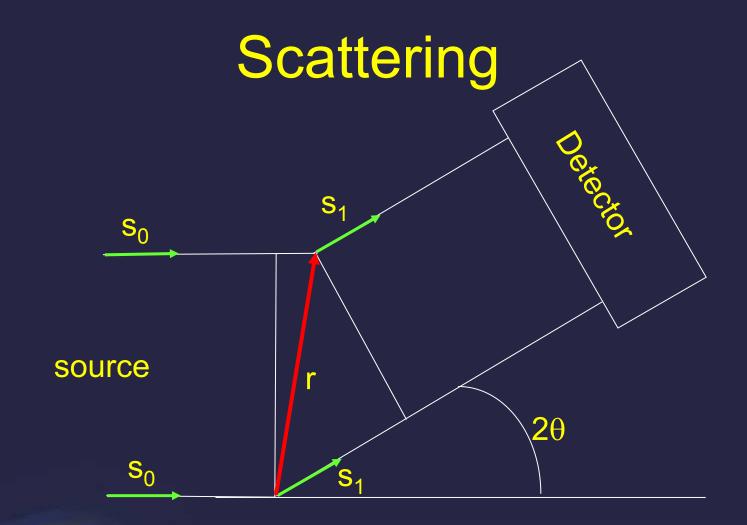
## Data Analysis Solution scattering

Marc Malfois – September 2010





Path difference:  $r.s_1-r.s_0 = r (s_1-s_0)$ 



### Scattering vector Scattered **S**<sub>1</sub> $|s_0| = |s_1| = 1/\lambda$ S Length 1/λ 2θ **S**<sub>0</sub> Length $1/\lambda$ s is called the scattering vector $s = |s| = 2 \sin\theta / \lambda$ Other notations: $q = h = "s" = 2\pi s$

SAS = SMALL ANGLE scattering => s  $\sim 2\theta/\lambda$ 



## Scattered amplitude

$$F(q) = \int_{V_r} \rho(\mathbf{r}) e^{irq} dV_r$$

Fourier Transform of the electron density  $\rho(r)$ 

The scattered intensity is

$$I(q) = F(q).F^*(q)$$

At s = 0; 
$$F(0) = \int_{V_r} \rho(r) dV_r$$

 $I(0) = (number of electrons)^2$ 



## Scattering intensity

 $I(q) = F(q).F^*(q)$ 

$$I(q) = \int_{V_r} \rho(r) e^{-i\vec{q}\vec{r}} dV_r \int_{V_r} \rho(-r) e^{i\vec{q}\vec{r}} dV_r$$

$$I(q) = \int_{V_r} \int_{V_{r'}} \rho(r) \rho(r') e^{-i\vec{q}(\vec{r}-\vec{r}')} dV_r dV_{r'}$$

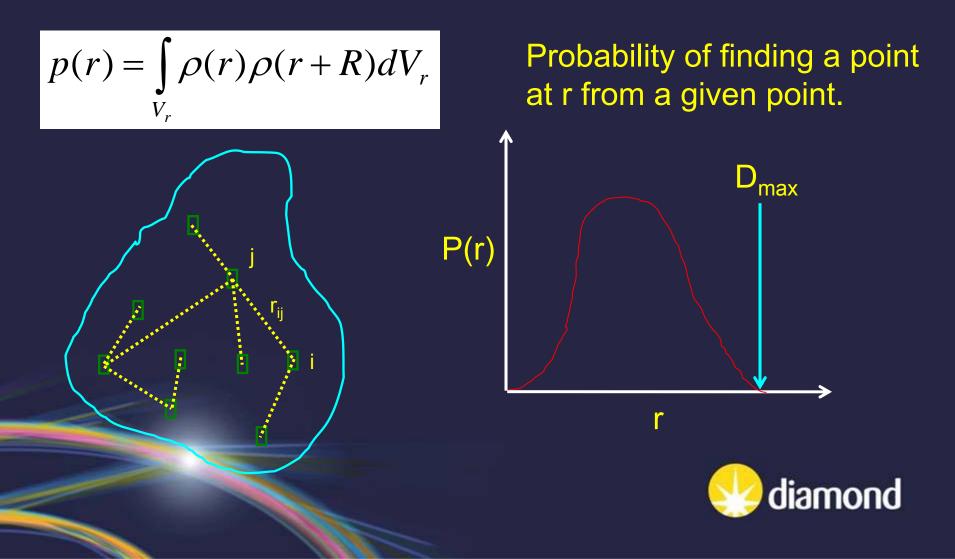
$$p(R) = \int_{V_r} \rho(r) \rho(r+R) dV_r$$

### Pair distribution function

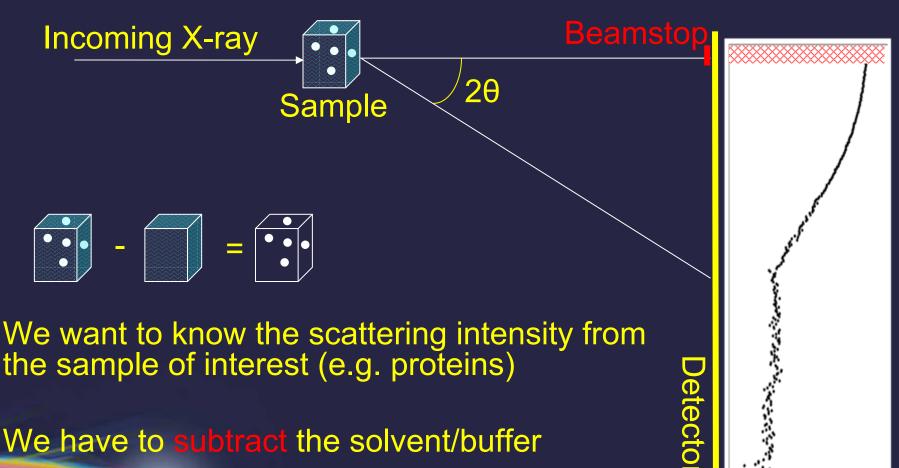
$$I(q) = \int_{V_r} p(R) e^{-i\vec{R}\vec{q}} dV_r \quad with \quad \vec{R} = \vec{r} - \vec{r}'$$



# Pair distance distribution function

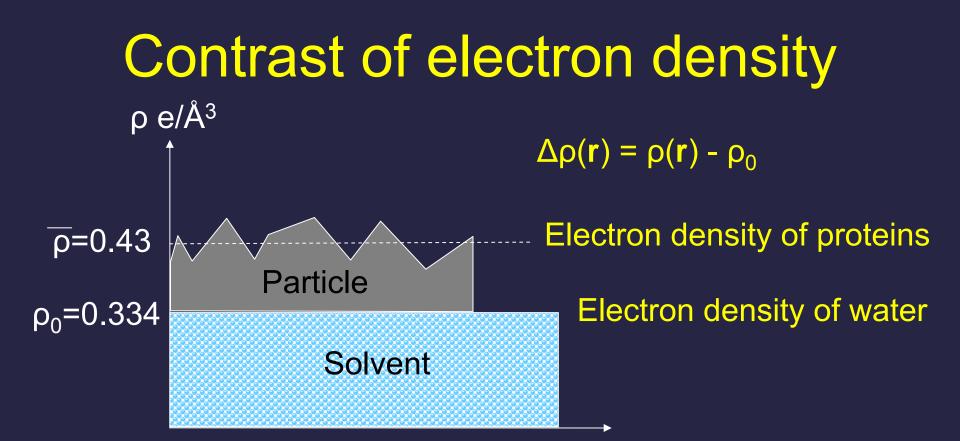


## **Experimental setup**



diamond

We have to subtract the solvent/buffer



The electron density for buffers with high salt quantity has to be calculated.

