

# **SAXS and Crystallography**

## **Comparison of information and limits**

**Small angle X-ray  
scattering - SAXS**

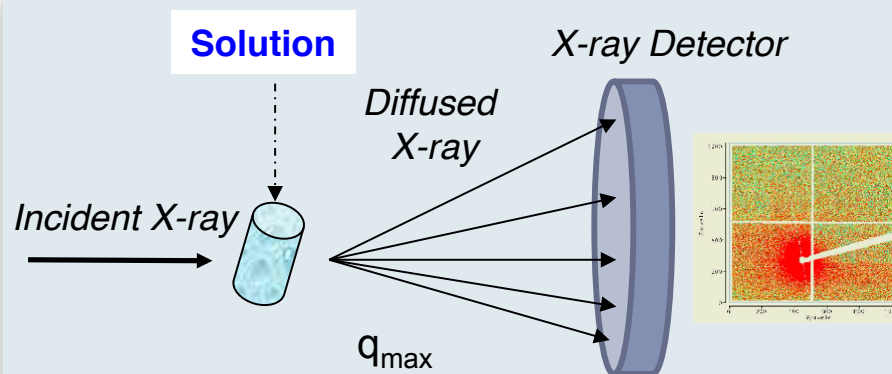
**Average conformation  
in solution**

**Crystallography**

**Crystal structure**

## SAXS

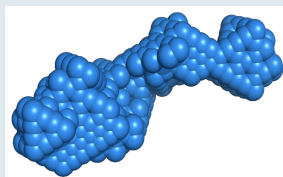
**Average conformation in solution**



**Data processing**

**Analysis**  
*Ab initio*  
 Molecular model

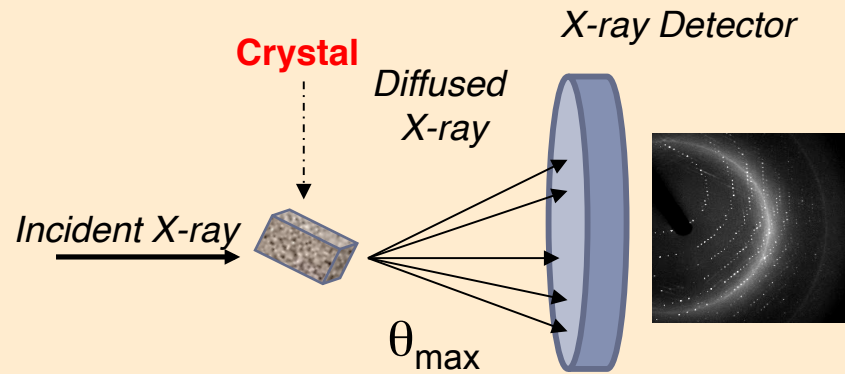
**Model or envelope**



Limiting factors:  
**Aggregation and heterogeneity**

## Crystallography

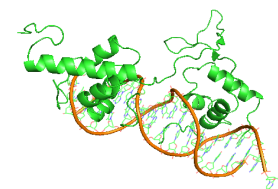
**Crystal structure**



**Data processing**

**Resolution**  
 Molecular replacement  
 MAD, SAD, MIR,...

**3D structure**



Bottle-neck:  
 crystallization

## SAXS



High concentration solution



Limitations

- Purity
- Quantity
- Concentration
- Solubility

Sample

## Crystallography



Frozen crystal



Limitations

- Purity
- Quantity
- Concentration
- Solubility
- Crystallization = **Bottle neck**

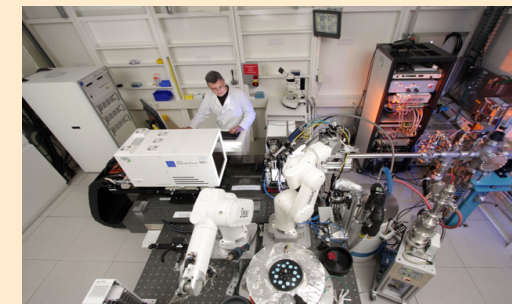
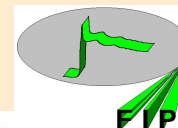
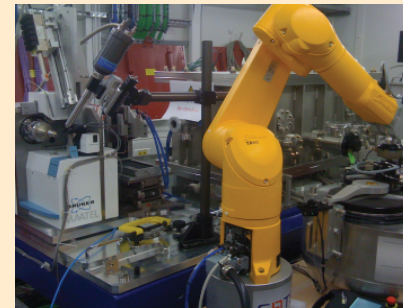
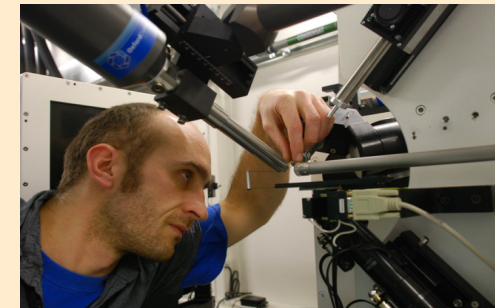
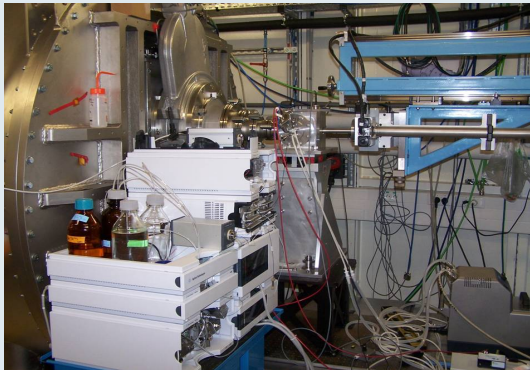
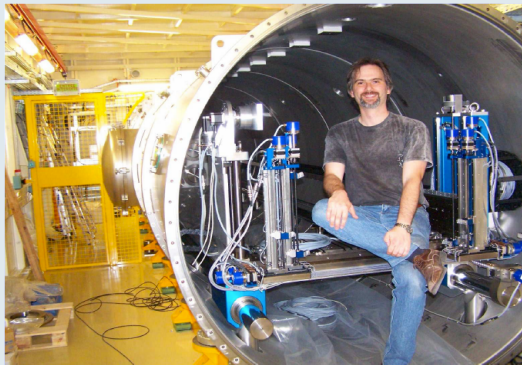
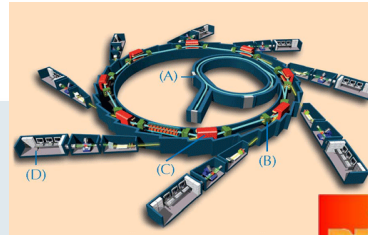
For a given project, if crystals can grow then the sample solution is compatible with SAXS constraints.

