CHEMISTRY
A EUROPEAN JOURNAL

FULL PAPER

DOI: 10.1002/chem.201302801

Allosteric Indicator Displacement Enzyme Assay for a Cyanogenic Glycoside

D. Amilan Jose,[a, b] Martin Elstner,[a] and Alexander Schiller*[a]

Abstract: Indicator displacement assays (IDAs) represent an elegant approach in supramolecular analytical chemistry. Herein, we report a chemical biosensor for the selective detection of the cyanogenic glycoside amygdalin in aqueous solution. The hybrid sensor consists of the enzyme β-glucosidase and a boronic acid appended viologen together with a fluorescent reporter dye. β-Glucosidase degrades the cyanogenic glycoside amygdalin into hydrogen cyanide, glucose, and benzaldehyde. Only the released cyanide binds at the allosteric site of the receptor (boronic acid) thereby inducing changes in the affinity of a formerly bound fluorescent indicator dye at the other side of the receptor. Thus, the sensing probe performs as allosteric indicator displacement assay (AIDA) for cyanide in water. Interference studies with inorganic anions and glucose revealed that cyanide is solely responsible for the change in the fluorescent signal. DFT calculations on a model compound revealed a 1:1 binding ratio of the boronic acid and cyanide ion. The fluorescent enzyme assay for β-glucosidase uses amygdalin as natural substrate and allows measuring Michaelis–Menten kinetics in microtiter plates. The allosteric indicator displacement assay (AIDA) probe can also be used to detect cyanide traces in commercial amygdalin samples.

Keywords: amygdalin · boronic acid · chemical biosensors · density functional calculations · fluorescence

Introduction

Selective and sensitive detection of biological and environmentally important agents in water is of significant interest in molecular biology, environmental monitoring, and food safety.[1] Hence, designing new sensors is one of the most important topics especially in analytical chemistry.[2] The receptor/spacer/reporter paradigm requires a receptor, which is covalently tethered to a reporter, albeit a chromophore or fluorophore. Often, analyte binding alters optical properties by a photoinduced electron transfer (PET) mechanism.[1d,3] However, supramolecular analytical chemists were fascinated of Anslyn's revitalized indicator displacement assays (IDAs).[4] They have become popular as supramolecular sensors because of their advantages over traditional receptor/spacer/reporter systems.[5] To date, a variety of IDAs have been introduced, such as colorimetric IDAs (C-IDA), fluorometric IDAs (F-IDA), metal complex IDAs (M-IDA), and enantiomeric IDAs (E-IDA).[6] Very recently, even a mechanically controlled indicator displacement assay (MC-IDA) has been presented by the groups of Anslyn and Ariga.[7] All mentioned types of displacement assays are based on the competition between an analyte and an indicator for binding to the receptor (host) at the same binding site. In contrast, a different system was reported by Singaram and co-workers,[8] in which the indicator is displaced by means of an allosteric interaction of an analyte with a receptor. Herein, the analyte binds at another site (allosteric site) of the host thereby inducing changes in the affinity of the indicator to the receptor. This can be called an allosteric indicator displacement assay (AIDA, Scheme 1).[9]

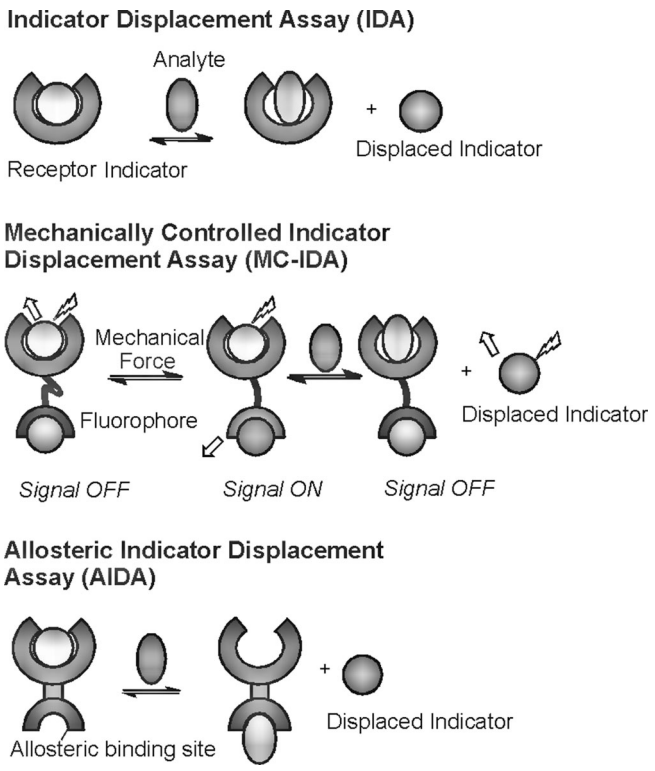
Indicator displacement assays have found prominent applications in supramolecular tandem enzyme assays in the group of Nau.[10] These assays rely on different binding affinities of indicator dye, substrate, and the corresponding product with the supramolecular receptor. They have been cleverly applied for monitoring enzymatic transformations involving amino acids, biogenic amines, amino aldehydes, and nucleotides.[11] Recently, supramolecular real-time fluorescent assays for the monitoring of the activity of carbohydrate active enzymes have been reported by Singaram group and our group. These were the first AIDA enzyme assays.[12] As a continuation of our ongoing endeavor to explore the AIDA system in real-time fluorescent enzyme assays, we identified cyanogenic glycosides as challenging substrates.[13]

Cyanogenic glycosides are of potential danger for mammals, because hydrogen cyanide (HCN) is produced by hydrolysis (spontaneous or enzymatically regulated reactions).[14] The amount of cyanogenic glycosides in plants is usually reported as the level of releasable HCN. The most familiar cyanogenic glycoside is amygdalin ([O-β-D-gluco-

[a] Dr. D. A. Jose, Dipl. Chem. M. Elstner, Prof. Dr. A. Schiller
Institute for Inorganic and Analytical Chemistry
Abbe Center of Photonics, Friedrich Schiller University Jena
Humboldtstrasse 8, 07743 Jena (Germany)
E-mail: alexander.schiller@uni-jena.de

[b] Dr. D. A. Jose
Current address: Department of Chemistry
National Institute of Technology
Kurukshetra, Haryana 136119 (India)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201302801.



