UNIVERSITÀ DI SIENA 1240

USiena air

Platinum Nanozyme-Enabled Colorimetric Determination of Total Antioxidant Level in Saliva

This is the peer reviewed version of the following article:

Original:

Pedone, D., Moglianetti, M., Lettieri, M., Marrazza, G., Pompa, P.P. (2020). Platinum Nanozyme-Enabled Colorimetric Determination of Total Antioxidant Level in Saliva. ANALYTICAL CHEMISTRY, 92(13), 8660-8664 [10.1021/acs.analchem.0c01824].

Availability:

This version is availablehttp://hdl.handle.net/11365/1278579 since 2024-11-20T14:40:52Z

Published:

DOI:10.1021/acs.analchem.0c01824

Terms of use:

Open Access

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. Works made available under a Creative Commons license can be used according to the terms and conditions of said license.
For all terms of use and more information see the publisher's website.

(Article begins on next page)

29 November 2024

analytical chemistry

pubs.acs.org/ac Letter

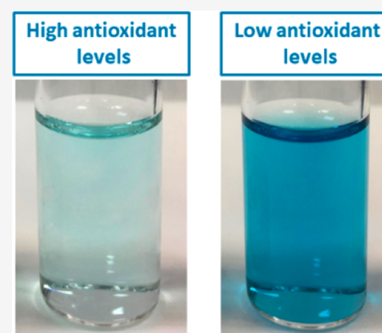
Platinum Nanozyme-Enabled Colorimetric Determination of Total Antioxidant Level in Saliva

Deborah Pedone,[⊥] Mauro Moglianetti,[*,⊥] Mariagrazia Lettieri, Giovanna Marrazza, and Pier Paolo Pompa[*]

Cite This: https://dx.doi.org/10.1021/acs.analchem.0c01824 Read Online

ACCESS |
Metrics & More |
Article Recommendations |
Supporting Information

ABSTRACT: Redox imbalance and oxidative stress-related biomarkers are raising increasing consensus in the scientific community for their significant role in a wide range of human disorders. In this framework, the total antioxidant capacity (TAC), namely, the overall pattern of both enzymatic and nonenzymatic antioxidant compounds within the body, represents an important bioanalytical parameter. To date, however, antioxidant assays require costly instrumentations, laboratory setups, and reagents, and they are invasive. Yet, their accuracy typically suffers from strong sensitivity to interfering matrices and inability to detect the complete pattern of physiological antioxidant molecules, due to the use of reaction schemes and probes/substrates that are not sensitive to the diverse range of relevant target species. Here, we exploit the enzyme-mimetic properties of platinum nanoparticles combined with hydroxyl radical probes produced at the particle surface to develop an effective detection scheme that is sensitive to both single electron transfer (SET) and hydrogen atom transfer (HAT) reactions, thus covering all the physiologically relevant antioxidant species. Importantly, the nanozyme-enabled method allows fast (5 min), accurate, and noninvasive evaluation of the body TAC through saliva via simple naked-eye or smartphone-based inspection.



Downloaded via 93.70.231.123 on June 13, 2020 at 12:41:42 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

