Computational design of small-molecule sensors/actuators

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Kortemme lab

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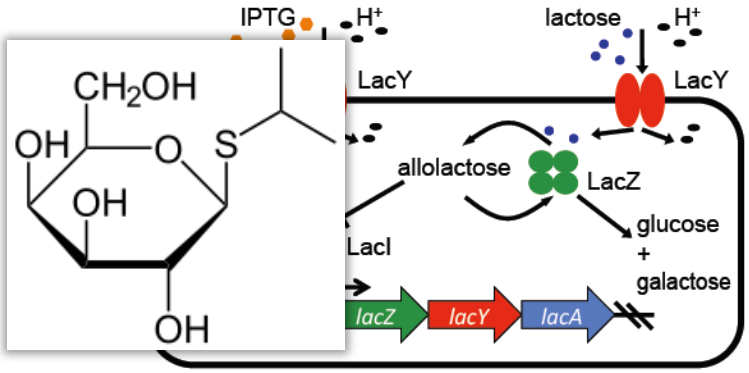
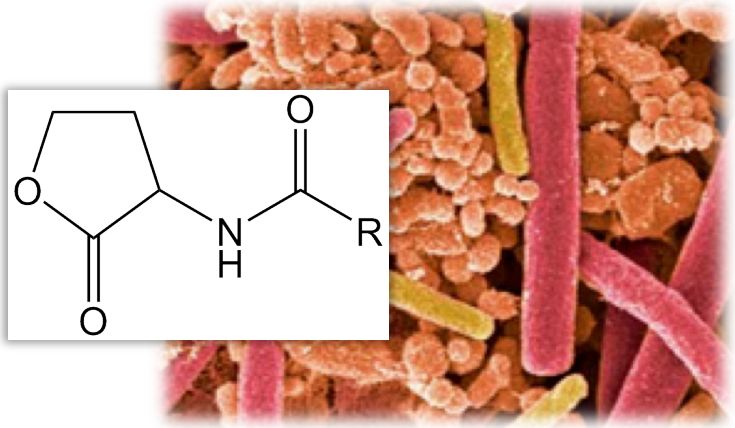
San Francisco

RosettaCon 2013

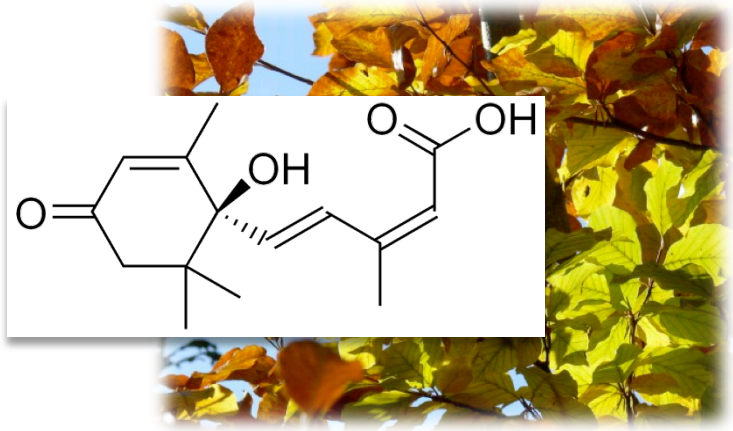
Detecting and responding to signals are fundamental to living systems



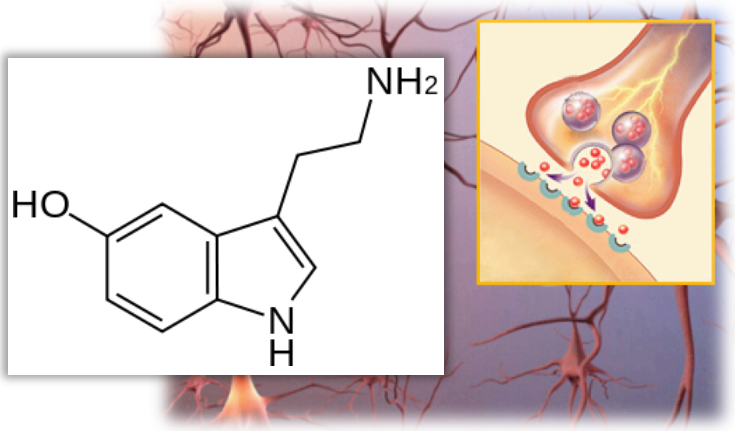
Controlling metabolism



Collective decision making

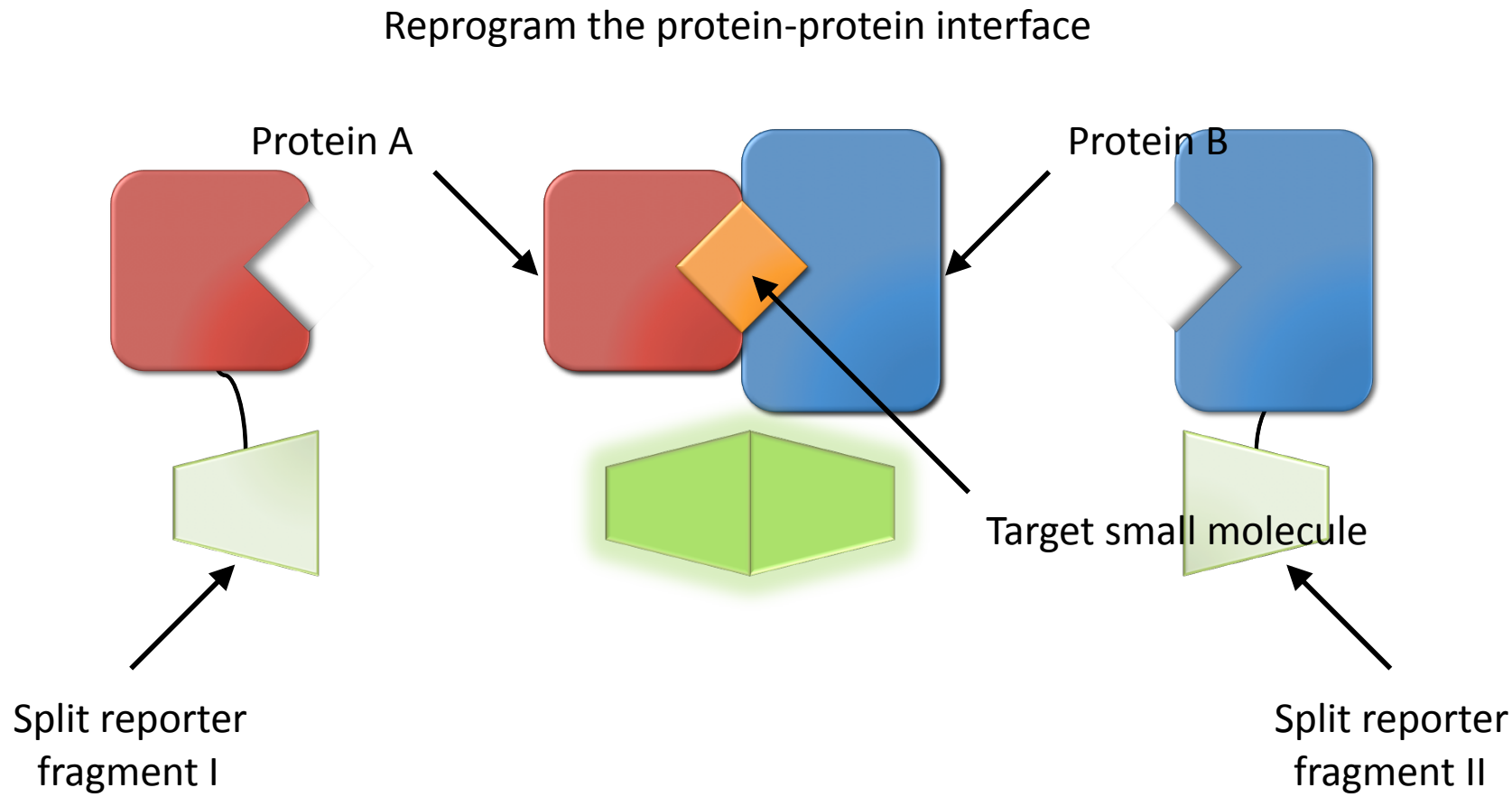


Adapting to the environment

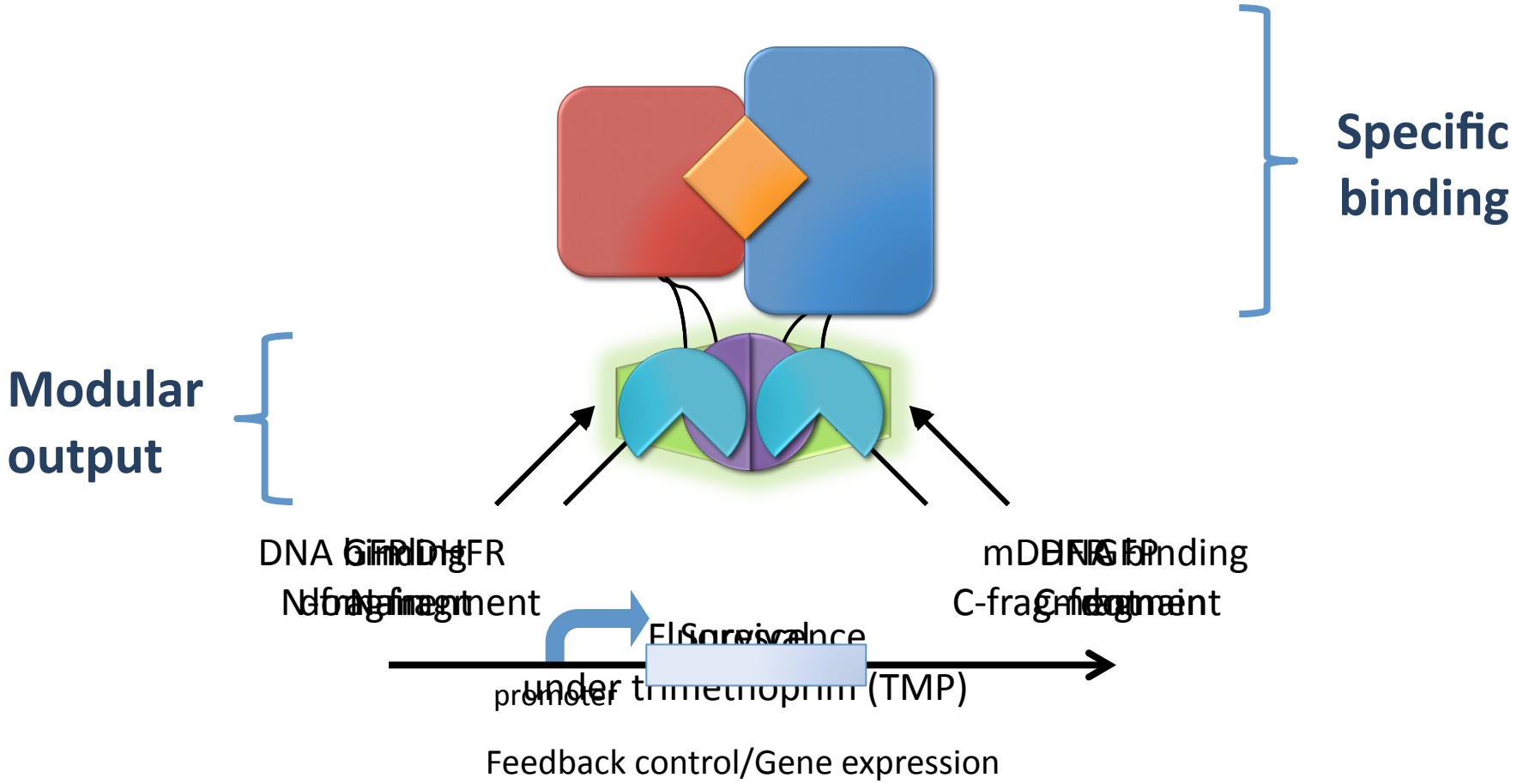


Mood and behavior

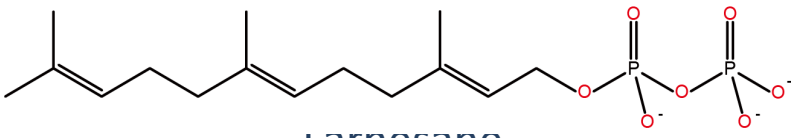
Design concept – Ligand induced dimerization



