ChemComm

COMMUNICATION

An electrochemical sensor for selective TNT sensing based on *Tobacco mosaic* virus-like particle binding agents†

Cite this: *Chem. Commun.*, 2014, 50, 12977

Received 27th August 2014, Accepted 8th September 2014

DOI: 10.1039/c4cc06735e

www.rsc.org/chemcomm

Faheng Zang,[a] Konstantinos Gerasopoulos,[a] Xiao Zhu Fan,[a] Adam D. Brown,[b] James N. Culver[c] and Reza Ghodssi*[a]

This paper presents a selective differential sensing method based on diffusion modulation of the target molecules through suspended *Tobacco mosaic* virus-like particles modified with binding peptides for TNT sensing in solution.

Sensing of trace amounts of small toxic compounds such as explosive chemicals in complex aqueous environments faces significant challenges, including low signal level from the target molecules, large background signal as well as the requirement for ultra-sensitive transduction systems suitable for operation in solution. Conventional approaches are based on immobilization of receptors such as polymers, peptides, or antibodies on the transducer surface where the target species attach or react. The generated signal can be measured using fluorescent tags or in a label-free manner, as has been shown with field-effect transistors.[1–6] While these sensors are selective, they require sophisticated microfabrication processes with high precision, surface functionalization steps before the measurements, or additional labelling after the binding event, limiting their potential use in practical applications.

In this work, we present an alternative approach for sensing of small molecules that is based on biomacromolecular receptors as binding agents in solution. While this method can be readily expanded to various small molecules with large contrast in size compared to biological binding agents, 2,4,6-trinitrotoluene (TNT) was selected as a testbed to demonstrate the sensing efficacy, primarily due to its importance in homeland security and environmental applications. TNT is a widely used explosive and also a toxic chemical for organisms from bacteria to humans.[7,8] The growing production and usage of TNT has resulted in contamination of soil and water in construction sites and weapon test grounds, increasing the need for reliable sensing in aqueous environments. When operating in solution, pulse voltammetric electrochemical sensors have been reported as one of the most sensitive approaches to quantify TNT concentration, by analysing the peak current fingerprints from the electrochemical reduction of nitro groups ($-NO_2$) to amine groups ($-NH_2$).[9,10] This method is not limited by the size of the analyte since it is based on charge transfer. However, selectivity is a bottleneck as the reduction peaks are very wide, and background subtraction is usually required to distinguish the TNT signal.

The method presented here addresses the aforementioned limitations by combining the fast response and high sensitivity of conventional electrochemical sensors with the selectivity of bioreceptors for rapid and label-free chemical detection. A unique feature of this sensing method is the use of modified *Tobacco mosaic* virus (TMV)-like particles (VLP) as free-floating binding agents in the bulk solution that modulate the target TNT diffusion coefficient. This allows selective TNT detection within minutes without the need for surface functionalization of the transducer, a typically time-consuming added step. This "TNT filtering" enabled by the biological binding agents is quantified by a differential current measurement method, where the reduction currents with and without VLP are measured. The specificity of the bioreceptors and the simplicity of use make the sensor suitable for TNT detection in complex aqueous environments in a rapid and selective manner. The VLP binding agents are used in microfabricated electrochemical sensors to study the compatibility of this sensing mechanism with on-chip microsensors for TNT detection in low-volume solutions.

TMVs are macromolecules with several attractive properties such as programmable affinity, structural rigidity and chemical stability in various environments. Recent advances in viral engineering have resulted in the reliable production of VLPs that preserve the cylindrical structure of TMV, formed by the helical arrangement of thousands of identical coat proteins, but without the viral RNA (Fig. 1a).[11] This enables faster production of modified

[a] *Department of Electrical and Computer Engineering, Institute for Systems Research, University of Maryland, College Park, USA. E-mail: ghodssi@umd.edu; Fax: +1 301 314 9920; Tel: +1 301 405 8158*

[b] *Fischell Department of Bioengineering, University of Maryland, College Park, USA*

[c] *Institute for Bioscience and Biotechnology Research and Department of Plant Science and Landscape Architecture, University of Maryland, College Park, USA*

† Electronic supplementary information (ESI) available: VLP genetic modification, characterization of diffusion coefficient, calculation of Faradaic current ratio, beaker and microscale experiment setups. See DOI: 10.1039/c4cc06735e

