Supporting Information for

Electrochemical DNA-based sensors for molecular quality control: continuous, real-time melamine detection in flowing whole milk

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Materials and methods.

Materials. Melamine, cyuaric acid, 6-Mercapto-1-hexanol, and tris(2-carboethyl)-phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were used as received. Undiluted whole milk was purchased from Costco, Inc. For electrode polishing, 2 and 7/8" microcloth, 1 μm monocrystalline diamond slurry (in a petroleum distillates-based slurry), and 0.05 μm alumina powder were obtained from Buehler (IL, USA). The methylene blue and thiol-modified DNA sequences were synthesized by LGC Biosearch Technologies, purified by C18 HPLC and PAGE, confirmed by HPLC profile and mass spectrometry. These were dissolved in TE buffer (1×) (10 mM tris(hydroxymethyl)aminomethane, 1 mM EDTA, pH 8.0) to a final concentration of 100 μM, aliquoted and stored at -20°C prior to use.

Sensor Fabrication. E-DNA biosensors were fabricated as previously described.[1] Briefly, we first polish 2 mm diameter gold disk electrodes (CHI instruments, Austin, TX) on a micro-cloth soaked with diamond slurry followed by sonication in ethanol for 5 min. We further polish the electrodes using a micro-cloth soaked in an aqueous suspension of 0.05 μm alumina followed by sonication in water for another 5 min. After this we clean the electrodes electrochemically by cycling 1000 times between -0.4 and -1.35 V (all potentials versus Ag/AgCl) at 2 V s^{-1} in aqueous 0.5 M NaOH using a three-electrode setup. The working electrodes are then rinsed thoroughly with water and transferred into 0.5 M H_2SO_4 where we apply an oxidizing potential of 2 V

for 5 s followed by a reducing potential of -0.35 V for 10 s followed by cycling between -0.35 and 1.5 V for 20 cycles at 4 V s^{-1} and for 4 cycles at 0.1 V s^{-1}.

Immediately prior to sensor fabrication we prepare a solution of thiol-and-methylene-blue-modified DNA in phosphate buffered saline buffer (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.0) by incubating a solution of 100 μM DNA and 20 mM tris-(2-carboxyethyl) phosphine hydrochloride (1:200) for 1 hr at room temperature followed by dilution with PBS to 200 nM as confirmed by UV-Vis spectroscopy. We then immerse freshly cleaned electrodes in this solution for 1 hr at room temperature. The resulting sensors are washed with deionized water and then incubated in 20 mM 6-mercaptohexanol solution in PBS overnight at 4°C before being rinsed with water prior to use.

Electrochemical measurements Electrochemical measurements were performed at room temperature using a CHI630C potentiostat with a CHI684 Multiplexer (CH Instruments, Austin, TX) and a standard three-electrode cell containing a platinum counter electrode and an Ag/AgCl (3M NaCl) reference electrode. Square wave voltammetry (SWV) was performed using a potential window of -0.05 to -0.4 V, a potential step of 0.001 V, an amplitude of 0.05 V at 500Hz.

The continuous, real-time measurements were conducted in a circulated system using a gear pump (Model: 74013-55, Cole-Parmer; IL, USA) through a closed loop consisting of a 30 mL sample reservoir and two 4 mm-diameter and 25 cm-long plastic tubes.[1]

Table S1. DNA sequences employed here for melamine recognition.

	DNA Sequence (from 5' to 3')
T12	HS-C6-TTTTCCCCTTTT-MB
T20	HS-C6-TTTTTTTTCCCCTTTTTTTT-MB
T28	HS-C6-TTTTTTTTTTTTCCCCTTTTTTTTTTTT-MB

Table S2. The binding properties of our three sensors.

	PBS buffer		Whole milk	
	$K_{1/2}$ (μM) [a]	n_H [a]	$K_{1/2}$ (μM) [a]	n_H [a]
T12	490 ± 10	1.9 ± 0.1	463 ± 10	1.2 ± 0.2
T20	241 ± 3	2.7 ± 0.1	272 ± 8	2.3 ± 0.1
T28	276 ± 4	2.9 ± 0.1	299 ± 5	2.8 ± 0.1

[a] Error range reflects that the uncertainty in the fit parameters when these response curves are fitted by Hill equation with 97% confidence intervals.

