Detection of Bacteria Using Inkjet-Printed Enzymatic Test Strips

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Supporting Information

Sensor Construction and Storage

The paper substrate was placed into the standard paper feed of the printer. For our work, patterning was done through the use of Microsoft PowerPoint software. A large checkerboard square pattern with two alternating materials was created to maximize the amount of sensors printed on each paper. In order to print only the channel of interest, the color of the letter has to match the channel printed. To print only the magenta channel, the RGB value must be set to (255,0,255). For cyan channel printing, (0,255,255) must be used and for yellow channel (255,255,0) must be used. The image color management (ICM) also must be turned off in the Advanced tab of the printer properties to ensure no mixing of the channels occurs. Before printing, the print heads were cleaned two times using the "Head Cleaning" function in the Maintenance tab of the printer properties to ensure that the channels were filled. After printing, the sensor sheet was cut into small circles through the use of a standard hole punch

and glued onto a 1 cm by 4 cm strip of card stock paper to provide rigidity to the sensor. Completed strips were kept refrigerated for up to week before being used in our study.

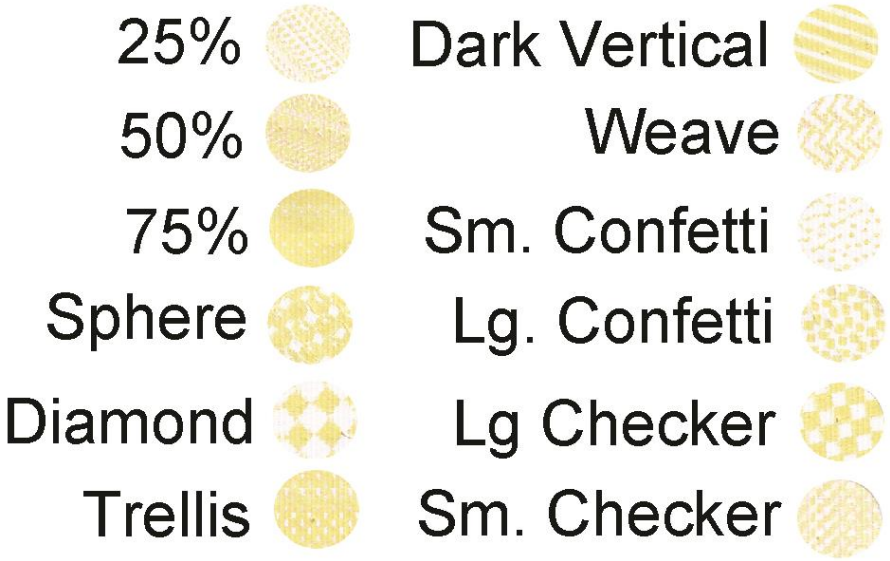

Figure S1. Representative patterned strips before immersion.

Profile Image Analysis

To quantitatively investigate the change in color across the sensor strip, one scanned strip image per pattern was analyzed using ImageJ software. By taking a representative 70 pixels of the image, a RGB Profile Plot was generated whose RGB values were converted to CMY color space. To compare the color response from pattern to pattern, we took the average yellow response of the strip before immersion and subtracted it from the average magenta response after dipping. Figure S2 shows that the small checker is as good as the diamond pattern but better than any of the other sensor designs. To quantify heterogeneity, we graphed the standard deviation of responses across the sensor surface. Figure S3 shows that the diamond pattern has by far the highest variation across the surface of all patterned surfaces. Taking these two graphs together indicates that the small checker pattern is the best to use for our system. The pattern that we used in PowerPoint can be seen in Figure S4.

