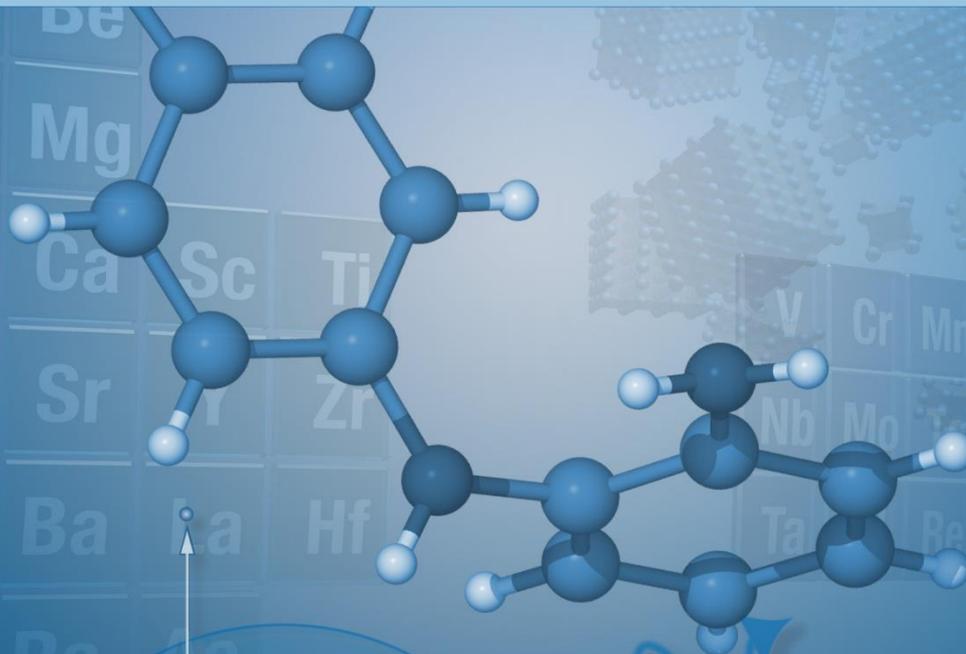




Bruker Webinar: SC-XRD

*Macromolecular Crystallography at the
Newcastle Structural Biology Lab*

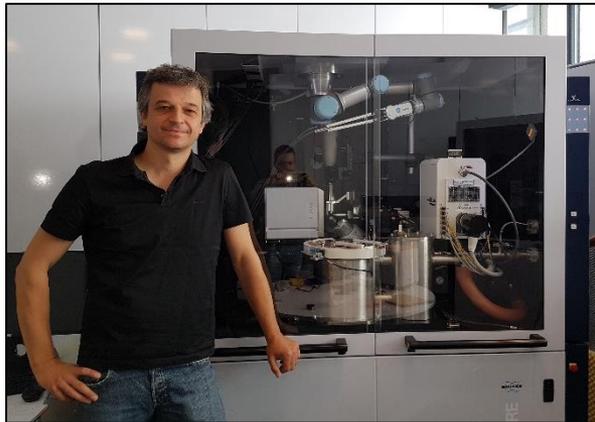


Dr. Arnaud Baslé
X-ray Facilities Manager
Biosciences Institute
Newcastle University, U.K.

Dr. Michael Mrosek
Application Scientist
Karlsruhe, Germany
Bruker AXS

June 23rd 2020

Who is talking?



Dr. Michael Mrosek:

- In Karlsruhe, Germany
- Application Scientist SCD
- Biological crystallography
- PhD in structural biology (Uni Basel)
- Joined Bruker 2017
- Michael.Mrosek@bruker.com



Dr. Arnaud Baslé

- In Newcastle upon Tyne, UK
- Senior X-ray facilities manager
- Macromolecular crystallography
- PhD in cell and molecular biology (Uni Houston, TX)
- Joined current post in 2009
- arnaud.basle@ncl.ac.uk

Crystallography at the Newcastle Structural Biology Laboratory with the Bruker D8 VENTURE home source

Dr Arnaud Baslé
Biosciences Institute
Bruker webinar 23/06/20

- Introduction
- Home source testing samples
- Experimental phasing with Gallium
 - Sulphur SAD
 - Cobalt SAD
- Drug discovery
- FragLites

- A user facility for Macromolecular X-ray Crystallography

Biosciences Institute

Prof. Bert van den Berg

Membrane protein structural biology

Dr. Paula Salgado

Structural microbiology of *C. difficile*
pathogenicity

School of Natural and
environmental Sciences

Dr. Jon Marles-Wright

Structural Studies of metabolic
compartmentalisation in Bacteria

Translational and Clinical Research
Institute

Prof. Jane Endicott

Mechanistic studies of complexes controlling the cell cycle
and transcription

Prof. Martin Noble

Biomolecular Structures and anti-cancer drug discovery

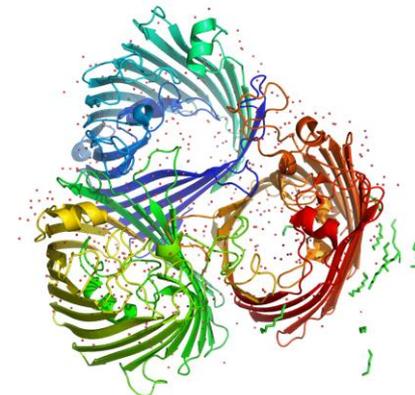
Many other labs (see acknowledgments)

2015-2019: 94 publications, 28 Ph.D. students (~40+ papers)

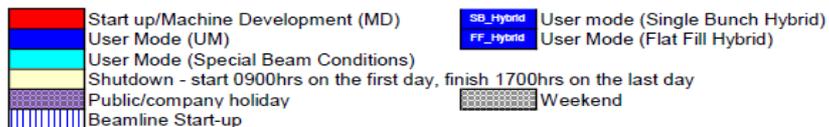
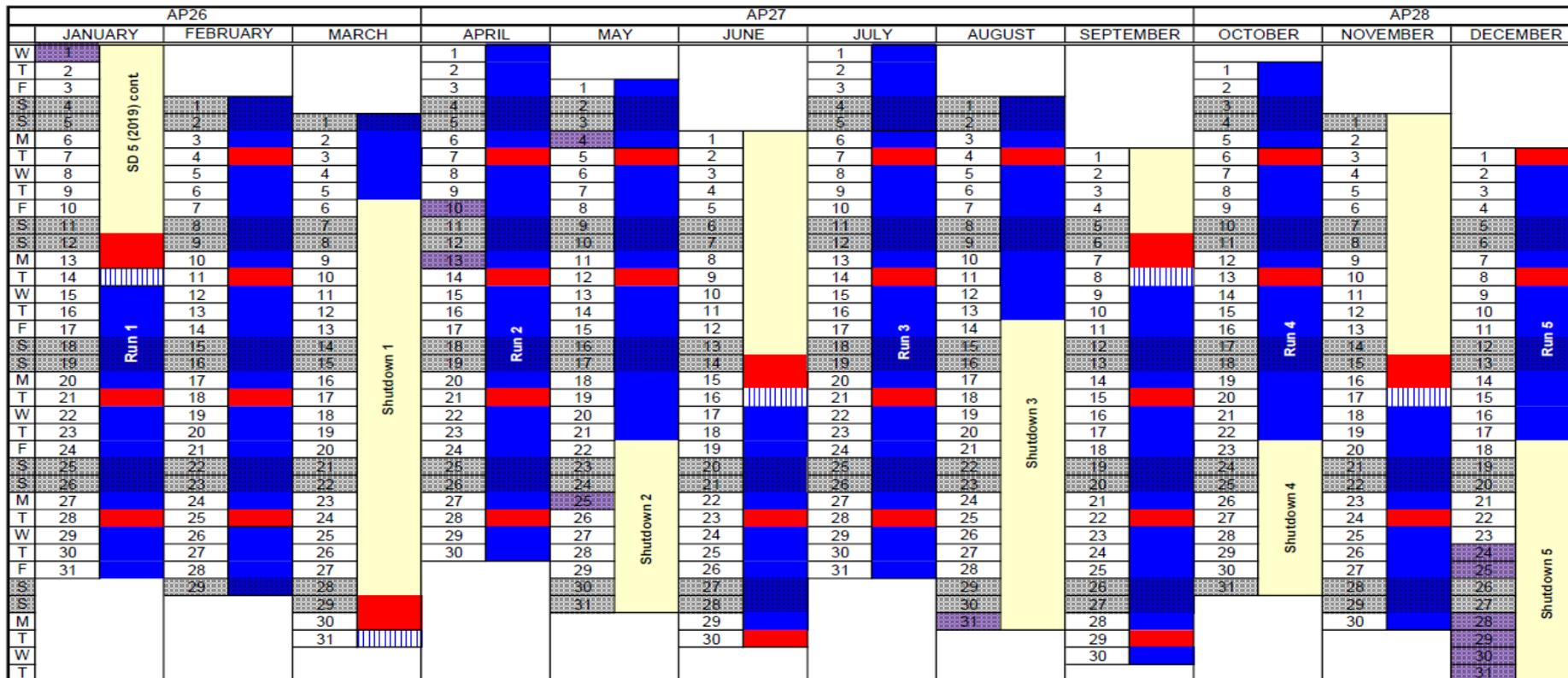
- 2009 Arnaud recruited by Prof. Rick Lewis as X-ray facilities manager
- Diamond User Committee representative (since 2017)
- CCP4 wg1, wg2, dev group member.