The increasing exposure to environmental pollutants, cigarette smoke, and UV radiations, along with drug abuse and stressful daily routine may alter redox homeostasis, one of the most important balances for our well-being and health.[1,2] The equilibrium between free radicals (reactive oxygen species, ROS, and reactive nitrogen species, RNS) and antioxidants in the body plays a key role in maintaining cell functionality and integrity.[1,2] On the other hand, ROS/RNS overproduction, as well as a decrease of antioxidant levels, have been recently related to oxidative stress, premature aging, and onset of a wide variety of disorders.[1−3] Total antioxidant capacity (TAC) is emerging as an important oxidative stress-related biomarker for monitoring the health status of organisms. TAC includes the overall pattern of both enzymatic and nonenzymatic endogenous antioxidant compounds able to scavenge ROS and RNS.[4,5] In recent years, several assays for measuring TAC in biological fluids have found commercial application.[6−9] They evaluate the ability of antioxidant molecules to (i) quench or reduce radical substrates (indirect methods), (ii) reduce metal ions (methods based on inhibited autoxidation), and (iii) competitively block fluorogenic or chromogenic radical probes (methods based on the competitive probe reaction).[6] Even though some of these assays have already been introduced in clinical analyses to measure antioxidant levels in blood, they present some limitations in measuring TAC. Indeed, some of them are inappropriate from a physicochemical perspective,[6] as they are not sensitive to thiol-containing molecules[7] and/or do not involve biologically relevant radicals in the measurements[8] and thus do not provide a reliable evaluation of all the physiological antioxidant species forming the body TAC. Moreover, they are instrumental and time consuming and require costly and unstable reagents and specialized personnel and equipped laboratories, and they are invasive.[7,10] All of these features impair their frequent and accurate application.

Recently, due to their low cost and rapidity, colorimetric methods based on nanomaterials have been proposed in diagnostics as an alternative to traditional approaches.[11−13] In this respect, the ability of antioxidants to modulate the size or aggregation of gold nanoparticles (AuNPs) and, consequently, their optical properties has been exploited to develop new colorimetric biosensors.[14,15] Other methods were based on the growth inhibition of AuNPs by hydrogen peroxide (H_2O_2)[16] or on the color change of ceria nanoparticles upon incubation with antioxidant solutions.[10] In this work, we report the development of a rapid and portable colorimetric assay for the naked-eye or smartphone-based evaluation of the TAC of

Received: April 28, 2020
Accepted: June 2, 2020
Published: June 2, 2020

 © XXXX American Chemical Society

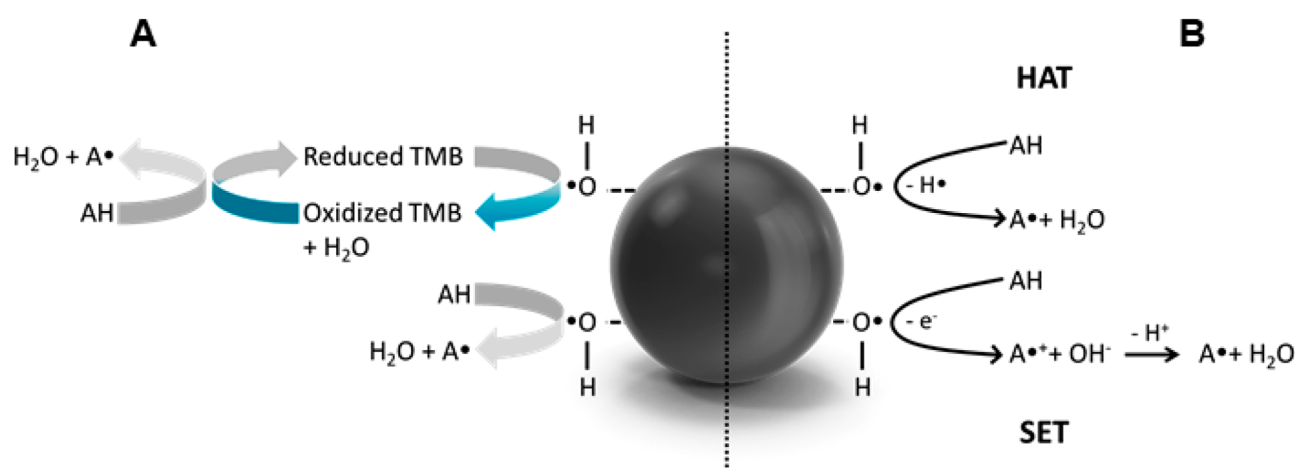


Figure 1. Working mechanism of Pt nanozyme-based colorimetric test for measurement of antioxidant species. (A) Scheme describes the competitive reaction of the TMB chromogenic substrate and antioxidants (AH) with the $OH^{\bullet}$ radical formed at the particle surface. This produces oxidized colored TMB, with an absorbance signal inversely proportional to the AH concentration (direct reaction of AH and oxidized TMB can also occur). (B) Scheme on the right shows that the combined nanozyme−$OH^{\bullet}$ system can promote both single electron transfer (SET) and hydrogen atom transfer (HAT) reactions, thus covering all the physiologically relevant antioxidant species.

