1992 Final Report 1 Dec 88 - 30 Nov 91

Defining Protein Electrostatic Recognition Processes N00014-89-J-1174

Elizabeth D. Getzoff
Victoria A. Roberts

The Scripps Research Institute
10666 North Torrey Pines Rd.
La Jolla, CA 92037

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



Distribution unlimited



The objective of this proposal is to elucidate the nature of electrostatic forces controlling protein recognition processes by using a tightly coupled computational and interactive computer graphics approach. We developed the TURNIP program to determine the most favorable precollision orientations for two molecules by systematic search of all orientations and evaluation of the resulting electrostatic interactions. TURNIP was applied to the transient interaction between two electron transfer metalloproteins, plastocyanin and cytochrome *c*. Our results suggest that the productive electron-transfer complex involves interaction of the positive region of cytochrome *c* with the negative patch of plastocyanin, consistent with experimental data. Application of TURNIP to the formation of the stable complex between the HyHEL-5 antibody and its protein antigen lysozyme showed that long-distance electrostatic forces guide lysozyme toward the HyHEL-5 binding site, but do not fine tune its orientation. Determination of docked antigen/antibody complexes requires including steric as well as electrostatic interactions, as we have done for the U10 mutant of the anti-phosphorylcholine antibody S107. We have enhanced the graphics program Flex, a convenient desktop workstation program for visualizing molecular dynamics and normal mode motions. Flex now has a user interface and has been rewritten to use standard graphics libraries, so as to run on most desktop workstations.

electrostatic recognition, computer graphics, protein binding, electron transfer, computational search

Unclassified Unclassified Unclassified UL

FINAL TECHNICAL REPORT

GRANT NUMBER: N00014-89-J-1174 R&T CODE 441n011

PRINCIPAL INVESTIGATOR: Elizabeth D. Getzoff

INSTITUTION: The Scripps Research Institute

GRANT TITLE: Defining Protein Electrostatic Recognition Processes

AWARD PERIOD: 1 December 1988 - 30 November 1991

OBJECTIVE: To elucidate the nature of electrostatic forces controlling recognition processes.

APPROACH: Tightly coupled computational and interactive computer graphics programs were designed and used to predict and visualize electrostatic interactions of proteins. Results were compared with other experimental data.

ACCOMPLISHMENTS:

Development of TURNIP, a Program for Calculating Precollision Orientations. The TURNIP program (Roberts et al., 1991) determines the most favorable precollision orientations for two molecules maintained at a specified distance by a systematic search of all orientations and evaluation of the resulting electrostatic interactions. A constant minimum separation distance between the two molecules was found to be essential for the algorithm to work robustly.

Precollision Orientations of an Electron-Transfer Complex. TURNIP was applied to the investigation of the transient interaction between two electron transfer metalloproteins, plastocyanin and cytochrome *c* (Roberts et al., 1991). The computational results were compared with the results found by visual alignment of electrostatic fields displayed with computer graphics. A computational search with TURNIP with a separation distance of 12 Å gave three energetically favorable families of orientations that positioned the the positive charges surrounding the heme edge on cytochrome *c* near the acidic patch surrounding Tyr 83 on plastocyanin. All dockings placed the heme edge of cytochrome *c* on the surface of plastocyanin and appeared to have no obstacles for further movement of cytochrome *c* towards the acidic patch of plastocyanin. None of the energetically favorable orientations placed the heme edge near His 87, the only exposed copper ligand of plastocyanin. In fact, dockings from the energetically favored precollision orientations indicated that movement of cytochrome *c* towards the His 87 region was sterically blocked by a solvent-exposed loop of conserved residues. Our results are consistent with the productive electron-transfer complex involving interaction of the positive region of cytochrome *c* with the negative patch of plastocyanin, which brings the heme edge into close proximity with Tyr 83, as indicated by experimental data. Thus, electrostatic forces appear to control the approach of these two electron-transfer partners during their transient interaction.

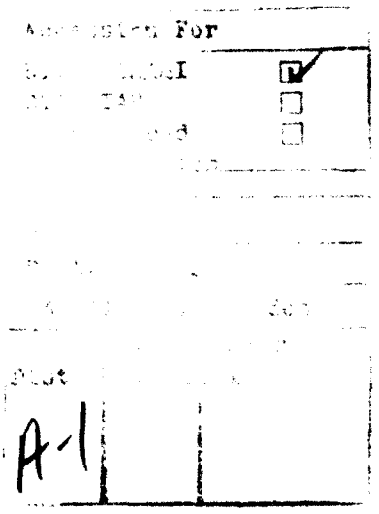
Investigation of Electrostatic Forces in Stable Binding Complexes. We have also completed our analysis of the U10 mutant of the

phosphorylcholine-binding antibody S107, (Chien et al., 1989; Behar et al., 1989). This mutant antibody results from a single-site mutation of Asp 101 to Ala, over 9 Å distant from the antigen binding site, which results in a complete loss of phosphorylcholine binding activity. A combination of molecular dynamics and minimization techniques indicated that the mutant antibody may fold so that Arg 94 of the heavy chain extends into the antigen binding pocket to electrostatically and sterically block the binding of the hapten.