Very low contrast for proteins in solution



## **Contrast of electron density**

$$I(q) = \int_{V_r} \int_{V_{r'}} \Delta \rho(r) \Delta \rho(r') e^{-i\vec{q}(\vec{r}-\vec{r}')} dV_r dV_{r'}$$

$$p(R) = \int_{V_r} \Delta \rho(r) \Delta \rho(r+R) dV_r$$



## Solution of particles



Solution=Motif (Protein)\*LatticeI(c,s)=F(0,s).S(c,s)Form factor<br/>of the particleStructure factor<br/>of the particle



## Solution of particles

### Assumption

Ideality : No intermolecular interactions Monodispersity : Identical particles

Particle in solution => thermal motion => Particles have a random orientation/x-ray beam. The sample is **isotropic**. Only the **spherical average** of the scattered intensity is experimentally accessible.

$$F_1(q) = \int_{V_r} \Delta \rho(\mathbf{r}) e^{irq} dV_p$$

Amplitude of 1 particle

$$I(q) = n.=n.I_1(q)$$

Intensity of n particles in solution



## Debye formula

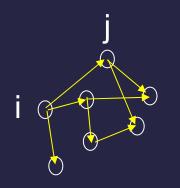
$$i_1(q) = < i_1(\vec{q}) > = \int_{\Omega} i_1(\vec{q}) d\Omega$$

$$i_1(q) = \int_{V_1} \int_{V_2} \Delta \rho(r_1) \Delta \rho(r_2) \frac{\sin(r_{12}q)}{r_{12}q} dr_1 dr_2$$

$$i_1(q) = 4\pi \int_0^\infty p(r) \frac{\sin(qr)}{qr} dr$$



## Debye formula



Particle : Discrete sum of elementary scatterers (Atoms).

$$\dot{t}_1(q) = \sum_{i}^{N} \sum_{j}^{N} f_i(q) f_j(q) e^{i\pi q(r_i - r_j)}$$

With  $f_i(q)$  the form factor of the elementary form factor Debye 1915

$$i_1(q) = \sum_{i}^{N} \sum_{j}^{N} f_i(q) f_j(q) \frac{\sin qr_{ij}}{qr_{ij}}$$

Widely used for model calculations **by** diamond

## Intensity at the origin

$$I(0) = \frac{I_0 r_e^2 cMd}{N_A a^2} \left[ \overline{\nu_p} (\rho - \rho_0) \right]^2$$

 $r_e^2$ : 7.95 10<sup>-26</sup> cm<sup>2</sup> scattering cross section of the e<sup>-</sup> c: concentration of the sample (mg/ml) d: Thickness of the sample  $I_0$ : Intensity of the direct beam  $v_p$ : partial specific volume

> The molecular weight can be determined from the intensity at origin



## Intensity at the origin

Determination of I<sub>0</sub>

- Well know reference sample (Glassy carbon on I22)
- Scattering by water( O. Glatter)

In case of **relative intensity**, it is possible to determine the molecular weight of the sample by measuring the scattering of a reference sample (Lysozyme or BSA)

$$Mw_s = \frac{I_r(0)}{I_s(0)} \cdot \frac{c_s}{c_r} Mw_r$$



## **Guinier Law**

$$I(q) = 4\pi \int_0^\infty p(r) \frac{\sin(qr)}{qr} dr$$

Taylor series expansion of

\_

$$\frac{\sin(qr)}{qr} = 1 - \frac{(qr)^2}{3!} + \frac{(qr)^4}{5!} - \dots$$

$$I(q) = 4\pi \int_{0}^{\infty} p(r) dr \left[ 1 - \left(\frac{q^{2}}{3!}\right) \frac{\int_{0}^{\infty} r^{2} p(r) dr}{\int_{0}^{\infty} p(r) dr} \right] = I(0) \left[ 1 - \frac{q^{2} R_{g}^{2}}{3} \right]$$
  
I(0) 
$$2R_{g}^{2}$$

Taylor series expansion of

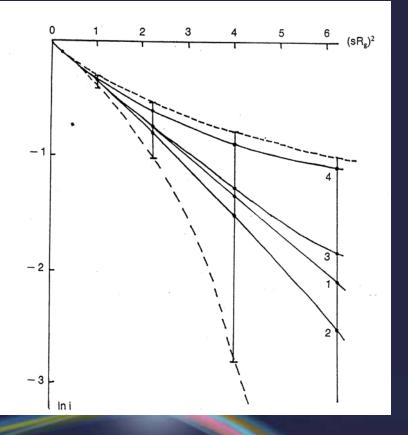
qR<sub>g</sub><<1

$$e^{x} = 1 + x + \frac{x^{2}}{2!} + \frac{x^{3}}{3!}$$
 with  $x = -\frac{q^{2}R_{g}^{2}}{3}$ 

$$I(q) = I(0)e^{-\frac{q^2 R_g^2}{3}}$$



### Accuracy of the Guinier law Valide for qR<sub>g</sub><< 1 but qR<sub>g</sub><1.3 in the literature



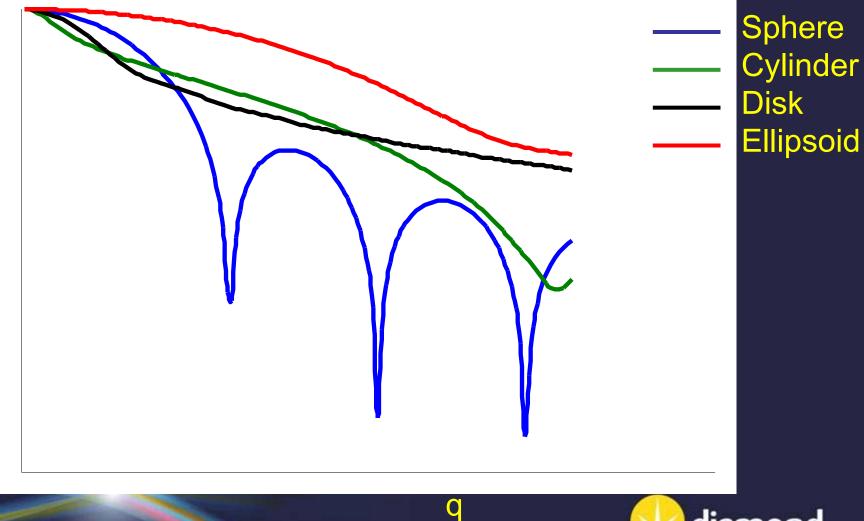
1 – Guinier law (exponential)
 2 – Sphere
 3 – Thin disk
 4 – long rod

## Depends on the shape of the particle

Structure Analysis by Small Angle X-ray and Neutron Scattering L.A. Feigin and D.I. Svergun (1987), Plenum Press.



