COFUND PhD project – Light in Paris

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

We are seeking a motivated PhD student with a background in neuroscience and/or physics, and a strong interest in the molecular mechanisms of cell biological phenomena. The project is co-directed by **Christian SPECHT** (NeuroBicêtre / DHNS, University Paris-Saclay) and **Ignacio IZEDDIN** (Institut Langevin, PSL University). The PIs have substantial experience in teaching and supervision at PhD and postdoctoral level. 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 is a neuroscientist with a longstanding interest in the molecular interactions and diffusion processes at synapses. Ignacio Izeddin is a physicist specialising in advanced imaging technologies. Together with **Clément CABRIEL** (Institut Langevin), he has pioneered the use of event-based sensors for single molecule imaging which is essential to this project.

The project has been designed to provide in-depth expertise in cutting-edge imaging techniques, as well as a broad range of cellular and molecular methodologies. Thus, upon completion of the PhD, the candidate will have specialized skills, while maintaining an interdisciplinary scope. The progress of the project will be monitored by close supervision. The PhD student will regularly present his/her results at departmental seminars. Participation and presentation at national or international conferences is expected. In addition, progress will be evaluated by annual meetings of the PhD monitoring committee with external experts. It is required that the PhD student will produce at least one major first-author publication during the duration of the fellowship. In addition, contributions to other projects and resulting publications are encouraged through interactions with other members of the laboratory and external collaborators.

Project outline

The protein α-synuclein (αSyn) 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, αSyn 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 αSyn at synapses and its mis-targeting in proteopathic diseases remains unknown. **The central premise of this project is that the diffusion and trapping of αSyn at synapses are essential to its physiological function and can also 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 αSyn 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 αSyn 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 (µs 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. Applying our expertise in event-based SMLM, the PhD project will analyse the diffusion dynamics of αSyn at synapses across multiple orders of magnitude, quantify its clustering behaviour, and explore the earliest consequences of αSyn aggregation in proteopathic diseases to address these key questions:

1. What is the absolute concentration of αSyn in neurons and the number of binding sites at synapses?

2. What is the biochemical strength of αSyn interactions at synapses as judged by event-based diffusion analysis?

3. How do pathogenic processes affect αSyn dynamics at resting state and in response to synaptic activity?

Our working model is that there is a discrete number of binding sites for αSyn at synapses (Fig. 1A). Occupancy of these binding sites depends upon the strength of the interactions, the concentration of free (soluble) αSyn, and its diffusion kinetics in the axonal compartment (Fig. 1B). If the binding of αSyn 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 αSyn 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).

U1195 Inserm-Université Paris-Saclay - 80 rue du Général Leclerc - 94276 KREMLIN-BICÊTRE CEDEX, France
Email : christian.specht@inserm.fr – Tél. : 33 (0)1 49 59 53 86

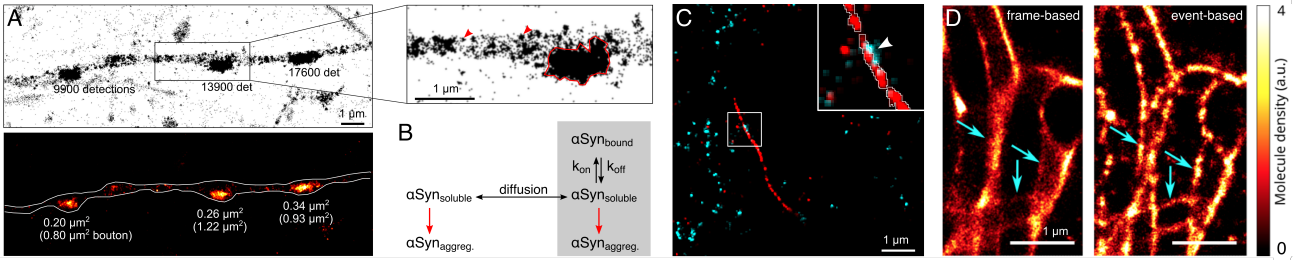

Figure 1. Quantitative SMLM of αSyn-Eos in cortical neurons. (A) Pointillist image showing the number of detections (top) and the reconstructed SMLM image with size measurements of the αSyn domain and synaptic boutons (bottom) [2]. (B) Dynamic model of αSyn trapping at synapses (grey area), axonal diffusion and aggregation (red arrows) [2]. (C) Aggregation of αSyn in axons (red cluster) in response to pathogenic αSyn 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 αSyn clustering and dynamics.

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

Using a newly developed knock-in mouse model expressing fluorescently tagged αSyn (KI-αSyn$^{Eos/Eos}$, unpublished) we will conduct quantitative event-based SMLM in brain slices to measure copy numbers and packing densities of αSyn 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 αSyn that can exceed several orders of magnitude (Fig. 1A,D). We will thus be able to measure absolute concentrations of αSyn in different sub-cellular compartments and conformational states. This information is important in view of the known effects of gene dosage on αSyn 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 αSyn at synapses using an event-based vision sensor

The intracellular mobility of αSyn 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 αSyn-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 αSyn at synapses that can be quantified in thermodynamic terms, as demonstrated in our previous studies with membrane receptors [5].

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

αSyn dissociates from synaptic vesicles in response to presynaptic activity [3, 6]. It is not known what effect αSyn aggregation has on the protein dynamics at synapses. To explore the mechanistic link between synaptic activity, αSyn dynamics at synapses, and protein aggregation, we will apply exogenous αSyn fibrils to hippocampal neurons (in collaboration with Luc Bousset, CNRS). The diffusion of αSyn 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 αSyn diffusion will be assessed using selective blockers. Though technically challenging, these recordings can provide direct evidence about the earliest effects of αSyn 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 αSyn in its native cellular environment and will provide insights into the first molecular changes in response to αSyn 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.**

Eligibility

To apply for this project, see COFUND criteria for eligibility, which include international mobility. For further information please contact christian.specht@inserm.fr

Bibliography

1. Burré, Sharma, Südhof (2018). Cell biology and pathophysiology of α-synuclein. *Cold Spring Harbor perspectives in medicine* 8:a024091.
2. Specht (2021). A quantitative perspective of alpha-synuclein dynamics - why numbers matter. *Front Synaptic Neurosci* 13:753462.
3. Verdier, Laurent, Cassé, Vestergaard, Specht, Masson (2023). Simulation-based inference for non-parametric statistical comparison of biomolecule dynamics. *PLoS Comput Biol* 19:e1010088.
4. Cabriel, Monfort, Specht, Izeddin (2023). Event-based vision sensor for fast and dense single-molecule localization microscopy. *Nature Photonics* 17:1105.
5. Masson, Dionne, Salvatico, Renner, Specht, Triller, Dahan (2014). Mapping the energy and diffusion landscapes of membrane proteins [...] using high-density single-molecule imaging and Bayesian inference. *Biophys J* 106:74.
6. Fortin, Nemani, Voglmaier, Anthony, Ryan, Edwards (2005). Neural activity controls the synaptic accumulation of alpha-synuclein. *J Neurosci* 25:10913.

U1195 Inserm-Université Paris-Saclay - 80 rue du Général Leclerc - 94276 KREMLIN-BICÊTRE CEDEX, France
Email : christian.specht@inserm.fr – Tél. : 33 (0)1 49 59 53 86