

How Can Next Generation Light Sources Impact Structural Biology and Molecular Biophysics?

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“Prediction is very difficult,
especially of the future.”

Niels Bohr

4th Generation Sources

- Broad spectral range & tunability
- High intensity
- Higher brightness
- Exquisitely short pulses
 - Reasonable repetition rate

WOW!

4GLS Opportunities

- Traditional:
 - Spectroscopy: $f(\lambda)$
 - Kinetics: $f(t)$
 - Microscopy: $f(x,y)$
- Now:
 - Multiparameter, hyperspectral, 3D, time resolved
 - $f_1(\lambda,x,y,z,t)$, $f_2(\lambda,x,y,z,t)$, ...

• ~~Crystallography~~

Why did Synchrotron Radiation Became Critical for Macromolecular X-ray Crystallography?

A μ Case Study

- Improved rate of data collection†
- MAD phasing †‡
- Prevention of radiation damage ‡
- Brightness critical for poor crystals and large structures †*
- Proteins from all organisms produced in *e coli* §

† Inherent property of synchrotron radiation

‡ Required development in hard-, soft- and/or wetware

* improved synchrotron capabilities and/or more demanding experiment

§ Resulted from independent developments in other fields

Components of Success

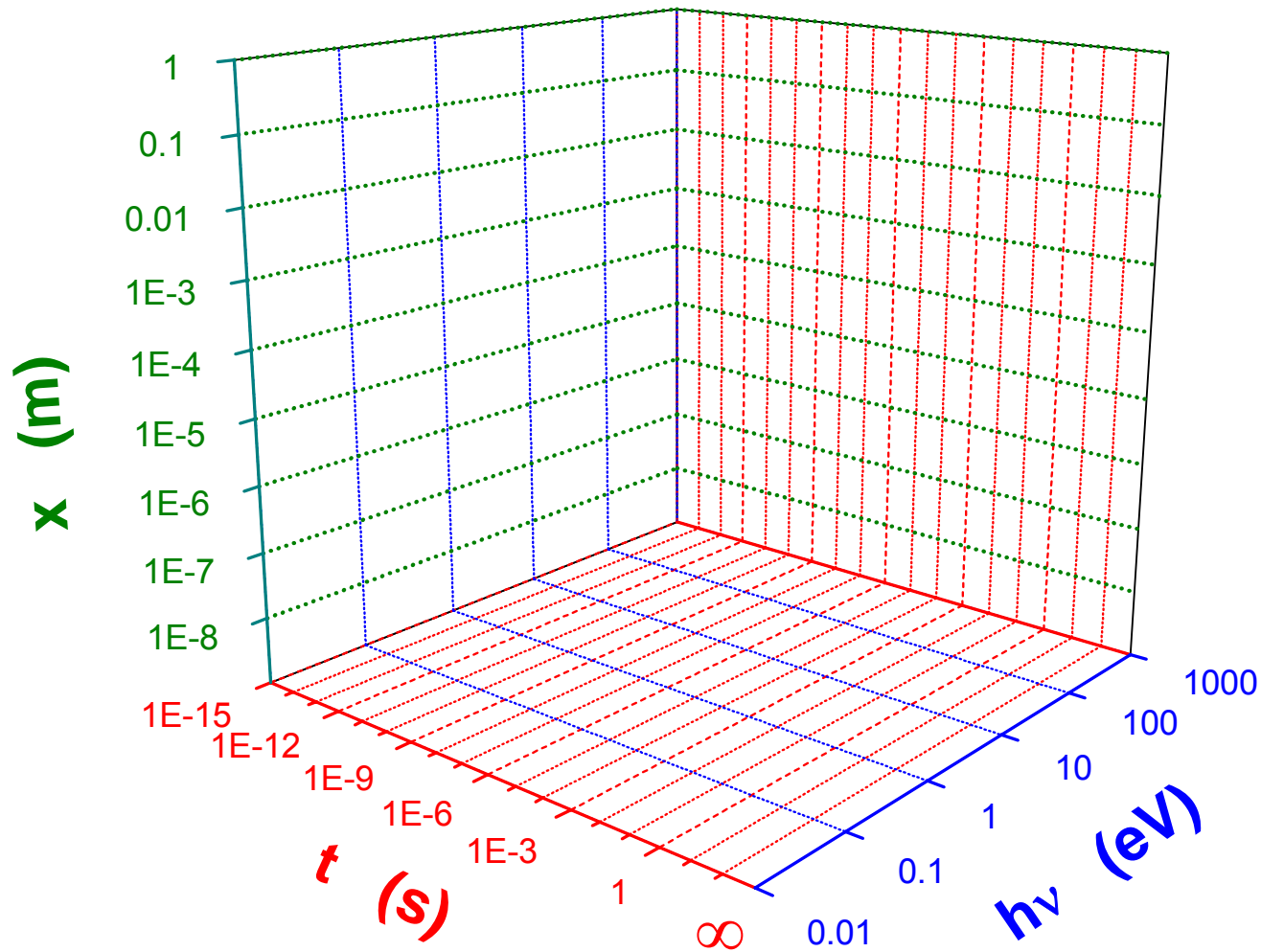
- Inherent properties of the source
 - Intensity
 - Tunability
 - Brightness
- Auxiliary support
 - Hardware
 - Software
 - Wetware
- Developments in other fields
 - Electronics
 - Computers
 - Molecular & cellular biology, genetics, ...
- **User community**

