

Computation-Assisted Nanopore Detection of Uranyl Ions

G. M. Roozbahani, X. Chen, Y. Zhang and X. Guan

Illinois Institute of Technology, Chicago, IL, USA, xguan5@iit.edu

ABSTRACT

Uranium is one of the most common radioactive contaminants in the environment. As a major nuclear material in production, the environmental samples (like soil and groundwater) can provide signatures on uranium production activity inside the facility. Thus, developing a new and portable analytical technology for uranium in aqueous media is significant not only for environmental monitoring, but also for non-proliferation. In this work, a label-free method for the detection of uranyl (UO_2^{2+}) ions is developed by monitoring the translocation of a peptide probe in a nanopore. Based on the difference in the number of peptide events in the absence and presence of uranyl ions, nanomolar concentration of UO_2^{2+} ions could be detected in minutes. The method is highly selective; micromolar concentrations of Cd^{2+} , Cu^{2+} , Zn^{2+} , Ni^{2+} , Pb^{2+} , Hg^{2+} , Th^{4+} , Mg^{2+} and Ca^{2+} would not interfere with the detection of UO_2^{2+} ions. In addition, simulated water samples were successfully analyzed.

Keywords: nanopore sensing, uranyl ion, peptide, biosensor, chelation

1 INTRODUCTION

Because of uranium mining, nuclear power production, nuclear weapon development, as well as other industrial and medical application, uranium has been one of the most common radioactive contaminants in the environment, which raises concerns about its environmental impact and risk for human health [1]. Thus far, various analytical techniques have been utilized to detect uranyl ions, including radiospectrometry [2], inductively coupled plasma mass spectrometry [3], fluorescence [4], colorimetric [5] and complexometric titration [6]. However, most of these techniques are laborious, time-consuming, and require the use of sophisticated instruments or fluorescent labels / dyes. In addition, environmental samples like soil and groundwater near a uranium processing facility can provide significant signatures about its production activities inside the facility, and to detect such early signatures at declared or undeclared areas is significant for preventing nuclear materials from wrong people [7]. Therefore, development of other fundamentally different techniques, which are especially label-free, easy to operate, and potentially field-deployable, for uranyl detection is highly desirable not only for environmental monitoring but also for non-proliferation of nuclear materials or weapons.

Nanopore stochastic sensing has attracted substantial interest as an emerging label-free and amplification-free technique for measuring single molecules [8]. Under an applied voltage bias, the movement of an analyte in a nanopore produces a measurable ionic current blockage. The identity and the concentration of the analyte could be revealed from its characteristic current signatures such as the event residence time, amplitude, frequency and even the shape of the blockage. In addition to its biosensing application, nanopore sensing technology has been successfully applied to study a variety of other research areas, for example, covalent and non-covalent bonding interactions, biomolecular folding and unfolding, and enzyme kinetics. It should be noted that nanopore biosensing is generally achieved by modifying the nanopore interior to introduce binding sites for molecular recognition of target analytes. Recently, Wang and coworkers reported a coordination chemistry-based stochastic nanopore sensing method for the detection of Cu^{2+} ions by using a peptide as a ligand probe [9]. The detection was based on the effect of Cu^{2+} on peptide translocation in a nanopore. Briefly, in the absence of Cu^{2+} , the translocation of the copper-chelating agent in the nanopore produced only one major type of events. In contrast, in the presence of Cu^{2+} ions, they interacted with the copper-chelating agent to form copper chelates, which produced a new type of events in the nanopore. By taking advantage of these new events, quantitative detection of Cu^{2+} ions could be achieved. In this work, based on the similar metal ion-chelating agent interaction principle but with a different detection mechanism, we developed a new nanopore method for the detection of UO_2^{2+} in aqueous media.

2 EXPERIMENTAL SECTION

2.1 Materials and Reagents

Peptide HH_{14} , a 14-amino acid peptide with a sequence of HHHHHHKHHHYHHH, was obtained from WatsonBio sciences (Houston, TX). Other chemicals were bought from Sigma (St. Louis, MO). All the chemicals, including the HH_{14} peptide, were dissolved in HPLC-grade water (ChromAR, Mallinckrodt Baker). The stock solutions of the peptide and metal salts were prepared at concentrations of 10 mM each, and were kept at $-20\text{ }^\circ\text{C}$ before and after use. Lipid 1,2-diphytanoylphosphatidylcholine was purchased from Avanti Polar Lipids (Alabaster, AL). Teflon film was obtained from Goodfellow (Malvern, PA). The α -hemolysin

(α HL) (M113F)₇ protein pores was made according to our previous work [10].

