3DEM Data Archiving & Validation, Challenges

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NYSBC CryoEM Course

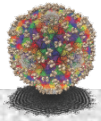
April 10, 2017

EMDataBank

Unified Data Resource for 3DEM

3DEM Data Archives for Structural Biology

- PDB – coordinates - managed by wwPDB
- EMDB – maps - managed by EMDataBank
- EMPIAR – raw images - managed by EBI



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Protein Data Bank (PDB)

- Single global archive of 3-D macromolecular structures (>125,000 entries) est. 1971
- 1990s: First EM structures deposited
- 2017: ~1500 EM structures
- Managed by the world-wide PDB organization

EM Data Bank (EMDB)

- Global archive for maps produced using 3DEM methods, est. 2002 @ EBI
- Since 2007, managed by EMDataBank partners, with support from NIGMS
- We expect to reach 5000 entries by end of 2017

NCMI National Center for Macromolecular Imaging

RCSB PDB PROTEIN DATA BANK

PDBe Protein Data Bank in Europe

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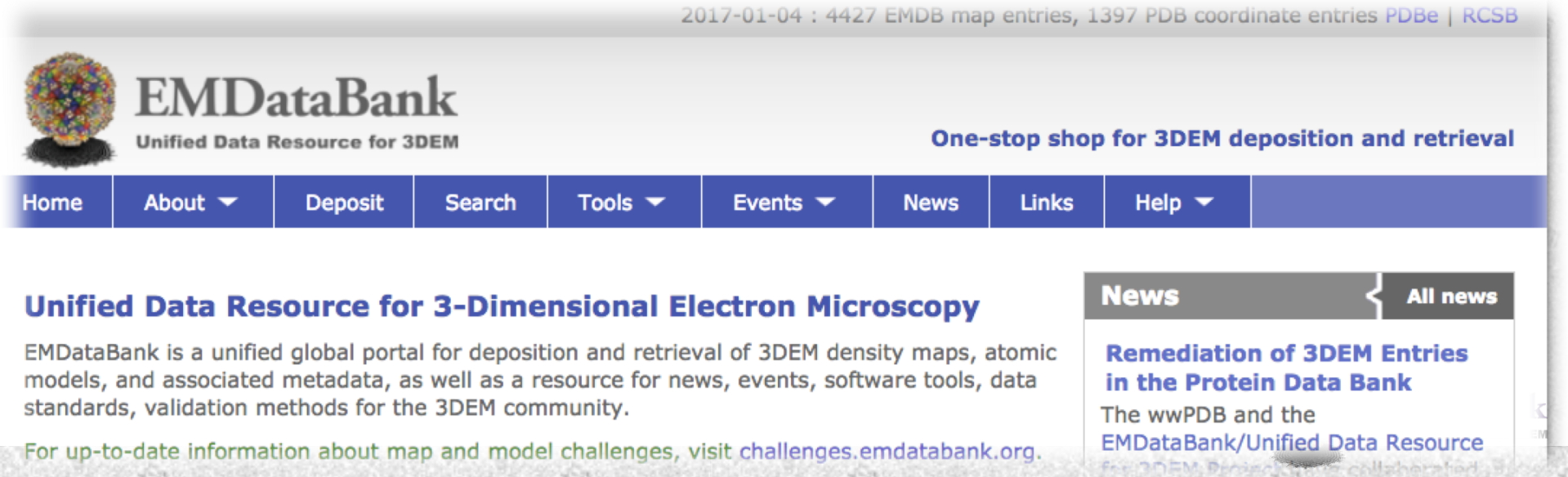
EMPIAR (pronounced empire)

