Engineering the surface properties of a zwitterionic polymer brush to enable simple fabrication of inkjet printed point-of-care immunoassays

Cassio M. Fontes[1]†, Rohan K. Achar[1]†, Angus Hucknall[1], Daniel Y. Joh[1], Imran Ozer[1], Somnath Bhattacharjee[1], Ashutosh Chilkoti[1]*

Affiliations

[1]Department of Biomedical Engineering, Pratt School of Engineering, Duke University, Durham NC 27708 USA.

†*These authors contributed equally to this work.*

Corresponding Author(*): Ashutosh Chilkoti / Department of Biomedical Engineering, PO Box 90281, Duke University, Durham NC 27708-0281 / Tel: (919) 660-5373 / Email: chilkoti@duke.edu

Table of Contents:

Figure S1: D4 assay on POEGMA coated slides……p2

Figure S2: Capture and detection spots on POEGMA and PSBMA substrate imaged prior and post sample incubation……..….p3

Figure S3: X-ray photoelectron spectra of surface after intermediate synthesis steps and after surface PCBMA synthesis under different conditions…….…..…… p4

Figure S4: D4 assay dose-response curves……..…p5

SUPPORTING INFORMATION

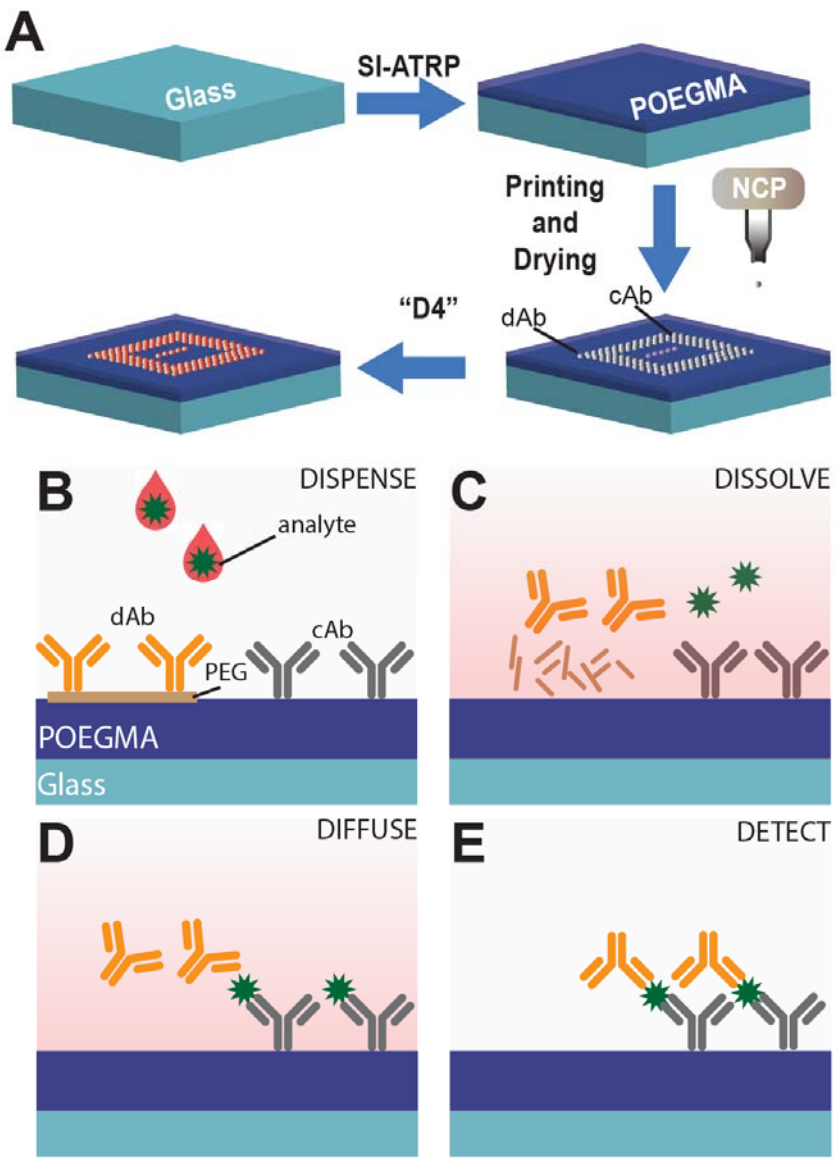

Figure S1: D4 assay on POEGMA coated slides. (A) *D4 assay fabrication strategy where a glass slide has a POEGMA polymer brush grown from the surface through SI-ATRP followed by inkjet printing of capture and detection reagents on the polymer surface. After a mild dessication process that ensures the brushe's biofunctionalization, the test device is ready to be used.