Modeling Plant Biosensors for Heavy Metal Detection with a Case Study of a Nickel Sensing Circuit

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Abstract

Plant biosensors could help monitor heavy metal contamination by reporting the bioavailable fraction of a contaminant in living tissue. Nickel is a useful test case because plants need trace amounts of nickel, but higher exposure can damage growth and physiology. This manuscript presents a computational framework for a proposed plant nickel biosensor based on the bacterial nickel regulator NikR. The framework treats sensing as a dynamic process rather than a direct conversion from external nickel to fluorescence. The model separates nickel uptake, intracellular nickel availability, NikR activation, promoter occupancy, reporter transcription, translation, fluorophore maturation, reporter degradation, and optical background. Simulations show that sampling time, reporter maturation, promoter strength, and parameter uncertainty can strongly change the apparent dose response. We compare the mechanistic model with a simpler Hill approximation, identify influential parameters, and outline the measurements needed to test the circuit experimentally. The result is a clear modeling workflow for evaluating plant nickel sensing circuits before making claims about sensitivity, specificity, or environmental use.

1 Introduction

Heavy metal pollution remains challenging to monitor continuously because sensitive analytical methods such as inductively coupled plasma mass spectrometry and atomic absorption spectroscopy require sample collection, preparation, trained personnel, and centralized instrumentation [13]. Living biosensors offer a complementary strategy because engineered cells can integrate bioavailable contaminant exposure over time and convert molecular recognition into an optical, electrical, or growth output [8]. Plants are attractive hosts for environmental biosensing because they naturally interface with soil, water, and agricultural environments, but plant biosensors must account for plant development, tissue architecture, metal transport, chlorophyll autofluorescence, containment, and environmental variability [7, 10].

Nickel is a biologically meaningful target for a prototype biosensor. Plants require nickel as a cofactor for urease, yet excess nickel can inhibit photosynthesis, disrupt nutrient balance, induce oxidative stress, and reduce growth [14, 4, 5]. Nickel hyperaccumulator species demonstrate that plants can tolerate and compartmentalize high nickel burdens, but hyperaccumulation is not the same as sensing [6, 15]. A biosensor must report bioavailable nickel reproducibly, preferably below concentrations that cause broad toxicity or nonspecific stress responses.

The design considered here contains two conceptual modules. In the sensory module, nickel alters the activity of NikR. In the reporter module, a synthetic promoter drives a measurable output. The overall architecture is plausible, but quantitative claims require a more explicit measurement model. Environmental nickel is not equivalent to nuclear nickel. Mature fluorescence is not equivalent to transcription. Detection limit is not a fixed property of a promoter alone. This manuscript therefore presents a computational framework, simulated dose response results, and validation criteria rather than claiming that the biosensor has already been built or validated.

2 Results

2.1 Design Objectives and Scope

A credible plant nickel biosensor should satisfy four criteria. First, it should respond to bioavailable nickel over a concentration range relevant to environmental or agricultural monitoring. Second, it should distinguish nickel regulation from generic metal stress. Third, it should produce a measurable output with a defined response time and statistical limit of detection [11]. Fourth, it should be usable in contained settings before any environmental release is considered.

This work does not claim that these criteria have already been met. Instead, it specifies the circuit architecture, model structure, controls, and calibration experiments needed to test them. This framing keeps the circuit from being presented as a validated biosensor before sensitivity, specificity, and response time measurements.

2.2 Biological Circuit Design

2.2.1 Nickel Recognition by NikR

NikR is a bacterial transcription factor that responds to nickel and is found in organisms including *Escherichia coli* and *Helicobacter pylori*. It regulates nickel homeostasis genes by changing DNA binding activity in response to nickel binding [1, 2, 3]. For plant use, NikR should be optimized for plant codon usage, expressed from a promoter suitable for plant expression, and targeted to the nucleus with a nuclear localization signal. A synthetic plant promoter containing NikR operator sequences would then control the reporter gene.

This system should be treated as a new plant synthetic regulatory part, not as a direct bacterial operon transplant. Chromatin context, transcriptional initiation, nuclear protein concentration, operator spacing, and interference from plant transcription factors may all affect transferability. A promoter library should therefore vary operator copy number, operator spacing, minimal promoter strength, untranslated regions, and genomic integration site. This modular testing strategy is consistent with general genetic circuit design principles [16, 9].

