IDENTIFICATION OF 15 PHTHALATE ESTERS IN COMMERCIAL CHEESE POWDER VIA CYCLODEXTRIN-PROMOTED FLUORESCENCE DETECTION

Benjamin B. Cromwell  
*University of Rhode Island, Bcromwell@my.uri.edu*

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IDENTIFICATION OF 15 PHTHALATE ESTERS IN COMMERCIAL CHEESE POWDER VIA CYCLODEXTRIN-PROMOTED FLUORESCENCE DETECTION

BY

BENJAMIN B. CROMWELL

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

UNIVERSITY OF RHODE ISLAND

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BENJAMIN CROMWELL

APPROVED:

Thesis Committee:

Major Professor       Mindy Levine
                       Brett Lucht
                       Soni Pradhanang
                       Geoffrey Bothun

Nasser H. Zawia
DEAN OF THE GRADUATE SCHOOL

UNIVERSITY OF RHODE ISLAND
2019
ABSTRACT

Human exposure to toxicants such as environmental pollutants, carcinogens, and endocrine disruptors occurs via a variety of mechanisms, including through the use of commercial products that contain these toxicants. Among such commercial products, many classes of food products have been found to contain toxicants, including phthalate and phthalate esters, which have traditionally been detected using mass spectrometry-based techniques. Reported herein is the use of gas chromatography mass spectrometry and cyclodextrin-promoted fluorescence detection to accomplish the sensitive (0.124 µM limits of detection) detection of 15 phthalate esters in commercial cheese powder used in macaroni and cheese products. These results highlight the versatility of the cyclodextrin platform to operate in highly complex sample matrices, as well as the potential to use such platforms for the development of practical sensing devices for toxicants in commercial products.
ACKNOWLEDGMENTS

I’d like to thank the University of Rhode Island for allowing me to learn, study, and grow as a student over my time here. I’d like to thank Dr. Mindy Levine for accepting me into her group and supporting me throughout my career. I’ve learned a lot about independence and what it means to stand on my own feet, those lessons I will take with me forever. To the Levine group members who came before me, Dr. Dan Jones, Dr. Sauradip Chaudhuri, and Ben Smith, thank you for your guidance and honest input. To the current members of the Levine group, I thank you for putting up with me for these years and respecting me as an equal, mentor, and friend. I’d like to thank Teresa Mako and Joanie Raciocot especially for helping to create a productive and supportive environment, as well as their friendship. I’d like to thank Mara Dubnicka for working directly alongside me this past year and being a wonderful mentee. A special thank you to my collaborators throughout my time who have allowed me to mentor and educate a variety of students and tinker with instruments. To the professors who have taught beside me, I thank you for fueling my passion for teaching and your support throughout.

To my family, thank you. I could not do any of this without the constant love and support you gave me. Mom and Dad, thank you for never being more than a phone call away and being helpful even if it was just to be there talk to.
PREFACE

This dissertation is presented in manuscript format according to the guidelines of the graduate school of the University Rhode Island. Two manuscripts will be presented in this thesis. Chapter 1 is being submitted for publication to ACS Sensors with authors Benjamin Cromwell, Mara Dubnicka, Sage Dubrawski, and Mindy Levine. Chapter 2 is published in Supramolecular Chemistry with authors Sauradip Chaudhuri, Dana J. DiScenza, Molly Verderame, Benjamin Cromwell and Mindy Levine.
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CHAPTER 1

Manuscript in preparation for submission to ACS Sensors
Identification of 15 Phthalate Esters in Commercial Cheese Powder via
Cyclodextrin-Promoted Fluorescence Detection

Benjamin Cromwell, Mara Dubnicka, Sage Dubrawski and Mindy Levine*\textsuperscript{a}
Department of Chemistry, University of Rhode Island, Kingston, RI, USA

Corresponding Author:
Mindy Levine, Ph.D.
Department of Chemistry
University of Rhode Island
ABSTRACT

Human exposure to toxicants such as environmental pollutants, carcinogens, and endocrine disruptors occurs via a variety of mechanisms, including through the use of commercial products that contain these toxicants. Among such commercial products, many classes of food products have been found to contain toxicants, including phthalate and phthalate esters, which have traditionally been detected using mass spectrometry-based techniques. Reported herein is the use of gas chromatography mass spectrometry and cyclodextrin-promoted fluorescence detection to accomplish the sensitive (0.124 µM limits of detection) detection of 15 phthalate esters in commercial cheese powder used in macaroni and cheese products. These results highlight the versatility of the cyclodextrin platform to operate in highly complex sample matrices, as well as the potential to use such platforms for the development of practical sensing devices for toxicants in commercial products.

Keywords: phthalate esters, cyclodextrin, fluorescence spectroscopy, linear discriminant analysis

INTRODUCTION

Toxic chemicals have been found in a wide variety of commercial products (Georgescu and Georgescu 2013), including processed foods (Baskar and Aiswarya 2018) and beverages (Ordonez et al 2016). Examples of such chemicals include phthalates (Erythropel et al 2014), phenol and phenol derivatives (Wilmart et al 2016), and a variety of other organic and inorganic analytes (Tsatsakis et al 2016). Exposure to these chemicals is concerning because of their known and suspected toxicity (Brusick 2005), which can cause deleterious health effects to humans (Benjamin et al
and other species exposed to the chemicals (Staples et al 2011). Moreover, the long-term environmental persistence of such chemicals means that potential exposure can occur long after the usage of the chemicals and the initial release into the environment (Chen et al 2016). Current methods to detect these compounds include gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) (Caballero-Casero et al 2016), although newer methods such as electrochemically-based methods (Kashefi-Kheyabadi et al 2018) and spectroscopic (including Raman-based) methods (Wu et al 2018) have also been reported.

Our group has recently developed a fundamentally new method for toxicant detection in complex environments, using cyclodextrin-promoted energy transfer from the toxicant of interest to a high quantum yield fluorophore, for photophysically active analytes (Serio et al 2013; Serio et al 2015) (Figure 1A), and cyclodextrin-promoted fluorescence modulation, for non-photophysically active analytes (DiScenza and Levine 2016a; DiScenza and Levine 2016b) (Figure 1B). These proximity-induced, non-covalent interactions between the cyclodextrin host, toxicant analyte, and fluorophore reporter result in highly analyte-specific fluorescence read-out signals, which have been used for toxicant detection in a broad variety of complex environments. Such environments include human plasma (Serio et al 2014), breast milk (DiScenza et al 2018), and urine (DiScenza et al. 2016; DiScenza, Lynch and Feder, et al 2018), as well as in extracts collected from oil (Serio, Chanthalyma, et al 2013; Serio and Levine 2015) and fuel spills (DiScenza, Verderame and Levine 2016), in contaminated marine environments (DiScenza, Lynch and Miller, et al 2017), and in commercial milk products and plant milk alternatives (DiScenza, Lynch and
Verderame, et al 2018). Compared to most currently utilized methods, fluorescence-based toxicant detection has the potential to lead to more rapid read-out signals, highly portable devices, and improved detection sensitivity and selectivity (Mako, Racicot, and Levine 2019).

**Figure 1.** Schematic illustration of (A) cyclodextrin-promoted analyte-to-fluorophore fluorescence energy transfer and (B) cyclodextrin-promoted analyte-specific fluorescence modulation.

Detection of toxicants in complex food matrices such as macaroni and cheese products using cyclodextrin-promoted fluorescence detection has not been reported to date, although such detection is expected to be of significant interest, as popular news reports have indicated that high concentrations of phthalates are found in the cheese powder of commercial macaroni and cheese products (Kounang 2017). Of note, there are a significant number of commercial macaroni and cheese products available, and the composition of the cheese powder in these products is variable. Moreover, the
concentration of available phthalates in the cheese powder will depend on the cooking method, the chemical composition of the cookware, and other highly consumer-specific parameters, which means that developing detection methods that can be operated by the end user are of significant interest. Current methods for toxicant detection in macaroni and cheese products are time-consuming and require specialized laboratory equipment, which precludes individual consumers from analyzing their own macaroni and cheese products at a time and place of their choosing, i.e. after the food has been prepared (Mannion et al 2019).

Reported herein is the fluorescence-based detection of phthalate esters in commercial cheese powder, which operates with high sensitivity (0.124 µM limits of detection) and general applicability (for operating in complex environments, and for correctly identifying individual analytes and analyte mixtures). In parallel to the fluorescence results, detailed GC-MS analysis of the samples provides additional insight about the presence of known toxicants. Together, the two analytical methods provide a detailed picture of the samples in question, and lead to significant insight about potential toxicant exposure from such food consumption.

MATERIALS AND METHODS

Fluorescence measurements were recorded on a Shimadzu RF-5301PC spectrophotofluorimeter with 1.5 nm excitation and 1.5 nm emission slit widths. All analytes and fluorophores (compounds 1-17, Figure 2) were purchased from Sigma-Aldrich Chemical Company and used as received. All cyclodextrins were purchased from Tokyo Chemical Industry and used as received. Computational experiments were performed using Spartan 18 software for electrostatic potential maps and Molecular
Operating Environment for system modelling. All GC-MS measurements were obtained using a Shimadzu GC-MS QP-2020 gas chromatograph-mass spectrometer.

**Figure 2.** Structures of all phthalate ester analytes (1-15), control analyte (16) and high quantum yield fluorophore (17) used in these investigations.

**General Procedure for GC-MS Characterization Experiments**

**Extraction Procedure**

5 grams of each cheese powder sample were placed in an 8 mL vial with 3 mL of deionized water and vortexed for 5 minutes. 3 grams of sodium chloride was added to the solution along with 3 mL of analytical grade acetonitrile.

In a glass vial, 5 grams of sample were dissolved in 3 mL of deionized water followed by vortexing for 3 minutes and sonicating for 5 minutes. 3 mL of acetonitrile was then added to the solution along with 5 grams of sodium chloride. Samples were exposed to 5 minutes of further sonication and vortexing, followed by refrigeration for 10 minutes. Centrifugation at 5,500 rpm yielded a two-layer system with the top layer
collected into a round bottom flask. The process was repeated 2 times, and subsequent extractions were collected into the same round bottom flask. The combined extracts were evaporated to dryness using a rotary evaporator and brought to a final volume of 1 mL which was transferred into an analysis vial. A standard sample doped with 15 nM of analytes 1-15 in tetrahydrofuran was also put through the same extraction procedure as a positive control for retention time, spectra and viability.

**GC-MS Procedure**

The oven temperature was set at 150°C for 1.00 minute followed by a 5.00°C/min ramp to 270°C with a 5.00-minute hold. The injection temperature was set to 100°C, with a splitless injection, with a 150°C ion source temperature and 230°C interface. A 4.00-minute solvent cut time was used with the m/z ratio range set to 35.00 m/z (low mass cutoff) to 500 m/z (high mass cutoff).

**Procedure for equilibration experiments**

In a quartz cuvette, 1.25 mL of a 10 mM cyclodextrin solution dissolved in phosphate buffered saline (PBS, buffered to pH 7.4) and 1.25 mL of a cheese powder sample (5 g/L in water) were combined with 100 µL of a 0.1 mg/mL solution of fluorophore 17 and set on a shaker table for single minute increments prior to sampling. Spectra were obtained by fluorescence analysis at a 460 nm excitation wavelength. The sampling continued until the change in the fluorescence signal was less than 5% over 5 minutes, as calculated by Equation 1, below:

\[
\text{Signal change} = \frac{(Fl_{t+5 \text{ minutes}} - Fl_{\text{initial}})}{Fl_{\text{initial}}} \quad (\text{Eq. 1})
\]

where \(Fl_{t+5 \text{ minutes}}\) represents the integrated fluorescence emission five minutes after the initial time point, and \(Fl_{\text{initial}}\) represents the integrated fluorescence emission at the
initial time point.

**General Procedure for Fluorescence Modulation Experiments**

In a quartz cuvette, 1.25 mL of a 10 mM cyclodextrin solution dissolved in PBS and 1.25 mL of a cheese powder sample (5 g/L in water) were combined and mixed thoroughly by shaking on a shaker station for 1 hour. Next, 100 µL of a 0.1 mg/mL fluorophore 17 solution in tetrahydrofuran (THF) was added, and the solution was excited four times using a 460 nm excitation wavelength. Then, 50 µL of analytes 1-16 (1.0 mg/mL in THF) were added to the mixture, and the solution was again excited four times at 460 nm. The fluorescence emission spectra were integrated vs. wavenumber on the X-axis using OriginPro software, and the degree of fluorescence modulation was determined using Equation 2, below:

Fluorescence modulation = $\frac{F_{\text{analyte}}}{F_{\text{blank}}}$

where $F_{\text{analyte}}$ is the integrated emission of the fluorophore in the presence of the analyte and $F_{\text{blank}}$ is the integrated emission of the fluorophore in the absence of the analyte. Fluorescence modulation ratios greater than 1 indicate an enhancement of fluorescence emission of the fluorophore in the presence of analyte, fluorescence modulation ratios less than 1 indicate a decrease in fluorescence emission of the fluorophore in the presence of analyte, and fluorescence modulation ratios close to 1 indicate minimal change in the fluorescence emission of the fluorophore in the presence of analyte.

**General Procedure for Limit of Detection Experiments**

Limit of detection experiments were conducted following literature-reported procedures (Cheng et al 2016). In brief, 1.25 mL of γ-cyclodextrin and 1.25 mL of 5
g/L cheese powder in water were added to a quartz cuvette and mixed thoroughly. Next, 100 µL of fluorophore 17 was added to the cuvette and the solution was excited six times at 460 nm. Next, 10 µL of analyte was added, and again the solution was excited six times at 460 nm. This step was repeated for 20 µL of analyte, 30 µL of analyte, 40 µL of analyte, 50 µL of analyte, 60 µL of analyte, 70 µL of analyte, 80 µL of analyte, 90 µL of analyte, and 100 µL of analyte.

All fluorescence emission spectra were integrated vs. wavenumber on the X-axis, and calibration curves were generated. The curves plotted the analyte concentration, measured in µM, on the X-axis and the fluorescence modulation ratio on the Y-axis. The curve was fitted to a straight line and the equation of the line was determined. The limit of detection was calculated according to Equation 3, below:

\[
\text{Limit of detection} = 3(SD_{\text{blank}})/m
\]  
(Eq. 3)

Where \(SD_{\text{blank}}\) is the standard deviation of the blank sample and \(m\) is the slope of the calibration curve. In all cases, the limit of detection was calculated as a concentration in µM.

