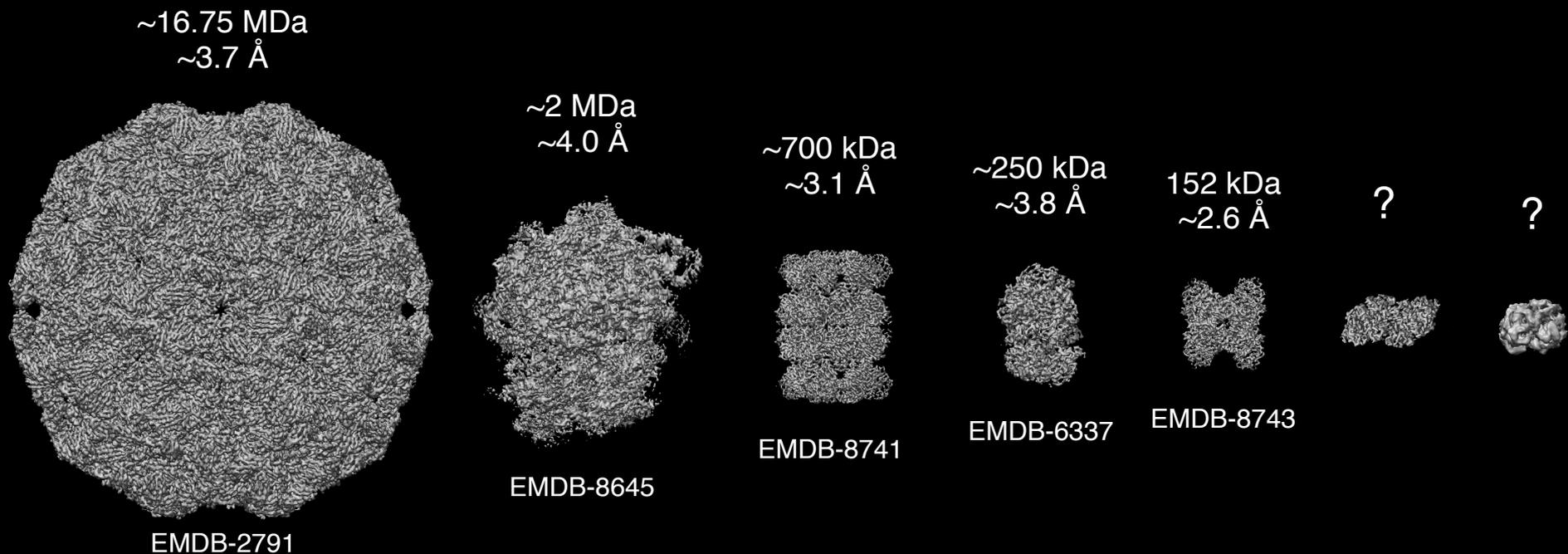


Exploring the Size and Resolution Limits of Conventional Single-Particle cryo-EM at 200 keV

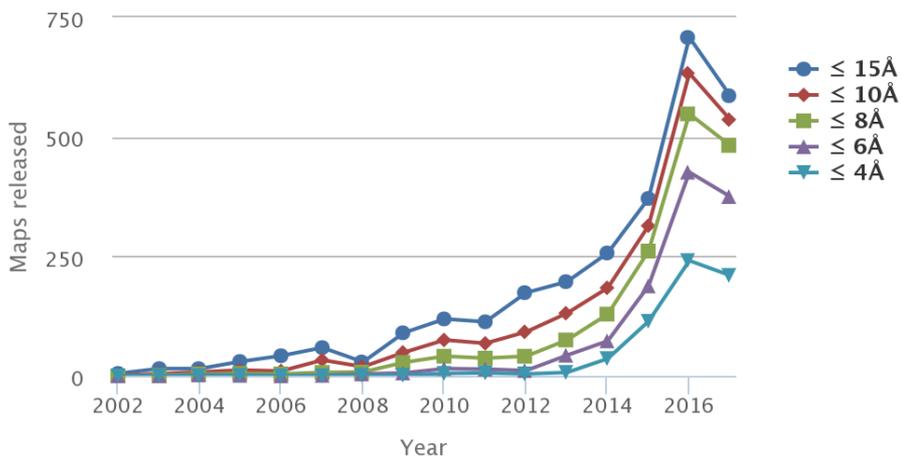


Mark Herzik

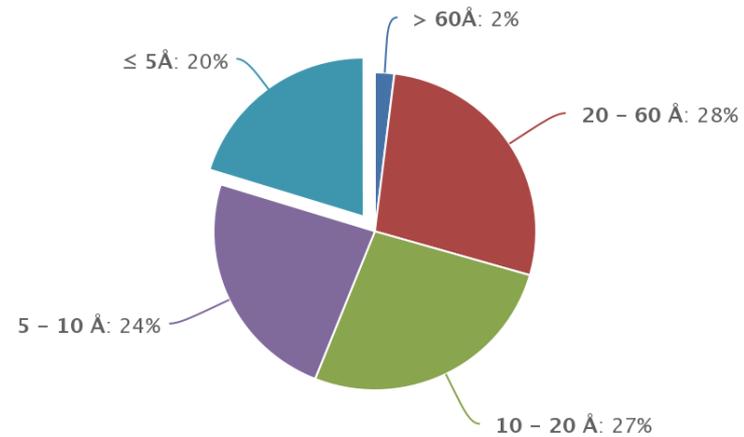
Laboratory of Dr. Gabriel Lander
The Scripps Research Institute

