ENTASIS THERAPEUTICS

An Adventure in Antibacterial Drug Discovery

Competing Methyl Effects on Biochemical Potency and Cell Accumulation

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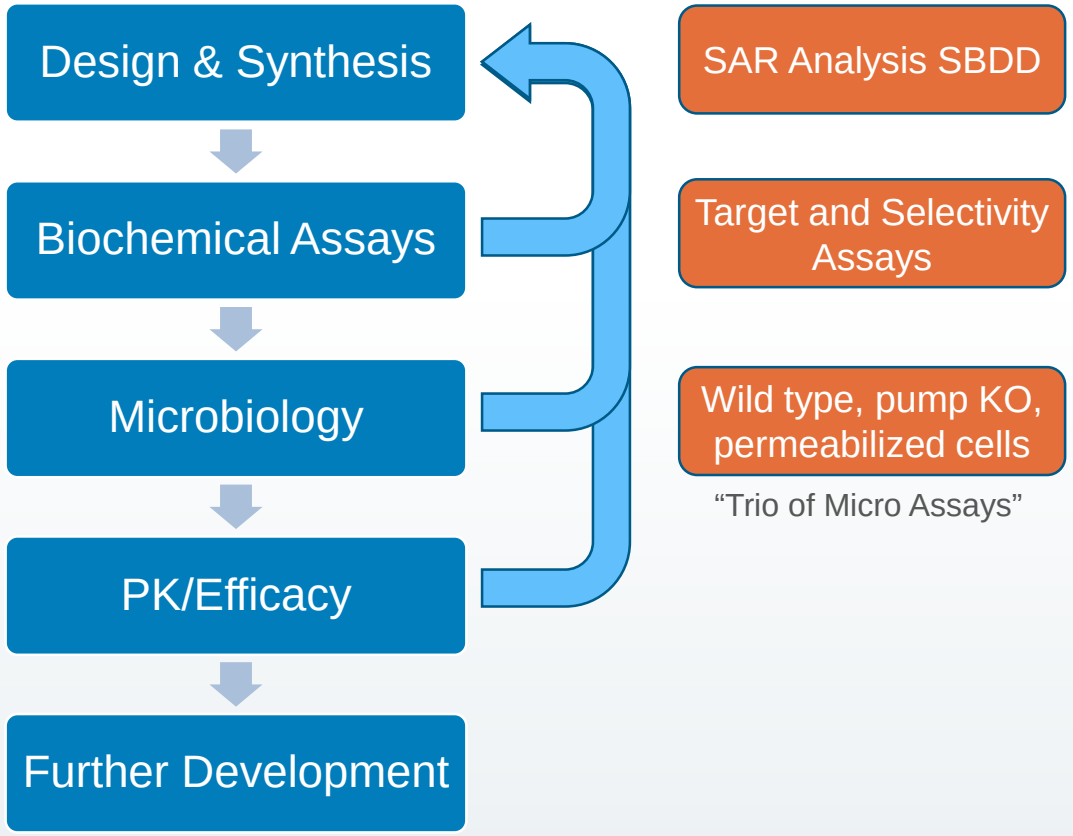
Disclosures

- Ruben Tommasi: Full-time Employee; Share Holder; Entasis Therapeutics.

The ‘Typical’ Drug Discovery Flowchart

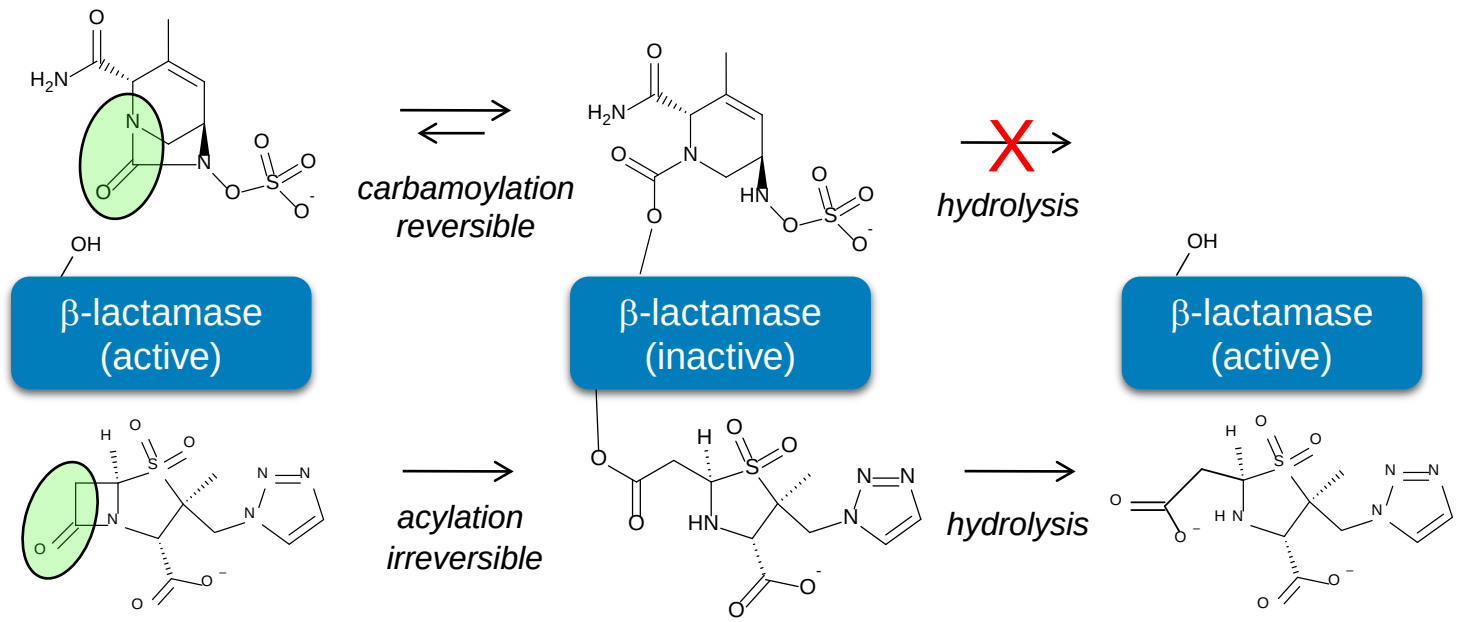


- Lead Optimization cycle with early focus on biochemical activity followed closely by microbiology is typical
  - Med Chem focus on optimizing biochemical potency
  - Classical Micro tools rather uninformative:
    - Typical chemist comment using “trio of micro assays”:
    - “My compound is effluxed” or “My compound doesn’t permeate well”
- Optimization is quite difficult as the data team obtains does not guide the team on how to fix the issue
  - What if your favorite biochemical enhancement affects the way your entire series permeates?
  - Wouldn’t you want to know?
  - Early on so you could do something about it…

