Into the future – a structure biologist's dreams

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Structural Biologist's dreams

Cells are the basis of life

Wanted -

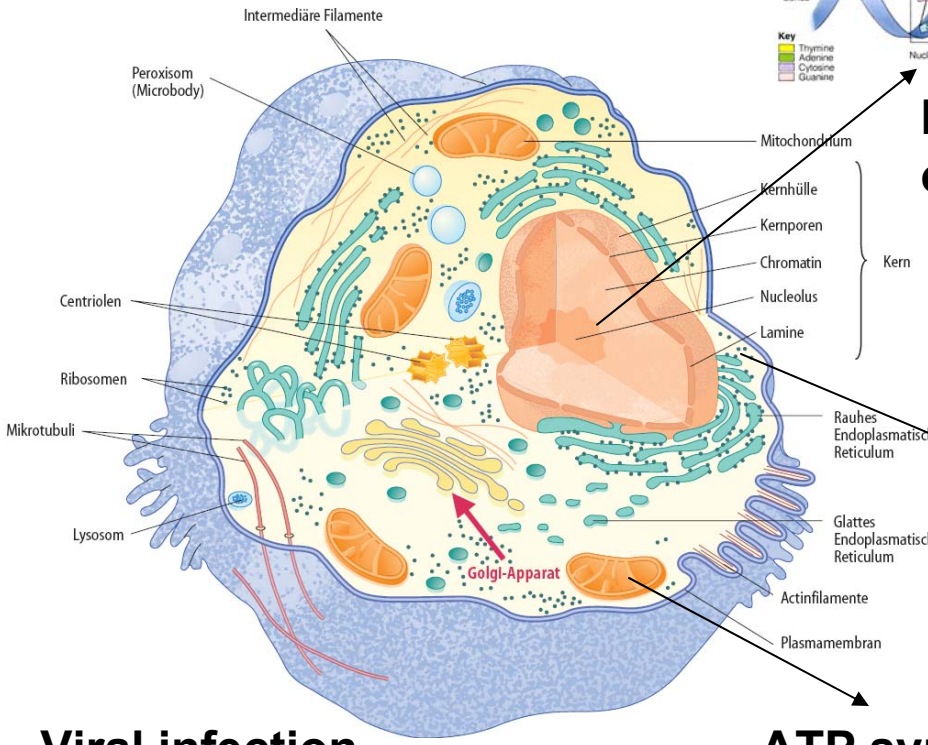
High resolution temporal & spatial inventory of cells

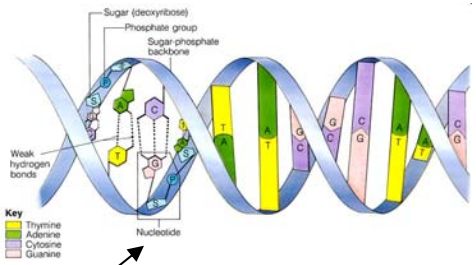
Who is where, when, why

Comprehensive characterization of the players

- 3D structures of macromolecules & assemblies
- how do they function, structural changes
-how do they acquire their structures, i.e. fold

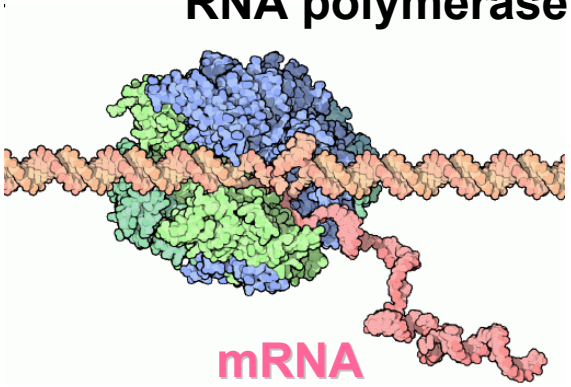
Cells are the basis of life



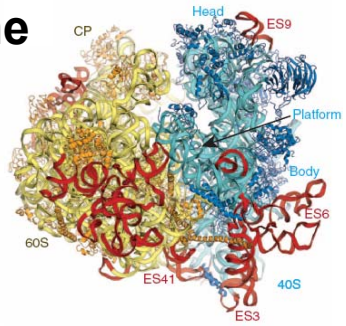


DNA: storage of genetic info

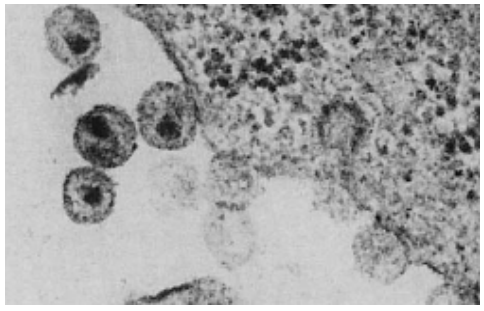
Transcribed by RNA polymerase



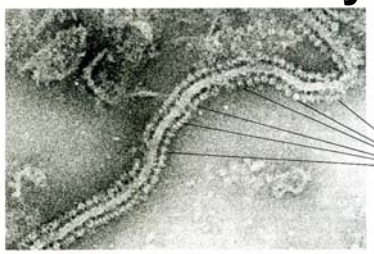
Translated and synthesized in protein: ribosome



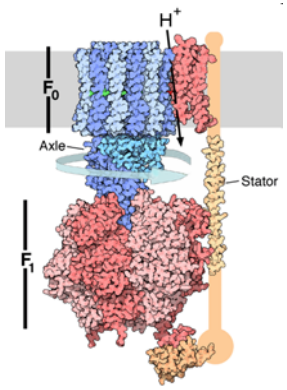
Viral infection



ATP synthesis



F_1/F_0 ATP synthase



Some open issues in structural biology

Structures of big (weakly binding) complexes
membrane proteins
transient intermediates, folding
chromatin/ genome structure
cellular organization at high resolution

Current limitations of crystallography,
electron microscopy
X-ray microscopy

Sample preparation (biochemistry)
Crystals for crystallographic approaches
Data collection, radiation damage
Computational approaches dealing with disorder

ERLs (and FELs) have properties that open new possibilities

High brightness
High coherence
Small source size
Short pulse length
High repetition rate (except XFEL)
Stability shot-to-shot (except seeded)

- Which questions can be addressed ?
  What resolution is required/useful?
- What is needed to make this work?

What component is where, when, why ?