Experiments:

Big Bang vs. Bread & Butter

- Big Bang
 - May be of high importance
 - May not be repeated often
 - *e.g.* DNA double helix
- Bread & Butter
 - Initial demonstration may be of high importance
 - Later, importance may be in aggregation of data
 - Will be repeated many times with minor variations (*e.g.* different samples)
 - *e.g.* 3D protein structures

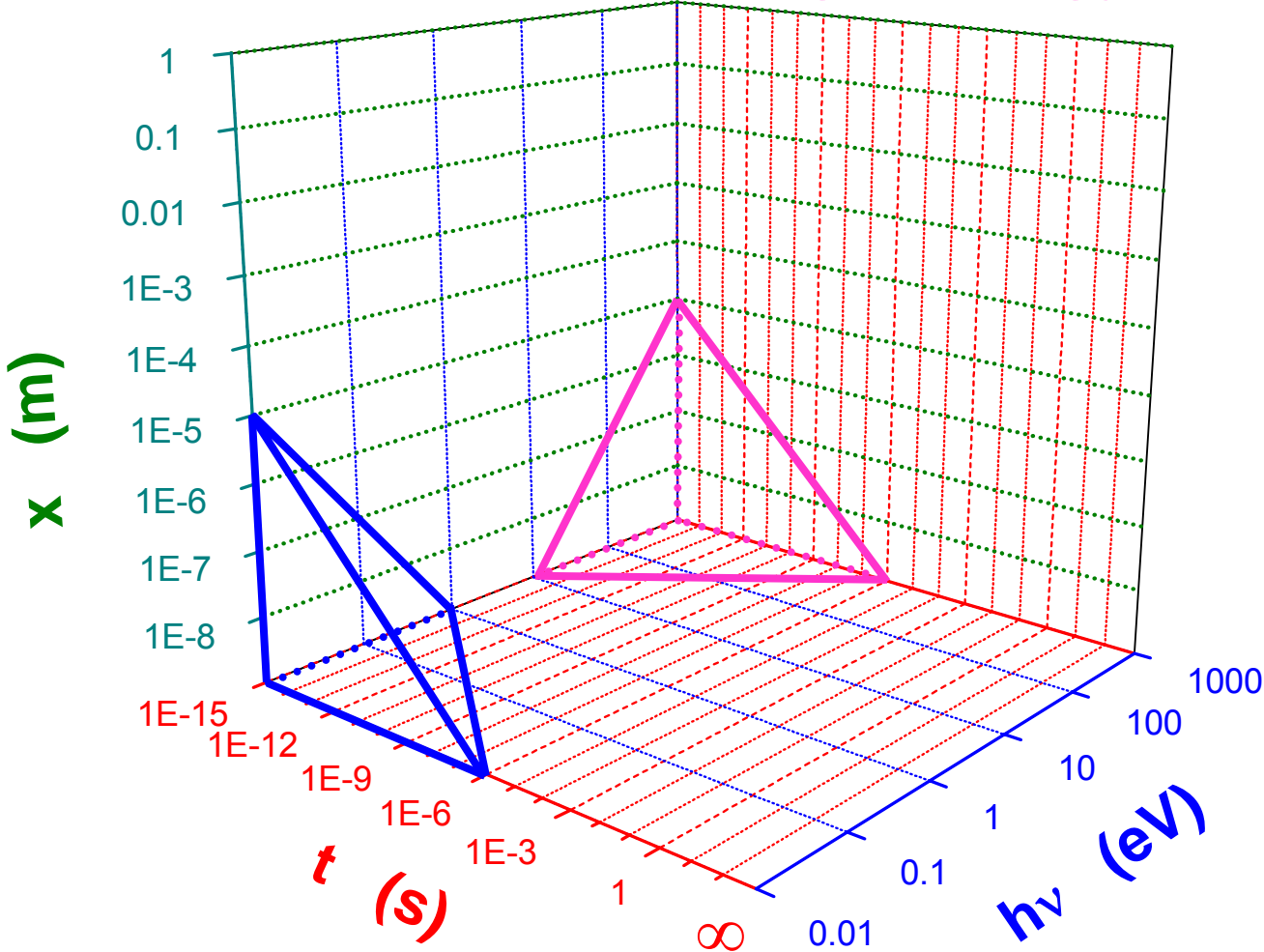
4GLS Parameter Space



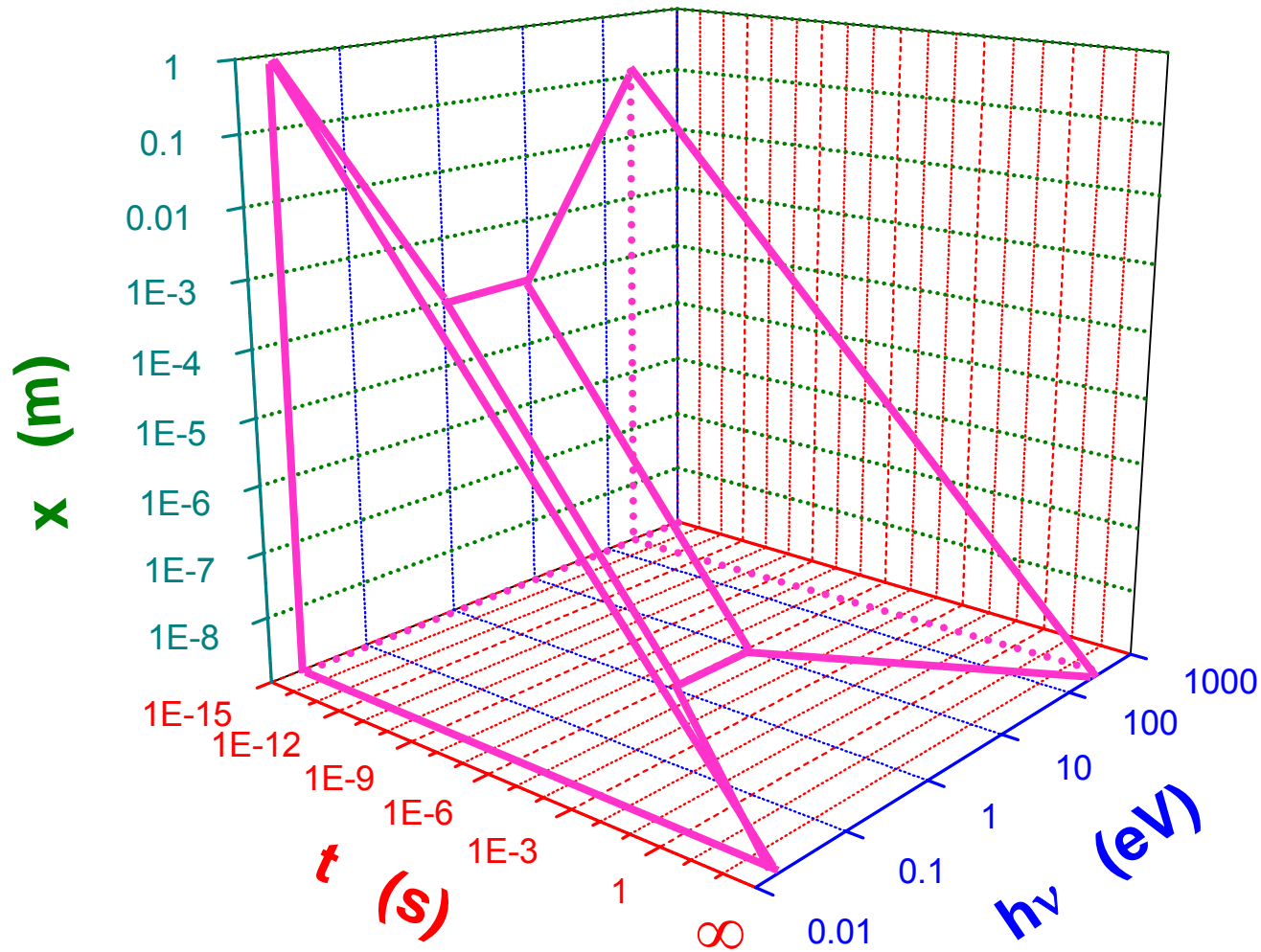
Groundbreaking Capabilities:

