Detection of Explosive Compounds

Using Photosystem II -based Biosensor

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Abstract

The efficacy of a Photosystem II (PS II) -based biosensor for the detection of explosive compounds has been explored. The idea is based on the close similarities in the chemical structures of the widespread explosives and herbicides, with the latter known to inhibit functioning of the PS II by attaching to the binding site of the Q_B mobile plastoquinone electron acceptor. The gold screen-printed electrodes (Au-SPE) functionalized with PS II -enriched particles were used for the detection of explosives in a droplet biosensor configuration. A crude preparation of PS II produced from spinach leaves, known as BBY particles, was employed to modify the Au-SPE working electrode employing BSA-glutaraldehyde-based immobilization procedure. Inhibition of the PS II functioning was detected by photo-electrochemical measurements in the presence of a mediator (either non-native quinone or ferricyanide). The biosensor was highly responsive to herbicides (as expected) as well as to picric acid, with limits of detection in the nanomolar range, but trace detection of trinitrotoluene (TNT) was not effective. The detection limit for picric acid was 25 nM as compared to ~400 nM for TNT with duroquinone mediator. Low affinity of PS II to TNT has been corroborated by means of DCPIP assay; possible reasons for low affinity are discussed.

Keywords: Photosystem II, Herbicides, picric acid, TNT, Screen-printed electrode.

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1. Introduction

Nitric explosives (e.g. TNT, RDX, nitrophenols) comprise one of the largest classes of explosives and are frequently used by terrorist organizations around the world because of their ease of manufacture and because of their relative accessibility due to the extensive use by the military and commercial enterprises. Various methods for explosives detection have been developed or proposed so far. Unfortunately, the most sensitive of them, such as liquid or gas chromatography, are relatively slow and require instrumentation that is too complex and expensive for use in the field. Various other approaches have been recently proposed. For example, quenching of the fluorescence of pyrene [1], Nile Red [2] or semiconductor organic polymers [3] by nitric explosives was described. The latter approach is currently being commercialized [4]. Selective explosives detection using immunosensors is based on the high affinity and selectivity of the analyte / antibody interaction that is detected using either plasmon resonance [5] or changes in the fluorescence or bioluminescence of the reporter proteins incorporated into the antibody [6]. However, antibodies are difficult to prepare, are very analyte-specific and too sensitive to the environment. Summarizing, there is an apparent need to continue the development of new methods of explosives detection, with the long-term objective of making them faster, less expensive, more sensitive and more reliable. One should also note that for the purposes of reducing the number of the false positives, it would be beneficial to introduce systems with several "orthogonal" (i.e. utilizing different physical, chemical or biological principles) detection technologies.

In this paper we discuss the ability of a biosensor based on inhibition of the Photosystem II (PS II) to sense trace levels of explosive compounds. The idea for such

sensor is inspired by recent progress in the development of inexpensive and sensitive herbicide biosensors based on photosynthetic reaction centers (RC). Since using PS II for herbicides detection was proposed more than 20 years ago [7] this subject has been explored extensively [8-15]. It is important to note that the chemical structures of nitric explosives are fairly similar to those of the herbicides detectable by these biosensors (Figure 1); moreover, nitric explosives are known to be strong electron acceptors. Thus, detection of explosives by natural photosynthetic RC-based sensors seems quite promising. Photosystem II is a trans-membrane protein complex responsible for the water splitting and oxygen evolution; it is a part of the electron transfer chain in photosynthesis. In PS II sunlight energy is utilized for charge separation starting with the formation of $P680^{+}$–$Pheo^{-}$. The electron then travels from the pheophytin to immobile plastoquinone Q_A to mobile plastoquinone Q_B. The latter accepts two electrons (and two protons), transforms to quinol and carries the electrons away. P680 is re-reduced by electrons originating from water. The mechanism of inhibition of photosynthetic reaction centers by herbicides in vivo involves herbicide molecules attaching to the Q_B binding site and preventing plastoquinone from binding. The exposure of the PS II-based biosensor to the inhibitor results in a decrease of the photoinduced current in an electrical circuit containing the photosynthetic reaction centers, as the mediator (replacement for plastoquinone) cannot bind to the Q_B site. [Suggested location of Figure 1]

Detection of TNT and picric acid will be considered in this manuscript. Herbicide data obtained with the same biosensor will be reported for comparison. Picric acid (2,4,6-trinitrophenol) is a yellow crystalline solid, easily soluble in water. It was widely employed as an explosive during the World War I. It is also used for various laboratory

purposes, such as staining biological samples and preservation of specimen. Some optical sensors for the detection of picric acid have been reported [16-19]. The first evidence of picric acid being an inhibitor of photosynthetic electron transport in Photosystem II (PS II) has been reported a while ago [20]. TNT is used in organic synthesis but is best known as an explosive material with convenient handling properties. Among the commonly used methods for detection of TNT are spectrophotometric [21,22], immunoassay [5,23] and electrochemical [24]. TNT is a very strong electron acceptor and this property was used recently for detection employing quenching of photoluminescence of polysiloles [25]. The solubility of TNT in water is about 100 mg/L at room temperature [26]. TNT is known to inhibit the growth of the plants and to affect PS II fluorescence [27], although the exact mechanism of these effects was not elucidated.

