

Discovery of DNA–Carbon Nanotube Sensors for Serotonin with Machine Learning and Near-infrared Fluorescence Spectroscopy

Payam Kelich,[▽] Sanghwa Jeong,[▽] Nicole Navarro,[▽] Jaquesta Adams, Xiaoqi Sun, Huanhuan Zhao, Markita P. Landry,* and Lela Vuković*



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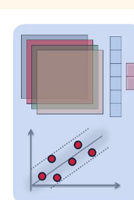


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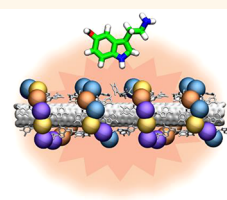
ABSTRACT: DNA-wrapped single walled carbon nanotube (SWNT) conjugates have distinct optical properties leading to their use in biosensing and imaging applications. A critical limitation in the development of DNA–SWNT sensors is the current inability to predict unique DNA sequences that confer a strong analyte-specific optical response to these sensors. Here, near-infrared (nIR) fluorescence response data sets for ~100 DNA–SWNT conjugates, narrowed down by a selective evolution protocol starting from a pool of ~10¹⁰ unique DNA–SWNT candidates, are used to train machine learning (ML) models to predict DNA sequences with strong optical response to neurotransmitter serotonin. First, classifier models based on convolutional neural networks (CNN) are trained on sequence features to classify DNA ligands as either high response or low response to serotonin. Second, support vector machine (SVM) regression models are trained to predict relative optical response values for DNA sequences. Finally, we demonstrate with validation experiments that integrating the predictions of ensembles of the highest quality neural network classifiers (convolutional or artificial) and SVM regression models leads to the best predictions of both high and low response sequences. With our ML approaches, we discovered five DNA–SWNT sensors with higher fluorescence intensity response to serotonin than obtained previously. Overall, the explored ML approaches, shown to predict useful DNA sequences, can be used for discovery of DNA-based sensors and nanobiotechnologies.



10¹⁰ DNA candidates



computational ML tools



discovery of DNA–SWNT optical sensors for serotonin

KEYWORDS: single-walled carbon nanotubes, DNA–nanotube conjugates, optical sensors, serotonin, machine learning

INTRODUCTION

Single walled carbon nanotubes (SWNT) are constituent parts of many hybrid material systems designed for nanotechnology applications, such as sensing, biological imaging, electronics, and gene delivery.^{1–10} Noncovalent polymer adsorption is a widely used method to functionalize SWNTs while also solubilizing them in aqueous environments by forming a “corona phase” on the SWNT surface. A variety of polymers have been used for SWNT functionalization, including nucleic acids, peptides, surfactants, lipids, and peptoids.^{11–20} Among those, nucleic acid functionalized SWNT conjugates are the most ubiquitous and arguably the most technologically useful in important applications, including optical sensing of biologically important analytes,^{1,2} polynucleotide (DNA/RNA) delivery for genetic transformation applications,^{7,21} and for chirality sorting of multichirality SWNT samples into chirality-pure constituents.^{22–27}

DNA sequence plays an essential role in DNA–SWNT conjugates that optically sense analytes and is solely responsible for analyte-specific molecular recognition. An

effective sequence must simultaneously bind with high affinity to the analyte and the underlying SWNT surface to result in a significant selective change of the SWNT near-infrared (nIR) fluorescence response, $\Delta F/F$, in the presence of the target analyte. Prior work has found that as little as a single nucleotide substitution in a DNA sequence can abolish sensor response to a target analyte.²

Most DNA–SWNT-based sensors are generated using either pre-existing molecular recognition elements^{28,29} or low-throughput screening approaches, in which fewer than 100 DNA sequences are screened for fluorescence modulation by target analytes.^{2,30} The latter approach, characterized by random successful parameter hits, relies upon the fortuitous

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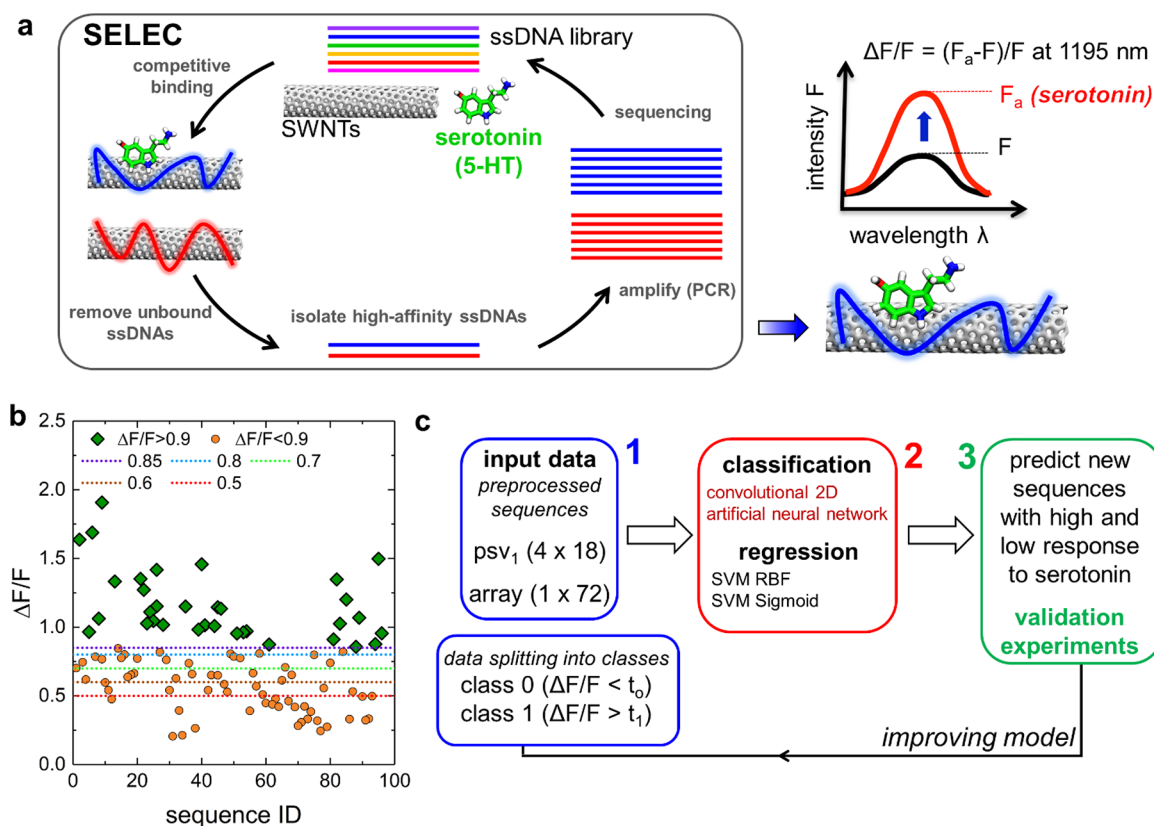


