ANALYSIS OF SUMOYLATED PROTEINS *IN VIVO*

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UPStream EUROPEAN RESEARCH TRAINING IN THE UBIQUITIN PROTEASOME SYSTEM

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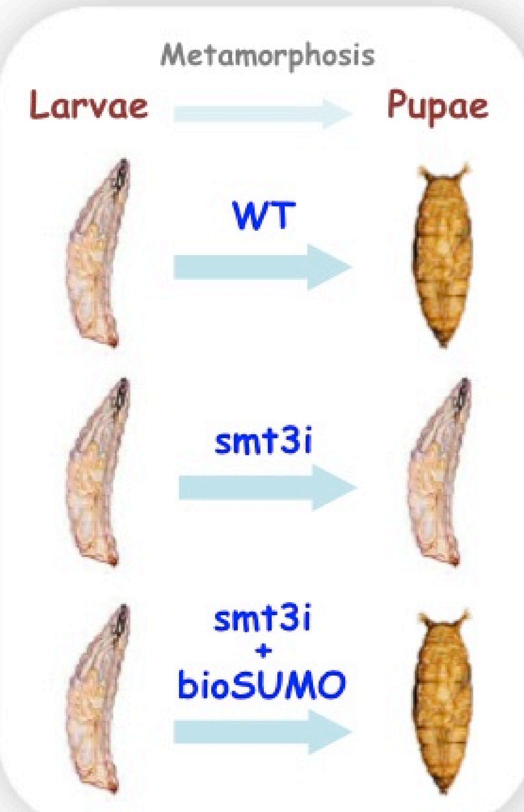
INTRODUCTION

SUMOylation is a post-translational modification characterized by the covalent and reversible binding of the Small Ubiquitin-like Modifier (SUMO) to a target protein. SUMOylation regulates many cellular processes, including transcription, DNA damage repair, protein-protein interactions, protein localization and trafficking. We are interested on studying the *in vivo* role of *Drosophila* SUMO (**Smt3**) implicated in the steroid biosynthetic pathway and required for metamorphosis in flies.

We are developing a novel strategy to isolate **tissue-specific** SUMOylated proteins, based on the *in vivo* biotinylation of a variant of Smt3 (bioSUMO).

II. TISSUE-SPECIFIC ISOLATION *IN VIVO*



Is the bioSUMO construct functional *in vivo*?

SUMOylation is necessary for steroid synthesis and steroid hormones are necessary for metamorphosis. When by RNAi we remove SUMO from the gland that produces steroid hormones, the larvae cannot enter in metamorphosis.

We use the silenced SUMO larvae as a tool to test bioSUMO functionality. Our test shows that the bioSUMO molecule is fully functional *in vivo*.

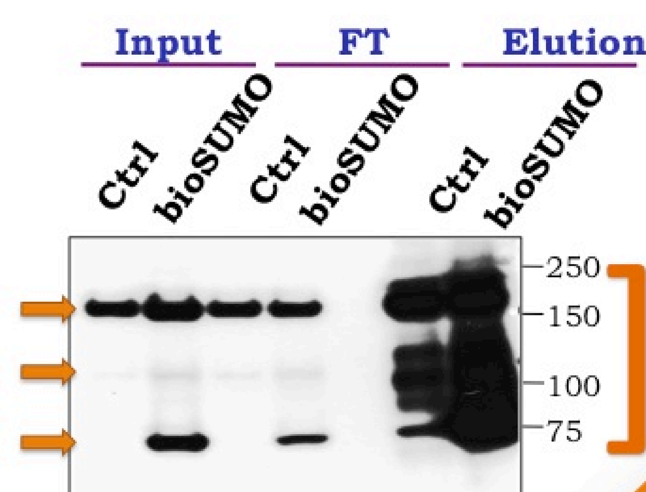
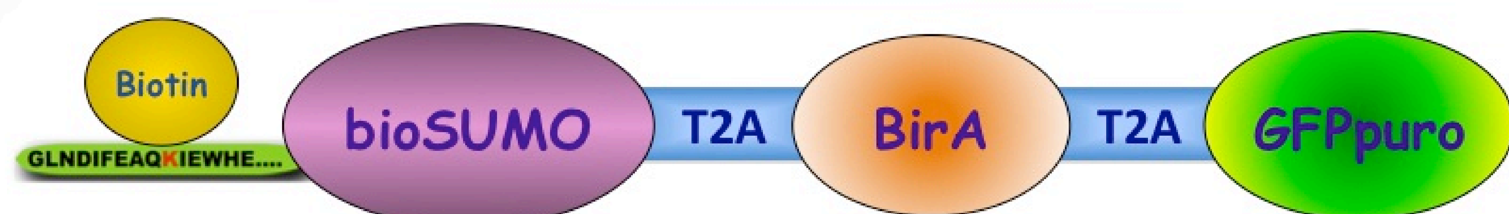


