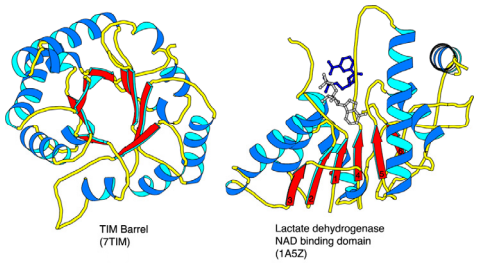
Protein Structure Determination



How are these structures determined?

Why Bother With Structure?

- The amino acid sequence of a protein contains interesting information.
- A protein sequence can be compared to other protein sequences to establish its evolutionary relationship to other proteins and protein families.
- However, for the purposes of understanding protein function, the 3D structure of the protein is far more useful than the sequence.

Protein Sequences Far Outnumber Structures

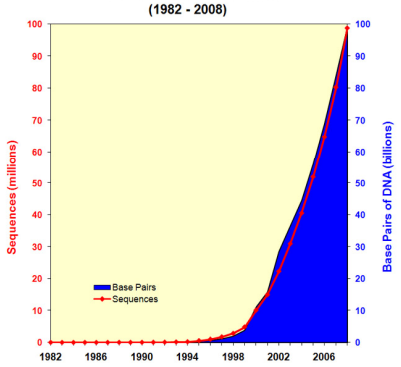
- Only a small number of protein structures have been experimentally determined.

  PDB **~64,623 protein structures**
  Genebank **~61,132,599 sequences**

- Of the 64,623 structures, only **15,702** are dissimilar in sequence (<30% ID).

Growth of GenBank

Now over 100M sequences and 100B base pairs from 223K species

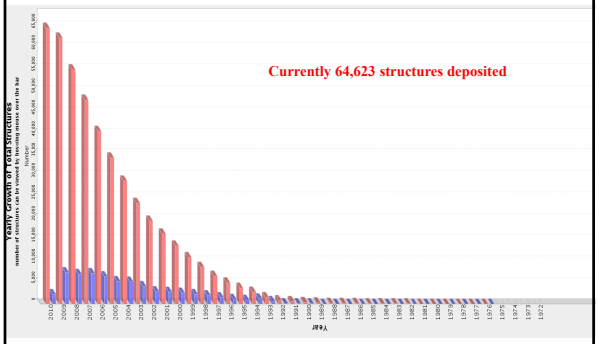


Exponential Increase

http://www.ncbi.nlm.nih.gov/Genbank/genbankstats.html

http://www.ncbi.nlm.nih.gov/Sitemap/Summary/statistics.html

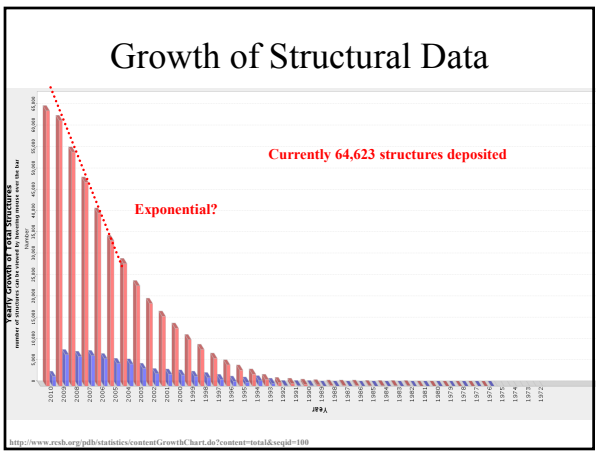
Growth of Structural Data



Currently 64,623 structures deposited

http://www.rcsb.org/pdb/statistics/contentGrowthChart.do?content=total&seqid=100

Growth of Structural Data



Currently 64,623 structures deposited

Exponential?

http://www.rcsb.org/pdb/statistics/contentGrowthChart.do?content=total&seqid=100

Structural Proteomics

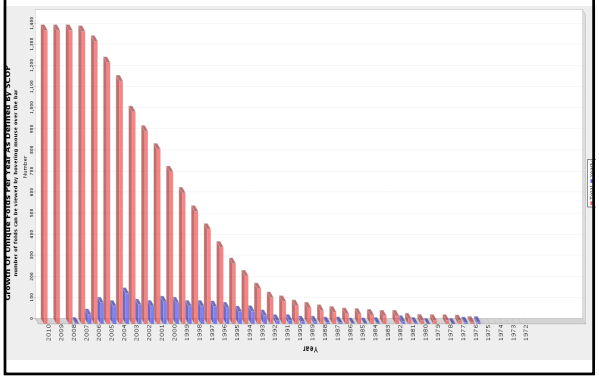
- Use experimentally determined structures to model the structures of similar proteins
  - Threading
  - Homology Modeling
  - Fold recognition

  Avoids *Ab initio* structure determination
- Need representative protein structures for the total repertoire of protein folds
- Provide 3D portraits for all proteins in an organism
- Goal: Use structure to infer function.
  - More sensitive than primary sequence comparisons

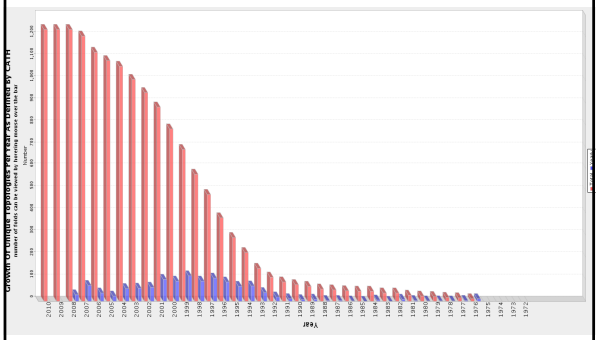
Redundancy in PDB (20 April 10)

