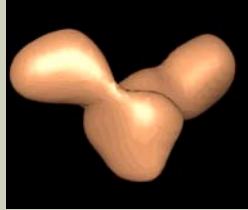


### Electron tomography: theory and applications

Cryo-electron tomography allows 3D imaging of volumes to about 2 nm resolution.

The method is also used to study cells and very large macromolecules.

This show exemplifies the expansion of the cellular cryo-electron tomography method to include also studies of individual protein molecules.



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ET-Group, Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden

### Electron tomography process



Preparation

Microscopy

Reconstruction

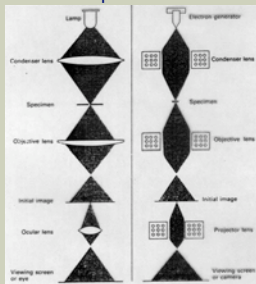
Visualization

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### Similarity light – electron microscope

Light microscope      Electron microscope



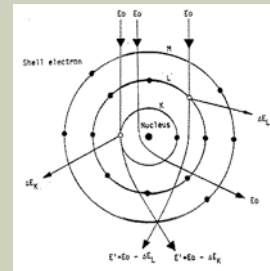
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### Electron scattering

Elastic scattering – without energy loss

Inelastic scattering – energy loss depending on hit electron



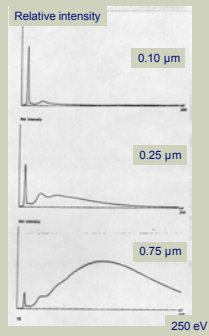
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## Inelastic scattering

### 1. Use thin specimen

The amount of inelastic scattering depends on the specimen thickness

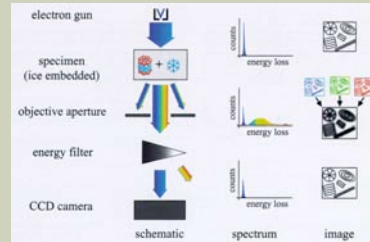


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## Inelastic scattering

Inelastically scattered electrons blur the image.

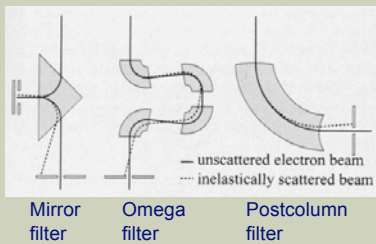


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## Inelastic scattering

### 2. Remove by filter



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## Inelastic scattering

### 3. Increase the acceleration voltage

The 3 MeV Hitachi microscope in Osaka

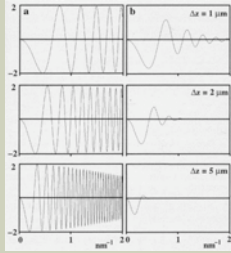


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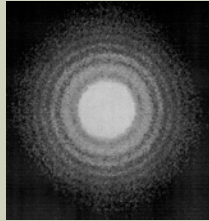
8

## Transfer function

Field emission gun  
LaB<sub>6</sub> filament



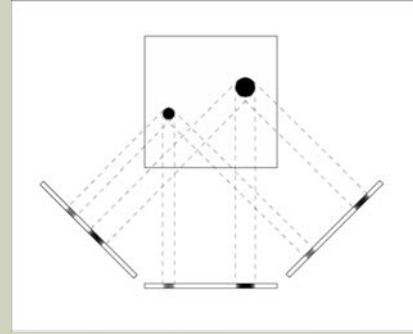
Power spectrum



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## PROJECTION OF AN OBJECT

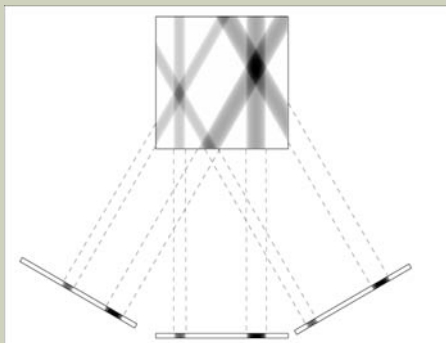


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## "BACKPROJECTION" of PROJECTIONS

