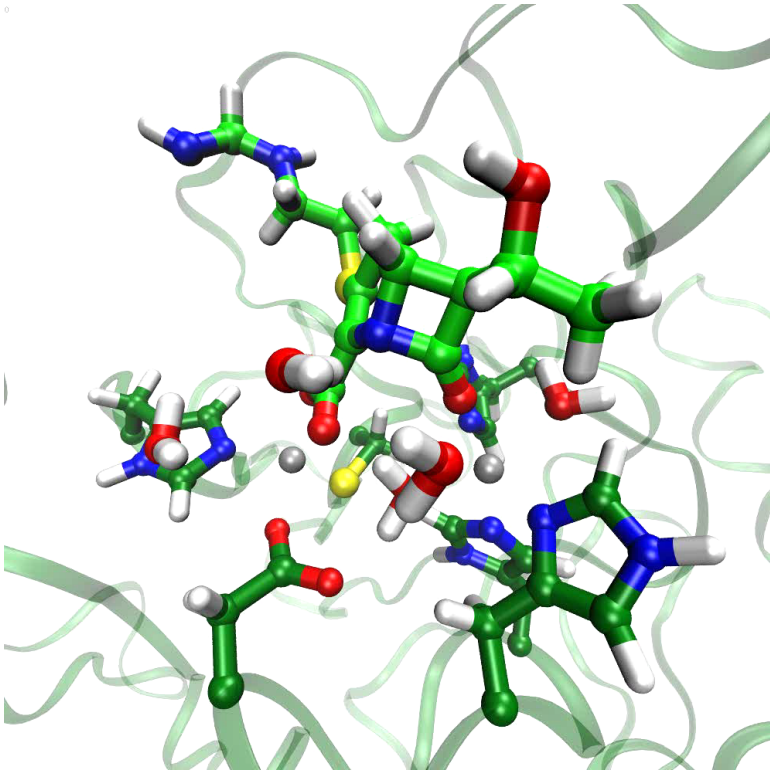
# Structural Biology Using Light Sources Helps Combat Antibiotic Resistance and Infectious Diseases

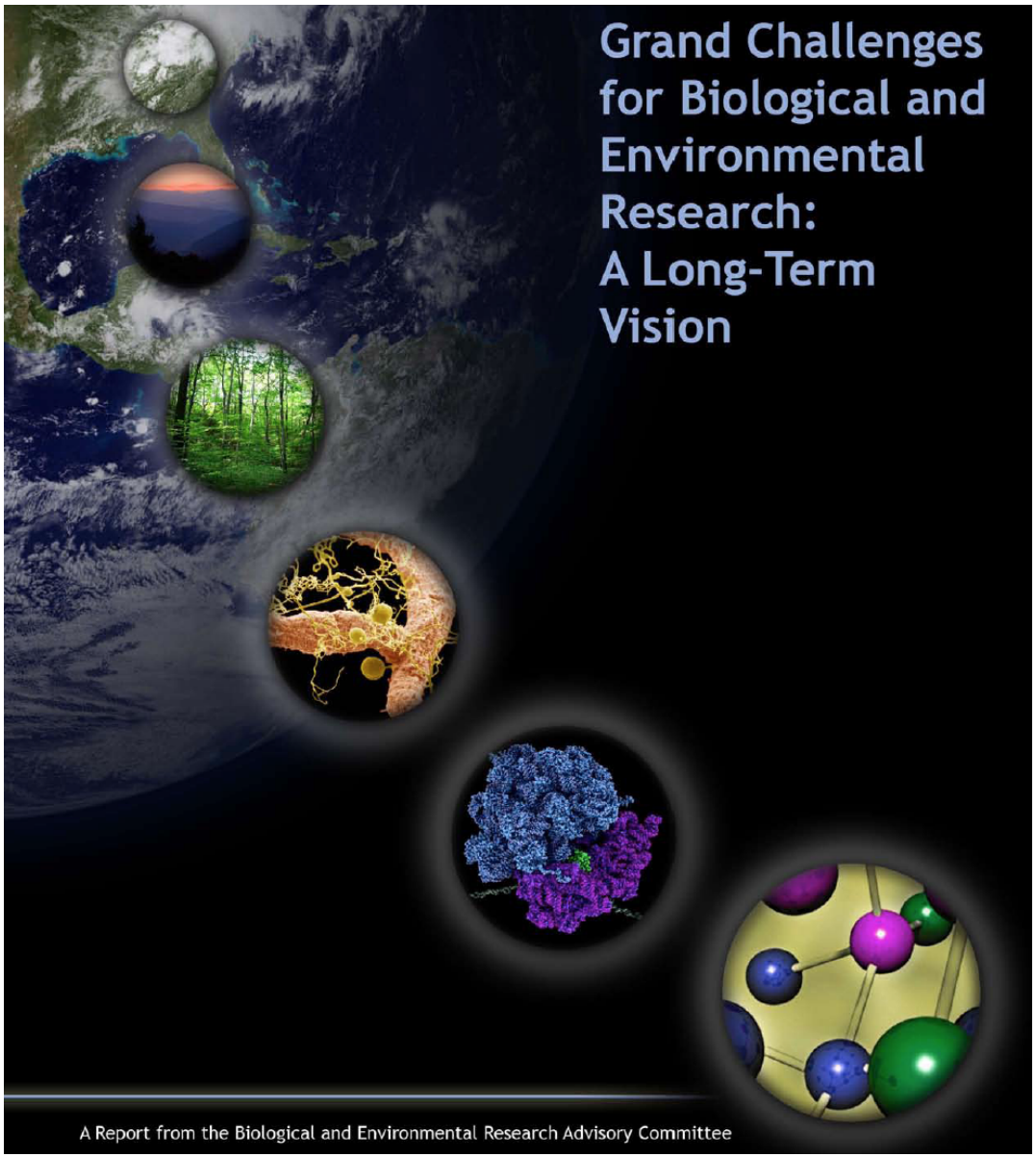

**Andrzej Joachimiak**

***Argonne National Laboratory***

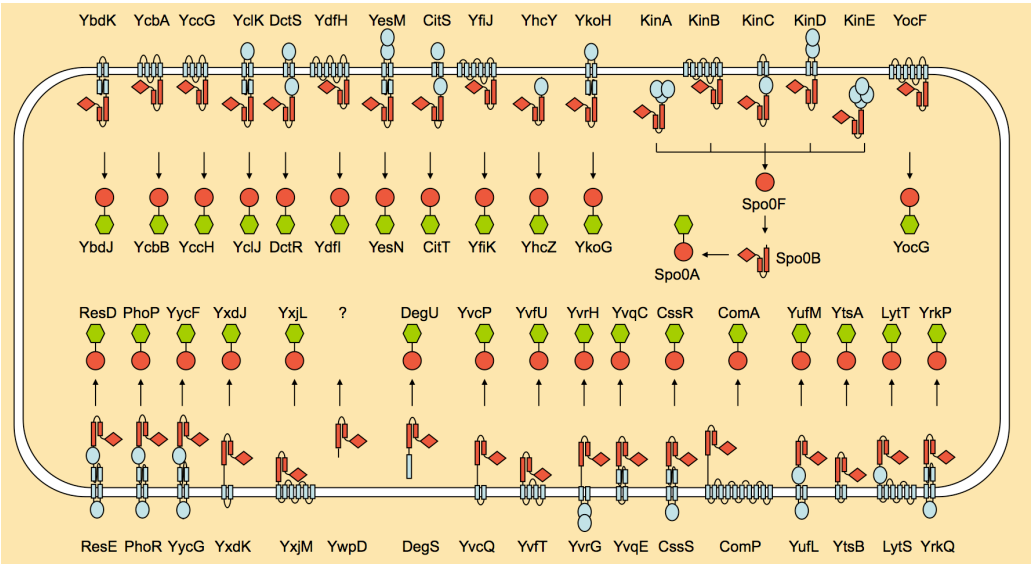

**BERAC 2014, Washington DC**



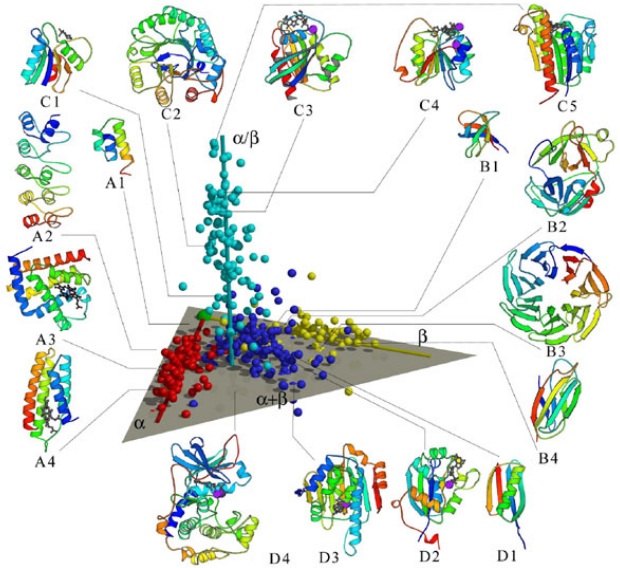
# Addressing Complexity of Biological Systems



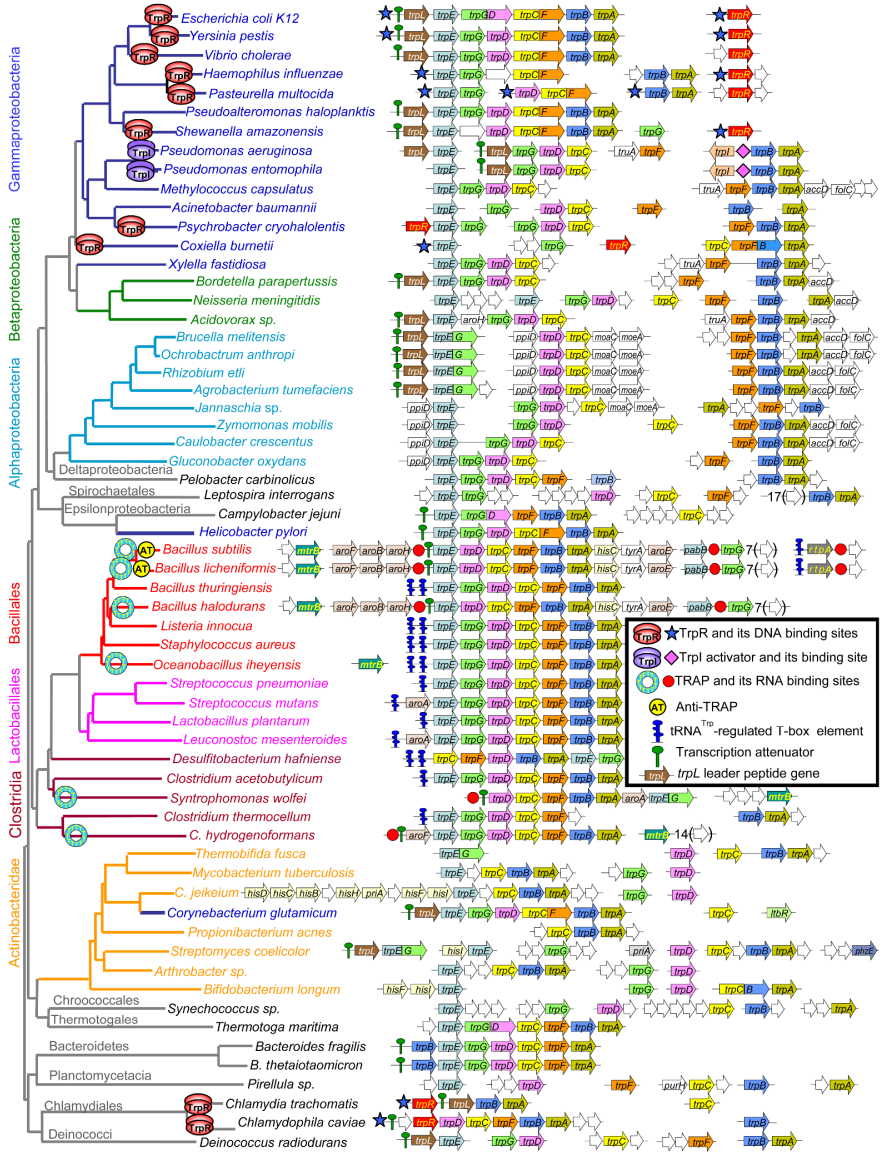
# Addressing Complexity of Biological Systems




H. Szurmant



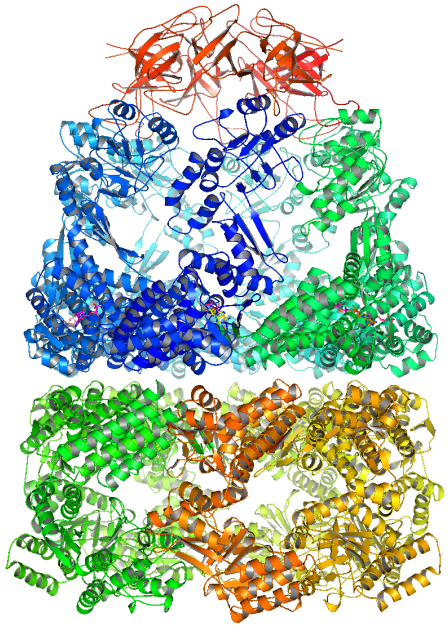

Choi & Kim, *PNAS*, 2006



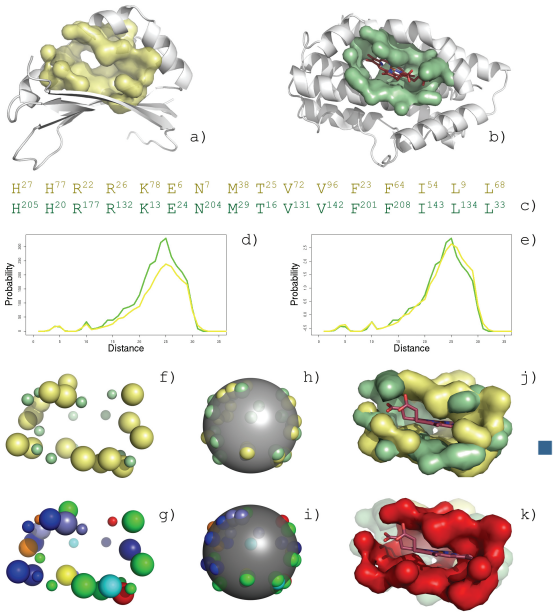

Merino *et al. Curr Opin Microbiol. 2008*

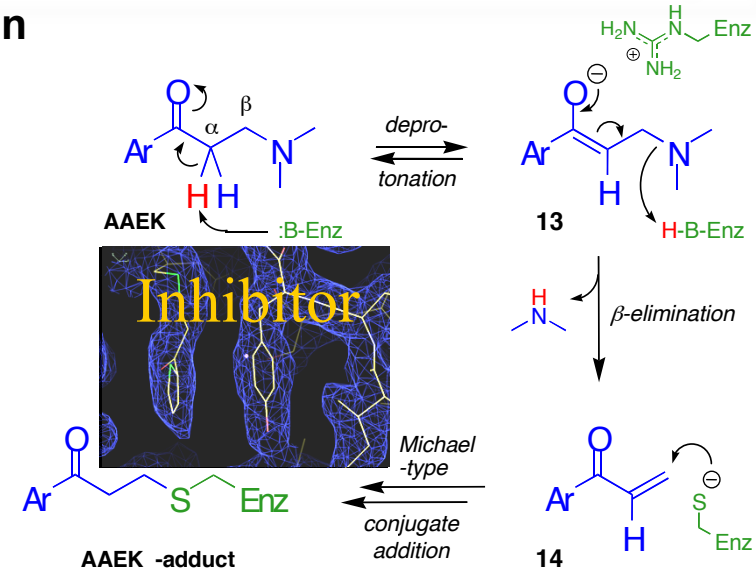
# Biology Rests on Macromolecular Structures
## Conceptually Functionally Mechanistically

- **Proteins fold inside chaperonin cage**

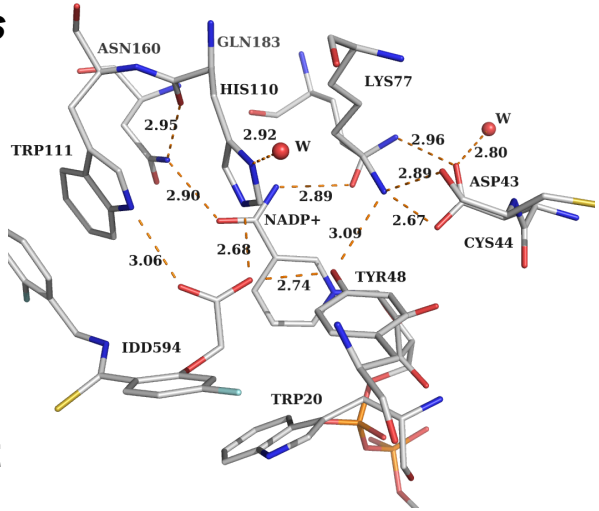


- **Identical active sites found in two different protein scaffolds**

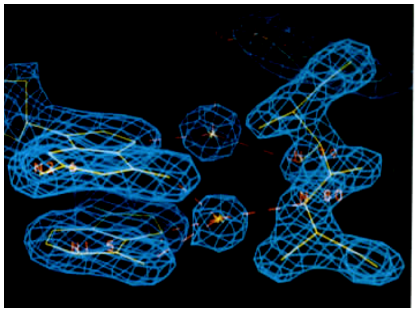


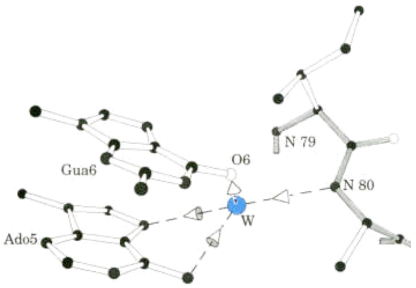




- **Structure shows how the inhibitor blocks activity of sortase B from *B. anthracis***




- **Water mediates specific protein–DNA interactions**






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

# Macromolecular Crystallography Vision

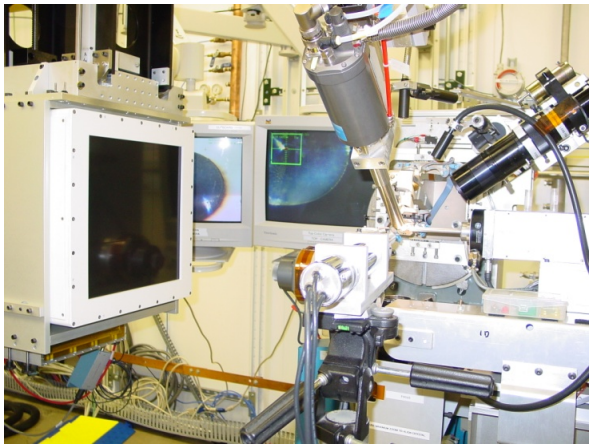
- **Challenging biological problems**
  - Protein-protein and protein-nucleic acid complexes
  - Large transmembrane proteins
  - Large multi-component assemblies and multi-domain eukaryotic proteins
  - Viral particles and assemblies
  - Cells and cell microcompartments
  - Crystallography *in cellulo*
- **Nano and micro X-ray crystallography**
- **Low resolution structures**
- **Application of advanced hybrid methods**
- **HTP crystallography** – rapid response to emerging diseases
- **Atomic and subatomic resolution structures**
- **Radiation damage mitigation/correction**
- **SAD/MAD phasing**

# Biology User Facilities at U. S. Light Sources Support Basic Research in Many Important Areas

| Bioenergy | Environmental Studies | Human Health |
|---|---|---|
| Biofuels | Climate change | Infectious Diseases |
| Biomass deconstruction | Carbon cycling | Antibiotic Resistance |
| Systems engineering | Microbial Dark Matter | |

The Advanced Photon Source and the Advanced Protein Characterization Facility at Argonne National Laboratory

# Structure Determination Using SBC 19-ID/19-BM

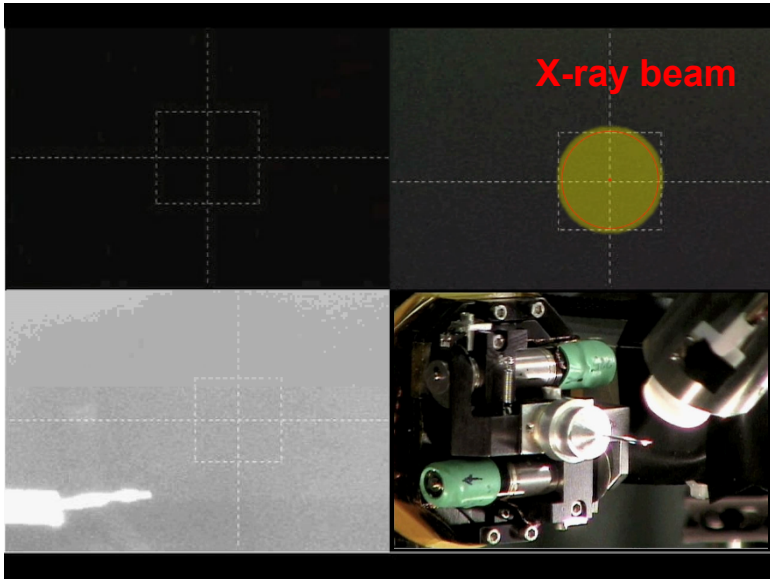


Two highly productive beamlines
4,289 PDB deposits
1,488 publications

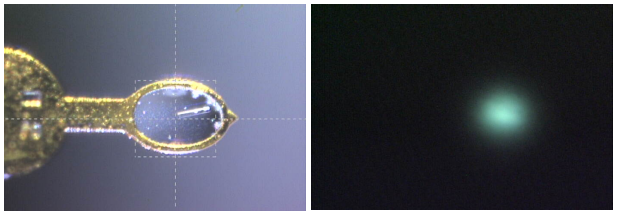
HKL3000 semi-automated structure determination:
>1,500 PDB deposits
>300 citations

Highly flexible beam size from 5, 10, 15, and 20 × 20 µm to 200 × 200 µm

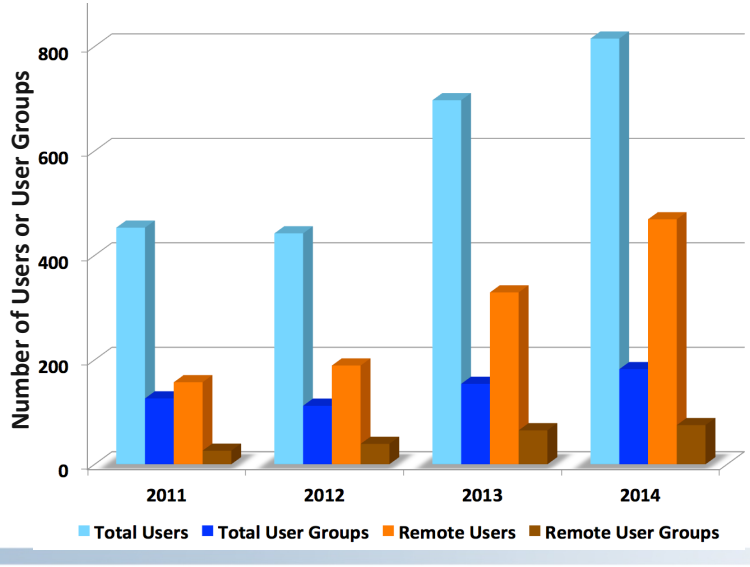
Loop auto-centering
Crystal "point-and-click" centering
**Remote access**