**BUT**, a sample solution compatible with SAXS may never crystallize.

SAXS

Crystallography

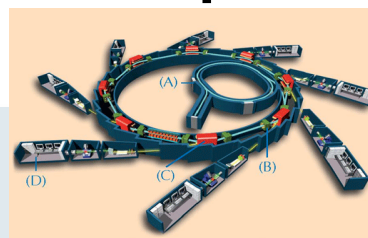
## Data acquisition



# SAXS

# Crystallography

## Data acquisition



|                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|-------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Gamme d'énergie</b>              | Between ~5 and 17 keV                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Résolution en énergie</b>        | ~2 eV                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Source</b>                       | In-vacuum U20 undulator.<br>Source Size (sigma, $\mu\text{m}$ ): 388 (H) x 8.1 (V)<br>Source Divergence (sigma, $\mu\text{rad}$ ): 14.5 (H) x 4.6 (V)                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Optiques</b>                     | Diaphragm at 11.7 m ( $1 \times 0.5 \text{ mm}^2$ )<br>Fixed exit DCM Si <sub>111</sub> at 20 m<br>Fixed incidence focusing KB at 22.5 m<br>Sample position : 30 - 32 m<br>Detector / Sample Distance : 0.5 - 8 m                                                                                                                                                                                                                                                                                                                                                  |
| <b>Environnements d'échantillon</b> | X / Z precision table<br>Stopped flow device for chemistry<br>Online HPLC for proteins in solution ( <a href="#">SAXIER</a> project  )<br>High throughput sampler for proteins in solution ( <a href="#">SAXIER</a> project  )<br>Couette Cell (collaboration with LPS, Orsay)<br>GISAXS chamber<br>Automatic sample changer (50 samples, thermostated)<br>Linkam heating stage THMS600<br>... |
| <b>Taille du faisceau</b>           | 450x20 $\mu\text{m}^2$ FWHM dans la cabane expériences                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <b>Flux sur l'échantillon</b>       | $8.10^{12}$ ph/s @7keV, $8.10^{11}$ ph/s @16keV (à 400 mA de courant anneau)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>Détecteurs</b>                   | SAXS : PCCD170170 (AVIEX), Gain > 3ADU/ph, Noise≈2ADU<br>WAXS (2014) : Détecteur à pixels hybrides                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <b>Chambre de détection</b>         | Vide primaire, positions du détecteur SAXS :<br>- 0.20 / + 0.20 m (horiz), -0.20 / +0.20 (vert), 0.5 m / +6 m (le long du faisceau).                                                                                                                                                                                                                                                                                                                                                                                                                               |

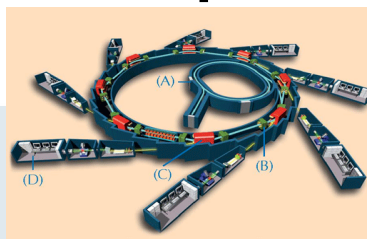
### Données techniques

|                                     |                                                                                                                                                                                                                                           |
|-------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Energy range</b>                 | Between 5 KeV and 15 KeV                                                                                                                                                                                                                  |
| <b>Energy Resolution</b>            | ~0.000075 (Si 311) - ~0.0002 (Si 111)                                                                                                                                                                                                     |
| <b>Source</b>                       | U20 In-vacuum undulator                                                                                                                                                                                                                   |
| <b>Flux @ first optical element</b> | White beam – depends on undulator settings                                                                                                                                                                                                |
| <b>Optics</b>                       | Kirkpatrick-Baez pair of bi-morph mirrors plus channel cut cryogenically cooled monochromator crystal                                                                                                                                     |
| <b>Sample Environment</b>           | 3 circle $\kappa$ goniostat (10 $\mu\text{m}$ sphere of confusion)<br>6 axis robot sample changer (MSC/Rigaku ACTOR)<br>Oxford Cryosystems cryostream<br>Si drift diode energy dispersive detector plus MCA for fluorescence measurements |
| <b>Beam size at sample</b>          | Variable between $100 \times 100 \mu\text{m}^2$ to $250 \times 250 \mu\text{m}^2$                                                                                                                                                         |
| <b>Flux on sample</b>               | > $2.0 \times 10^{12}$ Phot/s/0.02%bw for 500 mA stored current.                                                                                                                                                                          |
| <b>Detectors</b>                    | ADSC Q315r                                                                                                                                                                                                                                |
| <b>Polarization</b>                 | Linear                                                                                                                                                                                                                                    |

