ELECTRONIC SUPPLEMENTARY INFORMATION

Fluorescent DNAs Printed on Paper: Sensing Food Spoilage and Ripening in the Vapor Phase

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CONTENTS:

Synthesis of oligodeoxyfluoroside. Syntheses of monomers Y, E, B, and K were carried out using previous protocols (See Figure 1 in the main text for structure).[1] Spacer phosphoramidite (S) and 5,6-dihydro-dT phosphoramidite (H) were purchased from Glen Research. Using standard phosphoramidite chemistry, the tetrameric ODFs were synthesized on 1 µmol scale using 3-phosphate CPG solid support (Glen Research) as previously described.[2] Coupling times of 15 min were used in an ABI 394 DNA/RNA synthesizer. After cleavage from the solid support (0.05 M potassium carbonate in methanol, 24 h), the sequences were purified using a Shimadzu 10 Series HPLC equipped with a C4 column (Alltech Platinum EPS) and diode array absorbance detector. Acetonitrile and 0.05 M tetraethylammonium acetate were used as eluents. The sequences were confirmed using MALDI-TOF mass spectrometry and characterized by absorption and fluorescence emission spectroscopy (full spectra available).[2] The results are summarized in Table S1.

Charging ink cartridge with ODF and printing. HP Ink 60 black cartridges (Hewlett-Packard) were cut open manually at 1 cm from the top using a serrated blade (Figure S1, B and C). The absorbent sponge containing black ink was removed, and the empty cartridge was cleaned thoroughly by rinsing multiple times with water and finally with ethanol. Upon seeing no residual black ink, the cartridge was air dried and 70 µL of 50 µM of ODF solution (containing 5% PEG8000) was placed on top of the nozzle (Figure S1, red arrow in C) using a pipette. The top was placed back and secured using tape, after which the nozzle was gently wiped with a wet tissue paper until the ODF solution began to bleed outside. The cartridge was placed inside an HP Deskjet F4280 thermal inkjet printer, and 100% cotton (cellulose) paper (Canson Infinity Rag Photographique, 210 gsm, letter size) was placed in the printer feeder.

A 2 cm black square was designed with Illustrator 10 (Adobe) in CMYK color mode and printed in black and white mode with “best” quality. After printing, the paper with deposited ODF was repositioned back to the printer feeder and reprinted five more times at the same position. After printing, the prints were air-dried in dark at room temperature for two days and stored under the same condition up to six days. The printed ODFs showed bright colors against the background under a portable mercury lamp at 366 nm (UVP UVGL-58 Mineralight Lamp) and definite boundaries indicating good alignment throughout six rounds of printing (Figure S1, D and E).

Description of food spoilage and ripening. Detailed description of the foods and condition of spoilage and ripening is summarized below (Table S2). Initial changes in appearance or odor of the foods are also noted. Spoilage and ripening conditions can be found in the main text. To summarize, each food (20 g) was placed in a glass container topped with rubber septum under air, and they were allowed to spoil for a period of two weeks at room temperature (one batch of ground beef was spoiled at 4 ℃). Fresh food (20 g) from the same batch was also equilibrated in air-tight glass flasks for two hours before sampling. Chilled ground beef sample was allowed to warm to room temperature for 10 min prior to measurements with ODF. Whole fruits were allowed to ripen at room temperature for one week before being sliced into 1 cm^3 cubes (20 g) and placed in glass containers with septum for two hours before testing. Fresh fruits from the same lot were prepared in the similar way. 1 mL headspace gas from each food samples were drawn by an air-tight syringe, and injected slowly into the air-tight cuvette containing ODFs.

ODF image acquisition and data processing. Setup of ODFs in a cuvette and acquisition of epifluorescence images are described in the main text. Briefly, the ODFs on paper were cut into 1 mm squares and placed in a 5 mL quartz fluorescence cuvette with septum cap (Starna Cells) where volatile samples were injected by syringe slowly and equilibrated for 15 min (Figure S2, A). The fluorescence from ODFs was observed when placed under a portable mercury lamp at 366 nm (UVP UVGL-58 Mineralight Lamp). Examples of epifluorescence microscope images from ground beef spoiled at room temperature are shown (Figure S2, B). Quantitatively measurements of color changes were accomplished by averaging RGBL values (8-bit) over 200-pixel square at the center of the sensor using Photoshop CS5 (Adobe) (Figure S2, B and C).

Screening of paper substrate and polymer additive for ODF ink. Various solid substrates (glass, plastics, and paper-based) were tested for fluorescence quality, background signal, and drying characteristics. For testing, three 0.2 µL drops of 100 µM aqueous solutions of YSES or YSYK were placed on the substrate and air-dried in dark for 5 h. The dried spots were imaged under an epifluorescence microscope (Nikon Eclipse E800 equipped with Ushio USH-102DH short-arc mercury lamp, 4X objective, λ_{ex} = 340-380 nm, λ_{em} > 420 nm) using a Spot RT digital camera and Spot Advanced Imaging software. The images of dried spots and qualitative analysis are shown below (Figure S3 and Table S3). ODFs spotted on glass slides showed little fluorescence and minimal background. The diminished fluorescence can be due to dried ODFs

forming non-fluorescent precipitates against the polar surface. Plastic sheets yielded mostly faint to low ODF fluorescence and varied drying characteristics. Cellulose acetate (HP) and polyester films (Inkpress, 5 milli-inch thickness) showed some light-scattering bluish background. Cellulose acetate and cello (polypropylene, Cindus, transparent) allowed fast spreading of aqueous ODF drops, leading to coffee-ring behavior. Cellophane (Pacon Corp, transparent), polystyrene (Plastruct, clear, 0.3 mm thickness), and polyester sheets showed slower spreading and thus aggregated ODF residues. Paper-based substrates – loose leaf (Hilroy) and glossy photo paper (HP) showed higher background fluorescence, from optical-brightener additives, while black construction paper (Wausau) yielded least background and ODF fluorescence due to the black pigments absorbing much of the excitation light. Optical brightener-free papers showed high fluorescence to background ratio, and 100% cotton (pure cellulose) paper (Canson Infinity, 210 gsm), which fared better in drying characteristics compared to 75% wood pulp and 25% cotton paper (Inkpress, 220 gsm), were chosen as the standard paper.

