

A combined systems and structural modeling repositions antibiotics for *Mycoplasma genitalium*

Denis Kazakiewicz^{1,2,*}, Jonathan R. Karr^{3,*}, Karol M. Langner^{4,5},
Dariusz Plewczynski^{6,7}

¹ Center for Statistics, Universiteit Hasselt, Hasselt BE3500, Belgium

² Center for Innovative Research, Medical University of Białystok, Białystok 15-089, Poland

³ Department of Genetics & Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York NY 10029, USA

⁴ Department of Molecular Physiology & Biological Physics, University of Virginia, Charlottesville VA 22908, USA

⁵ Current address: Google Inc., Mountain View CA 94043, USA

⁶ Centre of New Technologies, University of Warsaw, Warsaw 02-106, Poland

⁷ The Jackson Laboratory for Genomic Medicine, Farmington CT 06030, USA

* Authors contributed equally to this work

Prequel...

End-user's requirements¹ to the whole-cell model

- Extensive documentation, where both Biology and Simulation are clearly explained
- Explicit list of parameters that can be changed and separate list of hard-coded values
- Possibility to change parameters easily
- Script interface
- Computationally fast simulations using parts of the whole model

¹ Biased

A combined systems and structural modeling
repositions antibiotics for *Mycoplasma genitalium*

Novel antibiotics against *Mycoplasma genitalium* are needed

- It infects 1-3% of all individuals
- The second most common cause of non-gonococcal urethritis in men¹
- Increasingly common cause of cervicitis, endometritis, and pelvic inflammatory disease in women
- Traditional anti-Mycoplasma antibiotics: macrolides, fluoroquinolones, tetracyclines²
- Resistance as high as 40% in some regions³
- New anti-Mycoplasma targets and drugs are urgently needed

¹L E Manhart. "Mycoplasma genitalium: An emergent sexually transmitted disease?" In: *Infect. Dis. Clin. North Am.* 27.4 (2013), pp. 779–792

²D Taylor Robinson. "Diagnosis and antimicrobial treatment of *Mycoplasma genitalium* infection: sobering thoughts". In: *Expert Rev. Anti. Infect. Ther.* 12.6 (2014), pp. 715–722

³K Salado-Rasmussen and J S Jensen. "Mycoplasma genitalium testing pattern and macrolide resistance: a Danish nationwide retrospective survey". In: *Clin. Infect. Dis.* 59.1 (2014), pp. 24–30

Novel methods for drug development are needed

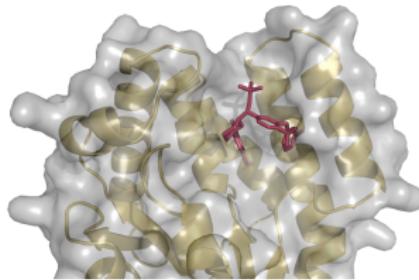
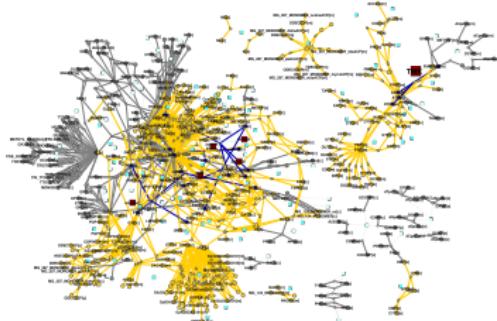
- Development rate of new molecular entities has declined by 20% ¹
- New computational methods are needed to identify potential repositioning candidates to increase the productivity of the drug development pipeline²

¹ I Khanna. "Drug discovery in pharmaceutical industry: productivity challenges and trends". In: *Drug Discov. Today* 17.19–20 (2012), pp. 1088–1102

² M C Rosales-Hernández and J Correa-Basurto. "The importance of employing computational resources for the automation of drug discovery". In: *Expert Opin. Drug Discov.* 15 (2015)

Systems modeling and molecular modeling for drug discovery

- Often in practice: Systems and structure 2 different realms
- Growing understanding of the value of combination

