Research Article

Ratiometric Sensing of Hydrogen Peroxide Utilizing Conformational Change in Fluorescent Boronic Acid Polymers

Kan Takeshima,¹ Kanako Mizuno,¹ Hitoshi Nakahashi,¹
Hiroshi Aoki,² and Yasumasa Kanekiyo¹

¹Department of Biotechnology and Environmental Chemistry, Kitami Institute of Technology, 165 Koen-cho, Kitami, Hokkaido 090-8507, Japan

²National Institute of Advanced Industrial Science and Technology (AIST), 16-1 Onogawa, Tsukuba, Ibaraki 305-8569, Japan

Correspondence should be addressed to Yasumasa Kanekiyo; kanekiyo@mail.kitami-it.ac.jp

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We demonstrate that the copolymers containing boronic acid and pyrene units can be utilized for the fluorometric sensing of hydrogen peroxide (H_2O_2) in aqueous solutions. The copolymer exists in a relatively extended conformation in the absence of H_2O_2 , whereas the polymer chain is contracted by the reaction of boronic acid moieties with H_2O_2 to form phenol groups. This conformational change induces aggregation of the originally isolated pyrene groups. As a result, relative intensity of excimer emission with respect to monomer emission increases with H_2O_2 concentration. Accordingly, the present methodology enables us to measure H_2O_2 by means of ratiometric fluorescence change in the range of 0–30 μM .

1. Introduction

Boronic acids are known to form boronate esters with saccharides through reversible boronic acid-diol interactions (Scheme 1(a)) [1, 2]. By utilizing this nature, a variety of saccharide-sensing systems have been developed [3–5]. Boronic acids are also known to react with reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) to generate the corresponding phenol derivatives (Scheme 1(b)) [6–12]. This reaction has been utilized for creating colorimetric [13–15] and fluorescent [16–20] probes for sensing H_2O_2 by directly connecting boronic acid moiety with chromophoric or fluorophoric compounds. When these probes react with H_2O_2 , spectrophotometric changes are induced due to the alteration of the electronic state within the probes caused by the conversion of boronic acid moiety into phenol group. The boronic acid-based H_2O_2 probes are advantageous over the conventional enzyme-based sensors [21] in terms of durability and reproducibility because they do not use unstable bioorganic substances. One of the drawbacks of the boronic acid-based method may be the necessity of complicated organic synthesis for obtaining the probes.

Our group has been developing saccharide-sensing systems utilizing polymeric boronic acids. Previously, we had reported a novel saccharide detection strategy utilizing saccharide-induced conformational changes in fluorescent boronic acid polymers, which was synthesized from a radical copolymerization of a boronic acid monomer and a pyrene monomer [22]. This polymer takes a relatively contracted conformation in an aqueous solution due to hydrophobic aggregation of pyrene groups. On binding with saccharides, the boronic acid moieties become negatively charged, and the polymer chain changes its conformation to an extended state due to intramolecular electrostatic repulsion between the boronate groups. Thus, the pyrene groups are enforced to be dissociated. These conformational changes can be conveniently detected by monitoring the excimer to monomer intensity ratio in the fluorescence spectra. It occurred to us that a similar mechanism may be applied for developing sensing systems for H_2O_2 . If the charge state of the boronic acid groups in the fluorescent polymer is altered by the reaction with H_2O_2 , the conformation of the polymer chain will also be varied due to change in electrostatic interaction within the polymer. Thus, we expect that the concentration of

SCHEME 1: Reaction of boronic acid with saccharide (a) and hydrogen peroxide (b).

H_2O_2 can be fluorometrically monitored using boronic acid polymers.

Here, we report our result on the endeavor for novel fluorometric sensing method for H_2O_2 . Accordingly, we prepared copolymers containing boronic acid, acrylamide, and pyrene units with different compositions. After the reaction of the polymers with H_2O_2 in aqueous solutions, fluorescence spectral changes of the solutions were measured. As a result, we found that the relative intensity of excimer emission with respect to monomer emission increases with H_2O_2 concentration, and the present methodology enables us to measure H_2O_2 by means of ratiometric fluorescence change in the range of 0–30 μM .

