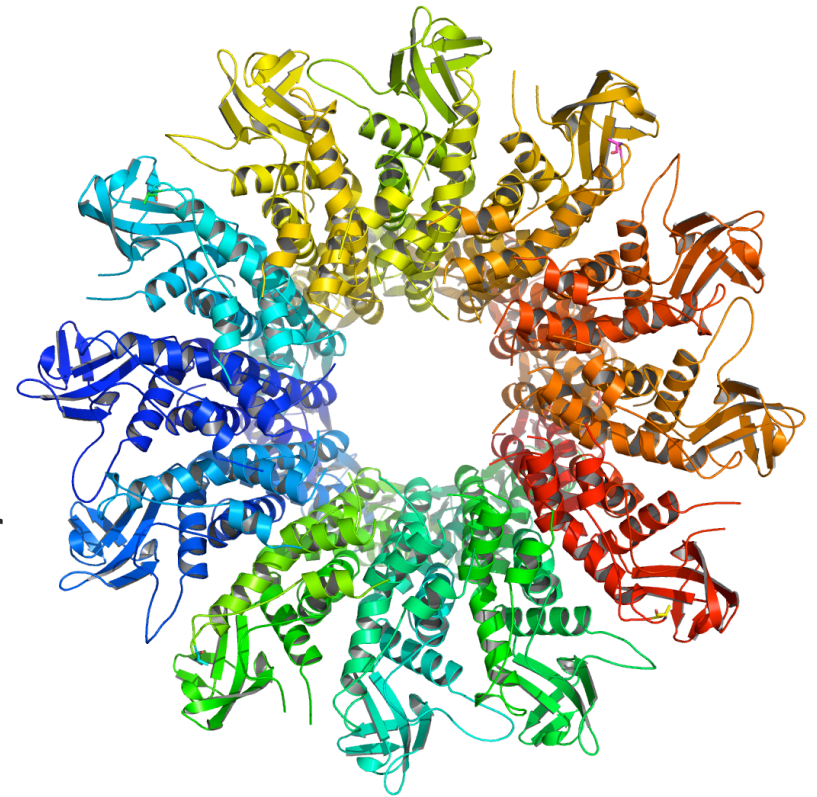


Update on the new Advanced Protein Crystallization Facility



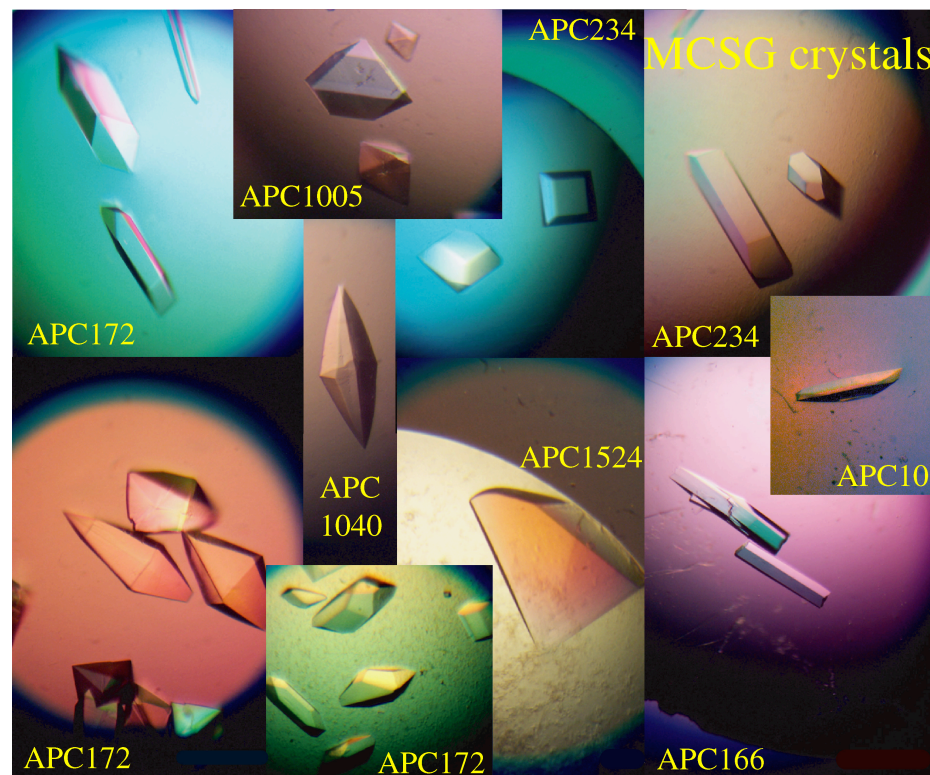
Concept

- The Advanced Protein Crystallization Facility (APCF) will establish a state-of-the-art highly automated laboratory, integrated with a scientific collaboration facility, for production and characterization of proteins and protein crystals in order to take full advantage of Argonne's capabilities for determining the three-dimensional structures of proteins and characterization of their functions.



