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**Multi-level Omics Analysis Provides Insight to the *Ignicoccus hospitalis*—*Nanoarchaeum equitans* Association**

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## Abstract

**Background:** Studies of interspecies interactions are inherently difficult due to the complex mechanisms which enable these relationships. A model system for studying interspecies interactions is the marine hyperthermophiles *Ignicoccus hospitalis* and *Nanoarchaeum equitans*. Recent independently-conducted ‘omics’ analyses have generated insights into the molecular factors modulating this association. However, significant questions remain about the nature of the interactions between these archaea.

**Methods:** We jointly analyzed multiple levels of omics datasets obtained from published, independent transcriptomics, proteomics, and metabolomics analyses. DAVID identified functionally-related groups enriched when *I. hospitalis* is grown alone or in co-culture with *N. equitans*. Enriched molecular pathways were subsequently visualized using interaction maps generated using STRING.

**Results:** Key findings of our multi-level omics analysis indicated that *I. hospitalis* provides precursors to *N. equitans* for energy metabolism. Analysis indicated an overall reduction in diversity of metabolic precursors in the *I. hospitalis*—*N. equitans* co-culture, which has been connected to the differential use of ribosomal subunits and was previously unnoticed. We also identified differences in precursors linked to amino acid metabolism, NADH metabolism, and carbon fixation, providing new insights into the metabolic adaptations of *I. hospitalis* enabling the growth of *N. equitans*.

**Conclusions:** This multi-omics analysis builds upon previously identified cellular patterns while offering new insights into mechanisms that enable the *I. hospitalis*—*N. equitans* association.

**General Significance:** Our study applies statistical and visualization techniques to a mixed-source omics data set to yield a more global insight into a complex system, that was not readily discernable from separate omics studies.

## Introduction

Benefits from systems-level analysis of transcriptomics, proteomics, and metabolomics data have yielded fruitful insights into complex systems and provide a more comprehensive analysis of cellular activity [1–5]. In addition to analyzing single cell types or single organisms, systems-level ‘omics’ data analysis is particularly beneficial when attempting to better characterize interspecies relationships and associations. While systems biology research has made great strides in strengthening our understanding of complex biological systems, detailed knowledge of fundamental molecular interaction mechanisms, cellular communication, and nutrient exchange within microbial communities is still limited. Multi ‘omics’ studies provide additional insights into critical cellular pathways and mechanisms of interspecies communication within a microbial community. These insights are however not readily accessible through single ‘omics’ data interpretation. Recent studies employing systems-level analyses have demonstrated the potential of these methods to better understand the fundamental properties of microbial communities in soil, water treatment plants, the human body, and the nature of interspecies interactions [6–9].

A microbial system whose complex cellular organization is lacking in comprehensive understanding of its molecular networks, and would benefit from a systems-level multi-omics analysis to better understand the nature of its interspecies interactions, is the archaeal system comprised of *Ignicoccus hospitalis* and *Nanoarchaeum equitans*. *I. hospitalis* is a hyperthermophilic chemolithoautotroph isolated from hydrothermal marine vents located off the coast of Iceland. This organism derives its energy from the reduction of elemental sulfur to hydrogen sulfide, and utilizes carbon dioxide as its sole carbon source [10]. *I. hospitalis* has one of the smallest genomes of any free-living organism, with just under 1,500 protein-coding genes,

