FRET in nucleic acids using base analogues

L. Marcus Wilhelmsson

Chalmers University of Technology, Department of Chemistry and Chemical Engineering, Chemistry and Biochemistry, SE-41296 Gothenburg, Sweden

E-mail: marcus.wilhelmsson@chalmers.se

Fluorescent base analogues are important for investigating DNA- and RNA-containing systems.[1] A major challenge in developing such molecules with excellent absorptive and emissive properties is how the need for base-pairing and available space in the native nucleic acid structure restrict their shape, size, charge as well as hydrogen bond donor and acceptor positions. Not being discouraged by these challenges, we have put considerable efforts into the development of bright analogues for the natural nucleobases with special emphasis on applications in Förster resonance energy transfer (FRET)[2,3].

Currently we are developing adenine analogue FRET-pairs. In ongoing investigations the qANs[4,5] (Fig. 1, bottom **2**) show promising properties as FRET-donors inside nucleic acids. We have shown that they are excellent adenine analogues in DNA-systems and have a considerable brightness inside DNA. We are also developing a qA-derivative that has shown to be an excellent adenine analogue and, in preliminary measurements, to be a useful FRET-acceptor to the qANs inside DNA (Fig. 1, top).

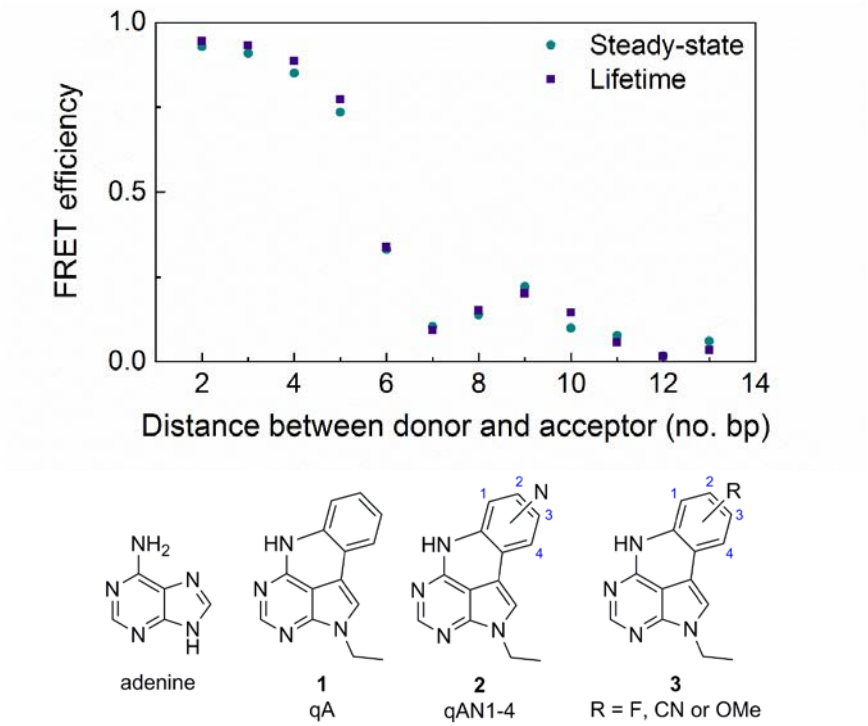

Figure 1. Top: FRET efficiency dependence on number of bases separating novel analogue FRET-donor (qAN1) and –acceptor inside B-form DNA. Bottom: Adenine and members of quadracyclic adenine (qA) family.

Importantly, the photophysical properties of the novel adenine FRET-donor and -acceptor fit well the corresponding properties of our previously developed FRET-pair tC^{O}-tC_{nitro}[6,7,8] making it possible to perform FRET between our new A-analogues and the members of the commercially available tC-family[6,7,8]. Recently we also developed the RNA phosphoramidite of tC^{O} and incorporated it into RNA for the possible future use in FRET in RNA.[9] Moreover, lately we are exploring a novel A-analogue whose brightness is significantly higher than any of our previous internal nucleic acid fluorophores. With our collection of new base analogues we increase the flexibility of base-base FRET-studies from only cytosine positions to adenine and combined adenine-cytosine positions as well as, importantly, the sensitivity of fluorescent base analogues, now approaching single molecule levels.

Funding: This work was supported by the Swedish Research Council (VR) [2013-4375] and the Swedish Foundation for Strategic Research (SSF) [ID14-0036 and IS14-0041].

Acknowledgement: AstraZeneca, Gothenburg, is acknowledged for the fruitful recent collaboration on the development of fluorescent base analogues. Past and present members of my group are acknowledged for their contributions to the work presented here.

References:

[1] L. M. Wilhelmsson, *Q. Rev. Biophys.* **2010**, 43, 159
[2] Y. Shi, A. Dierckx, P. H. Wanrooij, et al., *P. Natl. Acad. Sci. USA*, **2012**, 109, 16510
[3] B. Dumat, A. F. Larsen, L. M. Wilhelmsson, *Nucleic Acids Res.* **2016**, 44, e101
[4] B. Dumat, M. Bood, M. S. Wranne, et al., *Chem. Eur. J.* **2015**, 21, 4039
[5] A. F. Larsen, B. Dumat, M. S. Wranne, et al., *Sci. Reports* **2015**, 5, 12653
[6] P. Sandin, L. M. Wilhelmsson, P. Lincoln, et al., *Nucleic Acids Res.* **2005**, 33, 5019
[7] P. Sandin, K. Börjesson, H. Li, et al., *Nucleic Acids Res.* **2008**, 36, 157
[8] K. Börjesson, S. Preus, A. H. El-Sagheer, et al., *J. Am. Chem. Soc.* **2009**, 131, 4288
[9] A. F. Füchtbauer, S. Preus, K. Börjesson, et al., *Sci. Reports* **2017**, in press