Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information (ESI) for Detection of Cystic Fibrosis Transmembrane Conductance Regulator ΔF508 Gene Mutation Using a Paper-Based Nucleic Acid Hybridization Assay and a Smartphone Camera

K. Malhotra, M. O. Noor, U. J. Krull*

Chemical Sensors Group, Department of Chemical and Physical Sciences, University of Toronto Mississauga, 3359 Mississauga Road North, Mississauga Ontario L5L 1C6, Canada. Corresponding author: ulrich.krull@utoronto.ca

1 Materials and Methods

1.1 Reagents and Oligonucleotides

Green-emitting CdS_xSe_{1-x}/ZnS (core/shell) quantum dots (gQD with peak photoluminescence (PL) of 525 nm) capped with oleic acid in toluene were obtained from Cytodiagnostics (Burlington, ON, Canada). Whatman® cellulose chromatography papers (Grade 1, 20 cm x 20 cm), tetramethylammonium hydroxide solution (TMAH, 25% w/w in methanol), L-glutathione (GSH, reduced, ≥98%), sodium (meta)periodate ($NaIO_4$, ≥99%), formamide (F, ≥99.5%), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), chloroform (CH_3Cl, anhydrous ≥99%), granular lithium chloride (LiCl), sodium cyanoborohydride ($NaCNBH_3$, 95%), 1-(3-aminopropyl)imidazole (API, 98%) sodium dodecyl sulfate (SDS, ≥98.5%), and 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES, ≥99.5%) were obtained from Sigma-Aldrich (Oakville, ON, Canada). Buffer solutions were prepared using a water purification system (Milli-Q, 18 $M\Omega cm^{-1}$) and were autoclaved prior to use. Synthetic oligonucleotide sequences were obtained from AGCT Technologies (Toronto, ON, Canada) and Integrated DNA Technologies (IDT, Coralville, Iowa, U.S.A).

1.2 Preparation of GSH-QDs

Green-emitting CdS_xSe_{1-x}/ZnS (core/shell) quantum dots (gQDs, peak PL = 525 nm) capped with oleic acid in toluene were made water-soluble via a ligand exchange reaction with glutathione (GSH). In a typical reaction, 200 mg of L-glutathione was dissolved in 600 µL of tetramethylammonium hydroxide solution (TMAH). The solution of L-glutathione in TMAH was added dropwise to a solution of 0.35 µM organic gQDs dissolved in 2 mL of chloroform. The resulting solution was agitated for 5 minutes using a vortex mixer and stored overnight in darkness at room temperature. Glutathione modified gQDs (GSH-QDs) were extracted into aqueous solution using borate buffer saline solution. 100 µL of 50 mM borate buffer saline (BBS, pH 9.25, 100 mM NaCl) solution was added to the GSH-QD solution and agitated for 1 minute using a vortex mixer. The organic (bottom) layer was discarded and ethanol was added to the aqueous (top) layer at a 1:1 ratio. Ethanol-buffer precipitations were performed as follows; the mixture was centrifuged at 8000 rpm for 5 minutes to obtain a pellet of GSH-QDs. The resulting supernatant was discarded, and the pellet was dissolved in 150 µL of BBS solution. These ethanol-buffer precipitations were done two additional times and the resulting QD pellet was dissolved in 200 µL of 50 mM borate buffer (BB, pH 9.25).[1] The concentration of GSH-QDs was determined using UV-vis absorption spectroscopy and GSH-gQDs (ε = 2.1x10^5) were stored at 4 °C.[2]

1.3 Preparation of QD-probe Oligonucleotide Conjugates

GSH-QDs were conjugated with wild type or mutant type (WT or MT, respectively) oligonucleotide probe strands via self-assembly of oligonucleotide probes terminated with a single or double disulfide (DTPA) moiety at the 5’ terminus. Self-assembly was accomplished via

in-situ reduction of the disulfide moiety of probe strands to dithiol using Tris(2-carboxyethyl)phosphine hydrochloride (TCEP). In a typical reaction, GSH-QDs (400 nM) were incubated with 40 times molar excess of CFTR probes (16.5 μM) and 500 times molar excess of TCEP (8.3 mM) in 50 mM borate buffer saline (BBS, pH 9.25, 100 mM NaCl). The mixture was agitated overnight via an orbital shaker. After overnight incubation, the QD-probe conjugates were subjected to "salt aging". The concentrations of NaCl and TCEP were increased in small increments over a period of 2 hours to 400 mM and 9.7 mM, respectively. The mixture was subsequently shaken overnight using an orbital shaker. The solution containing QD-probe conjugates was used without further purification (unless otherwise stated) and stored at 4 °C.[1]