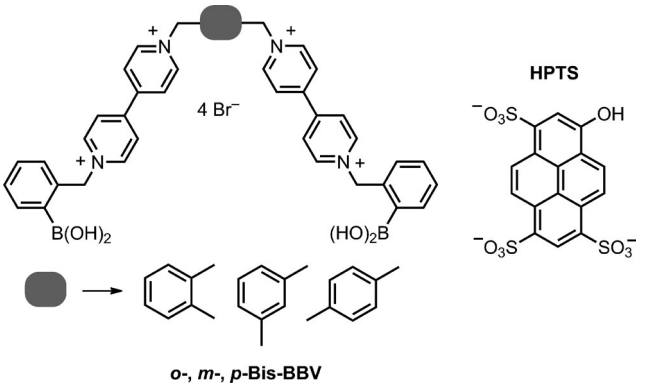
Scheme 1. Recent developments in the field of indicator displacement assays (IDA), such as mechanically controlled IDA[7] and allosteric IDA (AIDA).[9a]

pyranosyl-(1-6)-β-D-glucopyranosyloxy]benzeneacetonitrile).[15] Amygdalin can be found in kernels of food plants, such as apples, almonds, peaches, cherries, and apricots.[16] Although amygdalin is not toxic itself, HCN, liberated as a result of hydrolysis by acids or enzymes, causes acute toxicity.[17] Isolated amygdalin is widely sold as an antitumor natural product.[18] However, recent studies showed cyanogenic compounds to be dangerously toxic, as well as amygdalin itself as clinically ineffective in the treatment of cancer.[19] Taken orally, amygdalin is potentially lethal, because internal β-glucosidase releases HCN upon metabolism. β-Glucosidase is a glycoside hydrolase that acts upon β-1−4 bonds linking two glucose or glucose-substituted molecules. It is an exocellulase with specificity for a variety of β-D-glycoside substrates, for example, cellobiose. Recently, β-glucosidases have been used for enzymatic glycosylation of terpenoids.[13,20] Because of the potential health hazard associated with the ingestion of cyanide through consumption of cyanogenic plants and food, several analytical methods, such as colorimetric methods, HPLC, GC-MS, potentiometric, and electrochemical methods have been developed for detection of cyanogenic glycosides and their metabolites. Most of these labor-intensive methods need sophisticated analytical instruments.[21] Hence, the development of rapid and robust detection methods is a very important task. Herein, we report a boronic acid appended bis-viologen-based hybrid sensor system for the detection of amygdalin and cyanide in water by using fluorescence spectroscopy. The sensing system works with the AIDA approach; cyanide ions, produced during an enzymatic reaction of amygdalin, can be detected in situ.

Results and Discussion

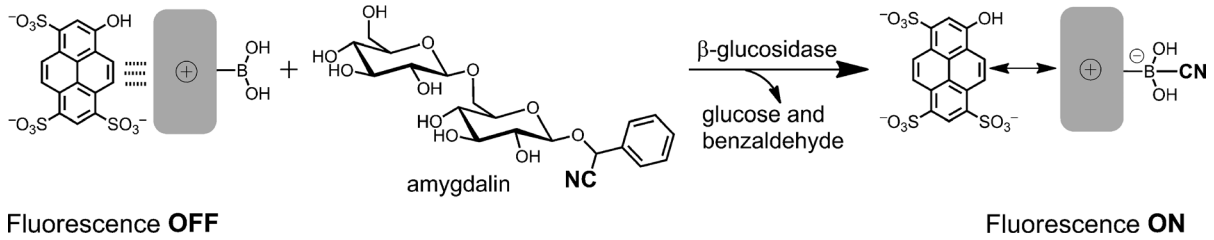
Selection of the receptor and experimental design: The Singaram group has generated a two-component sensing system for continuous monitoring of the blood-glucose level.[8b,22] The system is originally composed of 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) as the reporter unit and derivatives of bis-boronic acid-appended mono viologens (4,4′-*o*-BBV and 3,3′-*o*-BBV) as receptors for saccharides.[23] In collaboration with the Singaram group, we have implemented 4,4′-*o*-BBV/HPTS and 3,3′-*o*-BBV/HPTS in selective enzymatic assays for sucrose phosphorylase (SPO) and phosphoglucomutase (PGM).[12] The sensing system is perfectly suited for the screening of glycosylation reactions with SPO and sucrose as donor, because fructose is always released as product.[9c,13]

Bis-viologens are superior quenchers compared to monoviologen derivatives (Scheme 2).[24] In general, boronic acid based receptors have been utilized for the detection of



Scheme 2. Chemical structures of isomers of bis-boronic acid receptors (*o*-Bis-BBV, *m*-Bis-BBV and *p*-Bis-BBV) and indicator dye (HPTS) used for the two-component sensor system.

anionic species including fluoride and cyanide ions.[25] Thus, we used the second-generation bis-viologen isomers for our study (Scheme 2). All bis-viologens were synthesized by a previously reported procedure and characterized by standard analytical methods.[24] Modification in the synthesis protocol can be found in the Supporting Information. The quenching efficiency and preliminary binding studies of monosaccharides with the Bis-BBV isomers has been reported by Singaram and co-workers,[9a,24] but the application of *o*-, *m*-, *p*-Bis-BBV receptors in enzyme assays with cyanogenic glycosides and for the detection of cyanide anions at physiological conditions is not known.



