In-Silico Nanobio-Design. A New Frontier in Computational Biology

Raul E. Cachau[1,*], Fernando D. Gonzalez-Nilo[2], Oscar N. Ventura[3] and Martin J. Fritts[4]

[1]*ABCC, SAIC-Frederick, Inc., National Cancer Institute at Frederick, Frederick, MD, USA;* [2]*CBSM, Universidad de Talca, Chile;* [3]*CCPG-DETEMA, Facultad de Química, Universidad de la República, Montevideo, Uruguay;* [4]*Nanotechnology Characterization Laboratory, SAIC-Frederick, Inc., National Cancer Institute at Frederick, Frederick, MD*

Abstract: Nanobiology is a fast-emerging discipline that brings the tools of nanotechnology to the biological sciences. The introduction of new techniques may accelerate the development of highly specific biomedical treatments, increase their efficiency, and minimize their side effects. Introducing foreign bodies into the complex machinery of the human body is, however, a great and humbling challenge, as past experience has shown. In order for nanobiology to reach its full potential, we must devise a means to alter the properties of nanoparticles, as expressed in the human body, in a predictable manner. Computer-aided methods are the natural option to speed up the development of these technologies. Yet, the procedures for annotation and simulation of nanoparticle properties must be developed and their limitations understood before computational methods can be fully exploited. In this review we will compare the state of development of nanoscale simulations in the biological sciences to that of the computer-aided drug design efforts in the past, tracing a historical parallel between both disciplines. From this comparison, lessons can be learned and bottlenecks identified, helping to speed up the development of computer-aided nanobiodevice design tools.

INTRODUCTION

The twin goals of biomedicine are the improvement of human life and the understanding of the fundamental processes that govern biological machineries. The two goals are inextricably linked to one another. This is particularly true in contemporary biomedicine where the goals have advanced to the treatment of very complex illnesses, including cancer, the correction of body imbalances resulting from aging, and the treatment and prevention of illnesses resulting from genetic predisposition. These aims will ultimately result in the development of highly personalized biomedical treatments [1].

Molecular microdevices will one day assist biomedical procedures in the same way as the surgeon uses mechanical instruments today [2, 3]. However, if we are at the dawn of a new era in biosciences, it is probably more due to nanobiology than to any other discipline [4-7]. The nanobiosciences encompass a body of disciplines that, with their bottom-up approach to biology, may finally unlock the full potential of human creativity in the biological universe, allowing the manipulation of biological systems using artifacts at the molecular scale [2, 4].

The current state of development of computational approaches for nanodevice design resembles that of the early days of computer-aided drug design. The road ahead is, however, long and treacherous. To our advantage, computational tools have grown in power, as has our ability to use them to their full potential. However, as we begin developing rational approaches to nanobiodevice design, those procedures must be developed, tested, and improved before the full power of this emerging discipline can be harnessed. We can apply our experiences in computer-aided drug design to the development of nanobiological drug design.

Nearly 30 years ago, the expression *rational drug design* was coined to describe a set of procedures for improving the pharmacological properties of chemicals, based on the knowledge of the three-dimensional arrangement of the molecule [8]. Indeed, the term *rational* was a carry-over expression from the physical sciences, which largely dominated the computer-driven efforts in drug design at the time. In physics, the nature of a system is entirely described by the positions of the elements that constitute the system and their associated momentum and energy.

At the time, *rational drug design* was frequently associated with the application of state-of-the-art computational tools for the calculation of quantum mechanical equations. In practice, this meant using largely unvalidated algorithms running on the fairly inefficient and underpowered computers then available. However, the difficulties encountered during the early efforts in computer-assisted drug design were not entirely due to the lack of computational power or accurate programs. They were also the result of our lack of understanding of the many ways in which a foreign molecule interacts with the extremely complex environment of a living system.

Thus, the expectation was that the knowledge of the three-dimensional structure of a molecule and its exploration using first-principle techniques would be sufficient to fully characterize its biological properties and predict the ways in which the molecule could be modified to optimize them. This was understood, simply, as an extension of the application of the methods of molecular physics to the biosciences. Biomedicine, it turned out, was quite a bit more complicated.

We should remember that out of every 6,200 designed and synthesized compounds, merely 7 are tested in humans and only 3 reach phase III studies. Out of those three, only 1 will successfully make it to the drugstore and at a cost of nearly $1,000,000,000 U.S. for the entire effort [9]. Most of the waste is due to toxicity, solubility, and bioavailability defects. Those properties are not, however, entirely predictable from the *isolated* structure of the molecule, but are the result of the complex interplay between the molecule and the cell and tissue environment.

