Imaging of RNA delivery to cells by thiazole orange as a fluorescent RNA base substitution†

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Interstrand thiazole orange (TO) dimers in RNA show a yellow colored emission that can be distinguished from the green TO monomer emission by confocal microscopy inside CHO cells.

In contrast to the fluorescent toolbox for imaging proteins,1 the development of fluorescent probes to image RNA in cells remains a challenge.2 Currently, RNA is detected by fluorescence in situ hybridization (FISH)3–5 or by molecular beacons.6 Moreover, fluorescent base pairs respond to RNA hybridization and base mismatches.4,6–8 RNA has also been imaged in living cells by templated reactions.9 The readout of all these assays is a change of emission intensity, preferably an enhancement. However, undesired quenching inside cells could cause artifacts. Hence, RNA probes that change their emission maximum (=color) as the readout represent an important alternative for imaging. This has been realized by FRET processes e.g. in the form of wavelength-shifting10–12 molecular beacons or binary probes.13–15

Thiazole orange (TO) is extensively used as a non-covalently binding staining agent for nucleic acids.16 Moreover, TO was linked covalently to oligonucleotides,17–19 DNA-binding peptides10 and as a base surrogate into PNA.18 The latter PNA was applied to detect SNPs.18 Recently, we reported about TO attached via its quinoline as an artificial DNA base.19 If two TO chromophores are incorporated via their thiazoles as artificial DNA bases, their optical properties are altered significantly.20 The interstrand TO dimers exhibit a red-shifted emission and hence display DNA hybridization by a color change. Herein, we present the transfer of this concept from DNA to RNA, and report preliminarily about imaging of RNA delivery to cells.

First, we synthesized RNA1 and the counterstrand RNA2 bearing a single TO chromophore as an artificial base surrogate, and the strands RNA3 and RNA4 without any modification (Scheme 1). In the duplex that is formed by RNA1 and RNA2 (RNA1-2) the TO chromophores are forced into close contact with each other. The duplex RNA1-3 bears only one TO modification and serves as a reference for any changes of the optical properties. As expected, the absorption spectra of the single TO chromophore in RNA1-3 and RNA4-2 exhibit the TO-typical absorption bands at 482/509 nm that can be assigned to the 0 → 1 and 0 → 0 vibronic transitions (Fig. 1, left). In comparison, the absorption differences of RNA1-2 can be interpreted by strong excitonic interactions between the two TO chromophores. Interestingly, some groundstate interactions exist already in the single strand (RNA2). It is important to point out, however, that these interactions can be interrupted by increasing the temperature (70 °C) or by hybridization with RNA4. The duplex RNA4-2 has nearly the same optical properties as the single TO-modified RNA1-3.

Fig. 1 Left: UV/Vis absorption spectra of RNA1, RNA2, RNA1-2, RNA1-3 and RNA4-2; right: fluorescence spectra of RNA1, RNA2, RNA1-2, RNA1-3 and RNA4-2; excitation at 490 nm; 2.5 μM in 10 mM Na-P buffer, 250 mM NaCl, pH 7, 20 °C.

When the duplexes RNA1-3 and RNA4-2 are excited at 490 nm, the fluorescence shows a maximum at ~530 nm that corresponds...
to the typical green emission of the TO dye (Fig. 1, right). The fluorescence of the duplex RNA1-2 is dominated by a broad band at ~573 nm as a result of excitation of the TO dimer complex. Similar to the TO dimers in DNA,26 the emission has a yellow color. It is important to note, that the existence of excitonic interactions between two TO dyes does not automatically yield a color. It is important to note, that the existence of excitonic interactions between two TO dyes does not automatically yield a color. It is important to point out, however, that this destabilization is less compared to single glycol modifications with normal nucleobases instead of chromophores.25 And moreover, the second TO modification in duplex RNA1-2 does not introduce an additional destabilization (4.5 °C per modification). Taken together, not only the interactions of TO with the adjacent base pairs in RNA1-3 regain some of the lost thermal stability due the glycol linker but also the interstrand hydrophobic interactions between two TO chromophores in RNA1-2. Hence, the interstrand TO dimer in RNA could be regarded as a hydrophobically and diagonally interacting base pair that shows a fluorescence readout signal for RNA hybridization. The quantum yields in the range of 20% together with a large "virtual" "Stokes'-shift" of nearly 100 nm make this fluorescence emission persisted even inside cells. The ratio of the fluorescence of the green to the yellow channel was 0.8 from 500 to 550 nm (green channel) and from 570 to 640 nm (yellow channel) (Fig. 2). Remarkably, the difference in the fluorescence of the duplex RNA1-2 and RNA1-3, 100 nM in cell culture medium (Ham’s F12), before and after particle formation with LipofectamineTM 2000; excitation at 488 nm.

In conclusion, we have shown that, similar to DNA, the TO dimer in RNA could be regarded as a hydrophobically interacting base pair that shows a red-shifted fluorescence readout signal for hybridization and can be applied to monitor RNA delivery to cells using Lipofectamine™ 2000. These results are promising for imaging delivery of interference RNA to cells. This allows the discrimination between single and double stranded RNA inside cells. Since both strands carry the same fluorophore, dehybridization can be monitored using a single excitation wavelength. The TO base pair can potentially be placed in a hairpin stem for molecular beacons or to monitor RNA folding equilibria.

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Notes and references