JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Electrochemical Control of Rapid Bioorthogonal Tetrazine Ligations for Selective Functionalization of Microelectrodes

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Supporting Information

ABSTRACT: We demonstrate that bioorthogonal tetrazine ligations can be utilized to rapidly modify electrode surfaces, both with redox probes and enzymes. Furthermore, we show that the redox-active nature of 1,2,4,5-tetrazines can be exploited to gain electrochemical control over surface modification. To our knowledge this is the first demonstration of controlling a tetrazine ligation by changing the redox state of one of the reactants. We utilize the redox-switchable feature of tetrazine ligations for the site-selective functionalization of a 10 μm spaced interdigitated array of microelectrodes. In addition, we were able to achieve potential controlled ligation of the redox enzyme horseradish peroxidase to a macroscopic planar electrode. The rapid kinetics, bioorthogonal reactivity, and electrochemical control provided by tetrazine ligations should lead to numerous applications related to electrode functionalization.

Microelectrode arrays (MEA) with immobilized biomolecules are employed in numerous research areas, including high-throughput screening,[1] drug discovery,[2] and biosensor technology.[3] A popular strategy for electrode immobilization is through covalent attachment via surface coupling reactions.[4] Although less common, a particularly valuable class of covalent coupling reactions are those that can be electrochemically controlled, enabling site-specific functionalization of electrode arrays.[5] Over the years, a variety of ingenious redox-controllable coupling methods have been developed.[6] Applications include the formation of DNA microarrays,[7] tissue engineering,[8] protein modification,[9] and polymer fuctionalization.[10] The utility of these systems encourages the search for novel coupling approaches that may possess unique features. In the field of chemical biology, various ligation reactions with high performance have recently been developed.[11] Recently, there has been increasing interest in the use of bioorthogonal tetrazine ligations for bioconjugation. The ligation is based on the inverse-electron demand Diels–Alder reaction (IEDDAR) between tetrazines and strained alkenes such as *trans*-cyclooctene (TCO) (Figure 1A). Tetrazine ligations benefit from tunable and rapid kinetics, bioorthogonal reactivity, and no requirement for additional catalysts. For these reasons, tetrazine ligations have become a popular conjugation method with a wide range of outstanding applications, such as live cell imaging,[12] in vivo drug delivery,[13] materials functionalization,[14] and nucleic acid detection.[15]

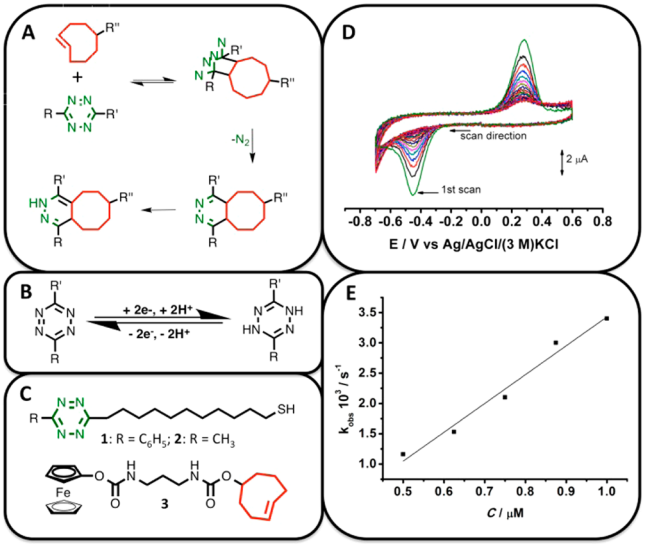

Figure 1. (A) IEDDAR between a 1,2,4,5-tetrazine and a TCO. (B) Redox behavior of 1,2,4,5-tetrazines. (C) Synthesized tetrazine and TCO compounds. (D) Sequential CVs of a mixed SAM during a 20 min functionalization period. Electrolyte 0.1 M phosphate buffer (pH = 7) and 1 μM TCO-PEG$_3$-amine. (E) Plot of k_{obs} versus the concentration of TCO-PEG$_3$-amine for a mixed SAM.

