CONSIGLIO NAZIONALE DELLE RICERCHE

PROGRAMMA SHORT TERM MOBILITY 2014

Il Fruitore: **Giancarlo TRIA**

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Istituzione ospitante: **Istituto di Cristallografia (IC) sede di Bari**

Titolo del programma: **Interpretazione e analisi di dati sperimentali Bio-SAXS presso XMI-Lab, IC-CNR**

Within the short term mobility program 2014 the scientist Giancarlo Tria has been invited by the Institute of Crystallography (Bari outstation) for a period of two weeks. The guest is currently a member of the BioSAXS group (EMBL Hamburg c/o DESY) and under the supervision of Dr. Dmitri Svergun is one of the developers of the ATSAS package – the most widely used collection of software for the analysis and interpretation of SAS data. The main goal of the collaboration was to use the counselling of the guest for the analysis and interpretation of experimental Small Angle X-ray Scattering (SAXS) data for Ubiquitin and diUbiquitin collected at BioSAXS beamlines BM29 (ESRF, Grenoble) and P12 (PETRA III, Hamburg). Moreover, the collaboration was also intent to the measurement of biomaterial using a first-generation-synchrotron-class X-ray laboratory microsource, coupled to a three-pinhole camera, which has been recently installed in our laboratory XMI-Lab (at CNR Bari). This in house machine allows SAXS as well as WAXS (wide-angle X-ray scattering) images to be acquired simultaneously, and scanning SAXS/WAXS microscopy to be carried out.

Ubiquitin

Experimental data for Ubiquitin measured in its classical buffer (MES 20mM pH 6.0) are quite good and fully analyzable. No concentration effect was observed. Although the data show some noisy at high angle – mainly due to the fact that the protein is rather small – the overall parameters can be estimated from the experimental curve (Table I). The theoretical scattering computed by CRYSOL using the public available crystal structure of Ubiquitin (PDB code 3EHV) fits reasonably well the experimental curve (χ = 1.055) (Figure 1). Moreover, the ab initio reconstruction computed using DAMMIF agrees rather well with the crystal too (Figure 2). The particle has been also measured using different buffer conditions with different concentrations of Zn ((i) ZnAc2 25mM, MES 20mM pH 6.0; (ii) ZnAc2 200mM, MES 20mM pH 6.0) and no significant changes were observed. The protein seems to be rather stable in both the buffers and no oligomerization occurs within 10 minutes. Nevertheless, it is important to highlight that the very last two measurements (1 and 2 mg/ml) of Ub in the buffer (i) did shown some aggregation. The measurement for these two samples took place about 10 minutes later than the protein was seated in the buffer. However, the quality of the Guinier region for these two curves discourage the is not good enough for proceeding with the analysis and the data cannot be analyzed. What observed for the last two curves might suggest the speculation that Ub needs few minutes before to start the oligomerization process in presence of Zn. Further SAXS measurements might be therefore planned in such a way that different measurements of the sample can be done after n-minutes time, simulating so a time-resolved measurement. In this way, potential oligomerization process might be fully monitored.

Table I. Data collection and SAXS-derived parameters for Ubiquitin

Data-collection parameters	
Instrument (detector)	PILATUS 1M pixel (67 x 420 mm^2)
Beam geometry (mm^2)	0.2 x 0.12
Wavelength (Å)	1.24
s range (nm^{-1})	0.05-5
Exposure time (sec)	1 (20 frames x 0.05sec)
Concentration range (mg ml^{-1})	1 – 10
Temperature (K)	293.15
Structural parameters	
$I(0)$ (cm^{-1}) [from $P(r)$]	407±5
R_g (nm) [from $P(r)$]	1.3±0.2
$I(0)$ (cm^{-1}) [from Guinier approximation]	413±5
R_g (nm) [from Guinier approximation]	1.3±0.2
D_{max} (nm)	3.7±0.5
Porod volume estimate (nm^3)	10.5±5
Dammif excluded volume (nm^3)	15.7±5
Molecular-mass determination	
Molecular mass M_r (kDa) [from *Porod invariant*]	7±3
Molecular mass M_r (kDa) [from *excluded volume*]	8±3
Molecular mass M_r (kDa) [from $I(0)_{BSA} = 4107.93$]	7±3
Calculated monomeric M_r (kDa) [from sequence]	~8.5
Software employed	
Primary data reduction	PIPELINE
Data processing	PRIMUS
Ab initio analysis	DAMMIF
Validation and averaging	DAMAVER
Computation of model intensities	CRYSOL
Three-dimensional representations	PYMOL

Figure 1. CRYSOL theoretical scattering (red) from the crystal (PDB code 3EHV) against the experimental data (blue) of Ubiquitin ($\chi = 1.055$).