2. Materials and methods

2.1 Reagents

All chemicals except TNT were purchased from Sigma-Aldrich; TNT was purchased from ChemService, USA. Organic baby spinach leaves were obtained from local food retailers.

2.2 Isolation of PS II-containing particles

The BBY particles (in honor of Berthold, Babcock and Yocum [28]) are PS II-enriched membrane fragments. They are obtained by treating thylakoids with Triton X-100 and centrifugation and are mostly devoid of Photosystem I (PS I). Note that the hydrophobic mediator may travel within the remaining membrane to reach its binding

site. The oxygen evolving capacity is also preserved. The particles were isolated from spinach leaves according to [29]. All the steps of extraction were carried out in dim green light at low temperature (samples on ice or procedure in cold room). The leaves were washed, depetiolated and then ground with blender in homogenising buffer (20 mM MES (2-(*N*-morpholino)ethanesulfonic acid), pH 6.0, 15 mM NaC1, 5 mM $CaC1_2$). After filtering through cheesecloth (16 layers) the material was centrifuged for 10 min at 10,000 g. Pellet (containing chloroplasts) was resuspended in homogenising buffer to a concentration of 2 mg Chl/ml. The chlorophyll concentration was determined by the method of Arnon [30]. Triton X-100 solution was added to a final concentration of 25 mg/mg Chl. After 15 min of gentle stirring the suspension was centrifuged for 25 min at 40,000 g. The particles were resuspended in homogenising buffer supplemented with 0.4 M sucrose, then centrifuged for 5 min at 2,000 g to remove starch, then sedimented once more for 25 min at 40,000 g. The prepared particles were stored at −70 °C until used. The oxygen evolution activity as measured by DCPIP (Dichlorophenolindophenol; see Section 2.5) assay for BBY sample was ~90 (μmol/mg Chl/h).

A different protocol, not involving enriching the samples with PS II was employed for comparison. Deveined spinach leaves were crushed in a blender with homogenizing buffer. The homogenate was filtered through cheesecloth and the filtrate was centrifuged for 2 min at 2500 g at 4 °C. The resulting pellet was then resuspended in the homogenising buffer diluted 20 times. This step was used to lyse the chloroplast membranes. The resulting solution was then crushed in a Wheaton mixer and centrifuged for 3 min at 3500 g. The resulting pellet contained the purified membranes. They were then resuspended in a measuring buffer (15 mM MES, pH 6.5, containing 0.5 M

mannitol, 0.1 M NaCl, 5 mM $MgCl_2$, and $5 \cdot 10^{-5}$ M chloramphenicol) to obtain a final concentration of chlorophyll-containing thylakoid membrane fragments between 2 and 3 mg/ml.

2.3. Biosensor preparation

The screen-printed electrodes were purchased from DropSens Inc. (model DRP-220). The electrode assembly consists of a gold working electrode (with the area of ~13 mm^2), a graphite counter-electrode and an Ag/AgCl reference electrode. The choice of the immobilization technique was based on its ability to preserve the biological sample in its active form for long periods of time. The procedure for bovine serum albumin (BSA)-glutaraldehyde matrix-based immobilization was similar to that described earlier [7,8,11,31-34]. Briefly, a 10 % solution of BSA in measuring buffer was mixed with equal amount of BBY particles. For cross-linking purpose a 10 % glutaraldehyde solution was added to make a final glutaraldehyde concentration of 0.3 %. 2.5 µl of the mixture was spread over the working electrode and incubated for 30 min at 4 ^oC for the matrix formation. The entrapment of BBY particles in a BSA-glutaraldehyde resulted in a very stable matrix formation on top of the central / working screen-printed electrode. The immobilized photosynthetic material was visible as a green deposition on the electrodes. The electrodes functionalized with PS II were stored at –20 °C until used.