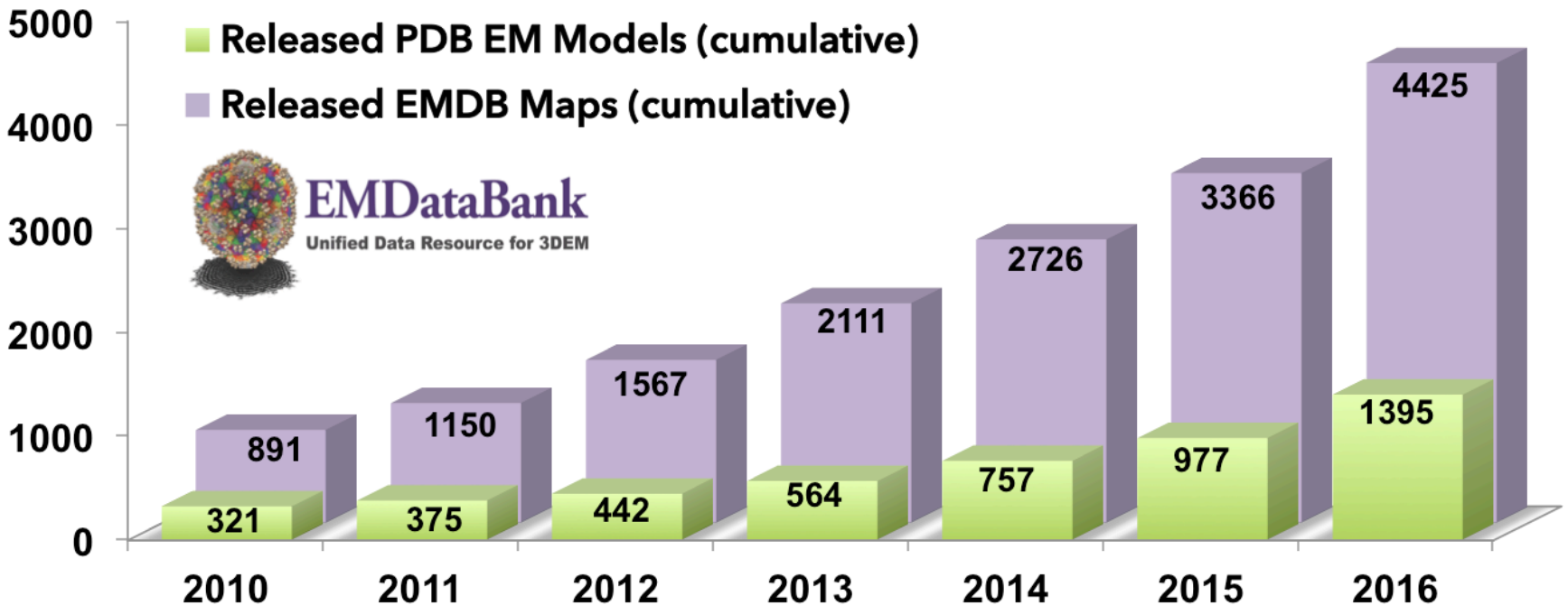
- Archive for raw electron microscopy image data est. @ EBI/PDBe in 2013
- ~100 entries
- Supported by MRC, BBSRC

EMDataBank.org

- Global portal for deposition and retrieval of 3DEM density maps, atomic models, and associated metadata
- Resource for news, events, software tools, data standards, validation methods for the 3DEM community



Growth of 3DEM Archives



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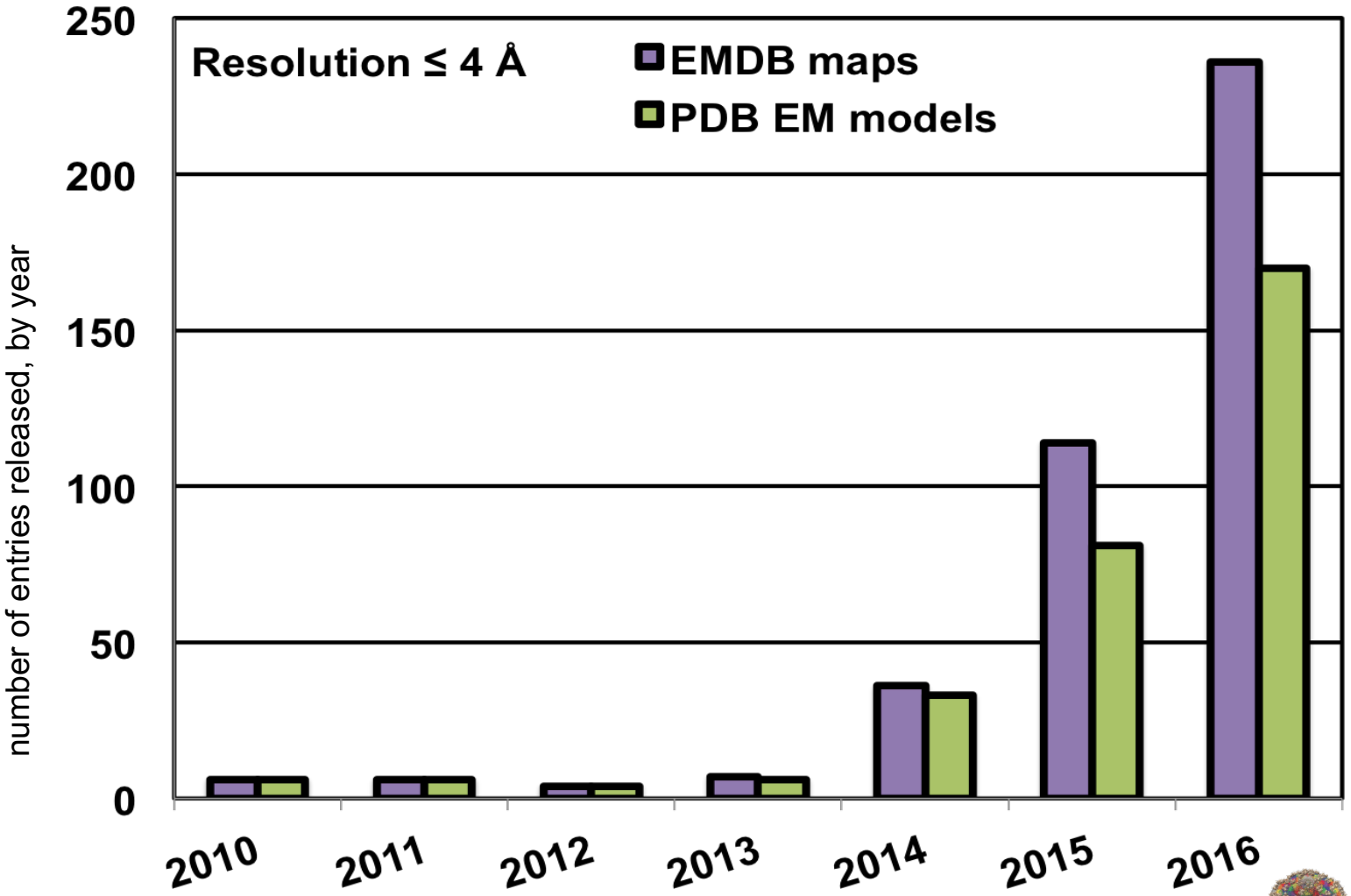
EM Structures @ 4 Å or better