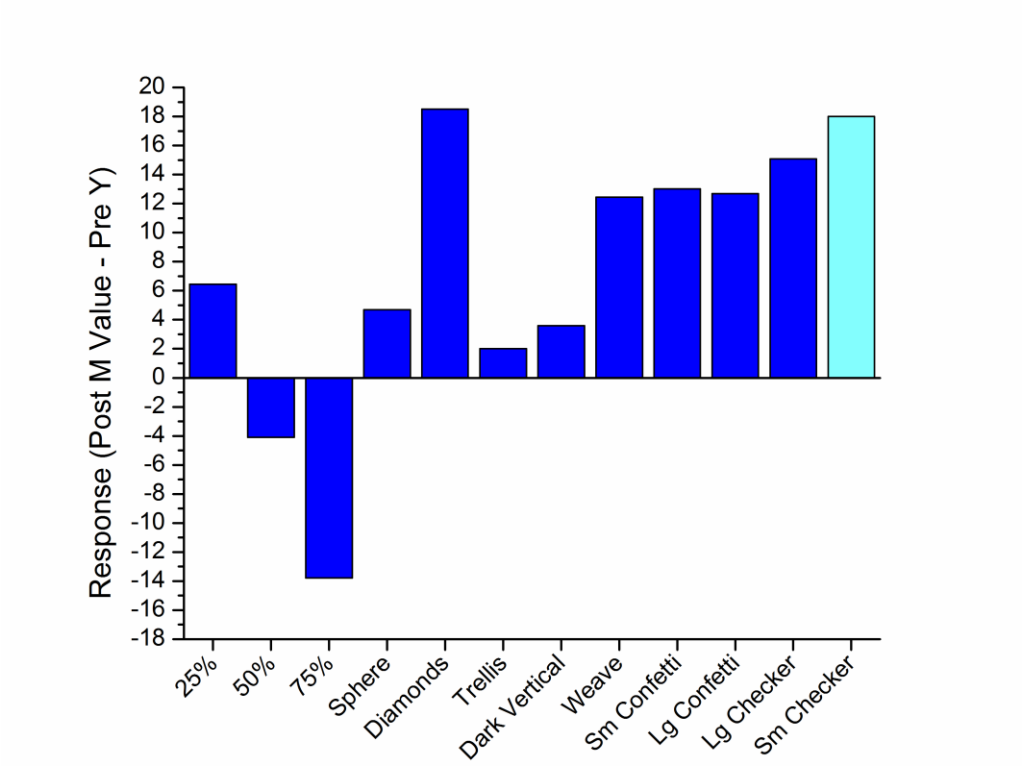

Figure S2. Change in color response of the 12 sensor patterns after immersion.

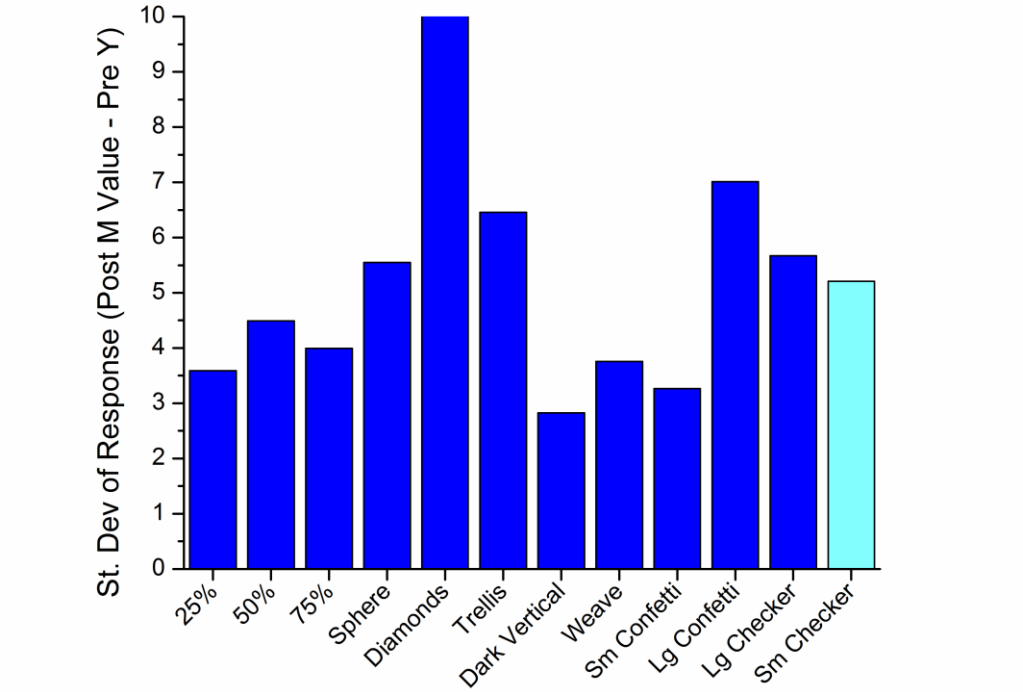

Figure S3. Variance in the color change of the 12 sensor designs.

