

A ¹⁸F Radiolabelled Zn(II) Sensing Fluorescent Probe

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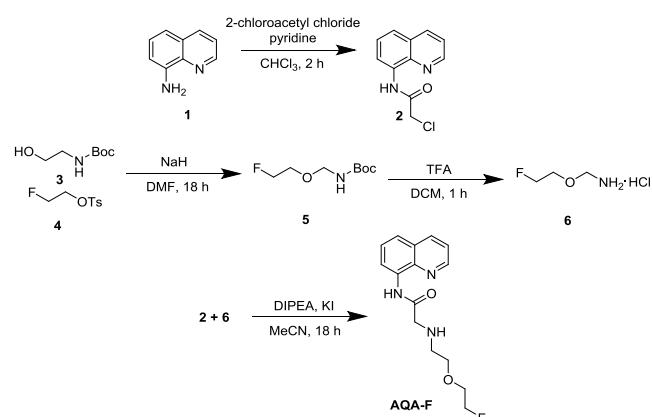
A selective fluorescent probe for Zn(II), AQA-F, has been synthesized. AQA-F exhibits a ratiometric shift in emission of up to 80 nm upon binding Zn(II) ([AQA-F] = 0.1 mM, [Zn(II)Cl₂] = 0–300 μM). An enhancement of quantum yield from Φ = 4.2% to Φ = 35% is also observed. AQA-F has a binding constant, K_d = 15.2 μM with Zn(II). This probe has been shown to respond to endogenous Zn(II) levels in vitro in prostate and prostate cancer cell lines. [¹⁸F]AQA-F has been synthesized with a radiochemical yield of 8.6% and a radiochemical purity of 97% in 88 minutes. AQA-F shows the potential for a dual modal PET/fluorescence imaging probe for Zn(II).

Prostate cancer (PCa) is the 2nd most common cancer worldwide for males, with >161,000 new cases in the US in 2017 (19% of male cancer cases; 10% of total cancer cases).¹ Diagnosis and active surveillance of PCa involves measurement of serum levels of prostate specific antigen (PSA), with invasive biopsies used for determination of the prognostic Gleason Score. Novel, simple diagnostic tests are needed to catch the disease early and this may be achieved by assessing endogenous Zn(II) levels.

Zn(II) has been identified as a key metal in a range of biological functions;^{2,3} its homeostasis is maintained by a number of proteins including Zrt-like Irt-like proteins (ZIP) and cation diffusion facilitators (CDF).⁴ Overabundance of Zn(II) is implicated in a number of diseases (e.g. Alzheimer's disease,⁵ diabetes⁶ and PCa⁷). Zn(II) has emerged as a promising diagnostic target in PCa progression as the homeostasis of Zn(II) is disrupted in diseased tissue.^{8,9}

The largest concentration of Zn(II) is found in the prostate gland (total Zn(II) concentration = 800–1500 μM)¹⁰ where uptake from extracellular fluids is controlled by the Zrt-like Irt-like Protein 1 (ZIP1) transporter.⁷ Mutation of the tumor suppressor gene SLC39A1 (encoding ZIP1) downregulates ZIP1 protein levels in PCa tissue, leading to 62–75% lower levels of Zn(II) accumulation.⁹ This significant decrease in malignant tissue allows for the potential diagnosis of prostate cancer through measurement of the Zn(II) concentration in the prostate.

Over the last two decades, research into probes which give an indication of disease progression by responding to different concentrations of Zn(II) has become a key area of interest.^{11,12,13} A plethora of optical probes for Zn(II) have been developed;^{14–23} typically by modifying organic fluorophores to include Zn(II) sensing units. Modification of fluorescein by addition of dipicolylamine (DPA) units produced a high affinity fluorescence based probe, ZP1.²⁴ Further development led to ZPP1 which demonstrates a ratiometric based detection of zinc with a 10 nm shift in emission.²⁵ Compartmentalisation of zinc in diseases has been highlighted by similar probes.²⁶



Scheme 1: Synthesis of AQA-F

Combining these optical probes with an additional imaging modality can improve the properties of the probe.^{27,28} In particular, the pairing of PET and optical imaging can allow for

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direct cellular validation of the PET probe.^{27,29} Whilst radiolabelling of optical probes is well reported,^{29–33} few examples of “smart” dual modal optical-PET probes are present in the literature.^{31,34,35}

The quinoline-based AQZ probe developed by Zhang *et al.*³⁶ is an organic fluorophore and Zn(II) chelator showing excellent ratiometric fluorescence properties upon Zn(II) binding. AQZ has recently been shown attached to a MRI chelate as a dual-modal probe for diagnosis of diabetes.³⁷ The Zn(II) binding properties of AQZ were retained after functionalization through the terminal alcohol group.

