

COFUND PhD project – Light in Paris

Synuclein diffusion analysis in neurons using event-based super-resolution microscopy (*SEVEN*)

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The protein α -synuclein (α Syn) is widely expressed in the central nervous system. Under physiological conditions α -synuclein is thought to act as a regulator of synaptic vesicle release [1, 2]. It is enriched in the presynaptic terminal, where it has two dynamic states: free soluble monomers and transiently bound forms, creating a dynamic equilibrium between bound and diffusing molecules [3]. However, α Syn can also be recruited to pathological aggregates in the axon or in the soma, which is a common mechanism in several neurodegenerative diseases including Parkinson disease (PD), dementia with Lewy bodies (DLB) and multiple system atrophy (MSA). The relationship between the diffusion behaviour of α Syn at synapses and its mis-targeting in proteopathic diseases remains unknown. **The central premise of this project is that the diffusion and trapping of α Syn at synapses are essential to its physiological function, and can provide insights into its pathological behaviour.**

Super-resolution imaging using single molecule localisation microscopy (SMLM) is ideal to explore these processes, because the detection of individual fluorophores can yield ultra-structural information about the numbers and distribution of α Syn at synapses, in the cytosol and in axonal aggregates [2](Fig. 1A). The tracking of fluorophores in subsequent image frames in live SMLM recordings gives access to diffusion and binding parameters of proteins in their cellular context. The major difficulty of quantitative and dynamic SMLM is the huge variability of α Syn concentration and mobility (from freely diffusing molecules to densely packed aggregates), which poses unique challenges in terms of spatial and temporal resolution.

We have recently introduced an entirely new approach to SMLM using event-based sensors, also known as neuromorphic vision sensors [4]. Unlike traditional frame-based cameras, these sensors feature asynchronous pixels that respond only to intensity changes, significantly improving their capacity to image densely packed molecules such as protein clusters at high spatio-temporal resolution (μ s instead of ms). Event-based sensors therefore hold exceptional promise for studying multi-scale processes in space and time, and we are developing a new methodology for an improved analysis of protein mobility in living samples. We will apply our expertise in event-based SMLM to study the diffusion dynamics of α Syn at synapses across multiple orders of magnitude, quantify its clustering behaviour, and to explore the earliest consequences of α Syn aggregation in proteopathic diseases by addressing these key questions:

1. What is the absolute concentration of α Syn in neurons and the number of binding sites at synapses?
2. What is the biochemical strength of α Syn interactions at synapses as judged by event-based diffusion analysis?
3. How do pathogenic processes affect α Syn dynamics at resting state and in response to synaptic activity?

Our working model is that there is a discrete number of binding sites for α Syn at synapses (Fig. 1A). Occupancy of these binding sites depends upon the strength of the interactions, the concentration of free (soluble) α Syn, and its diffusion kinetics in the axonal compartment (Fig. 1B). If the binding of α Syn to synaptic vesicles gives rise to oligomers [1], a degree of cooperativity can be expected. The formation of axonal aggregates is also dependent on the concentration of free α Syn and is assumed to be irreversible [2], which could lead to a depletion of the cytosolic pool and to non-equilibrium dynamics (Fig. 1B,C).

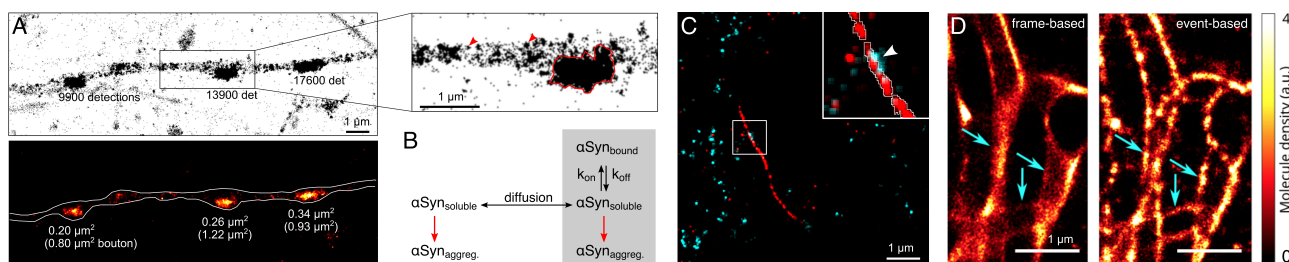


Figure 1. Quantitative SMLM of α Syn-Eos in cortical neurons. (A) Pointillist image showing the number of detections (top) and the reconstructed SMLM image with size measurements of the α Syn domain and synaptic boutons (bottom) [2]. (B) Dynamic model of α Syn trapping at synapses (grey area), axonal diffusion and aggregation (red arrows) [2]. (C) Aggregation of α Syn in axons (red cluster) in response to pathogenic α Syn fibrils (cyan) [Jaubert et al. in preparation]. (D) High-density SMLM using an event-based sensor (right) compared to camera-based SMLM (left) [4]. The capacity of event-based sensors to detect densely packed molecules will be used to quantify α Syn clustering and dynamics.

