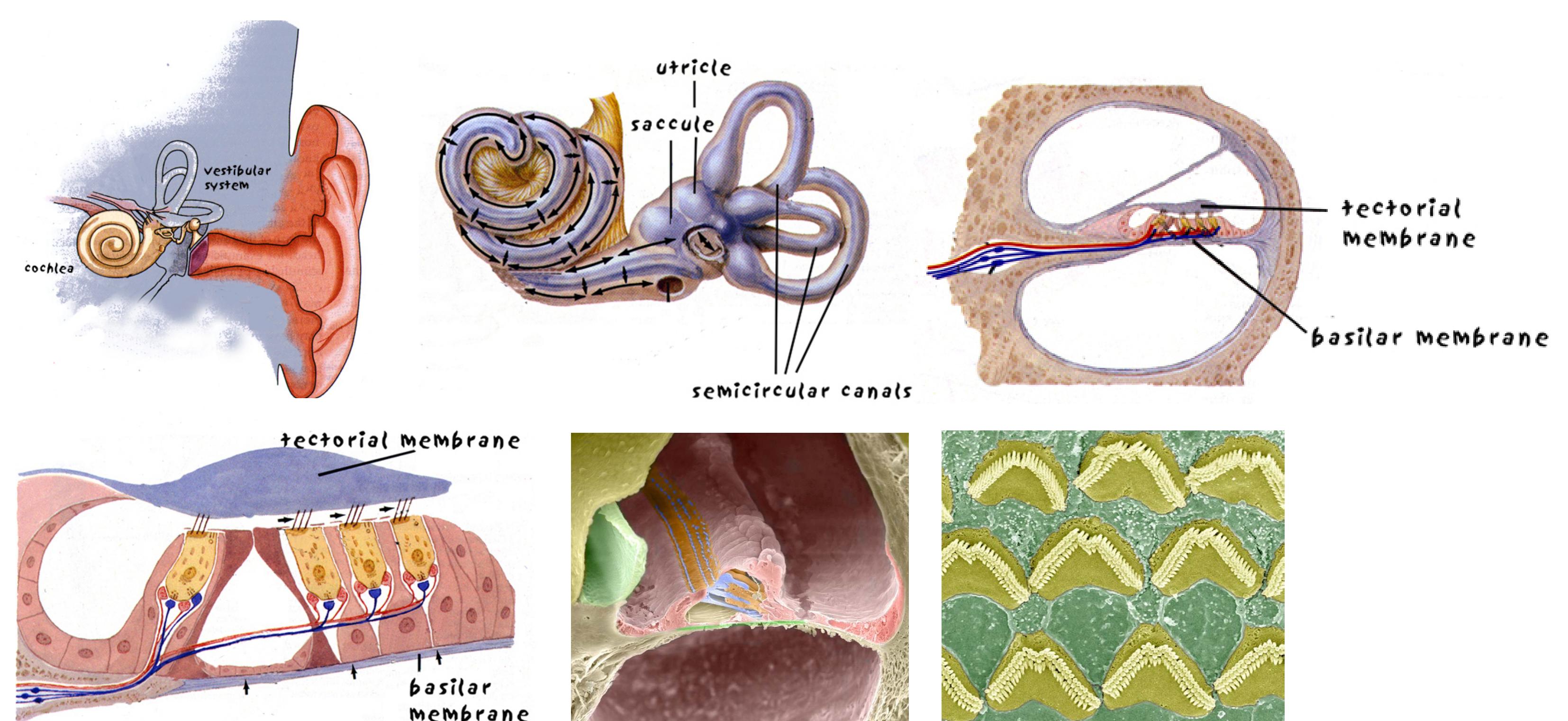
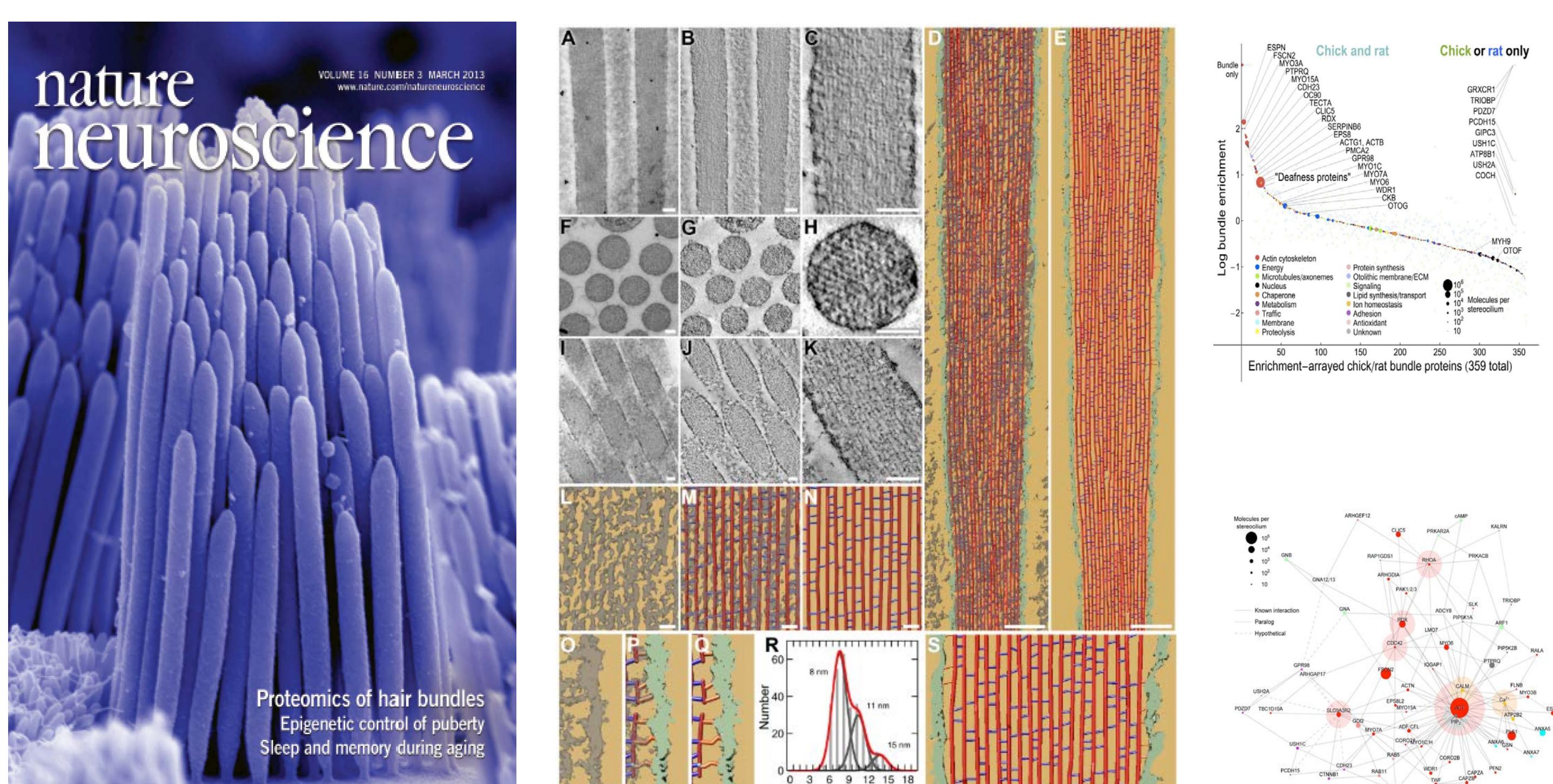


ABSTRACTS

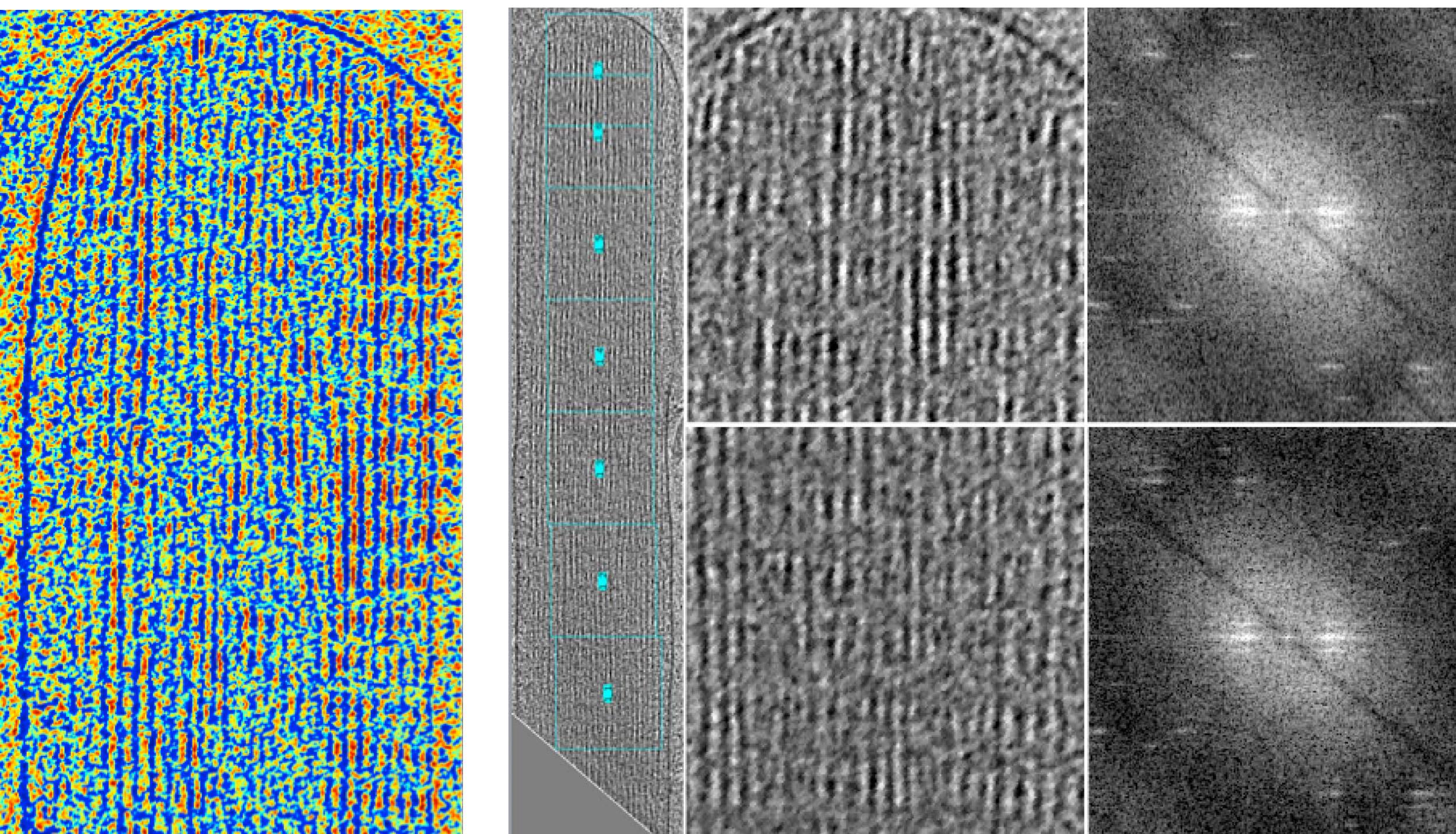
Our senses of hearing and balance rely on the function of hair cells that reside in the sensory epithelia of the cochlea, sacculus, utricle and semicircular canals. The hair bundle protruding from the apical surface of hair cells, consists of stereocilia, which are actin-bundle-based, membrane-enclosed organelles that are connected by tip link proteins. Cryo-EM/tomography of unstained frozen-hydrated intact stereocilia revealed that the membrane reseals at the point of insertion into the cell body. Judging by visual inspection as well as 2D Fourier analysis of tomographic slabs, wild type stereocilia contained both regions of high order, as well as regions of low order. We found evidence of gaps and forking in the actin core, suggesting that the actin filaments were structured more like a gel rather than a paracrystalline monolith. Given the higher order of the actin core in *Pls1*^{-/-} knockout mice (*Pls1*^{-/-}) (2), we imaged *Pls1*^{-/-} stereocilia, which are currently used for development of automated filament tracking approaches. We have obtained simplified volumetric models of the actin core as well as an unexpected 3D volumetric arrangement of the actin in the taper and rootlet region. We are currently probing the space between the actin core and the membrane for unconventional myosin macromolecules.