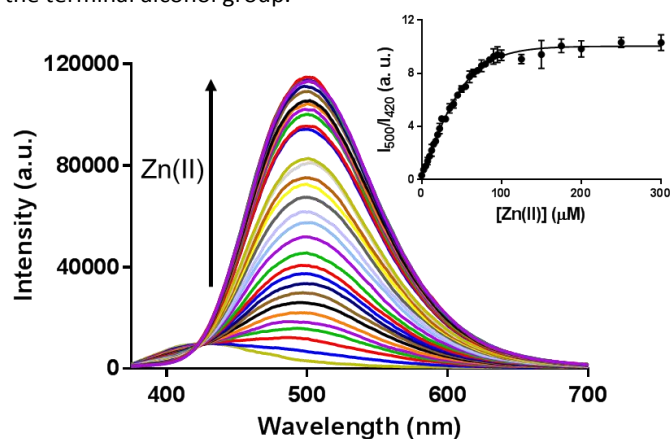


Figure 1. Fluorescence spectra of **AQA-F** (0.1 mM, $\lambda_{ex} = 320$ nm) in HEPES buffer (10 mM, pH 7.68) solution in the presence of increasing concentrations of Zn(II) (0-3 equivalents, 2.5-300 μ M, $n = 3$).

We herein report the development of a fluorinated fluorescent probe, **AQA-F**, which upon binding Zn(II) exhibits a change in emission profile allowing endogenous Zn(II) levels to be monitored. Furthermore, we have undertaken preliminary radiolabeling studies to demonstrate the feasibility of producing [¹⁸F]**AQA-F**. This Zn(II) sensor is designed to set the foundation for a dual modal PET/fluorescence probe for prostate cancer diagnosis.

AQA-F was synthesized in 4 steps from 2-fluoroethanol (Scheme 1). The fluorinated chain 2-(2-fluoroethoxy)ethan-1-amine hydrochloride (**6**) was prepared by condensation of *tert*-butyl (2-hydroxyethyl)carbamate (**3**) with 2-fluoroethyl 4-methylbenzenesulfonate (**4**) under basic conditions to yield **5** followed by deprotection under acidic conditions.³⁸ Conjugation of **6** to 2-chloro-N-(quinol-8-yl)-acetamide (**2**), under the same conditions reported for the synthesis of AQZ,³⁶ yielded **AQA-F** in an 80% yield.

AQA-F demonstrated an excitation maximum of 320 nm and an emission maximum of 420 nm (Figure S4) with quantum yield, $\Phi = 4.2\%$ (Table S4). Upon Zn(II) binding an 8-fold enhancement in fluorescence intensity was observed (Figure 1). This fluorescence enhancement ($\Phi = 35\%$) was also accompanied by a red shift in the emission maxima of 80 nm to 500 nm.

Three isosbestic points at 243, 278 and 323 nm were observed in the absorption spectra for **AQA-F** upon addition of Zn(II) (Figure 2). The ratio of the absorbance at 260 and 278 nm (Inset, Figure 2) highlighted the binding of Zn(II) until the addition of

one equivalent; following this, the ratio began to plateau. This indicates that **AQA-F** binds Zn(II) in a 1:1 molar ratio with a K_d of 15.2 μ M.

A decrease in fluorescence intensity was observed at acidic pH due to protonation of the secondary amine ($pK_a = 6.62$, Figure 3). This protonation also limits Zn(II) binding; there is no increase in fluorescence intensity in the presence of Zn(II) at acidic pH. The fluorescence intensity begins to increase at pH 6 ($pK_a = 6.86$), with the maximum intensity observed in the physiological pH window which is promising for biological

