Visual Molecular Dynamics Investigations of the Impact of Hydrophobic Nanoparticles on Prognosis of Alzheimer's Disease and Cancers

I.C. Baianu*, M. Charles and V.I. Prisecaru

*University of Illinois at Urbana, ACES College,

FSHN, Agricultural & Food Chemistry NMR & NIR Microspectroscopy Facility,

350 Burnsides research Laboratory, 1208 Pennsylvania Ave, Urbana, IL. 61801, USA

email**: i-baianu@uiuc.edu**

ABSTRACT

The possible impact of hydrophobic lectin nanoparticles on the prognosis and progression of Alzheimer's disease (AD) and cancers was investigated by Visual Molecular Dynamics (VMD) computer modeling programs available from the Beckmann Advanced Research Institute at the University of Illinois at Urbana. Our results indicate the possibility of impeding pathological aggregation of certain proteins such as modified tau- or beta-amyloid that are currently being considered as possible causes of Alzheimer's disease. VMD programs serve as useful tools for investigation hydrophobic protein aggregation that may play a role in aging of human populations.

* Corresponding Author.

KEY WORDS:

Visual Molecular Dynamics (VMD) computer modeling applications in Nanomedicine; novel vaccines from plants; medical applications of Lectin nanoparticles for diagnostics and treatments; Nuclear Magnetic Resonance (NMR) , MRI/ MRM; PET scanning; Visual Molecular Dynamics (VMD)investigations
VMD applications in Nanomedicine
Prognosis of Alzheimer's Disease (AD)
hydrophobic protein nanoparticles
hydrophobic lectin nanoparticle structure
hydrophobic lectin nanoparticle dynamics
lectins clinical trials in cancer

1. **INTRODUCTION**

Visual Molecular Dynamics (VMD) computer modeling is a readily available, intuitive and powerful tool for studying complex molecular interactions such as protein aggregation and its effect on protein activity and physiological functions. We are reporting the first VMD investigation of the aggregation process of selected proteins, such as hydrophobic lectin nanoparticles, with potentially important applications in Nanomedicine for the prognosis and possibly therapy as well of cancers and Alzheimer's Disease (AD). In the case of AD there is a large body of accumulated evidence that hydrophobic aggregation of modified proteins such as the τ— **(or tau**-) and **beta-amyloid (or a-β) abeta** proteins may actually be the cause of AD**.** Any attempt at treatment of AD is however impeded by a lack of understanding of the highly complex interactions involved in the initiation and progression of AD, although- in the very rare cases of AD with a hereditary component- progress has been made through detailed genetic population models aided by computer statistical analysis. VMD has not been employed so far to unravel the very difficult complexity problems posed by the aggregation of hydrophobic proteins. As discussed in this report, the lectin plant proteins present both a useful model for studying hydrophobic nanoparticle aggregation and a possible approach to improving the prognosis and outcomes of AD as well as certain types of cancers not yet investigated through clinical trials. Therefore, VMD applications in Nanomedicine have the potential for aiding the design and implementation of successful clinical trials in cancer and AD.

2. **LECTIN APPLICATIONS TO CANCER DETECTION AND TREATMENT**

Lectins are proteins, or more precisely glycoproteins, that agglutinate erythrocytes of some or all blood groups *in vitro* (Sharon 1998). They are an important group of bioactive proteins and glycoproteins found in most organisms (Baianu and Prisecaru, 2011), including plants, vertebrates, invertebrates, bacteria and viruses, and have several important applications to the fields of health food and medical biotechnology. Lectins are used as tools in the fields of biochemistry, cell biology and immunology, as well as for diagnostic and therapeutic purposes in cancer research (Sharon and Lis 2002). Lectin aggregation can be employed on a large-scale basis for the commercial production of biologically active proteins (Takamatsu et al, 1999). Lectins have been used in glycoprotein purification, oligosaccharide analysis, as well as in cell-selection processes. Lectins can bind reversibly with free sugars or with sugar residues of polysaccharides, glycoproteins or glycolipids (Goldstein and Poretz, 1986). There has been

