STRUCTURAL AND CONFORMATIONAL DYNAMICS OF A DISORDERED PROTEIN MOTIF

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BACKGROUND

Bai et al.(1) identified a WH2-domain-containing protein, SALS (sarcomere length short) in *Drosophila* as an important regulator of the assembly of sarcomeric actin structures. It contributes to the establishement of sarcomere length and organization by promoting the growth of the pointed end of actin filaments. The absence of SALS is already lethal in the embryonic age. This may be due to the shortening of the length of sarcomeric actin filaments, or the disruption of their order.

SALS is a relatively large protein, consisting of 935 amino acids. According to our bioinformatic analysis it is an intrinsically disordered protein (IDP). IDPs are biologically active proteins, that however do not have a well-defined three dimensional structure. They possess specific physico-chemical properties, different from those of ordered proteins (e.g. hydropholic/charged:hydrophobic amino acid ratio, thermal stability, electrophoretic mobility). A lot of well-known proteins that are involved in key cellular processes, and/or are affected by diseases, are partially or completely disordered.

In the case of SALS previous studies have revealed only two motifs consisting of a few 10 amino acids, called WH2 domains, that are also disordered protein regions (IDR) of low structural complexity. Considering their role, they possess actin-binding properties. Depending on the number and sequence of domains, proteins containing WH2 show multifunctional properties. During our previous research we completed the functional analysis of the SALS WH2 domains (SALS-WH2)(2). Based on our results both of the SALS WH2 domains interact with actin, and through their activities shift the monomer:filament ratio towards monomeric actin.

(1) Bai J, Hartwig JH, Perrimon N. SALS, a WH2-domain-containing protein, promotes sarcomeric actin filament elongation from pointed ends during Drosophila muscle growth. Dev Cell.2007 Dec;13(6):828-42.

(2) Tóth MÁ, Majoros AK, Vig AT, Migh E, Nyitrai M, Mihály J, Bugyi B. Biochemical Activities of the Wiskott-Aldrich Syndrome Homology Region 2 Domains of Sarcomere Length Short (SALS) Protein. J Biol Chem. 2016 Jan 8;291(2):667-80.

Abbreviations

SALS: **sa**rcomere **l**ength **s**hort
SALS-WH2: WH2 domain-containing construct
WH2: **W**iskott-Aldrich syndrome protein **h**omology domain **2**
IDP**:** **i**ntrinsically **d**isordered **p**rotein
IDR: **i**ntrinsically **d**isordered **r**egion
G-actin: monomeric actin
F-actin: filamentous actin
Trp: tryptophan residues
ANS: 1-anilino-naphthalene-8 sulfonic acid
GuHCl: guanidine hydrochloride

AIMS

The structural and conformational dynamic properties of the WH2 domains of SALS are not yet known, and we further aimed to perform:

- **Bioinformatics analysis and comparison of SALS-WH2 to a protein with a well-defined structure, actin monomer.**
- **Experimental verification of in silico predicted results.**

CONCLUSIONS

In silico studies, we have shown that:

- **The SALS-WH2 adopt conformations likely representing a transition of globular and swollen coil-like conformations.**
- **The structure of SALS-WH2 is highly context-dependent: it may adopt a more ordered structure after binding to a partner molecule.**
- **The single tryptophan residue in SALS-WH2 has a higher surface availability than that of the sum of the four tryptophans in actin.**

In experimental studies, we have shown that:

- **The intrinsic Trp and the extrinsic ANS fluorescence emission of SALS-WH2 differs from that of G-actin even in the native state.**
- **SALS-WH2 has different spectral responses to chemical and thermal denaturation as compared to G-actin.**
- **SALS WH2 domains do not lose activity after heat treatment, showing thermal stability.**
- **The exothermic peak and baseline shift appeared in the DSC thermogram of SALS-WH2.**

The combination of in silico and experimental studies support the IDR nature of the WH2 domains of SALS

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RESULTS

1. IN SILICO ANALYSIS

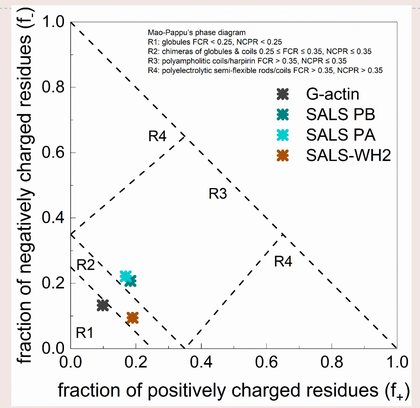

FIGURE 1.1. Das-Pappu phase diagram plot depicting distinct conformational classes intrinsically disordered proteins. The characteristics of G-actin and SALS are indicated (Classification of Intrinsically Disordered Ensemble Regions; CIDER, http://pappulab.wustl.edu/CIDER/). FCR, total fraction of charged residues; NCPR, net charge per residue.

