Multicomponent covalent dye assembly for tight binding and sensitive sensing of L-DOPA†

Xuan-Xuan Chen, Xin Wu, Peng Zhang, Miao Zhang, Bing-Nan Song, Yan-Jun Huang, Zhao Li and Yun-Bao Jiang*

A mixture of two simple perylene-containing receptors was found to bind bifunctional L-DOPA synergistically and tightly via orthogon al boronate ester and imine bond formation in neutral aqueous solutions, the resulting three-component assembly forming optically active long fibrous aggregates.

The development of molecular sensors often involves efforts to increase the affinity of molecular recognition events for achieving higher sensitivity and/or selectivity for the analyte of interest.¹ A commonly employed strategy for binding multifunctional analytes is to design a complementary multifunctional receptor so that the binding affinity is enhanced through the multivalency effect.² Examples of this approach include sensors for the neurotransmitter dopamine or its precursor L-DOPA, which typically contain a boronic acid group and an aldehyde group or an ionic group.³ This necessitates elaborate molecular design in placing the binding groups in appropriate positions and orientations in the sensor molecule. Simplification of the sensor structure and thus the ease of sensor synthesis are expected if a mixture of monofunctional receptors could bind multifunctional analytes with high affinity. Challenging, however, is achieving a synergistic effect required to overcome the entropic cost of forming such multicomponent assemblies. We demonstrate herein our successful exploitation of dye aggregation that makes possible the synergistic binding and highly sensitive sensing of L-DOPA by using mixtures of simple monofunctional receptors.

Our L-DOPA sensing ensemble consists of two perylenebisimide (PBI) based receptors – 1, a chirality sensor for α-hydroxy acids,⁴ and a novel aldehyde 2 (Fig. 1). It should be noted that the aldehyde appears as a poor amine binder without stabilization by an intramolecular hydrogen bond and electron-withdrawing groups,⁵ yet needs to bind L-DOPA in neutral aqueous solutions when the amine group of L-DOPA (pKa 8.7) exists mostly in protonated form. It was assumed that formation of an otherwise unstable boronic acid–aldehyde–L-DOPA ternary complex would be stabilized by PBI aggregation driven by the strong aromatic π–π stacking interaction between PBI receptors 1 and 2.

The L-DOPA-dependent self-assembly of 1, 2 and a 1:1 mixture of them was initially investigated by UV-Vis absorption, circular dichroism (CD) spectroscopy and dynamic light scattering (DLS) measurements. 1 and 2 were found to exist predominantly as small oligomeric H-type aggregates in water (pH 7.5, with 50 mM HEPES) whether alone or mixed with each other at a total dye concentration of 50 μM, as judged from the shape of the UV-Vis absorption intensity and increase of aggregate size, Fig. 2) and guest-induced supramolecular chirality (resulting in induced CD signals from the PBI chromophore, Fig. S22 and S23 in the ESI†) occurred for both 1 and 2 (50 μM) upon their respective interaction with L-DOPA. Therefore, binding of L-DOPA led to the formation of larger and optically active PBI aggregates of 1 or 2, similar to the reported interaction of 1 with α-hydroxy carboxylates.⁶ Both receptors however show only modest interactions for L-DOPA as determined from UV-Vis absorption titrations (Fig. S11 and S12 in the ESI†), and weak CD signals induced by L-DOPA.

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† Electronic supplementary information (ESI) available: Compound synthesis, UV-Vis absorption spectra, CD spectra, binding constant determination, Job plots, and selectivity study. See DOI: 10.1039/c5cc03495g

Fig. 1 Structures of L-DOPA receptors 1 and 2, and control compounds 3–6.
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A mixture of 1 (25 μM) and 2 (25 μM), in comparison, displays superior sensing performance. The dependence of absorption spectra (Fig. 2a), aggregate size (Fig. 2b) and CD intensities (Fig. 3a) of the 1–2 mixture on L-DOPA concentration shows significantly sharper slopes at low L-DOPA concentrations, indicating higher affinity of the mixture for L-DOPA compared to a single dye component. Interestingly, inspection of Fig. 3a reveals a higher magnitude and an opposite sign for the L-DOPA induced CD signal of the 1–2 mixture compared to the 1 mixture, which has been supported by CD titration (Fig. S22, ESI†). Refer to ESI† for a detailed discussion.

