Principal Investigator: Caroline Smet-Nocca
Grant Title: Structure and function of O-GlcNAc/phosphorylation cross talk in tau protein

Abstract:

The *O*-β-linked N-acetylglucosaminylation (*O*-GlcNAcylation) is an important posttranslational modification involved in regulation of the pathophysiology of the neuronal tau protein which in its hyperphosphorylated form, constitutes the intraneuronal fibrillar inclusions associated with several neurodegenerative disorders including Alzheimer's disease (AD). *O*-GlcNAcylation has been involved in modulation of tau phosphorylation levels and inhibition of tau aggregation properties while a decrease of *O*-GlcNAcylation could be involved in tau hyperphosphorylation. However, the molecular mechanisms at the basis of these observations remain to be defined. Our study aims to decipher the role of *O*-GlcNAcylation in the regulation of tau phosphorylation, conformation and aggregation.

We used high resolution NMR spectroscopy and molecular dynamics (MD) simulations to describe the direct *O*-GlcNAcylation/phosphorylation crosstalk in the longest isoform of tau protein and investigate their effect on peptide conformation. We described for the first time the *O*-GlcNAc modification pattern at the quantitative level in the full-length tau protein by NMR spectroscopy. Then, we showed by a systematic examination of the quantitative modification patterns that phosphorylation slightly increases tau *O*-GlcNAcylation by OGT while *O*-GlcNAcylation has no effect on site-specific tau phosphorylation by the kinase activities of ERK2 or a rat brain extract (Figure 1) except for Ser404 for which a crosstalk with Ser400 *O*-GlcNAcylation highlighted a regulation of the AD-relevant PHF-1 phospho-epitope (pSer396/pSer404) by O-GlcNAcylation (1). Our data suggest that indirect mechanisms act in the reciprocal regulation of tau phosphorylation and *O*-GlcNAcylation *in vivo* involving regulation of the enzymes responsible of phosphate and *O*-GlcNAc dynamics. Hence, we are investigating the modulation by *O*-GlcNAcylation of kinases involved in tau phosphorylation with a potential impact on its aggregation properties *in vitro*. Furthermore, we have shown by all-atom explicit solvent MD simulations that the PHF-1 peptide in absence of post-translational modifications forms transient helix from residues Ser404-Gln410, in particular hydrogen bond between backbone atoms of Leu408 and Ser404 was found to be present in these helical conformations. Our advanced simulations combined with NMR in peptides around the PHF-1 phospho-epitope show that phosphorylation and *O*-GlcNAcylation both disrupt the turn-like conformation.

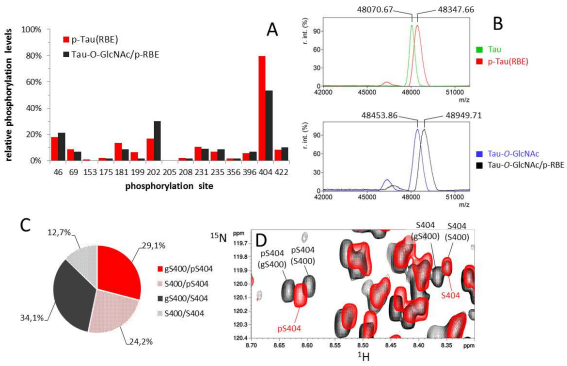

Figure 1: Impact of *O*-GlcNAcylation on phosphorylation of tau protein by kinase activity of a rat brain extract investigated by NMR spectroscopy.

(1) Bourré, G., Cantrelle, F.-X., Kamah, A., Chambraud, B., Landrieu, I. and **Smet-Nocca, C.** Direct crosstalk between O-GlcNAcylation and phosphorylation of tau protein investigated by NMR spectroscopy. Front. Endocrinol. - Molecular and Structural Endocrinology 9. doi: 10.3389/fendo.2018.00595.