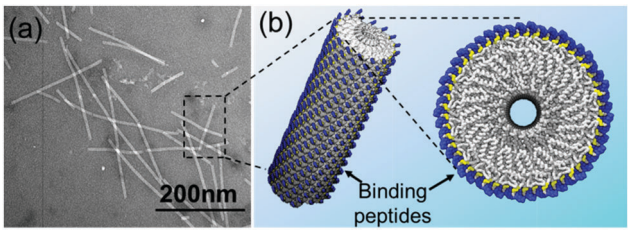

Fig. 1 (a) TEM image of purified VLPs, and (b) VLP structure and peptide mutation of the coat proteins.

particles directly from bacteria cultures at a higher yield compared to the conventional virus purification from plants. Previously, both TMVs and VLPs have been used as nanostructured scaffolds or genetically modified receptors for energy and biosensing applications.[12–15] In these devices, the particles were self-assembled directly on substrates. When the target molecules are significantly smaller than the receptors however, the sensitivity of a sensor can be dramatically reduced.

In this work, we leverage the tremendous contrast in size between the VLP and the TNT to develop a new sensing technique suitable for selective sensing of small target molecules in solution. We utilize a previously identified TNT binding peptide[16] to genetically modify the TMV coat protein for expression and assembly in *Escherichia coli* cells. As a result, assembled VLPs displaying TNT binding peptides (VLP-bp-TNT) were produced (see ESI†). This peptide sequence has been shown to exhibit very high selectivity to TNT *versus* other competing molecules such as 2,4-dinitrotoluene, demonstrating its suitability for selective TNT sensing in a complex solution.

The sensing methodology employed here utilizes square wave voltammetry due to its fast scan rate and high sensitivity. The peak current signatures of TNT without VLP binding agents were first characterized using conventional square wave voltammetry where a 25 mV amplitude square wave was superimposed on a 4 mV step function at 50 Hz (Fig. 2a). Three characteristic current peaks are observed in the voltage range from −0.3 V to −0.9 V (*vs.* Ag/AgCl). Among these, the first peak at −0.53 V arises directly from the reduction of the nitro groups in the TNT molecule, and was used for quantifying the TNT concentration.[4] For reactions on planar electrodes, the net peak current i_p can be expressed as,

$$i_p = nFS\sqrt{D_{eff}}\Delta\Phi_p f^{1/2} c^*$$

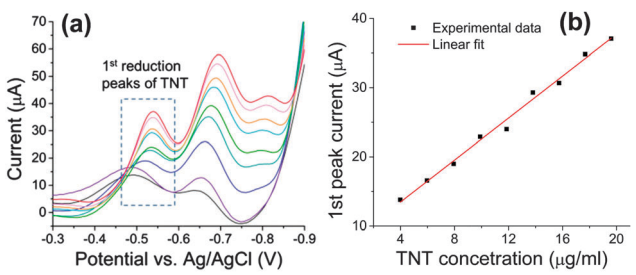

Fig. 2 (a) Three characteristic peaks of TNT obtained using square wave voltammetry, and (b) the correlation between the 1st peak currents at −0.53 V (*vs.* Ag/AgCl) and TNT concentration.

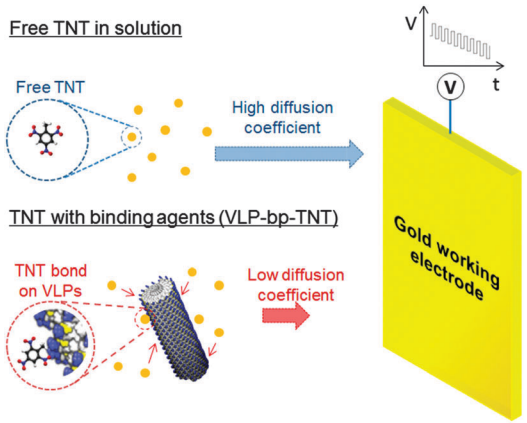

Fig. 3 Diffusion modulations of TNT using VLP-bp-TNT binding agents.

which shows the current is linear to the concentration c^* and the square root of the diffusion coefficient D of TNT in the bulk solution.[14,17] TNT in acetonitrile was introduced in an electrolyte containing 5 ml of 0.1 M NaCl and 0.01 M sodium phosphate. The TNT concentration was varied from 0 to 20 μg ml^{-1}, and the relationship between current and concentration was verified to be linear (Fig. 2b).

