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Reconstruction and modeling protein translocation and compartmentalization in *Escherichia coli* at the genome-scale

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**Abstract**

**Background:** Membranes play a crucial role in cellular functions. Membranes provide a physical barrier, control the trafficking of substances entering and leaving the cell, and are a major determinant of cellular ultra-structure. In addition, components embedded within the membrane participate in cell signaling, energy transduction, and other critical cellular functions. All these processes must share the limited space in the membrane; thus it represents a notable constraint on cellular functions. Membrane- and location-based processes have not yet been reconstructed and explicitly integrated into genome-scale models.

**Results:** The recent genome-scale model of metabolism and protein expression in *Escherichia coli* (called a ME-model) computes the complete composition of the proteome required to perform whole cell functions. Here we expand the ME-model to include (1) a reconstruction of protein translocation pathways, (2) assignment of all cellular proteins to one of four compartments (cytoplasm, inner membrane, periplasm, and outer membrane) and a translocation pathway, (3) experimentally determined translocase catalytic and porin diffusion rates, and (4) a novel membrane constraint that reflects cell morphology. Comparison of computations performed with this expanded ME-model, named *i*JL1678-ME, against available experimental data reveals that the model accurately describes translocation pathway expression and the functional proteome by compartmentalized mass.

**Conclusion:** *i*JL1678-ME enables the computation of cellular phenotypes through an integrated computation of proteome composition, abundance, and activity in four cellular compartments (cytoplasm, periplasm, inner and outer membrane). Reconstruction and validation of the model has demonstrated that the *i*JL1678-ME is capable of capturing the functional content of membranes, cellular compartment-specific composition, and that it can be utilized to examine the effect of perturbing an expanded set of network components. *i*JL1678-ME takes a notable step towards the inclusion of cellular ultra-structure in genome-scale models.

**Keywords:** Constraint-based modeling, gene expression, metabolism, protein translocation, compartmentalization

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**Background**

Compartmentalization provided by membranes is essential for life. Compartmentalization allows unique internal microenvironments, permits harvestable energy gradients, provides organizational structure, protects the cell, and more. Membranes also represent significant physical barriers. Thus, cells have evolved pathways that allow molecule transport between compartments. As a gram-negative bacterium, *Escherichia coli* has two membranes: An inner, tightly regulated membrane and an outer, more porous membrane (see [1,2] for review). In order to achieve desired membrane functions, *E. coli* has evolved a system to translocate proteins into their appropriate locations.

There is a wealth of scientific information on protein translocation processes, but holistic studies on their system-wide effects are lacking. Such genome-wide studies...
are important as protein translocation enables key cellular functions. These functions need to be put into context of all other cellular functions to understand their energetic requirements, general interactions and balance with the rest of the cell. To do so, one must take a systems approach, where comprehensive molecular processes and interactions are reconstructed into a self-consistent and computable format. A couple of recently published studies have taken steps in this direction. In a comprehensive approach to cellular processes, the recent whole-cell model of *Mycoplasma genitalium* incorporates a SecA + Sec translocase pathway into one of its protein formation modules [3]. In this model, translation is uncoupled from translocation, even though the two processes can happen concurrently [4]. Furthermore, protein translocation rates are not calculated de novo but are instead based on user-inputted gene expression levels and energy-carrier metabolite concentrations (calculated prior from a separate module). Thus, set expression levels of protein translocation cases operate as a constraint on other processes; for example, metabolism uptake is dependent on the number of transporters. Additionally, membrane lipid formation is driven by a biomass objective function [3], whereas a computation based on a cell’s surface area might be more appropriate. In another study, a larger effort was focused on the genome-scale reconstruction of the protein secretion pathway in *Saccharomyces cerevisiae* [5]. This model of protein secretion is ‘stand-alone’ and is not integrated with additional cellular processes. It can be used as a scaffold on which omics data (e.g., RNA-seq) can be overlaid to estimate effects of protein abundance and metabolic costs of translocation on the cell. Although these models contain some detail about protein translocation, both are reliant on expression data input and are not dependent on the demands of cellular events. Finally, another notable model incorporated membrane space into a genome-scale model of *E. coli* to demonstrate that while the membrane may cap certain fluxes, leading to simultaneous respiration and fermentation at high growth rates, metabolic demands drive the membrane proteome. Although this model lacks the process of protein translocation and has only four integral proteins, it demonstrated that the consequence of protein translocation, namely compartment formation, truly constrains cellular events [6].

A recent genome-scale model of metabolism and gene-expression of *E. coli*, called a ME-model [7] or specifically, the retroactively named iOL1650-ME model (following a previous naming convention [8]), affords us the opportunity to integrate protein translocation seamlessly with cellular processes. Although iOL1650-ME describes the synthesis of all the proteins in the proteome, the proteins are not compartmentalized. In this work, we significantly expanded the validated iOL1650-ME model [7] to include a comprehensive reconstruction of protein translocation pathways. The expanded iOL1650-ME includes a reconstruction of lipoprotein biogenesis, the incorporation of four distinct protein compartments (cytoplasm, periplasm, the inner and the outer membrane), published enzymatic rates of the translocases and diffusion rates of outer membrane porins, and a membrane constraint based on cell morphology all integrated into one reconstruction. The expanded model, hereafter referred to as jL1678-ME, allows for de novo prediction of enzyme abundances and their cellular location as well as the constraining effects of membrane production. We apply jL1678-ME to show how it is predictive of compartmentalized cellular content for validation, describe its utility and limitations, and show how it can be applied to examine a broadened scope of applications including targeted inhibition of proteins.