Small, Fast & Low Energy

Small, Fast & High Energy



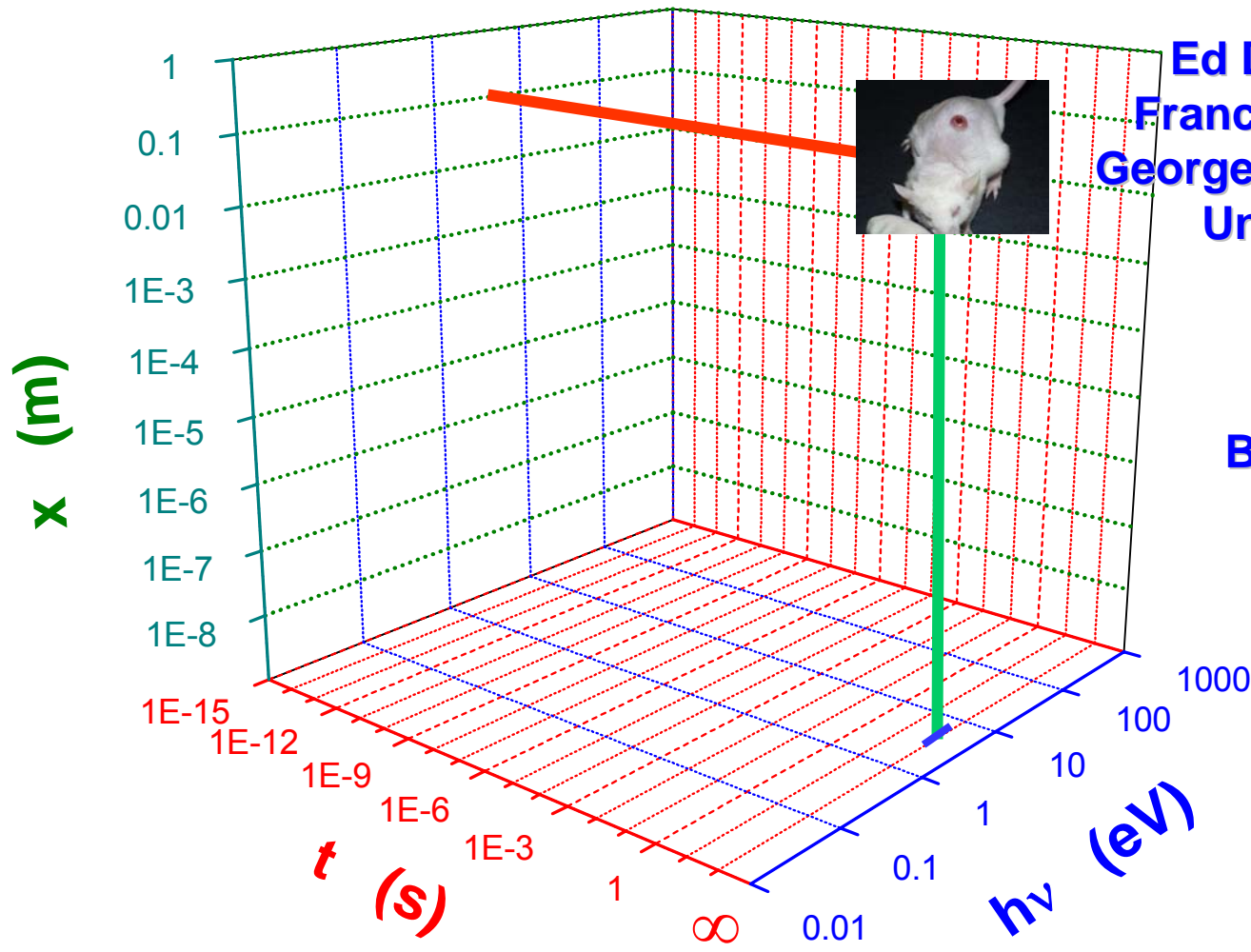
Emphasis of Small Structures, Rapid Events and Very High or Low Photon Energies