The integration of VLP binding agents in the TNT-containing solution changes the peak current by modulation of the effective diffusion coefficient, which is inversely proportional to the hydrodynamic radii of the molecules. VLP-bp-TNT is a large macromolecule assembled from thousands of individual coat protein subunits (molecular weight ∼17.5 kDa), which are significantly larger than free TNT (∼227.3 Da). The hydrodynamic radius of TMV was previously reported to be approximately 42 nm[18] while that of TNT is less than 0.4 nm. The suspended VLP bioreceptors bind free TNT on their surface displayed receptor peptides and form "composite" macromolecules with extremely low diffusion coefficient (Fig. 3). This selective biological binding event reduces the effective diffusion coefficient in the sensor to D_{eff}', and generates a unique differential current ($\Delta i_p \propto (D_{eff})^{1/2} - (D_{eff}')^{1/2}$). In practice, the differential current is calculated from the peak current in the presence of VLP-bp-TNT binding agents and a negative control (VLP-1cys, a construct without binding peptides) from two independent experiments in an identical experimental set-up. This method eliminates the requirement for background subtraction as in conventional electrochemical methods and enables enhanced TNT detection selectivity in unknown samples.

The change of diffusion coefficient due to VLP and TNT biological binding was studied using chronoamperometry, where the current response was measured when applying a step potential from 0.2 V to −0.6 V (Fig. 4). Faradaic currents from TNT reduction were recorded from 0.6 s to exclude the initial charging effect. The results were analyzed using the Cottrell equation, which correlates the Faradaic current i_F, diffusion coefficient D and time t ($\Delta i_F(t) \propto (D/\Delta t)^{1/2}$). The diffusion coefficient is inversely proportional to the change of $1/i_F^2$ over time.[19] Based on the above, the diffusion coefficients

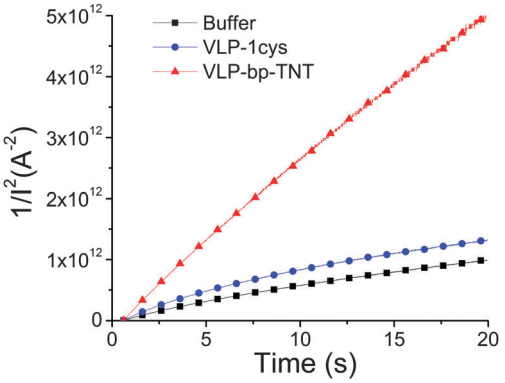

Fig. 4 Chronoamperometry of 20 μg ml^{-1} TNT solution in the presence or absence of VLP-bp-TNT binding agents.

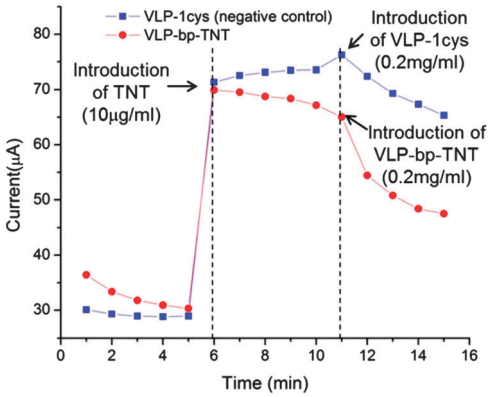

Fig. 5 Dynamic responses of the peak current with sequential introduction of 10 μg ml^{-1} TNT and 0.2 mg ml^{-1} VLPs in solution.

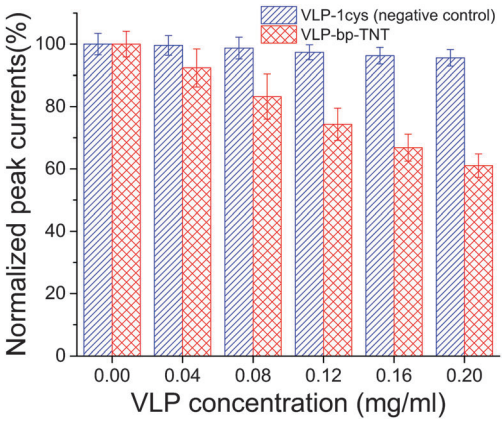

Fig. 6 Normalized peak currents *vs.* VLP concentration obtained from the reduction of 10 μg ml^{-1} TNT in solution.