The schematics of the biosensor are presented in Figure 2A. [Suggested location of Fig.2]

2.4 Photo-electrochemical measurements

The electrochemical response of PS II-functionalized electrodes was investigated using the CHI 630C electrochemical workstation from CH Instruments, USA. I-t curves were measured at room temperature, with a 50 µl droplets of the measuring buffer solution placed onto the working area covering the three electrodes. During the measurement the electrodes were illuminated with red light from a 7 mW 670 nm LED (Sanyo DL3149-057). In case of duroquinone (DQ) the working electrode was polarized at 0.62 V and for ferricyanide (FeCy) the electrode was at 0.36 V [11]. The mediator concentrations were 0.2 mM and 1 mM, respectively. As seen in part B of Figure 2, containing an example of the experimental data, in the absence of light only small dark current is registered. (This background/ dark current differed somewhat from sensor to sensor.) Illuminating the sensor leads to a significant increase in the detected current which is due to charge separation in the PS II. Turning the light off results in current returning to the pre-illumination values. Addition of photosynthesis inhibitors results in a decrease of the magnitude of the photo-induced current peak. The difference between maximal photo-induced current within the peak and the pre-illumination dark current (double-ended arrow) was utilized in the calculations of relative activities presented below. The excitation wavelength dependence of the photocurrent closely matched the absorption spectrum of PS II, indicating that PS II remained intact and active after immobilization and that it was indeed the source of the observed photocurrent.

2.5 DCPIP assay

DCPIP (Dichlorophenolindophenol) is a dye which changes color depending on its redox state. DCPIP assay was performed to determine the activity of PS II and to

confirm the low inhibition effect of TNT on PS II (in the absence of the BSA-glutaraldehyde matrix). Reduction of DCPIP by PS II was monitored by following a decrease in DCPIP absorbance at 592 nm. The assay buffer contained 20 mM Tricine, pH 7.5, 0.2 mM sucrose, 3 mM $MgCl_2$ and 10 mM KCl. Absorption spectra were measured with a Cary 5000 UV-VIS-NIR spectrophotometer at 22 °C.

3. Results and discussion

Quality of the PS II preparation is of crucial importance for biosensor performance. Both intact thylakoids [7,8,32,34,35] and PS II-enriched BBY particles [10,11,31] have been used in electrochemical cells and herbicide biosensors. At low temperature (4 ^{o}C) and in the dark both BBY particles [31] and thylakoid membranes [36] can be stored for hundreds of hours. At room temperature the half-life of the immobilized BBY particles and thylakoids is reduced to tens of hours [10,11,34]. Our biosensor exhibited approximately 35% decrease of the photocurrent within 7 hours. Thus, the stability of our biosensor is slightly better than that reported in [11]. Somewhat reduced lifetime of the screen-printed electrode biosensor as compared to [10,34] is ascribed to release of Ag+ ions from the reference electrode [11]. The performance of biosensors employing both types of PS II preparations was explored. Biosensor with thylakoid membrane fragments exhibited slower response and longer recovery time as well as poorer signal-to-noise ratio as compared to the sensor with BBY particles. Thus, BBY preparation was selected as better suited for biosensor applications. In case ferricyanide (FeCy) was used as a mediator, large residual activity at high inhibitor concentrations was observed due to the nonspecific nature of the FeCy binding, i.e. FeCy

most probably can accept electrons from sites other than the Q_B site. This result is in agreement with [11]. Therefore, DQ was considered a preferred mediator, as better dynamic range results in somewhat better limits of detection. Nevertheless, data obtained with FeCy as a mediator is still reported for comparison.

3.1 Detection of explosives and herbicides

In vivo the herbicides compete with the Q_B plastoquinone for its binding site on the D1 protein thus leading to disruption of electron transfer from Q_A to Q_B and further along the electron transfer chain. In our experiments binding of the herbicide or explosive molecule to the Q_B site prevents the mediator from accepting electrons from the site and hence the process of electron transfer from PS II to the mediator and from the mediator to the electrode is stalled. The detection is based on the decrease of the photocurrent in the presence of analyte. A baseline current change value was first obtained without the addition of any analyte. Then the effect of introducing additional droplets per se has been explored. A droplet (50 µl) of measuring buffer containing the mediator was allowed to spread over the electrodes covered with immobilized PS II and the photocurrent generated from the biosensor was measured with the illumination time of 20 sec after 15 min of incubation. This process was repeated with new droplets to ascertain the behavior in photocurrent response over a period of time. Addition of new droplets without any inhibitor did not lead to any changes beyond the slow natural decay of the photosynthetic protein material over time.

For measurements of the effect of the herbicides or explosives the biosensor was subjected to a droplet containing the analyte and mediator in measuring buffer, and the

light-induced current change was measured after 15 min. This incubation time has been judged necessary in case of BSA-glutaraldehyde immobilization as the analyte molecule has to diffuse to its binding site and that process is slow for gel-matrix system [13]. Before applying next, higher, concentration of the analyte, the sensor was washed with measuring buffer. Alternatively, a fresh sensor was used to obtain the response at a particular analyte concentration. On starting the measurements with a fresh biosensor the steady state current was higher and the photocurrent signal measurements showed higher standard deviations during the first 25-30 min of the measurement. This may be referred to as a preconditioning phase. Thereafter the signal was largely stable over a period of 3 hours during which the different analyte concentrations could be tested using 15 min incubation time.

