Single nanopore to follow Tau protein aggregation induced by heparin

Nicoletta Giamblanco[a], Yann Fichou[b], Emmanuel Balanzat[c], Jean-Marc Janot[a], Songi Han [b], Sébastien Balme[a]

[a] Institut Européen des Membranes, UMR5635 université de Montpellier ENSCM CNRS-, Place Eugène Bataillon, 34095 Montpellier cedex 5, France
[b] Institute 2, City, Postal code, Country
[c] Centre de recherche sur les Ions, les Matériaux et la Photonique, UMR6252 CEA-CNRS-ENSICAEN, 6 Boulevard du Maréchal Juin, 14050 Caen Cedex 4, France 3
e-mail: sebastien.balme@umontpellier.fr

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Amyloid fibrils are formed by the proteins assembly into highly ordered β-sheet structures. They are involved in several neurodegenerative diseases. The mechanism of aggregation is still totally solve due to the lack of method which allows one to follow under continuous measurement amyloid fibril formation, maturation through amyloid shape analysis. Thus, developing such analytical methods is particularly important to understand the process of amyloid formation. This methods should allow to identify the different intermediates (aggregate and protofibril) which are often more toxic than fibril.
Solid-state nanopores are versatile tools for single molecule sensing. Because their sizes can be tuned from few nm until hundreds nanometers, they allow to characterize biomacromolecule assembly such as amyloid [1]. Typically, the experiments consist to record the ionic transport trough a single nanopore as a function of the time. The translocation of object (here protein aggregates) induces a current perturbation characterized by a dwell time and the amplitude of current blocade. Despite of the simplicity of the concept, three main limitations have to be overcome (i) the unspecific adsorption because the protein aggregation usually enhances the adsorption at solid/liquid interfaces (ii) the nanopore stability and reusability (iii) the possibility to detect object longer than several hundred nanopore without clog the nanopore [2-3]. Recently we propose an alternative strategy to characterize the amyloid using conical track-etched nanopores functionalized to prevent the fouling. The advantage is that the chemical etching, the functionalization and the detection can be operated “one-pot” with a high success rate. Using conical nanopore with different size we have follow the kinetic of β-lactoglobulin amyloid growth. We have detected and discriminated the different intermediates (protofibril and “end-off” aggregate) produced during the amyloid formation. Such strategy as allow us to characterize the effect of promoter and inhibitor of fibrillation process as well as the enzymatic degradation of amyloid [4].
Following this prove of concept, we have applied our strategy to characterize the protein tau aggregation induced by heparin. We have considered ΔTau187 truncated and a Mutant P301L well-known to form amyloid. Using nanopore sensor, we have differentiate three different population of aggregate and determine for each one the kinetic parameters of growth. The analysis of the current blockade fluctuation allows us to formulate new assumptions about the flexibility and autofragmentation of Tau amyloid.

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