Other Capabilities May be critical for Some Experiments

Action Spectrum for Melanoma Induction

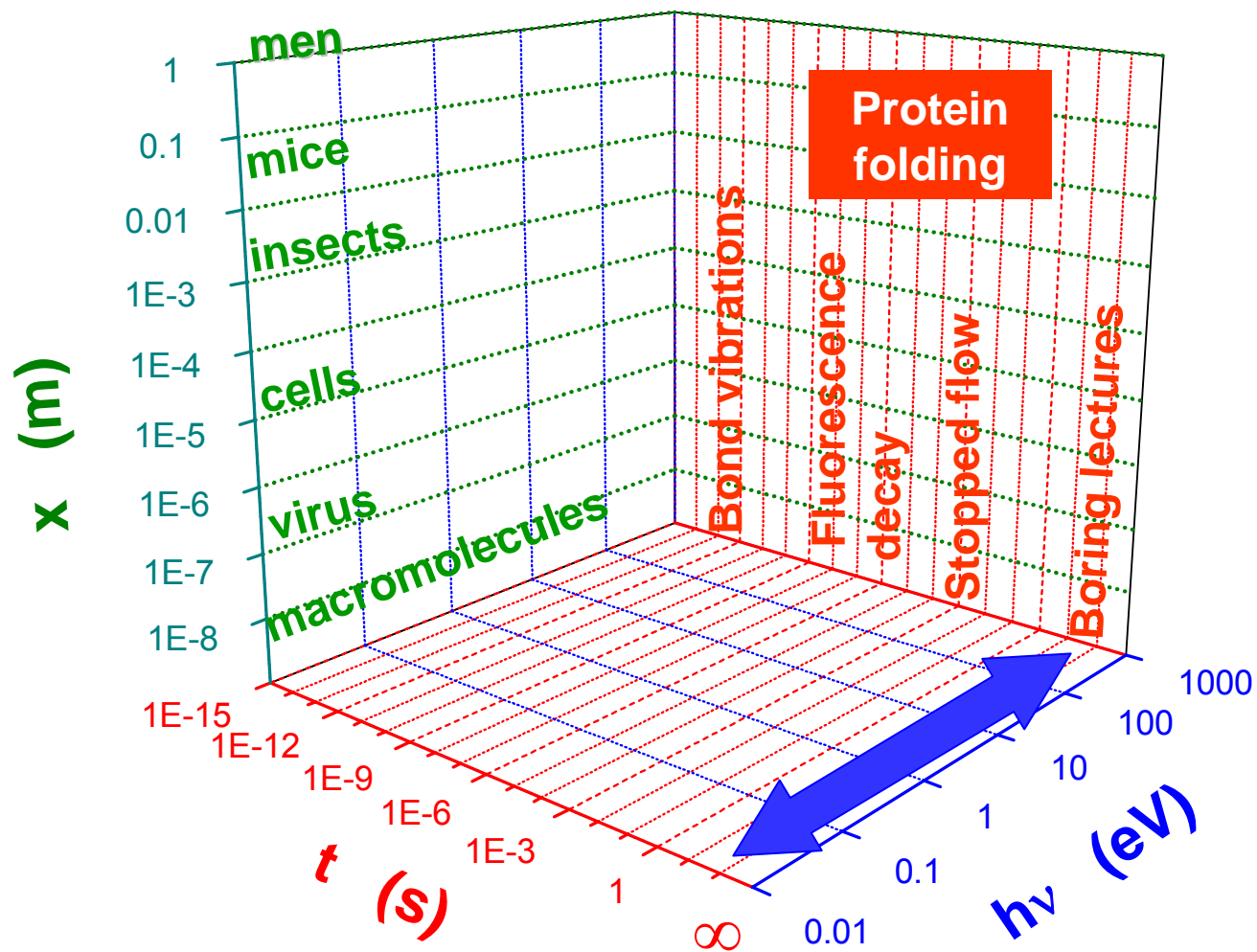
Large, UV & Long Exposures



Ed DeFabo &
Frances Noonan
George Washington
University

BIG BANG!

4GLS Parameter Space



Which Biomolecules?

- DNA
- RNA
- Proteins
- Lipids
- Polysaccharides
- Small molecules (cofactors & drugs)

