TITLE: Exploring the structure-function relationship in mt-tRNA and their fragments

Summary: Mitochondria contain a minimum set of only 22 different types of mt-tRNAs where a disruption in the correct function of a singular mt-tRNA can significantly reduce its functionality. Consequently, mt-tRNA genes are known hotspots for pathological mutations with more than 300 documented mutations linked to wide range of human diseases. Additionally, mt-tRNAs can engage in a still poorly understood biogenesis, to generate tRNA fragments which participate in important molecular processes such as gene silencing, RNA processing, protein translation, epigenetic regulation and cell differentiation. High-resolution structural information is lacking for both the mt-tRNAs and their fragments. Understanding the structural details of mt-tRNAs will allow us to identify which structural motifs are crucial for their normal function and how mutations lead to diseased states. Structurally characterizing mt-tRNA fragments will provide much needed information on how their structure plays a role in the complex web of their metabolic interactions and reveal best ways to intervene for treatment purposes.

Research techniques used: NMR spectroscopy-main method (1D, 2D, 3D spectra, utilizing different NMR active nuclei, in liquid phase and aligned media, kinetics and dynamics); structural calculations using AMBER/CYANA software; RNA synthesis and purification (synthesizer utilizing solid support RNA chemistry-enables site specific labeling (isotopic enrichment, florescence dye, paramagnetic probe)), enzymatic in-vitro T7 RNA synthesis (enables residue specific labeling (modified or isotopically enriched)); fluorescence spectroscopy. With cooperation of dr. Marjetka Podobnik (D11) lab: protein expression and **c**ryo-electron microscopy.

The reason why the topic is innovative: High-resolution structures of mt-tRNAs and their fragments are severely lacking because they are difficult to crystalize due to their dynamics and structural plasticity which makes our detailed NMR approach highly innovative and of great interest to the wider scientific community. The approach to use cell lysates to achieve biologically relevant folds of mt-tRNA as well as to follow maturation disruption when observing their pathologically mutated versions is also cutting edge in studying structure-function relationship in biological/pathological states with obvious therapeutic potential.

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