Several polymer and small-molecule additives at different concentrations were screened for optimal fluorescence output. To 100 μM aqueous YSES solution, each additive at the specified concentration (w/v for polymers and v/v for small-molecules) was dissolved. All polymers and ethylene glycol were purchased from Sigma-Aldrich, while ethanol, isopropanol, and acetone were purchased from Fisher Scientific. Polymer characteristics are described in Figure S4. The solution was printed three times as 4 mm squares on 100% cotton paper (same setup as above), dried for two days, and imaged (24-bit) under an epifluorescence microscope (Nikon Eclipse E800 equipped with Ushio USH-102DH short-arc mercury lamp, 4X objective, λ_{ex} = 340-380 nm, λ_{em} > 420 nm) using a Spot RT digital camera and Spot Advanced Imaging software. The average luma values were extracted from 100-pixel square at the center of the image and compared to each other (Figure S4). Alcohols (ethanol and isopropanol) and acetone-containing ODF solutions spread quickly into the paper, causing some smearing in some cases. Cellulose was the only polymer that did not dissolve fully and the solid suspension was used instead, leading to decrease in overall fluorescence due to clogging of the ink cartridge nozzle. 5% PEG8000 was chosen due to its yielding the brightest fluorescence, and the slight increase in viscosity of the solution (1-3 cP) led to better jetting of the dyes from the nozzle of the ink cartridge.[3]

Effect of storage of printed ODFs on response to moisture. To see the effect of storage on ODF response, moisture was selected as the reference analyte. 1 mL headspace gas from standing water (saturated water vapor at 20 ℃) was used. The experimental setup and data acquisition are described above. Each ODF aged two to six days from the same batch of printing (50 μM, printed six times on 100% cotton paper) measured the response to moisture against air background, and the sum of the absolute changes in ΔRGB values was plotted (Figure S5). The responses were generally constant for all ODFs from two to six days, ensuring that storage had little effect on the sensitivity of ODFs to volatile samples.

Time course measurements of ground beef spoilage. Two different sources of ground beef (see Table S2) were allowed to spoil as described in the main text at room temperature and 4 ℃. 1 mL of headspace gas was tested against the six printed sensors for fifteen days (every day for the first week and once every two days after). Ground beef spoiled at 4 ℃ was allowed to warm to room temperature briefly (10 min) before drawing headspace gas. Each measurement represents average of signals from triplicate trials. Measurements at day 0 were taken after a two-hour equilibration in the sealed glass flasks. ΔRGBL values calculated from day 0 were subtracted from ΔRGBL values for each subsequent day. Sum of the absolute changes in RGB channels are plotted against time (see Figures 5 in the main text), and channel-specific responses are also given for ground beef at room temperature (Figure S6) and 4 ℃ (Figure S7).

Time course measurements of milk spoilage. The milk sample (see Table S2 for description) was split into three aliquots (20 g each) and these were allowed to spoil separately but in the same location at room temperature as described in the main text. Care was taken in treating each aliquot in the same manner to approximate equal bacterial exposure. Visible curdling and sour smell were noticeable at the same time (day 4). 1 mL of headspace gas was tested against the six printed sensors for fifteen days (every day for the first week and once every two days after). Each aliquot was measured once with each ODF sensor. Measurements at day 0 were taken after a two-hour equilibration in the sealed glass flasks. ΔRGBL values calculated from day 0 were subtracted from ΔRGBL values for each subsequent day. Sums of the absolute changes in RGB channels are plotted against time (see Figure 6 in the main text), and channel-specific responses for each aliquot are also given (Figure S8).

Sequence	*Calculated Mass*	*Found*[a]	λ_{max}, *abs (nm)*[b]	λ_{max}, *em (nm)*[c]
YSES	1188.23	1189.9	345	488
YSYK	1473.37	1471.45	344	394
YYEK	1719.42	1722.73	345	479
EKEH	1695.42	1697.69	443	447
BBBK	1819.45	1820.56	378	411
YYSB	1388.29	1389.34	345	491

Table S1. Characterization of ODF sequences, listed from 5' to 3' direction.[2] [a]MALDI-TOF mass spectrometry data. [b]Absorbance spectra of 1 µM ODF in 1x phosphate-buffered saline solution (pH 7.4) using a Cary 100 Bio UV-vis spectrometer at room temperature. [c]Fluorescence emission spectra of 1 µM ODF in 1x phosphate-buffered saline solution (pH 7.4) using a Jobin Yvon-Spex Fluorolog 3 spectrometer at room temperature (ex = 345 nm, em > 375 nm, slit width = 5 nm).

