Experimental report

13/05/2019

Proposal: 8-02-799 **Council:** 4/2017

Title: Single membranes interaction with Phenylalanine to the understanding of Phenylketonuria disease mechanisms

Research area: Biology

This proposal is a resubmission of 9-13-692

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Samples: phenylalanine
deuterated phospholipids, cholesterol, glycolipids for model membranes
Doxycycline

Instrument	Requested days	Allocated days	From	To
D17	6	3	22/05/2018	25/05/2018

Abstract:

Phenylketonuria (PKU OMIM 261600) is one of the most common inherited metabolic disorders (1:10,000 births). Its onset and progression is connected to an excess of phenylalanine (Phe) concentration in blood and tissues at the basis of neuropsychological deficits. Recent studies indicated the presence of amyloid-like assemblies in the brains of transgenic mouse models and patients with PKU. The co-localization of typical hallmarks of amyloid-type structures and Phe presence was used as an indication that selfassembled structures of the excess Phe could accumulate in the brain and potentially cause neurological illness.If Phe aggregates are toxic, mixing them with interfering molecules that affects either the structure or the stability of aggregates, is effective in reducing toxicity. We observed that the extent and effect of interaction of Phe with model membranes depends on membrane composition. We intend to go on in the study of the type and extent of the interaction of Phe, also mixed with an interfering molecule, with different model membranes, composed by phospholipids, cholesterol and glycolipids in different proportions.

Phenylketonuria (PKU OMIM 261600) is one of the most common inherited metabolic disorders (1:10,000 births). Its onset and progression is connected to an excess of phenylalanine (Phe) concentration in blood and tissues.
We were interested in the study of the extent and effect of Phe interaction with membrane lipids, to assess their mutual structural interaction and understand the conditions for Phe aggregation on membrane surface.
Calorimetry, SAXS, WAXS and SANS measurements showed the propensity of Phe to interact with raft-like membranes affecting lipid structure and thermotropic behaviour.
Preliminary reflectivity measurements on supported model membranes revealed that even at very low concentrations Phe interacts with lipids, up to the hydrophobic core of the membrane (Fig 1). We also observed that the interaction is stronger when the target membrane contains cholesterol.

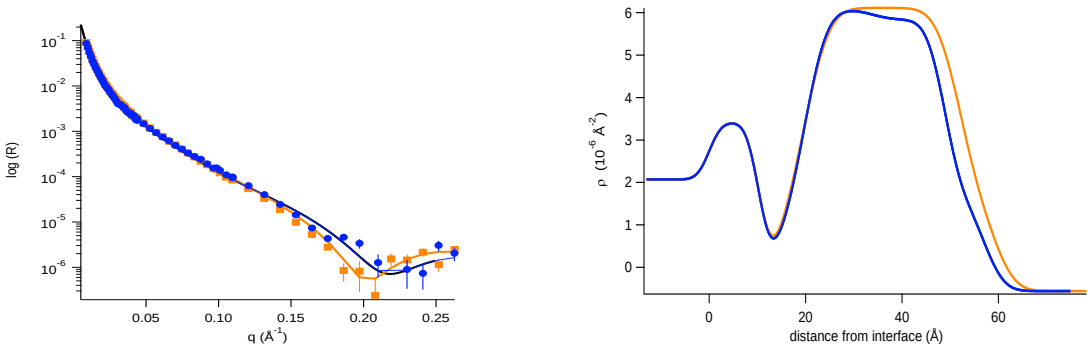

Fig 1 *Left:* reflectivity curves of a d_{62}DPPC membrane before (orange) and after (blue) the interaction with Phe at 20 µM concentration in H_2O. Dots and squares are the experimental points, lines refer to the best fits of the data. *Right:* scattering length density profiles of the d_{62}DPPC membrane before (orange) and after (blue) the interaction with Phe.

Thanks to the possibility offered by the PSCM laboratories tu deposit even complex asymmetric membranes, we went further in our investigation investigating whether the presence of raft domains promotes phe-membrane interaction.
GM1 ganglioside has been deposited in the outer leaflet of a cholesterol containing phospholipid membrane.
Results indicate that both propensity and extent of interaction of Phe with membranes depend on membrane lipid composition. Phe interaction with the hydrophobic core of the bicomponent membranes (DPPC+cholesterol and DPPC+GM1 ganglioside) is more favoured. Notably no interaction is observed with the raft model membrane.
We also investigated the interaction of Phe with a DPPC floating membranes, which, even less stable, is not affected by the presence of the rigid silicon support.
We found that after interaction the effect was the same as if the floating membrane coverage increased (Fig. 2), which in principle is not possible. We hypothesize that before interaction the fluid membrane forms dispersed protruding structures. This would take material out from the silicon and supported membrane proximal layers, which give rise to the reflectivity signal, interpreted as a poorly covering membrane, as if the membrane presents holes. Actually, the amount of total lipids is higher than

visible, but if the protrusions are long and dispersed enough, they would not have a significant contrasting mass to be detected by reflectivity. When Phe comes and anchors to the lipid heads, membrane rigidity is increased and such protrusions are not allowed, being high convex local curvatures no longer permitted. As a result we observe an apparent increase in membrane coverage.

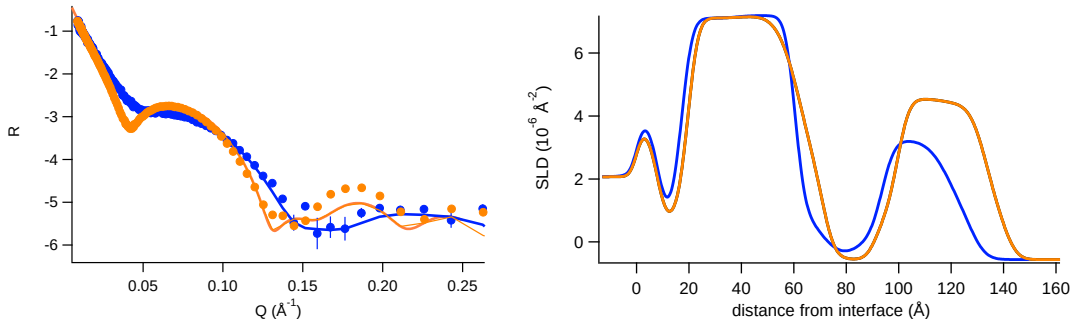

Fig 2 *Left:* reflectivity curves of a d_{62}DPPC membrane floating on top of a d_{83}DSPC membrane before (blue) and after (orange) the interaction with Phe at 20 µM concentration in H_2O. Dots are the experimental points, lines refer to the best fits of the data. *Right:* scattering length density profiles of the system before (blue) and after (orange) the interaction with Phe.