Scheme 3. Working principle of cyanide detection from amygdalin by an allosteric indicator displacement enzyme assay.

Enzyme assay with the cyanogenic glycoside amygdalin: Enzyme assays are necessary to evaluate the performance of enzyme variants in high-throughput screening.[12b] Almond β-glucosidase has been used for the enzymatic glycosylation of terpenoids. However, reaction conditions and enzyme variants have to be still screened and improved for higher product yields.[13,20] Thus, real-time enzyme assays for β-glucosidases are of high interest. Natural substrates, such as cyanogenic glycosides, liberate cyanide through enzymatic hydrolysis by β-glucosidase.[26] Upon reaction of the cyanogenic glycoside amygdalin, the products glucose, benzaldehyde, and HCN are formed (Scheme 3).

The reaction of amygdalin with β-glucosidase in the presence of *o*-Bis-BBV/HPTS was studied by time-dependent emission intensity measurements (F/F_0; Figure 1). Fluorescence experiments were carried out by using a microtiter plate reader in 384-well plates with 100 μL as a working volume (time resolution 30 s). The receptor isomer *o*-Bis-BBV (4×10^{-5} M) containing HPTS (4×10^{-6} M) dye was freshly prepared in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (100 mM, pH 7.20). In the absence of β-glucosidase, the fluorescence of *o*-Bis-BBV/HPTS with amygdalin (60.5 mM) was in a quenched state and virtually stable. In presence of β-glucosidase, the fluorescence intensity increased significantly, and the reaction was followed in regular time intervals (Figure 1). Similar results were obtained by performing the enzyme assay in a classical fluorescence spectrometer with a better time resolution. We show also the change in emission intensity at different enzyme concentrations (0 to 1.19 UN mL^{-1}). A hyperbolic saturation curve was obtained by plotting the initial velocities V_i against the concentration of the enzyme (Figure 1, inset). For the enzyme assay, we used the *o*-Bis-BBV isomer due to the very low glucose response compared to the *m*-and *p*-Bis-BBV isomers.[24]

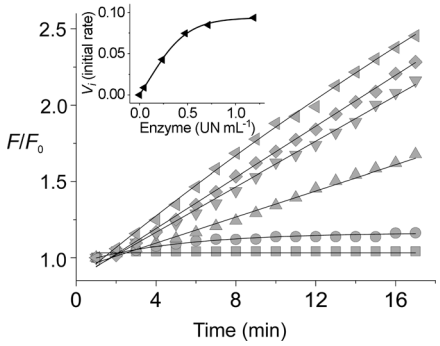


Figure 1. Time-dependent fluorescence measurements of *o*-Bis-BBV/HPTS ($4\times10^{-5}/4\times10^{-6}$ M) in the presence of amygdalin (60.5 mM) with varying concentrations of β-glucosidase (from down to top 0, 0.048, 0.238, 0.476, 0.714, and 1.19 UN mL^{-1}) in water (100 mM HEPES, pH 7.20) at 37 °C. Inset: plot of initial rate V_i versus β-glucosidase concentration.

We presume that the only enzymatic product, which binds to the boronic acid receptor, is cyanide. It displaces the indicator HPTS from the two-component sensing ensemble and the fluorescence turns on. This allows real-time monitoring of the β-glucosidase-mediated reaction (Figure 1). In contrast, existing enzyme assays for β-glucosidase use labeled substrates with fluorogenic or chromogenic groups, such as resorufin β-D-glucopyranoside, fluorescein di-(β-D-glucopyranoside, and *p*-nitrophenyl-β-D-glucopyranoside.[27] Upon enzymatic hydrolysis, the substrates split into glucose and reporter agent. The signaling mechanism in our system involves ground-state complex formation between dye and a cationic boronic acid receptor that facilitates electron transfer from the dye to the bipyridinium salt, resulting in a fluorescence quenching of the dye (Scheme 3). When the enzymatic product cyanide strongly interacts with the boronic acid moieties of the ground-state complex at physiological pH, the cationic viologen is partially neutralized. This weakens the complex and its quenching efficiency. As a consequence, an increase in fluorescence intensity is observed.

In principle, the hydrolysis products of amygdalin glucose and cyanide, or both of them, could be responsible for the increase of fluorescence intensity. Thus, we have carried out several experiments to elucidate unambiguously the "working" analyte. Among receptors for the recognition of fluoride and cyanide ions developed to date, boron compounds are shown to be one of the most effective molecular platforms due to their high affinity toward nucleophilic anions.[25,28] Many chemical receptors are available for the detection of cyanide ions.[1d,29] However, most of them failed to bind in pure water, and cyanide binding often interferes with other anionic species. Therefore, the search for a highly selective cyanide sensor in pure water is still a challenging task.[25b,28,30]

Anion binding in water: We have investigated the binding properties of the isomers *o*-, *m*-, *p*-Bis-BBV, and HPTS (4×