Figure 1. Approach to learning which DNA sequences in DNA–SWNT conjugates provide high response to serotonin. (a) A selective evolution protocol, SELEC, performed for up to six rounds, experimentally identified ssDNAs with high affinity for SWNTs and, separately, SWNTs in the presence of serotonin. Some of the high-affinity ssDNAs are selected for follow-up fluorescence emission spectroscopy experiments of ssDNA–SWNT conjugates before and after the addition of 100 μM of serotonin. (b) The optical response, $\Delta F/F$, of 96 unique ssDNA–SWNT conjugates to 100 μM serotonin; the data also include duplicate measurements for four of the sequences. (c) The main computational approach. DNA sequences are preprocessed into either binary psv₁ format or a simple binary array. The sequences are split in two classes of optical response, according to $\Delta F/F$ -based threshold values. The sequences and their $\Delta F/F$ values are used to train classification and regression models. The models of highest quality are used to predict other sequences with high and low response to serotonin, which are tested in validation experiments. The obtained experimental data are then used to generate further models.

discovery of candidate sensors. While this approach can be useful for starting new directions of research, it is not a sustainable method for optimizing identified sensing technologies or to develop sensors for elusive analytes. In a recent advance, we started addressing this challenge: we developed a method (called SELEC) by which to “evolve” ssDNA–SWNT-based molecular recognition toward an analyte of interest, with selectivity that increases with each round of evolution.³¹ In this approach, $\sim 10^{10}$ unique ssDNA strands can be evolved for molecular recognition of target analytes while still adsorbed to the surface of a nanomaterial.

The data sets generated by the SELEC approach contain rich information on DNA sequences that confer analyte selectivity and SWNT binding affinity. Herein, we leverage these unique data sets to guide our selection of a data set of ~ 100 DNA–SWNT conjugates for which we determine $\Delta F/F$ nIR fluorescence response to the chosen analyte. The resulting data set is used to develop machine learning (ML) models that learn and make predictions of useful ssDNA sequences, which previously eluded experimental validation, that bind to and optically sense the chosen analyte on SWNT surfaces. The model predictions are examined in validation experiments, the results of which are then used to retrain models and predict DNA sequences that produce higher $\Delta F/F$ response to the target analyte. While our approach could be applied to other

analytes, we here demonstrate our approach for serotonin (5-hydroxytryptamine, 5-HT), a neurotransmitter with many roles in the central nervous system³² and outside the brain.³³ As serotonin biosensing is of great importance, many recent efforts have been devoted to its sensor development.^{28,31,34,35}

RESULTS AND DISCUSSION

Classifying DNA Sequences in DNA–SWNT Conjugates Based on Their Optical Response to Serotonin.

We first sought to train and test classifier models for predicting 18-nucleotide (nt) long ssDNA sequences with a high relative nIR fluorescence response to serotonin following conjugation to SWNT. First, models were trained on an initial data set of 96 unique ssDNA sequences, identified by previous SELEC experiments. This initial data set was selected from a library of all possible $\sim 10^{10}$ 18-nt ssDNA sequences that competitively bind to either SWNT (control samples) or SWNT in the presence of serotonin (experimental samples).³¹ The SELEC protocol, schematically shown in Figure 1a, was performed for several selection rounds, each of which provided data sets of selected DNA sequences and their abundance. The 96 sequences, primarily the most abundant sequences from the experimental and control groups from SELEC rounds 3 to 6, were chosen for follow-up serotonin response spectroscopic measurements, thus forming the initial data set for model

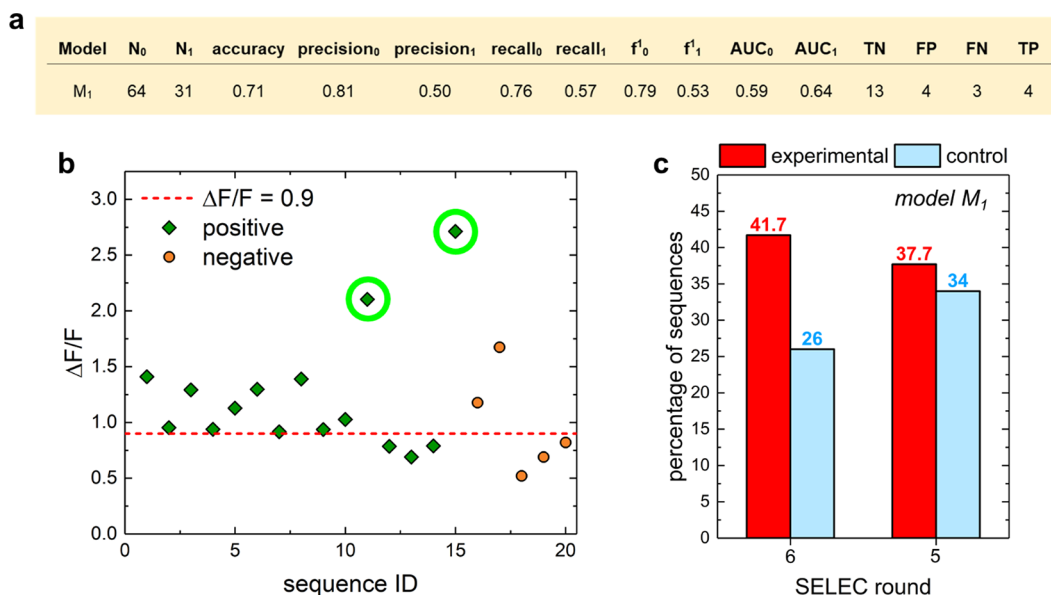


Figure 2. Performance of a representative CNN model trained on the initial data set. (a) Evaluation of a representative CNN M_1 model trained on the initial data set, using $t_1 > 0.9$ and $t_0 < 0.85$. (b) The optical response, $\Delta F/F$, of ssDNA–SWNT conjugates to $100 \mu\text{M}$ serotonin, obtained for 20 other ssDNA sequences, predicted by model M_1 to have high response (labeled positive) and low response (labeled negative) to serotonin. Sequences with $\Delta F/F$ values exceeding 1.9, the highest values in the initial data set, are marked with green circles. (c) Percentage of sequences in R6E, R5E, R6C, and R5C SELEC data sets predicted by model M_1 to be high responders to serotonin. The percentage is calculated for the first 300 SELEC data sequences in the given experimental/control data set that are not in the corresponding control/experimental data set.