The inhibition data for various analytes is plotted as residual activity (in percent) versus concentration (on a logarithmic scale) in Figure 3. The residual activity is calculated as [photocurrent with inhibitor]/[photocurrent without inhibitor] x100%. Experimental data were fitted to a logistic equation describing a sigmoidal binding curve:

$$R = Min + \frac{Max - Min}{1 + ([I]/IC50)^{H}} \qquad (1)$$

Here *Max* is the maximal activity before adding any analyte and *Min* is the minimum residual activity, when sensor is saturated by the inhibitor; *H* is the Hill slope, and *[I]* is the inhibitor concentration. The *IC5*0 is the concentration corresponding to the point midway between top and bottom of the sigmoidal curve. We found this generalized logistic equation to provide somewhat better fits to the experimental data than the Langmuir adsorption isotherm [11] usually used in case of competitive binding to one

site. Equation 1 is equivalent to that of [11] for *H=1* and *Min=0* and has been used previously by other authors, for example in [35]. Although the value of *H* is quite sensitive to various measurement errors and usually is not used to make quantitative inferences, in principle *H* values smaller than one may indicate the presence of several binding sub-sites in the same domain. The limit of detection, LOD, is the concentration upon measuring which one can tell with certainty (usually 99% confidence interval is employed) that the analyte is indeed present; it was calculated as

$$LOD = IC50\left(\frac{2.6\sigma}{Max - Min - 2.6\sigma}\right)^{1/H} \qquad (2)$$

which, again, is reduced to expression from [11] for *H*=1 and *Min*=0. The factor of 2.6 corresponds to 99% confidence interval. Figure 3 shows the experimental data obtained with DCMU herbicide (3-(3,4-dichlorophenyl)-1,1-dimethylurea), picric acid and TNT, and their fits using Equation 1. As can be seen from the Figure 3 the biosensor is highly responsive to classical herbicides such as DCMU (as expected) and only slightly less responsive to picric acid. Essentially, the sigmoidal curve shifts towards higher concentrations for picric acid, and shifts still further for TNT (note the different concentration ranges in different frames of Figure 3). The range of recognition extends up to almost 10 μM for DCMU, but for the picric acid the results exhibit low S/N ratio for concentrations higher than 1 μM, which affects the recognition range. In case of TNT the biosensor shows almost no inhibition of photocurrent for concentrations up to ~1 μM, but inhibition effect becomes apparent upon further increasing the TNT concentration. The inhibition is almost complete for a saturated solution of TNT (~$5 \cdot 10^{-4}$ M). Note that due to poor solubility of TNT in water the location of the lower plateau of the sigmoidal curve (*Min* in Eqs. 1 and 2) is somewhat ambiguous. The values reported in this work

were obtained constraining the fits to *Min* being equal to residual activity at maximal TNT concentration. Such constraining seems justified as for other analytes *Min* was not zero. On the other hand, allowing *Min* to decrease to zero results in approximately 1.5-fold increase in respective *IC50* and LOD. The biosensor was also employed to detect another nitric explosive, tetryl (2,4,6-trinitrophenylmethylnitramine). Although tetryl inhibited the functioning of the PS II at very low concentrations, ~10 nM (not depicted), the data scatter was large and the effect was not fully reversible, possibly indicating permanent damage to the PS II. Thus it is not clear if the mechanism of inhibition by tetryl involves Q_B site binding. (The effect of TNT was reversible, see below.)
[Suggested location of Figure 3]

Table 1 summarizes the data on the limits of detection (LOD) *IC50* and other fit parameters for various substances. The LOD for DCMU was comparable to values previously reported in BSA-glutaraldehyde matrix [11], and better than in [35]. The LOD for picric acid previously reported using luminescence quenching method is 2 μM [36] and by fluorescence emission of hexaphenysilole-chitosan film ~21 nM [37]. Thus, the present detection limits for picric acid of 25 nM (with DQ) and 29 nM (with FeCy, data not shown) constitute a significant improvement over that for luminescence quenching, and is comparable to that of the fluorescence detection method. Interestingly, the LOD for picric acid is significantly better than that reported in [11] for several nitrophenolic herbicides, indicating that BSA-glutaraldehyde matrix does not impede diffusion of picric acid to such a degree. The LOD and *IC50* observed for picric acid in this work fall into the same range as observed for nitrophenolic herbicides using Clark electrode [10]. The

IC50 value observed in this work is also in reasonable agreement with 0.15 μM reported in [20]. [Suggested location of Table 1]

