



HKL-3000

Toward the Future of Structural Biology

Wladek Minor

Structural Biology
for the ~~high-throughput~~ high-output era

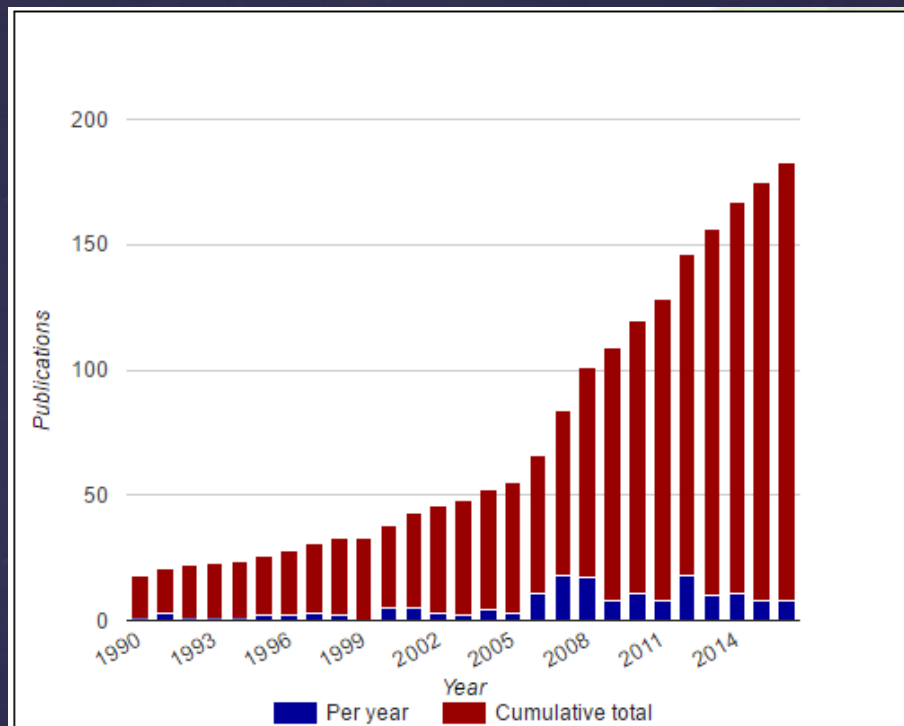
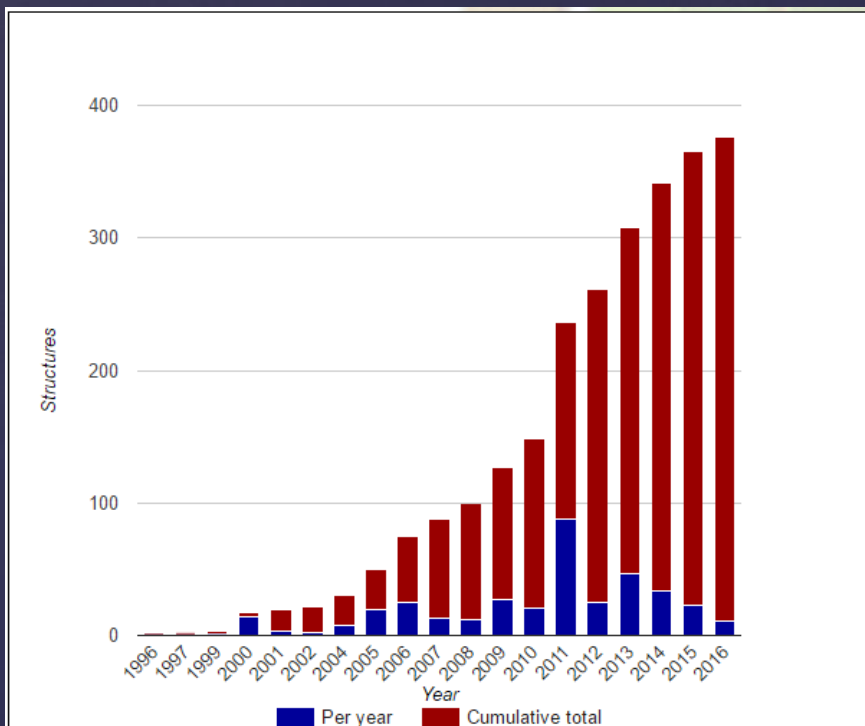
High throughput SB

- Automatic cloning
- HT automatic expression
- HT automatic purification
- HT automatic crystallization
- HT automatic data collection
- HT automatic structure solution/refinement

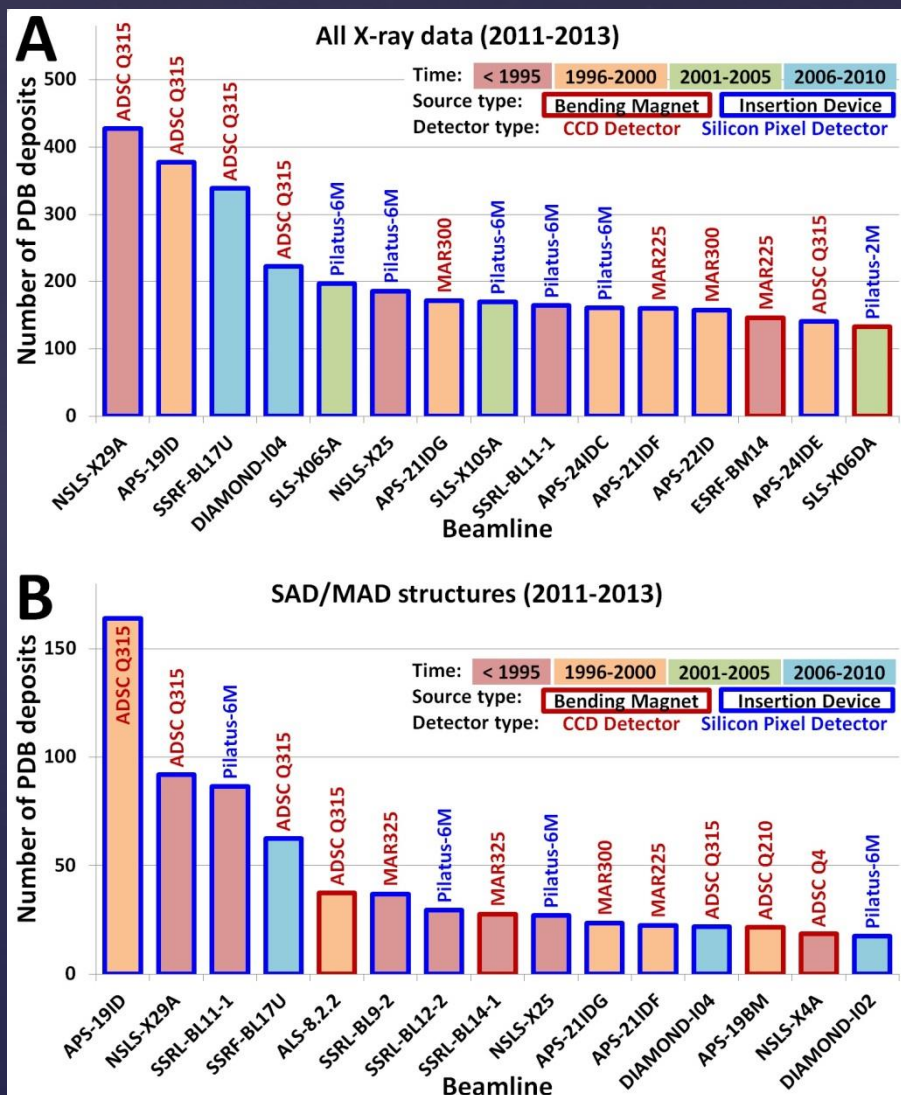
High throughput SB

- Automatic cloning
- HT automatic expression
- HT automatic purification
- HT automatic crystallization
- HT automatic data collection
- HT automatic structure solution/refinement
- **Automatic paper writing**

User perspective



Where we should collect data ?



Diffraction experiment - the last experiment before deposition to PDB

Dataset – 2minutes, sample change 2minutes -> 10minutes

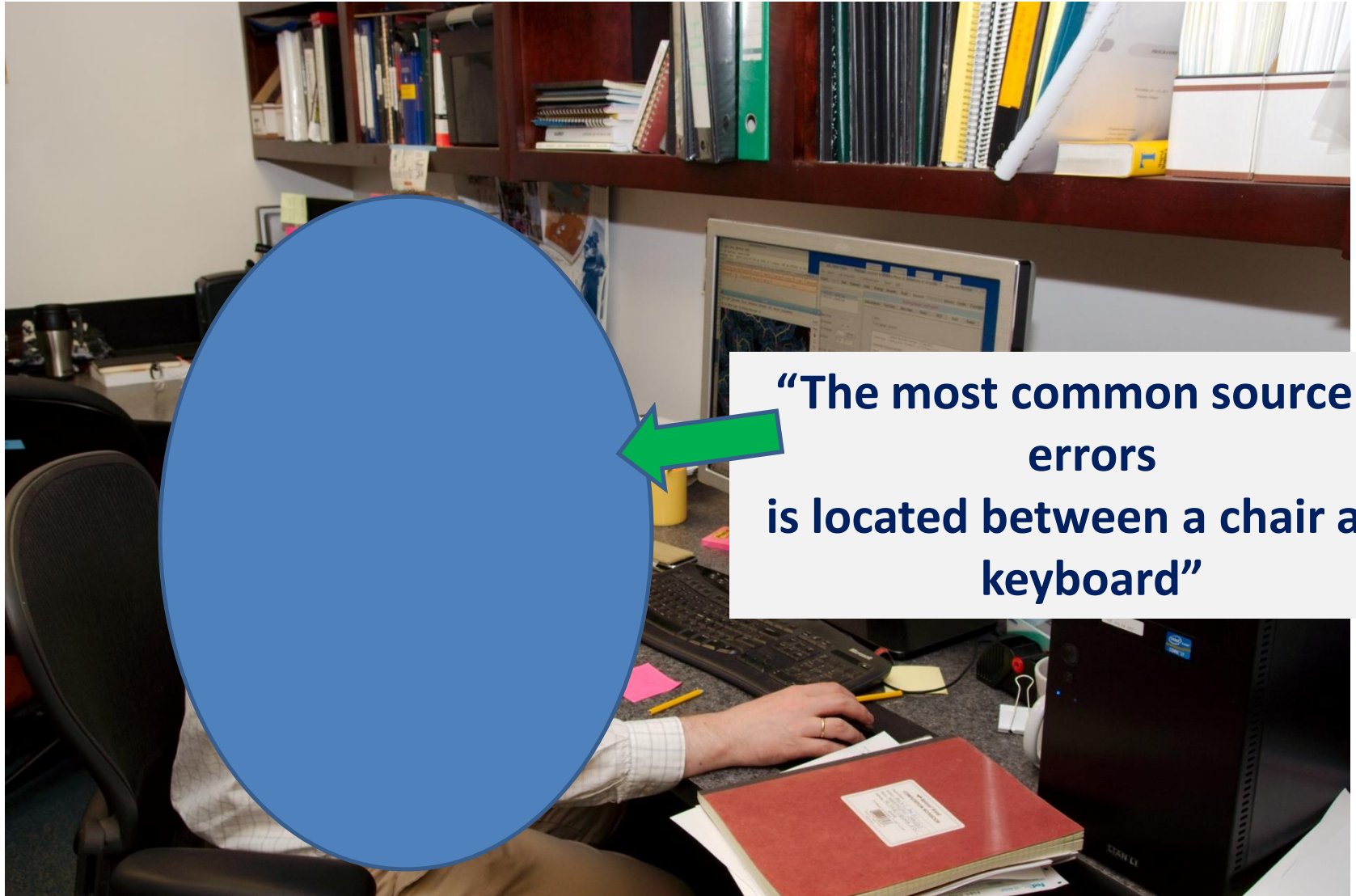
6 datasets/hour -> 144 datasets/day

180 days -> 25920 datasets/day -> 2.5 PDB

125 synchrotron stations -> 324 PDB

Efficiency -> 0.3%

User from a Programmer Point of View

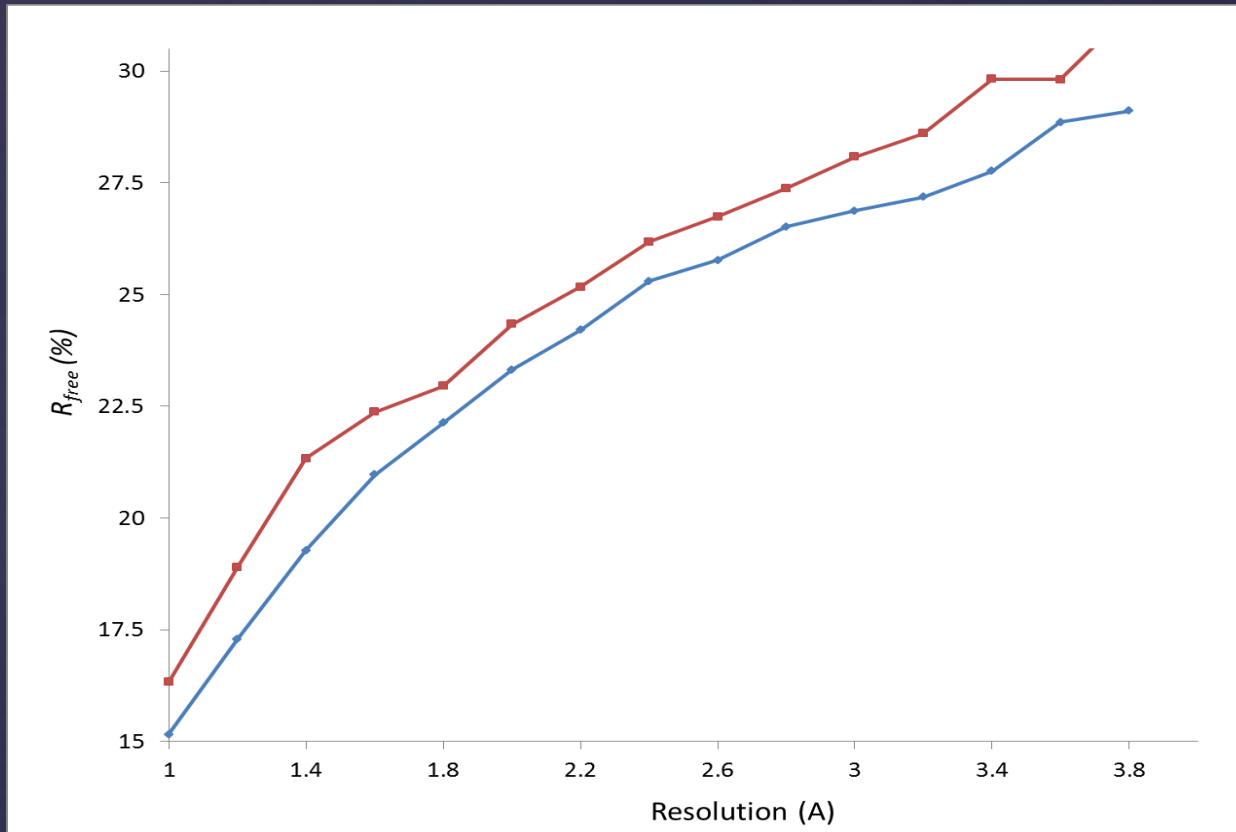


“The most common source of errors is located between a chair and keyboard”

What experimenters know about data collection ?

