Multi Crystal Data Collection

A New Synchrotron Serial Crystallography Method

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Users'Meeting 2015

Acknowledgements

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  - Ekatarina Round

Introduction

Serial Crystallography

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

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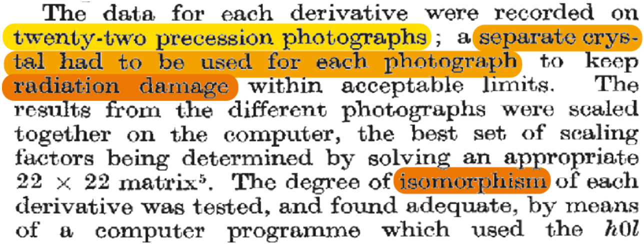

The data for each derivative were recorded on twenty-two precession photographs; a separate crystal had to be used for each photograph to keep radiation damage within acceptable limits. The results from the different photographs were scaled together on the computer, the best set of scaling factors being determined by solving an appropriate 22 × 22 matrix[5]. The degree of isomorphism of each derivative was tested, and found adequate, by means of a computer programme which used the *h0l*

Kendrew et al. (1960) Structure of Myoglobin

SFX: Serial femtosecond crystallography

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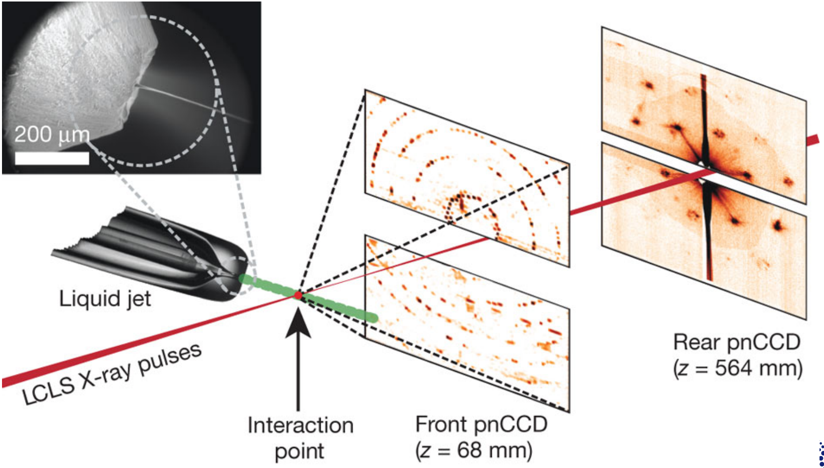
Femtosecond nanocrystallography (Chapman *et al.*, Nature 2011)



SSX: Synchrotron serial crystallography

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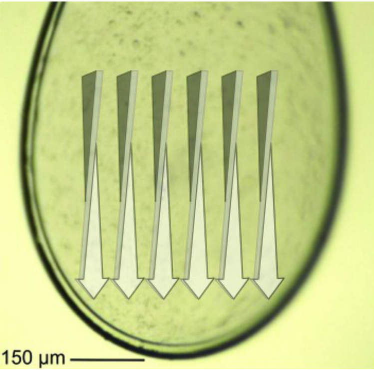
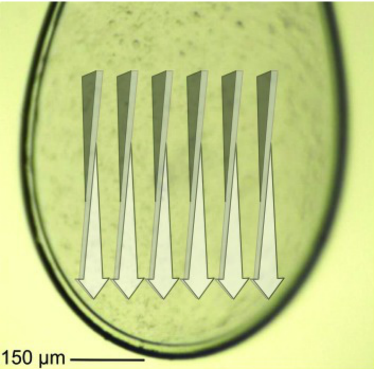
Structure of *Trypanosoma brucei* Procathepsin B at 3Å

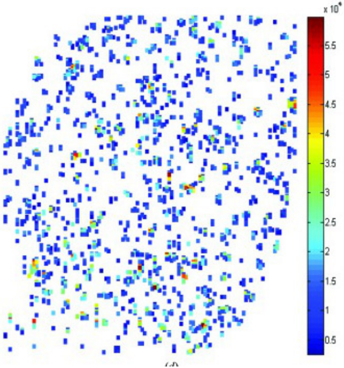


Gati *et al.*, IUCrJ 2014: Serial crystallography on *in vivo* grown microcrystals using synchrotron radiation