The poor limit of detection observed for TNT suggests that TNT binding to the Q_B site is weak. Alternatively, low sensitivity to TNT may be attributed to low permeability of the BSA-glutaraldehyde matrix to TNT. DCPIP assay was employed to test the latter possibility. In case of the intact electron transfer chain the introduction of DCPIP interrupts the passage of electrons between PS II and PS I, most likely by accepting electrons from the reduced mobile plastoquinone after it leaves the PS II complex. In our experiment, when the duroquinone is displaced by the inhibitor and not reduced, the subsequent reduction of DCPIP is impossible. Measurements involving DCPIP were performed with BBY particles in suspension, without BSA-glutaraldehyde gel matrix present. Results quantitatively similar to those shown in Figure 3C were observed that clearly indicate that the choice of a matrix system does not pose significant limitations to the interaction of TNT with the herbicide binding site of the PS II. The question may also be posed if the inhibition of PS II by TNT is accompanied by some permanent damage to the PS II. When saturated solution of TNT (i.e. the maximum concentration possibly achievable in the experiments on whole plants, [27]), was employed, the photocurrent was reduced to ~10 % of the maximal value. Washing the electrode surface with the buffer solution completely (although gradually) restored the magnitude of the photocurrent. Thus, binding of TNT to PS II is reversible and no permanent damage to PS II occurs.

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3.2 Possible mechanism of DCMU, TNT and picric acid binding at the Q_B Site

The PS II-based biosensor shows high inhibition effect for classical herbicides such as DCMU, the effect slightly decreases for picric acid and is strongly reduced for TNT. The occurrence of a methyl group in TNT instead of hydroxyl group could pose significant limitations to the ability of TNT to bind to the Q_B site, as sufficient number of hydrogen bonds may not be formed. To test these ideas AutoDock 4 software [38,39] has been employed for modeling of the docking of the above ligands to the Q_B binding site. AutoDock takes into account dispersion-repulsion, electrostatics, hydrogen bonds and desolvation terms. Q_B plastoquinone was removed from the cyanobacterial PS II complex structure (pdb code 3BZ1 [40]; the high-resolution structure of PS II from spinach is not available) using PyMol to allow for modeling of binding of herbicides and explosives to the empty Q_B site. The latter was defined as the vicinity of the following D1 residues: HIS 215, PHE 255, SER 264 and PHE 265 which are most involved in herbicide binding according to mutation studies ([41] and references therein). These residues, along with MET 214, LEU 218, ALA 251 and LEU 271 comprise the Q_B site according to structure data [40,42]. Surprisingly, the calculated binding affinities of all of the compounds studied are comparable and are not correlated with their ability to inhibit electron transfer in our biosensor. The optimal positions of DCMU, picric acid and TNT in the Q_B binding pocket are depicted in Figure 4. Note that TNT still appears to form hydrogen bonds with the Q_B pocket residues. As discussed above with respect to the DCPIP experiment, the BSA-glutaraldehyde matrix does not limit the accessibility of the Q_B site to TNT. We are left with several non-trivial possibilities. First, one could argue that the native membrane, present in both biosensor and DCPIP experiments and for both BBY particles and thylakoid membrane particles not enriched with PS II, adversely affects the accessibility

of the Q_B binding site to TNT. Alternatively, one could suggest the presence of an alternative site for TNT docking, with very high affinity, and with binding to this site not affecting electron transport. In both cases the effective concentration of TNT experienced by the Q_B site would be significantly lower than TNT concentration in solution. With respect to the second mechanism we note that a TNT binding site with similar affinity has been discovered on the CP43 protein of PS II by accident in the course of our modeling study. However, a site with the affinity just comparable to that of the Q_B site cannot alone explain our observations. Finally, one could also note (see [43,44] for the reviews) that nitrophenolic herbicides, to whose class picric acid obviously must be assigned, likely exhibit somewhat different PS II inhibition mechanisms and binding sites as compared to DCMU and triazine derivatives. The concept of several binding sub-sites in the same binding domain has been developed [45-47] to address the differences between nitrophenolic and other herbicides. Our modeling results indeed indicate that picric acid and DCMU bind to the Q_B site in a somewhat different manner (see supplemental information). It has also been suggested that the mechanism of action of nitrophenolic herbicides additionally involves interaction with the electron donor side of the PS II and the respective binding site was proposed ([48] and references within). In the latter case the similar sensitivity of the biosensor to DCMU and picric acid would be purely coincidental. Exploring these possibilities further is beyond the scope of this manuscript, focused on the feasibility of simple and easy to handle PS II-based biosensor for explosives. More light on these issues can be shed by performing similar measurements on isolated reaction centers of purple bacteria. The latter exhibit inhibition by herbicides using mechanism similar to that in PS II, but are much more stable in isolated form.

