

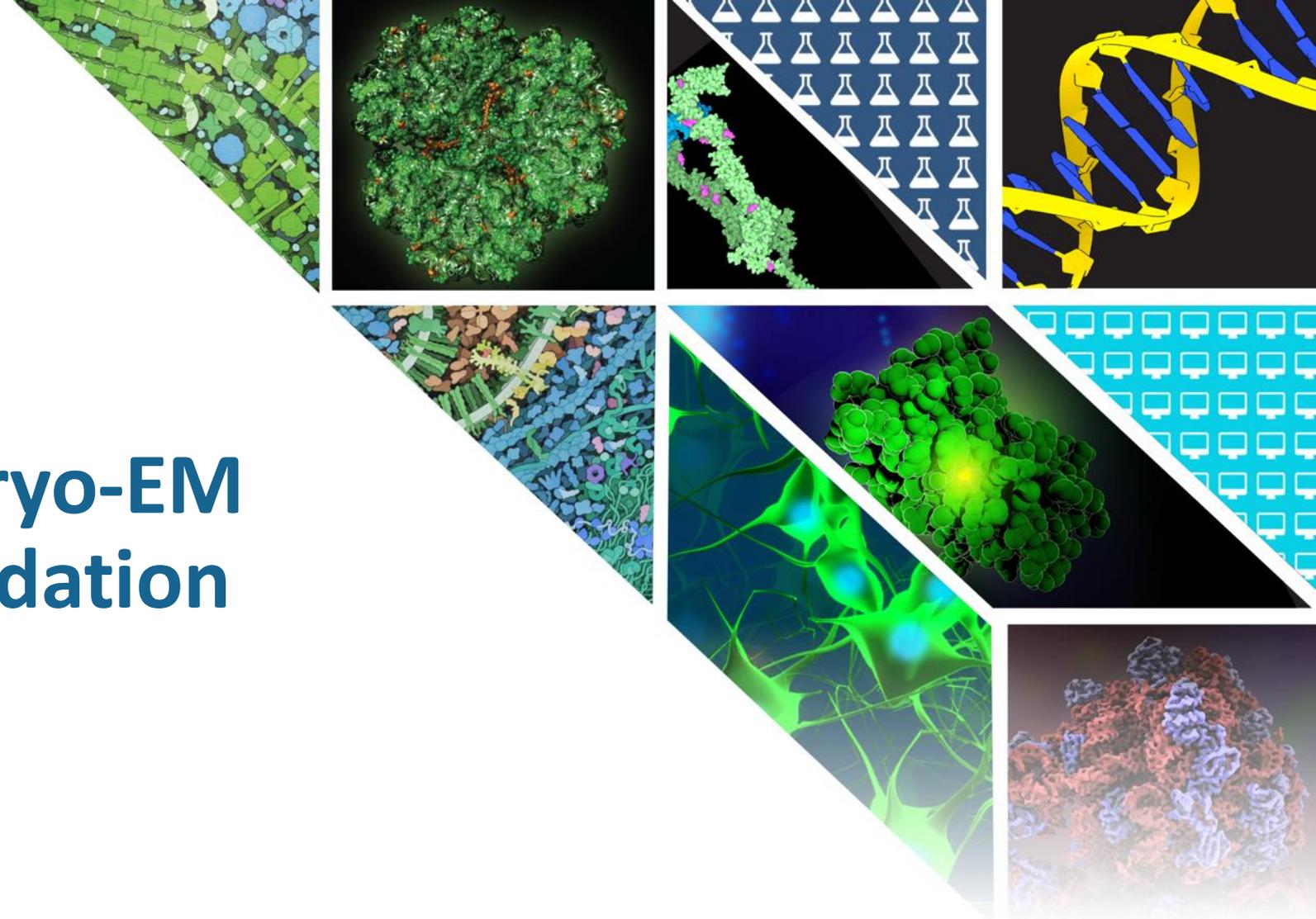
# Best Practices for Cryo-EM Deposition and Validation

Justin W. Flatt, Ph.D.

Biocurator, RCSB Protein Data Bank

Rutgers, The State University of New Jersey

NYSBC Single Particle Short Course March 20, 2026



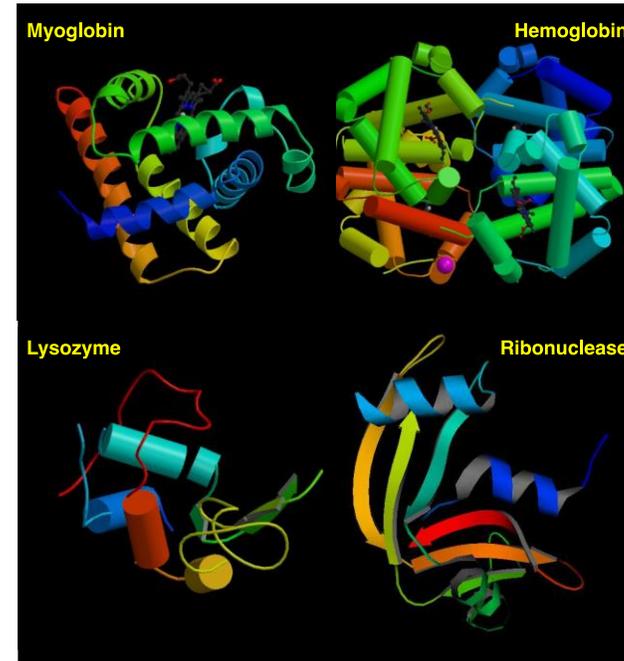
# Outline

- I. Frozen in Motion: 3DEM Then, Now, and Beyond – wwPDB milestones and evolution
- II. Meeting the Data Deluge Challenge – Extended PDB IDs, PDBx/mmCIF, OneDep
- III. Quality at Scale: Validation of 3DEM structures – Insights, Best practices, What's Next

- I. Frozen in Motion: 3DEM Then, Now, and Beyond – wwPDB milestones and evolution

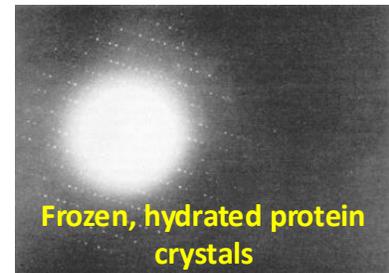
# Protein Data Bank (Established 1971)

- A single global archive for structural biology data
- Started with just 7 protein structures
- Now over 250,000 structures freely available!
- Jointly managed by wwPDB partnership started in 2003
- Members: RCSB PDB (US), PDBe (Europe), PDBj (Japan), and PDBc (China); plus EMDB (3DEM) and BMRB (NMR)

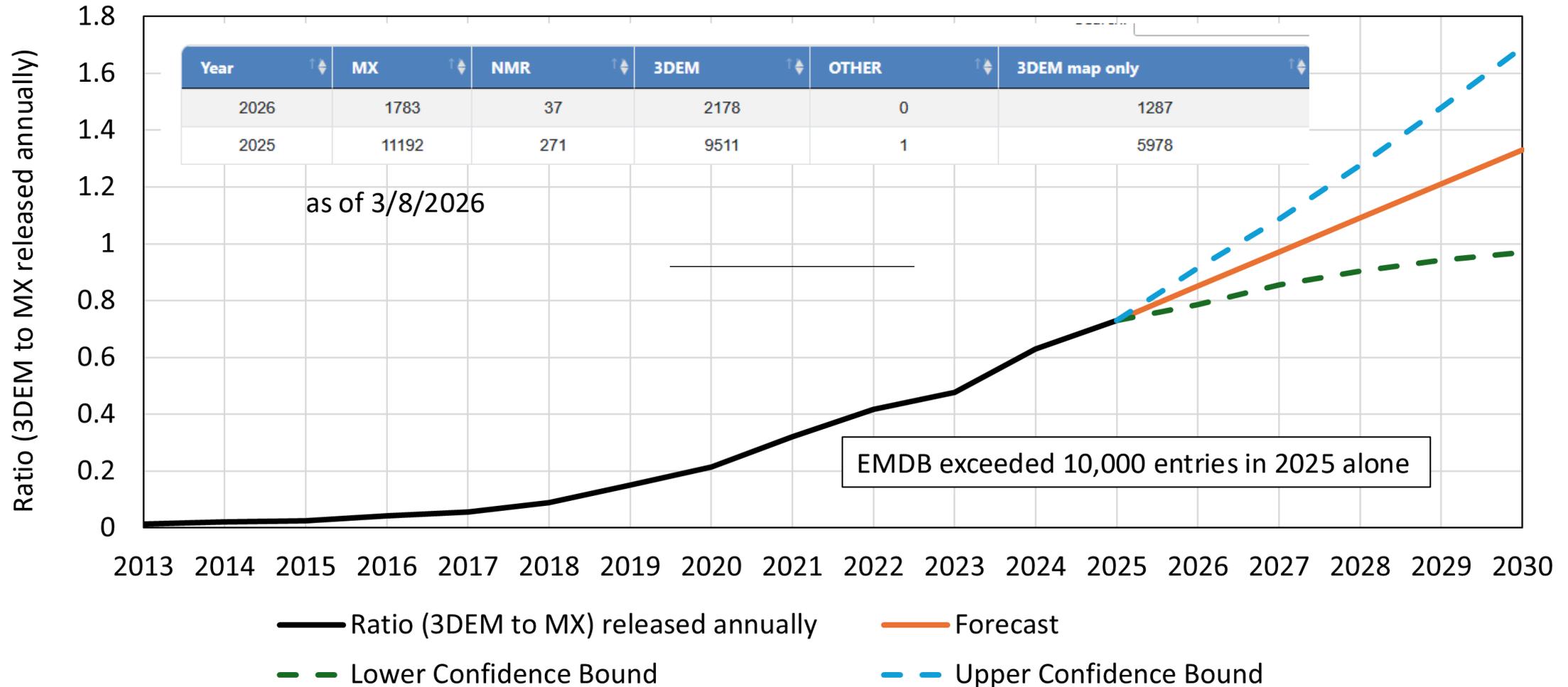


X-ray structures led to the launch of archive

Cryo-EM not yet born when the PDB began



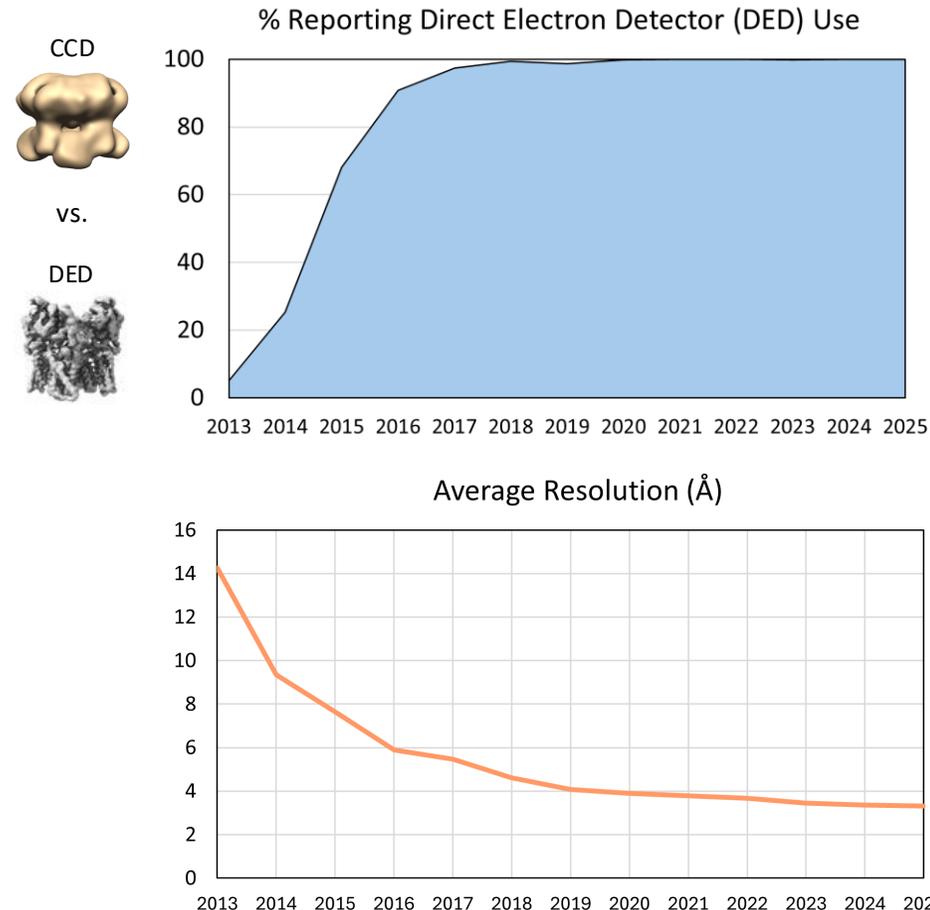
# Annual PDB Structure Releases for 3DEM ~~Will Soon Eclipse~~ Macromolecular Crystallography



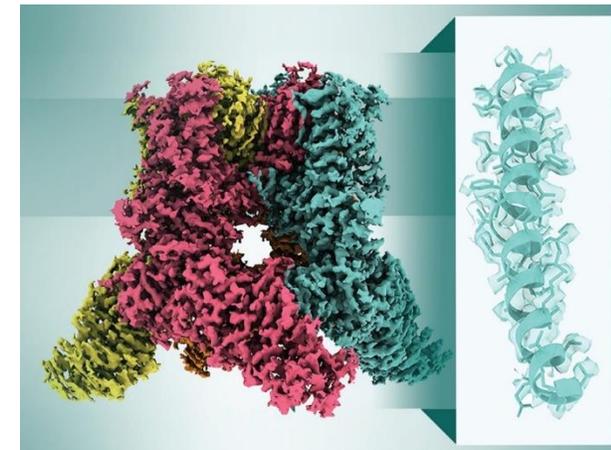
# The Cryo-EM Resolution Revolution as a Data Revolution in Structural Biology

## Facilitated by

- Direct electron detectors
- Modern electron optics
- Improved data collection software
- Sophisticated image processing with improved algorithms
- FAIR and sustainable data management



300 keV • Direct Detector •  
RELION (1.9 Å)



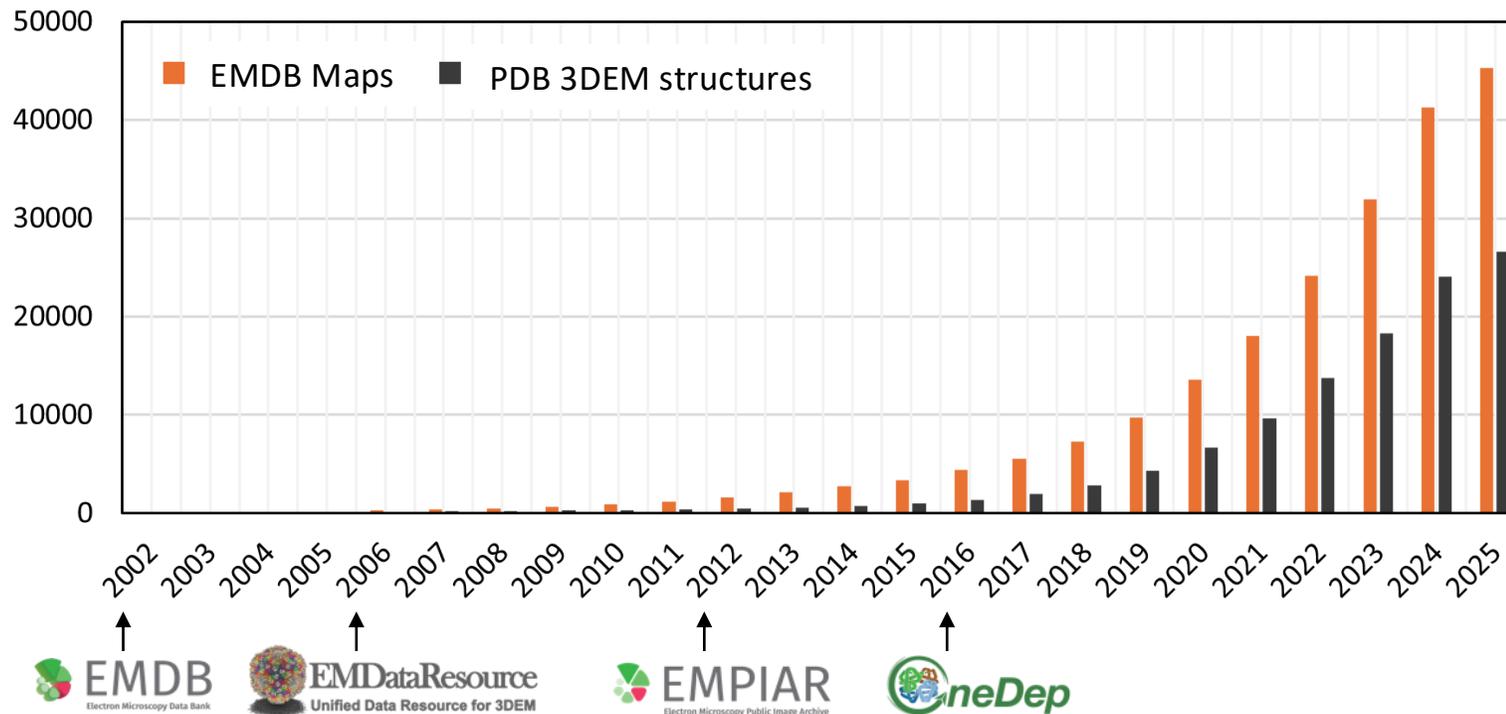
EMD-23853, pdb\_00007mij

Community-wide deposition and validation standards critical for data quality, consistency, and trust

