

Practical aspects of MX

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Who am I?

Initial training

- M.S. degree from the ECP (mechanics, electrotechnics,...), major: Accelerators and High Energy Physics
- M.S. degree in Chemistry - Physics (Paris VI University, France)
- Ph.D. degree in Physics (Paris XI University, France): *"Study of the non linear dynamic of the Free Electron Laser spectrum in the Compton regime"*

Experience

- construction of beamline D2AM at the ESRF
- construction of beamline FIP at the ESRF
- 2-year at the Salk Institute, USA (structure of CHS, STS, TAA1, ...)

Present situation

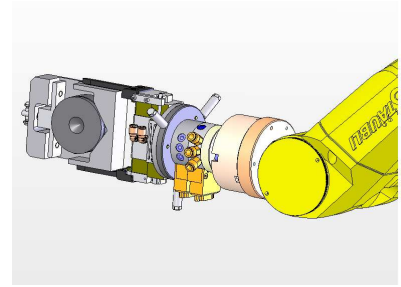
- head of the Synchrotron Group at the "Institut de Biologie Structurale" (Grenoble)
- head of beamline FIP-BM30A at the ESRF

Crystal handling

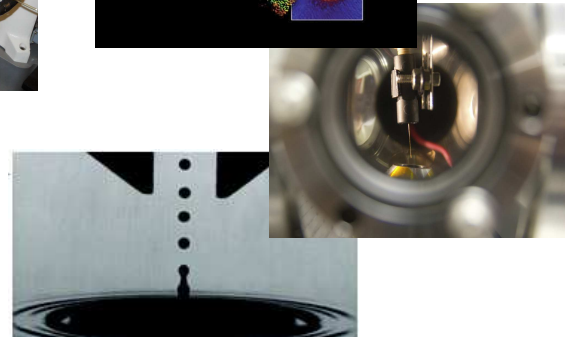
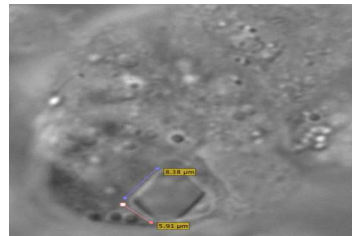
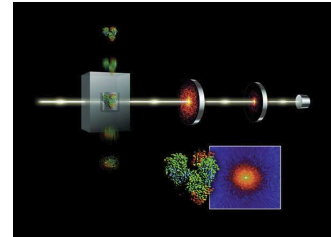
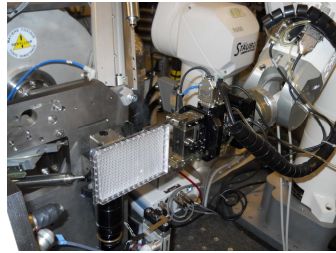
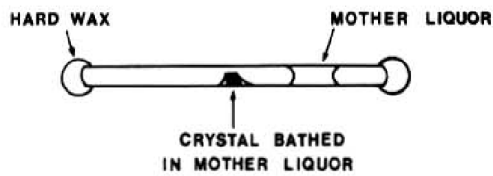
Flash-frozen crystals



High pressure



Room temperature



The Harvesting Station



Data collection strategy

Images - What to collect?

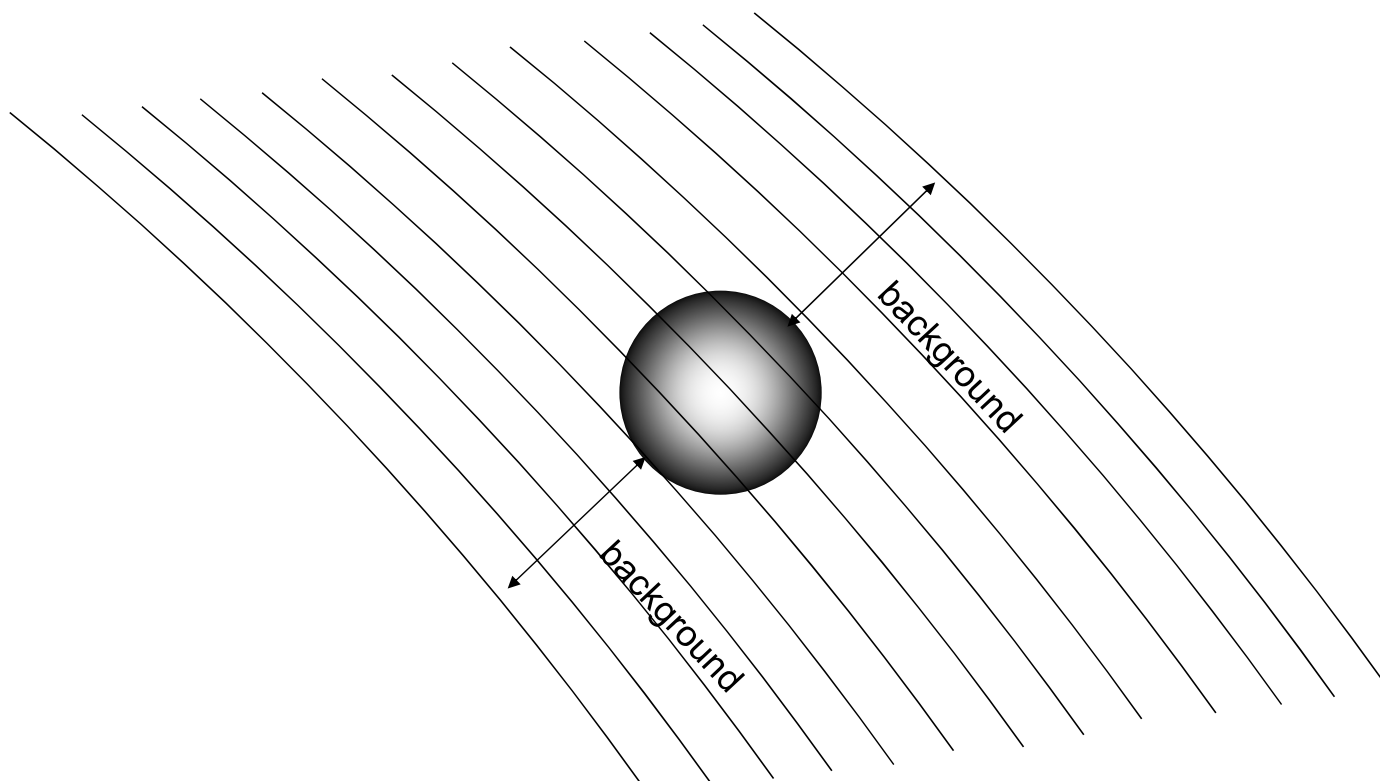
- Overall start and end
- Rotation increment
- Exposure time
- Depends on crystal, spacegroup, mosaicity
- Spatial overlaps
- Expected statistics

Classes of error in MX

Dependence on signal

Time	Dependence on signal		
	none	sqrt	proportional
	none	CCD Read-out Photon counting	Detector calibration attenuation partiality Non-isomorphism Radiation damage
	1/sqrt		Beam flicker
1/prop.			Shutter jitter Sample vibration