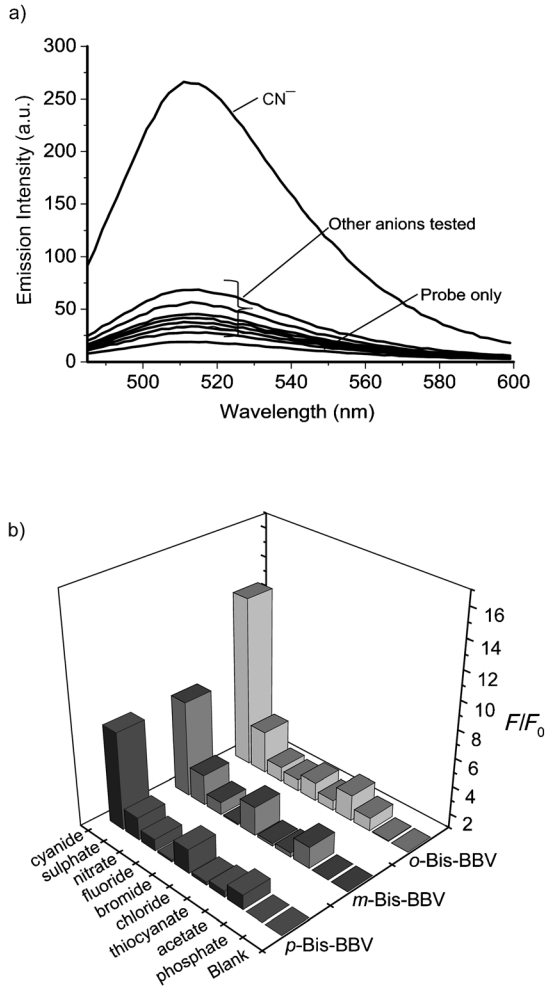


Figure 2. a) Fluorescence spectra of *o*-Bis-BBV/HPTS = $4\times10^{-5}/4\times10^{-6}$ M with inorganic anions, such as F^-, CN^-, Br^-, Cl^-, SO_4^{2-}, $H_2PO_4^-$, NO_3^-, SCN^-, and AcO^- (5×10^{-3} M); b) fluorescence emission intensity modulation (F/F_0) of *o*-, *m*-, and *p*-Bis-BBV/HPTS with anions. Reaction conditions: water with 100 mM HEPES, pH 7.20 at room temperature.

$10^{-5}/4\times10^{-6}$ M) with a collection of inorganic anions, such as F^-, CN^-, Br^-, Cl^-, SO_4^{2-}, $H_2PO_4^-$, NO_3^-, SCN^-, and AcO^- (5×10^{-3} M) in water at pH 7.20 by using fluorescence spectroscopy measurements.

Without addition of anions, the fluorescence intensity of the *o*-Bis-BBV/HPTS system was quenched (Figure 2). A strong fluorescence signal modulation (F/F_0) was only observed in the presence of the cyanide anion. Inorganic anions obtain high free energies of hydration ΔG_{Hydr}. Therefore, anion hosts have to compete more effectively with the surrounding medium. That makes it especially difficult to bind fluoride, sulfate, and phosphate anions in aqueous solution.[31] It is also known from literature that cationic ammonium boranes can be used for cyanide complexation because of favorable Coulombic receptor-cyanide attractions.[32]

A stepwise OH^-/CN^- exchange process of a boronic acid with the cyanide ion in aqueous solution is possible.[25a] Thus, we performed DFT energy calculations on the reaction of cyanide and hydroxyl ions with a boronic acid moiety. For computational simplicity, bis-BBV receptors have been modeled as phenyl boronic acid ($pB(OH)_2$; Figure 3). Previously, Larkin and co-workers showed the usability of different DFT methods for energy calculations with boronic acids.[33] Because of the known problems to estimate dative bond energies with the B3LYP functional, we have also used the M06-2X functional.[34] It has been shown that the M06-2X functional predicts accurately interaction energies in boron–nitrogen adducts and Raman spectra of protected boronic acids.[35] The calculated results show that the formation of an anionic $pB(OH)_3^-$ through the addition of a hydroxyl ion is an exergonic process with a Gibbs energy of -187.4 kJ mol^{-1}. In contrast, this reaction is with -51.8 kJ mol^{-1} less exergonic in the PCM model calculations (in the case of the M06-2X functional, it is -91.7 kJ mol^{-1}, see Table S1 in the Supporting Information).

Addition of a single cyanide ion to $pB(OH)_2$ forms a tetrahedral species $pB(OH)_2CN^-$ in an exergonic reaction, with ΔG of -48.6 kJ mol^{-1}. It changes to slightly endergonic values in aqueous-phase calculations ($\Delta G = 33.7$ kJ mol^{-1} (B3LYP) and $\Delta G = 13.7$ kJ mol^{-1} (M06-2X)). However, the substitution of three hydroxide groups by three cyanide ions is a strong endergonic process in all calculated cases (Table S1 in the Supporting Information). These theoretical calculations indicate for the first time that threefold cyanide-coordinated boron complex $pB(CN)_3^-$ is not favorable as shown in earlier reports,[25a,b] and formation of one cyanide-coordinated boron complex $pB(OH)_2CN^-$ is the most favorable.[36]

The generation of an anionic boronate displaces the HPTS molecule, which is responsible for the fluorescent enhancement (Scheme 3). Other anions show very small to negligible changes in the emission intensity (Figure 2). They could not form anionic boronates in water. Fluorescence titration experiments were carried out to determine the sensitivity of the cyanide sensing system *o*-Bis-BBV/HPTS. From these experiments, we found that the probe is able to detect selectively cyanide in the millimolar range with a detection limit of around 4 mM in water (Figure S4 in the Supporting Information). In a similar way, fluorescence titrations of *m*- and *p*-Bis-BBV with anionic substrates were conducted (Figures S5 and S6 in the Supporting Information). Interestingly, from the three isomers of the boronic acid receptor, *o*-Bis-BBV isomer showed the strongest fluorescence intensity modulation (F/F_0). Fitting of fluorescent spectral data with a nonlinear binding isotherm[8b] gave similar apparent binding constants of 207 ± 20, 135 ± 20, and 137 ± 20 M^{-1} for *o*-Bis-BBV, *m*-Bis-BBV, and *p*-Bis-BBV isomers, respectively (Figures S4–S6 in the Supporting Information). We were not able to calculate the binding affinities with other anions, because they showed very small changes in fluorescence emission (Figure 2 and S2 and S3 in the Supporting Information).