3D-imaging of cells

- Depth resolution

  2D projection of 3D object

  - tomographic approaches
    - multi-angular imaging using split beams
    - curvature of Ewald sphere, small objects or sectioning

  (Bergh et al., Quart. Rev.Biophys.2008)

- What does one see?

  cells are very crowded

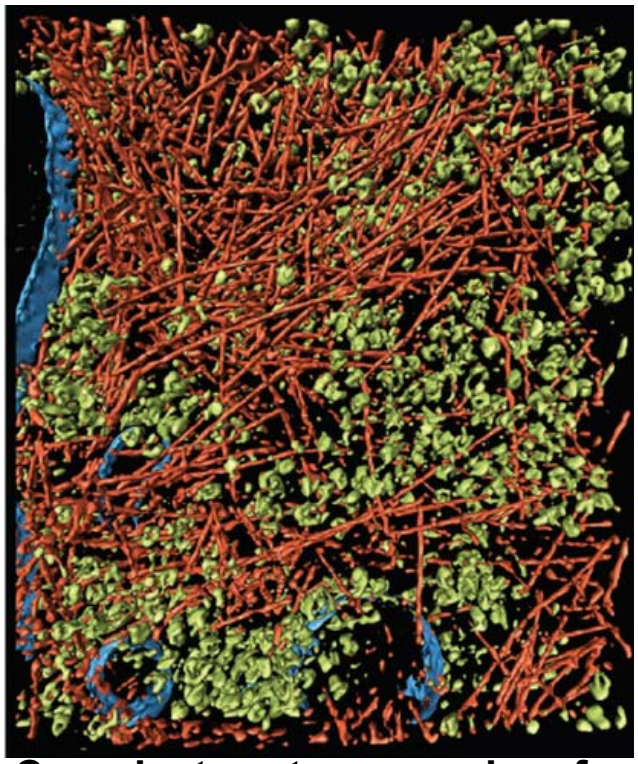
  - identification of particles (superposition, contrast, shape)

Actin filaments
Ribosomes,
membranes

- Correlation with function

  - correlation with light microscopy, fluorescence labels, nonlin. nanocrystals (20-100nm, phasing?)

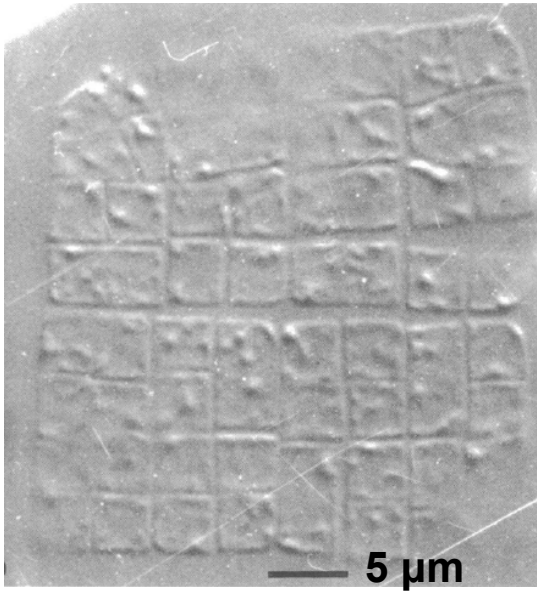
  (Pantazis et al PNAS 107:13535 (2010))

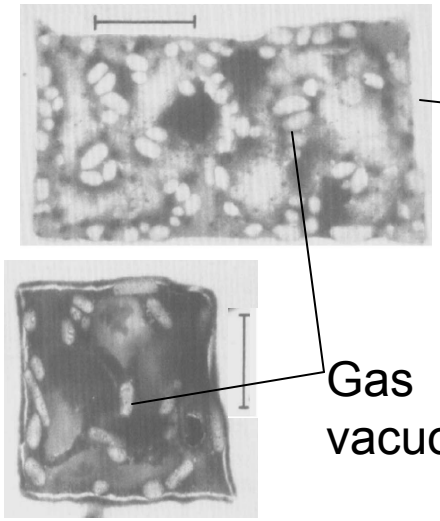


Cryoelectron tomography of *Dictyostelium* cells
815 x 870 x 97 nm
Science 298:1209 (2002)

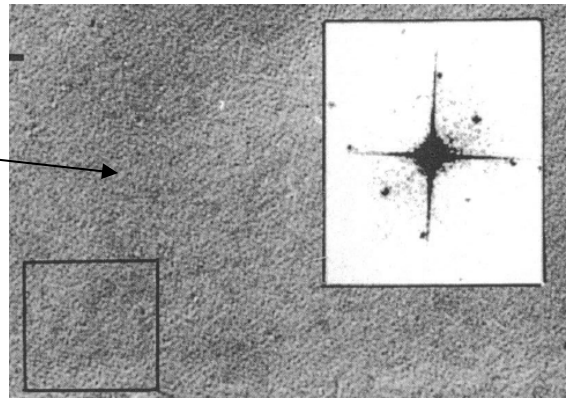
3D imaging of cells

Square bacterium (Walsby, Nature 283:69(1980), 150 nm thick)





Gas vacuole



Hexagonal and tetragonal arrangement of cell wall subunits

J. Bact. 150:851 (1982)

Unicellular green alga *Ostreococcus tauri*

Smallest free-living eukaryote, a picoplancton, mean length 1±0.3 µ, width 0.7±0.2 µ
naked, nonflagellated cell with a single mitochondrion and chloroplast

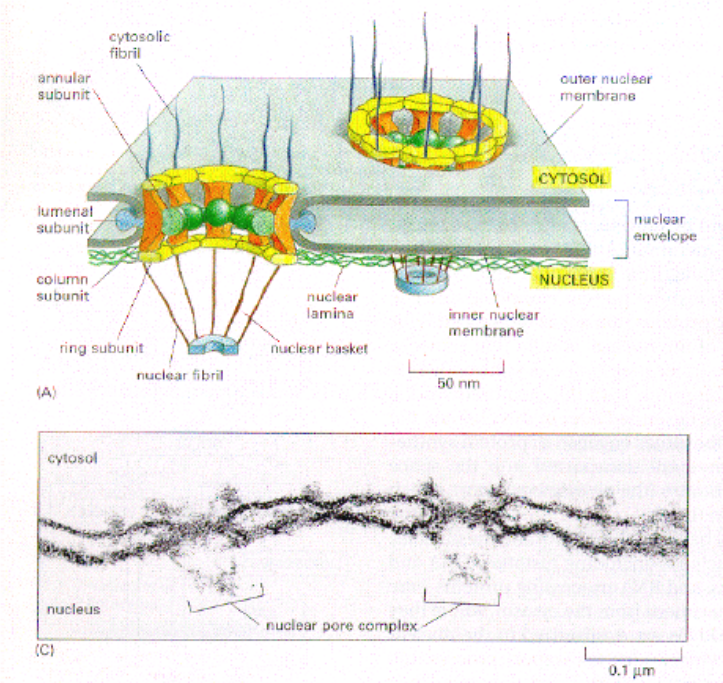
Courties et al Nature 370:255 (1994)