# SAXS

# Crystallography

## Data acquisition



|                                     |                                                                                                                                                                                                                                                                                                                                                   |
|-------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Gamme d'énergie</b>              | Between ~5 and 17 keV                                                                                                                                                                                                                                                                                                                             |
| <b>Résolution en énergie</b>        | ~2 eV                                                                                                                                                                                                                                                                                                                                             |
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| <b>Taille du faisceau</b>           | 450x20 $\mu\text{m}^2$ FWHM dans la cabane expériences                                                                                                                                                                                                                                                                                            |
| <b>Flux sur l'échantillon</b>       | $8.10^{12}$ ph/s @7keV, $8.10^{11}$ ph/s @16keV (à 400 mA de courant anneau)                                                                                                                                                                                                                                                                      |
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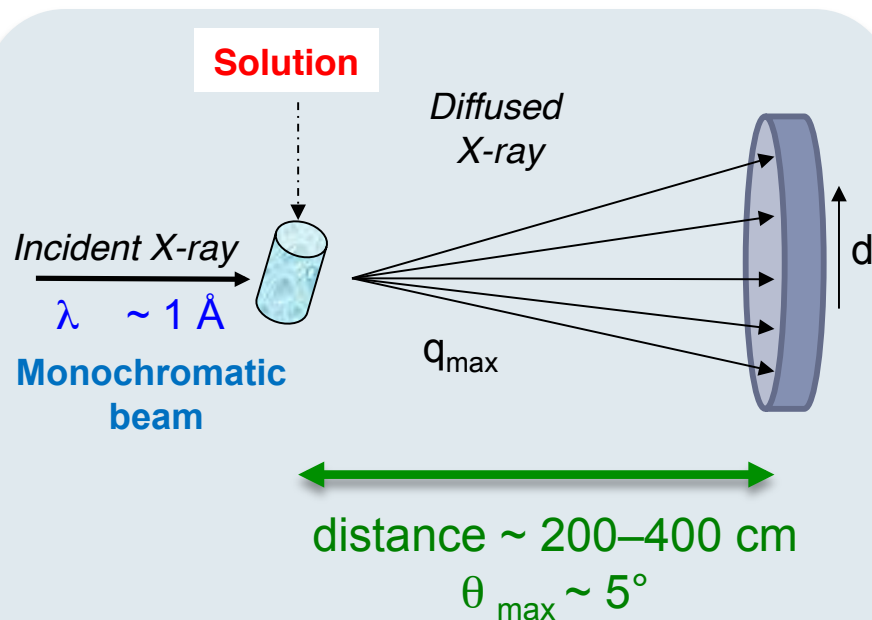
### Données techniques

|                                     |                                                                                                                                                                                                                                           |
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| <b>Energy range</b>                 | Between 5 KeV and 15 KeV                                                                                                                                                                                                                  |
| <b>Energy Resolution</b>            | ~0.000075 (Si 311) - ~0.0002 (Si 111)                                                                                                                                                                                                     |
| <b>Source</b>                       | U20 In-vacuum undulator                                                                                                                                                                                                                   |
| <b>Flux @ first optical element</b> | White beam - depends on undulator settings                                                                                                                                                                                                |
| <b>Optics</b>                       | Kirkpatrick-Baez pair of bi-morph mirrors plus channel cut cryogenically cooled monochromator crystal                                                                                                                                     |
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| <b>Beam size at sample</b>          | Variable between 100x100 $\mu\text{m}^2$ to 250x250 $\mu\text{m}^2$                                                                                                                                                                       |
| <b>Flux on sample</b>               | > 2.0 e+12 Phot/s/0.02%bw for 500 mA stored current.                                                                                                                                                                                      |
| <b>Detectors</b>                    | ADSC Q315r                                                                                                                                                                                                                                |
| <b>Polarization</b>                 | Linear                                                                                                                                                                                                                                    |

# SAXS

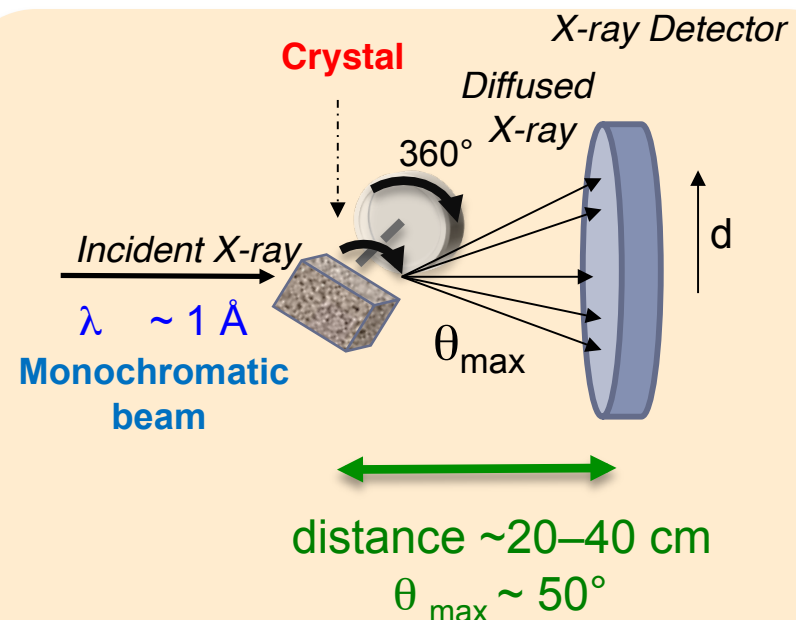
# Crystallography

## Data acquisition



$$d_{\max} \sim 6 \text{ \AA}$$

The notion of resolution in SAXS should be modulated because we obtain an average of conformations



