System-level analysis of *Salmonella* metabolism during infection
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Infectious diseases represent a major threat to human health. To develop urgently needed new control strategies, a transition from research focusing on individual factors to a more integrated system-level analysis might be needed. Such an approach faces great challenges and might require development of new concepts in large-scale data analysis. Here, I discuss for the well-characterized model pathogen *Salmonella*, how extensively studied metabolism can be used as a training field for infection biology at the systems level. Extensive experimental data can be analyzed in context using metabolic network visualization tools and in silico modeling based on genome-scale metabolic reconstructions. Suitable approaches to obtain still missing comprehensive quantitative data on *Salmonella* nutrition in infected host tissues are described. Such an integrated investigation of *Salmonella* metabolism during infection will enable an unprecedented large-scale understanding of pathogen in vivo activities, help to evaluate concepts and strategies for system-level analysis of host/pathogen interactions in general, and provide a basis for rational development of novel antimicrobials and efficacious live vaccines.

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**Introduction**
Infectious diseases represent a major worldwide threat to human health [1]. Novel strategies to combat infectious disease are urgently needed because of rising resistance of pathogens to antimicrobial therapy, an increasing number of immunosuppressed patients that are highly susceptible to infection, increasing travel that enhances worldwide transmission of novel and re-emerging pathogens, and potential bioterrorism threats.

The substantial progress in infection biology research in the last two decades could provide a basis for novel control strategies. In particular, genomes of all major human pathogens as well as human and animal model hosts have been sequenced, a large number of pathogen virulence factors as well as antimicrobial immune mechanisms have been identified and understood in molecular to atomic detail, and pathogen and host responses have been studied using global approaches including transcriptomics and proteomics [2–8]. However, it has remained difficult to translate this extensive knowledge into effective new control strategies [9]. Specifically, efficacious vaccines are still lacking for major pathogens [10–12], and the dramatic decline in the development of novel antimicrobials over the last 20 years [13] is likely to substantially reduce treatment options in the near future [1].

**The need for system-level infection biology**
One potential reason why it is so difficult to translate basic research to effective strategies for combating infectious diseases could be the prevailing focus on the action of individual pathogen or host components. While this reductionist approach was highly successful to identify and characterize key virulence and immune factors [14,15], it cannot explain the course of complex multifactorial infectious diseases involving hundreds of interacting pathogen and host factors. Integration of the vast existing knowledge and development of appropriate methodology to analyze interacting host/pathogen networks will be required to facilitate rational development of new control strategies [16].

Such a system-level approach is technically challenging since hundreds of pathogen and host factors need to be simultaneously analyzed. An integrated approach to infection is also conceptually difficult because two systems (pathogen and host) and their interactions need to be understood. The new field Systems Biology is providing novel concepts and methodology that could help to meet these challenges. In fact, infection is a particularly fascinating research area for Systems Biology since one can study interactions of two coevolved systems with major importance for human health [16,17].

**Metabolism: a suitable subsystem**
A comprehensive system-level description of infection is currently unfeasible as complete in silico models are even lacking for the extensively studied, relatively simple bacterium *Escherichia coli*. However, one could start with a well-characterized subsystem to develop appropriate research concepts and technology. Metabolism is a particularly well-suited subsystem that has been studied in prokaryotes and eukaryotes for many decades at the level of enzymes, reactions, metabolites, and fluxes [18]. Because of this knowledge it is now possible to build in...
silico models of E. coli metabolism that describe quantitative interactions of more than 1200 metabolic enzymes, more than 2000 reactions, and more than 1000 metabolites [19]. Importantly, these models correctly predict more than 90% of several thousand experimentally determined E. coli metabolic mutant phenotypes. Analogous complex models for eukaryotic metabolic networks are similarly successful [20]. These data demonstrate that metabolism is by far the best understood biological network, and thus provides an excellent framework to investigate host-pathogen interactions at the system level.

Metabolic host–pathogen interactions have not been a focus of infection research in the past [21], but interest has been markedly stimulated by recent findings that demonstrate a major impact of metabolism on both acute and persistent infections [22,23,24], and a direct link to virulence [25–27]. Moreover, metabolic enzymes are attractive targets for the development of novel antimicrobials [9]. Complementary experimental approaches to analyze pathogen metabolism in infected cells/tissues at the system level are being developed [28,29].

