Situation, utilization and visualization of cryo-EM structure data

クライオ電子顕微鏡データの現状、見方、使い方

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BSJ2017 luncheon seminar
2017-09-21, Kumamoto univ.

"The Resolution Revolution"

Werner Kühlbrandt, *Science,* 2014

"*Liberty Leading the People*"
painted by Eugène Delacroix

Two viewpoints

Cryo-EM (3DEM) data in databanks

1. **Comparison to crystallography**
   Methodology outline
   [raw data] → [map] → [atomic model]

2. **Comparison to NMR**
   Ensemble structure of molecule in solution

Background:

Cryo-EM data in databanks

Yearly statistics of EMDB & PDB deposition

Resolution

Year

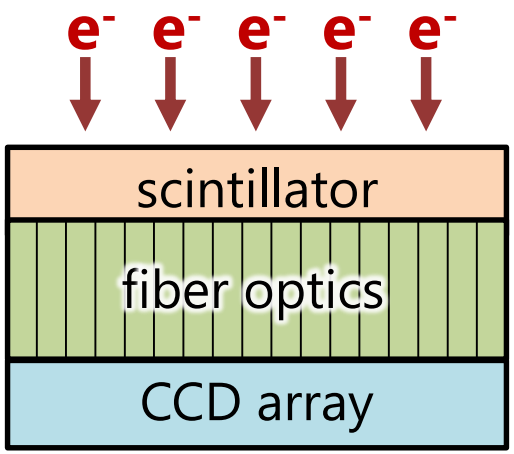
Submission Year	~ 2 A	~ 3 A	~ 5 A	~ 7 A	~ 10 A	~ 15 A	~ 23 A	~ 34 A	~ 51 A	~ 77 A	~ 115 A	Total
2017	1	24	433	122	106	38	46	69	19	5	1	900
2016	20	34	**668**	210	235	90	153	100	46	3	8	1597
2015	4	21	333	70	127	81	128	83	43	7	3	973
2014	1	4	105	55	145	71	143	139	57	76	2	817
2013		1	45	41	107	94	144	96	70	19		653
2012			18	31	87	63	121	75	58	9		493
2011			10	11	80	78	89	46	13	9	3	355
2010		1	23	23	59	69	53	54	16		3	327
2009		1	16	13	43	46	43	21	5			198
2008			8	8	39	36	52	36	15	1		214
2007			3	3	46	37	40	26	12	1		175
2006			1		23	37	44	38	7	2		156
2005	1		1		20	26	34	20	1	2		106
2004		1	2	3	11	23	16	3			1	60
2003			3	1	9	21	10	26	3			75
2002					4	18	7	3	1	11		45