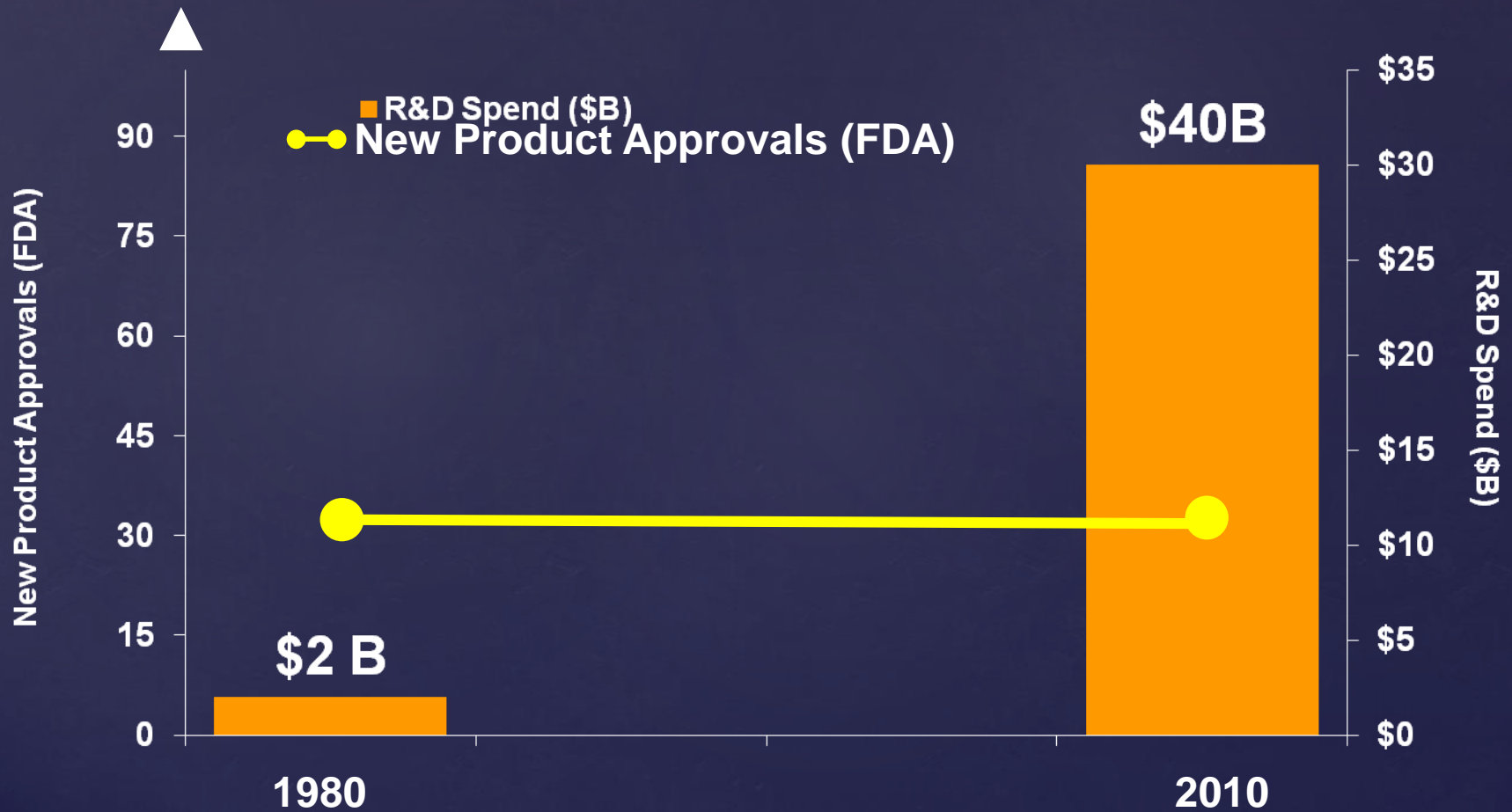
```
REMARK 3 ESTIMATED OVERALL COORDINATE ERROR.
REMARK 3 ESU BASED ON R VALUE (A) : NULL
REMARK 3 ESU BASED ON FREE R VALUE (A) : NULL
REMARK 3 ESU BASED ON MAXIMUM LIKELIHOOD (A) : NULL
REMARK 3 ESU FOR B VALUES BASED ON MAXIMUM LIKELIHOOD (A**2) : NULL
REMARK 3
REMARK 3 RMS DEVIATIONS FROM IDEAL VALUES.
REMARK 3 DISTANCE RESTRAINTS. RMS SIGMA
REMARK 3 BOND LENGTH (A) : NULL ; NULL
REMARK 3 ANGLE DISTANCE (A) : NULL ; NULL
REMARK 3 INTRAPLANAR 1-4 DISTANCE (A) : NULL ; NULL
REMARK 3 H-BOND OR METAL COORDINATION (A) : NULL ; NULL
REMARK 3
REMARK 3 PLANE RESTRAINT (A) : NULL ; NULL
REMARK 3 CHIRAL-CENTER RESTRAINT (A**3) : NULL ; NULL
REMARK 3
REMARK 3 NON-BONDED CONTACT RESTRAINTS.
REMARK 3 SINGLE TORSION (A) : NULL ; NULL
REMARK 3 MULTIPLE TORSION (A) : NULL ; NULL
REMARK 3 H-BOND (X...Y) (A) : NULL ; NULL
REMARK 3 H-BOND (X-H...Y) (A) : NULL ; NULL
REMARK 3
REMARK 3 CONFORMATIONAL TORSION ANGLE RESTRAINTS.
REMARK 3 SPECIFIED (DEGREES) : NULL ; NULL
REMARK 3 PLANAR (DEGREES) : NULL ; NULL
REMARK 3 STAGGERED (DEGREES) : NULL ; NULL
REMARK 3 TRANSVERSE (DEGREES) : NULL ; NULL
REMARK 3
REMARK 3 ISOTROPIC THERMAL FACTOR RESTRAINTS. RMS SIGMA
REMARK 3 MAIN-CHAIN BOND (A**2) : NULL ; NULL
REMARK 3 MAIN-CHAIN ANGLE (A**2) : NULL ; NULL
REMARK 3 SIDE-CHAIN BOND (A**2) : NULL ; NULL
```


Unexpected correlation?

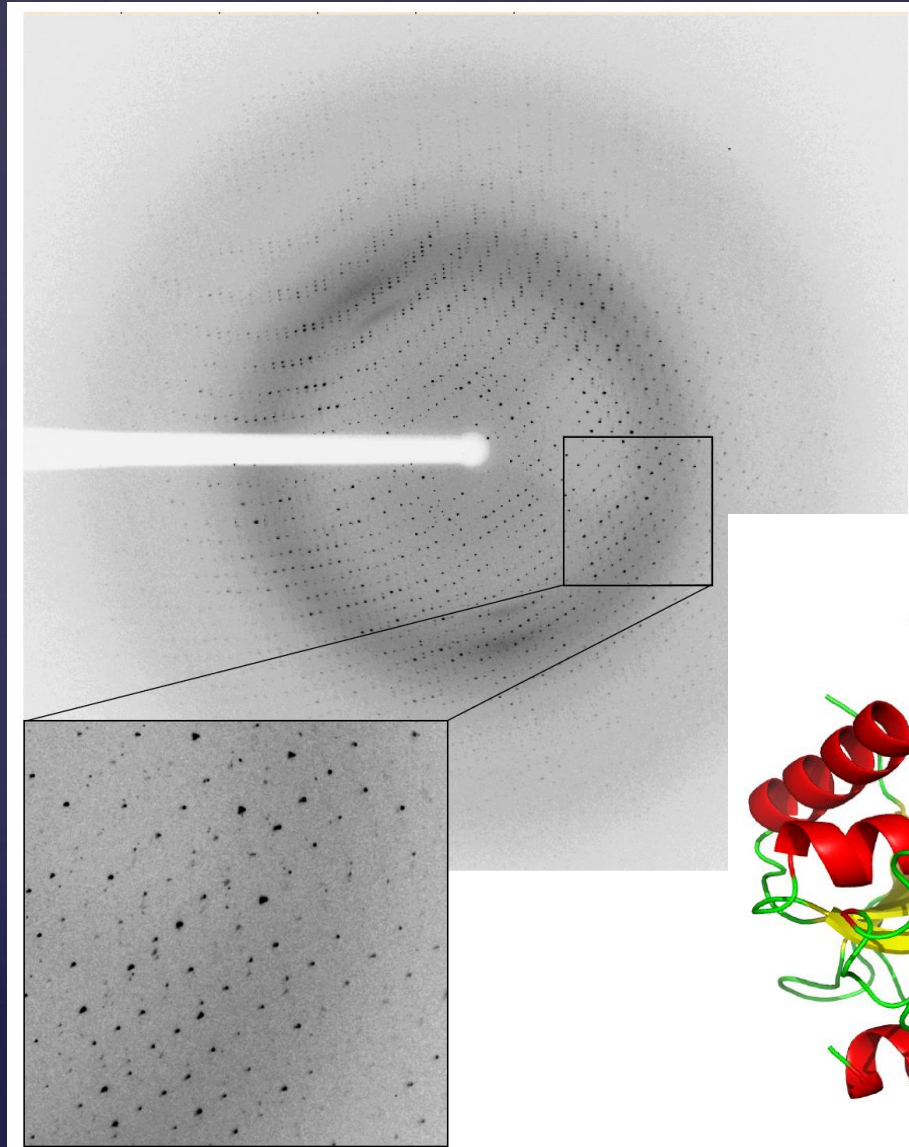


Average R_{free} by resolution bin (with a width of 0.2 Å for X-ray crystallography PDB structures deposited after January 1, 2001, divided into two groups by the number of missing data items (“NULLs”) in the PDB file. The means for “high-completion” deposits (20 NULLs or less) are shown in blue, and the means for “low-completion” deposits (50 or more NULLs) are shown in red.

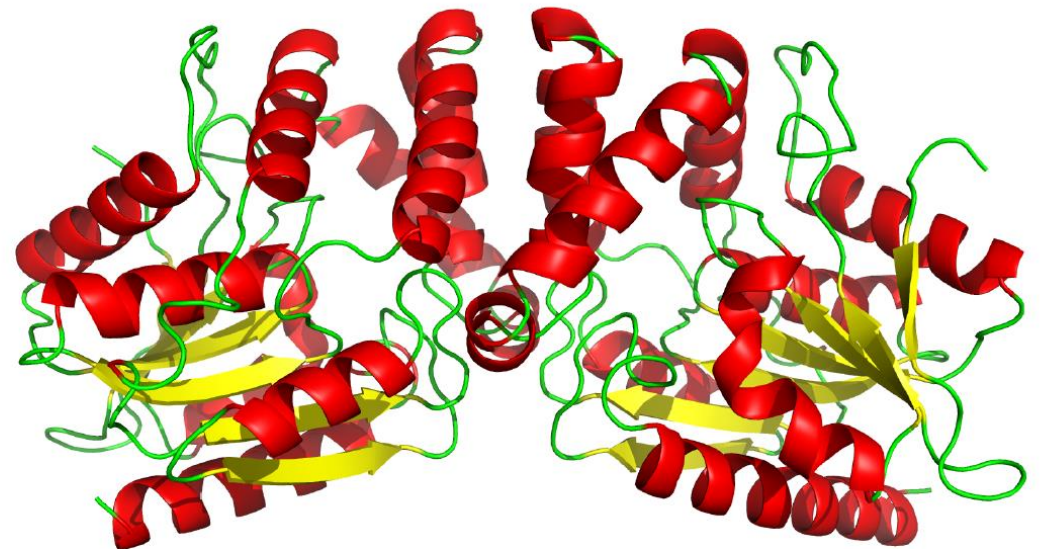
Industry R&D Spend Compared to New Product Approvals



... number of crystals



RutD from *E. coli*



Data collection

- **What is beneficial for particular type of experiment (MR, SAD, MAD, Ligand screening) ?**
- **What is possible ?**

Examples:

- **Wide sectors *vs.* narrow sectors**
- **Inverse beam experiment**
- **Crystal orientation**

Theory *vs.* Experiment

In theory,

there is no difference between
theory and practice.

Theory *vs.* Experiment

In theory,

there is no difference between
theory and practice.