4. Conclusions

An application of a biosensor based on Photosystem II-enriched BBY particles from spinach leaves and inexpensive commercially available screen-printed electrodes to detection of various photosynthesis inhibitors is described. The biosensor with BBY particles immobilized with BSA-glutaraldehyde is capable of detecting not only the herbicides, as earlier reported in the literature, but also some explosives. While picric acid was detected in concentrations similar to those of the widespread herbicides (and with similar concentration dependence likely indicating Q_B site binding), the limit of detection for TNT is significantly higher (worse). Thus, from the viewpoint of explosives detection, the PS II-based biosensor reported in this work, although not impressive as a stand-alone device for TNT detection, could be employed as one component of the "orthogonal" detection schemes involving several methods of detection based on different physical, chemical or biological principles. The screen-printing technology allows for the mass production of identical low-cost disposable biosensor components of such schemes (these components, once prepared, can be stored at low temperatures for extensive periods of time until used). It is important to point out that this sensor is intended for early warning applications and as such is not capable of distinguishing between different explosives if they are present in unknown concentrations. Similar statement can be made concerning application of the PS II-based biosensors for herbicide detection [7-15]. The present biosensor also may be employed in environmental protection and pharmaceutical applications for rapid screening of picric acid in water samples [18,19]. Determination of the reasons for low sensitivity of the PS II-based

biosensor to TNT requires extended research, including that involving isolated bacterial reaction centers as well as other types of explosives resembling different classes of herbicides (e.g. RDX explosive versus atrazine herbicide).

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Vitae: Vijayender Bhalla

Vijayender Bhalla obtained his Ph.D. in bio-nanotechnology jointly from CSIO and Panjab University in Chandigarh, India, in 2006. His graduate research on DNA-based biomolecular electronics was supervised by Dr. Lalit Bharadwaj. After graduating Vijayender first joined the group of Dr. Bruno Samori at the University of Bologna, and later moved to Montreal to join Valter Zazubovich at Concordia. Currently Dr. Bhalla is a Pool Scientist at the Biosensors Laboratory, Microbial Research Institute in Chandigarh. His research is focused on development of label-free immunosensors for herbicides detection.

Vitae: Xin Zhao

Xin Zhao obtained her B.Sc. and M. Sc. degrees in Chemistry from the University of Tianjin, China, under supervision of Dr. Yang Qui Hua. Her research project was devoted to the studies of nano-perovskite photocatalysts. In 2008 Xin joined Concordia Department of Chemistry and Biochemistry as a graduate student and since then has been involved in the development of biosensors for explosives detection under supervision of Dr. Zazubovich. She was awarded another M. Sc. degree in 2010.

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Table 1: Comparison of the biosensor parameters for DCMU herbicide, picric acid and TNT.

	Min (%)	*IC50* (nM)	R	LOD (nM)	σ (%)	H	Range of Recognition
DCMU (DQ)	15.2	87	0.998	1.1	1.25	0.74	1 nM-10μM
DCMU (FeCy)	36.2	116	0.995	14	2.66	0.98	10 nM-10 μM
Picric acid (DQ)	13.2	434	0.999	25	0.94	1.21	10 nM-1 μM
Picric Acid (FeCy)	25.7	1057	0.990	29	0.76	0.98	10 nM-10 μM
TNT (DQ)	10.0*	9900	0.988	378	4.58	0.58	1 μM-0.5 mM
TNT (FeCy)	13.7*	11300	0.985	635	5.77	0.54	1 μM-0.5 mM

* For TNT the *Min* value is somewhat ambiguous due to poor solubility of TNT in water. The TNT data in this table was obtained with constrained values of *Min*. Allowing *Min* to further decrease to zero results in approximately 1.5-fold increase of *IC50* and LOD. See text for details.

Figure captions:

Figure 1. Chemical structures of some herbicides and explosive compounds. A: DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea); B: phenolic herbicide DNOC, 4,6-dinitro-o-cresol; C: picric acid, 2,4,6- trinitrophenol and D: TNT, 2,4,6-trinitrotoluene.

Figure 2. A: The schematics of the biosensor. Vertically elongated green ellipses: PS II-containing particles; yellow circles: BSA; black circles: mediator. B: Representative signal (in the absence of any inhibitor). Illumination of the biosensor results in the photocurrent peak. The magnitude of the peak is indicated by a double-ended arrow. In the presence of the inhibitor the peak magnitude is reduced.

Figure 3. Residual activity of the BBY particle-based biosensor for DCMU (A), picric acid (B) and TNT (C) versus inhibitor concentration. DQ was used as a mediator. In Frame C data from separate experiments is combined.

Figure 4. Orientation of DCMU (A), picric acid (B) and TNT (C) molecules in the Q_B binding site of the PS II according to AutoDock modeling. The inhibitor molecule is depicted in sticks. Yellow dashed lines represent the hydrogen bonds; the numbers associated with these bonds are their lengths in Å.