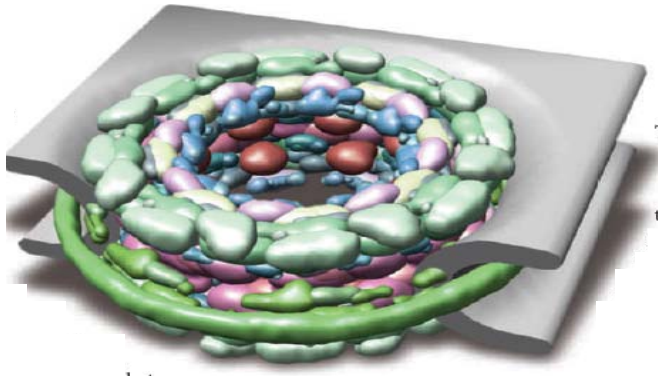
STRUCTURES OF DESIRE

What do protein crystallographers dream of? The eukaryotic ribosome, the spliceosome, the nuclear-pore complex, the HIV trimer and almost any transmembrane protein, finds **Ananyo Bhattacharya**.

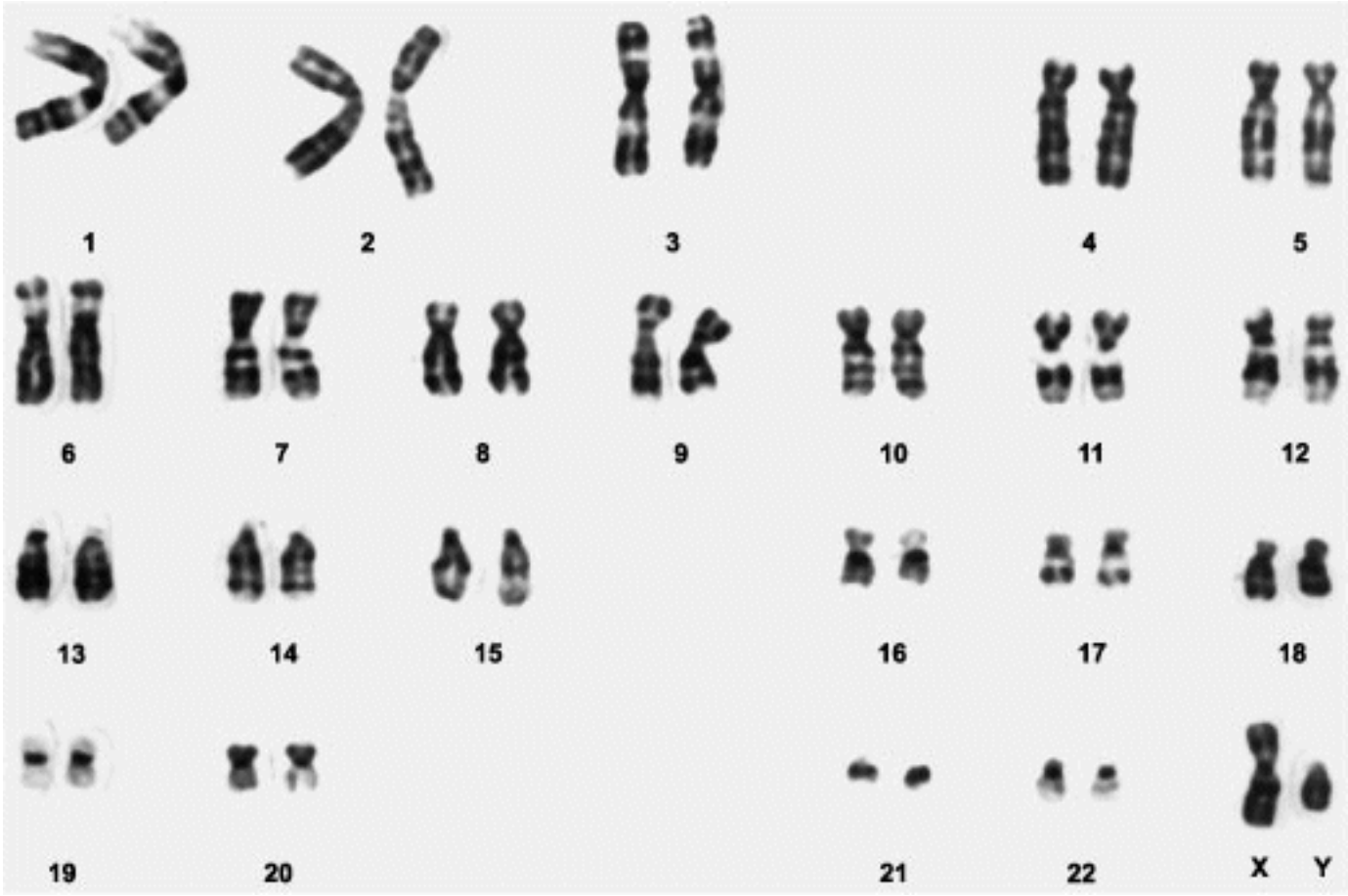
Nature 459, (2009)

Nuclear pore: 100 nm wide, 50 MDa, 200 pores/yeast cell
456 constituent proteins,
30 distinct ones