Task 1. Quantification of endogenous α Syn molecule numbers and concentrations across brain regions

Using a newly developed knock-in mouse model expressing fluorescently tagged α Syn (KI- α Syn^{Eos/Eos}, unpublished) we will conduct quantitative event-based SMLM in brain slices to measure copy numbers and packing densities of α Syn across different brain regions. The advantage of using an event sensor [4] is that we will be able to record precise detection numbers despite the steep concentration differences of diffuse, clustered and aggregated forms of α Syn that can exceed several orders of magnitude (Fig. 1A,D). We will thus be able to measure absolute concentrations of α Syn in different sub-cellular compartments and conformational states. This information is important in view of the known effects of gene dosage on α Syn aggregation [2], making it possible to predict disease progression and the vulnerability of certain neuron types (e.g. dopaminergic neurons) in Parkinson disease.

Task 2. Tracking-free diffusion analysis of α Syn at synapses using an event-based vision sensor

The intracellular mobility of α Syn in axons and presynaptic terminals has been previously measured by calculating diffusion properties based on the tracking of individual molecules [3]. There are severe limitations to this approach due to the need for trajectory reconstruction and the attainable detection density. To bypass these difficulties, we will adopt a new analytical strategy based on the use of an event-based sensor [4]. The spatio-temporal fluctuations of single molecule detections of α Syn-Eos will be recorded in cultured hippocampal neurons to deduce protein concentrations and mobility without performing any tracking, thereby avoiding errors associated with trajectory reconstruction. Our aim is to extract effective trapping energies of α Syn at synapses that can be quantified in thermodynamic terms, as demonstrated in our previous studies with membrane receptors [5].

Task 3. Pathophysiological implications: α Syn diffusion-trapping at synapses during pathogenic insults

α Syn dissociates from synaptic vesicles in response to presynaptic activity [3, 6]. It is not known what effect α Syn aggregation has on the protein dynamics at synapses. To explore the mechanistic link between synaptic activity, α Syn dynamics at synapses, and protein aggregation, we will apply exogenous α Syn fibrils to hippocampal neurons (in collaboration with L. Bousset, MirCEN). The diffusion of α Syn in axons and at synapses will be recorded simultaneously using event-based SMLM. Action potentials will be triggered by electrical stimulation, and the effects of Ca^{2+} influx and vesicle fusion on α Syn diffusion will be assessed using selective blockers. Though technically challenging, these recordings can provide direct evidence about the earliest effects of α Syn pathology on synapse function and plasticity.

The development of dynamic super-resolution imaging through event-based SMLM in this project constitutes a significant technological improvement. Together, the experiments are designed to yield quantitative information about the diffusion-trapping of α Syn in its native cellular environment and will provide insights into the first molecular changes in response to α Syn pathology. Furthermore, molecule counting using event-based SMLM adds a new, quantitative perspective on the role of gene dosage in proteopathic diseases [2]. **The novelty of the project therefore lies in the application of a new analytical strategy to explore an important biomedical topic.**

Partnership

The project brings together two interdisciplinary groups with complementary expertise in the fields of Neuroscience and Physics, in line with the 3i requirement of the COFUND call. **Christian Specht** (Inserm U1195, UPSaclay), is a neuroscientist with a longstanding interest in the molecular interactions and diffusion processes at synapses [2]. **Ignacio Izeddin** and **Clément Cabriel** (Institut Langevin, ESPCI, Université PSL) are physicists specialising in advanced imaging technologies. They have pioneered the use of event-based sensors for single molecule imaging which is essential to this project [4]. The project also has the technical support of our industrial partner **Abbelight** (Cachan) for hardware development.

Available means

Advanced imaging equipment is available at the two partner laboratories, including a Zeiss Elyra PS1 microscope for super-resolution imaging, a state-of-the-art SAFe360 module (Abbelight) for 3D and multi-colour SMLM in living and fixed neurons and brain slices, as well as an event-based SMLM microscope suitable for live-cell experiments. The host laboratory (Inserm U1195, UPSaclay) has cell culture and animal facilities (holding the colony of KI- α Syn^{Eos/Eos} mice), shared infrastructure for electron microscopy, biochemistry and molecular biology, work benches and office space. Experiments in the partner lab (Institut Langevin) will focus on new analytical techniques and software development. Our research is supported by external funding (to CS and II). Both PIs (CS and II) have substantial experience in teaching and supervision at PhD and postdoctoral level.

Bibliography

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