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Fluorescence Turn-On Sensor to Characterize Quaternary Folding Structure of Transthyretin

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Protein misfolding contributes to the pathogenesis of neurodegenerative and chronic diseases, such as Non Alcoholic Fatty Liver Disease (NAFLD). NAFLD has become the most common liver diseases in the majority of developed countries. Though the mechanisms underlying the disease have yet to be fully understood, obesity and insulin resistance are known contributing factors. We hypothesize that excess free fatty acids, which are characteristic of obesity, provoke protein misfolding and contribute to disease pathogenesis. Investigators have developed small-molecule, fluorescence turn-on sensors to detect misfolded proteins. In our study, we describe use of a fluorescence sensor based on a coumarin derivative to detect the folding state of transthyretin (TTR) in liver cells. This model was selected because liver cells are known to secrete TTR and protein misfolding in liver cells may have a role in the development of NAFLD. TTR mRNA expression in H4IIE rat and HepG2 human liver cells was determined by qPCR, TTR protein expression was measured by Western Blot, and TTR protein misfolding was assessed using a fluorescence turn-on sensor specific for the properly folded and assembled quaternary TTR protein.