Multidisciplinary approach
Biophysics and proteomics
modeling
Alber et al., Nature 450 (270)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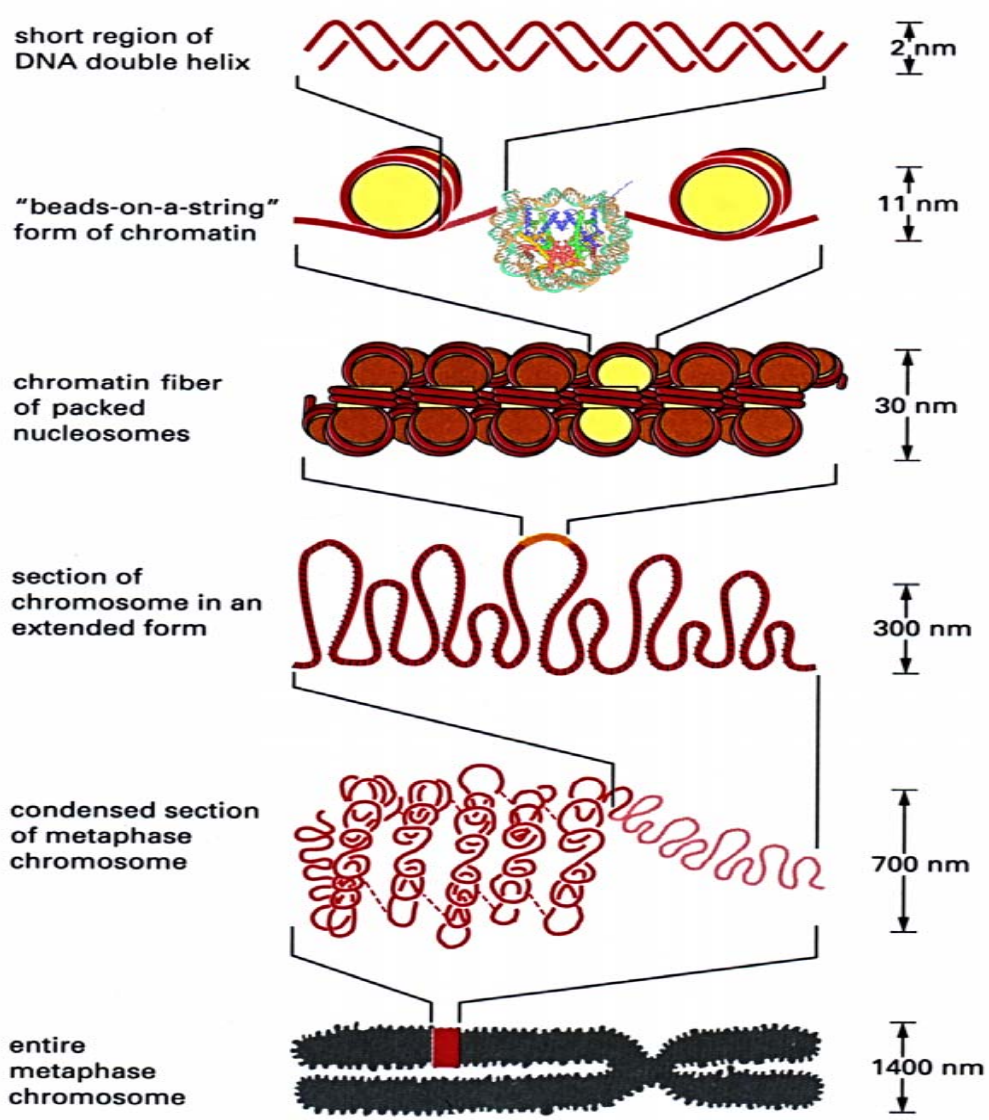
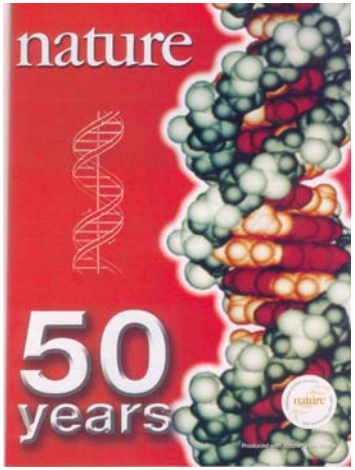
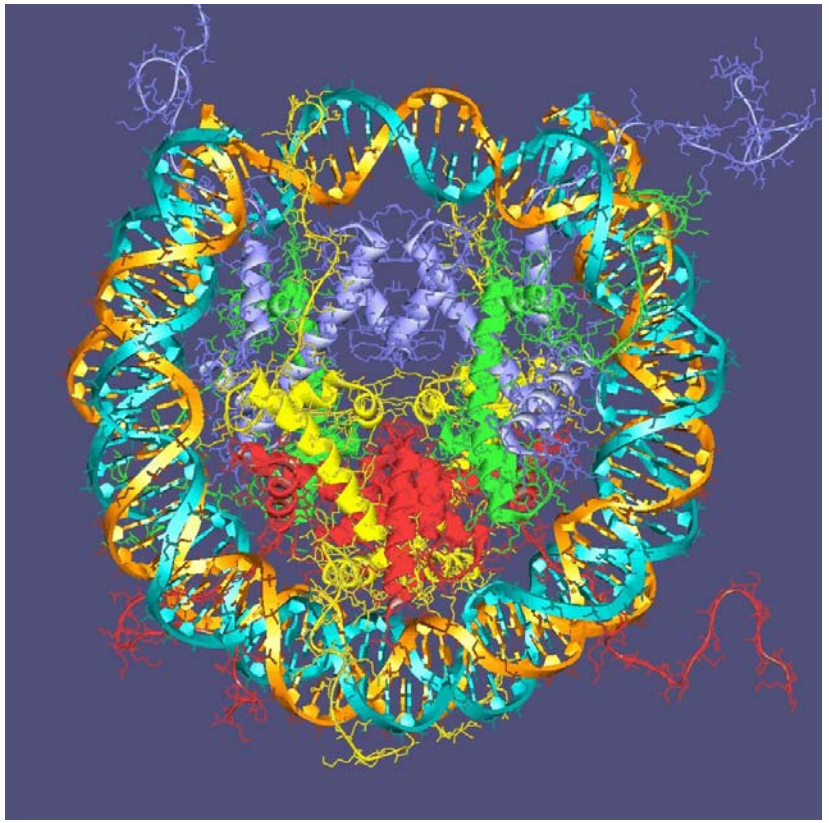
Chromatin structure

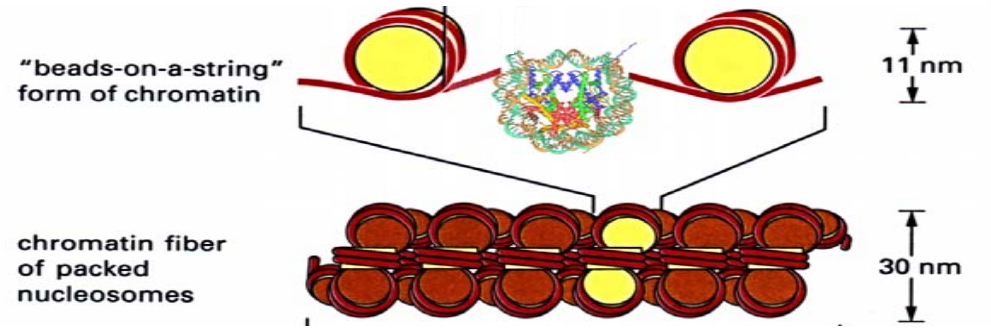


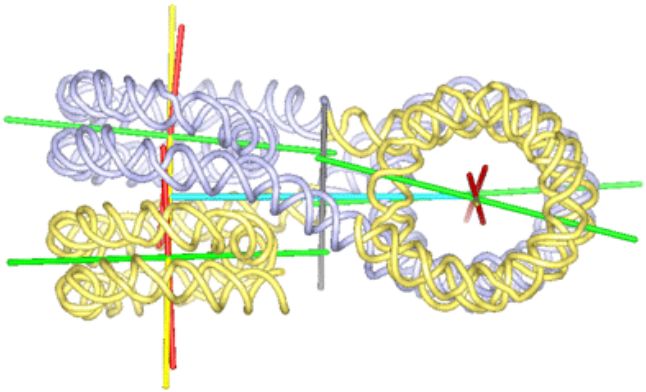
adapted from Alberts, Bray, Lewis, Raff, Roberts & Watson, 1994





