Proposal for MSC - M2_PhD fellowship:

"Mechanical toxicity: a possible origin for Alzheimer's disease."

AD is characterized by impairment of short-term memory, cognitive disorders and ultimately neuronal cell loss. The disease is associated with overproduction of the β-amyloid (Aβ) peptide in the brain. Whereas "senile plaques", i.e. deposit of aggregated Aβ are the hallmark for AD, the specific mechanisms underlying AD onset are still unknown. Aβ initially associates as soluble oligomers which form higher-molecular-weight protofibrillar oligomers, converted into the insoluble fibrils that make up amyloid plaques. The prefibrillar intermediates appear to be the primary toxic species. Such "amyloid" behavior occurs for a wide range of polypeptides/proteins that can self-assemble into ordered nanostructures.

Over these last years, studies on *post-mortem* human brains, AD animal models and on cellular systems have shown an amazing interplay between numerous aspects of AD and lipid composition and metabolism. A significant role of both cholesterol and gangliosides (negatively charged glycosphingolipids) appears likely. Two other sphingolipids sphingosine (Sph) and sphingosine-1-phosphate (S1P) have also been linked to AD. Numerous studies on cells and model systems indicate that the role of lipids in the development of AD is at least in part related to Aβ/biomembrane interactions. Aβ, an amphipathic peptide, binds to bilayers and specific lipids act as heterogeneous seeds for its polymerization.

The activity of our team concerns the diversity of the lipid composition of biomembranes and its physicochemical and biological consequences on membrane heterogeneity and mechanical properties. In relation to AD, using model membrane lipid vesicles, we have studied the interplay between Aβ membrane binding and a specific type of lateral lipid heterogeneity: Lo microdomains, as mimics for biological "rafts". Lipid rafts are domains enriched in cholesterol and sphingolipids (e.g. gangliosides) that play essential roles in biomembrane lateral compartmentalization. Unexpectedly, Aβ binds to GM1 only in the non-raft Ld phase, but thereby modulates the dynamics of Lo domain formation and coarsening through line tension effects (Seigneuret et al., in preparation). We have also found that Sph and S1P act antagonistically on the membrane binding of Aβ and that such binding involves "gel-like" microdomains (Watanabe et al. 2015, 2016). Such results emphasize an important role of lipid composition and of lipid lateral heterogeneity on Aβ membrane binding that may have consequences for AD.

The pursuit of our work on the membrane physicochemical aspects of AD will be devoted to the investigation of an hypothesis which is unique to our team: the possible mechanical origin of the pathology. Our proposal is that Aβ, through its interaction with specific lipids and its amyloid aggregation properties, alters the mechanical properties of the neuron plasma membrane and thereby causes neuronal dysfunction (e.g. in axon growth and synapse formation). We call this putative effect "mechanical toxicity".

This idea is based on a series of unpublished experiments (described below) as well as on known properties of the amyloid state. The latter is an energetically stable alternative state for many proteins and peptides. Such structures, under certain physicochemical conditions, form ordered nanomaterials, such as hydrogels. Indeed, a particular fragment of the Aβ peptide can undergo gelation.

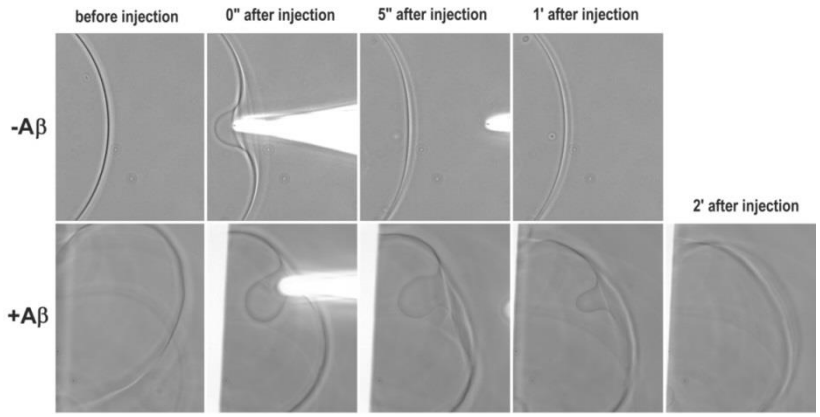
We suggest the possible formation in AD of a quasi-2D Aβ hydrogel at the neuron plasma membrane interface, due to peptide aggregation and specific lipid interactions. This hydrogel could also involve headgroups of glycolipids such as GM1 and, more generally, the glycocalyx of the plasma membrane. This hydrogel layer could alter mechanical properties of the neuronal membrane and hinder neuron functions.

