Using nanobodies against CNPase as a crystallisation tool

Christine Touma[1*], Matti Myllykoski[1], Arne Raasakka[2], Felipe Opazo[3], Gopinath Muruganandam[4] and Petri Kursula[2]

[1]*Faculty of Biochemistry and Molecular Medicine and Biocenter Oulu, University of Oulu, Oulu, Finland*
[2]*Department of Biomedicine, University of Bergen, Bergen, Norway*
[3]*Institute for Physical Chemistry, Goettingen University, Goettingen, Germany*
[4]*VIB VUB Center for Structural Biology, Vlaams Institute Biotechnology, Brussels, Belgium*
**christine.touma@oulu.fi*

2', 3'-Cyclic Nucleotide 3'-phosphodiesterase (CNPase) can be found anchored to the membrane in the cytoplasmic space of myelin. One of its many functions is to act as an antagonist to Myelin Basic Protein to maintain the Major Dense Line and allow for correct compaction of the myelin sheath[1]. CNPase is comprised of an N-terminal polynucleotide kinase-like (PNK-like) domain and a C-terminal 2H domain. There are differences in these domain structures across all domains of life, suggesting an evolutionary shift from an inherited tRNA ligase-type activity to a unique domain assembly for human CNPase[2]. The 2H or phosphodiesterase domain has been extensively studied and its structure solved[3,4]. Unfortunately, the N-terminal domain is not as well characterised and has proven to be difficult to produce and crystallise. Here, nanobodies are used to encourage formation of crystals that otherwise would be difficult to grow. Nanobodies against CNPase were produced in collaboration with Dr Felipe Opazo (Goettingen University, Germany) and are only the VHH domain of a canonical antibody. The exact functional nature of CNPase in myelin is currently unknown; there are many hypotheses about its function and many of these reduce the N-terminal domain as a remnant of evolution that may no longer be catalytically required in humans. By revealing the full-length structure of CNPase it will provide an insight into its function and provide some understanding on whether its two domains play a physiological or a more structural role in myelin elaboration and compaction.

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