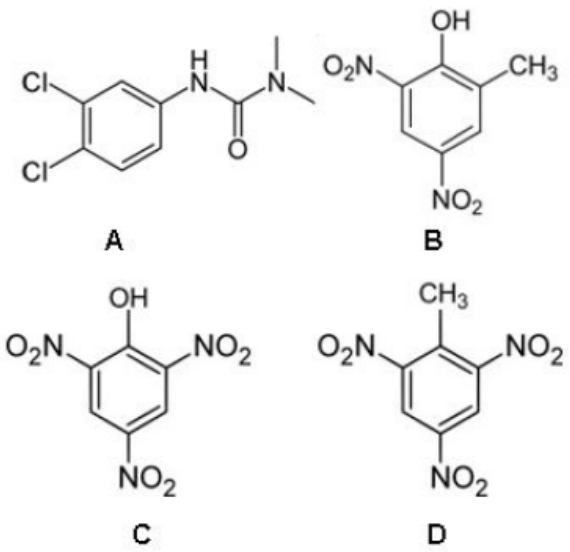

Figure 1

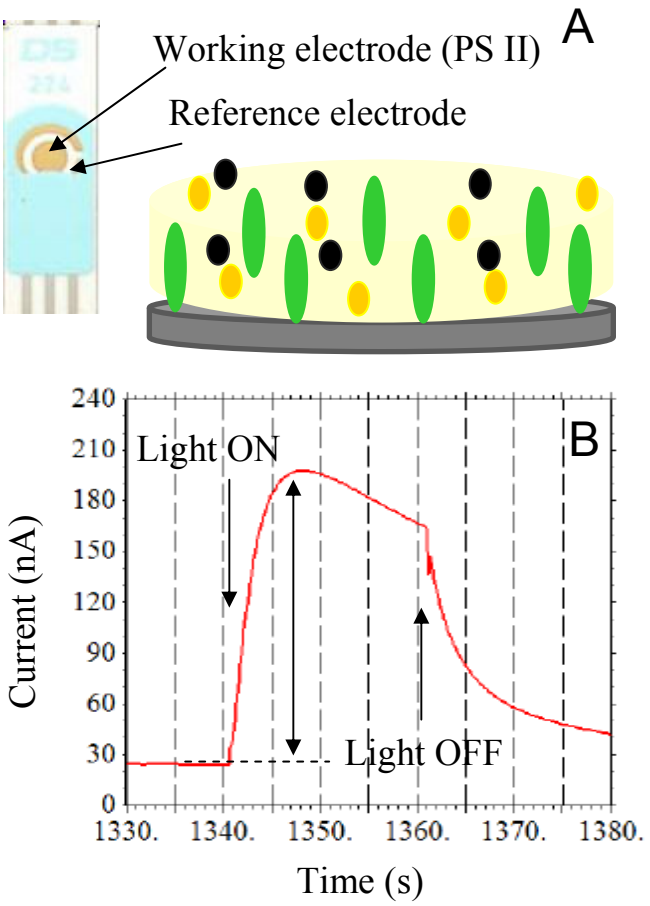
A
Working electrode (PS II)
Reference electrode
B
Light ON
Light OFF
Current (nA)
Time (s)
240
210
180
150
120
90
60
30
0
1330.
1340.
1350.
1360.
1370.
1380.

Figure 2

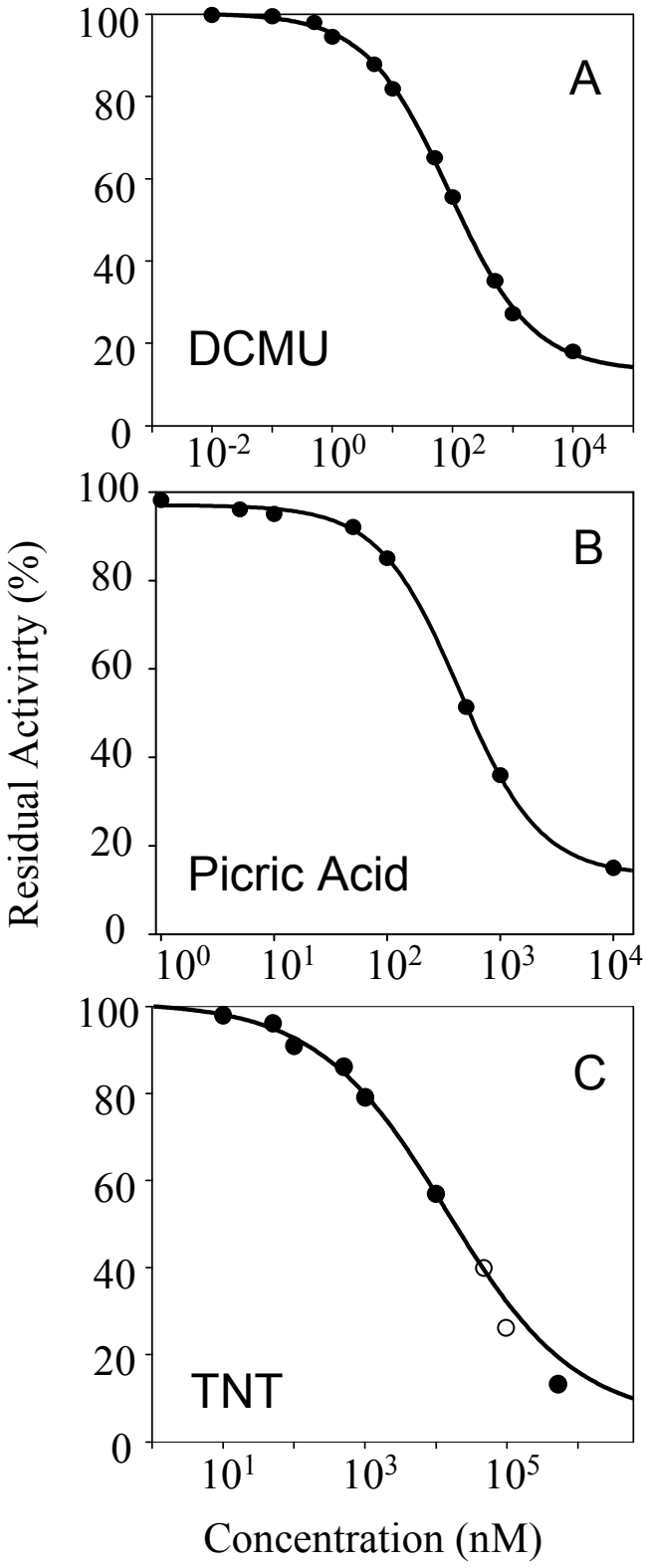
A
DCMU
B
Picric Acid
C
TNT
Residual Activirty (%)
Concentration (nM)
100
80
60
40
20
0
10^{-2}
10^{0}
10^{2}
10^{4}
10^{1}
10^{3}
10^{5}

