A. SPECIFIC AIMS

Computational. The Specific Aim 1 continues to be the development of computational tools for molecular and supramolecular (Aim 1A), microphysiological or subcellular (Aim 1B) and cellular systems (Aim 1C) levels of complex biological processes. Our specific aims 2 and 3 also remain unchanged as the integration of these multiscale methods, and the development and implementation of computational tools for the organization and visualization of the data and output.

Biomedical. The three originally proposed developmental projects (DPs) on complex cell signaling and regulatory processes remain to be the major focus of the Biomedical Aims. The respective topics of concentration are effect of NO on apoptotic response (DP1), DNA damage recognition and signaling (DP2) and ligand interactions with signaling molecules (DP3).

B. STUDIES AND RESULTS

Specific Aim 1A. Progress: (ii) the initial development of a hybrid method that combines molecular dynamics (MD) simulations and Gaussian network model (GNM) analysis, applied to hemoglobin T → R transition (collaboration between Bahar (Center for Computational Biology and Bioinformatics (CCBB), Pitt) and Ho (Biology, CMU)) (Xu et al., 2003) and still in progress, (i) the extension of the GNM to predict the dynamics of large assemblies, supported by its recent application to ribosomal dynamics (Yang et al., 2004). These studies have been conducted with the partial support from Pitt School of Medicine (SOM) provided to the CCBB, and two new grant proposals have been submitted for pursuing these studies (See other support pages).

Specific Aim 1B. The software for spatially realistic simulation of microphysiology (MCell and DReAMM; http://www.mcell.psc.edu) were originally designed to simulate the 3-D reaction/diffusion aspects of neurotransmission, and a critical first aim was/is to expand MCell's computational kernel and modeling language to more general signaling pathways such as those of the DPs on apoptosis or DNA damage signaling. A new prototype code base for MCell has been produced to incorporate the interactions between different diffusing molecules, rather than just between diffusing molecules and stationary molecules located on surfaces representing cell and/or organelle membranes. We began preliminary testing of molecule-molecule interactions with the new Monte Carlo (MC) algorithms. See (Stiles et al., 2004) for more details.

Specific Aim 1C. New features have been added to the XPP/XPPAUT software, which currently allows for solving ~600 differential equations (http://www.math.pitt.edu/~bard/xpp/xpp.html) (Ermentrout's lab). Additionally, a logical network model of apoptosis has been constructed in Ta'asan lab (CMU, Math), building on their existing framework that allows for stochastic modeling of thousands of reactions and population variations. The modeling is agent based where molecules are considered at a few levels of abundance, representing no expression, low and high expression. Likewise, reaction rates are discretized as slow, moderate and fast. The developed model of apoptosis includes multiple compartments as cell surface, mitochondria, and nucleus, and focuses on caspase activation through FAS, TNFR, mitochondrial pathways including stress activation. The apoptosis model is found to be robust to the changes in most of the parameters, and rapid activation of caspase-3 is ensured only by a small subset of reactions consistent with previous experimental observations (Rehm et al., 2002; Tyas et al., 2000).

Specific Aim 2. Major progresses towards the integration of different tools and combined use of experimental and computational data include; initial tests of a hybrid methodology that jointly exploits the results from MD simulations and GNM calculations to predict conformational changes induced by allosteric effects (Xu et al., 2004); integration of NMR relaxation data and GNM computations to assess the molecular mechanism of conformational transitions (Temiz et al., 2004); assessment of stability and folding/unfolding kinetics by combined analysis of GNM and FIRST computations (Rader & Bahar, 2004) and site-directed mutagenesis experiments (Rader et al., 2004); comparison of dynamic MC simulations and dielectric self energy Poisson-Nernst-Planck continuum theory (Graf et al., 2004) towards the design of an algorithm that combines both methods; continued development of MCell's input language to handle the combinatorics problem of fully general molecular complex formation.

