Are presynaptic proteins involved in epilepsy SUMOylated?

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Background and Aims

Epilepsy is one of the most common neurological disorder. In UK, **600,000 people** are diagnosed with epilepsy and shockingly there are 21 epilepsy-related deaths every week. It is characterized by frequent seizures which are caused by excessive electrical activity within the neuronal network in the brain.

During brain development, crucial sequential processes take place to form a functional neuronal network. Communication between neurons occurs in specialized structures called synapses. The organization and function of synapses rely on protein-protein interactions which need to be regulated both in time and space. This is mostly achieved by post-translational modifications (PTMs). Among them, SUMOylation is found to be a key regulator for brain development and neuronal communication (Fig. A).

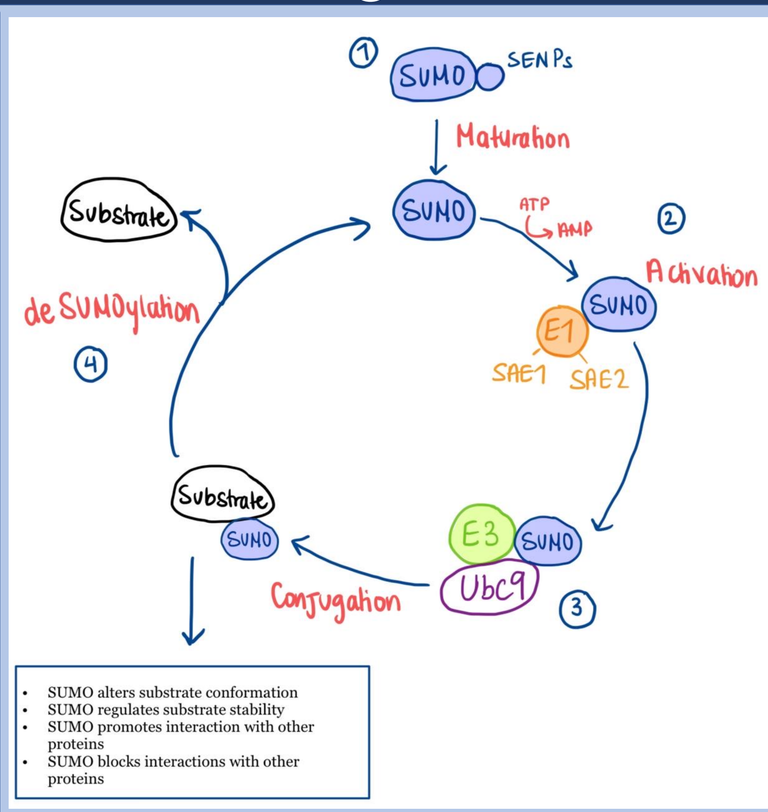


Figure A: Diagram of the SUMO pathway. To synthesize mature SUMO, it has to be first cleaved by SUMO proteases (SENPs) (1). The next step is activation of SUMO by E1 enzyme with SEA1/SAE2 subunits (2). SUMO is passed to conjugating enzyme Ubc9 which mediates conjugation of SUMO to a lysine residue in substrate protein. During conjugation, SUMO has a number of consequences (3).

Through modification of ion channels in neurons, SUMOylation exerts an essential role in controlling membrane potential and cell excitability, which are biophysical markers relevant to epilepsy (Qi, Wang, et al., 2014). In addition, **803 SUMO2/3-modified synaptic proteins** have been identified which represent about 18% of the synaptic proteome (Pronot et al., 2021). 50% are presynaptic proteins involved in synaptic vesicle transport and exo/endocytosis and **51 of those SUMO candidates have been associated to Epilepsy** (Pronot et al., 2021).

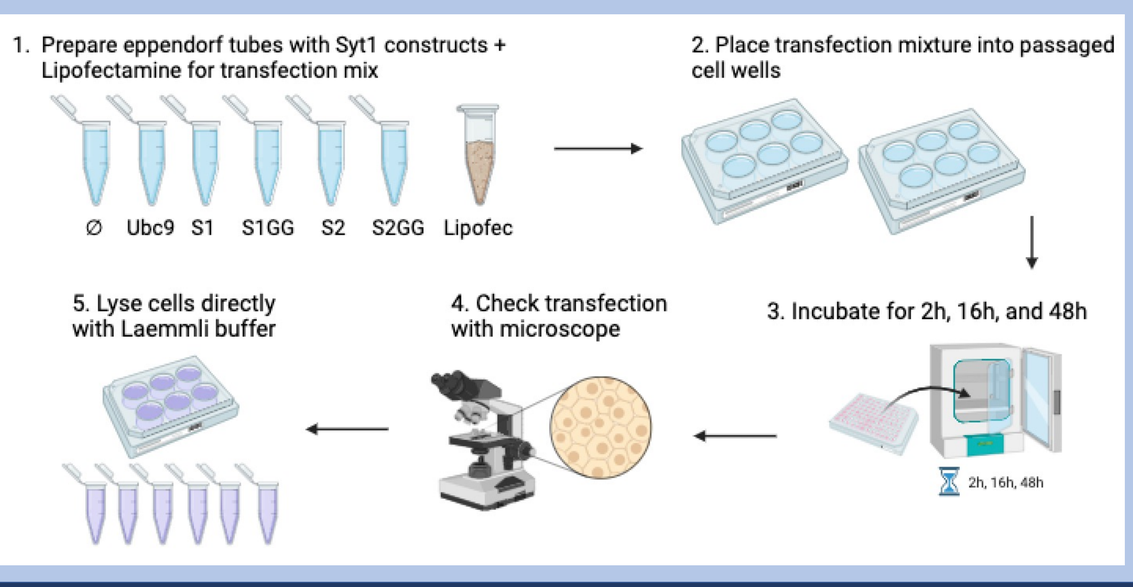
It raises the hypothesis that SUMOylation could be a regulator of presynaptic function. It is now crucial to define the physiological role of SUMOylation in presynaptic proteins and how disruption in SUMOylation contributes to the aetiology of epilepsy.

