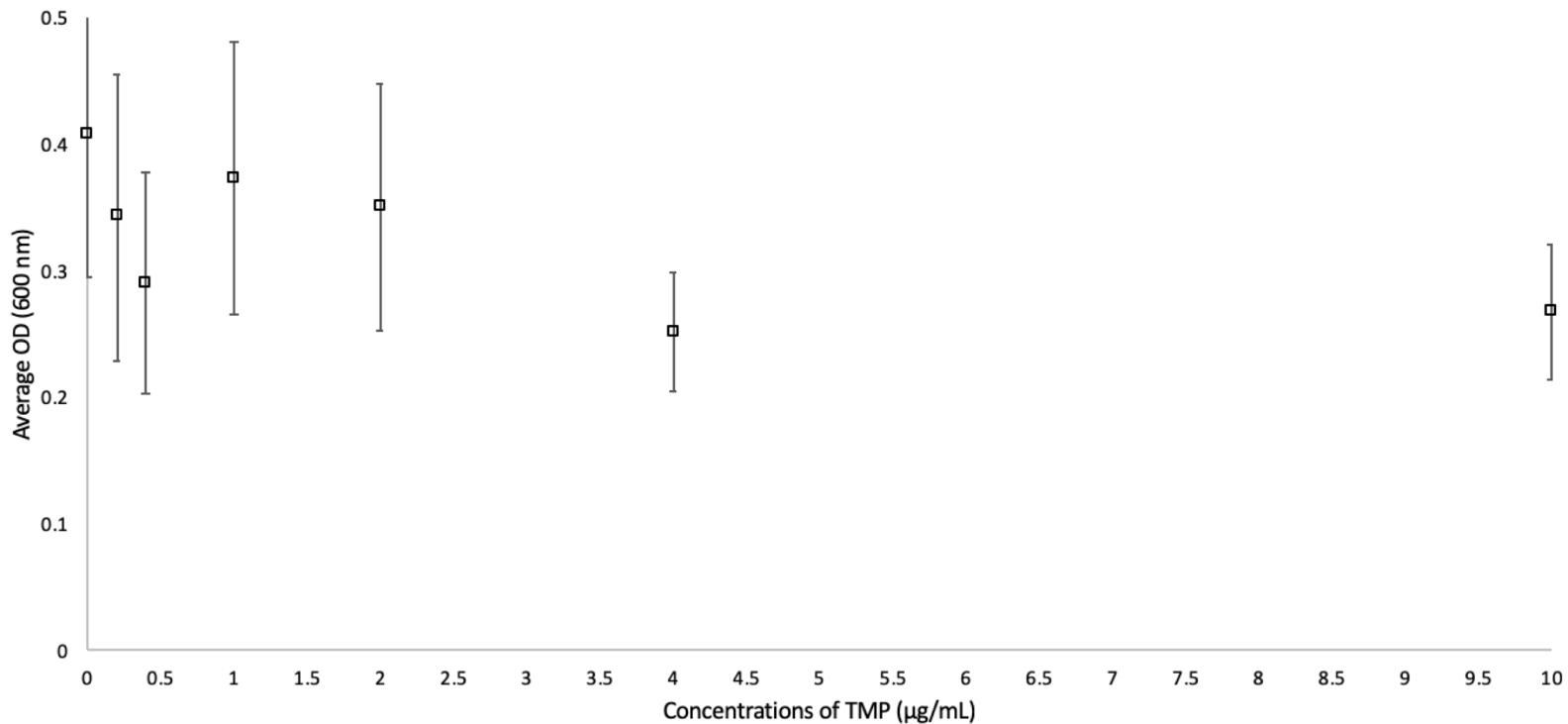


2Lo6 Project

Christy Au-Yeung, Meea Fogal,
Bushra Haque, Megan Kwong,
Joshua Ling, Peipei Wang

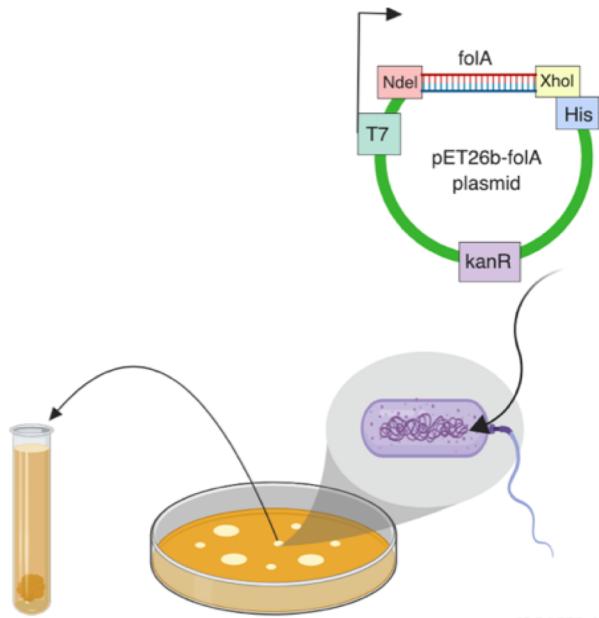
Dose Response Curve

Average Optical Density Values of *E. coli* BL21 (DE3) Cells Measured with Varying Concentrations of TMP

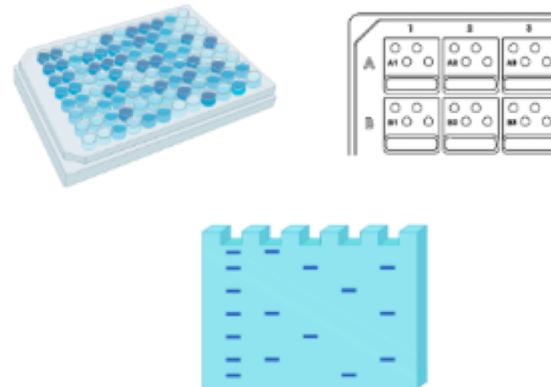


2L06 Project Overview

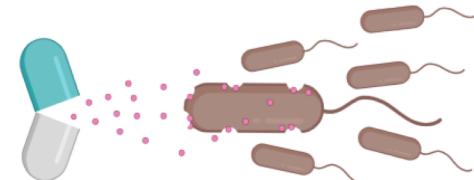
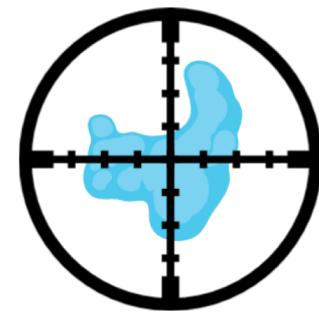
1. Insertion of pET26b-*folA* plasmid into *E. coli* DH5a cells