Specific Aim 3. Three major developments are: (1) A new website has been constructed for automated release and visualization of the results from GNM analysis of PDB structures (http://ignm.ccbb.pitt.edu/). (2) To optimize DReAMM for models composed of thousands of mesh objects, we have begun modifying the underlying OpenDX (http://www.opendx.org) source code (~one million C/C++ lines) to improve a number of critical user interface design features, and we have also written a set of critical C code modules that now handle manipulation of meshes, wireframes, boundaries, and molecules, as well as custom depth cueing and color mapping of the different objects. A new prototype version of DReAMM incorporates these changes, and is orders of magnitude faster than the original version. (3) A new detailed tutorial has been posted on the XPP/XPPAUT website (http://www.math.pitt.edu/~bard/bardware/tut/start.html)

Developmental Project 1 (DP1). We started with the apoptosis model proposed by Fussenegger et al. (2000) and modified it to improve the realism of cytochrome *c* release, and include p53 interactions and truncation of Bid to tBid. We also created the first generation model of the reaction pathways of NO, coupled to apoptosis, which includes the formation of peroxynitrites, p53 induction via DNA damage, formation of S-nitrosative species and their inhibition of caspases, and inhibition of cyt *c* release by cGMP. Our mathematical model yielded results in reasonable agreement with experimental data (Billiar's group) on the dependence of NO effects on cellular environment. It also suggested that the induction of apoptosis in macrophages as opposed to suppression in hepatocytes is related to the different iron levels in these two cell types. The results are summarized in a recent review (Vodovotz et al., 2004).

Developmental Project 2 (DP2). Wood's group recently isolated DNA polymerase θ, a new enzyme encoded by the human *POLQ* gene, with an exceptional ability to replicate past an AP site, inserting A with 22% of the efficiency of a normal template, and then continuing extension as avidly as with a normally-paired base (Seki et al., 2003). POLQ preferentially incorporates A opposite an AP-site. On non-damaged templates POLQ makes frequent errors, incorporating G or T opposite T about 1% of the time. This low fidelity distinguishes POLQ from other A-family polymerases. Mammalian POLQ has three unusual sequence insertions. Comparative modeling of POLQ by Bahar's group suggested that one insert of ~22 residues at the tip of the polymerase thumb domain confers considerable flexibility and additional DNA contacts to reduce the enzyme fidelity and enhance its processive ability, as summarized in a manuscript (Seki et al., 2004).

Developmental Project 3 (DP3). Using high throughput screening, Lazo's group discovered a novel inhibitor of CDC25B (referred to as 5169131) with an IC_{50} of 10.4 μM. The inhibitor shows competitive inhibition with the CDC25B substrate, OMFP. Co-crystallization of the protein with the inhibitor is difficult since the inhibitor tends to precipitate. Bahar's group performed simulations for docking the inhibitor on the protein using MOE package. In addition, a model for CDCD25B complexed with its substrate was generated. Superimposition of the CDC25B-inhibitor and CDC25B-OMFP models showed that the protein sites that bind the inhibitor and the substrate overlap, consistent with the experimentally observed competitive inhibition. The results from this collaborative study between Lazo, Wipf and Bahar labs are summarized in (Brisson et al, 2004).

C. SIGNIFICANCE.

Quantitative modeling of the complex interactions involved in cell signaling and regulation is important in understanding the origin and mechanism of deregulation processes and identifying new targets for molecular therapies. A productive cooperation has been initiated between scientists specialized in different aspects of these complex processes, evidenced by several manuscripts recently submitted/accepted, which support the synergistic effect of coordinating experimental and computational studies.

D. PLANS

Specific Aim 1a. Three future directions of research are: (1) further developing and testing hybrid models that combine different methods. We will devise models where different structural regions are represented at different levels of complexity (i.e. catalytic regions at atomic details, rigidly moving domains as elastic networks, etc.). (2) loop *structure* prediction and *flexible binding*, particularly at DNA/drug binding regions, in the light of the problems that emerged in modeling damaged DNA recognition (DP2) and competitive binding of inhibitors (DP3).

Specific Aim 1b. Further development MCell and DReAMM to bridge between molecular and cellular simulations is a key component of the multiscale computations aims of our pre-Center activities. MCell models can easily grow to include hundreds to thousands of separate mesh objects representing different cells or parts of cells, and thus significant scalability issues arise. We will adopt a hierarchical molecule/complex naming convention combined with a binary notation for the presence and state of different binding sites, and use wild-card characters to enable different binding events in any arbitrary order.

Specific Aim 1c. We are now taking a more detailed approach in which concentrations and rate constants reported in previous studies are incorporated. An important improvement will be to restore the resistance of the cells to small pro-apoptotic perturbations (i.e. maintain the stability of the resting state (Siehl et al., 2002). To this end, we will assume Hill-type cooperative kinetics for the binding of cyt *c* or procaspase-9 to Apaf-1. Preliminary studies already show that this leads to a bistable behavior depending on low or high apoptotic stimuli (e.g. death ligand concentration), consistent with the concept of a threshold value to induce apoptosis.