**General Procedure for Molecular Operating Environment**

Molecular models of the analytes, fluorophores, and cyclodextrin host were generated using the build function within the Molecular Operating Environment (MOE) software. Analytes were defined as the ligands and the \(\gamma\)-cyclodextrin host was defined as the receptor. The ligand-receptor system underwent a “quick preparation,” which is an initial energy minimization function, followed by a more in-depth dynamics calculation using a Berendsen velocity/position scaling algorithm and Amber 14: EHT forcefield. The calculations done in solvent were prepared using a 90-angstrom cubic
cell populated with roughly 2500 water molecules in order to display the most likely conformation in aqueous environments.

**General Procedure for Spartan 18’ Electrostatic Potential Maps**

Computational models of the molecules were built in Spartan 18 and underwent a ground state geometry calculation using semi-empirical PM3 methods in the gas phase followed by an electrostatic potential map, ESP, surface calculation. The values for the ESP maxima and minima energies were normalized across all samples in order to better compare polarities and electrostatic characters.

**RESULTS**

**System Component Selection**

Cheese samples were bought from a popular grocery store in order to provide a store brand sample that would have the largest potential buyers. With the growing popularity of “organic” foods and the marketing around the health benefits, we decided to include organic and regular cheese samples to cover a wider variety and possible provide insight into differences between organic and non-organic products. Analyte selection of phthalate esters was based on recent news reports. Our group has a well-published history of working with cyclodextrin hosts and BODIPY fluorophores, leading to the experimental design around this system. Each of these components are discussed in more detail, below.

**Cheese Sample Selection**

News reports highlighted the presence of phthalate esters in one commercial cheese powder product (Kounang 2017). In order to research the prevalence of such phthalates in cheese powders, we selected multiple powders produced from a variety...
of companies. These powders include both organic cheese powder companies and non-organic cheese powder manufacturers, as popular culture generally associates organic food with less chemical contamination, and we were interested in determining if the organic cheese powder contained less toxicants.

**Analyte Selection**

The selection of phthalate esters was driven by the recent news reports that have found such esters in a commercial cheese powder. Analyte selection was driven by a desire to confirm the accuracy of that report, elucidate the scope of phthalate esters that are found in such powders, and identify where such esters are found in other commercial cheese powder samples produced by other manufacturers. The broad variety of phthalate esters selected is due to the fact that these esters can have widely disparate toxicities despite their highly similar structures (Gardner et al 2016). We find that phthalates 2, 5, 6-8, and 10 are present in powdered cheese samples, although other phthalates have been detected in a variety of cheese related food products (Serrano et al 2014).

**Cyclodextrin Selection**

The use of cyclodextrin as a supramolecular host for promoting intermolecular interactions has been well documented, both by our group and others (Cova et al. 2018; Kfoury, Landy, and Fourmentin 2018). In the system reported herein, the role of cyclodextrin is to act as a supramolecular scaffold that promotes favorable interactions between the phthalate ester analyte and the high quantum yield fluorophore acceptor, resulting in highly analyte-specific changes in the fluorescence emission. Unmodified commercially available γ-cyclodextrin was used for this purpose. The choice of γ-
cyclodextrin was made due to its large cavity size its well-documented ability to form ternary complexes with two small molecule guests, which provides the opportunity for both the phthalate ester analyte and fluorophore to bind in the cavity simultaneously (Hamai 2010, Hamai 2006).

Fluorophore Selection

For these experiments, a commercially available BODIPY derivative with a methyl substituent was selected, based on the well-documented ability of BODIPY fluorophores to display high quantum yield and robust performance under a variety of experimental conditions (Pliquett et al 2019; Chansaenpak et al 2018). Commercial availability of the fluorophore provides additional advantages in ensuring widespread applicability and adaptation of this system in potential commercial detection devices.

Cheese Sample Characterization

GC-MS Experiments

GC-MS was used to characterize the undoped (i.e. analyte-free) cheese samples (Figure 3). As expected, all cheese samples showed peaks in the chromatograms which corresponded to various fatty acid compounds typically found in dairy products, predominantly hexanoic acid and octanoic acid. Interestingly, almost all of the samples, except the regular store brand, showed a peak that corresponded to dicyclohexyl phthalate 6, a chemical contaminant and known plasticizer that is not part of the food composition. All phthalates found in undoped cheese samples are shown in Table 1. Moreover, the regular (i.e. non-organic) brands showed a higher concentration of the phthalate compared to the organic brands (5.43% of the total chemical composition in the non-organic brands compared to 0.24% of the total
composition in the organic brand). The identity of all peaks shown in the electronic support information (Tables S2-S5) was confirmed using the NIST14 library as well as by matching the retention times and mass spectra to known standards (Castle, Mayo, and Gilbert 1989).

**Table 1.** Phthalates detected in cheese powder samples via gas chromatography-mass spectrometry (GC-MS analysis)\(^a\)

| Analyte | regular | organic |
|---------|---------|---------|
|         | Name Brand | Store Brand | Name Brand | Store Brand |
| 1       | X        |         | X          |
| 2       | X        |         |            |
| 3       |          |         |            |
| 4       | X        | X       | X          |
| 5       | X        |         |            |
| 6       | X        | X       | X          |
| 7       | X        | X       |            |
| 8       | X        |         |            |
| 9       |          |         |            |
| 10      | X        |         |            |
| 11      |          |         |            |
| 12      |          |         |            |
| 13      |          |         |            |
| 14      |          |         |            |

\(^a\)
The presence of an “X” indicates that the phthalate analyte has been found in the particular cheese sample investigated.

**Fluorescence Experiments**

**Analyte-Doped Cheese Sample Results**

In order to prove viability of the technology along with getting proper limits of detection, a series of doping studies were done, in which known amounts of the analytes of interest were added to the cheese powders and the ability of our technology to detect those compounds was measured. Before that could be done, however, an equilibration study was performed, as cheese powders are notably turbid and require time and thorough mixing prior to analysis.

**Equilibration study**

Due to the complex nature of the processed cheese powder, we conducted detailed equilibration studies to determine when an ideal time for analysis was reached. Results of these studies indicated that one hour of equilibration was required (Figure 3 and Table 2). Of note, the most dramatic change happened in the first 15 minutes of equilibration, with the difference between \( t = 0 \) min and \( t = 6 \) minutes being more than 75%. One hour was used as the standard equilibration time, as the difference in the signal between 55 minutes and 60 minutes was less than 1%. These changes were calculated as a change in emission, \( \Delta \text{Emission} \), using Equation 4 below, where \( E_i \) is the initial emission and \( E_{i+5\text{minutes}} \) is the emission after five minutes.

\[
\frac{(E_i - E_{i+5\text{minutes}})}{E_i} \times 100\% = \Delta \text{Emission} \quad \text{(Eq. 4)}
\]
Figure 3. Fluorescence spectra results of the timed equilibration experiment showing average emission over 6-minute intervals

Table 2. Equilibration study change in emission signal over 60 minutes\(^\text{a}\) using Eq. 4

| Time (mins) | ΔEmission | Time (mins) | ΔEmission |
|------------|-----------|-------------|-----------|
| 0          | 0%*       | 32          | 11%       |
| 1          | -4%*      | 34          | 5%        |
| 2          | -4%*      | 35          | 4%        |
| 3          | -5%*      | 36          | 4%        |
| 6          | 43%       | 37          | 2%        |
| 7          | 50%       | 38          | 4%        |
| 8          | 55%       | 39          | 8%        |
| 9          | 60%       | 42          | 6%        |
| 12         | 62%       | 43          | 8%        |
| 14         | 63%       | 44          | 7%        |
|   |   |   |   |
|---|---|---|---|
| 15 | 59% | 45 | 2% |
| 16 | 53% | 49 | 5% |
| 18 | 37% | 50 | 2% |
| 19 | 25% | 51 | 4% |
| 20 | 23% | 52 | 5% |
| 21 | 22% | 53 | 4% |
| 23 | 15% | 54 | 4% |
| 24 | 13% | 55 | 2% |
| 25 | 13% | 56 | 2% |
| 26 | 11% | 57 | 1% |
| 29 | 9%  | 58 | 1% |
| 30 | 10% | 59 | 2% |
| 31 | 10% | 60 | -1% |

* values are compared to the T = 0 minute emission until 5 minutes have passed; after that, values are compared to the emission 5 minutes previously

**Fluorescence Modulation**  
Because phthalates were detected in all of the cheese powder samples investigated, we then decided to dope known amounts of a broader variety of phthalates into the cheese powders, with the goal of understanding how the presence of the phthalates affects the fluorescence emission signal of fluorophore 17 and how such analyte-induced changes can be used for phthalate detection. This phenomenon was true independent of the identity of the cheese powder matrix investigated. Furthermore, the simplicity of this fluorescence-based system allows for rapid results generation compared with the more tedious GC-MS analysis. This fact, combined with advances in portable fluorescence spectrometers, lays the ground work for future rapid, highly portable fluorescence-based detection systems.
Investigating differences in the fluorescence modulation results obtained in different brands of cheese powder revealed that most cheese powders had fluorescence modulation ratios greater than 1 (Table 3), although variability between different cheese powder sources was observed. All samples had a significant number of fatty acids (*vide supra*), including hexanoic acid and octanoic acid. Such fatty acids are able to self-associate into higher order architectures that provide an environment with increased hydrophobicity as well as increased steric constraints, both of which facilitate increased fluorescence emission (Li et al. 2012). Other notable trends observed for analyte-induced fluorescence modulation include:

(a) All samples showed fluorescence modulation values greater than 1, meaning that the presence of the analyte led to increased fluorescence emission of the fluorophore. This phenomenon is due in part to the large cavity size of \( \gamma \)-cyclodextrin, which enables it to bind (or partially bind) the analyte and the fluorophore in the cavity simultaneously. This ternary complexation, in turn, leads to reduced free bond rotation and a concomitant increase in the observed fluorescence emission. Moreover, the continued interaction between the analyte and the fluorophore afforded by the ternary complex facilitates low limits of detection for all phthalate ester analytes (*vide infra*).

(b) Compounds 10 and 11 showed markedly higher modulation values compared to all the other analytes investigated. Computational modelling of these analytes in the \( \gamma \)-cyclodextrin complex showed an improved steric complementarity between the larger analytes and the \( \gamma \)-cyclodextrin host cavity (*vide infra*), which in turn results in a more substantial analyte-induced
fluorescence change.

(c) Overall, organic brand cheese powder samples displayed analyte-induced fluorescence increases that were slightly higher than those observed in the non-organic brands. This may be due to the fact that these brands have a wider variety of ingredients due to the nature of their processing, or lack thereof, while regular brands have large quantities of additives diluting the original components. The mass spectrometry data revealed more that there is more than twice the number of organic components in organic cheese powder samples compared to the organic components found in non-organic cheese matrices.
Table 3. Fluorescence modulation ratios obtained with fluorophore 17 and analytes 1-16\(^a\)

| Compound | Regular Cheese Powder | Organic Cheese Powder |
|----------|-----------------------|-----------------------|
|          | Name Brand | Store Brand | Name Brand | Store Brand |
| 1        | 1.53 ± 0.01 | 1.25 ± 0.00 | 1.32 ± 0.01 | 1.42 ± 0.01 |
| 2        | 1.69 ± 0.01 | 1.30 ± 0.01 | 1.27 ± 0.00 | 1.37 ± 0.01 |
| 3        | 1.50 ± 0.01 | 1.78 ± 0.01 | 2.02 ± 0.02 | 1.80 ± 0.02 |
| 4        | 1.56 ± 0.01 | 1.70 ± 0.04 | 1.48 ± 0.01 | 2.32 ± 0.00 |
| 5        | 1.45 ± 0.01 | 3.27 ± 0.04 | 1.97 ± 0.01 | 1.99 ± 0.01 |
| 6        | 1.32 ± 0.01 | 1.32 ± 0.01 | 1.87 ± 0.01 | 1.34 ± 0.01 |
| 7        | 1.28 ± 0.01 | 2.26 ± 0.03 | 1.66 ± 0.00 | 1.69 ± 0.00 |
| 8        | 1.91 ± 0.01 | 1.24 ± 0.01 | 2.37 ± 0.01 | 1.73 ± 0.01 |
| 9        | 2.16 ± 0.01 | 1.64 ± 0.01 | 1.57 ± 0.00 | 2.01 ± 0.001 |
| 10       | 4.02 ± 0.04 | 4.80 ± 0.02 | 4.59 ± 0.10 | 4.00 ± 0.03 |
| 11       | 3.61 ± 0.02 | 2.56 ± 0.01 | 2.34 ± 0.02 | 4.63 ± 0.03 |
| 12       | 1.35 ± 0.00 | 1.18 ± 0.01 | 1.28 ± 0.00 | 1.38 ± 0.00 |
| 13       | 1.40 ± 0.01 | 1.24 ± 0.01 | 1.32 ± 0.01 | 1.27 ± 0.00 |
| 14       | 1.06 ± 0.01 | 1.20 ± 0.02 | 1.25 ± 0.01 | 1.40 ± 0.00 |
| 15       | 1.12 ± 0.00 | 1.14 ± 0.00 | 1.31 ± 0.01 | 1.23 ± 0.01 |
| 16       | 1.10 ± 0.00 | 1.22 ± 0.01 | 1.44 ± 0.01 | 1.36 ± 0.01 |

\(^a\) Fluorescence modulation values were calculated according to Equation 2, above, and each value represents the average of at least 6 trials.
Limits of Detection

In addition to using fluorescence modulation to validate the compositional differences in the samples, we also used it to quantify system sensitivity for each analyte. The sensitivity of the system was determined by calculating limits of detection (LODs) for analytes 1-15 in each cheese sample, using γ-cyclodextrin as a supramolecular host (see Table S1 in the ESI for more details), with LODs ranging from 11.893-0.124 μM, depending on the identity of the analyte, cheese powder, and fluorophore selected. For comparison, the Consumer Product Safety Committee reports that the maximum acceptable intake of phthalate esters ranges from 152 mg/kg-day for diisononylphthalate to as low as 0.5 mg/kg-day in the case of dibutylphthalate. Based off of a one cup serving size of macaroni and cheese, and an approximate adult human weight of 63 kg, our highest LOD (i.e. least sensitive detection capability) is still an order of magnitude lower than what can be safely consumed. In general, the LODs of the analytes in organic brand cheese powder were significantly lower than the LODs obtained in non-organic cheese powder and the store brand samples had lower limits of detections than the brand names. This may be due to the additives in the regular brands having interfering interactions with the system. Of note, the strong complementarity between analytes 10 and 11 and the cyclodextrin cavity, which resulted in strong fluorescence modulation values, also led to lower LOD values, with compound 11 having the lowest limit of detection observed (0.124 μM). In contrast, the analytes with higher LOD values (i.e. those with less sensitive detection) tended to be the smaller structures, with dimethylphthalate and monomethylphthalate having much higher average LODs across all samples. Moreover, the mono-substituted
phthalates (compounds 12-15) were shown to have larger LODs compared to their disubstituted analogues. This trend is likely due to weaker binding of the mono-substituted analogues in the cyclodextrin cavity, due to increased hydrophilicity and decreased steric complementarity, which in turn weakens this hydrophobically promoted complexation (Figure 4). Options to improve the sensitivity in the smaller analytes include the use of a smaller cyclodextrin host, such as α- or β-cyclodextrin, and such experiments are currently under investigation in our laboratory.

