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Short Communication

# Fluorescence Correlation Spectroscopy as a New Method for the Investigation of Aptamer/Target Interactions*

**Heike Schürer[1], Andrej Buchynskyy[2], Kerstin Korn[1], Michael Famulok[3,**], Peter Welzel[2,**] and Ulrich Hahn[1,**]**

[1] Universität Leipzig, Institut für Biochemie, Talstr. 33, D-04103 Leipzig, Germany
[2] Universität Leipzig, Institut für Organische Chemie, Johannisallee 29, D-04103 Leipzig, Germany
[3] Rheinische Friedrich-Wilhelms-Universität, Kekulé-Institut für Organische Chemie und Biochemie, Gerhard-Domagk-Str. 1, D-53121 Bonn, Germany

* Dedicated to Professor Volker A. Erdmann on the occation on his 60th birthday

** Corresponding authors

**Fluorescence correlation spectroscopy is an attractive tool for monitoring molecular interactions in solution. We report here a new and highly sensitive method for studying the interaction of aptamers with their targets using this technique. *In vitro* selection technology is a combinatorial method for the generation of nucleic acid receptors (aptamers) that are capable of binding to various target molecules. Using the *in vitro* selection approach we isolated RNAs which bind to the antibiotic moenomycin with high affinity. The formation of RNA-moenomycin complexes was studied by fluorescence correlation spectroscopy with a tetramethylrhodamine-labeled derivative of moenomycin.**

*Key words:* Aptamers / FCS / Moenomycin / SELEX.

In recent years the technique of fluorescence correlation spectroscopy (FCS) has been developed into a highly sensitive method for the investigation of molecular interactions (Rauer *et al.*, 1996; Rigler *et al*., 1999; Rigler and Elson, 2000). During the last few years technologies for the combinatorial synthesis and the screening of compound libraries have been developed (Bacher and Ellington, 1998). One of the combinatorial techniques is SELEX (Systematic Evolution of Ligands by Exponential Enrichment), the screening of randomized nucleic acid libraries for active molecules, so-called aptamers, which specifically bind to target compounds with high affinity (Osborne and Ellington, 1997; Famulok, 1999). Using this *in vitro* selection/amplification procedure we isolated nuclease-resistant 2'-aminopyrimidine RNA aptamers from a library of approximately 7 x $10^{14}$ different oligonucleotides that bind to the antibiotic moenomycin A with high affinity (Schürer *et al*., 2001).

Moenomycin (**1a**) is an inhibitor of the transglycosylation reaction, which is one of the last steps in peptidoglycan biosynthesis of the bacterial cell wall, and is therefore a highly promising target for the design of new antibiotics (El-Abadla *et al*., 1999). Different fluorescence-based approaches such as fluorescence quenching or depolarization assays have been applied during the last years for the quantitative determination of aptamer/target interactions (Wang and Rando, 1995; Wang *et al*., 1996; Gilbert *et al*., 1997). In the present study we demonstrate aptamer/target binding by measuring the binding reaction of selected RNA-aptamers with a fluorescent moenomycin derivative using FCS. For this study the decarbamoyl derivative **1b** of moenomycin (obtained from **1a** by reaction with butylamine in methanol, Vogel *et al.*, submitted), which has previously been shown to bind to the aptamers, was labeled with the fluorophore tetramethylrhodamine isothiocyanate (in 10:1 DMF-pyridine, 12 h at 20 °C), yielding the fluorescent derivative **2** (Figure 1). Furthermore we have verified that the selected aptamers do not bind to that part of the moenomycin molecule that has been used for the attachment of the fluorescence chromophore (Schürer *et al.*, 2001).