But, in practice, there is.

http://www.proteindiffraction.org

The screenshot shows a web browser window with the URL `proteindiffraction.org`. The browser's address bar and tabs are visible at the top. Below the browser window, the website's navigation menu includes links for Home, About, Browse, Statistics, and Submit data. The main content area features a featured article on the left with the NIH logo and a diffraction pattern image. To the right of the image is the article title and a paragraph of text. Below the article is a search bar and a list of search examples. At the bottom right, there are three icons representing Browse & search, Statistics, and Submit data.

proteindiffraction.org

Home About Browse Statistics Submit data

 National Institutes of Health
Office of the Director
Data Science at NIH

Integrated Resource for Reproducibility in Macromolecular Crystallography

This project is being funded by the [Targeted Software Development](#) award 1 U01 HG008424-01 as part of the [BD2K \(Big Data to Knowledge\)](#) program of the National Institute of Health. The project is developing tools for "wrangling" data from protein diffraction experiments. We are also creating a growing repository of diffraction experiments used to determine protein structures in the [PDB](#), contributed by the [CSGID](#), [SSGICD](#), [JCSG](#), [MCSG](#), [SGC](#) and other large-scale projects, as well as individual research laboratories.


Currently indexed diffraction experiments: **2920**


[Read more...](#)


Search diffraction experiments

Search examples

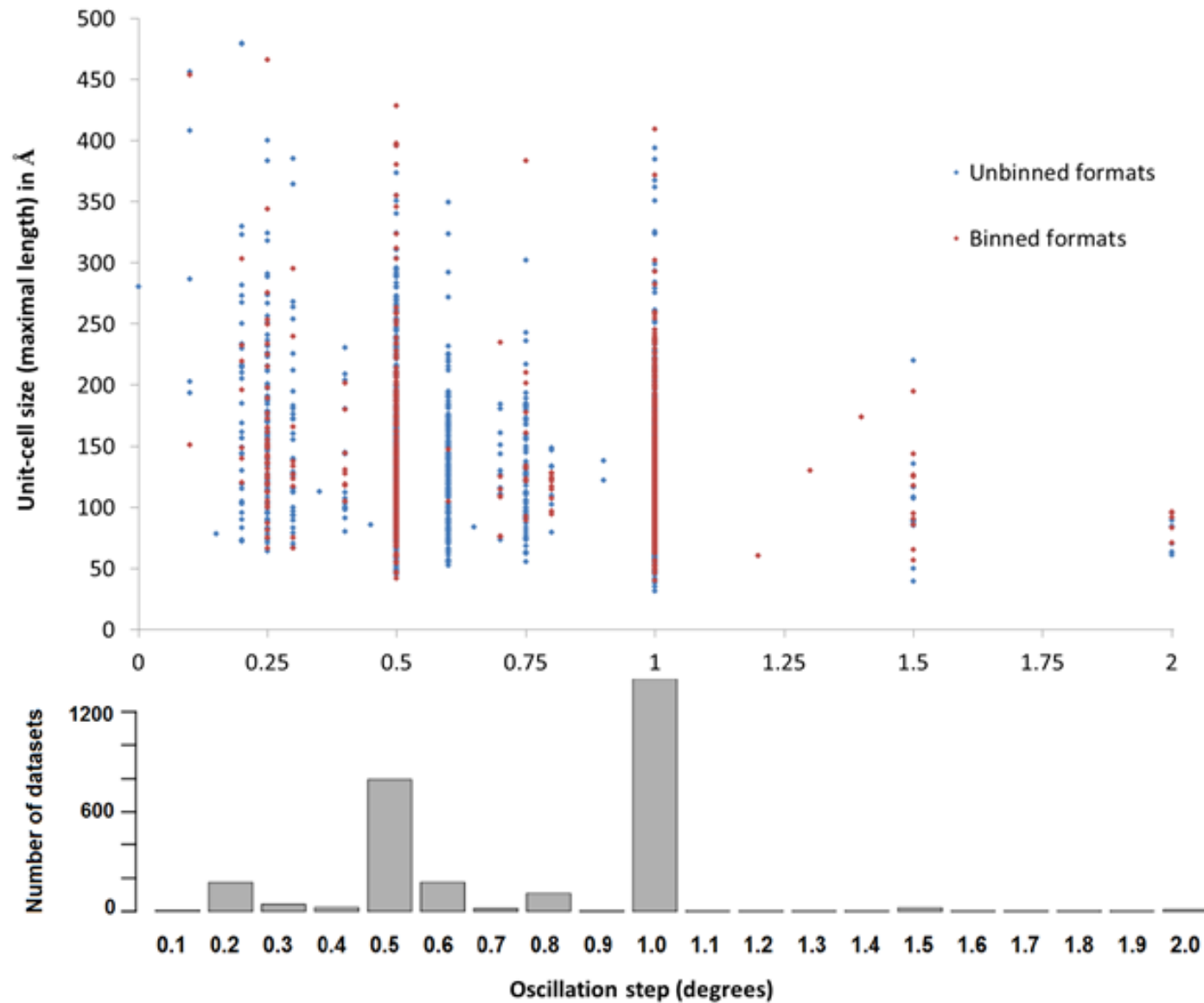
- Find a specific PDB ID: [4K6A](#)
- Free format search: ['potential drug target'](#)
- Combining searches: [drug AND cholera](#)
- Specific beamline: [beamline=21-ID-G](#)
- Fuzzy search: [authors ~ Shabalin](#)
- Resolution limit (Angstroms): [resolution<1.25](#)
- Search by tag: [workshop](#)

 Browse & search

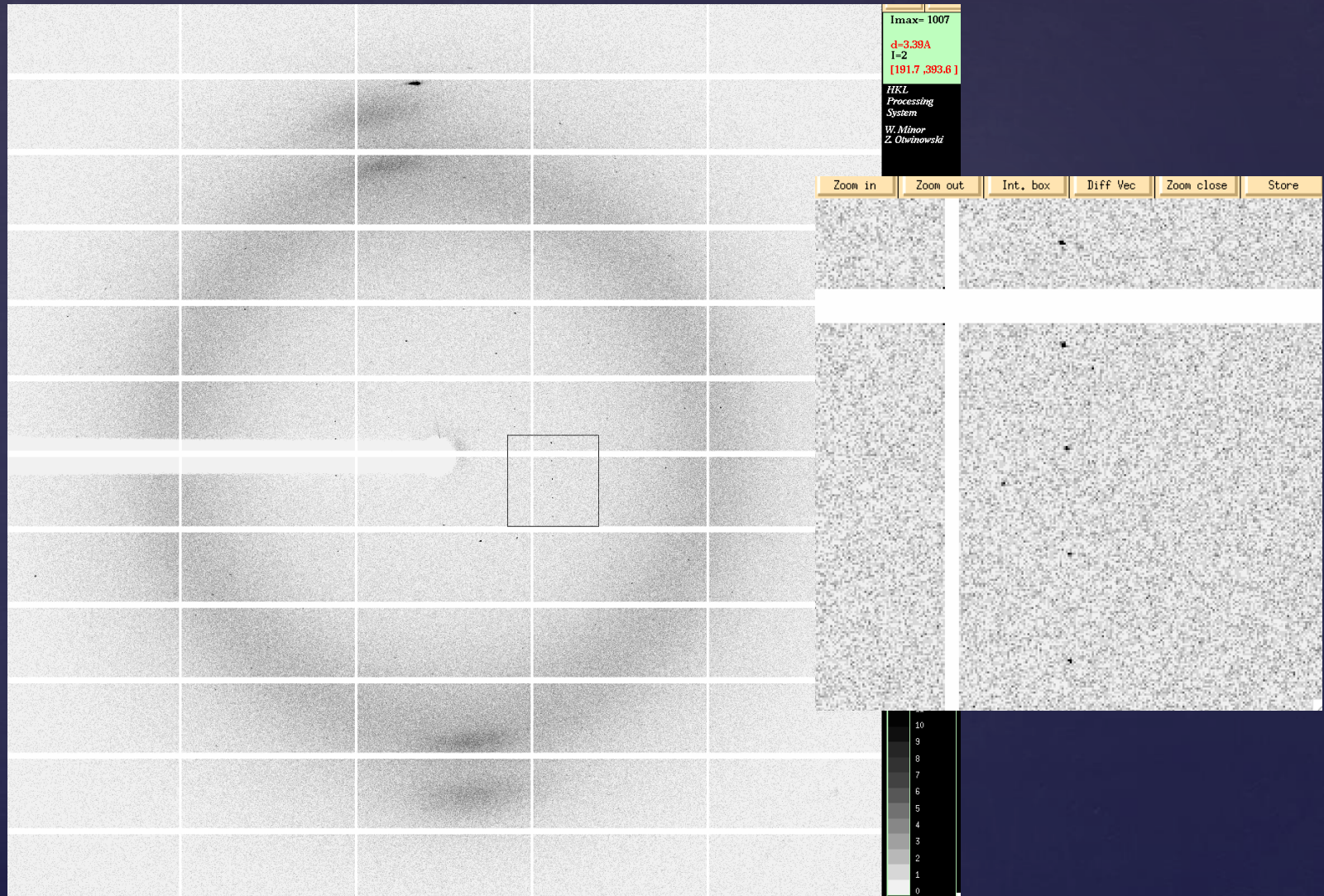
 Statistics

 Submit data

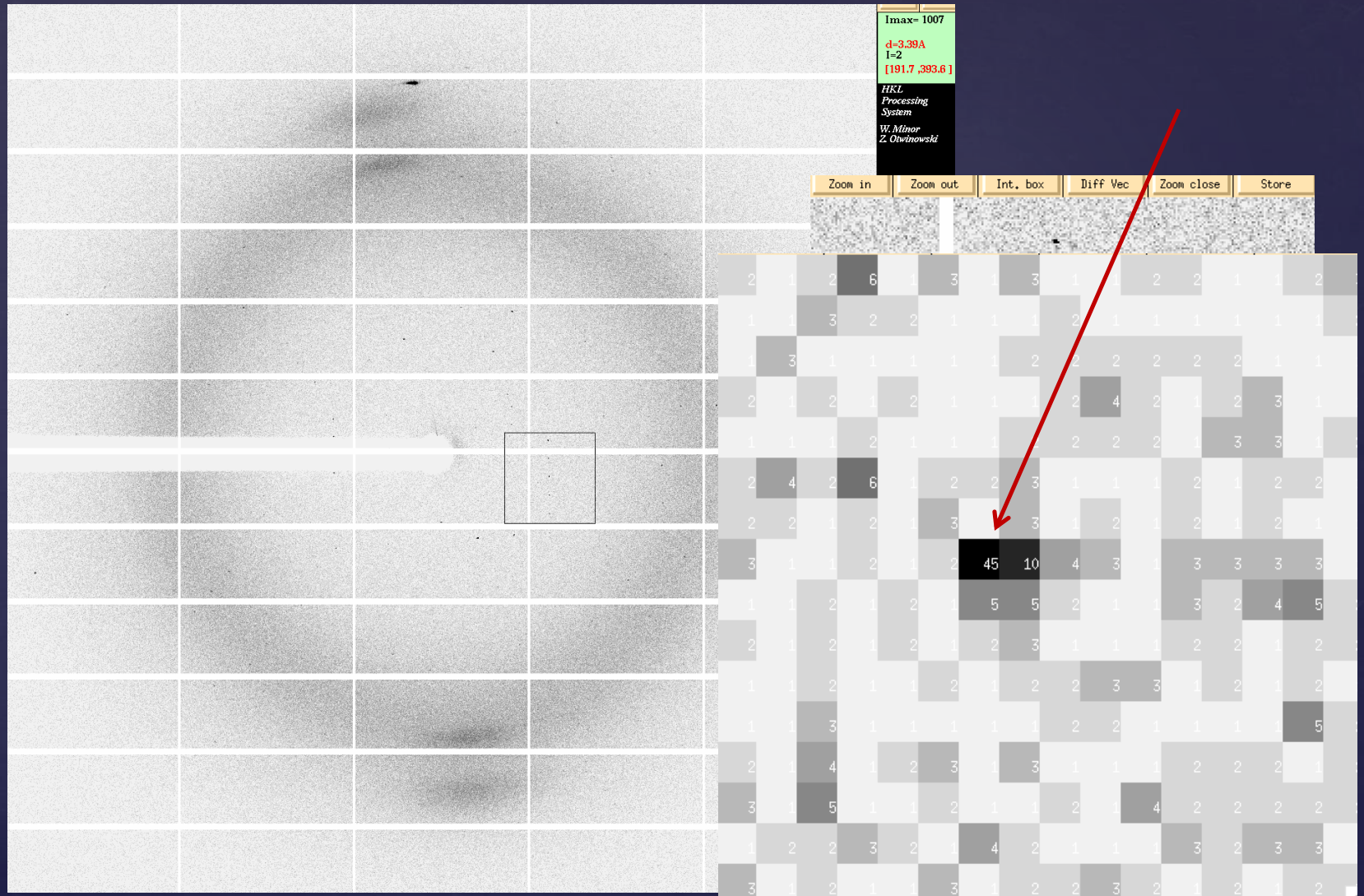
Data collection strategy



Optimal data collection ?

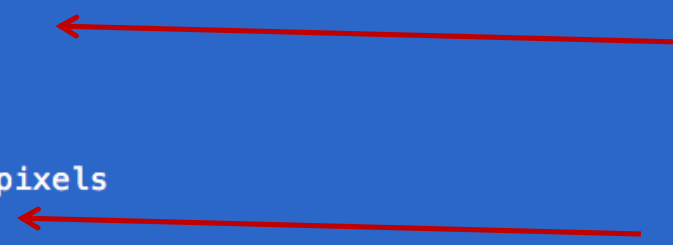


Optimal data collection ?



