Electronic Supporting Information

A G-quadruplex-selective luminescent switch-on probe for the detection of sub-nanomolar human neutrophil elastase†

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Experimental section

Materials. Reagents, unless specified, were purchased from Sigma Aldrich (St. Louis, MO) and used as received. Calf-thymus DNA (ct DNA) was purchased from Sigma Aldrich (St. Louis, MO) and purified by literature method.[1] Iridium chloride hydrate ($IrCl_3.xH_2O$) was purchased from Precious Metals Online (Australia). All oligonucleotides were synthesized by Techdragon Inc. (Hong Kong, China).

General experimental. Mass spectrometry was performed at the Mass Spectroscopy Unit at the Department of Chemistry, Hong Kong Baptist University, Hong Kong (China). Melting points were determined using a Gallenkamp melting apparatus and are uncorrected. Deuterated solvents for NMR purposes were obtained from Armar and used as received.

^{1}H and ^{13}C NMR were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C). ^{1}H and ^{13}C chemical shifts were referenced internally to solvent shift (CD_3CN: ^{1}H, δ 1.94, C δ 118.7; d_6-DMSO: ^{1}H δ 2.50, C δ 39.5). Chemical shifts (δ) are quoted in ppm, the downfield direction being defined as positive. Uncertainties in chemical shifts are typically ±0.01 ppm for ^{1}H and ±0.05 for ^{13}C. Coupling constants are typically ± 0.1 Hz for ^{1}H-^{1}H and ±0.5 Hz for ^{1}H-^{13}C couplings. The following abbreviations are used for convenience in reporting the multiplicity of NMR resonances: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. All NMR data was acquired and processed using standard Bruker software (Topspin).

Photophysical measurement. Emission spectra and lifetime measurements for complex **1** were performed on a PTI TimeMaster C720 Spectrometer (Nitrogen laser: pulse output 337 nm) fitted with a 380 nm filter. Error limits were estimated: λ (±1 nm); τ (±10%); φ (±10%). All solvents used for the lifetime measurements were degassed using three cycles of freeze-vac-thaw.
Luminescence quantum yields were determined using the method of Demas and Crosby[2] $[Ru(bpy)_3][PF_6]_2$ in degassed acetonitrile as a standard reference solution (Φ_r = 0.062) and calculated according to the following equation:

$$\Phi_s = \Phi_r(B_r/B_s)(n_s/n_r)^2(D_s/D_r)$$

where the subscripts s and r refer to sample and reference standard solution respectively, n is the refractive index of the solvents, D is the integrated intensity, and Φ is the luminescence quantum yield. The quantity B was calculated by $B = 1 - 10^{-AL}$, where A is the absorbance at the excitation wavelength and L is the optical path length.

Synthesis

The precursor complex $[Ir_2(ppy)_4Cl_2]$[3] and 2,9-diphenyl-1,10-phenanthroline (dpp)[4] were prepared according to the reported literature methods. They were characterized by ^{1}H NMR, ^{13}C NMR and high resolution mass spectrometry (HRMS).

$[Ir(ppy)_2(dpp)]PF_6$ (1). A suspension of $[Ir_2(ppy)_4Cl_2]$ (0.1 mmol) and 2,9-diphenyl-1,10-phenanthroline (0.22 mmol) in a mixture of DCM:methanol (1:1, 20 mL) was refluxed overnight under a ntirogen atmosphere. The resulting solution was then allowed to cool to room temperature, and was filtered to remove unreacted cyclometallated dimer. To the filtrate, an aqueous solution of ammonium hexafluorophosphate (excess) was added and the filtrate was reduced in volume by rotary evaoration until precipitation of the crude product occurred. The precipiate was then filtered and washed with several portions of water (2 × 50 mL) followed by diethyl ether (2 × 50 mL). The product was recrystallized by acetonitrile:diethyl ether vapor diffusion to yield the titled compound as a dark yellow solid.

Yield: 69%. 1H NMR (400 MHz, CD3CN) d 8.68 (d, *J* = 8.0 Hz, 2H), 8.26 (s, 2H), 7.79-7.75 (m, 4H), 7.73-7.70 (m, 2H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.13 (d, *J* = 8.0Hz, 2H), 7.01 (t, *J* = 8.0 Hz, 2H), 6.96–6.93 (m, 2H), 6.73 (t, *J* = 8.0 Hz, 4H), 6.61(br s, 4H), 6.47 (t, *J* = 8.0Hz, 2H), 6.13 (t, *J* = 8.0 Hz, 2H), 5.20 (d, *J* = 8.0Hz, 2H); 13C NMR (400 MHz, CD3CN) d 169.4, 167.3, 152.0, 149.7, 148.5, 143.6, 140.5, 139.8, 139.4, 132.0, 131.9, 130.5, 130.4, 129.4, 129.2, 128.9,128.4, 125.4, 123.2, 122.0, 121.0; MALDI-TOF-HRMS: Calcd. for $C_{49}H_{40}IrN_4$ $[M–PF_6]^+$: 832.9901 Found: 832.9689. Quantum yield (Φ) = 0.16, Emission lifetime (τ) = 3.73 μs.