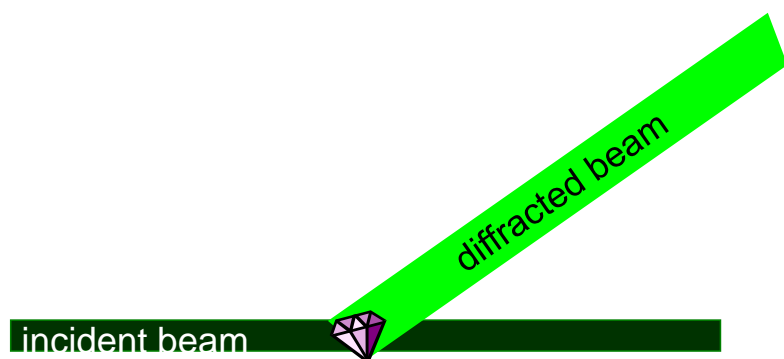
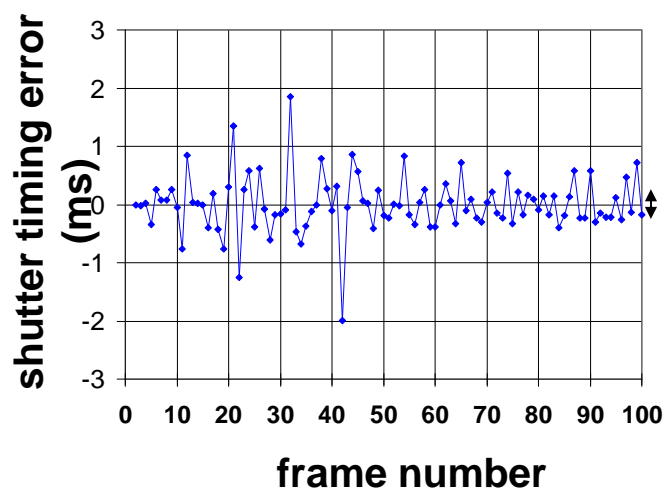
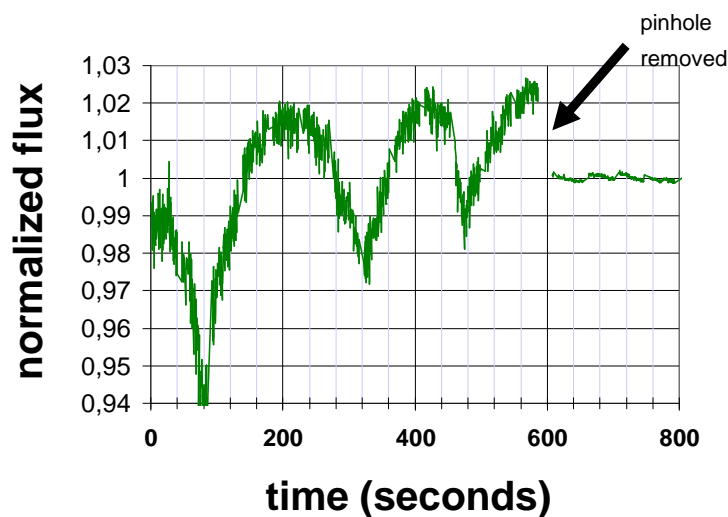
Fine Slicing



Pflugrath, J. W. (1999). "The finer things in X-ray diffraction data collection", *Acta Cryst. D* **55**, 1718-1725.

(J. Holton, ALS)

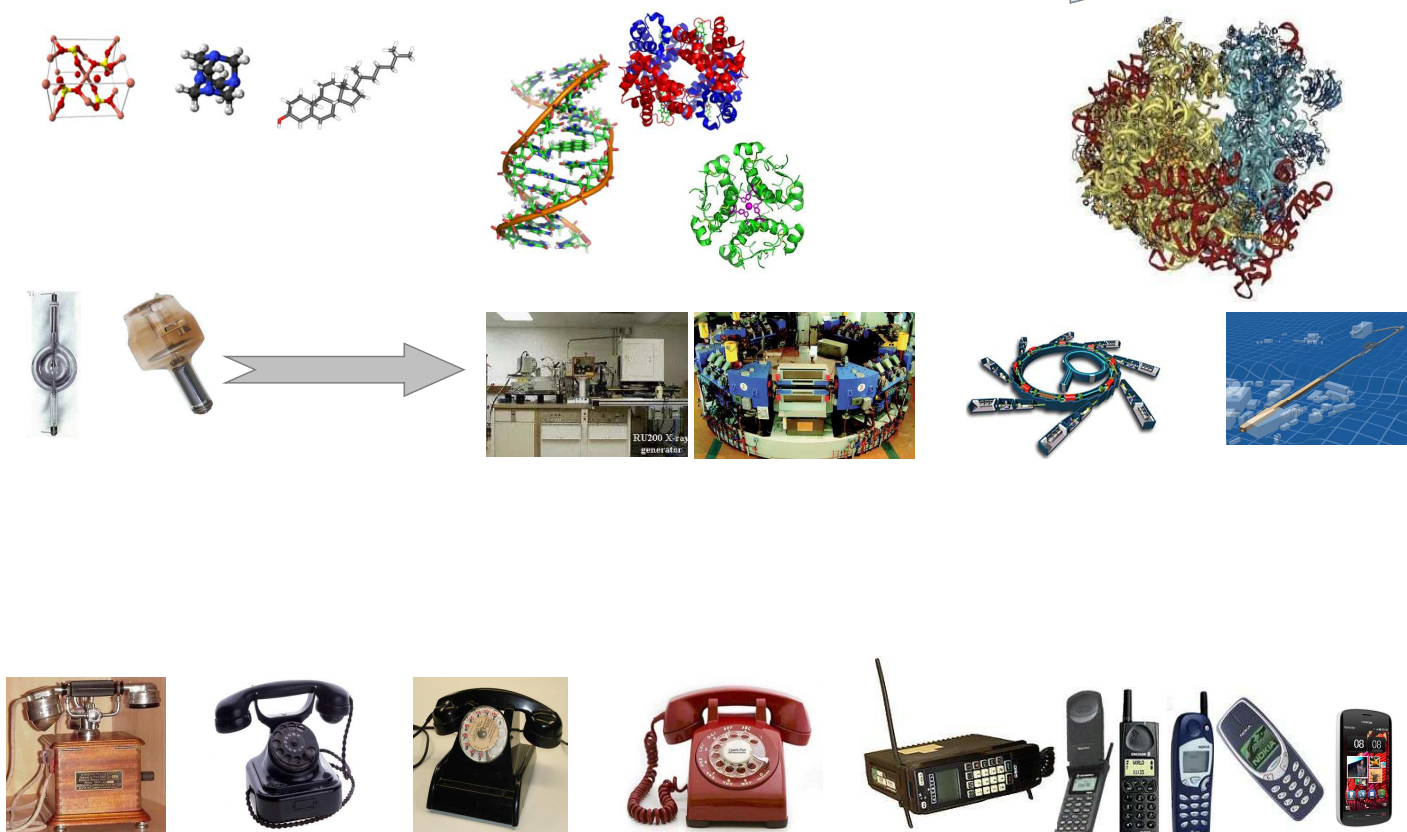
Beam flicker/Shutter jitter/Xtal vibration



- reduce flux and increase exposure
- OR:
- vibrate at frequencies **different** from experiment
 - average over it

(J. Holton, ALS)

1910 1920 1930 1940 1950 1960 1970 1980 1990 2000 2010



Data processing

- ❖ Indexing (finding the unit cell, orientation & space group)
- ❖ Integrating (determining the intensities of each spot)
- ❖ Merging (scaling data, averaging data & determining data quality)
- ❖ Calculating structure factor amplitudes from merged intensities

(Auto-) Indexing Methods

1. **Fourier Method** Bricogne (1986), Rossmann (1999), used in Mosflm (and most likely Denzo/HKL2000)

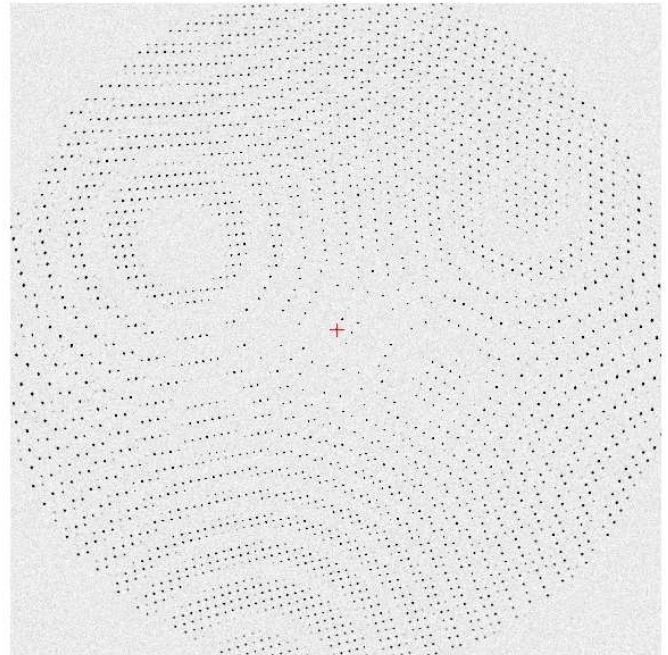
In very brief: Fourier Transformation along one correct lattice direction has main frequency at length of lattice constant (plus higher harmonics), for other directions peaks are smaller

⇒ test many directions (mosflm: about 7300 steps)

2. **Difference Vectors** Kabsch (1993), used in XDS

Basics:

- (a) Calculation of reciprocal lattice points from reflection data.
- (b) Fitting of parameters based on **differences** of locally close lattice points
- (c) Using small differences reduces the effect of systematic errors