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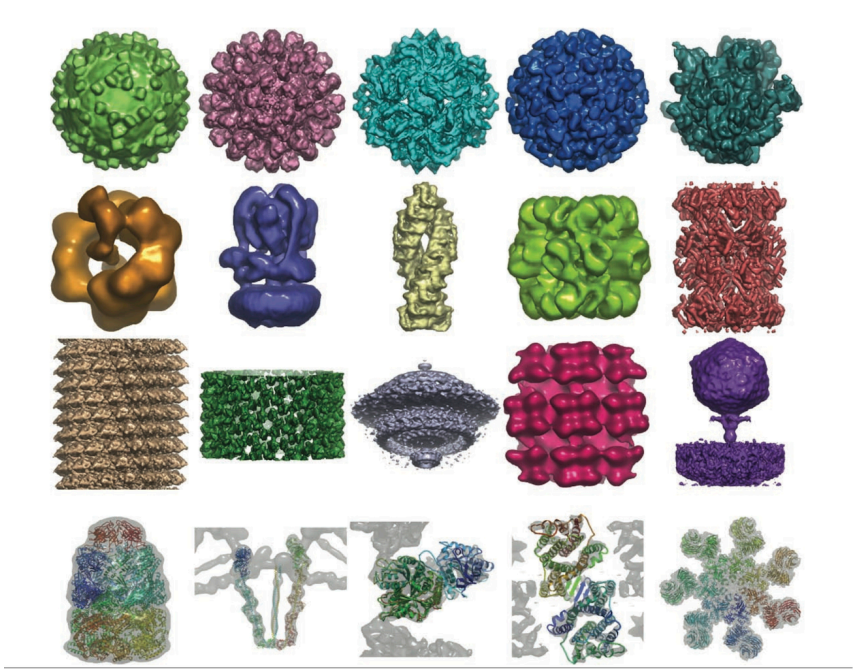
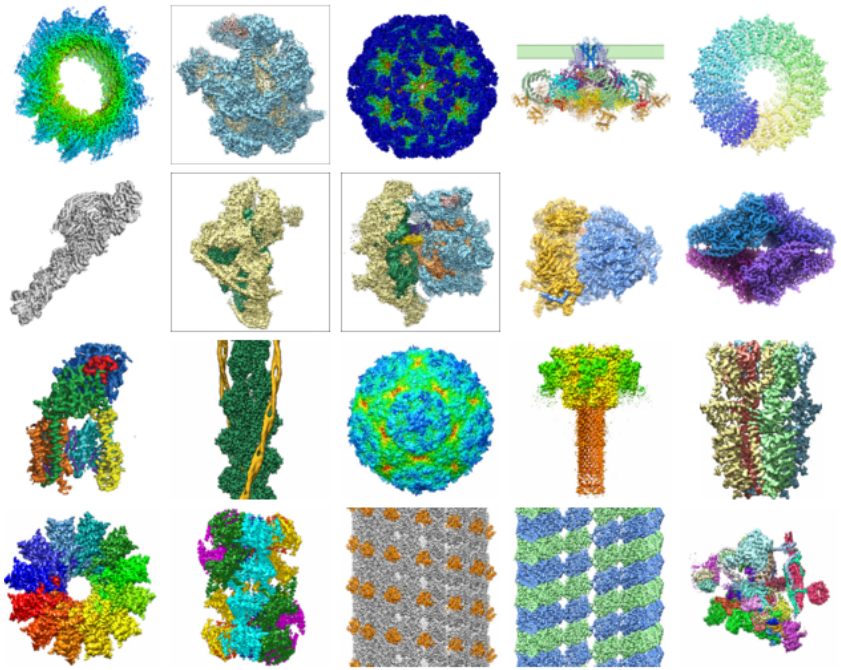
EM Structures 2010 vs 2015

