Plant Nanobionic Sensors for Arsenic Detection

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Arsenic is a highly toxic heavy-metal pollutant which poses a significant health risk to humans and other ecosystems. In this work, the natural ability of wild-type plants to pre-concentrate and extract arsenic from the belowground environment is exploited to engineer plant nanobionic sensors for real-time arsenic detection. Near-infrared fluorescent nanosensors are specifically designed for sensitive and selective detection of arsenite. These optical nanosensors are embedded in plant tissues to non-destructively access and monitor the internal dynamics of arsenic taken up by the plants via the roots. The integration of optical nanosensors with living plants enables the conversion of plants into self-powered autosamplers of arsenic from their environment. Arsenite detection is demonstrated with three different plant species as nanobionic sensors. Based on an experimentally validated kinetic model, the nanobionic sensor could detect 0.6 and 0.2 ppb levels of arsenic after 7 and 14 days respectively by exploiting the natural ability of Pteris cretica ferns to hyperaccumulate and tolerate exceptionally high level of arsenic. The sensor readout could also be interfaced with portable electronics at a standoff distance, potentially enabling applications in environmental monitoring and agronomic research.

The abundance of arsenic compounds in the environment poses a serious threat to human health and ecosystems.\(^1,2\) Long-term exposure to arsenic in humans is associated with cardiovascular diseases, birth defects, severe skin lesions, and various types of cancer.\(^3,4\) Anthropogenic activities such as mining, smelting, irrigation with arsenic-contaminated water, and the extensive use of arsenic-based pesticides in the past decades have led to significant arsenic accumulation in underground water and agricultural soils.\(^5-7\) Elevated levels of arsenic in the soils not only inhibit plant growth and result in substantial losses in crop production, but also lead to higher arsenic uptake by crops and contamination of the food chain.\(^7-9\) These concerns over arsenic exposure prompted the World Health Organization and Food and Agriculture Organization of the United Nations to set the maximum contaminant level of arsenic in drinking and irrigation water to 10 and 100 ppb respectively.\(^10\)

Arsenic exists primarily as arsenite (\(\text{As}^{3+}\)) and arsenate (\(\text{As}^{5+}\)) in aqueous environment.\(^11\) In anaerobic conditions such as paddy soils, arsenite is the predominant chemical form of arsenic and it can be efficiently taken up by plants via different mechanisms.\(^12,13\) However, there is a lack of reliable techniques capable of rapidly assessing the uptake of arsenic in plants or the arsenic content within agricultural soil. The conventional method to determine the arsenic level in plants and soil is based on regular field sampling, plant tissue digestion, extraction, and analysis using mass spectrometry.\(^14-17\) Such sampling procedure requires extensive sample pre-treatment, bulky, and expensive instrumentation, and does not allow for real-time monitoring of arsenic contamination in the field.\(^18\) Reflectance spectroscopy and hyperspectral imaging have been proposed as alternatives to monitor arsenic level in plants at a remote distance.\(^19-21\) However, these methods are non-specific toward arsenic contamination, and they rely on slow phenotypic changes of stressed plants such as significant reduction in chlorophyll concentration, destruction of leaf cellular structure and appearance of chlorotic symptoms.\(^19,22\) Electrochemical and optical arsenic detection using nanoparticles have been demonstrated in vitro and in contaminated water samples.\(^21-27\) but their application to monitor the arsenic uptake within plants in real time remains unexplored.

In this work, we demonstrate the use of living plants, interfaced with specifically designed nanomaterials, to serve as self-powered and naturally occurring detectors of arsenic present in belowground environment. This plant nanobionic approach enables real-time monitoring of arsenite taken up by the roots of wild-type plants at a standoff distance. A pair of single-walled carbon nanotube (SWNT)-based near-infrared (NIR) fluorescent nanosensors was rationally designed to selectively recognize arsenite via modulation of their emission intensity. These nanosensors were embedded in the leaf mesophyll of living plants, enabling the detection of arsenite molecules as they are taken up by the roots, transported along the plant vasculature and pre-concentrated in the leaf lamina. The integration of our nanosensors with Cretan brake fern (Pteris cretica), a fern species capable of hyperaccumulating high levels of arsenic in their tissues, enabled the standoff detection of arsenite at the low ppb level, well below the regulatory limit of arsenic in drinking and irrigation water. By harnessing the unique optical properties of nanomaterials and the natural properties of plants to pre-concentrate and hyperaccumulate arsenic, we show the engineering of living plants as autonomous microfluidic samplers capable of real-time, non-destructive, and ultrasensitive detection of arsenite in the environment.

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Herein, we use the corona phase molecular recognition (CoPhMoRe) technique, which we have previously introduced,[28] to develop SWNT-based optical nanosensors for selective detection of arsenite. In this technique, an adsorbed heteropolymer phase on the SWNT surface, called the corona, provides synthetic molecular recognition sites that can bind or interact with the target analyte. Such interaction translates into modulations in the NIR fluorescence spectrum of SWNTs, and enables the detection of a variety of target analytes including small signaling molecules and nitroaromatic compounds in living plants.[29–31] SWNTs offer unique advantages for long-term sensing applications in planta because they fluoresce in the near-infrared region away from the chlorophyll autofluorescence and do not photobleach.[32,33] In addition, their surface properties can be engineered to target different plant organs or subcellular organelles.[34–36]

