Fluorescent Probes

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Emergent Molecular Recognition through Self-Assembly: Unexpected Selectivity for Hyaluronic Acid among Glycosaminoglycans

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Abstract: *Oligophenylenevinylene (OPV)-based fluorescent (FL) chemosensors exhibiting linear FL responses toward polyanions were designed. Their application to FL sensing of glycosaminoglycans (heparin: HEP, chondroitin 4-sulfate: ChS, and hyaluronic acid: HA) revealed that the charge density encoded as the unit structure directs the mode of OPV self-assembly: H-type aggregate for HEP with 16-times FL increase and J-type aggregate for HA with 93-times FL increase, thus unexpectedly achieving the preferential selectivity for HA in contrast to the conventional HEP selective systems. We have found that the integral magnitude of three factors consisting of binding mechanism, self-assembly, and FL response can amplify the structural information on the target input into the characteristic FL output. This emergent property has been used for a novel molecular recognition system that realizes unconventional FL sensing of HA, potentially applicable to the clinical diagnosis of cancer-related diseases.*

Self-assembly is ubiquitously seen in nature, ranging from biological systems to artificial nanomaterials, which is strongly associated with the origin of the functionality.[1,2] For example, self-assembly of protein subunits into well-defined superstructures is a prerequisite for vital biological functions such as transcription, translation, metabolic, and immune systems.[3] Therein, the biological functions are driven by a key signal (initial input), whereas the mutation in protein structures causes fatal biofunctional disorders. Such a critical impact of the initial input evokes the emergent phenomena.[4] Learning from the biological systems, we can acquire a chance to apply this emergent property to the creation of self-assembly-based functional materials. Although there exists one report describing the emergent property of molecular self-assembly,[4] its utilization toward practical applications still remains unexplored.

Recently, we reported a molecular recognition system coupled with self-assembly and fluorescence (FL) signalling by using an oligophenylenevinylene (OPV)-based FL chemosensor.[5] Therein, a subtle difference in guest structures dramatically alters the resulting FL responses. For example, L- and *meso*-tartarate afforded the distinct FL difference in the OPV sensor as well as the totally different self-assembly morphologies: fibrous structure with L-tartarate and finite particle with *meso*-tartarate. It is known that the preferable conformations of L- and *meso*-tartarate in water are *anti* and *gauche* conformers, respectively. Therefore, we demonstrated that only a conformational preference originating from their stereochemical difference determines the J- or H-type self-assembly path of the OPV sensor, which leads to the characteristic self-assembly morphologies and the consequent FL responses. In other words, an initial input of guest structural information into the OPV sensor critically directs the OPV self-assembly process, leading to a FL response characteristic to the guest input (incommensurable output). This schematic concept highlights a key feature of the emergent property in the self-assembly-based FL sensory system, and more importantly, achieves precise molecular recognition that has never been accomplished by the conventional molecular recognition based on the traditional key-and-lock[6] binding technique. In this study, we utilize this emergent property for the design of a novel FL sensing system applicable to biologically relevant polymers, glycosaminoglycans (GAGs) such as heparin (HEP), chondroitin 4-sulfate (ChS), and hyaluronic acid (HA), as shown in Figure 1.

GAG is an important class of biopolymer that plays a key role in the biofunctional regulation including cell growth and differentiation, angiogenesis, and immune response.[7] For clinical purposes, HEP has been mostly utilized as a responsible anticoagulant during surgery, and its efficient and reliable sensing methodologies have been developed through an appropriate design of assembly-based binders and colorimetric or fluorescent chemosensors.[8,9] More recently, application of cationic aggregation-induced emission dyes[10] was offered because of their strong cohesive force with the polyanions.[11] In any case, most reports described the selective detection of the most anion-charged HEP owing to the preferential association of cationic chemosensors. Their applications were therefore limited to HEP sensing and have rarely been explored to FL sensing of other GAGs such as HA in spite of the clinical importance related to cancer diagnosis involving its overexpression and excretion.[12] In this context, we have developed a novel polyanion sensing system

