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Structural characterization of a mitochondrial intermembrane domain

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Our object of study is a human protein anchored at the mitochondrial inner membrane. This protein consists of three domains whose function is still unknown. The N-terminal domain (N) spans through the intermembrane space, whereas the C-terminal domain (C) faces the mitochondrial matrix, both regions being connected by a transmembrane helix embedded in the inner membrane. Several constructs of both the N and C domains were generated to obtain stable protein fragments suitable for expression and crystallization. However, only a few N-terminal constructs were soluble, but most of them aggregated during purification. Construct N4 showed the highest expression levels in *Escherichia coli* and could be reasonably purified. Nevertheless, once pure, N4 had a high tendency to precipitate, which led us to explore new approaches to improve its stability. We will present the re-design approaches of the constructs to improve solubility and solve aggregation and precipitation.

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