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RESEARCH ARTICLE

REVISED Lovastatin lactone may improve irritable bowel syndrome with constipation (IBS-C) by inhibiting enzymes in the archaeal methanogenesis pathway [version 2; peer review: 1 approved, 2 approved with reservations]

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Abstract

Methane produced by the methanoarchaeon *Methanobrevibacter smithii* (*M. smithii*) has been linked to constipation, irritable bowel syndrome with constipation (IBS-C), and obesity. Lovastatin, which demonstrates a cholesterol-lowering effect by the inhibition of HMG-CoA reductase, may also have an anti-methanogenesis effect through direct inhibition of enzymes in the archaeal methanogenesis pathway. We conducted protein-ligand docking experiments to evaluate this possibility. Results are consistent with recent clinical findings.

METHODS: F420-dependent methylenetetrahydromethanopterin dehydrogenase (*mtd*), a key methanogenesis enzyme was modeled for two different methanogenic archaea: *M. smithii* and *Methanopyrus kandleri*. Once protein models were developed, ligand-binding sites were identified. Multiple ligands and their respective protonation, isomeric and tautomeric representations were docked into each site, including F420-coenzyme (natural ligand), lactone and β -hydroxyacid forms of lovastatin and simvastatin, and other co-complexed ligands found in related crystal structures.

RESULTS: 1) Generally, for each modeled site the lactone form of the statins had more favorable site interactions compared to F420; 2) The statin lactone forms generally had the most favorable docking scores, even relative to the native template PDB ligands; and 3) The statin β -hydroxyacid forms had less favorable docking scores, typically scoring

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in the middle with some of the F420 tautomeric forms. Consistent with these computational results were those from a recent phase II clinical trial (NCT02495623) with a proprietary, modified-release lovastatin-lactone (SYN-010) in patients with IBS-C, which showed a reduction in symptoms and breath methane levels, compared to placebo.

CONCLUSION: The lactone form of lovastatin exhibits preferential binding over the native-F420 coenzyme ligand *in silico* and thus could inhibit the activity of the key *M. smithii* methanogenesis enzyme *mtd* *in vivo*. Statin lactones may thus exert a methane-reducing effect that is distinct from cholesterol lowering activity, which requires HMGR inhibition by statin β -hydroxyacid forms.

Keywords

IBS , IBS-C , Lovastatin , homology modeling , multi-site docking

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REVISED Amendments from Version 1

Typographical errors in the abstract and introduction sections fixed. Mention of other considered templates in the modeling of the A5UMI1 sequence described in the Protein modeling and site identification section.

See referee reports

Introduction

Irritable bowel syndrome (IBS) affects as many as 45 million people in the United States, and up to 23% of the worldwide population¹. Depending on the region, as many as 43.3% of these patients will have irritable bowel syndrome with constipation (IBS-C)². The illness affects both men and women; however, two-thirds of diagnosed sufferers are women. Studies have linked methane production to the pathogenesis of constipation and IBS, as well as obesity³. Methanogens – i.e. anaerobes that respire hydrogen to produce methane – are found in many habitats supporting anaerobic biodegradation of organic compounds, including human and animal intestinal tracts^{4,5}. Archaea are the only confirmed, naturally occurring biological sources of methane. *Methanobrevibacter smithii* (*M. smithii*) is the predominant methanogen in the human intestine accounting for 94% of the methanogen population³.

The isoprenoid biosynthesis for the main cell membrane components in archaea (archaeol) relies on the same enzyme that catalyzes the biosynthesis of the isoprenoid cholesterol in humans – HMG-CoA reductase (mevalonate pathway)⁶. It has been previously suggested that statins, i.e. known HMG-CoA reductase inhibitors, can also interfere with the biosynthesis of the archaeal cell membrane and thus inhibit archaeal growth⁷. Statins, specifically lovastatin, have been shown to lower methanogenesis in human stool samples⁸ and can inhibit archaeal cell membrane biosynthesis without affecting bacterial numbers as demonstrated in livestock and humans. Lovastatin is a secondary metabolite produced during fungal growth and is found in oyster mushrooms⁹, red yeast rice¹⁰, and Pu-erh¹¹.

Humans and archaea utilize the HMGR-I isoform for isoprenoid biosynthesis¹². Mevastatin and lovastatin were both shown to inhibit growth of several rumen *Methanobrevibacter* isolates in the ~10 nmol/ml range³. While it is believed that statins inhibit methane production via their effect on cell membrane biosynthesis mediated by inhibition of HMG-CoA reductase, there is accumulating evidence for an alternative or additional mechanism of action where statins inhibit methanogenesis directly¹³. In one case, *in silico* molecular docking of the methanogenic enzyme F420-dependent NADP oxidoreductase (*fno*) showed that both lovastatin and mevastatin had higher affinities for the F420 binding site on *fno* than did F420 itself. It has been suggested that lovastatin may act as an inhibitor of *fno*¹⁴.