and is the only identified and characterized host for the small, hyperthermophilic archaeal organism, *N. equitans* [11,12]. The *N. equitans* genome is too small to support life independently, containing only 556 protein-coding genes [13], and notably lacking enzymes catalyzing bioenergetic pathways essential for independent growth. *N. equitans* is thus incapable of survival without physical attachment to and co-existence with *I. hospitalis* [11,14,15]. Furthermore, while the attachment of *N. equitans* slows the growth of *I. hospitalis*, causing early entry into stationary phase, *N. equitans* does not appear to have significant deleterious effects on *I. hospitalis*'s physiology and survival [14]. Physical attachment and co-culture growth appear to satisfy *N. equitans*'s requirement for anabolic precursors, whereby the import of metabolites and energy-rich small molecules from *I. hospitalis* compensates for cellular processes that cannot be independently supported by the cellular components encoded within *N. equitans*'s genome. However, the molecular mechanisms enabling transfer or exchange of nutrients and cellular precursors between the two species have remained elusive [16,17]. A similar nanoarchaeal symbiotic system recently isolated from a terrestrial geothermal system indicates that this type of interspecies interactions is not restricted to *Ignicoccus* and *Nanoarchaeum* species, and may be widespread in high temperature environments [18].

Recent transcriptomics, proteomics, and metabolomics analyses have focused independently on better understanding the nature of the inter-species interactions between *I. hospitalis* and *N. equitans*. These studies have characterized molecular differences between cell cultures of *I. hospitalis* grown in the absence or in co-culture with *N. equitans* [12,15,16,19]. Through these efforts, insights into energy transfer, growth processes, and metabolic coupling have been identified, suggesting that *N. equitans* exploits *I. hospitalis* primarily to obtain molecules that can be used as a source of metabolic energy rather than for *N. equitans* biomass

production. While these studies have been informative, much remains to be elucidated about the molecular mechanisms that enable this unusual inter-species association and the overall physiological coupling between these two organisms. Systems-wide analysis of multi-omics datasets presents an opportunity to strengthen our understanding of the molecular mechanisms driving the inter-species interactions shared between *I. hospitalis*—*N. equitans*.

In this study, we have utilized data generated from multiple functional genomics platforms, i.e. transcriptomics, proteomics, and metabolomics, to specifically uncover interaction mechanisms that were not apparent in previous studies that analyzed the data from each omics technology independently. Using the bioinformatics resources, Database for Annotation, Visualization, and Integrated Discovery (DAVID) and Search Tool for Retrieval of Interacting Genes/Proteins (STRING), transcriptomics, proteomics, and metabolomics datasets recorded on *I. hospitalis* and *N. equitans* were combined and analyzed at the systems-level [20,21]. DAVID is a web-based tool designed to extract biological meaning from large lists of genes and proteins by identifying functionally related groups through enrichment analysis, and has been widely used because of its versatility in handling various data formats as well as its efficient analysis of large datasets [22–24]. STRING complements DAVID by generating interaction maps highlighting and visualizing relationships between genes, proteins, and metabolites based on genomic context, experimental evidence, conserved gene co-expression, and text mining [25]. By combining transcriptomics, metabolomics, and proteomics data into an integrated dataset, a better understanding of the biological processes characterizing the *I. hospitalis*–*N. equitans* association has been achieved.

## **Methods**

## *Omics Data Sets*

The datasets used in this study were generated from separate transcriptomics, proteomics, and metabolomics studies performed previously. Detailed experimental methods are reported in the original publications [16,19], and a table of experimental details for each ‘omics’ study, outlining the number of replicates, is included in supplementary material (Supplementary Table 1). Briefly, each of these ‘omics’ studies were performed on microbial cells collected from *I. hospitalis*-only cultures and *I. hospitalis* – *N. equitans* co-cultures at late log phase of growth [19]. For transcriptomics analysis, cell pellets from each culture were homogenized in Trizol and purified using a PureLinkRNA Kit (Invitrogen, Carlsbad, CA, USA). RNA was converted to cDNA using a ds-cDNA Synthesis Kit (Invitrogen) and cDNA abundance was assessed using a high-density gene expression microarray (1plex, 385k) manufactured by Roche NimbleGen Inc. All arrays were performed in triplicate and were scanned with a SureScan HR DNA Microarray Scanner and quantified using NimbleScan 2.6 [19]. 1404 *I. hospitalis* transcripts were identified and expression abundances were normalized using Loess normalization [19]. The gene abundances for both cultures were compared for differentially expressed gene levels by an analysis of variance and were corrected for multiple comparisons (data accessible at <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57033>). These abundances were used to calculate fold changes for each gene between the two cultures [19].