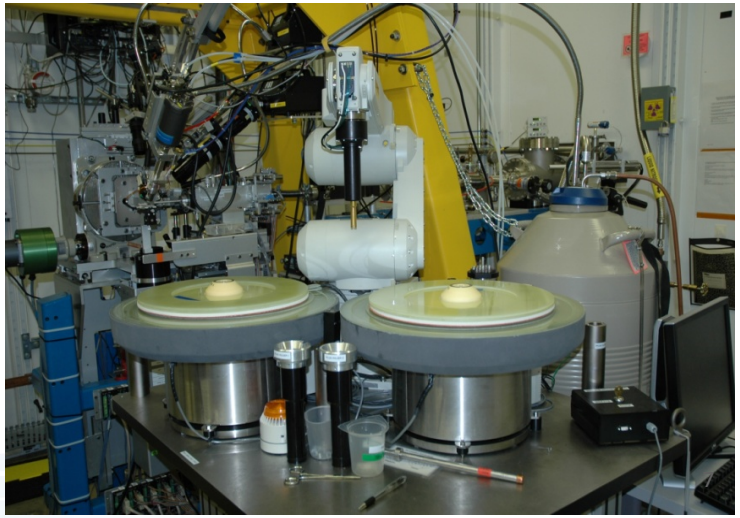



Sample centering



Microcrystals and mini-beams



# SBC Science Highlights

## The Nobel Prize in Chemistry 2009

"for studies of the structure and function of the ribosome"

Photo: MRC Laboratory of Molecular Biology

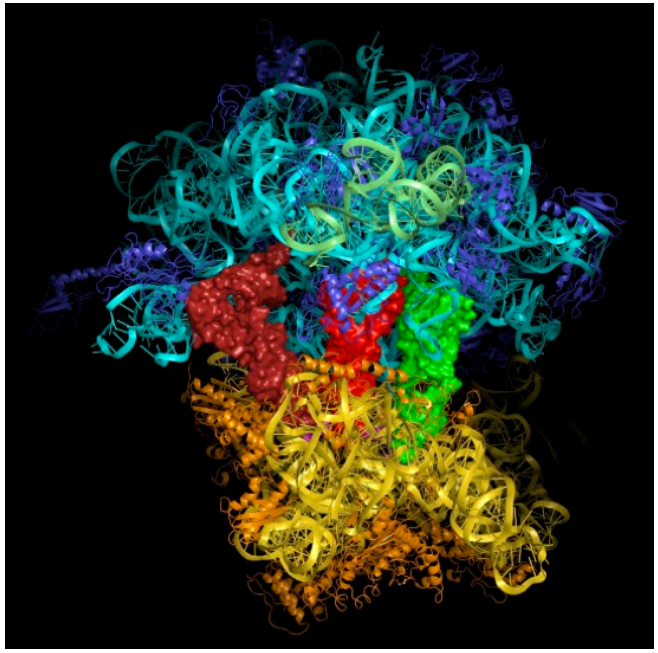
**Venkatraman Ramakrishnan**

Credits: Michael Marsland/Yale University

**Thomas A. Steitz**

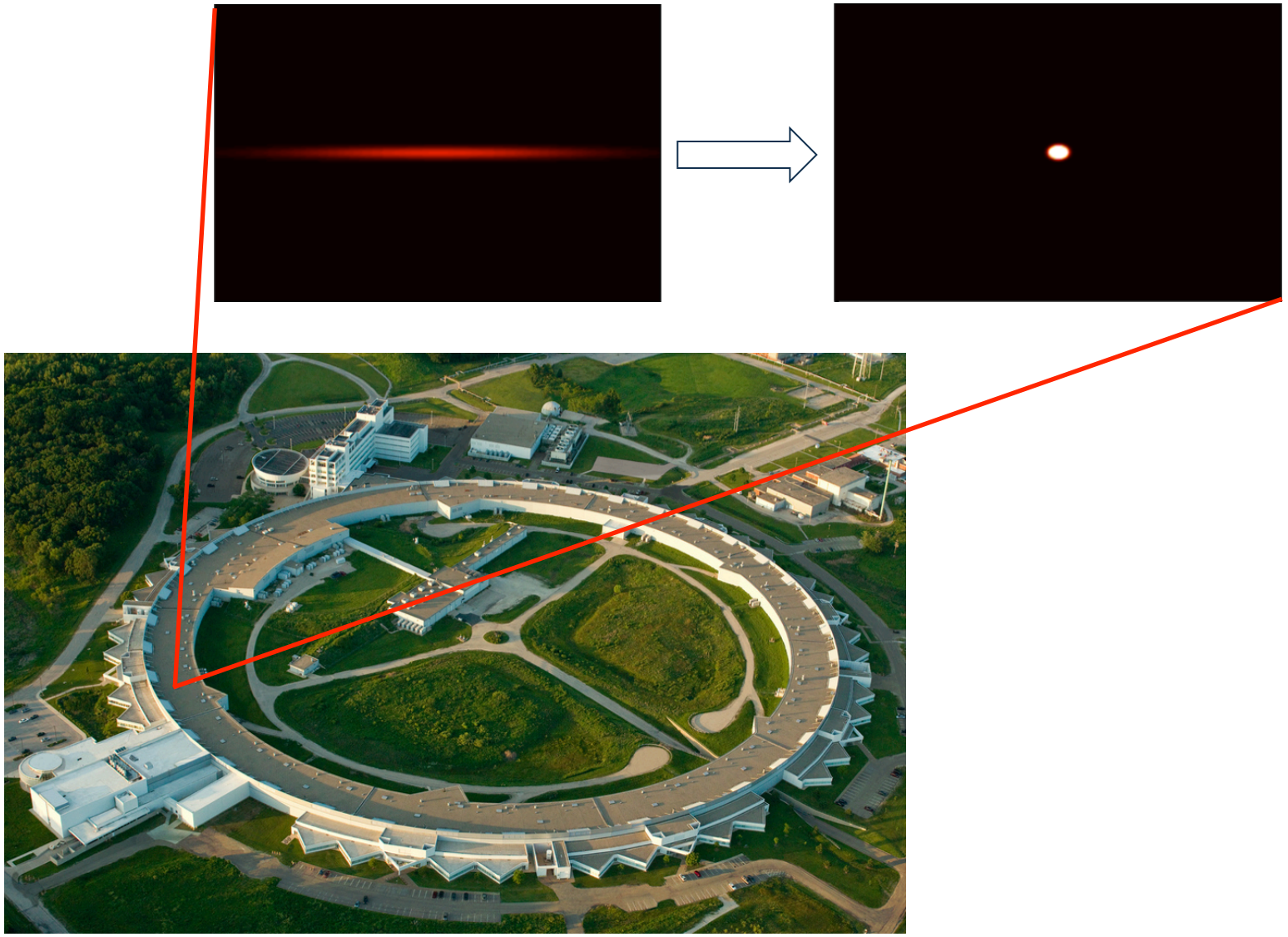
Credits: Micheline Pelletier/Corbis

**Ada E. Yonath**

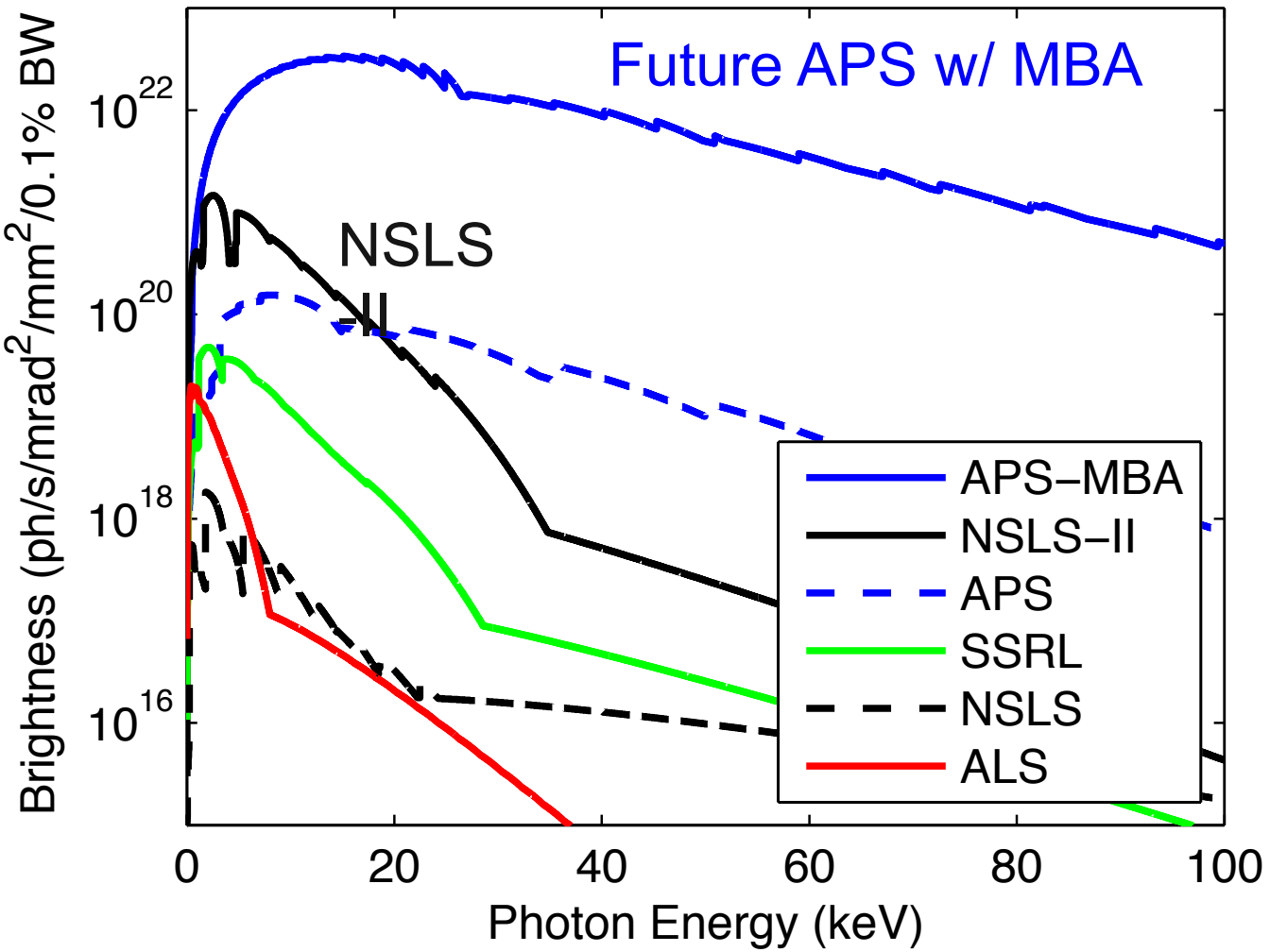


Yonath – 24 structures, 13 publications
Steitz – 19 structures, 10 publications
Ramakrishnan – 5 structures, 3 publications

# An Multi Bend Achromat Lattice at APS: a New Generation

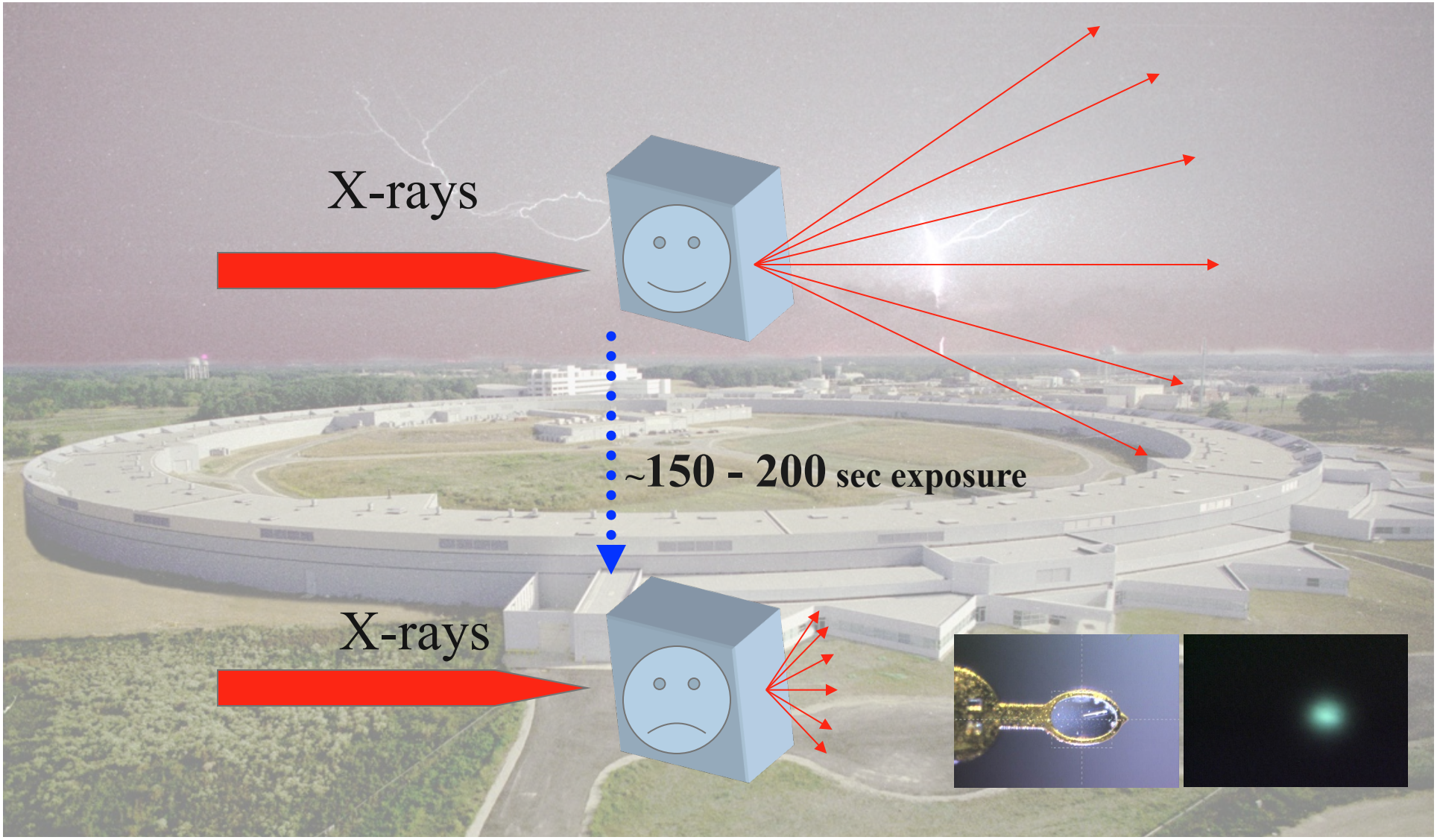


# APS: High Energy Star in US X-ray Facility Constellation




Brightness vs. X-ray energy at top beamlines among BES synchrotron facilities

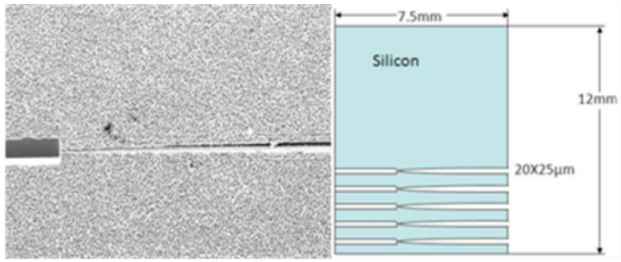
# 19-ID Beamline at APS



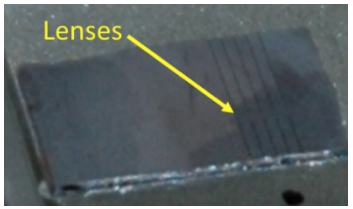

SMALL CRYSTALS
MICRO- & MINI-BEAMS

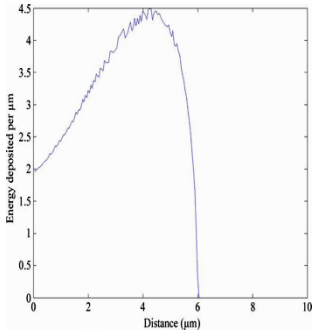
# Mitigation of Radiation Damage Using Line Focus Beam

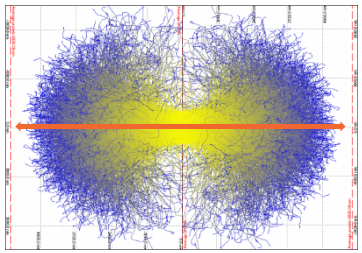
- A new strategy to reduce primary X-ray damage in macromolecular crystallography uses the basic principle to separate as much as possible the X-ray irradiated region, where the diffracted signal originates, from the region where damage accumulates.
- The photo electrons causing radiation damage accumulate predominantly outside the irradiated region of the crystal exposed with a line focused beam leading to a 4.5 factor decrease of radiation damage.



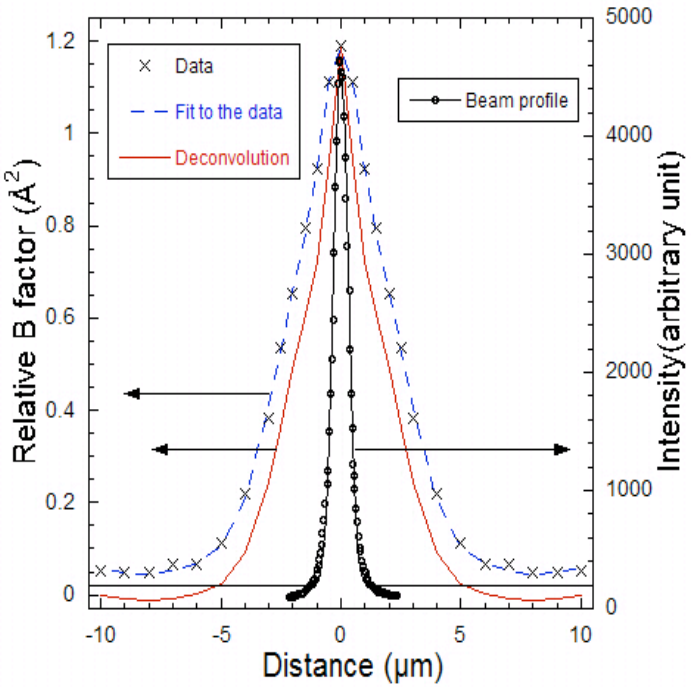

Silicon kinoform lens (Evans-Lutterrodt, BNL)



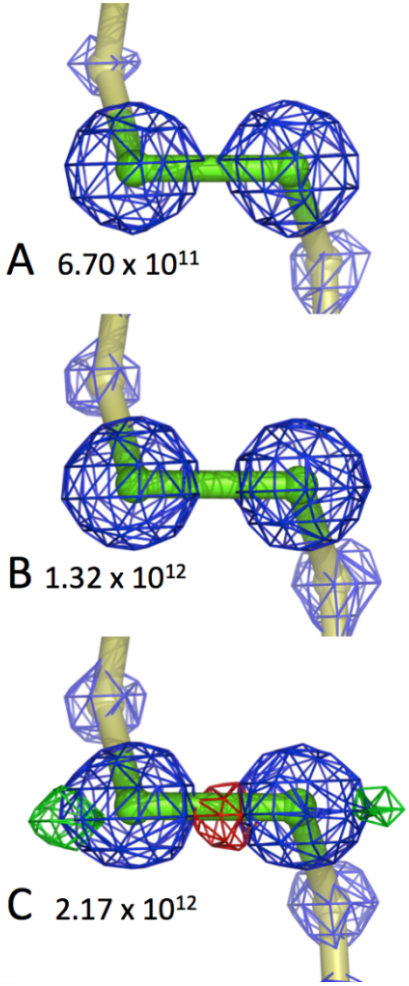



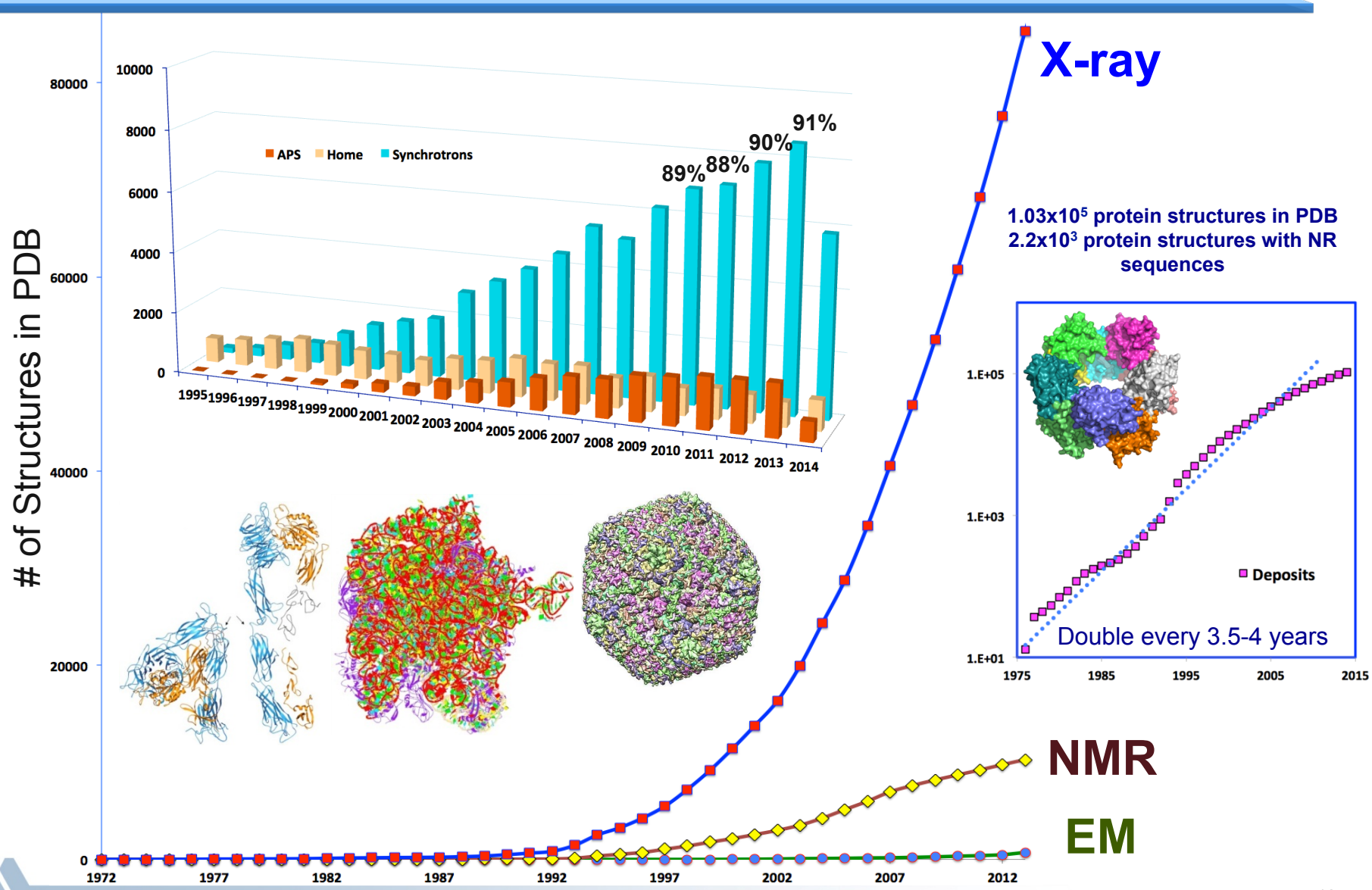
Experiment




Stern *et al. Acta Crys. D*, 2009
Finfrock *et al. Acta Crys. D,* 2010
Finfrock *et al. Acta Crys. D,* 2013

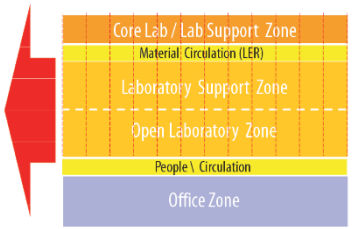


# Impact of Synchrotrons and APS on Structure Determination

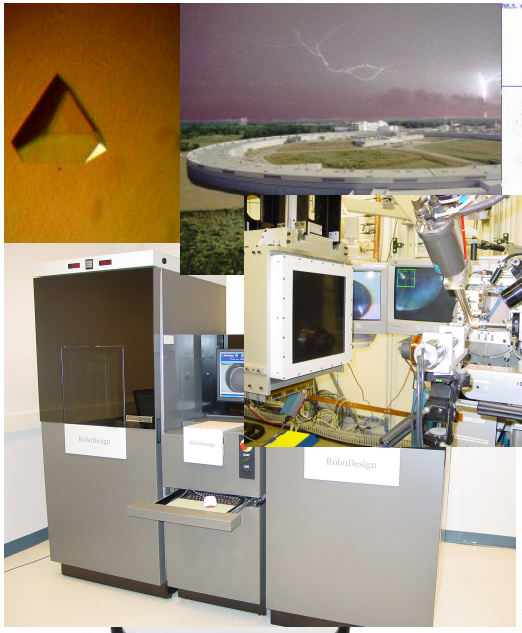


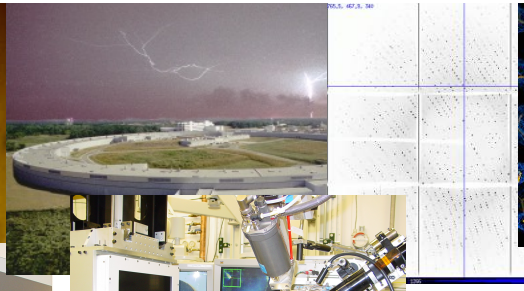
# Advanced Protein Crystallization Facility (APCF)

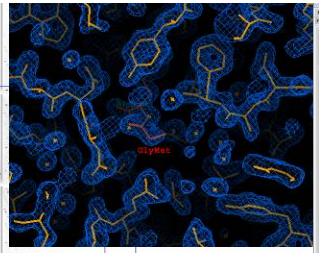
- The APCF is a state-of-the-art highly automated crystallization laboratory attached to the APS at sector 19 next to the MX beamlines.
- The facility includes laboratories with HTP robot-assisted technologies for the production and structural and functional characterization of proteins and macromolecular complexes.
- The facility combines expertise, technology and advanced methods used for the crystallization of macromolecules.
- The APCF enables collaborative research by providing research facilities for community projects, by hosting visiting scientists and by developing informatics gateways to facilitate collaborations with the biology community.