In relation to this hypothesis, the proposed PhD subject will combine 2 approaches where the candidate will study both reconstituted membrane systems and actual neuronal cells in response to Aβ stimulation. Reconstituted systems will allow the candidate to vary lipid composition and extract the mechanical response of well-defined membranes (part 1) whereas living cells will be used to characterize a biological cell system (part 2).

Part 1: Influence of Aβ on the mechanical properties of model bilayers in relation to lipid composition.

The experiments that led us to the "mechanical toxicity" hypothesis are as follows (see figure). GUVs (giant unilamellar vesicle) containing GM1 were deformed by a flow from a micropipette. Without Aβ pretreatment, after stopping the flow, the shape of the GUVs recovered rapidly, emphasizing the elastic behavior of the bilayer. On the other hand, when the GUVs were incubated with Aβ prior to the deformation, the GUV showed a plastic response,

keeping the deformed shape without flow for about 1 min. This shows that the mechanical properties of membranes can be significantly modified by the interaction with Aβ.



Relaxation dynamics of a GUV submitted to a mechanical deformation. untreated (top) or pre-treated with "aged" Aβ solution (bottom) PC/GM1 9/1 mol%, T = 25°C, pH 7.4

The candidate will acquire these experiment and further them into a systematic study: (1) role of lipid composition and lateral heterogeneity; (2) influence of Ab aggregation state (monomer, oligomer, protofibril, fibril, modulated by pre-"aging" of the preparation); (3) quantification. These experiments will be done in collaboration with G. Staneva (Inst. Biophysique, Sofia). **The results will be discussed with the team of J.B. Fournier (MSC)** with whom we previously developed a systematical approach to study membrane deformations triggered by local chemical changes (Bitbol et al. 2011, 2012). This protocol with be adapted to flow-induced deformations in order to extract relevant parameters such as the membrane bending rigidity, the area expansion modulus and the intermolecular friction coefficient.

Part 2: Influence of Aβ on the mechanical properties of neurons

In this part of the project, **the candidate will interact with the team of A. Asnacios (MSC)**, learning the single-cell microplate deformation assay and adapting it to neurons. Human neurons, iPSC (induced pluripotent stem cells)-derived, will be provided in the framework of a collaboration with A. Yamada (ENS Dpt. Chemistry, Paris). In this assay, cells are caught between two glass microplates: one rigid and one flexible, with a calibrated bending stiffness, and the resulting deformation is measured (Bufi et al. 2015). The setup can be used in continuous deformation mode, to measure the static modulus, or in oscillatory mode to measure dynamical storage and loss moduli. The candidate will adapt this approach to measurements on the cellular body of neurons. He/she will also attempt to adapt to axons a recent modification of the setup for small sizes, that uses a spherical glass cantilever (Saitakis et al, 2017). The influence of Aβ pretreatment of neurons (monomer, oligomer, protofibril, fibril) will be studied.

Expected outcomes: In all, these 2 approaches will allow us to test our hypothesis of a lipid-related mechanical origin of AD with possible consequences on therapeutic perspectives. Besides, the physicochemical study of bilayer-hydrogel interactions has reaches other topics such as cell adhesion or membrane-nanomaterial interactions.

Theoretical counterparts: J.B. Fournier is interested in the possible development theoretical framework for the description of the mechanical properties of a lipid bilayer/quasi-hydrogel “superbilayer”.

Possible developments/improvements: (1) Design of a high-throughput microfluidic method for measurement of flow-induced deformations on GUVs (combining trapping by elastic energy gradient and the removable wall techniques). **(2)** Adaptation of the cell microplate deformation assay to GUVs (optimization of adhesion conditions).

External collaborations: G. Staneva (Inst. Biophysique, Sofia), A. Yamada (ENS Dpt. Chemistry, Paris)

Supervisor: Miglena I. ANGELOVA; e-mail: miglena.angelova@univ-paris-diderot.fr; Bât. Condorcet, 8e étage, p.849A; Tel : 00 33 1 57 27 70 82.

Previous PhD supervisions and participations at MSC: Nada KHALIFAT (supervisor M.Angelova), Anne-Florence BITBOL (supervisor J-B Fournier), Chiho WATANABE (supervisor M. Angelova),

Know-hows at MSC: -Our team: preparation of model membrane systems (GUV), video-microscopy, microinjection, control of Aβ aggregation.
-Asnacios team: single-cell microplate deformation assays.
-Fournier team: theoretical analyses