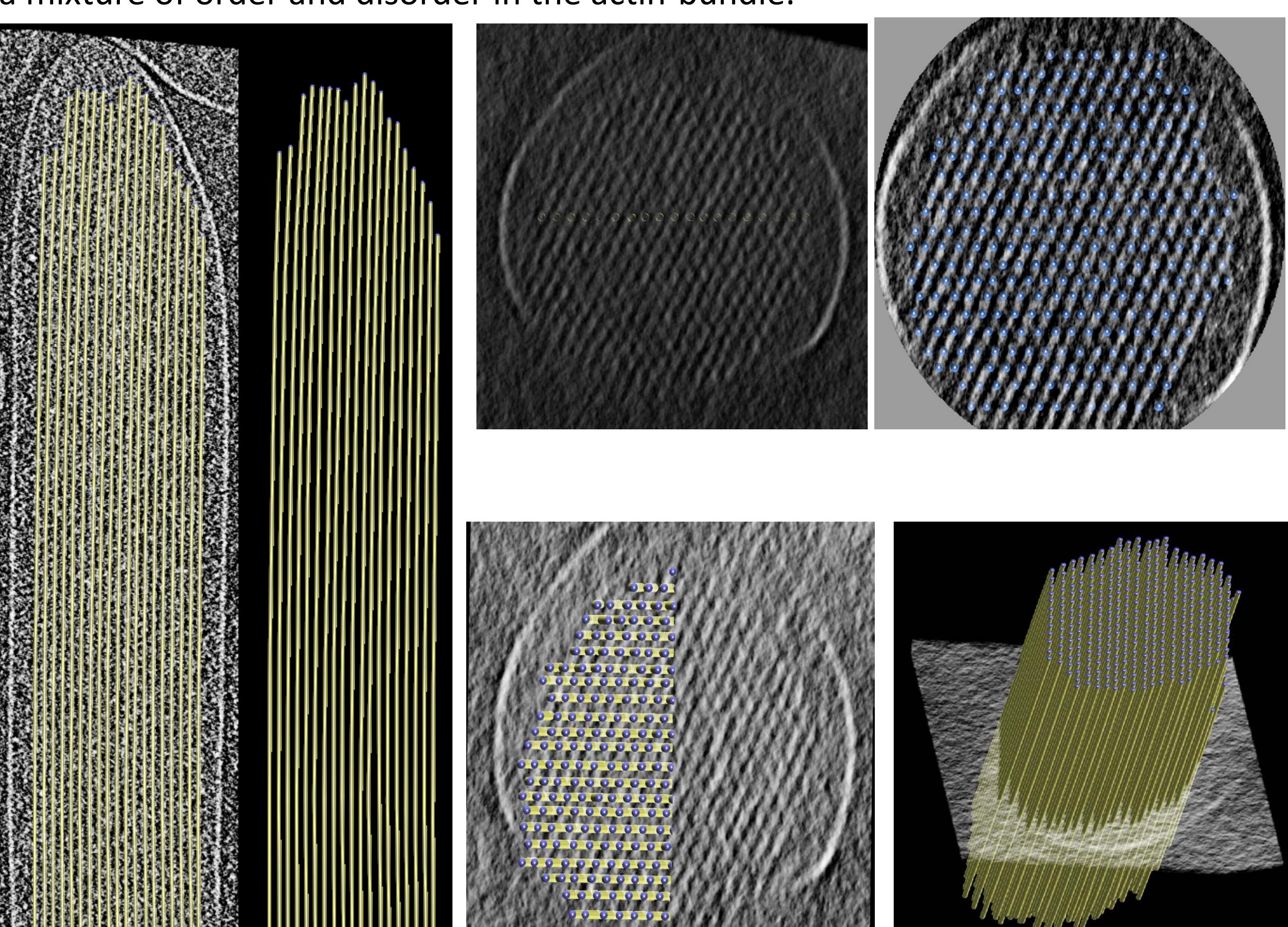
Our senses of hearing and balance reside in the auditory and vestibular organs of the inner ear, namely the cochlea, which detects sound, the sacculus, utricle and semicircular canals, which detect vibration, linear as well as angular acceleration, respectively. A cross-section through the cochlea reveals the sensory epithelium consisting of hair cells with their characteristically shaped hair bundles, the organelle of mechano-electrical transduction.