![Figure 2](image-url)

**Fig. 2** Absorption quenching (AA/ΔA) at 501 nm (a) and average aggregate size (hydrodynamic diameter, \(D_h\)) (b) of 1 (50 μM), 2 (50 μM) or a mixture of 1 (25 μM) and 2 (25 μM) versus concentration of L-DOPA in pH 7.5 HEPES buffer. Note that the aggregate size of 1 shows a more complicated, non-monotonic dependence on L-DOPA concentration. We propose that this is due to different aggregation structures formed throughout the titration, which has been supported by CD titration (Fig. S22, ESI†).

![Figure 3](image-url)

**Fig. 3** (a) CD spectra of 1 (50 μM), 2 (50 μM) or a mixture of 1 (25 μM) and 2 (25 μM) in the presence of L-DOPA (0.1 mM) in pH 7.5 HEPES buffer. (b) CD intensity at 480 nm and 525 nm of a mixture of 1 (25 μM) and 2 (25 μM) versus concentration of L-DOPA in pH 7.5 HEPES buffer. (c) Cartoon representation showing aggregates formed by the 1–2–L-DOPA complex. (d) TEM image taken from a sample prepared from a solution of 1 (25 μM), 2 (25 μM) and L-DOPA (0.1 mM) in pH 7.5 HEPES buffer.

Table 1 shows a much higher maximum \(D_h\) of the aggregates formed by the 1–2 mixture (4980 nm), compared to that of the aggregates of 1 (1347 nm) or 2 (1796 nm). Transmission electron microscopy (TEM) images show long fibrils formed by the 1–2 mixture in the presence of L-DOPA (Fig. 3d), while the aggregates formed by 1 or 2 alone in the presence of L-DOPA were not stable enough for observation by TEM. These observations suggest a completely different type of L-DOPA binding to the 1–2 mixture. The higher affinity of the 1–2 mixture for L-DOPA leads us to hypothesize that one molecule of 1 and one molecule of 2 bind to the same L-DOPA molecule, *via* a boronate ester linkage and an imine linkage (Fig. 3c), with synergy presumably resulting from aromatic stacking-facilitated aggregation of 1 and 2. This could be seen as multivalent binding, regarding a stacked 1–2 “dimer” as a divalent host, leading to stabilization of the ternary 1–2–L-DOPA complex. Such a hypothesis has been supported by Job plot experiments that reveal the dye to L-DOPA stoichiometry to be 2 : 1 (Fig. S28, ESI†) and the 1 to 2 stoichiometry to be 1 : 1 (Fig. S29, ESI†).

In the case of 1 or 2 alone, the low affinity for L-DOPA does not allow stoichiometry determination by Job plots. In the case of the 1–2 mixture, the CD intensity of the aggregates starts to decline at L-DOPA concentration beyond 0.2 mM (Fig. 3b), with a concomitant decrease of \(D_h\) (Fig. 2b). Given the 2 : 1 (dye to L-DOPA) stoichiometry revealed by the CD-based Job plot, this change can be attributed to binding of more L-DOPA molecules to the aggregates than the 2 : 1 stoichiometry, possibly disrupting the compact aggregate structure formed by the 2 : 1 complex. To evaluate the binding affinity, apparent binding constants were calculated from part of the UV-Vis binding isotherm (due to multiple equilibria) and tabulated in Table 1. Although the values should be regarded as relative values, they support the existence of strong synergism.

