Supporting Information

Biofunctional Paper via Covalent Modification of Cellulose

Arthur Yu[1†], Jing Shang[1†], Fang Cheng[1], Bradford A. Paik[2], Justin M. Kaplan[3], Rodrigo B. Andrade[3], Daniel M. Ratner[1]*

[1]Department of Bioengineering, University of Washington, 3720 15th Ave NE, Seattle, 98195. Email: arthuryu@u.washington.edu (Arthur Yu), shangj@u.washington.edu (Jing Shang), ffcheng@dlut.edu.cn (Fang Cheng), dratner@u.washington.edu (Daniel M. Ratner)

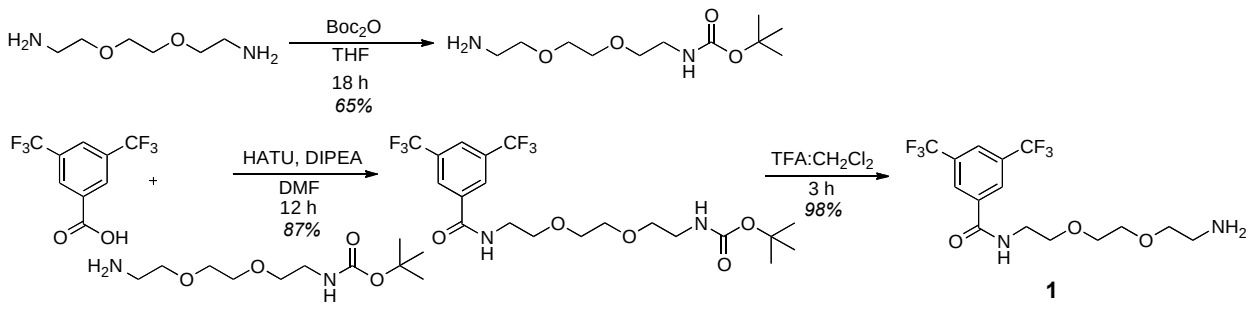
[2]Department of Chemical Engineering and Materials Science, University of Minnesota, 421 Washington Ave SE, Minneapolis, MN 55455-0132, USA. Email: bradford.paik@gmail.com (Bradford. A. Paik)

[3]Department of Chemistry, Temple University, Beury Hall 130, 1901 N. 13th Street, Philadelphia, PA, 19122, USA. Email: jkaplan1@temple.edu (Justin M. Kaplan); randrade@temple.edu (Rodrigo B. Andrade).

[†]These authors contributed equally to this work.

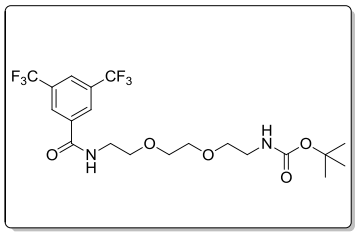
*To whom correspondence should be addressed. Address: 3720 15th AVE NE, Box 355061, Seattle, WA, 98195, USA, Phone: 206-543-1071, Fax: 206-685-3925, E-mail: dratner@u.washington.edu.

Scheme S1. Synthesis of hexafluorobenzamide **1** (F-Tag)



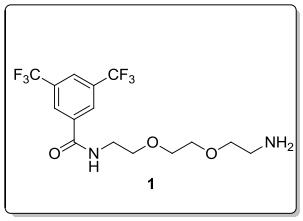
The synthesis of (2-[2-(2-Amino-ethoxy)-ethoxy]-ethyl)-carbamic acid *tert*-butyl ester was prepared according to reported procedures[1] and confirmed by NMR spectroscopy.

Formation of the amide bond by coupling 3,5-bis(trifluoromethyl)benzoic acid to the amino *tert*-butyl ester was achieved using the peptide coupling agent HATU with Hünig's base at room temperature for 12 hours. Then removal of the Boc protecting group using trifluoroacetic acid (TFA) afforded hexafluorobenzamide **1** in excellent yield.



To an oven dried 25 mL round-bottomed flask was added 3,5-bis(trifluoromethyl)benzoic acid (1.20g, 4.52 mmol) and anhydrous DMF (22mL) under an argon atmosphere. To the solution was added HATU (1.72g, 4.52 mmol), DIPEA (1.50mL, 9.04 mmol) and was allowed to stir for 15 minutes to activate the acid. After 15 minutes, amino *tert*-butyl ester (1.10g, 4.52 mmol) was added and allowed to stir overnight at room temperature. Dilute using ethyl acetate (40 mL) and wash with sat. NH_4Cl (40 mL), sat. $NaHCO_3$ (40 mL), H_2O (2 x 40 mL), brine (40 mL) dried (Na_2SO_4) and filtered. The solvent was concentrated under reduced pressure, and the residue was purified by flash column chromatography eluting with EtOAc/hexanes (3:7) to afford 1.91 g (87%) of Boc protected benzamide as a pale yellow oil.