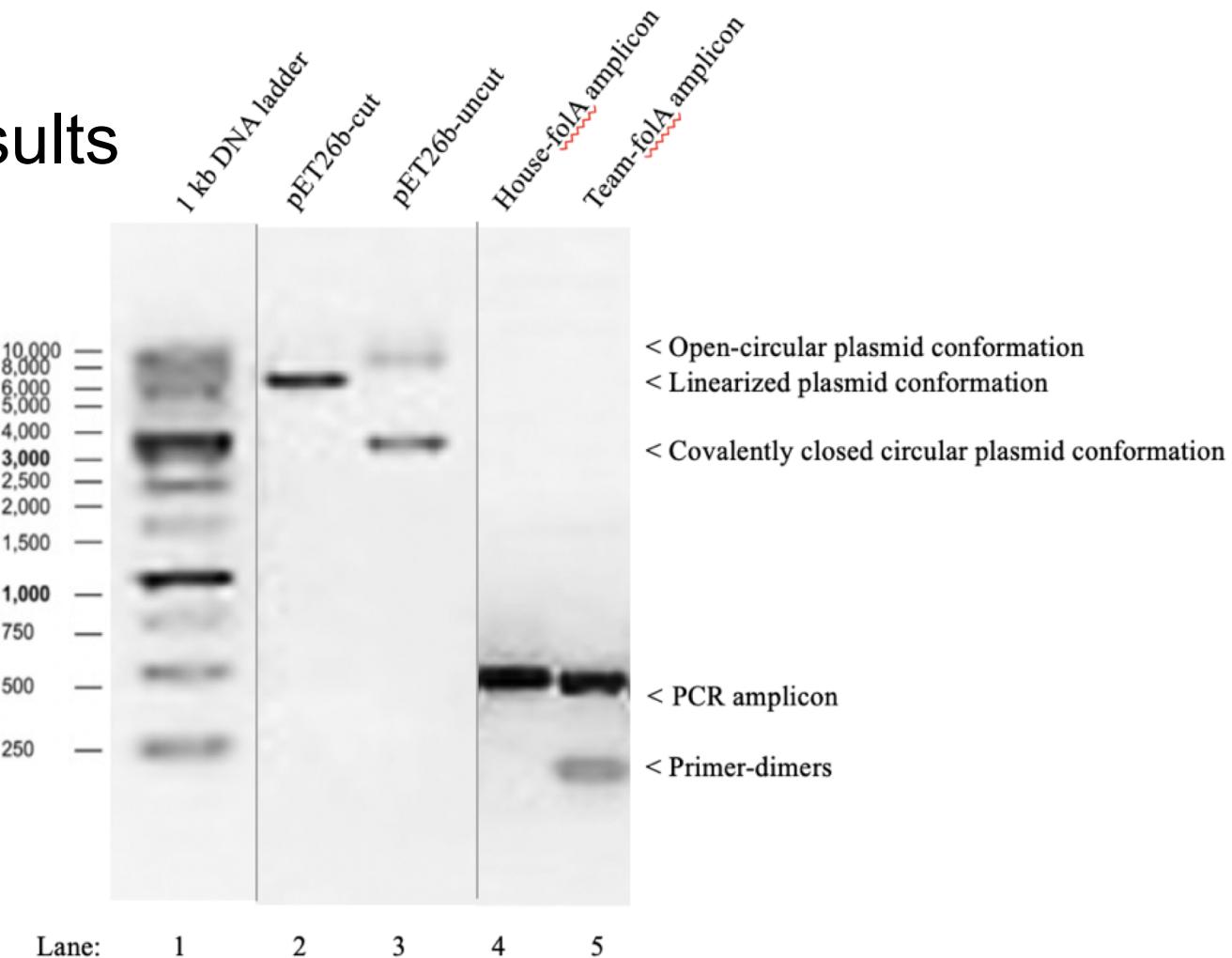
2. Expression, purification, quantification and visualization of DHFR protein product