* ***(B-E)*** *Sequence of events that entail the use of the D4 assay. First, sample is* ***d****ispensed (B) on the chip's surface. This* ***d****issolves (C) the excipient that was printed with the* Ab_d*s that* ***d****iffuse (D) and bind to the* Ab_c*-bound analyte. Following a rinse step, the fluorescence signal can be* ***d****etected (E) through a tabletop fluorescence scanner or a cell-phone based fluorescence reader.*

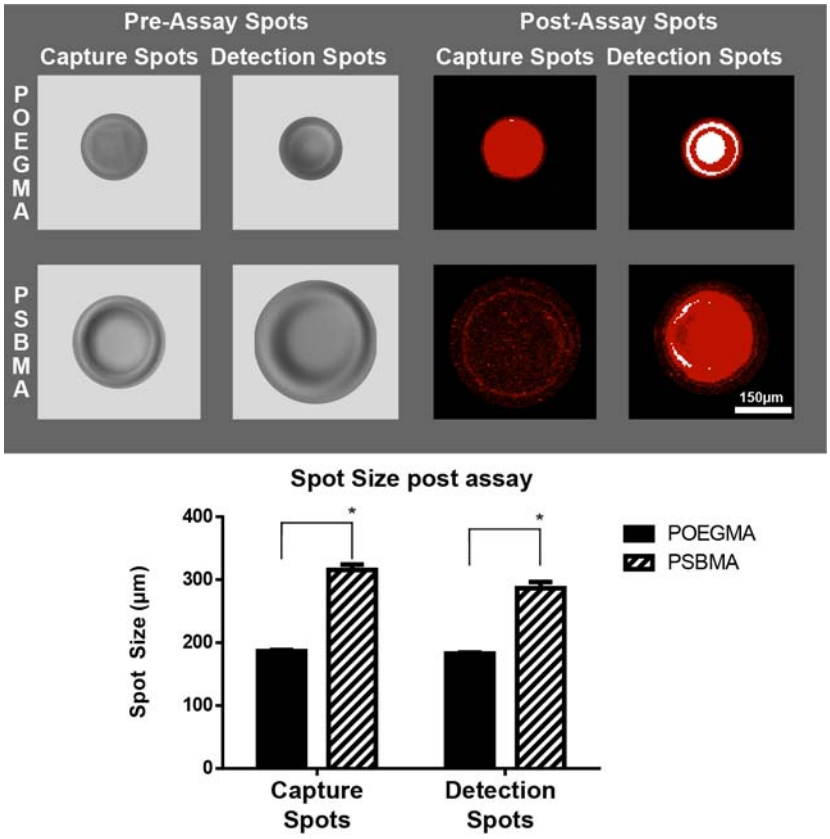

Figure S2: Capture and detection spots on POEGMA and PSBMA substrate imaged prior and post sample incubation. (A) *Optical and Fluorescence images obtained prior and post sample incubation depicting the difference in caoture and detection spot morphology for D4 assays on POEGMA and PSBMA substrate. Both substrate were inkjet printed in the same print run, with drops of 350 picoliters in volume for capture and 300 for detection spots.* ***(B)*** *Spot size evaluated for D4 assays fabricated on POEGMA and PSBMA spot sample incubation. Independent measurements were performed in 5different chips. There was statistically signicant differences between group means as determined by two-way ANOVA ($p<0.001$) folowed by Sidak's post-hoc test. All comparissons between POEGMA and PSBMA substrate were deemed statistically significant. Means with lines between bars and marked with a * are significantly different.*