Emission response of 1 towards different forms of DNA

The G-quadruplex DNA-forming sequences (HTS and H21) were annealed in Tris-HCl buffer (20 mM Tris, 100 mM KCl, pH 7.0) and were stored at –20 °C before use. Complex **1** (1 μM) was added to 5 μM of ss DNA, ct DNA or G-quadruplex DNA in Tris-HCl buffer (20 mM Tris, pH 7.0) containing. Emission spectra were recorded in 500−700 nm range using an excitation wavelength of 320 nm.

Emission measurement for the detection of HNE in buffered solution

5 μM of HNE aptamer was annealed with 5 μM of its partially complementary sequence in annealing buffer (20 mM Tris, pH 7.0) at 95 °C and the reaction mixture was allowed to cool to room temperature and stored at –20 °C before use. For emission measurement, 100 μL of annealed DNA duplex and the indicated concentrations of HNE or other proteins were added into Tris-HCl buffer (20 mM Tris, pH 7.0). The solutions were incubated for 1 h, followed by the addition of complex **1** at a final concentration of 3 μM. Emission spectra were recorded in the 500–700 nm range, after equilibration at 25.0 °C for 10 min. Excitation wavelength = 320 nm.

Fig. S1 Normalized excitation and emission spectra of complex **1** (20 μM) in acetonitrile solution at 298K.

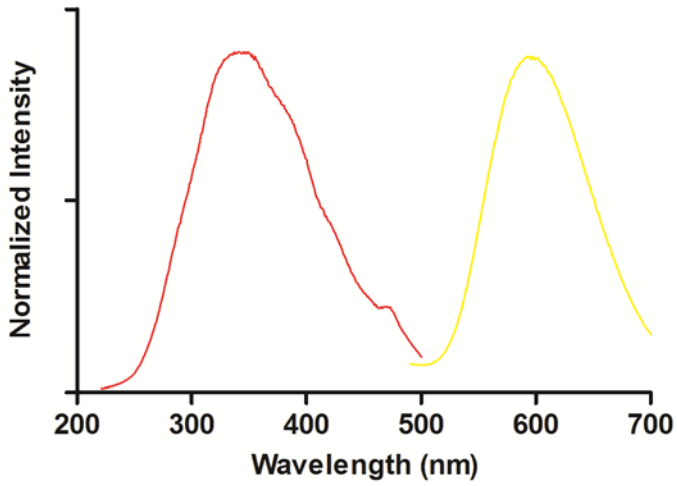


Fig. S2 Relative intensity change at 585 nm of the system ([aptamer duplex] = 1 μM, [HNE] = 30 nM) in the presence of varying concentration of complex **1** (1, 3 and 6 μM) in aqueous buffered solution (20 mM Tris, pH 7.0). Error bars represent the standard deviations of the results from three independent experiments.

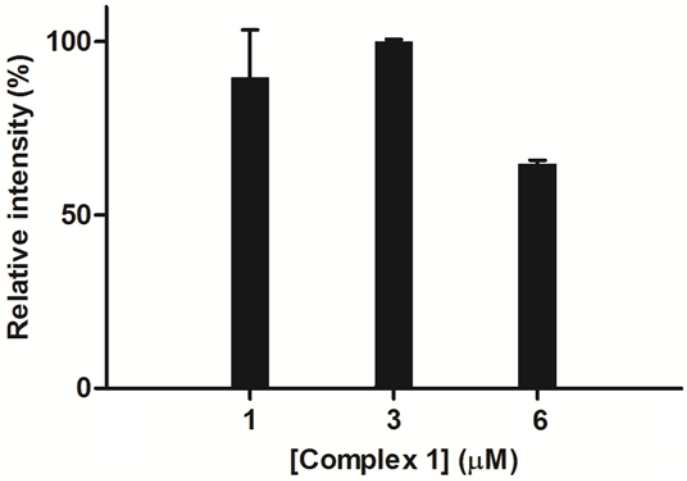


Fig. S3 Relative intensity change at 585 nm of the system ([**1**] = 3 μM, [HNE] = 42 nM) in the presence of varying concentrations of aptamer duplex (0.1, 0.5 and 1 μM) in aqueous buffered solution (20 mM Tris, pH 7.0). Error bars represent the standard deviations of the results from three independent experiments.