Interference study: A disadvantage of many optical chemosensors for cyanide is that they are easily disturbed by other anions. Most of the systems that are based on boron deriva-

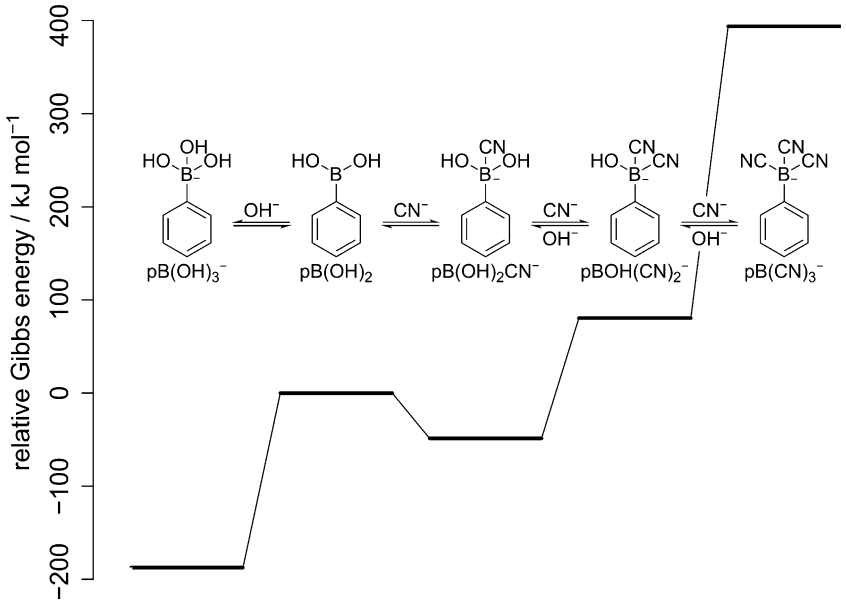


Figure 3. Possible OH^-/CN^- exchange process in phenyl boronic acid ($pB(OH)_2$) used for the DFT calculations and comparison of relative Gibbs energy (ΔG) for different species of phenyl boronic acid obtained upon OH^-/CN^- exchange process, the values were calculated by using B3LYP/aug-cc-pVTZ.

tives show high competition between cyanide and fluoride.[32,37] To investigate the anion interference in cyanide binding, we have measured the fluorescence response of cyanide (8.0×10^{-3} M) together with common anions, such as F^-, Br^-, Cl^-, SO_4^{2-}, $H_2PO_4^-$, NO_3^-, SCN^-, and AcO^-. These anions were added in excess (4.0×10^{-2} M). Very small or negligible fluorescence changes can be observed in the presence of all mentioned anions (Figure S7 in the Supporting Information). These results indicate that the sensing system is highly selective towards cyanide over other anions in water. We have also investigated the interference of monosaccharides with cyanide. The monosaccharides glucose and galactose did not show any fluorescence changes at concentrations of 4.0×10^{-2} M, fructose showed very little interference with cyanide (Figure S7 in the Supporting Information). These findings further validate that cyanide is solely responsible for the change in emission intensity of the β-glucosidase assay (Figure 3).

In addition, we performed control experiments with the substrates cellobiose, lactose, and maltose (Figure S11 in the Supporting Information). β-Glucosidase has also the ability to hydrolyze these substrates and produce glucose as one of the products.[38] As shown in Figure 4, the sensing system *o*-Bis-BBV/HPTS did not show any detectable changes in the fluorescence spectra even when measuring the fluorescence after 6 h. These results clearly demonstrate that glucose, produced during hydrolysis of cellobiose, lactose, or maltose, has no or a very weak effect on the fluorescence signal (Figure 4). As a control, amygdalin did not show any fluorescent change in absence of β-glucosidase (Figure S10 in the Supporting Information). These experiments demonstrate how amygdalin can be selectively determined of other glycosides in aqueous solution. The detection limit of amygdaline is 9 mM after 30 min of enzyme reaction.

We investigated the Michaelis–Menten (MM) behavior of the β-glucosidase assay in the presence of *o*-Bis-BVV/HPTS by measuring the kinetics with varying amygdalin concentrations (Figure S8 in the Supporting Information). Initial rate constants were calculated within the first five minutes of the β-glucosidase assay. The rate constants were plotted against the concentration of amygdalin (Figure S9 in the Supporting Information). The plot followed a typical MM saturation curve and gave apparent K_m and V_{max} values of 23.8 ± 1 mM and 0.05 ± 0.001 mM min^{-1}, respectively. These measured kinetic values for β-glucosidase activity are different from literature values ($K_m=1.45$ mM at 50 °C in 50 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer, pH 6.2).[38b,39] Changes in assay conditions and the use of a different monitoring method could be the reason.

