Experimental report

23/09/2021

Proposal: DIR-209 **Council:** 4/2020

Title: Photoinduced oligomerization of the transcription factor EL222

Research area: Biology

This proposal is a new proposal

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Experimental team:

Local contacts: Anne MARTEL

Samples: EL222

Instrument	Requested days	Allocated days	From	To
D22	3	1	23/09/2020	24/09/2020

Abstract:

Light controls the structure and assembly of photosensory proteins like cryptochromes, phytochromes and light-oxygen-voltage (LOV) proteins. For instance, EL222 is a light-activated LOV-containing transcription factor that interacts with its target DNA only when illuminated with blue light. EL222 is known to form multimers upon light irradiation but the molecular mechanism and kinetics are largely unknown. Here we propose to employ small-angle neutron scattering (SANS) to uncover the oligomeric intermediates in the photocycle of EL222. SANS will be utilized to render low-resolution structural models of the photo-oligomers as well as to gain insight into the thermodynamics and kinetics of cluster formation and dissociation. We expect that our results will pave the way for the study of light-induced protein oligomerization phenomena. Moreover, the acquired knowledge may help in the design of photo-oligomerization systems for regulating protein-protein interactions with light.

Photoinduced oligomerization of the transcription factor EL222

1. 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).
Samples were cleaned-up (to remove aggregates formed during freezing or thawing) and buffer-exchanged (to D_2O-based buffer) by size-exclusion chromatography (SEC) in a Superdex 75 Increase 10/300 column. The main peak was pooled and concentrated using centrifugal filters of 10 kDa molecular weight cut-off. EL222 was quantified by UV/Vis spectroscopy using Nanodrop spectrometer (ε=13000 $M^{-1}cm^{-1}$ at 450 nm). A stock solution was kept at 4 °C and diluted as needed. All protein handling steps were done under dim light.

2. Results (SANS@D22).

Cells: 300 microliters circular cuvettes, 1 mm pathlength, 2.5 cm^2 irradiation area.
Illumination: LED M455L3 (Thorlabs) emitting at 450 nm (provided by us): 40 mW at maximum (with collimator).
Sample: EL222 (hydrogenated) in D-buffer (except for 2 measurements in H-buffer) at 2 different concentrations (0.08 and 0.8 mM) and 3 different powers (0.6, 6 and 40 mW). The corresponding irradiances are 2, 20 and 130 µW/mm^2. The largest possible sample-to-detector distance at D22 (17.6 meters) was used in order to accurately determine the size of the largest particles.
Data collection procedure:

a) 30 minutes @ 17.6 m (dark-state)
b) 15 minutes @ 1.5 m (dark-state)
c) 30 minutes @ 17.6 m (lit-state): 30 frames x 1 minute
d) 15 minutes @ 1.5 m (lit-state)
e) 120 seconds @17.6 m (dark recovery): 100 frames x 5 seconds
f) 15 minutes @ 17.6 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 ($N_{OLIGOMER}$)
- At "high" concentration, *I(0)* increases with illumination time until a steady level is reached, and the decreases after ceasing illumination returning to the initial (dark-adapted) value.
- At "low" concentration, no changes in I(0) are observed, regardless of the irradiance.

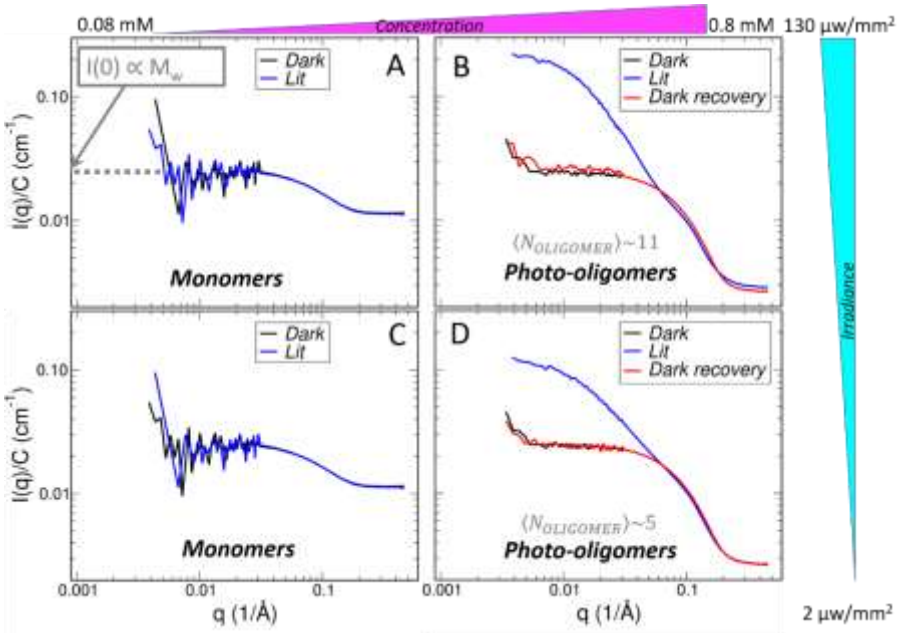

Figure 1. *Concentration- and dose-dependent EL222 oligomerization. A) 0.08 mM and 130 µW/mm². B) 0.8 mM and 130 µW/mm². C) 0.08 mM and 2 µW/mm². D) 0.8 mM and 2 µW/mm².*

- The radius of gyration (R_G) and maximum particle diameter (D_{max}) of the "clusters" could be quantified.