Number: EMDB & PDB entries

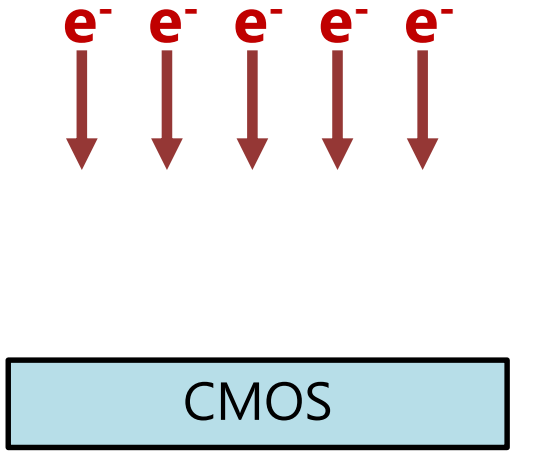
EM Navigator statistics

https://pdbj.org/emnavi/

Direct electron detector

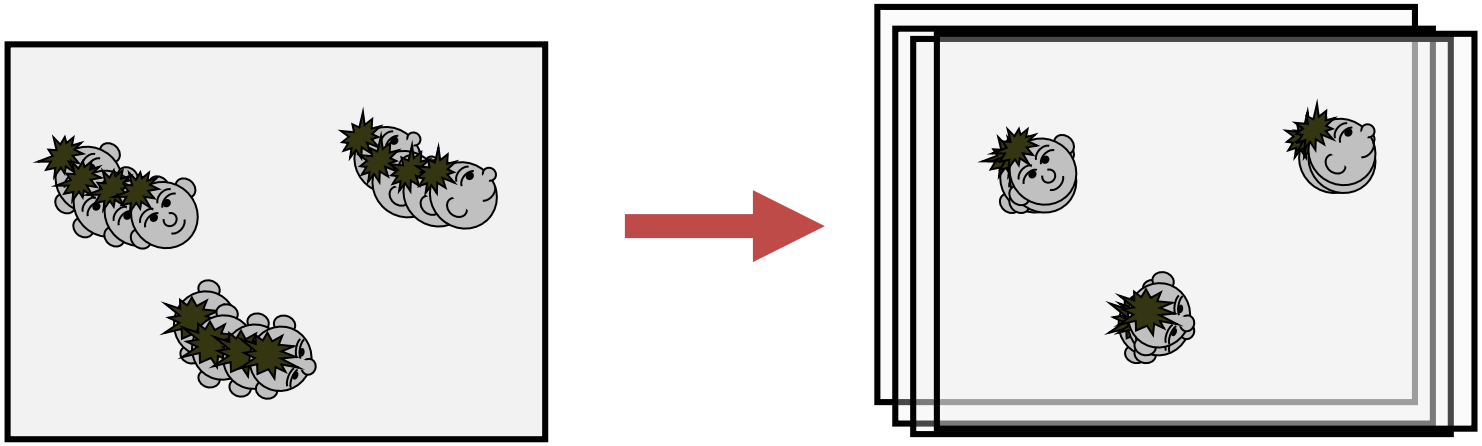


Legacy CCD camera



Direct electron detector

➔ Resolution, sensitivity, & speed improved



Micrographs as movie

motion correction

High resolution detectors

Resolution

Detector

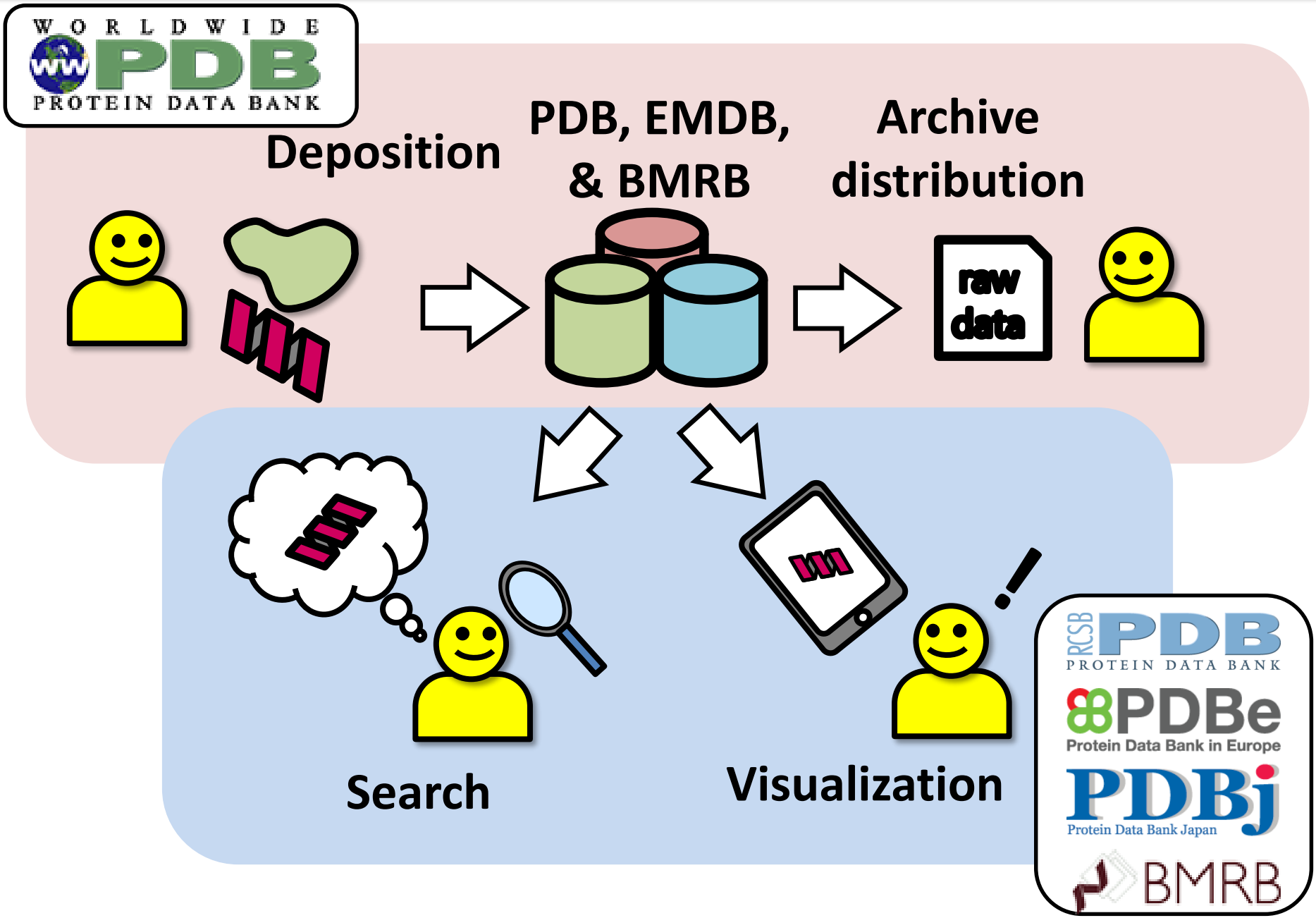
Detector	~ 2 A	~ 3 A	~ 5 A	~ 7 A	~ 10 A	~ 15 A	~ 23 A	~ 34 A	~ 51 A
GATAN K2		27	**507**	160	171	36	21	34	35
FEI FALCON II		9	**493**	130	96	58	43	20	5
GATAN K2 SUMMIT		14	372	89	82	22	12	13	3
KODAK SO163 FILM		4	98	76	367	353	318	204	36
GATAN K2 QUANTUM	2	3	45	8	53	4	10	6	2
OTHER		6	43	9	20	10	6	20	4
GATAN ULTRASCAN 4000			31	37	112	133	241	116	60
DIRECT ELECTRON DE-12			12	6	7		2	1	1
GATAN GENERIC	1	3	12	7	26	33	56	38	16
FEI FALCON I		2	10	18	14	6	5	2	9
n/a	1		10	3	38	36	82	50	32
GENERIC FILM			8	2	6	4	17	4	
DIRECT ELECTRON DE-20		4	6		2	1	2	2	
GENERIC CCD		2	5	4	6	18	32	37	18
TIETZ TEMCAM F415			5	7	30	28	38	40	22