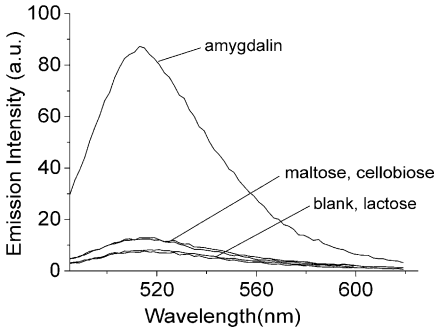


Figure 4. Fluorescence spectra of *o*-Bis-BBV/HPTS ($4\times10^{-5}/4\times10^{-6}$ M, blank) with 60.5 mM of substrates amygdalin, maltose, cellobiose, and lactose in water (HEPES, 100 mM, pH 7.20) at 37 °C in presence of enzyme β-glucosidase (0.356 UN mL^{-1}). The emission spectra were recorded after 6 h.

Cyanide contamination in commercial amygdalin sources: Amygdalin has been used as an anticancer treatment in humans worldwide. Although many anecdotal reports are available, no controlled clinical trials of amygdalin have ever been conducted.[23,24] However, commercial preparations of amygdalin are still sold through internet. These problematic products may contain contaminations of CN^- and/or glucosidases. To quantify the amount of CN^-, fluores-

cent measurements with potassium cyanide (KCN)-spiked amygdalin samples were carried out. The fluorescence intensity at $\lambda = 518$ nm increased in a linear fashion with a higher percentage of KCN in the amygdalin samples (0–40%).

A similar change in emission intensity was also observed with enzymatic hydrolysis of amygdalin. The content of the total cyanide content present upon decomposition of amygdalin can be estimated with a calibration curve generated from the cyanide-spiked amygdalin (Figure 5, inset). The detection limit of CN^- in amygdalin is around 2 wt%. In addition, we were able to discriminate different amygdalin samples by their purity. We used samples from the following commercial sources: Aldrich (purity 96%), Alfa–Aesar (purity 98%), and TCI-Europe (purity 99%). We measured the fluorescence intensities at different amygdalin concentrations (22, 55, 82 mM) in independent triplicates. It is important to note that we are analyzing traces of CN^- in these "pure" commercial samples. The dataset was analyzed by linear discriminant analysis.[8c,23a] A clear separation of all amygdalin samples was obtained with a small variance (Figure S13 in the Supporting Information). This shows that the current AIDA probe not only works well in a fluorescent β-glucosidase assay and for the detection of amygdalin among other oligosaccharides. They also offer a high potential of being used in the detection of cyanide in amygdalin samples and other cyanogenic glycosides.

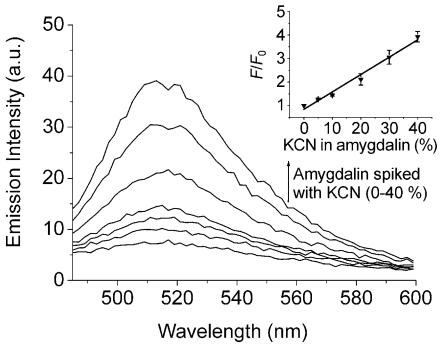


Figure 5. Fluorescence intensity change of *o*-Bis-BBV/HPTS ($4\times10^{-5}/4\times10^{-6}$ M) with amygdalin (75 mM) spiked with KCN in water (100 mM HEPES, pH 7.20). Inset: plot of spiked percentage of KCN in amygdalin versus change in emission intensity at $\lambda = 518$ nm.

Conclusion

We have described the ability of boronic acid appended bisviologen quenchers (*o*-, *m*-, *p*-Bis-BBV) to be used as probes for monitoring the enzymatic reaction of amygdalin with β-glucosidase. We confirmed that among different enzymatic products, cyanide is exclusively responsible for the change in emission intensity of the sensing system *o*-Bis-BBV/HPTS at physiological conditions. It is the first fluorescent displacement assay to detect the cyanide ion released from amygdalin. The cyanide sensing mechanism works under allosteric indicator displacement assay. The binding behavior of cyanide with boronic acid receptors was investigated by comparative DFT methods to confirm a single OH^-/CN^- exchange process in a model compound. We showed that threefold cyanide-coordinated boronate complex is not favorable. The sensing mechanism has the potential of being used in amygdalin detection and in the monitoring of different levels of cyanide contamination in the commercial amygdalin samples by fluorescent spectroscopy. We anticipate that allosteric indicator displacement enzyme assays will be used in future as powerful tools for the detection of complex bioanalytes and for the evaluation of new enzyme variants.

Acknowledgements

All authors thank the EC for financial support through the FP7-project "Novosides" (grant agreement nr. KBBE-4-265854; www.novosides.eu). A.S. is grateful to the Carl Zeiss Foundation for a Junior Professor fellowship. We also acknowledge the support of the Center of Medical Optics and Photonics (CeMOP) and the Jena Center of Soft Matter (JCSM) at the Friedrich Schiller University Jena.

[1] a) V. M. Mirsky, A. K. Yatsimirsky, *Artificial Receptors for Chemical Sensors*, Wiley-VCH, Weinheim, **2011**, p. 469; b) O. S. Wenger, *Chem. Rev.* **2013**, *113*, 3686–3733; c) J. Rebek, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 10423–10424; d) R. Martínez-Mañez, F. Sancenon, *Chem. Rev.* **2003**, *103*, 4419–4476; e) A. W. Czarnik, *Nature* **1998**, *394*, 417–418; f) S. W. Thomas, G. D. Joly, T. M. Swager, *Chem. Rev.* **2007**, *107*, 1339–1386.

[2] a) A. P. Umali, E. V. Anslyn, *Curr. Opin. Chem. Biol.* **2010**, *14*, 685–692; b) X. Lou, D. Ou, Q. Li, Z. Li, *Chem. Commun.* **2012**, *48*, 8462–8477; c) J. Chan, S. C. Dodani, C. J. Chang, *Nat. Chem.* **2012**, *4*, 973–984.