EM Map Resolution Distribution Across the EMDB

Maps achieving given resolution levels

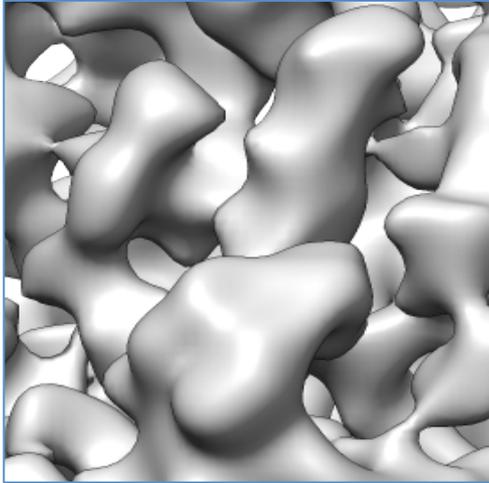


Resolution distribution for released maps

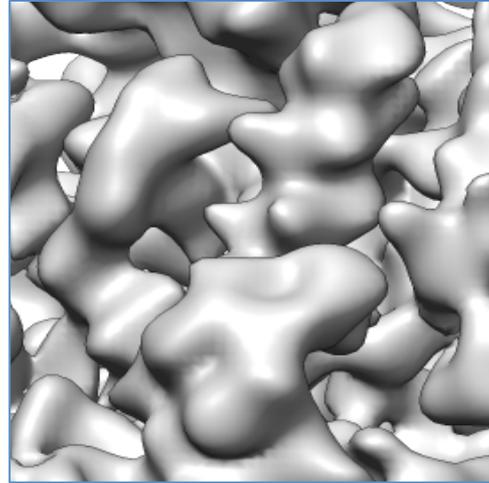


The Impact of ~ 0.5 Å Gain in Resolution – Why We Chase Higher-Resolution

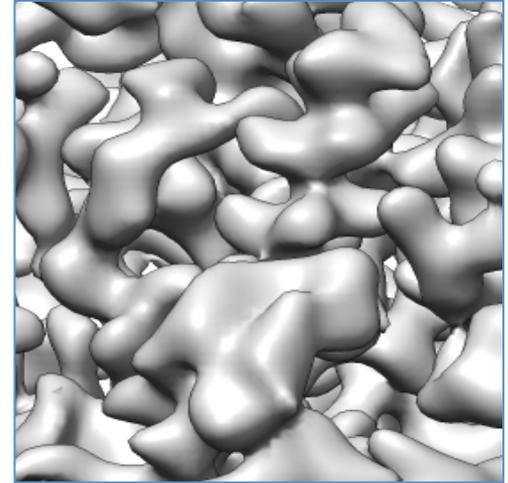
~ 4.9 Å



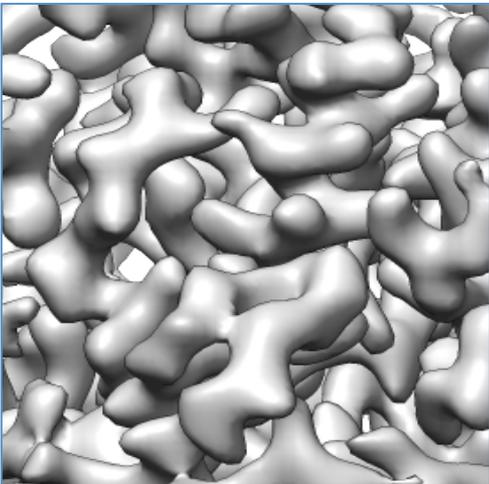
~ 4.4 Å



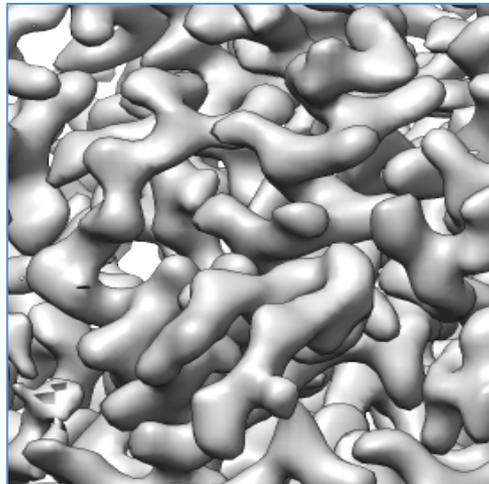
~ 3.9 Å



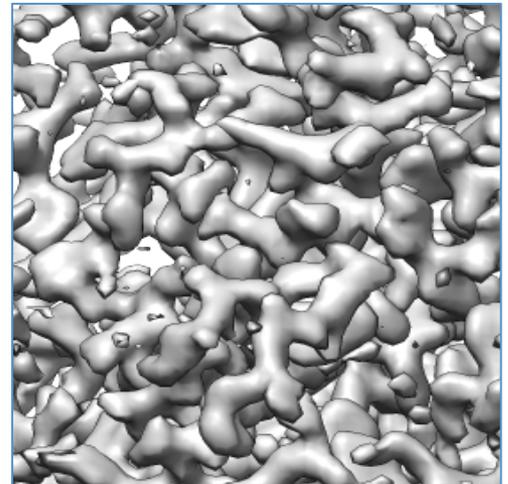
~ 3.5 Å



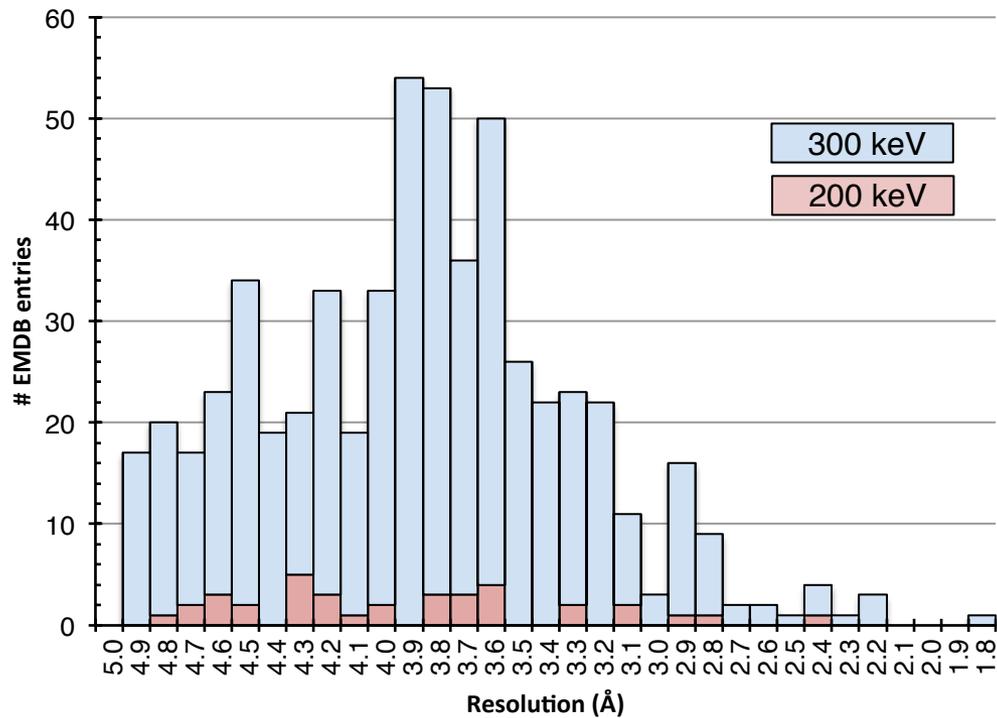
~ 3.0 Å



~ 2.2 Å



Structures in the EMDB Resolved to Better than 5 Å Resolution



>98% of EMDB entries better than 5 Å resolution have been imaged at 300 keV



[The Krios is the "go-to" microscope for high resolution. Why is this?]

- 3 Constant power condenser lenses
- Very stable optics
- Customization
- First microscope with autoloader



[The Krios is the "go-to" microscope for high resolution. Why is this? Is access to a Krios necessary for a lab to compete in the cryo-EM field?]

- 3 Constant power condenser lenses
- Very stable optics
- Customization
- First microscope with autoloader

- NO



[The Krios is the "go-to" microscope for high resolution. Why is this? Is access to a Krios necessary for a lab to compete in the cryo-EM field? Can conventional cryo-EM be used to solve small structures?]

- 3 Constant power condenser lenses
- Very stable optics
- Customization
- First microscope with autoloader
- NO
- YES!

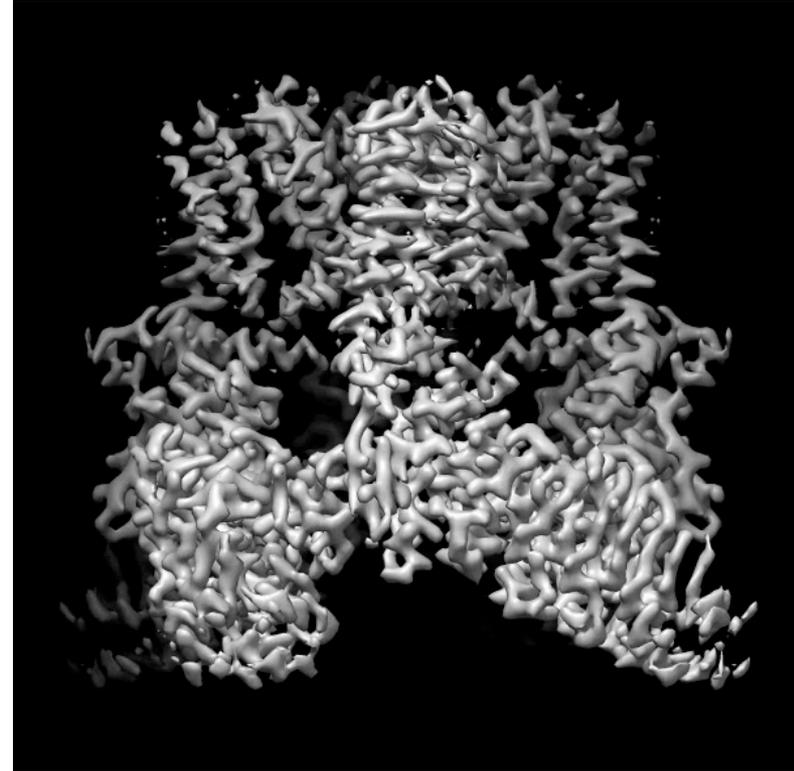


TSRI's 200 keV Workhorse – Base Model FEI/ThermoFisher Talos Arctica



No phase plate
No energy filter
K2 camera

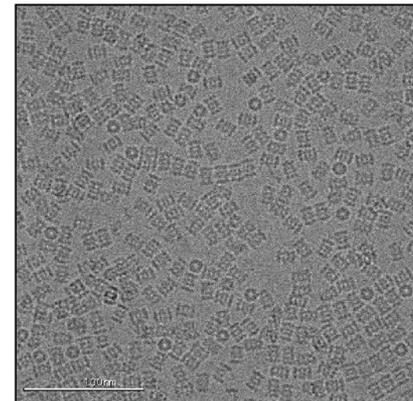
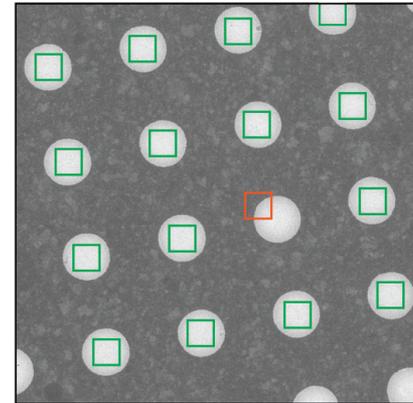
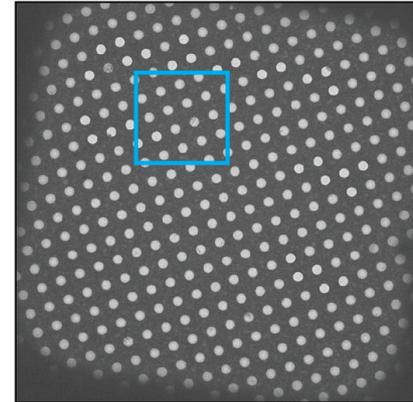
TSRI's 200 keV Workhorse – Base Model FEI/ThermoFisher Talos Arctica



TSRI's 200 keV Workhorse – Base Model FEI/ThermoFisher Talos Arctica



- Manually frozen
- Leginon
- Appion
- MotionCorr2
- CTFFIND4/gCTF
- RELION 1.4/2.0/2.1



NATURE METHODS | BRIEF COMMUNICATION



Achieving better-than-3-Å resolution by single-particle cryo-EM at 200 keV

Mark A Herzik Jr, Mengyu Wu & Gabriel C Lander

Affiliations | Contributions | Corresponding author

Nature Methods **14**, 1075–1078 (2017) | doi:10.1038/nmeth.4461

Received 22 May 2017 | Accepted 29 August 2017 | Published online 09 October 2017

PROTOCOL EXCHANGE | COMMUNITY CONTRIBUTED

Setting up the Talos Arctica electron microscope and Gatan K2 direct detector for high-resolution cryogenic single-particle data acquisition