**Results and discussion**

All proteins in *E. coli* are synthesized in the cytoplasm, but over 20% of *E. coli*’s protein-coding open reading frame (pORF) are annotated to encode protein with non-cytoplasmic functions, and an estimated 15% of cellular protein mass is in the cell envelope [9,10]. These proteins are assisted by translocase complexes to get to their cellular destinations. Depending on their final location and biochemical properties, the translocation route taken for a particular protein involves one of three integral inner membrane translocases (Sec, Tat, and YidC) and perhaps an outer membrane translocase (LolB and Bam) (see [1,11] for review). The most-studied and ubiquitous translocase is the Sec complex [12]. The channel-forming Sec protein has two chaperone pathways that converge on it. One, the SRP/Sec pathway, brings nascent peptides to the Sec complex and primarily uses the kinetic energy of translation to drive protein integration into the inner membrane [4,13,14]. Sometimes, the mediator YidC binds to Sec complex to enhance proper membrane integration, but on its own, YidC is an insertase that translocates a couple of essential proteins [15-17]. Alternatively, proteins moving to the periplasm and beyond generally follow the SecB/Sec pathway which uses an ATPase, SecA, to thread chaperoned, unfolded proteins through the Sec complex and into the periplasm [18-21]. Furthermore, non-cytoplasmic, folded proteins which often contain cofactors take the Tat translocase, a dynamic protein complex that recruits TatA subunits to adjust its channel size appropriately and is driven by an electrochemical gradient [22-24]. To get to the outer membrane, proteins must first cross the inner membrane, then take one of the two pathways: Lol or Bam. The Lol pathway excises lipoproteins from the inner membrane and incorporate them into the outer membrane [25,26]. In the Bam pathway, unfolded β-barrels are chaperoned in the periplasm, typically by SurA [27,28], to the Bam complex, which facilitates their proper
Figure 1 (See legend on next page.)
The incorporation of protein translocation pathways requires proteins to have defined compartmentalization. First, two new compartments, inner and outer membranes, were added to the three existing compartments in iOL1650-ME (cytoplasm, periplasm, and extra-cellular) [7]. Using the protein databases EchoLocation [37], Uniprot [38], and Ecocyc [39] as well as the bioinformatic programs PSORTb [40] and TMHMM [9], the 1,568 pORFs included in the reconstruction were assigned to compartments (Figure 1C). pORFs with a transmembrane component or a lipid membrane anchor were assigned to either the inner or outer membrane; otherwise, pORFs were either cytoplasmic or periplasmic. Proteins composed of multiple pORFs were assigned to the compartment of its components (Additional file 2: Table S2), but if any of its pORFs was in a membrane then the entire complex was assigned to that membrane, with the outer membrane taking precedence over the inner (e.g., AcrAB-ToIC multidrug efflux system is assigned to the outer membrane). For example, ATP synthase has pORFs located in the inner membrane (AtpB, AtpC, AtpE, AtpF) and cytoplasm (AtpA, AtpD, AtpG, AtpH), but the synthase itself is assigned to the inner membrane so that it may interact with metabolites in both the cytoplasm and periplasm.

The compartment assignment resulted in 71% of pORFs being assigned to the cytoplasm, 21% to the inner membrane, 6% to the periplasm, and 2% to the outer membrane.

Assigning translocated proteins to pathways
Protein translocation reactions were formulated for each pORF. Using a set of rules based on experimental data, protein location, and physical properties (Additional file 2: Table S3), non-cytoplasmic annotated pORFs were assigned to translocation pathways (Figure 1D). The developed template reactions allowed for the methodological creation of each pORF's translocation reactions and their subsequent incorporation into the reconstruction. Additional pathway development steps included determining the amount of ATP hydrolyzed by SecA for each pORF (i.e., 1 ATP per ~25 amino acids) [41], assigning 23 pORFs to lipoprotein biogenesis [37], and calculating the number of TatA's needed for each Tat-translocated pORF [23] (Additional file 1, Additional file 2: Table S1, Additional file 3: Figure S1).

Published translocase k_{cat} values were associated with appropriate proteins in the translocation pathways.
These values [42-46] were incorporated into the model through coupling constraints [36,47], which account for turnover rates by linking gene expression to metabolism through the dependence of reaction fluxes on enzyme concentration (Figure 1D) [35]. Additionally, outer membrane porins were represented to behave as passive-diffusion channels [2] in the reconstruction. Instead of identical turnover rates for all outer membrane porins in the cell, incorporation of porin-specific coupling constraints allowed the model to account for individualized solute diffusion rates based on effective porin radius, hydrodynamic solute radius, membrane thickness, and growth rate (see Additional file 2: Table S4 for list of solutes, which are also exchange metabolites). This formulation represents the cross-sectional area a solute can pass through and distance a solute had to travel to reach the periplasm [48] (Additional file 1). Without these coupling constraint updates, the model was unable to predict accurate translocase levels (Additional file 3: Figure S2).