$$d_{\max} \sim 0.6 \text{ \AA}$$

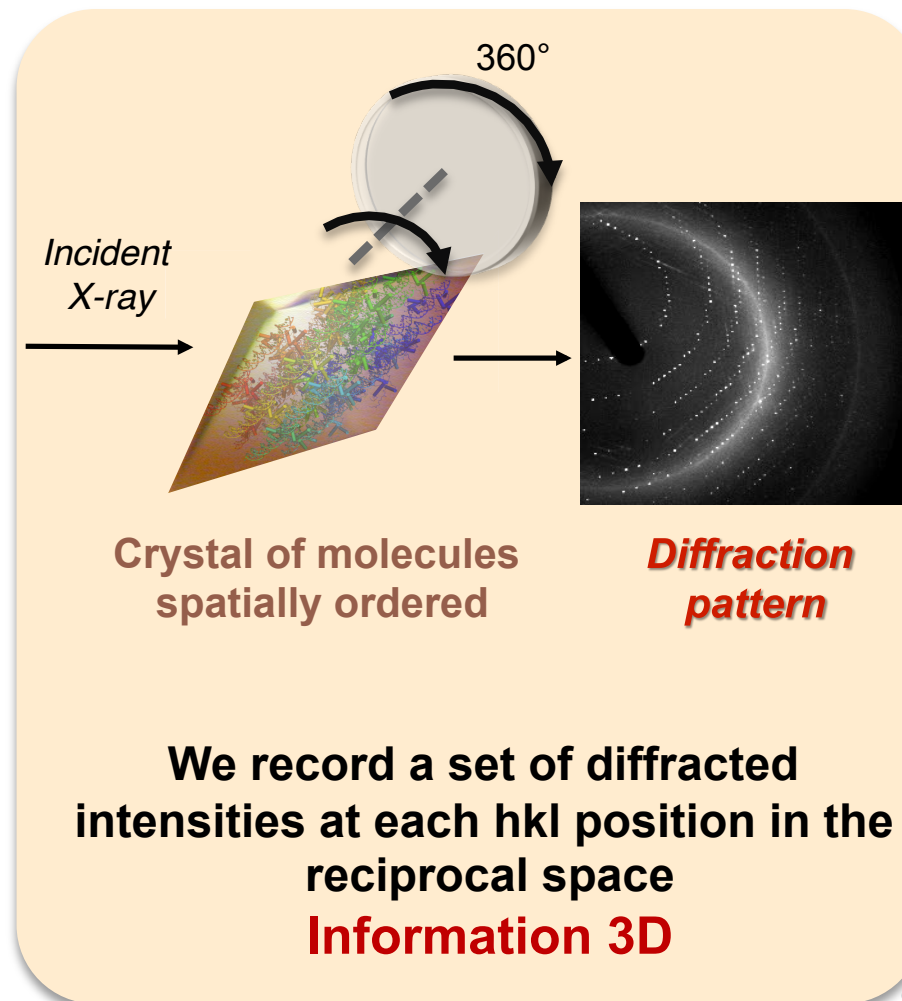
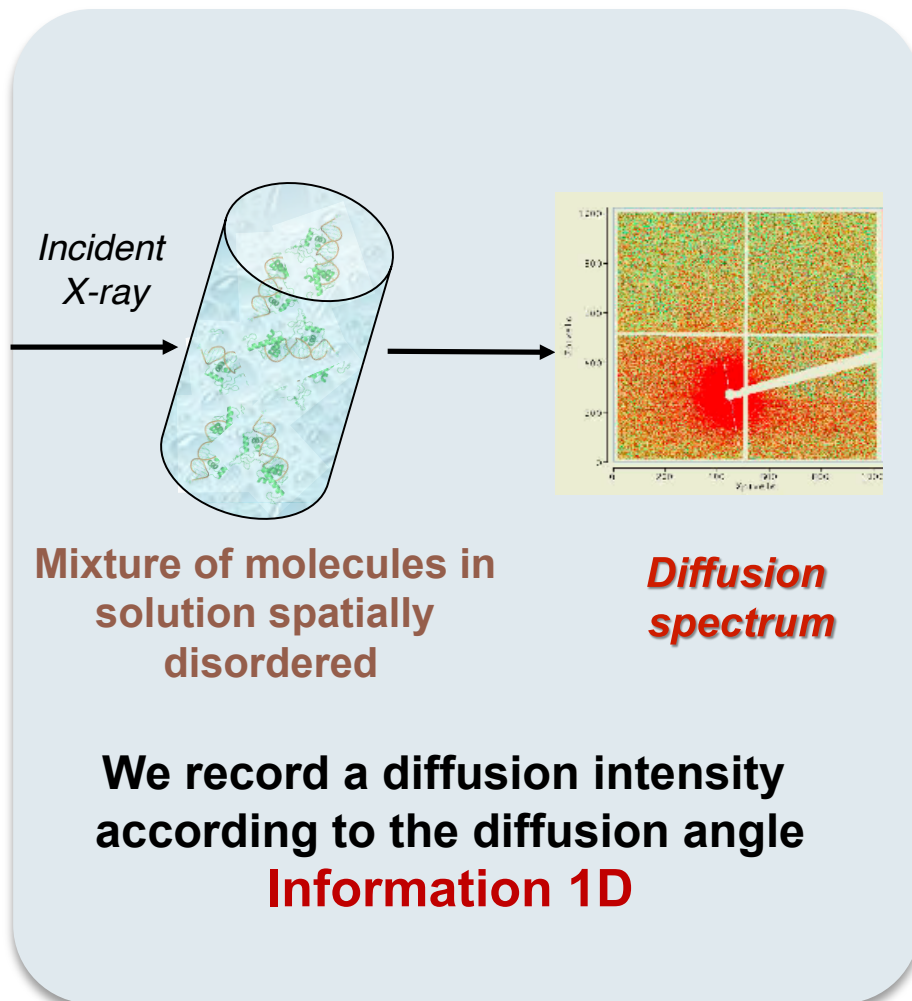
$$2 d \sin \theta = \lambda$$

