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Design of a Hypersensitive pH Sensory System Created by a Combination of Charge Neutralization and Aggregation-Induced Emission (AIE)

Daisuke Yoshihara[a], Takao Noguchi[b], Bappaditya Roy[b], Junji Sakamoto[a], Tatsuhiro Yamamoto[a], and Seiji Shinkai*[a, b]

Abstract: In our bodies, a slight pH change causes remarkable activation or serious damage in the biological processes and continuously keeps biological homeostasis. Detection of such a slight pH change has been a constant demand in searching unusual biological events. In this paper, we demonstrate a novel pH sensory system that has been achieved through a combination of charge neutralization by a slight pH change with aggregation-induced emission (AIE). We selected a cyano-functionalized oligo(phenylene-vinylene) (cyanoOPV) backbone for AIE and introduced ammonium-tethered boronic acid groups as a pH-dependent function. The self-assembling of these dyes (**OPV-Cn**) was readily achieved by pH-dependent charge neutralization at the neutral pH region. This sensory system showed unusually sensitive pH responsiveness in a narrow pH range. Moreover, this pH change was observed in a biologically important neutral pH region. We therefore believe that this system is broadly applicable to detect the slight pH change occurring in the biological events.

The pH value plays decisive roles in many biological events and maintains the biological homeostasis at the moderate conditions. As a matter of cause, therefore, it is highly important to monitor a slight pH change occurring in our bodies.[1] In order to attain this purpose, we usually use a pH meter or a chemical pH indicator.[2] Recently, a fluorescence-based pH indicator has been paid more attention because of its easy recognition by naked eyes. In fact, various fluorescence-based pH indicators have been explored so far.[3] In addition, the fluorescence-based sensory system features the very high sensitivity,[4] so that one may consider that this system is more advantageous in many cases than the UV-vis absorption-based pH sensory system.

In general, the principal of the traditional unimolecular pH sensor is based on dissociation or neutralization of a functional group integrated in the concerned dye molecule. Hence, its color change is basically same as the pH titration curve, which can be analyzed by a Henderson–Hasselbalch equation.[5] This manifests the limitation of this method: that is, the unimolecular pH sensory system is not suitable for sensitive detection of a slight pH change. To monitor the slight pH change, one must explore some new pH sensory systems on the basis of the mechanism different from the traditional unimolecular pH sensory mechanism.

Aggregation-induced emission (AIE) has recently been raised as an active target of research.[6] The phenomenon is caused by the suppression of the molecular motion (i.e., conformational change) by the supramolecular stacking,[6a] but not by the conventional structural change (i.e., configurational change).[7] We reported a few novel fluorescence sensory systems by the marriage of molecular recognition and AIE.[8] For example, guanidinium-tethered cyano-functionalized oligo(phenylene-vinylene) (cyanoOPV) recognized the structural difference among anionic polysaccharides (heparin, chondroitin sulfate, and hyaluronic acid) and resulted in the aggregate formation with different fluorescence intensity.[8a] In addition, we explored a D-glucose sensing system by the combination of AIE and dynamic covalent bond formation between boronic acid and D-glucose, resulting in ratiometric fluorescence sensing of D-glucose.[8b] We considered that a very sensitive pH sensory system might be explored by utilizing this AIE-based molecular recognition.

There are a few reports about the relationship between a pH change and an AIE phenomenon.[9] Apparently, some of them showed a sharp fluorescence change in a narrow pH region,[9a] and those results were obtained mostly in the polymeric system where the cooperative actions were operative.[9b] Moreover, the pH range in action is only in acidic or basic regions. Taking the application to biological systems (e.g., to the healthcare research field) into consideration, the sharp fluorescence change occurring at neutral pH region has been a constant need of demand.[10]

As described above, we already reported a sugar sensing system through the combination of AIE and boronic acid.[8b] This system worked well in the neutral pH region. Boronic acid is well-known as a functional group that changes the charge between neutral and anionic states by a pH change (Figure 1).[11] One can design the boronic acid group so that it may change the charge state at the neutral pH region. This change in the charge state should affect the aggregation properties of a boronic acid-tethered AIE dye molecule. As the aggregation of the AIE dye tends to occur in a cooperative fashion, this cascade change, pH – charge state – aggregation – AIE should lead to a novel pH-sensitive sensing system around neutral pH region.