Salmonella as a model pathogen
Salmonella is a prime model organism for Systems Biology of Infection because it is highly similar to well-characterized E. coli, Salmonella microbiology and molecular biology are simple, excellent mouse disease models for human salmonellosis have been developed, and diverse aspects of Salmonella biology and virulence properties are covered by extensive literature.

Salmonella can cause either diarrhea or a life-threatening systemic disease called typhoid fever [30], which kills more than 200,000 children each year [31]. Cell culture infection models reproduce important aspects of host cell invasion and intracellular Salmonella replication, which are hallmarks of human diarrhea and typhoid fever. On the other hand, not all relevant conditions in infected tissues can be reproduced in cell culture, and diverging in vitro/in vivo mutant phenotypes [32] can result in misinterpretation. Animal infection models more closely mimic the complex and dynamic environment in human patients. Importantly, available Salmonella mutant phenotypes in human patients correlate well with data from animal infections [33].

Animal disease models for human diarrhea have been developed in large animals such as cattle [30] and more recently, also in mice [34]. More extensive experimental data, however, are available for the mouse model of typhoid fever [30] and this review will, therefore, focus on this model. Mice are natural hosts for systemic Salmonella infection with histopathological features highly similar to human typhoid fever. Commonly used inbred mouse lines such as BALB/c or C57B/6 are highly susceptible to systemic salmonellosis because of a dys-
functional slc11a1 allele, while mice carrying a wild-type slc11a1 allele are rather resistant [35]. Resistant mice are probably more suitable for investigating pathogen/host coevolution, while susceptible mice enable analysis of rapidly progressing, ultimately lethal disease. Moreover, because of a traditional focus on BALB/c mice as host model, by far more data are available for salmonellosis in susceptible compared to resistant mice. As a consequence, system-level analysis is currently more easily done in susceptible mice.

**Experimental data**

Multiple sources of experimental data provide information on *Salmonella* metabolism during infection.

**Biochemistry**

More than 20 currently available complete genome sequences of *Salmonella* strains provide a comprehensive list of metabolic enzymes principally encoded by *Salmonella*. There are different levels of functional characterization among these enzymes.

Ninety-one *Salmonella* enzymes have been purified and biochemically characterized according to the BRENDA enzyme database [36]. In many cases, kinetic parameters were measured for multiple possible substrates. This detailed functional information is highly valuable to understand and predict biochemical capabilities of *Salmonella* strains. However, even this high-quality characterization might still be incomplete. In particular, important natural substrates might have been missed in some cases, kinetic parameters measured *in vitro* in dilute buffers might differ from more relevant *in vivo* conditions with very high protein concentrations, and enzymes might have additional not yet discovered activities in unrelated pathways or even outside metabolism (‘catalytic promiscuity’ and ‘moonlighting activities’) [37].

Some 1053 *Salmonella* enzymes have closely related *E. coli* orthologs for which manually curated, continuously updated high-quality annotations are available in the EcoCyc database [38*]. This database contains summaries and direct links to available protein structures, enzymatic properties, and regulation of each enzyme. Additional information is available in other databases such as KeGG [39]. In the vast number of cases, orthologous enzymes in *Salmonella* and *E. coli* share similar properties and functions.

In addition to these well-characterized enzymes, *Salmonella* genomes contain several hundred genes with sequence properties typical for enzymes, but unknown specificity. *In silico* prediction of potentially catalyzed reactions is difficult, since single amino acid substitutions around the active site can dramatically alter substrate recognition. Experimental data based on large-scale mutant phenotyping [18] as well as high-throughput biochemical assays using purified proteins [40] can be informative, but in most cases in-depth biochemical analysis will be required to identify likely natural substrates and reactions. Although most such ‘novel’ enzymes will affect only peripheral pathways (most central reactions have been extensively characterized), accurate annotation will be required to obtain comprehensive metabolic network models.

**Enzyme expression (transcriptomics/proteomics)**

Enzymes encoded in the *Salmonella* genome constitute the metabolic network that is principally available for nutrient utilization and biosynthesis. Under any given specific environment, however, only a subset of these enzymes is usually synthesized and this imposes an important constrain [41] on *Salmonella* metabolic capabilities. Large-scale *Salmonella* transcriptome and proteome data sets have recently been obtained for a variety of conditions. Some data are available for *Salmonella* interacting with mammalian host cells in cell culture models [42–44] or infected animals [29**], providing an increasingly comprehensive picture about what enzymes and pathways are used by *Salmonella* during infection. Interestingly, these data reveal hundreds of highly *in vivo* expressed metabolic enzymes indicating allocation of major *Salmonella* resources into metabolism during infection.