2010: Molecular Shapes



0.5% of all entries in PDB
(332 of 67500)

2015: Traceable Densities



0.8% of all entries in PDB
(905 of 112400)

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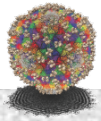
3DEM Structure Validation



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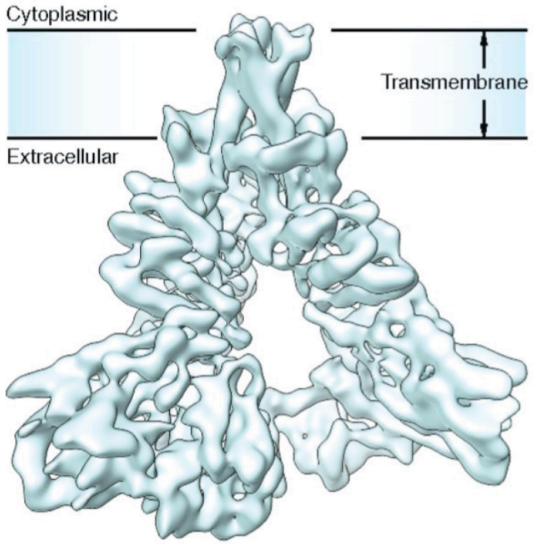
Importance of Validation

- **J. Cohen, Is High-Tech View of HIV Too Good to Be True?** *Science* 341, 443-444 (2013)
- **R.M. Glaeser, Replication and validation of cryo-EM structures** *J. Struct. Biol.* 184, 379-380 (2013)
- **R. Henderson, Avoiding pitfalls of single particle cryo-electron microscopy: Einstein from noise,** *PNAS* 110, 18037-41 (2013)
- **M. van Heel , Finding trimeric HIV-1 envelope glycoproteins in random noise,** *PNAS* 110, E4175-7 (2013)
- **S. Subramaniam, Structure of trimeric HIV-1 envelope glycoproteins,** *PNAS* 110, E4172-4 (2013)



EMD-5418 Y Mao, JG Sodroski *et al*. Molecular architecture of the uncleaved HIV-1 envelope glycoprotein trimer *PNAS* 110, 12438-12443 (2013)

Community Input for Validation

Task Force	Meeting/ Workshop	Chair(s)/Membership	Outcome
X-ray Validation Task Force	2008 (2015)	Randy Read (Univ of Cambridge) 17 members	(2011) *Structure* 19: 1395-1412
NMR Validation Task Force	2009, 2011, 2013 (x2), 2015	Gaetano Montelione (Rutgers) Michael Nilges (Institut Pasteur) 10 members	(2013) *Structure*, 21: 1563-1570
3DEM Validation Task Force	**2010**	**Richard Henderson (MRC-LMB) Andrej Sali (UCSF) 21 members**	**(2012) *Structure* 20: 205-214**
Small-Angle Scattering Task Force	2012, 2014	Jill Trewhella (Univ Sydney) 6 members	(2013) *Structure* 21: 875-881
Hybrid Methods Workshop	2014	Andrej Sali (UCSF), Torsten Schwede (Univ Basel), Jill Trewhella (Univ Sydney) 27 members	(2014) *Structure* 23: 1156-1167

EM Validation Task Force

Henderson *et al*. (2012) ***Structure 20***, 205-214
http://www.ncbi.nlm.nih.gov/pubmed/22325770

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EM VTF Recommendations

- Main recommendations: EM maps
  - Standards for assessing resolution and accuracy of a map need to be developed
  - Structural features in a map should be in accordance with the claimed resolution
- Main recommendations: models fitted into EM maps
  - Criteria for assessing models in context of map-model fit need to be developed
  - Capability to archive coarse-grained representations of models is needed
- More research and development needed!

EM VTC on Resolution

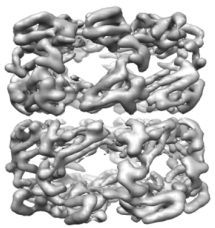
- Resolution via FSC: “Deposition of a published map should include its full FSC curve to the Nyquist frequency on a linear spatial-frequency scale.”
  - Masking: discouraged
  - Fully independent refinement: encouraged
- Low resolution structures (where secondary structure elements cannot be identified) can be validated using tilt-based methods

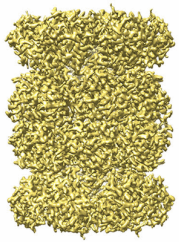
2015-Ongoing: Map, Model Challenges

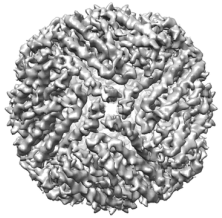
- Goals: Develop benchmarks, encourage development of best practices in 3DEM reconstruction and model fitting, **evolve criteria for validation**, compare and contrast different approaches
- Developed by expert committees
- Results discussion via Participant Workshops/Journal Special Issues
- http://challenges.emdatabank.org