SSX: Synchrotron serial crystallography

Structure of *Trypanosoma brucei* Procathepsin B at 3Å





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

Method

Multi Crystal Data Collection

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☐ Sample on mesh loop

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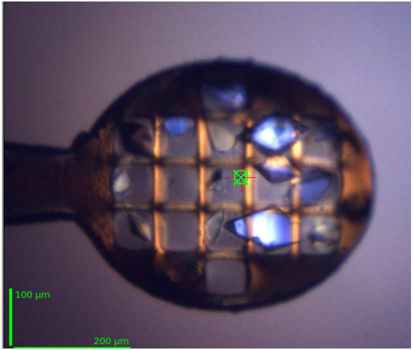
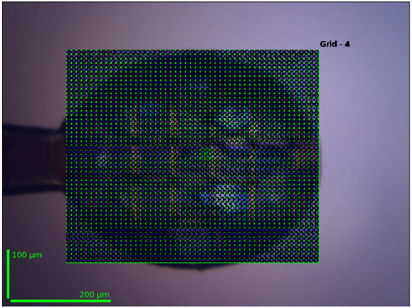
☐ Sample on mesh loop



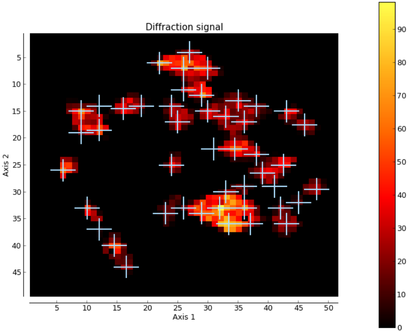
Multi Crystal Data Collection

- ☐ Sample on mesh loop
- ☐ Mesh scan of sample



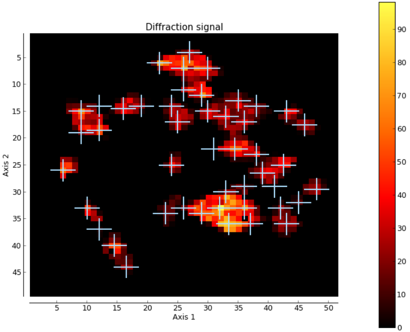
Multi Crystal Data Collection

- ☐ Sample on mesh loop
- ☐ Mesh scan of sample
- ☐ Detection of protein diffraction



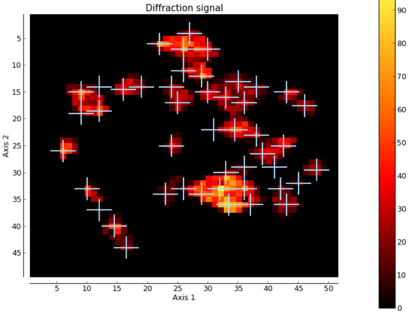
Multi Crystal Data Collection

- ☐ Sample on mesh loop
- ☐ Mesh scan of sample
- ☐ Detection of protein diffraction
- ☐ Series of partial data collection



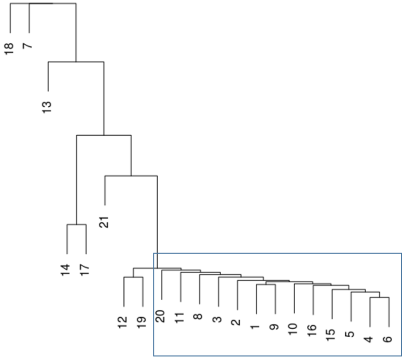
Multi Crystal Data Collection

- ☐ Sample on mesh loop
- ☐ Mesh scan of sample
- ☐ Detection of protein diffraction
- ☐ Series of partial data collection
- ☐ Integration of partial sets

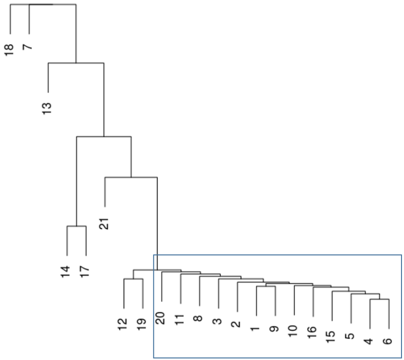


Multi Crystal Data Collection

☐ Sample on mesh loop
☐ Mesh scan of sample
☐ Detection of protein diffraction
☐ Series of partial data collection
☐ Integration of partial sets
☐ Hierarchical cluster analysis



Multi Crystal Data Collection



- ☐ Sample on mesh loop
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- ☐ Detection of protein diffraction
- ☐ Series of partial data collection
- ☐ Integration of partial sets
- ☐ Hierarchical cluster analysis
- ☐ Data merging