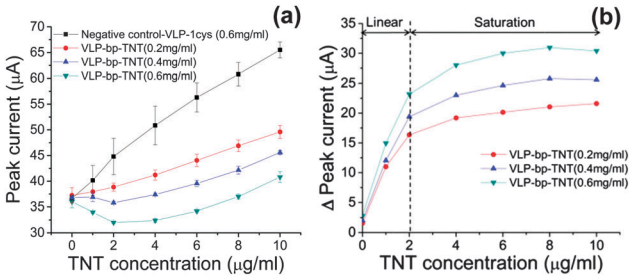

Fig. 7 (a) Absolute peak currents and (b) differential currents of TNT reduction in the presence or absence of binding agents.

for each case shown in Fig. 4 can be calculated (see ESI†). The calculated TNT diffusion coefficient in buffer was 5.70 × 10^{-6} cm^2 s^{-1}, in good agreement with previously reported values[20] (6.71 × 10^{-6} cm^2 s^{-1}). The diffusion coefficient of 4.40 × 10^{-6} cm^2 s^{-1} for unmodified VLPs (VLP-1cys) was 4.04 times higher than that of VLP-bp-TNT (1.09 × 10^{-6} cm^2 s^{-1}). This indicates a Faradaic current ratio of 2.01 between control experiments and the bioreceptor VLP-bp-TNT at a 20 μg ml^{-1} TNT concentration.

The dynamics of VLP-TNT binding were studied by monitoring the peak current in response to the sequential introduction of TNT and VLPs in solution (Fig. 5). The current decrease for the VLP-bp-TNT was significantly higher compared to the VLP-1cys. In both cases, the currents stabilized after three minutes post introduction of the biomolecules. The instability of the current level in the first 3 minutes is a combination of biological binding and fluidic turbulence. Therefore, in later experiments, the TNT peak current was measured after 3 minutes of stabilization from the introduction of VLPs.

The effective diffusion coefficient of the electroactive molecules was controlled by the ratio of bound and free floating TNTs in solution. The TNT peak current in the presence of increasing concentration of VLPs is shown in Fig. 6 (see ESI† for experimental setup). The current decreases to 61.0% as the concentration of binding agents increases to 0.2 mg ml^{-1}. In the control experiment with VLP-1cys, the peak current is stable with only a 4.4% variation. The strong correlation between the current and binding agent concentration enables tunability of the proposed sensor by adjusting the sensitivity and resolution through the differential current.

The response of the developed VLP-based sensor to increasing concentration of TNT was also studied (see ESI† for detailed setup). Due to TNT and VLP-bp-TNT binding, the otherwise linear response of the current *vs.* TNT concentration that was observed in the negative control experiments (Fig. 7a) is modified. The peak currents with VLP-bp-TNT are subtracted from the peak currents of the control (Fig. 7a), resulting in the differential peak currents (ΔI_{peak}) of Fig. 7b. The differential current showed saturation when all the peptide binding sites on the VLPs were gradually occupied by TNT molecules. When the concentration of binding agent increased from 0.2 mg ml^{-1} to 0.6 mg ml^{-1}, the saturation point moved from 2 μg ml^{-1} to approximately 4 μg ml^{-1} TNT concentration. The slope of the differential current *vs.* TNT concentration increases with the concentration of binding agents, indicating an increase in sensor sensitivity. The Faradaic current ratio of VLP-1cys (0.6 mg ml^{-1}) to VLP-bp-TNT (0.2 mg ml^{-1}) is 2.03. This matches well with the Faradaic current ratio calculated from the Cottrell equation in Fig. 4 (see ESI† for details), which validates that the nonlinearity in the current is due to the difference in diffusion coefficient between the two systems.

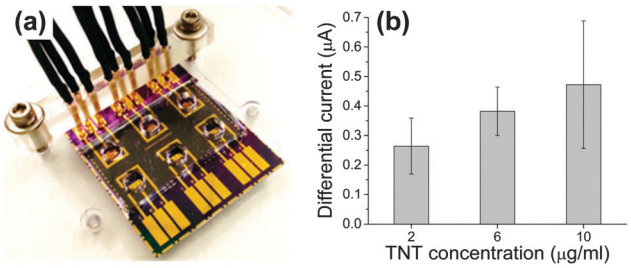

Fig. 8 (a) Optical image of on-chip electrochemical sensor, and (b) differential currents for varying concentrations of TNT.

Field applications outside laboratory settings require sensors that are portable and compatible with limited amount of reagents without compromising fast response and high selectivity. The feasibility of applying the VLP-based TNT sensing method in miniature sensors was investigated through the development and characterization of an on-chip electrochemical sensor. Gold working, counter and pseudo reference electrodes integrated with 30 μl Polydimethylsiloxane reaction chambers were microfabricated forming a sensor array (Fig. 8a). An interdigitated electrode geometry optimized in previous work[13] (50 μm finger width and spacing) was selected (see ESI† for detailed setup). The differential current between sensors in the presence of VLP-bp-TNT and the control VLP-1cys was measured for varying concentrations of TNT (Fig. 8b). As in the case of the experiments in bulk solution (Fig. 7b), the differential current increases with TNT concentration. A clear saturation was not observed, suggesting that the linear region can be extended in a microscale sensor. These results demonstrate that the developed sensing method is applicable for microscale sensors for low-volume TNT detection.