Number: EMDB & PDB entries

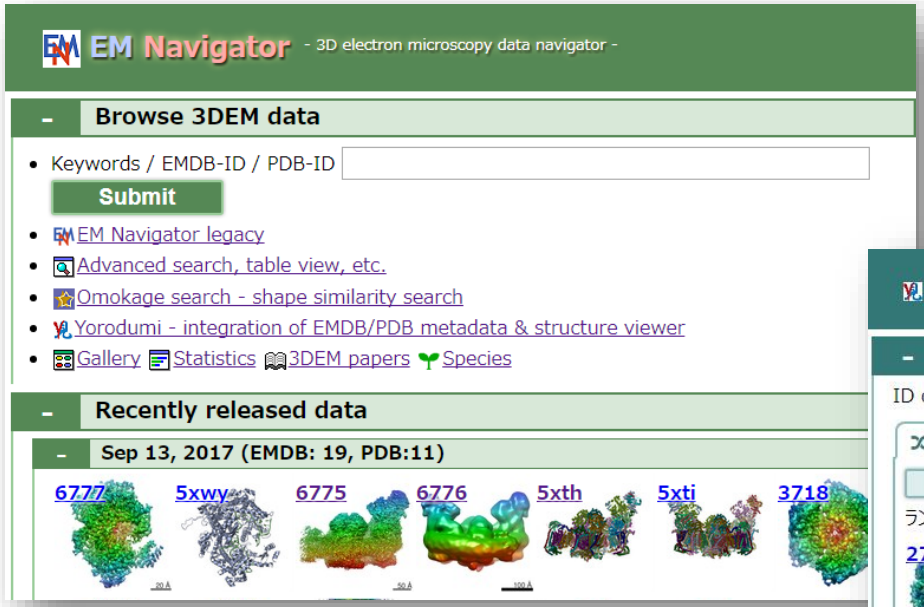
EM Navigator statistics

https://pdbj.org/emnavi/

wwPDB collaboration

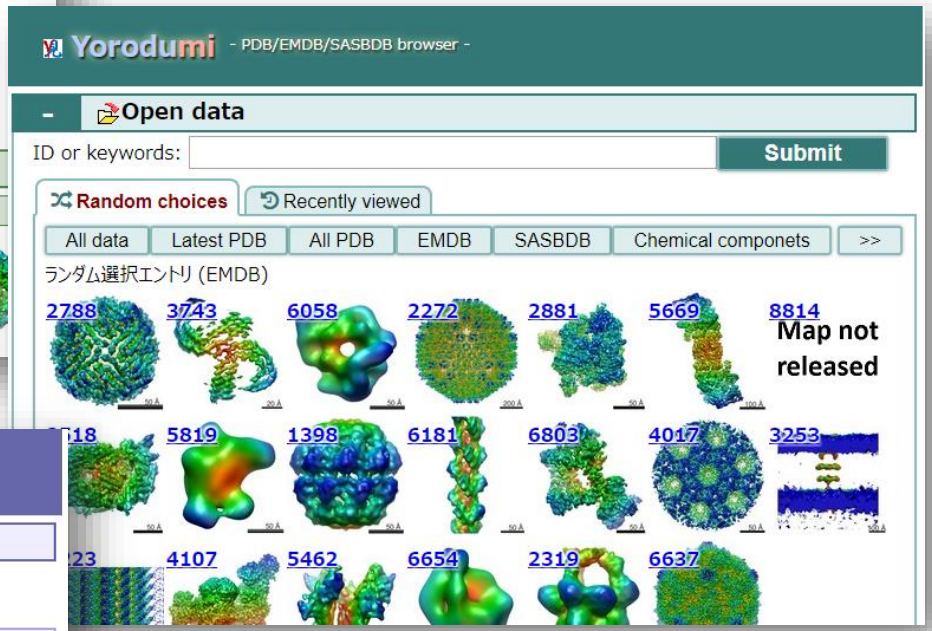


EM Navigator *et al.*

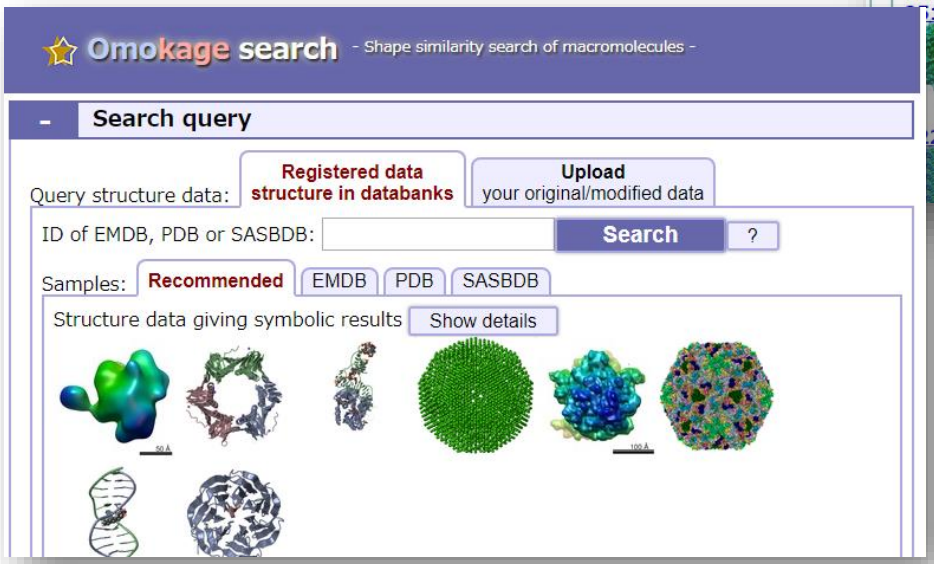


EM Navigator

https://pdbj.org/emnavi/



Yorodumi



Omokage search

PDBx version 5

WORLDWIDE PDB
PROTEIN DATA BANK

03/22/2017

Archival PDBx/mmCIF Version V4 to V5 Update in the Protein Data Bank

The wwPDB is preparing the update of PDBx/mmCIF model files for all entries in the PDB archive to V5 version of the PDBx/mmCIF dictionary. When completed, all PDB model files will have better organized content and will conform to the revised data model used within the wwPDB OneDep System. A list of changes will be available at the wwPDB website (https://www.wwpdb.org/documentation/remediation). Since January

News in wwPDB website
www.wwpdb.org

Comparison

to crystallography

Similarity in methods

		Crystallography	3DEM
Raw data	Aligned?	Yes	**No**
	Phase?	**No**	Yes
↓		Initial model/phase required	Initial map can help
	Density map	**Refine**	**Refine**
↓			Initial model required for low-res. data
	Atomic model		**Refine**

EM data in *Yorodumi* (万見)

PHENIX v2411 is used for **refinement**

Processing

Software	Name: PHENIX / Version: dev_2411: / Classification: refinement
CTF correction	Type: PHASE FLIPPING AND AMPLITUDE CORRECTION
3D reconstruction	Resolution: 3.2 Å / Resolution method: FSC 0.143 CUT-OFF / Number of particles: 238758 / Symmetry type: POINT

Refine

Refine ID	Highest resolution
1	3.20
ELECTRON MICROSCOPY	

Refine LS restraints

Refine ID	Type	Dev ideal	Number
ELECTRON MICROSCOPY	f_bond_d	0.010	10528
ELECTRON MICROSCOPY	f_angle_d	1.201	14384
ELECTRON MICROSCOPY	f_dihedral_angle_d	11.771	8615
ELECTRON MICROSCOPY	f_chiral_restr	0.063	1619
ELECTRON MICROSCOPY	f_plane_restr	0.005	1642