organisms through a small sample of saliva, exploiting the nanozyme functionality of platinum nanoparticles (PtNPs).[17]

Our approach allows overcoming current technological limitations (costs, stability, and portability) arising from the use of biological enzymes, such as peroxidase, thanks to the high efficiency and stability of the Pt nanocatalysts in a wide range of pH and temperature conditions, as well as their insensitivity to proteases.[13,17,18] In particular, the proposed method is based on the reaction between antioxidants and hydroxyl radicals ($OH^{\bullet}$) produced at the surface of PtNPs, enabling the detection of all the single species as well as of the global TAC pattern, representing the complete panel of physiologically relevant antioxidant molecules. The colorimetric test was also applied to the evaluation of the TAC in real samples of saliva from healthy volunteers and validated against standard instrumental techniques.

We designed an innovative approach for the rapid and visual evaluation of the body TAC through saliva. Saliva represents an emerging diagnostic fluid in the field of point-of-care devices, due to the easy and noninvasive sample collection.[19,20] Moreover, a strong correlation between the TAC of saliva and blood has been reported in the literature, with saliva containing all the relevant antioxidant species (see also below).[5,19−22] Our colorimetric method is based on the reaction between antioxidants and H_2O_2 in the presence of Pt nanozymes (Figure S1) as peroxidase mimics and 3,3′,5,5′-tetramethylbenzidine (TMB) as a chromogenic probe (Figure 1A). In particular, a redox reaction catalyzed by PtNPs occurs. First, H_2O_2 is activated, leading to the formation of the $OH^{\bullet}$ radical on the particle surface.[23−26] This is followed by oxidation of the TMB substrate by $OH^{\bullet}$,[25,26] with the release of water and formation of a colored TMB radical, with a consequent color change of the solution from transparent to dark blue in a few minutes. Antioxidants compete with the TMB substrate in the interaction with $OH^{\bullet}$ or reduce the oxidized TMB back[27] (Figure 1A and Figure S2), leading to a concentration-dependent color change of the solution. The color intensity is thus indirectly proportional to the level of antioxidants, so the assay can be applied for the naked-eye measurement of the antioxidant level of the sample. As a proof of principle, time-dependent absorbance curves recorded in the absence or in the presence of a well-known antioxidant compound (i.e., glutathione, GSH) are reported in Figure 2. While in the

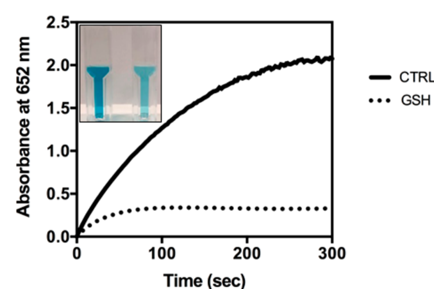


Figure 2. Time-dependent absorbance signals at 652 nm of TMB after incubation with platinum nanozymes and H_2O_2 in the presence of water (CTRL) or a 14 mM glutathione (GSH) aqueous solution. Inset: effect of the presence of antioxidants on the colorimetric response of the sensor. In the presence of antioxidants, the color intensity decreases as a function of the antioxidant molecule concentration.

control sample the solution turns into dark blue in 5 min, due to the complete oxidation of TMB, a pale blue color is observed upon incubation with GSH. Importantly, the proposed strategy based on the combination of Pt nanozymes and $OH^{\bullet}$ radical substrates allows detection of both single electron transfer (SET) and hydrogen atom transfer (HAT) redox reactions, unlike several standard methods (Figure 1B). This is a crucial feature to enable measurement of all the antioxidant compounds that are physiologically relevant, namely, both HAT working species, such as thiol-containing molecules (e.g., glutathione, cysteine), and electron-donating SET species (e.g., uric acid), which all together contribute to the body TAC.