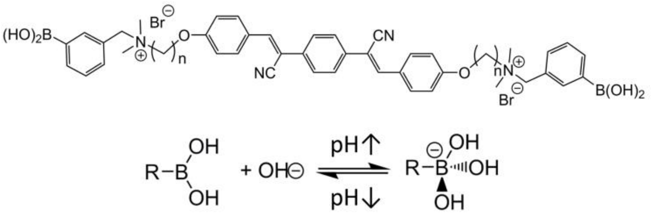

Figure 1. Molecular structure of **OPV-Cn** (n = 3 or 4) and neutral and anionic states of the boronic acid group.

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In this paper, we propose a novel approach toward a fluorescence-based pH sensing system around neutral pH region through a combination of charge neutralization and AIE. We have found that a very sharp fluorescence response emerges from charge pH-dependent neutralization followed by self-assembling of AIE molecules. Once the self-assembling action starts, it proceeds cooperatively. This auto-accelerative behavior leads to the sharp increase in the fluorescence intensity. Hence, one can expect that this system is useful to sense a very slight pH change, compared with that obtained from the conventional unimolecular pH sensors.

We selected a cyanoOPV backbone for AIE[12] and introduced ammonium-tethered boronic acid groups as a pH-dependent function. A sharp fluorescence change was observed around pH 6-7, indicating that a pH change can be amplified through the molecular assembling. Moreover, the pH responsive range was easily adjustable by the spacer length between the cyanoOPV and the ammonium group. We also investigated the correlation between the medium pH and the resultant morphology. The results revealed that an interesting relation exists among medium pH, fluorescence, and morphology. To the best of our knowledge, this is the first report on a hypersensitive fluorescence pH sensory system around neutral pH region.

We prepared two cyanoOPV derivatives (**OPV-C3**, **OPV-C4**) bearing ammonium-tethered phenylboronic acid groups (Figure 1). In order to examine their pH dependence, the fluorescence spectra were measured at various pH conditions (Figure 2). These two compounds showed a very sharp fluorescence change around pH 6-7. The results clearly support the view that our system working at neutral pH region is suitable for the biological purposes. The fluorescence increased as the increase in the medium pH, indicating that the charge state is changing from more soluble cationic species to less soluble zwitterionic species. Interestingly, the pH responsive range was different between **OPV-C3** and **OPV-C4**. This finding implies that one can adjust the pH responsive range by the spacer length. As the fluorescence intensity in the present system is associated with the aggregation properties, one may consider that the change in the pH responsive range is tuned by the hydrophobic effect and/or the odd-even number effect of the alkyl chain.

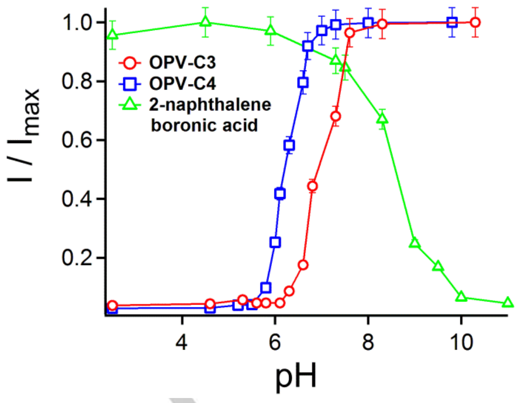

Figure 2. Plots of the normalized fluorescence intensity vs. pH in MeOH-aquaous MES buffer solution (0.10 M, 1:3 v/v) at 25 °C with 365 nm (**OPV-Cn**) and 278 nm (2-naphthaleneboronic acid) excitation. Circle; I_{465}/I_{max} in **OPV-C3**, square; I_{465}/I_{max} in **OPV-C4**, triangle; I_{345}/I_{max} 2-naphthaleneboronic acid. The error bars indicate 95 % confidence interval.

As a reference experiment, we measured the pH dependence of 2-naphthaleneboronic acid which should show the unimolecular pH responsive behavior (Figure 2, triangle). The fluorescence intensity for 2-naphthaleneboronic acid was changed by the addition of OH^- to boronic acid, leading to the emergence and the inhibition of intramolecular charge transfer through pH change.[13] As shown in Figure 2, the fluorescence intensity decreases as the pH increases. Basically, this curvature is equivalent to the unimolecular titration curve.