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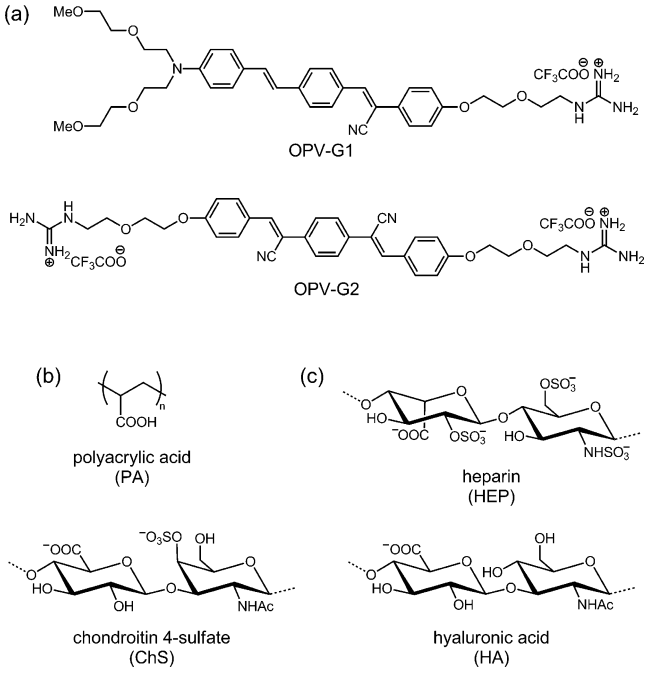

Figure 1. Chemical structures of a) assembly-based fluorescent chemosensors: OPV-G1 and OPV-G2, b) polyacrylic acid (PA), and c) representative glycosaminoglycans: heparin (HEP), chondroitin 4-sulfate (ChS), and hyaluronic acid (HA).

using new self-assembly-based FL chemosensors (OPV-G1 and OPV-G2,[5] Figure 1a) for a representative polyanion, polyacrylic acid (PA, Figure 1b), that exhibits a linear FL response toward the PA concentration. In the course of its application to GAG (Figure 1c) sensing, we unexpectedly found that OPV-G2 shows the selective FL detection and sensing of HA, in contrast to the conventional HEP-selective sensing systems.[8,9,11] Here, we wish to emphasize that such unexpected selectivity does appear when the emergent property of self-assembly is integrated as a novel molecular recognition technique.

OPV-G1 was water-soluble and showed a weak red FL emission maximum at 604 nm with excitation wavelength at 400 nm. The FL intensity was enhanced by 5.0-fold by the addition of PA, the FL intensity being observable by our naked eyes (Figure S1 in the Supporting Information (SI)). In order to test the performance level of OPV-G1 for sensing purposes, a FL titration experiment was performed. Upon increasing concentration of PA, the FL intensity increased linearly and reached a plateau at ca. 1.0 equivalent per repeat unit of PA (Figures 2a,b and S2 in the SI). This linearity was also confirmed in the UV/Vis titration result. Upon increasing concentration of PA, the absorbance of OPV-G1 at 414 nm decreased with increasing absorbance at 406 nm (Figure 2c). This spectral change indicates that π-stacked aggregates are formed. The ratiometric plot (A_{406}/A_{414}) also revealed a linear relationship (Figure 2d), correlating with the FL titration result (Figure 2b). These results support that the FL response with the linear relationship stems from the formation of π-stacked aggregates of OPV-G1 on the polyanionic platform of PA.

Here one fundamental question arises: why is the linear FL response observed in spite of the self-assembly system? This result is rather surprising for us because we have reported, to date, the nonlinear FL response accompanied with the molecular self-assembly process.[5,13] In order to address this query, we performed a titration experiment where PA concentration was fixed at 30 μM and OPV-G1 concentration was varied. Upon increasing concentration of OPV-G1, the FL intensity was increased nonlinearly and saturated at 30 μM which is equivalent to PA concentration (Figure S3a,c in the SI). When PA is absent, the FL intensity of OPV-G1 was increased linearly with its increasing concentration (Figure S3b,c in the SI). These results indicate that the nonlinear FL response stems from the cooperative association[14] of OPV-G1 to PA. According to Hill equation,[14] the apparent global association constant ($\mathrm{Log}\,K_a$) was estimated to be 8.65 (Figure S4 in the SI). As the nonlinear FL increase was saturated at 1.0 equivalent (Figure S3c in the SI), we could apply this $\mathrm{Log}\,K_a$ value to explain why the linear FL response is observable for the PA sensing shown in Figure 2b: OPV-G1 quantitatively associates PA with $\mathrm{Log}\,K_a = 8.65$ until the plot reaches the plateau (Figure 3a). Owing to this linear relationship, the detection range of PA concentration becomes adjustable by the initial OPV-G1 concentration