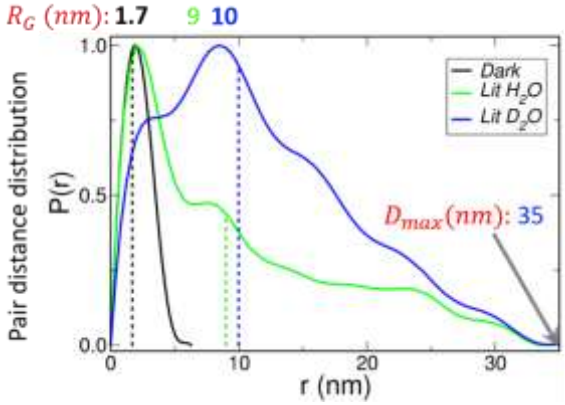

Figure 2. *Pair distance distribution function derived from the scattering curves (GNOM). Radii of gyration (R_G) and maximum particle dimensions (D_{max}) are indicated.*

- The higher the dose the larger the oligomer size but there is a "saturation" effect i.e. beyond 6 mW further increases in light power do not lead to larger photo-oligomers.
- Photo-association and photo-dissociation kinetics are largely independent of concentration i.e. the reactions are zeroth order.

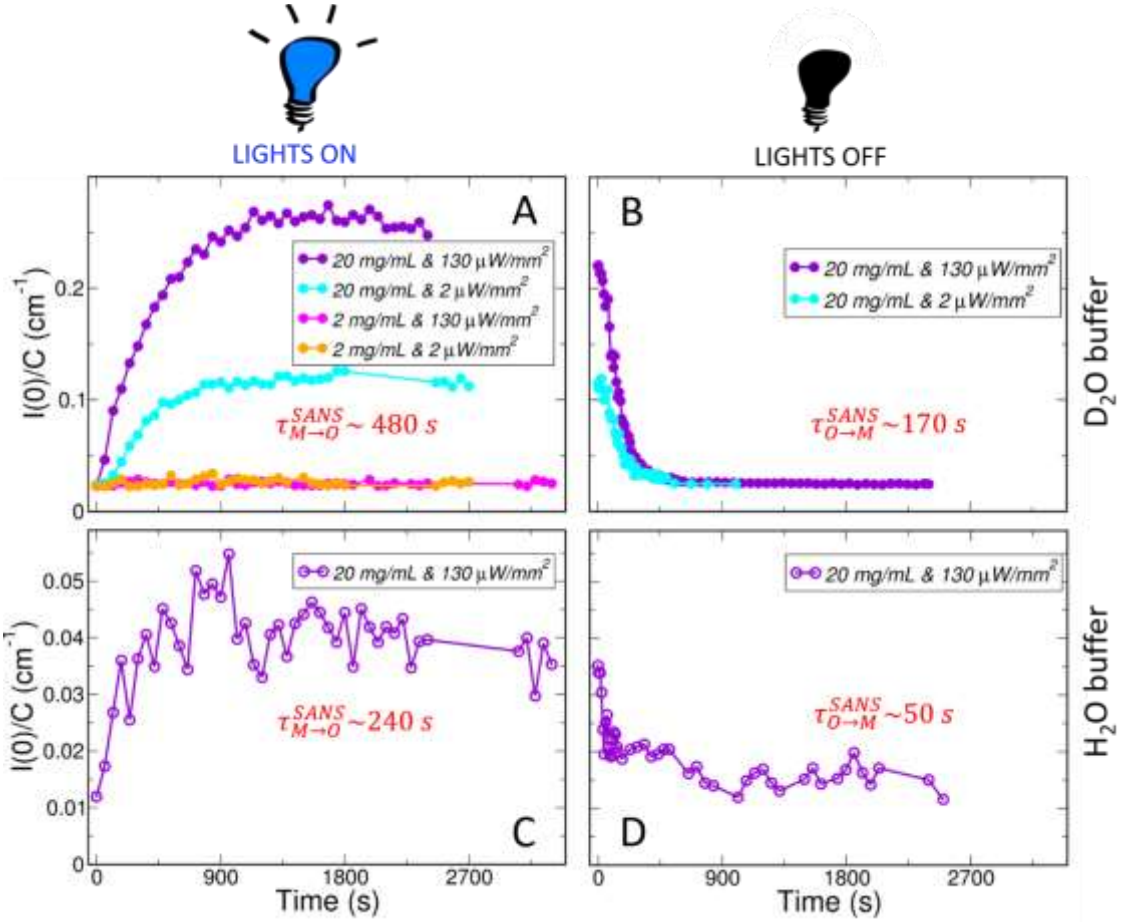

Figure 3. *Variation in the scattering intensity at zero angle as a function of time upon switching lights on (left panels) or off (right panels). A) Photo-oligomerization kinetics in D-buffer. B) Dark disassembly kinetics in D-buffer. C) Photo-oligomerization kinetics in H-buffer. D) Dark disassembly kinetics in H-buffer.*

3. Conclusions.

- ✓ EL222 forms oligomers beyond the dimeric state upon blue-light irradiation (only dimers have been reported in the literature). Photobodies have been described for other photoreceptors like plant cryptochromes and phytochromes.
- ✓ The light-induced oligomerization of EL222 is essentially fully reversible.
- ✓ Photo-oligomers assemble slowly (~8 minutes of continuous blue-light illumination) and dissociate quickly (~3 minutes after turning lights off). Oligomer disassembly seems to share a similar timescale with adduct rupture.
- ✓ Kinetic isotope effect: Photo-association kinetics are 2 times faster in D-buffer (than in H-buffer), while dark recovery kinetics are 3 times faster in D-buffer.
- ✓ The overall size/shape of the EL222 clusters may be modeled with ATSAS software.

4. Future directions

- ✓ It would be good to have more concentration points and/or irradiances.
- ✓ What is the effect of DNA? Does it prevent massive oligomerization?