2.2.2 Reporter and Measurement Strategy

A fluorescent reporter such as GFP, mNeonGreen, or a destabilized GFP variant can support non-destructive imaging. Stable GFP is useful for cumulative exposure but can obscure rapid changes because the fluorescent protein persists after transcription decreases. A destabilized reporter should improve temporal resolution, although at the cost of lower signal amplitude [12].

A ratiometric design is recommended. Green fluorescence induced by nickel can be normalized to constitutive red fluorescence in the same tissue. This ratio reduces artifacts caused by leaf thickness, illumination, camera exposure, and general expression capacity. Imaging should also include untransformed plants to estimate autofluorescence, plants carrying the reporter but not NikR to estimate promoter behavior that does not depend on NikR, and constitutive reporter controls to estimate variation in tissue imaging.

2.3 Corrected Dynamical Model

2.3.1 State Variables

The model separates environmental nickel exposure from the intracellular regulatory state. This follows the broader principle that biological circuits should be interpreted with explicit dynamic models rather than only input and output curves at steady state [17, 18]. Let N_e denote external bioavailable nickel, N_i intracellular or nuclear nickel available to NikR, R free NikR protein, C active NikR bound to nickel, P fractional promoter occupancy by active NikR, m reporter mRNA, G_u immature reporter protein, G mature fluorescent reporter, and F measured fluorescence.

A minimal model is

$$\frac{dN_i}{dt} = k_{in}N_e - k_{out}N_i - k_{seq}N_i, \tag{1}$$

$$\frac{dR}{dt} = \alpha_R - \delta_R R - k_f R N_i + k_r C, \tag{2}$$

$$\frac{dC}{dt} = k_f R N_i - k_r C - \delta_C C, \tag{3}$$

$$P = \frac{C^h}{K_C^h + C^h}, \tag{4}$$

$$\frac{dm}{dt} = \alpha_0 + \alpha_1 P - \delta_m m, \tag{5}$$

$$\frac{dG_u}{dt} = \beta m - k_{mat}G_u - \delta_G G_u, \tag{6}$$

$$\frac{dG}{dt} = k_{mat}G_u - \delta_G G, \tag{7}$$

$$F = sG + F_0. \tag{8}$$

Here k_{in} and k_{out} summarize uptake and export. The parameter k_{seq} represents sequestration into compartments or chelated pools. The parameter α_R is NikR production, k_f and k_r are nickel association and dissociation rate constants, h is empirical cooperativity, α_0 is promoter leak, α_1 is inducible transcription, β is translation, k_{mat} is fluorophore maturation, δ terms are dilution or degradation rates, s is instrument sensitivity, and F_0 is autofluorescent background.

2.3.2 Response Time

The response time is controlled by the slowest relevant step among nickel transport, NikR activation, transcription, mRNA turnover, translation, fluorophore maturation, and reporter degradation. Stable GFP can require hours before fluorescence approaches a new steady state, making it poorly suited for detection on the scale of minutes. Destabilized fluorescent proteins or luminescent reporters may reduce memory, but they require more sensitive measurement. Claims such as "rapid response" should therefore be supported by time course data and fitted half times rather than inferred from promoter logic.

2.4 Simulated Dose Response Behavior

Original simulations of the ODE model produced dose response curves that changed over time rather than a single static calibration relationship (Figure 1). Across the simulated range, fluorescence increased monotonically with external nickel and with sampling time. At the highest simulated nickel concentration, fold induction increased from approximately 6.4-fold at 6 h to 34.1-fold at 48 h. This result illustrates why sampling time must be treated as part of the measurement definition. The same circuit can appear weak, moderate, or strong depending on when fluorescence is read.