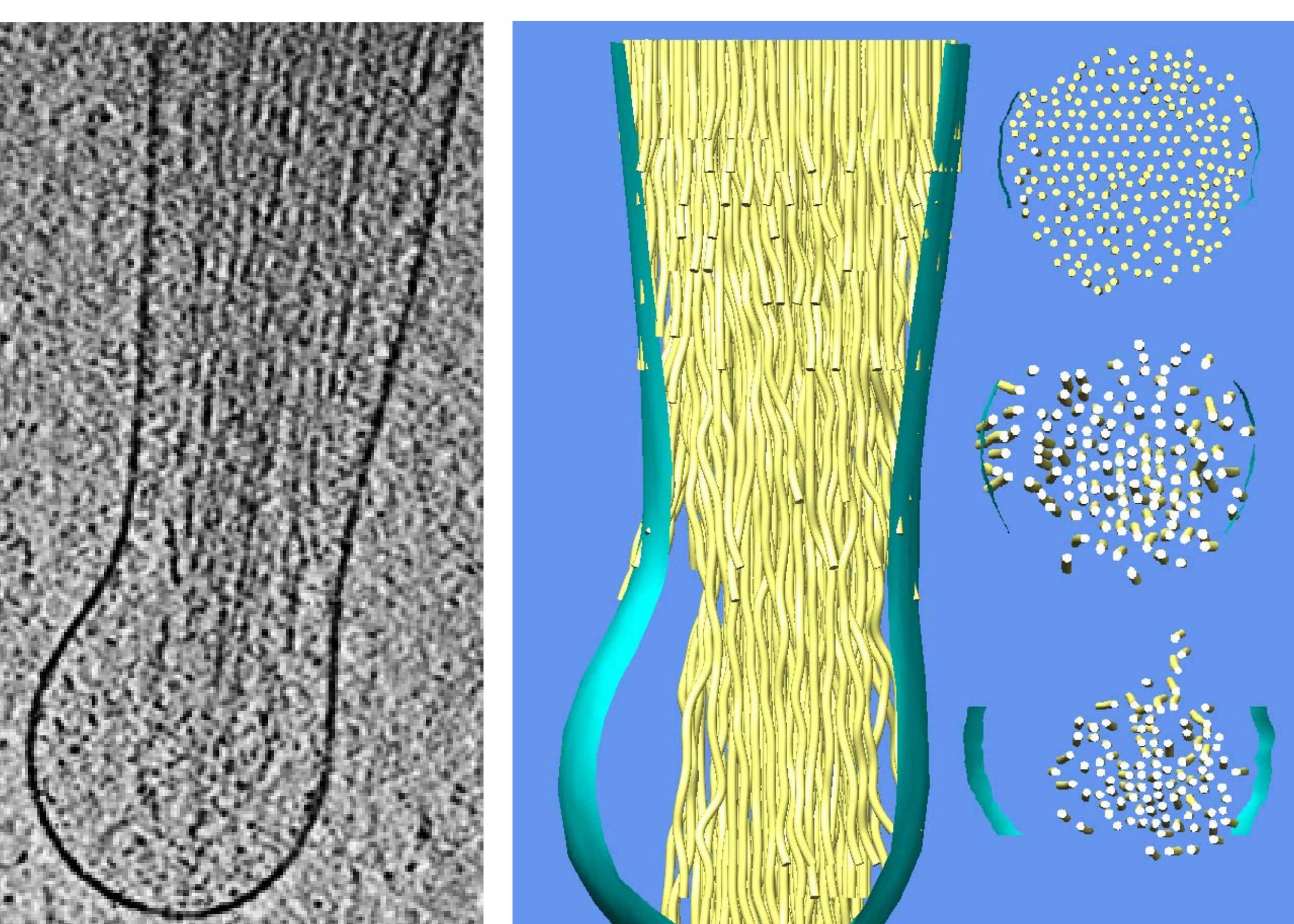
The hair bundle consists of individual stereocilia, with adjacent stereocilia being linked via the so-called tip link protein, whose identity remains somewhat controversial. Our 2013 Nature Neuroscience paper featured a correlative quantitative proteomics and electron tomographic analysis of stereocilia, providing a quantitative proteome inventory, which also serves as a starting point towards a high resolution pseudoatomic model of stereocilia.