(Tim Grüne, Gottingen)

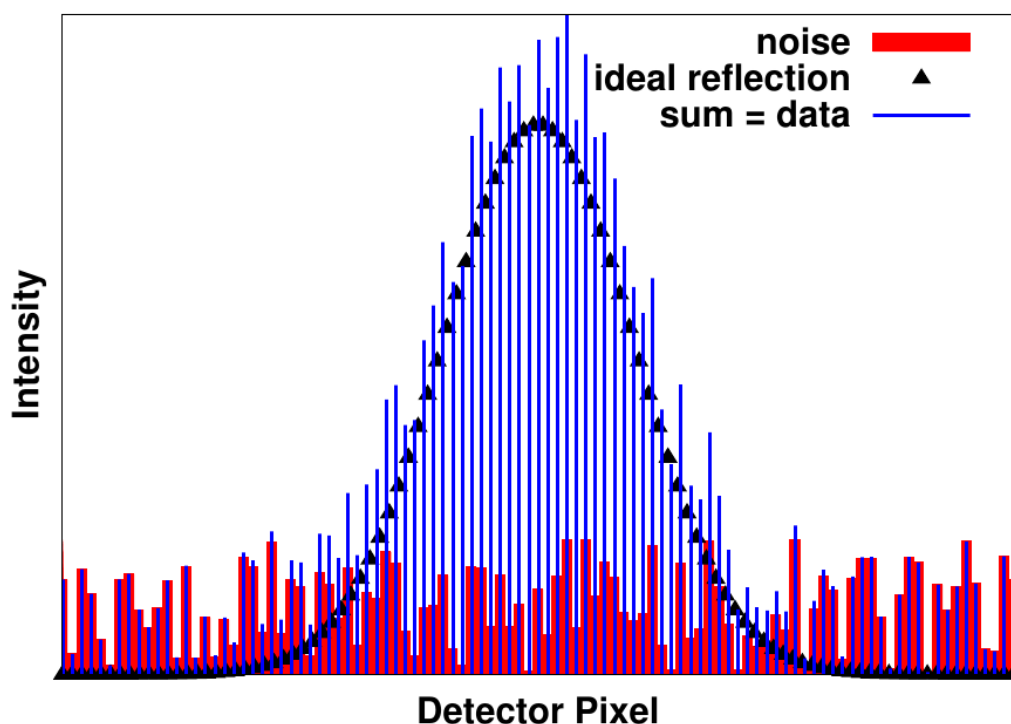
Data Integration = Data Reduction

Finding Spots per Frame:

1. Calculate expected spot positions (X_{calc}, Y_{calc}) from experimental parameters
2. Extract pixel intensities
 - Spot size/ extent (2D or 3D)
 - Background
 - Border between background and spot region
3. Calculate spot centroid (X_{obs}, Y_{obs})
4. Comparison between (X_{obs}, Y_{obs}) and (X_{calc}, Y_{calc}) allows refinement of experimental parameters.

(Tim Grüne, Gottingen)

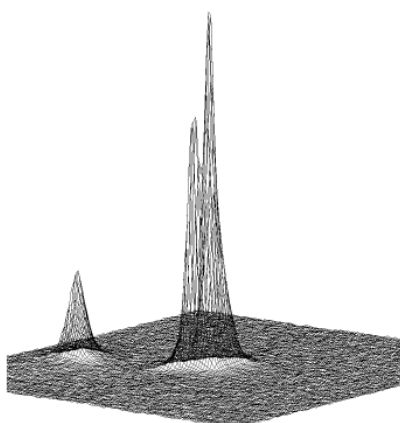
Spot & Background Noise



(Tim Grüne, Gottingen)

Strong Spots

3D profile



Numeric values per pixel

475	482	488	494	499	504	509	514	519	524	529	534	539	544	549	554	559	564	569	574	579	584	589	594	599	604	609	614	619	624	629	634	639	644	649	654	659	664	669	674	679	684	689	694	699	704	709	714	719	724	729	734	739	744	749	754	759	764	769	774	779	784	789	794	799	804	809	814	819	824	829	834	839	844	849	854	859	864	869	874	879	884	889	894	899	904	909	914	919	924	929	934	939	944	949	954	959	964	969	974	979	984	989	994	999	1004	1009	1014	1019	1024	1029	1034	1039	1044	1049	1054	1059	1064	1069	1074	1079	1084	1089	1094	1099	1104	1109	1114	1119	1124	1129	1134	1139	1144	1149	1154	1159	1164	1169	1174	1179	1184	1189	1194	1199	1204	1209	1214	1219	1224	1229	1234	1239	1244	1249	1254	1259	1264	1269	1274	1279	1284	1289	1294	1299	1304	1309	1314	1319	1324	1329	1334	1339	1344	1349	1354	1359	1364	1369	1374	1379	1384	1389	1394	1399	1404	1409	1414	1419	1424	1429	1434	1439	1444	1449	1454	1459	1464	1469	1474	1479	1484	1489	1494	1499	1504	1509	1514	1519	1524	1529	1534	1539	1544	1549	1554	1559	1564	1569	1574	1579	1584	1589	1594	1599	1604	1609	1614	1619	1624	1629	1634	1639	1644	1649	1654	1659	1664	1669	1674	1679	1684	1689	1694	1699	1704	1709	1714	1719	1724	1729	1734	1739	1744	1749	1754	1759	1764	1769	1774	1779	1784	1789	1794	1799	1804	1809	1814	1819	1824	1829	1834	1839	1844	1849	1854	1859	1864	1869	1874	1879	1884	1889	1894	1899	1904	1909	1914	1919	1924	1929	1934	1939	1944	1949	1954	1959	1964	1969	1974	1979	1984	1989	1994	1999	2004	2009	2014	2019	2024	2029	2034	2039	2044	2049	2054	2059	2064	2069	2074	2079	2084	2089	2094	2099	2104	2109	2114	2119	2124	2129	2134	2139	2144	2149	2154	2159	2164	2169	2174	2179	2184	2189	2194	2199	2204	2209	2214	2219	2224	2229	2234	2239	2244	2249	2254	2259	2264	2269	2274	2279	2284	2289	2294	2299	2304	2309	2314	2319	2324	2329	2334	2339	2344	2349	2354	2359	2364	2369	2374	2379	2384	2389	2394	2399	2404	2409	2414	2419	2424	2429	2434	2439	2444	2449	2454	2459	2464	2469	2474	2479	2484	2489	2494	2499	2504	2509	2514	2519	2524	2529	2534	2539	2544	2549	2554	2559	2564	2569	2574	2579	2584	2589	2594	2599	2604	2609	2614	2619	2624	2629	2634	2639	2644	2649	2654	2659	2664	2669	2674	2679	2684	2689	2694	2699	2704	2709	2714	2719	2724	2729	2734	2739	2744	2749	2754	2759	2764	2769	2774	2779	2784	2789	2794	2799	2804	2809	2814	2819	2824	2829	2834	2839	2844	2849	2854	2859	2864	2869	2874	2879	2884	2889	2894	2899	2904	2909	2914	2919	2924	2929	2934	2939	2944	2949	2954	2959	2964	2969	2974	2979	2984	2989	2994	2999	3004	3009	3014	3019	3024	3029	3034	3039	3044	3049	3054	3059	3064	3069	3074	3079	3084	3089	3094	3099	3104	3109	3114	3119	3124	3129	3134	3139	3144	3149	3154	3159	3164	3169	3174	3179	3184	3189	3194	3199	3204	3209	3214	3219	3224	3229	3234	3239	3244	3249	3254	3259	3264	3269	3274	3279	3284	3289	3294	3299	3304	3309	3314	3319	3324	3329	3334	3339	3344	3349	3354	3359	3364	3369	3374	3379	3384	3389	3394	3399	3404	3409	3414	3419	3424	3429	3434	3439	3444	3449	3454	3459	3464	3469	3474	3479	3484	3489	3494	3499	3504	3509	3514	3519	3524	3529	3534	3539	3544	3549	3554	3559	3564	3569	3574	3579	3584	3589	3594	3599	3604	3609	3614	3619	3624	3629	3634	3639	3644	3649	3654	3659	3664	3669	3674	3679	3684	3689	3694	3699	3704	3709	3714	3719	3724	3729	3734	3739	3744	3749	3754	3759	3764	3769	3774	3779	3784	3789	3794	3799	3804	3809	3814	3819	3824	3829	3834	3839	3844	3849	3854	3859	3864	3869	3874	3879	3884	3889	3894	3899	3904	3909	3914	3919	3924	3929	3934	3939	3944	3949	3954	3959	3964	3969	3974	3979	3984	3989	3994	3999	4004	4009	4014	4019	4024	4029	4034	4039	4044	4049	4054	4059	4064	4069	4074	4079	4084	4089	4094	4099	4104	4109	4114	4119	4124	4129	4134	4139	4144	4149	4154	4159	4164	4169	4174	4179	4184	4189	4194	4199	4204	4209	4214	4219	4224	4229	4234	4239	4244	4249	4254	4259	4264	4269	4274	4279	4284	4289	4294	4299	4304	4309	4314	4319	4324	4329	4334	4339	4344	4349	4354	4359	4364	4369	4374	4379	4384	4389	4394	4399	4404	4409	4414	4419	4424	4429	4434	4439	4444	4449	4454	4459	4464	4469	4474	4479	4484	4489	4494	4499	4504	4509	4514	4519	4524	4529	4534	4539	4544	4549	4554	4559	4564	4569	4574	4579	4584	4589	4594	4599	4604	4609	4614	4619	4624	4629	4634	4639	4644	4649	4654	4659	4664	4669	4674	4679	4684	4689	4694	4699	4704	4709	4714	4719	4724	4729	4734	4739	4744	4749	4754	4759	4764	4769	4774	4779	4784	4789	4794	4799	4804	4809	4814	4819	4824	4829	4834	4839	4844	4849	4854	4859	4864	4869	4874	4879	4884	4889	4894	4899	4904	4909	4914	4919	4924	4929	4934	4939	4944	4949	4954	4959	4964	4969	4974	4979	4984	4989	4994	4999	5004	5009	5014	5019	5024	5029	5034	5039	5044	5049	5054	5059	5064	5069	5074	5079	5084	5089	5094	5099	5104	5109	5114	5119	5124	5129	5134	5139	5144	5149	5154	5159	5164	5169	5174	5179	5184	5189	5194	5199	5204	5209	5214	5219	5224	5229	5234	5239	5244	5249	5254	5259	5264	5269	5274	5279	5284	5289	5294	5299	5304	5309	5314	5319	5324	5329	5334	5339	5344	5349	5354	5359	5364	5369	5374	5379	5384	5389	5394	5399	5404	5409	5414	5419	5424	5429	5434	5439	5444	5449	5454	5459	5464	5469	5474	5479	5484	5489	5494	5499	5504	5509	5514	5519	5524	5529	5534	5539	5544	5549	5554	5559	5564	5569	5574	5579	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XDS Characteristics