Specific Aim 2. Perhaps the most challenging aspect is a real integration of the activities conducted by different groups. We will continue the progress made already in new tool development, and work toward integrating new algorithms at coarse-grained molecular scales (Bahar), microphysiological scales (Stiles), and cellular/tissue scales (Ta'asan and Ermentrout).

Specific Aim 3. Spatially realistic models at different scales present substantial difficulties at the stage of interactive user design, visualization, and animation, so the pre-Center activities on the development of visualization and model design tools will be continued, some of which are already ongoing with new funding (Stiles).

DP1-3. Significant progress leading to publishable results was made during the preliminary studies performed within the scope of the three DPs, each constituting a good starting point for future progress in a Center of Excellence, and each being an example of *a newly initiated productive collaboration between experimental and computational labs.* We will pursue these studies towards a molecular understanding of factors affecting the response of cells in response to different pro- or anti-apoptotic stimuli (DP1), the DNA damage recognition or by-passing properties of DDB proteins (DP2), the competitive binding of different inhibitors to phosphatases (DP3). The current apoptosis model will be expanded to include heat shock proteins, the effects of different proteins inhibition and DNA damage, in accord with the aims of DP2 and DP3.

Other DPs. In addition to these three DPs, four projects are illustrative of new research areas that are directly related to the goals of the pre-Center, and could also evolve into driving projects for a full center: **(1)** *Mathematical analysis (binomial probability analysis) of single pixel calcium imaging data at neuromuscular junctions*, undertaken by J. Stiles in collaboration with S. Meriney (Neuroscience, Pitt), to predict the number and average opening probability of voltage-gated calcium channels at the frog neuromuscular junction, which already led to a publication (Washman et al., 2004); **(2)** *Monte Carlo simulation of presynaptic calcium dynamics and neurotransmitter release*, directed by J. Stiles and carried out by John Pattillo, a post-doc in Stiles lab. Potential-activation of voltage-gated calcium channels, stochastic calcium ion entry and diffusion, calcium binding to sensor sites on arrays of synaptic vesicles, and vesicle fusion and resulting transmitter release are simulated to obtain novel predictions for the number of calcium-binding sites on synaptic vesicles. **(3)** *Dynamics of ligand-gated ion channels that contribute to neuronal function, with focus on glycine receptor (GlyR) as a prototype*. These channels play a fundamental role in fast electrical signaling in the nervous system, and channel dysfunction or pharmacological modulation by drugs or anesthetics have profound effects. A multi-faceted collaboration is conducted with the leadership of Coalson (Chem, Pitt), Kurnikova (Chem, CMU) and Cascio (Mol Gen & Biochem, SOM, Pitt) labs, with both experimental and theoretical/modeling components, to elucidate structure/function relations in the GlyR. **(4)** *Mechanism of inhibition of HIV-1 reverse transcriptase (RT)*, a structural, computational and experimental study of the NNRTI-induced conformational changes in HIV-1 RT conducted by

Bahar (CCBB, Pitt), Madura (Chem & Biochem, Duquesne) and Sluis-Kremer (Medicine, Pitt) labs (Sluis-Kremer et al., 2004; Zhou & Madura, 2004a; 2004b)

E. PUBLICATIONS. Publications related to this grant are presented in Appendix I. A total of 22 publications (21 papers + 1 Book chapter) are listed, of which 15 are published/accepted and 7 submitted. Soft copies of most of the published/accepted papers are made accessible in the pre-NPEBC website http://www.health.pitt.edu/pcbc (website currently being updated).

F. SPECIAL REQUIREMENTS

F.1. Description of activities. Research activities have been described above. Organizational and educational activities are described in F.2 and F.4, respectively.

F.2. Organizational structure implemented. The organizational structure and activities are:

1. *Formation of an Executive Committee (EC)* also serving as an Internal Scientific Committee, composed of Bahar (Chair, Comp Biol & Bioinformatics, Pitt), Stiles (Co-Chair, PSC/CMU), Brown (Co-Chair, Biology, CMU), Madura (Co-Chair, Chem & Biochem, Duquesne), Ermentrout (Mathematics, Pitt), Rosenberg (Biology, Pitt), and Ho (Biology, CMU). These individuals are committed to these positions for the duration of the pre-NPEBC. The PI's of the DPs, Billiar (Surgery, Pitt), Wood (Molecular Oncology, UPCI), and Lazo (Pharmacology, Pitt), are serving as adjunct members. The EC Chair and/or Co-Chairs serve as a liaison with the Chairs (or Directors) of the participating Departments (or Centers). Quarterly meetings were held by the EC members.

