Optimising the Diamond experience from a user's perspective

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John Innes Centre

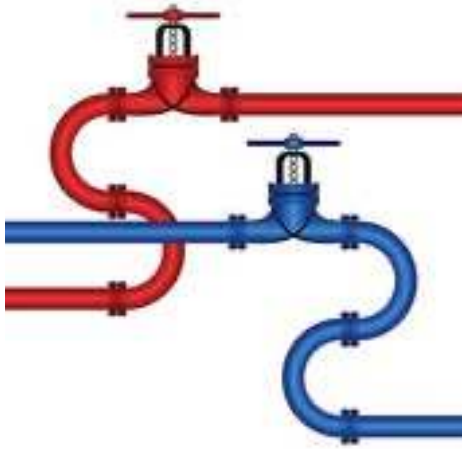
Unlocking Nature's Diversity

UKRI Biotechnology and Biological Sciences Research Council

Strategy

Tools

Pipelines



Remote

- Maximizing efficiency
- Minimizing time commitment
- Managing your data
- How to collect data

“Routine” data collection:

- on non-hazardous samples
- at cryogenic temperatures
- on pre-cooled crystals in pucks
- at conventional wavelengths
- not unattended data collection (UDC)

Primary goals (data quantity **AND** quality…)

- To collect as much data as possible…
- To collect the best possible data…
- To collect the data that will enable me to:
  - Solve my structure
  - Extend the resolution of my structure
  - Show that my ligand is bound

Secondary goal (make your life easier!)

Primary goals (data quantity **AND** quality…)

- To collect as much data as possible…
- To collect the best possible data…
- To collect the data that will enable me to:
  - Solve my structure
  - Extend the resolution of my structure
  - Show that my ligand is bound

Secondary goal (make your life easier!)

- Try to answer these questions **during** your beamtime:
  - Can I solve my structure?
  - Can I extend the resolution of my structure?
  - Is my ligand bound?
- Make best use of time and minimise the amount of follow-up work…

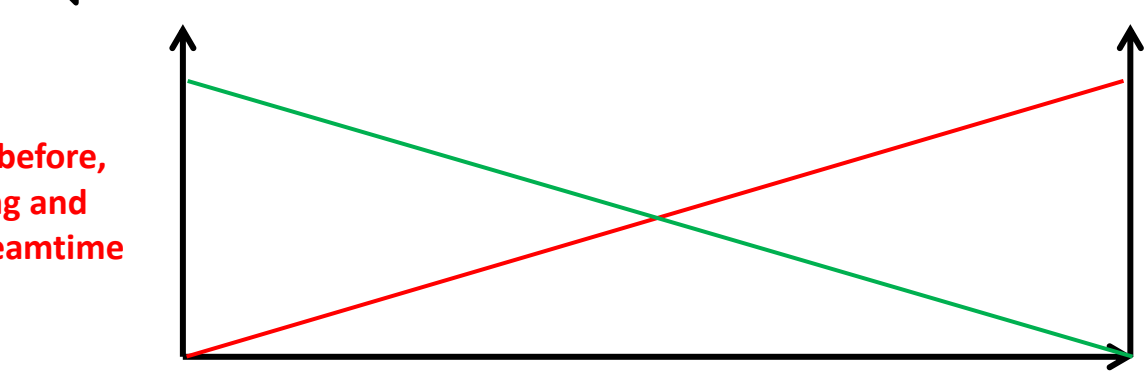
How many samples to prepare per project…

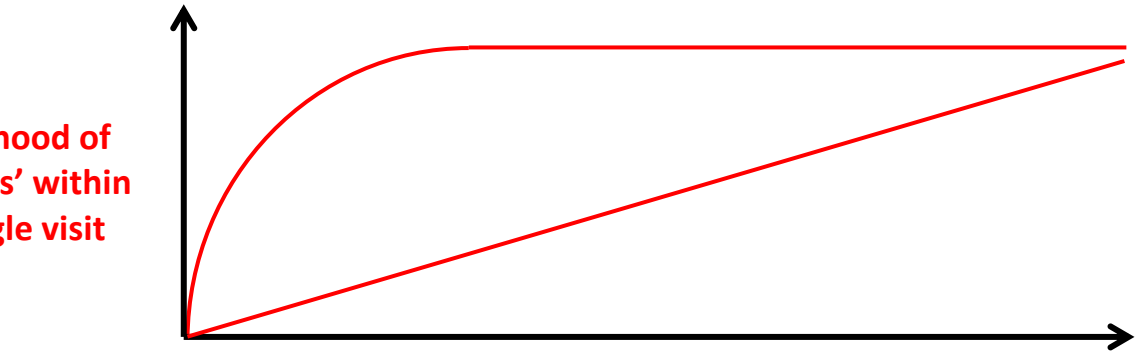
One crystal

High visit frequency

Low visit frequency

A dewar full of crystals (112)





Data collection is always a compromise…

How much data do I need?

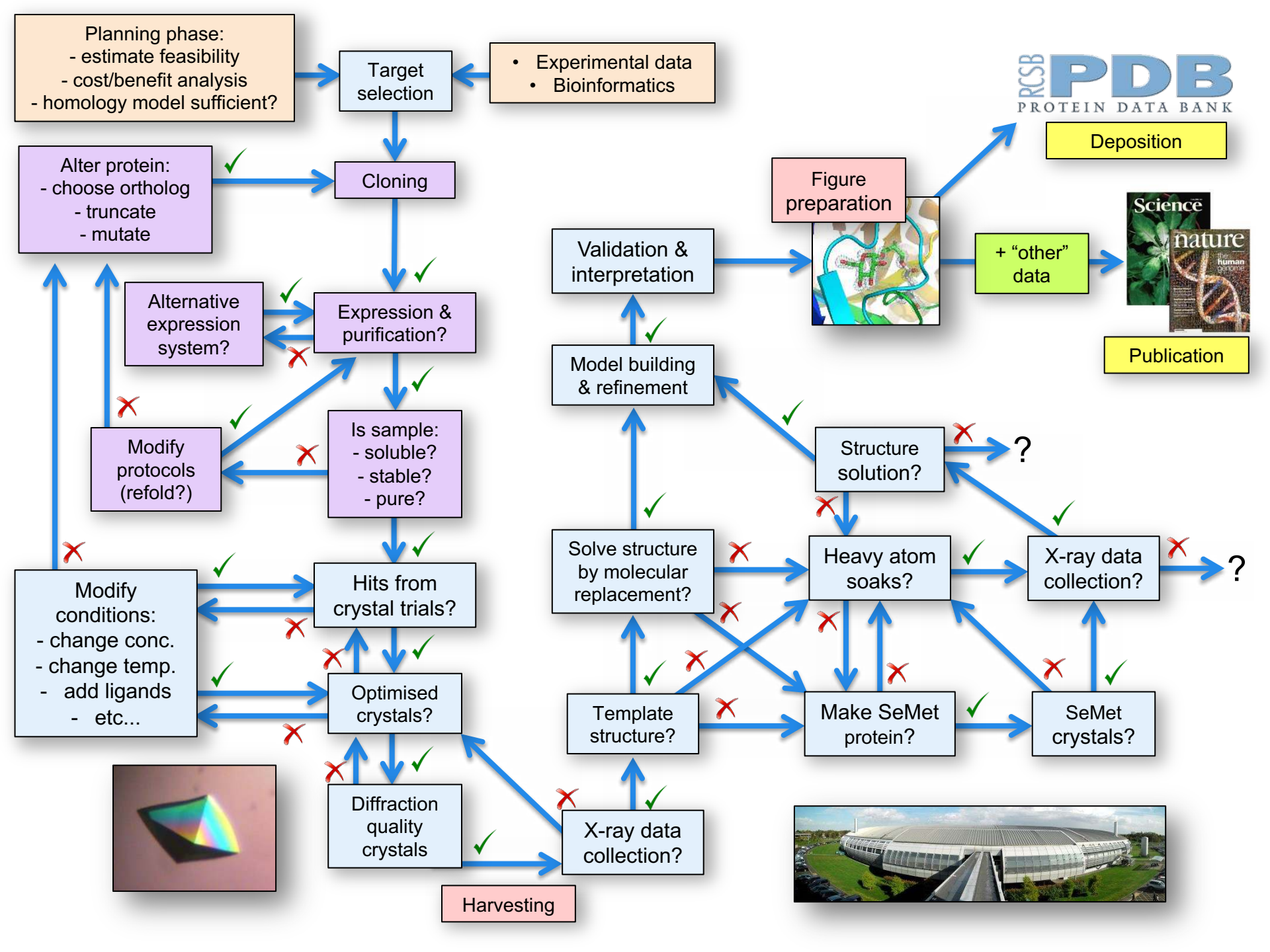
What resolution do I need?

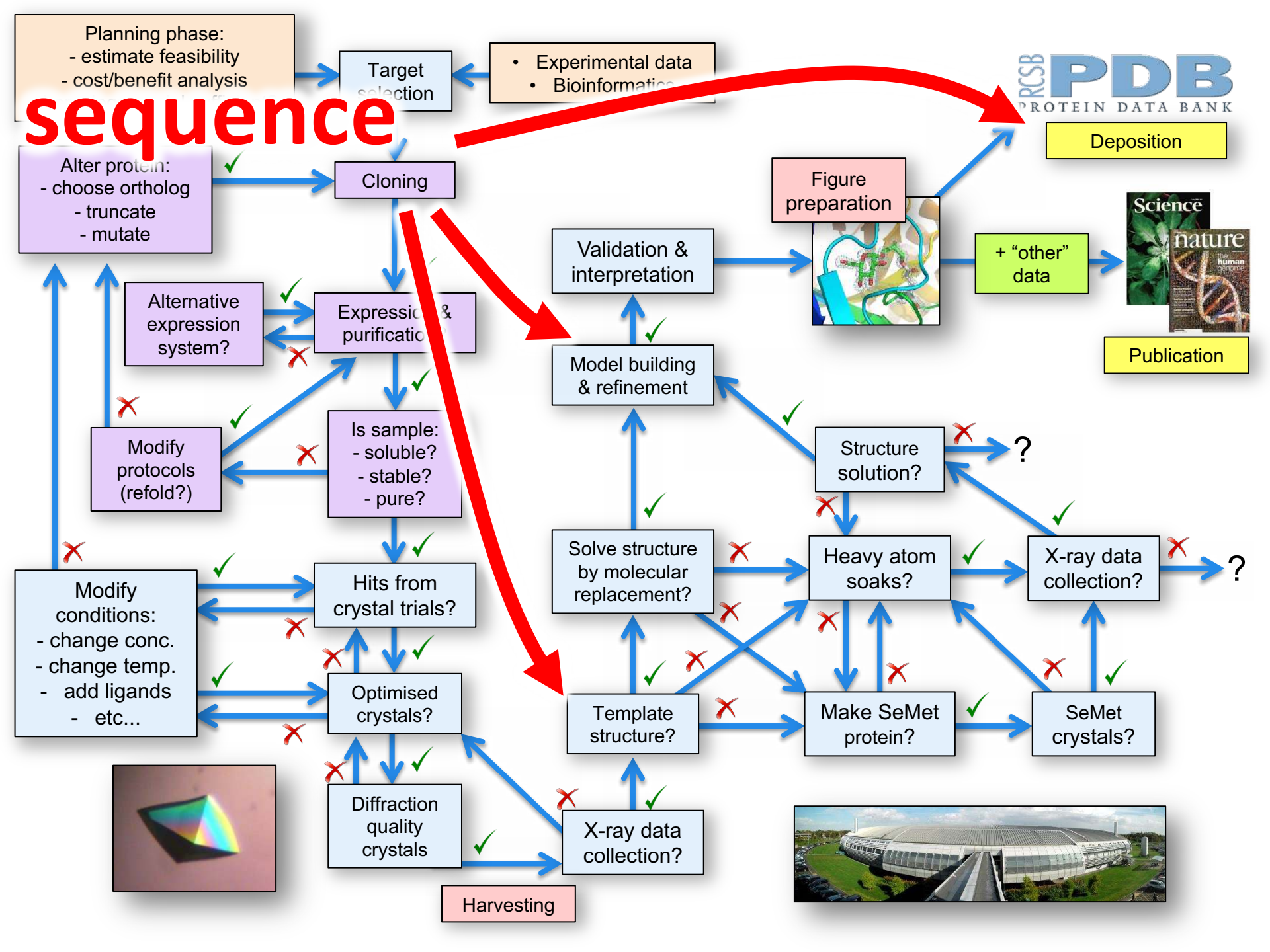
What are the data for?

How much time do I have?

How many crystals do I have?

Think about this **before** your beamtime!


Planning phase:
- estimate feasibility
- cost/benefit analysis
- homology model sufficient?
Target selection
• Experimental data
• Bioinformatics
RCSB PDB
PROTEIN DATA BANK
Deposition
Alter protein:
- choose ortholog
- truncate
- mutate
Cloning
Figure preparation
Validation & interpretation
+ "other" data
Science
nature
Publication
Alternative expression system?
Expression & purification?
Model building & refinement
Modify protocols (refold?)
Is sample:
- soluble?
- stable?
- pure?
Structure solution?
?
Solve structure by molecular replacement?
Heavy atom soaks?
X-ray data collection?
?
Modify conditions:
- change conc.
- change temp.
- add ligands
- etc...
Hits from crystal trials?
Optimised crystals?
Template structure?
Make SeMet protein?
SeMet crystals?
Diffraction quality crystals
X-ray data collection?
Harvesting


Planning phase:
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- cost/benefit analysis
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- mutate

Cloning

Alternative expression system?

Expression & purification?

Modify

Is sample:
- soluble?
- stable?
- pure?

Hits from crystal tri...?

- change pH
- change temp.
- add ligands
- etc...

Optimised crystals?

Diffraction quality crystals

Harvesting

X-ray data collection?

Template structure?

S... structure molecular placement?

Model building & refinement

Validation & interpretation

Struct... solutio...

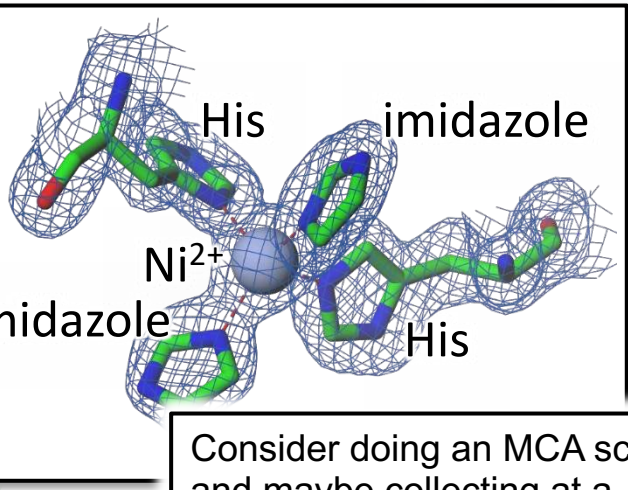
Heavy a... soaks?

collection?

