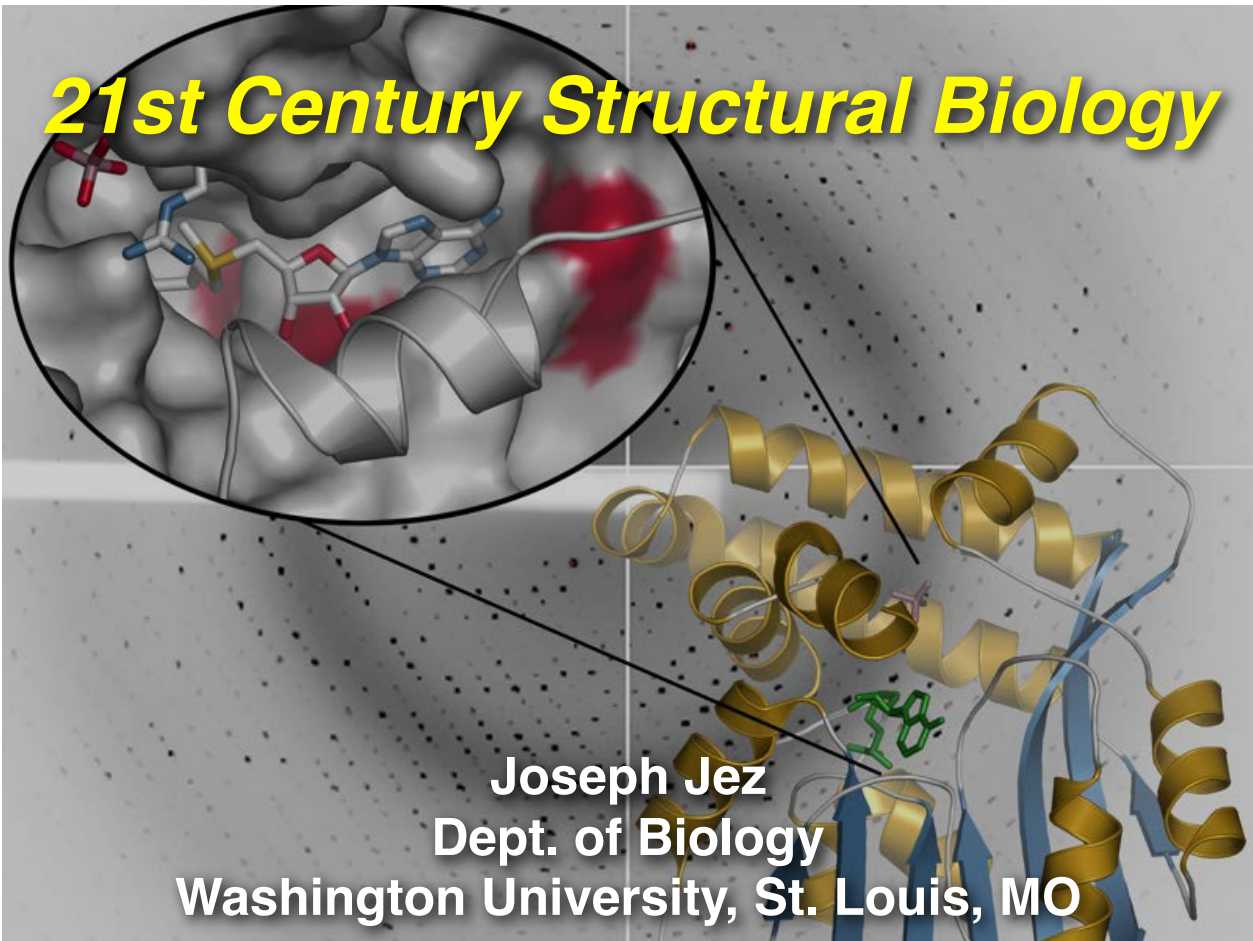


# 21st Century Structural Biology



Joseph Jez  
 Dept. of Biology  
 Washington University, St. Louis, MO

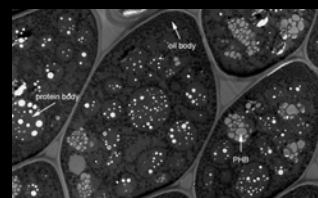
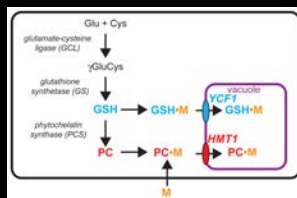
## Jez Lab - Exploring Nature's Machinery

Environment & Application



Metabolism

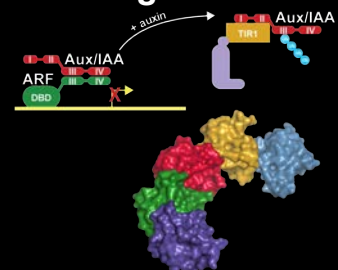
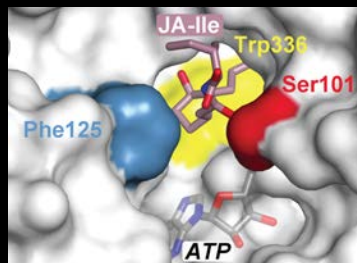
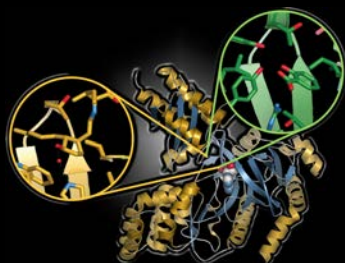
Cellular



Structure

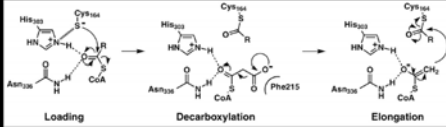
Mechanism

Regulation

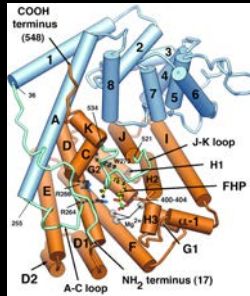


# Armed with the knowledge of where every atom is ...

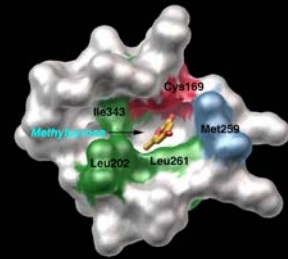
## Chemical Mechanism



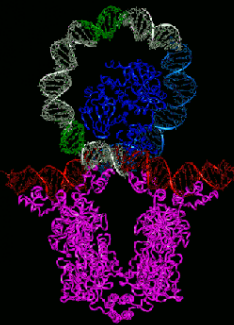
## Evolutionary Relationship



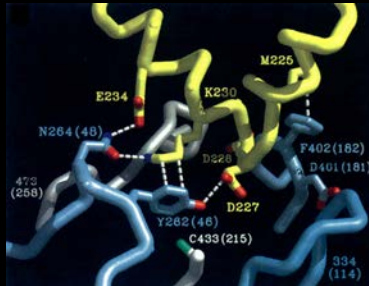
## Protein Engineering



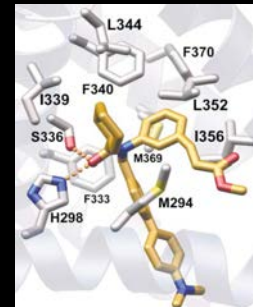
## Gene Regulation



## Protein-Protein



## Drug Design



## 20th Century - Setting the Stage

1935: First Protein Crystal Diffraction  
(Crowfoot, Fankuchen, Bernal)

