THE
UNIVERSITY
OF RHODE ISLAND

University of Rhode Island
DigitalCommons@URI

Chemistry Faculty Publications

Chemistry

2014

Cyclodextrin-promoted energy transfer for broadly applicable small-molecule detection

Nicole Serio
University of Rhode Island

Chitapom Chanthalyma
University of Rhode Island

See next page for additional authors

Follow this and additional works at: https://digitalcommons.uri.edu/chm_facpubs

This is a pre-publication author manuscript of the final, published article.

Authors

Nicole Serio, Chitapom Chanthalyma, Lindsey Prignano, and Mindy Levine

Cyclodextrin-promoted energy transfer for broadly applicable small-molecule detection

Nicole Serio, Chitapom Chanthalyma, Lindsey Prignano, and Mindy Levine*

Department of Chemistry, University of Rhode Island, Kingston, RI 02881, USA

51 Lower College Road, Kingston, RI 02881; phone: 401-874-4243; email: mlevine@chm.uri.edu

Cyclodextrin-promoted energy transfer for broadly applicable small-molecule detection

Reported herein is the development of non-covalent, proximity-induced energy transfer from small-molecule toxicants to organic fluorophores bound in the cavity of γ-cyclodextrin. This energy transfer occurs with exceptional efficiency for a broad range of toxicants in complex biological media, and is largely independent of the spectral overlap between the donor and acceptor. This generally applicable phenomenon has significant potential in the development of new turn-on detection schemes.

1. Introduction

The accurate detection of small-molecule organic toxicants in complex environments has significant implications for public health. Such toxicants are potentially significant contributors to human disease,[1-3] and are found in food supplies,[4-6] water supplies,[7] and in commercial products.[8] Current methods for the detection of these chemical toxicants generally require multiple steps: (a) extraction of the toxicants from the environment;[9] (b) purification of the toxicants via high-performance liquid chromatography[10] or gas chromatography;[11] and (c) detection of the toxicants by mass spectrometry[12] or fluorescence spectroscopy.[13] Such detection methods are limited in their ability to distinguish toxicants with identical molecular weights or similar fluorescence spectra.

Small-molecule toxicants can also be detected through fluorescence energy transfer-based methods. Such fluorescence energy transfer, which has been used extensively for biomolecule detection,[14-16] often requires significant spectral overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor to achieve efficient energy transfer (*i.e.* a Förster-type mechanism).[17] This overlap ultimately compromises the sensitivity of the system, as even in the absence of the

target analyte there is residual donor emission.[18] Efficient energy transfer that is independent of the spectral overlap (*i.e.* a Dexter-type mechanism) has the potential to lead to improved sensitivities in fluorescent detection schemes.[19,20]

Reported herein is a highly efficient, practical approach for small-molecule detection: using the small molecules directly as energy donors in a non-covalent, macrocycle-promoted energy transfer scheme.[21] In such a scheme, both the toxicant and the fluorophore are bound in the interior of γ-cyclodextrin (Figure 1). The enforced proximity of the two molecules allows for non-covalent energy transfer to occur, with excitation of the toxicant (energy donor) resulting in energy transfer to and emission from the fluorophore (energy acceptor). The energy transfer is independent of the spectral overlap between the donor and the acceptor, and has the potential to lead to improved sensitivities in turn-on detection schemes.

We recently reported that cyclodextrin-promoted energy transfer occurred from polycyclic aromatic hydrocarbons (PAHs) (compounds **1-5**, Figure 2) and polychlorinated biphenyls (PCBs) (compounds **14-19**, Figure 2) to three fluorophores (two of which are shown in Figure 3).[22-24] Proximity-induced energy transfer between the analytes and the fluorophores occurred in the cavity of γ-cyclodextrin, resulting in up to 35% energy transfer efficiencies.

Reported herein is a substantial expansion of this preliminary report to include (a) a wide range of small-molecule toxicants as energy donors (Figure 2);[25] (b) energy transfer efficiencies as high as 100%; and (c) examples of successful energy transfer in complex media: coconut water, plasma,[26] breast milk,[27] and seawater. The general and highly efficient energy transfer reported herein highlights the robust nature of this phenomenon and the strength of the intermolecular interactions that allow for such energy transfer to occur.

