Published in final edited form as:

Food Anal Methods. 2018 September ; 11(9): 2419–2430. doi:10.1007/s12161-018-1228-8.

Cyclodextrin-Promoted Fluorescence Detection of Aromatic Toxicants and Toxicant Metabolites in Commercial Milk Products

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Abstract

The detection of polycyclic aromatic hydrocarbons (PAHs) and their metabolites in food and in agricultural sources is an important research objective due to the PAHs' known persistence, carcinogenicity, and toxicity. PAHs have been found in the milk of lactating cows, and in the leaves and stems of plants grown in PAH-contaminated areas, thereby making their way into both cow milk and plant milk alternatives. Reported herein is the rapid, sensitive, and selective detection of 10 PAHs and PAH metabolites in a variety of cow milks and plant milk alternatives using fluorescence energy transfer from the PAH to a high quantum yield fluorophore, combined with subsequent array-based statistical analyses of the fluorescence emission signals. This system operates with high sensitivity (low micromolar detection limits), selectivity (100% differentiation even between structurally similar analytes), and general applicability (for both unmodified lipophilic PAHs and highly polar oxidized PAH metabolites, as well as for different cow and plant milk samples). These promising results show significant potential to be translated into solid-state devices for the rapid, sensitive, and selective detection of PAHs and their metabolites in complex, commercial food products.

Keywords

polycyclic aromatic hydrocarbons; cyclodextrin; commercial milk; fluorescence spectroscopy

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitous organic pollutants known for their long-term environmental persistence (Manzetti 2013; Marni et al. 2013), suspected carcinogenicity (Jarvis et al. 2014), and well-documented toxicity to humans and animals (Balcioglu 2016; Hayakawa 2016). PAHs are released into the environment via the incomplete combustion of petroleum products through natural and anthropogenic events, including forest fires (Wang et al. 2017), oil spills (Allan 2012; Forth et al. 2017), vehicular

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

No human subjects were involved in this study.

Informed consent: Not applicable

Supporting Information

Synthesis of fluorophore **11** and summary tables and figures for all experiments can be found in the supporting information.

emissions (Ma et al. 2017), and tobacco products (Vu et al. 2015). Once released, PAHs spread widely throughout the environment, persisting in the water (Zgheib 2008), air (Brown et al. 2013), soil (Ruby et al. 2012), and sediment (Wang et al. 2015). PAHs are known to bioaccumulate and biomagnify in the food chain, and can cause substantial toxicity to humans (Wang et al. 2017) and other organisms (Mearns et al. 2015).

Human exposure to PAHs through the aforementioned events has been well-documented, and individuals who have been exposed to PAHs often contain oxidized PAH metabolites in their breast milk (Madhavan et al. 1995), blood (Qin et al. 2011), and urine (Jongeneelen et al. 1987). To understand the extent of human exposure to PAHs, sensitive, selective, and reliable detection methods are necessary. Currently used methods for PAH detection rely on the use of separation techniques such as liquid chromatography (Nieva-Cano et al. 2001) or gas chromatography (Khalili et al. 1995) followed by detection via mass spectrometry (Aiken et al. 2007) or fluorescence spectroscopy (Delgado et al. 2004). Although these methods are highly sensitive, they are time-consuming and expensive (Frysiner et al. 2003), and thus limit the practical applicability of these methods as well as the ability to conduct rapid, high throughput assays of large populations to measure widespread toxicant exposure.

Previous work in our laboratory has focused on the use of cyclodextrin-promoted energy transfer for the rapid, sensitive, and selective fluorescence detection of aromatic toxicants (Mako et al. 2012; Serio et al. 2013b; Serio et al. 2014b; Serio et al. 2015c). We have shown that such detection operates successfully in human urine (DiScenza et al. 2016a; Serio et al. 2014a) and breast milk (DiScenza et al. 2017a), that it can be used for the detection of non-polar aromatic toxicants and highly polar toxicant metabolites (Serio et al. 2015b), and that it can form part of oil spill remediation strategies using cyclodextrin-promoted PAH extraction followed by fluorescence detection (Serio et al. 2013a; Serio et al. 2015a; Serio and Levine 2015; Serio and Levine 2016). Non-photophysically active analytes have also been detected using a modified cyclodextrin-promoted fluorescence modulation (DiScenza and Levine 2016a,b; DiScenza et al. 2016b; DiScenza et al. 2017b).

One fluid that is particularly important as a target for toxicant detection is cow milk, because of its well-documented prevalence in the human diet (Haug et al. 2007; Rozenberg et al. 2016). Cow milk has a high fat content, which enables non-polar lipophilic PAHs to readily accumulate (Abou-Arab 2014; Girello et al. 2014). In fact, researchers have found that lactating cows in PAH-contaminated regions excrete PAHs and PAH metabolites in their milk (Srogi 2007). In addition to widespread cow milk consumption in the human diet, plant milk consumption (*i.e.* almond milk, cashew milk, and soy milk) has become increasingly popular (Amini 2010; Massey 2003), and these plants can also contain PAH contamination. Such contamination is exacerbated by the presence of waxy substances on leaves that promote the accumulation of hydrophobic compounds, including PAHs (Howsam et al. 2001; Srogi 2007). The presence of PAHs and PAH metabolites in cow milk and plant milk alternatives and subsequent consumption by humans can lead to exposure-related diseases, although the correlation between toxicant exposure and the development of exposure-related disease remains poorly understood. Obtaining better methods for detecting such exposure is a crucial first step in understanding the relationship between PAH exposure and the development of such disease (Srogi 2007).

Reported herein is the rapid, sensitive, and selective detection of 10 PAHs and oxidized PAH metabolites in cow milk and plant milk alternatives using cyclodextrin-promoted energy transfer to high quantum yield fluorophores. This system has been proven to operate with high sensitivity (micromolar detection limits), selectivity (100% differentiation even between structurally similar analytes), and general applicability (for both unmodified lipophilic PAHs and highly polar oxidized PAH metabolites, as well as for different cow and plant milk samples).

MATERIALS AND METHODS

¹H NMR spectra were obtained using a Bruker 300 MHz spectrometer. UV-visible spectra were obtained using a Shimadzu UV-3600 Plus UV-Vis-NIR spectrophotometer. Fluorescence spectra were obtained using a Shimadzu 5301PC spectrofluorimeter. GC-MS measurements were obtained using a Shimadzu GC-MS-QP2020 gas chromatograph-mass spectrometer. All toxicants and toxicant metabolites (compounds **1–10**, Figure 1) were purchased from Sigma Aldrich and used as received. Fluorophore **11** was synthesized following literature-reported procedures (Shepherd et al. 2004). Fluorophores **12** and **13** were purchased from Sigma Aldrich and used as received. Cow milk (whole and organic whole) and plant milk alternative products (almond milk, cashew milk, soy milk) were purchased from a local grocery store. Raw milk was purchased from a local farm. All milk samples were frozen and stored in a freezer until needed.

