Supporting Information (SI) Appendix for

Inkjet Printed Point-of-Care Immunoassay on a Nanoscale Polymer Brush Enables Sub-Picomolar Detection of Analytes in Blood

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1. Sample Volume and Internal Calibration Spots

For quantitative diagnostic platforms, variations in sample volume can affect assay results. The assay geometry shown in this work accommodates volumes between approximately 35 to 150 μL of sample. For our experiments in which volumes were not essential to the measurement, blood was transferred directly to the D4 assay from a fingerstick. For example, Fig. 2 in the main text (IgG/IgM detection) represents an assay for which (1) results are either positive or negative and (2) concentrations of analyte are high; thus, control of sample volumes is not critical for assay readout. However, for assays in the manuscript where volume control was critical for quantitation, we elected to transfer 50 μL of blood/serum using an adjustable volume pipette to the D4 assay chip.

However, the requirement for strict volume control (and the need for pipettes) can be eliminated by the use of positive control calibration spots, which are shown in the images in Fig. 3A and Fig. S1 below. These positive-control spots are comprised of anti-dAb Abs (targeting the Fc portion of dAbs) printed in the center of D4 arrays alongside anti-analyte cAb spots. In a manner similar to LFIA control lines (which also contain Abs targeting the Fc portion of labelled dAbs), these positive-control spots indicate to the user whether dAbs were successfully localized to the active area of the assay. Importantly, these positive-control spots also help quantitatively correct against inter-assay variation, as their intensity reflects variation in (i) concentration of soluble dAbs in the sample, which depends on both the *sample volume* and on the *dissolution efficiency* of soluble dAb, and (ii) *fluorescence efficiency* of labelled dAbs. Thus, normalizing the intensity of cAb spots against those from positive-control spots effectively controls for these variables.

Fig. S1B shows data from individual representative leptin-D4s both exposed to 1 ng/mL and 8 ng/mL of leptin analyte, whereby arrays were exposed to a range of sample volume from 50 to 150 μL. The absolute intensity of the cAb spots vary across replicates at each analyte

concentration, and can be attributed to variations in the factors described above. However, as shown in Figs. S1C-D, the inter-assay variability in readout is addressed after normalizing the fluorescence intensities of the cAb by those from their respective positive-control spots.