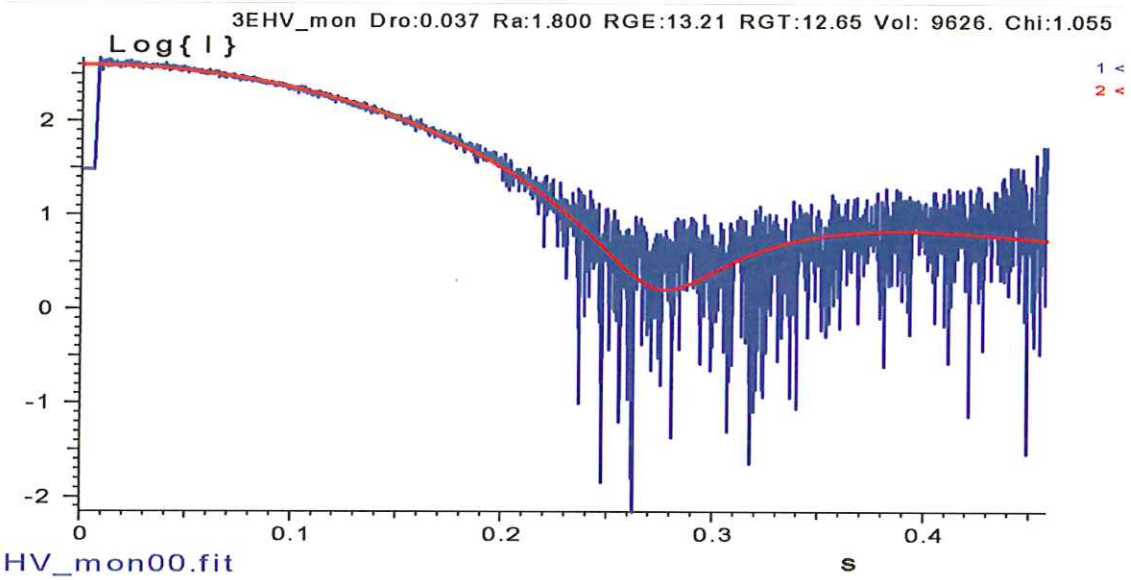
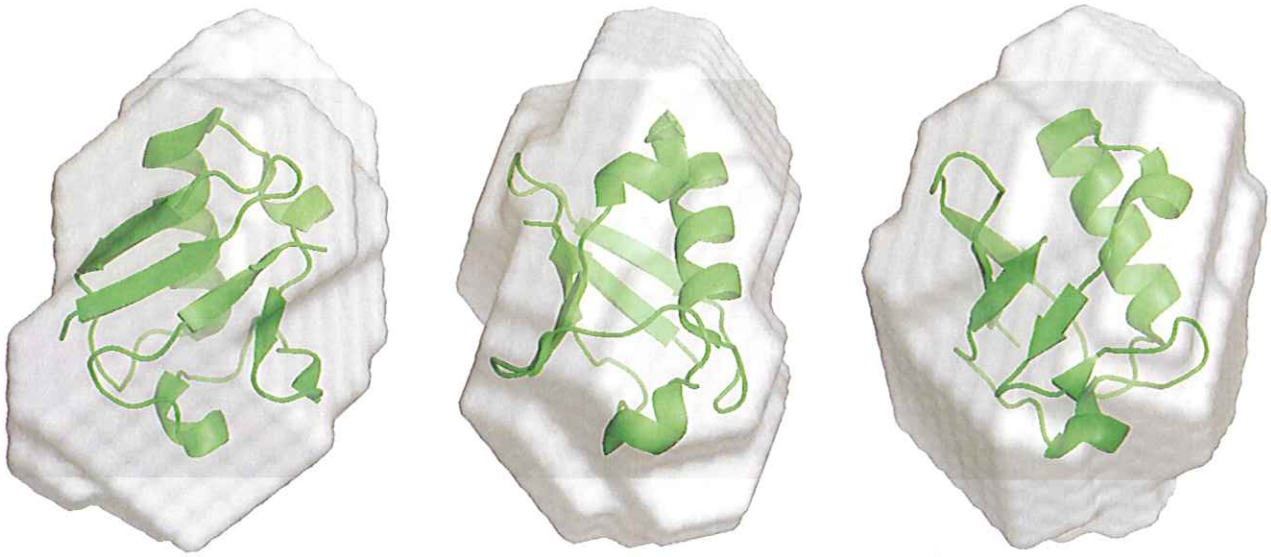