To rule out more complex assemblies formed by a mixture of symmetrically functionalized dyes 1 and 2 with L-DOPA, mono-functionalised perylene dyes 5 and 6 (Fig. 1) were synthesized and their interactions with L-DOPA examined. CD titrations show similar synergistic interactions of 5 and 6 with L-DOPA (Fig. S31–34, ESI†), with indeed the same 1 : 1 stoichiometry (indicated by Job plots, Fig. S35 and S36, ESI†) as with the 1–2 mixture. Interestingly, the L-DOPA affinity of the 5–6 mixture was even higher than that of the 1–2 mixture, as CD titration of

<table>
<thead>
<tr>
<th>Guest</th>
<th>Host</th>
<th>1-DOPA</th>
<th>Dopamine</th>
<th>Catechol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>(3.4 ± 0.6) (\times) 10^2</td>
<td>nd^b</td>
<td>nd^b</td>
</tr>
<tr>
<td>2</td>
<td>1 and 2</td>
<td>(3.2 ± 0.5) (\times) 10^4</td>
<td>(1.7 ± 0.2) (\times) 10^3</td>
<td>(3.3 ± 0.7) (\times) 10^2</td>
</tr>
<tr>
<td>1 and 3</td>
<td>(1.2 ± 0.2) (\times) 10^4</td>
<td>(3.3 ± 0.7) (\times) 10^2</td>
<td>(4.8 ± 1.3) (\times) 10^3</td>
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^a Different aggregate structures formed throughout titration indicated by CD studies, therefore \(K\) cannot be determined with reliability. However the dependence of PBI absorption and aggregate size on 1-L-DOPA concentration (Fig. 2) does indicate weaker interaction of 1 with L-DOPA compared to the 1–2 mixture. ^b Not determined. ^c A PBI “dimer” consisting of one molecule of 1 and one molecule of 2 or 3 is regarded as the host.
the 5–6 mixture by L-DOPA shows the maximum CD intensity exactly at the 2 : 1 (dye to L-DOPA) ratio (Fig. S34, ESI†), while a three-fold excess of L-DOPA was required to reach the maximum CD intensity in the case of the 1–2 mixture (Fig. 3b).

With the stoichiometry for the 1–2–L-DOPA complex confirmed to be 1 : 1 : 1 and binding of only one set of the functional groups verified by results with the monofunctionalised control compounds 5 and 6, we examined the affinity of the 1–2 mixture respectively for dopamine, catechol, L-tyrosine and L-phenylalanine, structurally partly similar to L-DOPA, by UV-Vis titrations (Table 1), to test the involvement of a boronate ester and an imine linkage. The interaction of L-tyrosine and L-phenylalanine was too weak for binding constant determination (Fig. S16 and 17, ESI†), supporting the role of a boronate ester linkage. Appreciably lower affinities were found for catechol (Table 1). Although dopamine also contains a catechol moiety and an amine group and therefore it may bind the 1–2 mixture in the same manner as L-DOPA, its affinity for the 1–2 ensemble is even lower than that of catechol (Table 1). This may seem contrary to the assumed role of imine formation in the strong binding of the 1–2 ensemble to L-DOPA. A possible explanation is that the 1–2–L-DOPA complex is additionally stabilized by ion pairing between the carboxylate group of L-DOPA and the quaternary ammonium group of either 1 or 2, which is absent in the case of dopamine binding. The lower affinity of dopamine compared to catechol for the 1–2 ensemble might be explained by the higher hydrophobicity of catechol, which outweighs the potential effect of an imine bond in the case of dopamine binding. This is possible since a positive correlation has been found between the binding affinity and the guest hydrophobicity in the case of binding of 1 with 2-hydroxy-carboxylate guests.4 In this regard it needs to be confirmed whether the interaction between 2 and L-DOPA is merely ionic or actually involves an imine bond. To that end, negative control compounds 3 and 4 (Fig. 1) were synthesized and tested. Compared to the 1–2 mixture, the 1–3 mixture shows substantially lower affinity for L-DOPA and dopamine, whereas higher affinity for catechol (Table 1). The higher affinity for catechol of the 1–3 mixture is likely due to the higher hydrophobicity of 3 than 2. In the case of L-DOPA binding, despite the relatively unfavourable hydrophobicity of 2, the 1–2 mixture binds more strongly than the 1–3 mixture. The higher affinity for L-DOPA of the 1–3 mixture compared to 1 alone could be due to a similar 1 : 1 : 1 1–3–L-DOPA complex, with the carboxylate group of L-DOPA bound electrostatically to the quaternary ammonium group of 3. Stoichiometry determination of Job plots is not feasible due to the low CD signal of the 1–3–L-DOPA complex. In addition to the higher affinity for L-DOPA of the 1–2 ensemble compared with the 1–3 ensemble, another piece of evidence supporting the presence of an imine bond is the different kinetics of chirality induction observed with these two systems. The CD intensity increases with time over the course of 1 h for the 1–2–L-DOPA complex (Fig. S27, ESI†), which can be ascribed to the relatively slow formation of imine. This slow kinetic profile was not observed for the 1–3–L-DOPA mixture (Fig. S46, ESI†). The 1–4 mixture, despite a higher electron density on the carbonyl oxygen of ketone 4, behaves similarly to the 1–3 mixture, showing weaker interaction with L-DOPA compared to the 1–2 mixture (Fig. S47 and S48, ESI†), thereby ruling out hydrogen bonding between the aldehyde carbonyl group and L-DOPA amino/ammonium group responsible for binding of L-DOPA to 2. In this case, the degree of absorption quenching of the PBI chromophore is too small for accurate binding constant determination. With the 1–3 and 1–4 mixtures, interestingly, L-DOPA was unable to induce the same extent of absorption quenching (Fig. S48, ESI†) and CD induction (Fig. S45 and S49, ESI†) in the PBI chromophore as observed with the 1–2 mixture, again highlighting the importance of the imine bond.