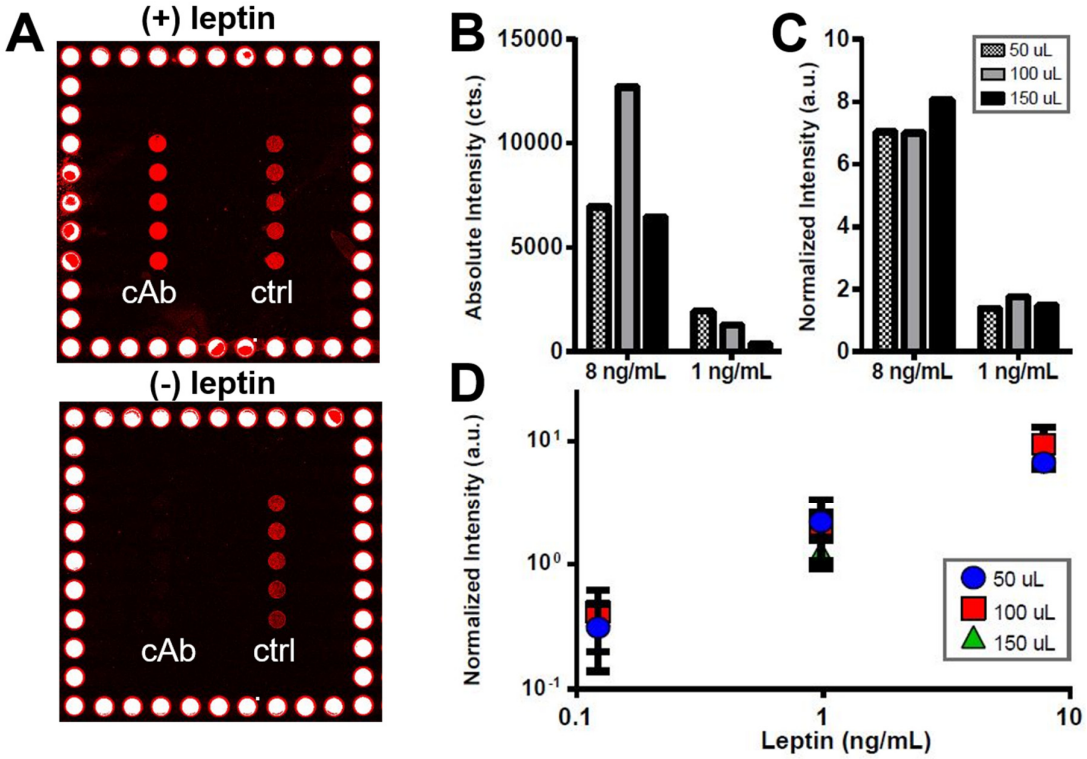

Fig. S1: D4 arrays with control spots to reduce inter-assay variability. **(***A*) Results of leptin-D4 assay performed with (top) and without (bottom) leptin Ag in the presence of control spots. The center of the array contains cAb spots against (i) leptin on the left and (ii) the Fc portion of dAbs, which were used as controls to normalize intensity measurements. (*B*) Absolute D4 intensities for representative leptin-D4 arrays run at different concentrations and volumes, showing significant variation in signal. (*C*), Results for the same representative assays shown in (B) after normalization against their respective control spots indicate a reduction in inter-assay variability. (*D*) Average values of normalized cAb spot intensities across duplicate runs

at the indicated concentrations and sample volumes – Results show expected dose-dependence and acceptable inter-assay variation with normalization. (Error bars: s.d.)