Header – is CBF header a MAH ?

```
# 2015/May/06 10:30:40
# Pixel_size 172e-6 m x 172e-6 m
# Silicon sensor, thickness 0.001 m
# Oscillation_axis omega
# Excluded_pixels: badpix_mask.tif
# Chi 0.0000 deg.
# Angle_increment 0.1000 deg.
# Polarization 0.99
# file_comments
# N_oscillations 2500
# Beam_xy (1223.03, 1256.56) pixels
# Exposure_time 0.020000 s
# Phi 0.0020 deg.
# Energy_range (0, 0) eV
# Start_angle 160.6000 deg.
# Detector_distance 0.617619 m
# Detector_Voffset 0.0000 m
# Alpha 0.0000 deg.
# Flat_field: (nil)
# Threshold_setting 7619 eV
# Exposure_period 0.020950 s
# N_excluded_pixels: = 321
# Kappa 0.0020 deg.
# Tau = 0 s
```



Do you like this image?



Do you like this image ?



How expensive is bright lens ?



See more choices

Canon EF 85mm f1.2L II USM Lens
for Canon DSLR Cameras - Fixed
by Canon

\$1,999.00 ✓Prime

Get it by **Monday, Aug 24**

More Buying Choices

\$1,999.00 new (22 offers)

\$1,499.99 used (24 offers)

Trade-in eligible for an Amazon gift card

★★★★★ ▾ 159



See Style Options

Canon EF 85mm f/1.8 USM Medium
Telephoto Lens for Canon SLR
Cameras - Fixed
by Canon

\$369.00 ✓Prime

Get it by **Monday, Aug 24**

More Buying Choices

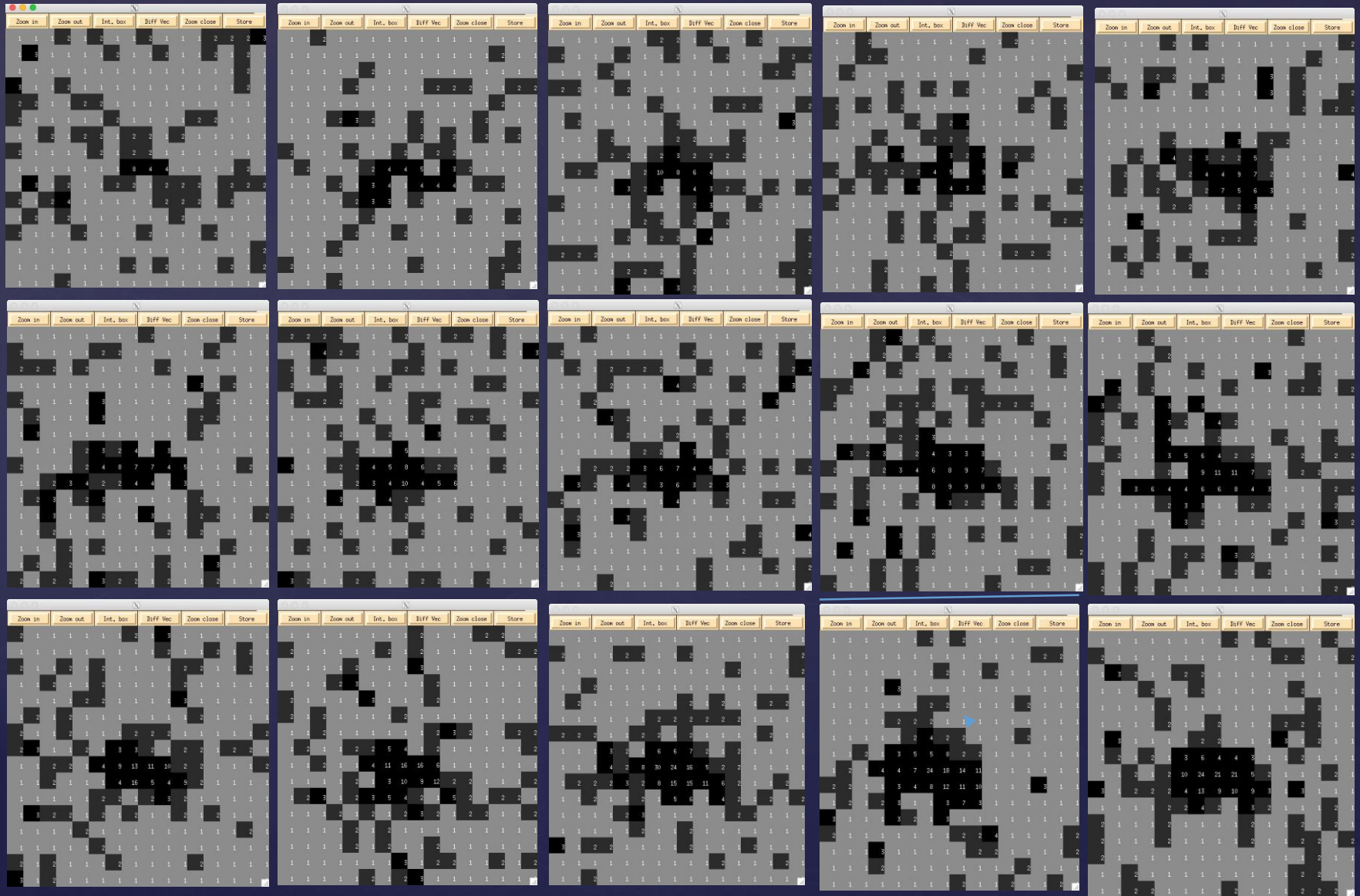
\$369.00 new (27 offers)

\$298.00 used (26 offers)

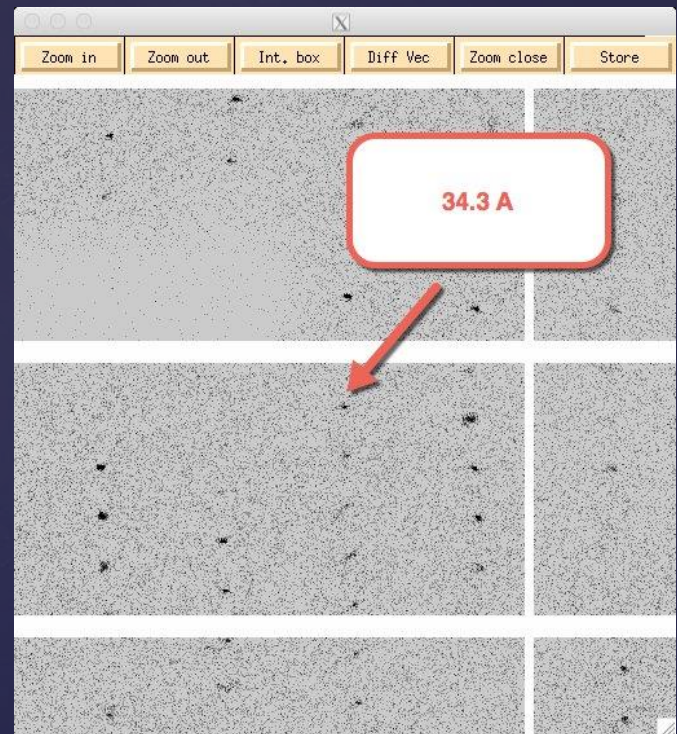
Trade-in eligible for an Amazon gift card

★★★★★ ▾ 770

Thin slicing, 0.01s/frame

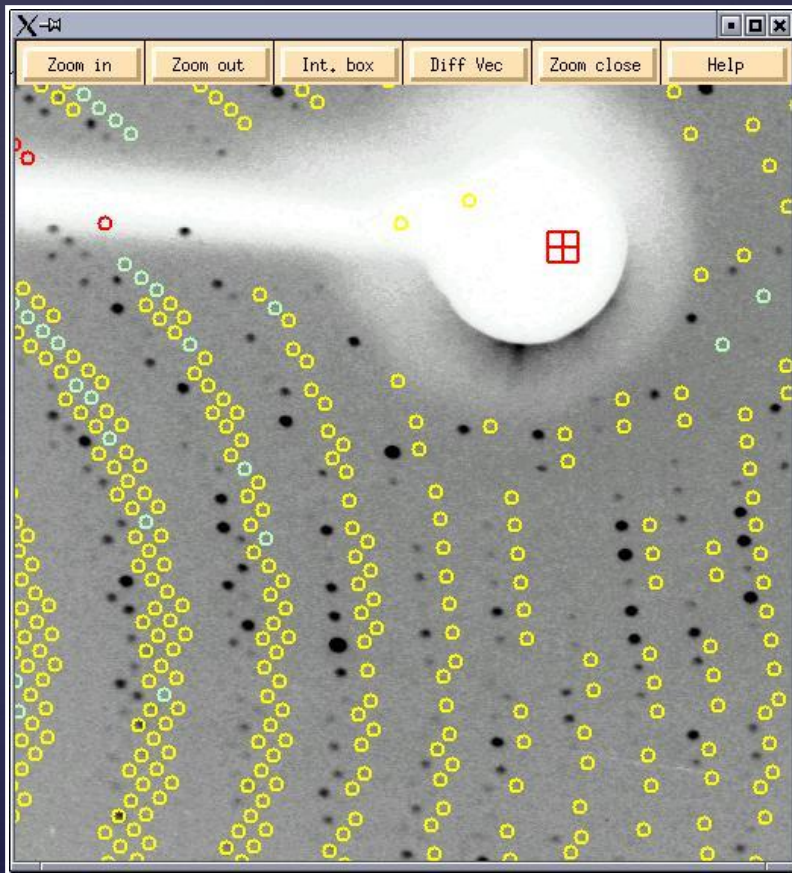


VS, S, W, VW

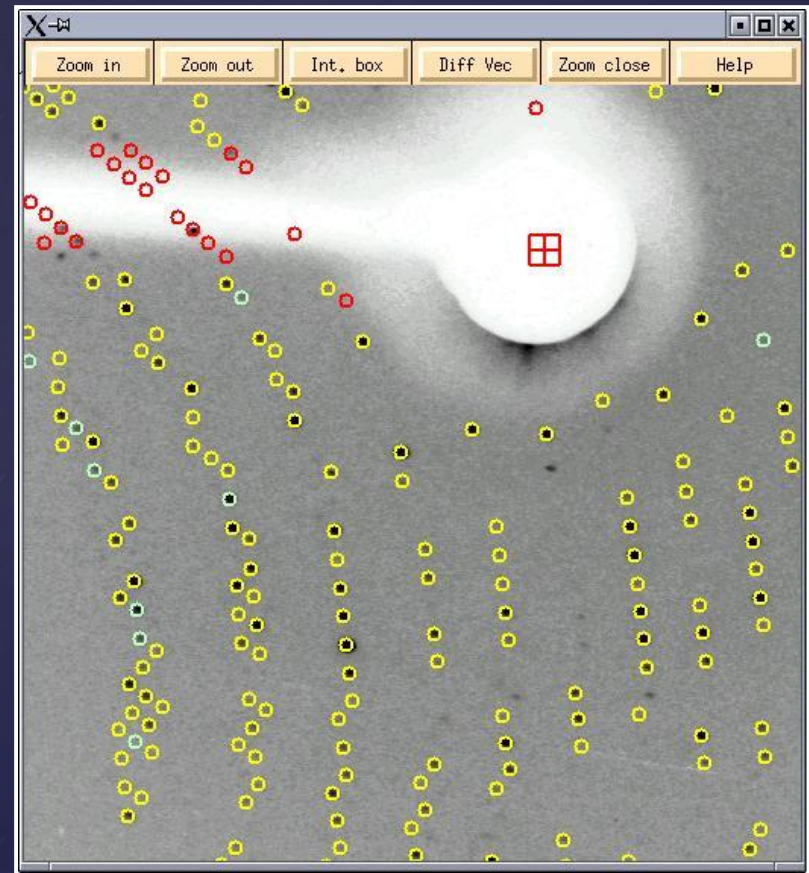


Xbeam, Ybeam

150.6 151.2



150.0 150.0



Crystal orientation

Crystal orientation software interface showing a diffraction pattern and refinement parameters.

Refinement Information

Space Group: **P222**
Resolution: 50.00 - 0.70

Positional: 70 X- χ^2 : 0.37 Y- χ^2 : 1.12 (0)
Partiality: 68 χ^2 : 0.51 (0)

X Beam: 125.064 Y Beam: 321.677

a: 5.13 b: 14.02 c: 14.88
 α : 90.00 β : 90.00 γ : 90.00

Crystal Rotation X: -79.777 0.000 0.020
Crystal Rotation Y: 0.210 0.000 0.017
Crystal Rotation Z: 175.101 0.000 0.024

Detector Rotation X:
Detector Rotation Y:
Detector Rotation Z:

Crystal Alignment

Space Group: **P222** Update

Axis Spindle a* Vertical c*

Distance:	X	Y	Z	Avail
Mosaicity:				
Y Scale:	82.262	-4.824	-92.494	?
Skew:	-97.738	4.824	87.506	?
Refinement Sigma Cut:	82.262	175.176	92.494	?
3D Window 1:	-97.738	-175.176	-87.506	?
Peak Search:	82.262	175.176	-87.506	?
Display:	-97.738	-175.176	92.494	?

