Experimental report

17/02/2020

Proposal: 8-03-967 **Council:** 10/2018

Title: Light-state solution structure of the photocontrolled DNA-binding protein EL222

Research area: Biology

This proposal is a new proposal

Main proposer: Gustavo FUERTES

Experimental team: Gustavo FUERTES
Aditya CHAUDHARI

Local contacts: Anne MARTEL

Samples: EL222-hybrid
DNA33
EL222-hydrogenated
EL222-matchout labelled

Instrument	Requested days	Allocated days	From	To
D22	5	2	27/09/2019	29/09/2019

Abstract:

The transcription factor EL222 regulates gene expression in a light-dependent manner and, as a consequence, has found widespread use in optogenetic applications. At the molecular level, the protein displays a simple architecture consisting of a flavin binding light-oxygen-voltage (LOV) domain and a helix-turn-helix (HTH) DNA binding domain. The crystal structure of dark-state EL222 shows that LOV and HTH domains are tightly packed against each other thereby blocking the recognition of DNA. Upon excitation of the flavin moiety with blue light, EL222 undergoes quick conformational changes that lead to protein dimerization and DNA complexation. However, our knowledge of the light-adapted states remains incomplete. We propose to employ small-angle scattering techniques, SAXS and SANS, in order to model the structural ensembles of EL222 in solution. Specifically, light-activated EL222 will be measured both alone and bound to DNA. The gathered information is expected to shed light on the activation mechanism of LOV photoreceptors and might help in the design of new variants of EL222 with altered optogenetic features.

Light-state solution structure of the photocontrolled DNA-binding protein EL222

1. Samples.

1.1. Protein.

EL222(17-225) recombinantly produced in *E. coli* BL21(DE3). Two versions:
H-EL222: hydrogenated (^{1}H) expressed and purified by us at the Institute of Biotechnology CAS (Czech Republic), 2 mL at 17 mg/mL.
D-EL222: matchout labelled (^{2}H) produced at the D-lab (ILL) and purified by us. Two batches: 110 microliters at 6 mg/mL (from flasks), 30 microliters at 4.7 mg/ml (from fermentation). It is obvious that fermentation did not work as expected!

1.2. DNA.

33 bp fragment showing nanomolar affinity for EL222. Purchased from Sigma.

1.3. Buffers.

Prepared at the Chemistry lab of ILL (recall that pD = pH + 0.4):

H-buffer: MES 50 mM NaCl 100 mM pH=6.8 (H_2O-based, pH adjusted with NaOH)
D-buffer: MES 50 mM NaCl 100 mM pD=6.8 (D_2O-based, pD adjusted with NaOD)

Samples were cleaned-up and buffer-exchanged by size-exclusion chromatography (SEC) in Superdex 75 Increase 10/300 column. These experiments were performed at EMBL. Protein and DNA quantification was done using a Nanodrop UV/Visible spectrometer (EMBL)

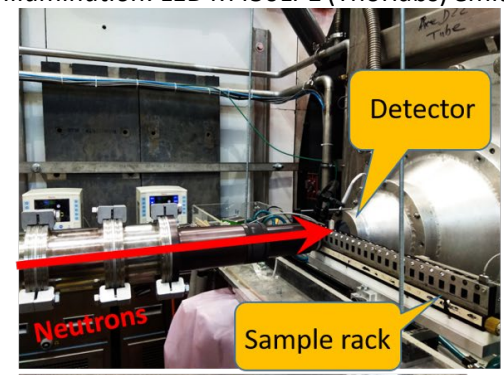
2. Results (analysis as of 17/02/2020).

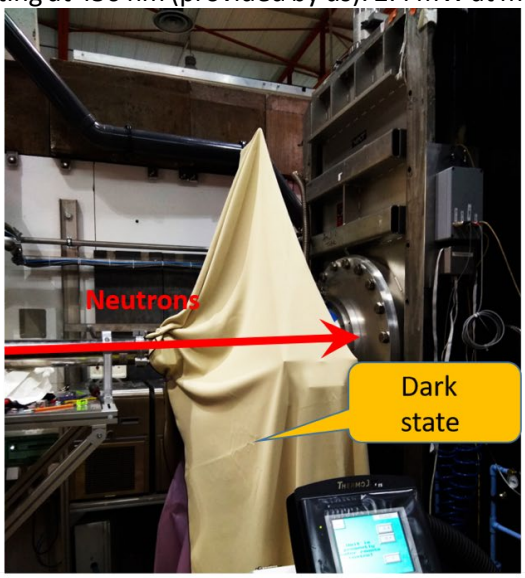
2.1. SANS in rectangular cuvettes.