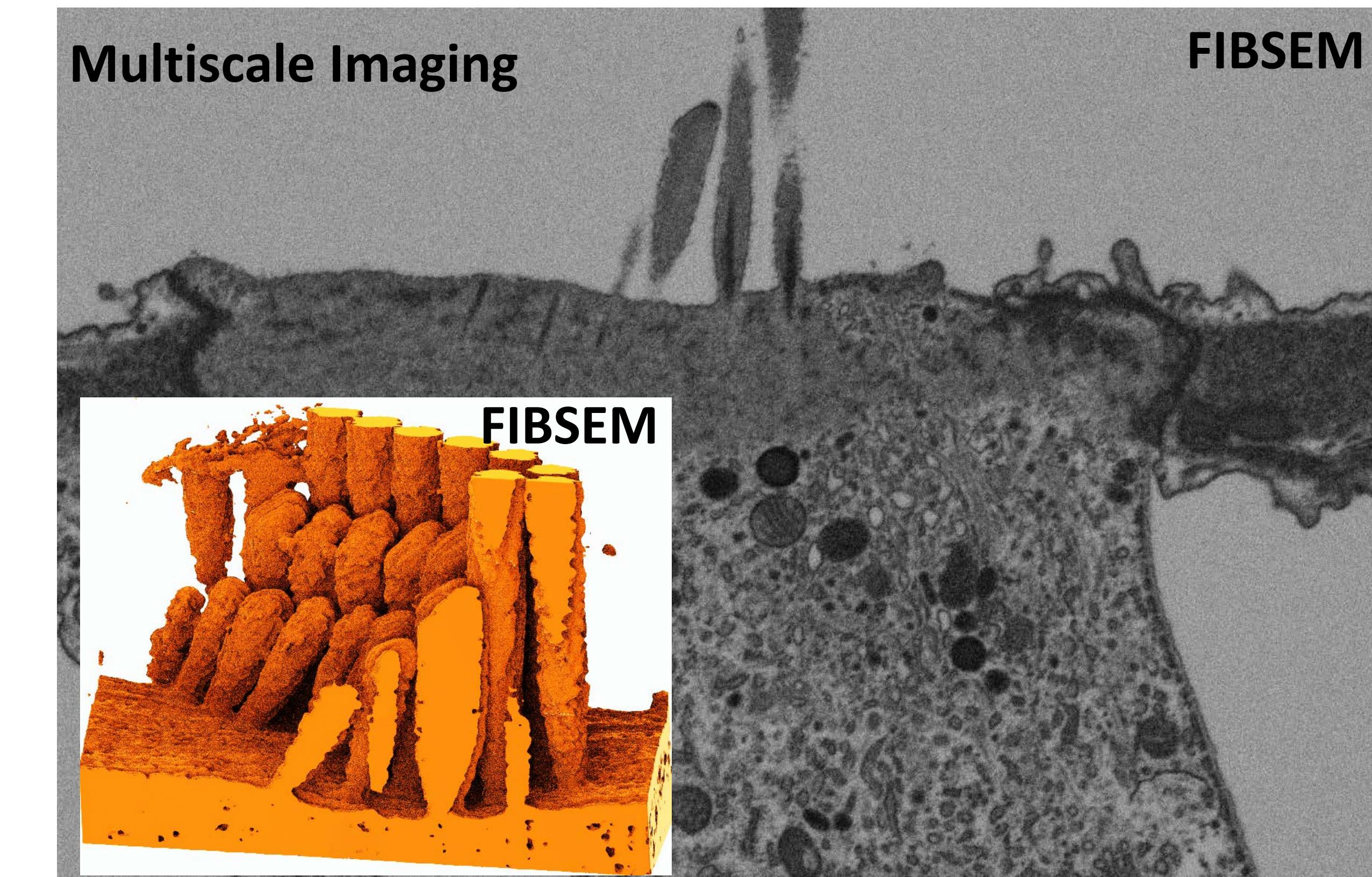
Cryo-Electron Tomography of frozen-hydrated, intact individual stereocilia, showing a mixture of order and disorder in the actin-bundle.



