Experimental report

18/03/2022

Proposal: 8-03-1045 **Council:** 10/2020

Title: Lit-state solution structure of EL222 in complex with DNA

Research area: Biology

This proposal is a resubmission of 8-03-1027

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Samples: DNA33
EL222 hydrogenated
EL222 matchout labelled
Hybrid EL222

Instrument	Requested days	Allocated days	From	To
D22	3	1	17/03/2021	18/03/2021

Abstract:

The transcription factor EL222 regulates gene expression in a light-dependent manner and, as a consequence, has found widespread use in optogenetic applications to control gene expression patterns in vivo. At the molecular level, the protein displays a simple architecture consisting of a flavin-binding light-oxygen-voltage (LOV) domain and a DNA-binding helix-turn-helix (HTH) module. The crystal structure of dark-adapted 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 conformational changes that lead to its association with DNA. However, our knowledge of the light-adapted states remains incomplete. We propose to employ an integrative structural biology approach including small-angle scattering techniques, SAXS and SANS, among others in order to model the solution structure of EL222/DNA complexes. The gathered information is expected to shed light on the signal transduction mechanism of LOV photoreceptors and might help in the design of photocontrolled protein-DNA interactions.

Lit-state solution structure of EL222 in complex with DNA

1. Goals

A. Modeling of EL222-DNA complex after irradiation.
B. Modeling of EL222-DNA complex under continuous irradiation

2. Buffer, protein and sample prep.

H-buffer: MES 50 mM NaCl 100 mM pH=6.8
D-buffer: MES 50 mM NaCl 100 mM pD=6.8
EL222(17-225) recombinantly produced in *E. coli* BL21(DE3).
DNA33: high-affinity DNA fragment
Frozen samples were shipped in dry ice as usual. All handling steps were done under dim light.

3. Results (SANS@D22, remote operation).

PROBLEM: EL222 samples started precipitating upon thawing or after mixing with DNA. Re-annealing of DNA (by melting it at 95 degrees and slowly cooling it down (1 °C/min) at room temperature did not solve the problem.
CONCLUSION: The new batch of EL222 was unexpectedly unstable and tended to aggregate. This may have been a consequence of slowly thawing the protein on ice.
SOLUTION: To use an old batch of protein that was kept at 4 °C since last experiments (September 2021, DIR-209).

3.1. SEC-SANS (Size-exclusion chromatography coupled to SANS).

Set-up: Superdex 200 Increase 10/300 run at 0.4 mL/min. 200 microliters containing approximately 20 mg/ml of protein were injected. 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 preparation prior to SEC-SANS: EL222 and DNA33 were mixed in 1:2 protein:DNA molar ratio in 1 mm path-length circular cuvette and 2.5 cm^2 irradiation area. Mixtures were irradiated for 10 minute at 40 mW (with collimator) with a LED M455L3 (Thorlabs) emitting at 450 nm (provided by us)
Runs:

1) EL222 + DNA in D2O buffer.
2) EL222 + DNA in H2O buffer.
3) EL222 alone (Control). Note: direct injection without prior illumination
4) EL222 + DNA in H2O buffer.

Data collection procedure:
Step 1: When the EL222-DNA complex (or the protein alone) starts eluting as seen by the increase in absorbance at 450 nm, stop the flow and acquire SANS (ca. 30 minutes)
Step 2: Lights ON: measure the photo-aggregation kinetics of EL222-DNA complexes (ca. 30 minutes, LED with collimator at maximum power, approx. distance 13 cm.)
Step 3: Lights OFF: measure the recovery kinetics of the EL222-DNA complex (ca. 10 minutes)
Step 4: Resume the flow and finalize the SEC-SANS run.
Problem: the chromatograms were not recorded! In general, little or no signal were detected in the scattergrams. This is surprising since we injected more than 10 mg/ml and a typical dilution factor of 6 should still give plenty of signal.
Results: The SANS traces were analyzed with Chromix. Only the first two runs gave some signal.

