

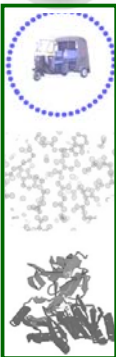
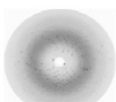
# Has my experiment worked?

Santosh Panjika

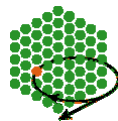
EMBL



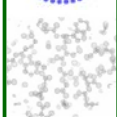
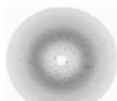
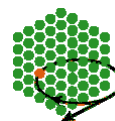
## Experiment ?



- SAD/MAD
- RIP
- MR



## SAD/MAD

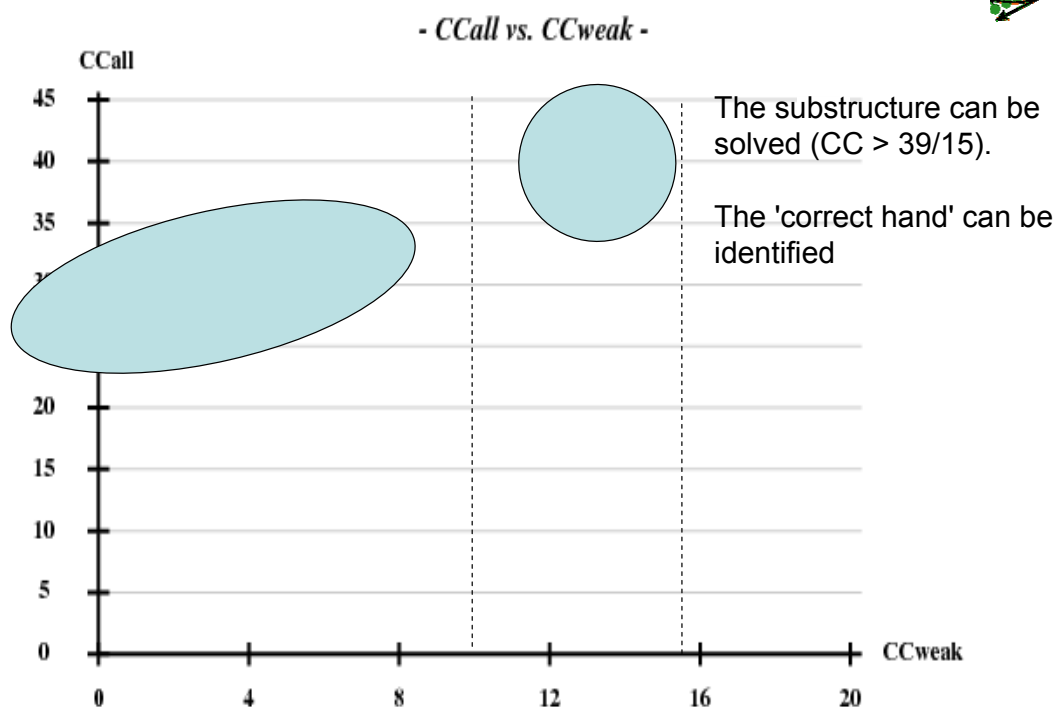
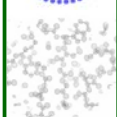
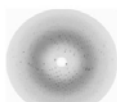


- Anomalous signal can be measured from the crystal
- The original data are complete ( $>90\%$ , better  $>95\%$ )
- The original data are sufficiently strong ( $I/\sigma(I)_{\text{hires}} > 5$ , better  $>10$ )
- The original data have no/few 'problems' (ice-rings, missing data, beam stop shadows etc.)
- The anomalous differences have been measured with sufficient accuracy

Anomalous data  $\rightarrow$  sites  $\rightarrow$  phases  $\rightarrow$  map  $\rightarrow$  model  $\rightarrow$  refinement  $\rightarrow$  validation

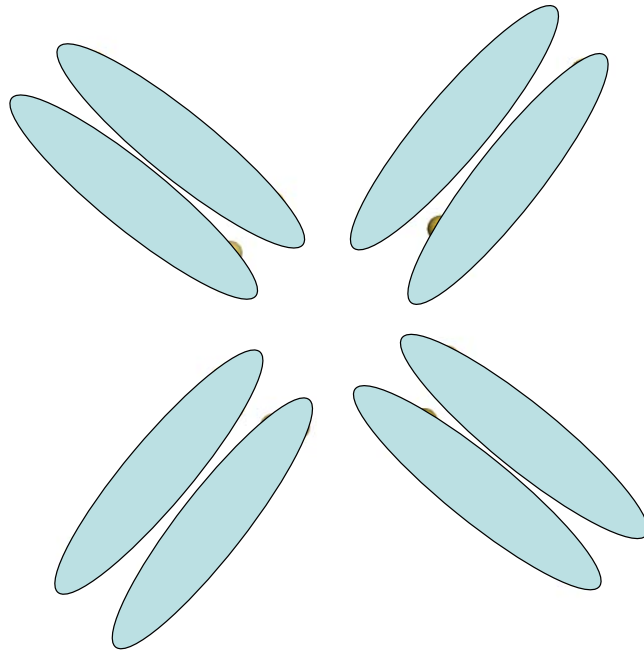
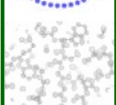
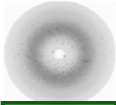
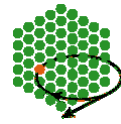
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## First step: Substructure determination



