Synchrotron Serial Crystallography

Opportunities and recent results

Ulrich Zander

ESRF Structural Biology Group

2016-02-08

Acknowledgements

- EMBL Hamburg
  - Gleb Bourenkov
- ESRF
  - Alexander Popov
  - Christoph Müller-Dieckmann
  - Olof Svenson
  - Daniele De Sanctis
  - Gordon Leonard
  - Igor Melnikov
  - Gianluca Santoni
- EMBL Grenoble
  - Max Nanao
  - Andrew McCarthy
- IBS
  - Ivan Chushchin
  - Valentin Gordeliy
  - Ekatarina Round

ESRF

Introduction

Serial Crystallography

‘

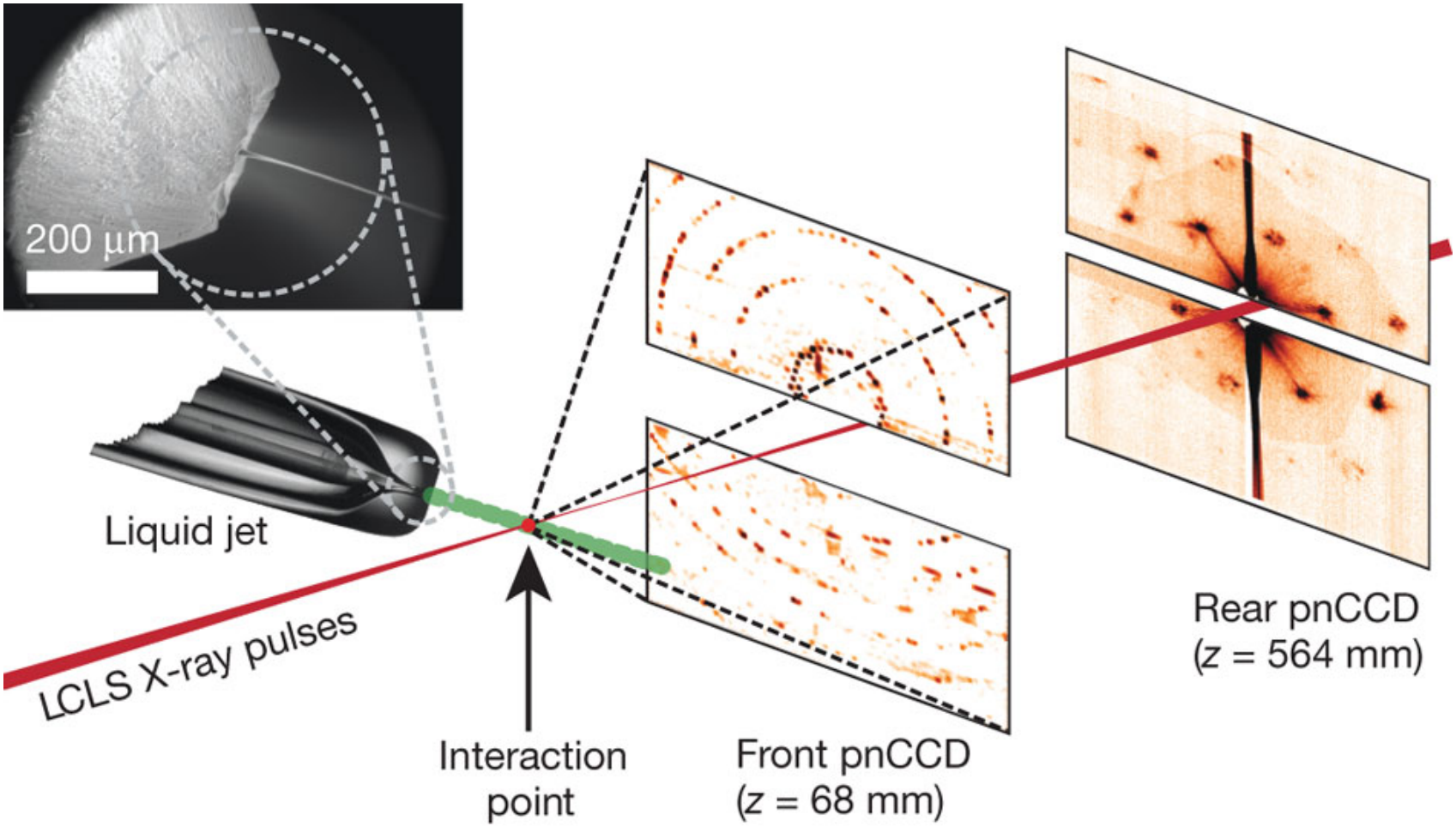
Serial Crystallography

Serial collection of partial datasets of different crystal(position)s

Serial Crystallography at XFEL: SFX

Serial Crystallography at XFEL: SFX

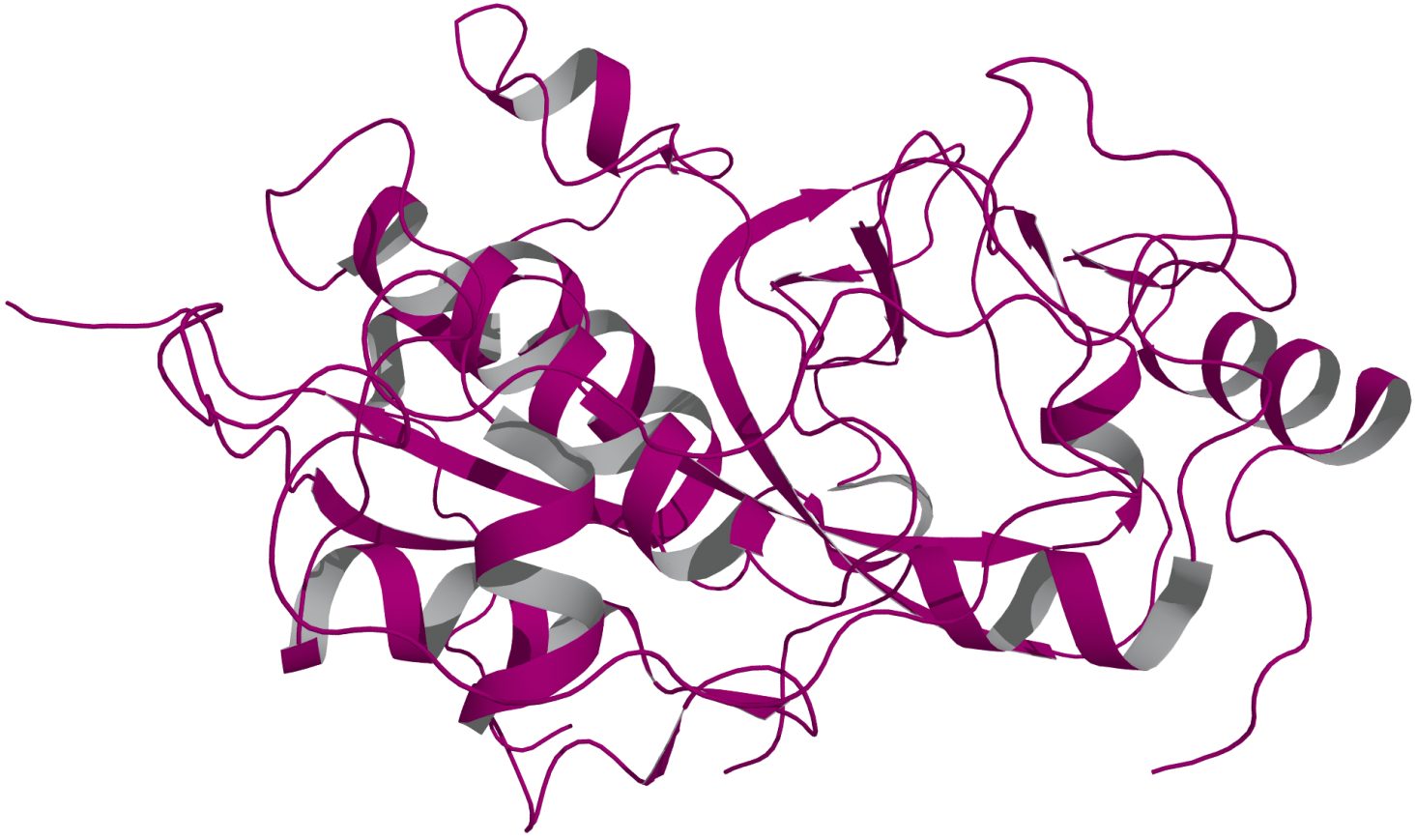
Femtosecond nanocrystallography (Chapman *et al.*, Nature 2011)



Serial Crystallography at Synchrotrons: SSX

Serial Crystallography at Synchrotrons: SSX

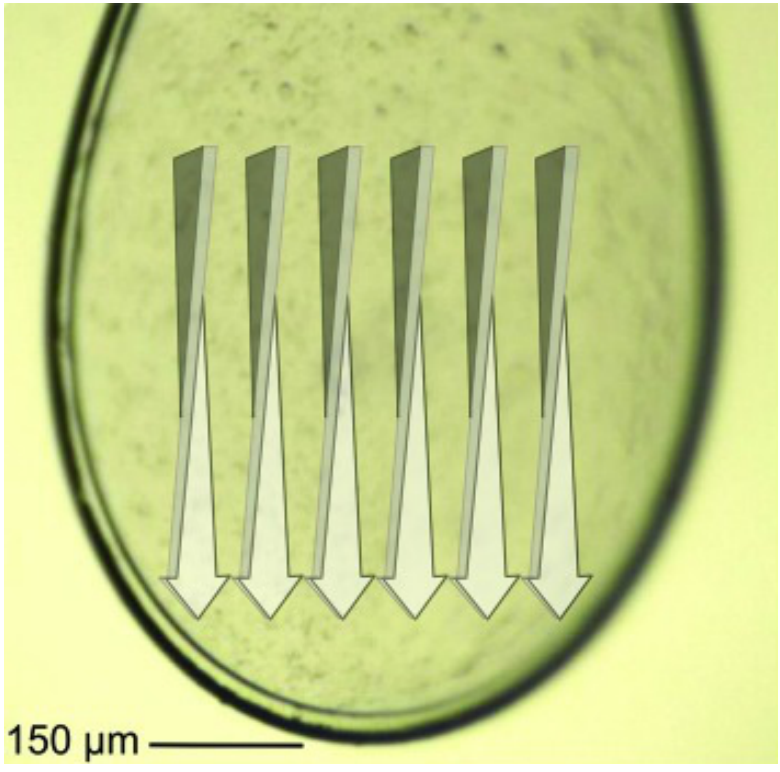
Structure of *Trypanosoma brucei* Procathepsin B at 3Å



Gati *et al.*, IUCrJ 2014: Serial crystallography on *in vivo* grown microcrystals using synchrotron radiation

Serial Crystallography at Synchrotrons: SSX

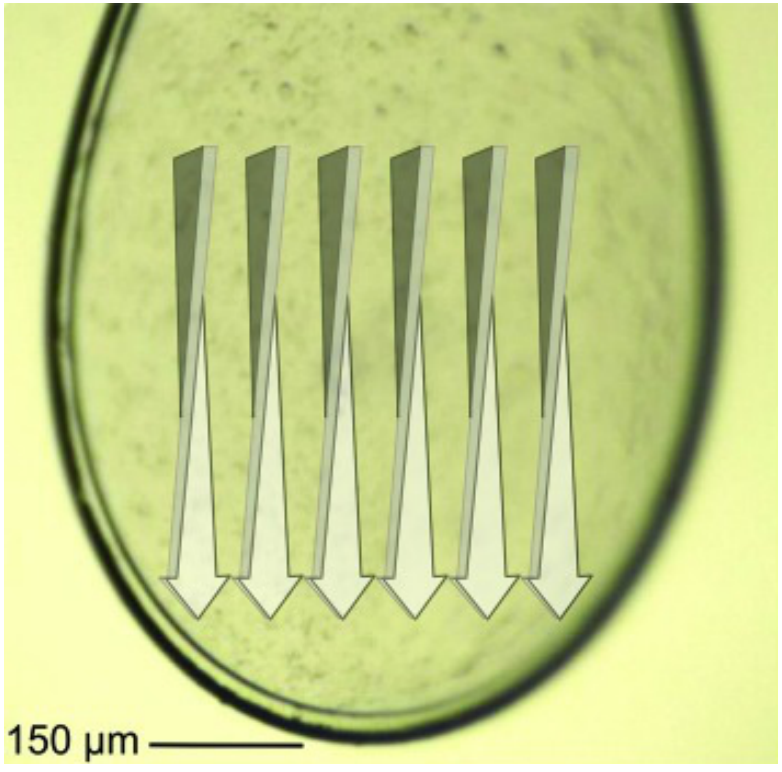
Structure of *Trypanosoma brucei* Procathepsin B at 3Å

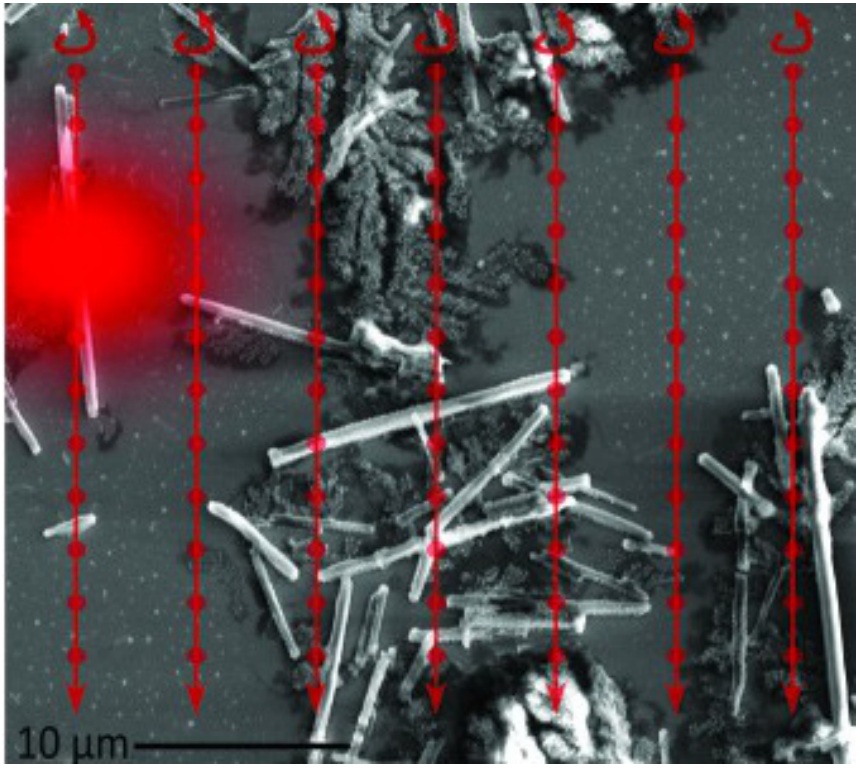


Gati *et al.*, IUCrJ 2014: Serial crystallography on *in vivo* grown microcrystals using synchrotron radiation

Serial Crystallography at Synchrotrons: SSX

Structure of *Trypanosoma brucei* Procathepsin B at 3Å

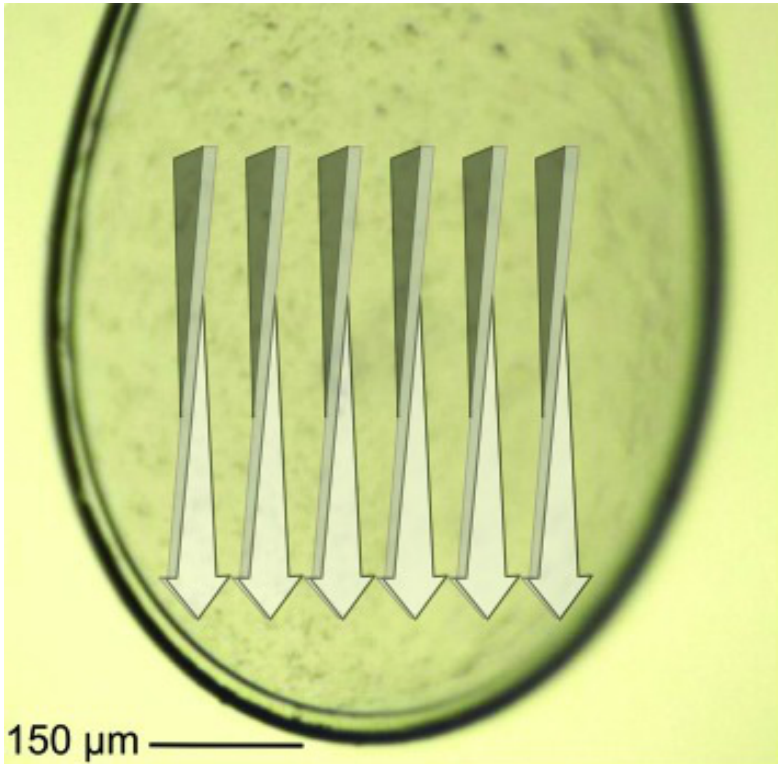


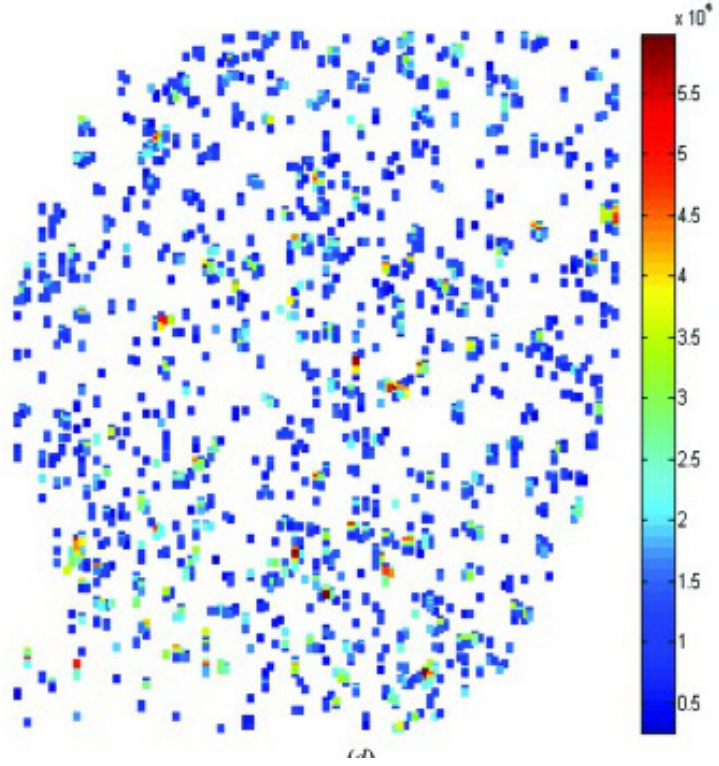


Gati *et al.*, IUCrJ 2014: Serial crystallography on *in vivo* grown microcrystals using synchrotron radiation

Serial Crystallography at Synchrotrons: SSX

Structure of *Trypanosoma brucei* Procathepsin B at 3Å

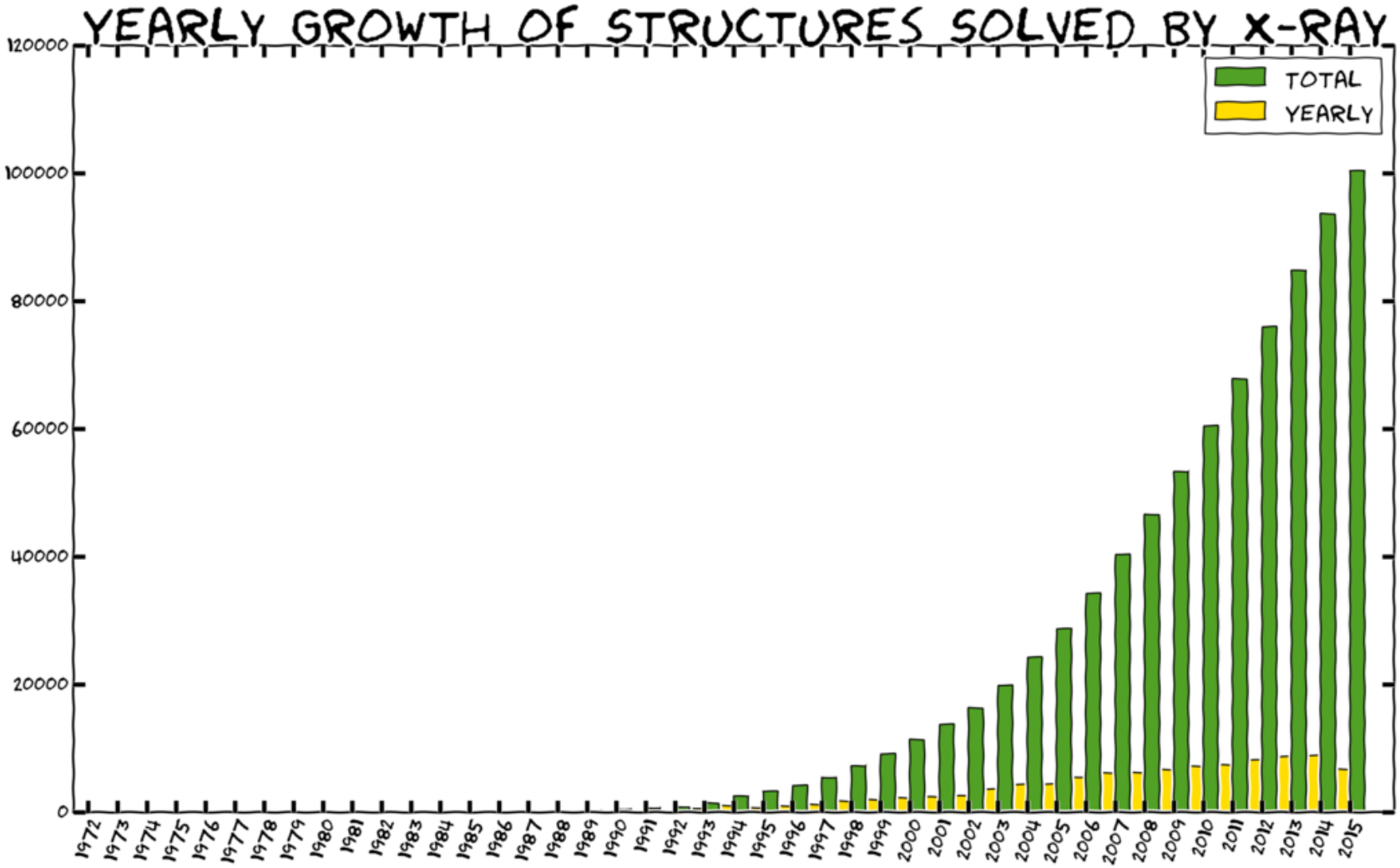




Gati *et al.*, IUCrJ 2014: Serial crystallography on *in vivo* grown microcrystals using synchrotron radiation

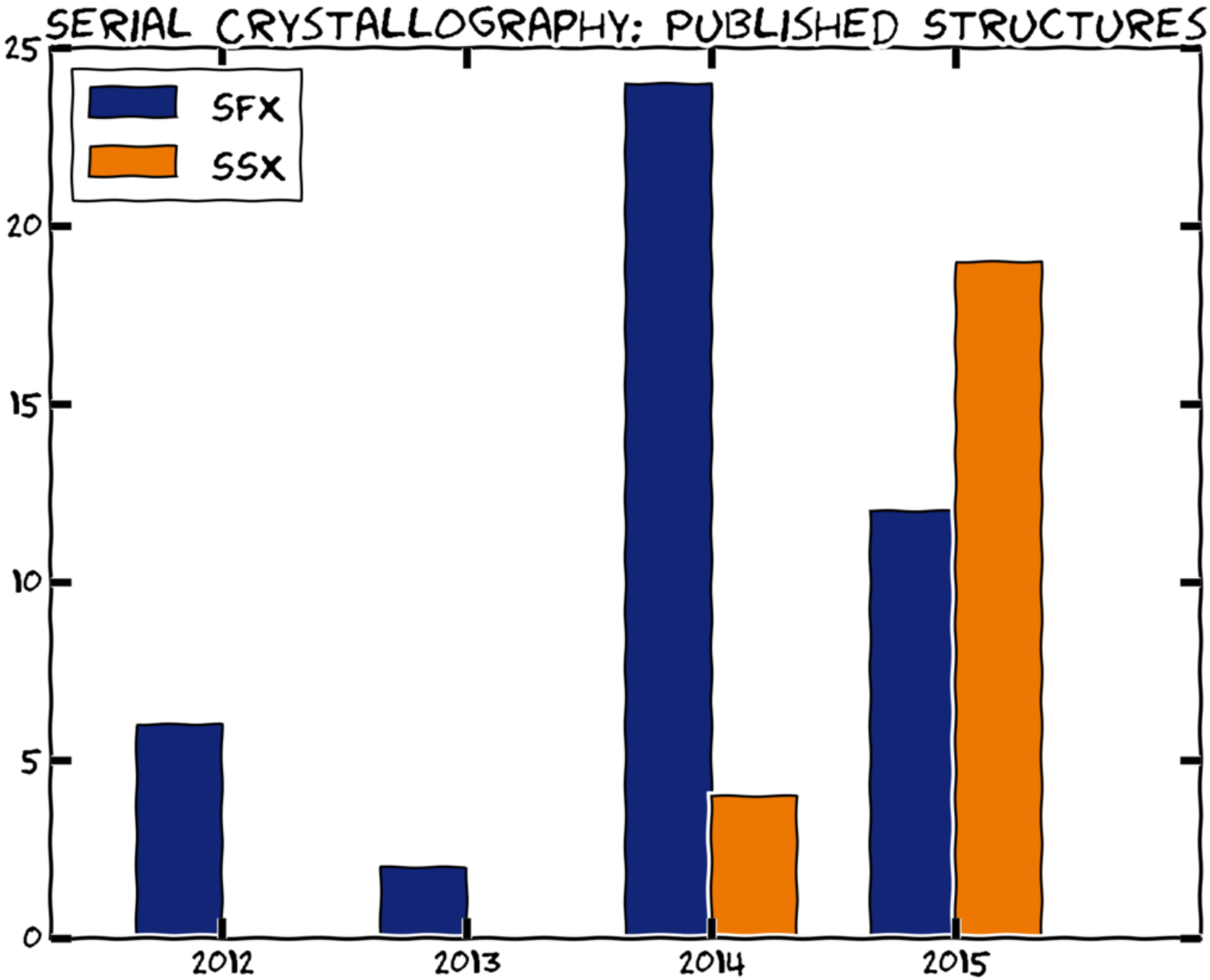
Some numbers...