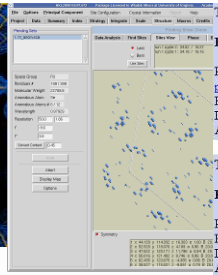


# HTP Structure Determination Pipeline at APCF









Target APC24875

PDB 1TZ0 PDBsum

Protein BC2969 (111 aa) COG2329 related proteins

Bacillus cereus

Deposited: 09 Jul 2004

Authors: Y. Kim, L. Lezondra, A. Joachimiak

1TZ0

Target APC24929

PDB 1T06 PDBsum

Protein BC3264 (235 aa) related proteins

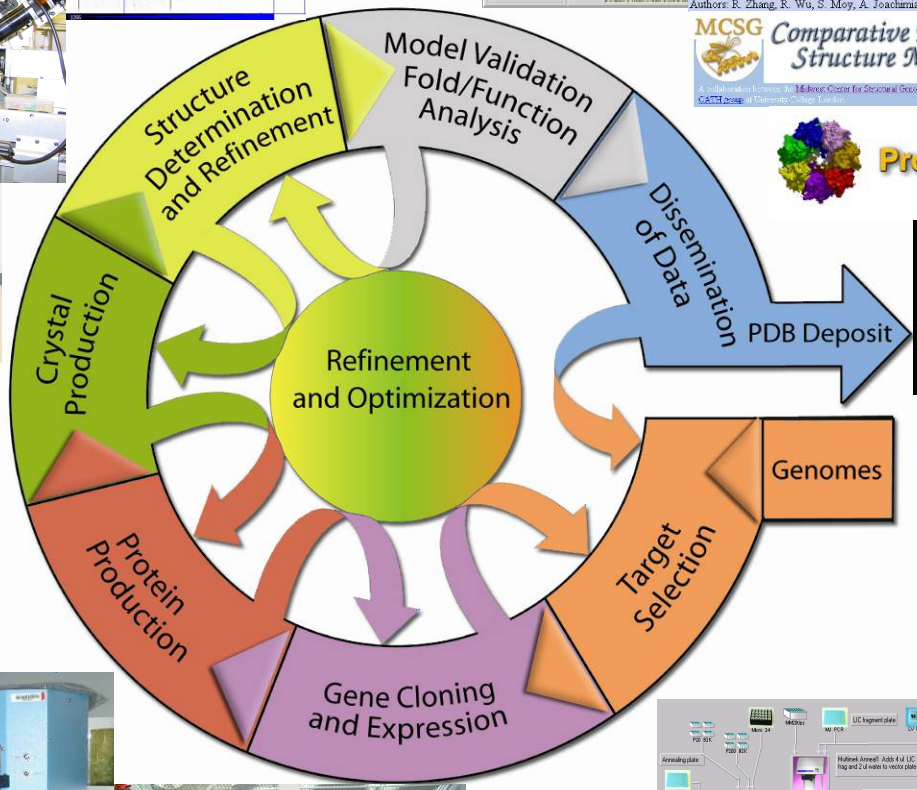
Bacillus cereus

Deposited: 07 Apr 2004

Authors: R. Zhang, R. Wu, S. Moy, A. Joachimiak

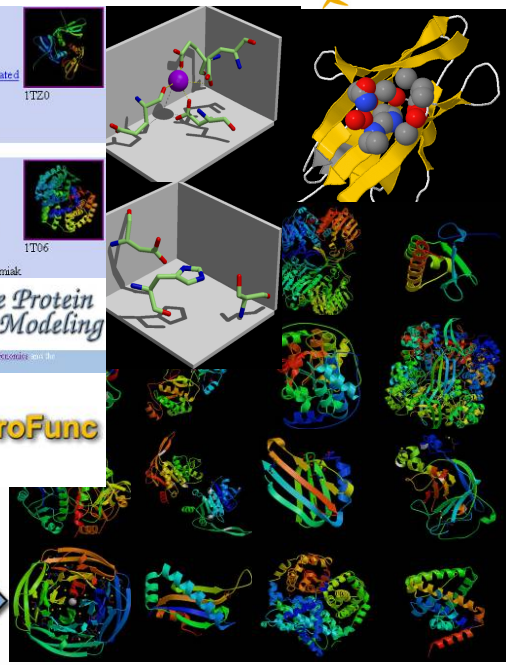
1T06






MCSG Comparative Protein Structure Modeling

ProFunc



ATGAGAATGAAGCGATTTTTAA
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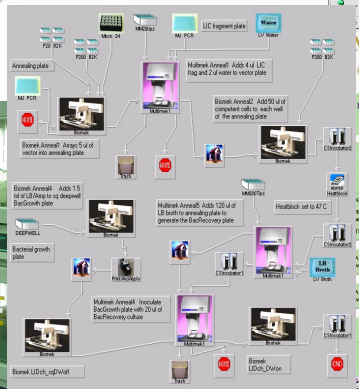
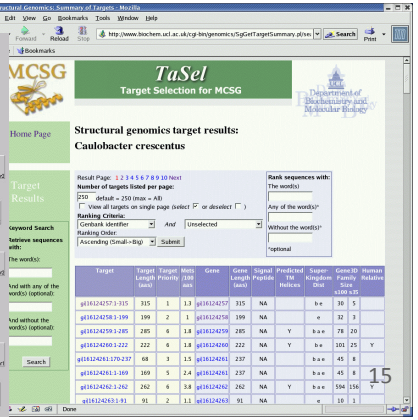
AAATTACAACAAAAATTTCAAA
TTTGAATCACTTGAAAAAATAA
TCATTAAATTATCCAGTACTAC
GAGCGAGAACATCGACGTAAAG

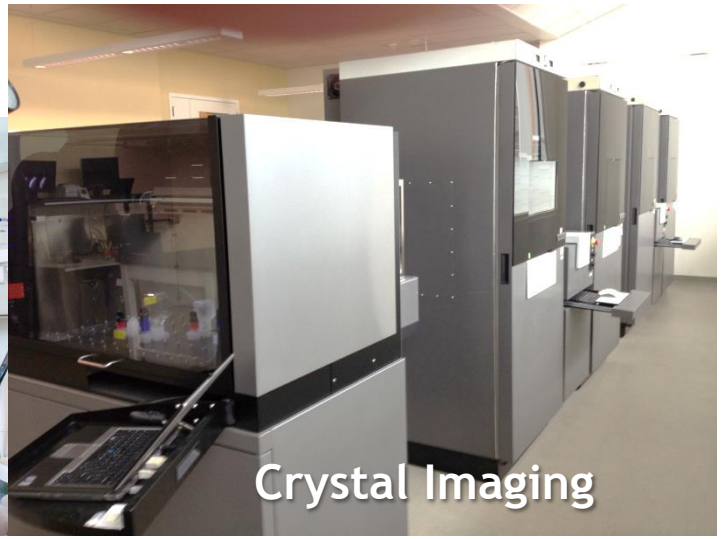
| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| APC35529 | RBSTP0061 | | - | | | | | | | Active |
| APC35531 | RBSTP1011 | + | + | + | + | | | | | Active |
| APC35532 | RBSTP2755 | + | + | + | + | - | | | | Stopped (failed trials) |
| APC35534 | RBSTP1017 | + | + | + | | | | | | Active |
| APC35536 | RBSTP2758 | + | + | + | + | + | + | SAD | 1XA0 | In PDB |
| APC35540 | RBSTP2765 | + | + | + | + | | | | | Suspended |
| APC35541 | RBSTP1026 | + | + | + | + | | | | | Stopped-homolog solved by others |
| APC35544 | RBSTP1038 | | - | | | | | | | Active |
| APC35545 | RBSTP1039 | + | /+ | + | + | | | | | Active |
| APC35547 | RBSTP0099 | + | + | + | | | | | | Active |





# Advanced Protein Characterization Facility

- **Robotic Gene Cloning**: ~100 overexpression cloning vectors for proteins used for crystallization and protein characterization, capacity 20,000 constructs per year.
- **Protein Expression:** bacterial, insect cell, mammalian expression systems at small and large scale, capacity 10,000 proteins per year.
- **Semi-automated Protein Purification:** parallel workstation systems for multiple, highly efficient large-scale purification, capacity 7,000 proteins per year.
- **Characterization:** enzymatic activity, ligand binding, protein-protein/protein-nucleic acid interaction (96-well format DLS, UV/VIS/fluorescence spec, thermal shift instruments, ITC, OCTET). Capacity 8,000 proteins per year.
- **Semi-automated Crystallization and Imaging:** robotic crystallization, incubation and monitoring system, capacity 7,000 proteins per year.
- **Database Management and Bioinformatics:** all experimental data captured, stored and analyzed.



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| Bioenergy | Environmental Studies | Human Health |
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| Biofuels | Climate change | Infectious Diseases |
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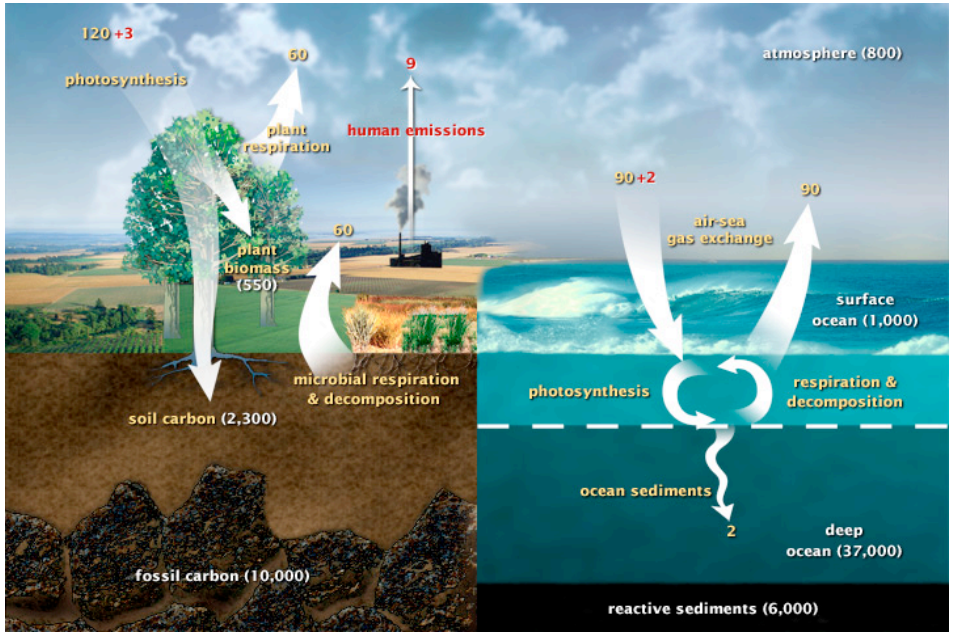
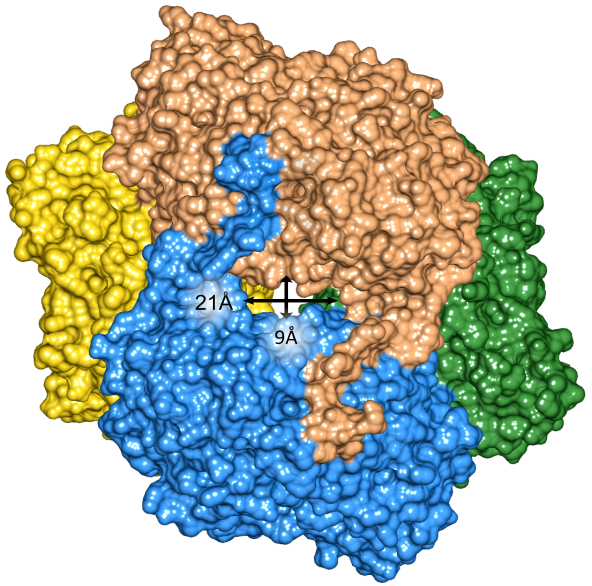
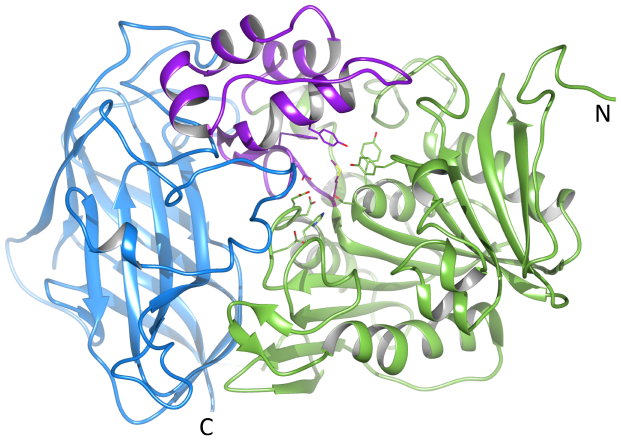
**Microbial biology as we know it is strongly biased toward cultured organisms inhabiting less populated environments**



# First Protein Structure Solved from a Single-Cell Genome of an Uncultured Marine Sediment Archaea

- Sedimentary marine archaea identified through single-cell genomics may play a vital role in C (and N) cycling; their genetic information appears to encode a set of putative extra- and intracellular proteases to hydrolyze detrital proteins. But are these really proteases and do we find them elsewhere?
- Clone, express, purify, and characterize biochemically and structurally putative proteases from newly sequenced archaea.
- Analyze marine deep sediment metagenomic data to determine how common and widespread these proteases are in marine microbiota and understand their impact on global geochemical processes.
- First structure of bathyaminopeptidase (BAP) from *Thaumarchaeota archaeon* SCGC AB-539-E09T determined.
- BAP is a true example of an enzyme found only in the uncultured species; sequenced cultured organisms do not contain a close homolog. A search of available genomes revealed highly similar (84-96% at the amino acid level) sequences only from estuarine sediment in North Carolina, USA.
- *The ability to perform detailed characterizations of enzymes from native subsurface microorganisms, without requiring growth in pure culture, promises understanding of key C transformations in the environment and identification of new enzymes for biotechnological applications.*



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| Biofuels | Climate change | Infectious Diseases |
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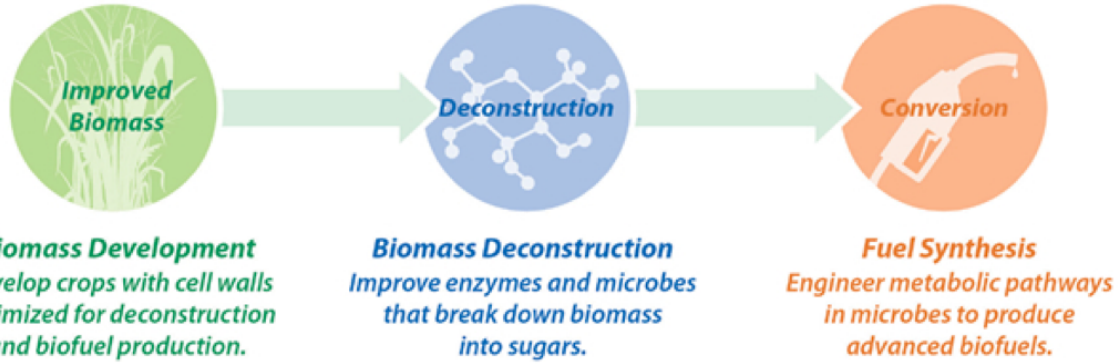
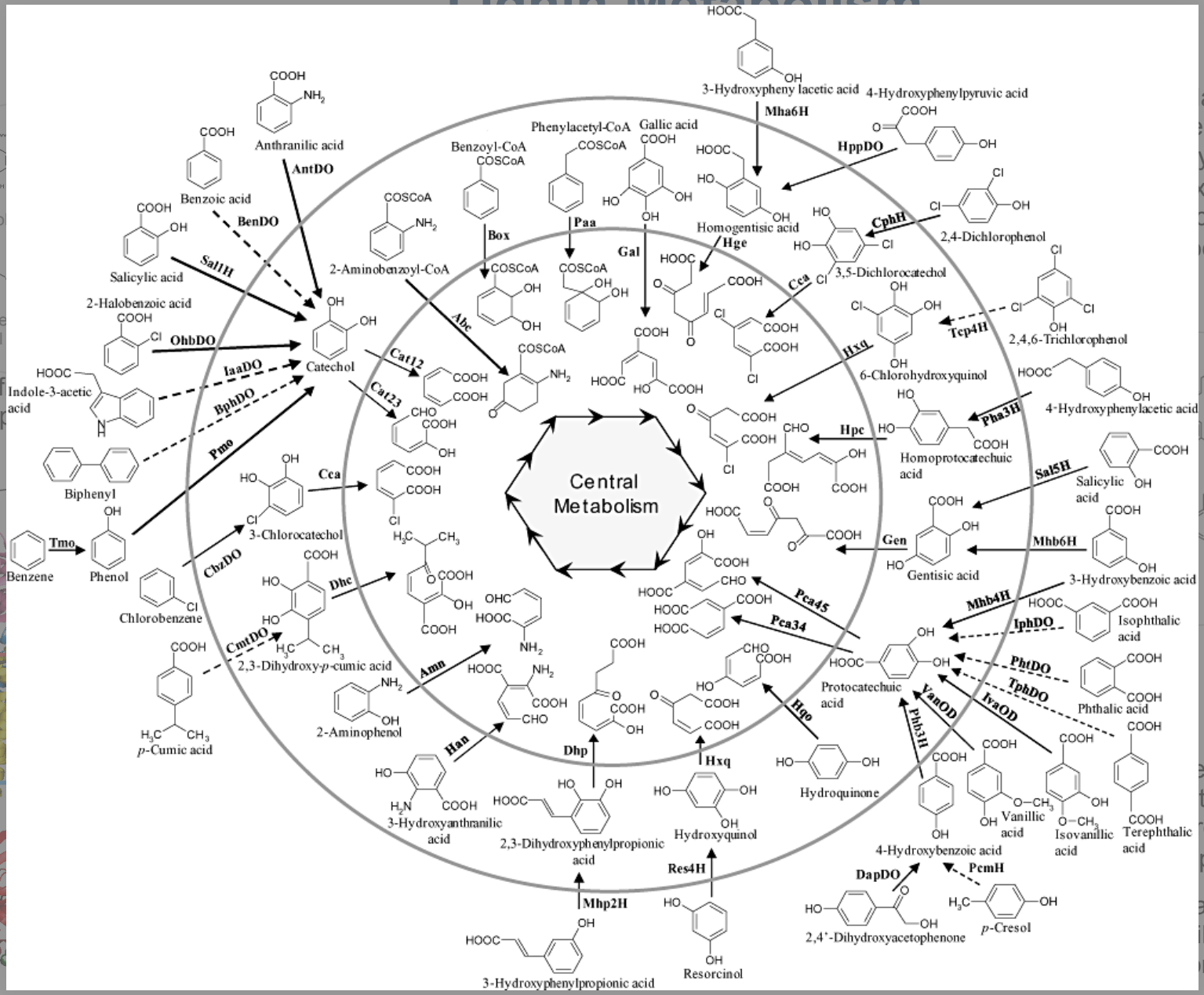
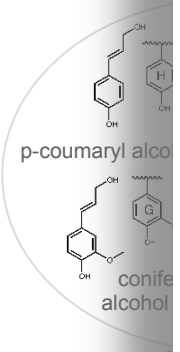
DOE Bioenergy Research Centers

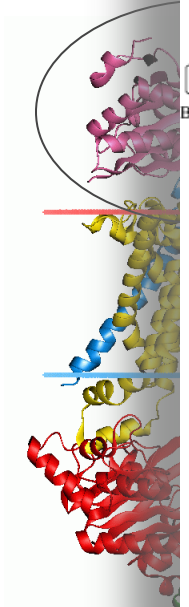


# Lignin Metabolism

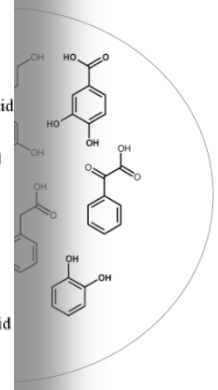



Produced f
of diverse p



ation leads to
eight products
ylic acids,
xylic acids,
carboxylic
ducts).