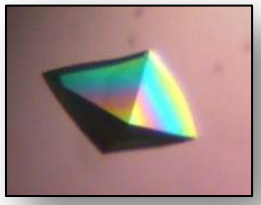
Make SeMet protein?

SeMet crystals?

potential ligands



Consider doing an MCA scan and maybe collecting at a different wavelength – could help with:
- phasing
- map interpretation



Methods

Planning phase:
- estimate feasibility
- cost/benefit analysis
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Target selection

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Alter protein:
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Cloning

Alternative expression system?

Expression & purification?

Modify protocols (refold?)

Is sample:
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Modify conditions:
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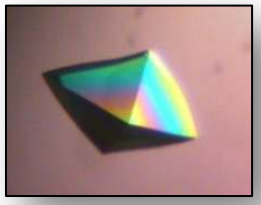
- etc...

Hits from crystal trials?

Optimise crystals?



Diffraction quality crystals

Harvesting

X-ray data collection?

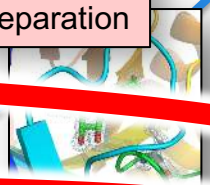
Template structure?

Solve structure by molecular replacement

Model building & refinement

Validation & interpretation

Figure preparation



RCSB PDB PROTEIN DATA BANK

Deposition

+ "other"

Publication

Structure solution?

Heavy atom soaks?

Make SeMet protein?

X-ray data collection

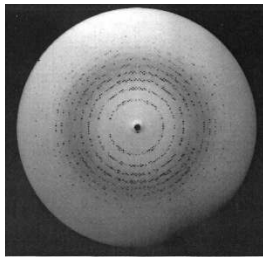
?

Detector types

hours

readout time

1 ms



Film

Image plate

CCD (charge-coupled device)

360° data sets possible in <10 s!

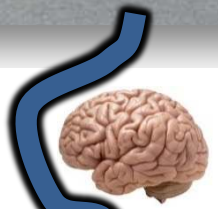
HPC (hybrid photon counting)

1990 2000 2010 2020

MX data collection has become faster…

20th century data collection

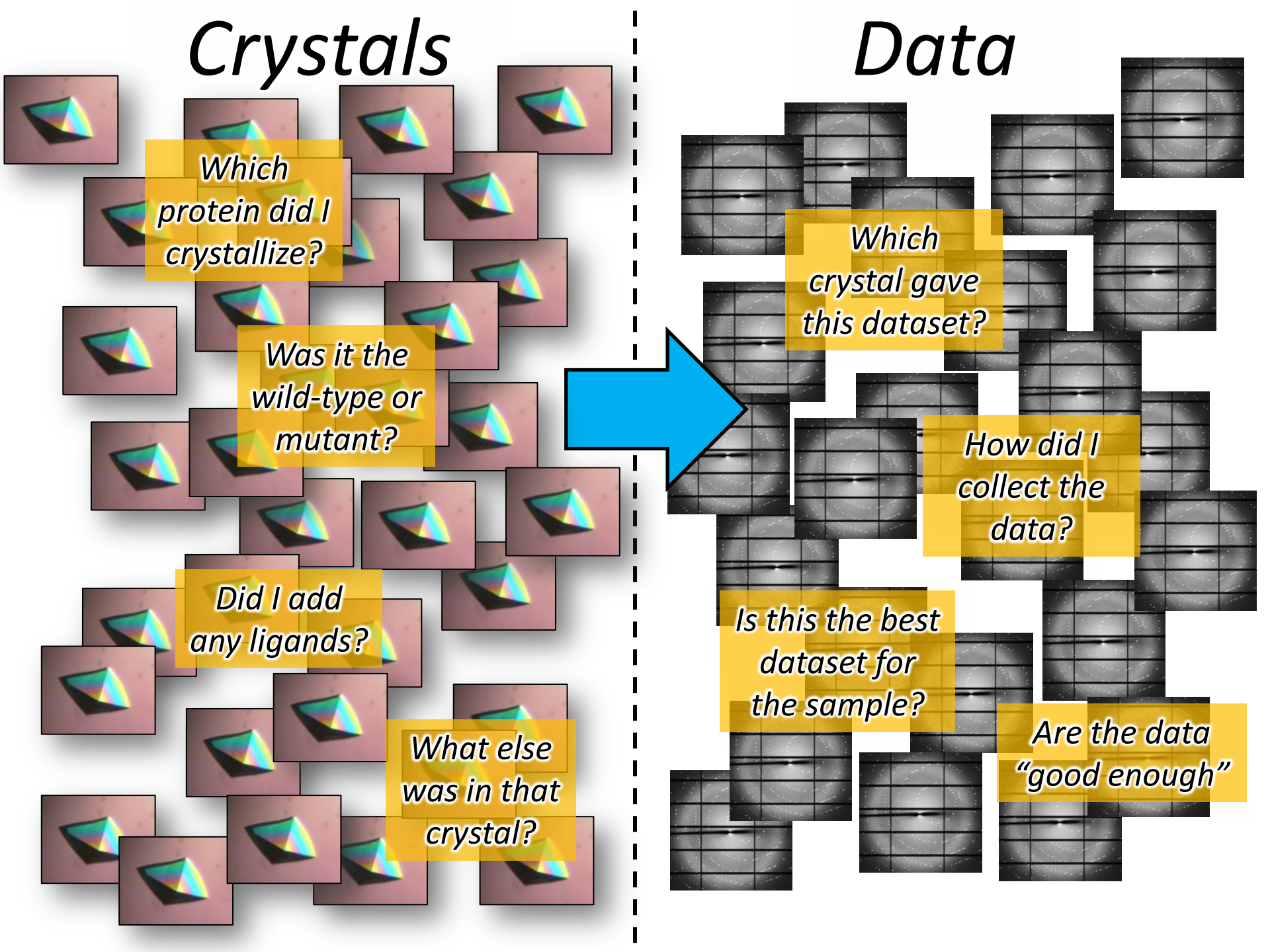
21st century data collection



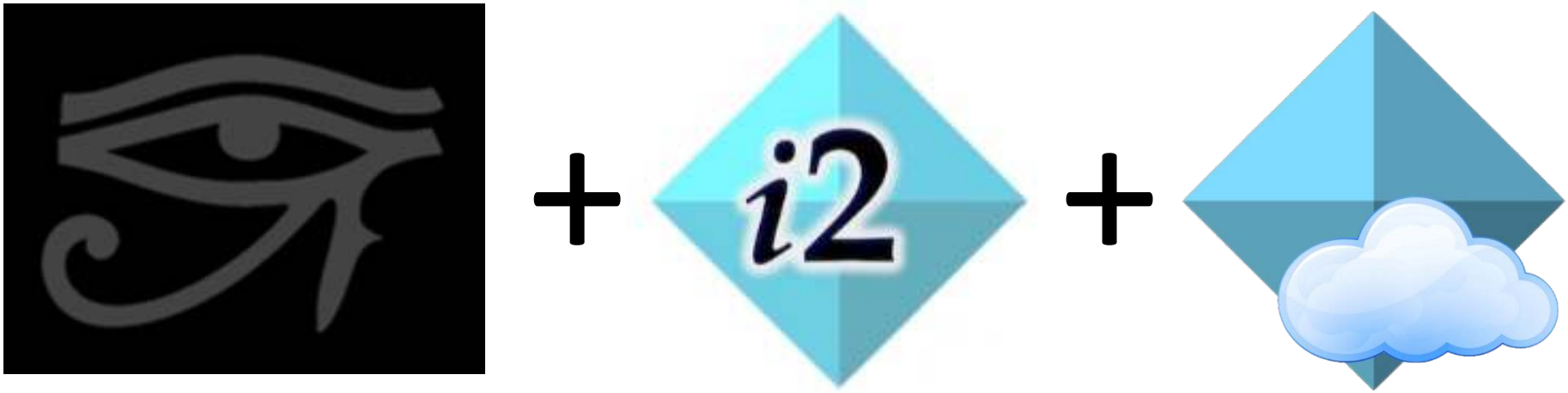
MX data collection has become much faster…

- In principle, could collect one dataset every 5 mins

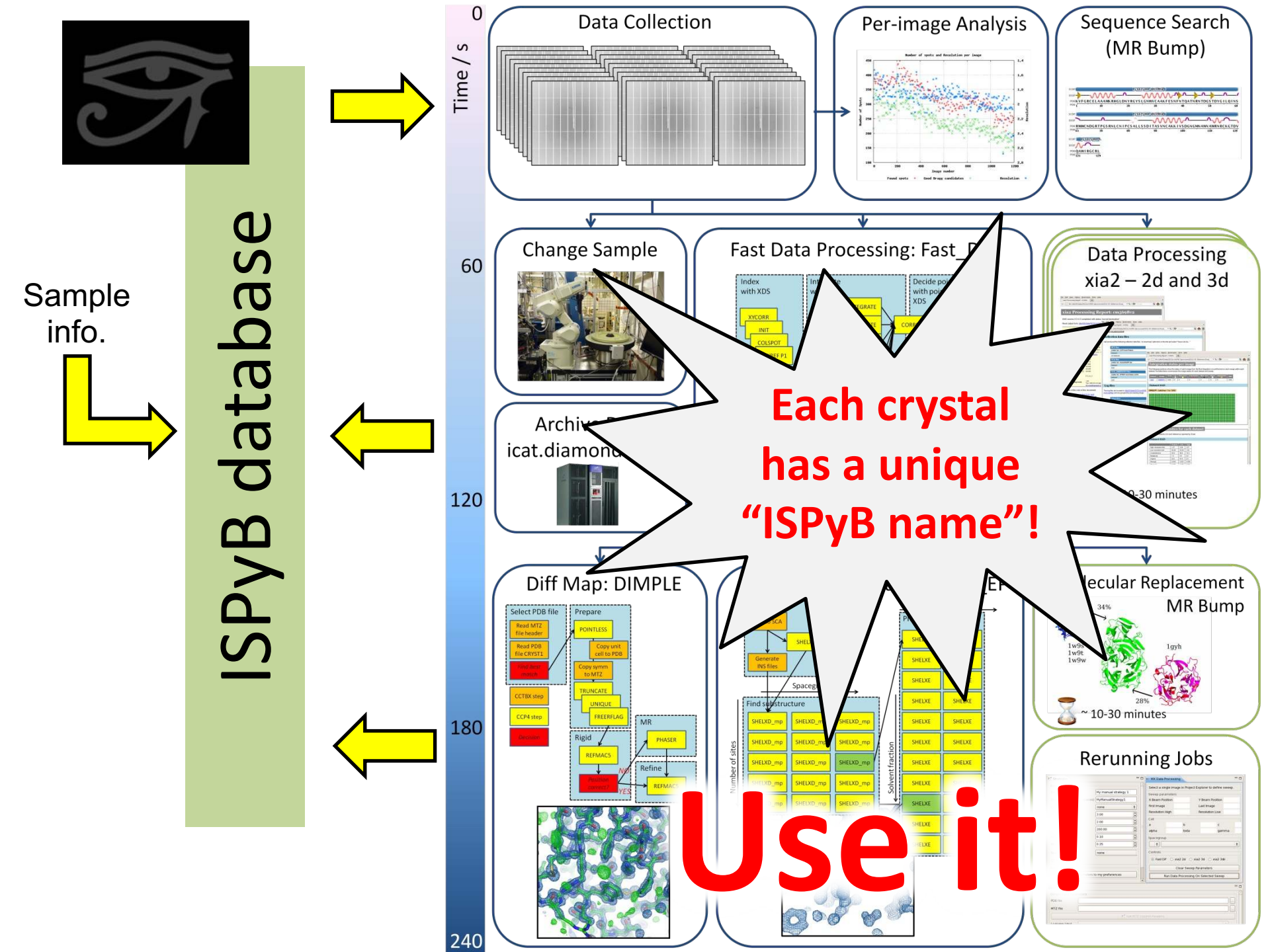
  (mounting/aligning + evaluating test images + collection)
- In practice, ~5 “useful” data sets per hour is good
  - includes screening for “best” crystal
  - …and thinking!
- But - beamtime is still in high demand
  - users are generating crystals more rapidly
  - can get away with “marginal” samples
- Each session is often split between several groups
  - therefore need to be efficient and organised...

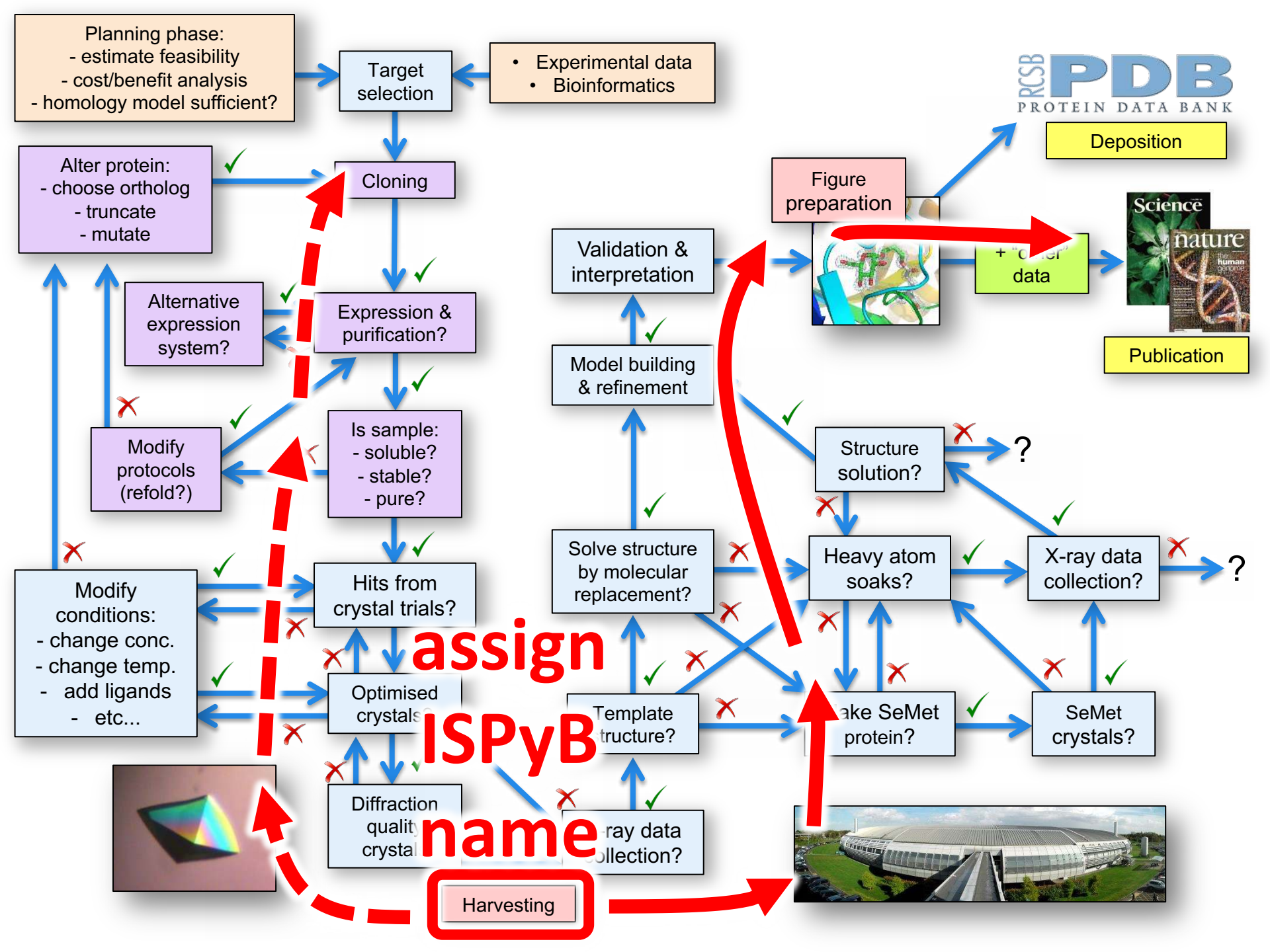

Crystals
Data
Which protein did I crystallize?
Was it the wild-type or mutant?
Did I add any ligands?
What else was in that crystal?
Which crystal gave this dataset?
How did I collect the data?
Is this the best dataset for the sample?
Are the data "good enough"

Taking control...



