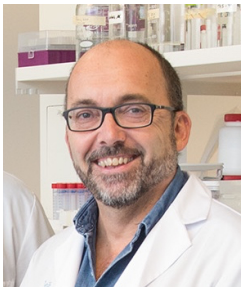
Principal Investigator: José Abad Rodríguez
Grant Title: Negatively charged axonal glycans in myelination patterning

Abstract



Myelinated axons present non-myelinated discontinuities required for fast electric impulse conduction, or long unmyelinated tracts in external layers of brain cortex, supporting plasticity and complex cognitive functions. We showed that neurons display axonal membrane segments (G4Ds) containing galectin-4 (Gal-4) on their surface that are incompetent for myelination. Gal-4 binding affinity and its capacity to cross-link glyco-conjugates and form high-order molecular networks on the membrane, motivated our hypothesis that negatively-charged glycoconjugates are organized in G4Ds by interaction with galectins and drive the correct spatial-temporal distribution of myelin.
Objectives:
1- To define the regulation of G4D during neuron differentiation
1- To study the localisation in G4Ds of charged glycoconjugates
2- To identify which glycosylated components of the G4Ds are relevant for the inhibition of local myelination.
Methods:
- Primary neuron and oligodendrocyte cultures.
- In vitro myelination on microfluidic-driven patterned substrata (striped carpets)
- Immunofluorescence and conventional/confocal microscopy
- Quantitative image analysis
- Gradient ultracentrifugation and ionic-exchange chromatography
Results:
- Neurons regulate G4D dimensions and disposition independently of OLGs, and define variable axon segments incompetent for myelination.
- G4Ds contain HS proteoglycan and molecules bearing CS D/E glycosaminoglycans, among others. Poly-sialic PSA-NCAM and Neu5Ac/Gc2-3-Gal-bearing molecules are segregated from them.
- Heavy fractions of neuron extracts obtained by density gradients present myelination inhibitory activity when tested in striped carpet assays
- Full-covered substrata assays are not suitable to measure myelination inhibitory activity.