Despite the recent surge of applications, the tetrazine ligation has not been utilized for the functionalization of electrode surfaces. This is surprising, considering the extremely fast kinetics of the tetrazine ligation when employing *trans*-cyclooctene (TCO) as a dienophile,[16] which would be useful for rapid bioorthogonal surface functionalization with low concentrations of reagents. Furthermore, although the redox behavior of 1,2,4,5-tetrazines (Figure 1B) is well established in coordination chemistry and materials science,[17] the redox-active nature of tetrazines has not previously been used for switching the reactivity of IEDDARs. Here we report the first example of tetrazine ligation at an electrode surface. Additionally, we demonstrate that the redox-active nature of tetrazines can be exploited to electrochemically control surface modification, allowing independent functionalization of band electrodes on an interdigitated array (IDA) as well as potential controlled modification of electrodes with redox enzymes.

To examine the tetrazine coupling reaction at an electrode surface, we utilized self-assembled monolayers (SAMs) of alkyl

Received: March 31, 2015
Published: July 1, 2015

DOI: 10.1021/jacs.5b03371
J. Am. Chem. Soc. 2015, 137, 8876–8879

thiols on gold.[18] One likely reason why the tetrazine ligation has not previously been applied for the functionalization of SAMs is the synthetic challenge of synthesizing the necessary asymmetrically substituted 1,2,4,5-tetrazines. We recently demonstrated that a Lewis acid-catalyzed synthetic methodology enables one-pot access to a variety of asymmetric 1,2,4,5-tetrazines.[19] We adapted this approach to synthesize the thiolated tetrazines **1** and **2** (Figure 1C). Mixed tetrazine-terminated SAMs were then formed by immersing gold-coated silicon wafers into ethanolic solutions of **1** or **2**, respectively, containing 1-nonanethiol as a diluent (see Supporting Information for full experimental details).

To examine the redox behavior and the reactivity of the surface bound tetrazines, cyclic voltammetry (CV) experiments on SAMs were conducted in 0.1 M phosphate buffer (pH = 7). Mixed monolayers of **1** and **2** ($\chi_{1,2}$ = 0.05) display a similar redox behavior with a reduction at E_{pc} = −0.47 V (**1**) and E_{pc} = −0.46 V (**2**), respectively, and an associated oxidation at E_{pa} = 0.26 V (**1**) and E_{pa} = 0.28 V (**2**), respectively, versus Ag/AgCl/(3 M)KCl. The lack of change in the signal shape and intensity after multiple cycles indicates a chemically reversible redox process, which we attribute to an interconversion between the 1,2,4,5-tetrazine and its reduced and protonated 1,4-dihydro-1,2,4,5-tetrazine species.[17b,c] This behavior exhibits similarity to the quinone/hydroquinone redox couple.[20] CVs recorded at various scan rates between 0.1 and 0.5 V/s show a linear dependency between the peak current and the scan rate (Figure S4) and thus reflect the behavior of a surface-bound redox-active substrate. Exposure of tetrazine monolayers to a solution of TCO dienophile, which is known to react with tetrazines via IEDDAR, rapidly diminishes the tetrazine redox signal. The rate constant of the IEDDAR can therefore be determined by CV. Sequential CVs at a scan rate of 0.5 V/s were recorded once every minute, while mixed monolayers of **1** and **2** ($\chi_{1,2}$ = 0.05) were contacted to solutions of TCO-PEG$_3$-amine (Figure S1) in 0.1 M phosphate buffer (pH = 7). The concentration of TCO-PEG$_3$-amine was varied between 0.5 and 1 μM. Figure 1D depicts the successive decrease in signal intensity of surface bound **1** during the course of its reaction with a solution of 1 μM TCO-PEG$_3$-amine. To obtain the kinetic data for the IEDDAR on the SAM, the decrease in relative peak current I_t/I_0 (where I_t is the peak current at time t and I_0 is the initial peak current), was plotted against the reaction time (Figure S5). The pseudo-first-order rate constants k_{obs} were determined from an exponential fit, and second-order rate constants of k = 4800 M^{-1} s^{-1} (**1**) and k = 5200 M^{-1} s^{-1} (**2**) were estimated by plotting k_{obs} against the concentration of TCO-PEG$_3$-amine and applying a linear fit (Figure 1E). It is worth comparing this rate to other chemoselective reactions that have been performed on SAMs. The TCO tetrazine ligation is about 20 times faster than the fastest normal-electron demand Diels–Alder reaction between pentamethylcyclopentadiene[21] and hydroquinone (k = 220 M^{-1} s^{-1}) and about 5 times faster than the copper(I)-catalyzed azide alkyne cycloaddition (CuCAAC) (k = 1000 M^{-1} s^{-1}) when a large excess of copper catalyst is used.[22] It is important to point out that the tetrazine ligation does not require the use of a transition-metal catalyst, such as copper, which can lead to the degradation of biological molecules[23] through production of reactive oxygen species or can potentially adsorb onto electrode surfaces.[24] It is also likely that a further increase in the already outstanding rapid reaction rate could be achieved by the use of more reactive TCO derivatives[16c] or by substituting the 6-position of **1** and **2** with a more electron-withdrawing substituent.[25]