Ready

Controls

Refine for 5 cycles

Bravais Lattice Check Mosaicity

Abort Refinement Reference Zone Crystal Alignment

Integrate Integration Setup

Set Beam Position Set Blind Region Set Shadow Region

Reject Criteria Check in PDB

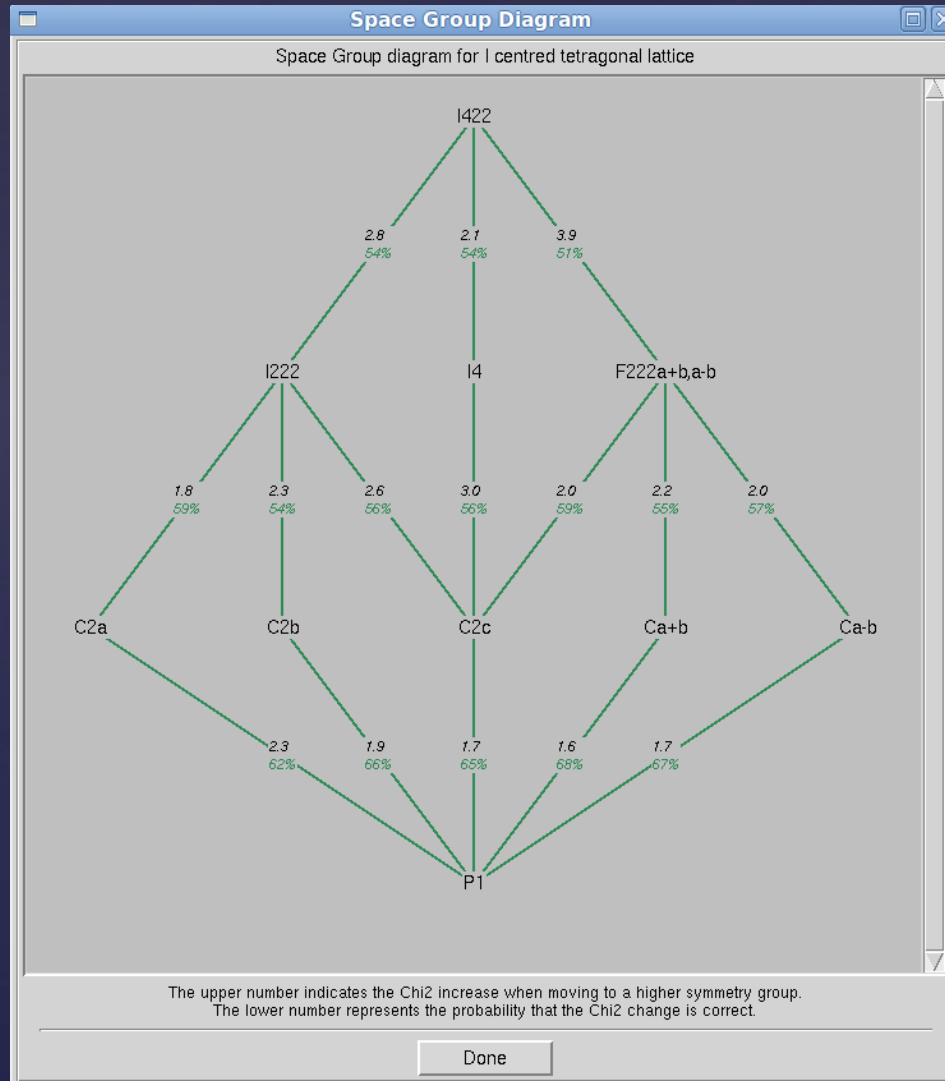
Peak Search

hkl = (1, 5, -1)

peak pos = [3550.6 , 1131.3]
intensity = 4460.2 ± 92.9
Dist. to prev. peak=356.1

```
putdebug: setactgroup 1
putdebug: DG.pendsets_set_select num 1
putdebug: suser kill
putdebug: DG.get detector format: raxis3s wide
putdebug: xdisp command: /home/marcel/HKL/hklsetup/hklbin/xdisp raxis3s wide /da
ta/marcel/Rigaku/crystal_align_test/crystal_test_5_0###.osc 1 -distance=127.38 -
lambda=0.71074
putdebug: save DLIST distance 127.38
putdebug: peaks fn /home/marcel/.hkl_230/peaks.file 12277
putdebug: SET(1,exp,distance.refined) 127.38
putdebug: DG.get detector format: raxis3s wide
putdebug: using Denzo version 2.3.1
putdebug: SG_change2...0
```

Space Group Determination Laue class diagram



Space Group Determination

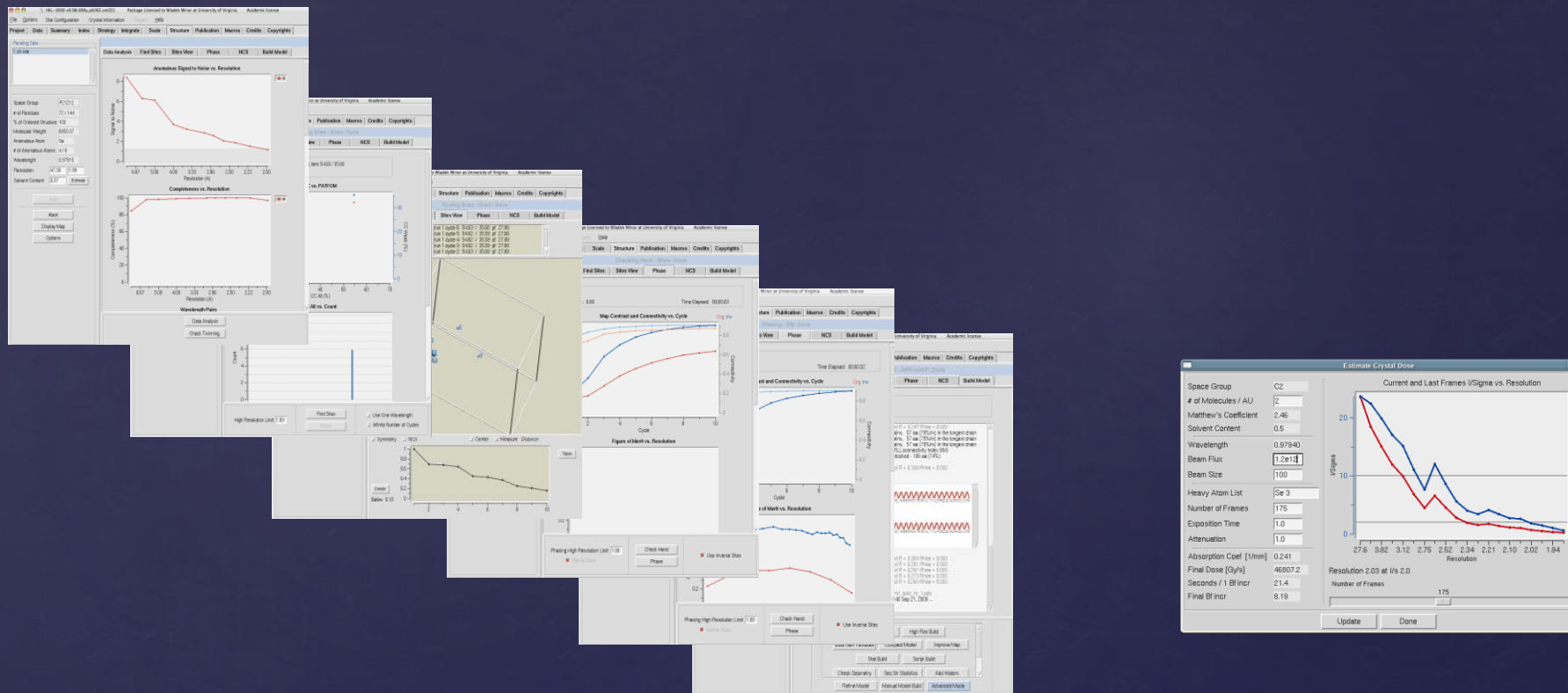
The screenshot displays the HKL3000 software interface. The main window shows a diffraction pattern with axes labeled 'l' and 'h'. A green box indicates $d=1.35\text{\AA}$, $I=0$, and $[0.0, 1.1]$. The software version is 7068. The HKL Processing System logo and the name of the user, W. Minor, are visible. A graph titled 'Low Resolution Completeness vs. Resolution' shows a plot of completeness versus resolution. The 'Space Group' is determined to be $P2_12_12$. The 'Log File' is `hhr_crystal1_maks2_scale.log`. The 'Done' button is highlighted. The 'Controls' section includes buttons for 'Scale Sets', 'Adjust Error Model', 'Edit Rejection File', 'Show Redundancies', 'Abort', 'Reindex', 'Show Log File', 'Reciprocal Lattice', 'Repr', 'Diagnostics', 'Exclude Frames', 'Check Space Group', and 'Space Group Diagram'.

Resolution (Å)	Completeness (%)
42.2	50
38.8	35
35.4	45
32.0	80
28.6	100
25.2	70
21.8	85
18.4	95
15.0	98
11.6	99
8.2	99
4.8	99

$P2_12_12$

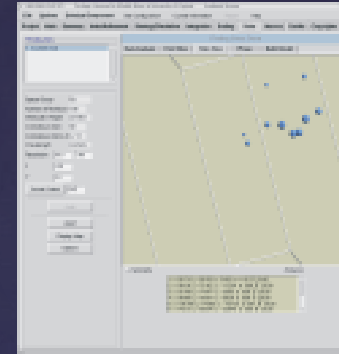
An inset diffraction pattern showing a different view of the data, with axes labeled 'l' and 'k'. The pattern consists of a grid of diffraction spots.

Where are the real bottlenecks ?



Database-controlled pipeline

lab e-book

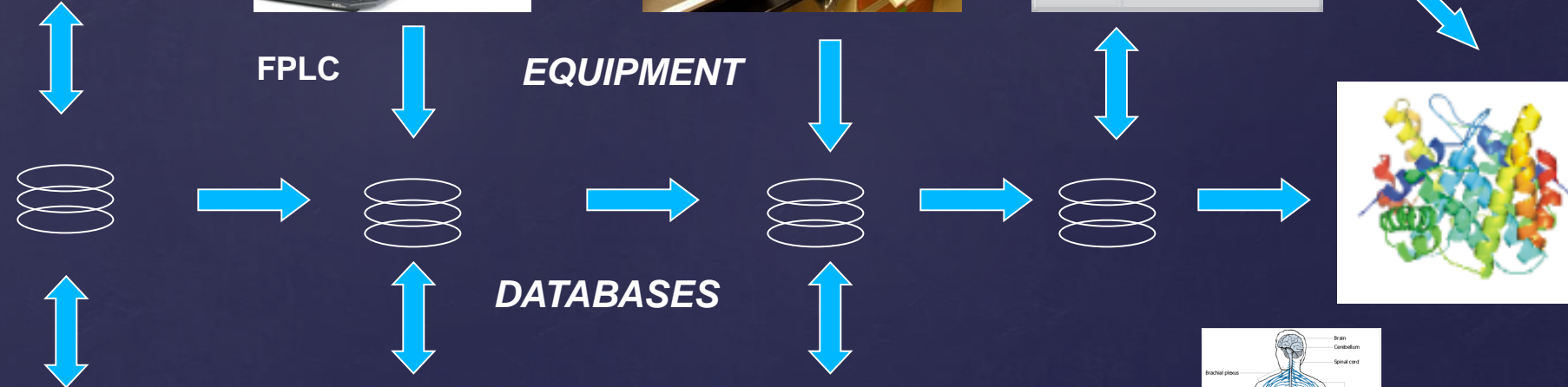


HKL-3000

FPLC

EQUIPMENT

DATABASES



WET LAB DATABASE

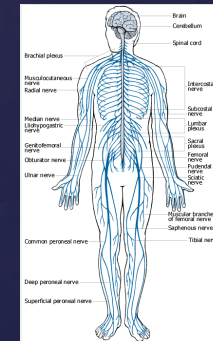
Sample ID	Sample Name	Sample Type	Sample Date	Sample Location	Sample Status
1001	Sample 1001	Sample Type 1	2001-01-01	Sample Location 1	Sample Status 1
1002	Sample 1002	Sample Type 2	2001-01-02	Sample Location 2	Sample Status 2

PeptideDB @ UNIVERSITY OF VIRGINIA

Peptide ID	Peptide Name	Peptide Type	Peptide Date	Peptide Location	Peptide Status
2001	Peptide 2001	Peptide Type 1	2001-01-01	Peptide Location 1	Peptide Status 1
2002	Peptide 2002	Peptide Type 2	2001-01-02	Peptide Location 2	Peptide Status 2

peptide list

Peptide ID	Peptide Name	Peptide Type	Peptide Date	Peptide Location	Peptide Status
3001	Peptide 3001	Peptide Type 1	2001-01-01	Peptide Location 1	Peptide Status 1
3002	Peptide 3002	Peptide Type 2	2001-01-02	Peptide Location 2	Peptide Status 2



Big brother?

Statistics / Progress in Minor Lab LIMS by researcher

Last week (17 Apr 2015 - 24 Apr 2015)

Person	Clones	Exprs	Purifs	Macro preps	Plates	Drops	Crystals	Datasets processed	Structure refs	Kinetic assays	Thermal shift assays
Cooper, David	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	0	<u>23</u>	<u>18</u>	<u>0</u>	0	0
Handing, Katarzyna	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	0	<u>51</u>	<u>53</u>	<u>13</u>	0	0
Hou, Jing	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>1</u>	30	<u>0</u>	<u>1</u>	<u>1</u>	0	0
Kowiel, Marcin	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	0	<u>1</u>	<u>8</u>	<u>3</u>	0	0
Shabalin, Ivan	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	0	<u>125</u>	<u>14</u>	<u>9</u>	0	0
Shumilin, Igor	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	0	<u>0</u>	<u>3</u>	<u>2</u>	0	0
Szlachta, Karol	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	0	<u>34</u>	<u>20</u>	<u>3</u>	0	0

Last month (25 Mar 2015 - 24 Apr 2015)

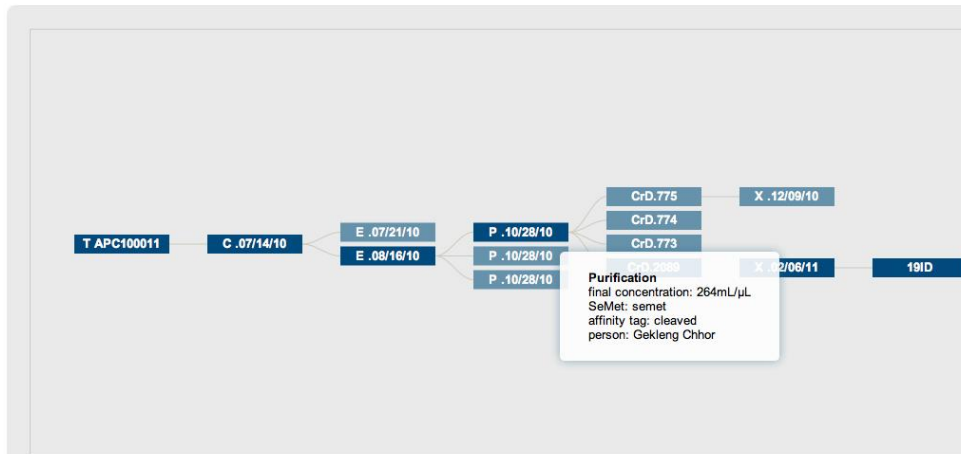
Target status and path to success



Midwest Center
for
Structural Genomics



- Consortium
- Overview
- MCSG Structural Biology Partnerships
- Investigators
- PSI Programs
- NIH Report
- Homologs in PDB
- PSI: Biology Targets
- MCSG PSI-2 Database
- PSI-2 Structures
- X-ray Structures
- NMR Structures
- Publications
- Resources
- Related Sites
- Progress
- Collaborators
- Technology
- Contact Us
- Log In