The ability of the Pt nanozyme-based assay to detect the wide variety of antioxidant species was demonstrated by probing the antioxidant molecules that primarily constitute the TAC, namely, glutathione (GSH), cysteine (Cys), homocysteine (Hcy), thiocyanate (KSCN), uric acid (UA), ascorbic acid (AA), and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, the hydrosoluble analogue of vitamin

https://dx.doi.org/10.1021/acs.analchem.0c01824
Anal. Chem. XXXX, XXX, XXX−XXX

E). All the measurements were performed at room temperature, recording the absorbance of the solution 5 min after incubation. As reported in Figure 3A and Figure S3, all the

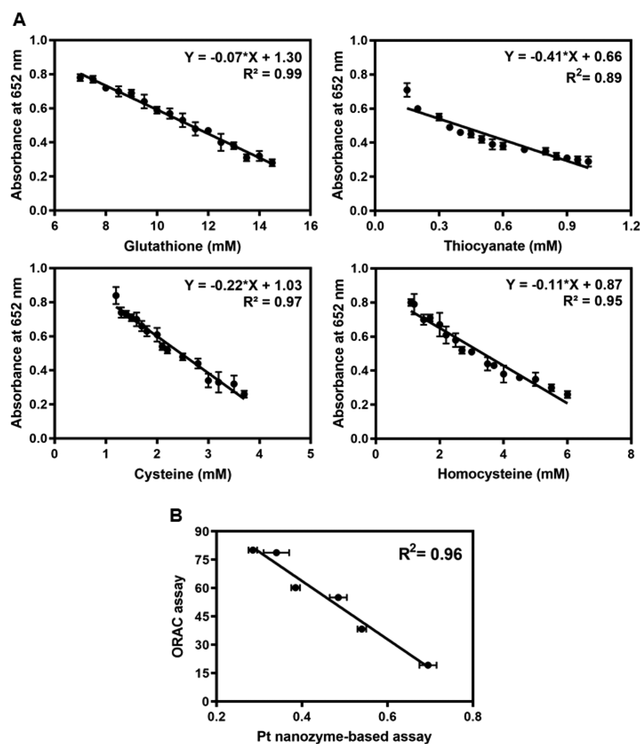


Figure 3. (A) Absorbance signal of the Pt nanozyme-based antioxidant assay as a function of different antioxidant molecules. (B) Validation of the colorimetric test versus the standard ORAC assay, probed for the TAC in samples of artificial saliva.

tested species showed a concentration-dependent colorimetric readout, indicating that the proposed nanozyme-based assay can efficiently detect all the relevant antioxidant compounds (both SET and HAT reactions). We also assessed the ability of the assay to detect antioxidant enzymes (e.g., catalase), and the test was proven to work efficiently (Figure S3). Notably, in the experimental conditions optimized herein, we observed a linear correlation between the absorbance intensity and the antioxidant concentration (with R^2 values always higher than 0.89), in the range of about 0.2−0.8 absorbance units, namely, in an optical window easy to be detected by the naked eye. The detection limit was specific for each antioxidant compound, ranging from 0.012 mM for catalase to 32 mM for uric acid, in such optical window. As anticipated, we exploited a PtNP concentration that magnifies the test response, allowing a visual determination of the TAC. On the other hand, the test is highly versatile, as the limit of detection and the analyte concentration ranges could be modified by simply changing the concentration of the artificial enzyme as well as of the other reagents.