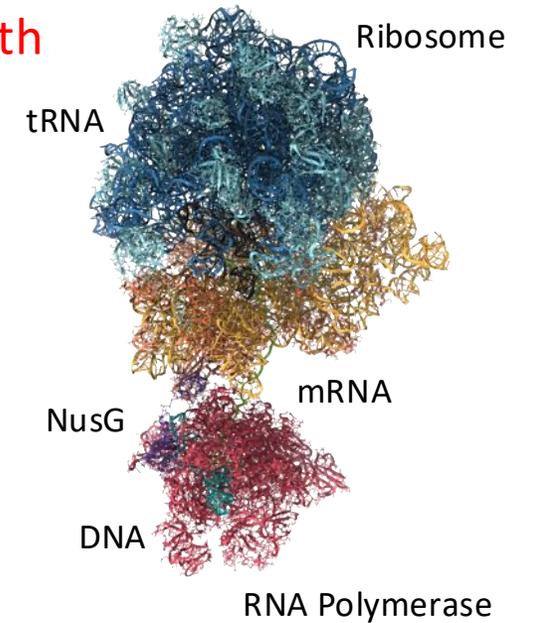
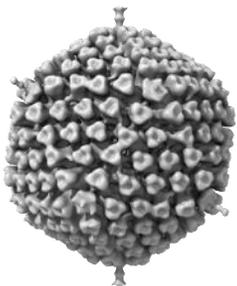
# Frozen In Motion: Then, Now, and Beyond

Ongoing Challenge: Flat funding with fixed biocuration resources despite continued growth

### Growth of 3DEM Archives



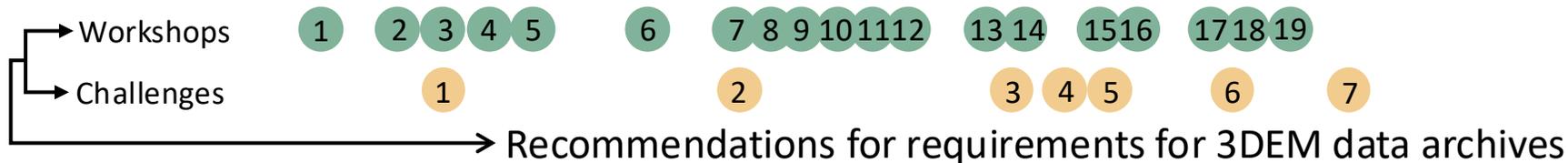
Human Adenovirus 2  
EMD-1016 (25 Å)  
Released 2002



NusG coupled expressome  
EMD-11418, pdb\_00006ztj (3.4 Å)  
Released 2020

Archives and Access

Data Standards



# AI-Fueled Future Needs Experts Now More than Ever



Cryo-EM Deposition Workshop  
2004  
Rutgers, USA



3DEM Validation Task  
Force 2010  
Rutgers, USA



Cryo-EM Joint Map and Model Challenges  
Workshop 2017  
SLAC, Stanford, USA

> [Nat Methods](#). 2024 Jun 25. doi: 10.1038/s41592-024-02321-7. Online ahead of print.

## Outcomes of the EMDDataResource cryo-EM Ligand Modeling Challenge

> [IUCrJ](#). 2024 Mar 1;11(Pt 2):140-151. doi: 10.1107/S2052252524001246.

## Community recommendations on cryoEM archiving and validation

## Depositors as Part of the Solution

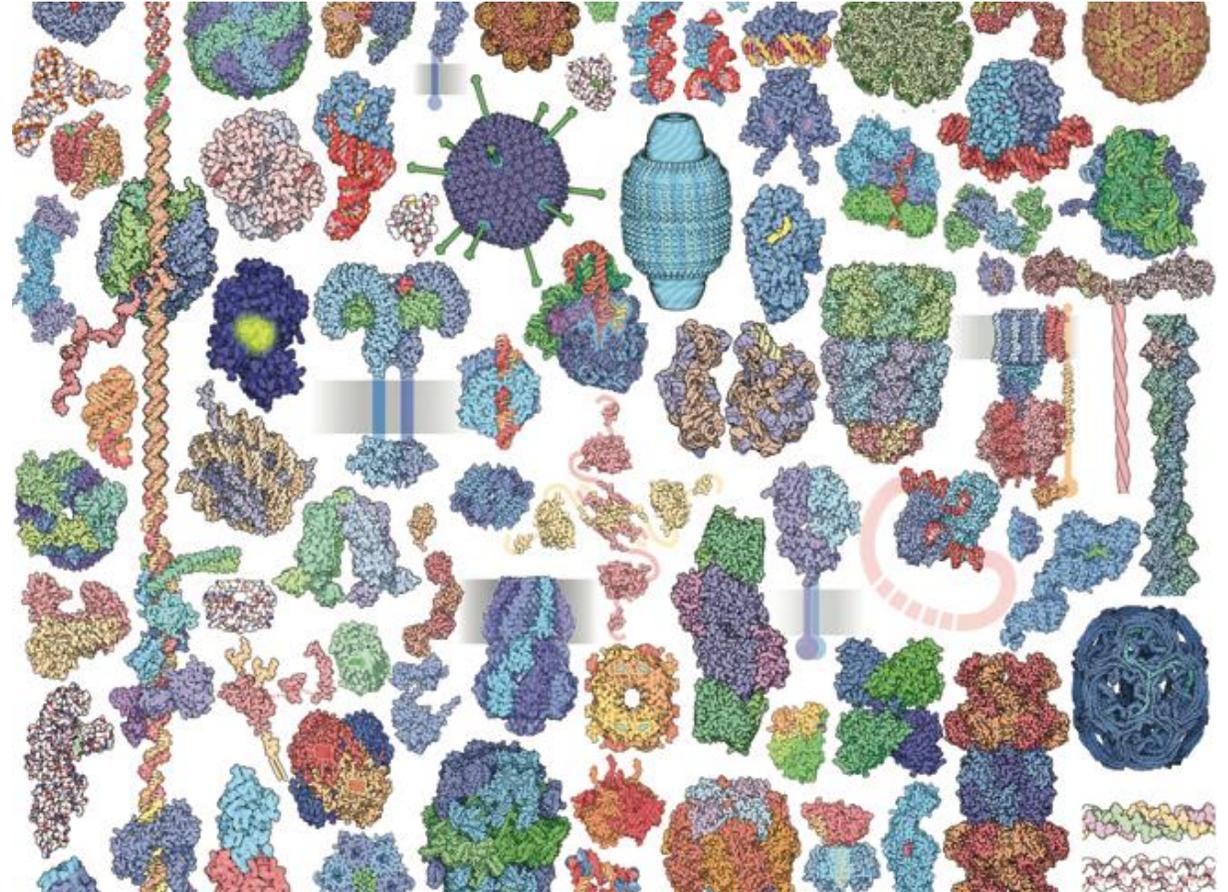
- Follow community standards
- Provide complete, accurate data
- Engage early and stay informed
- Enable high-quality annotations

# wwPDB: Data Stewardship by Domain Experts

## wwPDB as the Other Part of the Solution

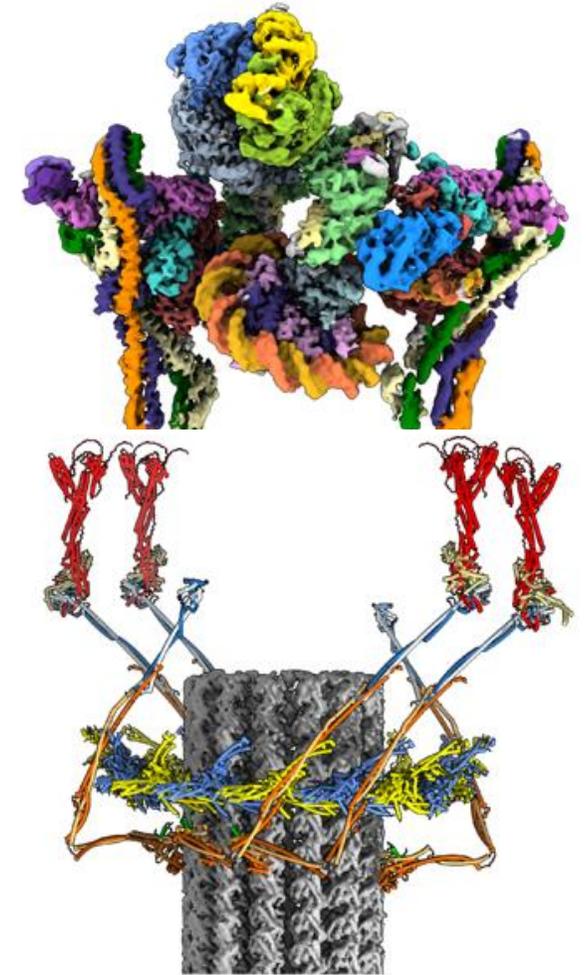
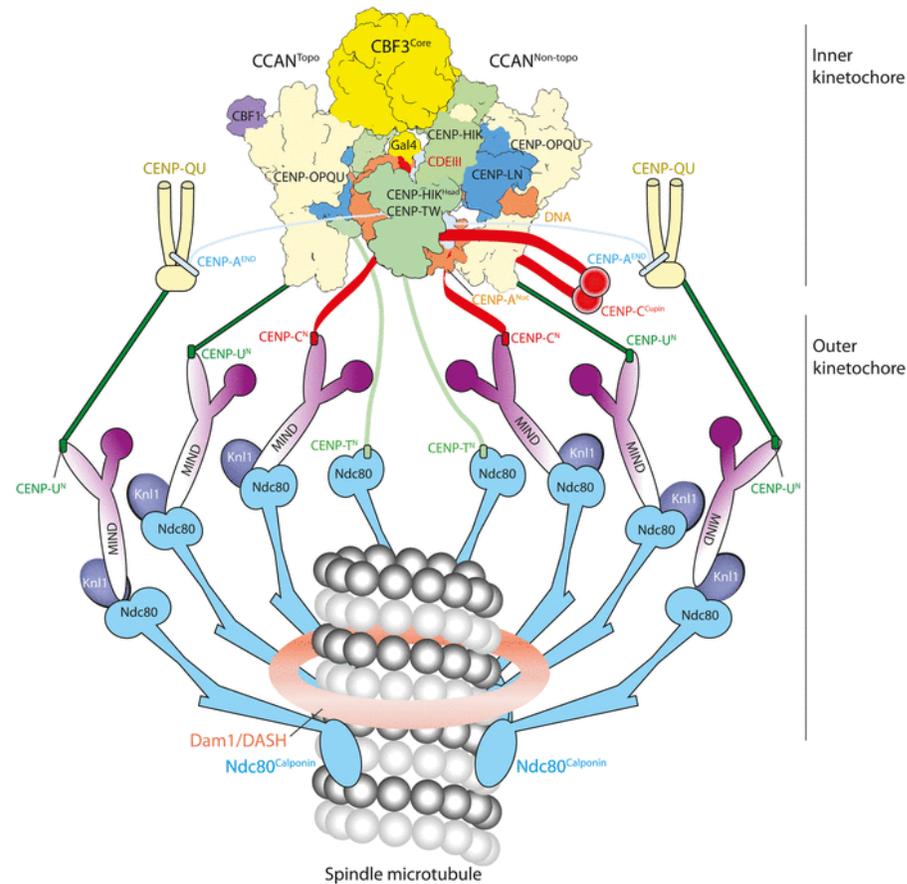
- Steward the global archive
- Centralize deposition & validation (OneDep)
- Curate and annotate expertly
- Guide depositors with tools and feedback

We must work together to keep pace with rapid growth and evolving standards!