Mark A. Herzik, Jr., Mengyu Wu & Gabriel C. Lander

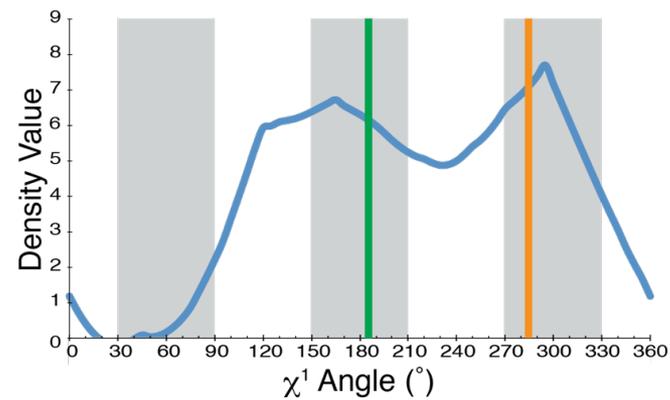
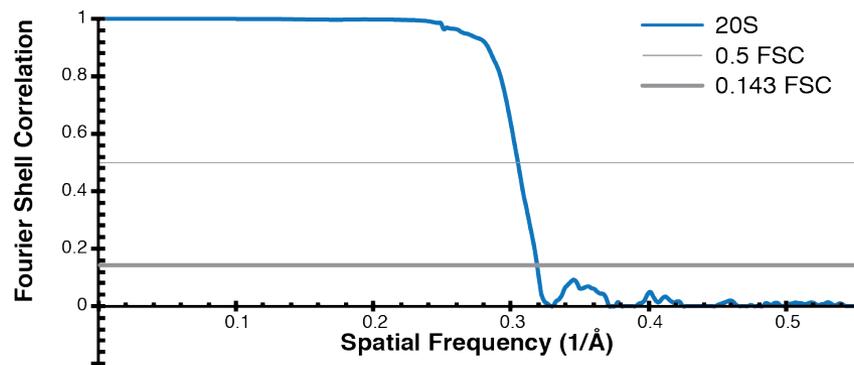
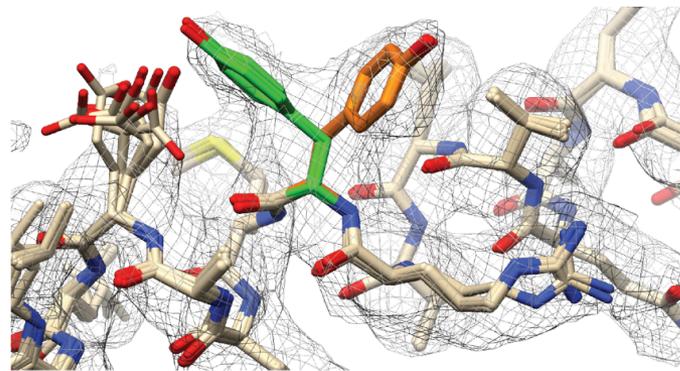
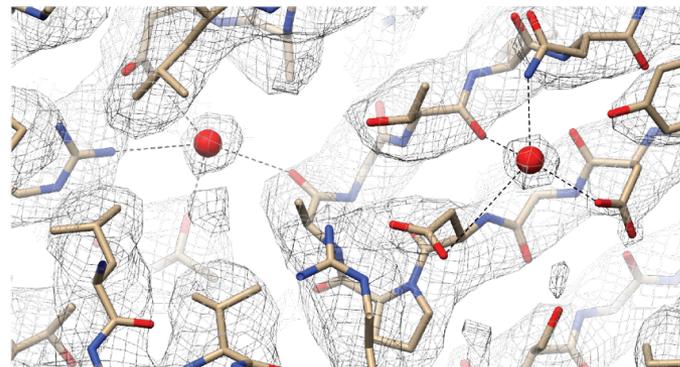
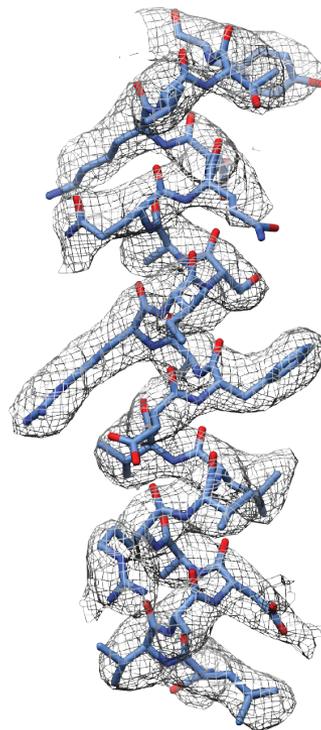
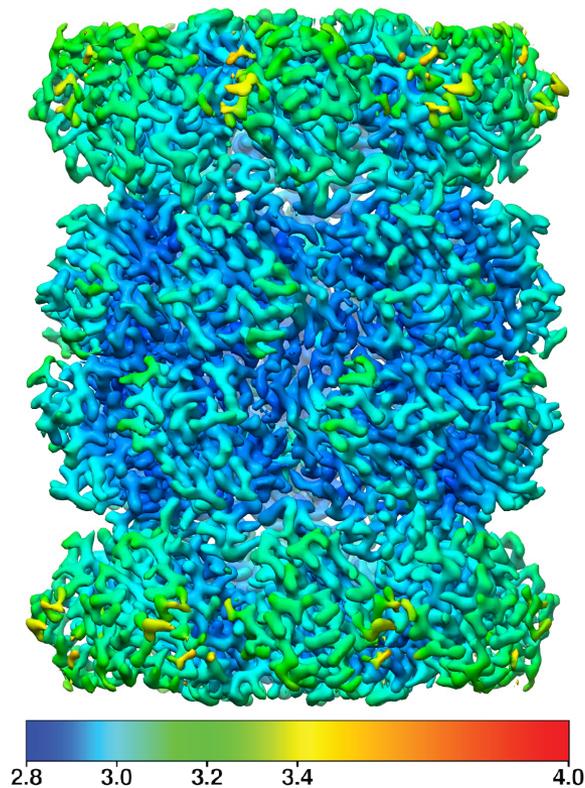
Lander Lab, The Scripps Research Institute

Protocol Exchange (2017) | doi:10.1038/protex.2017.108

Published online 12 October 2017

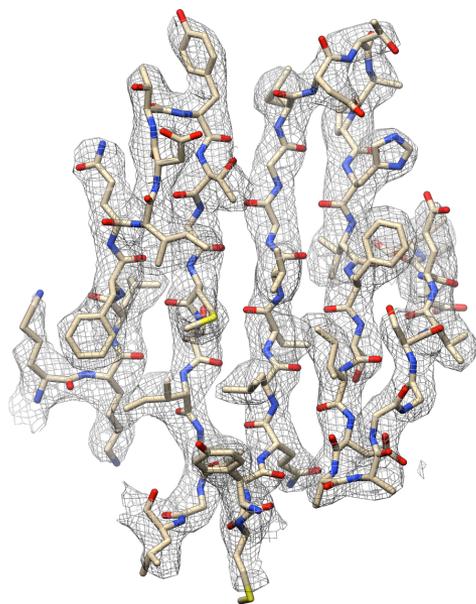
T. Acidophilum 20S Proteasome Core at ~3.1 Å Resolution

T. Acidophilum 20S Proteasome Core at ~ 3.1 Å Resolution

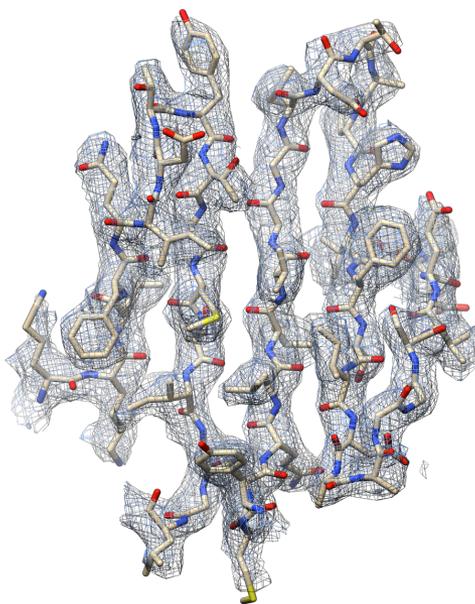


Comparison of 20S EM Density – Arctica vs. Krios

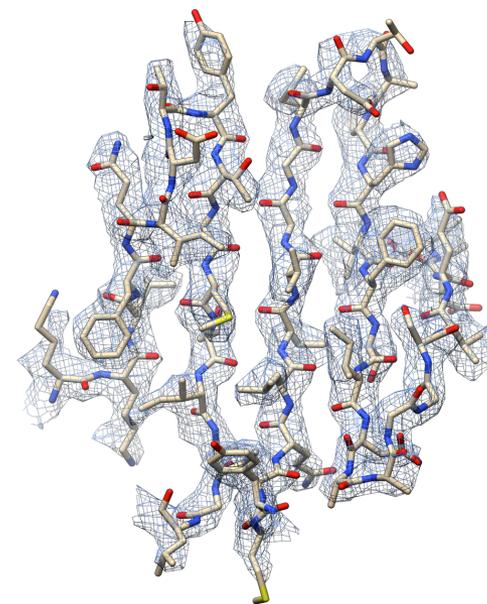
Arctica
~3.1 Å



Arctica/Krios
Overlay



Krios (EMDB-6287)
~2.8 Å



Ideal Structural Target <200 kDa? – Rabbit Muscle Aldolase

A2714 SIGMA

Aldolase from rabbit muscle

lyophilized powder, ≥8.0 units/mg protein

Synonym: D-Fructose-1,6-bisphosphate-D-glyceraldehyde-3-phosphate-lyase, Fructose-diphosphate Aldolase

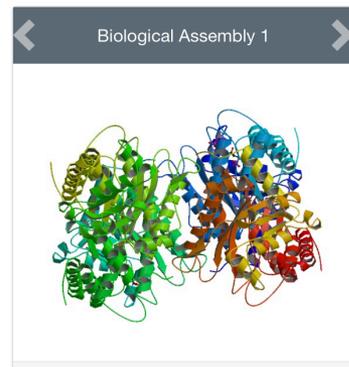
SDS

SIMILAR PRODUCTS

CAS Number [9024-52-6](#) | EC Number [232-781-0](#) | Enzyme Commission (EC) Number [4.1.2.13](#) ([BRENDA](#) | [IUBMB](#))

MDL number [MFCD00130453](#)

POPULAR DOCUMENTS: [SPECIFICATION SHEET \(PDF\)](#)



6ALD

RABBIT MUSCLE ALDOLASE A/FRUCTOSE-1,6-BISPHOSPHATE COMPLEX

DOI: [10.2210/pdb6ald/pdb](#)

Classification: [LYASE](#)

Deposited: 1998-12-23 Released: 2000-01-05

Deposition author(s): [Choi, K.H.](#), [Mazurkie, A.S.](#), [Morris, A.J.](#), [Utheza, D.](#), [Tolan, D.R.](#), [Allen, K.N.](#)

Organism: [Oryctolagus cuniculus](#)

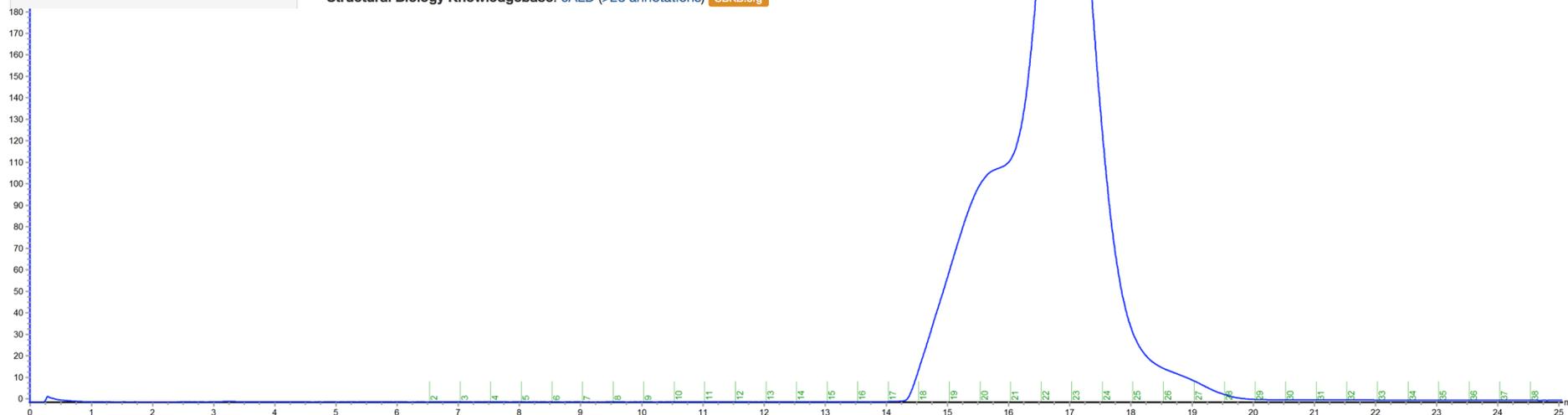
Expression System: Escherichia coli

Mutation(s): 1

Structural Biology Knowledgebase: [6ALD \(>23 annotations\)](#) [SBKB.org](#)