IR (neat) 3325, 3115, 2960, 2919, 2843, 1700, 1545, 1430, 1380, 1311, 1192 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.31 (bs, 1H), 8.27 (s, 1H), 7.88 (s, 1H), 7.66 (bs, 1H), 5.05 (bs, 1H) 3.45-3.63 (m, 10H), 3.44 (q, J = 5.2 Hz, 2H) 1.32 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.4, 155.9, 136.6, 131.7 (q, $^2J_{CF}$ = 33.6 Hz) 127.6, 124.5, 122.9 (q, $^1J_{CF}$ = 271.0 Hz) 79.2, 70.2, 70.0, 69.3, 41.5, 40.1, 28.1; HRMS (FAB) calc'd for $C_{20}H_{26}F_6N_2O_5$+Na^+ = 511.1644, found 511.1652.



1

A solution of TFA (3.0 mL) in CH_2Cl_2 (3.0 mL) was added to Boc protected hexafluorobenzamide (1.20g, 2.46 mmol). The mixture was stirred for 3 hrs at room temperature at which time the reaction mixture was cooled to 0^oC and ammonium hydroxide was added drop-wise until the solution was basic. The solution was then diluted with CH_2Cl_2 (35 mL) and washed with sat. $NaHCO_3$ (20 mL), H_2O (20 mL), brine (20 mL) and dried (Na_2SO_4) and filtered. The solvent was concentrated under reduced pressure, and the residue was purified by flash column chromatography eluting with MeOH/CH_2Cl_2 (2:8) to afford 0.955 g (98%) of hexafluorobenzamide **1** as a pale yellow oil.

IR (neat) 3300, 3091, 2918, 2897, 1682, 1477, 1295, 1130 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.35 (m, 1H), 8.32 (s, 2H), 7.88 (s, 1H), 3.53-3.64 (m, 8H), 3.44 (t, J = 5.2 Hz, 2H) 2.76 (m, 2H) 2.30 (bs, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.7, 136.6, 131.6 (q, $^2J_{CF}$ = 33.5 Hz) 127.7, 124.5, 122.9 (q, $^1J_{CF}$ = 271.3 Hz) 72.3, 70.2, 69.8, 69.5, 41.0, 40.1; HRMS (FAB) calc'd for $C_{15}H_{18}F_6N_2O_3+H^+$ = 389.1300, found 389.1310.

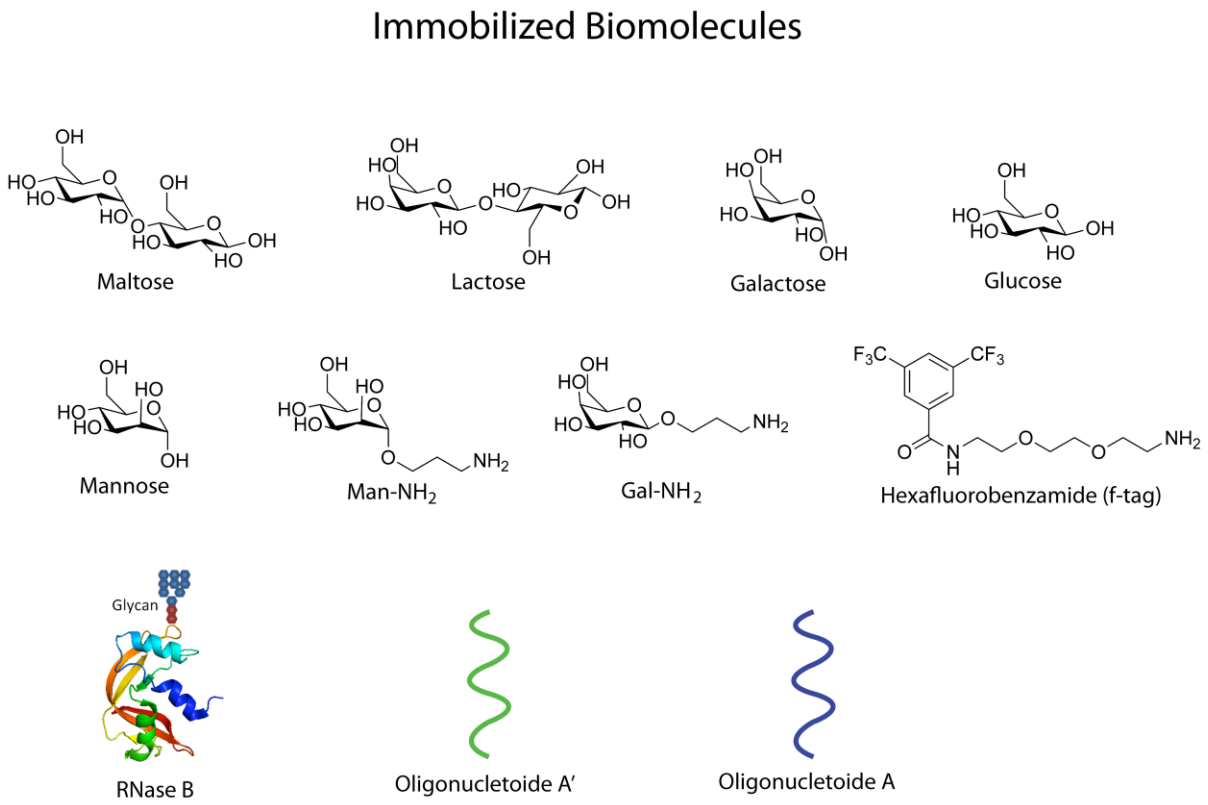
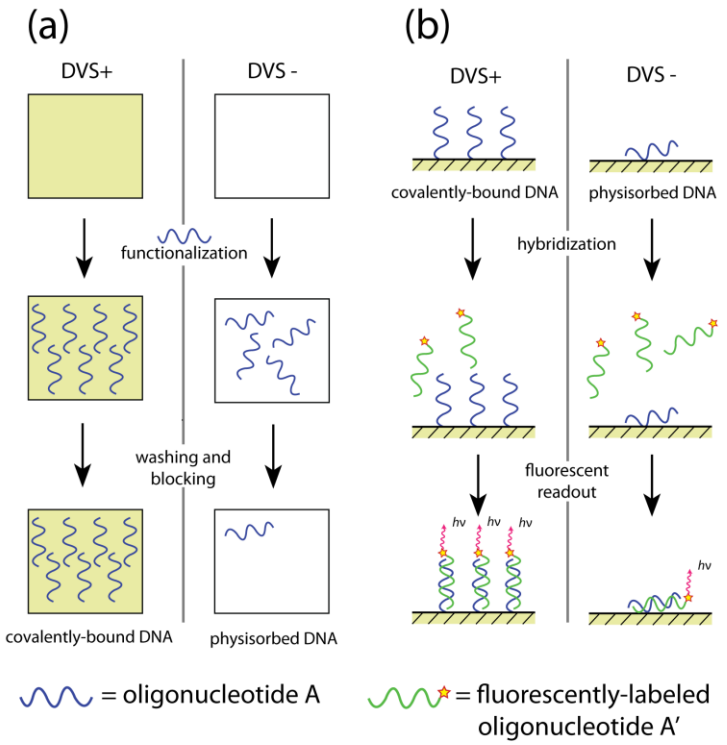


