# Hydrodynamic Properties and Interactions of Proteins Involved in Gene Expression by Fluorescence Correlation Spectroscopy (FCS)

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## Background and Objectives

Gene expression is a fundamental process regulated at many different levels. MicroRNAs are short fragments of RNA that regulate protein expression at post-transcriptional level. One of the mechanisms responsible for the regulation is strictly dependent upon interactions between disordered protein glycine-tryptophan protein of 182 kDa **(**GW182) and structured protein (CNOT1). This interactions are crucial for both initial translational inhibition and deadenylation process thus play an important role in gene expression[1,2].

Fluorescence correlation spectroscopy (FCS) is one of biophysical methods used to determine hydrodynamic properties of fluorescently labeled molecules that diffuse in aqueous solutions. This technique makes it possible to perform observations of intermolecular interactions by measuring of fluctuations in the fluorescence intensity signal.

The goal of this work was to determine translational diffusion coefficients and hydrodynamic radii of the folded and intrinsically disordered proteins and perform fluorescence titration at FCS to observe formation of binary complexes between proteins involved in miRNA-mediated gene silencing.

## Biological Relevance

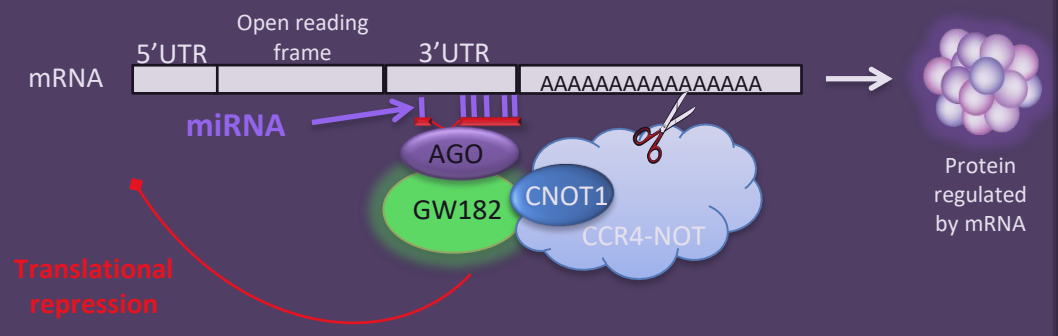


**Figure 1.** Schematic representation of post-transcriptional regulation of gene expression. The core of miRNA-induced silencing complex (miRISC) is formed along with Argonaute (Ago) and GW182 proteins, which are recruited to the 3' untranslated region (3'UTR) of target mRNAs[1,3].

## Matherials and Methods

Experimental work were performed on several folded and many different variants of intrinsically disordered proteins, that were overexpressed in *E. coli* or *Rosetta 2* and purified on His-Trap columns on an FPLC or in ion-exchange chromatography or size exclusion chromatography. The concentration of proteins used for the experiments was 200 nM, in 50 mM TRIS, 150 mM NaCl, 0,5 mM EDTA, 1 mM DTT/TCEP pH=8.0.

Proteins were labeled with, fluorescent probe Alexa Fluor 488 or in fusion with fluorescent proteins: mαEGFP or mCherry.

Fluorescence Correlation Spectroscopy experiments were run on the confocal microscope Axio Ovserver 780 Zeiss ConfoCor3 with beamsplitters MBS488 and 488/561 with filters BP495-555 nm and LP580 nm. Surface of glass plates was passivated with solution of 20 mM TRIS, 150 mM NaCl, 0,1% w/v Triton X-100 and 3 % w/v BSA. The actual temperature in the droplet was measured with a 0.5 mm thermocouple before and after of each measurement and respectively was 20 °C (± 0.5 °C).

Diffusion time ($\tau_d$) was derived from the one and two-component model that was fitted to the autocorrelation curves. This model take into account blinking or triplet state and diffusion in three dimensions respectively.

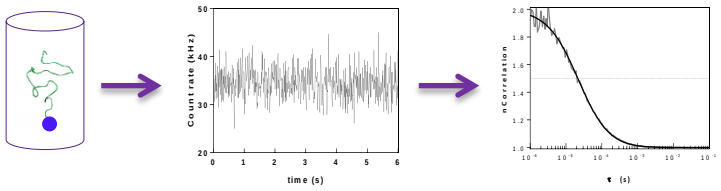


**Figure 2.** Principles of Fluorescence Correlation Spectroscopy experiment. Diffusion of a fluorescent molecules in detection volume generates intensity fluctuations, which are recorder over time. The fluctuations contains information on the diffusion coefficient and the number of particles present in the confocal volume that can then be directly obtained from the autocorrelation function.