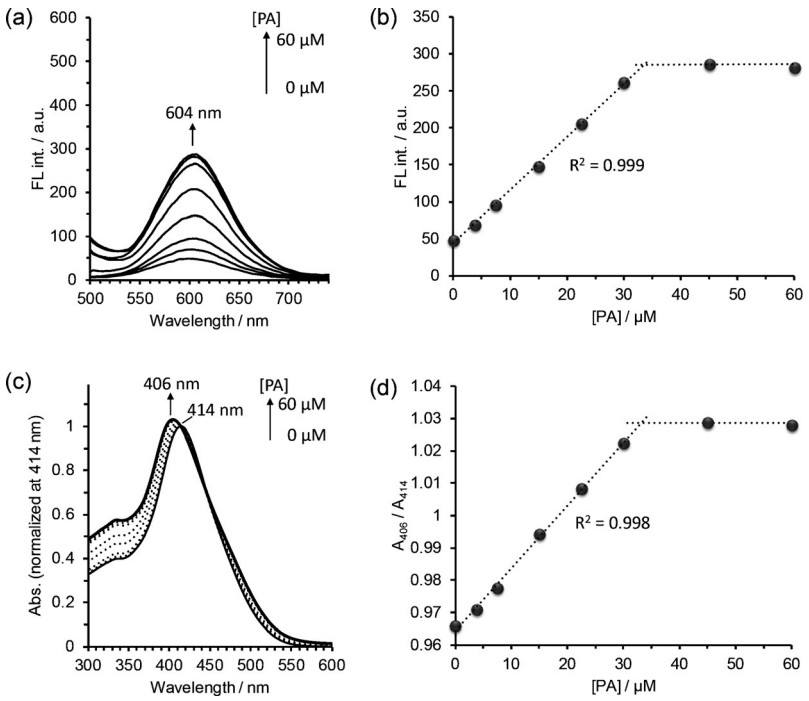

Figure 2. a) Fluorescence titration ($\lambda_{ex} = 400$ nm) of OPV-G1 upon increasing concentration of PA (0–60 μM) in HEPES buffered water. b) Plot of the changes in the fluorescence intensity at 604 nm. c) UV/Vis titration of OPV-G1 upon increasing concentration of PA (0–60 μM) in HEPES buffered water. d) Ratiometric plot of A_{406}/A_{414}. Conditions: [OPV-G1] = 30 μM, [HEPES] = 10 mM (pH 7.4), 25 °C.

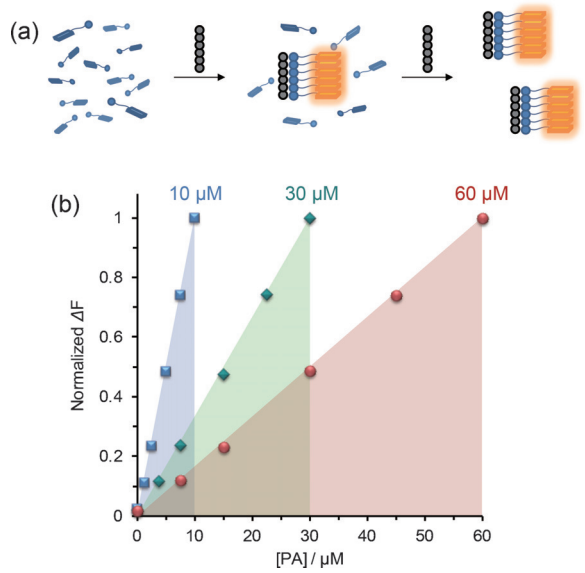

Figure 3. a) Schematic illustration of PA sensing that exhibits apparent 1:1 linear relationship. b) Dependence of OPV-G1 concentration (blue: 10 μM, green: 30 μM, red: 60 μM) on the detection range toward PA (0–1.0 equivalent).

(Figures 3b and S5 in the SI). From these results, we can rationalize a novel polyanion sensing system utilizing the assembly-based OPV-G1 chemosensor, where the multiple electrostatic interactions resulting from the cooperative binding mechanism dominate over the formation of π-stacked OPV/PA aggregates quantitatively (Figure 3a). This mechanism is the reason why the system achieves an apparent 1:1 linear relationship in the FL response, as shown in Figures 2b and 3b.

