SINGLE MOLECULE STUDIES OF THE RED AUTOFLUORESCENT PROTEIN DsRED

Maria F. Garcia-Parajo[1], Marjolein Koopman[1], Erik M.H.P. van Dijk[1], Vinod Subramaniam[2], Niek F. van Hulst[1]; [1]University of Twente, Postbus 217, Enschede, 7500AE Netherlands, [2]Max Plank Institute for Biophysical Chemistry, Germany

Recent studies on the newly cloned red fluorescence protein DsRed from the Discosoma genus have shown its tremendous advantages: bright red fluorescence and high resistance against photobleaching. However, it has also become clear that the protein forms closely packed tetramers and there is indication for incomplete protein maturation with unknown proportion of immature green species. We have applied single molecule methodology to elucidate the nature of the fluorescence emission in the DsRed. Real time fluorescence trajectories have been acquired with polarization sensitive detection. Our results indicate that energy transfer between identical monomers occurs efficiently with red emission arising equally likely from any of the chromophoric units. Photodissociation of one of the chromophores weakly quenches the emission of adjacent ones. Dual color excitation (at 488 nm and 568 nm) single molecule microscopy has been performed to reveal the number and distribution of red vs. green species within each tetramer. We find that 86 per cent of the DsRed contain at least one green species with a red-to-green ratio of 1.2 – 1.5. Based on our findings, oligomer suppression would not only be advantageous for protein fusion but it will also increase the fluorescence emission of individual monomers.