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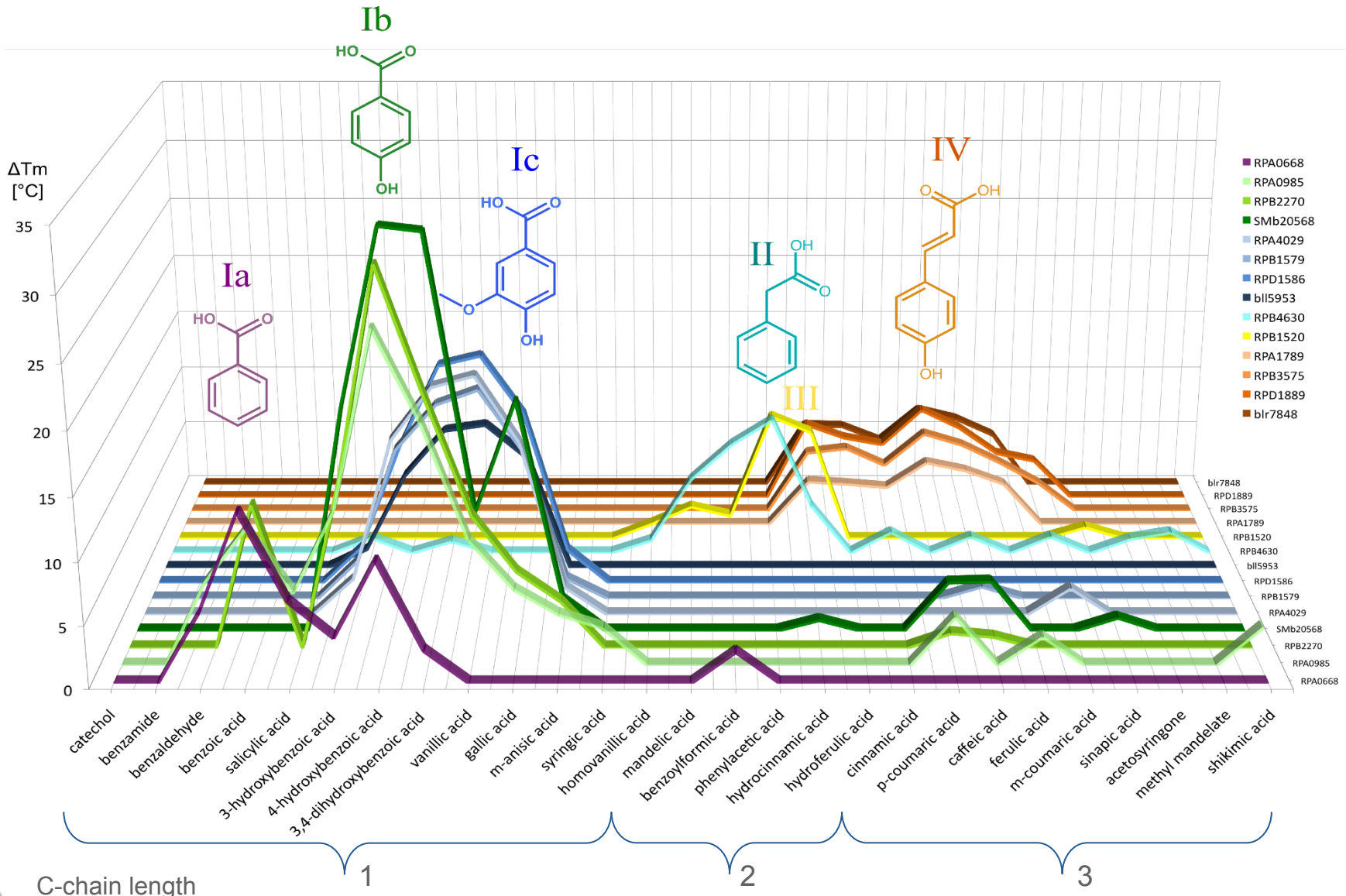
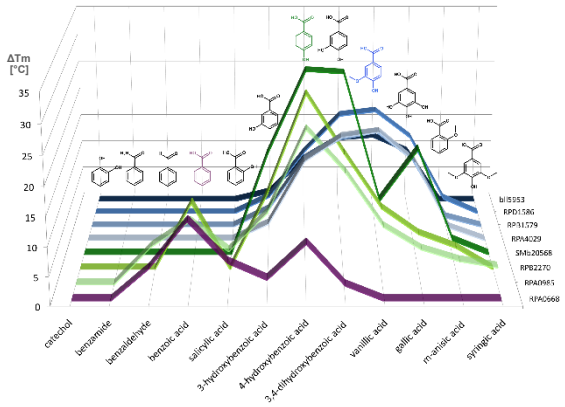
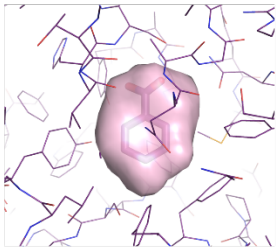
# Ligand-binding Profile



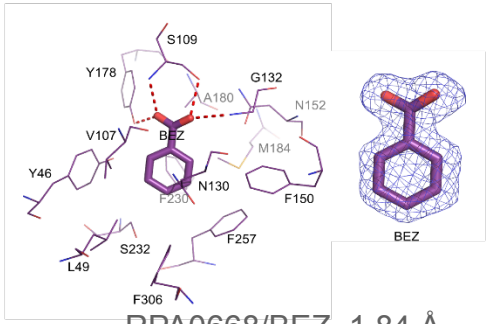
# Ligand-binding – Cluster I

Ia

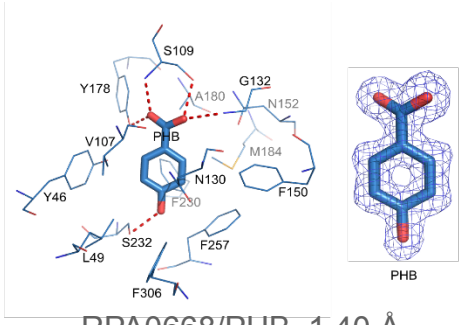


RPA0668 cavity 139 Å$^3$

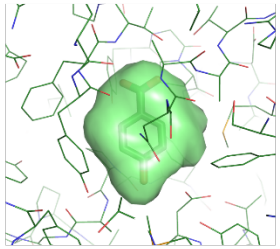



RPA0668/BEZ, 1.84 Å

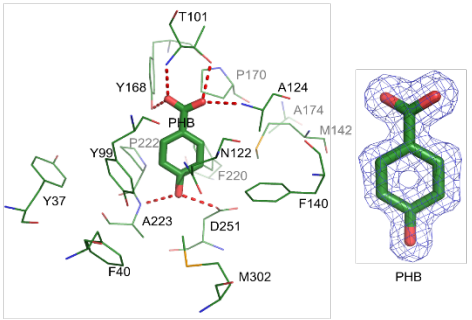



RPA0668/PHB, 1.40 Å

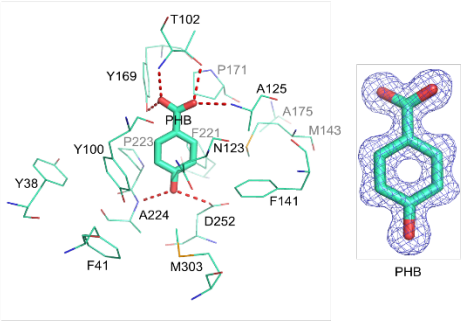
Ib



RPA0985 cavity 154 Å$^3$

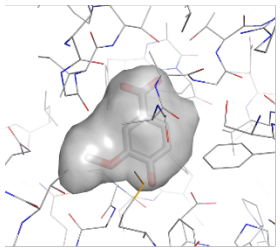



RPA0985/PHB, 1.45 Å

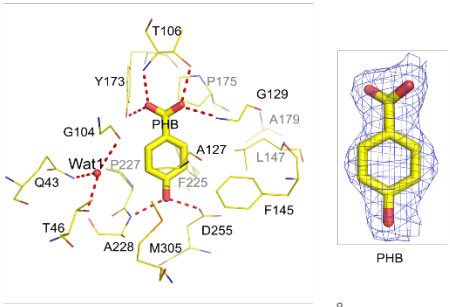



RPB2270/PHB, 1.30 Å

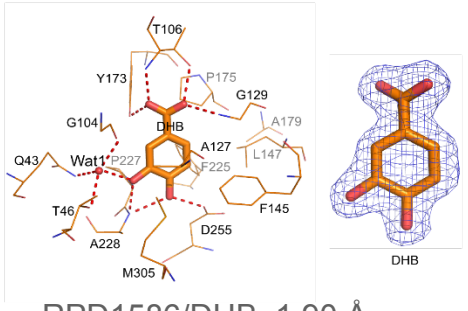
Ic



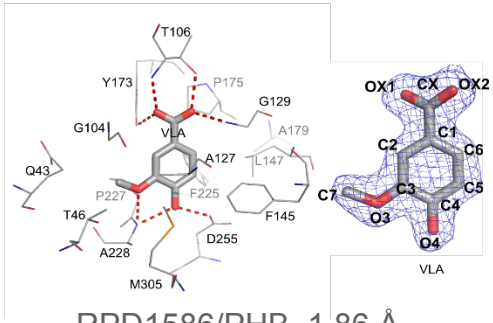
RPD1586 cavity 175 Å$^3$




RPD1586/PHB, 2.22 Å




RPD1586/DHB, 1.90 Å




RPD1586/PHB, 1.86 Å

# Biology User Facilities at U. S. Light Sources Support Basic Research in Many Important Areas

## Bioenergy

- Biofuels
- Biomass deconstruction
- Systems engineering

## Environmental Studies

- Climate change
- Carbon cycling
- Microbial Dark Matter

## Human Health

- Infectious Diseases
- Antibiotic Resistance

REPORT TO THE PRESIDENT ON COMBATING ANTIBIOTIC RESISTANCE

| Microbe | Description | Threat Level | U.S. Infections /Year | U.S. Deaths/Year |
|---|---|---|---|---|
| ***Clostridium difficile*** | *Clostridium difficile* causes life-threatening diarrhea, most frequently in people who have had both recent medical care and antibiotics. | Urgent | 250,000 | 14,000 |
| **Carbapenem-resistant Enterobacteriaceae (CRE)** | CRE are a family of bacteria (that includes pathogens such as *Salmonella* and *E.coli*.) resistant to the carbapenem[99] family of antibiotics. CRE have become resistant to all or nearly all the antibiotics currently available. | Urgent | 9,300 | 610 |
| **Drug-resistant *Neisseria gonorrhoeae*** | Multidrug- or cephalosporin-resistant gonorrhea is a sexually transmitted infection that can cause permanent reproductive health problems. | Urgent | 246,000 | <5 |

Executive Office of the President
President's Council of Advisors on Science and Technology

September 2014

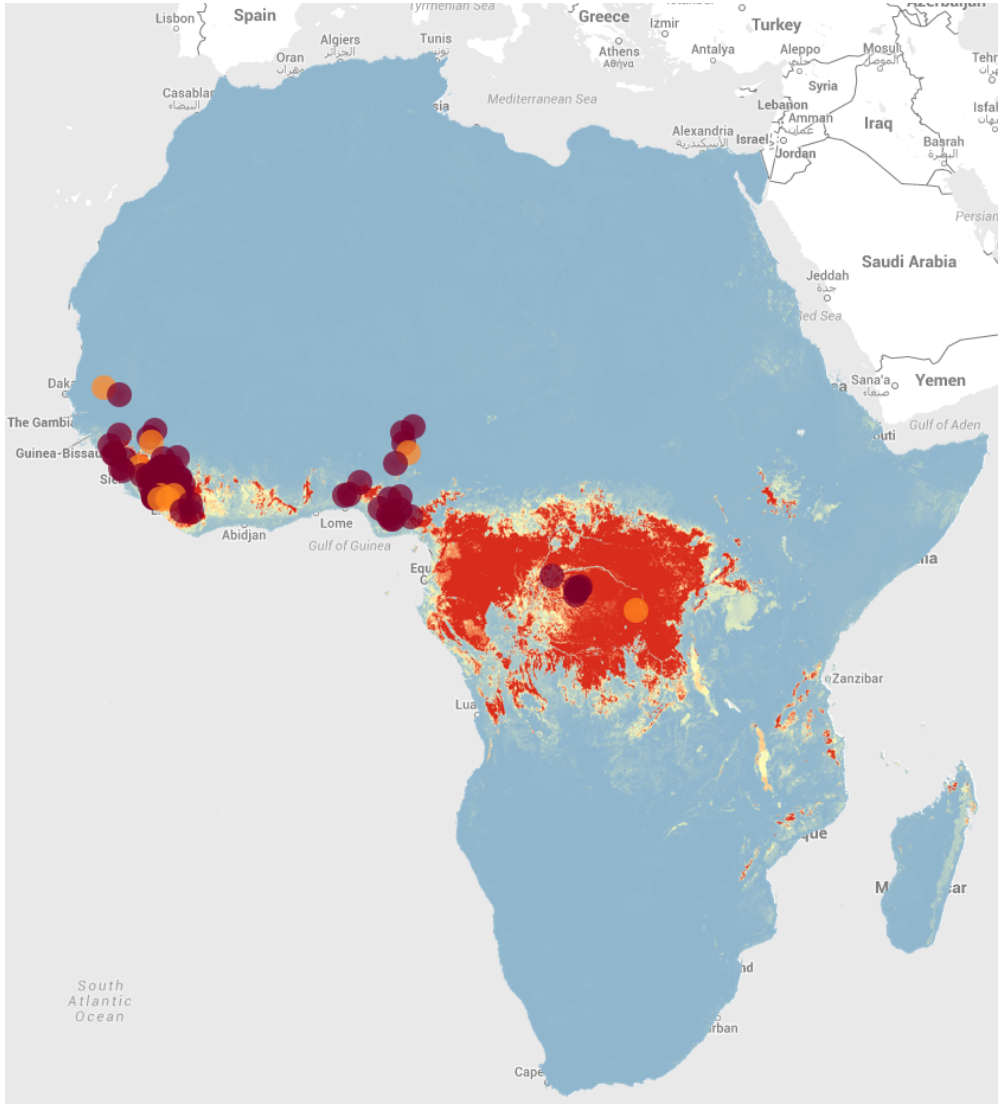
NATIONAL STRATEGY FOR COMBATING ANTIBIOTIC-RESISTANT BACTERIA

*Vision: The United States will work domestically and internationally to prevent, detect, and control illness and death related to infections caused by antibiotic- resistant bacteria by implementing measures to mitigate the emergence and spread of antibiotic resistance and ensuring the continued availability of therapeutics for the treatment of bacterial infections.*

September 2014

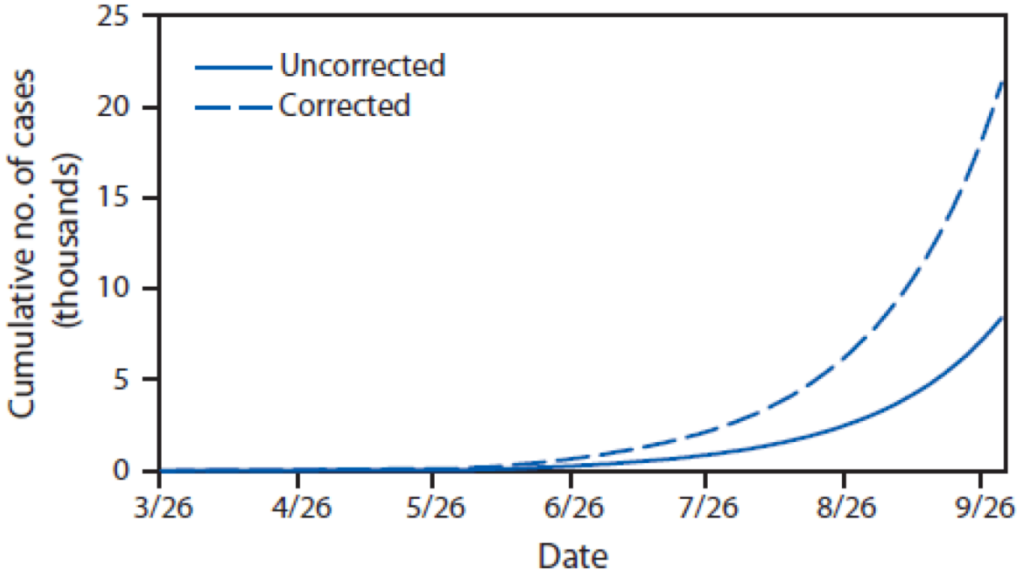
# Ebola Outbreak Cases and a Zoonotic Transmission Niche Across Central and West Africa

- Ebola virus infects bats and primates.
- At-risk areas are inhabited by 22 million people, however the rarity of human outbreaks emphasizes the very low probability of transmission to humans.
- Increasing population sizes and international connectivity by air since the first detection of Ebola virus in 1976 suggest that the dynamics of human-to-human secondary transmission in contemporary outbreaks will be very different to those of the past.

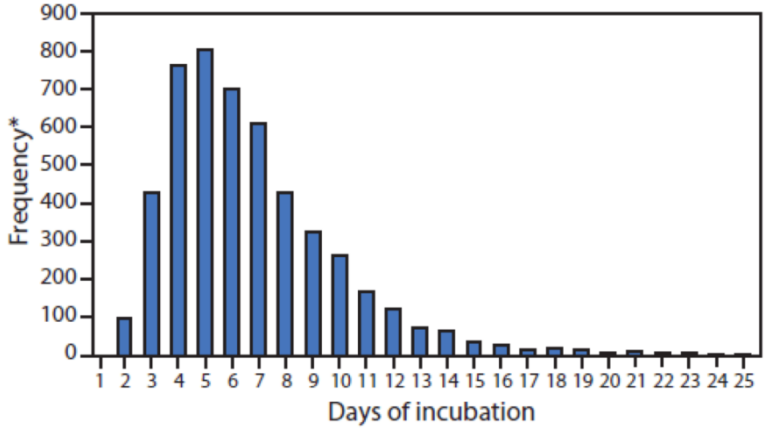



Pigott *et al. eLIFE*, 2014

# Estimated Number of Ebola Cases, with and without Correction for Underreporting, through September 30, 2014 in Liberia and Sierra Leone



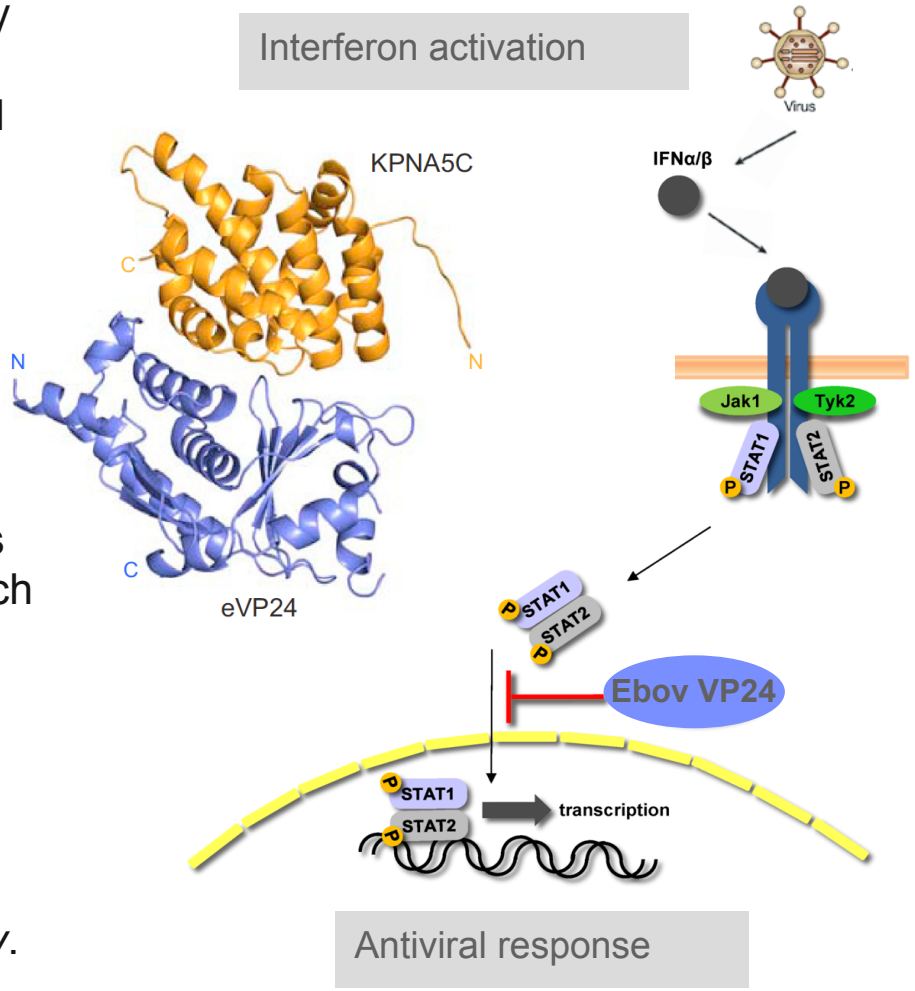

Meltzer *et al. Morbidity and Mortality Weekly Report*, 2014




Legrand *et al. Epidemiol Infect* 2007
Eichner *et al. Osong Public Health Res Perspect* 2011

# SBC-based Structures are Critical to Understanding of the Ebola Virulence. SBC has Contributed to 47% of all Ebola Virus Protein Structures and 16 Publications.

- High mortality of Ebola virus is caused by its ability to disrupt the body's immune response to the infection. Understanding that mechanism is crucial for the development of treatment.
- Using data collected at SBC, researchers with Dr. Gaya Amarasinghe's Lab determined the crystal structure of Ebola VP24 in complex with human KPNA5.
- Structure revealed how the VP24 protein disrupts the cell's innate immune response. VP24 operates by preventing the transcription factor STAT 1, which in conjunction with STAT 2 carries interferon's antiviral message, from entering the nucleus and initiating an immune response.
- *Structural information solved the mechanism by which Ebola virus controls the body's immune response and provides insight valuable for therapy*.



# Ebola Virus Studies Time Line

2014

- Structure-based *in silico* screening, biochemical and structural characterization of small molecules that target VP35.
- VP24 protein actively blocks the antiviral effects of interferon.

2013

- New aptamers bind VP35 with high affinity and specificity:  potential inhibitors.
- Structure of VP40 determined and shows its role in the virus life cycle.
- MARV VP35 inhibits interferon production in the signaling pathways.

2012

- Structure of VP24 determined.
- Development of templates for antiviral drugs for viral targets.

2011

- Simultaneous recognition of dsRNA by Ebola VP35 antagonizes host dsRNA sensors and immune responses.
- Structure of VP35 and its complex with dsRNA determined.

2010

- The potential of multifunctional Ebola VP35 as a therapeutic target.
- VP35 masks key recognition sites of molecules such as RIG-I, MDA-5, and Dicer to silence viral dsRNA in infection.

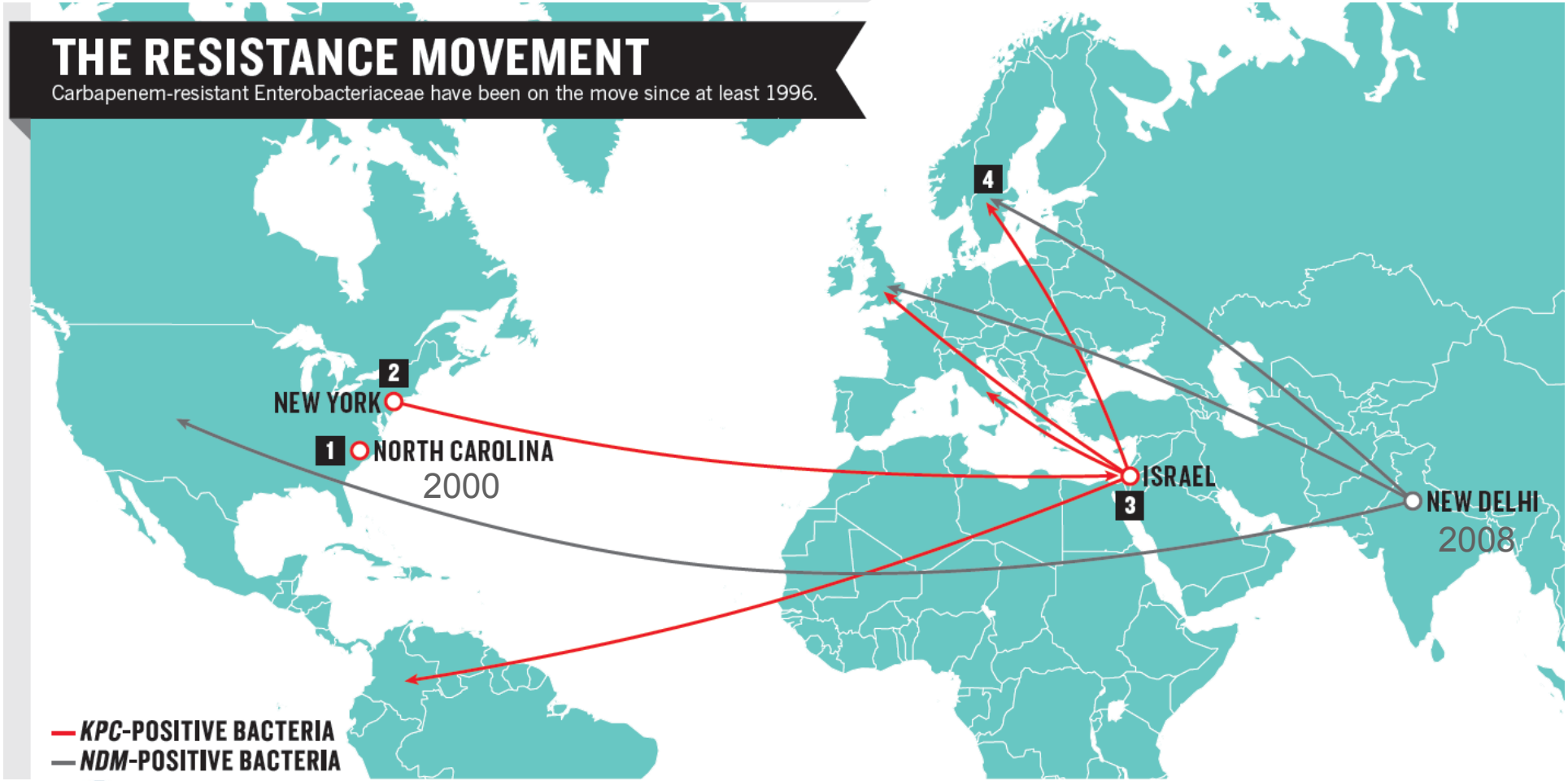
# Bacterial Pathogens and Humans - Rising Antibiotic Resistance

- The introduction of antibiotics in medicine gave false hope for the control of all infectious diseases.
- Dispersion of successful clones of multidrug resistant bacteria is common, often via the movement of people.
- The continued evolution of a complex array of antibiotic-resistance genes presents a formidable challenge.
- An equally troubling trend is the sophistication of pathogenic bacteria that continually evolve complex genetic systems for acquiring and regulating antibiotic-resistance mechanisms.
- Efforts to develop new antimicrobials have over the past two decades lagged behind the rapid evolution of resistant genes.
- Antibiotic resistance is growing relentlessly and is now a global problem - the dramatic spread in the US of the now common community strain of *Staphylococcus aureus* USA300 serves as an example.
- The evolution of antibiotic resistance is now occurring at an alarming rate and is outpacing the development of new countermeasures capable of thwarting infections in humans. This situation threatens patient care, economic growth, public health, agriculture, economic security, and national security.