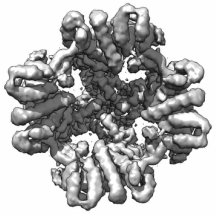
Benchmark Datasets

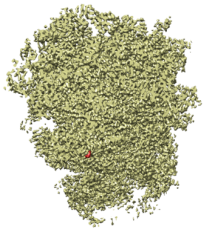
Map Challenge Targets: Raw Images @ EMPIAR

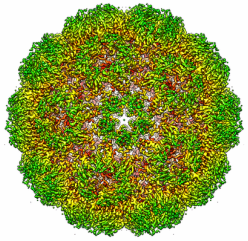

GroEL

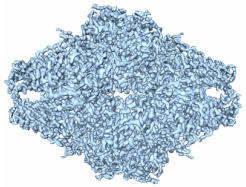

T20S Proteasome


Apo-Ferritin

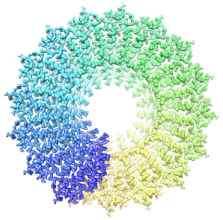

TrpV1 channel

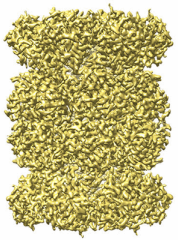

80S Ribosome

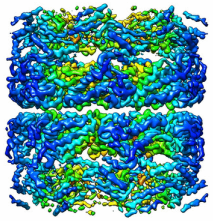

Brome Mosaic Virus

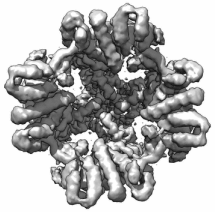

ß-galacto-sidase

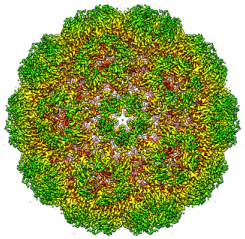
Model Challenge Targets: Maps @ EMDB

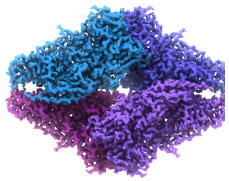

Tobacco Mosaic Virus

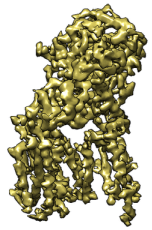

T20S Proteasome

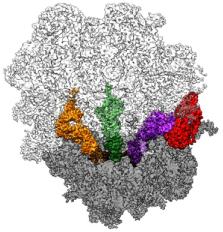

GroEL


TrpV1 channel


Brome Mosaic Virus


ß-galacto-sidase


γ-Secretase


70S Ribosome

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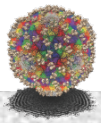
Challenges: Progress

- Map Challenge:
  - 27 participants submitted 66 maps
- Model Challenge:
  - 16 participants submitted 106 models