[3] C. R. Wade, A. E. J. Broomsgrove, S. Aldridge, F. P. Gabbai, *Chem. Rev.* **2010**, *110*, 3958–3984.

[4] a) S. L. Wiskur, H. Ait-Haddou, J. J. Lavigne, E. V. Anslyn, *Acc. Chem. Res.* **2001**, *34*, 963–972; b) D. Leung, S. O. Kang, E. V. Anslyn, *Chem. Soc. Rev.* **2012**, *41*, 448–479.

[5] a) E. V. Anslyn, *J. Org. Chem.* **2007**, *72*, 687–699; b) D.-J. Oh, K.-M. Kim, K.-H. Ahn, *Chem. Asian J.* **2011**, *6*, 2034–2039; c) M. Hu, G. Feng, *Chem. Commun.* **2012**, *48*, 6951–6953; d) S.-K. Sun, K.-X. Tu, X.-P. Yan, *Analyst* **2012**, *137*, 2124–2128; e) X. Zhang, E. V. Anslyn, X. Qian, *Supramol. Chem.* **2012**, *24*, 520–525.

[6] B. T. Nguyen, E. V. Anslyn, *Coord. Chem. Rev.* **2006**, *250*, 3118–3127.

[7] K. Sakakibara, L. A. Joyce, T. Mori, T. Fujisawa, S. H. Shabbir, J. P. Hill, E. V. Anslyn, K. Ariga, *Angew. Chem. Int. Ed.* **2012**, *51*, 9643–9646.

[8] a) S. Gamsey, N. A. Baxter, Z. Sharrett, D. B. Cordes, M. M. Olmstead, R. A. Wessling, B. Singaram, *Tetrahedron* **2006**, *62*, 6321–6331; b) S. Gamsey, A. Miller, M. M. Olmstead, C. M. Beavers, L. C. Hirayama, S. Pradhan, R. A. Wessling, B. Singaram, *J. Am. Chem. Soc.* **2007**, *129*, 1278–1286; c) A. Schiller, R. A. Wessling, B. Singarum, *Angew. Chem.* **2007**, *119*, 6577–6579; *Angew. Chem. Int. Ed.* **2007**, *46*, 6457–6459.

[9] a) A. Schiller, B. Vilozny, R. A. Wessling, B. Singaram, *Rev. Fluoresc.* **2011**, *8*, 155–191; b) M. Elstner, K. Weisshart, K. Muellen, A. Schiller, *J. Am. Chem. Soc.* **2012**, *134*, 8098–8100; c) B. Pignataro, A. Schiller in *Molecules at Work: Going Beyond Glucose Sensing with Boronic Acid Receptors*, Wiley-VCH, Weinheim, **2012**, pp. 315–338.