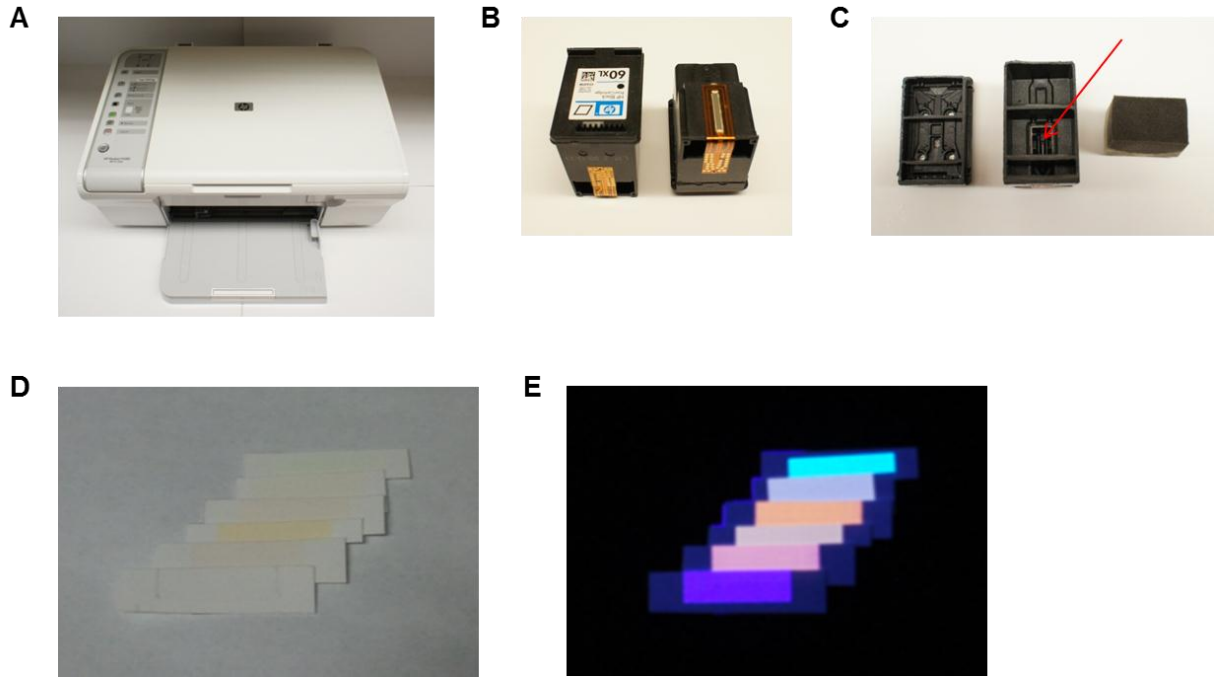

Figure S1. Setup of ODF printing using a thermal inkjet printer. (**A**) HP Deskjet F4280 was used. (**B**) HP Ink 60 black cartridge shown upright (left) and upside-down exposing the nozzle, where microdroplets of ODF solutions are expelled (right). (**C**) Ink cartridge after cutting open from the top. The top piece (left) uncovers to expose the absorbent sponge containing black ink (right), which after removing, the chamber facing the nozzle (red arrow) is unmasked. Aqueous solutions containing ODF are pipetted where the red arrow is marked. (**D**) Strips (2 cm in length)

of ODFs printed on 100% cotton paper six times and arranged linearly. (From the top: YSES, YYEK, YSYK, EKEH, BBBK, and YSYB). (**E**) The printed ODFs are clearly visible under a portable mercury lamp (366 nm, photographed by a digital camera). Notice the fluorescence output of ODF against faint blue background. The colors are imaged with higher quality under epifluorescence microscope (see Figure 2 in the main text and Figure S2).

Food	*Description*	*Lot/Identifier #*	*Temp (°C)*	*Spoilage Time (week)*	*Ripening Time (week)*	*First Visual/Olfactory Observation (day)*
Ground beef	Safeway Extreme Value 20% fat, fresh	Safeway Store #2719	rt	2		browning, bubbling, putrid smell (2)
Ground beef (second batch)	Safeway Extreme Value 20% fat, fresh	Safeway Store #705	4	2		bubbling, putrid smell (6)
Shrimp	Waterfront Bristro, raw, medium 51/60 per lb, tail & shell on	S2584 2021	rt	2		reddening, putrid smell (2)
Milk	Lucerne, reduced fat (2%) pasteurized	A4134 284	rt	2		curdling, bubbling, sour smell (4)
Cheese	Lucerne, sharp cheddar, natural block (8 oz)	S1339F 297 04	rt	2		mold - white (10)
Orange juice	Tropicana, 100% pure, no pulp pasteurized	48KD 0832	rt	2		cloudy (10)
Lettuce	Tanimura & Antle (USA)	#4061, A3 1058	rt	2		slime - green (12)
Bread	Safeway Kitchens, white	R3 1058	rt	2		mold - dark blue (7)
Peach	Flown Ripe (Chile), yellow-flesh	#4044	rt		1	soft, ripened smell (7)
Banana	Chiquita (Ecuador), yellow	#4011	rt		1	brown-yellow, ripened smell (5)
Tomato	Red Sun (Mexico), red round	#4664	rt		1	dark red, soft, ripened smell (7)

Table S2. Description of foods spoiled and ripened. First sign of visual or olfactory change and the corresponding day was noted. All foods except the second batch of ground beef (spoiled at 4 ℃) were purchased from the same local supermarket.