Advanced Protein Crystallization Facility Scope

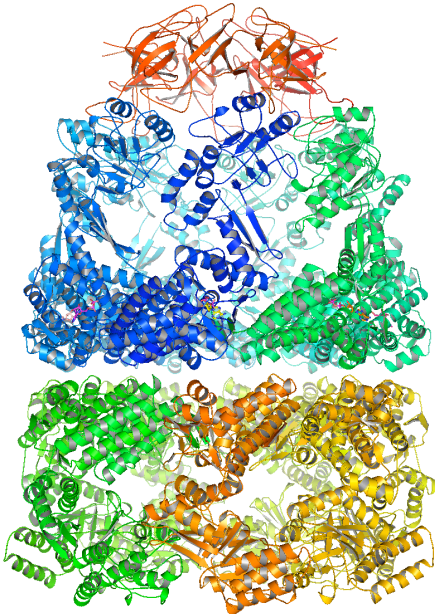
- The APCF will host Structural Genomics, Structural Biology and Biological Systems Science Programs:
 - It will enhance APS capabilities in Biology and Life Sciences,
 - It will serve as a user facility for Illinois Academic and Research Institutions,
 - It will be a resource for APS Biology and Life Sciences Community.



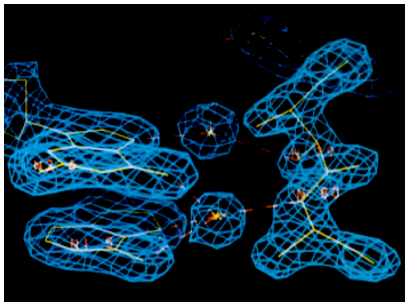
Biology Rests on Structural Observations

Conceptually
Functionally
Mechanistically

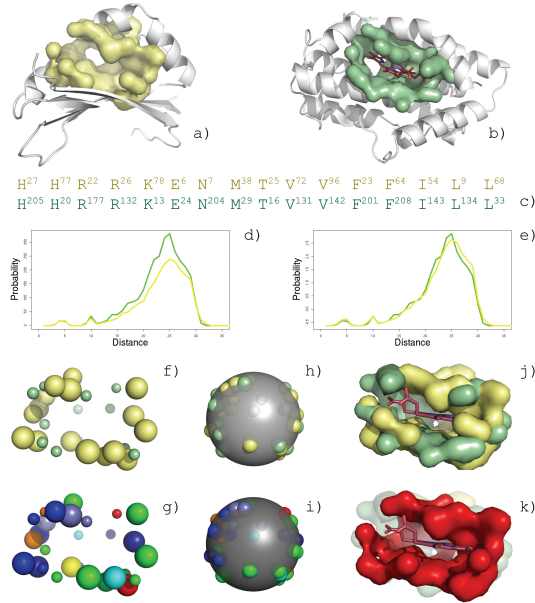
- Proteins fold inside chaperonin cage



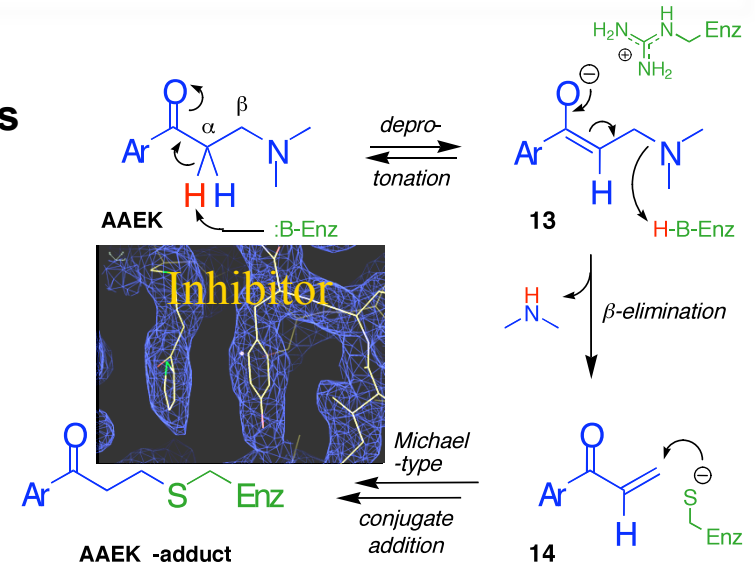
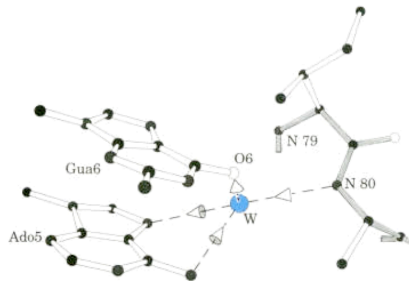
- Water mediates specific protein-DNA interactions



