Electronic Supplementary Material (ESI) for Nanoscale.

Towards Label-free, Wash-free and Quantitative B-type Natriuretic Peptide Detection for Heart Failure Diagnosis

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Supporting Information

1. Chemicals and Materials

Iron(III) acetylacetonate (51003), molybdenum(II) acetate (2320761), cobalt(II) acetate (3999731), fumed silica (7 nm in diameter; S5130), BNP (B5900), bovine serum albumin (BSA; A2153), Trizma base (T6066), hydrochloric acid (258148), phosphate buffer solution (P3619), 4-morpholineethanesulfonic acid (MES; M8250), and StabilCoat® Immunoassay Stabilizer (S0950) were all purchased from Sigma Aldrich. Phosphate buffered saline (PBS) buffer (10×) was purchased from VWR LLC. Hydrofluoric acid (HF; HX0621) was obtained from EMD. Silicon wafer (P/Boron dopant, 20000 angstroms thermal oxide) was from Silicon Valley Microelectronics. Compressed hydrogen and argon were from Praxair. Polyethylene terephthalate (PET; Melinex 329/1000, 10 mm thickness) was purchased from Tekra. Removable vinyl sticker (3 mm thickness) was purchased from Digital Graphic Inc./Sign Supply Canada. 3M optically clear adhesive (Type 8213, 3 mm thickness) was from 3M. Poly(methyl methacrylate) sheet (PMMA; 4.75 mm thickness) was obtained from McMaster-Carr. Antibody 50E1 was obtained from Abcam, while 24E11 and 24C5-biotin were obtained from Novus Biologicals. NT-proBNP, and BNP-free Plasma (BNFP) were obtained from Hytest Ltd. Patient plasma was purchased from BioreclamationIVT. COOH-coated polystyrene plates were purchased from Greiner-Bioworld. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was purchased from GBiosciences. N-Hydroxysuccinimide (NHS) was purchased from Alfa Aesar. Eu-Streptavidin and DELFIA® Enhancement Solution was purchased from Perkin Elmer.

2. CNT-TF synthesis

Briefly, a cocktail of metal catalysts including iron(III) acetylacetonate (3.0 mg), molybdenum(II) acetate (0.75 mg) and cobalt(II) acetate (4.6 mg) was sonicated with silica fume nanoparticles (50 mg) in ethanol (10 mL, anhydrous) for two hours. The mixture was then spin-coated at 2500 rpm for 20 seconds on a 2 × 3 cm^2 rectangular

piece of SiO_2/Si wafer (pre-cleaned with piranha solution). The wafer was transferred into a 2" CVD quartz tube furnace and heated to 850ºC under a constant flow of carrier gas mixture (600 sccm argon and 18 sccm hydrogen). After the temperature stabilized at 850ºC, the carrier gas flow was switched to bubble through ethanol (0ºC) to introduce ethanol vapor into the furnace for 20 minutes. Then the carrier gas was switched to bypass the ethanol bubbler and the CVD system was cooled down to room temperature.

3. NanoBot strip fabrication

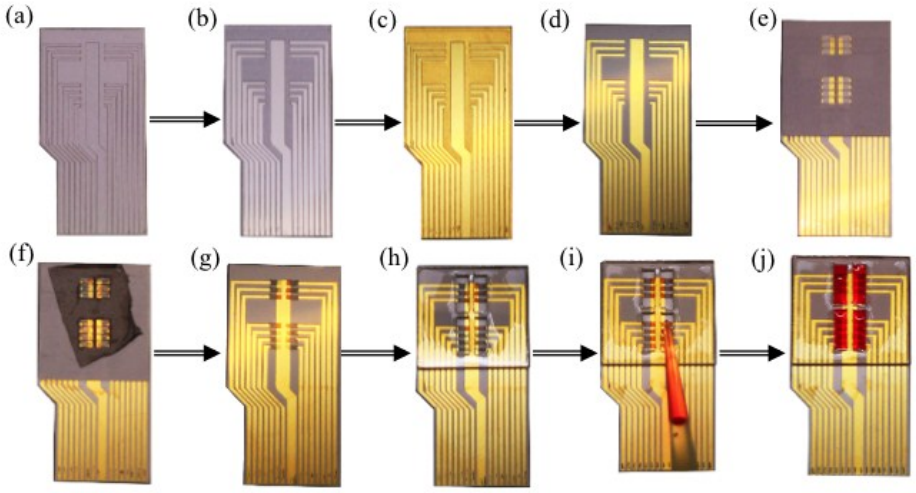

Figure S1. Optical images that corresponds to the schematic illustration in Figure 1B.