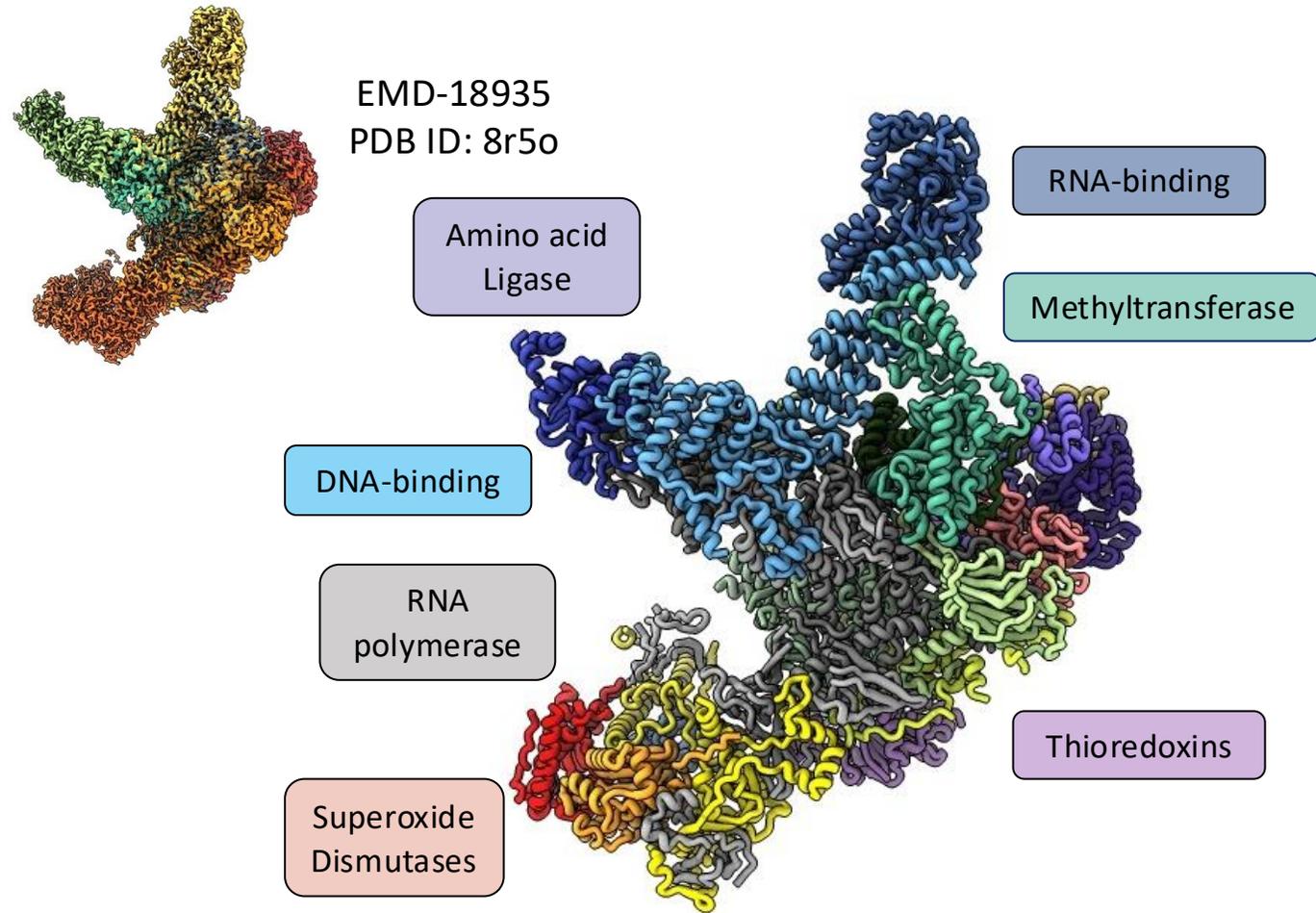
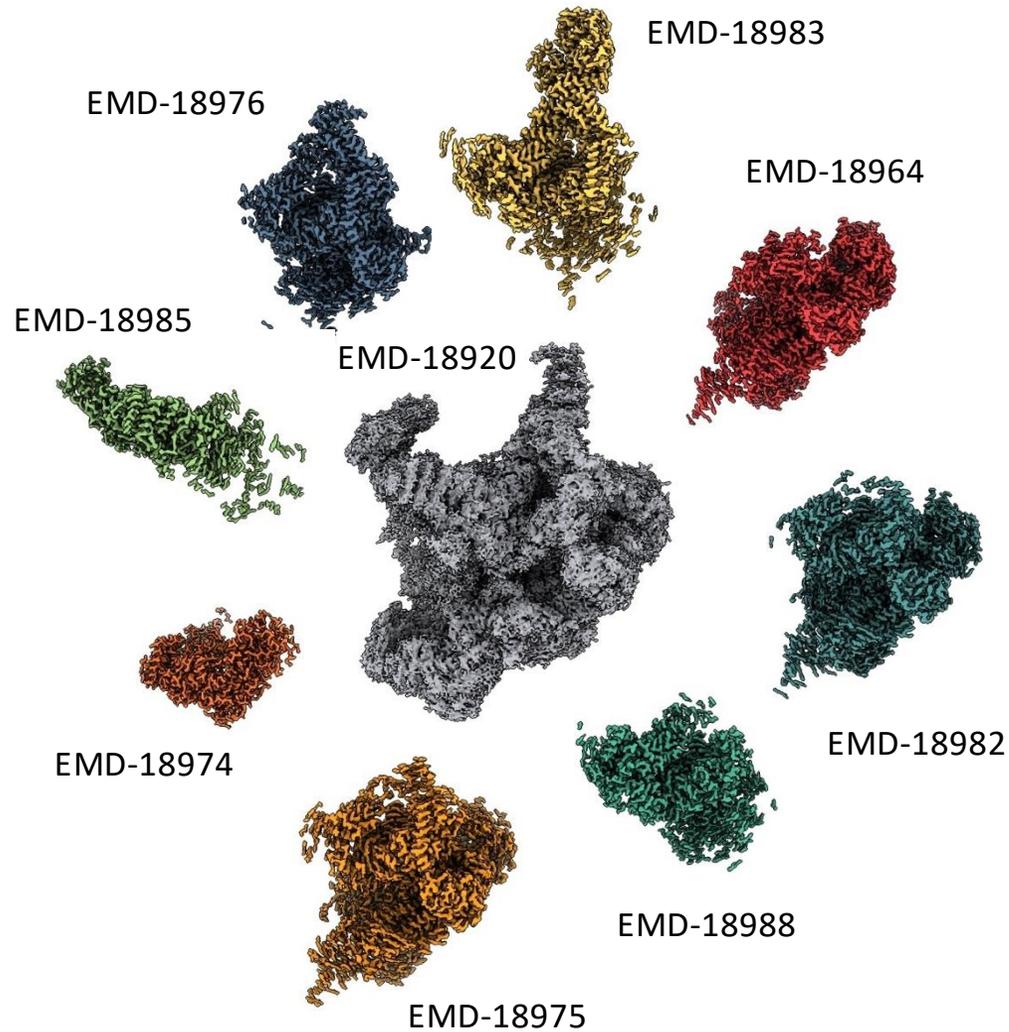
# A New Era of Unprecedented Structural Complexity

- Previously intractable systems can often be studied at resolutions suitable for atomic model building
- AI/ML predictions can help (but be careful!)
- Experimental structures are essential for understanding biology



Dendooven T. et al. Science Advances 9(30), eadg7480 (2023). doi:10.1126/sciadv.adg7480  
Muir K. W. et al. Science 382(6675), 1184–1190 (2023). doi:10.1126/science.adj8736

# A New Era of wwPDB Data Management

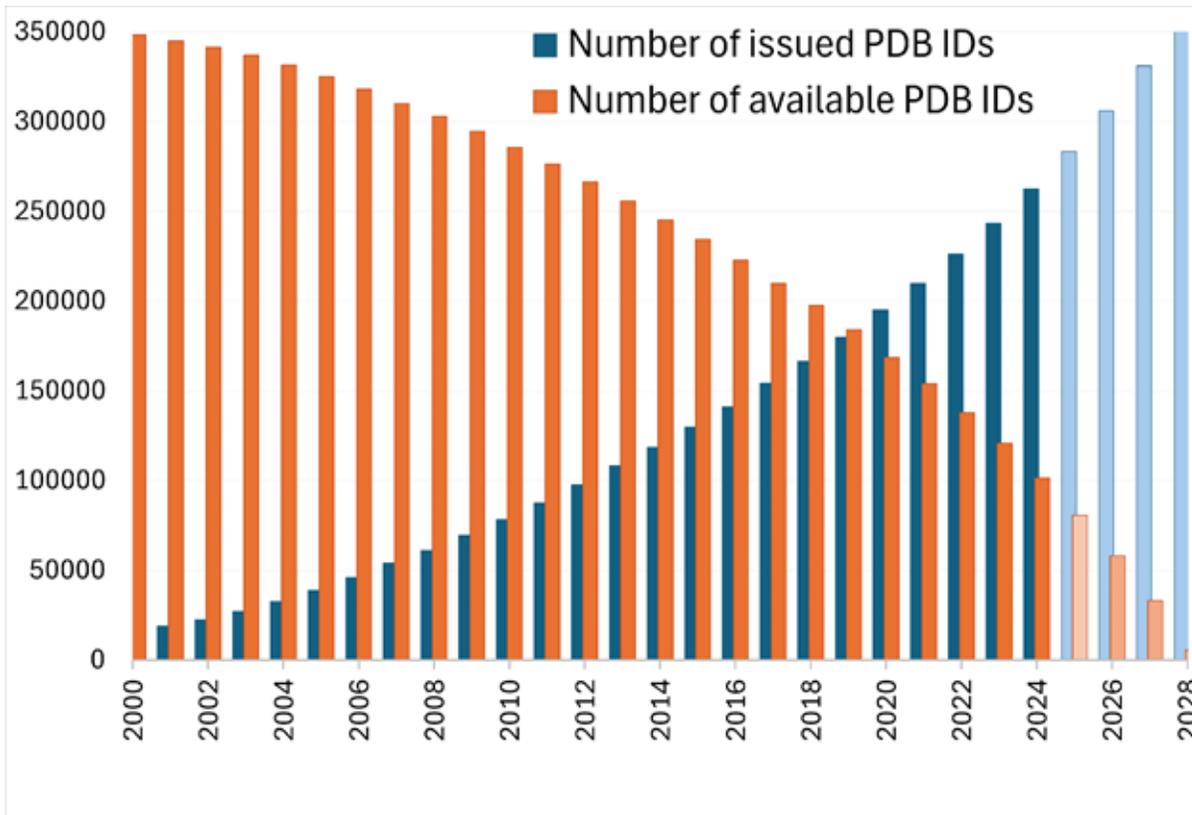


Supporting Consensus and Focused Maps

Composite Structure

## II. Meeting the Data Deluge Challenge – Extended PDB IDs, PDBx/mmCIF, OneDep

# Transitioning to Extended PDB IDs and PDBx/mmCIF Format to Support Growth



## Challenges:

- Since 1971, over 240,000 entries have used 4-character PDB IDs
- Software frequently built around legacy PDB file format cannot support extended IDs
- Users, journals, and software developers need to adopt to new PDB IDs and PDBx/mmCIF format now

New PDB ID has 12-character length with “pdb\_” prefix: `pdb_[a-z0-9]{8}`

Example: `pdb_00006vxx`

# Available Transition Resources

## Dedicated webpage describing the project

Documentation, FAQs, software tools, etc.

<https://www.wwpdb.org/documentation/new-format-for-pdb-ids>



## Extended IDs in the public files – ready for testing

For the current entries, the extended PDB IDs are generated by adding prefix “pdb\_0000” to their 4-character IDs: **4HHB / pdb\_00004hhb**

All current archival files have been updated with the extended PDB ids, users can start testing /adopting the extended IDs now:

<i>_database_2.database_id</i>	<i>PDB</i>
<i>_database_2.database_code</i>	<i>4HHB</i>
<i>_database_2.pdbx_database_accession</i>	<i>pdb_00004hhb</i>

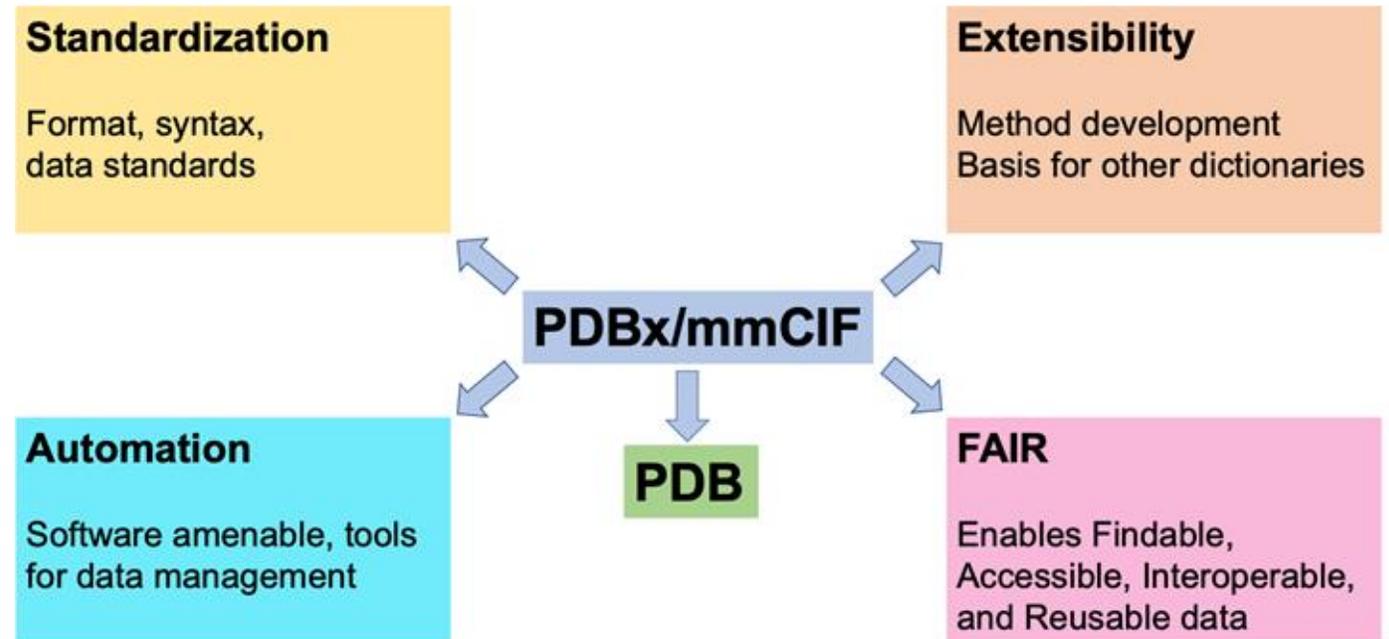
## Example files at GitHub – ready for testing

<https://github.com/wwPDB/extended-wwPDB-identifier-examples>

# PDBx/mmCIF Data Format

## PDBx/mmCIF: The master archival format of the PDB since 2014

- Community-developed
- Supports structures of any size and complexity
- Defines data types, boundary conditions, enumerations

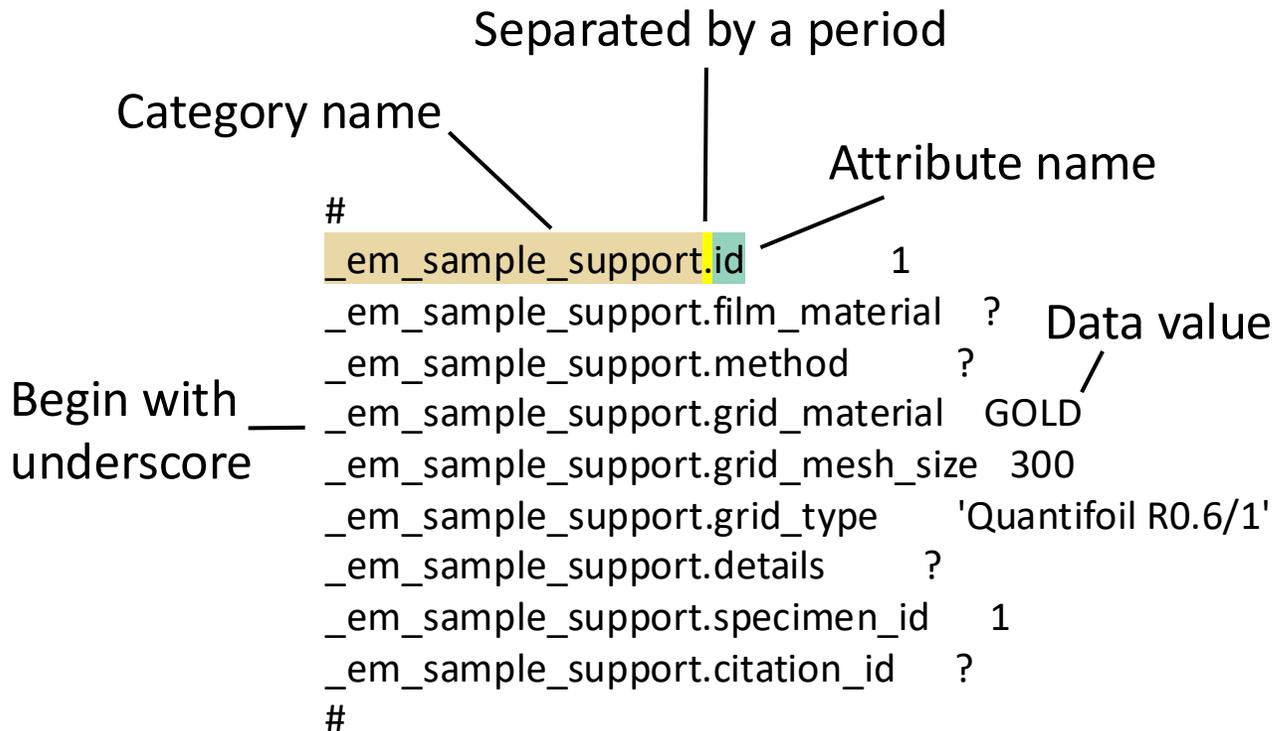


Entries with extended PDB IDs will be available only in PDBx/mmCIF format

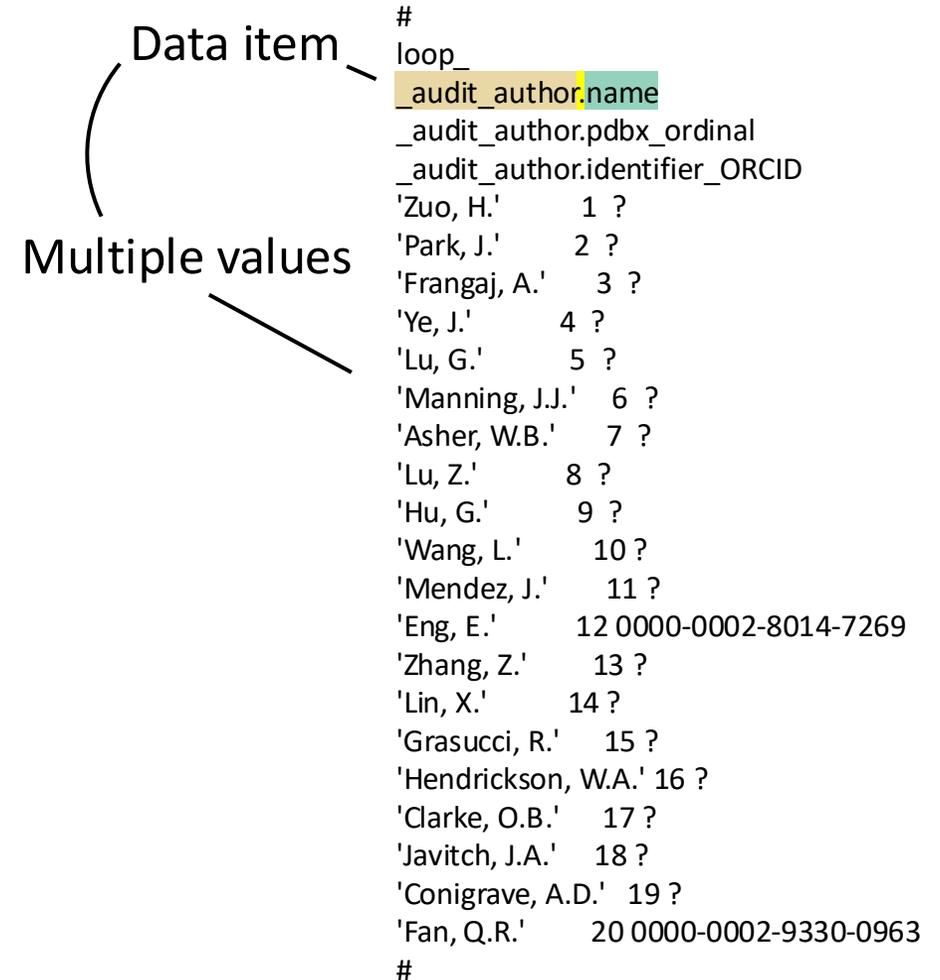
# How to read a PDBx/mmCIF File

Key-value style:

Tabular style (multiple values for single data item):



Category + attribute = Data item





# PDBx/mmCIF Powers Deeper Insight and Wider Impact for 3DEM

## Workflow:

Experiment

Sample  
Preparation

Grid

Imaging

Processing

Reconstruction

Modeling & Validation

## EM extension dictionary:

`_em_experiment.reconstruction_method, etc.`

`_em_vitrification.instrument, etc.`

`_em_sample_support.grid_type, etc.`

`_em_imaging.microscope_model, etc.`

`_em_ctf_correction.type, etc.`

`_em_3d_reconstruction.software, etc.`

`_em_3d_fitting_list.type, etc.`

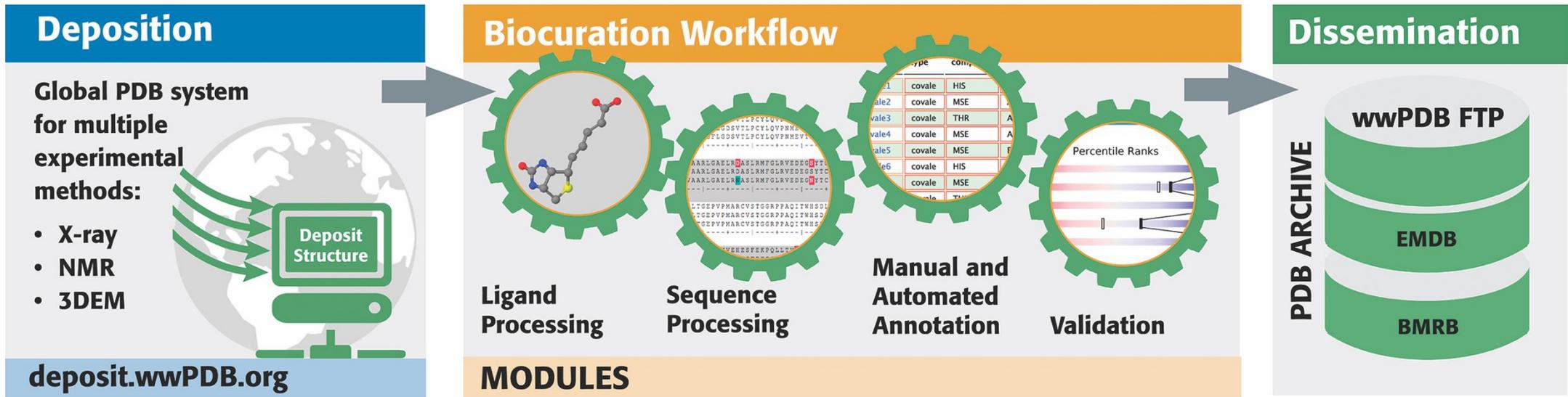
## Your data, fully realized:

- No limits on atom count or polymer chains
- Continuously updated data dictionary
- wwPDB validation reports now available in PDBx/mmCIF format

Example:

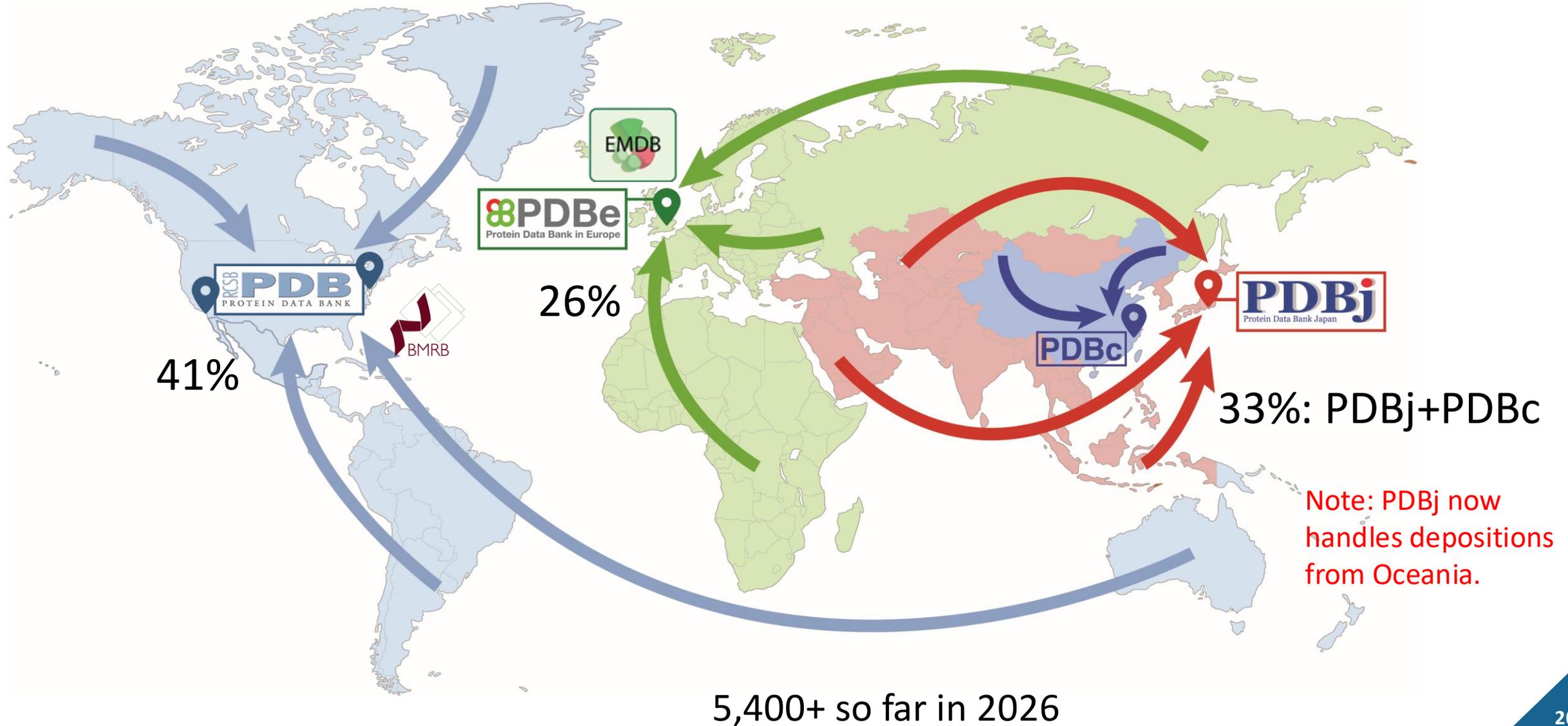
```
_em_vitrification.instrument  
'CRYOSOL VITROJET'
```

# OneDep: wwPDB's Unified Deposition System



- Supports all major experimental techniques
- Enhanced with extended metadata, more enumerations, and stronger data checks
- Prevents incomplete depositions
- Distributes responsibilities among wwPDB data centers
- Provides equal service to depositors worldwide
- Simplifies data exchange and archival updates
- Allows reliable remediation with version control

# OneDep: A Global Tool for a Global Community



# Depositions Become “Easier” Inside OneDep

- Direct data harvesting from uploaded PDBx/mmCIF files
- Dep UI collects metadata missing from uploaded files
- Issues PDB IDs for 3DEM models and EMDB IDs for 3DEM maps

wwPDB Deposition: D\_8000213910 -- Requested IDs: PDB, EMDB

**Completeness indicated** →

**Pages missing mandatory items flagged** →

**Mandatory fields highlighted in red**

**! The value must be positive when describing defocus**

**Warns when values fall outside expected ranges**

The screenshot displays the OneDep deposition form for wwPDB Deposition: D\_8000213910. The interface is divided into a sidebar and a main form area. The sidebar includes a 'List requirements' section with a progress bar for 'All items' and 'Mandatory items'. Below this is a 'Navigation' menu with various categories, including 'Microscopy', which is highlighted in red. The main form area contains several input fields for specimen preparation and microscope parameters. A red warning message is displayed: 'No value present for this mandatory item.' and a yellow warning message states: '! The value must be positive when describing defocus'. The form also includes a 'Log out' button at the bottom.

# Tips for a Smoother Deposition Process

- Register for training events
- Prepare files and validate before deposition
- Ask questions anytime for guidance and support

General: [deposit-help@mail.wwpdb.org](mailto:deposit-help@mail.wwpdb.org)

Specific: Log in to your session and select the Communication Page

- Provide accurate info for sequences and ligands
- Carefully review the validation report and address errors
- Share as you want others to share with you

Additional EM map (MRC/CCP4 format) ▾  
Pixel spacing (Å)\*: 1.1573  
Contour level\*: 0.3  
Short description:  
Unsharpened map for for dimeric motor domains of phi-like dynein-1 bound to a Lis1 dimer under Nde1\_Lis1 condition



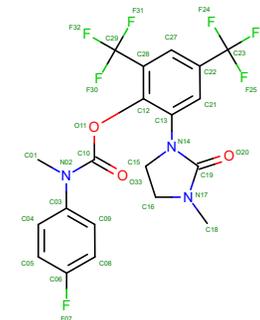
File	Description	Details
<a href="#">emd_47378_additional_1.map.gz</a>	Unsharpened map for for dimeric motor domains of phi-like dynein-1 bound to a Lis1 dimer under Nde1_Lis1 condition	+

Register for  
**Thursday September 25, 2025**  
1pm ET | 10am PT

Virtual Office Hour  
**PDB Policies for  
Deposition and  
Biocuration**

Sutapa Ghosh  
Irina Persikova

Navigation  
✓ Instructions  
✓ **Communication**  
✓ Re-upload files  
✓ Upload summary  
Admin  
Contact us



# Simplify OneDep Login and Entry Tracking with ORCID

- Authenticate as a contact author without sharing passwords
- ORCID login provides a summary table of your entries
- Traditional deposition ID/password still available

Depositions available to 0000-0001-5406-8156 (Flatt, Justin)

Deposition ID ↕	Entry ID ↕	Entry Title ↕	Method ↕	Created ↕	Site ↕	Status ↕	Last login ↕	Hold Date ↕
<a href="#">D_8000213312</a>	1395 / EMD-90412	Human collagen prolyl processing enzyme complex, P3H1/CRTAP/PPIB heterotrimer, in its apo state	EM	2024/09/18	RCSB	REL	2024/11/15	-
<a href="#">D_8000213465</a>	1462 / EMD-90437	In situ structure of the Caulobacter crescentus S-layer	EM	2025/01/16	RCSB	REL	2025/01/22	-
<a href="#">D_8000213466</a>	EMD-90438	SARS-CoV-2 Wuhan Spike ectodomain in complex with NHP polyclonal antibody NVX-NHPSA2 (NVX-CoV2373/rS-Beta immunized)	EM	2025/01/16	RCSB	REL	2025/01/27	2026/01/15
<a href="#">D_8000213523</a>	1497	Coxsackievirus B4 in complex with capsid binder compound 48	EM	2025/01/27	RCSB	PROC	2025/01/27	2026/01/26
<a href="#">D_8000213539</a>	1514	EM model only entry	EM	2025/01/31	RCSB	PROC	2025/02/25	2026/02/20
<a href="#">D_8000213565</a>	1517 / EMD-90463	Cryo-EM structure of RNA device 43 truncation mutant 3 (U100C), apo state	EM	2025/02/25	RCSB	PROC	2025/02/25	2026/02/24
<a href="#">D_8000213580</a>	EMD-90464	This is a test	EM	2025/03/05	RCSB	PROC	2025/03/05	2026/03/04
		Crystal structure of a stem-loop DNA aptamer complexed with SARS-CoV-2 nucleocapsid protein RNA-binding domain	XRAY	2025/03/05	RCSB	PROC	2025/03/05	2026/03/04
		This is a test	EM	2025/03/05	RCSB	PROC	2025/03/05	2026/03/04
		High resolution cryoEM structure of HPV16 bound to heparin	EM	2025/04/21	RCSB	PROC	2025/05/20	2026/04/20

Deposition ID

Password

[Log in](#)

[Forgot Password](#)

[Sign in with ORCID](#)

ORCID login improves access, but some features are still being improved

# Define Your Deposition Type Carefully at the Start As Requirements and IDs Cannot Be Changed

- Define the deposition type at the start
- OneDep uses these selections to determine entry requirements and validation
- Once you click “Start deposition”, ID settings cannot be changed

Errors in defining an entry require withdrawing it and starting a new deposition from scratch

Experimental method

X-Ray Diffraction

Electron Microscopy

Helical

Single particle — Select EM method

Subtomogram averaging

Tomography

Solution NMR

Neutron Diffraction

Electron Crystallography

Solid-state NMR

Fiber Diffraction

Are you depositing coordinates with this submission? — A PDB ID will be issued

No, experimental data only

Yes

Has the associated map been deposited previously? — An EMD ID will be issued

No

Yes

Is this a composite map deposition? — “Yes” requires compliance with composite map acceptance criteria

No

Yes

Requested accession codes

PDB  EMDB  BMRB

Please copy this code: 17449

Privacy policy

Tick to indicate that you have read and accepted the wwPDB policy

[Start deposition](#)

# Once Entry Type is Defined, OneDep Sets File Requirements

File	When Required	In Validation Report?
Coordinates (PDB/mmCIF)	Map+Model, Model-only	Yes
Primary map (MRC/CCP4)	Map+Model, Map-only	Yes
Half-maps (MRC/CCP4)	SPA/Helical/STA	Yes
EMDB public image	Required for all 3DEM entries	No
FSC curve (XML)	Optional (recommended)	Yes
Mask (MRC/CCP4)	Optional (recommended)	Yes
Additional maps (MRC/CCP4)	Optional (upload any relevant supporting maps)	No
Metadata (mmCIF)	Optional (Map-only; Recommended for Batch Submissions)	Some

Depositing raw cryo-EM image data to EMPIAR is best practice as it preserves and shares raw data for you study

Please provide the DOI for a related raw data set (e.g. diffraction image data with a DOI, not related PDB entries or related citations).