General Sample Preparation Procedure for Fluorescence Experiments

Frozen cow milk was thawed overnight in the refrigerator. The milk was then allowed to come to room temperature on the lab bench and shaken vigorously to ensure a uniform suspension. 1875 µL of this suspension and 20 µL of an analyte solution (1 mg/mL analyte in tetrahydrofuran (THF), compounds **1–10**, Figure 1) were added to a centrifuge tube. The solution was shaken and allowed to stand for 10 minutes. 938 µL of absolute ethanol and 938 µL of a 10 mM γ -cyclodextrin solution in phosphate buffered saline (PBS) were added to each tube, and the tubes were shaken and allowed to sit overnight in the refrigerator. The solution was centrifuged at 3000 rpm for 10 minutes, after which time it separated into three layers.

2.5 mL of the middle layer was transferred to a quartz cuvette and the fluorescence of the solution was measured as a result of excitation at the analyte's excitation wavelength and a result of excitation at the fluorophore's excitation wavelength. Then, 100 µL of a fluorophore solution (0.1 mg/mL in THF, compounds **11–13**, Figure 1) was added to the cuvette, and the solution was again excited at the analyte's excitation wavelength and at the fluorophore's excitation wavelength.

The same procedure was followed but without the addition of an analyte to obtain data in undoped samples.

General Procedure for Energy Transfer Experiments

The efficiency of the energy transfer was quantified as the ratio of the integrated emission of the fluorophore via analyte excitation to the integrated emission of the fluorophore via direct excitation:

$$\text{Energy transfer efficiency} = \frac{I_{DA}}{I_A} \times 100 \% \quad (\text{Eq. 1})$$

Where I_{DA} is the integration of the fluorophore from analyte excitation in the presence of analyte and I_A is the integrated fluorophore emission from direct excitation.

Analyte comparison ratios were calculated for each experiment according to Equation 2:

$$\text{Analyte comparison} = \frac{I_{\text{analyte}}}{I_{\text{blank}}} \quad (\text{Eq. 2})$$

where I_{analyte} is the integrated emission of the fluorophore from excitation at the analyte's excitation wavelength in the presence of the analyte, and I_{blank} is the integrated emission of the fluorophore from excitation at the analyte's excitation wavelength in the absence of the analyte. Analyte comparisons greater than 1 indicate that the fluorophore emission via excitation at the analyte wavelength is higher in the presence of the analyte, indicating legitimate energy transfer is occurring. Analyte comparison ratios less than 1 indicate that the fluorophore emission via excitation at the analyte wavelength is lower in the presence of the analyte, indicating analyte-driven fluorescence quenching. Analyte comparisons near 1 indicate little to no interaction of the analyte with the fluorophore, resulting in a fluorescence emission signal that is essentially unchanged with the addition of the analyte (the putative energy donor).

General Procedure for Limit of Detection Calculations

The limit of detection (LOD) is defined as the lowest concentration of analyte at which a signal can be detected. These experiments were conducted following literature-reported procedures (Cheng et al. 2016). To determine this value, the following steps were performed for each analyte-fluorophore combination:

1. Undoped, analyte-free milk samples were prepared according to the general sample preparation procedure for fluorescence experiments.
2. 2.5 mL of the middle layer was transferred to a cuvette. 100 μL of a 0.1 mg/mL solution of fluorophore **11** in THF was added to the cuvette, and the solution was excited at the fluorophore's excitation wavelength and analyte's excitation wavelength and fluorescence spectra were recorded. Six repeat measurements were taken.

3. 5 μL of a 1.0 mg/mL analyte solution in THF was added to the cuvette and the solution was again excited at the fluorophore's excitation wavelength as well as at the analyte's excitation wavelength. Six repeat measurements were taken.
4. Step 2 was repeated, adding analyte in 5 μL increments until a final volume of 40 μL was added. In each case, the solution was excited at the fluorophore's excitation wavelength and analyte's excitation wavelength and the fluorescence emission spectrum was recorded six times.
5. All fluorescence emission spectra were integrated vs. wavenumber, and calibration curves were generated with the analyte concentration on the X-axis (in μM) and the energy transfer ratio ($I_{\text{DA}}/I_{\text{A}}$) on the Y-axis. The curve was then fitted to a straight line and an equation for the line was determined.
6. For each case, the solution before any analyte was added was also excited at the excitation wavelength for the fluorophore and excitation wavelength for the analyte and the fluorescence emission spectra were recorded (as per step 1). These measurements are referred to as the "blank."
7. The limit of detection is defined according to Equation 4:

$$\text{LOD} = \frac{3(\text{SD}_{\text{blank}})}{m} \quad (\text{Eq. 4})$$

Where SD_{blank} is the standard deviation of the blank and m is the slope of the calibration curve. In cases where the slope of the trend line was negative, the absolute value of the slope was used to calculate LOD. In all cases, the LOD was calculated in μM .

General Procedure for Array Generation Experiments

Array-based analysis was performed using SYSTAT 13 statistical computing software with the following settings:

- a. Classical Discriminant Analysis
- b. Grouping variable: Analytes
- c. Predictors: γ -cyclodextrin/BODIPY, γ -cyclodextrin/Rhodamine 6G, γ -cyclodextrin/Coumarin 6
- d. Long-range statistics: Mahal

General Procedures for Undoped Characterization Experiments

GC-MS Experiments—Sample preparation experiments for GC-MS were conducted following literature-reported procedures (Baduel et al. 2015). In brief, 3 mL of each milk sample were added to 8 mL centrifuge tubes. Each sample was vortexed for one minute and allowed to settle for 15 minutes. 3 mL of acetonitrile were added, and each tube was shaken vigorously by hand. Then 1.2 grams of MgSO_4 and 0.3 grams of NaCl were added to each centrifuge tube and shaken for 1 minute. Samples were placed in the freezer for 10 minutes,

followed by centrifugation at 3700 rpm for 10 minutes. The supernatant was removed from each sample and analyzed by GC-MS.

All GC-MS measurements were obtained on a Shimadzu-QP2020 gas chromatograph-mass spectrometer following literature-reported procedures (Pfannkoch et al. 2010). The GC-MS operating conditions were as follows: column, Shimadzu SH-Rxi-5SiMS (30 m × 0.25 mm × 0.25 μm); carrier gas, helium at 2.0 ml/min; oven temperature, 60 °C (1 min) → 15 °C/min 325 °C (3 min); injection temperature, 250 °C; splitting ratio, splitless; electron impact (EI) ionization mode; MS ion source temperature, 230 °C; interface temperature, 150 °C; total run time, 22 minutes.

Fluorescence Experiments—Defrosted milk samples (3 replicates from each milk type) were prepared following the general sample preparation procedures. 100 μL of fluorophores **11–13** were added, and each sample was excited at various wavelengths characteristic of photophysically active organic contaminants. Energy transfer efficiencies were calculated using Equation 1, and arrays were generated for each sample following experimental procedures for array generation experiments.