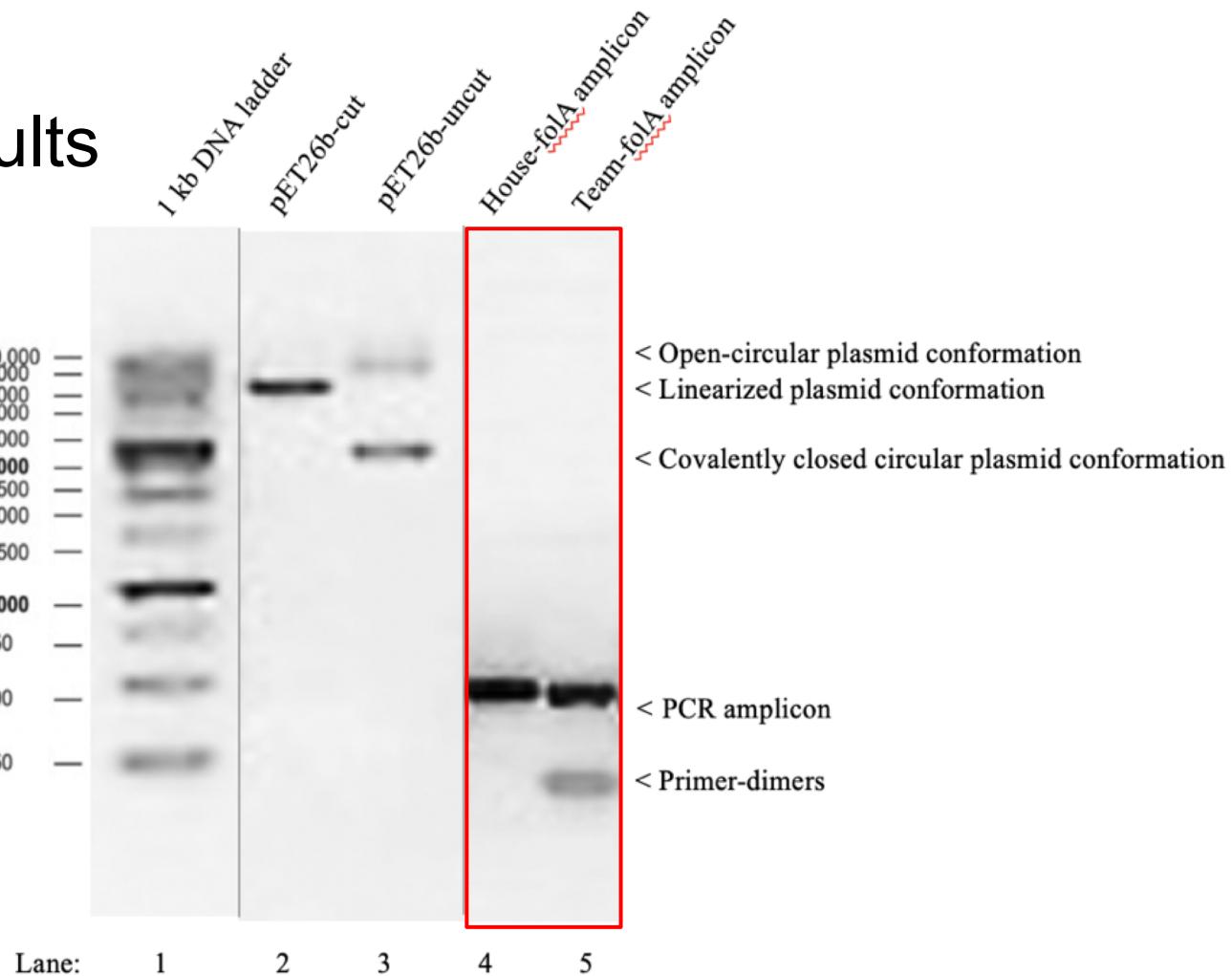
3. Design novel inhibitory drug



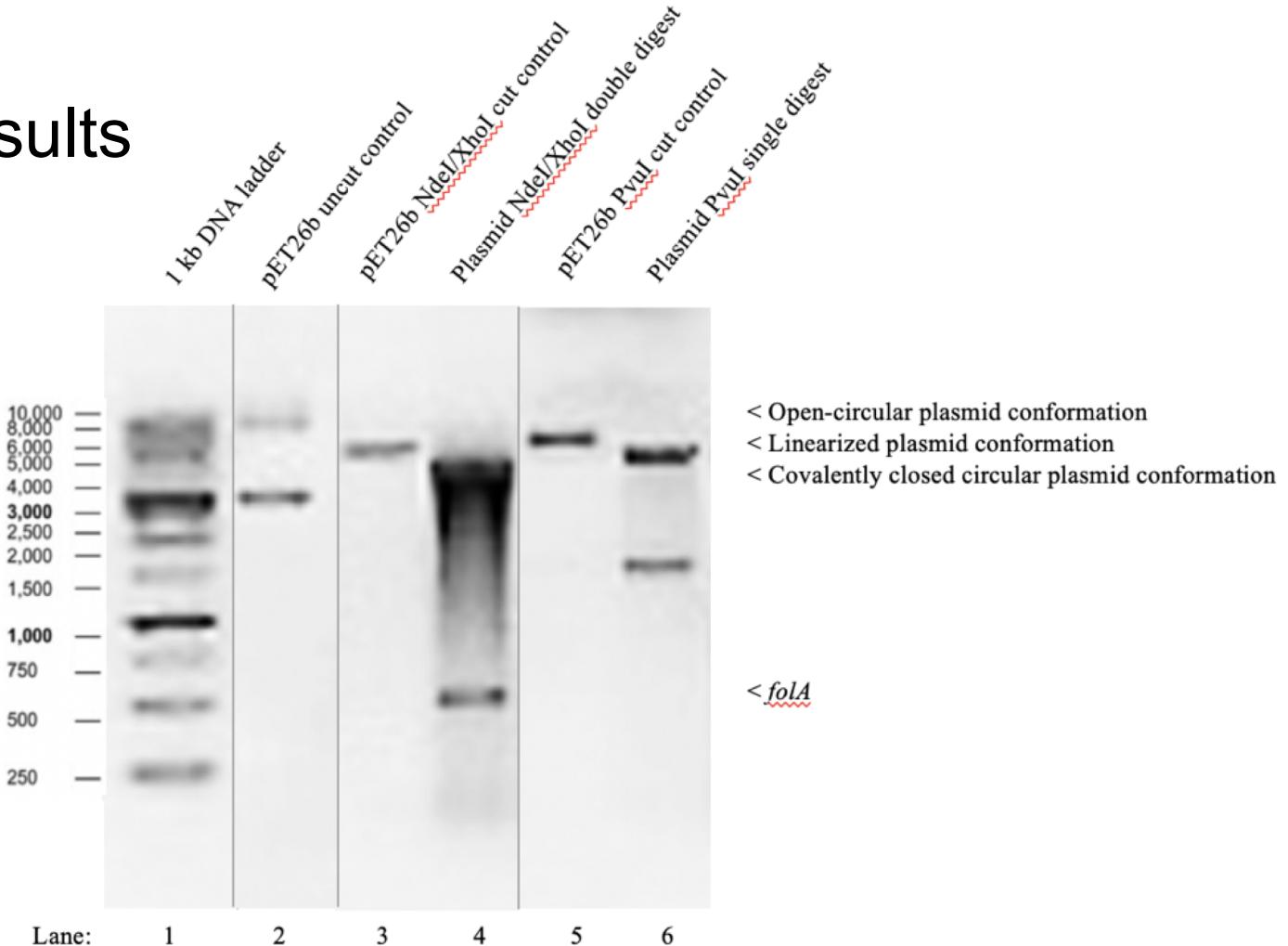
Term 1 Results



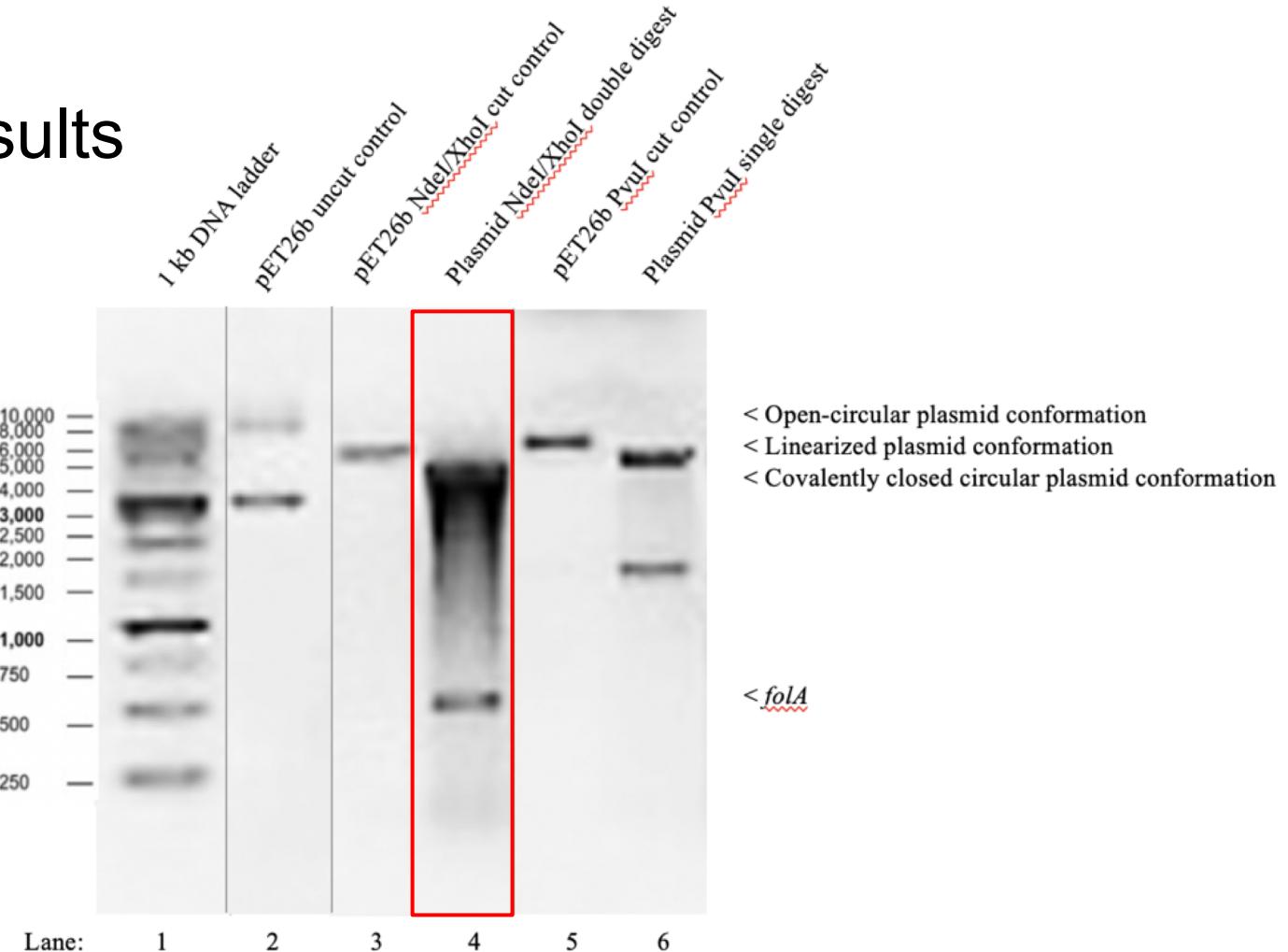
Term 1 Results



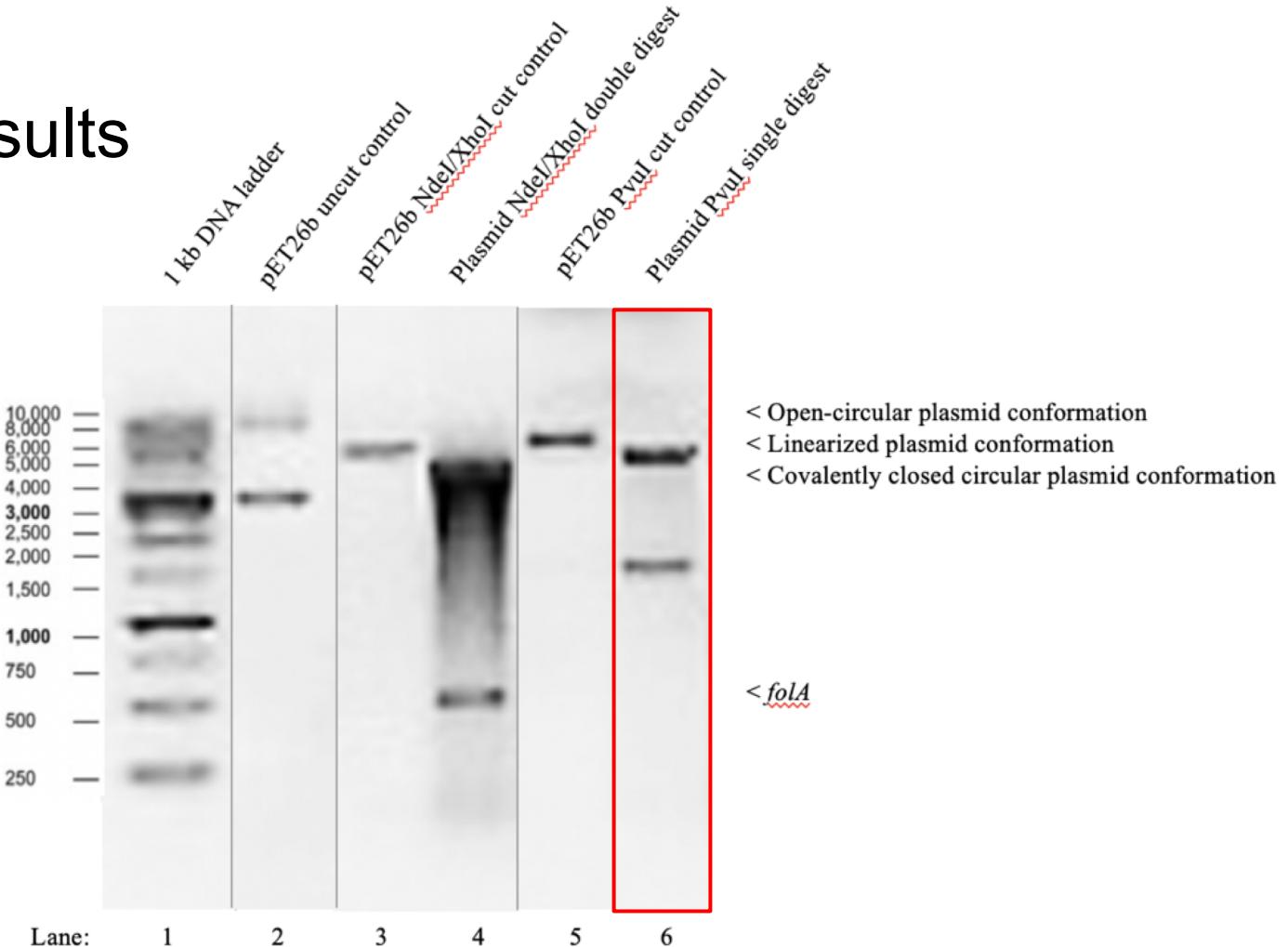
Term 1 Results



Term 1 Results

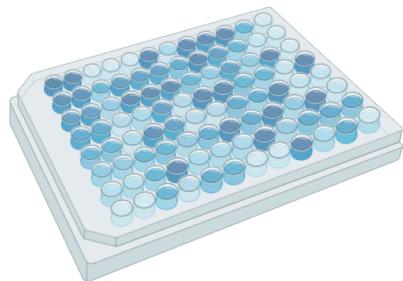


Term 1 Results

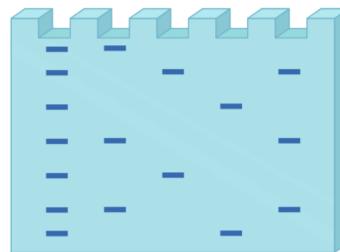


Term 2

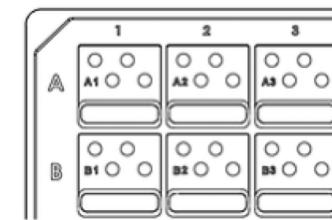
- We were able to collect data using the following techniques:



Bradford Assay



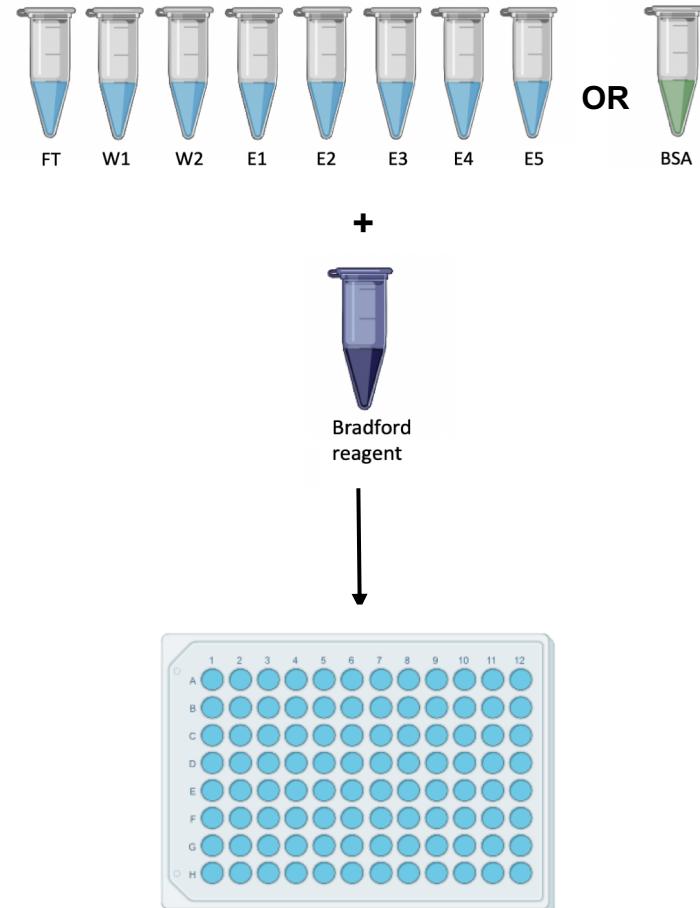
SDS-PAGE



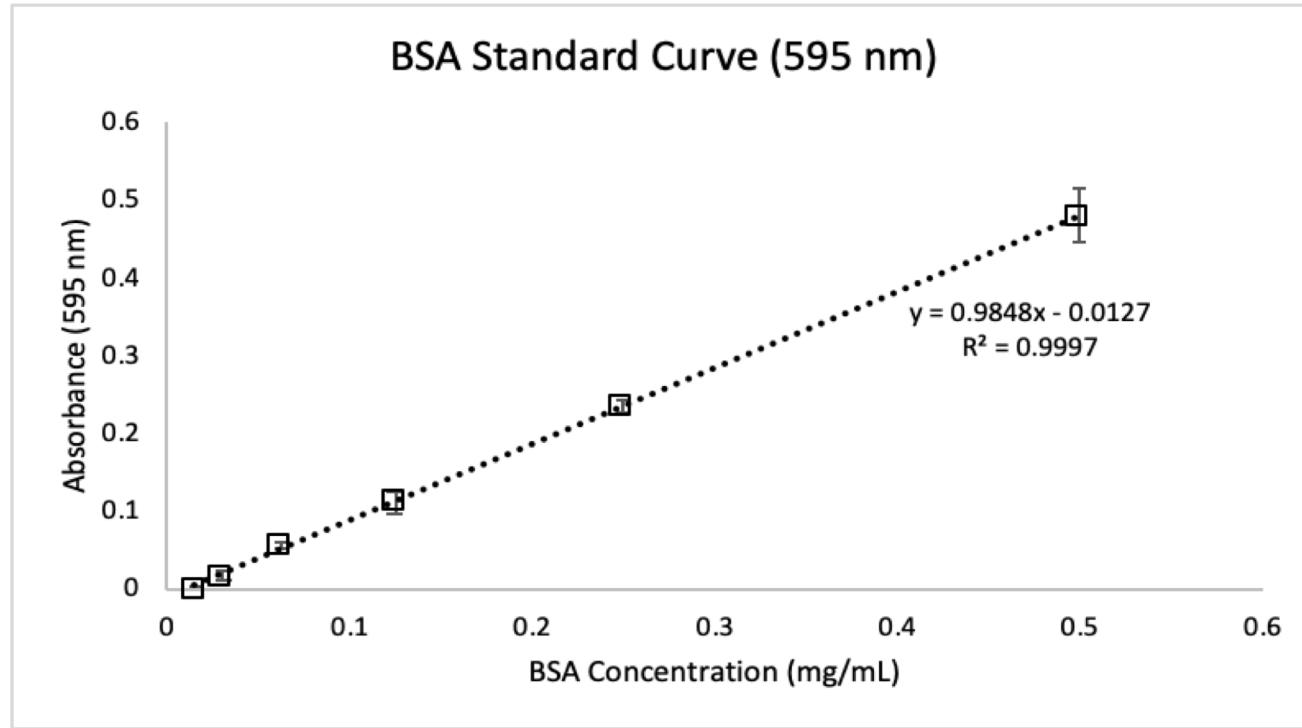
**Protein Crystallization
(sitting-drop method)**

Bradford Assay

- **Purpose:** quantifying DHFR
- Bovine serum albumin (BSA) as standard
- Bradford Reagent:
 - Coomassie Brilliant Blue G-250



Bradford Assay



- Linear Relationship: Beer-Lambert Law

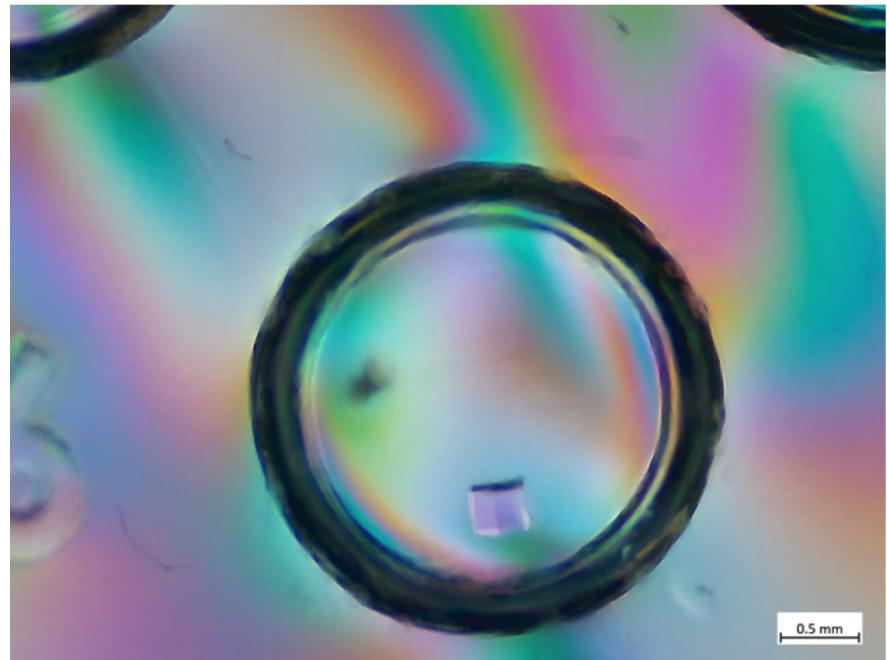
SDS-PAGE

- Clear DHFR protein bands found at ~20 kDa
- DHFR is present in:
 - Wash fractions (W1, W2, W3)
 - Elution fraction (E1)



Lysozyme Protein Crystallization

- **Method:** Sitting drop
 - Vapor diffusion method
- Positive control
- Crystal ranking: 3