NANOBIOLOGY AND COMPUTER AIDED DRUG DESIGN

The full *rational* approach to drug design was thus frustrated by our inability to exert the same level of control at every step of the process, from delivery to metabolism and secretion. We were, however, very successful at engineering *specificity*. In the words of Gregory Petsko, *hundreds* of the 6,200 pre-selected compounds are potentially very good drugs [9]. Those drugs may not get to the cell because they are not soluble enough, but at a basic level, those compounds could be perfectly functional for the inhibition of the targets they were designed against. This is particularly true for drugs resulting from structure-guided efforts where there are countless examples of the rapid development of highly specific lead compounds.

This situation has frustrated the computer-modeling community for decades. The *separation* of the target inhibitory function (drug) from the mechanisms used for its delivery and possibly for metabolite removal (carried out by nanoparticles) has the potential to change this entire situation.

*Address correspondence to this author at the SAIC-Frederick, 430 Miller Dr. Frederick, MD 21702; Tel: (301)-846-6062;
E-mail: cachau@ncifcrf.gov

1568-0266/07 $50.00+.00

Thus, we can envision two immediate uses for nanovessels: drug delivery, where the nanovessel is used to carry a payload to a specific destination; and imaging, where the payload has a detectable property [1]. In either case, the nanovessel properties would be responsible for the control of the delivery, solubility, and general toxicity of the system [1]. If this approach to drug delivery proves successful, thousands of previously discarded compounds could be reconsidered for encapsulation in nanovessels [1].

Although the introduction of a separate mechanism for delivery and solubility seems liberating for those involved in chemical design efforts, in some sense all we have done is pass the problems along. For instance, think about the computational cost of computing the quantum mechanical properties of a common drug (less than a thousand atoms.) Most *simplified* quantum mechanical algorithms will have a computational cost that grows with the square of the number of atoms. In the past, we applied those techniques with mixed success to *molecules*, but now we need to apply them to *nanoparticles*, molecular systems including thousands to millions of atoms. Even specialized quantum mechanical algorithms designed to deal with large numbers of atoms (with order N computational cost) have an entry cost which is fairly steep. Again, this is reminiscent of the early days of computer-aided drug design; using oversimplified algorithms in seriously limited hardware.

The problem is further complicated by the difficulties in obtaining experimental structures for many nanobioparticles. Most nanobioparticles do not crystallize, nor are they easily tractable by standard structural methods. This is particularly true for liposomes, dendrimers, and many metal particles, which tend to aggregate in rather unpleasant ways instead of forming lattices. Their structures are frequently highly symmetric, further confusing in-solution methods. Thus, in order to compute the properties of nanobioparticles from their structures, we must rely on *models* (guessed 3D coordinates) *and* property prediction methods. Choosing the best combination of model building and property prediction method constitutes a problem in itself.

Therefore, we need a strategy to tackle the complex set of problems presented by the study of nanobioparticles. In our favor, organic chemists have extensive experience in developing passivated materials and can incorporate motifs that interact with the biological system via cell receptors, antibodies, or a plethora of other mechanisms. The combined use of both passivation and specific biomarkers has done much to rationalize, simplify, and systematize the basic procedures employed for the *general* design of nanobioparticles.

NANOPARTICLES AS A DESIGN PROBLEM: LOOKING FOR PATTERNS

One of the most advanced areas of nanoparticle research is that of dendrimer design [10]. Dendrimers are archetypical nanobioparticles, completely artificial human creations, not present in nature. A dendrimer is a regularly branched, fully synthetic polymer molecule which resembles the branches of a tree. In fact, the name comes from the Greek *dendron*, meaning *tree*. The first dendrimers were described by Vögtle [11] in 1978; by Denkewalter and coworkers at Allied Corporation as polylysine dendrimers in 1981; by Tomalia at Dow Chemical [12], and by Newkome [13] in 1985. In the 1990s dendrimers caused an explosion of scientific interest because of their unique molecular architecture. This resulted in over 5,000 scientific papers and patents published by the end of 2005.

