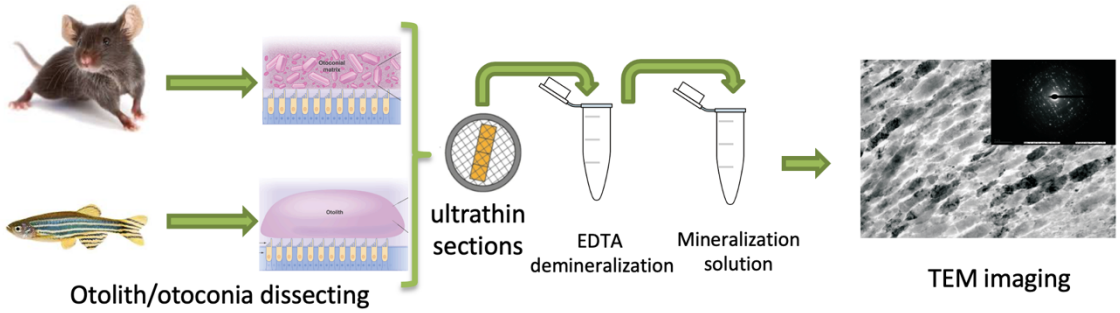
Native organic matrix based *in vitro* model for macromolecules role in the inner ear otolith/otoconia mineralization and regeneration.

Otoliths and otoconia are biominerals in the inner ear of vertebrates responsible for sense of balance and spatial orientation. Degeneration or displacement of otoconia is common age-related balance disorder called benign proximal positional vertigo (BPPV). In addition, commonly used drugs, such as aminoglycoside antibiotics, can lead to disruption of otoconial structure and function. Vestibular problems, like (BPPV), are reported in about 9% of people 65 years of age or older moreover balance-related falls account for more than half of the accidental deaths in the elderly. Despite such clinical significance, relatively little information has been compiled about the development and maintenance of otoconia.

The aim of this project is better understanding the molecular controls of otolith and otoconia biomineralization in the inner ear, to inform a novel approach to otoconia regeneration.

Motivation for the study: Most studies of otolith/otoconia mineralization are based on oversimplified *in vitro* mineralization assays with lack of a native macromolecular context. In this project, a novel Native Organic Matrix (NOM)-based *in vitro* model will be developed. It will allow evaluation of different molecular factors separately, with preserving the native context, yet reducing the complexities associated with *in vivo* studies. In NOM-based model ultrathin sections of otolith/otoconia will be used which allow ultrastructure analysis of crystal and organic matrix with transmission electron microscopy (TEM). Sections will be re-mineralized *in vitro* to test variety of factors controlling their formation (see figure below).



Description of the study: Otoconia and otoliths are made of organic matrix (1-10%) and calcium carbonate crystal. Organic matric (proteins, saccharides) control size, shape, and morphology of crystal. Most of otolith/otoconia proteins are extensively post-translationally modified including phosphorylation, glycosylation and glycosaminoglycans (GAGs) attachment, but their role in mineralization is unclear regardless their abundance. Comprehensive analysis of otoliths/otoconia organic matrix will be performed to identify major post-translational modifications and compare organic matrix composition of otoconia and otoliths.

NOM-based *in vitro* mineralization model will be used to study the role of post-translational modifications, highly acidic intrinsically disordered proteins (IDPs) and test antibiotic ototoxicity in mineralization of otolith and otoconia.

Based on outcome of experiments in this project some macromolecular candidates will be proposed for otoconia regeneration. We hypothesize that GAGs and acidic IDPs modulated by phosphorylation might promote nucleation and can be used in regeneration strategies. Dissected whole mouse otoconia subjected to partial dissolution will exposed mineralization solution with some additives to see if they can induce otoconia regeneration *in vitro*.

Expected outcomes: The long-term outcome of this project will result in development of strategies for treatment of human vestibular dysfunction and protective strategies against antibiotic ototoxicity. Direct outcome of that project will be better understanding of crucial factors affecting otolith/otoconia mineralization in model organisms and proposing of novel otoconia regeneration strategies.