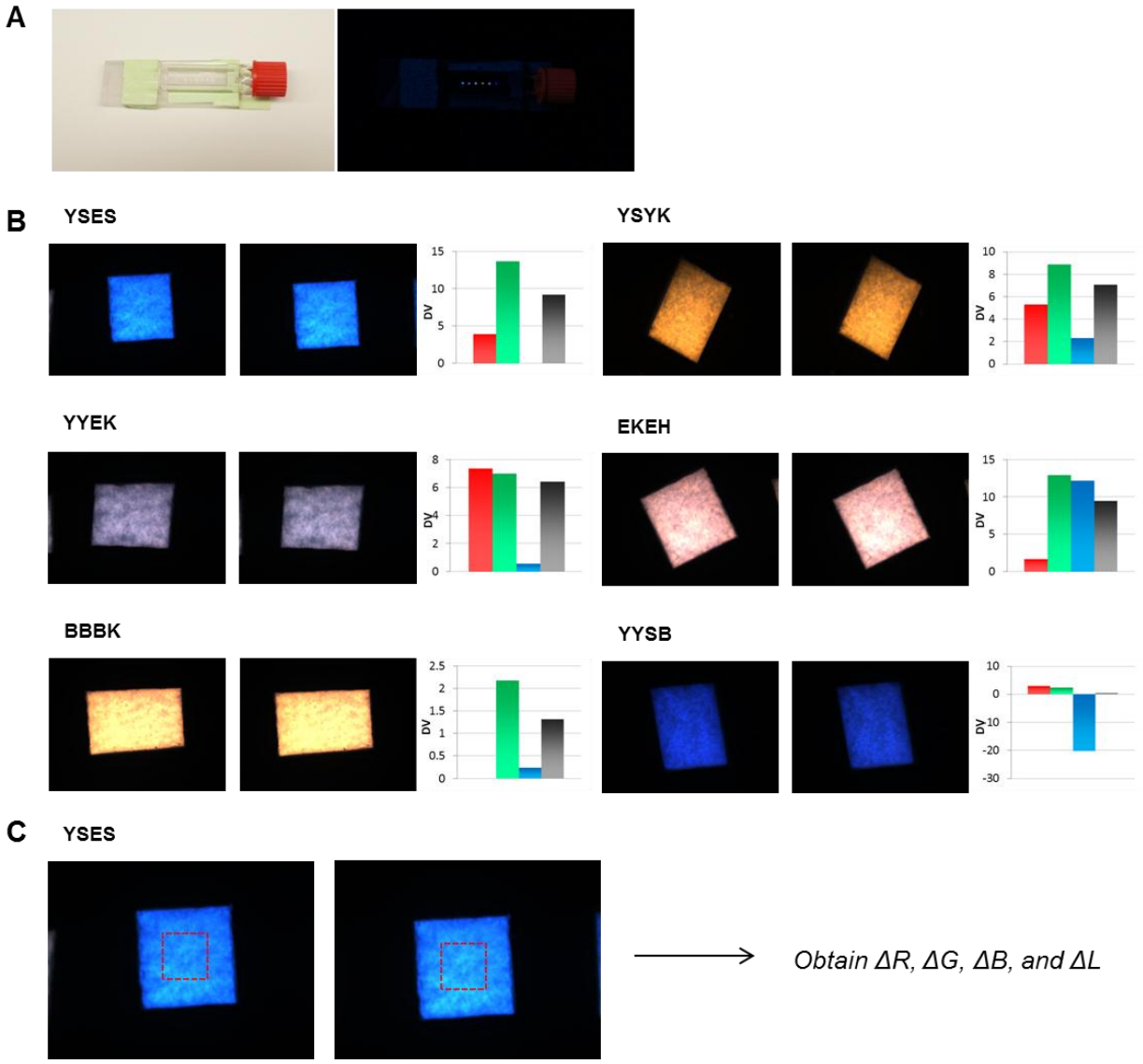

Figure S2. Imaging and data processing for ODFs printed on cotton paper. (**A**) *Left*: setup of ODFs (50 μM, printed six times on 100% cotton paper) in a fluorescence cuvette. *Right*: fluorescence is evident under a portable mercury lamp (366 nm, photographed). (**B**) Epifluorescence microscopy images using color CCD camera. All ODFs are excited by a single light source (340-380 nm). *Left*: images before exposure (air background). *Center*: after fifteen-minute exposure to headspace gas (1 mL) of ground beef spoiled at room temperature for two weeks. *Right*: Difference in digital values (DV) of red, green, blue, and luma channels (ΔRGBL) after exposure to gas sample. The values were averaged within 200-pixel square at the center of image and are shown in red, green, blue, and grey bars, respectively. (**C**) Illustration of a 200-pixel square selection (shown in red) at the center of ODF where average RGBL values were extracted. *Left*: YSES before exposure to the ground beef headspace gas as described in (B). *Right*: after fifteen-minute exposure. The two RGBL values before and after exposure yielded ΔRGBL values, a quantitative measure of ODF response to volatiles.