4. CNT-TF characterization

For Raman and AFM, the CNT-TF grown on a SiO_2/Si substrate was immersed in 1% HF for 1 min and lifted off in a water bath,[25] before being transferred onto a clean silica wafer. Raman spectra with a wavenumber range between 1000 cm^{-1} to 1800 cm^{-1} were acquired using a Horiba Jobin Yvon LabRAM HR 800 Raman spectrometer with a 532 nm excitation laser. AFM images were collected using a Nanoscope MultiMode™ AFM instrument (Veeco) in tapping mode with a silicon probe tip at a resonant frequency of 300 kHz. For SEM characterization, the grown CNT-TF was transferred onto gold-coated PET and mounted on an SEM sample stage. Images were taken using a Zeiss ULTRA Plus scanning electron microscope with a 10 kV accelerating voltage under 100 kX magnification. CNT-TF was transferred onto a silicon oxide wafer and imaged with a

Zeiss UltraPlus scanning electron microscope, illustrating the percolation of nanotubes in a film. The high tube density in the CNT-TF can be observed from the SEM image.

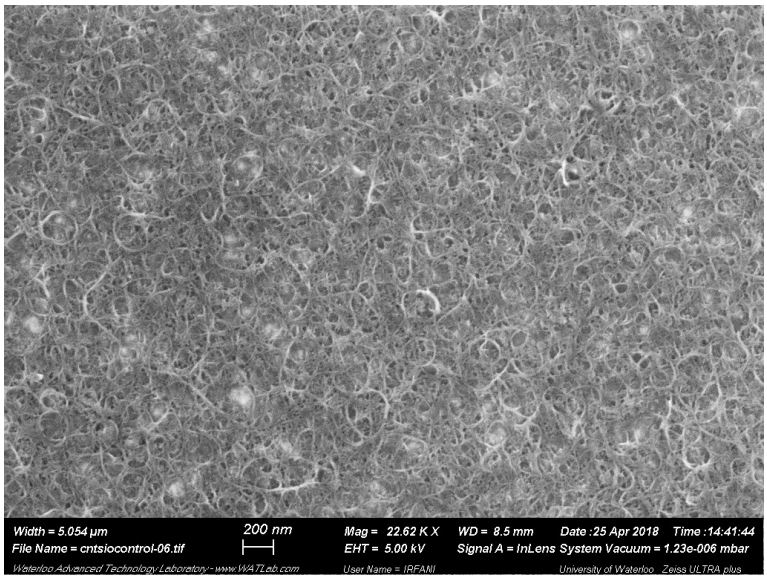

Figure S2. SEM image of a CNT-TF on a silica piece.

5. BNP and NT-proBNP fluorescent immunoassay (FIA)

Capture antibody (50E1) was covalently immobilized onto COOH-coated polystyrene plates through EDC-NHS chemistry. The capture antibody at a concentration of 66.9 nM in phosphate buffer (0.1 M, pH 7.4) was mixed with 2 mM EDC and 5 mM NHS both in MES buffer (0.05 M, pH 6) for 2 hours at room temperature. After washing with PBS-Tween (PBS-T) three times, the wells were blocked with 5% BSA overnight at 4^{o}C. The next day, the wells were washed twice with PBS-T. Standard BNP 10× concentrations were prepared in BNFP at 0, 5, 7.5, and 10 ng/mL. BNP samples diluted in BNFP containing 6.66 nM 24C5-biotin detection antibody were prepared at a concentration of 50, 100, 250, 500, 750, and 1000 pg/mL. Each sample was then added to the wells at 100 µL/well and incubated for one hour at room temperature. The wells were washed with 20 mM Tris-HCl buffer three times and incubated with 56 nM Eu-Streptavidin diluted in 50 mM Tris-HCl for 30 minutes at room temperature. The wells were then washed with 50 mM Tris-HCl and incubated with DELFIA® enhancement solution for 1 hour at room

temperature. Fluorescent signals were recorded using Spectramax M2e on the time-resolved fluorescence setting with 340 nm excitation and 615 nm detection wavelengths. To retrieve the calibration curve of NT-proBNP, the same protocol was implemented with NT-proBNP samples; however, 24E11 was used as the capture antibody and 5B6-biotin was used as the detection antibody. The NT-proBNP samples were prepared at the concentrations of 500, 1000, 2000, and 4000 pg/mL.

