Experimental report

03/04/2019

Proposal: 8-04-838 **Council:** 4/2018

Title: On the structural dynamics of multi-domain proteins

Research area: Biology

This proposal is a new proposal

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Samples: D2O
NaCl
MgCl2
HEPES
Atto647N
Heat shock protein 90
Adenosinediphosphate
Atto532
Adenylyl-imidodiphosphate

Instrument	Requested days	Allocated days	From	To
IN16B	2	2	06/10/2018	08/10/2018
IN15	3	3	01/10/2018	04/10/2018

Abstract:

The molecular chaperone Heat shock protein Hsp90 is one of the most abundant proteins in eukaryotic cells. Its function is related to a characteristic interconversion between an open and closed conformational state. Single-molecule FRET (smFRET) studies and MD simulations show that its dynamics are correlated over a large timescale ranging from local dynamics of ns to large conformational changes on the sec timescale. However, the underlying mechanism of the interconversion between the two states and the correlation over the timescales is still unclear. On the example of the Hsp90 system we wish to establish a comprehensive dynamical picture across timescales by using high-resolution neutron spectroscopy and smFRET data. This would in addition be an important step towards integrative modelling approaches. Finally, we also propose to investigate how the internal dynamics of proteins is impacted by fluorescent labelling, which is crucial for smFRET studies. This research is part of the PhD projects of Christian Beck and Benedikt Sohmen.

Experimental Report

1 Sample preparation

Wild type yeast Hsp90 and a variant of Hsp90 where the amino acid at position 452 was replaced by a cysteine was produced in *E.Coli* and purified according to elaborated protocols. The fluorophore Atto550 was attached at position 452 of the Hsp90 variant via cysteine-maleimide chemistry. Solvent exchange from a H_2O based buffer to the D_2O measurement buffer (150 mM KCl + 10 mM $MgCl_2$ in D_2O) was achieved by dialysis over night at 4°C. Directly before the experiment the samples were centrifuged for 20 min at 12857 g and 8°C in order to get rid of aggregates. Nucleotides were added directly before the experiments.

2 Measurements

A summary of all measured samples at IN15 and IN16B is shown in Table 1.

Table 1: Summary of all samples measured at IN15 and IN16B in the experiment 8-04-838. The title of the proposed experiment was 'On the structural dynamics of multi-domain proteins'.

Date	Instrument	Sample
01/10/2018 to 04/10/2018	IN15	50 mg/ml yHsp90
01/10/2018 to 04/10/2018	IN15	50 mg/ml yHsp90 + 2 mM ADP
01/10/2018 to 04/10/2018	IN15	50 mg/ml yHsp90 + 2 mM AMP-PNP
01/10/2018 to 04/10/2018	IN15	50 mg/ml yHsp90-D452C-Atto550
01/10/2018 to 04/10/2018	IN15	20 mg/ml yHsp90 + 2 mM ADP
01/10/2018 to 04/10/2018	IN15	20 mg/ml yHsp90 + 2 mM AMP-PNP
06/10/2018 to 08/10/2018	IN16B	Vanadium
06/10/2018 to 08/10/2018	IN16B	D_2O buffer
06/10/2018 to 08/10/2018	IN16B	50 mg/ml yHsp90 + 2 mM ADP
06/10/2018 to 08/10/2018	IN16B	50 mg/ml yHsp90 + 2 mM AMP-PNP

3 Preliminary results

3.1 NSE measurements

As a first analysis, the initial slope was analyzed for the scattering vectors q for each investigated sample. For Fourier times $\tau < 20$ ns the experimental data can be fitted by a single exponential decay.

$$I(q,\tau) = e^{-\Gamma_1 \tau} \tag{1}$$

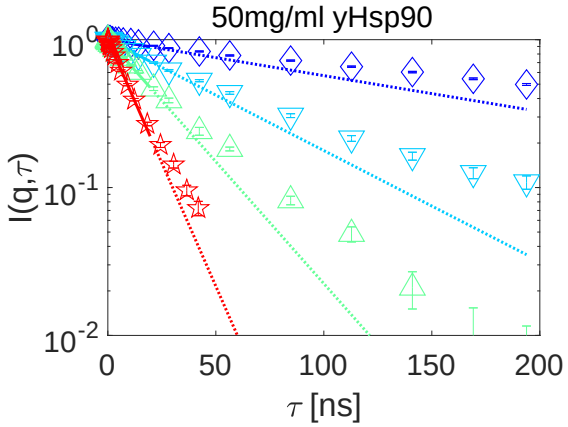
As it can be seen in Figure 1a, clear deviations of the mono-exponential decay are visible for longer Fourier times pointing towards well observable internal dynamics. Effective diffusion coefficients were obtained as D_{eff}=Γ_1/q^2. Figure 1b shows the effective diffusion coefficients as a function of q for the different samples. While the q dependence of the different curves seems to follow the same trend, the different absolute values show systematic offsets.