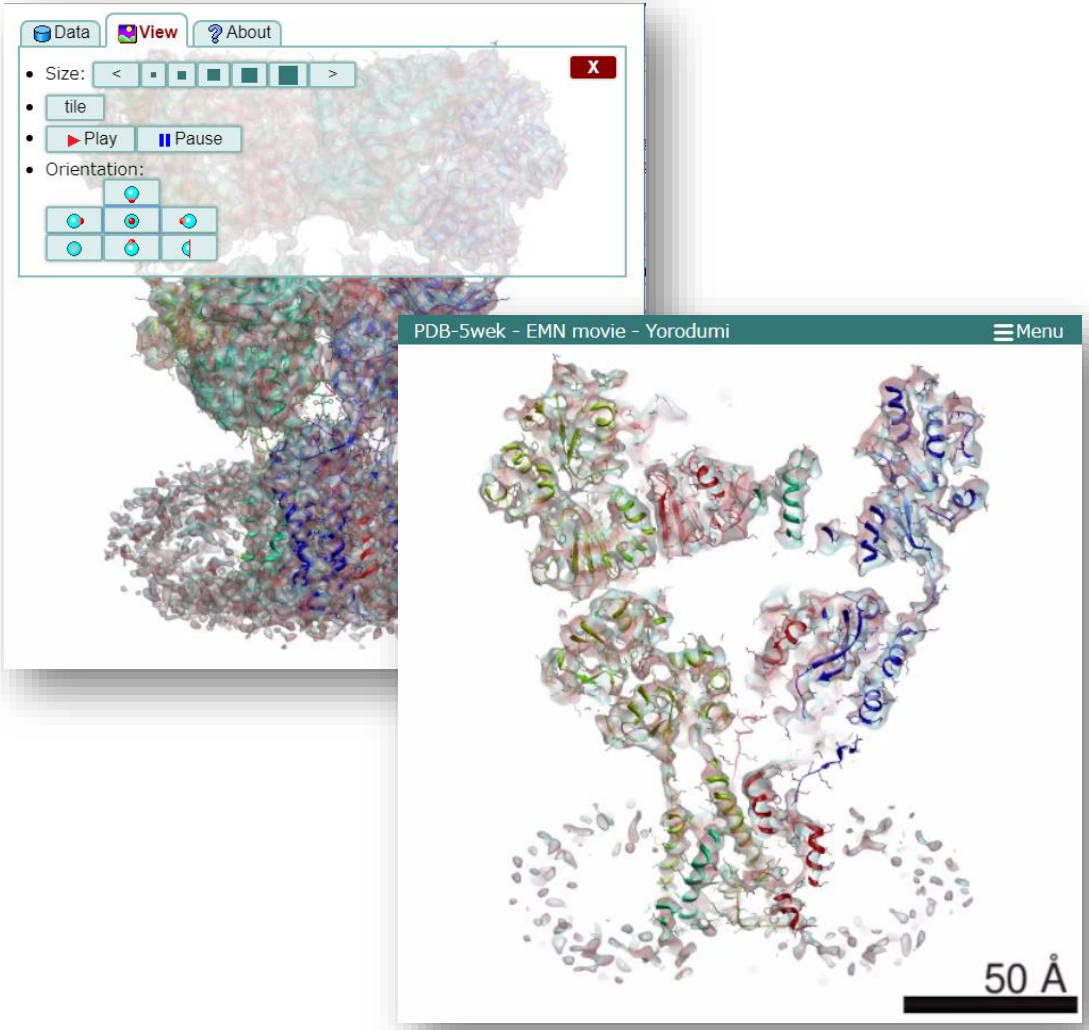
Restraint parameters during refinement

Screenshot of *Yorodumi*
data: PDB-5ool

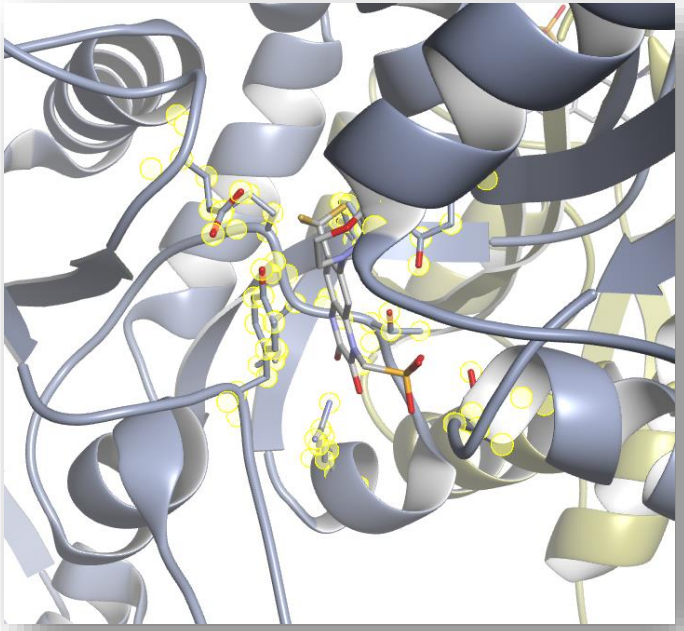
Difference in methods

		Crystallography	3DEM
Raw data	Aligned?	Yes	No
	Phase?	No	Yes
↓		Initial model/phase required	Initial map can help
	Density map	Refine	Refine
↓			Initial model required for low-res. data
	Atomic model	Refine	Refine

Map + model view of EM data



Map-model fitting as movie



Yorodumi-Molmil

Ligand binding site focus view

EMDB-8819

PDB-5wek

New PDB data in Yorodumi

Buffer solution	pH: 8
Buffer component	

ID	Conc.	Units	Name	Formula	Buffer ID
1	150	mM	sodium chloride	NaCl	1
2	20	mM	Tris	C4H11NO3	1
3	0.1	% w/v	digitonin	digitonin	1

Buffer solution details

EM software

ID	Name	Category	Imaging ID	Fitting ID	Image processing ID
2	SerialEM	IMAGE ACQUISITION	1		
7	COOT	MODEL FITTING		1	
8	Chimera	MODEL FITTING		1	
11	PHENIX	MODEL REFINEMENT		1	
15	Relion	RECONSTRUCTION			1

Software details

PDB-5vot

Databanks for each

	Crystallography	3DEM (incl. electron crystallography)
Raw data	-	**EMPIAR**
Density map	**PDB** (structure factor)	**EMDB**
Atomic model	**PDB**	

EM Navigator

Databank for EM raw images

EMBL-EBI | Services | Research | Training | About us

EMPIAR

Electron Microscopy Public Image Archive

EMPIAR home | Deposition | REST API | FAQ | About EMPIAR | Share | Feedback

EMPIAR, the Electron Microscopy Public Image Archive, is a public resource for raw, 2D electron microscopy images. Here, you can browse, upload, and download and reprocess the thousands of raw, 2D images used to build a 3D structure. More ...

Deposit your data in EMPIAR to share it with the structural biology community.

Browse and download EMPIAR datasets using the table below.

Show 50 entries Search:

Dataset	Title	Authors	Related EMDB/PDB entries
EMPIAR-10115	Tilt-series of e. coli carrying the ple7 plasmid	Swulius MT, Jensen GJ [Pubmed: 22904287] [DOI: 10.1128/jb.00505-12]	

Quick links

- EMDB
- PDBe
- EMDataBank
- EMPIAR: Quick tour

EMPIAR citations

Quantitative analysis of 3D alignment quality: its impact on soft-validation, particle pruning and