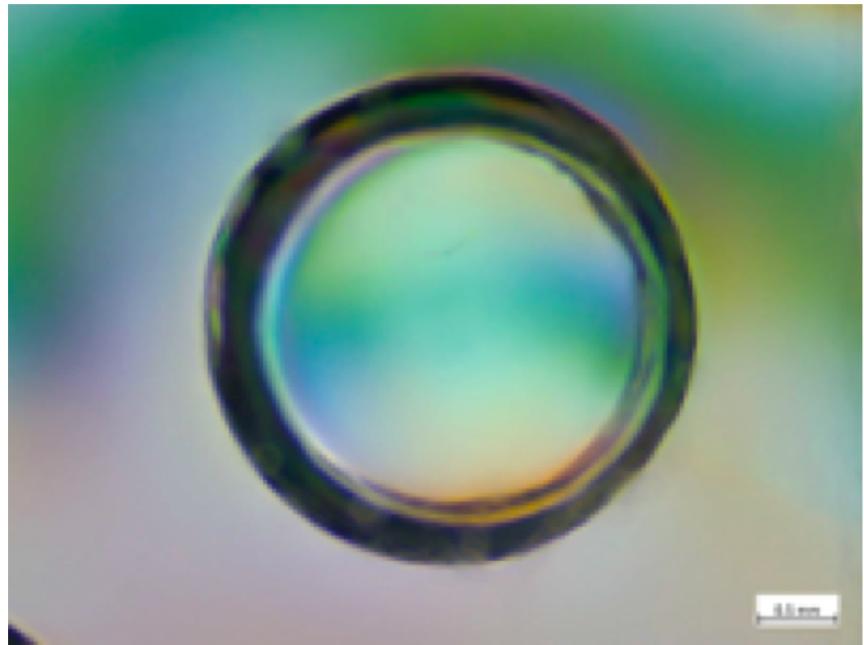


DHFR Protein Crystallization

- No observed DHFR crystals

Troubleshooting:

- Increased protein purity and mass
- Compromised DHFR homogeneity



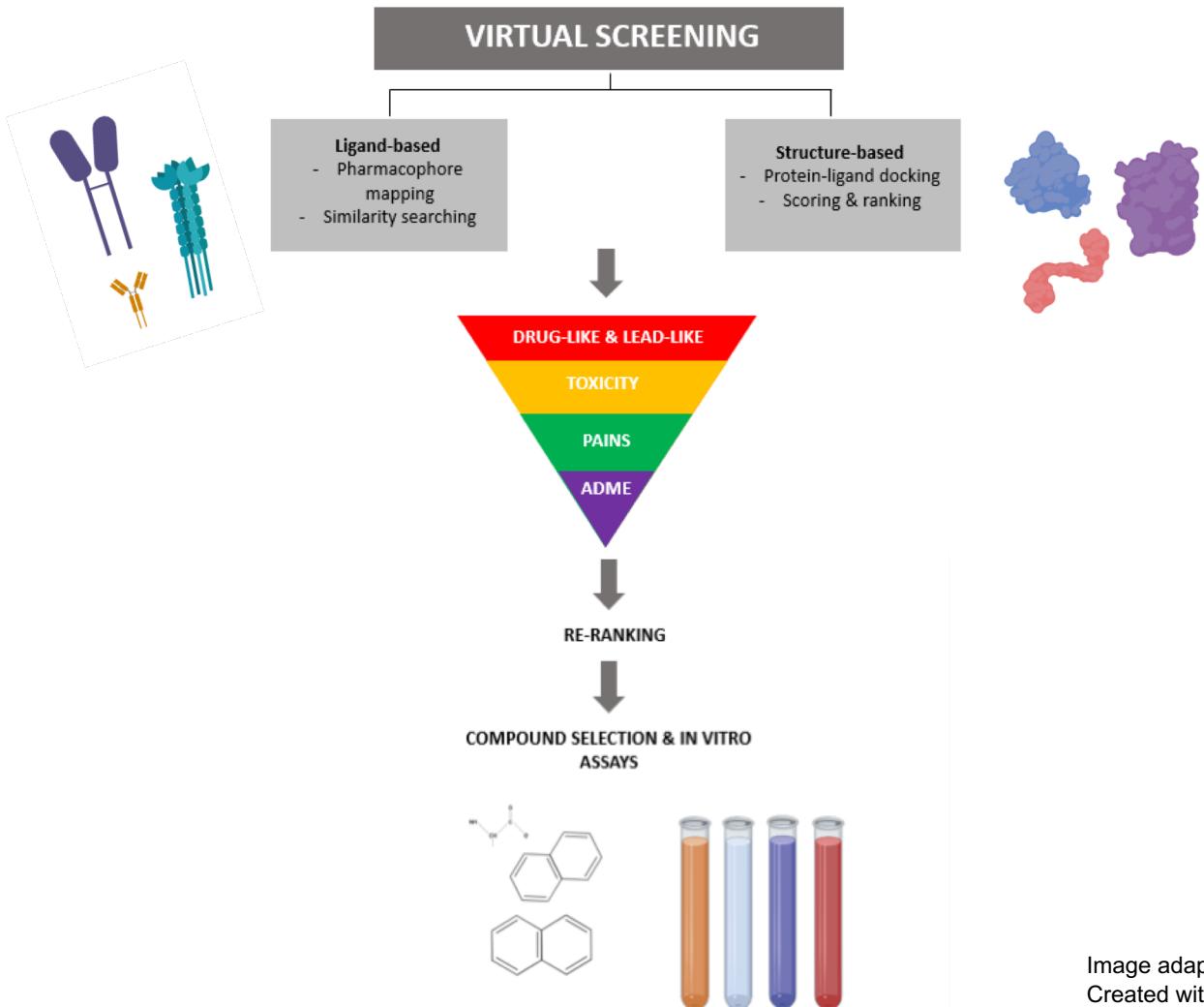
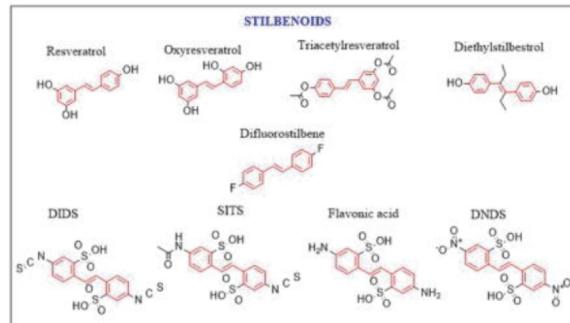
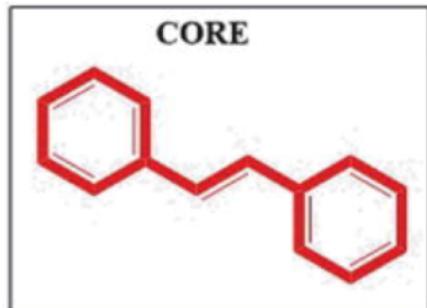
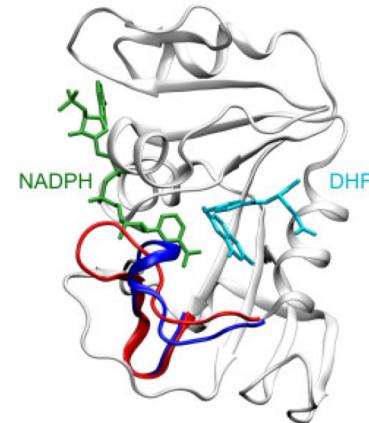