Some of the special features of XDS:

- Very flexible
- Large number of supported detector formats
- 3-dimensional spot integration
- Correction for Radiation Damage
- Optimised for new Pilatus Detector
- Command-line program
- Parallelised: Fast!
- Simple to read documentation

(Tim Grüne, Gottingen)

xds, xds_par Main program for data integration

XDS

xscale scaling program for multiple datasets. Can be replaced by other scaling programs

- scala (CCP4)
- aimless (CCP4)
- scalepack (HKL Research)
- sadabs (Bruker AXS)

N.B.: Data integration makes corrections which scaling program must not repeat. Otherwise: corruption of standard deviations (σ_I). Can hamper phasing

xdscnv converts scaled data file to other formats

XYINIT writes files for positional corrections of the detector plane. Most modern detectors provide already corrected images so that these to files are normally flat.

INIT determines initial detector background

COLSPOT locates strong diffraction spots and saves their centroids

IDXREF indexing: unit cell dimensions and crystal orientation

DEFPIX set active detector area (exclude resolution cut-off, beam stop shadow, ...)

(XPLAN, **optional**) generate "strategy" table with data completeness depending on

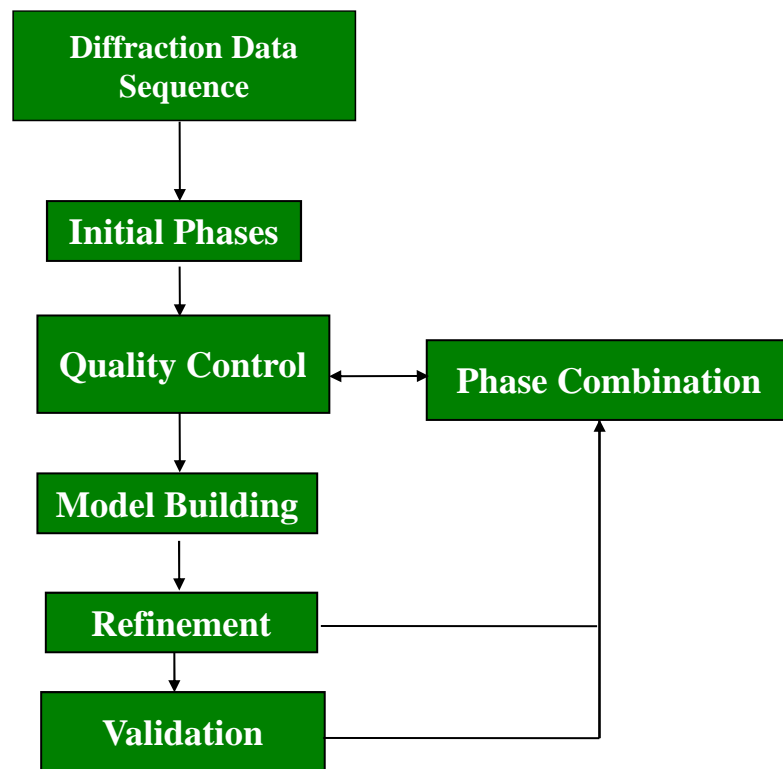
- starting angle
- total scan width

INTEGRATE determine reflection intensities

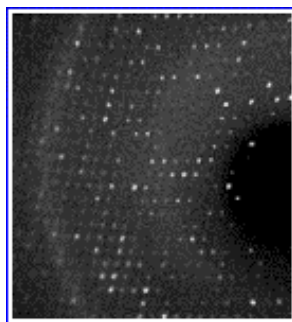
CORRECT applies corrections (polarisation, Lorentz-correction, ...), scales reflections, reports data statistics

(Tim Grüne, Gottingen)

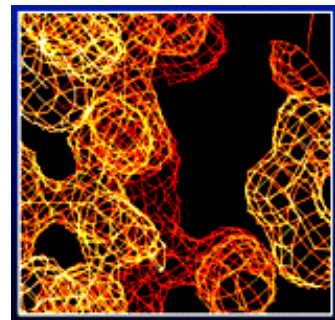
The steps to solve the macromolecular crystal structure



Phasing Methods in Macromolecular Crystallography



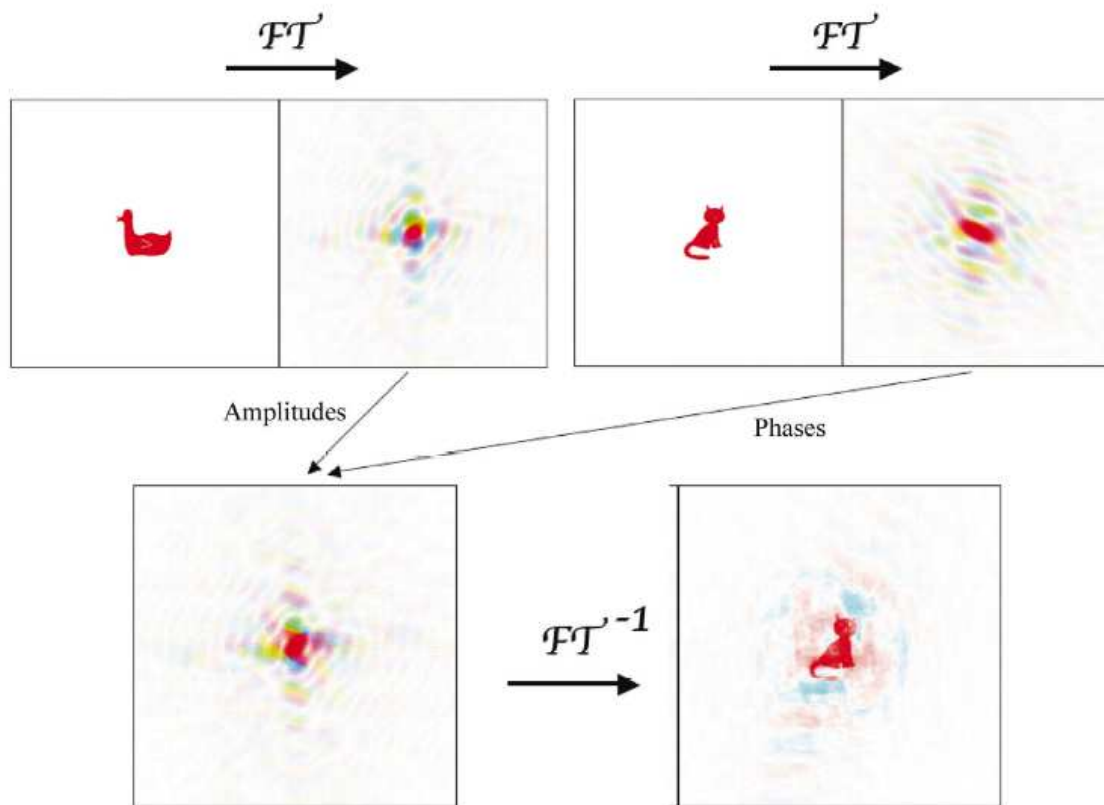
Fourier Transform



To get from the diffraction pattern to the electron density, you have to use a Fourier Transform.