Arsenite is chosen as the target analyte because it is the predominant form of arsenic in anaerobic paddy soils which can be taken up efficiently by crops through silicon transporters in the roots.[12,37,38] Previous studies have shown that guanine (G) and thymine (T) nucleotides can form strong hydrogen bonds with the hydroxy (−OH) groups of arsenite.[39,40] To exploit the ability of certain DNA bases to interact with arsenite, we first constructed a library of single stranded DNA (ssDNA)-wrapped SWNT with oligonucleotides of varying lengths and G-/T- compositions. The optical sensor responses of DNA-wrapped SWNT constructs were recorded following a 30-min incubation of 100 µM arsenite in 0.1 M NaCl solution buffer. SWNT wrapped with oligonucleotide sequences containing high G-/T- content, such as (GT)₅-SWNT where N = 5 to 30, exhibit a significant increase in fluorescence intensity (I/I₀) by as much as 650% for the (9,4) SWNT chirality upon the addition of arsenite (Figure 1a). Substitution of G-/T- nucleotides with adenine (A) or cytosine (C), such as (GTAA)₇-SWNT and (GTCG)₇-SWNT, diminishes the DNA-SWNT sensor response toward arsenite. Oligonucleotide sequences which do not contain G-/T- bases, such as C₁₀-SWNT and (AC)₁₅-SWNT, remain largely non-responsive when exposed to arsenite (Figure 1a). The decrease in sensor sensitivity as the G-/T- composition in the corona phase is reduced confirms previous findings which show that G-/T- nucleotides are potential binding sites with arsenite.[39,40] In addition, we found that the oligonucleotide length of the (GT)₅-SWNT construct significantly affected the fluorescence intensity modulation from arsenite. Shorter (GT)ₙ carriers of (GT)₅-SWNT, polynucleotides whose length is longer than the oligonucleotide sequence or if other short 10-mer sequences also exhibit similar sensitivity. We found that substitution of G-/T- bases with C nucleotides, which have a high affinity to bind onto SWNT sidewall,[44,45] decreased the sensor sensitivity toward arsenite (Figure S1, Supporting Information). This similar trend observed among longer oligonucleotide sequences and suggests that the specific oligonucleotide chemistry is responsible for the sensor sensitivity and selectivity (Figure 1a).

The large intensity increase of (GT)₅-SWNT observed upon arsenite interaction can be attributed to the low baseline fluorescence of SWNT chiralities with larger diameter (Figure 1c). The excitation-emission map of (GT)₅-SWNT also confirmed that the largest intensity modulations induced by arsenite were exhibited by large-diameter SWNT chiralities (Figure S2, Supporting Information). Recent studies suggested that short (GT)ₙ polymers form highly-ordered ring structures on the SWNT surface, creating a periodic charge distribution which effectively provides an effective doping of SWNT.[46] This doping effect suppresses the radiative exciton relaxation and activates non-radiative exciton relaxation mechanisms, giving rise to the quenched baseline fluorescence of short (GT)₅-SWNT constructs. The corona structure of adsorbed DNA on the SWNT surface is influenced by the solution microenvironment such as ionic strength and pH.[46] Thus, we further tested the response of (GT)₅-SWNT nanosensors in MES and TES buffers which are commonly used for plant infiltration.[11,47] (GT)₅-SWNT constructs maintain their turn-on response upon arsenite exposure, with (GT)₅-SWNT exhibiting the highest sensitivity of 218% and 195% in MES and TES buffer respectively (Figure S3, Supporting Information). The responses of (GT)₅-SWNT against different concentrations of arsenite could be fitted to a kinetic adsorption model to yield a sensor dissociation constant (Kₛ) of 26 µM (Figure 1d).[48] The limit of detection of (GT)₅-SWNT, calculated from the arsenite concentration which resulted in a signal-to-background ratio ≥ 3, was estimated to be 122 nM. In addition, the (GT)₅-SWNT sensor response toward arsenite can be reversed with the introduction of ethylenediaminetetraacetic acid, a common metal chelating agent (Figure S4, Supporting Information). (GT)₅-SWNT complexes were also selective toward arsenite over other heavy-metal ions which may be present as contaminants in the soil (Figure 1e). Taken together, the high sensitivity, selectivity, and compatibility in biologically relevant environment motivates the application of (GT)₅-SWNT to probe arsenite level in planta.

Spinach plants (Spinacia oleracea) were turned into an autonomous detector of arsenite by interfacing with a SWNT-based ratiometric sensor platform consisting of a reference and an active sensor. In this platform, (GT)₅-SWNT served as the active sensor which would exhibit a turn-on response upon the addition of 100 µM arsenate for all DNA-SWNT hybrids tested in this study (Figure 1b). Among (GT)₅-SWNT, (GT)₅-SWNT shows the minimal turn-on response of 15% while (GT)₅-SWNT shows a 110% response toward arsenate. The weaker response of (GT)₅-SWNT elicited by arsenate compared to arsenite may be attributed to the presence of ketone group in arsenite, which can disrupt the formation of hydrogen bonds with the amine groups of G-/T- nucleotides.[43] As (GT)₅-SWNT shows the highest sensitivity and selectivity toward arsenite, we further investigated if such sensor performance is unique to (GT)₅ sequence or if other short 10-mer sequences also exhibit similar sensitivity. We found that substitution of G-/T- bases with C nucleotides, which have a high affinity to bind onto SWNT sidewall,[44,45] decreased the sensor sensitivity toward arsenite (Figure S1, Supporting Information). This is similar to the trend observed among longer oligonucleotide sequences and suggests that the specific oligonucleotide chemistry is responsible for the sensor sensitivity and selectivity (Figure 1a).
upon arsenite detection, while C10-SWNT was selected as the reference sensor which would remain invariant upon arsenite exposure. The DNA-SWNT constructs were infiltrated into two different regions of a leaf lamina of spinach plants, separated by the midrib, via syringe infiltration at the adaxial side (Figure 2a). Arsenite solution was then introduced to the root environment and as transpiration occurs, arsenite would be taken up by the roots and transported to the leaf via the plant vasculature where they would eventually accumulate and come into contact with the embedded nanosensors. The NIR fluorescence of both sensor complexes were monitored at a standoff distance of 1 m with a 2D array InGaAs detector. (GT)5-SWNT fluorescence intensity started to increase ≈30 min after the introduction of 10 µM arsenite solution to the roots of spinach plants (Figure 2b). After 5 h, an 11% increase in the average (GT)5-SWNT fluorescence intensity was observed—this intensity modulation corresponds to ≈0.3 µM change in leaf arsenite concentration (Figure 2c). In contrast, the fluorescence intensity of the control sensor, C10-SWNT, remained relatively invariant throughout the experiment as expected.