Towards a Novel Class of Gram-negative Agents: Diazabicyclooctanone (DBO)

ETX2514: stable to β-lactamase hydrolysis

Tazobactam: hydrolyzed by β-lactamases

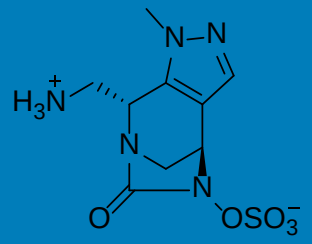


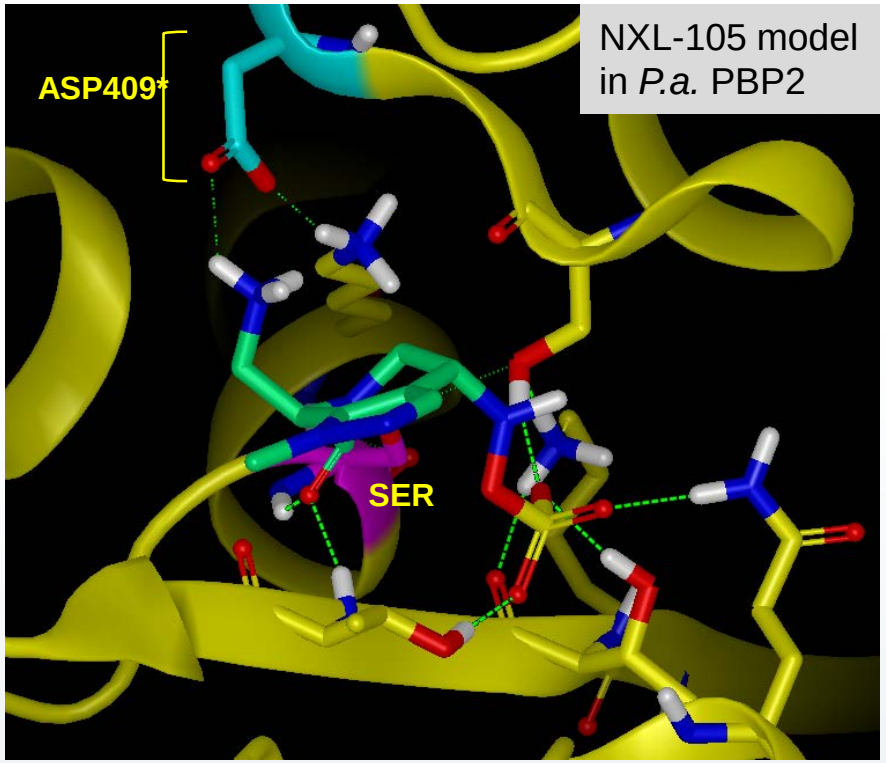
- In addition to acting as potent inhibitors of diverse β-lactamases, certain DBO analogs have intrinsic antibacterial activity
- Unique mechanism of inhibition and action of this class demonstrated by ETX2514*
- Several other DBO analogs have also exhibited PBP2 activity (*i.e.* nacubactam, zidebactam, NXL-105) but this feature has not yet been optimized

GOAL: Discover single I.V. DBO (no BLI required) to treat infections caused by *P. aeruginosa*, including MDR strains

*Reversibility of β-lactamase inhibition first exemplified with avibactam in: Ehmann D. *et al.*, PNAS (2012) *109*, 11663

NXL-105 Shows Excellent Activity against *P. aeruginosa in vitro*

NXL-105 **PBP2 Inhibitor**	
P.a. PBP2 acylation rate $k_{(on)}$ ($M^{-1}.s^{-1}$)	5,200
P.a. PBP3 acylation rate $k_{(on)}$ ($M^{-1}.s^{-1}$)	11
P.a. PBP1a acylation rate $k_{(on)}$ ($M^{-1}.s^{-1}$)	2
P.a. MIC (ARC6347, OXA-486, PDC-24) (mg/L)	0.25
P.a. MIC_{90} (N=302) (mg/L)	1



NXL-105 model in *P.a.* PBP2

*ASN in *P.a.* PBP1a, PBP1b and PBP3

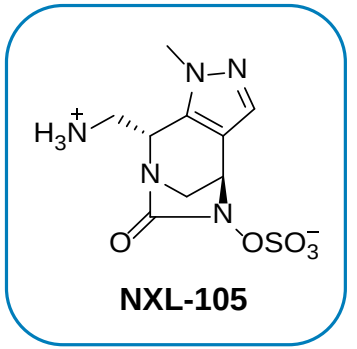
PBP2 selectivity rationalized by key salt bridge with ASP409