- Equipment management
- Purchasing
- User training (wide range of skills set)
 - Equipment and sample handling
 - Software and Crystallography
- Synchrotron data collection (Organisation/Sample management)
- IT
 - Hardware
 - Software (CCP4, Phenix, Globalphasing and more)

- Collaboration with groups not primarily structural biologist
76 PDB models deposited from 0.79 to 3.5 Å.
(73 from Newcastle)



2020 OPERATIONS CALENDAR



Introduction: NSBL facility



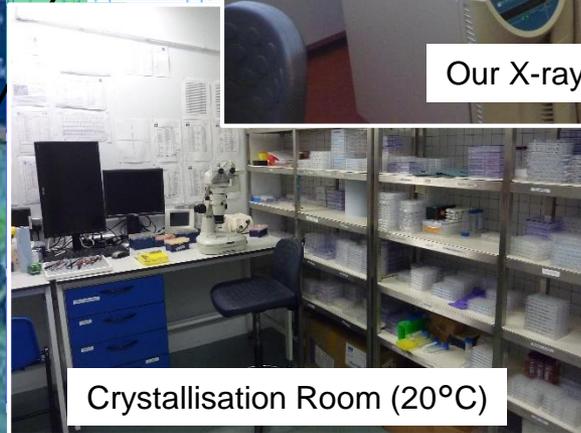
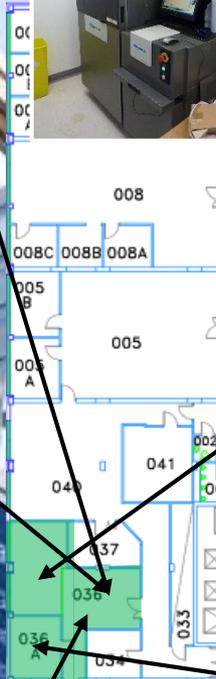
4°C plate Hotel

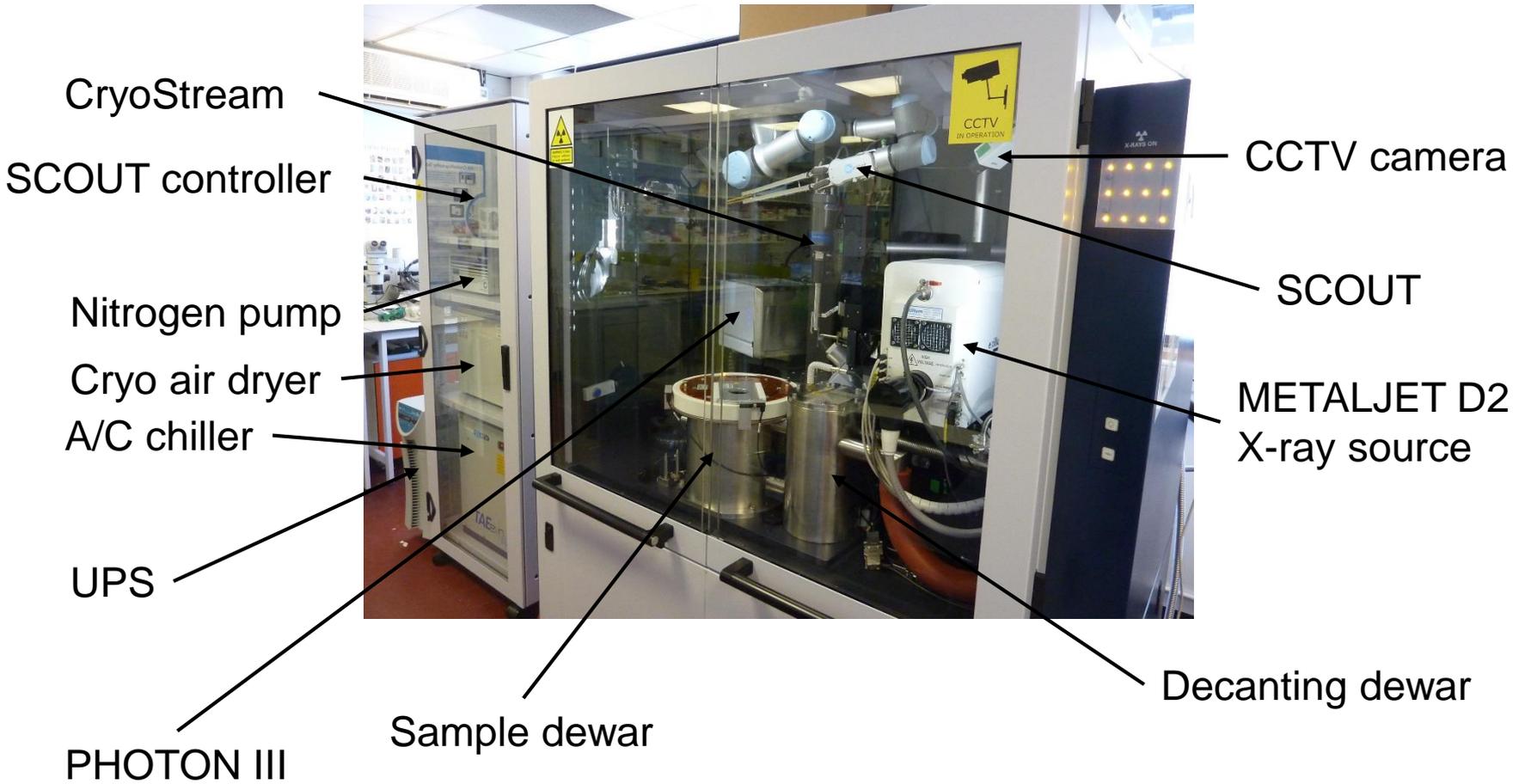


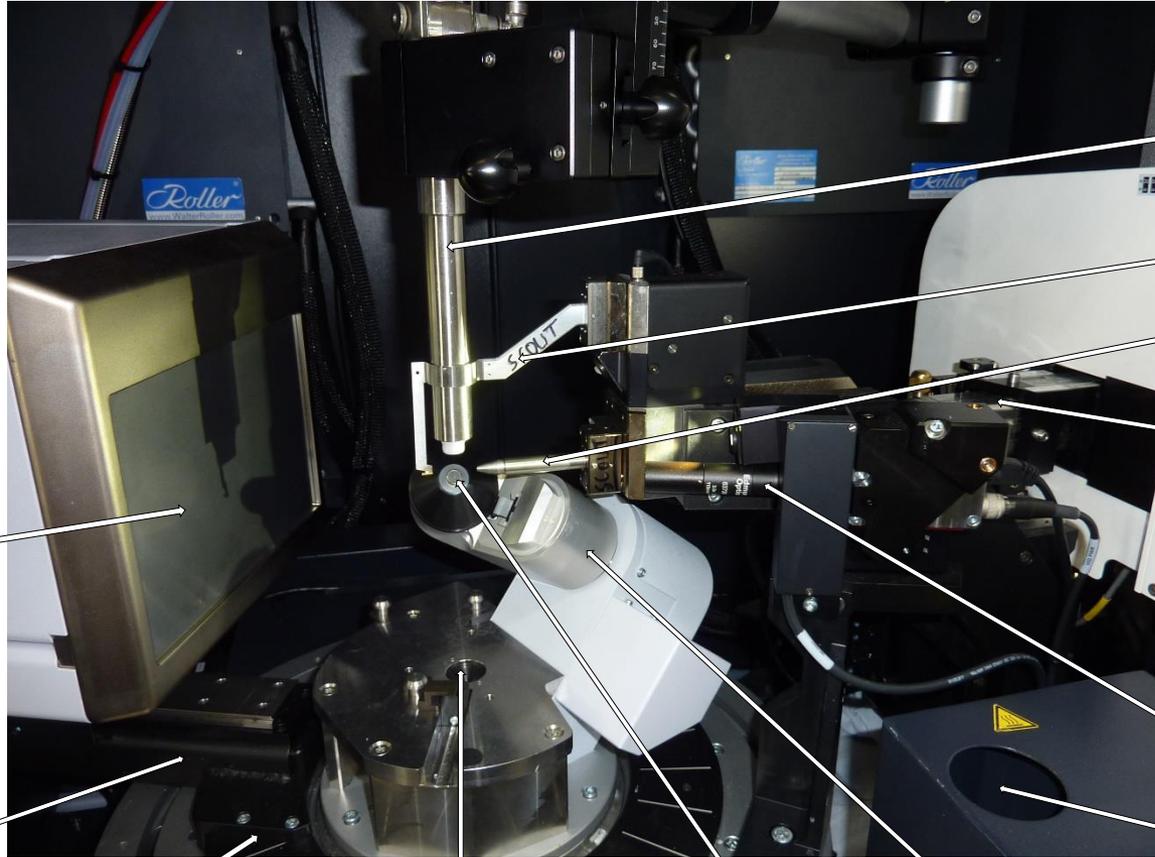
Servers



Freezers







PHOTON III
CPAD

Distance

2 Theta

Omega

AGH (phi)

