Experimental report

14/02/2017

Proposal: 8-03-895 **Council:** 10/2016

Title: Testing the role of exosomes in the spread of neurodegenerative deseases

Research area: Biology

This proposal is a new proposal

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Samples: Protein
Lipids

Instrument	Requested days	Allocated days	From	To
D11	2	1	30/01/2017	31/01/2017
D22	0	0		

Abstract:

Exosomes are nanosized extracellular lipid vesicles secreted by cells and circulating in biological fluids used for long-distance intercellular communication by transporting and delivering different type of biological cargoes. Exosomes might have a role in the Alzhaimer diseases (AD), since the propagation of AD through the brain could be linked to exosome transport of beta-amyloid peptides (Ab) and more importantly of its precursors protein (APP). We found that neural exosomes bind to other neurons with a certain degree of specificity, in particular APP enriched exosomes target only neurons in contrast with those not enriched with APP. Thus, it appears that APP and its pathogenic fragments have the ability to hijack a specific population of exosomes and thereby acquire specificity to target brain neurons. If confirmed, this could elucidate possible mechanisms of spreading of Ab throughout the brain, and could pave novel ways towards curing of AD. With this proposal we want to study nanostructural details of the two kinds of exosomes, and assess their properties as colloidal system by probing their interaction with model lipid membrane mimicking those of neurons and glia

Experimental Report 8-03-895 "Testing the role of exosomes in the spread of neurodegenerative deseases"

Experimental Team: Marco Maccarini, Karine LAULAGNIER
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Scientific Context. Exosomes are nanosized extracellular lipid vesicles (~100nm) secreted by cells and circulating in biological fluids such as plasma. Exosomes are used for long-distance intercellular communication by transporting and delivering different type of biological cargoes (proteins, lipids, nucleic acid) between cells and organs.[i] Cellular uptake of exosomes can modify protein and gene expression of the targeted cell.[ii] Exosomes have important implications in health. It has been hypothesized that exosomes represents the substrate for the spreading of pathogenic proteins within the brain causing neurodegenerative pathological conditions such as Parkinson's or Alzheimer's disease (AD). We found that neural exosomes bind to other neurons with a certain degree of specificity. In particular, exosomes from neuroblastoma cells bind indiscriminately to neurons and glial cells and can be taken up preferentially by glial cells. Moreover, we also produced exosomes from Neuroblastoma cells overexpressing APP. Remarkably, these APP-enriched exosomes target only neurons, in striking contrast to those not enriched with APP.

Experimental Results. With this experiment, we wanted to characterise from the structural point of view the two particular types of exosomes (APP-enriched and not APP-enriched) and assess their properties as colloidal system by probing their interaction with model lipid membrane mimicking those of neurons and glia (see proposal 76700). However, the concentration of the exosomes produced was very low, and we needed all the allocated time of 1 day just to perform the SANS scans on the exosomes with good statistics. Therefore of the tasks 1 - 6 of the proposal, we performed only the first two.
The SANS measurements at two contrasts are shown in Figure 1.

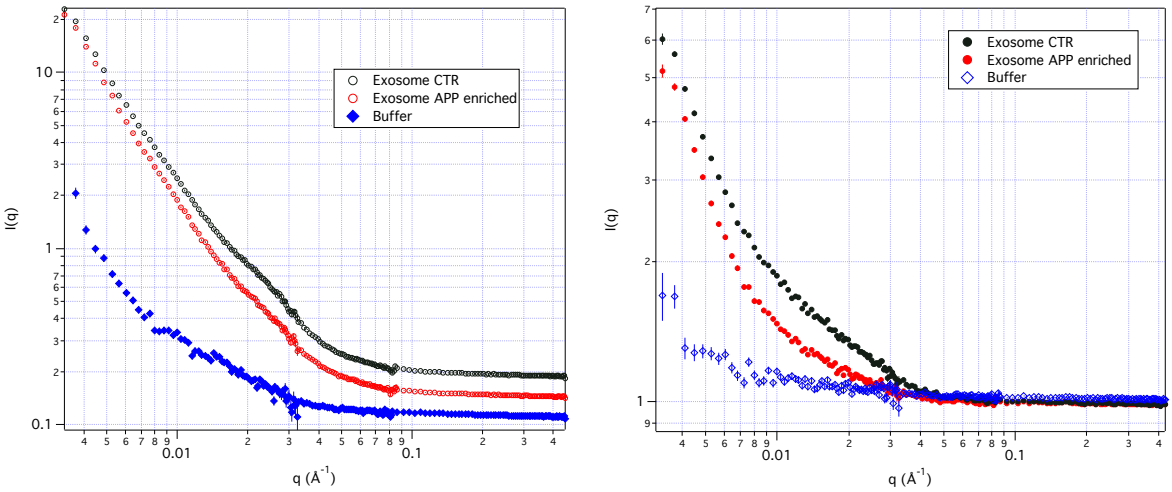

Figure 1 *SANS data of the control and APP enriched exosomes in D2O (left) and H2O (right).*

Light scattering experiments indicated gyration radius around 820 Å. With this size the Guinier regime (Q<1.3/Rg) is not fully reached. The Guinier analysis in this case gives a Rg slightly underestimated of 790 and 760 Å for the control and APP enriched exosomes, respectively. Interestingly in the region of Q ~0.02 $Å^{-1}$ a bump in the SANS profile is noticeable that might help the structural characterisation of the exosomes. A more detailed analysis is on-going.

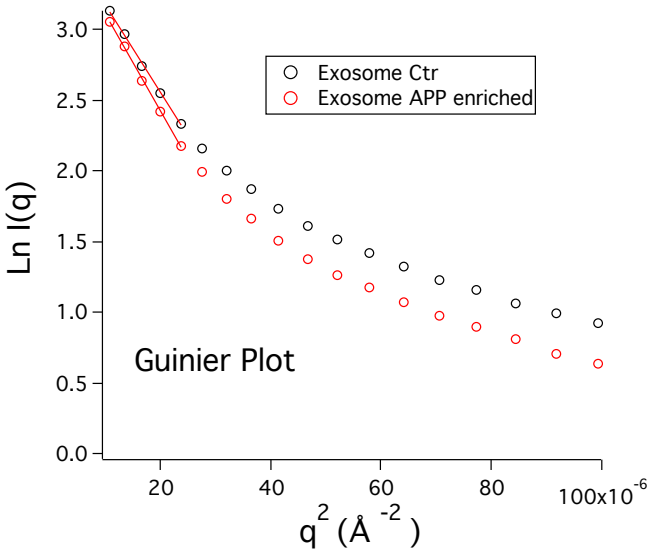

Figure 2. *Linear Fit in the Guinier Regime.*

[i] Lässer *et al.* Journal of Translational Medicine (2011) 9:9 ; van Niel *et al.* J. Biochem. (2006) 140, 13–21

[ii] Valadi *et al*. Nature cell Biology (2007) 9(6) 654 ; Colombo *et al.* Annu. Rev. Cell Dev. Biol. 2014. 30:255–89