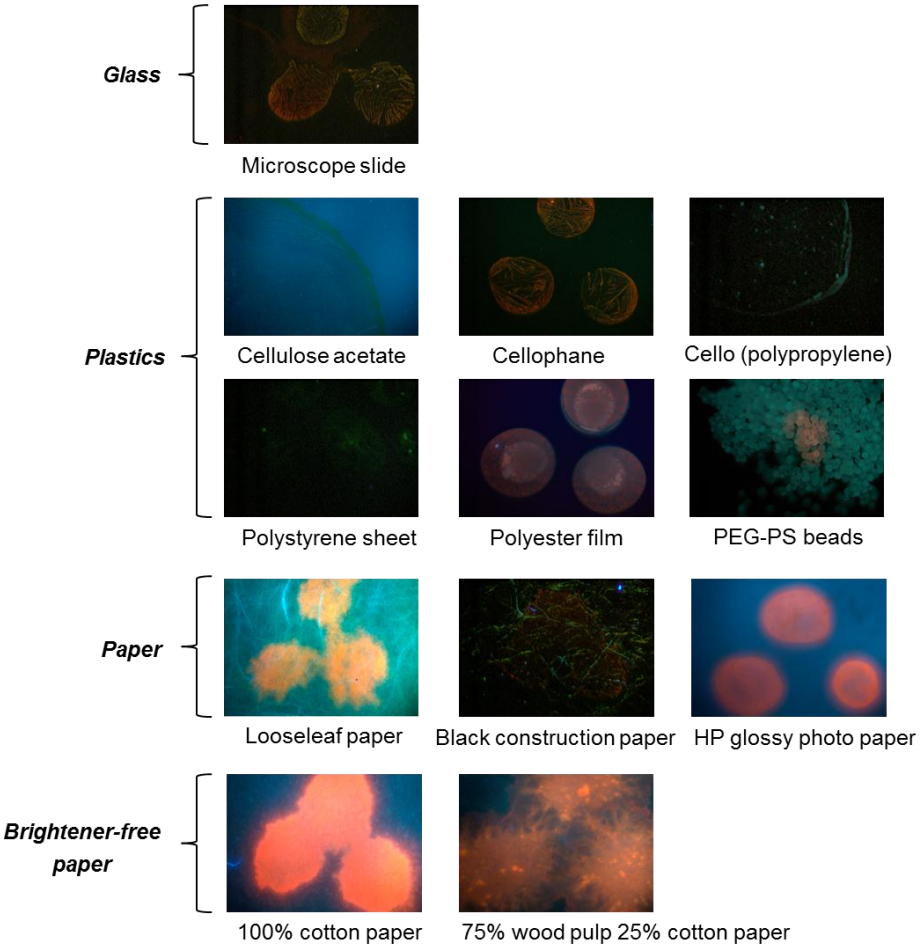

Figure S3. ODFs spotted on various solid surfaces for substrate screening. Three drops (0.2 μL) of 100 μM YSYK (reddish fluorescence color) or YSES (greenish fluorescence color) was spotted and dried (room temperature, dark, 5 h) and imaged under an epifluorescence microscope.

Substrate	*Intensity of ODF fl.*	*Background*	*Drying Characteristics*		*Comment*
			Spreading	*Dried shape*	
Glass, microscope slide	Faint/low	Faint	Large	Circular, uneven	Weak fluorescence
Cellulose acetate	Faint	Strong blue	Large	Ring-stain	Coffee-ring effect, high background
Cellophane	Low	Faint	Small	Uneven residues	Crystalline residue
Cello	Faint	Faint	Large	Circular, uneven	Weak fluorescence
Polystryene sheet	Low	Weak blue	Small	Even, some streaks	Best plastic tested
PEG-PS beads	Moderate	Weak blue	Large	Adheres to beads	Weaker fluorescence than covalent attachment
Looseleaf paper	Strong	Strong light blue	Large	Uneven absorption	Bright background
Black construction paper	Faint	None	Large	Adheres to fiber of paper	Paper absorbs excitation light
HP glossy photo paper	Strong	Strong light blue	Small	Even, some streaks	Even drying on glossy surface, bright background
75% wood pulp, 25% cotton paper	Moderate	Weak blue	Large	Non-circular absorption	Fast absorption but uneven, low background for paper
100% cotton paper	Strong	Weak blue	Small	Even, circular	Best paper tested

Table S3. Qualitative analysis of ODFs spotted on various substrates. See Figure S3 for details.

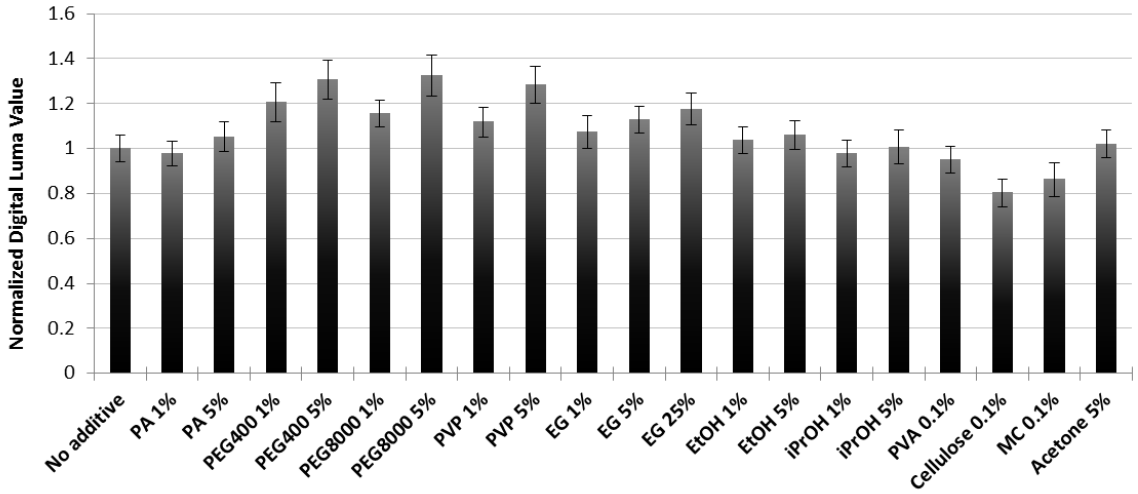

Figure S4. Comparison of fluorescence output of YSES with various polymer and small-molecule additives. 100 µM YSES with each additive was printed three times on 100% cotton paper. Average luma values over 100-pixel square of triplicate experiments were normalized and graphed. Abbreviations and polymeric characteristics are as follows: PA = polyacrylamide, M_w = 10,000; PEG = polyethylene glycol; PVP = polyvinylpyrrolidone, M_w = 10,000; EG = ethylene glycol, EtOH = ethanol; iPrOH = isopropanol; PVA = polyvinyl alcohol, 87-89% hydrolyzed, M_w = 31,000-50,000; cellulose = cellulose powder, 20 µm microcrystalline; MC = methylcellulose, viscosity 15 cP, 2% in water at 20 ℃. Error bars indicate standard deviation from triplicate measurements.