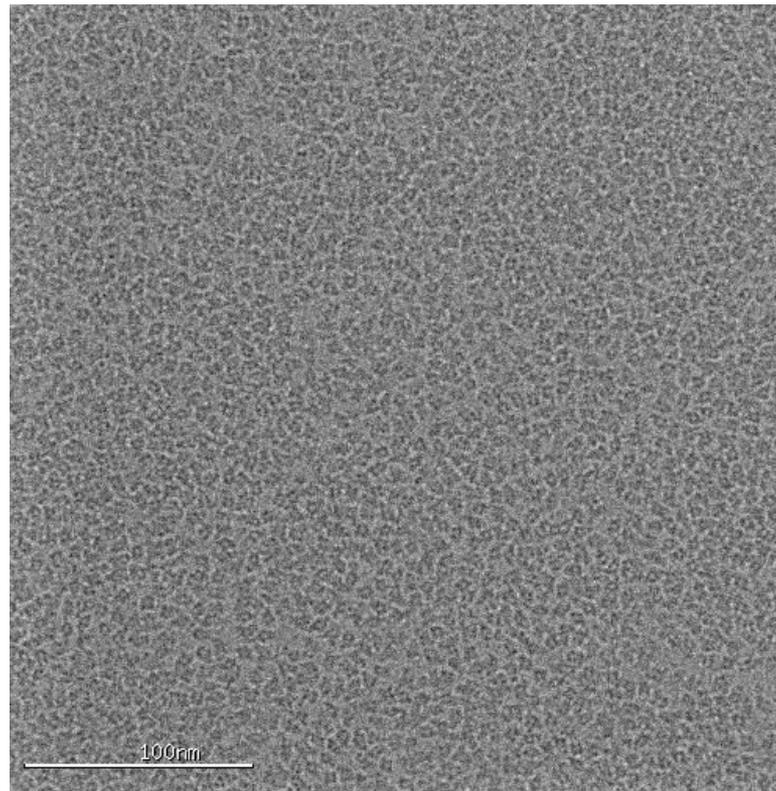
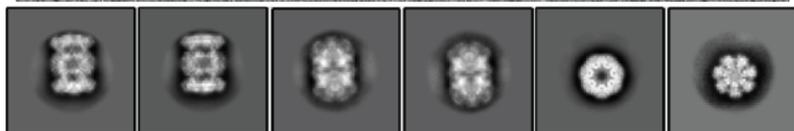
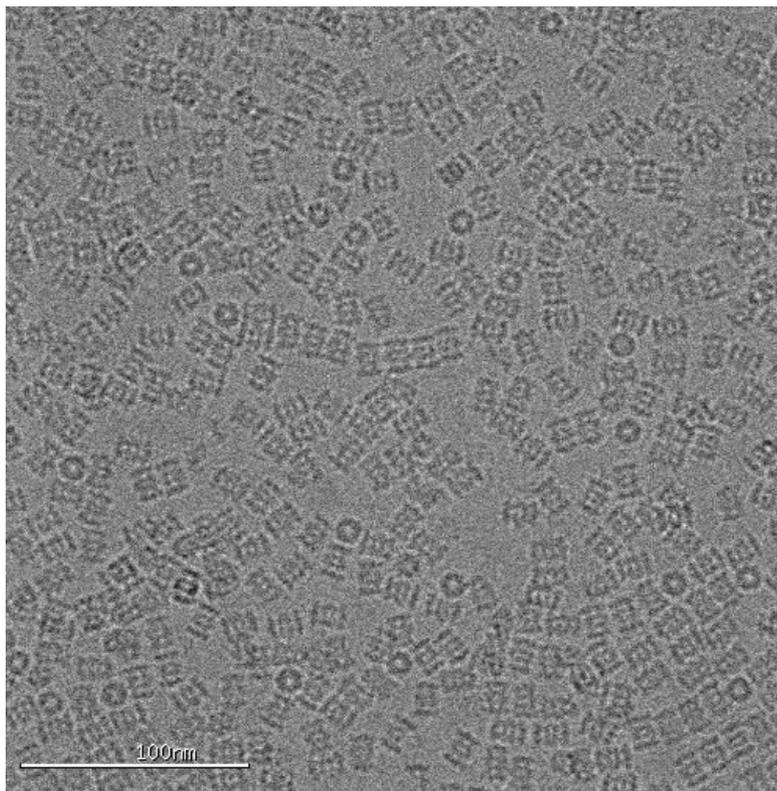
Display Files

Download Files



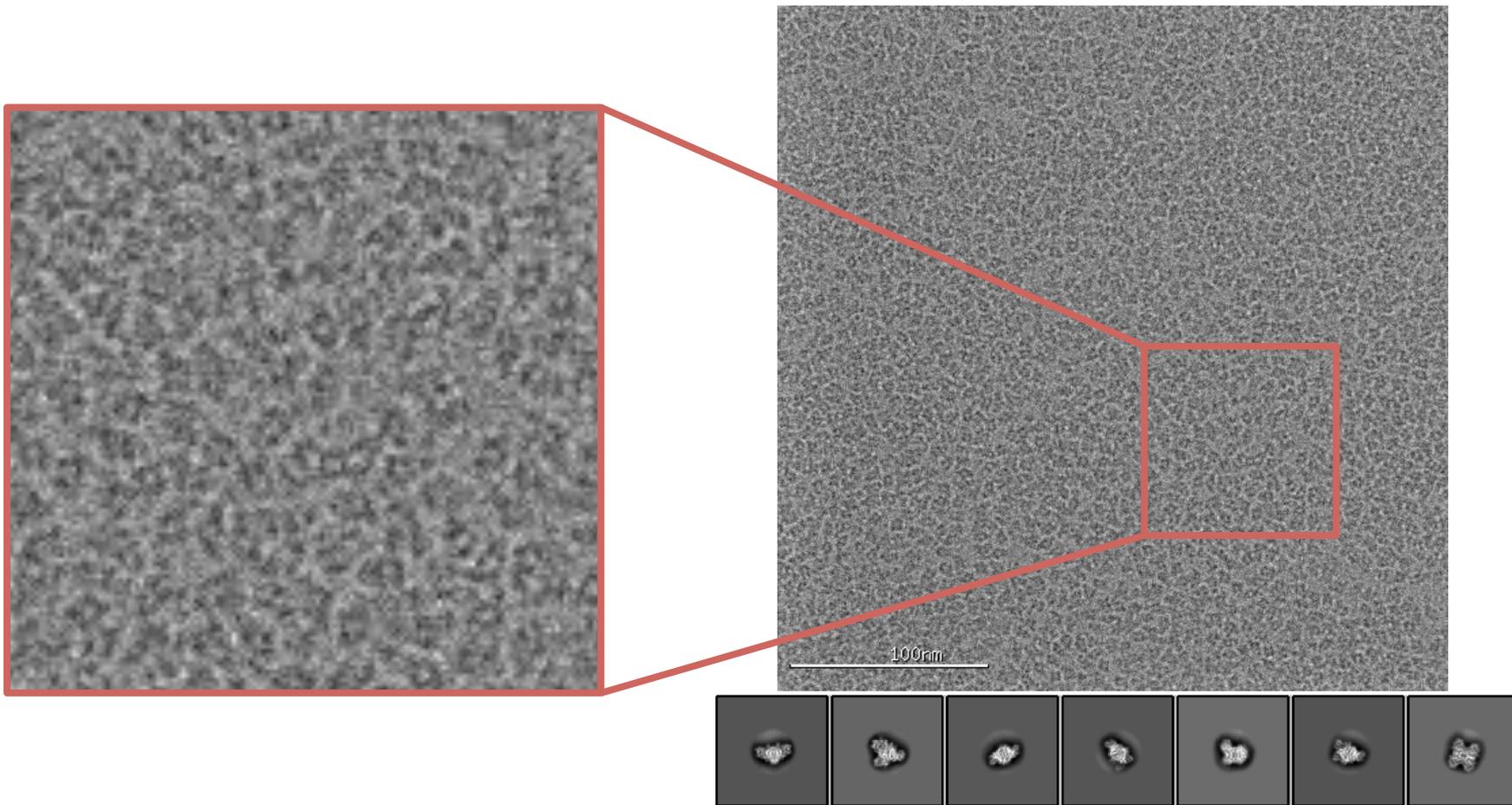
20S vs. Aldolase – Comparison of Particle Size and Particle Density