Organization of nucleosomes in DNA fibers





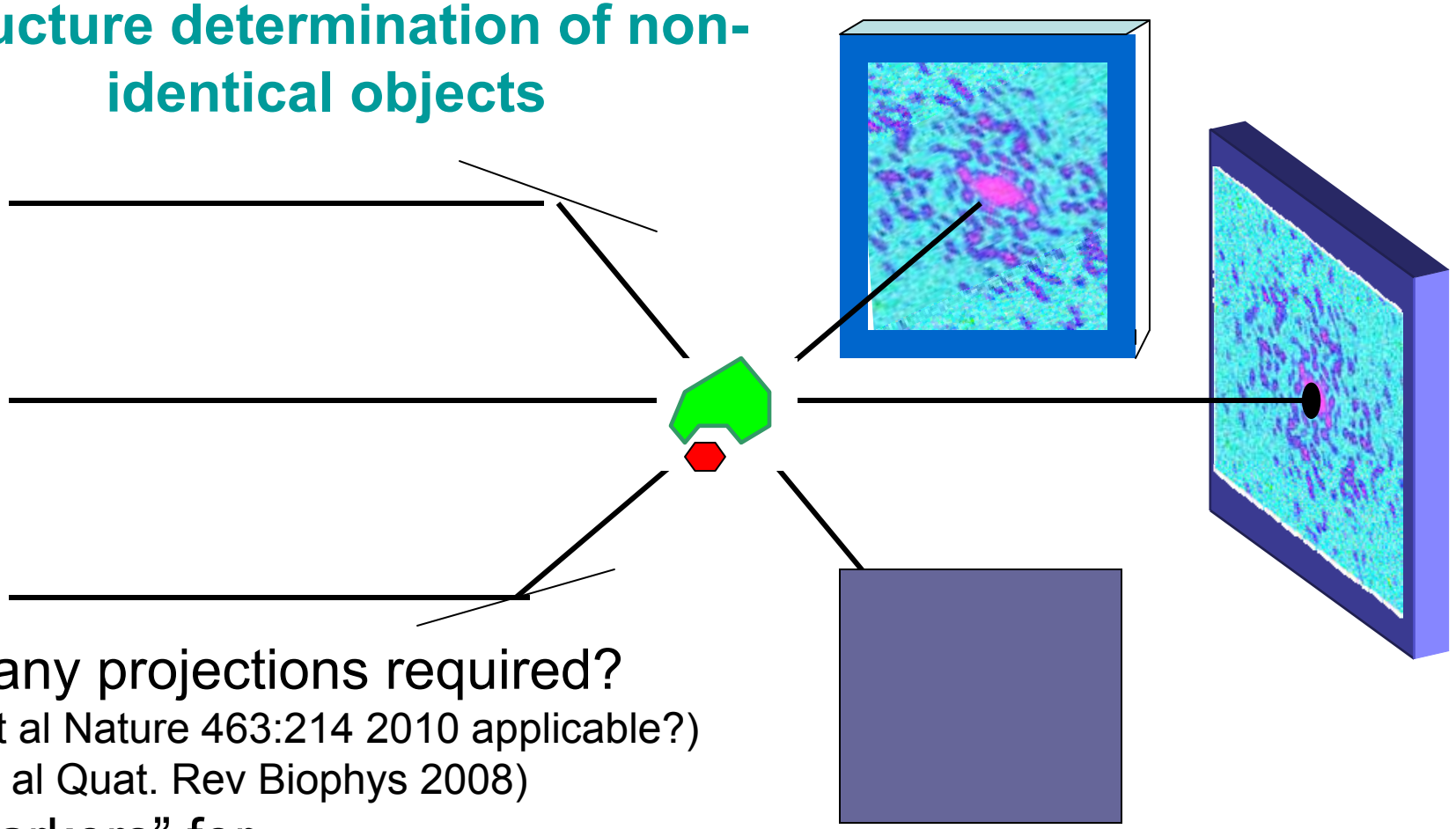
Schalch et al., Nature (2005)
Dorigo et al., Science 2004

Helical ribbon, two-start helix
Woodcock et al., J Cell Biol 99:42(1984)

- Higher order chromatin structure (> 30 nm fiber)
- in vivo structure, changes associated with active/inactive states
- function: modification of histone tails, complexes with e.g. remodeling factors,
- Correlation sequence/ structure

(first model of yeast genome
Duan et al., Nature (2010)

Structure determination of non-identical objects



How many projections required?

(Raines et al Nature 463:214 2010 applicable?)

(Bergh et al Quat. Rev Biophys 2008)

Add ”markers” for

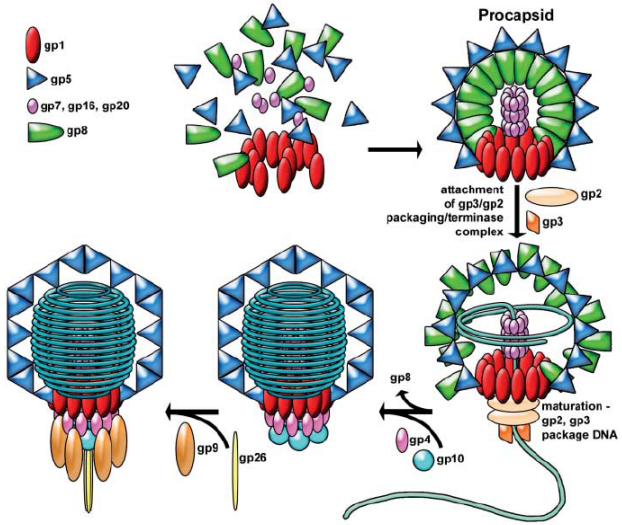
phasing (large clusters, non-linear nanocrystals)

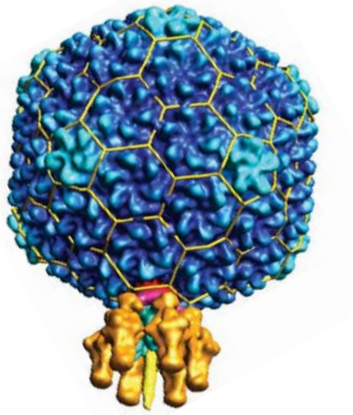
site specific labeling for averaging of reproducible structures in unique objects (?)