To estimate the sensitivity against the pH change quantitatively, we tried to calculate apparent pK_a values and n values from a linear relationship in modified Henderson-Hasselbalch equation [Eq. (1)],[14] where α is the degree of dissociation.

$$pH = pK_a - n\log\{(1-\alpha)/\alpha\} \qquad (1)$$

This equation is frequently used to estimate apparent pK_a value of pH responsive polymer systems instead of unimolecular pK_a value.[14] The feature of this equation is that “n” is appended to the usual Henderson-Hasselbalch equation. When this n value is 1, the molecule shows the unimolecular pH-dependent dissociation behavior (normal Henderson-Hasselbalch equation) (see n=1 line in Figure 3). When the n value is larger than 1, the molecule shows lower pH responsiveness than the unimolecular behavior. In general, most pH responsive polymer systems have the n values larger than 1.[14a] In contrast, when the n value is smaller than 1, the molecule shows higher pH responsiveness than the unimolecular behavior (see n<1 line in Figure 3).[14b] We evaluated apparent pK_a values and n values of three compounds from the fluorescence spectra. As summarized in Table 1, the apparent pK_a values for **OPV-C3**, **OPV-C4**, and 2-naphthaleneboronic acid were 6.92±0.12, 6.22±0.04, and 8.41±0.21, respectively. These results clearly indicate that being different from conventional boronic acids, those in **OPV-C3** and **OPV-C4** can work around neutral pH region. We also evaluated n values from Equation 1, the n values for **OPV-C3**, **OPV-C4**,

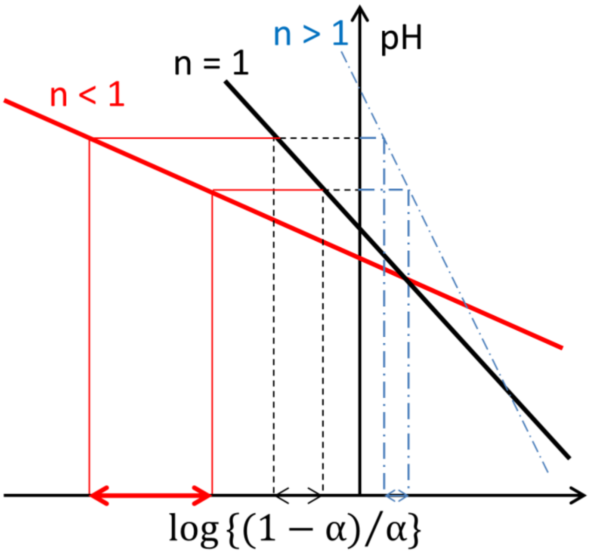

Figure 3. Schematic diagram for comparing the difference by the n value in Modified Henderson-Hasselbalch equation.

Table 1. Comparison of the parameters calculated by modified Henderson-Hasselbalch equation.

	pK_a[a]	n[a]
OPV-C3	6.92±0.12	0.58±0.10
OPV-C4	6.22±0.04	0.50±0.04
2-naphthalene-boronic acid	8.41±0.21	1.04±0.22

[a] The 95% confidence interval is included to display the uncertainty of the sample estimates.

and 2-naphthaleneboronic acid being 0.58±0.10, 0.50±0.04, and 1.04±0.22, respectively. The n value for 2-naphthaleneboronic acid was about 1. In contrast, the n values for **OPV-C3** and **OPV-C4** were considerably smaller than 1, indicating that the pH-dependence of these two compounds is much sharper than that of 2-naphthaleneboronic acid.

We also checked the reversibility of the pH-dependent fluorescence change in **OPV-C3** and **OPV-C4**. These two compounds clearly displayed a reversible fluorescence change between pH 5.3 and pH 7.3 (Figure 4).

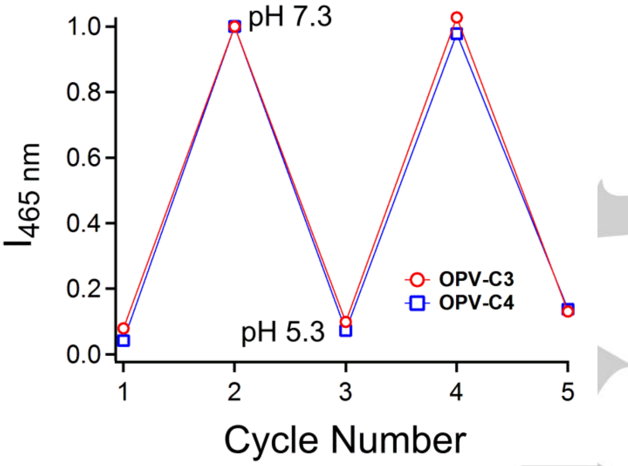

Figure 4. Recycle tests of the pH-responsive **OPV-Cn** in MeOH-aquaous MES buffer solution (0.10 M, 1:3 v/v) at 25 °C with 365 nm (**OPV-Cn**) excitation. The pH value of the solution was varied reversibly between 5.3 and 7.3.

