DNA CONDENSATION CHARACTERISED BY FLUORESCENCE CORRELATION SPECTROSCOPY (FCS)

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The condensation of DNA involves a dramatic decrease in the volume occupied by a DNA molecule and is of immense biological importance. Such structural, physico–chemical and energetic aspects of the condensation continue to receive much attention and have led to the development of non-viral gene therapy (NVGT) delivery vectors. NVGT protocols require reproducible and efficient delivery systems especially for the introduction of plasmid DNA into target cells (e.g. cancer, CNS, cystic fibrosis and inflammation). The NVGT vectors normally carry positive charges which interact with DNA phosphate, leading to DNA condensation into nanoscale complexes. This DNA condensation gives both protection from nuclease hydrolytic enzymes and provides particles of the appropriate size to enter cells (50-150 nm).

FCS is a technique where fluorescence fluctuations detected from an illuminated spot of the volume of a femtoliter, mainly arising from Brownian diffusion of fluorescently labelled single molecules, are analysed. This analysis yields the read-out parameters count rate, diffusion time, and particle number (PN) which can (in the ideal case) be converted into brightness, diffusion coefficient and concentration of the labelled species, respectively. Since FCS is a method which is sensitive to volume changes of macromolecules, it has been the aim of several international research groups to establish this single molecule technique for direct monitoring of the condensation process and characterization of the resulting condensing agent-DNA nanoparticle.

In 2002, we reported for the first time that FCS is indeed able to visualize DNA condensation induced by the natural DNA condensing agent spermine as well as by the positively charged amphiphilic compound HTAB [Kral, T. *et al.* **Biol. Chem.** 383 (2002) 331, Kral, T. *et al.* **Biophys. Chem.** 95 (2002) 135 and Kral, T. *et al.* **Cell. Mol. Biol. Lett.** 7 (2002) 203]. Plasmids of different sizes have been stained by the DNA intercalating agent propidium iodide (ProI) and ethidium bromide (EthBr). Since the condensation processes were characterised by apparent increases in the diffusion coefficients up to a factor 10, this single molecule technique proofed to be a very sensitive tool for studying DNA conformational changes resulting from polycations-mediated condensation.

However, it was found that both stains have large effects on the diffusion coefficients of DNA as well as on the critical condensing agent concentration. Additionally the condensation process leads to ProI and EthBr release from DNA and this to a significant reduction in fluorescence, making the interpretation of the FCS analysis more complicated.

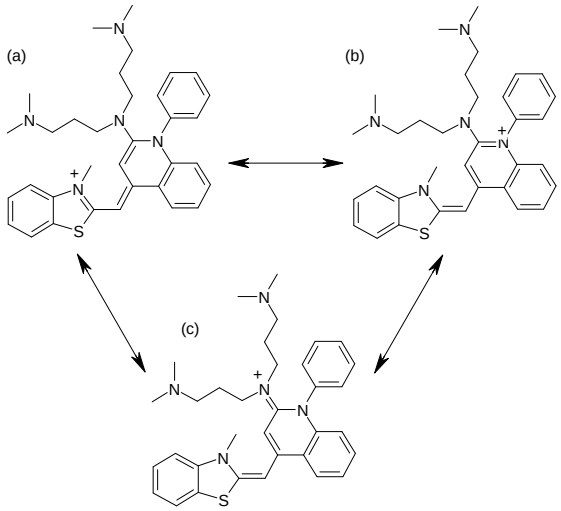

Fig. 1. Three mesomers of PicoGreen® (PG), an unsymmetrical monomethine cyanine dye containing a polyamine side-chain to improve DNA-binding affinity.

In recent contributions [Kral, T. *et al.* **J. Fluor.** (2005), in press., Kral, T. *et al.* submitted and Adjimatera, N. *et al.* submitted], we have shown that increasing amounts of labelling by PicoGreen® (PG; Fig. 1) do not influence the determined diffusion coefficients. Due to the high quantum yield and absorption coefficient of this dye, 10-times lower dye loading is needed for FCS studies when compared to ProI and EthBr. Moreover, count rate is much less sensitive to the condensation process, indicating that dye release is (if at all) only to a minor extent interfering with the condensation process. This latter advantage of PG staining led to a better understanding of the PN/condensing agent titration curves. In the case of the uncondensed DNA, the concentration calculated from the apparent PN is up to 20-fold larger than the expected one. On the other hand, when the condensation process is essentially completed, the PN reaches its predicated theoretical value for efficient cationic condensing agents (non-viral vectors). In the uncondensed form, the size of the circular plasmid DNA molecule is considerably bigger than the confocal element and segmental motions of the multiple labelled DNA molecule as a possible additional cause for fluorescence fluctuations have to be considered [Kral, T. *et al.* submitted and Adjimatera, N. *et al.* submitted]. On the other hand, the PN, which is the most accurate read-out parameter of a FCS experiment, gives quantitative information

on the packing density of DNA-condensing agent aggregates. Thus, direct information on the quality of condensing molecules has been derived.

In our recent applications this methodology has been used to study the interactions of different sized DNA molecules (4.7, 10 and 13 kilobase pairs) with liposomes formulated from cationic lipid [Kral, T. *et al.* submitted] as well as with two newly synthesized lipopolyamines [Adjimatera, N. *et al.* submitted]. In particular the FCS data shows that N^4,N^9-dioleoylspermine (Fig. 2) efficiently condenses DNA molecules by forming DNA-lipopolyamine nanoparticles [6].

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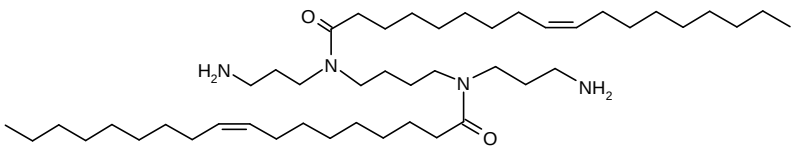

Fig. 2. N^4,N^9-Dioleoylspermine (spermine conjugated with two chains of C18 fatty acid, with two positive charges at physiological pH).