ACS Combinatorial Science

Research Article

pubs.acs.org/acscombsci

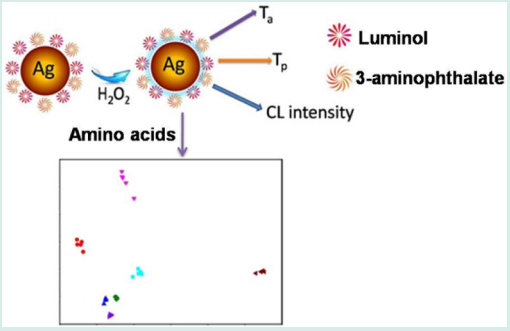
Simple and Sensitive Discrimination of Amino Acids with Functionalized Silver Nanoparticles

Yi He,* Yun Liang, and Haili Yu

School of National Defence Science & Technology, Southwest University of Science and Technology, Mianyang, 621010, P. R. China

Supporting Information

ABSTRACT: A chemiluminescence (CL) sensing method for amino acid discrimination based on luminol functionalized silver nanoparticles (LumAgNPs) has been developed. Luminescence emission in the presence of hydrogen peroxide under neutral conditions was characterized in three ways: the time required for the signal to appear (T_a), the time required to reach maximum luminescence (T_p), and CL intensity. These factors were found to change upon interaction of the nanoparticles with various amino acids, leading to distinct response patterns characteristic of each analyte. Seven amino acids (L-cysteine, L-proline, L-phenylalanine, L-arginine, L-threonine, L-glutamic acid, and L-tyrosine) were identified at a concentration of 10 ng/mL. This sensitivity is about 3 orders of magnitude better than that of recently reported methods based on fluorescent sensor arrays using cucurbit[n]uril and comparable to high-performance liquid chromatography. Application to 27 unknown samples gave a 96.3% success rate at the 10 ng/mL level.



KEYWORDS: *sensor, amino acid, chemiluminescence, functionalized silver nanoparticles*

INTRODUCTION

The detection of individual amino acids is important in a variety of applications, such as in diagnosis of such disorders as neural tube defect, pancreatitis, and Alzheimer disease.[1] Various methods for amino acid determination have been reported, such as high-performance liquid chromatography, spectrophotometry, electrochemistry, mass spectrometry, and capillary zone electrophoresis.[2,3] However, these methods can suffer from high cost, low sensitivity and specificity, and complicated sample pretreatment.

As an alternative to single-readout methods, such as those listed above, "chemical nose/tongue" strategies can have some advantages. In this approach, differential interactions of analytes with a receptor array give a distinct pattern for each analyte as a fingerprint, which is employed for classification and identification. Array-based sensors can therefore discriminate various analytes with high throughput and accuracy,[4] but few attempts have been reported for amino acids. One promising system using cucurbit[n]uril has been described,[5] but it suffers from high detection limits, elaborate molecular design, and multistep organic synthesis. Thus, the development of simple, rapid, and sensitive sensor with a single sensing element for classification and identification of amino acids is highly desirable.

Compared with ordinary spectrum methods, chemiluminescence (CL) assays do not need an external excitation light source, which can improve sensitivity and signal-to-noise owing to the lack of light source interference.[6] Luminol, a common CL reagent, has previously been shown to reduce silver nitrate to form luminol functionalized silver nanoparticles (LumAgNPs),[7] on which luminol molecules are immobilized via Ag−N covalent interaction. The hydrogen peroxide-driven CL behavior of LumAgNPs is dynamically tunable by regulating experimental conditions, such as H_2O_2 concentration and buffer pH.[8] We expected that a single experimental operation could generate multidimensional information, including the time required for the signal to appear (T_a), the time required to reach maximum luminescence (T_p), and CL intensity. The use of luminol and H_2O_2 alone or luminol−H_2O_2 in the presence of unfunctionalized AgNPs, does not provide this capability. In the present work, we report on the use of these factors (T_a, T_p, and CL intensity) as a novel sensing method for simple and sensitive discrimination of various amino acids. These particles could be used in this way to discriminate amino acids in the presence of human urine.