2. *The organizational structure of research activities* exactly follows the diagram presented in the original proposal (Figure B.2.) with the only exception of B. Ermentrout replacing C. Chow (who moved to NIH) as the computational group leader in DP1.

3. *The selection of development projects* was already made in the original submission. We also proposed a mechanism for the review and elimination/selection of old/new DPs. The progress made in each of the original DPs supports their continuation, while other collaborations (other DPs; see above) have also emerged in the past year. The limited budget of the pre-NPEBC does not presently permit us to allocate more funds to support these other DPs and these are currently being conducted using other sources. Our plan is to apply for a full Center in the coming year, and include/request support for these newly emerging collaborative projects, as appropriate.

4. *External Advisory Committee.* Ronald Levy (Rutgers), Douglas Lauffenberger (MIT), Angela Gronenborn (NIH) and Robert L. Jernigan (Iowa State U) were contacted to serve as members of the EAC, who kindly accepted. The visits of the EAC members to Pittsburgh has been coordinated with the pre-NPEBC workshop and seminar series (see below).

5. *The first Pre-NPEBC workshop* was held on Feb 3, 2004 at the Pittsburgh Supercomputing Center, and was attended by over 50 researchers (PIs, postdoctoral fellows, and students) from Pitt, CMU, PSC, and Duquesne. The goal was to bring together researchers within the local community and to present the types of existing problems and data/methods that could benefit from an efficient collaboration between the computational and experimental groups. The workshop was organized in five 1-hour sessions, one on each of the existing developmental projects, one on “other” possible projects (with emphasis on membranes, signaling and neurobiology problems), and one on the integration of computational and mathematical models and methods. It concluded with breakout sessions for brainstorming within groups, and a final summary session where plans for advancing/continuing collaborative research were outlined. An outline of the workshop program can be found in Appendix III. A second pre-NPEBC workshop has been scheduled for Fall 2004, where speakers from universities outside the Pittsburgh area, including the EAC members will be invited to present their research and discuss the state-of-the-art research in multiscale modeling of complex biological processes.

6. *New pre-NPEBC Investigators*. New Faculty that contributed to pre-NPEBC activities are **J. Klein-Seetharaman** (Pharmacology, SOM, Pitt), **D. Zuckerman** (CCBB, Pitt) who joined Pitt after

the submission of the revised pre-NPRBC proposal, N. Sluis-Kremer (Medicine, SOM, Pitt), **S. Meriney** (Neuroscience, Pitt), **R. Rosenfeld** (CS, CMU), and **M. Kurnikova** (Chem, CMU).

6. *New Recruitments*. A major development was the recruitment of new Faculty who would contribute to the pre-NPEBC efforts. Our first goal was to strengthen the systems biology group, and we have been successful in recruiting an excellent scientist, **Dr. Ivan V. Maly** (PhD at Northwestern U with G. Borisy and postdoc with Doug Lauffenberger at MIT), specialized in cellular self-assembly and its coupling to cellular networks. Dr. Maly will be joining the CCBB as a tenure-tract Faculty in July 1, 2004, and will be an active member of the pre-NPEBC multiscale modeling efforts. Another Faculty recruitment is **Dr. David Swigon** (Rutgers), by the Math Dept at Pitt, originally a mathematician, specialized in modeling DNA elasticity and the regulation of transcription, who is expected to be involved in DP2. The CCBB is in also at the final stages of recruiting **Dr. Carlos Camacho** (Res Assoc in Vajda's lab, Boston U) at Associate Prof level, an expert in structure prediction and protein-protein docking, who will contribute to DP3.

7. *Starting a new Department of Computational Biology at Pitt SOM.* The CCBB at Pitt SOM recently submitted a proposal for becoming a department at the SOM, Pitt, which has already been approved by the SOM Executive Committee and is currently awaiting the approval of Planning and Budgetary Committee. It is expected that the organizational and educational activities of the pre-NPEBC and the possible future Center will significantly benefit from the establishment of a department (to be chaired by Dr. Bahar) fully dedicated to the development, implementation and integration of computational and theoretical methodologies in cooperation and coordination with experimental groups/studies.

