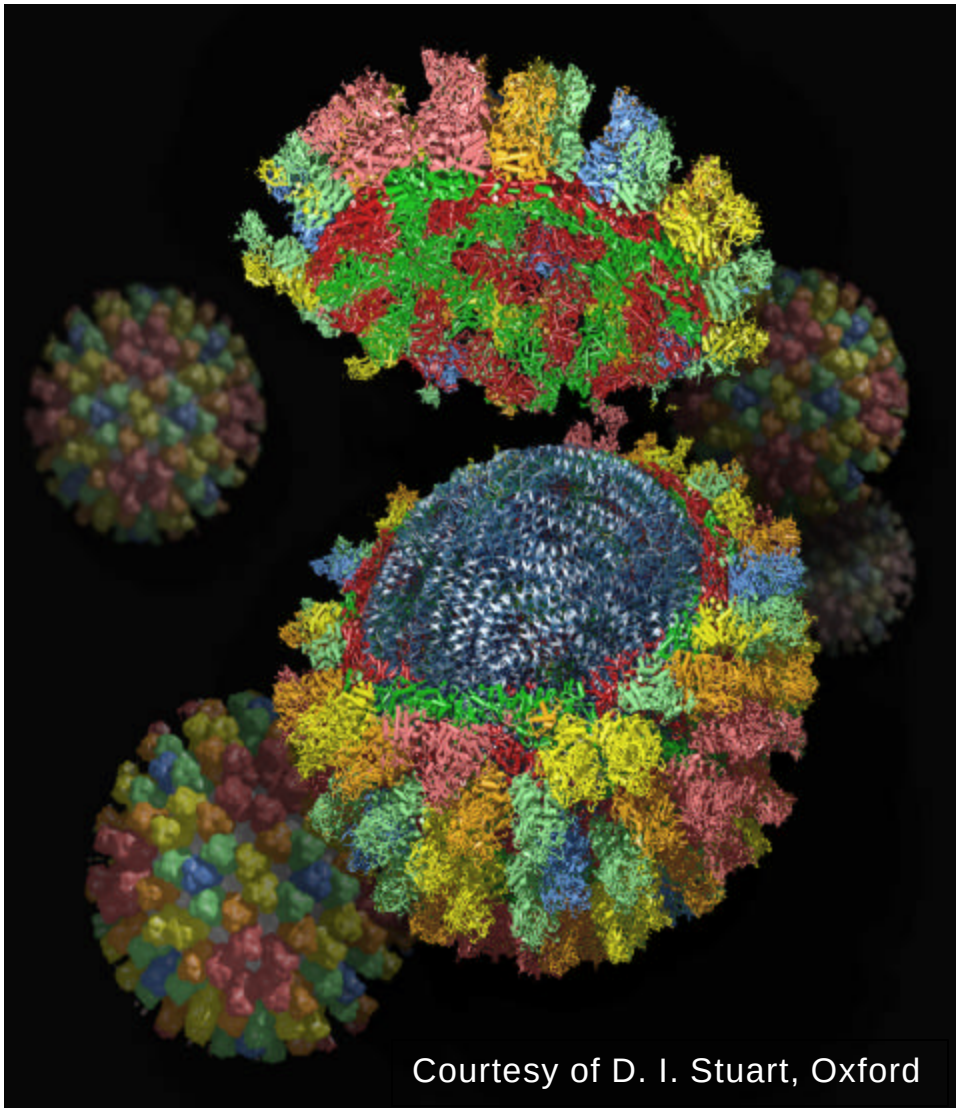



A Planar Section across the Molecular Transform of TBSV
2 Å resolution

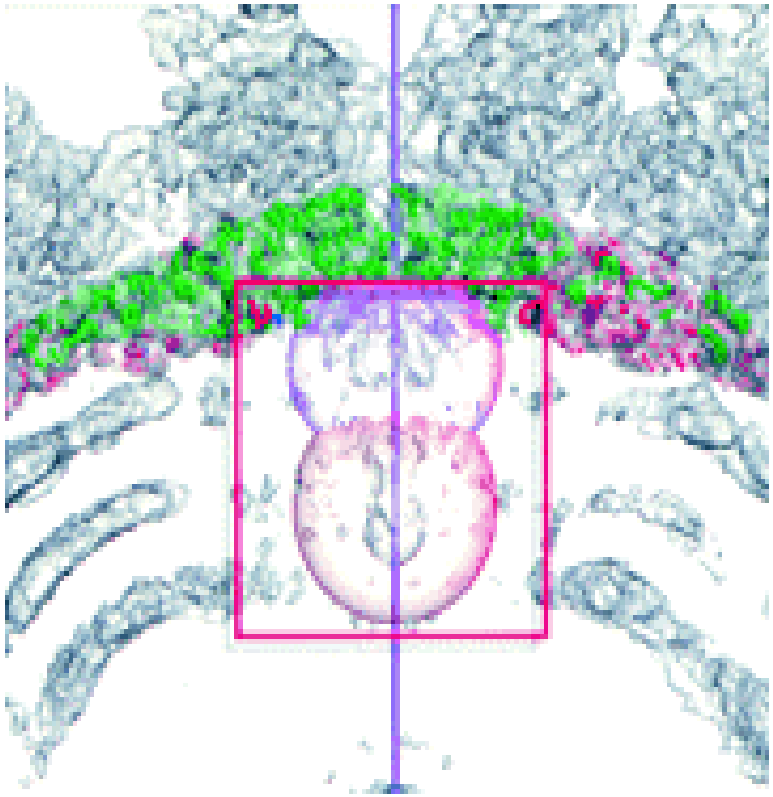

A Planar Section across the Molecular Transform of TBSV
8 Å resolution

- **Continuous molecular transforms (oversampling)**
- **Tools of classical crystallography (these should work with reproducible structures)**
- **Holography**