ISPyB/SynchWeb *CCP4i2* *CCP4 Cloud*


Sample info.
ISPyB database
Time / s
0
60
120
180
240
Data Collection
Per-image Analysis
Sequence Search (MR Bump)
Change Sample
Fast Data Processing: Fast_
Data Processing xia2 – 2d and 3d
Archiv
icat.diamond
Each crystal has a unique "ISPyB name"!
Diff Map: DIMPLE
Select PDB file
Prepare
POINTLESS
TRUNCATE
UNIQUE
FREERFLAG
MR
PHASER
Rigid
REFMAC5
Refine
Find substructure
Molecular Replacement MR Bump
~ 10-30 minutes
Rerunning Jobs
Use it!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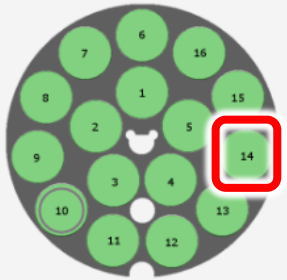
assign
Optimised crystals
Template structure?
ISPyB
SeMet protein?
SeMet crystals?
Diffraction quality crystal
name
ray data collection?
Harvesting

Date: 29-JUL-2017
Beamline: i03 Diamond
Visit ID: mx13467-41
Protein acronym: NmADH9
ISPyB name: NmADH9_23
Dataset name: NmADH9_23_1

Harvest your crystals and enter sample info into ISPyB

Shipment	JIC_260717_I03
Dewar	DLS-MX-0002
Container Type	Puck
Registered Container	DLS-442 [View]
Barcode	Click to edit
Automated Collection	+ Queue this container for Auto Collect
Comments	Click to edit



Location History

Date	Status	Location	Beamline
08-09-2017 10:49	at facility		
04-08-2017 15:58	at DLS		
29-07-2017 11:26	processing	7	i03
21-07-2017 12:04	at DLS		

10 ▾ Page « ‹ 1 › »

this is **UNIQUE**

+ Extra Fields

Location	Protein Acronym	Abundance	Components	Name	Spacegroup	Barcode	Comment	Status
9	NmADH9			NmADH9_18				
10	NmADH9			NmADH9_19				
11	NmADH9			NmADH9_20				
12	NmADH9			NmADH9_21				
13	NmADH9			NmADH9_?				
14	NmADH9			NmADH9_23				

Required for UDC

Basic | Extra Fields | Unattended (UDC)

Loc	Protein Acronym	Name	Sample Group	Anomalous	Barcode	Comment	Status
1	LYS	JIC_LYS_OCT	-				

turns on EP pipelines

Use a spreadsheet…

crystals_MX13467-41_i03_29-July-2017

Puck number – DLS442

Posi-tion	Person	Protein name	IspyB_name	Plate	Well	Conditions	Soak/cryo	Space group	Test	Reso-lution	Comments
9	Benjy	NmADH9	NmADH9_18	MCBL0004	A11.1	"	Cis cis nepetalactol+ 20%EG				10mM soak for approx 1 h
10	Benjy	NmADH9	NmADH9_19	MCBL0004	A11.1	"	8 oxogeranial+ 20%EG				10mM soak for approx 1 h
11	Benjy	NmADH9	NmADH9_20	MCBL0004	A11.1	"	8 oxogeranial+ 20%EG				10mM soak for approx 1 h
12	Benjy	NmADH9	NmADH9_21	HD01	B6	PEG 4k 29%, 0.1M Mes pH 6.5, 1 mM NAD	8-oxocitronellal+ 20%EG				5mM soak for approx 1.5 h
13	Benjy	NmADH9	NmADH9_22	HD01	B4	"	8-oxocitronellal+ 20%EG				5mM soak for approx 1.5 h
14	Benjy	NmADH9	NmADH9_23	HD01	B3	"	Cis cis nepetalactone+ 20%EG				5mM soak for approx 1.5 h
15	Benjy	NmADH9	NmADH9_24	HD01	B2	"	Cis cis nepetalactol+ 20%EG				5mM soak for approx 1.5 h
16	Benjy	NmADH9	NmADH9_25	HD01	B1	"	8 hydroxygeraniol+ 20%EG				5mM soak for approx 1 h

annotate hardcopy during data collection – helps decision making

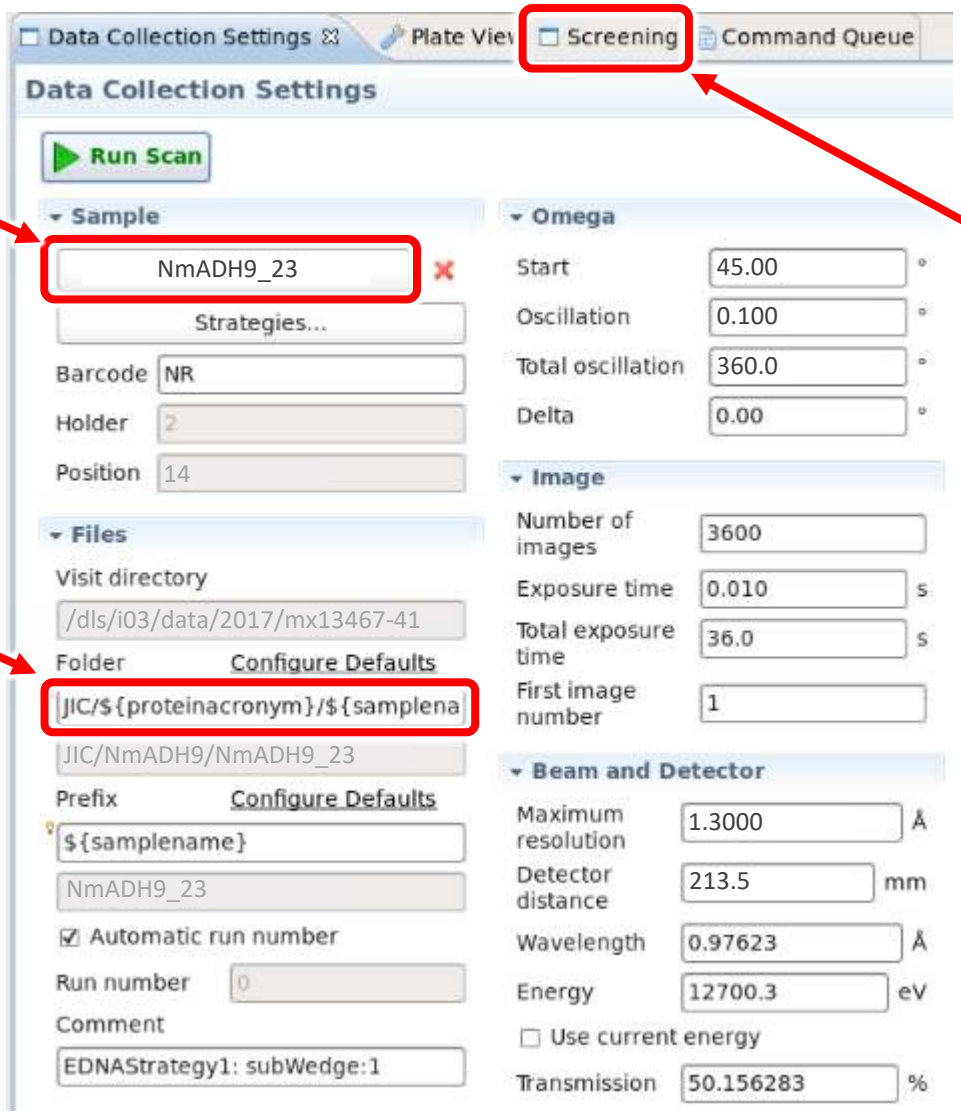
Data collection setup in GDA

Select required sample from drop-down menu:

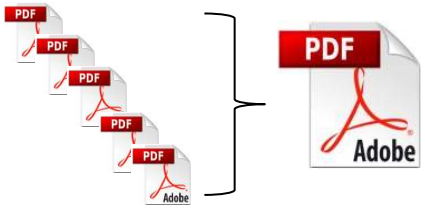
- no need to enter sample information or specify sample location
- less likely to get the wrong sample

Put your data into your own directory....

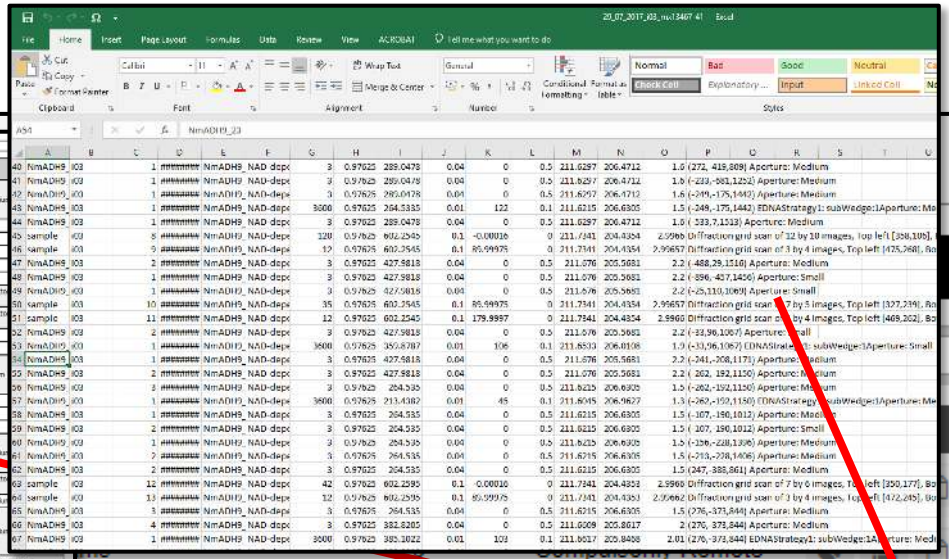
...especially important if there are multiple users from several institutions – simplifies backing up too....

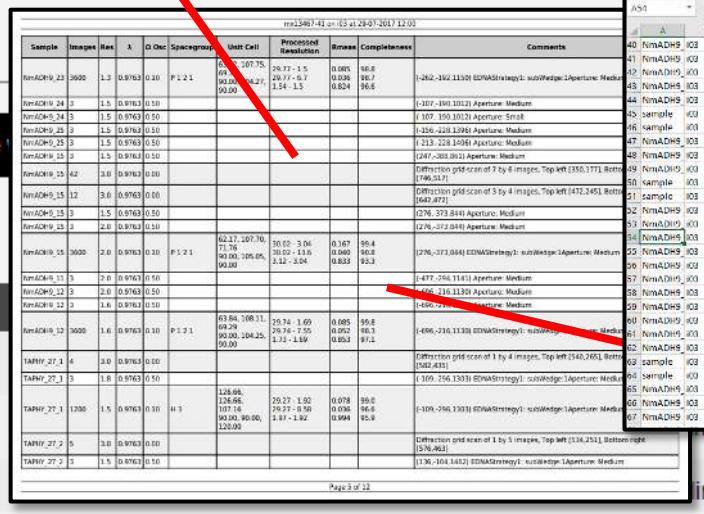


use the "screening" tab for test images – keeps them separate from datasets



simple to search this for an ISPyB name



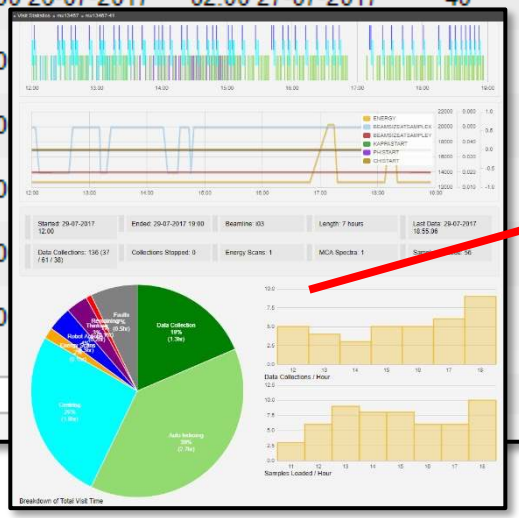


Visit List

This page lists the

Start						
19:00 15-09-2017						
02:00 11-09-2017						
10:00 05-08-2017					395	
12:00 29-07-2017	19:00 29-07-2017	41	i03	Dr Katherine McAuley	136	Compulsorily Remote
19:00 26-07-2017	02:00 27-07-2017	40	i03	Mr Mark Williams	71	Compulsorily Remote
02:0			i03	Dr Neil Paterson	38	Compulsorily Remote
17:0			i03	Dr Neil Paterson	76	
19:0			i04	Dr Melanie Vollmar	69	Compulsorily Remote
12:0			i04	Dr Dave Hall	106	Compulsorily Remote
02:0			i03	Dr Neil Paterson	82	Compulsorily Remote

10 5 6 7 › »



What you did...