**Incorporating cell-size and membrane constraints**

Cell envelope production was fundamentally changed to reflect the cell's shape and composition more accurately. The previously-developed iOL1650-ME accounts for production of kdo 2 lipid IV, phospholipids, and murein through growth rate dependent demands scaled to cell size [7]. These demands were identified as key areas for improvement to a more mechanistic description in JL1678-ME. Changes to the model included adding murein recycling, a lipoprotein demand, and a membrane spatial constraint. The peptidoglycan layer protects the cell from lysis by providing a physical structure, and it also dynamically renews its components by using enzymes located in all compartments of the cell (see [49] for review). To reflect this renewal process, AmpG permease transports anhydro-muropeptides to equal 45% of the murein demand, which causes a murein recycling loop [50]. Lipoproteins are also important for structural integrity, and the number of lipoproteins that have been estimated in a cell, 7×10^5, is a significant amount of mass [10], so a growth-rate scalable lipoprotein demand, using Braun's lipoprotein [51], was added. Finally, because there are inner and outer membrane compartments, membrane demands and composition can be more explicitly described with the genome-scale model. Membrane surface area, which is a function of growth rate, is required to be occupied completely by proteins and lipids (see Methods). The surface area of integral proteins was calculated from their mass, except for lipoproteins which were set to the approximate cross-sectional area of their lipid moieties (Additional file 2: Table S5) [10,23,52]. The rest of the outer membrane outer leaflet is filled in with kdo 2 lipid IV while the other three membrane leaflets are occupied by a mixed composition of phospholipids (see Methods for mathematical formulation of the membrane constraint) [53,54]. This novel membrane constraint not only allows a variable membrane proteome, but it also ensures that the cell is completely covered by two membranes.

**Updating model parameters**

Two model parameters were updated to reflect the new reconstruction content. The growth-associated maintenance (GAM) was updated from 35 to 34.98 mmol ATP gDW⁻¹ to account for the ATP spent translocating proteins out of the cytoplasm, which is small compared to the cell's total energy production but expensive per non-cytoplasmic protein (0.02 for translocating 2.3×10⁻³ mmol protein gDW⁻¹, or 85.7 ATP for each non-cytoplasmic protein). Also, the out-of-scope protein proportion of proteome, a parameter introduced in iOL1650-ME to account for proteins expressed in vivo but not actively utilized by the network reconstruction [7,55], was changed. As JL1678-ME includes more pORFs, this parameter's value had to be reduced by the expressed mass of new protein content. Thus, the out-of-scope protein proportion was changed from 0.45 to 0.36 to reflect JL1678-ME's increased comprehensiveness.

Taken in whole, the improved network reconstruction demonstrated that there is enough scientific literature to accurately reconstruct protein translocation in a genome-scale model. As a result of having this reconstruction, it was possible to compute physiological aspects of the cell envelope, which converges to a fully comprehensive in silico model of E. coli (Additional file 4).

**Proteomic shifts highlight the significance of new content in JL1678-ME**

iOL1650-ME and JL1678-ME enable quantitative predictions of genome-scale proteome abundances. Instead of requiring input expression data, these models calculate the proteins necessary to maximize growth rate through a metabolism-centered network. However, not only does JL1678-ME contain more reconstructed content, but it also has a reformulated cell envelope representation that requires more membrane production, phospholipid variety, and murein recycling.

To demonstrate the difference between the two ME-models, the computed protein expression fluxes in glucose M9 minimal media were compared (Figure 2, in silico media composition given in Additional file 2: Table S6). Although the majority of pORFs (1475) were approximately the same in both model simulations, 32 of the genes were differentially expressed, and a number of proteins were uniquely expressed (Figure 2A). Clearly, accommodating protein translocation has a systemic effect on the computed proteome.
Looking first at pORFs expressed in both models, the largest outlying subgroup is the cell membrane and envelope related proteins. This differential expression was due to the addition of murein recycling, which increases overall murein production (145%) and associated ATP expenditure (140%, which is 2.3% of all ATP production in overall murein production (145%) and associated ATP generation through oxidative phosphorylation. As consequence, expression of TCA cycle proteins and succinate dehydrogenase was greater. Finally, the collective increase in protein expression due to the expanded scope of iJL1678-ME caused a notable change in protein expression levels, and these shifts can be directly attributed to isozymes employed (e.g., AcnA verses AcnB in iJL1678-ME).

In summary, the increased scope of modeled genes in iJL1678-ME caused a notable change in protein expression levels, and these shifts can be directly attributed to model updates and constraints derived from biochemical knowledge available in literature. The resulting proteomic content was examined further.