1958: Experimental Phasing  
First Protein Structures  
(Perutz, Kendrew)



1965: First Enzyme Structure  
(Philips)



1970: Protein Data Bank

1972: Recombinant DNA

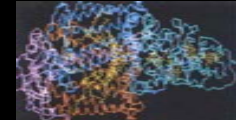
1976: Synchrotrons

1978: Computer Graphics



1981: SAD/MAD Phasing

1985: First Membrane Protein  
(Deisenhofer, Huber, Michel)



1986: HTS Screening



1995: accessible 3D graphics

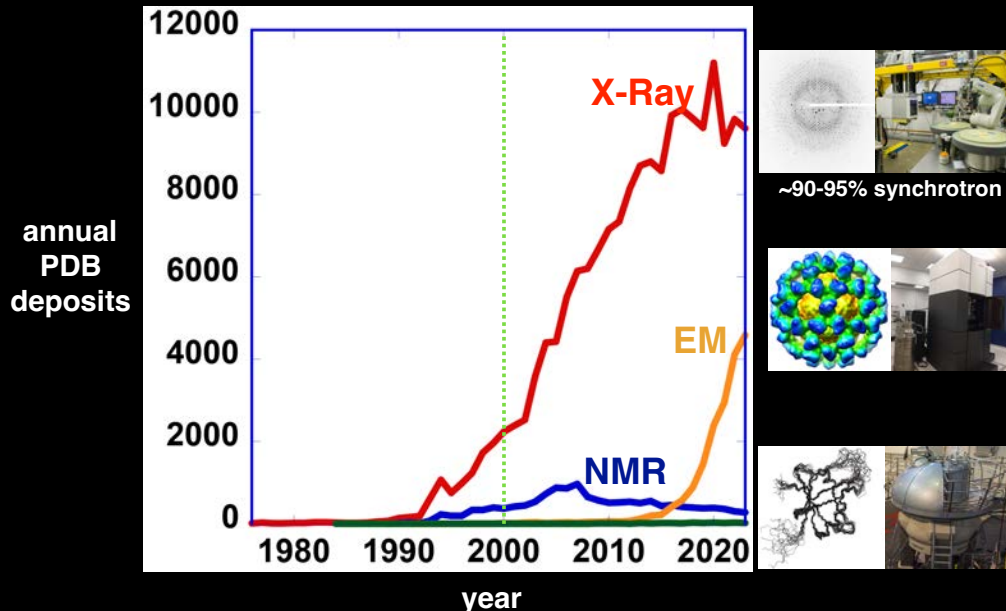


2000: Protein Structure Initiative



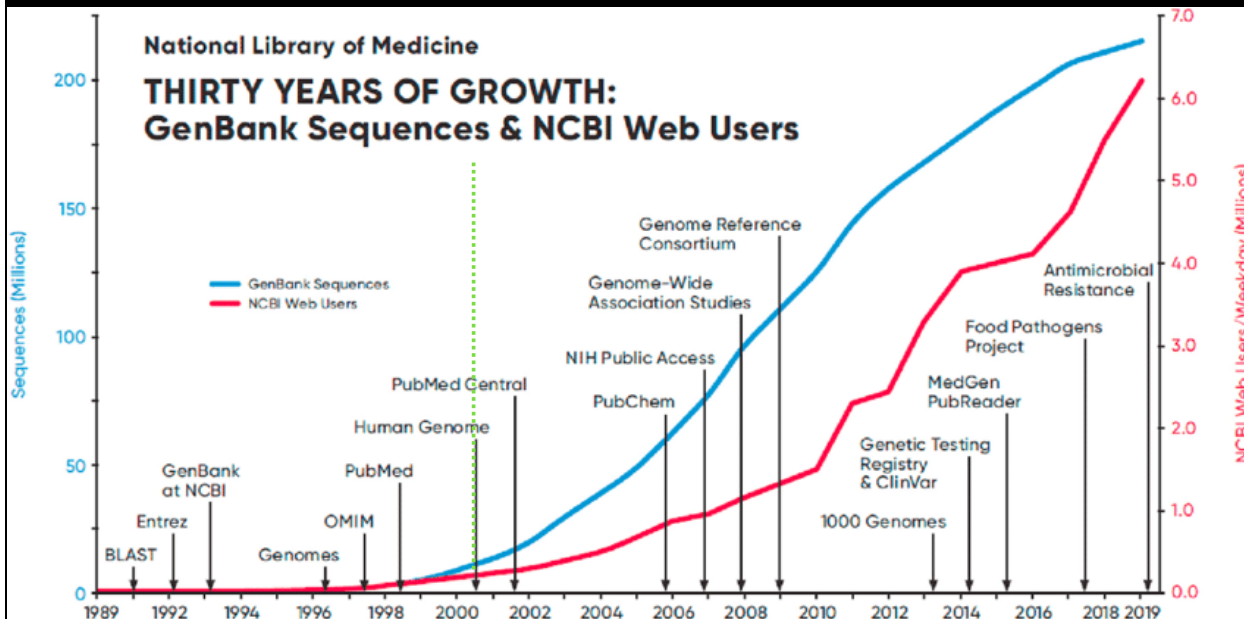
# 21st Century - Structural Explosion

5 Apr 24: 217,966 structures deposited



<https://www.rcsb.org/stats/all-released-structures>

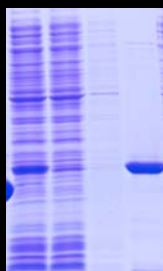
# 21st Century - Sequence Explosion



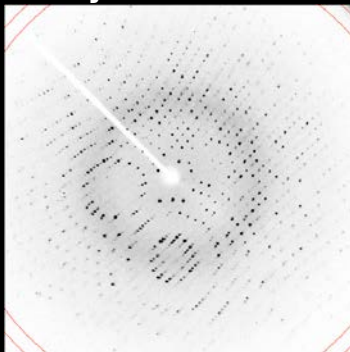


# Crystallography in 5 Steps

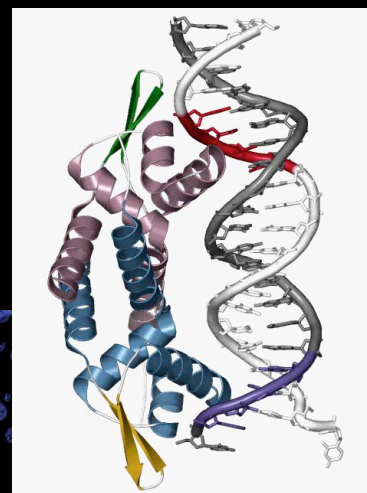
## 1. Purify Protein



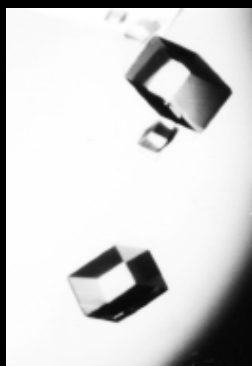
## 3. X-ray Diffraction Data



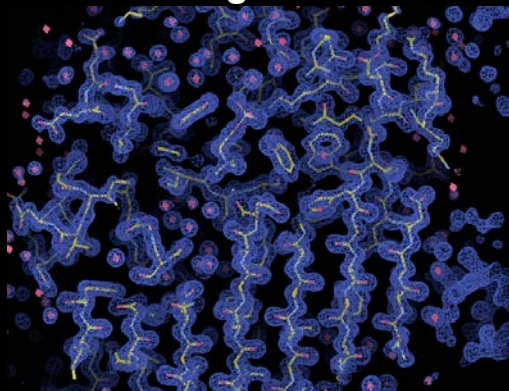
## 5. Building and Refining the Model



## 2. Grow Crystals

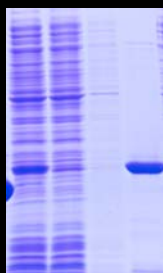


## 4. Phasing the Structure



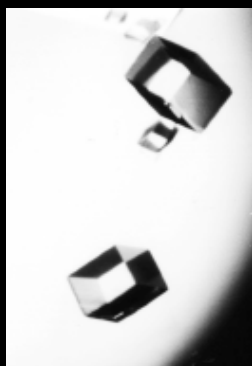