Lots of Data Received! – assessments now in progress

3DEM Structure Deposition



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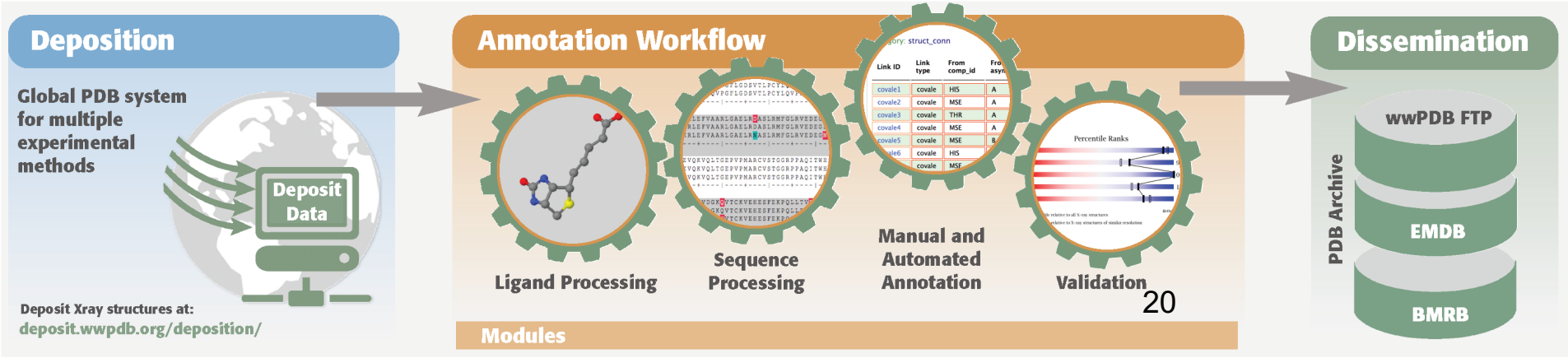
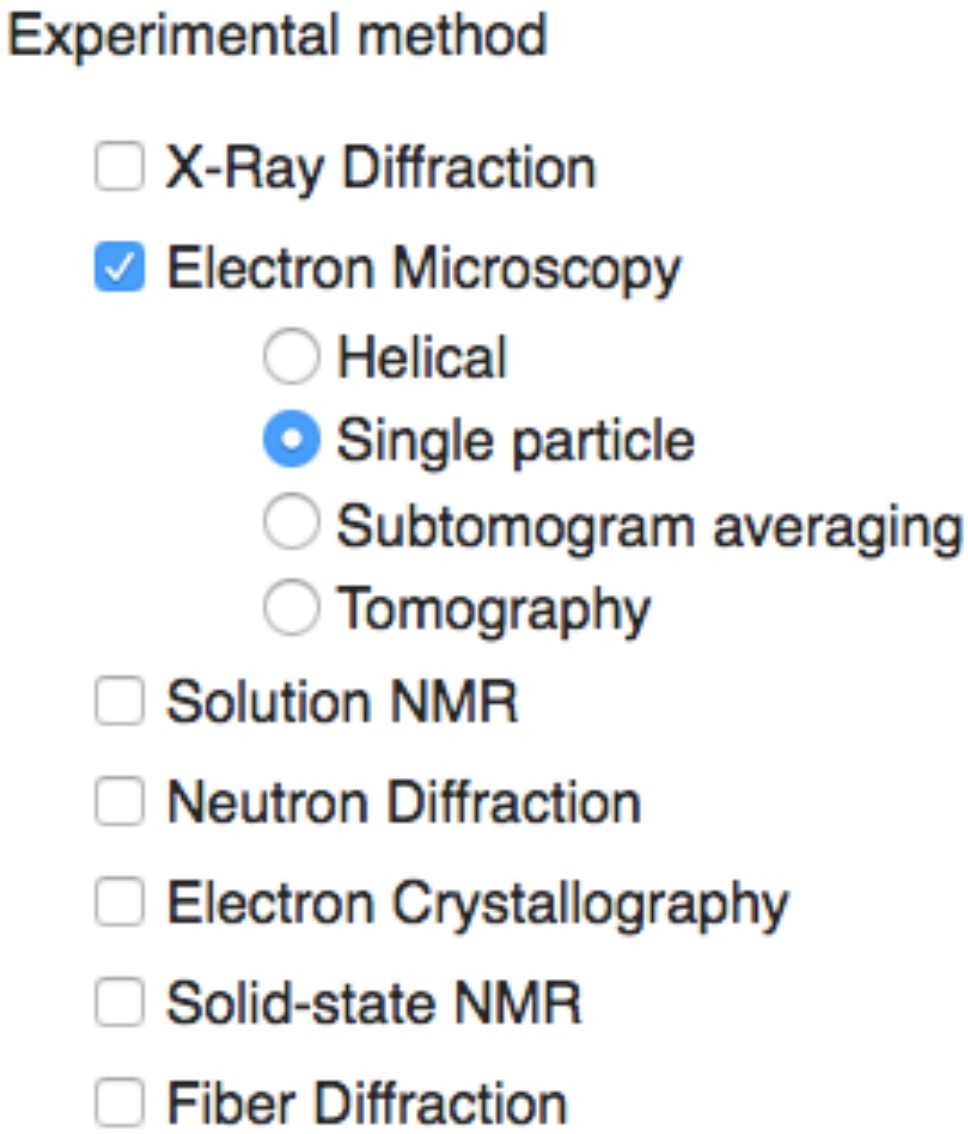
wwPDB OneDep System

- New Version for depositing structures from X-ray, NMR, and EM Launched January 2016
- Deposit map to EMDB with associated model to PDB in single session
- Validation report provided