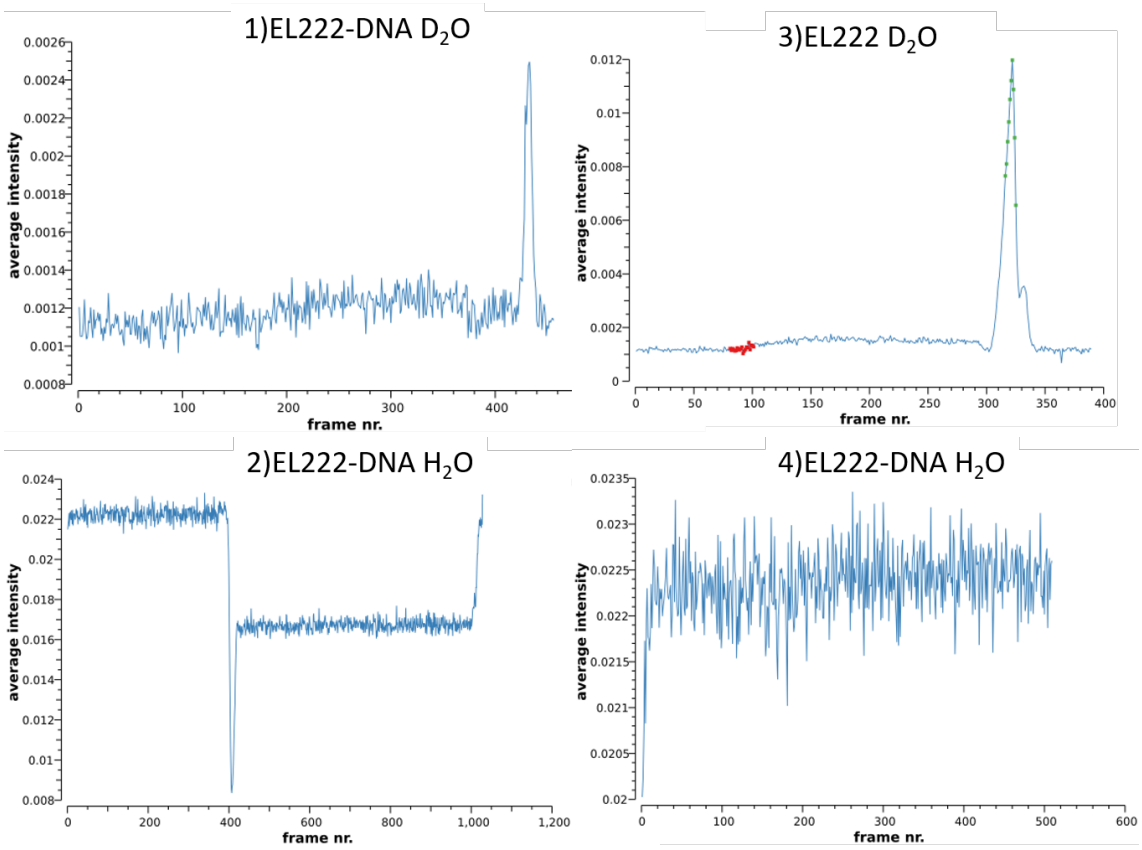

Figure 1. *SEC-SANS scattergrams.*

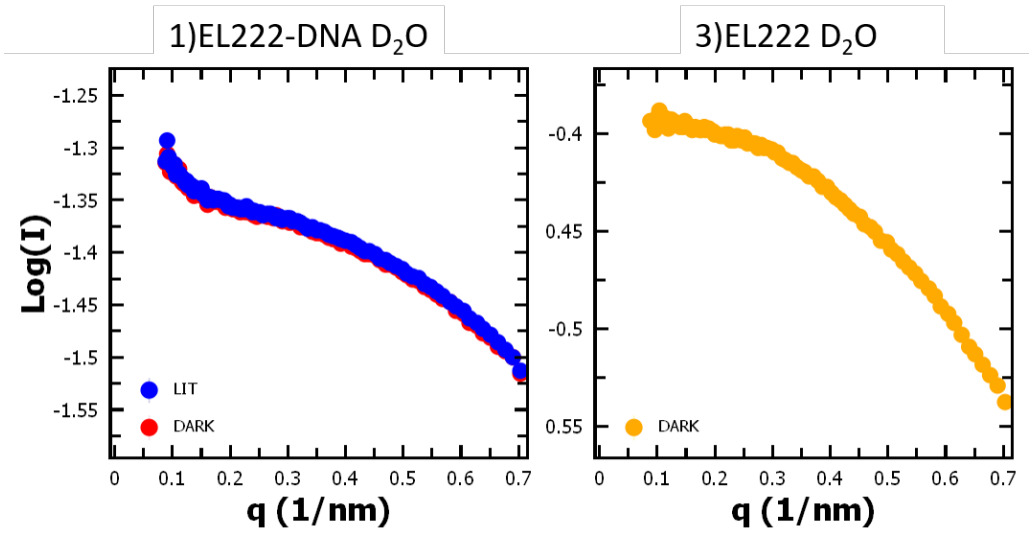

Figure 2. SANS profiles of the eluting peaks. EL222-DNA dark (frames 232-272), EL222-DNA lit (frames 274-331), and EL222 only (frame 316-325).

Sample	Rg (nm)
1) EL222+DNA DARK	1.46
1) EL222+DNA LIT	1.46
3) EL222	1.44

Table 1: Size of the investigated proteins. Radius of gyration (Rg) were obtained using the Guinier approximation.

4. Conclusions.

- ✓ The radius of gyration of EL222 is smaller than the expected for full-length EL222.
- ✓ In presence of DNA, no light-induced oligomerization of EL222 is detected. However, in the absence of DNA, EL222 seems also not to be able to aggregate. The latter observation is in clear conflict with our previous results.
- ✓ Subsequent SDS-PAGE and mass spectrometry experiments suggested the presence of truncated versions of EL222. In fact, EL222 is known to undergo proteolysis in the linker region between the light-sensitive and the DNA-binding domains, which could be enhanced by prolonged storage in the fridge. The existence of truncated versions would explain both the smaller size and the absence of photo-induced aggregation.

5. Future directions

- ✓ It is necessary to repeat these experiments with fresh EL222 samples
- ✓ The SANS profile of the complex between the helix-turn-helix domain of EL222 and DNA may be included in the modeling of EL222/DNA complexes.