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increasing demand for novel diagnostic and medical cancer therapies that utilize non-traditional sources. Epidemiological studies indicate that the consumption of a plant-based diet is strongly associated with a reduced risk of developing several types of cancer (Block et al, 1992). Plants contain numerous phytochemicals that can alter cancer-associated biochemical pathways. One such group that was investigated examined for its role in cancer chemoprevention is lectins. Lectins were reported to bind preferentially to cancer cell membranes, or their receptors, causing cytotoxicity, apoptosis and/or tumor growth inhibition. Lectins were thought to become internalized into cells, and some lectins were claimed to cause cancer cell agglutination and/or aggregation. Present in common foods, some lectins resist acid and/or enzymatic digestion and also were reported to enter the bloodstream in an intact, and biologically active, form. Lectins possess a spectrum of beneficial, as well as harmful, effects both *in vitro* and *in vivo*. Ingestion of lectins also sequesters the available body pool of polyamines, thereby claimed to thwart cancer cell growth. They have also been reported to affect the immune system by altering the production of various interleukins, or by activating certain protein kinases. Lectins were also reported to bind to ribosomes and thus inhibit protein synthesis. Lectins may also modify the cell cycle by inducing non-apoptotic G_1-phase accumulation mechanisms, G_2/M phase cell cycle arrest, and apoptosis, and might activate the caspase cascade. Lectins were also reported to down-regulate telomerase activity and inhibit angiogenesis. Lectins could inhibit cell adhesion, proliferation, colony formation and hemagglutination, and were reported to have cytotoxic effects on human tumor cells. Lectins could function as surface markers for tumor cell recognition, cell adhesion, signal transduction across the membrane, mitogenic cytotoxicity and apoptosis. Also, lectins were reported to modulate the growth, proliferation and apoptosis of premalignant and malignant cells both *in vitro* and *in vivo*.

For many years, lectins have been considered to be toxic substances to both cells and animals, mainly because of the observed agglutination of erythrocytes and other cells *in vitro*. On the other hand, it has also been reported that lectins have an inhibitory effect on the growth of tumors. Their potential for clinical applications has been investigated only in recent years. Lectins are now being considered for use both in the diagnostics and therapeutics of cancers. Thus, lectins are quite versatile biomarkers and have been utilized in a variety of studies involving histochemical, biochemical and functional techniques for cancer cell characterization (Munoz *et al*, 2001). Lectins may also be very useful tools for the identification of cancers and the degree of metastasis, or cancer development stage. Recently, there has been a tendency to shift lectins use from cancer detection to actual use in combating cancer. The reason for this shift is mainly

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caused by recent research that indicated the cytotoxic and apoptosis/necrosis-inducing effects of certain lectins, combined with the hypothesis that dietary lectins enter the systemic circulation intact (Wang et al, 1998).

One important feature appears to be that lectins stimulate the human immune system. Lectins were thus reported to exhibit antitumor and anticarcinogenic activities that could be of substantial benefit in cancer treatment. Extracts of *Viscum album* (mistletoe) are widely used as complementary cancer therapies in Europe. Mistletoe has been used parenterally for more than 80 years as an anticancer agent with strong immuno-modulating action. The quality of life of patients with late-stage pancreatic cancer was reported to be improved on account of exposure to mistletoe lectin (Friess et al, 1996). Immuno-modulation using recombinant ML was reported to influence tumor growth in breast cancer patients (Stein et al, 1998). Bladder carcinoma was reported to be significantly reduced, and survival times were reported to be prolonged in mice as the concentration of ML was increased from 3 to 30 ng. ML increased the life span, decreased the tumor growth and decreased hyperplasia of mice and rats with lymphoma and lung cancer (Kuttan et al, 1997).

A lectin purified from mesquite seed was reported to have an anti-proliferative effect on the cervical human tumor (HeLa) cells and on cell adhesion. *Vicia faba* agglutinin (VFA), a lectin from broad beans was reported to aggregate, stimulate the morphological differentiation of, and reduce the malignant phenotype of colon cancer cells (Jordinson et al, 1999). Wheat germ agglutinin (WGA) proved to be highly toxic to human pancreatic carcinoma cells *in vitro*. WGA exposure induced chromatin condensation, nuclear fragmentation and DNA release, consistent with apoptosis. The binding of the snail lectin Helix pomatia agglutinin (HPA), which recognizes N-acetylgalactosamine and N-acetylglucosamine sugars, is considered to be a strong predictor of metastasis and unfavorable prognosis in a number of human adenocarcinomas, including breast cancer (Brooks and Carter, 2001). Because of their carbohydrate bio-recognition properties, lectins may also be used as carriers for targeted drug delivery, in a manner similar to liposomes (Wroblewski et al, 2001), provided the possible side effects of such treatments could be minimized.

