

Other ways to solve structure *ab initio*  
*MXSchool2010*

Daniele de Sanctis



- History of RIP phasing
- Use of UV RIP phasing on ID23EH1
- Latest results and perspectives



- Radiation damage destroys your crystal
- Radiation damage prevents to get complete data
- Radiation damage depletes the anomalous signal
- The devil is not so black as he is painted

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## Specific Radiation Damage Can Be Used to Solve Macromolecular Crystal Structures

Raimond B.G. Ravelli,<sup>1\*</sup>  
Hanna-Kirsti Schröder Leiros,<sup>2</sup> Baocheng Pan,<sup>2</sup>  
Martin Caffrey,<sup>2</sup> and Sean McSweeney<sup>1</sup>  
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A number of robotic systems are under development to

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## Radiation-induced site-specific damage of mercury derivatives: phasing and implications

Udupi A. Ramagopal,<sup>a\*</sup> Zbigniew Dauter,<sup>b</sup> Radhakannan Thirumuruhan,<sup>c</sup> Elena Fedorov<sup>a</sup> and Steven C. Almo<sup>a,d,e\*</sup>

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The behavior of mercury-derivatized triclinic crystals of a 60 kDa protein target from the New York Structural Genomix Research Consortium provides novel insights into the mechanism of heavy-atom-specific radiation damage and its potential exploitation for *de novo* structure solution. Despite significant anomalous signal, structure solution by classic SAD and MAD phasing approaches was not successful. A detailed analysis revealed that significant isomorphous variation of the diffracted intensities was induced by X-ray irradiation. These intensity changes allowed the crystal structure to be solved by the radiation-damage-induced phasing (RIP) technique. Inspection of the crystal structure and electron-density maps demonstrated that the covalent S-Hg bonds at all four derivatized cysteine sites were much more susceptible to radiation-induced cleavage than other bonds typically present in native proteins. A simple diagnostic is described to identify the fingerprint of such decay at the time of

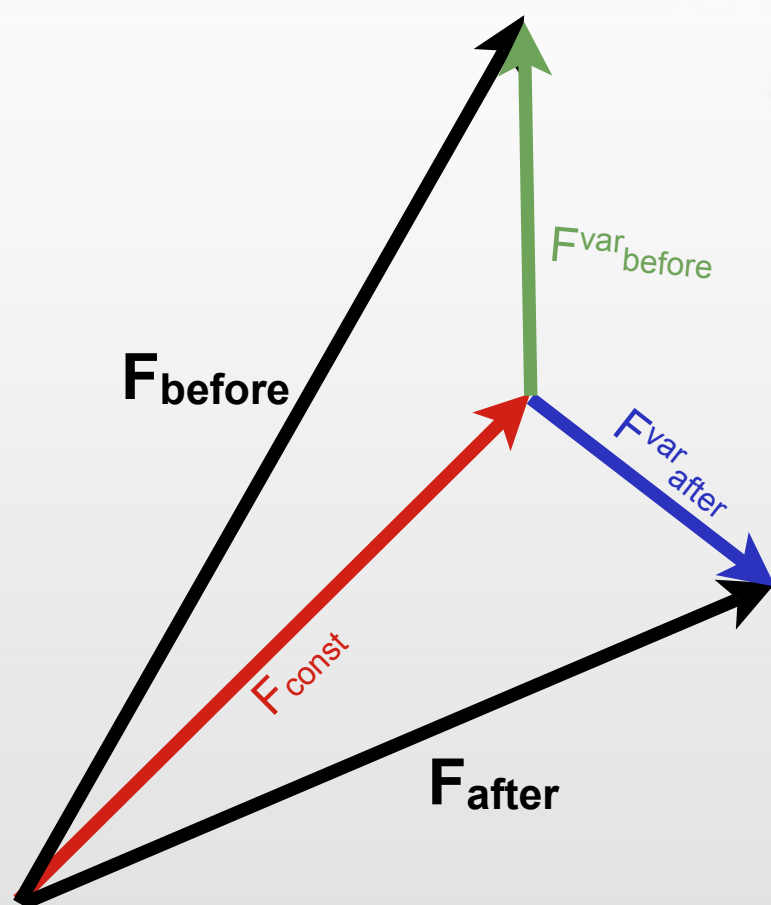
## Structural effects of radiation damage and its potential for phasing

Sankaran Banumathi,<sup>a</sup> Petrus H. Zwart,<sup>b</sup> Udupi A. Ramagopal,<sup>a\*</sup> Mirosława Dauter<sup>b</sup> and Zbigniew Dauter<sup>a\*</sup>

<sup>a</sup>Synchrotron Radiation Research Section, MCL, National Cancer Institute, Brookhaven National Laboratory, Upton, NY 11973, USA, and <sup>b</sup>SAJC Frederick Inc, Basic Research Program, Brookhaven National Laboratory, Upton, NY 11973, USA  
Correspondence e-mail: [dauter@bnl.gov](mailto:dauter@bnl.gov)

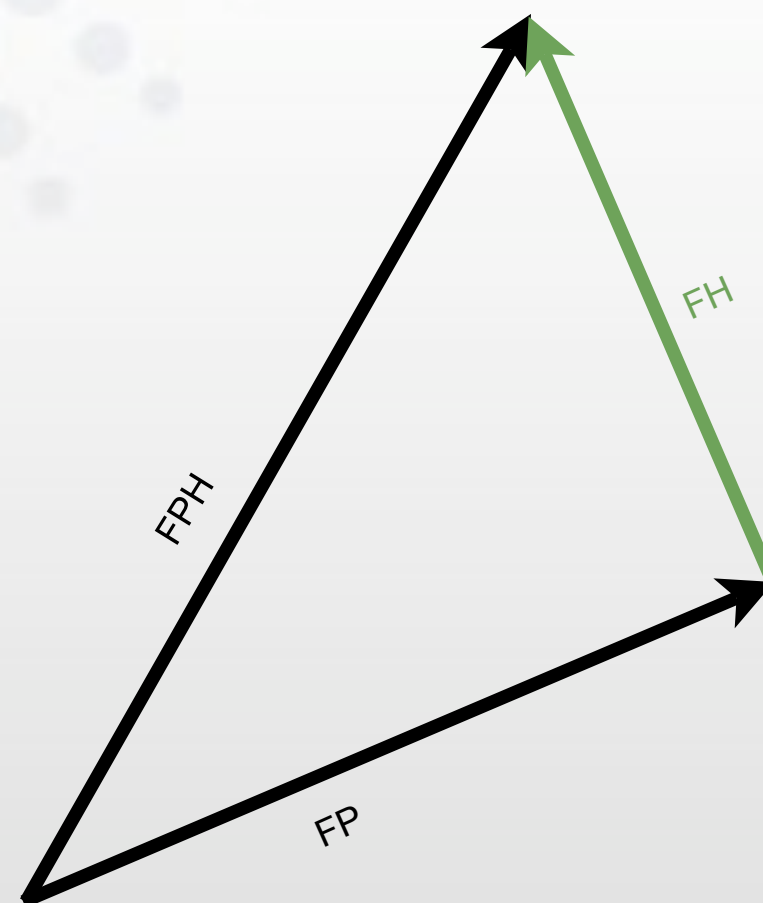
A detailed analysis of radiation-damage-induced structural and intensity changes is presented on the model protein thaumatin. Changes in reflection intensities induced by irradiation display a parabolic character. The most pronounced structural changes observed were disulfide-bond breakage and associated main-chain and side-chain imine residues. The structural changes induced on the hase estimates via an RIP procedure. Results obtained the partial structure may play an important role in

Radiation damage



$\approx$

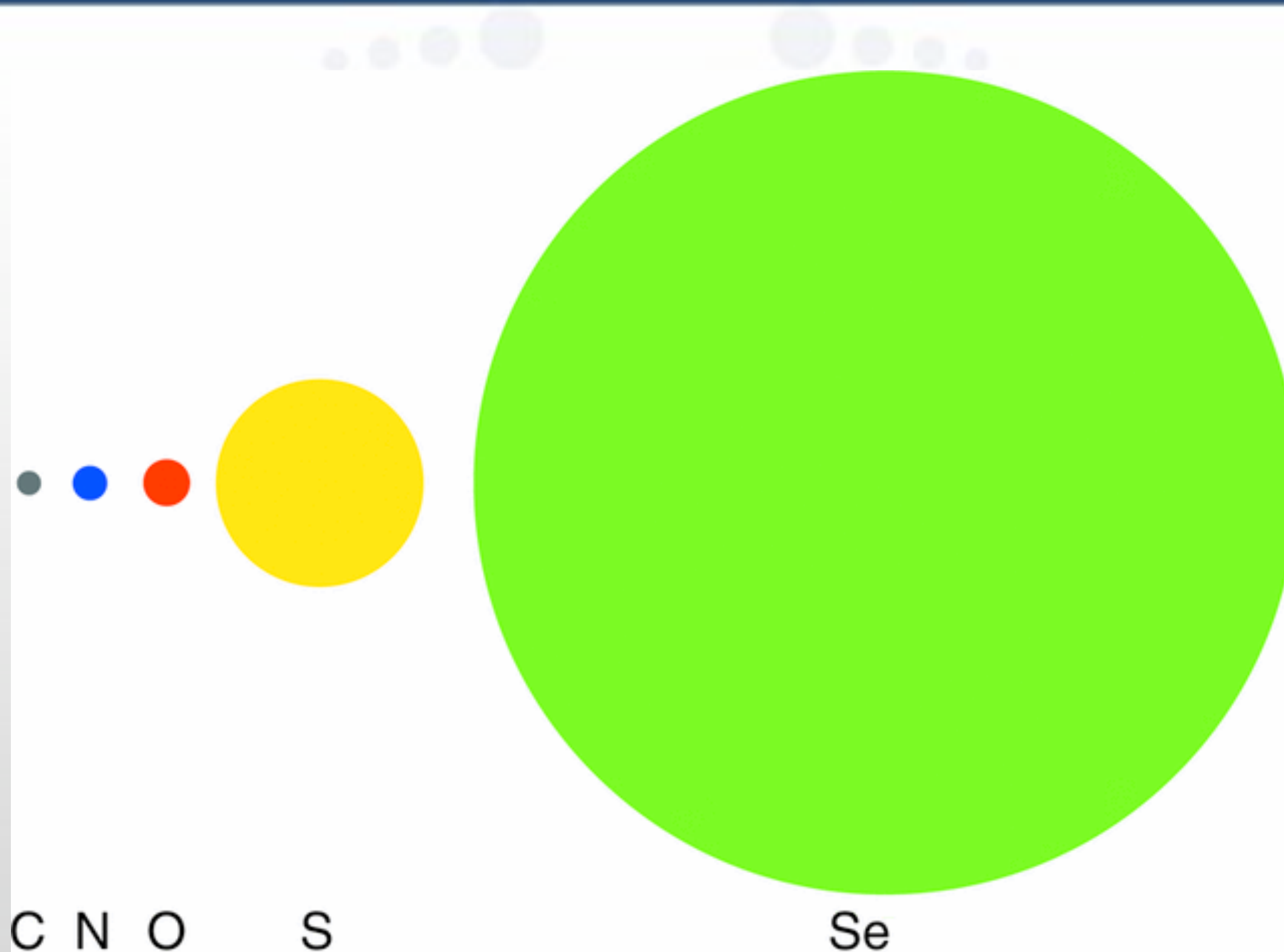
Isomorphous replacement



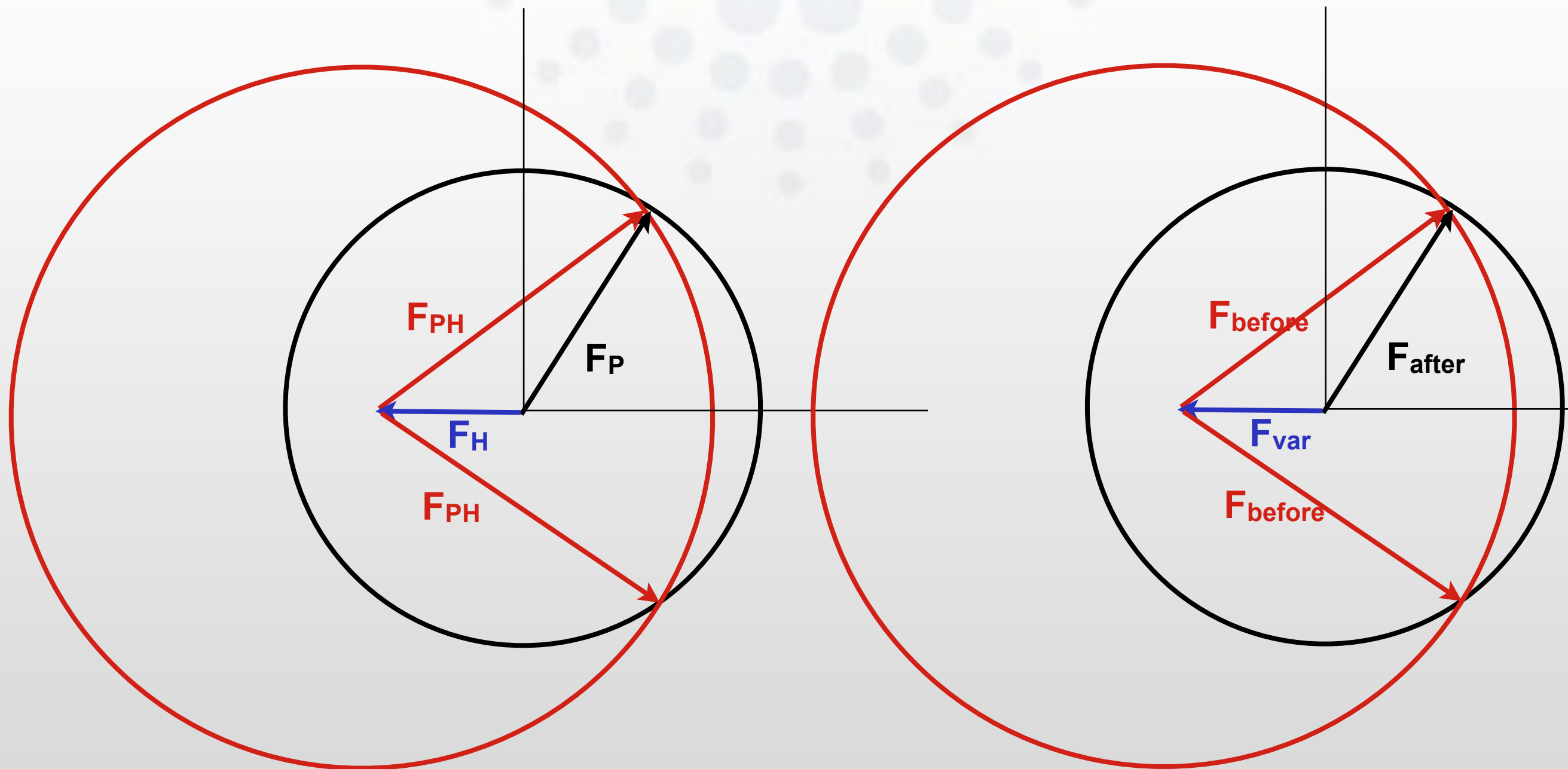
The Structure Factor is dependent on the X-ray dose. With a appropriate experiment planning an  $\mathbf{F}_{\text{before}}$  and an  $\mathbf{F}_{\text{after}}$  can be identified

If some particularly radiation sensitive site are identified, the difference between the two data collection can be treated as a derivative





Relative photoelectric cross sections of carbon, nitrogen, oxygen, sulfur and selenium at 13.114 keV



## research papers

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Crystallography 2004

## Radiation-damage-induced phasing with anomalous scattering: substructure solution and phasing

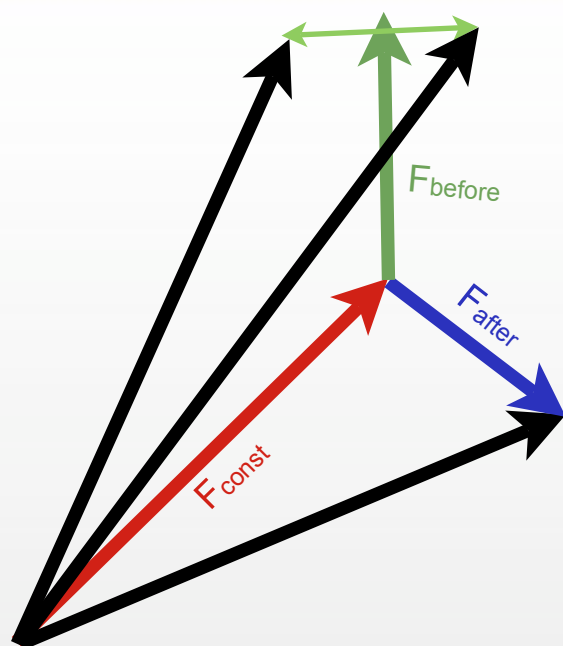
Petrus H. Zwart,<sup>a</sup> Sankaran Banumathi,<sup>b</sup> Mirosława Dauter<sup>a</sup> and Zbigniew Dauter<sup>b\*</sup>

<sup>a</sup>SAC-Frederick Inc., Basic Research Program, Brookhaven National Laboratory, Upton, NY 11973, USA, and <sup>b</sup>Synchrotron Radiation Research Section, MCL, National Cancer Institute, Brookhaven National Laboratory, Upton NY 11973, USA  
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Substructure-solution and phasing procedures using a combination of [anomalous scattering](#) and radiation-damage-induced isomorphous differences have been investigated. The tyrosine residues in thaumatin were iodinated with *N*-iodosuccinimide in the crystalline form as well as prior to crystallization. Several data sets were collected from both forms and used for substructure solution and phasing using various protocols, employing anomalous, isomorphous or both these signals. It was shown that combination of the anomalous and isomorphous signals in the form of the RIPAS (radiation-damage-induced phasing with anomalous scattering) strategy is beneficial for both locating the substructure and subsequent phasing.