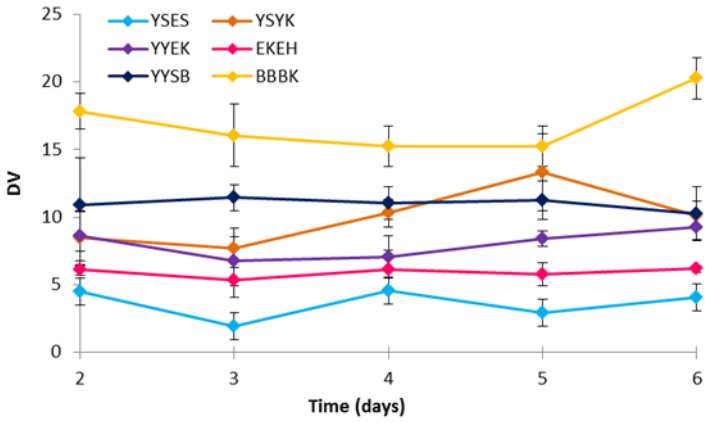

Figure S5. Plot showing nearly flat response of ODFs to 20% saturated water vapor during a storage period. The magnitude of response was represented by the sum of absolute changes in RGB digital values (DV) after exposure (DV = |ΔR| + |ΔG| + |ΔB|). Error bars indicate standard deviation after triplicate measurements.

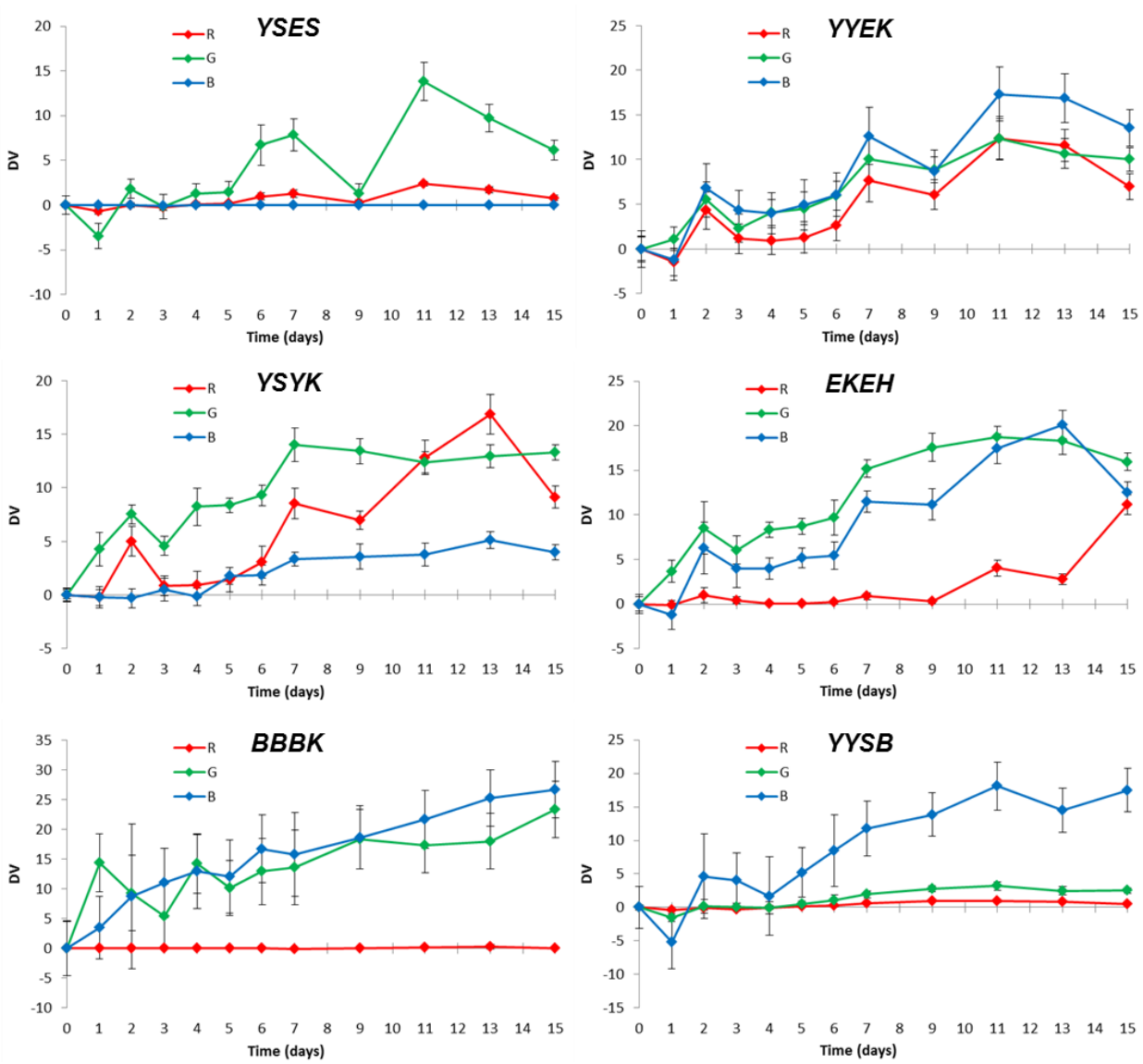

Figure S6. Time course ODF response to room temperature ground beef, broken into R,G,B channel responses. The y-axis represents difference between ΔR, ΔG, ΔB, and ΔL digital values (DV) of each day and the start (day 0). Error bars represent standard deviation of triplicate measurements.