$$\Leftrightarrow d = \lambda / 2 \sin \theta$$

## SAXS

## Crystallography

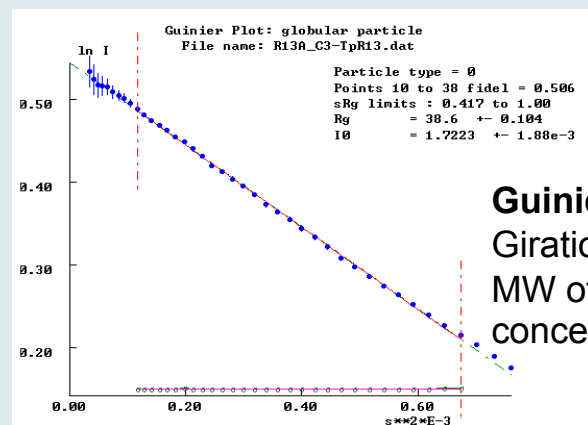
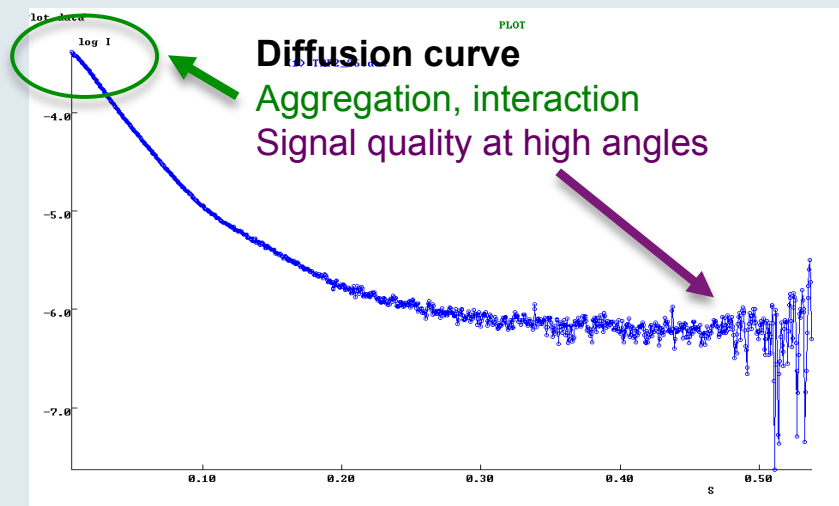
### Data acquisition



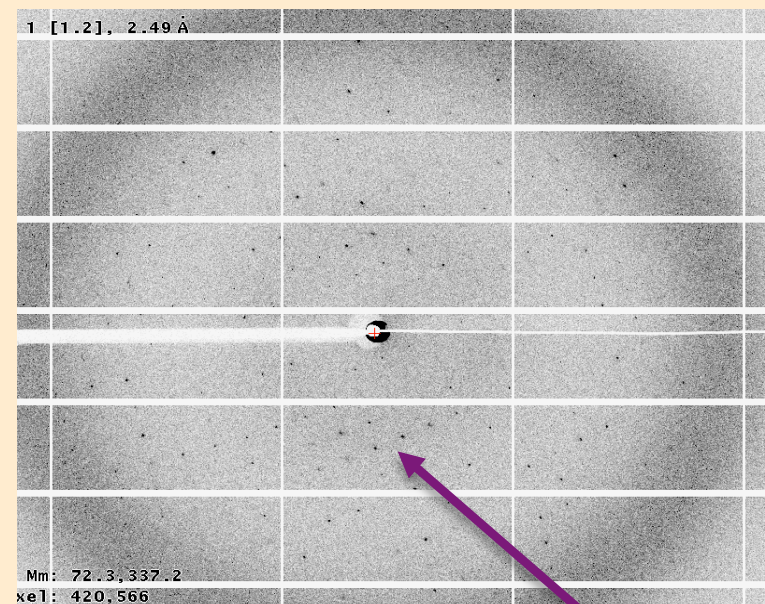
# SAXS

# Crystallography

## Direct informations



Diffraction quality  
 (monocrystal)