Sequence identity	Number of non-redundant chains
90%	25615
70%	23116
50%	20306
30%	15702

Unique folds in PDB (SCOP)



Unique topologies in PDB (CATH)



New Topologies and Folds Becoming Rare

Structural Genomics

Initiated in 1999 by NIH
Phase I included 9 large centers for high throughput structure determination
Phase I ran from ~2000 - 2005

Goal

The long-range goal of the Protein Structure Initiative (PSI) is to make the three-dimensional atomic-level structures of most proteins easily obtainable from knowledge of their corresponding DNA sequences.

http://www.nigms.nih.gov/psi/mission.html

Structural Genomics

Benefits

Structural descriptions will help researchers illuminate structure-function relationships and thus formulate better hypotheses and design better experiments.

The PSI collection of structures will serve as the starting point for structure-based drug development by permitting faster identification of lead compounds and their optimization.

The design of better therapeutics will result from comparisons of the structures of proteins that are from pathogenic and host organisms and from normal and diseased human tissues.

The PSI collection of structures will assist biomedical investigators in research studies of key biophysical and biochemical problems, such as protein folding, evolution, structure prediction, and the organization of protein families and folds.

Technical developments, the availability of reagents and materials, and experimental outcome data in protein production and crystallization will directly benefit all structural biologists and provide valuable assistance to a broad range of biomedical researchers.

Structural Genomics Centers in US

PSI-1 Winners

The Joint Center for Structural Genomics (JCSG)

During PSI-2, the JCSG has contributed to the overall goal of maximizing structural coverage of protein families with no structural representation and has continued to developm and disseminate innovative new technologies for structural biology. The JCSG consortium theme is the "central machinery of life" — proteins that are conserved in all kingdoms of life.

The Midwest Center for Structural Genomics (MCSG)

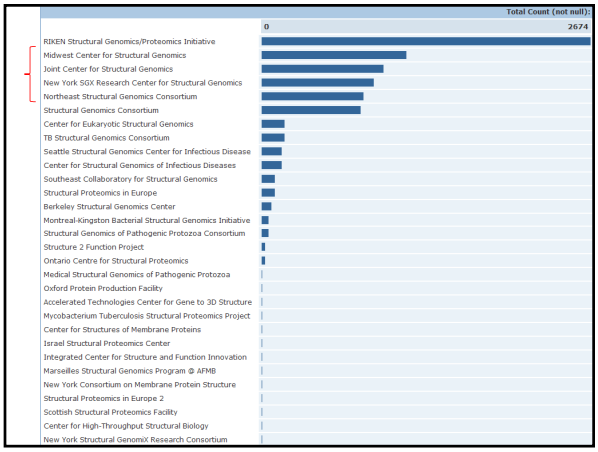
In PSI-2, the multi-institutional consortium is rapidly determining the structures of large numbers of strategically selected proteins using x-ray crystallography both to provide structural coverage of major protein superfamilies and to elucidate the entire protein folding space.

The New York Structural Genomics Research Consortium (NYSGRC)

During PSI-2, the consortium's individual project focuses on new targets, principally protein phosphatases and mul tidomain eukaryotic proteins.

The Northeast Structural Genomics Consortium (NEGS)

In PSI-2, the consortium is solving both prokaryotic and eukaryotic structural representatives from the major domain families constituting the eukaryotic proteome.



Structural Genomics Centers in US

PSI-1 Losers

Center for Eukaryotic Structural Genomics (CESG)

The CESG was founded as a collaborative effort to develop the technologies needed for economical high-throughput structure determination of biologically important eukaryotic proteins and to extend the knowledge of fold-function space. This project also aims to further the research of biologically important proteins in Arabidopsis. The protein structures are being determined via X-ray crystallography or NMR spectroscopy.

The Berkeley Structural Genomics Center (BSGC) The BSGC is pursuing an integrated structural genomics program designed to obtain a near-complete structural complement of two minimal genomes, Mycoplasma genitalium and Mycoplasma pneumoniae, two related human and animal pathogens. Both NMR spectroscopy and X-ray crystallography are being used for structural determination.

The Southeast Collaboratory for Structural Genomics (SECSG)

The objective of the SECSG is to develop and test experimental and computational strategies for high throughput structure determination of proteins by X-ray crystallography and NMR methods and to apply these strategies to scan the entire genome of an organism at a rapid pace. The eukaryotic organisms, *Caenorhabditis elegans*, *Homo sapiens* and an ancestrally-related prokaryotic microorganism having a small genome, *Pyrococcus furiosus*, have been selected as representative genomes.

Structural Genomics of Pathogenic Protozoa Consortium (SGPP)

The SGPP consortium aims to determine and analyze the structures of a large number of proteins from major global pathogenic protozoa including *Leishmania major*, *Trypanosoma brucei*, *Trypanosoma cruzi* and *Plasmodium falciparum*. These organisms are responsible for the diseases: leishmaniasis, sleeping sickness, Chagas' disease and malaria. X-ray crystallography is being used for structural determination.

The TB Structural Genomics Consortium (TB)

The goal of the TB consortium is to determine the structures of over 400 proteins from M. tuberculosis, and to analyze these structures in the context of functional information that currently exists and that is generated by the project. These structures will include about 40 novel folds and 200 new families of protein structures. The protein structures are being determined using X-ray crystallography.