Many enzyme activities are regulated by post-translational modifications such as phosphorylation with important consequences for metabolite fluxes. Although this has not yet been comprehensively investigated in *Salmonella*, suitable technologies to analyze protein modifications in bacteria are now available [45,46].

**Mutant phenotypes**

Even if an enzyme is expressed and active, it might not be functionally relevant for *Salmonella* virulence. To determine functional relevance, one needs to assess virulence phenotypes of *Salmonella* mutants lacking a specific enzyme. A large number of such mutants have already been constructed and tested in animal disease models [29**,47], and large unbiased transposon mutant libraries and, most recently, a large collection of defined mutants have been screened for avirulent clones using negative selection in infected animals [48,49,50*,51*]. Virulence attenuation in metabolic mutants suggests that in wild-type *Salmonella*, the respective enzyme is expressed and functionally relevant at least at some stage of infection.

In addition to engineered mutants, the rapidly rising number of complete *Salmonella* genome sequences has revealed hundreds of pseudogenes, which are unlikely to encode functional enzymes because of frameshift mutations or premature stop codons. Such pseudogenes in virulent strains suggest dispensability of the corresponding enzymes during infection.
There are some caveats with mutant phenotype interpretation. In particular, allelic exchange might cause polar effects on genes downstream of the mutated locus, and transposon insertions can result in detrimental gene fusions [52,53]. In both cases, artificial virulence phenotypes could lead to misinterpretation. Successful complementation with wild-type alleles resulting in phenotype reversion minimizes such risks. Even then, attenuation of enzyme mutants might not always simply indicate the high relevance of an associated enzymatic reaction but could also reflect moonlighting activities, dysregulation of other pathways, or accumulation of toxic upstream metabolites (see below).

On the other hand, the lack of detectable phenotypes does not prove absence of any in vivo fitness gain. Most animal infection studies fail to reveal growth rate defects of less than 5% because of limited sensitivity and experimental variation. Moreover, pseudogenes in field isolates might cause slight fitness defects but still become fixed because of small population bottlenecks during infection. Collectively, such weakly contributing enzymes could still have substantial impact on Salmonella virulence and disease progression.

Despite these caveats, the numerous available data clearly suggest that rather few metabolic enzymes have crucial importance for Salmonella virulence [29**]. This has important practical consequences by limiting the number of promising targets for urgently needed new antimicrobials. Surprisingly, a large majority of enzymes that are detectable by ex vivo proteomics, only moderately contributes to virulence (and thus have little potential as antimicrobial targets). Some detectable enzymes and a large number of enzymes with abundance levels below the current proteomics sensitivity threshold have even no detectable virulent phenotype at all. This apparent discrepancy between large protein resources allocated to metabolism (see above), but limited functional relevance of metabolic enzymes for Salmonella virulence remains a major open question.

### Integrated network analysis

The rapidly accumulating experimental data sets on Salmonella metabolism during infection are highly informative, and also pose substantial challenges for understanding and interpretation. Specifically, there is no simple, intuitive approach to extract biological meaning from lists with hundreds of enzymes and virulence phenotypes.

### Network visualization

A useful first step for integrative analysis of metabolism data is their visualization in a metabolic network chart. Among several possibilities, the freely accessible database BioCyc offers a particularly useful visualization platform that allows comprehensive highlighting of metabolites and enzymes detected in large-scale experimental data sets [29**,38]. Such visualization enables one to identify pathways that are represented by several highly in vivo abundant enzymes and thus might be relevant for virulence. As an example, many Salmonella fatty acid biosynthesis enzymes were highly expressed in vivo. On the basis of this finding, a fabC mutant that is unable to synthesize unsaturated fatty acids was constructed and found to be avirulent in the mouse typhoid fever model [29**]. Network visualization also helps to identify enzymes that are involved in several pathways potentially complicating phenotype interpretation. Finally, integration of expression data and mutant phenotypes in a common metabolic network chart facilitates identification of well-investigated data-rich versus poorly understood aspects. An updated overview of Salmonella metabolism in systemically infected mice highlights cofactor biosynthesis, anaerobic energy metabolism, and degradation of diverse nutrients as topics that merit further exploration (data not shown).