**Keywords:** [anomalous scattering](#); radiation damage; RIP; RIPAS.

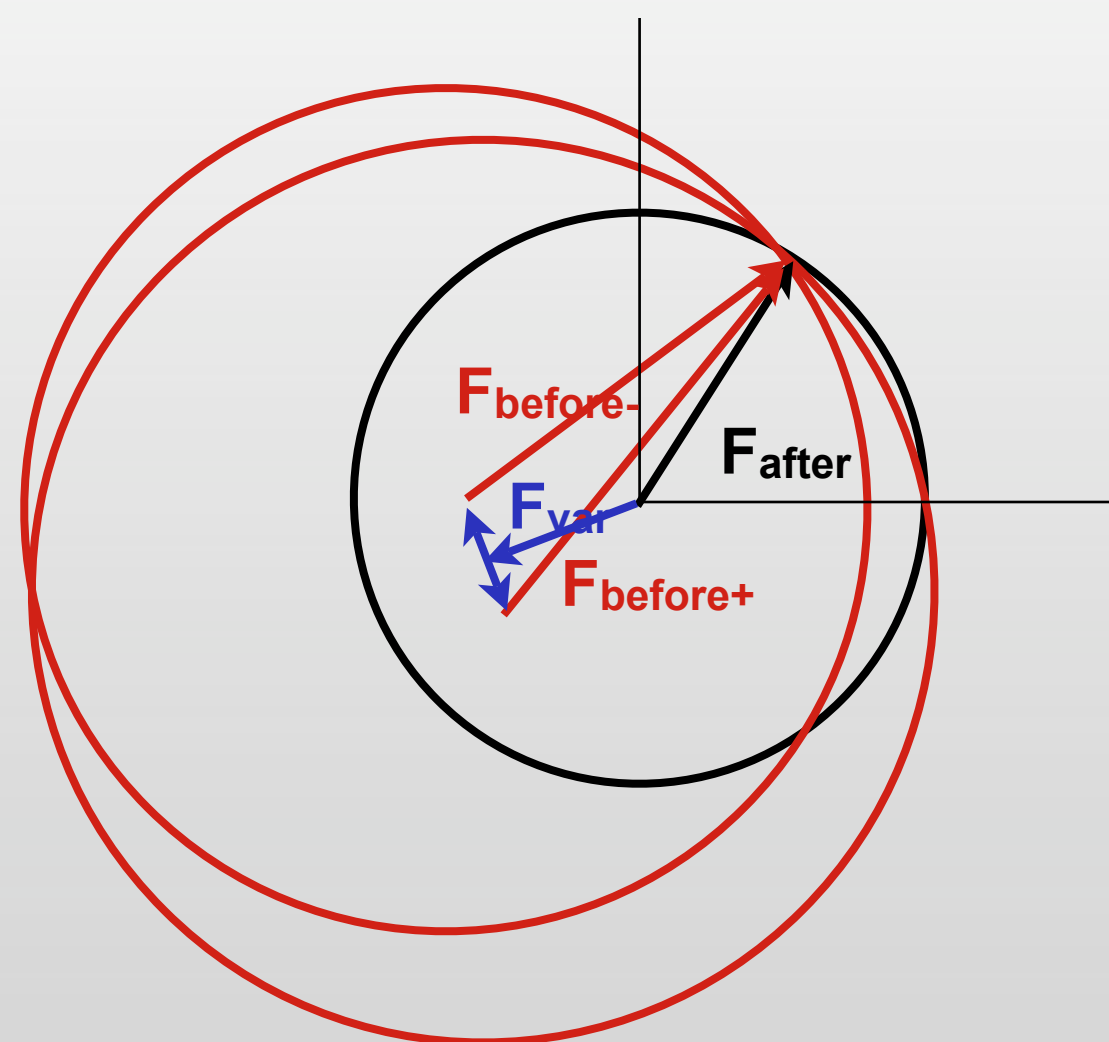
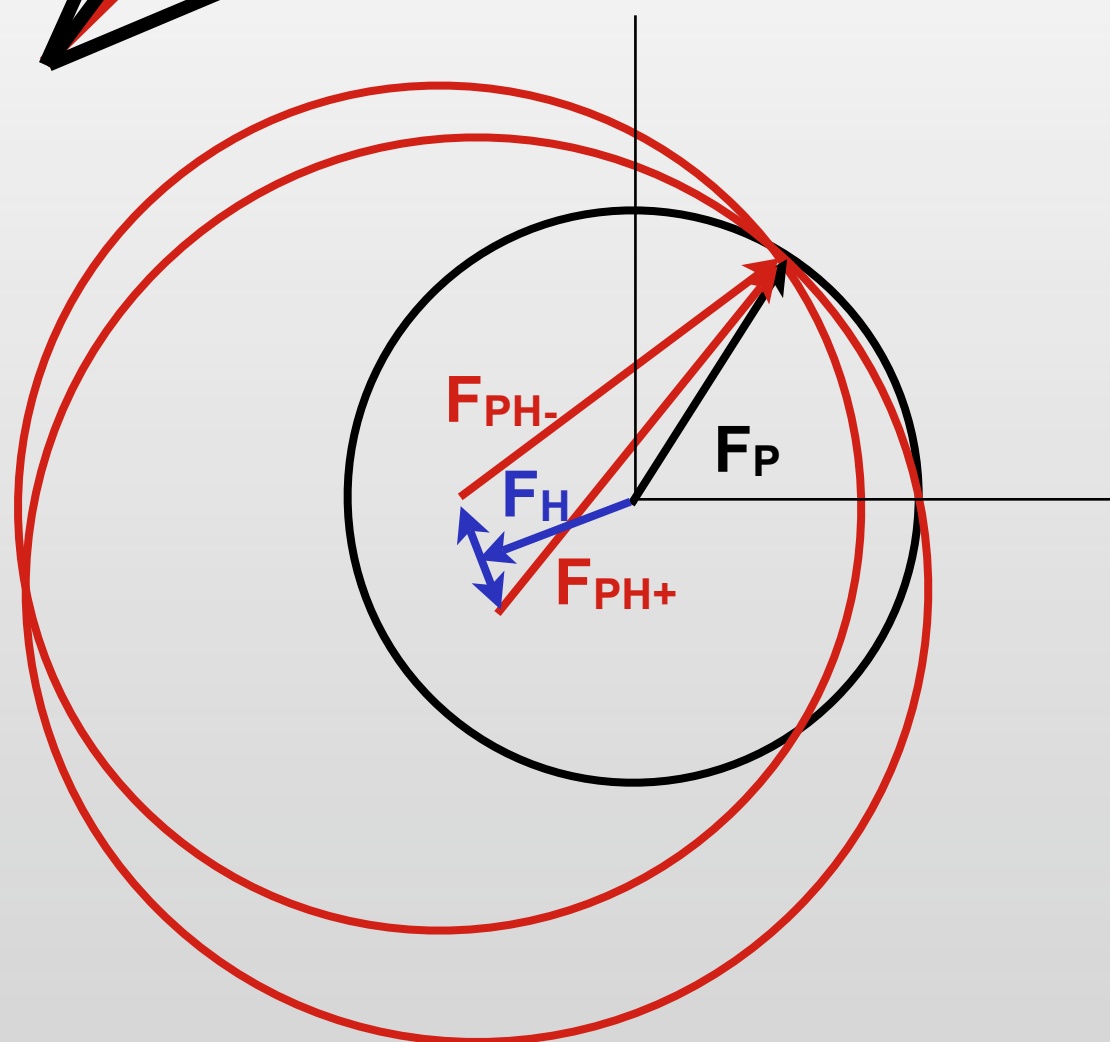




In the presence of an anomalous scatters,  $F_{\text{before}}$  will contain the anomalous contribution, while  $F_{\text{after}}$  will be collected away from the peak, or will contain much less anomalous signal because of depletion of the scatters

“(...) In fact, many structures have been solved unintentionally with a helping hand from RIP! In a MAD experiment, provided that the 'inflection point' dataset is collected last from the same crystal, the radiation damage has the effect of making  $f'$  for the MAD element at this wavelength even more negative than usual, enhancing the dispersive part of the MAD signal.”

[http://strucbio.biologie.uni-konstanz.de/ccp4wiki/index.php/SHELX\\_C/D/E](http://strucbio.biologie.uni-konstanz.de/ccp4wiki/index.php/SHELX_C/D/E)



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## PDB references:

elastase, 2blo, r2blosf

2blq, r2blosf

insulin, 2bn3, r2bn3sf

2bnl, r2bnlsf

lysozyme, 2blx, r2blxsf

2bly, r2blysf

ribonuclease A, 2blp,

## Improving radiation-damage substructures for RIP

Max H. Nanao,<sup>a</sup> George M. Sheldrick<sup>b</sup> and Raimond B. G. Ravelli<sup>a\*</sup>

<sup>a</sup>EMBL, 6 Rue Jules Horowitz, 38042 Grenoble, France, and <sup>b</sup>Georg-August Universität, Tammannstrasse 4, D-37077 Göttingen, Germany

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Specific radiation damage can be used to solve macromolecular structures using the radiation-damage-induced phasing (RIP) method. The method has been investigated for six disulfide-containing test structures (elastase, insulin, lysozyme, ribonuclease A, trypsin and thaumatin) using data sets that were collected on a third-generation synchrotron undulator beamline with a highly attenuated beam. Each crystal was exposed to the unattenuated X-ray beam between the collection of a 'before' and an 'after' data set. The X-ray 'burn'-induced intensity differences ranged from 5 to 15%, depending on the protein investigated. X-ray-susceptible substructures were determined using the integrated direct and Patterson methods in *SHELXD*. The best substructures were found by downscaling the 'after' data set in *SHELXC* by a scale factor *K*, with optimal values ranging from 0.96 to 0.99. The initial substructures were improved through iteration with *SHELXE* by the addition of negatively occupied sites as well as a large number of relatively weak sites. The final substructures ranged from 40 to more than 300 sites, with strongest peaks as high as 57 $\sigma$ . All structures except one could be solved: it was not possible to find the initial substructure for ribonuclease A, however, *SHELXE* iteration starting with the known five most susceptible sites gave excellent maps. Downscaling proved to be necessary for the solution of elastase, lysozyme and thaumatin and reduced the number of *SHELXE* iterations in the other cases. The combination of downscaling and substructure iteration provides important benefits for the phasing of macromolecular structures using radiation damage.

**Keywords:** radiation damage; synchrotron radiation; SAD; MAD; SIR; MIR; RIP; RIPAS.

“The best substructure were found by downscaling the ‘after’ data set in SHELXC...”



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## Phasing Macromolecular Structures with UV-Induced Structural Changes

Max H. Nanao<sup>1,2</sup> and Raimond B.G. Ravelli<sup>1,2,\*</sup>

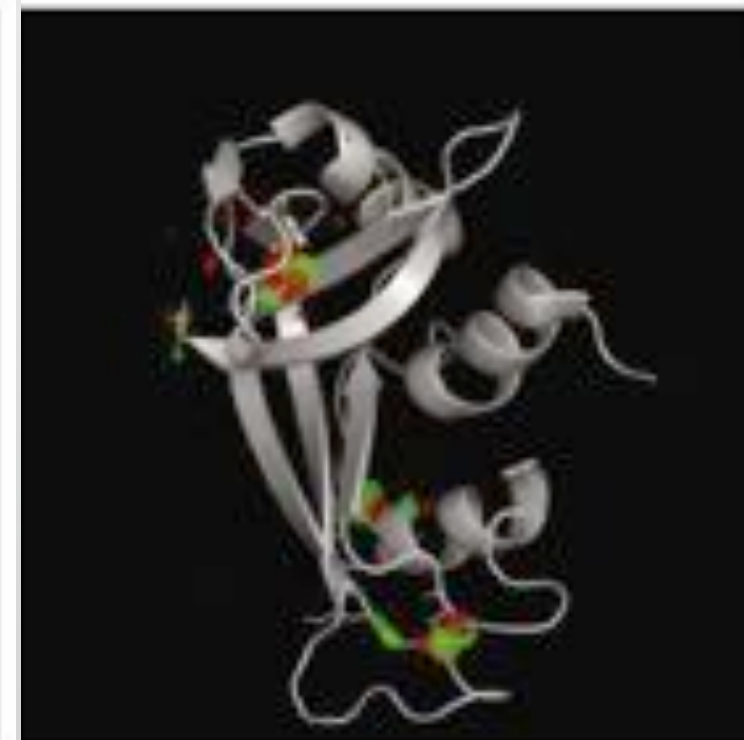
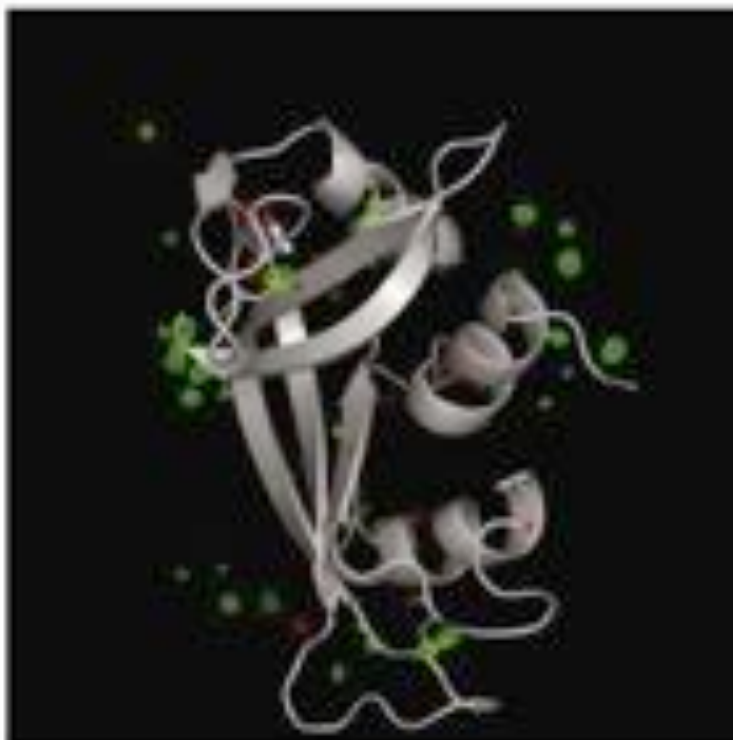
<sup>1</sup> European Molecular Biology Laboratory (EMBL)  
Grenoble Outstation  
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B.P. 181  
38042 Grenoble Cedex 9  
France

### Summary

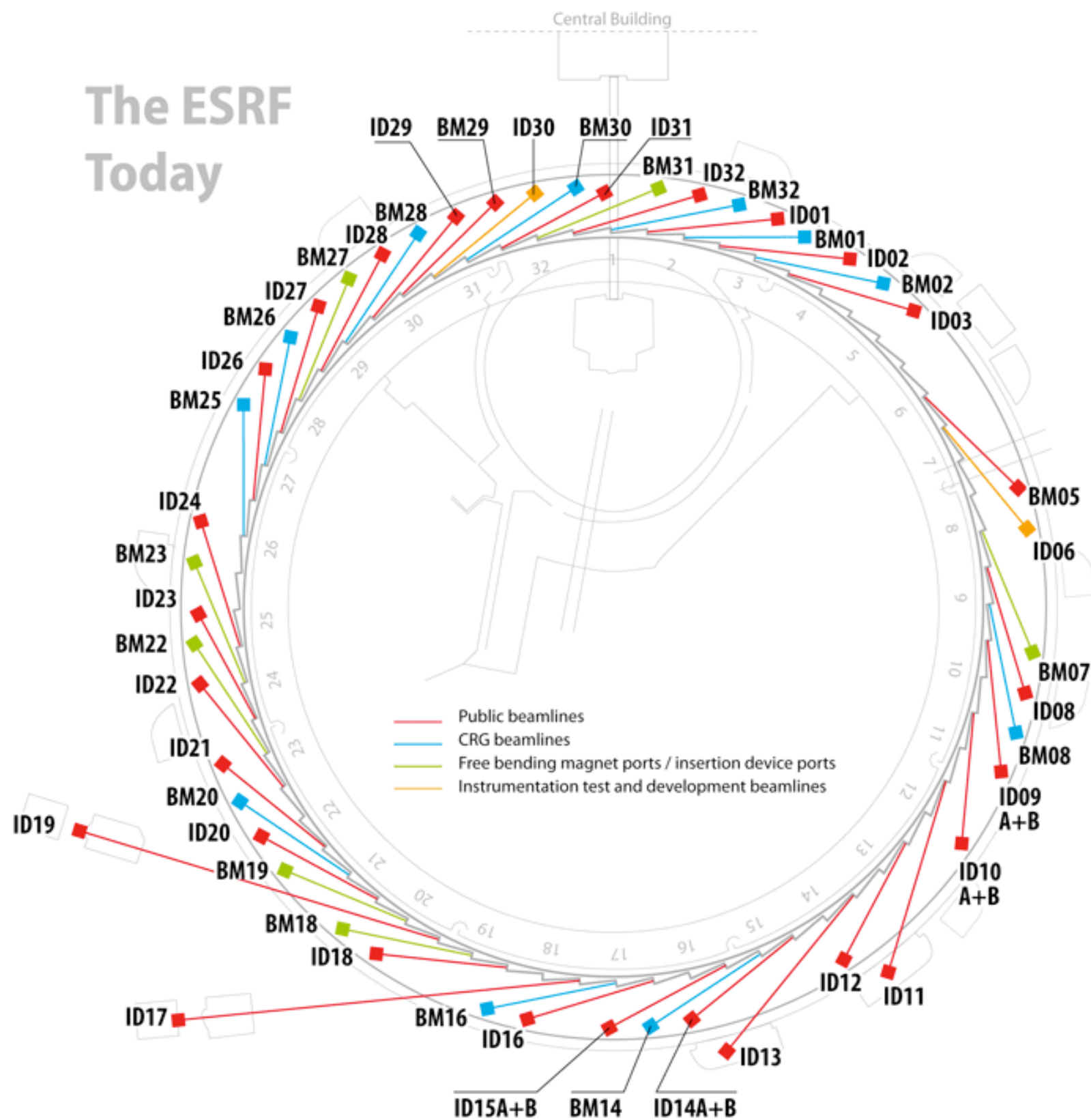
Experimental phasing of macromolecular crystal structures relies on the accurate measurement of two or more sets of reflections from isomorphous crystals, where the scattering power of a few atoms is different for each set. Recently, it was demonstrated that X-ray-induced intensity differences can also contain phasing information, exploiting specific structural changes characteristic of X-ray damage. This method (radiation damage-induced phasing; RIDP) has the advantage that

using macromolecular crystallography; such a method would save on labor, synchrotron time, toxic heavy atom solutions, and costs. Interesting concepts such as brute force molecular replacement, iterated projections (Elser, 2003), free energy minimization (Castleden, 1992; Scheres and Gros, 2004), and three-beam X-ray diffraction (Weckert and Hummer, 1997) remain in rather early stages of development. In contrast, the use of weak anomalous scatterers within native proteins, sometimes enhanced by naturally bound heavy atoms, holds great promise as a generally useful method. Small anomalous differences for sulfur and phosphorus can be accurately

measured with a weak beam, and phosphorus is a common element in nucleic acids and phospholipids. The use of these elements as anomalous scatterers presents a challenge