Figure 1. Pulldown of *Drosophila* flies that express bioSUMO in their heads. Input, Flowthrough (FT) and Elution, blotted and hybridized with anti-Biotin antibodies are shown. SUMOylated proteins are indicated with and orange bracket. Arrows indicateendogenous biotynilated proteins.

III. SYSTEM OPTIMIZATION IN CELLS



We adapted the bioSUMO system for the isolation of SUMOylated proteins in cultured cells. We designed a multicistronic approach using novel vectors containing viral-derived 2A like peptides (named CHYSEL), allowing the simultaneous expression of multiple proteins and leading to improved SUMO incorporation.

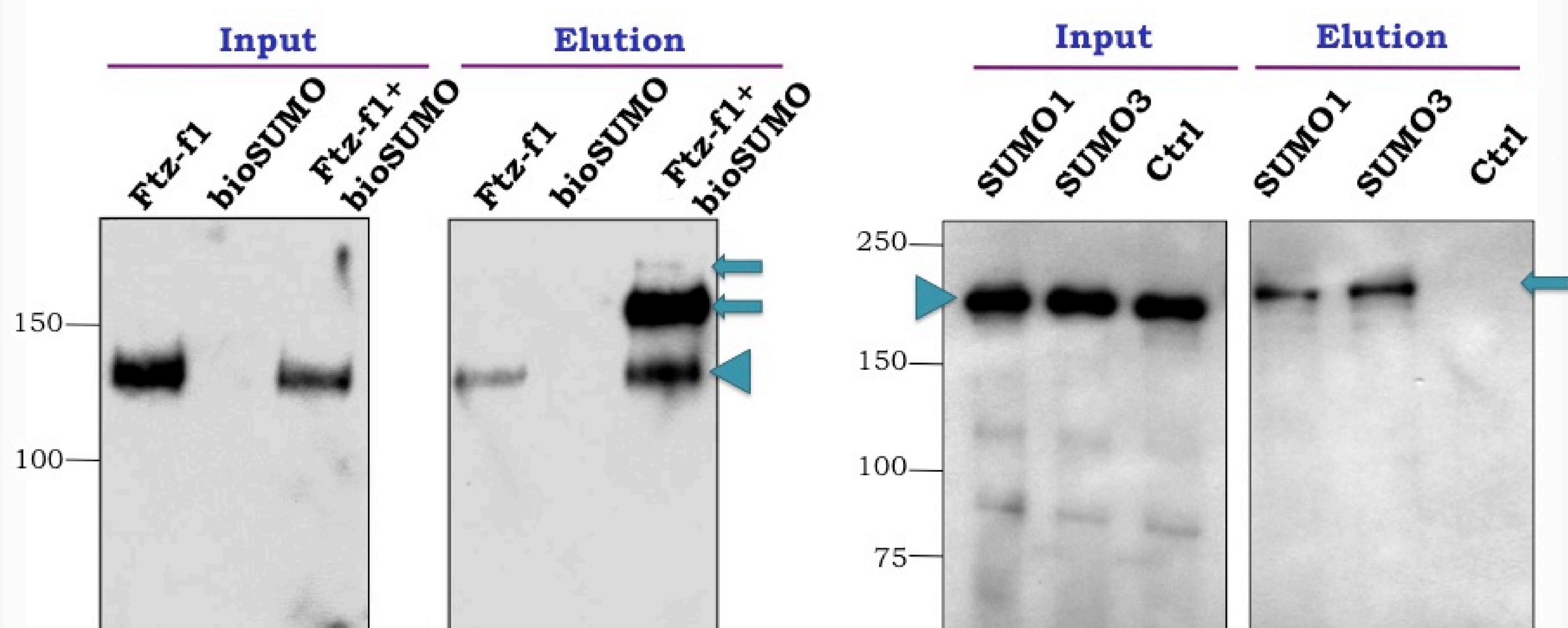
