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Interactive comment on "Group additivity calculation of the standard molal thermodynamic properties of aqueous amino acids, polypeptides and unfolded proteins as a function of temperature, pressure and ionization state" *by* J. M. Dick et al.

J. M. Dick et al.

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Perhaps the most noticeable effect of revising the manuscript has been to shorten it. The length of the paper published in *BGD* we estimate at about 36 pages in journal format; the present revision stands at about 26. The changes have produced a more focused and accessible paper. Here, we summarize the major features of the revision, reply to specific comments brought forward in the interactive discussion, and give a list of technical corrections.

Summary of revision

1. The title was changed to: **Temperature, pressure, and electrochemical constraints on protein speciation: Group additivity calculation of the standard molal thermodynamic properties of ionized unfolded proteins**. This conveys the essential meaning of the previous title, while putting major keywords up front. The words “electrochemical” and “speciation”, which are not in the original title, set the stage for the discussion of both chemical and biological speciation of proteins. Two examples of protein speciation calculations in this paper are the calculations of protein ionization state and its consequences (chemical speciation) and calculations of protein metastability limits (biological speciation; see Reply to the Referee and Editor, below).

2. The appendix on crystalline compounds was removed. The derivation of the properties of aqueous compounds does not depend on it, and it is notable that a reference to anything crystalline was not found in the title, abstract, or introduction of the manuscript published in *BGD*. The points brought up by Anonymous Referee #3 regarding the regression of heat capacity data for crystalline Leu (and the resulting sidechain group contributions) can be addressed by considering the heat capacities of all the crystalline amino acids, as well as crystalline poly(amino)acids (see, e.g., http://www.prz.rzeszow.pl/athas/databank/aminoaci/plleu/plleu-cp.gif), in a manner similar to the present study’s regression and comparison of data for many aqueous amino acids and tripeptides. The investigation of the properties of crystalline proteins and their biogeochemical consequence is now left for future work.

3. The other appendices were merged with the main body of the text. A discussion with L. L. Glaser helped to initiated this change. As a result, the continuity of the manuscript was improved. The reader now becomes familiar early on with the revised HKF equations, which are referenced throughout the paper.

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4. The duality between [UPBB] (unfolded protein backbone) and [PPBB] (polypeptide backbone) was eliminated by adopting a single designation, [PBB] (polypeptide or unfolded protein backbone), to replace both. A discussion of the only instance where we can deduce a quantitative distinction in the additivity properties of unfolded proteins and polypeptides (i.e., in the value of c_1) remains in the text.

5. The text was edited extensively in order to remove redundant explanations and to improve the tone and flow. The discussion of uncertainty was simplified by taking account of estimated uncertainties in C_P°, V° and κ_T° that are approximately *in*dependent of temperature, a provision that is validated by the general trend of the regression lines for amino acids and tripeptides.

6. In parallel with the textual changes, most of the multi-panel figures were redrawn with a space-saving strategy in mind. Subfigure labels (*a*, *b*, *c*, ...) were added. The amino acid and Gly–X–Gly tripeptide regression plots were merged into a single figure. Missing temperature keys in the protein net charge and Gibbs energy plots were implemented with a simple color scheme. Calculated values from the additivity algorithms used by previous authors were added to the protein heat capacity plot to aid in its interpretation.

7. As with the figures, many of the tables were restructured in order to offer a more direct presentation of the data. The table of symbols and abbreviations was removed in favor of the new structure figure, which contains both the one- and three-letter abbreviations for the amino acids. Other specific tabular adjustments were made: Values of c_1, c_2, ω and C_P° of [AABB$^+$] were corrected in the table of group properties (the figure showing calculated values of $\Delta C_{P,ion}^\circ$ already used the correct ones). A missing entry for Gly$^+$ was added to the table of amino acid properties.

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Reply to Anonymous Referee #3 and the editor

We would like to thank Anonymous Referee #3 and editor, J. Middelburg, for their helpful comments. Many of the revisions described above took account of their general suggestions. Their more specific comments were also addressed:

1. A figure showing the structures of amino acids, sidechain groups, and the backbone groups was added to the Introduction, along with supporting text. (referee, editor)
2. The relevance of this work for a broader audience is described with the aid of a new figure and section in the Discussion. The figure illustrates a connection between environmental chemical potentials (quantified in this case by temperature and the electrochemical variables Eh and pH) and the evolution of proteins in different biological species (quantified by the metastability limits of proteins from model organisms for soil and hydrothermal vent environments). The calculations also support a few remarks on the relative contributions of protein folding reactions to the outcome of thermodynamic calculations. (referee, editor)
3. Fixed butane-1,4-diol plot so that the curve now goes near the points. The plot calculations were incorrect; no change to the parameters was required. (referee)
4. Text labels on the amino acid and Gly–X–Gly tripeptide regression plots were made bigger, and separated for better readability. (referee)
5. We could not adopt the referee's suggestion of removing the HKF equations altogether, because the development of the rest of the text depends on specific references to many of them. However, the summary of the revised HKF equations of state has been made shorter by removing the equations for ΔH° and S°, which were were not referenced elsewhere in the text, and by changing some of the equations to inline format. (referee)

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Technical corrections

1. The technical errors raised by the referee and the editor and found in our source files were addressed. A number of technical errors found by the referee can not be traced in our source files; they may have appeared during production at *BGD* (the referee's comments are reproduced below, followed by our notes in brackets):

    - Page 1554, line 10: There is a missing left parenthesis.
    - Page 1572, lines 1-2: Check the title of this article to see if it is correct. [It is incorrect as shown; the listing in the bibliography file is correct.]
    - Figure 2 caption, line 6: The name "Hedwig" is misspelled. [The spelling should be controlled by the bibliography file.]
    - Figure 17 caption, lines 4 and 5: The same symbol is listed for values from two different references. One of them should be a filled circle? [Yes. Inglese and Wood, 1996 should be indicated by a filled circle.]

2. All tilde symbols were changed to the LaTeX math symbol `\sim`; this should prevent their being printed at a height above that of the rest of the characters.

3. The term "Gibbs free energy" was replaced by "Gibbs energy", in accord with IUPAC recommendations.

4. Reaction numbers were set in the style of the `reaction` environment provided in the `egu.cls` file.

5. Updates to protein names made in the Swiss-Prot database were applied in the manuscript: LYC_CHICK and RNP_BOVIN were changed to LYSC_CHICK and RNAS1_BOVIN, respectively. Swiss-Prot accession numbers were included in the protein information table.

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Interactive comment on Biogeosciences Discussions, 2, 1515, 2005.