We can draw some general patterns from dendrimer design studies. For instance, poly-ethylene glycol termination ensures a dendrimer will be well behaved, and the inclusion of antibodies or motifs recognized by cell receptors produce dendrimers that are readily accepted by the cell [1]. Despite these over-generalizations (for a comprehensive review on the subject see Tomalia [5, 14]), we can recognize trends that translate into a modular design of nanobioparticles. Knowing the trends should help us build modules in our computational tools that correspond to the modular structure of the nanoparticle. What happens under the dendrimers' canopy is a different story. A delicate balance of forces must be optimized in order to secure the drug uptake and its timely release at the proper site. Too tight a grip on the drug, and its release may be impeded. Too loose an interaction or too permeable the canopy, and the compound may leak during transport. The analysis of the drug–dendrimer interaction presents similarities with the old fashioned drug–enzyme interaction studies. However, our interest when studying dendrimer–drug interactions is as much in the path of uptake/release as in the mechanism of in-situ interaction. The main bottleneck in mapping the release path of a molecule from its binding site is computational time. Steered dynamics [15] is a new tool that has been successfully applied to this type of study in other areas of biomolecular engineering. In addition, parallel replica dynamics [16] and hyperdynamics [15] are other algorithms that can be used to explore rare, infrequent events.

The main conclusion we can draw from this analysis is that the software used to compute the dendrimer properties (and by extension the properties of other nanoparticles) should have a layered structure, with a core region describing the particle at an atomic detail, and an external shell that accepts a coarser treatment. Since the properties of nanoparticles are largely the result of their specific dimensions, what constitutes a molecular module within a nanoparticle may not be obvious. We can, however, make a comparison with the organization of natural biomolecules [17].

A first look at how biomolecules are organized suggests that sugars, nucleotides, peptides, and lipids are among the basic building blocks of the cell. However, further inspection reveals that the true nature of the molecular organization is coarser [17]. Folding constraints force the repetition of secondary structure elements among proteins. These patterns are so regular that in a few years, current initiatives in structural genomics are expected to have mapped out all the main structural blocks that form macromolecular structures.

Atom-by-atom manipulation allows a larger amount of creative freedom in the production of nanobioparticles, enabling the production of particles with unique properties. However, the same freedom used to create these large aggregates without the constraints imposed by the use of standard components (i.e., amino acids, nucleotides, etc.) makes the identification of the intermediate complexity modules that give raise to the nanoparticles' peculiar properties a real challenge. We could ask, for instance, what would be the structural equivalent, at a functional level, of an alpha helix in a dendrimer?

One valuable lesson from our previous efforts with small molecules is that even at that small scale, there are recognizable motifs that trigger specific responses from the biological system. If the biological response information is mapped onto the 3D scaffold of the molecule, an *ad hoc* function-structure map can be built. These maps can be used to establish what are frequently called structure–activity relationships (SAR) and has been one of the most useful ways in which structural information can be used to guide the design of novel compounds [18]. By identifying the structural motifs that give rise to the molecular properties, new and improved leads can be designed. We can envision a similar use of experimental information in combination with structural analysis in nanobiology [19].

The identification of functional motifs in nanoparticles will have other benefits for computer-aided nanoparticle design. The computation of large molecular aggregates carries risks associated with computational error propagations, which can take many forms. Multi-layered approaches may be a better option when dealing with extremely large problems.

The approaches discussed so far do not include an explicit description of the cell system and even less for tissues or the general environment. As we previously mentioned, this has been one of the main difficulties in computing toxicity, solubility, and ADME parameters in the past.

The simplest way in which we can introduce a phenomenological description of the biological environment is by means of average fields. This was done in past computer-aided drug design efforts in myriad ways, which are too extensive to review here. This general approach, however, had limited success beyond the computation of solubility parameters which are more tractable in this manner (i.e., by Poison Boltzman and reaction field treatments). We can try to extend and improve this type of modeling treatment by re-examining what makes nature's constructs as well as approved drugs successful.

