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Accelerating fluorescent sensor discovery: unbiased screening of a diversity-oriented BODIPY library†

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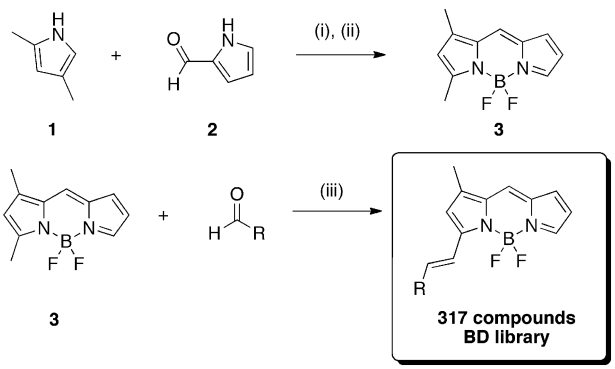
Herein, we report the first systematic and unbiased evaluation of the BODIPY fluorophore library against a wide panel of biologically relevant molecules, and discoveries of 2 novel fluorescent probes for BSA and dopamine.

4,4-Difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) dyes have attracted much attention in fluorescence imaging due to their outstanding photophysical properties (*e.g.* high extinction coefficient and photostability, narrow bandwidth, *etc*).[1] In particular, high fluorescence quantum yield and environmentally insensitive character made BODIPY fluorophore one of the popular labelling agents to visualize biomolecules,[2] including ceramides,[3] peptides,[4] and various metabolites.[5] On the other hand, there are limited numbers of BODIPY based chemosensors. These sensors were generally designed by connecting the fluorophore to recognition motifs (*e.g.* ion chelators) and exploit the photon electron transfer (PET) mechanism to monitor the binding process by fluorescence changes.[6] Despite the usefulness, the scope of these approaches is restricted to a few number of recognition events. In view of the collective limitations to design new binding modes and turn them into a fluorescence response, unbiased high-throughput screenings can be a practical strategy to accelerate the development of novel fluorescent probes.[7] Herein, we report the first systematic and unbiased analysis of the BODIPY fluorophores against a wide panel of biomolecules. The screening of a 317-member library against 94 biomolecules led to the discovery of two novel BODIPY probes for BSA and dopamine.

Fluorescent sensors must ideally display a moderate quantum yield that allows the observation of their fluorescence intensity increase/decrease upon interaction with the corresponding binding partners. Our group previously reported the combinatorial synthesis of 160 monostyryl-BODIPY dyes, which exhibited an average fluorescent quantum yield around 0.32.[8] In view of the potential of mono-styryl BODIPY dyes as both fluorescent turn-on and turn-off probes, we extended the mono-styryl BODIPY library (**BD**) using 505 aldehyde building blocks and the asymmetric 1,3 dimethyl-BODIPY scaffold in a Knoevenagel condensation reaction (Scheme 1). A total of 317 **BD** dyes with purities over 95% were collected for further evaluation, and showed broad absorbance and emission profiles (λ_{abs}: 525–616 nm, λ_{flu}: 540–656 nm), and an averaged quantum yield of 0.49 (for chemical structures and full spectroscopic characterization, see Tables S1 and S2 in Supporting Information†).

High-throughput unbiased screening strategies have been successfully used in drug discovery,[9] but they are still at an early stage in fluorescent sensor development. Unbiased sensor discovery screenings must enclose a number of biomolecule targets that embody broad biological events/interests and resemble the conditions in which the sensor will be eventually applied. Therefore, a total of 94 biomolecules in the range of their endogenous or effective concentrations were systematically combined with the **BD** compounds, and the resulting fluorescence pattern was examined. HEPES buffer was chosen as the vehicle since it can simulate better the intracellular conditions, and the 94 biomolecules were categorized in 10 sets: pH and



Scheme 1 Synthesis of the **BD** library. Reagents and conditions: (i) $POCl_3$, DCM, −5 °C for 3 h, then r.t. for 3 h; (ii) $BF_3{\cdot}OEt_2$, DIEA, 3h, r.t.; (iii) pyrrolidine/AcOH, R–CHO, ACN, microwave irradiation, (700 W).