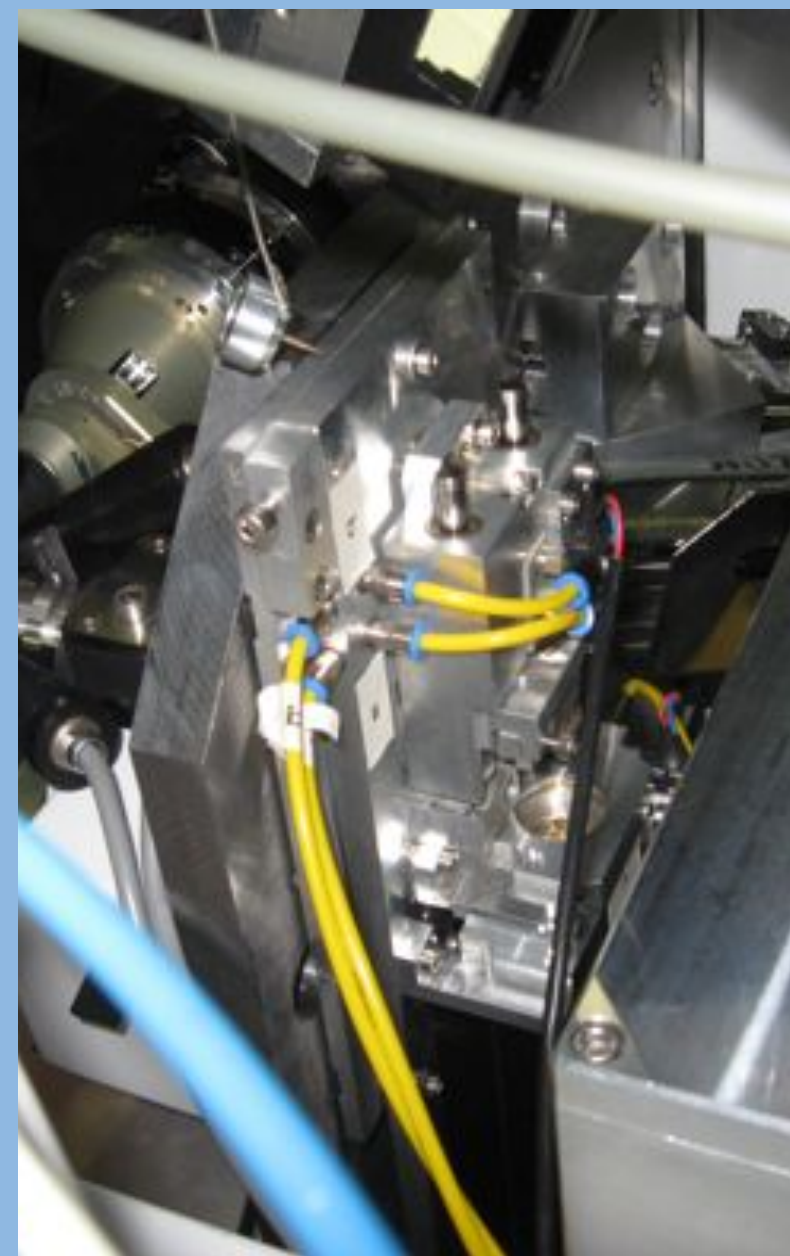
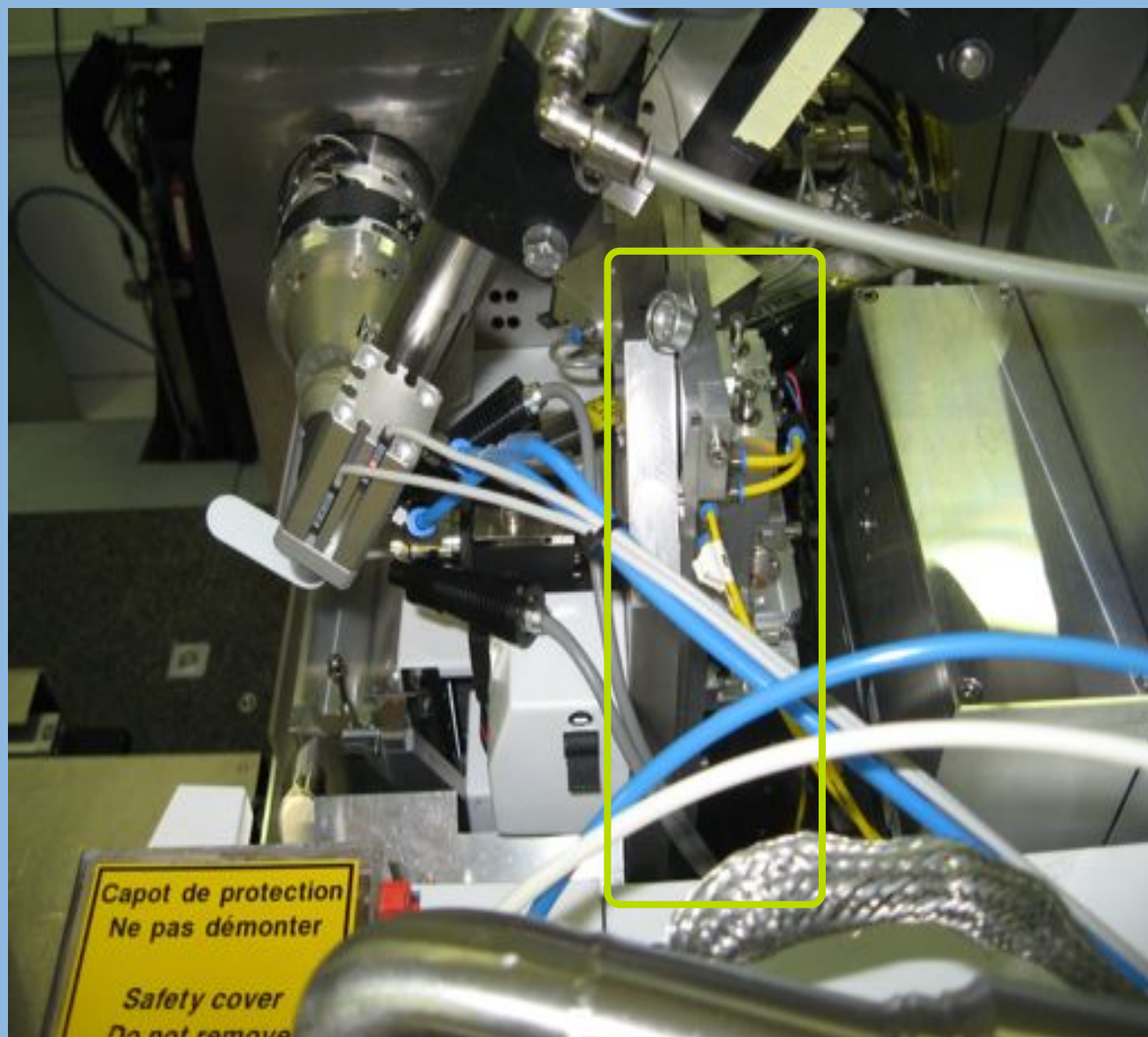


## The ESRF Today

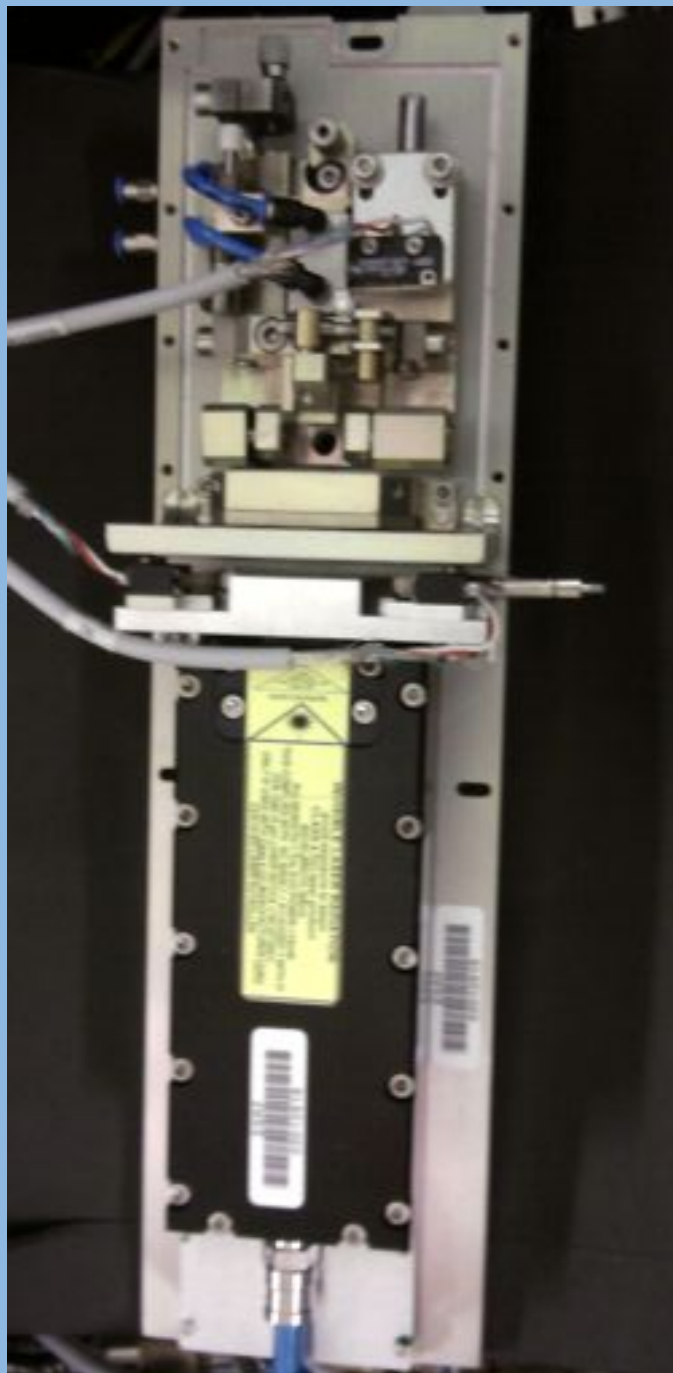




A UV laser is already installed on ID23EH1 since the early days...



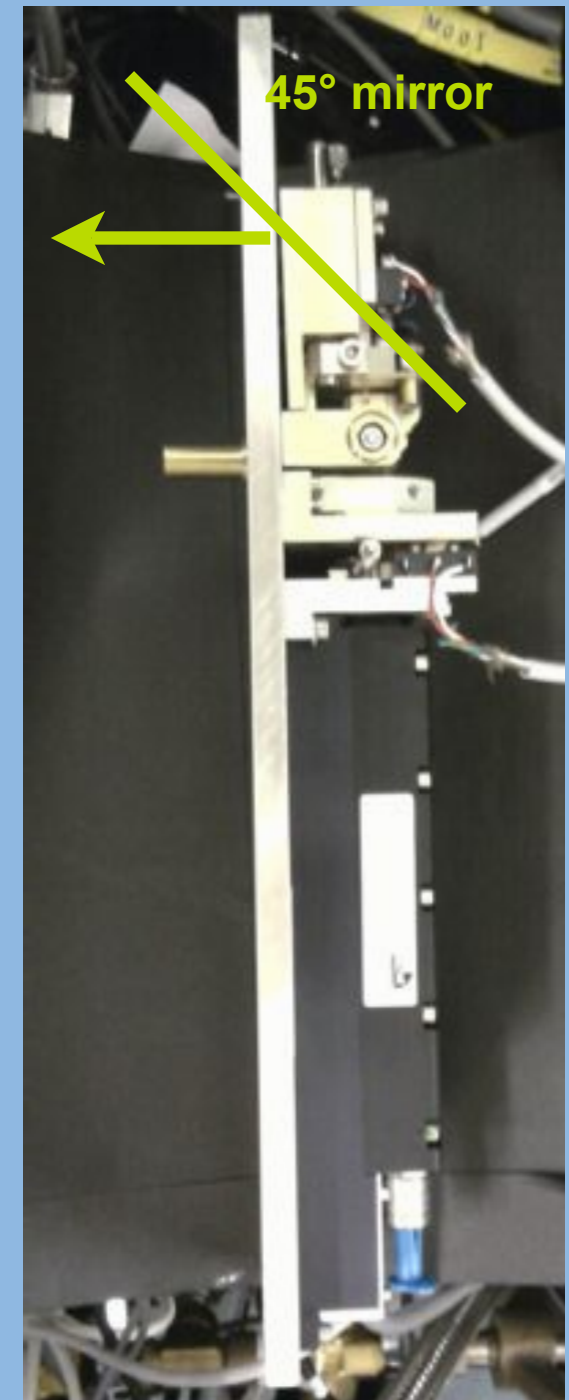


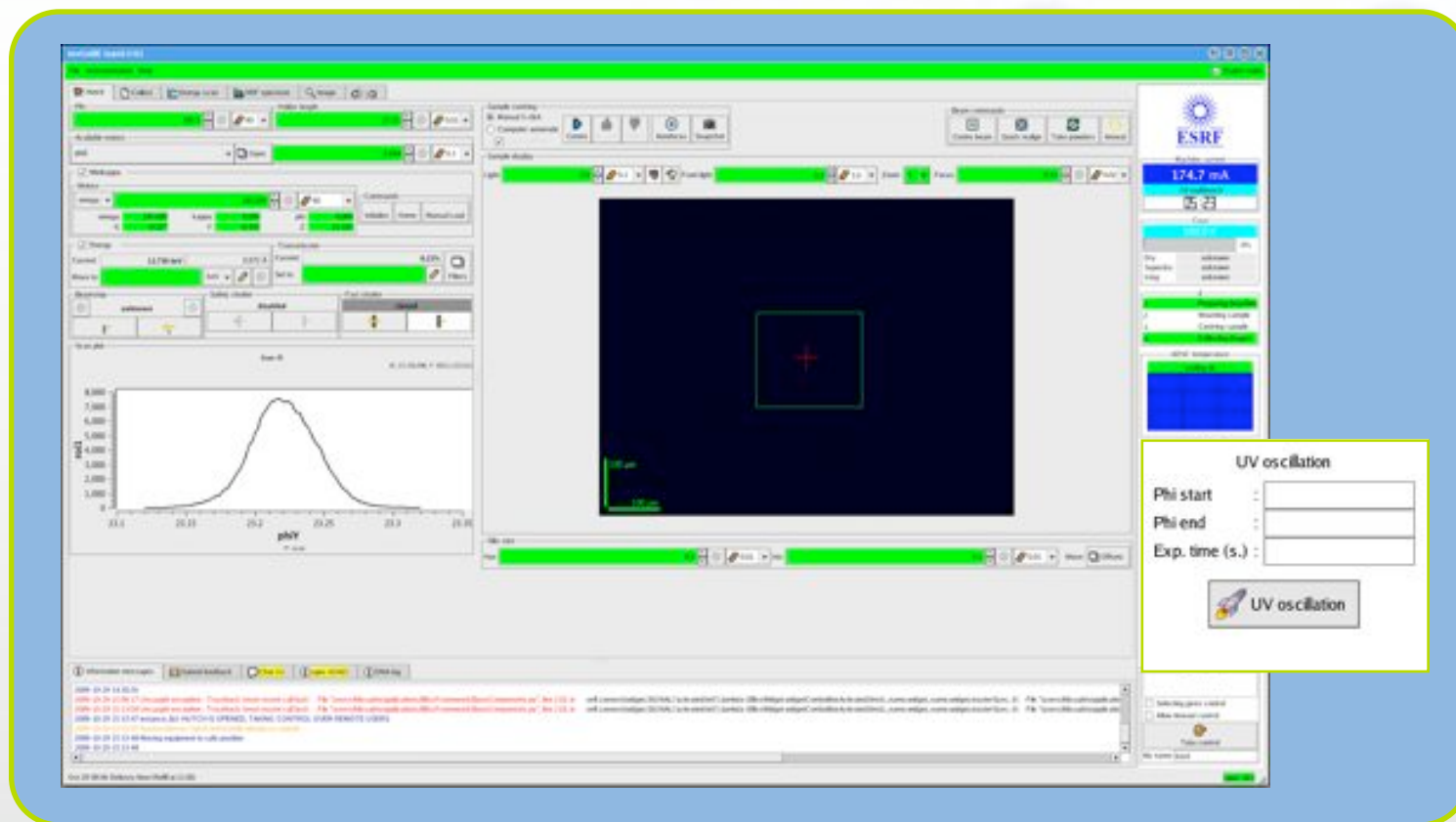


Teem photonics laser

Wavelength (nm)	266
Peak Power (kW)	1.4
Average Power (mW)	5
Repetition rate (kHz)	7
Pulse Width (ps)	400
Energy/Pulse (J)	0.7

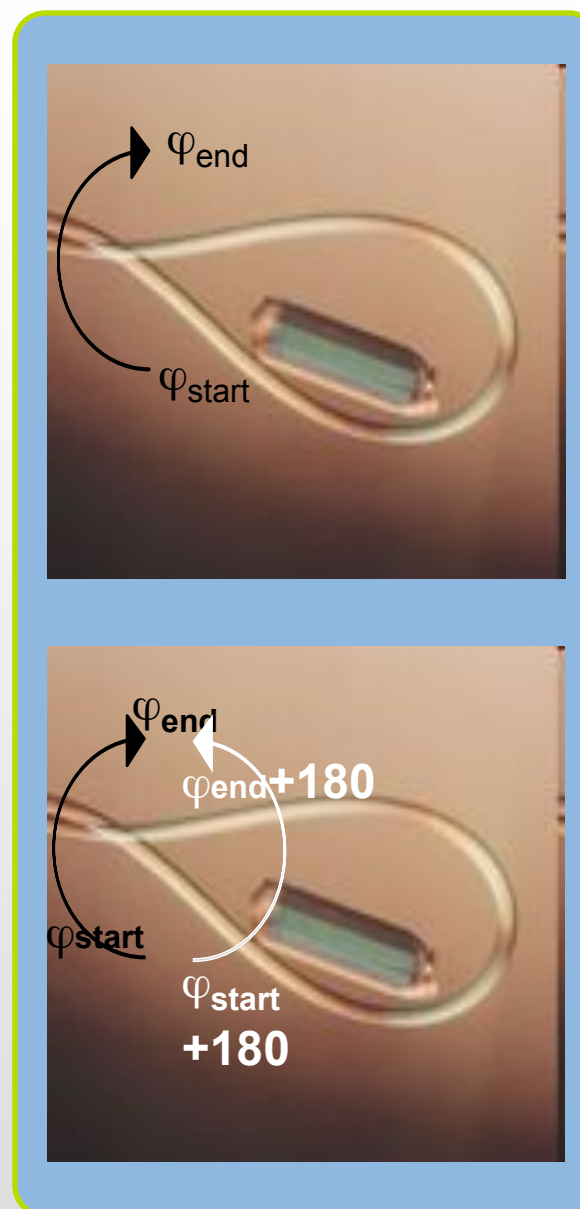
Average Power (measured at sample position ~1.6 mW)