Some numbers...



Source: www.pdb.org

Some numbers...

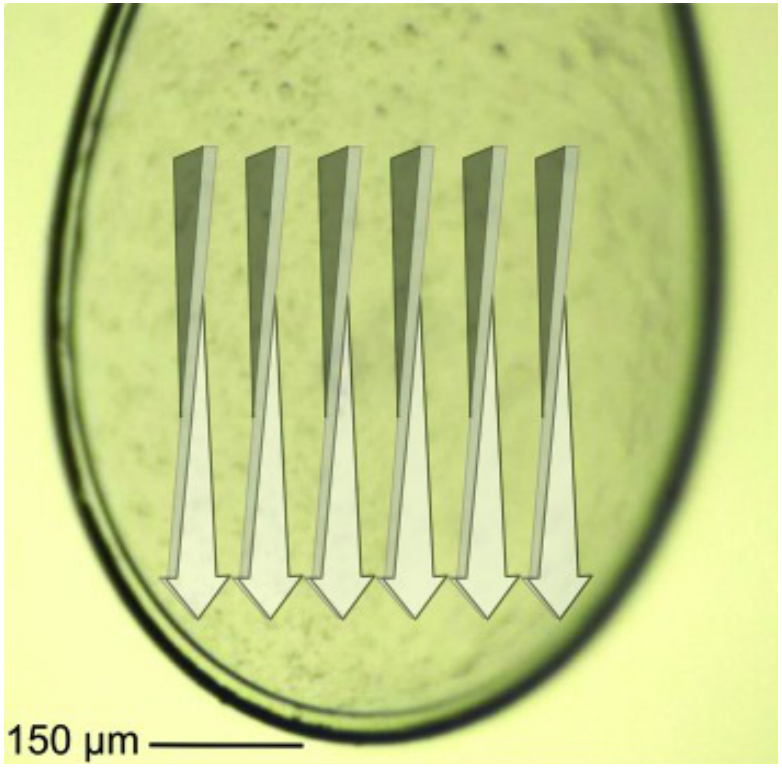


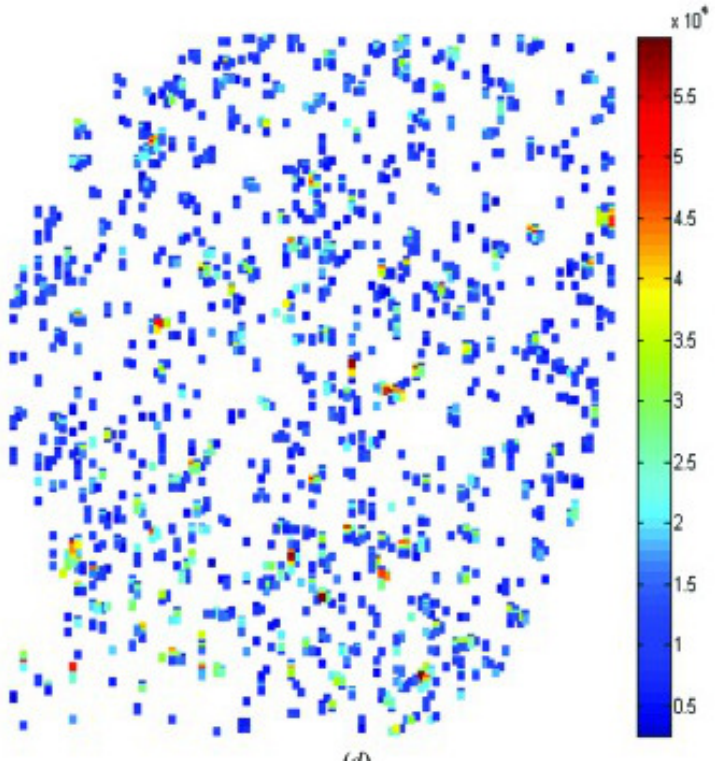
Source: www.pdb.org

Method

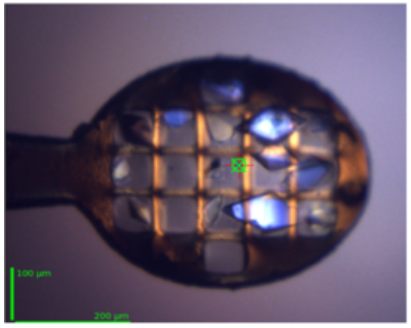
ESRF MeshAndCollect

ESRF MeshAndCollect



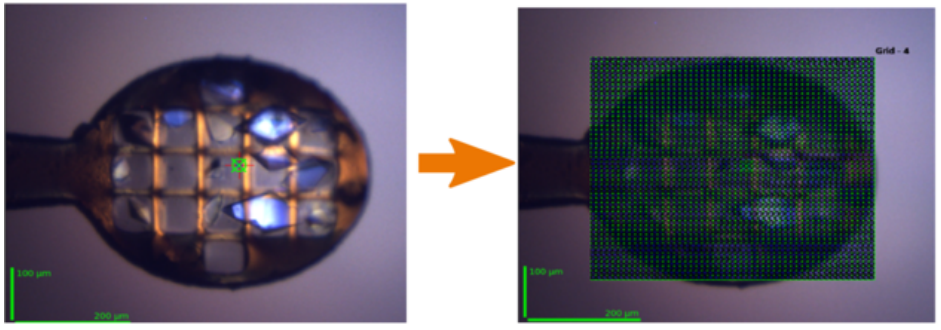


ESRF MeshAndCollect



Crystals on a mesh loop

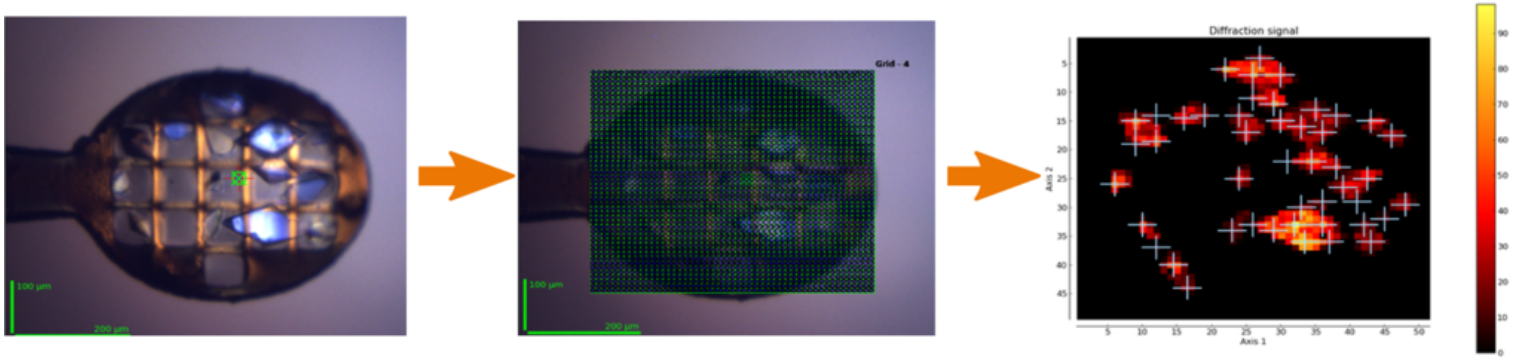
ESRF MeshAndCollect



Crystals on a mesh loop

Mesh scan of sample

ESRF MeshAndCollect

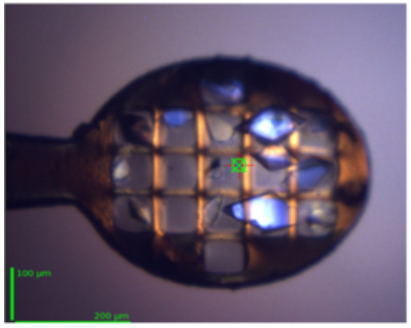


Crystals on a mesh loop

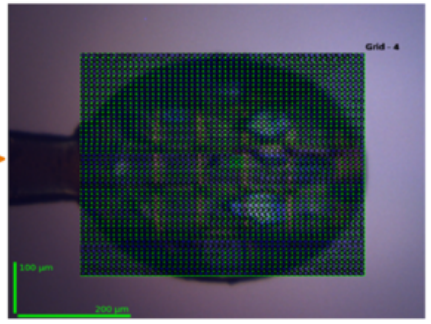
Mesh scan of sample

Determination of crystal positions
Partial data collections

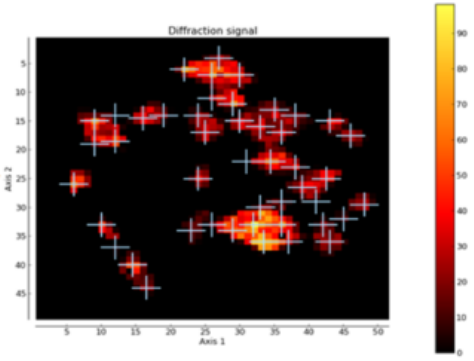
ESRF MeshAndCollect



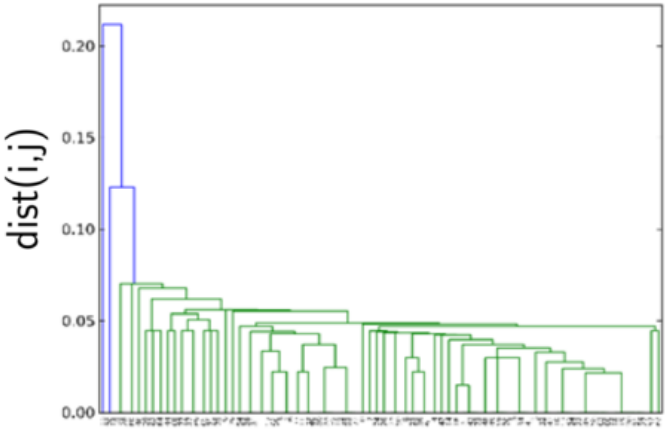
Crystals on a mesh loop



Mesh scan of sample



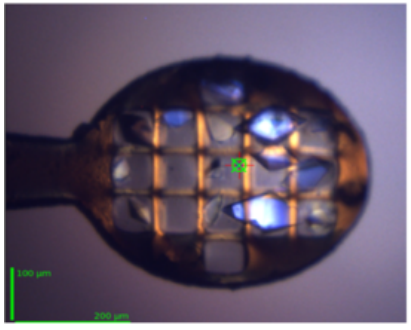
Determination of crystal positions
Partial data collections



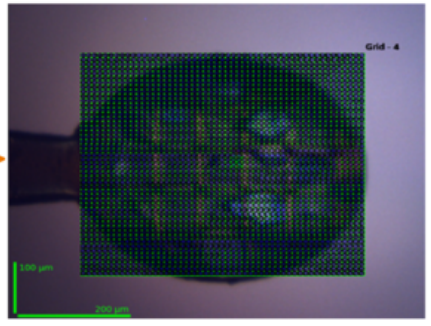
Dataset combination:
hierarchical cluster analysis

ESRF

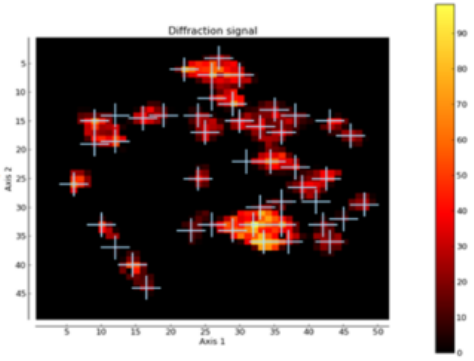
ESRF MeshAndCollect



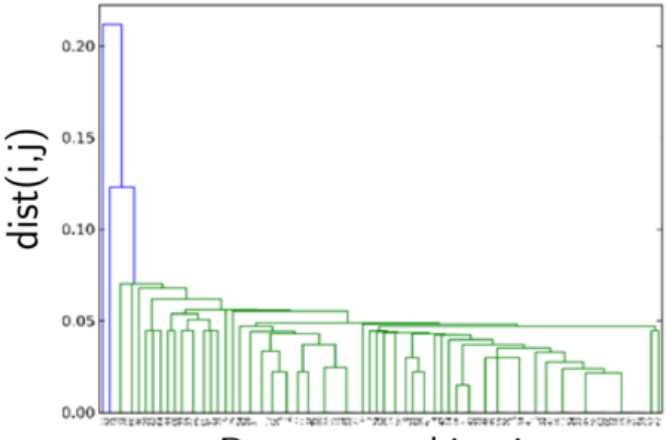
Crystals on a mesh loop



Mesh scan of sample



Determination of crystal positions
Partial data collections



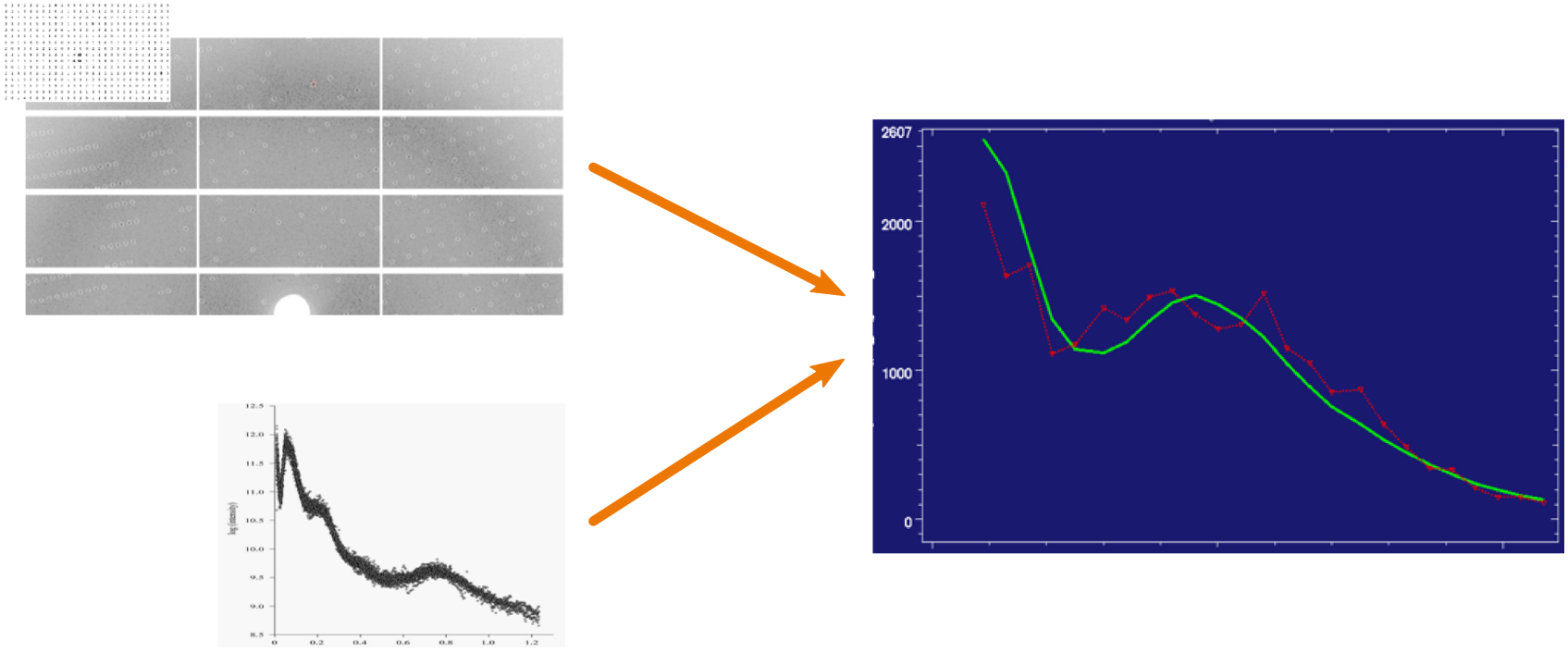
Dataset combination:
hierarchical cluster analysis

Protein Structure

Software

Evaluating diffraction signal with DOZOR

Evaluating diffraction signal with DOZOR



- Use Wilson plot as a prior
- Use all pixels, not just the local maxima
- score = total scattered intensity x radial shape similarity

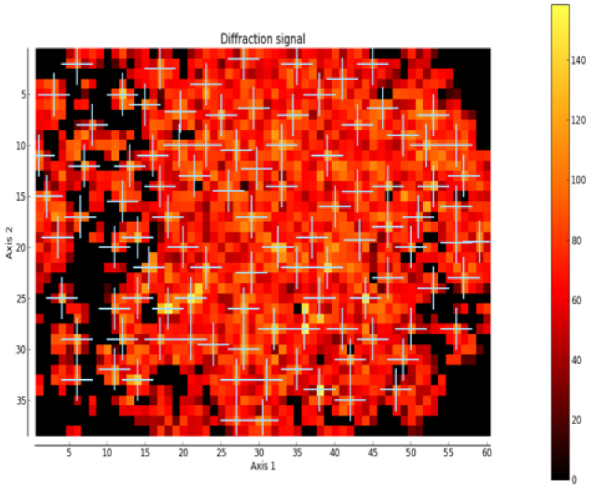
DOZOR: extensions

DOZOR: extensions

Dozor detects single crystals in the crystal mess as well as crystal overlapping

DOZOR: extensions

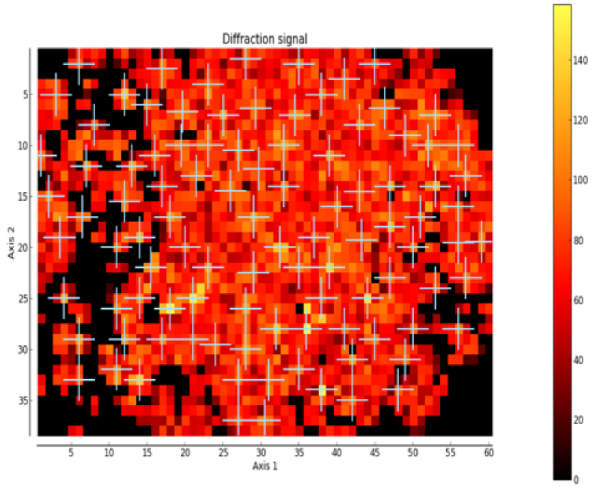
Dozor detects single crystals in the crystal mess as well as crystal overlapping



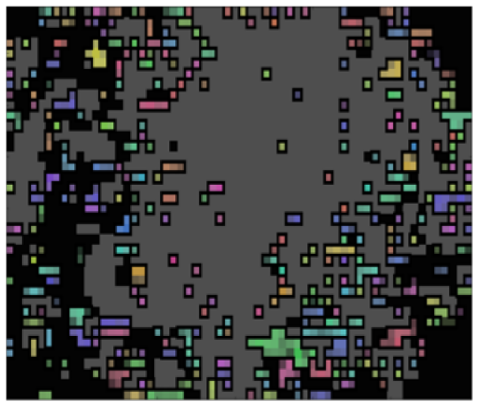
Initial crystal diffraction map

DOZOR: extensions

Dozor detects single crystals in the crystal mess as well as crystal overlapping



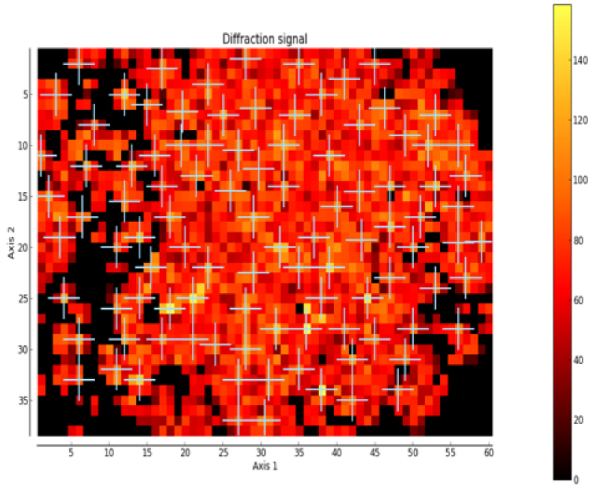
Initial crystal diffraction map



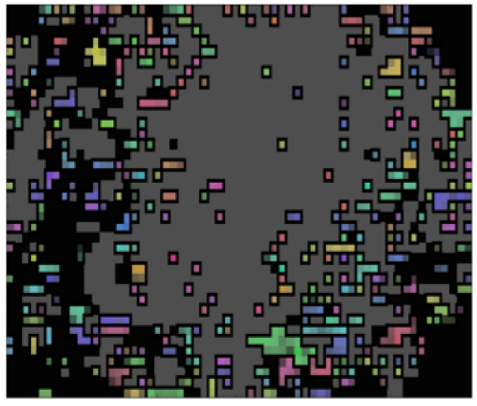
Crystal map after Dozor analysis

DOZOR: extensions

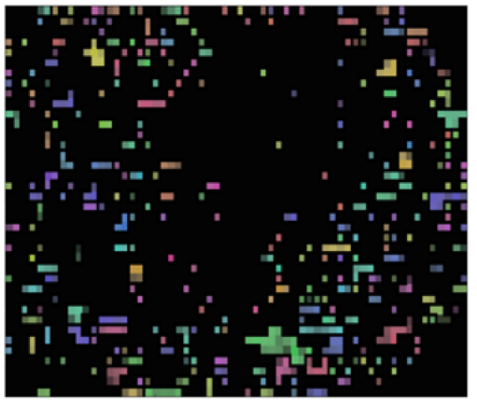
Dozor detects single crystals in the crystal mess as well as crystal overlapping