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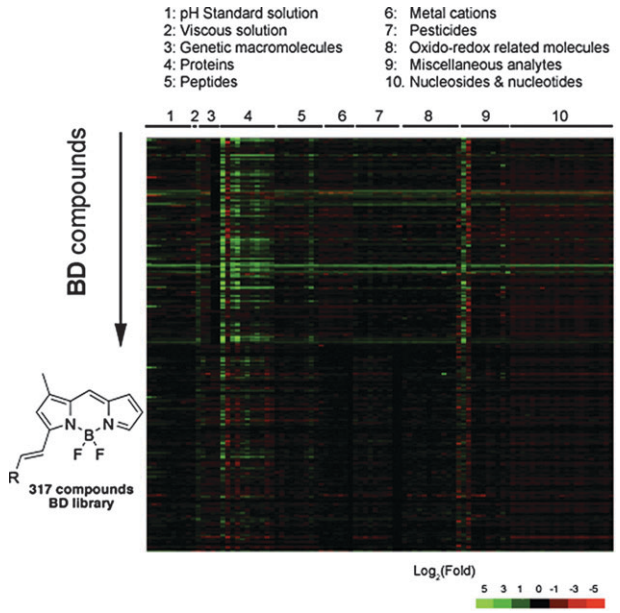

Fig. 1 Heatmap plot of the fluorescence pattern of 317 **BD** compounds against 94 biomolecules. Green and red colors indicate a fold change increase and decrease respectively at λ_{em}^{max} of each compound (Fold = $F_{analyte}/F_{vehicle}$).

viscous solutions, nucleotides and nucleosides, genetic macromolecules, peptides, proteins, metal cations, oxidation–reduction related molecules, pesticides, and other miscellaneous analytes (see Table S3 for details†). The fluorescence spectra of every compound was recorded at four serial analyte concentrations (94 analytes × 4 concentrations × 317 **BD** compounds = 119 192 data points), and the quality of the screening data was cross-validated by comparing dose-response patterns for a given biomolecule so that the number of false positive hits could be minimized.[10] The overall fluorescence responses of the 317 **BD** compounds were combined using a heatmap plot (Fig. 1). Heatmap plot represents fold change values of fluorescence emission as two color codes (green for turn-on and red for turn-off), which facilitated the deduction of the general behaviour of **BD** compounds as fluorescent probes: (1) derivatives containing a *p*-aminobenzyl or a heterocyclic group showed a very high fluorescence increase at low pH (Table S5†); (2) proteins showed the highest response amongst all analyte classes, and (3) many **BD** compounds showed a fluorescence intensity decrease in the presence of negatively charged biomolecules, such as genetic macromolecules (DNA, RNA) and nucleotides/nucleosides.

In addition to characterizing the general fluorescence profile of the **BD** library, the analysis of the unbiased screening data identified two compounds (**BD-101** and **BD-187**) with an outstanding turn-on/off responses (BSA and dopamine, respectively), and we further studied their application as fluorescent probes. **BD-101** showed a remarkable fluorescence turn-on effect upon binding to bovine serum albumin (BSA). Serum albumins are the major constituent of blood plasma,[11] and play a major role in transporting diverse endogenous biomolecules and drugs.[11,12] Several studies have been aimed at describing BSA drug binding sites [13] and their structural characterization,[14] as well as developing fluorescence probes for the different binding pockets.[15] However, the design of ligands that are species-selective is still challenging,[16] since serum albumins share a high sequence homology that makes them undistinguishable even by crystallographic methods.[14,17] Few BSA-selective fluorescent sensors are available, and to the best of our knowledge the most sensitive BSA probe (FA: 2-(6-diethylaminobenzo[b]furan-2-yl)-3-hydroxychromone) shows a limited 6-fold fluorescence increase.[18] **BD-101** underwent an exceptional 212-fold fluorescence increase with a dissociation constant (K_D) of 5.24 μM when binding to BSA (Fig. 2a, S2†), and the unbiased screening asserted its excellent selectivity profile with a negligible response against all other tested biomolecules (Fig. 2b). Furthermore, **BD-101** showed a clear selectivity for BSA when comparing 5 different serum albumins (bovine, human, rat, porcine, and sheep: Fig. S1†), being the most responsive BSA fluorescent sensor reported to date.

