

## **Research Excellence: Simultaneous Determination of Molecular Size and Molecular Shape of Nanoparticles and Proteins in Solution Using Fluorescence Correlation Spectroscopy (FCS)**

### **Roles of Individual Student Researchers**

The specific research activities of this project will be divided among the three students supported by the RE award. The planned division of labor will reflect the relative levels of skill and knowledge of the three student researchers. Their individual research activities will be as follows

**Michael Stewart:** Michael's initial work will be the FCS analysis of translational diffusion coefficients for a fluorescent protein that assumes a non-spherical shape under specific solution conditions. Proteins of this type are available commercially and the protein he will employ in his studies starting in first month of the project, is fluorescently-tagged concanavalin A (Con A). This protein undergoes a well-characterized pairwise self-association of protein monomers to form a non-spherical protein dimer that can further pairwise self-associate into a non-spherical protein tetramer. Michael will systematically vary the pH and ionic strength of Con A solutions to prepare individual solutions enriched in a desired monomer, dimer, or tetramer concentration. In follow up studies, he will compare his FCS findings with complementary measurements of the same solutions using fluorescence polarization analysis. The latter determination will provide an independent analysis of the rotational diffusion coefficients for the proteins. In the latter phase of his research, he will re-analyze his FCS data with the aid of computer programs developed by another member of the research team, Cody Graham, to obtain FCS-based rotational diffusion coefficients and the corresponding dimensions of the ellipsoids modelling the shape of the different Con A species. The outcome of Michael's research will be a protocol for performing FCS-based shape analyses of non-spherical proteins. This new protocol will enable our ability to

participate in future research contracts with industrial research partners interested in developing and commercializing new protein drugs.