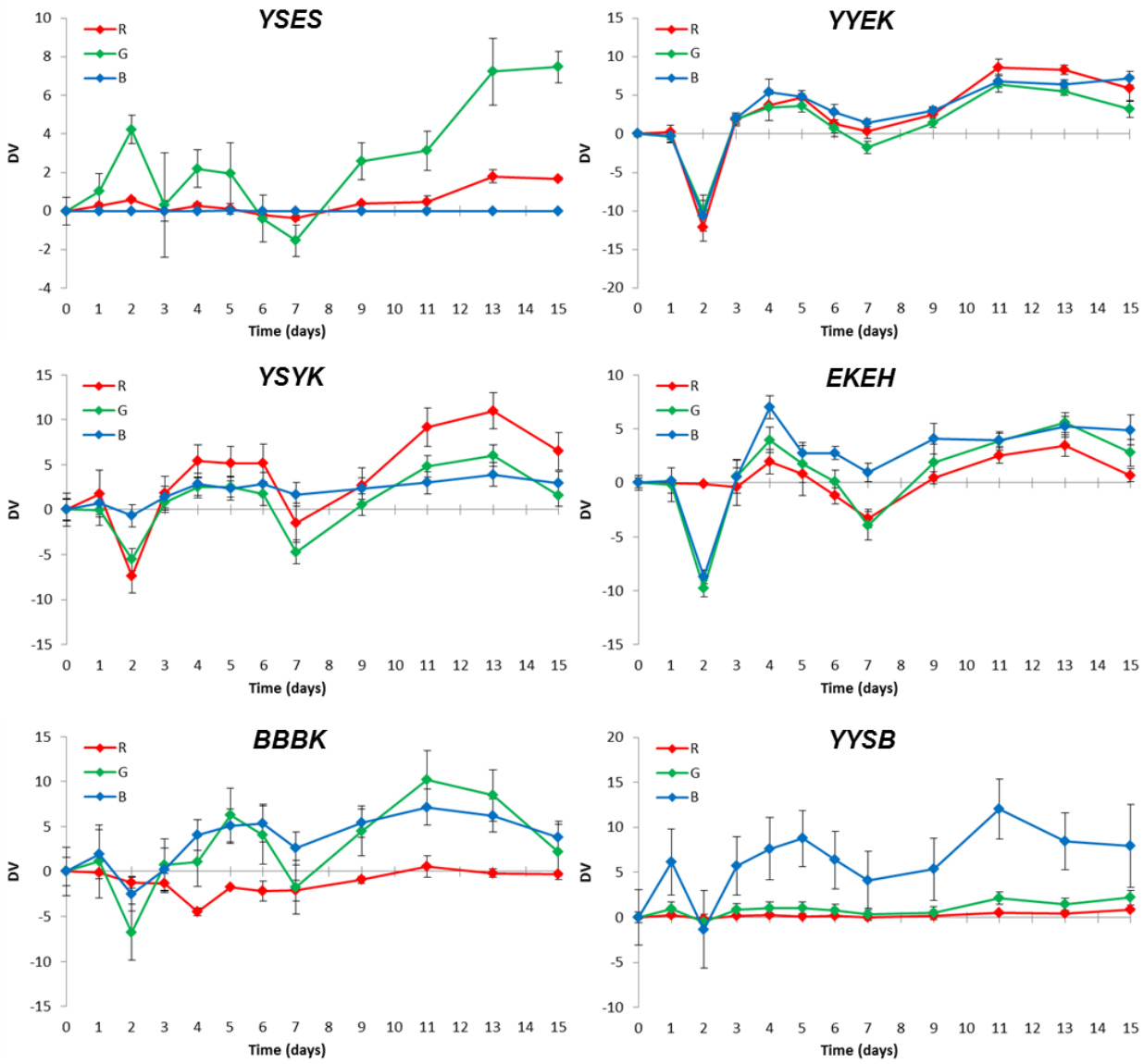

Figure S7. Time course ODF response to 4 ℃ ground beef, broken into R,G,B channel responses. The y-axis represents difference between ΔR, ΔG, ΔB, and ΔL digital values (DV) of each day and the start (day 0). Error bars represent standard deviation of triplicate measurements.