Figure S1. Biomolecules used in this work. The carbohydrates used were maltose, lactose, galactose, glucose, mannose, aminopropyl mannoside (Man-NH_2), and aminopropyl galactose (Gal-NH_2). Glycoprotein used was RNase B. Oligonucleotide sequences used were labeled A and A'.

Scheme S2. (a) Aminated oligonucleotide A was spotted onto DVS+ and DVS- membranes, illustrating covalent attachment to the DVS+ substrate and physisorption to the unmodified (DVS-) cellulose. Washing and blocking with PBS-T removes the majority of physisorbed oligonucleotide, while covalently-bound DNA remains on the DVS+ membrane. (b) A fluorometric DNA hybridization assay utilized Dylight 649-labeled probe sequence A' in hybridization buffer. DVS+ bound DNA on the DVS+ membranes would respond more strongly.



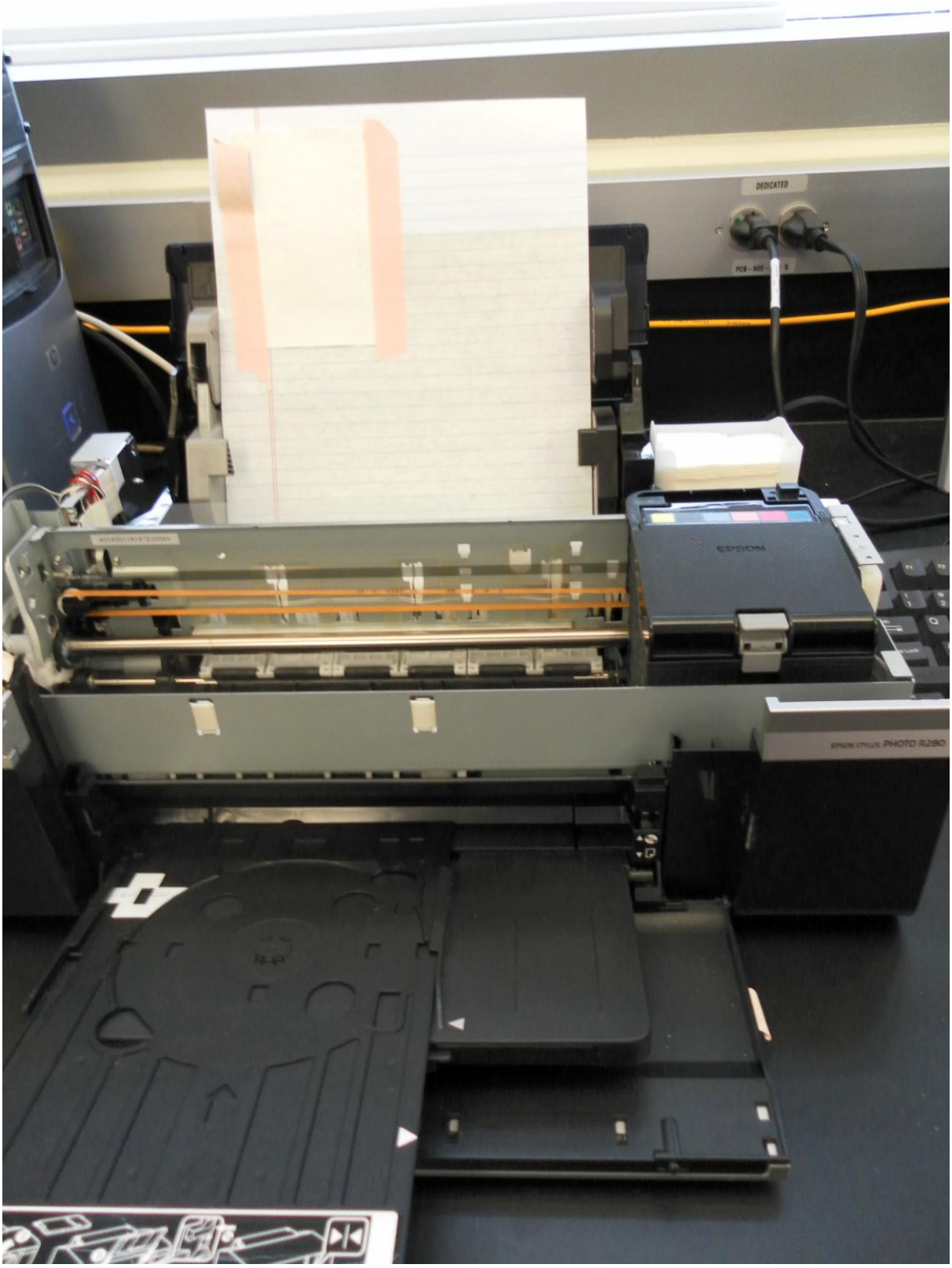


Figure S2. EPSON R280 inkjet modifications for printing carbohydrate bio-inks; uncovered printing mechanism including cartridges, inkjet, and DVS-activated sheet taped onto normal printer paper, ready to be printed with bio-ink.

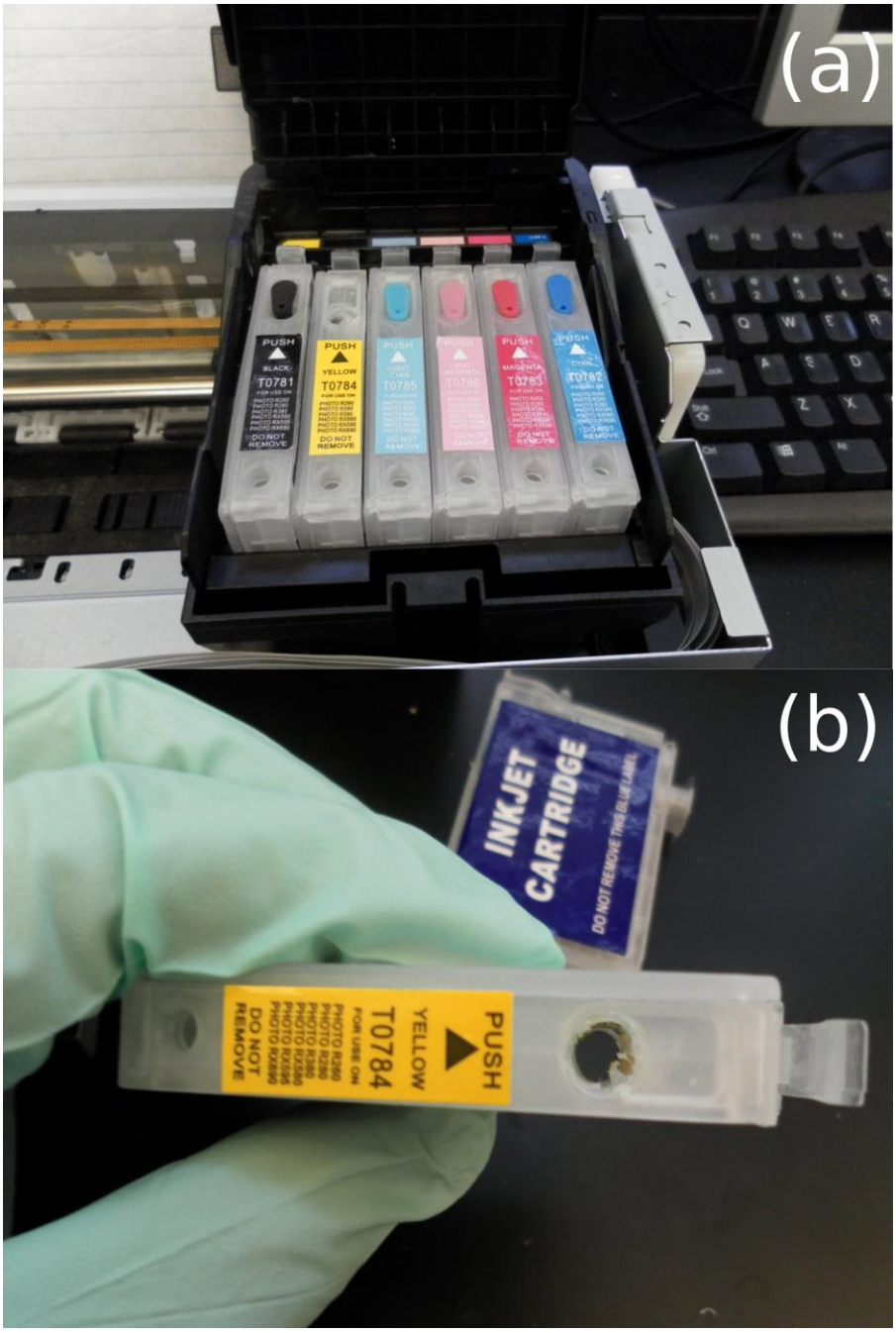


Figure S3. (a) Six empty refill cartridges replaced the original ink cartridges that shipped with the R280. This was necessary to accommodate the modifications made to one cartridge in (b). **(b)** Milled circular channel in the cartridge allowed room for insertion of pipette tip onto the printhead in lieu of the ink channel in the cartridge itself.