It has been observed that mucosal expression of terminal unsubstituted galactose is increased in colon pre-cancerous conditions and cancer, and that it allows interaction with mitogenic galactose-binding lectins of dietary or microbial origin. Based on this observation, an interesting

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hypothesis was postulated whereby galactose might be able to prevent cancer by binding and inhibiting such lectins from interacting with colon cancer cells (Evans et al, 2002). D-galactose treatment was reported to be effective in liver lectin blocking to prevent hepatic metastases in colorectal carcinoma patients (Isenberg et al 1997). Epithelial cancer cells showed an increased cell surface expression of mucin antigens with aberrant O-glycosylation, notably Thomsen-Friedenreich Antigens (TFA). TFA is a carbohydrate antigen with a proven link to malignancy (Irazoqui et al 2001). Immunoassays could be utilized for antigens such as TFA in order to determine the metastatic potential of breast and colon cancer cells. Molecular changes in the membrane surface in the case of both stomach and colon cancer cells occur during the progression to carcinogenesis. Carbohydrate patterns displayed on the cellular membrane exterior are *molecular signatures* with *unique* biological characteristics related to oncogenesis and metastasis, and could be used to determine the appropriate chemotherapeutic and surgical procedures for each specific cancer.

Lectins have already a demonstrated potential for the treatment, prevention and diagnosis of chronic diseases such as cancer. However, further research is required to elucidate the effects of purified and dietary lectins and their potential for defense against tumors.

3. COMPUTATIONAL BIOLOGY, MOLECULAR MODELING AND THEIR BIOMEDICAL APPLICATIONS IN NANOMEDICINE

Computational Biology has a very wide range of applications currently thought of 'belonging' to Biotechnology (Baianu, 1986) and Bioinformatics (Baianu, 2011), even though the development of such computations has preceded modern Biotechnology by many decades. Our approach is to focus on a few selected examples in greater depth, by considering molecular modeling techniques that have very wide applications, ranging from 'pure' chemistry to Biochemistry, Molecular Biology, Biotechnology, Nanomedicine, Pharmacology and Pharmacogenomics.

3.1. *Molecular Modeling Techniques*

Molecular modeling is a group of techniques that employ computer-generated images of chemical structures that show the relative positioning of all the atoms present in the molecule being studied, and/or the simulated dynamics of such molecules together with their ordering through space-time. Such techniques are of considerable help for understanding many

physicochemical properties of molecules, and may also provide clues about their possible role(s), that is, their *function*, in the organism. They can be thus especially valuable tools for investigating *structure-function* relationships. Proteins --within a given protein family-- have, in theory, similar sequences and generally share the same basic structure. Thus, once the structure for one member of the protein family is determined, molecular modeling computations can help determine the structure for other members of the same protein family. Such a homology technique when applied to protein structure may allow scientists to gain additional insight into protein structure, especially for those proteins for which the available experimental data is scarce.

3.1.1 Tasks in Molecular Modeling

In order to obtain optimal results, the National Center by Biotechnology (NCBI) suggested that protein sequences should be organized in protein families. Such readily searchable databases (Table 4) are currently available for many proteins (NCBI -2003).

Table 4. Databases for Molecular modeling

Protein Data Bank (**PDB**);

The molecular modeling database (**MMDB**) at NCBI

Clusters of Orthologous Group of proteins (**COG**s) with the COGNITOR program
The Basic Local Alignment Search Tool (**BLAST**)
Vector Alignment Search Tools (**VAST**)
The Conserved Domain Database(**CDD**)
Domain Architecture Retrieval Tool (**DAR**T).

(Source: **NCBI 2003**: "*A Science primer*").