In order to demonstrate that the polyanion sensing system has a feature of apparent 1:1 linear relationship, we next applied this OPV-G1 sensor to the FL sensing of GAGs (HEP, ChS, and HA shown in Figure 1c). As expected, OPV-G1 showed a linear FL response in the FL titration experiment (Figures S6 and S7 in the SI) and revealed that its selectivity is correlated with the anionic charge density: that is, HEP > ChS > HA (Figures 4a and S8 in the SI) as the order conventionally described in the previous reports.[8,9,11] As we reported previously,[13d] the selectivity in this class of measurements can be improved by the influence of the salt interference and medium pH. We thus applied this procedure to the present system. No remarkable influence of salt interference on the selectivity was confirmed, the FL intensity decreasing only by 8% under the salt condition ([salt] = 150 mM, salt: NaCl, CsCl, $CaCl_2$, shown in Figure S9 in the SI). Although the selectivity for HEP was not improved so much, one can claim that OPV-G1/GAG complexes with multiple electrostatic interactions have superior tolerance to the highly competitive salt interference. In contrast, the influence of medium pH appeared conspicuously. When the medium pH decreased to pH 1.5, the FL intensity of OPV-G1/HA decreased and reached the level of the background (Figure S10a in the SI) because of the protonation of carboxylate anions in HA while that of OPV-G1/HEP was still observable, indicating that HEP selectivity is significantly improved (Figure S10b in the SI). These results provide us an insight that the adjustment of the medium pH is very effective for improving the selectivity for GAGs.

A dramatic alteration in selectivity was observed when OPV-G2 sensor was used.[15] In its application to GAG sensing, we observed the unexpected selectivity for HA, the maximum FL increase being 93-fold (Figures 4b and S12 in the SI). This selectivity trend of HEP < ChS < HA is in direct contrast to the conventional selectivity trend of HEP > ChS > HA shown in Figure 4a. This marked selectivity inversion suggests that the affinity is not simply governed only by the electrostatic interactions as is the conventional case. The UV-vis titration provided a clear view that in the OPV self-assembly two different aggregation modes exist: that is, H-type aggregate with HEP and J-type aggregate with HA (Figures 4c and S13 in the SI) as supported by previous literatures.[5,13d,16] As mentioned above, one can consider that the selectivity of OPV-G2 for the GAGs is controllable by adjusting the medium pH condition. When the medium pH was adjusted to pH 1.5, the FL intensity of OPV-G2/HA indeed reached the level of the background. As a result, ChS selectivity is most enhanced (Figure S14 in the SI). These results give us a new rationale to explain the selectivity: the lower charge density in the unit structure of the GAGs directs the J-type mode of OPV-G2 self-assembly, affording an intense FL response.[17]

More generally, one can regard that structural information on target (in the present case, unit structure of GAGs) is amplified, via self-assembly of the OPV sensor, into a FL response characteristic to the structural information input (incommensurable output). We believe, therefore, that this is a striking feature of molecular self-assembly known as a sort of emergent property. This emergent property has now been utilized for unconventional FL sensing of GAGs, especially HA, by the integration of the self-assembly concept as a novel molecular recognition system.

In conclusion, we have developed a novel FL sensing system orchestrated by binding mechanism, self-assembly, and FL response, that exhibits apparent 1:1 linear FL response toward the polyanion concentration. In its application to GAG sensing, OPV-G1 shows the conventional HEP selectivity whereas the unconventional selectivity for HA has been displayed by OPV-G2 self-assembly. Owing to this unconventional selectivity, ChS is also detectable by adjusting the medium pH condition. These results provide us a new perspective that the selectivity corresponding to the GAG structural features can be tailored by utilizing self-assembly phenomena. Together with our previous report,[5] we wish to propose herein a novel molecular recognition system that realizes unconventional FL sensing via the emergent property of self-assembly.