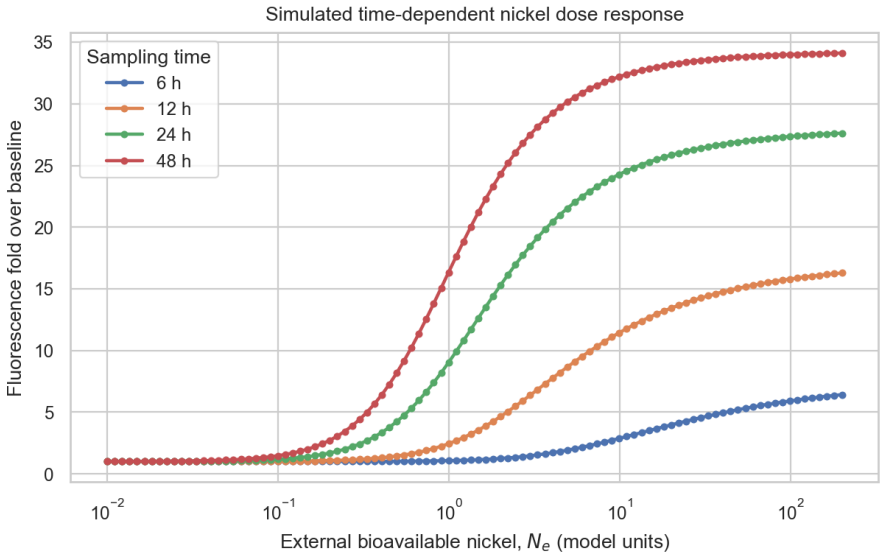

Figure 1: Simulated dose response behavior of the proposed nickel sensing circuit. The mechanistic model predicts that apparent sensitivity and dynamic range depend strongly on the imaging time point because nickel transport, transcription, translation, fluorophore maturation, and reporter degradation introduce delays between exposure and measured fluorescence.

2.5 Comparison with an Instantaneous Hill Approximation

A direct Hill function approximation produced a different apparent calibration curve from the mechanistic model at 24 h (Figure 2). The mechanistic model includes transport and reporter

dynamics, whereas the instantaneous model maps external nickel directly to fluorescence. This comparison demonstrates the main reason for using the ODE framework. A fitted static curve may hide delay, background, maturation, and degradation effects that matter for real biosensor interpretation.

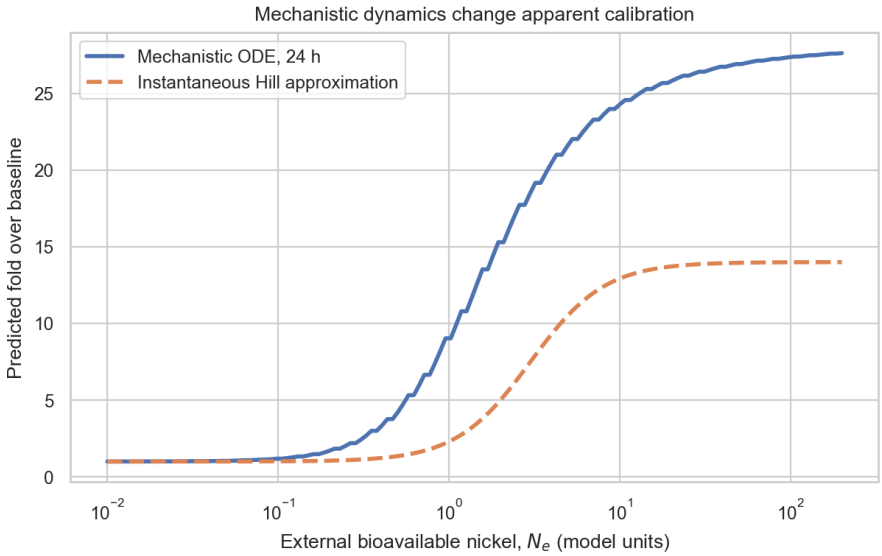

Figure 2: Comparison between the mechanistic ODE model and an instantaneous Hill approximation. The instantaneous approximation treats external nickel as directly converted into fluorescence, whereas the mechanistic model includes intracellular nickel availability, regulator activation, promoter occupancy, reporter expression, maturation, degradation, and background signal.

2.6 Uncertainty and Sensitivity Analysis

Monte Carlo simulations and sensitivity analysis based on rank correlations identified promoter induction strength, nickel uptake, and regulator activation threshold as major determinants of predicted output (Figure 3). At $N_e = 1$, the strongest rank correlations were observed for K_C ($\rho = -0.61$), k_{in} ($\rho = 0.58$), and α_1 ($\rho = 0.53$). At higher nickel concentrations, α_1 became increasingly dominant, consistent with saturation of the upstream sensing module. These results prioritize experimental measurements. Promoter induction strength and regulator threshold should be estimated early because uncertainty in these quantities strongly affects predicted fluorescence.

