# An Integrated Computational and Zebrafish Platform for Neuroactive Drug Discovery


UMass Chan MEDICAL SCHOOL

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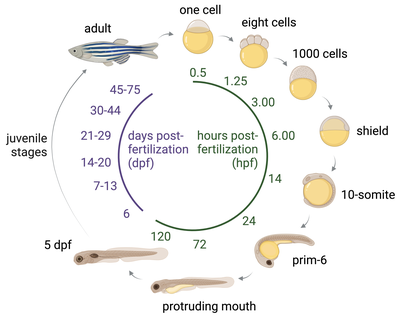
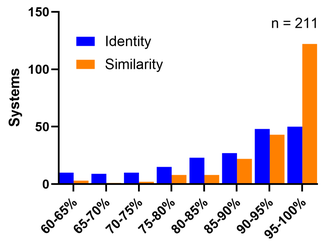
## Introduction

- Enhancing cognitive performance, wakefulness, stress resilience, and social motivation is critical to warfighter readiness.
- CNS drug discovery is limited by low-throughput rodent studies and poor translation from in vitro systems. Zebrafish offer a high-throughput, whole-animal vertebrate model with conserved neural circuitry and quantifiable behavioral outputs.
- Zebrafish are small, rapidly developing, and transparent until two weeks of age.

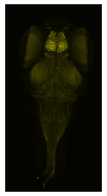



- Zebrafish share >70% of human genes.
- The binding pockets of >200 GPCRs, as important class of proteins for neuroactive drug discovery, are highly conserved[1]:



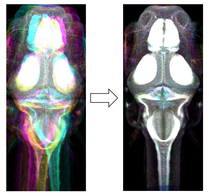

## Zebrafish brain imaging

**Whole-brain activity mapping**[2]: Calcium influx in active neurons leads to Erk phosphorylation

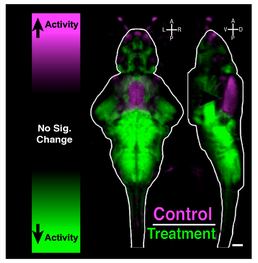


Rapidly fix brain state and stain freely swimming fish

p-Erk



Computational morphing



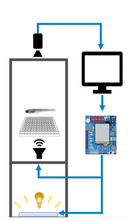

Activity differences for drugs, behavioral responses, or genetic mutants

Compare to Z-Brain[2] anatomical map of 294 regions

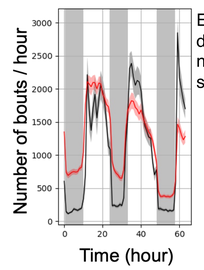
## Zebrafish behavior assays

Mutants Siblings

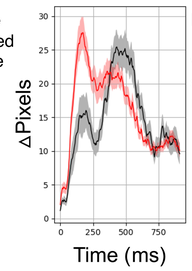
### Sleep, visual/acoustic startle (96-well plate, larvae, >1,000 fish / day)



Home-built behavioral systems, adaptable to many assays and custom tracking algorithms[3]

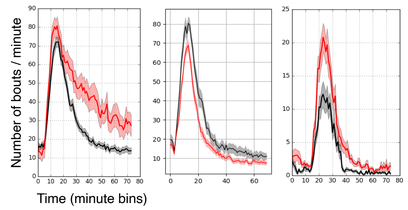



Example decreased nighttime sleep



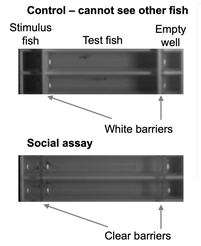

Example increased visual startle response

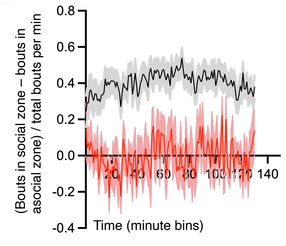
### Heat response, stress (96-well plate, larvae, >1,000 fish / day)




- Differential behavioral responses to a 10°C increase in water temperature[4]
- Assay for stress response, passive coping (giving up), and/or thermal tolerance
- Cold stress also produces a characteristic movement response[5]

### Social affiliation (20-well plate, juvenile, >200 fish / day)

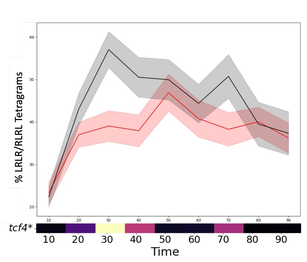



Example of eliminated social interaction in zebrafish mutant for an ortholog of autism risk gene

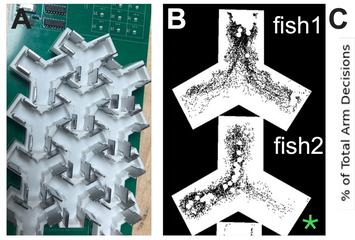
Autism risk mutations manuscript[6]

### Spatial learning / memory (12-well plate, juvenile, >100 fish / day)[7]




- A preference for an alternating turn search strategy in a maze is conserved across humans, mice, and fish[8]
- Example of abnormal search strategy, indicative of reduced spatial working memory, in zebrafish mutant for gene that causes intellectual disability

### Fear conditioning (12-well plate, juvenile, >100 fish / day)

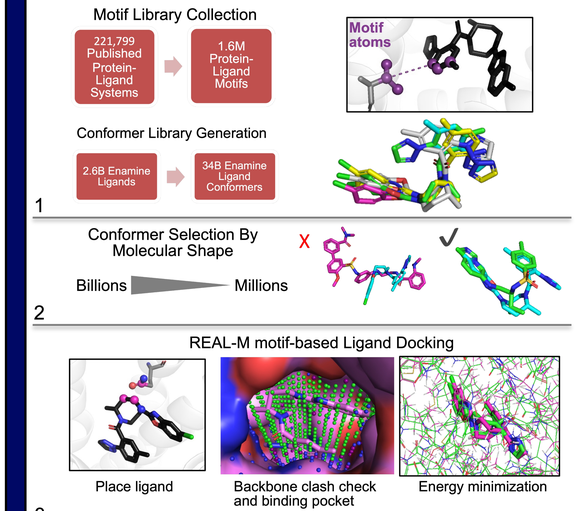


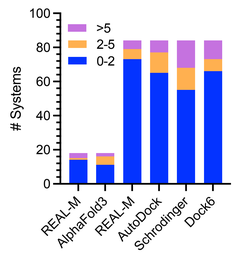

- Published associative fear conditioning paradigms are on single animals without a consistent apparatus
- Our new prototype is built on the Y-maze and would be the first highly scaled-up version of a vertebrate training assay

**A**) Y-maze shock assay with steel connected to a PCB through pogo pins. **B**) Black tracks for the 20 min following a 10 min shock period for fish2 versus unshocked fish1. Fish are shocked when they enter the *arm. **C**) Entrances in *arm pre-shock (blue) and 20 min after (orange), from 0-100%.

## Computational drug discovery

### Rosetta Engine for Anchoring Ligands with a Motif (REAL-M)[1]

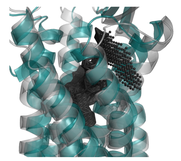


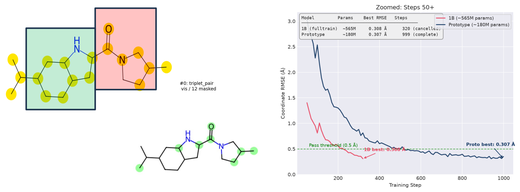

REAL-M with motifs outperforms classic docking and AF3, and it can rediscover molecules from published docking campaigns.

REAL-M manuscript[1]

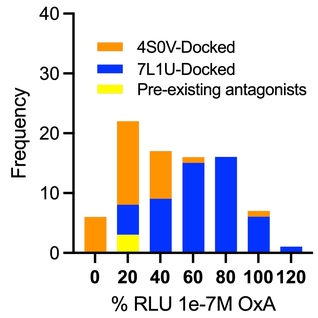
### Molecular dynamics to discover cryptic pockets and model activated states



### A new AI model for generating realistic, synthesizable ligand structures (in progress)

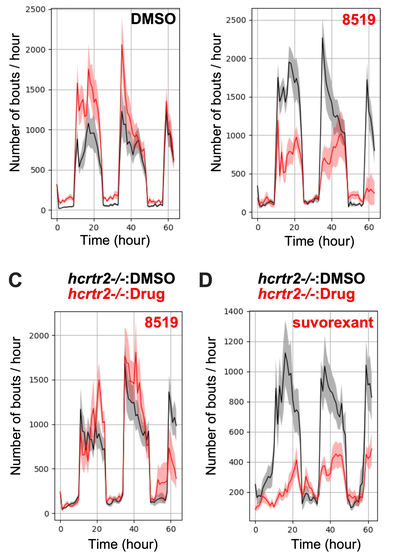



## Hypocretin receptor REAL-M test case




- A conserved receptor involved in sleep regulation, with preexisting approved drugs
- Using a GPCR cell-based assay (Presto Tango), dozens of high-efficacy antagonists were discovered[1]

### Zebrafish testing of best antagonists for behavioral regulation and off-target effects[1]




**A**) Heat shock increase in activity from hypocretin using transgenic line

**B**) Reduction in hypocretin-induced hyperactivity from one new compound (multiple were identified)

**C**) No effect of compound (20 μM) on behavior in receptor mutant, indicating specificity

**D**) Strongly reduced activity from FDA-approved suvorexant (5 μM) in mutant indicates toxicity and behavioral effects not through receptor

**Conclusion**: Zebrafish are ideally suited for screening predictions from computational discovery campaigns. This model enables rapid, scalable identification of compounds targeting performance-relevant behaviors, reducing risk prior to mammalian studies and expanding target accessibility

## Literature Cited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*Our work


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NIH National Institute of Neurological Disorders and Stroke

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## Contact

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