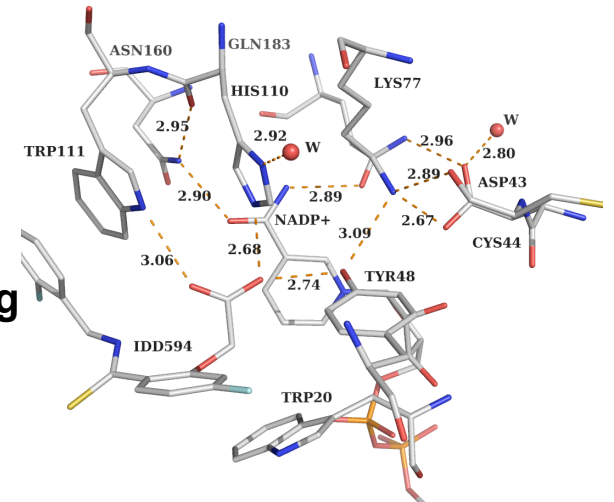
- Identical active sites found in two different protein scaffolds



- Deuterium atoms and proton network in the structure of human aldose reductase using a combined X-ray and neutron diffraction experiment



- Structure shows how inhibitor blocks activity of sortase B from *B. anthracis*



Protein Structure Determination Starts with Genome (DNA) Sequence

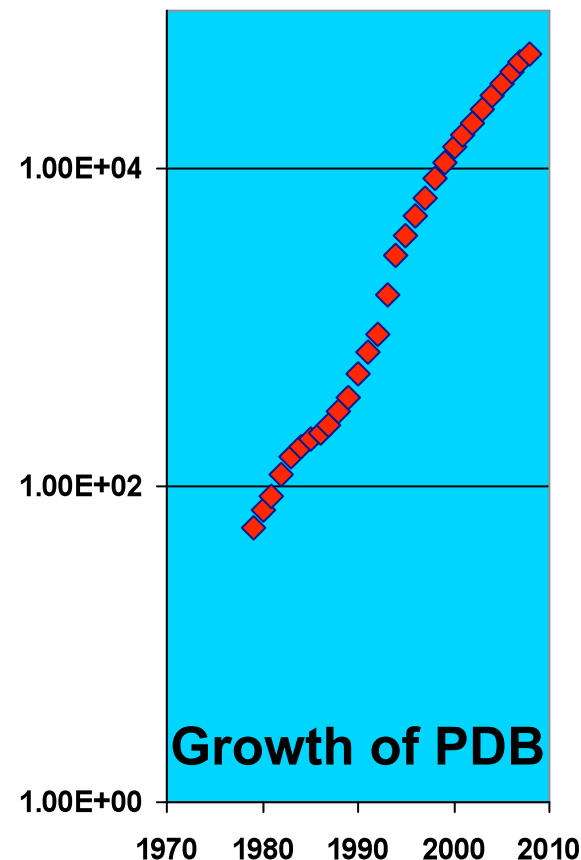
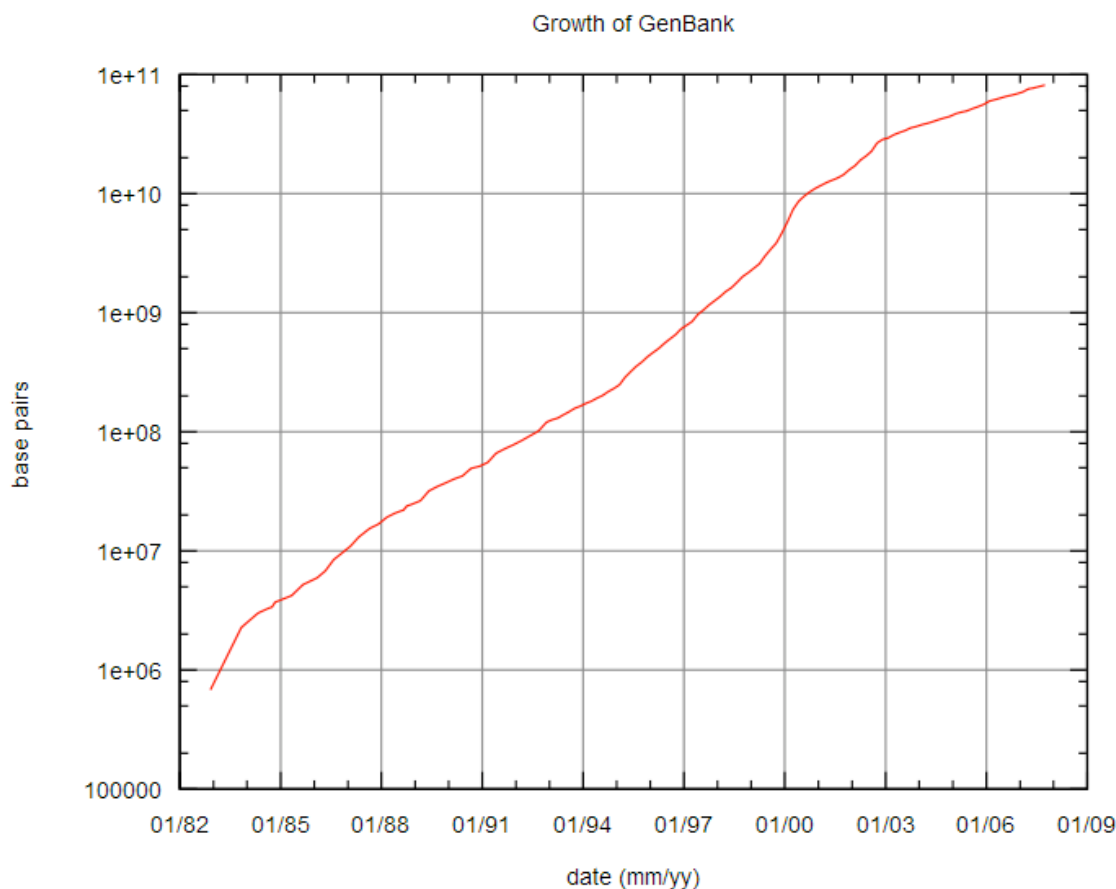
- The Human Genome Sequencing efforts of a decade ago gave rise to the technology that currently allows genome sequences to be determined in days rather than years.
- Each genome sequence describes the predicted primary structure of thousands of proteins for each organism.
- As the technology used to determine genome sequences becomes more broadly affordable, more laboratories are sequencing a variety of environmental sites as well as microbial communities within humans (microbiomes).
- The number of proteins for which there is no three dimensional structure determined or function known is increasing at a precipitous rate.
- In order to keep pace with the generation of genome sequence data, approaches to increase the rate of protein and crystal production as well as structure determination and function validation must be advanced in a comparable fashion.
- Crystallization of macromolecules is a major bottleneck in structure determination.