Design concept – Ligand induced dimerization



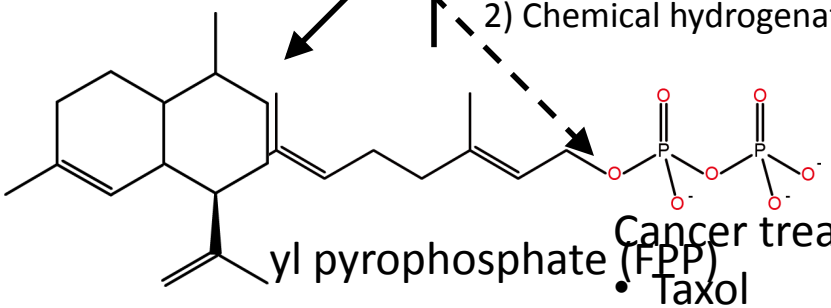
The first target – Farnesyl pyrophosphate, FPP



Farnesane

Farnesyl pyrophosphate (FPP)

1) Farnesene synthase
2) Chemical hydrogenation



yl pyrophosphate (FPP)

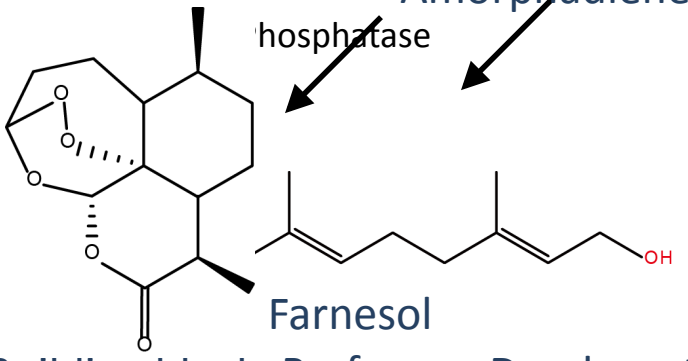
Amorphadiene

Cancer treatment:

- Taxol
- Irufloven

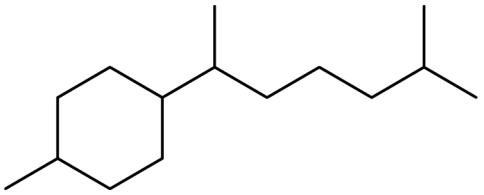
1) Bisabolene synthase
2) H_2; Pd catalyst

hosphatase



Farnesol

(Building block, Perfumes, Deodorants)



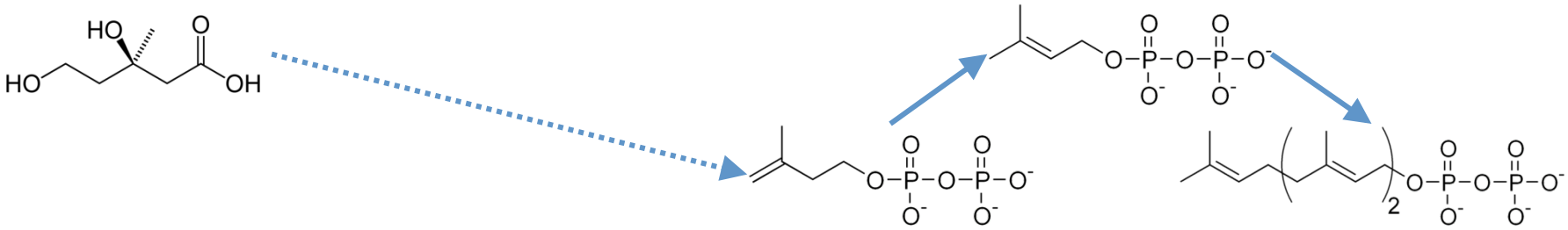
Bisabolane (Diesel alternative)

Artemisinin (Malaria)

Engineered metabolic pathway for FPP production in *E. coli*

Mevalonate pathway:

Mevalonate (MEV) → MEV-P → MEV-PP → IPP → DMAPP → FPP

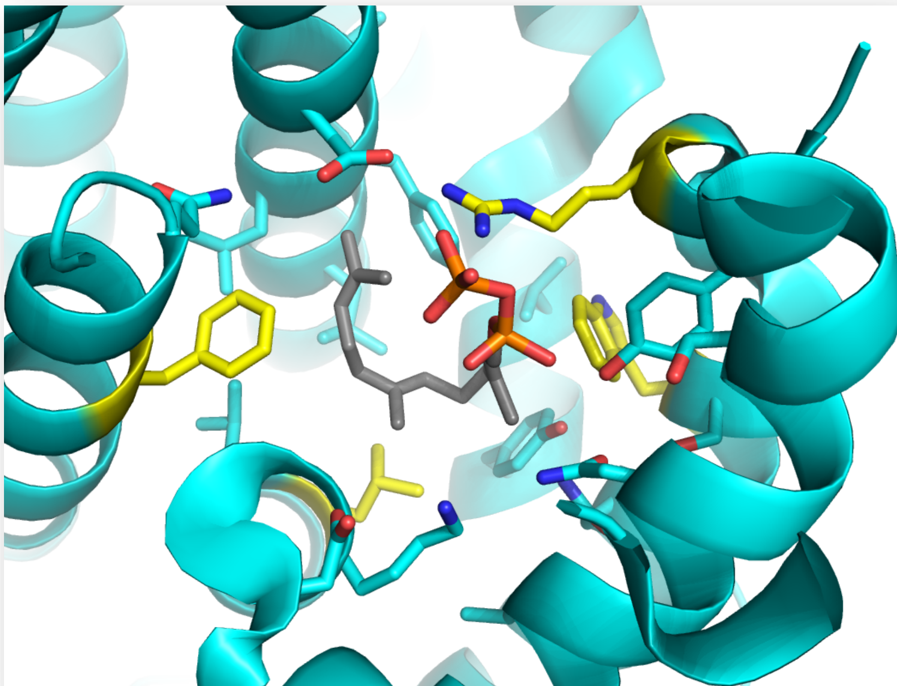


- 15-carbon farnesyl group with charged diphosphate – a flexible ligand
- *In vivo* production by engineered metabolic pathway
- Limited production in µM range

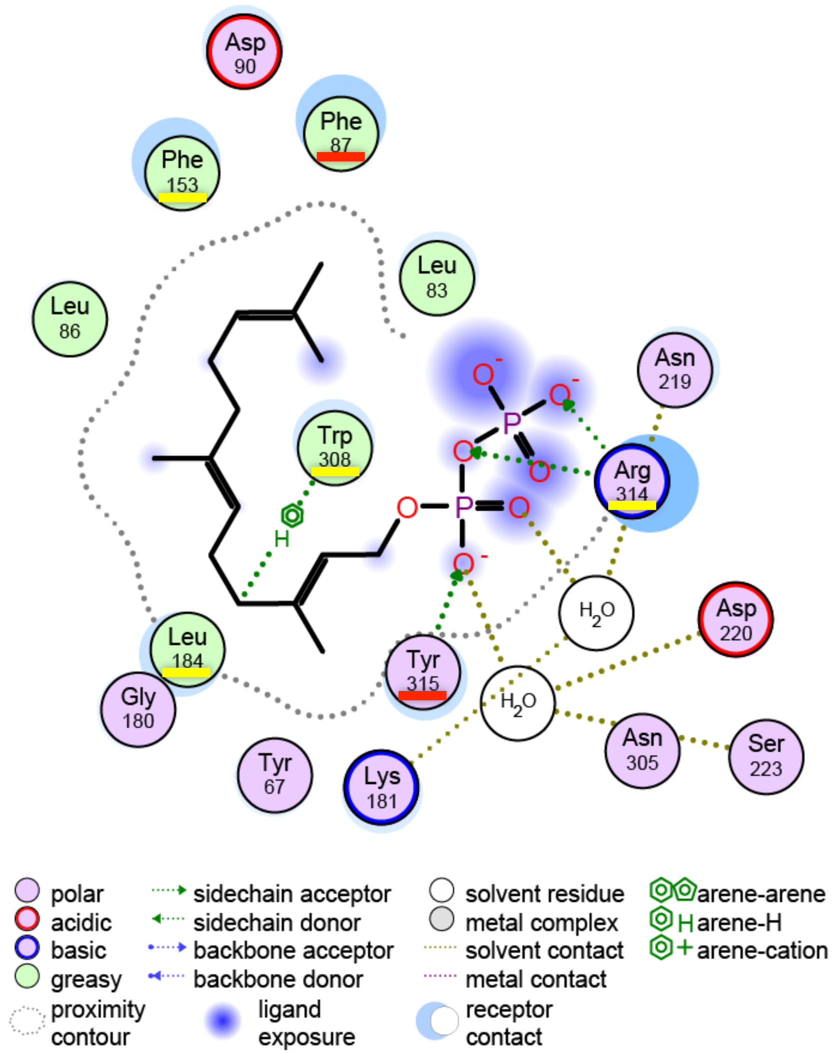
Keasling lab, UC Berkeley

Binding motif of FPP

Aristolochene synthase (3BNX)

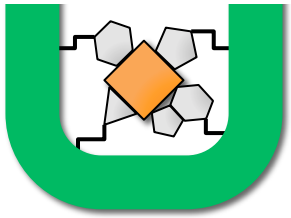


12,13-difluorofarnesyl diphosphate
$K_i \approx 0.8 \mu M$

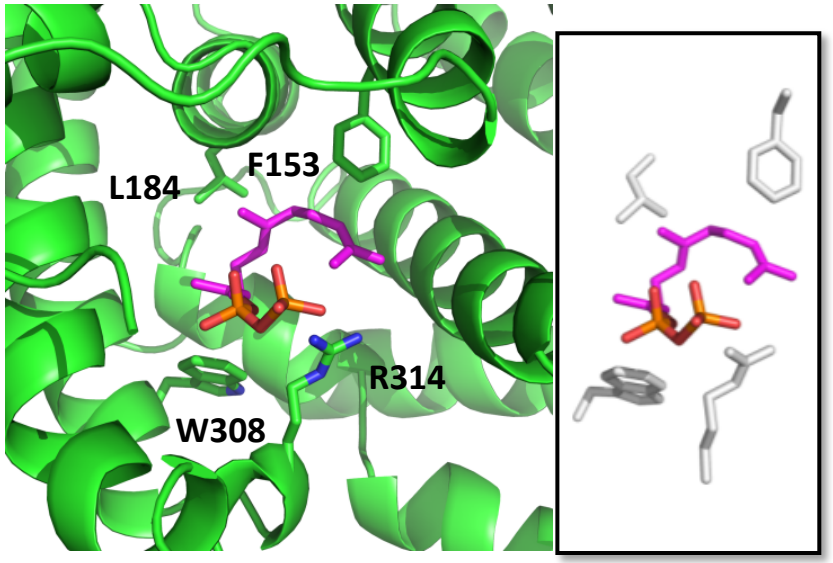


Strategy – motif directed design in three steps

I. Define the binding site geometry



Native binder



Aristolochene synthase (3BNX)

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Strategy – motif directed design in three steps