2. D4 data for cancer, cytokine, endocrine, and infectious disease markers.

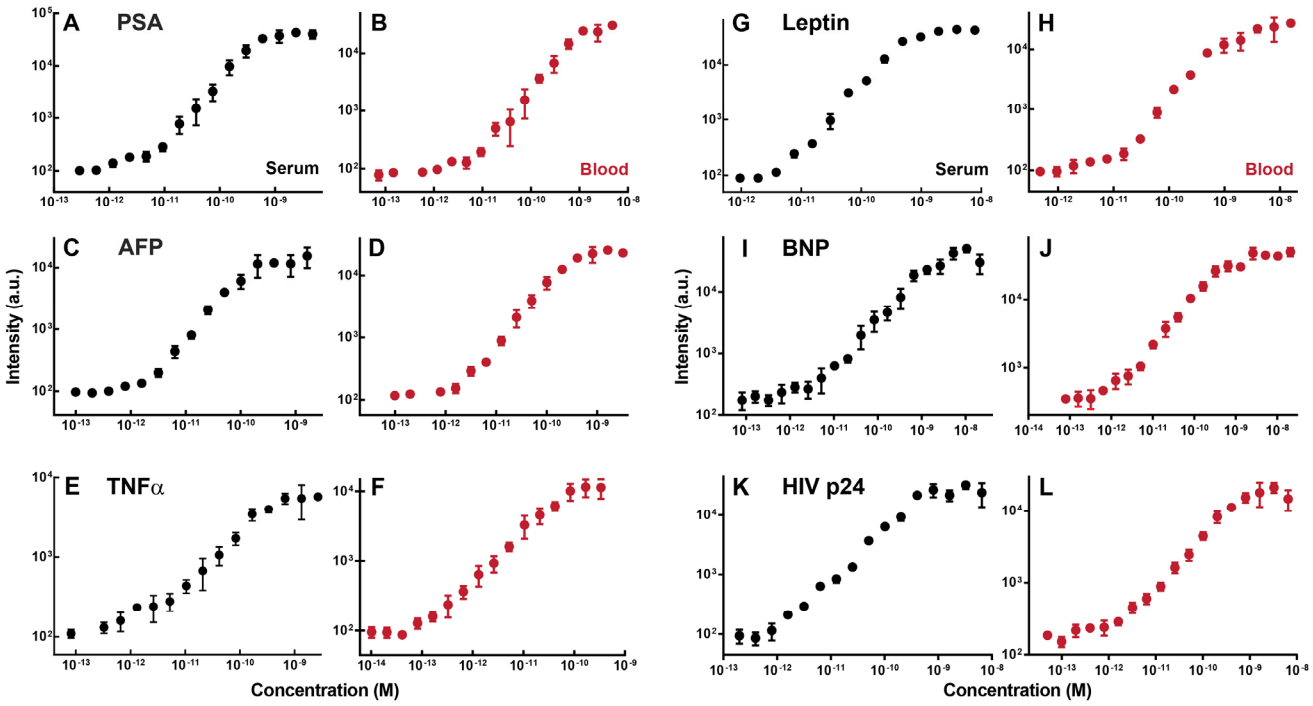

Fig. S2. Dose-response curves for single analyte D4 for cancer, cytokine, endocrine, cardiology, and infectious disease markers. Single-analyte dose-response curves show that the D4 assay can detect virtually any analyte for which Ab pairs are available. Analytes pertinent to cancer (PSA and AFP), endocrinology (leptin and ACRP30), cytokines (TNFα), and infectious disease (HIV p24) are shown here as representative examples. For each analyte, dose-response curves are shown from experiments performed in calf serum (black circles) and whole chicken blood (red circles). Each data point represents mean ± s.d. from three separately run D4 arrays.

3. D4 Incubation Time

We chose 90 minutes for the assays shown in Table 1 as we focused on achieving high sensitivities (via longer equilibration times) and showcasing similarities in performance between our D4 assays and that of standard ELISA. However, depending on the application, shorter incubation times may be desirable. Fig. S3 shows the performance of leptin-D4 using 15 minutes and 60 minutes of incubation time. These incubation times translate to analytical sensitivities (LOD) of 57 pg/mL and 43 pg/mL, respectively, and compare well with the 38 pg/mL LOD for a D4 assay carried out with a 90 min incubation period.. As shown in Fig. S3, an incubation time of 15 min is suitable for applications requiring ~100 pg/mL analytical sensitivity. On the other hand, if required, a longer incubation time can be implemented for assays that require a lower LOD.

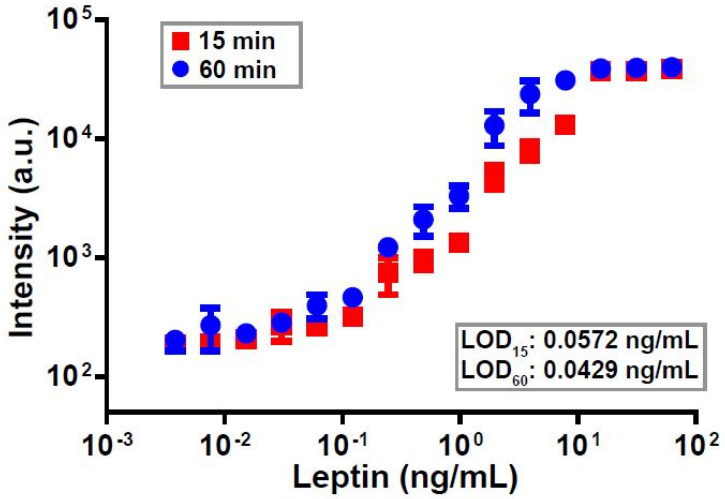

Fig. S3: The effect of incubation time on assay sensitivity. Dose-response curve of leptin-D4s using incubation lengths of 15 min (red square) and 60 min (blue circle), with corresponding LODs (LOD_{15} and LOD_{60}, respectively). Values represent average of duplicate runs +/- s.d.