2. Results and Discussion

The full chart of examined energy donors is shown in Figure 2. This chart contains several compounds that have been classified as known carcinogens (Group 1) according to the International Agency for Research on Cancer (IARC) (compounds **3**, **6-10**),[28] as well as a variety of other toxicants.[29-32] These structures also contain a wide variety of functional groups, steric bulk, and photophysical properties, which allows us to probe the donor features necessary for efficient energy transfer.

Energy transfer experiments were conducted by mixing the analyte and fluorophore in a 10 mM γ-cyclodextrin solution in phosphate-buffered saline (PBS), coconut water, seawater, human plasma, or human breast milk. The resulting solution was excited near the analyte's absorption maximum (defined as "analyte excitation") and near the fluorophore's absorption maximum (defined as "fluorophore excitation"). The energy transfer efficiencies were calculated according to Equation 1:

$$\% \text{ Energy Transfer} = I_{DA}/I_A \times 100\% \tag{1}$$

where I_{DA} is defined as the integrated fluorophore emission from indirect excitation and I_A is the integrated fluorophore emission from direct excitation. A graphical depiction of I_{DA} and I_A is shown in Figure 4.

Control experiments were also conducted to determine whether the observed fluorophore peaks from analyte excitation were due to legitimate energy transfer rather than a result of the fluorophore having non-zero absorption at the excitation wavelength of the analyte. In these experiments, the fluorophore was mixed with cyclodextrin and excited at the excitation wavelength of the analyte (but in the absence of any analyte). That fluorophore emission was compared to the emission of the fluorophore via analyte

excitation in the presence of the analyte. The ratio of these two emissions, defined as the "Fluorophore ratio" was calculated according to Equation 2:

$$\text{Fluorophore ratio} = I_{\text{fluorophore-control}}/I_{\text{fluorophore-analyte}} \qquad (2)$$

Where $I_{\text{fluorophore-analyte}}$ is the integration of the fluorophore emission in the presence of the analyte; and $I_{\text{fluorophore-control}}$ is the integration of the fluorophore emission in the absence of the analyte. Fluorophore ratios substantially less than 1 indicate that the fluorophore emission increases with analyte addition as a result of energy transfer.

The final concentrations of the toxicants were somewhat higher than literature-reported concentrations of contaminated biological samples,[33-35] although such literature reports vary widely depending on the toxicant identity, biological fluid, and sample population. Full results for all donor-acceptor combinations in all media are reported in the Electronic Supporting Information. Particularly exciting results were found using energy donors **7**, **8**, **11** and **12** with acceptor **20**.

2a. In Phosphate-Buffered Saline (PBS):

The energy transfer from analytes **7**, **8**, **11** and **12** to BODIPY **20** in 10 mM γ-cyclodextrin in PBS was exceptionally efficient, with greater than 100% efficiencies observed in all cases (Figure 5). Control experiments with 0 mM γ-cyclodextrin in PBS showed substantially less energy transfer than the 10 mM γ-cyclodextrin solution (Table 1), highlighting the beneficial role of γ-cyclodextrin in promoting energy transfer.

2b. In coconut water:

The composition of coconut water is remarkably similar to that of human plasma, and it has been used as a plasma surrogate during emergencies.[36,37] Analytes **7**, **8**, **11** and **12** demonstrated efficient energy transfer in 10 mM γ-cyclodextrin dissolved

in coconut water (Table 2), albeit with diminished efficiencies compared to energy transfer in pure PBS.

2c. In biological media:

The ability to achieve cyclodextrin-promoted energy transfer in biological media can provide significant benefit for the detection of toxicants. Efficient energy transfer from compounds **7**, **8**, **11** and **12** to fluorophore **20** occurred in both human plasma samples and human breast milk samples that were doped with 10 mM γ-cyclodextrin (Table 2).