NXL-105 is not Efficacious against *P. aeruginosa in vivo*

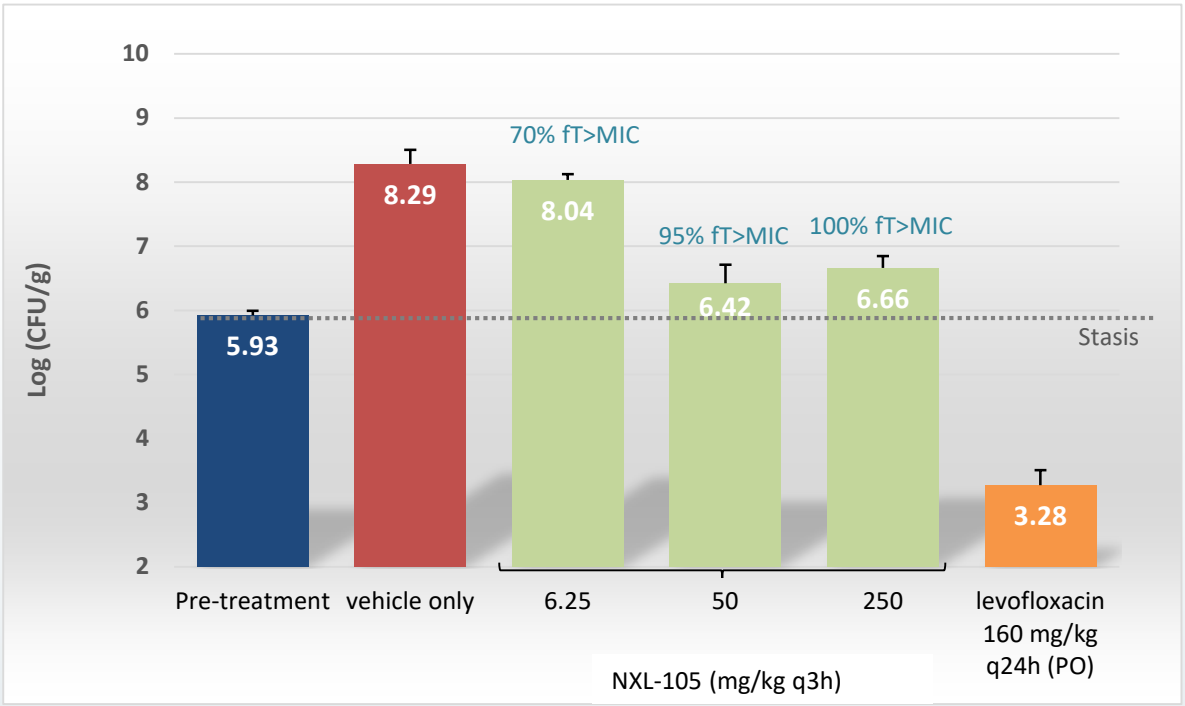
Murine neutropenic thigh infection model

MDR *P. aeruginosa* Clinical Isolate (ARC6347: OXA-486, PDC-24)

MIC (imipenem) > 4 mg/L, MIC (NXL-105) = 0.25 mg/L

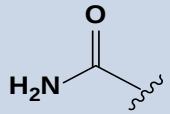
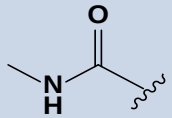
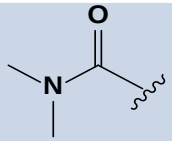


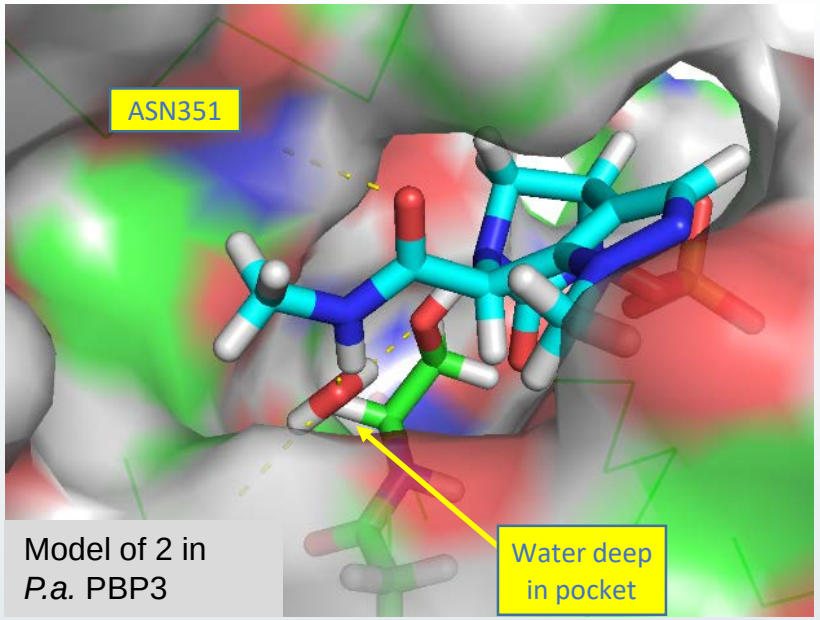
NXL-105



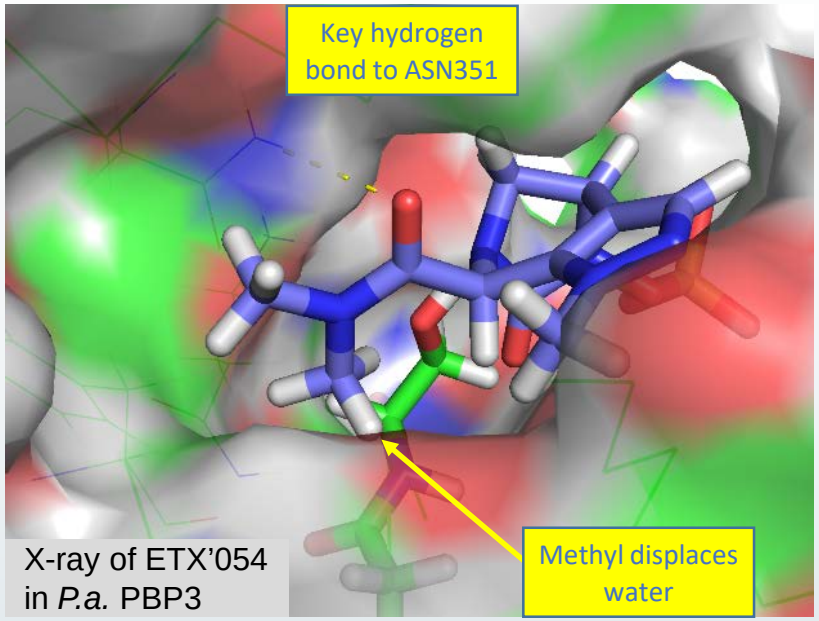
- Despite excellent *P.a.* MICs *in vitro* and exposures of up to 100% fT/MIC, NXL-105 is devoid of *in vivo* efficacy in a murine infection model
- NXL-105 also suffers from high frequency of resistance ($2x10^{-5}$ to $7x10^{-6}$ at 4xMIC, with >64-fold MIC increase) and severe inoculum effect
- **Hypothesis:** Is this driven by PBP spectrum and can we improve on that? (Yes!)