In order to demonstrate that the decrease of the CV signal is due to the tetrazine ligation, a monolayer of **1** was exposed to a 1 μM aqueous solution of a ferrocene modified TCO compound **3** for 30 min, and CVs were recorded before and after the reaction. The disappearance of the tetrazine signal along with the appearance of a surface ferrocene signal (Figure S6) verified the ligation reaction between the tetrazine and the TCO. Moreover, a comparison of the integrated CVs of the tetrazine reactant and ferrocene product reveals the expected two electron transfer involved in the redox process of the tetrazine.

After the successful implementation of the tetrazine ligation chemistry on SAMs, we next determined if electrochemically reducing the tetrazine to the 1,4-dihydro-1,2,4,5-tetrazine species would abrogate reactivity toward TCO functionalized molecules. Initially, we tested potential dependent IEDDAR on macroscopic planar electrodes. For this purpose a monolayer of **1** was contacted to a 1 μM solution of **3** in phosphate buffer (pH = 7), while the potential was held for 15 min at −600 mV versus Ag/AgCl/(3 M)KCl. After removal o the TCO, we first reoxidized any present 1,4-dihydro-1,2,4,5-tetrazine. We then performed CV to determine if any ferrocene had been immobilized. The absence of a ferrocene signal indicates that the electrode was not functionalized with **3**. The identical electrode was then again contacted for 15 min to a 1 μM solution of **3** in phosphate buffer (pH = 7), while the potential was held at open circuit. The appearance of a ferrocene signal indicates that the electrode was then functionalized with **3** (Figure S8).

Based on our initial experiments with planar electrodes, we hypothesized that the potential-dependent reactivity of tetrazines could be utilized to control functionalization on a microarray of independently addressable electrodes. Compared to microelectode modification using CuAAC, the absence of a soluble catalyst in the IEDDAR would be an advantage, since the copper(I) catalyst, employed in the CuAAC, can diffuse and possibly lead to nonspecific functionalization.[6d] We performed a potential controlled coupling of **3** on a pair of gold IDA band electrodes (Figure 2). The IDA (Abtech Scientific) was composed of two electrodes each consisting of 50 gold fingers 5 mm long by 10 μm wide, with an interelectrode spacing of 10 μm. Identical monolayers of **1** were formed on both gold electrodes. Initially, CVs of both electrodes were recorded at

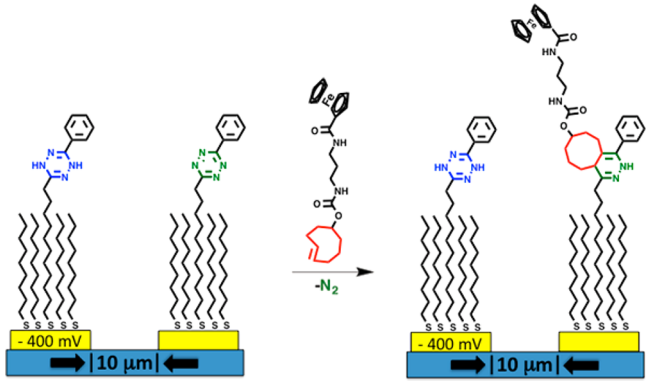

Figure 2. Electrochemically controlled TCO tetrazine ligation at a SAM of **1**, for site-selective functionalization of a 10 μm spaced IDA of independently addressable microelectrodes.

DOI: 10.1021/jacs.5b03371
J. Am. Chem. Soc. 2015, 137, 8876–8879

potentials near the ferrocene standard reduction potential. The IDA was then contacted to a 1 μM solution of **3** in phosphate buffer (pH = 7). Electrode 1 was held at −400 mV versus Ag/AgCl(gel), while electrode 2 was left at open circuit. After 15 min the IDA was rinsed, and CVs were recorded in a buffered 0.1 M NaCl solution (pH = 4). As expected, only electrode 2 was modified. To demonstrate that the method could possibly be employed to pattern electrode arrays, we repeated the reaction with the partially functionalized IDA under the same conditions, except that electrode 1 was left at open circuit and electrode 2 was held at −400 mV. Electrode 1 became functionalized with approximately the same amount of **3** as Electrode 2 (Figure 3), while electrode 2 did not undergo further functionalization.