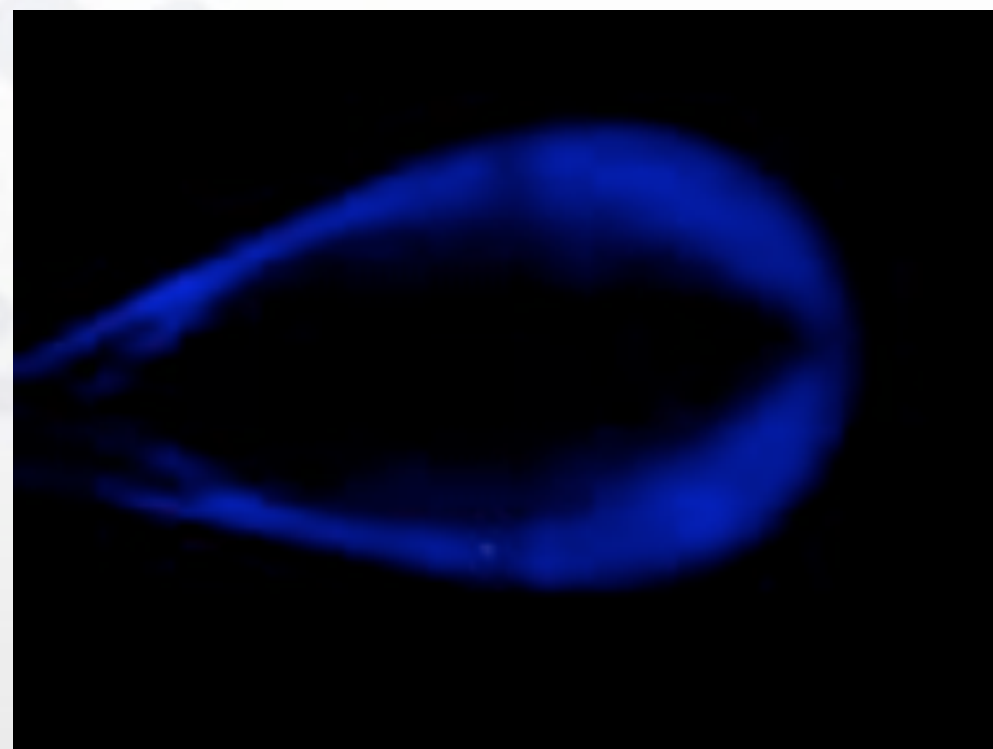


UV exposure is controlled through mxCuBE  
 No special intervention is needed by the user  
 (except usual type&click)  
 Needed informations are exposing  $\Delta\phi$  and time

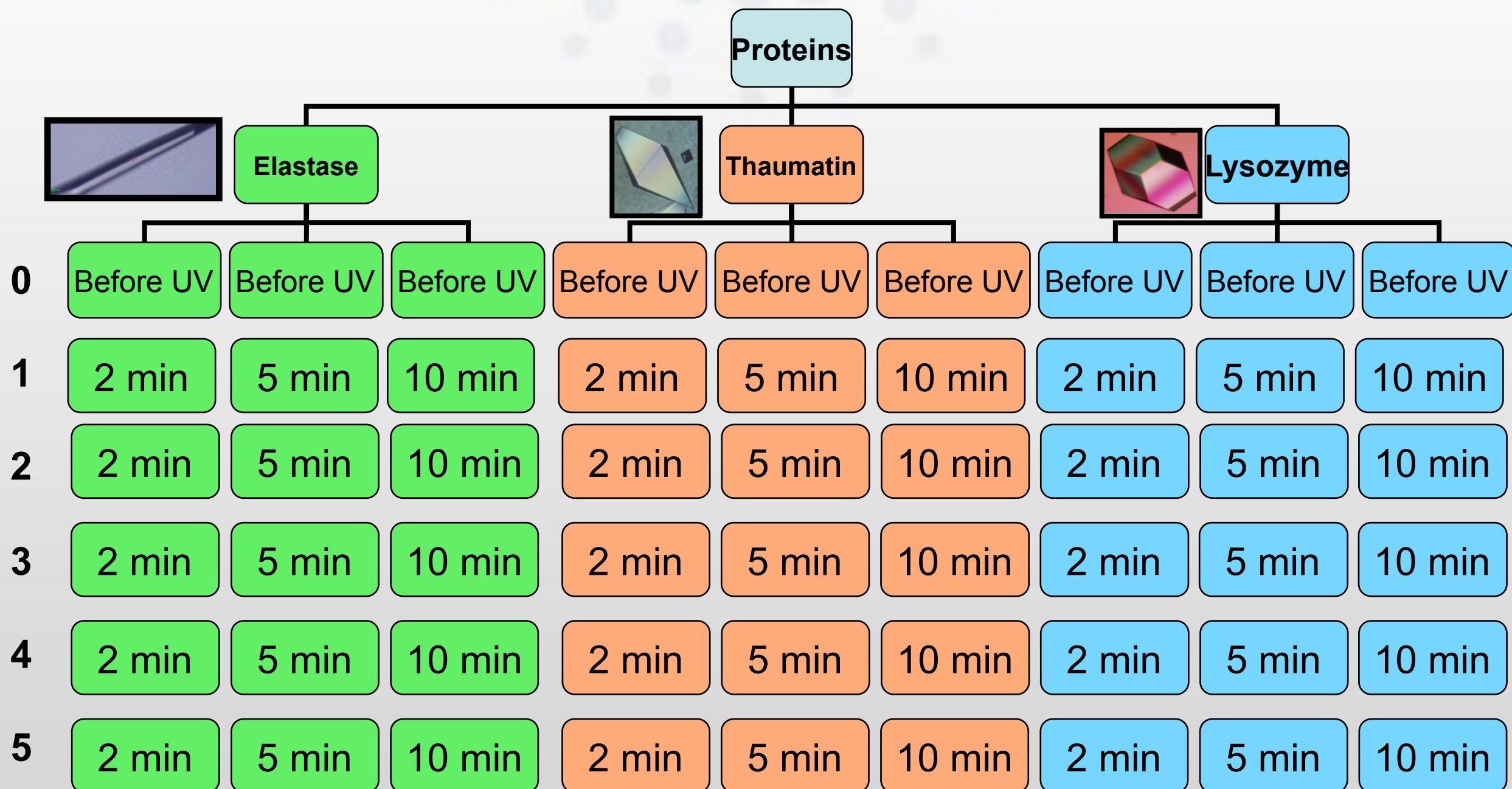
At the beginning one oscillation was performed  
 between chosen values  
 Now the two side of the crystal are exposed to  
 maximize exposed volume

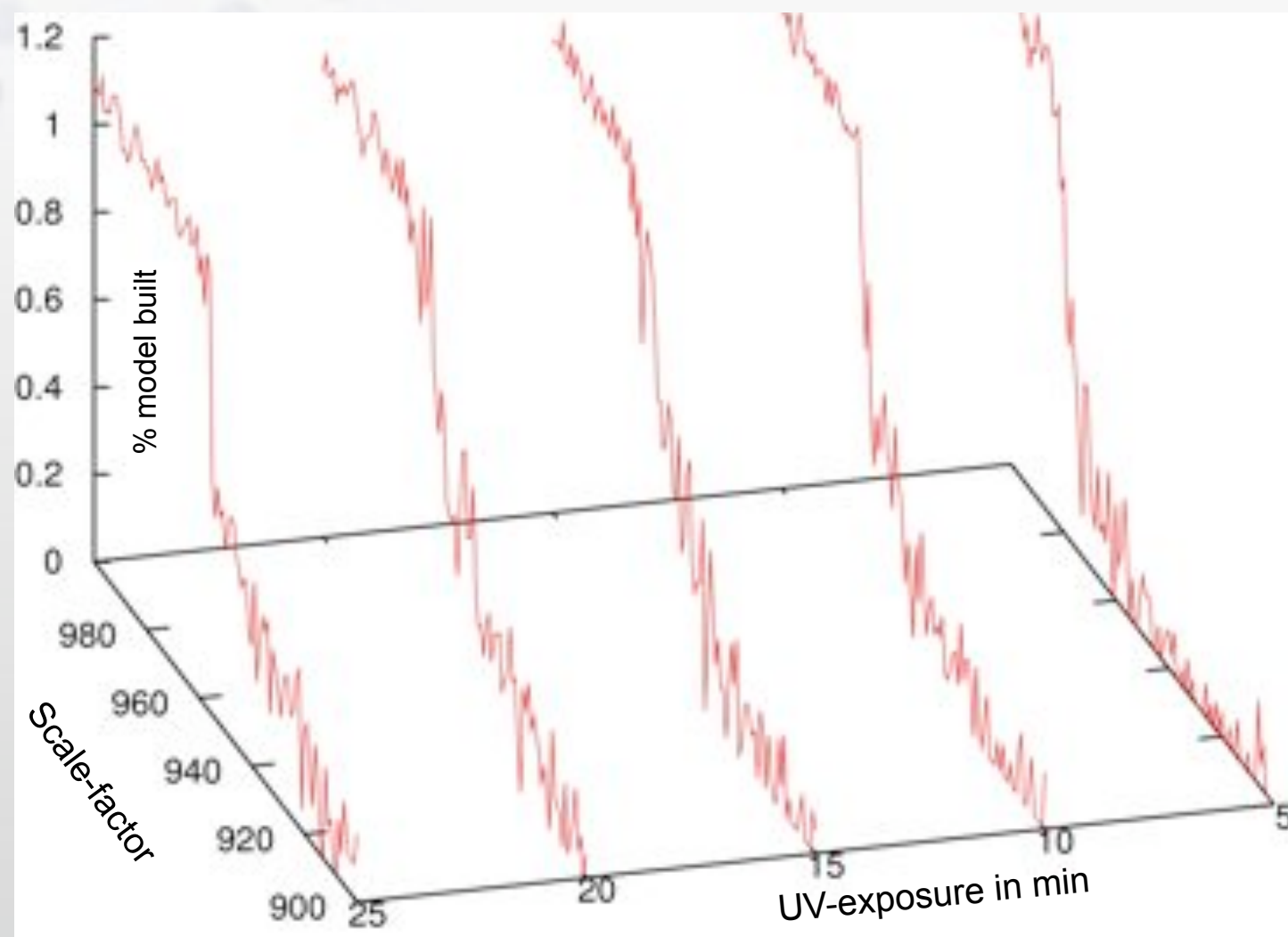
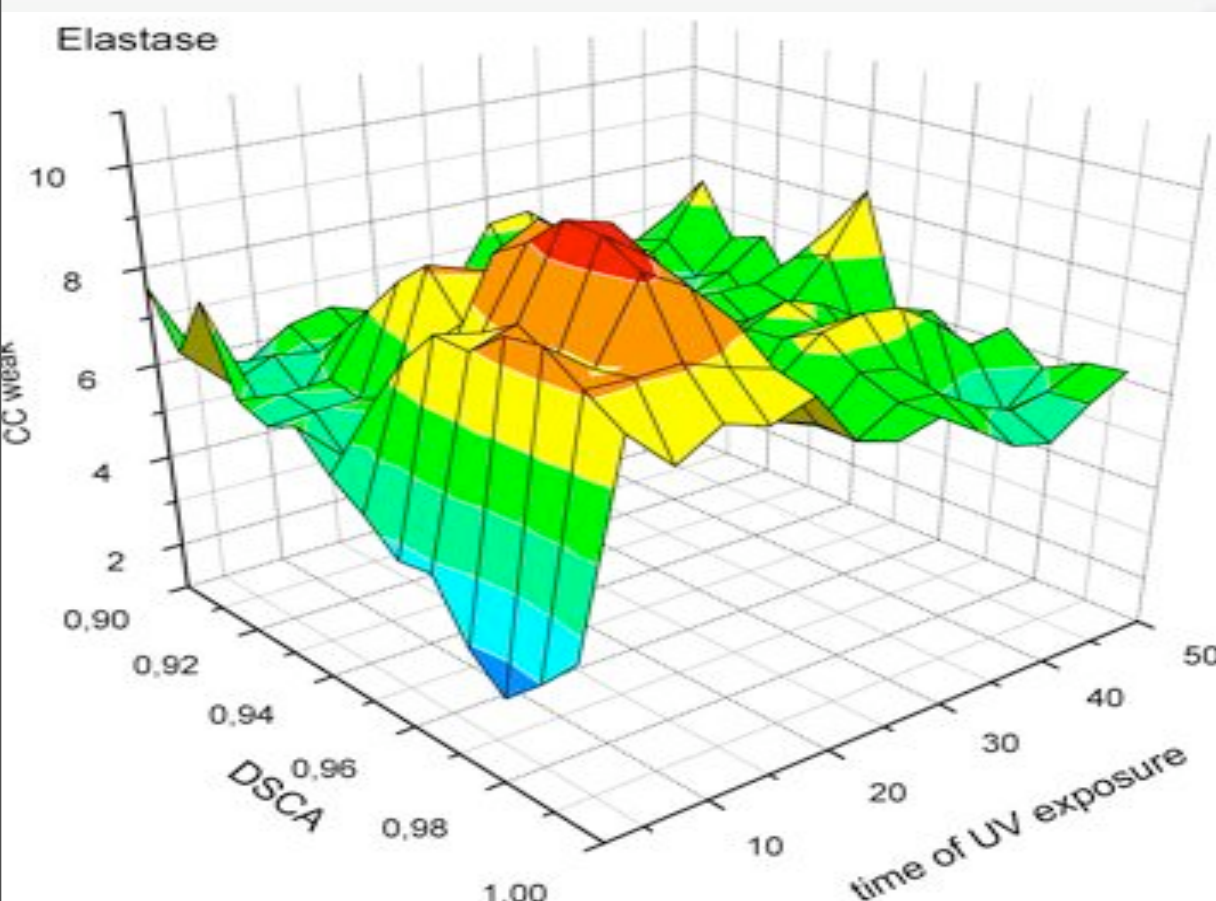




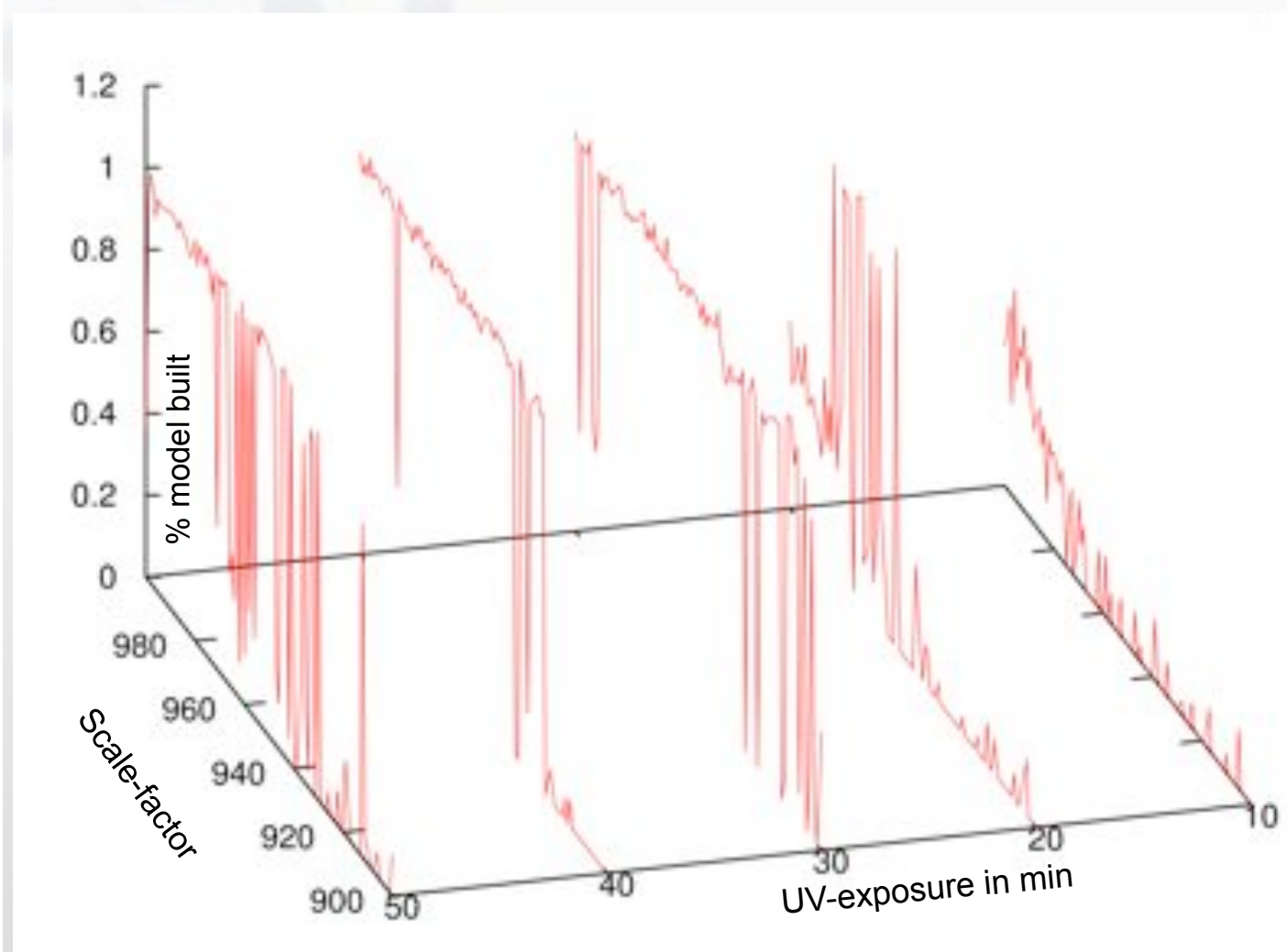
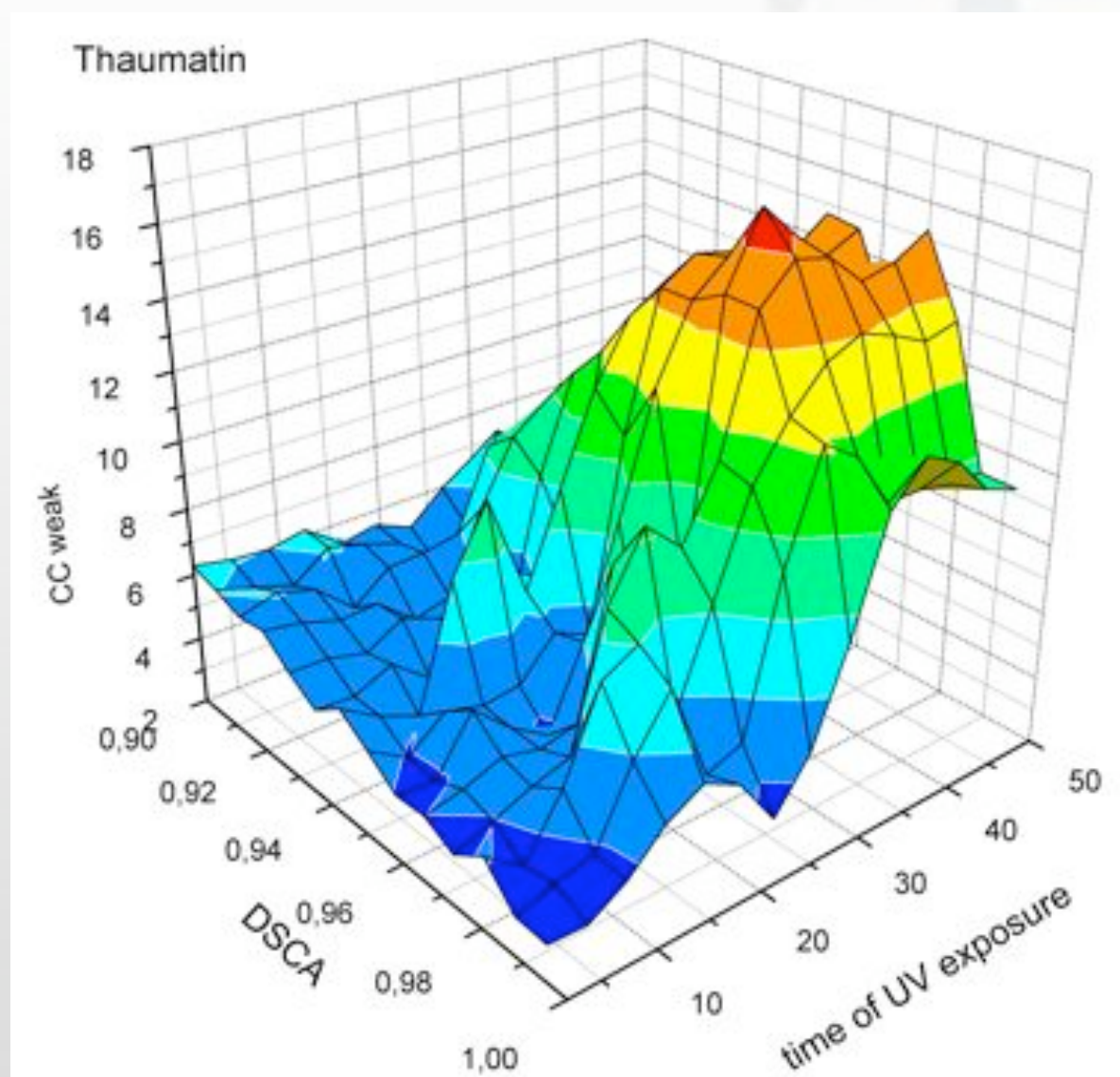