2. Experimental

2.1. Materials and Reagents. Boronic acid monomer (**1**) was synthesized according to the literature procedure [23] by the reaction between 3-aminophenylboronic acid and acryloyl chloride. Acrylamide (**2**) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Pyrene monomer (**3**) was synthesized by a similar procedure described in the literature [24] by reacting 1-aminomethylpyrene and acryloyl chloride. Aqueous hydrogen peroxide (30 wt%) was purchased from Wako. Reagents for preparing buffer solutions [N-cyclohexyl-2-aminoethanesulfonic acid (CHES) and N-cyclohexyl-3-aminopropanesulfonic acid (CAPS)] were supplied from Dojindo Laboratories (Kumamoto, Japan).

2.2. Synthesis of Polymers. The outline for the preparation of the fluorescent boronic acid polymers is illustrated in Scheme 2. The radical copolymerization of **1**, **2**, and **3** was carried out in homogeneous solutions in DMSO/ H_2O mixed solvent in the presence of a polymerization initiator (AIBN) at 70°C for 12 h under nitrogen atmosphere. For the monomer compositions, see Table 1. The obtained copolymers were purified by reprecipitation using acetone as a nonsolvent and then dried under vacuum. NMR spectra of the copolymers are shown in Figures S1–S5 (see Supplementary Material available online at <https://doi.org/10.1155/2017/7829438>). From these spectra, successful introduction of boronic acid

TABLE 1: Monomer compositions of copolymers^a.

Polymer	Monomer (μmol)		
	1	2	3
B-10	800	0	40
B-11	400	400	40
B-12	300	600	45
B-13	200	600	40
B-01	0	800	40

^aSolvent: DMSO 2 mL + H_2O 0.1 mL.

SCHEME 2: Preparation of fluorescent boronic acid polymers.

and pyrene units was ascertained. Compositions of monomer units in the copolymers were estimated by elemental analysis. The observed C/N ratios approximately agreed with the calculated values as shown in Table S1. By using these data, molar ratios of monomer units were estimated (Table S2). It was confirmed that the molar ratios were roughly agreed with compositions in the feed solutions. Molecular weights (Mw) of these copolymers are supposed to be in the range of 13,000–22,000 [22].

FIGURE 1: Fluorescence spectra for aqueous solutions of polymer B-11 (1 mg L^{-1}) buffered at pH 10.9 by 10 mM CAPS at various H_2O_2 concentrations at 25°C . The spectra are normalized at 397 nm. Excitation: 348 nm.

2.3. Measurement of Fluorescence Spectra. The fluorescent boronic acid polymer was dissolved in methanol to be the polymer concentration of 0.1 g L^{-1} . An aqueous solution containing H_2O_2 ($0\text{--}100 \mu\text{M}$) and buffer (10 mM) was prepared and 8 mL of this solution was poured into a screw-capped bottle. To this solution was added $80 \mu\text{L}$ of the polymer solution and stirred at 25°C for 60 min. After that, the fluorescence emission spectra of the solution were recorded using an excitation light at 348 nm. Other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan). The following buffers were used for setting pH of the solutions: 10 mM HEPES (pH 7.4), 10 mM CHES (pH 9.3–10.1), and 10 mM CAPS (pH 10.5–11.3).

2.4. Apparatus. Steady-state fluorescence spectra were recorded on Hitachi F-2500 fluorescence spectrophotometer. UV absorption spectra were obtained by using JASCO V-650 spectrophotometer. pH values were measured using Metrohm 827 pH lab. Aqueous solutions were prepared with distilled water purified by Yamato WG202 system.

3. Results and Discussion

3.1. Fluorometric Response against H_2O_2 . The fluorescence spectroscopic characteristics of the synthesized copolymers were investigated in aqueous buffered solutions containing various concentrations of H_2O_2 . The obtained fluorescence spectra are shown in Figures S6–S15. In the absence of H_2O_2 , polymer B-11 having a 1:1 molar ratio between the boronic acid and acrylamide units showed a broad excimer emission in the range of 430–600 nm in addition to a monomer emission in the range of 370–410 nm (Figure 1). The excimer emission is derived from the dimerization between excited-state and ground state pyrene groups. The excitation spectra were also measured (Figure S16) and it was found that the spectrum monitored at the excimer emission wavelength (486 nm) was remarkably different

FIGURE 2: Visible change of the emission color from the aqueous solutions of polymer B-11 (10 mg L^{-1}) containing or not containing H_2O_2 .

from that monitored at monomer emission wavelength (397 nm). The observed excitation spectra indicate that the pyrene groups form aggregations in the ground state probably due to hydrophobic and π - π stacking interactions [25].