![Figure 4](image.png)

**Figure 4.** Electrostatic potential maps of: (A) compound 1 and (B) compound 2 on the same electrostatic potential scale

**Molecular operating environment**

Due to the unique behavior of analytes 10 and 11, we decided to investigate their complexation computationally. In a system with analytes 10 and 11 together with fluorophore 17 and γ-cyclodextrin, the MOE-derived computations showed a ternary complex as the most energetically favorable structure, with particularly close-range contacts observed between the analytes and fluorophore. In contrast, minimizing the energy of the complex of cyclodextrin with other analytes (i.e. compound 8) clearly
show fluorophore 17 being ejected from the cavity and replaced solely with analyte. This indicates that there is likely to be limited interaction between the fluorophore and the analyte in such cases, resulting in minimal fluorescence modulation. Modulation in the ternary complex, by contrast, is noticeably higher due to the continued close-range interactions. Visualizations of these complexes can be seen in Figure 5.

**Figure 5.** Molecular Operating Environment images of γ-cyclodextrin in purple, fluorophore 17 in blue, and phthalate analytes: (A) Compound 8; (B) compound 11; and (C) compound 10 in green

**CONCLUSIONS**

In conclusion, reported herein is the cyclodextrin-promoted fluorescence-based detection of 15 phthalate ester analytes in commercial cheese powder samples. Compared to GC-MS based methods, this fluorescence modulation strategy allows for a broad substrate scope and rapid results generation, while maintaining high sensitivity and simple sample preparation procedures. GC-MS analysis, by contrast, was not able to detect all phthalate ester analogues and required significant time for method development and individual sample analysis, although it did provide identification of individual phthalate analytes as a good complement to fluorescence-based detection. In parallel with these experimental methods, computational experiments provided
unique insight about the nature of the cyclodextrin-promoted interaction, especially for the analytes that provided the greatest modulation values and lowest limits of detection. Current efforts in our laboratory are dedicated towards decreasing detection limits further and exploring additional cyclodextrin-fluorophore combinations. Results of these and other investigations will be reported in due course.

ASSOCIATED CONTENT

Supporting Information

All experimental procedures; summary tables and figures for all fluorescence modulation experiments, equilibration studies, limit of detection analyses; details of computational experiments. This information can be found in the electronic supporting information (ESI) which accompanies this manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

No human subjects were involved in this study.

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Electronic Supplementary Information For

Identification of 15 Phthalate Esters in Commercial Cheese Powder via Cyclodextrin-Promoted Fluorescence Detection

Benjamin Cromwell, Mara Dubnicka, Sage Dubrawski and Mindy Levine
MATERIALS AND METHODS

All analytes and cyclodextrins were obtained from Sigma-Aldrich chemical company. All fluorescence measurements were performed using a Shimadzu RF-6000 fluorimeter. The slit width was 1.5 nm for both excitation and emission. All fluorescence spectra were integrated vs. wavenumber on the X-axis using OriginPro Version 9.6.0.172. Gas chromatography-mass spectrometry (GC-MS) was done on a Shimadzu QP2020. Mass spectra were analyzed using the GC-MS Post Run analysis software equipped with the NIST14 library. Computational modelling was done using Molecular Operating Environment software and the electrostatic potential maps were generated via Spartan ’18 software.

**Figure 6.** Structure and identity of all analytes and fluorophores used
EXPERIMENTAL DETAILS

Experimental details for fluorescence equilibration experiments

In a quartz cuvette, 1.25 mL of a 10 mM cyclodextrin solution dissolved in phosphate buffered saline (PBS, buffered to pH 7.4) and 1.25 mL of a cheese powder sample (5 g/L in water) were combined with 100 µL of a 0.1 mg/mL solution of fluorophore 17 and set on a shaker table for single minute increments prior to sampling. Spectra were obtained by fluorescence analysis using a 460 nm excitation wavelength. The sampling continued until the change in the fluorescence signal was less than 5% over 5 minutes, as determined by Equation 1, below:

\[
\text{Signal change} = \frac{(Fl_{t+5} - Fl_{\text{initial}})}{Fl_{\text{initial}}} \quad \text{(Eq. 1)}
\]

where \( Fl_{t+5} \) minutes refers to the integrated fluorescence emission 5 minutes after the specified time, and \( Fl_{\text{initial}} \) refers to the integrated fluorescence emission at the initial time period.

Experimental details for fluorescence modulation experiments

In a quartz cuvette, 1.25 mL of a 10 mM cyclodextrin solution dissolved in PBS and 1.25 mL of a cheese powder sample (5 g/L in water) were combined and mixed thoroughly by shaking on a shaker station for 1 hour. Next, 100 µL of a 0.1 mg/mL solution of fluorophore 17 in tetrahydrofuran (THF) was added, and the solution was excited four times using a 460 nm excitation wavelength. Then, 50 µL of analytes 1-
16 (1.0 mg/mL in THF) were added to the mixture, and the solution was again excited four times at 460 nm. The fluorescence emission spectra were integrated vs. wavenumber on the X-axis using OriginPro software, and the degree of fluorescence modulation was determined using Equation 2, below:

\[ \text{Fluorescence modulation} = \frac{F_{\text{analyte}}}{F_{\text{blank}}} \]  

(Eq. 2)

where \( F_{\text{analyte}} \) refers to the integrated fluorescence emission in the presence of the analyte and \( F_{\text{blank}} \) refers to the integrated fluorescence emission in the absence of an analyte.

**Experimental details for limit of detection experiments**

Limit of detection experiments were conducted following literature-reported procedures (Cheng et al. 2016). In brief, 1.25 mL of γ-cyclodextrin and 1.25 mL of 5 g/L cheese powder in water were added to a quartz cuvette and mixed thoroughly. Next, 100 µL of fluorophore 15 was added to the cuvette and the solution was excited six times at 460 nm. Next, 10 µL of analyte was added, and again the solution was excited six times at 460 nm. This step was repeated for 20 µL of analyte, 30 µL of analyte, 40 µL of analyte, 50 µL of analyte, 60 µL of analyte, 70 µL of analyte, 80 µL of analyte, 90 µL of analyte, and 100 µL of analyte.

All fluorescence emission spectra were integrated vs. wavenumber on the X-axis, and calibration curves were generated. The curves plotted the analyte concentration, measured in µM, on the X-axis and the fluorescence modulation ratio on the Y-axis.
The curve was fitted to a straight line and the equation of the line was determined. The limit of detection was calculated according to Equation 3, below:

\[
\text{Limit of detection} = \frac{3(\text{SD}_{\text{blank}})}{m} \quad \text{(Eq. 3)}
\]

where \( \text{SD}_{\text{blank}} \) refers to the standard deviation of the blank (i.e. analyte-free) sample, and \( m \) refers to the slope of the line that is generated from the best curve fit.

**Experimental details for electrostatic potential maps**

Computational models of the molecules were built in Spartan 18’ and underwent a ground state geometry calculation using semi-empirical PM3 methods in the gas phase followed by an electrostatic potential map surface calculation (ESP). The values for the ESP maximum and minimum energies were normalized across all samples in order to better compare the polarities of analytes. Once calculations were completed a scale was selected to best visualize the differences in polarities by taking into account the lowest electrostatic potential and highest electrostatic potential value across all samples. In this case a scale from -250 kJ/mol to 250 kJ/mol was selected.

**Experimental details for computational modelling**

Molecular models of the molecules were generated using the build function within the Molecular Operating Environment (MOE) software. Analytes were defined as the ligands and the \( \gamma \)-cyclodextrin host was defined as the receptor. The ligand-receptor system underwent a “quick preparation,” which is an initial energy minimization function, followed by a more in-depth dynamics calculation using a Berendsen velocity/position scaling algorithm and Amber 14: EHT forcefield. The calculations
done in solvent were prepared using a 90-angstrom cubic cell populated with roughly 2500 water molecules in order to display the most likely conformation. Computer specifications for these experiments were as follows: Intel®Core™ i7-2600 CPU @3.40GHz with 16.0 GB of RAM. SUMMARY FIGURES FOR FLUORESCENCE EQUILIBRATION EXPERIMENTS

![Figure 7. Equilibration time studies, with the figure on the left showing the fluorescence emission in single-minute increments, and the figure on the right showing fluorescence emission of the fluorophore in 6-minute increments.](image)
SUMMARY FIGURES FOR FLUORESCENCE MODULATION EXPERIMENTS

Store brand organic sample fluorescence modulation graphs

Analyte 1 - fluorophore 17

Figure 8. Fluorescence modulation experiment for analyte 1 and fluorophore 17 in store-brand organic cheese powder

Analyte 2 - fluorophore 17

Figure 9. Fluorescence modulation experiment for analyte 2 and fluorophore 17 in store-brand organic cheese powder
Analyte 3 - fluorophore 17

Figure 10. Fluorescence modulation experiment for analyte 3 and fluorophore 17 in store-brand organic cheese powder

Analyte 4 - fluorophore 17

Figure 11. Fluorescence modulation experiment for analyte 4 and fluorophore 17 in store-brand organic cheese powder
Analyte 5 - fluorophore 17

Figure 12. Fluorescence modulation experiment for analyte 5 and fluorophore 17 in store-brand organic cheese powder

Analyte 6 - fluorophore 17

Figure 13. Fluorescence modulation experiment for analyte 6 and fluorophore 17 in store-brand organic cheese powder
Analyte 7 - fluorophore 17

Figure 14. Fluorescence modulation experiment for analyte 7 and fluorophore 17 in store-brand organic cheese powder

Analyte 8 - fluorophore 17

Figure 15. Fluorescence modulation experiment for analyte 8 and fluorophore 17 in store-brand organic cheese powder
Analyte 9 - fluorophore 17

**Figure 16.** Fluorescence modulation experiment for analyte 9 and fluorophore 17 in store-brand organic cheese powder

Analyte 10 - fluorophore 17

**Figure 17.** Fluorescence modulation experiment for analyte 10 and fluorophore 17 in store-brand organic cheese powder
Analyte 11 - fluorophore 17

Figure 18. Fluorescence modulation experiment for analyte 11 and fluorophore 17 in store-brand organic cheese powder

Analyte 12 - fluorophore 17

Figure 19. Fluorescence modulation experiment for analyte 12 and fluorophore 17 in store-brand organic cheese powder
Analyte 13 - fluorophore 17

Figure 20. Fluorescence modulation experiment for analyte 13 and fluorophore 17 in store-brand organic cheese powder

Analyte 14 - fluorophore 17

Figure 21. Fluorescence modulation experiment for analyte 14 and fluorophore 17 in store-brand organic cheese powder
Analyte 15 - fluorophore 17

**Figure 22.** Fluorescence modulation experiment for analyte 15 and fluorophore 17 in store-brand organic cheese powder

Analyte 16 - fluorophore 17

**Figure 23.** Fluorescence modulation experiment for analyte 16 and fluorophore 17 in store-brand organic cheese powder
Store brand regular cheese powder fluorescence modulation graphs

Analyte 1 - fluorophore 17

**Figure 24.** Fluorescence modulation experiment for analyte 1 and fluorophore 17 in store-brand regular cheese powder

Analyte 2 - fluorophore 17

**Figure 25.** Fluorescence modulation experiment for analyte 2 and fluorophore 17 in store-brand regular cheese powder
Analyte 3 - fluorophore 17

\[\text{Figure 26.} \text{ Fluorescence modulation experiment for analyte 3 and fluorophore 17 in store-brand regular cheese powder}\]

Analyte 4 - fluorophore 17

\[\text{Figure 27.} \text{ Fluorescence modulation experiment for analyte 4 and fluorophore 17 in store-brand regular cheese powder}\]
Analyte 5 - fluorophore 17

Figure 28. Fluorescence modulation experiment for analyte 5 and fluorophore 17 in store-brand regular cheese powder

Analyte 6 - fluorophore 17

Figure 29. Fluorescence modulation experiment for analyte 6 and fluorophore 17 in store-brand regular cheese powder
Figure 30. Fluorescence modulation experiment for analyte 7 and fluorophore 17 in store-brand regular cheese powder

Figure 31. Fluorescence modulation experiment for analyte 8 and fluorophore 17 in store-brand regular cheese powder
Analyte 9 - fluorophore 17

Figure 32. Fluorescence modulation experiment for analyte 9 and fluorophore 17 in store-brand regular cheese powder

Analyte 10 - fluorophore 17

Figure 33. Fluorescence modulation experiment for analyte 10 and fluorophore 17 in store-brand regular cheese powder
Analyte 11 - fluorophore 17

Figure 3.4. Fluorescence modulation experiment for analyte 11 and fluorophore 17 in store-brand regular cheese powder

Analyte 12 - fluorophore 17

Figure 3.5. Fluorescence modulation experiment for analyte 12 and fluorophore 17 in store-brand regular cheese powder
Analyte 13 - fluorophore 17

![Fluorescence modulation experiment for analyte 13 and fluorophore 17 in store-brand regular cheese powder](image)

**Figure 36.** Fluorescence modulation experiment for analyte 13 and fluorophore 17 in store-brand regular cheese powder

Analyte 14 - fluorophore 17

![Fluorescence modulation experiment for analyte 14 and fluorophore 17 in store-brand regular cheese powder](image)

**Figure 37.** Fluorescence modulation experiment for analyte 14 and fluorophore 17 in store-brand regular cheese powder
Analyte 15 - fluorophore 17

**Figure 38.** Fluorescence modulation experiment for analyte 15 and fluorophore 17 in store-brand regular cheese powder

**Name brand regular cheese powder fluorescence modulation graphs**

Analyte 1 - fluorophore 17

**Figure 39.** Fluorescence modulation experiment for analyte 1 and fluorophore 17 in name-brand regular cheese powder
Analyte 2 - fluorophore 17

Figure 40. Fluorescence modulation experiment for analyte 2 and fluorophore 17 in name-brand regular cheese powder