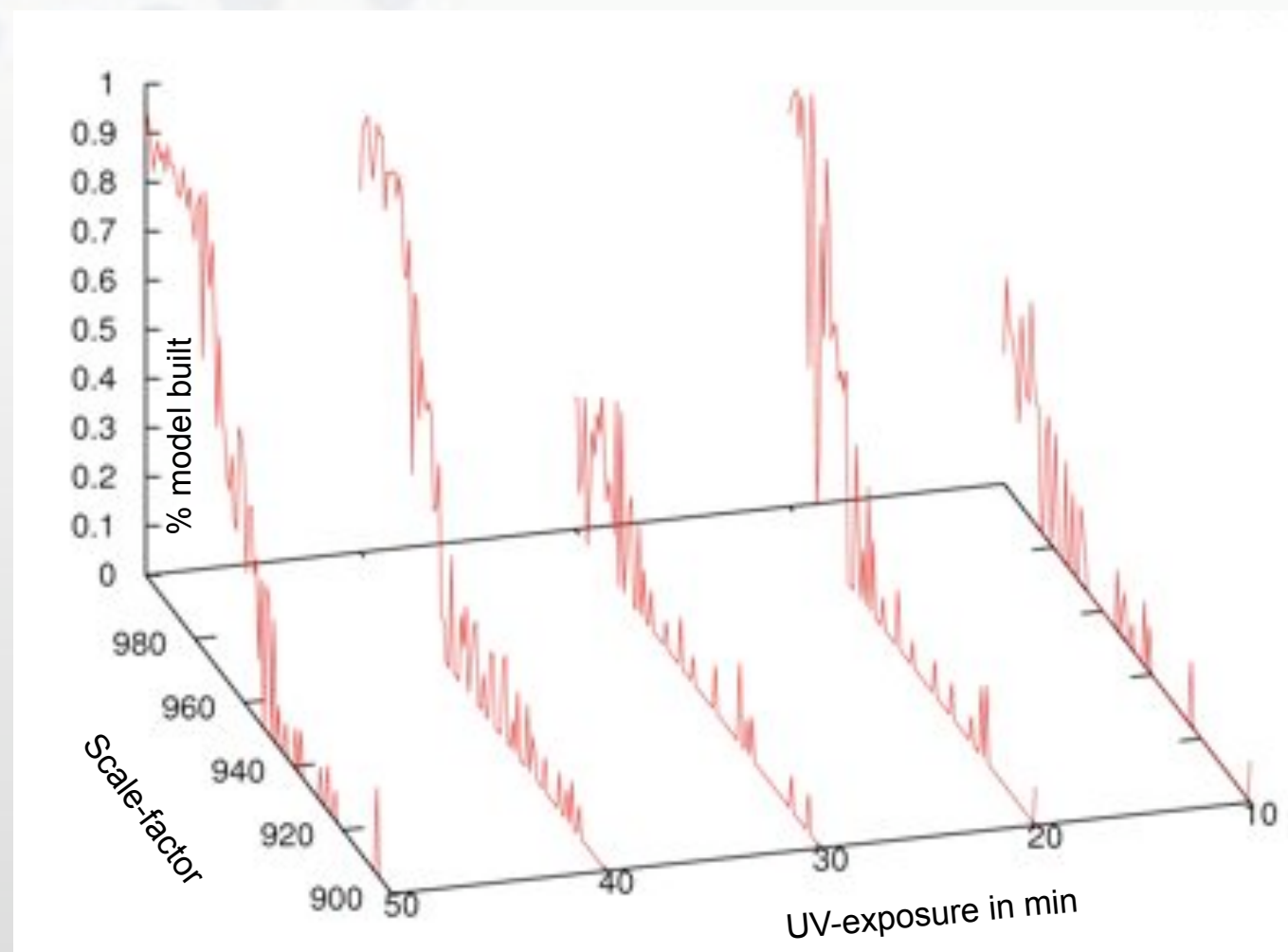
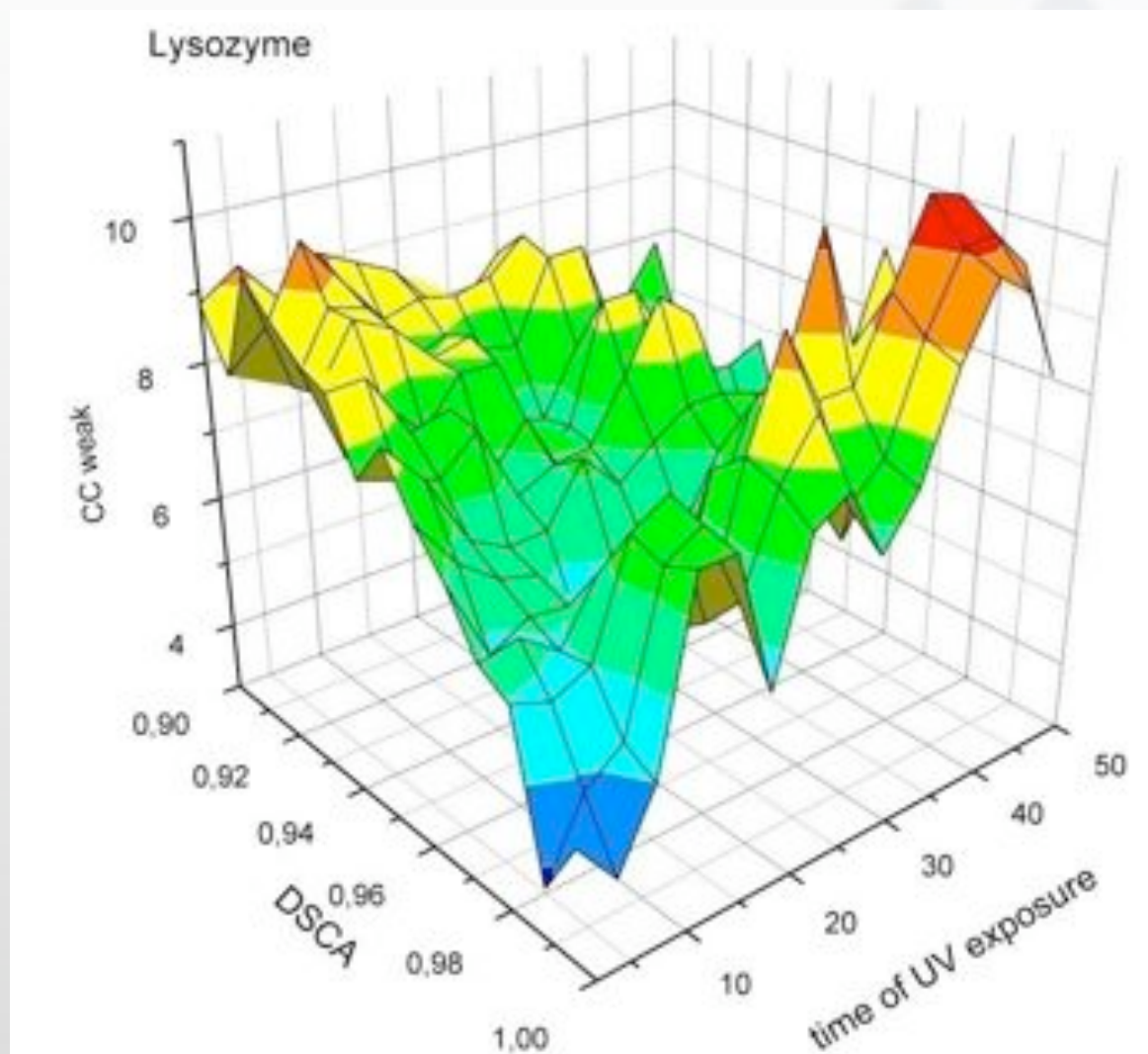
As the experimental setup was not designed for this kind of experiment, we made some tests to verify its performance







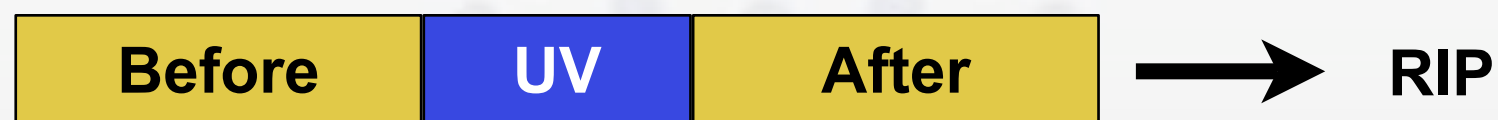




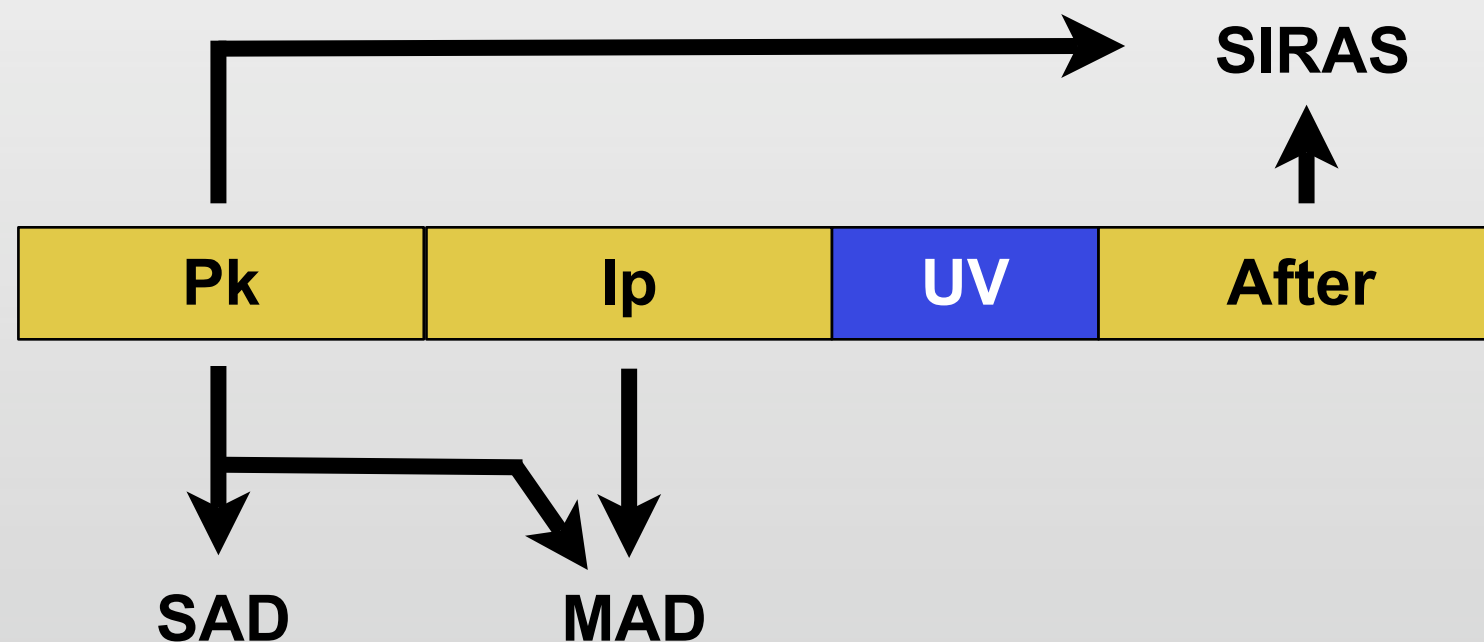
- All these test cases have disulphide bridges
- This commercial protein crystals are too good to really prove the technique
- Some minor UV damage was observed by Nanao&Ravelli on Methionine on PYP protein
- So we decide to play with Selenium labelled proteins....

For all cases UV exposure was 50 mins around collecting rotation

1. RIP **before** and **after** datasets were collected @ 12 KeV



2. SAD/MAD A **pk** and **ip** datasets were collected before irradiation  
SIRAS was done using **pk** and an **after** data from the same crystal



All data sets were processed with XDS and all structures were solved using Autorickshaw



FERULOYL ESTERASE DOMAIN OF XYNV  
FROM CLOSTRIDIUM THERMOCELLUM  
1GKK

300 resi - 8 Selenium labelled methionine + 5 Cd ions  
Space group P212121  
2 mol/au  
58% solvent



## FAE Before

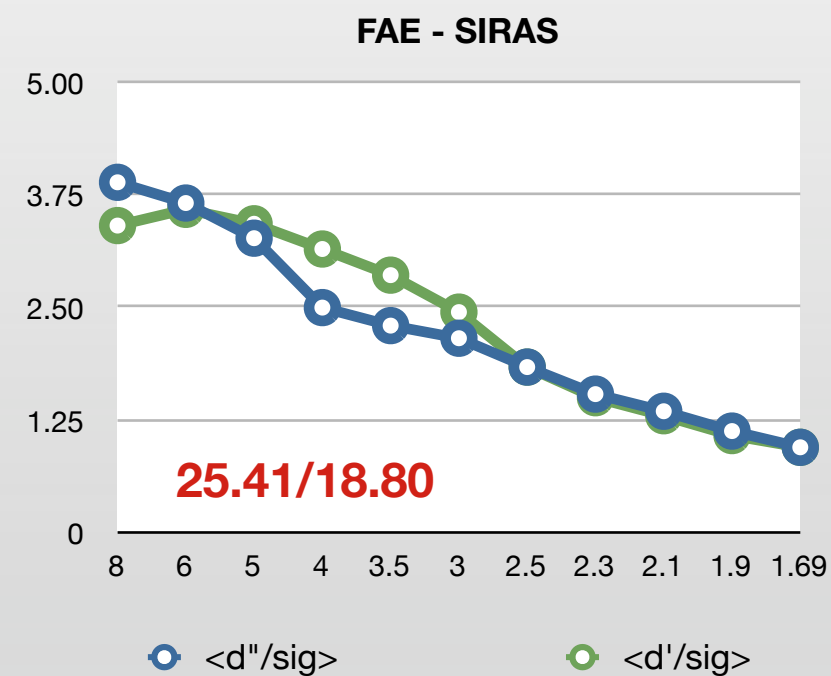
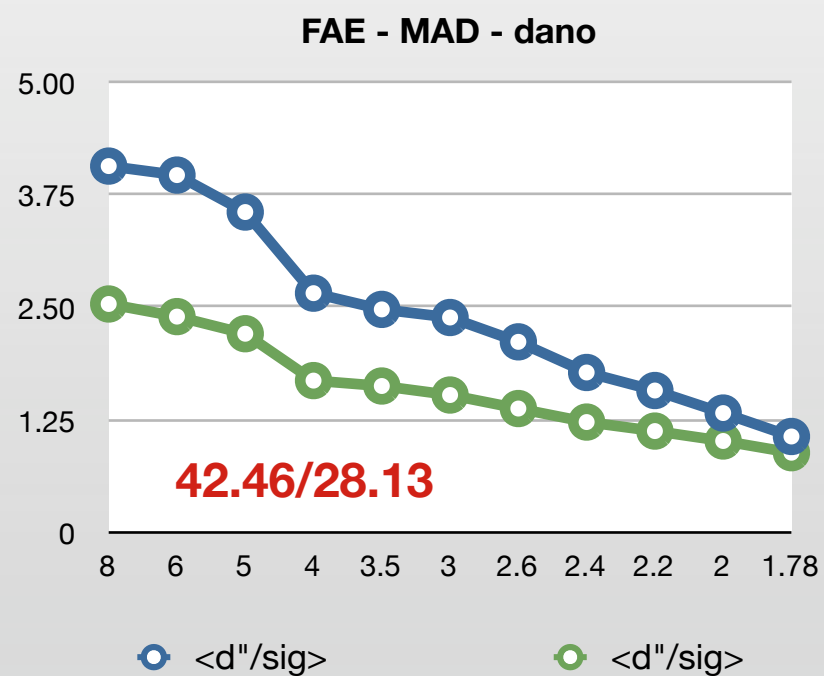
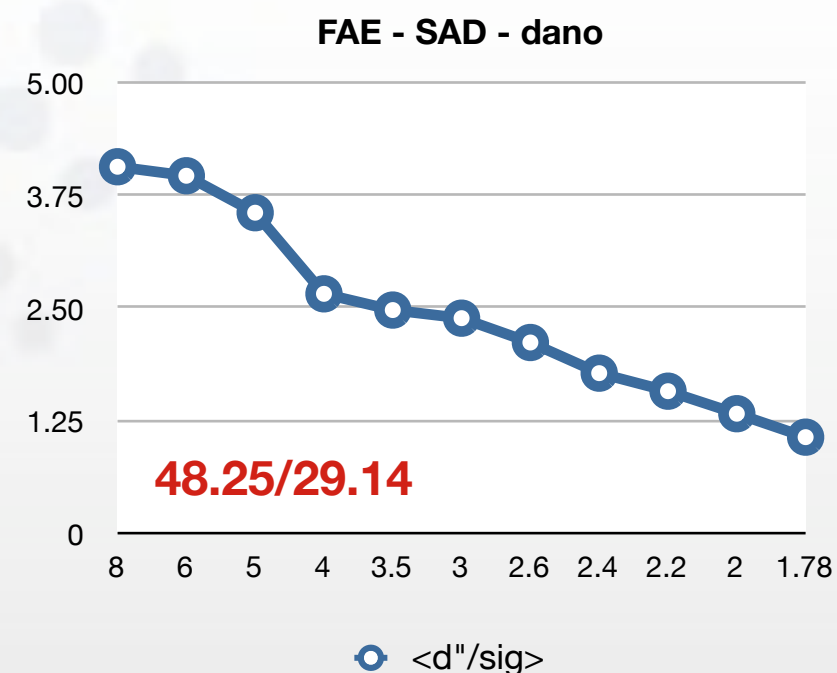
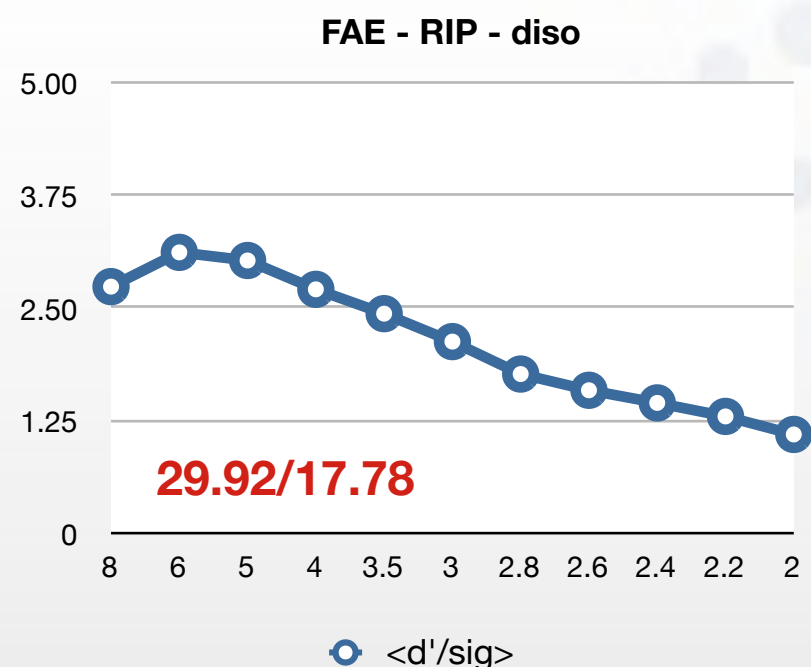
SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE  $\geq -3.0$  AS FUNCTION OF RESOLUTION