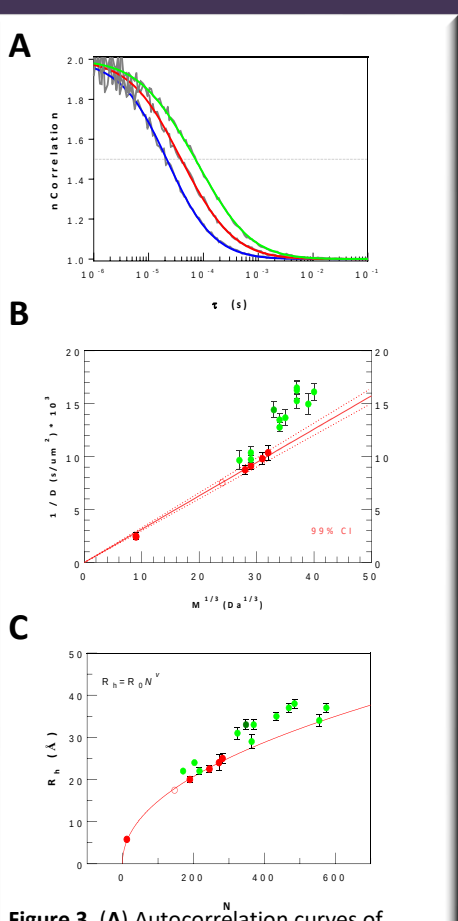


**Figure 3.** (**A**) Autocorrelation curves of ● fluorescent probe (644 a.m.u), ● folded protein (21000 a.m.u.) and ● intrinsically disordered protein (18800 a.m.u.).
(**B**) Relation between inverse of the diffusion coefficients and the cube root of the molar masses. (**C**) Values of hydrodynamic radii ($R_h$) determined from the diffusion coefficients compared with the number of amino acid residues (N). The red solid line is a fit to the power law: $R_h = R_0 N^{\nu}$ for the folded proteins.

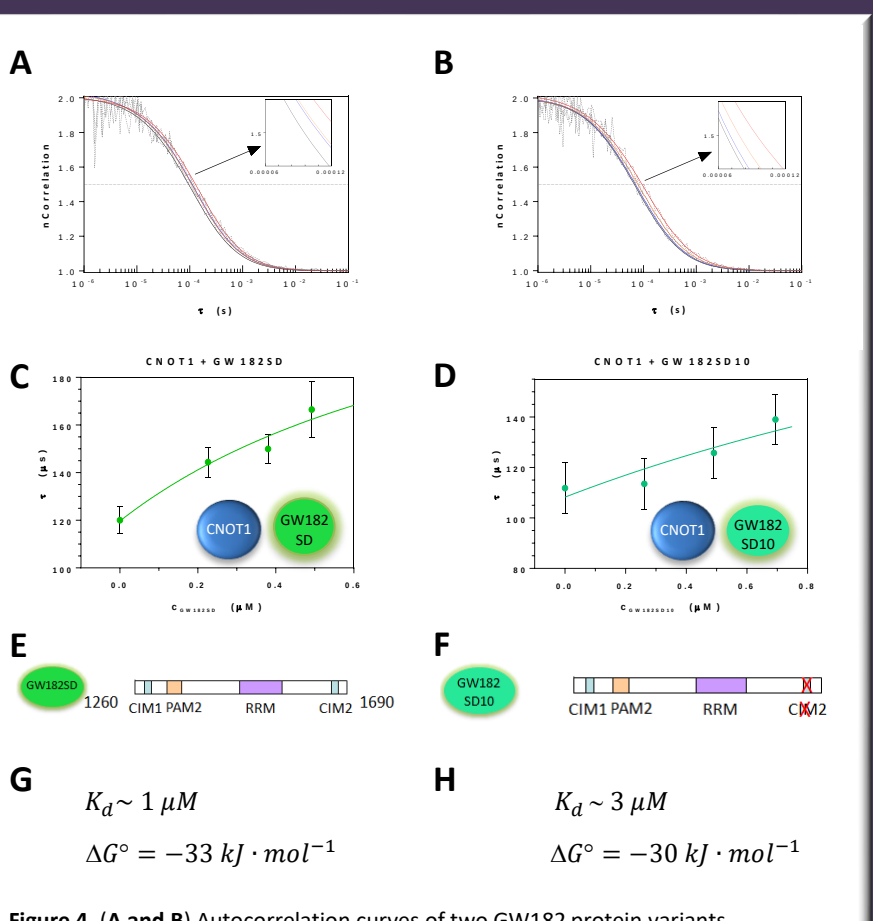


**Figure 4.** (**A and B**) Autocorrelation curves of two GW182 protein variants (● GW182SD and ● GW182SD10).
(**C and D**) Fluorescence titrations at FCS. Relation between diffusion times ($\tau_d$) of GW182 variants at increasing concentration (0 nM - 700 nM) of the ● CNOT1 protein.
(**E and F**) Schematic diagrams of the GW182 protein variants that were used in fluorescence titrations experiments at FCS.
(**G and H**) Estimated equilibrium dissociation constants ($K_d$) and changes in Gibbs free energy ($\Delta G°$) at 20 °C for binary complexes derived from the diffusion times ($\tau_d$).

## Results and discussion

1. Knowledge about hydrodynamic properties of intrinsically disordered proteins (Fig. 3) is nessesary for investigation of non-covalent interactions. Despite tremendous effort[4-6] to describe fully the properties of these group of proteins there is still a strong need for an accurate, fast and universal theoretical model.
2. Fluorescence Correlation Spectroscopy (FCS) enables us to estimate equilibrium constants, as well as changes in Gibbs free energy of binary complexes of intrinsically disordered proteins. Other protein-consuming methods are less effective and more time-consuming [4-6].
3. Fluorescence titrations at FCS (Fig. 4C-4D) gives information about potential binding partners of intrinsically disordered proteins involved in miRNA-mediated gene silencing. It also can reveal their key structural features that can affect binding with their partners.

## References

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