Intracellular nicotinamide adenine dinucleotide (NADH) is a key cofactor in energy metabolism pathways and a myriad of oxidation-reduction reactions in living cells. The crowded milieu of these cells with organelles and macromolecules influences many biological processes such as biomolecular diffusion, protein-protein and protein-substrate interactions, and protein folding. In this contribution, I will highlight our recent findings on the role of macromolecular crowding on biochemical reaction between NADH and selected dehydrogenases in both living cells and in controlled macromolecules-rich environment. In addition, multiscale diffusion (rotational and translational) of a small fluorophore will be used to understand the role of non-specific binding, heterogeneity in microenvironmental viscosity in crowded solutions. Our experimental approach is a combination of fluorescence lifetime imaging microscopy, time-resolved anisotropy and fluorescence correlation spectroscopy. The broader impacts of these results will be discussed within the context of energy metabolism and biophysics in the crowded milieu of living cells.