

# **BIOPHYSICAL CHEMISTRY LAB**

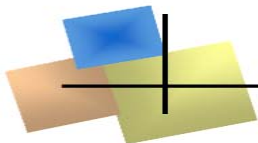
## **ANNUAL REPORT 2009**



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**Institute of Biochemistry and Biophysics  
Tehran, Iran**

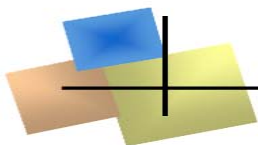
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# FOREWORD

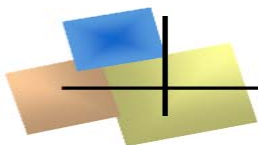
*The Laboratory of Biophysical Chemistry (LBC) was established in 1986 in the Institute of Biochemistry and Biophysics (IBB) and now functions as the main base and the mother of Biophysical Chemistry in Iran. This laboratory is well-known in the national and international level. It is also famous worldwide in the research area of Thermodynamics of Protein Denaturation and Protein-Ligand Interaction.*

*This laboratory enjoys from advanced facilities and is equipped with advanced apparatuses for the research on Biothermodynamics and Biomacromolecular Interactions. LBC is equipped with Nano and Micro Differential Scanning Calorimeters (DSC); Nano and Micro Isothermal Titration Calorimeters (ITC); modern Circular Dichroism (CD) Spectropolarometry, Sensitive Densitometer and Tensiometer; Fluorescence and Uv-vis Spectrophotometers, Microviscometers and Biochemical and Biophysical methods as well as and Computational facilities.*

*LBC is an appropriate place for the promotion of the research and science in the field of Biochemistry, Nanobiophysics, Biotechnology and Biophysical Chemistry. LBC is a suitable laboratory for training PhD students and postdoctorate researchers, associate researchers and sabbatical leaves for faculty members at national and international levels.*

*Up to the present time, 40 Ph.D and 60 Master students have developed their theses in this laboratory and graduated from the university. Faculty members, postdoctorates and students and foreign research associates using the facilities of this laboratory which have published hundreds of full research articles in international prestigious journals. LBC is an appropriate laboratory for supporting and promoting the research of scientists and researchers at national and international levels.*





## *Biophysical Chemistry Lab*

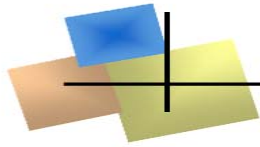


### **Accession of Biophysical Chemistry laboratory (BCL) to Iran Nanotechnology Laboratory Network**

A laboratory facility in the field of nanotechnology (such as equipment, technicians and operators, maintenance facilities, etc.) in many cases is beyond the power of individual researchers and investors. Therefore laboratory networking technology is particularly important in various branches of nanotechnology. Nanotechnology Laboratory Network was formed in Iran in early 1383 AH with the aim of creating appropriate laboratory to researchers and foundation of industrial laboratories to use better the country capacities. So far, during the network activity, more than 39 laboratory collections are accepted the members of the created firm network. Biophysical Chemistry laboratory (BLC) at Institute of Biochemistry and Biophysics (IBB), University of Tehran, also recently is joined to this network. This laboratory has become accessible to the Nano site and is ready to bring forward suitable services in various aforementioned areas. For more information, visit:

[www.ibb.ut.ac.ir/bcl](http://www.ibb.ut.ac.ir/bcl)





## *SUPERVISORS*



Ali A. Moosavi-Movahedi

(left person)

Professor of Biophysics

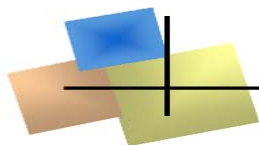
*Protein Thermodynamics*

Ali A. Saboury

(right person)

Professor of Biophysics

*Protein- Ligand Binding*



## Lab Colleagues

### **Professor M. Shamsipur**

Razi University  
Department of Chemistry

### **Professor P. Norouzi**

University of Tehran  
College of Science, Department of Chemistry

### **Professor. B. Moshiri**

University of Tehran  
Dept. of Electrical and Computational Eng.

### **Professor A. Niasari-Naslaji**

University of Tehran  
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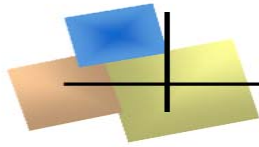
Medical Science University of Tehran  
Department of Pharmacy

### **Dr. G. Ataei**

Medical Science Shahid Beheshti University  
Department of Premedical Science

### **Dr. M. Amani**

Medical University of Ardebil



## *INTERNATIONAL COLLABORATION*

**★Professor G. Floris:**

Department of Applied Science in Biosystem, University of Cagliari, Cagliari, Italy