- **Molecular Replacement Method (MR)**
- **Isomorphs Replacement Method (MIR, SIR)**
- **Anomalous Dispersion Method (MAD, SAD, SIRAS)**
- **Direct Method**
- **Other Methods**

Phases critically impact model quality



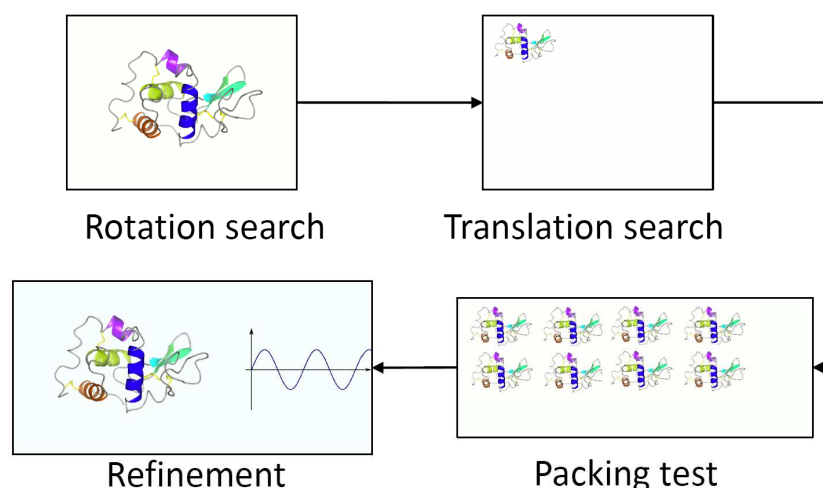
(Univ. North Carolina)

MR

The calculation involves a 6 dimensional search over all possible orientations and translations (EPMR, Genetic Algorithm).

This calculation is generally too time consuming to perform in full, so it is usually split into two parts:

- A 3 dimensional search over all possible orientations to determine the orientation of the model.
- A 3 dimensional search over all possible translations to determine the position of the orientated model.

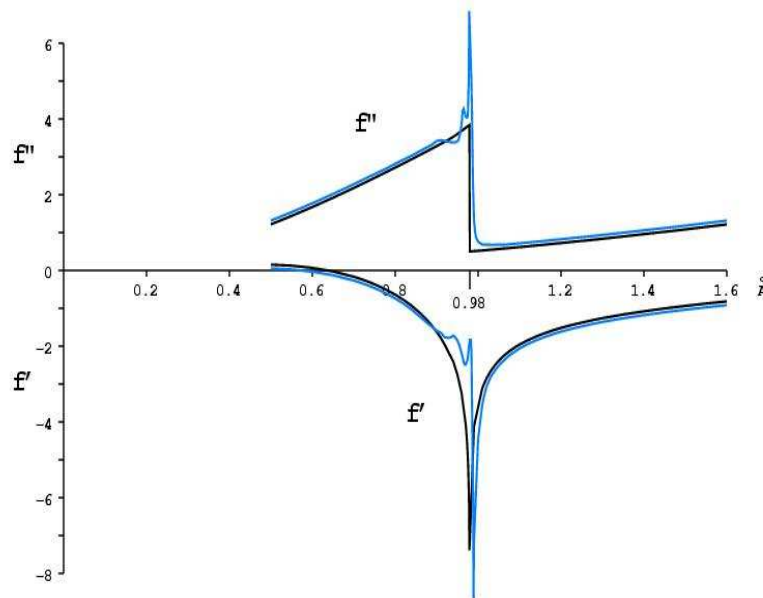


The anomalous signal

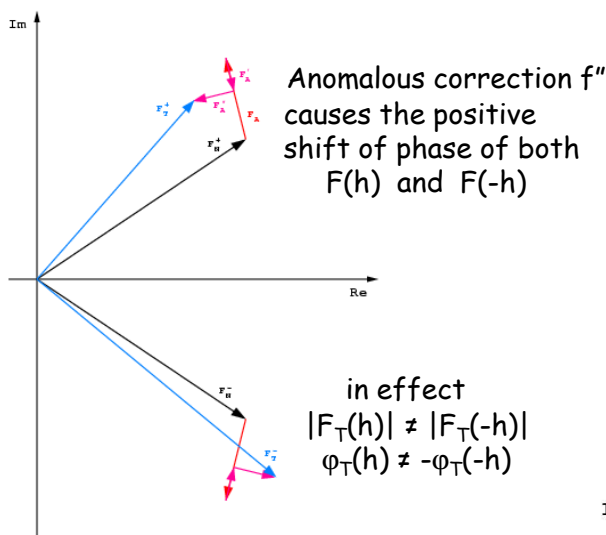
$$F(h) = \sum_j f_j \cdot \exp(2\pi i h \cdot r_j)$$

$$f_j = f_j^0(\theta) + f'_j(\lambda) + i \cdot f''_j(\lambda)$$

Anomalous correction f'' is proportional to absorption and fluorescence and f' is its derivative



Phasing with anomalous signal



Analytical MAD approach
(Karle & Hendrickson)

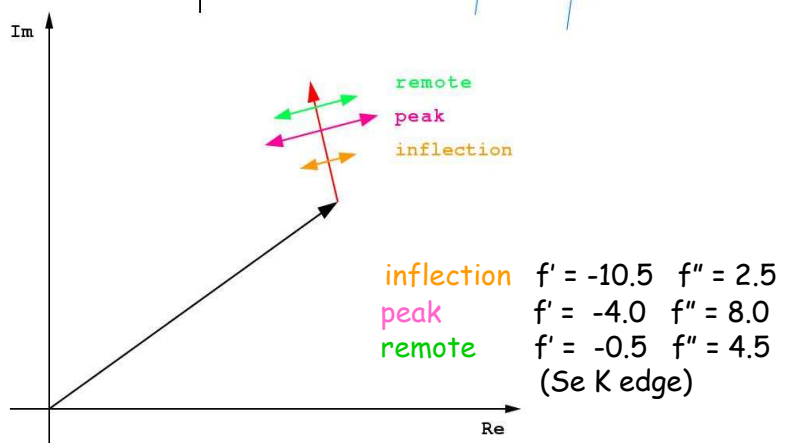
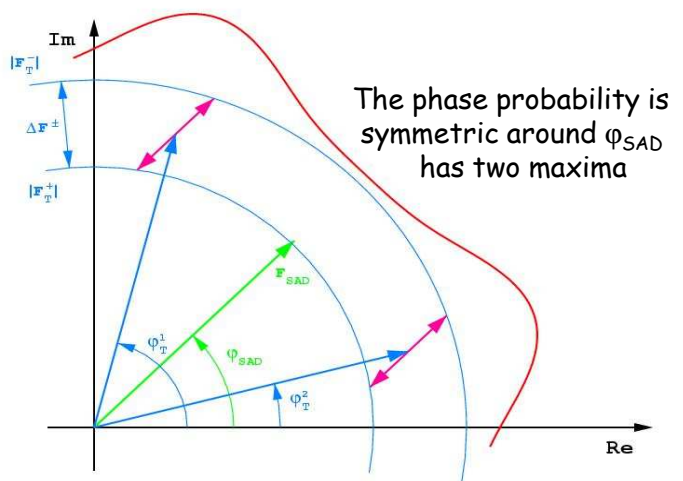
$$F_T(\pm)^2 = F_T^2 + a(\lambda) \cdot F_A^2 + b(\lambda) \cdot F_T \cdot F_A \cdot \cos(\varphi_T - \varphi_A) \pm c(\lambda) \cdot F_T \cdot F_A \cdot \sin(\varphi_T - \varphi_A)$$