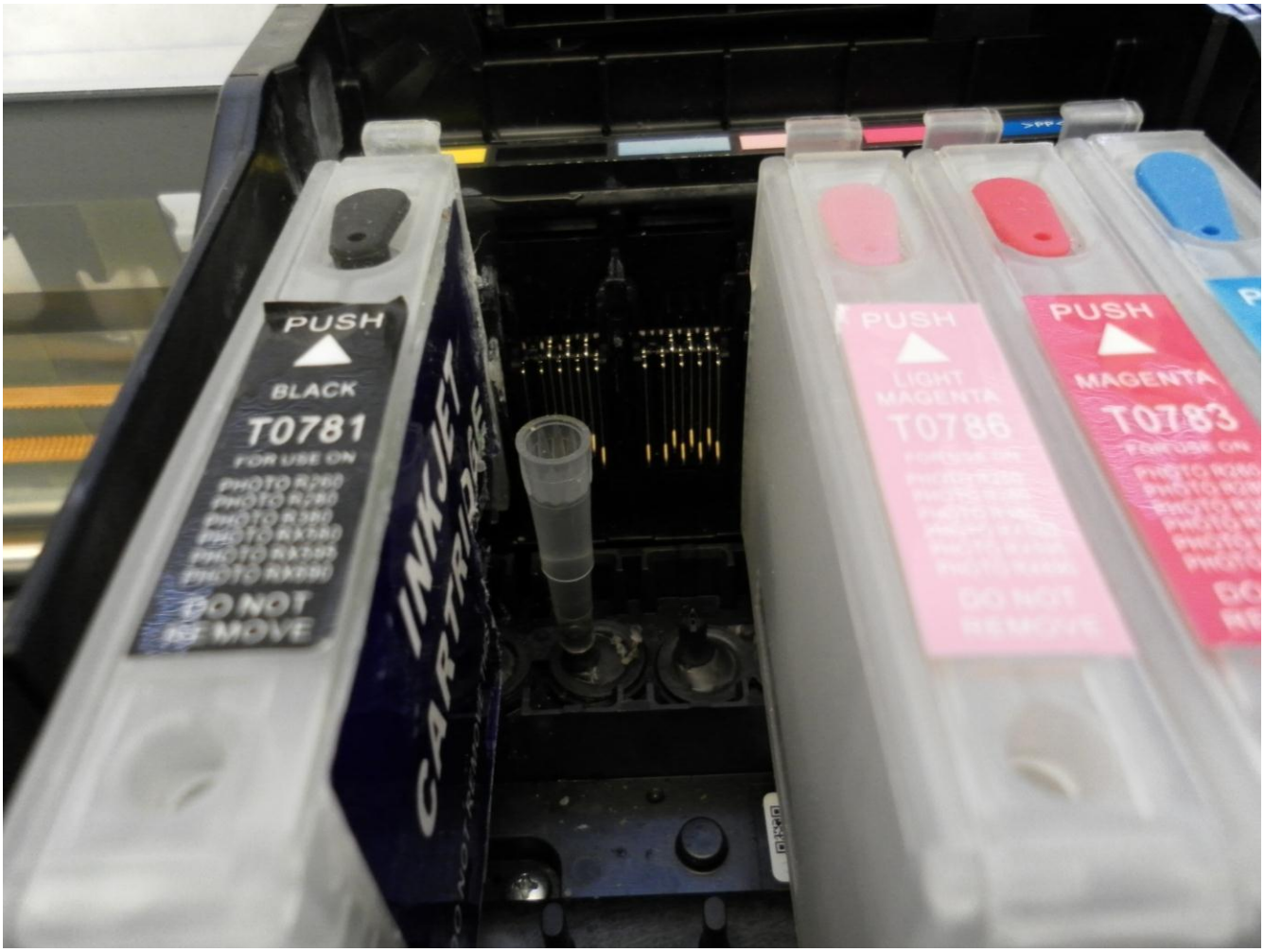


Figure S4. A pipette tip reservoir was fabricated by truncation of a 200 µL pipette tip so that the tip could be inserted onto the printhead. During printing preparation the reservoir is filled with either priming or bio-ink solution.