## Solution of particles



Scattering intensity



## **Radius of Gyration**

$$R_g^2 = \frac{\int_{V_r} \Delta \rho(r) r^2 dV_r}{\int_{V_r} \Delta \rho(r) dV_r}$$

R<sub>g</sub> is the quadratic mean of distances to the centre of mass weighted by the contrast of electron density

### Rg is an index of non sphericity

Sphere: 
$$Rg = \sqrt{\frac{3}{5}R}$$
 Cylinder (D,H)  $R_g = \sqrt{\frac{D^2}{8} + \frac{H^2}{12}}$ 

Smallest  $R_q$  for a given volume

Ellipsoid of revolution (a,b)

$$R_g = \sqrt{\frac{2a^2 + b^2}{5}}$$

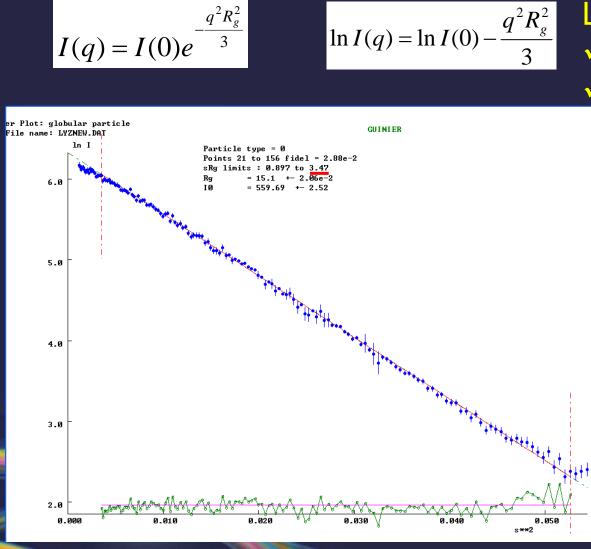


## **Guinier plot**

$$\ln I(q) = \ln I(0) - \frac{q^2 R_g^2}{3}$$

 $R_{g} = 15.1$ Å





It is very unlikely that any considerable progress will be made in the future.

## André Guinier on theory of small-angle scattering (Guinier 1969)



## Pair distribution function

$$p(r) = \frac{1}{(2\pi)^2} \int_0^\infty I(q) qr \sin(qr) dq$$

In theory, very easy calculation

Problem:

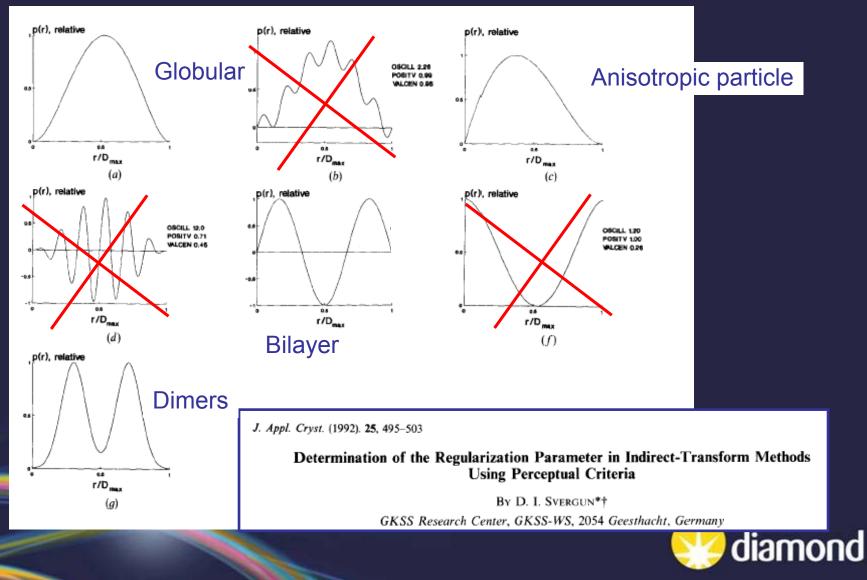
The intensity :

- only known over qmin-qmax (Detector size)
- ✓ affected by experimental errors
- $\Rightarrow$  Fourier transform of incomplete and noisy data is a ill-posed problem

Solution: Indirect Fourier Transform



## Pair distance distribution function



## Radius of Gyration and I(0)

$$I(0) = 4\pi \int_0^\infty p(r) dr$$

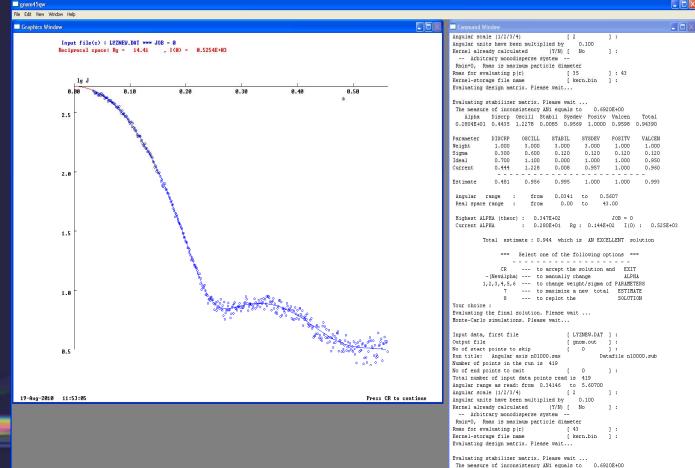
$$R_g^2 = \frac{1}{2} \frac{\int_0^\infty r^2 p(r) dr}{\int_0^\infty p(r) dr}$$

This estimate of Rg makes use of the whole scattering curve And is less sensitive to interactions or to the presence Of a small fraction of oligomers

Cross-check with the Guinier plot



### http://www.embl-hamburg.de/ExternalInfo/Research/Sax/gnom.html



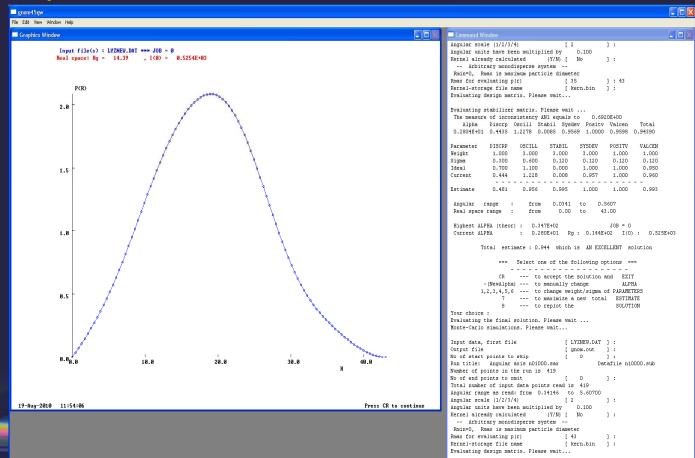
Valuating Stabilizer matrix. Please Walt ... The measure of inconsistency ANI equals to 0.6920E+00 Alpha Discrp Oscill Stabil Sysdev Positv Valcen Total 0.2804E+01 0.4435 1.2278 0.0085 0.9559 1.0000 0.9598 0.94390 Input file: ASCII Output file: Ascii but results appended to the file Number of point to omit: Due to subtraction issues closed to the beamstop Or to low concentration at higher q

*Rmax:* Maximum distance of the particle Here, Rmax=43Å

Other parameters, default values.