FCS measurements were performed using a ConfoCor® 1 (Carl Zeiss GmbH, Jena; Evotec Biosystems GmbH, Hamburg) device with a helium-neon laser (543.5 nm). The size of the confocal volume element was determined by calibration measurements with rhodamine-6G to be about 1 fl. For determination of binding properties the concentration of tetramethylrhodamine-labeled moenomycin derivative **2** was kept constant at 10 nM and the concentration of aptamers was varied in the range of 10 nM to 10 µM. The formation of complexes between **2** and the aptamers was identified by the change in the autocorrelation function (Figure 2). The autocorrelation function led to the corresponding diffusion times of the free and bound fluorescently labeled moenomycin derivative **2**. Binding of aptamers to **2** could be followed by an increase of the diffusion time from = 0.07 ms for **2** to = 0.4 ms for the **2**/aptamer complex. Due to the differences of diffusion times of free **2** *versus* the **2**/aptamer complex we were able to calculate the percentages of the formed complexes, using the 2-component model of the FCS

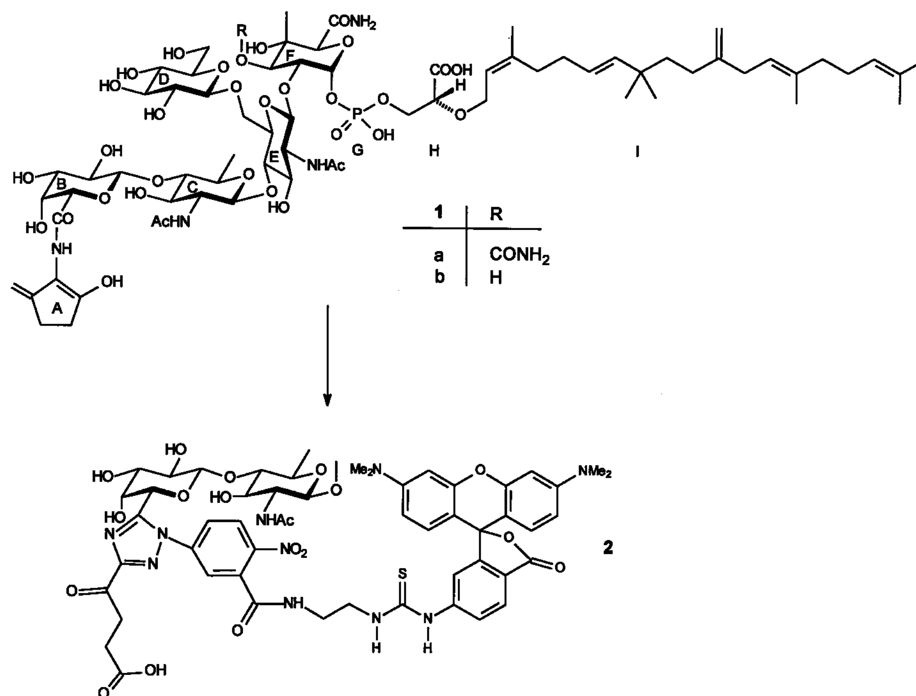


**Fig. 1** Structure of the Tetramethylrhodamine-Labeled Moenomycin A Derivative (**2**).

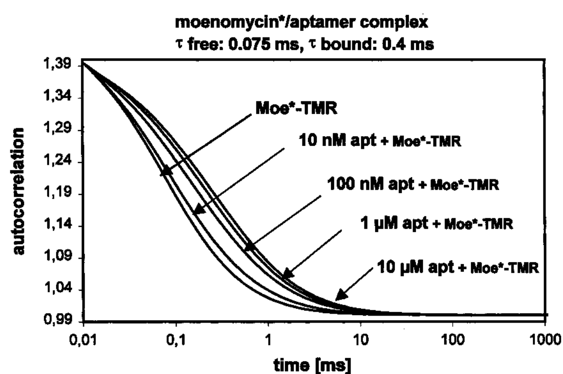


**Fig. 2** Normalized Autocorrelation Functions of TMR-Labeled Moenomycin Derivative **2**, Free and Complexed with Increasing Amounts of Moenomycin Binding Aptamers.

Increasing aptamer/**2** ratios lead to increasing complex formation as indicated by the corresponding correlation functions. The diffusion times for free **2*** and for **2***/aptamer –complexes are indicated in the Figure.