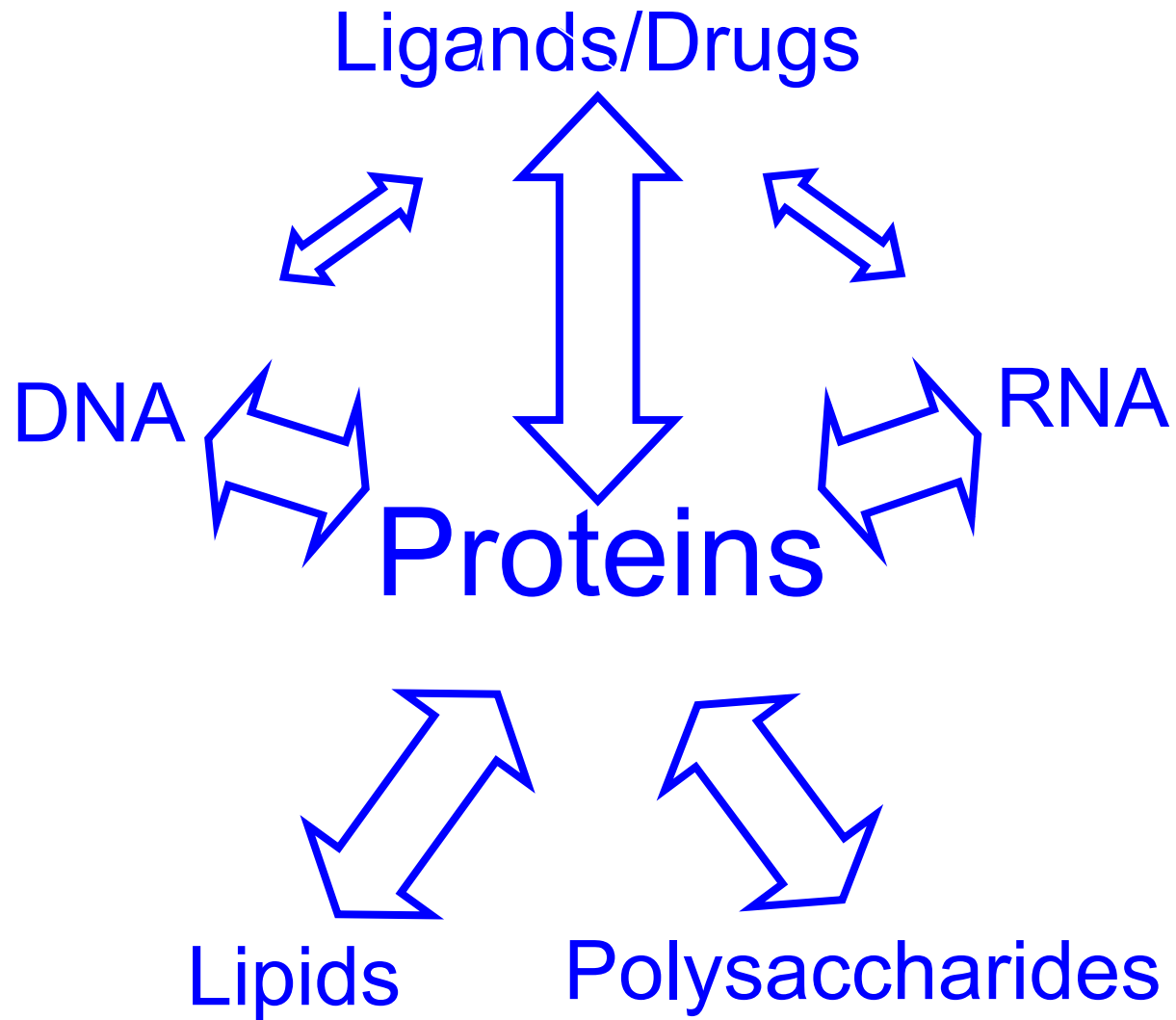
- DNA

- 4 structural subunits → limited structures: A,B,Z
- Many spectroscopic “hooks”
- Greatest current interest in information content, *i.e.* the sequence of bases, not 3D structure.
- Structural biology interest in interactions with other molecules
 - Proteins
 - RNA
 - Drugs and small molecules

- RNA
 - Four structural subunits
 - Informational & Structural roles
 - Interacts with
 - Proteins
 - DNA
 - Drugs

- Proteins
 - 20 subunits → great complexity
 - Huge range of structures and functions
 - Multiple conformations of a single protein
 - 3D structure critical to function
 - How do they fold?
 - Interact with: DNA, RNA, other proteins, lipid membranes, carbohydrates & small molecules
 - Frequent target of drug action
 - Can be produced from DNA sequence
 - **Essentially an infinite number**