Figure 3

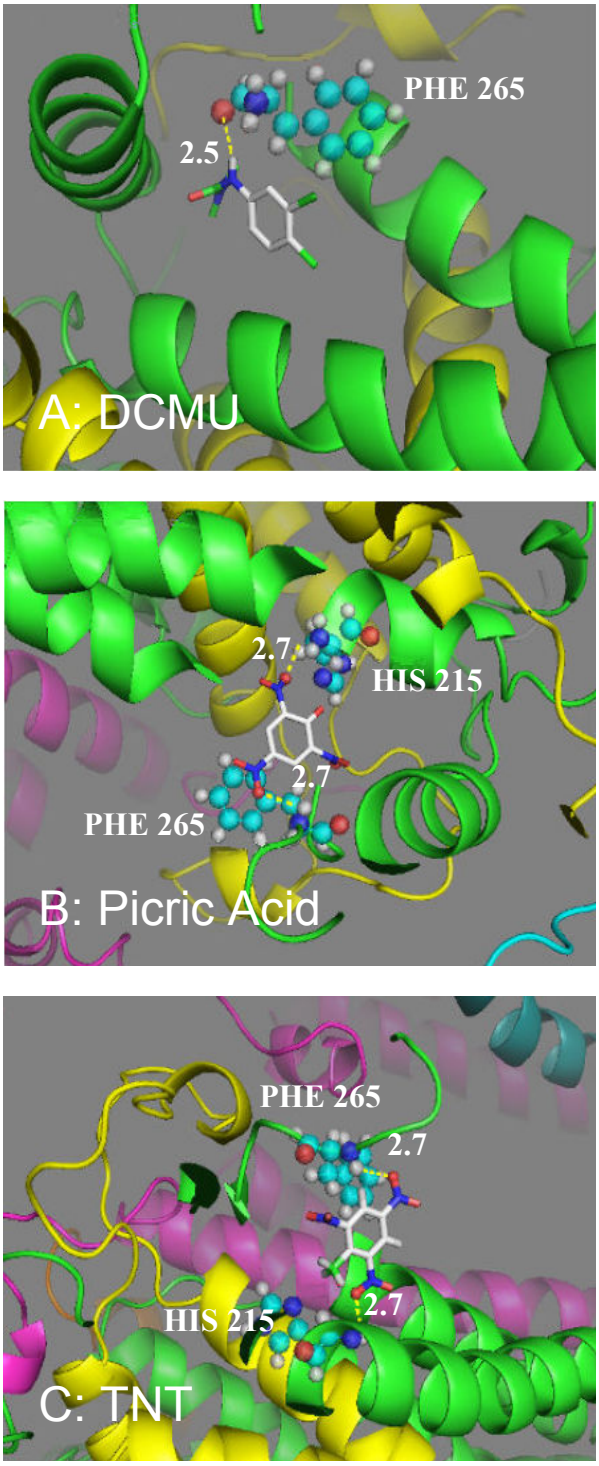
PHE 265
2.5
A: DCMU
2.7
HIS 215
2.7
PHE 265
B: Picric Acid
PHE 265
2.7
2.7
HIS 215
C: TNT

Figure 4

Valter Zazubovich

Vijayender Bhalla

Xin Zhao