TURNIP was applied to the investigation of formation of the stable complex between the HyHEL-5 antibody and its protein antigen lysozyme. Computer graphics analysis of the electrostatic fields suggested that the association of these two molecules might be substantially aided by electrostatic forces. Unlike the transient electron-transfer interaction between plastocyanin and cytochrome *c*, however, the formation of this stable antibody-antigen complex appears to be only partially directed by electrostatic forces. Long-distance electrostatic forces guide lysozyme toward the HyHEL5 binding site, but do not fine tune its orientation.

Computer Graphics Program Development. We have also continued the development of the graphics program *Flex* (Pique et al., 1991), a convenient desktop workstation program, for the display of molecular dynamics and normal mode motions (Fisher et al., 1990). The program now has a user interface that allows multiple molecules to be displayed and manipulated separately, assisting the analysis of the multiple favorable orientations found by TURNIP. New features have been added, including main-chain ribbons and depth cueing, that increase the usefulness of the program (Pique et al., 1991). *Flex* has been rewritten to use the XView 2-D and PHIGS 3-D graphics libraries under the X-Windows protocol (developed at MIT), so that it runs on any machine with X, for example Silicon Graphics, Stardent, and Sun SPARC graphics workstations. The X-Windows protocol allows rapid communications between machines such that *Flex* can be run on other machines, such as our Cray YMP or Convex C2 supercomputers, and viewed on a desktop workstation.

We have also begun preliminary investigations of the graphics program AVS, which provides an environment for creating innovative customized applications, with the goal of converting our analysis programs into modules that would run inside the AVS environment. To explore the feasibility of this approach, we have written a template searching program that runs outside AVS but communicates with AVS to display results.



PUBLICATIONS:

Chien, N. C., Roberts, V. A., Giusti, A. M., Scharff, M. D., and Getzoff, E. D. (1989) ''Significant Structural and Functional Change of an Antigen Binding Site by a Distant Amino Acid Substitution: Proposal of a Structural Mechanism'', *Proc. Natl. Acad. Sci. USA*, **86** 5532-5536.

Behar, S. M., Chien, N. C., Corbet, S., Diamond, B., Getzoff, E. D., Lustgarten, D., Roberts, V. A., Scharff, M. D., and Shin, S.-U. (1989) ''Impact of Somatic Mutation on the S107(T15) Heavy-Chain V Region of Antibodies Reactive with Self and Nonself'', *Cold Spring Harbor Symp. Quant. Biol.*, 54 921-931.

Fisher, C. L., Tainer, J. A., Pique, M. E., and Getzoff, E. D. (1990) ''Visualization of Molecular Flexibility and Its Effects on Electrostatic Recognition'', *J. Mol. Graph.*, 8, 125.

Roberts, V. A., Freeman, H. C., Olson, A. J., Tainer, J. A., and Getzoff, E. D. (1991) ''Electrostatic Orientation of the Electron-Transfer Complex Between Plastocyanin and Cytochrome *c*'', *J. Biol. Chem.*, **266**, 13431-13441.

Pique, M. E., Macke, T. J., and Arvai, A. S. (1991) ''Flex: A Light-Weight Molecular Display Program'', *J. Mol. Graph.*, **9**, 40-41.

PATENTS:

None.

## Distribution List for Final Reports

Attach a copy of the REPORT DOCUMENTATION PAGE (DD FORM 1473) to your final report as the first page and mail two copies (including the postcard labelled DTIC FORM 50 ) to:

Defense Technical Information Center
Building 5, Cameron Station
Alexandria, VA 22314

This allows other investigators to obtain copies of your report directly from DTIC. DTIC will fill out the postcard DTIC ACCESSION NOTICE (DTIC FORM 50) and return it to you with their number for your report. When you refer people to DTIC to get a copy of your report, give this number to expedite the request.

Mail one copy to each of the following and attach **this very page** to the back of your report – otherwise the folks below will think they have mistakenly received a copy meant for the Molecular Biology Program ):

(a) Dr. Michael Marron
ONR Code 1141
Molecular Biology Program
800 N. Quincy Street
Arlington, VA 22217-5000

(b) Administrative Grants Officer
ONR Resident Representative
(address varies - see copy of your grant/contract)

(c) Director,
Applied Research Directorate
ONR Code 12
800 N. Quincy Street
Arlington, VA 22217-5000

(d) Director
Office of Naval Technology
Code 22
800 N. Quincy Street
Arlington, VA 22217-5000

(e) Director
Chemical and Biological Sci Div
Army Research Office
P. 0. Box 12211
Research Triangle Park, NC 27709

(f) Life Sciences Directorate
Air Force Office of Scientific Res
Bolling Air Force Base
Washington, DC 20332

(g) Director
Naval Research Laboratory
Technical Information Div
Code 2627
Washington, DC 20375

Encl (1)

3/12/91