X-ray Co-crystal Structure (*P.a.* PBP3) Unveils Key Features and Helps Guide Analog Design

acylation rate k_{on} ($M^{-1}s^{-1}$)	NXL-105	1	2	ETX'054
R_1 group				
P.a. PBP2	5,200	131	110	<8
P.a. PBP3	11	230	610	582,000



Model of 2 in *P.a.* PBP3



X-ray of ETX'054 in *P.a.* PBP3

- Introduction of second methyl in **ETX'054** results in ~1000-fold increase in PBP3 k_{on}!
- Significantly faster on rate than carbapenems:
  - Meropenem: k_{on} = 49,000 $M^{-1}s^{-1}$
  - Imipenem: k_{on} = 1,100 $M^{-1}s^{-1}$
- Second methyl group displaces water deep in the pocket resulting in PBP3 potency enhancement

Antibacterial Activity of ETX'054 is Unaffected by β-lactamases in a *P. aeruginosa* Isogenic Panel

- β-Lactams lose their activity in presence of β-lactamases as depicted below for piperacillin, ceftazidime and imipenem
- In contrast, ETX'054, our DBO lead maintains activity in the presence of all 4 classes of β-lactamases tested

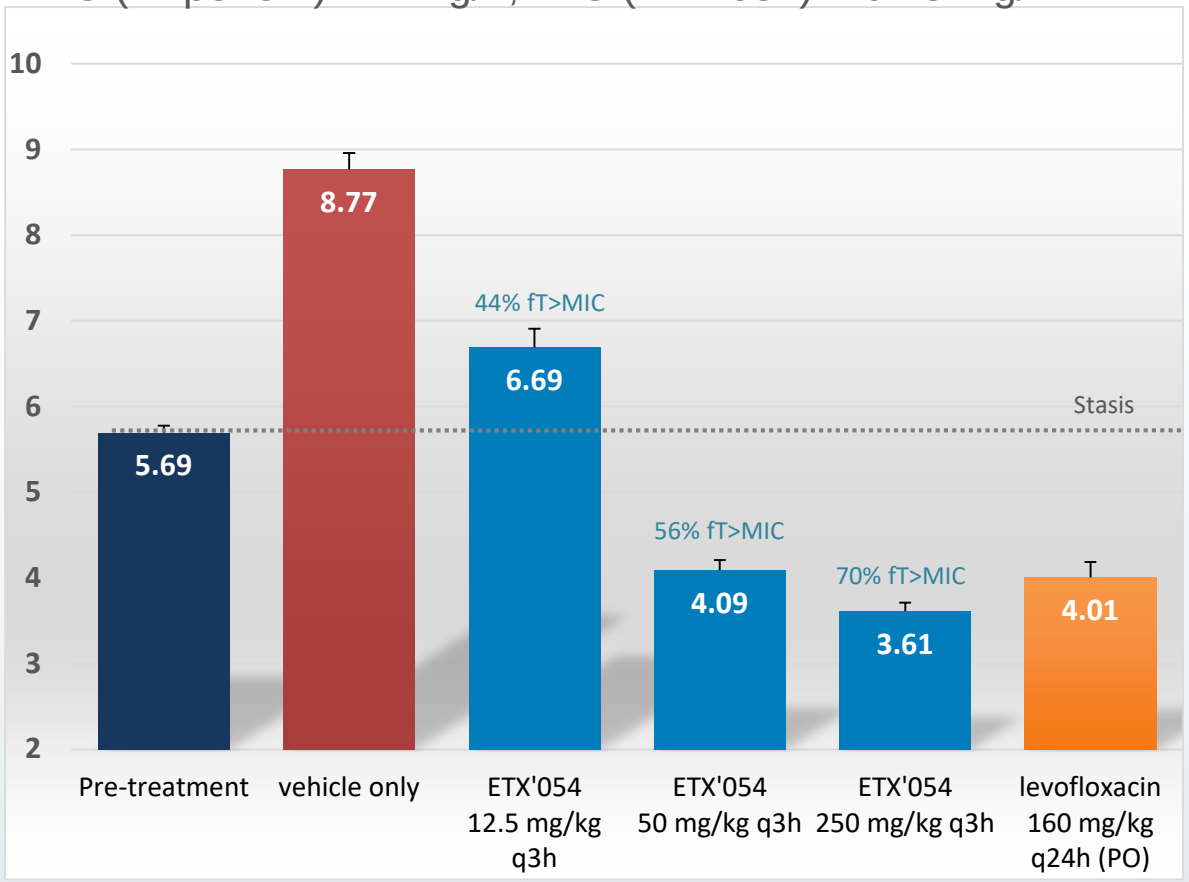
	MIC (mg/L) against *P. aeruginosa* isogenic strains expressing individual β-lactamases													
	parent	Class A				Class B			Class C		Class D				
Compound	PAO1	CTX-M-15	KPC-2	SHV-2a	TEM-1	NDM-1	VIM-1	VIM-2	AmpC	P99	OXA-10	OXA-23	OXA-40	OXA-48	OXA-58
piperacillin	**4**	**>256**	**>256**	**>256**	**>256**	**128**	**>256**	**128**	**256**	**128**	**>256**	**256**	**256**	**256**	**>256**
ceftazidime	**2**	**64**	**>64**	**32**	**4**	**>64**	**>64**	**>64**	**32**	**64**	**4**	**16**	**2**	**2**	**2**
imipenem	**1**	**1**	**32**	**1**	**1**	**16**	**64**	**64**	**1**	**1**	**1**	**16**	**32**	**64**	**8**
ETX'054	**4**	**4**	**8**	**4**	**4**	**8**	**4**	**4**	**8**	**8**	**8**	**4**	**4**	**8**	**4**