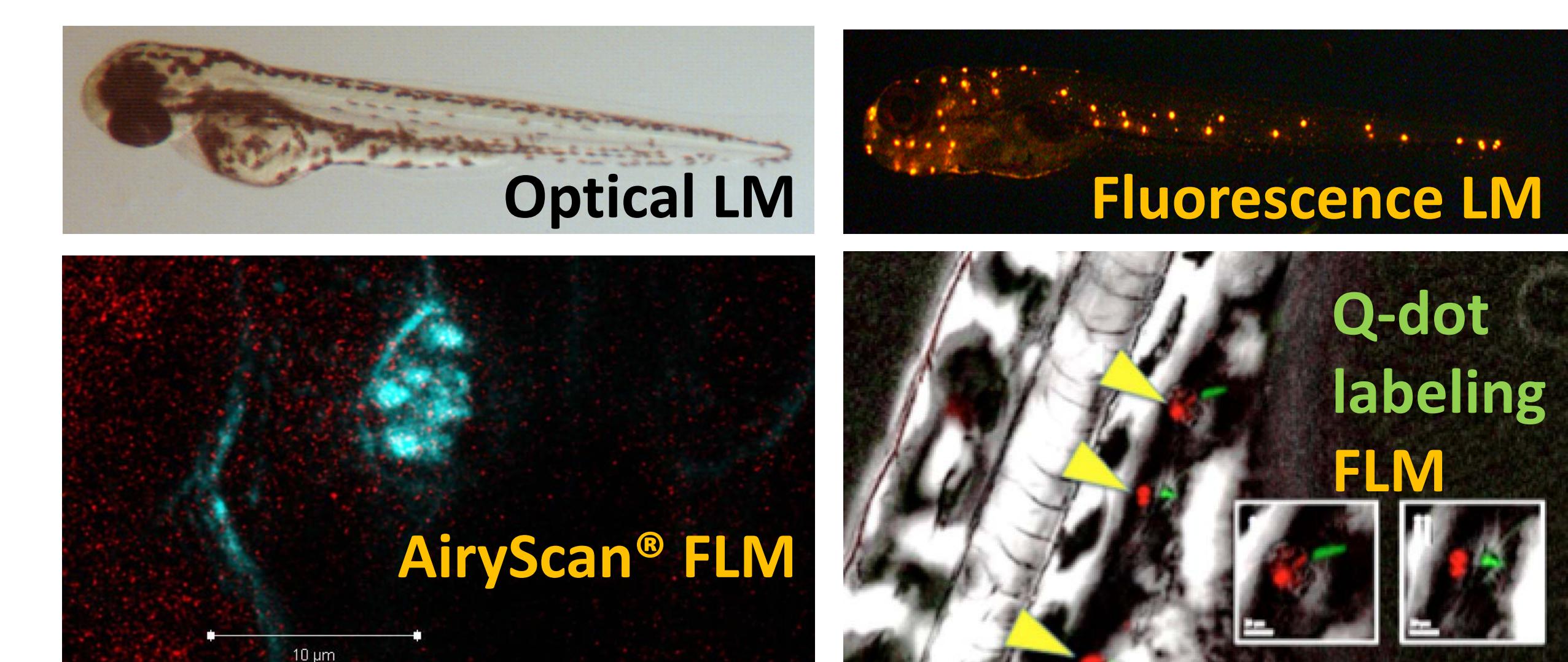
Manual placement of simplified volumetric models into the cryo-EM density maps of the tip and shaft regions of the stereocilia. Initial model position is refined through the use of Y-axis averaged density maps, that strengthen the signal.



