

High-throughput Design and Screening of Biological Sensors to Detect Small molecules

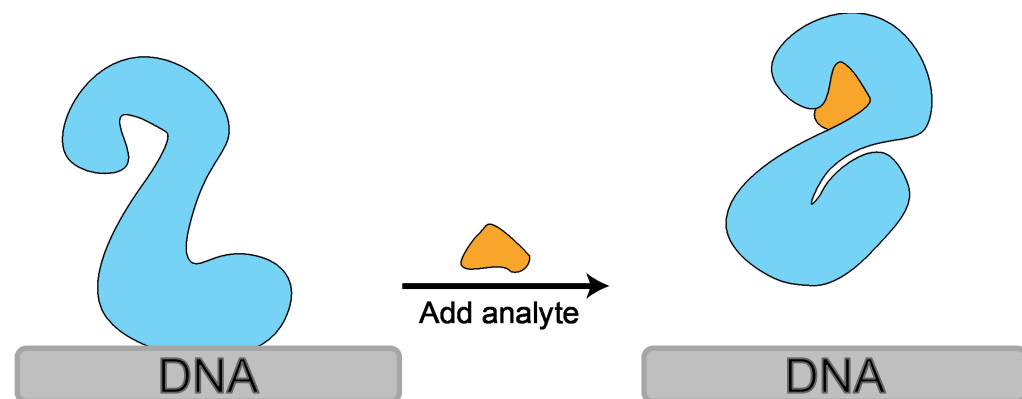
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BACKGROUND

Allosteric transcription factors are chemical sensors:

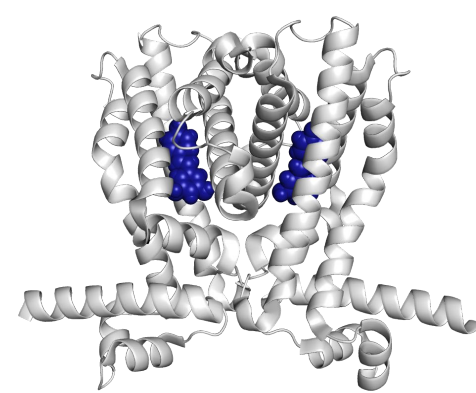
In this work we engineer protein sensors for a panel of non-native targets, demonstrating sensitivity to diverse molecules and laying the groundwork for future development of field deployable water quality assays. Bacterial allosteric transcription factors (aTFs) possess a DNA binding interaction that can be modulated by the addition of chemical inducers. These aTFs can be engineered to alter their specificity toward new targets of interest. (a)



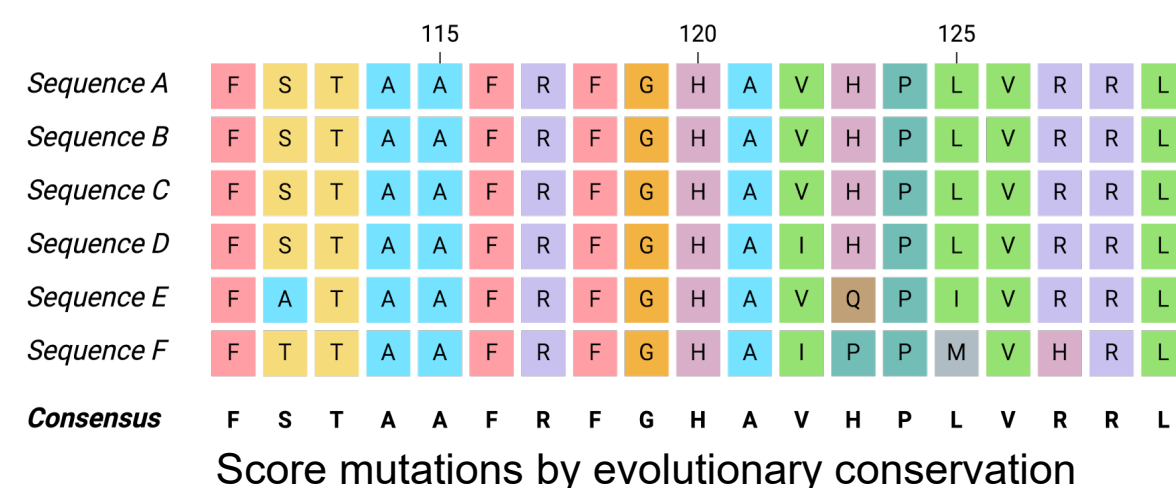
METHODS OVERVIEW

Evolution and structure guided design of a bacterial transcription factor (TtgR):

We generated a **17,000-member mutant library** of the aTF TtgR using a combination of biophysical stability predictions and evolutionary conservation



Mutate and score with Rosetta



We developed SensorSeq, a method that allows for the determination of aTF ligand sensitivity through the sequencing of an RNA barcode, which is mapped to a variant aTF sequence (b)



References:

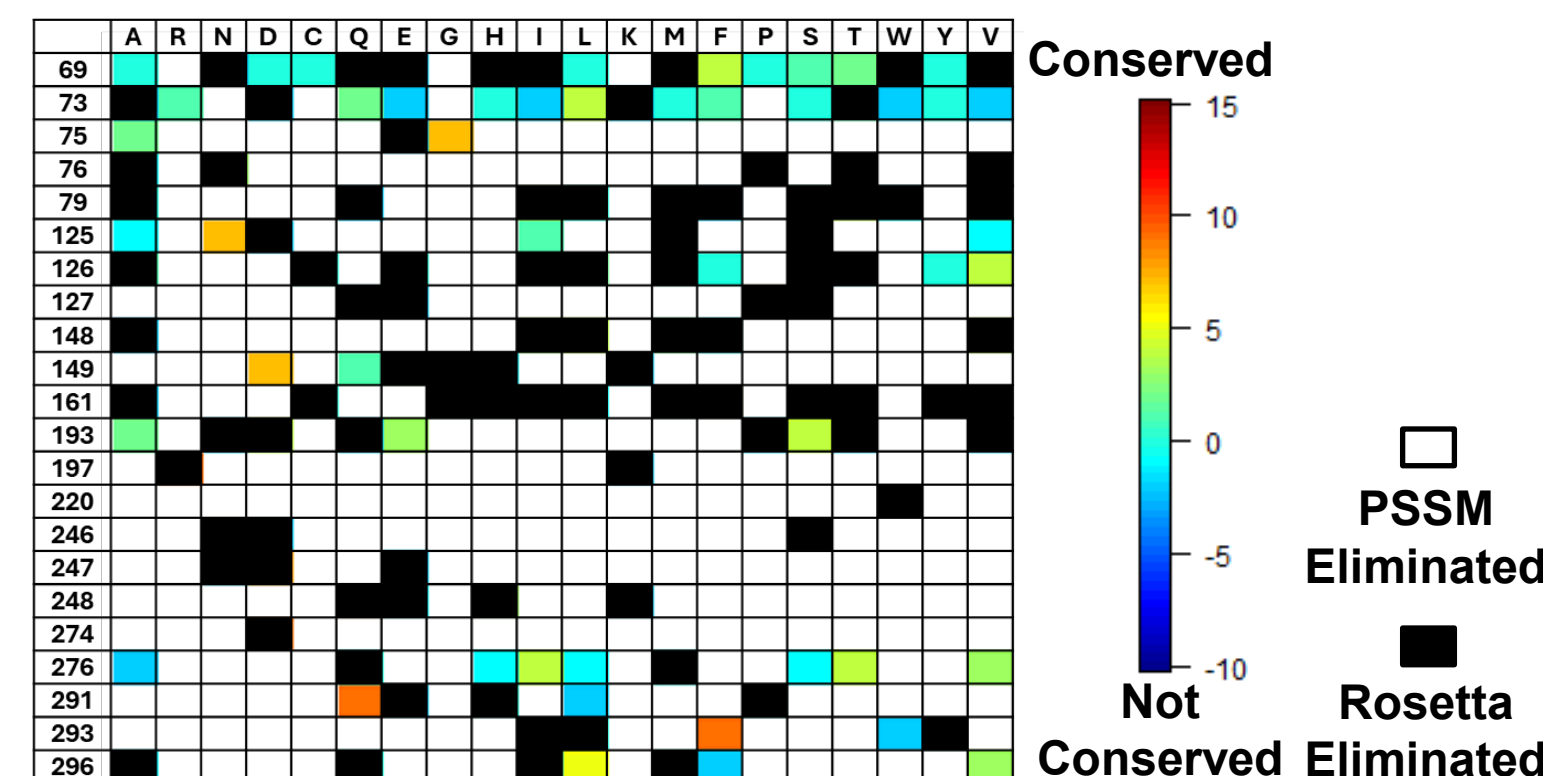
- (a) Nishikawa et al., Nat. Comm. (2021)
(b) Nishikawa and Chen, et al., bioRxiv (2024)



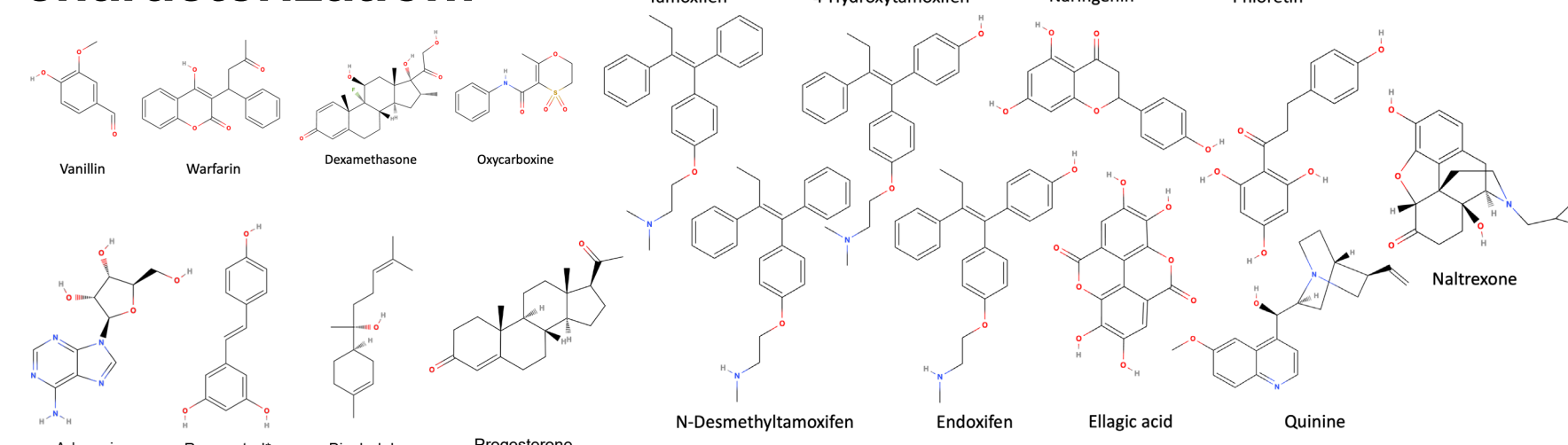
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SensorSeq was applied to screen for novel or enhanced function against 30 diverse ligands:



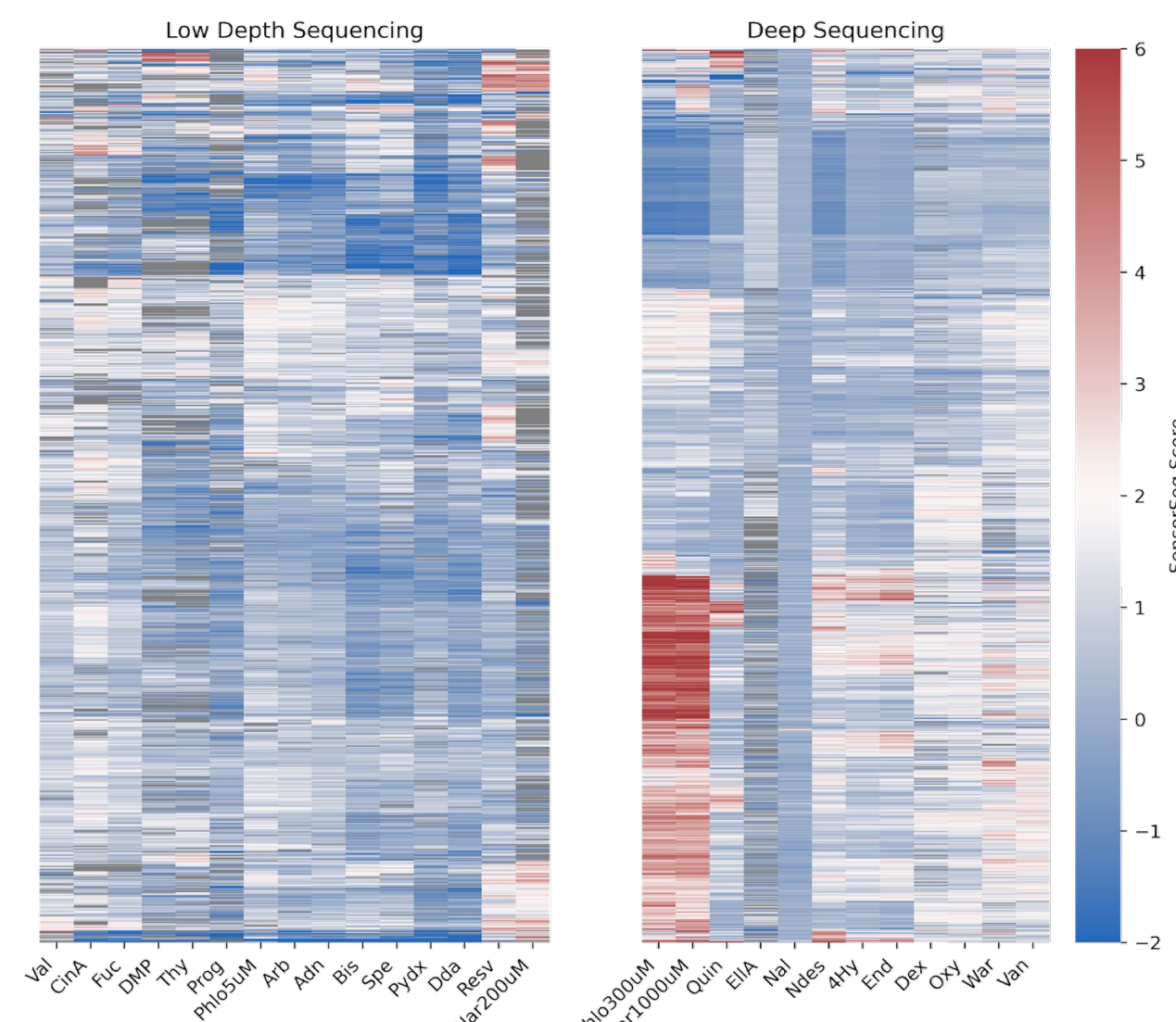
High-throughput experimentation enables many-target characterization:



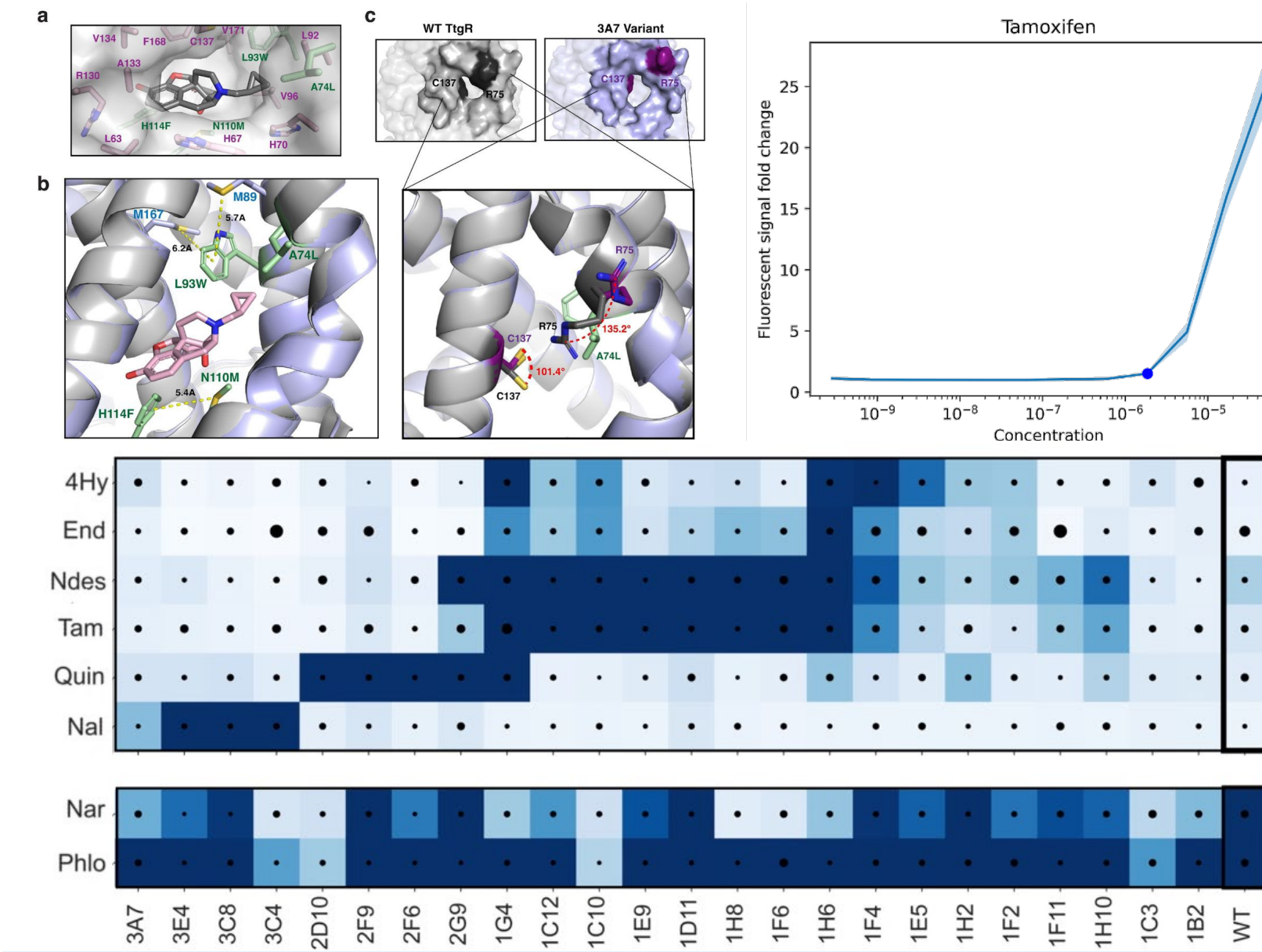
RESULTS

Sequencing results show new or enhanced sensors for 20 molecules:

Based on high throughput SensorSeq results, we observe variants with new or improved function for 20/30 ligands.



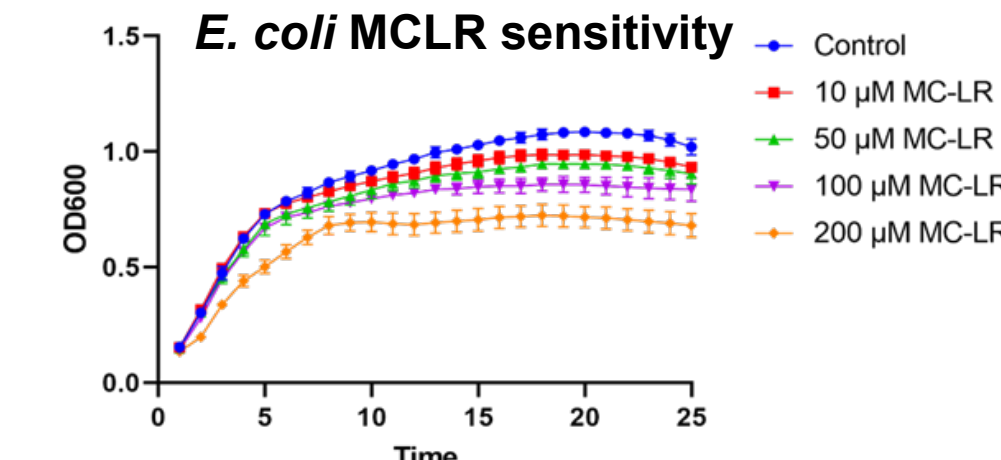
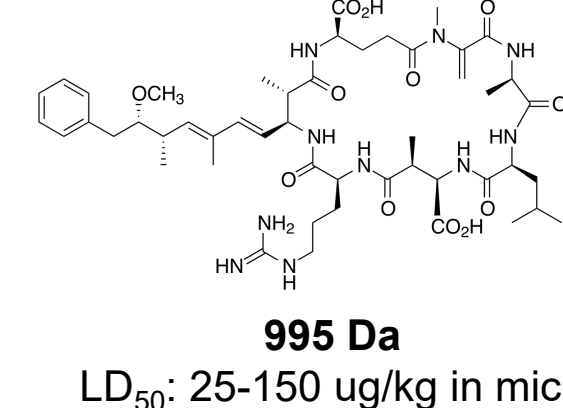
Secondary validation shows high consistency with sequencing assay



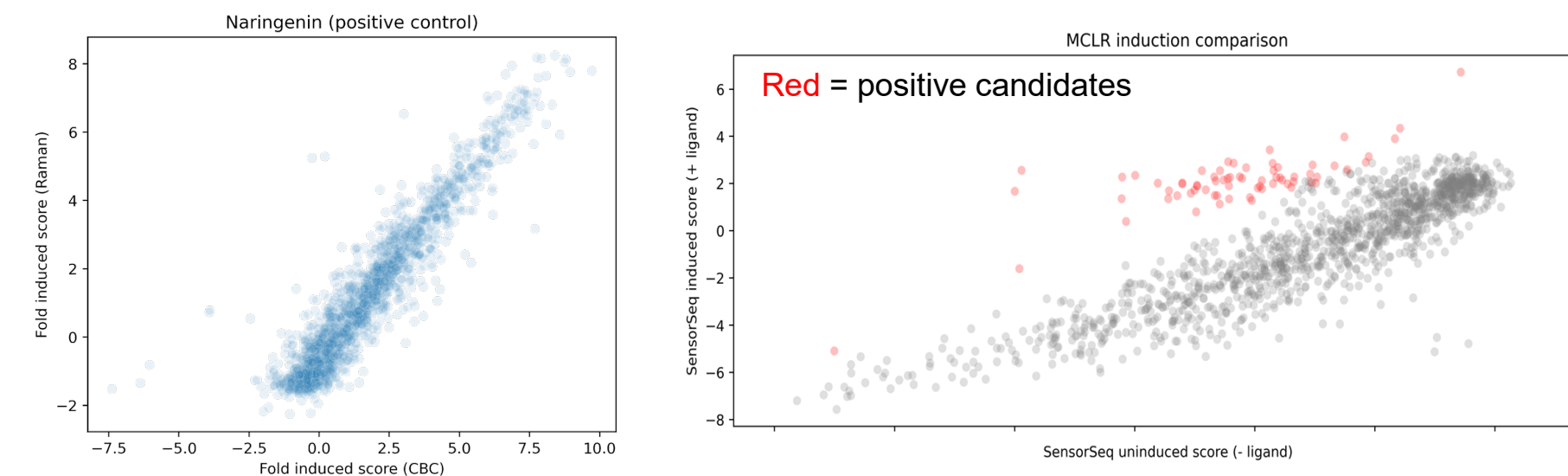
MICROCYSTIN-LR AS A LIGAND

Microcystin is a hepatotoxin that is found in freshwater lakes and ponds throughout the world.

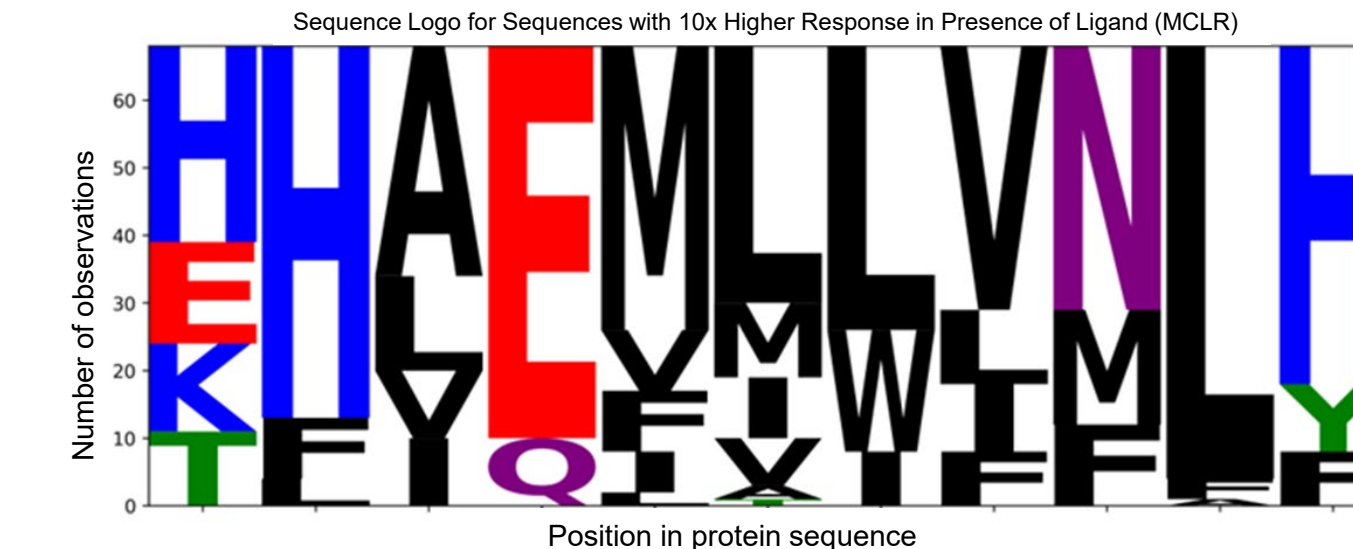
MCLR)is the most prevalent aquatoxin in the USA, with places like Lake Okeechobee having levels reported at >400 ug/L of water



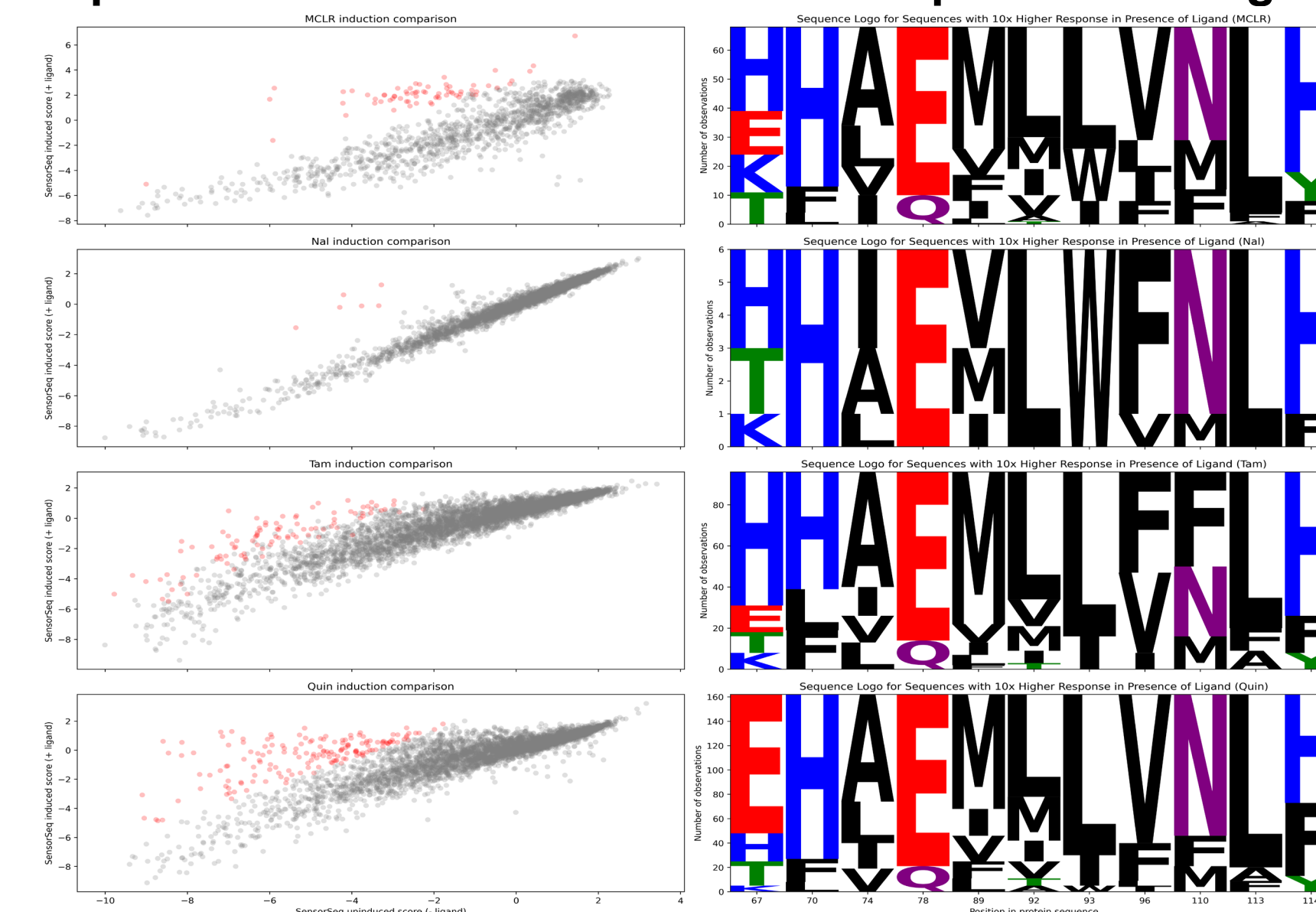
Positive control screen conducted at DEVCBC is consistent with Raman lab control:



Screen revealed set of candidate sensors for microcystin

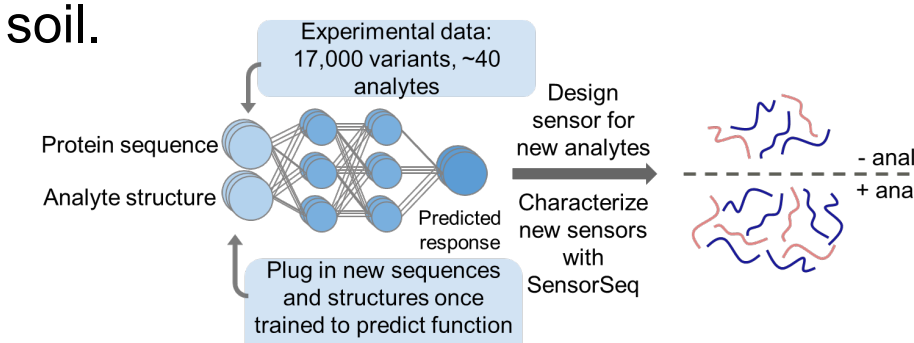


Sequence-fitness determinants are unique for each ligand



FUTURE DIRECTIONS

Utilizing the vast amounts of data collected with SensorSeq, we are in the process of a second iteration of TtgR design where mutation selection is guided by machine learning. This approach could be applied to several other aTF scaffolds with diverse native ligand sensitivity. Optimized aTFs have a range of applications, notably detection of toxins and pollutants in water, wastewater, and soil.



Acknowledgements: This work is a collaboration between DEVCBC and U. Wisconsin-Madison. The microcystin-LR SensorSeq work was made available through the Department of Defense (DoD) Funding Laboratory Enhancements Across (X) Four Categories (FLEX 4) in accordance with the Army, Navy, and Air Force efforts funded under the authority in 10 U.S.C. § 4123. DEVCBC internal Thank you to the UW-Madison biotechnology center for providing sequencing services. Thank you to Kelley Betts for assistance with poster formatting.