2d. Energy transfer in seawater:

The detection of toxic oil components in seawater has significant applications in the aftermath of environmental disasters such as the Deepwater Horizon oil spill of 2010[38] and the Colorado floods of 2013.[39] Such components include PAHs **1-5,** which we have previously shown can participate in energy transfer in purified PBS solution.[23] Cyclodextrin-promoted energy transfer using these donors occurred in seawater taken from Narragansett Bay (Rhode Island), with fluorophore **20** as an energy acceptor. All PAHs (**1-5**) exhibited some degree of energy transfer to fluorophore **20** (Figure 6) under these conditions.

For all complex fluids, the energy transfer efficiencies were somewhat lower than the efficiencies in pure PBS. These results are not surprising, considering the complex nature of coconut water,[40] human plasma,[41-44] and breast milk,[45,46] and the high salt content and complex nature of seawater.[47,48] That γ-cyclodextrin-promoted energy transfer from carcinogens to the fluorophores occurred successfully in such complex environments highlights the robust nature of this detection method and the underlying enabling supramolecular interactions.

In contrast to the results obtained in PBS solution, where cyclodextrin clearly promotes efficient energy transfer, many of the analyte-fluorophore pairs in complex media demonstrate equivalent or even greater energy transfer efficiencies in the absence of γ-cyclodextrin compared to the efficiencies in the presence of cyclodextrin. These results are likely due to two possible phenomena:

(a) For cases where the energy transfer efficiencies are roughly equivalent in the presence and absence of cyclodextrin, it is likely that the donor and acceptor associate without cyclodextrin due to the hydrophobic effect.[49] This association leads to energy transfer efficiencies that are essentially identical regardless of the cyclodextrin concentration. Previous research in our laboratory has shown some degree of cyclodextrin-free association as well.[23]

(b) For cases where the energy transfer efficiencies are lower in the presence of cyclodextrin, the cyclodextrin might bind one of the two small-molecules selectively, thus removing it from the proximity of the second molecule. This removal of one of the energy transfer partners lowers the observed energy transfer efficiencies.

2e. Comparison to Published Methods:

The ability to detect toxicants via non-covalent energy transfer has a number of advantages compared to previously-reported methods, including the ability to tune the emission signal of a single analyte throughout the spectral region through choosing a variety of fluorophores. To achieve this "tuning" ability, preliminary experiments were conducted using a third fluorophore: commercially available coumarin 6 (compound **22**) as a fluorescent energy acceptor with selected analytes (10 mM γ-cyclodextrin, PBS solution) as energy donors. Good energy transfer efficiencies were observed for many cases (Table 3), and in most cases the energy transfer efficiencies were substantially higher in the presence of γ-cyclodextrin compared to in its absence.

Moreover, the use of multiple fluorophores allows for the tuning of the fluorescence signal from a single analyte. For this experiment, analyte **12** was mixed with fluorophores **20**, **21**, and **22** in three vials (in 10 mM γ-cyclodextrin in PBS). Excitation of each solution at 320 nm (the excitation wavelength of the analyte) resulted in three distinct fluorophore signals at 515, 530, and 555 nm for fluorophores **20**, **22**, and **21**, respectively (Figure 7). This tuning of the toxicant signal via judicious choice of fluorophore provides maximum flexibility in developing toxicant detection schemes.

One key challenge of this method compared to published methods for toxicant detection is the difficulty in obtaining quantitative data through non-covalent energy transfer. Preliminary experiments have demonstrated that the fluorescence signal obtained via energy transfer is **not** proportional to the concentration of the analyte; this is line with literature reports that demonstrate a complicated relationship between fluorescence energy transfer signals and the concentration of the donor and acceptor.[50,51] This relationship is affected by a multitude of other intermolecular interactions, including donor-donor interactions,[52] fluorophore dimerization and aggregation,[53] and undesired fluorophore self-quenching.[54]

2f. General discussion

There are a number of factors that determine whether a particular analyte participates efficiently in cyclodextrin-promoted energy transfer, and the results reported herein provide crucial information towards deconvoluting some of these factors. High energy transfer efficiencies occur in cases where the analyte-fluorophore pairs (a) form ternary complexes in the cyclodextrin cavity with high affinities and (b) participate in proximity-induced energy transfer. The binding affinities in cyclodextrin are determined by the molecules' steric and electronic characters,[55] and the participation in energy transfer schemes is determined by steric and electronic complementarity

between the donor and acceptor,[56] molecular orientations of the two guests,[57] and the degree of spectral overlap with the fluorophore acceptor.[58]