The morphological change was evaluated through observation with a transmission electron microscope (TEM). The size of the aggregates was increased with the increase in pH (Figure 5). The result is highly compatible with the pH-dependent fluorescence change. The trend supports the view that the fluorescence increase observed with the pH increase is originated from the aggregate formation.

From the foregoing results, we can propose a consistent relationship among fluorescence, pH, and aggregate size (Figure 6). In a pH region lower than pK_a, **OPV-C3** and **OPV-C4** behave as cationic species. Their higher solubility does not allow them to form aggregates and the solutions are nonfluorescent. As the pH increases, boronic acids are changed to anionic state, and the molecules form less soluble zwitterionic species by the intermolecular charge neutralization. This triggers the aggregate formation. In a pH region higher than pK_a, the aggregates are fully formed and the solutions become strongly fluorescent. In our system this transition occurs cooperatively, which leads to a hypersensitive pH sensory system.

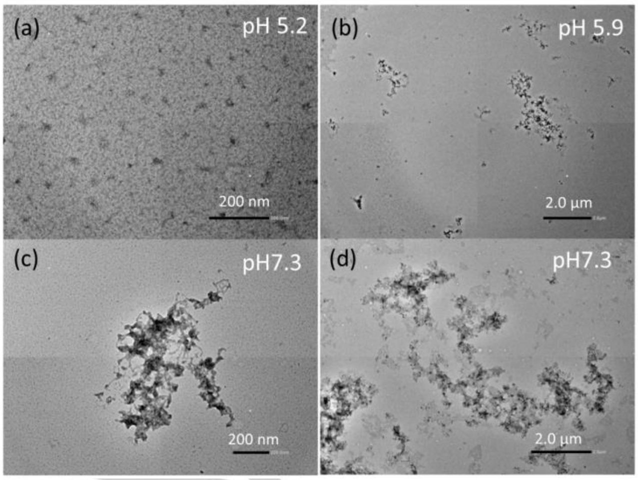

Figure 5. TEM images of **OPV-C4** at (a) pH 5.2, (b) pH 5.9, and (c), (d) pH 7.3. Concentration: [**OPV-C4**] = 10 µM. All images were taken after staining with 4.0 wt% phosphotungstic acid. Black lines are scale bars.

In summary, we have succeeded in designing a novel highly sensitive pH sensory system with the sensible combination of an AIE-core with the concept of charge neutralization between ammonium and pH-responsive boronic acid. The design concept utilized herein is totally different from that utilized in the traditional unimolecular sensors. This sensory system showed the hypersensitive pH response useful around neutral pH region. The relative sensitivity can be compared quantitatively by applying modified Henderson-Hasselbalch equation. Furthermore, the responsive pH region can be easily adjusted by changing the spacer length. We believe, therefore, that the present system has a large potential to detect biological events that may accompany a slight pH change.

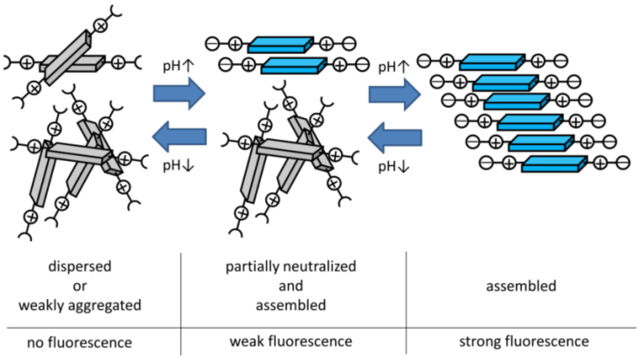

Figure 6. Schematic illustration of proposed our pH responsive fluorescence sensory system.

Acknowledgements

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Keywords: aggregation-induced emission • self-assembly • boronic acid • pH sensor • charge neutralization

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Layout 1:

COMMUNICATION

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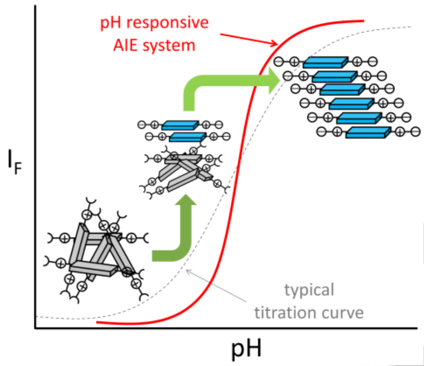

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