Test Cases

LCP Membrane Protein: Bacteriorhodopsin

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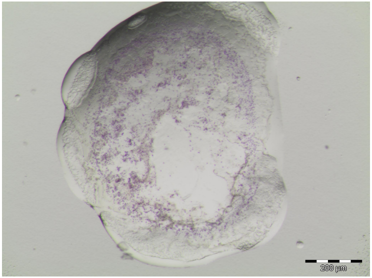
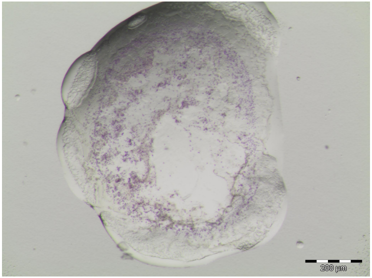


LCP Crystallization Drop, crystal size ca. 5 μm

LCP Membrane Protein: Bacteriorhodopsin



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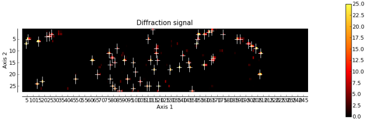
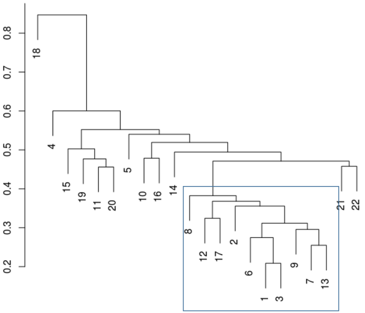
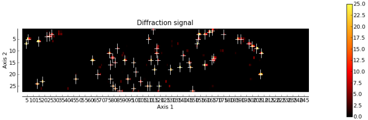


Heat Map after Mesh Scan: 59 spots picked for partial data collection

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
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LCP Membrane Protein: Bacteriorhodopsin

Statistics:

Space group: P 63

Resolution: 19.73-2.57 (2.71-2.57)

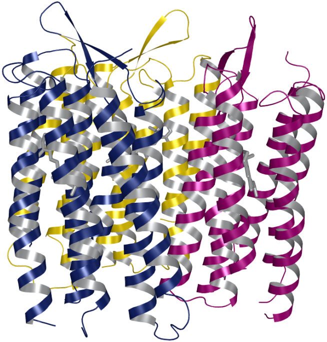
$R_{p.i.m.}$: 0.127 (0.546)

Completeness: 97.7 (87.1)

I/Sigma(I): 6.7 (1.8)

R_{work}: 0.18983

R_{free}: 0.20547



Bacteriorhodopsin: Biological Assembly

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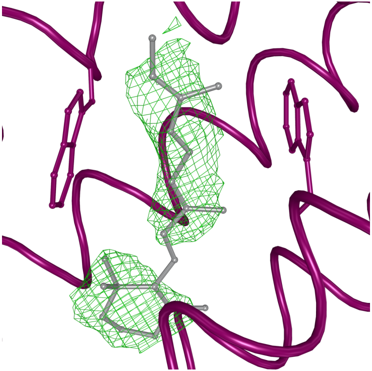

Retinal Cofactor in Fo-Fc map (cutoff 2σ)

Low Symmetry: Monoclinic Lysozyme

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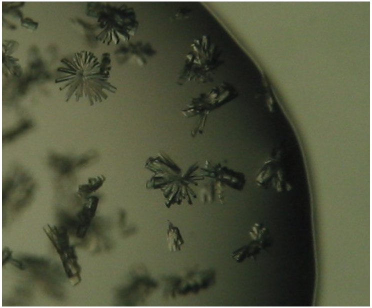
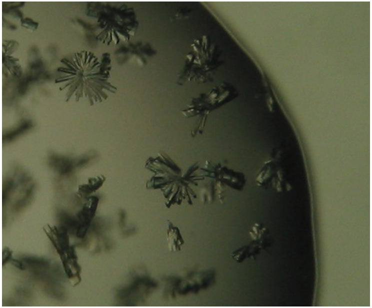
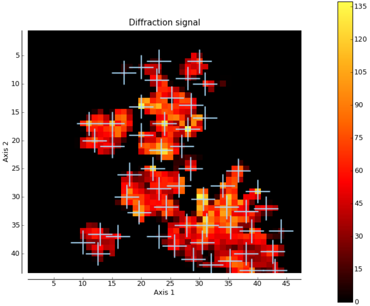