1.4 Fabrication of Paper Zones and Chemical Modification of Paper with Imidazole

Paper reaction zones were created on 20 cm x 20 cm sheets of Whatman cellulose chromatography paper (Grade 1) using an array pattern designed with AutoCAD 2012 software. The design was an array pattern consisting of 32 circular zones (diameter *ca.* 3 mm) in a 4 by 8 format. The dimensions of the paper sheets containing the reaction zones were 25 mm by 60 mm. Wax printing was done using a Xerox XolorQube 8570DN solid ink printer. Further details regarding paper processing can be found elsewhere.[1,3] After fabrication, wax was melted into the paper by placing paper sheets in an oven at 120 °C for 2 minutes.

Modification of paper was based on a two-step reaction. First, cellulose was oxidized to yield aldehyde groups, and then an imidazole functionality was added via reductive amination.

Oxidation of paper with aldehyde functionality was based on periodate oxidation of cellulose.[1] In a typical procedure, 0.18 g of LiCl and 0.06 g of $NaIO_4$ was dissolved in 6 mL of Milli-Q water and vortexed. Next, the solution was spotted onto paper zones in 5 μL aliquots. Papers were then placed in an oven at 50 °C until dry. Spotting and drying was repeated two more times after which the papers were washed. Washing was accomplished by placing the papers in Milli-Q water and agitating for 2 minutes, after which the papers were dried in a desiccator overnight.

Imidazole functionality was added to the aldehyde modified paper via reductive amination with sodium cyanoborohydride ($NaCNBH_3$). Briefly, a solution of 200 mM $NaCNBH_3$, and 160 mM 1-(3-aminopropyl)imidazole (API), in 100 mM 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES, pH 8.0) buffer was prepared. Next, 2 μL aliquots of the solution were spotted onto the aldehyde modified paper zones and allowed to react at room temperature over an hour.

1.4.1 Note on Troubleshooting Leaking of Paper Zones

A challenge that arose was leakage from hydrophilic zones defined by wax barriers in the paper (Figure S1). To minimize this issue, papers were heated for no more that 2 min at 120°C. In addition to this, previous protocols for paper modification have reported the use of a 10 min wash in BB buffer solution containing 0.1 % sodium dodecyl sulfate (SDS) after spotting with imidazole solution. This step was modified to a BB wash for 10 min.

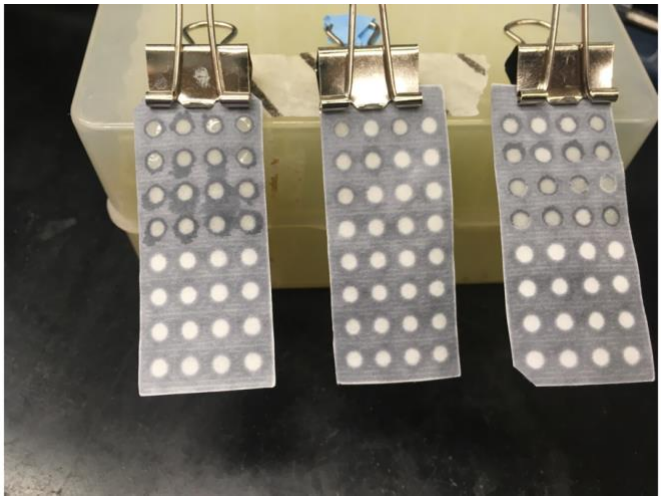

Figure S1. Image of buffer solution leakage from hydrophilic paper zones.

1.5 Solution Phase Hybridization Assays

Solution phase hybridization assays were conducted in triplicate using the direct assay format (Figure 1). For a typical FRET assay, aliquots of 3' Cy3 labelled oligonucleotide targets (CFTR WT Cy3 TGT or CFTR MT Cy3 TGT) were diluted in borate buffered saline solution (BBS, pH 9.25). Next, aliquots of gQD-probe conjugates were added to the solution and incubated at room temperature for 15 minutes before PL measurement were taken.