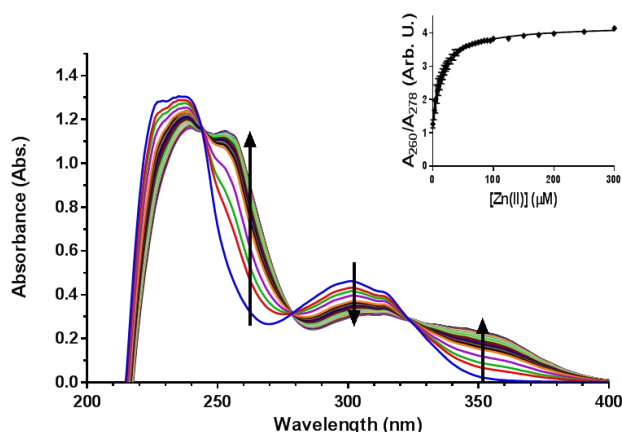


Figure 2. Absorption spectra of **AQA-F** (0.1 mM) in HEPES buffer (10 mM, pH 7.68) in the presence of increasing concentrations of Zn(II) (0-3 equivalents, 2.5-300 μ M, $n = 3$).

applications. Above pH 8.5 a reduction in fluorescence intensity of probe bound to Zn(II) was also observed ($pK_a = 9.68$). This decrease could be a result of a deprotonation of a water molecule bound to the metal centre.³⁹

The fluorescent response of **AQA-F** demonstrates specificity for Zn(II) over other divalent metal ions, especially those found in biological environments (Figure 4). A small enhancement in the emission at 500 nm of **AQA-F** is seen in the presence of Ca(II). A significant increase in fluorescence emission was observed for Zn(II). It must be noted that **AQA-F** has the highest affinity for Zn(II) compared to all of these metals (Table S3, Figure S1).

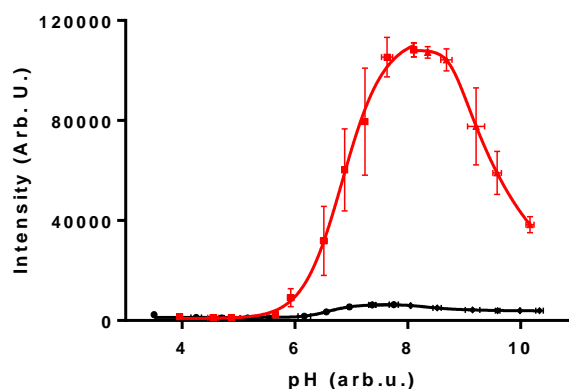


Figure 3. Fluorescence intensity of **AQA-F** (0.1 mM, $\lambda_{ex} = 320$ nm, $\lambda_{em} = 500$ nm) in KCl (0.1 M) solution with variation of

solution pH. Black = **AQA-F**, Red = **AQA-F** + 1 equivalent ZnCl_2 . ($n = 3$)

To validate **AQA-F** *in vitro* it was incubated with a healthy epithelial prostate cell line (RWPE-1) and a grade IV prostate adenocarcinoma cell line (PC-3) (Figure 5, Figure S3). **AQA-F** is internalized by cells following a 30 minute incubation period. The change in the emission profile of **AQA-F** upon binding Zn(II) could be used to assess the relative level of Zn(II) in cells. The ratio of emission at $\lambda_{em} = 500 \text{ nm}$ to $\lambda_{em} = 420 \text{ nm}$ (shown in Figure 5) is directly related to the Zn(II) concentration (Figure 1). This ratio is greater in the healthy prostate cells than in the PC-3 cell line (Table S5), indicating a greater Zn(II) concentration in the RWPE-1 cell line. This reflects the reduced Zn(II) uptake of prostate cancer cells due to the downregulation of the ZIP-1 Zn(II) transporter.^{7,9,40} In both cell lines, the ratio increases when incubated with additional Zn(II) (Table S5).

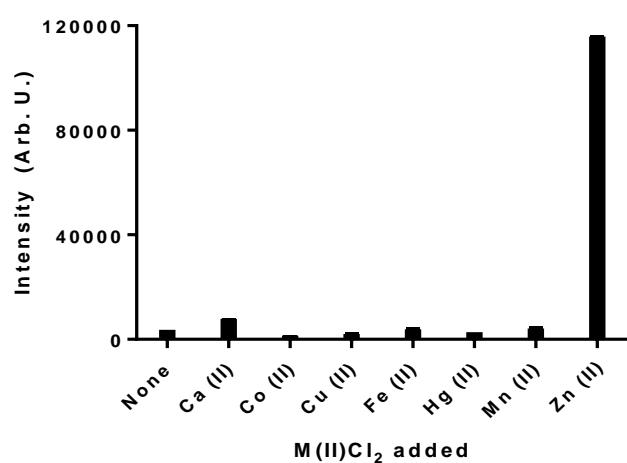
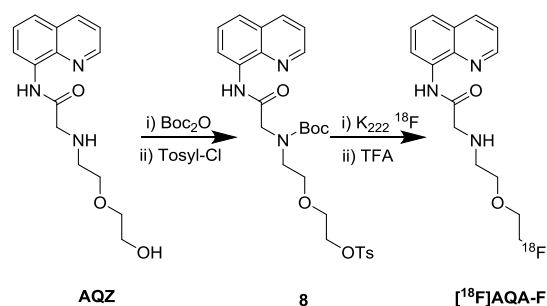