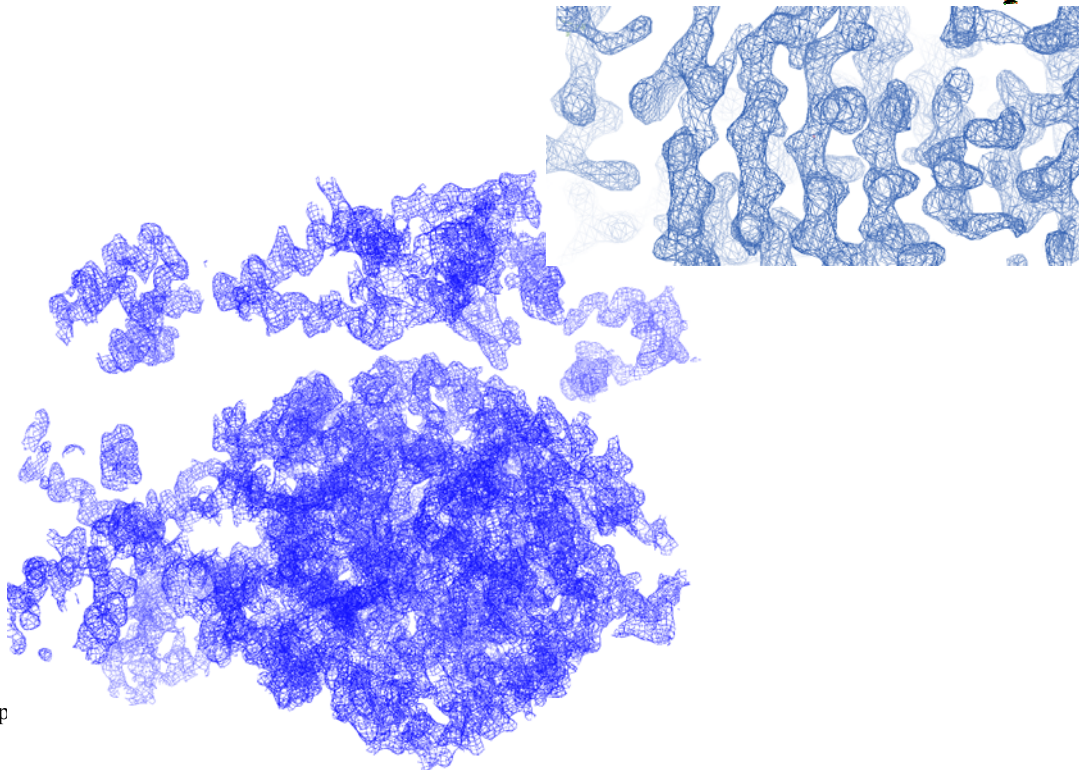
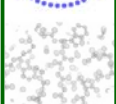
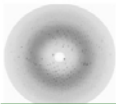
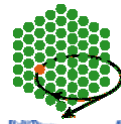
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## NCS determination from substructure



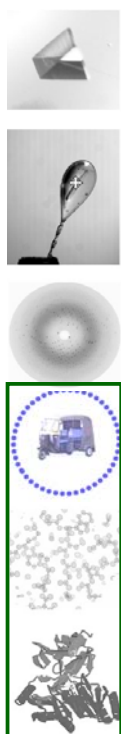
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## Interpretable electron density

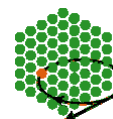
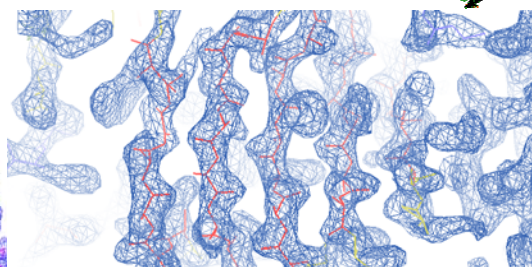
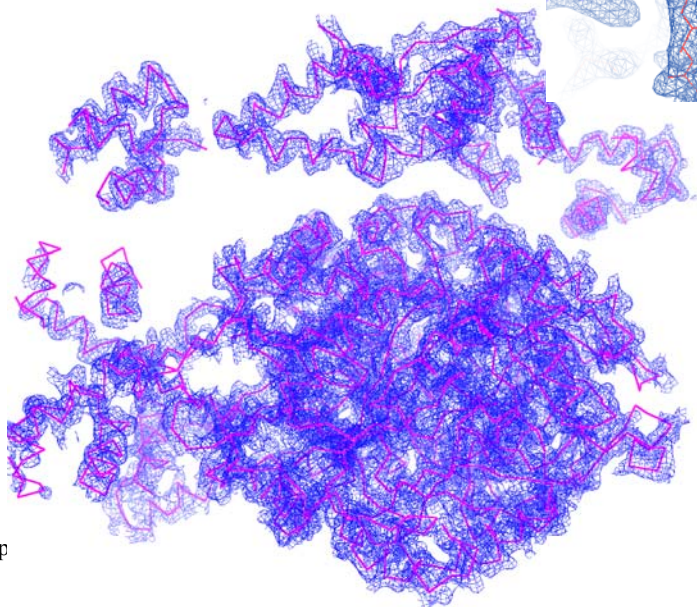


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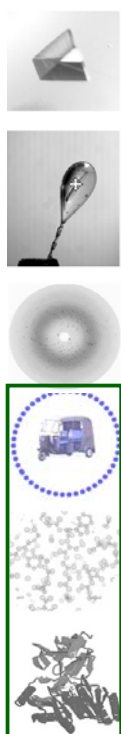
## Partial model



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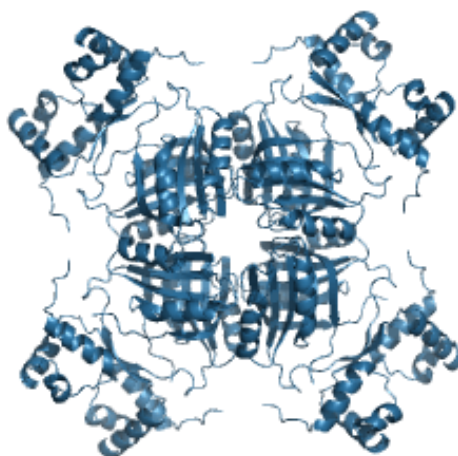


## Ultimate validation of X-ray diffraction experiment



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**Partially** refined structure



Quality of the **partially** refined structure

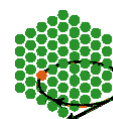
Space group	P2 <sub>1</sub>
Resolution (Å)	2.15
R <sub>cryst</sub> (%)	25.93
R <sub>free</sub> (%)	31.66
Estimated coordinate error (Å)	0.38