Secondly, a *target* must be selected. A *target* is a protein structure that has been determined *via* experiments. Thirdly, one must generate a purified protein for analysis of the chosen target and then determine the protein structure by analytical techniques such as X-ray crystallography and/or 2D-FT NMR.

The experiments described next, for example, studied apolipoprotein *A-1* by employing molecular modeling techniques in order to understand the interaction of proteins in food systems and complex organisms.

3.1.2. Apolipoprotein Structures

Lipoprotein in mammals have evolved as the primary transport vehicles for lipids. This role leads to the importance of lipoproteins in several diseases, such as atherosclerosis and cardiovascular disease. Lipoprotein particles consist of a core of neutral lipids, stabilized by a surface monolayer of polar lipids complexed with one or more proteins.

Apolipoprotein A-I and apo B are respectively, the major protein components of high-density (HDL) and very low density (VLDL) lipoproteins. Thus, understanding apolipoproteins is very important for medical and health-related fields, such as medical biotechnology, as well as food science and human nutrition.

3.1.3. Methods of Structure Determination applied to Apolipoprotein Molecules

The process of biosynthesis, the physical characteristics and the metabolism of apolipoproteins have been intensely studied. However, because of the non-crystalline, or *paracrystalline* structure of many apolipoproteins (Baianu, 2012) , it has been difficult to obtain structural data at the molecular, or atomic level. Therefore, methods combining the amino acid sequence with molecular methods are now being introduced. Thus, overall structures may be derived from correlations of global secondary structures determined from polarized light studies combined with local structures predicted from amino acid sequences. The known amino acid sequence of apolipoprotein from the position 7 to 156 of apo Lp-III was first used to design an Apo A-I template, that could be then approached by 'standard' molecular modeling techniques.

3.1.4. Molecular Modeling of Apolipoproteins A-I

Target: Apolipoprophorin III is chosen as a target for apolipoprotein A-I.(apo Lp A-I) . The structure of Apolipophorin III has been determined in a crystal at 2.5 Å resolution for the 18-kDa apo Lp III from the African migratory locust, *Locusta migratoria* and the 22-kDa N-terminal, receptor-binding domain of human apo E.

Template for Apo A-I: Lp IIIa is designed by using molecular software *IALIGN* from Lp III by inserting alanine for template of sequences of apo A-I (using the program **SYBYL**, v.5.5). Alanine residues are inserted at each of the gap position identified by **IALIGN**, an interactive

alignment program distributed with the Protein Identification Resource (**PIR**; Eleanor M.B, 1994) This model was compared with DgA-I, HuA-I and ChA-I resprsenting canine, human and chicken Apo A-I respectively. Results were then compared using a "strip of the helix" template (Vazquez et al 1992) by scoring 1 or 0 for residues that did , or did not, fit into the template.

Modeling Results.

A. *Sequence Comparison*. The five long α-helices connected by short loops in amino acid residues 7-156 of apo Lp-III is used as template for LpIIIa, Dig A-I, DgA-I, HuA-I. Amphipathic potential (AP) is used to detect if the predict structure is suitable for a lipid-aqueous interface in its stable condition. The results are shown in **Table 5**.

Table 5: **Amphipathic potentials** of predicted helical segments in apolipoprotein models.**

Model										
	# res	AP	# res	AP	# res	AP	# res	AP	# res	AP
Lp-III	19	0.79	25	0.8	22	0.77	27	0.7	21	0.67
Lp-IIIa	19	0.79	25	0.8	24	0.67	33	0.61	22	0.64
DgA-I	19	0.58	25	0.84	24	0.75	33	0.48	22	0.68
HuA-I	19	0.53	25	0.88	24	0.71	33	0.45	22	0.73
ChA-I	19	0.53	25	0.88	24	0.58	33	0.52	22	0.82

**Amphipathic potentials (AP) are the best average value for a helical segment of (# res) residues measured with the "strip of helix".(Vazquez, et al. 1992)