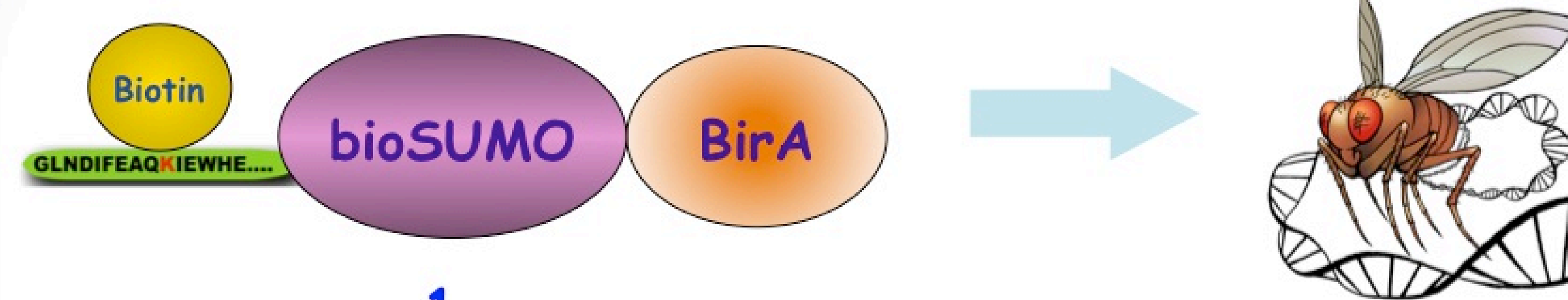


Figure 2a. SUMOylation assay of *Drosophila* transcription factor Fushi-Tarazu Factor1, Ftz-f1. Flag-Ftz-f1 is SUMOylated in presence of bioSUMO, as shown by the higher molecular weight forms (arrows). Arrowhead indicates unmodified protein. Anti-Flag Antibodies were used for the Western blot.

Figure 2b. SUMOylation assay of human SALL1 in HEK293 mammalian cells. We adapted the bioSUMO for mammalian SUMO molecules. SALL1-GFP is SUMOylated in presence of bioSUMO1 or bioSUMO3. Control is BirA alone. Arrowhead indicate the unmodified protein in the input and the Arrow the SUMOylated form in the elution. Anti-GFP antibodies were used for the Western blot.