The analytes that demonstrated highly efficient energy transfer in the various media included compounds **7**, **8**, **11**, and **12** (discussed herein) as well as compounds **1-3** (reported in previous publications). The fact that compounds **11** and **12** were efficient energy donors compared to compound **5** is likely due to the presence of the nitrogen substituents, which either enhance the electron donating ability of the analyte and/or provide favourable electrostatic interactions with the highly polarized fluorophore acceptors. Directly comparing the absorbance spectra, fluorescence spectra, and quantum yields of compounds **5**, **11**, and **12** indicate similar photophysical properties for the three compounds,[59,60] which rules out spectral overlap as a substantial contributing factor.

The success of compound **7** compared to structurally similar compound **6** may be a result of additional amino group enabling compound **7** to form more electrostatic interactions or to bind in cyclodextrin with higher affinities. The similarities in the spectral properties of compounds **6** and **7** again rule out spectral overlap as a significant factor.[61,62] The fact that the photophysical properties of the toxicant energy donors play only a limited role in determining energy transfer efficiencies strongly supports our hypothesis that proximity-induced energy transfer in the cyclodextrin cavity occurs via a Dexter-type, direct orbital overlap mechanism.

One of the most surprising results was the successful use of compound **8** as an energy donor in combination with fluorophore acceptors. Compound **8** has been used as a fluorescence quencher of other small molecules,[63,64] and is only weakly fluorescent. Nonetheless, the weak photophysical activity (455 nm emission maximum from 340 nm excitation) was sufficient for it to participate in proximity-induced energy transfer. The

free hydroxyl groups of the molecule likely allow for the formation of hydrogen bonds to the highly polarized fluorophore acceptors. Comparing the results obtained with compound **8** to those of compound **10** (which was relatively inefficient as an energy donor) highlight possible steric constraints (compound **10** is substantially larger than compound **8**) and functional group requirements (compound **10** lacks the free hydroxyl moieties) that are necessary for cyclodextrin-promoted energy transfer.

3. Conclusion

In conclusion, highly efficient energy transfer from a variety of organic toxicants occurred to multiple fluorophore acceptors when bound in the cavity of γ-cyclodextrin. The fact that this approach is successful in many environments with a variety of analytes is very beneficial. The robust nature of this approach leaves a wide range of opportunities to expand the scope of the analytes that can be detected, as well as the environments that they can be detected in. Indeed, the only requirement is that the analyte be (at least) weakly fluorescent. Furthermore, sample preparation is simple compared to current methods, as most media simply require dilution with PBS.

The fact that γ-cyclodextrin can bind analytes within its cavity in complex environments means that it can simultaneously isolate the analytes and promote energy transfer so that the analytes can be reliably identified. This method is a significant contribution to the facile and reliable detection of toxic analytes. The ability to tune the emission signal for a particular analyte by varying the choice of fluorophore provides substantial flexibility, and can be used in the development of array-based detection schemes. The development of such an array is currently under investigation, and results of these and other experiments will be reported in due course.

Experimental Section

All chemicals were obtained from Sigma-Aldrich chemical company or Fisher Scientific and used as received. BODIPY fluorophore **20** was synthesized following literature-reported procedures.[65] Human plasma was obtained from Innovative Technologies. Human breast milk was obtained from an anonymous donor. Seawater was obtained from the Narragansett Beach in Rhode Island. Coconut water (VitaCoco 100% Pure Coconut Water) was obtained from CVS Pharmacy.

The human plasma, seawater, and coconut water were used as received. The breast milk was prepared by separating all solids via filtration and centrifugation, followed by dilution with phosphate-buffered saline (PBS). UV-Visible spectra were recorded on an Agilent 8453 spectrometer. Fluorescence measurements were recorded on a Shimadzu RF 5301 spectrophotometer with slit widths of 1.5 nm excitation and 1.5 nm emission slit widths. All fluorescence spectra were integrated vs. wavenumber on the X-axis, using OriginPro Version 8.6.