4. Multiplexed D4 against cancer markers

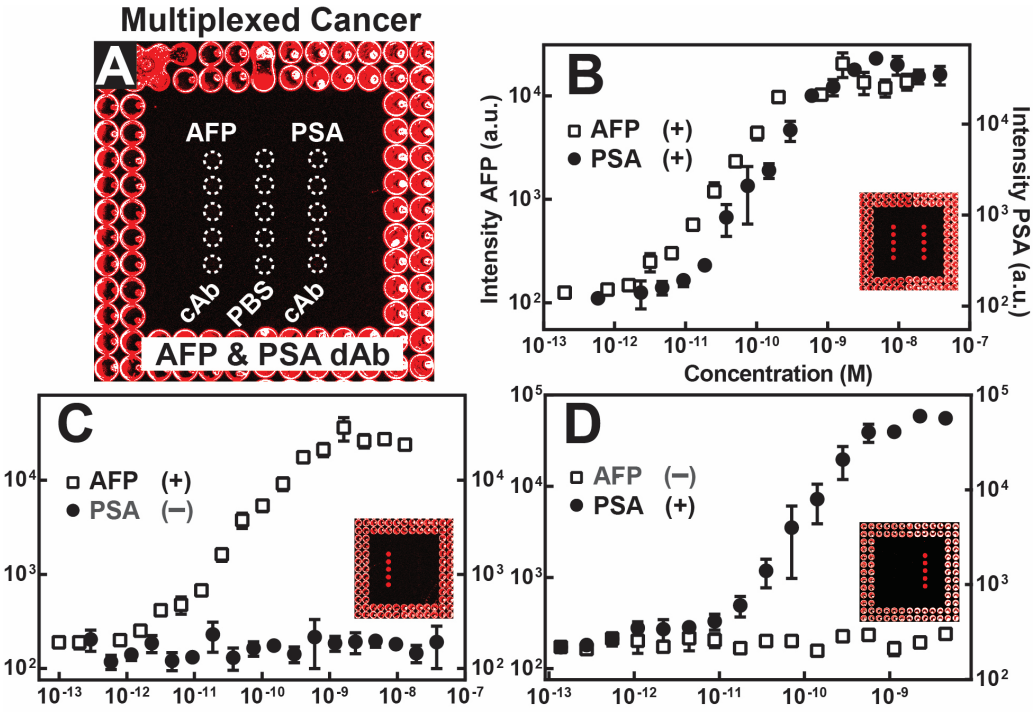

Fig. S4. Multiplexed assays against cancer markers AFP and PSA in whole blood. Fluorescent dAbs against both analytes are co-printed with PEG excipient as outer spots. Spots of cAb against each analyte are printed in the center of the array. (*A*) D4 image after incubation with whole chicken blood alone (without analyte). Spots for cAb and PBS are outlined in white dashes for clarity. (*B*) Dose-response curves after exposure to whole blood spiked with a mixture of both AFP and PSA analytes. (*C*-*D*) Dose-response curves when D4 arrays exposed to varying concentrations of (*C*) AFP only, or (*D*) PSA only. Insets for panels (*B*-*D*) show D4 image data. Data points are mean ± s.d. from three separately run D4 arrays.