I. THE STRATEGY



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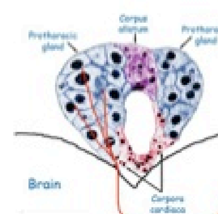
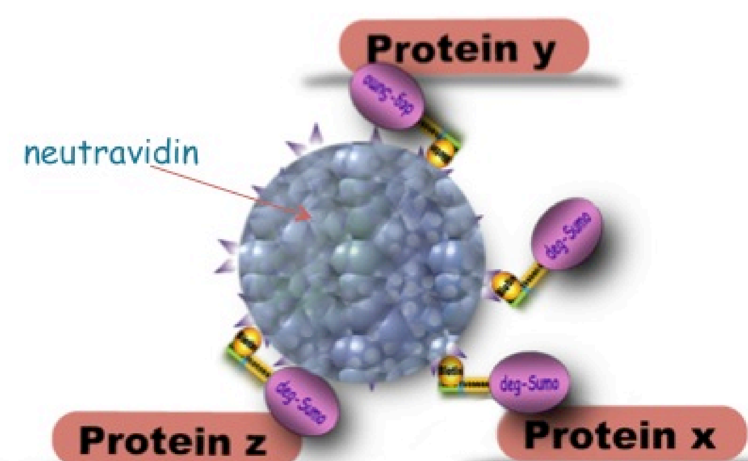
bioSUMO, a degenerated form of *Drosophila* Smt3, was fused to BirA, an *E. coli* enzyme able to attach a biotin moiety to a specific recognition sequence cloned at the N-terminus. This construct was prepared to be expressed in cultured cells and also to make transgenic animals (UAS/Gal4 system).

2

We generated transgenic flies carrying a UAS-bioSUMO-BirA fusion. We can express the transgen using tissue-specific Gal4 drivers. We are interested on expressing the UAS-bioSUMO in steroidogenic tissues, such as the prothoracic gland.





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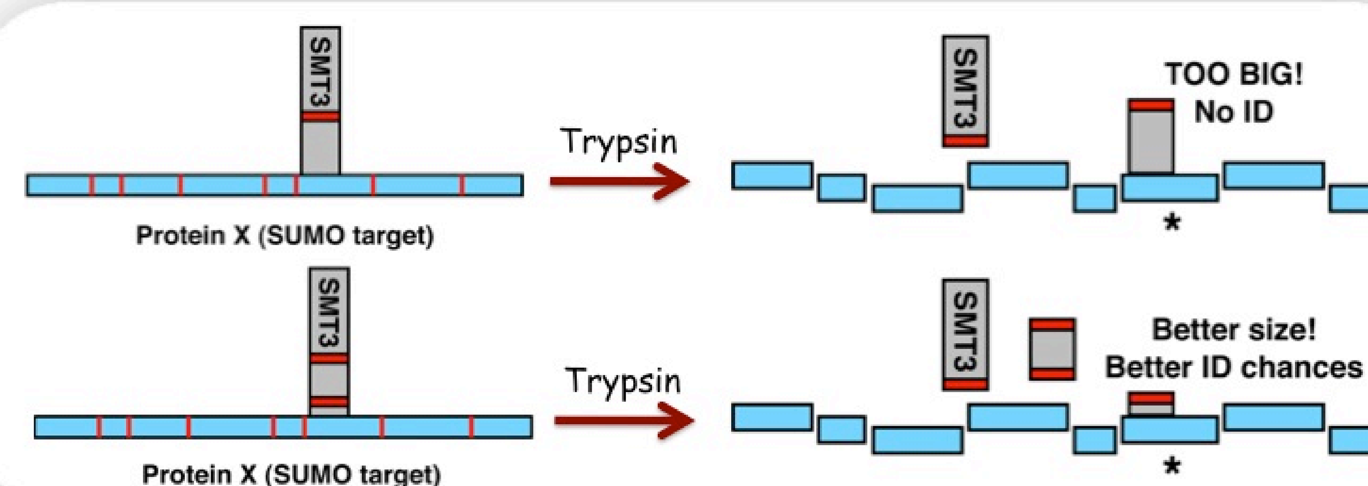
Pulldown using neutravidin beads allows the isolation of biotinylated proteins that are, therefore, SUMOylated.

IV. SYSTEM OPTIMIZATION FOR MASS SPECTROMETRY ANALYSIS



Mass spectrometry analysis requires proteins to be digested by trypsin. To identify the SUMOylation site, peptides generated after trypsinisation should not be too big. Therefore, we designed bioSUMO variants placing an Arginin close to the C-terminal part of the protein.