Figure 1. Screening and characterization of SWNT-based arsenite sensors. a) Comparison of sensor responses \( \left( \frac{I - I_0}{I_0} \right) \) of (GT)_N-SWNT, where \( N = 5-30 \), and other DNA-SWNT constructs against arsenite. Data represent mean ± standard deviation (s.d.) from \( n = 3 \) independent experiments. b) DNA-SWNT responses toward arsenate. Data represent mean ± s.d. from \( n = 3 \) independent experiments. c) NIR fluorescence spectra of (GT)_5-SWNT, (GT)_25-SWNT, and C10-SWNT before and after exposure to 100 µM arsenite. d) Calibration curve of (GT)_5-SWNT against different concentrations of arsenite. Fitting with kinetic adsorption model is shown in black. e) Selectivity of (GT)_5-SWNT against other heavy-metal cations commonly present in the soil or groundwater. Data represent mean ± s.d. from \( n = 3 \) independent experiments.
intensity of \((\text{GT})_5\)-SWNT to C\textsubscript{10}-SWNT \((I_{\text{G/C-SWNT}})\) was defined as the readout of the ratiometric sensor approach. When water was introduced to the roots of spinach plants as a control, \(I_{\text{G/C-SWNT}}\) remained relatively constant over 5 h (Figure 2d). The difference in the responses of the nanosensors confirmed that our ratiometric platform enabled the selective detection of arsenite as they were taken up by the roots and transported to the leaf lamina. The embedded nanosensors could tap into plants’ internal state and allow the interfacing of such information to electronics, enabling plants to serve as nanobionic devices which can communicate the information they receive from the environment to detectors easily interpreted by human.

The nanobionic approach can also be extended to other plant species to convert any wild-type plants into arsenic detectors. The nanosensor platform was applied to monitor arsenic uptake in rice plants (\(Oryza sativa\)). As a staple food for half of the global human population, rice is a major dietary source of arsenic\(^{[49,50]}\). Previous reports have shown that rice accumulates arsenite more efficiently than other cereal crops such as barley (\(Hordeum vulgare\)) or wheat (\(Triticum aestivum\)), elevating the concerns of arsenic contamination of the human food chain.\(^{[54,55]}\) The introduction of 10 \(\mu\)M arsenite to the roots of 6-week old rice plants resulted in an average of 15% increase in the \(I_{\text{G/C-SWNT}}\) profile after 5 h (Figure 2e,f). The \(I_{\text{G/C-SWNT}}\) level remained relatively constant in the absence of arsenite. The variance in the sensor response dynamics between rice and spinach plants may be due to differences in biological factors such as the vascular structure between monocotyledonous (e.g., rice) and dicotyledonous plants (e.g., spinach), distribution of arsenic uptake channels in the roots, as well as the leaf surface area which affects the transpiration rate. Nonetheless, these results suggest that the nanosensors can be applied to probe the arsenite uptake in both monocotyledonous and dicotyledonous plant species, such as rice and spinach respectively. This provides a unique practical advantage in contrast to genetic engineering methods to produce biosensors for analyte detection in planta, which are only feasible in a limited number of plant species.\(^{[33]}\)

We further demonstrated the versatility of our nanosensor probe for imaging in both the NIR range as well as the visible spectra. To enable imaging of the probe in the visible region, we prepared self-assembled nanostructures comprising of SWNT, single-stranded \((\text{GT})_5\) sequence and TO-PRO-1 (TP), a cyanine dye that intercalates with DNA. Unlike common fluorescent dyes which are typically quenched in the proximity of SWNT, TP switches from a non-fluorescent state to a highly fluorescent state when constrained in a conformationally restrictive environment.\(^{[53]}\) Upon tip-sonication, the three components readily self-assemble to form TP-(\(\text{GT})_5\)-SWNT nanoconstructs which are fluorescent in both the NIR range, enabled by the SWNT backbone, and the visible range, enabled by the TP dye. Successful incorporation of TP into the \((\text{GT})_5\)-SWNT construct
was confirmed the appearance of a distinct peak at 515 nm in TP-(GT)₅-SWNT absorbance spectrum, which corresponds to the absorption maximum of TP dye (Figure 3a). TP-(GT)₅-SWNT still maintain well-defined NIR fluorescence profile and, more importantly, the nanoconstructs show similar intensity modulation toward arsenite with and without TP intercalation (Figure 3b). Additionally, the fluorescence of TP-(GT)₅-SWNT in the visible range decreases in response to arsenite with a comparable sensitivity range as that of (GT)₅-SWNT in the NIR range (Figure 3c). To determine if this intensity modulation in the visible region is caused by the specific interaction between arsenite and (GT)₅ sequences, we also prepared TP-C₁₀-SWNT and monitored its response toward arsenite. The fluorescence of TP-C₁₀-SWNT in the visible range remained unaffected upon the introduction of arsenite at different concentrations (Figure 3c). These findings suggest that the interaction between the (GT)₅ wrapping and arsenite may induce a conformational change in the SWNT corona phase and the bound TP molecules, leading to intensity modulation of the dye-labeled nanoconstructs.