B. ***Energy minimized models***. In this model, electrostatic interactions contributes the most to favorable energies. Alanine was chosen as the spacer residue in building apo Lp-IIIa because of its function of small, non-ionic side chain and serves as a helix- stabilizing residue. Although it has a high probability of being found in helical structures, it does not participate through electrostatic interactions. Results of this model are discussed for potential energetic evaluation and amphipathic analysis of energy refined helices (see **Table 6 and Table 7**). The structure obtained through this molecular modeling is illustrated in **Figure 6**.
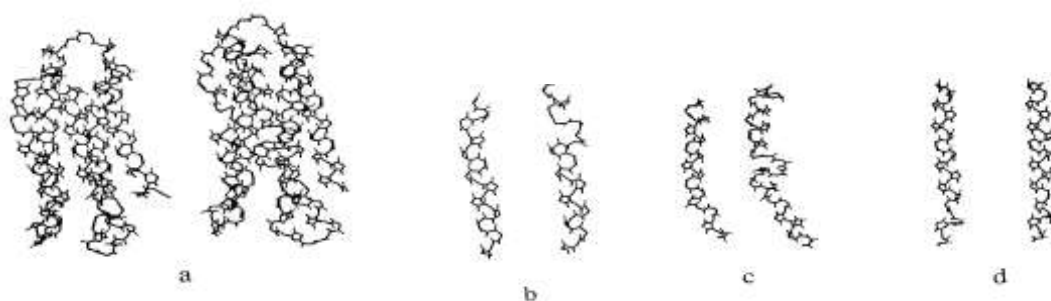
Figure 6. Energy Refined models of apo-Lp III and the template apo IIIa constructed by inserting alanine residues into gap positions identified when the sequences were aligned with the canine, human, and chicken apo A-1 sequences. Backbone structures for this complete models are in panel (a). The effects of inserted alanine residues on H3, H4, H5 are displayed in panels (b), (c), (d) respectively. In each display, the apo Lp III is shown to the left of the apo Lp-IIIa. (SOURCE: Brown et al, 1994).

Table 6. Energetic evaluation of the refined models

	Lp-in	Lp-lUa	DgbjA-I	HuA-I	ChA-I
Number of residues	150	165	165	165	165
Energy, kcal					
Bond stretching	20.7	23.1	28.9	29.2	31.8
angle bending	123.4	154.0	189.6	208.5	212.2
torsional	198.1	235.7	308.3	324.6	340.9
out of plane	26.1	35.8	37.7	41.5	44.5
1-4 van der Waals	218.7	235.6	247.2	260.2	270.3
van der Waals	-993.0	-1094.3	-1074.8	-1131.8	-1155.6
1-4 electrostatics	1684.0	1813.3	1497.1	1566.7	1524.1
electrostatic	-4497.8	-4567.3	-5214.7	-5182.6	-5208.4
H-bond	-69.3	-70.2	-64.5	-69.8	-76.3
Total Energy, kcal	-3289.0	-3234.3	-4045.2	-3953.6	-4016.4
kcal/mol/residue	-21.9	-19.6	-24.5	-24.0	-24.3
H1 (residues)	(7-25)		(72-90)	(73-88)	(72-90)
kcal/mol/residue	-16.1		-18.5	-16.6	-17.4
H2 (residues)	(35-59)		(100-124)	(101-125)	(100-124)
kcal/mol/residue	-16.1		-20.1	-19.4	-19.2
H3 (residues)	(70-91)	(70-91)	(135-158)	(136-159)	(135-158)
kcal/mol/residue	-17.6	-20.7	-18.4	-16.2	-17.6
H4 (residues)	(96-121)	(96-121)	(166-196)	(167-197)	(166-196)
kcal/mol/residue	-15.5	-13.8	-16.7	-16.9	-17.6
H5 (residues)	(136-155)	(136-155)	(207-235)	(208-236)	(207-235)
kcal/mol/residue	-15.8	-15.4	-14.0	-13.4	-14.9

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These energy calculations, based on the sequence segments initially assigned to HI -H5 (see **Tables 6 and 7**), are given to illustrate the stability of the structures. Sequences for helices 3 to 5 (H3, H4, H5) of apo Lp-IIIa contain inserted alanine residues. (*Source*: Brown, 1994).