Several reviews have appeared describing the reduction of CO₂ to CH₄ in methanoarchaea¹⁵. Considering other mechanisms by which statins may inhibit methanogenesis directly, we have explored two important dehydrogenases in the main methanogenesis pathway, including F420-dependent methylenetetrahydromethanopterin dehydrogenase of *M. smithii* [A5UMI1- 275 amino acid residues],

and evolutionarily related F420-dependent methylenetetrahydromethanopterin (methylene-H(4)MPT) dehydrogenase (*mtd*) of *Methanopyrus kandleri* [Q02394 – 358 amino acid residues]. Both only leverage F420 as a coenzyme, which assisted our computational analyses by avoiding issues associated with an NADP induced fit. The Q02394 sequence does not have crystallographic structural information in the Protein Data Bank (PDB)¹⁶, so we needed to identify acceptable templates to model this sequence. The A5UMI1 sequence, however matched the 3IQZ co-complex with methylenetetrahydromethanopterin (H4M) having 52% sequence homology. While both sequences required modeling, we needed to identify one or more acceptable templates for Q02394. After modeling and receptor site identification, we docked and rank-ordered multiple ligand variations across several modeled receptor sites to evaluate preferential binding characteristics for the ligands in question.

Methods

Protein sequences were extracted from UniProt¹⁷. Many protein structure modeling methods have been developed and are available with most performing well given crystallographic template(s) sharing sufficient sequence homology with target sequences of interest¹⁸. The Eidogen StructFast^{19,20} technology is well suited for this type of modeling. StructFast can operate in an automated mode where the best PDB template is automatically selected, or in a directed mode where modeling is guided based on a suggested PDB template^{21,22}.

Once models for A5UMI1 and Q02394 were developed with StructFast, ligand binding sites were identified by inference from the respective PDB templates used in modeling and from the Eidogen SiteSeeker algorithm²³. SiteSeeker looks for concave, surface features sufficiently exposed to enable ligand binding while also considering evolutionary conservation of sequence. In addition to sites identified by SiteSeeker, other sites were manually inferred within PyMOL v1.8 after aligning models and templates containing their respective co-complexed ligands. Residues on model structures with a 7Å cutoff of co-complexed ligands within the templates were exported and also processed as sites.

Ligands were carefully prepared considering different protonation states, isomers, and tautomers. We standardized charges, added missing hydrogens, enumerated ionization states, ionized functional groups, generated tautomers and isomers, and generated starting-point 3D coordinates for each ligand using BIOVIA's (Accelrys') Pipeline Pilot technology v8.5²⁴. Ligands were finally prepared into mol2 format²⁵. Each representation was then docked into each identified site and scored using AutoDock Vina v1.1.2²⁶, an open docking technology that utilizes grid-based energy evaluation and efficient search of ligand torsional freedom.

The AutoDock Vina system requires that receptor site files be formatted in the PDBQT [Protein Data Bank, Partial Charge (Q), & Atom Type (T)] molecular structure file format. The MGLTools v1.5.4²⁷ were used for this file format conversion. Additionally, AutoDock Vina requires a defined grid box surrounding the receptor site residues. Here, we identified the center of mass of each receptor site using all atoms in the receptor site PDB file. We then calculated within Pipeline Pilot the maximum distance between any

atom in the receptor site and the centroid in each x,y,z-direction. The lengths of each grid box were configured with these maximums. To insure reproducibility and comparability of docking simulations, we initiated each AutoDock Vina run with the same random seed value of 1162467901.

Results and discussion

Dataset 1. Raw data for ‘Lovastatin lactone may improve irritable bowel syndrome with constipation (IBS-C) by inhibiting enzymes in the archaeal methanogenesis pathway’

<http://dx.doi.org/10.5256/f1000research.8406.d117917>

Includes developed models (pdb format), ligands (mol2 format), sites (pdb format), and vina config files

Dataset 2. Lovastatin-lactone v. F420 in the A5UMI1 site

1 Data File

<http://dx.doi.org/10.6084/m9.figshare.3126538>

Protein modeling and site identification

We identified three different PDB templates that had sufficient sequence homology to model the **Q02394** sequence, by identifying other PDB co-complexes containing ligands with high 2D similarity to the H4M ligand. The top three PDBs showing significant sequence homology to **Q02394** included: 3F47 (57%), 3H65 (57%), and 4JJF (52%). Each template was used to model **Q02394**. In each case, ligand-binding sites were readily inferred from the ligand binding sites found in the respective template structures.