### http://www.embl-hamburg.de/ExternalInfo/Research/Sax/gnom.html



Evaluating stabilizer matrix. Please wait ... The measure of inconsistency ANI equals to 0.6920E400 Alpha Discrp Oscill Stabil Sysdev Positv Valcen Total 0.2804E401 0.4435 1.2278 0.0085 0.9569 1.0000 0.9598 0.94390 Rg=14.39Å I(0) = 525.0

Guinier plot: Rg=15.1Å I(0)=559.0

### P(r): •Smooth at Rmax •Only positive •Peak maximum centered



http://www.embl-hamburg.de/ExternalInfo/Research/Sax/gnom.html

Participation       Control finition       Control finiii finiii finition       Contro finition	e goonf5qw	
	File Edit Yew Window Help	
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In contrast - 1.00 with the dot		
Image: State Stat	Highest ALFHA (theor) : 0.347E+02 JOB = 0 Current ALFHA : 0.280E+01 Rg : 0.144E+02 I(0) : 0.525E+03	Bei
	Total estimate : 0.944 which is AN EXCELLENT solution	
Image: Discrete transmission		
No of state planes of state No of state planes of state in the state model of state in the state No of state planes of state in the state model of state in the state No of state planes of state in the state of state in the	CR to accept the solution and EXIT -(Newlpha) to nanually change ALPHA 1.2.2.5 to change of DENETRY	1 is
The measure of inconsistency ANI equals to 0.6320E+00         Alpha Discry Decill Stall System Postry Valcem Total         0.2804E+01       0.4435       1.2278       0.0085       0.9569       0.94390         Parameter       DISCRP       0SCILL       STALL       STALL       NUCEN         Weight       1.000       0.000       0.9598       0.94390         Sigma       0.300       0.000       0.000       0.9598       0.94390         Bigma       0.700       0.000       0.000       1.000       0.000         Sigma       0.300       0.600       0.010       0.120       0.120       0.120         Ideal       0.700       1.000       0.560       1.000       0.560       0.001       0.00       0.993         Angular       range :       from       0.001 to       43.00       0.993         Angular       range :       from       0.001 to       43.00       0.993         Mugular       range :       from       0.001 to       43.00       0.5567         Real space range :       from       0.001 to       43.00       0.5557         Current       0.444       1.228       0.001 to       43.00       0.5557	No of start points to skip [ 0 ]: Run tile: Angular axis noll000.sub Number of points in the run is 419 No of end points to cont [ 0 ]: Total number of input data points read is 419 Angular range as read; from 0.34146 to 5.60700 Angular scale (1/2/3/4) [ 2 ]: Angular range as read; from 0.34146 to 5.60700 Angular units have been multiplied by 0.100 Kernel aiready calculated (Y/N) [ No ]: - Arbitrary monodisperse system Rmine0, Rmax is maximum particle diameter Rmax for evaluating p(r) [ 43 ]: Kernel-atorage file name [ Kern.hin ]:	sol
Weight       1.000       3.000       3.000       3.000       1.000       1.000         Sigme       0.000       0.000       1.000       0.120       0.120       0.120         Ideal       0.700       1.000       0.000       1.000       0.950       0.000       0.000       0.993         Ideal       0.700       1.000       0.957       1.000       0.993       0.995       1.000       0.993         Angular range       ffcm       0.031       to       0.5607       0.5607         Peal space range       ffcm       0.0341       to       0.5607         Current ALPRA       :       0.2802401       Fg       0.1414402       1(0):       0.525E403         Total estimate:       0.944       which is       WEXCHLENT       0.0525E403         Current ALPRA       :       0.2802401       Fg       0.1412402       1(0):       0.525E403         Total estimate:       0.944       which is       WEXCHLENT       0.01100       ===       Select one of the following options       ===         CR       to accept the solution and EXIT       -(Reskipha)       to maximize a new total ESTIMATE       1.2,3,4,5,6	The measure of inconsistency ANM equals to 0.6920E+00 Alpha Discrp Oscill Stabil Sysdev Positv Valcen Total	
Angular range : from 0.0341 to 0.5607 Real space range : from 0.00 to 43.00 Highest ALPHA (theor) : 0.347E+02 JOB = 0 Current ALPHA : 0.280E+01 kg : 0.148E+02 I(0) : 0.525E+03 Total estimate : 0.944 which is IN EXCELLENT bolution === Select one of the following options === CR to accept the solution and EXIT -(NexAlpha) to manually change ALPHA 1.2.3,4.5,6 to change wight/sigma of PARAINTERS 7 to maximize a new total ESTIMATE 8 to repiot the Solution wight	Weight         1.000         3.000         3.000         1.000         1.000           Sigma         0.300         0.600         0.120         0.120         0.120         0.120           Ideal         0.700         1.000         1.000         1.000         0.950           Current         0.444         1.228         0.008         0.957         1.000         0.960	
Real space range :       from       0.00       to       43.00         Highest ALPHA (theor) :       0.347E+02       JOB = 0       OC         Current ALPHA :       0.280E+01       Rg :       0.144E+02       I(0) :       0.525E+03         Total estimate :       0.944       which is in EXCELLENT collution       ==       Select one of the following options ===       CR       to accept the solution and EXIT       -/// ALPHA       J.23,4-5,6       to manually change       ALPHA       J.2,3,4-5,6       to maximize a new total ESTIMATE       SOLUTION       SOLUTION	Estimate 0.481 0.956 0.995 1.000 1.000 0.993	
Current ALPHA : 0.280F+01 Kg : 0.144E+02 I(0) : 0.525E+03 Total estimate : 0.944 which is ON EXCELLENT colution Select one of the following options CR to accept the solution and EXIT -(NewAlpha) to change with/sigms of ALPHATERES 1,2,3,4,5,6 to change with/sigms of ALPHATERES 7 to maximize a new total ESTIMATE 8 to repiot the SOLUTION		
Select one of the following options CR to accept the solution and EXIT -(RewAlpha) to manually change ALPHA 1,2,3,4,5,6 to change weight/sigma of PARAMETERS 7 to maximize a new total ESTIMATE 8 to repiot the SOLUTION		
CR to accept the solution and EXIT -(RevAlpha) to manually change ALPHA 1,2,3,4,5,6 to change weight/sigma of PARAHTERS 7 to maximize a new total ESTIMATE 8 to repiot the SOLUTION	Total estimate : 0.944 which is (NI EXCELLENT) solution	
CR to accept the solution and EIIT -(NevAlpha) to manually chapte ALPHA 1,2,3,4,5,6 to change wight/sigma of PARAMETERS 7 to meximize a new total ESTIMATE 8 to repiot the SOLUTION		
Your choice :	CR to accept the solution and EXIT -(NewAlpha) to manually chapted ALPHA 1,2,3,4,5,5 to chapted wight/signe of PAAMETERS 7 to maximize a new total ESTIMATE 8 to replace the SOUTHINE	
		~

Total estimate: Between 0 and 1

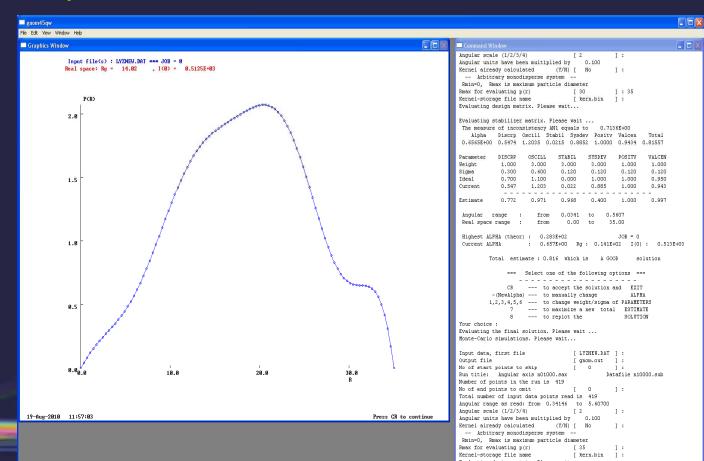
## 1 is the best solution



Evaluating design matrix. Please wait ... Evaluating stabilizer matrix. Please wait ...