Current PSI Centers

Large-Scale Centers
- Joint Center for Structural Genomics
- Midwest Center for Structural Genomics
- New York SGX Research Center for Structural Genomics
- Northeast Structural Genomics Consortium

Specialized Centers
- Accelerated Technologies Center for Gene to 3D Structure
- Center for Eukaryotic Structural Genomics
- Center for High-Throughput Structural Biology
- Center for Structures of Membrane Proteins
- Integrated Center for Structure and Function Innovation
- New York Consortium on Membrane Protein Structure

Homology Modeling Centers
- Joint Center for Molecular Modeling
- New Methods for High-Resolution Comparative Modeling

Resource Centers
- PSI-Materials Repository
- PSI Knowledgebase

60,000 plasmid clones

2008 Structural Genomics Progress

Status	Total Number of Targets	(%) Relative to "Cloned" Targets	(%) Relative to "Expressed" Targets	(%) Relative to "Purified" Targets	(%) Relative to "Crystallized" Targets
Cloned	61522	100.00	-	-	-
Expressed	39540	64.27	100.00	-	-
Soluble	18221	29.62	46.08	-	-
Purified	14031	22.81	35.49	100.00	-
Crystallized	5616	9.13	14.20	40.03	100.00
Diffraction-quality Crystals	2909	4.73	7.36	20.73	51.80
Diffraction	2429	3.95	6.14	17.31	43.25
NMR Assigned	1051	1.71	2.66	7.49	-
HSQC	1890	3.07	4.78	13.47	-
Crystal Structure	2291	3.72	5.79	16.33	40.79
NMR Structure	953	1.55	2.41	6.79	-
In PDB	2849	4.63	7.21	20.31	35.15
Work Stopped	14137	-	-	-	-
Test Target	4	-	-	-	-
Other	10	-	-	-	-

~40% of structures are from SG in Europe and Asia

http://targetdb.pdb.org/statistics/TargetStatistics.html

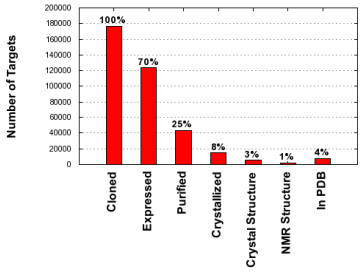
2010 Structural Genomics Progress

Status	Total Number of Targets	(%) Relative to "Cloned" Targets	(%) Relative to "Expressed" Targets	(%) Relative to "Purified" Targets	(%) Relative to "Crystallized" Targets
Cloned	176710	100.0	-	-	-
Expressed	123905	70.1	100.0	-	-
Soluble	47572	26.9	38.4	-	-
Purified	43609	24.7	35.2	100.0	-
Crystallized	14641	8.3	11.8	33.6	100.0
Diffraction-quality Crystals	7708	4.4	6.2	17.7	52.6
Diffraction	6628	3.8	5.3	15.2	45.3
NMR Assigned	2154	1.2	1.7	4.9	-
HSQC	3929	2.2	3.2	9.0	-
Crystal Structure	5215	3.0	4.2	12.0	35.6
NMR Structure	2050	1.2	1.7	4.7	-
In PDB	7569	4.3	6.1	17.4	38
Work Stopped	38962	-	-	-	-
Test Target	93	-	-	-	-
Other	8178	-	-	-	-

~36% of structures are from SG in Europe and Asia

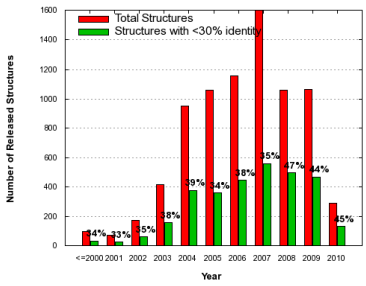
http://targetdb.pdb.org/statistics/TargetStatistics.html

Project Attrition



http://targetdb.pdb.org/statistics/TargetStatistics.html

Unique Folds?



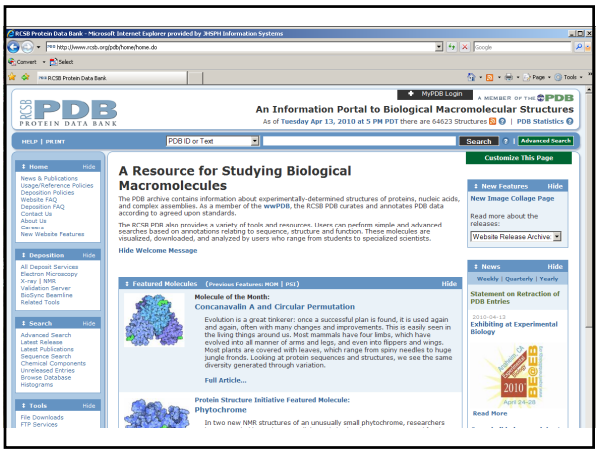
http://targetdb.pdb.org/statistics/TargetStatistics.html

Protein Structure Databases

- **Where does protein structural information reside?**
  - **PDB**:
    - http://www.rcsb.org/pdb/
  - **MMDB**:
    - http://www.ncbi.nlm.nih.gov/Structure/
  - **FSSP**:
    - http://www.ebi.ac.uk/dali/fssp/
  - **SCOP**:
    - http://scop.mrc-lmb.cam.ac.uk/scop/
  - **CATH**:
    - http://www.biochem.ucl.ac.uk/bsm/cath_new/