 - Membrane bound proteins difficult to crystallize
 - Dynamics important in function

Proteins are Central



Major Areas for Biological Research at 4GLS

- Themes:
 - Electron & proton transfer & enzyme reactions
 - Photosynthesis & energy transduction
 - Membrane transport
- Approaches:
 - Imaging
 - Spectroscopy
 - Kinetics
 - All of the above – together!
- **Need biology specific infrastructure**

Hyperspectral Microscopic Imaging

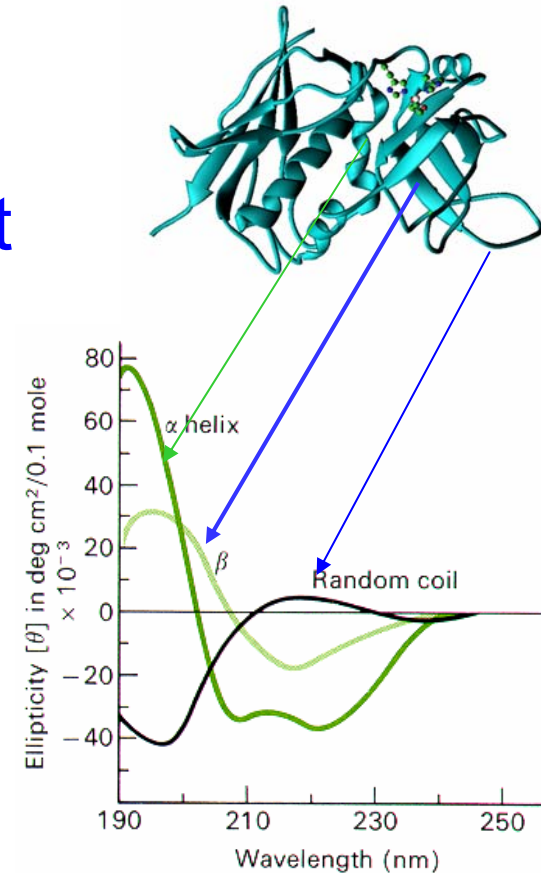
- UV/visible → 3D morphology, dynamic distribution of specific fluorescent markers
- Infrared → molecular distribution
- Far Infrared → molecular distribution?
- **ALL AT ONE FACILITY!**

Biophysical Spectroscopy

- Spectral range
 - Far IR (THz)
- Temporal resolution
 - Energy transfer
 - Membrane dynamics
 - Protein dynamics
- Polarization