The modeling of sequence **A5UMI1** was straightforward given its high 52% sequence homology to 3IQZ. Other templates (e.g. 1U6I, 1U6J, 1U6K, and 1QV9) were possibilities, but each had slightly lower resolutions, earlier deposit dates, and/or were in apo form. Each 3IQZ chain (A-F) was considered, given the possibility that one template-chain might offer additional or different insight into possible ligand binding locations. The Eidogen SiteSeeker algorithm identified only one site when template chains A, C, D were used, while two sites were identified in models leveraging template chains B, E, F. Unfortunately, the H4M site from the 3IQZ template was not easily inferred into any of the **A5UMI1**/3IQZ-based models, because 3IQZ has multiple chains involved in H4M binding.

Modeling sequences from PDB templates is done with individual chains. Quaternary modeling using models of individual chains can be very challenging. We manually modeled the H4M site as described in **Figure 1**. Since our aim was to dock all ligands across all possible ligand binding sites, we included the sites identified by inference (i.e. where ligands were present in templates), by the SiteSeeker algorithm run across single chain models, and by manually modeled sites as described by **Figure 1**. A total of 10 ligand-binding sites (**Table 1**) were identified across all the **Q02394** and **A5UMI1** models.

Ligand processing

The key ligands for this effort included lovastatin (lactone and hydroxyacid forms), F420, and simvastatin (lactone and

Figure 1. Modeled quaternary structure of **A5UMI1**/3IQZB (cyan) and **A5UMI1**/3IQZF (pink) after respective alignments onto chain-B and chain-F of 3IQZ within PyMOL²⁶. 3IQZ's chain-F is highlighted in silver. Dual chain model site residues (blue surface) were inferred from residues in chain-B and chain-F models that are within 7 Å of the 3IQZ ligand (H4M - white). 3IQZ's chain-B and chain-F form a quaternary structure with two different H4M binding sites (bottom).

Table 1. Ligand binding sites identified and inferred from models. Four sites from the **A5UMI1** modeling and six sites from **Q02394** modeling were used in the docking simulations.

A5UMI1	Q02394
3IQZB (H4M 7Å), 1 chain	3F47 (I2C)
3IQZB (SiteSeeker1)	3F47 (SiteSeeker)
3IQZB (SiteSeeker2)	3H65 (H4M)
3IQZB (H4M)_3IQZF (7Å)	3H65 (I2C)
	4JJF (FE9)
	4JJF (SiteSeeker)

hydroxyacid forms) and processed ligands that were found in the PDB templates used to model each sequence: 803, F42, H4M, I2C, FE9, SIM, 116, HMG, and 882. The latter four (SIM, 116, HMG, 882) were ligands found in the positive control templates for completeness.

The PDB often contains problematic ligand structures, so we processed both PDB ligands and ligands extracted from PubChem²⁹ for lovastatin (lactone and hydroxyacid forms), F420, and simvastatin (lactone and hydroxyacid forms). It should be noted, the PDB considers 803 as lovastatin (lactone form), F42 as coenzyme-F420, and SIM as simvastatin (hydroxyacid form), though their actual structural forms may vary depending on the PDB entry. This is why we also use PubChem structural representations for lovastatin, F420, and simvastatin.

It is well established that the β -hydroxyacid form and not the closed-ring lactone form of lovastatin is the active HMGR-binding form of the molecule³⁰. Simvastatin and lovastatin are commercially available in the lactone form; they behave as prodrugs which inhibit HMGR only after the opening of the lactone ring into the hydroxyacid form^{31,32}. The degree of hydrophobicity of imidazole derivatives correlates with improved activity against human methanogenic archaea³³.

Each ligand was computationally processed in the same way prior to docking. BIOVIA's (Accelrys') Pipeline Pilot was used for this ligand preparation. First, stereochemistry and charges were standardized, then ionized at pH 7.4, then tautomers (if present) were enumerated, and finally initial 3D models were determined. AutoDock Vina explores ligand 3D conformation, so the initial 3D models were simple starting points. Additionally, ligands were processed without the above standardization, ionization, and tautomer exploration. Each ligand representation was considered in the docking runs. Ligands processed with the standardization sequenced contained the prefix "STD_" and ligands without standardization contained the prefix "RAW_". Together, these expanded ligand representations can help gauge the docking algorithm's sensitivity to the ligand's structural representation.

Docking multi-ligand variations/multi-receptor sites

A total of 88 ligand variations were systematically docked into the 10 identified binding sites across all the **A5UMI1** and **Q02394** models for a total of 880 docking simulations. Even though AutoDock Vina achieves two orders of magnitude speed-up and significantly improves the accuracy of the binding mode predictions compared to AutoDock 4, 880 docking simulations could have taken several weeks to complete. To accelerate the effort, we requisitioned a compute cluster in the Amazon EC2³⁴ cloud environment for approximately three days at a cost under \$60.