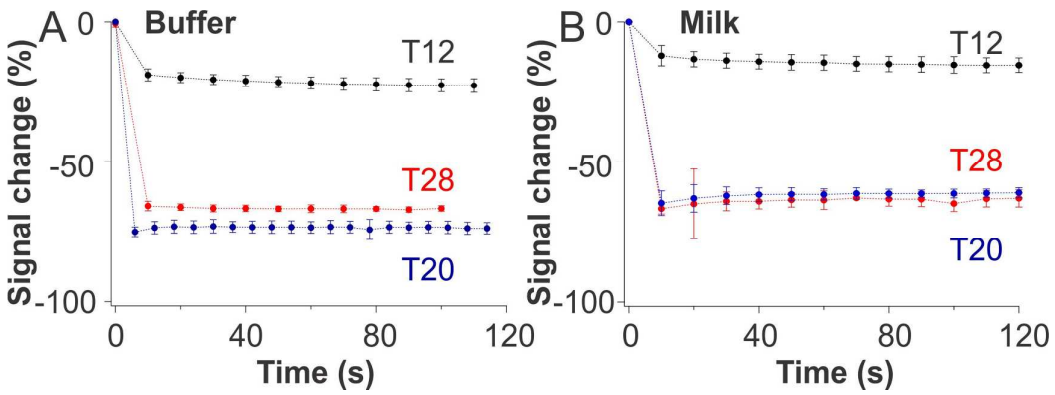

Figure S1. Sensors built from all three constructs are effectively fully equilibrated within the few seconds required to perform the first square-wave scan, whether in (A) a simple buffered solution or (B) in undiluted whole milk.

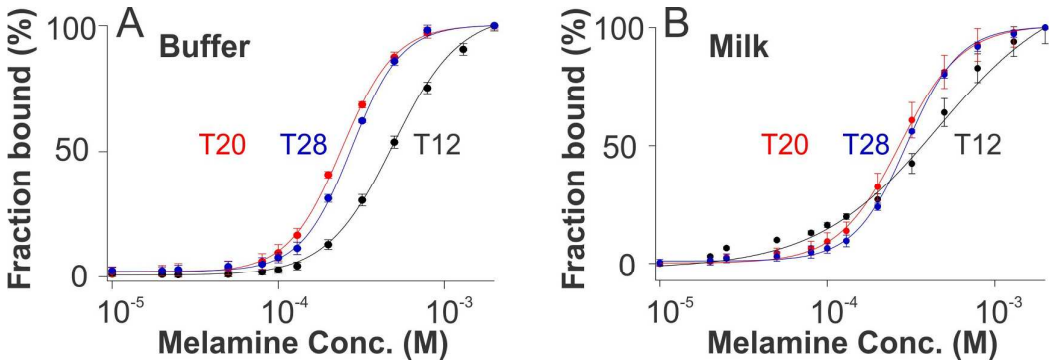

Figure S2. "Fraction bound curves" for sensors fabricated using T12, T20 and T28 in (A) simple buffer or (B) undiluted whole milk. Due to their thymine rich nature, these constructs are cooperative, steepening their binding curves. They are less cooperative, however, when challenged in milk rather than in the simpler sample matrix.

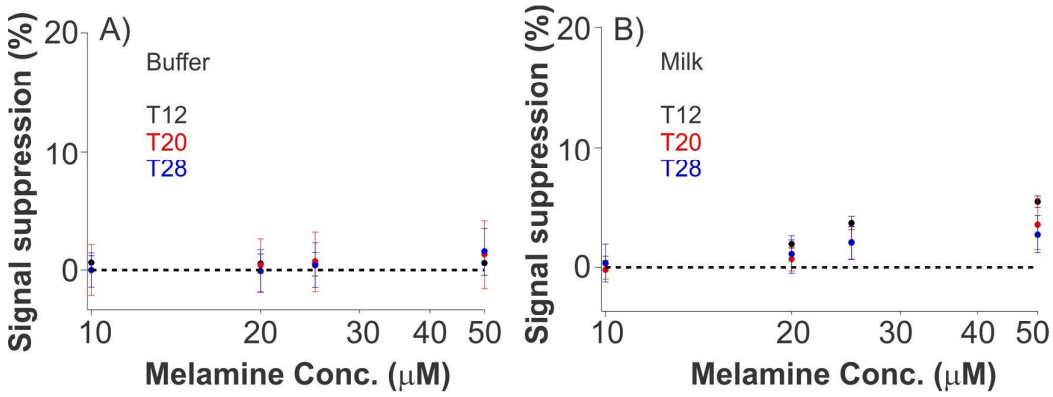

Figure S3. Signal response of sensors fabricated using T12, T20 and T28 at low concentration range in (A) simple buffer and (B) undiluted whole milk.

Reference:

1. Li, H.; Arroyo-Currás, N.; Kang, D.; Ricci, F.; Plaxco, K. W. *J. Am. Chem. Soc.* 2016, **138**, 15809-15812.