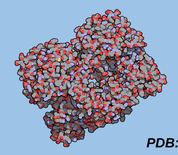
LynD Substrate Tolerance for Thiazole Installation in mRNA Display

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Emma Steude | Bowers Lab

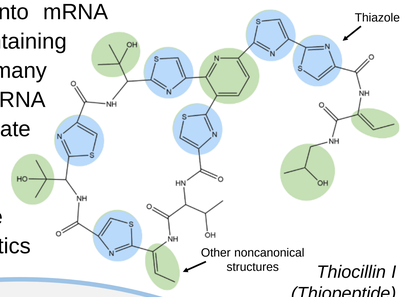


LynD
***PDB:** 4V1V*

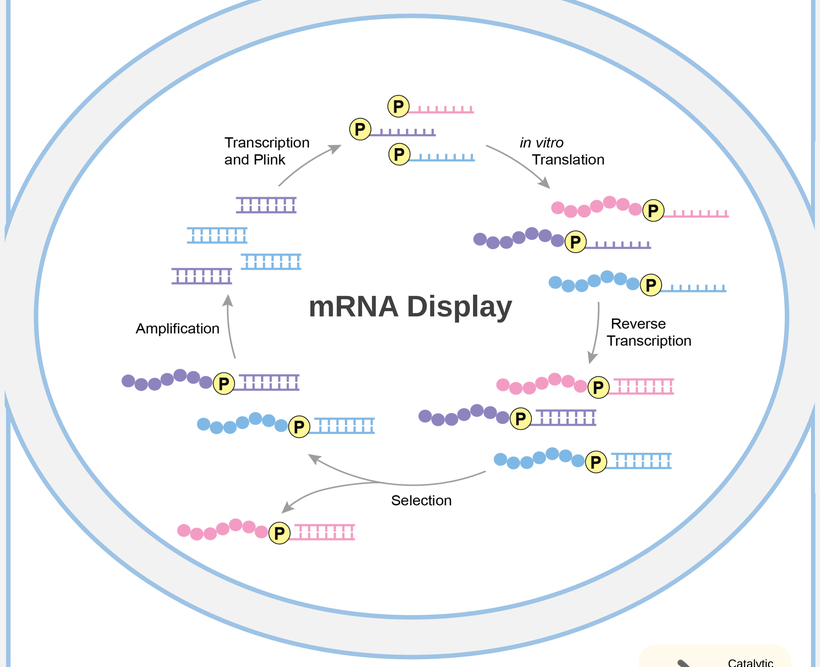
Introduction

Abstract:

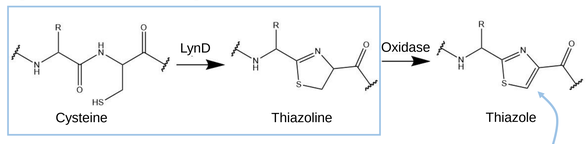
mRNA display is a powerful high-throughput screening technique used to identify potential therapeutics from diverse libraries of peptides.[1] Natural products provide insights into therapeutically relevant moieties that may be incorporated into mRNA display peptide libraries. For example, thiazoles are amide isosteres with beneficial drug-like properties that are found in many natural products.[2,3] LynD, the cyclodehydratase enzyme in aesturamide biosynthesis,[4] may be integrated into mRNA display to develop thiazole-containing libraries, but LynD must tolerate many diverse substrates to function with mRNA display. By exploring the substrate tolerance of LynD, more diverse mRNA display screening libraries may be created, expanding the potential of peptide-based therapeutics in drug discovery.



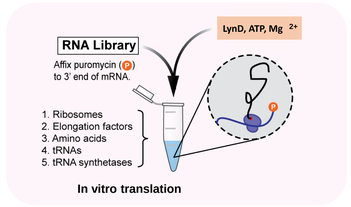
Thiocillin I (Thiopeptide)

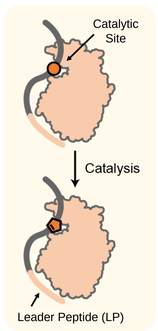


LynD to Incorporate Thiazoles:



- Amide Isostere
  - Planar
  - Reduced H-bonding

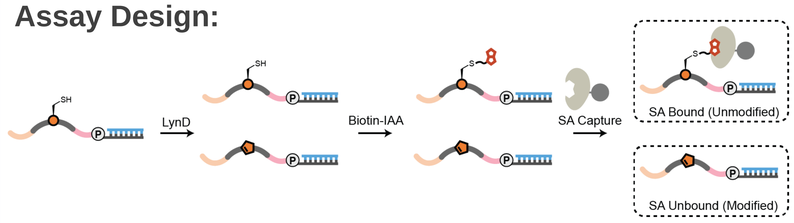




Will LynD modify libraries in mRNA Display?

Assay Development

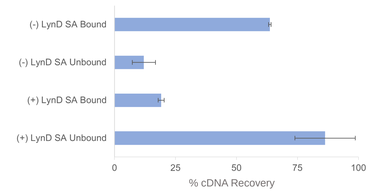
Assay Design:



Assay Validation through qPCR Quantification:

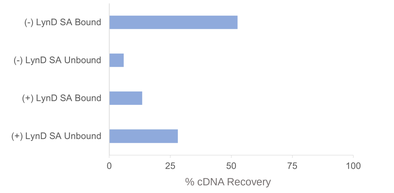
Model of Natural Substrate:

MLAELSEEALGGNGVDASACMPCYPSYDGSYPYDVPDYAGELARP



Randomized Library:

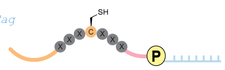
MLAELSEEALRDTVALPENGASXXXCXXXAQPYPYDVPDYAGELARP



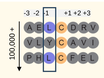
Substrate Tolerance

Fixed Cysteine Library NGS Results:

MLAELSEEAL-XXXCXXX-YPYDVPDYA-*mRNA Tag*
LynD LP | Core | HA Tag

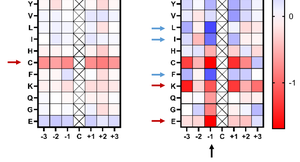


$$f_{mut} = \frac{c_{mut}}{c_{tot}}$$



$$Y_{mut} = \frac{f_{mut}(Modified)}{f_{mut}(Unmodified)}$$

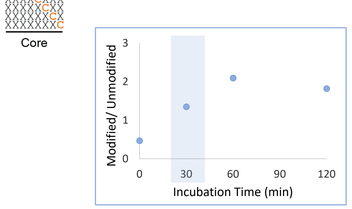
Unmodified Modified

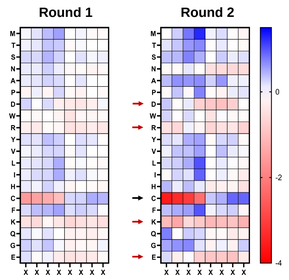


Plot: $Log_2(Y_{mut})$
>0 = SA Unbound (Modified)
<0 = SA Bound (Unmodified)

Expanded Library Initial NGS Results:

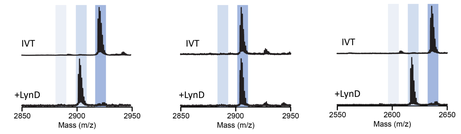
MLAELSEEAL-CXXXXXXX-YPYDVPDYA-*mRNA Tag*
LynD LP | HA Tag

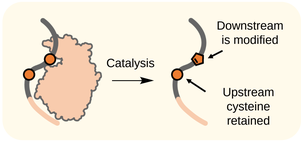




Leader Peptide to Cysteine Distance Dependance:

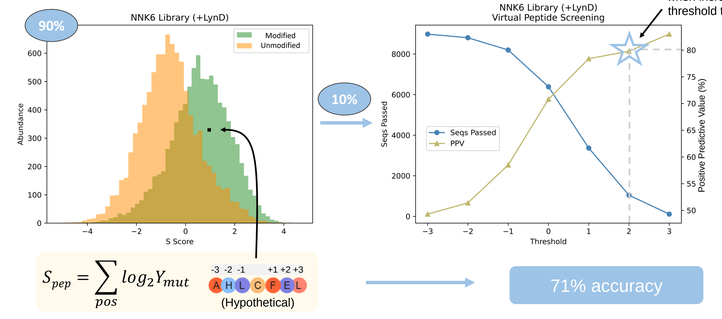
LP_CVDASACYPSYDG LP_CVDASASYPSYDG LP_CSACYPSYDG





Computational Analysis

S-Score Predictive Model:

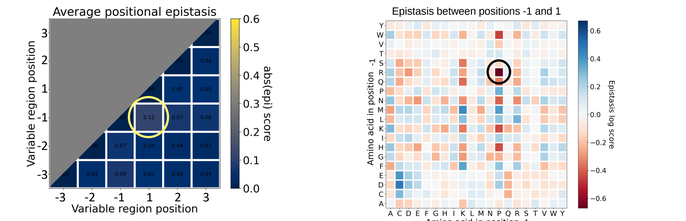


$$S_{pep} = \sum_{pos} log_2 Y_{mut}$$

-3 -2 -1 +1 +2 +3
A H L C F E L
(Hypothetical)

71% accuracy

Machine Learning Model for Epistatic Interactions:



Machine Learning Model:
Multilayer perceptron neural network

73% accuracy

Conclusions

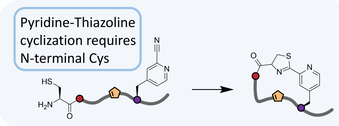
Summary:

- Validated LynD in mRNA display
- Determined high substrate tolerance!
  - Dislikes charged residues at -1 position
  - Prefers hydrophobic at -1 position
  - Low overall epistasis
- Developed model to predict LynD substrates with >70% accuracy
- Showed Cys distance from leader peptide dependance

Future Directions:

- Additional rounds of display for machine learning model
- Incorporate cyclization method into thiazoline library
- Run selection with library

Pyridine-Thiazoline cyclization requires N-terminal Cys



References & Acknowledgements

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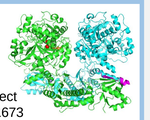

1. Iskandar, S. E.; Haberman, V. A.; Bowers, A. A. Expanding the Chemical Diversity of Genetically Encoded Libraries. *ACS Comb. Sci.* **2020**, 22, 712, DOI: 10.1021/acscombsci.0c00179
2. Siodlak, D.; Stas, M.; Broda, M. A.; Bujak, M.; Lis, T. Conformational properties of oxazole-amino acids: Effect of the intramolecular N-H···N hydrogen bond. *J. Phys. Chem. B* **2014**, 118, 2340– 2350, DOI: 10.1021/jp4121673
3. Wipf, P.; Fritch, P. C.; Geib, S. J.; Sefler, A. M. Conformational studies and structure-activity analysis of lissoclinamide 7 and related cyclopeptide alkaloids. *J. Am. Chem. Soc.* **1998**, 120, 4105– 4112, DOI: 10.1021/ja973580h
4. Koehnke, J.; Mann, G.; et al. Structural analysis of leader peptide binding enables leader-free cyanobactin processing. *Nat. Chem. Biol.* **2015**, 11, 558–563. DOI: 10.1038/nchembio.1841