kappa

cryostream

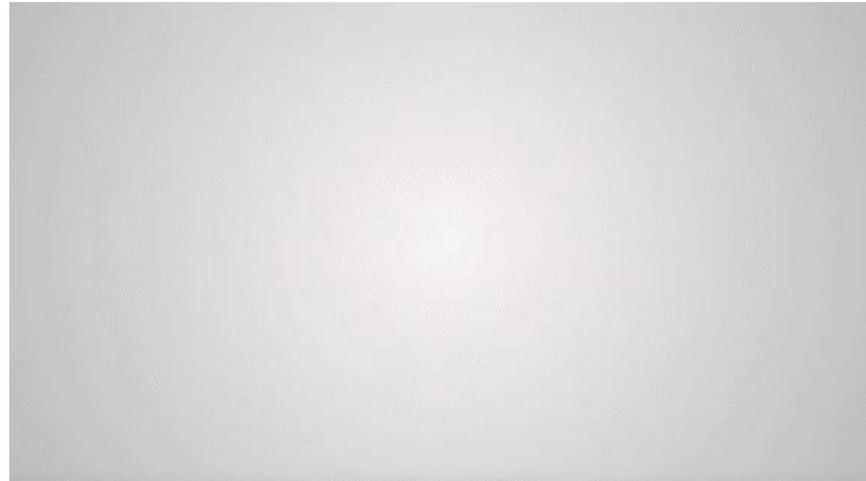
beamstop

collimator

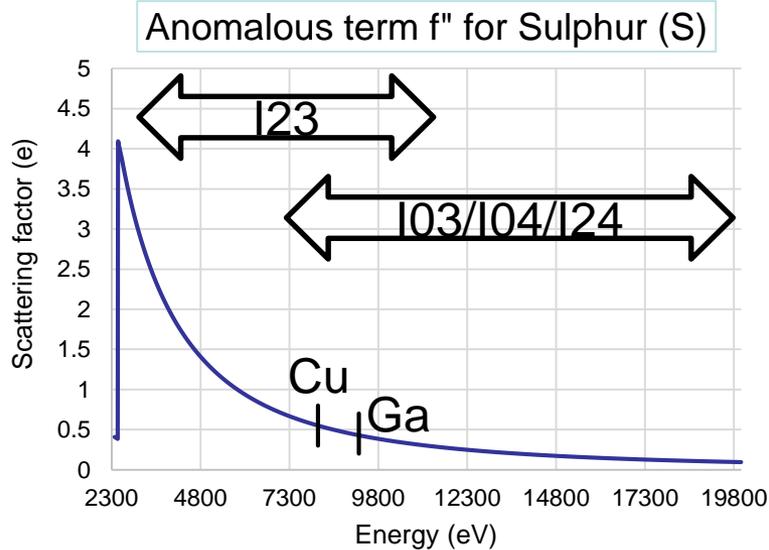
X-ray optics

Sample camera

Dryer

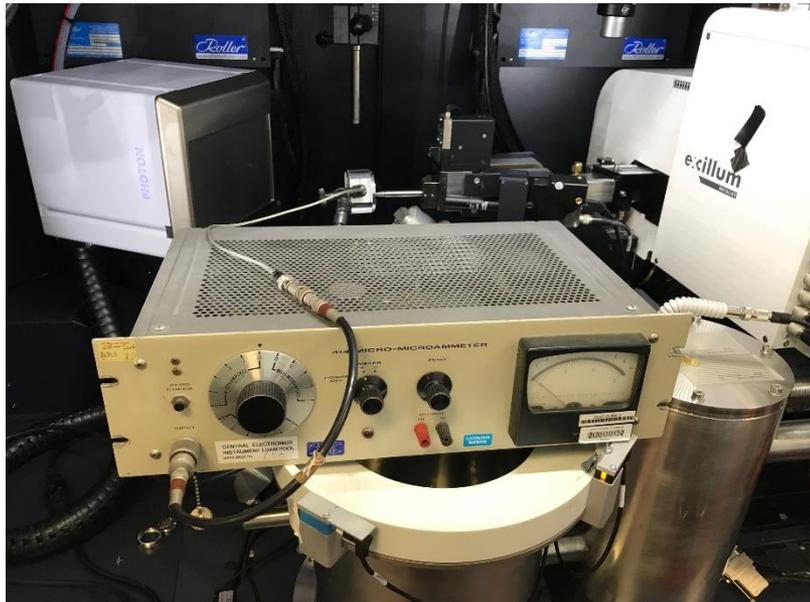


From Excillum, METALJET promotional video



Search Youtube for gallium

	Cu (1.54 Å)	Ga (1.34 Å)
f'' S (e)	~0.56	~0.43



picoammeter

70 kV and 2.857 mA (200 W)
 3.27×10^9 ph/s (70 μ m on sample)

Diamond I04-1: 3.5×10^{11} ph/s
(70 μ m aperture)

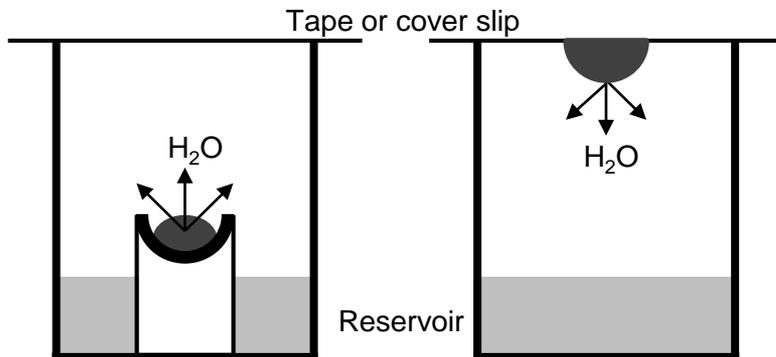
METALJET D2+
70 kV and 3.571 mA (250W)



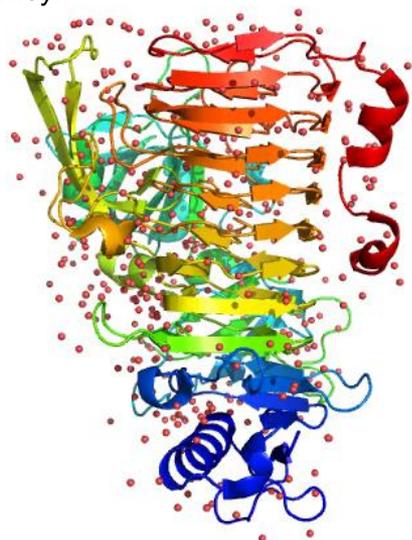
Pin diode mounted in front of the collimator