Figure 2. Ab initio reconstruction using DAMMIF (grey) superimposed to the crystal structure (PDB code 3EHV) using SUPCOMB.



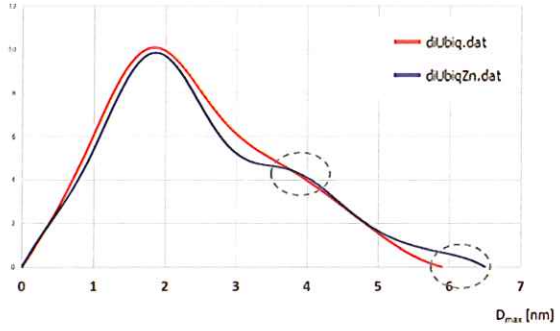
diUbiquitin

Dimeric Ubiquitin (diUbiquitin) in complex, and not, with Zn were also measured. The main goal of this characterization is to look for conformational rearrangement of the particle upon add of Zn. The experimental curves from diUbiquitin in its classical buffer (MES 20mM pH 6.0) are fully analysable although some noisy at high angle are observed. Overall parameters can be estimated from the experimental curves (Table II). *Ab-initio* reconstruction of both constrains do not show drastic differences. However, a slight difference is observed in the pair distance distribution (call P(r) function) comparison (Fig. 3) and conformational changes can be speculated from it. Rigid body modelling analysis were then performed using the software SASREF (ATSAS package) with the contact between the LYS48 of one molecule of Ubiquitin and C-terminal of the other Ubiquitin (PDBe 2LVQ) imposed as only restrain.

Table II. Data collection and SAXS-derived parameters for diUbiq and diUbiqZn

Data-collection parameters		
Instrument (detector)	PILATUS 1M pixel (67 x 420 mm^2)	
Beam geometry (mm^2)	0.2 x 0.12	
Wavelength (Å)	1.24	
s range (nm^{-1})	0.05-5	
Exposure time (sec)	1 (20 frames x 0.05sec)	
Concentration range (mg ml^{-1})	1 – 10	
Temperature (K)	293.15	
Structural parameters	**diUbiq**	**diUbiqZn**
$I(0)$ (cm^{-1}) [from $P(r)$]	~350	~350
R_g (nm) [from $P(r)$]	1.9±0.2	2.0±0.2
$I(0)$ (cm^{-1}) [from Guinier approximation]	~350	~350
R_g (nm) [from Guinier approximation]	1.9±0.2	2.0±0.2
D_{max} (nm)	6±1	6.5±1
Porod volume estimate (nm^3)	~23.5±3	~23.5±3
Dammif excluded volume (nm^3)	30±5	30±5
Molecular-mass determination		
Molecular mass M_r (kDa) [from *Porod invariant*]	15±3	
Molecular mass M_r (kDa) [from *excluded volume*]	15±3	
Calculated monomeric M_r (kDa) [from sequence]	~15	
Software employed		
Primary data reduction	PIPELINE	
Data processing	PRIMUS	
Ab initio analysis	DAMMIF	
Validation and averaging	DAMAVER	
Rigid body modelling	SASREF	
Three-dimensional representations	PYMOL	

Figure 3. P(r) functions comparison. The main differences are highlighted with dashed circles.