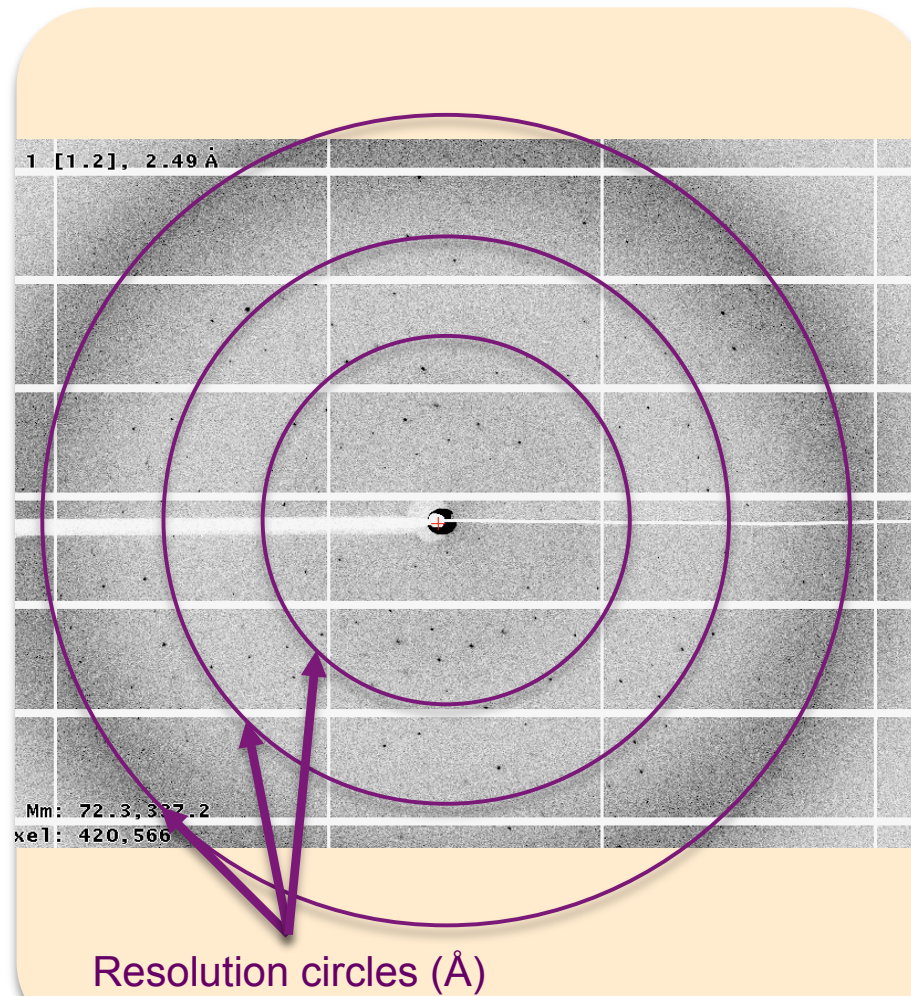
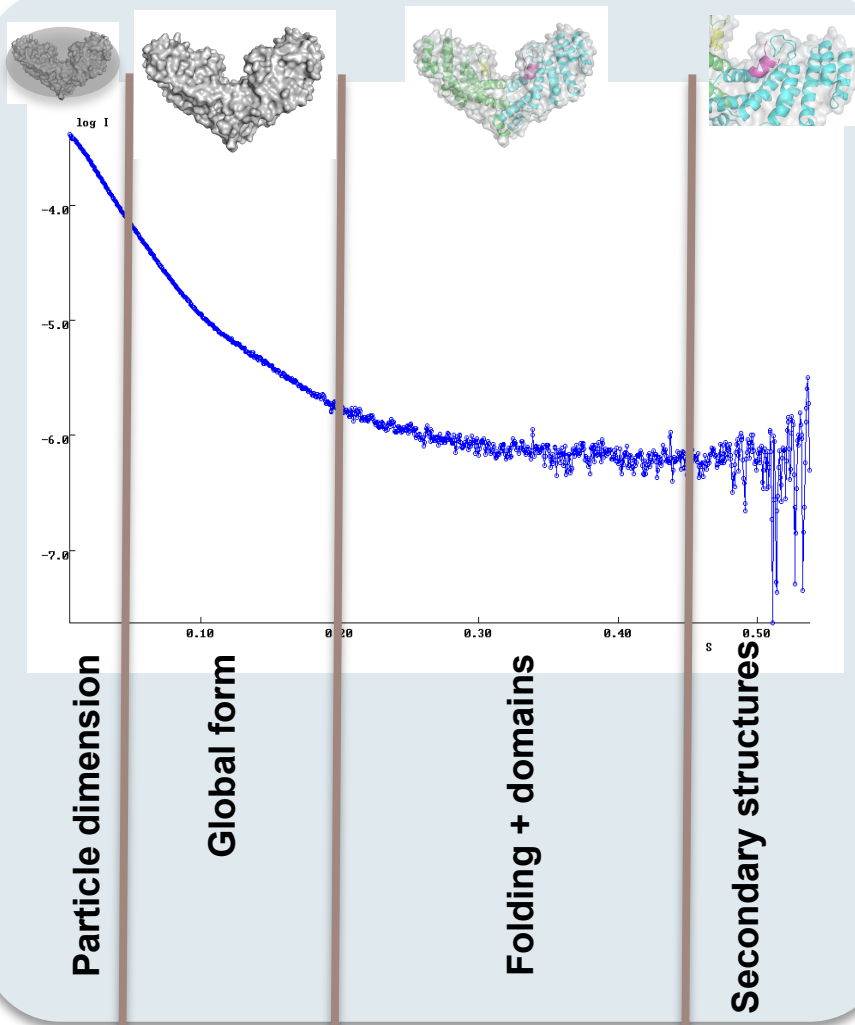
Unit cell parameters