### Geometry

R.m.s.d bonds (Å)	0.020
R.m.s.d bonds angle (°)	1.920

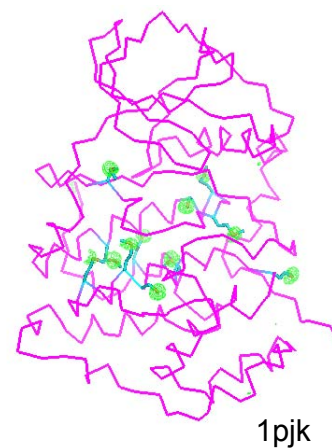
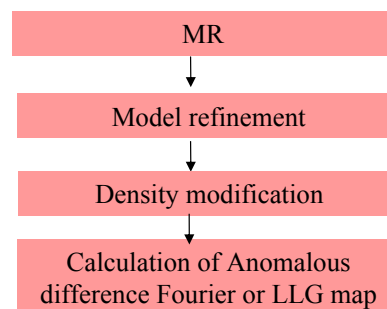
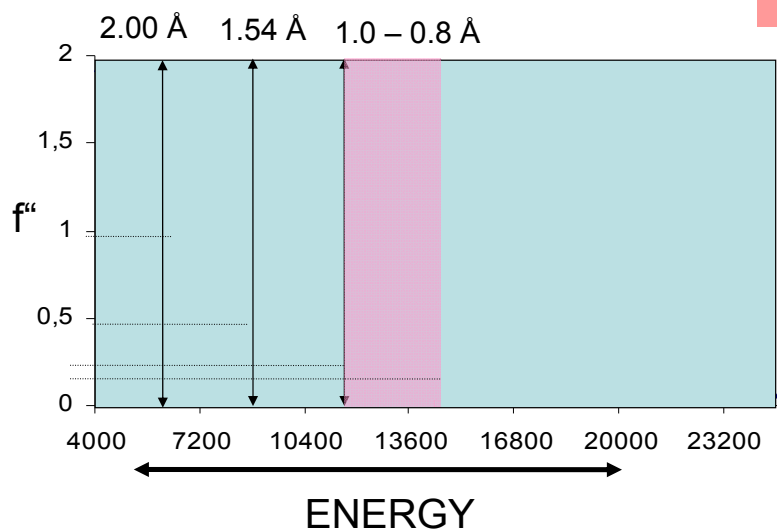
### Ramachandran plot, residues in

Most favoured regions (%)	94.3
Additionally allowed regions (%)	5.5
Generously allowed regions (%)	0.1
Disallowed regions	0.1

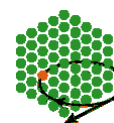
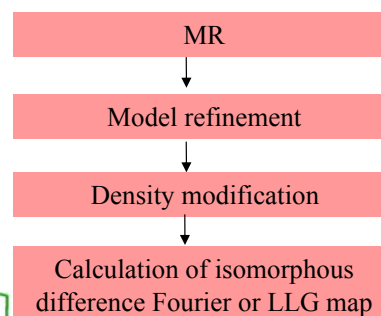
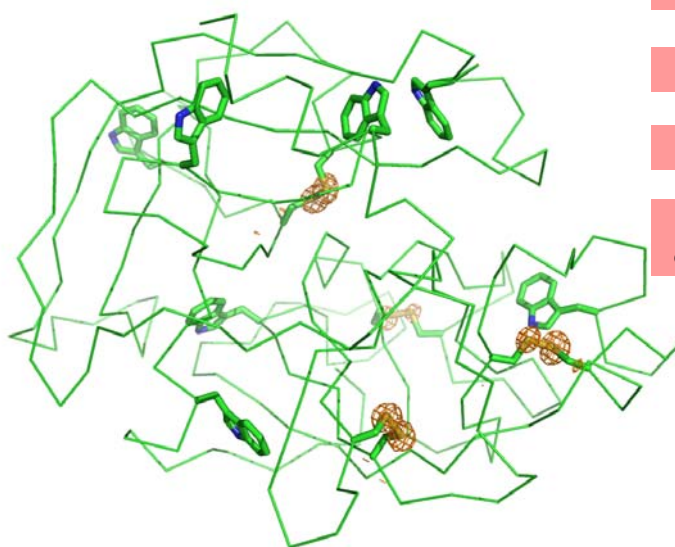
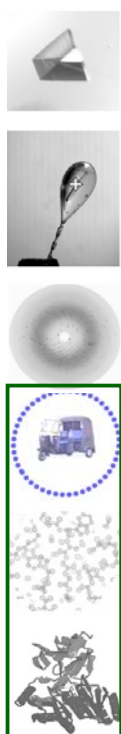


## Long wavelength data for validation of MR

- Collection of Anomalous data

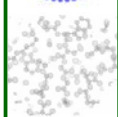
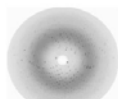
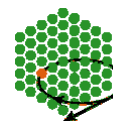


## UV-RIP data for validation of MR





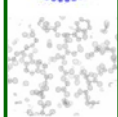
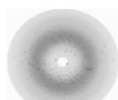
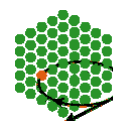
# Automated Structure Determination



- AUTOSHARP/SHARP (Vonrhein et al 2006 /de la Fortelle & Bricogne, 1997)
- SOLVE/RESOLVE (Terwilliger & Berendzen, 1999)
- CHART (Emsley, 1999)
- BnP (Weeks et al., 2001)
- HKL2MAP (Schneider, 2003)
- AcrS (Brunzelle et al., 2003)
- ADSP (BNL)
- ELVES (Holton et al., 2004)
- CRANK (Pannu et al., 2004)
- PHENIX ([www.phenix.org](http://www.phenix.org))
- **Auto-Rickshaw (Panjekar et al., 2005)**
- HKL3000 (Minor et al., 2006)

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## Why ?

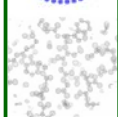
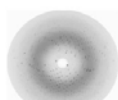
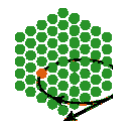


**Primary aim:** To achieve an interpretable electron density map and a partial structure in the minimal time in order **to confirm the success of the experiment** at the synchrotron while the crystal is still at or near the beam line

**Ultimate aim:** To achieve a model, which is correct and as complete as possible

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# The Auto-Rickshaw Approach