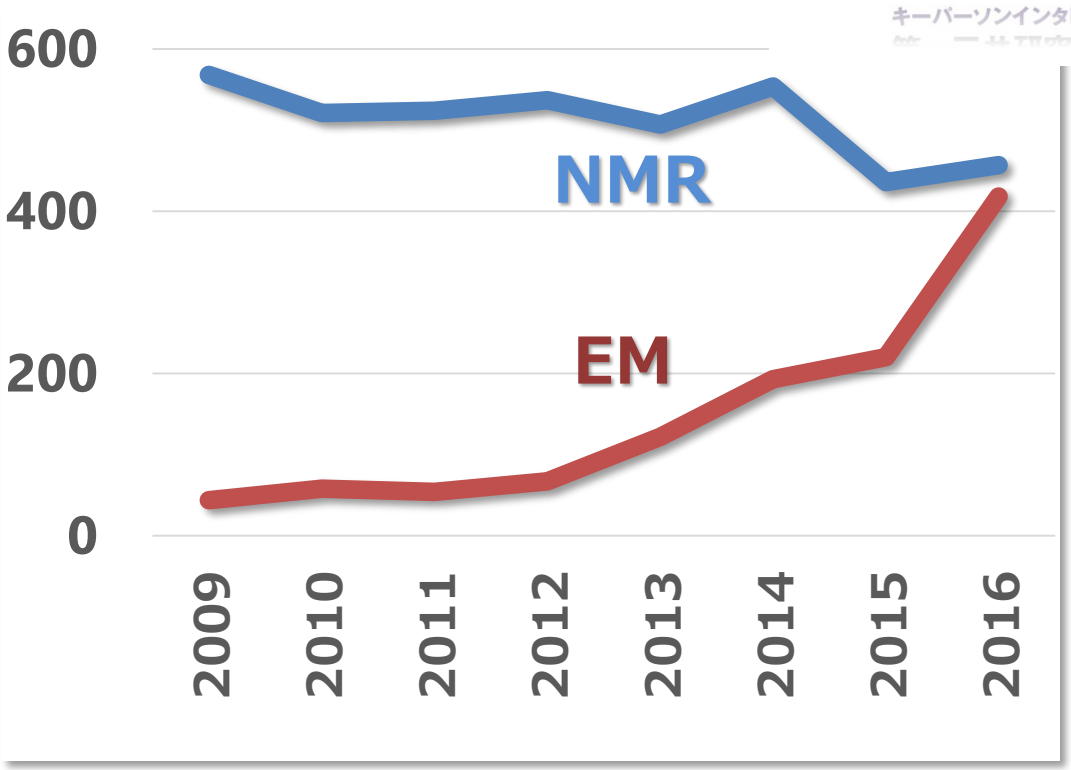
EMPIAR webpage by EMBL-EBI

Comparison

to NMR

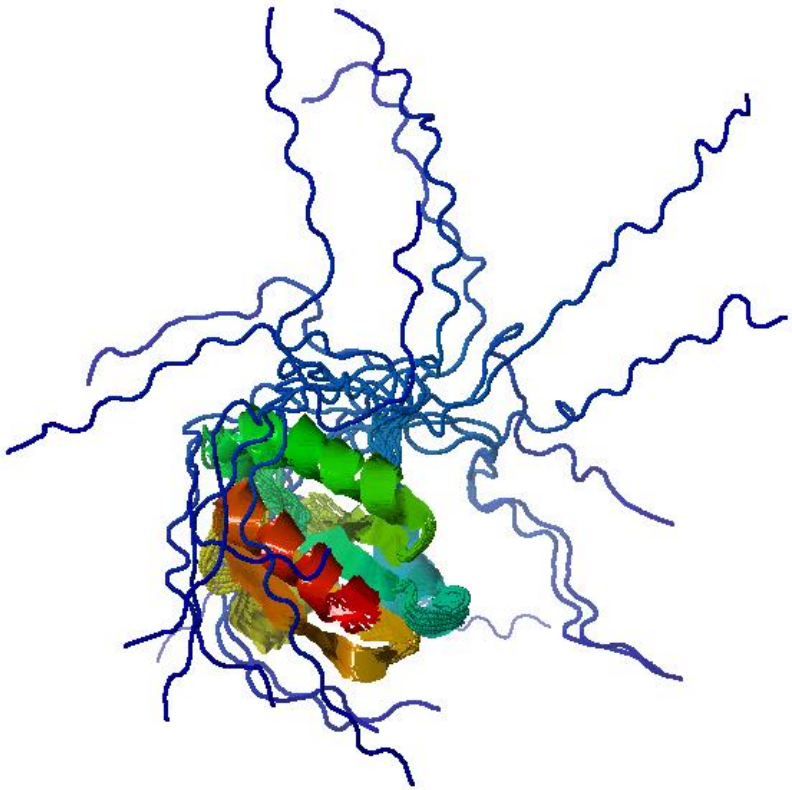
PDB deposition NMR vs. 3DEM

Nikkei biotech
cryo-EM issue
Feb, 2007



PDB yearly deposition
NMR vs. EM

Structure ensembles by NMR



Experiment

1 sample condition

100 conformers

Deposition