45000x, 0.91 Å/pixel



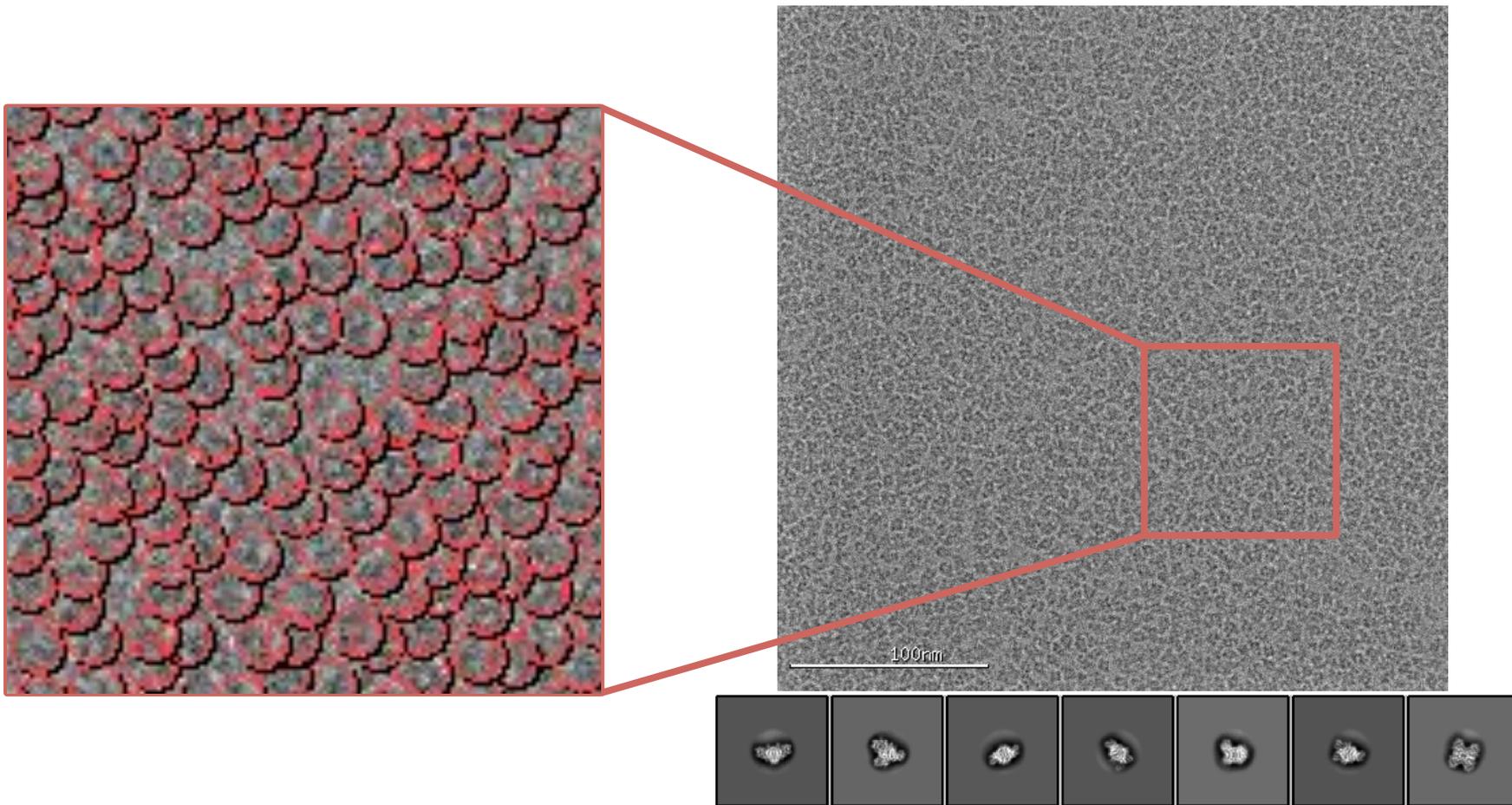
20S vs. Aldolase – Comparison of Particle Size and Particle Density

45000x, 0.91 Å/pixel

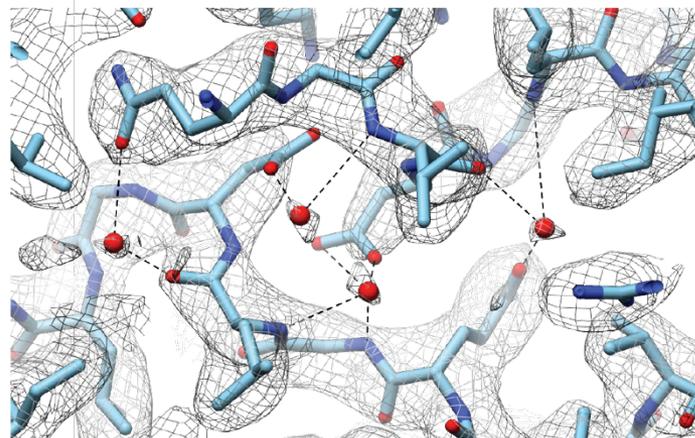
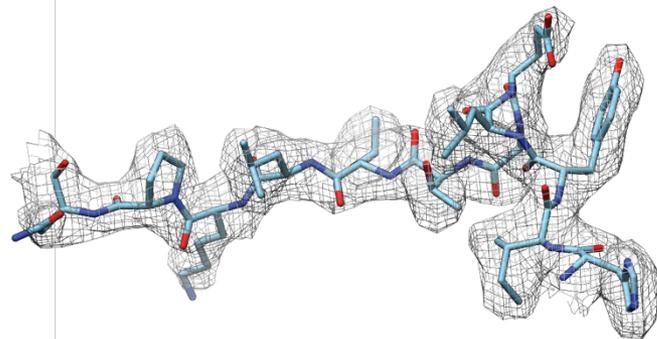
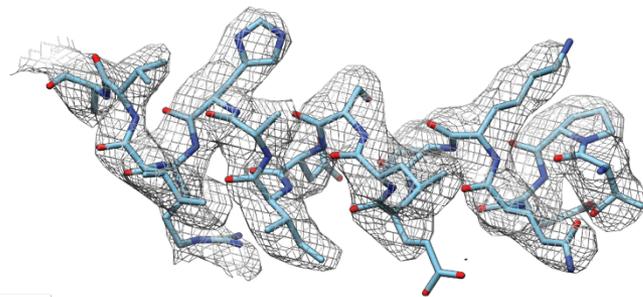
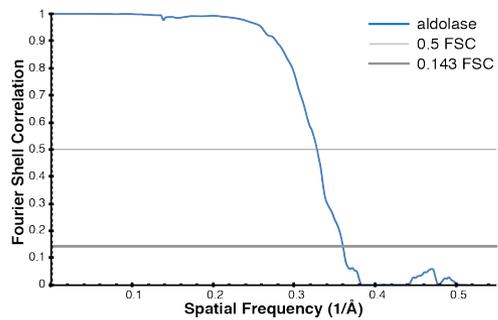
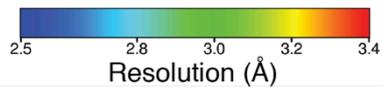
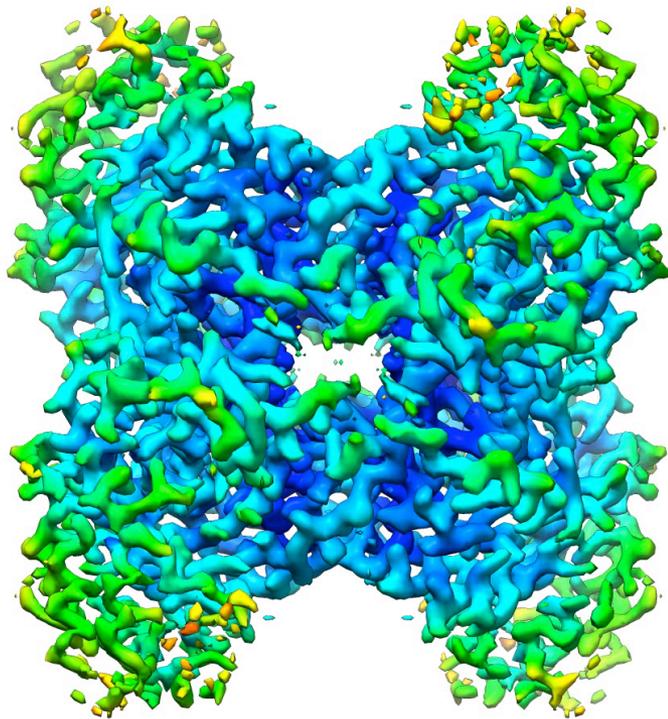


20S vs. Aldolase – Comparison of Particle Size and Particle Density

45000x, 0.91 Å/pixel



~150 kDa Rabbit Muscle Aldolase at ~2.6 Å Resolution



Ideal Structural Target <100 kDa? – Horse Liver Alcohol Dehydrogenase

55689 SIGMA

Alcohol Dehydrogenase equine

recombinant, expressed in *E. coli*, ≥ 0.5 U/mg

Synonym: ADH

SDS

SIMILAR PRODUCTS

CAS Number [9031-72-5](#) | EC Number [232-870-4](#) | Enzyme Commission (EC) Number [1.1.1.1](#) ([BRENDA](#) | [IUBMB](#))

MDL number [MFCD00081305](#)

Biological Assembly 1



2JHF

Structural evidence for a ligand coordination switch in liver alcohol dehydrogenase

DOI: [10.2210/pdb2jhf/pdb](#)

Classification: [OXIDOREDUCTASE](#)

Deposited: 2007-02-22 Released: 2007-04-24

Deposition author(s): [Meijers, R.](#), [Adolph, H.W.](#), [Dauter, Z.](#), [Wilson, K.S.](#), [Lamzin, V.S.](#), [Cedergren-Zeppezauer, E.S.](#)

Organism: [Equus caballus](#)

Experimental Data Snapshot

Method: X-RAY DIFFRACTION

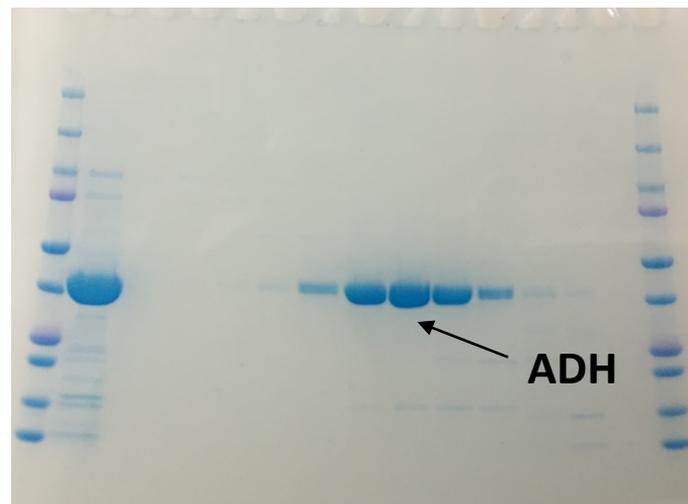
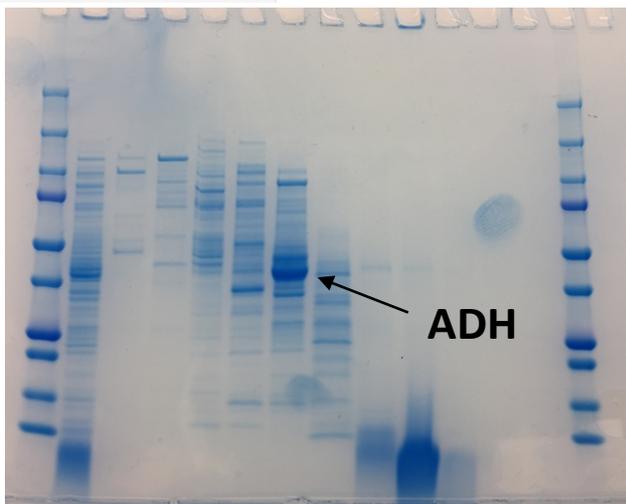
Resolution: 1.0 Å