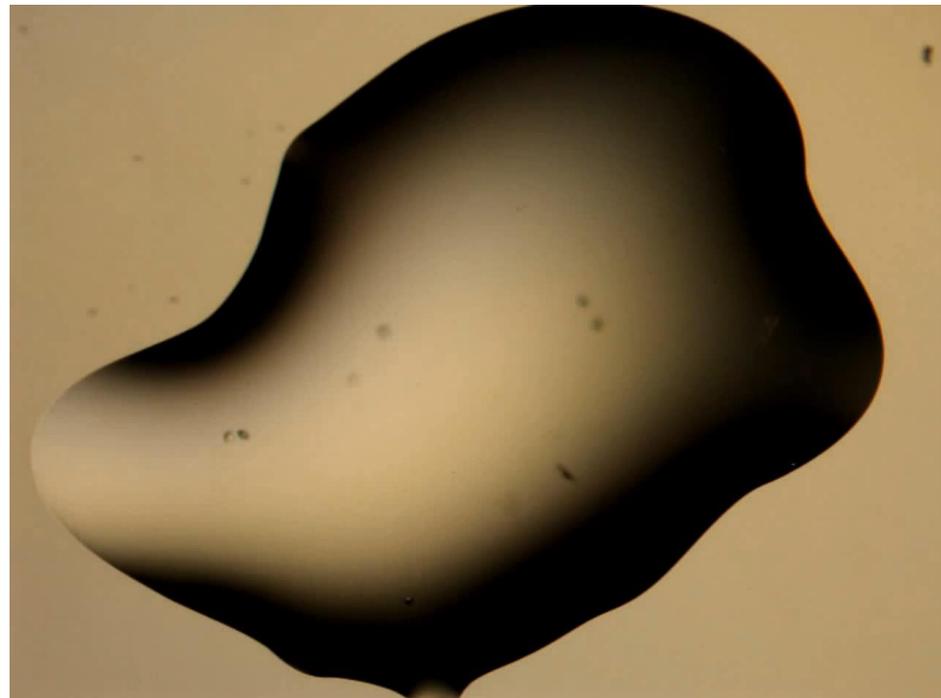
Prof. Elspeth Garman, University of Oxford



From Basle and Lewis, Principles and practice un Macromolecular Xray Crystallography. 2019. Biomolecular and Bioanalytical Techniques. Wiley.



5mqp. BT1002, lyase
2.0 Å



Lysozyme crystals growing

Problem is dual

- Water makes hexagonal ice when frozen
- Many small molecules are not water soluble

Introduction: sample handling

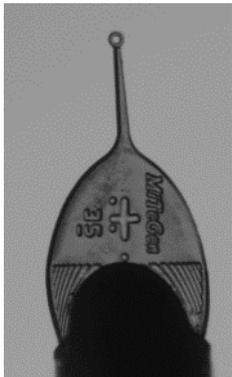
To reduce radiation damage we collect at 100 K
Therefore the samples need to be cryo-protected

- 20 % PEG 400
- 25 % Ethylene Glycol
- Paratone N oil
- Saturated salt solutions

Hexagonal ice

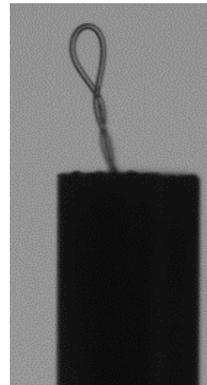


glycerol

Mitegen
Kapton

Always the same
Fragile
Easier to handle



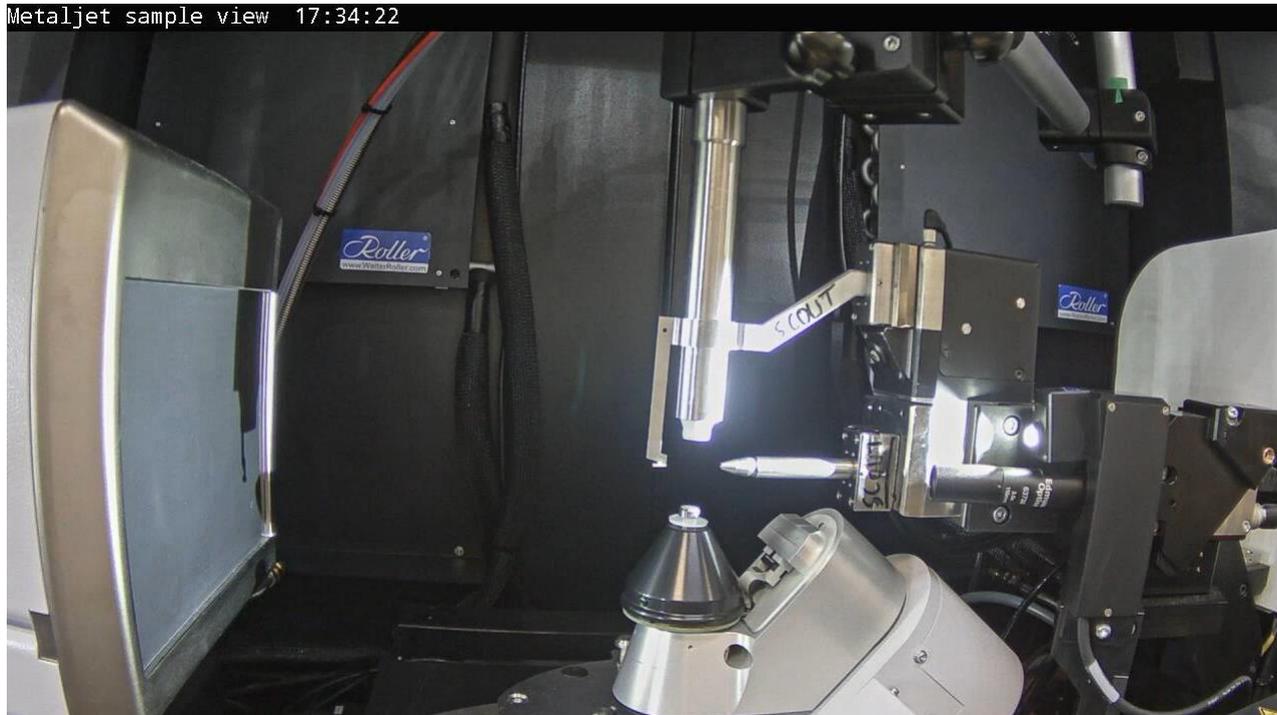
Hampton research
Nylon

Size
Robust

Weeks, months of cloning,
purifying and getting crystals



Uni Puck cover and plate with mounts

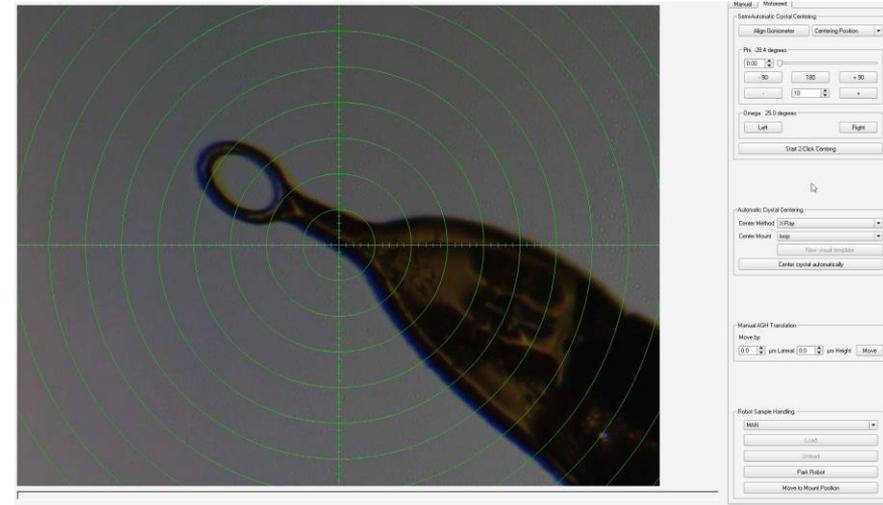


Collimators can be changed to reduce the divergence



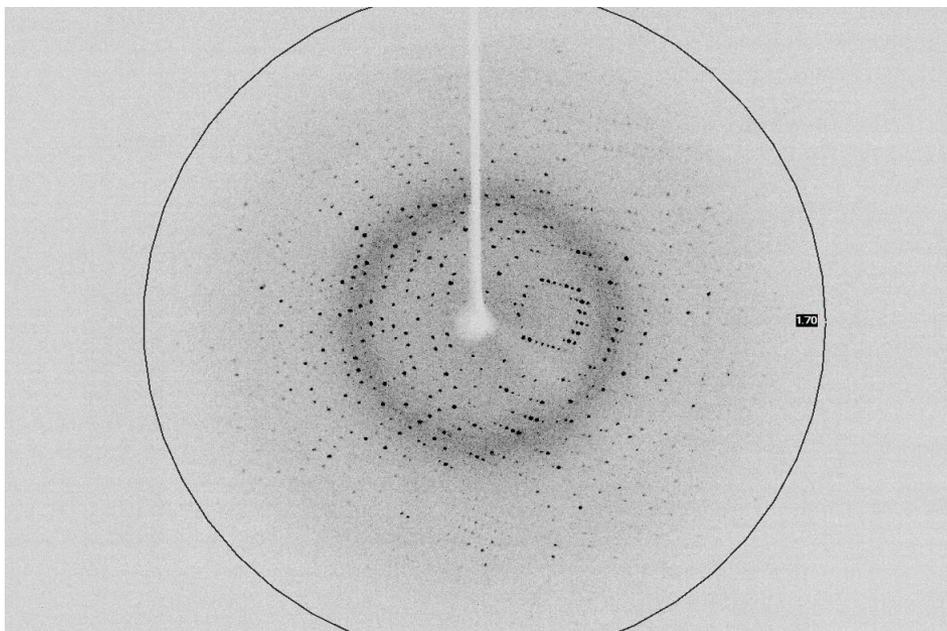
Sample loader from dewar to measurement

- Load time: 40 s (7 s out of LN2/cryojet)
- Centering time:
 - manual few seconds
 - Few minutes
- Drying time: < 5 min (also when collecting)



