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Supporting Information

Effect of Cholesterol Anchoring Group on the Properties of G-Quadruplex-Based FRET Probes for Potassium Ion Chemosensors 2014, 2, 267-286

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HPLC Analysis of FRET Probes

The Breeze 2 HPLC System with a 2998 photodiode array detector and 2475 multi-λ fluorescence detector (Waters Corp., Milford, MA, USA) was used to verify the analytical quality and purity of received probes. Oligonucleotides were separated using a 4.6 × 50 mm XBrige OST C18 column packed with 2.5-µm particles (average pore diameter 130 Å). The mobile phases were composed of 5% acetonitrile with 5 mM triethylammonium acetate, pH 7.5 (Mobile Phase A), and 80% acetonitrile (Mobile Phase B). The flow rate was 0.5 ml/min. For HPLC gradient and other conditions see Table S1. The oven temperature was set at 55 ºC.

Table S1. HPLC method conditions.

Column	**XBridge OST, 4.6 × 50 mm, 2.5 µm**
Mobile Phase A	5/95 acetonitrile/water with 5 mM triethylammonium acetate, pH = 7.5
Mobile Phase B	80/20 acetonitrile/water
Flow Rate	0.5 ml/min
Gradient	**Time** **Profile** (min) A% B% 0 100 0 20 0 100 25 100 0
Inj. Volume	25 µL
Column Temp	55 ºC
Detections	▪ Waters 2998 Photodiode Array Detector (PDA) Detector ▪ Waters 2475 Multi-λ Fluorescence Detector

The fluorescent oligonucleotides were dissolved in mobile phase A (5% acetonitrile with 5 mM triethylammonium acetate, pH 7.5) and injected (25 µl injection) onto the column. Figure S1 shows the chromatograms for four probes (F19T, ChF19T, 16F19T and Ch16F19T) monitored via the fluorescence signal of FAM (λ_{ex} = 490 nm, λ_{em} = 520 nm). The retention peaks appear around 4.5 min (16F19T probe), 6.0 min (F19T probe), 16.0 min (Ch16F19T probe), and 16.6 min (ChF19T probe). In the latter case, a longer retention times were due to the presence of cholesterol moiety that interacted strongly with the C18 stationary phase. The same retention times were observed when monitoring TAMRA fluorescence (λ_{ex} = 560 nm, λ_{em} = 585 nm) or with spectrophotometric detection at 260 nm. These results demonstrated that contaminations from partially labeled or unlabeled oligonucleotides were not present in the investigated samples.

Figure S1. Chromatograms of fluorescent probes monitored with fluorescence of FAM (λ_{ex} = 490 nm, λ_{em} = 520 nm). Separation conditions are given in Table S1.