2.2 Electrical Recording

Single channel recordings were carried out at $24 \pm 1^\circ \text{C}$ in a two-compartment chamber, which is separated by a Teflon septum having a $150 \mu\text{m}$ diameter aperture. Briefly, the planar bilayer was formed on the aperture of the Teflon film using 1,2-diphytanoylphosphatidylcholine. Unless otherwise noted, the experiments were performed under symmetrical buffer conditions, with the α HL proteins added to the grounded *cis* compartment, while metal ion salts and the peptide probe were introduced to the *trans* side of the chamber device. Currents were recorded with an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA, USA), filtered with a built-in four-pole low-pass Bessel filter at 10 kHz, and then sampled at 50 kHz with a Digidata 1440 A/D converter (Molecular Devices).

2.3 Data Analysis

The signatures of current blockage events were obtained using Clampfit 10.5 software (Molecular Device). Specifically, the conductance values and the mean residence time (τ_{off}) for the HH₁₄ peptide were derived from the amplitude and the residence time histograms by fitting the distributions to Gaussian and single exponential functions, respectively. The change (Δn) in the number of peptide HH₁₄ events after addition of metal ions, including UO_2^{2+} , to the solution was calculated by using the equation: $\Delta n = n_0 - n_1$, where n_0 represented the number of HH₁₄ events in the absence of metal ions, while n_1 depicted the number of peptide HH₁₄ events in the presence of metal ions. Therefore, a positive value of Δn indicated a reduction in the number of peptide events after addition of metal ions to the solution. Each single-channel current trace was recorded for 10 minutes. At least three separate experiments, in each of which a new protein nanopore was used, were performed for each sample

3 RESULTS AND DISCUSSION

3.1 Detection of UO_2^{2+} Ions using Peptide HH₁₄

Since uranyl ions themselves could not produce any current modulation events in the nanopore, in order to detect UO_2^{2+} , we utilized a 14-amino acid peptide (i.e., HH₁₄) as the ligand probe. The three histidines in the 6-, 13- and 14-positions of the peptide HH₁₄ sequences were designed based on the finding that, in the Cu(II)-A β complex, the Cu^{2+} ions were coordinated by three histidine amino acids (i.e., His-6, His-13 and His-14) in the A β peptide [11]. The other nine histidines were introduced to increase the coordination possibility between the peptide ligand and the target metal ion. Similar to the Cu^{2+} sensor reported

previously, the peptide probe HH₁₄ produced only one major type of events (Fig. 1a). However, unlike the Cu^{2+} sensor, no new events were observed after addition of UO_2^{2+} to the peptide solution. Instead, the number of peptide events decreased. Furthermore, we noticed that, with an increase in the concentration of added UO_2^{2+} , the peptide events become fewer and fewer. Specifically, when $0.5 \mu\text{M}$ UO_2^{2+} ions were added to the peptide HH₁₄ ($40 \mu\text{M}$) solution, the number of peptide events decreased by $56.5 \pm 2.4 \%$ (Fig. 1b). As the concentration of uranyl ions increased to $10 \mu\text{M}$, $92.8 \pm 2.2 \%$ of the HH₁₄ peptide events disappeared (Fig. 1c). Since the I-V Curves of HH₁₄, UO_2^{2+} , and their mixtures showed that the existence of uranyl in the nanopore didn't rectify ionic current, one possible reason for our observation that addition of UO_2^{2+} to the peptide HH₁₄ solution did not produce new types of events, but only decreased the peptide event count is because the interactions between peptide HH₁₄ and uranyl ions led to formation of UO_2^{2+} -HH₁₄ complexes, which passed through the nanopore too rapidly to be captured by the nanopore sensor ($\sim 200 \mu\text{s}$ resolution). Note that the isoelectric point of histidine is around 7.5, while that of