- **SALS-WH2 falls into the R2 region: the proteins adopt conformations likely representing a transition of globular and swollen coil-like conformations.**

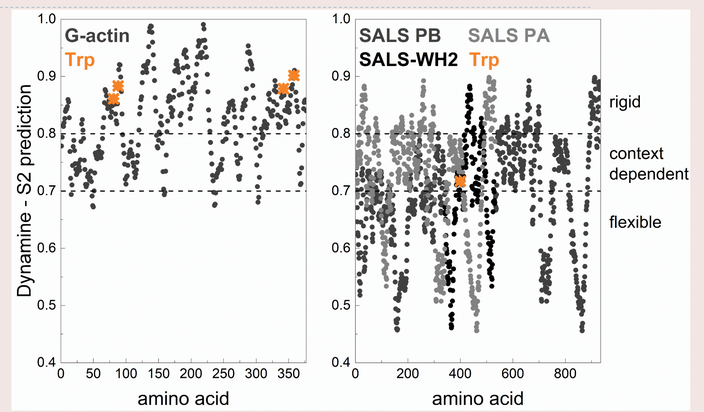

FIGURE 1.2. Protein backbone dynamics analysis of G-actin and SALS (DynaMine, https://bio2byte.be/dynamine/#). The S^2 order parameter predicted from the amino acid sequence is related to the rotational angle of the N-H bond vectors of the protein backbone; the larger the S^2 the more rigid the protein backbone.

- **The structure of SALS-WH2 is dominated by flexible/context-dependent regions: it may adopt a more ordered structure after binding to a partner molecule.**

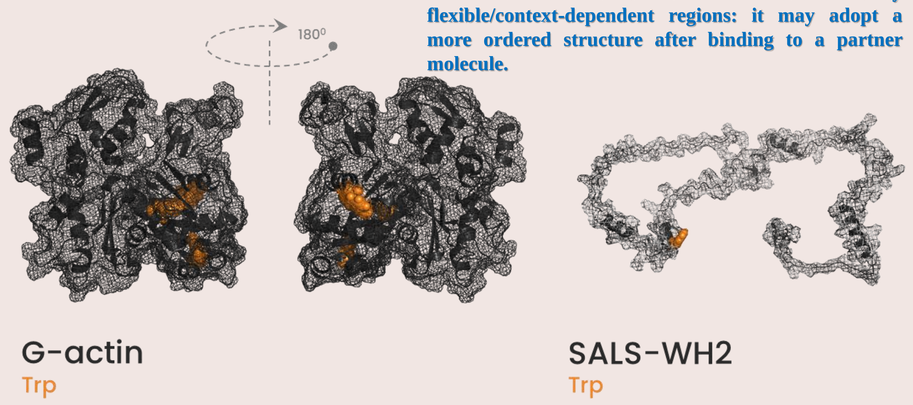

FIGURE 1.3. Structure of monomeric actin and structural model of SALS-WH2. The protein structure is shown as ribbon (secondary structure) and mesh (surface). Tryptophans are shown as orange spheres. The figure was prepared by PyMol. The figure is based on the X-ray structure of G-actin (PDB 1ATN) and the predicted sturcture of SALS (Alphafold, https://alphafold.ebi.ac.uk/). G-actin (*O. cuniculus*, UniProt ID P68135), SALS-PB (*D. melanogaster*, Flybase ID FBpp0081875), SALS-PA (*D. melanogaster*, Flybase ID: FBpp0081876)

G-actin		SALS-WH2	
residue	surface (Å^2)	residue	surface (Å^2)
Trp^{81}	57.529	Trp^{400}	205.976
Trp^{88}	0.598		
Trp^{342}	0		
Trp^{358}	18.063		

TABLE 1.1. Trp surface availability of G-actin and SALS-WH2. The calculation was prepared by PyMol. The surface of Trp residues was given in Å^2.

The single tryptophan residue in SALS-WH2 has a higher surface availability than that of the sum of the four tryptophans in actin.