Type of experimental data    DOI for related data set    DOI for metadata

EMPIAR             

# Strive To Go Beyond The Minimum When Sharing your Data

Upload file	Define type	Provide details
<input checked="" type="checkbox"/> <a href="#">Browse...</a> 0jpx.cif	Coordinates	Coordinates (mmCIF format)
<input checked="" type="checkbox"/> emd_36488.map	Primary map	EM map (MRC/CCP4 format) Pixel spacing (Å): 0.93889004 Contour level: 0.23 Short description:
<input checked="" type="checkbox"/> emd_36488.png	EMDB Image	Entry image for public display Image must include the entire primary map 
<input checked="" type="checkbox"/> emd_36488_half_map_1.map	Half-map	EM half map (MRC/CCP4 format) Pixel spacing (Å): 0.93889004 Contour level: 0.23 Short description:
<input checked="" type="checkbox"/> emd_36488_half_map_2.map	Half-map	EM half map (MRC/CCP4 format) Pixel spacing (Å): 0.93889004 Contour level: 0.23 Short description:

Continue deposition

Upload file

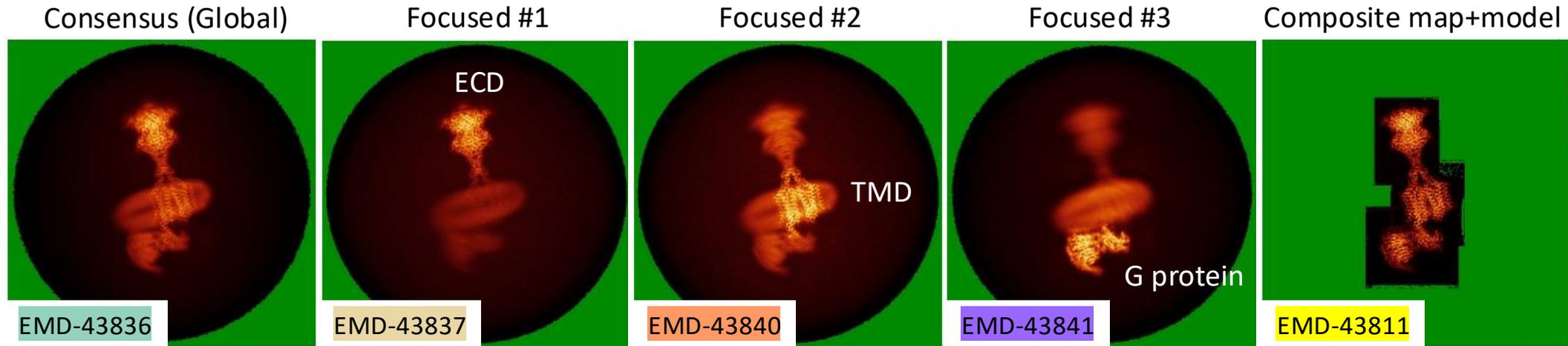
Define type

Provide details

Include all helpful files and provide sufficient descriptions to maximize validation, reuse, and future discovery

# Composite Map Entries Must Meet Additional Acceptance Criteria

All supporting maps must be deposited as separate entries



Each supporting map (e.g., consensus or focused EM volume) must be clearly defined and linked to the composite model entry

```

loop_
  _pdbx_database_related.db_name
  _pdbx_database_related.details
  _pdbx_database_related.db_id
  _pdbx_database_related.content_type
  EMDB 'Structure of human calcium-sensing receptor in complex with chimeric Gq (miniGisq) protein' EMD-43811
  'associated EM volume'
  EMDB 'Consensus map of human CaSR-miniGisq complex in nanodiscs' EMD-43836
  'consensus EM volume'
  EMDB 'Local refinement map of CaSR extracellular domain in nanodisc-reconstituted human CaSR-miniGisq complex' EMD-43837
  'focused EM volume'
  EMDB 'Local refinement map of CaSR transmembrane domain in nanodisc-reconstituted human CaSR-miniGisq complex' EMD-43840
  'focused EM volume'
  EMDB 'Local refinement map of G protein in nanodisc-reconstituted human CaSR-miniGisq complex' EMD-43841
  'focused EM volume'
  
```

# Composite Map Deposition Workflow

1. Review the EMDB Composite Map Deposition Guide:  
[https://www.ebi.ac.uk/emdb/documentation/deposition/composite\\_map](https://www.ebi.ac.uk/emdb/documentation/deposition/composite_map)
2. Create supporting depositions for:
  - The consensus volume
  - Each focused map
3. Create a deposition for the composite map
4. Link all related entries in the depUI or request annotator assistance during processing



## Composite Map Guide

### Introduction

The deposition of a composite map entry set follows the [doi.org/10.48550/arXiv.2311.17640](https://doi.org/10.48550/arXiv.2311.17640). In accordance with the

Are you depositing coordinates with this submission?

No, experimental data only

Yes

Has the associated map been deposited previously?

No

Yes

Is this a composite map deposition?

No

Yes

Provide related entries if any (e.g. PDB entries for the same citation)

Content type	Database name	Accession ID	Details
associated EM volume	EMDB	EMD-43811	Structure of human calcium
consensus EM volume	EMDB	EMD-43836	Consensus map of human C
focused EM volume	EMDB	EMD-43837	Local refinement map of C
focused EM volume	EMDB	EMD-43840	Local refinement map of C
focused EM volume	EMDB	EMD-43841	Local refinement map of G

# Associating Multiple Atomic Models With One EM Map

- Avoid depositing the same map multiple times
- Start by submitting one model as a Map+Model deposition
- Submit additional models as EM-model-only
- Link all models to the original Map+Model entry

Note: EM-model-only validation - coordinates assessed, map fit not yet included

Recommended:

Resource

## A Multi-model Approach to Assessing Local and Global Cryo-EM Map Quality

[Mark A. Herzik Jr.](#)<sup>1</sup>, [James S. Fraser](#)<sup>2</sup>, [Gabriel C. Lander](#)<sup>1,3</sup>  

[Show more](#) 

 Add to Mendeley  Share  Cite

<https://doi.org/10.1016/j.str.2018.10.003> 

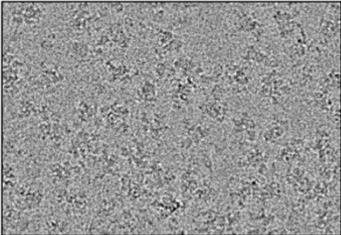
[Get rights and content](#) 

Watch for evolving best practices;  
EMDB is available for guidance

# Typing the Same Metadata Across Large Related Submissions Is a Major Burden

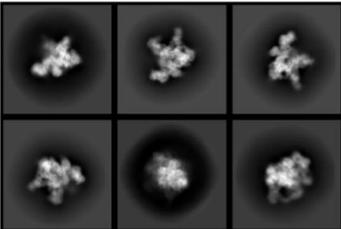
## Experimental Workflow

Download model coordinates from the PDB to create a 3D reconstruction of the structure. Test if provided coordinates.



30 nm

Download map and prepare a 3D reconstruction of the structure. Download the map and use it to create a 3D reconstruction of the structure. Download the map and use it to create a 3D reconstruction of the structure.



20 nm



Choose the specimen preparation used for this microscopy session\*:

Microscope model\*:

Illumination mode\*:

Imaging mode\*:

Electron source\*:

Acceleration voltage (kV)\*:

C2 aperture diameter (µm):

Spherical aberration coefficient (Cs in nm):

Nominal minimum defocus (nm):

Calibrated minimum defocus (nm):

Nominal maximum defocus (nm):

Calibrated maximum defocus (nm):

Nominal magnification:



**Submit deposition**

All items

Mandatory items

**Navigation**

- ✓ Instructions
- ✓ Communication
- ✓ Re-upload files
- ✓ Upload summary
- Admin
  - ✓ Contact information
  - ✓ Grant information
  - ✓ Release status
  - ✓ Entry title & author
  - ✓ Citation information
- Macromolecules
  - ✓ 1) RPA32 subunit of the hetero-
  - ✓ 2) DNA polymerase II small sub
  - ✓ 3) DNA polymerase II large sub
- EM sample
  - ✓ Overall sample description
- EM experiment
  - ✓ Specimen preparation
  - ✓ Microscopy

**Log out**

Common metadata must be entered across related entries

Upload Structure

Input Metadata

Submit

# Prepare Reusable Metadata for Multiple Structure Submissions Using pdb\_extract

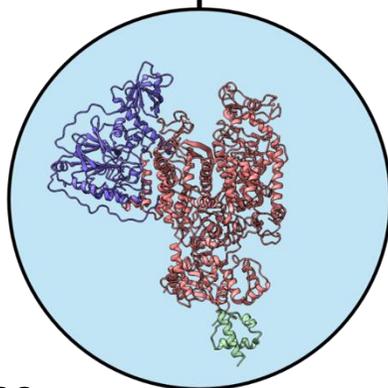


em\_imaging

entry_id*	id*	microscope_model*
× TO BE ASSIGNED, DO NOT CHANGE	1	TFS KRIOS

accelerating_voltage!	illumination_mode*
300	SPOT SCAN

mode*
BRIGHT FIELD



Microscope model\*: TFS KRIOS

Illumination mode\*: SPOT SCAN

Imaging mode\*: BRIGHT FIELD

Electron source\*: FIELD EMISSION GUN

Acceleration voltage (kV)\*: 300

Composite (Map+Model)

Consensus (Map-only)

Focused (Map-only)

Reduce effort,  
minimize errors

Enter metadata only once  
Generate deposition-ready mmCIF files

Upload to OneDep  
Data-entry pages auto-populated

# pdb\_extract Makes Metadata Preparation Simple

New and improved version



For now use: <https://pdb-extract-dev.wwpdb.org/> instead of <https://pdb-extract.wwpdb.org/>

1. Prepare reusable EM metadata template



2. Make files deposition-ready



3. Upload to OneDep



map-only

or

coordinates

Select file type...  
0) Metadata:  
Metadata (mmCIF format)  
1) Main map (mandatory)

Select file type...  
0) Coordinates:  
Coordinates (mmCIF format)  
Coordinates (PDB format)

- Create from a method-specific template:
- Load and edit a previously prepared metadata file:
- Extract metadata from an mmCIF file upload:  No file selected.
- Fetch metadata from PDB or EMDB by ID:

Select Experimental Method  X-Ray  NMR  EM

Select Type of Upload File  PDB  mmCIF

Upload Structure Model Coordinates File  No file selected.

Process large batches (e.g., 50+) quickly using standalone command-line version

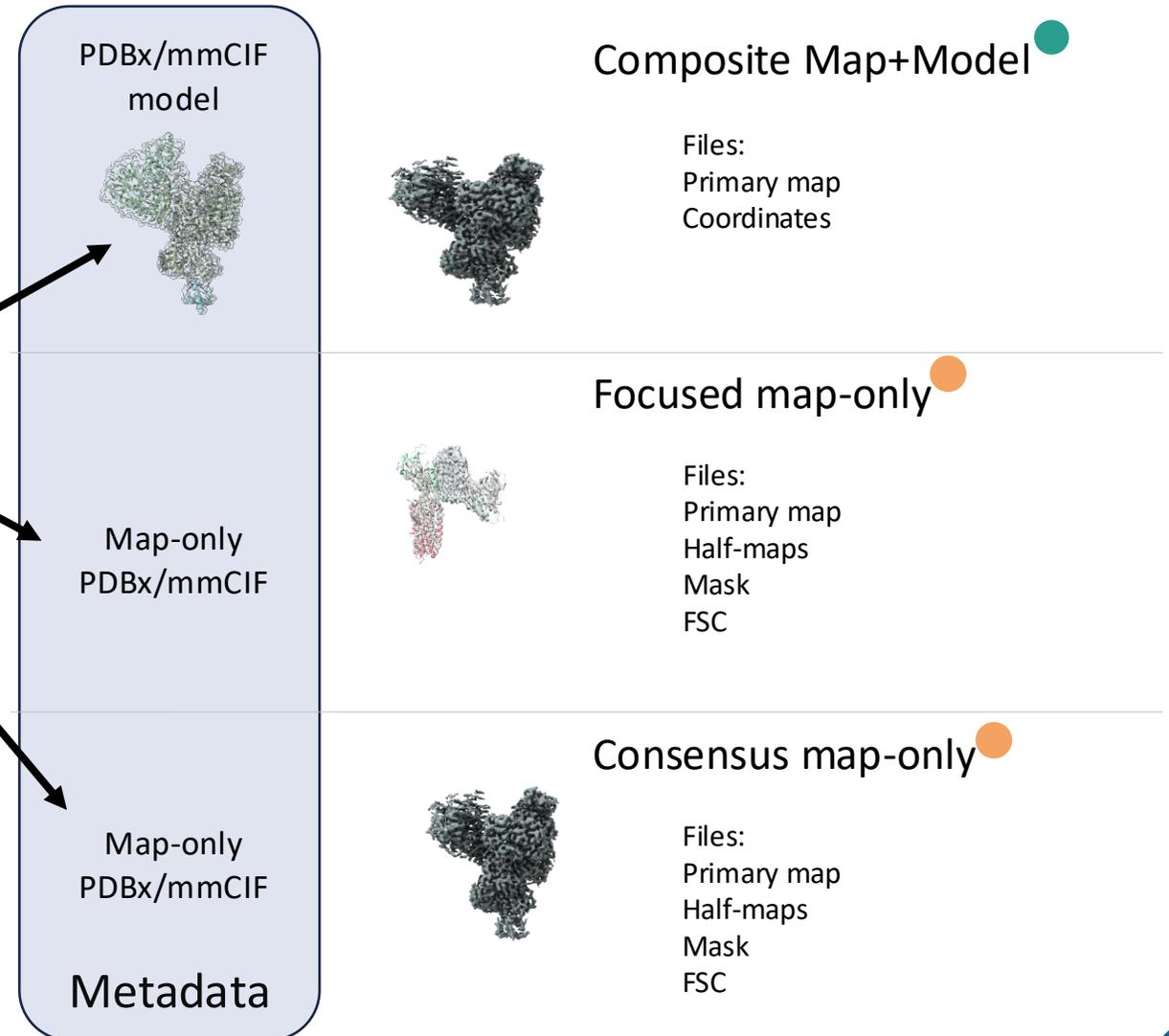
# pdb\_extract Reduces the Burden of Batch Submissions to OneDep

Simple composite case:

Download model coordinates mmCIF file for OneDep PDB deposition and validation of this structure

Download map-only-metadata mmCIF file for another OneDep map-only deposition related to this structure

For help please email  
deposit@deposit.rcsb.org,  
chenghua.shao@rcsb.org, or  
justin.flatt@rcsb.org

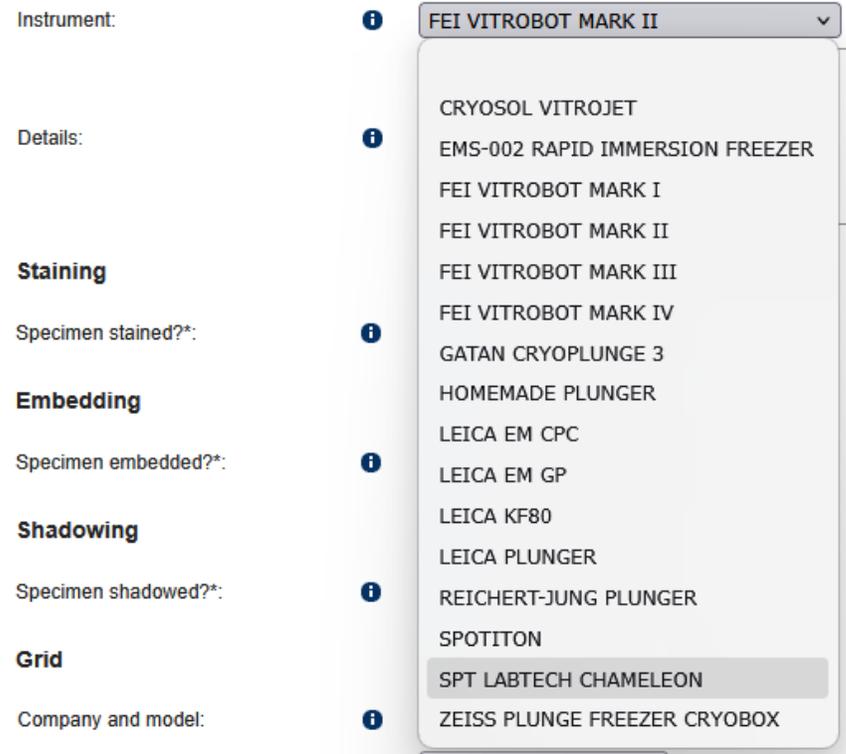


# Working Together For Accurate Records

Please report any needed changes upon noticing!