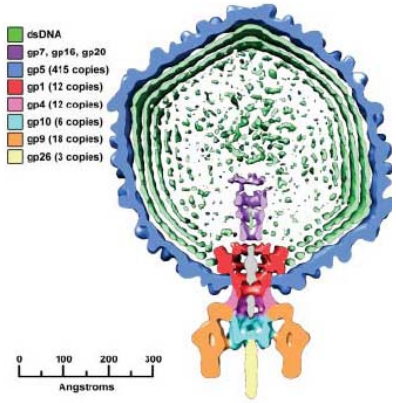
Cryogenic sample mount similar to EM. Holey carbon, graphene?

Egg-carton like structure by surface modification

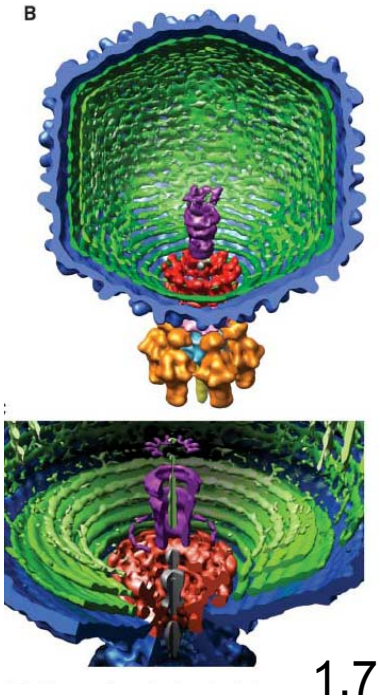
3-dimensional genome organization in viruses



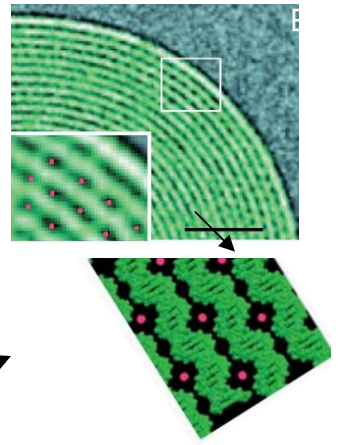




Science 312: 1791 (2006)



PNAS 2009



Electron microscopy:
resolution of genome structure limited by **dynamic scattering**, radiation damage

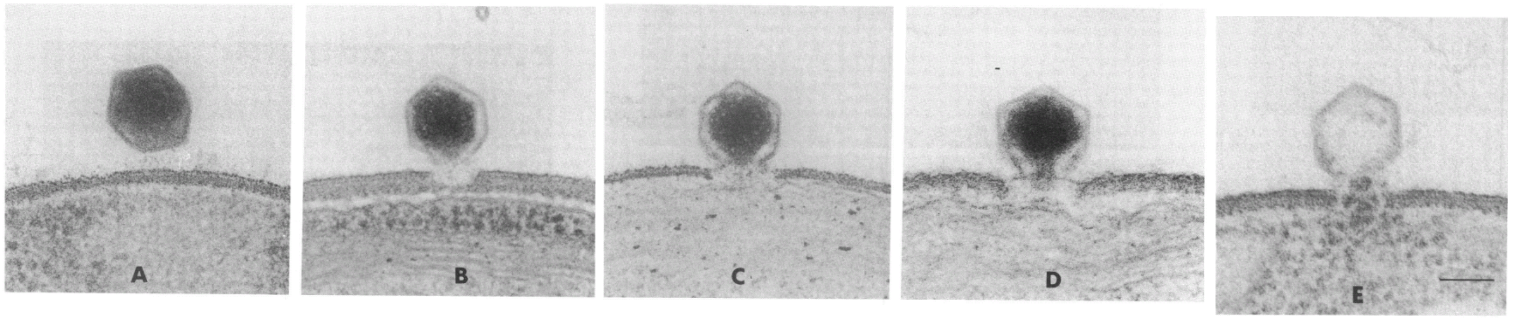
X-ray:
greater penetration depth
Classification
(averaging with internal break in symmetry)

1.7 nm resolution, 26422 particles

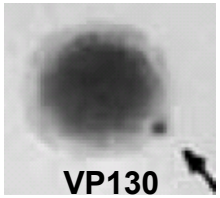
Complex unique vertex decorated by a spike in a giant algal virus

Paramecium bursaria Chlorella virus-1

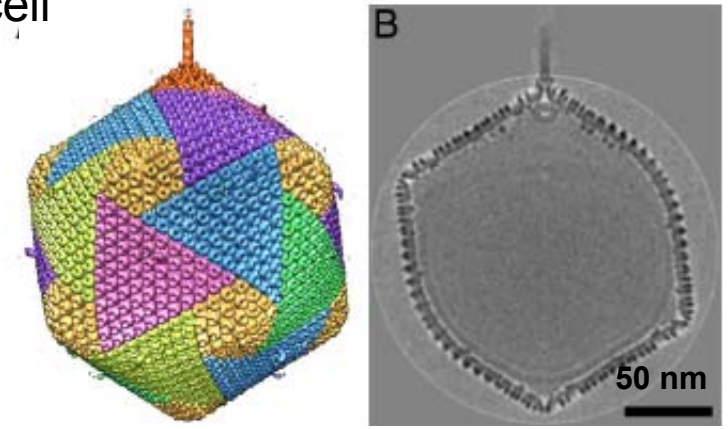
Genome 331-kbp codes for 11 tRNAs and 365 putative proteins, of which more than 100 are present in the mature virion.