Initial crystal diffraction map



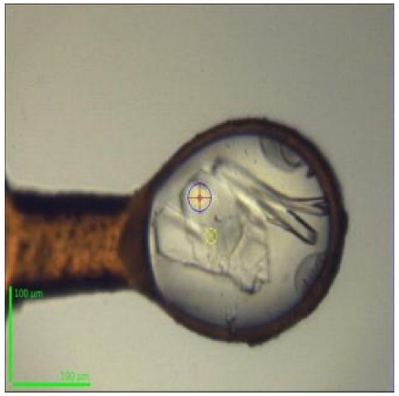
Crystal map after Dozor analysis



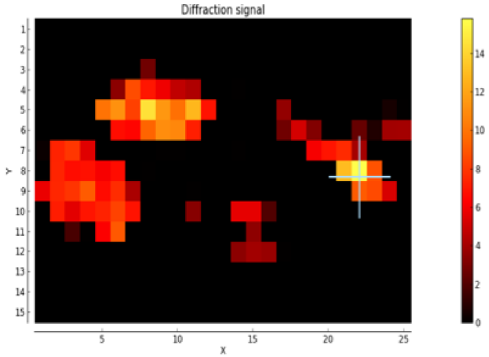
Crystal map omitting regions with overlaps

DOZOR: extensions

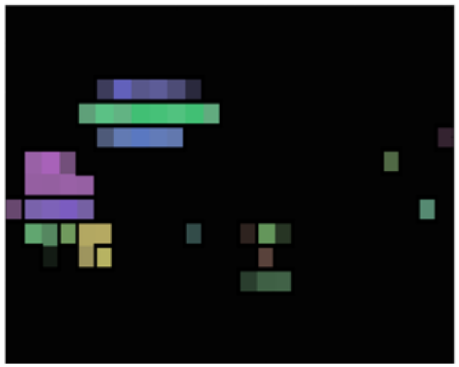
Dozor determines regions of crystal homogeneity and size



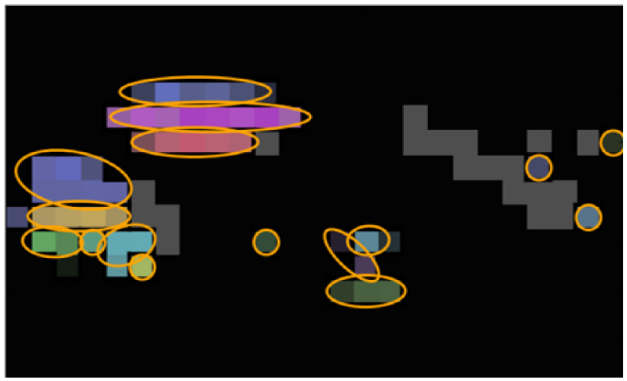
Snapshot of the sample



Initial diffraction map, showing diffracting regions on the sample



Crystal map ommiting regions of overlapping



fit crystal shapes corresponding to crystal integrity

Data Processing

Data Processing

- XDS

Data Processing

- XDS
- ccCluster: Hierarchical cluster analysis

Data Processing

- XDS
- ccCluster: Hierarchical cluster analysis
- Aimless

Data Processing

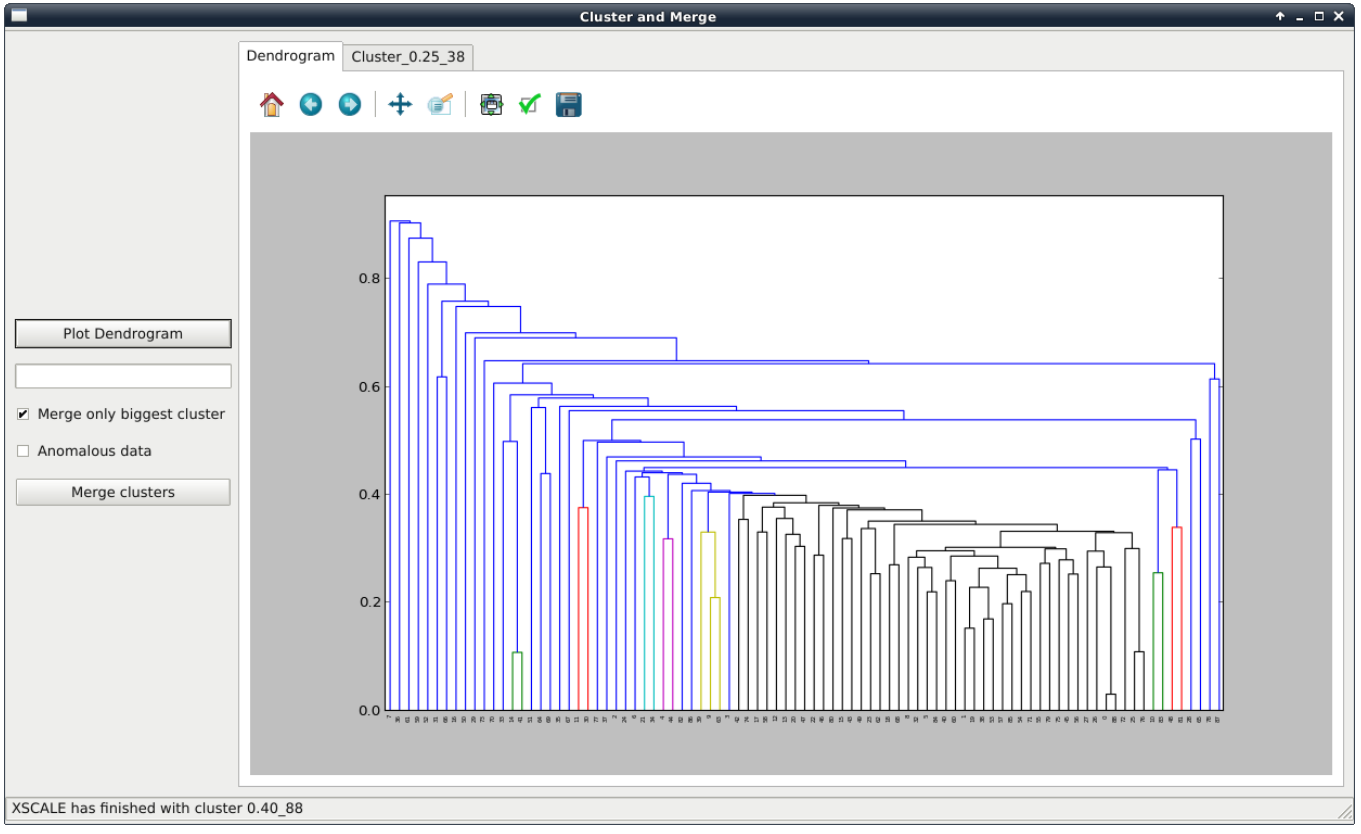
- XDS
- ccCluster: Hierarchical cluster analysis
- Aimless
- MR: Dimple

Data Processing

- XDS
- ccCluster: Hierarchical cluster analysis
- Aimless
- MR: Dimple
- SAD: Crank2

Hierarchical Cluster Analysis (HCA)

Hierarchical Cluster Analysis (HCA)



Results

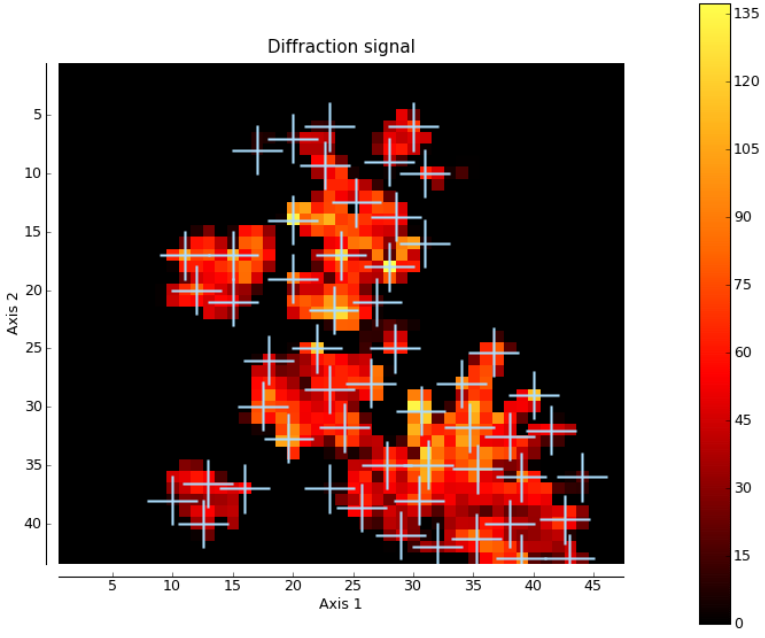
Low Symmetry: Monoclinic Lysozyme

Low Symmetry: Monoclinic Lysozyme

Crystallization Drop

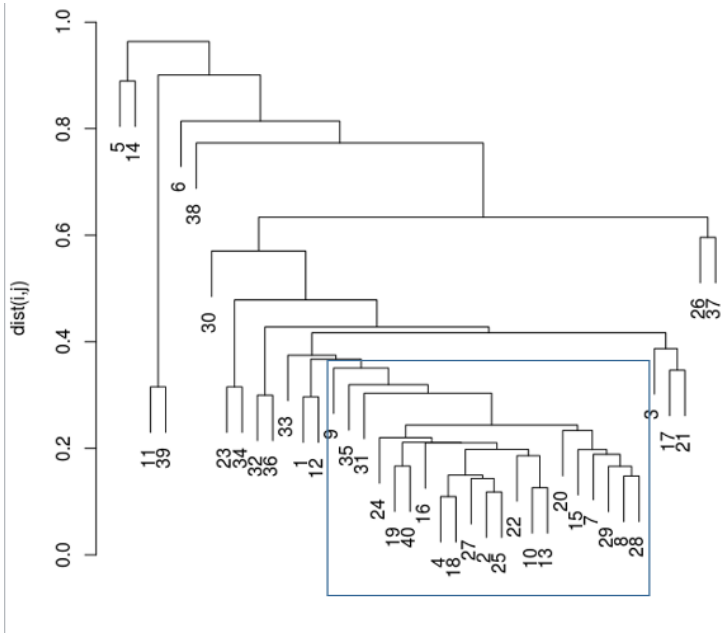
Low Symmetry: Monoclinic Lysozyme

Crystallization Drop

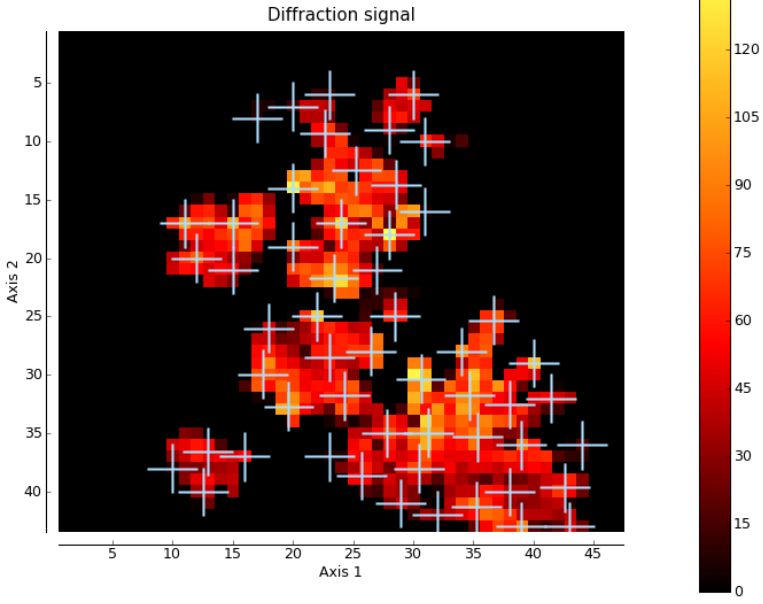


Heat Map after Mesh Scan:
54 spots picked for partial data collection

Low Symmetry: Monoclinic Lysozyme



hierarchical cluster analysis:
21 out of 40 integrated datasets selected for merging



Heat Map after Mesh Scan:
54 spots picked for partial data collection

Low Symmetry: Monoclinic Lysozyme

Statistics:

Space group: P21

Resolution: 19.73-1.59 (1.68-1.59)

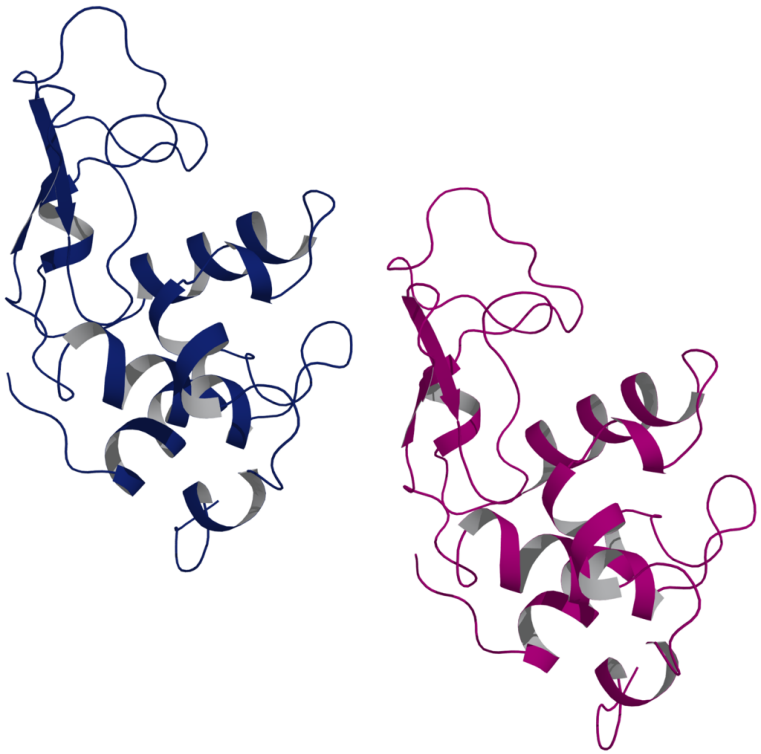
$R_{p.i.m.}$: 0.080 (0.486)

Completeness: 85.0 (82.1)

$< I > / \sigma < I >$: 8.0 (2.2)