6. Dissociation constant of 50E1-BNP

In antibody-antigen complexes, the dissociation constant (K_D) illustrates the binding strength or affinity of the antibody to its antigen at equilibrium given by the following equation:

$$K_D = \frac{[A][B]}{[AB]} = \frac{k_{dissociation}}{k_{association}}$$

At equilibrium, the ratio of the dissociation and association rates is equal to K_D. Studies have demonstrated that K_D can be estimated when the receptor is at 50% occupancy.[33] Through the 50E1-BNP binding isotherm acquired by FIA, we have determined that the K_D for 50E1 is 3.59 nM, which is consistent with the affinity constants of antibodies in literature. This data illustrates that 50E1 has a strong affinity to the BNP antigen.[27]

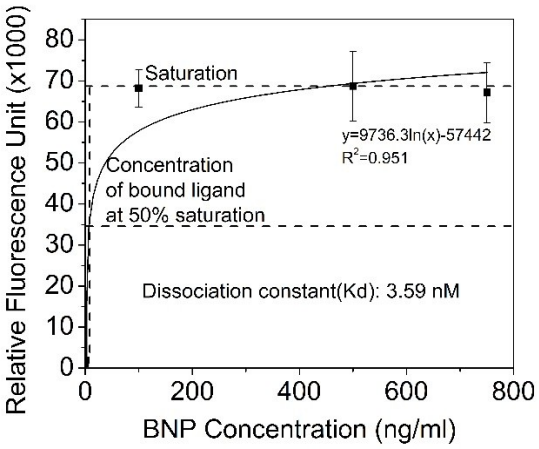

Figure S3. 50E1-BNP binding isotherm for the determination of K_D.

7. NanoBot reader block diagram

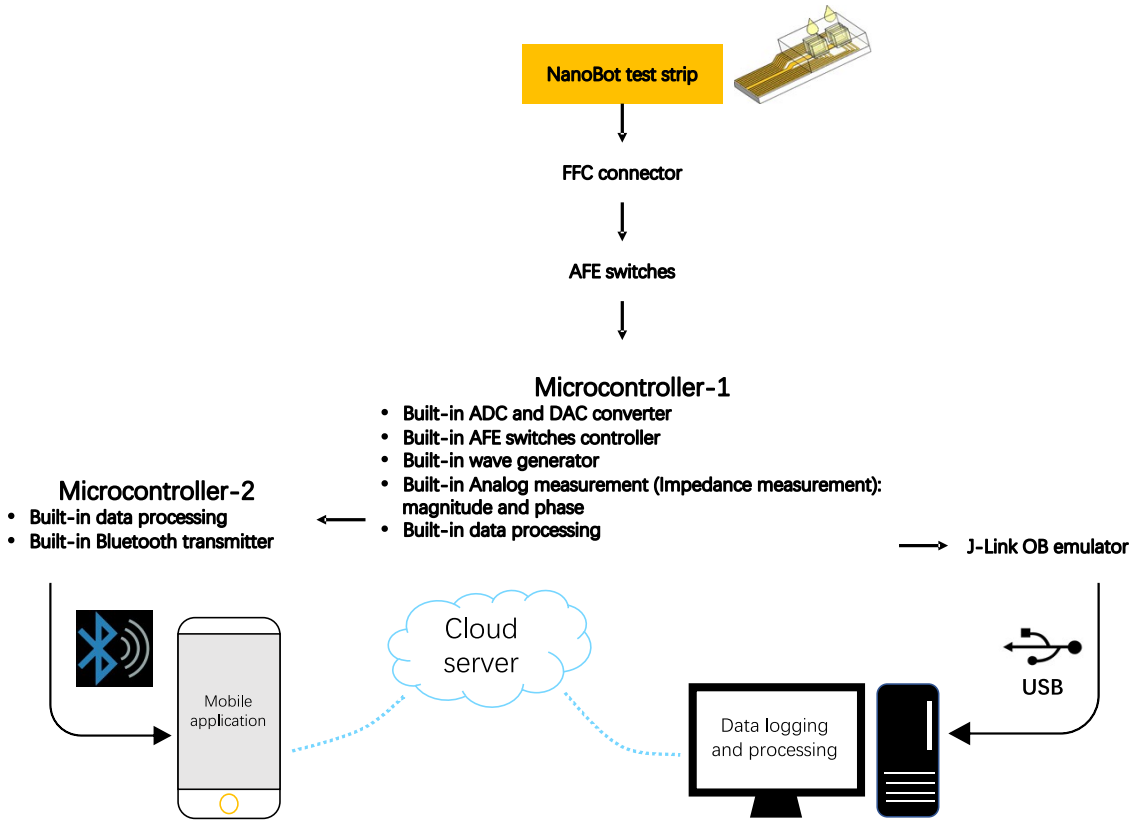

Figure S4. Block diagram of the NanoBot reader.

The readout unit is designed to imitate the AC impedance function of a standard bench-top potentiostat such as CHI650A, which can be used to apply a stable AC potential (less than 20 mV) across the CNT thin film bridging two electrodes and measure the impedance and phase values in a range of frequencies.