RESULTS AND DISCUSSION

Undoped Milk Characterization

Sample characterization experiments using gas chromatography-mass spectrometry (GC-MS) and fluorescence spectroscopy were performed on undoped (analyte-free) milk samples to identify key differences in the composition of the cow milk and plant milk samples.

Fluorescence Experiments—Analyte-free samples to which small amounts of fluorophores **11–13** were added demonstrated unique, sample-specific fluorescence response patterns from excitation at a wide variety of wavelengths. These specific wavelengths were chosen to represent excitation wavelengths characteristic of aromatic toxicants. Samples were excited at various excitation wavelengths to measure how the signals change in the presence of inherent aromatic toxicants. Linear discriminant analyses of these fluorescence responses generated distinct pattern identifiers for all wavelengths in all milk samples (Figure 2). The goal of linear discriminant analysis with jackknifed reclassification is to maximize the distance between different samples (i.e. 360 nm vs. 365 nm) while minimizing the distance between the four trials of each specific sample to result in 100% differentiation between samples (Bajaj et al. 2010). Array-based analysis has been used by our research group (Serio et al. 2015b) and a number of other research groups to measure selectivity of detection systems (Creran et al. 2015; Hizir et al. 2017; Miranda et al. 2010; Tao et al. 2017).

Each milk sample demonstrated a unique visual response pattern, likely due to the differences in the fatty acid composition of each sample (see GC-MS section for more details) (Leroux et al. 2016), which lead to different local micro-environments for fluorophores **11–13** that are detectable spectroscopically. Analogous sensitivities to local environments for BODIPY (Jiang et al. 2013; Marfin et al. 2014; Zhang et al. 2013),

Rhodamine (Ji et al. 2017; Ren et al. 2016), and Coumarin (Phatangare et al. 2014; Sarkar et al. 2015) fluorophores have been reported previously.

Some visual similarities between the group of plant milks (soy, almond, cashew) and the group of cow milks (organic, whole, and raw) were also observed. In particular, the arrays generated for whole milk and organic whole milk appear to be mirror images of each other, as do the arrays generated from almond and cashew milk. Moreover, the location of the signals from 380 nm excitation and 385 nm excitation are remarkably similar in the whole milk and raw milk arrays, as well as in comparing the arrays generated from almond and soy milk. Interestingly, even as little as a 5 nm difference in excitation wavelength (360 vs. 365 or 380 vs. 385 nm) resulted in 100% differentiation between response signals using array-based analysis. This shows the ability of our system to differentiate between extremely small variations.

Energy transfer efficiencies in the analyte-free milk solutions (to which small amounts of fluorophores **11–13** were added) were calculated according to Equation 1, and the results are summarized in Table 1. These results show that the energy transfer efficiencies were highest in the presence of fluorophore acceptor **11**, which is a consequence of the high quantum yield of that fluorophore (0.96 in tetrahydrofuran) (Zhang et al. 2016; Zhao et al. 2017), its electrostatic potential surface which is strongly complementary to that of the aromatic analytes (see ESI for more details), and its demonstrated ability to bind in or near the γ -cyclodextrin cavity and facilitate proximity-induced energy transfer (Milles et al. 2013). In contrast, the structure of fluorophore **12** makes it less able to penetrate the cyclodextrin cavity (both because of the large steric size as well as its central twisted biphenyl axis) (Zhao et al. 2010), and the quantum yield of fluorophore **13** is lower than that of fluorophore **11** (Rodionova et al. 2008). The energy transfer efficiencies observed in the soy milk samples were markedly lower than those observed in the other milk samples, likely as a result of soy protein aggregation (*vide infra*).

GC-MS—Differences between classes of cow milk and plant milk samples were further confirmed by GC-MS analysis (see ESI for full details). The cow milk samples (whole, organic, raw) showed peaks corresponding to fatty acid compounds typically found in cow milk, with raw milk showing a higher number and higher intensity of peaks (Figure 3A). The GC-MS spectra from plant milk samples showed peaks that indicated the presence of fatty acids, nut shell or plant protein components, flavorings, and other additives commonly found in plant milk (Figure 3B) (Fagbemi 2009; Scilewski da Costa Zanatta et al. 2017; Valdes et al. 2015). Notably, soy milk showed the highest number of peaks, which can be attributed to the hydrolysis of soy proteins into amides that occurs during soy milk preparation procedures (Jaiswal et al. 2015). Other peaks in the soy milk GC-MS spectra correspond to furfurals, which are known agricultural byproducts, and a variety of alcohols, glycols, and stearates which are commercially used de-foaming agents (Kamath et al. 2011).

Analyte Selection

The analytes selected include PAHs that have been found in dairy products (compounds **2**, **6**, **8**) (Santonicola et al. 2017), known PAH metabolites (compounds **1**, **3**, **4**, **7**, and **9**) (Lee et

al. 2015), an aromatic amine that is known to cause bladder cancer (compound **5**) (Zhao et al. 2015), and a partially hydrogenated aromatic amine that has been investigated for pesticide activity (compound **10**) (Choudhury and Das 1983; Das et al. 1998).

Analyte-Doped Energy Transfer

To enable the efficient detection of specific analytes in the complex milk environments, the milk samples were doped with micromolar concentrations of analytes **1–10** (in addition to micromolar concentrations of the fluorophores) prior to sample preparation and centrifugation procedures. Doping analytes and fluorophores at this early stage provides a mechanism to assess the extent to which preparation procedures for all milk samples retain the innate toxicants of interest. The amount of analyte and fluorophore that remained after sample preparation was quantified using UV-visible spectroscopic measurements, and the results indicated that significant amounts of all analytes and fluorophores remained in the analyzed layer (see ESI for more details).

Table 2 shows energy transfer efficiencies from analytes **1–10** to fluorophore **11** in analyte-doped milk samples (data obtained using the other fluorophore acceptors are shown in the ESI). The efficiency of the energy transfer was quantified as the ratio of the integrated emission of the fluorophore from analyte excitation to the integrated emission of the fluorophore from direct excitation, and is shown as a percentage value (Equation 1). The extent to which the observed fluorescence signals represent legitimate energy transfer was determined from the analyte comparison ratios (Equation 2, values shown in parentheses in Table 2), with ratios less than 1 indicating the presence of “legitimate” energy transfer rather than a result of exciting the toxicant donor at a wavelength where it has a notable absorption cross-section.