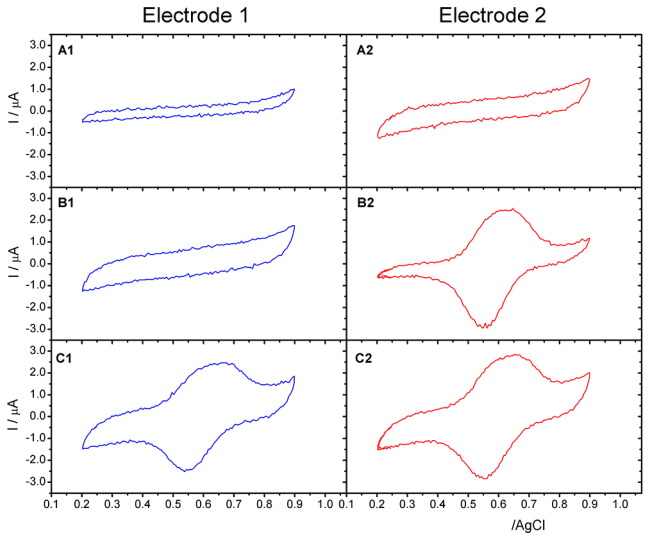

Figure 3. CVs (2 V/s scan rate) recorded before and after the selective functionalization of identical SAMs of **1** on a pair of gold IDA microelectrodes. Panels A1 and A2 depict the initial CVs before the exposure of electrodes to **3**. Panels B1 and B2 depict CVs after a 15 min functionalization in a solution of 1 μM of **3**. During the functionalization period, electrode 1 was held at −400 mV, whereas electrode 2 was left at open circuit. In panels C1 and C2 the potentials were switched. During the 15 min functionalization in a 1 μM solution of **3** electrode 1 was left at open circuit, whereas electrode 2 was held at −400 mV.

One of the major advantages of tetrazine ligations is their bioorthogonal reactivity profile. The redox-switchable tetrazine ligation could be a powerful tool to control the covalent ligation of biomolecules to an electrode surface. In particular, it would be interesting to utilize the chemistry with redox enzymes such as horseradish peroxidase (HRP), as they are frequently employed in conjunction with electrochemical detection on sensors.[26] To demonstrate the applicability of tetrazine ligation to the immobilization of biomolecules on electrodes, we immersed a mixed monolayer of **1** (SAM1) with a 1 μM solution of TCO-modified HRP in phosphate buffer (pH = 6.5) for 15 min. To ensure removal of any physically adsorbed HRP, the monolayer was rinsed and sonicated[27] for 5 min in a 1 M KCl solution.[28] SAM1 was then immersed in a solution containing 0.01% v/v H_2O_2 and 4-chloro-1-naphthol, a commonly utilized substrate for HRP that forms a dark precipitate upon oxidation.[26b] The experiment was then repeated identically, except that the second monolayer (SAM2) was held at −500 mV versus Ag/AgCl(gel), while immersed in the solution of TCO modified HRP. We observed significant brown precipitate on SAM1 (Figure 4Ba), indicating

