Alpha-synuclein affects differently the internal and external leaflet of the lipid membranes

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Report

Introduction

Peptides and proteins possess an inherent tendency to convert from their native functional states into intractable amyloid aggregates. This phenomenon is associated with a range of increasingly common human disorders, including Alzheimer and Parkinson's diseases, type II diabetes, and a number of systemic amyloidosis[1]. Amyloid toxicity is associated to the interaction between aggregates of protein and cell membrane in particular with the lipid part of the cell membrane. By simplifying the system, it is possible to systematically study the interaction of such protein aggregates with lipid membranes. Atomic Force Microscopy (AFM) is an approach to study topographical changes at the nanoscale including local biomechanical properties. As well, AFM is powerful biophysical approach for the study of planar supported lipid bilayers (SLB) of variable composition as membrane model systems[2].

Research activity

During my research activity I used a protocol that has been developed for preparing defect-free planar bilayers with the coexistence of both fluid and gel lipid phases [3].
In particular, I employed two different lipid mixtures for mimicking the composition of both the external and internal leaflet of the neuronal cell membranes.
The main difference between the two mixtures lies in the localization of the lipid head-group negative charge, confined in the gel phase and in the fluid phase for external and internal membrane, respectively.
Among all the possible amyloidosis, here my focus is on Parkinson's disease (PD).
In this context I found that the interaction with α-synuclein, the main peptide involved in PD, induced significant damages in SLBs with different extent in the two investigated lipid mixtures. These results highlighted the fine interplay between protein aggregates characteristic and membrane composition and organization as a key factor in the cytotoxicity of amyloid aggregates.

Materials and methods

A. MATERIALS

• Lipid solution in chloroform
• MilliQ grade water
• 1X Phosphate buffer saline(1X PBS)
• Clean and smooth substrate (mica)

Glass syringes
• Vortex mixer
• Vacuum oven or desiccator
• Extrusion set
• Pipettes
• Incubator

B. PROTOCOL

Small unilamellar lipid vesicle solution preparation

I calculated the amount of lipid chloroform solution and the amount of 1X PBS buffer needed to obtain the desired lipid bilayer compositions. (The stock lipid vesicle solution used is typically 1mM). Glass syringes were used to transfer appropriate amount of lipid chloroform solution for each lipid component into a small glass vial that has been cleaned appropriately. Then the chloroform in the mixture was vapurated very gently in the glass vial, by rotating the vial undernitrogen flow and The lipid cakes were formed on the walls of the glass vial. I Leaved the lipid cakes under vacuum (vacuum oven) for at least 2 hours (usually overnight) for complete solvent removal. The solution was extruded eleven times through a 100 nm porous polycarbonate filtering membrane at a temperature above the highest transition temperature of the lipids in the mixture. The resulting solution would contain SUVs of approximate 100 nm in diameter.

Supported lipid bilayer formation

I Diluted 30µl of 1 mg/ml lipid vesicle solution with 30µl MilliQ and 10µl $CaCl_2$. This is to prevent too many vesicles from binding to the SLB surfaces. And then I Cleaned the substrates (mica) with tape to have a fresh layer exposed. I Put the substrates in a petri dish, and covered the substrate surface with diluted SUV solutions. And then I Left the sample in a moisture box, and incubated at a temperature that is above the lipid transition temperature for 15 minutes (mEXT) and 25 minutes (mINT). (For example, DOPC can be incubated at room temperature, and POPS is suggested to be incubated at 60 °C, as its transition temperature is 55 °C.)

Washing the sample

After incubation, I washed the SLB in petri dishes with MilliQ at least 3 times to remove the vesicles in excess. This is done by creating liquid flow in the solution (pipetting the solution up and down 3 times) and substitute with fresh MilliQ after each wash (removing part of the solution and adding fresh solution in for 3 times).

Results obtained

Ordered and disordered domains co-exist in lipid membrane. The typical aspect of SLBs are shown in the upper images for both the lipid mixtures under investigation.

Alpha synuclein damages SLBs, creating holes preferentially located in the fluid phase domains. The process of destabilization is evident at 40 nM (Fig. 3e,4k), but , for external composition only, 1 nM concentartion of Alpha-syn is sufficient to create significant damage. The last result indicates that the external membranes is more sensitive to the presence of Alpha-syn with respect to the internal.