Crystallization Drop

Low Symmetry: Monoclinic Lysozyme

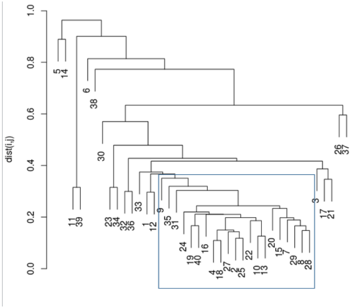


Crystallization Drop

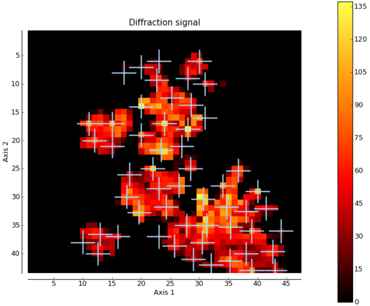


Heat Map after Mesh Scan: 54 spots picked for partial data collection

Low Symmetry: Monoclinic Lysozyme



hierarchical cluster analysis:
20 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)

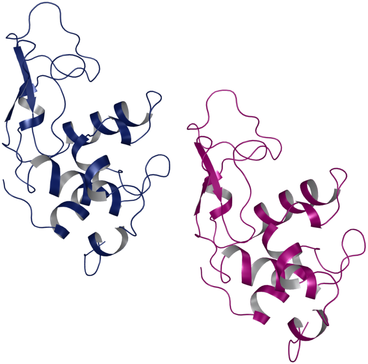
$R_{p.i.m.}$: 0.080 (0.486)

Completeness: 85.0 (82.1)

I/Sigma(I): 8.0 (2.2)

R_{work}: 0.21291

R_{free}: 0.26489



mC Lysozyme: Secondary Structure

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)

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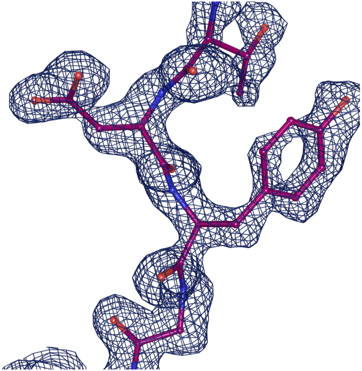

2Fo-Fc Map Example (cutoff 1 σ)

Experimental Phasing: Thermolysin

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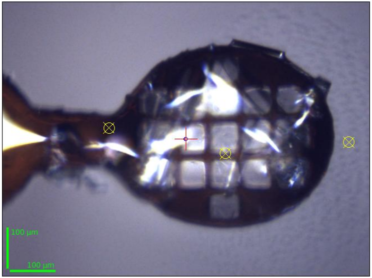
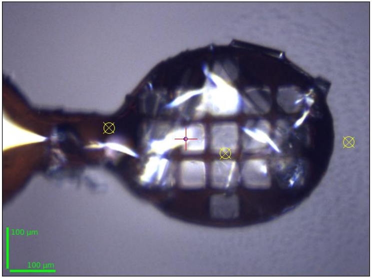
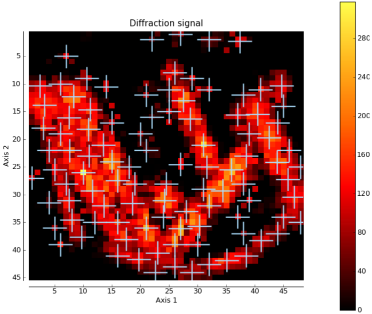


Crystals on Mesh Loop

Experimental Phasing: Thermolysin

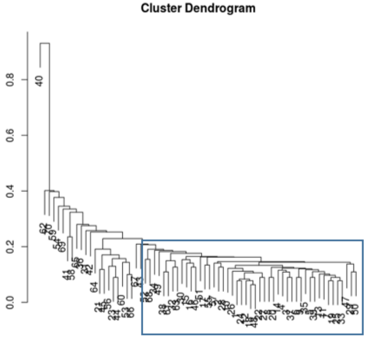


Crystals on Mesh Loop

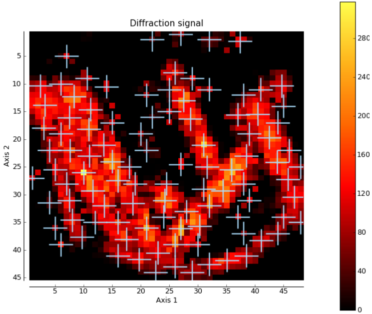


Heat Map after Mesh Scan:
96 spots picked for partial data collection

Experimental Phasing: Thermolysin

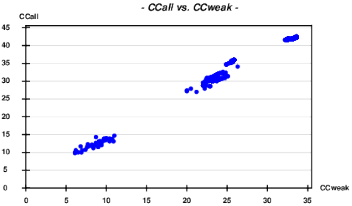


hierarchical cluster analysis:
49 out of 77 integrated datasets
selected for merging