\textbf{In silico computations recapitulate in vivo data}

To estimate the accuracy of the iJL1678-ME \textit{in silico} proteome, glucose M9 minimal media simulation results were compared to experimental data (Additional file 2: Table S6). Unlike iOL1650-ME, iJL1678-ME calculates a compartment-specific proteome with absolute protein levels. Although this ability may be especially useful in studying the membrane proteome, an area plagued by hardship due to its hydrophobic and amphiphilic nature, it has also created difficulty in comprehensively

\textbf{Figure 2 Proteome expression comparison between iOL1650-ME and iJL1678-ME.} The difference that the protein translocation reconstruction brings to iOL1650-ME is compared through computed protein expression in glucose M9 minimal media conditions. (A) Protein translation flux between iJL1678-ME and iOL1650-ME. The majority of pORF expression (93.5%) are approximately the same in both model simulations, but 4.1% are uniquely expressed in iJL1678-ME, and 0.4% is uniquely expressed in iOL1650-ME (points along the -inf line). 2.0% of the proteins are differentially expressed; the majority of which are expressed to a greater extent in iJL1678-ME than in iOL1650-ME, but two proteins involved in small carbon metabolism (EutD and PurT) are expressed lower. (B) Histograms detailing the functional annotations of the uniquely expressed genes within the two models.
evaluating iJL1678-ME’s results. Even though the correlation between the transcriptome and proteome is poor on a protein-to-transcript level [56,57], RNA-seq is a robust currently-available omic data-source which covers genome-scale expression in all compartments. Assuming that discrepancies in transcript-to-protein ratios are reduced through averaging, RNA-seq data (GEO accessions: GSE48324 [58] and GSE61327 [59]) was assumed as a one-to-one proxy for protein levels. Protein masses were calculated from amino acid sequences and normalized by relative fractional proteome mass. Once a comprehensive quantitative proteomics dataset is available, it will be important to validate that the same functional groups are under-predicted.

Since the network reconstruction expanded the scope of iOL1650-ME, we sought to validate the new features of the genome-scale model. The computed mass of all proteins associated with a translocation pathway (color labeled in Figure 1B) as a fraction of total cellular protein mass is largely similar to in vivo data (Figure 3A, Additional file 3: Figure S3). The most notable outlier is the Tat pathway. The difference between in silico and in vivo expression may be due to the fact that a TatBC complex forms multiple channels to simultaneously translocate substrates [60,61], but in iJL1678-ME model, each TatBC complex translocates a single substrate at any point in time. To explore the possibility of a different representation for TatBC, the mass of TatBC was adjusted by four-fold (the maximum demonstrated number of bound precursor proteins) and this improved the in vivo to in silico correlation ($R^2 = 0.897$ to 0.925, p-value = 0.014 to 0.009), which hints at the possibility TatBC commonly forms multiple channels per complex in vivo. These results demonstrate that bottom-up reconstruction approaches and constraint-based modeling can estimate relative protein levels when incorporated with turnover rates and metabolic demands and serves as validation of the reconstructed content (see Additional file 3: Figure S2 for translocation without $k_{cat}$).

iJL1678-ME’s ability to accurately compute protein amounts extends to compartmentalization, which is enabled due to protein translocation (Figure 3B). Simulation results predict that the mass of cytoplasmic proteins constitute approximately 79% of the proteome, while the inner membrane protein masses are 10%, periplasmic 1.0%, and outer membrane 10%. Calculating these same values for in vivo measurements gave 76.6%, 10.6%, 4.9%, and 7.9%, respectively. In a complementary analysis, iJL1678-ME estimated outer membrane protein values closer to published numbers than in vivo (RNA-seq) data’s approximation of the outer membrane proteome. The in silico protein numbers reflect experimental published amounts at $7.2 \times 10^5$ lipoproteins verses $7 \times 10^5$ and $1.5 \times 10^5$ porins verses $2 \times 10^5$ [10], which implies that the RNA-to-protein ratio is not one-to-one for outer membrane proteins. As there are less proteins in the non-cytosolic compartments, the averaging effect of large groups is less effective, which may explain the discrepancy.

Where do the similarities and differences between the computed and measured compartment-specific protein mass arise from? To answer this question, the protein

![Figure 3](http://www.biomedcentral.com/1752-0509/8/110)
masses were broken down into smaller subgroups, as labeled in iJO1366 which used EcoCyc and GO annotations [39,62]. All 1,568 pORFs were categorized by functional annotation as opposed to a gene-by-gene comparison, with the assumption that a larger sample size would reduce the discrepancies between protein and RNA abundances. A comparison between computational predictions and experimental data was performed using linear regression of log-log values with zero values being removed from further calculations (Figure 4). A normal probability plot of the standardized residuals of the initial model (Additional file 3: Figure S4) revealed that while most points could be described by a normal distribution, five points describing lowly-expressed functions in iJL1678-ME were out of range (Figure 4A). These five points were separated for further analysis while the reduced set of points was refitted, resulting in a more accurate linear model (Figure 4B).