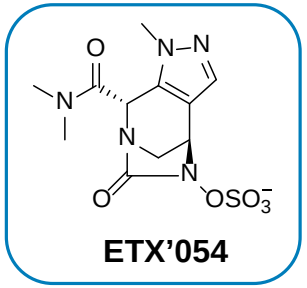
ETX’054 is Efficacious *in vivo* Against a *P.a.* Clinical Isolate

Murine neutropenic thigh infection model

MDR *P. aeruginosa* Clinical Isolate (ARC6347: OXA-486, PDC-24)

MIC (imipenem) > 4 mg/L, MIC (ETX’054) = 0.25 mg/L





ETX’054

- PK profile similar to other members of DBO class
- Robust activity achieved in neutropenic murine thigh efficacy model
- However, high MIC_{90} against recent *P.a.* clinical isolates required further investigation

Compound	N	Min	Max	MIC_{50}	MIC_{90}
ETX’054	599	0.12	>32	8	32
Imipenem	599	0.06	>32	2	32

- Outcome: High MIC driven by poor cell permeation!

Troubleshooting High MIC_{90} of ETX'054

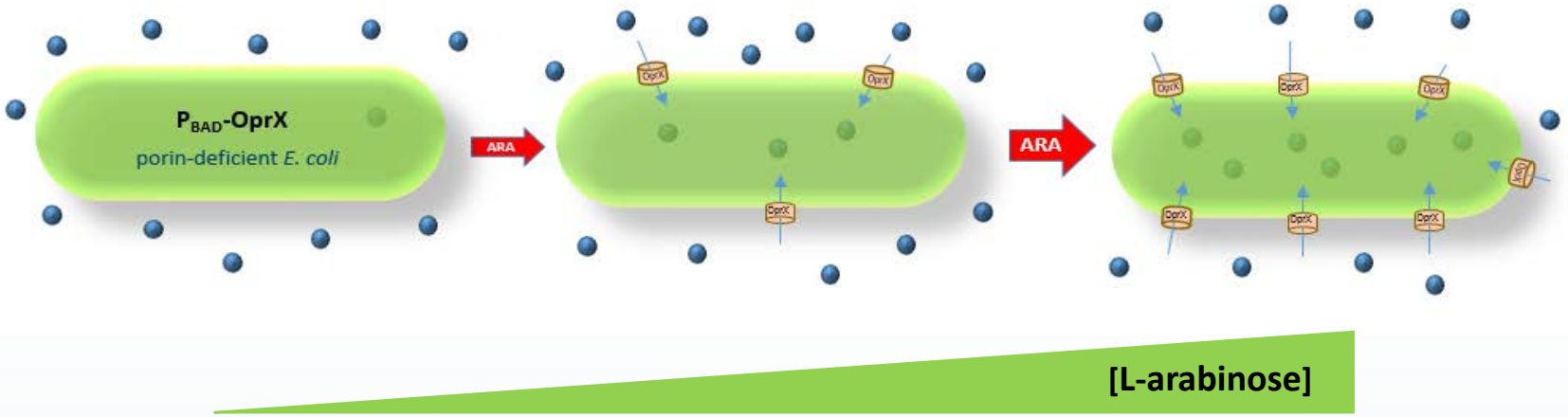
- Not influenced by binding to low MW PBPs
- Not influenced by AmpC regulation
- Not related to stringent response adaptation (like NXL-105)
- Not linked to inhibitor degradation (stability or enzyme-mediated)

- Confirmed both efflux and low permeation contribute to high MIC_{90}

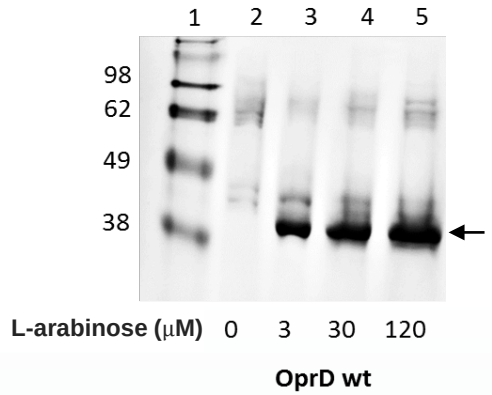
Potential Cause	Conclusion	Rationale
Primary target = PBP3	Not a liability	FOR is < 1 $x10^{-9}$; unable to isolate stable resistant mutants in lab strains, those raised in a less susceptible clinical isolate only increase by 2-4x and did not map to PBPs (mapped to frameshift mutations in aroB, involved in NADH metabolism)
Stringent response adaptation (similar to NXL-105)	Not a liability	NBPs are bactericidal and are not cross-resistant to $NXL\text{-}105^{R}$ mutants which map to stringent response genes
Compound degradation	Not a liability	- No evidence of β-lactamase-mediated degradation (panel of purified enzymes, isogenic bLA panel, clinical isolates) - High MICs do not change upon addition of BLI (ETX2514) - Does not occur due to changes in media pH during growth
Efflux	Likely liability	- 4x decrease in MIC for efflux-deficient strains are observed - TNseq analysis shows enrichment of mexR mutants - Correlation of high efflux phenotype and higher ETX'054 MICs in clinical isolates
Influx/Uptake	Likely liability	- High MICs in clinical isolates are reduced to a greater extent by addition of PMBN than in wt PA01 (rifampicin as comparator) - No porin-mediated uptake in TOMAS panel of porins - TNseq analysis did not enrich for any porin mutants, but did identify mutants in polysaccharide/capsule biosynthesis