Some basic properties are common among all commercially available drugs. For instance, a drug should not rapidly and spontaneously hydrolyze or its half-life would be too short to be useful. These and other criteria have been used to develop the loosely crafted concept of *druggicity*—the quality of a chemical compound to be more like a drug than another. Similarly, we could ask what makes a protein a biologically compatible molecule, unlike other polypeptides that we can synthesize in the laboratory. If we analyze the microenvironment of a natural macromolecule or a drug throughout its life in the body, we will notice that, although the average values of temperature (or pH or ionic strength) may be well-established, these are macroscopic parameters and large fluctuations are observed in the microenvironment. Furthermore, many biomolecules have a ubiquitous presence across tissues. In other words, rapid changes in the microenvironment and multiple locations demand that biologically compatible molecules be fairly resilient to short time insults from the surrounding environment. Some of the mechanisms used by macromolecules to defend themselves are quite evident. For example, pepsin (326aa) carries its own buffer in the form of 36 aspartic and glutamic residues in order to survive the extremely aggressive environment of the stomach [17]. We can generalize this observation by saying that a well-behaved biomolecule must have built-in mechanisms to remain stable against small fluctuations in the macroscopic parameters (pH, μ, T etc. .) Sensitivity analysis has been pioneered by Roberta Susnow [20, 21] and proven a great asset in the determination of difficult molecular geometries. In practical terms, we have applied this analysis to nanobiomolecules by studying the variability of molecular parameters when challenged by electric fields, simulated changes in pressure, etc. A well-behaved nanoparticle must behave in a stable and predictable manner within a large range of challenges. An extension of this analysis would include the decomposition of the information obtained in terms of contributing motifs, which leads us back to multi-layered approaches.

THE COMPUTATIONAL CHALLENGE

Ultimately, more realistic and better-integrated approaches covering the whole scale of the problem from whole body scale down to the atomic detail will be needed. Many elements of the nanobiology modeling toolbox will be based on prior developments in structural biology and material sciences.

Quantum mechanics–molecular mechanics (QM-MM) hybrid methods are a well-established way to extend the use of standard quantum mechanical techniques to larger systems [22]. *Fujitsu* has introduced a simplified quantum mechanical code (MOZYME/COSMO) for whole macromolecular simulations. SIESTA [23] and similar codes, based on localized plane waves density functional treatments (LPW-DFT), are an alternative to more computationally expensive approaches when more accurate treatments are needed. The application of LPW-DFT to gold nanoparticle-thiol interactions provided a first look at the way in which the thiol linker modifies the arrangement of the gold atoms in nano-scale structures, a key piece of information on our under-standing of how coating agents interact with nanoparticles [24]. Molecular mechanics-based calculations have also shown a steady improvement in recent years. NAMD allows the computation of molecular properties of very large molecular aggregates (millions of atoms) in highly distributed computational environments and the visualization and analysis of the modeling results (VMD) [25]. The combination of these new, faster programs with more efficient algorithms like replica dynamics [16] and hyperdynamics [15] is opening new possibilities in the simulation of larger time scale and more complex molecular events. Meso-scale calculations are now feasible, too [26]. MesoDyn is an interesting tool for this kind of study [27]. Meso-scale/atomic scale hybrid treatments have also been developed [28], but less successfully than in the case of QM-MM, with meso-hybrids still being prone to artifacts [29].

A very important problem unresolved by the above-mentioned treatments is the prediction of a particle's body location. This problem is on a much larger scale and is of critical importance to nanoparticle modeling studies, since knowing the location of the nanoparticles could drastically simplify our need to compute nanoparticle properties in multiple environments. Computational Fluid Dynamics (CFD) approaches may be the answer to some of those concerns. *CFD Research* has put forth an interesting multi-scale, mechano-biological model of nanoparticle toxicity, based on such approaches. The goal of *CFD Research* is to build a unified quantitative understanding of nanoparticle deposition and partitioning within the body (lung, liver, spleen, and brain). For example, nanoparticle deposition in the lung is site-specific and depends, among other factors, upon the aerodynamic size and electrostatic charge distributions. Once inhaled, nanoparticles can reach the sensitive alveolar regions and stay there for long periods of time. Models describing cellular response upon nanoparticle contact can then be used to determine the level of tissue response (i.e., inflammation, in the case of toxic effects). More importantly, the knowledge of the location of the particle retention sites can be used to analyze which tissue-specific receptors may be involved, in order to enhance or avoid the nanoparticle uptake at that site.

Establishing the relations between these ill-connected procedures will depend on our ability to characterize the molecular motifs that give rise to the nanoparticle properties at each scale. The lack of such level of description in the current literature is hardly surprising, given the paucity of systematic characterization of nanobioparticles at the molecular level. Current efforts at relating meso-scale descriptions of nanoparticles with their structural, physico-chemical, and toxicological properties demonstrate the feasibility of such approaches [30, 31]. Yet more needs to be done at the structural level of annotation of nanobioparticles to identify functional motifs, build reasonable SAR protocols and, ultimately, develop the required software to jump-start the computer-aided design of nanobioparticles.