(1) ISPyB interface

(2) Visit PDF (combine with others...)

mx13467-41 on i03 at 29-07-2017 12:00

Sample	Images	Res	λ	Ω Osc	Spacegroup	Unit Cell	Processed Resolution	Rmeas	Completeness	Comments
NmADH9_23	3600	1.3	0.9763	0.10	P 1 2 1	63.92, 107.75, 69.36 90.00, 104.27, 90.00	29.77 - 1.5 29.77 - 6.7 1.54 - 1.5	0.085 0.036 0.824	98.8 98.7 96.6	(-262,-192,1150) EDNAStrategy1: subWedge:1Aperture: Medium

(3) Visit Excel sheet (annotate...)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	
1		mx13467-41_i03_29-JUL-2017 - 7 hr - Pilatus3 6M detector (100 Hz)																			
2																					
3	data	Image prefix	Run	Sta	Sam	Prot	# imag	Wavele	Dista	Exp.	Phi st	Phi ra	Xbea	Ybea	Det	auto/m	resoln	space gr	cell	twinnin	comments
54		NmADH9_23	2	##	NmA	NAD	3	0.9763	428	0.04	0	0.5	212	206	2.2						
55		NmADH9_23	3	##	NmA	NAD	3	0.9763	265	0.04	0	0.5	212	207	1.5						
56	12	NmADH9_23	1	##	NmA	NAD	3600	0.9763	213	0.01	45	0.1	212	207	1.3	a/3dii	1.37	P21	64 108 69 / 90 104 90	2.09	Binary complex with NAD - best data so far
57		NmADH9_24	1	##	NmA	NAD	3	0.9763	265	0.04	0	0.5	212	207	1.5						
58		NmADH9_24	2	##	NmA	NAD	3	0.9763	265	0.04	0	0.5	212	207	1.5						

Ideal scenario during session:

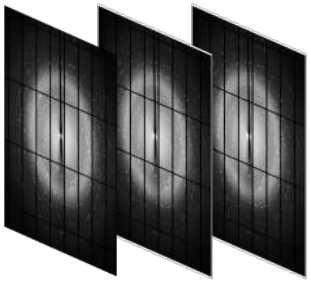
- Load first sample
- Collect test images
- Based on these, decide:
  - to collect...
  - not to collect...
  - to revisit later...
- For a “suitable” sample:
  - devise a data collection strategy
  - collect data set
- Analyse data as they are collected
- Based on this analysis, revise plans if appropriate
- Move on to next sample…

– not efficient

without automation

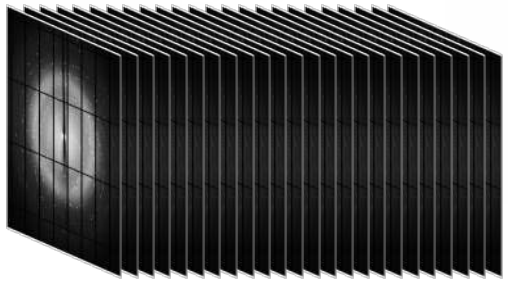
robotic sample exchange

align crystal in X-ray beam



collect test images

devise data collection strategy based on test images (and what you want to use the data for)



collect dataset

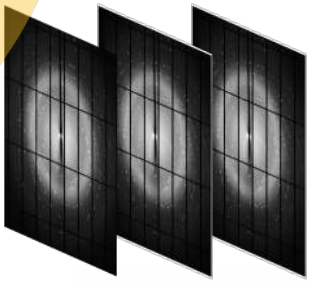
typically 3,600 x 0.1° images

analyse data on-the-fly

Don't forget to think!

robotic sample exchange

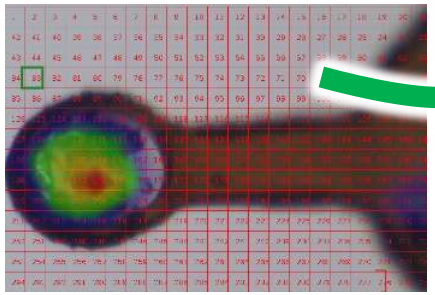
align crystal in X-ray beam

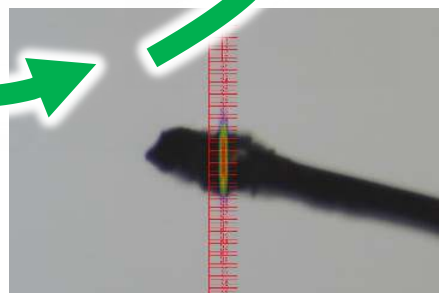


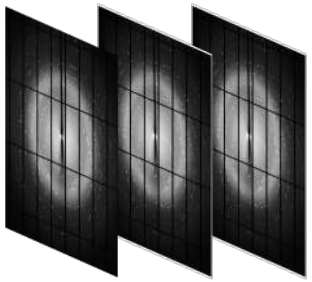
collect test images

May be more efficient to screen your samples in batches, then decide what to do…

- do this automatically with X-ray centring
- also an opportunity to grab a coffee!

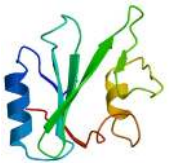






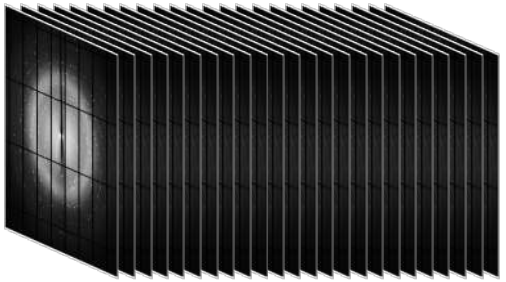
test images

before session...



template

sequence



dataset

Look at images (e.g. in ADXV) - can they be indexed?

ISPyB

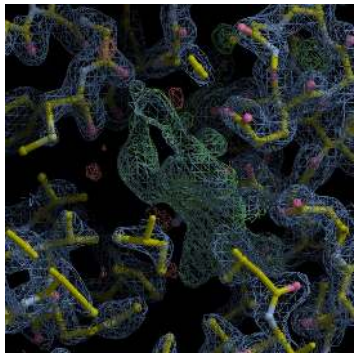
< 2 min

Fast DP

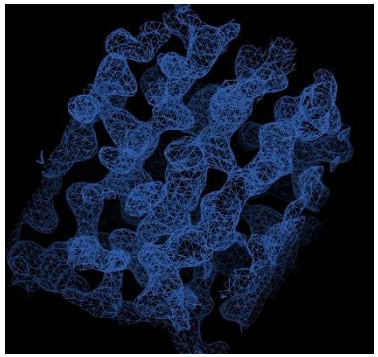
h	k	l	Iplus	SIGIplus	Iminus	SIGIminus
-45	0	4	-1.00	1.20	-1.00	1.20
-45	0	5	-0.03	1.82	-0.03	1.82
-45	0	6	2.17	2.01	2.17	2.01
-45	0	7	-0.22	1.24	-0.22	1.24
-45	0	8	0.63	1.33	0.63	1.33
-45	0	9	1.46	1.40	1.46	1.40
-45	0	10	0.11	1.34	0.11	1.34
-45	0	11	2.02	1.41	2.02	1.41

< 5 min

Quick feedback

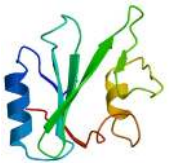


Dimple



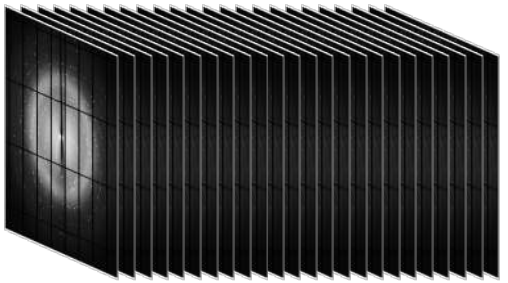
Fast EP

before session...



template

sequence

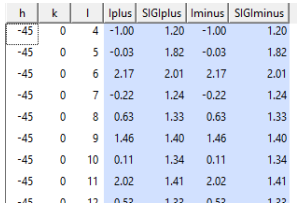


dataset

ISPyB

20-30 min

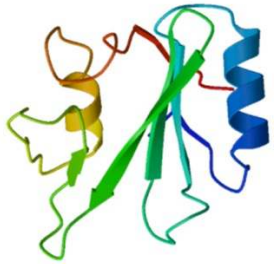
Xia2
DIALS
autoPROC
Xia2.multiplex



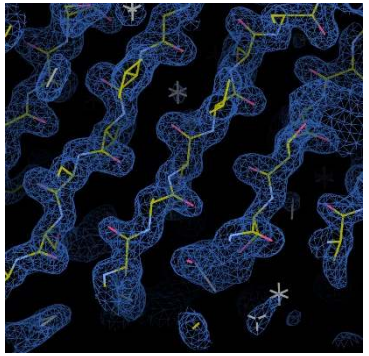
h	k	l	Iplus	SIGIplus	Iminus	SIGIminus
-45	0	4	-1.00	1.20	-1.00	1.20
-45	0	5	-0.03	1.82	-0.03	1.82
-45	0	6	2.17	2.01	2.17	2.01
-45	0	7	-0.22	1.24	-0.22	1.24
-45	0	8	0.63	1.33	0.63	1.33
-45	0	9	1.46	1.40	1.46	1.40
-45	0	10	0.11	1.34	0.11	1.34
-45	0	11	2.02	1.41	2.02	1.41