Jon

Ingo

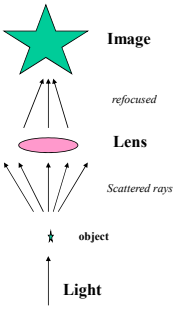


PDB Contents 20 April 2010

Exp.Method	Proteins	Nucleic Acids	Protein/NA Complexes	Other	Total
X-RAY	52212	1206	2401	17	55836
NMR	7279	896	154	7	8336
ELECTRON MICROSCOPY	195	17	76	0	288
HYBRID	16	1	1	1	19
Other	123	4	4	13	144
Total	59825	2124	2636	38	64623

X-ray Crystallography

Optical Microscope



Atomic Resolution

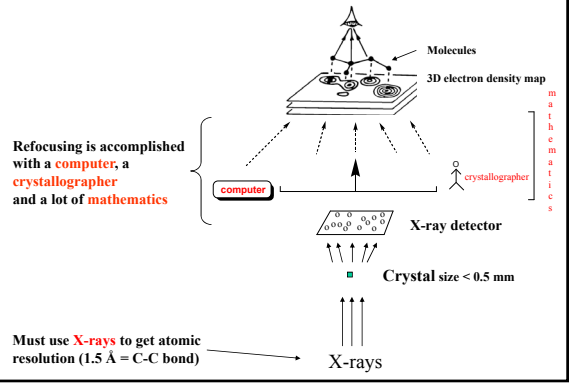
We want to resolve inter-atomic distances (~1.5 Å, 0.15 nM)

Visible light has a wavelength of ~ 500 nm (5000 Å)

Electron beam: λ_c ~ 0.001 Å (if e^- is moving at c)
Electron velocity is less in electron microscopes
Typical resolution is ~10 Å, but can be improved

X-ray generators produce photons of λ = 0.5 – 2.5 Å
Use λ = 1.542 Å

X-ray Crystallography



X-Ray Crystallography

1. Make crystals of your protein
   0.3-1.0mm in size
   Proteins must be in an ordered, repeating pattern.
2. X-ray beam is aimed at crystal and data is collected.
3. Structure is determined from the diffraction data.

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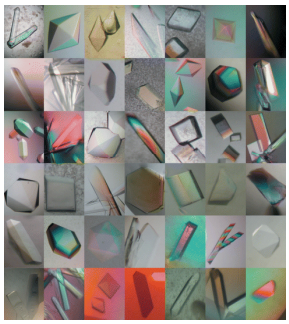
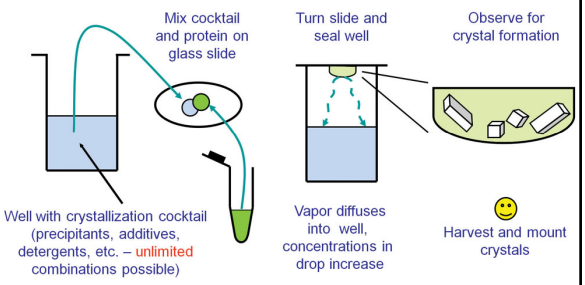
Protein Crystals



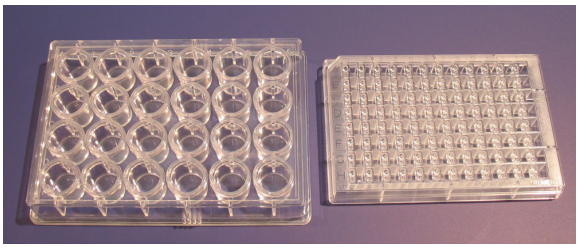
Schmid, M. Trends in Microbiolgy, **10**:s27-s31.

Protein Crystals



http://www.ruppweb.org/Garland/gallery/Ch1/index.htm#5

Protein Crystals



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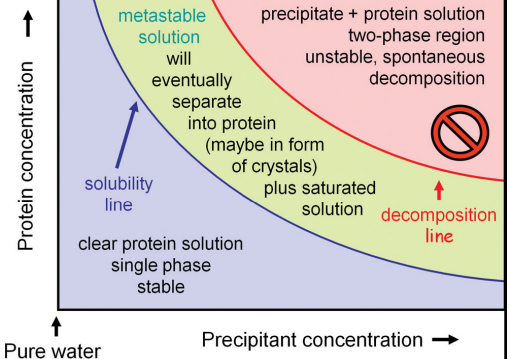