R_{work}: 0.21291

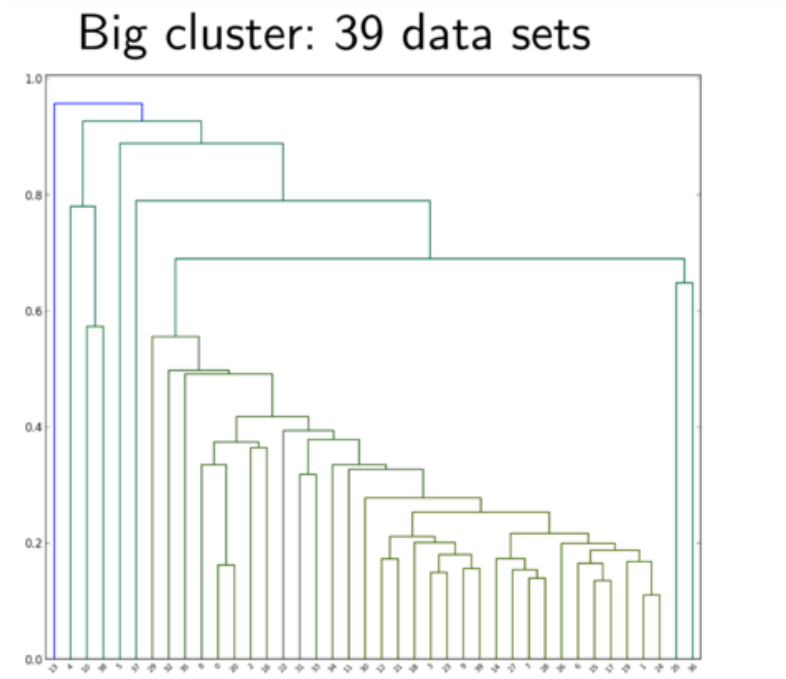
R_{free}: 0.26489



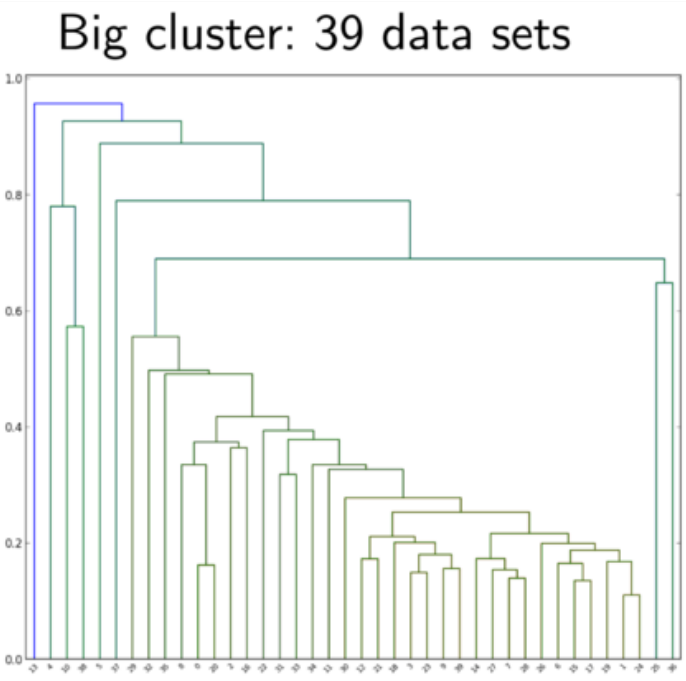
mC Lysozyme: Secondary Structure

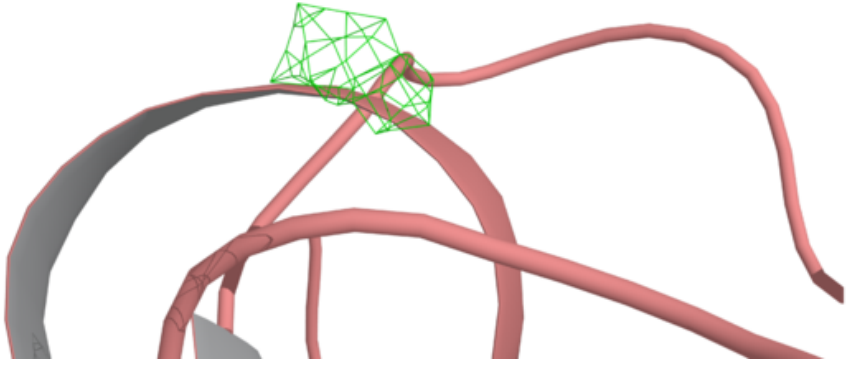
mC Lysozyme: Isomorphism Problem

mC Lysozyme: Isomorphism Problem



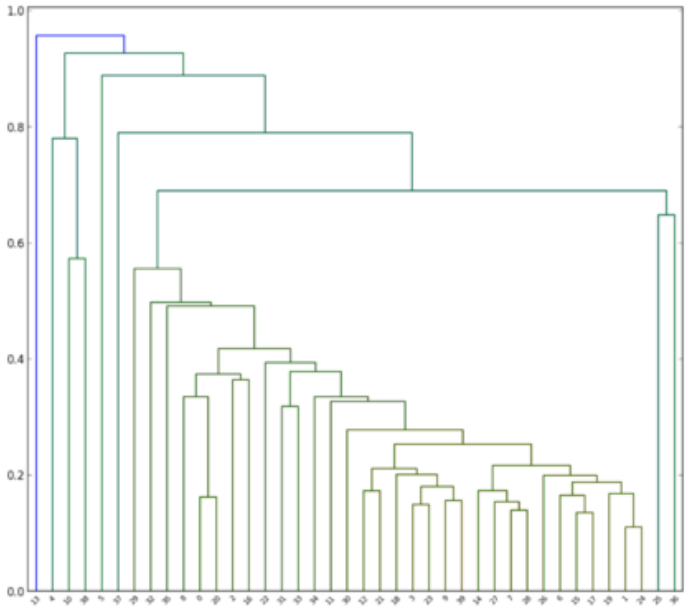
mC Lysozyme: Isomorphism Problem



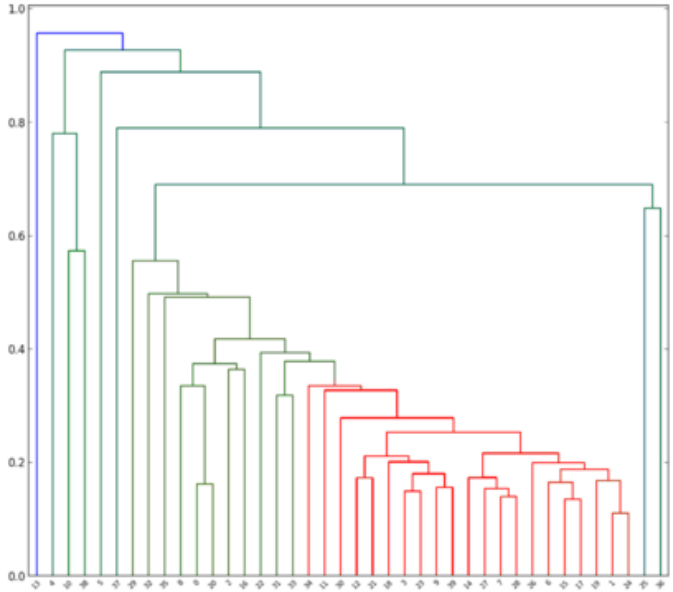


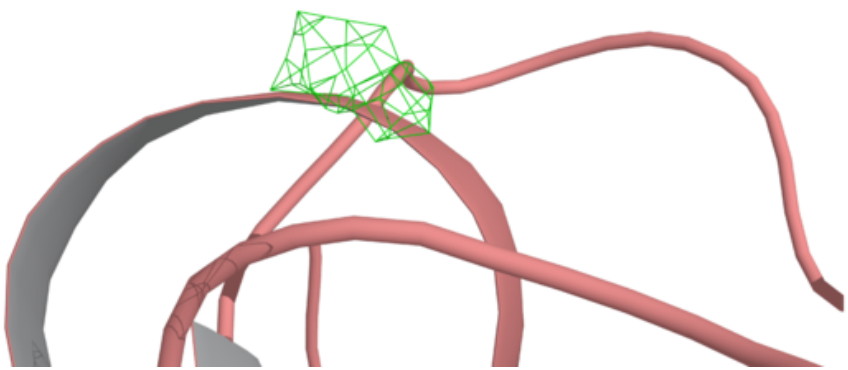
mC Lysozyme: Isomorphism Problem

Big cluster: 39 data sets



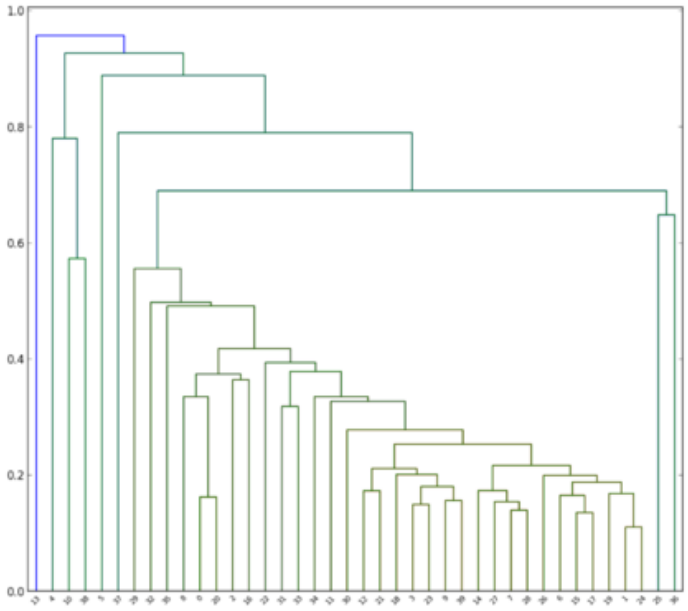
Small cluster: 21 data sets



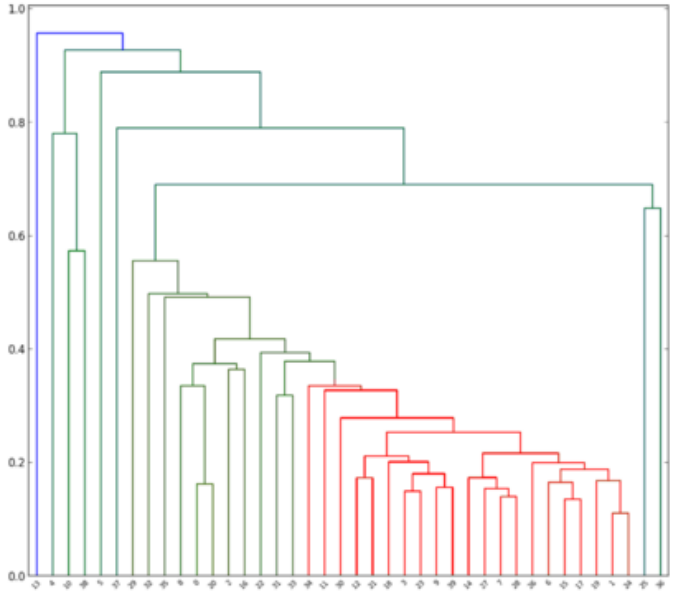


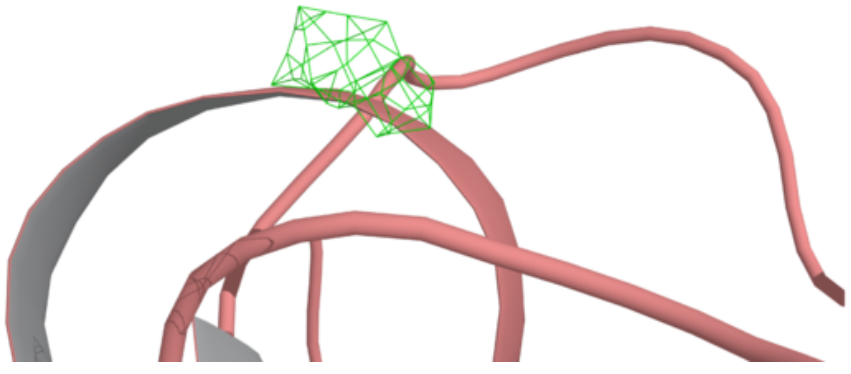
mC Lysozyme: Isomorphism Problem

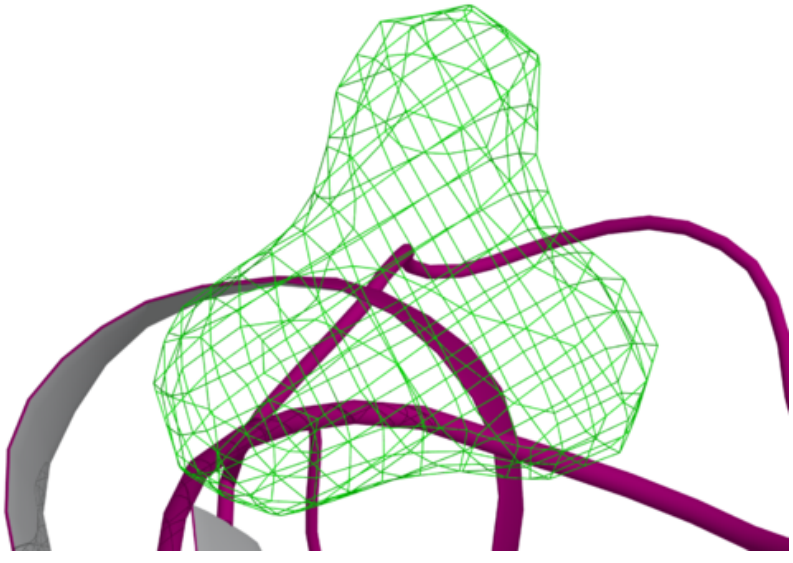
Big cluster: 39 data sets



Small cluster: 21 data sets

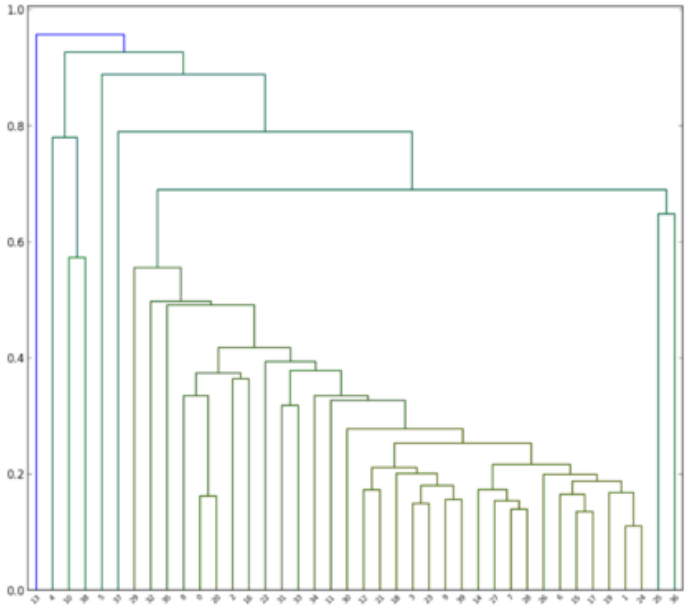




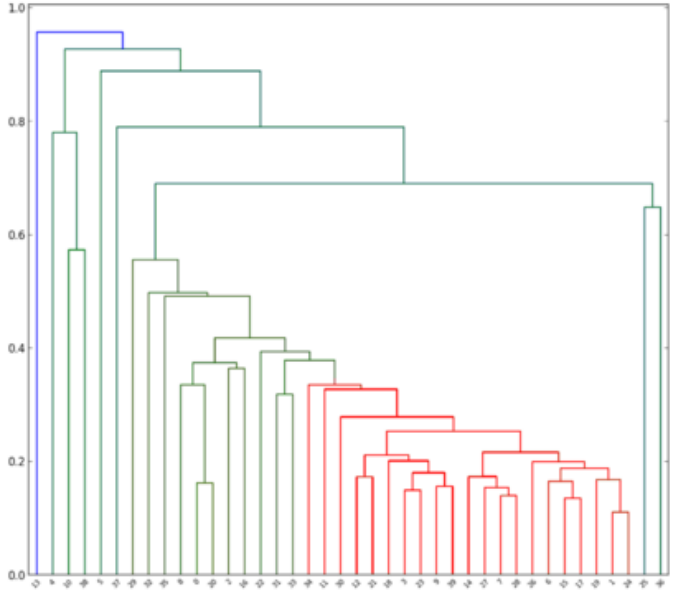


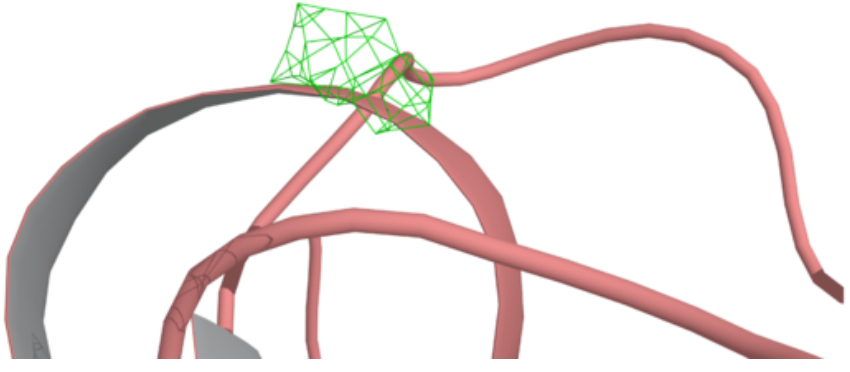
mC Lysozyme: Isomorphism Problem

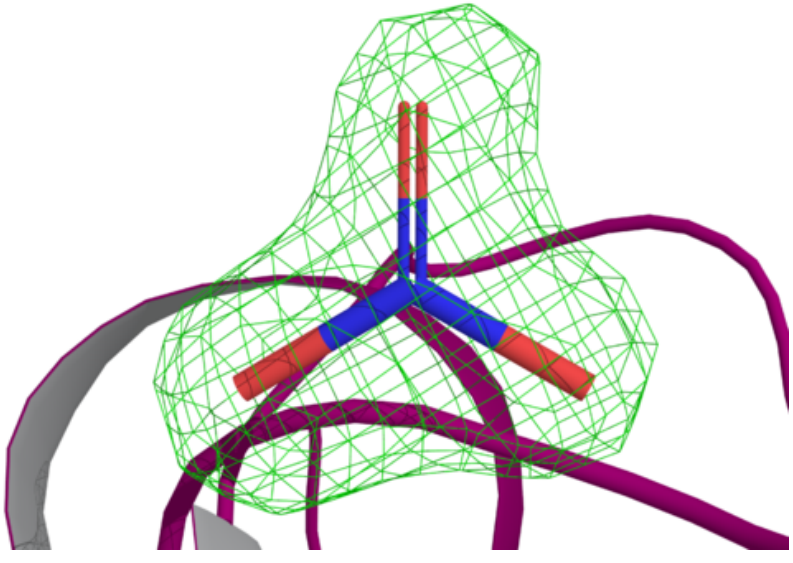
Big cluster: 39 data sets



Small cluster: 21 data sets

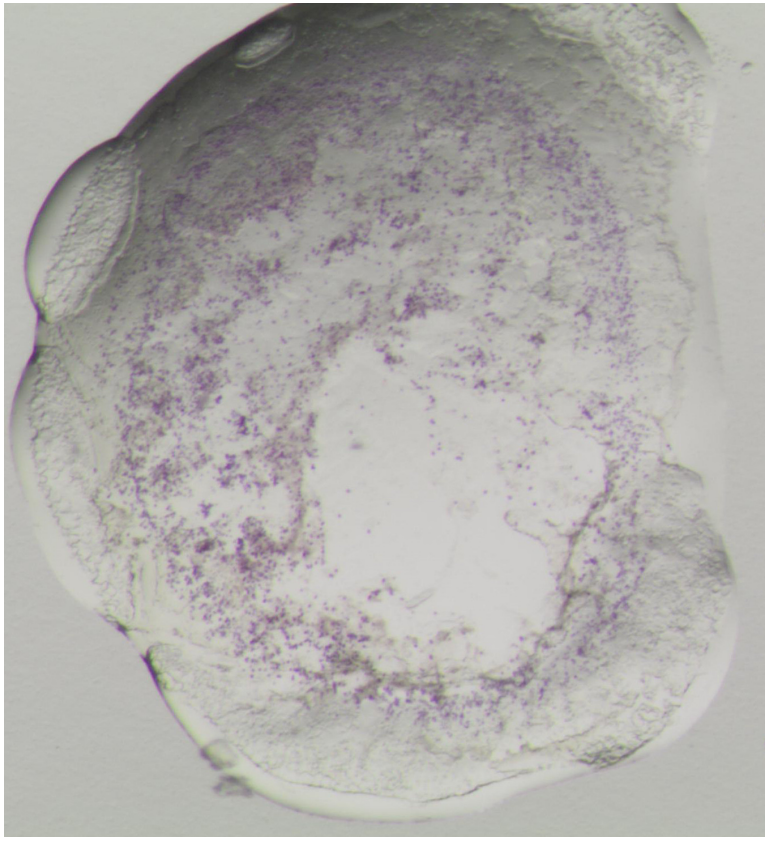






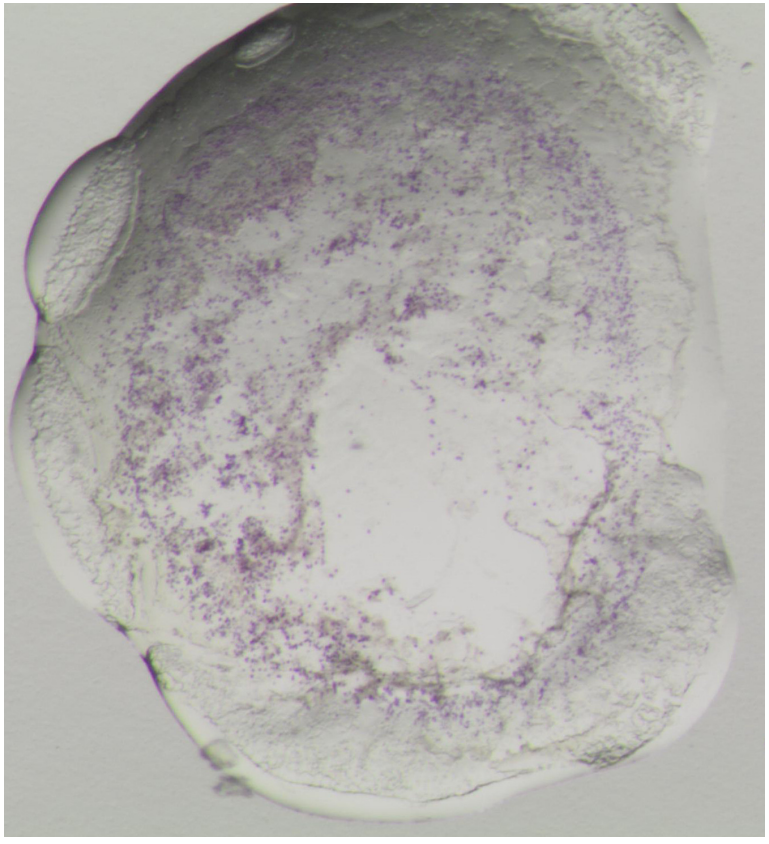
LCP Membrane Protein: Bacteriorhodopsin

LCP Membrane Protein: Bacteriorhodopsin

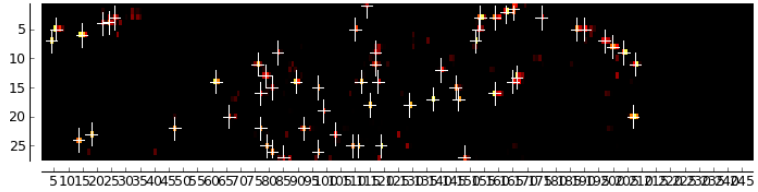


LCP Crystallization Drop, crystal size ca. 5 μm

LCP Membrane Protein: Bacteriorhodopsin

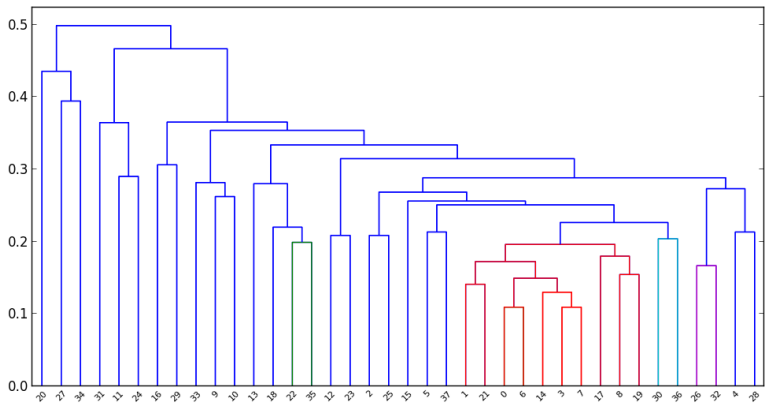


LCP Crystallization Drop, crystal size ca. 5 μm

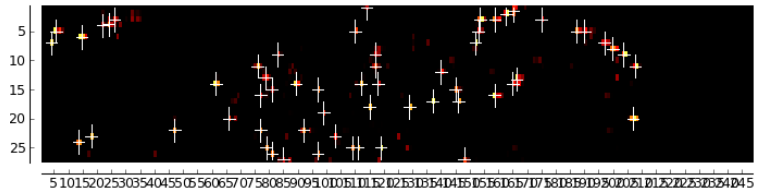


Heat Map after Mesh Scan:
59 spots picked for partial data collection

LCP Membrane Protein: Bacteriorhodopsin



hierarchical cluster analysis:
10 out of 38 integrated datasets
selected for merging



Heat Map after Mesh Scan:
59 spots picked for partial data
collection

LCP Membrane Protein: Bacteriorhodopsin

Statistics:

Space group: P 63

Resolution: 19.73-2.57 (2.71-2.57)

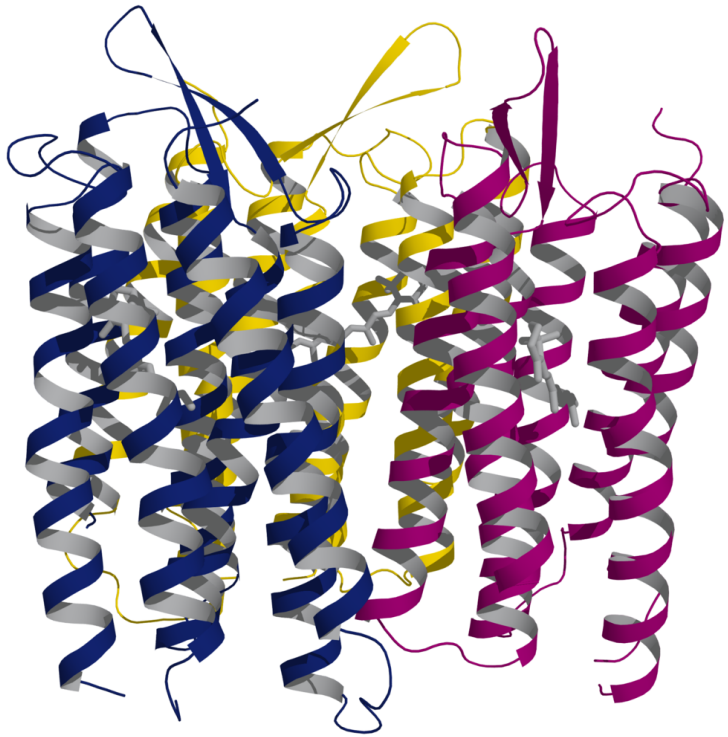
$R_{p.i.m.}$: 0.127 (0.546)

Completeness: 97.7 (87.1)

$< I > / \sigma < I >$:: 6.7 (1.8)

R_{work}: 0.18983

R_{free}: 0.20547



Bacteriorhodopsin:
Biological Assembly

LCP Membrane Protein: Bacteriorhodopsin

Statistics:

Space group: P 63

Resolution: 19.73-2.57 (2.71-2.57)

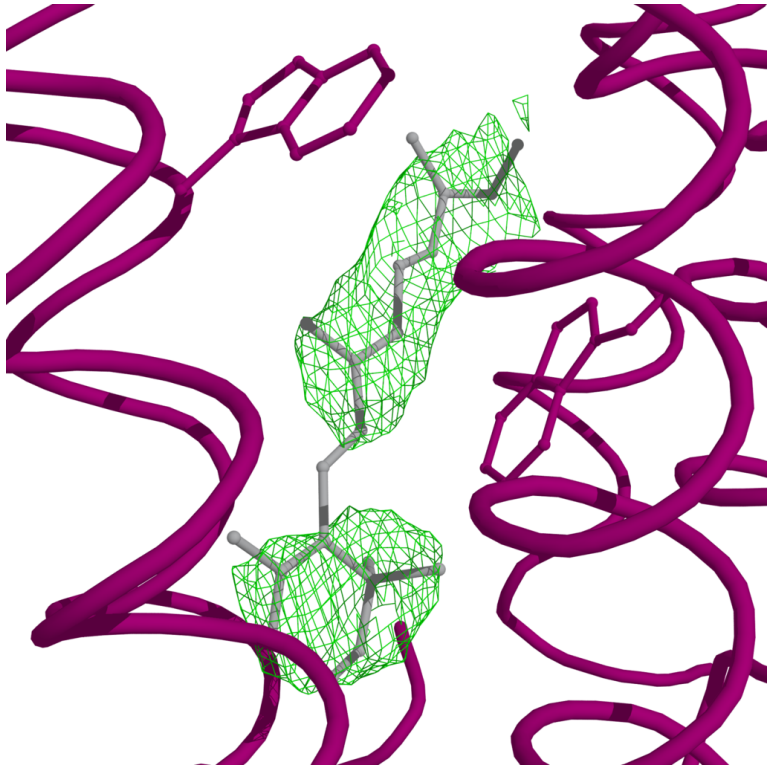
$R_{p.i.m.}$: 0.127 (0.546)

Completeness: 97.7 (87.1)

$< I > / \sigma < I >$:: 6.7 (1.8)

R_{work}: 0.18983

R_{free}: 0.20547



Retinal Cofactor in Fo-Fc map (cutoff 2σ)