F.3. DPs that have been initiated and progress made in each of them. See the paragraphs DP1, DP2 and DP3 in Section **B** (Studies and Results), and DP1-3 and Other DPs in Section **D**.

F.4. Progress toward developing educational opportunities in biomedical computing.
1. Planning a cross-campus PhD program in Computational Biology. A cross-campus PhD program in computational biology is planned between Pitt and CMU with the leadership of Drs. Bahar (CCBB, SOM, Pitt), Carbonell (Director of Language Techn Institute, Comp Sci, CMU) and Stiles (PSC/CMU). The vision is to offer a first-class program that capitalizes on the excellence of CMU in computer science and on the expertise and strength of Pitt in biomedical sciences. While the organization and administration of a joint program is a challenging task, both groups are enthusiastic and encouraged by productive collaboration already initiated within the scope of the present pre-NPEBC award and an NSF-ITR grant (PIs: Raj Reddy (CS, CMU) and J. Klein-Seetharaman (SOM, Pitt; Bahar and Carbonell are co-PIs). Three major tracts are conceived: bioinformatics, computational structural/molecular biology and system biology.
2. *Biomedical and Bioinformatics Summer Institute (BBSI)*. This is an NSF/NIH funded program that started in Sept 2002, offering a 10-week summer program to undergraduate (junior and senior) and graduate (1^{st} and 2^{nd}year) students interested in computational biology. The major theme of the program is 'Multiscale Modeling' in line with the pre-NPEBC goals, and six PI playing a key role in the pre-NPEBC (Bahar, Stiles, Coalson, Madura, Ermentrout and Meirovitch) serve as the course instructors and co-PIs of the program. 20+ Faculty from Pitt, CMU, PSC and Duquesne take part in mentoring the research activities of the students.
3. *Other programs and courses*. A new undergraduate degree-granting program in *Bioinformatics* has been designed with the leadership of Pitt CS and Biology departments, which is expected to admit students in Fall 2006. A PhD program in Molecular Biophysics with a strong component in Comp Bio has been approved at Pitt and CMU, starting in Fall 2004. CMU is currently offering B.S and M.S. programs in *Comp Bio & Chem*. A new graduate course has been designed at Duquesne, entitled "Simulation and Visualization", and another graduate course "Simulation Methods" jointly taught by Duquesne Chem and CS departments will be offered in Spring 2005.
4. *Postdocs and students* are involved in the pre-NPEBC activities, and thus have the opportunity to directly learn/apply up-to-date concepts and methods in biomedical computing, and take part in multidisciplinary research activities. See Appendix IV for the list of postdocs/students who have actively contributed to the computational and experimental activities of the pre-NPEBC in the past year, all being funded by 'other' sources.

Appendix I: Publications by pre-NPEBC investigators

(The pre-NPEBC participants (Faculty) and their postdocs/students are written in boldface. The publications labeled as (*) refer to developmental projects DP1-DP3 and the other DPs (see Section D)

Aronson Jr., Nathan N.; Halloran, B. A.; Alexyev, M. F.; Amable, L.; **Madura, J. D.;** Pasupulati, L.; Worth, C.; Van Roey, P. (2004). Mechanism of Chitin Oligosaccharide Hydrolysis by the Family 18 Chitinase A from *Serratia marcescens Biochem. J.*, in press.

Brisson, M., T Nguyen, **A Vogt, J** Yalowich, A Giorgianni, **D Tobi, I Bahar,** CRJ Stephenson, P **Wipf and JS Lazo**. 2004. Discovery and characterization of novel small molecule inhibitors of human Cdc25B dual specificity phosphatase *Molecular Pharmacology*, submitted. (*)

Chen, S-C. and **I Bahar**. 2004. Mining frequent patterns in protein structures: a study of protease families. *Bioinformatics* **20** Suppl. 1, i1-19.

Dalal, P.D., Robertson, K, Aronson, N.N.Jr**., Madura, J.D.** (2004). Isothermal Calorimetric Study of Oligosaccharide Binding to *Sm*ChiA E315L Mutant, *FEBS Letts*, submitted.

Dalal, P.D., Robertson, K, Aronson, N.N.Jr., **Madura, J.D.** (2004). Fluorescence Study of Oligosaccharide Binding to *Sm*ChiA E315L Mutant, *FEBS Letts,* submitted.