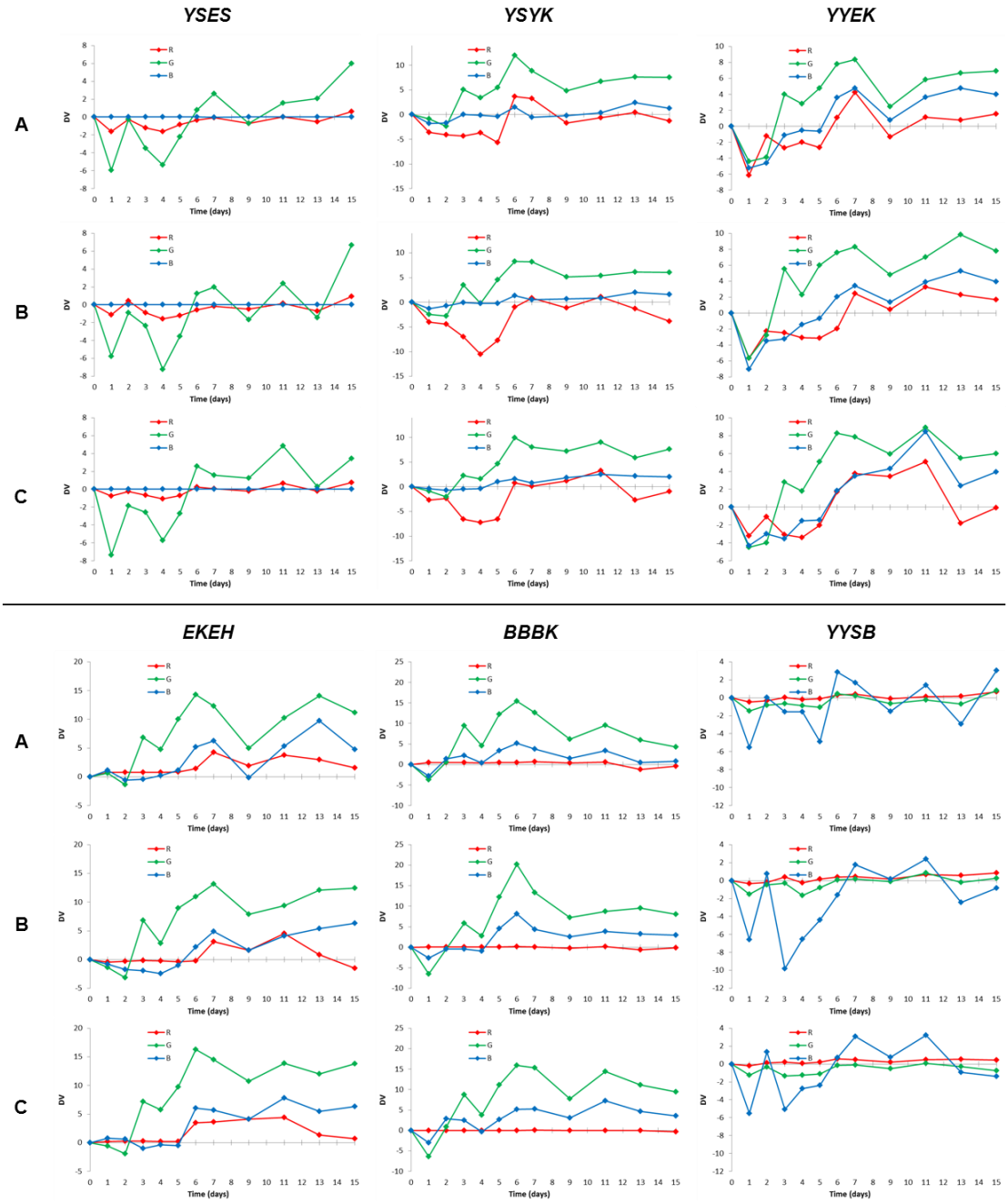

Figure S8. Time course ODF response to three aliquots of room temperature milk, broken into R,G,B channel responses. Three aliquots (**A**, **B**, and **C**) were measured once with each ODF sensor on paper at each time point. The y-axis represents difference between ΔR, ΔG, ΔB, and ΔL digital values (DV) of each day and the start (day 0).

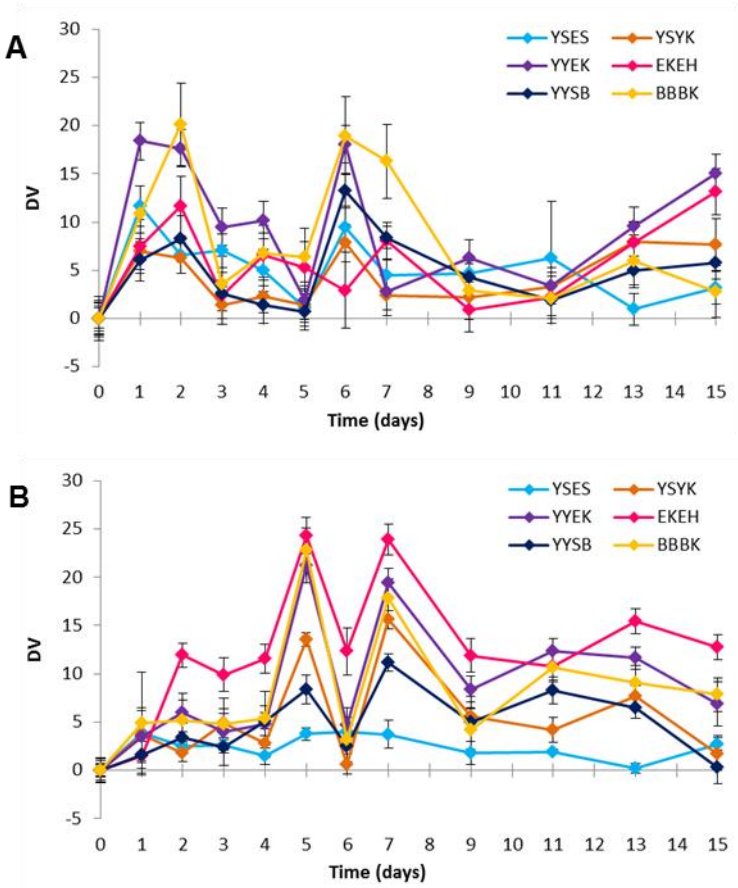

Figure S9. Time course ODF response to two batches of room temperature milk from different production lots and spoiled at different times, showing differences in responses, apparently due to differences in bacterial populations. Two samples (**A** and **B**) from different batches of the same brand were measured in triplicate with each ODF sensor at each timepoint. The y-axis represents sum of absolute changes ΔR, ΔG, ΔB, and ΔL digital values (DV = $|\Delta R| + |\Delta G| + |\Delta B|$) from each day to the start (day 0).

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