Publication

BIOLOGICAL
CRYSTALLOGRAPHY

ISSN 1399-0047

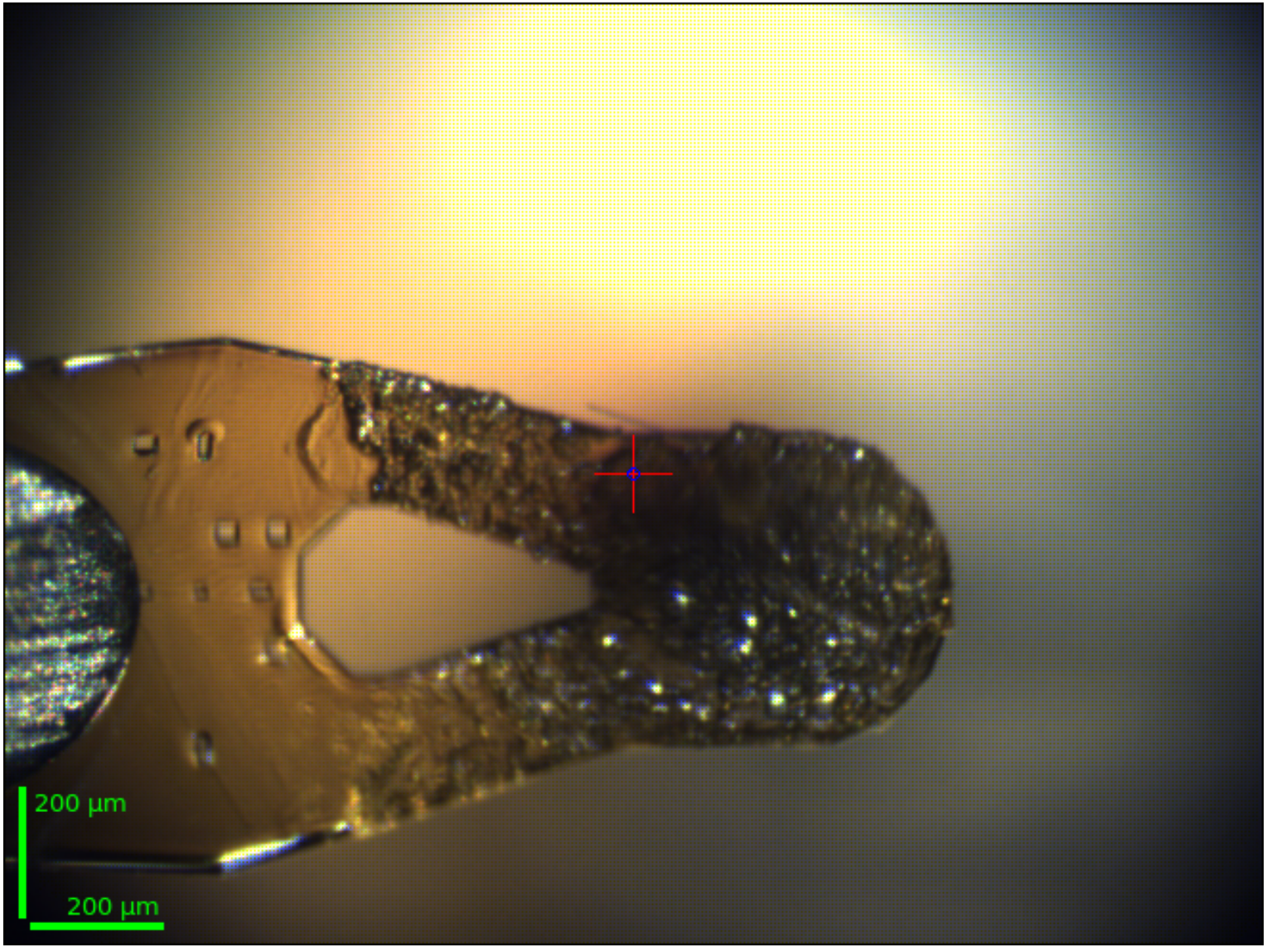
MeshAndCollect: an automated multi-crystal data-collection workflow for synchrotron macromolecular crystallography beamlines

Ulrich Zander,[a] Gleb Bourenkov,[b] Alexander N. Popov,[a] Daniele de Sanctis,[a] Olof Svensson,[a] Andrew A. McCarthy,[c,d] Ekaterina Round,[e,f,g,h,i] Valentin Gordeliy,[e,f,g,h,i] Christoph Mueller-Dieckmann[a] and Gordon A. Leonard[a]*

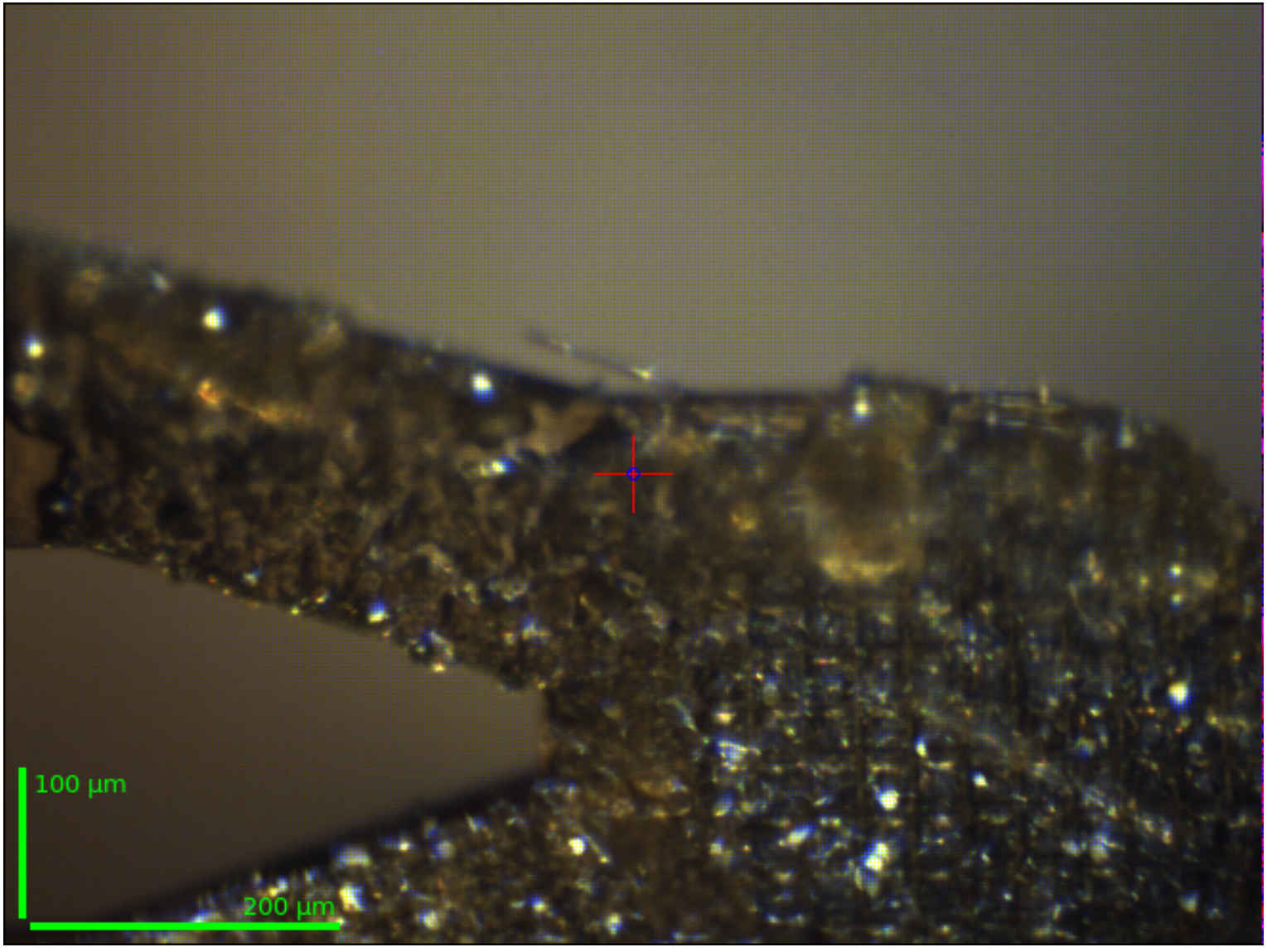
Received 19 June 2015
Accepted 24 September 2015

Proteinase K SAD by halide soaking: NaBr

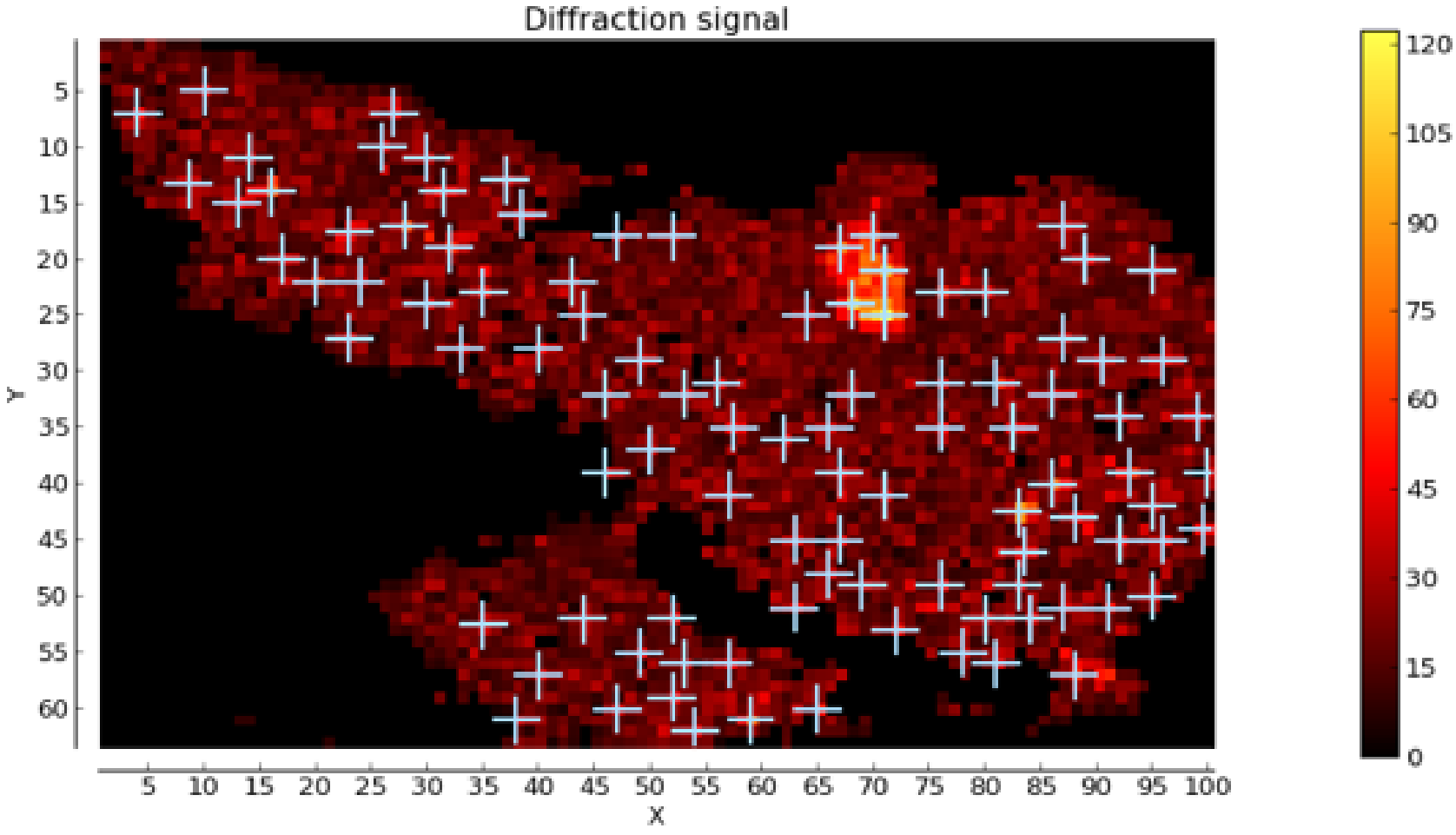
Proteinase K SAD by halide soaking: NaBr



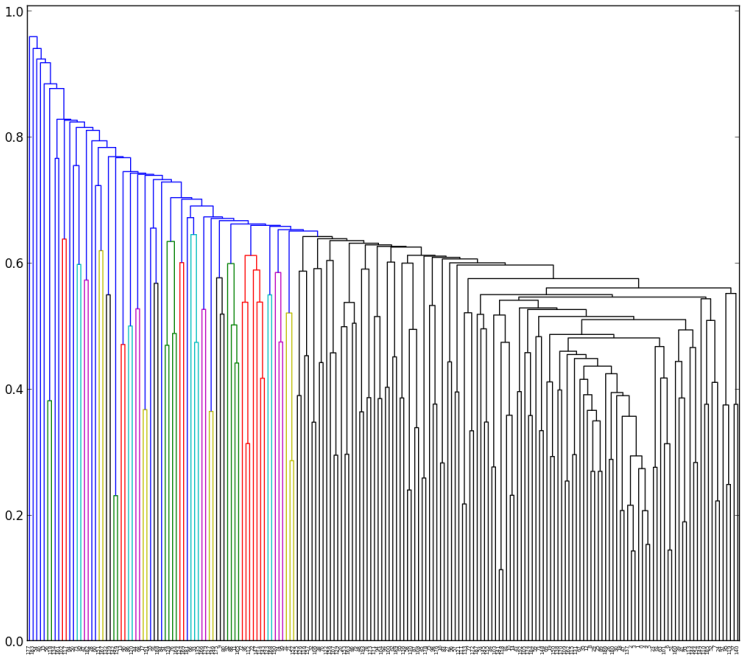
Proteinase K SAD by halide soaking: NaBr



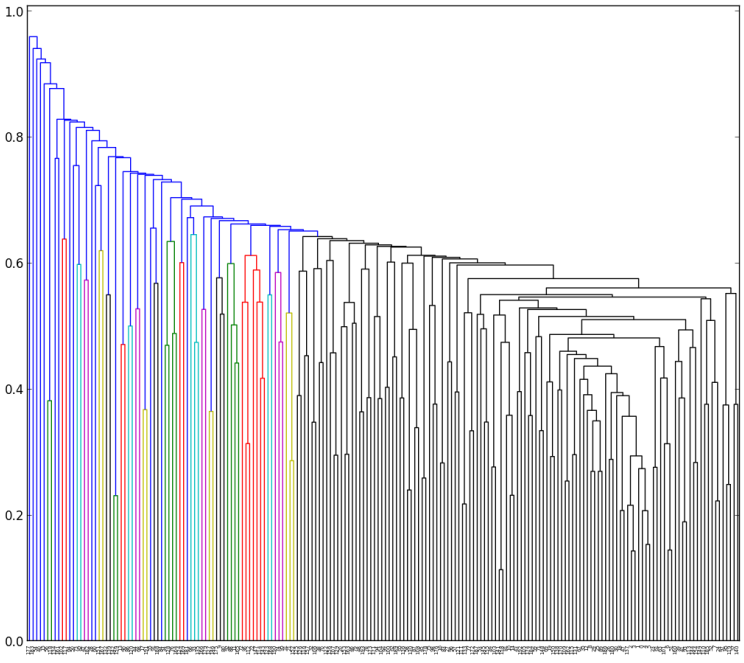
Proteinase K SAD by halide soaking: NaBr



Proteinase K SAD by halide soaking: NaBr

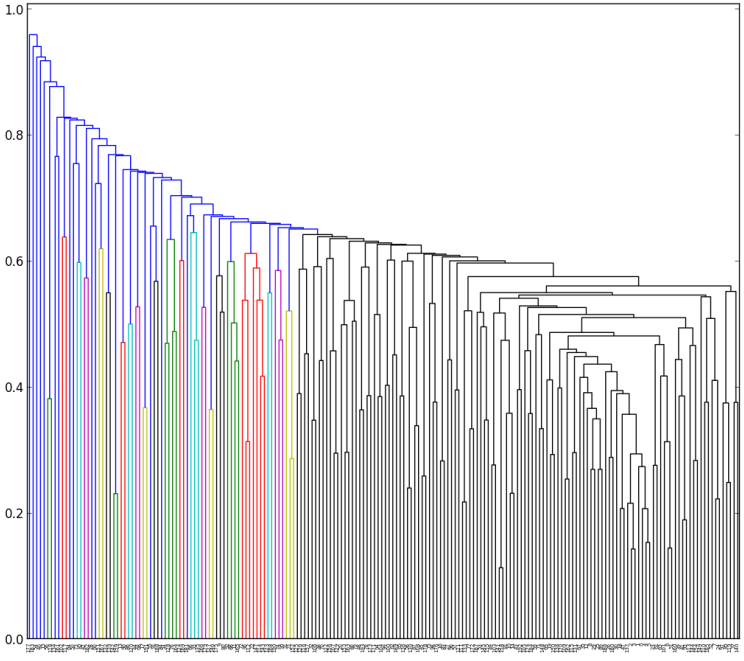


Proteinase K SAD by halide soaking: NaBr



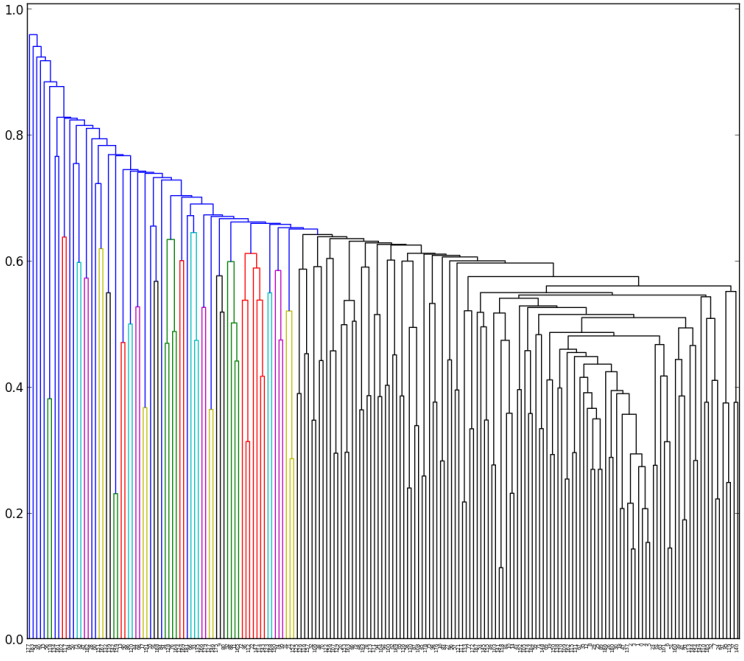
- High multiplicity: 122 datasets

Proteinase K SAD by halide soaking: NaBr



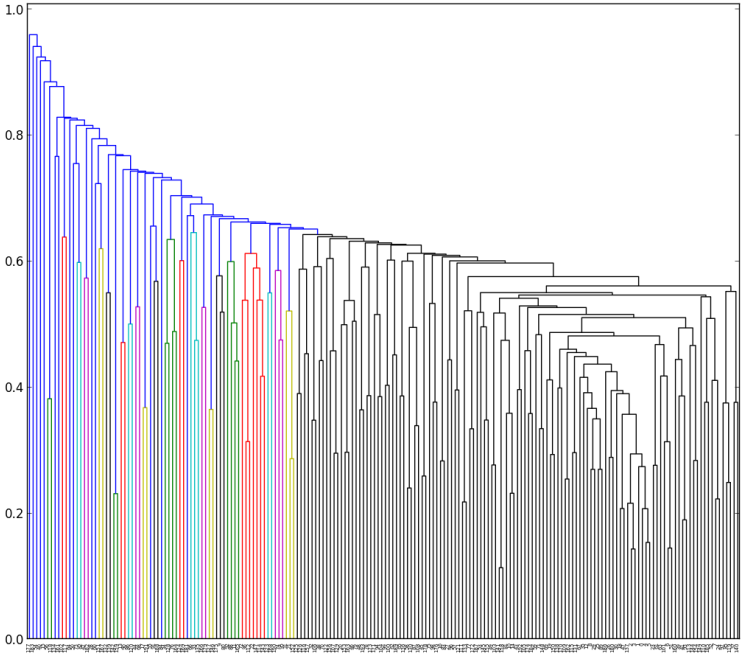
- High multiplicity: 122 datasets
- Substructure determination, Initial phasing

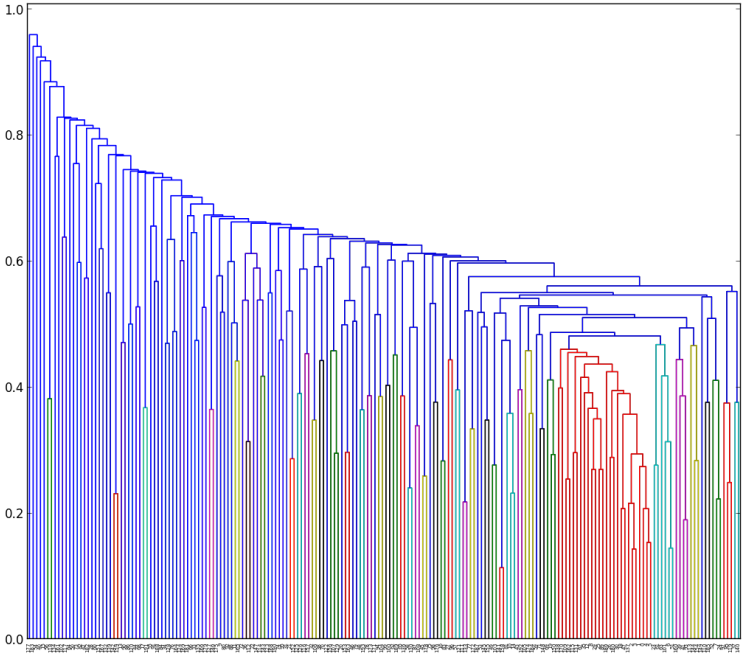
Proteinase K SAD by halide soaking: NaBr



- High multiplicity: 122 datasets
- Substructure determination, Initial phasing

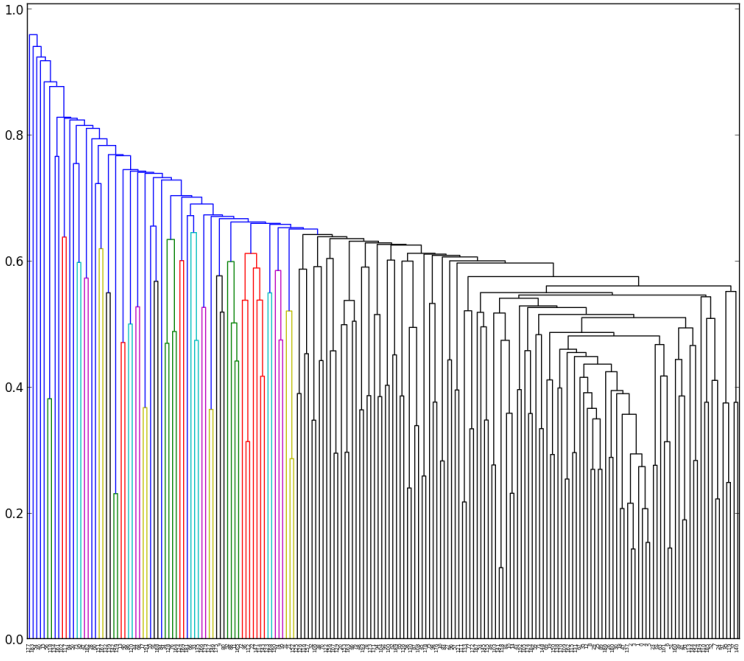
Proteinase K SAD by halide soaking: NaBr