5. Mobile phone attachment

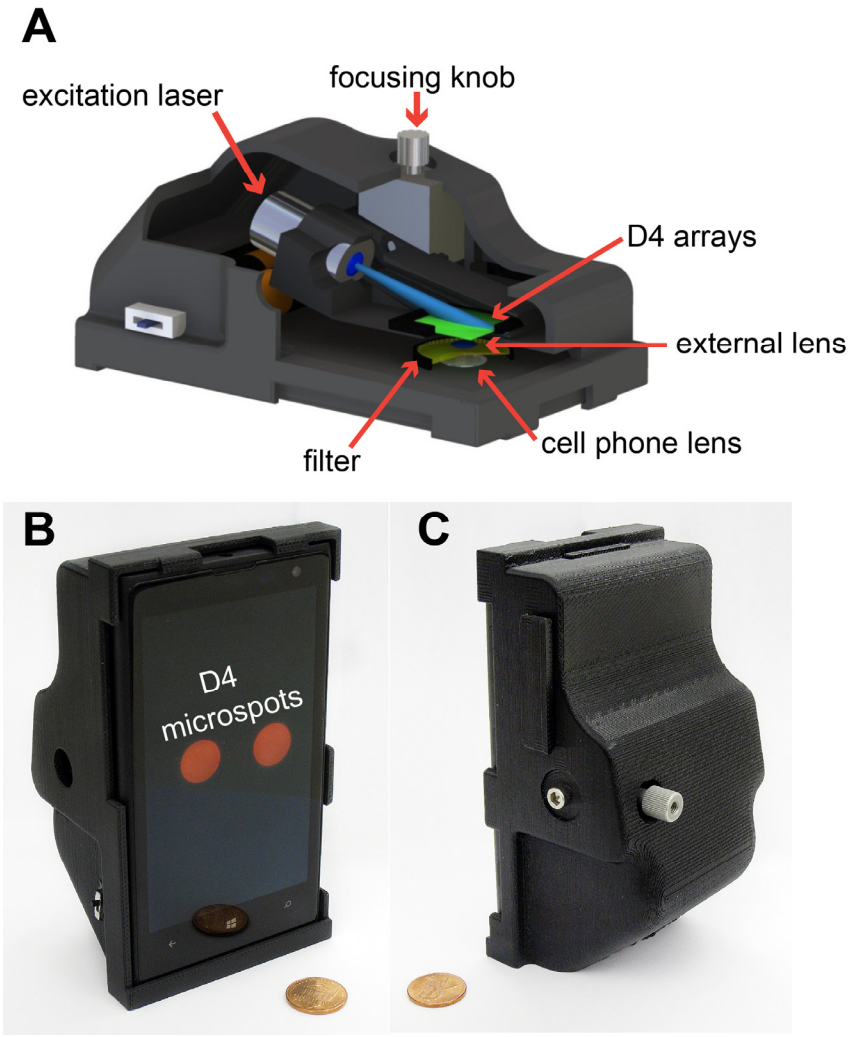

Fig. S5. Schematic diagram of the mobile phone attachment for fluorescence imaging and quantification of D4 arrays. (*A*) A 3D illustration of the mobile phone opto-mechanical attachment is shown here. For fluorescence analysis of D4 arrays, we created a compact and cost-effective imaging system that utilizes an external lens, in addition to the existing lens of the mobile phone camera, as well as an oblique illumination angle of ~75° to increase the signal-to-noise ratio of the acquired fluorescence images on the phone. (*B-C*) Photographs of the actual mobile phone imaging platform used for this experiment. A penny is included in the images for scale. A picture of leptin-D4 microarray spots is seen on the screen of the phone in (*B*).

6. D4 Cost Estimate

Materials for SI-ATRP on Glass	**Cost ($) per *Individual* Assay**
Glass	0.14
Ethanol	0.00372
3-Aminopropyltriethoxysilane	0.00925
Dichloromethane	0.00414
Triethylamine	0.000519
alpha bromoisobutyryl bromide	0.00222
Monomer for POEGMA brushes	0.0109
Copper (II) bromide	0.00023
1,1,4,7,10,10-Hexamethyltriethylenetetramine	0.00086
Antibody Reagent Cost	
capture antibody (1.5 ng per assay)	0.0009
detection antibody (43 ng per assay)	0.026
TOTAL ($ per assay)	**0.199**

Table S1. Cost estimate for individual D4 assays. Our method involves batch processing of a large number of D4 chips in parallel—thus the estimates provided are based on prices we paid for materials/reagents per single large fabrication batch, and then subsequently dividing this value by the number of assays in the fabrication batch. Production of D4 assays involves two major steps: (1) polymerization of POEGMA brushes on glass chips and (2) printing of antibody reagents. This table itemizes the cost of reagents/materials per assay for each step (including solvents and substrate costs). Notably, the cost of antibody reagents per assay is low given the compact assay dimensions and low microspot printing volumes (300 picoliters per spot). This estimate excludes the cost of equipment and labor costs associated with assay fabrication.