Some traditional antioxidant tests are only able to detect antioxidants acting through electron transfer, and hence, they poorly perform in determining the amount of thiolated antioxidants (Figure S4(A,B)).[7,8] Indeed, the reaction between antioxidants and free radicals occurs by either a SET or HAT process, depending on the antioxidant chemical structure and physicochemical properties, such as redox potential, polarity, solubility, and hydrophobic/hydrophilic character.[7,9,28] As thiols mainly react by the HAT mechanism in acidic environments, SET-based methods are poorly sensitive to their presence.[7,8] However, from a biological point of view, thiol-containing molecules represent the first class of antioxidant compounds consumed within the body by free radicals, so changes in their levels may be indicative of the onset of oxidative stress conditions.[29]

We observed different reaction kinetics for each antioxidant compound (Figure S5). Radical chain-breaker antioxidants, such as AA and Trolox, instantaneously react with $OH^\bullet$,[6,8,30] causing a complete inhibition of the TMB oxidation in the early phase (for a period time that depends on the antioxidant concentration), followed by a rapid absorbance increase. On the contrary, UA and thiol-containing scavengers only cause a partial inhibition of TMB oxidation compared to the control, due to their relatively slower reaction rate.[6,8] As a consequence, the proposed method is characterized by high versatility. Indeed, while punctual measurements at a given time allow titrating the total antioxidant content of a sample, a kinetic investigation might provide information about its antioxidant species, in terms of reactivity toward radicals.[6]

The analytical performance of the assay was evaluated by measuring intraday and interday precision (repeatability and reproducibility, respectively). We recorded the same absorbance responses repeating the measurement six times for the same sample (Figure S6A) or using 10 samples prepared on different days (Figure S6B), obtaining a relative standard deviation of ca. 6% for both the experiments. Moreover, to validate the nanozyme-based antioxidant test, the response of the sensor to the physiological range of TAC was analyzed in artificial saliva samples, obtained modulating antioxidant levels in a wide range,[31−35] as reported in Table S1. Figure S7A shows a clear concentration-dependent optical response of the assay as a function of the TAC, confirming its ability to detect the total panel of physiologically important antioxidant molecules. The colorimetric test was also evaluated in comparison to a standard instrumental technique based on fluorescence measurements (ORAC test, see Figure S7B). Interestingly, the colorimetric nanozyme-based assay showed excellent agreement with the reference test, with a correlation R^2 of 0.96 (Figure 3B).

The colorimetric method was then applied to analyze real samples of saliva. As a preliminary control experiment, we verified that the test results are not significantly affected by the complex biological matrix (Figure S8). In particular, a test performed in a saliva sample supplemented with an increasing amount of KSCN confirmed the concentration-dependent colorimetric response, proving the possibility to use the method also in this biological fluid without major interferences. Hence, the test was exploited to measure the saliva TAC from 83 healthy volunteers.

As shown in Figure 4, the optimized conditions of the nanozyme-based assay allow obtaining a clear colorimetric characterization of the different TAC levels in a wide range of absorbance (ca. 0.2−0.8, in line with the previous TAC measurement in simulated saliva samples, see Figure S7A). In particular, the donors' TAC was characterized by a broad Gaussian distribution centered around 0.5 absorbance, consistent with the corresponding average value of salivary TAC (1.20 mM, see Figure S7A and Table S1).[34] A fraction (ca. 30%) of the tested population fell in the regions of high or low TAC, the lower TAC level suggesting onset of oxidative stress (15% population). Interestingly, such wide colorimetric

https://dx.doi.org/10.1021/acs.analchem.0c01824
Anal. Chem. XXXX, XXX, XXX−XXX

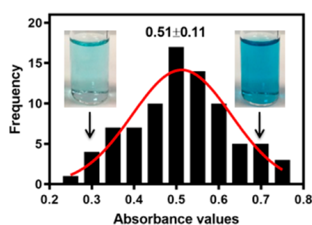


Figure 4. TAC levels of saliva samples collected from 83 healthy volunteers, aged between 20 and 50 years, measured by the nanozyme-based assay. Insets: representative photographs of two vials corresponding to different levels of salivary antioxidants (0.3 and 0.7 absorbance), showing a clear visual readout.

response can be also evaluated by simple visual inspection, thus enabling a fast and instrument-free semiquantitative assessment of this interesting biomarker (see photos in the insets of Figure 4).