Figure 4. Metal binding assay showing the fluorescence response of **AQA-F** (0.1 mM, $\lambda_{ex} = 320 \text{ nm}$, $\lambda_{em} = 500 \text{ nm}$) in HEPES buffer (10 mM, pH = 7.68) upon addition of 1 equivalent of metal chloride. ($n = 3$)



Scheme 2. Synthesis of $[^{18}\text{F}]\text{AQA-F}$

A preliminary study to produce $[^{18}\text{F}]\text{AQA-F}$ (Scheme 2) was performed in a two-step process from **8**. The first step introduces the ^{18}F isotope through nucleophilic substitution at the terminal tosylate leaving group, with a radiochemical conversion (RCC) of 82.6%, in 15 minutes at $110 \text{ }^\circ\text{C}$ in anhydrous MeCN (Figure S12). Following this, hydrolysis of the Boc

protecting group under acidic conditions gave $[^{18}\text{F}]\text{AQA-F}$ with a 54.3% RCC (Figure S13). The radiochemical purity after purification by semi-preparative HPLC is recorded at 97%, (Figure S10), with an isolated radiochemical yield of 8.6% with a total synthesis time of 88 minutes, thus showing the possibilities for a PET tracer for Zn(II) .

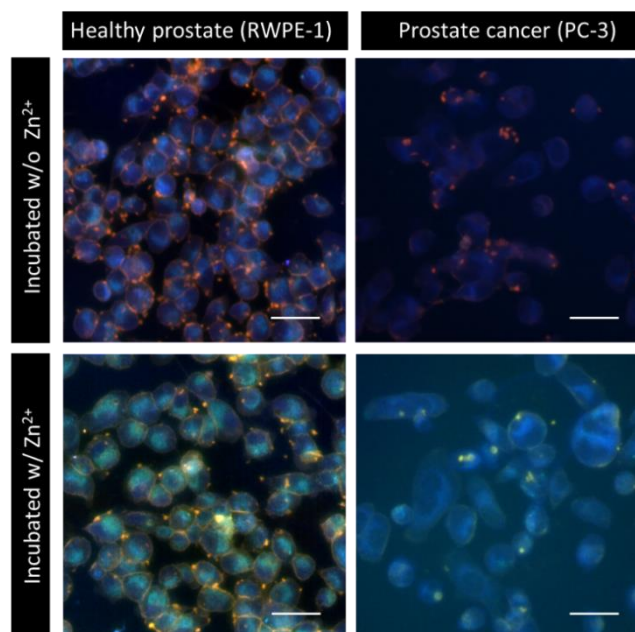


Figure 5. A collection of micrographs showing RWPE-1 and PC-3 cells that have been incubated with $100 \mu\text{M}$ **AQA-F** and Rhodamine Concanavalin A. Scale bar = $50 \mu\text{m}$.

AQA-F demonstrates a red shift of 80 nm from 420 nm to 500 nm when binding Zn(II) . This novel probe has been shown to bind Zn(II) in a 1:1 ratio with the highest affinity of all the metals tested, possessing a K_d of $15.2 \mu\text{M}$. **AQA-F** exhibits a fluorescence maximum at physiological pH and has been shown to be internalized by healthy and cancerous prostate cells within 30 minutes. The shift from 420 nm to 500 nm when binding Zn(II) allows for differentiation of free probe and Zn(II) -bound probe through fluorescent microscopy. $[^{18}\text{F}]\text{AQA-F}$ was radiolabelled in 8.6% RCY with radiochemical purity of 97%, thus showing the potential for a dual modal PET/fluorescence imaging probe for Zn(II) . The fluorescent probe, **AQA-F**, which has the potential for the *in vitro* determination of endogenous Zn(II) levels has been synthesized as both a cold standard and radiolabelled analogue $[^{18}\text{F}]\text{AQA-F}$.

Conflicts of interest

There are no conflicts to declare.

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