GenBank Sequence Data Growth

~ 12.6×10^6 protein sequences
in NR database

~ 1.8×10^4 protein structures
with NR sequences in PDB



Data double every 12-15 months

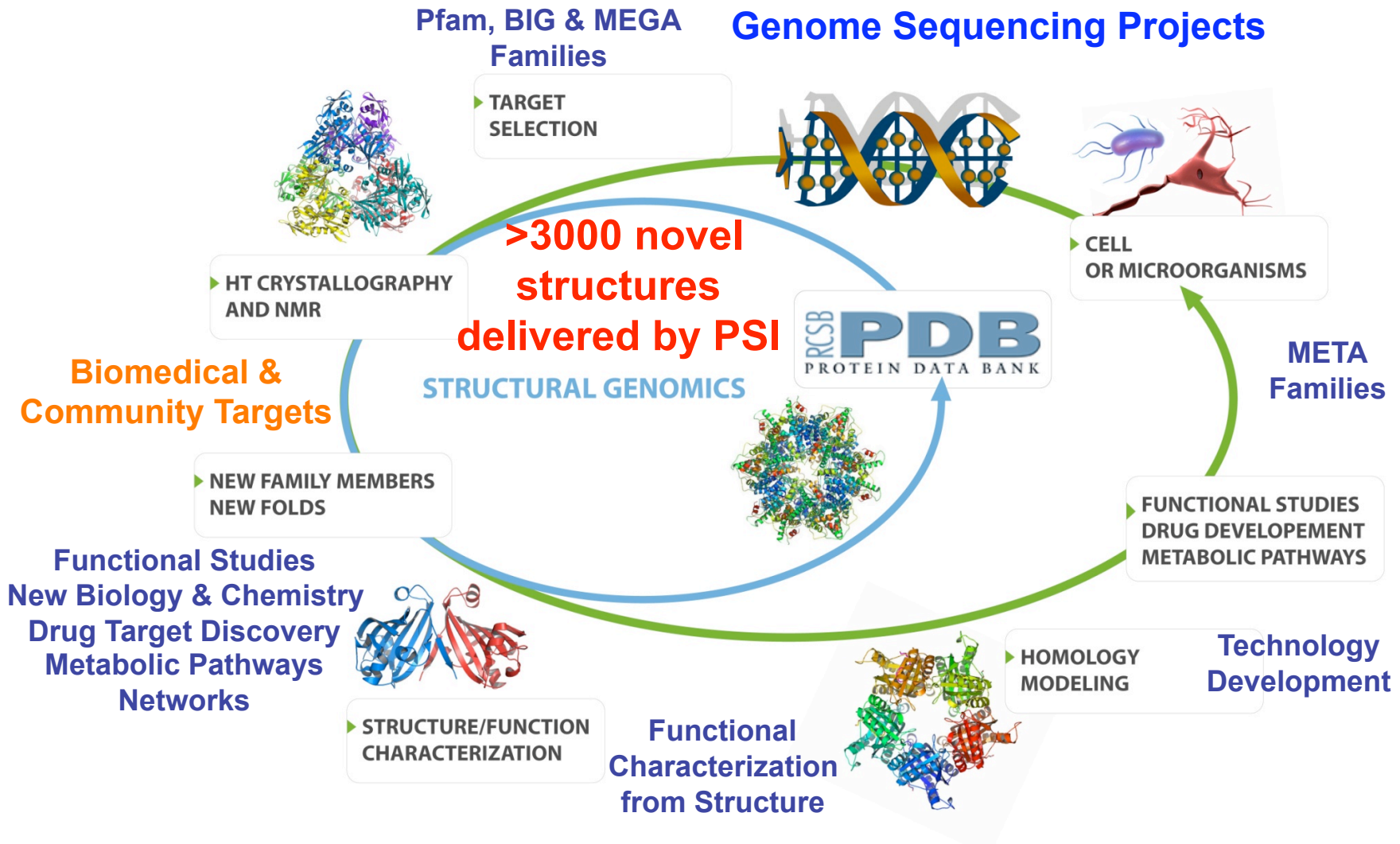
~ 7.3×10^4 protein
structures in PDB



Protein Structure Initiative: To make the three-dimensional atomic level structures of most proteins easily available from knowledge of their corresponding DNA sequences



From Genomics to Structural Biology to Biology - Exploring the Unknown
Fold → Family → Organism → New Biology & Chemistry



Objective

- Proteins are the molecular machines of the body, responsible for virtually everything that we do.
- The structures being discovered are as diverse as the many functions performed by proteins across the biological world.
- The primary objective is to determine the structures of important protein targets nominated by the broad biology community.
- The long-term goals are to provide high-quality structural models for a significant number of biomedically and biologically important proteins and protein families, to improve the structure determination pipeline so that it can also be applied to challenging proteins and protein complexes, and to develop advanced protein production and crystallization methods for these protein families.