hours

Slower feedback

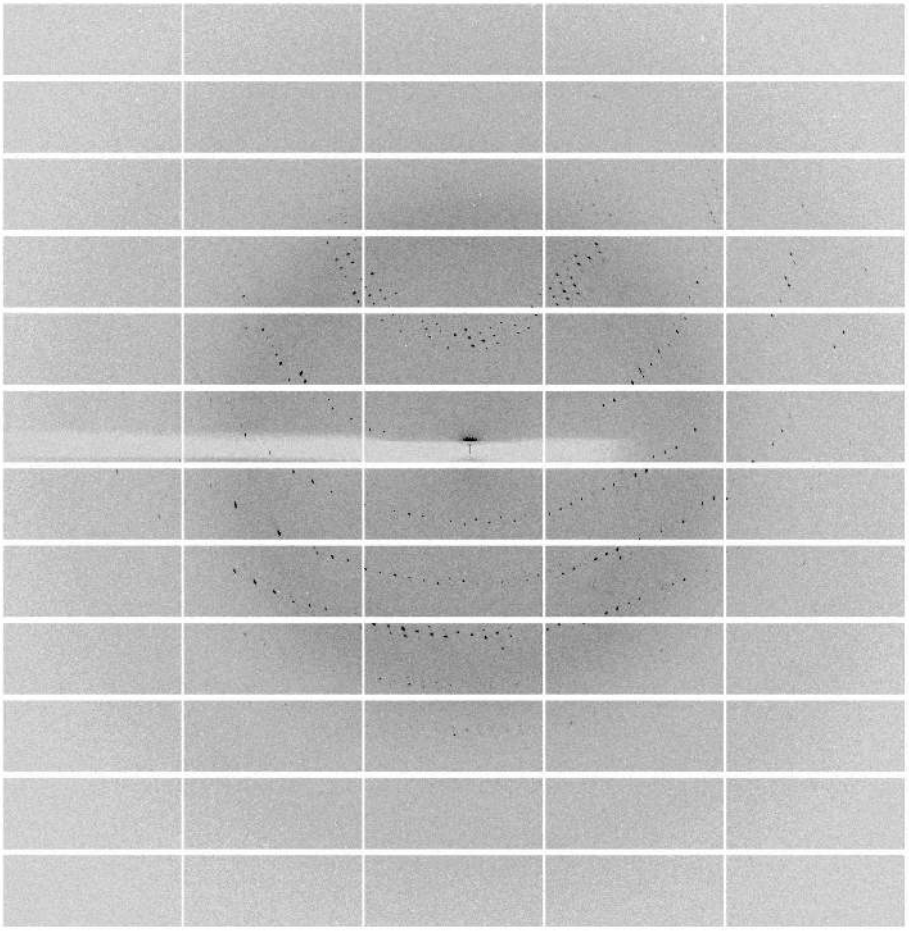


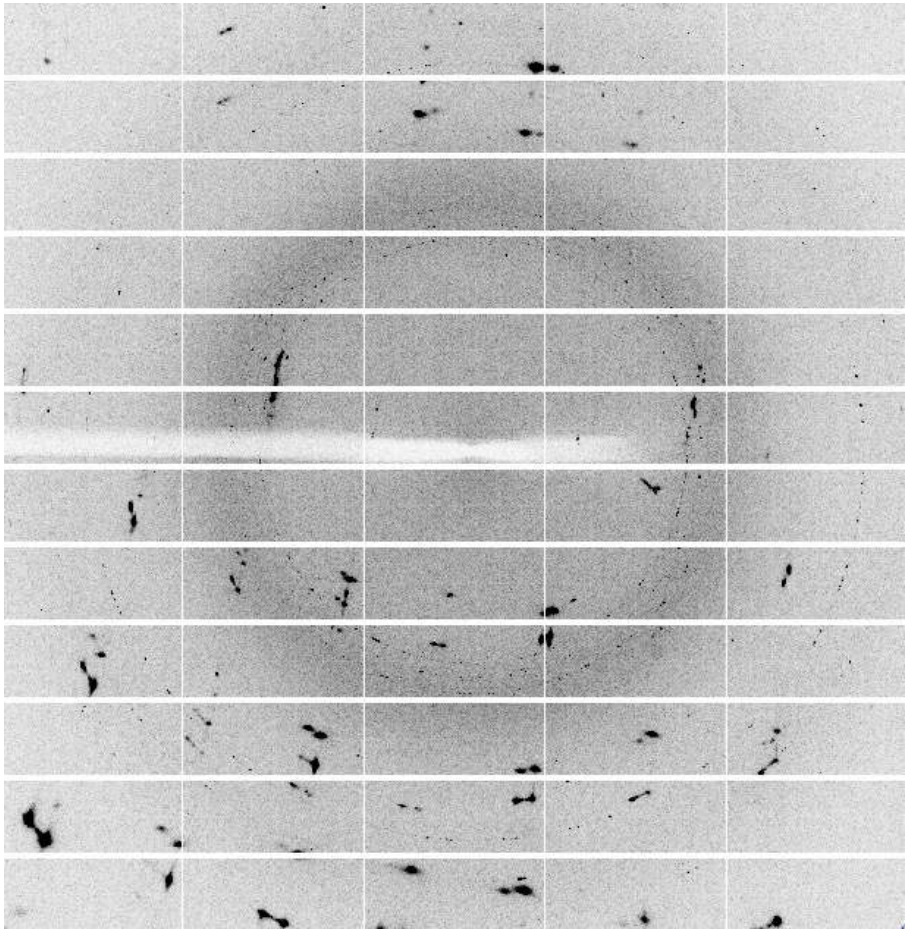
MrBUMP



autoSHARP/Crank2/
AutoBuild

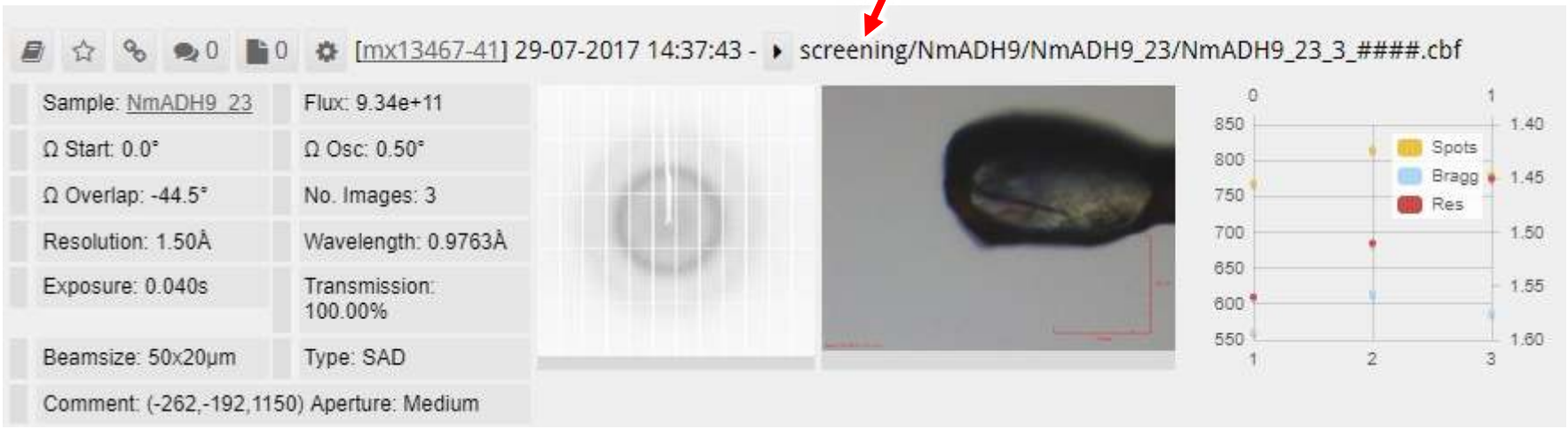
LOOK at your images!!!!





Screening…

(using the “screening” tab...)



Screening…


0
0
[mx13467-41] 29-07-2017 14:37:43 - screening/NmADH9/NmADH9_23/NmADH9_23_3_####.cbf
Sample: NmADH9_23
Flux: 9.34e+11
Ω Start: 0.0°
Ω Osc: 0.50°
Ω Overlap: -44.5°
No. Images: 3
Resolution: 1.50Å
Wavelength: 0.9763Å
Exposure: 0.040s
Transmission: 100.00%
Beamsize: 50x20µm
Type: SAD
Comment: (-262,-192,1150) Aperture: Medium
0
1
850
800
750
700
650
600
550
1.40
1.45
1.50
1.55
1.60
1
2
3
Spots
Bragg
Res
Strategies
Mosflm:
EDNA:
Xia2:

Screening…

Have I seen this crystal form before?

Strategies — Mosflm: ✔ EDNA: ✔ Xia2: ✔

xia2.strategy

Space Group	A	B	C	α	β	γ
P 1 2 1	63.87	107.69	69.31	90.00	104.25	90.00

Lookup Cell

Strategy	Description	Ω Start	Ω Osc	Res (Å)	Ran… Res	Rel Trn (%)	Abs Trn (%)	Exposure (s)	No. Images
anomalous Wedge1	Standard Anomalous Dataset Multiplicity=3 I/sig=2 202s	0	0.15	1.51	1.1…	4.0	4	0.010	2214
gentle Wedge1	Gentle: Target Multiplicity=2 I/sig=2 Maxlifespan=20s	6	0.15	1.51	1.2…	4.0	4	0.010	2000
high multiplicity Wedge1	Strategy with target multiplicity=16 I/sig=2 202s	0	0.15	1.51	1.1…	4.1	4.1	0.010	2400
native Wedg…	…Native Dataset Multiplicity=3 I/sig=2 …2s	162	0.15	1.51	1.…	2.2	2.2	0.010	1947

EDNA MX…

Space Group	A	…	γ
P2	63.99	107.4…	

Lookup Cell

Strategy		Ω Start	Ω Osc	Res (Å)	Ranking Res (Å)	Rel Trn (%)	Abs Trn (%)	Exposure (s)	No. Images
Strategy1 Wedge1	Standard Native Dataset Multiplicity=3 …		0.10	1.51	1.21	11.0	11	0.010	1630
Strategy2 Wedge1	Standard Anomalous Dataset Multiplicity=3 I/sig=2 Maxlifespan=202 s				1.24	12.4	12.4	0.010	2740
Strategy3 Wedge1	strategy with target multiplicity=1… s					5.1	5.1	0.010	3600
Strategy4 Wedge1	Gentle: Target Multiplicity=2 and … s		0.10				14.8	0.010	1110
Strategy5 Wedge1	UnderDEV Anomalous Dataset, …		0.10	1.51	1.24			0.010	2740

mosflm

Space Group	A	B	C	α	…
P2	63.80	107.65	69.44	90.00	10…

…okup Cell

Strategy	Description	Ω Start	Ω Osc	Res (A)	Ranking Res (Å)	Rel Trn (%)	Abs Trn (%)	Exposure	No. Images
anomalous Wedge1		196	0.20	1.46	0.00	0.0	0	0.000	525
native Wedge1		211	0.20	1.46	0.00	0.0	0	0.000	525

Strategies are currently not reliable

Notes about strategies…

Despite what your supervisor or colleague advises…

- always collect a minimum of 360° (unless you have a very good reason not to)
- consider multiple 360° passes if you have low symmetry (ideally rotating around a different axis for each – see data collection talks…)

If your test images don't index, but the crystal is diffracting reasonably well…

- collect a data set anyway – it might be useful!
  - inspect screening images in ADXV
  - look for highest resolution spots (NOT ice or salt spots!)
  - subtract 0.5 from this resolution value. E.g. spots at 2.5 Å → collect to 2.0 Å
  - collect 3,600 x 0.1° images with a total dose of 1-2 MGy

Checking the results…

Checking the results…

Auto Processing — Fast DP: ✔ Xia2: ✔ ✔ ✔ MultiXia2: ? ? autoPROC: ✔

Type	Resolution	Spacegroup	I/sig(I)	Rmeas Inner	Rmeas Outer	Completeness	Cell	Status
fast_dp	29.77 - 1.50	P 1 2 1	5.2	0.036	0.824	98.8	63.92 107.75 69.36 90.00 104.27 90.00	
xia2 3d	28.52 - 1.37	P 1 21 1	0.9	0.038	1.688	98.4	63.92 107.75 69.36 90.00 104.27 90.00	
xia2 3dii	42.04 - 1.37	P 1 21 1	0.8	0.038	1.685	98.5	63.92 107.75 69.36 90.00 104.27 90.00	
xia2 dials	107.77 - 1.30	P 1 21 1	.9	0.039	1.753	98.2	63.94 107.77 69.37 90.00 104.26 90.00	
autoPROC 1.0.5 (see: http://www.globalphasing.com/autoproc/)	107.76 - 1.50	P 1 21 1	3.3	0.038	0.877	98.8	63.93 107.76 69.37 90.00 104.26 90.00	

fast_dp | xia2 3d | xia2 3dii | xia2 dials | autoPROC 1.0.5 (see: http://www.globalphasing.com/autoproc/)

Plots | Archive | Logs & Files | Lookup Cell

Beam Centre	X	Y
Start	211.60	206.96
Refined	211.50	206.92
Δ	0.10	0.04

Space Group	A	B	C	α	β	γ
P 1 21 1	63.92	107.75	69.36	90.00	104.27	90.00

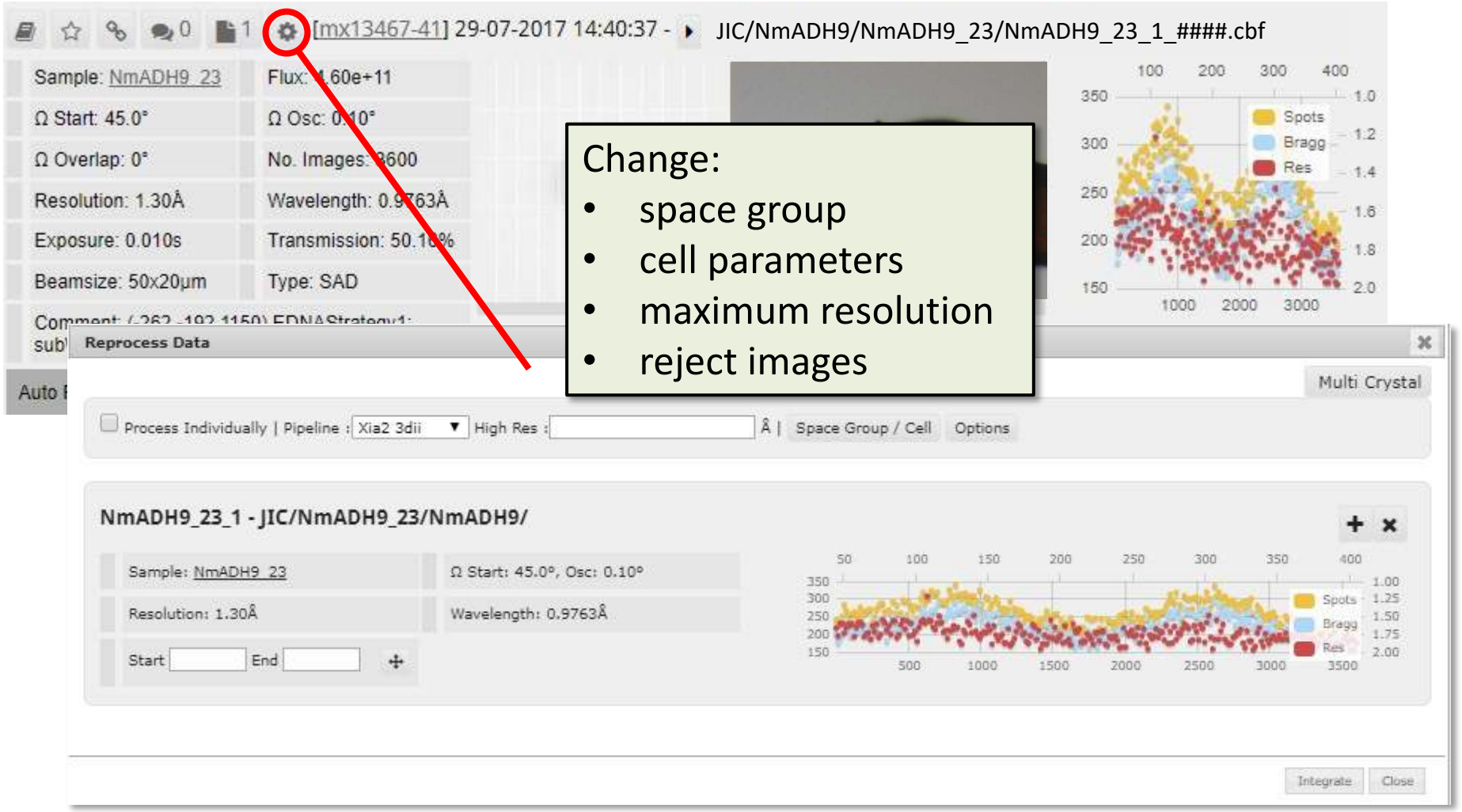
Shell	Observations	Unique	Resolution	Rmeas	I/sig(I)	CC Half	Completeness	Multiplicity	Anom Completeness	Anom Multiplicity	CC Anom
outerShell	64205	9220	1.37 - 1.39	1.685	1.1	0.5	96.7	7.0	96.0	3.5	0.0
innerShell	64845	9660	3.72 - 42.06	0.038	44.2	1.0	99.9	6.7	98.6	3.5	0.1
overall	1285570	187412	1.37 - 42.04	0.099	10.8	1.0	98.5	6.9	98.0	3.5	0.1

Consistent indexing gives more confidence in the results…

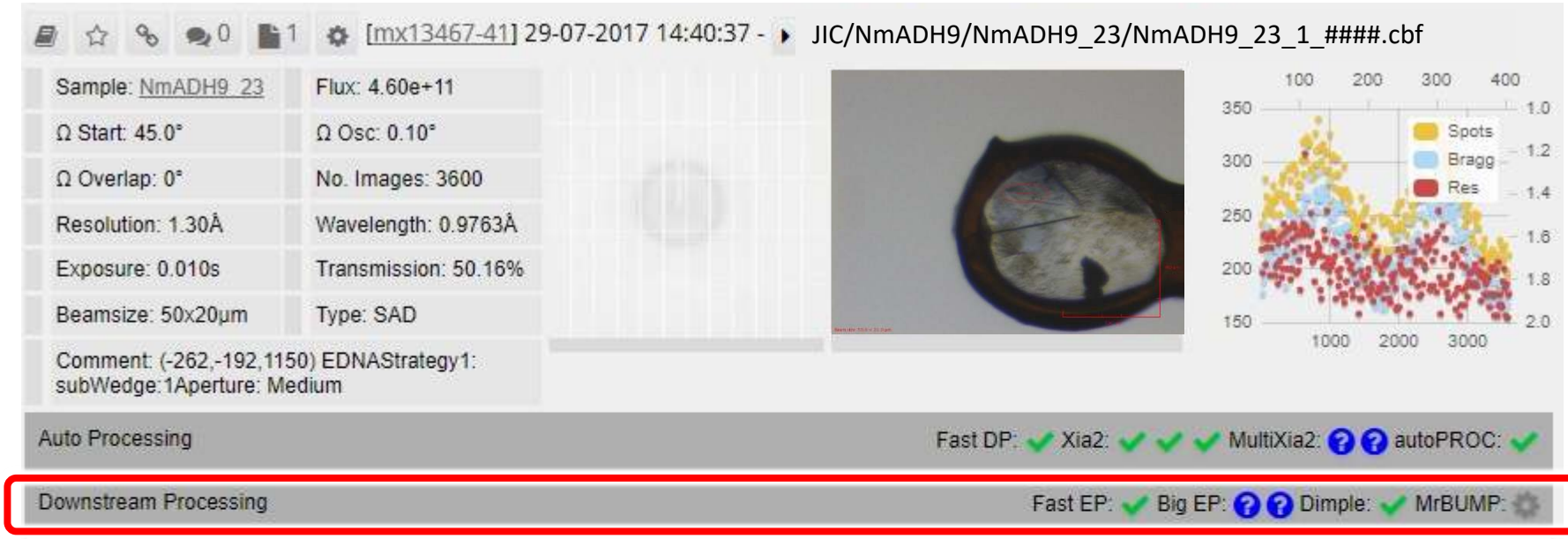
… but treat space group assignment as only a hypothesis at this stage! (see later talks)