For the whole and organic cow milks, legitimate energy transfer (as indicated by analyte comparison ratios greater than 1) was observed for analytes **2**, **3**, and **8**. In the case of the other analytes, although no energy transfer is occurring, it is noteworthy that the introduction of the analyte led to noticeable changes in the fluorophore emission signal (as indicated by analyte comparison ratios that are substantially different from 1). Previous work in our group has demonstrated the ability of several aromatic and non-aromatic toxicants to modulate the fluorescence emission of fluorophores **11–13** (DiScenza and Levine 2016a, b; DiScenza et al. 2016b; DiScenza et al. 2017b). Analyte **8** is a particularly important target for detection due to its known toxicity (Chepelev et al. 2015), carcinogenicity (Ceccaroli et al. 2015), and teratogenicity (Wells et al. 1997). Moreover, its strong binding in the hydrophobic γ -cyclodextrin cavity has been well-documented in the literature (Ghosh et al. 2012; Mousset et al. 2014; Munoz de la Pena et al. 1991). In the case of raw milk, only analytes **1** and **4** act as legitimate energy donors likely due to the complex nature of raw milk (see GC-MS section above).

More cases of legitimate energy transfer were observed for the nut-based almond and cashew milks, with all analytes except compounds **9** and **10** acting as legitimate energy donors in this context. This differential behavior is likely a result of the increased non-covalent interactions due to the composition of these milks (see GC-MS section above). In the case of soy milk, low energy transfer values were observed for all analytes investigated.

These lower values compared to the other milk samples are likely a result of the aggregation of large soy proteins upon centrifugation and subsequent binding of PAHs in the aggregated proteins, a phenomenon that has been well-documented in the literature (Nik et al. 2008).

Both the fluorescence modulation or legitimate energy transfer occurring from doped analytes to fluorophore **11** in organic whole milk, whole milk, raw milk, almond milk, and cashew milk can be attributed to amphiphilic fatty acids in milk that are known to form micelles with hydrophobic interiors (Bahri et al. 2017). These micelles work synergistically with the cyclodextrin to promote PAH inclusion (Dai et al. 2017), leading to significant energy transfer efficiencies. The energy transfer efficiencies observed for all cow milks were similar in magnitude, which highlights the robust nature of this cyclodextrin-promoted detection system to operate successfully in multiple environments. These results represent improvements over our previous results obtained in purified buffer solution where many of the presumed cases of cyclodextrin-promoted energy transfer were determined to be a result of directly exciting the analyte at a wavelength where it has noticeable absorption rather than a result of legitimate energy transfer. These improved results can be attributed to the differences in media composition between the aqueous buffer and lipophilic milk, as previously described above.

Practical Considerations

Sensitivity—The sensitivity of the system was determined by calculating limits of detection for all analytes in all milk samples using literature-reported procedures (Table 3). Both aromatic toxicants as well as toxicant metabolites can be detected at micromolar concentrations, which are comparable (after mathematical conversion of the micromolar values to parts-per-million) to the reported permissible exposure limits of 0.2 ppm for compounds **2**, **6**, and **8** (Occupational Safety and Health Administration 2012). The only notable exceptions to such low LODs were the values obtained in raw milk, which is likely due to the complex nature of that environment. Current efforts in our laboratory are focused on lowering all detection limits to obtain optimal detection sensitivities. To that end, we are exploring the possibility of incorporating a pre-concentration step to obtain higher effective concentrations and enable practical detection systems.

Selectivity—The selectivity of the system in correctly identifying analytes was determined using array-based statistical analysis of the integrated fluorophore emission signals. Linear discriminant analysis (LDA) of this data resulted in 100% success in the differentiation of all 10 analytes (Figure 4), even among the structurally similar compounds (i.e. compounds **1** vs. **6**, and compounds **3** vs. **4**). Such high selectivity is particularly relevant for consumer safety detection applications, because structurally similar analytes often have widely disparate toxicities. One way to understand this high level of selectivity is to look at the fluorescence spectra of each analyte-fluorophore-milk combination, which shows clear differentiation and leads to a unique ‘fingerprint’ for each combination (see ESI for more details). These results highlight the uniqueness of this approach for achieving a high level of selectivity and differentiation between structurally similar analytes.

Generality—The general applicability of this detection method was demonstrated by comparing the fluorescence response signals of the toxicant analyte donors in the six different milk samples, all of which have significant differences in their chemical compositions, including notable differences in their lipid contents. For these experiments, we selected a benzo[*a*]pyrene (compound **6**) as a high-impact target for detection due to its significant toxicity. The integrated fluorescence responses from analyte **6** in the presence of the six different milk samples yielded six unique response signals (Figure 5), with the statistical analysis providing 100% success in identifying the milk environment in which the analyte was found. The distinct arrays seen for each milk sample highlight the potential for profiling samples based on such identification, and creating milk-specific patterns for accurate sample identification.

In conclusion, cyclodextrin-promoted, proximity-induced fluorescence modulation and energy transfer from 10 PAHs and PAH metabolites to high quantum yield fluorophores **11–13** enabled successful detection in multiple types of cow milk and plant milk alternatives. This fluorescence energy transfer operates with high sensitivity (micromolar detection limits), selectivity (100% differentiation between analytes and 100% selectivity in accurate identification of the milk environment), and general applicability (for both unmodified lipophilic PAHs and highly polar oxidized PAH metabolites, as well as for samples with varying chemical composition). These results show significant potential in the development of practical detection systems for consumer safety applications. Current efforts in our laboratory are focused on the development and optimization of such systems, and results of these and other investigations will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

FUNDING

Funding is acknowledged from the National Cancer Institute (grant number: CA185435) and from the University of Rhode Island Project Completion Grant Program.

References

- Aiken AC, DeCarlo PF, Jimenez JL. Elemental analysis of organic species with electron ionization high-resolution mass spectrometry. *Anal Chem*. 2007; 79:8350–8358. [PubMed: 17914892]
- Allan SE, Smith BW, Anderson KA. Impact of the Deepwater Horizon oil spill on bioavailable polycyclic aromatic hydrocarbons in Gulf of Mexico coastal waters. *Environ Sci Technol*. 2012; 46:2033–2039. [PubMed: 22321043]
- Amini M, Esmailzadeh A, Shafaeizadeh S, Behrooz J, Zare M. Relationship between major dietary patterns and metabolic syndrome among individuals with impaired glucose tolerance. *Nutrition*. 2010; 26:986–992. [PubMed: 20624672]
- Baduel C, Mueller JF, Tsai H, Ramos MJG. Development of sample extraction and clean-up strategies for target and non-target analysis of environmental contaminants in biological matrices. *J Chromatogr A*. 2015; 1426:33–47. [PubMed: 26643725]