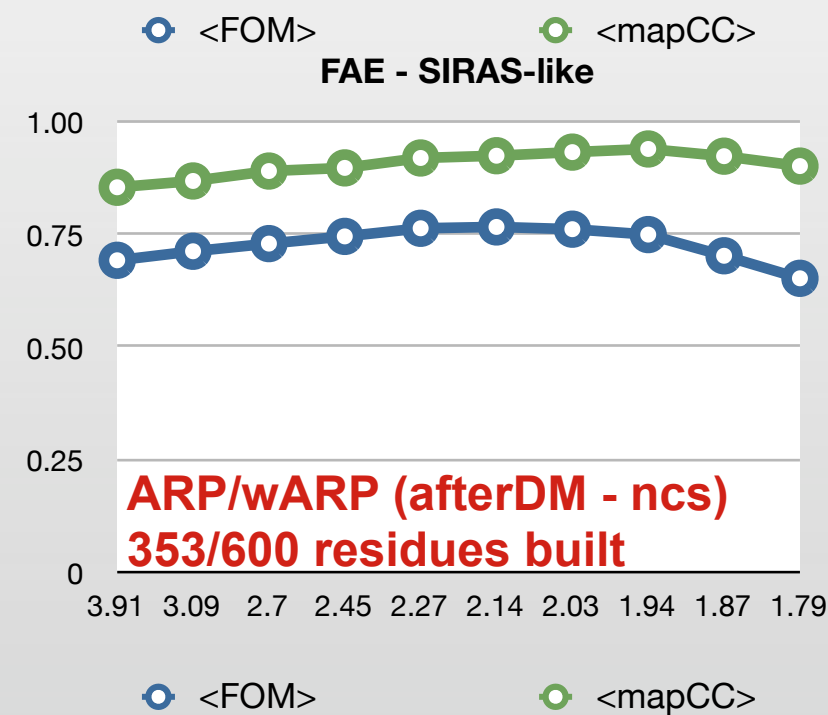
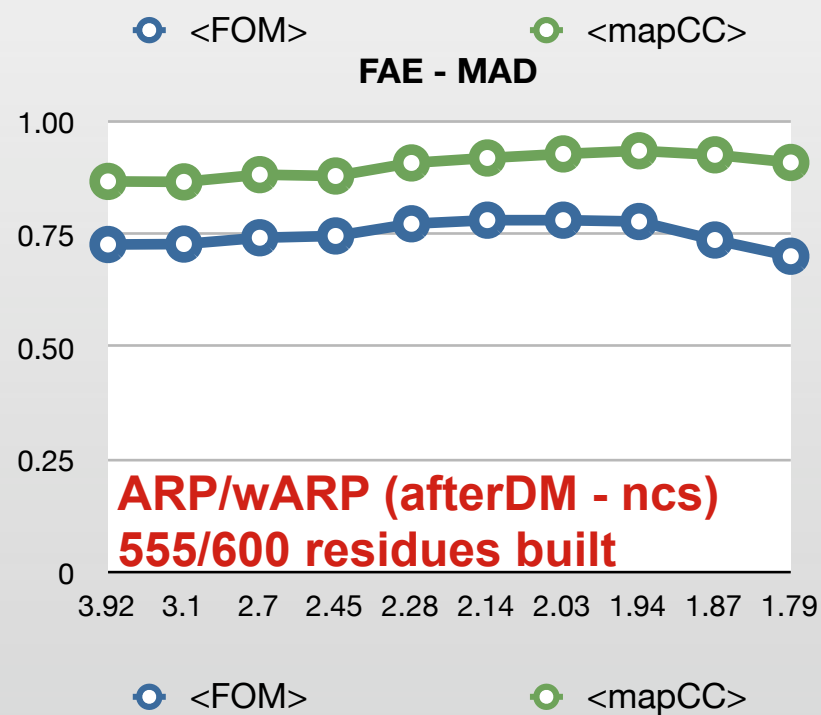
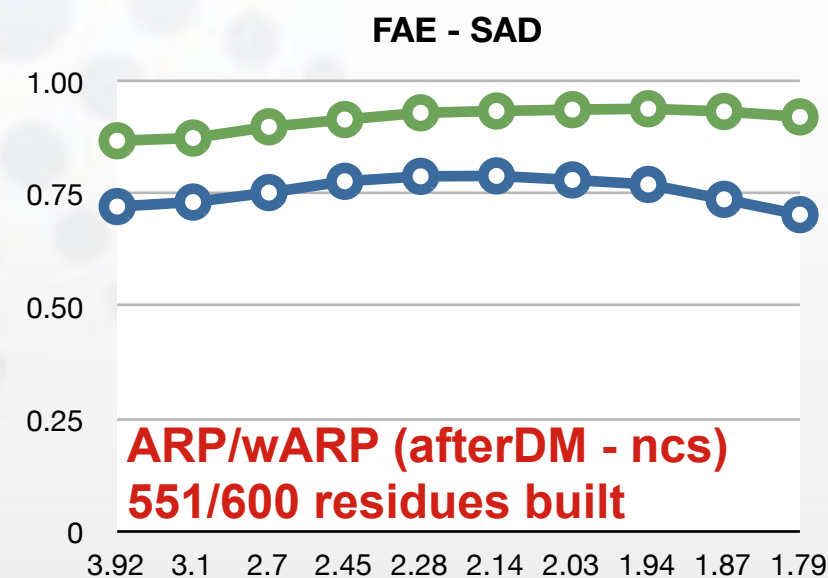
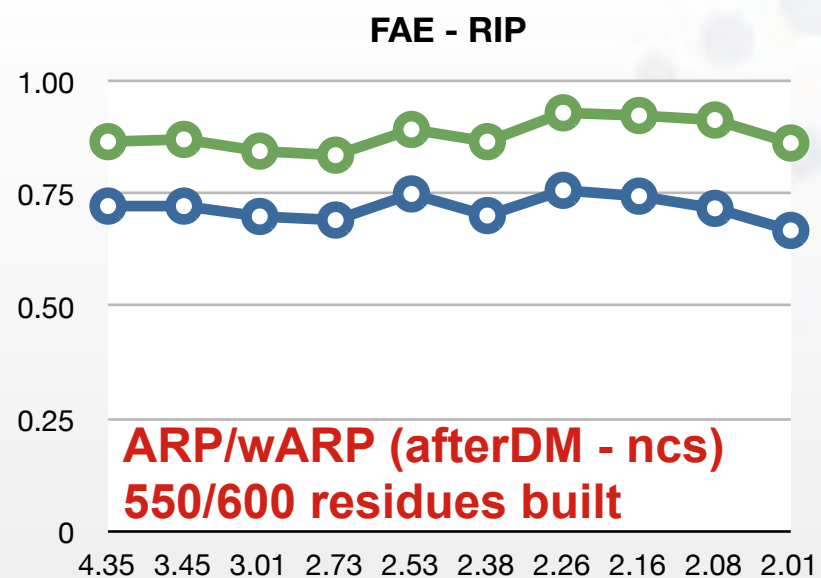
RESOLUTION LIMIT	NUMBER OF REFLECTIONS			COMPLETENESS		R-FACTOR	R-FACTOR	COMPARED	I/SIGMA	R-meas	Rmrgd-F	Anomal Corr	SigAno	Nano
	OBSERVED	UNIQUE	POSSIBLE	OF DATA	observed expected									
5.08	13502	3568	3568	3603	99.0%	3.2%	3.3%	13445	34.57	3.7%	2.4%	36%	1.084	2349
3.60	23948	6143	6143	6164	99.7%	3.4%	3.4%	23839	34.43	3.9%	2.5%	19%	0.984	4020
2.94	31137	7833	7833	7858	99.7%	4.3%	4.0%	31031	28.06	5.0%	3.5%	11%	0.956	5088
2.55	37304	9250	9250	9261	99.9%	5.8%	5.5%	37183	21.25	6.7%	5.2%	10%	0.908	6065
2.28	42097	10377	10377	10397	99.8%	7.0%	6.8%	41990	17.80	8.1%	6.5%	6%	0.869	6729
2.08	46382	11417	11417	11438	99.8%	8.6%	8.6%	46282	14.53	9.9%	8.6%	6%	0.838	7257
1.93	50313	12399	12399	12413	99.9%	11.8%	12.3%	50188	10.71	13.6%	12.4%	4%	0.795	7701
1.81	53353	13270	13270	13289	99.9%	17.1%	18.2%	53151	7.52	19.7%	18.2%	0%	0.761	7937
1.70	55821	14099	14099	14192	99.3%	26.1%	28.9%	55446	4.85	30.2%	28.9%	1%	0.725	8011
total	353857	88356	88356	88615	99.7%	6.7%	6.7%	352555	15.88	7.7%	8.7%	7%	0.848	55157

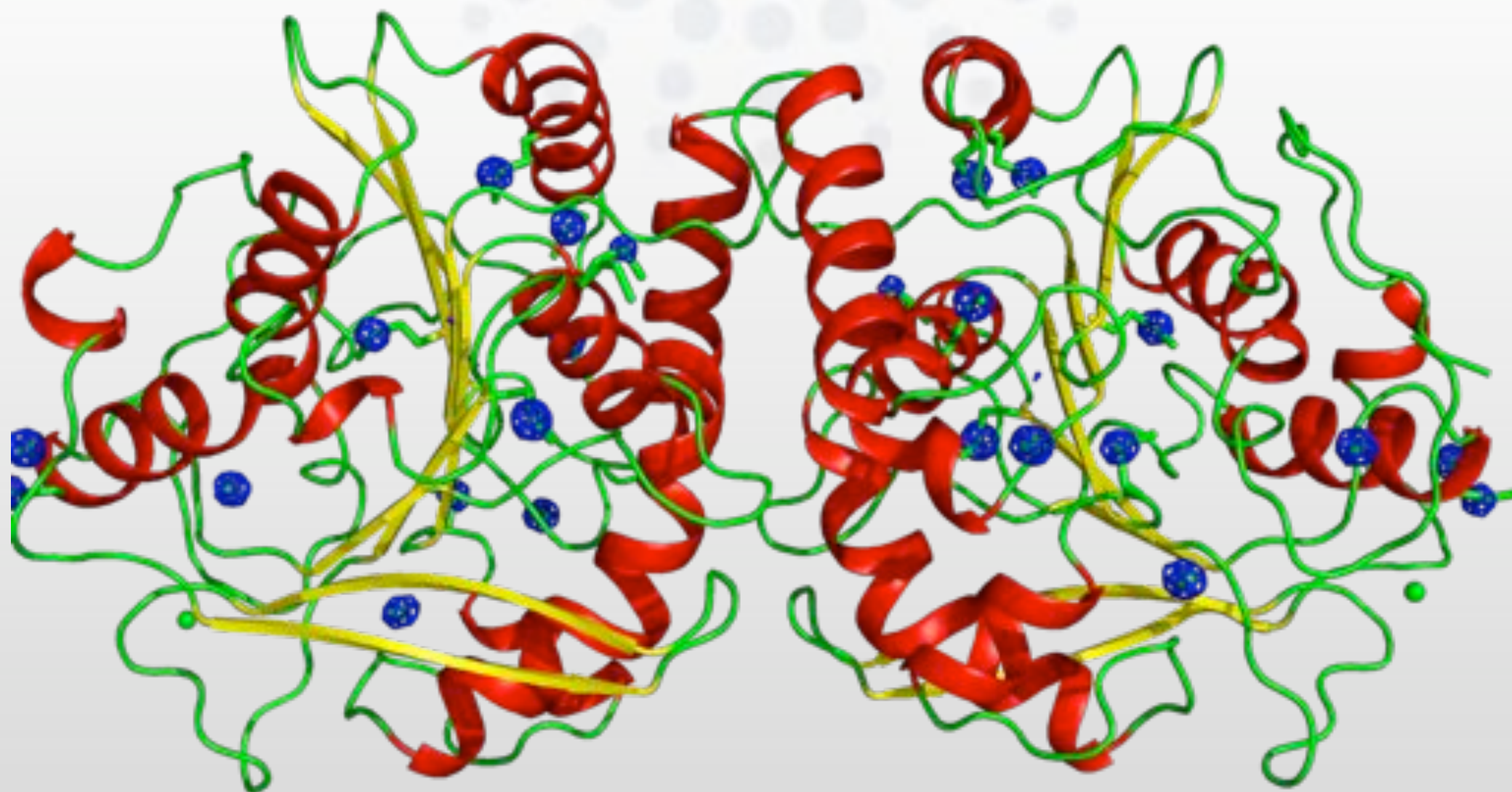
## FAE After 50 mins exposure


SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE  $\geq -3.0$  AS FUNCTION OF RESOLUTION

RESOLUTION LIMIT	NUMBER OF REFLECTIONS			COMPLETENESS		R-FACTOR	R-FACTOR	COMPARED	I/SIGMA	R-meas	Rmrgd-F	Anomal Corr	SigAno	Nano
	OBSERVED	UNIQUE	POSSIBLE	OF DATA	observed expected									
5.09	13482	3564	3564	3598	99.1%	3.3%	3.4%	13423	33.46	3.9%	2.5%	34%	1.059	2362
3.61	23851	6127	6127	6155	99.5%	3.5%	3.5%	23741	32.84	4.1%	2.6%	15%	0.971	3978
2.95	31202	7813	7813	7837	99.7%	4.6%	4.3%	31099	26.62	5.3%	3.7%	12%	0.962	5158
2.55	37211	9213	9213	9223	99.9%	6.1%	5.9%	37090	20.05	7.1%	5.6%	9%	0.913	6097
2.28	42059	10356	10356	10370	99.9%	7.7%	7.5%	41948	16.33	8.9%	7.3%	5%	0.862	6740
2.09	46449	11420	11420	11432	99.9%	9.8%	9.9%	46342	12.96	11.3%	9.9%	6%	0.828	7312
1.93	50218	12377	12377	12395	99.9%	13.8%	14.4%	50089	9.35	15.9%	14.6%	4%	0.788	7694
1.81	53259	13233	13233	13253	99.8%	20.8%	22.5%	53062	6.24	24.0%	22.5%	-1%	0.744	7946
1.70	52098	13826	13826	14155	97.7%	34.1%	38.1%	51645	3.63	39.7%	40.6%	-3%	0.709	6885
total	349829	87929	87929	88418	99.4%	7.3%	7.4%	348439	14.54	8.4%	10.2%	6%	0.842	54172









**Mycobacterium tuberculosis Chorismate synthase**  
2011

**400 resi - 11 Selenium labelled methionine**  
**Space group P6422**  
**1 mol/au**  
**73% solvent**



## ChSynt Before

SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE  $\geq -3.0$  AS FUNCTION OF RESOLUTION