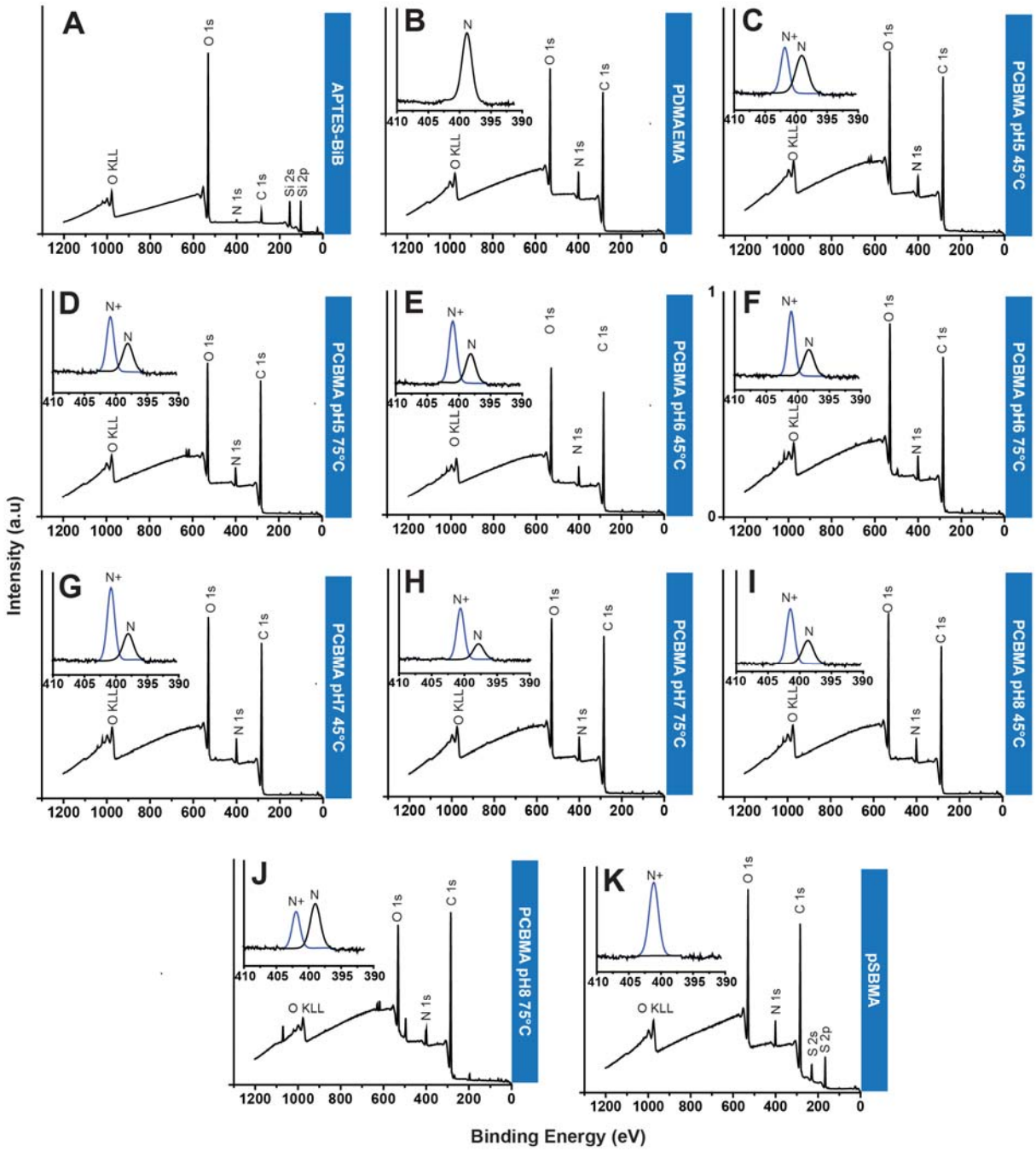

Figure S3: X-ray photoelectron spectra of surface after intermediate synthesis steps and after surface PCBMA synthesis under different conditions. (A) *Survey spectra of APTES-BiB (initiator) grafted from SiO_2 surface.* ***(B)*** *Survey spectra of PDMAEMA. Inset: High resolution N 1s spectra with peak deconvolution following SI-ATRP, showing the prevalence of tertiary amines consistent with PDMAEMA brushes.* ***(C-J)*** *Survey spectra of surfaces after PCBMA synthesis under different conditions. Inset: High resolution N 1s spectra with peak deconvolution following nucleophilic substitution, showing the prevalence of quaternary amines consistent with the formation of zwitterionic brushes.* ***(K)*** *Survey spectra of PSBMA coated surface. Inset: High*

resolution N 1s spectra with peak deconvolution following nucleophilic substitution, showing the sole presence of quaternary amines consistent with the brushe's chemical constitution.