The 1–2 ensemble allows detection of L-DOPA by CD spectroscopy with a sensitivity down to 5 μM (Fig. 3b). Moreover, the CD intensity is found to be linear with the enantiomeric excess (ee) of the DOPA guest (Fig. S26, ESI†), showing the potential for sensing ee of DOPA. Selectivity of the 1–2 mixture for L-DOPA over potential interferents was investigated (Fig. S30, ESI†). The interference of D-glucose, phenylalanine and tyrosine was minor up to 5 mM. The CD response of the 1–2 mixture to L-DOPA was significantly reduced by the coexistence of D-fructose, catechol and dopamine, which is consistent with their high affinity for boronic acids.7 However, since they induce little to no CD signals, qualitative detection of L-DOPA is possible in the presence of low concentration of them.

In summary, we have shown that by PBI aggregation, the three-component L-DOPA–boronic acid–aldehyde complex can be formed at low concentrations in aqueous solutions, with a strong synergistic effect between two orthogonal dynamic covalent bonds. This allows sensitive CD-based sensing of L-DOPA at micromolar concentrations by mixing two simple receptors, which is different from reported approaches of using a bifunctional sensor, yet the affinity for L-DOPA is exceptional compared to other reported synthetic sensors for L-DOPA or dopamine in aqueous solutions.5 The high affinity is proposed to result from stabilization by aromatic π-stacking between 1 and 2. Remarkable also is the formation of the otherwise weak imine bond at micromolar concentrations in neutral aqueous solutions without resorting to imine stabilization by intra-molecular hydrogen bonds or placing electron-withdrawing groups. Although the benzaldehyde moiety is less reactive, leading to a relatively slow response that might be disadvantageous for practical sensing applications, further improvements can be expected by using a more electrophilic aldehyde group such as pyridine aldehydes.8 The divalent binding of L-DOPA to the 1–2 ensemble, interestingly, results in the formation of long fibrous PBI aggregates with a significantly larger size and stronger induced CD signals compared to aggregates formed by the 1–1–L-DOPA or 2–2–L-DOPA complexes in which only monovalent binding of L-DOPA is possible. Despite the proofing of the principle in the L-DOPA example, the strategy of utilizing dye aggregation in building multicomponent systems has implications for sensor design in which multivalent guest binding is useful.

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Notes and references