

# ***Interactive comment on “Uncovering chemical signatures of salinity gradients through compositional analysis of protein sequences” by Jeffrey M. Dick et al.***

## **Anonymous Referee #2**

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Dick et al explore (meta)genomic and (meta)transcriptomic datasets to identify a thermodynamic-based metric to characterize the effect of redox and, perhaps to a greater extent, salinity on the composition of proteins, with the argument being that minimizing energetic demands associated with protein synthesis should be a selectable phenotype. The authors compare one of several metrics, including the average oxidation state of carbon in proteins and their stoichiometric hydration state and compare these to several existing indices that have previously been shown to track with salinity gradients including the GRAVY index and the average protein isoelectric point. The results suggest that in many systems (but not all) the stoichiometric hydration state outperforms the other metrics in describing variation in proteome composition across

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salinity gradients.

In general, I found the paper to be another interesting read from the primary author. However, as a microbiologist that is interested in understanding how energy availability and demand affect the distribution of microorganisms and their evolution, I would appreciate seeing a more robust effort to link the thermodynamics way of thinking (as presented here) to physiological process or mechanism that could then be used to gauge why such patterns may exist. More or less, I think this is a missed opportunity that, if executed effectively, could elevate the utility of this paper and this way of thinking. Thus, I strongly suggest the authors attempt to explain their observations at a level that makes sense to the more biologically oriented reader. As I was reading this, I could not help but think to myself how any one or several observations made sense from the level of phenotype and natural selection. The authors might consider asking themselves this same question and then speculating where possible to make this body of work a greater utility for the community.

The following list of minor comments is meant to further improve this work:

Line 1: For the average reader – what is the connection between thermodynamics and environmental variation. Lead in with this first.

Line 8: Replace “behave” with something more valid. The metric does not correlate for XXX in hypersaline environments. . .

Line 15: Communities do not adapt, populations of individuals do.

Line 26: I would not call this complementary but rather an interrelated approach since selection (imposed as an argument in previous paragraph) can and should act on the energetic demand of protein synthesis.

Line 39-40: What about the authors own work on the communities inhabiting the out-flow channel at Bison Pool, Yellowstone?

Line 44-45: While I don't disagree with this assumption, at least as a first order con-

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straint, it would be useful to relate to the reader why this assumption is made. Perhaps to avoid this confusion, the authors move this statement to below where they describe and justify their approach.

Line 58-62: This paragraph seems out of place. I suggest moving the discussion of what you did previously up in the introduction and add the last sentence of this paragraph to the end of the preceding paragraph.

Line 67: alternatives to what?

Line 305-310: I don't understand the reasoning here? Why did eukaryotes start to become important in these systems? Are there actually eukaryotes in these systems? The authors have the data to evaluate this and should evaluate it to see if the logic makes sense.

Line 315: Why would heterotroph proteomes have a lower hydration state?

Line 315-317: is there an argument to be made about why a major evolutionary transition favors a shift from higher to lower dehydration state? i.e., is this an adaptive feature that allows the latter to compete with the former from an evolutionary perspective?

Line 325: is it possible that diffusion limitation makes H<sub>2</sub>O less available to cells living nearer to a particle surface? Again, an explanation for what the observations might mean is warranted

Line 350: proteins in metagenomes

Line 360: Could this be due to aquaculture and introduction of more organic compounds/waste and its selection of heterotrophic taxa, that as stated earlier in the paper, tend to host proteomes with a lower hydration state

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