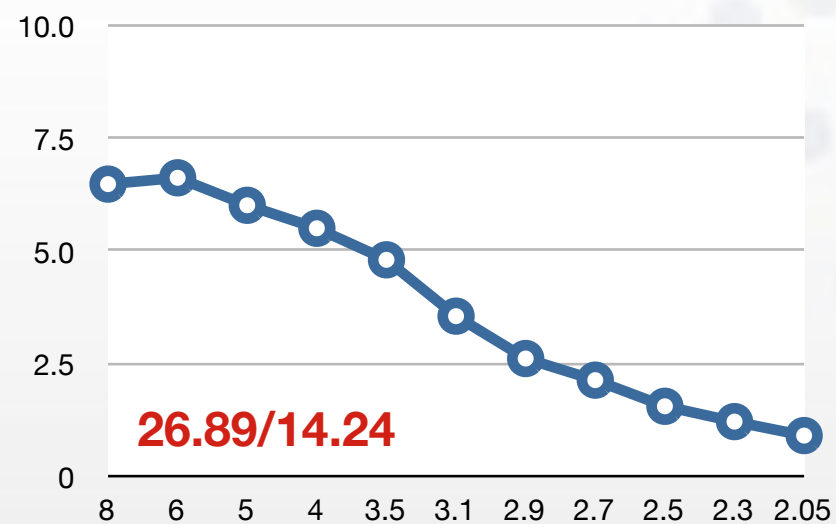
CROSS-SECTIONAL DATA WITH SIGNAL/NOISE RATIO AS FUNCTION OF RESOLUTION															
RESOLUTION	NUMBER OF REFLECTIONS			COMPLETENESS		R-FACTOR	R-FACTOR	COMPARED	I/SIGMA	R-meas	Rmrgd-F	Anomal	SigAno	Nano	
LIMIT	OBSERVED	UNIQUE	POSSIBLE	OF DATA	observed	expected						Corr			
6.11	16910	2168		2248	96.4%		3.7%	3.9%	16893	44.66	4.0%	2.0%	13%	0.891	1504
4.34	29590	3675		3722	98.7%		4.6%	4.4%	29553	39.62	4.9%	2.4%	6%	0.908	2975
3.55	38802	4668		4702	99.3%		5.0%	4.9%	38777	36.10	5.4%	2.7%	2%	0.856	3970
3.07	45891	5450		5480	99.5%		7.4%	7.3%	45884	25.01	7.8%	4.9%	0%	0.821	4749
2.75	52234	6166		6178	99.8%		12.8%	12.8%	52222	15.72	13.6%	8.7%	-1%	0.818	5462
2.51	57536	6804		6811	99.9%		22.9%	23.1%	57530	9.26	24.4%	16.3%	-1%	0.786	6090
2.32	61533	7345		7347	100.0%		35.6%	36.9%	61526	6.01	38.0%	25.9%	1%	0.768	6640
2.17	64796	7861		7862	100.0%		59.2%	62.0%	64790	3.64	63.1%	44.1%	1%	0.745	7152
2.05	43004	7902		8356	94.6%		86.9%	91.1%	42679	1.85	96.0%	87.0%	0%	0.715	5726
total	410296	52039		52706	98.7%		9.6%	9.8%	409854	15.27	10.3%	13.3%	1%	0.793	44268

## ChSynt After 50 mins exposure

SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE  $\geq -3.0$  AS FUNCTION OF RESOLUTION

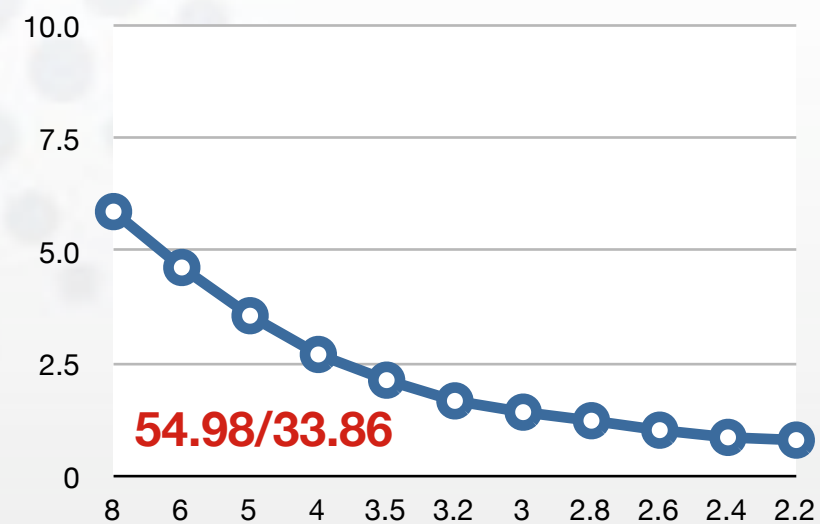
RESOLUTION	NUMBER OF REFLECTIONS			COMPLETENESS		R-FACTOR	R-FACTOR	COMPARED	I/SIGMA	R-meas	Rmrgd-F	Anomal	SigAno	Nano	
LIMIT	OBSERVED	UNIQUE	POSSIBLE	OF DATA	observed	expected						Corr			
6.11	16974	2177		2256	96.5%		4.1%	4.3%	16956	40.30	4.4%	2.2%	1%	0.848	1511
4.33	29596	3682		3728	98.8%		5.3%	5.0%	29558	34.71	5.6%	2.8%	1%	0.874	2980
3.54	38798	4675		4709	99.3%		6.0%	5.8%	38772	30.53	6.4%	3.4%	1%	0.871	3975
3.07	46004	5469		5498	99.5%		9.8%	9.6%	45996	19.66	10.4%	6.5%	-2%	0.802	4768
2.75	52332	6177		6190	99.8%		19.6%	19.5%	52320	10.96	20.8%	13.5%	-1%	0.796	5472
2.51	57629	6818		6826	99.9%		37.8%	38.3%	57624	5.89	40.3%	27.6%	1%	0.777	6109
2.32	61512	7352		7355	100.0%		61.5%	64.1%	61506	3.58	65.6%	43.9%	-2%	0.733	6643
2.17	64812	7882		7882	100.0%		109.2%	115.0%	64806	2.02	116.6%	80.5%	0%	0.705	7170
2.05	43070	7903		8376	94.4%		159.6%	169.3%	42745	0.99	176.2%	163.3%	1%	0.666	5746
total	410727	52135		52820	98.7%		12.7%	12.8%	410283	11.96	13.6%	19.7%	0%	0.767	44374

ChSynt - RIP - diso



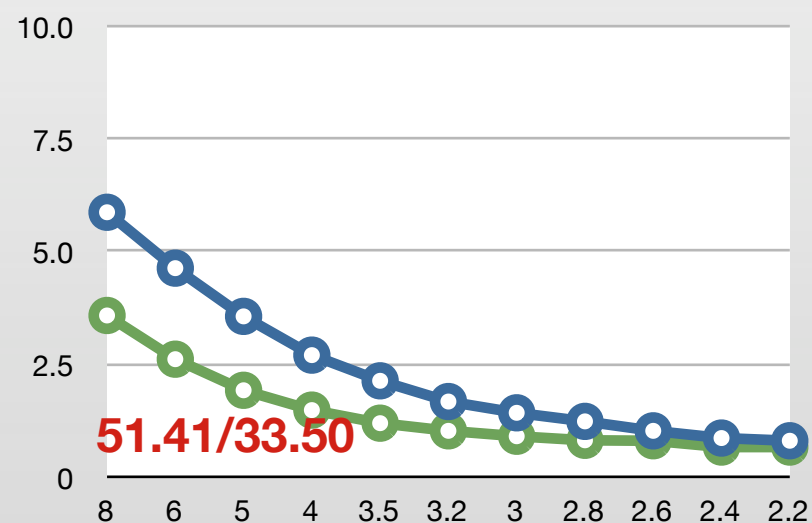
$\langle d'/\sigma \rangle$

ChSynt - SAD - dano



$\langle d''/\sigma \rangle$

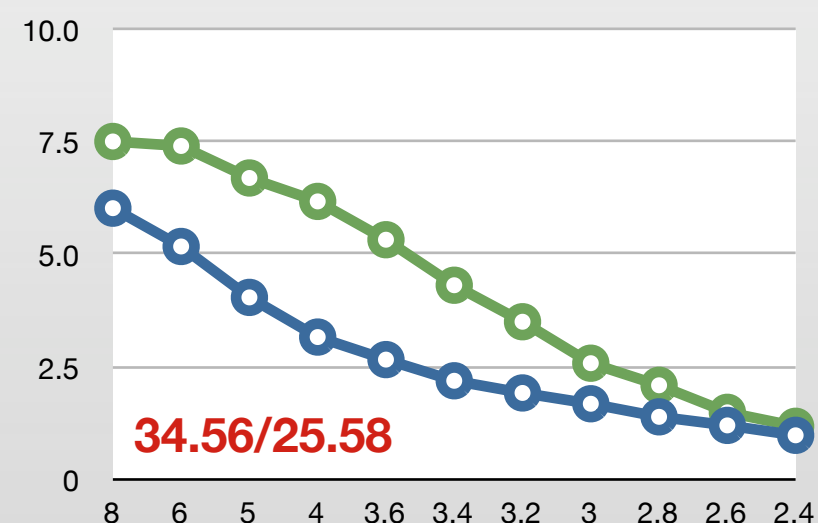
ChSynt - MAD Pk - dano



$\langle d''/\sigma \rangle$

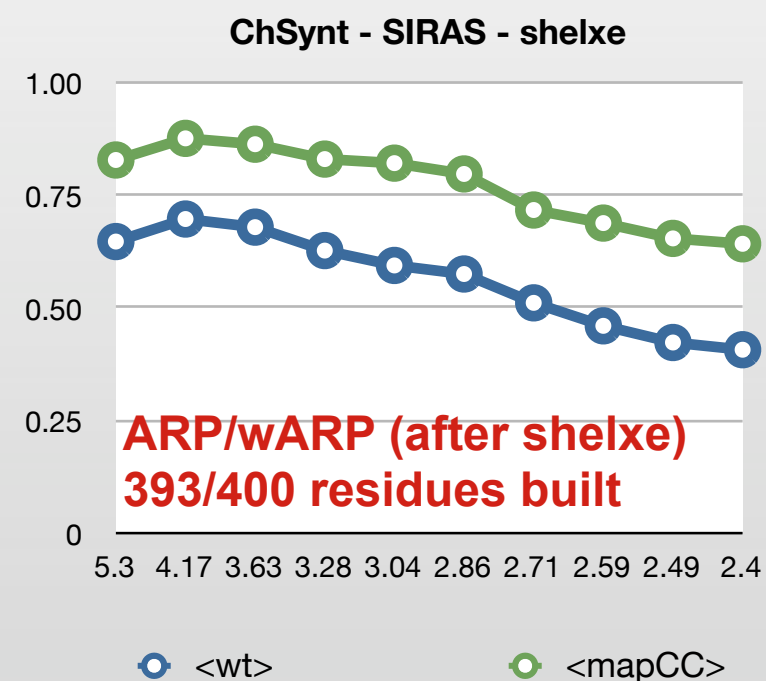
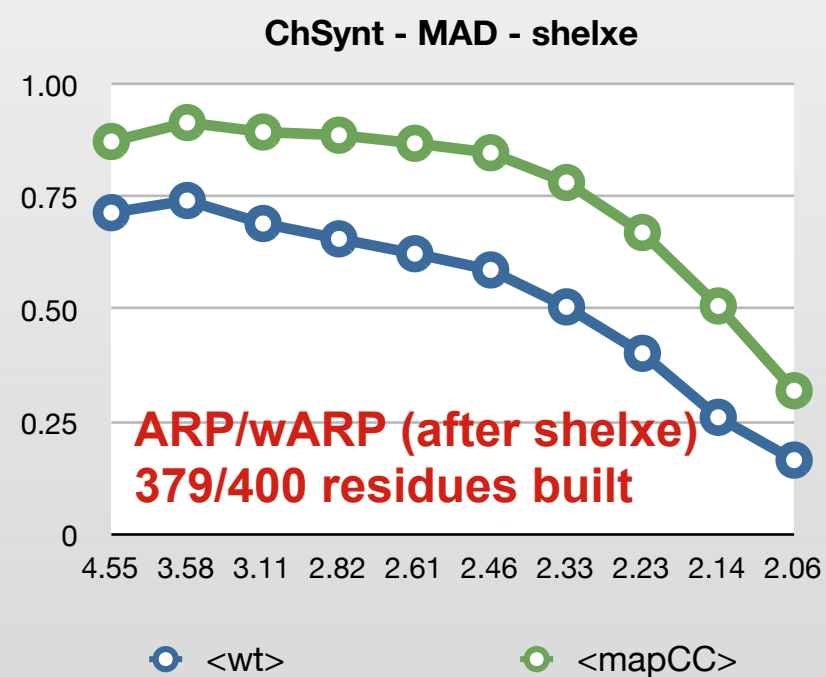
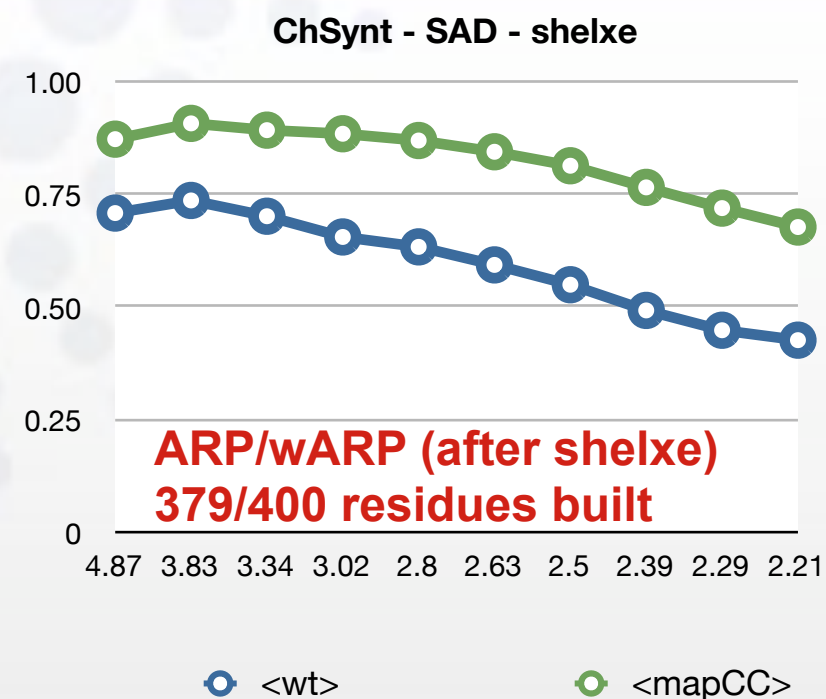
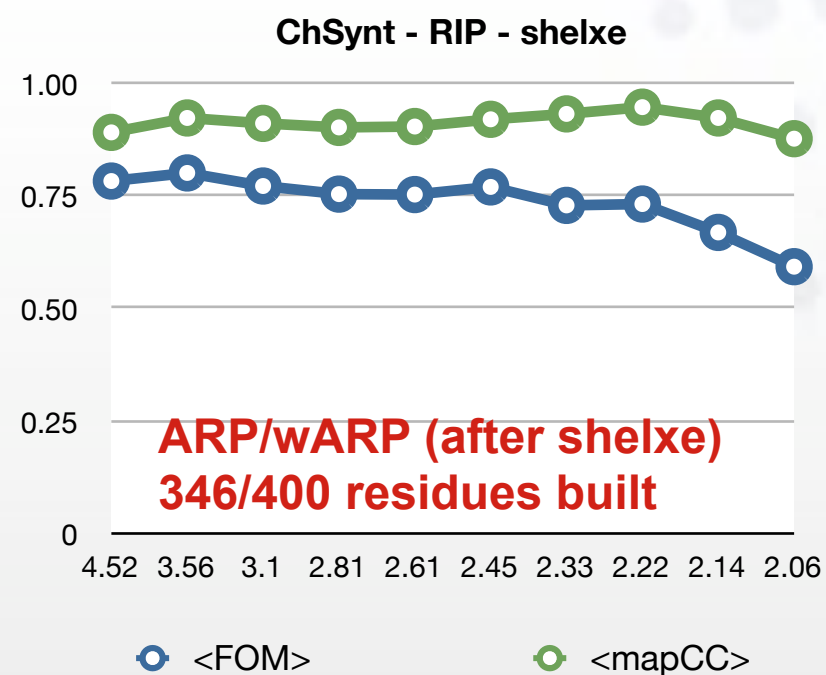
$\langle d'/\sigma \rangle$

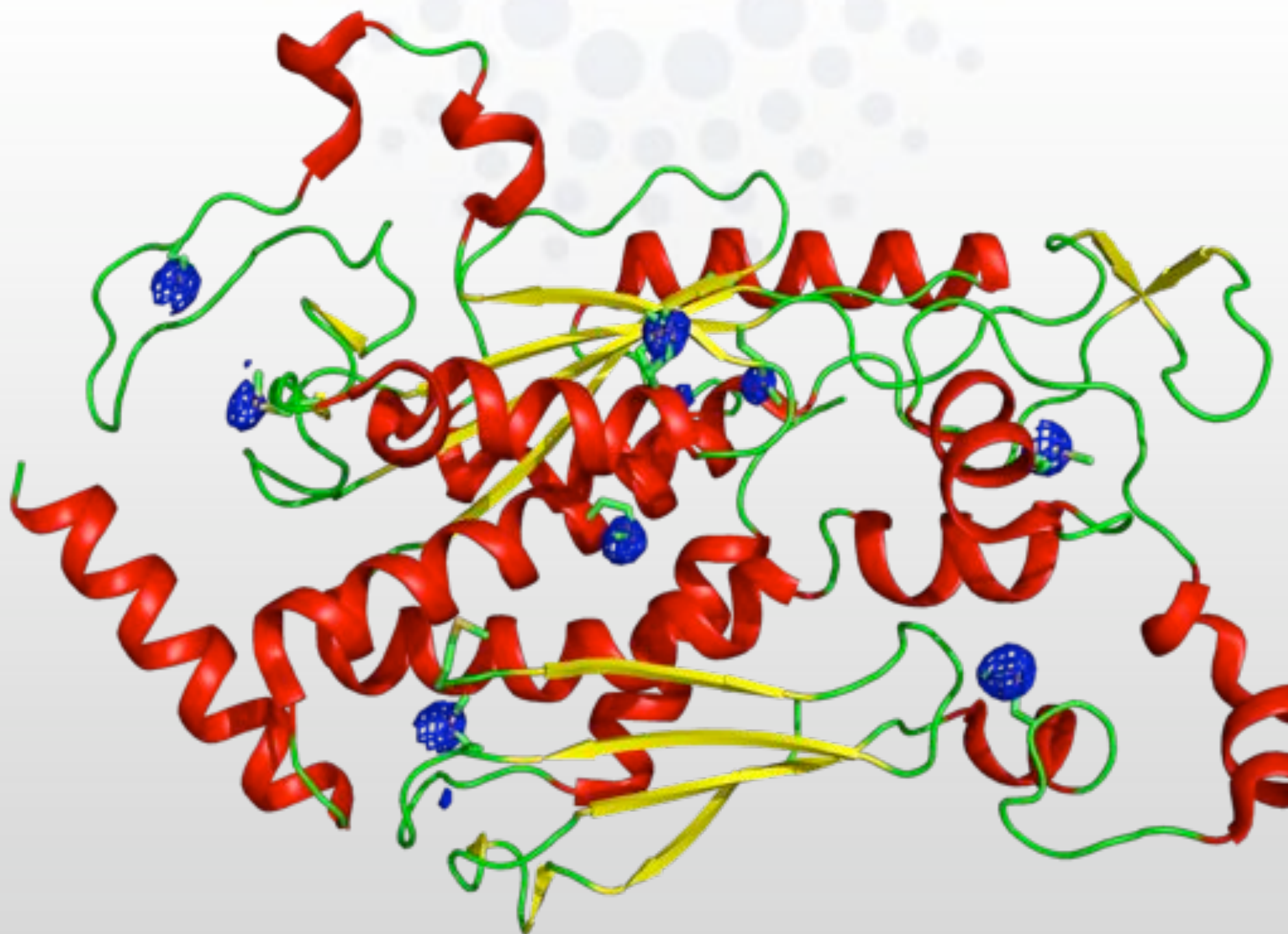
ChSynt - SIRAS diso/dano



$\langle d''/\sigma \rangle$

$\langle d'/\sigma \rangle$







H35

100 resi - 7 Selenium labelled methionine  
Space group P21212  
4 mol/au  
56% solvent



