FINAL PROGRESS REPORT

GRANT #: N00014-93-1-0405

PRINCIPAL INVESTIGATOR: Barry Honig

INSTITUTION: Columbia University

GRANT TITLE: Solvent Effects on the Stability of Biomolecules

AWARD PERIOD: 3/15/93 - 3/14/96

OBJECTIVES: The objective of this proposal is the development of rapid computational methods for the accurate calculation of conformational free energies, binding free energies and solvation free energies of biomolecules. Specific aims include: a) the development of parameters designed for continuum solvent calculations on proteins, b) applications to the prediction of protein conformation with particular emphasis on loop conformations.

APPROACH: The basic approach we have developed is to calculate accurate gas phase conformational energies which are then added to solvation free energies to obtain conformational free energies in solution. Gas phase energies are obtained with either quantum mechanical calculations or accurate potential energy functions. Solvent effects are treated with continuum methods. The DelPhi program, which solves the Poisson-Boltzmann equation numerically, is used for electrostatic properties while free energy/surface area relationships are used to treat nonpolar interactions. Parameters are developed by fitting to experimental data or based on high level quantum mechanical calculations.

ACCOMPLISHMENTS: We made major advances in the calculation of solvation free energies. A method and parametrization scheme which allows the accurate calculation of solvation free energies was described. Electrostatic contributions to solvation are derived from finite difference solutions to the Poisson equation while nonpolar contributions are obtained from a free energy surface area relationship. A new parameter set (PARSE) was developed which accurately reproduced solvation free energies for a test set of 67 molecules (average error of .4 kcal/mole).

In order to treat molecules in different environments, it is necessary to account for changes in charge distribution. To this end, we combined high level quantum mechanical calculations with a continuum description of the solvent . This allows us to carry out quantum mechanical calculations in very different protein/solvent environments. By using correlated wavefunctions at the generalized valence bond/perfect pairing level we were able to produce accurate gas phase charge distributions. These are then used to obtain solvation free energies in a self consistent formalism which cycles through quantum mechanical calculations in the solvent reaction field and continuum electrostatic

calculations using polarized solute charges. An average error of .7 kcal/mole for 29 molecules was obtained with only *one* adjustable parameter, the atomic radius of hydrogen.

Based on this work we developed; a) a physical model to describe the partitioning of organic solutes between any two solvents. b) a parameterization scheme to accurately calculate partition coefffcients between water an aqueous solvents, and c) a new quantum mechanical/continuum model to calculate solvation energies. This method corrects deficiencies in all existing force fields that implicitly assume that hydrogen bonding with solvent molecules in entirely electrostatic in nature. These three accomplishments have brought us to the point that we have the most comprehensive understanding and most accurate models of solvation that have been developed to date.

SIGNIFICANCE: The ability to predict solvation, conformational and binding free energies underlies many research areas. These include protein stability in non-aqueous solvents, biomimetics, biopolymeric materials, metal ion biosensors, molecular recognition and water at biological interfaces. More specifically, the availability of simple physical models that our approach offers, when combined with accurate computational methods to calculate solvent effects on stability, should provide a firm physical basis for the design of new proteins and biomimetics with desired solubilities and stabilities in different environments.

PUBLICATIONS:

1. D. Sitkoff, K. Sharp, B. Honig (1994) Accurate calculation of hydration free energies using continuum solvent models. J. Phys. Chem. 98:1978.

2. D. Tannor, B. Marten, R. Murphy, R. Friesner, D. Sitkoff, A. Nicholls, B. Honig and M. Ringnalda (1994) Accurate first principles calculation of molecular charge distributions and solvation energies from ab-initio quantum mechanics and continuum dielectric theory. J.Amer. Chem. Soc. (submitted).

3. K. Smith and B. Honig (1994) Evaluation of the conformational free energies of loops in proteins. PROTEINS: Structure, Function, Genetics, 18:119.

4. B. Honig and A. Nicholls (1995) Classical Electrostatics in Biology and Chemistry, Science 268:1144-1149.

5. Marten, B., Kim, K., Cortis, C., Friesner, R., Murphy, R.B., Ringnalda, M.N., Sitkoff, D. and Honig, B., (1996) A New Model for Calculation of Solvation Free Energies: Correction of Self-Consistent Reaction Field Continuum Dielectric Theory for Short Range Hydrogen-Bonding Effects, J.Phys. Chem. \plain \f2 (in press).

6. D. Sitkoff, N. Ben-Tal and B. Honig (1996). Calculation of alkane to water solvation free energies using continuum models. J. Phys. Chem. 100:2744-2762.

HIGHLIGHT PAGE

OBJECTIVES:

To develop rapid computational methods for the accurate calculation of conformational free energies, binding free energies and solvation free energies of biomolecules.

ACCOMPLISHMENTS:

The development of an accurate parameter set for the calculation of solvation free energies.

The development of a new method to carry out high level quantum mechanical calculations in different solvent/macromolecule environments.

The development of a new method to calculate alkane/water partition coefficients.

The development of a general quantum mechanical/continuum method to calculate solvation free energies.

SIGNIFICANCE:

The availability of simple physical models to calculate solvent effects on stability, should provide a firm physical basis for the design of new proteins and biomimetics with desired solubilities and stabilities in different environments.

LICENSED TECHNOLOGIES:

The DelPhi program is licensed by Columbia to Biosym Technologies in San-Diego, which distributes it to academic, government and industrial labs. Delphi was developed under partial ONR support. The PARSE parameter set, described in this Progress Report is now being distributed as part of the DelPhi program. PARSE was developed entirely under ONR support.

INDUSTRIAL CONNECTIONS:

Based on our success with PARSE and with promise it holds for the calculation of conformational and binding free energies, a number of companies have approached us to help them develop methods to predict the affinity of possible drugs to proteins of known structure. At present we are working with Upjohn but have entered into discussion with both Biosym and MSI to develop general purpose software for this purpose.

The algorithm developed for the joint quantum mechanical/solvation free energy calculations has been licensed by Columbia to Schrodinger Technologies, a start-up based in Oregon and New Jersey.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	4/19/99	FINAL REPORT 15June93-14June96

4. TITLE AND SUBTITLE

SOLVENT EFFECTS ON THE STABILITY OF BIOMOLECULES

5. FUNDING NUMBERS

N00014-93-1-0405

6. AUTHOR(S)

DR. BARRY HONIG

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Department of Biochemistry, BB221
Columbia University, Box 36
630 West 168th St.
New York, NY 10032

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Office of Naval Research
800 N. Quincy St.
Arlington, VA 22217-5000

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The goal of the proposal was to develop rapid computational methods for the accurate calculation of conformational free energies, binding free energies and solvation free energies of biomolecules. This was accomplished through (a) the development of an accurate parameter set for the calculation of solvation free energies, (b) the design of a new method to carry out high level quantum mechanical calculations in different solvent/macromolecule environments, (c) the design of a new method to calculate alkane/water partition coefficients, and (d) the development of a general quantum mechanical/continuum method to calculate solvation free energies. The availability of simple physical models to calculate solvent effects on stability should provide a firm physical basis for the design of new proteins and biomimetics with desired solubilities and stabilities in different environments.

14. SUBJECT TERMS

Solvation, proteins, energetics, electrostatics

15. NUMBER OF PAGES

3

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	