2. EXPERIMENTAL ANALYSIS

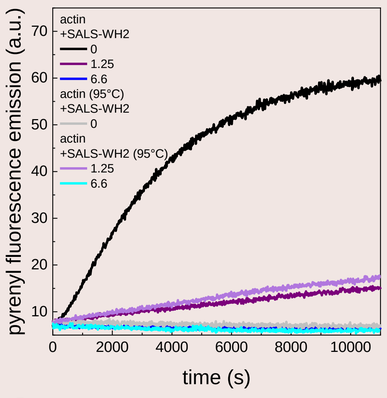

FIGURE 2.1. Thermal stability of SALS-WH2. Representative polymerization kinetics of actin (2.5 µM, containing 5% pyrenyl-actin) in the absence and presence of increasing amounts of SALS-WH2 and heat-treated SALS-WH2, as indicated. As a control, the gray curve shows the polymerization kinetics of heat-treated actin (2.5 µM, containing 5% pyrenyl-actin). a.u., arbitrary units. Heat treating conditions: the samples were heated at 95 °C for 20 min using a mini dry bath thermoblock. Immediately they were placed on ice and incubated with 10 mM DDT for 5-10 minutes before use.

- **Heat-treated actin monomers are not able to form a filament.**
- **SALS-WH2 and the heat-treated SALS-WH2 inhibit actin assembly in a concentration-dependent manner.**
- **SALS WH2 domains do not lose activity after heat treatment, showing thermal stability.**

INTRINSIC FLUORESCENCE

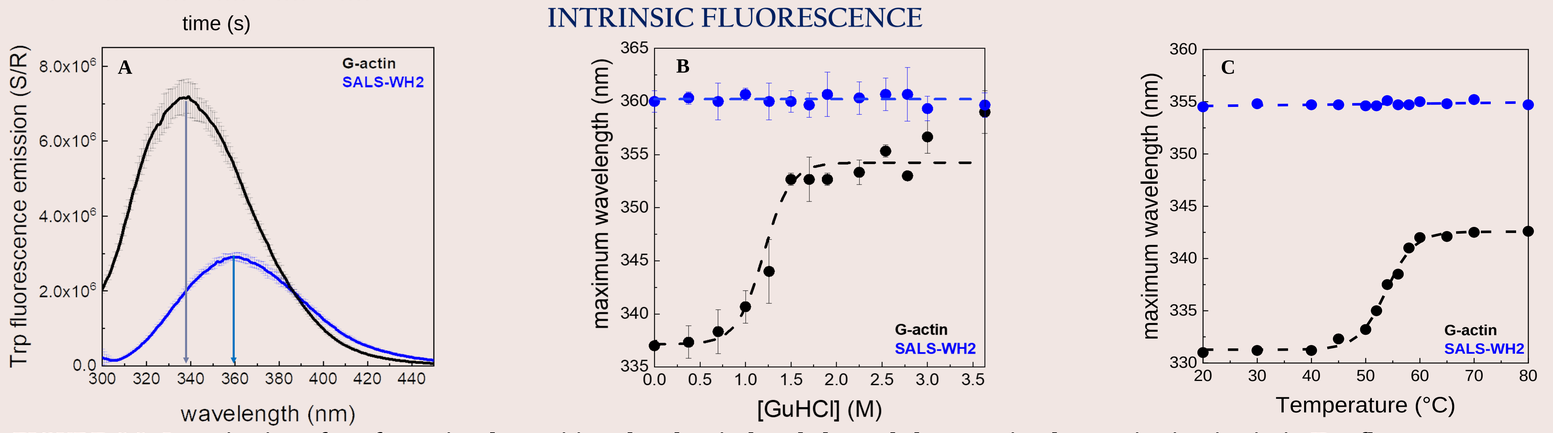

FIGURE 2.2. Investigation of conformational transitions by chemical and thermal denaturation by monitoring intrinsic Trp fluorescence. ***A,*** representative fluorescence emission spectra of Trp of G-actin and SALS-WH2 (0.4 mg/ml), as indicated. ***B, C,*** maximum emission wavelength of Trp plotted as a function of GuHCl concentration and - temperature, respectively. Trp was excited at 295 nm, and the emission was recorded between 300 nm and 450 nm. Dashed lines in the corresponding colors show the sigmoidal fits to the data. S/R is a signal in cps/reference in microAmpere. Error bars, standard deviation (n = 3).