# Protein & Crystallization Advances

## 1. Purify Protein



- **robotic gene cloning/gene synthesis**  
~100 over expression cloning vectors - 20,000 constructs per year
- **protein expression**  
bacterial, yeast, insect cell, mammalian cell expression systems  
cell-free systems
- **semi-automated protein purification**  
parallel workstations for multiple, large-scale

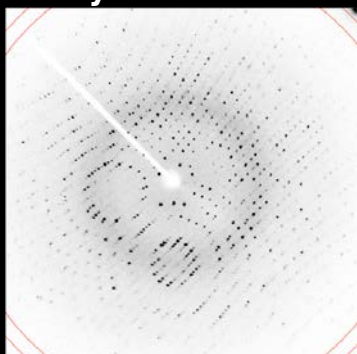
## 2. Grow Crystals



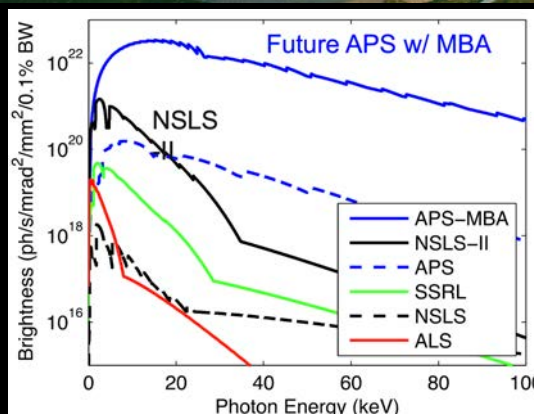
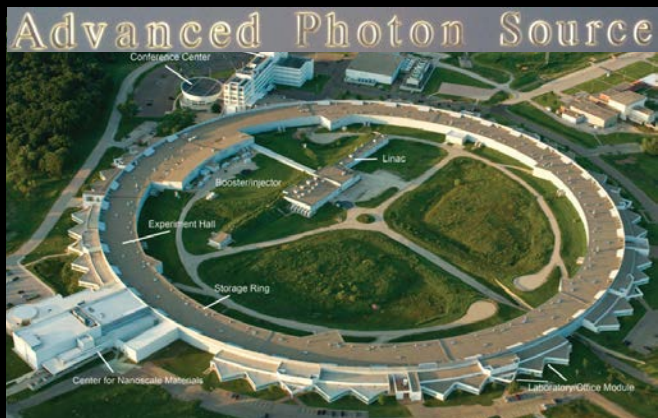
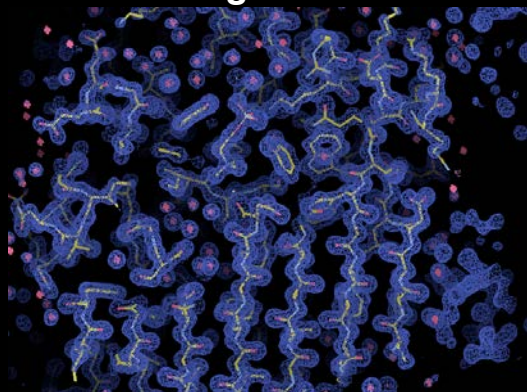
- **semi-automated crystallization and image**  
nL drops, robotic crystallization, incubation, monitoring  
~7,000 proteins per year

# Synchrotron Sources

## 3. X-ray Diffraction Data

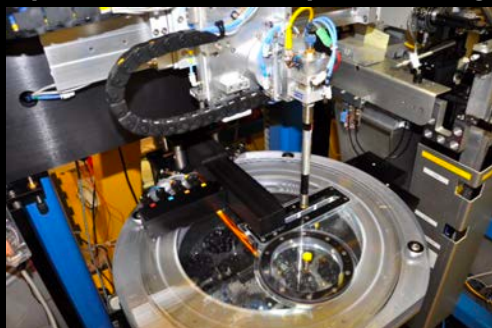


## 4. Phasing the Structure



# Beamline Technology

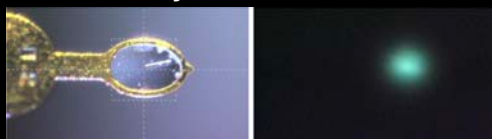
- remote data collection (robotics)
- point-and-click sample centering



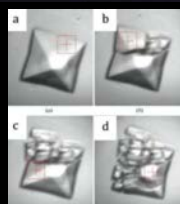
- detector tech - PILATUS - 100 images/sec
- robotic goniometer (rapid)



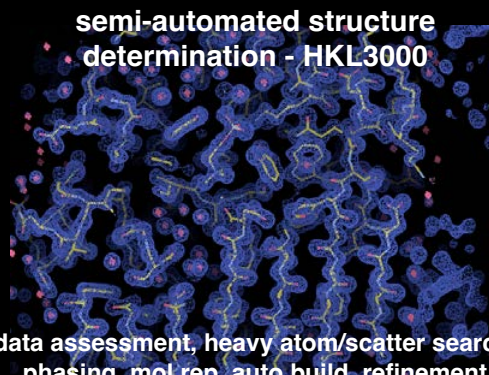
- microcrystals & mini-beams



high-intensity beam  
flexible beam size -  
1 x 1  $\mu\text{m}$  to  
200 x 200  $\mu\text{m}$