Optimized Cell-based Porin Over-expression Assay

Incorporation of Uptake Assay in Screening Cascade: An Unprecedented Approach



[L-arabinose] α [porin] α permeability

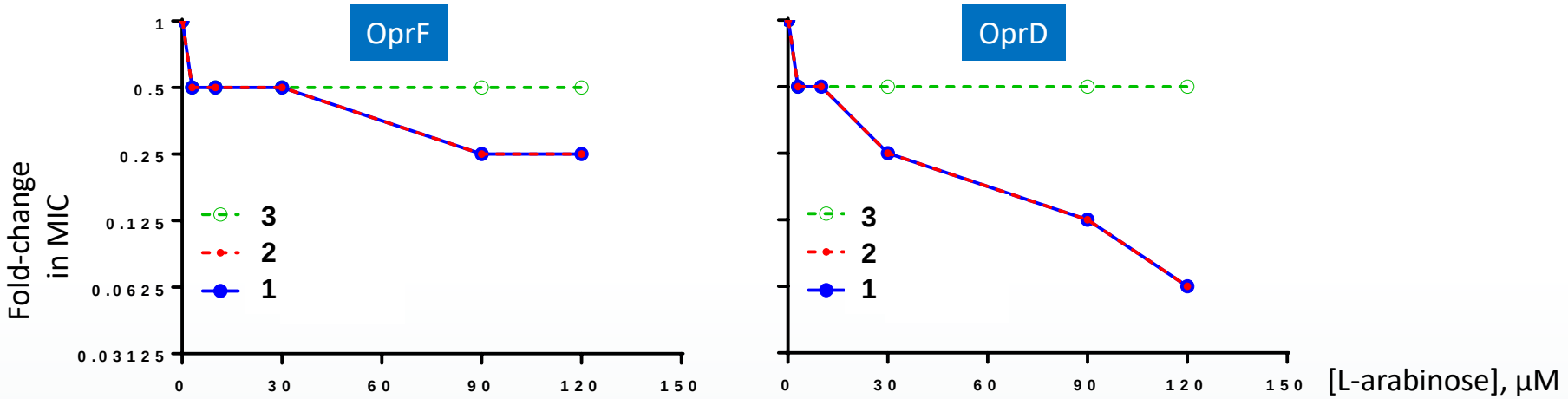


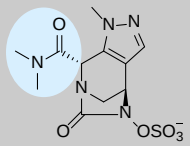
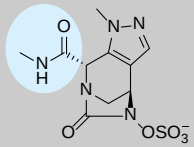
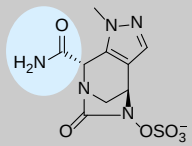
- Increasing porin concentration in the OM to increase OM permeability
- Tuning OM permeability with selective inducer in a controlled fashion
- Read out using fold-change in MIC, independent of activity level

- *E. coli* K-12 genetically modified for more sensitive and uniform response to inducer
- Deleted native porins (*ompF*, *ompC* and *ompA*)
- Deleted *tolC* to test porins with/without efflux
- Heterologous porin expression
  - 10 *P. aeruginosa*, 1 *A. baumannii* and 2 *K. pneumoniae* porins

Iyer R. *et al.*, ACS Infect. Dis. (2017) *3*, 310

Generating Structure-Porin Permeation Relationships (SPPR) to Improve Cellular Potency



Compound	3	2	1
Structure			
Porin permeation	none	++, multiple	+++, multiple
P.a. PBP3 $k_{(on)}$	582,000	610	230

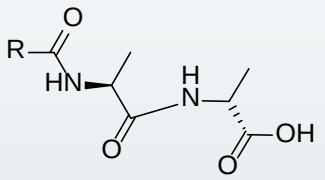
- While "Methyl Effect" for **ETX'054** provides ~1000-fold enhancement in PBP3 acylation,
- Porin permeation is abolished entirely across a set of porins with this substitution.
- Hydrogen bond donor is needed at R_1 to allow for permeation through various porins in *P. aeruginosa*
- **Lead Optimization efforts re-directed away from dimethyl amide.**

Structural Basis for Porin Permeation?

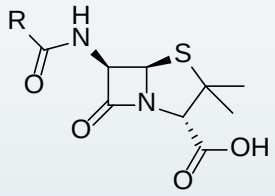
- We've used MD simulations in the past to explore structure basis of porin recognition
- Note the H-bonding on opposite side from the 'arginine wall'

- Our R1 analogs would be prime candidates for further analysis by MD simulation.
  - This is still a gap for us

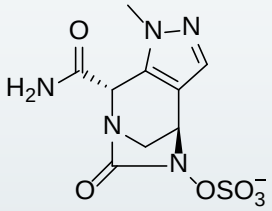
- One other consideration is that these compounds are dipeptide mimetics, recognition of H-bonding pattern not surprising