[10] R. N. Dsouza, A. Hennig, W. M. Nau, *Chem. Eur. J.* **2012**, *18*, 3444–3459.
[11] a) G. Ghale, N. Kuhnert, W. M. Nau, *Nat. Prod. Commun.* **2012**, *7*, 343–348; b) M. Florea, S. Kudithipudi, A. Rei, M. J. Gonzalez-Alvarez, A. Jeltsch, W. M. Nau, *Chem. Eur. J.* **2012**, *18*, 3521–3528; c) G. Ghale, V. Ramalingam, A. R. Urbach, W. M. Nau, *J. Am. Chem. Soc.* **2011**, *133*, 7528–7535; d) M. Florea, W. M. Nau, *Org. Biomol. Chem.* **2010**, *8*, 1033–1039; e) W. M. Nau, G. Ghale, A. Hennig, H. Bakirci, D. M. Bailey, *J. Am. Chem. Soc.* **2009**, *131*, 11558–11570.
[12] a) B. Vilozny, A. Schiller, R. A. Wessling, B. Singaram, *Anal. Chim. Acta* **2009**, *649*, 246–251; b) J.-L. Reymond, V. S. Fluxa, N. Maillard, *Chem. Commun.* **2009**, 34–46.
[13] T. Desmet, W. Soetaert, P. Bojarova, V. Kren, L. Dijkhuizen, V. Eastwick-Field, A. Schiller, *Chem. Eur. J.* **2012**, *18*, 10786–10801.
[14] J. Vetter, *Toxicon* **2000**, *38*, 11–36.
[15] A. V. Morant, N. Bjarnholt, M. E. Kragh, C. H. Kjaergaard, K. Joergensen, S. M. Paquette, M. Piotrowski, A. Imberty, C. E. Olsen, B. L. Moeller, S. Bak, *Plant Physiol.* **2008**, *147*, 1072–1091.
[16] D. A. Jones, *Phytochemistry* **1998**, *47*, 155–162.
[17] T. Tatsuma, K. Komori, H. H. Yeoh, N. Oyama, *Anal. Chim. Acta* **2000**, *408*, 233–240.
[18] C. Holden, *Science* **1976**, *193*, 982–985.
[19] a) J. D. Khandekar, H. Edelman, *JAMA J. Am. Med. Assoc.* **1979**, *242*, 169–171; b) E. S. Schmidt, G. W. Newton, S. M. Sanders, J. P. Lewis, E. E. Conn, *JAMA J. Am. Med. Assoc.* **1978**, *239*, 943–947.
[20] F. Rivas, A. Parra, A. Martinez, A. Garcia-Granados, *Phytochem. Rev.* **2013**, 1–13.
[21] a) T. Tatsuma, K. Tani, N. Oyama, H.-H. Yeoh, *J. Electroanal. Chem.* **1996**, *407*, 155–159; b) U. Backofen, F. M. Matysik, G. Werner, *Fresenius J. Anal. Chem.* **1996**, *356*, 271–273; c) H. H. Yeoh, V. D. Truong, *Food Chem.* **1993**, *47*, 295–298; d) H. H. Yeoh, *Biotechnol. Tech.* **1993**, *7*, 761–764; e) J. L. Lambert, J. Ramasamy, J. V. Paukstelis, *Anal. Chem.* **1975**, *47*, 916–918.
[22] a) D. B. Cordes, B. Singaram, *Pure Appl. Chem.* **2012**, *84*, 2183–2202; b) B. Vilozny, A. Schiller, R. A. Wessling, B. Singaram, *J. Mater. Chem.* **2011**, *21*, 7589–7595.
[23] a) A. Schiller, B. Vilozny, R. A. Wessling, B. Singaram, *Anal. Chim. Acta* **2008**, *627*, 203–211; b) J. N. Camara, J. T. Suri, F. E. Cappuccio, R. A. Wessling, B. Singaram, *Tetrahedron Lett.* **2002**, *43*, 1139–1141.
[24] Z. Sharrett, S. Gamsey, P. Levine, D. Cunningham-Bryant, B. Vilozny, A. Schiller, R. A. Wessling, B. Singaram, *Tetrahedron Lett.* **2008**, *49*, 300–304.
[25] a) R. Badugu, J. R. Lakowicz, C. D. Geddes, *J. Am. Chem. Soc.* **2005**, *127*, 3635–3641; b) R. Badugu, J. R. Lakowicz, C. D. Geddes, *Dyes Pigm.* **2005**, *64*, 49–55; c) J. V. Ros-Lis, R. Martinez-Manez, J. Soto, *Chem. Commun.* **2005**, 5260–5262; d) Y. Kim, H.-S. Huh, M. H. Lee, I. L. Lenov, H. Zhao, F. P. Gabbai, *Chem. Eur. J.* **2011**, *17*, 2057–2062.
[26] T. Yamashita, T. Sano, T. Hashimoto, K. Kanazawa, *Int. J. Food Sci. Technol.* **2007**, *42*, 70–75.
[27] a) G. M. King, *Appl. Environ. Microbiol.* **1986**, *51*, 373–380; b) A. K. Grover, D. D. Macmurchie, R. J. Cushley, *Biochim. Biophys. Acta Enzymol.* **1977**, *482*, 98–108.
[28] K. C. Song, K. M. Lee, N. V. Nghia, W. Y. Sung, Y. Do, M. H. Lee, *Organometallics* **2013**, *32*, 817–823.
[29] a) Z. Xu, X. Chen, H. N. Kim, J. Yoon, *Chem. Soc. Rev.* **2010**, *39*, 127–137; b) U. Reddy, G. P. Das, S. Saha, M. Baidya, S. K. Ghosh, A. Das, *Chem. Commun.* **2013**, *49*, 255–257; c) S. Saha, A. Ghosh, P. Mahato, S. Mishra, S. K. Mishra, E. Suresh, S. Das, A. Das, *Org. Lett.* **2010**, *12*, 3406–3409.
[30] a) Y.-K. Yang, J. Tae, *Org. Lett.* **2006**, *8*, 5721–5723; b) C. Männel-Croisé, F. Zelder, *Inorg. Chem.* **2009**, *48*, 1272–1274; c) C. Männel-Croisé, F. Zelder, *ACS Appl. Mater. Interfaces* **2012**, *4*, 725–729.
[31] Z.-h. Lin, S.-j. Ou, C.-y. Duan, B.-g. Zhang, Z.-p. Bai, *Chem. Commun.* **2006**, 624–626.
[32] T. W. Hudnall, F. P. Gabbaie, *J. Am. Chem. Soc.* **2007**, *129*, 11978–11986.
[33] a) J. D. Larkin, K. L. Bhat, G. D. Markham, B. R. Brooks, H. F. Schaefer, C. W. Bock, *J. Phys. Chem. A* **2006**, *110*, 10633–10642; b) J. D. Larkin, J. S. Fossey, T. D. James, B. R. Brooks, C. W. Bock, *J. Phys. Chem. A* **2010**, *114*, 12531–12539.
[34] a) H. A. LeTourneau, R. E. Birsch, G. Korbeck, J. L. Radkiewicz-Poutsma, *J. Phys. Chem. A* **2005**, *109*, 12014–12019; b) B. G. Janesko, *J. Chem. Theory Comput.* **2010**, *6*, 1825–1833.
[35] D. N. Reinemann, A. M. Wright, J. D. Wolfe, G. S. Tschumper, N. I. Hammer, *J. Phys. Chem. A* **2011**, *115*, 6426–6431.
[36] L. Feng, Y. Wang, F. Liang, W. Liu, X. Wang, H. Diao, *Sens. Actuators B* **2012**, *168*, 365–369.
[37] Z. Ekmekci, M. D. Yilmaz, E. U. Akkaya, *Org. Lett.* **2008**, *10*, 461–464.
[38] a) Y. B. Tewari, R. N. Goldberg, *J. Biol. Chem.* **1989**, *264*, 3966–3971; b) F. F. Zanoelo, M. d. L. T. d. M. Polizeli, H. F. Terenzi, J. A. Jorge, *FEMS Microbiol. Lett.* **2004**, *240*, 137–143.
[39] S. Yang, Z. Jiang, Q. Yan, H. Zhu, *J. Agric. Food Chem.* **2008**, *56*, 602–608.

Received: July 17, 2013
Revised: August 30, 2013
Published online: October 7, 2013