Proteomics data were generated from intracellular protein analyses using online 2D-LC-MS/MS of label-free peptides pre-digested with trypsin in duplicate for each culture as reported in Gianonne et al. (2011, 2014). Peptides were identified with Myrimatch [26] using the combined *I. hospitalis*-*N. equitans* FASTA protein database (GenBank CP000816.1,

AE017199.1), and semi-quantitative differential analysis was based on spectral counting as originally reported [15]. Proteins encoded by 1154 genes of the *I. hospitalis* genome were identified. Normalized spectral abundance factor (NSAF) values for each protein were calculated based on number of spectra collected and protein length, and each NSAF value was normalized over average total spectra count over all sample sets (data accessible at <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3149612/bin/pone.0022942.s003.xls>). These normalized spectral count values were used to calculate fold changes in protein abundance between the *I. hospitalis*-only and *I. hospitalis*-*N. equitans* co-cultures [15].

To generate metabolite profiles, metabolites were extracted from cell cultures grown in triplicate using an aqueous MeOH extraction and analyzed by LC-MS and <sup>1</sup>H 1D NMR [16]. For LC-MS analysis, normal and reverse LC phase chromatography was employed using an UHPLC system connected to a Q-TOF mass spectrometer run in positive ion mode. The data were processed using Masshunter Qualitative software (Agilent, Santa Clara, CA) and analyzed using software package MZmine 2.10. NMR metabolite analyses were performed on biological duplicate samples, and 1D <sup>1</sup>H NMR spectra were acquired using a Bruker 600 MHz (<sup>1</sup>H Larmor frequency) AVANCE III solution NMR spectrometer. Resulting 1D <sup>1</sup>H NMR spectra were processed and analyzed using the Chenomx<sup>TM</sup> software version 7.6 (Chenomx Inc., Edmonton, AB, Canada) [16]. Separate analyses of the MS and NMR metabolomics data resulted in the unambiguous identification of 35 metabolites in the *I. hospitalis*-*N. equitans* culture and 39 in the *I. hospitalis*-only culture, and when combined, resulted in doubling the number of unambiguous metabolite IDs (data accessible at <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4529127/table/T1/>) [16]. Fold changes were



calculated using mass spectral peak areas (MS) or metabolite concentrations (NMR) for each detected metabolite.

#### *Enrichment Analysis using DAVID*

Proteins, transcripts, and metabolites identified with a fold change of 1.5 or higher when comparing *I. hospitalis* cultures alone versus the *I. hospitalis*-*N. equitans* co-cultures were analyzed using DAVID (<https://david.ncifcrf.gov>) to identify enriched groups of functionally similar components as described by Huang et al. [27]. The combined dataset contained 807 transcripts / proteins / metabolites that were higher in abundance in the *I. hospitalis*-only samples and 604 in the *I. hospitalis*-*N. equitans* co-culture samples, all with adjusted p-values smaller than 0.05. DAVID connects each data input with a biological annotation based on gene ontology and biochemical pathway association, and then identifies groups that are overrepresented in the dataset based on  $X^2$  and Fisher's exact tests. This program uses genes and proteins as input data. As such, metabolic information was incorporated by using proteins involved in producing and/or processing each metabolite as proxies. The enzymes involved in processing metabolites of interest were identified using the Biocyc database for *I. hospitalis* [28]. Output of the DAVID analysis contains a list of functional groups with enrichment scores, p-values, as well as Benjamini p-values and false discovery rates to account for multiple comparisons between all features [27]. Through this process, we obtained lists of functionally-related groups that were enriched in the *I. hospitalis*-only culture and the *I. hospitalis*-*N. equitans* co-culture. An enrichment score cutoff with a p-value < 0.05 was applied.