Virus brings ion channel to reduce pressure of host cell to inject its DNA

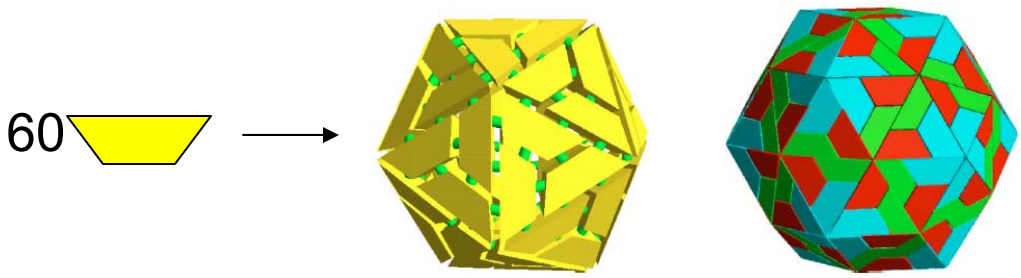


Unique vertex involved in infection



Cherrier et al PNAS 116:1105 2009

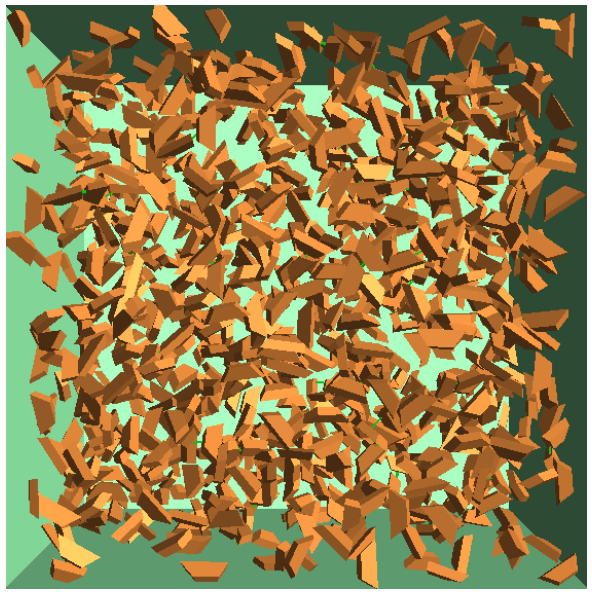
Self assembly of viruses



Icosahedral geometry
2, 3, 5 fold symmetry
20-sided solid,
each facet 3-fold symmetry
12 capsomers pentagons
20 capsomers hexamers
Quasiequivalence (Caspar, Klug 1962)

Max. enclosed volume for subunit size
Minimal “gene usage”
Self assembly
Disassembly mechanism (e.g. swelling)

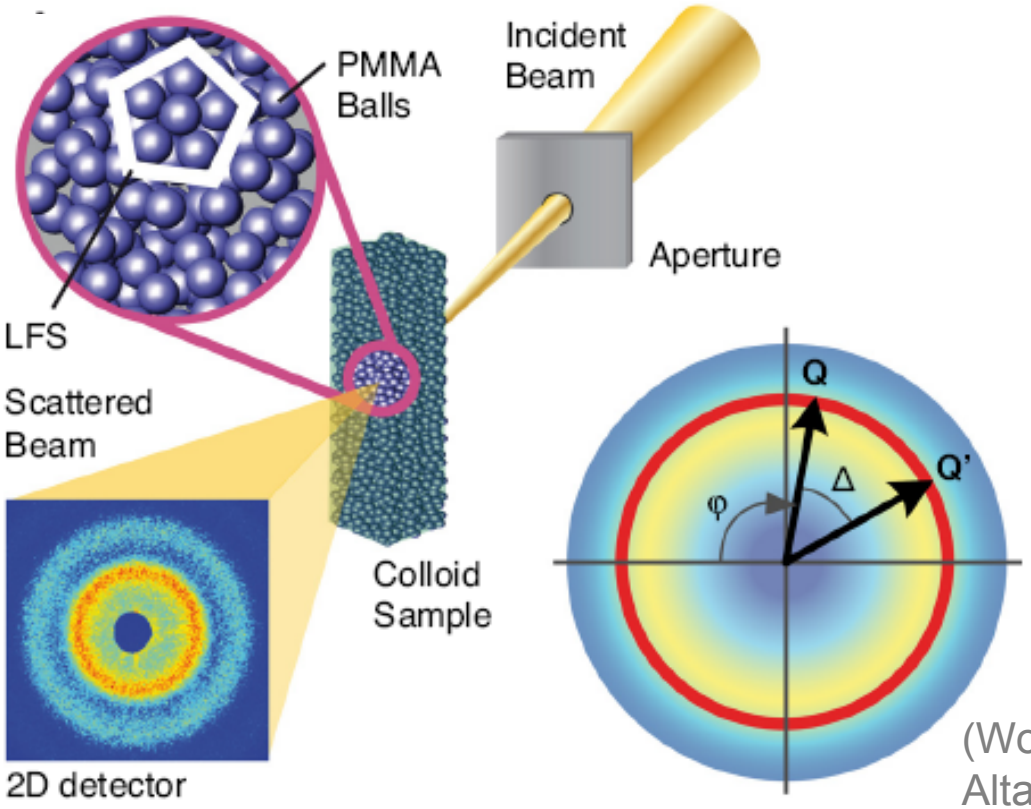
Assembly can be misdirected -> drugs
Poorly understood: retrovirus provirions
Nanoparticles