These are the following aims of the investigation:

A) Use bioinformatic analysis to identify key presynaptic proteins with highly probable SUMO sites
B) Set up SUMOylation assay in the lab
C) Validate the SUMOylation assay protocol on Synaptotagmin1 (Syt1), a known SUMO target

Methods

Part 1: Transfection of HEK293 cells



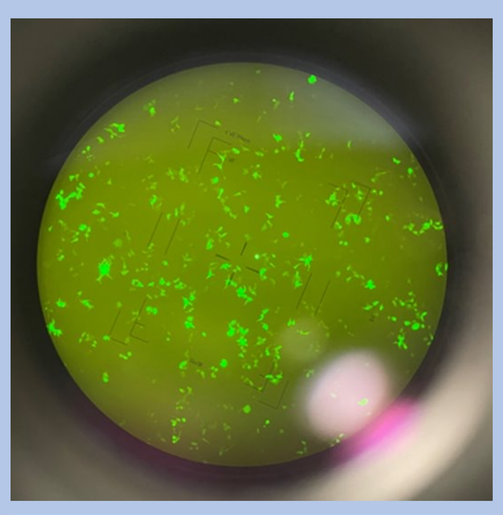
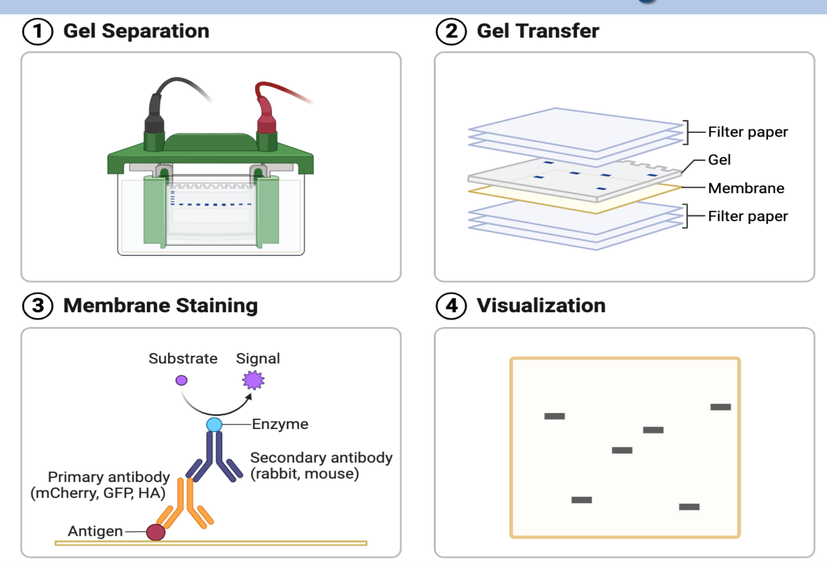


Figure 1: GFP-expressing HEK cells

Part 2: Western Blotting



Results

A) Bioinformatic analysis

Presynaptic Proteins	SUMO interaction	
	Human	Rat
CDKL5	High	High
Dynamin 2	High	High
Synaptotagmin 1	High	High
SV2A	Not found	High
Synaptophysin	Not found	High
Synaptogyrin	Not found	Not found
Syndapin1 (PACSIN1)	High	High
Syndapin2 (PACSIN2)	High	High
Rab5a	High	High
Rab10	High	High
SynGAP	High	Low

Table 1: Summary of presynaptic protein SUMO interaction. The bioinformatic tools used include SUMOgo, SUMOplot, JASSA, and GPS-SUMO.