EXPERIMENTAL PROCEDURES

Preparation of LumAgNPs. The LumAgNPs were prepared according to the published protocol.[7] Briefly, a silver nitrate aqueous solution (5 mM, 4 mL) was added to an absolute alcohol solution (10 mL) under magnetic stirring. After the temperature was increased to 60 °C, 0.5 mL of 0.01 M luminol dissolved in 0.1 M NaOH aqueous solution was injected into the mixture, causing a color change from light yellow to brownish yellow. After it was stirred for 2 h, the solution was cooled to room temperature, centrifuged twice at 12500 rpm for 15 min, and the soft sediment was redispersed

Received: February 2, 2015
Revised: May 19, 2015
Published: June 18, 2015

ACS Publications © 2015 American Chemical Society

DOI: 10.1021/acscombsci.5b00045
ACS Comb. Sci. 2015, 17, 409−412

with 5 mM phosphate buffer (PBS, 5 mM, pH 7.4). The resulting suspension was stored at 4 °C before being used. The concentration of redispersed LumAgNPs was estimated to be 0.1 nM by the standard method.[9]

Multidimensional Sensing of Amino Acids. PBS buffer (5 mM, pH 7.4), 0.1 mL of LumAgNPs aqueous solution, and various amounts of amino acids were mixed thoroughly for 10 min, followed by injection of 0.3 mL of 10 mM H_2O_2 and immediate monitoring of CL signal (T_a, T_p, and CL intensity). Five replicate measurements were performed for each amino acid. The raw data were normalized using the formula $I = (I - I_0)/I_0$, where I and I_0 were the values of T_a, T_p, or CL intensity in the presence and absence of target amino acid. The normalized data matrix (3 channels ×7 amino acids ×5 replicates) was further processed using principal component analysis (PCA) in Matlab 7.0 (the MathWorks, Inc., U.S.A.).

Identification of Unknown Samples. To test unknown amino acid samples, 27 samples were prepared randomized, and identified by separate researchers. CL responses of each amino acid were measured three times, averaged, and subjected to PCA using the above procedures. Identifications were achieved in terms of their Mahalanobis distance-square proximities to known group centers from the training matrix.

RESULTS AND DISCUSSION

Characterization of LumAgNPs. The LumAgNPs were prepared via the reported luminol-driven reduction of $AgNO_3$ (Figure 1).[7] It has been demonstrated that luminol and its

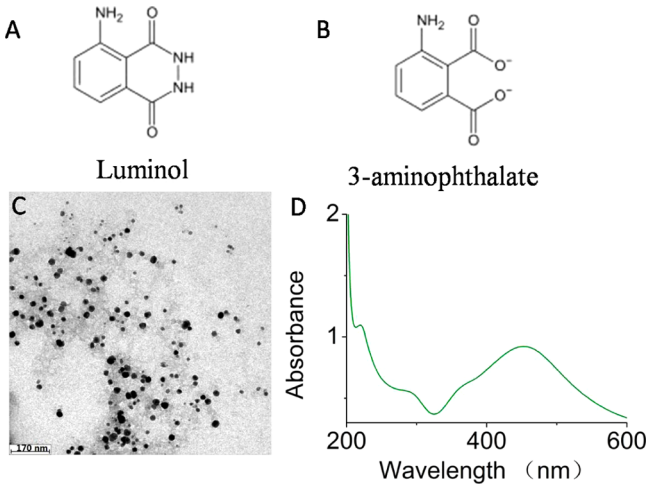


Figure 1. Molecular structures of luminol (A) and 3-aminophthalate (B). TEM image (C) and UV−vis spectrum (D) of LumAgNPs.

oxidation product (3-aminophthalate (Figure 1B)) coexist on the surface of LumAgNPs via Ag−N covalent interaction.[7] The resulting LumAgNPs were characterized by transmission electron microscopy (TEM) and UV−visible (UV−vis) adsorption spectroscopy, respectively. As shown in Figure 1C, the morphology of LumAgNPs was spherical, with an average diameter of approximately 35 nm. Their absorption spectrum shows two peaks (295 and 360 nm) characteristic of luminol.[10] The peak located at 450 nm is ascribed to surface plasmon resonance.[7]

Principle of Sensor Based on LumAgNPs−H_2O_2 CL System. The working principle of the CL sensor for amino acids is illustrated in Figure 2. Compared with traditional luminol−H_2O_2 system or luminol−H_2O_2 in the presence of