When $1 \mu\text{M}$ of H_2O_2 was added to the solution, the excimer emission was slightly intensified relative to the monomer emission. With increasing H_2O_2 concentration, the relative excimer intensity gradually increased and almost saturated above $30 \mu\text{M}$ as shown in Figure 1. This spectral change accompanied a clearly visible variation in the emission color from the solution as shown in Figure 2. By measuring the corresponding UV absorption spectra (Figure S17), it was found that a new absorption assignable to phenol moiety emerged at around 290 nm with increasing H_2O_2 concentration, and the absorbance leveled off above $30 \mu\text{M}$. To assess the reaction kinetics, time-course of the excimer to monomer intensity ratio was measured with $30 \mu\text{M}$ H_2O_2 (Figure S18). The intensity ratio gradually increased with increasing reaction time and then reached constant after 60 min. These observations indicate that the boronic acid groups were quantitatively converted into phenol moieties when H_2O_2 concentration is larger than $30 \mu\text{M}$, and the reaction of the boronic acid groups with H_2O_2 induced a conformational change in the copolymer from expanded to contracted state. It is calculated that the concentration of boronic acid unit in the measurement solutions of B-11 is ca. $3.5 \mu\text{M}$. (Polymer B-11 was synthesized from 76 mg (0.4 mmol) of **1**, 28.4 mg of **2**, and 11.4 mg of **3**. Supposing that the monomer composition in the copolymer is the same as in the feed solution, the number of moles of boronic acid per unit weight of the copolymer is calculated to be 3.45 mmol g^{-1} . Therefore, concentration of boronic acid unit in the measurement solutions containing 1 mg L^{-1} of B-11 is estimated to be $3.45 \mu\text{mol L}^{-1}$.) However, the detection curve exhibited saturation at much higher H_2O_2 concentration. This means that H_2O_2 does not quantitatively react with the boronic acid unit. Probably the decomposition reaction of H_2O_2 into H_2O and O_2 is competing, and/or the reaction is relatively slow so that the reaction with boronic acid

FIGURE 3: Comparison of the relative change in excimer (486 nm) to monomer (397 nm) emission intensity ratios against reactive oxygen species (30 μ M each) for aqueous solutions of B-11 buffered by 5 mM Na₂CO₃/5 mM NaHCO₃ (pH 10.2).

is uncompleted within 60 min at lower H₂O₂ concentrations.

The reaction of boronic acid with H₂O₂ to be converted into phenol was also confirmed by ¹H NMR measurements. Firstly, phenylboronic acid was used as a model compound (Figure S20). In an alkaline D₂O solution, phenylboronic acid showed signals in the range of 7.6–7.2 ppm. After the reaction with H₂O₂, it showed upfield shifts to 7.2–6.6 ppm that is assignable to the quantitative conversion to phenol. Then, copolymer B-11 was dissolved in alkaline D₂O and NMR spectra were measured in the absence and presence of H₂O₂ (Figure S21). In the absence of H₂O₂, it showed broad peaks assignable to the boronic acid moiety at around 7.2 ppm. In the presence of excess amount of H₂O₂, the peaks disappeared and new peaks attributable to phenol moiety emerged in the range of 7.0–6.2 ppm.

We also examined detection selectivity against reactive oxygen species [26]. In these cases, measurements were conducted at pH 10.2 with sodium carbonate buffer instead of Good's buffer to avoid causing side reactions of reactive oxygen species with buffer. As the results shown in Figure 3, hypochlorite induced change in the ratio of excimer to monomer emission intensities that is much smaller than the case of H₂O₂. Also, t-butyl hydroperoxide (TBHP) showed nearly negligible response.