# Rising Antibiotic Resistance in U. S.

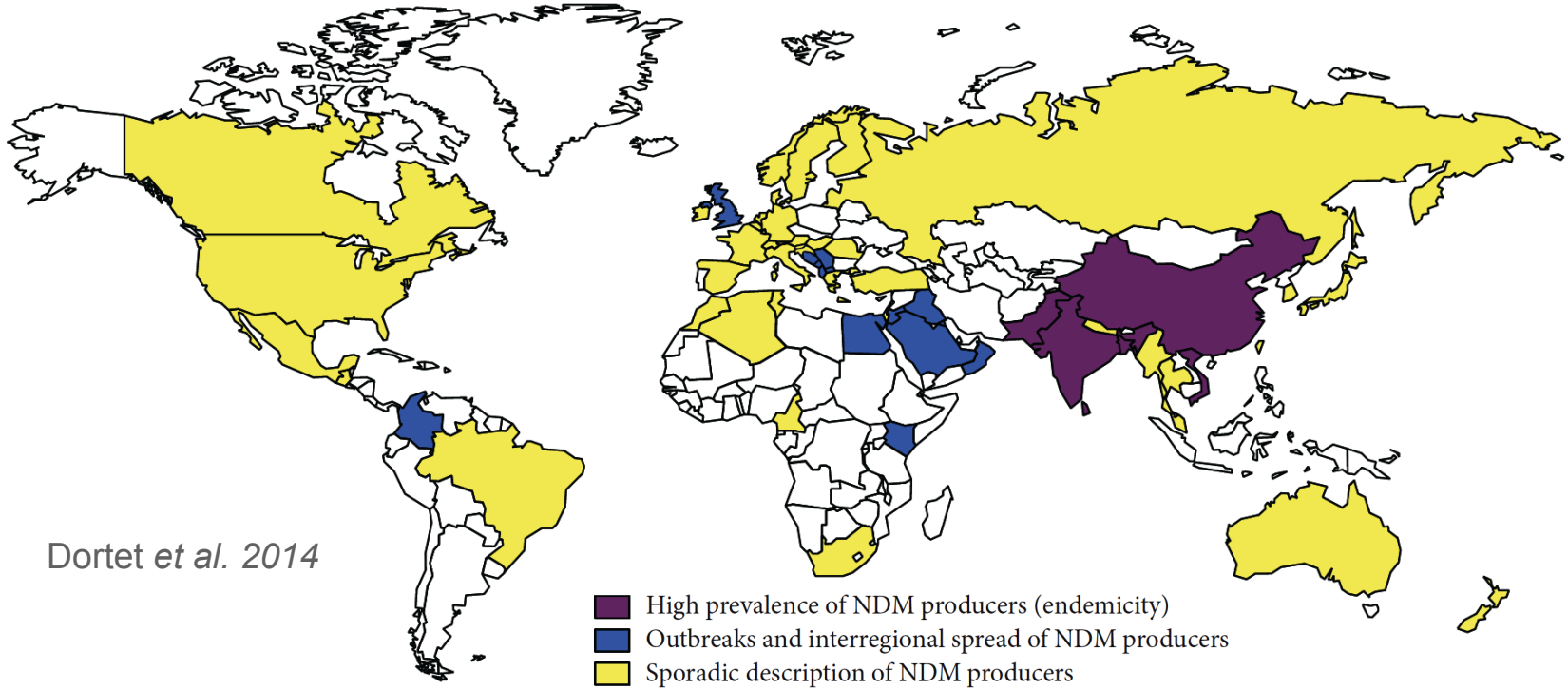
- **President's Council of Advisers on Science and Technology - Report to the President on Combating Antibiotic Resistance, Sep. 2014**
- **National Strategy for Combating Antibiotic Resistance Bacteria, Sep. 2014**
- **PCAST recommendations:**
  - **Improving our surveillance of the rise of antibiotic–resistant bacteria**
  - **Increasing the longevity of current antibiotics**
  - **Increasing the rate at which new antibiotics, as well as other interventions, are discovered and developed**
- **The President Issued an Executive Order on Combating Antibiotic-Resistant Bacteria, Sep. 18, 2014.**
- **Impact in U. S. :**
  - 2,299,000 – infections/year.
  - 8 million days in hospitals.
  - 37,830 – death/year.
  - *Streptococcus pneumoniae* is the leading cause of bacterial pneumonia and meningitis in the United States (>1.2M infections/year).
  - *Clostridium difficile* causes life–threatening diarrhea (>14k deaths/year).
- **The annual domestic impact of antibiotic-resistant infections to the U.S. economy has been estimated at $20 billion in excess direct health care costs.**
- **Additional costs to society for lost productivity as high as $35 billion per year.**

# THE LAST RESORT



# NDM-1 Dissemination

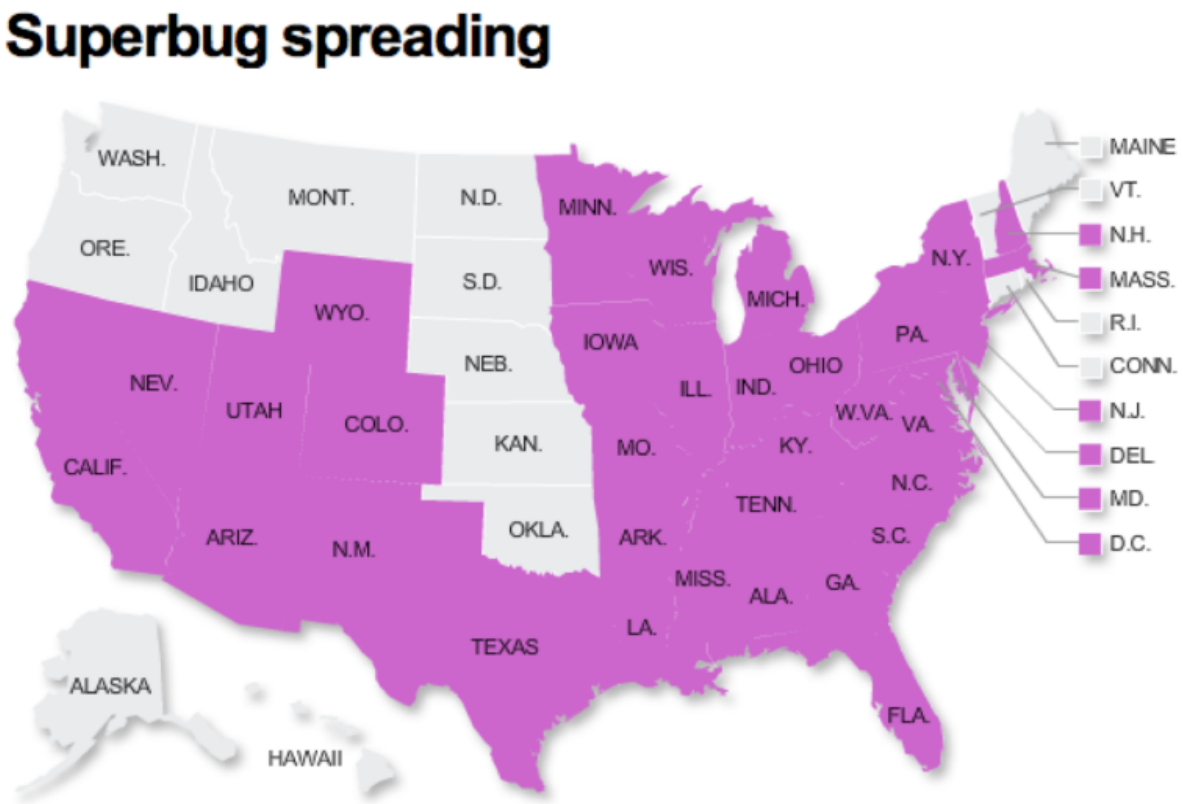
- Strains carrying *blaNDM-1* genes have been found in over 40 countries world-wide.
- The rapid emergence of NDM-1 has been linked to mobile plasmids (IncA/C, IncL/M, IncF) that move between different strains resulting in world-wide dissemination.




Dortet *et al. 2014*

*blaNDM-1* gene was found in *Escherichia coli, Klebsiella pneumoniae, K. oxytoca, Proteus mirabilis, Acinetobacter baumannii, A. iwoffii, A. pittii, Enterobacter cloacae, Citrobacter freundii, Morganella morganii, Pseudomonas spp., Providencia rettgeri, Shigella boydii, Vibrio cholerae, Aeromonas caviae,* and *Salmonella enterica.*

# Outbreak of Drug-Resistant Bacteria Linked to Lutheran General Hospital (2013)

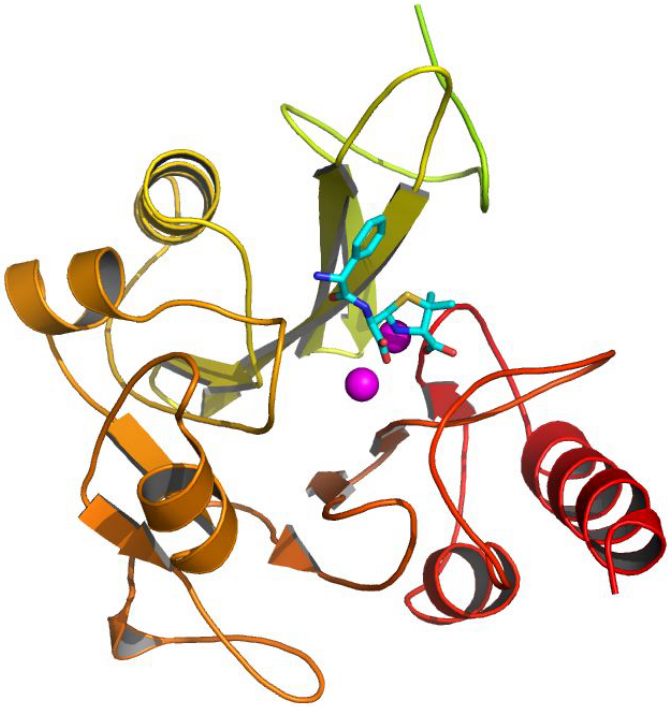


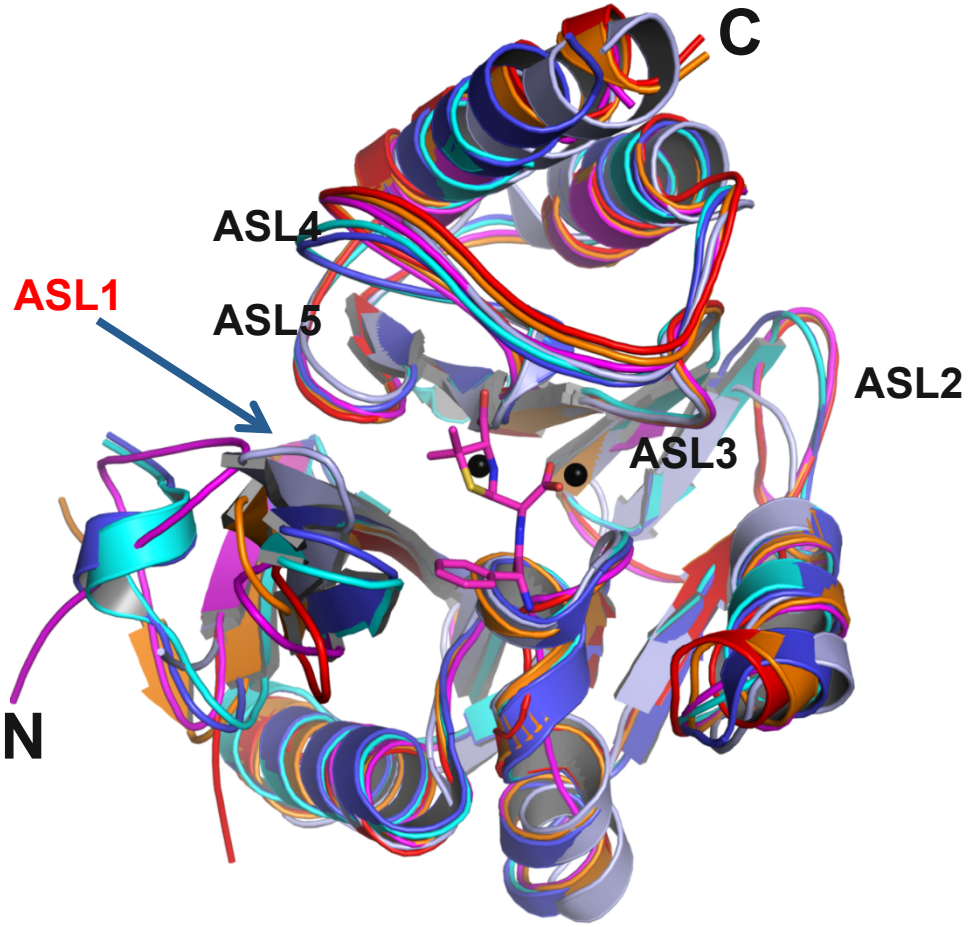

- The CDC has discovered 44 cases of a strain of bacteria called carbapenem-resistant enterobacteriaceae (NDM-1), or CRE, in northeast Illinois, including 38 confirmed cases involving patients at Advocate Lutheran General Hospital in Park Ridge who underwent an endoscopic procedure of the pancreas or bile ducts between January and September 2013.

# Rapid Response to Emerging Disease - the NDM-1 from *K. pneumoniae*

- Goals are to understand the broad range of binding specificity, mechanistic details and to ultimately develop inhibitors (drugs) against NDM-1.
- Synthetic NDM-1 gene was used.
- Over 120 structure-based constructs NDM-1 through NDM-8 (deletions, multiple and point mutants, different N- and C-terminal tags) were designed and cloned into 4 MCSG vectors.
- 60 constructs (60%) expressed at a high level and were purified in large scale multiple times.
- NDM-1 was co-crystallized in various combinations with metals (Zn, Co, Cd, Mn), 15 antibiotics, and 13 inhibitors.
- Crystals were obtained for 10 constructs and as a result, 20 structures were determined, including 4 isolated NDM-1 variants (NDM-3, NDM-4, NDM-5 and NDM-6).
- 11 structures were deposited to PDB, representing 45% of all deposits for this protein in PDB.

# The NDM-1 Structure and Comparison with other MBL Structures

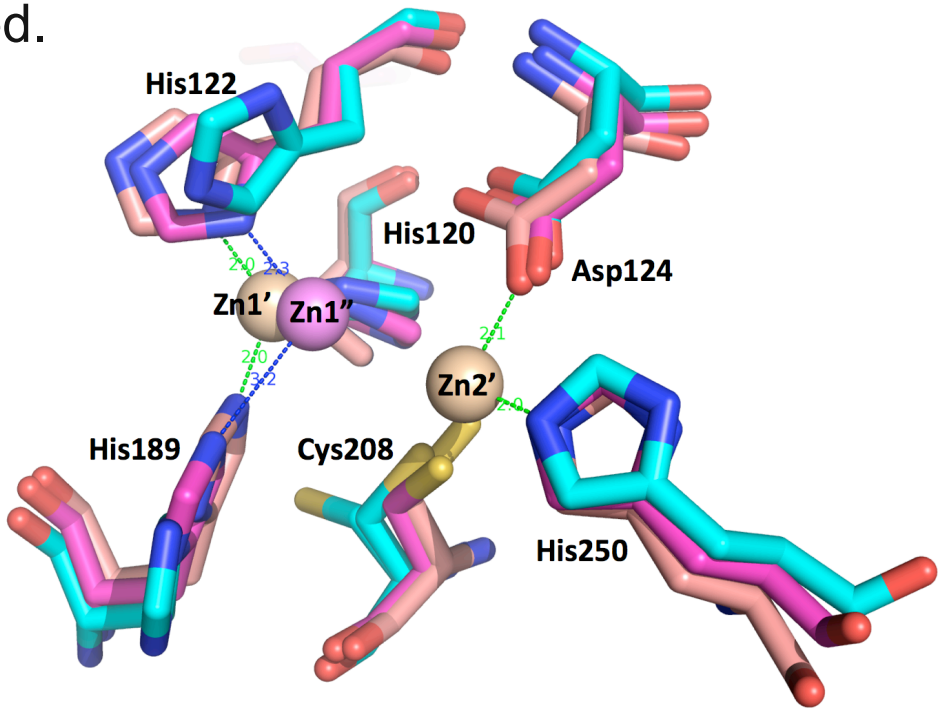


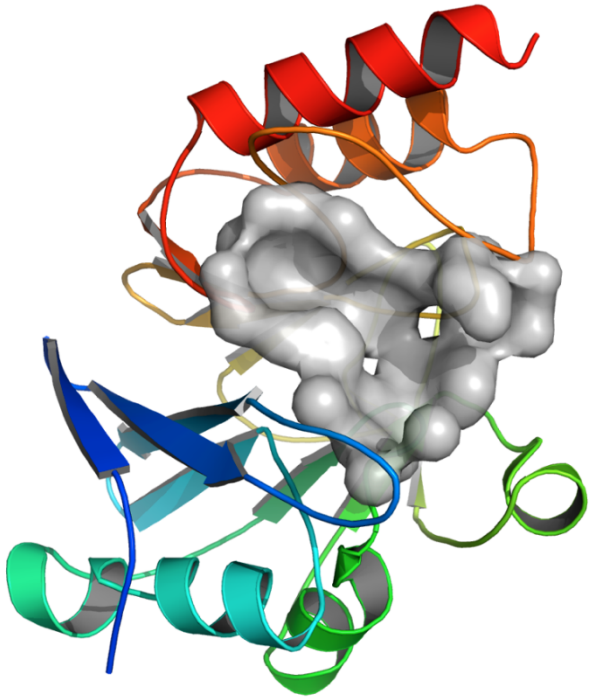



Δ38-NDM-1 (apo)
Δ30-NDM1 (dizinc)
Δ36-NY-NDM-1 (monozinc)
2YZ3-VIM-2
2WHG-VIM-4
1DD6-IMP-1

# The Active Site of NDM-1

- The active site is located in the middle of the molecule between two β-sheets.
- This well-defined hydrophobic and partly positively charged cavity is shaped by five active site loops. These loops provide key conserved side chains for coordinating metal ions as well as a proposed catalytic general acid/base.
- These residues appear to have well-defined conformations in the absence and presence of metal ions and this part of the active site is structurally well conserved.