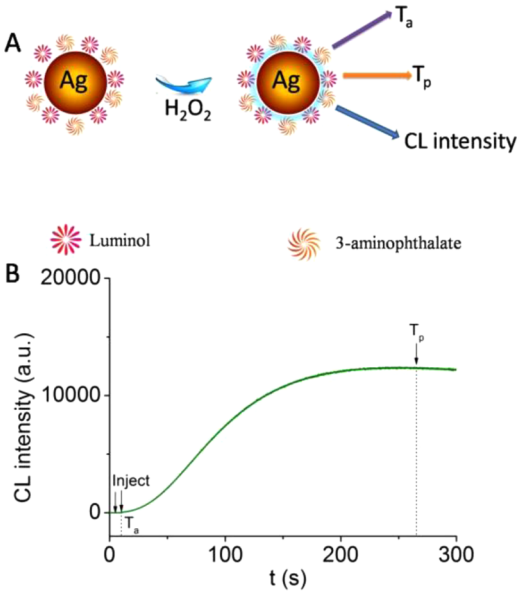


Figure 2. (A) Schematic illustration of triple-channel properties of LumAgNPs−H_2O_2 CL system. (B) CL kinetic curve of 0.1 mL of LumAgNPs and 0.3 mL of H_2O_2 (10 mM, pH 7.4) system.

unfunctionalized AgNPs under neutral environment (no emission or very weak emission, Supporting Information Figure S1), the CL of LumAgNPs-H_2O_2 system is strong and the appearance of luminescence intensity is relatively slow as shown in Figure 2B. When 0.3 mL of H_2O_2 (10 mM, pH 7.4) was injected to 0.1 mL of LumAgNPs solution, CL did not occur immediately. After 5s, CL emission was gradually observed and the corresponding CL intensity increased along with time, reaching maximum intensity at 260 s after injection. Therefore, this CL system was characterized in terms of triple-channel properties (T_a, T_p, and CL intensity) in each experiment.

The mechanism of chemiluminescence in this system involves three steps: (1) partial dissolution of LumAgNPs by H_2O_2, generating Ag^+ ions and $HO^\bullet$ radicals on the particle surface, (2) further reaction of hydroxyl radicals with luminol and HO_2^- to produce luminol radical and superoxide radical anion, and (3) electron-transfer reaction of luminol radicals and superoxide radical anion to generate excited-state 3-aminophthalate, producing CL emission.[8,11] The T_a parameter corresponds to the buildup of $HO^\bullet$ radicals, and T_p reflects the time required to achieve an equilibrium state of maximum CL intensity.

It has been demonstrated that various amino acids can be absorbed on the surface of AgNPs via Ag−N and Ag−S bonds, N−H···Ag and O−H···Ag hydrogen bonds, and electrostatic interaction.[12] The presence of amino acids is expected to influence the redox reaction of LumAgNPs and H_2O_2. Moreover, the active oxygen intermediates could react with adsorbed amino acids,[13] and luminol might directly interact with amino acids via hydrogen bonds. Hence, the presence of amino acids can alter the CL dynamic process of LumAgNPs−H_2O_2 and influence the triple-channel properties of the LumAgNPs−H_2O_2 CL system. Different amino acids, having different chemical and physical properties, could be expected to give distinct CL response patterns.

Fingerprints of Amino Acid Generated via the Triple-Channel Properties of LumAgNPs−H_2O_2 CL System. To demonstrate the feasibility of this multichannel readout, seven

DOI: 10.1021/acscombsci.5b00045
ACS Comb. Sci. 2015, 17, 409−412

amino acids having different molecular weight [L-cysteine (Cys), L-proline (Pro), L-phenylalanine (Phe), L-arginine (Arg), L-threonine (Thr), L-glutamic acid (Glu), and L-tyrosine (Tyr)] were chosen. The LumAgNPs-H_2O_2 triple-channel properties (T_a, T_p, and CL intensity) in the presence of these molecules were measured as shown in Supporting Information Figure S3, then the signal change was calculated and plotted as shown in Figure 3.