The energy transfer experiments were conducted as follows: 2.5 mL of a 10 mM solution of γ-cyclodextrin dissolved in the fluid of interest (PBS, coconut water, Narragansett Bay seawater, human plasma, or human breast milk) were measured into a cuvette. 20 µL of the analyte (1 mg/mL) and 100 µL of the fluorophore (0.1 mg/mL) were added. After thorough mixing, the solution was excited at two wavelengths: near the analyte's absorption maximum (defined as "analyte excitation") and near the fluorophore's absorption maximum (defined as "fluorophore excitation"). The energy transfer efficiencies were calculated according to Equation 1:

$$\% \text{ Energy Transfer} = I_{DA}/I_A \times 100\% \qquad (1)$$

where I_{DA} is defined as the integrated fluorophore emission from indirect excitation and I_A is the integrated fluorophore emission from direct excitation. A

graphical depiction of I_{DA} and I_A is shown in Figure 4. Experiments were also conducted where 0 mM of γ cyclodextrin were used for each fluid, analyte, and fluorophore combination, in place of the 10 mM cyclodextrin solution.

Control experiments were conducted as follows: (a) The fluorophore was mixed with γ-cyclodextrin and excited at the excitation wavelength of the analyte (but in the absence of any analyte); and (b) the fluorophore and analyte were both mixed in γ-cyclodextrin and excited at analyte excitation wavelength. The fluorophore emission that resulted from excitation at the analyte wavelength in the absence of the analyte was compared to the fluorophore emission from excitation at the analyte wavelength in the presence of the analyte. The ratio of these two emissions, shown as the "Fluorophore ratio" was calculated according to Equation 2:

$$\text{Fluorophore ratio} = I_{\text{fluorophore-control}}/I_{\text{fluorophore-analyte}} \qquad (2)$$

Where $I_{\text{fluorophore-analyte}}$ is the integration of the fluorophore emission in the presence of the analyte; and $I_{\text{fluorophore-control}}$ is the integration of the fluorophore emission in the absence of the analyte. Full tables of energy transfer efficiencies for all analyte-fluorophore combinations and summary figures of all analyte-fluorophore combinations are shown in the Supplementary Material.

Acknowledgements

Notes and references

Electronic Supplementary Information (ESI) available: Synthesis of fluorophore **20**, details of all energy transfer experiments and control experiments, details of all sample

preparation, summary tables and figures of all experiments.

[1] van den Berg, M.; Denison, M. S.; Birnbaum, L. S.; DeVito, M. J.; Fiedler, H.; Falandysz, J.; Rose, M.; Schrenk, D.; Safe, S.; Tohyama, C.; Tritscher, A.; Tysklind, M.; Peterson, R. E. *Toxicol. Sci.* **2013**, *133*, 197-208.

[2] Lock, E. A.; Zhang, J.; Checkoway, H. *Toxicol. Appl. Pharmacol.* **2013**, *266*, 345-355.

[3] Bohacek, J.; Mansuy, I. M. *Neuropsychopharmacol.* **2013**, *38*, 220-236.

[4] Barlow, S.; Schlatter, J. *Toxicol. Appl. Pharmacol.* **2010**, *243*, 180-190.

[5] Lachenmeier, D. W. *Open Toxicol. J.* **2009**, *3*, 30-34.

[6] Kuo, C.-Y.; Chang, S.-H.; Chien, Y.-C.; Chiang, F.-Y.; Wei, Y.-C. *J. Exposure Sci. Environ. Epidemiol.* **2006**, *16*, 410-416.

[7] Maslia, M. L.; Aral, M. M.; Faye, R. E.; Suarez-Soto, R. J.; Sautner, J. B.; Wang, J.; Jang, W.; Bove, F. J.; Ruckart, P. Z. *Water Quality, Exposure and Health* **2009**, *1*, 49-68.

[8] Mojska, H.; Gielecinska, I.; Stos, K. *Food Chem. Toxicol.* **2012**, *50*, 2722-2728.