Artist's view:



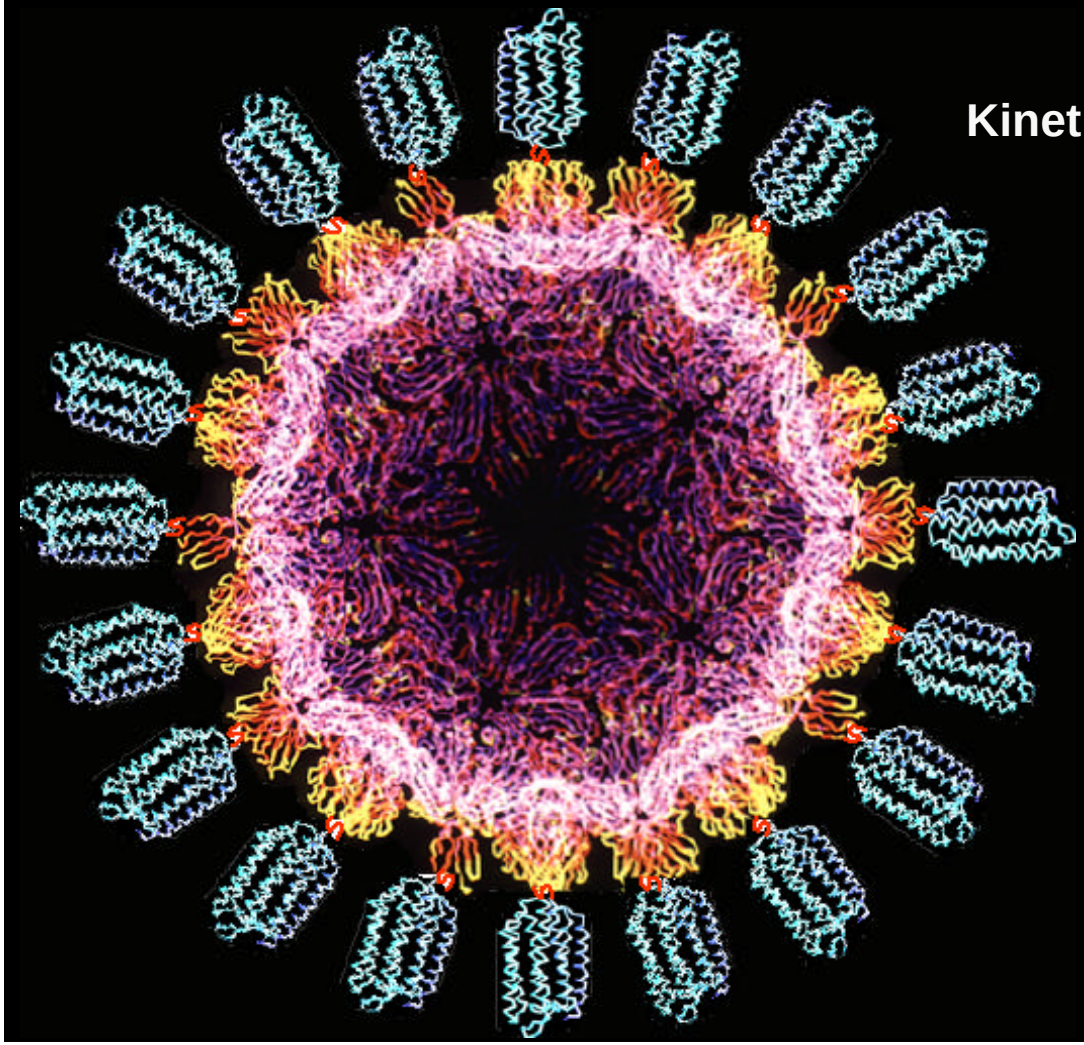
Nucleic acid is released at the right time in the right order



Experiment:

Gouet et al. *Cell* 97, 481-490 (1999)

Most biochemical processes involve diffusion of reactants



Kinetic studies in crystals suffer from "the mixing problem"

Decorated nanoclusters may help to overcome this limitation

Implications for Functional Genomics

MYCOPLASMAS

The smallest creatures capable of self-replication

- ~300 nm Ø (cell membrane: 8 nm)
- Solvent content: 60-70%

1 DNA (genome size: 600 - 1,300 kbp)
400 ribosomes
10,000 RNA molecules
50,000 protein molecules
400,000,000 water/solute molecules