The docking process scores ligand conformations based on ligand conformation and ligand-to-receptor interactions within a grid box. After the 880 docking simulations were complete, we rescored all docked ligand variations against their respective full model structures. This enabled a more realistic rank ordering given possible

overlap with a docked ligand and other portions of a model not represented in the rectangular box. This also served as an internal control, since rescoring was completed independently of the docking simulations.

Since it is unknown which (if any site) might actually engage the ligands of interest, we calculated the average, minimum, and maximum affinity of each ligand/variation for each of the 10 sites. The top-two sites (highlighted in bold in Table 2) were used to then rank order each ligand. Table 3 shows the rank ordered ligands using the AutoDock Vina overall score, which considers steric interactions (Gauss 1, Gauss 2, and steric), dispersion/repulsion, hydrophobic interaction between hydrophobic atoms, and, where applicable, hydrogen bonding.

Given the rank ordering in Table 3, several observations became evident:

- 1) Consistent with Sharma *et al.*¹⁴, the lactone form statins docked into each site with favorable site interactions (i.e. lower docking scores) as compared to F420 for the same sequence/site grouping.
- 2) The statin lactone forms generally had more favorable docking scores, even relative to the native template PDB ligands.
- 3) The statin hydroxyacid forms had less favorable docking scores and typically scored in the middle with some of the F420 forms.
- 4) The F420 scores were generally the lowest for each sequence/site models of **A5UMI1** and **Q02394**.

Table 4 (a,b) details the AutoDock Vina scoring metrics of lovastatin-lactone v. lovastatin-hydroxyacid across the top two modeled sites. The lovastatin lactone form had better AutoDock scores across each site as compared to the hydroxyacid form. Similarly, the calculated affinity (kcal/mol) of the lactone form was better within both modeled **A5UMI1** sites. Figure 2 depicts the best scoring lovastatin-lactone and -hydroxyacid poses in the **A5UMI1** modeled site (top) and the **Q02394** modeled site (bottom). The **A5UMI1** modeled site is more spherically form fitting while the **Q02394** modeled site is more elongated. The **A5UMI1** site also contains a greater concentration of hydrophilic residues (depicted in cyan in Figure 2). In each modeled site, the best scoring lactone and hydroxyacid form were docked roughly in the same position with similar interactions, however the lactone form contained more favorable intermolecular feature.

Figure 3 depicts lovastatin-lactone (top) v. F420 (bottom) docked into the top **A5UMI1** modeled site (see Dataset 2 helps to visualize and perceive additional detail depicted). Lovastatin-lactone had better AutoDock scores and more favorable calculated affinities – despite having fewer hydrogen bond interactions. Both ligands appear to be interacting with ARG-255, ARG-150, and GLN-153, though F420 seems to also interact with ARG-244. F420's fit is also considerably more constrained, which explains why its AutoDock Vina score is 4.4x worse than lovastatin-lactone's score.

Table 2. Average, minimum, and maximum affinity for each site. Affinities were computed from AutoDock Vina²⁶. The top two scoring sites from **A5UMI1** and **Q02394** are in bold. These sites were used to rank the ligands in Table 3.

Docking Site	Average Affinity (kcal/mol)	Min Affinity (kcal/mol)	Max Affinity (kcal/mol)
A5UMI1_3IQZB (SiteSeeker2)	-7.87	-9.01	-6.08
Q02394_4JJF (SiteSeeker)	-7.03	-9.07	3.88
Q02394_3F47 (I2C)	-6.34	-9.34	24.92
A5UMI1_3IQZB (H4M)_3IQZF (7Å)	-5.57	-8.56	6.29
Q02394_4JJF (FE9)	-0.22	-9.25	247.57
A5UMI1_3IQZB (H4M 7Å), 1 chain	0.18	-7.50	320.44
Q02394_3H65 (I2C)	0.80	-9.52	249.56
Q02394_3H65 (H4M)	3.90	-6.19	125.33
Q02394_3F47 (SiteSeeker)	168.88	-6.21	277.08
A5UMI1_3IQZB (SiteSeeker1)	214.07	-7.42	355.49

Table 3. Average AutoDock Vina scores over the top-two sites (see Table 2). Statin ligands highlighted in green are lactone form, or red if hydroxyacid form. F420 ligands are in blue. Tautomeric representations are included in each average. Standardized ligands are prefixed with "STD_," those without standardization are prefixed with "RAW_" (see text). Ligand names have suffixes containing either the PDB entry they were originally extracted from, or their respective PubChem²⁹ CIDs.