training. nIR fluorescence emission was measured for those 96 unique ssDNA–SWNT conjugates before and after the addition of $100 \mu\text{M}$ serotonin; these data were already reported in ref 31 and are provided in Figure 1b and Supporting Information (SI), Figure S9 and Table S1. The response of conjugates to serotonin was calculated from the fluorescence emission spectra for the (8,6) chirality dominant peak ($\sim 1195 \text{ nm}$ center wavelength) as $\Delta F/F = (F_a - F)/F$, where F is the fluorescence signal before addition of serotonin and F_a is the fluorescence signal after the addition of serotonin (Figure 1a). The measured $\Delta F/F$ values for sequences in the initial data set range from 0.2 to 1.9. The initial data set, with sequences colored according to the SELEC group they belong to (experimental, control, or neither), is also shown in SI, Figure S9.

Using the approach in Figure 1c, convolutional neural network (CNN) classifier models were trained and tested on the obtained data set of 96 DNA sequences and their corresponding $\Delta F/F$ values (SI, Table S1, Figure 1b). The data set was split into two classes of sequences based on the response to serotonin, namely, class 1 sequences with a strong response to serotonin ($\Delta F/F$ threshold $t_1 > 0.9$) and class 0 sequences with a low response to serotonin (variable threshold $\Delta F/F$ values of $t_0 < 0.85, 0.8, 0.7, 0.6$, and 0.5). The thresholds were selected to examine how we can best identify sequences that lead to DNA–SWNT conjugates with either an exceptionally high response or an exceptionally low response to target analyte, which is the information that one would ideally want for practical applications of DNA–SWNT sensors. The choice of threshold $t_1 > 0.9$ leads to class 1 containing 32% of sequences from the total data set (31 out of 96 sequences). The variable threshold t_0 allows examining the effects of data set size and balance on ML model quality and stability: larger thresholds should lead to more sequences in class 0, imbalanced classes, more stable models, and learning of

sequences with intermediate response to serotonin, while lower thresholds should lead to fewer data points in class 0, more balanced classes, less stable models, and learning of sequences with very low response to serotonin. Input for CNN models consisted of ssDNA sequences, converted to position specific vector (psv_1) form with binary values (example shown in SI, Figure S1). The output of trained CNN models are probabilities for the input sequences to belong to class 0 and class 1 (independent).

Quality parameters for one of the best CNN models trained on the initial data set, model M_1 , are provided in Figure 2a. For M_1 , values of the area under the receiver operating curve (AUC) were 0.59 for predicting class 0 sequences and 0.64 for predicting class 1 sequences, while precision/recall were 0.81/0.5 and 0.76/0.57 for predicting class 0 and class 1 ssDNA sequences, respectively. Models were sensitive to removal of several sequences from input, especially class 1 sequences, as observed when seeking a high-quality CNN model trained on a truncated initial data set of only 93 data points. Quality parameters for a representative model M_{1B} , prepared with 93 data points from the initial data set, are reported in SI, Figure S2. Overall, while the CNN approach is giving good but not exceptional quality parameters, we decided to use it for classifying DNA sequences because several other ML methods tested, including AdaBoost, logistic regression, support vector classification, and random forest, led to unusable models. These latter methods consistently led to class 0 and class 1 probabilities of 0.5 ± 0.2 , indicating a poor differentiation between sequences with high and low response to serotonin for models trained with these methods (SI, Table S2). Separately, we choose psv_1 encoding of sequences over other types of encoding reported by others³⁶ that we also tested herein. Specifically, we found that term frequency vectors with sequence patterns 1, 2, 3, and 4 nucleotides in length ($\text{tfv}_1, \text{tfv}_2, \text{tfv}_3$, and tfv_4) frequently resulted in zero values in confusion

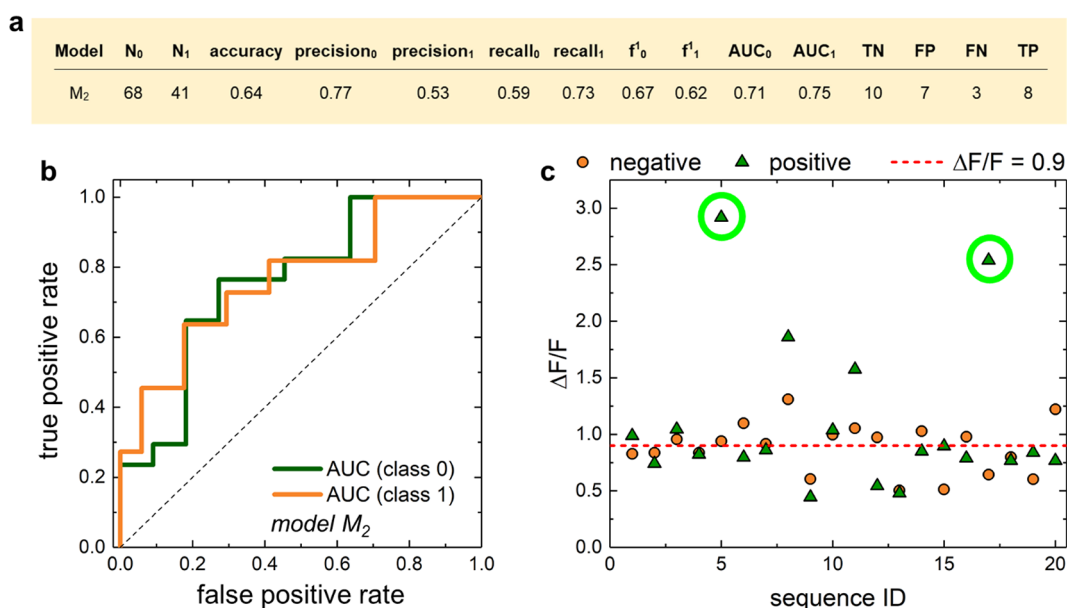


Figure 3. Performance of CNN models using the expanded data set. (a) Evaluation of a representative CNN M_2 model trained on the expanded data set, using $t_1 > 0.9$ and $t_0 < 0.85$. (b) ROC curves of model M_2 when predicting class 0 and class 1 sequences. (c) The optical response $\Delta F/F$ of ssDNA–SWNT conjugates to 100 μM serotonin, obtained for 40 ssDNA sequences. Sequences with $\Delta F/F$ values exceeding 1.9, the highest value in the initial data set, are marked with green circles.

matrices for testing sequences (simultaneous zero values for true positives and false positives). Null values in confusion matrices were noted in 90% of models for tf_{v1} , 100% of models for tf_{v2} and tf_{v3} , and 82% of models for tf_{v4} encoding (SI, Table S3).