Purification
final concentration: 264mL/μL
SeMet: semet
affinity tag: cleaved
person: Gekleng Chhor

- tree orientation**
- left
 - top
 - bottom
 - right
 - done

Target APC100011

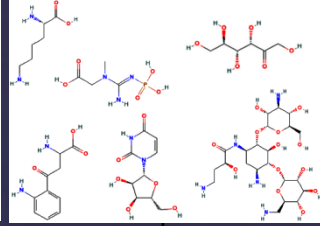
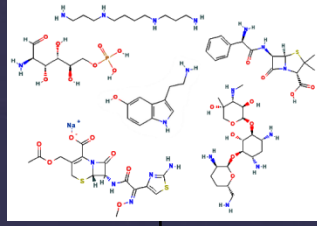
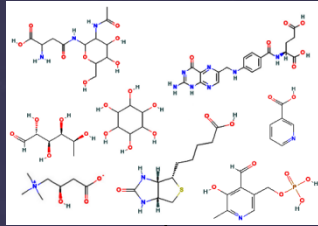
(simple view)

Stage	in_pdb
Locus Tag/Selection DB Lookup	AAur_4089
NCBI Protein Lookup	gi: 119963336 accession: YP_949757
Organism	Arthrobacter aurescens TC1
Common Name	beta-N-acetylhexosaminidase [Arthrobacter aurescens TC1]
Gene Name	nahA
NCBI Gene Lookup	4638054
NCBI Annotation	
TIGR Main Role/Annotations	
TIGR Sub Role	
Comment	
Selection Phase	MCSG::Biology::Human Microbiome

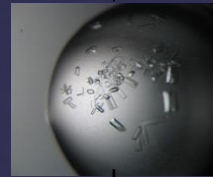
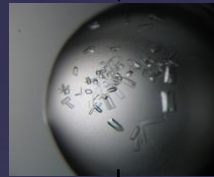
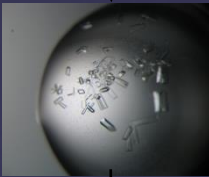
HKL-3000 at SBC



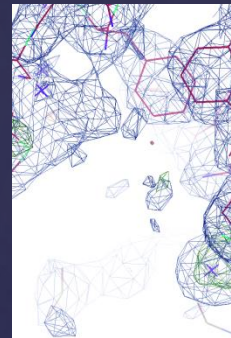
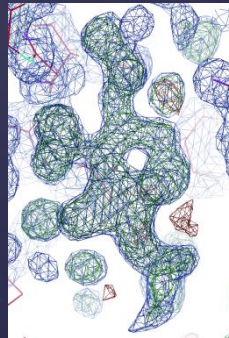
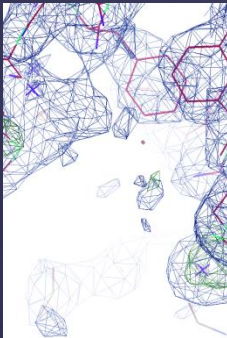
Crystallographic ligand screening



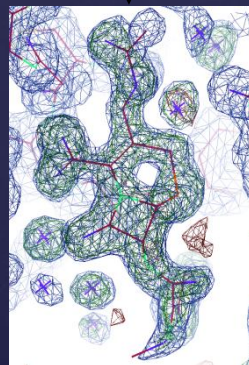
Cocktail solutions,
each composed of 5-10
potential ligands



Soaking protein crystals
with cocktail solutions

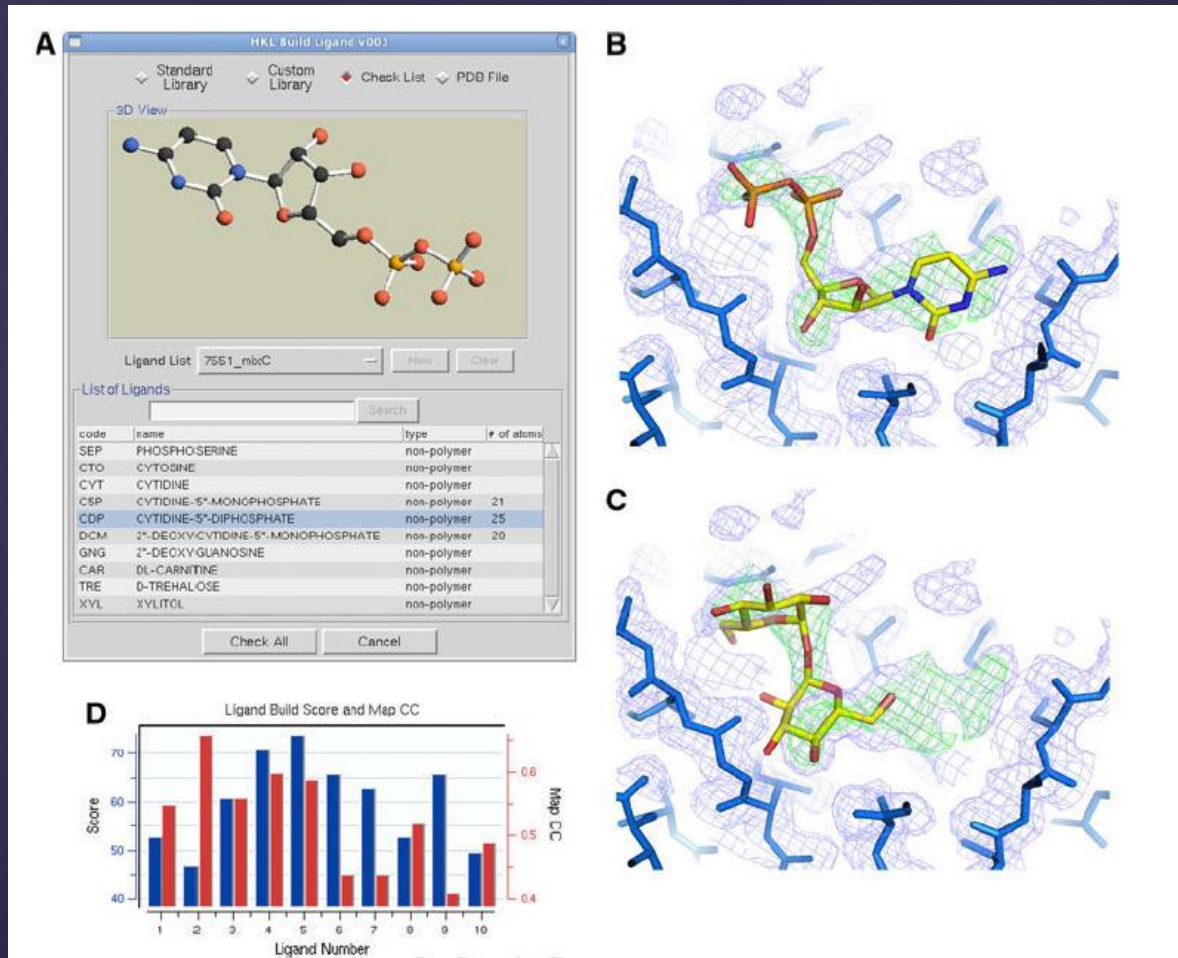


Data collection, structure
solution and identification of
additional electron density



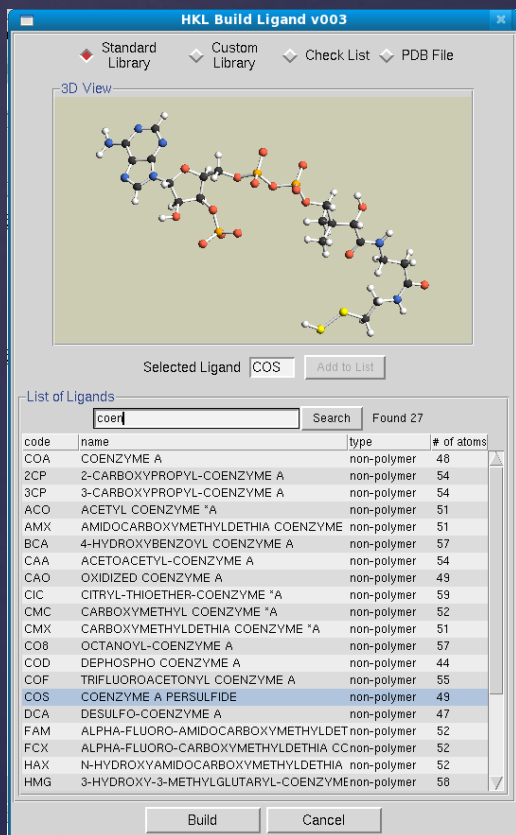
Identification of bound compound
based on the structures
of the cocktail components
and the difference electron density
map

Ligand fitting

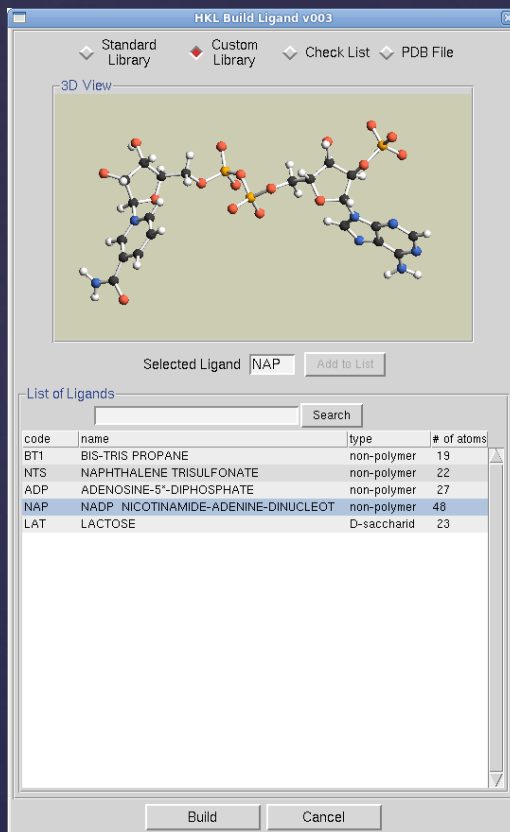


Automatic fitting of single ligand from particular library

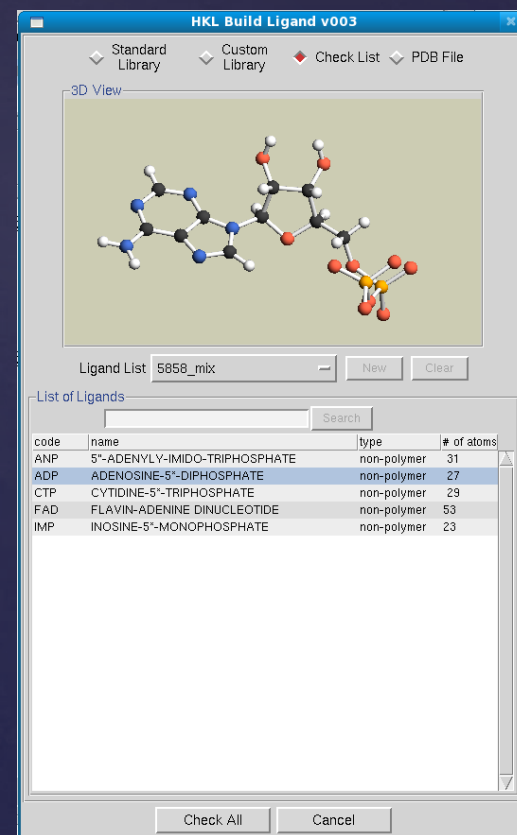
(a)



(b)