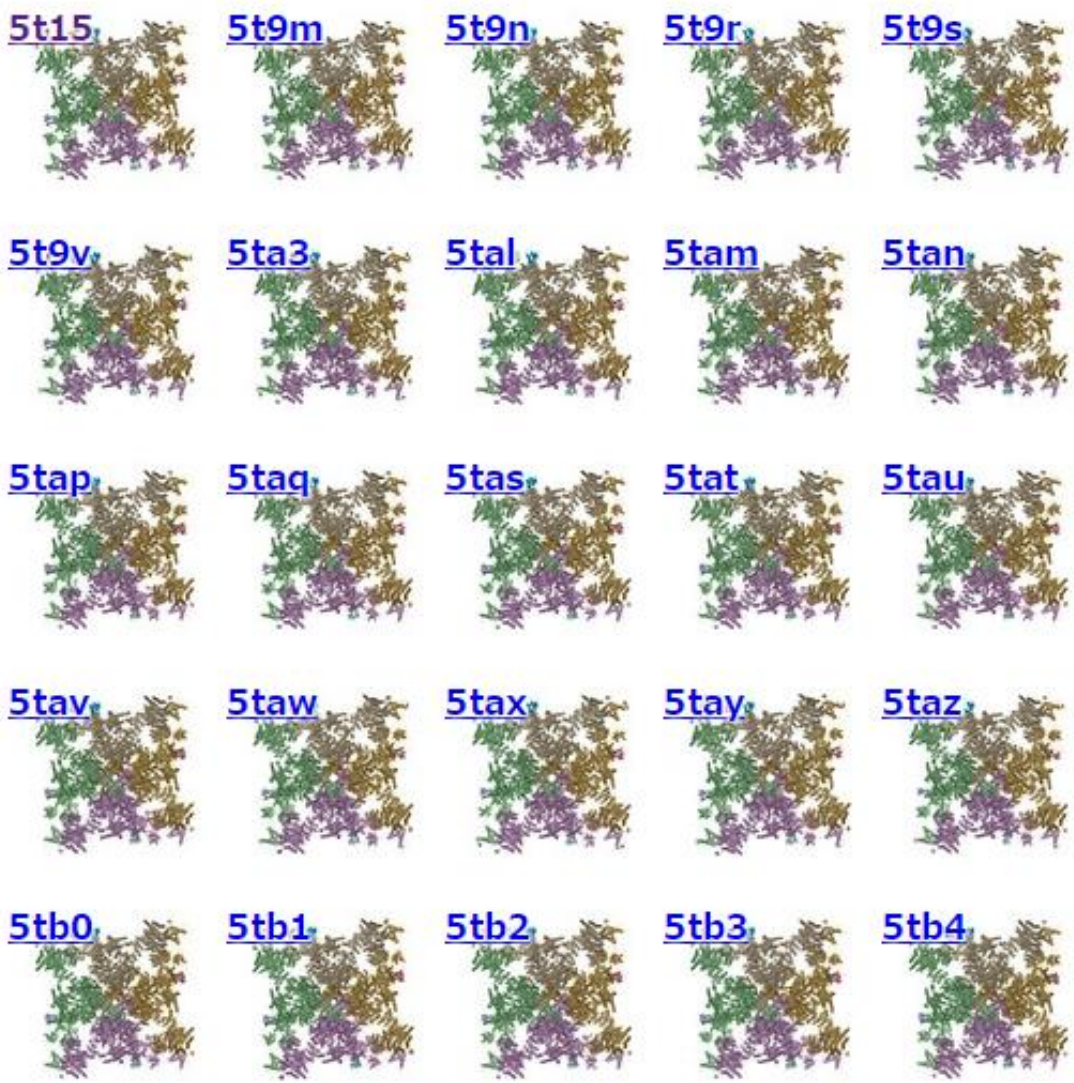
20 conformers

1 entry

PDB-6ali

putative thioredoxin

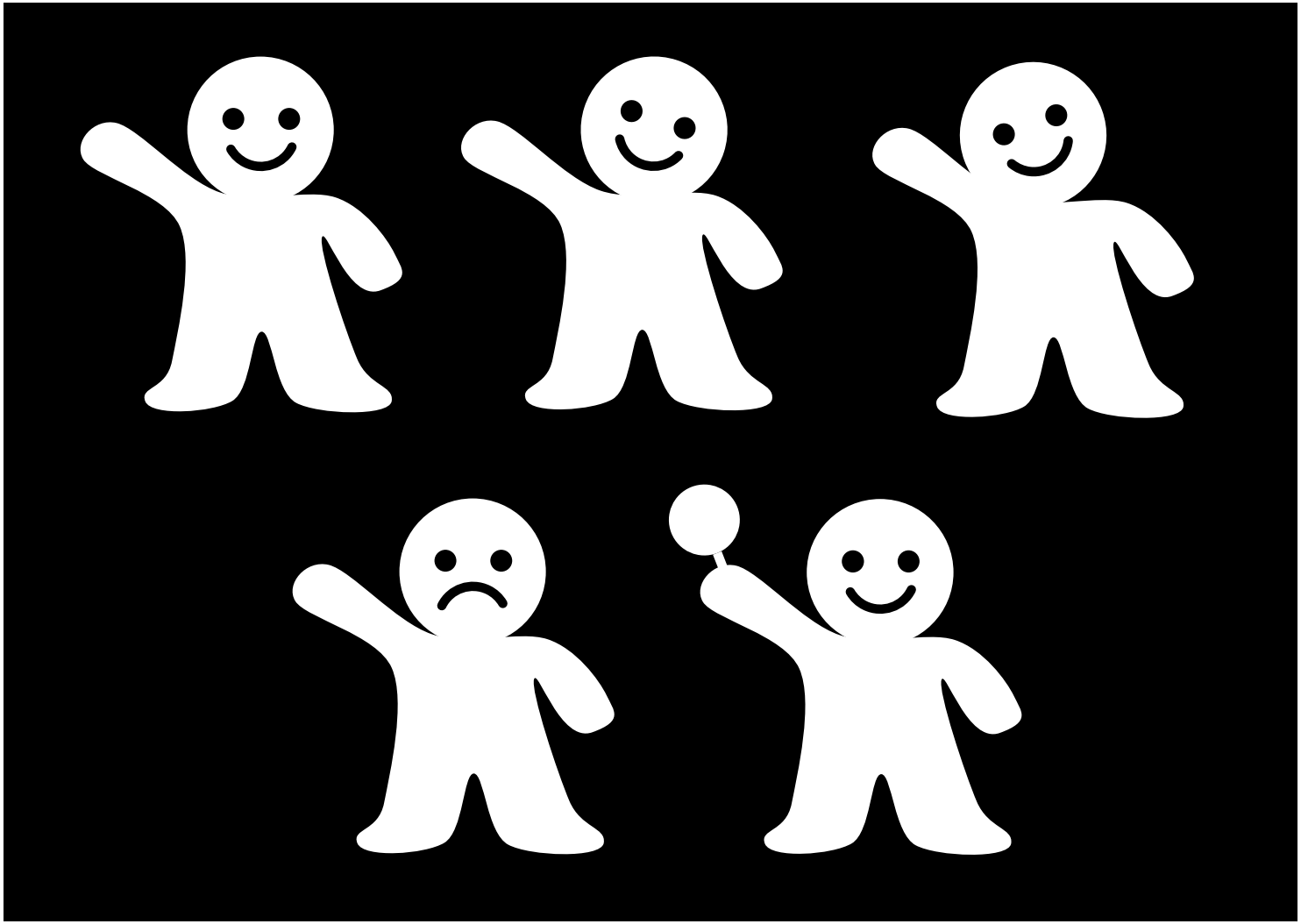
Structure ensembles by cryo-EM



5 samples
x (1 all + 4 classes)
= 25 PDB entries

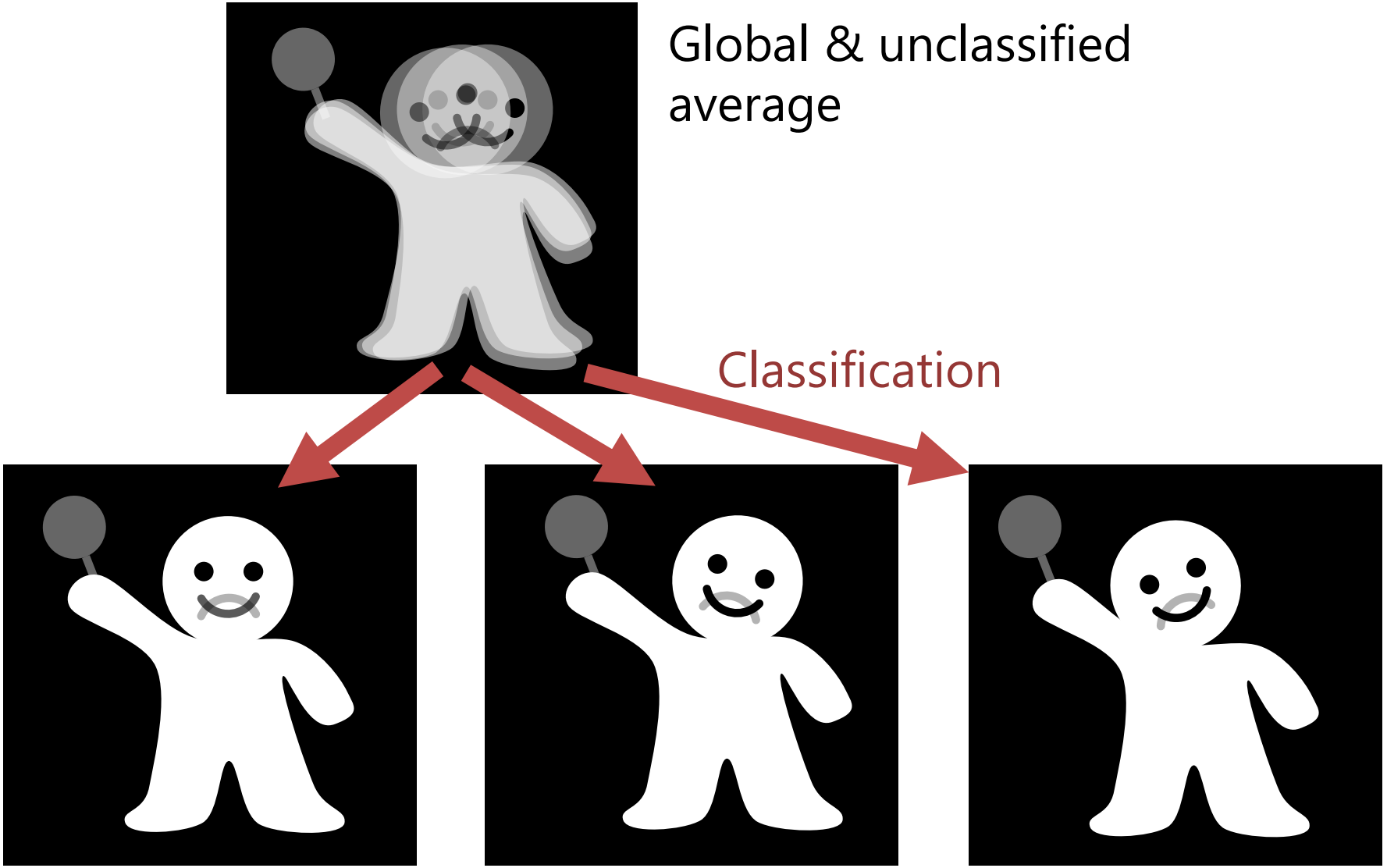
Ryanodine receptor (calcium channel) des Georges *et al.*, *Cell*, 2016