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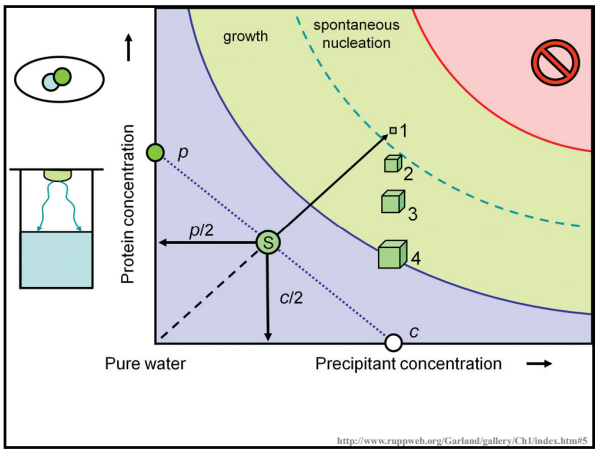
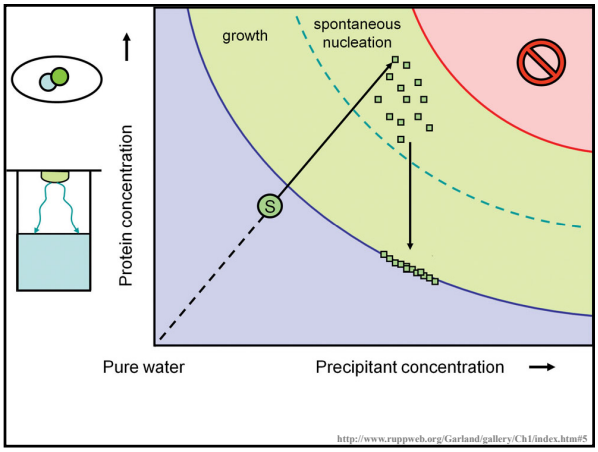
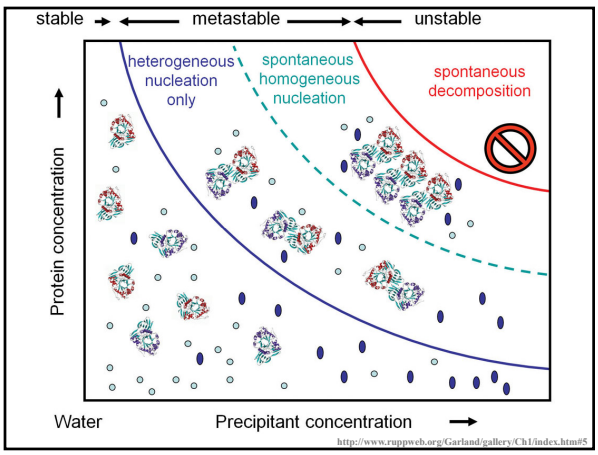
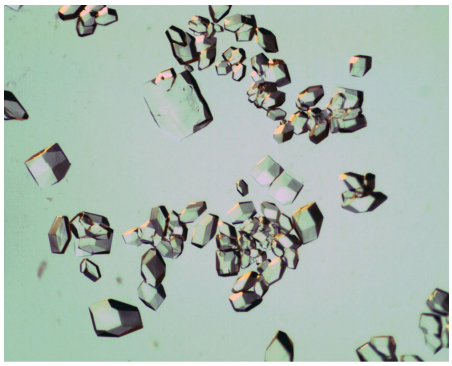

growth
spontaneous nucleation
Protein concentration
p
p/2
S
c/2
c
1
2
3
4
Pure water
Precipitant concentration
http://www.ruppweb.org/Garland/gallery/Ch1/index.htm#5


growth
spontaneous nucleation
Protein concentration
S
Pure water
Precipitant concentration
http://www.ruppweb.org/Garland/gallery/Ch1/index.htm#5


stable
metastable
unstable
heterogeneous nucleation only
spontaneous homogeneous nucleation
spontaneous decomposition
Protein concentration
Water
Precipitant concentration
http://www.ruppweb.org/Garland/gallery/Ch1/index.htm#5

Protein Crystals



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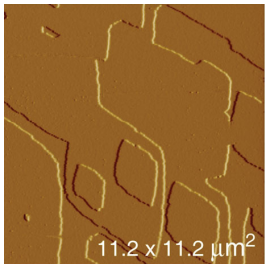
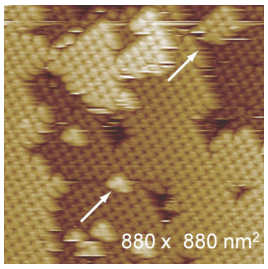
Protein Crystals





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X-Ray Crystallography

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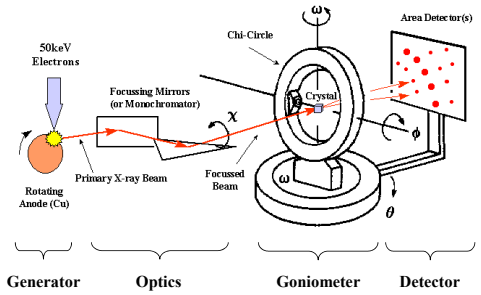
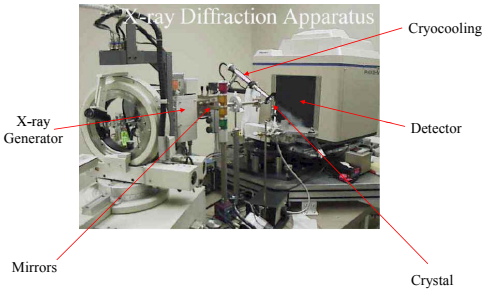
X-Ray Diffraction Experiment

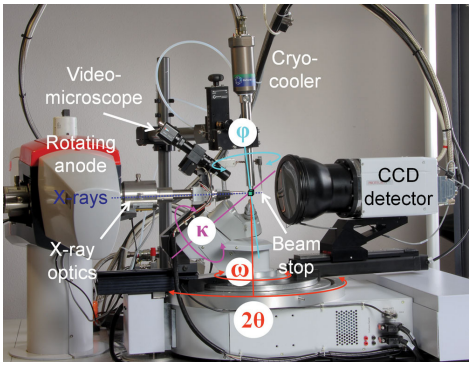


Optional: Cryo for protein samples

X-ray Crystallography Equipment



X-ray Crystallography Equipment



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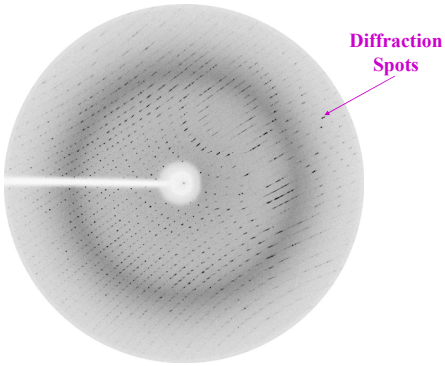
Protein Diffraction Image



Why Spots?