Table 7. Amphipathic analysis of energy refined helices

Model	Lp-III	Lp-IIIa	gA-I	HuA-I	ChA-I
HI	N7-E25	N7-E25	D72-E90	D73-K88	E72-E90
Hb sector	140°	140°	100°	60°	100°
Av. Hb	0.98	0.98	0.78	0.7	0.81
H2	P35-S59	P35-S59	L100-E124	L101-E125	L101-E124
Hb sector	100°	100°	100°	100°	100°
Av. Hb	0.8	0.8	0.7	0.89	0.95
H3	S70-T91	S70-S85	Q137-R150	E136-R149	L135-L151
Hb sector	140°	120°	100°	100°	120°
Av. Hb	0.93	0.79	0.95	0.88	0.82
H4					
H4A	A96-S121	Q98-T107	D168-K181	Q172-E179	D167-R181
Hb sector	100°	140°	80°	100°	80°
Av. Hb	0.56	0.91	1.1	0.94	1.18
H4B	_____	Q117-al21d	S187-S196	A190-A194	P186-V195
Hb sector	_____	60°	100°		60°
Av. Hb	_____	0.45	0.45	_____	0.66
H5	E136-V156	L134-A155	L213-I232	L214-T237	E212-L235
Hb sector	120°	80°	100°	100°	100°
Av. Hb	0.74	0.53	1.05	0.96	0.86

Table 7. The size of the hydrophobic (Hb) sector of a helix was determined from helical Wheel projections (Schiffer et al 1967) The average hydrophobicity (Av. Hb), of this sector was calculated by the method of Eisenberg (1984*)* . Single letter designations are used for Amino acid residues, (al21d) designates the fourth alanine residue inserted between residues 121 and 122 of Lp-III. (*Source*: Brown, 1994).

After evaluating the potential energy for this model, the lateral view structures of apolipoproteins for apo lipophorin III(residues 7-156), canine apolipoptrotein A-I (residues 72-236), human apolipoprotein A-I (residues 73-237) and chicken apolipoprotein A-I (residues 72-236). The computation results are shown in **Figure 7** (according to Brown, 1994).
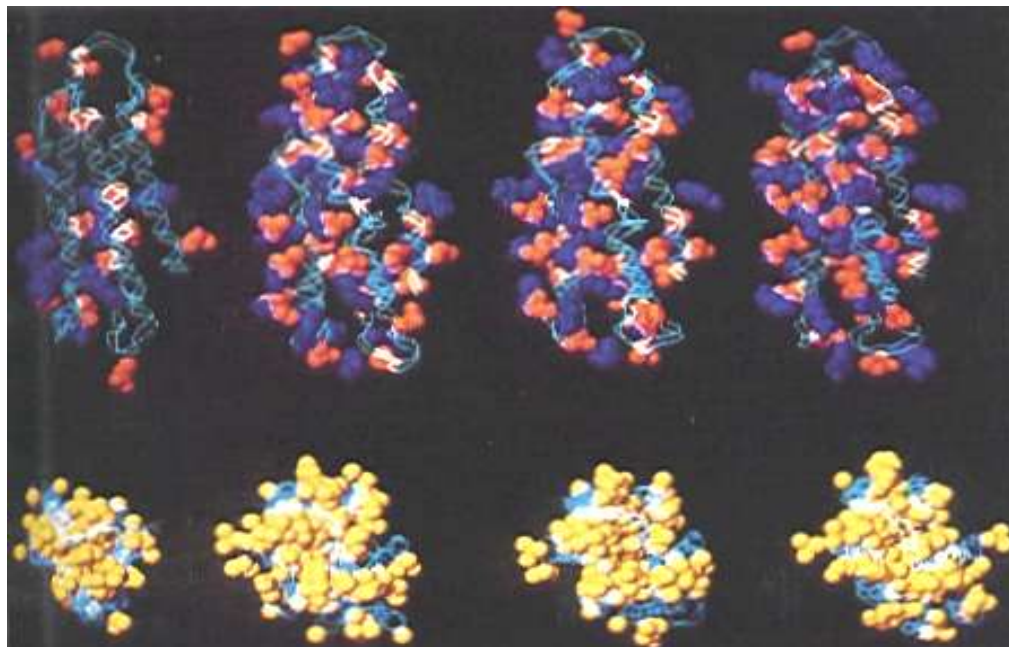
Figure 7. Energy refined models of from left to right apo lipophorin III(residues 7-156) canine apolipoptrotein A-I (residues 72-236), human apolipoprotein A-I (residues 73-237) and chicken apolipoprotein A-I (residues 72-236). Peptide backbones are represented by a double stranded ribbon. In the lateral view, only ionizable sidechains are displayed with acidic groups in red and basic groups in blue. In the end-on view of the molecules only the hydrophobic sidechains are displayed in orange. (*Source*: Brown, 1994)