CONCLUSIONS

We can assert, with little doubt, that nanobiology will have an ever-increasing role in improving our capacity to do *in-vivo* imaging and intervention or *ex-vivo* analysis. Nanoparticles will allow the use of combinations of agents to deal with the causative agents of disease and in targeting intervention sites accurately, avoiding biological barriers, minimizing collateral effects on healthy tissue, and possibly even monitoring the course of treatment in real time [1, 6, 32].

If past experience with drug design efforts is an indication, the speedy success of these novel techniques will require our understanding of the basic motifs responsible for the nanoparticle function. Structural activity relationships (SAR) can improve nanobioparticles while uncovering new, unexpected properties, but

the tools for structure modeling and analysis of nanoparticles must be developed and validated.

The history of computer-aided drug design shows successes in designing specificity and problems in correctly predicting ADME parameters, especially absorption (solubility, etc.) and PK/PD. Nanoparticles are a logical extension of previous delivery systems on a smaller scale; they can encapsulate or bind drugs for delivery, circumventing some ADME and PK/PD problems.

On the other hand, nanoparticles are much larger structures, when compared to small drugs, and impose a bigger demand on computational methods. Advances in computing power, algorithms, and codes may help surmount some of those difficulties, but modeling the interactions of drugs and/or carriers with a realistic biological matrix remains a "grand challenge" problem. Furthermore, nanobioparticles can exhibit a far greater range of structural variability than small moieties, and the attempt to characterize and standardize their structures is just beginning.

Therefore, we expect the structure of nanobiology modeling software will be layered to handle the large computational demands imposed by the complexity of the problem. The modeling protocols should also be flexible, since the whole body scale (particle location) down to quantum mechanical scale problems will have to be integrated during the design cycle. The software flexibility should accommodate the links between layers, which will be more easily implemented through SAR parametric relations.

Ultimately, controlling and predicting the properties of designed nanobioparticles will require a concerted effort to develop SAR-based design loops to guide their design. Thus, the road ahead will demand joint efforts in rational design of nanoparticles *and* in their physical, *in vitro*, and *in vivo* characterization to standardize well-characterized delivery systems, provide data to validate the models and codes, and, finally, develop the associated SARs which will be used to accelerate the design/synthesize/characterize loop.

To reach this goal, we must begin collaborative efforts to attack the grand-challenge, the problem of modeling nanomaterials in a realistic biological environment; to accelerate the introduction of improved instrumentation to aid in more accurate and efficient characterization of nanomaterials; and to introduce nanocomputing and structural annotation as a pervasive tool throughout the design cycle.

Early examples of such collaborative efforts are work of the Nanotechnology Characterization Laboratory in cataloging nanoparticles and their properties, and the nanoHUB and nanoHIVE initiatives which should bring together the software components required for the development of integrated simulation packages.

ACKNOWLEDGMENT

This work has been funded in part with funds from the NCI-NIH (Contract No. NO1-CO-12400) and Programa Bicentenario de Ciencia y Tecnología de Chile (project No. ACT24). The contents of this publication do not necessarily reflect the views or policies of the DHHS, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

REFERENCES

[1] McNeil, S.E. Nanotechnology for the biologist. *J. Leukocyte Biol.*, **2005**, *78*, 585-594.

[2] Ferrari, M. Cancer nanotechnology: Opportunities and challenges. *Nat. Rev. Cancer*, **2005**, *5*(3), 161-171.

[3] Duncan, R. The dawning era of polymer therapeutics. *Nat. Rev. Drug Discov.*, **2003**, *2*(5), 347-360.

[4] Peck, J.C. *et al*., Big Ideas and Small Technologies for the Pharmaceutical Industry's Research and Development Future. Business Briefings: Pharmatech, **2003**: pp. 72-76.

[5] Tomalia, D.A. Birth of a new macromolecular architecture: dendrimers as quantized building blocks for nanoscale synthetic polymer chemistry. *Prog. Polymer Sci*., **2005**, *30*(3-4), 294-324.

[6] Rosi, N.L.; Mirkin, C.A. Nanostructures in biodiagnostics. *Chem. Rev.*, **2005**, *105*(4), 1547-1562.

[7] Mirkin, C.A. The beginning of a small revolution. *Small*, **2005**, *1*(1), 14-16.

[8] The Practice of Medicinal Chemistry, ed. C.G. Wermuth. 2003, Oxford: Academic Press.

[9] Strømgaard, K. Chemistry meets Biology : Chemical Approaches to the Study of Biology. *ChemBioChem*, **2004**, *5*, 1601-1603.