- The ultimate goal is to build, together with the structural biology community, a foundation for 21st century structural biology where the structures of virtually any protein or protein complex is available to the biology community through the Protein Data Bank.

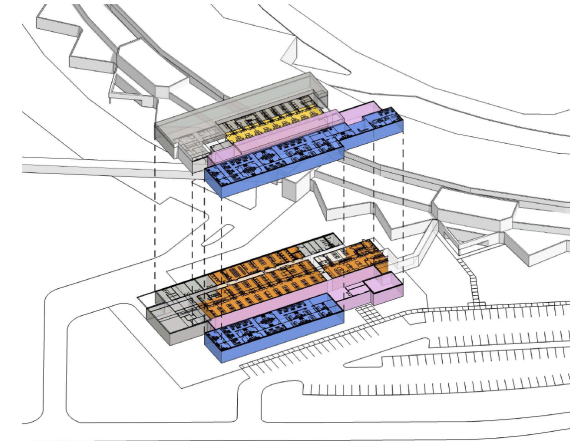
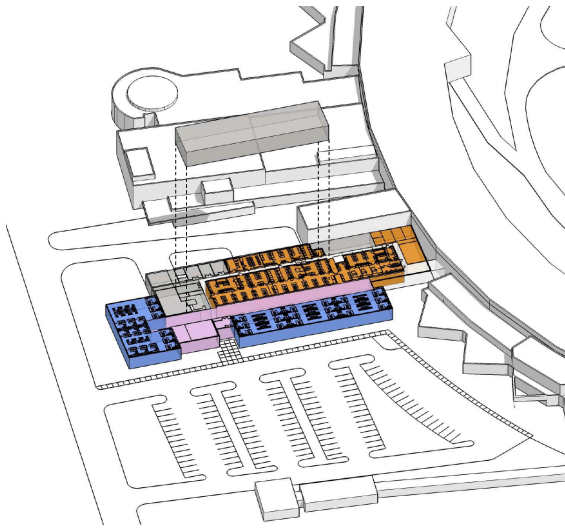
APCF Collaborative Research

- Enable collaborative research by providing research facilities for community-nominated projects, by hosting visiting scientists and by developing informatics gateways to facilitate collaborations with the biology community.
- Develop of high-throughput technologies in protein expression, purification and crystallization to tackle all classes of proteins and protein complexes.
- Develop high-throughput technologies for automated structure determination using synchrotron radiation that includes real-time structure solution, automated model building, accelerated structure refinement, rapid model validation, and deposition.
- Develop and apply advanced sequence- and structure-based bioinformatics tools to annotate solved structures.
- Disseminate the information and materials to the biology community.

APCF Components

- Bioinformatics,
- Gene Cloning and Protein Expression,
- Eukaryotic and Viral Proteins Expression,
- Purification and Crystallization,
- Data Analysis,
- Structure Determination,
- Databases and LIMS.