Centering done remotely (i.e. home)

Drug discovery project
(complete to 1.6 Å in 6 minutes)



PHOTON III 360 degrees diffraction pattern
(x18 accelerated; 2 s / degree)

- We upgraded PHOTON II (10 x 14 cm) to PHOTON III (20 x 14 cm).
- Mixed-mode
 - Integration mode for strong
 - Photon-counting for weak
- Large detector size allow fast data collection

Aimless/Pointless plugin

Data Indexing and Integration

PROTEUM 3 (Saint +)

XDS (cbf)

DIALS

XIA2

Autoproc

Space Group determination and Processing Statistics

Pointless Aimless

CCP4i



CCP4i2



CCP4 cloud

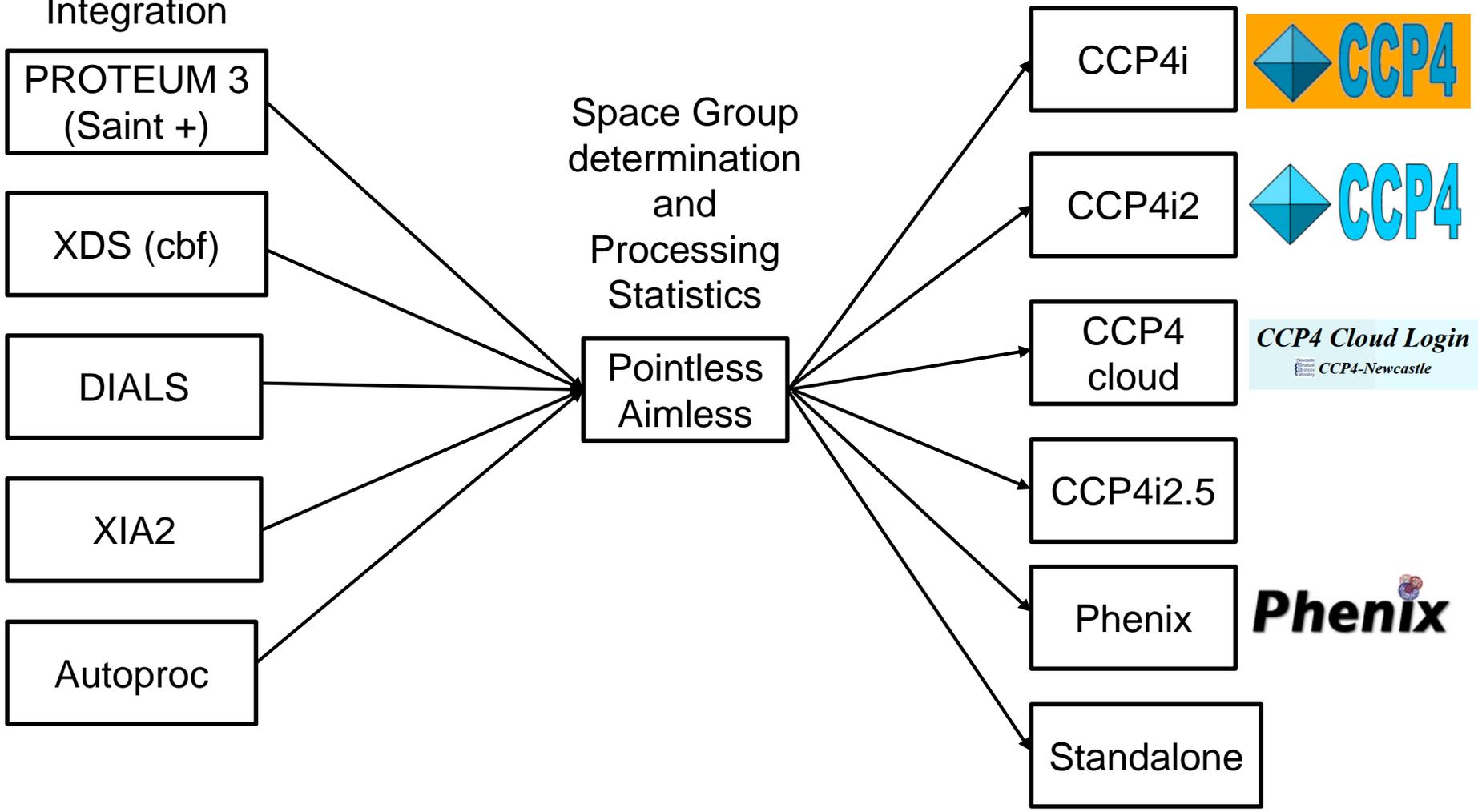


CCP4i2.5

Phenix



Standalone



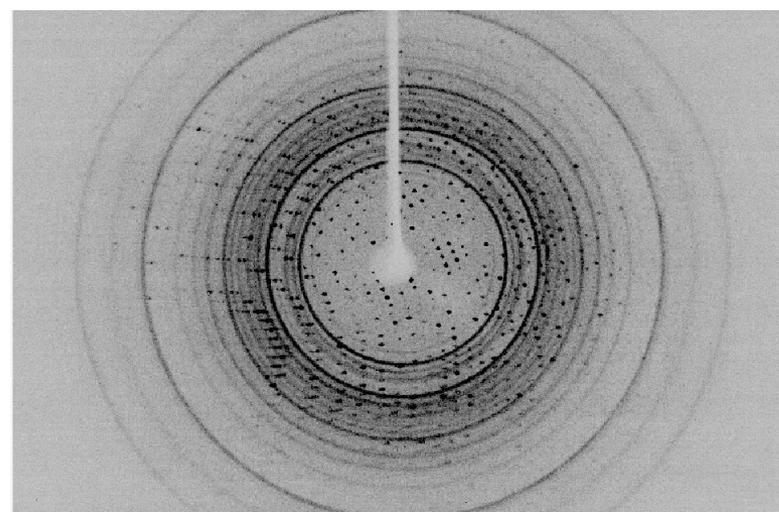
- Introduction
- Home source testing samples
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 - Cobalt SAD
- Drug discovery
- FragLites

- Salt
- Bad cryo
- Not diffracting
- Not having ligand

IMMEDIATE ANSWER with a home
source



Salt diffraction pattern



Poorly cryoed protein diffraction pattern

Of course we can test good samples and collect valuable information

- Unit cell parameters and Bravais
- Protein Complexes

- Introduction
- Home source testing samples
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CBM77 Carbohydrate binding module

121 AA, 12637 Da

P6₁,22

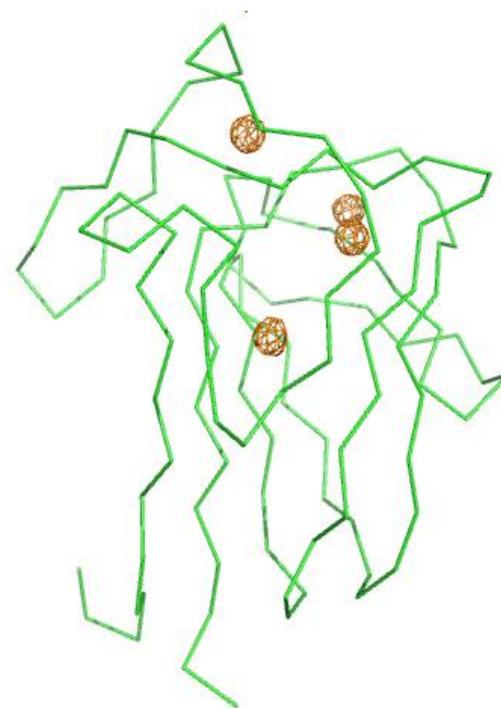
69.3, 69.3, 121.6, 90, 90, 120

~ 62 hours, inverted phi diffractometer

	sites	Solvent (%)
CBM77	5	39

Micromax 007 rotating Cu anode

	Overall	Outershell
res (Å)	43.05 – 1.93	1.99 - 1.93
Mean I/sd(I)	84.4	27.5
CC _{1/2}	1.000	1.000
Anom Comp	100.0	100.0
Anom Multiplicity	64.3	61.9