- Bahri A, Martin M, Gergely C, Pugniere M, Chevalier-Lucia D, Marchesseau S. Atomic force microscopy study of the topography and nanomechanics of casein micelles captured by an antibody. *Langmuir*. 2017; 33:4720–4728. [PubMed: 28481103]
- Bajaj A, Miranda OR, Phillips R, Kim I, Jerry DJ, Bunz UHF, Rotello VM. Array based sensing of normal, cancerous and metastatic cells using conjugated fluorescent polymers. *J Am Chem Soc*. 2010; 132:1018–1022. [PubMed: 20039629]
- Brown AS, Brown RJC, Coleman PJ, Conolly C, Sweetman AJ, Jones KC, Butterfield DM, Sarantaris D, Donovan BJ, Roberts I. Twenty years of measurements of polycyclic aromatic hydrocarbons (PAHs) in UK ambient air by nationwide air quality networks. *Environ Sci Process Impacts*. 2013; 15:1199–1215. [PubMed: 23636622]
- Ceccaroli C, Pulliero A, Geretto M, Izzotti A. Molecular fingerprints of environmental carcinogens in human cancer. *J Environ Sci Health C*. 2015; 33:188–228.
- Cheng D, Zhao W, Yang H, Huang Z, Liu X, Han A. Detection of Hg²⁺ by a FRET ratiometric fluorescent probe based on a novel BODIPY-RhB system. *Tetrahedron Lett*. 2016; 57:2655–2659.
- Chepelev NL, Moffat ID, Labib S, Bourdon-Lacombe J, Kuo B, Buick JK, Lemieux F, Malik AI, Halappanavar S, Williams A, Yauk CL. Integrating toxicogenomics into human health risk assessment: Lessons learned from the benzo[a]pyrene case study. *Critical Rev Toxicol*. 2015; 45:44–52. [PubMed: 25605027]
- Choudhury B, Das BP. Larvicidal activity of some reduced carbazoles and compounds containing the methylenedioxyphenyl ring. *Current Sci*. 1983; 52:1130–1132.
- Creran B, Bunz UHF, Rotello VM. Polymer-nanoparticle assemblies for array based sensing. *Curr Org Chem*. 2015; 109:1054–1062. [PubMed: 27134552]
- Dai L, Liu K, Wang L, Liu J, He J, Liu X, Lei J. Injectable and thermosensitive supramolecular hydrogels by inclusion complexation between binary-drug loaded micelles and α -cyclodextrin. *Material Sci Engineering C*. 2017; 76:966–974.
- Das BP, Das K, Chowdhury DN, Das GK, Choudhury B. Comparative pesticidal studies of 1,2,3,4-tetrahydrocarbazole and 2-methyl-1, 2, 3,4-tetrahydrocarbazole. *Environment Ecology*. 1998; 16:405–408.
- Delgado B, Pino V, Ayala JH, González V, Afonso AM. Nonionic surfactant mixtures: A new cloud-point extraction approach for the determination of PAHs in seawater using HPLC with fluorimetric detection. *Anal Chim Acta*. 2004; 528:165–172.
- DiScenza DJ, Levine M. Selective detection of non-aromatic pesticides via cyclodextrin-promoted fluorescence modulation. *New J Chem*. 2016a; 40:789–793.
- DiScenza DJ, Levine M. Sensitive and selective detection of alcohols via fluorescence modulation. *Supramol Chem*. 2016b; 28:881–889.
- DiScenza DJ, Gareau L, Serio N, Roque J, Prignano L, Verderame M, Levine M. Cyclodextrin-promoted detection of toxicants and toxicant metabolites in urine. *Anal Chem Lett*. 2016a; 6:345–355.
- DiScenza DJ, Verderame M, Levine M. Detection of benzene and alkylated benzene derivatives in fuel contaminated environments. *CLEAN: Soil, Air, Water*. 2016b; 44:1621–1627.
- DiScenza DJ, Lynch J, Verderame M, Serio N, Prignano L, Gareau L, Levine M. Efficient fluorescence detection of aromatic toxicants and toxicant metabolites in human breast milk. *Supramol Chem*. 2017a.
- DiScenza DJ, Lynch J, Miller J, Verderame M, Levine M. Detection of organochlorine pesticides in contaminated marine environments via cyclodextrin-promoted fluorescence modulation. *ACS Omega*. 2017b; 2:8591–8599. [PubMed: 30023587]
- Balcioglu EB. Potential effects of polycyclic aromatic hydrocarbons (PAHs) in marine foods on human health: A critical review. *Toxin Rev*. 2016; 35:98–105.
- Fagbemi TN. Effect of processing on chemical composition of cashew nut (*Anacardium occidentale*). *J Food Sci Technol*. 2009; 46:36–40.
- Forth HP, Mitchelmore CL, Morris JM, Lipton J. Characterization of oil and water accommodated fractions used to conduct aquatic toxicity testing in support of the Deepwater Horizon oil spill natural resource damage assessment. *Environ Sci Technol*. 2017; 36:1450–1459.

- Frysjer GS, Gaines RB, Xu L, Reddy CM. Resolving the unresolved complex mixture in petroleum-contaminated sediments. *Environ Sci Technol*. 2003; 37:1653–1662. [PubMed: 12731850]
- Ghosh P, Mandal S, Das T, Maity A, Gupta P, Purkayastha P. “Extra stabilization” of a pyrene based molecular couple by γ -cyclodextrin in the excited electronic state. *Phys Chem Chem Phys*. 2012; 14:11500–11507. [PubMed: 22806481]
- Girello AM, Sperati D, Tarola AM. Determination of polycyclic aromatic hydrocarbons in Italian milk by HPLC with fluorescence detection. *Food Addit Contam*. 2014; 31:703–710.
- Haug A, Hostmark AT, Harstad OM. Bovine milk in human nutrition- a review. *Lipids Health Dis*. 2007; 6:25–41. [PubMed: 17894873]
- Hayakawa K. Environmental behaviors and toxicities of polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons. *Chem Pharm Bull*. 2016; 64:83–94. [PubMed: 26833435]
- Hizir MS, Robertson NM, Balcioğlu M, Alp E, Rana M, Yigit MV. Universal sensor array for highly selective system identification using two-dimensional nanoparticles. *Chem Sci*. 2017; 8:5735–5745. [PubMed: 28989614]
- Howsam M, Jones KC, Ineson P. PAHs associated with the leaves of three deciduous tree species. II: uptake during a growing season. *Chemosphere*. 2001; 44:155–164. [PubMed: 11444296]
- Jaiswal P, Jha SN, Borah A, Jindal G. Detection and quantification of soymilk in cow-buffalo milk using Attenuated Total Reflectance Fourier Transform Infra-Red Spectroscopy (ATR-FTIR). *Food Chem*. 2015; 168:41–47. [PubMed: 25172681]
- Jarvis IW, Dreij K, Mattsson A, Jernstrom B, Stenius U. Interactions between polycyclic aromatic hydrocarbons in complex mixtures and implications for cancer risk assessment. *Toxicol*. 2014; 321:27–39.
- Ji H, Xi K, Zhang Q, Jia X. Photodegradable hydrogels for external manipulation of cellular microenvironments with real-time monitoring. *RSC Adv*. 2017.
- Jiang N, Fan J, Liu T, Cao J, Qiao B, Wang J, Gao P, Peng X. A near-infrared dye based on BODIPY for tracking morphology changes in mitochondria. *Chem Commun*. 2013; 4:10620–10622.
- Jongeneelen FJ, Anzion RBM, Henderson PT. Determination of hydroxylated metabolites of polycyclic aromatic hydrocarbons in urine. *J Chromatography Biomed Appl*. 1987; 413:227–232.
- Kamath S, Webb RE, Deeth HC. The composition of interfacial material from skim milk foams. *J Dairy Sci*. 2011; 94:2707–2718. [PubMed: 21605740]
- Khalili NR, Scheff PA, Holsen TM. PAH source fingerprints for coke ovens, diesel and gasoline engines, highway tunnels, and wood combustion emissions. *Atmosphere Environ*. 1995; 29:533–542.
- Lee SY, Lee JY, Shin HS. Evaluation of chemical analysis method and determination of polycyclic aromatic hydrocarbons content from seafood and dairy products. *Toxicol Res*. 2015; 31:265–271. [PubMed: 26483885]
- Leroux C, Bernard L, Faulconnier Y, Rouel J, de la Foye A, Domagalski J, Chilliard Y. Bovine mammary nutrigenomics and changes in the milk composition due to rapeseed or sunflower oil supplementation of high-forage or high-concentrate diets. *J Nutrigenet Nutrigenomics*. 2016; 9:65–82. [PubMed: 27318968]
- Ma Y, Liu A, Egodawatta P, McGree J, Goonetilleke A. Assessment and management of human health risk from toxic metals and polycyclic aromatic hydrocarbons in urban stormwater arising from anthropogenic activities and traffic congestion. *Sci Total Environ*. 2017; 579:202–211. [PubMed: 27847187]
- Madhavan ND, Naidu KA. Polycyclic aromatic hydrocarbons in placenta, maternal blood, umbilical cord blood and milk of Indian women. *Human Experiment Toxicol*. 1995; 14:503–506.
- Mako T, Marks P, Cook N, Levine M. Fluorescent detection of polycyclic aromatic hydrocarbons in ternary cyclodextrin complexes. *Supramol Chem*. 2012; 24:743–747.
- Manzetti S. Polycyclic aromatic hydrocarbons in the environment: Environmental fate and transformation. *Polycyclic Aromatic Comp*. 2013; 33:311–330.
- Marfin YS, Merkushev DA, Levshanov GA, Rumyantsev EV. Fluorescent properties of 8-phenyl-BODIPY in ethanol-ethylene glycol mixed solutions. *J Fluoresc*. 2014; 24:1613–1619. [PubMed: 25169768]