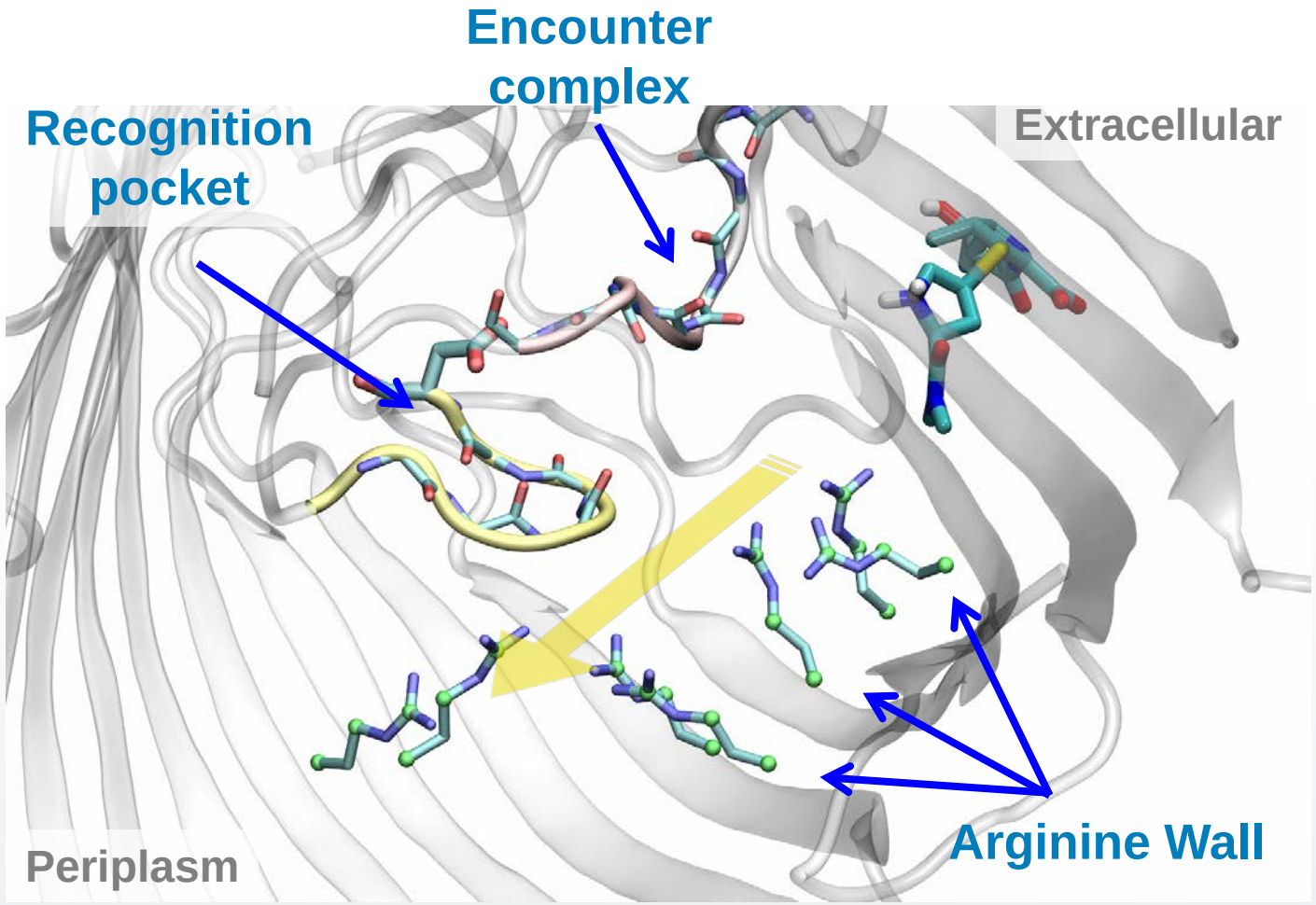
D-ala-D-ala terminus



Penicillin core



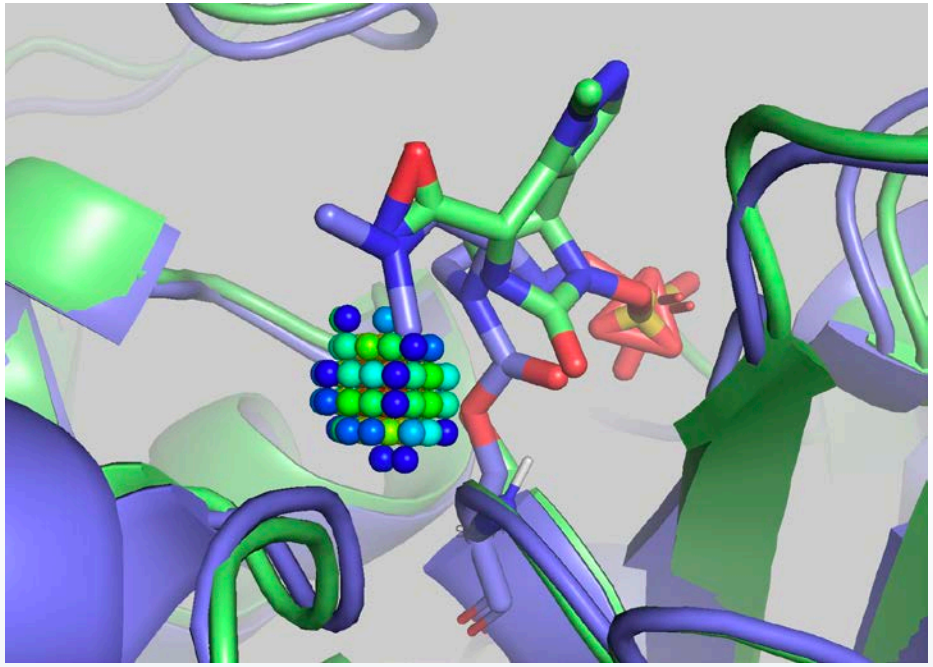
DBO



Meropenem trajectory through *P.a.* OprD, umbrella sampling

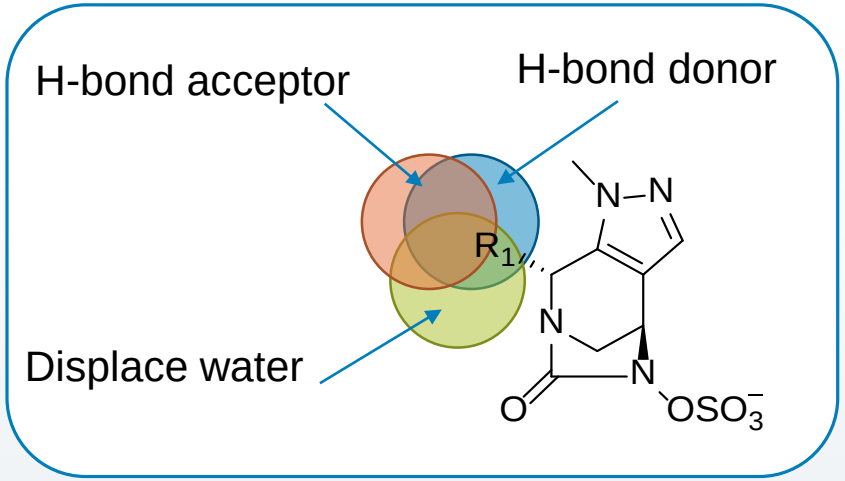
Revised Lead Optimization Strategy

Inclusion of pharmacophore requirements for uptake!