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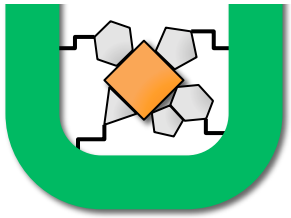
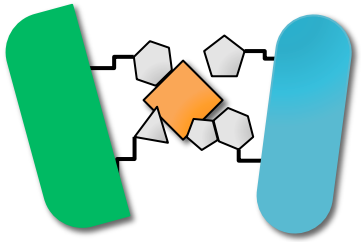
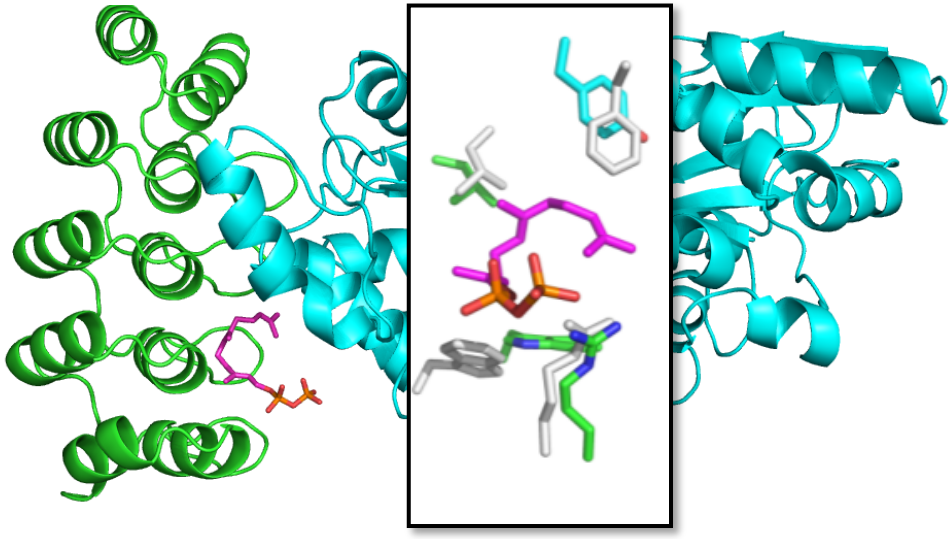


II. Search for matches in >1,000 protein interfaces



Heterodimer match



Ankyrin repeat protein-Maltose binding protein (1SVX)

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Strategy – motif directed design in three steps

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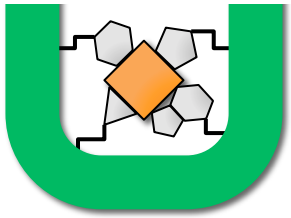

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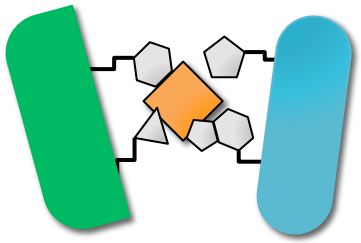
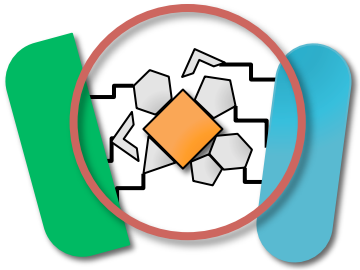


III. Stabilize binding site & predict “tolerated” sequences



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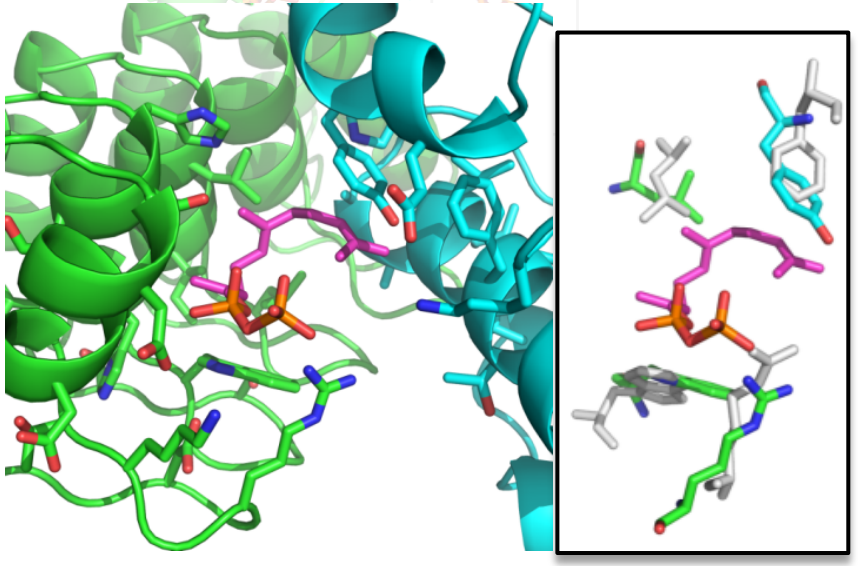
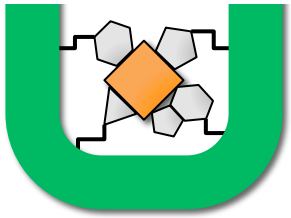
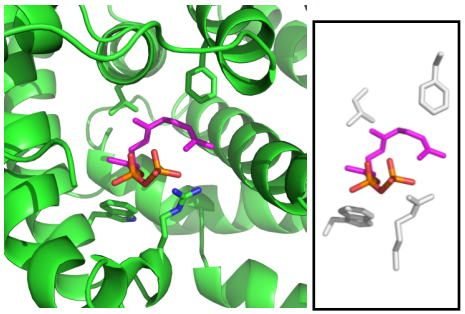
Single and bb ensemble designs



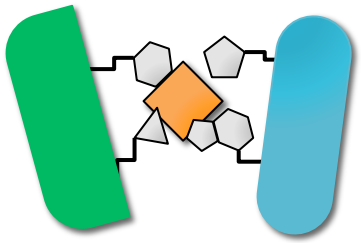
Design strategy – active site grafting

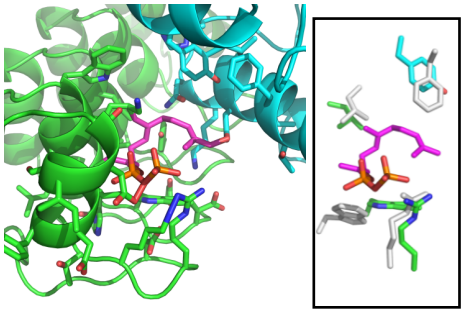
I. Define the binding site geometry



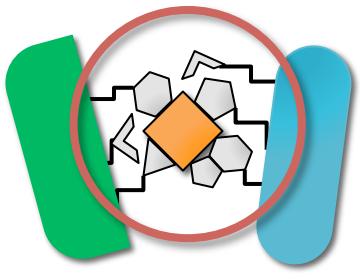


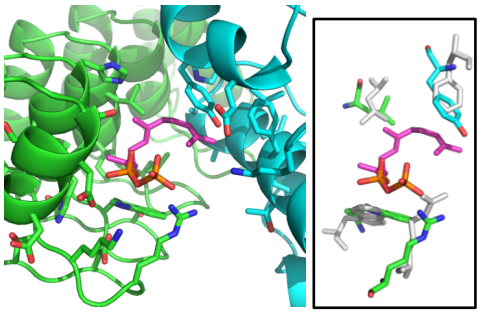
II. Search for matches in >1,000 protein interfaces





III. Stabilize binding site & predict “tolerated” sequences





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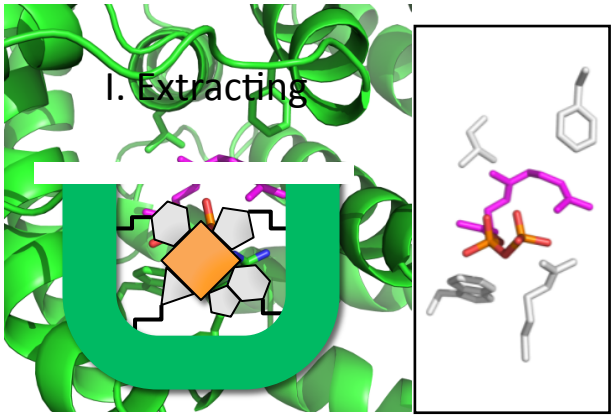
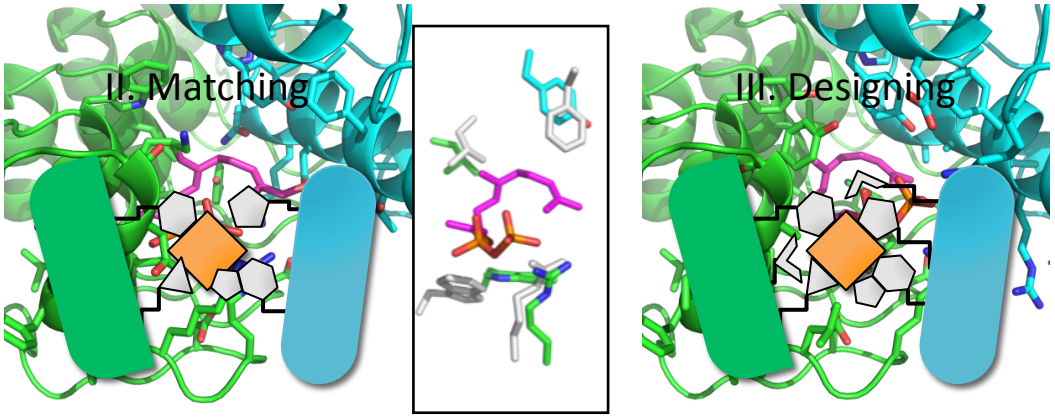
Design strategy overview

I. Native binder



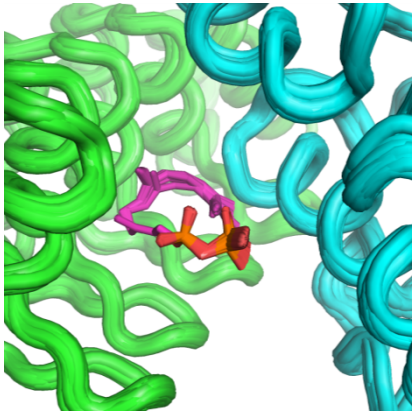
Aristolochene synthase (3BNX)

II. Heterodimer candidate

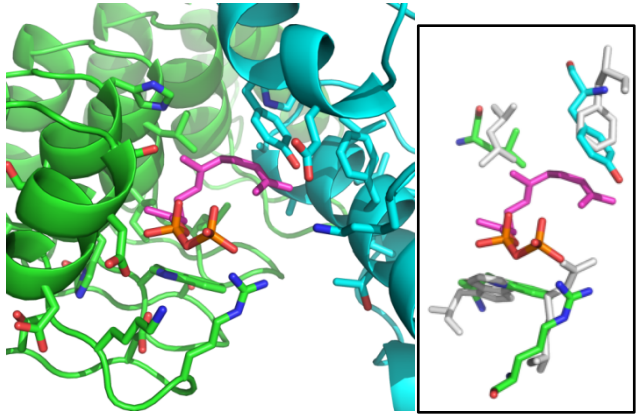


Ankyrin protein-Maltose binding protein (1SVX)

