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# Novel Quantitative FRET Technologies for the Determinations of Protein Interaction Dissociation Constant, $K_d$, and Protease Kinetics, $K_{cat}/K_M$ Determinations


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**Location: Bourns A265**
**Time: 11:10am**


## Abstract:

Förster resonance energy transfer (FRET) technology has been widely used in biological and biomedical research. We recently developed a novel quantitative FRET analysis method and applied this method to determine protein interaction affinities and protease kinetics in the SUMOylation cascade. The novel methodology is based on the quantitative analysis of the FRET signal from the total fluorescent signal at acceptor emission wavelength, which consists of three components: donor emission, acceptor emission and FRET signal during the digestion process. SUMOylation is an important protein post-translational modification, which is carried out by multi-step enzymatic cascade reactions for peptide activation and substrate conjugation, and plays critical roles in diverse physiological processes, including transcriptional regulation, signal transduction, cell survival and death and DNA damage response. We developed a new theoretical and experimental procedure for protein interaction Kd determination of SUMO1 and its E2 ligase, Ubc9, and individual

interaction in the full SUMOylation cascade by FRET assay. The Kd values are also consistent with those determined with other traditional approaches, such as surface plasmon resonance(SPR) and isothermal titration calorimetry(ITC). We have also developed a novel quantitative FRET-based protease assay for SENP1 kinetics parameter determination. The novel theoretical and experimental procedures to determinethe kinetics parameters,kcat, KM and catalytic efficiency (kcat/KM) of catalytic domain SENP1 towards preSUMO1. These results are superior than those obtained from biochemical assays. These developments represent a novel methodology of biosensor based on FRET, which can be used in general for protein-protein interaction dissociation constant and protease kinetics determinations.

**Abstract:**
Jiayu Liao has completed his Ph.D in 1999 from the University of California at Los Angeles. He did his post-doc. training with Peter G. Schultz at the Scripps Research Institute. After he finished his post-doc. training, he joined GNF as Principle Investigator and Founding Scientist of GPCR platform. His collaboration work with Prof. Hugh Rosen on the discovery of EDG1-specific agonist as novel immunosuppressant at the Scripps Research Institute led to the award of the Scripps Molecular Screening Center from NIH Roadmap Initiative. His collaborative work with Prof. Mingwei Wang on the discovery of the first non-peptide agonist for Glucagon-like peptide 1 (GLP1) receptor led to the establishment of Chinese Nation Compound Library in Shanghai. He joined the University of California at Riverside in 1999 as founding faculty of Department of Bioengineering. He has published more than 30 papers in reputed journals with more than 2000 citations and serving in several important review panels in USA and China.