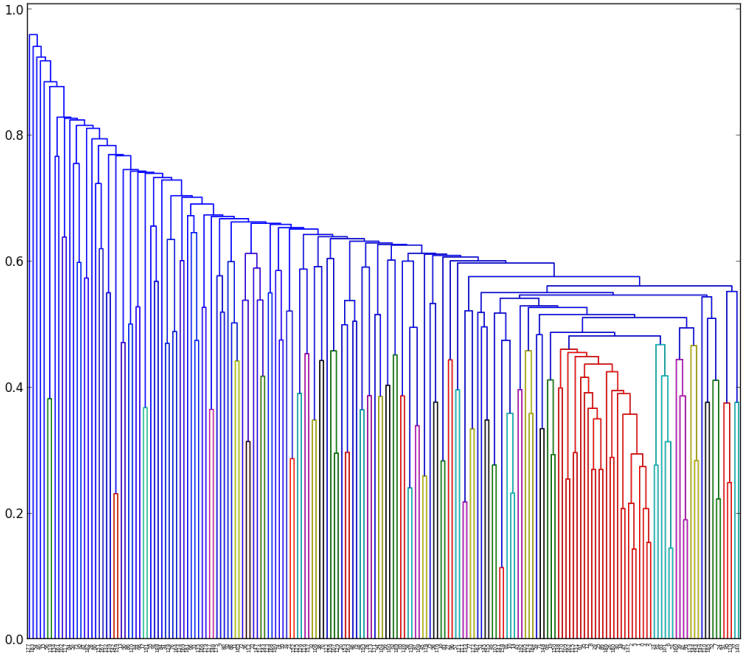


- High multiplicity: 122 datasets
- Substructure determination, Initial phasing

Proteinase K SAD by halide soaking: NaBr

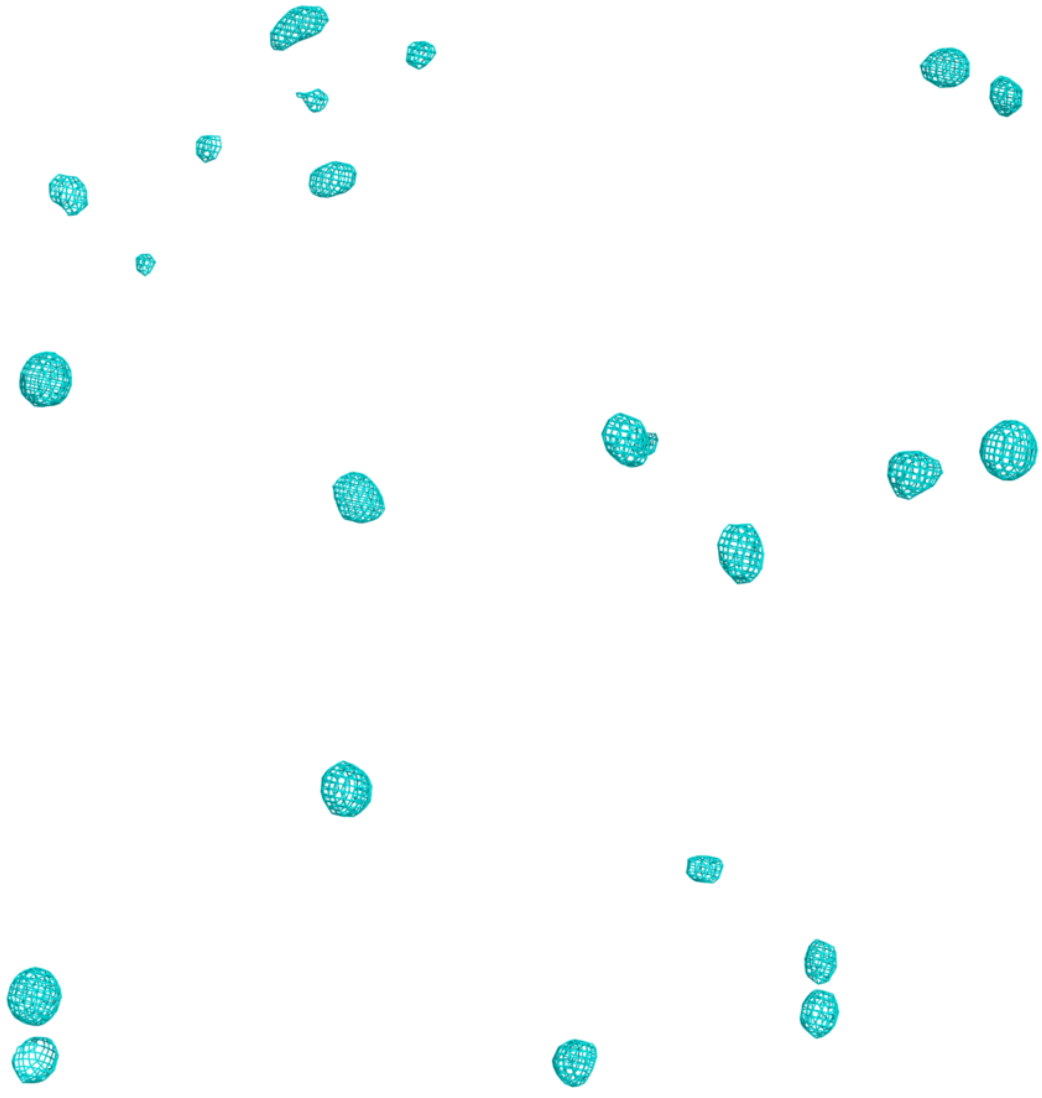


- High multiplicity: 122 datasets
- Substructure determination, Initial phasing

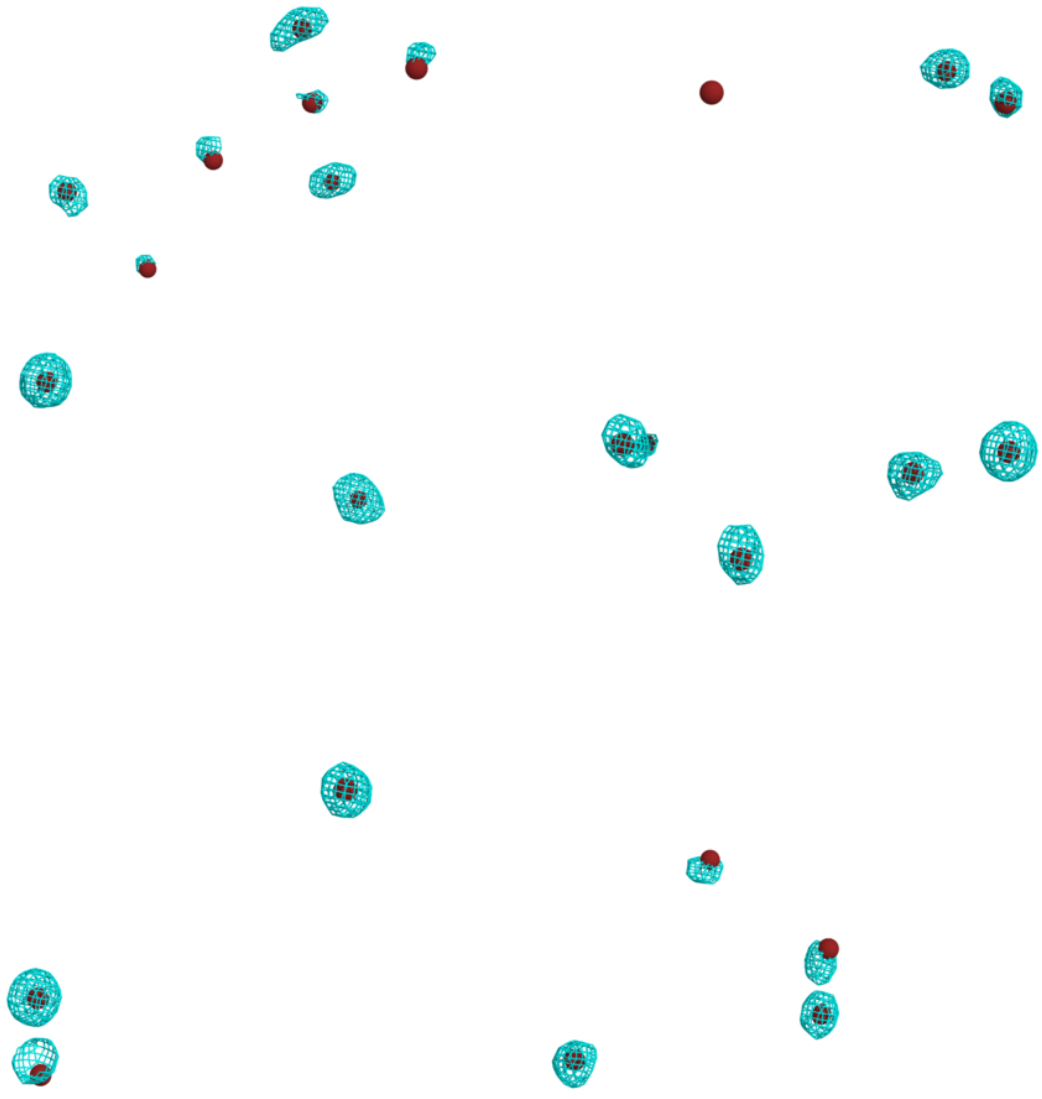


- High isomorphism: 26 Datasets

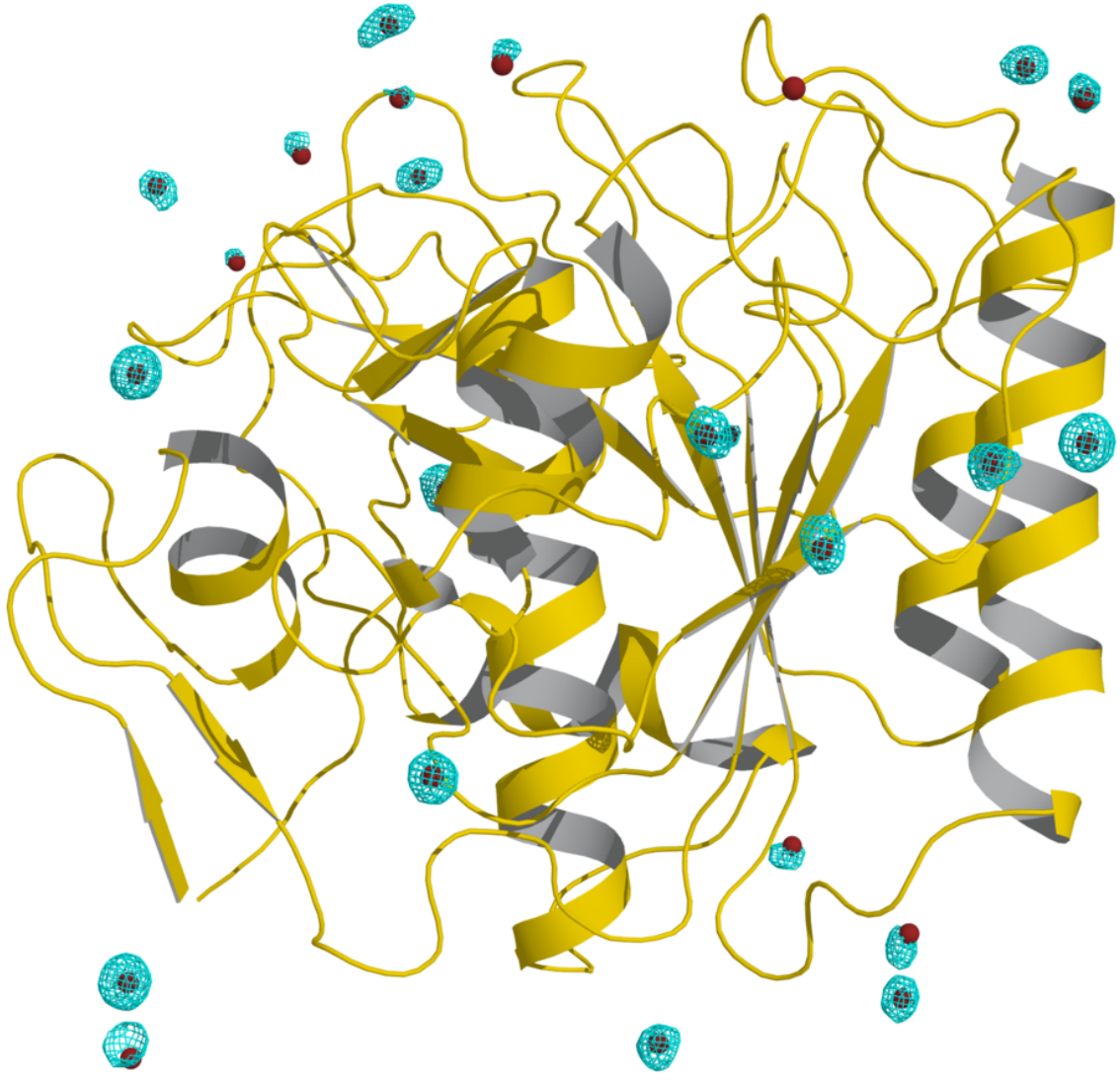
Proteinase K SAD by halide soaking: NaBr



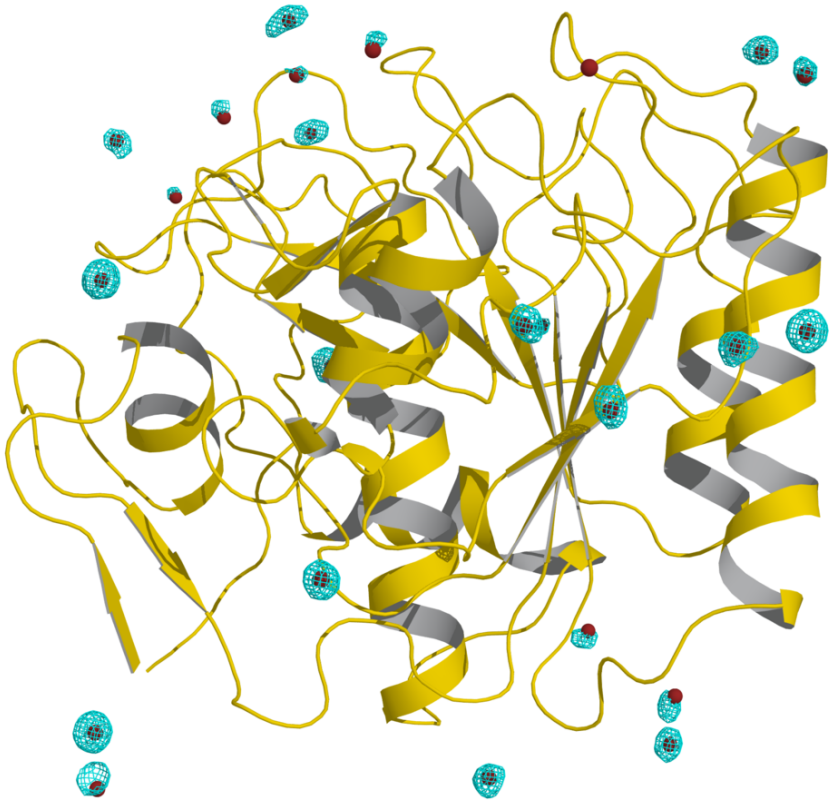
Proteinase K SAD by halide soaking: NaBr



Proteinase K SAD by halide soaking: NaBr



Proteinase K SAD by halide soaking: NaBr



Proteinase K SAD by halide soaking: NaBr

Statistics:

Space group: P 43212

Resolution: 19.79-1.40 (1.42-1.40)

$R_{p.i.m.}$: 0.060 (0.307)

Completeness: 99.7 (100)

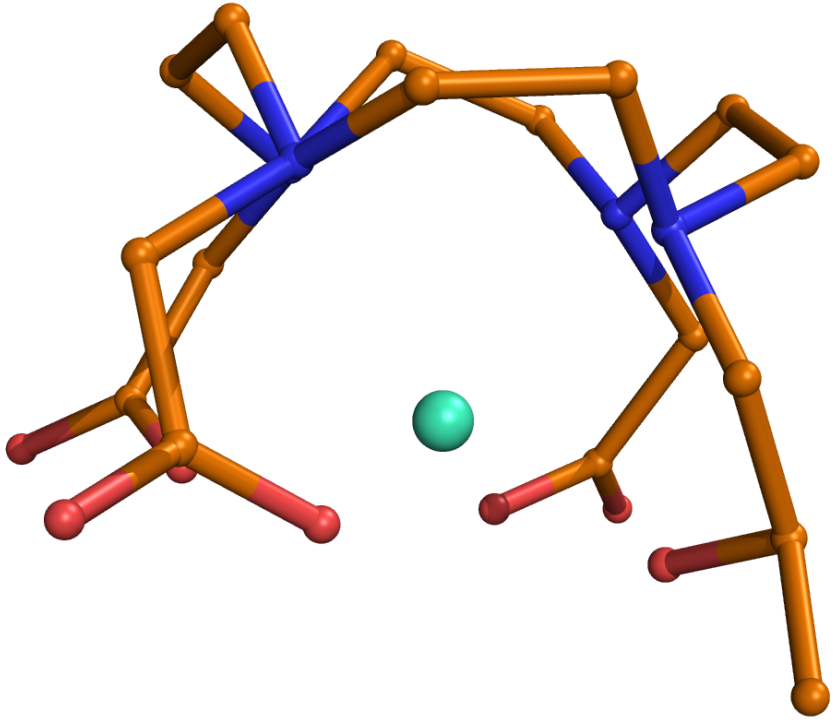
$< I > / \sigma < I >$: 11.9 (5)

R_{work}: 0.15085

R_{free}: 0.17166

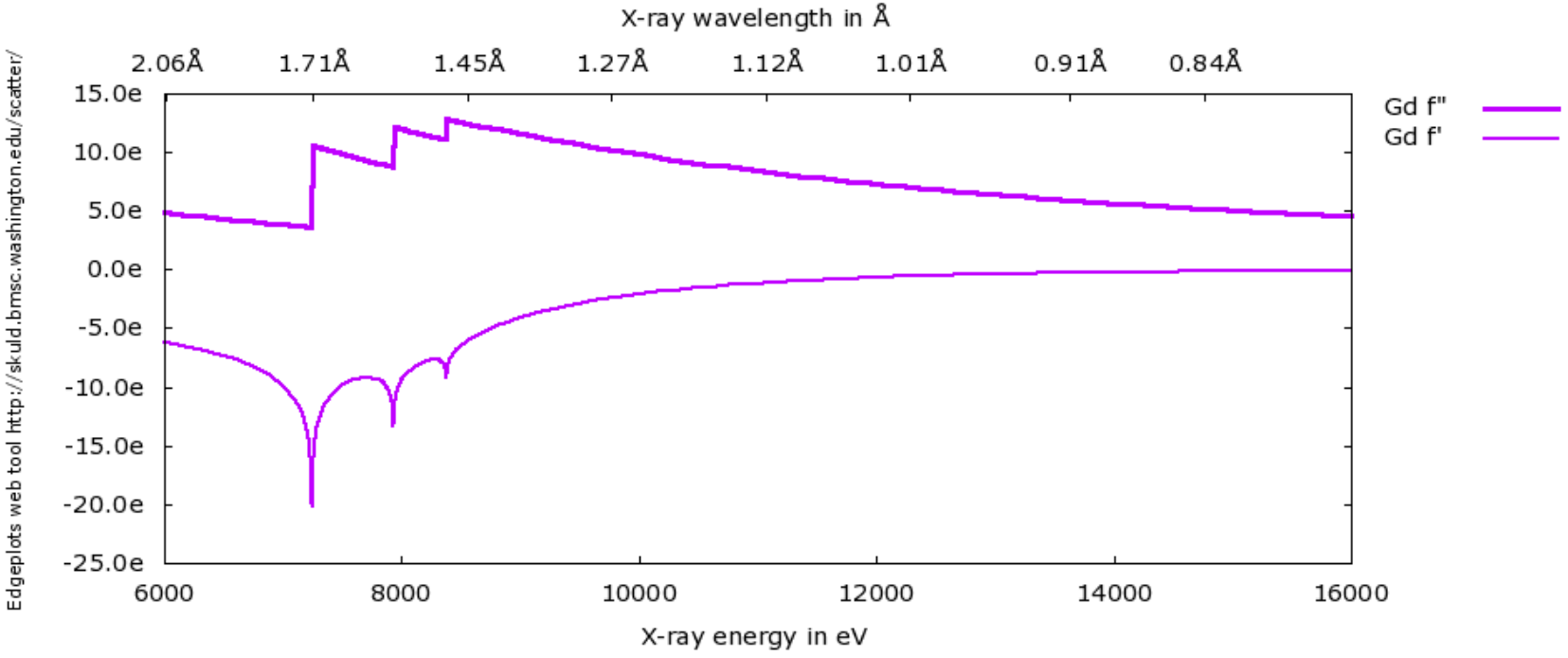
Lysozyme Gd-SAD

Lysozyme Gd-SAD

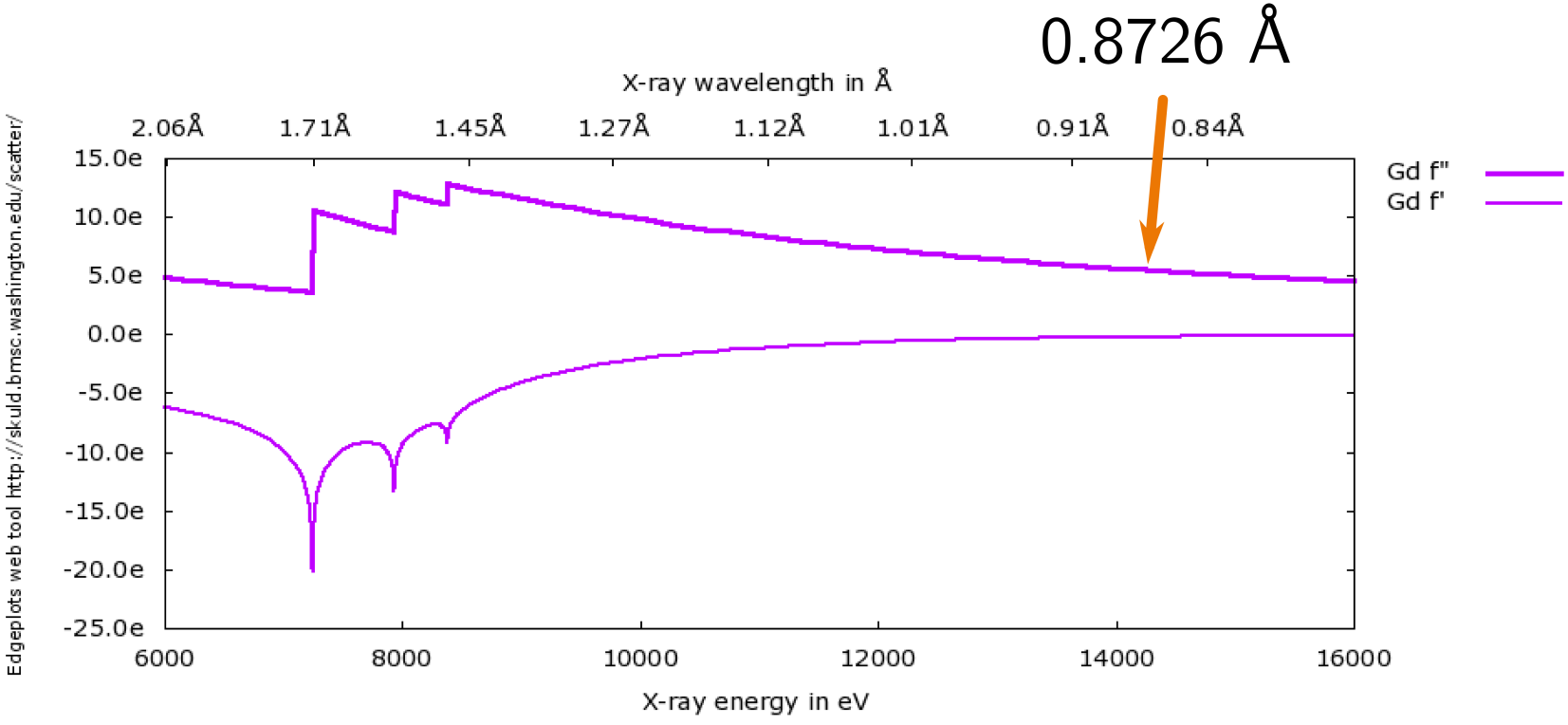


Gd-HPDO3A (Jena Bioscience)