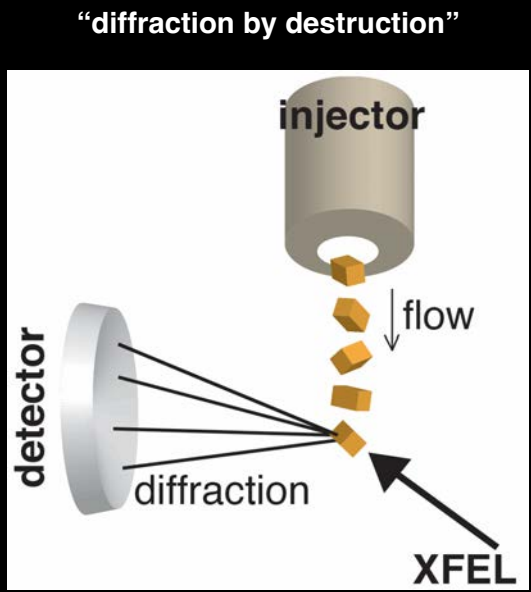
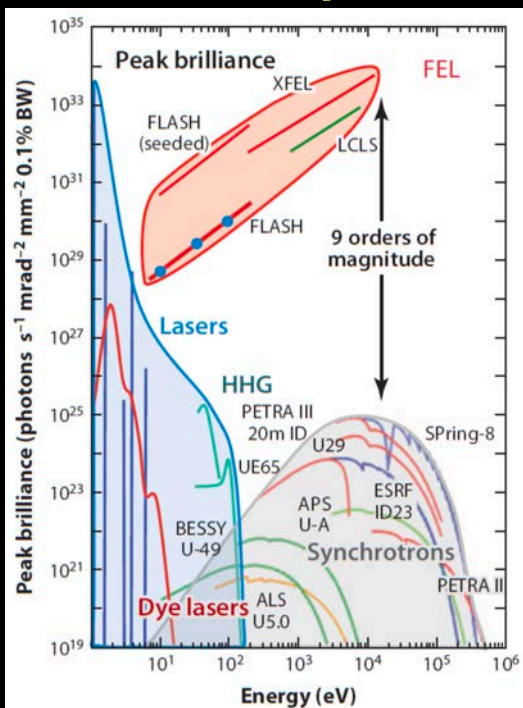
- semi-automated structure  
determination - HKL3000



data assessment, heavy atom/scatter search,  
phasing, mol rep, auto build, refinement



# Next? X-ray Free Electron Lasers (XFEL)

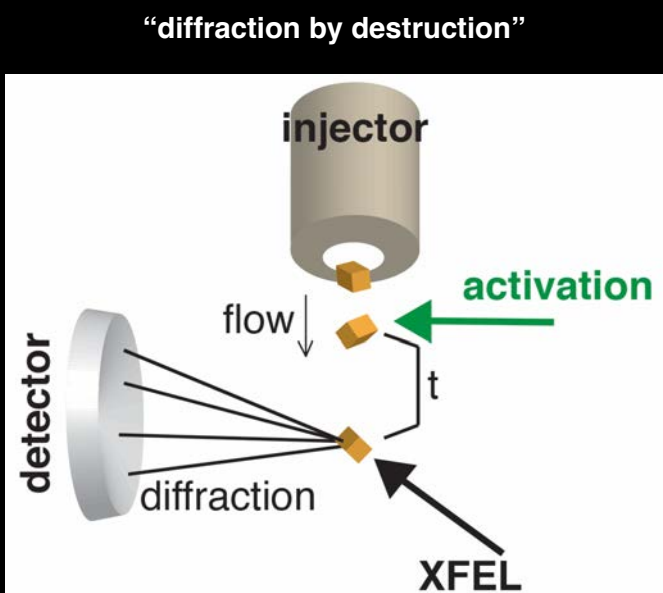
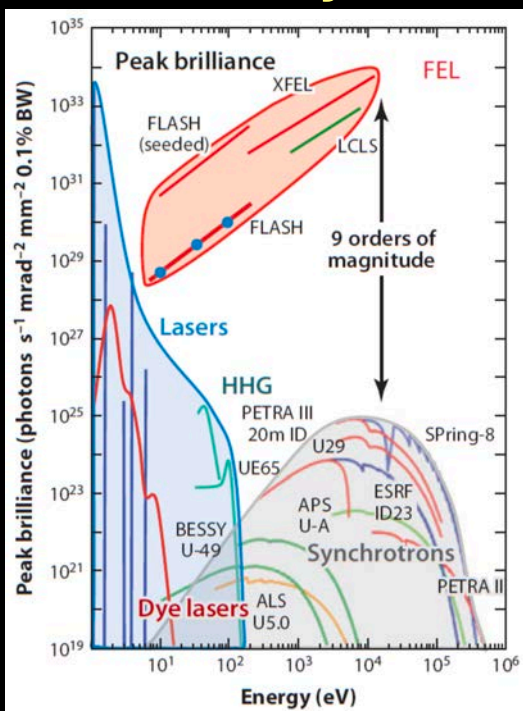


XFEL serial crystallography

- based on linear electron accelerator
- high brightness with femtosecond pulse
- fully coherent light

Ullrich et al (2012) Ann Rev Phys Chem

# Next? X-ray Free Electron Lasers (XFEL)



laser activation = time resolution

- based on linear electron accelerator
- high brightness with femtosecond pulse
- fully coherent light

Ullrich et al (2012) Ann Rev Phys Chem

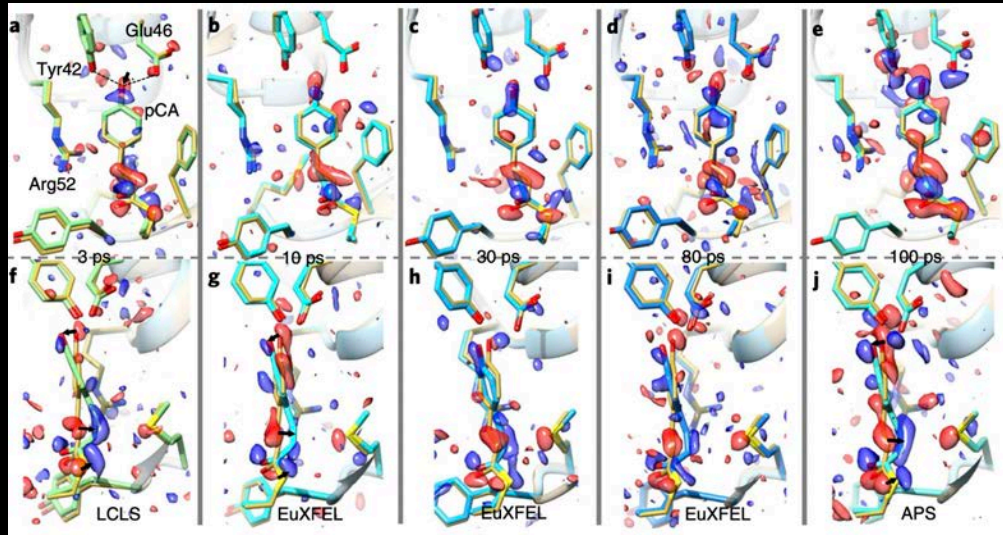
# Next? X-ray Free Electron Lasers (XFEL)