1.6 Immobilization of QD-Probe Oligonucleotide Conjugates, and Solid Phase Hybridization Assays

Solid phase hybridization assays were conducted in two formats; direct assay and sandwich assay (See Figure 1). For direct assay, first QD-probe conjugates were immobilized on paper zones, dried at room temperature, and then washed with BB for 5 minutes. Next, 3' Cy3 labelled oligonucleotide targets (CFTR WT TGT Cy3 or CFTR MT TGT Cy3) were spotted onto the paper zones and dried at room temperature before washing with BBS for 30 sec. Papers were

then dried for an hour under vacuum before imaging using a smartphone camera. Depending on the desired investigation (i.e. wash conditions for stringency), a further wash step was done followed by drying under vacuum for an hour before imaging with a smartphone. For sandwich based assays, first QD-probe conjugates were immobilized on paper zones, dried at room temperature, and then washed with BB for 5 minutes. Next, oligonucleotide targets (CFTR WT TGT or CFTR MT TGT) were spotted onto the paper zones, dried at room temperature, and then 3' Cy3 labelled reporter sequences were spotted and dried at room temperature before being washed with BBS for 30 sec. Papers were then dried for an hour under vacuum before imaging with a smartphone. Depending on the desired investigation (i.e. wash conditions for stringency), a further wash step was done followed by drying under vacuum for an hour before imaging with a smartphone camera.

1.7 Instrumentation

- Ultraviolet-visible (UV-vis) absorption spectra were obtained using the VWR UV-1600PC Scanning Spectrophotometer (VWR International, Radnor, PA).
- Solution-phase PL intensity measurements were obtained using the QuantaMaster Photon Technology International spectrofluorimeter (London, ON, Canada).
    - The excitation source was a xenon arc lamp (Ushio, Cypress, CA)
    - The detector was a red-sensitive R928P photomultiplier tube (PMT, Hamamatsu, Bridgewater, NJ).
- Digital PL images from paper substrates were acquired using an iPhone SE (Apple, Cupertino, CA, USA) in a dark room. PL images were acquired using the built-in camera

application for the iPhone and the camera software was used with default settings (no alterations were made with respect to exposure time and detector sensitivity). For the collection of all PL images, and to prevent saturation of the detector, a neutral density (ND) filter 16 was placed in front of the iPhone camera.

- Paper substrates were illuminated using at the long wavelength setting (365 nm) for a handheld ultraviolet (UV) lamp (UVGL-58, LW/SW, 6W; The Science Company, Denver, CO, USA).
- Paper substrates were imaged in parallel with a row of four control spots consisting of only immobilized QD-probe conjugates (no target added). Using these control spots as reference, the background R/G ratio could be calculated. Due to dark room imaging, the control sports served as the brightest spots and all software-based image adjustments such as contrast, exposure time and gamma correction etc., were made with respect to these control spots. Hence, consistency of imaging was possible.
- The acquired images were processed using ImageJ software (version 1.49v, National Institutes of Health, Bethesda, MB, USA).

2. Data Analysis

2.1 Förster Formalism of gQD-Cy3 FRET Pair

Solution based measurements were done to determine the Förster distance (R_o) using Equation S1 where n refers to the refractive index of the surrounding medium (in this investigation, a value of 1.33 was used), κ^2refers to the orientation factor (in this investigation, a random orientation was assumed and a value of 2/3 was assigned). The quantum yield (QY, Φ_D) of glutathione modified green quantum dots (GSH-gQDs) was taken to be 65% (previously reported[4]). Finally, the spectral overlap interval (J) was determined using Equation S2.

$$R_o^6 = 8.79\ x\ 10^{-28} mol\ x\ (n^{-4}\kappa^2\Phi_D J) \qquad \textbf{Equation S1}$$

In Equation S2, F_D is the fluorescence intensity associated with the donor as a function of wavelength (λ), ε_A is the molar extinction coefficient associated with the FRET acceptor as a function of λ.

$$J = \frac{\int F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda}{\int F_D(\lambda) d\lambda} \qquad \textbf{Equation S2}$$

The Förster distance was calculated to be 4.7 nm. The normalized absorption and emission spectra for the gQD-Cy3 FRET pair are shown in Figure S2 to provide a descriptive means of expressing the spectral overlap of the FRET pair.