Although the resolution of the technique might not clearly highlight rearrangement of such a small particle (dimer ~15kDa), the presence of Zn does show a different rearrangement of the particle. The two single Ubiquitin molecules prefer to be in a row (Fig. 4) in absence of Zn whereas in presence of Zn the two molecules face each other (Fig. 5). The arrangement in presence of Zn might suggest a double interaction between both the Lys48 and the C-terminal.

Figure 4. Rigid body modelling for diUbiquitin (PDBe 2LVQ) in absence of Zn ($\chi = 0.86$).

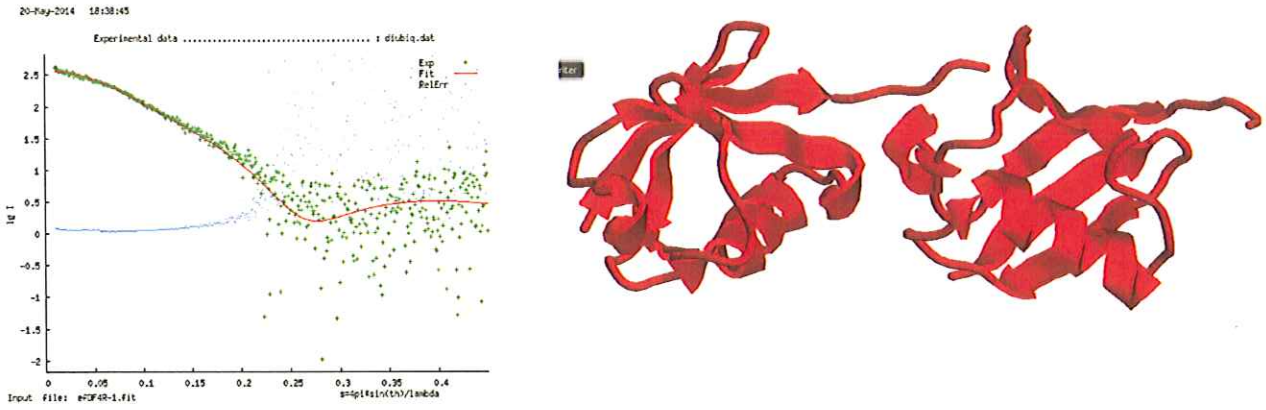
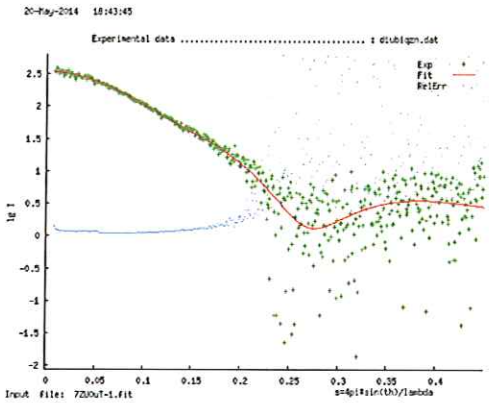
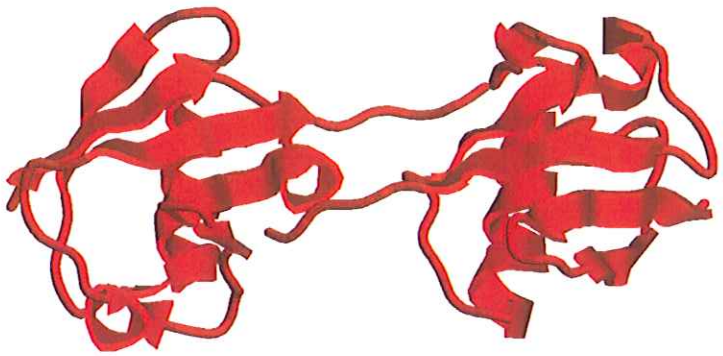


Figure 5. Rigid body modelling for diUbiquitin (PDBe 2LVQ) in presence of Zn ($\chi = 0.80$)..





Bari, 26 Maggio 2014

Il proponente (Dritan SILIQI)

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