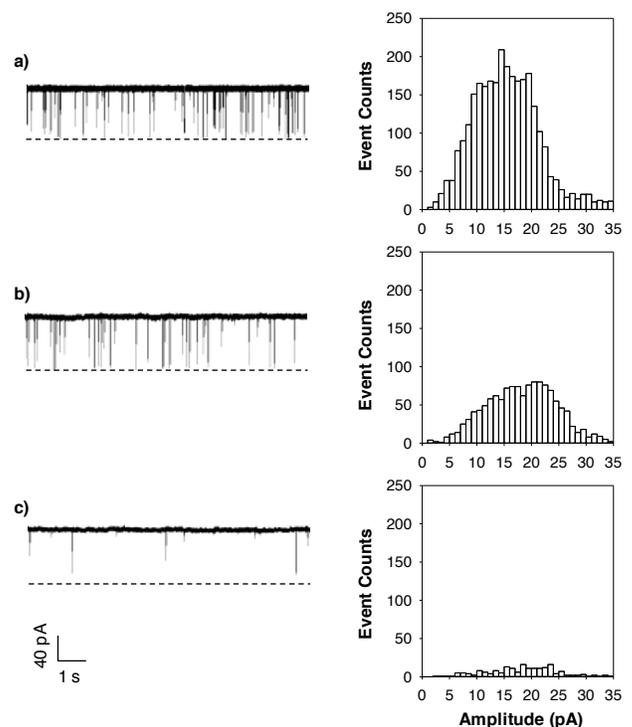


Figure 1: Nanopore detection of UO_2^{2+} ions using peptide HH₁₄. (a) $40 \mu\text{M}$ HH₁₄; (b) $40 \mu\text{M}$ HH₁₄ + $0.5 \mu\text{M}$ UO_2^{2+} ; and (c) $40 \mu\text{M}$ HH₁₄ + $10 \mu\text{M}$ UO_2^{2+} . (Left) Typical single-channel current recording trace segments; and (Right) the corresponding event amplitude histograms. The experiments were performed at +100 mV with the (M113F)₇ α HL pore in an electrolyte solution containing 1.0 M NaCl and 10 mM Tris•HCl (pH 6.5). Both the peptide and uranyl ions were added to the *trans* compartment of the nanopore sensing chamber. Dashed lines represent the levels of zero current.

lysine is ~ 9.7 . Therefore, under our experimental conditions, peptide HH₁₄ was positively charged. After chelation with UO₂²⁺, the net positive charge of the peptide-uranyl ion complex increased, and hence, the complex would be electrophoretically driven through the nanopore more rapidly than the uncomplexed peptide. Alternatively, the metal ion-peptide complexes might have larger molecular sizes than the nanopore opening so that they could not enter and pass through the pore. However, stoichiometric consideration of the UO₂²⁺ - HH₁₄ interaction could not explain such a large (56.5%) reduction in the peptide HH₁₄ events after addition of 0.5 μ M to 40 μ M HH₁₄. Furthermore, dynamic light scattering experiment (data not shown) demonstrated that uranyl would not induce HH₁₄ aggregation. Therefore, the most likely mechanism behind our finding was that the binding of uranyl to the peptide HH₁₄ enabled other uncomplexed peptide molecules to undergo conformational change.

3.2 pH Effect on the Sensitivity of the Nanopore Sensor

To achieve highly sensitive detection of UO₂²⁺, translocation of peptide HH₁₄ in the α HL nanopore was carried out in a series of electrolyte solutions with different pH values (from pH 4.5 to 7.5) and different buffer components. Our experimental results showed that, as the pH value of the electrolyte solution decreased from pH 7.5 to pH 4.5, the frequency and the mean residence time of the peptide events decreased by ~ 10 -folds, and ~ 60 -folds, respectively. The results were not unreasonable considering the net charges of peptide HH₁₄ at these various pH values. As discussed in the previous section, the isoelectric point of histidine is ~ 7.5 , while that of lysine is ~ 9.7 . Therefore, in our various investigated buffer solutions of different pH values (from pH 4.5 to pH 7.5), peptide HH₁₄ had net positive charges. By systematic calculation of the charge state of HH₁₄, we found that peptide HH₁₄ had a +1.05, +4.05, +10.31, +12.69 charge at pH 7.5, pH 6.5, pH 5.5, and pH 4.5, respectively. Therefore, under a positively applied potential bias, in theory, a decrease in the pH of the electrolyte solution would lead to a decrease in the peptide event residence time and an increase in the peptide event frequency. However, due to the resolution of the single channel recording setup, most of the rapid peptide events (e.g., at pH 4.5 and pH 5.5) were missed under our experimental conditions. The electrolyte buffer solution of pH 6.5 rather than pH 7.5 was used in the remaining experiments because a larger percent reduction in the number of peptide events after addition of uranyl ion (1 μ M) to the peptide solution (10 μ M) was obtained at this pH. Specifically, after addition of uranyl, the number of peptide events was reduced by 83.0% in the pH 6.5 solution compared to 6.2% for the pH 7.5 solution, again suggesting that the net charge of the uranyl-peptide complex played an important role in the disappearance of the biomolecule events.