Re-running jobs:

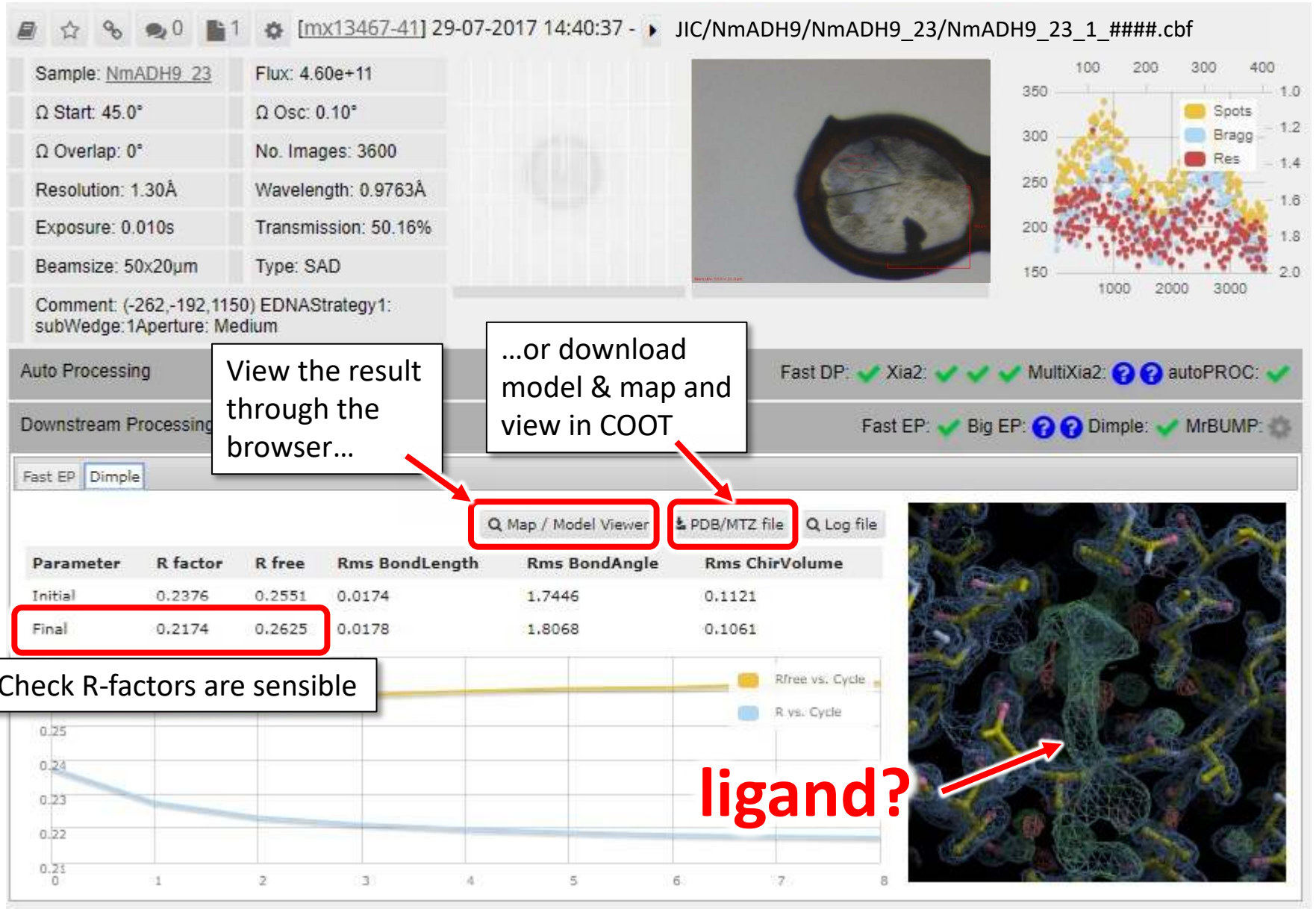
- Most pipelines will run from the command line (Terminal window)
- Also through ISPyB interface...



Checking the results…



Checking the results…



Ideal scenario after session:

- Each data set characterised as:
  - useful/may be useful/not useful
- Some datasets processed to your satisfaction
- You may have interpretable experimental maps
- You may have preliminary structures
- You **will** have less follow-up work to do!

What to do with all the data...

Raw data (images)
- removed from disk after 30 days – still available through TopCAT (tape archive)

"Meta" data – all the other "stuff" – removed from disk after 30 days – not backed up

>90% of useful datasets derived from **Meta data** rather than going back to **Raw data**

...just be thankful it's not cryo-EM!

Getting your data home…

- FTP data home (use an App or a script)

Quite slow...

globus

Faster...

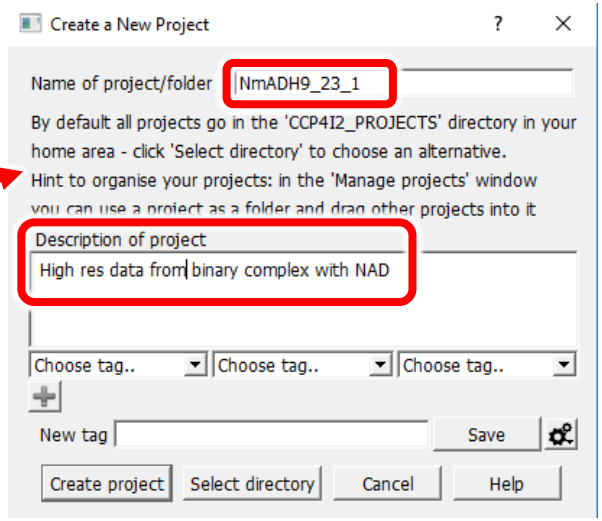
In the meantime:

- use autoprocessing output or...
- (re)process data remotely on DLS computers and transfer output only
- copy and archive raw data later

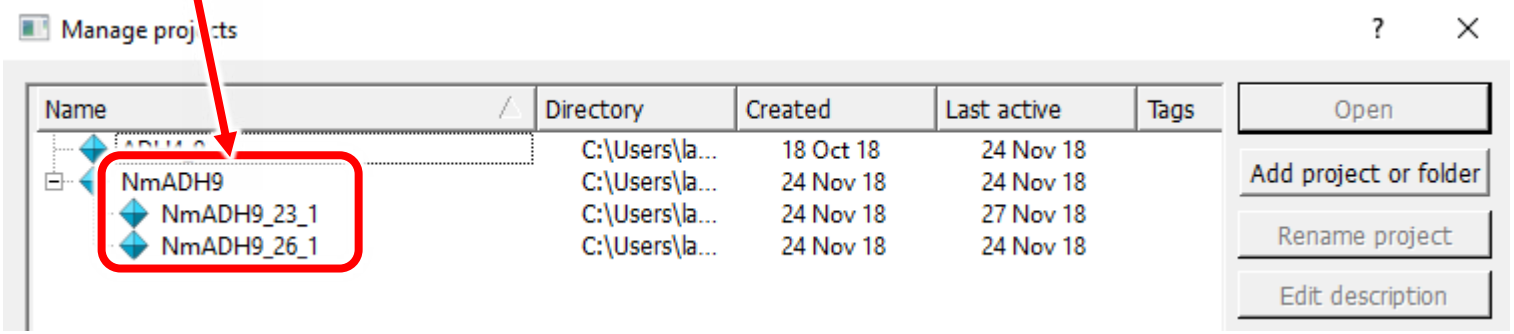
Retain ISPyB name in CCP4i2...

CCP4i2

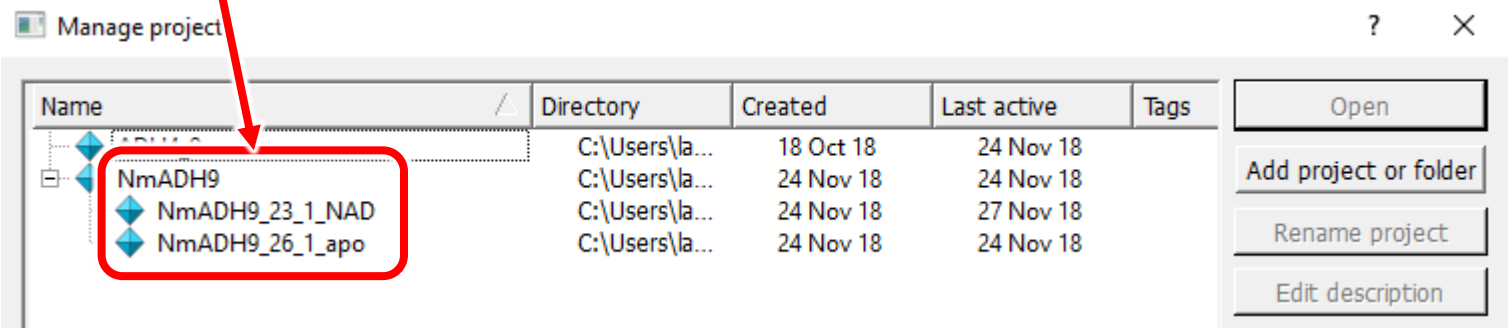
Create a separate "project" for each dataset



If you have more than one dataset for a given protein – create a separate "dummy project" and group the "dataset projects" under this:



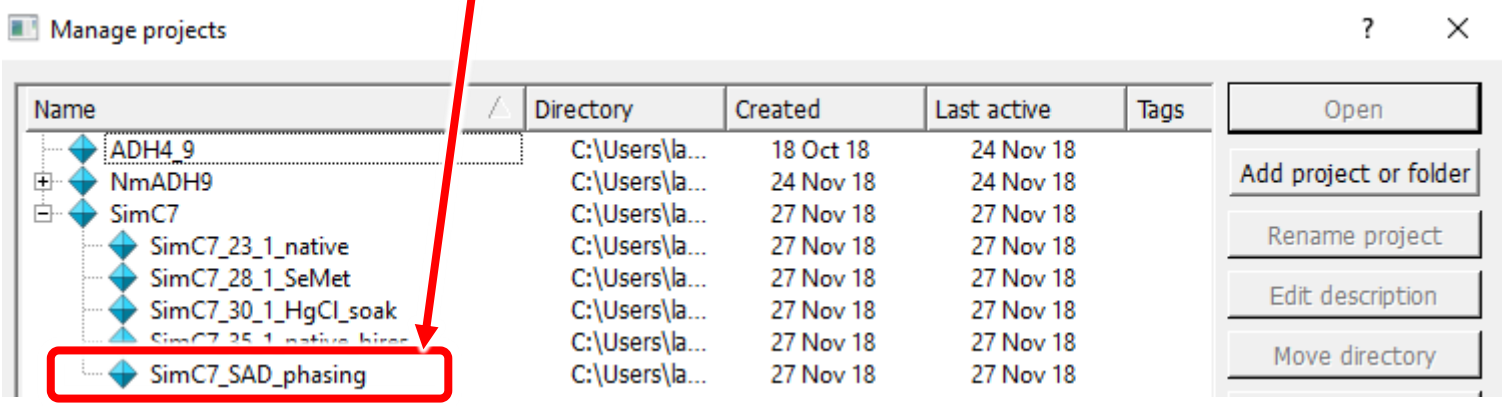
Can rename "projects" later... (when you know more...)*



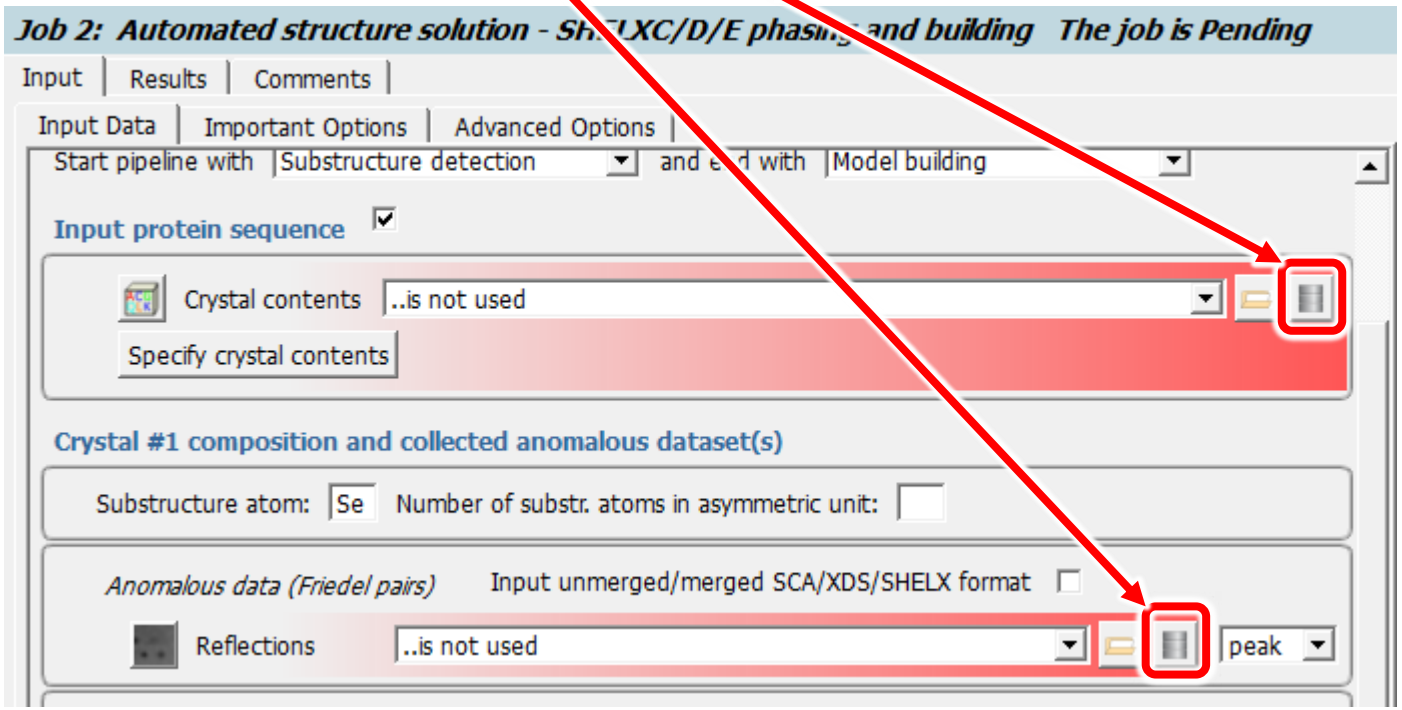
*but directory names remain the same

CCP4i2

Could create “non-dataset projects” for specific activities such as phasing...