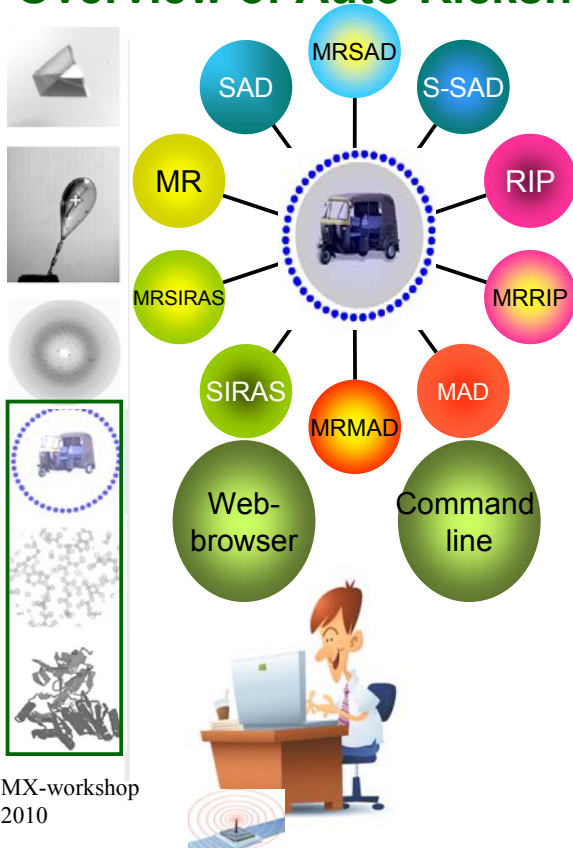
- Try to Mimic What An Experienced Crystallographer would Do

- Try To Be As Fast As Possible ... And (Just) As Good As necessary

- Minimise User Input

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## Overview of Auto-Rickshaw



Version

Beamline  
Advanced

Quick  
Longer



Auto-Rickshaw server

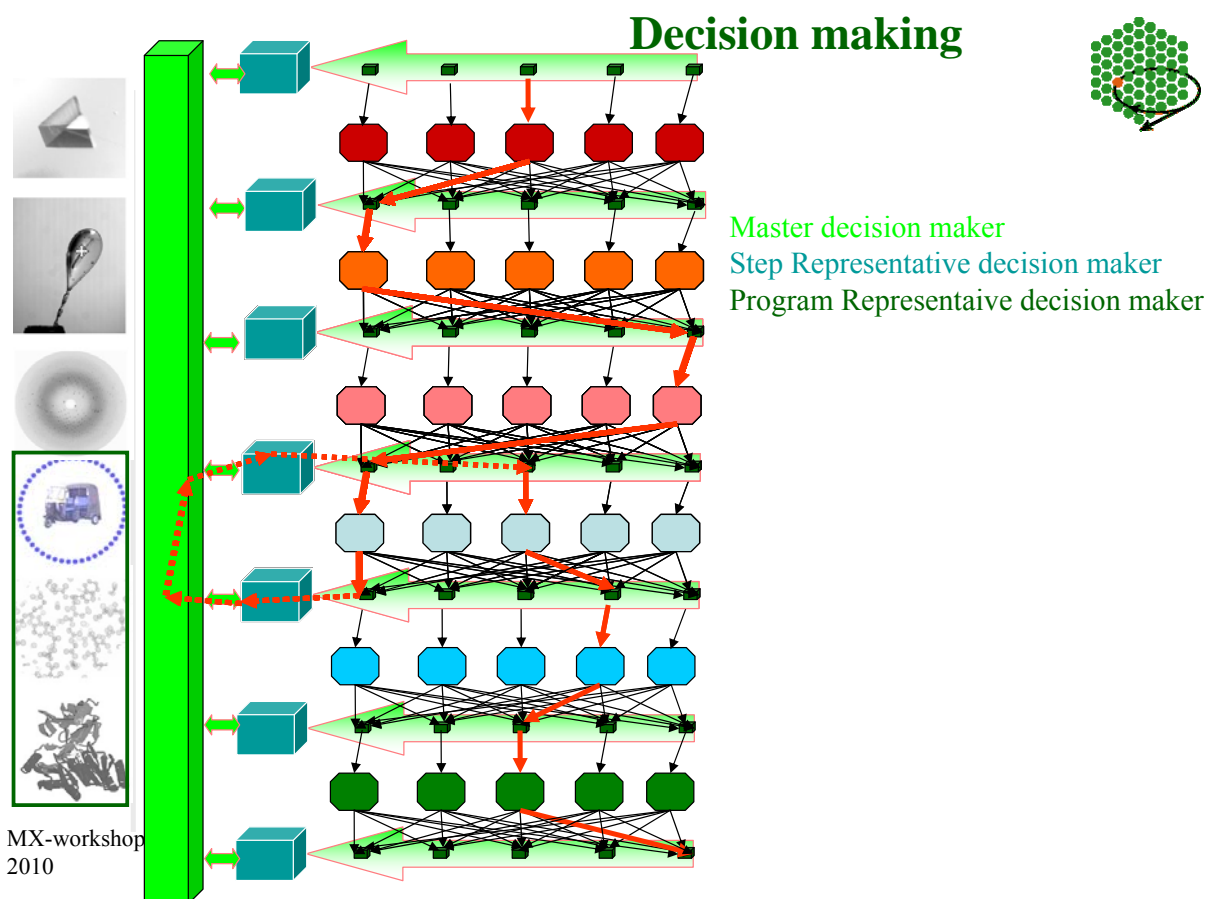
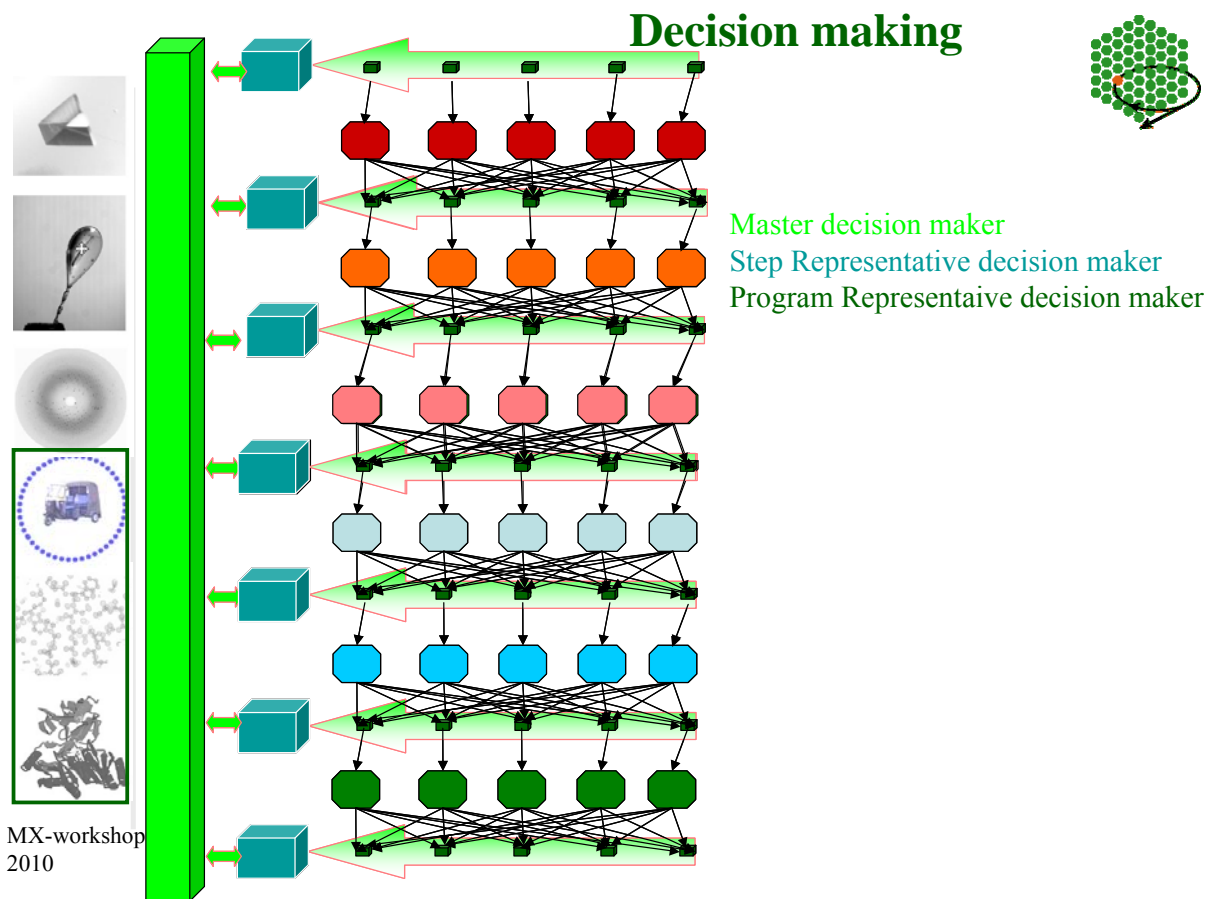
<http://www.embl-hamburg.de/Auto-Rickshaw>