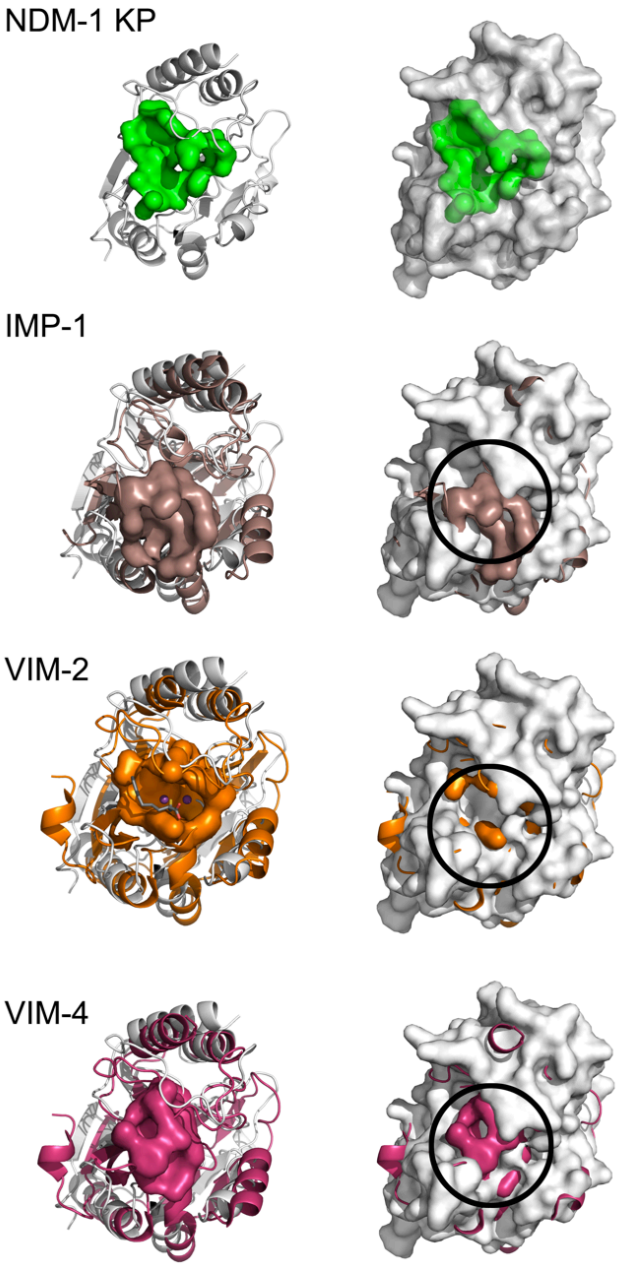





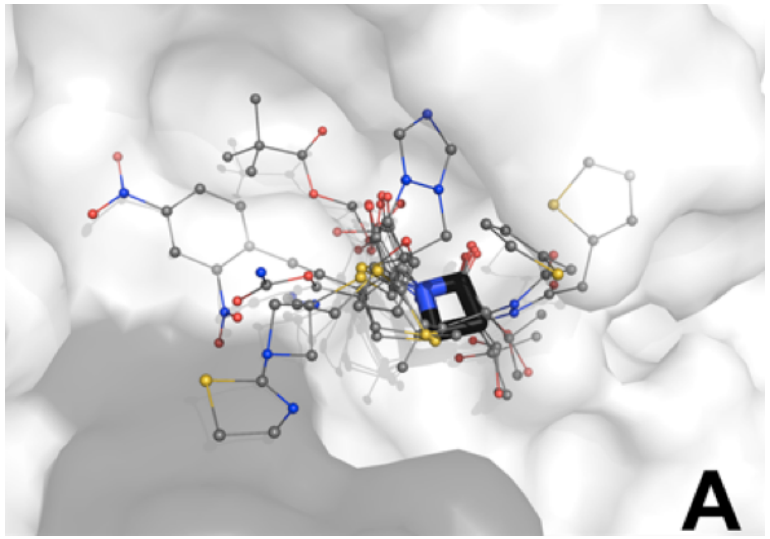
Kim, Y. *et. al. PLOS One* 2011

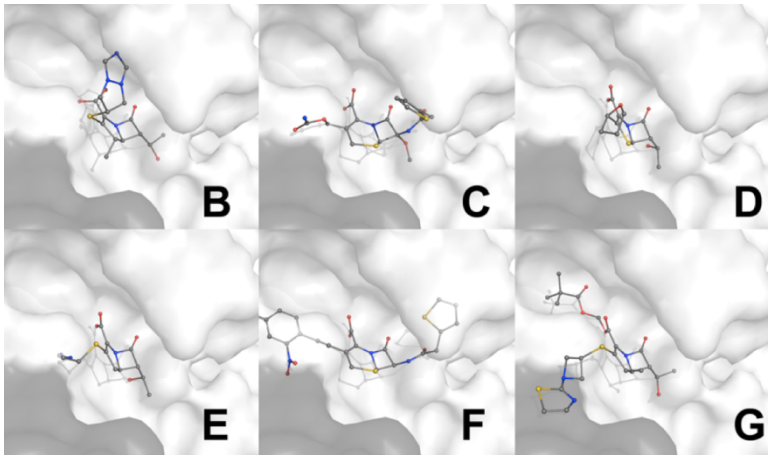
# The Active Site of NDM-1 is Enlarged

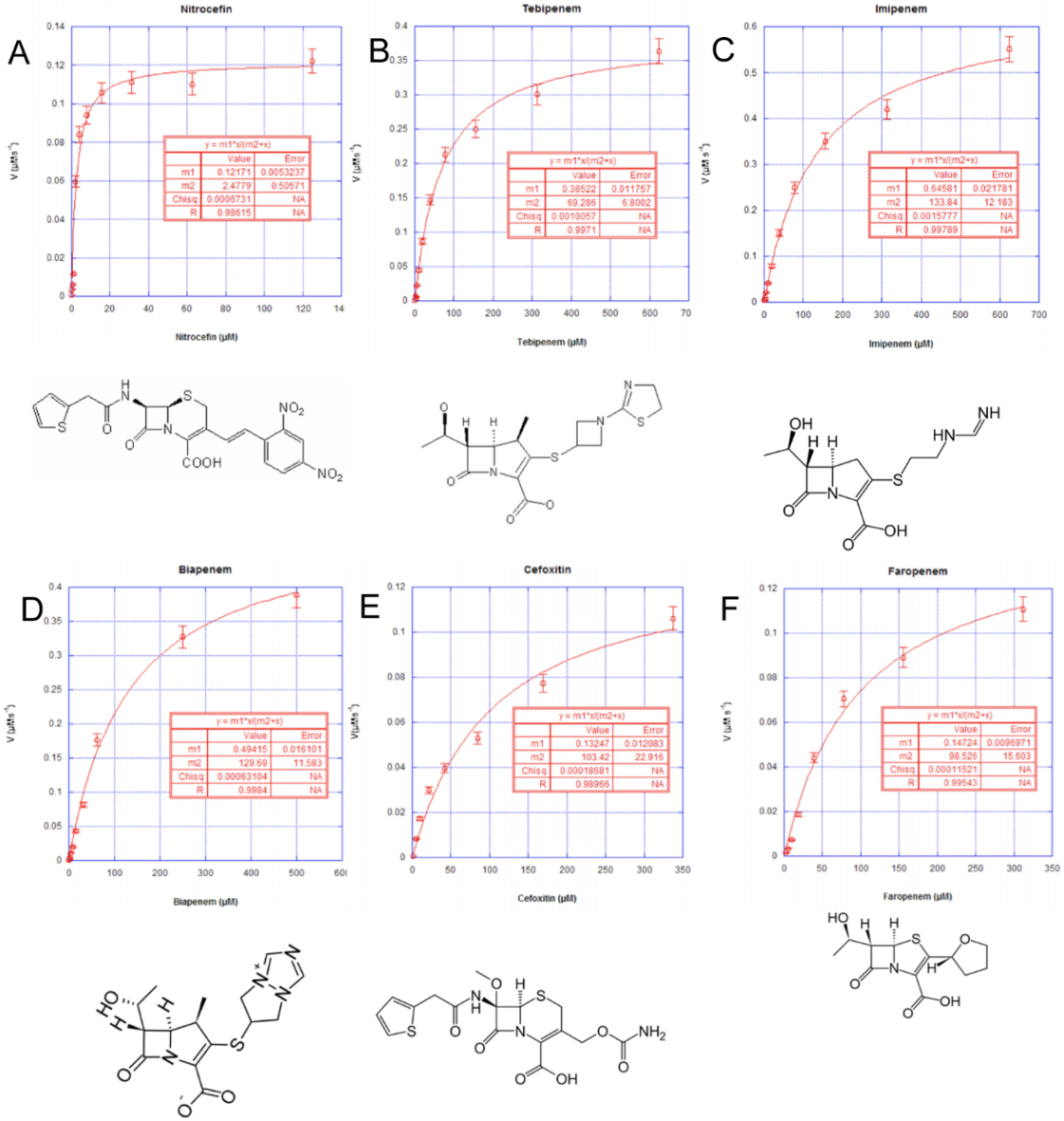
- The structures revealed an enlarged and flexible active site capable of accommodating many β-lactam substrates by having many of the active site residues on flexible loops, which explains the NDM-1 extended spectrum activity.
- The most important difference between the NDM-1 and other MBL structures is a significantly larger active site cavity.
- Size of the binding pocket is doubled (591 vs. 45-303 Å$^3$)



# NDM-1 Broad ß-Lactam Specificity

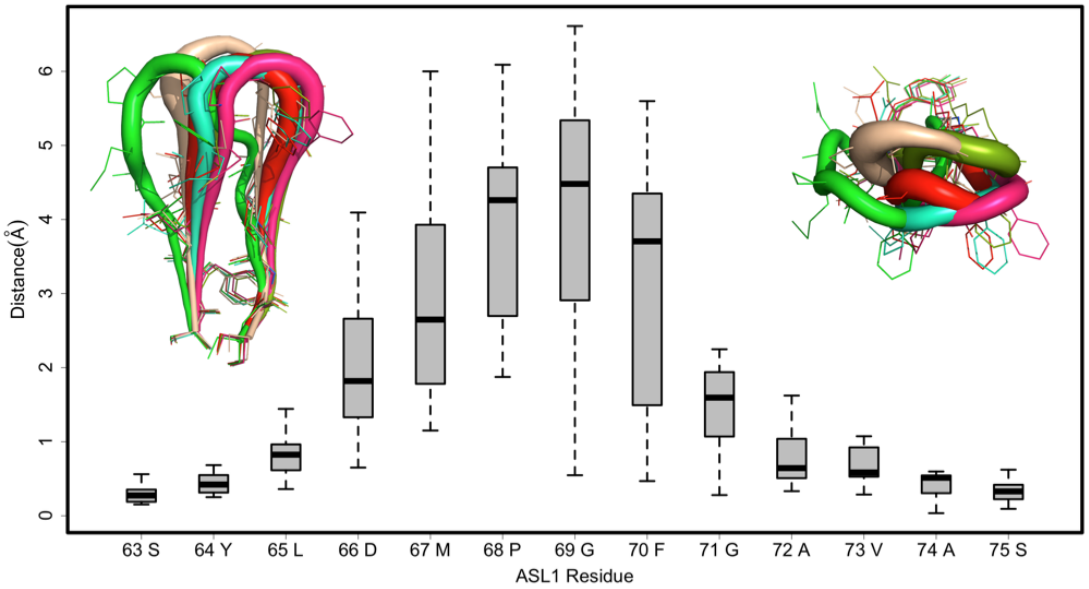


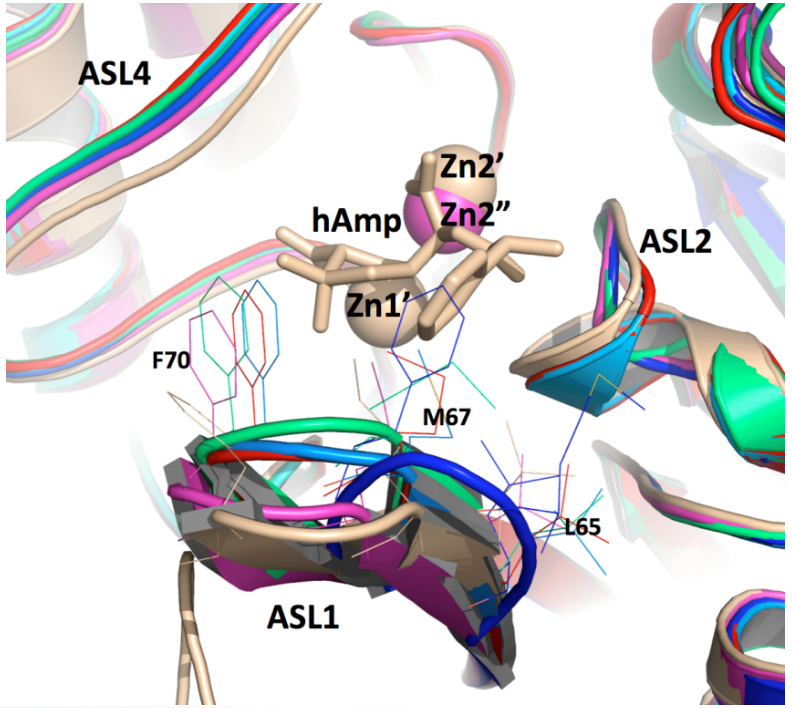



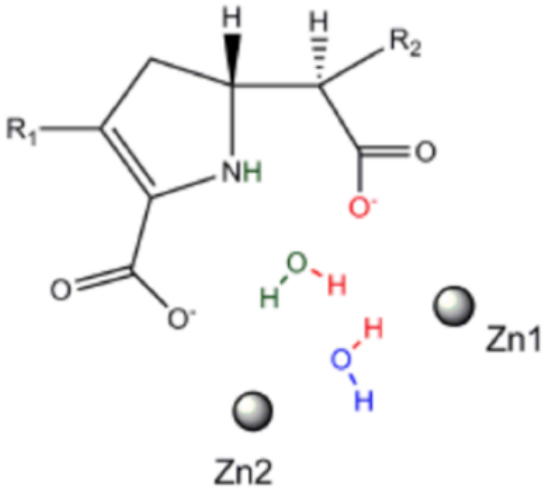
# The NDM-1 Active Site Loop 1

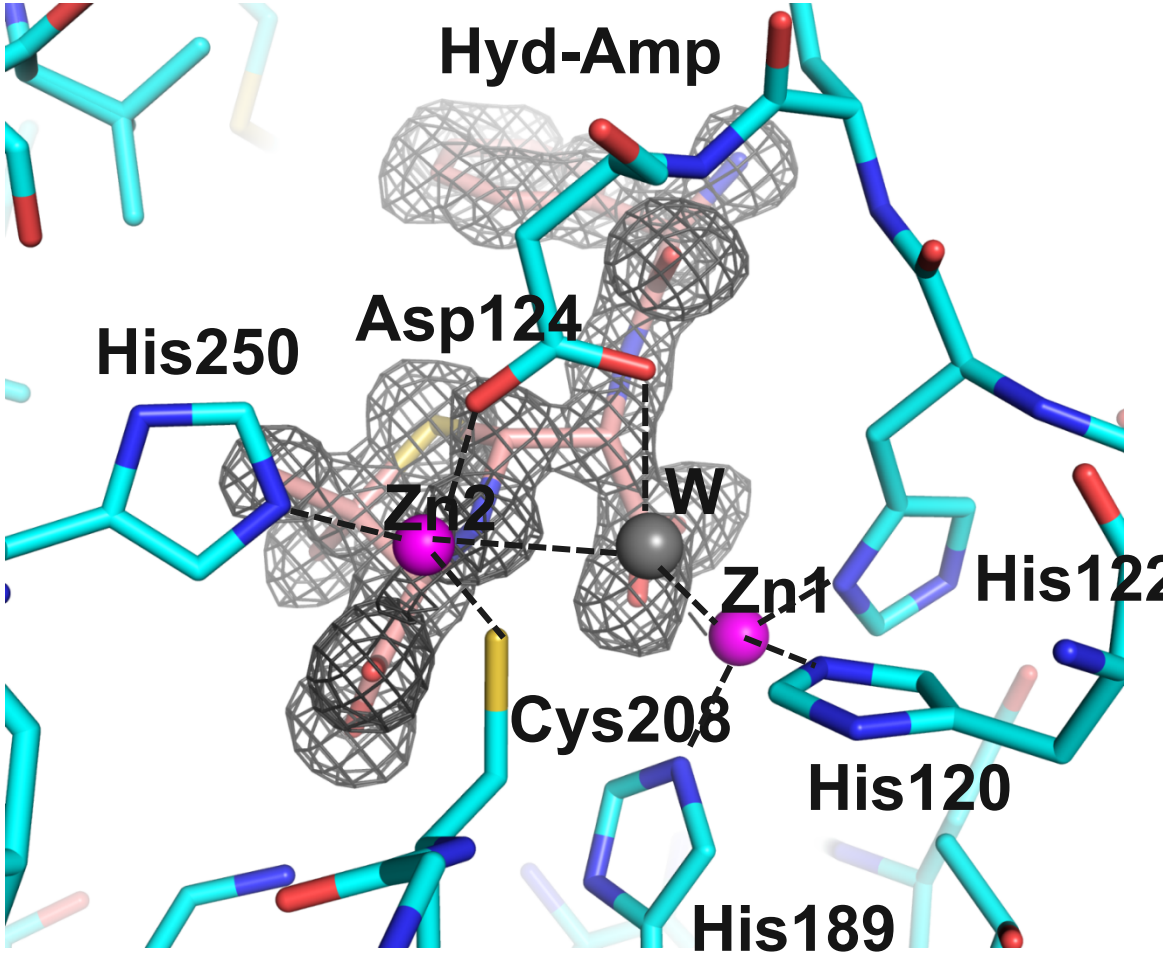
- Enlarged, more open and flexible binding site (ASL1 and ASL4).
- Additional residues from these loops, in particular ASL1, seem to also participate in the positioning of ligands in the active site in an orientation suitable for the hydrolysis of the β-lactam ring.
- NDM-1 seems to specifically recognize the β-lactam moiety; the rest of the ligand is recognized by mainly hydrophobic residues in the pocket.



# NDM-1: Crystal Structure with Hydrolyzed Ampicillin - Implications to Catalytic Mechanism



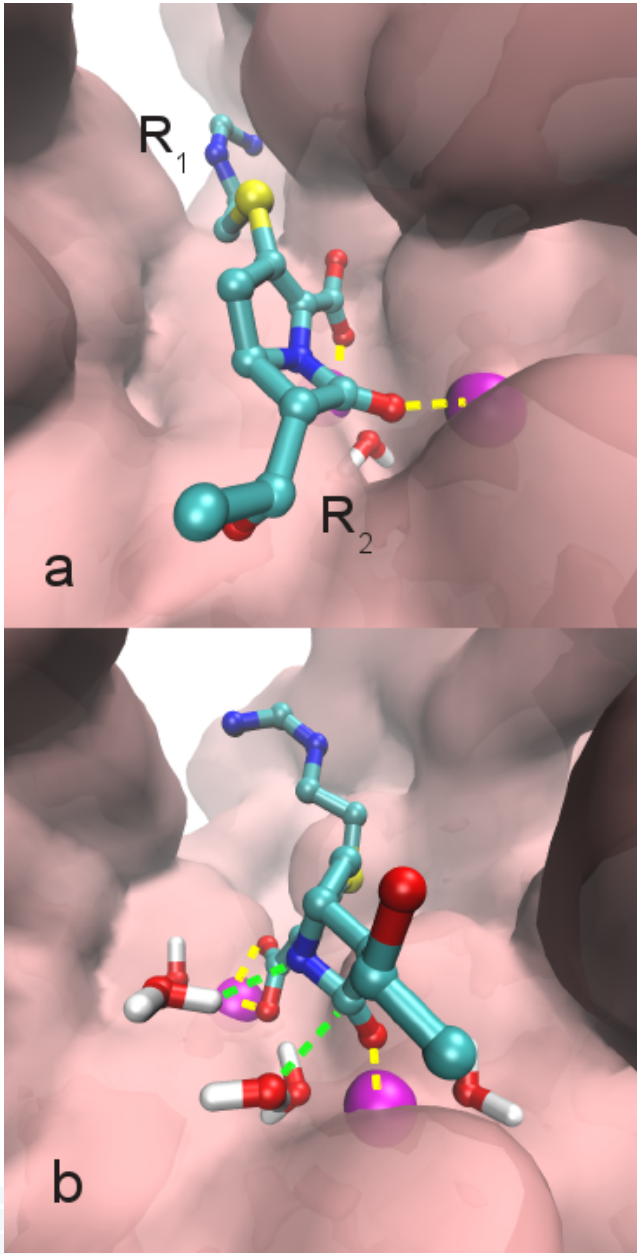

Resolution 1.05 Å
R/Rfree – 13.3/15.7%
pH=5.5



Kim, Y. *et. al. FASEB J.* 2013

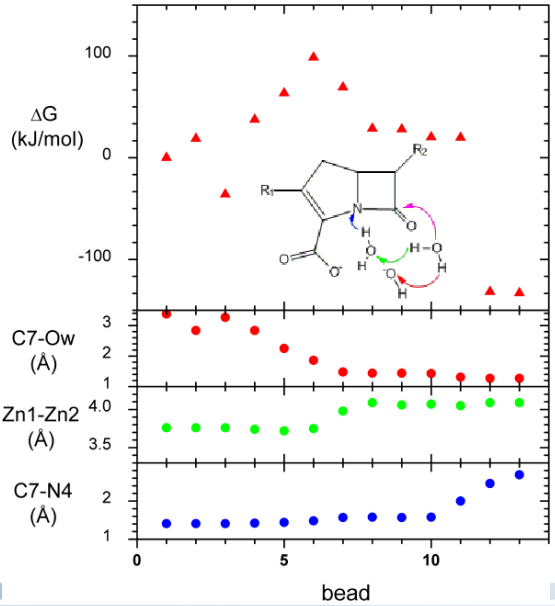
# Simulation of NDM-1 Active Site

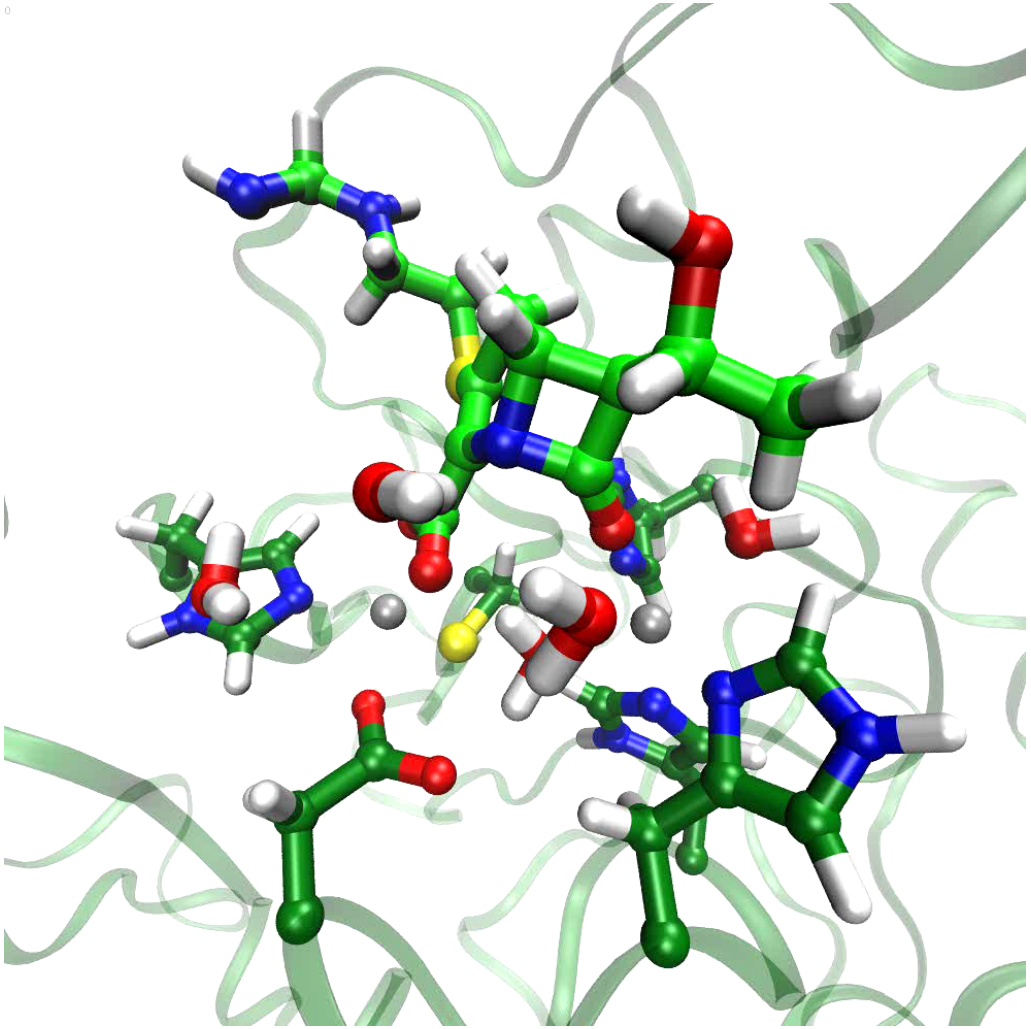
- NDM-1 substrate recognition is provided by interactions between the zinc ions and the carboxyl and carbonyl oxygen atoms of the substrate and nonspecific, generally hydrophobic interactions with the protein.
- The oriented water molecule occupies a pocket between the zinc ions.
- When the mobile loops move, a thin film of water surrounds the substrate, including two water molecules that serve as the nucleophile and as the source of the final proton required for ring cleavage.



# Bulk Water Attack – the Oriented Hydroxide Ion Case

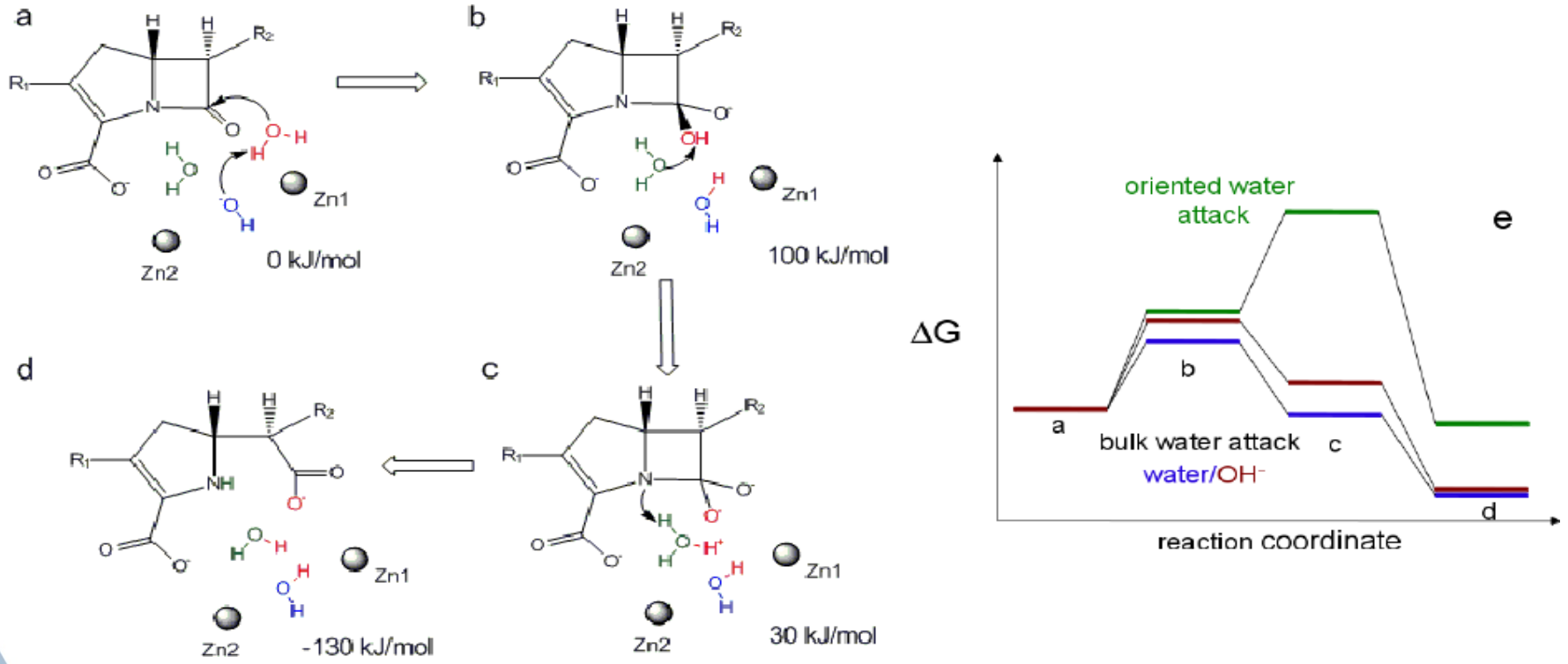
- The oriented hydroxide ion serves as the general base.
- The carbonyl (C7) carbon-water (Ow) oxygen distance decreases during the initial attack and the distance between zinc ions increases, reflecting the change in the charge state when a proton is transferred to the hydroxide ion.
- The nitrogen (N4)-carbon (C7) bond is cleaved in the final state.