**★Professor T. Haertle**

National Research Institute of Agronomique, 44316 Nantes Cedex 03, France

**★ Professor F. Ahmad**

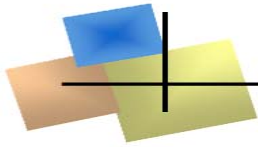
Director, Centre for Interdisciplinary Research in Basic Sciences, Jamia Millia Islamia (A Central University), Jamia Nagar, NEW DELHI - 110 025, India

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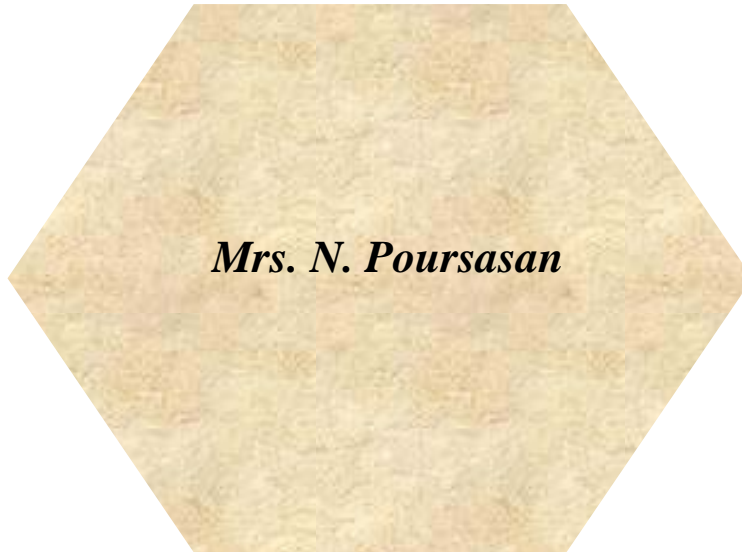
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**★Dr. N. Sheibani**

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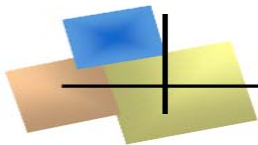


*LAB ASSISTANTS:*



***Mrs. N. Poursasan***

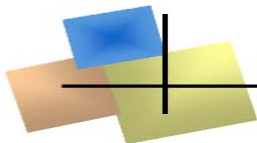




**P. Daneshgar**



**M. Salami**



## *STUDENTS (Year 2009)*

### Doctor of Philosophy (Ph.D)

\*\*\*\*\*

\*\*\*\*\*

- \* M. Alijanianzadeh
- \* M. Atri
- \* S. Bagheri
- \* M. Bohlooli
- \* M. Falahati
- \* A. Fallahbagheri
- \* F. Farivar
- \* L. Fotouhi
- \* F. Ghamari
- \* H. Hadi-Alijanvand
- \* M. A. Hekmat
- \* Y. Sefidbakht
- \* H. Sepasi-Tehrani
- \* A. Sharifizadeh
- \* F. Taghavi
- \* H. Zare
- \* S. Zolghadr Jahromi

### Master of Science (MSc)

\*\*\*\*\*

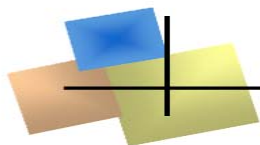
- ☆ H. Ahmadizadeh
- ☆ L. Alaie
- ☆ R. Amany
- ☆ M. J. Bagheri-Arabi
- ☆ P. Bazzi
- ☆ H. Derakhshankhah
- ☆ S. Ebrahim Damavandi
- ☆ M. Ghasemi Aliabadi
- ☆ M. Goodarzi
- ☆ E. Kachooei
- ☆ R. Malekjani
- ☆ M. Naderi
- ☆ A. Rashidnia

### **Associate researcher**

\*\*\*\*\*

✓ **Faezeh Moosavi-Movahedi**



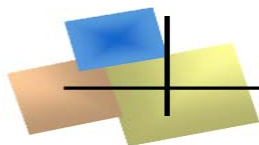


## *Alumni (Year 2009)*



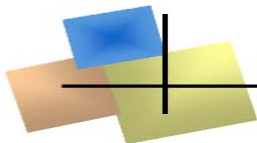
<i>Name</i>	<i>Topic of Thesis</i>
<b><u>Ph.D:</u></b>	
<b>J. Badraghi</b>	Comparative structural and chaperone studies on phosphorylated and dephosphorylated $\alpha$ S1-casein from camel and bovine milks
<b>S.J. Mousavy</b>	Effects of mobile phone radiofrequency on the structure and function of the normal and beta thalassemia human hemoglobin and in the presence of deferasirox
<b>M. Salami</b>	Bioactive peptide production and fractionation from hydrolysis of camel milk proteins
<b><u>MS.C</u></b>	
<b>E. Amin</b>	Effect of some synthetic dithiocarbamates on the structure and function of mushroom tyrosinase
<b>M. Rohban</b>	Thermodynamical investigation on the Effects of silver nanoparticles on the structure of calf thymus DNA and determination of its cytotoxicity on K562 cell line
<b>M. Esmaeili</b>	The Study of Nanomicelle-Beta Casein and its Peptides from Camel Milk upon Interaction with Curcumin;Antioxidant Activity