Network visualization is a powerful tool to interrogate diverse experimental data sets, but the large number of fine details can obscure general aspects of network architecture and its functional implications for Salmonella infection biology. To understand Salmonella in vivo metabolism at a more integrated level, it can be useful to group enzymes and pathways into broader categories. One popular higher level concept represents metabolism as a bow–tie network [54]: multiple nutrient degradation pathways form input channels that provide small building blocks and energy currencies. These inputs are interconverted in a central intermediary metabolism core. From this core, each component of a new cell can be generated through biosynthetic pathways. While such a concept oversimplifies metabolism, it still captures important features of the network better than simple scale-free abstractions [55,56].

Interestingly, grouping of Salmonella metabolic enzymes according to this scheme revealed that almost all crucially important enzymes appear to be involved in one particular subclass of biosynthetic (output) pathways [29**]. Specifically, this subclass yields products that are not available from the mammalian host. This more integrated analysis thus reveals a simple rule to identify one Achilles heel of Salmonella in vivo metabolism for antimicrobial chemotherapy. On the other hand, it also reveals a remarkable resilience against perturbations in large parts of the metabolic network. Although this is fully compatible with the low number of strong metabolic mutant phenotypes in unbiased large-scale genetic screens (see above), the ultimate reasons for this remarkable robustness are not immediately obvious.

It is clear that Salmonella can adapt to a wide variety of environmental conditions. For example, it can use more than 80 different carbon sources [57] including many that
are not available in mammalian tissues (e.g. melibiose). *Salmonella* can also use multiple electron acceptors for anaerobic respiration, again including compounds with low abundance in mammalian tissues. It is not surprising that corresponding utilization and respiration pathways might be dispensable in systemically infected mice. Such condition-dependent enzyme essentiality/dispensability might account for a major part of metabolic robustness as it does in other microbial systems [58**] but so far, a quantitative analysis for *Salmonella* is lacking.

More interestingly, even many enzymes that are synthesized and active during infection (based on proteomics data and mutant phenotypes), contribute only weakly to *Salmonella* virulence. This limited functional relevance might account for a major part of metabolic robustness as it does in other microbial systems [58**] but so far, a quantitative analysis for *Salmonella* is lacking.

**In silico** modeling

Because of the vast complexity of metabolic networks, a system-level understanding cannot easily be achieved using manual analysis of each individual enzyme. Instead, *in silico* simulation of genome scale metabolic models is a powerful approach to systematically interrogate all possible perturbations and to understand potential compensatory mechanisms (see ‘Introduction’ section). High-quality genome scale models have been generated for large number of bacterial pathogens, including a very recent *Salmonella* reconstruction [60]. On the basis of the most recent *E. coli* model [19**], we and others are currently generating a next generation, fully compartmentalized *Salmonella* metabolic network reconstruction.

Such reconstructions can be used to evaluate consequences of enzyme inactivation for defined biochemical capabilities of *Salmonella*. By analyzing metabolites flux distributions across the perturbed network one can rapidly identify compensatory mechanisms that buffer mutation phenotypes. However, to exploit the full power of integrated *in silico* network analysis, a realistic *in vivo* metabolism model that includes quantitative estimates about nutrient availability in host microenvironments, is required.

**Closing knowledge gaps: *Salmonella* in vivo nutrition**

Nutrient availability in host environments is a crucial aspect of further research on host–pathogen interactions and a prerequisite for powerful modeling approaches. Unfortunately, *in vivo* nutrition is still poorly understood for most bacterial pathogens [21**]. This is also true for *Salmonella* that replicates intracellularly in phagosomes of macrophages during systemic infection.

Recent cell culture data suggest that external metabolites that enter host cell endocytotic pathways become rapidly available in the *Salmonella*-containing phagosome [61]. It is thus possible that *Salmonella* nutrition reflects interstitial fluid composition in infected tissues. However, endosomes carry potent nutrient transport systems that will selectively deplete endosomal luminal contents [62,63]. Direct experimental analysis of endosomal nutrient content in cells from infected tissues is exceedingly difficult. On the other hand, indirect approaches based on nutrient-inducible promoter activities or virulence phenotypes of auxotrophic and nutrient utilization mutants, might still yield informative semi-quantitative data.