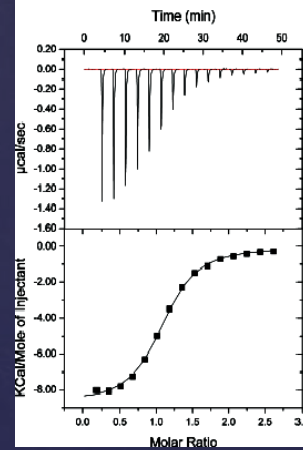
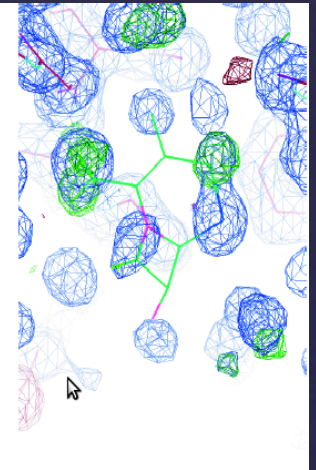
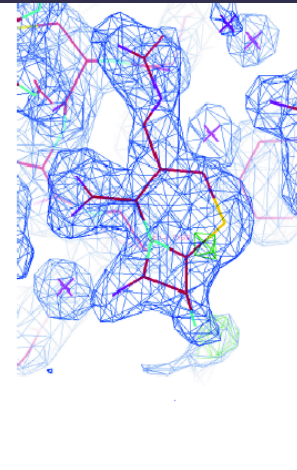
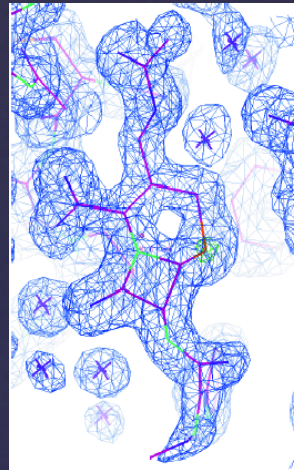
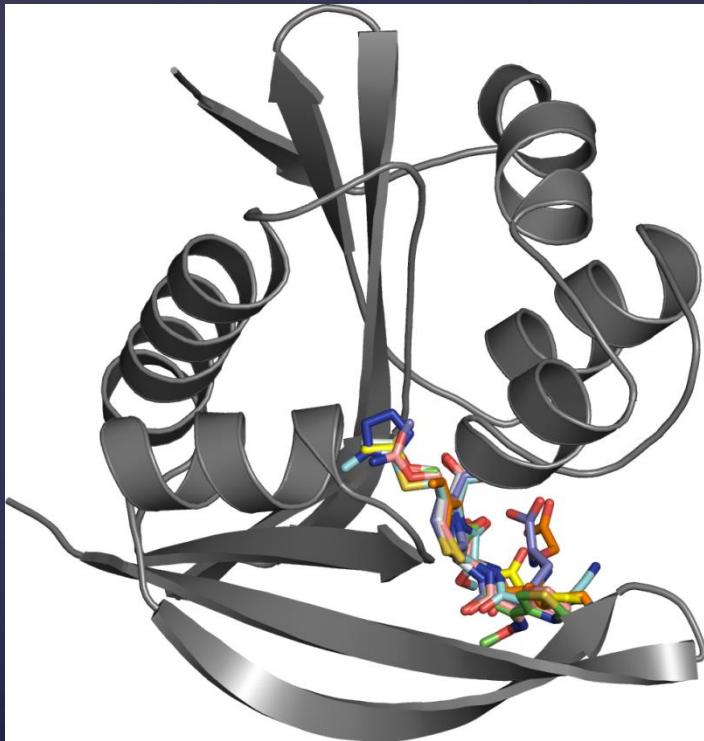


(c)

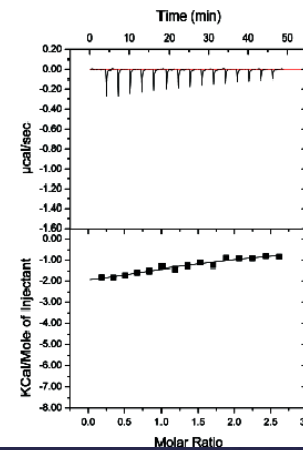


Ligands evaluation using crystallography and ITC

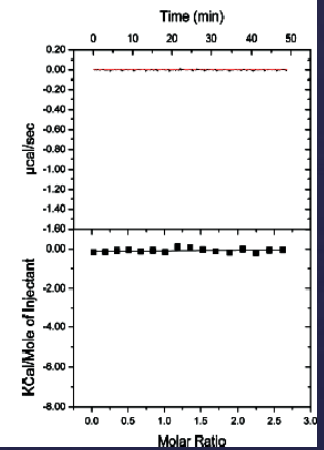
The search is extended to compounds showing similarity to the first hits.



Cefotaxime
 $K_d = 3.5 \pm 0.2 \mu\text{M}$



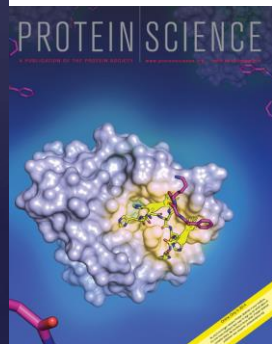
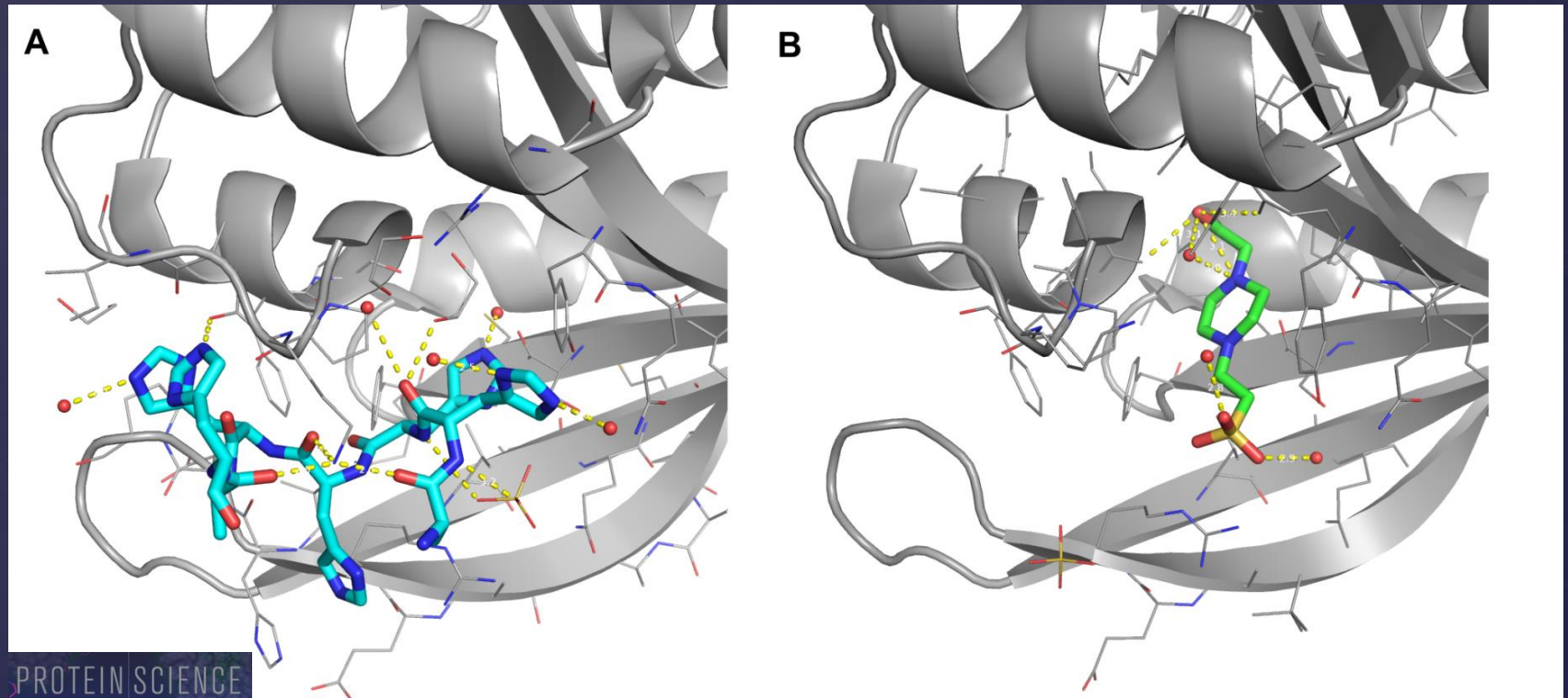
7-aminocephalosporanic acid



7-aminodesacetoxycephalosporanic acid

Evaluation of identified ligands using ITC allows to determine the binding affinity of the ligands and to identify which compounds bind with the highest affinity.

Structures of PA4794 with His-tag (A) and HEPES (B) bound to substrate binding site

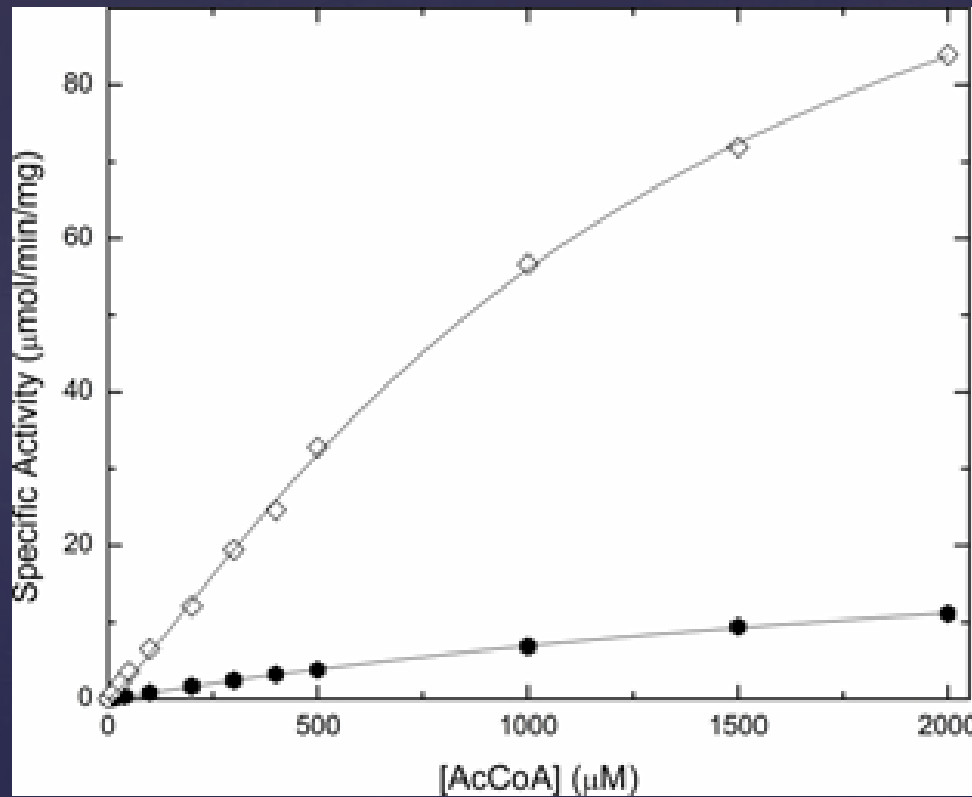


Double trouble—Buffer selection and His-tag presence may be responsible for nonreproducibility of biomedical experiments



Karolina A. Majorek,^{1,2,3,4} Misty L. Kuhn,^{4,5} Maksymilian Chruszcz,^{1,3,4,6}
Wayne F. Anderson,^{3,4,5} and Wlodek Minor,^{1,3,4*}

Effect of His-tag on SpeG



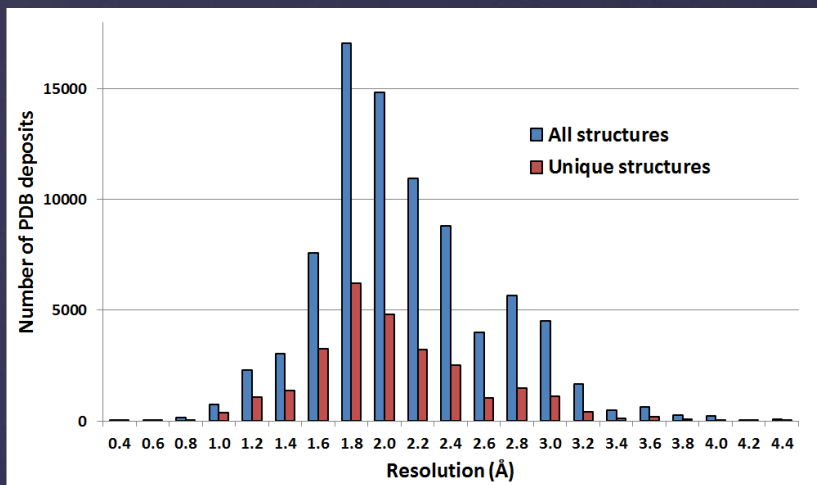
Protein purification and crystallization artifacts: The tale usually not told



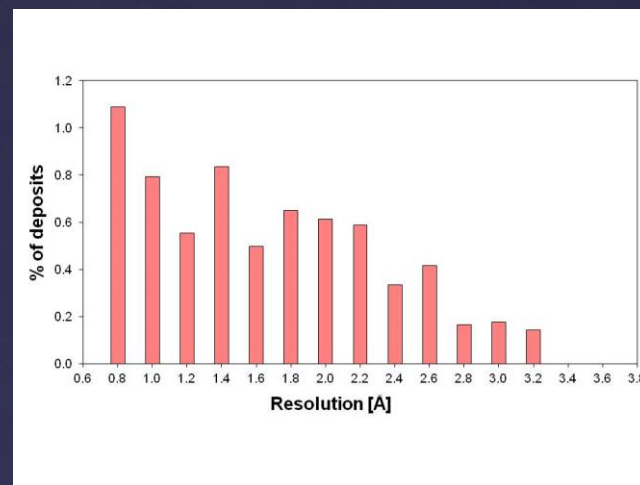
Table 1. Known Structures of Proteins that Have Been Identified as Common Purification and Crystallization Artifacts

	Name of the protein	Molecular weight (kDa)	PDB ID
Affinity, solubility, anti-aggregation tags	Maltose-binding protein (MBP)	43	1LLS, 1MPB, 3PUW, 3SEU, 4KYC
	Glutathione-S-transferase (GST)	24	4ECB
	Thioredoxin (Trx)	11	1F6M, 2AJQ, 2H73, 4HU9, 4X43
	N-Utilization substance (NusA)	55	1U9L, ^a 1WCN, ^b 2KWP, ^c 4MTN ^d
<i>E. coli</i> native proteins	Small ubiquitin related modifier 1 (SUMO1)	12	2UYZ, 1Z5S, 4WJQ, 2I02
	Haloalkane dehalogenase	33	4E46
	Metal-binding lipocalin (YodA)	25	1OEJ, 4TNN
	Carbonic anhydrase (YadF)	25	2ESF
	Ferric uptake regulator (Fur)	16	2FU4
	cAMP-regulatory protein (CRP)	24	1CGP, 2CGP, 2GZW, 3FWE, 3HIF, 3N4M, 3QOP, 4FT8, 4HZF, 4I0A, 4I0B, 4N9H, 4N9I
	Glucosamine-6-phosphate synthase (GlmS)	67	4AMV, 1JXA, 3OOJ, 2J6H
	Glycogen synthase (GlgA)	53	2QZS
	Component 1 of the 2-oxoglutarate dehydrogenase complex (ODO1)	105	2JGD
	Component E2 of dihydroliipoamide succinyltransferase (ODO2)	44	1C4T
Proteases	Formyl transferase (YfbG, AmA)	46	1U9J, 1YRW, 1Z7E, 2BLN, 4WKG, 1ESO
	Cu/Zn-superoxide dismutase (Cu/Zn-SODM)	16	1Q23
	Chloramphenicol-O-acetyl transferase (CAT)	26	1Q23
	Host factor-I protein (Hfi)	11	3VU3
	Tobacco etch virus (TEV)	28	1LVM
	Rhinovirus 3C protease	48	1CQQ
	SUMO protease C-terminal domain	26	2HL9
	Enterokinase	26	1EKB
	Trypsin	26	3UY9
	Chymotrypsin	26	1GGD
Exogenous proteins	Thrombin (active form)	36	3SQE, 1MH0, 4H6T
	Thermolysin	60	4D9W
	Proteinase K	40	3DVS
	Pepsin	41	5PEP
	Neutrophil elastase	29	5ABW
	LysN Peptidyl-Lys metalloendopeptidase	44	1GE7
	Lysyl endopeptidase	28	4NSY
	Factor Xa	55	1KIG
	Lysozyme	16	4TWS, 4PRQ, 1AKI
	DNase protein	31	2A40