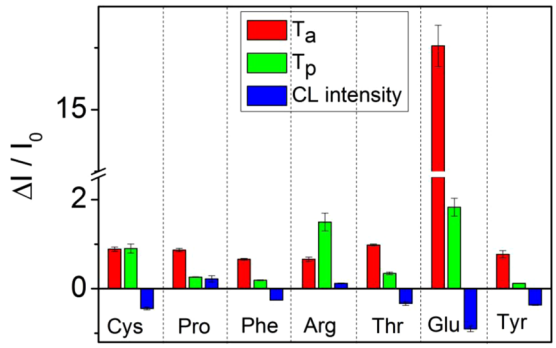


Figure 3. Fingerprints of seven amino acids based on the patterns for the corresponding values of $\Delta I/I_0$. $\Delta I = I - I_0$, where I and I_0 were the values of T_a, T_p, or CL intensity in the presence and absence of target amino acid. The amino acid concentration was 10 ng/mL.

The various amino acids generally increased values of both T_a (Figure 3, red bars) and T_p (green bars). CL intensity was mostly diminished, except in two cases (blue bars). In general, glutamic acid caused the greatest magnitude of response in all three parameters.

PCA for Discrimination of Amino Acids by LumAgNPs-Based Sensor. To establish unique triple-channel response fingerprints, PCA analysis was performed as a statistical method. Two extracted principal components accounted for 95% of the variance, and were employed to generate a two-dimensional (2D) plot as shown in Figure 4. At an amino acid concentration of 10 ng/mL (midnanomolar), PCA confirmed that the canonical CL response patterns (3 channels × 7 amino acids × 5 replicates) of the amino acids were clearly clustered into seven groups, which corresponded to each specific amino acid, and the five duplicated measurements of each amino acid were in a narrow distribution in the PCA plots. These results demonstrated that the CL properties of LumAgNPs had excellent capability for discriminating these seven amino acids.

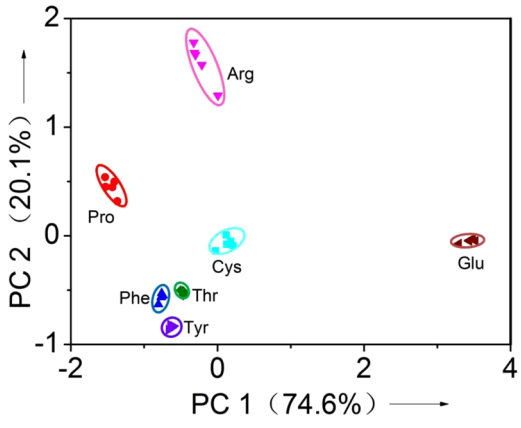


Figure 4. PCA plot for the discrimination of seven amino acids (10 ng/mL) based on the triple-channel CL properties of LumAgNPs–H_2O_2 system.

The robustness of the developed sensor was also checked by using unknown samples randomly taken from the training set. The identification accuracy of the unknown samples was found to be 96.3% (26 out of 27) at the 10 ng/mL level. This sensitivity is about 3 orders of magnitude higher than that of the recently proposed methods based on the fluorescent sensor arrays using cucurbit[n]uril,[5] and comparable to high-performance liquid chromatography.[14]

The triple-channel CL responses were further evaluated for discrimination of amino acids at various concentrations. Pro and Tyr as the model amino acids were measured at three different concentrations (5, 10, and 20 ng/mL). As shown in Figure 5, the PCA plot for Pro and Tyr with different concentrations were not random, but followed certain patterns and could be differentiated from each other.

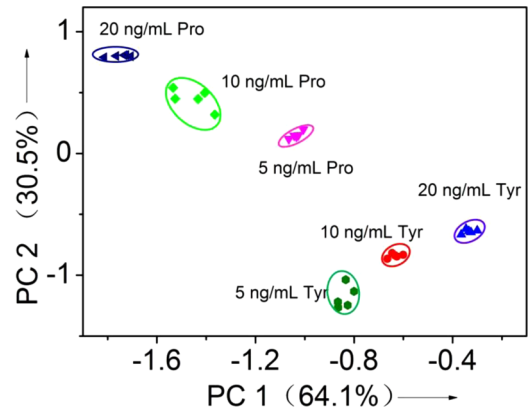


Figure 5. PCA plot for the discrimination of Tyr and Pro at different concentrations based on the triple-channel properties of LumAgNPs–H_2O_2 system.