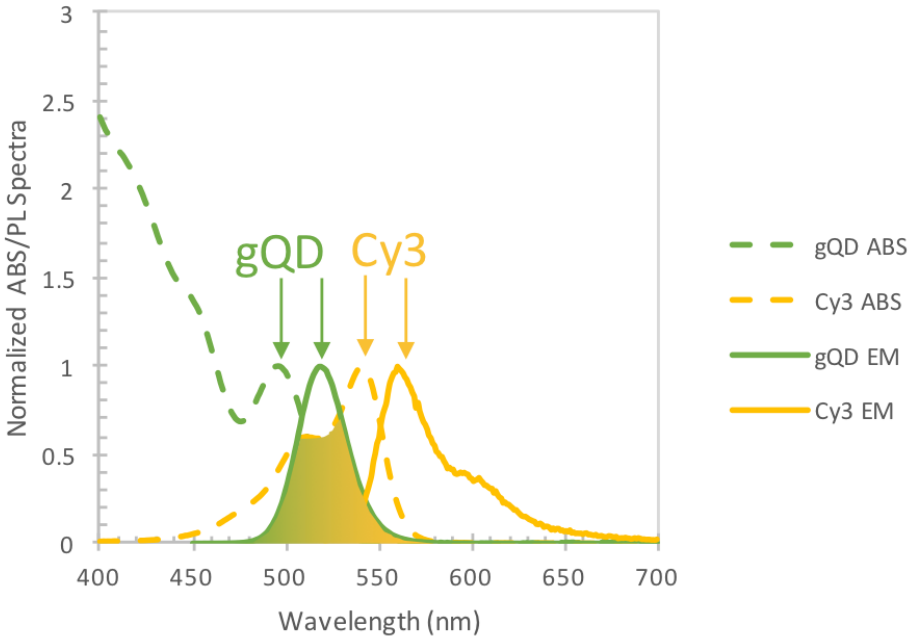

Figure S2. Normalized absorption and emission spectra for the gQD and Cy3 FRET pair. The spectral overlap is represented by the shaded region. Absorption is shown as dashed lines and emission is shown as solid lines.

2.2 Hybridization Assays in Solution by Fluorimetric Detection

Investigation of the FRET pair in solution was accomplished via spectral analysis to obtain a ratiometric value for the FRET interaction. To accomplish this, normalized and background corrected spectra were mathematically processed via Equation S3 to obtain a ratio corresponding to the energy transfer process.

$$R_{PL} = \left(\frac{\sum_{\lambda=560}^{590} PL(\lambda)}{\sum_{\lambda=510}^{540} PL(\lambda)}\right)_{DA} - \left(\frac{\sum_{\lambda=560}^{590} PL(\lambda)}{\sum_{\lambda=510}^{540} PL(\lambda)}\right)_{D} \qquad \textbf{Equation S3}$$

Background correction was accomplished using Equation S3, where the wavelength range of 560 nm to 590 nm corresponded to the emission spectra of Cy3 dye, and the wavelength range of 510 nm to 540 nm corresponded to the emission spectra of gQDs. A ratio of the Cy3 emission to gQD emission was taken for samples containing both donor and acceptor (i.e. subscript DA for

donor-acceptor) and the background donor emission was subtracted (i.e. subscript D for donor). The ratios obtained from this processing were further averaged using three measurements in total.

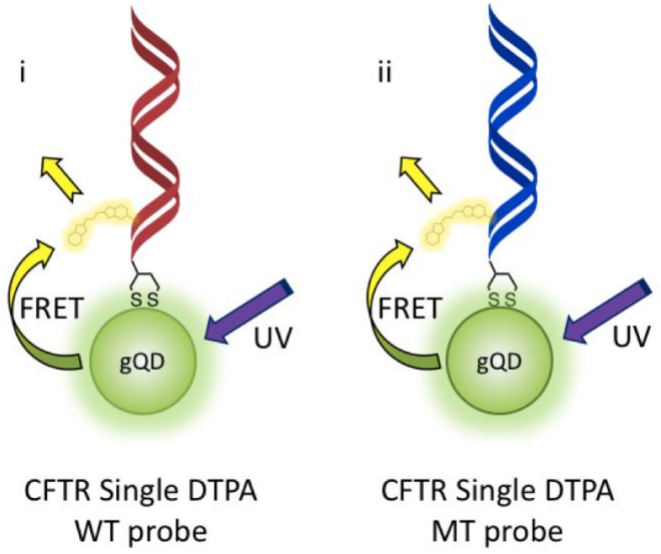

Figure S3. Representations of the two different direct assay formats investigated in solution phase. gQDs were modified with i) CFTR Single DTPA WT probe, ii) CFTR Single DTPA MT probe, and were mixed with complementary CFTR WT Cy3 target strands and CFTR MT Cy3 target strands. Hybridization resulted in proximity of gQDs and Cy3, which resulted in FRET.

A range of stoichiometric concentrations for gQDs-probe conjugates and targets were investigated to obtain concentration-response curves for the different gQD-probe conjugates. In total, two different types of conjugates were investigated in solution including, gQD-WT probe conjugates and gQD-MT probe conjugates (shown visually as Figure S3i and ii, respectively). The response curves generated for the two conjugates are shown in Figure S4Ai to Figure S4Aii. For each of the conjugates, hybridization of two different types of targets were investigated. Data points shown in orange correspond to CFTR MT Cy3 TGTs and data points shown in blue correspond to CFTR WT Cy3 TGTs. For gQD-probe conjugates with WT probes, the FRET signals

for CFTR WT Cy3 TGTs (fully complementary, FC) were expected to be greater than that for CFTR MT Cy3 TGTs (partially complementary, PC) due to formation of more stable oligonucleotide hybrids. Similar results were also expected for gQD-probe conjugates with MT probes (i.e. greater FRET signals from samples of FC hybrids vs. PC hybrids).

