Lovastatin lactone may improve irritable bowel syndrome with constipation (IBS-C) by inhibiting enzymes in the archaeal methanogenesis pathway [version 3; peer review: 2 approved, 1 approved with reservations]

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Abstract
Methane produced by the methanomicrobe 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 ofLovastatin 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 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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Introduction

Irritable bowel syndrome (IBS) affects as many as 45 million people in the United States, and up to 23% of the worldwide population. Depending on the region, as many as 43.3% of these patients will have irritable bowel syndrome with constipation (IBS-C). 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 obesity. Methanogens – i.e. anaerobes that respire hydrogen to produce methane - are found in many habitats supporting anaerobic biogradation of organic compounds, including human and animal intestinal tracts. 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 population.

The isoprenoid biosynthesis for the main cell membrane components in archaea (archaeal) relies on the same enzyme that catalyzes the biosynthesis of the isoprenoid cholesterol in humans - HMG-CoA reductase (mevalonate pathway). 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 growth. Statins, specifically lovastatin, have been shown to lower methanogenesis in human stool samples 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 mushrooms, red yeast rice, and Pu-erh.

Humans and archaea utilize the HMGRI-I isoform for isoprenoid biosynthesis. Mevastatin and lovastatin were both shown to inhibit growth of several rumen Methanobrevibacter isolates in the ~10 nmol/ml range. 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 directly. 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.

Several reviews have appeared describing the reduction of CO₂ to CH₄ in methanoarchaea. Considering other mechanisms by which statins may inhibit methanogenesis directly, we have explored two important dehydrogenases in the main methanogenesis pathway, including F420-dependent methylenetetrahydrodethanopertin dehydrogenase of M. smithii [A5UMI - 275 amino acid residues], and evolutionarily related F420-dependent methylenetetrahydrodethanopertin (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), so we needed to identify acceptable templates to model this sequence. The A5UMI sequence, however matched the 3IQZ co-complex with methylenetetrahydrodethanopertin (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 UniProt. 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 interest. The Eidogen StructFast 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. Once models for A5UM1 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 algorithm. 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’s) Pipeline Pilot technology v8.5. Ligands were finally prepared into mol2 format. Each representation was then docked into each identified site and scored using AutoDock Vina v1.1.2, an open docking technology that utilizes grid-based energy evaluation and efficient search of ligand torsional freedom. AutoDock Vina has been tested against the Directory of Useful Decoys, performing frequently better than many commercially available docking programs.

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 v1.5.4 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 archael 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 ASUM11 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 ASUM11 was straightforward given its high 52% sequence homology to 3IQZ. Other templates (e.g. 1U6J, 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 each 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 ASUM11/3IQZ-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 ASUM11 models.

Ligand processing

The key ligands for this effort included lovastatin (lactone and hydroxyacid forms), F420, and simvastatin (lactone and 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 PubChem 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 molecule. 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. The degree of hydrophobicity of imidazole derivatives correlates with improved activity against human methanogenic archaea.

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 ASUM11 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 EC2 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.\textsuperscript{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 grouping.

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

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**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.

<table>
<thead>
<tr>
<th>A5UMI1</th>
<th>Q02394</th>
</tr>
</thead>
<tbody>
<tr>
<td>3IQZB (H4M 7Å), 1 chain</td>
<td>3F47 (I2C)</td>
</tr>
<tr>
<td>3IQZB (SiteSeeker1)</td>
<td>3F47 (SiteSeeker)</td>
</tr>
<tr>
<td>3IQZB (SiteSeeker2)</td>
<td>3H65 (H4M)</td>
</tr>
<tr>
<td>3IQZB (H4M)_3IQZF (7Å)</td>
<td>3H65 (I2C)</td>
</tr>
<tr>
<td></td>
<td>4JJF (FE9)</td>
</tr>
<tr>
<td></td>
<td>4JJF (SiteSeeker)</td>
</tr>
</tbody>
</table>

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**Table 2**

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**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 PyMOL\textsuperscript{48}. 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 2. Average, minimum, and maximum affinity for each site. Affinities were computed from AutoDock Vina\(^26\). The top two scoring sites from ASUMI1 and Q02394 are in bold. These sites were used to rank the ligands in Table 3.

<table>
<thead>
<tr>
<th>Docking Site</th>
<th>Average Affinity (kcal/mol)</th>
<th>Min Affinity (kcal/mol)</th>
<th>Max Affinity (kcal/mol)</th>
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<td>Q02394_3F47 (I2C)</td>
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<td>-9.34</td>
<td>24.92</td>
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<td>ASUMI1_3IQZB (H4M)_3IQZF (7Å)</td>
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<td>-8.56</td>
<td>6.29</td>
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<td>ASUMI1_3IQZB (H4M 7Å), 1 chain</td>
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<td>214.07</td>
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</table>

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 PubChem\(^29\) CIDs.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Average AutoDock Vina Score</th>
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<tbody>
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<tr>
<td>STD_803_1cqp</td>
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<tr>
<td>RAW_Simvastatin_pubchem_54454</td>
<td>14.34</td>
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<tr>
<td>STD_Simvastatin_pubchem_54454</td>
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<tr>
<td>RAW_Lovastatin_pubchem_53232</td>
<td>14.42</td>
</tr>
<tr>
<td>STD_Lovastatin_pubchem_53232</td>
<td>14.42</td>
</tr>
<tr>
<td>RAW_FE9_4jjfA</td>
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<td>RAW_FE9_4yt4A</td>
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<td>STD_I2C_3f47A</td>
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Table 4 (a,b). Lovastatin-lactone (a) v. lovastatin-hydroxyacid (b) metrics across the top two modeled receptor sites. AutoDock4.1Score is a weighted sum of steric interactions (Gauss 1, Gauss 2, and steric), repulsion, hydrophobic interaction between hydrophobic atoms, and, where applicable, hydrogen bonding26.