**Nutrient-induced gene regulation**

Specifically, several *Salmonella* promoters are known to respond to the presence of specific nutrients in the environment. Quantitative measurements of *in vivo* promoter activities using reporter fusions [64], as well as analysis of corresponding transcript or protein levels can thus provide hints about nutrient *in vivo* availability. However, regulatory networks can be complex making simple interpretations difficult. In particular, metabolic enzymes usually respond to both specific and general signals such as catabolite repression, and many degradative enzymes are highly expressed even in complete absence of the corresponding nutrient [65]. Transcriptomics and proteomics thus offer limited information on nutrient availability.

**Auxotrophic mutants**

Mutants that depend on external supplementation with defined nutrients (auxotrophs) are more informative. As an example, a *Salmonella purH* mutant requires an external source of purines for growth. The fact that this mutant can grow in infected mice and cause lethal infection [66] indicates that purines must be available in host microenvironments. In addition to this qualitative evidence, it is even possible to derive coarse estimates for purine influx into *Salmonella* cells *in vivo*. Specifically, a *Salmonella* cell contains approximately $58 \times 10^6$ purine molecules (based on *E. coli* data [19**]). Combined with the *in vivo* generation time of some 8.7 hours for a purH mutant [29**], this suggests that *Salmonella* obtains on average at least about 1800 purine molecules cell$^{-1}$ s$^{-1}$ from the host environment. Obviously, this is only a lower limit as some purines might be lost to wasteful side reactions in the *Salmonella* cell. Similarly, the widely used strain SL1344 is a histidine auxotroph [67]. On the basis of its generation
time of about six hours [29**], and the demand of about
12 × 10^6 histidine molecules per cell [19**], this
suggests a supply of at least 500 histidine molecules
cell⁻¹ s⁻¹ from the host environment. At present, quan-
titative growth data are available for 22 auxotrophic
Salmonella mutants from which limits of incoming
fluxes for about 10 different host nutrients can be
estimated. Because of substantial variation in animal
experiments and uncertainties in actual nutrient
demand, such estimates have substantial error margins
but still provide useful starting points for in silico
analysis.

Impact of nutrient utilization defects
Salmonella can utilize at least 82 different metabolites as
carbon sources [57] and 69 of these are potentially avail-
able in mouse tissues. Salmonella expresses degradative
enzymes for many of these nutrients during infection
[29**] suggesting that at least some of them might be
relevant in vivo. Many potential carbon sources are them-
selves nonessential for Salmonella. As a consequence,
there are no auxotrophic Salmonella mutants to interro-
gate availability of such nutrients in host microenviron-
ments. Instead, one can measure the in vivo growth rate of
Salmonella mutants unable to utilize a particular carbon
source. If that carbon source is an important contributor to
Salmonella nutrition during infection, such a mutant
should have a lower in vivo growth rate compared to
wild-type Salmonella.

To block nutrient utilization one could delete non-redu-
dant enzymes involved in the corresponding cytoplasmic
degradation pathway. However, such mutants can have
complex phenotypes because of accumulation of toxic
intermediates. As an example, mutants that lack UDP-
galactose-4-epimerase (GalE) cannot utilize galactose as a
carbon source but in addition, are even galactose-sensi-
tive because of accumulation of toxic galactose-1-phos-
phate [68] (which is rapidly degraded in wild-type
Salmonella containing functional GalE). Attenuation of
galE mutants could thus be caused by the loss of galactose
as an exploitable important carbon source, or by small
(nutritionally irrelevant) galactose levels in the host
microenvironment that result in accumulating toxic galac-
tose-1-phosphate if further degradation is blocked.
Accumulating toxic intermediates have also been
observed for other carbohydrate degradation pathway
mutants [69]. These non-informative phenotypes prevent
carbon source evaluation.

A more appropriate strategy to identify relevant carbon
sources involves mutations that block nutrient uptake. As
the nutrient does not enter Salmonella, it cannot be
converted into a potentially toxic intermediate. Even if
minor quantities of that nutrient are taken up through
some other transporters, a functional cytosolic degra-
dation pathway would prevent accumulation of toxic
intermediates. Although transporter mutant phenotypes
are thus more informative, suitable inactivation strategies
are still demanding because of a large number of uptake
mechanisms for almost all potential carbon sources. As an
example, glycerol-phosphate can be taken up by transporters
[UgpC]₄[UgpA][UgpE][UgpB] (ABC transporter) and GlpT (major facilitator superfamily) but could also be
dephosphorylated in the periplasm to glycerol that could
enter through GlpF (major intrinsic protein channel) or
even freely diffuse through the inner membrane. As
another example, glucose can be transported not only
by the glucose PTS system but also by the mannose PTS
system as well as GalP and [MglA]₂[MglC]₂[MglB] galac-
tose transporters. Inactivation of single transporters is
thus insufficient for determining the relevance of glycerol
or glucose as carbon sources. Instead, multiple mutations
inactivating all potentially involved transporters are
required. We and others [70] currently use such multiple
mutation strategies to determine the in vivo relevance of
diverse carbohydrates.