7. Process Control of D4 Assays

Fig. S6 below shows a flowchart summarizing process control of D4 assays. To assess the quality of POEGMA coatings after SI-ATRP, we first use reflective mode spectroscopic ellipsometry (Woollam M-88) to determine the thickness of polymer films. Ellipsometry is performed on reflective Si wafers having a 20 nm thermal oxide layer that are processed in parallel with glass chips. This step is important given previous work showing that only POEGMA films thicker than ~10 nm are reliably resistant to non-specific binding [*Langmuir* 22(8):3551 & *Adv Mat* 16(4):338]. Next, x-ray photoelectron spectroscopy (XPS) is used to analyze representative POEGMA-coated glass chips from a batch to confirm the chemical composition of POEGMA coatings. Representative spectra are shown in Fig. S7, whereby underlying Si peaks are absent from survey spectra (Fig. S7A), and the deconvolved high-resolution C 1s peaks are consistent with the approximately 1:3:10 (COOR:CH_x:COR) stoichiometry of POEGMA (assuming 4-5 ethylene glycol sidechain units).

After inkjet printing of Abs, assay performance is assessed by generating dose-response curves on representative D4s for each print batch. Assay FOMs are calculated and compared to previously characterized and accepted values. We accept a maximum of 10% deviation from accepted FOM. Furthermore, as shown in Section 5 (and Fig. S5) above, we also include printing internal calibration spots that adjust for variations in sample volume and dissolution/diffusion efficiency of detection reagents.

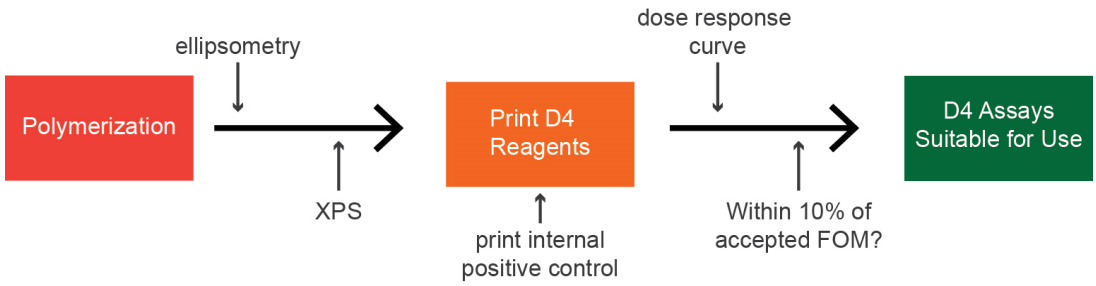

Fig. S6: Flowchart of process control.

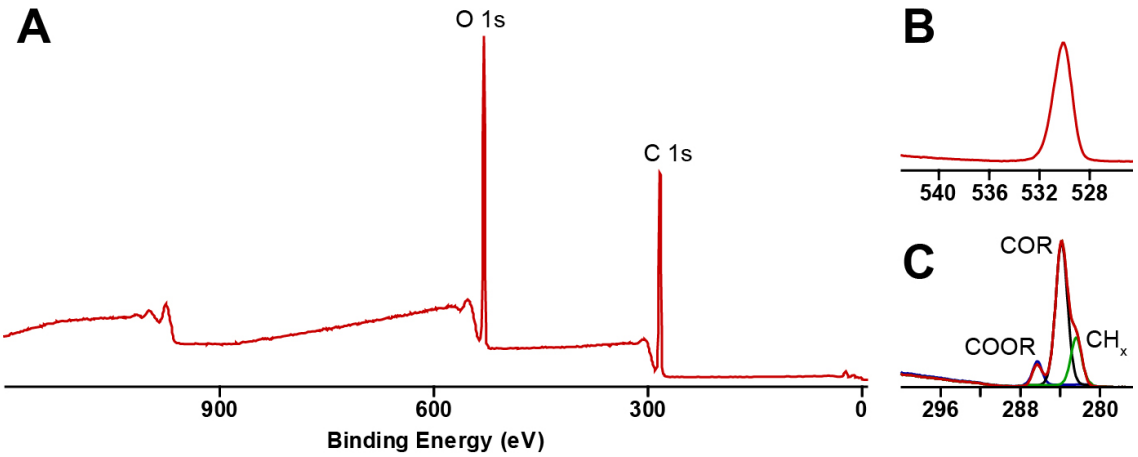

Fig. S7: Representative XPS spectra of POEGMA. (**A**) Survey spectrum (**B**) high resolution O1s spectrum (**C**) high-resolution C1s spectrum, with deconvolved CH_x, COR, and COOR peaks shown.