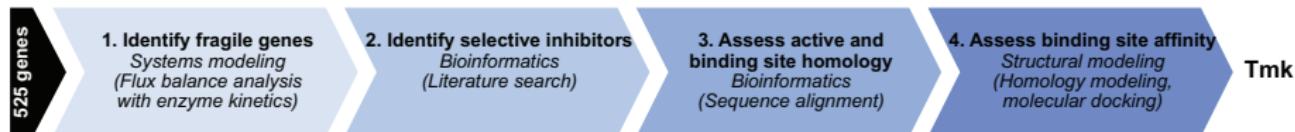


M Duran-Frigola, R Mosca, and P Aloy. "Structural systems pharmacology: the role of 3D structures in next-generation drug development". In: *Chem. Biol.* 20.5 (2013), pp. 674–684

Aims

- To identify potential new anti-Mycoplasma therapeutics by repositioning existing drugs from another species
- Combination of quantitative systems modeling, structural modeling, and bioinformatics
- Attempt to develop a drug repositioning approach, which is simple in implementation and useful in practice

Methods Summary



- Kinetically constrained FBA systems model to identify fragile metabolic enzymes
- Literature search for selective inhibitors of homologs of the top scoring enzymes
- Sequence alignment to verify that the inhibitor binding site is conserved among bacteria and not shared with humans
- Homology modeling and molecular docking to verify that the inhibitor binding site is conserved across bacteria and more energetically favorable than its human homolog

Implementation

- The model of the *M. genitalium* metabolism is a part of whole-cell model represents 146 of 525 genes, 104 enzymes, 568 metabolites, and 645 reactions including 57, 67, and 102 kinetically constrained enzymes, genes, and reactions, respectively
<https://simtk.org/home/wholecell>
- Find fragile nodes: apply perturbation to the system (partial inhibition)
- MODELLER, AutoDock Vina

Top list of potentially effective drug targets

Table: Predicted protein expression levels at which growth is 50% of that of the wild type strain (EC₅₀) for the most growth-sensitive proteins

Symbol	Name	Locus	EC ₅₀ (Rel)
Tmk	Thymidylate kinase	MG006	0.5000
MetK	S-adenosylmethionine synthetase	MG047	0.1771
AckA	Acetate kinase	MG357	0.0753

Piperidinylthymines inhibit Gram-positive bacterial Tmk

- Piperidinylthymines inhibit Gram-positive bacterial Tmk (*S. aureus*)¹
- *S. aureus*: Tmk inhibitor binding sites Arg53, Phe70, Arg74, Ser101, and Gln105²
- Piperidinylthymines exhibited activity in an in vivo mouse model of *S. aureus* MRSA252 infection³

¹ G Martínez-Botella et al. "Sulfonylpiperidines as novel, antibacterial inhibitors of Gram-positive thymidylate kinase (TMK)". In: *Bioorg. Med. Chem. Lett.* 23.1 (2013), pp. 169–173

² G Martínez-Botella et al. "Discovery of selective and potent inhibitors of Gram-positive bacterial thymidylate kinase (TMK)". In: *J. Med. Chem.* 55.22 (2012), pp. 10010–10021

³ T A Keating et al. "In vivo validation of thymidylate kinase (TMK) with a rationally designed, selective antibacterial compound". In: *ACS Chem. Biol.* 7.11 (2012), pp. 1866–1872

Tmk inhibitor binding sites are conserved between *M. genitalium* and *S. aureus*

