

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

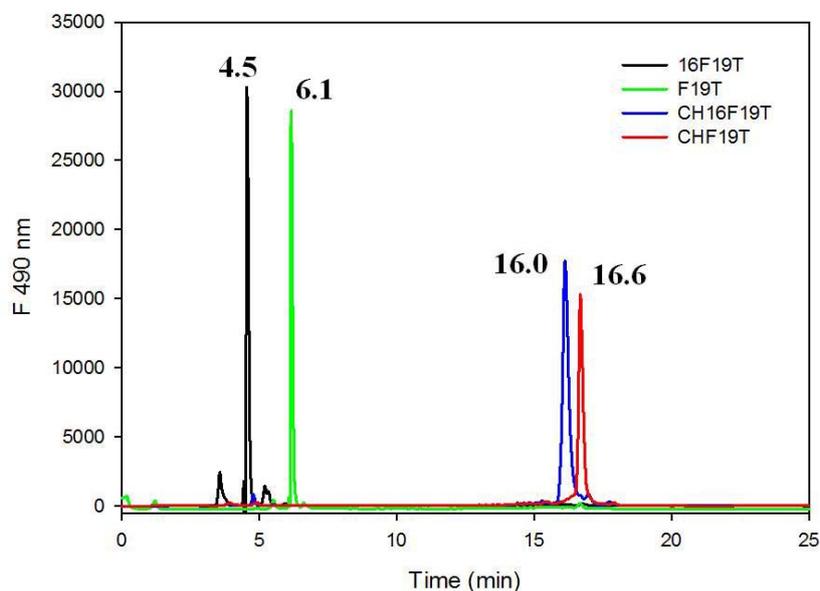
The Breeze 2 HPLC System with a 2998 photodiode array detector and 2475 multi- $\lambda$  fluorescence detector (Waters Corp., Milford, MA, USA) was used to verify the analytical quality and purity of received probes. Oligonucleotides were separated using a  $4.6 \times 50$  mm XBridge OST C18 column packed with 2.5- $\mu$ m 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 °C.

**Table S1.** HPLC method conditions.

<b>Column</b>	<b>XBridge OST, 4.6 × 50 mm, 2.5 μm</b>		
<b>Mobile Phase A</b>	5/95 acetonitrile/water with 5 mM triethylammonium acetate, pH = 7.5		
<b>Mobile Phase B</b>	80/20 acetonitrile/water		
<b>Flow Rate</b>	0.5 ml/min		
<b>Gradient</b>	<b>Time</b>	<b>Profile</b>	
	(min)	A%	B%
	0	100	0
	20	0	100
	25	100	0
<b>Inj. Volume</b>	25 μL		
<b>Column Temp</b>	55 °C		
<b>Detections</b>	<ul style="list-style-type: none"> <li>▪ Waters 2998 Photodiode Array Detector (PDA) Detector</li> <li>▪ Waters 2475 Multi-<math>\lambda</math> Fluorescence Detector</li> </ul>		

The fluorescent oligonucleotides were dissolved in mobile phase A (5% acetonitrile with 5 mM triethylammonium acetate, pH 7.5) and injected (25  $\mu$ l injection) onto the column. Figure S1 shows the chromatograms for four probes (F19T, ChF19T, 16F19T and Ch16F19T) monitored via the fluorescence signal of FAM ( $\lambda_{\text{ex}} = 490$  nm,  $\lambda_{\text{em}} = 520$  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 ( $\lambda_{\text{ex}} = 560$  nm,  $\lambda_{\text{em}} = 585$  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 ( $\lambda_{\text{ex}} = 490$  nm,  $\lambda_{\text{em}} = 520$  nm). Separation conditions are given in Table S1.



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