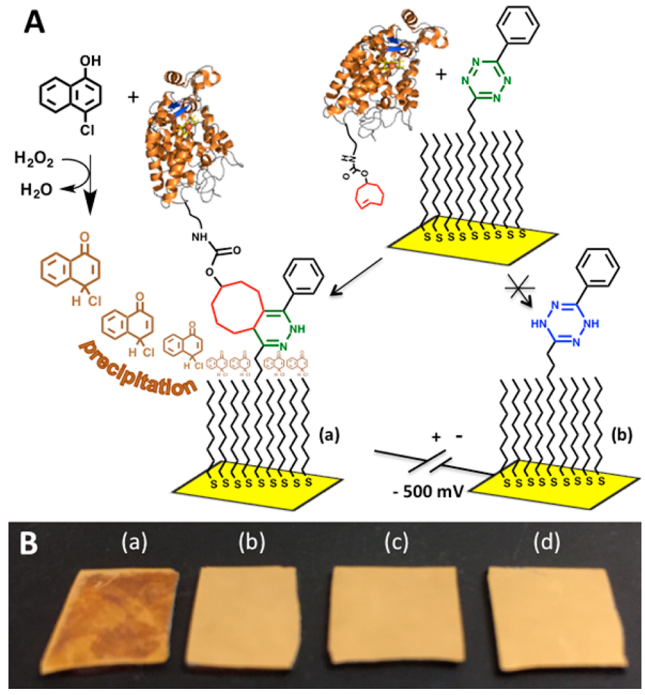

Figure 4. (A) Electrochemically controlled immobilization of TCO-HRP on mixed SAMs (χ_1 = 0.05). (B) Experimental results of electrochemically controlled immobilization of TCO-HRP (a and b) and control experiments (c and d). Conditions including type of protein/composition of SAM/applied potential: (a) **1** and $CH_3(CH_2)_8SH$ (χ_1 = 0.05)/TCO-HRP/open circuit. (b) **1** and $CH_3(CH_2)_8SH$ (χ_1 = 0.05)/TCO-HRP/−400 mV. (c) **1** and $CH_3(CH_2)_8SH$ (χ_1 = 0.05)/HRP/open circuit. (d) $CH_3(CH_2)_8SH$/TCO-HRP/open circuit. Graphical representation of HRP adapted from PDB: 1w4y.

surface functionalization with HRP, whereas no brown precipitate was observed on SAM2 (Figure 4Bb). Additionally, we performed control experiments where a monolayer of **1** was immersed in a solution of HRP lacking TCO (Figure 4Bc) and a second monolayer of 100% 1-nonanethiol was immersed in a solution of TCO modified HRP (Figure 4Bd). Both SAMs were not functionalized with HRP, as indicated by the lack of precipitate formation. For future studies requiring protein or cell immobilization, polyethylene glycol terminated alkanethiols could be employed as diluent for the mixed SAMs, to better prevent nonspecific adsorption.[29]

In summary, we have demonstrated the applicability of tetrazine ligations for the functionalization of electrode surfaces. The surface coupling proceeds with extremely rapid reaction rates under biocompatible conditions. Moreover, the IEDDAR at a tetrazine-modified SAM can be switched on and off by electrochemically controlling the redox-state of the tetrazine. We exploited this property to independently address electrodes on a MEA, and we were able to achieve addressable functionalization of 10 μm spaced microelectrodes on an IDA. The bioorthogonal nature of the reaction also enabled rapid and electrochemically controlled modification of electrodes with biomolecules such as redox enzymes. Site-selective functionalization in the absence of catalyst and confining agent facilitates the patterning of high-density MEAs without the

DOI: 10.1021/jacs.5b03371
J. Am. Chem. Soc. 2015, 137, 8876−8879

need for time-consuming optimization procedures. In addition to site-selective microelectrode functionalization, tetrazine-terminated SAMs could also be attractive for patterning electrodes used in sensor technology, optoelectronic devices, microcantilevers, or redox catalysis.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures and additional voltammetric data. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b03371.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the University of California, San Diego for financial support. Furthermore, we thank Ali Hosseini for his helpful comments.

■ REFERENCES

(1) (a) Gurard-Levin, Z. A.; Scholle, M. D.; Eisenberg, A. H.; Mrksich, M. *ACS Comb. Sci.* **2011**, *13*, 347. (b) Shi, M.; Peng, Y.; Zhou, J.; Liu, B.; Huang, Y.; Kong, J. *Biosens. Bioelectron.* **2006**, *21*, 2210. (c) Min, D.-H.; Tang, W.-J.; Mrksich, M. *Nat. Biotechnol.* **2004**, *22*, 717.

(2) Stett, A.; Egert, U.; Guenther, E.; Hofmann, F.; Meyer, T.; Nisch, W.; Haemmerle, H. *Anal. Bioanal. Chem.* **2003**, *377*, 486.

(3) (a) Puri, N.; Sharma, V.; Tanwar, V.; Singh, N.; Biradar, A.; Rajesh. *Prog. Biomater* **2013**, *2*, 5. (b) Vasilyeva, E.; Lam, B.; Fang, Z.; Minden, M. D.; Sargent, E. H.; Kelley, S. O. *Angew. Chem., Int. Ed.* **2011**, *50*, 4137.