The nanosensors' visible fluorescence enables the visualization of nanosensor dynamics within plant cells at a subcellular resolution with visible confocal microscopy. TP-(GT)₅-SWNT complexes are localized along the cell membrane in the spinach mesophyll layer after syringe infiltration to the adaxial side of a spinach leaf (Figure 3d). As shown in Figure 3d, the addition of 10 µm arsenite decreases the visible fluorescence intensity of the nanoconstructs with different magnitude at various subcellular locations. Three randomly-selected locations of TP-(GT)₅-SWNT showed quenching magnitudes between 30% and 60% (Figure 3e). The heterogeneous sensor dynamics between different subcellular locations may be caused by the spatial profile of arsenite transport within plant cells, or the distribution of nanosensors within the leaf mesophyll. This demonstration highlights the facile modification that can be employed to engineer versatile SWNT-based probes, allowing application in the NIR range for whole plant imaging as well as in the visible region for subcellular arsenite detection as shown in this work.

Plants exhibit natural diversity in their adaptive responses to thrive in arsenic-containing soils. Some species of plants,
primarily ferns from the *Pteris* genus, have naturally evolved the exceptional capability to accumulate and tolerate a high concentration of arsenic in their aboveground biomass.[54–56] For example, the Chinese brake fern *Pteris vittata*, the first known arsenic hyperaccumulating fern, can concentrate as much as 5131 ppm arsenic in the fronds when grown in soil containing 50 ppm arsenic over 2 weeks.[57] In this work, we harnessed the hyperaccumulating capability of the Cretan brake fern *P. cretica*, a previously identified arsenic hyperaccumulator,[54,58] to enhance the sensitivity of plant nanobionic sensors for arsenic detection. Both (GT)₅-SWNT and C₁₀-SWNT were applied on separate sides of the costa, the midrib of the fern leaflet (Figure 4a). Upon exposure to 10 µm arsenite solution at the roots, the NIR fluorescence intensity of (GT)₅-SWNT showed a steady increase over 7 days relative to the initial value, while that of C₁₀-SWNT remained relatively invariant (Figure 4b). The ratiometric sensor response (Δ*I/I₀) of *P. cretica* plants is consistently higher throughout the 7-day period than that of spinach or rice plants, showing a significant increase of 74% relative to the initial level. (Figure 4c). There was no significant difference in the chlorophyll concentration between plants infiltrated with MES buffer and plants treated with DNA-SWNT and 10 µm arsenite over the 7-day period (Figure S5, Supporting Information). Inductively coupled plasma mass spectroscopy (ICP-MS) analysis was also performed on the treated plant samples to construct a calibration curve which allows the translation of sensor intensity modulation to actual changes in the frond arsenic concentration (Figure S6, Supporting Information). The nanosensor intensity modulation can also be captured by a portable Raspberry Pi platform equipped with a charge-coupled

![Figure 4. Arsenite detection with nanobionic sensor based on arsenic hyperaccumulator *Pteris cretica*. a) Bright-field image of *Pteris cretica* frond infiltrated with (GT)₅-SWNT and C₁₀-SWNT under 785-nm excitation. Scale bar: 0.5 mm. b) Time-lapse images showing the intensity changes of embedded nanosensors upon arsenite exposure. Time denotes the time points after arsenite introduction via root uptake. c) Fluorescence intensity changes of SWNT nanosensors embedded in spinach, rice and *Pteris cretica* plants exposed to 10 µm arsenite root medium. Data represent mean ± s.e.m. from n = 5 independent biological samples. d) Arsenite concentration in *Pteris cretica* frond treated with 10, 5, 1, 0.1 µm arsenite solution and deionized water. The concentrations in mmol g⁻¹ DW are translated from sensor intensity responses. Data represent mean ± s.e.m. from n = 5 independent biological samples. e) Contour plot of plant nanobionic sensor’s limit of detection as a function of uptake solution volume and root fresh weight after 7 days. Cross indicates the minimum detection limit of 4.7 nm (0.6 ppb). f) Contour plot of plant nanobionic sensor’s limit of detection as a function of uptake solution volume and root fresh weight after 14 days. The cross indicates the minimum detection limit of 1.6 nm (0.2 ppb).](image-url)
device (CCD) camera, a similar technology to a commercial smartphone-based camera (Figure S7a, Supporting Information). Analysis of images collected through the CCD camera showed a similar ratiometric sensor response as that captured with the InGaAs detector with a 64% increase over 7 days (Figure S7b, Supporting Information), demonstrating the feasibility of interfacing the plant nanobionic sensor with inexpensive, portable electronic devices. The plant nanobionic sensor was then treated with lower concentrations of arsenite in the root uptake solution down to 0.1 µM. As expected, the arsenic concentration in P. cretica fronds, obtained from monitoring the nanosensor fluorescence intensity, decreased with lower arsenite concentrations in the root uptake solution across the 7-day experiment duration (Figure 4d). We note that there may be slight differences in the sensor response dynamics if arsenite is introduced into the soil instead of the root uptake solution, due to factors such as soil porosity, tortuosity, and gravimetric water content. Nonetheless, our work demonstrates that plants can be engineered as living environmental sensors for sensitive arsenite detection from the belowground environment.