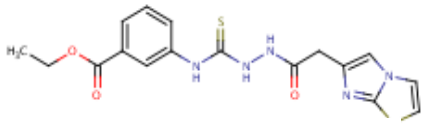
# NDM-1 Reaction Mechanism

- The oriented hydroxide ion (or water molecule) serves as a general base to accept a proton from a bulk water molecule.
  - A proton from the intermediate state is transferred to another bulk water molecule.
  - The proton is then shuttled to the N4 nitrogen of the substrate.
  - The nitrogen (N4) - carbon (C7) bond is cleaved in the final state. For each step free energy values are estimates of QM/MM calculations.
- The pathway energetics with a bulk water as the nucleophile (red, blue) or the oriented water acting as the nucleophile (green) are compared.

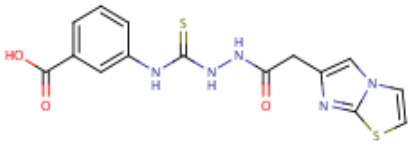


# The NDM-1 Novel Inhibitors

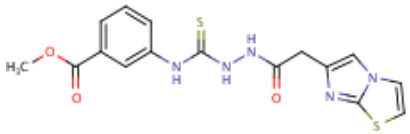
- A high-throughput screen of diverse small molecule library of approximately 50,000 compounds against purified NDM-1 enzyme revealed several inhibitors with low micromolar $IC_{50}$s.
- Focus on thiourea compounds: very potent, with low human cell cytotoxicity and straightforward synthetic chemistry.
- A structure-based approach is used to develop a lead molecule through synthetic chemistry efforts.



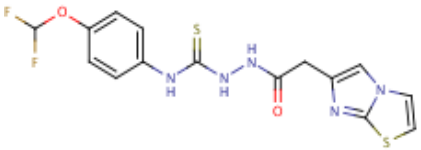
0.15 μM



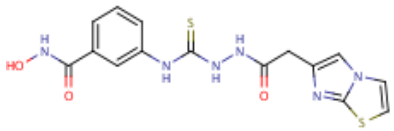
78 μM



9.8 μM

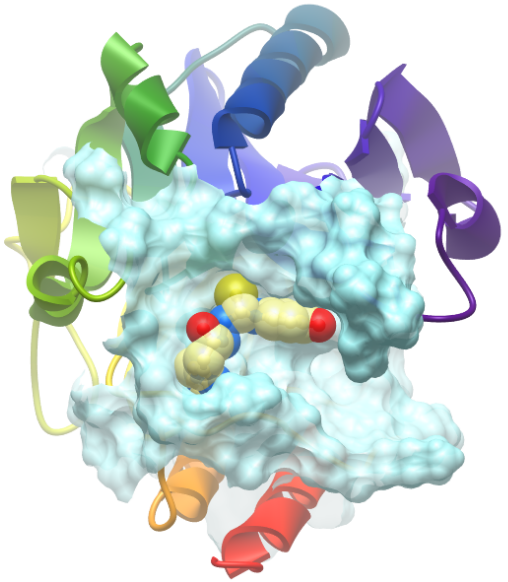


0.6 μM

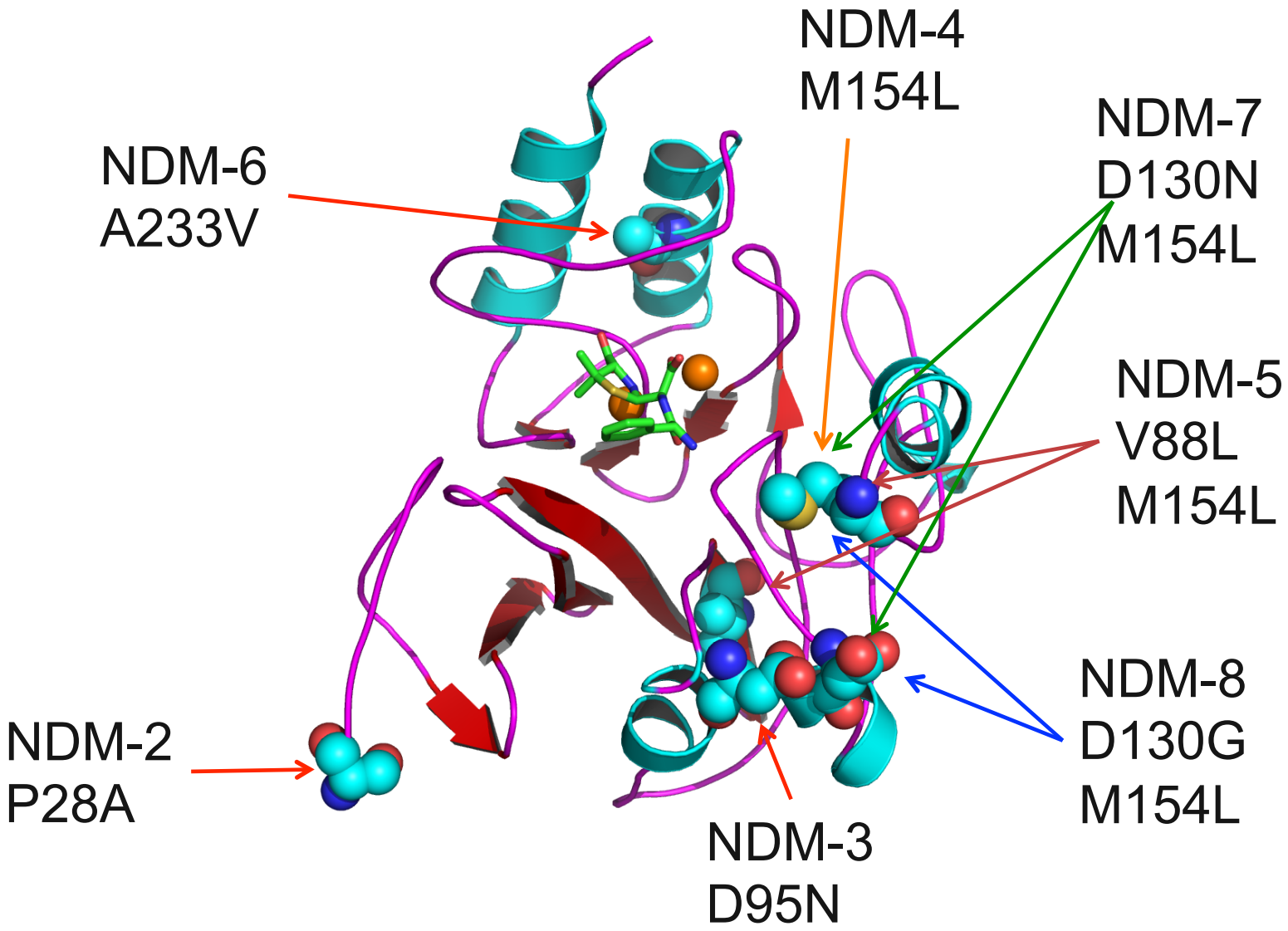


39 μM

initial hit



# Naturally Occurring Variants of NDM (12)

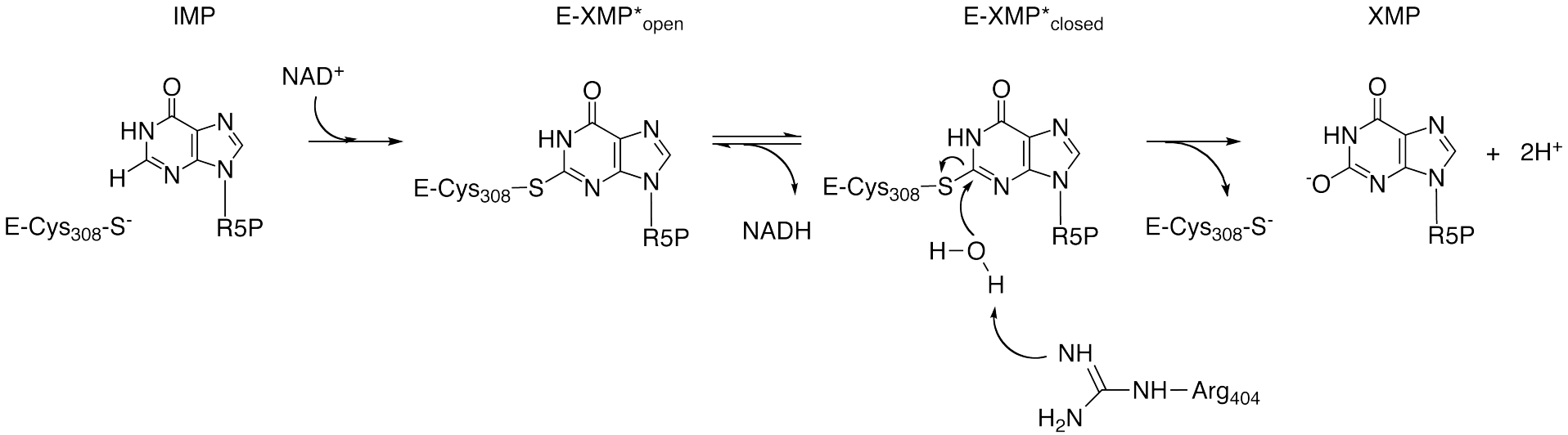


# The NDM-1 Enzyme can be Considered the Ultimate Example of Enzyme Promiscuity

- It shows lack of specificity for substrate recognition, with exception of the β-lactam moiety.
- Can use multiple metal cofactors for ligand binding and catalysis.
- Can switch catalytic mechanisms based on pH.

# Inosine 5'-Monophosphate Dehydrogenase (IMPDH)

- IMPDH is a universal and essential enzyme found in all three kingdoms of life.
- IMPDH catalyzes the oxidation of inosine 5'-monophosphate (IMP) to xanthine 5'-monophosphate (XMP) with the concomitant reduction of $NAD^+$ to NADH.
- The reaction is a unique branch point between adenine and guanine nucleotide biosynthesis, and a rate-limiting step of *de novo* GMP biosynthesis.
- IMPDH is crucial for DNA and RNA synthesis, signal transduction, differentiation, and other processes involved in cell proliferation.



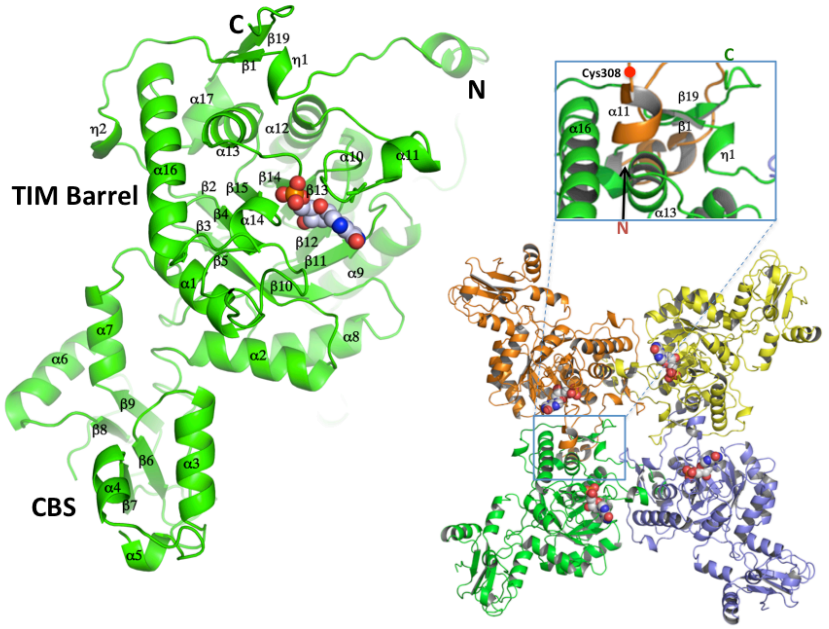

- Inhibitors of bacterial IMPDHs are known to inhibit pathogenic bacteria.

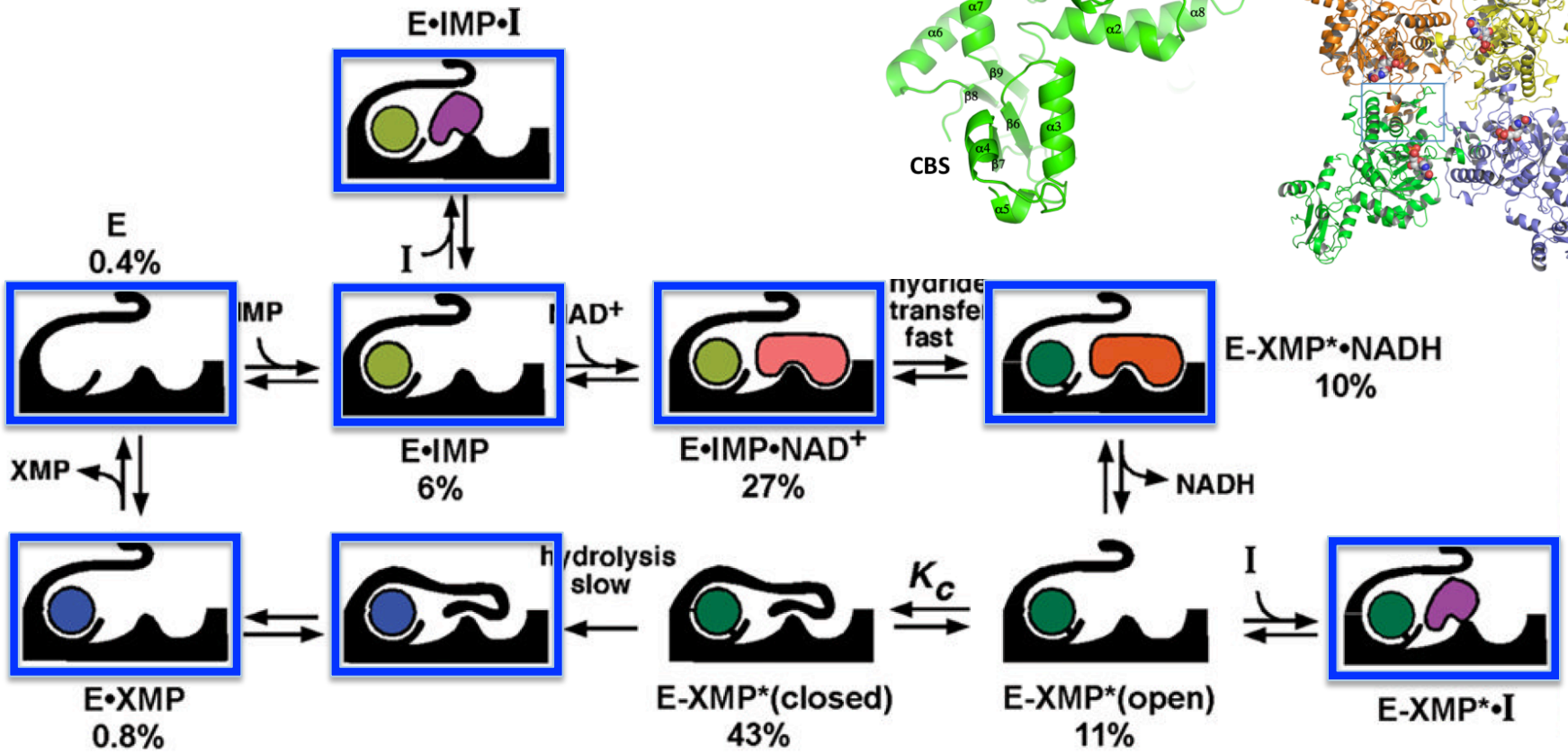
# IMPDH as a Drug Target

- **Human type II IMPDH is actively targeted in drug development programs** for immunosuppressive, anticancer, and antiviral chemotherapy. Ribavirin and mycophenolic acid (MPA) are known inhibitors of the human IMPDH enzyme.
- Several parasites have been targeted with inhibitors.
- **Structural and kinetic differences between mammalian and microbial enzymes** - most drugs that are successful in the inhibition of mammalian IMPDH are far less effective against the microbial forms of the enzyme.
- **Goal - exploit differences to design drugs that target microbial IMPDH**. With greater knowledge of the structure and catalytic mechanism of the microbial enzymes, an effective and selective inhibitor of microbial IMPDH can be developed for use as a drug against bacteria including multi-drug resistant strains.

# IMPDH Reaction Mechanism

- Random addition of substrates and ordered release of cofactor and product.

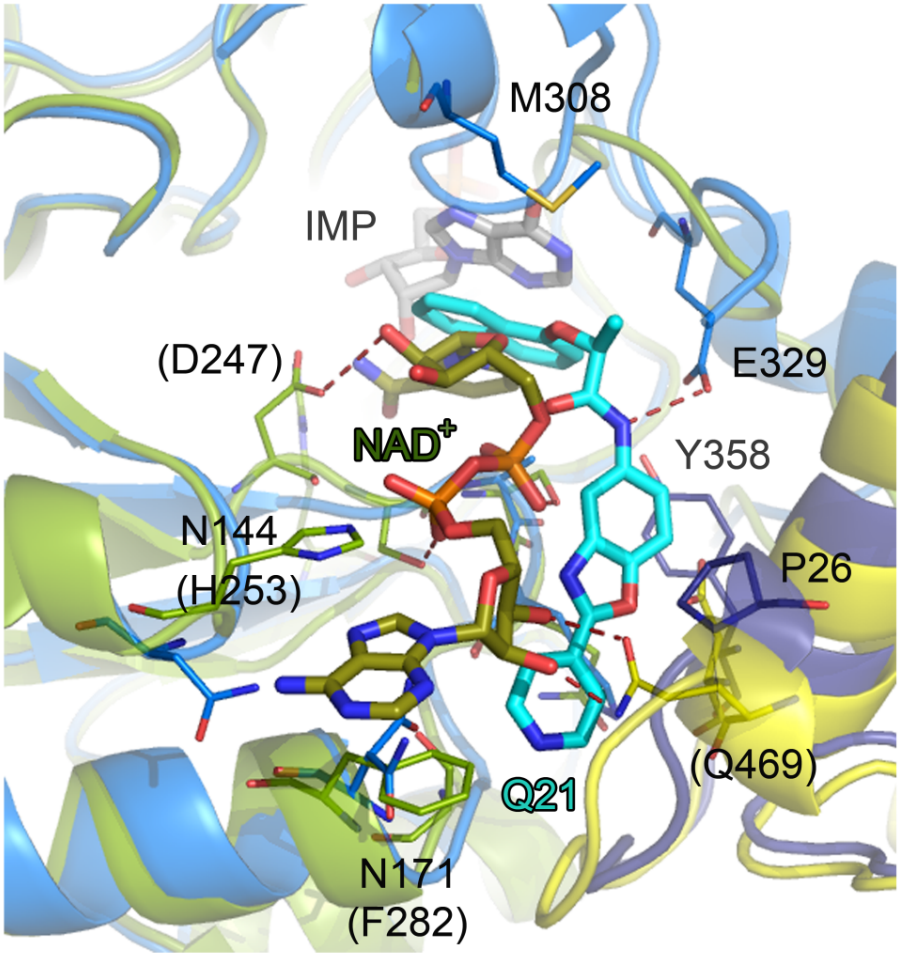



*Cryptosporidium parvum*

Umejiego *et al., Chem. Biol. (2008)*

# Unexpected Inhibitor Binding Mode Observed in *C. parvum* IMPDH



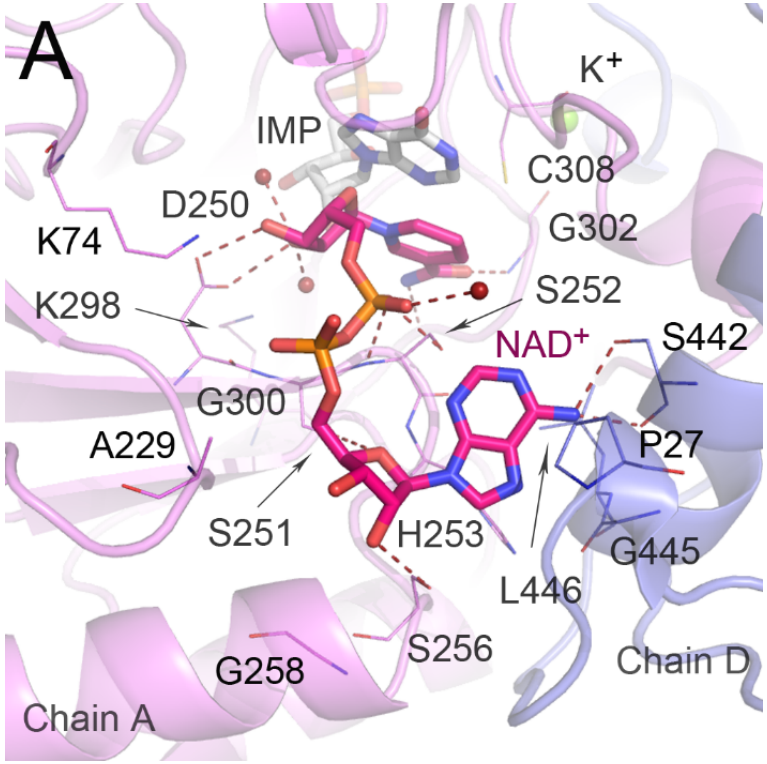

- Inhibitors were designed to mimic the $NAD^+$ binding based on the structures of human type II enzyme.
- $NAD^+$ interaction with the human type II IMPDH are mainly within one subunit.
- Important stacking interactions are present in the human type II enzyme for the adenosine ring of $NAD^+$.
- Structures with *C. parvum* enzyme revealed an unexpected inhibitor binding mode.
- No residues capable of adenine stacking in the *C. parvum* IMPDH.
- Possibility of different cofactor binding modes in human and bacterial enzymes.