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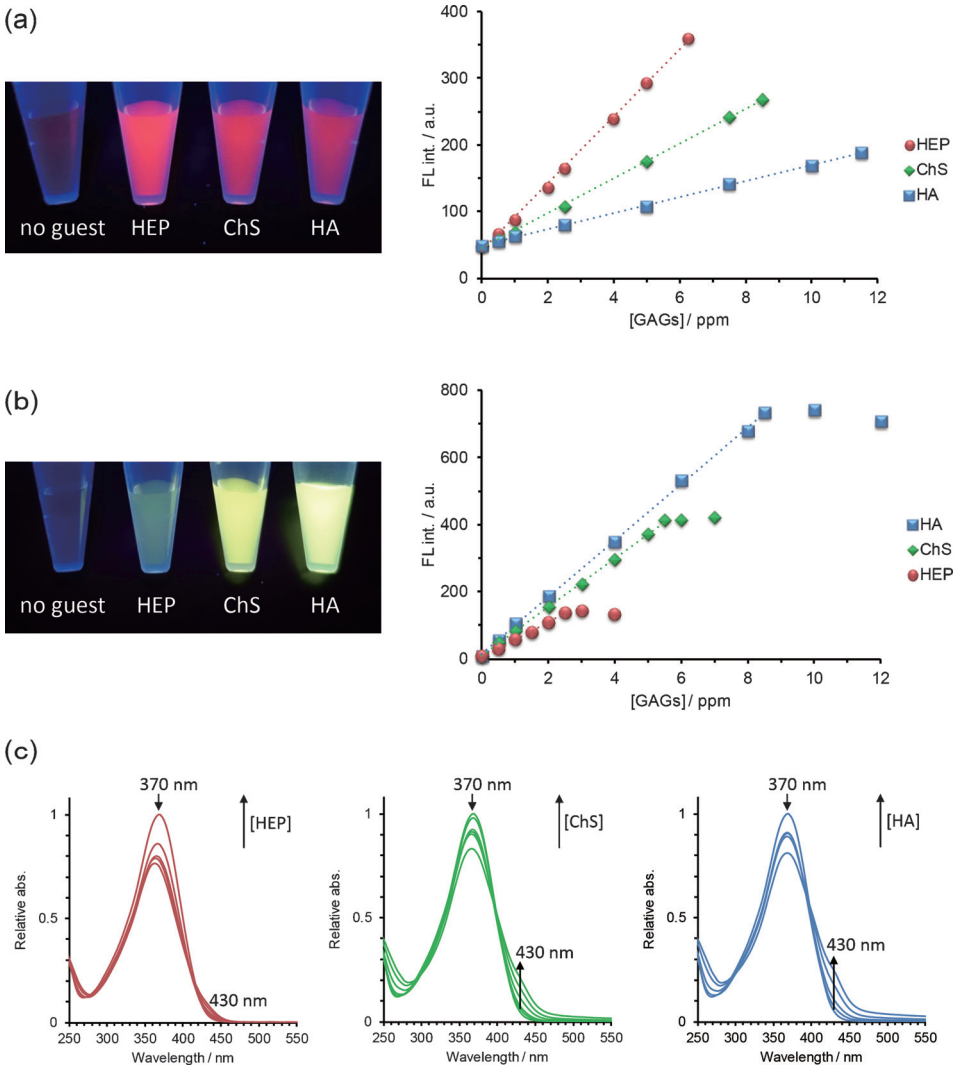

Figure 4. a) Photograph of OPV-G1 in the absence and presence of HEP (6.25 ppm), ChS (8.50 ppm), and HA (11.5 ppm), and changes in the fluorescence intensity of OPV-G1 upon increasing concentrations of HEP (red), ChS (green), and HA (blue). Conditions: [OPV-G1] = 30 μM, [HEPES] = 10 mM (pH 7.4), 25 °C, λ_{ex} = 400 nm. b) Photograph of OPV-G2 in the absence and presence of HEP (2.5 ppm), ChS (5.5 ppm), and HA (8.5 ppm), and changes in the fluorescence intensity of OPV-G2 upon increasing concentrations of HEP (red), ChS (green), and HA (blue). c) UV/Vis titration of OPV-G2 upon increasing concentrations of HEP (red), ChS (green), and HA (blue) in HEPES buffered water. Conditions: [OPV-G2] = 10 μM, [HEPES] = 10 mM (pH 7.4), 25 °C, λ_{ex} = 388 nm.

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[17] This is further supported by the dilution experiment of the HEP charge with HA; see Figure S15 in the SI.

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