In summary, a differential sensing method using genetically modified macromolecules as binding agents for selective chemical sensing in aqueous environments was developed. The free-floating, suspended VLPs modulated the effective diffusion coefficient of the target TNTs within 3 minutes and contributed to unique differential current signatures that are proportional to TNT concentration. The genetically modified VLP bioreceptors were successfully tested in both beaker-scale and on-chip electrochemical sensors. This sensing method enables rapid label-free detection of TNT and can be expanded to a variety of electroactive species that have great size contrast with the programmable biologically engineered receptor macromolecules.

The work was supported by the Army Research Office Biochemistry Program (Grant No. W911NF1110138) and National Science Foundation Nanomanufacturing Program (Grant No. NSF-CMMI 0927693). The authors acknowledge the support of the Maryland NanoCenter and its FabLab.

Notes and references

1 K. Bratin, P. T. Kissinger, R. C. Briner and C. S. Bruntlett, *Anal. Chim. Acta*, 1981, **130**, 295–311.
2 L. Feng, H. Li, Y. Qu and C. Lu, *Chem. Commun.*, 2012, **48**, 4633–4635.
3 S. Z. Nergiz, N. Gandra, M. E. Farrell, L. Tian, P. M. Pellegrinob and S. Singamaneni, *J. Mater. Chem. A*, 2013, **1**, 6543–6549.
4 J. L. Pablos, M. Trigo-López, F. Serna, F. C. García and J. M. García, *Chem. Commun.*, 2014, **50**, 2484–2487.
5 T. H. Kim, B. Y. Lee, J. Jaworski, K. Yokoyama, W. Chung, E. Wang, S. Hong, A. Majumdar and S. W. Lee, *ACS Nano*, 2011, **5**, 2824–2830.
6 E. R. Goldman, I. L. Medintz, J. L. Whitley, A. Hayhurst, A. R. Clapp, H. T. Uyeda, J. R. Deschamps, M. E. Lassman and H. Mattoussi, *J. Am. Chem. Soc.*, 2005, **127**, 674–675.
7 J. Yinon, *Toxicity and metabolism of explosives*, CRC press, 1990.
8 E. R. Travis, N. K. Hannink, C. J. van der Gast, I. P. Thompson, S. J. Rosser and N. C. Bruce, *Environ. Sci. Technol.*, 2007, **41**, 5854–5861.
9 J. Wang, R. K. Bhadab, J. Lub and D. MacDonald, *Anal. Chim. Acta*, 1998, **361**, 85–91.
10 J. Wang, *Electroanalysis*, 2007, **19**, 415–423.
11 A. D. Brown, L. N. Naves, X. Wang, R. Ghodssi and J. N. Culver, *Biomacromolecules*, 2013, **14**, 3123–3129.
12 X. Z. Fan, E. Pomerantseva, M. Gnerlich, A. Brown, K. Gerasopoulos, M. McCarthy, J. Culver and R. Ghodssi, *J. Vac. Sci. Technol., A*, 2013, **31**, 050815.
13 F. Zang, X. Z. Fan, K. D. Gerasopoulos, H. Ben-Yoav, A. D. Brown, J. N. Culver and R. Ghodssi, *IEEE Sens. J.*, 2013, 1408–1411.
14 K. Gerasopoulos, E. Pomerantseva, M. McCarthy, A. Brown, C. Wang, J. N. Culver and R. Ghodssi, *ACS Nano*, 2012, **6**, 6422–6432.
15 K. Gerasopoulos, X. Chen, J. N. Culver, C. Wang and R. Ghodssi, *Chem. Commun.*, 2010, **46**, 7349–7351.
16 J. W. Jaworski, D. Raorane, J. H. Huh, A. Majumdar and S. W. Lee, *Langmuir*, 2008, **24**, 4938–4943.
17 A. A. M. Bond and F. Scholz, *Electroanalytical methods: guide to experiments and applications*, Springer, 2010, p. 125.
18 S. P. Wargacki, B. Pate and R. A. Vaia, *Langmuir*, 2008, **24**, 5439–5444.
19 A. J. van Stroe and L. J. J. Janssen, *Anal. Chim. Acta*, 1993, **279**, 213–219.
20 S. Taylora, J. H. Levera, J. Faddena, N. Perrona and B. Packerb, *Chemosphere*, 2009, **77**, 1074–1081.