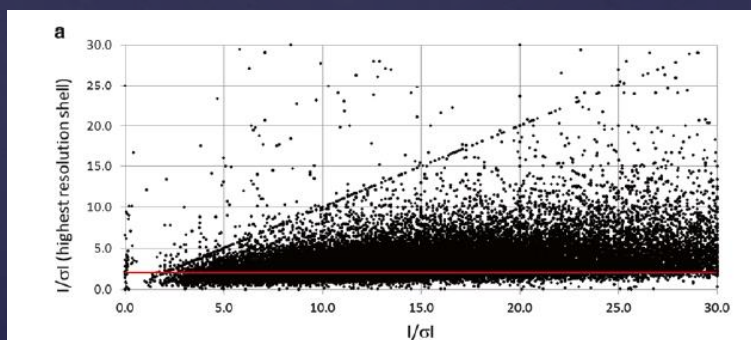
Resolution and ligands in PDB



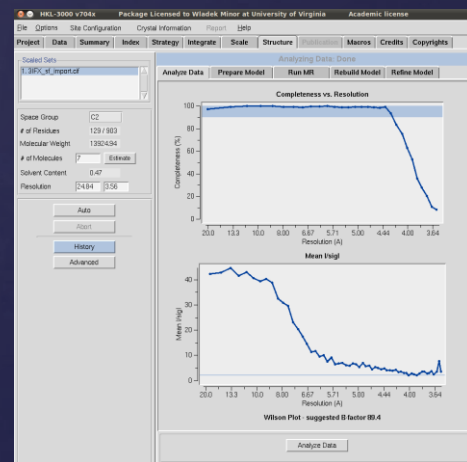
Cooper et al, *Expert Opinion on Drug Discovery* (2011) 6: 771-782



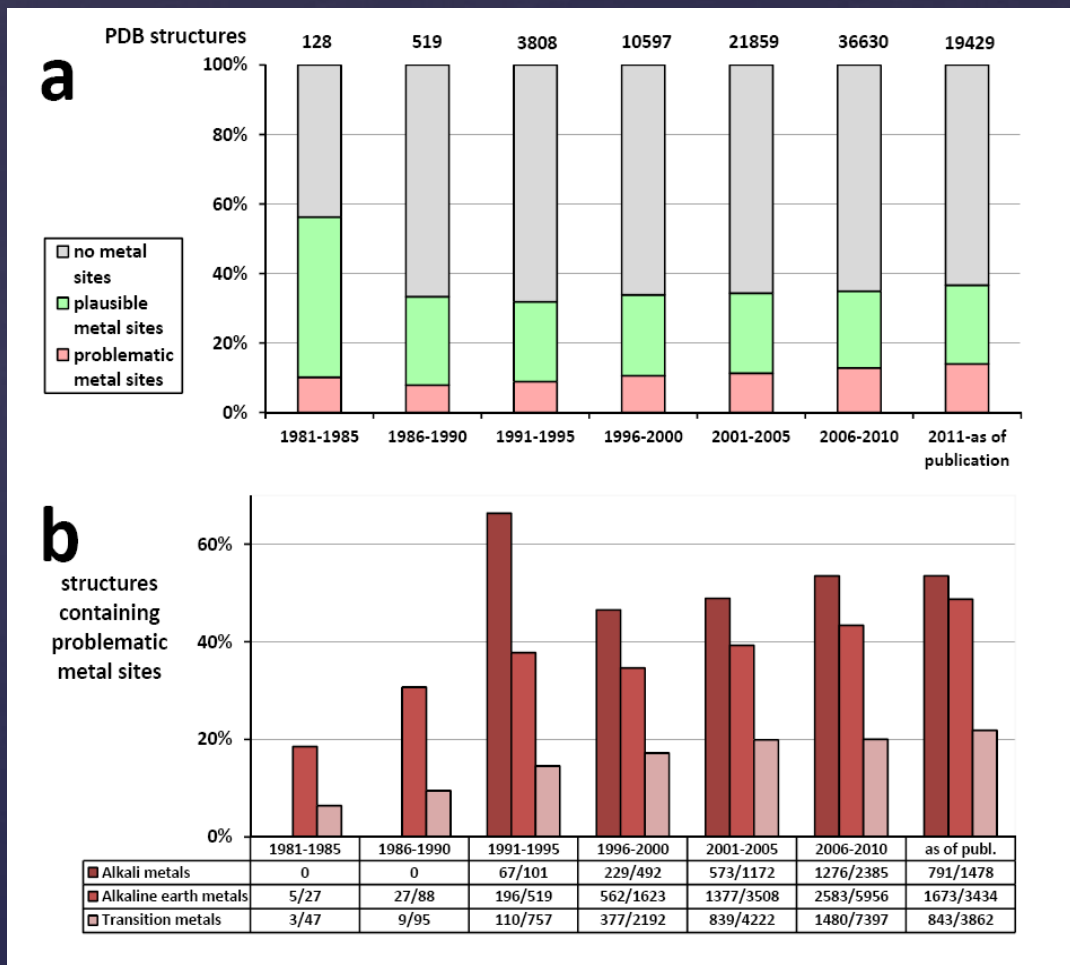
Cooper et al, *Expert Opinion on Drug Discovery* (2011) 6: 771-782



Domagalski et al. *Methods in Molecular Biology* (2014) 1091: 297-314



Metals in PDB



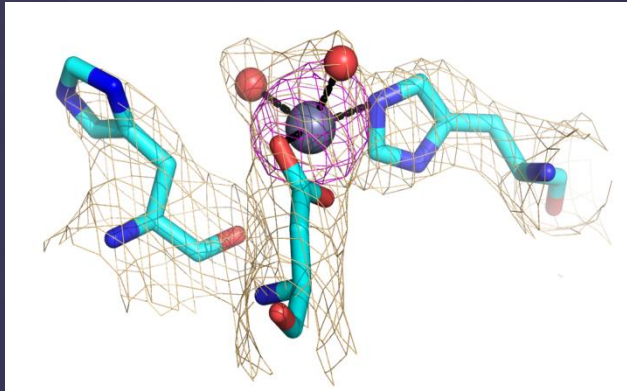
Metal binding site validation: *CheckMyMetal* server

ID	Res	Atom	Valence	BV symmetry	Geometry	RMSD geometry angles	Missing vertices	Bidentate	CBVS	Alternative metal
400:A	_MG	MG--	2.1	0.111	Octahedral	5.36	0	0	4.41	
400:B	_MG	MG--	2.06	0.079	Octahedral	4.13	0	0	4.32	

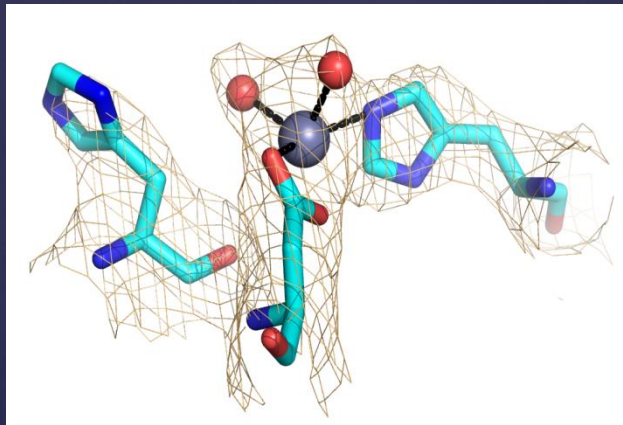
Mouse click action:	Basic controls:	Use the buttons below to control the view
<input checked="" type="radio"/> None <input type="radio"/> Center <input type="radio"/> Distance <input type="radio"/> Label <input type="button" value="Zoom In"/> <input type="button" value="Zoom Out"/> <input type="button" value="Center"/>	Left-Click to rotate Shift-Left-Click up & down to zoom Right-Click for Jmol's context menu	Residue Name: <input checked="" type="radio"/> On <input type="radio"/> Off Metal Distances: <input type="radio"/> On <input checked="" type="radio"/> Off Protein Cartoon: <input type="radio"/> On <input checked="" type="radio"/> Off Spin: <input type="radio"/> On <input checked="" type="radio"/> Off Antialiasing: <input checked="" type="radio"/> On <input type="radio"/> Off

Legend	Explanation
Valence	Summation of bond valence values for an ion binding site
BV symmetry	Summation of bond valence vectors, weighted by bond valence values. Increase when the coordination sphere is not symmetrical due to incompleteness.
Geometry	Ion binding site geometry, as calculated by the NEIGHBORHOOD algorithm
RMSD geometry angles	R.M.S. Deviation of observed geometry angles (L-M-L angles) compared to ideal geometry, in degrees
Missing vertices	Number of sites that is not occupied in the coordination sphere for the assigned geometry
Bidentate	Number of residues that form a bidentate interaction instead of being considered as multiple ligands
CBVS	Calcium Bond Valence Sum, used for alternative metal(s) prediction [Muller, P. et al. (2003) Is the bond-valence method able to identify metal atoms in protein structures? Acta Crystallogr. D Biol. Crystallogr., 59, 32-37.]
Alternative metal	A list of alternative metal(s) is proposed in descending order of confidence, assuming metal environment is accurately determined

The way to prove presence of metal



Metal binding site A
9668eV



Metal binding site A
9618eV

Data collected below and above zinc
absorption edge - APS 19BM

Dissonance in Science

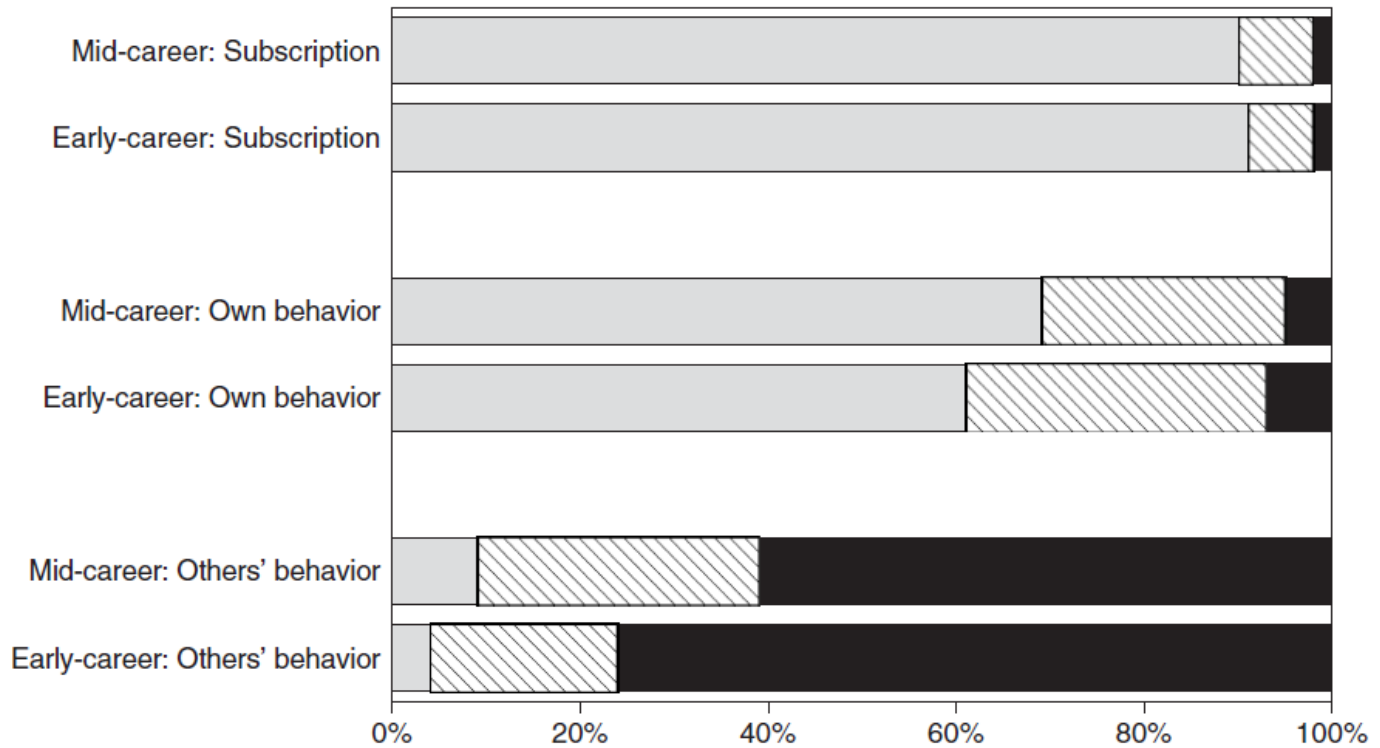


FIG. 3. Norm versus Counternorm Scores: Percent with Norm > Counternorm (dotted), Norm = Counternorm (striped), Norm < Counternorm (solid).

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