Circular Dichroism

- The difference in the absorption of left- and right-circularly polarized light
- Can be either positive or negative → more information than absorption
- Requires molecular asymmetry
 - Water has **NO** circular dichroism
 - Proteins, DNA, RNA, polysaccharides have large, characteristic CD that depends on structure
 - Particularly useful for characterizing proteins



CD of poly-L-lysine in different conformations.
Greenfield and Fasman, *Biochemistry* **8**, 4108 (1969)

Synchrotron Radiation CD vs Conventional CD

- Synchrotron Radiation
 - Spectral range extended to about 160 nm for samples in water
 - And to < 140 nm for samples not in water
- SRCD increases
 - increases “information content”
 - Improves signal/noise at all wavelengths
 - Decreases time to record data “high throughput”

Why Use Synchrotron Radiation?

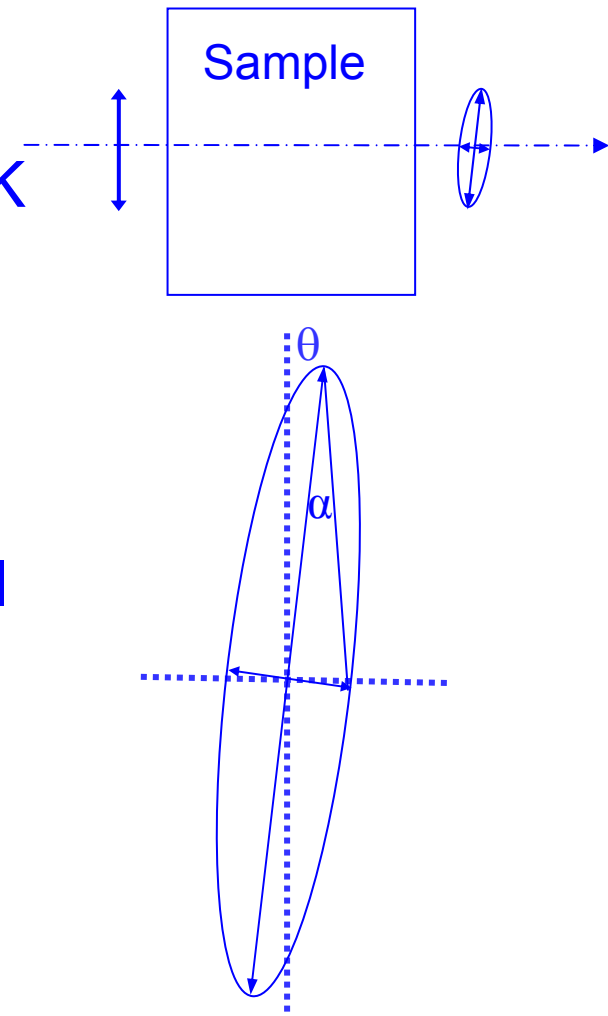
- Important information on proteins and polysaccharides in the far- and vacuum UV (λ from 160 to 240 nm)
- Laboratory CD Spectrometers use Xe arc sources
 - Intensity drops for $\lambda < 200$ nm

CD at 4GLS?

- Static CD spectra of proteins and other biomolecules classic “bread & butter” experiment
- Proteins are not “static” molecules
- Time resolved CD to ~ 1 ms achieved with “stopped flow” mixing and present optics & electronics (SRS Daresbury pioneer)
 - Many “slow” steps in protein reorganization accessible in this time domain
 - Starting to see from biochemistry community

Time Resolved Optical Activity at 4GLS

- For $\Delta t < \approx 1$ ms, must resort to other approaches
- CD and optical rotation related by KK transform
- There are methods for measuring rotation of polarization with ns resolution (D. Kliger at UCSC)
- Rapid synchronized initiation of structural changes in proteins critical
- Light pulses are the best approach
- 4GLS has unique capabilities for mixed start/probe combinations
- Will biologists find it interesting?



The Way Forward

- 4GLS offers an unprecedented combination of capabilities
- Challenges:
 - Building the facility
 - Developing experimental capabilities
 - Building user communities
- None are trivial
- All are necessary