## SAXS



### Direct informations

## Crystallography



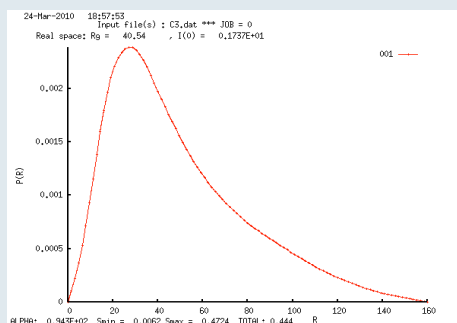
## SAXS

### Calculated informations (Fourier transform)

#### P(R)

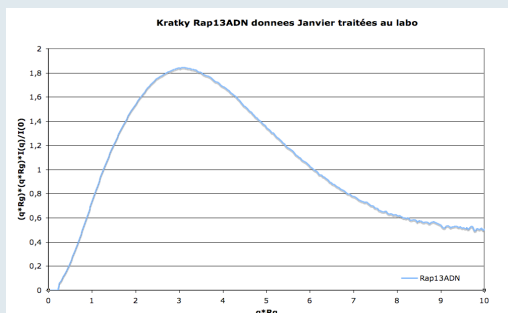
R<sub>g</sub>, D<sub>max</sub>

First evaluation of the particle shape



#### Kratky plot

Evaluation of the structuration level of the particle



## Crystallography

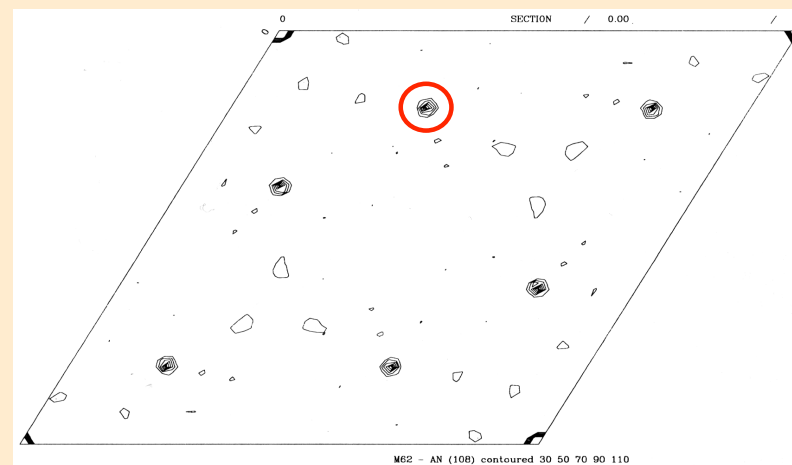
#### Patterson function

Patterson difference map

Position of heavy atoms or anomalous peaks

We calculate a Patterson difference map with the coefficients:

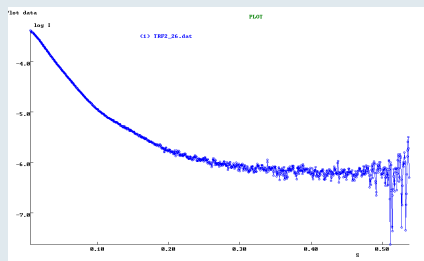
$$\Delta F_{\text{iso}} \text{ ou } \Delta F_{\text{ano}}$$



## SAXS

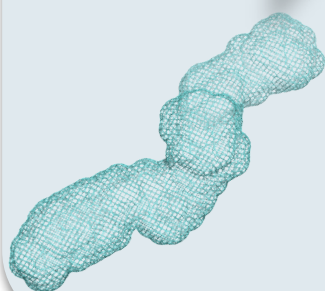
## Crystallography

### Data analysis



*ab initio*  
Approaches  
envelope  
calculation

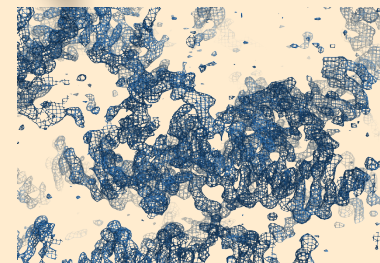
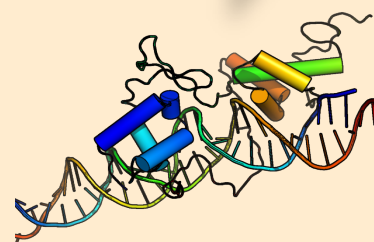
3D model  
calculation



Molecular  
replacement

Structure  
resolution

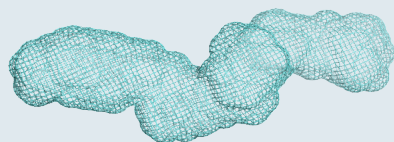
Experimental  
approaches  
MIR, SAD, MAD,  
SIRAS,...



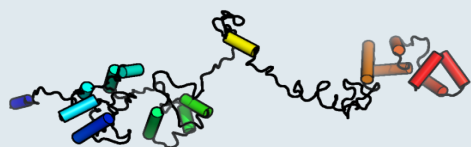
## SAXS



**Envelope or 3D model**



**3D average conformation in solution**



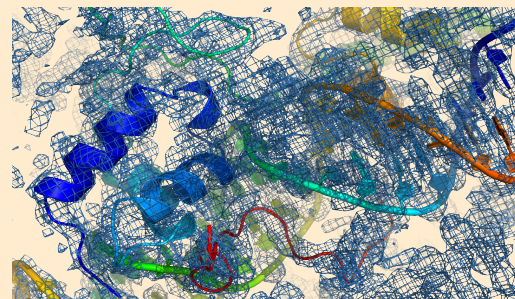
*Global properties analysis of the particle in solution: proportions, flexibility, assembly,...*