R-Value Free: 0.152

R-Value Work: 0.125

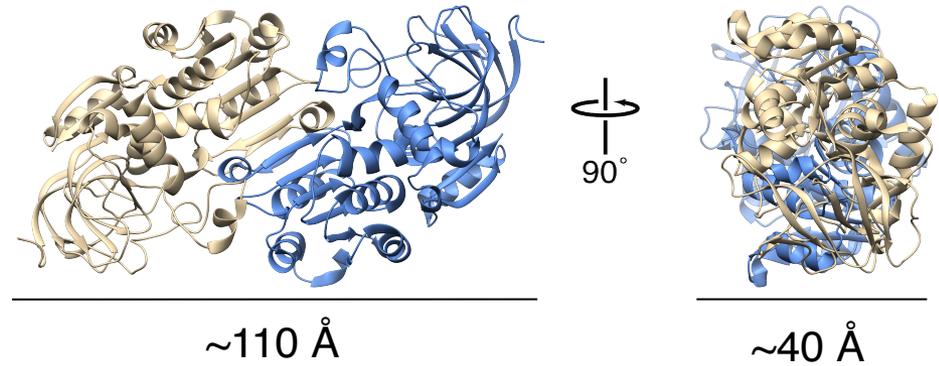
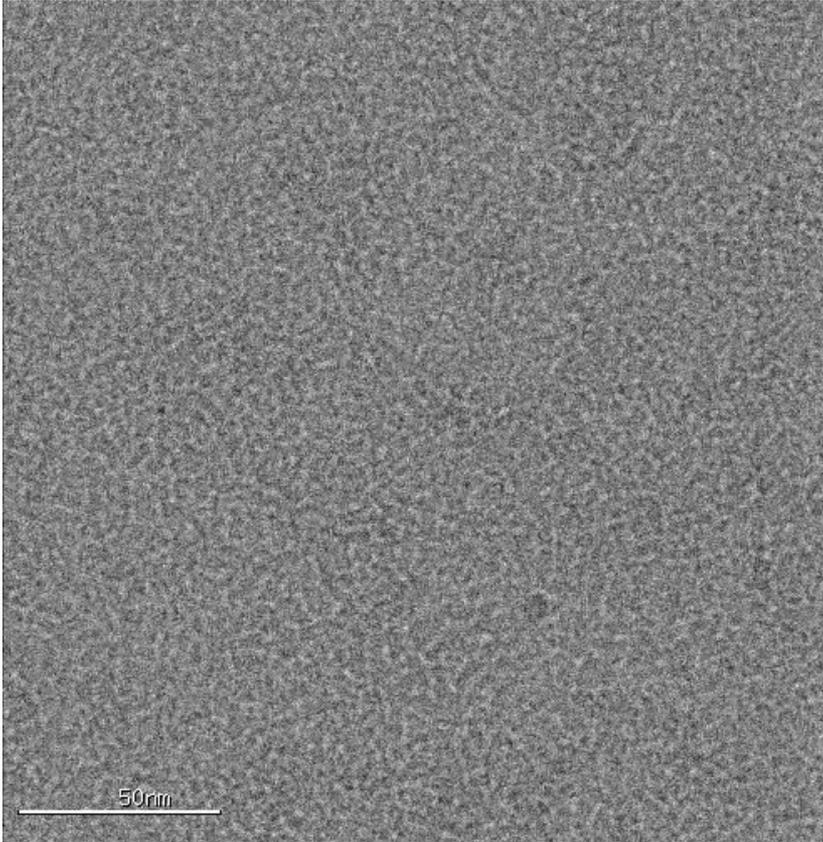
Display Files

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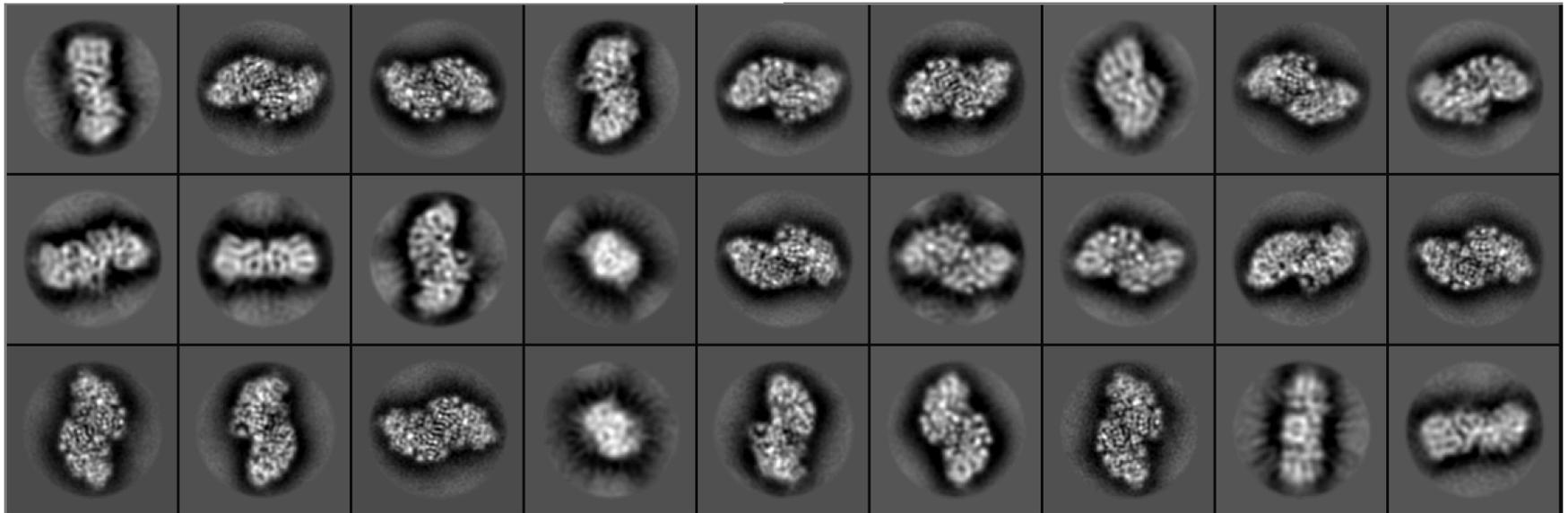
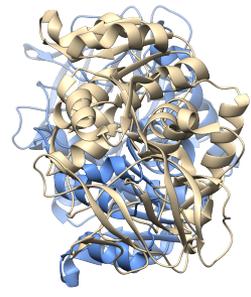
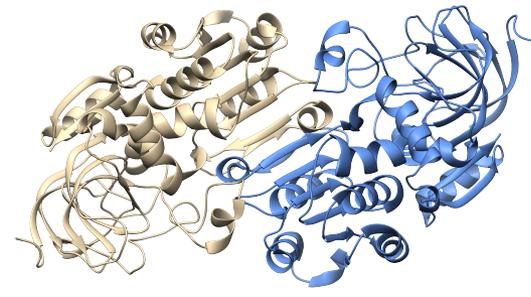
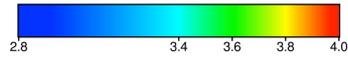
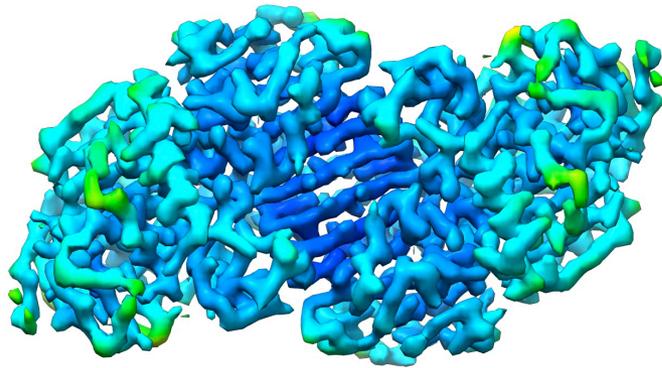