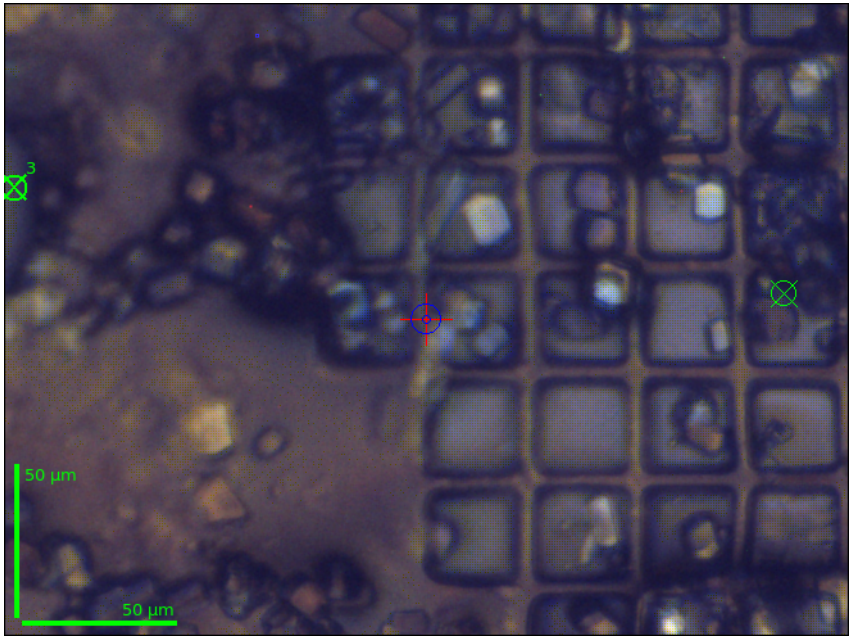
Lysozyme Gd-SAD



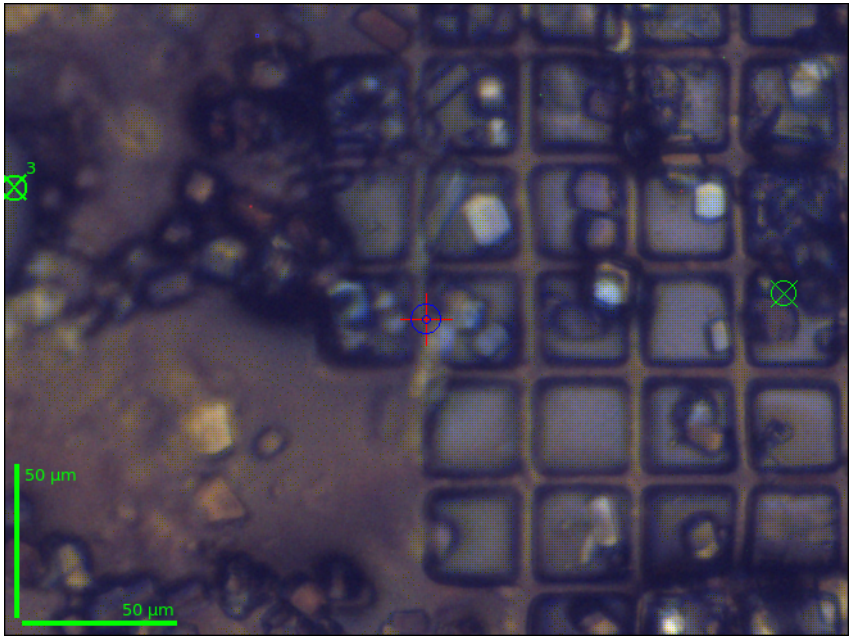
Lysozyme Gd-SAD

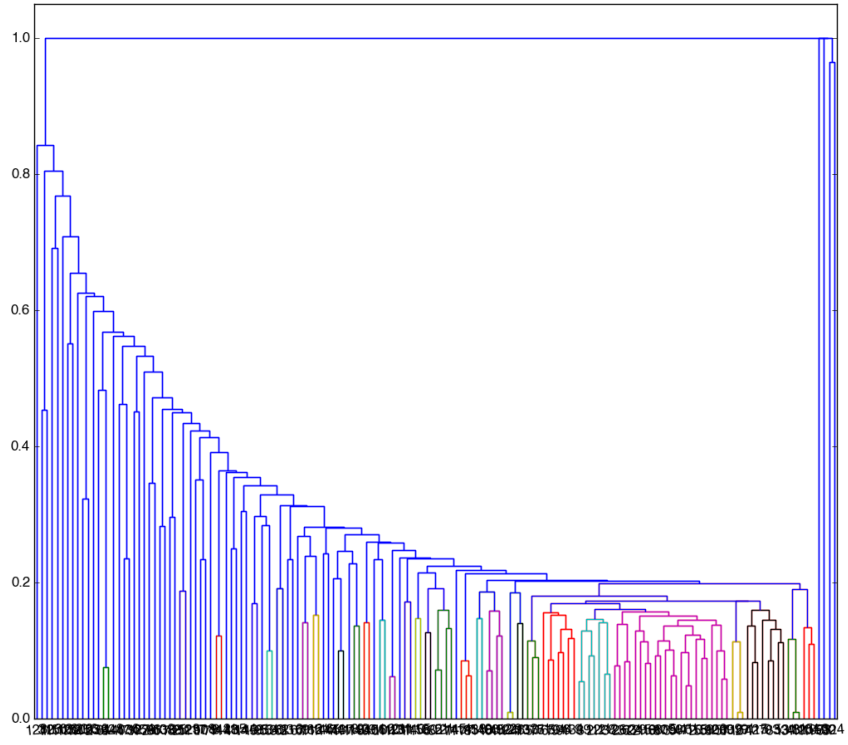


Lysozyme Gd-SAD

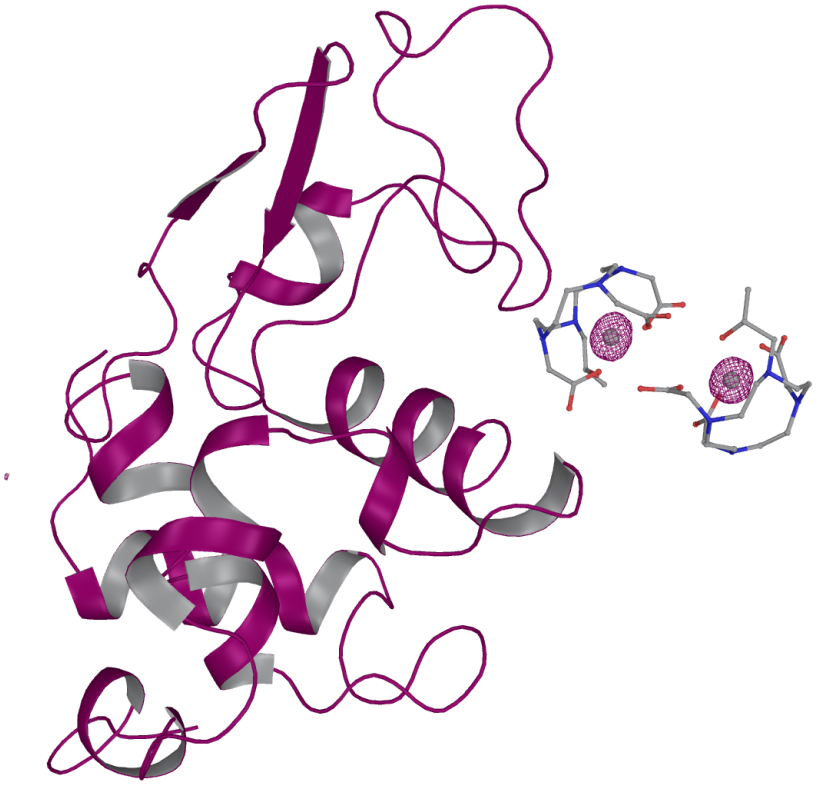


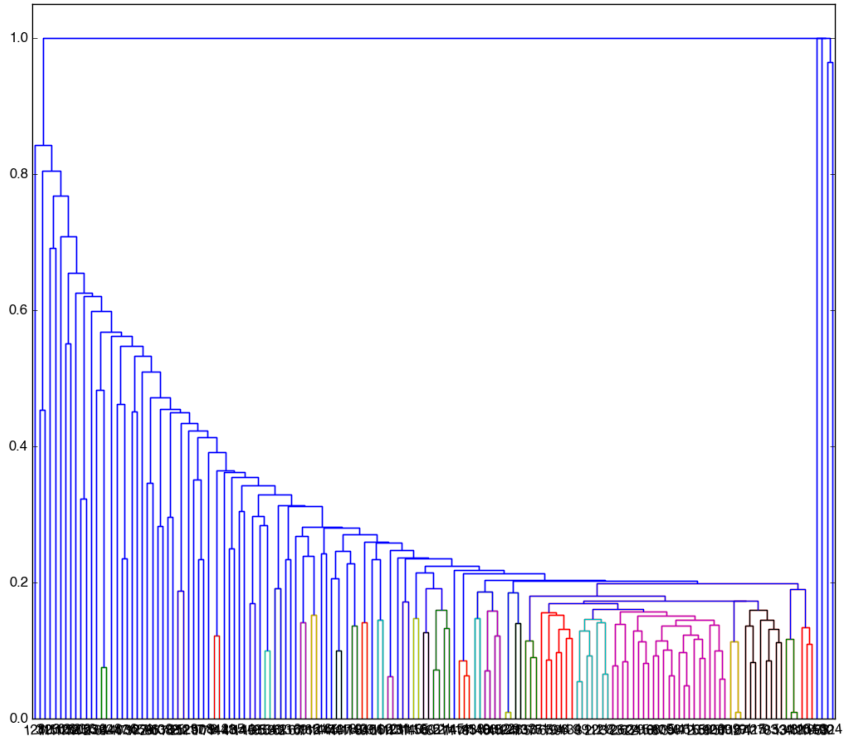
Lysozyme Gd-SAD



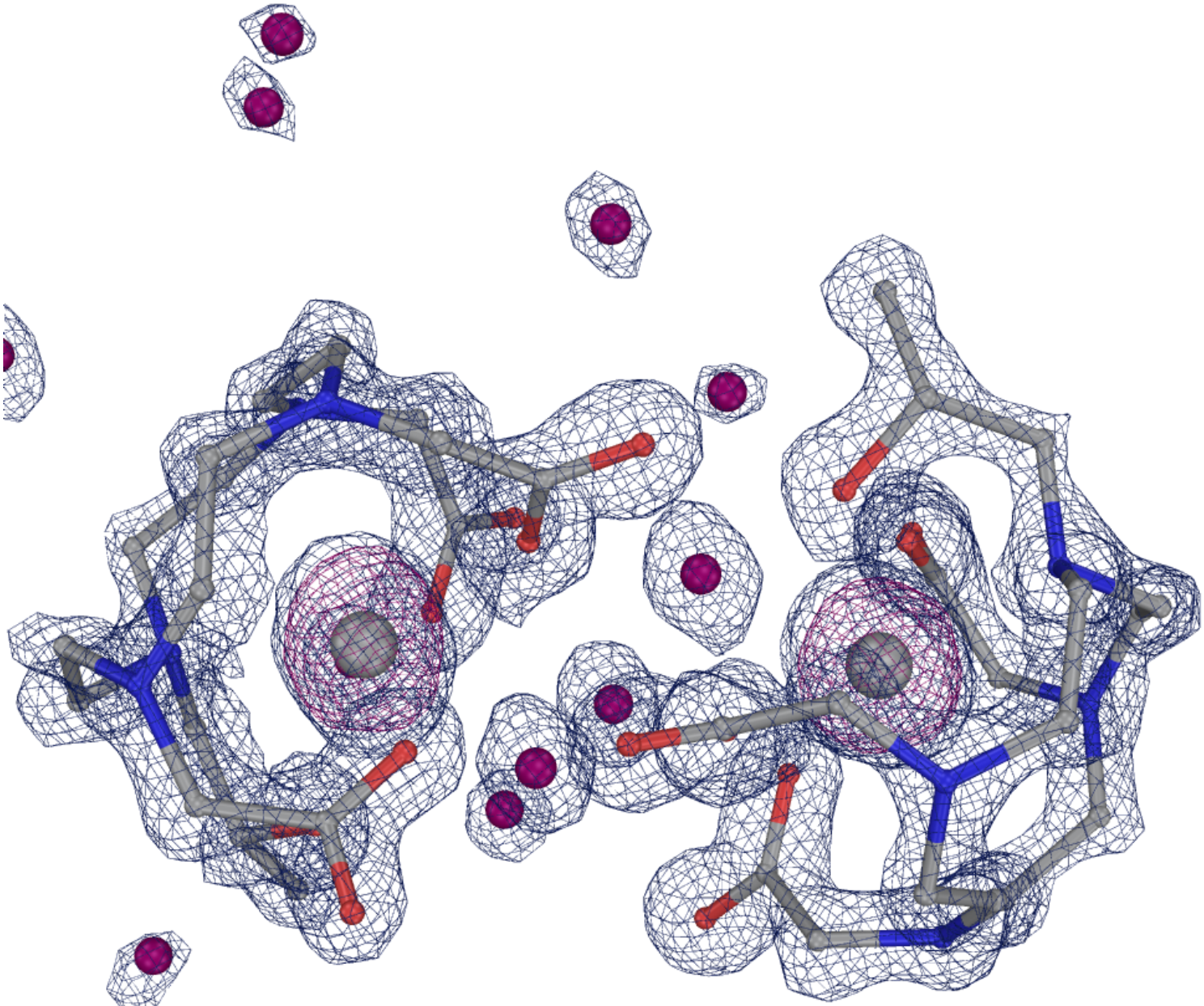


Lysozyme Gd-SAD

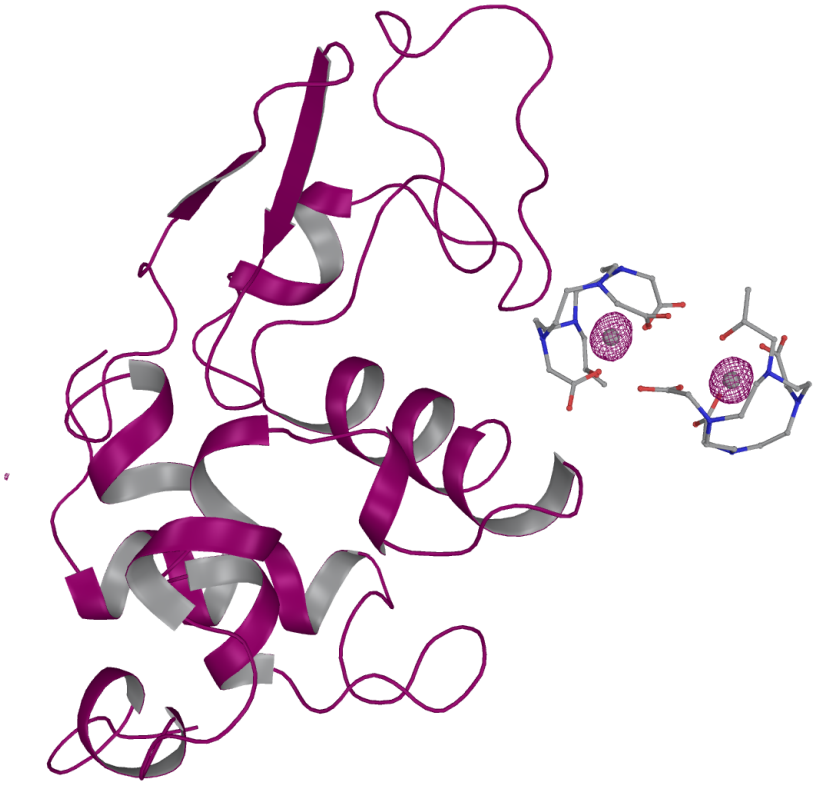




Lysozyme Gd-SAD



Lysozyme Gd-SAD



Statistics:

Space group: P 43212

Resolution: 19.45-1.2 (1.22-1.20)

$R_{p.i.m.}$: 0.040 (0.766)

Completeness: 100.0 (99.8)

Multiplicity: 15.8 (11.8)

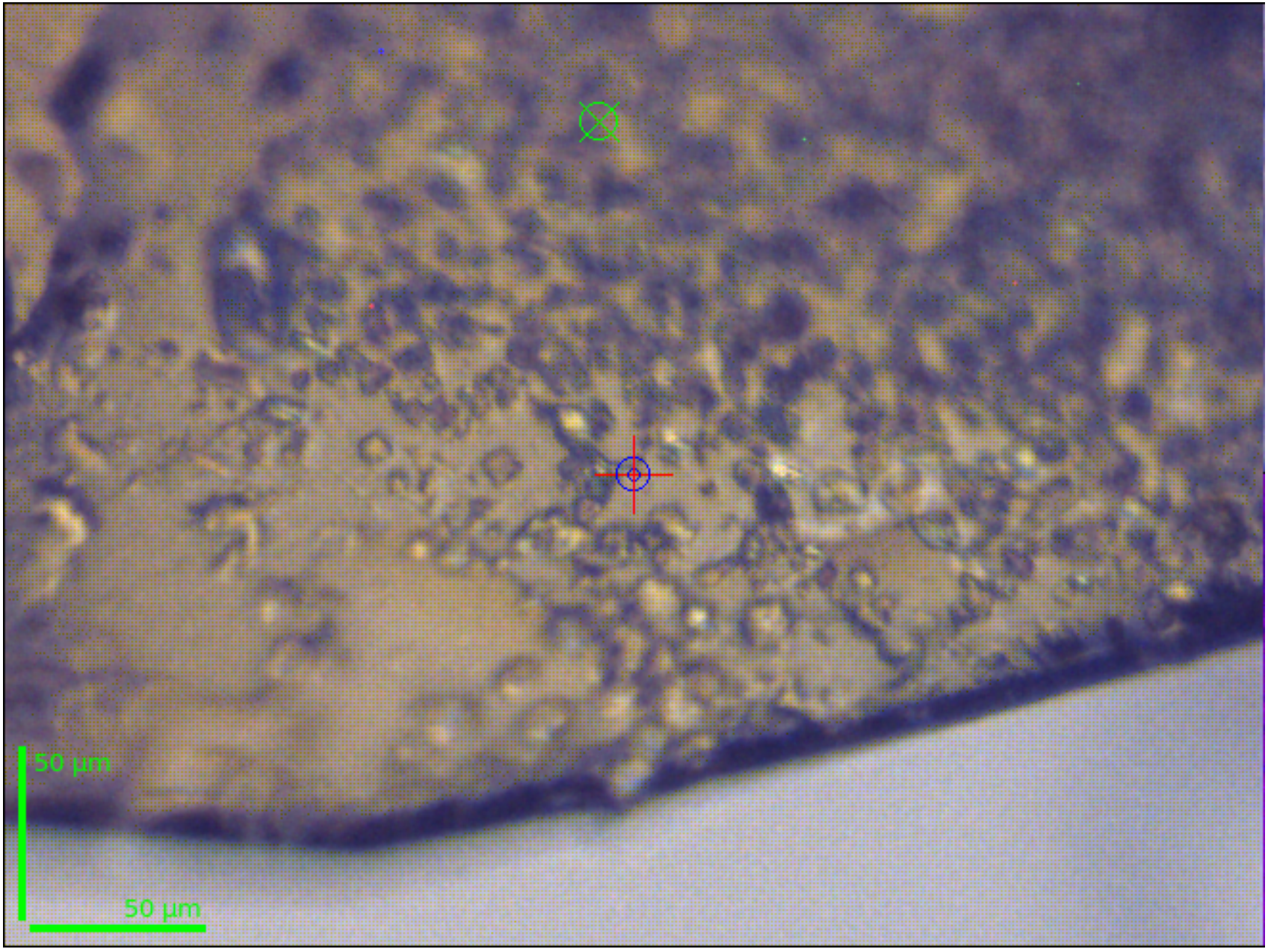
$< I > / \sigma < I >$: 11.5 (1.5)