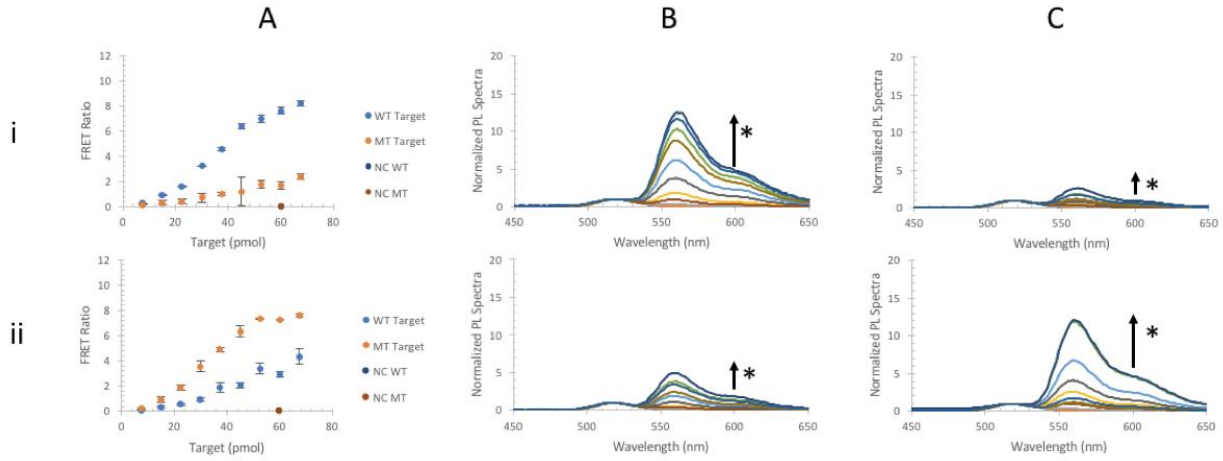

Figure S4. Hybridization of the gQD-probe strands were investigated in solution by fluorescence spectroscopy. gQD-probe conjugates with i) CFTR single DTPA WT probe, ii) CFTR single DTPA MT probe were hybridized with CFTR Cy3 WT and CFTR Cy3 MT target strands. The concentration-response curves for the different gQD-probe conjugates are shown A. WT Cy3 labelled target strands are seen in blue and MT Cy3 labelled target strands are seen in orange. Normalized PL spectra for the calibration curves are shown for B) CFTR WT Cy3 labelled target strands and C) CFTR MT Cy3 labelled target strands (* indicates increasing target concentration).

2.3 Hybridization Assays in Paper Substrates Using Smartphone Detection

Investigation of the fluorescence response caused by hybridization within paper substrates was accomplished by image analysis to obtain a ratiometric value for the FRET process.

$$R_{PL} = \left(\frac{EM_{Red}}{EM_{Green}}\right)_{DA} - \left(\frac{EM_{Red}}{EM_{Green}}\right)_{D} \qquad \textbf{Equation S4}$$

Background correction was accomplished using Equation S4, where the intensity of signal in the paper zone for the red color channel (i.e. EM_{Red}) corresponded to emission of Cy3 and the intensity of signal for the green color channel (i.e. EM_{Green}) corresponded to emission of gQD. A ratio of the Cy3 emission to gQD emission was taken for samples containing both donor and acceptor (i.e. subscript DA for donor-acceptor) and the background donor emission was subtracted (i.e. subscript D for donor) for each sample spot. The data was further processed by obtaining an average value of four background corrected paper zones for each sample concentration (example of image processing provided as Figure S5).