At the same time, a quantitative portable sensor for TAC measurements can be obtained by a smartphone-based readout. To this aim, we used a commercially available smartphone app. As a proof of principle, we performed hue, saturation, and lightness (HSL) analysis on captured images, quantifying the color change of the solution in the presence of different amounts of GSH (Figure 5). As shown, the

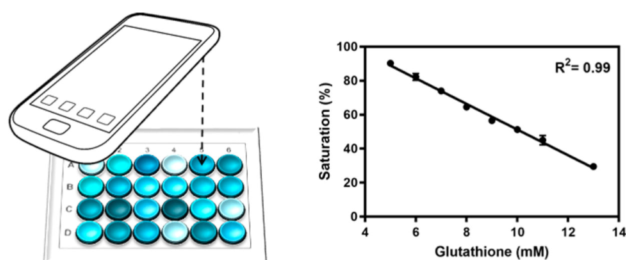


Figure 5. Smartphone-based measurement of antioxidant levels. Colorimetric response of the Pt nanozyme-based assay measured as saturation percentage by hue, saturation, and lightness (HSL) analysis on captured images versus glutathione concentration.

antioxidant compound elicited a distinct color decrease in the solution as a function of its concentration. Remarkably, plotting S values obtained by HSV analysis against GSH concentration, we observed an excellent linear relationship, with an R^2 value of 0.99, in close agreement with spectral analysis in terms of accuracy and precision. Accuracy, rapidity, easy of use, and low cost make the proposed colorimetric analysis approach a valid alternative to laboratory methods, enabling also unspecialized people to measure their body TAC.

In conclusion, the method described in this letter combines the advantages of the instrumental antioxidant tests while overcoming their major limitations. This is due to the combination of PtNPs as artificial enzymes and the reactivity of the $OH^{\bullet}$ radical formed at the particle surface to measure both SET and HAT reactions as well as the global TAC pattern.[8] The assay was optimized to give a wide colorimetric response, allowing straightforward evaluation by naked-eye or smartphone-based quantitative analysis. The test can be performed in 5 min at room temperature, allowing the same accuracy as instrumental techniques. The possibility to use the nanozyme-based test on real saliva samples was demonstrated by analyzing the TAC of 83 donors, envisaging the possibility to exploit it for frequent noninvasive screenings as well as in specialized studies deepening the role of TAC biomarkers in a wide range of sectors, including sports performance, nutrition, wellness, aging, and others.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.0c01824.

Additional figures and table for characterization of PtNPs and of the colorimetric reaction and Materials and Methods (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Mauro Moglianetti − *Istituto Italiano di Tecnologia, Nanobiointeractions & Nanodiagnostics, 16163 Genova, Italy; Istituto Italiano di Tecnologia, Nanobiointeractions & Nanodiagnostics, Center for Bio-Molecular Nanotechnologies, 73010 Arnesano, Lecce, Italy;* orcid.org/0000-0003-0747-7963; Email: mauro.moglianetti@iit.it

Pier Paolo Pompa − *Istituto Italiano di Tecnologia, Nanobiointeractions & Nanodiagnostics, 16163 Genova, Italy; Istituto Italiano di Tecnologia, Nanobiointeractions & Nanodiagnostics, Center for Bio-Molecular Nanotechnologies, 73010 Arnesano, Lecce, Italy;* orcid.org/0000-0001-7549-0612; Email: pierpaolo.pompa@iit.it