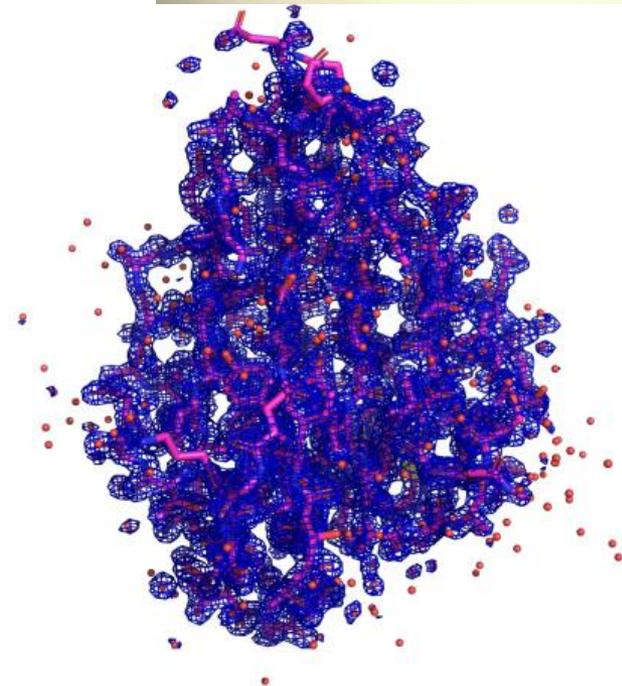
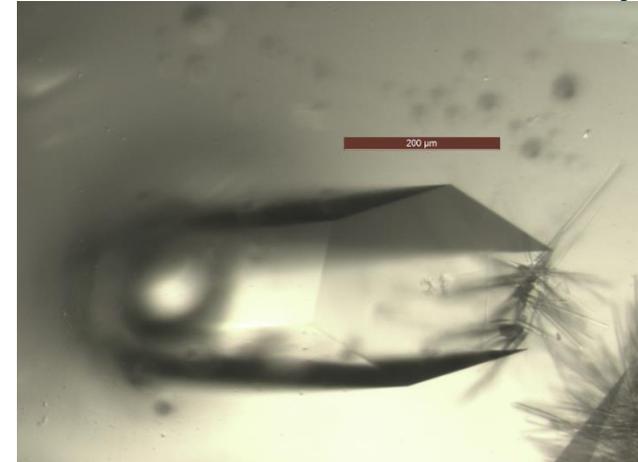


Ana Luis, Prof. Harry Gilbert, FRS

I. Venditto *et al.* Complexity of the *Ruminococcus flavefaciens* cellulosome reflects an expansion in glycan recognition. 2016. PNAS

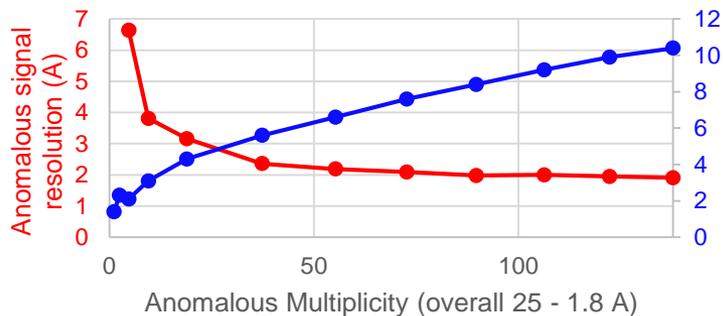
- 11 datasets of 720 degrees
- 5 sec / 0.5 degree (2 hours datasets)
- Phi rotation of 720°, chi 5°, 2 theta 20°, 130 mm
- Photon II

	Overall	Outershell
Res (Å)	24.31 – 1.66	1.69 – 1.66
Mean I/sd(I)	87.4	1.4
CC _{1/2}	1.000	0.620
Anom Comp	99.4	88.0
Anom Multiplicity	111.7	6.0



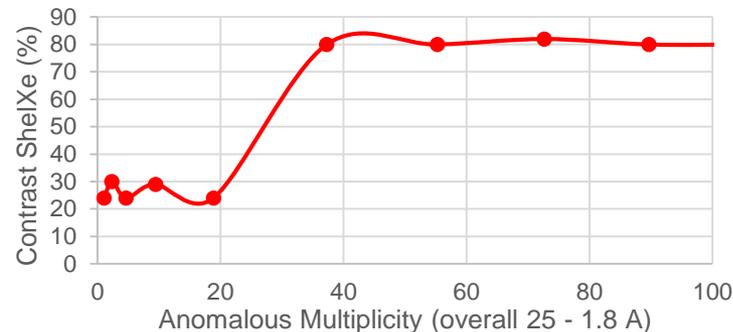
Sulphur SAD how much redundancy do you need?