## H35 Before

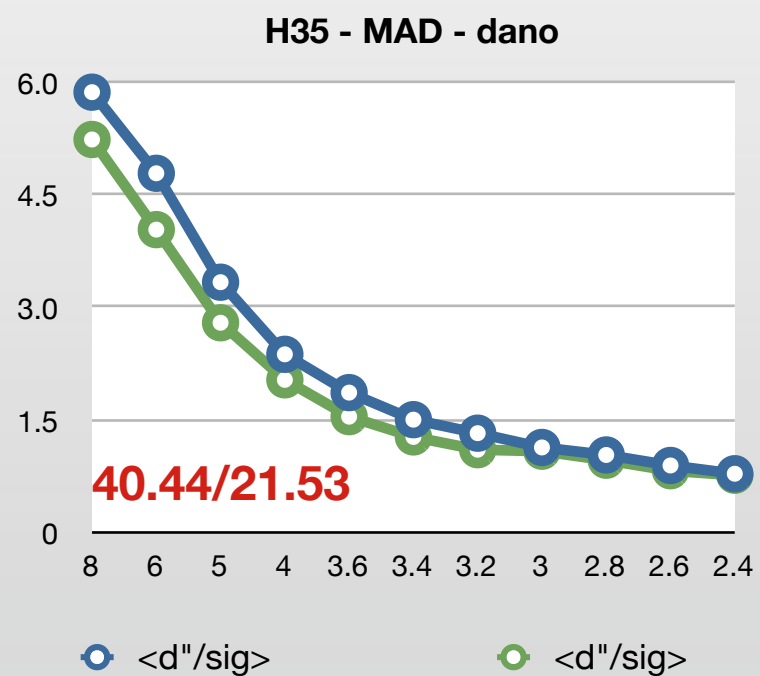
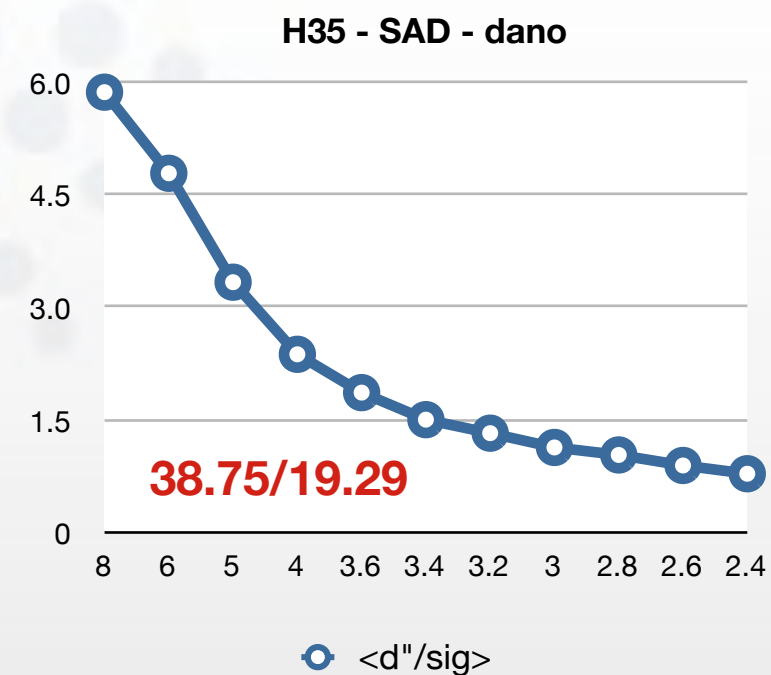
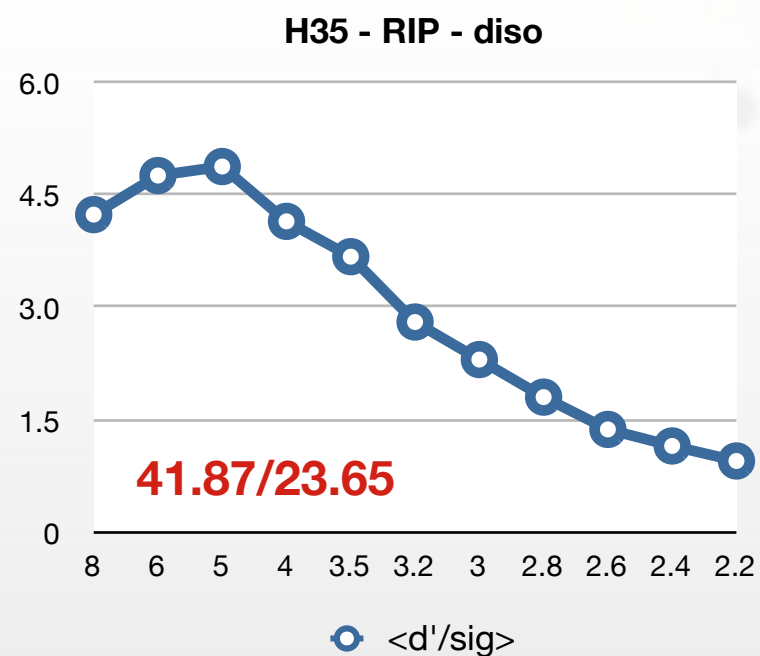
SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE  $\geq -3.0$  AS FUNCTION OF RESOLUTION  
 RESOLUTION NUMBER OF REFLECTIONS COMPLETENESS  
 LIMIT OBSERVED UNIQUE POSSIBLE OF DATA observed expected

R-FACTOR	R-FACTOR	COMPARED	I/SIGMA	R-meas	Rmrgd-F	Anomal Corr	SigAno	Nano
2.9%	3.4%	4181	30.55	3.4%	2.6%	6%	0.818	517
4.4%	4.2%	7277	25.33	5.0%	4.1%	-4%	0.841	918
4.5%	4.3%	9557	26.41	5.1%	3.9%	-5%	0.815	1238
7.2%	7.0%	11395	17.32	8.1%	7.3%	-3%	0.830	1456
13.8%	13.8%	13008	9.69	15.6%	16.1%	0%	0.801	1621
25.1%	25.8%	14357	5.32	28.4%	32.8%	-2%	0.753	1779
46.2%	47.7%	15591	2.99	52.1%	59.5%	-1%	0.734	1928
81.5%	84.6%	16719	1.71	91.9%	99.6%	1%	0.723	2043
142.3%	149.2%	17489	0.96	160.5%	179.0%	3%	0.679	2138
10.4%	10.6%	109574	9.88	11.8%	23.7%	0%	0.762	13638

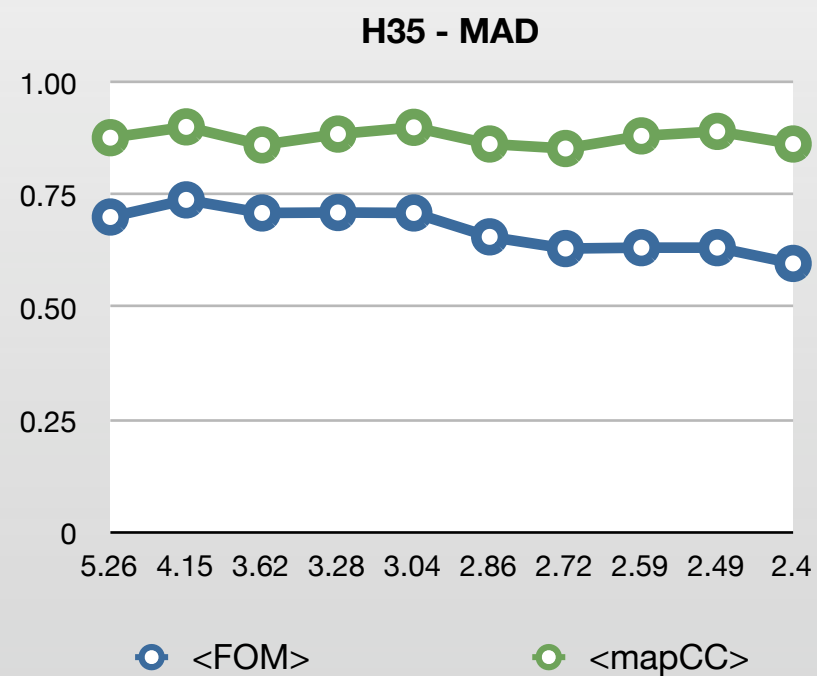
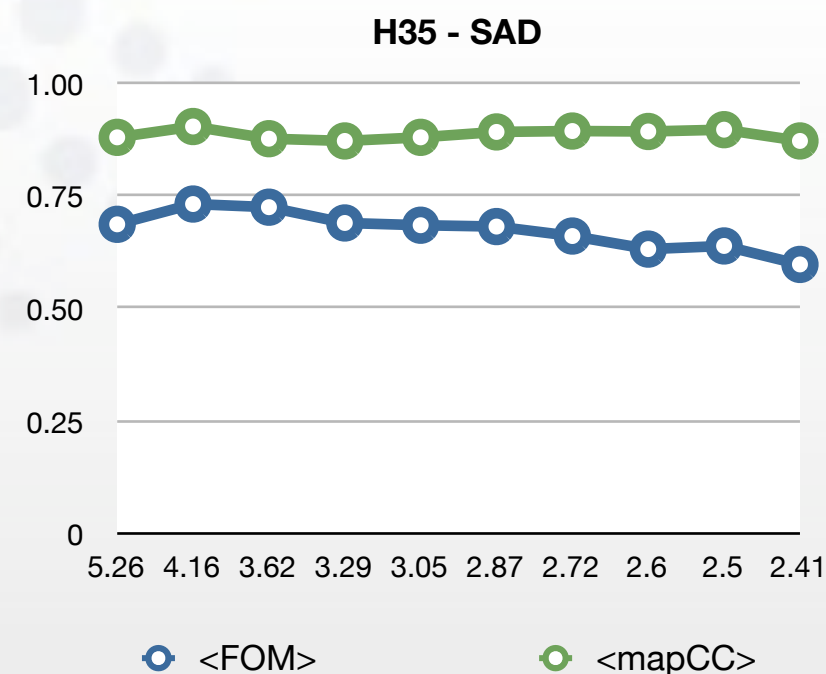
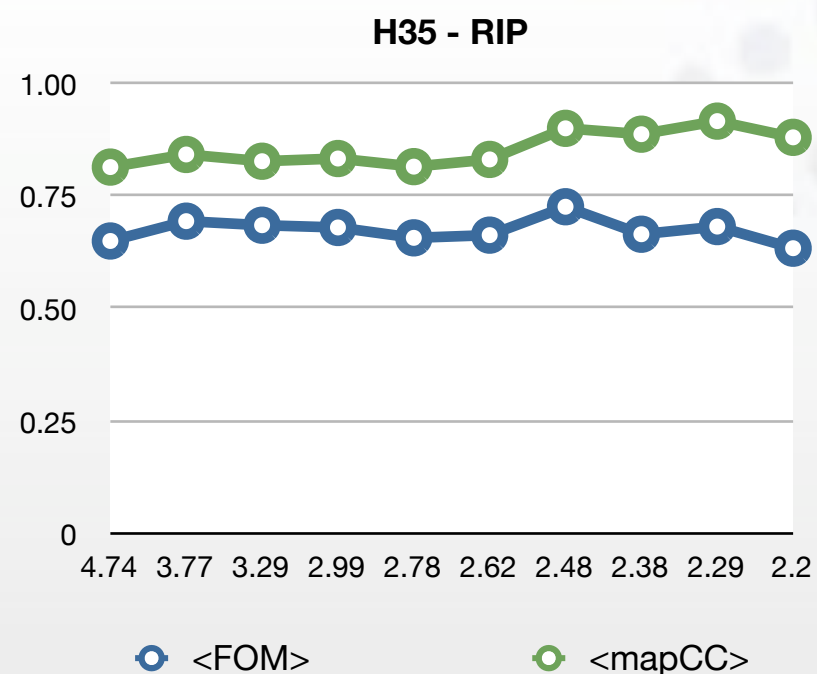
## H35 After 50 mins exposure

SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE  $\geq -3.0$  AS FUNCTION OF RESOLUTION  
 RESOLUTION NUMBER OF REFLECTIONS COMPLETENESS  
 LIMIT OBSERVED UNIQUE POSSIBLE OF DATA observed expected

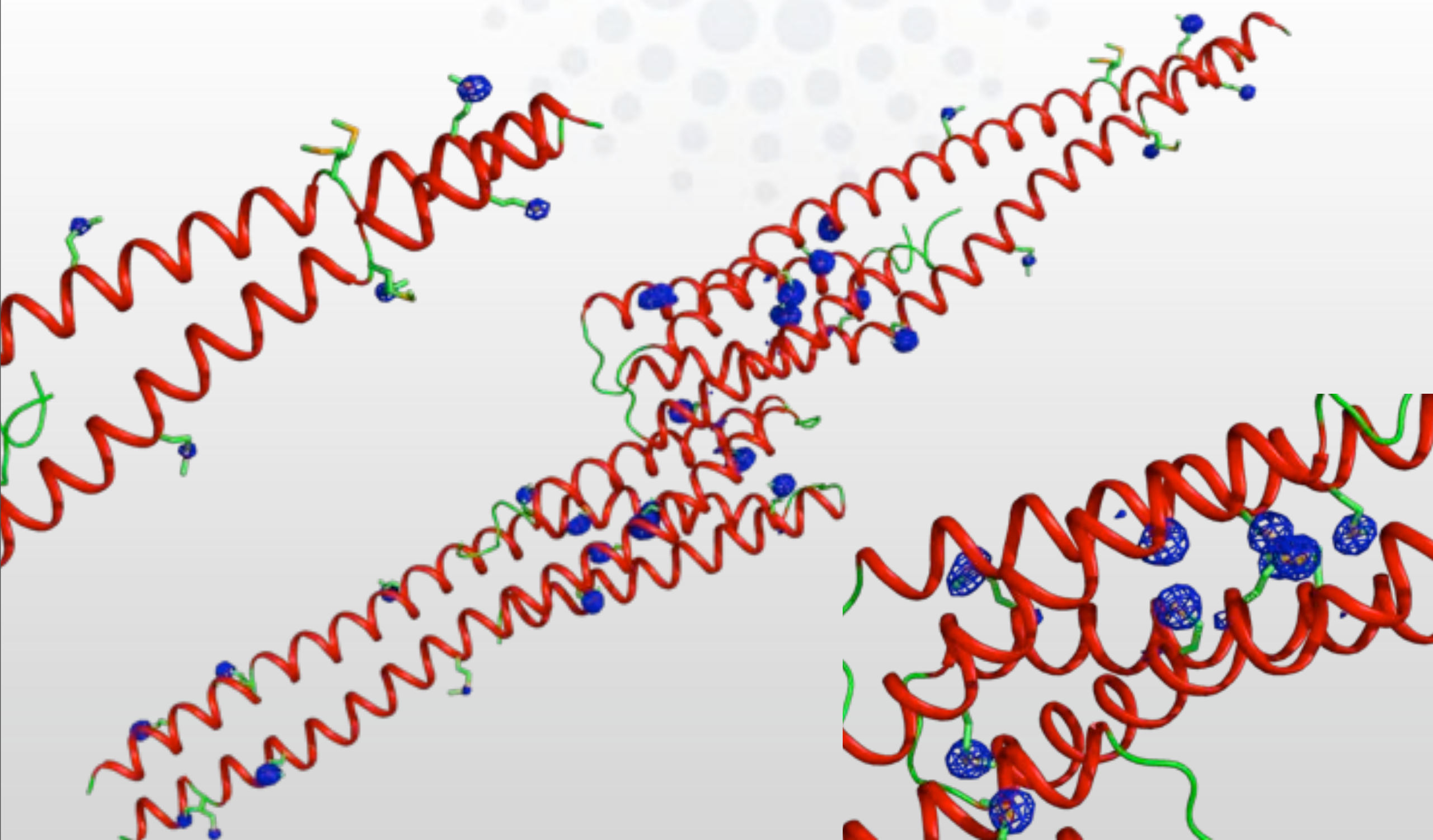
R-FACTOR	R-FACTOR	COMPARED	I/SIGMA	R-meas	Rmrgd-F	Anomal Corr	SigAno	Nano
3.0%	3.5%	4209	29.28	3.5%	2.8%	9%	0.867	529
4.7%	4.6%	7354	22.90	5.3%	4.6%	-7%	0.805	942
4.9%	4.6%	9598	24.23	5.5%	4.3%	-11%	0.801	1240
8.7%	8.6%	11551	14.69	9.9%	9.1%	-3%	0.808	1473
18.5%	18.4%	13079	7.63	20.9%	21.4%	2%	0.797	1639
36.0%	36.4%	14424	3.87	40.6%	46.2%	0%	0.765	1798
69.3%	72.1%	15742	2.04	78.1%	86.8%	-1%	0.711	1950
126.5%	131.1%	16804	1.13	142.6%	151.0%	-1%	0.673	2056
218.8%	226.0%	17588	0.64	246.7%	266.0%	-2%	0.641	2143
12.7%	12.9%	110349	8.49	14.4%	30.4%	-2%	0.742	13770



?



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Why H35 SIRAS didn't work????

I already had exposed that crystal to UV and it looks like no additional damage is possible, but strange enough, the peak anomalous signal was still enough to phase....more work to do!!!

- the SIRAS approach is working at its best because damaged sites are the anomalous scatters
- More complicated (and maybe more interesting) scenario are foreseen in case of combination of independent phases
- Still there is room to investigate the effect of UV on
  - Metalloproteins
  - DNA
  - protein-DNA complexes
  - Nucleotide binding proteins
  - ....



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