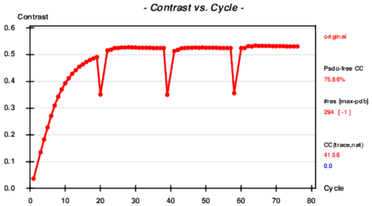


Heat Map after Mesh Scan:
96 spots picked for partial data
collection

Experimental Phasing: Thermolysin



ShelxD Results



ShelxE Results

Experimental Phasing: Thermolysin

Statistics:

Space group: P 6122

Resolution: 19.88-1.27 (1.33-1.27)

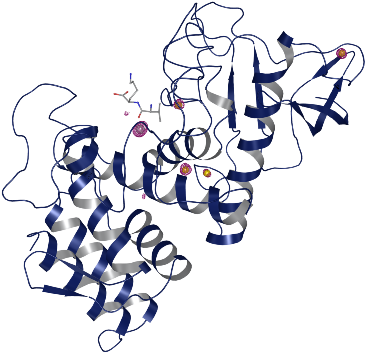
R$_{p.i.m.}$: 0.017 (0.344)

Completeness: 92.0 (53.4)

I/Sigma(I): 25.4 (2.7)

R$_{work}$: 0.16989

R$_{free}$: 0.19703



Thermolysin Secondary Structure, Zn-Atom and Ca-Atoms in anomalous map (4.5 σ)

First Results

Sanofi - AV

AV151

Sanofi - AV

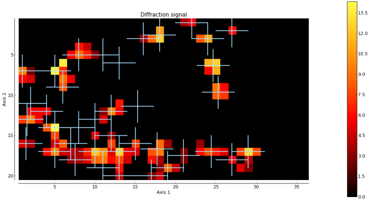
AV151

On-axis camera snapshot

Sanofi - AV

AV151

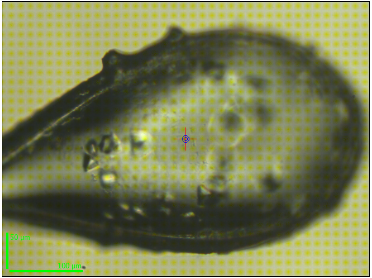
On-axis camera snapshot



Mesh scan: 38 positions, 34 sets integrated

Sanofi - AV

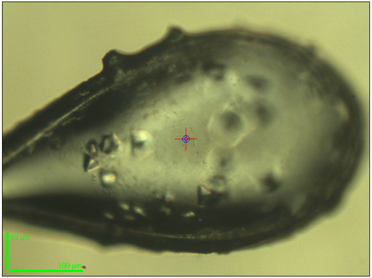
AV56



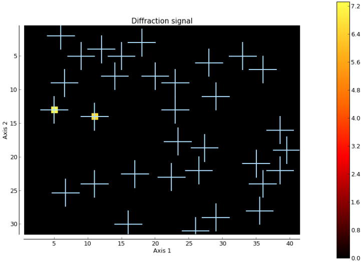
On-axis camera snapshot

Sanofi - AV

AV56



On-axis camera snapshot



Mesh scan: 32 positions, 30 sets integrated

Sanofi - AV

Statistics

AV151 (19 sets):
Resolution: 20.00-2.1 (2.16-2.10)
$R_{p.i.m.}$: 0.064 (0.532)
Completeness: 99.5(99.5)
I/Sigma(I): 10.5 (2.4)

AV56 (19 sets):
Resolution: 20.0-2.20 (2.27-2.00)
$R_{p.i.m.}$: 0.086 (0.688)
Completeness: 99.6 (99.9)
I/Sigma(I): 9.5 (2.1)

Perspectives

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- ☐ Characterization: Estimate Resolution

Perspectives

☐ Characterization: Estimate Resolution ✓

Perspectives

- ☐ Characterization: Estimate Resolution ✓
- ☐ Burn Strategy

Perspectives

- ☐ Characterization: Estimate Resolution ✓
- ☐ Burn Strategy
- ☐ Room Temperature

Perspectives

- ☐ Characterization: Estimate Resolution ✓
- ☐ Burn Strategy
- ☐ Room Temperature
- ☐ In situ

Fin.