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to eliminate incorrect regulatory models. The models, in turn, provide feedback and guidance to our experimental work to better understand the roles that sRNA plays in the cellular response.

[1] Fire et al., ''Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans'', Nature (1998)
[2] Raj et al., ''Imaging individual mRNA molecules using multiple singly labeled probes'', Nature Methods (2008).
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889-Pos Board B675
Optimizing Photophysical Conditions in Confocal Fluorescence Correlation Spectroscopy (FCS)
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FCS is a powerful method to measure diffusional and rotational properties of molecules as well as intramolecular transitions (e.g. triplet transition rates and isomerization) and intermolecular interactions (e.g. protein-protein, DNA-protein, etc.). Since the introduction of the confocal measuring principle by Rigler et al. and single photon counting avalanche photodiodes, FCS techniques attained a tremendous revival due to an increased sensitivity enabling even the study of single fluorescent molecules traversing the open, optically confined volume element. Despite these improvements it still remains desirable to further enhance the most important parameter in FCS measurements, i.e. the count rate per molecule (CRpM), in order to further improve the overall performance of FCS measurements (e.g. to enhance signal-to-noise and signal-to-background ratios, and, simultaneously, to minimize acquisition times).
To this end we present here investigations showing an increase of the CRpM of > 200 kHz for Rhodamine Green (excit. laser power > 0.4 mW, 488 nm) along with an effective confocal volume in the subfemtoliter range (0.76 fl). However, photophysical processes like photobleaching, saturation, Rayleigh and Raman scattering as well as triplet transitions and/or isomerization have to be taken into account to perform optimized FCS measurements. Under these conditions we succeeded to yield signal-to-background ratios (i.e. CRpM/BGCR with BGCR background count rate due to scatter) of > 400 at relatively low laser power (0.1-0.3 mW). Further increasing the laser power results in increased triplet transition rates, bleaching and linearly increasing BGCR whereas CRpM levels out into a plateau. Thus, higher excitation energies (above 0.5mW) are in case of Rhodamine Green of counterproductivity. Finally, thanks to the improved single molecule detection sensitivity, we demonstrate that pico- and even femtomolar concentrations of fluorophores can be detected by confocal FCS under optimized photophysical conditions.

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Heterogeneity of Anti-VEGF Aptamer : A Single Molecule Study
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Age-related macular degeneration (AMD) is a major cause of severe visual loss worldwide. Among the key regulators, inhibition of vascular endothelial growth factor (VEGF) serves as a promising candidate for controlling angiogenesis in AMD. The emergence of aptamers as anti-VEGF (aV) provides a new pathway for targeting VEGF to control the disease. aV exhibits conformational dynamics and heterogeneity, implying a rugged energy landscape. Characterization of the heterogeneity of aptamers by single molecule fluorescence energy transfer (smFRET) has not been extensively elucidated. smFRET efficiency distributions in single-molecule experiments contain both structural and dynamical information. Extraction of this information from these distributions requires a careful analysis of unwanted contributions from dye-photophysics. We used cross-correlation analysis to characterize each time series trajectory and distinguish it from contributions of unwanted dye photophysics. NMR and analytical centrifugation were used to complement the dynamic smFRET conformational analyses. Wavelet denoising and state-finding algorithms were used to process the smFRET data to remove shot-noise and find among the ensembles of DNA aptamer conformational states. Binding of VEGF was found to induce a shift in the equilibrium conformation of the DNA aptamer to two conformations. Increased ionic strength resulted in a decrease of the binding ability of the aV to the VEGF.

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Photostability without pH Drop - An Alternative Oxygen Scavenging System for Sinlge-Molecule FRET Experiments
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Single-molecule FRET has become a powerful tool to study conformational changes in cellular machines and many other biomolecules. Labeled with fluorophores, distance changes can be tracked with high temporal and spatial resolution. The performance of such fluorophores in experiments, however, is limited by the average fluorophore lifetime before bleaching among other factors. Oxygen-scavenging systems can create oxygen-depleted conditions in fluorescence experiments and thereby reduce the bleaching due to free singlet oxygen.
The most common enzymatic system used for oxygen scavenging is glucose-oxidase and catalase (GOC). One of the pitfalls of this combination, however, lies in the creation of acidic conditions by the production of gluconic acid. This can lead to a significant drop in pH over the course of an experiment affecting many biomolecules and fluorophores.
Here, we present an alternative enzymatic system for oxygen scavenging whose performance exceeds that of GOC with two commonly used fluorophores, the cyanine dyes Cy3 and Cy5. The average molecule lifetime before bleaching is extended and no change in pH value is observed. We compare our observations with other common oxygen scavenging systems.

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The Stability and Dynamics of E-Coli Beta-Clamp by Single Molecule and Fluorescence Correlation Spectroscopy
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The sliding clamp is an important component of DNA polymerase III. In the presence of the γ complex and ATP, the β clamp, which consists of two monomeric units, forms a ring-like structure around double stranded DNA. The rest of the DNA replication machinery binds to the β clamp and continues replication. This process increases the rate and the processivity of replication by orders of magnitude.
In this project, we investigated the dimerization equilibrium of the beta-clamp dimer, and the existence of rapid spontaneous conformations that involve the opening of the dimer interface. The β-clamp was labeled with TMR dyes. In a closed conformation, the dyes can form a H-dimer and quench the fluorescence. However, in an open conformation, the dyes are further apart from each other and their fluorescence increases. Thus, if the β-clamp dissociates at low concentrations it should result in more fluorescence. This was investigated through single molecule spectroscopy, and the data was used to find the dissociation constant of the β-clamp. In addition, the kinetics of the rapid spontaneous fluctuation between open and closed conformations of the β-clamp is studied using fluorescence correlation spectroscopy.

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Investigating the Stoichiometry of RuBisCO Activase by Fluorescence Fluctuation Methods
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Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is an enzyme that catalyzes the carboxylation of the substrate Ribulose-1,5-bisphosphate . The notorious inefficiency of RuBisCO to catalyze carboxylation is due to inhibition by various metabolites. RuBisCO activase, an ancillary enzyme is needed to foster the activity of RuBisCO. Activase has been recognized as a member of the AAA+ family of the ATPases. It facilitates the removal of firmly bound sugar phosphates thereby restoring RuBisCO activity.
The stoichiometry and oligomerization kinetics of fluorescently tagged RuBisCO activase was investigated in a wide range of concentrations using Fluorescence Correlation Spectroscopy (FCS) in conjunction with Photon counting Histogram (PCH) analysis. Experiments revealed that Activase exists as a monomer at sub-micro molar concentrations, and assembles into oligomers (possible hexamers) at higher concentrations. The analysis of the concentration-dependent diffusion coefficient revealed that the binding between the subunits occurs in steps involving intermediates. The pathway of assembly taken by Activase was found to be independent of the presence of ATP-γS or ADP in the system.