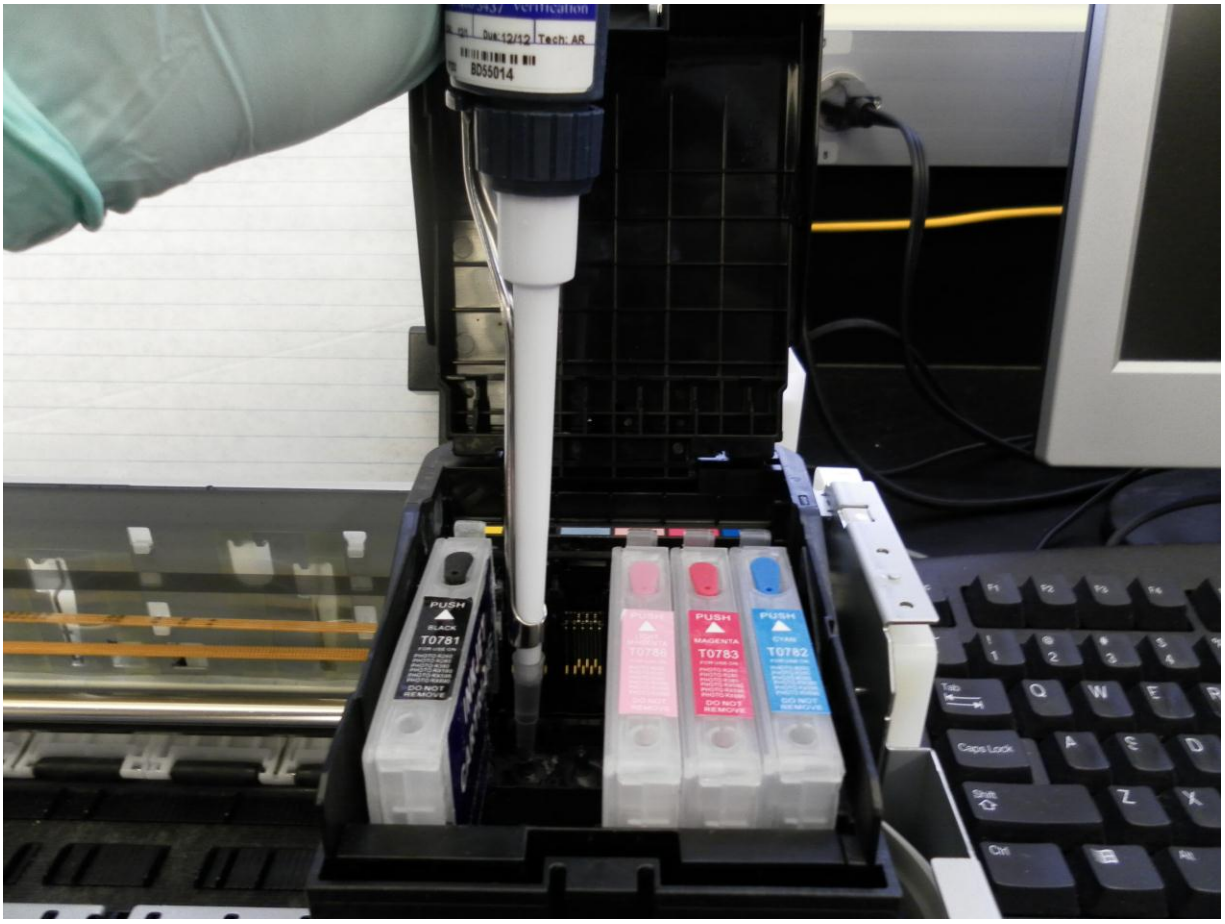


Figure S5. A pipettor was used to apply pressure and push through 50-100 µL of the bio-ink solution to fill the printhead and eliminate air bubbles which would interfere with the production of droplets in the inkjet.