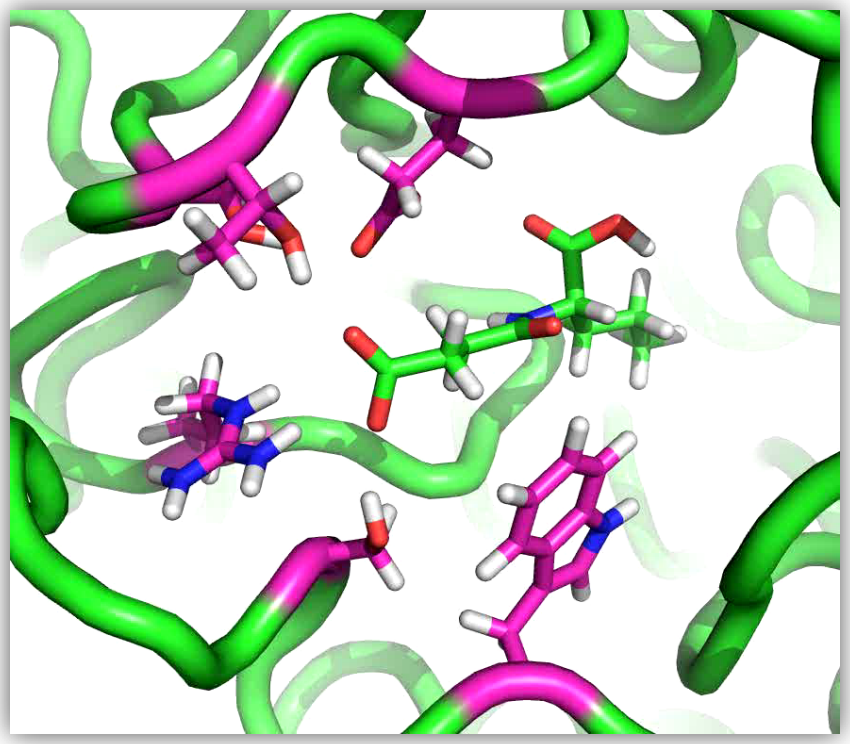
III. Modeling backbone flexibility



IV. Redesigning sequences



Improved methods for more predictive design



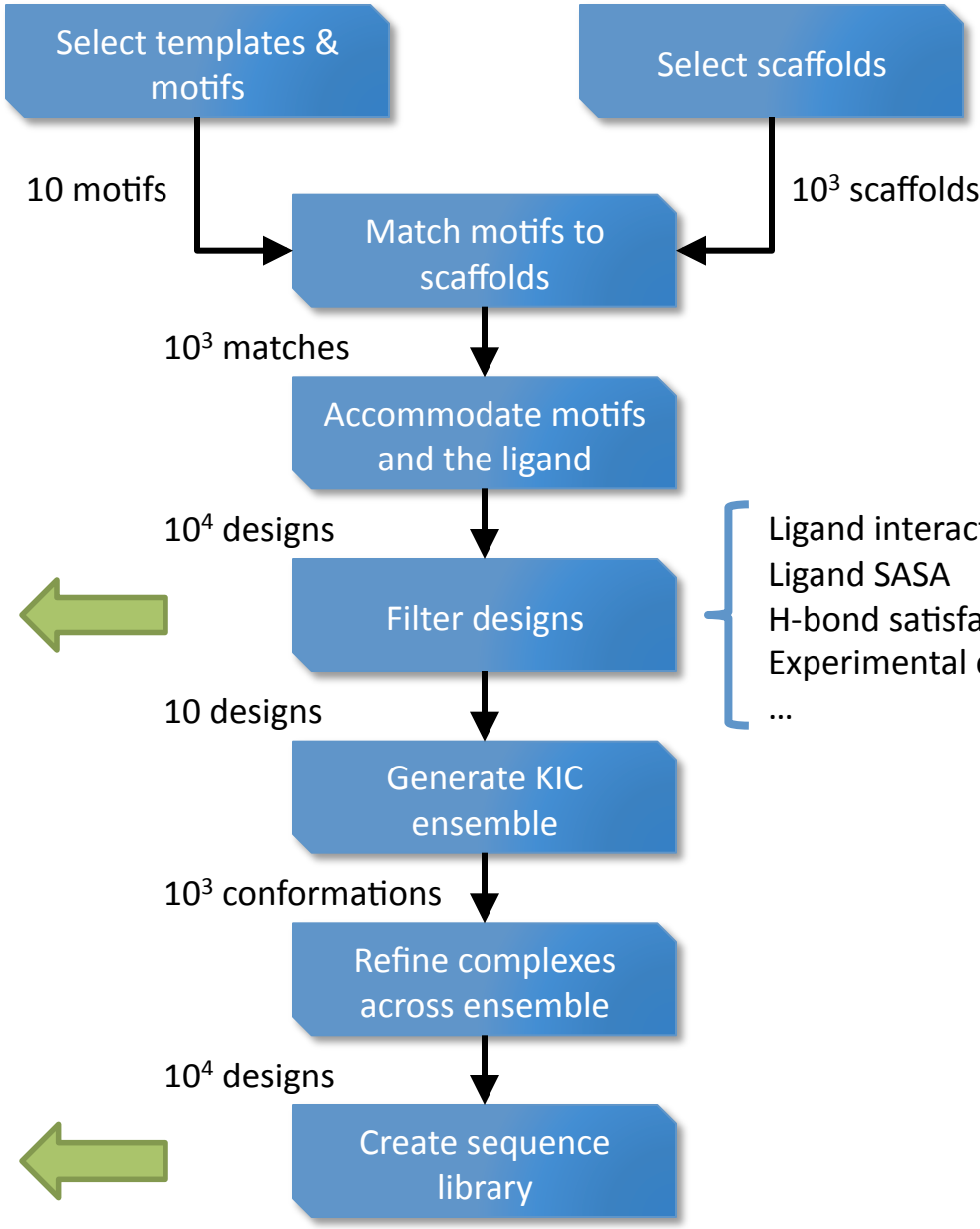
- New sequence tolerance protocol:

  Coupling changes of sequences, backbone, side chains & ligands

"Computational redesign of enzyme substrate specificity using coupled side-chain backbone moves"

Noah Ollikainen

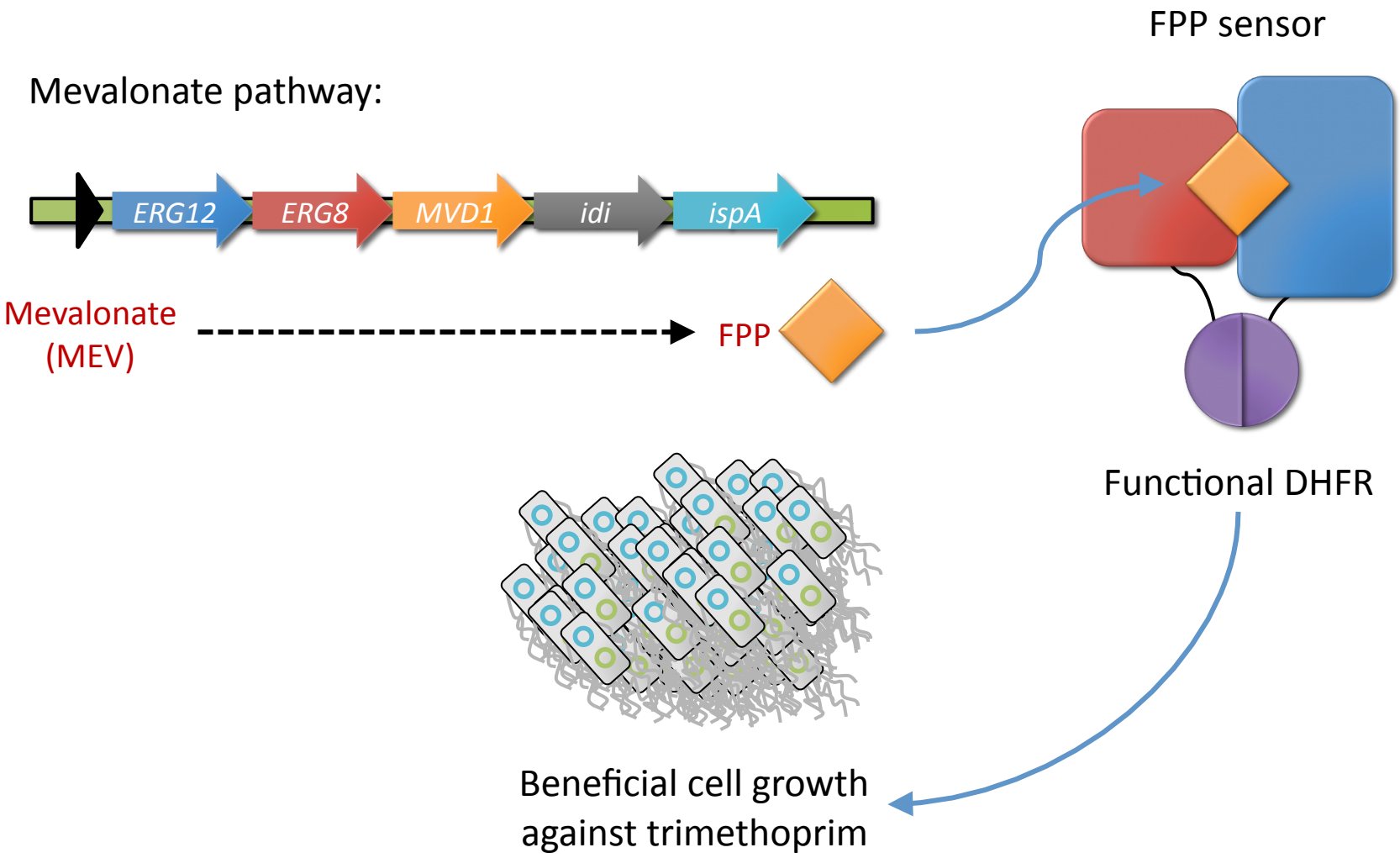
Validation



Single design
+ manipulations

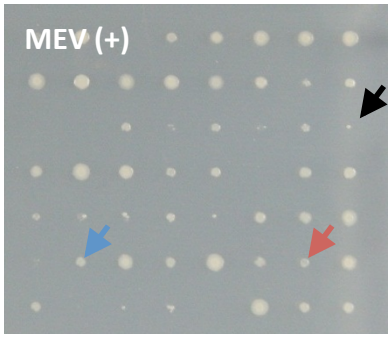
bb ensemble design
theoretical complexity: 10^6

Designed sensors are confirmed by split DHFR growth assays

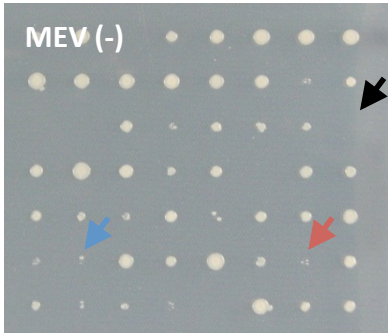


Designed sensors are confirmed by split DHFR growth assays

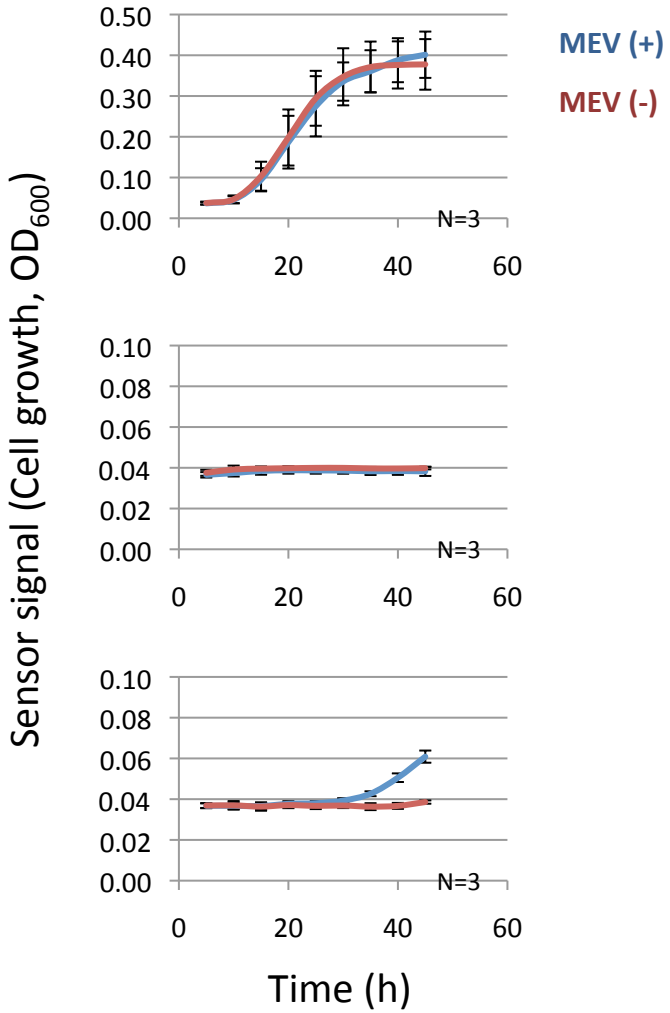
Ligand (+)



