Proteins generally have to be stably folded to execute their functions inside cells, an environment where a plethora of factors can influence their folding behavior. In particular, macromolecular chaperones, specific interaction partners, ligands and even non-specific interactions affect the nature of the popularly described funnel shaped landscape of protein folding. While much success has been achieved in vitro, biophysical studies of protein folding and stability inside cells remain relatively scarce.

To this end we are building a folding sensor capable of reporting on thermodynamic stability of a protein inside a cell or in cell lysate, which exploits the “mutually exclusive folding” (MEF) concept\(^1\). In this design our test protein of interest (P\(_2\)) is inserted into a surface loop of the host protein (P\(_1\)), in such a manner that both cannot fold to their native state. Addition of a ligand that stabilizes P\(_1\) creates a folding tug-of-war between the two proteins. The winner—the protein with greater thermodynamic stability—folds at the expense of the loser. Moreover, titration of P\(_1\) stability yields a melting curve for P\(_2\) as a function of ligand. The last necessary element to use MEF as an in-cell folding sensor is a readout that reports on the folding state of either P\(_1\) or P\(_2\). In our development of this system, we are using *E. coli* dihydrofolate reductase as P\(_1\) and ubiquitin as P\(_2\). Our readout reports on the folding state of ubiquitin, based on enhanced accessibility of a tetra-Cys sequence for FlAsH binding upon ubiquitin unfolding.