The measure of inconsistency AN1 equals to 0.7136E+00

http://www.embl-hamburg.de/ExternalInfo/Research/Sax/gnom.html

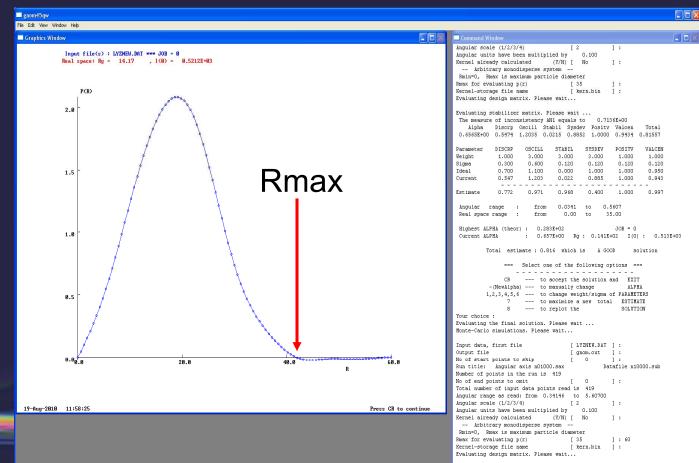


Rmax = 35Å

P(r) not smooth at **Ŕmax** => Rmax must be bigger



http://www.embl-hamburg.de/ExternalInfo/Research/Sax/gnom.html



Evaluating stabilizer matrix. Please wait ... The measure of inconsistency MNI equals to 0.09958400 Alpha Discrp Oscill Stabil Sysdev Positv Valcen Total 0.1660E-01 0.4076 1.7159 0.0210 0.9569 1.0000 0.8827 0.75599 P(r) negative a high r  $\Rightarrow$  The particle must be smaller. Rmax is likely to be at the intercept of the p(r) with the x-axis



## Using crystallographic structure

- To validate theoretical model
- To analyse similarities/difference in solution and in the crystal
- To build a complex from subunits



CRYSOL
$$I(q) = \langle |F(q)|^2 \rangle_{\Omega} = \langle |A(q) - \rho_s E(q) + \partial \rho_b B(q)|^2 \rangle_{\Omega}$$
 $\land$ A(s): Atomic scattering in vacuum $\land$ E(s): Atomic scattering in vacuum $\land$ E(s): Scattering from the excluded volume $\land$ B(s): scattering from the hydration shell $\land$ Svergun D.I., Barberato C. & Koch M.H.J.  
(1995) J. Appl. Cryst., 28, 768-773.

## CRYSOL

Number of experimental points ..... : 419

(2)

Angular units multiplied by ..... : 0.1000

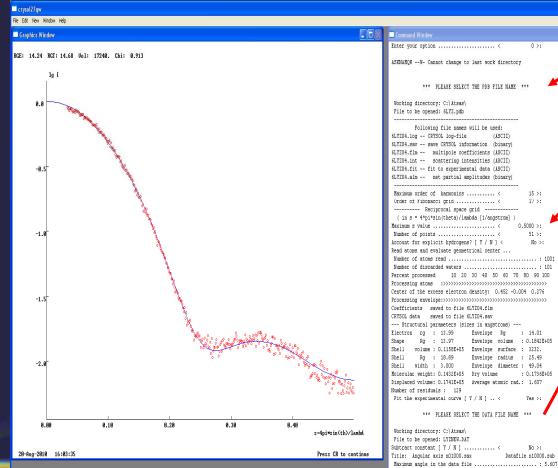
Number of points after regriding ...... : 201 Electron density of the solvent, e/1\*\*3 < 0.3340 >: Number of experimental points used ..... : 201 ----- Fitting the experimental data ... ---

2.5:

Yes >:

Angular units in the input file: 4\*pi\*sin(theta)/lambda [1/angstrom] (1) 4\*pi\*sin(theta)/lambda [1/nm]

2 \* sin(theta)/lambda [1/angstrom] (3) 2 \* sin(theta)/lambda [1/nm] (4) ..... <



### Input file: PDB file

### q-range: must be checked in the data file.

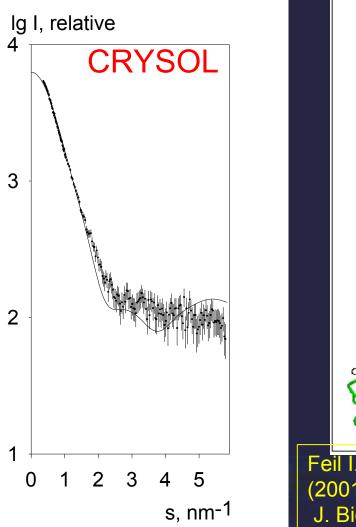
### Input file: Ascii file

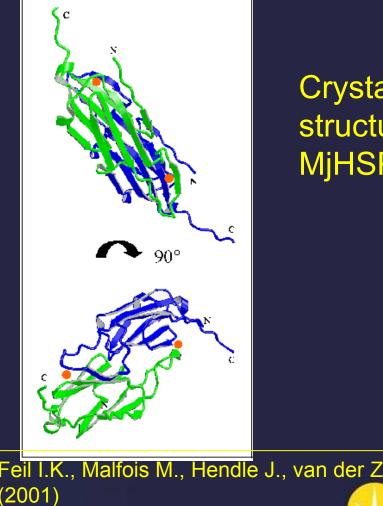
*Electron density:* to be calculated if highly salted buffer, By default, electronic density of water.