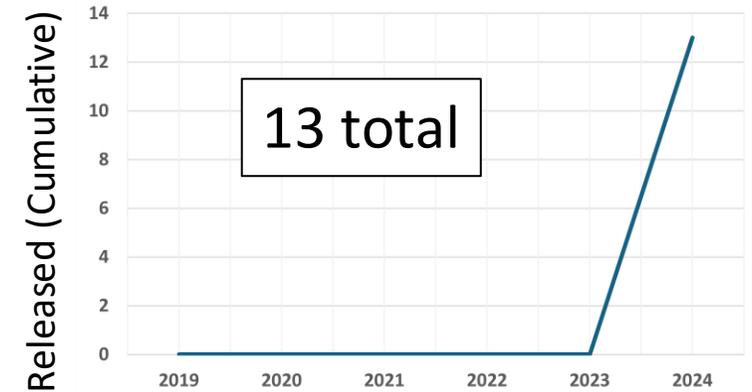


SPT Labtech introduced the Chameleon in 2019

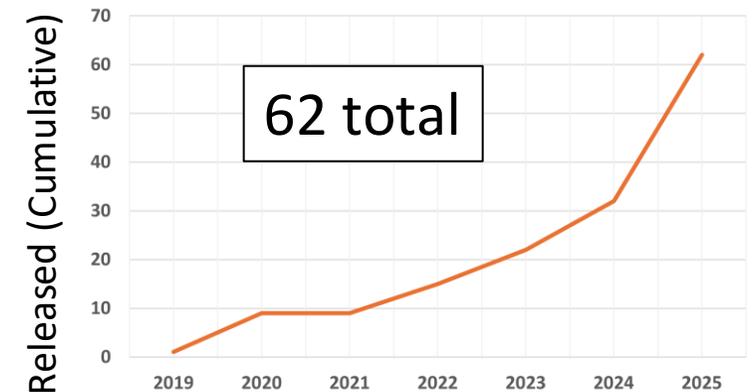


Request was not received to add the device to the pull-down until 2024

Entries with Chameleon Vitrification



vs. Spotiton with Chameleon in Title or Details

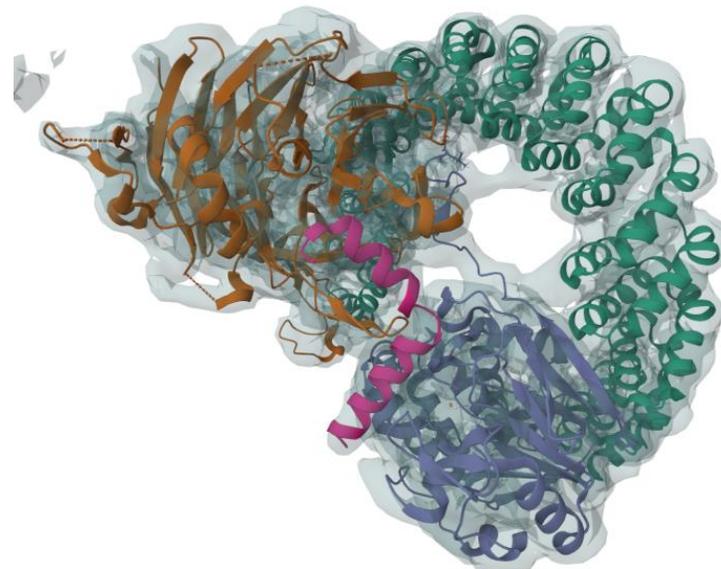
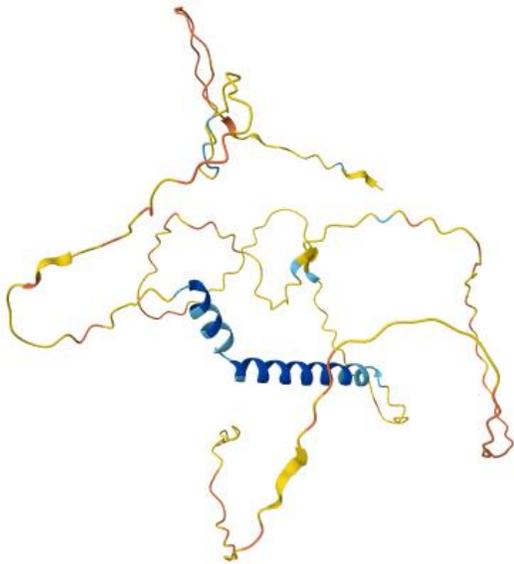


As a result, existing entries need to be updated to specify chameleon

# Complete Workflow Descriptions Enable Learning and Broader Adoption of More Robust Methods

## Starting Model(s)

Initial Refinement Model(s)		
Type	Source	Accession Code
experimental model	PDB	<a href="#">3DW8</a>
in silico model	AlphaFold	Q96E09



PDB ID: 8so0/EMD-40644

EM Software		
Task	Software Package	Version
PARTICLE SELECTION	RELION	4.0
PARTICLE SELECTION	Topaz	0.2.5
IMAGE ACQUISITION	EPU	3.0
CTF CORRECTION	CTFFIND	4.1.14
CTF CORRECTION	RELION	4.0
MODEL FITTING	UCSF ChimeraX	1.4
INITIAL EULER ASSIGNMENT	RELION	4.0
FINAL EULER ASSIGNMENT	RELION	4.0
RECONSTRUCTION	RELION	4.0
MODEL REFINEMENT	PHENIX	1.20.1-4487
MODEL REFINEMENT	Coot	0.9

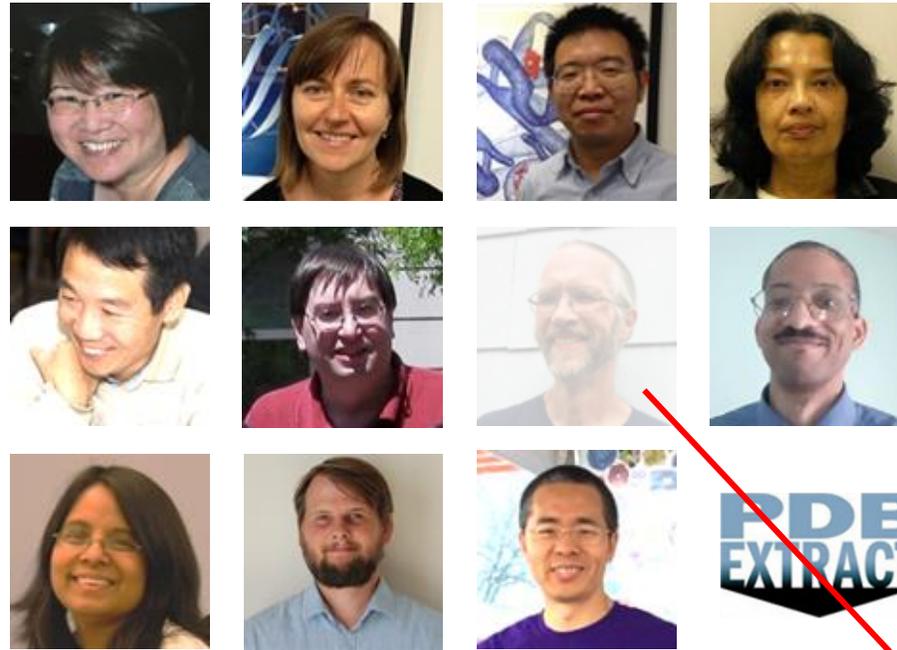
Map-Model Fitting and Refinement	
Id	1 (3DW8)
Refinement Space	REAL
Refinement Protocol	FLEXIBLE FIT
Refinement Target	Cross-correlation
Overall B Value	
Fitting Procedure	
Details	Iterating between manual refinement in Coot and automated real-space refinement in Phenix

# RCSB PDB Support for 3DEM Structure Deposition



RCSB team handles all depositions submitted from Americas

## Team



RCSB PDB has processed over 17,600 EM entries. Always happy to help the 3DEM community!

## Advisors



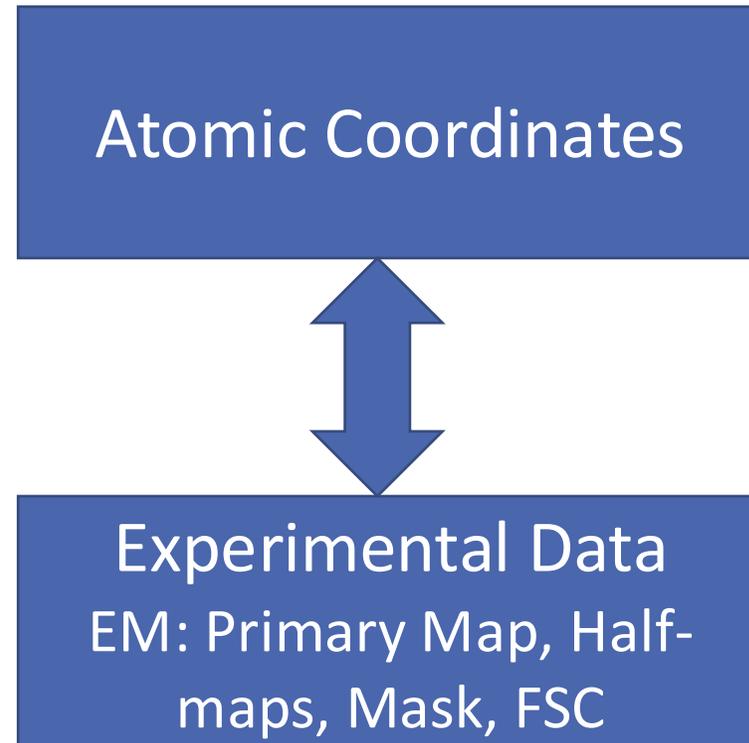
Brian retired (Feb 2026); position open for EM expert!

### III. Quality at Scale: Validation of 3DEM structures – Insights, Best practices, What's Next

# Evolving Validation Standards for 3DEM

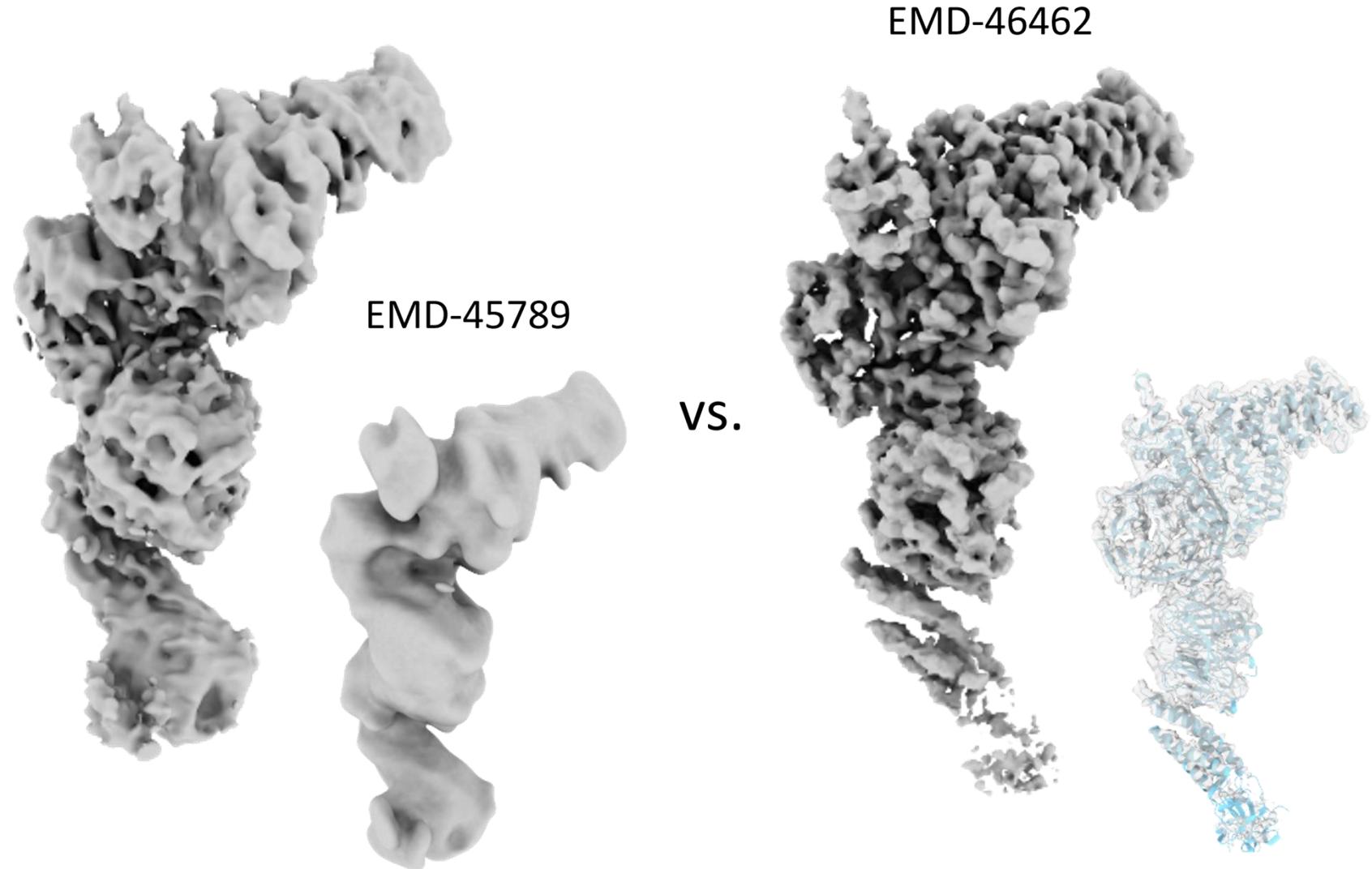
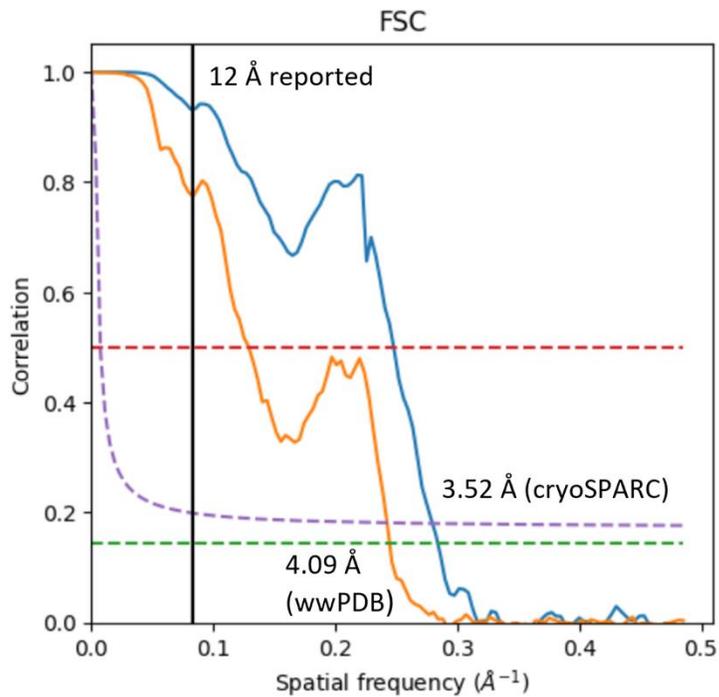
## What Gets Validated:

- Molecular geometry quality against established chemical references
- Experimental data quality
- Goodness-of-fit between atomic coordinates and experimental data