#### *Interaction Maps using STRING*

The enriched groups from DAVID were then run through the STRING (<http://string-db.org/>) software to obtain network visualizations identifying the relationships between cellular components based on genomic characteristics [genes occur in similar genetic context in different species (genomic neighborhood), gene products are fused in the genome (gene fusion), proteins occur or have similar function in the same metabolic pathway (gene co-occurrence), genes are co-expressed (conserved co-expression)], high-throughput omics experimental evidence, and previous knowledge, i.e. databases and text mining [20,25,27]. All networks retained for analysis were constructed using the high confidence criterion as defined by STRING as the association score “S” > 0.75 [25]. Resulting clusters of predicted associations were thus produced and spatially arranged according to high confidence scores as defined in STRING with no further manipulation of the clusters.

## Results

To gain a better understanding of the cellular mechanisms mediating the interactions between *I. hospitalis* and *N. equitans*, previously acquired transcriptomics, proteomics, and metabolomics datasets for *I. hospitalis* grown alone and in co-culture with *N. equitans* were combined to undertake an extensive, systems-level multi-omics analysis [16,19]. DAVID analysis yielded a list of functionally related groups that were enriched in the data for each type of *I. hospitalis* and *I. hospitalis*-*N. equitans* cell cultures. These enriched groups were then used to construct interaction maps in STRING connecting proteins and genes based on genome proximity (i.e. encoded on same operon), related biochemical pathways, co-expression, and text mining [25,27].

The STRING-generated network maps revealed several significant observations with respect to inter-species interactions between *I. hospitalis* and *N. equitans* that were not previously identified in the single ‘omics’ platform analyses (Figures 1 and 2). In each of these maps, the largest and most connected cluster in the network is a group of strongly associated ribosomal proteins (Figure 3). While both clusters contain small and large subunit ribosomal proteins, each cluster is comprised of subsets of ribosomal genes and proteins specific to each type of cell culture (Table 1). Rather than representing a simple increase or decrease in ribosomal gene transcription levels, these cluster patterns are in all likelihood evidence of “ribosomal tuning” and differential usage of specific ribosomal proteins [29–32]. A comparison of *I. hospitalis*-*N. equitans* co-cultures to *I. hospitalis*-only cell cultures showed that 8 ribosomal protein mRNAs and 5 ribosomal proteins had fold changes greater than 10. The prominence and distinctness of the two ribosomal clusters clearly distinguish the *I. hospitalis*-only culture from the *I. hospitalis*-*N. equitans* co-culture.

The interaction maps also highlighted prominent clusters associated with amino acid metabolic pathways. Enrichment analyses of the *I. hospitalis*-only cell cultures included pathways involved in amino acid charging of transfer RNAs for incorporation of specific amino acids into polypeptide chains, and involved the biosynthetic pathways for proline, histidine, methionine, valine, tyrosine, tryptophan, glycine, aspartate, and serine (Figure 2 and Table 2). In contrast, *I. hospitalis* -*N. equitans* co-cultures showed enrichments in the data for metabolic pathways involving asparagine, aspartate, glutamine, and phenylalanine (Figure 1 and Table 2). The DAVID analysis also identified enzyme enrichments in the co-culture corresponding to the up-regulation of leucine and isoleucine biosynthetic pathways. These clusters thus revealed

significant differences in amino acid requirements when *I. hospitalis* is grown in co-culture with *N. equitans* compared to when *I. hospitalis* is grown in the absence of *N. equitans*.