26-Jan



## <u>aB-crystallin domain</u>



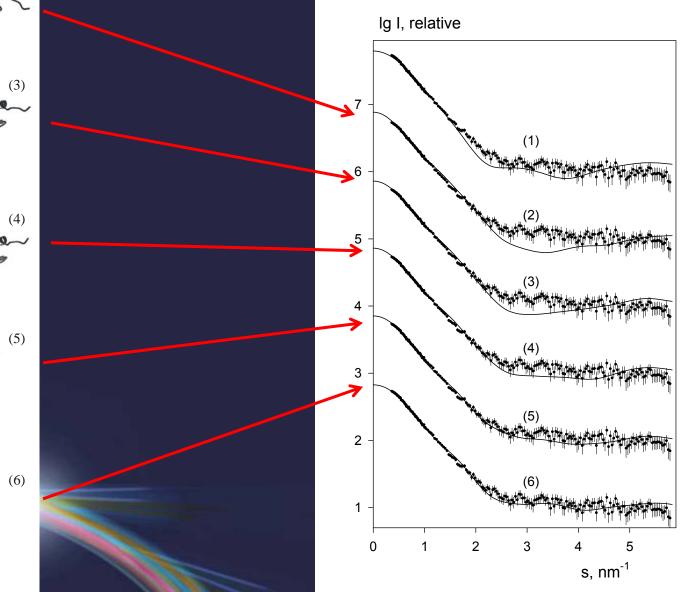


Crystallographic structure of MjHSP 16.5

Feil I.K., Malfois M., Hendle J., van der Zandt H., Svergun D.I. (2001) J. Biol. Chem., **276 (15)**, 12024-1202 **diamond** 

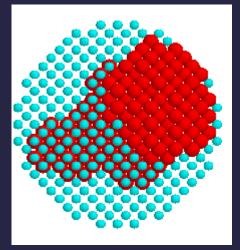


## Dimer modelling



#### http://www.embl-hamburg.de/ExternalInfo/Research/Sax/dammin.html

From Dmax determined in GNOM, a sphere is created.



•The sphere is filled by dummy atoms.

The dummy atoms belong to the protein or belong to the solvent.

The number of dummy atoms is about 1000

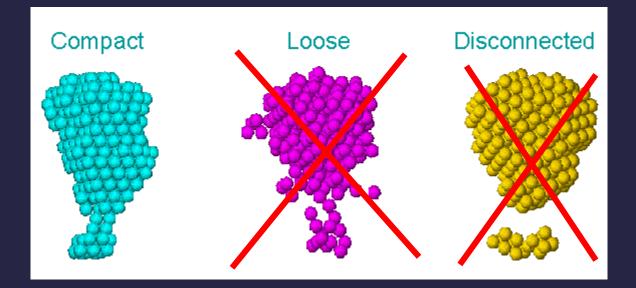
Can describe complex shape

No unique shape restoration unless contraints

Svergun, D.I. (1999) Biophys. J. **76**, 2879-2886

## DAMMIN CONSTRAINTS

http://www.embl-hamburg.de/ExternalInfo/Research/Sax/dammin.html





http://www.embl-hamburg.de/ExternalInfo/Research/Sax/dammin.html

Command Window	
***       Ab inito shape determination by simulated       ***         ***       annealing using a single phase dummy atoms model       ***         ***       Win 9x/NT, UNIX/Lunux/Mac release version 5.3       ***         ***       Last modified        06/05/09 19:00       ***         ***       Last modified        06/05/09 19:00       ***         ***       Please reference: D.Svergun (1999). Biophys. J.       ***         ***       76, 2879-2886.       ***         ***       Copyright (c) ATSAS Team       ***         ***       EMBL, Hamburg Outstation, 1999 - 2009       ***	
====== DAMMIN53 started on 20-Aug-2010 16:20:44	
Mode: <[F]>ast, [S]low, [J]ag, [K]eep, [E]xpert < Fast >: Log file name < .log >: t1 *** PLEASE SELECT THE INPUT FILE NAME ***	
Working directory: C:\Atsas\ File to be opened: gnom.out Project identificator: t1 Enter project description: : Random sequence initialized from: : 162052 ** Information read from the GNOM file ** Data set title: Angular axis n01000.sax Datafile n10000.sub	0
Raw data file name:       LYZNEW.DAT         Maximum diameter of the particle       : 43.00         Solution at Alpha = 0.280E+01 Rg : 0.144E+02 I(0) : 0.552E+03         Radius of gyration read       : 14.40         Number of GNOM data points       : 446         Angular units in the input file:       447pi*sin(theta)/lambda [1/nmgstrom] (1)         4*pi*sin(theta)/lambda [1/nm ] (2) < 1 >:         Maximum s value [1/angstrom]       : 0.5607         Number of Shannon channels       : 7.675         Portion of the curve to be fitted       : 1.000 >:         Number of knots in the curve to fit       : 2.0         *** Warning: constant reduced to avoid oversubtraction       : 2.537         Maximum order of harmonics       : 10         Initial DAM: type S for sphere [default],       E for ellipsoid, C for cylinder, P for parallelepiped         or start file name       :	

Input name: GNOM file Dmax has to be determined before running dammin/dammif

**Initial shape** 

Symetry

### Particle shape



### http://www.embl-hamburg.de/ExternalInfo/Research/Sax/dammin.html

File Edit View Window Help Graphics Window 19 Les: 8.1887 DisCog: 8.8116 Scale = 8.1648-85	
139 Los: 0.1087 DisCog: 0.0116 Scale = 0.164E-05	🗖 🔀 🗖 Command Window
2-fog-281 1525.86	<pre>4*pi*sin(thets)/lambda [1/angstrom] (1) 4/pi*sin(thets)/lambda [1/angstrom] (2) &lt; 1 &gt;1 Maximum svalue svalue (2) &lt; 1 &gt;1 Maximum (2)</pre>

nond

http://www.embl-hamburg.de/ExternalInfo/Research/Sax/dammin.html

Shape restoration: Equivalent to the excluded volume in CRYSOL

$$I(q) = \left\langle \left| F(q) \right|^2 \right\rangle_{\Omega} = \left\langle \left| A(q) - \rho_s E(q) + \partial \rho_b B(q) \right|^2 \right\rangle_{\Omega}$$

DAMMIN/DAMMIF DO NOT take into account the hydration shell

The atomic scattering in vacuum is subtracted from the experimental data A constant is subtracted from the data to force the s<sup>-4</sup> decay of the intensity at higher angles. Green curve is the curve to be fitted.