nature **methods**

ARTICLES

<https://doi.org/10.1038/s41592-019-0628-z>

## Time-resolved serial femtosecond crystallography at the European XFEL

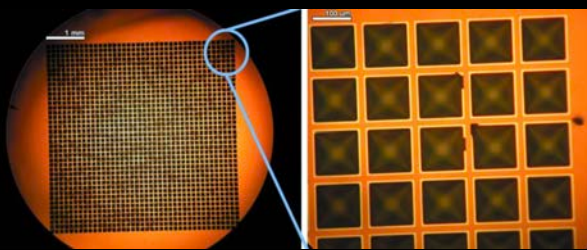


reaction progress - 3, 10, 30, 80, 100 ps

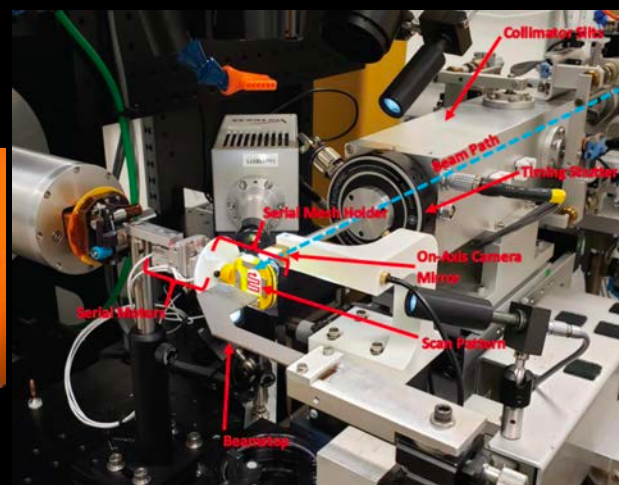
Pandey et al (2020) *Nature Methods*

# Next? Serial Data Collection

crystallization in microgrid



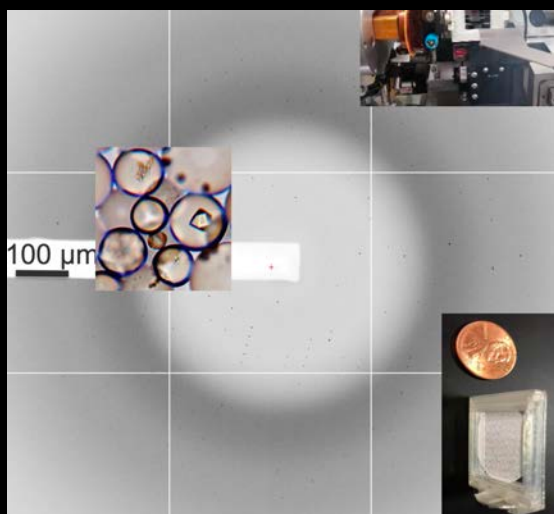
serial data collection



protein dynamics and reaction kinetics

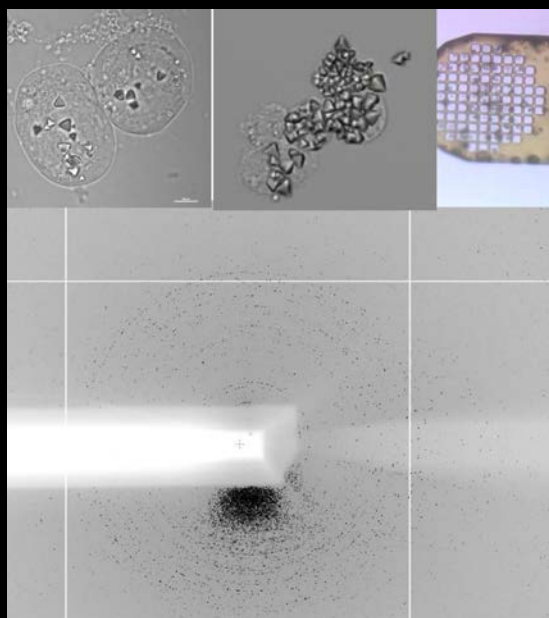
# Next? Data Collection *in situ* and *in cellulo*

crystallization and in situ data collection  
in microfluidic devices



APS/SBC - G Babnigg/Y Kim/B Nocek

diffraction in cellulo  
- insect cells



APS/SBC - G Babnigg/Y Kim

# Next? Fragment Screening in Crystals

“come with crystals, leave with hits”

robotic crystallization  
+ fragment library

fast crystal soaking  
100 cpds in 30 sec

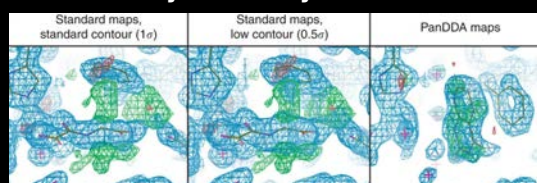


shifter harvesting  
100 xtals/hr

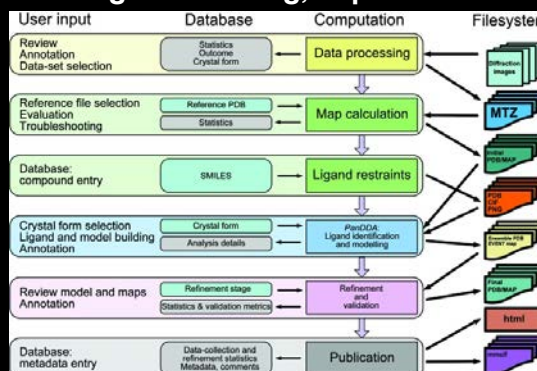
automated data collection  
2-5 min/sample  
~600 datasets/day



multi-crystal analysis - PanDDA



bulk data processing -  
XChemExplorer for bulk refinement,  
ligand building, deposition





# Next? Fragment Screening in Crystals

PNAS

RESEARCH ARTICLE

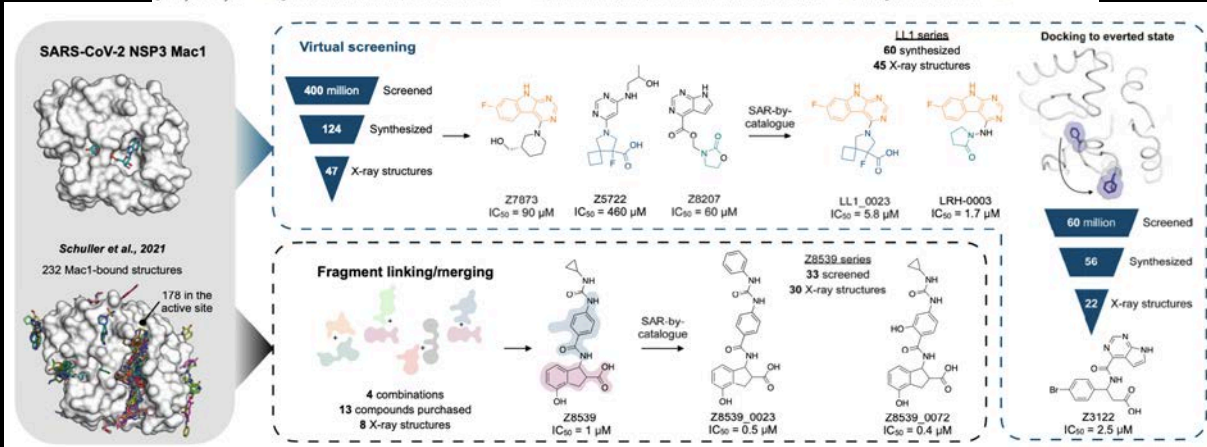
BIOPHYSICS AND COMPUTATIONAL BIOLOGY

OPEN ACCESS



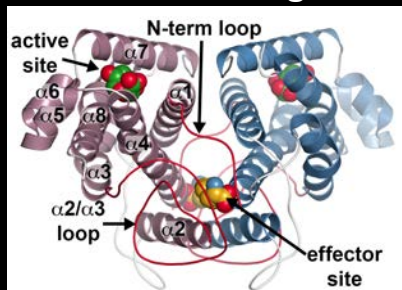
## Iterative computational design and crystallographic screening identifies potent inhibitors targeting the Nsp3 macrodomain of SARS-CoV-2