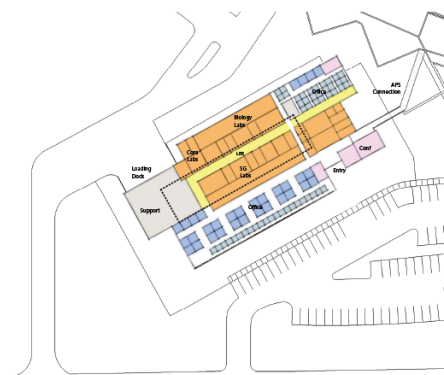
Evolution of APCF Concept



Alternate 3

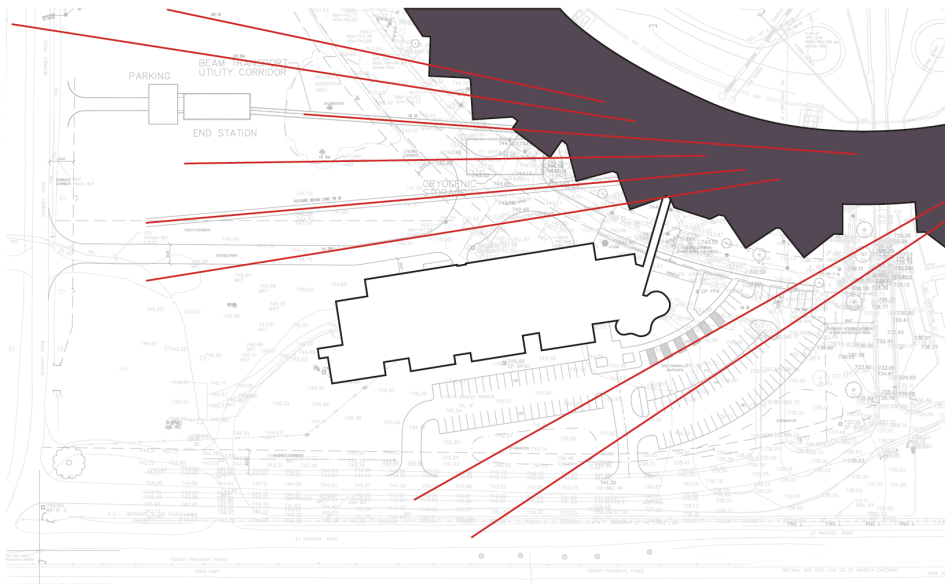


Alternate 1



Alternate 2

Association with APS



APCF Summary

Project Data

- 55,486 GSF
- 28,519 NSF
- Building Population - 70 persons
- Private Offices - 33 total
- Open Workstations - 37 total
- Conference Rooms - 5 total
 - (1) 40-person
 - (1) 20-person
 - (1) 10-person
 - (2) 4-person
- Break Room

Offices

- Admin Suite 595sft
- MCSG 4,582sft
- MPEG + ESR-SFA 1,434sft
- Core Labs 176sft

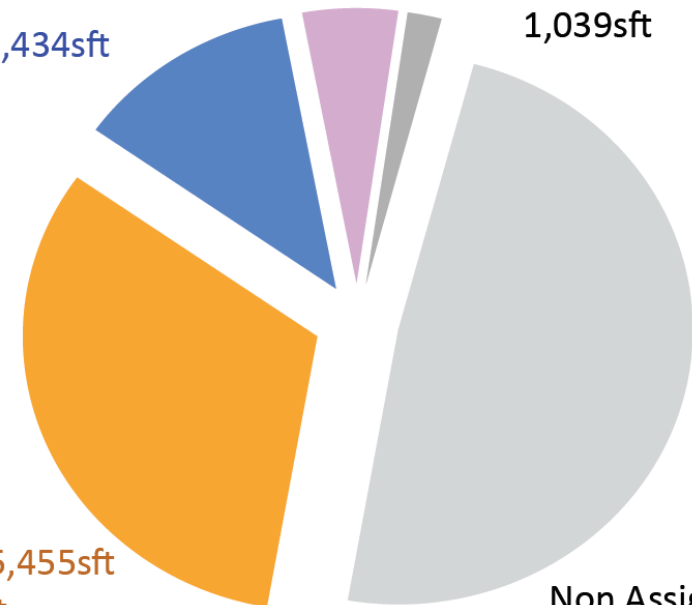
Conference Areas

2,897 sft

Misc Bldg Support
1,039sft

Lab Spaces

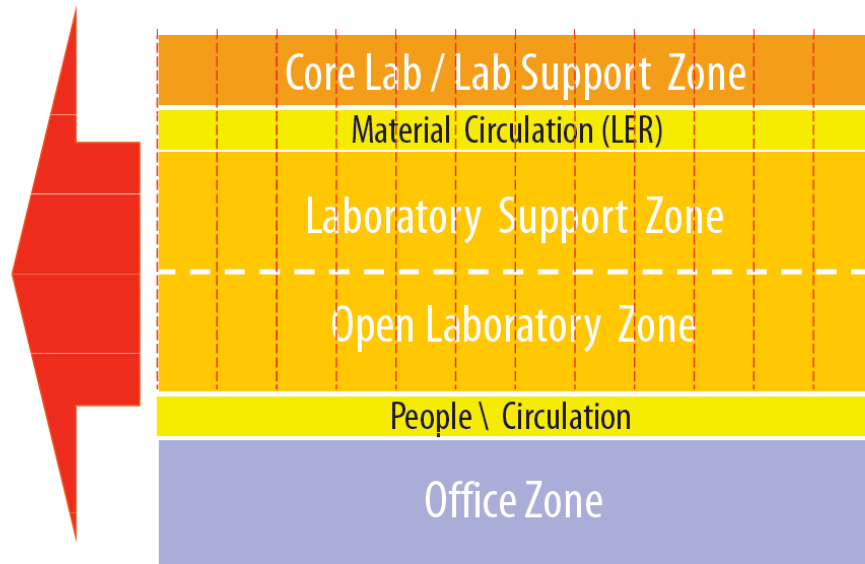
- MCSG 10,368sft
- MPEG + ESR-SFA 5,455sft
- Core Labs 1,973sft