We described the uptake of arsenite in P. cretica with a kinetic model to obtain a theoretical limit of detection of the plant nanobionic sensor. For arsenite molecules to be detected by the nanosensors embedded in the frond, they have to be taken up by transporters in the roots and translocated to the frond via the xylem before coming into contact with the nanosensors. At the frond, arsenite may be sequestered into the vacuole for long-term storage and detoxification.[61] The exchange of arsenite between these different compartments can be summarized as a series of reactions:

\[ \text{As}_{\text{sol}}^{3+} \rightarrow \text{As}_{\text{root}}^{3+} \rightarrow \text{As}_{\text{frond}}^{3+} \rightarrow \text{As}_{\text{seq}}^{3+} \]  

where \( \text{As}_{\text{sol}}^{3+} \), \( \text{As}_{\text{root}}^{3+} \), \( \text{As}_{\text{frond}}^{3+} \), and \( \text{As}_{\text{seq}}^{3+} \) denote the arsenite species present in the uptake solution, roots, frond, and sequestration compartment respectively. The nanosensor fluorescence intensity indicates the level of \( \text{As}_{\text{frond}}^{3+} \). In P. vittata, another arsenic hyperaccumulating species in the Pteridaceae family, the transporter-mediated influx of As\(^{3+}\) from the uptake solution into the roots (\( \text{As}_{\text{root}}^{3+} \rightleftharpoons \text{As}_{\text{frond}}^{3+} \)) has been shown to follow Michaelis–Menten kinetics, with a maximum net influx rate of 8–10 nmol As g\(^{-1}\) root fresh weight (FW) h\(^{-1}\) at saturating conditions.[63] The translocation of As\(^{3+}\) from the roots to the fronds (\( \text{As}_{\text{root}}^{3+} \rightarrow \text{As}_{\text{frond}}^{3+} \)) in P. vittata is mainly driven by transpiration, with a mean transpiration rate of 5–7 g H\(_2\)O g\(^{-1}\) frond FW d\(^{-1}\) under normal conditions.[62] Accounting for the plant biomass used in this study (~23 g root FW and 15 g frond FW) and the average arsenite concentration in the xylem sap of hyperaccumulators,[61] we estimated the arsenite root-to-frond translocation rate and the maximum root influx rate to be 29–38 µmol As d\(^{-1}\) and 4.3–5.4 µmol As d\(^{-1}\) respectively. We further defined a modified Damköhler number (\( Da \)) as the ratio between the compartmental exchange rates:

\[ Da = \frac{\text{root influx rate}}{\text{root-to-frond translocation rate}} \]  

When \( Da \gg 1 \), the temporal changes of \( \text{As}_{\text{frond}}^{3+} \) are controlled primarily by the root-to-frond translocation rate, while \( Da \ll 1 \) indicates that the root influx rate is the rate-determining step. The \( Da \) for our plant nanobionic system is ~0.11–0.18, which indicates that the arsenite influx from the uptake solution into the roots is the rate-determining step. As such, assuming the root uptake follows a Michaelis–Menten kinetic model and the sequestration process (\( \text{As}_{\text{frond}}^{3+} \rightarrow \text{As}_{\text{seq}}^{3+} \)) follows a first-order reaction, the mass balances of \( \text{As}_{\text{frond}}^{3+} \) and \( \text{As}_{\text{sol}}^{3+} \) can be described with the following ordinary differential equations:

\[ \frac{d\text{As}_{\text{frond}}^{3+}}{dt} = \frac{\text{FW}_{\text{root}} \text{I}_{\text{max}} \text{C}_{\text{sol}}}{\text{K}_{\text{m}} + \text{C}_{\text{sol}}} - k_d \text{As}_{\text{frond}}^{3+} \]  

\[ \frac{d\text{As}_{\text{sol}}^{3+}}{dt} = -\frac{\text{FW}_{\text{root}} \text{I}_{\text{max}} \text{C}_{\text{sol}}}{\text{V}_{\text{sol}} (\text{K}_{\text{m}} + \text{C}_{\text{sol}})} \]

where \( \text{As}_{\text{frond}}^{3+} \) denotes the amount of arsenite in the frond, \( \text{FW}_{\text{root}} \) is the root fresh weight, \( \text{I}_{\text{max}} \) is the maximum net influx rate of arsenite into the roots, \( \text{C}_{\text{sol}} \) is the arsenite concentration in the uptake solution, \( \text{K}_{\text{m}} \) is the Michaelis–Menten constant which is an inverse measure of the root transporters’ affinity toward arsenite, and \( k_d \) is the first-order sequestration rate constant of arsenite in the frond, and \( \text{V}_{\text{sol}} \) is the uptake volume solution. The proposed kinetic model can describe changes in P. cretica frond arsenite concentration, obtained from the nanosensor intensity profile, upon exposure to 10, 5, 1, and 0.1 µM arsenite at the roots with high fidelity (Figure 4d). The fitting process yields three kinetic parameters for the P. cretica nanobionic system: \( \text{K}_{\text{m}} \) of 5.84 ± 1.63 µM, \( \text{I}_{\text{max}} \) of 3.65 ± 0.22 nmol g\(^{-1}\) root FW h\(^{-1}\), and \( k_d \) of 0.0012 ± 0.0004 h\(^{-1}\). The \( \text{K}_{\text{m}} \) value estimated from our kinetic data is similar to those obtained for P. vittata plants previously reported by other groups (Table 1). It is ~30 times lower than the \( \text{K}_{\text{m}} \) of arsenite transporters in rice roots (180 µM),[64] indicating a higher affinity of P. cretica roots than rice roots toward arsenite. The lower value of \( \text{I}_{\text{max}} \) to that of P. vittata indicates a slightly slower arsenite net uptake rate into the roots of P. cretica than P. vittata. The low value of \( k_d \) suggests that while the nanosensor detection mechanism is reversible, P. cretica hyperaccumulator takes up arsenite almost irreversibly and the arsenite detection by plant nanobionic can therefore be considered irreversible.