B) Set up of SUMOylation assay

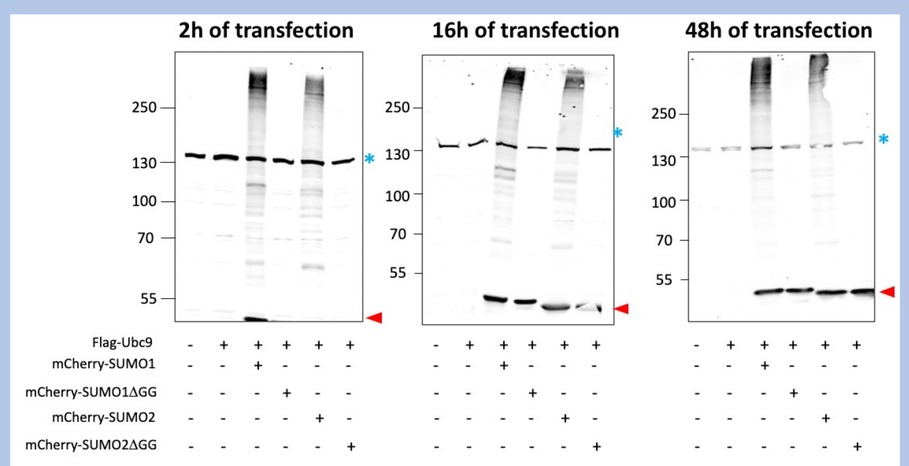


Figure 2: Immunoblot of SUMOylation levels using mCherry antibody on HEKs cells lysates expressing or not Flag-Ubc9, mCherry SUMO moieties WT or mutated for either 2h of transfection, 16h of transfection or 48h of transfection. The red arrow indicates free mCherry-SUMO moieties. Blue star shows an unspecific band present in all the conditions.

C) Identification of SUMO Targets

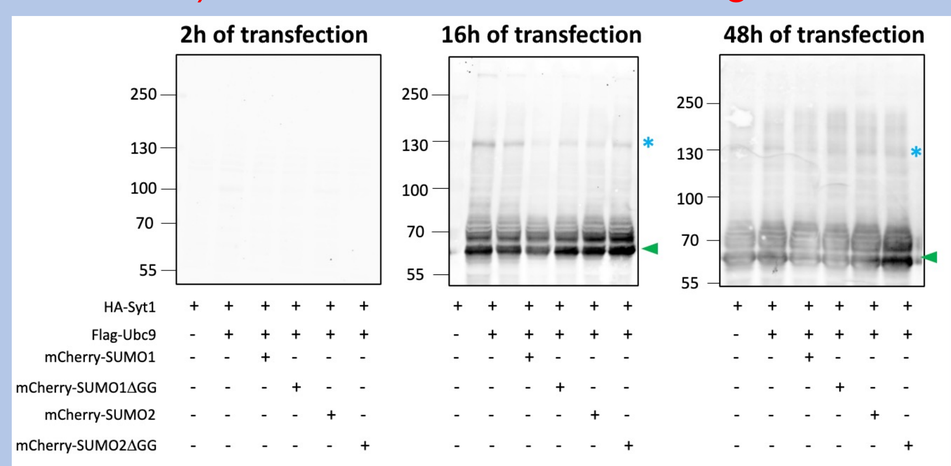


Figure 3: Immunoblot using HA-Tag antibody of HEKs cells lysates expressing HA-Synaptotagmin1 in presence or absence of Flag-Ubc9, mCherry SUMO moieties WT or mutated for either 2h of transfection, 16h of transfection or 48h of transfection. The green arrow indicates the unmodified form of HA-Synatotagmin1. Blue star shows an unspecific band present in all the conditions.

Discussion

SUMOylation assay successful → Results indicate high expression of SUMO proteins in mCherry-tagged SUMO1 and SUMO2 conditions after 2h, 16h, 48h (Fig.1)

Syt1 is not SUMOylated in vitro → Absence of SUMO-modified band in Syt1 condition after 2h, 16h, and 48h → Results contradict publications showing Synaptotagmin1 as a SUMOylation target in mouse brain (Fig.2)

Several explanations :

- SUMOylation is a dynamic PTM and only a small number of proteins are SUMOylated. It is challenging to detect the SUMO-modified band
- SUMO-modified band lost in the lysis process or the western blotting machine is not sensitive enough to detect it
- HEK293T cells which highly overexpress our protein of interest and the SUMO machinery is not sufficient enough to SUMOylate all of the proteins
- *An alternative cell line could be COS7 cells which could give a milder protein overexpression and have also been used to validate the SUMOylation assay*

Qi Y, Wang J, Bomben V C, Li D-P, Chen S-R, Sun H, Xi Y, Reed J G, Cheng J, Pan H-L, Noebels J. L., Yeh E.T.H. (2014) Hyper-SUMOylation of the Kv7 Potassium Channel Diminishes the M-Current Leading to Seizures and Sudden Death. *Neuron*, doi:10.1016/j.neuron.2014.07.042
Pronot, M., Kieffer, F., Gay, A.-S., Debayle, D., Forquet, R., Poupon, G., Schorova, L., Martin, S. and Gwizdek, C. (2021). Proteomic Identification of an Endogenous Synaptic SUMOylome in the Developing Rat Brain. *Frontiers in Molecular Neuroscience*, doi:10.3389/fnmol.2021.780535.

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