Since 15th April 2008 the AR server is available to world wide crystallographic community

### Necessary Input required

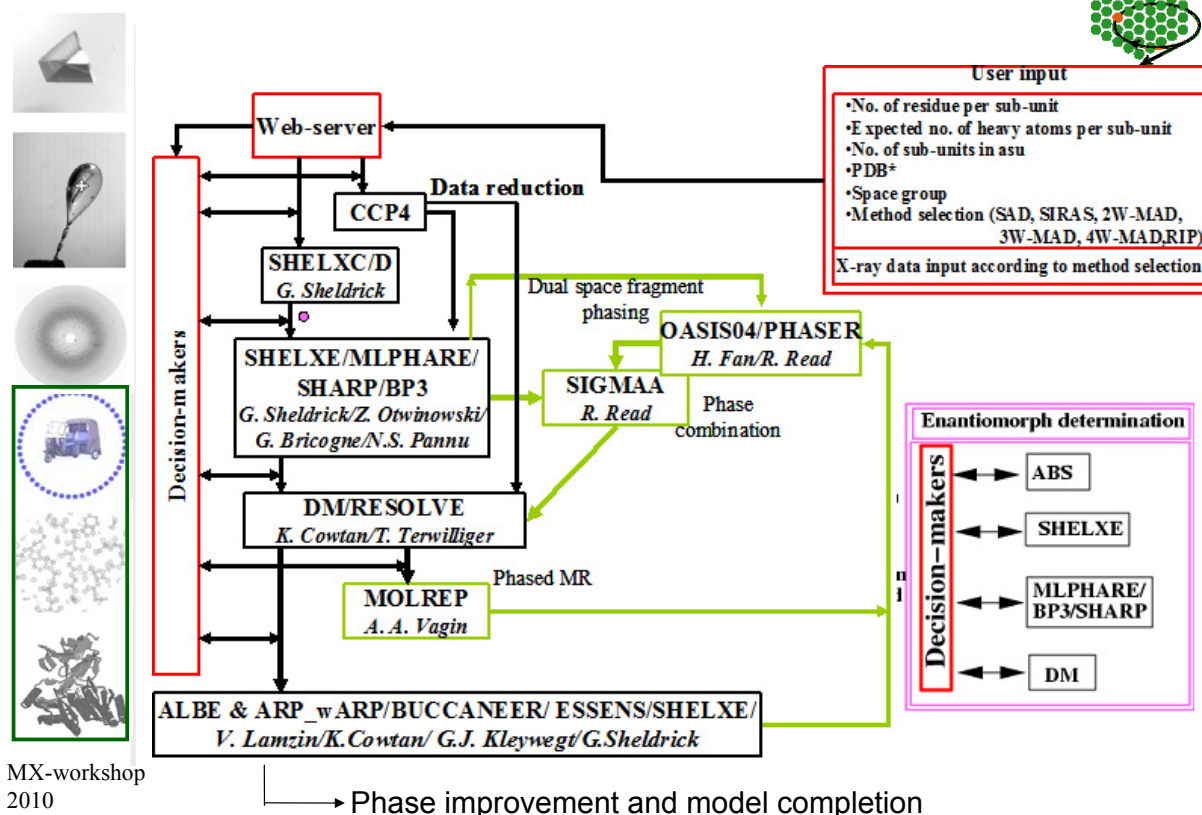
- No. of residues per monomer
- No. of expected heavy atom sites per monomer
- No. of monomers per a.u.
- Space group
- X-ray data
- Sequence file (optional)
- Model file PDB-format (optional)
- Email address