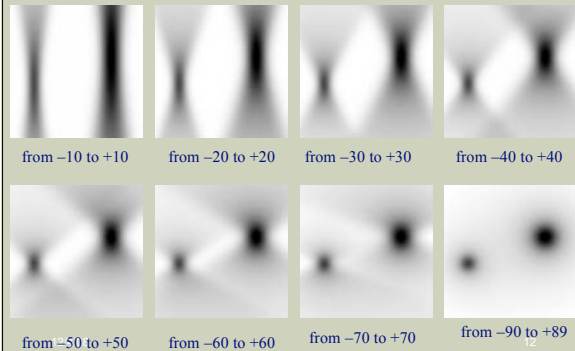


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"Missing data" problem: elongation of the reconstruction in the beam direction due to missing images at high tilts



© Sergei Masch

### A real reconstruction

1 projection at 0 degree

3 projections from -30 to +30 degrees

7 projections from -60 to +60 degrees

13 projections from -60 to +60 degrees

121 projections from -60 to +60 degrees

180 projections from -90 to +89 degrees

© Sergio Alvarez

### Three-dimensional reconstruction

Three-dimensional reconstruction by filtered back projection

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### Principle of 3D-reconstruction

**The Object and its Transform**

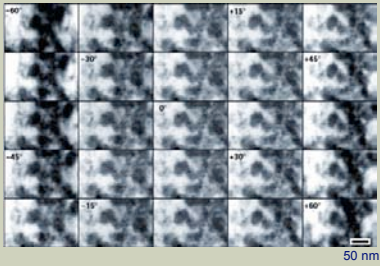
Since all tilt images are projections of objects, they are central sections in the F.T. of the objects.  
 $\pm 90^\circ$  tilts cause a wedge of missing data in the F.T. of the objects.

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### Medium/High-dose data (stained section)

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### Tilt series



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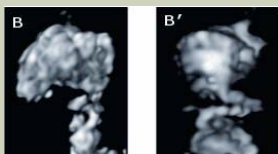
### Three-dimensional reconstruction



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### Three-dimensional reconstruction

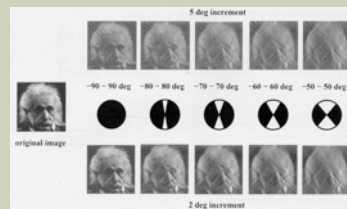


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### Three-dimensional reconstruction

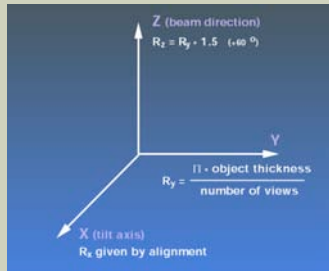
The effect of missing data



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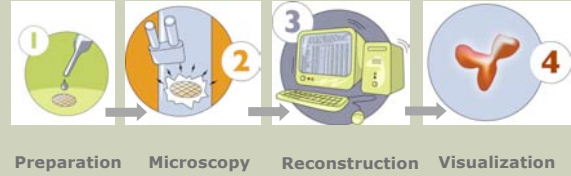
## Resolution



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## Electron tomography process

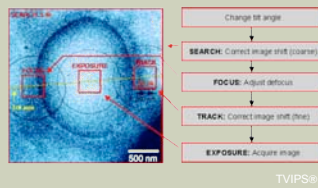


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## Cryo-electron microscopy: data collection strategy

- Automated data collection
- Tilt  $-60^\circ$  to  $+60^\circ$
- Record 121 images
- Total dose  $20 \text{ e}^-/\text{Å}^2$



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## Experimental setup

**Equipment**  
 Philips CM200 FEG  
 TVIPS 2k x 2k CCD camera

**Specimens**  
 Flash-frozen solutions on holey carbon, unstained  
 Cryo-sections, 70 – 100 nm thick, PVA-embedding (Tokuyasu) and light uranyl acetate staining