Figure S4. Checkboard pattern used in Microsoft PowerPoint to generate the test strip functionality.

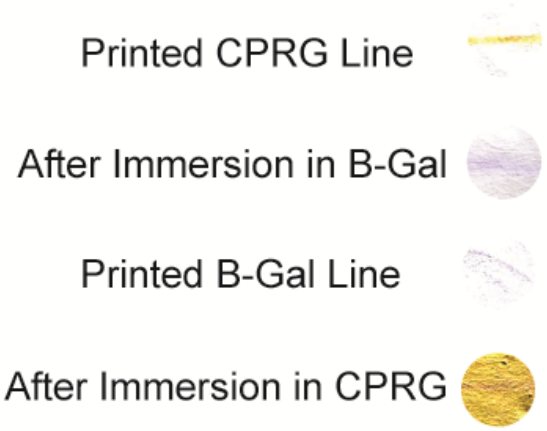

Figure S5. Motility experiments of both substrate and enzyme along the paper substrate.

CPRG Leaching Analysis

To quantitate the amount of CPRG that could possibly leach out into the analyte mixture, we incubated a completed test strip with 50 µL of MilliQ water. After five minutes, the strip was removed and 50 µL of a 0.5 nM β-galactosidase solution was added to the solution. The solution was incubated for 5 minutes and the color change was assessed at the 595 nm wavelength similar to our previous work[1]. A control solution was also analyzed containing enzyme and MilliQ water. Visually, both solutions appeared clear and no significant difference was seen in the absorbance values.

Solution	Absorbance at 595 nm
Control (No Strip)	.07±.01
Strip Incubated Water	.08±.01

Table S1. Absorbance values of the analyte solution after incubating with the sensor