[9] Zhao, W.-j.; Chen, X.-b.; Fang, L.; Li, C.-l.; Zhao, D.-y. *J. Agriculture Food Chem.* **2013**, *61*, 1804-1809.

[10] Morimoto, N.; Otsuka, Y.; Nishi, S.; Kobayashi, A.; Kakehi, K. *Polycyclic Aromatic Compounds* **2012**, *32*, 503-514.

[11] Retamal, M.; Costa, C.; Suarez, J. M.; Richter, P. *Int. J. Environ. Anal. Chem.* **2013**, *93*, 93-107.

[12] Jobst, K. J.; Shen, L.; Reiner, E. J.; Taguchi, V. Y.; Helm, P. A.; McCrindle, R.; Backus, S. *Anal. Bioanal. Chem.* **2013**, *405*, 3289-3297.

[13] Okparanma, R. N.; Mouazen, A. M. *Appl. Spectroscopy Rev.* **2013**, *48*, 458-486.

[14] Li, H.; Bazan, G. C. *Adv. Mater.* **2009**, *21*, 964-967.

[15] Lv, F.; Wang, S.; Bazan, G. C., in *Conjugated Polyelectrolytes*, ed. B. Liu and G. C. Bazan, Wiley-VCH, Weinheim, **2013**, pp. 201-229.

[16] Woo, H. Y.; Nag, O. K.; Kim, J.; Kang, M.; Bazan, G. C. *Molec. Cryst. Liq. Cryst.* **2008**, *486*, 244-249.

[17] Feron, K.; Belcher, W. J.; Fell, C. J.; Dastoor, P. C. *Int. J. Molec. Sci.* **2012**, *13*, 17019-17047.

[18] Singh, H.; Bagchi, B. *Curr. Sci.* **2005**, *89*, 1710-1719.

[19] Liao, D. W.; Cheng, W. D.; Bigman, J.; Karni, Y.; Speiser, S.; Lin, S. H. *J. Chinese Chem. Soc.* **1995**, *42*, 177-187.

[20] Zheng, J.; Swager, T. M. *Chem. Commun.* **2004**, 2798-2799.

[21] Biedermann, F.; Rauwald, U.; Cziferszky, M.; Williams, K. A.; Gann, L. D.; Guo, B. Y.; Urbach, A. R.; Bielawski, C. W.; Scherman, O. A. *Chem. Eur. J.* **2010**, *16*, 13716-13722.

[22] Mako, T.; Marks, P.; Cook, N.; Levine, M. *Supramol. Chem.* **2012**, *24*, 743-747.

[23] Serio, N.; Miller, K.; Levine, M. *Chem. Commun.* **2013**, *49*, 4821-4823.

[24] Radaram, B.; Potvin, J.; Levine, M. *Chem. Commun.* **2013**, *49,* 8259-8261.

[25] IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. http://monographs.iarc.fr/ (28 June 2013, date last accessed).

[26] Fong, Z. V.; Winter, J. M. *Cancer J.* **2012**, *18*, 530-538.

[27] Mannetje, A.'t; Coakley, J.; Bridgen, P.; Brooks, C.; Harrad, S.; Smith, A. H.; Pearce, N.; Douwes, J. *Sci. Total Environ.* **2013**, *458-460*, 399-407.

[28] Waters, M. D.; Stack, H. F.; Jackson, M. A. *IARC Scientific Publications* **1999**, *146*, 499-536.

[29] Jain, V.; Hilton, B.; Lin, B.; Patnaik, S.; Liang, F.; Darian, E.; Zou, Y.; MacKerell, A. D.; Cho, B. P. *Nucleic Acids Res.* **2013**, *41*, 869-880.

[30] Shimada, T.; Murayama, N.; Yamazaki, H.; Tanaka, K.; Takenaka, S.; Komori, M.; Kim, D.; Guengerich, F. P. *Chem. Res. Toxicol.* **2013**, *26*, 529-537.

[31] Koleva, Y.; Georgieva, S. *Oxidative Commun.* **2013**, *36*, 225-234.