Analyte 3 - fluorophore 17

Figure 41. Fluorescence modulation experiment for analyte 3 and fluorophore 17 in name-brand regular cheese powder
Analyte 4 - fluorophore 17

Figure 42. Fluorescence modulation experiment for analyte 4 and fluorophore 17 in name-brand regular cheese powder

Analyte 5 - fluorophore 17

Figure 43. Fluorescence modulation experiment for analyte 5 and fluorophore 17 in name-brand regular cheese powder
Analyte 6 - fluorophore 17

**Figure 44.** Fluorescence modulation experiment for analyte 6 and fluorophore 17 in name-brand regular cheese powder

Analyte 7 - fluorophore 17

**Figure 45.** Fluorescence modulation experiment for analyte 7 and fluorophore 17 in name-brand regular cheese powder
Analyte 8 - fluorophore 17

**Figure 46.** Fluorescence modulation experiment for analyte 8 and fluorophore 17 in name-brand regular cheese powder

Analyte 9 - fluorophore 17

**Figure 47.** Fluorescence modulation experiment for analyte 9 and fluorophore 17 in name-brand regular cheese powder
Analyte 10 - fluorophore 17

Figure 48. Fluorescence modulation experiment for analyte 10 and fluorophore 17 in name-brand regular cheese powder

Analyte 11 - fluorophore 17

Figure 49. Fluorescence modulation experiment for analyte 11 and fluorophore 17 in name-brand regular cheese powder
Analyte 12 - fluorophore 17

![Graph](image1.png)

**Figure 50.** Fluorescence modulation experiment for analyte 12 and fluorophore 17 in name-brand regular cheese powder.

Analyte 13 - fluorophore 17

![Graph](image2.png)

**Figure 51.** Fluorescence modulation experiment for analyte 13 and fluorophore 17 in name-brand regular cheese powder.
Figure 52. Fluorescence modulation experiment for analyte 14 and fluorophore 17 in name-brand regular cheese powder

Figure 53. Fluorescence modulation experiment for analyte 15 and fluorophore 17 in name-brand regular cheese powder
Analyte 16 - fluorophore 17

**Figure 54.** Fluorescence modulation experiment for analyte 16 and fluorophore 17 in name-brand regular cheese powder

**Name brand organic cheese powder fluorescence modulation graphs**

Analyte 1 - fluorophore 17

**Figure 55.** Fluorescence modulation experiment for analyte 1 and fluorophore 17 in name-brand organic cheese powder
Analyte 2 - fluorophore 17

![Fluorescence modulation experiment for analyte 2 and fluorophore 17 in name-brand organic cheese powder](image)

**Figure 56.** Fluorescence modulation experiment for analyte 2 and fluorophore 17 in name-brand organic cheese powder

Analyte 3 - fluorophore 17

![Fluorescence modulation experiment for analyte 3 and fluorophore 17 in name-brand organic cheese powder](image)

**Figure 57.** Fluorescence modulation experiment for analyte 3 and fluorophore 17 in name-brand organic cheese powder
Analyte 4 - fluorophore 17

![Graph showing fluorescence modulation experiment for analyte 4 and fluorophore 17 in name-brand organic cheese powder.]

**Figure 58.** Fluorescence modulation experiment for analyte 4 and fluorophore 17 in name-brand organic cheese powder

Analyte 5 - fluorophore 17

![Graph showing fluorescence modulation experiment for analyte 5 and fluorophore 17 in name-brand organic cheese powder.]

**Figure 59.** Fluorescence modulation experiment for analyte 5 and fluorophore 17 in name-brand organic cheese powder
Analyte 6 - fluorophore 17

Figure 60. Fluorescence modulation experiment for analyte 6 and fluorophore 17 in name-brand organic cheese powder

Analyte 7 - fluorophore 17

Figure 61. Fluorescence modulation experiment for analyte 7 and fluorophore 17 in name-brand organic cheese powder
Analyte 8 - fluorophore 17

**Figure 62.** Fluorescence modulation experiment for analyte 8 and fluorophore 17 in name-brand organic cheese powder

Analyte 9 - fluorophore 17

**Figure 63.** Fluorescence modulation experiment for analyte 9 and fluorophore 17 in name-brand organic cheese powder
Analyte 10 - fluorophore 17

Figure 64. Fluorescence modulation experiment for analyte 10 and fluorophore 17 in name-brand organic cheese powder

Analyte 11 - fluorophore 17

Figure 65. Fluorescence modulation experiment for analyte 11 and fluorophore 17 in name-brand organic cheese powder
Analyte 12 - fluorophore 17

Figure 66. Fluorescence modulation experiment for analyte 12 and fluorophore 17 in name-brand organic cheese powder

Analyte 13 - fluorophore 17

Figure 67. Fluorescence modulation experiment for analyte 13 and fluorophore 17 in name-brand organic cheese powder
Analyte 14 - fluorophore 17

![Graph 1](image1)

**Figure 68.** Fluorescence modulation experiment for analyte 14 and fluorophore 17 in name-brand organic cheese powder

Analyte 15 - fluorophore 17

![Graph 2](image2)

**Figure 69.** Fluorescence modulation experiment for analyte 15 and fluorophore 17 in name-brand organic cheese powder
Analyte 16 - fluorophore 17

**Figure 70.** Fluorescence modulation experiment for analyte 16 and fluorophore 17 in name-brand organic cheese powder

**SUMMARY FIGURES FOR LIMIT OF DETECTION EXPERIMENTS**

Name brand regular cheese powder limit of detection graphs

**Figure 71.** Limit of detection plot for dimethylphthalate 1
Figure 72. Limit of detection plot for diethylphthalate 2

Figure 73. Limit of detection plot for dibutylphthalate 3
Figure 74. Limit of detection plot for diisobutylphthalate 4

Figure 75. Limit of detection plot for dihexylphthalate 5
Figure 76. Limit of detection plot for dicyclocyclohexylphthalate 6

Figure 77. Limit of detection plot for dioctylphthalate 7
Figure 78. Limit of detection plot for diisononylphthalate 8

Figure 79. Limit of detection plot for diphenylphthalate 9
**Figure 80.** Limit of detection plot for diallylphthalate 10

**Figure 81.** Limit of detection plot for benzylbutylphthalate 11
Figure 82. Limit of detection plot for monomethylphthalate 12

Figure 83. Limit of detection plot for monobutylphthalate 13
Figure 84. Limit of detection plot for monocyclohexylphthalate 14

Figure 85. Limit of detection plot for monobenzylphthalate 15
**Figure 86.** Limit of detection plot for tetrahydrofuran 16

Store brand regular cheese powder limit of detection graphs

**Figure 87.** Limit of detection plot for dimethylphthalate 1
Figure 88. Limit of detection plot for diethylphthalate 2

Figure 89. Limit of detection plot for dibutylphthalate 3
Figure 90. Limit of detection plot for diisobutylphthalate 4

Figure 91. Limit of detection plot for dihexylphthalate 5
Figure 92. Limit of detection plot for dicyclohexylphthalate 6

Figure 93. Limit of detection plot for dioctylphthalate 7
Figure 94. Limit of detection plot for diisononylphthalate 8

Figure 95. Limit of detection plot for diphenylphthalate 9
Figure 96. Limit of detection plot for diallylphthalate 10

Figure 97. Limit of detection plot for benzylbutylphthalate 11
Figure 98. Limit of detection plot for monomethylphthalate 12

Figure 99. Limit of detection plot for monobutylphthalate 13
Figure 100. Limit of detection plot for monocyclohexylphthalate 14

Figure 101. Limit of detection plot for monobenzylphthalate 15
Figure 102. Limit of detection plot for tetrahydrofuran 16

Store brand organic cheese powder limit of detection graphs

Figure 103. Limit of detection plot for dimethylphthalate 1
Figure 104. Limit of detection plot for diethylphthalate 2

Figure 105. Limit of detection plot for dibutylphthalate 3
Figure 106. Limit of detection plot for diisobutylphthalate 4

Figure 107. Limit of detection plot for dihexylphthalate 5
Figure 108. Limit of detection plot for dicyclohexylphthalate 6

Figure 109. Limit of detection plot for dioctylphthalate 7
Figure 110. Limit of detection plot for diisononylphthalate 8

Figure 111. Limit of detection plot for diphenylphthalate 9
Figure 112. Limit of detection plot for diallylphthalate 10

Figure 113. Limit of detection plot for benzylbutylphthalate 11
Figure 114. Limit of detection plot for monomethylphthalate 12

Figure 115. Limit of detection plot for monobutylphthalate 13
Figure 116. Limit of detection plot for monocyclohexylphthalate 14

Figure 117. Limit of detection plot for monobenzylphthalate 15
**Figure 118.** Limit of detection plot for tetrahydrofuran 16

Name brand organic cheese powder limit of detection graphs

**Figure 119.** Limit of detection plot for dimethylphthalate 1
Figure 120. Limit of detection plot for diethylphthalate 2

Figure 121. Limit of detection plot for dibutylphthalate 3
Figure 122. Limit of detection plot for diisobutylphthalate 4

Figure 123. Limit of detection plot for dihexylphthalate 5
Figure 124. Limit of detection plot for dicyclohexylphthalate 6

Figure 125. Limit of detection plot for dioctylphthalate 7
**Figure 126.** Limit of detection plot for diisononylphthalate 8

**Figure 127.** Limit of detection plot for diphenylphthalate 9
Figure 128. Limit of detection plot for diallylphthalate 10

Figure 129. Limit of detection plot for benzylbutylphthalate 11
Figure 130. Limit of detection plot for monomethylphthalate 12

Figure 131. Limit of detection plot for monobutylphthalate 13
Figure 132. Limit of detection plot for monocyclohexylphthalate 14

Figure 133. Limit of detection plot for monobenzylphthalate 15
Figure 134. Limit of detection plot for tetrahydrofuran 16
SUMMARY FIGURES FOR GAS CHROMATOGRAPHY EXPERIMENTS

**Figure 135.** Store-brand regular cheese powder sample GC-MS overlay of the relative intensities for both undoped and doped samples

**Figure 136.** Name-brand regular cheese powder sample GC-MS overlay of the relative intensities for both undoped and doped samples
**Figure 137.** Store-brand organic cheese powder sample GC-MS overlay of the relative intensities for both undoped and doped samples

**Figure 138.** Name-brand organic cheese powder sample GC-MS overlay of the relative intensities for both undoped and doped samples
SUMMARY FIGURES FOR ELECTROSTATIC POTENTIAL MAP GENERATION

Electrostatic maps were generated in Spartan ’18. A ground state calculation using semi-empirical PM3 method allowed for an electrostatic potential map surface to be created. The maps were then normalized to put all analytes on the same scale.

Figure 139. Electrostatic potential map of analyte 1

Figure 140. Electrostatic potential map of analyte 2
Figure 141. Electrostatic potential map of analyte 3

Figure 142. Electrostatic potential map of analyte 4

Figure 143. Electrostatic potential map of analyte 5
**Figure 144.** Electrostatic potential map of analyte 6

**Figure 145.** Electrostatic potential map of analyte 7

**Figure 146.** Electrostatic potential map of analyte 8
Figure 147. Electrostatic potential map of analyte 9

Figure 148. Electrostatic potential map of analyte 10

Figure 149. Electrostatic potential map of analyte 11

Figure 150. Electrostatic potential map of analyte 12
Figure 151. Electrostatic potential map of analyte 13

Figure 152. Electrostatic potential map of analyte 14

Figure 153. Electrostatic potential map of analyte 15

Figure 154. Electrostatic potential map of analyte 16
Figure 155. Electrostatic potential map of fluorophore 17
Summary Figures for Computational Studies

The following images were generated in Molecular Operating Environment software.

Water is colored grey to allow for visualization of the systems inside.

Figure 156. 90 angstrom water cube

Figure 157. Guide to ligand interaction figure
Figure 158. Benzylbutyl phthalate 11 in pink, water in grey, γ-cyclodextrin in blue, and fluorophore 17 in green

Figure 159. Ligand interactions of benzylbutyl phthalate 11
**Figure 160.** Diallylphthalate 10 in blue, fluorophore 17 in green, γ-cyclodextrin in pink and water in grey

**Figure 161.** Ligand interactions of diallylphthalate 10
**Figure 162.** Diisononyl phthalate 8 in green, fluorophore 17 in blue, γ-cyclodextrin in pink and water in grey

**Figure 163.** Ligand interactions of diisononyl phthalate 8
### SUMMARY TABLES

**Summary Table for Limit of Detection Experiments**

Table 4. Summary of the limit of detection experiments

| Analyte | name brand organic | store brand organic | store brand regular | name brand regular |
|---------|--------------------|---------------------|--------------------|-------------------|
| 1       | 5.722              | 11.515              | 6.253              | 3.878             |
| 2       | 4.156              | 3.381               | 3.876              | 4.035             |
| 3       | 1.014              | 1.222               | 1.097              | 2.422             |
| 4       | 0.245              | 1.584               | 0.990              | 2.971             |
| 5       | 0.992              | 1.494               | 0.306              | 1.265             |
| 6       | 1.020              | 1.706               | 4.094              | 4.386             |
| 7       | 1.123              | 1.055               | 0.443              | 2.157             |
| 8       | 1.170              | 0.486               | 4.159              | 0.850             |
| 9       | 0.722              | 1.066               | 2.532              | 0.837             |
| 10      | 0.236              | 0.254               | 0.238              | 1.489             |
| 11      | 0.124              | 0.464               | 0.527              | 0.797             |
| 12      | 2.375              | 1.933               | 10.333             | 11.893            |
| 113     | 2.183              | 3.127               | 7.631              | 5.321             |
| 14      | 4.496              | 2.953               | 7.733              | 8.115             |
| 15      | 5.751              | 2.432               | 3.283              | 8.034             |
Table 5. Mass spectra peak identification for name-brand organic cheese powder

| Retention Time (min) | Area   | Height | Relative concentration | Compound name                                      |
|----------------------|--------|--------|------------------------|----------------------------------------------------|
| 13.245               | 74897  | 2921   | 0.13                   | Methyl formate                                     |
| 14.324               | 77412  | 28816  | 0.13                   | Serine, methyl ester                               |
| 16.11                | 82396  | 16442  | 0.14                   | Serine, methyl ester                               |
| 13.412               | 108913 | 62435  | 0.18                   | 2-Dodecanone                                       |
| 9.465                | 113124 | 25826  | 0.19                   | Butanoic acid, 3-methyl-                           |
| 15.84                | 125161 | 33876  | 0.21                   | L-Proline, N-valeryl-, octadecyl ester             |
| 15.224               | 127827 | 21127  | 0.22                   | diisobutyl phthalate                               |
| 8.64                 | 135807 | 17763  | 0.23                   | Butanoic acid, 4-chloro-                           |
| 14.823               | 144241 | 35667  | 0.24                   | L-Proline, N-valeryl-, heptadecyl ester            |
| 17.437               | 139982 | 49956  | 0.24                   | .delta.-Nonalactone                                |
| 21.66                | 142464 | 73730  | 0.24                   | Phthalic acid, dodecyl octyl ester                 |
| 9.706                | 153634 | 15267  | 0.26                   | Butanoic acid, 3-methyl-                           |
| 20.476               | 176615 | 28859  | 0.3                    | Undecanoic acid, 10-bromo-                         |
| 6.324                | 200082 | 29949  | 0.34                   | Butanoic acid, 3-methyl-                           |
| 11.815               | 270973 | 102996 | 0.46                   | 2,4-Di-tert-butylphenol                            |
| 15.623               | 269489 | 113380 | 0.46                   | 2H-Pyran-2-one, 6-hexyltetrahydro-                 |