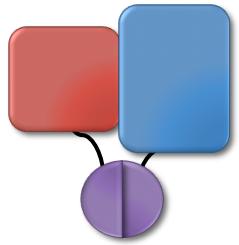
Ligand (-)



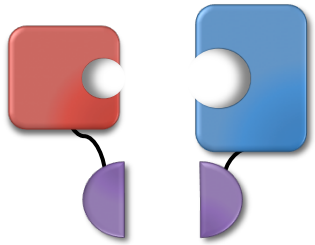
"Colony printing"



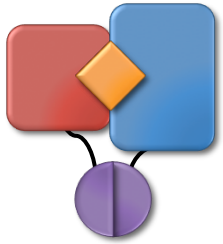
wtAR-wtMBP



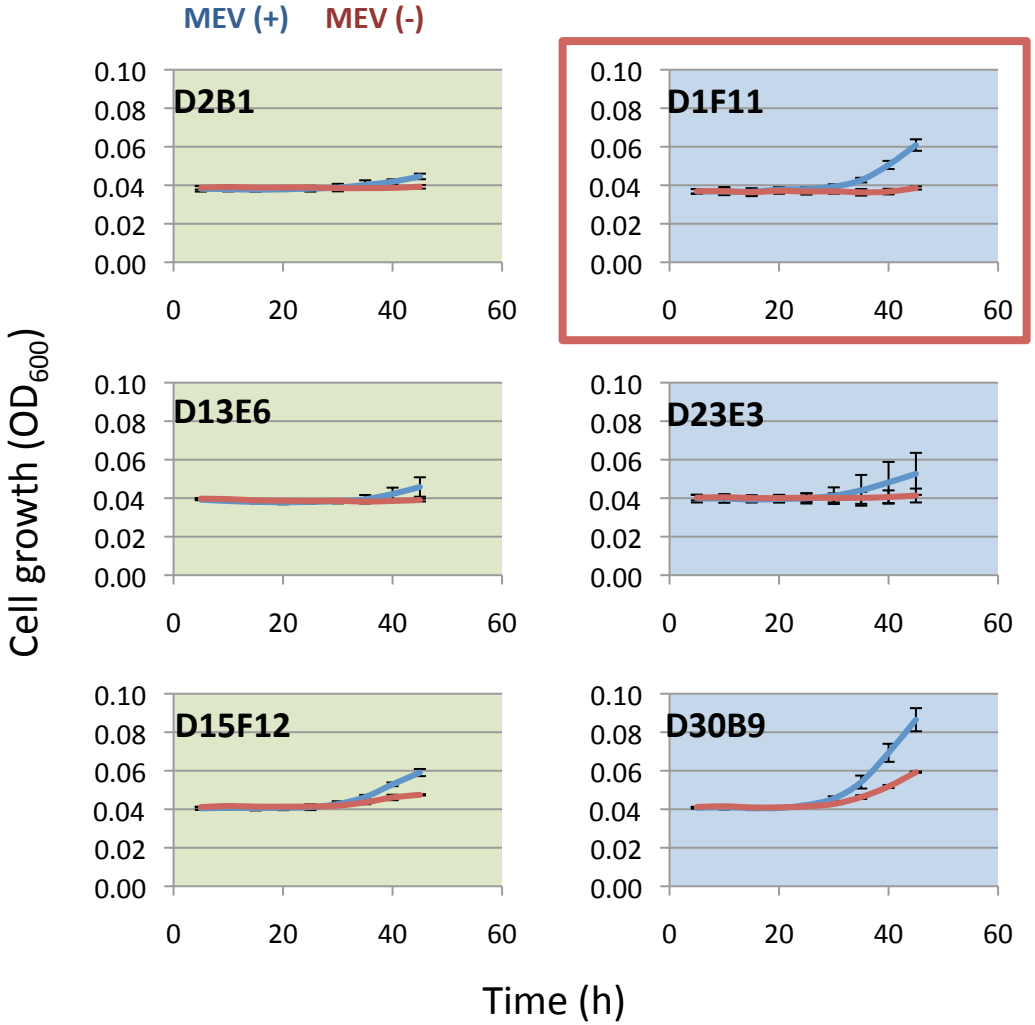
negative control



FPP sensor

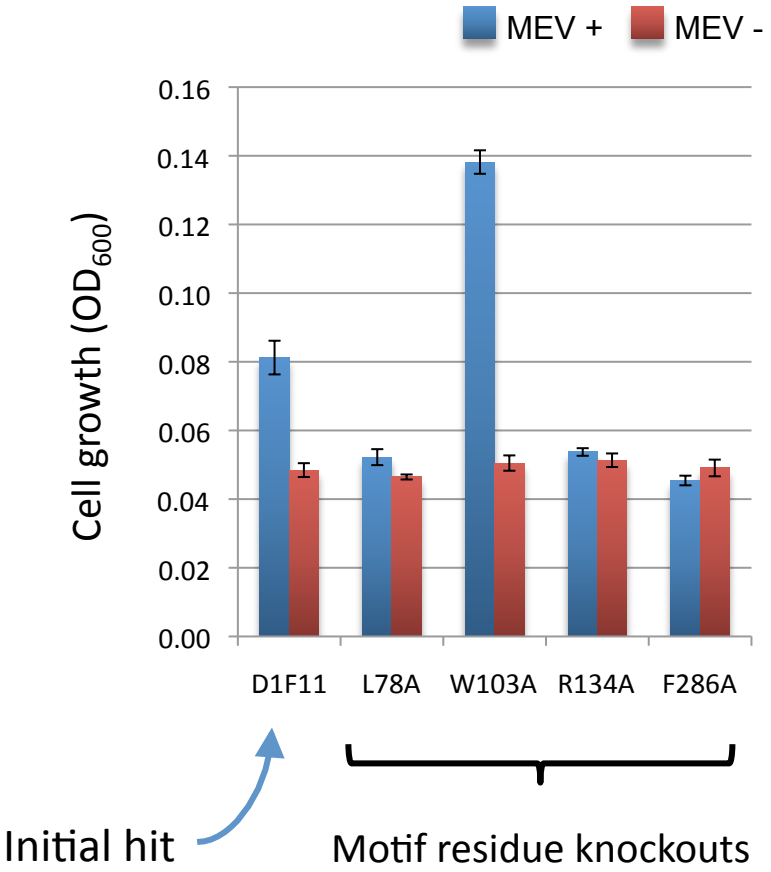


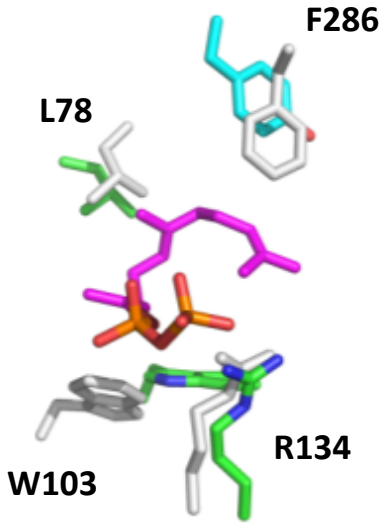
Initial screen: ~1% of surviving clones are sensitive to FPP production

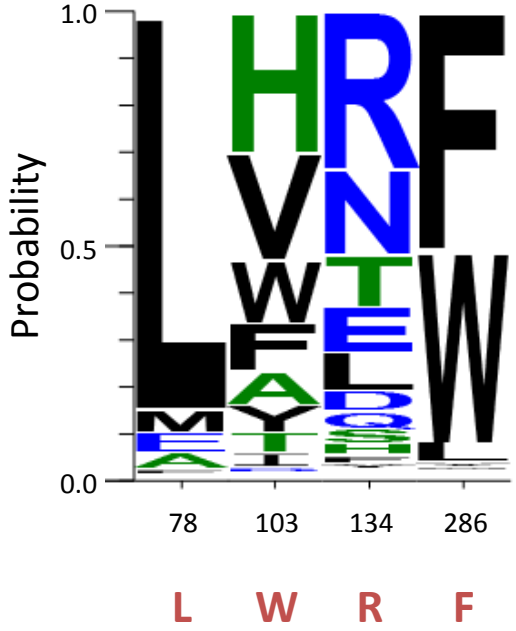


- 27 initial hits confirmed
  - 7 from the library design
  - 20 from the *in vitro* evolved single design