X-ray scattering from individual proteins is diffuse

Spots arise from a phenomenon called diffraction that is based on the crystal lattice

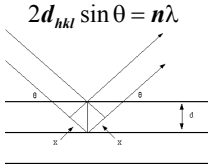
Location of reflections indicates how an object crystallized
230 possibilities

Intensity of reflections contains information about the structure of the object in the crystal

Bragg's Law

Why do we get spots (reflections) and not a diffuse pattern of scattered x-rays?

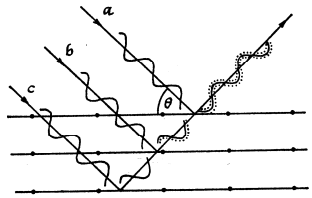
$$2d_{hkl} \sin\theta = n\lambda$$



sin θ = x/d
2d sin θ = 2x

Difference in path (2x) must equal integral number of wavelengths (nλ)

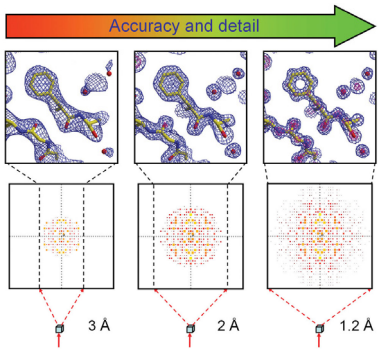
Constructive Interference



d = resolution

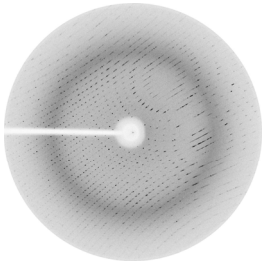
- Condition for reflection

Resolution



http://www.ruppweb.org/Garland/gallery/Ch1/index.htm#5

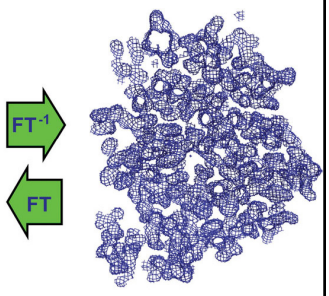
Phase Problem



Every diffraction spot (reflection) has a phase and intensity
- **-The intensities are recorded by the detector**
- **-The phases are lost**
- **-Must have both to reconstruct the image (structure)**

Phase Problem

h	k	l	F(hkl)	φ(hkl)
2	0	0	228.0	180.0
1	0	1	10.4	90.0
2	0	1	901.8	270.0
1	1	1	367.0	332.1
1	2	3	149.3	37.8
8	9	1	97.9	255.1
7	7	2	111.5	139.7

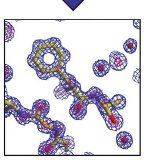


Electron Density

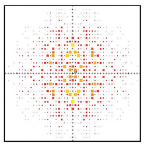
http://www.ruppweb.org/Garland/gallery/Ch1/index.htm#5

Phase Problem

$$\rho(x,y,z) = \frac{1}{V}\sum_{-h}^{h}\sum_{-k}^{k}\sum_{-l}^{l} F_{hkl}\exp[-2\pi i(hx+ky+lz-\alpha_{hkl})]$$



Electron Density



Intensities

The crystallographic phase problem

Phases

http://www.ruppweb.org/Garland/gallery/Ch1/index.htm#5

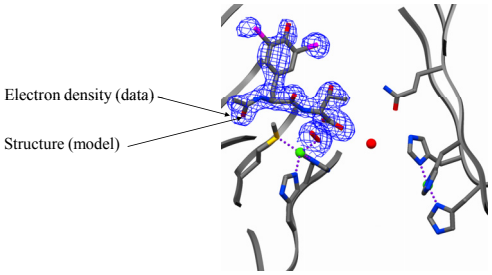
Solutions to the Phase Problem

Molecular replacement
- -Use known structure of close homologue
- -Rotational and translational search for solution

Heavy atom labeling
- -Label the protein with electron dense atoms (Hg)
- -Compare independent datasets collected from native and labeled protein
- -Heavy atom substructure provides initial phases

Anomalous diffraction
- -Crystal must contain atoms with absorption edges between 0.5 and 2.5 Å
- -Compare independent datasets collected at pre-edge and post-edge x-ray energies

Model Building



Crystallography Pros/Cons

Advantages
- -can be "fast" – down to a few months
- -large structures possible (ribosome)
- -very low resolution (down to 0.5 Å)
- -observables typically > refinement parameters

Disadvantages
- -requires crystal formation
- -non-physiological conditions
- -crystal contacts can limit protein motion

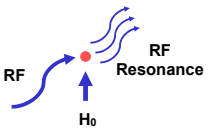
Nuclear Magnetic Resonance

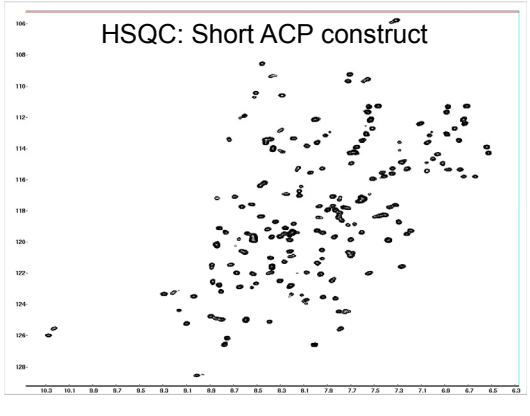
Nuclear Magnetic Resonance

Magnetically align unpaired proton spins (H_0)

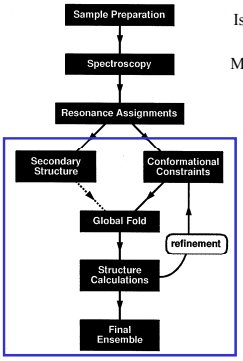
Probe with radio frequency (RF)

Observe resonance





NMR Overview



Isotopic labeling (^{15}N, ^{13}C)

Multiple experiments (pulse sequences)

Positional refinement typically not possible. Dihedral angles used.