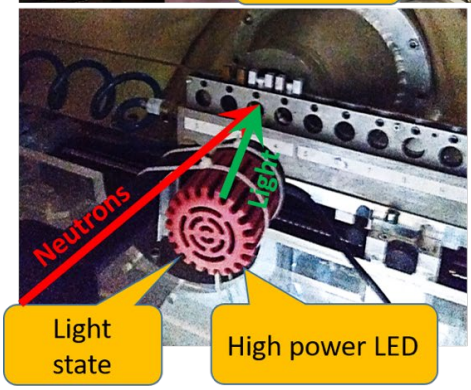
Cells: 200 microliters, 1 mm pathlength, 10 x 7 mm aperture.
Illumination: diode laser emitting at 405 nm (provided by ILL).
Sample: H-EL222 in D-buffer at various concentrations (5-10 mg/mL)
Problem: no difference was seen in light vs dark samples most likely due to low output power: 0.2 mW at maximum as measured by power-meter PM100D (Thorlabs, provided by ILL).

2.2. SANS in circular cuvettes.

Cells: 300 microliters, 1 mm pathlegnth, 2.54 cm^2 irradiation area, 12 mm aperture (diameter).
Illumination: LED M450LP1 (Thorlabs) emitting at 450 nm (provided by us): 2.4 mW at maximum.







Advantages of SANS (neutrons) vs SAXS (X-rays):
✓ Easy to illuminate
✓ No radiation damage
✓ Contrast variation: H_2O vs D_2O

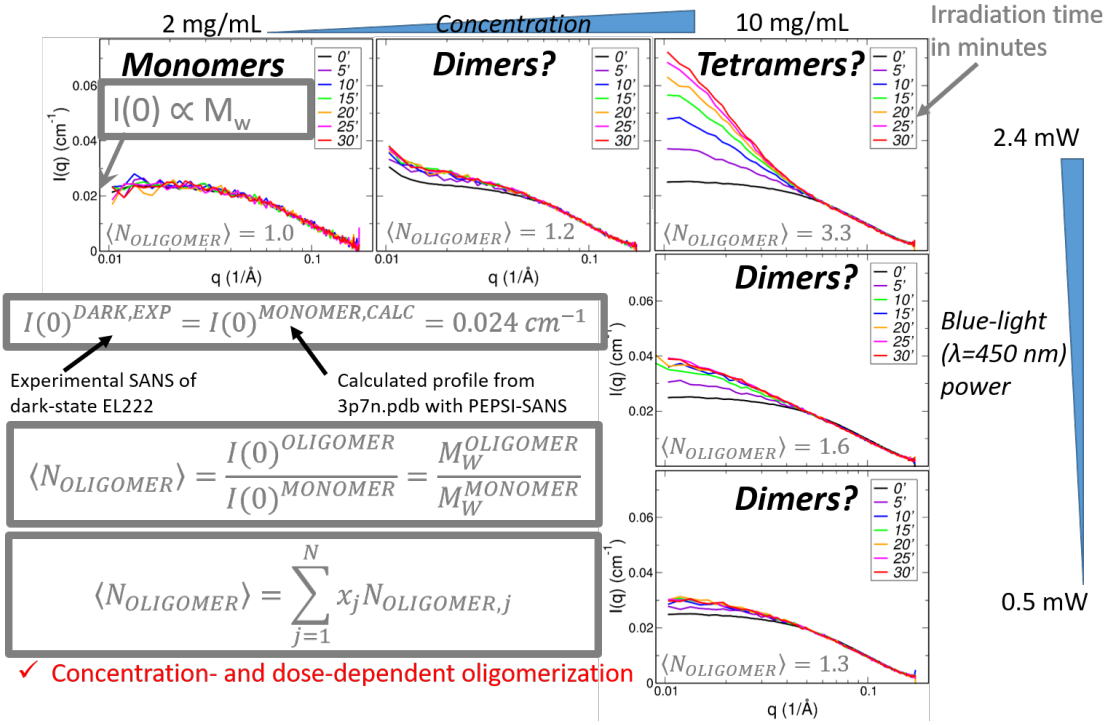
Sample: H-EL222 in D-buffer at 3 different concentrations (2, 5 and 10 mg/mL) and 3 different powers (2.4, 1.2 and 0.5 mW). Power was found to linearly depend on applied voltage (e.g. 5 V corresponds to 2.4 mW measured at 13 cm from the light source)
Data collection procedure:

a) 30 minutes @ 5.6 m (dark-state)
b) 15 minutes @ 1.5 m (dark-state)
c) 30 minutes @ 5.6 m (lit-state): 30 frames x 1 minute
d) 15 minutes @ 1.5 m (lit-state)
e) 200 seconds @5.6 m (dark recovery): 100 frames x 2 seconds
f) 30 minutes @ 5.6 m (dark recovery): 15 frames x 2 minutes
g) 15 minutes @ 1.5 m (dark recovery)

Results: Concentration- and dose-dependent light-induced EL222 oligomerization.

- The intensity at zero angle, *I(0)*, is proportional to the molecular weight and hence to the weight-averaged number of subunits in the oligomer ($N_{OLIGOMER}$)
- *I(0)* increases with illumination time until a steady level is reached.
- *I(0)* decreases after ceasing illumination returning to the initial (dark-adapted) value.
- The higher the concentration of EL222 the larger the oligomer size.
- The higher the dose the larger the oligomer size.