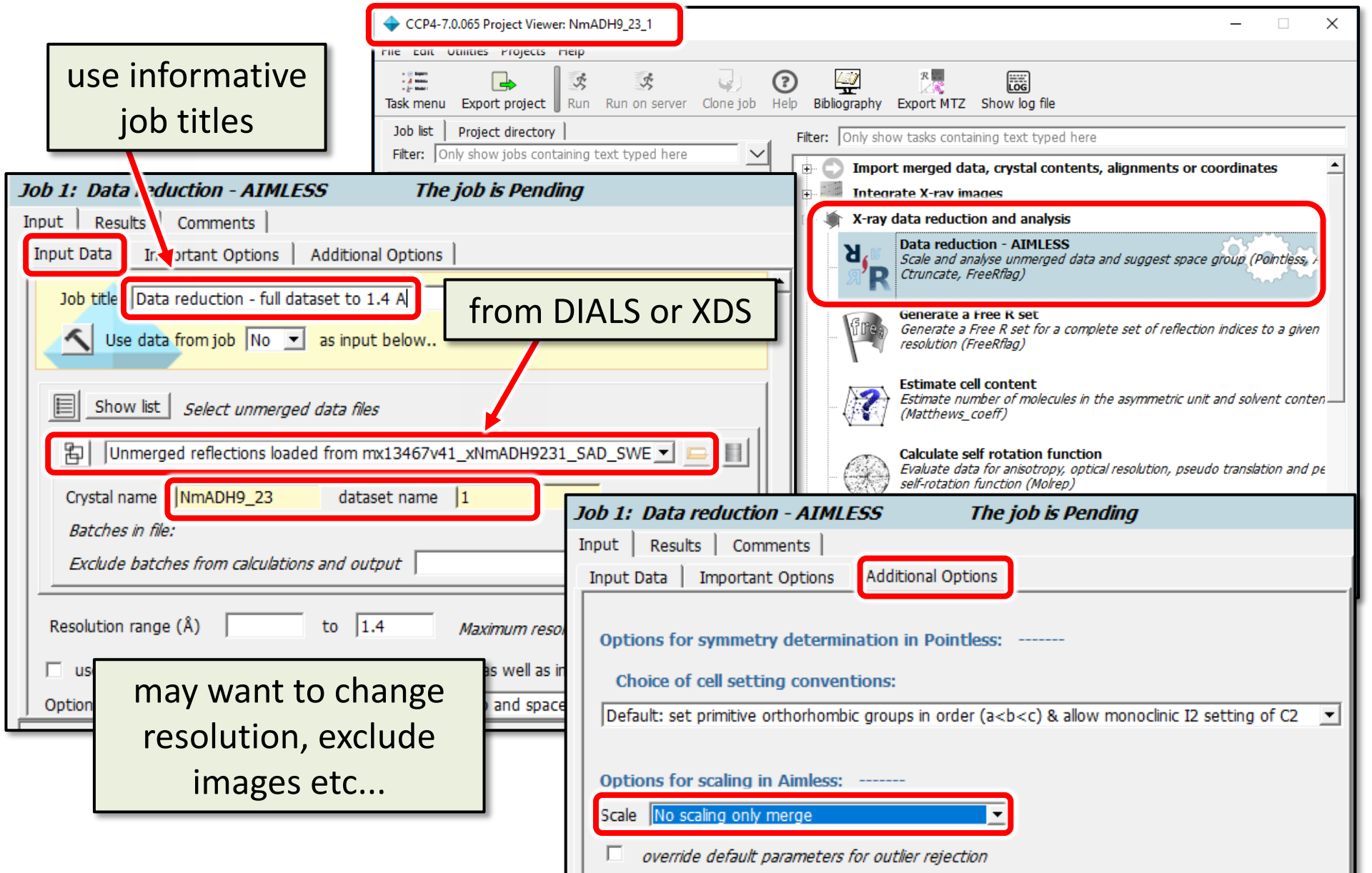


...then “borrow” files from other projects...



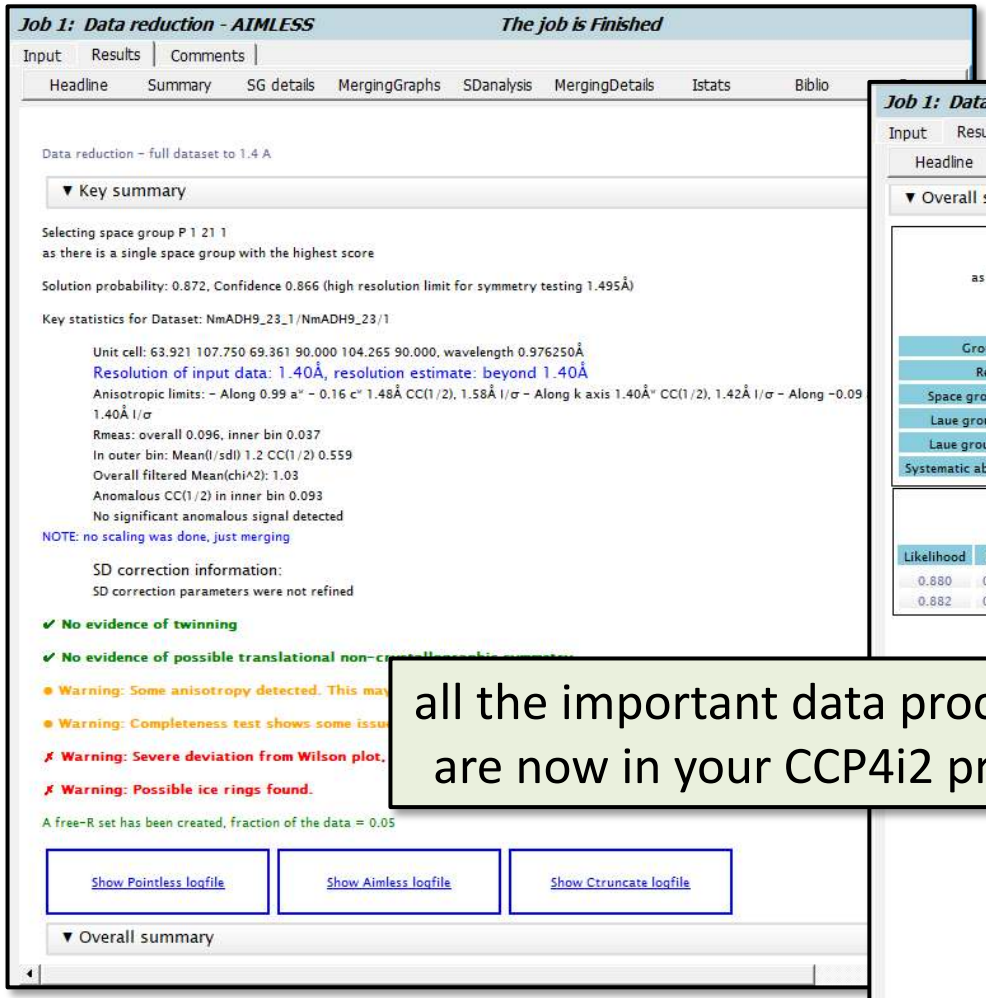
My preferred option for processing...

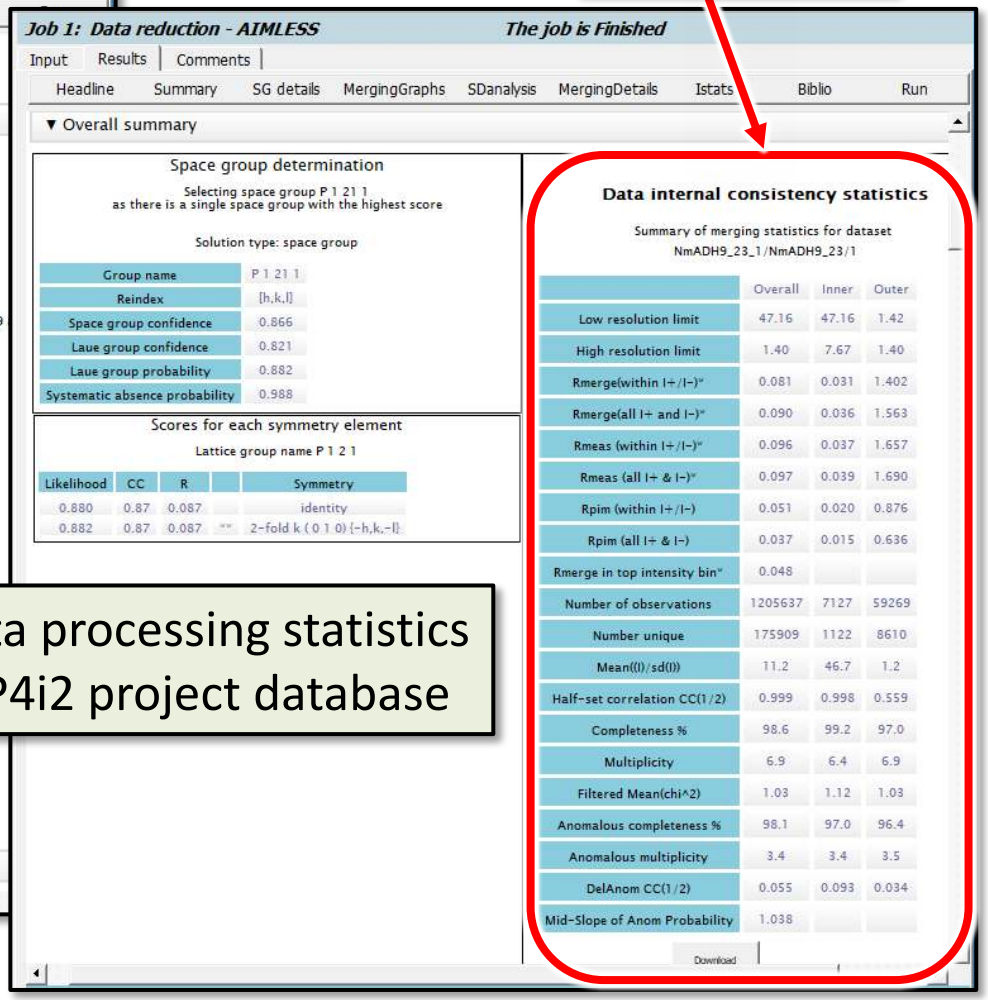
- where the pipelines have done a good job – take part-processed dataset (from the "Meta" data) and re-run the merging step in CCP4i2



Gives lots of output – useful for troubleshooting...

for your paper/thesis





all the important data processing statistics are now in your CCP4i2 project database

...or reprocess from scratch using DIALS or XDS…

Summary - why use ISPyB & pipelines?

- Faster sample changing (select by ISPyB name)
  - essential for remote…
- Informs the decision making process
  - make decisions sooner
  - revise strategy on the fly
    - (e.g. recollect dataset x... no more data required for project y...)
  - make better overall use of beamtime
- Reduces amount of post-beamtime follow-up work
- Simple to keep track of your samples and data

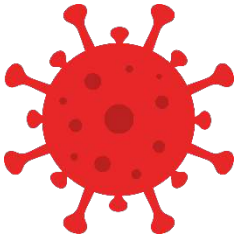
Remote data collection

not going to cover unattended data collection (UDC)…

On-site data collection can be a big time commitment…

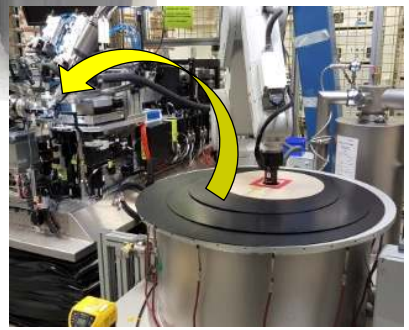
Remote data collection saves you time…

…and the planet



…and is covid-secure

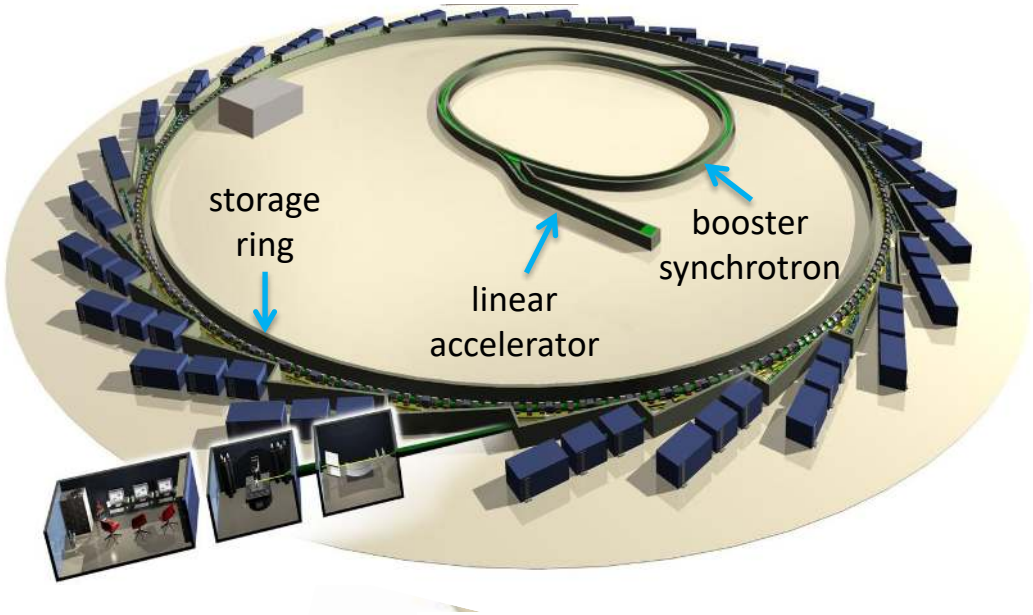
For routine data collection at 100 K:

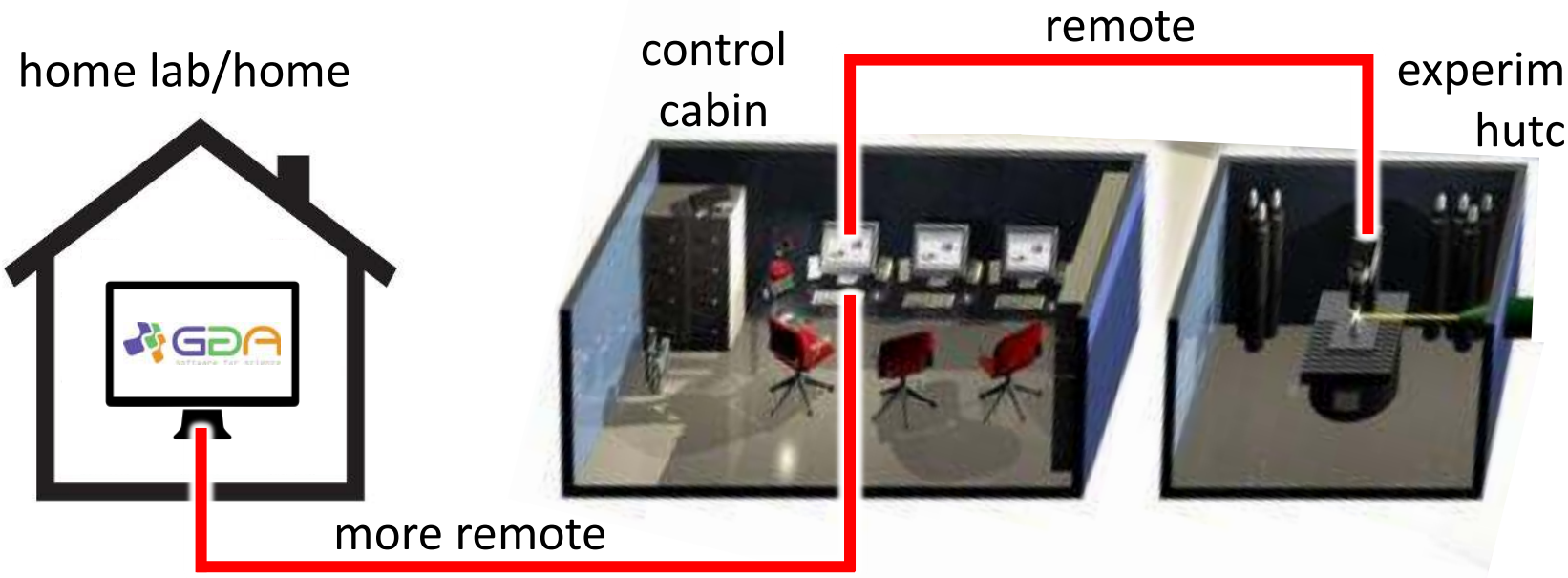


- Samples prepared and cryo-cooled in home lab
- Sample information entered into ISPyB database
- Transported to Diamond in dry shipping dewars
- Samples mounted robotically
- Data collection controlled through GDA interface
- Only manual operation at DLS: loading/unloading pucks
  - Up to 592 samples can be loaded at once
- Everything else computer driven…

Therefore you don’t need to be there!

What is remote data collection?

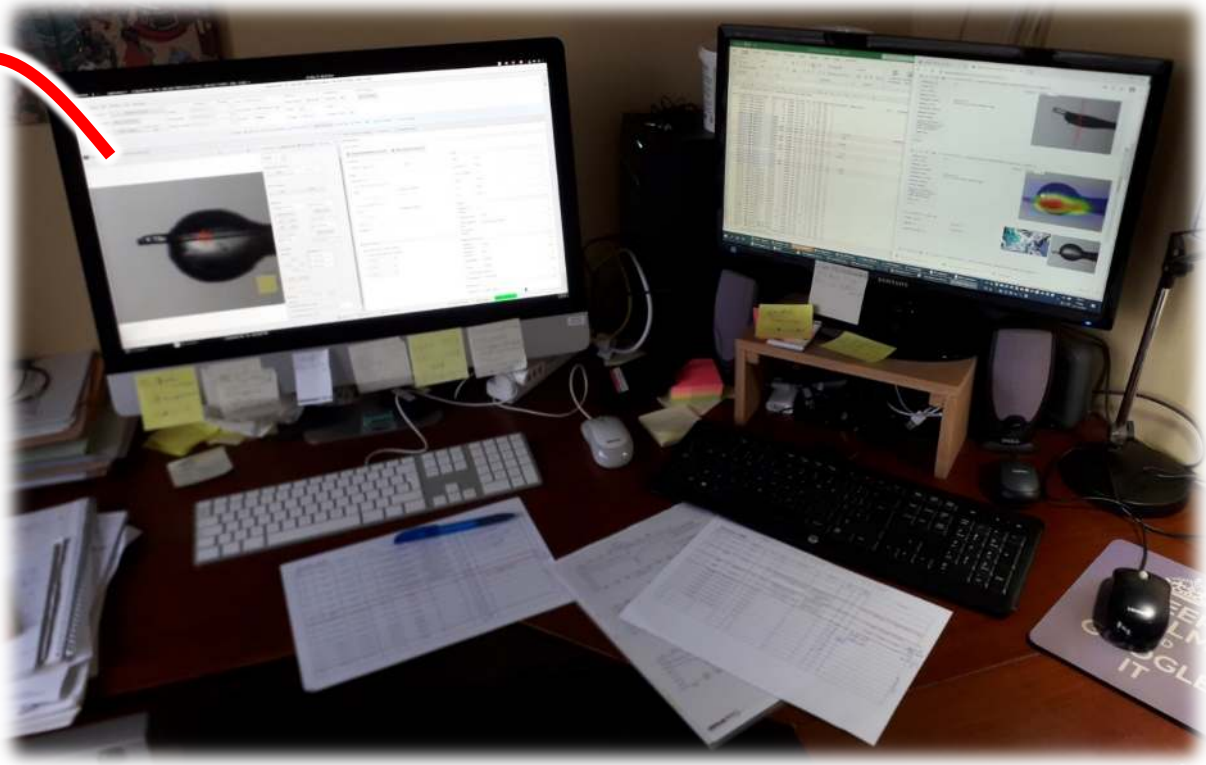




Remote data collection lockdown style!

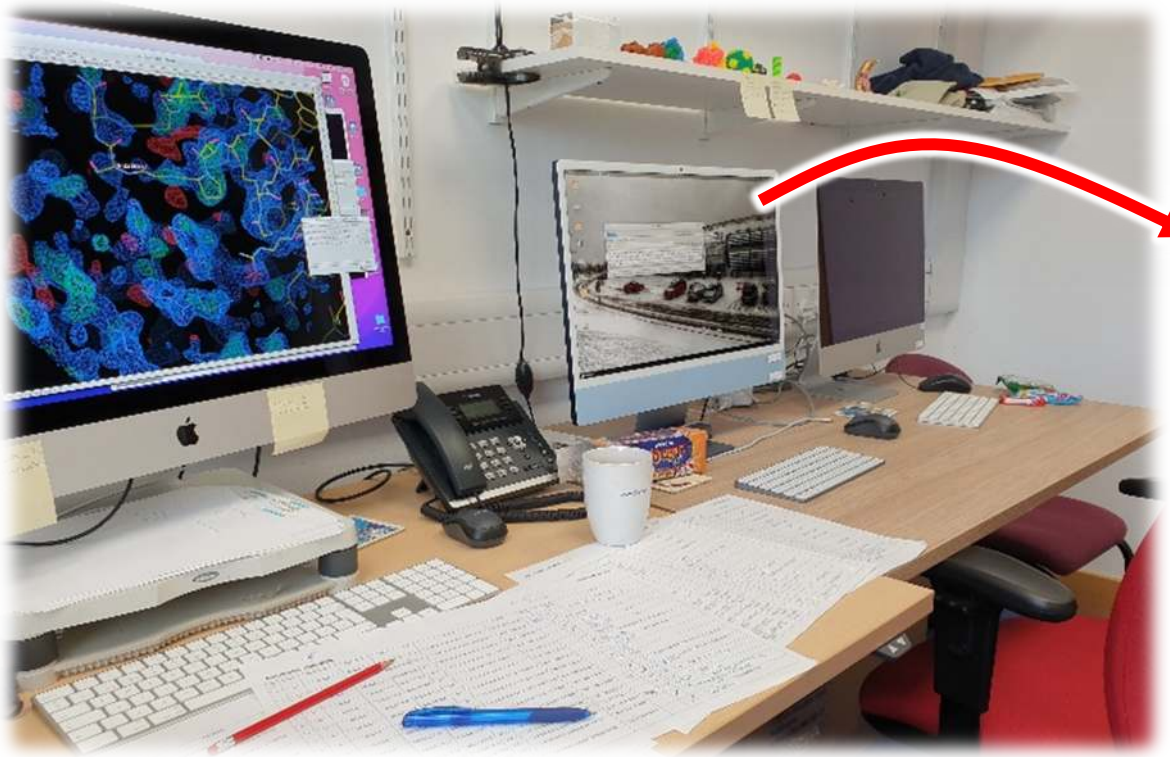
…from here

share screen
with others



Remote data collection – normality resumed

…from here



share screen
with others

Do these 3 things well BEFORE your session

1.

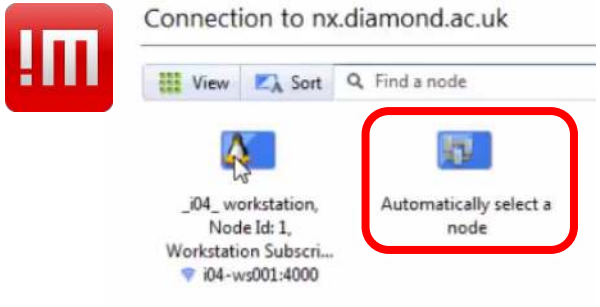
Think about the experiments that you want to run

- discuss with your local contact if necessary

2.

Check that your remote NX connection works

- if you are given the option to “automatically select a node”, choose this (unless you are going to collect data)
- if intending to use a 2nd monitor – check it works



3.

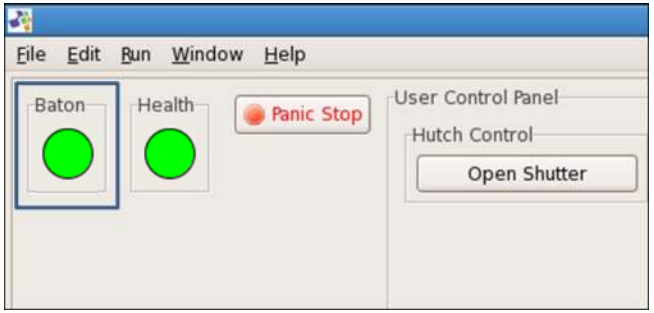
While you are connected, read the beamline “message of the day”

- open a Terminal window and type:
- more /dls_sw/<beamline_name>/etc/motd

Timeline for fully remote data collection

Sometime prior to shift:

- LC loads pucks into beamline dewar and enters puck positions into ISPyB database

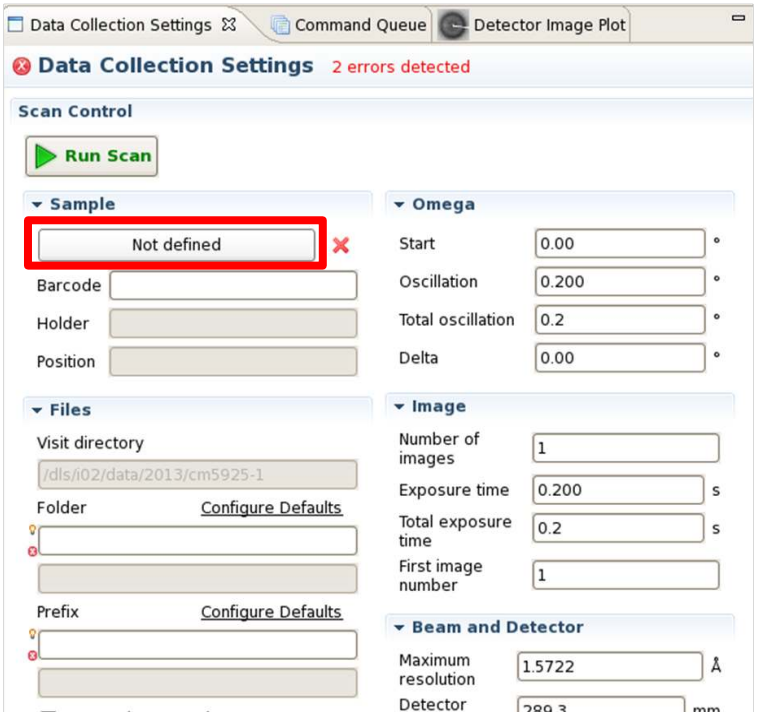


Shortly before start of shift (or your slot in running order):

- connect to beamline computer using NX client
- start GDA

Zero hour:

- take the baton, select sample from menu, collect data!



When things go wrong…

Can **you** fix things?

- probably not!

On-site user:

- During normal working hours – call local contact
- Out of hours – call EHC

Remote user:

- During normal working hours – call local contact
- Out of hours – call EHC

Therefore you don’t need to be there!

Check the webcams!

If you suspect a problem – call the EHC!

If you see no diffraction – 3 main causes:

…could be all 3 😖

(1) there is a problem (any number of things…)

(2) you are doing something wrong/stupid

(3) your sample is rubbish!

Pop a couple of test crystals into one of your pucks (something you know will diffract e.g. lysozyme)

Advantages of remote data collection:

- Beamtime is fully used
- Users collect data on their own crystals
- Users stay at home labs (or at home)
  - time commitment is low
  - your boss/collaborator can observe data collection
  - useful for training non-experts
- Time can be used flexibly
- Less stressful
- Difficult to “break” the beamline

Are there any disadvantages of remote data collection?

- miss the “wow factor” of being at a synchrotron
- lose face-to-face interactions with Diamond staff
  - do BAG training
  - go to User meeting
  - get in touch online

Take home messages

- make full use of ISPyB (use ISPyB name!)
- exploit the MX software tools/pipelines (collect 360°!)
- use remote data collection for routine experiments
- think before and during data collection
- this is may be your last experiment – don't mess it up!

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- Access to MX beamlines at Diamond
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  - Beamline staff
  - EHC/Control Room staff
  - User Office
- Software developers
- BBSRC

John Innes Centre

UKRI Biotechnology and Biological Sciences Research Council