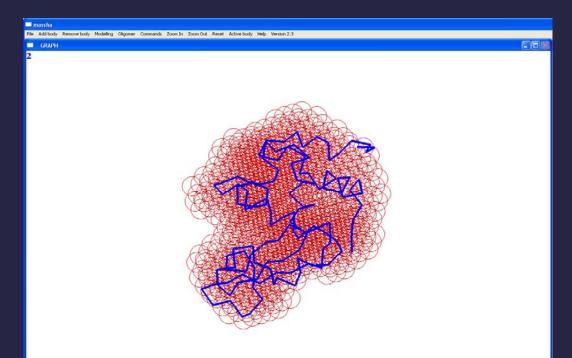


### http://www.embl-hamburg.de/ExternalInfo/Research/Sax/dammin.html

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File Edit View Window Help		
Graphics Window		
138 Los: 0.0135 DisCog: 0.0000 Scale = 0.160E-05	Rf: 0.00235 Los:0.0352 Dis:0.0000 Per: 0.4544 Sca: 0.234E-05         j: 68 T: 0.860E-06 Suc: 1519 Eva: 2250539 CPU: 0.109E+04 SqF: 0.0146         Rf: 0.00162 Los:0.0350 Dis:0.0000 Per: 0.4535 Sca: 0.233E-05         j: 69 T: 0.774E-06 Suc: 1519 Eva: 2259543 CPU: 0.110E+04 SqF: 0.0139         Rf: 0.00152 Los:0.0317 Dis:0.0000 Per: 0.4559 Sca: 0.221E-05         j: 70 T: 0.696E-06 Suc: 1519 Eva: 2268902 CPU: 0.111E+04 SqF: 0.0136         Rf: 0.00176 Los:0.0317 Dis:0.0000 Per: 0.4559 Sca: 0.221E-05         j: 70 T: 0.696E-06 Suc: 1519 Eva: 2268902 CPU: 0.111E+04 SqF: 0.0136         Rf: 0.00176 Los:0.0301 Dis:0.0000 Per: 0.4559 Sca: 0.221E-05         j: 70 T: 0.697E-06 Suc: 1519 Eva: 2269934 CPU: 0.111E+04 SqF: 0.0136         Rf: 0.00176 Los:0.0301 Dis:0.0000 Per: 0.4529 Sca: 0.217E-05         j: 70 T: 0.627E-06 Suc: 1519 Eva: 223934 CPU: 0.112E+04 SqF: 0.0130	
2.5	<ul> <li>J. 11. 0.028-L003 04. ISLE EVAL. 22/594 CF0. 0.112EF04 Sqt. 0.0106</li> <li>Rf: 0.00088 L003:0.2079 Disc.0.0000 Per:: 0.4535 Scat. 0.210E-05</li> <li>J: 72 T: 0.554E-06 Suc: 1519 EVAL 2290153 CFU: 0.113EH04 SqT. 0.0130</li> <li>Rf: 0.00089 L003:0.2079 Disc.0.0000 Per:: 0.4535 Scat. 0.210E-05</li> <li>J: 73 T: 0.508E-06 Suc: 1519 EVAL 2302043 CFU: 0.114E+04 SqT. 0.0126</li> <li>Rf: 0.00183 L003:0.258 Disc.0.0000 Per:: 0.4530 Scat. 0.203E-05</li> <li>T/4 T: 0.457E-06 Suc: 1519 EVAL 2302043 CFU: 0.115E+04 SqT. 0.0120</li> </ul>	
2.0	Rf: 0.00140 Los:0.0236 Dis:0.0000 Per: 0.4528 Sca: 0.200E-05 j: 75 T: 0.411E-06 Suc: 1509 Eva: 2329922 CPU: 0.116E+04 Sqf: 0.0116 Rf: 0.00110 Los:0.0222 Dis:0.0000 Per: 0.4532 Sca: 0.195E-05 j: 76 T: 0.370E-06 Suc: 1458 Eva: 2345119 CPU: 0.117E+04 Sqf: 0.0114 Rf: 0.00120 Los:0.0215 Dis:0.0000 Per: 0.4526 Sca: 0.196E-05 j: 77 T: 0.333E-06 Suc: 1243 Eva: 2360316 CPU: 0.118E+04 Sqf: 0.0111	
1.5	Rf: 0.00133 Los:0.0204 Dis:0.0000 Per:       0.4536 Sca: 0.188E-05         j: 78 T:       0.300E-06 Suc:       1134 Vexi       237513 CPU;       0.11984-04 SqF: 0.0108         Rf: 0.00083 Los:0.0194 Dis:0.0000 Per:       0.4542 Sca:       0.1984E-05       0.1006         j: 79 T:       0.270E-06 Suc:       103 Vexi       239710 CPU;       0.120E+04 SqF: 0.0106         Rf: 0.00096 Los:0.0166 Dis:0.0000 Per:       0.4593 Sca:       0.181E-05       0.122E+04 SqF: 0.0105         Rf: 0.00093 Los:0.0164 Dis:0.0000 Per:       0.4530 Sca:       0.180E-05         Rf: 0.00093 Los:0.0164 Dis:0.0000 Per:       0.4530 Sca:       0.180E-05	
	j: 81 T: 0.2188-06 Suc: 647 Eva: 2421104 CPU: 0.1238-04 Sqf: 0.0103 Rf: 0.00074 Los:0.0176 Dis:0.0000 Per: 0.4539 Sca: 0.1798-05 j: 82 T: 0.1978-06 Suc: 508 Eva: 2436301 CPU: 0.1248-04 Sqf: 0.0100 Rf: 0.00102 Los:0.0165 Dis:0.0000 Per: 0.4529 Sca: 0.1748-05 j: 83 T: 0.1778-06 Suc: 351 Eva: 2451948 CPU: 0.1258+04 Sqf: 0.0098 Rf: 0.00079 Los:0.0160 Dis:0.0000 Per: 0.4530 Sca: 0.1738-05 j: 83 T: 0.1589-06 Suc: 351 Eva: 2451965 CPU: 0.1258+04 Sqf: 0.0097	
8.5	Rf: 0.00058 Los:0.0157 Dis:0.0000 Per: 0.4533 Sca: 0.166E-05         j: 057 10.0143E-06 Suc: 275 Eva: 2481692 CPU: 0.128E+04 SqF: 0.0096         Rf: 0.00072 Los:0.0153 Dis:0.0000 Per: 0.4532 Sca: 0.166E-05         j: 86 T: 0.129E-06 Suc: 203 Eva: 2497089 CPU: 0.129E+04 SqF: 0.0095         Rf: 0.00080 Los:0.0149 Dis:0.0000 Per: 0.4535 Sca: 0.166E-05         j: 87 T: 0.116E-06 Suc: 203 Eva: 2497089 CPU: 0.152E+04 SqF: 0.0095         Rf: 0.00080 Los:0.0149 Dis:0.0000 Per: 0.4535 Sca: 0.166E-05         j: 87 T: 0.116E-06 Suc: 167 Eva: 2512286 CPU: 0.130E+04 SqF: 0.0094         Rf: 0.00096 Los:0.0144 Dis:0.0000 Per: 0.4535 Sca: 0.1655 Sca	
0.0 0.00 0.10 0.20 0.30 0.40 0.50 s, 1/A	RE: 0.00096 L0830.0144 J1810.0000 PET: 0.4035 Scat 0.1652-05         1: 88 T: 0.1042-06 Suc: 124 Eva: 2527483 CFU: 0.10181404 SqT: 0.0092         RE: 0.00042 L0830.0142 D1830.0000 PET: 0.4534 Scat 0.164E-05         1: 99 T: 0.9408-07 Suc: 69 Eva: 254260 CFU: 0.10181404 SqT: 0.0092         RE: 0.00062 L0830.0140 D1830.0000 PET: 0.4533 Scat 0.162E-05         1: 90 T: 0.446E-07 Suc: 61 Eva: 2557877 CFU: 0.1341404 SqT: 0.0092         RE: 0.00037 L0830.0139 D1830.0000 PET: 0.4533 Scat 0.162E-05         1: 91 T: 0.762E-07 Suc: 61 Eva: 2557074 CFU: 0.1351404 SqT: 0.0092         RE: 0.00037 L0830.0139 D1830.0000 PET: 0.4533 Scat 0.162E-05         1: 91 T: 0.762E-07 Suc: 51 Eva: 2557074 CFU: 0.1351404 SqT: 0.0092         RE: 0.00037 L0830.0139 D1830.0000 PET: 0.4533 Scat 0.162E-05         1: 92 T: 0.066E-07 Suc: 51 Eva: 2557074 CFU: 0.1351404 SqT: 0.0092         RE: 0.00037 L0830.0139 D1830.0000 PET: 0.4533 Scat 0.162E-05         1: 92 T: 0.066E-07 Suc: 51 Eva: 257374 CFU: 0.1351404 SqT: 0.0092         RE: 0.00037 L0830.0139 D1830.0000 PET: 0.4533 Scat 0.162E-05         1: 92 T: 0.066E-07 Suc: 51 Eva: 257371 CFU: 0.1361404 SqT: 0.0091	
28-Aug-2818 16:49:44	<pre>]; 92 1: 0.6008-00' Suc: 36 EVa: 23062/1 CPU 0.1506404 Sqr: 0.0091 Rf: 0.00045 Los:0.0137 Dis:0.0000 Per: 0.4537 Sca: 0.161E-05 ]: 93 T: 0.617E-07 Suc: 39 EVa: 2603468 CPU: 0.137Ho4 Sqr: 0.0091 Rf: 0.00045 Los:0.0137 Dis:0.0000 Per: 0.4537 Sca: 0.161E-05 ]: 94 T: 0.555E-07 Suc: 33 EVa: 2618665 CPU: 0.139EH04 Sqr: 0.0090 Rf: 0.00035 Los:0.0135 Dis:0.0000 Per: 0.4540 Sca: 0.160E-05 ]: 95 T: 0.450E-07 Suc: 16 EVa: 2633862 CPU: 0.140E+04 Sqr: 0.0090 Rf: 0.00038 Los:0.0135 Dis:0.0000 Per: 0.4538 Sca: 0.161E-05 ]: 96 T: 0.4580E-07 Suc: 16 EVa: 2633862 CPU: 0.141E+04 Sqr: 0.0090 Rf: 0.00038 Los:0.0135 Dis:0.0000 Per: 0.4538 Sca: 0.160E-05</pre>	
	Final SQRT(Chi) against raw data : 0.8238	
	===== DAMMIN53 finished at 20-Aug-2010 16:49:44 Press CR to terminate the program	