3.2. Effect of pH. We further investigated the effect of pH on the responsiveness towards H₂O₂. Figure 4 shows the pH dependency of the excimer to monomer intensity ratio in the absence and presence (30 μ M) of H₂O₂. At pH 7.4, there was no significant difference of intensity ratios in the absence and presence of H₂O₂. Unfortunately, further decrease in pH resulted in precipitation of the copolymer due to lack of boronate negative charges at acidic conditions. In the absence of H₂O₂, the relative intensity of excimer emission was almost constant with the increase in pH and then decreased when pH exceeded 10. The decrease in the excimer intensity may be due the extension of the polymer

FIGURE 4: Ratio of excimer (486 nm) to monomer (397 nm) emission intensities for aqueous solutions of polymer B-11 containing (30 μ M) or not containing H₂O₂ as a function of pH at 25°C.

chain that was induced by the ionization of the boronic acid groups into negatively charged boronate groups [27] by which electrostatic repulsion was created within the polymer chain. This idea is supported by the study made by Chandar et al. on the conformations of a pyrene-labeled poly(acrylic acid) using fluorescence of the excimer/monomer emissions. They found that the polymer changed from coiled to extended form with increasing pH [28]. In the presence of 30 μ M of H₂O₂, the relative excimer intensity markedly increased with the increase in pH until 10.9, and it later decreased at pH 11.3. The increase in the excimer intensity suggests that the polymer chain bearing phenol moieties is contracted with the increase in pH. The phenol groups are expected to be partially dissociated at around pH 10 since pK_a values of monomeric phenols are typically around 10. We therefore speculated that the contraction was caused by the hydrogen bonding between the dissociated phenolate group and the undissociated phenol group [29]. This bonding should be stronger than the hydrogen bonding between phenols only since stronger electrostatic interaction occurs.

To confirm whether the above-mentioned interpretation is appropriate, effect of ionic strength on the response against H₂O₂ was evaluated (Figure S19). The response was largely suppressed in the presence of NaCl, which is attributable to disruption of the electrostatic interaction at the higher ionic strength. This result supports the view that the electrostatic attraction between phenol and phenolate is important for the response. The further increase in pH converts the undissociated phenol groups into dissociated phenolate groups, resulting in the creation of electrostatic repulsion between the negatively charged phenolate groups. Thus, the extension of the polymer chain is induced and the excimer emission

SCHEME 3: Conformational change of the fluorescent boronic acid polymer in response to H_2O_2 .

FIGURE 5: Relative change in excimer (486 nm) to monomer (397 nm) emission intensity ratios as a function of H_2O_2 concentrations for aqueous solutions of the polymers buffered by 10 mM CAPS (pH 10.9).

intensity decreases. According to these results, it was found that the H_2O_2 -responsive change in the fluorescence intensity ratio is maximal at pH 10.9.

3.3. Effect of Monomer Composition. In order to evaluate the effect of boronic acid density on the polymer chain, copolymers having different monomer compositions (Table 1) were used. To compare responses of these polymers towards H_2O_2 , the magnitude of increase in the excimer to monomer intensity ratio was plotted against H_2O_2 concentration, as shown in Figure 5. When compared with the response of polymer B-11, it is apparent that the decrease in the boronic acid

content resulted in depression of the change in the excimer to monomer emission intensity ratios. Polymer B-12, which has a 1:2 molar ratio between the boronic acid and acrylamide units, exhibited a slightly diminished response. Moreover, polymer B-13 showed a further diminished response. In the case of polymer B-01, which does not have boronic acid unit, no virtual fluorescence response was induced. These observations clearly support the view that the existence of boronic acid group is indispensable for the occurrence of the responsiveness, and the reaction with H_2O_2 causes conformational alterations in the copolymer, resulting in the fluorescence spectral changes. Unexpectedly, polymer B-10, which has the highest boronic acid content, showed a much lower response as compared to polymer B-11. Polymer B-10 mainly consists of boronic acid units with a small content of pyrene units and no acrylamide unit. Since the boronic acid unit is sterically bulkier than acrylamide unit, the conformational flexibility of the polymer chain should be relatively restricted due to the steric hindrance between neighboring boronic acid moieties. This could be the reason for the suppression of response against H_2O_2 .

4. Conclusions

In conclusion, we established that the copolymers containing boronic acid and pyrene units can be utilized for the fluorometric sensing of H_2O_2 . The copolymer exists in a relatively extended conformation in the absence of H_2O_2 , whereas the polymer chain is contracted by the reaction of the boronic acid moieties with H_2O_2 . This conformational change induces aggregation of the originally isolated pyrene groups as illustrated in Scheme 3. As a result, relative intensity of excimer emission with respect to monomer emission increases with H_2O_2 concentration. Accordingly, the present methodology enables us to measure H_2O_2 by means of

ratiometric fluorescence changes in the range of 0–30 μM with the detection limit of 1 μM .