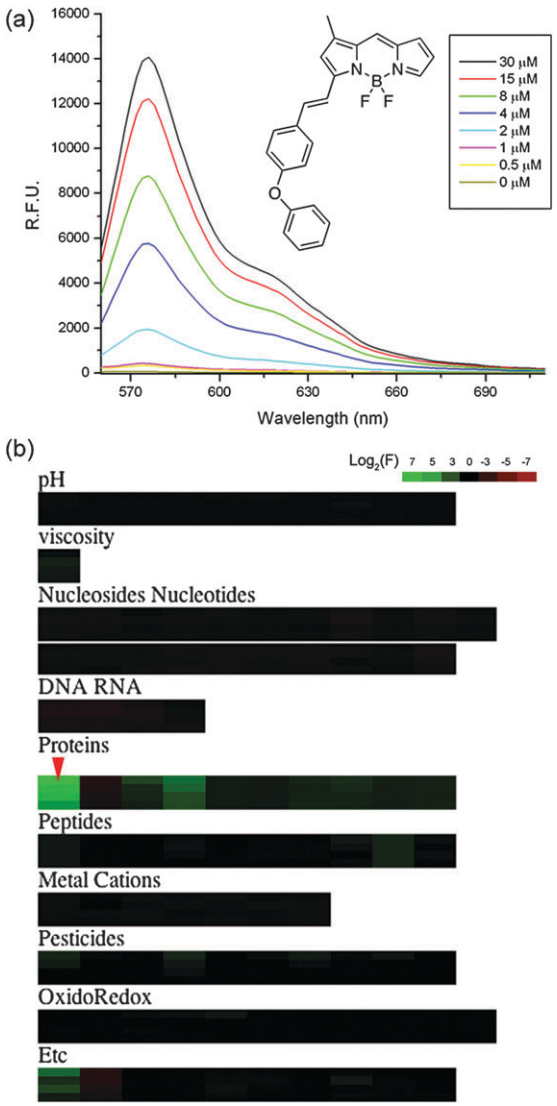

Fig. 2 (a) Fluorescence emission spectra of **BD-101** in the presence of serial concentrations of BSA ($\lambda_{excitation}$: 530 nm); (b) Fluorescence response profile of **BD-101** (10 μM) upon unbiased screening (detail decoding table is in Table S4†), and BSA was marked with a red arrow.

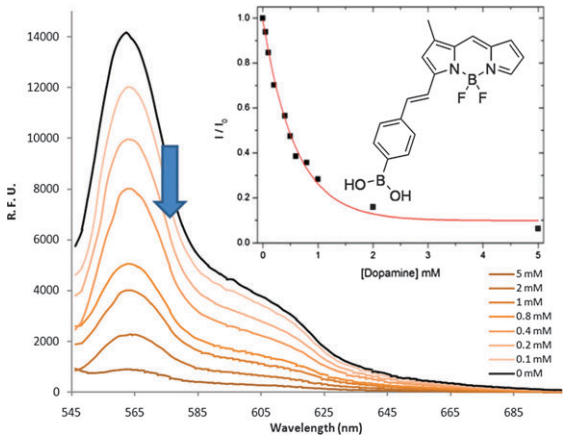

Fig. 3 Fluorescence quenching of **BD-187** spectra (10 μM) upon interaction with serial concentrations of dopamine (λ_{ex}: 520 nm). Fluorescence intensity was measured at 562 nm for quenching curve.

The diverse quantum yields of the **BD** library enabled the discovery of compounds behaving as fluorescent turn-off probes. **BD-187**, **BD-404** and **BD-405** displayed a significant fluorescence quenching upon interaction with dopamine, a catecholamine neurotransmitter.[19] These three **BD** compounds were the only library members containing a boronic acid group, which can potentially interact with the catechol moiety of dopamine.[20] Interestingly, the fluorescence quenching effect showed a strong dependency on the position of the boronic acid. Whereas the *ortho*-substituted analogue, **BD-405**, exhibited the weakest quenching effect, the *para*-substituted compound **BD-187** showed an exceptional 10-fold fluorescence emission decrease with a dissociation constant (K_D) of 0.49 μM (Fig. 3, S3†). **BD-187** quenching followed a linear correlation with dopamine concentration up to 1 mM, which exceeded the sensitivity of the IDA and dopamine sensors reported so far.[21]

In summary, we have systematically examined the fluorescence response of a diversity-oriented BODIPY library (**BD**) using an unbiased *in vitro* screening strategy. Based on the quantum yield changes against a variety of biomolecules, we could infer general trends of the BODIPY fluorescence response pattern, and discover two novel fluorescent probes: **BD-101** behaved as a turn-on sensor for BSA, while **BD-187** showed a remarkable fluorescence quenching upon interaction with dopamine. With this first unbiased screening of the fluorescence pattern of the BODIPY structure, we demonstrated the power and broad applicability of this approach for accelerating fluorescent sensor discovery, without prior implementation of a pre-established binding motif in the fluorophore structure.

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