2.7 Validation Requirements

2.7.1 Construct Assembly

The circuit should be assembled in stages. First, NikR expression and nuclear localization should be verified using a tagged NikR variant. Second, synthetic promoters containing NikR operator sequences should be screened in transient expression assays, such as *Nicotiana benthamiana* leaf infiltration, before stable plant transformation. Third, the best promoter and reporter combinations

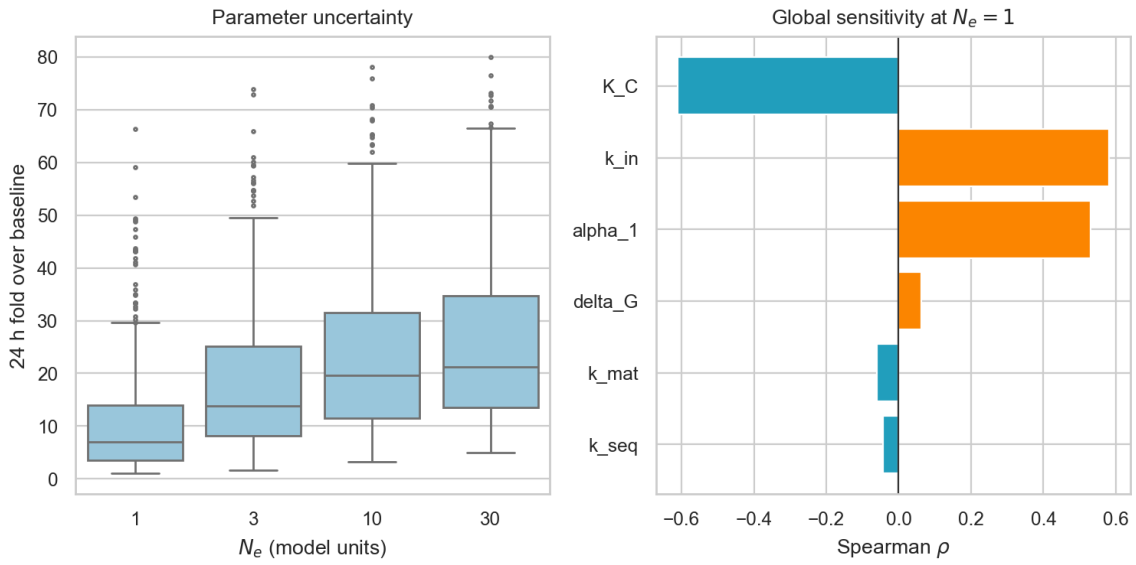

Figure 3: Parameter uncertainty and global sensitivity analysis. Left, Monte Carlo simulations show uncertainty in the 24 h fold response across representative nickel concentrations. Right, Spearman rank correlations at $N_e = 1$ identify parameters that most strongly influence model output. Positive values indicate that increasing the parameter increases predicted fluorescence. Negative values indicate that increasing the parameter decreases predicted fluorescence.

should be integrated into a model plant such as *Arabidopsis thaliana* for dose response and stability testing.

2.7.2 Calibration and Controls

Plants or leaf discs should be exposed to a nickel concentration series and imaged at multiple time points. Essential controls include samples without nickel, untransformed plants, constitutive reporter plants, reporter plants without NikR, promoter constructs without the reporter, and plants exposed to potentially interfering metals such as cobalt, copper, zinc, and cadmium. Nickel accumulation should be measured independently for a subset of samples using analytical chemistry so that fluorescence can be related to tissue nickel rather than only the external dose.

2.7.3 Model Fitting and Validation

Model fitting should proceed hierarchically. Reporter maturation and degradation can be measured independently using inducible expression or translation inhibition assays. Promoter parameters can then be fit from background corrected fluorescence time courses normalized by the ratiometric reporter. Validation should use nickel concentrations held out from fitting and independent biological replicates. Parameter uncertainty should be reported because multiple parameter combinations can generate similar dose response curves.

3 Materials and Methods

3.1 Computational Model Implementation

The simulated results were generated from the ODE model defined in the Results section. External nickel, N_e, was treated as a fixed exposure condition for each simulation. Intracellular nickel availability, regulator state, promoter occupancy, reporter mRNA, immature reporter protein, mature fluorescent reporter, and measured fluorescence were then propagated through the coupled equations. The simulations should be interpreted as model based design evaluations, not as experimental plant measurements.

3.2 Parameterization

The model uses dimensionless or normalized parameters because measurements of NikR promoter behavior in plants are not yet available. Baseline values were chosen to produce biologically plausible qualitative behavior, including low basal fluorescence, monotonic induction with nickel, delayed fluorescence accumulation, and saturation at high nickel exposure. The parameter names and interpretations are listed in Table 1. These values are placeholders for future fitting, not claims about a measured plant line.

Table 1: Model parameters and biological interpretations.