Ligand	Average AutoDock Vina Score A5UMI1_3I QZB (SiteSeeker2) + Q02394_4 JJF (SiteSeeker)
RAW_803_1cqp	13.86
STD_803_1cqp	13.86
RAW_Simvastatin_pubchem_54454	14.34
STD_Simvastatin_pubchem_54454	14.34
RAW_Lovastatin_pubchem_53232	14.42
STD_Lovastatin_pubchem_53232	14.42
RAW_FE9_4jifA	16.31
RAW_FE9_4yt4A	19.91
RAW_I2C_3f47A	22.35
STD_F42_3iqe	26.34
RAW_F42_3iqe	26.99
STD_SimvastatinAcid_pubchem_64718	27.23
STD_SIM_1hw9	27.66
STD_LovastatinAcid_pubchem_64727	27.92
RAW_SimvastatinAcid_pubchem_64718	29.52
RAW_LovastatinAcid_pubchem_64727	29.89
RAW_SIM_1hw9	30.68

Ligand	Average AutoDock Vina Score A5UMI1_3I QZB (SiteSeeker2) + Q02394_4 JJF (SiteSeeker)
STD_882_2q1l	33.31
RAW_882_2q1l	33.87
STD_116_1hwj	34.04
STD_H4M_1y60	39.54
RAW_116_1hwj	39.92
RAW_H4M_1y60	40.86
RAW_F42_3b4yA	54.70
STD_F420_pubchem_123996	56.39
RAW_H4M_3h65A_1607662	58.46
STD_F42_4qvb	61.33
RAW_F42_4qvb	62.72
RAW_F420_pubchem_123996	64.00
STD_F420_pubchem_123996	64.00
RAW_F42_1jayA	67.77
STD_F420_pubchem_123996	69.50
STD_H4M_3h65A	72.91
STD_HMG_1dq9	107.17
STD_FE9_4jifA	111.50
STD_FE9_4yt4A	286.45
STD_F42_3b4yA	571.39
STD_F42_1jayA	835.97
RAW_HMG_1dq9	2671.39
STD_I2C_3f47A	12109.50

Figure 3. Lovastatin-lactone 1: (top) [Calculated affinity: -7.2 (kcal/mol); AutoDock4.1Score: 14.3]; 2: (bottom) F420 [Calculated affinity: -6.99 (kcal/mol); AutoDock4.1Score: 63.3] docked into A5UMI1_3IQZB_SiteSeeker2. Hydrogen bond interactions are denoted with yellow dotted lines.

Conclusions

Given the large number of ligand-to-site docking scenarios, we were able to observe several key trends that together suggest that statin binding is likely for the two key targets in question **A5UMI1** and **Q02394**. In most cases, the lactone form appears to have preferential binding over the hydroxyacid form and F420. And in many cases, lovastatin/lactone and simvastatin/lactone appear to have preferential binding to even the native ligands found in the PDB templates used to model **Q02394** and **A5UMI1**.

The docking simulations are consistent with those from a recent phase II clinical trial ([NCT02495623](#)³⁵) with a proprietary, modified-release lovastatin-lactone (SYN-010) in patients with constipation-predominant, irritable bowel syndrome, which showed a reduction

in symptoms and breath methane levels compared to placebo. Given that the lactone form seems to preferentially bind, the next stage of the project is to identify molecules with similar features to lovastatin-lactone that also show similar or better receptor-site interaction potential.

Data availability

F1000Research: Dataset 1. Raw data for 'Lovastatin lactone may improve irritable bowel syndrome with constipation (IBS-C) by inhibiting enzymes in the archaeal methanogenesis pathway', [10.5256/f1000research.8406.d117917](#)³⁶

Figshare: Lovastatin-lactone v. F420 in the A5UMI1 site. doi: [10.6084/m9.figshare.3126538](#)³⁷

Author contributions

SM: Finalized analysis workflow, suggested additional hypotheses, performed the experiments, and wrote first and subsequent drafts of the manuscript. JS: Provided many helpful suggestions during the manuscript preparation. JK: Made many helpful suggestions during the initial planning and during manuscript review. MP: Provided insights into methane microbiology and reviewed all drafts. VW: Co-developed the research question and design of experiments and reviewed all drafts. KG: Originated

the research idea, the major hypotheses and a draft analysis plan and reviewed all manuscript drafts. All authors approved the final manuscript.

Competing interests

No competing interests were disclosed.