As many as 20 structures produced

NMR Experimental Observables

- **Backbone conformation from chemical shifts (Chemical Shift Index- CSI)**
- **Distance constraints from NOEs**
- **Hydrogen bond constraints**
- **Backbone and side chain dihedral angle constraints from scalar couplings**
- **Orientation constraints from residual dipolar couplings**

NMR Pros/Cons

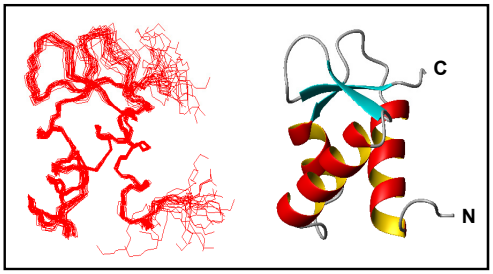
Advantages

-no crystal formation needed
-more physiological conditions

Disadvantages

-results in a set of models that are compatible with data
-size limitation to 200-300 residues (extended recently)
-must label protein with ^{15}N and ^{13}C
-observables typically < refinement parameters

Precision
NMR vs. X-ray



RMSD of the ensemble **Mean coordinate error**

A PDB File

Header contains information about protein and structure
date of the entry, references, crystallographic data,
contents and positions of secondary structure elements

```
HEADER    OXIDOREDUCTASE                  03-OCT-02   1MXT
TITLE     ATOMIC RESOLUTION STRUCTURE OF CHOLESTEROL OXIDASE
TITLE    2 (STREPTOMYCES SP. SA-COO)
COMPND    MOL_ID: 1;
COMPND   2 MOLECULE: CHOLESTEROL OXIDASE;
COMPND   3 CHAIN: A;
COMPND   4 SYNONYM: CHOD;
COMPND   5 EC: 1.1.3.6;
COMPND   6 ENGINEERED: YES;
COMPND   7 OTHER_DETAILS: FAD COFACTOR NON-COVALENTLY BOUND TO THE
COMPND   8 ENZYME
```

A PDB File

Header contains information about protein and structure
date of the entry, references, crystallographic data,
contents and positions of secondary structure elements

```
SOURCE    MOL_ID: 1;
SOURCE   2 ORGANISM_SCIENTIFIC: STREPTOMYCES SP.;
SOURCE   3 ORGANISM_COMMON: BACTERIA;
SOURCE   4 GENE: CHOA;
SOURCE   5 EXPRESSION_SYSTEM: ESCHERICHIA COLI;
SOURCE   6 EXPRESSION_SYSTEM_COMMON: BACTERIA;
SOURCE   7 EXPRESSION_SYSTEM_STRAIN: BL21(DE3)PLYSS;
SOURCE   8 EXPRESSION_SYSTEM_VECTOR_TYPE: PLASMID;
SOURCE   9 EXPRESSION_SYSTEM_PLASMID: PCO202
```

A PDB File

Header contains information about protein and structure
date of the entry, references, crystallographic data,
contents and positions of secondary structure elements

```
AUTHOR   A.VRIELINK,P.LLARIO
REVDAT   1   25-FEB-03 1MXT   0
JRNL      AUTH   P.LLARIO,N.SAMPSON,A.VRIELINK
JRNL      TITL   SUB-ATOMIC RESOLUTION CRYSTAL STRUCTURE OF
JRNL      TITL 2 CHOLESTEROL OXIDASE: WHAT ATOMIC RESOLUTION
JRNL      TITL 3 CRYSTALLOGRAPHY REVEALS ABOUT ENZYME MECHANISM AND
JRNL      TITL 4 THE ROLE OF FAD COFACTOR IN REDOX ACTIVITY
JRNL      REF   J.MOL.BIOL.                V. 326  1635 2003
JRNL      REFN   ASTM JMOBAK  UK ISSN 0022-2836
```

A PDB File

Header contains information about protein and structure
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contents and positions of secondary structure elements

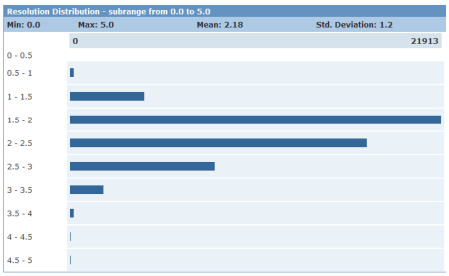
```
REMARK   3  DATA USED IN REFINEMENT.
REMARK   3   RESOLUTION RANGE HIGH (ANGSTROMS) : 0.95
REMARK   3   RESOLUTION RANGE LOW  (ANGSTROMS) : 28.00
REMARK   3   DATA CUTOFF            (SIGMA(F)) : 0.000
REMARK   3   COMPLETENESS FOR RANGE        (%) : 94.1
REMARK   3   CROSS-VALIDATION METHOD           : FREE R
REMARK   3   FREE R VALUE TEST SET SELECTION   : RANDOM
REMARK   3
REMARK   3  FIT TO DATA USED IN REFINEMENT (NO CUTOFF).
REMARK   3   R VALUE   (WORKING + TEST SET, NO CUTOFF) : 0.110
REMARK   3   R VALUE           (WORKING SET, NO CUTOFF) : 0.110
REMARK   3   FREE R VALUE                (NO CUTOFF) : 0.132
REMARK   3   FREE R VALUE TEST SET SIZE (%, NO CUTOFF) : 5.000
REMARK   3   FREE R VALUE TEST SET COUNT   (NO CUTOFF) : 13180

REMARK   3   TOTAL NUMBER OF REFLECTIONS   (NO CUTOFF) : 263551
```