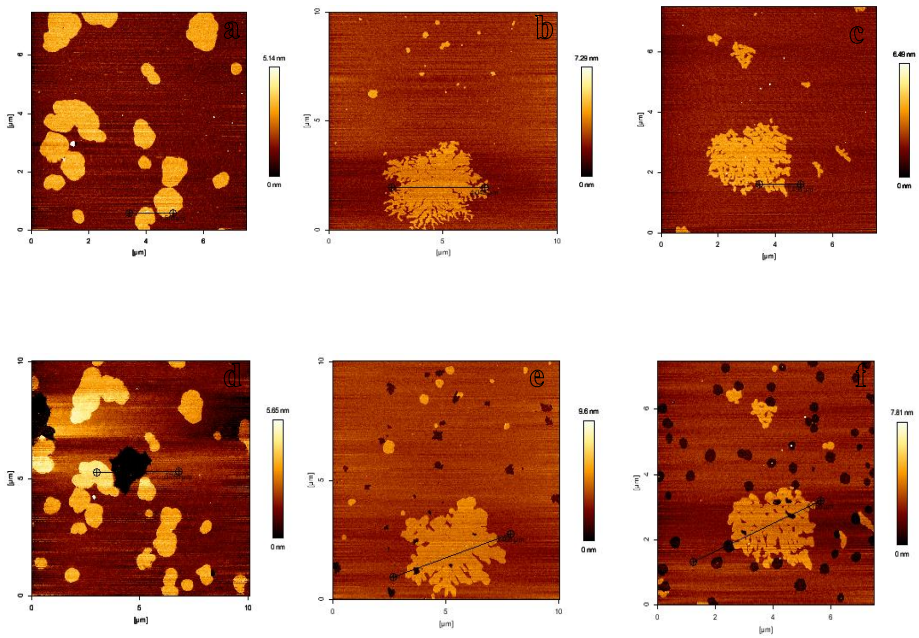

Figure 1. Representative AFM images of mEXT (a,b,c), alpha-syn+ mEXT at concentration 1 nM (d), 40 nM (e) and 200 nM (f).

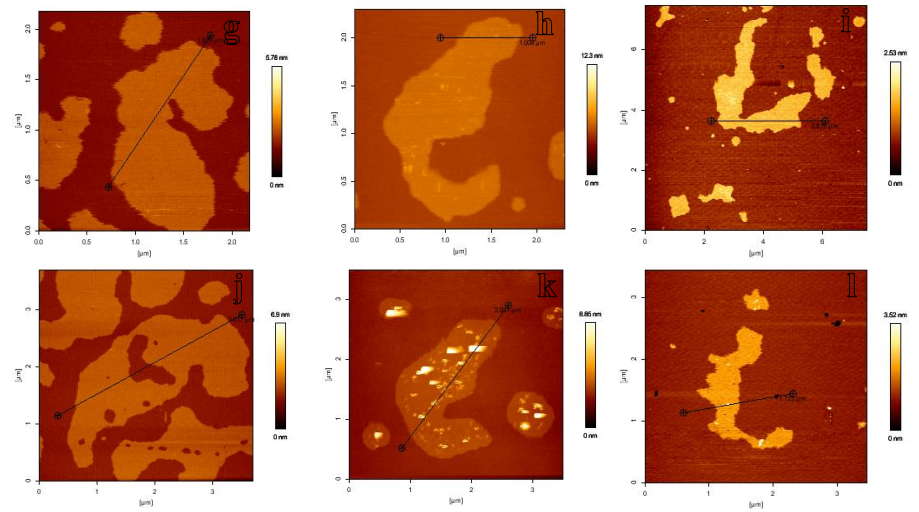

Figure 2. Representative AFM images of mINT (g,h,i), alpha-syn+ mINT at 1 nM (j), 40 nM (k) and 200 nM (l).

Future plans

AFM has the unique ability to investigate biological samples without labelling or fixation in their native conditions at the single-molecule level. It means that it is difficult to locate two or more types of components on the cell membranes by the AFM technique; and AFM cannot be used to image intracellular molecules because it is only used to investigate the sample surface.
Optical microscopy is an indispensable tool in biological research. By labelling samples with fluorophores, optical microscopy provides a way to identify specific components and to investigate interactions among different components. Due to the diffraction limit of light, conventional fluorescence microscopies, such as confocal scanning microscopy have a limited resolution, a lateral resolution of about 250 nm and a vertical resolution of about 500 nm, can not be used to investigate biological samples at the single-molecule level. Therefore, coupling AFM with super resolution optical microscopy such as Stimulated Emission Depletion (STED) is very interesting for us because it provides an excellent opportunity to study the distributions of specific components and the interactions among different components at nanometer resolution. Although optical microscopy has abilities to simultaneously identify several specific components and to deeply visualize a cell, it cannot provide the high-resolution topography of the cell which reveals the cell structure.

References

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Formation activity

I followed and passed these courses:

1) Advanced microscopy and spectroscopy for the study of surfaces and interfaces

Prof. Buzio, Prof. Gerbi,Prof. Savio

2) Advanced Computational Physics

Prof. Ferrando, Dr. Rossi

3) Training school (Nanoscale Optical Microscopy 11-14 June 2019, Venice, Italy)

4) Atomic Force Microscopy: Imaging & Force Mapping, 1 CFU

Dr. Marco Salerno

Comunication at conference:

14th Multinational Congress on Microscopy (MCM2019) in Belgrade, Serbia, 15-20 September 2019

oral talk

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