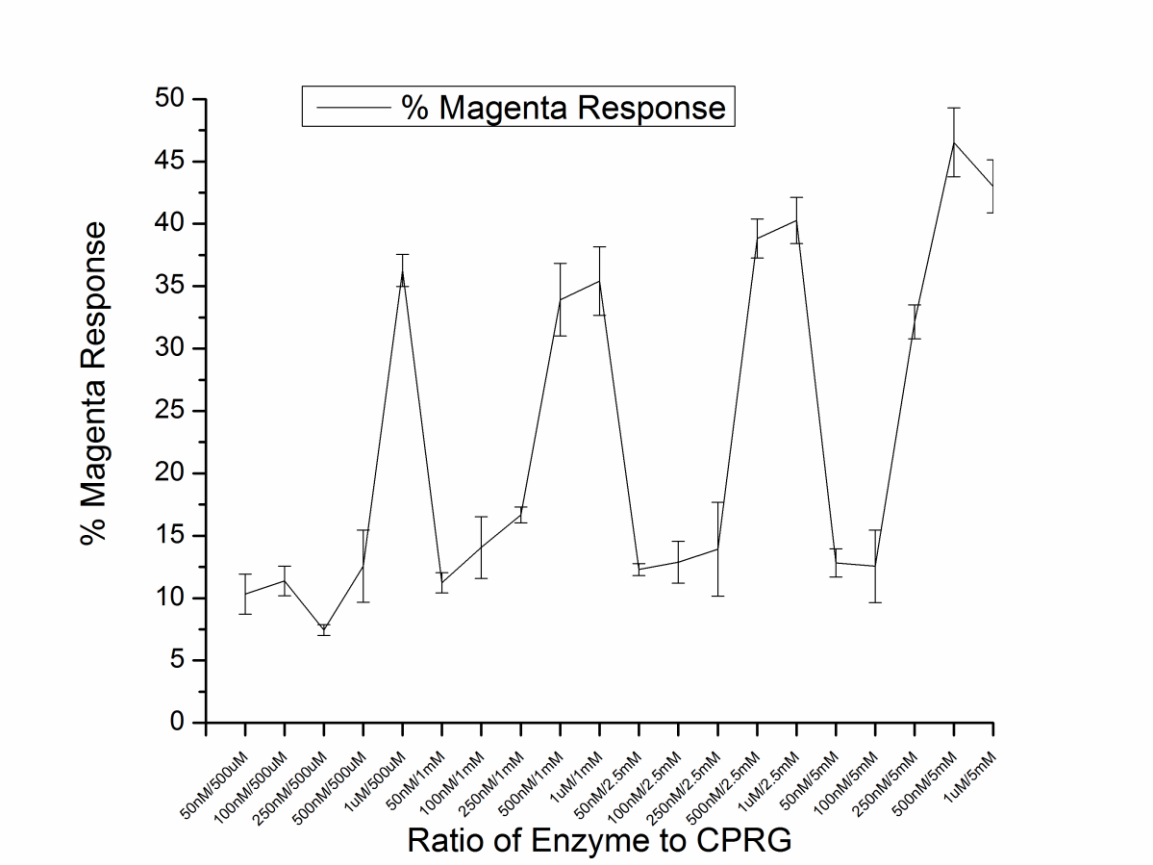

Figure S6. – Magenta response of the scanned test strips against the ratio of enzyme to printed CPRG. Error bars represent 6 measurements of each test strip.

Figure S7. NP-free test strip response against pH buffered MilliQ water solutions.

Figure S8. NP-free test strip response against pH buffered drinking water solutions at relevant pH levels.

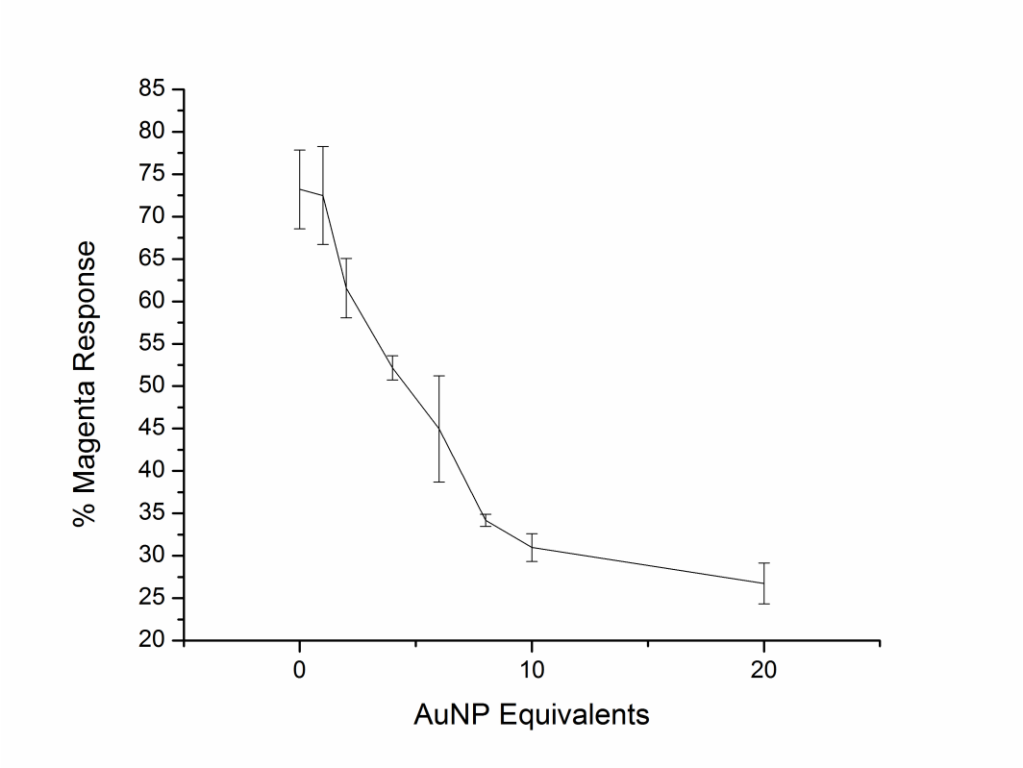

Figure S9. Magenta response against the amount of AuNPs equivalents needed to inhibit the enzyme. Error bars represent 6 measurements of each test strip.

Bacteria Culturing

Strains of both Escherichia coli (E. coli XL1 Blue; Gram-negative) and Bacillus subtilis (B. subtilis; Gram-positive) bacteria were cultured in lysogeny broth (LB) growth medium and successively washed via centrifugation with 5 mM PBS (pH 7.4). Both samples of each bacteria were adjusted to an OD of 1.0 at the 600nm wavelength, which relates to ~10^8 bacteria/mL. These bacteria were used within 48 hours after purification to ensure a viable sample.