Energy production pathways were also highlighted in the interaction maps, including NADH metabolism and carbon flow through catabolic reactions. Differences in NADH metabolism between the *I. hospitalis*-only and the *I. hospitalis*-*N. equitans* co-culture samples were highlighted by differences in enzyme components of NADH dehydrogenases and ferredoxins (Figure 4). Inversely correlated patterns of gene and protein expression result in gene identifiers appearing in both the *I. hospitalis*-*N. equitans* co-culture and the *I. hospitalis*-only culture clusters. Carbon assimilation in *I. hospitalis* occurs through an autotrophic dicarboxylate/4-hydroxybutyrate cycle that was characterized by Huber et al. [33]. This pathway employs reduced metabolic intermediates for efficient CO<sub>2</sub> fixation. A cluster of enzymes responsible for the catalysis of intermediary steps of this CO<sub>2</sub> fixation pathway was identified in the interaction map of the *I. hospitalis*-only cell cultures (Table 3, Supplementary Figure 1). Examination of up-regulated transcripts/high protein levels in the *I. hospitalis*-*N. equitans* co-culture compared to the *I. hospitalis*-only culture confirmed that very few of the enzymes of the dicarboxylate/4-hydroxybutyrate pathway were represented. Since this pathway was not highlighted in either DAVID or STRING for the *I. hospitalis*-*N. equitans* co-culture samples, this suggests that carbon may be diverted from the dicarboxylate/4-hydroxybutyrate cycle of *I. hospitalis* to generate intermediates needed for *N. equitans* growth and survival (Figure 5). The findings regarding NADH metabolism and carbon fixation illustrate differences in energy production between the two types of cell growth.

Functional themes, as identified in the DAVID and STRING analyses, emphasize cellular changes occurring when *I. hospitalis* was grown by itself or in co-culture with *N. equitans* (Table

4). The *I. hospitalis*-only cell cultures reveal enrichment of proteins catalyzing a broad range of cellular activities including nucleotide metabolism, gene transcription, membrane transport, energy production, and protein translation. In contrast, regulated pathways in the *I. hospitalis* – *N. equitans* co-cultures appear dominated by proteins involved in membrane transport and energy production, including those regulating gene transcription and protein translation processes as presented above. The cellular processes modulating the association of *I. hospitalis* and *N. equitans* are summarized in Figure 5, which builds upon previous knowledge and includes the additional insights gained here and described above. Integrated analyses of the multi-omics data thus permit a broader global assessment of cellular processes, drawing attention to metabolic enzymes necessary for energy production in *I. hospitalis*-*N. equitans* co-cultures, consistent with previously reported transcriptomics and proteomics analyses [19].

## **Discussion**

Application of a systems-wide approach to analyze multi-level omics datasets generated supporting evidence and strengthened biological trends observed in previous studies [15,16,19]. The study also generated novel insights into the molecular mechanisms forming the basis of the *I. hospitalis*-*N. equitans* association. The data analyses presented in Table 4 suggest that *I. hospitalis* shuttles energy-producing metabolic precursors to *N. equitans*, as evidenced by enrichment sets related to increased levels of enzymes involved in energy production pathways specific to the *I. hospitalis*-*N. equitans* co-culture growth conditions. Reduction in the diversity of enriched cellular processes when comparing the profiles of the *I. hospitalis*-only cultures and the *I. hospitalis*-*N. equitans* co-cultures (Figures 1 and 2) corroborates a previous hypothesis that metabolic diversity is reduced in *I. hospitalis*-*N. equitans* co-culture samples due to *I.*

*hospitalis*'s need to redirect its metabolism toward energy-producing metabolic pathways to sustain the growth of *N. equitans* [15]. In addition to this functional adaptation, identification of specific network connections involving ribosomal proteins, enzymes engaged in amino acid and NADH metabolism, and carbon flow through the *I. hospitalis* dicarboxylate/4-hydroxybutyrate cycle has generated new insights about specific molecular processes mediating *I. hospitalis*-*N. equitans* inter-species interactions (summarized in Figure 5).

The omics datasets used in this study originate from growth conditions that were not identical; therefore, comparison of the reported transcriptomics, proteomics, and metabolomics profiles from the original publications must be scrutinized. The variation in sampling time-points is a direct result of the high degree of difficulty in culturing *N. equitans*, which only grows in the presence of *I. hospitalis*. Nevertheless, proteomics analyses resulting from *I. hospitalis*-*N. equitans* co-cultures samples at different growth stages of *I. hospitalis* have failed to detect significant differences in protein profiles [15,19]. The interaction network connections presented here are based on the comparisons performed between matching conditions, however, it is likely that the different state of the cultures at the time of sampling contributed to an under-representation of physiological changes.