<i>Mycoplasma genitalium</i> G-37	1 MN--KGVFVIEGVGDAGAKTALIEGFKKLYPTKFL--NYQLTYTREPG-GTLLAEKIRQLLN	R48
<i>Staphylococcus aureus</i> MRSA252	1 M---SAFITFEGPEGSGKTTVINE---VYHRLVK--DYDViMTREPG-GVPTGEEIRKIVLE	53
<i>Escherichia coli</i> K12	1 MR---SKYIVIEGLEGAGKTTARNV---VVETLEQLGIRDVFTREPG-GTQLAEKLRSVLID	56
<i>Homo sapiens</i>	1 MAARRGALIVLEGVDRAKGSTQSRK---LVEALCAA-GHRAELLRFPERSTEIGKLSSYLQK	59
	F66 R70	
<i>Mycoplasma genitalium</i> G-37	59 E-----TMEPLTEAYLFAAARTEHISKLIKPAIEKEQLVISDRFVFSEFAYOGLSKKIGIDI	115
<i>Staphylococcus aureus</i> MRSA252	54 GN-----DMDIRTEAMLFAASRREHLVLKVIPALKEGKVVLCRDYIDSSLAYOGYARGIGVEE	111
<i>Escherichia coli</i> K12	57 IKSGVDEVITDKAEVLMFYAARVQLVETVIKPALANGTWIGDRHDLSIQAQYGGGRGIQDHM	119
<i>Homo sapiens</i>	60 KS-----DVEDHSVHLLESANRWEQVP-LIKEKLSQGVTLVVDRYAFSVAFTGAKENFSLDW	116
	S97 Q101	
<i>Mycoplasma genitalium</i> G-37	116 VQKINHHALRNMMNPNTFILDCNFKEALQRQMKGNDNLLDEFIKGKNDFTVRSYLLSDV	177
<i>Staphylococcus aureus</i> MRSA252	112 VRALNEFAINGLYPDLTITYLNVSAEVGRERIIKNSRDQNRLDQ-EDLK-FHEKVIEGYQEIIH	172
<i>Escherichia coli</i> K12	120 LATLRDAVLGDFRPDLTLYLDVTPEVGLKRARARG-ELDRIEQ-ESFD-FFNRTRARYLELAA	179
<i>Homo sapiens</i>	117 CKQP---DVGLPKPDVLFLQLQLADAARK---G-AFGHRY-ENGA-FQERALRCFHQLMK	169
	178	
<i>Mycoplasma genitalium</i> G-37	-KK-NCFLINGDNKQEHLEKFIE-LL-----TRCLQQPTHY	210
<i>Staphylococcus aureus</i> MRSA252	173 NESQRFKSVNADQPLEVNVEDTYQT-----IKYLEK---I	205
<i>Escherichia coli</i> K12	180 -QDKSIHTIDATQPLEAVMDAIRTT-----THWVKEL-DA	213
<i>Homo sapiens</i>	170 DTTLNWKMVDAKSIEAVHEDIRVLSEDAIRTATEKPLGEL-WK	212

Figure: Multiple sequence alignment of *M. genitalium*, *S. aureus*, *E. coli*, and *H. sapiens* Tmk shows that the reported inhibitor binding site (highlighted residues numbering according to *S. aureus* Tmk; is conserved among bacteria and divergent from *H. sapiens*.