The proposed kinetic model can be used to predict the arsenite concentration in the frond as a function of plant root biomass, uptake solution volume and uptake duration (Figure S8, Supporting Information). We further utilized the model to estimate the theoretical detection limit of P. cretica-based nanobionic arsenite sensor, defined as the minimum arsenite concentration in the root uptake solution that can be

| Table 1. Kinetic parameters estimated from the time profile of arsenite uptake in Pteris cretica reported by nanosensors. Values obtained from model fitting are in agreement with reported values from previously published reports. |
|---|---|---|---|
| Kinetic parameters | Fitting values | Reported values\(^a\) | References |
| \( K_{\text{m}} [\mu M] \) | 5.84 ± 1.63 | 6–25 | [77,78] |
| \( \text{I}_{\text{max}} [\mu M \cdot g^{-1} \cdot \text{root FW h}^{-1}] \) | 3.35 ± 1.02 | 8–10 | [61] |
| \( k_d [\text{h}^{-1}] \) | (1.23 ± 0.42) × 10\(^{-3}\) | N.A. | |

\(^a\) Reported values were based on kinetic data on Pteris vittata plants.
detected by the plant nanobionic sensor. The minimum frond arsenite concentration that gave a signal-to-background ratio ≥3 was determined to be 110 nmol g⁻¹ frond dry weight (DW). The kinetic model was then utilized to compute the limit of detection in the uptake solution that results in this level of arsenite in the frond under different experimental conditions and root biomass. Considering an uptake period of 7 days, a limit of detection of 4.7 nm (0.6 ppb) could be achieved with roots of 30 g FW and uptake solution volume of 5 L (Figure 4e). This detection limit suggests that the plant nanobionic sensor can be used to monitor arsenite levels well below the regulatory limit of arsenic in drinking water (10 ppb) and in irrigation water (100 ppb). This figure of merit is also lower than the detection limit of the (GT)₅-SWNT nanosensor alone (122 nm; 15.8 ppb), highlighting the ability of P. cretica to pre-concentrate and hyperaccumulate arsenite to increase the detection sensitivity of a nanobionic sensor. A lower detection limit can be achieved at longer uptake duration with a larger root biomass and a higher uptake solution volume (Figure S9, Supporting Information). When the uptake period is extended to 14 days, the detection limit of the plant nanobionic sensor can be reduced to 1.6 nm (0.2 ppb) with roots of 30 g FW and uptake solution volume of 5 L (Figure 4f). While this limit of detection may not apply to rice or spinach plants tested earlier, these species may still constitute useful plant-based sensors to monitor arsenic accumulation in heavily-contaminated areas, as well as in edible plants for food safety evaluation and plant science studies.

The ability of ferns in the Pteridaceae family to tolerate and hyperaccumulate exceptionally high levels of arsenic appears to result from the presence of certain genes and proteins recently identified in P. vittata. Arsenic antiporter gene ACR3 was found to be necessary for arsenic tolerance in P. vittata gametophytes by mediating the vacuolar sequestration of arsenite.[59] Similarly, the GAPCI, GSTF1, and OCT4 proteins are required for the import and reduction of arsenate inside the cells.[65] However, the uptake pathway of arsenite in Pteridaceae ferns has not been fully elucidated, partly due to the difficulty in generating transgenic ferns.[66] In rice, the uptake of arsenite into rice roots is primarily facilitated by OsNIP2;1 (Ls1), a member of the nodulin-26 like intrinsic proteins (NIPs) that is also responsible for the uptake of silicon (Si).[12,67] In P. vittata, the aquaporin tonoplast intrinsic protein 4 (TIP4) is the only channel to date that has been shown to mediate arsenite uptake.[68] It is unknown if arsenite uptake in Pteridaceae ferns share the same pathways or transporters as those responsible for Si influx into the roots. In this study, we used the optical nanosensors to investigate the effect of Si on arsenite uptake in P. cretica. As expected, the fluorescence intensity of (GT)₅-SWNT showed a steady increase upon exposure to 10 µM arsenite for 5 h, while that of C₁₀-SWNT remained invariant (Figure 5a). The addition of silicic acid to the medium suppressed arsenite uptake by P. cretica, as shown by the slower and insignificant change in embedded (GT)₅-SWNT intensity after 5 h of treatment (Figure 5a). Image analysis showed that while the mean relative intensity of (GT)₅-SWNT to C₁₀-SWNT increased by ≈15% after 5-h exposure to arsenite, the presence of silicic acid in the arsenite uptake medium led to negligible arsenite accumulation in P. cretica. To ascertain that this competitive inhibition effect can be attributed uniquely to silicic acid, the fern roots were also subjected to an uptake medium containing both arsenite and phosphate. Extensive physiological data across plant species have shown that phosphate and arsenate uptake are mediated by the same transporters.[61,69–72] The presence of phosphate did not inhibit arsenite uptake in P. cretica as monitored by our nanosensors (Figure 5a). The sensor response was similar in terms of magnitude and temporal profile to the case where only arsenite was present in the medium. The average relative intensity of (GT)₅-SWNT to C₁₀-SWNT increased by 13% after 5-h exposure to the root uptake medium containing both phosphate and arsenite (Figure 5b). As an example of the novel utility of the sensors introduced in this work, these results indicate that the arsenite and Si uptake in P. cretica

Figure 5. Application of nanosensors to investigate the arsenite uptake pathway in Pteris cretica. a) False-colored images showing the embedded nanosensor response upon the exposure of Pteris cretica roots to medium containing only arsenite, arsenite and silica, or arsenite and phosphate. The green and orange arrows correspond to (GT)₅-SWNT and C₁₀-SWNT respectively. Scale bars: 0.5 mm. b) Time profile of normalized nanosensor intensity upon exposure to the different root media. The shaded region represents s.e.m. from n = 5 independent biological samples.
may share the same transport systems previously identified in rice.\textsuperscript{12,13,73} The application of our nanosensors to investigate mechanisms of arsenite uptake in \textit{P. cretica} further illustrates the versatility of our plant nanobionic approach, which can be utilized for the creation of a new class of sensors as well as to aid botany research.