S-SAD Anomalous Signal and I/SigI

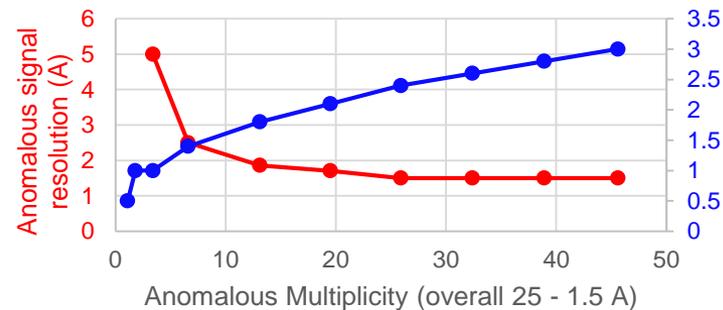


CBM77

S-SAD

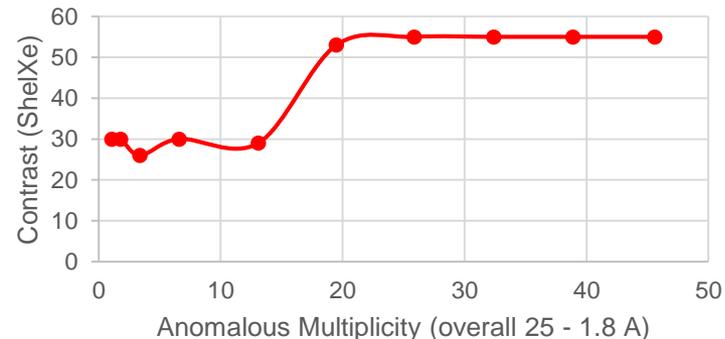


S-SAD Anomalous Signal and I/SigI

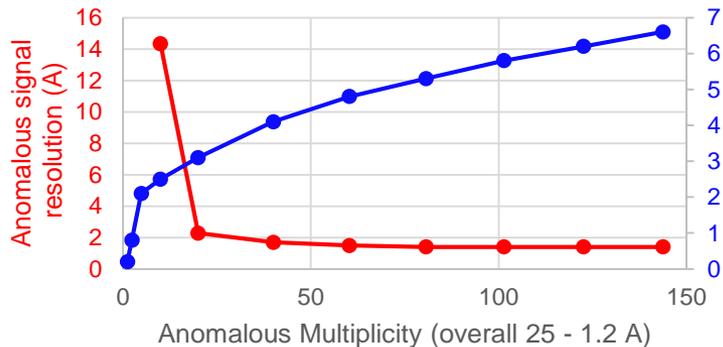


Protein

S-SAD



S-SAD Anomalous Signal and I/SigI

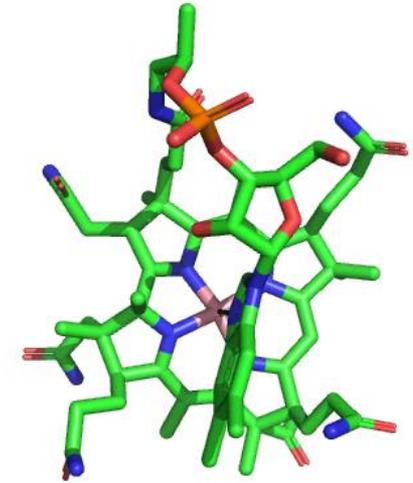


Lysozyme

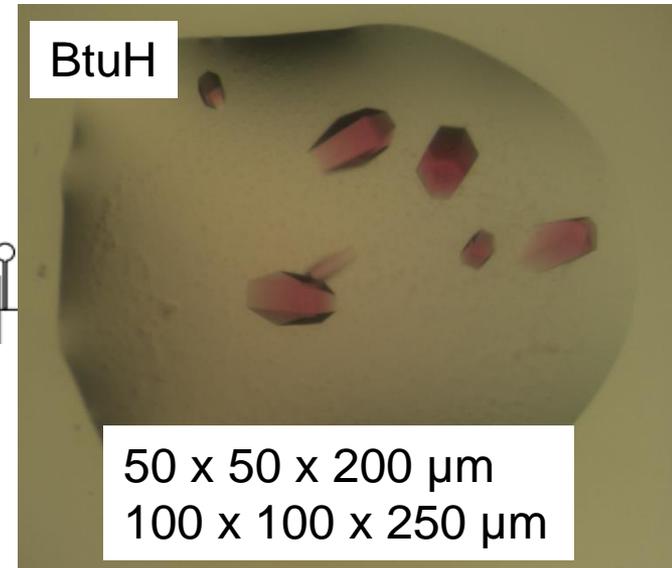
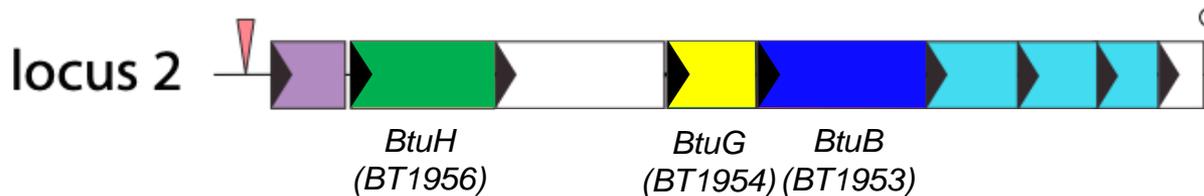
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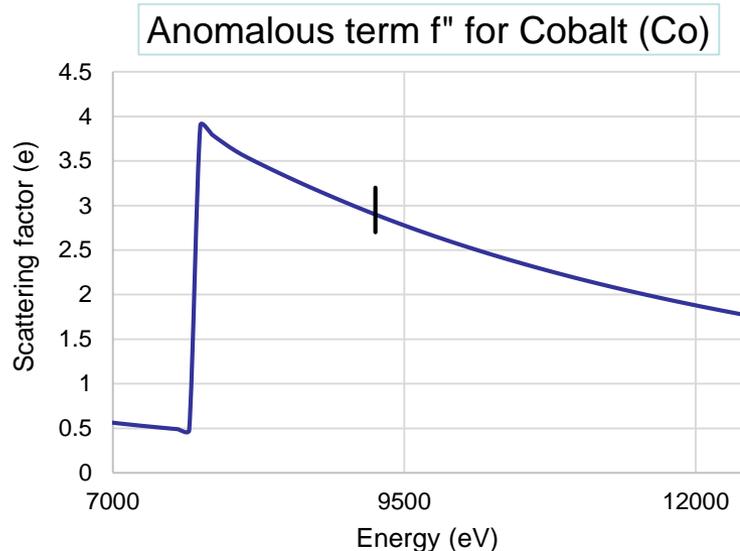
Co-SAD on the metaljet BtuH

- The OM membrane transporter BtuB in *E. coli* is responsible for the vitamin B12 uptake
- Vitamin B12 is an essential micronutrient for the gut microbiota
- Efficient uptake of B12 essential for gut fitness
- In *Bacteroides thetaiotaomicron* 3 loci
- locus 2 important *in vivo*



Vitamin B12





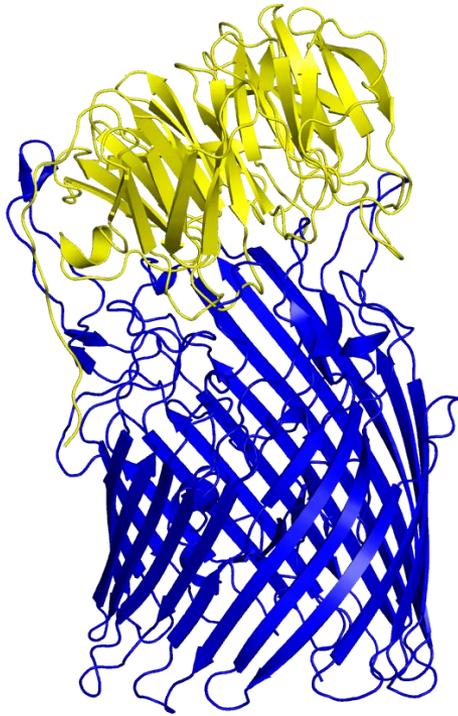
Unit Cell: 129.91 235.31 137.72
Bravais: oC SG: C222

2 Co sites

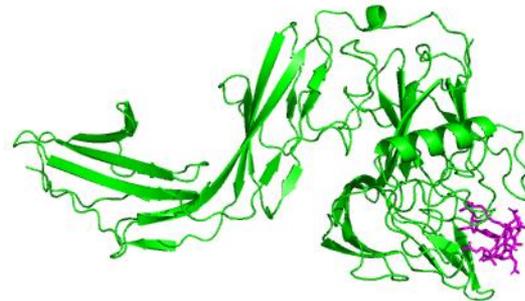
Strategy: let the software decide but
as of high multiplicity to collect over
the weekend
(36 runs, 24799 images, 60 sec
exposure / degree)
Photon III

	Overall	Outershell
Low res (Å)	24.96	1.93
High res (Å)	1.90	1.90
Mean I/sd(I)	15.3	2.2
CC _{1/2}	0.994	0.558
Anom Comp	100.0	100.0
Anom Multiplicity	33.6	15.0

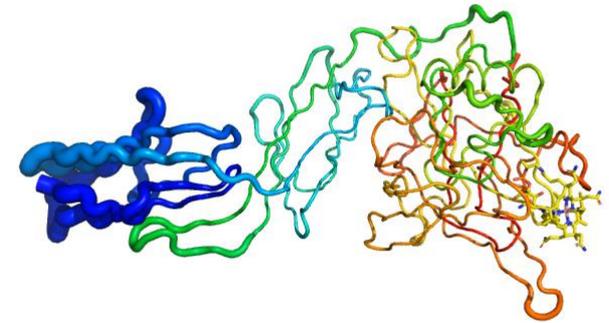
Co-SAD on the metaljet BtuH



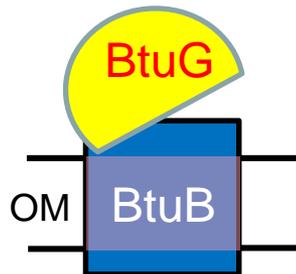
BtuB in complex with BtuG



BtuH in complex with cyanocobalamin



BtuH with cyanocobalamin



- Why does *B. theta* need another surface-exposed protein?
- What is the role of BtuH?

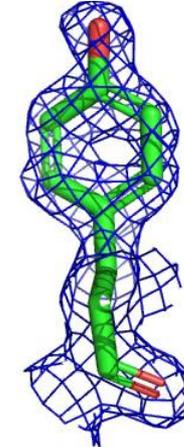
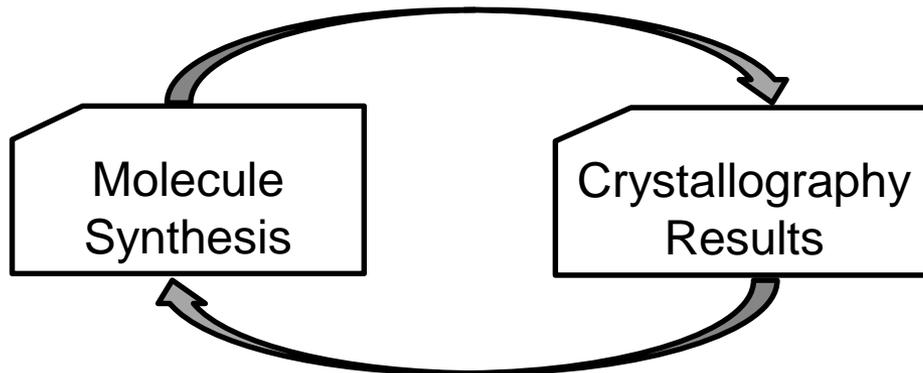
- Introduction
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- FragLites

- Sample need to be reproducible
- Not too many not too few
- Not too big not too small
- Reasonable resolution
- Binding pocket accessible
- DMSO resistant
- Target is oP but close to tP so manual strategy
- 6 minutes data collection, 360 degrees, 1 sec per image (0.5 degrees width)
- Photon III

	Overall	Outershell
Low res (Å)	23.68 – 1.58	1.61 – 1.58
Mean I/sd(I)	19.2	1.6
CC _{1/2}	0.999	0.534
Comp	100	100
Multiplicity	12.2	7.6

Drug discovery how fast can we go?