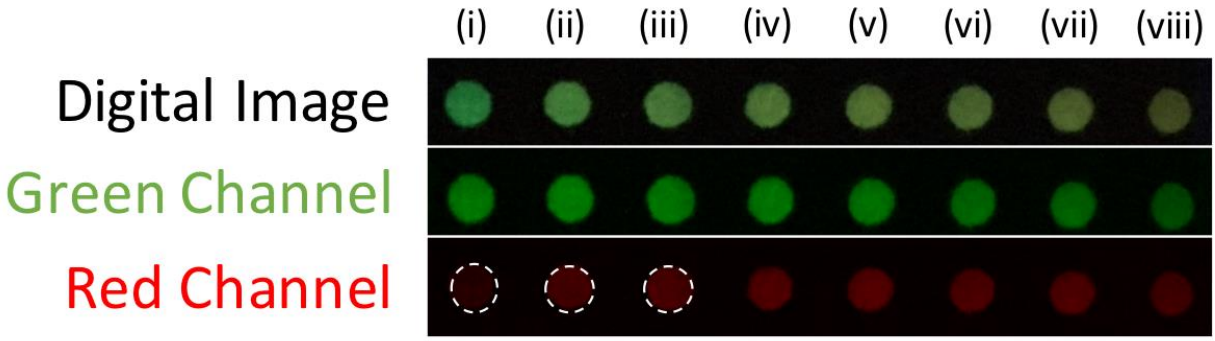

Figure S5. Digital smartphone image and the accompanying post-processing PL images (post processing included R-G-B color splitting yielding pseudocolored images), gQD-WT probe conjugates with green channel (gQDs) and red channel (Cy3) for varying concentrations of Cy3 labelled target; (i) 0 pmol, (ii) 2.4 pmol (iii) 3 pmol (iv) 3.9 pmol (v) 4.8 pmol (vi) 6 pmol (vii) 7.5 pmol (viii) 9 pmol of CFTR Cy3 TGT. The white dashed circle indicates locations of spots that may not be visible otherwise.

2.4 Optimization of Wash Conditions for Direct and Sandwich Based Assay by Smartphone Imaging

2.4.1 Labelled Target (Direct Format)

To determine the optimized conditions of stringency required to achieve selectivity for the fully complementary oligonucleotide hybrids, wash conditions were explored where

selectivity was controlled as a function of time and added formamide (%v/v). The resulting ratiometric values were summarized in Table S1 for gQD-WT probe – WT Target hybrids, Table S2 for gQD-WT probe – MT Target hybrids, Table S3 for gQD-MT probe – MT Target hybrids, and Table S4 for gQD-MT Probe – WT Target hybrids. Based on the predicted energies of hybridization (Figure 3 and 4), FC hybrids were expected to be more stable and to retain more signal under stringent wash conditions than PC hybrids.

For WT probe, the wash condition that offered the greatest signal for FC hybrids (Table S1) and the least signal for PC hybrids (Table S2, i.e. within noise) was chosen as the wash condition to continue further investigations. Similarly, for MT probe, the wash conditions offering the greatest signal for FC hybrids (Table S3) and the least signal for PC hybrids (Table S4, i.e. within noise) was chosen as the wash condition to continue further investigations.

For WT probe, the wash conditions meeting the criteria for mismatch discrimination were BB+5% formamide at 10 min, and BB+10% formamide at 5 and 10 min. For MT probe, the wash conditions meeting the criteria for mismatch discrimination were BB+5% formamide at 10 min, BB+7.5% formamide at 10 min, and BB+10% formamide at 5 and 10 min.

Table S1. Summary of R/G Ratiometric Signals for gQD-WT probe – WT Target hybrids

WT Probe - WT Target R/G Ratio Signal		BB+X% Wash Times (minutes)		
		0	5	10
Amount of Formamide Added (% v/v)	0	1.08 ± 0.03	1.01 ± 0.03	0.94 ± 0.02
	5	1.05 ± 0.03	0.96 ± 0.03	0.79 ± 0.02
	7.5	1.02 ± 0.02	0.81 ± 0.03	0.80 ± 0.02
	10	0.99 ± 0.01	0.7 ± 0.1	0.5 ± 0.1

Table S2. Summary of R/G Ratiometric Signals for gQD-WT probe – MT Target hybrids

WT Probe - MT Target R/G Ratio Signal		BB+X% Wash Times (minutes)		
		0	5	10
Amount of Formamide Added (% v/v)	0	0.98± 0.02	0.20 ± 0.04	0.10 ± 0.01
	5	0.92 ± 0.03	0.13 ± 0.02	0.00 ± 0.02
	7.5	0.96 ± 0.02	0.12 ± 0.03	0.10 ± 0.02
	10	0.93 ± 0.03	0.05 ± 0.01	0.02 ± 0.01

Table S3. Summary of R/G Ratiometric Signals for gQD-MT probe – MT Target hybrids

MT Probe - MT Target R/G Ratio Signal		BB+X% Wash Times (minutes)		
		0	5	10
Amount of Formamide Added (% v/v)	0	0.91 ± 0.05	1.04 ± 0.05	1.03 ± 0.02
	5	0.87 ± 0.06	0.90 ± 0.01	0.68 ± 0.01
	7.5	1.03 ± 0.03	0.91 ± 0.02	0.81 ± 0.03
	10	1.01 ± 0.03	0.78 ± 0.03	0.62 ± 0.03