Due to their departure from normalcy, the five outliers in Figure 4A were examined to identify reasons for modeled discrepancies. The five points covered genes involved with inorganic ions, cofactor and prosthetic groups, protein maturation, and metabolite transportation. Not only is the available knowledge of metal ion and cofactor requirements sparse [63], but the model demands the incorporation of only the most necessary groups into proteins. As result, expression of inorganic ion, cofactor, and prosthetic related pORFs are low. Similarly, protein maturation pORFs are required for proper inclusion of ions and groups; they also assist mis-folded proteins, whose possibility are not computed in optimal situations. Lastly, iJL1678-ME predicts a lower periplasmic mass for small metabolite transportation as compared to in vivo data. Closer examination of this functional group revealed that the model has severely decreased the diversity of ABC transporters to five protein species. However, E. coli produces multiple species of ABC transporters in preparation for environmental changes [64]. This readiness to consume a variety of substrates improves the cell’s overall fitness, but when confronted with glucose as the sole carbon source.
substrate, the varied over-expression limited the predicted optimal growth rate, according to \( \text{iJL1678-ME} \).

**Applications predict the effect of molecular perturbations**

Genome-scale models of metabolism have enjoyed many successes in elucidating interactions, metabolic engineering, drug targeting, and more. Up to this point in time, perturbations in genome-scale models are often focused on gene knockouts and constraining a particular reaction to a bound \[65\]. \( \text{iJL1678-ME} \) can be used to provide new insights which cannot be currently be achieved with existing models; that is, \( \text{iJL1678-ME} \) can be used to estimate the detailed effects of molecular processes and physical parameters and on a much broader scale. This ability of \( \text{iJL1678-ME} \) will be demonstrated through two examples: Membrane crowding and Sec pathway inhibition.

**Assessing the consequences of membrane crowding**

Molecular crowding in the finite space of cells limits metabolic activity \[6,66\]. Such crowding constraints are found both in the volume of the cell (also called 'packing' constraints) as well as the surface area of its membranes. \( \text{iOL1650-ME} \), and consequently \( \text{iJL1678-ME} \), implicitly considers volume crowding effects because density is constrained based on the overall growth rate \[7\]. Limited surface area in the membranes are thought to constrain major aspects of metabolism and physiology; for example, it may force \( \text{E. coli} \) to employ a mixture of respiration and fermentation to maximize growth rate \[6,67\]. Thus, as part of the reconstruction process, a constraint on the fraction of protein in the membranes was incorporated into \( \text{iJL1678-ME} \) (Additional file 1). This membrane constraint is mechanistic and imposed on a genome-scale, thereby representing a unique opportunity for a detailed assessment of the consequences of limited membrane space. The results of restricting the total surface area of integral membrane proteins in the model are described.

Computations of growth optimization were performed with constraints on the protein-to-lipid surface area ratios in both the inner and outer membranes. These computations revealed that the maximum growth rate was achieved when the fraction of membrane surface area occupied by protein was 42% and 25% for the inner membrane and outer membrane, respectively. Furthermore, over- and under-production of membrane proteins did not affect the maximum growth rate with the same severity. The uneven slopes from the apex at 42% and 25% indicates that over-expression of membrane proteins may be less taxing on growth rate than under-expression, suggesting that it may in the cell's favor to over-produce membrane proteins than under-produce (Figure 5A).

As the inner membrane contains a diverse set of proteins that are important for metabolism, \( \text{iJL1678-ME} \) was used to examine the effects of spatial limitations on the inner membrane proteome. Although oxidative phosphorylation is much more efficient than alternate energy producing pathways, \( \text{E. coli} \) at high growth-rates and in excess glucose also employs fermentation pathways \[68\]. The electron transport system (ETS) is embedded in the membrane, and limited membrane space for the ETS may be why \( \text{E. coli} \) resorts to the mixed energy-production strategy \[6\]. \( \text{iOL1650-ME} \), on the other hand, predicted that such a phenomenon occurs based on the trade-off between ATP generation and protein production costs \[7\].

In \( \text{iJL1678-ME} \), acetate secretion has been almost eliminated compared to \( \text{iOL1650-ME} \) (8.1 to 1.5 mmol gDW\(^{-1}\) h\(^{-1}\)), due to the porin constraint. Differences in diffusion rates for each metabolite allowed the model to recognize that gases diffuse faster than solubilized carbon molecules, and complete metabolism of a carbon source becomes a better investment. However, fermentation returned when the inner membrane protein surface area decreased below 50%, as demonstrated by the increased secretion of acetate (Figure 5B). Within these regions of constraining protein-occupied surface area, the cell model produced less oxidative phosphorylation products, which includes the ETS, instead of glucose PTS permeases and transporters for continued and increased glucose uptake, as previously hypothesized (Figure 5B & C) \[6\]. At extremely low surface areas allocated to proteins (≤10%), there was not enough room to accommodate NADH dehydrogenase in the membrane. Instead, alternate dehydrogenases were expressed. Thus, to maximize growth rate, \( \text{iJL1678-ME} \) chose to increase fermentation rates with decreased membrane space.

Once membrane space permits complete metabolism of glucose influx at ~50% protein-occupied surface area, fermentation pathways are no longer heavily employed which improves metabolic efficiency, hence the drop in in glucose uptake and increase oxygen uptake (Figure 5B). However, beyond 50%, \( \text{iJL1678-ME} \) makes a trade-off between producing more ETS, an expensive investment, to alternative proteins (Figure 5C). This shift in protein expression to accommodate the trade-off of ETS may play out similarly for proteins not required for metabolism, protein translocation, or metabolite transport but are essential for other processes (e.g., expression of flagella for locomotion).