- Marini M, Frapiccini E. Persistence of polycyclic aromatic hydrocarbons in ocean sediments. *Chemosphere*. 2013; 90:1839–1846. [PubMed: 23127723]
- Massey LK. Dietary animal and plant protein and human bone health: A whole foods approach. *J Nutrition*. 2003; 133:862S–865S. [PubMed: 12612170]
- Mearns AJ, Reish DJ, Oshida PS, Ginn T, Rempel-Hester MA, Arthur C, Rutherford N, Pryor R. Effects of pollution on marine organisms. *Water Environ Res*. 2015; 87:1718–1816. [PubMed: 26420104]
- Milles S, Meyer T, Scheidt HA, Schwarzer R, Thomas L, Marek M, Szenté L, Bittman R, Herrmann A, Guenther Pomorski T, Huster D, Mueller P. Organization of fluorescent cholesterol analogs in lipid bilayers - Lessons from cyclodextrin extraction. *Biochim Biophys Acta*. 2013; 1828:1822–1828. [PubMed: 23583923]
- Miranda OR, Creran B, Rotello VM. Array-based sensing with nanoparticles: ‘chemical noses’ for sensing biomolecules and cell surfaces. *Curr Opin Chem Biol*. 2010; 14:728–736. [PubMed: 20801707]
- Mousset E, Oturan MA, Van Hullebusch ED, Guibaud G, Esposito G. Soil washing/flushing treatments of organic pollutants enhanced by cyclodextrins and integrated treatments: state of the art. *Critical Rev Environ Sci Technol*. 2014; 44:705–795.
- Munoz de la Pena A, Ndou T, Zung ZB, Warner IM. Stoichiometry and formation constants of pyrene inclusion complexes with .beta.- and .gamma.-cyclodextrin. *J Phys Chem*. 1991; 95:3330–3334.
- Nieva-Cano MJ, Rubio-Barroso S, Santos-Delgado MJ. Determination of PAH in food samples by HPLC with fluorimetric detection following sonication extraction without sample clean-up. *Analyst*. 2001; 126:1326–1331. [PubMed: 11534600]
- Nik AM, Tosh S, Poysa V, Woodrow L, Corredig M. Physicochemical characterization of soymilk after stepwise centrifugation. *Food Res Int*. 2008; 41:286–294.
- Occupational Safety & Health Administration (OSHA). Chemical Sampling Information: Coal Tar Pitch Volatiles (Benzene Soluble Fractions). Occupational Safety & Health Administration; 2012. https://www.osha.gov/dts/chemicalsampling/data/CH_229000.html [Accessed Jul 5, 2017]
- Pfannkoch EA, Stuff JR, Whitecavage JA. High Throughput method for the determination of PAHs in seafood by QuEChERS-SBSE-GC-MS. AppNote Global Analytical Solutions. 2010 AN/2010/6b-1/AN/2010/6b-11.
- Phatangare KR, Lanke SK, Sekar N. Fluorescent coumarin derivatives with viscosity sensitive emission - synthesis, photophysical properties and computational studies. *J Fluoresc*. 2014; 24:1263–1274. [PubMed: 24882602]
- Qin YY, Leung CKM, Lin CK, Leung AOW, Wang HS, Giesy JP, Wong MH. Halogenated POPs and PAHs in blood plasma of Hong Kong residents. *Environ Sci Technol*. 2011; 45:1630–1637. [PubMed: 21207933]
- Ren L, Kim YJ, Park SY, Lee S, Lee J, Park CP, Lim YT. Rapid fluorescence detection of hypoxic microenvironments by nitro-benzyl conjugated chitosan nanoparticles encapsulating hydrophobic fluorophores. *J Mater Chem B*. 2016; 4:4832–4838.
- Rodionova OA, Puzyk MV, Balashev KP. Effect of cyclopalladation on the spectroscopic properties of a Coumarin dye. *Opt Spectrosc*. 2008; 105:62–66.
- Rozenberg S, Body JJ, Bruyere O, Bergmann P, Brandi ML, Cooper C, Devogelaer JP, Gielen E, Goemaere S, Kaufman JM, Rizzoli R, Reginster JY. Effects of dairy products consumption on health: Benefits and beliefs- a commentary from the Belgian Bone Club and the European Society for clinical and economic aspects of osteoporosis, osteoarthritis, and musculoskeletal diseases. *Calcif Tissue Int*. 2016; 98:1–17. [PubMed: 26445771]
- Ruby MV, Lowney YW. Selective soil particle adherence to hands: Implications for understanding oral exposure to soil contaminants. *Environ Sci Technol*. 2012; 46:12759–12771. [PubMed: 23148503]
- Santonicola S, Albrizio S, Murru N, Ferrante MC, Mercogliano R. Study on the occurrence of polycyclic aromatic hydrocarbons in milk and meat/fish based baby food available in Italy. *Chemosphere*. 2017; 184:467–472. [PubMed: 28622644]
- Sarkar I, Prakash RHS, Desai A, Mishra AK. Utilizing an aggregate forming microenvironment sensitive coumarin-cholesterol conjugate as a sensor of pluronic organization and micro-polarity. *RSC Adv*. 2015; 5:97279–97288.