FCS measurements were carried out in binding buffer (20 mM HEPES, pH 7.4, 150 mM NaCl, 5 mM $MgCl_2$) using 10 nM of **2** and varying concentrations of aptamers in the range of 10 nM to 10 µM. For the binding reaction 20 µl of the respective aptamer solution (10 nM to 10 µM) and 1 µl of **2** (final concentration about 10 nM) were preincubated for 15 min at 25 °C. All FCS measurements were carried out at 25 °C in eight-chamber cover glasses (NUNC®), the data acquisition time was 30 s for each experiment. Data were fitted using the FCS ACCESS Evaluation software (2-component fit).

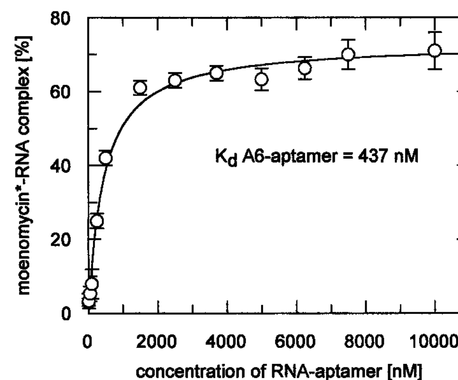


**Fig. 3** Determination of the Binding Poperties of Aptamer/**2** Interaction by FCS.

The percentages of the complexes formed were calculated on the basis of the different diffusion times of free **2** (0.075 ms) and **2**/aptamer complexes (0.4 ms) using the FCS ACCESS 2.0 evaluation software. The dissociation constant for the A6-aptamer was determined to be 437 nM.

**Table 1** Comparison of $K_d$ Values for a Moenomycin Binding Aptamer (A6) Using Different Methods for $K_d$ Determination.

| Method for $K_d$ determination | Dissociation constant $K_d$ (nM) |
|---|---|
| Affinity chromatography (isocratic elution)[1] | 320 |
| FCS | 437 |

[1] According to Arnold *et al.* (1986).

ACCESS 2.0 evaluation software. The higher the aptamer concentration, the more moenomycin/aptamer complex was formed. In a next step we plotted the percentages of the moenomycin/aptamer complexes *versus* the aptamer concentrations. From the resulting data a dissociation constant of 437 nM was determined *via* non-linear regression using the GraFit software (Erithaceus Software Ltd. UK; see Figure 3 and Table 1).

To date, most methods for $K_d$ determination are based on protocols involving radiolabeling. The most widely applied current methods for the investigation of aptamer/target interactions are affinity chromatography (Arnold *et al.*, 1986), filter binding assays (Klug *et al.*, 1997) and surface plasmon resonance (Hendrix *et al*., 1997). Using affinity chromatography we determined a $K_d$value of 320 nM for the binding of the A6-aptamer, one representative of a collection of selected moenomycin A-specific aptamers. This result is in good agreement with the FCS results, where a $K_d$ of 437 nM for binding of the A6- aptamer to **2** was determined (Table 1). Thus FCS yields results with a similar accuracy as a conventional method like affinity chromatography. Gilbert *et al.* (1997) compared $K_d$ values for two farnesyl-Ras aptamers using either a column binding assay or fluorescence binding studies, yielding differences of about one order of magnitude. With respect to these data our results obtained by affinity chromatography and FCS do not differ significantly from each other.

The advantages of FCS are (i) much more rapid recording of data with a (ii) higher sensitivity and (iii) without a need for radioactive labeling.

In conclusion, we have shown that FCS is a fast method for the determination of binding properties of aptamer/target complexes. We were able to demonstrate that results of FCS measurements are comparable with those obtained with conventional methods and that FCS opens the door for a high throughput screening of large numbers of aptamers. As already proposed by Rigler (1995), FCS allows the screening of large numbers of individual molecules of a combinatorial library. RNA molecules showing the desired properties could be selected out of large quantities based on their interaction with a fluorescent target molecule.

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