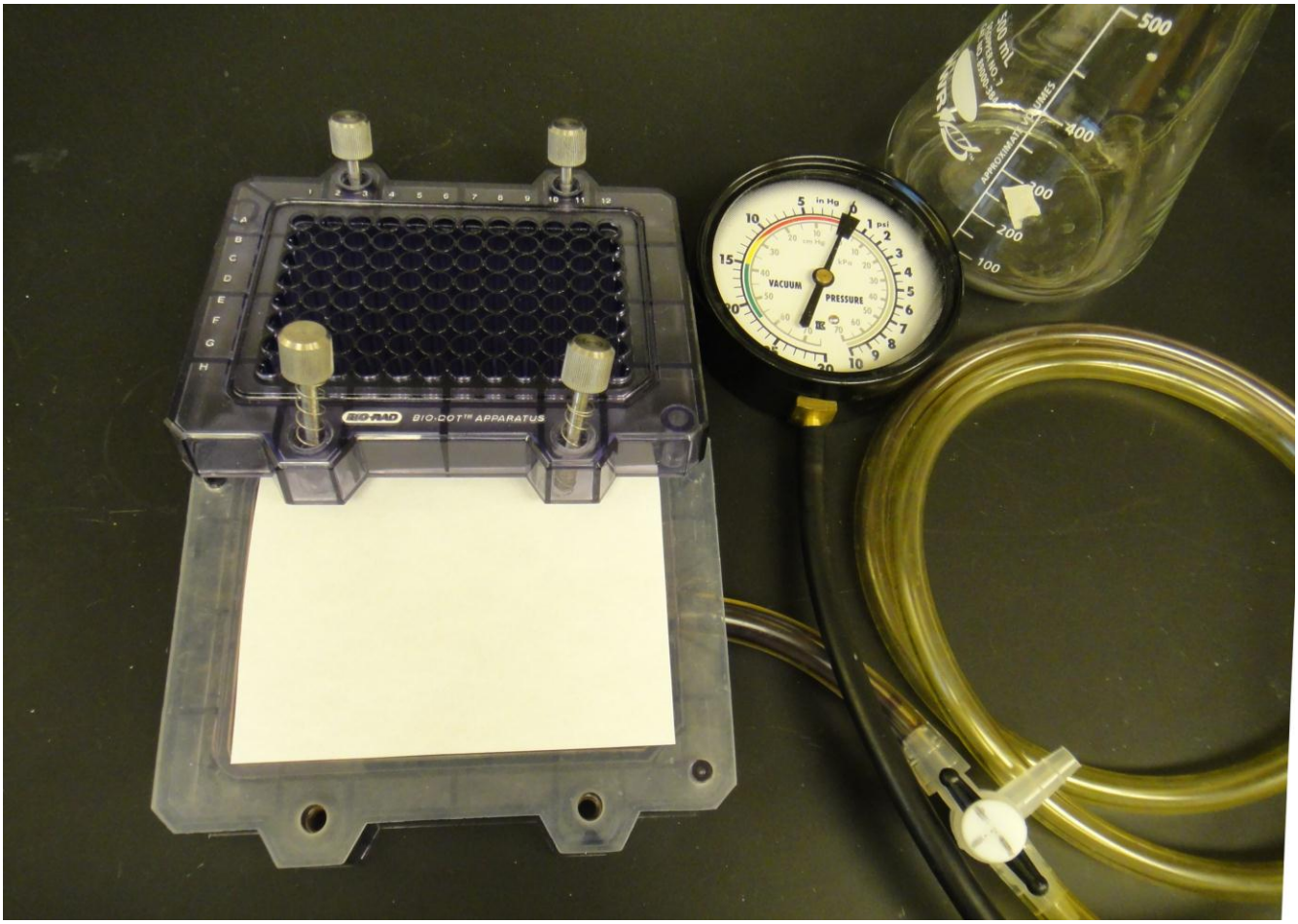


Figure S6. Bio-Dot apparatus with 96-well sample template that secures to the vacuum manifold via screws, a cellulose membrane for assaying, rubber sealing gasket sandwiched between the template and vacuum manifold, and vacuum tube extending out from the manifold. Here the tube connects to an intermediate waste container in which the assay waste solutions from the Bio-Dot are deposited.

Intensity Analysis. Colorimetric assay results were scanned as .tiff images at 600 dpi. The images were imported into ImageJ software, converted to grayscale, and inverted. Because the 96-well format of the Bio-Dot apparatus imprinted the shape of the circular well walls into the intensity profile of each spot after assay operation, the region of interest (ROI) was easy to identify as an outlined circle on the grayscaled and inverted image. Each ROI is exactly the same area in size; as the ROI Manager in ImageJ allows the duplication of a selected ROI, only the first ROI was manually selected. All of the ROIs in the ROI manager can be analyzed at the same time. The IDs were then exported to Microsoft Excel.

Fluorometric assay results were scanned using the Storm 865 system, and analyzed using GE Healthcare's ImageQuant TL software. ROI selection was conducted in the same fashion as it was done in ImageJ, as the fluorescent assays on the Bio-Dot produced similarly imprinted well shapes onto the intensity profile. The resultant fluorescent intensities were exported to Microsoft Excel.

