FLUORESCENCE AND RESONANCE ENERGY TRANSFER OF 3,4-BIS (2,4-DIFLUOROPHENYL)-MALEIMIDE Katya Nacheva, William A. Maza, Randy W. Larsen, Roman Manetsch*

3,4-Diaryl-maleimides (DMs) have been previously reported to possess interesting fluorescent properties such as strong extinction coefficients (ε > 50,000 $M^{-1}cm^{-1}$), high fluorescent quantum yields, and large Stokes shifts (≥ 100 nm). Nevertheless, to date, DMs still remained unexplored and were used primarily for material science applications.

Herein we report the identification and synthesis of a novel fluorophore, 3,4-Bis(2,4-difluorophenyl)-maleimide. Our DM probe displays a large Stokes shift of 139nm (λ_{abs} 341 nm and λ_{em} 481nm), a high fluorescent quantum yield in DCM (Φfl=0.61) and an extinction coefficient of 48,400 $M^{-1}cm^{-1}$ at 320nm. Furthermore, as a proof-of-concept, we designed a Förster resonance energy transfer (FRET) system where our molecule and a compatible dark quencher were appended to a peptide substrate of the membrane-anchored protease $\beta-$secretase, which is an important therapeutic target for Alzheimer's disease. In addition we developed a second FRET system by employing another fluorophore as a suitable acceptor and demonstrated 2.5 fold increase in the FRET efficiency as a function of the distance between the FRET partners.