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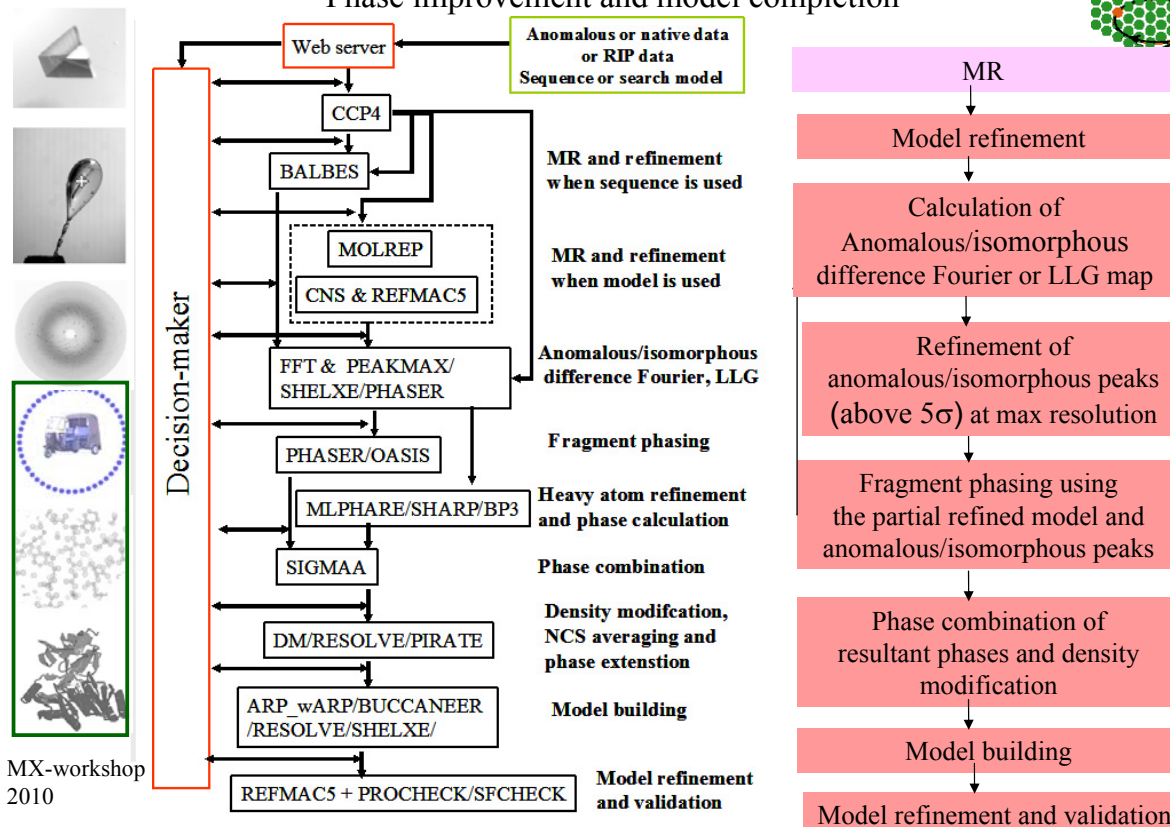


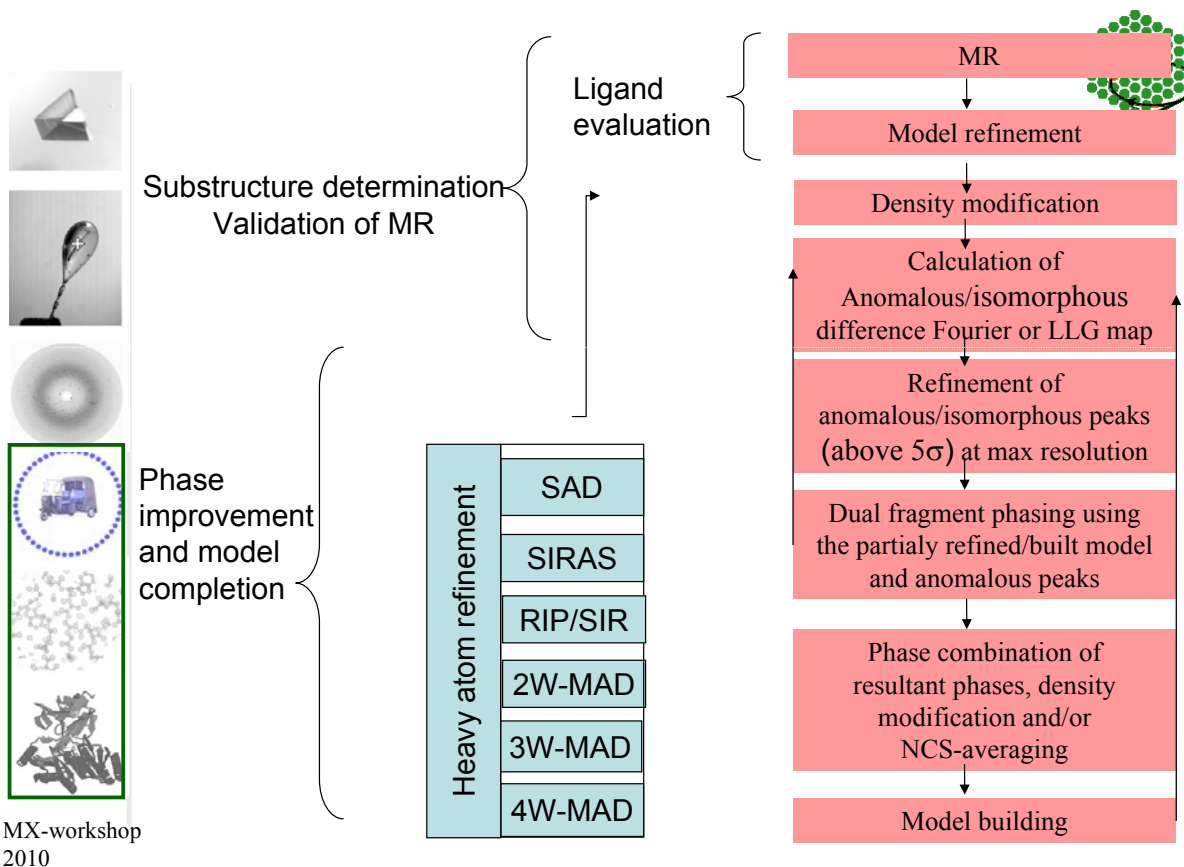
# The Auto-Rickshaw System for experimental phasing



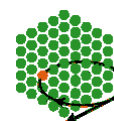
## Software pipeline for combination of MR and experimental phasing

### Phase improvement and model completion





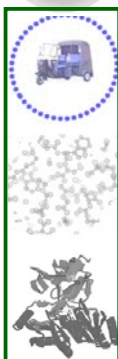
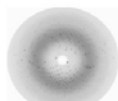
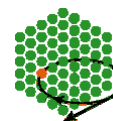
## How long Auto-Rickshaw takes to complete the job



