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Application for Cryo-EM time at ESRF – Experimental Method

This document should consist of a **maximum** of **four A4 pages** (including references) with a minimal font size of **12 pt**.
The last 2 pages are reserved for images/data proving the suitability of the samples for Cryo-EM measurements.

Proposal Summary (should state the aims and scientific basis of the proposal):

High-resolution structure of the TH:14-3-3 complex

Tyrosin hydroxylase (TH), a homotetramer (224 kDa), is an enzyme involved in catalysing the hydroxylation of L-Tyr to L-dihydroxyphenylalanine (L-DOPA), the rate-limiting reaction in the synthesis of dopamine and other catecholamines like noradrenaline and adrenaline, which modulate blood pressure and other functions (Haavik et al., 2008). In collaboration with the group of Aurora Martínez (Bergen University. Norway), we have succeeded in determining by cryoEM the structure of the human TH at 3.8 Å resolution (Fig. 1A) with data obtained in our recent session at the ESRF facility (MX-2163). Our next goal is to study the TH:14-3-3 complex, formed by TH and the cofactor 14-3-3, a dimeric protein that binds to and modulates a large number of proteins, including kinases and phosphatases (Obsil et al., 2001).
We have already taken cryoimages of the TH:14-3-3 complex (Fig. 1B) that reveal that the sample is ready for data to be acquired in a state-of-the-art cryoelectron microscope, ideally again in this ESRF facility. The 3D reconstruction of the complex at the highest resolution possible, ideally below 4Å, could help us to understand the mechanism by which 14-3-3 modulates TH activity.

Scientific background:
The aromatic amino acid hydroxylases (AAAHs) constitute a family of enzymes that catalyse the hydroxylation of aromatic amino acids using tetrahydrobiopterin (BH4) as cofactor. There are four genes coding for these enzymes in mammals, phenylalanine hydroxylase (PAH), tyrosine hydroxylase (TH) and tryptophan hydroxylase 1 and 2 (TPH1 and TPH2). TH is fundamentally localised in the central nervous system (CNS), peripheral sympathetic neurons and the adrenal medulla. Mutations in TH are linked to TH deficiency, a rare autosomal recessive disorder characterised either by progressive, hypokinetic-rigid syndrome and dopa-responsive dystonia, or by a more severe phenotype and complex encephalopathy that is less responsive to L-DOPA treatment (Haavik et al., 2008; Willemsen et al., 2010).

Experimental technique(s), required set-up(s), measurement strategy, sample details (quantity...etc):
Samples will be vitrified and mounted in our Institute and will be sent to Grenoble prior to our visit. We want to acquire more data using a K2 detector in counting mode, with a sampling resolution around 1 Å/pixel.

Beamline(s) and beam time requested with justification:

If possible, we request three days (9 slots) of data acquisition to accumulate enough data to generate a very high-resolution 3D reconstruction of the TH:14-3-3 complex.

Results expected and their significance in the respective field of research:
Illnesses caused by TH malfunction, in most of cases due to mutations, have important biomedical implications. A complete understanding of the complex structure, catalytic mechanism, and regulatory properties of human TH , in this case in complex with its modulator 14-3-3, requires high-resolution information, preferably at atomic resolution.

References
- Bezem MT, Baumann A, Skjaerven L, Meyer R, Kursala P, Martinez A, Flydal MI (2016) Stable preparations of tyrosine hydroxylase provide the solution structure of the full-length enzyme. Sci. Rep. 6:30390.
- Haavik J, Blau N and Thony B (2008) Mutations in human monoamine-related neurotransmitter pathway genes. Human Mutation 29: 891–902.

- Obsil T, Ghirlando R, Klein DC, Ganguly S, Dyda F (2001). Crystal structure of the 14-3-3zeta:serotonin N-acetyltransferase complex. a role for scaffolding in enzyme regulation". Cell. 105 (2): 257–267.
- Willemsen MA,Verbeek MM, Kamsteeg EJ et al. (2010) Tyrosine hydroxylase deficiency: a treatable disorder of brain catecholamine biosynthesis. Brain 133: 1810–1822.