Where do in vivo cells fall along this scan across inner membrane occupancy? The calculated in vivo surface area of 28.5%, based on RNA-seq data (Additional file 1), puts a cell below optimal membrane occupancy. Within this range of in vivo surface area, the increased acetate secretion hints that membrane space constraints may indeed be why cells employ combinatorial energy production pathways at maximum growth rates, as Zhuang et al. had hypothesized \[6\]. Furthermore, oxygen uptake drops severely when the protein surface area approaches the
in vivo value of 28.5% (17 mmol gDW$^{-1}$ h$^{-1}$ which is close to the measured values of 15 mmol gDW$^{-1}$ h$^{-1}$ [69] and 18 mmol gDW$^{-1}$ h$^{-1}$ [70]). This finding implies that a finite inner membrane protein surface area can limit the oxygen uptake and usage rate, thereby lowering the growth rate to less than the maximum potential.

**Perturbations in network performance by changing enzymatic efficiency**

The Sec pathway is a key pharmaceutical target due to its ubiquity and essentiality. For example, SecA is particularly attractive since it does not have a human homologue, and a recent non-cellular assay for SecA activity was developed specifically for drug discovery [71]. However, effects of decreased Sec translocase activity on a cell are largely unknown. While reactions in metabolic models can be capped to mimic protein inhibition, iJL1678-ME takes this ability further by targeting enzymatic efficiencies, similar to the effects of drugs. Thus, the impact of inhibiting Sec translocation on overall cellular phenotype was analyzed with iJL1678-ME by targeting key enzymes. SecA is the energy driver for the SecB/Sec pathway, and the ribosome is the energy driver for the SRP pathway. Together, these two pathways meet at SecYEGDF (Figure 1B). Due to their importance, these three proteins were inhibited.

When the \(k_{\text{cat}}\) values of SecA, SecYEGDF, and the ribosome were reduced in a step-wise manner, growth rate was affected differently in each situation (Figure 6A). The relationship between ribosome inhibition and growth rate is nearly linear. SecA and SecYEGDF, on the other hand, behave in a hyperbolic manner. Thus, unlike ribosome, the activity of SecA or SecYEGDF must be nearly eliminated (i.e., SecA < 2.5%, SecYEGDF < 5%) to reduce the growth rate by half. A closer look at these extremely low enzymatic rates reveals that the in silico membrane proteome was dominated by SecYEGDF. Therefore, membrane occupancy was capped at 50%, as done by Zhuang et al. [6], to determine whether spatial limitations may change the overall behavior to Sec pathway perturbations. The
inhibition simulations were repeated, showing that ribosome was not affected by membrane limitations, while effects were observed when SecA and SecYEGDF’s turnover rates dropped below two amino acids per second (Additional file 3: Figure S5). However, regardless of membrane space, both SecA and SecYEGDF must be severely inhibited to significantly decrease growth rate. This example of targeting Sec translocation shows that iJL1678-ME can be used to discover cellular effects of selected perturbations. Other molecular behaviors, like combinatorial drug effects, may find similar answers through iJL1678-ME. For example, simultaneously targeting the two chaperone pathways for SecYEGDF, namely SecA and ribosome, is not a synergistic approach, and SecA must still be targeted for complete inhibition to significantly lower the growth rate (Figure 6B).

Conclusions
Taken in whole, iJL1678-ME stoichiometrically represents the wealth of knowledge known for protein translocation of E. coli in an integrated and computable format. For the first time, a bottom-up stoichiometric reconstruction (with turnover rates) predicted protein levels without expression data as inputs and imposed constraints. Furthermore, the ability to explicitly model protein translocation and compartmentalization of proteins is a significant advancement for genome-scale models, as it alleviates the need for fixed demands for the newly reconstructed content. In combination with the membrane constraint, proteomic predictions represent a milestone for constraint-based modeling. As an example, iJL1678-ME could be utilized for designing fine-tuned engineered strains by identifying how the membrane proteome may react to overexpression of non-cytoplasmic proteins and for determining ways to counteract undesired effects through selective gene manipulation. Through exploration of modeled membrane formation contextualized within protein translocation and metabolism, iJL1678-ME demonstrated that bottom-up systems-biology can be used to predict and analyze cellular physiology, thereby providing an opportunity to assist and supplement research on fundamentally challenging areas which may otherwise be difficult to study.

Improvements in iJL1678-ME are likely to come through further experimental evidence. For example, more elucidation is required on the exact stoichiometry of TatA proteins per substrate and complex before such information can be incorporated into iJL1678-ME. Other ME-model based reconstructions may include a module to simulate plasmid induction and subsequent protein secretion. Finally, iJL1678-ME’s predictive capabilities could be improved by incorporating data types such as ribosome profiling, quantitative proteomics, and additional kcat values. In conclusion, ME-models with compartmentalization and membrane constraints open exciting new avenues for the use of genome-scale models to interpret biological functions, to form the basis for strain designs, and understand infectious disease.

Methods
Reconstruction
A metabolism and gene expression model of E. coli, retroactively named here iOL1650-ME following an established convention [8], was used as the starting basis on which protein translocation reconstruction was built upon [7].