Flexibility in biomolecule

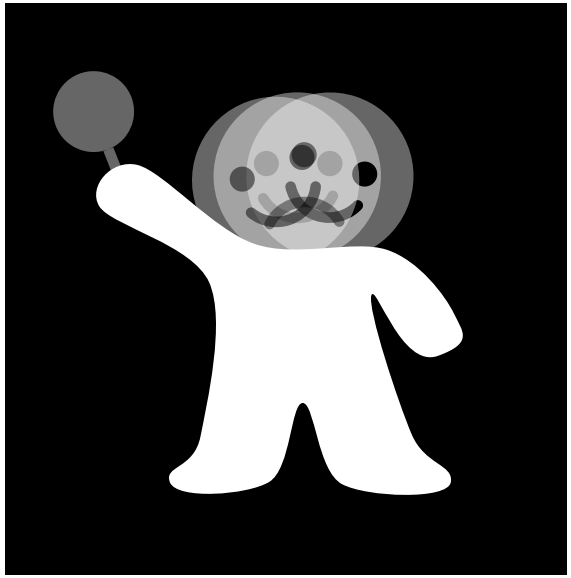


flexibility, heterogeneity & inoccupancy
in molecular assembly

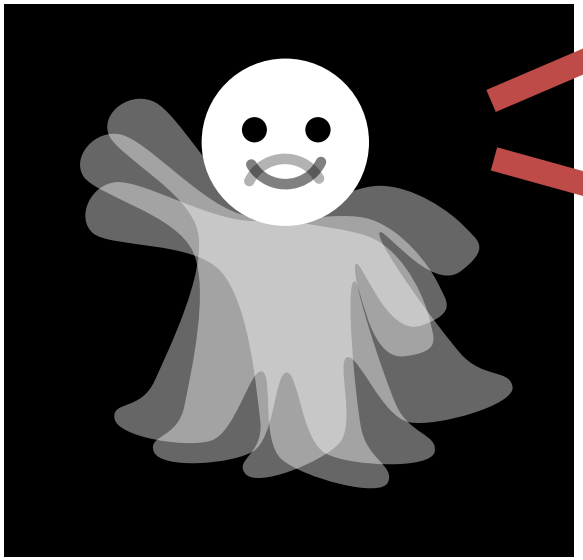
Classification



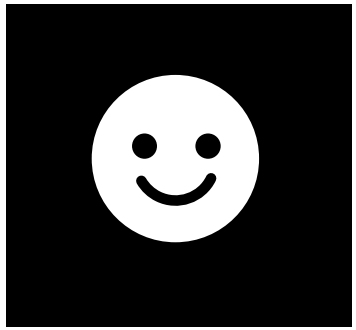
Local averaging & classification



Local (masked) average



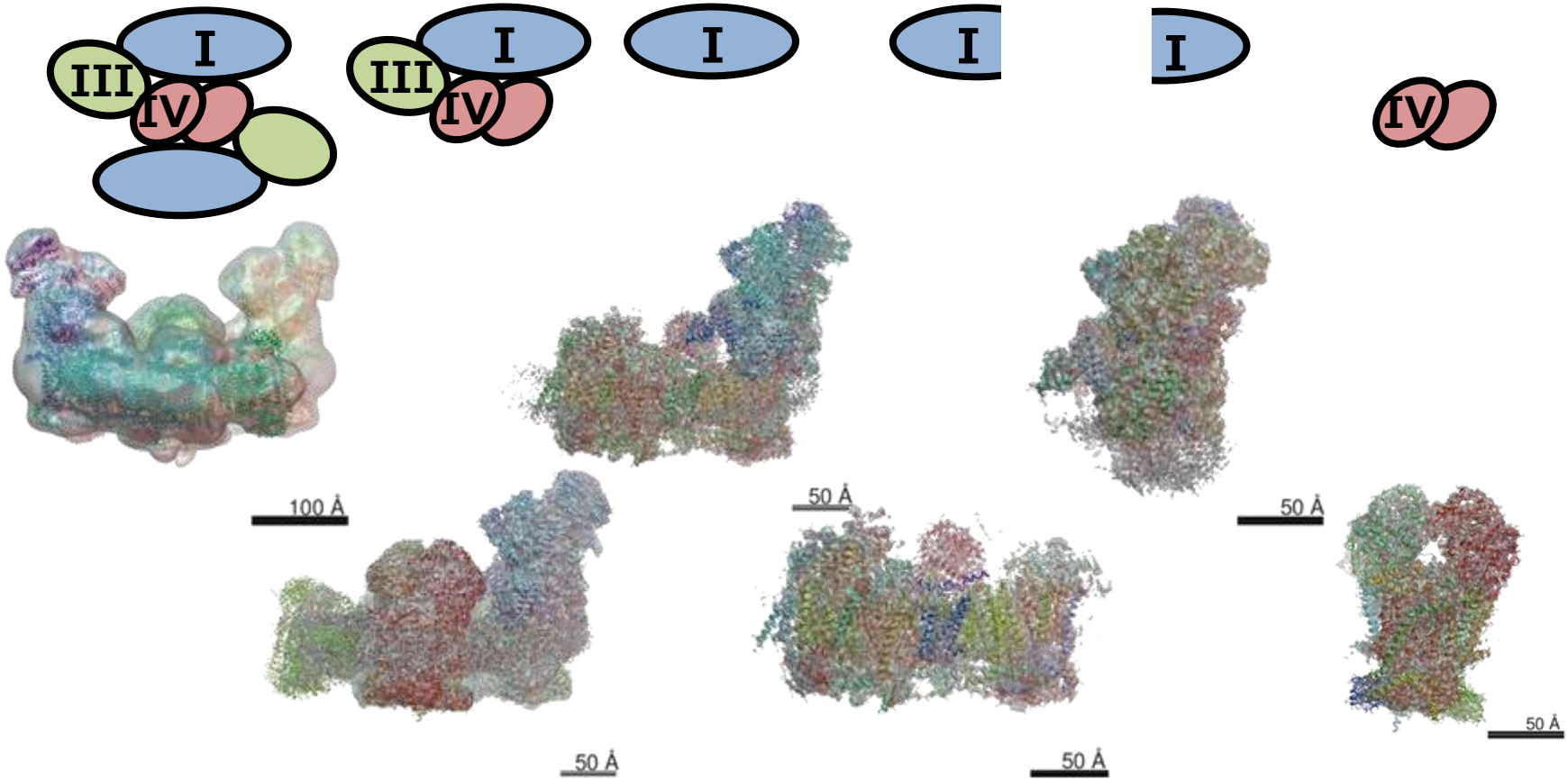
Classification



Local (masked) classification

Respiratory "megacomplex"

EMDB	6776	6776	6773	6772	6771	6774
PDB	5xti	5xth	5xtd	5xtc	5xtb	5xte
res. (Å)	17.4	3.9	3.7	3.7	3.4	3.4



Yorodumi Papers (万見文献)

Database of citation of structure data

Search result

- List of structure papers

Showing 1 - 20 of 281 items for **keywords**: respiratory

- Architecture of Human Mitochondrial Respiratory Megacomplex I2III2IV2.