- Scilewski da Costa Zanatta T, Manica-Berto R, Ferreira CD, Cardozo MMC, Rombaldi CV, Zambiasi RC, Dias ARG. Phosphate fertilizer and growing environment change the phytochemicals, oil quality, and nutritional composition of roundup ready genetically modified and conventional soybean. *J Agricultural Food Chem.* 2017; 65:2661–2669.
- Serio N, Levine M. Efficient extraction and detection of aromatic toxicants from crude oil and tar balls using multiple cyclodextrin derivatives. *Marine Poll Bull.* 2015; 95:242–247.
- Serio N, Levine M. Solvent effects in the extraction and detection of polycyclic aromatic hydrocarbons from complex oils in complex environments. *J Inclusion Phenom Macrocyclic Chem.* 2016; 84:61–70.
- Serio N, Chanthalya C, Prignano L, Levine M. Cyclodextrin-enhanced extraction and energy transfer of carcinogens in complex oil environments. *ACS Appl Mater Interfaces.* 2013a; 5:11951–11957. [PubMed: 24187939]
- Serio N, Miller K, Levine M. Efficient detection of polycyclic aromatic hydrocarbons and polychlorinated biphenyls via three-component energy transfer. *Chem Commun.* 2013b; 49:4821–4823.
- Serio N, Chanthalya C, Prignano L, Levine M. Cyclodextrin-promoted energy transfer for broadly applicable small-molecule detection. *Supramol Chem.* 2014a; 26:714–721. [PubMed: 25937786]
- Serio N, Prignano L, Peters S, Levine M. Detection of medium-sized polycyclic aromatic hydrocarbons via fluorescence energy transfer. *Polycyclic Aromatic Comp.* 2014b; 34:561–572.
- Serio N, Chanthalya C, Peters S, Levine D, Levine M. 2-Hydroxypropyl beta-cyclodextrin for the enhanced performance of dual function extraction and detection systems in complex oil environments. *J Inclusion Phenom Macrocyclic Chem.* 2015a; 81:341–346.
- Serio N, Moyano DF, Rotello VM, Levine M. Array-based detection of persistent organic pollutants via cyclodextrin-promoted energy transfer. *Chem Commun.* 2015b; 51:11615–11618.
- Serio N, Roque J, Badwal A, Levine M. Rapid and efficient pesticide detection via cyclodextrin-promoted energy transfer. *Analyst.* 2015c; 140:7503–7507. [PubMed: 26436147]
- Shepherd JL, Kell A, Chung E, Sinclar CW, Workentin MS, Bizzotto D. Selective reductive desorption of a SAM-coated gold electrode revealed using fluorescence microscopy. *J Am Chem Soc.* 2004; 126:8329–8335. [PubMed: 15225076]
- Srogi K. Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review. *Environ Chem Lett.* 2007; 5:169–195. [PubMed: 29033701]
- Tao Y, Li M, Auguste DT. Pattern-based sensing of triple negative breast cancer cells with dual-ligand cofunctionalized gold nanoclusters. *Biomaterials.* 2017; 116:21–33. [PubMed: 27914264]
- Valdes A, Vidal L, Beltran A, Canals A, Garrigos MC. Microwave-assisted extraction of phenolic compounds from almond skin byproducts (*Prunus amygdalus*): a multivariate analysis approach. *J Agricultural Food Chem.* 2015; 63:5395–5402.
- Vu AT, Taylor KM, Holman MR, Ding YS, Hearn B, Watson CH. Polycyclic aromatic hydrocarbons in the mainstream smoke of popular U. S. cigarettes. *Chem Res Toxicol.* 2015; 28:1616–1626. [PubMed: 26158771]
- Wang L, Xiang Z, Stevanovic S, Ristovski Z, Salimi F, Gao J, Wang H, Li L. Role of Chinese cooking emissions on ambient air quality and human health. *Sci Total Environ.* 2017; 589:173–181. [PubMed: 28262368]
- Wang X, Thai PK, Mallet M, Desservettaz M, Hawker DW, Keywood M, Mijevic B, Paton-Walsh C, Gallen M, Mueller JF. Emissions of selected semivolatile organic chemicals from forest and savannah fires. *Environ Sci Technol.* 2017; 51:1293–1302. [PubMed: 28019099]
- Wang YB, Liu CW, Kao YH, Jang CS. Characterization and risk assessment of PAH-contaminated river sediment by using advanced multivariate methods. *Sci Total Environ.* 2015; 524–525:63–73.
- Wells PG, Kim PM, Laposa RR, Nicol CJ, Parman T, Winn LM. Oxidative damage in chemical teratogenesis. *Mutation Res.* 1997; 396:65–78. [PubMed: 9434860]
- Zgheib S, Moilleron R, Chebbo G. Screening of priority pollutants in urban stormwater: Innovative methodology. *WIT Trans Ecol Environ.* 2008; 111:235–244.
- Zhang L, Chen Y, Jiang J. Solid state fluorescent functionalized-triphenylamine Bodipy detector for HCl vapor with high stability and absolute fluorescent quantum yield. *Dyes Pigments.* 2016; 124:110–119.

- Zhang X, Wang Z, Yue X, Ma Y, Kieseewetter DO, Chen X. pH-Sensitive fluorescent dyes: Are they really pH-sensitive in cells? *Mol Pharm*. 2013; 10:1910–1917. [PubMed: 23464828]
- Zhao L, Geng H, Liang ZF, Zhang ZQ, Zhang T, Yu DX, Zhong CY. Benzidine induces epithelial-mesenchymal transition in human uroepithelial cells through ERK1/2 pathway. *Biochem Biophys Res Commun*. 2015; 459:643–649. [PubMed: 25757908]
- Zhao N, Xuan S, Fronczek FR, Smith KM, Vicente MGH. Enhanced hypsochromic shifts, quantum yield, and π - π interactions in a meso, β -heteroaryl-fused BODIPY. *J Org Chem*. 2017; 82:3880–3885. [PubMed: 28231707]
- Zhao Y, Yang Y, Cun S, Hao Z, Hong Y, Huang R, Chi CM, Zhao Y. Synthesis of novel bis(β -cyclodextrin)s linked with glycol and their inclusion complexation with organic dyes. *Helv Chim Acta*. 2010; 93:1136–1148.

Figure 1.
Structures of analytes **1–10** and fluorophores **11–13**

Figure 2.