~81 kDa Equine Alcohol Dehydrogenase at ~2.9 Å Resolution

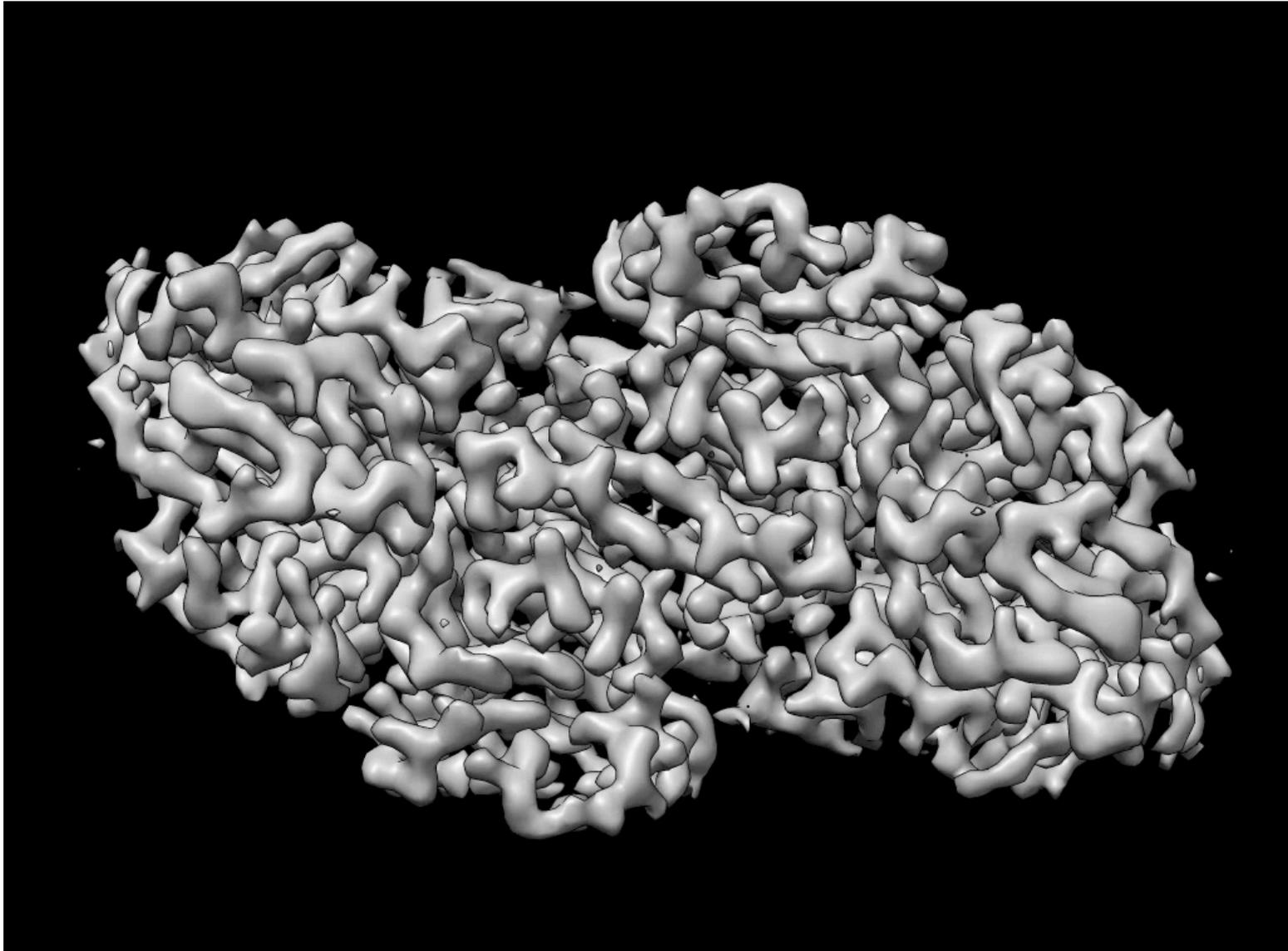
73000x, 0.556 Å/pixel



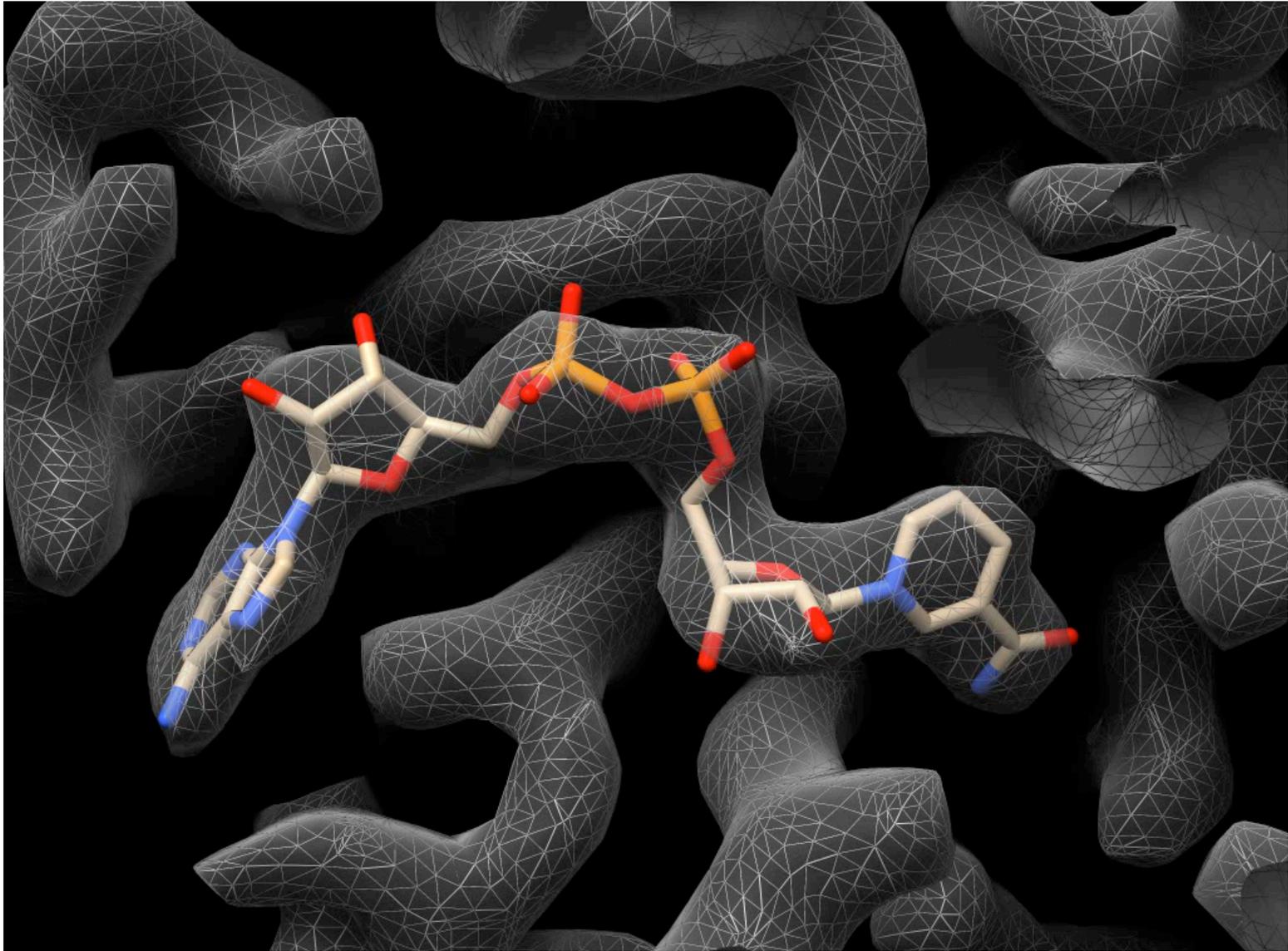
~81 kDa Equine Alcohol Dehydrogenase at ~2.9 Å Resolution



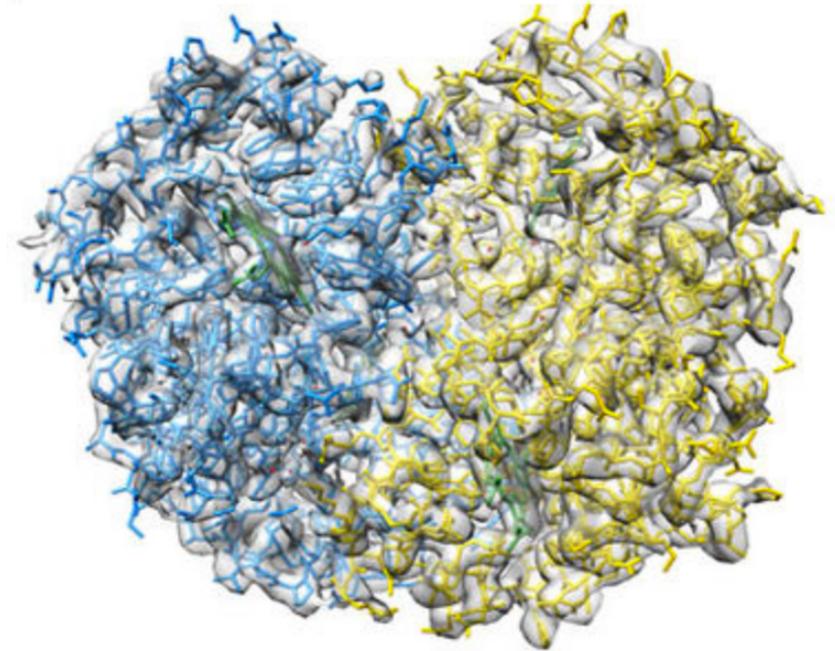
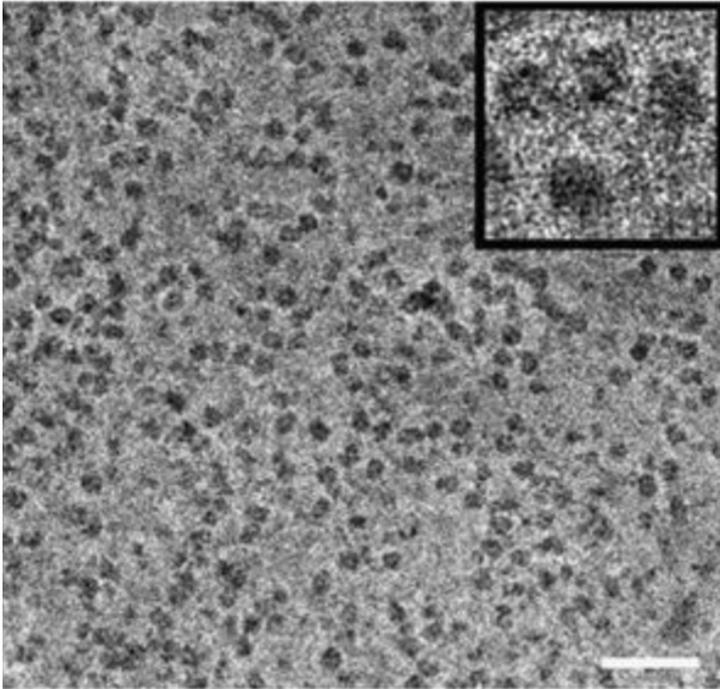
~81 kDa Equine Alcohol Dehydrogenase at ~2.9 Å Resolution



~81 kDa Equine Alcohol Dehydrogenase at ~2.9 Å Resolution

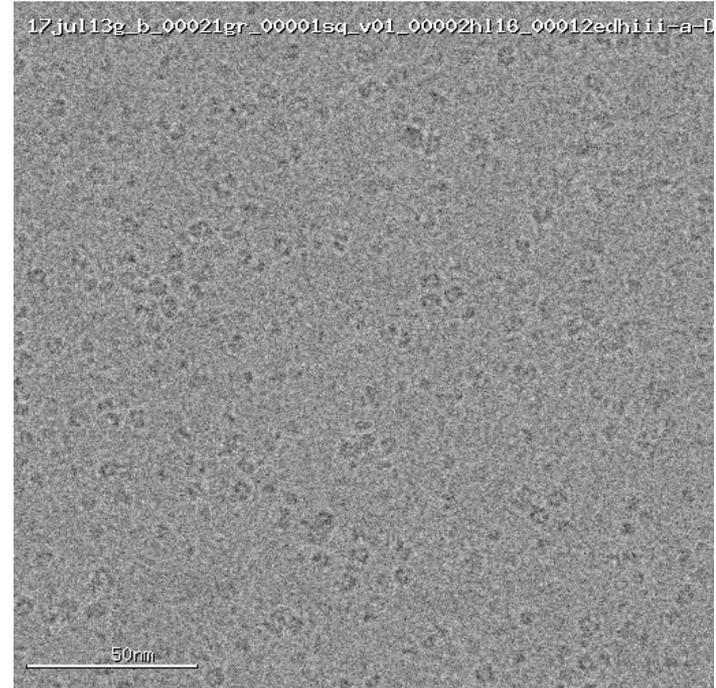
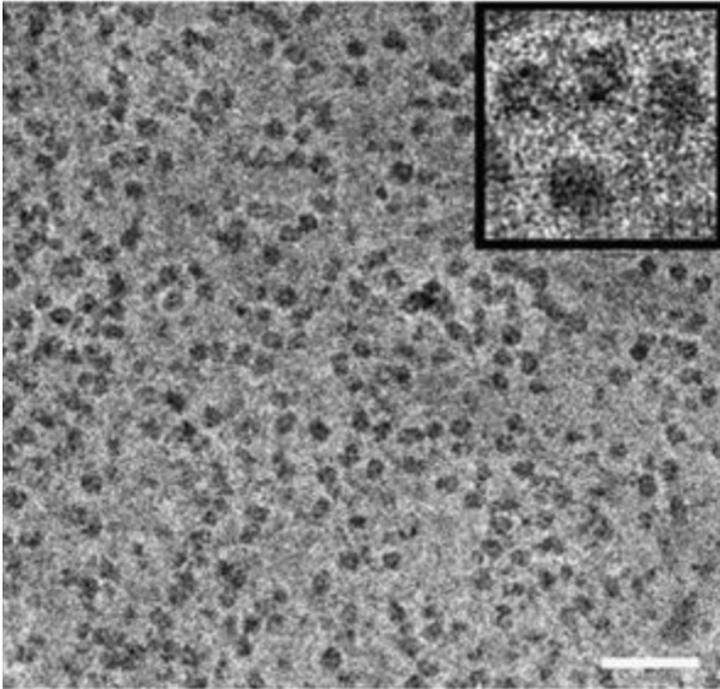