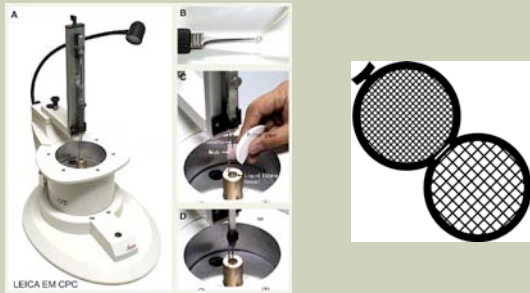
**Single-axis tilt series**  
 Magnification 27000x, pixel size  $\sim 5 \text{ \AA}$   
 Tilt range  $\pm 60^\circ$ , every degree  
 Dose per image 10 - 15  $\text{e}^-/\text{nm}^2$



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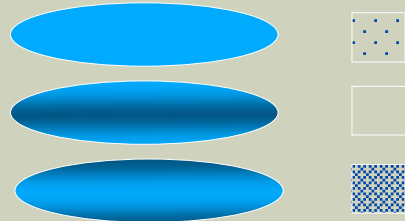
### Proteins in solution



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### From 1 mg/ml solutions, a grid may have tons of protein or none at all

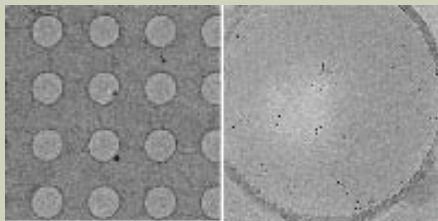


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### Tiny holes in a carbon film on the grid:

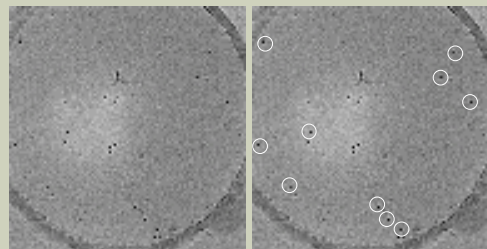
1 hole diameter = 1 micron



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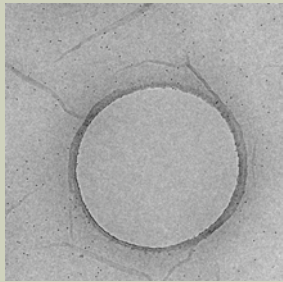
### The alignment gold must be evenly distributed



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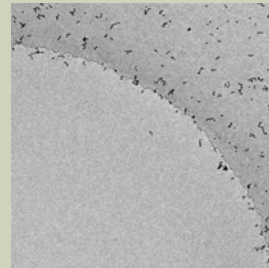
Non-ionic detergent (CHAPS)



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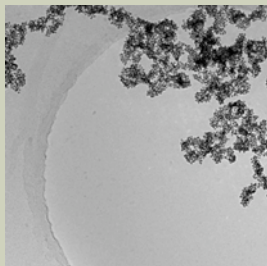
Glycerol



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Low pH (5)



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The number of molecules per volume can be calculated

1 mmole/150,000 mg

1 mg/ml

$6 \times 10^{20}$  molecules/mole

$4 \times 10^{15}$  molecules/ml

$\pi r^2 \times \text{depth}$

$3 \times (5 \times 10^{-5} \text{ cm})^2 \times 10^{-5} \text{ cm}$

$7.5 \times 10^{-14} \text{ ml}$

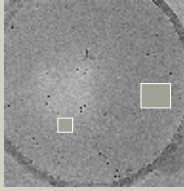
75 zeptoliters

$4 \times 10^{15} \text{ molecules/ml} \times 7.5 \times 10^{-14} \text{ ml} = 300 \text{ molecules}$

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The reconstruction boxes can have different sizes

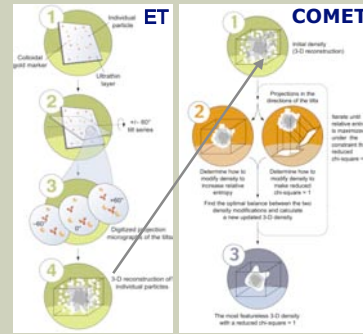


Entire volume 75 zeptoliters  
 Entire contents 300 molecules  
 Reconstruction box about 1%  
 Box contents about 3 molecules