[32] Baptista, J.; Pato, P.; Tavares, S.; Duarte, A. C.; Pardal, M. A. *Ecotoxicol. Environ. Safety* **2013**, *94*, 147-152.

[33] Riffelmann, M.; Muller, G.; Schmieding, W.; Popp, W.; Norpoth, K. *Int. Archives Occupational Environ. Health* **1995**, *68*, 36-43.

[34] Madhavan, N. D.; Naidu, K. A. *Human Experimental Toxicol.* **1995**, *14*, 503-506.

[35] Kishikawa, N.; Wada, M.; Kuroda, N.; Akiyama, S.; Nakashima, K. *J. Chromatography B* **2003**, *789*, 257-264.

[36] Prathapan, A.; Rajamohan, T. *J. Food Biochem.* **2011**, *35*, 1501-1507.

[37] Campbell-Falck, D.; Thomas, T.; Falck, T. M.; Tutuo, N.; Clem, K. *American J. Emergency Medicine* **2000**, *18*, 108-111.

[38] McNutt, M. K.; Chu, S.; Lubchenco, J.; Hunter, T.; Dreyfus, G.; Murawski, S. A.; Kennedy, D. M. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 20222-20228.

[39] http://www.nytimes.com/2013/09/27/us/after-the-floods-a-deluge-of-worry-about-oil.html

[40] Prades, A.; Dornier, M.; Diop, N.; Pain, J.-P. *Fruits* **2012**, *67*, 87-107.

[41] Quehenberger, O.; Armando, A. M.; Brown, A. H.; Milne, S. B.; Myers, D. S.; Merrill, A. H.; Bandyopadhyay, S.; Jones, K. N.; Kelly, S.; Shaner, R. L.; Sullards, C. M.; Wang, E.; Murphy, R. C.; Barkley, R. M.; Leiker, T. J.; Raetz, C. R. H.; Guan, Z.; Laird, G. M.; Six, D. A.; Russell, D. W.; McDonald, J. G.; Subramaniam, S.; Fahy, E.; Dennis, E. A. *J. Lipid Res.* **2010**, *51*, 3299-3305.

[42] Itakura, H.; Yokoyama, M.; Matsuzaki, M.; Saito, Y.; Origasa, H.; Ishikawa, Y.; Oikawa, S.; Sasaki, J.; Hishida, H.; Kita, T.; Kitabatake, A.; Nakaya, N.; Sakata, T.; Shimada, K.; Shirato, K.; Matsuzawa, Y. *J. Atherosclerosis Thrombosis* **2011**, *18*, 99-107.

[43] Anderson, N. L. *Clinical Chem.* **2010**, *56*, 177-185.

[44] Jude, I. C.; Catherine, I. C.; Frank, O. C. *Pakistan J. Nutrition* **2010**, *9,* 103-105.

[45] Ruhaak, L. R.; Lebrilla, C. B. *Adv. Nutrition* **2012**, *3*, 406S-414S.

[46] Kim, J.; Friel, J. *Lipid Technol.* **2012**, *24*, 103-105

[47] Marion, G. M.; Millero, F. J.; Camoes, M. F.; Spitzer, P.; Feistel, R.; Chen, C. T. A. *Marine Chem.* **2011**, *126*, 89-96.

[48] Sathya Devi, V.; Chidi, O. O.; Coleman, D. *Spectroscopy* **2009**, *23*, 265-270.

[49] Breslow, R. *J. Phys. Org. Chem.* **2006**, *19*, 813-822.

[50] Andrew, T. L.; Swager, T. M. *J. Polym. Sci. B Polym. Phys.* **2011**, *49*, 476-498.

[51] Levine, M.; Song, I.; Andrew, T. L.; Kooi, S. E.; Swager, T. M. *J. Polym. Sci. A Polym. Chem.* **2010**, *48*, 3382-3391.

[52] Marushchak, D.; Johansson, L. B.-A. *J. Fluorescence* **2005**, *15*, 797-803.

[53] Morrison, L. E. *Molecular Biotechnol.* **2010**, *44*, 168-176.