Stefan Gahbauer<sup>1,1</sup>, Galen J. Correy<sup>1,1</sup>, Marion Schuller<sup>2</sup>, Matteo P. Ferla<sup>1,6</sup>, Yagmur Umay Doruk<sup>1</sup>, Moira Rachman<sup>1</sup>, Taiasean Wu<sup>1,1</sup>, Morgan Diolaiti<sup>1</sup>, Siyi Wang<sup>1</sup>, R. Jeffrey Neitz<sup>1</sup>, Daren Fearon<sup>1,1</sup>, Dmytro S. Radchenko<sup>1,1</sup>, Yurii S. Moroz<sup>1,1</sup>, John J. Irwin<sup>1</sup>, Adam R. Renslo<sup>1,1</sup>, Jenny C. Taylor<sup>1,6</sup>, Jason E. Gestwicki<sup>1</sup>, Frank von Delft<sup>1,1,1,1</sup>, Alan Ashworth<sup>1</sup>, Ivan Ahelf<sup>1</sup>, Brian K. Shoichet<sup>1,2</sup>, and James S. Fraser<sup>1,2</sup>



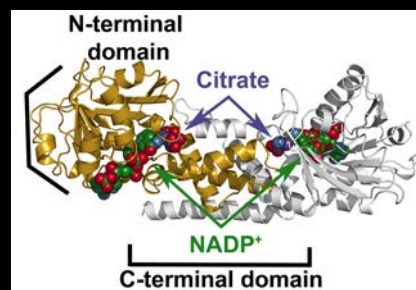
# Next? Fragment Screening in Crystals

## effector-based regulation



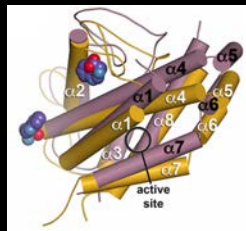
fragment screening for natural & non-natural effectors

## feedback-based regulation



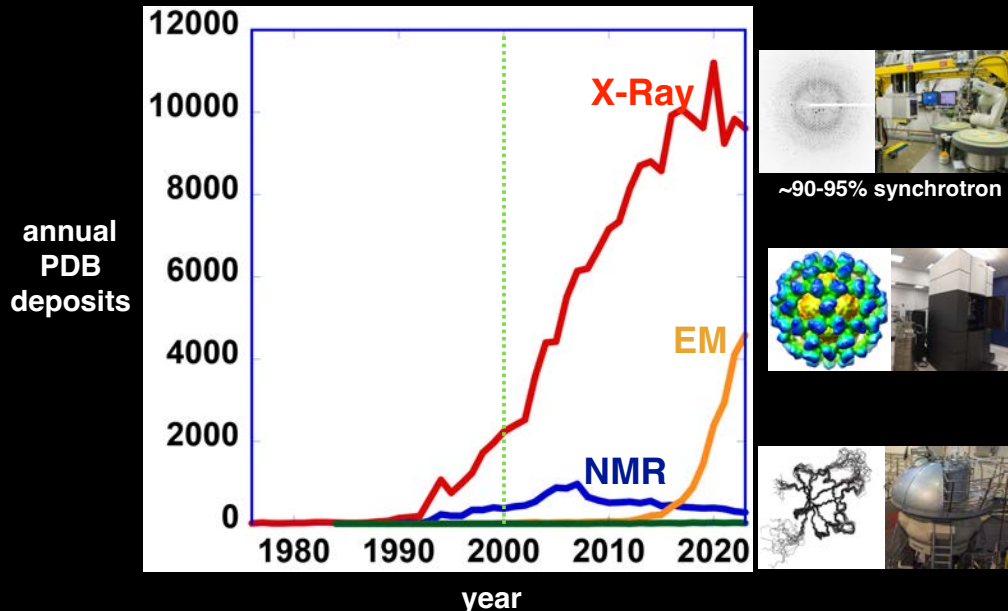
design variants to release or modulate regulation