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Electron Tomography and COMET



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Basic equations

The relative entropy function is maximized:

$$S = - \sum_{\vec{X}} \mathbf{F}(\vec{X}) \cdot \ln \left[ \frac{\mathbf{F}(\vec{X})}{\mathbf{m}(\vec{X})} \right]$$

The chi-square merit function is minimized:

$$\chi_i^2 = \sum_{\vec{x}^{(i)}} \frac{1}{\sigma^2} \left[ b^{(i)} \cdot \mathbf{F}_{calc}(\vec{x}^{(i)}) - \mathbf{F}_{obs}(\vec{x}^{(i)}) \right]^2$$

The result is the most featureless density whose projections fit the observations within their variances.

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Basic equation

The iterative solution to the constrained maximum entropy tomography reconstruction can be formulated as

$$F_{new}(\vec{X}) = F_{old}(\vec{X}) + \alpha * u(\vec{X}) + \beta * v(\vec{X})$$

The  $\alpha$  and  $\beta$  is determined by solving a generalised eigenvalue problem making use of the Hessian matrix of the equation system to predict optimal values for the shifts.

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### Invisible structures made visible with COMET

Low-dose tilt series

Filtered back projection

COMET-refined

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### Electron Tomograms of IgG

Filtered Backprojection at high and low cutoff-levels

After Comet Processing

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### Computer programs

<p><u>Programs</u></p> <ul style="list-style-type: none"> <li>• XPIX</li> <li>• REFINE</li> <li>• IMAGE-EXTRACT</li> <li>• BACKPROJ</li> <li>• COMET</li> <li>• BOB, AVS, VMD ...</li> </ul> <p><u>Platforms</u></p> <ul style="list-style-type: none"> <li>• Compaq OSF1</li> <li>• Linux RedHat, SUSE</li> <li>• Mac OSX</li> </ul>	<ul style="list-style-type: none"> <li>Pick marker coordinates</li> <li>Geometry alignment</li> <li>Data extraction</li> <li>Filtered back projection</li> <li>Post refinement</li> <li>Visualization</li> </ul>
---	--

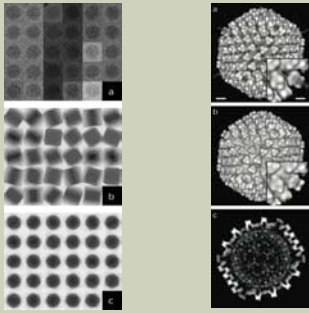
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### COMET refinement

Noise reduction during 10 cycles of refinement of an IgG using COMET

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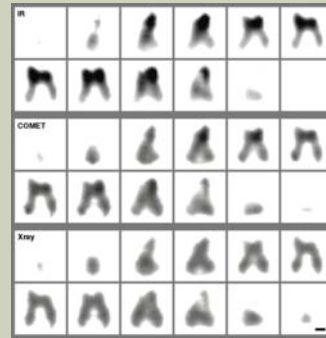
COMET on Adeno virus data



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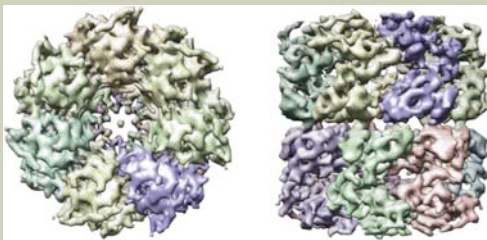
Hexon density improvement with COMET



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GroEL at 6Å



Two rings, each containing 7 identical subunits.