Three out of four motif knockouts eliminate the sensor activity

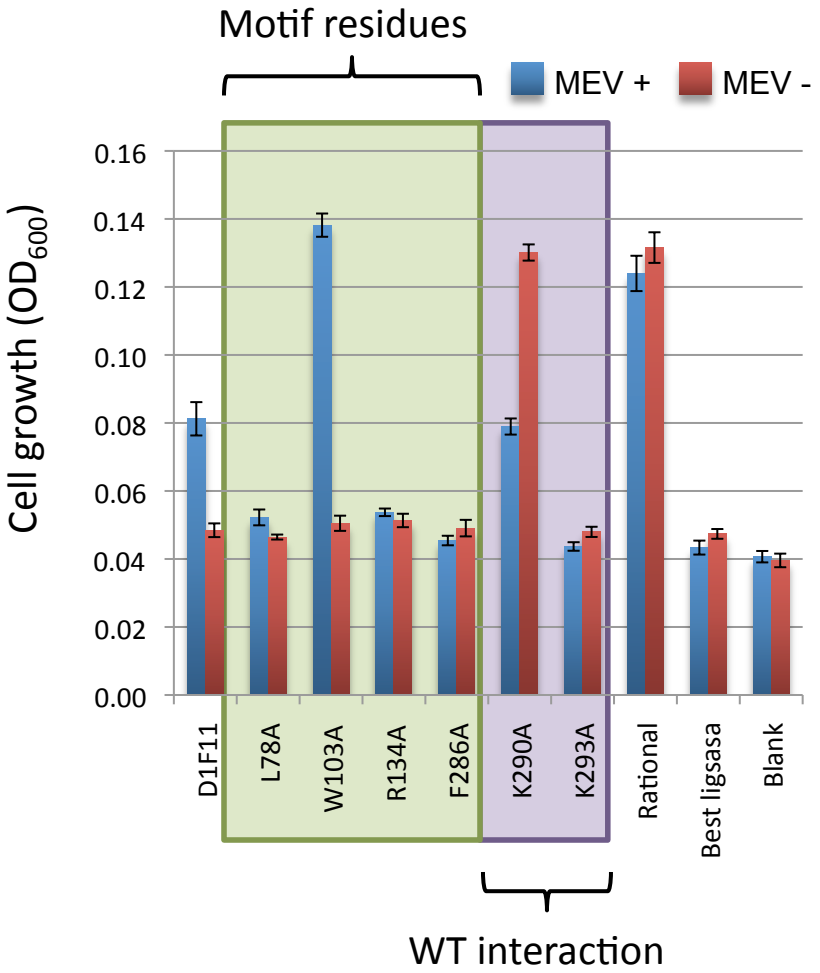






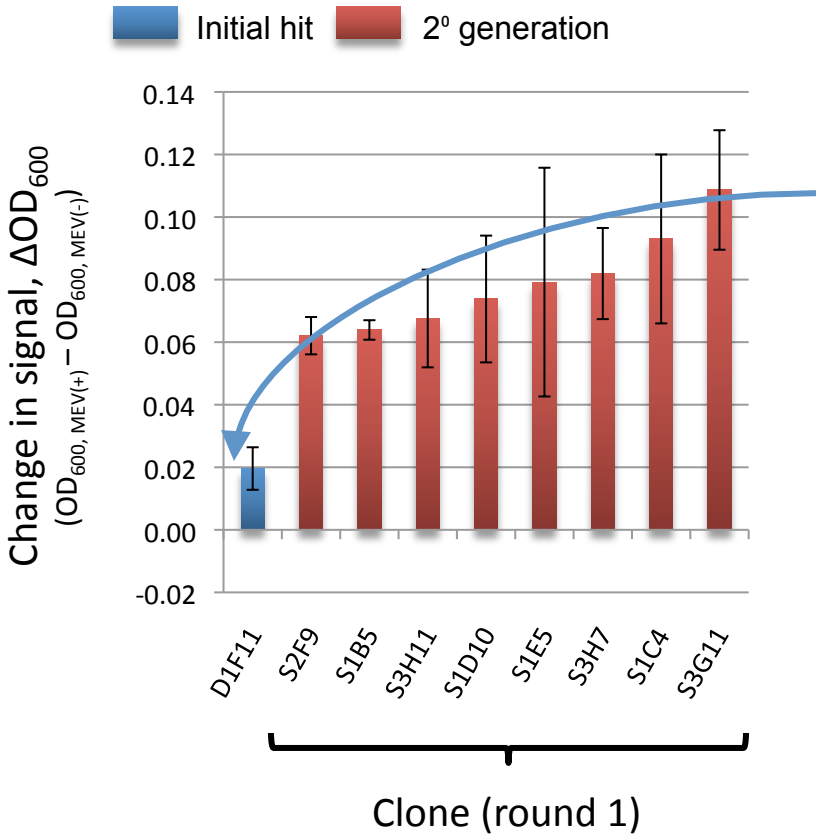
Predicted preference of motif residues

Motif knockouts confirm design validity



- Three out of four designed motif residues are confirmed to be essential to the binding.
- Improvement of W103A suggests possible overpacking of the binding site and/or the ligand movement.
- Remaining interactions from WT scaffold seem to be required in the initial hit.

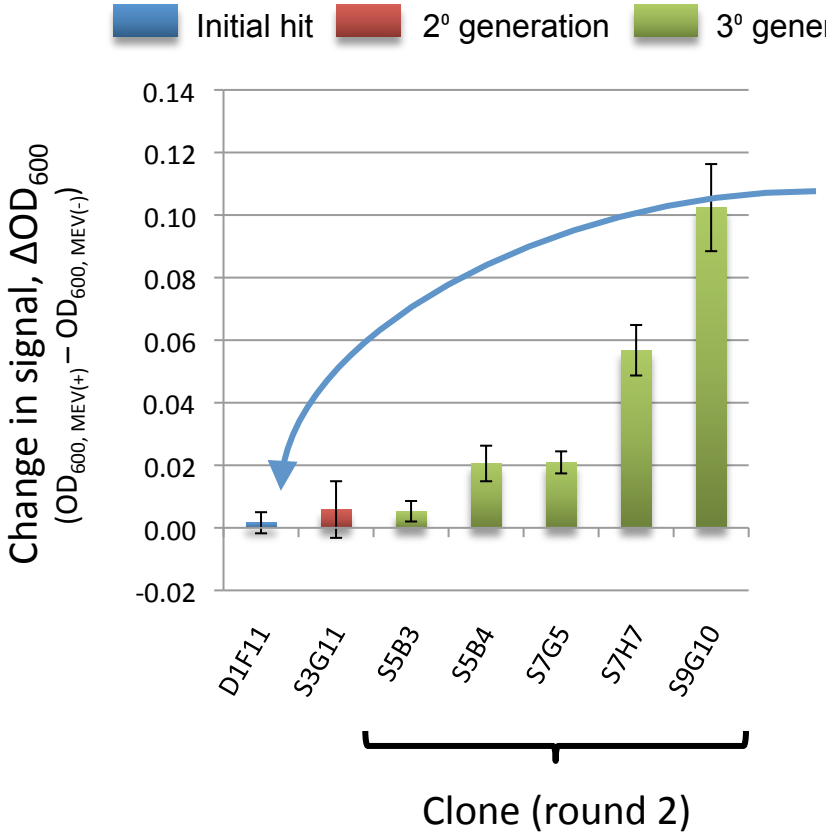
Initial sensor activity can be optimized



The initial hit had 18 mutations.

- Iterative saturation mutagenesis (ISM)
  - 11 positions are selected around the active site

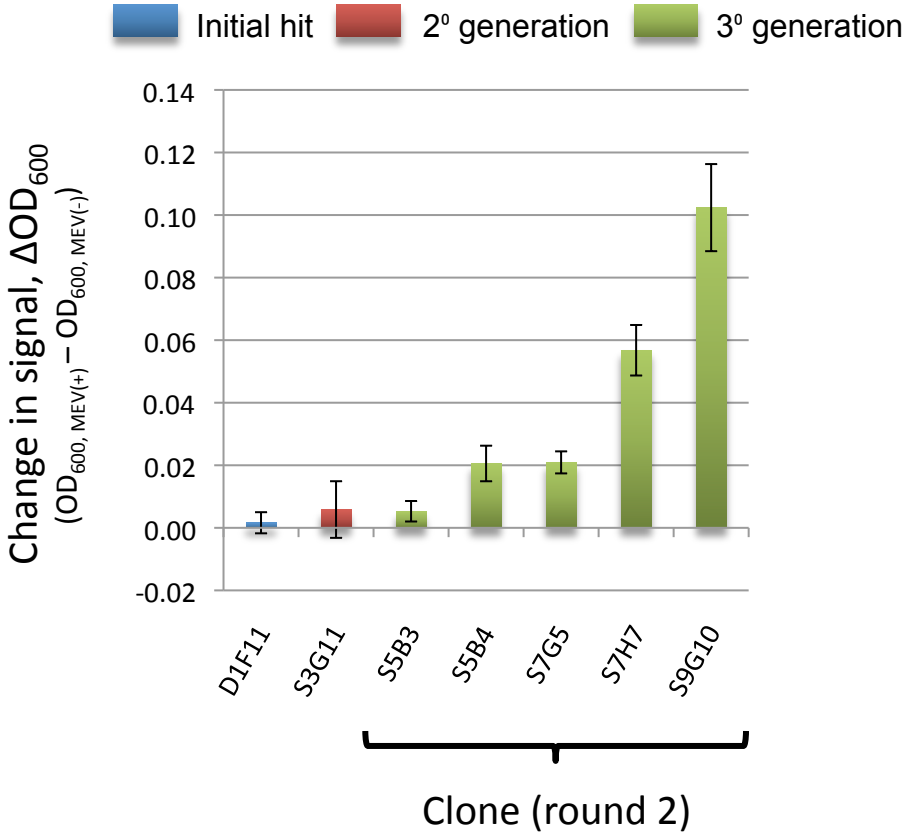
Initial sensor activity can be optimized

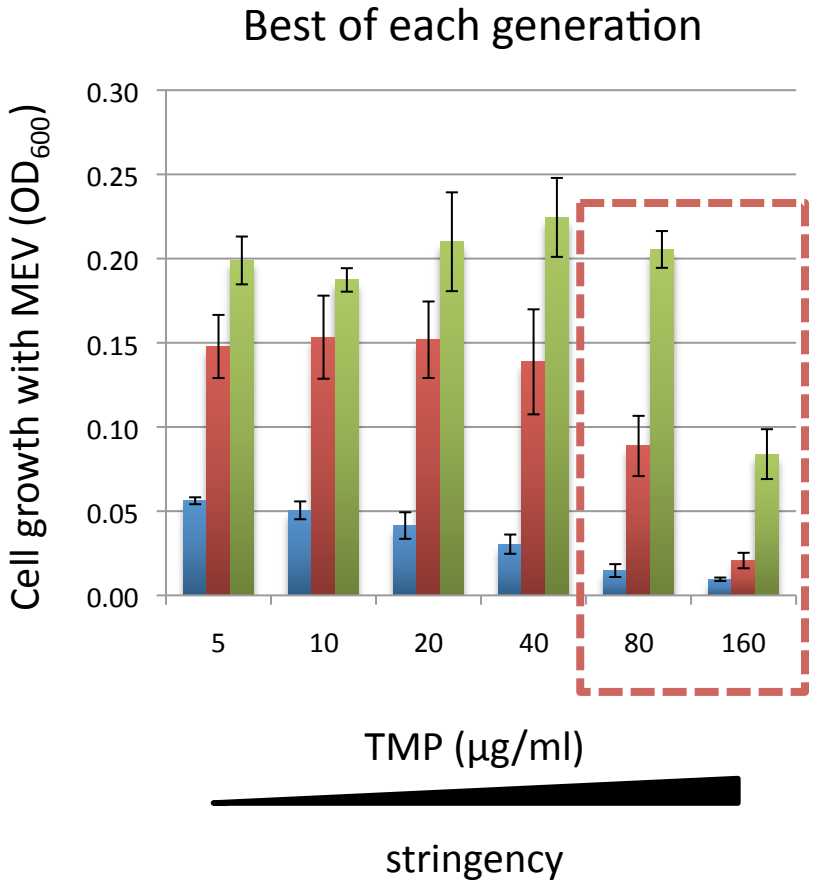


First round, best clone under more stringent condition

- Iterative saturation mutagenesis (ISM)
  - 11 positions are selected around the active site

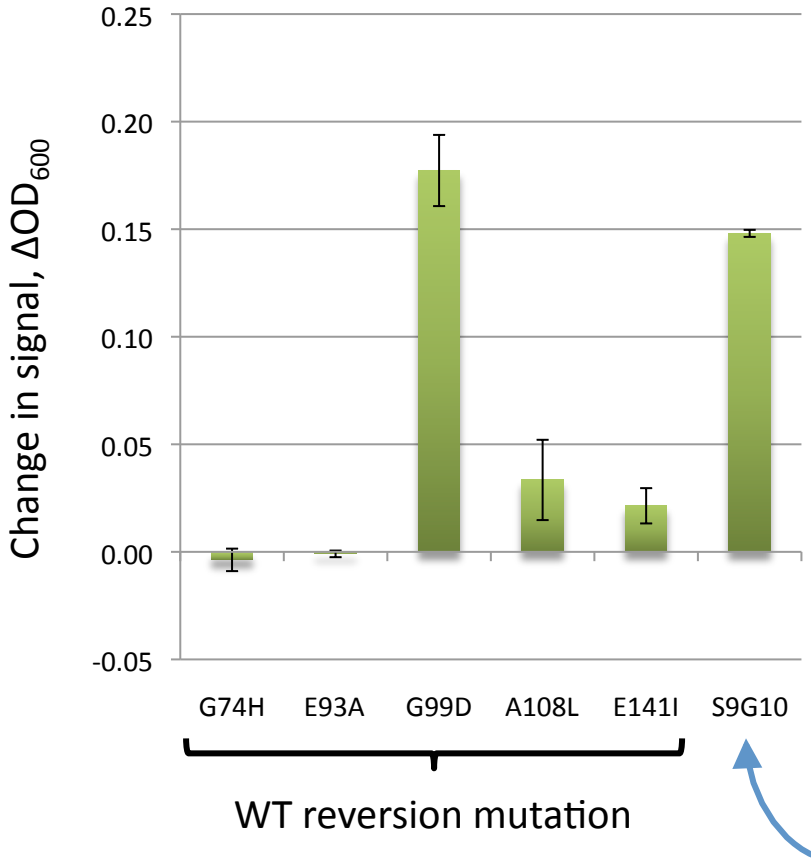
Initial sensor activity can be optimized