We next examined the predictions of model M_1 for the most abundant DNAs from control and experimental SELEC data sets. We used model M_1 to classify the 300 most abundant DNA sequences from round 6 and round 5 experimental (R6E/R5E) and control (R6C/R5C) SELEC data sets, excluding any overlapping sequences found in both experimental and control data sets from the same rounds. Model M_1 predicts 41.7%/37.7% of R6E/R5E sequences and 26%/34% of R6C/R5C sequences to have high $\Delta F/F$ response to serotonin (Figure 2c). Because experimental data set sequences were selected based on their high affinity for SWNTs in the presence of serotonin, in contrast to the control data set sequences, our expectations were that experimental data sets contain more of the serotonin responsive sequences. These expectations agree with the predictions reported in Figure 2c.

To test the quality of model M_1 predictions, 20 DNA sequences were selected from the 300 most abundant sequences in the R6E SELEC data set for experimental validation measurements. According to model M_1 probabilities for these sequences to be in classes 0 or 1, 15 of the sequences are predicted to have high response to serotonin, while the remaining 5 are predicted to have low response to serotonin (SI, Table S4). Figure 2b and SI, Table S4, provide the experimentally measured $\Delta F/F$ values for the selected DNA sequences. Interestingly, 12 out of 15 predicted high-response sequences had $\Delta F/F$ values greater than the class 1 threshold, $t_1 > 0.9$ (80%, obtained from 12 out of 15 sequences). Furthermore, the validation experiments identified two sequences with $\Delta F/F$ values of 2.1 and 2.7, and thus a higher response to serotonin than observed for all the sequences within the initial data set. These sequences correspond to

ID#90 (8 reads, $\Delta F/F = 2.1$) and ID#115 (7 reads, $\Delta F/F = 2.7$), based on the read numbers in the R6E data set. Separately, 3 out of 5 predicted low-response sequences measured $\Delta F/F$ values lower than the class 0 threshold, $t_0 < 0.85$. Overall, predicted probability and experimental $\Delta F/F$ values do not have a statistically significant correlation (SI, Figure S3).

Performance of Single CNN Models Trained on Our Expanded Data Set. To examine if inclusion of additional experimental data points can produce more predictive models, we trained a second representative CNN model M_2 on the expanded data set of 113 sequences, which combined the initial data set (singly measured sequences in Figure 1b and SI, Table S1) and the additional experimental data from the first set of validation experiments (Figure 2b and SI, Table S4). A representative model M_2 has an accuracy of 0.64, AUC values of 0.71 for predicting class 0 sequences, and 0.75 for predicting class 1 sequences, while precision/recall are 0.77/0.59 and 0.53/0.73 for predicting class 0 and class 1 ssDNA sequences, respectively. In addition to improved AUC values, the M_2 model has significantly improved ROC curves in comparison to models M_1 and M_{1B} (Figure 3b, and SI, Figure S2).

To test the quality of model M_2 predictions, 40 DNA sequences were selected from 280 untested most abundant sequences in the R6E data set for the next round of experimental validation measurements. Of those 40 DNA sequences, half were predicted by M_2 to have a low response (labeled as negative) and the other half were predicted to have a high response (labeled as positive) (SI, Figure S4). Figure 3c, and SI, Table S4 provide the experimentally measured $\Delta F/F$ values for the selected DNA sequences. Model M_2 overestimates false positive sequences because only 7 out of 20 sequences (35%) predicted to have high response to serotonin actually have $\Delta F/F$ greater than the class 1 threshold of $t_1 = 0.9$, while the remaining 13 out of 20 sequences (65%) have $\Delta F/F < 0.9$. Single models such as M_2 could potentially be predicting sequences with responses similar to randomly

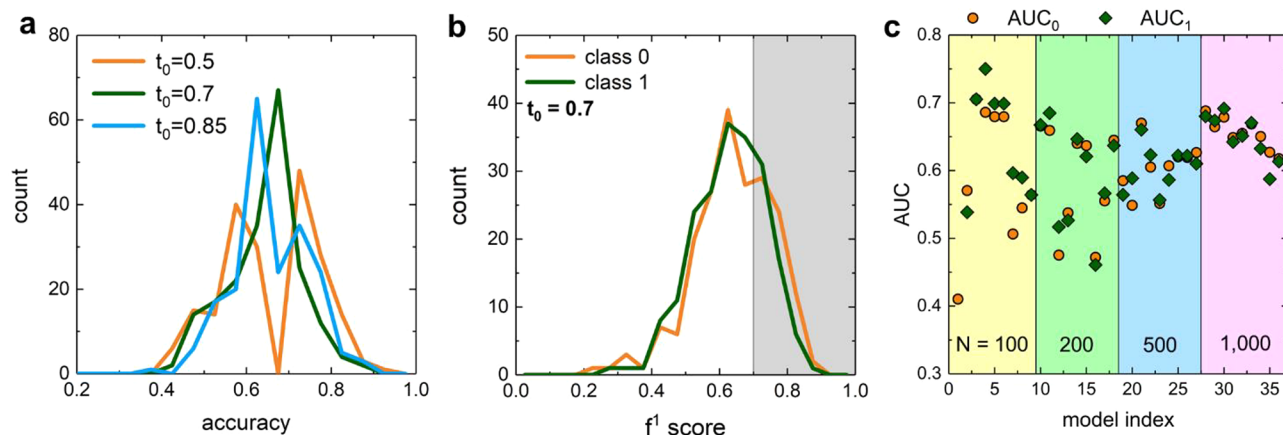


Figure 4. Stochasticity of CNN models. (a) Distribution of accuracy values for 200 CNN models with psv₁ input, obtained using different random states for several t_0 values. (b) Distribution of f^1 score values for 200 CNN models, obtained using different random states for $t_0 = 0.7$. (c) Dependence of model stability on data set size. AUC values for nine CNN models trained on 100, 200, 500, and 1000 sequences in each of two classes, extracted from R6C (class 0) and R6E (class 1) SELEC data sets. Each model has a different random state variable.

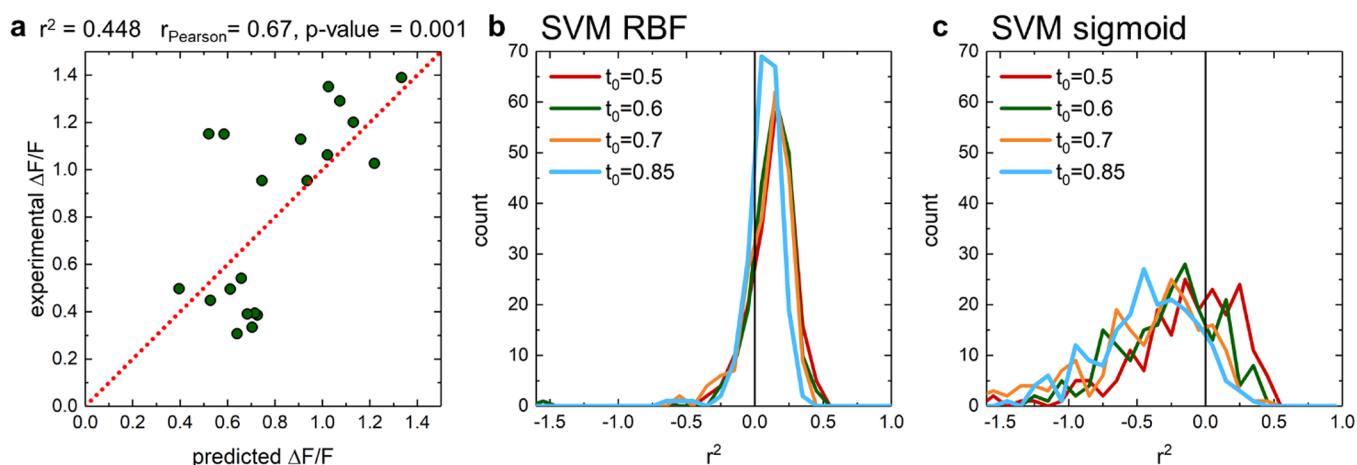


Figure 5. SVM regression models for predicting $\Delta F/F$ values of ssDNA–SWNT conjugates. (a) Comparison of experimentally measured $\Delta F/F$ values and $\Delta F/F$ values predicted by one of the best SVM RBF regression models, trained using the expanded data set and thresholds $t_1 > 0.9$ and $t_0 < 0.6$ for one selected random state variable. (b) Distribution of r^2 values for 200 SVM RBF models, obtained using different random state variables. (c) Distribution of r^2 values for 200 SVM sigmoid models, obtained using different random state variables.

chosen sequences. Of practical relevance, model M_2 predicted two previously undiscovered sequences from the R6E evolution group with a very strong response to serotonin, with $\Delta F/F$ values of 2.5 and 2.9. Interestingly, these sequences correspond to ID#264 (6 reads, $\Delta F/F = 2.5$) and ID#156 (7 reads, $\Delta F/F = 2.9$), based on the read numbers in the R6E evolution group. Separately, while all the sequences predicted to have a low response to serotonin had $\Delta F/F < 1.3$, only 9 out of 20 sequences (45%) have $\Delta F/F$ values smaller than the class 0 threshold of $t_0 = 0.85$.

Predicting High-Response DNAs from Combined Classification and Regression Models. When training models M_1 and M_2 , we noted their stochastic behavior and dependence on the random state variable (different training/testing data set splits) selected during the training procedure. To characterize the stochasticity of these models trained on our sparse data sets of ~ 100 sequences, we next analyzed their accuracy and f^1 scores. The analysis was performed on 200 CNN models trained on the expanded data set using different random state variables and several t_0 threshold values (0.5, 0.6, 0.7, 0.8, 0.85). Distributions of these models' accuracies and f^1

scores, shown in Figure 4a,b, and SI, Figure S5, range from 0.4 to 0.93 and 0.2 to 0.9, respectively. While these distributions span a wide range, most models have accuracies and f^1 scores higher than 0.5 and should thus be predictive. Furthermore, more than half of the models in Figure 4b have accuracy and f^1 scores higher than 0.6. Interestingly, the predictions of high response sequences are of higher quality for lower thresholds t_0 (SI, Figure S5 and Table S6). Model stochasticity decreases and model stability increases once the data sets contain 500 or more sequences per class (Figure 4c, input sequences selected from SELEC data sets).

With the objective of predicting DNA sequences with the highest $\Delta F/F$ values, we next trained regression models, which predict $\Delta F/F$ values based on the DNA sequence input. The regression models were trained using the support vector machine (SVM) regression algorithm with radial basis function (RBF) and sigmoid kernels, based on successful applications of these algorithms for sequence input.³⁷ One of the best SVM RBF regression models, trained on the expanded data set with sequences with $\Delta F/F > 0.9$ and $\Delta F/F < 0.6$, is shown in Figure 5a. There is a high correlation between $\Delta F/F$ values of test

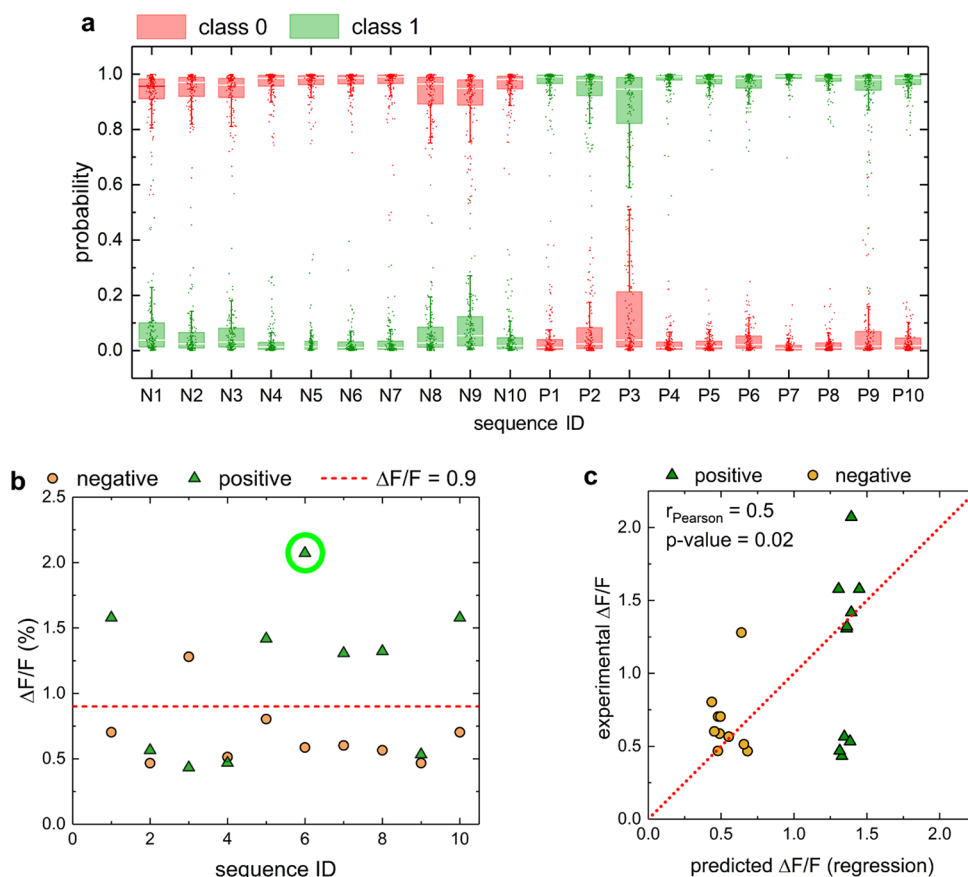


Figure 6. Predicting DNA sequence response to serotonin from multiple high-quality classification and regression models. (a) Probabilities of 20 DNA sequences to be high response (class 1) or low response (class 0) to serotonin; these sequences were selected based on predictions of multiple high-quality classification and regression models, as described in the text. (b) The optical response, $\Delta F/F$, of ssDNA–SWNT conjugates to 100 μM serotonin, obtained for the same 20 ssDNA sequences. The sequence with $\Delta F/F$ value exceeding 1.9, the highest value in the initial data set, is marked with a green circle. (c) Comparison of experimentally measured and predicted $\Delta F/F$ values for the same 20 ssDNA sequences.

sequences obtained experimentally and those predicted by this SVM model, with $r^2 = 0.448$ and the Pearson coefficient $r_{\text{Pearson}} = 0.67$ (p -value = 0.001). However, as with classification models, the quality of regression models also depends on the random state variable. This dependence is noted in distributions of r^2 values for 200 models obtained with SVM RBF and SVM sigmoid methods, different random state variables, and different t_0 values (Figure 5b,c). For both SVM RBF and SVM sigmoid methods, r^2 values range from negative values to 0.5, with SVM RBF models being on average of higher quality than SVM sigmoid models.

Given the large number of high-quality classification and regression models obtained, we next assessed whether combining the predictions of these models could be used to determine DNA sequences with high and low response to serotonin. For this purpose, CNN models were trained on input data with thresholds $t_1 = 0.9$ and $t_0 = 0.5$ or 0.6 , which resulted in f_0^1 and $f_1^1 > 0.6$. These were next used to predict high and low response sequences from a set of 3000 most abundant previously untested R6E sequences. Separately, the best regression models with $r^2 > 0.45$, trained using the expanded data set and sequences within thresholds $t_1 > 0.9$ and $t_0 < 0.5$, were used to predicted $\Delta F/F$ values for the same 3000 sequences. After ranking the sequences according to their regression-predicted $\Delta F/F$ values, we extracted the top 10 sequences that are also classified as high response with the

CNN models by having consistently high/low probabilities to be in class 1/class 0 (Figure 6a). For comparison, we also extracted the bottom 10 ranked sequences, which are also classified as low responders to serotonin based on having consistently low/high probabilities to be in class 1/class 0 (Figure 6a). The probabilities of these 20 sequences to be in class 1/class 0 were also determined by the ensemble of multilayer perceptron artificial neural network (MLP-ANN) models, shown in SI, Figure S8; the ensemble included the models trained on input data with thresholds $t_1 = 0.9$ and $t_0 = 0.7$, which have f_0^1 and f_1^1 scores greater than 0.6. The probabilities obtained from the MLP-ANN models (SI, Figure S8) closely followed the trends of probabilities from the CNN models (Figure 6a). The performance of the above top 10 and bottom 10 sequences, labeled as positive and negative, was then examined experimentally (Figure 6b, and SI, Table S7). Six out of 10 positive sequences (60%) had $\Delta F/F$ responses greater than the class 1 threshold of $t_1 = 0.9$, and one of them had $\Delta F/F = 2.1$ (SI, Figure S6), exceeding the highest value in the initial data set (1.9). Furthermore, 9 out of 10 negative sequences (90%) had $\Delta F/F$ response smaller than the class 1 threshold of $t_1 = 0.9$. There is a statistically significant correlation between experimentally measured and predicted $\Delta F/F$ values (Figure 6c), with Pearson correlation coefficient of $r_{\text{Pearson}} = 0.5$ and p -value of 0.02.

Table 1. DNA–SWNT Sensors for Serotonin Identified in the Present Study^a

sequence	$\Delta F/F$	ID in R6E dataset	no. of reads (sequencing)
CCCCCAAGGCAACCAGACGTCCGCCCC	2.103	90	8
CCCCCGACCCACACCAACCAGTGCCCC	2.713	115	7
CCCCCGACCCCTTACCACCAACTCCCC	2.917	156	7
CCCCCAACACAAGACAACGCGTGCCCC	2.538	264	6
CCCCCGACCCAAAGCCAACACCTCCCC	2.072	473	5

^aThe ID numbers and the numbers of reads are obtained from the R6E SELEC dataset.³¹

CONCLUSIONS

In this work, we apply machine learning methods toward discovery of DNA–SWNT sensors for serotonin with high nIR fluorescence response to serotonin. In prior sensor design efforts, we experimentally tested the serotonin response of 96 DNA–SWNT sensors, which were chosen by the abundance (read numbers in library) of each DNA sequence in the experimental and control libraries from SELEC experiments. This previous selection method assumes the best predictor of sensor sensitivity and selectivity is the sequence abundance, which excludes the characterization of low abundance DNA sequences. Here, we demonstrate that ML models can improve on this abundance-based selection. Specifically, ML models can autonomously learn the correlation between DNA sequences and fluorescence responses to analytes and thus assist and improve the selection of better sensor candidates. Choosing the abundant sequences from SELEC experiments increases the chance that the initial data set for ML model training will contain a large proportion of high response sequences, which is less likely to be obtained for a data set of completely randomly chosen sequences.

ML models can learn correlations from data sets of already experimentally tested sequences and predict promising DNA sequences. After testing multiple ML methods, we find that convolutional neural network classifier models provide the most meaningful results when trained on sparse data sets of ~100 DNA sequences, a size typical for sensor search efforts. At first, we train and test two single CNN classifier models and use them to predict high response DNA sequences for experimental validation. Even though the prediction accuracies calculated from testing data differ from the accuracies measured in experiments, these models still predicted multiple DNA sequences with higher response to serotonin than those previously achieved experimentally. Furthermore, we trained and tested regression models on DNA sequence input to predict the relative nIR fluorescence response of these sequences.

Through analyses of model quality parameters for multiple models obtained with different training/testing data splitting, we show that both classification and regression models trained on our sparse data sets are stochastic. However, the majority of models are predictive, because most models have accuracies greater than 50%. Further analyses of model stochasticity dependence on data set size indicate that model stability can be achieved with data sets of 500 molecules per class. Because obtaining such large data sets in experiments is difficult, we instead explore integrating the predictions of multiple highest quality artificial or convolutional neural network classifiers and SVM regression models, inspired by model ensembling approaches,³⁸ and demonstrate an effective increase in the success of this ML approach. We experimentally validate that our integrated approach leads to 60% correct predictions for high responding sequences and 90% correct predictions for

low responding sequences, supporting the utility of our method for predicting promising DNA sequences and accelerating sensor discovery. Separately, we show that a simpler principal component analysis (PCA) approach appears to be predictive in analysis plots but exhibits a poor correlation between predictions and validation experiments (SI, Figure S7), in contrast to the successful artificial and convolutional neural network classifiers and SVM regression models. Furthermore, many sequence patterns are reported in high-response sequences, but most have low abundance (SI, Table S8), further confirming the benefit of using ML models when making predictions of high response sequences from existing experimental data sets.

Overall, our ML approaches led to discovery of five serotonin DNA–SWNT sensors, identified in Table 1. Importantly, these sensors all had higher response than sensors previously identified experimentally using only manual screening of the highest-abundance sequences in the R6E SELEC library ($\Delta F/F = 1.9$). Furthermore, the ability to predict DNA sequences that do not respond to serotonin (or any analyte of interest such as interfering agents) with our models is also important for sensor design. Taken together, our results suggest ML approaches can rapidly identify DNA sequences that are great responders for the target analyte and could significantly expedite the development of technologies dependent on DNA–SWNT conjugates, including biosensors, bioelectronics, and chirality separation of SWNTs.

EXPERIMENTAL METHODS

Data Set Encoding and ML Models. Data sets used to train and test the initial ML models consisted of ssDNA sequences and their corresponding $\Delta F/F$ values, obtained in experiments reported and described in ref 31. This data set is also listed in SI, Table S1. ssDNAs consisted of 18-nt variable segment flanked by two C₆-mers from each side. Sequences of 18-nt variable segments of ssDNAs were considered as input data for our models. Several encodings of input data were considered, including position specific vectors (1-gram, labeled as psv₁ and shown in SI, Figure S1) and term frequency vectors (1-, 2-, 3-, or 4-gram, labeled as tfv₁, tfv₂, tfv₃, and tfv₄ and described in SI, Table S3). The data set from SI, Table S1 was split into binary classes, where class 0 contains DNA sequences with low response to serotonin and class 1 contains DNA sequences with high response to serotonin. The threshold value to define class 0, t_0 , was varied as a parameter ($t_0 < 0.85, 0.8, 0.7, 0.6, \text{ or } 0.5$). The threshold value to define class 1, t_1 , was held fixed at $t_1 = 0.9$. This threshold selection leads to a reasonable balance of class sequences, as required for model training and testing.

Performance of several ML classifier models was tested, including AdaBoost, logistic regression, support vector classification linear, and random forest. For these models, sequences were expressed as 1×72 binary arrays, obtained by sequential listing of psv₁ matrix columns into a one-dimensional array. Separately, we tested the performance of convolutional neural network (CNN) models on psv₁ and term frequency vector input, successful in previous predictions of DNA and RNA sequence specificities.³⁹ All our models were trained to predict

the probability of the input sequence having high or a low response to serotonin. ML models were trained using the Scikit-learn library, and CNN models were constructed with Keras and TensorFlow 2 used as backend. ML and CNN models were trained using the initial data set (SI, Table S1). Because the best performance was observed for CNN models with psv_1 encoding, the models for extended data sets were generated only with the CNN approach.

All of the codes for training ML classification and regression models are freely available on GitHub (<https://github.com/vukoviclab/DNAseensor>).

Evaluation Metrics. Our CNN models were trained to predict the response of 18-nt DNA sequences to serotonin, or equivalently, to predict these sequences' probabilities to belong to class 0 or class 1 molecules. In these models, probabilities for sequences to belong to class 0 and probabilities for sequences to belong to class 1 were evaluated independently. Predicted high response sequences were determined according to the criterion that normalized class 1 probabilities, defined as $\text{probability}(\text{class } 1)/[\text{probability}(\text{class } 0) + \text{probability}(\text{class } 1)]$, are greater than 0.5.

For each prepared model, we calculated multiple metrics including accuracy, precision, recall, f^1 score, ROC curves, and areas under the ROC curves (AUC), and monitored the number of true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN). TP/FP values are numbers of test sequences correctly/incorrectly predicted to have a high response to serotonin by the models, and TN/FN values are numbers of test sequences correctly/incorrectly predicted to have low response to serotonin by the models. Accuracy was calculated as $Ac = (TP + TN)/(TP + TN + FP + FN)$, precision was calculated as $Prec = TP/(TP + FP)$, and recall was calculated as $R = TP/(TP + FN)$, and f^1 score = $(2Prec - R)/(Prec + R)$, according to their standard definitions. For ML models, single values of precision, recall, and f^1 values were evaluated. For CNN models, two values of precision, recall, and f^1 scores were reported, allowing the independent assessment of prediction quality for sequences with low and high response to serotonin.

Performance of all the models was also examined with the receiver operating characteristic (ROC) curves and the areas under ROC curves (AUC). For each CNN model, two ROC curves and AUC values were obtained, one reporting the prediction quality for test sequences with low response to serotonin (AUC_0) and the other reporting the prediction quality for test sequences with high response to serotonin (AUC_1).

For most of the data sets (with defined encoding and classification thresholds), 200 models were generated with different random states (different training/testing data splitting). For some sets of the trained models, we report the following evaluations related to model quality metrics: mean, standard deviation, minimum, 25%, 50%, and 75% percentile values, and maximum.

PCA Analysis. The 200 most abundant sequences from R6E and R6C SELEC data sets were analyzed using principal component analysis (PCA) within Scikit-learn library. Locations of some of the experimentally tested sequences were then examined in the above determined PCA space.

Motif Search. Sequences from the expanded data set (used to train and test model M_2) were split in two classes: sequences that recognize serotonin (positive, $\Delta F/F > 0.9$) and sequences that do not recognize serotonin (negative, $\Delta F/F < 0.85$). These two classes were used to search for DNA sequence motifs associated with serotonin recognition using MERCI software.⁴⁰ In the search, minimal occurrence frequency for positive sequences f_P and the maximal occurrence frequency for negative sequences f_N were set to 3 and 0, and the maximum motif length was set to 18.

ssDNA–SWCNT Suspension Preparation. ssDNA-functionalized SWNT suspensions were generated with the following protocol: 1 mg of HiPCo SWNT (NanoIntegris) was added to 0.9 mL of PBS buffer, and the solution was mixed with 100 μL of 1 mM ssDNA. We prepared colloidal suspensions of SWNTs with the initial 96 ssDNA sequences (SI, Table S1) and all the subsequent sequences (SI, Tables S4, S5, and S7) comprising variable 18-nt sequences flanked by two C_6 -mers from each side. The resulting mixture was

bath-sonicated for 2 min and tip-sonicated for 10 min at 5 W power in an ice bath. After sonication, the black ssDNA–SWNT suspension was centrifuged for 30 min at 16 100g to precipitate nondispersed SWNT, and the supernatant containing solubilized ssDNA–SWNT was collected. The supernatant was spin-filtered with 100 kDa MWCO centrifugal filters at 6000 rpm for 5 min with DNase-free water to remove unbound ssDNA, and the purified solution at the top of the filter was collected. This spin filtration to remove unbound ssDNA was repeated three times. The ssDNA–SWNT suspension was diluted with PBS buffer and stored at 4 °C until use. The concentration of the ssDNA–SWNT suspension was calculated by measuring its absorbance at 632 nm with an extinction coefficient for SWNT of $0.036 (\text{mg/L})^{-1} \text{cm}^{-1}$.

Fluorescence Response Measurement of Sensors to Serotonin. Fluorescence spectra of 99 μL ssDNA–SWNT suspensions (10 mg/L) in PBS were measured before and 10 s after the addition of 1 μL of 10 mM serotonin solution for a final serotonin concentration of 100 μM . We analyzed the fluorescence intensity change of the (8,6) SWNT chirality peak (~ 1195 nm) in this study. $\Delta F/F$ was calculated as $\Delta F/F = (F_a - F)/F$ based on the baseline fluorescence intensity before analyte addition (F) and the fluorescence intensity 10 s after analyte addition for the (8,6) SWNT chirality (~ 1195 nm) (F_a). All of the values of $\Delta F/F$ for sequences in the initial and validation data sets were obtained as a mean of technical triplicates measurements, which were normally in high agreement. $\Delta F/F$ readout for DNA–SWNT sensors was based on (8,6) peak intensity rather than the integrated intensity, even though both choices lead to the same qualitative trend. Yet, (8,6) peak is more sensitive than the integrated intensity; for example, the $\Delta F/F$ for E3-P6 DNA sequence (SI, Table S7) is 207% when using the (8,6) peak and 86% when using the integrated intensity. $\Delta F/F$ values for the initial 96 ssDNA sequences, ranging from 0 to 1.9, represent the fluorescence response for the 96 most abundant sequences from the experimental and control groups for SELEC rounds 3 to 6. $\Delta F/F$ values of an additional 100 sequences, tested in validation experiments, are reported in SI, Tables S4, S5, and S7. We note that our data sets for building ML models do not distinguish the response of two enantiomers in DNA-wrapped (8,6) SWNT $\Delta F/F$ measurements. DNA sequences are classified as high response in the experimentally obtained data sets as long as at least one of the DNA-wrapped SWNT enantiomers has high response to serotonin. Therefore, if the ratio of (8,6) enantiomers in the SWNT sample is changed, DNA–SWNT samples made with the high-response sequences could have a different $\Delta F/F$ response to serotonin.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsnano.1c08271>.

The initial data set of DNA sequences and their optical response to serotonin, example of position specific vector encoding of a DNA sequence, performance of two representative convolutional neural network models, examples of probabilities/predictions for several machine learning algorithms, performance of convolutional neural network models with term frequency vector encoding of sequences, the validation data sets of DNA sequences and their optical response to serotonin, correlation between the experimental response and predicted probabilities for validation sequences, probabilities of validation sequences to be low or high responders to serotonin from single convolutional neural network models, distribution of f^1 score values for ensembles of convolutional neural network models, performance of convolutional neural network models for several different data set classifications, example spectra for one of the DNA–SWNT sensors, principal

component analyses of sequences from data sets, probabilities of validation sequences to be low or high responders to serotonin from multiple artificial neural network models, a plot of the initial data set of DNA sequences and their optical response to serotonin, analyses of motifs in DNA sequences with high response to serotonin (PDF)

AUTHOR INFORMATION

Corresponding Authors

Lela Vuković – Bioinformatics Program, University of Texas at El Paso, El Paso, Texas 79968, United States; orcid.org/0000-0002-9053-5708; Email: lvukovic@utep.edu

Markita P. Landry – Department of Chemical and Biomolecular Engineering and California Institute for Quantitative Biosciences, QB3, University of California, Berkeley, California 94720, United States; Innovative Genomics Institute, Berkeley, California 94702, United States; Chan–Zuckerberg Biohub, San Francisco, California 94158, United States; orcid.org/0000-0002-5832-8522; Email: landry@berkeley.edu

Authors

Payam Kelich – Bioinformatics Program, University of Texas at El Paso, El Paso, Texas 79968, United States

Sanghwa Jeong – School of Convergence Engineering, Pusan National University, Yangsan 50612, South Korea; orcid.org/0000-0003-3529-0346

Nicole Navarro – Department of Chemical and Biomolecular Engineering, University of California, Berkeley, California 94720, United States

Jaquesta Adams – Department of Chemical and Biomolecular Engineering, University of California, Berkeley, California 94720, United States

Xiaoqi Sun – Department of Chemical and Biomolecular Engineering, University of California, Berkeley, California 94720, United States

Huanhuan Zhao – Bioinformatics Program, University of Texas at El Paso, El Paso, Texas 79968, United States

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acsnano.1c08271>

Author Contributions

[†]P.K., S.J., and N.N. contributed equally

Notes

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