Graf, P., MG Kurnikova**, RD Coalson** and A Nitzan. 2004. Comparison of dynamic lattice Monte-Carlo simulations and dielectric self energy Poisson-Nernst-Planck continuum theory for model ion channels. *J. Phys. Chem. B* **108**, 2006-2015. (*)

Katpally, S.; Ghorab, M. K., **Madura, J. D**., Mosher, J., Thompson, D., Adeyeye, M. C. (2004). Characterization of Bile Salt/SBE-7? -cyclodextrin Interactions Using Isothermal Titration Calorimetry, submitted.

Rader, AJ and **I. Bahar**. 2004. Folding Core Predictions from Network Models of Proteins *Polymer*, 45, 659-668.

Rader, AJ., G. Anderson, **B. Isin,** H. G. Khorana, **I. Bahar**, and J. Klein-Seetharaman. 2004. Rhodopsin core residues stabilize the disulfide bond at the interface between transmembrane and extracellular domains" *Proc. Natl. Acad Sci USA,* in press.

Seki, M., C. Masutani, LW Yang, A Schuffert, S Iwai, **I Bahar and R Wood**. (2004) High efficiency bypass of DNA damage by a single human DNA polymerase, *EMBO J.,* submitted. (*)

Sluis-Cremer, N., NA Temiz, and **I Bahar.** (2004) Conformational changes in HIV-1 reverse transcriptase induced by nonnucleoside reverse transcriptase inhibitor binding. *Current Research in HIV* (in press). (*)

Stiles, JR, Ford, WC, Pattillo, JM, Deerinck, TE, Ellisman, MH, Bartol, TM, and Sejnowski, TJ, Spatially realistic computational physiology: past, present, and future, to appear in Parallel Computing: Software Technology, Algorithms, Architectures & Applications, ed. G. Joubert et al., Elsevier Press (2004, in press).

Tsonchev, S., **RD Coalson**, A Liu and TL Beck. 2004. Flexible Polyelectrolyte Simulations at the Poisson-Boltzmann Level: a Comparison of the Kink-Jump and Multigrid Configurational-Bias Monte Carlo Methods. *J. Chem. Phys.* **120** , 9817-9821.

Temiz, A., E. Meirovitch and **I. Bahar.** 2004. *E. coli* Adenylate Kinase Dynamics: Comparison of Elastic Network Model Modes with Mode-Coupling ^{15}N-NMR Relaxation Data, *Proteins: Structure, Function and Bioinformatics*, in press.

Thomas, L.L., Dalal, P.D., Aronson, N.N.Jr., **Madura, J.D**. (2004) Molecular Modeling of β(1→4) linked *N*-acetyl glucosamine in *Sm*ChiA Subsites, *J. Chem. Info. Comput. Sci.*, submitted.

Vodovotz, Y., P Kim, **Z Bagci**, **GB Ermentrout, CC Chow, I Bahar and T. Billiar**. 2004. Inflammatory modulation of hepatocyte apoptosis by nitric oxide: *in vivo*, *in vitro*, and *in silico* studies. *Current Molecular Medicine,* in press. (*)

Wachman, ES., RE Poage, **JR Stiles,** DL Farkas and **SD Meriney**. 2004. Spatial distribution of calcium entry evoked by single action potentials within the presynaptic active zone. *J. Neurosci.*, **24**, 2877-2885. (*)

Wang, Y. **Rader, AJ, Bahar, I.** and Jernigan, RL. 2004. "Global Ribosome Motions Revealed with Elastic Network Model" *J. Struct Biol*, in press.

Wierzbicki, A.; Dalal, P.; **Madura, J.D**. (2003) Molecular Dynamics Simulations of Crystal Induced Membranolysis *J. Phys. Chem.* 107, 12345-12351

Xu, C., Dror Tobi and **I. Bahar**. 2003. Allosteric Changes in Protein Structure Computed by a Simple Mechanical Model: Hemoglobin T --> R2 Transition". *J. Mol. Biol.* **333**, 153–168.

Zhou, Z. and **Madura, J. D.** (2004a) QSAR and CoMFA Study of HIV-RT Inhibitors *J. Chem. Inf. Comput. Sci.*, submitted. (*)

Zhou, Z. and **Madura, J. D**. (2004b) Relative Free Energy of Binding and Binding Mode Calculations of HIV-1 RT Inhibitors Based on Dock-MM-PB/GS *Proteins: Structure, Function and Bioinformatics*, in press. (*)

Other papers cited in the report:

M Fussenegger, JE Bailey, and J Varner. (2000). A mathematical model of caspase function in apoptosis. *Nature Biotechnology* **18**, 768-774.