Program Areas

APCF Laboratory Concept

Planning Concepts – Expandable



Planning Concepts

- State-of-the-Art
- Flexible
- Adaptable
- Modular
- Expandable
- Safe

Efficient Engineering & Sustainable Design

APCF Current Design



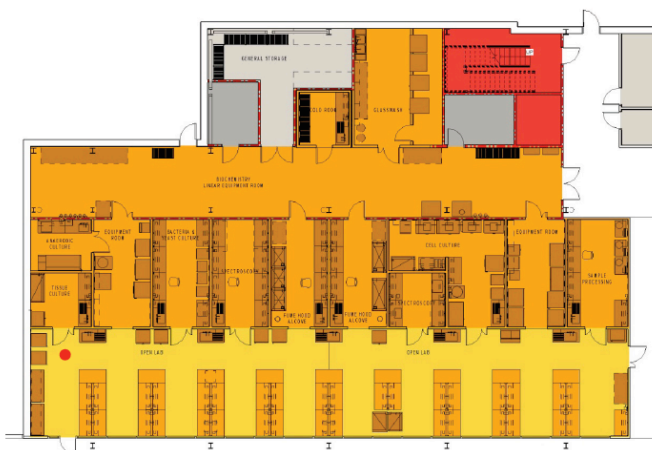
APCF - Focus on Biology Community at the APS



APCF Components



APCF Biochemical Laboratories



Partial Plan:
Membrane Protein Engineering Group (MPEG)
Environment Sensing & Response Scientific Focus Area Program (ESR-SFA)

B001 - Open Lab



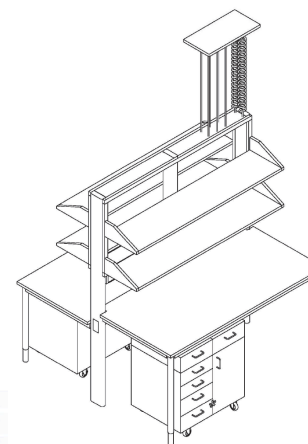
APCF Gene Cloning and Protein Expression Labs



Partial Plan
Midwest Center for Structural Genomics



SG001A – Open Lab

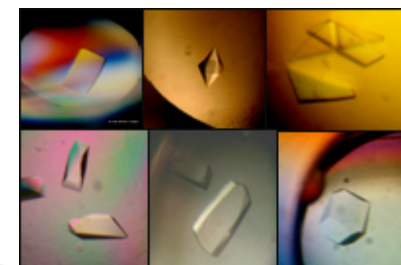




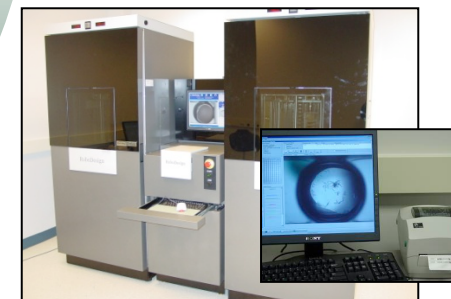
HTP Protein Crystallization



X-ray test &
Data collection



Crystal freezing



D
B

Optimized crystallization screens tested on thousands of proteins.
4 "best-96" MCSG-1 – MCSG-4 crystallization screens.

Commercial screens

Optimized droplet size for production data quality crystals.

Custom screens making

Cryo-condition database.

Reductive methylation and *in situ* proteolysis.

Dispensing of reservoirs

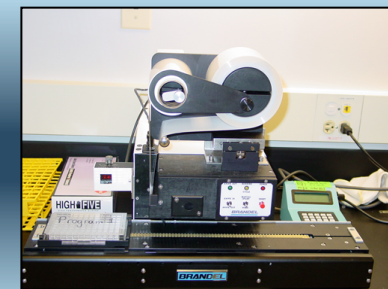
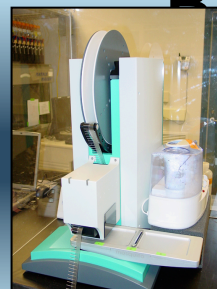
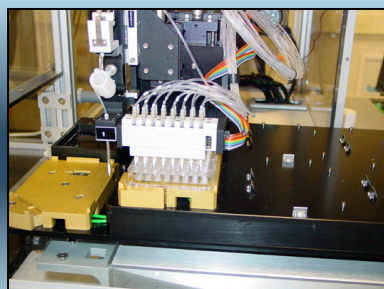
Affinity tag as a variable in crystallization.