* All LODs were calculated using the procedures listed above, and the results represent an average of at least 6 trials. **Summary Table for GC-MS Results**
| Retention Time (min) | Area    | Height  | Relative concentration | Compound name                                    |
|----------------------|---------|---------|------------------------|-------------------------------------------------|
| 19.283               | 290788  | 41006   | 0.49                   | Pentadecanoic acid                              |
| 12.295               | 331647  | 49059   | 0.56                   | Octanoic acid                                   |
| 9.242                | 348133  | 38233   | 0.59                   | Butanoic acid, 4-chloro-                        |
| 13.602               | 370417  | 163123  | 0.63                   | .delta.-Nonalactone                             |
| 20.777               | 380935  | 53869   | 0.64                   | Dicyclohexyl phthalate                          |
| 8.505                | 395105  | 36629   | 0.67                   | Butanoic acid, 3-methyl-                        |
| 11.269               | 738336  | 109232  | 1.25                   | 2-Nonanone                                      |
| 8.865                | 809725  | 76192   | 1.37                   | Acetaldehyde semicarbazone                      |
| 6.523                | 2014261 | 223460  | 3.4                    | Maltol                                          |
| 10.012               | 4553519 | 478861  | 7.69                   | n-Decanoic acid                                 |
| 7.583                | 1297244 | 190397  | 21.91                  | Octanoic acid                                   |
| 4.945                | 3291666 | 237756  | 55.6                   | Hexanoic acid                                   |

**Table 6.** Mass spectra peak identification for store-brand organic cheese powder
|       |        |         |   |                           |
|-------|--------|---------|---|--------------------------|
| 12.172| 78472  | 27548   | 0.7| 2-Methyltetrasane       |
| 11.29 | 83231  | 43783   | 0.74| 2,3-Dimethyldecan        |
| 11.339| 84127  | 40934   | 0.75| .delta.-Nonalactone      |
| 12.297| 85435  | 33736   | 0.76| Pentadecafluorooctanoic acid, tetradecyl ester |
| 19.074| 99400  | 27238   | 0.88| dihexyl phthalate        |
| 12.365| 101127 | 53781   | 0.9 | Hexadecane               |
| 8.39  | 107406 | 17912   | 0.95| 1-Decanol, 2-ethyl-      |
| 9.658 | 117476 | 26596   | 1.04| Dodecane, 4-methyl-      |
| 8.522 | 130109 | 43288   | 1.16| Heneicosane, 11-(1-ethylpropyl)- |
| 11.819| 161699 | 70896   | 1.44| 2,4-Di-tert-butylphenol  |
| 11.994| 168343 | 52132   | 1.5 | Eicosane                 |
| 9.471 | 177793 | 64732   | 1.58| Dodecyl nonyl ether      |
| 7.945 | 186292 | 50471   | 1.66| Dodecane                 |
| 20.472| 196704 | 49829   | 1.75| Dicyclohexyl phthalate   |
| 10.067| 231748 | 95002   | 2.06| 1-Tetradecene            |
| 11.559| 290883 | 157049  | 2.58| Butylated Hydroxytoluene |
| 6.61  | 301285 | 44250   | 2.68| Maltol                   |
| 8.935 | 436146 | 172156  | 3.87| Tetradecane              |
| 10.149| 463881 | 253131  | 4.12| Tetradecane              |
| 6.052 | 512374 | 61552   | 4.55| Bicyclo[3.2.1]oct-3-en-2-one, 4-methyl- |
| 7.556 | 689947 | 180507  | 6.13| Octanoic acid            |
| Retention Time (min) | Area     | Height   | Relative concentration | Compound name                                      |
|---------------------|----------|----------|------------------------|----------------------------------------------------|
| 7.642               | 139174   | 252365   | 12.36                  | Ether, dodecyl isopropyl                           |
| 4.936               | 3974537  | 302644   | 35.31                  | Hexanoic acid                                      |

**Table 7.** Mass spectra peak identification for store-brand regular cheese powder

| Retention Time (min) | Area     | Height   | Relative concentration | Compound name                                      |
|---------------------|----------|----------|------------------------|----------------------------------------------------|
| 11.277              | 40315    | 23968    | 0.63                   | 1-Propene, 3-propoxy-                              |
| 12.08               | 41153    | 17762    | 0.65                   | 17,21-Dimethylheptatriacontane                     |
| 5.26                | 43421    | 14492    | 0.68                   | Butanoic acid, 4-chloro-                           |
| 9.765               | 43907    | 17614    | 0.69                   | Succinic acid, 2,2-dichloroethyl tetrahydrofurfuryl ester |
| 15.225              | 44483    | 26111    | 0.7                    | Phthalic acid, hept-4-yl isobutyl ester           |
| 16.032              | 45658    | 25999    | 0.72                   | Phthalic acid, butyl 2-pentyl ester                |
| 9.562               | 50463    | 18579    | 0.79                   | Succinic acid, 2-methylpent-3-yl tetrahydrofurfuryl ester |
| 23.289              | 52307    | 7795     | 0.82                   | Docosanoic acid, 1,2,3-propanetriyl ester         |
| 12.357              | 69775    | 41635    | 1.1                    | Dodecane, 2,7,10-trimethyl-                        |
| 9.456               | 72412    | 26008    | 1.14                   | Nonane, 5-methyl-5-propyl-                         |
| 14.083              | 76810    | 34151    | 1.21                   | Diallyl phthalate                                  |
| 12.29               | 83012    | 49860    | 1.3                    | 1-Undecene, 9-methyl-                              |
| 9.248               | 83197    | 11620    | 1.31                   | Phthalamic acid                                   |
| 12.497              | 92751    | 49164    | 1.46                   | Diethyl Phthalate                                 |
| 19.281              | 102152   | 34892    | 1.6                    | Benzyl butyl phthalate                            |
| Retention Time (min) | Area    | Height  | Relative concentration | Compound name                                      |
|---------------------|---------|---------|------------------------|----------------------------------------------------|
| 9.585               | 47028   | 17954   | 0.54                   | 4-Methoxycarbonyl-4-butanolide                      |
| 12.303              | 56749   | 28354   | 0.65                   | L-Proline, N-(3-cyclopentylpropionyl)-, propyl ester |
| 12.002              | 59567   | 29330   | 0.69                   | Decane, 3,3,5-trimethyl-                            |
| 11.34               | 59691   | 36999   | 0.69                   | Acetaldehyde, diethylhydrazone                     |

Table 8. Mass spectra peak identification for name-brand regular cheese powder
|       |         |          |        |                                      |
|-------|---------|----------|-------|-------------------------------------|
| 9.275 | 63720   | 17041    | 0.73  | Phthalic anhydride                  |
| 13.203| 69962   | 45798    | 0.81  | aR-Turmerone                        |
| 11.823| 72963   | 39965    | 0.84  | Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters |
| 12.371| 73652   | 36987    | 0.85  | Borane, diethyl(decyloxy)-          |
| 8.945 | 75121   | 32054    | 0.86  | Borane, diethyl(decyloxy)-          |
| 13.42 | 74898   | 46829    | 0.86  | 1,2-Ethanediamine, N'-ethyl-N,N-dimethyl- |
| 15.625| 79495   | 49749    | 0.91  | Acetaldehyde, diethylhydrazone      |
| 8.887 | 81707   | 37217    | 0.94  | Oxirane, methyl-, (S)-              |
| 8.99  | 88734   | 35414    | 1.02  | Nonane, 5-methyl-5-propyl-          |
| 21.657| 89206   | 34904    | 1.03  | Di-n-octyl phthalate                |
| 13.608| 106496  | 43321    | 1.23  | Acetaldehyde, diethylhydrazone      |
| 9.485 | 111932  | 37921    | 1.29  | Heptane, 3,3,5-trimethyl-            |
| 11.286| 126288  | 71376    | 1.45  | 2-Dodecanone                        |
| 11.565| 138272  | 66923    | 1.59  | 2,4,6-Tris(1,1-dimethylethyl)-4-methylcyclohexa-2,5-dien-1-one |
| 19.267| 149402  | 50204    | 1.72  | Benzyl butyl phthalate              |
| 6.283 | 157898  | 24357    | 1.82  | 1-Butanol, 2-methyl-, (S)-          |
| 10.02 | 158315  | 53056    | 1.82  | Butanoic acid, 4-chloro-             |
| 19.072| 225516  | 84249    | 2.59  | Phthalic acid, hexyl hex-3-yl ester |
| 10.074| 314320  | 133735   | 3.62  | 3-Tetradecene, (Z)-                 |
| 10.153| 363715  | 158698   | 4.19  | Tetradecane                         |
|             |       |       |    |                                 |
|------------|-------|-------|----|---------------------------------|
| 20.471     | 472235| 100337| 5.43| Dicyclohexyl phthalate          |
| 7.559      | 931111| 210116| 10.71| Octanoic acid                   |
| 7.647      | 1003296| 145478| 11.54| Octanoic acid                   |
| 4.979      | 3019114| 239003| 34.74| Hexanoic acid                   |
CHAPTER 2

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Colorimetric detection of polycyclic aromatic hydrocarbons using supramolecular cyclodextrin dimer-squaraine constructs

Sauradip Chaudhuri, Dana J. DiScenza, Molly Verderame, Benjamin Cromwell, and Mindy Levine

Department of Chemistry, University of Rhode Island, Kingston, RI, USA

Corresponding Author:

Mindy Levine, Ph. D.

Department of Chemistry

University of Rhode Island

Kingston, Rhode Island 02881, USA

MLevine@chm.uri.edu
Abstract

The development of solution-state colorimetric detection methods for highly toxic, environmentally persistent polycyclic aromatic hydrocarbons (PAHs) is reported herein. This method relies on supramolecular chemistry, and in particular on the ability of the PAH analytes to displace a squaraine dye bound with high affinities in β-cyclodextrin dimer hosts. The displacement of the dye results in visible color changes that are highly specific to each target analyte, resulting in up to 100% successful differentiation among 30 PAH analytes using straightforward image processing procedures and linear discriminant analyses.
**Introduction**

The detection of toxic chemicals in the environment is an important research area because these chemicals can have a variety of deleterious health effects to humans\(^1\) and other species.\(^2\) The chemicals are released into the environment through large scale chemical,\(^3\) oil,\(^4\) and fuel spills,\(^5\) and are also released in much lower quantities through the use of consumer products that contain these chemicals.\(^6,7\) This second category of chemical release is potentially more concerning, as the release can occur continuously for years without the consumer's knowledge,\(^8\) and cumulative exposure to such chemicals can reach toxic levels.\(^9\) Toxic chemicals that can be released through consumer product usage include a diverse suite of small organic molecules, such as bisphenol A (BPA)\(^10\) and other BPA analogues,\(^11\) phthalate esters such as diethyl and dibutyl phthalate,\(^12\) and polycyclic aromatic hydrocarbons (PAHs).\(^13\)

Currently used methods to detect bisphenols, phthalates, and PAHs generally rely on mass spectrometry-based methods such as gas chromatography mass spectrometry (GC-MS)\(^14\) and liquid chromatography mass spectrometry (LC-MS),\(^15\) which provide outstanding sensitivity\(^16\) but have significant drawbacks in terms of lack of portability, the requirement for costly laboratory equipment, and the need for highly trained instrument operators. Newer methods have also been developed, including the use of highly portable mass spectrometry,\(^17\) electrochemical detection methods\(^18\) and Raman spectroscopy-based methods,\(^19\) although the general applicability of such methods has not yet been determined.

Our group has developed a fundamentally new approach for toxicant detection in complex environments, which relies on cyclodextrin to act as a supramolecular scaffold and promote non-covalent interactions between an analyte of interest and a
high quantum yield fluorophore. These interactions result in proximity-induced fluorescence energy transfer, for photophysically active analytes,\textsuperscript{20} or proximity-induced fluorescence modulation, for non-photophysically active analytes.\textsuperscript{21} This method has been used for highly sensitive and specific detection in a variety of complex environments, including human plasma,\textsuperscript{22} urine,\textsuperscript{23} saliva,\textsuperscript{24} and breast milk,\textsuperscript{28} as well as in extracts collected directly from an oil spill\textsuperscript{26} and fuel spill,\textsuperscript{27} in contaminated marine environments,\textsuperscript{28} and in cow milk and plant milk alternatives.\textsuperscript{29}

Although this fluorescence-based detection has a number of advantages compared to mass spectral methods, it still requires sample excitation in order to observe an output signal, meaning that a laboratory-grade fluorimeter or portable hand-held fluorimeter is required. More portability is possible using analyte-induced color changes (i.e. colorimetric detection), which can be visualized using naked-eye detection methods as well as through quantitative image processing. Recent work from our group has reported the ability of a squaraine dye to display a visually different color upon binding in the cavity of a β-cyclodextrin dimer. Displacement of the squaraine dye from the cavity by a benzo[a]pyrene analyte resulted in a color change to a solution that closely resembled the color of the free (i.e. uncomplexed) dye solution.\textsuperscript{30}

Reported herein is a significant expansion of cases in which displacement of squaraine dyes from the cavities of β-cyclodextrin supramolecular hosts by a wide variety of aromatic analytes results in visible color differences that are highly analyte-specific and rely on strong and specific binding of the analytes in the cyclodextrin cavity. These color changes can be quantified using image processing software, and
understood with the assistance of computational modeling. The resulting quantitative information processed via linear discriminant analysis successfully generated unique response patterns for each analyte and 100% accuracy in unknown sample identification.