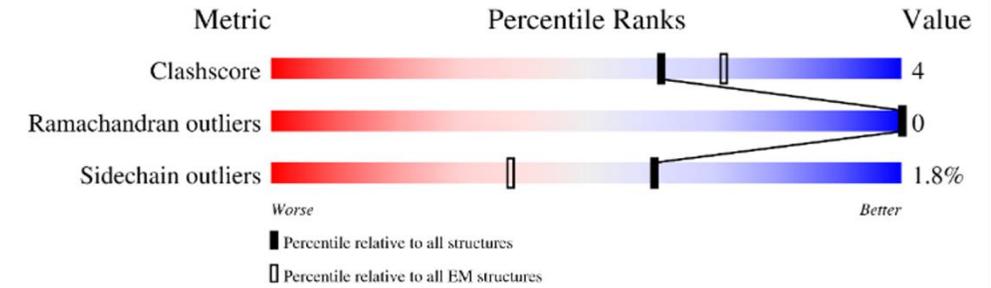
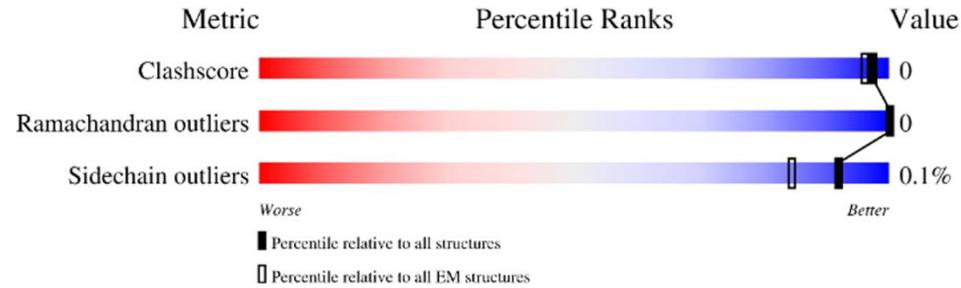
- High-quality PDB data essential for biomedical research
- Structure validation standards and tools developed by community experts
- 3DEM Validation Task Force publishes recommendations and consensus OneDep implementation

# Never Take the FSC at Face Value

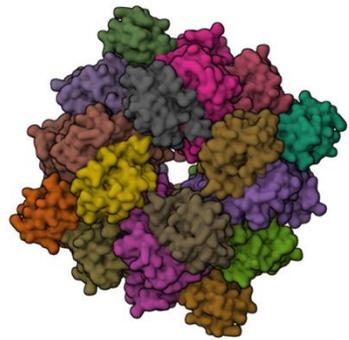


Always check local density and compare to similar-resolution maps

# Looking Good is not Always Being Right



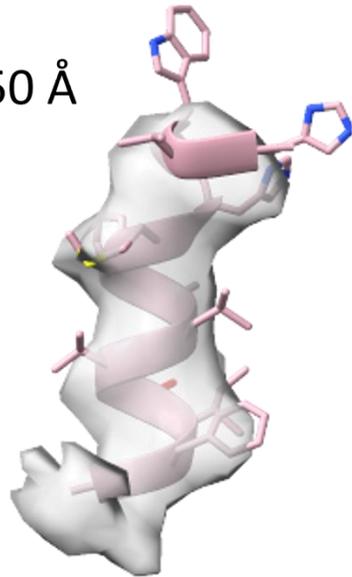
VS.



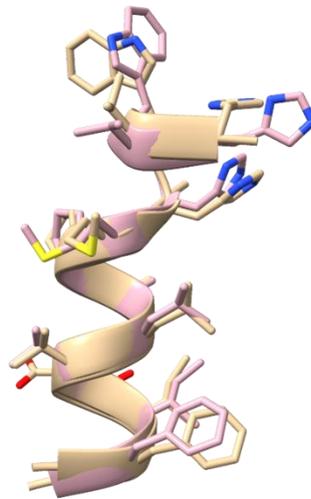
Subtomogram averaging  
9fwv/EMD-50836

Chain C: 383-HVWHMPALVEIFGG-395

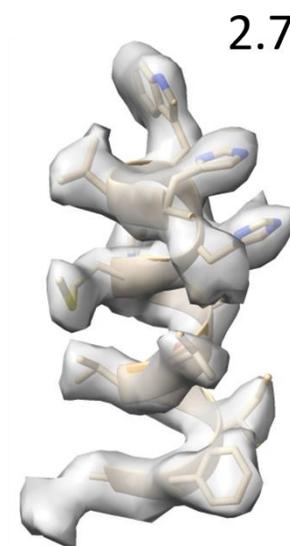
3.50 Å



Fit to map

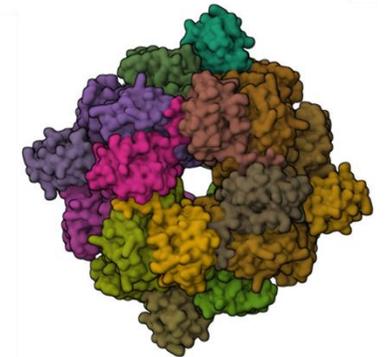


Superimposed on 6hbc



Chain C: 383-HVWHMPALVEIFGG-395

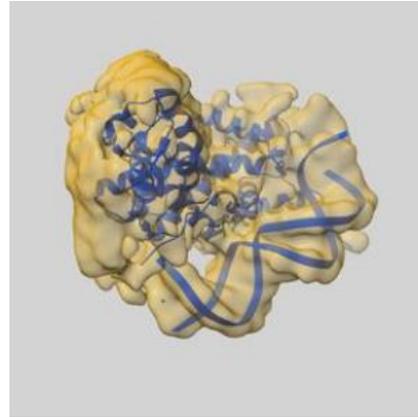
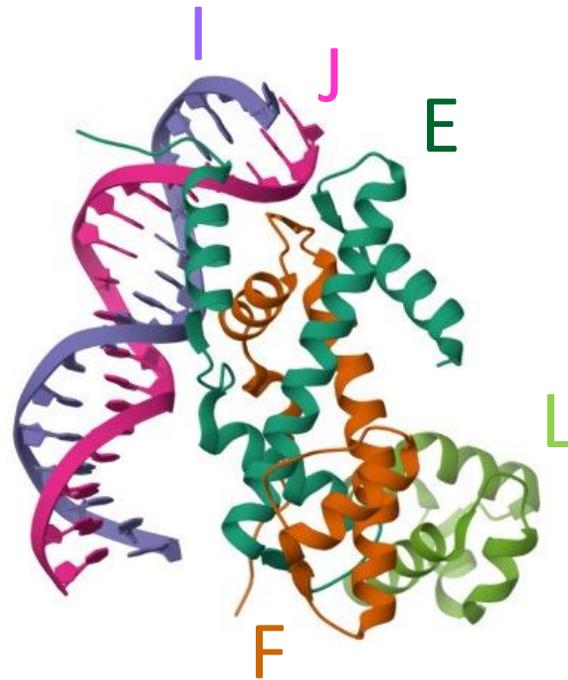
2.78 Å



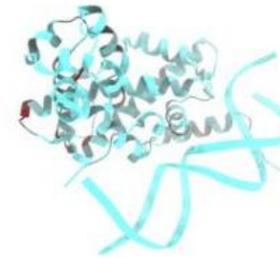
Single Particle  
6hbc/EMD-0180

Outliers can be refined away, creating deceptively perfect scores for imperfect structures

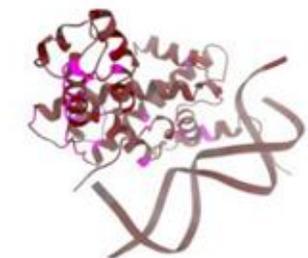
# Looking “Too Good” is Easier to Spot



Atom inclusion



Q-score



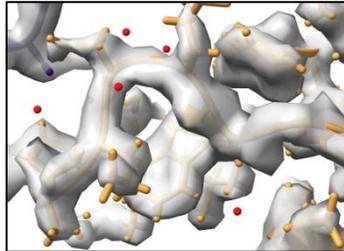
Chain	Atom inclusion	Q-score
All	0.8450	0.2190
E	0.7770	0.1630
F	0.7830	0.2130
I	1.0000	0.3320
J	0.9890	0.3210
L	0.8030	0.1510

Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	2.89	-	-
Author-provided FSC curve	2.89	3.23	2.94
Unmasked-calculated*	3.49	4.29	3.57

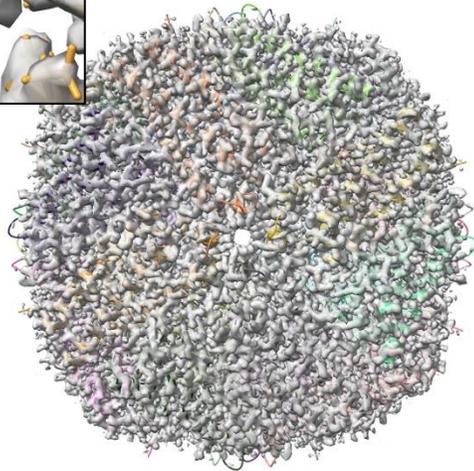
Pintilie, G., Zhang, K., Su, Z. *et al.* Measurement of atom resolvability in cryo-EM maps with Q-scores. *Nat Methods* **17**, 328–334 (2020). <https://doi.org/10.1038/s41592-020-0731-1>

# Good Compared to What?

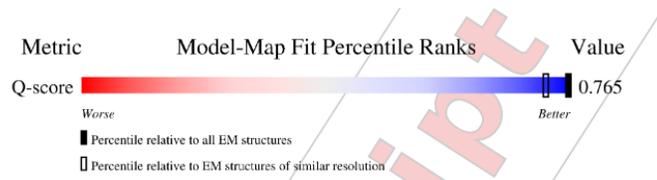
## Well-fitted



2.0 Å Reported  
2.26 Å Calculated

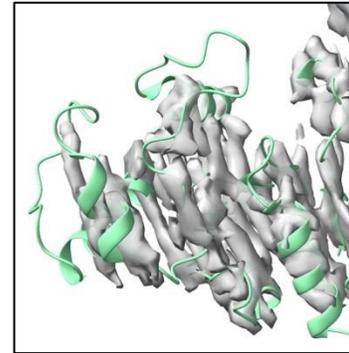


PDB ID: 6wx6  
EMD-21951

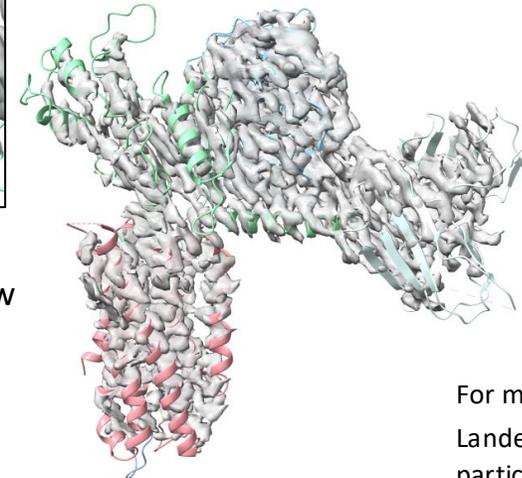


Compared to 25,397 EM structures  
1,659 structures at similar resolution (1.50-2.50)

## Preferred Orientation

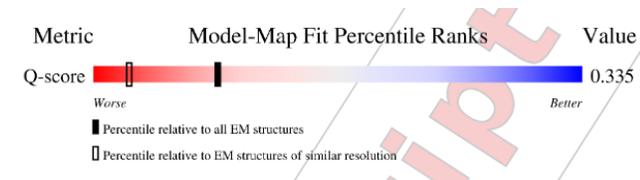


Rotated around X  
axis 45°



PDB ID: 7f8w  
EMD-31494

3.1 Å Reported  
No half-maps, no estimate

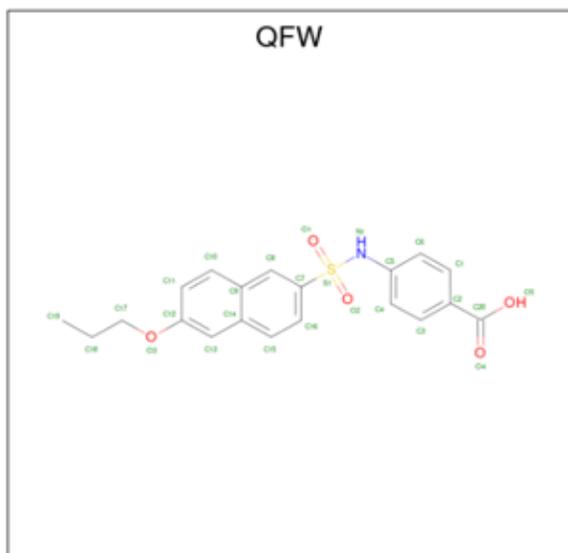


Compared to 25,397 EM structures  
14,724 structures at similar resolution (2.60-3.60)

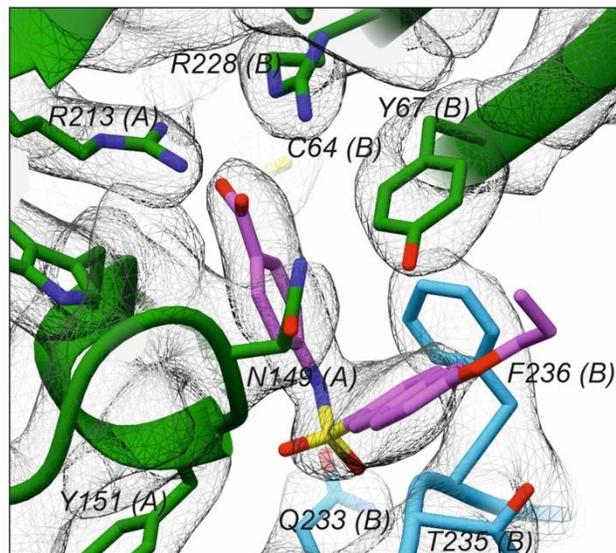
For more on this case see:  
Lander, G. C. (2024). Single  
particle cryo-EM map and model  
validation: It's not crystal clear.  
Current Opinion in Structural  
Biology, 89, 102918.  
<https://doi.org/10.1016/j.sbi.2024.102918>

# Modeling Ligands Are Double the Trouble

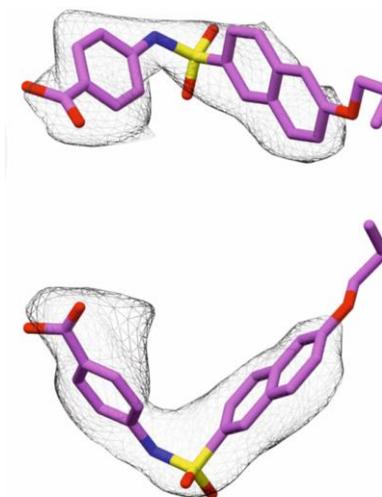
Ligand description



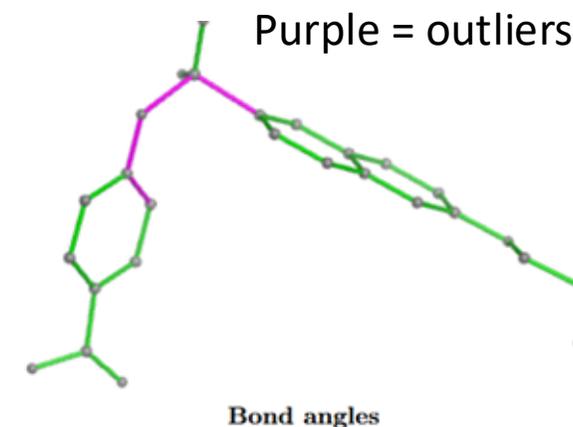
PDB 6zck/EMD-11165



Flatt, J.W., et al. (2020) *Commun Biol*  
<https://doi.org/10.1038/s42003-021-01779-x>

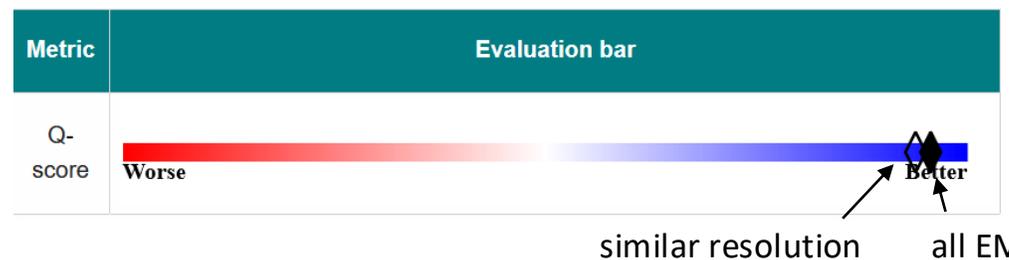


Avg. Q-score: 0.624  
 Ligand Q-score 0.544



Geometry validated against the Cambridge Structural Database (CSD); environmental clashes measured; per-ligand fit to experimental data assessed using Q-score

Always verify that map fit, stereochemistry, and interactions are chemically sensible.