$$a(\lambda) = (f'^2 + f''^2)/f_0^2$$

$$b(\lambda) = 2 \cdot f'/f_0$$

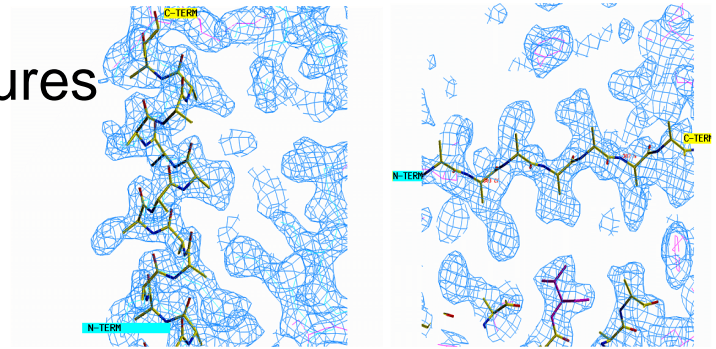
$$c(\lambda) = 2 \cdot f''/f_0$$

Three unknowns: F_T , F_A and $(\varphi_T - \varphi_A)$:
system can be solved

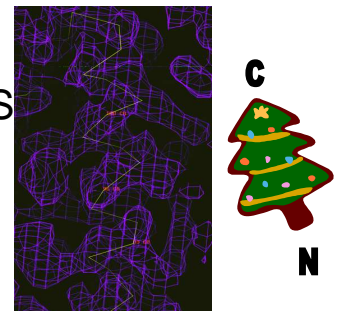


Model Building: Steps in making the first trace in electron density map

- Generating Ca chain trace
- recognize secondary structures
- Identifying chain direction
- sequence assignment
- add water molecules, etc...



- ↓
- The sulfur or Se-methionines are the perfect starting point for the sequence fitting if the map is from sulfur SAS or Se-MAD phases.
 - Tryptophan is so much larger than all the other amino acids it can often be recognized.
 - Hydrophilic side chains are often disordered.
 - A correct fitting should be easily extended in both directions.



Structure Validation and Deposition

Generate symmetry related molecules.
(contacts \geq Van der Waals packing distance)

Missing density is much better than extra density

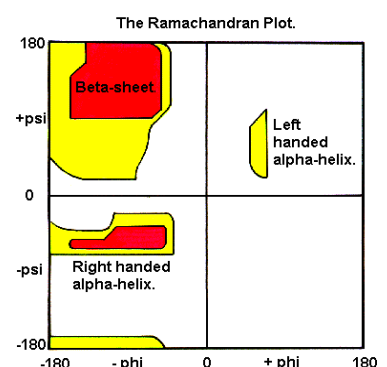
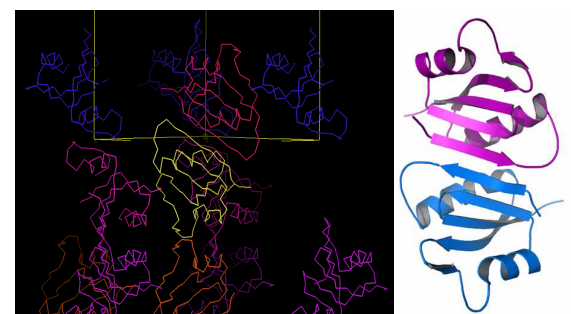
The model should make chemical sense

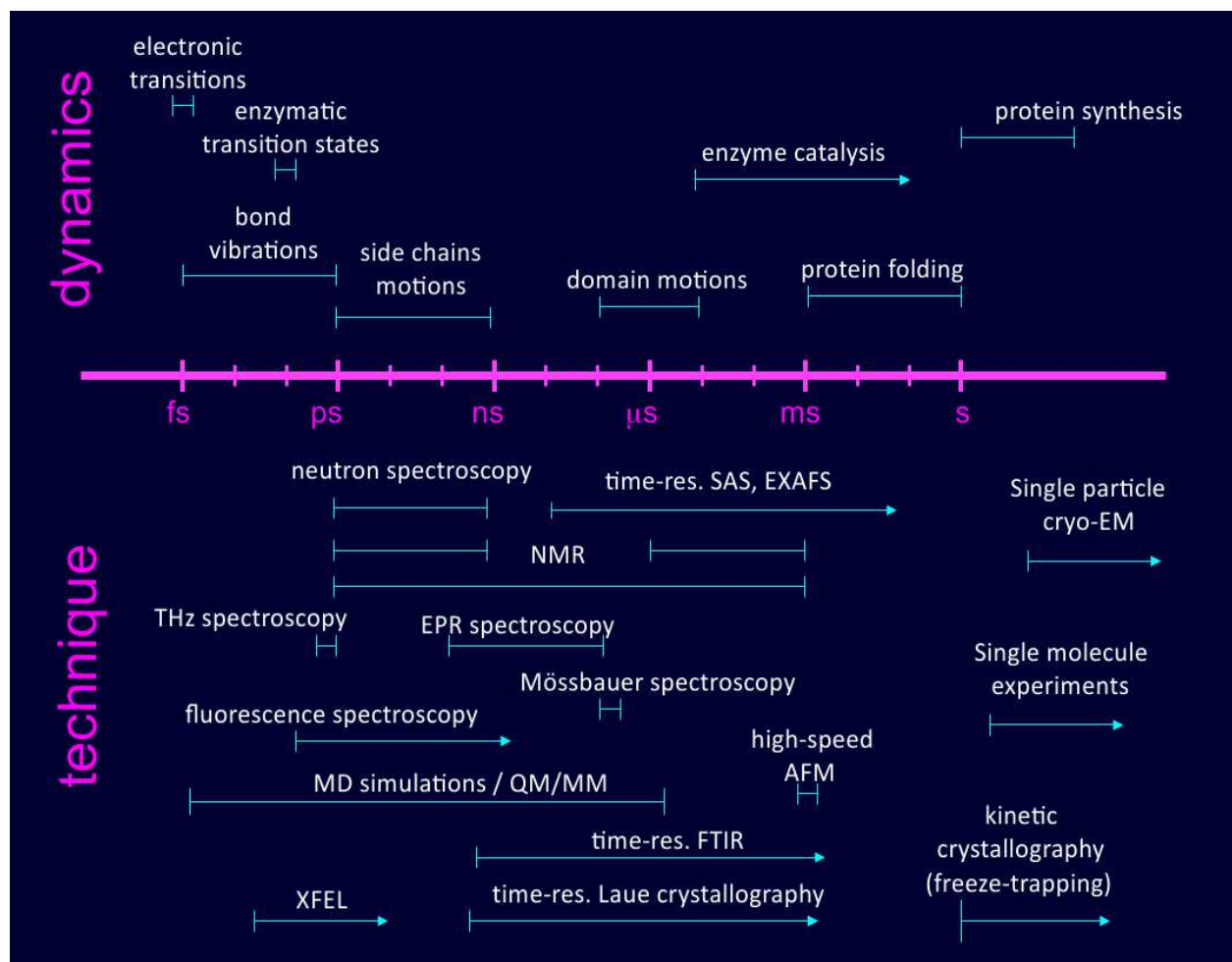
Residues identified as being in the active site:
are they close together in the model?