Seki, M., F. Marini, and **R.D. Wood** (2003) POLQ (Pol theta), a DNA polymerase and DNA-dependent ATPase in human cells. *Nucleic Acids Res.* **31**:6117-6126

Appendix II: Pre-NPEBC Seminar Series

Date	Speaker	Presentation Title
October 13, 2003	Arieh Warshel, Ph.D. University of Southern California	Computer Simulations of Protein Functions: From Enzymes to Ion Channels and Other Functioning Biological Systems
November 25, 2003	Qiang Cui, Ph.D. University of Wisconsin	Preliminary Insights Into Mechanochemical Couplings in Mysosin with Molecular Simulations
December 10, 2003	Ronald Levy, Ph.D. Rutgers University	Effective Potentials for Protein Folding and Binding with Thermodynamic Constraints
January 22, 2004	Gerhard Hummer, Ph.D. Laboratory of Chemical Physics National Institutes of Health	Water in Confinement: From Nanotubes to Proteins
February 12, 2004	John Tyson, Ph.D. Virginia Polytechnic Institute & State University	Regulation of the Eukaryotic Cell Cycle: Theory, Computation and Experiment
March 4, 2004	H. Steven Wiley, Ph.D Pacific Northwest Laboratory	Interrogative Cell Signaling: How Cells Perceive Their Context
April 15, 2004	Carlos J. Camacho, PhD Boston University, Dept of Biomedical Engineering	Folded to bind: *In silico* predictions of protein-protein interactions
October 2004	Douglas Lauffenberger MIT Biological Engineering Division, Dept of Biology and Chem Eng	*(to be announced)*

Appendix III: Pre-NPEBC Worskhop, February 3, 2004.

Time	Topic/Speaker
9 – 9.15 am	**Introduction** Dr. Ivet Bahar, (CCBB, Pitt) Dr. Joel Stiles (PSC/CMU)
9.15 - 10.15 am	**Session I** **Nitric Oxide and Apoptosis** Dr. Yoram Vodovotz (Surgery, UPMC) Dr. G. Bard Ermentrout (Math, Pitt)
9.15 – 9.25 am	Dr. Yoram Vodovotz (Surgery, UPMC) *Nitric oxide and apoptosis*
9.25 – 9.35 am	Dr. G. Bard Ermentrout (Math, Pitt) *Modeling NO and apoptosis: theory and strategy*
9.35 – 10.00 am	E. Zerrin Bagci (CCBB, Pitt) *Nitric oxide and apoptosis*
10.00 – 10.15 am	Discussion
10.15 – 11.15 am	**Session II** **DNA Damage Recognition and Signaling** Dr. Richard Wood (UPCI and Pharmacology, Pitt) Dr. Ivet Bahar (CCBB, Pitt)
10.15 – 10.25 am	Dr. Richard Wood (UPCI and Pharmacology, Pitt) *Function & mechanism of DDB: a protein from human cells that recognizes DNA damage*
10. 35 – 10.45 am	Dr. John Rosenberg (Biol Sci, Pitt)
10.25 – 10.35 am	Dr. Vesna Rapic-Otrin (MGB, Pitt) *From UV-DDB to Cullin-Containing Ubiquitin Ligase*
10.45 – 10.55 am	Discussion
10.55 – 11.15 am	Dr. Baskaran Rajasekaran (MGB, Pitt)
11.15 am – 12.15 pm	**Session III** **Signaling Ligands and Cascades** Dr. John Lazo (Pharmacology, Pitt) Dr. Daniel Zuckerman (CCBB, Pitt)
11.15 – 11.25 am	Dr. John Lazo (Pharmacology, Pitt) *Modeling ligand interactions with signaling molecules*