A combination of these and alternative approaches is
likely to yield a detailed and semi-quantitative picture
of Salmonella nutrition in infected host microenviron-
ments. Even at the current stage, available experimental
evidence suggests Salmonella access to a wide variety of
diverse host nutrients available at small but significant
quantities [29**]. This qualitatively rich (but quantitati-
vely poor) host environment contributes to Salmonella in
vivo robustness against metabolic perturbation. The
relative importance of environmental versus internal
causes of metabolic robustness can be assessed with
the genome-scale in silico metabolism network model
once we have comprehensive quantitative data on all
important host nutrients.

Translational aspects of Salmonella metabolism
Pathogens nutrition and metabolism are key aspects of
infectious diseases. A detailed and comprehensive under-
standing thus represents a fundamental goal of basic in-
fec tion biology. In addition to these basic research aspects,
comprehensive understanding of Salmonella in vivo metab-
olism might also help to develop new control strategies.

Predicting novel antimicrobial targets
In particular, large-scale experimental data combined
with in silico modeling will help us to understand why
only a very few among thousands of metabolic enzymes
can be used as suitable targets for antimicrobial che-
motherapy, and how this remarkable robustness might
be overcome. As an example, inhibition of enzyme A
might be buffered by an alternative pathway. In silico
simulations can rapidly identify this pathway and some of
its key enzymes that, when inhibited together with
enzyme A, could cause a synthetically lethal Salmonella
phenotype. In this way, in silico modeling can reduce the
enormous number of possible target combinations to a more manageable number of promising candidates for subsequence experimental validation. Novel antimicrobial combination targets could facilitate the development of urgently needed new antibiotics [71].

Fine-tuning of Salmonella live vaccines

In addition, a detailed quantitative understanding of Salmonella metabolism during infection could help to modify colonization capabilities of live attenuated Salmonella vaccines. Such vaccines can induce potent protective immune responses both against Salmonella antigens [72,73] as well as heterogeneously expressed antigens from other pathogens [74]. However, it is important to limit adverse effects such as fever or bacteremia in vaccinees and household contacts that may become infected by live Salmonella shed after vaccination. On the other hand, overattenuation will result in low bacterial loads and poor immunogenicity. Efficacious live Salmonella vaccines thus require a fine balance of Salmonella in vivo replication capabilities.

There is a long history of using metabolic mutations in Salmonella to generate promising vaccine strains [67,75]. One interesting recent example utilizes a manA mutation to terminate Salmonella in vivo replication after a few generations [76]. Salmonella manA is auxotrophic for mannose, which is present in rich media used for growing vaccine inoculum doses. However, once Salmonella manA enters mammalian tissues, it has no longer access to sufficient quantities of mannose from the environment [77]. It will thus use up internal mannose stores during a few division cycles, and then stop to grow. Large-scale experimental in vivo data sets and in silico modeling will enable systematic and comprehensive evaluation of alternative strategies to modulate in vivo replication of attenuated Salmonella vaccine strains.

Conclusion

Salmonella metabolism during infection offers a great training field for system-level analysis of host–pathogen interactions. In particular, we can learn how to interpret large-scale data on pathogen in vivo properties in context, how to identify data we are still missing for a deeper understanding, and how in silico modeling can enable us to comprehensively interrogate networks containing thousands of components. Ultimately, this approach could offer an unprecedented level of integrated understanding of hundreds of pathogen in vivo activities. On this basis, it might be possible to rationally develop urgently needed new strategies for the control of infectious diseases.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


This highly refined in silico genome-scale metabolic reconstruction of E. coli is valuable for comparison with Salmonella metabolism. Moreover, multiple ways to interrogate large metabolic networks are described.

One of the first studies demonstrating crucial importance pathogen metabolic activities in an important infectious disease.


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