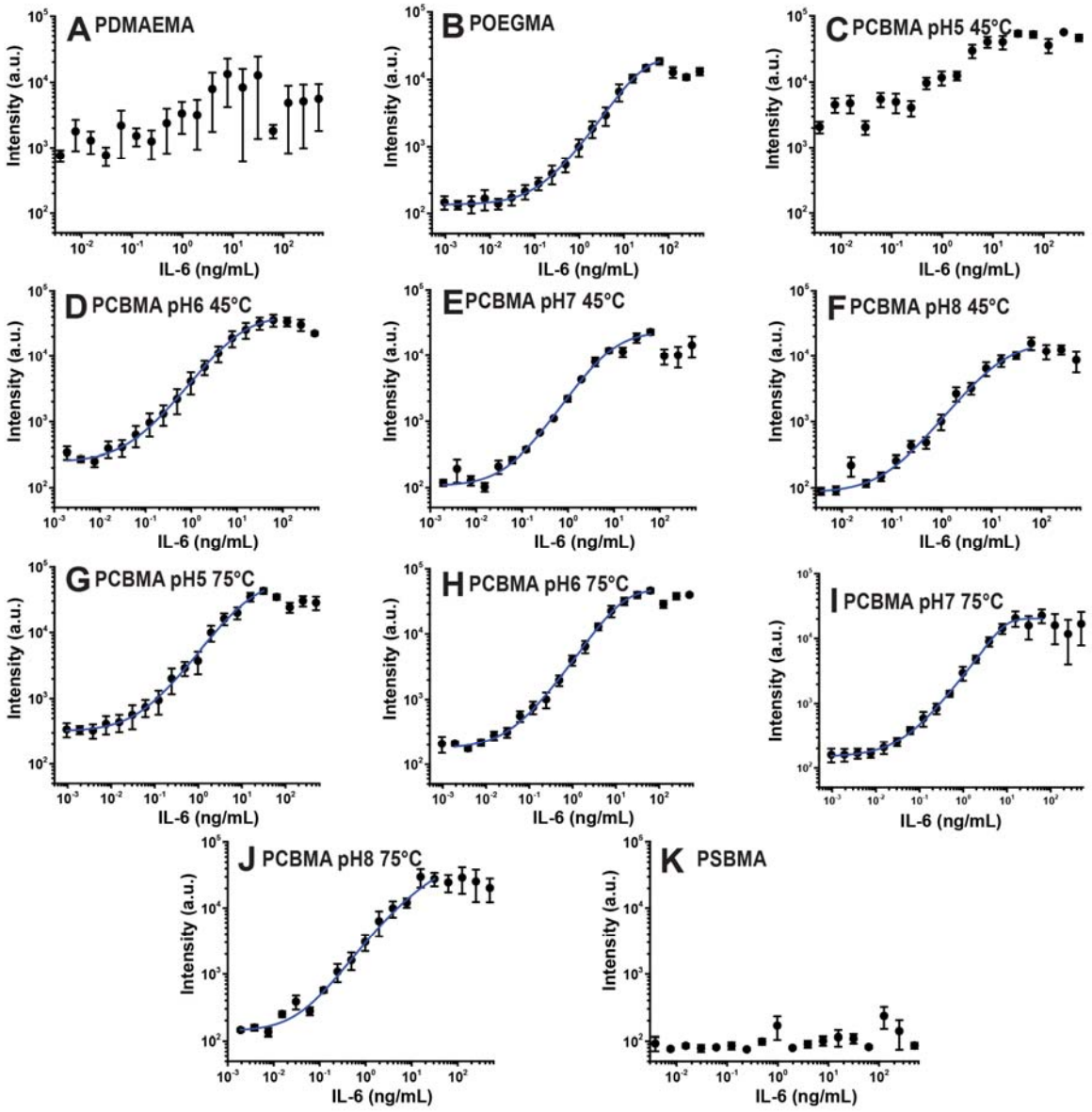

Figure S4: D4 assay dose-response curves. (A-K) *Dose-response curves of D4 assays fabricated on POEGMA, PSBMA, PDMAEMA and PCBMA-PDMAEMA brushes synthesized under different conditions of pH and temperature. Each point in the dose-response curve is the result of the average and s.e. of three independently conducted D4 assays.A 5-parameter logistic fit is shown by the blue curve.*