3DEM Deposition: Method

http://deposit.wwpdb.org/deposition/



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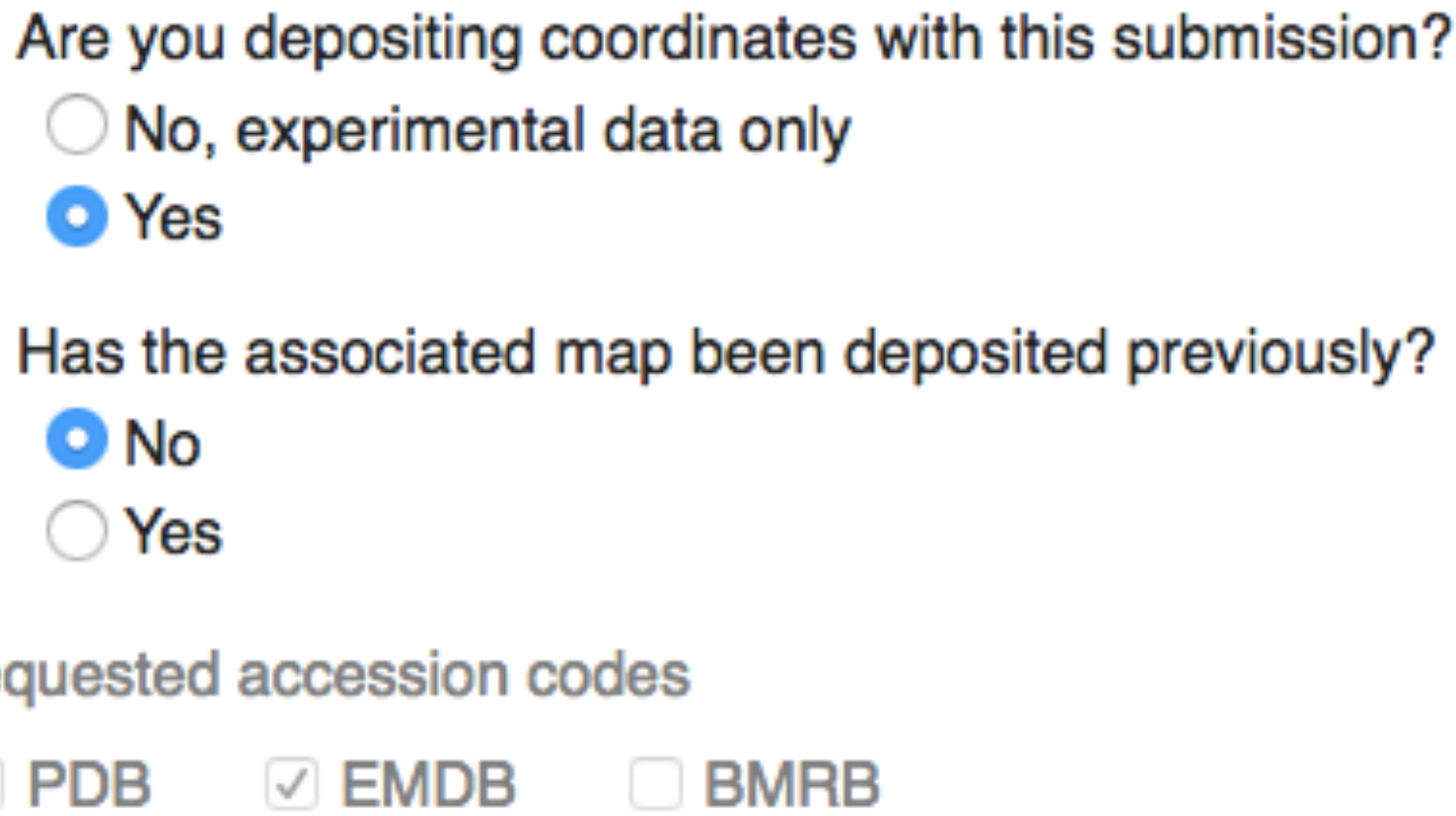
3DEM Deposition: IDs

http://deposit.wwpdb.org/deposition/



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File uploads: 3DEM map/model submission in OneDep

✓Select file type...

0) Coordinates

- Coordinates (mmCIF format)
- Coordinates (PDB format)

1) Main map (mandatory)

- EM map (MRC/CCP4 format)

2) Image for EMDB (mandatory)

- Entry image for public display

3) Additional maps

- Additional EM map (MRC/CCP4 format)

4) Masks

- EM mask (MRC/CCP4 format)

5) Half (even-odd) maps

- EM half map (MRC/CCP4 format)