Grant information

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References

1. **Facts About IBS.**
[Reference Source](#)
2. Sperber AD, Dumitrascu D, Fukudo S, *et al.*: **The global prevalence of IBS in adults remains elusive due to the heterogeneity of studies: a Rome Foundation working team literature review.** *Gut*. 2016; pii: gutjnl-2015-311240.
[PubMed Abstract](#) | [Publisher Full Text](#)
3. Pimentel M, Gonsalves RP, Rao SSC, *et al.*: **Methanogens in Human Health and Disease.** *Am J Gastroenterol Suppl*. 2012; 1(1): 28–33.
[Publisher Full Text](#)
4. Zhang H, Banaszak JE, Parameswaran P, *et al.*: **Focused-Pulsed sludge pre-treatment increases the bacterial diversity and relative abundance of acetoclastic methanogens in a full-scale anaerobic digester.** *Water Res*. 2009; 43(18): 4517–26.
[PubMed Abstract](#) | [Publisher Full Text](#)
5. Miller TL, Wolin MJ: **Methanogens in human and animal intestinal Tracts.** *System Appl Microbiol*. 1986; 7(2–3): 223–9.
[Publisher Full Text](#)
6. Jain S, Caforio A, Driessen AJ: **Biosynthesis of archaeal membrane ether lipids.** *Front Microbiol*. 2014; 5: 641.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
7. Miller TL, Wolin MJ: **Inhibition of growth of methane-producing bacteria of the ruminant forestomach by hydroxymethylglutaryl-SCoA reductase inhibitors.** *J Dairy Sci*. 2001; 84(6): 1445–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
8. Marsh E, Morales W, Chua KS, *et al.*: **Lovastatin Lactone Inhibits Methane Production in Human Stool Homogenates.**
[Reference Source](#)
9. Jahromi MF, Liang JB, Ho YW, *et al.*: **Lovastatin production by *Aspergillus terreus* using agro-biomass as substrate in solid state fermentation.** *J Biomed Biotechnol*. 2012; 2012: 196264.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
10. Liu J, Zhang J, Shi Y, *et al.*: **Chinese red yeast rice (*Monascus purpureus*) for primary hyperlipidemia: a meta-analysis of randomized controlled trials.** *Chin Med*. 2006; 1: 4.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
11. Zhao ZJ, Pan YZ, Liu QJ, *et al.*: **Exposure assessment of lovastatin in Pu-erh tea.** *Int J Food Microbiol*. 2013; 164(1): 26–31.
[PubMed Abstract](#) | [Publisher Full Text](#)
12. Pérez-Gil J, Rodríguez-Concepción M: **Metabolic plasticity for isoprenoid biosynthesis in bacteria.** *Biochem J*. 2013; 452(1): 19–25.
[PubMed Abstract](#) | [Publisher Full Text](#)
13. Gottlieb K, Wacher V, Sliman J, *et al.*: **Review article: inhibition of methanogenic archaea by statins as a targeted management strategy for constipation and related disorders.** *Aliment Pharmacol Ther*. 2016; 43(2): 197–212.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
14. Sharma A, Chaudhary PP, Sirohi SK, *et al.*: **Structure modeling and inhibitor prediction of NADP oxidoreductase enzyme from *Methanobrevibacter smithii*.** *Bioinformation*. 2011; 6(1): 15–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
15. Ferry JG: **Enzymology of one-carbon metabolism in methanogenic pathways.** *FEMS Microbiol Rev*. 1999; 23(1): 13–38.
[PubMed Abstract](#) | [Publisher Full Text](#)
16. **Protein Data Bank archive (PDB).**
[Reference Source](#)
17. UniProt Consortium: **UniProt: a hub for protein information.** *Nucleic Acids Res*. 2015; 43(Database issue): D204–12.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
18. **List of protein structure prediction software.**
[Reference Source](#)
19. Debe DA, Danzer JF, Goddard WA, *et al.*: **STRUCTFAST: protein sequence remote homology detection and alignment using novel dynamic programming and profile-profile scoring.** *Proteins*. 2006; 64(4): 960–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
20. Poleksic A, Danzer JF, Hambly K, *et al.*: **Convergent Island Statistics: a fast method for determining local alignment score significance.** *Bioinformatics*. 2005; 21(12): 2827–31.
[PubMed Abstract](#) | [Publisher Full Text](#)
21. Palmer B, Danzer JF, Hambly K, *et al.*: **StructSorter: a method for continuously updating a comprehensive protein structure alignment database.** *J Chem Inf Model*. 2006; 46(4): 1871–6.
[PubMed Abstract](#) | [Publisher Full Text](#)
22. Hambly K, Danzer J, Muskall S, *et al.*: **Interrogating the druggable genome with structural informatics.** *Mol Divers*. 2006; 10(3): 273–81.
[PubMed Abstract](#) | [Publisher Full Text](#)
23. **Eidogen-Sertanty SiteSeeker.** Eidogen-Sertanty, Inc.
[Reference Source](#)
24. **BIOVIA Pipeline Pilot.**
[Reference Source](#)
25. **Tripos Mol2 File Format.**
[Reference Source](#)
26. Trott O, Olson AJ: **AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading.** *J Comput Chem*. 2010; 31(2): 455–61.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
27. **Dallakyan S: MGLTools.**
[Reference Source](#)
28. **Schrodinger LLC: The PyMOL Molecular Graphics System.** Version 1.8. 2015.
[Reference Source](#)
29. Kim S, Thiessen PA, Bolton EE, *et al.*: **PubChem Substance and Compound databases.** *Nucleic Acids Res*. 2016; 44(D1): D1202–13.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
30. Duggan DE, Chen IW, Bayne WF, *et al.*: **The physiological disposition of lovastatin.** *Drug Metab Dispos*. 1989; 17(2): 166–73.
[PubMed Abstract](#)
31. Sirtori CR: **The pharmacology of statins.** *Pharmacol Res*. 2014; 88: 3–11.
[PubMed Abstract](#) | [Publisher Full Text](#)
32. Wood WG, Müller WE, Eckert GP: **Statins and neuroprotection: basic pharmacology needed.** *Mol Neurobiol*. 2014; 50(1): 214–20.
[PubMed Abstract](#) | [Publisher Full Text](#)
33. Khelaifia S, Brunel JM, Raoult D, *et al.*: **Hydrophobicity of imidazole derivatives correlates with improved activity against human methanogenic archaea.** *Int J Antimicrob Agents*. 2013; 41(6): 544–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
34. **Amazon Elastic Compute Cloud (Amazon EC2).**
[Reference Source](#)
35. Gottlieb K: **NCT02495623 - A Study of the Effect of SYN-010 on Subjects With IBS-C.** In: Inc. SB, editor. 2015.
[Reference Source](#)
36. Muskall S, Sliman J, Kokai-Kun J, *et al.*: **Dataset 1 in: Lovastatin lactone may improve irritable bowel syndrome with constipation (IBS-C) by inhibiting enzymes in the archaeal methanogenesis pathway.** *F1000Research*. 2016.
[Data Source](#)
37. Muskall S, Sliman J, Kokai-Kun J, *et al.*: **Lovastatin-lactone v. F420 in the A5UM11 site.** *Figshare*. 2016.
[Data Source](#)