Homology modeling. *M. genitalim* and *S. aureus*. Similar active site

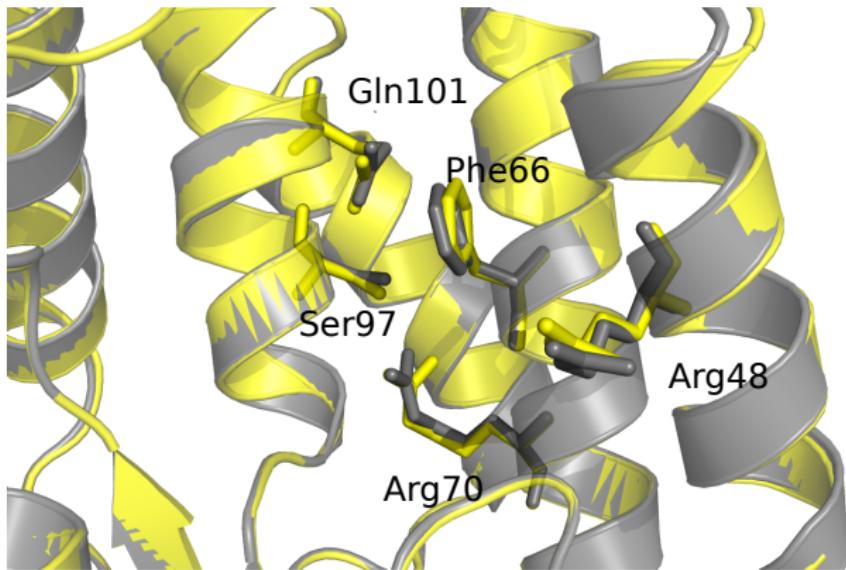


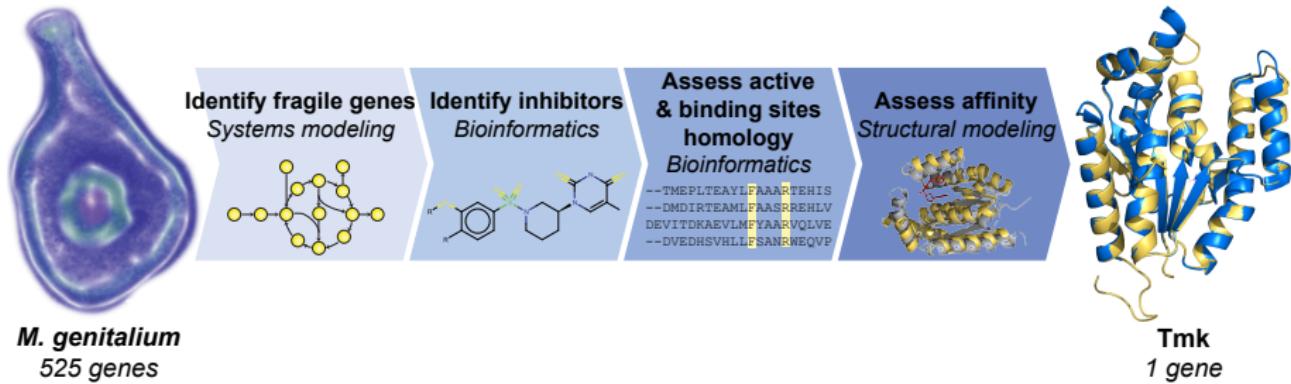
Figure: Superimposed structures of *M. genitalium* (yellow) and *S. aureus* (grey; PDB ID: 4QGG) Tmk shows that *M. genitalium* Tmk shares the same piperidinylthymine binding site; residues labeled according to *S. aureus*).

Table: Predicted interaction energies of seven piperidinylthymine compounds with *M. genitalium*, *S. aureus* and *H. sapiens* Tmk.

PDB Ligand ID	Predicted interaction energy (kcal mol ⁻¹)		
	<i>M. genitalium</i>	<i>S. aureus</i>	<i>H. sapiens</i>
32E	-12.2	-10.8	-10.4
32K	-14.8	-12.4	-13.0
0YB	-12.1	-11.3	-10.6
31Z	-12.2	-10.9	-11.1
T05	-12.2	-11.1	-11.2
16T	-11.8	-11.1	-10.9
13Y	-11.7	-10.3	-10.9

Conclusions

- Systems modeling combined with structural modeling and bioinformatics can be a powerful drug discovery approach
- Other researchers have shown, this combined strategy can be used to discover new targets and lead compounds. In addition, as we have demonstrated, it can also be used to reposition established drugs to new species.
- We extrapolated the effect of piperidinylthymines from *S. aureus* to the distantly related *M. genitalium*
- We envision that this repositioning approach could also be used to repurpose anticancer treatments between cancers
- Looking forward, we believe that broader systems models, will facilitate de novo target and drug discovery



Scripts and files

<https://simtk.org/home/wholecell>

Results in *Comput Biol Chem* pii, S1476-9271(15)30089-X (2015)

Corresponding author: Jonathan Karr karr@mssm.edu

Acknowledgements

Paweł Kafarski