Table S4. Summary of R/G Ratiometric Signals for gQD-MT probe – WT Target hybrids

MT Probe - WT Target R/G Ratio Signal		BB+X% Wash Times (minutes)		
		0	5	10
Amount of Formamide Added (% v/v)	0	0.87 ± 0.02	0.22 ± 0.02	0.11 ± 0.01
	5	0.86 ± 0.03	0.08 ± 0.03	0.05 ± 0.02
	7.5	1.00 ± 0.03	0.07 ± 0.01	0.05 ± 0.02
	10	0.95 ± 0.04	0.07 ± 0.01	0.04 ± 0.01

2.4.2 Target Determination by Sandwich Assay

The process for determining the optimal wash conditions for sandwich assays was similar to that used for direct assays. The relevant ratiometric values of signals are summarized in Table S5 for gQD-WT probe – WT Target hybrids, Table S6 for gQD-WT probe – MT Target hybrids, Table S7 for gQD-MT probe – MT Target hybrids, and Table S8 for gQD-MT Probe – WT Target hybrids. FC hybrids were expected to be more stable and to retain more signal under stringent wash conditions than PC hybrids. It is important to note that the gQD-MT probe – WT Target hybrid had a much larger ΔG_{max} than the other PC hybrids. Thus, it was expected to require more

stringent wash conditions to achieve discrimination of FC from PC sequences. As with direct assay, discrimination of the FC hybrids from the PC hybrids required wash conditions where ratiometric signal from FC hybrids was present and signal from PC hybrids was within the noise of the detector. Thus, for WT probe, the wash condition offering the greatest signal for FC hybrids (Table S5) and the least signal for PC hybrids (Table S6, i.e. within noise) was chosen as the optimal wash condition to continue further investigations. The wash conditions offering the greatest signal for FC hybrids (Table S7) and the least signal for PC hybrids (Table S8, i.e. within noise) was chosen as the optimal wash condition to continue further investigations.

For MT probe, the wash conditions meeting the criteria for mismatch discrimination are more limited than those for WT probe due to the stability of the PC hybrid (see the thermodynamic treatment of the hybrids in the main article). Of the various wash conditions, BB+5% formamide at 20 min wash, BB+7.5% formamide at 20 min, and BB+10% Formamide at 5, 10, 15 and 20 min meet the criteria for the assays. Of the different wash conditions for MT probe, only BB+5% formamide at 20 min met all the criteria because the BB+7.5% formamide and BB+10% formamide washes were rejected for WT Probe. Thus, BB+5% formamide at 5 min for gQD-WT probes and 20 min wash for gQD-MT probes was chosen for further characterization of the figures of merit for the assays.

Table S5 Summary of R/G Ratiometric Signal for gQD-WT probe – WT Target hybrids

WT Probe - WT Target R/G Ratio Signal		BB+X% Wash Times (minutes)				
		0	5	10	15	20
Amount of Formamide Added (% v/v)	0	0.62 ± 0.05	0.46 ± 0.02	0.40 ± 0.01	0.40 ± 0.02	0.37 ± 0.04
	1.25	0.697±0.006	0.62 ± 0.02	0.60 ± 0.01	0.53 ± 0.03	0.42 ± 0.06
	2.5	0.74 ± 0.01	0.63 ± 0.03	0.61 ± 0.01	0.46 ± 0.03	0.40 ± 0.02
	3.75	0.67 ± 0.03	0.56 ± 0.02	0.50 ± 0.03	0.43 ± 0.01	0.32 ± 0.03
	5	0.62 ± 0.04	0.33 ± 0.06	0.28 ± 0.03	0.26 ± 0.02	0.20 ± 0.04
	7.5	0.63 ± 0.02	0.19 ± 0.06	0.12 ± 0.04	0.13 ± 0.04	0.06 ± 0.02
	10	0.52 ± 0.06	0.02 ± 0.04	0.02 ± 0.03	0.01 ± 0.03	0.00 ± 0.04

Table S6 Summary of R/G Ratiometric Signal for gQD-WT probe – MT Target hybrids

WT Probe - MT Target R/G Ratio Signal		BB+X% Wash Times (minutes)				
		0	5	10	15	20
Amount of Formamide Added (% v/v)	0	0.51 ± 0.06	0.02 ± 0.04	0.02 ± 0.06	0.04 ± 0.04	0.02 ± 0.03
	1.25	0.59 ± 0.02	0.07 ± 0.04	0.00 ± 0.02	0.01 ± 0.01	-0.01 ± 0.04
	2.5	0.62 ± 0.07	0.07 ± 0.05	0.03 ± 0.03	0.01 ± 0.04	0.02 ± 0.04
	3.75	0.54 ± 0.04	0.03 ± 0.02	0.01 ± 0.03	0.00 ± 0.02	-0.01 ± 0.01
	5	0.48 ± 0.02	-0.02 ± 0.04	0.00 ± 0.02	0.00 ± 0.03	-0.01 ± 0.06
	7.5	0.43 ± 0.07	-0.01 ± 0.03	0.00 ± 0.05	0.02 ± 0.02	-0.03 ± 0.02
	10	0.37 ± 0.05	-0.05 ± 0.03	-0.04 ± 0.04	-0.04 ± 0.03	-0.02 ± 0.04