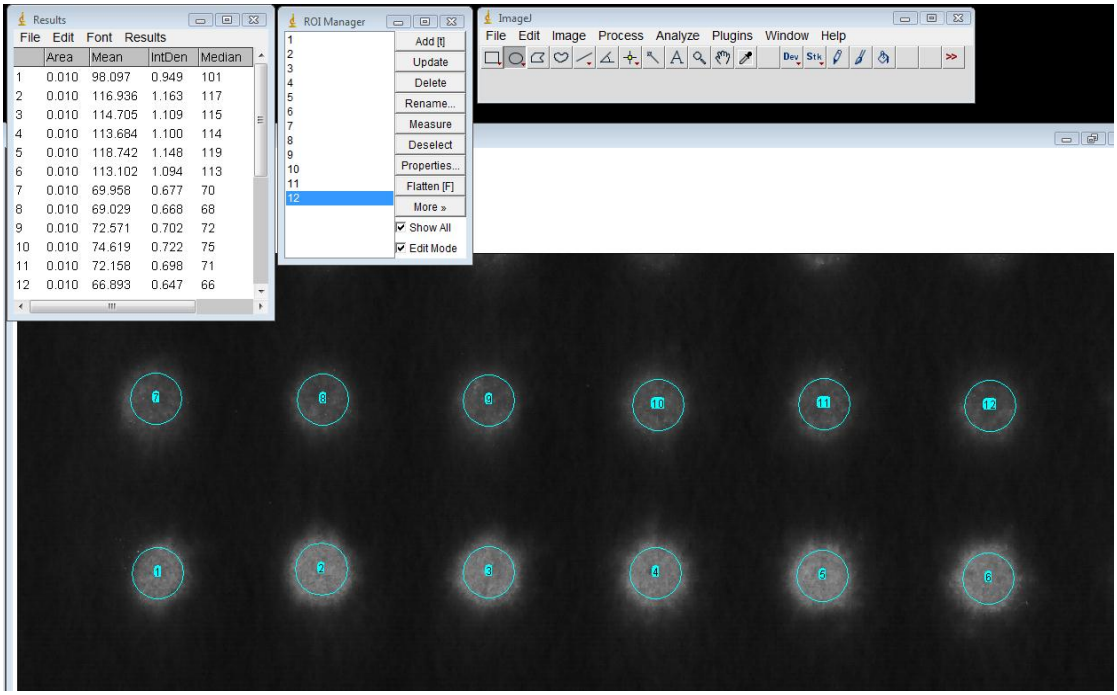


Figure S7. Desktop capture of the ImageJ interface used for ROI analysis of a colorimetric assay result. Twelve ROIs are selected via duplication of the first ROI selected using the ROI Manager. All of the ROIs can then be analyzed, and the Integrated Density ("IntDen") is reported in the Results window for each ROI.

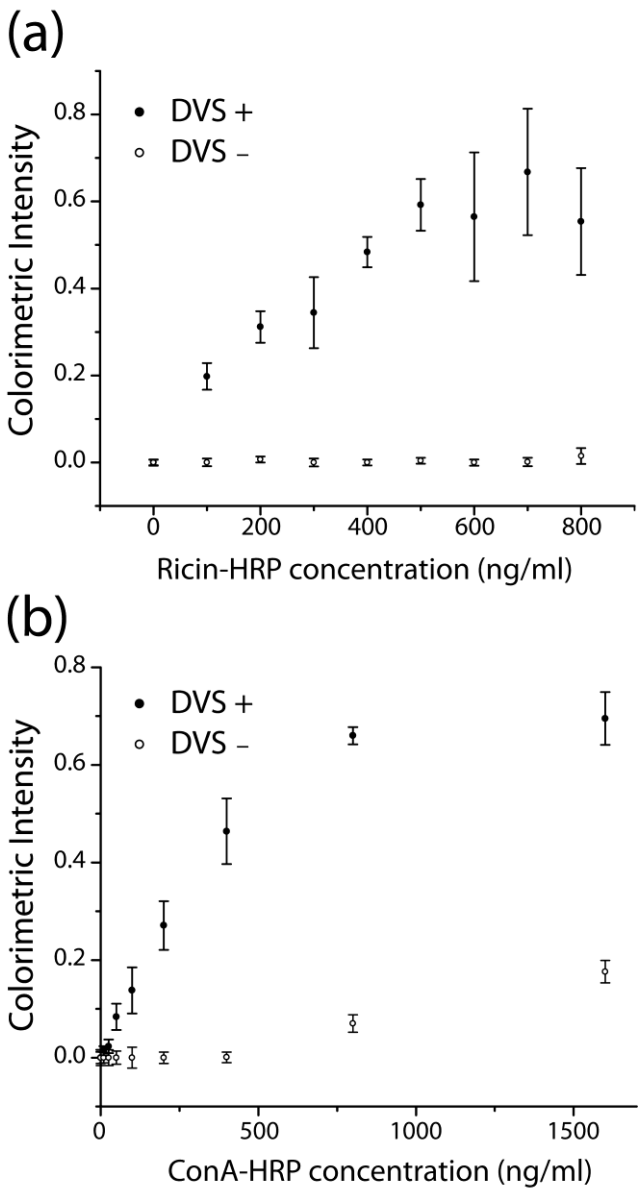


Figure S8. (a) Dose-dependent response of the RCA120-hrp-galactose interaction on DVS-activated cellulose functionalized with 555 mM galactose, for increasing RCA120-hrp concentrations. Response saturated at approximately 500 ng/mL, (4.8 nM), while the lowest signal was approximately 0.96 nM. (b) Dose-dependent response of the Con A-hrp-mannose interaction saturated at approximately 1000 ng/mL (96 nM), while the limit of detection was determined to be 10 ng (96 pM).

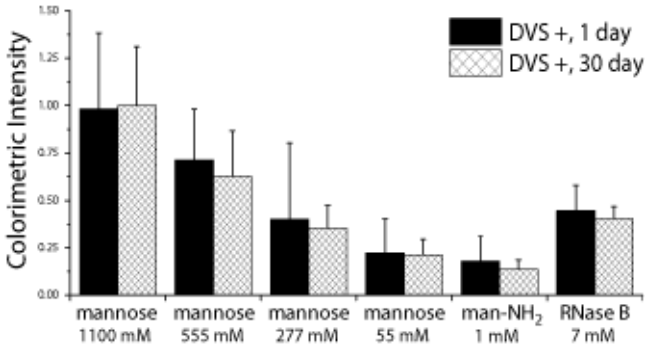


Figure S9. Colorimetric analysis of intensities of biomolecule spots on DVS-activated membranes does not reveal significant differences between membranes functionalized the day of DVS activation, and membranes functionalized 30 days after being stored in in aluminum foil. A Student's t-test with p = 0.05 did not yield any significant differences between any of the 1 day-30 day pairs.

References:

(1) Pastorin, G.; Wu, W.; Wieckowski, S.; Briand, J.P.; Kostarelos, K.; Prato, M.; Bianco, A. *Chem. Commun.* **2006**, 1182-1184.