Please add proof (raw images, 3D reconstruction, class averages etc.) justifying the need for cryo EM measurements on the Titan Krios:

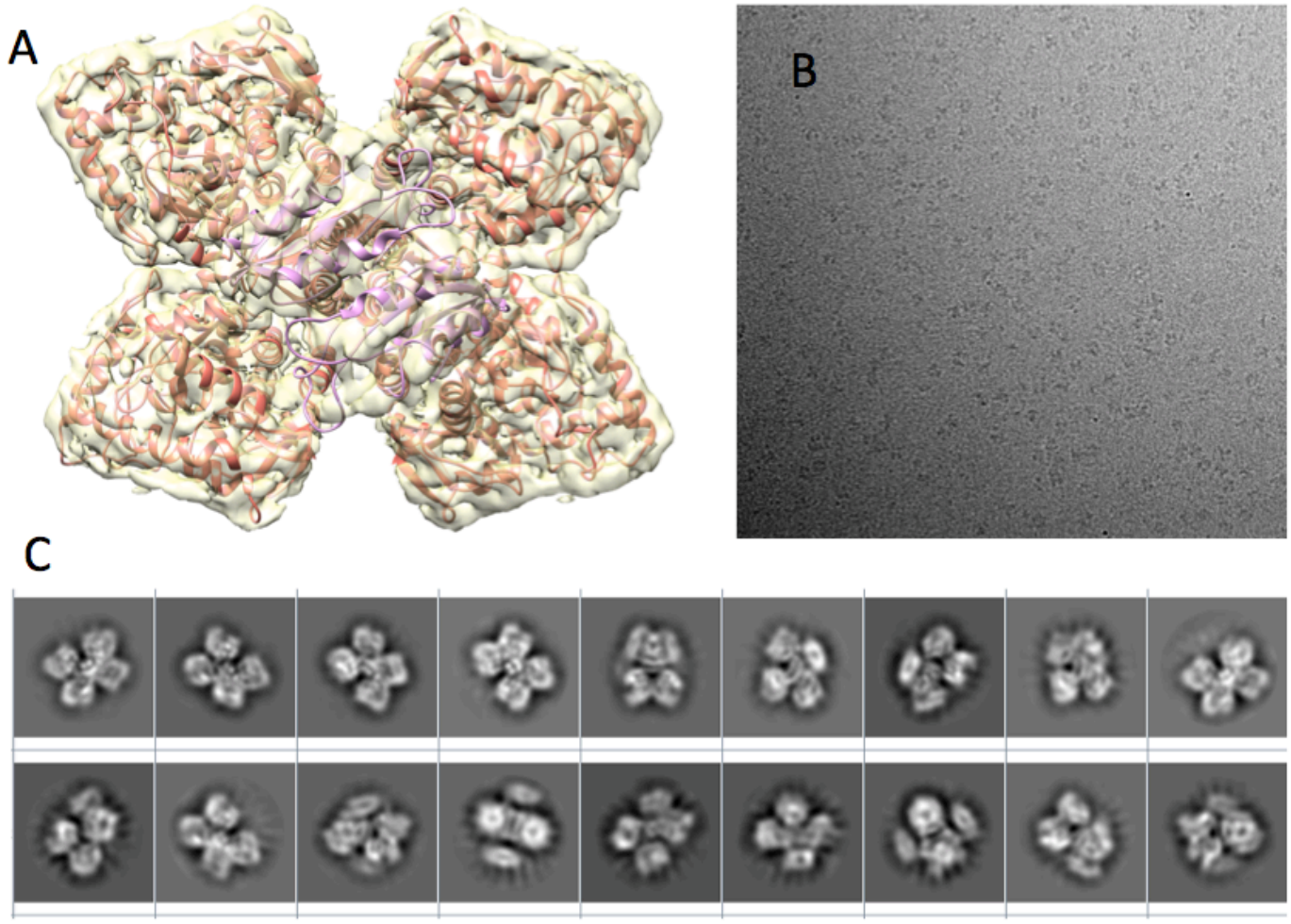

Figure 1. **(A)** 3D reconstruction of human TH and preliminary docking with the crystal structure of the catalytic domain (pdb 2XSN) and the solution structure of the rat regulatory domain (pdb 2MDA). **(B)** Image of vitrified human TH:14-3-3 complex recorded in a Talos Arctica equipped with a Falcon III electron direct detector. (**C**) Preliminary 2D classification carried out with 405.000 particles of the TH:14-3-3 complex