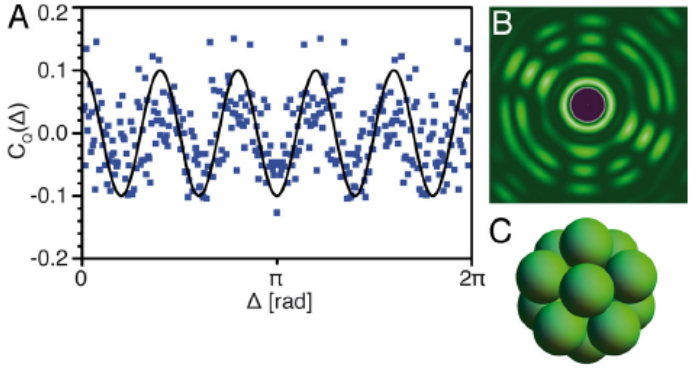


http://www.ph.biu.ac.il/~rapaport/anim_gif/capsid_s_anim.gif

Studying e.g. capsid self assembly

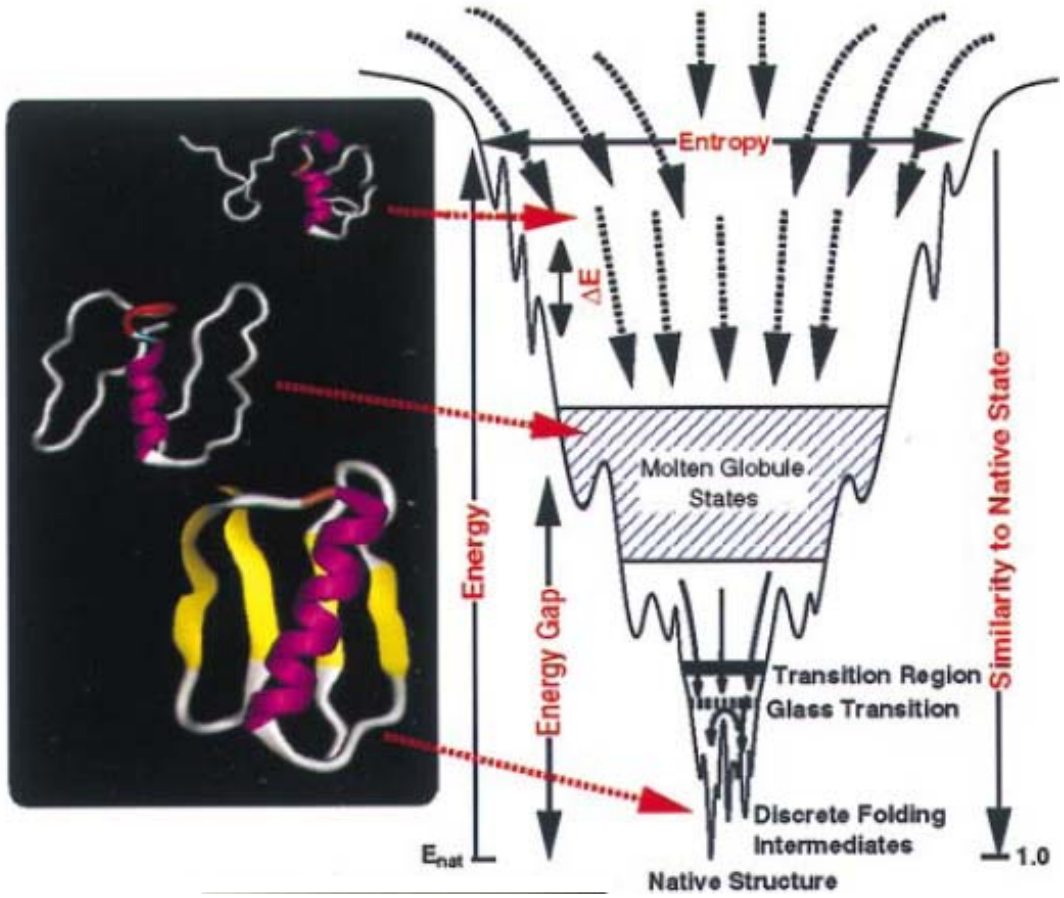
- Initiate assembly by rapid mixing
  continuous flow (nanofluidics) , droplet mixing
- Collect time-series of SAXS/WAXS data
- Analyze for angular cross correlations in intensity to analyze for local symmetries analogously to recent colloid study





(Wochner et al., PNAS 106:11511(2009)
Altarelli et al., PHYS. REV. B **82**, 104207 (2010)

David Phillips, Royal Institution, London, 5th Nov 1965



Protein folding

Funnel-shaped energy Landscape:

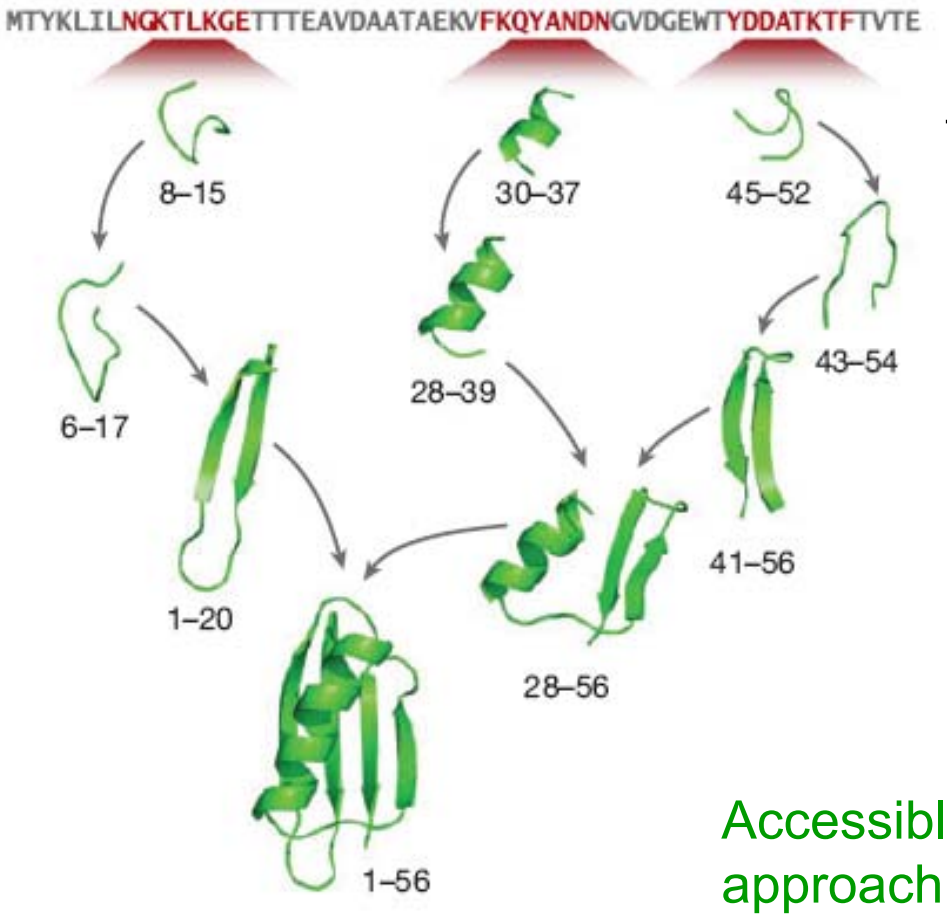
Many high-energy states rugged landscape

Few low-energy states

Stochastic process, initial hydrophobic collapse
Physical models *vs* bioinformatics approach of folding may allow to obtain deeper understanding of forces and dynamics that govern protein properties:

Predict conformational changes, e.g. induced fit
Refine models beyond homology structures
Improvement for multi-domain or domain swapped or low homology models

Zipping and assembly mechanism



On fast time scales (ps-ns) peptide fragments search for local meta-stable structures (loops, beta-turns, helices)

Few are stable enough to survive for longer time scales, grow/zip into larger and more stable structures

On longer time scales, pairs or groups of substructures assemble into larger and more native like structures

Accessible via fast mixing and correlation approach to yield structural information? Complement with parallel IR/CD measurements Information beyond ensemble? Needs new software. Serves as input for computational models on folding

Dill et al Annu Rev Biophys 2008

Solution studies have great potential

- Low and high resolution structural features in SAXS and WAXS data, need better methods to extract those.
  Use coherence to exploit angular cross correlations to study assembly reactions

- New mixing devices and high intensity, high repetition X-ray sources may allow routine microsecond studies, faster studies by temperature-jump reaction initiation

- Structural changes occurring during reactions, in particular folding. characterization of the molten globule, distribution study of large proteins in dilute solutions to prevent aggregation? Simultaneously with IR, VUV (CD) spectroscopy, correlations?

Combination with labeling, alternative methods such as double electron electron resonance (DEER) spectroscopy

Concluding remark

New sources provide new scientific options require development in both both hardware and software