Cell, Vol. 170, Issue 6, Page 1247-1257.e12, Year 2017
Runyu Guo / Shuai Zong / Meng Wu / Jinke Gu / Maojun Yang
Methods: EM (single particle)

6771 6772 6773 6774 5xtb 5xtc 5xtd 5xte

6775 6776 5xth 5xti

- Immunogenicity and structures of a rationally designed prefusion M

Proc. Natl. Acad. Sci. U.S.A., Vol. 114, Issue 35, Page E7348-E7357, Year 2
Jesper Pallesen / Nianshuang Wang / Kizzmekia S Corbett / Daniel Wrapp / Hannah L Turner / Christopher A Cottrell / Michelle M Becker / Lingshu Wang / Erica L Andres / Arminja N Kettenbach / Mark R Denison / James D Chappell / B Ward / Jason S McLellan
Methods: EM (single particle) / X-ray diffraction

8783 8784 8785 8787 8788 8789

Yorodumi Papers - Database of articles cited by EMDB/PDB/SASBDB data -

+ Search query

- Structure paper

Title	Architecture of Human Mitochondrial Respiratory Megacomplex I2III2
Journal, issue, pages	*Cell*, Vol. 170, Issue 6, Page 1247-1257.e12, Year 2017
Publish date	Aug 23, 2017
Authors	Runyu Guo / Shuai Zong / Meng Wu / Jinke Gu / Maojun Yang
External links	PubMed:28844695 / Publisher's page
Methods	EM (single particle)
Resolution	3.4 - 17.4 A

EMDB-6771: Movie | Structure viewer
Cryo-EM structure of human respiratory complex I matri

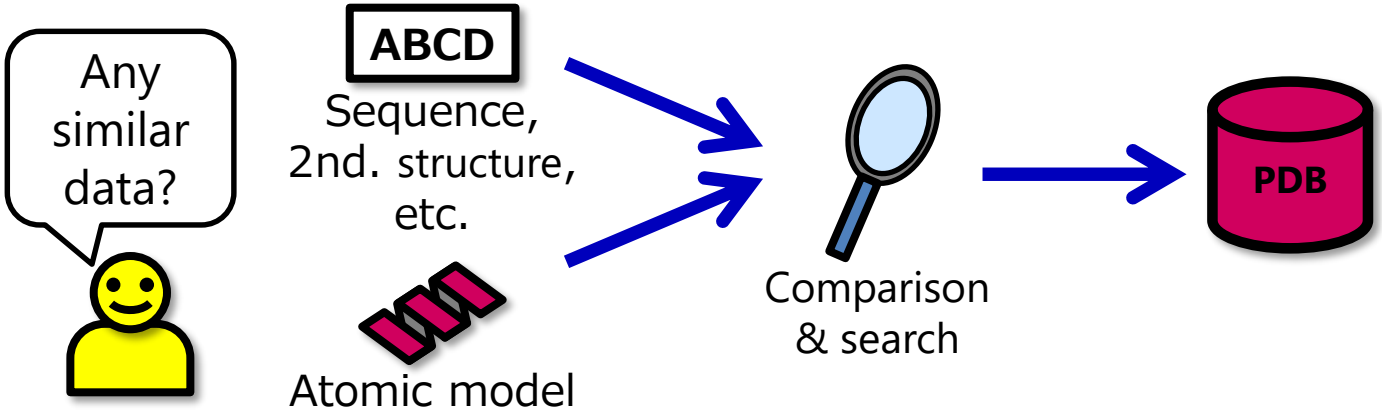
EMDB-6772: Movie | Structure viewer
Cryo-EM structure of human respiratory complex I trans

EMDB-6773: Movie | Structure viewer
Cryo-EM structure of human respiratory complex I

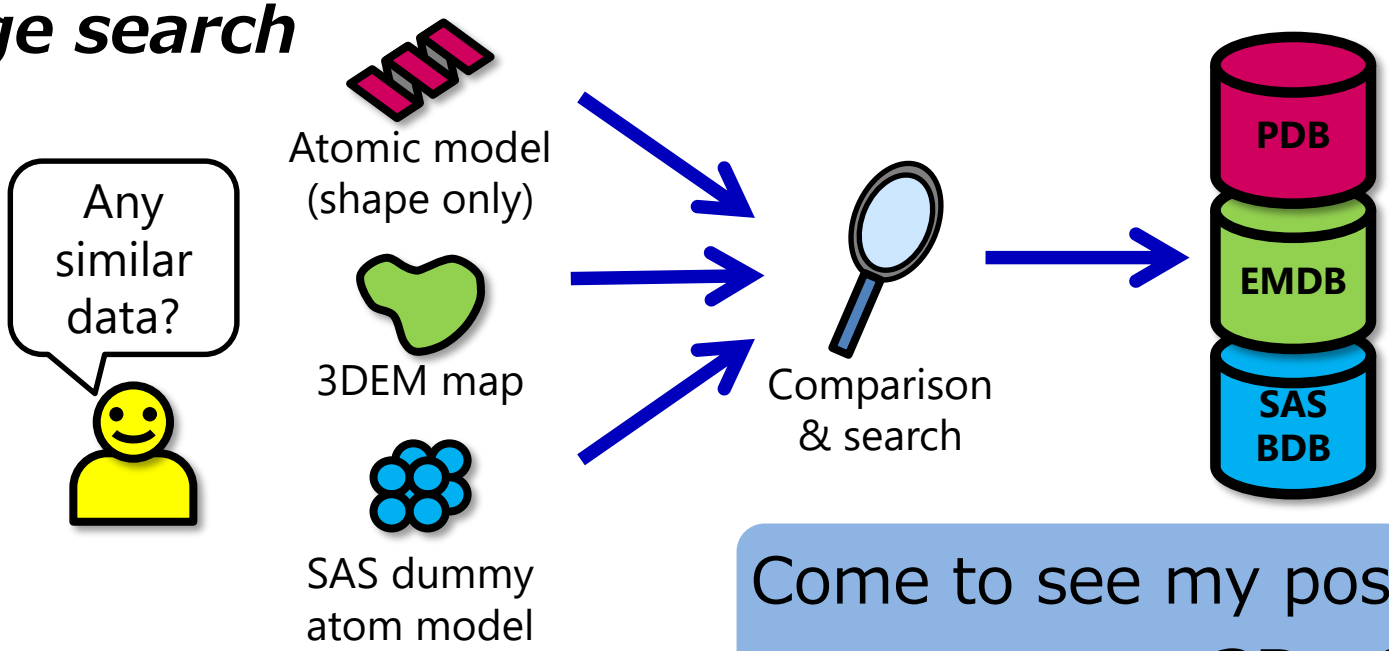
Details of data entries

Omokage search

Typical ones (*e.g. Structure Navigator* of PDBj)



Omokage search



Come to see my poster!

3Pos026

Enjoy cryo-EM data with PDBj services!

EM Navigator https://pdbj.org/emnavi/

Yorodumi https://pdbj.org/yorodumi/

Omokage search https://pdbj.org/omokage/