**Result**

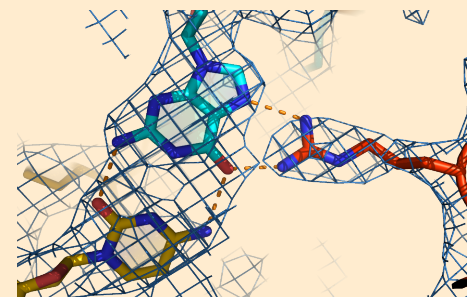
## Crystallography



**3D Structure**



**3D structure of the particle in the crystal**



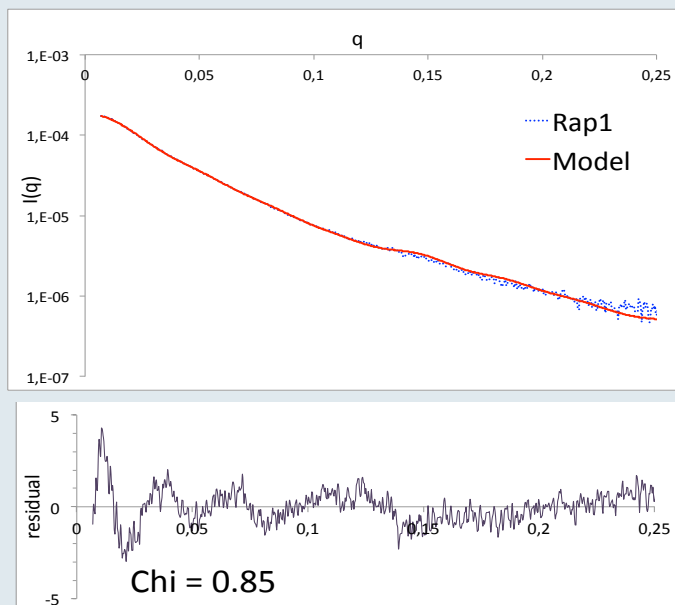
*Atomic scale analysis of the crystal structure: conformation, distance, electron delocalization,...*

## SAXS

### Results evaluation

## Crystallography

#### Agreement between calculated and experimental data



$$\text{Residual} = (I_{\text{th}} - I_{\text{exp}}) / \sigma(I_{\text{exp}})$$

$$\text{Chi} = 1 / (N - 1) \sum_1^N [(I_{\text{th}} - I_{\text{exp}}) / \sigma(I_{\text{exp}})]^2$$

Based on few hundreds of intensities

| Data collection                                      |                                               |
|------------------------------------------------------|-----------------------------------------------|
| Wavelength (Å)                                       | 0.98                                          |
| Space-group                                          | P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub> |
| Diffraction limits (last shell)                      | 2.95 Å (3.13 Å – 2.95 Å)                      |
| Unit cells (a×b×c×α×β×γ)                             | 40.6×102.9×116.8<br>90×90×90                  |
| R <sub>merge</sub>                                   | 0.138 (0.688)                                 |
| Number of unique reflections                         | 9831                                          |
| I/σ                                                  | 14,32 (3.39)                                  |
| Completeness                                         | 0.901 (0.688)                                 |
| Molecular replacement                                |                                               |
| Refinement                                           | Buster5                                       |
| Resolution                                           | 2.95 Å                                        |
| R <sub>work</sub>                                    | 0.1923                                        |
| R <sub>free</sub>                                    | 0.2650                                        |
| Figure of merit                                      | 0.869                                         |
| Number of residues                                   | 223                                           |
| Number of bases                                      | 62                                            |
| Number of water molecules                            | 6                                             |
| RMSD bond                                            | 0.0010                                        |
| RMSD angles                                          | 1.48                                          |
| Average B-factor (protein, Å <sup>2</sup> )          | 62                                            |
| Average B-factor (DNA, Å <sup>2</sup> )              | 100                                           |
| Average B-factor (residues 675-601, Å <sup>2</sup> ) | 69                                            |
| PDB entry                                            | 3UKG                                          |

Multiple evaluation parameters of the structure  
 Few thousand to hundred thousand of  
 diffraction intensities  
 A few thousand atoms  
**⇒ Reliability ++**

## SAXS



### Methodological limits

## Crystallography



#### Sample production

- Pur  
    ➡ > 95%
- Large quantity  
    ➡ in the order of mg
- High concentration solubility  
    ➡ in the order of mg/ml
- Resisting the irradiation during the experiment (no sample freezing)
- Not aggregating

#### Sample production

- Pur  
    ➡ > 95%
- Large quantity  
    ➡ in the order of mg
- High concentration solubility  
    ➡ in the order of mg/ml
- Able to crystallize
- Diffracting crystals
- Allowing to calculate an electron density map

## SAXS



### Information Limits

## Crystallography



**Envelope or 3D model  
Average 3D conformation in solution**

*Analysis of the global properties of  
the particle in solution: proportions,  
flexibility, assembly,...*

- ⇒ **Average model**
- ⇒ **No atomic scale information**
- ⇒ **No experimental structure**

**3D Structure of the crystallized  
particle**

*Atomic scale analysis:  
conformation, distance, electron  
delocalisation,...*

- ⇒ **No information in solution**
- ⇒ **No or very few information on  
the particle dynamics**

**SAXS**



**Crystallography**



**Complementarity**

*Average conformation in solution*

*Possible modelisation of flexible regions*

*Allow to verify which assembly is compatible with the diffusion curve ion*

*Allow to estimate the domain organisation flexibility*

*Solid state 3D structure*

*Ambiguity of multimers assembly*

*Ambiguity of domain organisation*

***Tutorials***

***Formation of 3-4 working groups***

**SAXS**

***Data analysis,...***

**Crystallography**

***« Beginners »  
Data analysis***

***Molecular replacement***

***Electron density map  
manipulation***

***« Advanced »  
Data analysis***

***Experimental phasing***

***Electron density map  
manipulation***