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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References

- [1] T. D. James, M. D. Philips, and S. Shinkai, *Boronic Acids in Saccharide Recognition*, RSC Publishing, Cambridge, UK, 2006.
- [2] Y. Kanekiyo and S. Shinkai, "CHAPTER 1: Supramolecular chemistry of boronic acids," *Monographs in Supramolecular Chemistry*, vol. 2016-, no. 16, pp. 1–43, 2016.
- [3] Z. Guo, I. Shin, and J. Yoon, "Recognition and sensing of various species using boronic acid derivatives," *Chemical Communications*, vol. 48, no. 48, pp. 5956–5967, 2012.
- [4] X. Wu, Z. Li, X.-X. Chen, J. S. Fossey, T. D. James, and Y.-B. Jiang, "Selective sensing of saccharides using simple boronic acids and their aggregates," *Chemical Society Reviews*, vol. 42, no. 20, pp. 8032–8048, 2013.
- [5] X. Sun and T. D. James, "Glucose Sensing in Supramolecular Chemistry," *Chemical Reviews*, vol. 115, no. 15, pp. 8001–8037, 2015.
- [6] H. G. Kuivila, "Electrophilic displacement reactions. III. Kinetics of the reaction between hydrogen peroxide and benzenboronic acid," *Journal of the American Chemical Society*, vol. 76, no. 3, pp. 870–874, 1954.
- [7] H. G. Kuivila and A. G. Armour, "Electrophilic displacement reactions. IX. Effects of substituents on rates of reactions between hydrogen peroxide and benzenboronic acid," *Journal of the American Chemical Society*, vol. 79, no. 21, pp. 5659–5662, 1957.
- [8] J. Zielonka, A. Sikora, M. Hardy, J. Joseph, B. P. Dranka, and B. Kalyanaraman, "Boronate probes as diagnostic tools for real time monitoring of peroxynitrite and hydroperoxides," *Chemical Research in Toxicology*, vol. 25, no. 9, pp. 1793–1799, 2012.
- [9] K. Sato, E. Abe, M. Takahashi, and J.-I. Anzai, "Loading and release of fluorescent dye from layer-by-layer film-coated magnetic particles in response to hydrogen peroxide," *Journal of Colloid and Interface Science*, vol. 432, pp. 92–97, 2014.
- [10] K. Sato, M. Takahashi, M. Ito, E. Abe, and J.-I. Anzai, "H₂O₂-induced decomposition of layer-by-layer films consisting of phenylboronic acid-bearing poly(allylamine) and poly(vinyl alcohol)," *Langmuir*, vol. 30, no. 31, pp. 9247–9250, 2014.
- [11] X. Sun, Q. Xu, G. Kim et al., "A water-soluble boronate-based fluorescent probe for the selective detection of peroxynitrite and imaging in living cells," *Chemical Science*, vol. 5, no. 9, pp. 3368–3373, 2014.
- [12] T. Yoshii, S. Onogi, H. Shigemitsu, and I. Hamachi, "Chemically reactive supramolecular hydrogel coupled with a signal amplification system for enhanced analyte sensitivity," *Journal of the American Chemical Society*, vol. 137, no. 9, pp. 3360–3365, 2015.
- [13] L.-C. Lo and C.-Y. Chu, "Development of highly selective and sensitive probes for hydrogen peroxide," *Chemical Communications*, vol. 9, no. 21, pp. 2728–2729, 2003.
- [14] C.-P. Lu, C.-T. Lin, C.-M. Chang, S.-H. Wu, and L.-C. Lo, "Nitrophenylboronic acids as highly chemoselective probes to detect hydrogen peroxide in foods and agricultural products," *Journal of Agricultural and Food Chemistry*, vol. 59, no. 21, pp. 11403–11406, 2011.
- [15] A. Matsumoto, R. Nishiyabu, and Y. Kubo, "Synthesis of a borylated boron-dibenzopyrromethene dye enabling the visual detection of H₂O₂ vapor," *RSC Advances*, vol. 4, no. 72, pp. 37973–37978, 2014.