Authors

Deborah Pedone − *Istituto Italiano di Tecnologia, Nanobiointeractions & Nanodiagnostics, 16163 Genova, Italy; Istituto Italiano di Tecnologia, Nanobiointeractions & Nanodiagnostics, Center for Bio-Molecular Nanotechnologies, 73010 Arnesano, Lecce, Italy*

Mariagrazia Lettieri − *Dipartimento di Chimica Ugo Schiff, 50019 Sesto Fiorentino, Fi, Italy*

Giovanna Marrazza − *Dipartimento di Chimica Ugo Schiff, 50019 Sesto Fiorentino, Fi, Italy; Istituto Nazionale Biostrutture e Biosistemi, 00136 Roma, Italy*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.analchem.0c01824

Author Contributions

The manuscript was written through contributions of all authors.

Author Contributions

⊥Deborah Pedone and Mauro Moglianetti contributed equally.

Funding

This work has been partially supported by ASI MARS-PRE Project.

Notes

The authors declare the following competing financial interest(s): Mauro Moglianetti and Pier Paolo Pompa have a patent application on this technology.

■ ACKNOWLEDGMENTS

We are grateful to Mariada Malvindi, Enrico Binetti, Vito Maggi, and Roberto Fiammengo for their help.

■ REFERENCES

(1) Nathan, C.; Cunningham-Bussel, A. *Nat. Rev. Immunol.* **2013**, *13* (5), 349−361.