<table>
<thead>
<tr>
<th>Site</th>
<th>A5UMI1 3IQZB (siteSeeker2)</th>
<th>Q02394 4JJF (siteSeeker)</th>
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<td>Gauss 2</td>
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<td>Hydrophobic</td>
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<tr>
<td>AutoDock4.1 Score</td>
<td>14.3</td>
<td>13.4</td>
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</table>

**a)** Lovastatin (lactone): RAW_803_1cqp

**b)** Lovastatin (hydroxyacid): mevinolinic acid; PubChem 64727

<table>
<thead>
<tr>
<th>Site</th>
<th>A5UMI1 3IQZB (siteSeeker2)</th>
<th>Q02394 4JJF (siteSeeker)</th>
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<td>Hydrogen</td>
<td>5.3</td>
<td>2.9</td>
</tr>
<tr>
<td>AutoDock4.1 Score</td>
<td>28.2</td>
<td>31.6</td>
</tr>
</tbody>
</table>

Figure 2. Best scoring lovastatin-lactone and -hydroxyacid poses in A5UMI1 3IQZB_SiteSeeker2 (top) and Q02394 4JJF_SiteSeeker (bottom). Lovastatin-lactone form is shown with green sticks and hydroxyacid form with red sticks. Residues within 5 angstroms of ligands are labeled. Hydrophilic site residues are shown in cyan and hydrophobic residues in gray.
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 4.4× worse than lovastatin-lactone’s score.

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 dehydrogenase targets in question A5UM1 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 A5UM1. While in vitro ligand binding experiments were not conducted, the docking simulations suggest that these dehydrogenases in the main methanogenesis pathway may be possible targets. These results are also consistent with those from a recent phase II clinical trial (NCT02495623) 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 archael methanogenesis pathway", 10.5256/f1000research.8406.d117917


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 The author(s) declared that no grants were involved in supporting this work.

References

1. Facts About IBS. Reference Source
Published Abstract | Publisher Full Text
Published Full Text
Published Abstract | Publisher Full Text
Published Full Text
Published Abstract | Publisher Full Text | Free Full Text
Published Abstract | Publisher Full Text
Published Abstract | Publisher Full Text | Free Full Text
Published Abstract | Publisher Full Text | Free Full Text
Published Abstract | Publisher Full Text
Published Abstract | Publisher Full Text
Published Abstract | Publisher Full Text | Free Full Text
Published Abstract | Publisher Full Text | Free Full Text
Published Abstract | Publisher Full Text | Free Full Text
16. Protein Data Bank archive (PDB). Reference Source
Published Abstract | Publisher Full Text | Free Full Text
18. List of protein structure prediction software. Reference Source
PubMed Abstract | Publisher Full Text
PubMed Abstract | Publisher Full Text
PubMed Abstract | Publisher Full Text
PubMed Abstract | Publisher Full Text
24. BIVOIA Pipeline Pilot. Reference Source
PubMed Abstract | Publisher Full Text | Free Full Text
27. Dallakyan S: MGLTools. Reference Source
PubMed Abstract | Publisher Full Text | Free Full Text
PubMed Abstract
PubMed Abstract | Publisher Full Text
PubMed Abstract | Publisher Full Text
PubMed Abstract | Publisher Full Text
34. Amazon Elastic Compute Cloud (Amazon EC2). Reference Source
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Dusica Vidović
Department of Pharmacology, University of Miami, Miami, FL, USA

The authors have responded to two questions from my previous report. One question was related to the choice of the docking program AutoDock Vina, while the other question was related to the target of SYN-010 in the clinical trial that authors referenced.

The authors have justified the use of AutoDock Vina as a well tested docking program that has been found to be "a strong competitor against other programs, and at the top of the pack in many cases".

Regarding the target of SYN-010 in the clinical trial, although the binding assays for the key dehydrogenases targets were not conducted, the presented docking study of lactone statins generally show preferential docking as compared to F420 (known to interact with both dehydrogenases targets). Based on these findings, the authors suggest that these targets may interact with the lactone form of the statins.

Competing Interests: No competing interests were disclosed.

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

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

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Reviewer Report 07 June 2016

https://doi.org/10.5256/f1000research.9304.r13719

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Dusica Vidovic
Department of Pharmacology, 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 have read this submission. I 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.

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**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 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](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+Pos](http://content.stockpr.com/syntheticbiologics/db/220/608/file/Lovastatin+in+Human+Stool-FINAL+Pos)) 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.
Rolf Thauer
Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

The manuscript describes modeling studies suggesting that Lovastin lactone, a statin, inhibits growth of methanogenic 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 Lovastin inhibits methane formation from H2 and CO2 in non-growing cell suspensions of M. smithii and/or that Lovastin inhibits Mtd activity in cell extracts of M. smithii of 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 have read this submission. I 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.

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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.