- [16] E. W. Miller, A. E. Albers, A. Pralle, E. Y. Isacoff, and C. J. Chang, "Boronate-based fluorescent probes for imaging cellular hydrogen peroxide," *Journal of the American Chemical Society*, vol. 127, no. 47, pp. 16652–16659, 2005.
- [17] F. He, F. Feng, S. Wang, Y. Li, and D. Zhu, "Fluorescence ratio-metric assays of hydrogen peroxide and glucose in serum using conjugated polyelectrolytes," *Journal of Materials Chemistry*, vol. 17, no. 35, pp. 3702–3707, 2007.
- [18] B. C. Dickinson, C. Huynh, and C. J. Chang, "A palette of fluorescent probes with varying emission colors for imaging hydrogen peroxide signaling in living cells," *Journal of the American Chemical Society*, vol. 132, no. 16, pp. 5906–5915, 2010.
- [19] W.-K. Oh, Y. S. Jeong, S. Kim, and J. Jang, "Fluorescent polymer nanoparticle for selective sensing of intracellular hydrogen peroxide," *ACS Nano*, vol. 6, no. 10, pp. 8516–8524, 2012.
- [20] K. Żamojć, M. Zdrowowicz, D. Jacewicz, D. Wyrzykowski, and L. Chmurzyński, "Fluorescent Probes Used for Detection of Hydrogen Peroxide under Biological Conditions," *Critical Reviews in Analytical Chemistry*, vol. 46, no. 3, pp. 171–200, 2016.
- [21] G. Fusco, P. Bollella, F. Mazzei, G. Favero, R. Antiochia, and C. Tortolini, "Catalase-based modified graphite electrode for hydrogen peroxide detection in different beverages," *Journal of Analytical Methods in Chemistry*, vol. 2016, Article ID 8174913, 12 pages, 2016.
- [22] Y. Kanekiyo, H. Sato, and H. Tao, "Saccharide sensing based on saccharide-induced conformational changes in fluorescent boronic acid polymers," *Macromolecular Rapid Communications*, vol. 26, no. 19, pp. 1542–1546, 2005.
- [23] Y. Kanekiyo, M. Sano, R. Iguchi, and S. Shinkai, "Novel nucleotide-responsive hydrogels designed from copolymers of boronic acid and cationic units and their applications as a QCM resonator system to nucleotide sensing," *Journal of Polymer Science, Part A: Polymer Chemistry*, vol. 38, no. 8, pp. 1302–1310, 2000.
- [24] J. Matsui, M. Mitsuishi, and T. Miyashita, "Characterization of the molecular environment of polymer Langmuir-Blodgett films using a pyrene fluorescent probe," *Macromolecules*, vol. 32, no. 2, pp. 381–386, 1999.
- [25] F. M. Winnik, "Photophysics of preassociated pyrenes in aqueous polymer solutions and in other organized media," *Chemical Reviews*, vol. 93, no. 2, pp. 587–614, 1993.
- [26] Q. Wang, C. Liu, J. Chang et al., "Novel water soluble styrylquinolinium boronic acid as a ratiometric reagent for the rapid detection of hypochlorite ion," *Dyes and Pigments*, vol. 99, no. 3, pp. 733–739, 2013.
- [27] M. A. Martínez-Aguirre, R. Villamil-Ramos, J. A. Guerrero-Alvarez, and A. K. Yatsimirsky, "Substituent effects and pH profiles for stability constants of arylboronic acid diol esters," *Journal of Organic Chemistry*, vol. 78, no. 10, pp. 4674–4684, 2013.

- [28] P. Chandar, P. Somasundaran, N. J. Turro, and K. C. Waterman, "Excimer fluorescence determination of solid-liquid interfacial pyrene-labeled poly(acrylic acid) conformations," *Langmuir*, vol. 3, no. 2, pp. 298–300, 1987.
- [29] A. M. Buytendyk, J. D. Graham, K. D. Collins, K. H. Bowen, C.-H. Wu, and J. I. Wu, "The hydrogen bond strength of the phenol-phenolate anionic complex: A computational and photoelectron spectroscopic study," *Physical Chemistry Chemical Physics*, vol. 17, no. 38, pp. 25109–25113, 2015.