[10] Svenson, S.; Tomalia, D.A. Commentary - Dendrimers in biomedical applications - reflections on the field. *Adv. Drug Deliv. Rev.*, **2005**, *57*(15), 2106-2129.

[11] Buhleier, E.; Wehner, W.; Vogtle, F. Cascade-Chain-Like and Nonskid-Chain-Like Syntheses of Molecular Cavity Topologies. *Synthesis-Stuttgart*, *1978*(2), 155-158.

[12] Tomalia, D.A. *et al*., A New Class of Polymers - Starburst-Dendritic Macromolecules. *Polymer J.*, **1985**, *17*(1), 117-132.

[13] Newkome, G.R. *et al*., Micelles .1. Cascade Molecules - a New Approach to Micelles - a [27]-Arborol. *J. Organ. Chem*., **1985**, *50*(11): 2003-2004.

[14] Tomalia, D.A.; Frechet, J.M. Introduction to "Dendrimers and dendritic polymers". *Prog. Polymer Sci*., **2005**, *30*(3-4), 217-219.

[15] Voter, A.F.; Montalenti, F.; Germann, T.C. Extending the time scale in atomistic simulation of materials. *Ann. Rev. Mater. Res.*, **2002**, *32*, 321-346.

[16] Liu, P. *et al*., Replica exchange with solute tempering: A method for sampling biological systems in explicit water. *Proc. Natl. Acad. Sci. USA*, **2005**, *102*(39), 13749-13754.

[17] Goodsell, D.S., Bionanotechnology: Lessons from Nature. 2004: Wiley-Liss, Oxford.

[18] Richards, W.G. Computer-Aided Drug Design. *Pure Appl. Chem.*, **1994**, *66*(8), 1589-1596.

[19] Cachau, R.E. *et al*., New modeling strategies for the computational characterization of nanobioparticles. *Biophys. J*., **2007** (in press).

[20] Susnow, R.; Senko N.; Ocain, T. Conformational Sensitivity Analysis of Fkbp-Fk506 Rapamycin Complexes. *J. Comput. Chem.*, **1994**, *15*(10), 1074-1090.

[21] Susnow, R. *et al*., Conformational Study of Dipeptides - a Sensitivity Analysis Approach. *J. Comput. Chem*., **1994**, *15*(9), 947-962.

[22] Friesner, R.A., Ab initio quantum chemistry: Methodology and applications. *Proc. Natl. Acad. Sci. USA*, **2005**, *102*(19), 6648-6653.

[23] Soler, J.M. *et al*., The SIESTA method for ab initio order-N materials simulation. *J. Physics-Condensed Matter.*, **2002**, *14*(11), 2745-2779.

[24] Garzon, I.L. *et al*., Do thiols merely passivate gold nanoclusters? *Phys. Rev. Lett.*, **2000,** *85*(24), 5250-5251.

[25] Phillips, J.C. *et al*., Scalable molecular dynamics with NAMD. *J. Comput. Chem*., **2005**, *26*(16), 1781-1802.

[26] Maiti, A.; Wescott, J.; Goldbeck-Wood, G. Mesoscale modelling: recent developments and applications to nanocomposites, drug delivery and precipitation membranes. *Int. J. Nanotechnol*., **2005**, *2*(3), 198-214.

[27] Fraaije, J.; Zvelindovsky, A.V.; Sevink, G.J.A. Computational soft nanotechnology with mesodyn. *Mol. Simulation*, **2004**, *30*(4), 225-238.

[28] Chang, R.; Ayton, G.S.; Voth, G.A. Multiscale coupling of mesoscopic- and atomistic-level lipid bilayer simulations. *J. Chem. Phys*., **2005**, *122*(24).

[29] Jakobsen, A.F.; Mouritsen, O.G.; Besold, G. Artifacts in dynamical simulations of coarse-grained model lipid bilayers. *J. Chem. Phys*., **2005**, *122*(20).

[30] Decuzzi, P. *et al*., A theoretical model for the margination of particles within blood vessels. *Ann. Biomed. Eng*., **2005**, *33*(2), 179-190.

[31] Decuzzi, P.; Ferrari, M. The adhesive strength of non-spherical particles mediated by specific interactions. *Biomaterials*, **2006**, *27*(30), 5307-5314.

[32] Chang, M.M.C. *et al*., Nanotechnologies for biomolecular detection and medical diagnostics. *Curr. Opin. Chem. Biol.*, **2006**, *10*(1), 11-19.

Received: January 12, 2007 Accepted: January 23, 2007