3.2 Å Resolution Human Hemoglobin Structure Using Volta Phase Plate

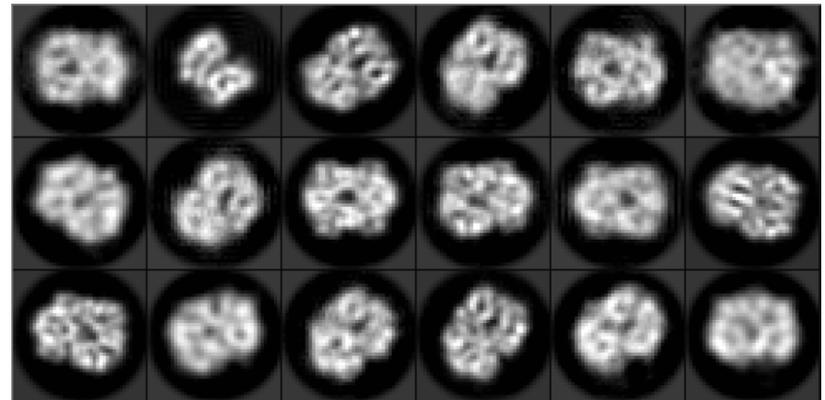
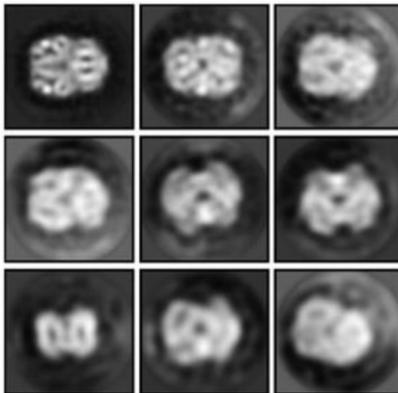


Khoshouei et al. Nature Comm 2017

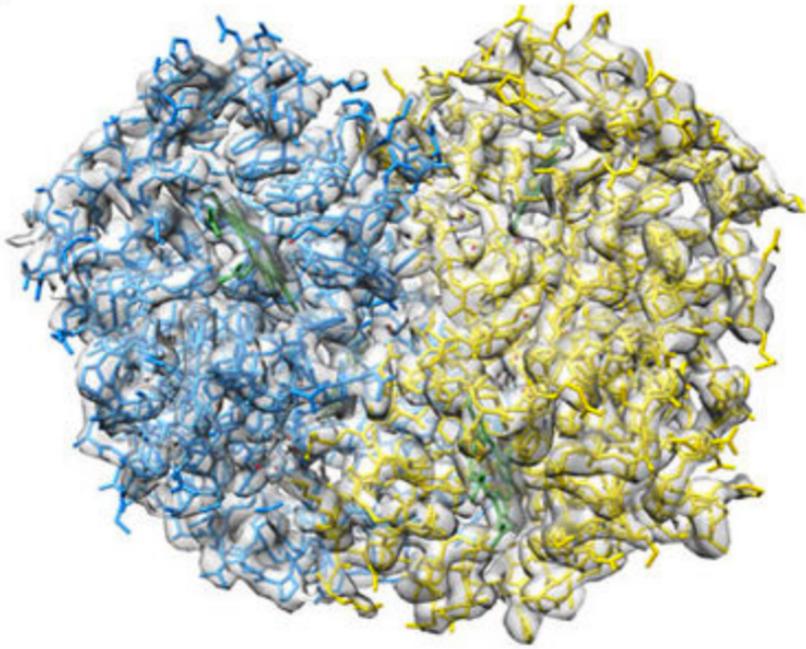
3.2 Å Resolution Human Hemoglobin Structure Using Volta Phase Plate



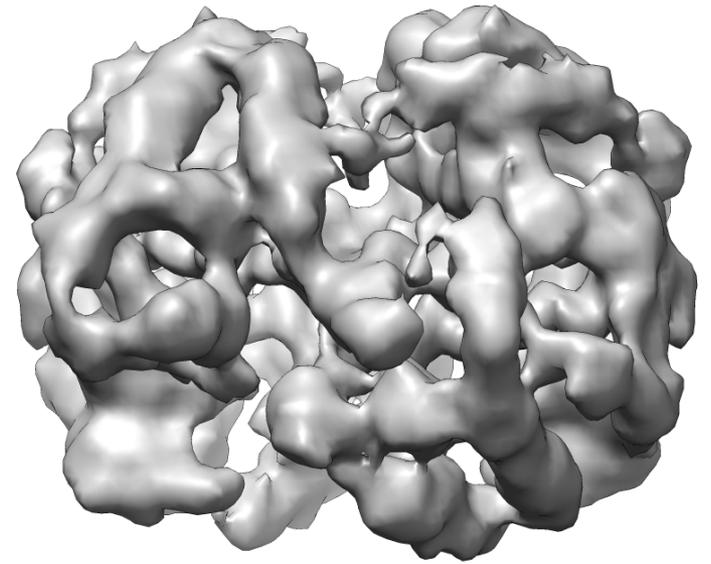
Khoshouei et al. Nature Comm 2017



3.2 Å Resolution Human Hemoglobin Structure Using Volta Phase Plate



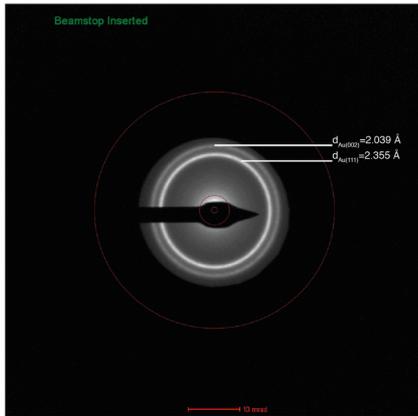
175,374 particles = 3.2 Å



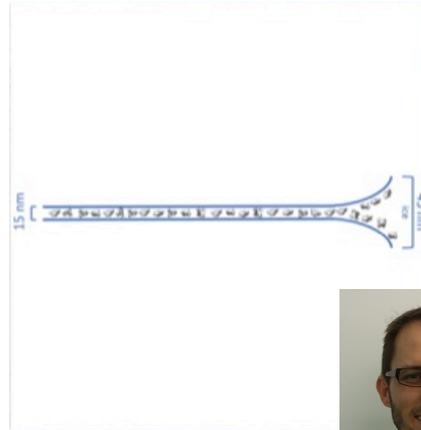
34,181 particles = ~4.5 Å

What do we think was important?

Parallel Illumination

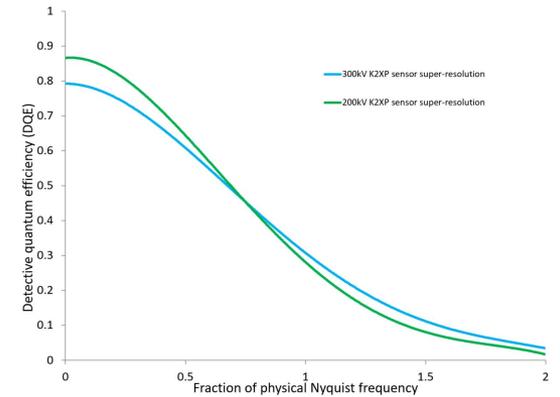


Ice Thickness/Particle Density



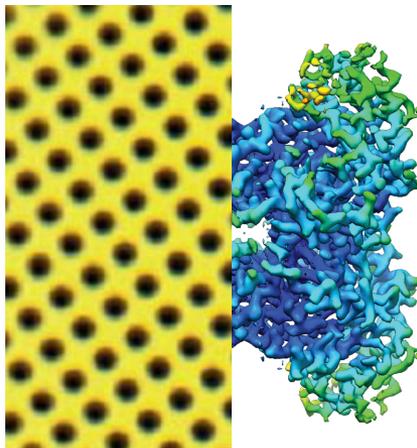
Alex Noble

Camera Settings



Paul Mooney

Specimen Stability



High-Throughput Data Collection and Processing

Leginon

Appion

What could we do better? What are the Anticipated Limitations?

What could we do better?

- How far can we image away from parallel illumination?
- Per-frame, per-particle CTF correction?
 - Minimize aberrant effects of Z-translation
- Alternate processing/refinement schemes?
 - Why didn't classification help the 20S data sets?
- Lower defocus range?
 - $\geq -1.5\mu\text{m}$ defocus did not contribute highest resolution information

What are the anticipated limitations?

- Particle heterogeneity
- Not all particles tolerate thin ice and high density
- Thin ice
- Slightly inflexible when choosing exposure rate

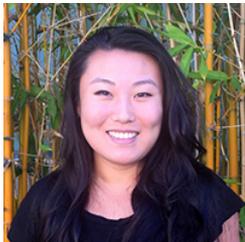
Acknowledgements



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The Scripps Research Institute

Mr. Bill Anderson

Jean-Christophe Ducom, Ph.D.

Lisa Dong

Charlie Bowman

Matthijn Vos (FEI/ThermoFisher)

Paul Mooney (Gatan)

Sjors Scheres (RELION, MRC LMB)

Yifan Cheng & Zanlin Yu (UCSF)



Funding:



Damon Runyon
Cancer Research
Foundation





[Advanced Search](#)

A multi-model approach to assessing local and global cryo-EM map quality

Mark A Herzik Jr.,  James Fraser, Gabriel C Lander

doi: <https://doi.org/10.1101/128561>

This article is a preprint and has not been peer-reviewed [what does this mean?].

Abstract

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[Metrics](#)

[Supplementary material](#)

 [Preview PDF](#)