S.J. Ludtke, D.-H. Chen, J.-L. Song, D.T. Chuang, and W. Chiu (2004) Structure 12, 1129-1136

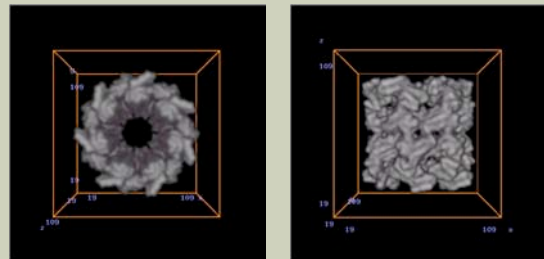
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The GroEL model

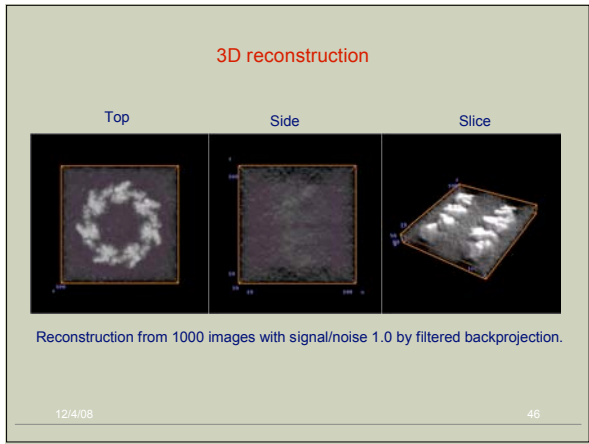
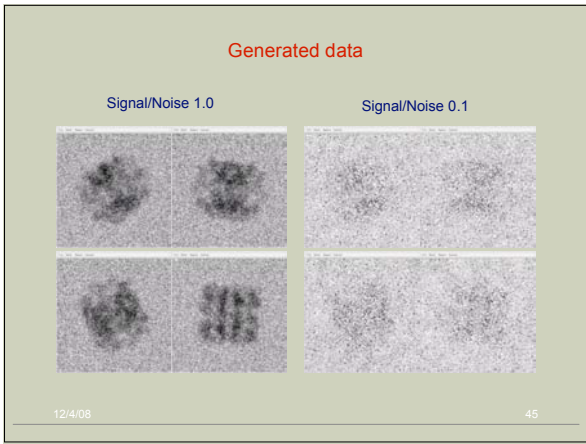
Top view

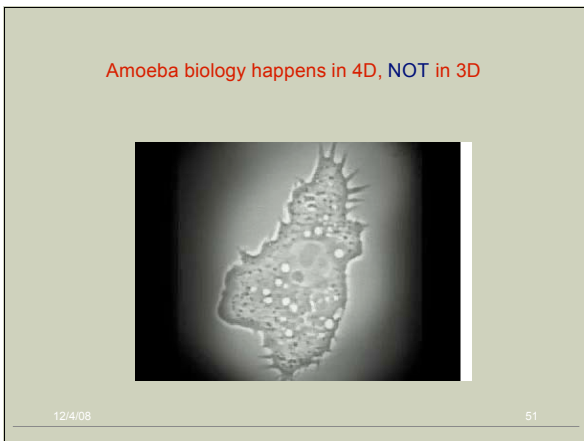
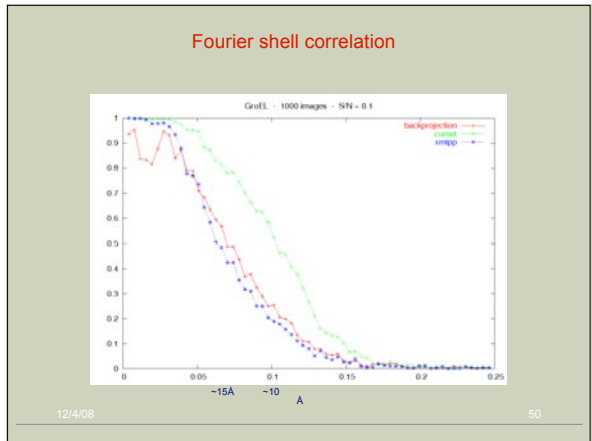
Side view



