Experiment title:
" SAXS studies of ELF3/ELF4 proteins - balancing the two arms of the plant circadian clock "

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BM 29

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REPORT:

The plant circadian clock is an endogenous time keeping mechanism that anticipates daily diurnal changes in the environment and is a master regulator of numerous metabolic, physiological, and developmental processes. This clock is periodically synchronized to day-night cycles by the external environmental cues of light and temperature [1]. This oscillator is a network of multiple-interlocked positive/negative transcriptional and translational feedback loops [2,3]. At the core are two related MyB-like morning transcription factors CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), and TIMING OF CAB EXPRESSION 1 (TOC1) that expresses in the evening [4,5]. Other genes critical for the core oscillator mechanisms are EARLY FLOWERING 3 and 4 (ELF3 and ELF4) and LUX ARRHYTHMO (LUX). ELF3 and ELF4 encode highly conserved plant-specific nuclear localized proteins with no similarity to known functional protein domain and are necessary for the clock to oscillate; their biochemical activies are enigmatic. LUX encodes a MyB-domain containing GARP transcription factor [6,7]. ELF3, ELF4, and LUX can assemble into a complex, termed the Evening Complex (EC), and the complex assembly provides the foundation of clock cycling [8,9,10]. Loss-of-function mutation studies of these components revealed total arrythmicity, which thus highlights the central requirement for the EC in mediating clock activity [6,7,11].

The aim of our project was to determine the basic structural parameters and low resolution structure in solution of proteins from the plant circadian clock - ELF4 (full length protein and N-terminal ELF4 fragment), as well as ELF3 middle fragment and ELF3 C-terminal fragment using small angle scattering of synchrotron radiation.

Solution scattering data for ELF3 and ELF4 recombinant proteins were collected on the BM29 BioSAXS Beamline of ESRF (Grenoble, France) using synchrotron radiation (wavelength λ =0.9919 nm). SAXS images were collected using a photon counting Pilatus 1 M pixel detector at a sample to detector distance of 2867 mm within the scattering vector range 0.08 $nm^{-1} > s > 3.8$ nm^{-1}

(where s = 4π sin θ/λ and 2θ is the scattering angle).

Exemplary SAXS data for the analyzed recombinant proteins are presented, as the Kratky plot, in Figure 1. The Kratky plot for globular protein shows an intense maximum due to a highly ordered inner structure, whereas unfolded proteins exhibit a plateau [12]. In Figure 1, for recombinant fusion proteins (containing His-NusA expression tags) ELF4 and ELF3 the Kratky plot showed such plateau, suggesting that the proteins might be unstructured and/or highly dynamic. However for the His-NusA-ELF4/His-NusA-ELF3 (middle fragment) complex (in molar ratio 1:1) is observed the strong maximum in Kratky plot. This maximum suggests the intermolecular protein-protein interaction, which can contribute to the structural stability of the proteins in the complex.

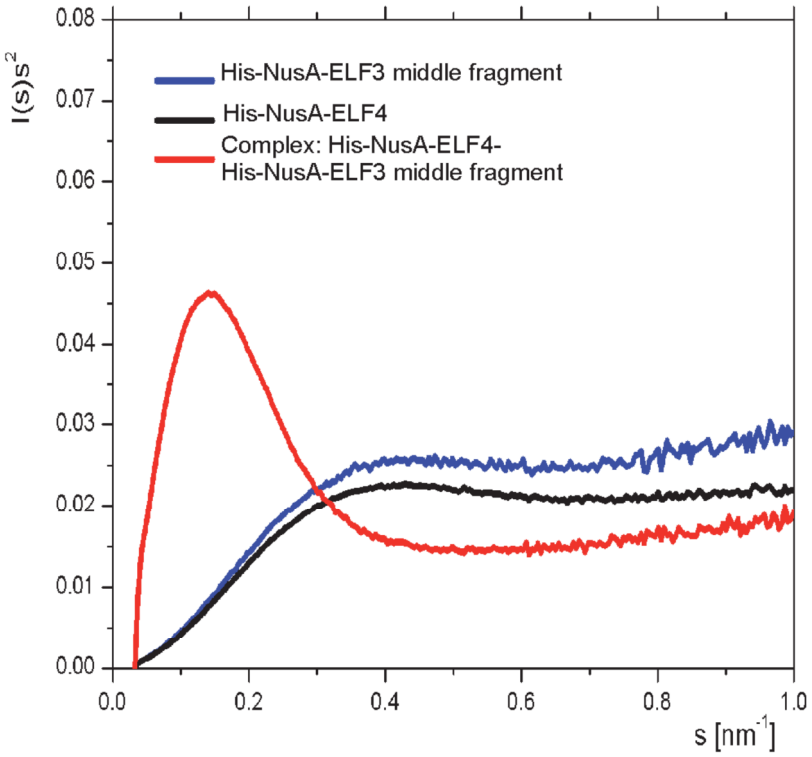

Figure 1. SAXS data Kratky plot for complex between the His-NusA-ELF4 and His-NusA-ELF3 middle fragment (molar ratio: 1:1) and recombinant fusion proteins: His-NusA-ELF3 middle fragment and His-Nus-ELF4.

References

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