(4) (a) Chechik, V.; Crooks, R. M.; Stirling, C. J. M. *Adv. Mater.* **2000**, *12*, 1161. (b) Haensch, C.; Hoeppener, S.; Schubert, U. S. *Chem. Soc. Rev.* **2010**, *39*, 2323. (c) Nicosia, C.; Huskens, J. *Mater. Horiz.* **2014**, *1*, 32.

(5) (a) Choi, I. S.; Chi, Y. S. *Angew. Chem., Int. Ed.* **2006**, *45*, 4894. (b) Graaf, M. D.; Moeller, K. D. *Langmuir* **2014**, DOI: 10.1021/la504254e. (c) Choi, I.; Yeo, W.-S. *ChemPhysChem* **2013**, *14*, 55.

(6) (a) Bunimovich, Y. L.; Ge, G.; Beverly, K. C.; Ries, R. S.; Hood, L.; Heath, J. R. *Langmuir* **2004**, *20*, 10630. (b) Zhuang, L.; Zuo, H.; Wu, Z.; Wang, Y.; Fang, D.; Jiang, D. *Anal. Chem.* **2014**, *86*, 11517. (c) Kim, K.; Jang, M.; Yang, H.; Kim, E.; Kim, Y. T.; Kwak, J. *Langmuir* **2004**, *20*, 3821. (d) Devaraj, N. K.; Dinolfo, P. H.; Chidsey, C. E. D.; Collman, J. P. *J. Am. Chem. Soc.* **2006**, *128*, 1794. (e) Hu, L.; Stuart, M.; Tian, J.; Maurer, K.; Moeller, K. D. *J. Am. Chem. Soc.* **2010**, *132*, 16610. (f) Fellet, M. S.; Bartels, J. L.; Bi, B.; Moeller, K. D. *J. Am. Chem. Soc.* **2012**, *134*, 16891. (g) Bartels, J.; Lu, P.; Maurer, K.; Walker, A. V.; Moeller, K. D. *Langmuir* **2011**, *27*, 11199. (h) Moeller, K. D. *Organometallics* **2014**, *33*, 4607. (i) Choi, I.; Kim, Y. K.; Min, D.-H.; Lee, S.; Yeo, W. S. *J. Am. Chem. Soc.* **2011**, *133*, 16718.

(7) (a) Canete, S. J. P.; Lai, R. Y. *Chem. Commun.* **2010**, *46*, 3941. (b) Furst, A.; Landefeld, S.; Hill, M. G.; Barton, J. K. *J. Am. Chem. Soc.* **2013**, *135*, 19099.

(8) Luo, W.; Yousaf, M. N. *J. Am. Chem. Soc.* **2011**, *133*, 10780.

(9) Mendes, P. M.; Christman, K. L.; Parthasarathy, P.; Schopf, E.; Ouyang, J.; Yang, Y.; Preece, J. A.; Maynard, H. D.; Chen, Y.; Stoddart, J. F. *Bioconjugate Chem.* **2007**, *18*, 1919.

(10) Hansen, T. S.; Daugaard, A. E.; Hvilsted, S.; Larsen, N. B. *Adv. Mater.* **2009**, *21*, 4483.

(11) (a) Sletten, E. M.; Bertozzi, C. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 6974. (b) Lim, R. K. V.; Lin, Q. *Chem. Commun.* **2010**, *46*, 1589. (c) Ramil, C. P.; Lin, Q. *Chem. Commun.* **2013**, *49*, 11007.

(12) (a) Devaraj, N. K.; Upadhyay, R.; Haun, J. B.; Hilderbrand, S. A.; Weissleder, R. *Angew. Chem., Int. Ed.* **2009**, *48*, 7013. (b) Devaraj, N. K.; Hilderbrand, S.; Upadhyay, R.; Mazitschek, R.; Weissleder, R. *Angew. Chem., Int. Ed.* **2010**, *49*, 2869. (c) Wu, H.; Yang, J.; Šečkutė, J.; Devaraj, N. K. *Angew. Chem., Int. Ed.* **2014**, *53*, 5805.

(13) Devaraj, N. K.; Thurber, G. M.; Keliher, E. J.; Marinelli, B.; Weissleder, R. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 4762.

(14) (a) Hansell, C. F.; Lu, A.; Patterson, J. P.; O'Reilly, R. K. *Nanoscale* **2014**, *6*, 4102. (b) Chen, C.; Allen, C. A.; Cohen, S. M. *Inorg. Chem.* **2011**, *50*, 10534.