3.2.   Combination of Molecular Modeling with Other Techniques

It is important to combine molecular modeling with other techniques in order to improve the accuracy of the modeling results. A recent study of Apolipoprotein has produced a high-resolution reconstruction of the structure of apolipoprotein through combination with a solution-phase X-ray technique. It was shown that Apoliproprotein A-1 is an 243-residue protein that contains a globular amino-terminal domain (residue 1-43) and a lipid-binding carboxyl-terminal domain (residues 44-243; Segrest et al, 1992).   The aqueous phase X-ray diffraction crystal structure was obtained at 4 Å (0.4 nm) resolution, which is moderate. This has suggested the accuracy of molecular modeling by combining the X-ray crystallographic with molecular modeling (**Figure 8**; from Segrest et al, 1992.; Borhani, 1997).
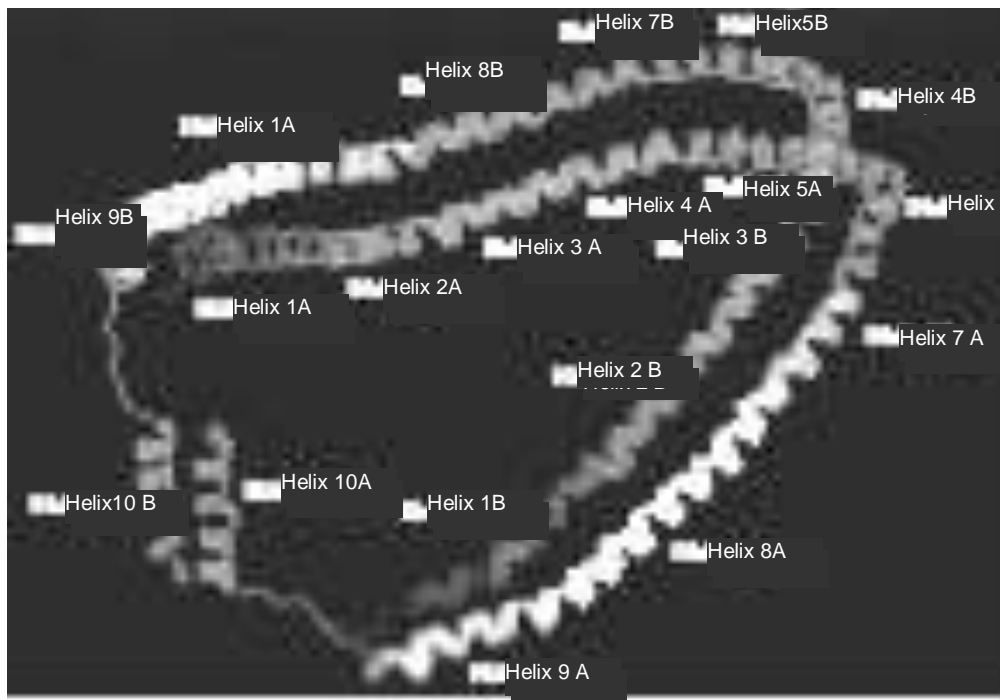
Figure 8. X-ray Diffraction Crystal structure of the Apolipoprotein A-I: Δ(1-43) dimer in solution. (Source: Borhani et al. 1997).

3.3. In-Depth Analyses of Molecular Structure

The determination of apolipoprotein A-I can be then further associated with lipid containing domains by employing other molecular modeling techniques, such as VMD (generated in The Beckman Research Institute at the University of Illinois at Urbana, IL, USA) . The 'belt model' is used to show the possible orientations of lipoprotein with its apolipoprotein inserted (**Figure 9**). The suggested structure can then serve as a template in other high density lipoproteins for their structure determination and also help in understanding the biological interaction.