Table S7 Summary of R/G Ratiometric Signal for gQD-MT probe – MT Target hybrids

MT Probe - MT Target R/G Ratio Signal		BB+X% Wash Times (minutes)				
		0	5	10	15	20
Amount of Formamide Added (% v/v)	0	0.65 ± 0.01	0.58 ± 0.07	0.58 ± 0.06	0.6 ± 0.1	0.55 ± 0.04
	1.25	0.80 ± 0.08	0.76 ± 0.03	0.79 ± 0.05	0.71 ± 0.05	0.69 ± 0.04
	2.5	0.7 ± 0.1	0.67 ± 0.06	0.71 ± 0.08	0.55 ± 0.06	0.56 ± 0.08
	3.75	0.77 ± 0.07	0.71 ± 0.04	0.73 ± 0.03	0.70 ± 0.05	0.59 ± 0.06
	5	0.72 ± 0.08	0.57 ± 0.06	0.49 ± 0.08	0.46 ± 0.07	0.37 ± 0.08
	7.5	0.71 ± 0.02	0.44 ± 0.04	0.35 ± 0.03	0.26 ± 0.02	0.21 ± 0.04
	10	0.73 ± 0.02	0.21 ± 0.05	0.1 ± 0.02	0.11 ± 0.04	0.10 ± 0.04

Table S8 Summary of R/G Ratiometric Signal for gQD-MT probe – WT Target hybrids

MT Probe - WT Target R/G Ratio Signal		BB+X% Wash Times (minutes)				
		0	5	10	15	20
Amount of Formamide Added (% v/v)	0	0.6 ± 0.1	0.44 ± 0.08	0.4 ± 0.01	0.4 ± 0.1	0.36 ± 0.06
	1.25	0.74 ± 0.05	0.68 ± 0.05	0.63 ± 0.04	0.46 ± 0.03	0.39 ± 0.01
	2.5	0.7 ± 0.1	0.63 ± 0.08	0.58 ± 0.08	0.37 ± 0.05	0.28 ± 0.06
	3.75	0.72 ± 0.02	0.62 ± 0.02	0.52 ± 0.02	0.33 ± 0.03	0.20 ± 0.03
	5	0.7 ± 0.1	0.19 ± 0.02	0.16 ± 0.04	0.15 ± 0.02	0.06 ± 0.05
	7.5	0.71 ± 0.01	0.11 ± 0.03	0.10 ± 0.04	0.06 ± 0.01	0.03 ± 0.03
	10	0.70 ± 0.01	0.04 ± 0.02	0.02 ± 0.01	0.04 ± 0.01	0.03 ± 0.03

2.4.3 Blind Assay for Detection and Quantification of CFTR Target Mixes

The performances of the direct and sandwich assays were investigated with a blind experiment with samples containing WT only, MT only, and mix of WT and MT targets. Samples were prepared in BBS buffer with a final concentration of 3.0 pmol for direct assay and 7.5 pmol for sandwich assay. Ratiometric signal was measured pre-and post-wash for sample identification. Signal from the assays and subsequent identification of samples were found to be in agreement, supporting applicability for clinical application (Table 9).

Table 9. Blind Assay for Direct and Sandwich Assays

Assay Format	Blind Sample	Spiked Samples	Signal		Sample Identification
			WT assay	MT assay	
Direct Assay	1	WT only	0.54 ± 0.03	0.02 ± 0.02	WT
	2	WT and MT	0.49 ± 0.01	0.58 ± 0.04	Mix
	3	MT only	0.00 ± 0.02	0.65 ± 0.06	MT
	4	MT only	0.01 ± 0.03	0.43 ± 0.02	MT
Sandwich Assay	1	MT only	0.02 ± 0.01	0.55 ± 0.03	MT
	2	WT and MT	0.24 ± 0.03	0.43 ± 0.03	Mix
	3	WT and MT	0.25 ± 0.02	0.40 ± 0.01	Mix
	4	MT only	0.03 ± 0.02	0.35 ± 0.05	MT

3. References

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