In this work, we demonstrate the integration of nanoparticles with living plants to engineer plant nanobionic sensors capable of real-time detection of arsenite in the sub-ground environment. DNA-wrapped SWNT nanosensors were rationally designed using the CoPhMoRe technique for selective and sensitive arsenite detection. These nanoconstructs can be incorporated into the tissues of wild-type plants and remained sensitive in vivo, enabling the conversion of living plants into microfluidic arsenite detectors capable of autosampling their surroundings through natural transpiration. Surface modification of DNA-SWNT constructs allows the versatile use of these sensors in both the NIR and visible region for whole plant and subcellular imaging. We also showed that the sensitivity of plant nanobionic sensors can be significantly enhanced by exploiting the hyperaccumulating capability of select species such as \textit{P. cretica}. Such plants exhibit high capacity in arsenite extraction from the sub-ground environment and its translocation to the fronds. In addition, hyperaccumulators can tolerate high concentrations of arsenite, promoting their use as sensitive sensing devices in their natural environment. The increased sensitivity of plant nanobionic sensors compared to optical nanosensors alone illustrates the synergistic properties of plant nanobionic devices by actively pre-concentrating specific analytes in vivo and enabling the communication of this analyte through an optical signal easily intercepted by electronic devices. We envision that the ability of select plants to pre-concentrate and hyperaccumulate specific analytes, resulting in a much higher internal concentration without showing any signs of toxicity, can be extended to engineer other plant nanobionic sensors for environmental monitoring applications. Hyperaccumulators of other metalloids or trace elements, an extensive list of which has been compiled,\textsuperscript{74} can be potentially converted into ultrasensitive detectors of their environment with the proposed plant nanobionic approach. The versatility of plant nanobionics was also shown through sensor application in plant science research to investigate the uptake pathways of arsenite in \textit{P. cretica}. This new class of nanobionic sensors should find immediate utility in environmental monitoring and agronomic studies.

**Experimental Section**

**Materials:** All reagents were purchased from Sigma Aldrich unless otherwise stated.

**Preparation of DNA-SWNT Nanoconstructs:** Raw HiPco SWNTs were obtained from Nanointegris (Lot #HR27-104). Single stranded DNA oligonucleotides were purchased from Integrated DNA Technologies. 1 mg of SWNT was mixed with 0.25 mg of ssDNA in 1 mL of 0.1 M NaCl. The mixture was sonicated with 3 mm probe tip (Cole–Parmer) at 40% amplitude for 20 min in an ice bath. The sample was then centrifuged at 30,000 g for 90 min to remove unsuspended SWNT aggregates. The collected supernatant was dialyzed against 0.1 M NaCl with a 20 kDa MWCO dialysis bag (Spectra-Por) for 3 days to remove excess ssDNA.

TP-(GT)$_5$-SWNT and TP-C$_{10}$-SWNT prepared according to previously published method with slight modification.\textsuperscript{78} Briefly, 1 mg of SWNT was mixed with 0.25 mg of ssDNA and TP solution at a dye:ssDNA ratio of 1:4 in 1 mL of deionized water. Tip-sonication and centrifugation were carried as described above in DNA-SWNT nanoconstruct preparation. The collected supernatant was dialyzed against deionized water with a 20 kDa MWCO dialysis bag (Spectra-Por) for 3 days.

**Absorption Spectra Measurement:** The UV–vis absorption spectra of DNA-SWNT and TP-labeled DNA-SWNT were collected using a quartz cuvette (Starna) with 1 cm path length in Shimadzu UV-3101PC spectrophotometer. All absorption spectra were background-subtracted using reference solutions. The concentration of the DNA-SWNT nanosensors was determined using its absorbance at 632 nm and extinction coefficient of 0.036 L mg$^{-1}$ cm$^{-1}$.

**Plant Growth:** Seeds of carmel spinach (\textit{Spinacia oleracea}) were purchased from David’s Garden Seeds. Seeds of indica rice cultivar (\textit{Oryza sativa}; IR24) were kindly donated by Professor Bing Yang laboratory (Donald Danforth Plant Science Center, St. Louis, MO). Cretan brake fern (\textit{Pteris cretica}) plants were obtained from Josh’s Frogs. Plant seeds were grown in Fafard Professional all-purpose blend potting soil in a Conviron Adapits 1000 growth chamber. Spinach plants and Cretan brake ferns were grown with a 14-h-light/10-h-dark photoperiod at 100 µmol s$^{-1}$ m$^{-2}$, 60% relative humidity, and day/night temperatures of 22 and 18 °C respectively. Rice seeds were first germinated at 37 °C for 4 days. Rice seedlings were then washed carefully with water and transplanted to potting soils in growth chamber with 12-h-light/12-h-dark photoperiod at 100 µmol s$^{-1}$ m$^{-2}$, 60% relative humidity, and day/night temperature of 28 and 25 °C respectively. Fertilizer (N:P:K = 15:9:12) was applied to the potting soil every 2 weeks.