The distinctive difference in ribosomal protein clusters between the two culture types (Figure 3, Table 1) suggests that *I. hospitalis* may switch to an alternative set of ribosomal proteins in response to the physiological load imposed by its interactions with *N. equitans*. A comparable stress coping mechanism has been shown to occur in *Mycobacterium tuberculosis* [32]. In this case, the organism utilizes an alternate set of ribosomal proteins as a function of the availability of zinc [32]. Other types of ribosomal protein tuning have been documented, whereby cells make adjustments to the levels, ratios, or types of ribosomal proteins in order to

facilitate optimal growth as a function of changing nutrient availability [29–31]. For *I. hospitalis*, these ribosomal proteins may reflect an adaptation to *N. equitans* attachment. Several different factors may be driving the tuning of ribosomal protein levels in the *I. hospitalis*-*N. equitans* co-culture samples. First, change in ribosomal protein composition could be employed by *I. hospitalis* to minimize the metabolic burden imposed on its growth in the presence of *N. equitans*. Mechanistically, this could result in selective translations of mRNA transcripts into subsets of proteins needed for *I. hospitalis*'s own maintenance and survival, while simultaneously coping for the diversion of *I. hospitalis* metabolic resources toward the metabolic needs of *N. equitans*. Such a response would represent an energy-cost minimization strategy toward protein synthesis, which could compensate for a potential energy drain resulting from *I. hospitalis*'s co-existence with *N. equitans* [34]. Additionally, it is possible that in the presence of *N. equitans*, these ribosomal proteins are involved in moonlighting functions (i.e. they perform more than one cellular functions), and thus would need to be present in higher abundance [35,36]. Distinct changes observed in ribosomal protein composition suggest the presence of important alternative cellular mechanisms that may enable *I. hospitalis* to optimize its cellular requirements in light of *N. equitans*'s metabolic requirements.

The multi-level omics analysis also highlights the importance of selecting different amino acid pathways when *I. hospitalis* is grown by itself compared to when it is cultured in the presence of *N. equitans* (Table 2). This difference is particularly noteworthy as it has been shown that in co-culture with *N. equitans*, the amount of protein produced by *I. hospitalis* does not significantly increase or decrease compared to when *I. hospitalis* is grown without *N. equitans* [15]. It is thus thought that *N. equitans*'s survival depends on *I. hospitalis* to supply metabolites and energy-generating precursors as a mechanism to compensate for *N. equitans*'s very small

genome and lack of metabolic enzyme-encoding genes, but may not necessarily require the physical transfer of amino acids between the two species [16,19]. The results of our system-level analysis support this hypothesis and provide insights into which metabolic precursors could be transferred to *N. equitans* upon co-culture growth with *I. hospitalis*.

It has also been shown that the protein expression profiles of *N. equitans*, when grown in co-culture with *I. hospitalis*, reflect enhanced levels of aromatic, hydrophobic, and positively-charged amino acids [37]. Of these amino acids, our analysis reveals protein enrichments corresponding to metabolic pathways involving phenylalanine, leucine, and isoleucine. Additionally, enzymes catalyzing the conversion of precursors to their respective amino acids are annotated in *N. equitans* for phenylalanine, leucine, and isoleucine [13]. Together these findings suggest that *I. hospitalis* shuttles specific amino acid precursors to *N. equitans*, as these cannot be independently synthesized by *N. equitans*.