Resolution:
Low > 3 Å
Mid 2-3 Å
High 1.5-2 Å
Very High < 1.5 Å

R factor (residual):
Low resolution ~ 27%
Mid resolution ~ 22 %
High resolution ~ 29 %
Very High res ~ 15%

Resolution



A PDB File

Header contains information about protein and structure
date of the entry, references, crystallographic data,
contents and positions of secondary structure elements

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HELIX   14  14 ALA A  289  THR A  304  1                                  16
HELIX   15  15 THR A  402  GLN A  405  5                                   4
HELIX   16  16 ASN A  406  GLY A  425  1                                  20
HELIX   17  17 ASP A  474  ILE A  478  5                                   5
HELIX   18  18 PRO A  486  VAL A  506  1                                  21
SHEET    1   A 6 HIS A 248  GLN A 255  0
SHEET    2   A 6 TYR A 261  LYS A 268 -1  O  GLU A 266   N  GLN A 249
SHEET    3   A 6 LEU A 274  LEU A 287 -1  O  LEU A 275   N  GLN A 267
SHEET    4   A 6 TYR A  10  ILE A  16  1  N  VAL A  14   O  PHE A 286
SHEET    5   A 6 THR A  36  GLU A  40  1  O  LEU A  37   N  VAL A  15

SHEET    6   A 6 VAL A 242  THR A 246  1  O  THR A 243   N  MET A  38
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A PDB File

Body of PDB file contains information about the atoms in the structure

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ATOM     76  N   PRO A  12      31.129  -4.659  43.245  1.00  9.00           N
ATOM     77  CA  PRO A  12      32.426  -4.662  42.542  1.00  9.00           C
ATOM     78  C   PRO A  12      32.423  -4.009  41.182  1.00  8.02           C
ATOM     79  O   PRO A  12      33.267  -3.177  40.892  1.00  8.31           O
ATOM     80  CB  PRO A  12      32.791  -6.126  42.592  1.00 10.02           C
ATOM     81  CG  PRO A  12      32.190  -6.663  43.857  1.00 10.12           C
ATOM     82  CD  PRO A  12      30.850  -5.927  43.925  1.00  9.87           C
ATOM     90  N   ALA A  13      31.485  -4.468  40.316  1.00  8.06           N
ATOM     91  CA  ALA A  13      31.357  -3.854  39.004  1.00  7.28           C
ATOM     92  C   ALA A  13      29.947  -3.309  38.814  1.00  7.21           C
ATOM     93  O   ALA A  13      28.969  -3.932  39.200  1.00  7.56           O
ATOM     94  CB  ALA A  13      31.636  -4.879  37.897  1.00  8.54           C
```

Atom number

Atom name

Residue name

Residue number

A PDB File

Body of PDB file contains information about the atoms in the structure

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ATOM     79  O   PRO A  12      33.267  -3.177  40.892  1.00  8.31           O
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ATOM     90  N   ALA A  13      31.485  -4.468  40.316  1.00  8.06           N
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ATOM     94  CB  ALA A  13      31.636  -4.879  37.897  1.00  8.54           C
```

Coordinates in Å

(X,Y,Z)

Mean coordinate error:

Low > 3 Å	.4 Å
Mid 2-3 Å	.3 Å
High 1.5-2 Å	.2 Å
Very High < 1.5 Å	.1 Å

A PDB File

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ATOM     79  O   PRO A  12      33.267  -3.177  40.892  1.00  8.31           O
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ATOM     82  CD  PRO A  12      30.850  -5.927  43.925  1.00  9.87           C
ATOM     90  N   ALA A  13      31.485  -4.468  40.316  1.00  8.06           N
ATOM     91  CA  ALA A  13      31.357  -3.854  39.004  1.00  7.28           C
ATOM     92  C   ALA A  13      29.947  -3.309  38.814  1.00  7.21           C
ATOM     93  O   ALA A  13      28.969  -3.932  39.200  1.00  7.56           O
ATOM     94  CB  ALA A  13      31.636  -4.879  37.897  1.00  8.54           C
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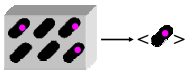

- Occupancy of 0.5

Fractional occupancy

A PDB File

Body of PDB file contains information about the atoms in the structure

```
ATOM     76  N   PRO A  12      31.129  -4.659  43.245  1.00  9.00           N
ATOM     77  CA  PRO A  12      32.426  -4.662  42.542  1.00  9.00           C
ATOM     78  C   PRO A  12      32.423  -4.009  41.182  1.00  8.02           C
ATOM     79  O   PRO A  12      33.267  -3.177  40.892  1.00  8.31           O
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ATOM     82  CD  PRO A  12      30.850  -5.927  43.925  1.00  9.87           C
ATOM     90  N   ALA A  13      31.485  -4.468  40.316  1.00  8.06           N
ATOM     91  CA  ALA A  13      31.357  -3.854  39.004  1.00  7.28           C
ATOM     92  C   ALA A  13      29.947  -3.309  38.814  1.00  7.21           C
ATOM     93  O   ALA A  13      28.969  -3.932  39.200  1.00  7.56           O
ATOM     94  CB  ALA A  13      31.636  -4.879  37.897  1.00  8.54           C
```

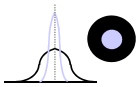

B-factor Å^2