(15) (a) Šečkutė, J.; Yang, J.; Devaraj, N. K. *Nucleic Acids Res.* **2013**, *41*, e148. (b) Wu, H.; Cisneros, B. T.; Cole, C. M.; Devaraj, N. K. *J. Am. Chem. Soc.* **2014**, *136*, 17942.

(16) (a) Blackman, M. L.; Royzen, M.; Fox, J. M. *J. Am. Chem. Soc.* **2008**, *130*, 13518. (b) Kurra, Y.; Odoi, K. A.; Lee, Y.-J.; Yang, Y.; Lu, T.; Wheeler, S. E.; Torres-Kolbus, J.; Deiters, A.; Liu, W. R. *Bioconjugate Chem.* **2014**, *25*, 1730. (c) Darko, A.; Wallace, S.; Dmitrenko, O.; Machovina, M. M.; Mehl, R. A.; Chin, J. W.; Fox, J. M. *Chem. Sci.* **2014**, *5*, 3770.

(17) (a) Schwach, M.; Hausen, H.-D.; Kaim, W. *Inorg. Chem.* **1999**, *38*, 2242. (b) Nickerl, G.; Senkovska, I.; Kaskel, S. *Chem. Commun.* **2015**, *51*, 2280. (c) Kaim, W. *Coord. Chem. Rev.* **2002**, *230*, 127.

(18) (a) Love, J. C.; Estroff, L. A.; Kriebel, J. K.; Nuzzo, R. G.; Whitesides, G. M. *Chem. Rev.* **2005**, *105*, 1103. (b) Vericat, C.; Vela, M. E.; Benitez, G.; Carro, P.; Salvarezza, R. C. *Chem. Soc. Rev.* **2010**, *39*, 1805.

(19) Yang, J.; Karver, M. R.; Li, W.; Sahu, S.; Devaraj, N. K. *Angew. Chem., Int. Ed.* **2012**, *51*, 5222.

(20) (a) Yousaf, M. N.; Mrksich, M. *J. Am. Chem. Soc.* **1999**, *121*, 4286. (b) Yousaf, M. N.; Chan, E. W. N.; Mrksich, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 1943.

(21) Gawalt, E. S.; Mrksich, M. *J. Am. Chem. Soc.* **2004**, *126*, 15613.

(22) Collman, J. P.; Devaraj, N. K.; Eberspacher, T. P. A.; Chidsey, C. E. D. *Langmuir* **2006**, *22*, 2457.

(23) Kazakov, S. A.; Astashkina, T. G.; Mamaev, S. V.; Vlassov, V. V. *Nature* **1988**, *335*, 186.

(24) Ciampi, S.; Le Saux, G.; Harper, J. B.; Gooding, J. J. *Electroanalysis* **2008**, *20*, 1513.

(25) (a) Liu, F.; Liang, Y.; Houk, K. N. *J. Am. Chem. Soc.* **2014**, *136*, 11483. (b) Karver, M. R.; Weissleder, R.; Hilderbrand, S. A. *Bioconjugate Chem.* **2011**, *22*, 2263.

(26) (a) Dill, K.; Ghindilis, A.; Schwarzkopf, K. *Lab Chip* **2006**, *6*, 1052. (b) Akter, R.; Rahman, M. A.; Rhee, C. K. *Anal. Chem.* **2012**, *84*, 6407. (c) Yoon, H. C.; Yang, H.; Kim, Y. T. *Analyst* **2002**, *127*, 1082.

(27) Graham, D. M. *Mater. Matters (Milwaukee, WI, U. S.)* **2006**, *1*, 18.

(28) Hobara, D.; Uno, Y.; Kakiuchi, T. *Phys. Chem. Chem. Phys.* **2001**, *3*, 3437.

(29) (a) Hodneland, C. D.; Mrksich, M. *J. Am. Chem. Soc.* **2000**, *122*, 4235. (b) Sigal, G. B.; Mrksich, M.; Whitesides, G. M. *J. Am. Chem. Soc.* **1998**, *120*, 3464. (c) Yeo, W.-S.; Hodneland, C. D.; Mrksich, M. *ChemBioChem* **2001**, *2*, 590.

DOI: 10.1021/jacs.5b03371
J. Am. Chem. Soc. 2015, 137, 8876−8879