R_{work}: 0.17319

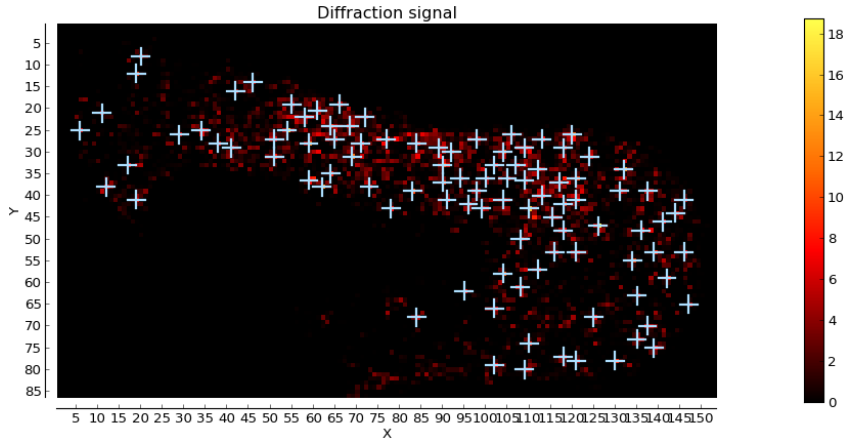
R_{free}: 0.17802

Insulin Gd-SAD

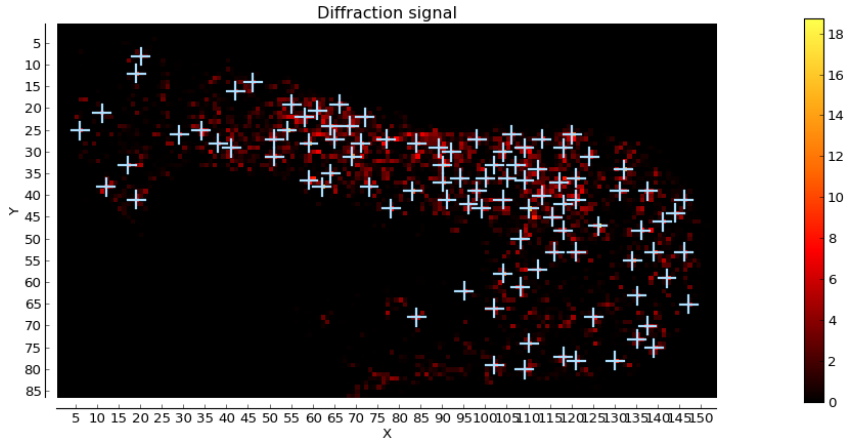
Insulin Gd-SAD

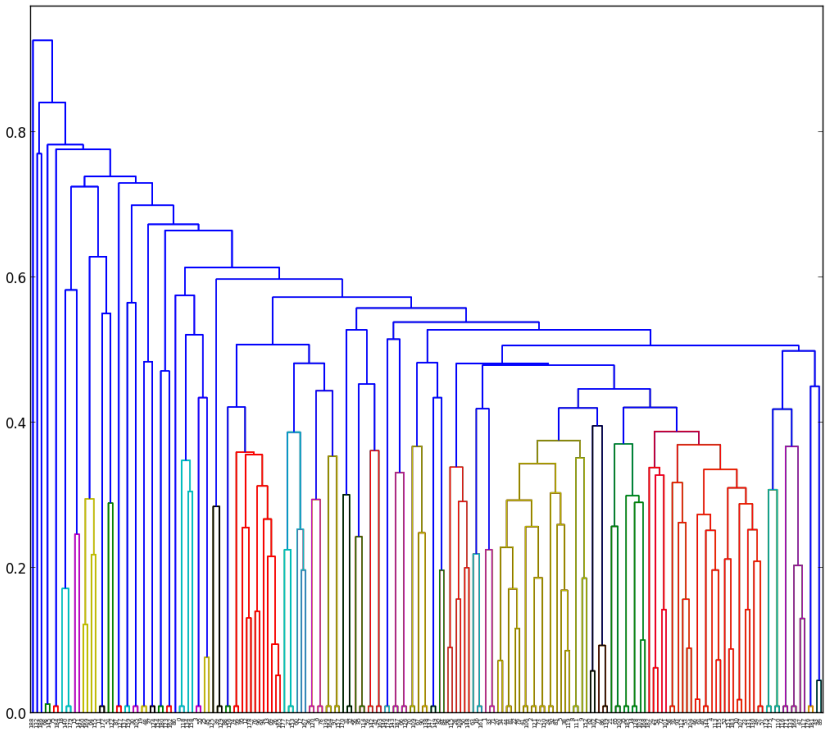


Insulin Gd-SAD

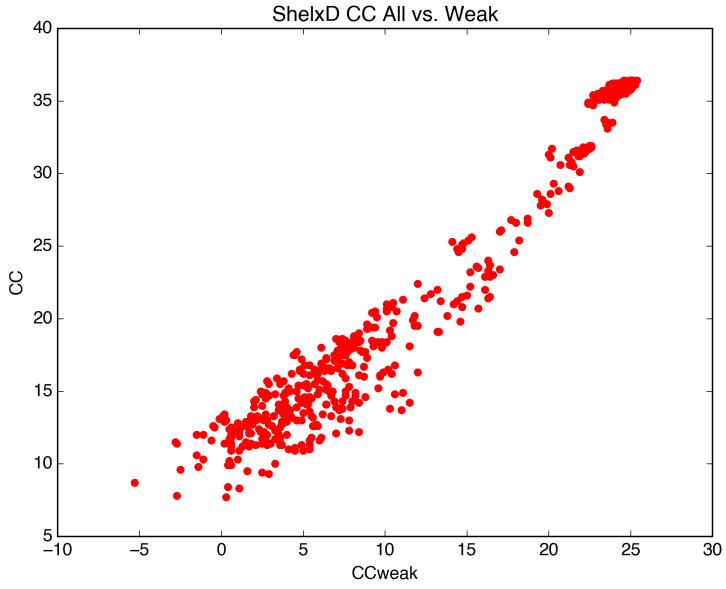


Insulin Gd-SAD

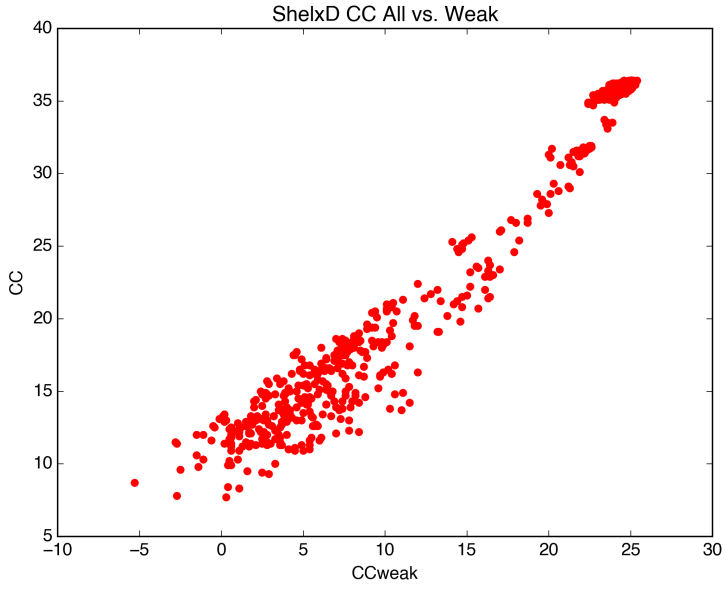


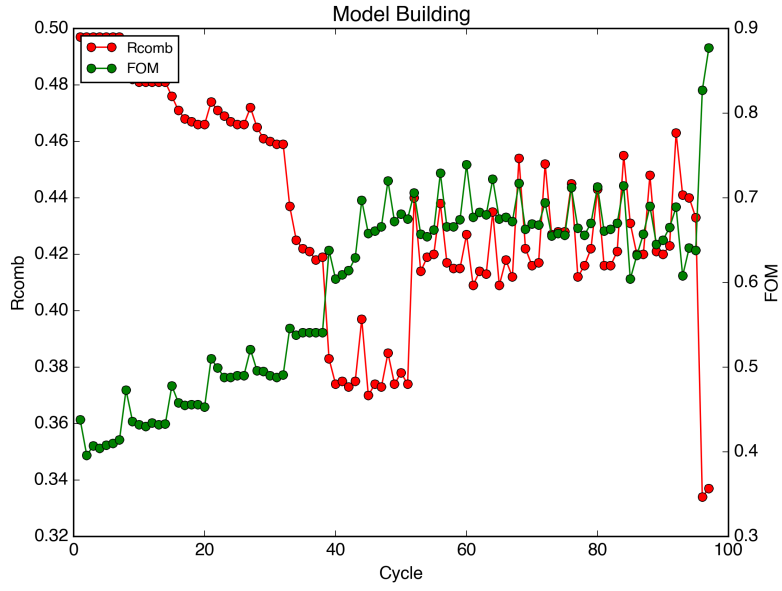


Insulin Gd-SAD

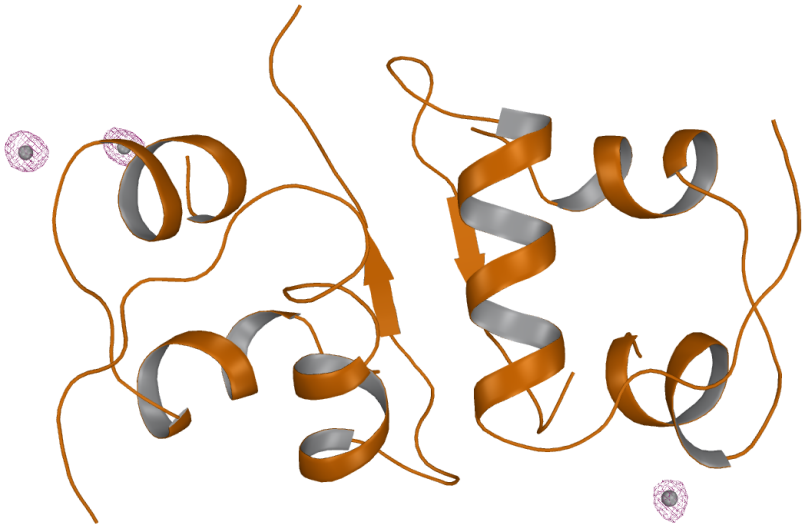


Insulin Gd-SAD

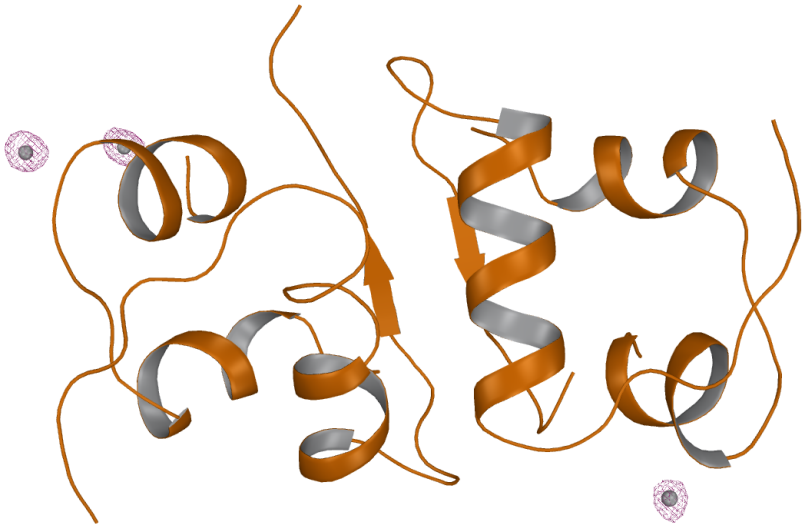


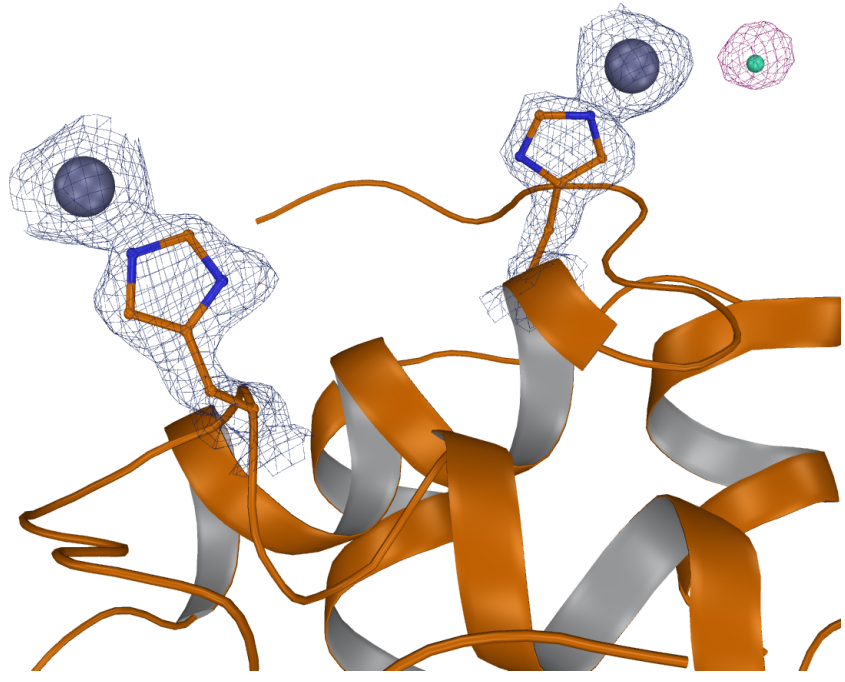


Insulin Gd-SAD

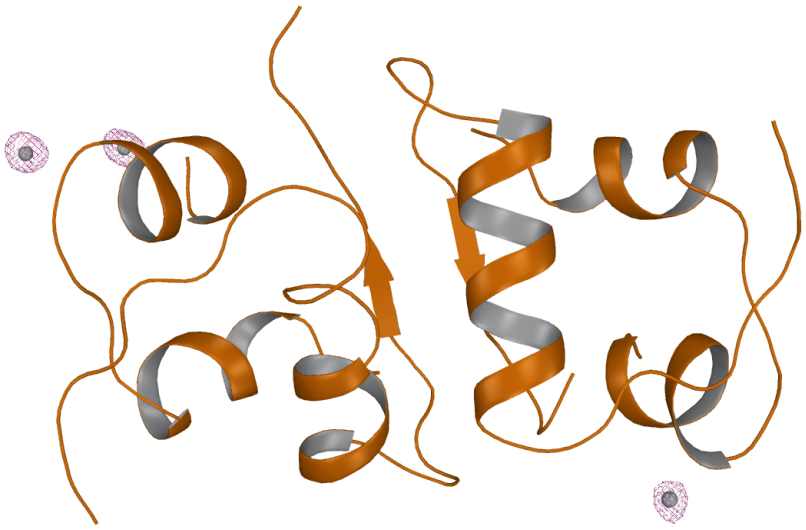


Insulin Gd-SAD





Insulin Gd-SAD



Statistics:

Space group: H 3

Resolution: 16.93-1.90 (1.94-1.90)

$R_{p.i.m.}$: 0.108 (0.745)

Completeness: 99.8 (99.5)

Multiplicity: 7.6 (6.9)

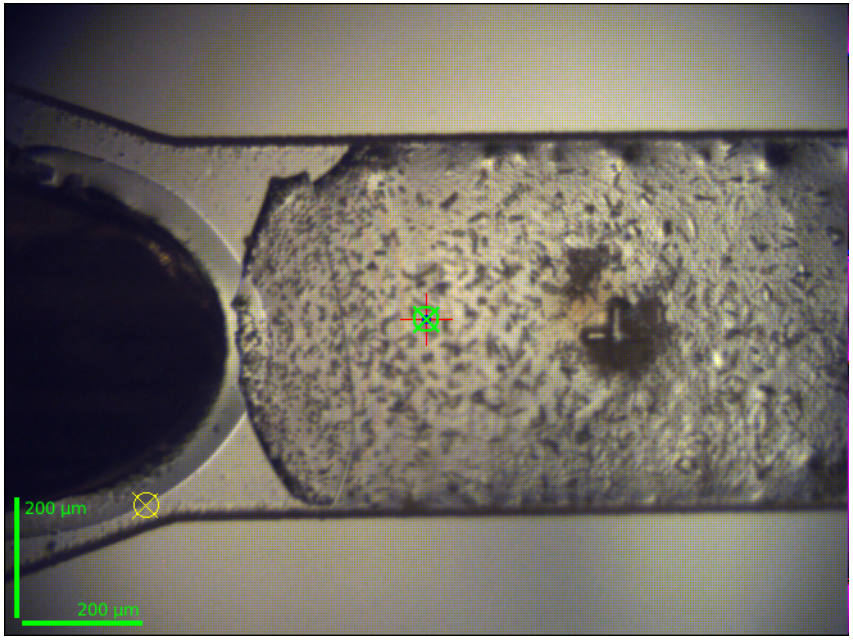
$< I > / \sigma < I >$: 10.0 (2.9)

R_{work}: 0.19787

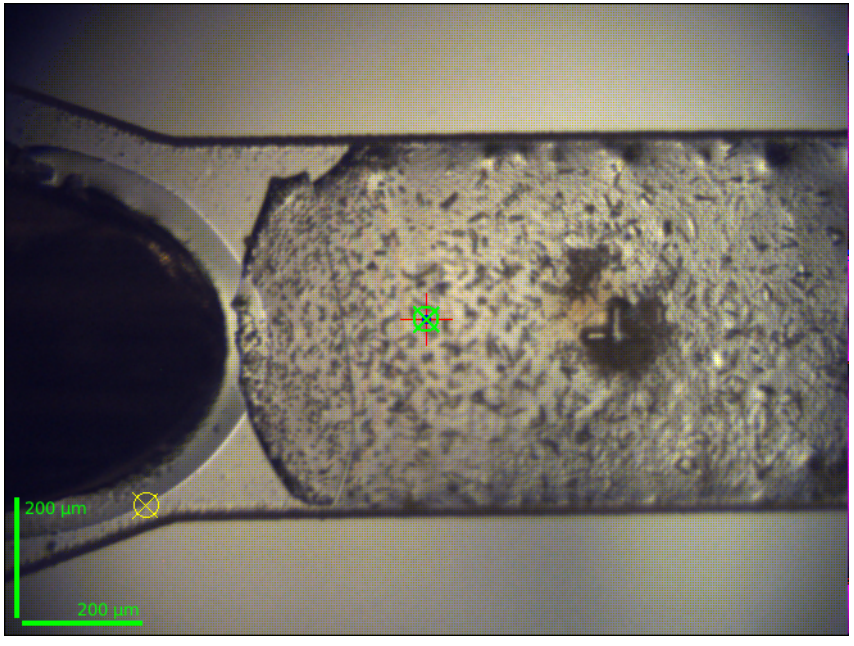
R_{free}: 0.24436

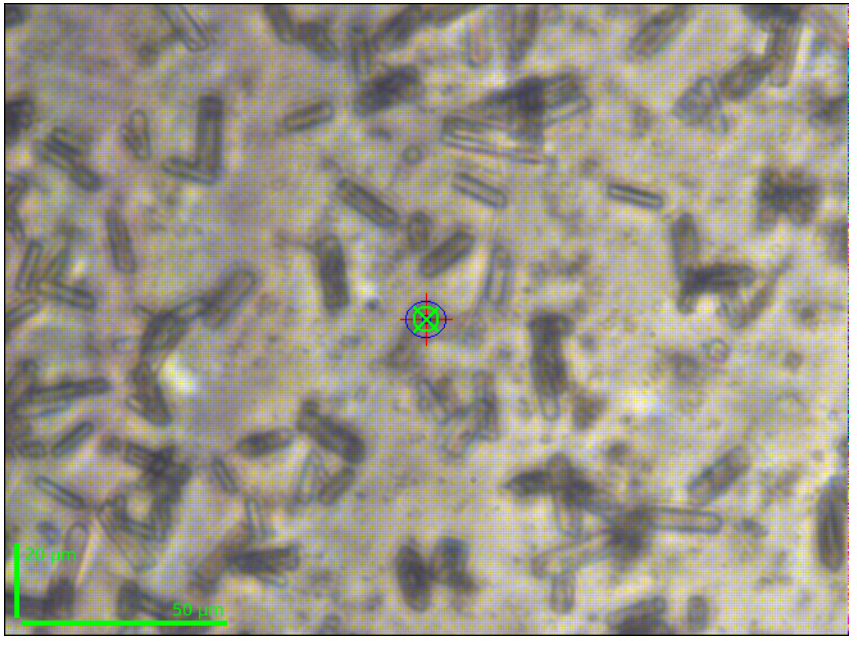
Neanderthal Adenylosuccinate Lyase (nADSL)

Neanderthal Adenylosuccinate Lyase (nADSL)

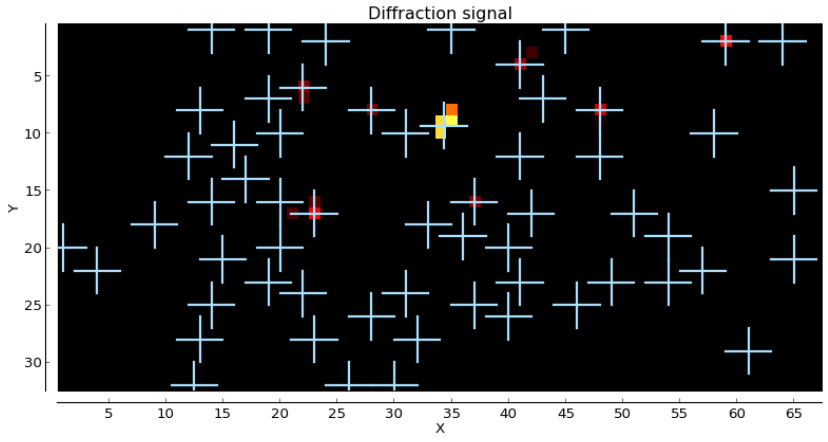


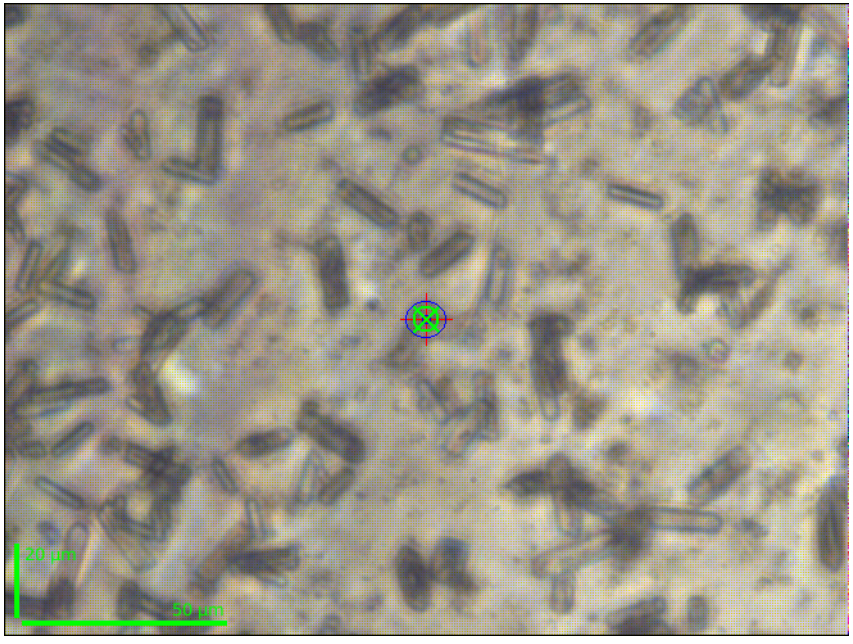
Neanderthal Adenylosuccinate Lyase (nADSL)



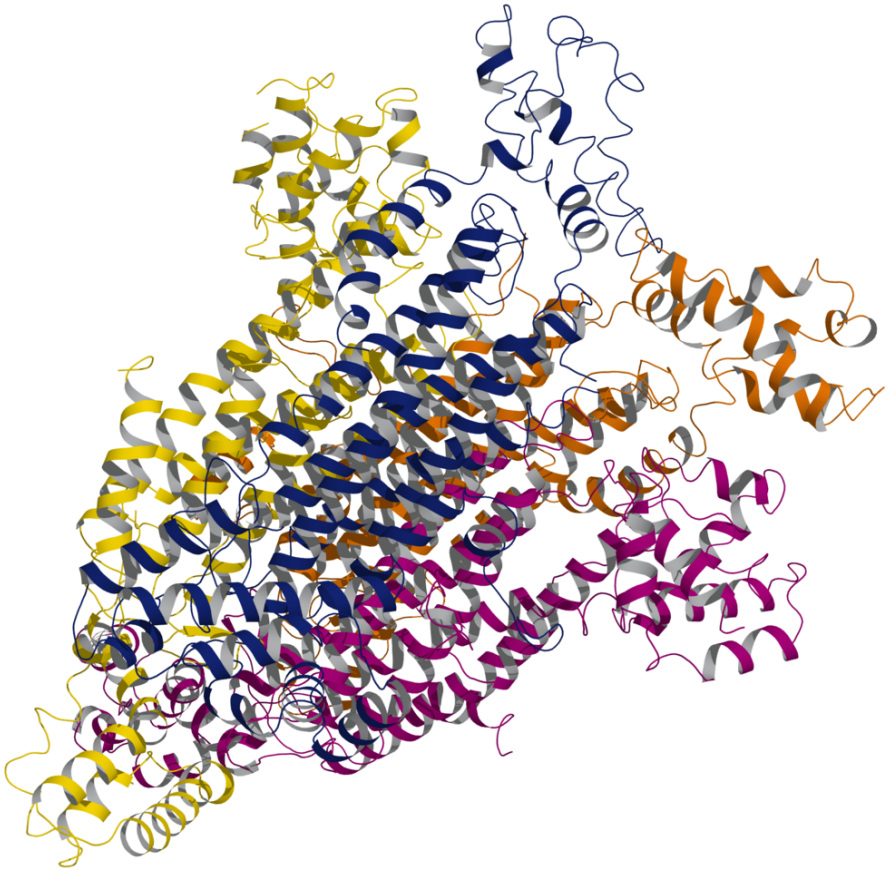


Neanderthal Adenylosuccinate Lyase (nADSL)





Neanderthal Adenylosuccinate Lyase (nADSL)



Statistics:

Space group: P 212121

Resolution: 20.07-2.90 (3.01-2.90)

$R_{p.i.m.}$: 0.168 (0.834)

Completeness: 99.7 (100)

$< I > / \sigma < I >$: 8.3 (2.7)

R_{work}: 0.178

R_{free}: 0.299

Perspectives

Perspectives

Perspectives

- room temperature/in situ experiments

Perspectives

- room temperature/in situ experiments
- more experimental phasing

Perspectives

- room temperature/in situ experiments
- more experimental phasing
- USER SAMPLES!!!

Fin.