[54] Morrison, L. E. *Methods Molecular Biol.* **2008**, *429*, 3-19.

[55] Szente, L.; Szeman, J. *Anal. Chem.* **2013**, *85*, 8024-8030.

[56] Yuan, L.; Lin, W.; Zheng, K.; Zhu, S. *Acc. Chem. Res.* **2013**, *46*, 1462-1473.

[57] Saini, S.; Srinivas, G.; Bagchi, B. *J. Phys. Chem. B* **2009**, *113*, 1817-1832.

[58] Bai, D.; Benniston, A. C.; Hagon, J.; Lemmetyinen, H.; Tkachenko, N. V.; Harrington, R. W. *Phys. Chem. Chem. Phys.* **2013**, *15*, 9854-9861.

[59] Canabate Diaz, B.; Schulman, S. G.; Segura Carretero, A.; Fernandez Gutierrez, A. *Anal. Chim. Acta* **2003**, *489*, 165-171.

[60] Dufresne, S.; Roche, I. U.; Skalski, T.; Skene, W. G. *J. Phys. Chem. C* **2010**, *114*, 13106-13112.

[61] Wang, Y.-Q.; Zhang, H.-M.; Zhang, G.-C.; Zhou, Q.-H.; Fei, Z.-H.; Liu, Z.-T.; Li, Z.-X. *J. Molec. Structure* **2008**, *886*, 77-84.

[62] Siskova, K.; Kubala, M.; Dallas, P.; Jancik, D.; Thorel, A.; Ilik, P.; Zboril, R. *J. Mater. Chem.* **2011**, *21*, 1086-1093.

[63] Manivannan, C.; Renganathan, R. *J. Luminescence* **2011**, *131*, 2365-2371.

[64] Anbazhagan, V.; Kandavelu, V.; Kathiravan, A.; Renganathan, R. *J. Photochem. Photobiol. A* **2008**, *193*, 204-212.

[65] Shepherd, J. L.; Kell, A.; Chung, E.; Sinclar, C. W.; Workentin, M. S.; Bizzotto, D. *J. Am. Chem. Soc.* **2004**, *126*, 8329-8335.

Table 1. Selected energy transfer efficiencies in PBS

Donor	Acceptor	In 10 mM cyclodextrin	In 0 mM cyclodextrin
7	**20**	121%	25%
8	**20**	107%	24%
11	**20**	168%	32%
12	**20**	119%	27%

Table 2. Selected energy transfer efficiencies in complex media[a]

Donor	In coconut water		In plasma		In breast milk	
	10 mM CD	0 mM CD	10 mM CD	0 mM CD	10 mM CD	0 mM CD
7	29%	29%	27%	30%	24%	26%
8	26%	26%	26%	27%	25%	24%
11	39%	31%	17%	22%	28%	30%
12	26%	18%	21%	16%	19%	30%

a CD = γ-cyclodextrin; fluorophore **20** used as the energy acceptor in all cases

Table 3. Selected energy transfer efficiencies with fluorophore **22**

Donor	10 mM CD	0 mM CD
7	24%	8%
8	30%	38%
11	28%	26%
12	56%	39%

Fig. 1 Schematic illustration of cyclodextrin-promoted energy transfer from organic toxicants to fluorophore acceptors

Fig. 2 Known and suspected toxicants investigated as energy donors

Fig. 3 Fluorophores investigated as energy acceptors

Fig. 4 Graphical illustration of I_{DA}/I_A for a generic donor-acceptor

Fig. 5 Energy transfer in PBS from (a) compound **7**; (b) compound **8**; (c) compound **11**; and (d) compound **12** to fluorophore **20**. The black line represents analyte excitation and the grey line represents direct fluorophore excitation.

Fig. 6 Energy transfer in seawater to fluorophore **20** from (a) analyte **1**; (b) analyte **2**; (c) analyte **3**; (d) analyte **4**; and (e) analyte **5**. The black line represents analyte excitation and the grey line represents direct fluorophore excitation.

Fig. 7 A comparison of the fluorophore emission peak from toxicant **12** to fluorophores **20-22** in 10 mM γ-cyclodextrin in PBS.