To investigate the performance of our developed CL sensor to analyze samples in more complex solutions, the particles were further used for discrimination of amino acids in the presence of diluted human urine (1% (v/v)). PBS buffer (control), diluted urine, and urine samples with added different amino acids (10 ng/mL each) were measured. As shown in Figure 6, the diluted human urine alone gave signals distinct from the buffer control, possibly due to amino acids present in diluted human urine. However, just as in the buffer studies, each of the amino acids in the presence of diluted human urine generated distinct responses, indicating that the developed CL sensor for discrimination of amino acids may be applied to realistic biological samples, such as diluted human urine.

CONCLUSION

In summary, we found that various amino acid analytes could influence the CL dynamic process of LumAgNPs–H_2O_2 system and alter the corresponding triple-channel CL properties (T_a, T_p, and CL intensity), producing distinct responses. This allows amino acids to be discriminated in solution via chemiluminescence for the first time. Significantly, this method successfully resolved amino acids in the presence of diluted human urine. The use of chemiluminescent nanoparticles provides potential advantages of easy synthesis of the sensor species, simple experimental operation, and greatly enhanced

DOI: 10.1021/acscombsci.5b00045
ACS Comb. Sci. 2015, 17, 409–412

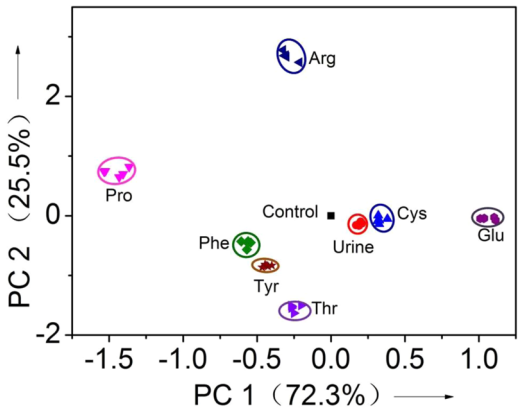


Figure 6. PCA plot for the discrimination of seven amino acids (10 ng/mL) in the presence of diluted human urine (1% (v/v)) based on the triple-channel properties of LumAgNPs–H_2O_2 system.

sensitivity over fluorescent methods. While the present proof-of-concept method cannot be used to recognize unknown amino acids at unknown concentrations in real samples, we believe that these results open up a new avenue for design and development of future sensors for the detection of amino acids and other analytes.

■ ASSOCIATED CONTENT

Supporting Information

Reagents and apparatus, CL kinetic curves of LumAgNPs–H_2O_2, luminol–H_2O_2, and luminol–H_2O_2–unfunctionalized AgNPs systems, molecular structures of target amino acids, CL kinetic curve of the sensor system in the absence and presence of different amino acids, training matrix of the response patterns against amino acids at 10 ng/mL, and detection and identification of unknown amino acids at 10 ng/mL. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscombsci.5b00045.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: yhe2014@126.com.

Author Contributions

The manuscript was written through contributions of all authors.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The support of this research by the Foundation of Science and Technology Department of Sichuan Province (Grant No. 2015JY0053) and the Doctoral Program of Southwest University of Science and Technology (Grant No. 14zx7165) is gratefully acknowledged.

■ REFERENCES

(1) Zhou, Y.; Yoon, J. Recent progress in fluorescent and colorimetric chemosensors for detection of amino acids. *Chem. Soc. Rev.* **2012**, *41* (1), 52–67.

(2) Heli, H.; Hajjizadeh, M.; Jabbari, A.; Moosavi-Movahedi, A. Fine steps of electrocatalytic oxidation and sensitive detection of some amino acids on copper nanoparticles. *Anal. Biochem.* **2009**, *388* (1), 81–90.

(3) (a) Baron, R.; Zayats, M.; Willner, I. Dopamine-, L-DOPA-, adrenaline-, and noradrenaline-induced growth of Au nanoparticles: assays for the detection of neurotransmitters and of tyrosinase activity. *Anal. Chem.* **2005**, *77* (6), 1566–1571. (b) Chen, Z.; Luo, S.; Liu, C.; Cai, Q. Simple and sensitive colorimetric detection of cysteine based on ssDNA-stabilized gold nanoparticles. *Anal. Bioanal. Chem.* **2009**, *395* (2), 489–494. (c) Strieglerová, L.; Kubáň, P.; Boček, P. Electromembrane extraction of amino acids from body fluids followed by capillary electrophoresis with capacitively coupled contactless conductivity detection. *J. Chromatogr. A* **2011**, *1218* (37), 6248–6255.