Array response patterns using linear discriminant analysis from undoped samples of: (A) whole milk, (B) organic whole milk, (C) raw milk, (D) almond milk, (E) cashew milk, and (F) soy milk using cyclodextrin-fluorophore predictors.

Figure 3.

GC-MS overlay of: (A) cow milk samples where the black line represents organic whole milk, the pink line represents whole milk, and the blue line represents raw milk, and (B) plant milk samples where the black line represents soy milk, the pink line represents cashew milk, and the blue line represents almond milk.

Figure 4. Array response patterns using linear discriminant analysis from analyte-doped samples of: (A) whole milk, (B) organic whole milk, (C) raw milk, (D) almond milk, (E) cashew milk, and (F) soy milk using cyclodextrin-fluorophore predictors.

Figure 5. Array response patterns using linear discriminant analysis of response patterns of analyte 6 in (A) cow milk, (B) plant milk, and (C) all milk samples using cyclodextrin-fluorophore predictors.

Table 1

Calculated energy transfer efficiencies in undoped milk samples^a

Fluorophore	Organic	Whole	Raw	Almond	Cashew	Soy
11	21.8 ± 0.1	21.3 ± 0.4	31.8 ± 2.0	21.7 ± 0.2	23.1 ± 0.2	5.1 ± 0.0
12	8.5 ± 0.0	4.3 ± 0.0	7.4 ± 0.0	7.4 ± 0.0	7.2 ± 0.0	2.6 ± 0.0
13	15.5 ± 0.0	14.8 ± 0.0	14.6 ± 0.0	7.1 ± 0.1	6.7 ± 0.0	3.3 ± 0.0

^aEnergy transfer efficiencies were calculated using Equation 1; λ_{ex} (analytes) = 360 nm; λ_{ex} (**11**) = 460 nm; λ_{ex} (**12**) = 490 nm; λ_{ex} (**13**) = 420 nm. Results presented in table represent an average of four trials.

Table 2

Calculated energy transfer efficiencies and analyte comparisons in analyte-doped milk samples with fluorophore **11**^a

Analyte	Whole	Organic	Raw	Almond	Cashew	Soy
1	19.1 ± 0.4 (0.87)	19.2 ± 0.3 (0.75)	20.7 ± 0.6 (1.04)	24.2 ± 0.0 (1.12)	23.7 ± 0.1 (1.79)	13.7 ± 0.5 (0.44)
2	23.3 ± 0.2 (1.14)	25.4 ± 0.1 (1.11)	19.8 ± 0.2 (0.99)	21.3 ± 0.2 (2.08)	18.3 ± 0.2 (1.25)	20.6 ± 1.5 (0.57)
3	22.4 ± 0.1 (1.11)	23.2 ± 0.3 (1.09)	20.0 ± 0.3 (0.99)	21.4 ± 0.2 (1.08)	17.4 ± 0.1 (1.14)	13.2 ± 1.1 (0.59)
4	22.8 ± 0.2 (1.04)	22.9 ± 0.4 (0.86)	20.6 ± 0.2 (1.06)	21.0 ± 0.3 (1.27)	16.7 ± 0.1 (1.10)	17.1 ± 0.7 (0.39)
5	23.5 ± 0.2 (1.12)	23.7 ± 0.5 (0.88)	17.6 ± 0.2 (0.76)	20.0 ± 0.1 (1.04)	16.8 ± 0.2 (1.14)	10.4 ± 0.1 (1.03)
6	18.3 ± 0.2 (0.89)	21.0 ± 0.2 (0.74)	20.9 ± 0.0 (0.92)	22.2 ± 0.1 (1.19)	16.9 ± 0.0 (1.12)	10.6 ± 0.3 (0.99)
7	19.8 ± 0.1 (0.95)	22.6 ± 0.3 (1.02)	17.6 ± 0.3 (0.91)	23.3 ± 0.1 (1.25)	25.3 ± 0.2 (1.61)	8.9 ± 0.6 (0.82)
8	23.3 ± 0.2 (1.15)	25.5 ± 0.3 (1.01)	20.0 ± 0.3 (0.99)	20.7 ± 0.3 (1.11)	17.0 ± 0.2 (1.25)	13.4 ± 1.4 (1.59)
9	31.0 ± 0.3 (0.52)	35.9 ± 0.6 (0.41)	23.0 ± 0.3 (0.49)	20.7 ± 0.1 (0.96)	15.9 ± 0.2 (0.88)	14.1 ± 0.9 (0.46)
10	13.8 ± 0.2 (0.65)	15.5 ± 0.2 (0.60)	13.7 ± 0.0 (0.62)	17.6 ± 0.1 (0.87)	13.1 ± 0.1 (0.98)	4.5 ± 0.4 (0.45)

^aEnergy transfer efficiencies were calculated using Equation 1; Analyte comparisons in parentheses were calculated using Equation 2; λ_{ex} (analytes) = 360 nm; λ_{ex} (**11**) = 460 nm; Results presented in table represent an average of four trials.

Table 3

Calculated limits of detection for analytes **1–10** with fluorophore **11** in cow milk and plant milk alternatives.^a

Analyte	Whole (µM)	Organic (µM)	Raw (µM)	Almond (µM)	Cashew (µM)	Soy (µM)
1	5.85 ± 0.00	3.43 ± 0.46	3.21 ± 0.00	0.09 ± 0.00	5.87 ± 0.06	2.30 ± 0.05
2	5.40 ± 0.00	1.33 ± 0.09	35.40 ± 0.00	0.38 ± 0.01	2.70 ± 0.00	1.85 ± 0.00
3	2.22 ± 0.37	2.30 ± 0.00	21.19 ± 0.00	3.58 ± 0.00	20.60 ± 3.46	31.00 ± 0.00
4	4.01 ± 0.00	6.54 ± 0.00	6.24 ± 0.00	6.50 ± 0.00	27.08 ± 10.26	4.30 ± 0.66
5	87.54 ± 11.67	10.97 ± 0.00	44.86 ± 0.00	3.38 ± 0.00	27.33 ± 8.24	45.11 ± 0.00
6	4.17 ± 0.13	17.26 ± 0.00	2.42 ± 0.00	0.24 ± 0.00	0.13 ± 0.00	0.07 ± 0.00
7	0.39 ± 0.00	1.76 ± 0.00	8.42 ± 0.00	1.53 ± 0.02	3.54 ± 0.13	6.79 ± 0.00
8	23.18 ± 0.00	8.13 ± 0.00	4.63 ± 0.00	2.24 ± 0.00	2.77 ± 0.07	4.70 ± 0.00
9	0.83 ± 0.07	0.33 ± 0.02	5.38 ± 0.00	3.39 ± 0.40	11.22 ± 1.39	3.10 ± 0.00
10	5.92 ± 0.00	4.34 ± 0.00	10.27 ± 0.00	2.74 ± 0.26	8.04 ± 0.00	33.44 ± 0.00

^aLimits of detection were calculated using the procedures in Chen 2016; see Electronic Supporting Information for more information.