- **Trp fluorescence emission of SALS-WH2 differs from that of G-actin even in the native state, which may indicate a structural difference.**
- **The different spectral responses of G-actin and SALS-WH2 to chemical and thermal denaturation further support that the two proteins have different conformational properties.**
- **For SALS-WH2, instead of a steep sigmoidal change, an almost straight line is obtained by plotting the maximum wavelengths as a function of GuHCl and temperature. This reflects the lack of cooperative conformational transitions during unfolding and may indicate the presence of a disordered structure.**

EXTRINSIC FLUORESCENCE

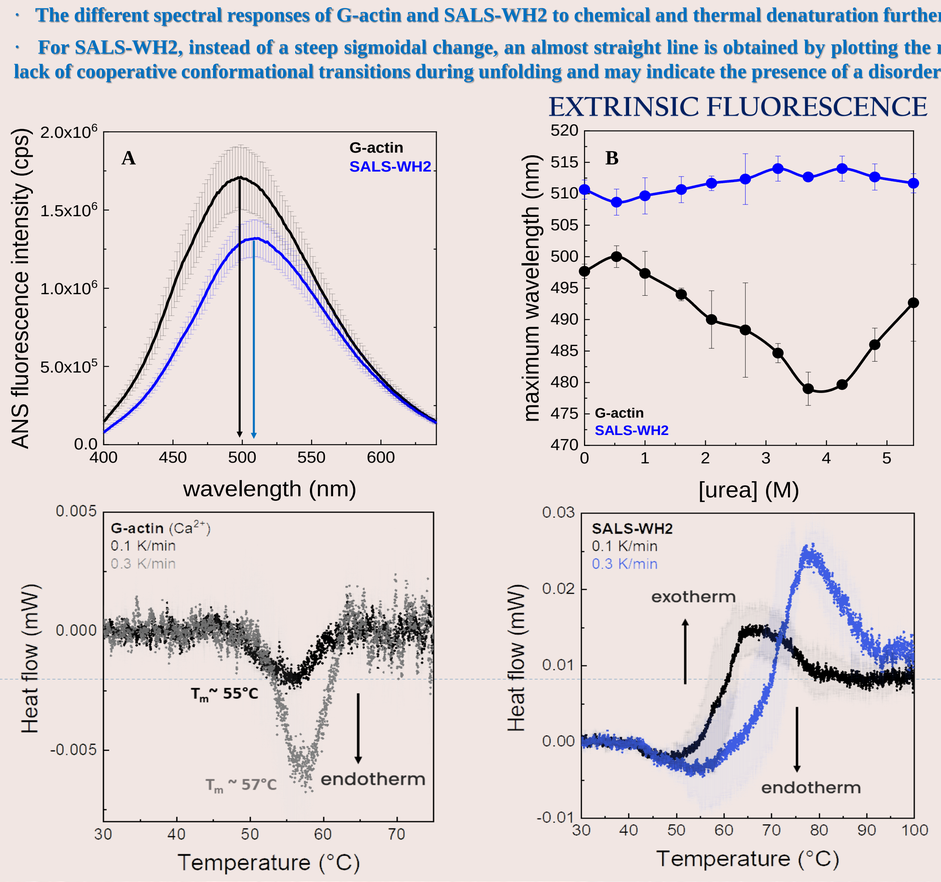

FIGURE 2.3. Availability of hydrophobic core following by extrinsic ANS fluorescence upon chemical denaturation. ***A,*** ANS (102 µM) fluorescence emission spectrum of G-actin and SALS-WH2 (15 µM), as indicated. ***B,*** the conformational transition of the emission maximum wavelength of ANS as a function of urea concentration. ANS was excited at 360 nm, and the emission was recorded between 400 nm and 640 nm. Error bars, standard deviation (n = 3).

- **The fluorescence intensity of ANS was higher for G-actin than for SALS-WH2, ANS is less able to bind to SALS-WH2 even in its native state.**
- **There is no structural transition during unfolding for the WH2 domains of SALS, which may also indicate a more disordered structure.**

FIGURE 2.4. Differential scanning calorimetry (DSC). The denaturation curves of ***A,*** G-actin (1 mg/ml) and ***B,*** SALS-WH2 (1 mg/ml) at a heating rate of 0.1K/min (black curves) and 0.3 K/min (gray and blue curves) using a Setaram MicroDSC III calorimeter. n=3. Error bars, standard deviation.

- **The faster heating rates cause greater heat flow change and push both endothermic and exothermic peaks towards higher temperatures.**
- **Endothermic and exothermic changes in SALS WH2 can also be observed during heat denaturation.**
- **The endothermic reaction can correspond to the unfolding of the alpha-helices of the SALS WH2 domains.**
- **The exothermic peak and baseline shift in the DSC curve may support the IDR nature of the SALS-WH2 domains.**