**Experimental Section**

All starting materials, solvents, and analytes were purchased from Sigma-Aldrich chemical company, Fisher Scientific, or Tokyo Chemical Industry (TCI), and used as received unless otherwise noted. All final products and all isolated chemical intermediates were analyzed using $^1$H NMR spectroscopy, $^{13}$C NMR spectroscopy, and high-resolution mass spectrometry, and details of these syntheses have been reported previously. All analytes were dissolved in tetrahydrofuran at a stock concentration of 1 mg/mL. Volumes of the stock analyte solutions were added to 2.5 mL aqueous solution of phosphate buffered saline (PBS, buffered at pH 7.4) containing hosts (H1-H3) and squaraine S1, with a final concentration of 30 µM. Of note, equimolar concentrations of analytes and hosts (30 µM) were made up by adding corresponding volumes of the stock solution into an aqueous PBS solution.

Photographs of the solution were taken using an iPhone 6 cellphone, with automatic camera settings. All images were processed using a web-based platform ([http://matkl.github.io/average-color/](http://matkl.github.io/average-color/)), which provided the average Red (R), Green (G), Blue (B), Hue (H), Saturation (S), and Luminescence (L) values for every image. Experiments were performed four times. Arrays were constructed using SYSTAT 13 software, using differences between the RGB-HSL values of samples and RGB-HSL
values of the control for each analysis, jack-knifed classification analysis for unknown sample identification, and long-range statistics of Mahal. Computed structures were energy-minimized using Spartan version 16. For energy minimization experiments, we used molecular dynamics calculations followed with molecular mechanics (optimized using an MMF94 force field). The structure was further minimized using semi-empirical level computations in gaseous media, with a PM3 force field. Electrostatic potential maps were generated using Spartan version 16. Images of host-guest complexes were calculated using an MOE 2018 operating system.31

Results and Discussion

Results previously reported by our group have concluded that squaraine S1 binds in cyclodextrin hosts H1-H3 with extremely high affinities (Table 9, Figure 164) as measured by fluorescence spectroscopy titrations,30,31 with particularly high affinities observed in the binding of squaraine S1 in host H1.

Table 9. Summary of Binding Constants of Squaraine S1 in Cyclodextrin Hosts H1-H3

| Host | Squaraine dye S1 |
|------|------------------|
| H1   | 1.7(0.4) x 10^5 M^-1(ref 30) |
| H2   | 4.2(1.0) x 10^5 M^-1(ref 30) |
| H3   | 2.4(0.6) x 10^6 M^-1(ref 30) |

*a* Binding constants were calculated via fluorescence titrations of the hosts (H1-H3) with increasing concentrations of the S1 guest measured in aqueous PBS solution.
Figure 164. Structures of cyclodextrin hosting (H1-H3) and squaraine dye S1 used in colormimetic detection schemes

The absorption spectrum of squaraine S1 in free solution is markedly different than its spectra in cyclodextrin, as a result of the fact that binding in cyclodextrin disrupts both H- and J-aggregate formation. These differences are also visible using naked eye detection (Figure 165), with clear differences that are reflected in different red (R), green (G), blue (B), hue (H), saturation (S), and luminescence (L) values. Starting from a complex of squaraine S1 and host H2, selected for its intermediate binding affinity compared to hosts H1 and H3, introduction of benzo[a]pyrene 11 (Figure 166) results in a solution color that closely resembles that of the unbound squaraine, as a result of the fact that benzo[a]pyrene displaces squaraine from the β-cyclodextrin cavity.
Figure 165. Changes in visible color that occur with the addition of analyte 11 to a solution-state complex of host H2 and guest S1

Figure 166. Structures of all aromatic analytes investigated

In addition to benzo[a]pyrene, many other polycyclic aromatic hydrocarbons (PAHs) have been reported to bind in β-cyclodextrin hosts,\textsuperscript{33,34} due to hydrophobically-driven inclusion between the hydrophobic guests and hydrophobic cyclodextrin interior cavity.\textsuperscript{35} Binding strengths of these PAH-cyclodextrin complexes
varies substantially depending on the PAH identity (Table 12), with high affinities observed in cases of strong steric complementarity between the guest and host (i.e. for naphthalene). Larger PAH guests are unable to fit completely inside the β-cyclodextrin cavity, and the resulting measured binding affinities are weaker as a result.

Table 10. Summary of Literature-Reported Binding Constants of PAH analytes in β-Cyclodextrin

| Analyte          | Literature-Reported Binding Constants in β-Cyclodextrin |
|------------------|--------------------------------------------------------|
| Benzene (1)      | 128 M⁻¹ (ref. 36)                                      |
| Naphthalene (2)  | 608 M⁻¹ (ref. 37)                                      |
| Anthracene (3)   | 32.4 M⁻¹ (ref. 38)                                     |
| Pyrene (10)      | 44 M⁻¹ (ref. 39)                                       |

*a Binding constants were reported in the literature

Introduction of small amounts of concentrated solutions of analytes 1-16 to solutions of cyclodextrin hosts H1-H3 bound with squaraine S1 resulted in distinctly different color changes for all analyte-cyclodextrin-squaraine combinations investigated (Figure 167). As expected, the colors of solutions that contained analytes were different from the analyte-free controls, as a result of analyte-induced displacement of the squaraine from the cyclodextrin cavities, with different degrees of displacement resulting in slightly different colorations. These color differences could be translated into quantitative color differences using a web-based image processing software, which provided notable differences among the RGB-HSL values for all solutions (see ESI for more details).
Figure 167. Illustration of visible color changes that result from introduction of analytes 1-16 t solutions of squaraine S1 with: (A) host H1; (B) host H2; and (C) host H3

The significant amount of data generated from such analyses can be processed using array-based statistical analysis to generate unique response patterns for each analyte. The use of statistical analysis to generate unique pattern identifiers for analytes in analogous situations is well-precedented, including in the detection of red wine blends,\textsuperscript{40} cancer cells,\textsuperscript{41,42} and amine vapors,\textsuperscript{43} and results in the Levine group have made ample use of linear discriminant analysis (LDA) to generate highly selective sensors.\textsuperscript{20} Error! Bookmark not defined. For this data, we explored a wide variety of ways to use the RGB-HSL values to generate meaningful arrays and highly selective colorimetric sensors.

\textit{RGB-HSL predictors}
When all RGB-HSL values were used as predictors for the arrays in combination with squaraine 4 as a signaling element with hosts H1, H2, and H3, each case led to 100% differentiation between structurally similar analytes (Figure 168). The percent differentiation was calculated using the jackknifed classification method, in which each analyte is individually reclassified to determine the ability of the array to differentiate between structurally similar analytes, and the percent differentiation refers to the percentage of the time in which that reclassification is correct (see Tables 23-34 in ESI for more details). The arrays for hosts H1, H2, and H3 were all visually different. The array for host H1 resulted in benzo[a]pyrene 11 and perylene 14 extremely well-separated from the other analytes, with their signals appearing on the opposite side of the array. This is likely due to the fact that both of these analytes can form 1:2 analyte: cyclodextrin complexes,44,45, which provides a unique signal pattern compared to the other analytes. The array for host H2 led to grouping of samples with samples 2, 12, 13, and 14 separated from the rest of the samples, which includes an optimally sterically matched analyte (compound 2), the only analyte with hydroxyl groups that can form hydrogen bonds to the cyclodextrin exterior rim (compound 12), and two moderately large PAHs (compounds 13 and 14) that can bind in β-cyclodextrin-based hosts with unique stoichiometries and geometries.46
Figure 168. Linear discriminant analysis results using RGB-HSL values for four trials of (a) host H1; (b) host H2; and (c) host H3 in combination with squaraine S1. Arrays were generated using SYSTAT 13 statistical software with the following settings: (a) classical discriminant analysis; (b) grouping variable: analytes, predictors: differences between control values and sample values of RGB-HSL; (d) long-range statistics: Mahal.

**RGB predictors**

When arrays were generated using only RGB values as predictors, the results using the three different hosts were noticeably different. For host H1, the array generated using only RGB values as predictors led to 100% differentiation between analytes. Similar to the array generated using RGB-HSL values as predictors, analytes 11 and 14 were well-separated from the rest of the analytes. Interestingly, analyte 12 was also well-separated from the other analytes, and grouped closely to analyte 4. The close spatial relationship between analytes 12 and 4 can be explained based on their structures, in particular the fact that they both contain heteroatoms and are capable of participating in hydrogen bonding with the cyclodextrin rim. The axes of the array generated using RGB predictors were a smaller range compared to the axes on the
array with RGB-HSL predictors, which means, unsurprisingly, that the analytes were better separated when more predictors were used. By contrast, the array for host H3 using RGB values as predictors led to only 88% differentiation between structurally similar analytes. Jackknifed classification analysis of these results showed that misclassification occurred only for analytes 5 and 7, which were misclassified as each other. Finally, for host H2 we were unable generate an array using RGB values alone. This is likely because the differences in RGB values between samples and control samples were not enough to lead to reasonable separation between analytes.

**HSL predictors**

Arrays were also generated using only HSL values as predictors. The array for host H1 with HSL values as predictors led to 100% differentiation between analytes. The visual array showed noticeable similarities to the array generated using RGB-HSL values as predictors, which can be attributed to the fact that differences in HSL values contributed more to the RGB-HSL array compared to the RGB predictors. The array for host H2 using HSL values as predictors led to 100% differentiation between analytes, again with notable visual similarities to the array generated using RGB-HSL predictors, which aligns with our hypothesis that RGB values do not contribute to the array for host H2. Finally, the array for host H3 with HSL values as predictors led to 100% differentiation between analytes, with an interesting mirror image relationship to the array generated using RGB-HSL values as predictors.

**Two-component predictors**

Arrays were also generated using various two-component combinations of RGB-HSL values as predictors. In the case of host H1, each combination (RG, RB, RH, GB, GH,
BH, HS, and SL) led to 100% differentiation between analytes. This is particularly exciting for our system because it shows we can still obtain maximum separation with less input data. The only combination that did not lead to 100% differentiation was the combination of HL, which led to 88% differentiation. This is likely due to the fact that differences in H and L values were not sufficient to lead to separation of analytes. For host H2, the only two-component array that could be generated was the combination of HL. This combination of predictors led to 100% differentiation between analytes. Visually, the two-component array using HL as predictors looked very similar to the three-component array using HSL as predictors, indicating that reducing input data can still lead to high observed selectivity. For host H3, each combination (RB, HS, HL) led to 100% differentiation between analytes, and the combination of RG and SL predictors led to 88% and 75% differentiation, respectively.

Computational Analysis

Further explanations for the observed trends in colorimetric array-based sensitivity was obtained through computational work, which highlighted the electron-rich nature of many of the fused aromatic ring analytes, in accordance with literature precedent (as indicated by the yellow color seen in the centers of the structures). The fact that there was some electron-deficiency along the exterior of the ring systems is also in accord with literature precedent, and is only interrupted by the presence of highly electron-rich heteroatoms. Notable differences among the electrostatic potential mapping of each analyte (Figure 169) were observed, especially for analytes containing heteroatoms (compounds 4, 5, and 12) and those containing non-aromatic components (compound 5), which translated into unique, analyte-specific array-based
response patterns. Quantitative computational data (see ESI for more details) indicates that the computed energies of the structures varied widely, with a range of more than 300 kJ/mol. Overall higher energies were observed for aromatic structures compared to those that lacked aromaticity (i.e. compound 5) as well as those with non-aromatic heteroatom substituents (i.e. compounds 5 and 12). Such widespread variability in turn leads to highly analyte-specific colorimetric responses in the interaction of the analytes with the squaraine-cyclodextrin complexes.

Figure 169. Electrostatic potential mapping of selected analytes. (A) Analyte 1; (B) Analyte 11; (C) Analyte 12; and (D) Analyte 14.
Figure 170. Computed structures of squaraine S1 in host H3. (a) Surface indicates the host area that is interacting with the guest. (B) Space-filling model of the host indicates significant inclusion of the squaraine guest; (C) Surface mapping of the guest shows protrusion of the terminal cyclohexyl groups from the cyclodextrin cavities.

Figure 171. Computed structures of analyte 11 in host H3. (A) Surface indicates the host area that is interacting with the guest. (B) Space-filling model of the host indicates significant inclusion of the guest. (C) Surface mapping of the guest shows virtually no protrusion of the surface area.

Additional computational support for strong supramolecular interactions in cyclodextrin dimer host: guest complexes was provided through using specialized docking software.\textsuperscript{31} Combining squaraine dye S1 with supramolecular host H3 led to a minimized geometry that showed close range interactions between the central squaraine cyclobutanone core and amides in the linker.
structure, raising the possibility of intermolecular hydrogen bonding as a stabilizing force (Figure 170A). Moreover, the squaraine was relatively well-encased by the cyclodextrin dimer host (Figure 170B), with some fraction of the cyclohexyl termini protruding from the cyclodextrin hosts on either side (Figure 170C). Binding of analyte 11 in host H3, by contrast, resulted in virtually complete inclusion of the benzo[a]pyrene guest (Figure 171A), with some interaction possible between the benzo[a]pyrene and aromatic anthracene linker (Figure 171B), and with strong inclusion of the benzo[a]pyrene surface area observed (Figure 171C). This degree of more complete inclusion of the benzo[a]pyrene guest in the cyclodextrin dimer host can explain how the analyte is able to displace the strongly bound squaraine fluorophore, through enabling even greater degrees of steric compatibility and complexation benefits.

A more detailed analysis of the quantitative computational data indicates that the computed energies of the analytes, hosts, and complexes varied widely, ranging from approximately 300 kJ/mol to 1300 kJ/mol (see ESI for more details). Overall, higher energies were observed for fully aromatic structures compared to those with some saturated component (i.e. compound 5) as well as those with heteroatom substituents (i.e. compounds 5 and 12). Such widespread variability in analyte energies results in greater variability in the energies of host-guest complexes, with a range of more than 1300 kJ/mol. Overall, the lowest energy value was measured for compound 6, the smallest analyte, and the largest energy value was measured for the relatively large compound 15. The hosts have to adopt particular conformations in order to accommodate each analyte, which results in highly variable energy
differences. These variations, in turn, result in highly analyte-specific colorimetric responses in the interaction of each analyte with the squaraine-cyclodextrin complexes.

**Conclusions**

Strongly analyte-dependent changes were observed in the squaraine guest-cyclodextrin host complex solution upon introduction of 16 different aromatic analytes. Quantitative array-based analysis generates unique signals for each target analyte, and attempts were made to use computational experiments to provide further insight into the mechanism of analyte-induced changes. The ease of use of this system, combined with the 100% success using 16 different structurally related compounds, provides substantial opportunity to generate practical and commercial colorimetric sensors. Efforts towards this goal and others are currently underway in our laboratory, and results of these efforts will be reported in due course.