engineer allosteric paths  
optimizing response curves



# 21st Century - Structural Explosion

5 Apr 24: 217,966 structures deposited



<https://www.rcsb.org/stats/all-released-structures>

# Deep Neural Network Applications

Article

## Highly accurate protein structure prediction with AlphaFold

<https://doi.org/10.1038/s41586-021-03819-2>

Received: 11 May 2021

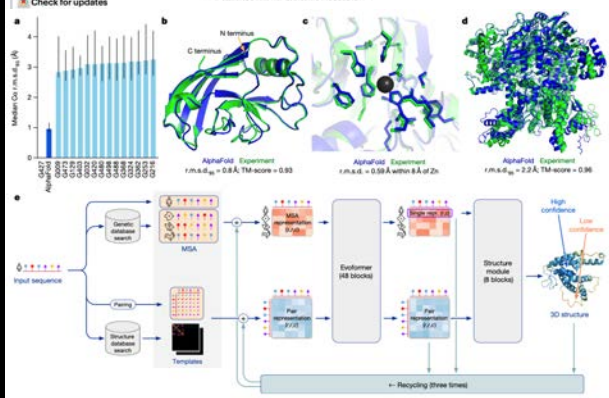
Accepted: 12 July 2021

Published online: 15 July 2021

Open access

Check for updates

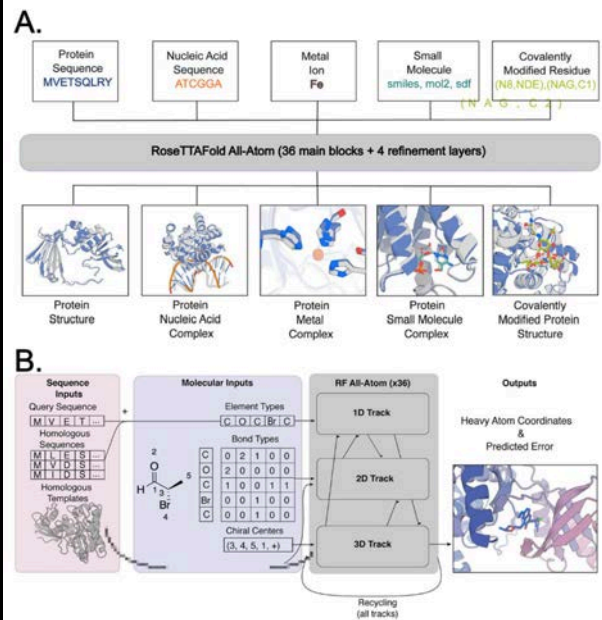
John Jumper<sup>1,2,3\*</sup>, Richard Evans<sup>1,2</sup>, Alexander Pritzel<sup>1,2</sup>, Tim Green<sup>1,2</sup>, Michael Figurnov<sup>1,2</sup>, Olaf Ronneberger<sup>1,2</sup>, Kathryn Tunyasuvunakool<sup>1,2</sup>, Russ Bates<sup>1,2</sup>, Augustin Židek<sup>1,2</sup>, Anna Potapenko<sup>1,2</sup>, Alex Bridgland<sup>1,2</sup>, Clemens Meyer<sup>1,2</sup>, Simon A. Kohli<sup>1,2</sup>, Andrew J. Ballard<sup>1,2</sup>, Andrew Cowie<sup>1,2</sup>, Bernardino Romera-Paredes<sup>1,2</sup>, Stanislav Nikolov<sup>1,2</sup>, Rishub Jain<sup>1,2</sup>, Jonas Adler<sup>1,2</sup>, Trevor Back<sup>1,2</sup>, Stig Petersen<sup>1,2</sup>, David Reiman<sup>1,2</sup>, Ellen Clancy<sup>1,2</sup>, Michal Zielinski<sup>1,2</sup>, Martin Steinegger<sup>1,2</sup>, Michalina Pacholska<sup>1,2</sup>, Tomas Berghammer<sup>1,2</sup>, Sebastian Bodenstein<sup>1,2</sup>, David Silver<sup>1,2</sup>, Oriol Vinyals<sup>1,2</sup>, Andrew W. Senior<sup>1,2</sup>, Koray Kavukcuoglu<sup>1,2</sup>, Pushmeet Kohli<sup>1,2</sup> & Demis Hassabis<sup>1,2,3\*</sup>



<https://www.nature.com/articles/s41586-021-03819-2>

## Generalized biomolecular modeling and design with RoseTTAFold All-Atom

Rohith Krishna<sup>1,2\*</sup>, Jue Wang<sup>1,2\*</sup>, Woody Ahern<sup>1,2,3\*</sup>, Pascal Sturmfeil<sup>1,2,3</sup>, Preetham Venkatesh<sup>1,2,3\*</sup>, Indrek Kalvet<sup>1,2,3\*</sup>, Gyu Rie Lee<sup>1,2,3\*</sup>, Felix S. Morey-Burrows<sup>1,2</sup>, Ivan Anishechko<sup>1,2</sup>, Ian R. Humphreys<sup>1,2</sup>, Ryan McHugh<sup>1,2,4</sup>, Dionne Vafeados<sup>1,2</sup>, Xinting Li<sup>1,2</sup>, George A. Sutherland<sup>1,2</sup>, Andrew Hitchcock<sup>1</sup>, C. Neil Hunter<sup>1</sup>, Alex Kang<sup>1</sup>, Evans Brackenbrough<sup>1</sup>, Asim K. Bera<sup>1</sup>, Minkyung Baek<sup>1</sup>, Frank DiMajo<sup>1,5</sup>, David Baker<sup>1,2,3,6\*</sup>



<https://www.science.org/doi/epdf/10.1126/science.adl2528>

# Deep Neural Network Applications

## Article

### Highly accurate protein structure prediction with AlphaFold

<https://doi.org/10.1038/s41586-021-03819-2>

Received: 11 May 2021

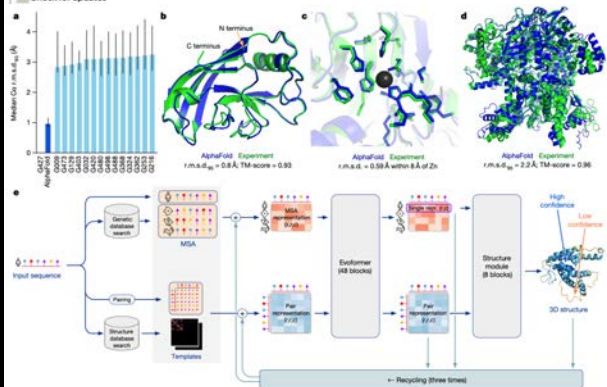
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Open access

Check for updates

John Jumper<sup>1,2,3</sup>, Richard Evans<sup>1,4</sup>, Alexander Pritzel<sup>1,5</sup>, Tim Green<sup>1,6</sup>, Michael Figurnov<sup>1,4</sup>, Olaf Ronneberger<sup>1,7</sup>, Kathryn Tunyasuvunakool<sup>1,8</sup>, Russ Bates<sup>1,9</sup>, Augustin Židek<sup>1,9</sup>, Anna Potapenko<sup>1,8</sup>, Alex Bridgland<sup>1,8</sup>, Clemens Meyer<sup>1,8</sup>, Simon A. Kohli<sup>1,8</sup>, Andrew J. Ballard<sup>1,8</sup>, Andrew Cowie<sup>1,8</sup>, Bernardino Romera-Paredes<sup>1,8</sup>, Stanislav Nikolov<sup>1,8</sup>, Rishub Jain<sup>1,8</sup>, Jonas Adler<sup>1,8</sup>, Trevor Back<sup>1,8</sup>, Stig Petersen<sup>1,8</sup>, David Reiman<sup>1,8</sup>, Ellen Clancy<sup>1,8</sup>, Michal Zieliński<sup>1,8</sup>, Martin Steinegger<sup>1,8</sup>, Michalina Pacholska<sup>1,8</sup>, Tomas Berghammer<sup>1,8</sup>, Sebastian Bodenstein<sup>1,8</sup>, David Silver<sup>1,8</sup>, Oriol Vinyals<sup>1,8</sup>, Andrew W. Senior<sup>1,8</sup>, Koray Kavukcuoglu<sup>1,8</sup>, Pushmeet Kohli<sup>1,8</sup> & Demis Hassabis<sup>1,8,10</sup>



<https://www.nature.com/articles/s41586-021-03819-2>

## Know Your Tools - Powerful Hypothesis Generator

nature methods



Article

<https://doi.org/10.1038/s41592-023-02087-4>

### AlphaFold predictions are valuable hypotheses and accelerate but do not replace experimental structure determination

Received: 30 January 2023

Accepted: 11 October 2023

Published online: 30 November 2023

Thomas C. Terwilliger<sup>1,2</sup>, Dorothee Liebschner<sup>3</sup>, Tristan I. Croll<sup>4</sup>, Christopher J. Williams<sup>5</sup>, Airlie J. McCoy<sup>4</sup>, Billy K. Poon<sup>3</sup>, Pavel V. Afonine<sup>3</sup>, Robert D. Oeffner<sup>4</sup>, Jane S. Richardson<sup>5</sup>, Randy J. Read<sup>4</sup> & Paul D. Adams<sup>3,6</sup>

Limited Scope (protein not systems)  
Conformations & Dynamics  
Experimental Accelerator