Ligand screening.

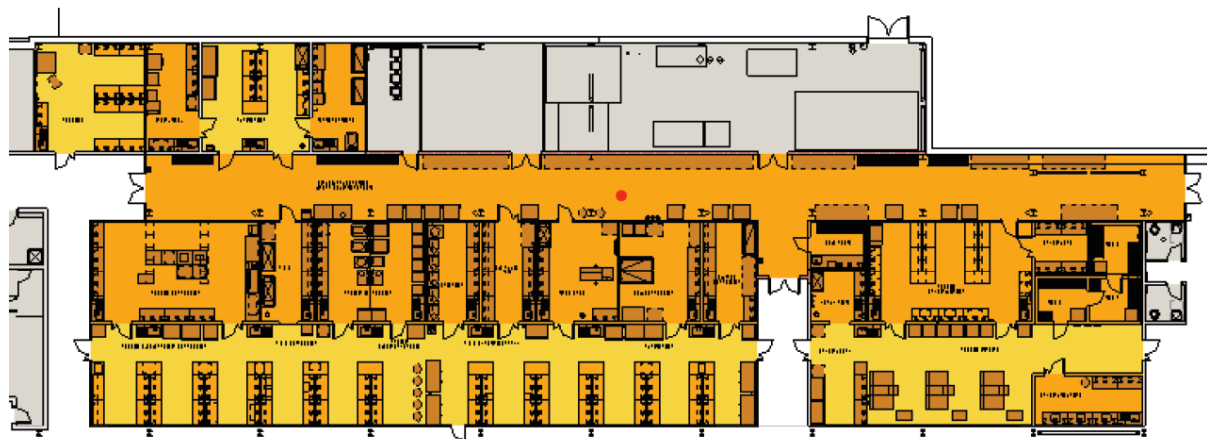
Set up crystallization

Visualization
Manual and robotic

Crystal optimization



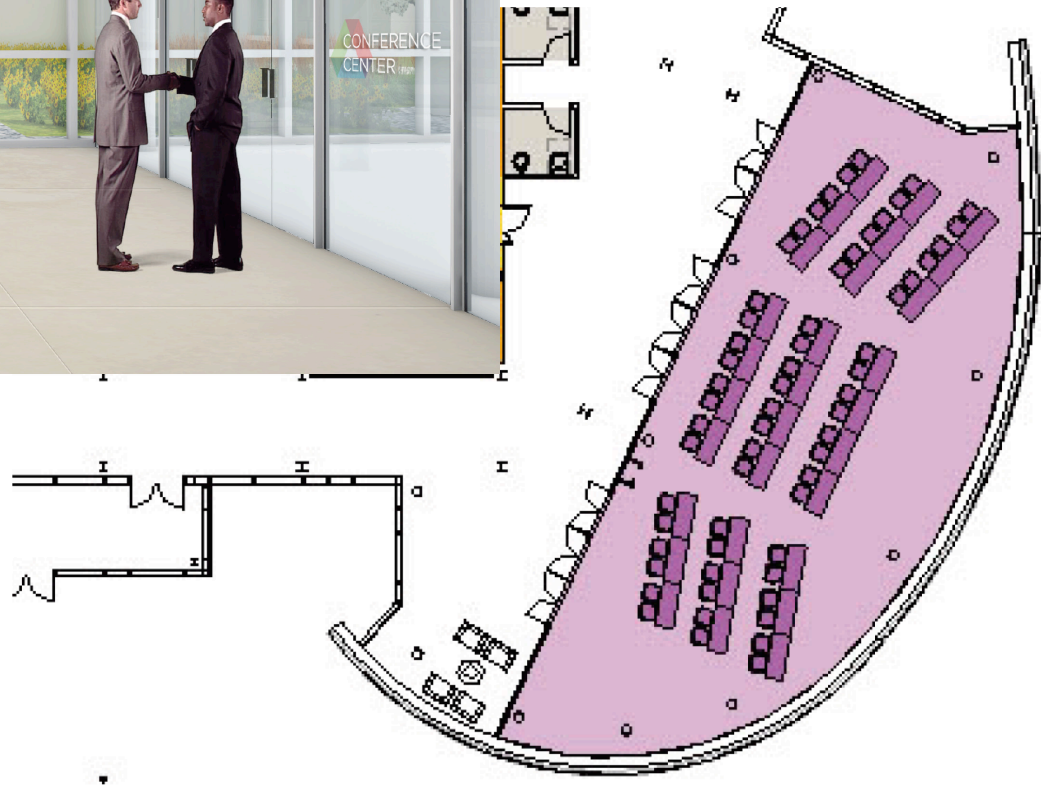
APCF Structural Genomics and Structural Biology Labs



Partial Plan
Midwest Center for Structural Genomics

B020 – Linear Equipment Room

APCF Conference Facilities



Thank you