3.3 Effect of Voltage Bias and Peptide Concentration on UO₂²⁺ Detection

To identify the optimum conditions needed to achieve the maximum nanopore resolution for the detection of UO₂²⁺, we further investigated the translocation of peptide HH₁₄ (without/with UO₂²⁺) in the nanopore at different voltages, ranging from +60 mV to +140 mV. Our experimental results showed that, in the absence of UO₂²⁺, both the frequency and the blockage amplitude of the peptide events increased, while the peptide event residence time decreased as the applied potential bias increased. After addition of UO₂²⁺ to the peptide HH₁₄ solution, the percent reduction in the number of peptide events first increased and then did not change significantly with an increase in the voltage. Although the percent event reduction at +100 mV was slightly smaller than that of +140 mV ($78.9 \pm 5.8\%$ vs. $79.4 \pm 5.7\%$), +100 mV was chosen as the optimum applied potential, and this voltage was used in the remaining experiments because the bilayer at +140 mV was not as stable as that at +100 mV. Note that, the principle for our nanopore sensor to detect UO₂²⁺ was based on the effect of uranyl on the frequency of the peptide HH₁₄ events. In order to achieve highly sensitive detection of UO₂²⁺, two conditions need to be satisfied: one is a large number of peptide events in the absence of UO₂²⁺; and the other is a large percent peptide event reduction in the presence of UO₂²⁺.

In addition, the effect of the peptide HH₁₄ concentration on the nanopore sensor resolution was examined. We found that the number of peptide events was linearly proportional to the peptide concentration, suggesting that the concentration of the peptide would not affect the sensitivity of the nanopore significantly. A concentration of 40 μ M HH₁₄ was used in the remaining experiments since it produced enough events for statistical data analysis within a relatively short recording time.

3.4 Sensitivity and Selectivity of the UO₂²⁺ Nanopore Sensor

Utilizing the current physical condition (i.e., pH 6.5, +100 mV applied potential bias, and 40 μ M HH₁₄ peptide), dose response curve for UO₂²⁺ detection was constructed by monitoring the interaction between peptide HH₁₄ and the nanopore in the presence of UO₂²⁺ ions at various concentrations, ranging from 25 nM to 500 nM. Our experimental results showed that the percent peptide event reduction was linearly correlated with the UO₂²⁺ concentration from 25 nM to ~ 200 nM (Fig. 2a). It was found that the detection limit (which is defined as the UO₂²⁺ concentration corresponding to three times the standard deviation of blank signal) in a 10-minute electrical recording was 10 nM, which is more than good enough for analyzing uranyl ion in natural water (note that the maximum contamination level for UO₂²⁺ in drinking water

defined by U.S. Environmental Protection Agency is 130 nM).

Nine metal ions, including Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Pb^{2+} , Hg^{2+} , Th^{4+} , Ca^{2+} , and Mg^{2+} , were selected as potential interfering species to examine the selectivity of the nanopore UO_2^{2+} sensor because of their similar chemical properties and/or abundances in water. With the exception of Th^{4+} ($5 \mu\text{M}$) and Ca^{2+} ($500 \mu\text{M}$), the concentrations of all the other metal ions used in this investigation were $20 \mu\text{M}$ each. Our single-channel recording experimental results suggested that these nine metal ions did interact with peptide HH_{14} to form metal-peptide complexes. However, the existence of these cationic species would not affect uranyl ion detection significantly. As shown in Fig. 2b, in the presence of Mg^{2+} , Cd^{2+} , Pb^{2+} , Hg^{2+} , and Th^{4+} , the number of peptide events increased by $0.5 \pm 3.1 \%$; $9.7 \pm 1.9 \%$, $13.9 \pm 3.8 \%$, $7.0 \pm 1.2 \%$, and $11.3 \pm 2.6 \%$, respectively. Similar to UO_2^{2+} , the existence of Ca^{2+} , Ni^{2+} , Cu^{2+} , or Zn^{2+} ions in the solution led to a decrease in the peptide event count. However, considering that only small event count decreases ($5.1 \pm 1.9\%$, $5.6 \pm 0.9\%$, $2.0 \pm 1.1\%$, and $9.6 \pm 3.7\%$ for Ca^{2+} , Ni^{2+} , Cu^{2+} , and Zn^{2+} , respectively) were obtained in the presence of relatively large (20 to 500 μM) concentrations of interfering metal ions, the effect is negligible (note that, in comparison, $56.5 \pm 2.4 \%$ decreases in the number of peptide events were observed after addition of $0.5 \mu\text{M}$ uranyl ions to the solution). Taking together, the combined results suggest that our nanopore sensor is highly selective to UO_2^{2+} .