The stereochemical parameters such as bond length, bond angle etc,
should within the standard deviation from their ideal values

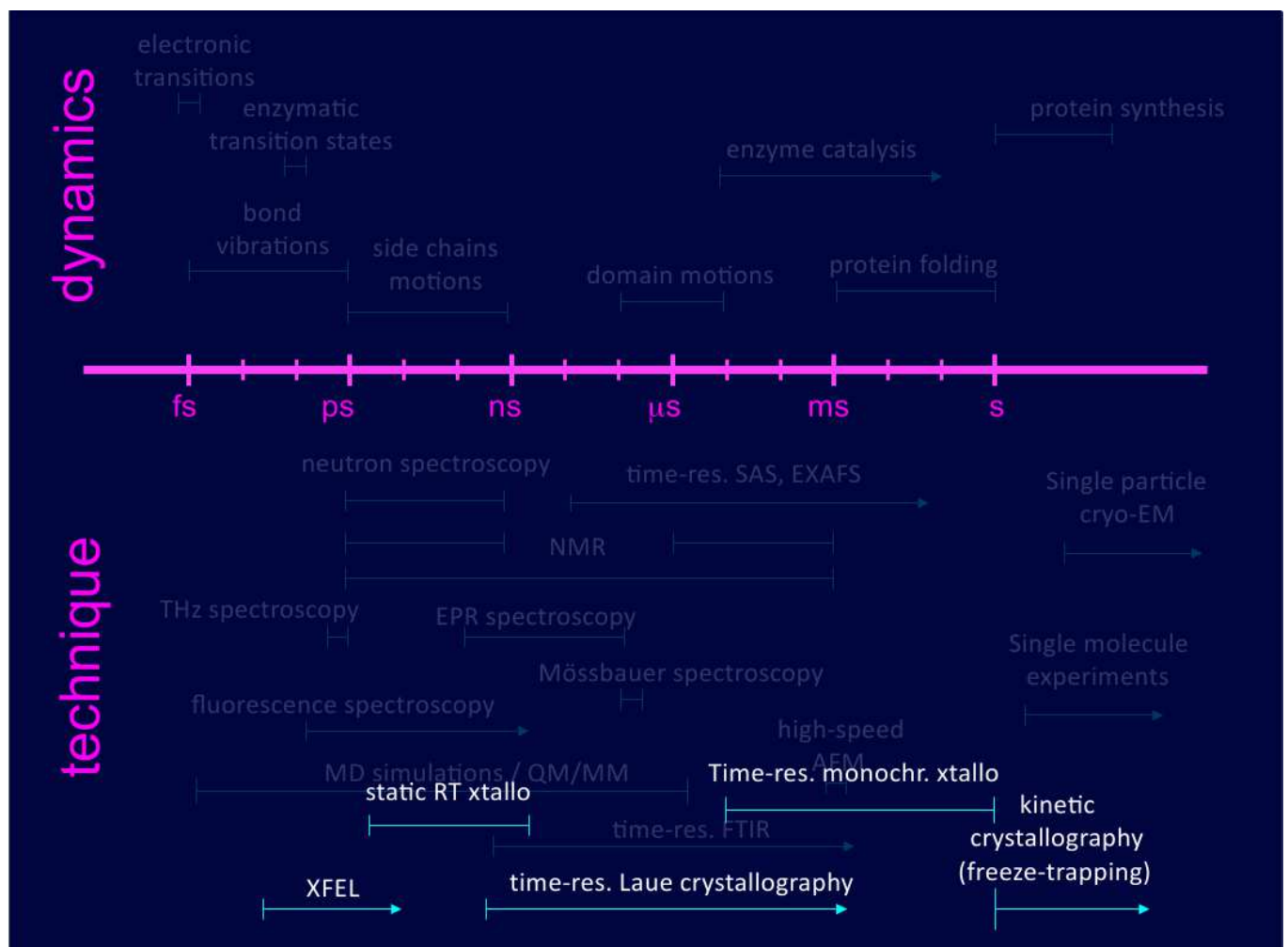
The Ramachandran Plot should be normal

==> WHATCHECK, MOLPROBITY, ...





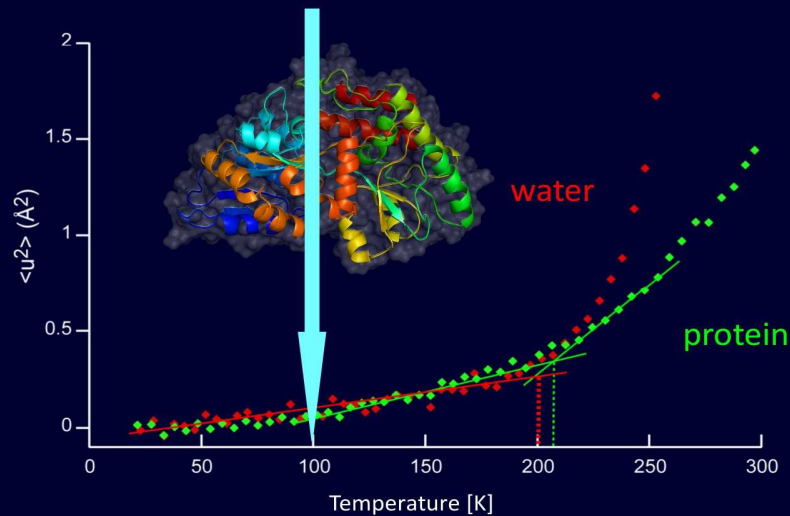
M. Weik, ESRF Users Meeting, 2014



M. Weik, ESRF Users Meeting, 2014

Temperature-dependent side-chain flexibility from neutron scattering

Cryo X-ray data collection



Wood, Frölich, Gabel, Moulin, Haertlein, Paciaroni, Zaccai, Tobias & Weik (2008) JACS 130, 4586

Cryo-cooling at 500 K / s : protein conformational changes quenched at 200 K

Halle (2004) PNAS 2004, 4793

M. Weik, ESRF Users Meeting, 2014

Room temperature crystallography

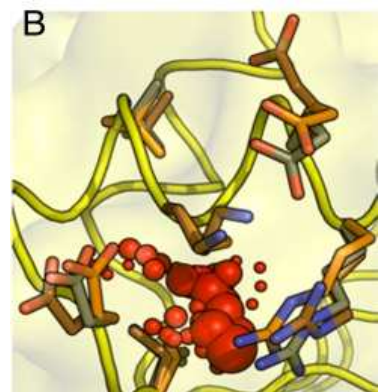
Flash cooling of protein crystals

- biases structural collective motions in protein crystals;
- remodels the conformation of > 35% of side chains;
- eliminates packing defects necessary for functional motions;
- induces bias toward smaller, overpacked, and unrealistically unique models.

Instead, **room-temperature** X-ray crystallography experiments, such as the *in situ* experiments, helps in revealing

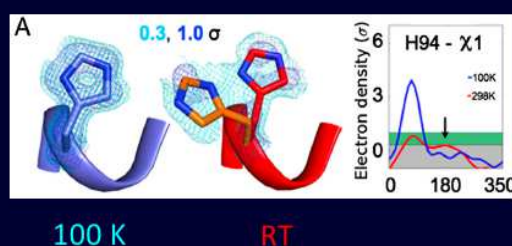
- motions crucial for catalysis,
- ligand binding,
- allosteric regulation.

In the signaling switch protein, H-Ras, an allosteric network consistent with fluctuations detected in solution by NMR was uncovered in the room-temperature, but not the cryogenic, electron-density maps (Fraser *et al.*, PNAS, 2011 (108), 16247-52).



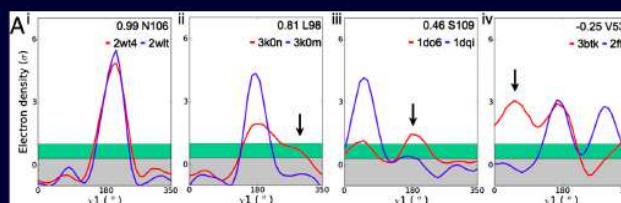
Protein conformational heterogeneity greater in RT than in 100 K structures

Fraser, van den Bedem, Samelson, Lang, Holton, Echols & Alber (2011) PNAS 108, 16247



Alternate conformation of H94
In H-Ras at RT, but not at 100 K

Cryo-cooling remodels
conformational distributions in
35% of all protein side-chains

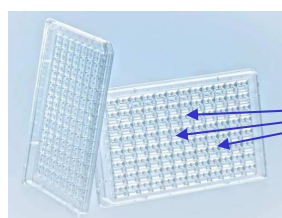


Tools to analyse conformational heterogeneity in crystal structures:

- **RINGER**: samples e- density around side-chain dihedrals below 1σ level (Lang *et al.* (2010) Protein Sci. 19, 1420)
- **qFit**: automates building of alternative polypeptide conformations (van den Bedem *et al.* (2009) Acta Cryst. D65, 1107)
- Time-averaged crystallographically restrained MD **refinement of ensembles** (Burnley *et al.* (2012) eLife 1, e00311)
- **END, RAPID**: place e- density maps on absolute scale and calculate noise at each position in the map (Lang *et al.* (2014) PNAS 111, 237)