Log (Concentration)	B. Sub	St Dev	E. Coli	St Dev
7	28.5	1.64317	38.5	5.95819
6	25.6667	2.87518	35.8333	7.19491
5	27.5	0.83666	33.3333	3.26599
4	26.3333	1.50555	34.1667	1.94079
3	14.3333	8.4538	30	1.67332
2	19.1667	3.0605	30.6667	1.8619
1	23	1.26491	29.1667	0.75277
0	22.3333	1.63299	28.6667	1.63299

Table S2. Cyan average percentages along with the standard deviation of 6 chosen spots taken from the scanned image of each test strip versus concentration of bacteria.

Log (Concentration)	B. Sub	St Dev	E. Coli	St Dev
7	67.6667	3.9833	80.5	2.73861
6	57.6667	2.58199	84.3333	2.80476
5	68	2.68328	63	4.38178
4	53.6667	2.50333	62.5	3.67423
3	41.6667	3.38625	50.6667	3.38625
2	22.3333	6.7429	33.1667	1.16905
1	26.3333	1.0328	31	0.89443
0	23.1667	1.47196	31.5	1.04881

Table S3. Magenta average percentages along with the standard deviation of 6 chosen spots taken from the scanned image of each test strip versus concentration of bacteria.

Log (Concentration)	B. Sub	St Dev	E. Coli	St Dev
7	9.66667	2.50333	23.3333	3.32666
6	11.5	4.03733	14.1667	9.9482
5	9.5	3.44964	35.1667	4.70815
4	24.5	2.16795	38.6667	1.50555
3	26	8.31865	46.1667	1.47196
2	50.8333	1.94079	63	7.58947
1	51.3333	2.87518	63.5	2.25832
0	58.1667	2.99444	63.6667	3.26599

Table S4. Yellow average percentages along with the standard deviation of 6 chosen spots taken from the scanned image of each test strip versus concentration of bacteria.

Concentration	% Cyan	St Dev
200	40.1667	0.98319
150	26.3333	5.20256
100	27.6667	1.21106
75	26.8333	2.99444
50	28.5	1.51658
25	21.1666	1.329
10	21.6667	6.121
0	14.3333	2.80476

Table S5. Cyan average percentages along with the standard deviation of 6 chosen spots taken from the scanned image of each test strip versus salt concentration.

Concentration	% Magenta	St Dev
200	78.6667	4.67618
150	74.6667	3.20416
100	38	1.89737
75	30.6667	4.2269
50	33.6667	2.42212
25	23.1667	.983192
10	22.3333	6.53197
0	16.6667	3.26599

Table S6. Magenta average percentages along with the standard deviation of 6 chosen spots taken from the scanned image of each test strip versus salt concentration.

Concentration	% Yellow	St Dev
200	28.6667	3.55903
150	16	8.50882
100	53.5	4.72229
75	63	5.32917
50	61.3333	4.17931
25	65.5	6.156
10	78.6667	9.75021
0	53.5	3.61939

Table S7. Yellow average percentages along with the standard deviation of 6 chosen spots taken from the scanned image of each test strip versus salt concentration.

Figure S10. Visual comparison of test strips where β-gal was not printed between a strip immersed in MilliQ water (Left) and concentrated *E. coli* XL1 bacteria (Right)

Metal Used	Inhibition Concentration	Maximum Acceptable Concentration for Drinking Water
Copper	5 mg/mL	1.3 mg/mL
Lead	>20000 ppb	15 ppb
Zinc	>20000 ppm	20 ppm
Cadmium	5 mg/mL	.005 mg/mL
Sulfate	>1 g/L	250 mg/L
Sodium Dodecyl Sulfate	>4 mg/L	1.0 mg/L

Table S8. Concentrations at which the test strips respond to several common water contaiminates as well as the current water regulations for that. Note that the maximum sodium dodecyl sulfate level is for all foaming agents in water and is not specific for this chemical.

References:

1.) Miranda, O. R.; Li, X.; Garcia-Gonzalez, L.; Zhu, Z.-J.; Yan, B.; Bunz, U. H. F.; Rotello, V. M. Colorimetric Bacteria Sensing Using a Supramolecular Enzyme–Nanoparticle Biosensor. *J. Am. Chem. Soc.* **2011**, *133*, 9650-9653.