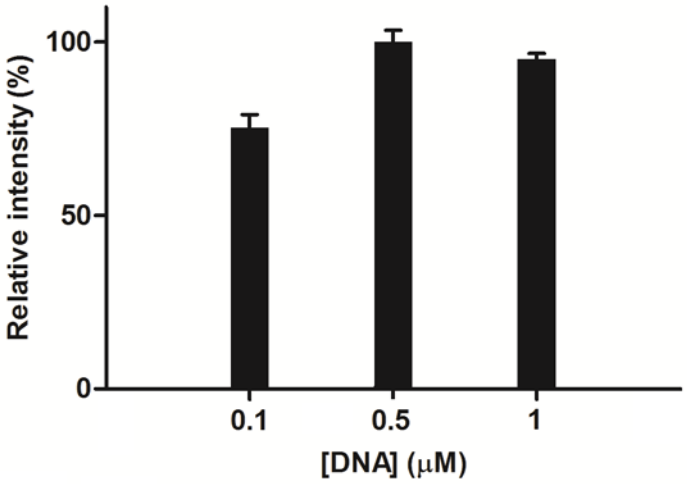


Fig. S4 Photograph image of the system ([**1**] = 3 μM, [aptamer duplex] = 0.5 μM) in Tris buffer (20 mM, pH 7.0) in the presence (left) or absence (right) of 6 nM HNE.

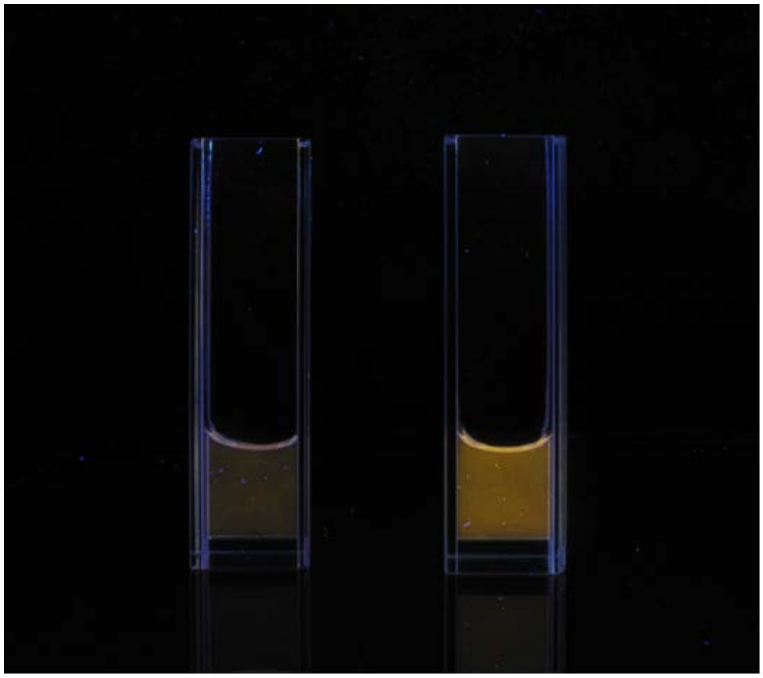


Fig. S5 Luminescence response of the system ([**1**] = 3 µM and [HNE aptamer] = 1 µM) in the presence of increasing concentration of HNE (0, 6, 18, 30 and 42 nM) in aqueous buffered solution (20 mM Tris, pH 7.0). Inset: luminescent intensity of the system at 585 nm vs concentration of HNE.

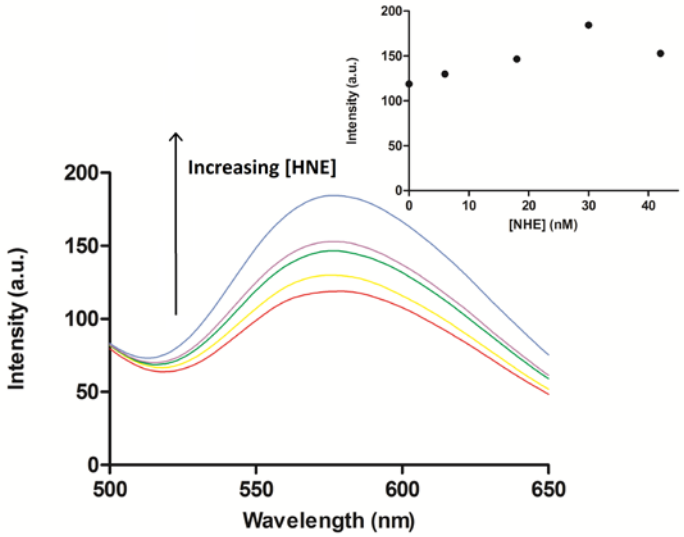


Fig. S6 Luminescence response of the system with the metal complex alone ([**1**] = 3 µM) in the presence of increasing concentrations of HNE (0, 30 and 60 nM) in aqueous buffered solution (20 mM Tris, pH 7.0).