11.25 – 11.35 am	Dr. Daniel Zuckerman (CCBB, Pitt) *Docking and binding computations: an overview*
11.35 – 11.45 am	Dr. Donald DeFranco (Pharmacology, Pitt) *Opposing roles of MAPK and Akt signaling pathways on neuronal cell survival: importance of temporal and spatial parameters*
11.45 am – 12.00 pm	Dr. Dror Tobi (CCBB, Pitt) *CDC25A / NSC663284 docking model*
12.00 noon – 12.15 pm	Dr. Billy Day (Pharmaceutical Sciences, Pitt) *Proteomics as a tool in drug discovery*
12.30 – 1.30 pm	**LUNCH**
1.30 – 2.30 pm	**Session IV** **Integration Session (Multiscale Modeling)** Dr. Joel Stiles (PSC/CMU) Dr. Jeffry Madura (Chem-Biochem, Duquesne)
1.30 - 1.45 pm	Dr. Jeffrey Evanseck (Chem-Biochem, Duquesne) (Molecular)
1.45 - 2.00 pm	Dr. Joel Stiles (PSC/CMU) (Subcellular) *Molecular-to-cellular modeling of complex biological processes*
2.00 - 2.15 pm	Dr. Shlomo Ta'asan (Math, CMU) (Cellular) *A framework for multiscale modeling of biological systems*
2.15 - 2.30 pm	Panel Discussion
2.30 – 3.30 pm	**Session V** **Other Projects** (membranes, signal transduction, hemoglobin) Dr. Michael Cascio (MGB, Pitt) Dr. Rob Coalson (Chem, Phys, Pitt)
2.30 – 2.40 pm	Dr. Roni Rosenfeld (CS/LTI, CMU) *Inferring property selection pressure from positional residue conservation*
2.40 pm – 2.50 pm	Dr. Michael Cascio (MGB, Pitt) *Nicotinicoid Ion Channel Structure*
2.50 – 3.00 pm	Dr. Rob Coalson (Chem, Phys, Pitt) *Modeling ion permeation through protein channels*
3.00 – 3.10 pm	Dr. Maria Kurnikova (Chem, CMU) *Hierarchica approaches to modeling of ion channels and receptors*
3.10 – 3.20 pm	Dr. Jeffry Madura (Chem-Biochem, Duquesne) *Bilayer simulations*

3.20 – 3.30 pm	Dr. Guillermo Romero (Pharmacology, Pitt) *Protein-lipid interactions*
3.30 – 4.00 pm	**Breakout Session**
4.00 – 4.30 pm	**Concluding Remarks**

Appendix IV: Postdocs and students who participated in pre-NPEBC activities

Name	Position	PI	Support Source
Z. Bagci	Graduate Student	Bahar	Pre-NPEBC
J. Chang	Scientific programmer	Stiles	NIH
S-C Chen	Graduate Student	Bahar	CCBB
Jennifer Dietrich	Undergraduate Student	Benos	NIH-NSF BBSI
R. Gandhlin	Postdoc	Ta'asan	Center for Nonlinear Analysis, Math, CMU
Paul Gera	Undergraduate Student	Tang	NIH-NSF BBSI
Scott Geyer	Undergraduate Student	Evanseck	NIH-NSF BBSI
Jennifer Greene	Undergraduate Student	B. W. Day	NIH-NSF BBSI
William Hawse	Graduate Student	Bahar	NIH-NSF BBSI
B. Isin	Graduate Student	Bahar	NSF ITR
John Jara	Graduate Student	Day	NIH-NSF BBSI
Natalie Kantz	Undergraduate Student	Meirovitch	NIH-NSF BBSI
Jeffrey Kidd	Undergraduate Student	Hicholas	NIH-NSF BBSI
R. Munshi	Postdoc	Bahar	CCBB
Kenneth Ober	Undergraduate Student	Klein-Seetharaman	NIH-NSF BBSI
J. Patillo	Postdoc	Stiles	NIH
Bridgette Payne	Graduate Student	Stiles	NIH-NSF BBSI
A. J. Rader	Postdoc	Bahar	CCBB
Mehrdad Safavian	Undergraduate Student	Bahar	NIH-NSF BBSI
Elisa Sandvik	Undergraduate Student	Deerfield	NIH-NSF BBSI
David Sivakoff	Undergraduate Student	Ermentrout	NIH-NSF BBSI
M. Seki	Postdoc	Wood	UPCI and NIH
N. A. Temiz	Graduate Student	Bahar	CCBB
D. Tobi	Postdoc	Bahar	NSF ITR / Pre-NPEBC
Kristin Wheeler	Undergraduate Student	Benos	NIH-NSF BBSI
C. Xu	Postdoc	Bahar	CCBB
Z. Zhou	Postdoc	Madura	State of PA DOH