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Electronic Supplementary Information for
Colorimetric Detection of Polycyclic Aromatic Hydrocarbons Using Supramolecular Cyclodextrin Dimer-Squaraine Constructs
Sauradip Chaudhuri, Dana J. DiScenza, Molly Verderame, Benjamin Cromwell, and Mindy Levine
MATERIALS AND METHODS

All dimeric cyclodextrin hosts and squaraine guests were synthesized following previously reported procedures. All polycyclic aromatic hydrocarbon analytes were purchased from Sigma Aldrich chemical company or from Fisher Scientific, and used as received. All solution-state complexes between the squaraine dyes and cyclodextrin dimer hosts were formed by combining 30 µL of the squaraine solution (24 µM final concentration) in an aqueous solution of 150 µL of the host (120 µM final concentration) and allowing the solution to equilibrate for several minutes, and photographing the resulting solutions. All images were processed using a web-based platform (http://matkl.github.io/average-color/), which provided the average Red (R), Green (G), Blue (B), Hue (H), Saturation (S), and Luminescence (L) values for every image. Arrays were constructed using Systat software, using differences between the RGBHSL values of samples and RGBHSL values of the control for each analysis. Electrostatic potential maps and energies were generated using Spartan ’18. Equilibrium geometry ground state energies were calculated using molecular mechanics with a Merk Molecular Force Field (MMFF) method in water. Electrostatic potential maps were generated using the semi-empirical Parameterized Model 3 (PM3) method in water. Electrostatic potential maps go from red (high electron density) to blue (low electron density).
DETAILS OF ANALYTES, HOSTS, AND SQUARAINES

Figure 172. Structures of hosts H1-H3

Squaraine guest:

Figure 173. Structure of squaraine S1
All analytes:

Figure 174 Structures of analytes 1-16
EXPERIMENTAL PROCEDURES

EXPERIMENTAL PROCEDURES FOR DIGITAL IMAGE PROCESSING

Each sample was isolated in its own image using cropping of the main photographs, taking care to stay away from the edges of the colored solution. Each cropped photograph was then subjected to analysis using the web-based platform, which provided average red, green, blue, hue, saturation, and luminescence values for the entire image.

EXPERIMENTAL PROCEDURES FOR ARRAY GENERATION

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

(a) Classical Discriminant Analysis

(b) Grouping variable: Analytes

(c) Predictors: Differences between control values and samples values of Red, Green, Blue, Hue, Saturation, and Luminescence

(d) Long-range statistics: Mahal
### SUMMARY TABLE FROM RGB-HSL (Red, Green, Blue – Hue, Saturation, Luminescence) VALUES

**Table 11.** RGB-HSL values for each combination

| Analyte | Host 1                  | Host 2                  | Host 3                  |
|---------|-------------------------|-------------------------|-------------------------|
| 1       | 189,184,196-31,6,65     | 154,154,181-216,15,66  | 225,124,224-105,7,69   |
| 2       | 169,164,180-259,10,67   | 164,164,187-235,14,69  | 237,134,219-23,33,69   |
| 3       | 156,154,175-246,12,65   | 152,154,188-228,21,67  | 234,134,221-28,24,69   |
| 4       | 159,154,178-243,11,66   | 163,164,191-229,18,69  | 227,124,219-30,12,67   |
| 5       | 166,164,184-243,12,68   | 178,174,194-229,12,63  | 222,124,220-0,3,67     |
| 6       | 167,164,184-240,11,69   | 177,174,193-218,11,63  | 223,124,220-40,4,67    |
| 7       | 165,164,186-234,13,69   | 139,134,175-220,18,62  | 218,114,217-60,1,65    |
| 8       | 164,164,177-235,8,67    | 145,144,171-217,13,62  | 223,124,215-53,11,66   |
|   | 172,174,189- | 150,154,183- | 220,124,221- |
|---|--------------|--------------|--------------|
| 9 | 226,11,61    | 218,19,65    | 150,3,67     |
| 10| 170,174,192-| 149,144,183-| 224,124,225-|
|   | 226,15,61    | 217,19,65    | 140,5,68     |
| 11| 174,174,197-| 153,154,182-| 229,124,230-|
|   | 227,17,63    | 213,17,66    | 140,6,60     |
| 12| 187,184,194-| 160,164,183-| 230,134,224-|
|   | 240,5,65     | 217,14,67    | 75,15,69     |
| 13| 173,174,185-| 152,154,180-| 229,124,225-|
|   | 245,8,60     | 223,16,65    | 60,7,69      |
| 14| 174,174,171-| 157,154,168-| 231,134,215-|
|   | 40,2,68      | 207,6,64     | 60,25,67     |
| 15| 178,174,190-| 154,154,176-| 232,134,240-|
|   | 245,9,62     | 221,12,65    | 225,21,63    |
| 16| 188,184,204-| 138,134,164-| 224,124,243-|
|   | 244,14,67    | 228,13,69    | 224,44,62    |

* RGB-HSL values were calculated using web-based software that measured the average values for all images*
### SUMMARY TABLES FROM ARRAY-BASED ANALYSES

#### SERIES 1

**Table 12.** Array results for differences from control sample using RGB-HSL values for Series 1

| Sample | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | % correct |
|--------|----|----|----|----|----|----|----|----|----|-----------|
|        | 100| 100| 100| 100| 100| 100|    |    |    |           |
| Sample | 1  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2  |           |
| 1      | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 10     | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 11     | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 12     | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 13     | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 14     | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 15     | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 16     | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 2      | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 3      | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 4      | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 5      | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 6      | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 7      | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 8      | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 9      | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| total  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 100       |

**Cumulative Proportion of Total Dispersion**

|              | 0.960 | 0.993 | 0.999 | 1.000 | 1.000 |

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Table 13. Array results for differences from control sample using RGB values for Series 1

| Sample | 1 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | % correct |
|--------|---|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|-----------|
| 1      | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 10     | 0 | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 11     | 0 | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 12     | 0 | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 13     | 0 | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 14     | 0 | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 15     | 0 | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 16     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 2      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 3      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 4      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 100       |
| 5      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 100       |
| 6      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 100       |
| 7      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 100       |
| 8      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 100       |
| 9      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 100       |
| total  | 4 | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 100       |

Cumulative Proportion of Total Dispersion

| 0.726 | 0.983 | 1.000 |
Table 14. Array results for differences from control sample using HSL values for Series 1

| Sample | 1 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | % correct |
|--------|---|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|-----------|
| 1      | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 10     | 0 | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 11     | 0 | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 12     | 0 | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 13     | 0 | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 14     | 0 | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 15     | 0 | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 16     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 2      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 3      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 0 | 0 | 100       |
| 4      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 0 | 100       |
| 5      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 100       |
| 6      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 100       |
| 7      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0 | 0 | 100       |
| 8      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0 | 100       |
| 9      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 4 | 100       |
| total  | 4 | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 100       |

Cumulative Proportion of Total Dispersion
Table 15. Array results for differences from control sample using RG values for Series 1

| Sample | 1  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | % correct |
|--------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----------|
| 1      | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 10     | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 11     | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 12     | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 13     | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 14     | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 15     | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 16     | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 2      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 3      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 4      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 5      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 100       |
| 6      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 7      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 100       |
| 8      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 100       |
| 9      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| total  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 100       |

Cumulative Proportion of Total Dispersion

| 0.964 | 1.000 |
Table 16. Array results for differences from control sample using RB values for Series 1

| Sample | 1 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | % correct |
|--------|---|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|-----------|
| 1      | 1 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 100       |
| 10     | 0 | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 11     | 0 | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 12     | 0 | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 13     | 0 | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 14     | 0 | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 15     | 0 | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 16     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 2      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 3      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 4      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 5      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 100       |
| 6      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 100       |
| 7      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 100       |
| 8      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 100       |
| 9      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 100       |
| total  | 4 | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 100       |

Cumulative Proportion of Total Dispersion

|                          | 0.624 | 1.000 |
|--------------------------|-------|-------|

Table 17. Array results for differences from control sample using GB values for Series 1

| Sample | 1 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | % correct |
|--------|---|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|-----------|
| 1      | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 10     | 0 | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 11     | 0 | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 12     | 0 | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 13     | 0 | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 14     | 0 | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 15     | 0 | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 16     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 2      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 3      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 4      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 100       |
| 5      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 100       |
| 6      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 100       |
| 7      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 100       |
| 8      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 100       |
| 9      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 100       |
| total  | 4 | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 100       |

Cumulative Proportion of Total Dispersion

|            | 0.635 | 1.000 |
|------------|-------|-------|
| Sample     |       |       |
|            |       |       |
**Table 18.** Array results for differences from control sample using HS values for Series

| Sample | 1 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | % correct |
|--------|---|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|-----------|
| 1      | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 10     | 0 | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 11     | 0 | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 12     | 0 | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 13     | 0 | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 14     | 0 | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 15     | 0 | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 16     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 2      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 3      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 4      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 5      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 100       |
| 6      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 100       |
| 7      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 100       |
| 8      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 100       |
| 9      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 100       |
| total  | 4 | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 100       |

**Cumulative Proportion of Total Dispersion**

|                  | 0.998 | 1.000 |
|------------------|-------|-------|

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Table 19. Array results for differences from control sample using HL values for Series

| Sample | 1 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | % correct |
|--------|---|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|-----------|
| 1      | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 10     | 0 | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 11     | 0 | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 12     | 0 | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 13     | 0 | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 14     | 0 | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 15     | 0 | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 16     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 17     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 18     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 19     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 100       |
| 20     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| total  | 4 | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 88        |
|        |   | Cumulative Proportion of Total Dispersion |   |   |   |   |   |   |   |   |   |   |   |   |   |   |          |
|        |   | 0.998 |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1.000     |

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Table 20. Array results for differences from control sample using SL values for Series 1

| Sample | 1  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | % correct |
|--------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----------|
| 1      | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 10     | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 11     | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 12     | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 13     | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 14     | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 15     | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 16     | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 2      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 3      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 4      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 5      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 100       |
| 6      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 100       |
| 7      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 100       |
| 8      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 100       |
| 9      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 100       |
| total  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 100       |

Cumulative Proportion of Total Dispersion

|                  | 0.564 | 1.000 |
|------------------|-------|-------|

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**Table 21.** Array results for differences from control sample using RH values for Series 1

| Sample | 1 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | % correct |
|--------|---|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|-----------|
| 1      | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 10     | 0 | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 11     | 0 | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 12     | 0 | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 13     | 0 | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 14     | 0 | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 15     | 0 | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 16     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 2      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 3      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 4      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 5      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 100       |
| 6      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 100       |
| 7      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 100       |
| 8      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 100       |
| 9      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 100       |
| total  | 4 | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 100       |

Cumulative Proportion of Total Dispersion

|                      | 0.991 | 1.000 |
|----------------------|-------|-------|

Table 22. Array results for differences from control sample using GH values for Series 1

| Sample | 1 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | % correct |
|--------|---|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|-----------|
| 1      | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 10     | 0 | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 11     | 0 | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 12     | 0 | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 13     | 0 | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 14     | 0 | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 15     | 0 | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 16     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 2      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 3      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 4      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 100       |
| 5      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 100       |
| 6      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 100       |
| 7      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 100       |
| 8      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 100       |
| 9      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 100       |
| total  | 4 | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4 | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 100       |

Cumulative Proportion of Total Dispersion

|                  | 0.982 | 1.000 |
Table 23. Array results for differences from control sample using BH values for Series 1

| Sample | 1 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | % correct |
|--------|---|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|-----------|
| 1      | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 10     | 0 | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 11     | 0 | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 12     | 0 | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 13     | 0 | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 14     | 0 | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 15     | 0 | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 16     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 2      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 3      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 4      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 100       |
| 5      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 100       |
| 6      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 100       |
| 7      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 100       |
| 8      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 100       |
| 9      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 100       |
| total  | 4 | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 100       |

Cumulative Proportion of Total Dispersion

| 0.985 | 1.000 |
**SERIES 2**

**Table 24.** Array results for differences from control sample using RGB-HSL values for Series 2

| Sample | 1 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | % correct |
|--------|---|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|-----------|
| 1      | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 10     | 0 | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 11     | 0 | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 12     | 0 | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 13     | 0 | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 14     | 0 | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 15     | 0 | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 16     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 2      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 3      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 4      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 5      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 100       |
| 6      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 100       |
| 7      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 100       |
| 8      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 100       |
| 9      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 100       |
| total  | 4 | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 100       |

**Cumulative Proportion of Total Dispersion**

|                  | 0.945 | 1.000 | 1.000 |
|------------------|-------|-------|-------|

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**Table 25.** Array results for differences from control sample using HSL values for Series 2

| Sample | 1  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | % correct |
|--------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----------|
| 1      | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 10     | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 11     | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 12     | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 13     | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 14     | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 15     | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 16     | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 2      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 3      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 4      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 100       |
| 5      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 100       |
| 6      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 100       |
| 7      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 100       |
| 8      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 100       |
| 9      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 100       |
| total  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 100  | 100   |

**Cumulative Proportion of Total Dispersion**

|                  | 0.909 | 0.984 | 1.000 |
|------------------|-------|-------|-------|
|                  |       |       |       |
Table 26. Array results for differences from control sample using HL values for Series 2

| Sample | 1 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | % correct |
|--------|---|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|----------|
| Sample | 1 | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 10     | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 11     | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 12     | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 13     | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 14     | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 15     | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 16     | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 2      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 3      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 4      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 5      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 100      |
| 6      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 100      |
| 7      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 100      |
| 8      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 100      |
| 9      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 100      |
| total  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 100      |

Cumulative Proportion of Total Dispersion

|                  | 0.961 | 1.000 |
|------------------|-------|-------|
| **Total**        |       |       |
**SERIES 3**

**Table 27.** Array results for differences from control sample using RGB-HSL values for Series 3

| Sample | 1 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | % correct |
|--------|---|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|----------|
| 1      | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 10     | 0 | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 11     | 0 | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 12     | 0 | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 13     | 0 | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 14     | 0 | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 15     | 0 | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 16     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 2      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 3      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 4      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 100       |
| 5      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 100       |
| 6      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 100       |
| 7      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 100       |
| 8      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| 9      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100       |
| total  | 4 | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 100       |

| Cumulative Proportion of Total Dispersion | 0.99 | 1.00 | 1.00 | 1.00 | 1.00 |
|------------------------------------------|------|------|------|------|------|