- As part of 2019 campaign
- 113 samples tested with 40 ligands bound (35.4 %).
- Resolution range 1.7 to 2.2 Å
- Most samples were not sent to synchrotron



Electron density map at 1.5 σ

3 minutes datasets

180 ° @ 1 s	Overall	Outershell
Low res (Å)	23.68 – 1.58	1.61 – 1.58
Mean I/sd(I)	14.0	1.2
CC _{1/2}	0.998	0.391
Comp	98.9	95.9
Multiplicity	6.1	3.9

360 ° @ 0.5 s	Overall	Outershell
Low res (Å)	23.68 – 1.75	1.78 – 1.75
Mean I/sd(I)	15.6	1.3
CC _{1/2}	0.998	0.503
Comp	100	100
Multiplicity	13.5	12.4

- Introduction
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 - Cobalt SAD
- Drug discovery
- **FragLites**

D. Wood *et al.* FragLites-Minimal, Halogenated Fragments Displaying Pharmacophore Doublets. An Efficient Approach to Druggability Assessment and Hit Generation. 2019. J Med Chem

Small molecules libraries

10 000s, 100 000s and more compounds
Initial screening small molecules 25 000

Fragment libraries

<250 Da
Lower affinities
Better solubilities

Fragment-based drug discovery to screen for druggable pockets

To help identification will lower occupancies

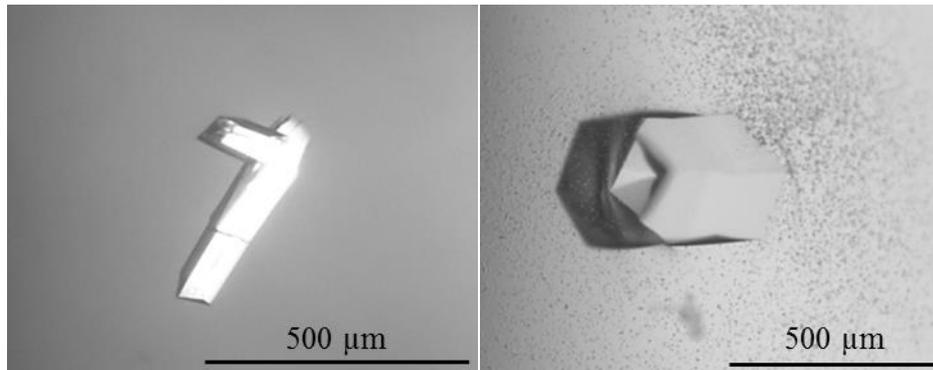
Introduction of an halogen atom to provide an anomalous signal

	Cu (1.54 Å)	Ga (1.34 Å)	Edge Peak
f ⁿ Br (e)	~1.27	~1.11	~3.89
f ⁿ I (e)	~ 6.93	~ 5.53	~13.54 (I23)

Fraglites

2 targets
(P2₁2₁2₁ and P6₅22)

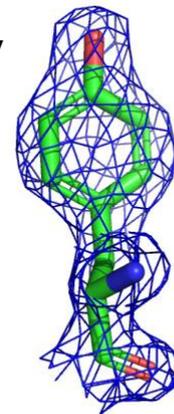
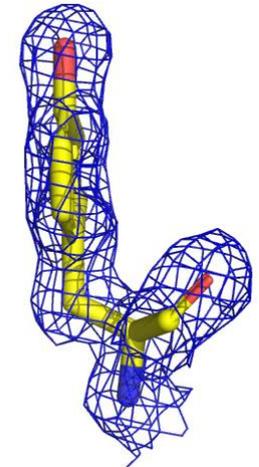
Automated collection
Anom. Multiplicity 5+



19 FragLites we dissolved in crystallisation condition at 50 mM
48 - 72 hours soaks

Cryo protection (30% EG addition to reservoir for one target or already cryoprotected for the other)

	Apo form	Soaks (19)	Hit rate	Sites
Target 1	1.5 Å	1.1 to 2.0 Å	26 %	3
Target 2	2.0 Å	1.6 to 2.8 Å	32%	4



Future work: merging, linking, growing the fragments to develop a drug candidate

Yi Min Ng, Dr Mathew Martin, Prof. Michael Waring, Prof. Martin Noble

Conclusion

- We use the D8 VENTURE in conjunction of synchrotron
 - Advance projects
 - Send well prepared samples to synchrotron
- Invaluable tool for training on sample handling
- Drive drug discovery efficiently
- SAD phasing can be done painlessly taking advantage of the large detector and multi-angle diffractometer

Future work

- Decouple data collection from processing
 - Proteum 3 is multiple licence (windows workstation)
 - Install linux version
- Script the data processing

All the Groups using the facility

B. van den Berg, D. Bolam, O. Davies, C. Dennison, J. Endicott, H. Gilbert, R. Lewis, E. Lowe, J. Marles-Wright, J. Munoz, H. Murray, M. Noble, T. Palmer, P. Salgado, W. Vollmer and K. Waldron



- Dr Vernon Smith
- Dr Mick Carr
- Dr Michael Mrosek
- Dr Holger Ott
- Dr Matthias Binkele
- Dr Jens Lubben
- Dr Tobias Stuerzer

Gary Hopkinson

Questions and Answers



Any questions?

Please type any questions you may have for our speakers in the [Q&A panel](#) and click Send.

Thank you!

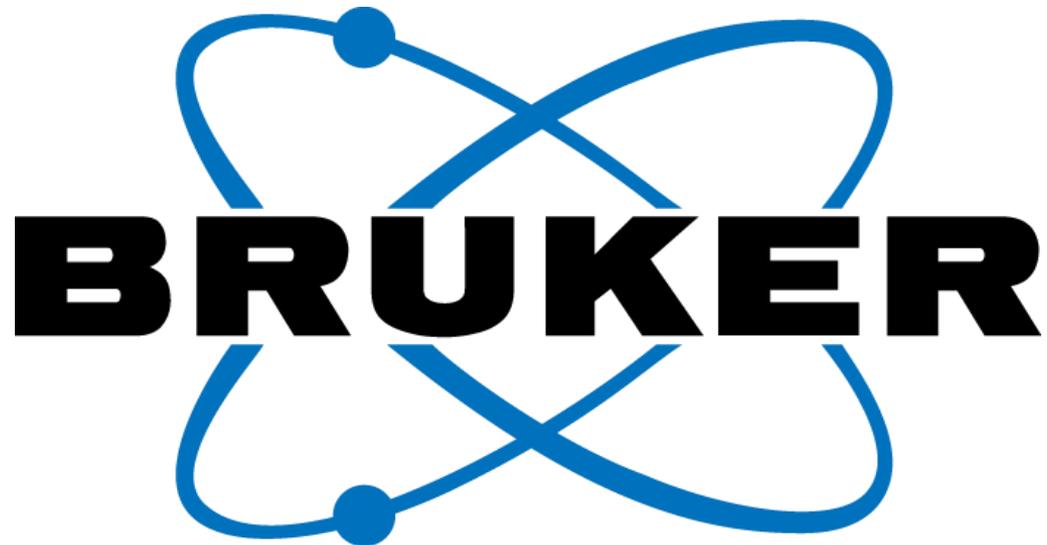


Forthcoming Webinar

July 8, 2020



The screenshot shows the Bruker website's webinar page. At the top is the Bruker logo and a navigation menu with links for Products, Applications, Service, News, Events, About Us, Login, and Contact. A search bar is also present. The main banner features images of Bruker diffractometers and a molecular structure, with social media icons for Facebook, Twitter, LinkedIn, and YouTube on the right. The breadcrumb trail reads: Home - Events - Webinars - Ways to Improve the Productivity of Your Single Crystal Diffractometer. The main title is "Ways to Improve the Productivity of Your Single Crystal Diffractometer" with a sub-label "Single Crystal X-ray Diffraction". A call to action says "Join us for an educational webinar". The "Overview" section contains text about the benefits of upgrading diffractometers. A registration box on the right shows the date "July 08th" (highlighted with a red box), two time options: "8 am CEST, Berlin (3 pm SGT, Singapore)" with a selected radio button, and "8 pm CEST, Berlin (11 am CDT, Chicago)" with an unselected radio button, and a "Register" button. The "Speakers" section lists "Dr. Martin Adam" (SC-XRD Product Manager, Bruker AXS) and "Dr. Vernon Smith" (Business Development Manager SC-XRD, Bruker AXS).



Innovation with Integrity