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Version 2

Reviewer Report 13 June 2016

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Obdulia Rabal

Small Molecule Discovery platform, Molecular Therapeutics Program, Center for Applied Medical Research, Navarra's Health Research Institute (IDISNA), University of Navarra, Pamplona, Spain

This paper provides insight on how statins inhibit methane production by direct inhibition of dehydrogenases using modelling studies. Previous work in this field (reference 14) focused on the F420-dependent oxidoreductase. Here, authors explore the potential binding of lovastatin and simvastatin into two F420-dependent methylenetetrahydromethanopterin dehydrogenases (mtd) of *M. smithii* and *Methanopyrus kandleri*. From modelling perspective, the paper is well-structured and it provides enough information to reproduce the results, highlighting common problems involving modelling techniques (e.g. different ligand structures in databases). Considerable computational effort was put into identifying the most probable binding site as well as the active form (lactone versus hydroxyacid) of the ligands. Maybe the major drawback is the lack of *in vitro* results confirming the results, what currently seems difficult to achieve on the basis of the previous authors' reply. Results from the clinical trial do not fully ensure that the mechanism goes via these targets, so I would suggest putting this correlation/clinical trial into context. Apart from that, I understand that *in vitro* validation of all modelling results is not always possible and the authors use a proper tone to expose their conclusions.

As a minor point, some comments (e.g. target description) on the PDB entries used to model Q02394 (page 4) would be useful.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 07 June 2016

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Dušica Vidović

Center for Computational Science, University of Miami, Miami, FL, USA

The authors modeled the structure of F420-dependent methylenetetrahydromethanopterin dehydrogenase (mtd) and systematically predicted possible binding sites in order to test docking of lactone form of statins vs. β -hydroxyacid form of statins, as well as the native ligand F420-coenzyme and other co-complexed ligands found in related structures. They used AutoDock Vina for docking and scoring and their results suggest that: for each modeled site the lactone form of the statins had more favorable site interactions compared to F420; the statin lactone forms generally had the most favorable docking scores, even relative to the native template PDB ligands; and the statin β -hydroxyacid forms had less favorable docking scores.

Can the authors explain why would the lactone form of lovastatin be a privileged ligand for the predicted binding sites when compared to the other tested ligands? Did the authors try to use another docking program to reproduce these findings?

Authors also suggest that the lactone form of lovastatin could inhibit the activity of the key *M. smithii* methanogenesis enzyme mtd in vivo. This is in agreement with the phase II clinical trial (NCT0249562335) the authors refer to. The clinical trial showed a reduction in symptoms and breath methane levels in patients treated with lovastatin-lactone (SYN-010) when compared to placebo. However, it is not clear from the clinical trial reference if SYN-010 inhibits mtd. It would be beneficial for this manuscript to add a reference that indicates the target of SYN-010.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 07 Jun 2016

Steven Muskal, Eidogen-Sertanty, Oceanside, USA

Thank you for your comments and questions.