6) Structure Factors

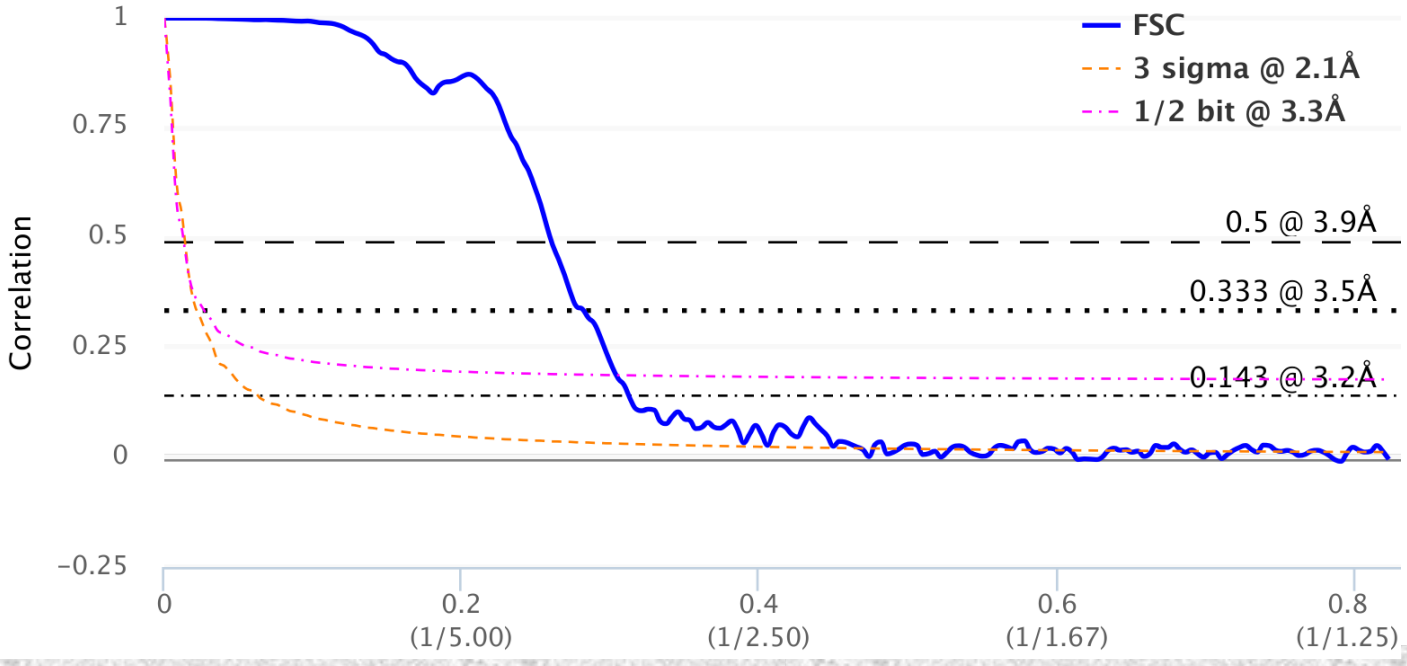
- mmCIF (structure factors)
- MTZ

Other Files

- FSC file (XML format)
- Ligand Image

FSC Curve Upload

- Create xml format file using a software package (e.g., Relion, EMAN)
- Use PDBe's Server: PDBe.org/FSC



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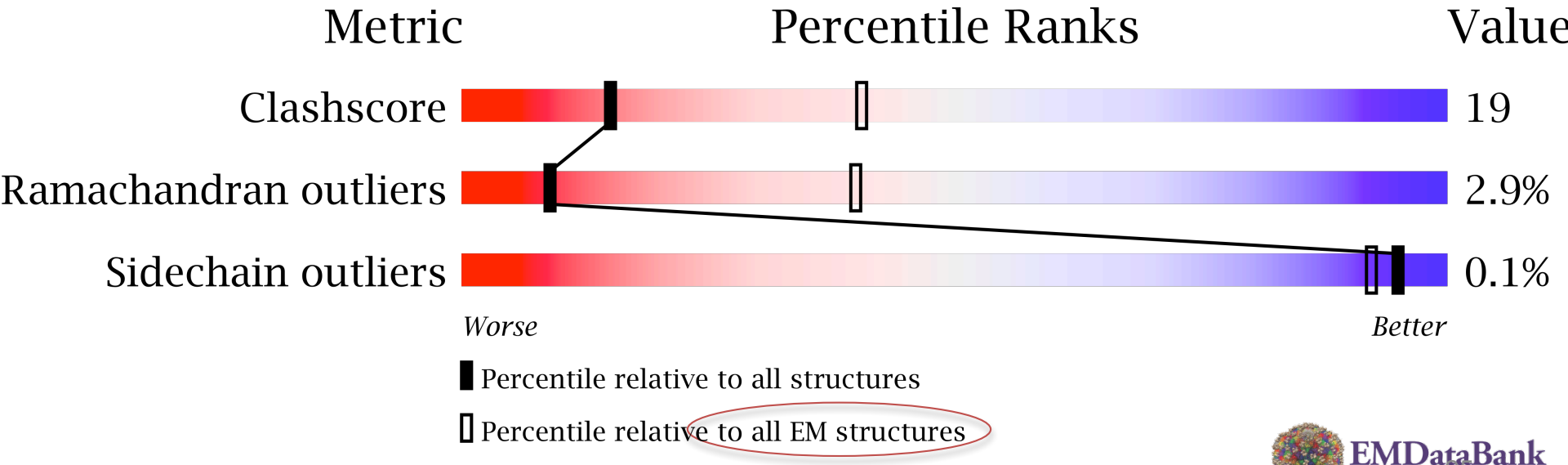
EM Validation Report

- "Table 1"
- Metrics of the EM model

Property	Value	Source
Reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	I	Depositor
Number of images	30000	Depositor
Resolution determination method	FSC 0.143	Depositor
CTF correction method	Not provided	Depositor
Microscope	JEOL 3200FSC	Depositor
Voltage (kV)	300	Depositor
Electron dose ($e^-/^2$)	Not provided	Depositor
Minimum defocus (nm)	500	Depositor
Maximum defocus (nm)	2000	Depositor
Magnification	50000	Depositor
Image detector	DIRECT ELECTRON DE-12 (4k x 3k)	Depositor



EM Validation Report: Proposed additions

- Table of Map Parameters
- Map images
- Plots
- Model to map fit
- Example: http://www.ebi.ac.uk/pdbe/entry/emdb/EMD-2984/analysis

Project Team

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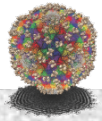
Eduardo Sanz Garcia
Ingvar Lagerstedt

Supported by NIH National Institute of General Medical Sciences



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