8. NanoBot and FIA Calibration Curves

Figures S5a and S5b depict the NanoBot and FIA calibration curves, respectively. A calibration curve is usually established as a standard curve to determine the concentration of a substance in an unknown sample. In our case, the NanoBot calibration curve for BNP had large error bars. The observed large variations could be due to the differences of the synthesized CNT-TFs such as different thickness, purity and defects. These can cause large signal variations for the same tested BNP sample. Furthermore, plasma proteins, which have different protein compositions for each individual, can adsorb onto the CNT-TF surface and cause variations in the slopes and intercepts of the NanoBot strip

calibration curve. Consequently, the calibration curve method was determined to be unreliable for the NanoBot and therefore could not be used (Fig. S5a). To circumvent this issue, we employed the standard addition method on the NanoBot strips to eliminate the variations caused by the different batches of NanoBot strips and human plasma. Variations between individual plate calibration curves were also observed in the FIA due to the different batches of plates and human plasma. However, the standard deviations observed in the FIA's standard calibration curve (Fig. S5b) were not as significant as those observed in the NanoBot system and thus were used to determine the unknown BNP concentrations.

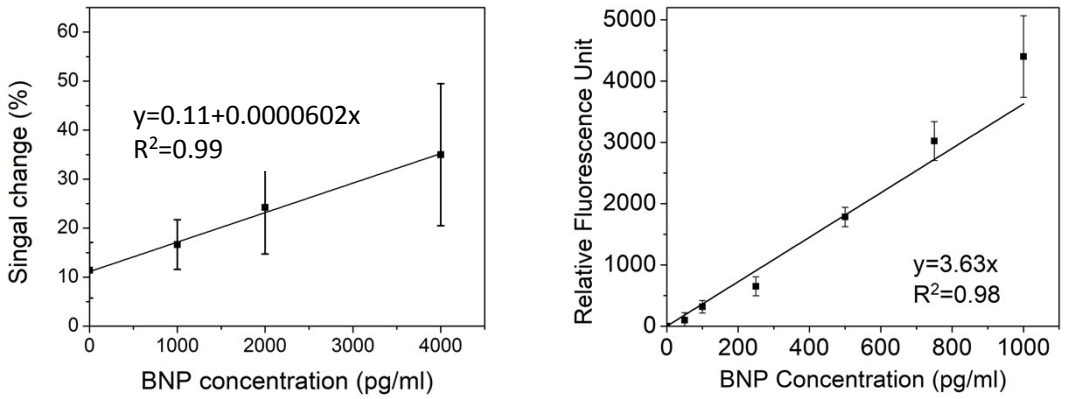

Figure S5. Calibration curves of the (a) NanoBot and (b) FIA acquired from different batches of NanoBot test strip and FIA plate, respectively.

9. NanoBot and FIA performance parameters

A few parameters were utilized to evaluate the performance of the NanoBot compared to the FIA. The following equation was used to calculate the percent error between the theoretical and experimentally derived BNP or NT-proBNP concentrations:

$$\%\ error = \frac{theoretical\ concentration - experimental\ concentration}{theoretical\ concentration} \times 100$$

For limit of detection (LOD), 10 BNFP samples were used as blanks and their experimental concentrations were determined using the NanoBot through the

aforementioned standard method. The LOD of the NanoBot was determined using the following equation per IUPAC guidelines:

$$LOD = mean + 2 \times standard\ deviation$$

The interclass correlation coefficient (ICC) used to compare the NanoBot and Alere Triage® performances was calculated with the following equation:

$$Correl\ (X,Y) = \frac{\sum (x - \bar{x})(y - \bar{y})}{\sqrt{\sum (x - \bar{x})^2 \sum (y - \bar{y})^2}}$$

10. NanoBot performance against the gold standard FIA

At the higher BNP concentrations of 1000 and 750 pg/mL, the NanoBot exhibited better CV at 4.32% and 7.16% compared to the FIA at 14.47% and 9.85%, respectively. However, the NanoBot's precision at 500 and 100 pg/mL at 19.19% and 24.40% were lower than the FIA at 8.05% and 20.13%, respectively, which could be attributed to the higher background signal relative to the BNP signal. Human error that occurred during these in-house assays also contributed both the NanoBot's and FIA's precision performance. On the other hand, the NanoBot's CV in detecting various NT-proBNP concentrations were lower than the FIA. Although the precision was highest at detecting 4000 pg/mL with a CV of 9.91% compared to the lower concentrations of 2000, 1000, and 500 pg/mL, the FIA exhibited higher precision at 4000 and 2000 pg/mL with CVs of 1.52% and 1.11%.

	Coefficient of Variation (%)				
	BNP			NT-proBNP	
Concentration (pg/mL)	Nanobot	FIA	Concentration (pg/mL)	Nanobot	FIA
1000	4.32	14.47	4000	9.91	1.52
750	7.16	9.85	2000	18.29	1.11
500	19.19	8.05	1000	18.34	27.85
100	24.40	20.13	500	29.83	10.68

Table S1. Precision of the NanoBot and FIA systems from 10 samples each of BNP and NT-proBNP spiked in human plasma.