The key dehydrogenases targets considered in this work [**A5UMI1** and **Q02394**] both interact with F420. However, *in vitro* statin lactone v. F420 binding experiments for these targets were not conducted. In the absence of this data, our modeling and subsequent docking of the lactone statins generally show preferential docking as compared to F420 - which suggests these targets may interact with the lactone form of the statins.

We selected AutoDock Vina not only because it is open-source and readily available to other researchers, but also because it has been well tested and found to be "a strong competitor against other programs, and at the top of the pack in many cases" (see

<http://vina.scripps.edu/index.html>). Researchers with access to commercial docking packages are welcome to compare the AutoDock Vina results and data provided with this paper.

Indeed, Synthetic Biologics' SYN-010 lowered breath methane and improved stool frequency in a Phase 2 Four Week Study in Patients with Irritable Bowel Syndrome with Constipation (IBS-C). This Phase 2 study did not identify the specific ligand-target interaction. However, work by Marsh *et al.* - Lovastatin Lactone Inhibits Methane Production in Human Stool Homogenates (<http://content.stockpr.com/syntheticbiologics/db/220/608/file/Lovastatin+in+Human+Stool-FINAL+Poster+%28ACG+2015%29.pdf>) does shed some light. While a messy culture, *M. smithii* was the predominant methanogen in the stool. In this work, lovastatin lactone was identified as the only effective methane inhibitor. Considering other mechanisms by which statins may inhibit methanogenesis, we suggested the dehydrogenases in the main methanogenesis pathway as possibilities.

Competing Interests: No competing interests were disclosed.

Version 1

Reviewer Report 11 April 2016

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Rolf Thauer

Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

The manuscript describes modeling studies suggesting that Lovastatin lactone, a statin, inhibits growth of methane-forming archaeon *Methanobrevibacter smithii* by inhibiting an F420 dependent-enzyme involved in CO₂ reduction to methane, namely F420-dependent methylene-tetrahydromethanopterin dehydrogenase (Mtd). Modeling studies had previously indicated that another F420-dependent enzyme, F420H₂:NADP oxidoreductase in methanogens could be a site of inhibition (reference 14). The results are interesting, however, before indexing the following information has to be added:

The authors must provide experimental evidence that their theoretical prediction is correct. Show that Lovastatin inhibits methane formation from H₂ and CO₂ in non-growing cell suspensions of *M. smithii* and/or that Lovastatin inhibits Mtd activity in cell extracts of *M. smithii* or better with the purified enzyme. The authors might want to team up with a lab experienced in the proposed experiments.

Without these experimental data the manuscript would contain nothing really new relative to the results published in reference 14.

The authors must clearly indicate that crystal structures of Mtd with and without substrates bound have been published and give reference to these publications. To only refer to PDBs without the enzyme name is not fair. To indicate in the Abstract, Methods, that there is “no tertiary protein structural information” is more than misleading and can be misunderstood.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 15 Apr 2016

Steven Muskal, Eidogen-Sertanty, Oceanside, USA

Thank you for your review and comments.

Based on your comments we fixed the misleading text in the Abstract that you noted, and also corrected a typographical swapping of the sequence IDs in the introduction.

We originally did not feel it necessary to mention all the possible templates considered in the modeling effort, but appreciate the value of doing so. Per your suggestion, we inserted additional text in the modeling section which refers to other relevant PDB templates - e.g. 1QV9 and 1U6I,J,K. 1QV9 - Coenzyme F420-dependent methylenetetrahydromethanopterin dehydrogenase (Mtd) from Methanopyrus kandleri: A methanogenic enzyme with an unusual quaternary structure, and 1U6I,J,K - The structures of native coenzyme F420-dependent methylenetetrahydromethanopterin dehydrogenase at various resolutions, and TLS refinement of the structure of Se-methionine labelled Coenzyme f420-dependent methylenetetrahydromethanopterin dehydrogenase (MTD) from Methanopyrus kandleri.

With respect to the experimental data comment, while this paper is a computational chemistry paper, we did note and reference the recently completed clinical trial - A Study of the Effect of SYN-010 on Subjects With IBS-C (<https://clinicaltrials.gov/ct2/show/NCT02495623>) as well as experimental work by Marsh et al. - Lovastatin Lactone Inhibits Methane Production in Human Stool Homogenates (<http://content.stockpr.com/syntheticbiologics/db/220/608/file/Lovastatin+in+Human+Stool-FINAL+Poster+%28ACG+2015%29.pdf>). In the latter, albeit a messy culture, *M. smithii* was the predominant methanogen in the stool, though the less common *Methanosphaera stadtmanae* may have been present as well.

Competing Interests: No competing interests were disclosed.

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