(4) (a) Chou, S. S.; De, M.; Luo, J.; Rotello, V. M.; Huang, J.; Dravid, V. P. Nanoscale graphene oxide (nGO) as artificial receptors: Implications for biomolecular interactions and sensing. *J. Am. Chem. Soc.* **2012**, *134* (40), 16725–16733. (b) Hu, J.; Jiang, X.; Wu, L.; Xu, K.; Hou, X.; Lv, Y. UV-induced surface photovoltage and photoluminescence on n-Si/TiO_2/TiO_2: Eu for dual-channel sensing of volatile organic compounds. *Anal. Chem.* **2011**, *83* (17), 6552–6558. (c) Kong, H.; Liu, D.; Zhang, S.; Zhang, X. Protein sensing and cell discrimination using a sensor array based on nanomaterial-assisted chemiluminescence. *Anal. Chem.* **2011**, *83* (6), 1867–1870. (d) Kong, H.; Lu, Y.; Wang, H.; Wen, F.; Zhang, S.; Zhang, X. Protein discrimination using fluorescent gold nanoparticles on plasmonic substrates. *Anal. Chem.* **2012**, *84* (10), 4258–4261. (e) Pei, H.; Li, J.; Lv, M.; Wang, J.; Gao, J.; Lu, J.; Li, Y.; Huang, Q.; Hu, J.; Fan, C. A graphene-based sensor array for high-precision and adaptive target identification with ensemble aptamers. *J. Am. Chem. Soc.* **2012**, *134* (33), 13843–13849.

(5) Minami, T.; Esipenko, N. A.; Zhang, B.; Isaacs, L.; Anzenbacher, P. "Turn-on" fluorescent sensor array for basic amino acids in water. *Chem. Commun.* **2013**, *50* (1), 61–63.

(6) Li, L. L.; Liu, K. P.; Yang, G. H.; Wang, C. M.; Zhang, J. R.; Zhu, J. J. Fabrication of graphene–quantum dots composites for sensitive electrogenerated chemiluminescence immunosensing. *Adv. Fun. Mater.* **2011**, *21* (5), 869–878.

(7) He, Y.; Liu, D.; He, X.; Cui, H. One-pot synthesis of luminol functionalized silver nanoparticles with chemiluminescence activity for ultrasensitive DNA sensing. *Chem. Commun.* **2011**, *47* (38), 10692–10694.

(8) Chen, H.; Gao, F.; He, R.; Cui, D. Chemiluminescence of luminol catalyzed by silver nanoparticles. *J. Colloid Interface Sci.* **2007**, *315* (1), 158–163.

(9) Sriram, M. I.; Kanth, S. B. M.; Kalishwaralal, K.; Gurunathan, S. Antitumor activity of silver nanoparticles in Dalton's lymphoma ascites tumor model. *Int. J. Nanomedicine.* **2010**, *5*, 753–762.

(10) Wang, W.; Xiong, T.; Cui, H. Fluorescence and electrochemiluminescence of luminol-reduced gold nanoparticles: photostability and platform effect. *Langmuir* **2008**, *24* (6), 2826–2833.

(11) Guo, J.-Z.; Cui, H.; Zhou, W.; Wang, W. Ag nanoparticle-catalyzed chemiluminescent reaction between luminol and hydrogen peroxide. *J. Photochem. Photobiol. A* **2008**, *193* (2), 89–96.

(12) Pakiari, A.; Jamshidi, Z. Interaction of amino acids with gold and silver clusters. *J. Phys. Chem. A* **2007**, *111* (20), 4391–4396.

(13) Li, S.; Tao, S.; Wang, F.; Hong, J.; Wei, X. Chemiluminescence reactions of luminol system catalyzed by nanoparticles of a gold/silver alloy. *Microchim. Acta* **2010**, *169* (1–2), 73–78.

(14) Wu, X.; Wang, R.; Jiang, Q.; Wang, S.; Yao, Y.; Shao, L. Determination of amino acid neurotransmitters in rat hippocampi by HPLC-UV using NBD-F as a derivative. *Biomed. Chromatogr.* **2014**, *28* (4), 459–462.

DOI: 10.1021/acscombsci.5b00045
ACS Comb. Sci. 2015, 17, 409–412