Investigation into NADH metabolism clusters (Figure 4) also reveals interesting differences between the two *I. hospitalis* growth environments. The only components that are shared between the two types of cell cultures are two subunits of a NADH dehydrogenase encoded by genes located within the same locus and which are thought to be part of the same transcriptional unit that comprises other NADH dehydrogenase elements [12] (Figure 4, Supplementary Figure 2). Proteins encoded by all but one of these genes were found in *I. hospitalis*-only culture, whereas the *I. hospitalis*-*N. equitans* co-culture cluster contains just the two shared genes mentioned above. Additionally, the clusters differ in that they have other NADH metabolism gene components located in a separate gene cassette (Figure 4, Supplementary Figure 3). Surprisingly, the co-culture cluster has roughly half of the components, while the *I. hospitalis*-only cluster contains the other half of the components



encoded by this gene cassette. This observation suggests that instead of entirely switching to an alternate set of NADH metabolic components, a rerouting of NADH oxidation pathways may render *I. hospitalis* better suited to adapt to the energetic coupling with *N. equitans* when grown as a co-culture (see summary Figure 5). Such a process could be controlled at the gene expression level by utilization of different promoters within these gene loci that are acted upon by different transcription factors. In this scenario, increased energy transfer could arise via coupling of *I. hospitalis*'s electron transport chain to ATP production in *N. equitans*. Analysis of the *N. equitans* genome reveals the most likely candidate for this ATP production machinery to be a putative sulfide:quinone oxidoreductase [12]. While the use of NADH as a substrate for this oxidoreductase remains to be confirmed, a shift to using an enzyme from *N. equitans* could rationalize why *I. hospitalis* decreases its production of NADH redox protein subunits when grown in co-culture with *N. equitans*.

Lower levels of protein components associated with the dicarboxylate/4-hydroxybutyrate carbon assimilation cycle as observed in the *I. hospitalis*-*N. equitans* network map suggest that elements of the CO<sub>2</sub> carbon fixation pathways are altered, diverted, or slowed when *I. hospitalis* is grown in co-culture with *N. equitans* (Table 3). Previous work has revealed that when grown with *N. equitans*, *I. hospitalis* does not actively replicate [14]. This observation strongly suggests that cellular materials and metabolic energy normally used to increase the cellular biomass of *I. hospitalis* is diverted to meet the metabolic needs of *N. equitans*. None of the proteins annotated in the *N. equitans* genome are believed to function in a dicarboxylate/4-hydroxybutyrate carbon assimilation cycle [12]. Lower abundance of CO<sub>2</sub> fixation enzymes in the STRING interaction maps of the *I. hospitalis*-*N. equitans* co-culture samples is consistent with previous observations suggesting that *N. equitans* acts as a parasite, consuming metabolites produced by *I. hospitalis*

[16]. This metabolic shift away from carbon assimilation suggests an alternate mechanism by which *I. hospitalis* adapts its growth patterns and metabolic responses to the presence of *N. equitans*. Previous analysis using proteomics-only data indicated higher protein abundance levels for carbon fixation enzymes. Our multi-level analysis provides a more complex view of the cellular changes occurring in *I. hospitalis* when grown in co-culture with *N. equitans*.

## **Conclusion**

Multi-level omics studies used to probe the biochemistry of complex organisms have been shown to strengthen our understanding of complex biological systems, and have provided insights into critical cellular mechanisms mediating interspecies interactions. Using an analysis of multi ‘omics’ datasets acquired independently, we have gained a deeper understanding of how the two archaeal organisms *I. hospitalis* and *N. equitans* interact with each other. This study clarifies previously described cellular networks and highlights cellular patterns that were not discernible previously from single transcriptomics, proteomics, or metabolomics analyses.

Results from this study suggest the presence of alternative pathways of ribosomal protein production and utilization, amino acid metabolism, and metabolic energy production, which together, point to possible adaptive mechanisms employed by *I. hospitalis* in response to *N. equitans* cells’ attachment, and concomitant growth of the two organisms. Such adaptive mechanisms appear to involve molecular switches that utilize less energy-demanding enzymes and cellular pathways. Findings from this study also provide evidence that metabolic precursors synthesized by *I. hospitalis* are shuttled to *N. equitans* to meet its cellular needs. This system-wide analysis applies statistical and visualization techniques to a multi-omics dataset to yield

additional insights into the cellular activity of a complex biological system that was not readily discernable from independent single ‘omics’ analyses.

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