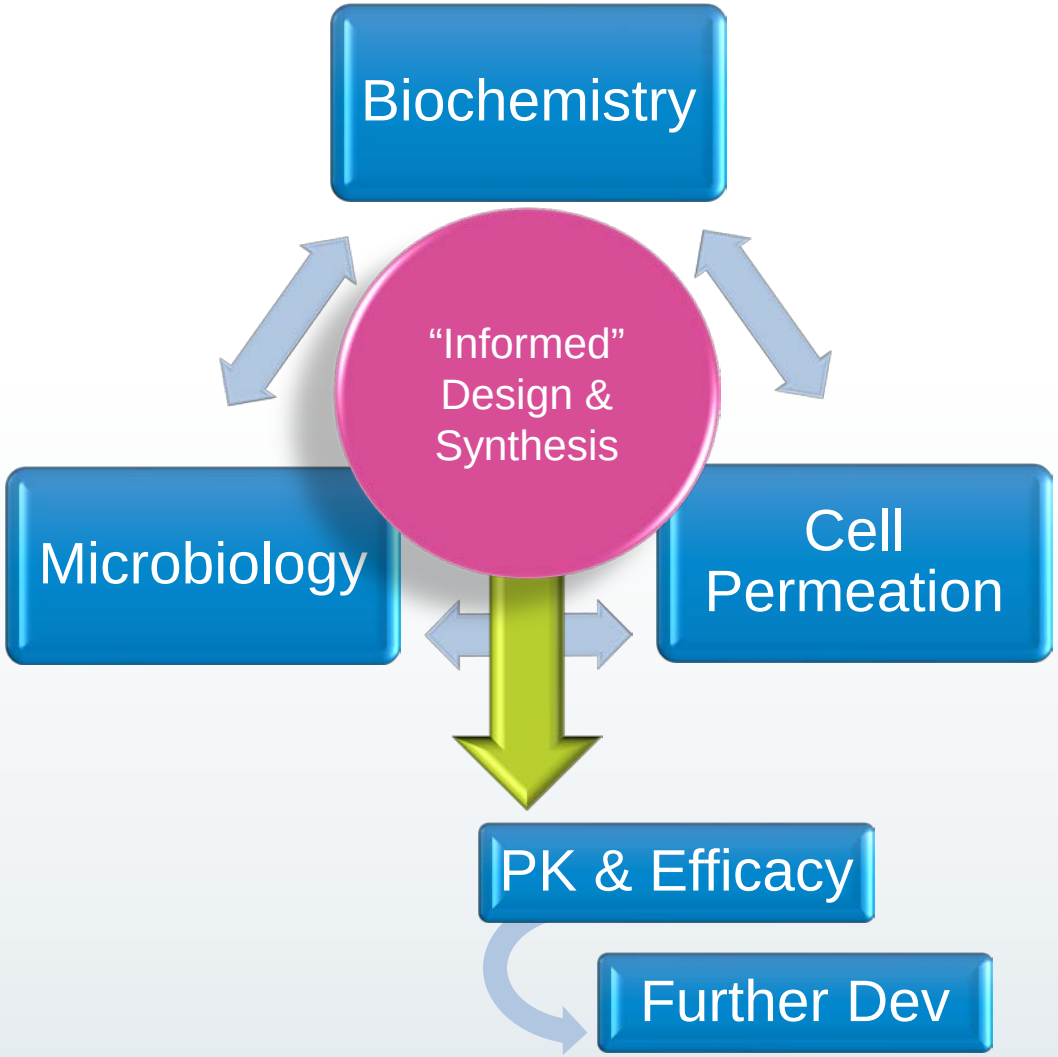
MD Simulations in P. aeruginosa PBP3

- R_1 Requirements:
  - Displacing water in pocket key to PBP3 potency
    - Room only for small group based on crystal structure
  - Hydrogen bond **acceptor** required to interact with ASN351
  - Hydrogen bond **donor** required for porin uptake



- Team identified novel substitutions at R_1 that meets all these criteria leading to candidate, ETX0462
  - *Manuscript with first disclosure in preparation*

‘Improved’ Antibiotic Drug Discovery Flowchart



- An integrated approach to optimization including target potency, microbiology and cell accumulation is recommended as early as possible in drug discovery project
  - This example illustrates that optimization path could be dead end for permeation (hence MIC)
  - Ideally, this would be without need for MIC
  - Exciting research in this area is continuing to evolve including mathematical models for permeation (Zgurskaya, Hergenrother, Ceccarelli, Winterhalter, others)
- Further efforts required to better understand structural requirements for porin uptake
- Subtlety of the ‘methyl effect’ for *P. aeruginosa* uptake via porins suggest that physicochemical rules-based approach will be very difficult.
- How often these subtle changes has affected hit to lead and lead optimization from HTS efforts is unknown!!

Conclusions and Future Directions

- Entasis is redefining antibacterial design by incorporating definition of the molecular drivers of compound uptake
  - *Unique multidisciplinary approach combining med chem, in vitro/in vivo biology and in silico tools*

- We discovered a novel class of non-β-lactam PBP3 inhibitors
  - Lead compounds maintain activity in the presence of all 4 classes of β-lactamases tested
  - Using structure-based drug design, selectivity shifted from PBP2 to PBP3/PBP1a
  - Translated into robust *in vivo* activity in neutropenic murine infection models
  - Uptake and efflux identified as features to increase potency against recent MDR *P. aeruginosa* clinical isolates
  - New candidate identified and manuscript is in preparation.

Acknowledgements

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- *Pharmaron*
- *JMI Laboratories*
- *Neosome Life Sciences*