Parameter	Interpretation	Role in model
k_{in}	Nickel uptake or nuclear availability rate	Converts N_e to N_i
k_{out}	Nickel export or loss rate	Removes intracellular nickel
k_{seq}	Sequestration or buffering rate	Reduces nickel accessible to NikR
α_R	NikR production rate	Sets regulator abundance
δ_R	NikR loss rate	Controls regulator turnover
k_f, k_r	Nickel association and dissociation rate constants	Determine active complex formation
K_C, h	Promoter activation threshold and cooperativity	Map active NikR to occupancy
α_0	Basal transcription rate	Determines leak expression
α_1	Induced transcription rate	Determines dynamic range
δ_m	mRNA degradation rate	Controls transcript memory
β	Translation rate	Converts mRNA to reporter protein
k_{mat}	Fluorophore maturation rate	Delays measured fluorescence
δ_G	Reporter degradation or dilution rate	Controls reporter persistence
s, F_0	Instrument sensitivity and background	Convert reporter to measured signal

3.3 Dose Response, Model Comparison, and Uncertainty Analyses

Dose response simulations were performed across logarithmically spaced nickel exposures and evaluated at 6, 12, 24, and 48 h. Fold response was calculated by normalizing fluorescence to the corresponding baseline without nickel at the same time point. The mechanistic model was com-

pared with an instantaneous Hill approximation to show how static calibration can differ from a dynamic reporter model. Parameter uncertainty was evaluated by Monte Carlo sampling of selected parameters, and global sensitivity was summarized using Spearman rank correlations between sampled parameter values and 24 h fold response [19].

3.4 Reproducibility

All numerical outputs used for the figures are included as CSV files in the `data/` directory. The manuscript figures were generated from these files without using experimental measurements. Future experimental versions should archive raw images, segmentation masks, instrument settings, growth conditions, nickel exposure metadata, analytical chemistry measurements, and model fitting scripts.

4 Discussion

The design is best understood as a framework for engineering metal sensing circuits designed for plants. Its strongest feature is modularity. The sensory regulator, synthetic promoter, reporter, and imaging method can be optimized independently. Its main risks are transfer failure of bacterial regulation into plant nuclei, weak specificity, slow response at the whole plant level, and poor quantitative calibration under environmental conditions.

The model is useful because it exposes these risks. A single Hill curve makes the system appear more predictable than it is. A compact mechanistic ODE model highlights the measurements needed to make the biosensor credible. These include intracellular nickel availability, promoter leak, induced expression strength, reporter maturation, degradation, background fluorescence, metal specificity, and biological variability. Once these quantities are measured, the model can guide promoter design, reporter choice, sampling time, and detection thresholds.

4.1 Limitations

This manuscript does not include wet lab measurements, so it cannot establish actual sensitivity, specificity, response time, or field performance. The model is intentionally low dimensional and omits transport from roots to shoots, spatial gradients, developmental changes, stochastic expression, metal competition at transporters, and transcriptional crosstalk caused by stress responses [20, 21]. NikR may also fail to regulate transcription efficiently in plant chromatin without extensive promoter engineering. These limitations should be addressed before presenting the platform as a validated environmental monitor.

4.2 Safety, Containment, and Intended Use

A plant biosensor must include biological and physical containment. For laboratory development, plants should be grown under institutional biosafety rules for transgenic plants. For any future field deployment, pollen flow, seed dispersal, ecological persistence, and regulatory approval would

need dedicated study. The safest near term use case is contained sensing with engineered plants, seedlings, or leaf tissues grown in controlled devices that receive environmental samples but are not released into the environment.

5 Conclusion

Engineering genetic circuits that sense nickel in plants is a plausible route toward low cost environmental biosensing, but it should be evaluated as a quantitative biological measurement system rather than as a simple fluorescence switch. This manuscript replaces an oversimplified relationship between nickel exposure and GFP output with coupled equations for uptake, regulator activation, transcription, translation, reporter maturation, degradation, and measurement. Experimental validation is still required before claims of deployable nickel biosensing can be made.

Data and Code Availability

No wet lab experimental data were generated for this manuscript. The simulated outputs for dose response, model comparison, half time, sensitivity, and uncertainty analysis are included as CSV files in the `data/` directory, and the manuscript figures are generated from those files. Future experimental versions should include raw fluorescence images, processed intensity tables, metal exposure metadata, analytical chemistry measurements, and fitting scripts.

Competing Interests

The author declares no competing interests.

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