WT	...IEVYQQQTGGR
T86R	...IEVYQQQRGGR
Q85R	...IEVYQQRTGGR
Q84/Q85R	...IEVYQERTGGR

Figure 3a. C-terminus sequence of different the different bioSUMO variants. Arginine residue **(R)** was placed close to the C-terminal part of the proteins.

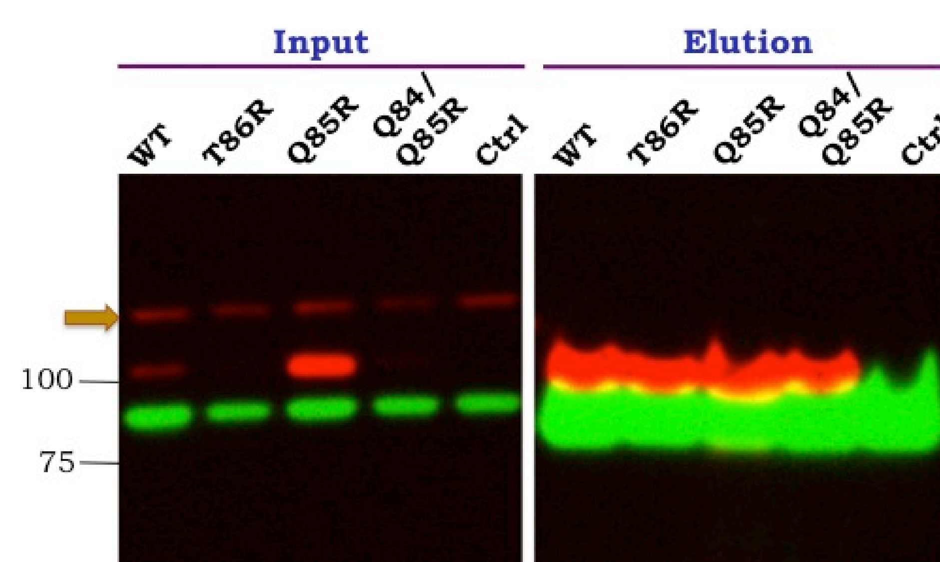


Figure 3b. SUMOylation assay of *Drosophila* GFP-Lola using different bioSUMO variants. Unmodified Lola protein is shown in green using anti-GFP antibodies. SUMOylated Lola is shown in red using anti-Biotin antibodies. Arrow indicates endogenous biotinylated proteins.

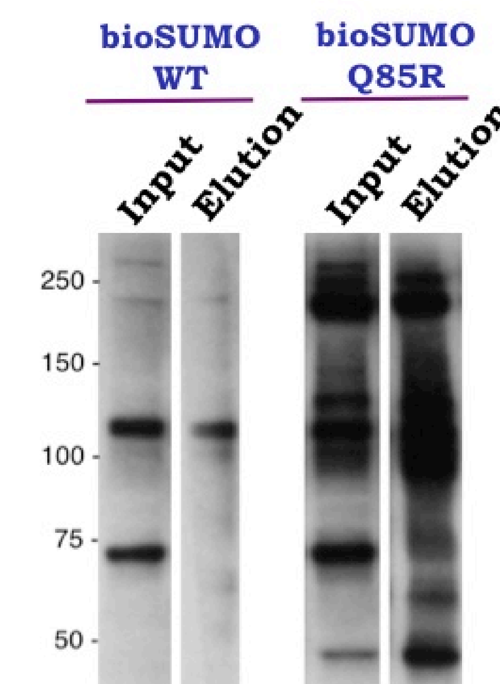


Figure 3c. SUMOylation assay in *Drosophila* cultured cells using bioSUMOwt or the variant bioSUMOQ85R. Efficiency of SUMOylation for the Q85R variant is higher than for WT.

SUMMARY

- A strategy based on biotynilation of SUMO could be used to isolate SUMOylated proteins in a tissue specific manner *in vivo*.
- We adapted this strategy to be used in cultured *Drosophila* or mammalian cells.
- Variants of bioSUMO were designed to improve the identification of bioSUMO conjugation sites in target proteins by mass spectrometry.