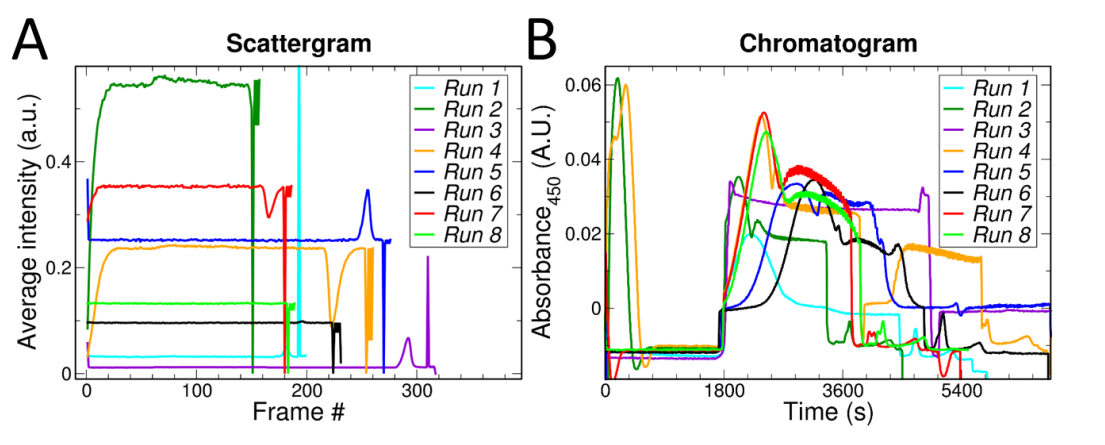
Problem: With the chosen sample-to-detector distances, there is no plateau at low scattering vector *q* (no good Guinier region) in some cases and therefore the size of the oligomers cannot be defined. See possible solutions in **Section 4.2.** below.



2.3. SEC-SANS (SEC coupled to SANS).

Set-up: Superdex 200 Increase 10/300 (provided by us) run at 0.4 mL/min (flow manually reduced to 0.04 mL/min when a peak appears). SANS curves and UV/Visible absorbance spectra at 4 distinct wavelengths - 260 (DNA), 280 (protein), 450 (FMN) and 800 nm (background) - were recorded as a function of time (or frames).
Sample: H-EL222 (10 mg/mL) mixed with DNA33 (16 mg/mL), i.e. 1:2 protein:DNA molar ratio, in 100 microliters total volume pre-irradiated for 30 minutes with 2.4 mW of blue-light power and immediately injected into the column. Note: for D-EL222 5 mg/ml of protein were mixed with 10 mg/mL of DNA.

8 runs: 4 D_2O ratios (0, 0.39, 0.59 and 1.0 as determined from **TEST-2928**) x 2 sample-to-detector distances (5.6 and 1.5 m).
Problem: little or no signal can be detected in the scattergrams. On the other hand, the chromatograms suggest EL222 concentrations below 1 mg/mL. Such low concentrations may explain the absence of scattering signal but it is unclear to us why the samples got diluted from 10 to <1 mg/mL? (a typical dilution factor is 1:3). See possible solutions in **Section 4.1.** below.



3. Conclusions.

- ✓ EL222 forms dimers and higher-order oligomers upon blue-light irradiation (only dimers have been reported in the literature).
- ✓ The light-induced oligomerization of EL222 is fully reversible. However, the radius of gyration of dark-adapted EL222 and dark-recovered EL222 are slightly different, suggesting distinct conformations i.e. closed vs open.
- ✓ Photo-oligomers assemble slowly (~15 minutes of continuous blue-light illumination) and dissociate quickly (~100 seconds after switching lights off). Oligomer disassembly seems slower than adduct rupture (~20 seconds). Need to check the kinetic isotope effect.
- ✓ The overall size/shape of the EL222 clusters can be modeled with ATSAS software.

4. Future directions

4.1. Lit-state structure of EL222 in complex with DNA.

- ✓ Increase the amount (volume and/or concentration) of EL222/DNA mixture injected into the column
- ✓ Purify the EL222/DNA complex by SEC, concentrate, put in the circular cuvettes and irradiate at 2.4 mW while exposing the sample to neutrons.

4.2. Light-induced oligomerization of EL222.

It is very interesting and challenging therefore deserving a separate proposal:

- ✓ Oligomerization state is ill-defined → Need to narrow down $N_{OLIGOMER}$ by measuring at lower *q* i.e. by moving the detector further away from the sample (At D22 the maximum distance is 17.6 meters which means q=0.001 $Å^{-1}$).
- ✓ Quantification thermodynamic (equilibrium constant of oligomerization: $K_{OLIGOMER}$ and kinetic (dark-to-light and light-to-dark rates: $k_{D\rightarrow L}$, $k_{L\rightarrow D}$) parameters
- ✓ Molecular basis (protein-protein interaction surfaces) → Different constructs e.g. LOV domain only and EL222Δα4.
- ✓ Decoupling between FMN photocycle and EL222 photocycle? → Measure dissociation kinetics in both H_20 and D_20. It would be helpful to measure by SANS a slow-cycling mutant AQTRIP (UV/Vis recovery time of 1800 s), does the oligomer also fall apart at a slower rate?