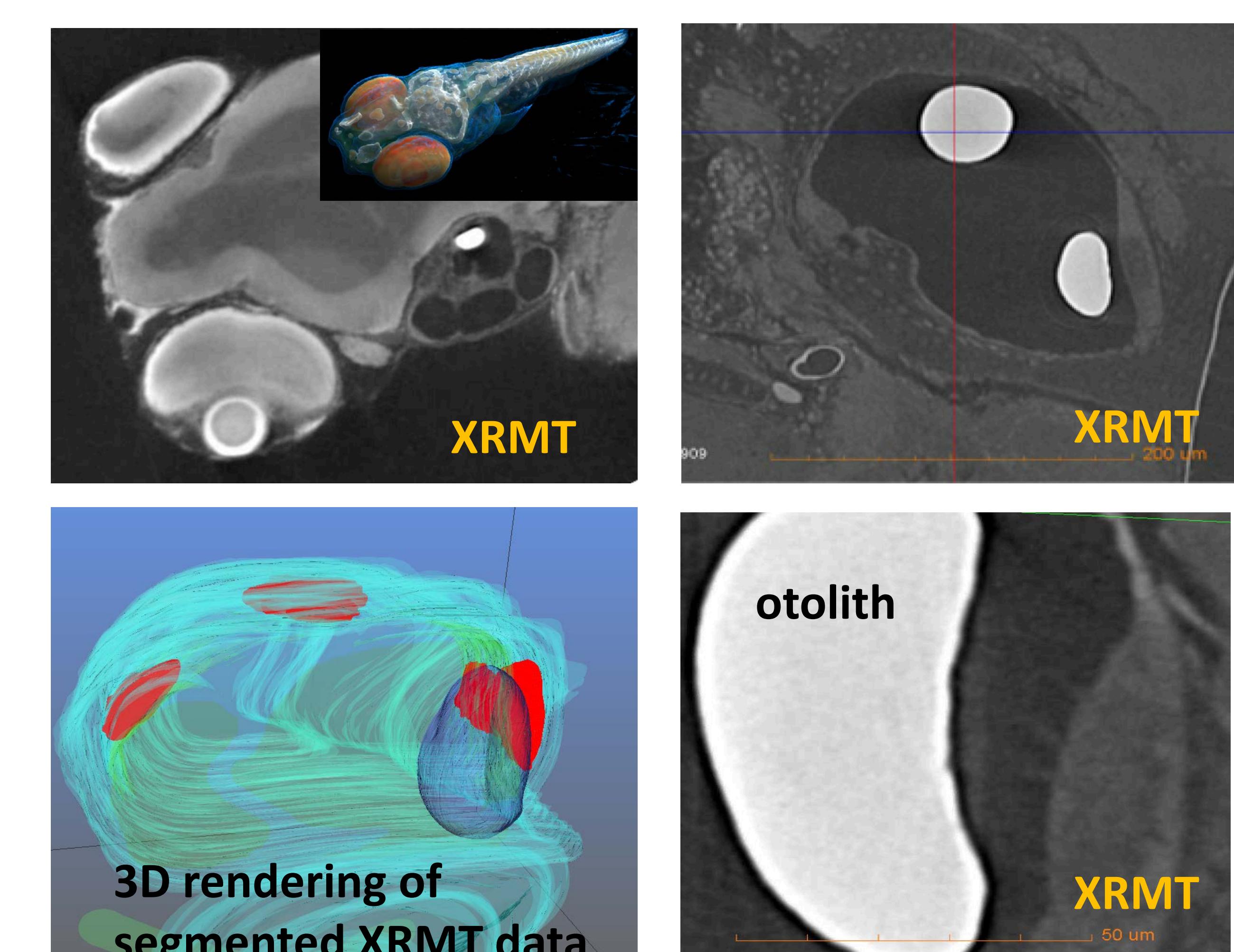
While actin filaments in the shaft along the axis remain largely parallel to one another, the pattern is significantly more complex in the taper region, where some filaments end on the plasma membrane while others twist into a more compact bundle conformation (also known as the rootlet).



Focused Ion Beam Scanning Electron Microscopy (FIBSEM) allows imaging of entire cells and small tissue volumes at ~10 nm resolution, visualizing large macromolecular complexes (like rootlets) and organelles (like the hair bundle).



A multiscale integrated bioimaging (e.g. of neuromast hair cells) will require a combination of AiryScan® confocal fluorescence light microscopy and FIBSEM, mediated by X-ray Microscope Tomography (XRMT).



X-ray Microscope Tomography (XRMT) is a powerful new technology that allows the visualization of entire (small) animals, such as zebrafish at the organ (inner ear), tissue (sensory epithelia), as well as cellular (hair cells) and subcellular (hair bundle). It also serves as an excellent bridging technology between (fluorescence) optical microscopy and high resolution FIBSEM imaging.