Literature review led to identification of five main translocation pathways plus three alternate assisting pathways.
proteins. These pathways were developed into template reactions to which each of JIL1678-ME’s pORFs could be applied to (Additional file 1).

Based on subcellular location annotations in Echolocat, EcoCyc and Uniprot (discrepancies and unknowns settled through PSORTb and TMHMM), all pORFs and protein complexes were assigned to one of four compartments: Cytosol, inner membrane, periplasm, and outer membrane [9,37-40]. The inner and outer membrane compartments are new additions to iOL1650-ME. New genes were also added to allow protein translocation and lipoprotein biogenesis. Reactions in iOL1650-ME were modified so that all proteins are compartmentalized. Furthermore, reactions were curated to ensure that reactions account for physical barrier membranes present. For example, if a reaction involves metabolites located in the cytoplasm and the periplasm, an inner membrane protein must be present for the reaction to occur.

Proteins with known experimental evidence were assigned to their respective translocase pathways. Based on these known peptides and current hypotheses, a set of rules was developed so that proteins without an experimentally-validated pathway could be assigned to one. These rules were established primarily by annotated subcellular location and secondarily by the type of protein (Additional file 2: Table S4). However, each pathway operates at its own speed. iOL1650-ME’s coupling constraints offer a solution for this problem, as the coupling constraints put limits on fluxes by linking reactions to enzyme degradation and the catalytic rate $k_{cat}$ (see Additional file 1 for basic formulation and example) [7]. Using this established constraint, turnover rates were applied to the translocase pathways to improve the model’s ability to predict the membrane proteome (see Additional file 3: Figure S2 for translocation without $k_{cat}$). Key proteins of each pathway had calculated proteome (see Additional file 3: Figure S2 for translocation without $k_{cat}$). Key proteins of each pathway had calculated turnover rates, and these $k_{cat}$ values were applied to all other enzymes in the pathway that have an interaction with that enzyme. The turnover rates of SecA, LolCDE, Bam, and Tat were all known from literature while the turnover rate for the SRP pathway was assumed to be equal to ribosome translation because of co-translational translocation [42-46]. For Tat-translocated proteins, a best fit polynomial equation for the number of TatA’s verses average channel diameter was used to calculate the number of TatA’s required for each [23]. Protein diameter was calculated by multiplying molecular weight by 1.21 to get volume and assuming a sphere shape [52]. Values were rounded up to the nearest integer.

Lipoprotein biogenesis was also determined to be relevant, and thus was included in the reconstruction process. The model has the flexibility to choose fatty acids from any available phospholipid. The proteins are modified by Lgt, Lsp, and Lnt to become lipoproteins (Additional file 1).

Murein demand was adapted from the original iOL1650-ME model. However, since it is known that 45% of murein is recycled, the model is forced to utilize the muropeptide transporter (AmpG), which has been implicated in the process of murein recycling [50], so that the flux of transported murein peptides is 45% of the murein demand (0.01389 mmol gDW$^{-1}$).

### Outer membrane porins

As many as $2 \times 10^5$ porins have been determined to be in the outer membrane [10]. Thus, to accurately account for these pathways, the outer membrane porins were coupled with diffusion rates [48,72,73]. In JIL1678-ME, the $k_{cat}$ values of the outer membrane porins are individualized for every combination of solute and porin, producing unique reactions reflecting effective diffusion rates based on diameters of solute and porin (Additional file 1, Additional file 2: Table S4). To calculate the concentration difference between the extra-cellular environment and the periplasm, only porins with calculated effective diameters remained in the model (Additional file 1). The diameters for all possible solutes were calculated using MarvinSketch assuming (1) the solutes were suspended in water (solvent radius: 1.4 Å) and (2) the solvent accessible surface area was a sphere, MarvinSketch 6.1.0, 2013, ChemAxon (http://www.chemaxon.com). With all the values known and inputted, this leaves the concentration difference between the extra-cellular ($C_o$) and periplasm ($C_p$), $C_o - C_p$, as the sole variable. Using an initial batch culture simulation in glucose M9 minimal media with the assumption $C_p << C_o$, the total flux of metabolite passage through outer membrane porins was calculated. Using JIL1678-ME’s flux results of outer membrane trafficking, the known number of porins ($2 \times 10^5$ per cell) [10], the solute diffusion rate through porins, and the porin constrain equations (Additional file 1), a series of simulations with varying total solute concentration differences were run to estimate the approximate difference to such that number of porins produced equals the experimental value [74]. This concentration difference, $6.5 \times 10^{-4}$ was incorporated into the porin diffusion rates as the default value, which may be adjusted by the user.

### Updating parameters

In order to determine how much more cellular mass JIL1678-ME explicitly accounts for, RNA-seq was first assumed to be a one-to-one proxy for protein expression levels, and in this dataset, the new pORFs and outer membrane proteins summed to 9.5% of all proteomic mass. As a comparison point, the outer membrane protein mass, i.e. lipoproteins and porins, was experimentally derived to be 7.4% of total proteomic mass [10]. Supplementing 7.4% with the estimated mass of protein translocases and lipoprotein biogenesis proteins from RNA-seq (as there were no experimental protein estimates available in literature)
summed to 9.2% of total proteomic mass, which is similar to 9.5%.