Fig 9a. Detailed Belt model displayed as a helical ribbon. C(NH$_2$)$_3$, blue, oxygen atom, red; phosphorous atom, yellow all other atom, black. (Source: Borhani 1997)
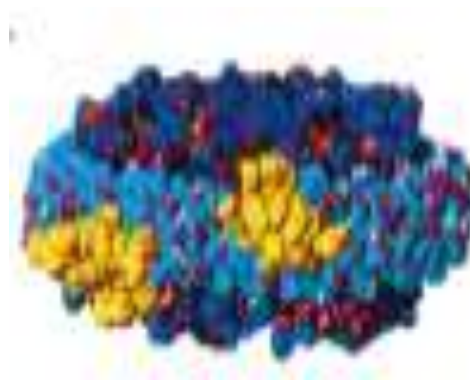
Fig 9b. Detailed Belt model displayed as all atom model, oriented in 9a. nitrogen atom, blue, oxygen atom, red; carbon atom, cyan, polar hydrogen atom, white. (Source: Borhani 1997)

3.4. Practical Applications of Molecular Modeling

Molecular modeling has been introduced for more than two decades ago. Increasingly, modeling software is available for a variety of industrial applications. Global markets for molecular modeling, in general, currently exceed 2 billion US $ annually (Fuji-Keizai, 2003).

3.4.1. Examples of Medical Applications of Molecular Modeling

Molecular modeling is especially helpful in the medical/biomedical and pharmacological fields, such as in nanoscale development of novel drugs of increased potency. Recent studies have shown the importance of using molecular modeling in medical, food sciences and nutrition (Food Ingredient first, 2003). The molecular modeling of Epigallocatechin Gallate (EGCG), and the HIV cell was undertaken by Shearer (2003). His report has inspired scientists in Japan who discovered the potential of green tea as an anti-HIV drug. The chemical compound that is found abundantly in the green tea called *Epigallocatechin Gallate* (EGCG) is reported to stop the HIV virus from binding to CD4 molecules and human T-cells.

3.4.2. Other Applications of Molecular Modeling

Other applications of molecular modeling to pharmacology, manufacturing, life sciences and chemistry greatly benefit from such molecular modeling programs. Nanotechnology has developed to a 30 to 40 million US $ market, and it also has the potential to grow to a 60 to 70 million US $ market within the next five years (Fuji-Keizai, 2003).

4. CONCLUSIONS

A summary and simplified overview of the selected applications of nanotechnology in the areas of Nanomedicine, foods, human nutrition and health- as well as the potential large-scale applications in the chemical industry- that were discussed in this report is presented in **Table 8**.

Table 8. Selected Biotechnology and Nanotechnology Tools and Their Applications

NOVEL TECHNIQUES	AREA	APPLICATION
Immobilized cells	Food Applications	Brewing industry Dairy Foods
	Human Nutition	Neutraceuticals
	Medical Applications	Regulation of fibrinolisis Detoxification of water
Genetic Manipulation	Food Applications	Synthesis of complex carbohydrates cloning of cellulose degrading enzyme
	Human Nutrition	Addition of vitamins in foods Reduction of ripening
	Medical applications	
	Other	Bioproduction of fuel
Liposomes in Microencapsulation	Food Applications	Flavor delivery Additives in bakery Chesee ripening
	Medical applications	protecion of DNA interactions Gene therapy Vaccines Drug carrier against Cancer and HIV
	Other	Creams Oil spills separations

The rapid development of biotechnological tools and 'quick' applications oriented towards immediate marketing may be responsible for generating legislative barriers against 'GMO-based' products. The complementary area of genetically manipulated microorganisms is adopting novel approaches to overcome such increasingly unprofitable legislative barriers and boost profits both in the short- and long-term.

The use of ultra-sensitive techniques and biocomputational modeling is essential in order to quantitate the physical and chemical properties of molecules and supra-molecular systems that are of primary interest to developments in nanotechnology and its applications to Nanomedicine.

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Policy changes may be therefore considered and implemented that would take advantage of such novel approaches to develop new niches and markets for both profitable and safe Nanotechnology applications worldwide, in the Chemical Industry, Agriculture, Health and Medicine.

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