Image adapted from (Profacgen, 2017)
Created with BioRender

E. coli DHFR: Allosteric binding sites

- Usual anti-DHFR drugs bind to active sites
- Small molecules in unique sites have potential

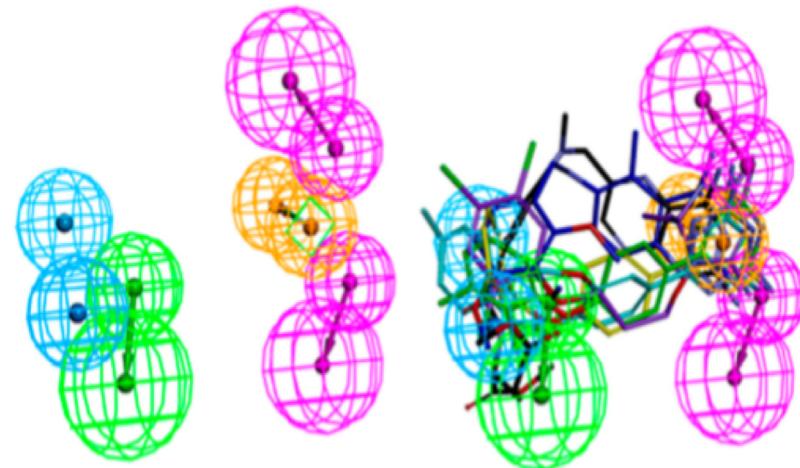
SAR - Structure-activity relationships, determining effects of new compounds from similar compounds



Srinivasan et al., Rational design of novel allosteric dihydrofolate reductase inhibitors showing antibacterial-effects on drug-resistant *E. coli* escape- 16 variants. *ACS chemical biology*, 2017 **12**, 7.

DHFR Inhibition and *Mycobacterium tuberculosis*

- Hybrid virtual screening:
pharmacophore model + docking
study and visual analysis
- Pharmacophore model of mtDHFR
inhibitors
- “G3” identified as most active



Wang et. al., Identification of novel DHFR inhibitors for treatment of tuberculosis by combining virtual screening with in vitro activity assay. *Journal of Biomolecular Structure and Dynamics*, 2019, **4**, 37.

BHAVYA FISHER

SCIENTIFIC

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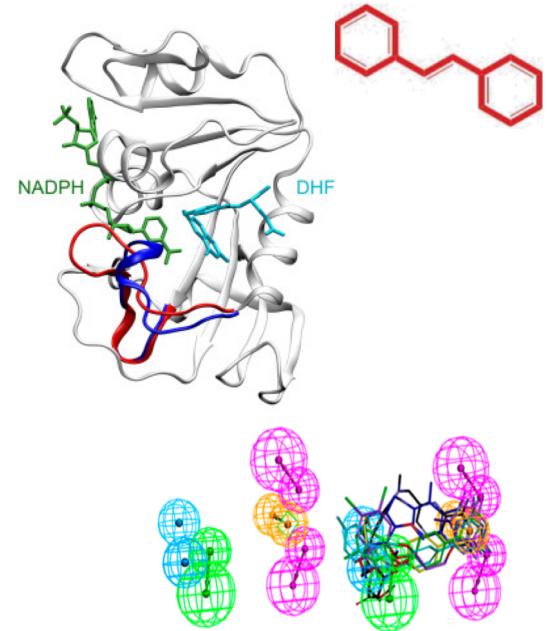
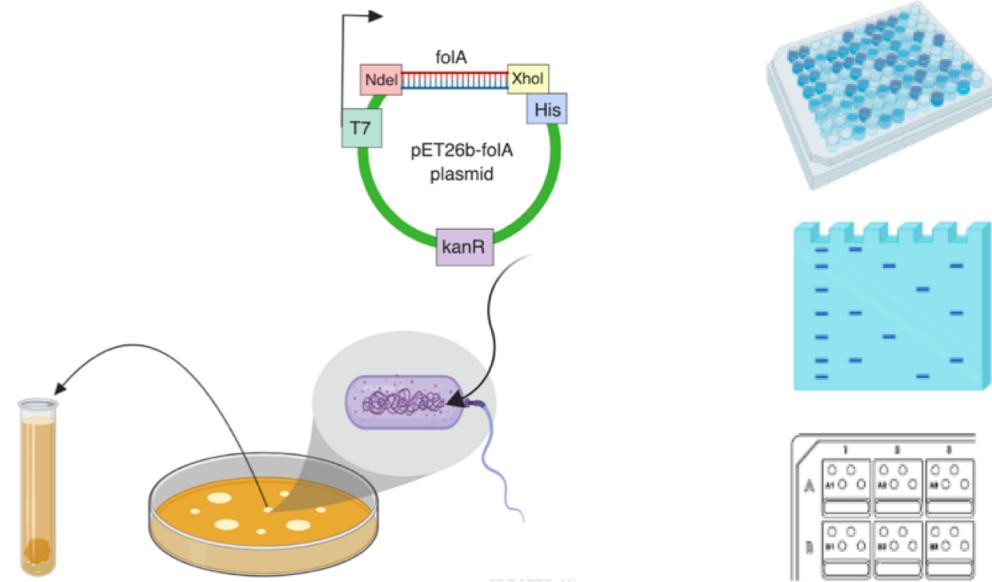
Teaching Assistants and 2L06 Peers

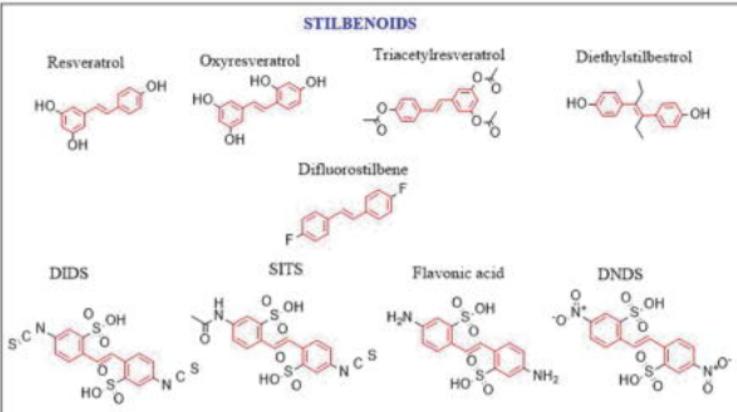
Summary

Molecular Cloning

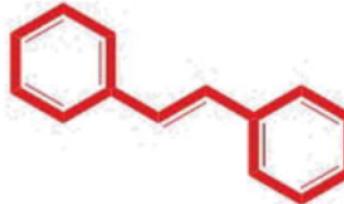
Expression, Purification and Analyses

Drug Design of Novel Inhibitors

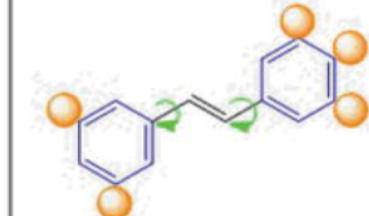
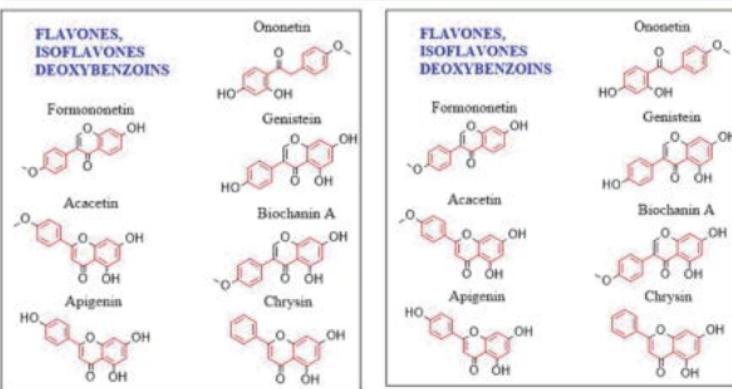