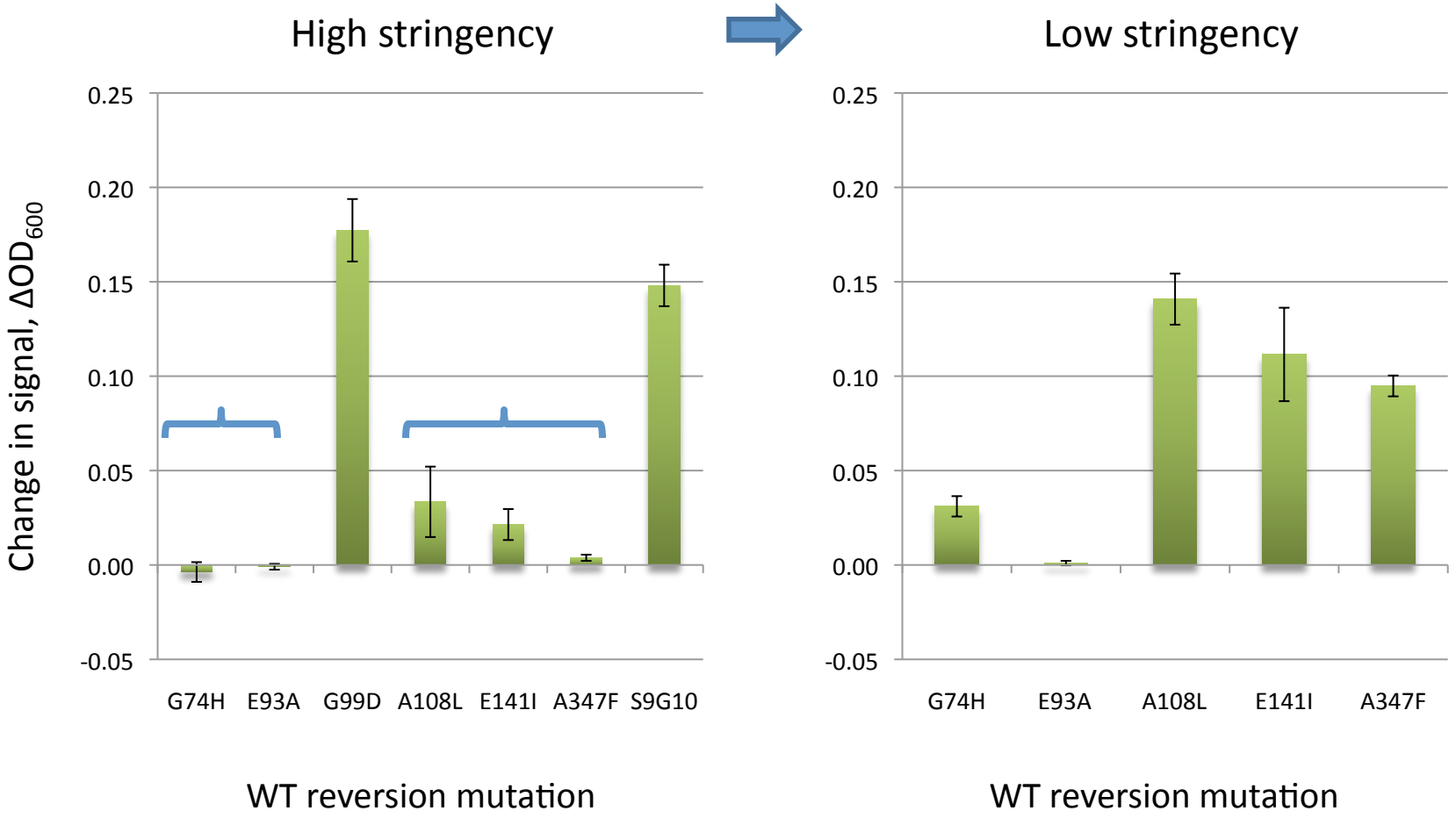
- Iterative saturation mutagenesis (ISM)
  - 11 positions are selected around the active site

Most mutations are essential for sensor activity

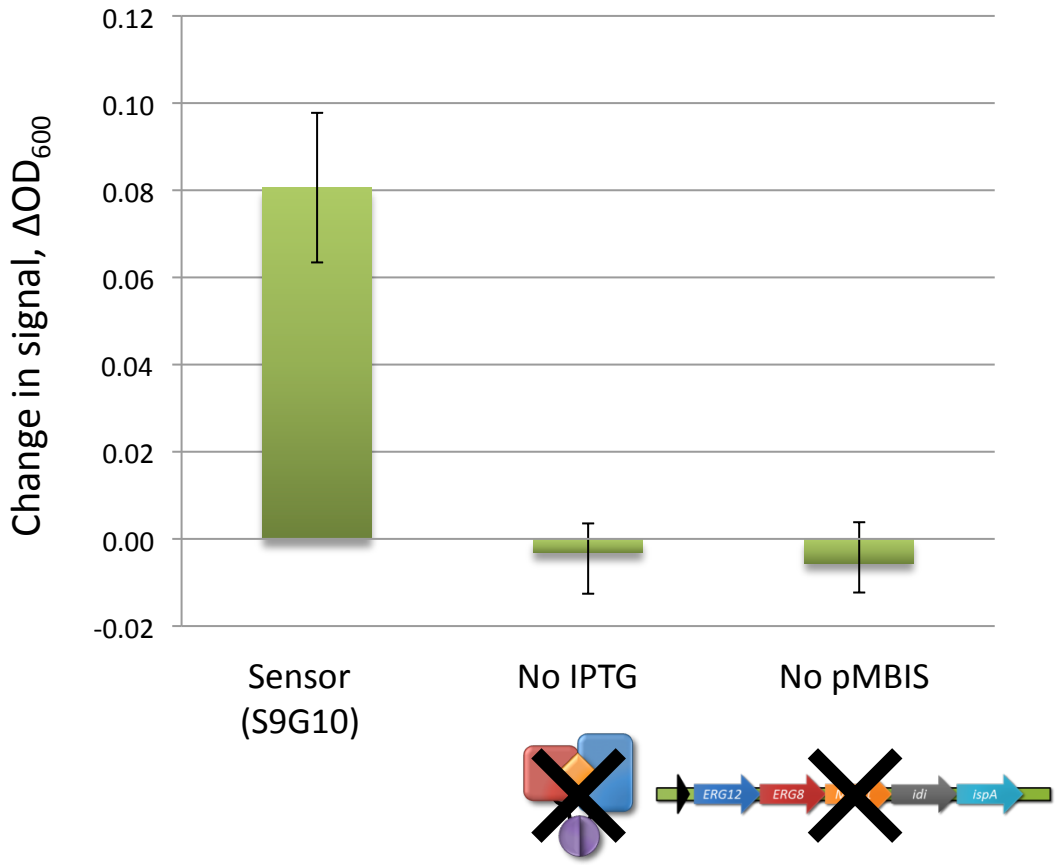


- Decreasing of melting temperature from 70°C to 40°C was observed for Ankyrin-repeat protein. (preliminary)
- WT reversion mutations were introduced to find unnecessary mutations.
- Mutations at conserved regions of AR were tested.

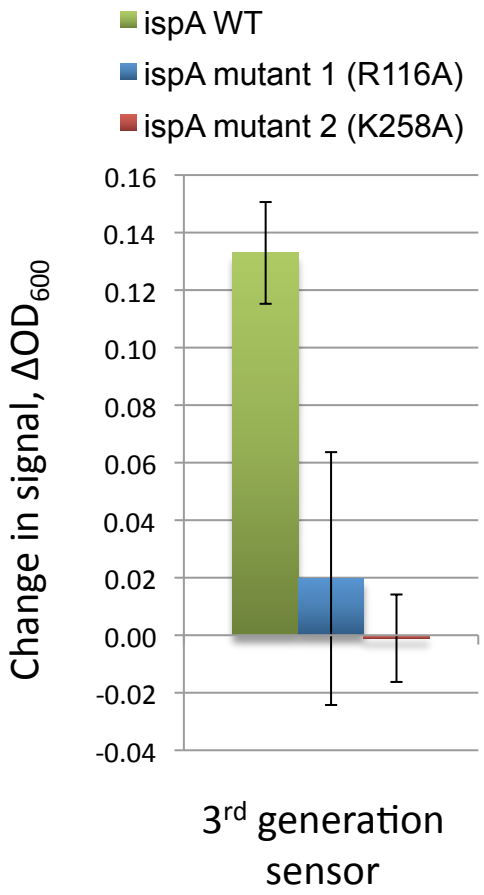
WT reversion



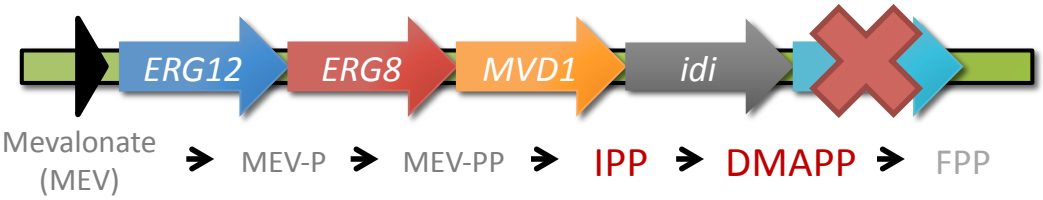
Sensor activity is dependent on the sensor expression and FPP pathway



Mutations in the last enzyme in the pathway eliminate sensor activity

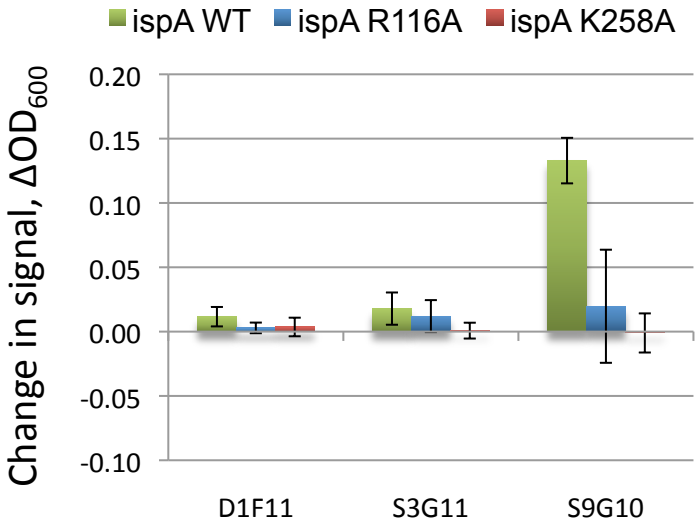


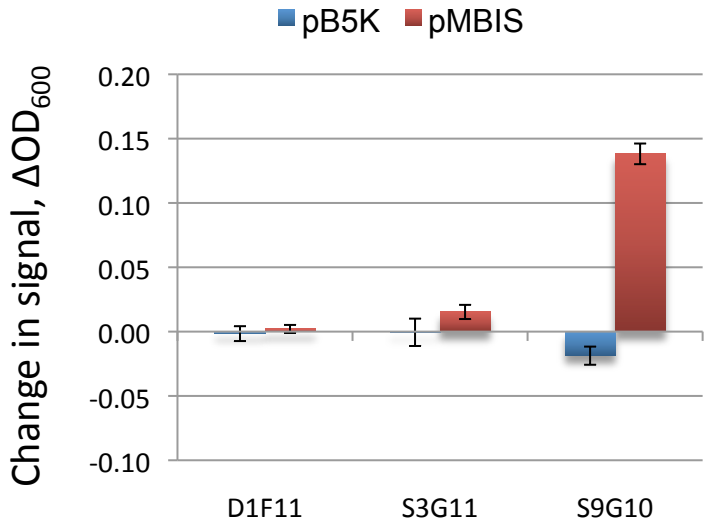
Mevalonate pathway:

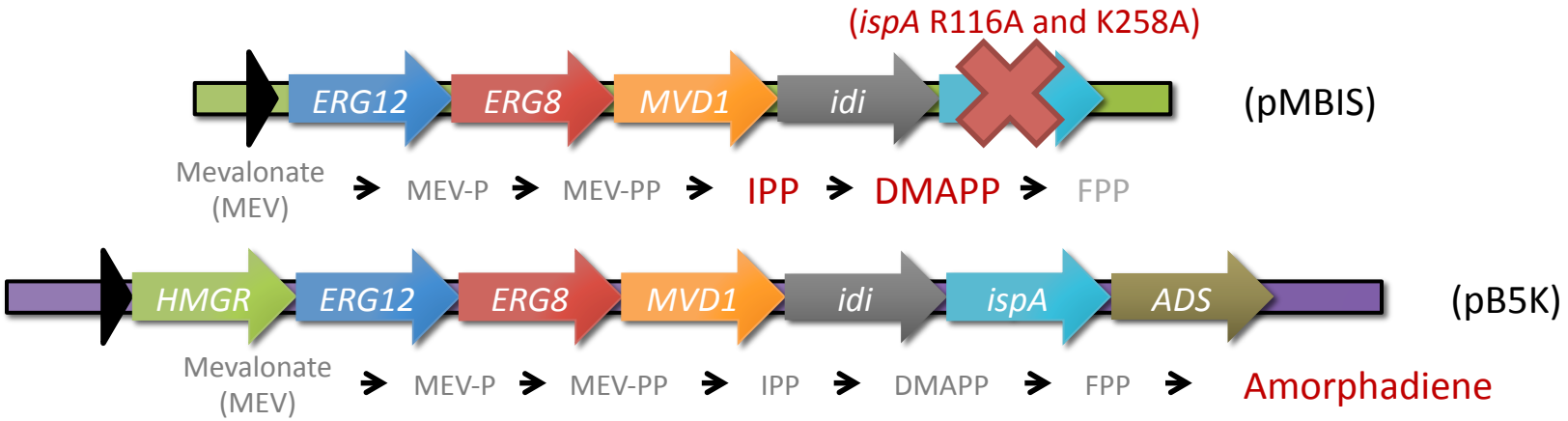


(*ispA* R116A and K258A)

Target authenticity

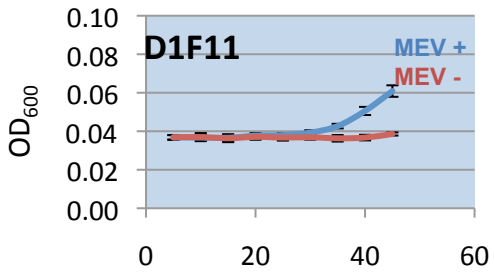


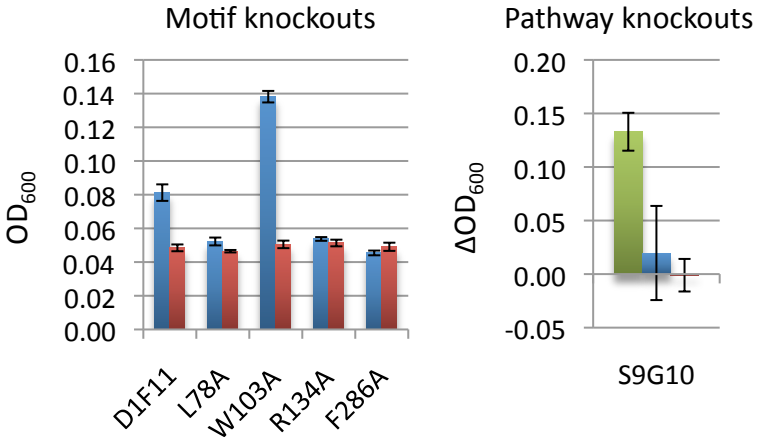




Conclusion & outlook

- Novel, functional sensors responding to FPP in cells are created by computational design.
- Binding site & pathway knockout mutations are consistent with predicted binding model & FPP sensing.
- Need *in vitro* quantification and structures of the complex.
- Explore the design fitness to learn about design successes and limitations.
- Test optimized sensors with modular outputs in metabolic flux control.





Where to go next?

Poster:

"Engineering small-molecule biosensors through computer-aided remodeling of protein-protein interfaces"

2012 Gen9 G-prize:
500 synthesized genes up to 1000 bp

Acknowledgments

W.M. KECK FOUNDATION

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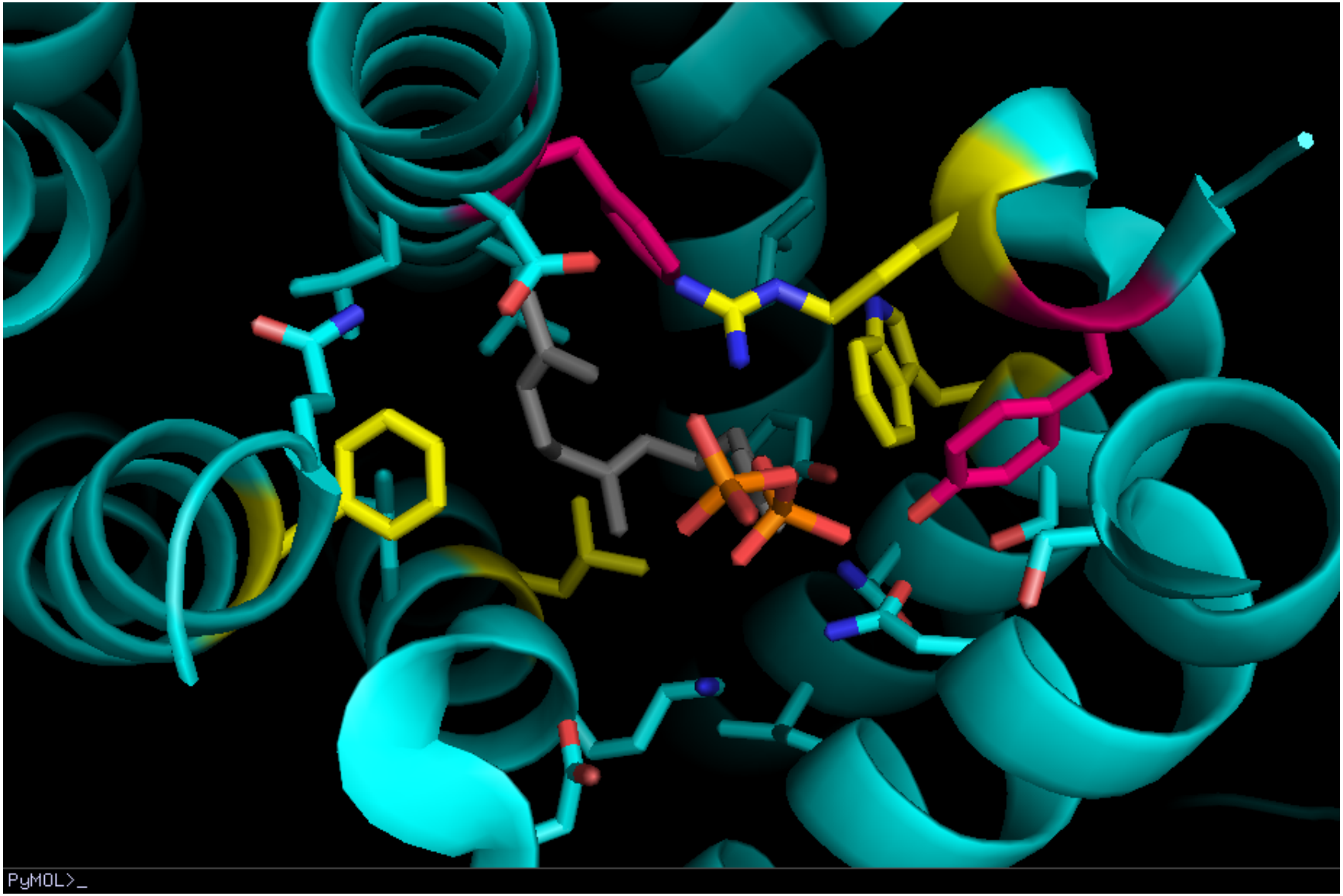
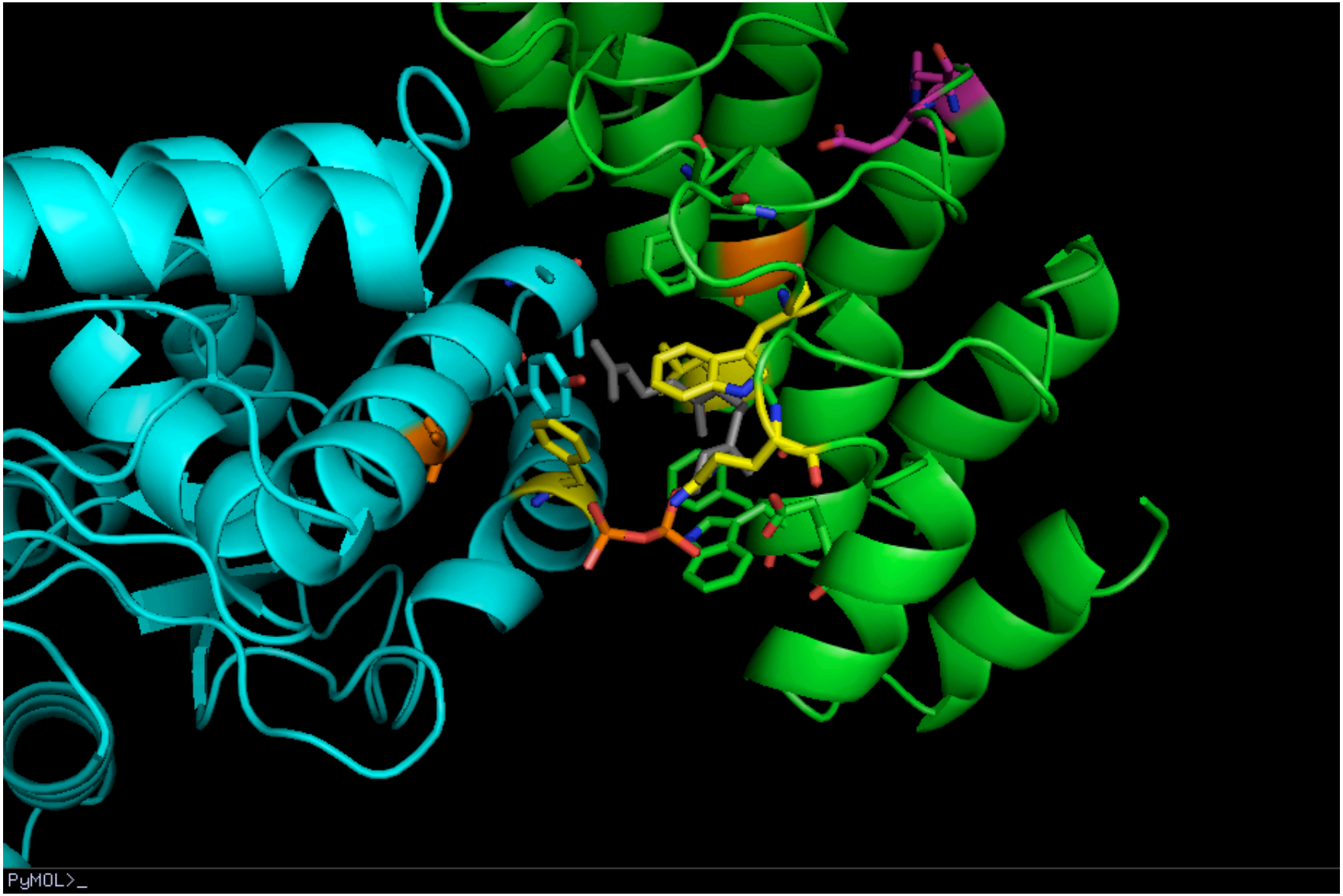

PyMOL>_


PyMOL>_

Saturation mutagenesis speculations

position	WT	design	change	
74*	H	F, I,L	G	overpacked? correlated with F70?
107	H	-	P,N,L,I	was not designed
108*	L	A	R,P	?
111	K	W,Y	V,L	overpacked? buries polar groups?
347*	F	(R**)	A,G,S,F	was not designed