Overlay of *C. parvum* IMPDH (blue/purple) with IMP and Q21 (PDB id 4IXH) with human type II IMPDH (olive/yellow) with CI-IMP and $NAD^+$ (PDB id 1NFB)

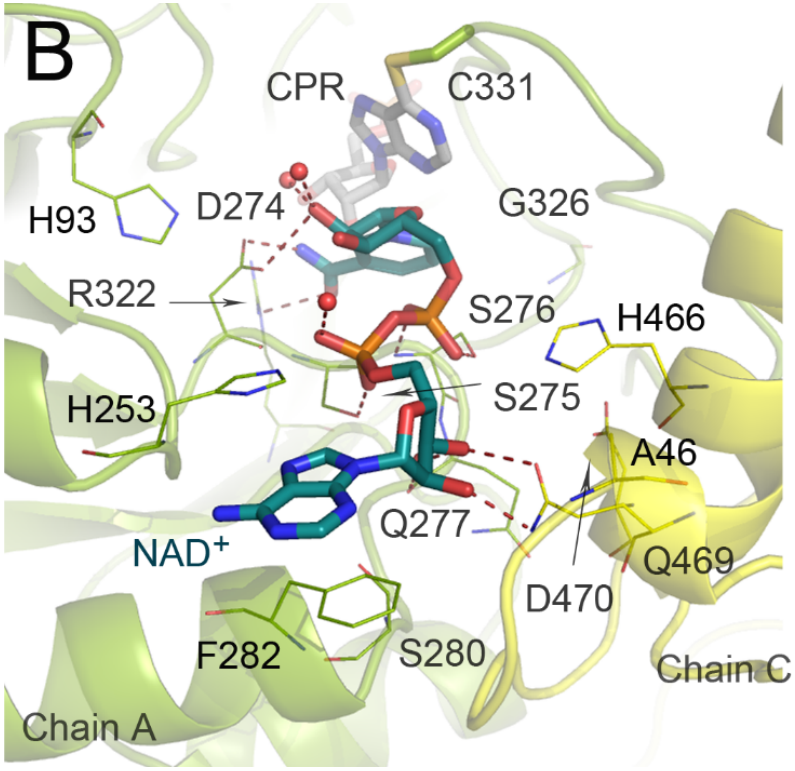
Gorla *et al. J. Med. Chem.* **2013**, *56*, 4028.

# Novel NAD$^+$ Binding Mode Found in Bacterial IMPDH

- First structure of prokaryotic IMPDH with bound NAD$^+$.
  - closed NAD$^+$ conformation.
  - adenine interacting with several residues from the adjacent monomer (S442, L446, P27).
- Human type II IMPDH structure with bound NAD$^+$.
  - open NAD$^+$ conformation.
  - adenine portion stacking between H253 and F282 within the same monomer.



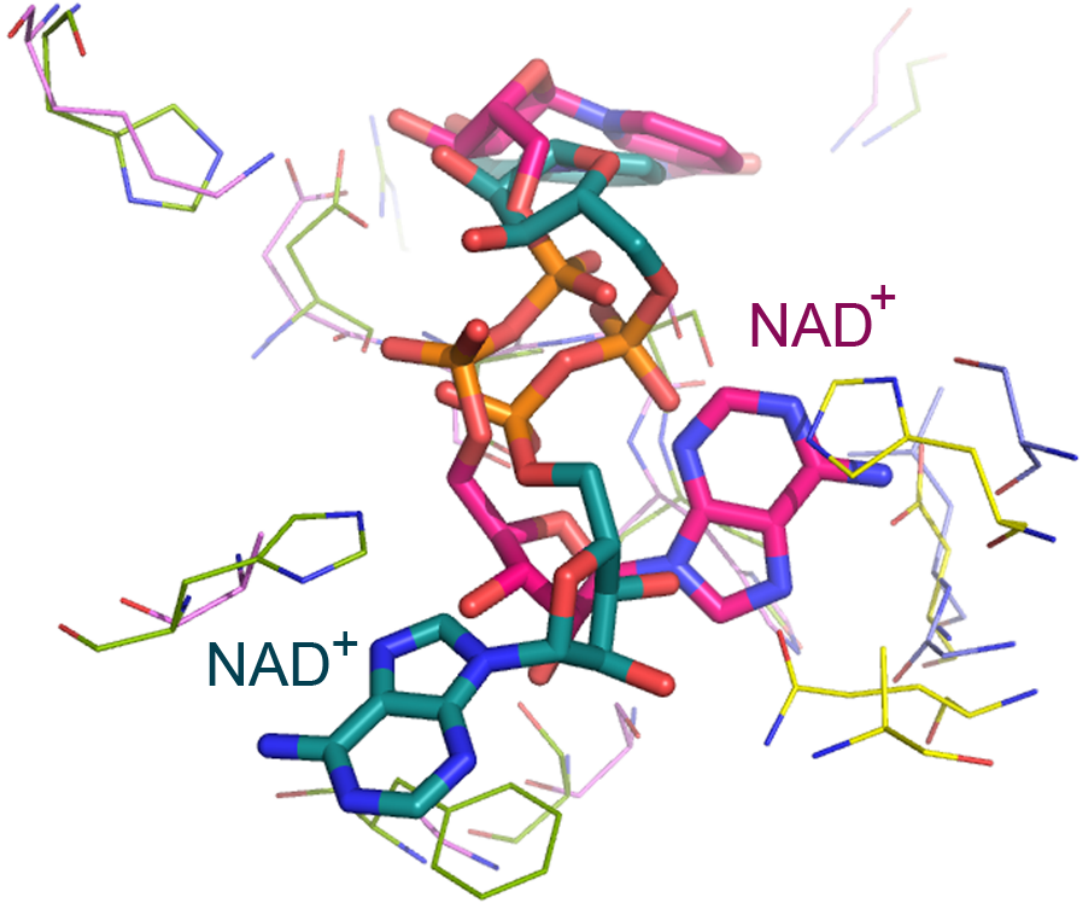

Closed NAD$^+$ conformation in *V. cholera* IMPDHΔCBS with IMP and NAD$^+$ (4HLV)




Open NAD$^+$ conformation in human type II IMPDH with 6-Cl-IMP (CPR) (1NFB)

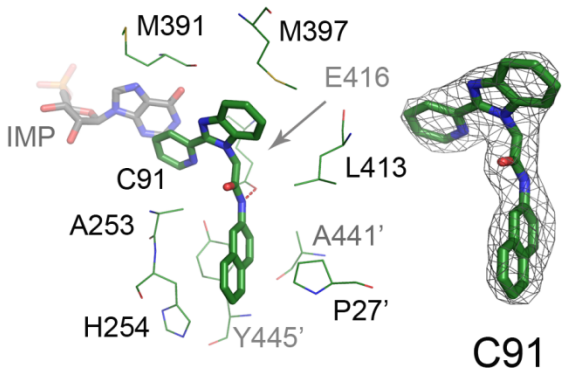
# Novel $NAD^+$ Binding Mode Found in Bacterial IMPDH

- There are two distinct eukaryotic ($A^E$) and bacterial ($A^B$) adenine subsites that can be explored.
- The binding mode of $NAD^+$ provides a rationale for the binding of inhibitors.

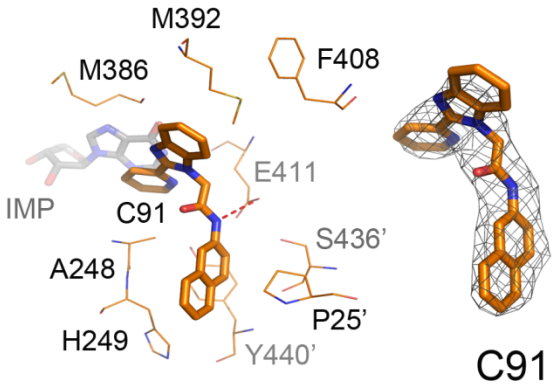


# Structures of the Bacterial IMPDH ΔCBS Mutants with Inhibitors

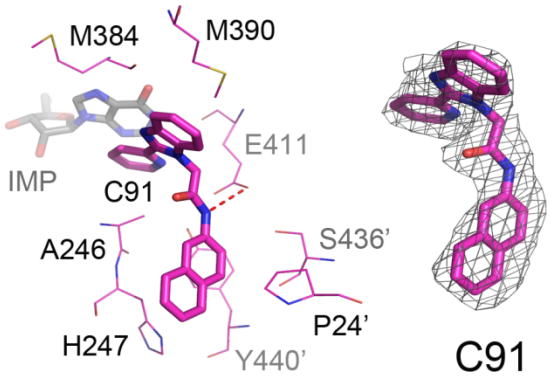
*B. anthracis* with IMP and C91



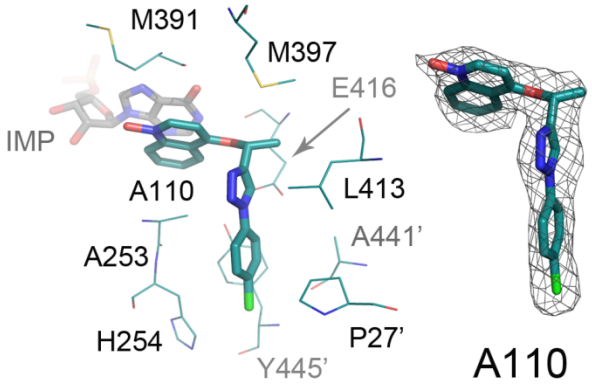

*C. perfringens* with IMP and C91



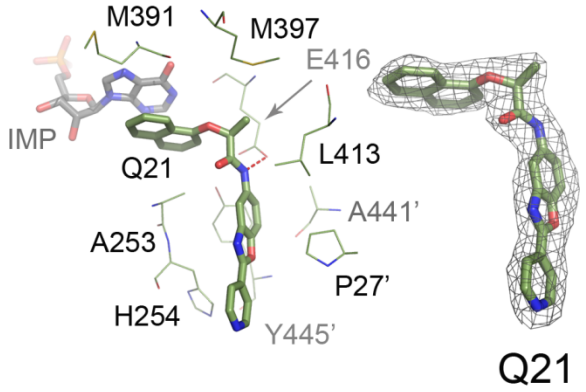

*C. jejuni* with IMP and C91



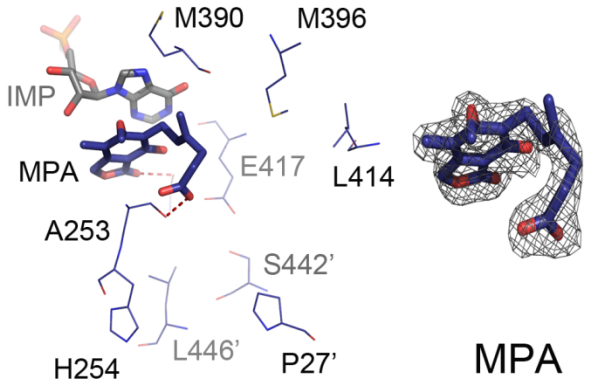

*B. anthracis* with IMP and A110




*B. anthracis* with IMP and Q21

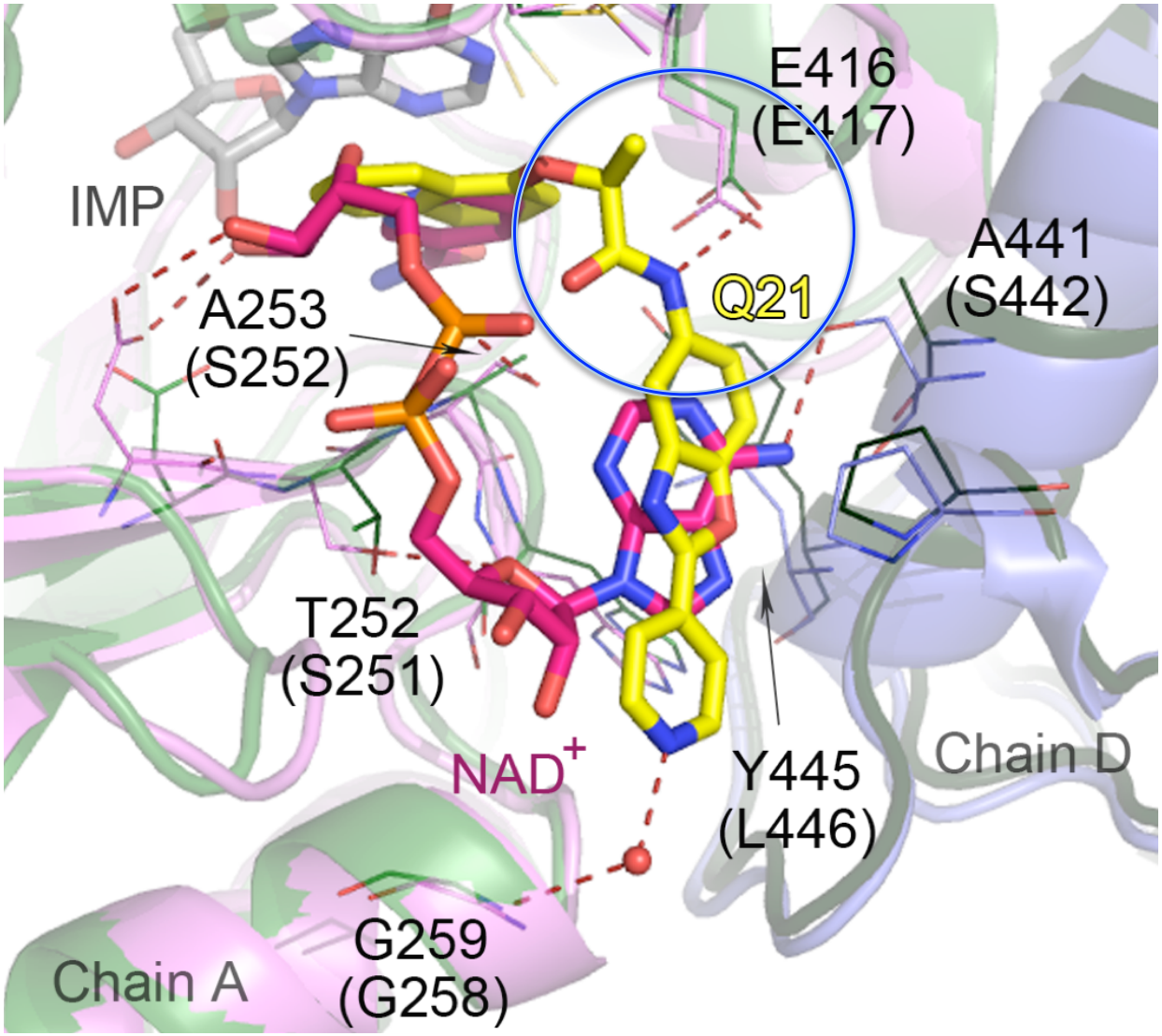


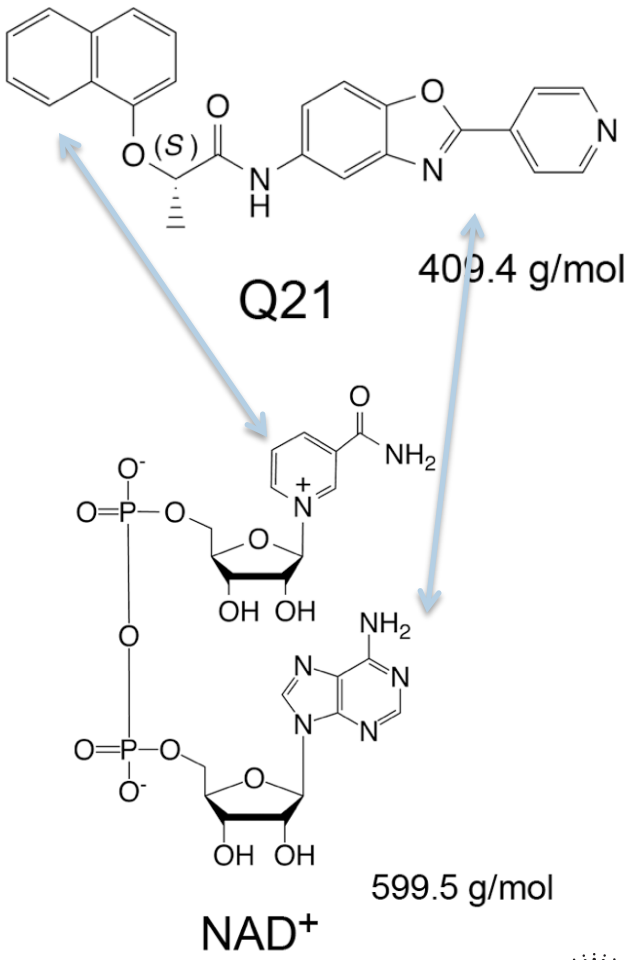

*V. cholerae* with IMP and MPA




$2F_0$-$F_c$ electron density map contoured at 1 σ for each ligand

# All Inhibitors Bind in the Same Mode Mimicking NAD+ Binding to Bacterial IMPDHs

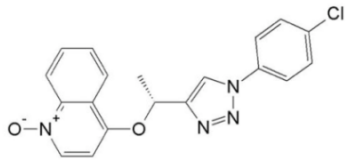


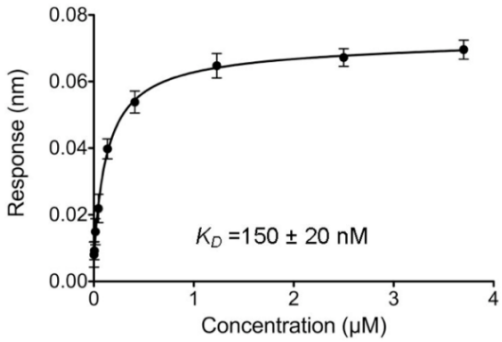



# Inhibitor Binding

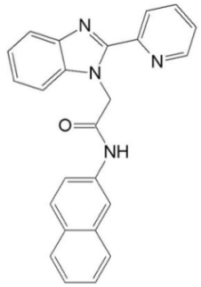
- IMPDHs from four different species show very different sensitivity to inhibitors.

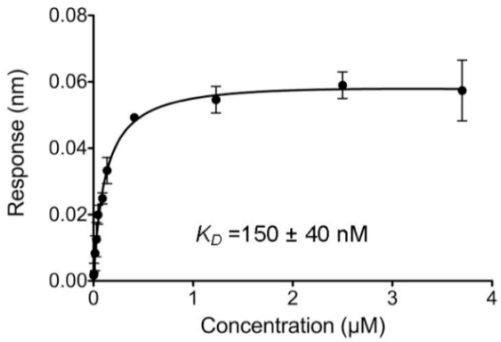
A110




C91



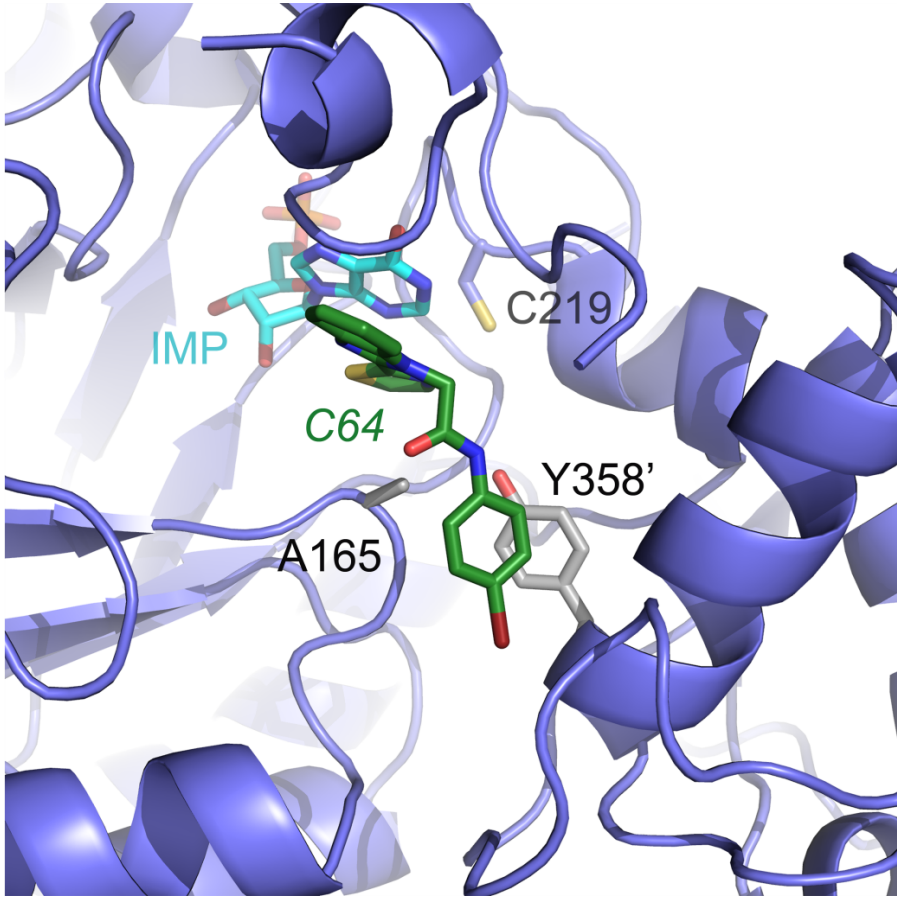




| | *B. anthracis* | | *C. jejuni* | | *C. perfringens* | | *V. cholerae* | |
|---|---|---|---|---|---|---|---|---|
| | $IC_{50}$ (nM) | $K_D$ (nM) | $IC_{50}$ (nM) | $K_D$ (nM) | $IC_{50}$ (nM) | $K_D$ (nM) | $IC_{50}$ (nM) | $K_D$ (nM) |
| **A110** | 57 ± 7 | 150 ± 20 | 120 ± 20 | 200 ± 18 | 280 ± 30 | 490 ± 42 | > 5000[a] | > 20000 |
| **C91** | 57 ± 1 | 150 ± 40 | 51 ± 9 | 140 ± 10 | 570 ± 20 | 1100 ± 62 | > 5000[a] | > 20000 |

Makowska-Grzyska et al. *Biochemistry* **2012**, *51*, 6148

# *C. parvum* Inhibitors and Structural Motif Required for Binding

| | Inhibitor minimal structural motif | |
|---|---|---|
| ***C. parvum*** | **A165** | **Y358'** |
| ***B. anthracis*** | **A253** | **Y445'** |
| ***C. jejuni*** | **A246** | **Y440'** |
| ***C. perfringens*** | **A248** | **Y440'** |
| ***M. tuberculosis*** | **A285** | **Y487'** |
| ***V. cholerae*** | **S252** | **L446'** |
| ***H. sapiens* II** | **S276** | **D470'** |




*MacPherson, et al. J. Am. Chem. Soc.* ***2010****, 132, 1230*
*Gorla, et al. J. Chem. Med.* ***2013****,* 56, 4028

# IMPDH Summary

- Multiple structures of IMPDHs from seven pathogenic bacteria have been determined, including structures of the protein-inhibitor complexes.
- The first structure of bacterial IMPDH with bound substrate and the $NAD^+$ cofactor shows a dramatically different mode of cofactor binding than the one observed for eukaryotic IMPDHs.
- This structure helps to explain the binding mode adapted by several different classes of inhibitors.
- Despite a high sequence similarity between bacterial IMPDHs, some important differences exist that can be exploited to design species-specific inhibitors - small changes in the active site translate into large changes in ligand binding.
- These structures provide the basis for exploring the inhibitor selectivity and offer a potential strategy for further ligand optimization that can be used to design more potent inhibitors of bacterial IMPDHs.

# Summation

- HTP technologies in molecular and structural biology can be applied to complex problems such as antibiotic resistance, human, animal, soil, and marine microbiomes and provide proteins for functional and mechanistic studies as well as structures of proteins, protein/ligand, protein/protein, and other complexes that are functionally important.
- Advanced structural studies, such as obtaining structures of protein complexes or more precisely identifying substrates, products and inhibitors of enzymes, are expected to contribute to the understanding of metabolic pathways, guide their engineering and contribute to the development of new treatments and drugs.
  - Basic research is essential for understanding the basis of antibiotic resistance and developing new approaches to antibiotic therapies as microbes evolve to evade existing antibiotics.
  - Combining genomic data with biological observations and HTP structural biology technologies can help advance our understanding of microbes, microbiomes, their evolution and adaptation, and their interactions.
  - Exploring the microbiomes' genomic potential may result in discoveries of new biology, new chemistry and metabolic pathways.

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