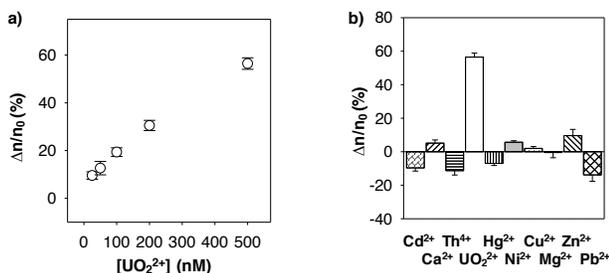


Figure 2: a) Dose-response curve; and b) selectivity of the UO_2^{2+} nanopore sensor.

3.4 Simulated Water Sample Analysis

To demonstrate the potential application of our nanopore sensor in real-world sample analysis, three simulated uranyl ion-contaminated water samples were created by spiking 100 nM uranyl ions to the tap water (obtained from our life science building), lake water (from Lake of Michigan), and Ice Mountain brand bottled spring water. The simulated water samples were analyzed by our nanopore sensor under the symmetrical buffer conditions. Our experimental results showed that the percent event reduction values ($22.7 \pm 2.3 \%$, $19.9 \pm 0.2 \%$, and $19.7 \pm 1.4 \%$) of the three simulated water samples were similar to that ($19.3 \pm 1.8 \%$) of the control sample (i.e., uranyl ion

standard solution), suggesting the matrix component in the water would not affect uranyl ion detection significantly.

4 CONCLUSIONS

In summary, a highly selective and sensitive nanopore sensor was successfully developed to detect UO_2^{2+} ions by using a peptide molecule as a chelating agent and taking advantage of peptide translocation in the nanopore. Although the formation of UO_2^{2+} -peptide complex did not produce new types of events in the nanopore, the percent reduction in the uncomplexed peptide translocation events could be utilized for UO_2^{2+} quantitation. The high selectivity for UO_2^{2+} of our nanopore sensor was supported by two experiments, i.e., the interference study and simulated water analysis. Our study showed that, in spite of their similar chemical properties and/or large concentration in the real-world samples, metal ions such as Cd^{2+} , Th^{4+} , Cu^{2+} , Zn^{2+} , Ni^{2+} , Pb^{2+} , Hg^{2+} , Mg^{2+} and Ca^{2+} , and other matrix components would not interfere with UO_2^{2+} detection significantly. Our developed nanopore sensor may find useful applications in detection of uranyl ions in natural water for environmental monitoring or for signatures on nuclear material production activity inside a processing facility.

REFERENCES

- [1] D. Brugge and V. Buchner, *Rev Environ Health.*, 26, 231, 2011.
- [2] J. Holzbeche and D. E. Ryan, *Anal. Chim. Acta.*, 119, 405, 1980.
- [3] M. Caddia and B. S. Iversen, *J. Anal. At. Spectrom.*, 13, 309, 1998.
- [4] G. I. Romanovskaya, V. I. Pogonin and A. K. Chibisov, *Talanta*, 34, 207, 1987.
- [5] J. H. Lee, Z. Wang, J. Liu and Y. Lu, *J. Am. Chem. Soc.*, 130, 14217, 2008.
- [6] M. Mlakar and M. Branica, *Marine. Chem.*, 46, 61, 1994.
- [7] A. Axelsson, D. M. Fischer and M. V. Penkin, *J. Radioact. Nucl. Chem.*, 282, 725, 2009.
- [8] G. Wang, L. Wang, Y. Han, S. Zhou and X. Guan, *Acc. Chem. Res.*, 46, 2867, 2013.
- [9] G. Wang, L. Wang, Y. Han, S. Zhou and X. Guan, *Biosens Bioelectron.*, 53, 453, 2014.
- [10] D. Wang, Q. Zhao, R. S. S. de Zoysa and X. Guan, *Sensor Actuat B.*, 139, 440, 2009.
- [11] V. Minicozzi, F. Stellato, M. Comai, M. D. Serra, C. Potrich, W. Meyer-Klaucke and S. Morante, *J. Biol. Chem.*, 283, 10784, 2008.