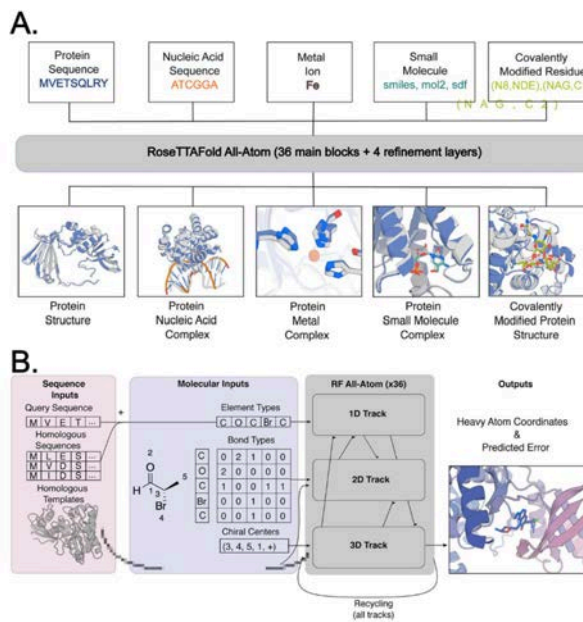


# What's Ahead - Molecular Design



## Generalized biomolecular modeling and design with RoseTTAFold All-Atom

Rohith Krishna<sup>1,2†</sup>, Jue Wang<sup>1,2†</sup>, Woody Ahern<sup>1,2,3†</sup>, Pascal Sturmfels<sup>1,2,3</sup>, Preetham Venkatesh<sup>1,2,3,4</sup>, Indrek Kalvet<sup>1,2,3</sup>, Gyu Rie Lee<sup>1,2,3</sup>, Felix S. Morey-Burrows<sup>1</sup>, Ivan Anishechenko<sup>1,2,3</sup>, Jan R. Humphreys<sup>1,2,3</sup>, Ryan McHugh<sup>1,2,3</sup>, Dionne Vafaei<sup>1,2</sup>, Xinting Li<sup>1,2</sup>, George A. Sutherland<sup>1</sup>, Andrew Hitchcock<sup>1</sup>, C. Neill Hunter<sup>4</sup>, Alex Kang<sup>1</sup>, Evans Brackenbrough<sup>1</sup>, Asim K. Bera<sup>1</sup>, Minkyung Baek<sup>1</sup>, Frank DIMajo<sup>1,2</sup>, David Baker<sup>1,2,3,4\*</sup>



<https://www.science.org/doi/epdf/10.1126/science.adl2528>

# What's Ahead - Molecular Design

## Geometric Deep Learning for Structure-Based Ligand Design

Alexander S. Powers, Helen H. Yu,<sup>○</sup> Patricia Suriana,<sup>○</sup> Rohan V. Koodli, Tianyu Lu, Joseph M. Paggi, and Ron O. Dror\*

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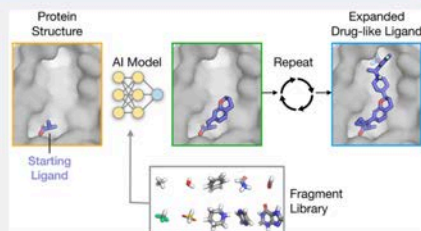
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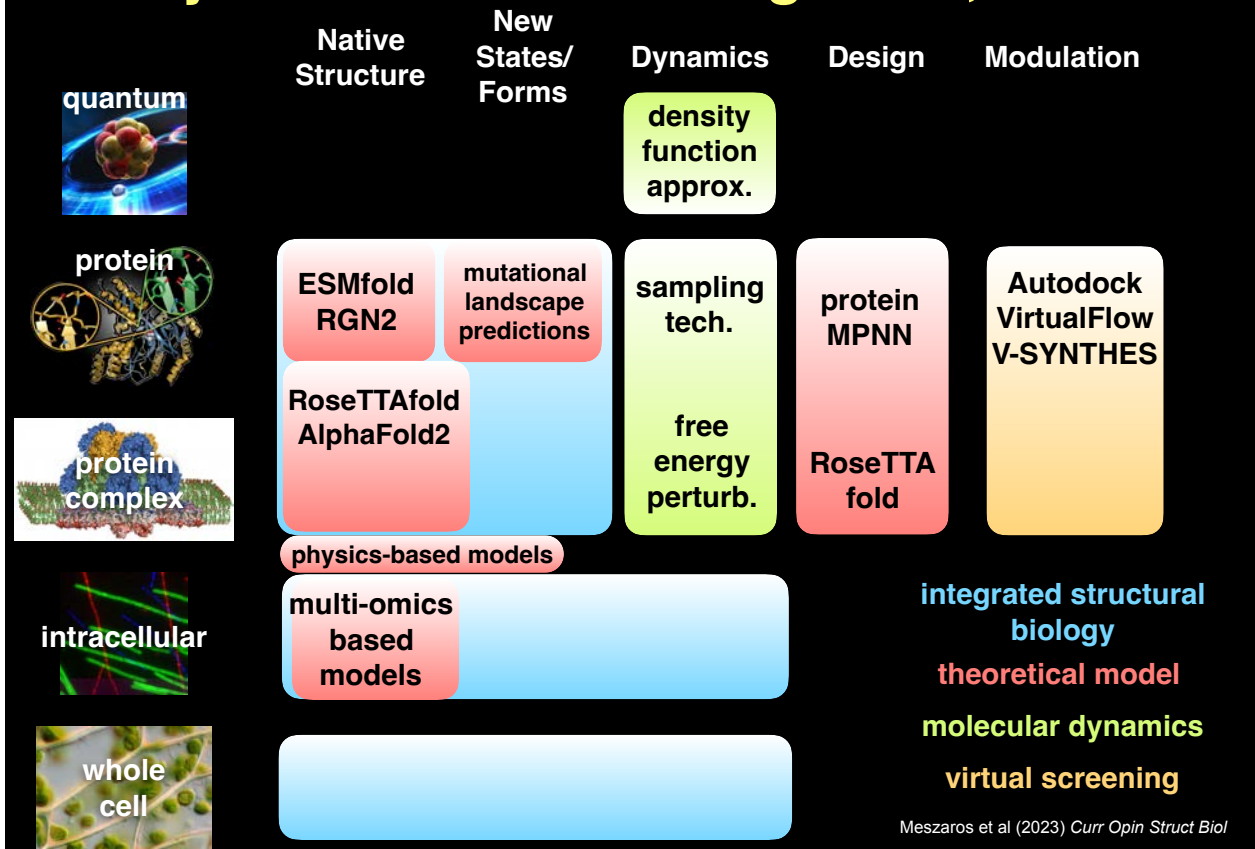
Article Recommendations

Supporting Information

**ABSTRACT:** A pervasive challenge in drug design is determining how to expand a ligand—a small molecule that binds to a target biomolecule—in order to improve various properties of the ligand. Adding single chemical groups, known as fragments, is important for lead optimization tasks, and adding multiple fragments is critical for fragment-based drug design. We have developed a comprehensive framework that uses machine learning and three-dimensional protein–ligand structures to address this challenge. Our method, FRAME, iteratively determines where on a ligand to add fragments, selects fragments to add, and predicts the geometry of the added fragments. On a comprehensive benchmark, FRAME consistently improves predicted affinity and



# Many Tools but Little Integration, So Far



# Tools at All Scales