2.0 GHz , 64 CPU-core cluster

Residues/ASU (Average)	Mol./ASU	Beamline version (approx. time)	Advanced version (approx. time)
500	1 or 2	3 min	90 min
1000	>2	15 min	180 min

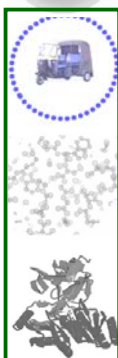
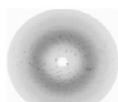
# Impact on Beamline Users



- Collect More Data Than Planned
- Terminate Data Collection Earlier

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## Example-1: Fewer Data Sufficient

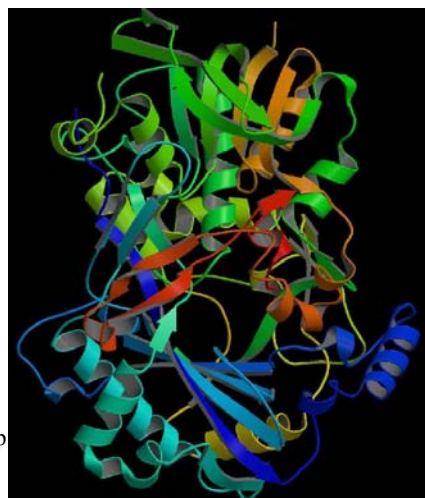


- A protein 593 residues long (solvent content 78%)
- Diffraction to a resolution of 2.55 Å
- Nine Se heavy atoms
- Space group  $P6_422$

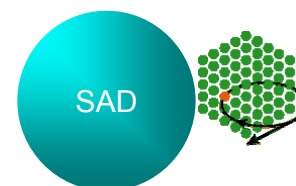
and

X-ray data      data collection

- peak (360°) - finished and processed
- inf (180°) - initiated and halted

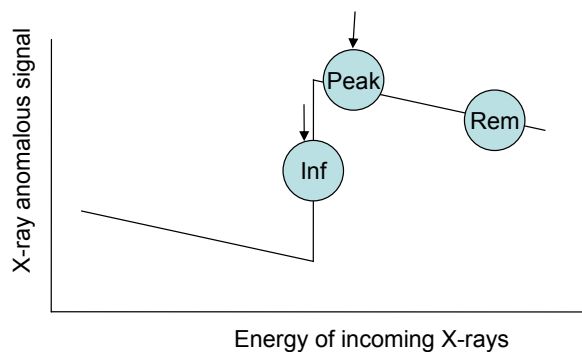


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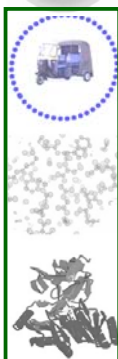
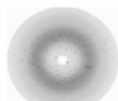
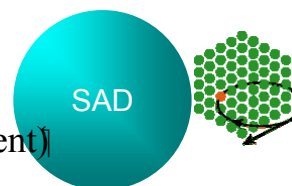


Auto-Rickshaw  
(CCP4+SHELXD+SHELXE+ARP/wARP)

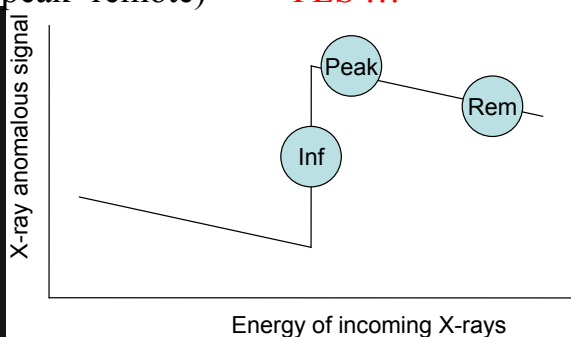
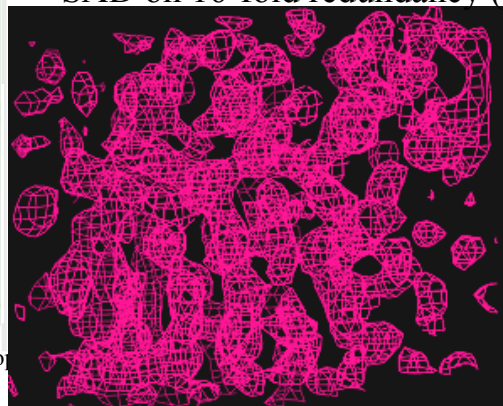
solved in 8 min (369  $\alpha$ -helical atom)  
130 residues traced at cycle 0 (30 min)  
561 residues traced after 2.5 hours



## Example-2: More Data Needed



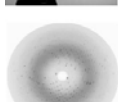
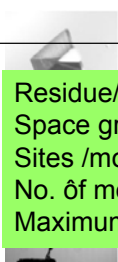
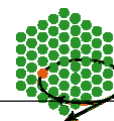
- A protein 250 residues long (48% solvent content)
- Diffraction to a resolution of 3 Å
- One Pt heavy atom expected
- but...
- SAD at peak - no solution
- 2W-MAD at peak and inflection - no solution
- 3W-MAD at peak, inflection and remote - no solution
- SAD on 16-fold redundancy (peak+remote) **YES !!!**



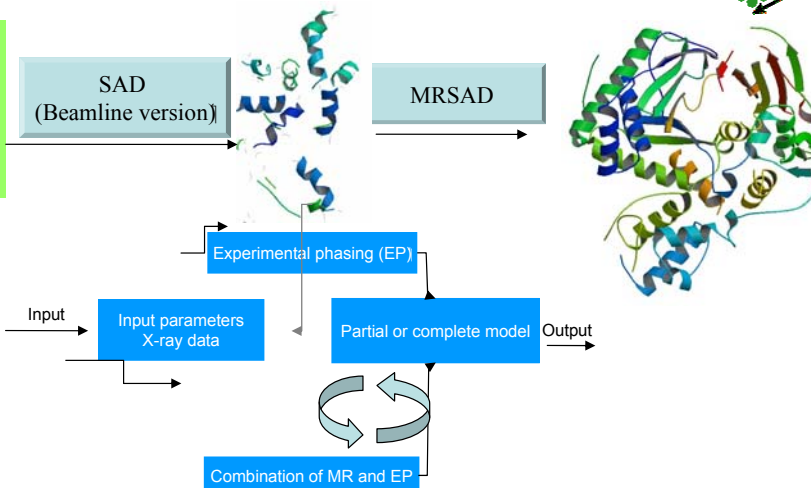
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## Example 3: MRSAD