3.2 QENS data analysis

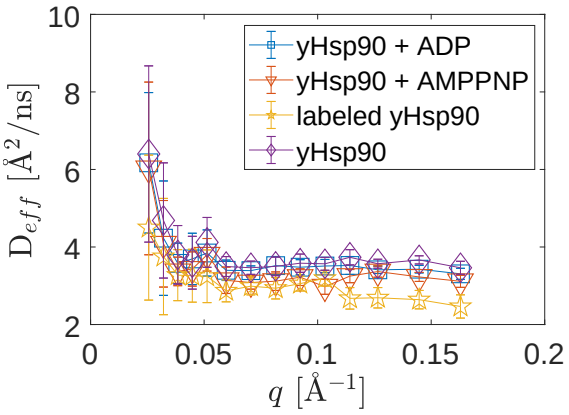
The QENS data was analyzed using two Lorentzians to describe the protein scattering function. The water background was modeled with one Lorentzian, which was fixed during the fit.

$$S(q,\omega) = \mathscr{R}(q,\omega) \otimes (\beta\,[A_0 \mathscr{L}_\gamma + (1-A_0)\mathscr{L}_{\Gamma+\gamma}] + \beta_{D_2O} S(q,\omega)) \tag{2}$$

The resolution function was fitted with two free Gaussian functions. In figure 2a, an example fit of the spectra obtained for the labeled protein is shown at $q = 1\,^{-1}$ with the different contributions. Figure 2b shows the FWHM of the Lorentzian functions as a function of q^2. It can be seen, that the one describing the apparent global diffusion (blue symbols) follows a q^2 dependence at small q, while modulations of the FWHM describing the internal dynamics point to additional hierarchical dynamics. Due to problems with the choppers, it was not possible to measure all planned samples with neutron backscattering.

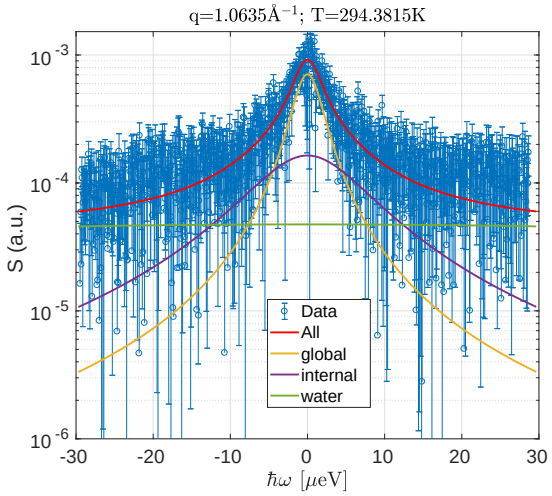


(a) Selected intermediate scattering functions measured at different scattering vectors ($q = 0.038, 0.070, 0.103, 0.144\,^{-1}$) for 50 mg/ml Hsp90 without nucleotides. Symbols represent the experimental measured scattering signal. The solid line represent a single exponential fit for $\tau < 20$ ns. The dashed lines show the extrapolation for higher τ. A clear deviation from the single exponential decay is visible indicating the presence of internal dynamics.

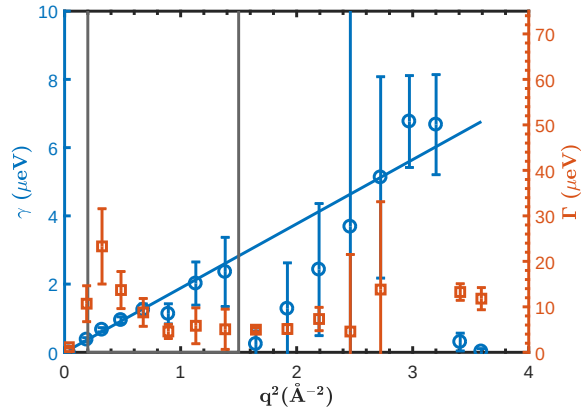


(b) Effective diffusion coefficients as a function of q for labeled yHsp90 and yHsp90 under various nucleotide conditions at a protein concentration of 50 $\frac{\text{mg}}{\text{ml}}$. The different samples show a similar q-dependence with a systematic offset in the absolute values.

Figure 1: Example fits and results of the IN15 measurements.



(a) Fit from the QENS fits. The different contributions of the fit of equation 2 is shown with the different lines.



(b) FWHM of the Lorentzian functions describing the apparent global diffusion (γ) and the internal dynamics (Γ) as a function of q^2 for 50 mg/ml labeled yHsp90.

Figure 2: Example fits and results of the IN16b measurements for the labeled protein.