The GAM (growth associated maintenance) was updated to account for the amount of ATP used in protein translocation. The ATP flux used in protein translocation by SecA and LolCDE was calculated and subtracted from the GAM value established in iOL1650-ME, reducing it from 35 to 34.98.

Membrane constraints

The combined surface area (SA) of membrane proteins, phospholipids (PE is phosphatidylethanolamine, PG is phosphatidylglycerol, and CLPN is cardiolipin), and lipopolysaccharides (LPS) must equal the total surface area of a cell (equation 1) times four membrane leaflets (equation 2) [54,75]. The surface area of each membrane molecule was determined by its classification (Additional file 2: Table S5). If the molecule was a protein, the protein was assumed to extend through the lipid bilayer and occupy twice the amount of calculated surface area. An additional constraint was imposed so that phospholipid composition would better reflect the diversity of known membranes (equation 3).

\[
SA(\mu) = 0.456\pi \times 2^{\frac{\ln(\mu)}{3}} \times \left(3.9 \times 2^{\frac{\ln(\mu)}{3}} - 0.456 \times 2^{\frac{\ln(\mu)}{3}}\right) + \left(0.912\pi \times 2^{\frac{\ln(\mu)}{3}}\right)^2
\]

\[4 \times SA(\mu) = \sum_{i \in \text{proteins}} SA \text{ of membrane}_{\text{protein}_i}
+ \sum SA \text{ of LPS}
+ \sum SA \text{ of phospholipids}
\]

\[
\sum SA \text{ of phospholipids} = 77\% \times \sum SA_{PE}
+ 18\% \times \sum SA_{PG}
+ 5\% \times \sum SA_{CLPN}
\]

Additional constraints in the iJL1678-ME include a variable maximum cap on protein surface area and the option to force the model to produce nonfunctional membrane protein.

This cell envelope demand for LPS and lipids originally appearing in iOL1650-ME was removed, which makes the production of these two types of molecules a function of growth rate, protein production, and membrane size. Membrane size was taken to growth-rate dependent as formulated by O’Brien et al. (see [7] supplemental materials).

Analyzing the model

The model was run using batch simulations, as described by O’Brien et al. using resources of the National Energy Research Scientific Computing Center, which is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC0205CH11231 [7]. For all analyses performed, the in silico media composition was M9 and an excess of glucose (4 g L\(^{-1}\)) (Additional file 2: Table S6).

Since membrane proteomics is difficult to study; it is even more difficult to obtain absolute numbers comparing relative ratios of protein amounts. Therefore, RNA-seq was used as an in vivo proxy for comparison (GEO accessions: GSE48324 [58] and GSE61327 [59]). A 1:1 ratio of protein expression levels to RNA-seq levels (FPKM normalized to overall expression) was assumed. Mass was calculated based on the atomic mass of the primary protein structure multiplied by the flux of protein being produced. In comparing in vivo data to in silico data, mass was summed up by compartment location, functional annotation, or both (Additional file 2: Table S2). Error bars are 1 standard deviation from two RNAseq runs.

The mass of compartmentalized functional annotations between in vivo and in silico data was compared on a log-log basis. A simple linear regression model was calculated between the two datasets. The standardized residuals (residual i / standard deviation of residual i) of the in silico data was plotted against a rankit score (expected values of the order statistics if the sample is normally distributed), creating a normal probability plot. A line passing through the first and third quartiles revealed points that deviated from a normal distribution (i.e. deviated from the quartile line). These points were removed from the dataset for further analysis and the simple linear regression model was recalculated for the reduced dataset.

Protein inhibition

To adjust the turnover rate of SecA, the coupling constraint was modified so that it would reflect numbers lower than the published value of 4.0 s\(^{-1}\) [43]. Similarly, all coupling constraints involved with SecYEGDF or ribosome were multiple by fractions to lower enzyme efficiencies. To limit membrane inner membrane protein surface area, the variable maximum cap (included as part of the membrane constraint formulation) was set to 0.5.

Ethics

This research did not involve human subjects, human material, human data, animals, or plants.

Additional files

Additional file 1: Supplemental Methods. In-depth details describing the methodology behind the reconstruction process.

Additional file 2: Supplemental Tables. Tables describing the changes and numbers used during reconstruction compiled into a single excel file. The table of contents is listed on the first sheet.
Additional file 3: Supplemental Figures. Supplementary figures and legends describing background analysis to accompany the figures in the paper.

Additional file 4: UJL1678-ME. The UJL1678-ME model in python pickle format along with sample python scripts and a README file to run a simulation.

Abbreviations

IOL1650-ME: A genome-scale metabolic and gene expression model of E. coli, IOL1678-ME: A genome-scale metabolic and gene expression model of E. coli including compartmentalization and protein translocation based of the IOL1650-ME model; pORF: Protein-coding open reading frame.

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

JKL: Conducted experiment, analyzed data, wrote manuscript. JAL: Provided research and analysis assistance, reviewed manuscript. EJO

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