https://dx.doi.org/10.1021/acs.analchem.0c01824
Anal. Chem. XXXX, XXX, XXX−XXX

(2) Marrocco, I.; Altieri, F.; Peluso, I. *Oxid. Med. Cell. Longevity* **2017**, *2017*, 32.
(3) Tartaglia, G. M.; Gagliano, N.; Zarbin, L.; Tolomeo, G.; Sforza, C. *Arch. Oral Biol.* **2017**, *78*, 34−38.
(4) Ziobro, A.; Bartosz, G. *Cell. Mol. Biol. Lett.* **2003**, *8*, 415−419.
(5) Wang, J.; Schipper, H. M.; Velly, A. M.; Mohit, S.; Gornitsky, M. *Free Radical Biol. Med.* **2015**, *85*, 95−104.
(6) Amorati, R.; Valgimigli, L. *Free Radical Res.* **2015**, *49* (5), 633−649.
(7) Prior, R. L.; Wu, X.; Schaich, K. *J. Agric. Food Chem.* **2005**, *53* (10), 4290−4302.
(8) Apak, R.; Güçlü, K.; Demirata, B.; Ozyürek, M.; Celik, S. E.; Bektaşoğlu, B.; Berker, K. I.; Ozyurt, D. *Molecules* **2007**, *12* (7), 1496−1547.
(9) Huang, D.; Ou, B.; Prior, R. L. *J. Agric. Food Chem.* **2005**, *53* (6), 1841−1856.
(10) Sharpe, E.; Frasco, T.; Andreescu, D.; Andreescu, S. *Analyst* **2013**, *138* (1), 249−262.
(11) Nitinaivinij, K.; Parnklang, T.; Thammacharoen, C.; Ekgasit, S.; Wongravee, K. *Anal. Methods* **2014**, *6* (24), 9816−9824.
(12) Bülbül, G.; Hayat, A.; Andreescu, S. *Sensors* **2015**, *15* (12), 30736−30758.
(13) Wang, X.; Hu, Y.; Wei, H. *Inorg. Chem. Front.* **2016**, *3* (1), 41−60.
(14) Scampicchio, M.; Wang, J.; Blasco, A. J.; Sanchez Arribas, A.; Mannino, S.; Escarpa, A. *Anal. Chem.* **2006**, *78* (6), 2060−2063.
(15) Basu, S.; Ghosh, S. K.; Kundu, S.; Panigrahi, S.; Praharaj, S.; Pande, S.; Jana, S.; Pal, T. *J. Colloid Interface Sci.* **2007**, *313* (2), 724−734.
(16) Ma, X.; Li, H.; Dong, J.; Qian, W. *Food Chem.* **2011**, *126* (2), 698−704.
(17) Pedone, D.; Moglianetti, M.; De Luca, E.; Bardi, G.; Pompa, P. P. *Chem. Soc. Rev.* **2017**, *46* (16), 4951−4975.
(18) Wu, J.; Wang, X.; Wang, Q.; Lou, Z.; Li, S.; Zhu, Y.; Qin, L.; Wei, H. *Chem. Soc. Rev.* **2019**, *48* (4), 1004−1076.
(19) Ngamchuea, K.; Chaisiwamongkhol, K.; Batchelor-McAuley, C.; Compton, R. G. *Analyst* **2018**, *143* (1), 81−99.
(20) Nunes, L. A. S.; Mussavira, S.; Bindhu, O. S. *Biochem Med. (Zagreb)* **2015**, *25* (2), 177−192.
(21) Astaneie, F.; Afshari, M.; Mojtahedi, A.; Mostafalou, S.; Zamani, M. J.; Larijani, B.; Abdollahi, M. *Arch. Med. Res.* **2005**, *36* (4), 376−381.
(22) Atsumi, T.; Iwakura, I.; Kashiwagi, Y.; Fujisawa, S.; Ueha, T. *Antioxid. Redox Signaling* **1999**, *1* (4), 537−546.
(23) Lin, S.; Zheng, D.; Li, A.; Chi, Y. *Anal. Bioanal. Chem.* **2019**, *411* (18), 4063−4071.
(24) Katsounaros, I.; Schneider, W. B.; Meier, J. C.; Benedikt, U.; Biedermann, P. U.; Auer, A. A.; Mayrhofer, K. J. J. *Phys. Chem. Chem. Phys.* **2012**, *14* (20), 7384−7391.
(25) Ma, M.; Zhang, Y.; Gu, N. *Colloids Surf., A* **2011**, *373* (1), 6−10.
(26) Wang, Z.; Yang, X.; Feng, J.; Tang, Y.; Jiang, Y.; He, N. *Analyst* **2014**, *139* (23), 6088−6091.
(27) Liu, X.; Wang, Q.; Zhang, Y.; Zhang, L.; Su, Y.; Lv, Y. *New J. Chem.* **2013**, *37* (7), 2174−2178.
(28) Müller, L.; Fröhlich, K.; Böhm, V. *Food Chem.* **2011**, *129* (1), 139−148.
(29) Balcerczyk, A.; Grzelak, A.; Janaszewska, A.; Jakubowski, W.; Koziol, S.; Marszalek, M.; Rychlik, B.; Soszynski, M.; Bilinski, T.; Bartosz, G. *BioFactors* **2003**, *17* (1−4), 75−82.
(30) Alberto, M. E.; Russo, N.; Grand, A.; Galano, A. *Phys. Chem. Chem. Phys.* **2013**, *15* (13), 4642−4650.
(31) Chandler, J. D.; Day, B. J. *Biochem. Pharmacol.* **2012**, *84* (11), 1381−1387.
(32) Moore, S.; Calder, K. A. C.; Miller, N. J.; Rice-Evans, C. A. *Free Radical Res.* **1994**, *21* (6), 417−425.
(33) Bald, E.; Głowacki, R. *Amino Acids* **2005**, *28*, 431−3.
(34) Benedetti, S.; Primiterra, M.; Finco, A.; Canestrari, F.; Cornelli, U. *Clin. Lab.* **2014**, *60*, 475−482.
(35) Sobaniec, H.; Sobaniec, W.; Sendrowski, K.; Sobaniec, S.; Pietruska, M. *Adv. Med. Sci.* **2007**, *52* (Suppl 1), 204−206.

https://dx.doi.org/10.1021/acs.analchem.0c01824
Anal. Chem. XXXX, XXX, XXX−XXX