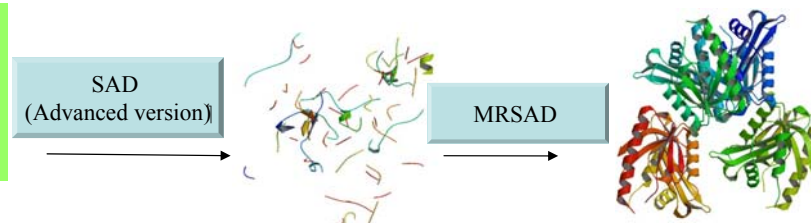
### Build your structure from a partial model



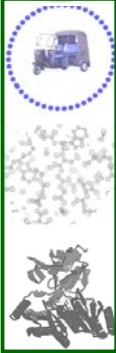
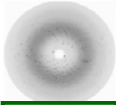
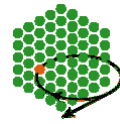
Residue/monomer: 547  
Space group:  $P4_32_12$   
Sites /monomer : 13  
No. of monomer in asu : 1  
Maximum resolution 2.5 Å



Residue/monomer: 261  
Space group:  $P2_1$   
Sites /monomer : 3  
No. of monomer in asu : 4  
Maximum resolution 2.30 Å



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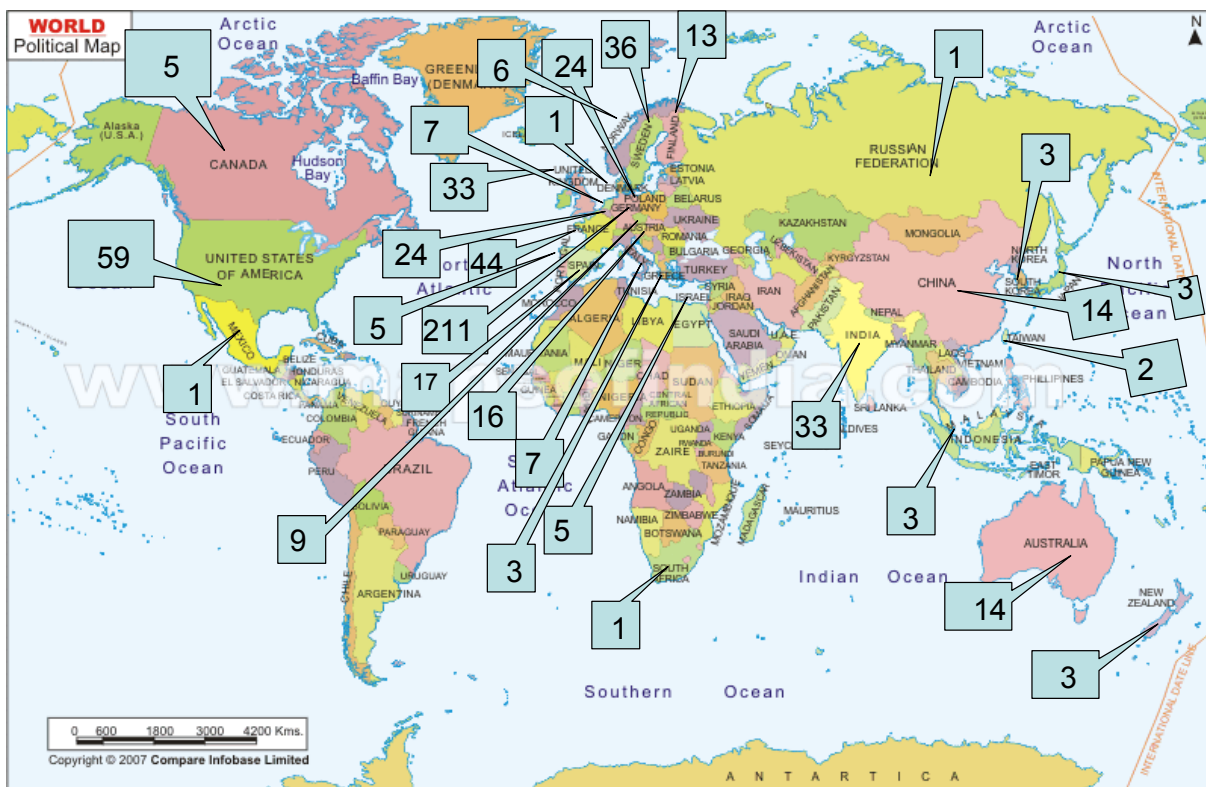
## An automatic output for the materials and methods

It includes also the references for all the programs which are used for the structure solution.

It guides the user how to acknowledge the pipeline along with the software used in the pipeline.

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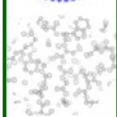
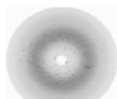
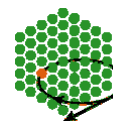
Geographic distribution of AR users



Total registered institutes : 257    Total unique users : 665



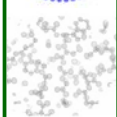
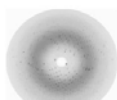
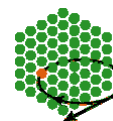
## Status and Highlights



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- More than 1300 novel structures solved
- Largest structure solved : asymmetric unit contains 7,500 residues
- Largest substructure solved: 140 heavy atoms sites in asymmetric unit
- A typical beamline user submits approximately 20-25 jobs with different phasing protocol during a data collection

## Auto-Rickshaw



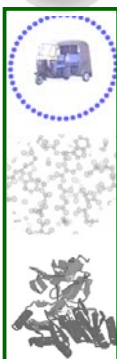
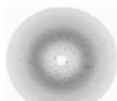
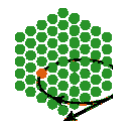
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### The Future



- Reduction of the number of input parameters
- Addition of other phasing protocols (i.e. MIR)
- Parallel computing at various steps of structure determination
- Scanning and improvement of decision making parameters
- Remote connection with data collection/processing software ( i.e. XIA2 and DNA)
- Auto-Rickshaw provides results in almost real time for data collection at home sources or second generation synchrotrons. Accelerating it further for third generation sources is a great challenge.

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More infos:

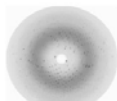
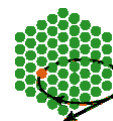


The Auto-Rickshaw Web Site:

<http://www.embl-hamburg.de/Auto-Rickshaw>

Google Auto-Rickshaw and EMBL

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Thank you for your attention