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Table 28. Array results for differences from control sample using RGB values for Series 3

| Sample | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | % correct |
|--------|----------|----------|----------|----------|----------|----------|----------|----------|----------|-----------|
| 1      | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 100       |
| 2      | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 100       |
| 3      | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 100       |
| 4      | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 100       |
| 5      | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 100       |
| 6      | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 100       |
| 7      | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 100       |
| 8      | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 100       |
| 9      | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 100       |
| total  | 4        | 4        | 4        | 4        | 4        | 4        | 4        | 4        | 4        | 100       |

Cumulative Proportion of Total Dispersion

0.868 1.000
Table 29. Array results for differences from control sample using HSL values for Series 3

| Sample | 1 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | % correct |
|--------|---|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|---------|
| 1      | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100     |
| 10     | 0 | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100     |
| 11     | 0 | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100     |
| 12     | 0 | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100     |
| 13     | 0 | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100     |
| 14     | 0 | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100     |
| 15     | 0 | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100     |
| 16     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100     |
| 2      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100     |
| 3      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100     |
| 4      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 100     |
| 5      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 100     |
| 6      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 100     |
| 7      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 100     |
| 8      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 100     |
| 9      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 100     |
| total  | 4 | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 100     |

Cumulative Proportion of Total Dispersion

| 0.982 | 1.000 | 1.000 |
Table 3. Array results for differences from control sample using RG values for Series 3

| Sample | 1 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | % correct |
|--------|---|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|----------|
| 1      | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 10     | 0 | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 11     | 0 | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 12     | 0 | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 13     | 0 | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 14     | 0 | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 15     | 0 | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 16     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 2      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 3      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 4      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 100      |
| 5      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0        |
| 6      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 100      |
| 7      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0        |
| 8      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 100      |
| 9      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 100      |
| total  | 4 | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4 | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 88       |

Cumulative Proportion of Total Dispersion

| 0.686 | 1.000 |
Table 31. Array results for differences from control sample using RB values for Series 3

| Sample | 1 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | % correct |
|--------|---|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|---------|
| 1      | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100     |
| 10     | 0 | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100     |
| 11     | 0 | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100     |
| 12     | 0 | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100     |
| 13     | 0 | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100     |
| 14     | 0 | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100     |
| 15     | 0 | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100     |
| 16     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100     |
| 2      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100     |
| 3      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 100     |
| 4      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 100     |
| 5      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 100     |
| 6      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 100     |
| 7      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 100     |
| 8      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 100     |
| 9      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 100     |
| total  | 4 | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4 | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 100     |

Cumulative Proportion of Total Dispersion

|                   | 0.724 | 1.000 |
|-------------------|-------|-------|

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Table 32. Array results for differences from control sample using HS values for Series 3

| Sample | 1 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | % correct |
|--------|---|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|------------|
| 1      | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100        |
| 10     | 0 | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100        |
| 11     | 0 | 0  | 0  | 4  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100        |
| 12     | 0 | 0  | 0  | 0  | 4  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100        |
| 13     | 0 | 0  | 0  | 0  | 0  | 4  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100        |
| 14     | 0 | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100        |
| 15     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100        |
| 16     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100        |
| 2      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100        |
| 3      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 100        |
| 4      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 100        |
| 5      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 100        |
| 6      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 100        |
| 7      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 100        |
| 8      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 100        |
| 9      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 100        |
| total  | 4 | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 100        |

Cumulative Proportion of Total Dispersion

|                   | 0.974 | 1.000 |
|-------------------|-------|-------|

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Table 3. Array results for differences from control sample using HL values for Series 3.

| Sample | 1  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | % correct |
|--------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----------|
| 1      | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 100       |
| 10     | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 100       |
| 11     | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 100       |
| 12     | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 100       |
| 13     | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 100       |
| 14     | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 100       |
| 15     | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 100       |
| 16     | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 100       |
| 2      | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 100       |
| 3      | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 100       |
| 4      | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 100       |
| 5      | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 100       |
| 6      | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 100       |
| 7      | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 100       |
| 8      | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 100       |
| 9      | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 100       |
| total  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 100       |

Cumulative Proportion of Total Dispersion

|              | 1.000 | 1.000 |
|--------------|-------|-------|
| total        | 100   | 100   |
Table 34. Array results for differences from control sample using SL values for Series 3

| Sample | 1 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | % correct |
|--------|---|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|---------|
| 1      | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100     |
| 10     | 0 | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100     |
| 11     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0       |
| 12     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100     |
| 13     | 0 | 0  | 4  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0       |
| 14     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0       |
| 15     | 0 | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100     |
| 16     | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100     |
| 2      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 4  | 0  | 0  | 0  | 0  | 0  | 0  | 100     |
| 3      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100     |
| 4      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0       |
| 5      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100     |
| 6      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100     |
| 7      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 100     |
| 8      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0       |
| 9      | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4       |
| total  | 4 | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4 | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 75      |

Cumulative Proportion of Total Dispersion

|                | Problem 1 | Problem 2 |
|----------------|-----------|-----------|
| 0.982          | 1.000     |
## SUMMARY TABLES FROM COMPUTATIONAL MODELING

Table 35. Results from computational modeling using Spartan for H1

| Analyte number | Host Computed Structural Energy (kJ/mol) | Analyte Computed Structural Energy (kJ/mol) | Host/Guest Complex Computed Structural Energy (kJ/mol) | Free Host and Free Guest Computed Structural Energy (kJ/mol) | Complexed vs Free Host and Guest Energy difference (kJ/mol) |
|----------------|------------------------------------------|---------------------------------------------|------------------------------------------------------|------------------------------------------------------------|----------------------------------------------------------|
| 1              | 4844.32                                  | 98.13                                       | 4801.62                                              | 4942.45                                                    | 140.83                                                   |
| 2              | 4844.32                                  | 170.18                                      | 4850.47                                              | 5014.50                                                    | 164.03                                                   |
| 3              | 4844.32                                  | 258.00                                      | 4826.50                                              | 5102.32                                                    | 275.82                                                   |
| 4              | 4844.32                                  | 230.72                                      | 4784.83                                              | 5075.04                                                    | 290.21                                                   |
| 5              | 4844.32                                  | 49.63                                       | 4812.27                                              | 4893.95                                                    | 81.68                                                    |
| 6              | 4844.32                                  | 296.45                                      | 5008.54                                              | 5140.77                                                    | 132.23                                                   |
| 7              | 4844.32                                  | 352.86                                      | 4929.68                                              | 5197.17                                                    | 267.50                                                   |
| 8              | 4844.32                                  | 311.55                                      | 5085.77                                              | 5155.87                                                    | 70.10                                                    |
| 9              | 4844.32                                  | 285.66                                      | 5126.74                                              | 5129.98                                                    | 3.24                                                     |
| 10             | 4844.32                                  | 268.36                                      | 5088.68                                              | 5112.68                                                    | 24.00                                                    |
| 11             | 4844.32                                  | 341.74                                      | 5132.22                                              | 5186.05                                                    | 53.84                                                    |
| 12             | 4844.32                                  | -4.09                                       | 4740.46                                              | 4840.23                                                    | 99.77                                                    |
| 13             | 4844.32                                  | 320.33                                      | 5192.63                                              | 5164.64                                                    | -27.98                                                   |
| 14             | 4844.32                                  | 343.17                                      | 5141.00                                              | 5187.49                                                    | 46.49                                                    |
| 15             | 4844.32                                  | 391.68                                      | 6114.77                                              | 5235.99                                                    | -878.77                                                  |
| 16             | 4844.32                                  | 349.03                                      | 5197.22                                              | 5193.34                                                    | -3.87                                                    |
Table 36. Results from computational modeling using Spartan for H2

| Analyte number | Host/Guest Complex Computed Structural Energy (kJ/mol) | Free Host and Guest Computed Structural Energy (kJ/mol) | Complexed vs Free Host and Guest Energy difference (kJ/mol) |
|----------------|-------------------------------------------------------|--------------------------------------------------------|----------------------------------------------------------|
| 1              | 4592.28                                               | 4657.35                                               | 65.07                                                    |
| 2              | 4667.53                                               | 4729.40                                               | 61.86                                                    |
| 3              | 4717.39                                               | 4817.22                                               | 99.82                                                    |
| 4              | 4666.17                                               | 4789.94                                               | 123.77                                                   |
| 5              | 4619.73                                               | 4608.85                                               | -10.88                                                   |
| 6              | 4825.60                                               | 4855.67                                               | 30.07                                                    |
| 7              | 4740.38                                               | 4912.07                                               | 171.69                                                   |
| 8              | 4806.57                                               | 4870.77                                               | 64.20                                                    |
| 9              | 4895.74                                               | 4844.88                                               | -50.86                                                   |
| 10             | 4790.67                                               | 4827.58                                               | 36.91                                                    |
| 11             | 4870.73                                               | 4900.95                                               | 30.22                                                    |
| 12             | 4852.96                                               | 4555.13                                               | -297.83                                                  |
| 13             | 4866.18                                               | 4879.54                                               | 13.36                                                    |
| 14             | 4913.89                                               | 4902.39                                               | -11.50                                                   |
| 15             | 5957.11                                               | 4950.89                                               | -1006.22                                                 |
| 16             | 4883.77                                               | 4908.24                                               | 24.47                                                    |
Table 37. Results from computational modeling using Spartan for H3

| Analyte number | Host Analyte number | Host/Guest Complex | Free Host and Free Guest | Complexed vs Free Host and Guest Energy difference |
|----------------|---------------------|--------------------|--------------------------|--------------------------------------------------|
|                | Computed Structural Energy (kJ/mol) | Computed Structural Energy (kJ/mol) | Computed Structural Energy (kJ/mol) | Computed Structural Energy (kJ/mol) |
| 1              | 4605.62             | 98.13              | 4668.09                  | 4703.75                                          | 35.67                                           |
| 2              | 4605.62             | 170.18             | 4758.43                  | 4775.80                                          | 17.37                                           |
| 3              | 4605.62             | 258.00             | 4798.65                  | 4863.62                                          | 64.97                                           |
| 4              | 4605.62             | 230.72             | 4753.42                  | 4836.34                                          | 82.92                                           |
| 5              | 4605.62             | 49.63              | 4700.87                  | 4655.25                                          | -45.62                                          |
| 6              | 4605.62             | 296.45             | 4939.78                  | 4902.07                                          | -37.71                                          |
| 7              | 4605.62             | 352.86             | 4914.56                  | 4958.48                                          | 43.91                                           |
| 8              | 4605.62             | 311.55             | 4892.69                  | 4917.17                                          | 24.49                                           |
| 9              | 4605.62             | 285.66             | 5025.72                  | 4891.29                                          | -134.43                                         |
| 10             | 4605.62             | 268.37             | 4907.45                  | 4873.99                                          | -33.46                                          |
| 11             | 4605.62             | 341.74             | 4941.16                  | 4947.36                                          | 6.20                                            |
| 12             | 4605.62             | -4.09              | 4926.55                  | 4601.54                                          | -325.01                                         |
| 13             | 4605.62             | 320.33             | 4964.01                  | 4925.95                                          | -38.06                                          |
| 14             | 4605.62             | 343.17             | 4999.15                  | 4948.80                                          | -50.35                                          |
| 15             | 4605.62             | 391.68             | 6075.54                  | 4997.30                                          | -1078.24                                        |
| 16             | 4605.62             | 349.03             | 4993.43                  | 4954.65                                          | -38.78                                          |
SUMMARY FIGURES

SUMMARY FIGURES FOR ARRAY GENERATION EXPERIMENTS

Series 1

**Figure 175.** Linear discriminant analysis of differences from control sample using RGB-HSL values for Series 1

**Figure 176.** Linear discriminant analysis of differences from control sample using RGB values for Series 1
**Figure 177.** Linear discriminant analysis of differences from control sample using HSL values for Series 1

**Figure 178.** Linear discriminant analysis of differences from control sample using RG values for Series 1
**Figure 179.** Linear discriminant analysis of differences from control sample using RB values for Series 1

**Figure 180.** Linear discriminant analysis of differences from control sample using GB values for Series 1
**Figure 181.** Linear discriminant analysis of differences from control sample using HS values for Series 1

**Figure 182.** Linear discriminant analysis of differences from control sample using HL values for Series 1
Figure 183. Linear discriminant analysis of differences from control sample using SL values for Series 1.

Figure 184. Linear discriminant analysis of differences from control sample using RH values for Series 1.
Figure 185. Linear discriminant analysis of differences from control sample using GH values for Series 1

Figure 186. Linear discriminant analysis of differences from control sample using BH values for Series 1
Figure 187. Linear discriminant analysis of differences from control sample using RGB-HSL values for Series 2

Figure 188. Linear discriminant analysis of differences from control sample using HSL values for Series 2
Figure 189. Linear discriminant analysis of differences from control sample using HL values for Series 2

SERIES 3

Figure 190. Linear discriminant analysis of differences from control sample using RGB-HSL values for Series 3
Figure 191. Linear discriminant analysis of differences from control sample using RGB values for Series 3

Figure 192. Linear discriminant analysis of differences from control sample using HSL values for Series 3
Figure 193. Linear discriminant analysis of differences from control sample using RG values for Series 3

Figure 194. Linear discriminant analysis of differences from control sample using RB values for Series 3
Figure 195. Linear discriminant analysis of differences from control sample using HS values for Series 3

Figure 196. Linear discriminant analysis of differences from control sample using HL values for Series 3
**Figure 197.** Linear discriminant analysis of differences from control sample using SL values for Series 3

**SUMMARY FIGURES OF COMPUTATIONAL MODELING**

**Figure 198.** Electrostatic potential map of analyte 1
**Figure 199.** Electrostatic potential map of analyte 2

**Figure 200.** Electrostatic potential map of analyte 3

**Figure 201.** Electrostatic potential map of analyte 4
Figure 202. Electrostatic potential map of analyte 5

Figure 203. Electrostatic potential map of analyte 6

Figure 204. Electrostatic potential map of analyte 7
Figure 205. Electrostatic potential map of analyte 8

Figure 206. Electrostatic potential map of analyte 9

Figure 207. Electrostatic potential map of analyte 10
Figure 208. Electrostatic potential map of analyte 11

Figure 209. Electrostatic potential map of analyte 12

Figure 210. Electrostatic potential map of analyte 13
**Figure 211.** Electrostatic potential map of analyte 14

**Figure 212.** Electrostatic potential map of analyte 15
Figure 213. Electrostatic potential map of analyte 16