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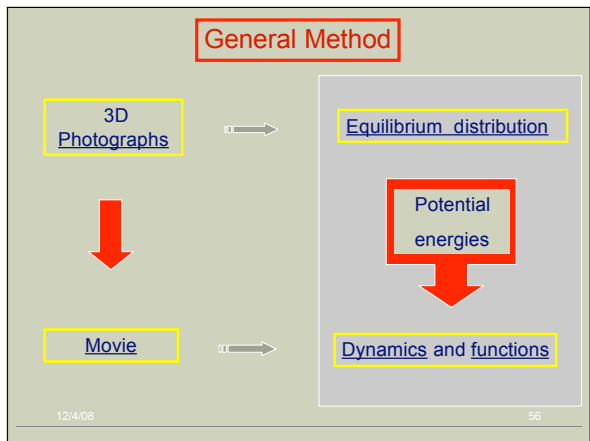
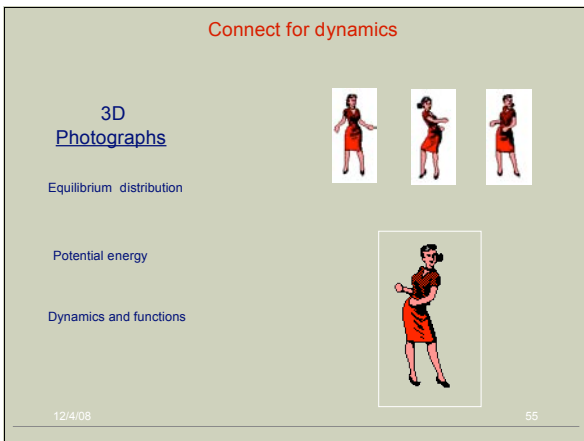
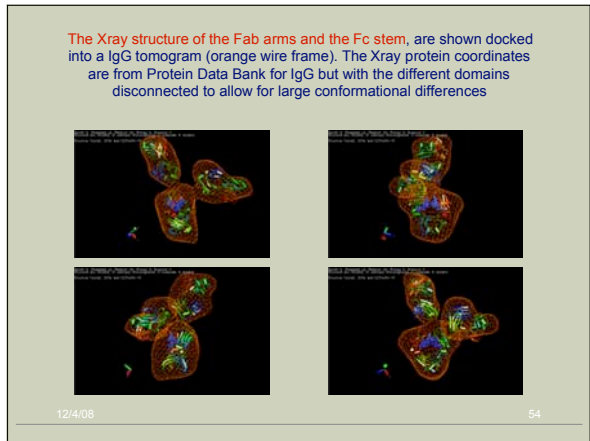
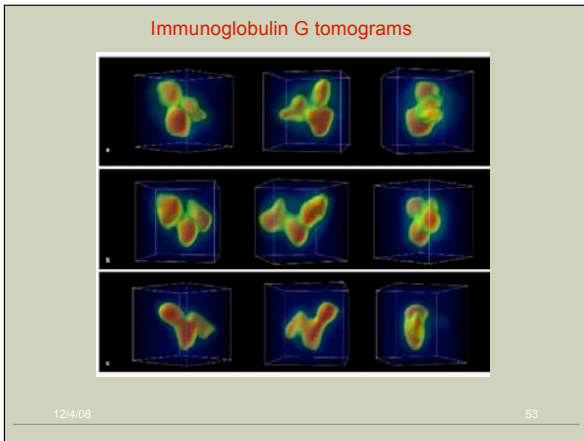




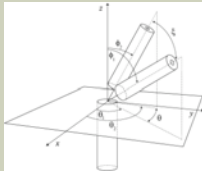
### Structural studies of Immunoglobulin G

Biochemical			TEM
X-ray			AFM
Single particle EM			CET

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## Model of IgG



Distribution of angles between IgG subunits

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## Large Scale Dynamics of IgG2a

### Antigen-antibody encounter reactions: simple laws explain complex dynamics

Simple animation  
Brownian dynamics (1000 steps of 1 nano second each)

L. Bongini, D. Fanelli, F. Piazza,  
P. De Los Rios, S. Sandin, M. Sanner, U. Skoglund

May 2005

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