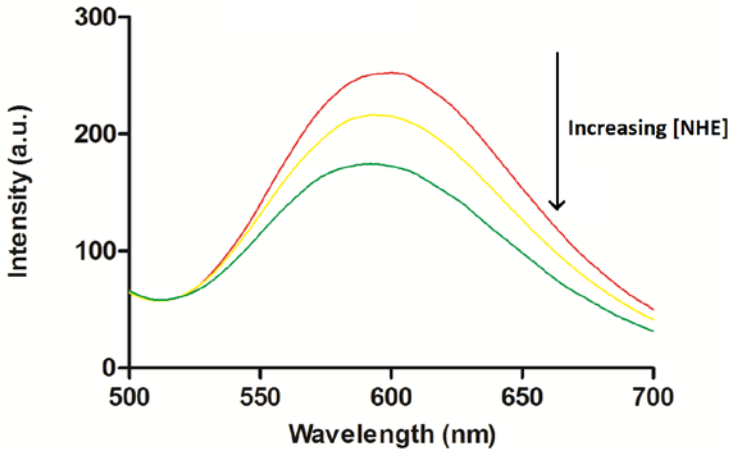
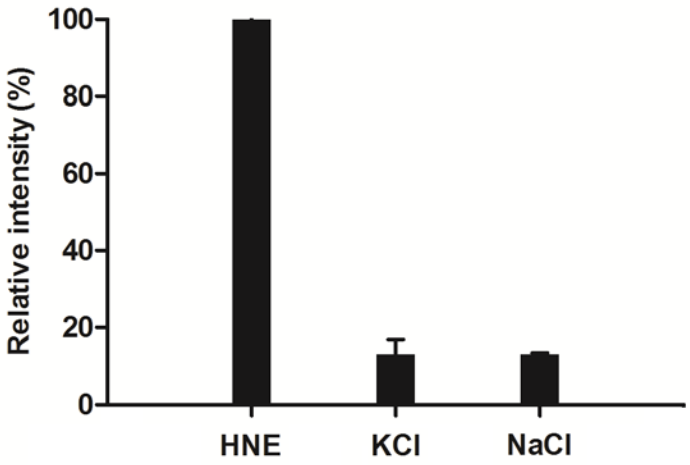


Fig. S7 Relative luminescence intensity of the system ([**1**] = 3 μM, [hybridized HNE aptamer] = 0.5 μM) in the presence of 3 nM HNE or 1 mM KCl or NaCl in Tris buffer (20 mM, pH 7.0). Error bars represent the standard deviations of the results from three independent experiments.



Scheme S1. Schematic representation of the single-stranded aptamer-based approach for the detection of human neutrophil elastase (HNE).

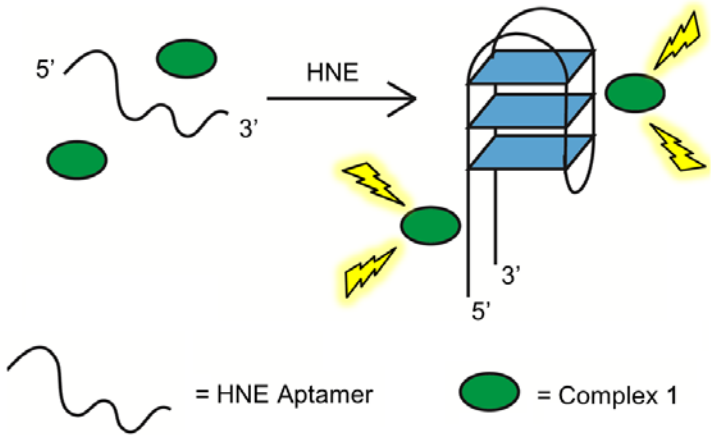


Table S1. Comparison of detection limit and range for the analytical techniques for HNE. References refer to those in the supporting information.

Method	*Detection limit*	*Range*	*Ref.*
Molecular beacon based approach	47 pM	0.34–68 nM	5
InnoZyme™ Human Neutrophil Elastase Immunocapture Activity Assay Kit (EMD Millipore, USA)	10.6 pM	0.0106–0.339 nM	
Human Neutrophil Elastase ELISA Kit (Hycult biotech, Netherlands)	14 pM	0.014–0.86 nM	
Our approach	40 pM	0.04–30 nM	

References

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