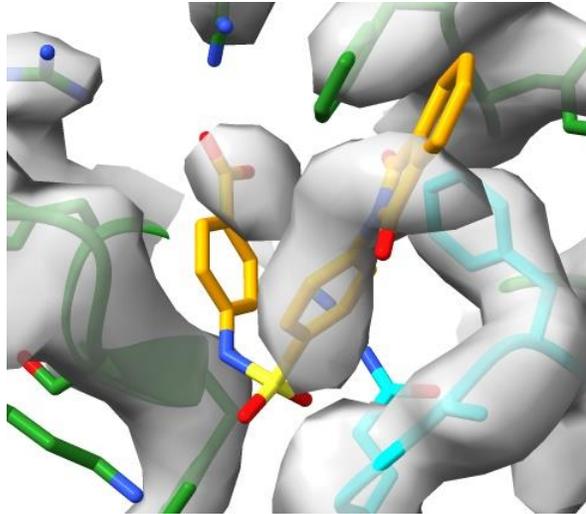
# Let Q-scores be a Guide to Your Ligands

PDB ID: 6gzv/EMD-0103

4.0 Å

Q Struct: 0.454

Q Ligand: 0.2



vs.

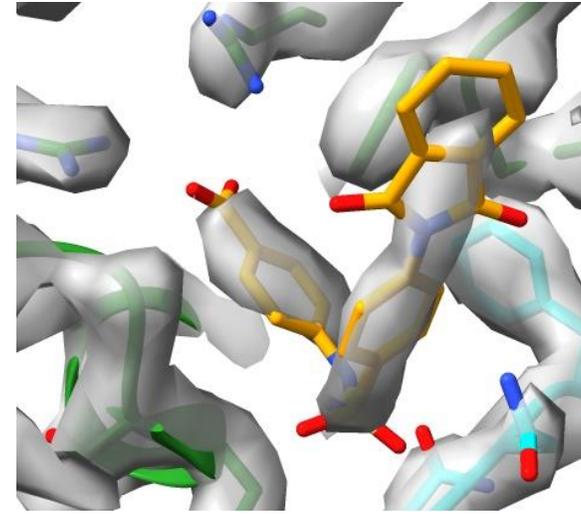
PDB ID: 6zcl/EMD-11166

Reprocessed and remodeled

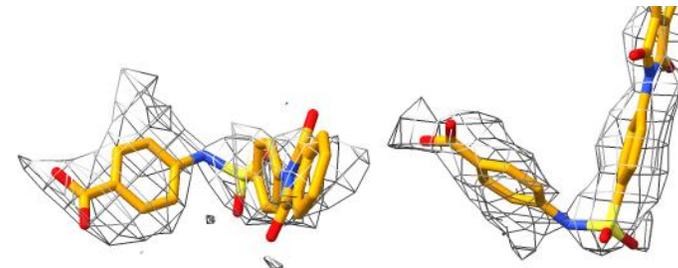
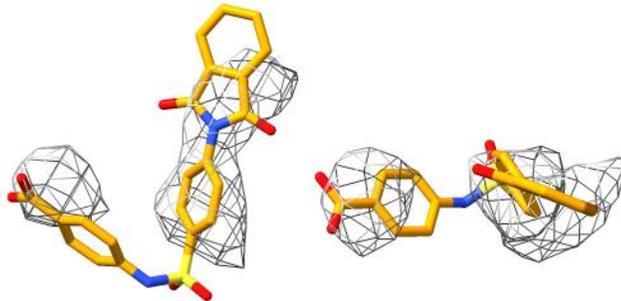
2.8 Å

Q Struct: 0.62

Q Ligand: 0.52

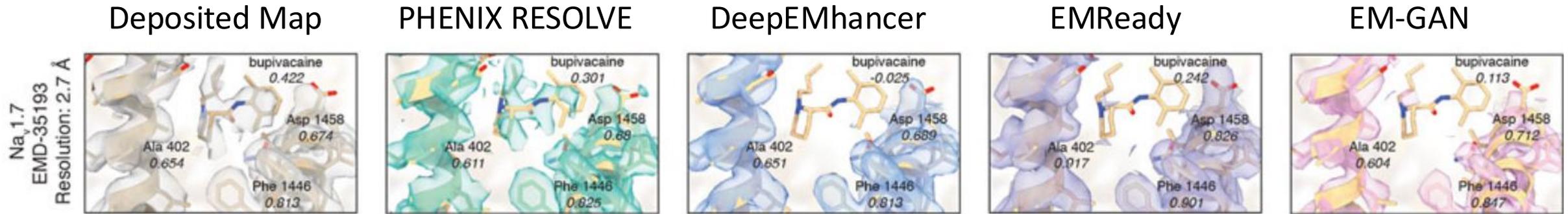


Fit 1



Fit 2

# Caution: ML Tools Can Distort Ligand Density

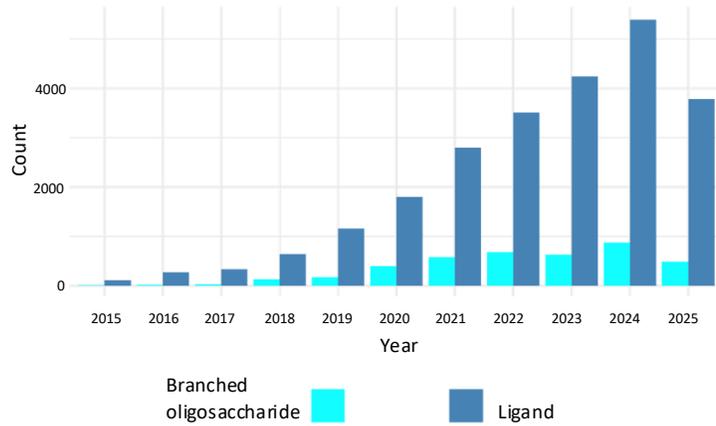


Berkeley, R.F., Cook, B.D., Herzik Jr, M.A. (2024). Machine learning approaches to cryoEM density modification differentially affect biomacromolecule and ligand density quality. *Front. Mol. Biosci, Sec. Structural Biology*. Vol 11, <https://doi.org/10.3389/fmolb.2024.1404885>.

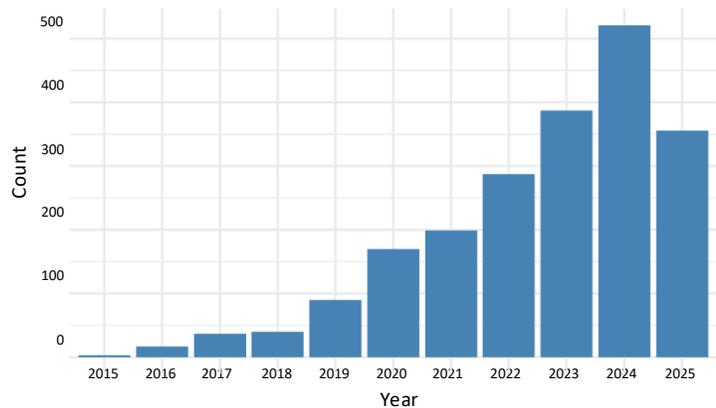
Extreme cases show ligands erased from the map

# Bird's Eye View of Ligand Quality Using Q-scores

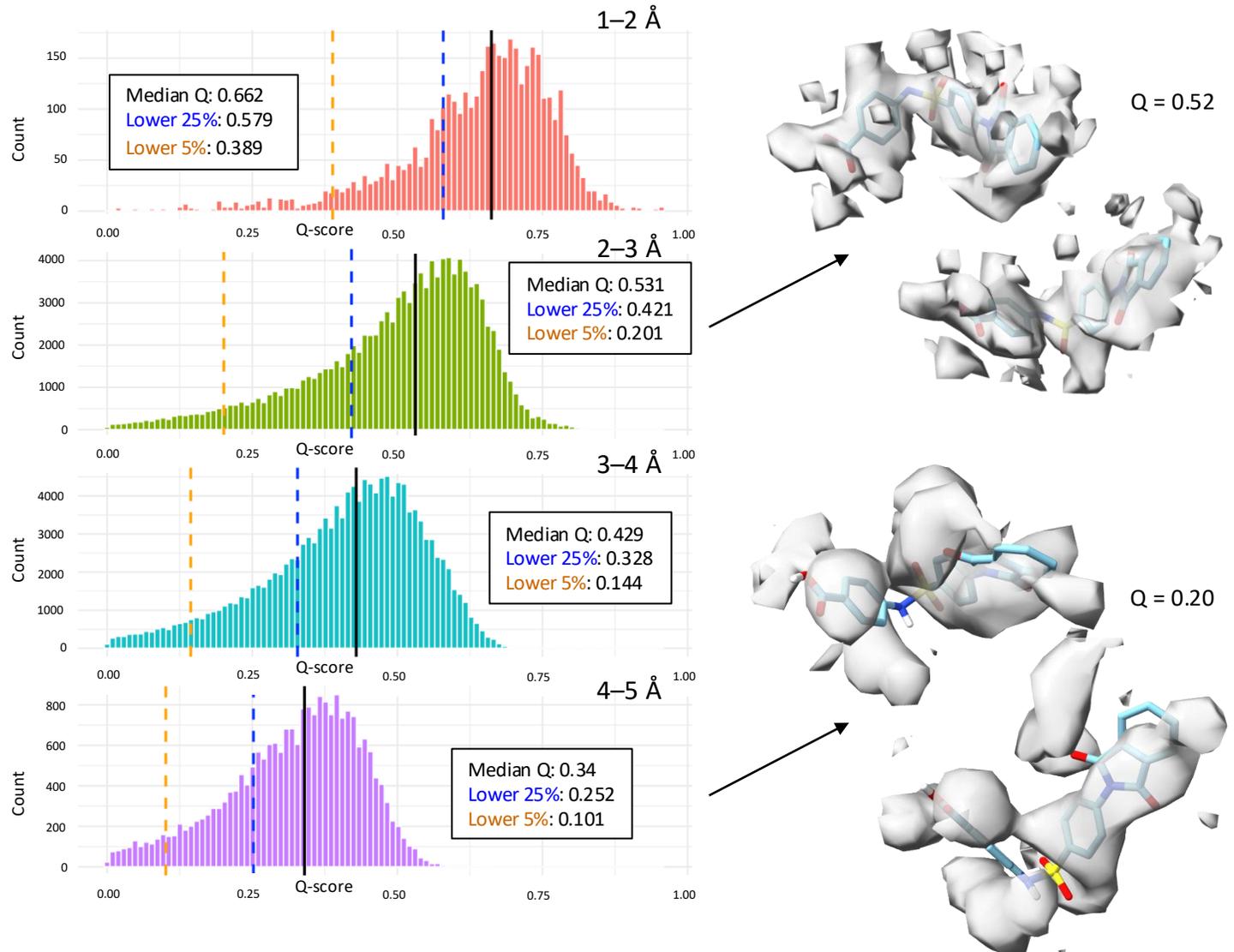
## Cryo-EM Depositions with Ligands or Branched Oligosaccharides



## New Ligands Entering the CCD



## Ligand Q-score Distribution Across Resolution Bins



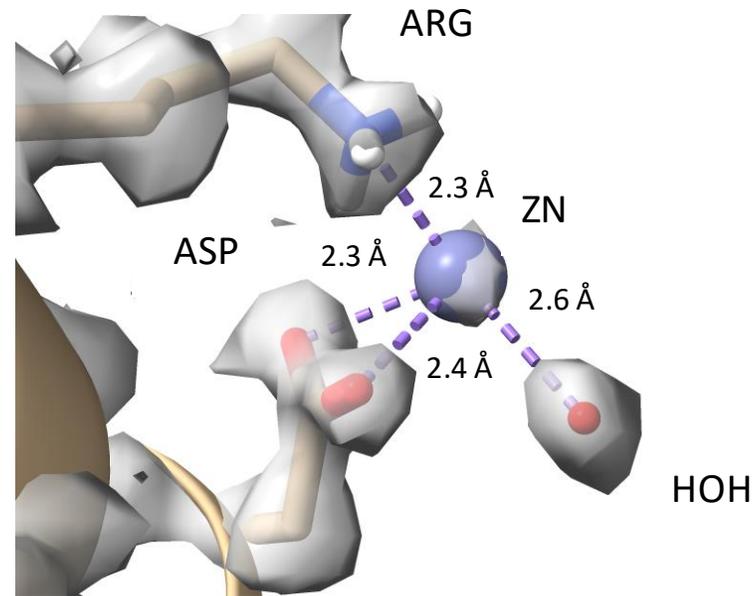
# Locking in Your Best Possible Model

- Validate early, validate often
- Leverage all available validation tools
- Model within the map's resolution limits
- Visually inspect local fit across the structure
- Review official validation report and any correspondence carefully
- Note: Coordinates can be updated post-release, but the primary map cannot

<https://validate.wwpdb.org>

Welcome to the wwPDB validation system!

This server performs the same validation as you would observe during the deposition process.



Version Number	Version Date	Version Type/Reason	Version Change
1.0	2023-10-11	Initial release	
1.1	2023-12-13		Data collection, Database references
2.0	2024-08-21	Coordinate replacement / Model orientation/position	Advisory, Atomic model, Data collection, Database references, Derived calculations, Experimental preparation, Refinement description, Source and taxonomy, Structure summary

The most valuable resource is the EM community



RCSB.ORG • info@rcsb.org

### Core Operations Funding

US National Science Foundation (DBI-2321666), National Institute of General Medical Sciences, National Institute of Allergy and Infectious Disease, and National Cancer Institute (NIH R01GM157729), and the US Department of Energy (DE-SC0019749)

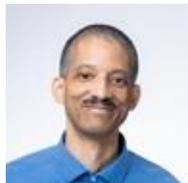
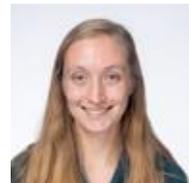
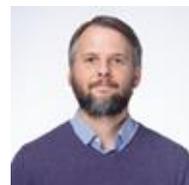
### Management



Follow us



Member of the Worldwide Protein Data Bank (wwPDB; [wwpdb.org](http://wwpdb.org))



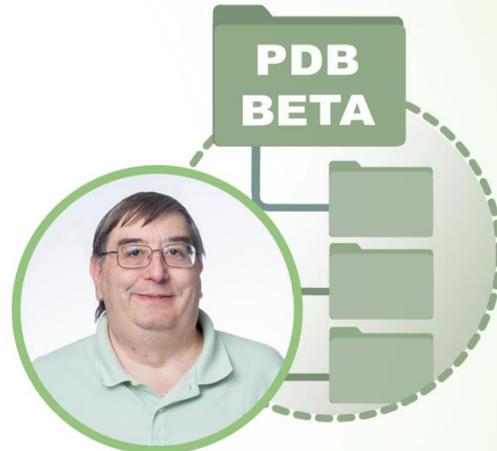
# Stay Connected with the RCSB PDB Team



**REGISTER FOR WEBINAR:**

## How to Use the PDB Beta Archive and Extended PDB IDs Today

**Thursday, March 26, 2026**  
1-2pm ET | 10-11am PT



**Ezra Peisach**  
Scientific Software Developer &  
PDBx/mmCIF Dictionary Manager



**REGISTER FOR OFFICE HOUR:**

## Exploring Biological Assemblies in the PDB

**Wednesday March 18, 2026**  
12-1pm ET | 9-10am PT



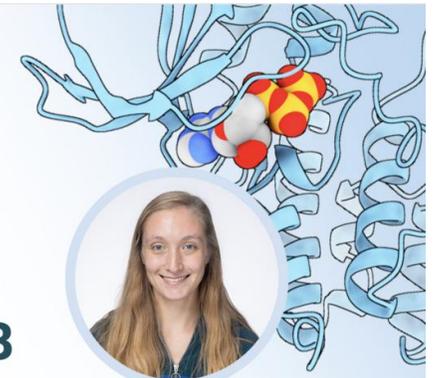
**Rachel Kramer Green**  
Scientific Support &  
Customer Service Lead



**REGISTER FOR WEBINAR:**

## Searching and Downloading Small Molecules in the PDB

**Thursday February 26, 2026**  
1-2pm ET | 10-11am PT



**Alison Biester**  
Scientific Researcher

Recordings posted at [PDB-101.rcsb.org](https://pdb-101.rcsb.org)