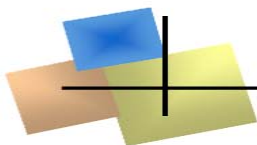


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- 2- A. Divsalar, A. A. Saboury, H. Mansoori-Torshizi, F. Ahmad, M. Islami-Moghaddam, F. Ahmad and G. H. Hakimelahi, "Comparative studies on the interaction between bovine  $\beta$ -lacto globulin type A and B and a new designed Pd(II) complex with anti-tumor activity at different temperatures", *Journal of Biomolecular Structure & Dynamics* **26** (2009), 587-598.
- 3- G. Rezaei-Behbehani, A. Divsalar, A. A. Saboury and A. Hekmat, "A thermodynamic study on the binding of PEG-stearic acid copolymer with lysozyme", *Journal of Solution Chemistry* **38** (2009), 219-229.
- 4- G. Rezaei-Behbehani, A. Divsalar, A. A. Saboury, F. Faridbod and M. R. Ganjali, "A high performance theory for thermodynamic study on the binding of human serum albumin with erbium chloride", *Chinese Journal of Chemistry* **27** (2009), 289-294.
- 5- P. Norouzi, R. Dinarvand, M. R. Ganjali, A. A. Moosavi-Movahedi, A. A. Saboury and A. Tamaddoni, "Application of adsorptive voltammetry for the detection of sub-nano molar cyclizine in biological fluids and tablets using fast Fourier transform continuous cyclic voltammetry in a flowing system", *Analytical Sciences* **25** (2009), 505-510.
- 6- J. Badraghi, R. Yousefi, A. A. Saboury, A. Sharifzadeh, T. Haertlé, F. Ahmad and A. A. Moosavi-Movahedi, "Effect of salts and sodium dodecyl sulphate on chaperone activity of camel alpha-S1-CN: Insulin as the target protein", *Colloids and Surfaces B: Biointerfaces*, **71** (2009), 300-305.
- 7- A. A. Saboury, "Enzyme inhibition and activation: A General Theory", *Journal of the Iranian Chemical Society* **6** (2009), 219-229.
- 8- S. Rezaei-Zarchi, A. Javed, M. J. Ghani, S. Ahmadian, H. Bari-Abarghouei, S. A. Hashemizadeh and A. A. Saboury, "Electrochemically different behaviors of cytochrome c in the presence of organic phosphates", *Analytical & Bioanalytical Electrochemistry* **1** (2009), 2-10.
- 9- M. Salami, R. Yousefi, M. R. Ehsani, S. H. Razavi, Jean-Marc Chobert, T. Haertlé, A. A. Saboury, M. S. Atri, A. Niasari-Naslaji, F. Ahmad and A. A.

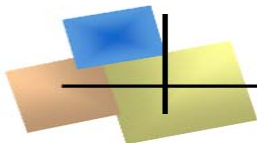


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- 13- F. Mohammadi, A. K. Bordbar, A. Divsalar, K. Mohammadi and A. A. Saboury, "Interaction of curcumin and diacetylcurcumin with the lipocalin member  $\beta$ -lactoglobulin", *The Protein Journal* **28** (2009), 117-123.
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- 16- A. A. Saboury, C. O. Aboluwoye and N. S. Sarraf, "pH Dependence study of the kinetic reaction of bovine carbonic anhydrase with 2,2'-dithiobispyridine in the absence and presence of surfactants", *Journal of Physical and Theoretical Chemistry* **6** (2009), 57-61.
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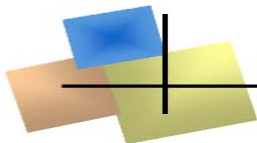


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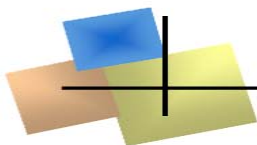




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- 29- K Nazari, A. Mahmoudi; M. Khosraneh, Z. Haghghian, A.A. Moosavi-Movahedi "Kinetic Analysis for Suicide-Substrate Inactivation of Microperoxidase-11: A Modified Model for Bisubstrate Enzymes in the Presence of Reversible Inhibitors" *Journal of Molecular Catalysis B: Enzymatic* 56, 61-69 (2009).
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- 31- A. Niasari-Naslaji, D. Nikjou, J. A. Skidmore, A. Moghiseh, M. Mostafaey, K. Razavi and A. A. Moosavi-Movahedi "Interspecies embryo transfer in camelids: the birth of the first Bactrian camel calves (*Camelus Bactrianus*) from dromedary camels (*Camelus dromedarius*)" *Reproduction, Fertility Development* 21, 333-337 (2009).
- 32- R.Mahinpour, G.h. Riazi, M.N. