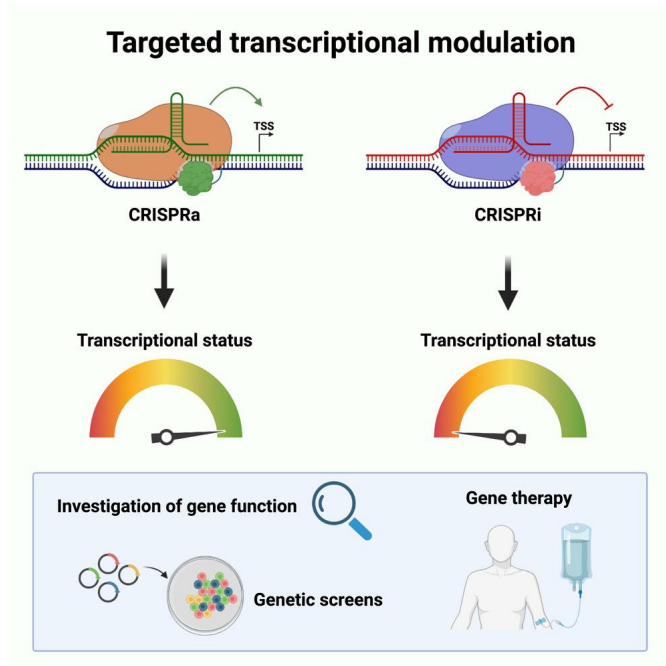


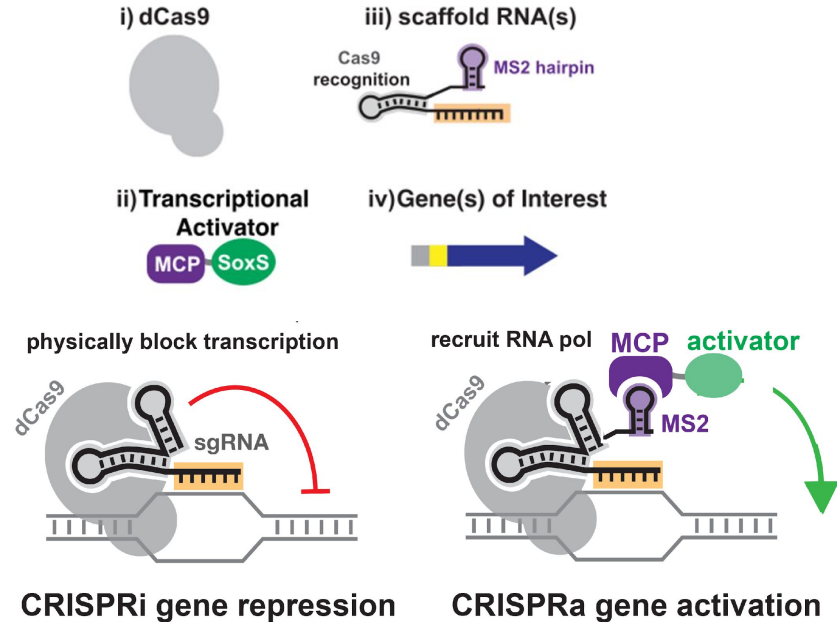
Predicting & Modeling CRISPRa functionality

Tommy G. Primo
December 4th, 2024
Bioengineering 537: Computational Biology

CRISPRa/i tools can be used to regulate metabolism



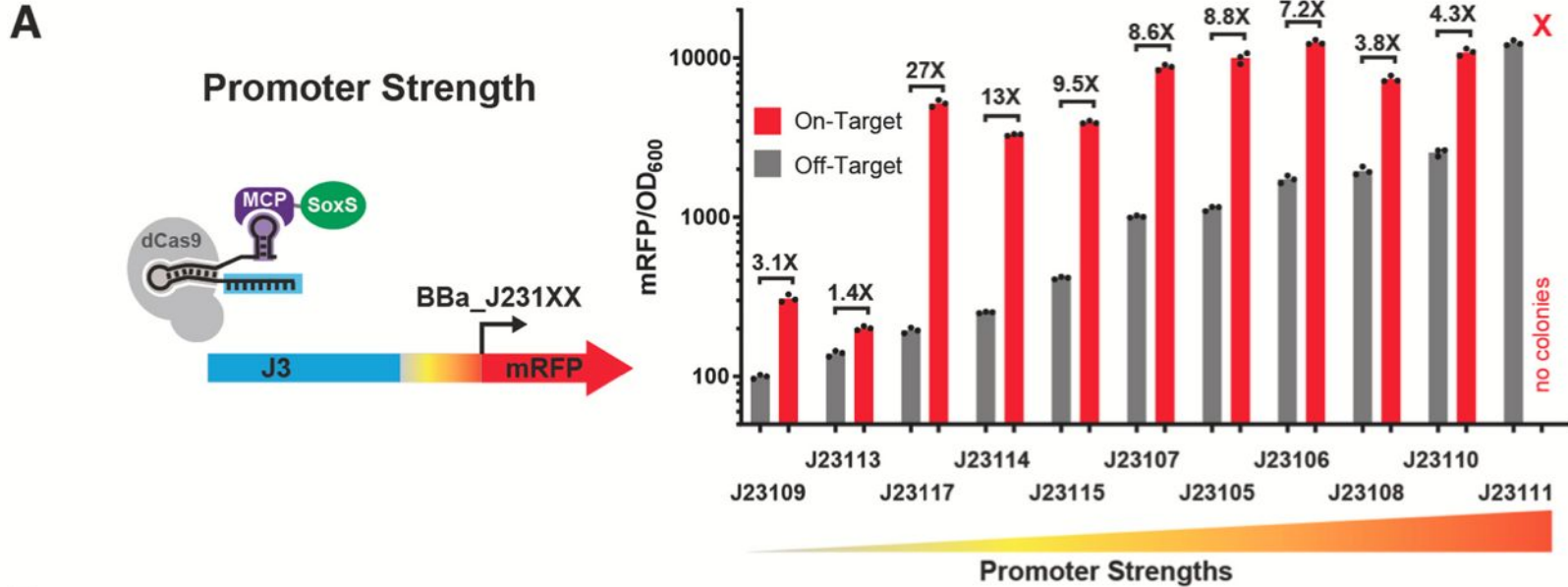
Bendixen *et al. Molecular Therapy* (2023)



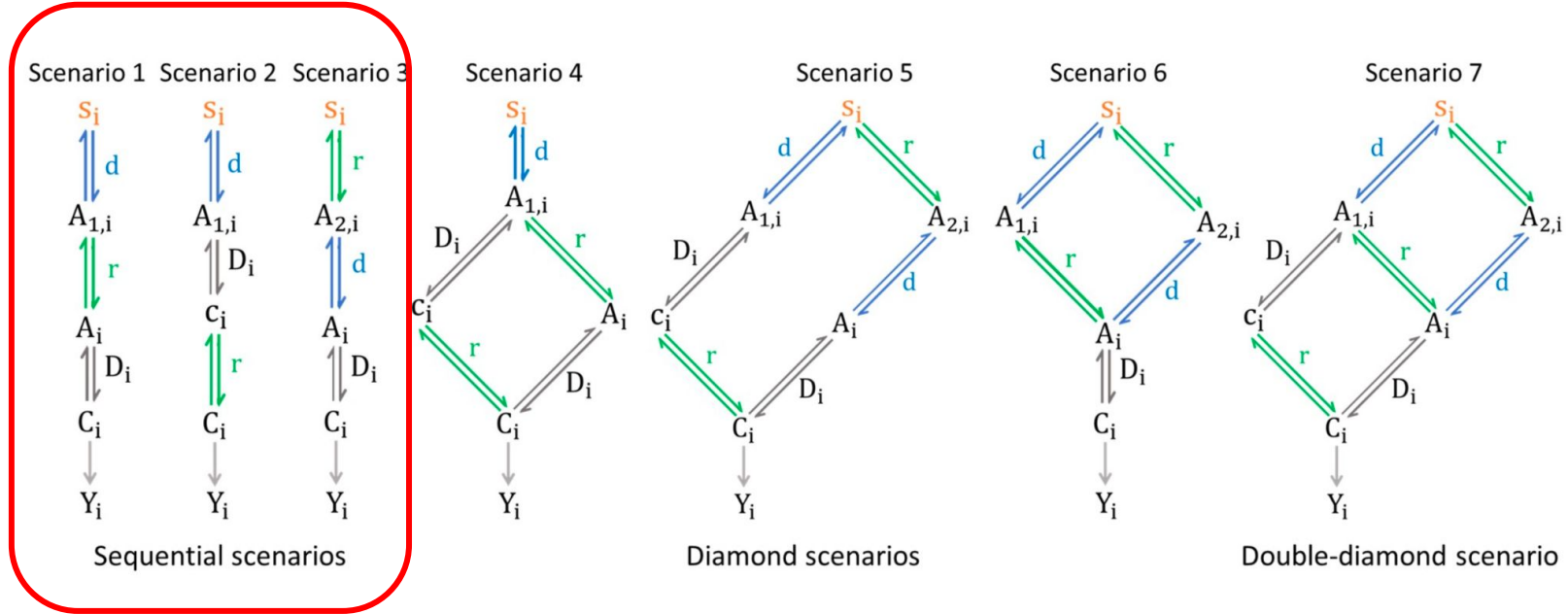
Dong *et al. Nat. Commun.* (2018)

Fontana & Dong *et al. Nat. Commun.* (2020)

Standardly Characterizing concentrations



Project Tool



Tool Allow biochemical modeling of CRISPRa-tethered complexes and allow researchers to understand I/O response when changing CRISPRa-machinery

Manoj et al. *IEEE. CDC* (2022)

Workflow of package

```
# Imports
import unittest
from crisprai_model import crisprai_model as cai

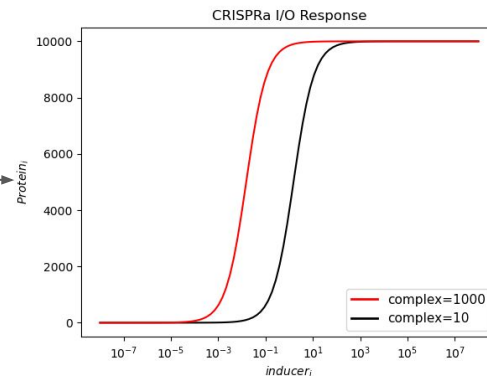
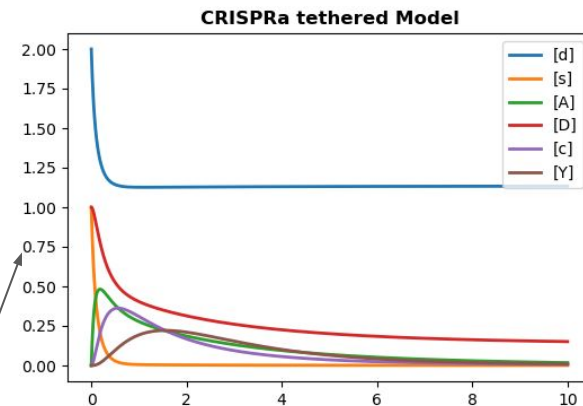
# Initialize the package and model
cai_circuit = cai()
cai_model = cai_circuit.get_crispra_model() # Test this by making sure you get a string returned
```

```
# Plot model
cai_simulation = cai_circuit.simulate_crispra_model(cai_model, time=8) # Test this to see if you're getting the array d
cai_circuit.plot_crispra_model(cai_simulation[0])

# Change concentration of machinery
updated_model = cai_circuit.change_concentration_of_machinery(cai_simulation[0], dcas_9=2, guide=1, target=1)
cai_circuit.plot_crispra_model(updated_model)
```

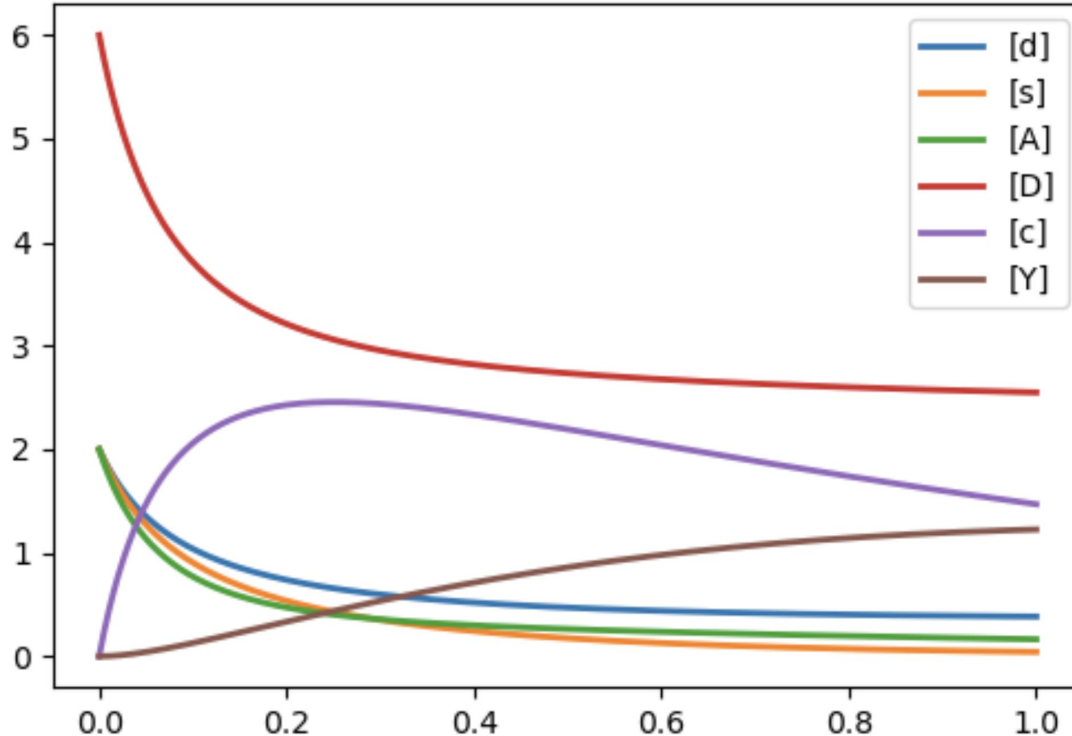
```
# IO visualization
io_model = cai_circuit.get_io_model()
io_simulation = cai_circuit.simulate_io_model(io_model)

cai_circuit.visualize_io_response(io_simulation[0], 8, [10,1000]) # Comparing
```



Simulating CRISPRa tethered Model

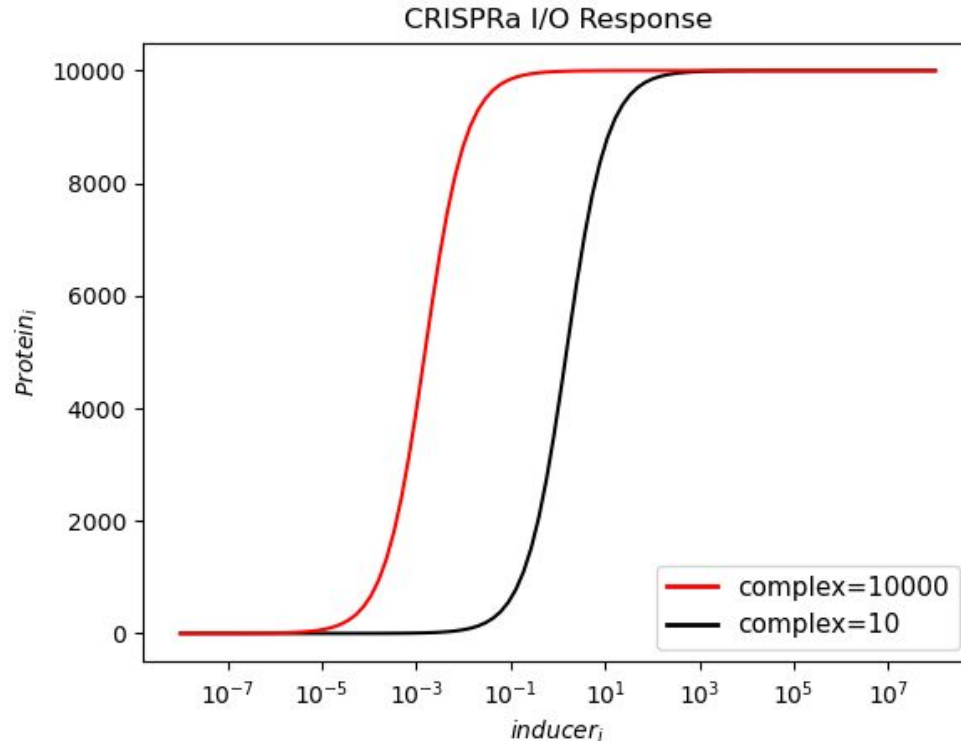
CRISPRa Modality I Model



Here Tool lets you modulate the concentrations of all the dynamics of your system.

- d - dCas9 protein
- s - gRNA
- A - Activator complex
- D - target DNA
- c - Activator bound to DNA complex
- Y - protein expression

I/O Response plot lets you see when you reach SS



Tool lets you see expression profile when you increase protein concentrations with changing inducer levels

- You can change complex concentrations and see by how much you need to increase your induction to reach a steady state of your protein concentration
- Depending on your inducer, IPTG or Rhamnose, this might change the inducer range

What's Left

- For BioE 537
 - Test functions & update comments
 - Make it Downloadable with PYPI
- After BioE 537
 - Add Cell growth into model to know when expression is too much
 - Introduce CRISPRa-machinery where the components are RNA recruited
 - Here the IO response may drop as the transcriptional activator isn't depended on Cas9 protein
 - Add guide competition
 - see what happens when you increase the number of guides and their effect on the system
 - Add autoregulation and other interesting dynamics like negative/positive feedback