**Nanosensor Screening and Selectivity Test:** DNA-SWNT nanoconstructs were diluted with 0.1 M NaCl, MES buffer (10 mm MES, 10 mm MgCl$_2$, pH 5.7), or TES buffer (10 mm TES, 10 mm MgCl$_2$, pH 7.5) to a concentration of 2 mg L$^{-1}$. Aliquots of SWNT suspensions were added to a 96-well plate for high-throughput screening. The fluorescence spectra of DNA-SWNT complexes were recorded with a custom-made NIR microscope array before and after a 30-min incubation of SWNT aliquots with 100 µm heavy-metal cations. Briefly, the 96-well plate was mounted on a motorized stage of a Zeiss AxioVision inverted microscope connected to a 1D InGaAs detector (Princeton instruments) with a PI Acton SP2500 spectrometer. The samples were excited with a 785-nm photodiode laser (Invictus) at the sample plane with 20× objective for a 10-s exposure time. The fluorescence intensity at 1128 nm wavelength, corresponding to the (9,4) chirality, was used to compare the sensor selectivity and response ($((I - I_0)/I_0)$, where $I_0$ is the initial fluorescence intensity before analyte addition and $I$ is the fluorescence intensity after analyte addition. The sensor responses of TP-(GT)$_5$-SWNT and TP-C$_{10}$-SWNT were measured in the NIR range using the method described above, and in the visible range using a Variank Scan flash microplate reader (Thermo Scientific). The fluorescence intensity corresponding to the maximum fluorescence peak at 540 nm was used to obtain the visible sensor response.

**Nanosensor Infiltration and Standoff Imaging of Arsenite Uptake:** Spinach, rice, and fern plants were infiltrated with both (GT)$_5$-SWNT and C$_{10}$-SWNT, which were prepared at 5 mg L$^{-1}$ concentration in MES buffer. Gentle pressure was applied to the abaxial side of the leaf to ensure no damage was inflicted during the needleless syringe infiltration of the nanosensors. For rice, a small puncture was introduced to the leaf surface using a pipette tip, after which DNA-SWNT was infiltrated through the puncture with gentle pressure during syringe infiltration. The nanosensors were infiltrated to opposite sides of the leaf midrib. The plant roots were then washed with deionized water carefully to remove the soils and transferred to a pretreatment solution containing 10 mM KCl and 5 mM MES of pH 6.0. A 785 nm laser was used to excite the embedded DNA-SWNT complexes. The NIR fluorescence intensity of both sensors was spatiotemporally monitored at a standoff distance of 1 m with a 2D InGaAs array (Princeton Instruments OMA V) equipped with a Nikon AF Micro-Nikkor 60 mm f/2.8D lens. Images were collected at a 2 s exposure time unless otherwise stated. A 900 nm long-pass
filter was placed in front of the camera lens to eliminate chlorophyll autofluorescence and the reflected excitation beam. After 20 min, the pretreatment solution incubating the roots was then replaced with a solution containing 10 or 0.1 µm of sodium meta-arsenite (NaAsO₂) unless otherwise stated.

For 7-day experiments, ten images were taken daily at a specific time during the day and the fluorescence intensity was averaged from the ten images for a daily profile. The excitation laser was turned off when images were not collected. Roots of treated plants were weighed to obtain their fresh weights. To calibrate the nanosensor fluorescence intensities in Pteris cretica, the front arsenic concentration was analyzed with ICP-MS by Galbraith Laboratories Inc. (Knoxville, TN, USA). Briefly, frond samples were cut at specific time points after arsenite treatment, rinsed with deionized water, and dried at 60 °C for 48 h. They were then weighed, ground to fine powder and analyzed by ICP-MS.

**Image and Data Analysis:** Image and data analysis were performed with ImageJ and Matlab R2018a. The sensor response was obtained from imaging experiments by normalizing the SWNT fluorescence with the corresponding initial value prior to the introduction of arsenite. 100 brightest pixels in a sensor spot were averaged to obtain a mean fluorescence intensity value. The normalized (GT)₅-SWNT intensity profile was divided with that of CₓCₓ-SWNT to yield a ratiometric sensor profile (IₓCₓ/SWNT). In Figure 4, the change in ratiometric sensor profile relative to the initial value is denoted as ΔI/Ip. Snapshots of false-colored images were generated by subtracting the first image collected at the time point of arsenite introduction from subsequent images. Numerical simulation of the kinetic model to fit arsenite concentration profile in Pteris cretica was performed with ode45 solver in Matlab R2018a. The sensor limit of detection was also simulated using Matlab R2018a.

**Confocal Microscopy:** Confocal images were obtained using a Zeiss LSM 710 microscope. 20 µL of TP-(GT)₅-SWNT in MES buffer was infiltrated into spinach leaves as attached to living plants. The leaf was excised 1 h after infiltration and a leaf disc (5 mm) was prepared using a cork borer. The leaf disc was then transferred to a glass slide with a cover slip and imaged with a 40× water-immersion objective. A final concentration of 10 µm arsenite solution was introduced into the chamber medium from the side of the cover slip with a pipette. TP-(GT)₅-SWNT nanosensors were excited with a 514 nm laser with the chamber medium from the side of the cover slip with a pipette. Chlorophyll autofluorescence imaging was obtained by excitation at 633 nm with emission channel between 660 and 750 nm. Confocal images of TP-(GT)₅-SWNT and chloroplast autofluorescence were captured every 5 min.

**Silicon, Phosphate, and Arsenite Interaction Experiments:** Pteris cretica plants were used in these experiments. The roots of the plants were gently removed from the soil and carefully washed with water. The plants were then transferred to a beaker containing a pretreatment solution of 10 mM KCl and 5 mM MES at pH 6.0. After nanosensor infiltration, plants were incubated in the growth chamber for 1 h before imaging. At the start of the experiments, the pretreatment solution was replaced with solutions containing 10 µm arsenite, 10 µm arsenite, and 100 µm phosphate (supplied as KH₂PO₄), or 10 µm arsenite and 100 µm silicic acid which was prepared from SiO₂ according to a previously published method.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Author Contributions**

T.T.S.L. and M.S.S. conceived the project, designed the study and wrote the manuscript. M.P. and J.C. assisted with data analysis and nanoparticle characterization.

**Keywords**

arsenic, carbon nanotubes, molecular recognition, nanoparticles, optical sensors, plant nanobionics

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