M. Weik, ESRF Users Meeting, 2014

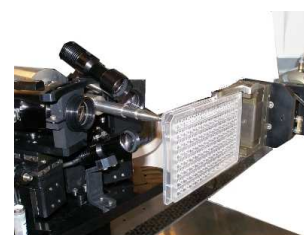
Data collection at RT



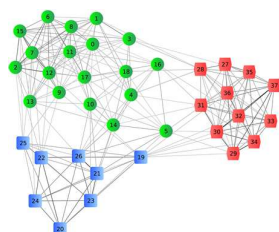
Same condition reproduced all over the plate
Or multiple crystals in capillaries



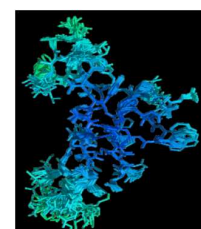
Data collection on selected crystals



Automated clustering of data set + merging:
Similar cell param. (BLEND) or CC

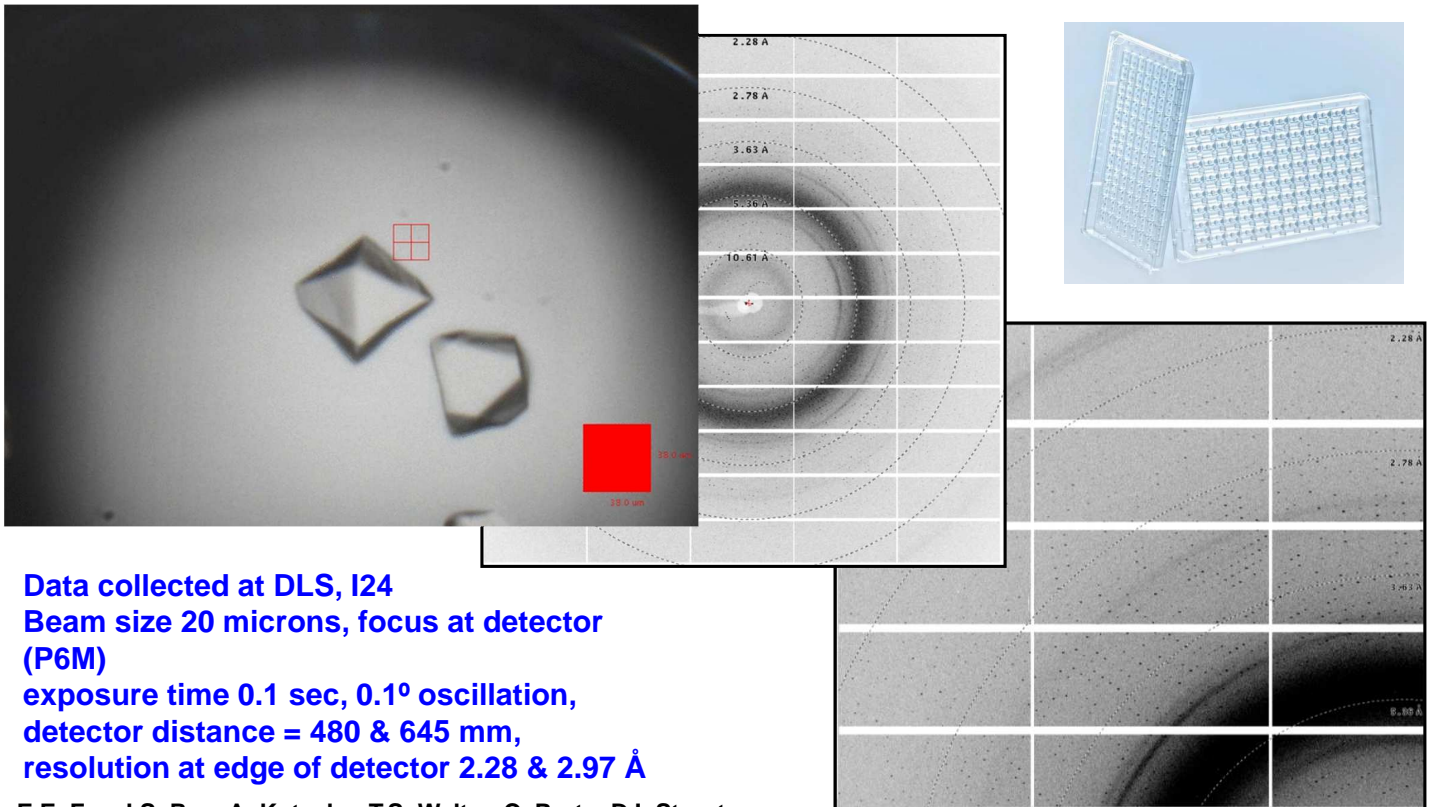


« ensemble » refinement (Phenix)



Bovine enterovirus 2

Crystallization plate screening on I24

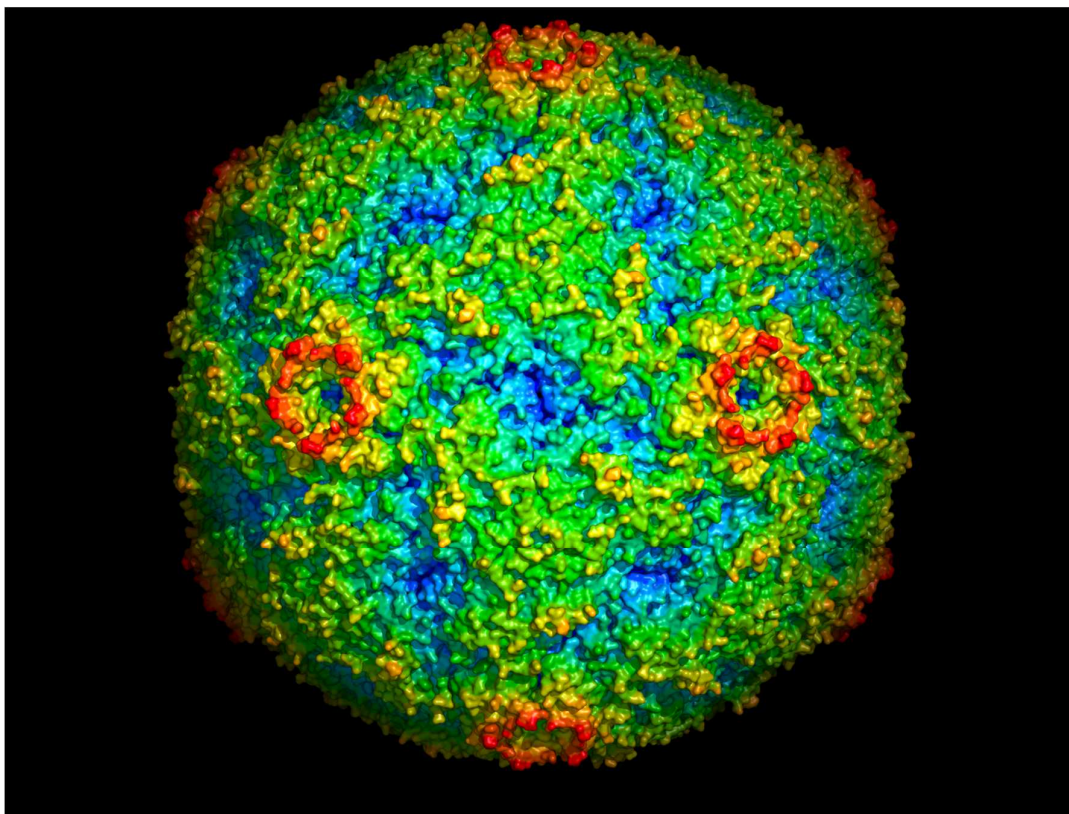


Data collected at DLS, I24
Beam size 20 microns, focus at detector (P6M)
exposure time 0.1 sec, 0.1° oscillation,
detector distance = 480 & 645 mm,
resolution at edge of detector 2.28 & 2.97 Å

E.E. Fry, J.S. Ren, A. Kotecha, T.S. Walter, C. Porta, D.I. Stuart,
The Wellcome Trust Centre for Human Genetics, University of Oxford (UK),
D.J. Rowlands, Institute of Molecular and Cellular Biology, University of Leeds (UK) and
Gwyndaf Evans, Robin Owen, Danny Axford, Jun Ashima, I24, Diamond Light Source (UK)

A new virus structure: Bovine enterovirus 2

Crystallization plate screening on I24 (DLS)



E.E. Fry, J.S. Ren, A. Kotecha, T.S. Walter, C. Porta, D.I. Stuart,
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