CORE



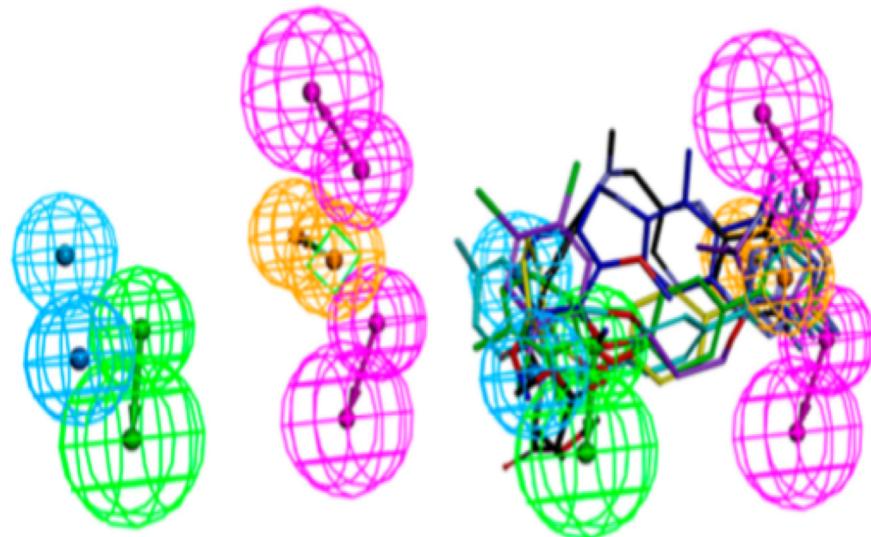
CHEMICAL FEATURES AIDING INHIBITION



- **H-bond Donor/Acceptor**
- **Rotatable bonds**
- **Two benzene rings**

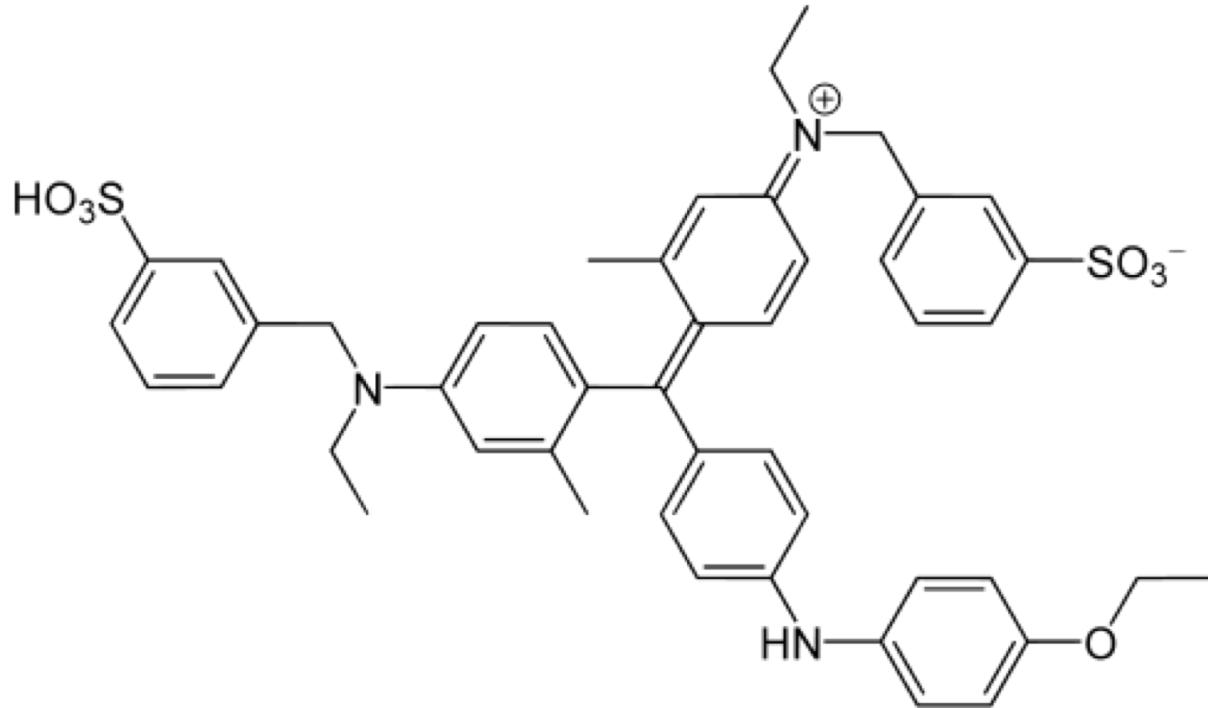
Hypo1 Pharmacophore Model

- Colour-coated features:
 - **Green** = hydrogen bond acceptor
 - **magenta** = hydrogen bond donor
 - **Cyan** = aromatic hydrophobic feature
 - **Dark blue**= hydrophobic aliphatic feature
 - **Orange** = aromatic ring



Wang et. al., Identification of novel DHFR inhibitors for treatment of tuberculosis by combining virtual screening with in vitro activity assay. *Journal of Biomolecular Structure and Dynamics*, 2019, **4**, 37.

Coomassie Brilliant Blue G-250



References

- Lionta, E., Spyrou, G., Vassilatis, D.K. and Cournia, Z., 2014. Structure-Based Virtual Screening for Drug Discovery: Principles, Applications and Recent Advances. *Current Topics in Medicinal Chemistry*, 14(16), pp.1923–1938.
- Srinivasan, B., Rodrigues, J.V., Tonddast-Navaei, S., Shakhnovich, E. and Skolnick, J., 2017. Rational design of novel allosteric dihydrofolate reductase inhibitors showing antibacterial-effects on drug-resistant *E. coli* escape-variants. *ACS chemical biology*, 12(7), pp.1848–1857.
- Wang, N., Ren, J.-X. and Xie, Y., 2019. Identification of novel DHFR inhibitors for treatment of tuberculosis by combining virtual screening with in vitro activity assay. *Journal of Biomolecular Structure and Dynamics*, 37(4), pp.1054–1061.