Running Input pending in Command Window

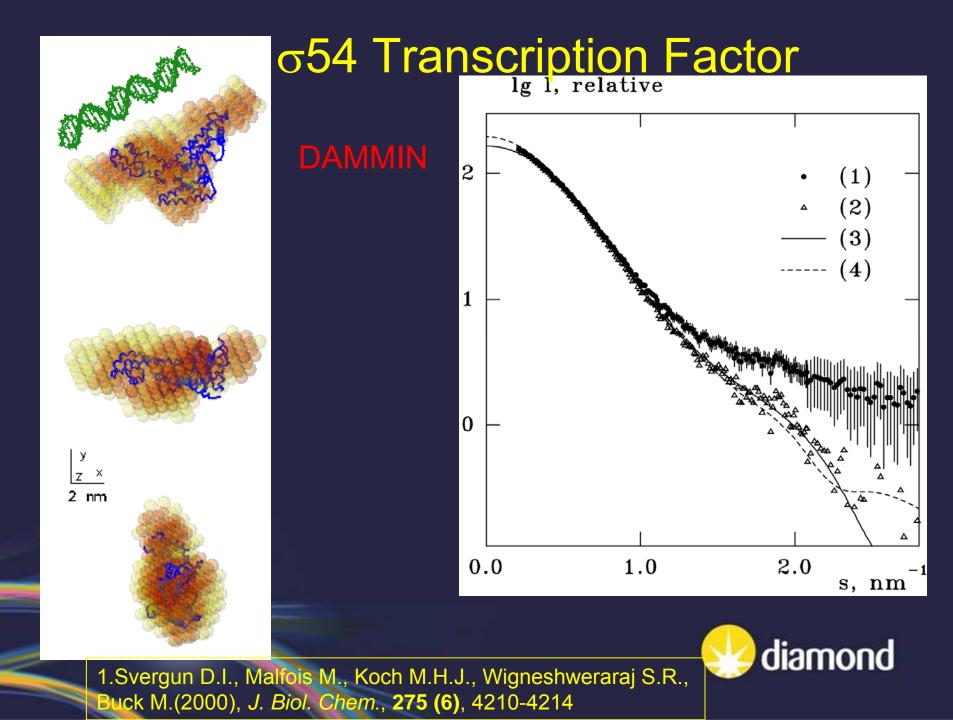
http://www.embl-hamburg.de/ExternalInfo/Research/Sax/dammin.html



### Output file: PDB file

Results can be visualise with MASHA





## **Experimental Considerations**

$$I_{particle}(q) = \frac{1}{c} \left[ \frac{I_{solution}(q)}{It_{solution}} - \frac{I_{background}(q)}{It_{background}} \right] \frac{1}{D(q)}$$

c: concentration (mg/ml) D(q): Detector response

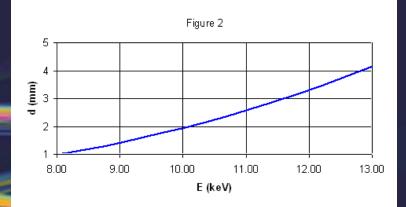
We measure ➤ The solution containing the particles of interest ➤ The background (Cell with the solvent/buffer) Eliminate contributions from parasitic background



## Thickness of the sample

The maximum scattering intensity is achieved by selecting the optimal thickness of the sample. The scattering intensity can be formulated as:  $I(q) \propto de^{-\mu d}$ 

d: the thickness of the sample  $\mu$ : linear absorption coefficient Optimal thickness  $\frac{\partial I}{\partial d} = k(1 - \mu d)e^{-\mu d} \Rightarrow d_{optimal}$ 



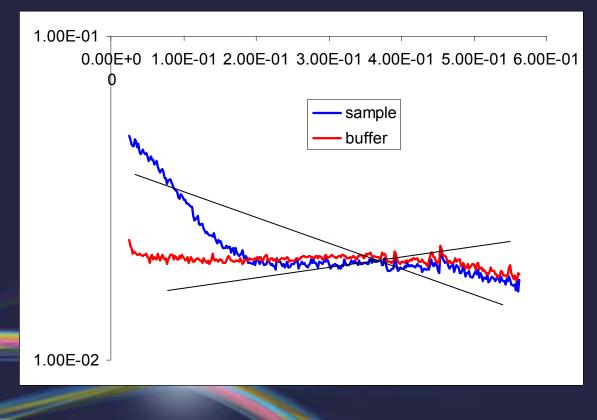
The linear absorption coefficient can be calculated or measured. Optimal thickness of the sample (water in this case) as a function of the energy.



μ

## Experiment

### Liquid cell or capillaries? Liquid cell (Same windows and same thickness) Capillaries (Different thickness)



Capillary thickness smaller for the sample than for the buffer

The scattering of the sample must be higher or equal to the scattering of the buffer



## Liquid cell or capillaries?

$$I(q) \propto de^{-\mu d}$$

Can be measured by a diode located in the beamstop

Unknown

For the liquid cell, the thickness is the same for the sample or for the buffer so there is no need to measure an empty cell. The empty capillary for the sample and capillary for the buffer must be measured => 2 extra measurements



## **Experimental Considerations**

Sample volume: 0.120ml with liquid cell

Sample volume: 0.05ml with HPLC

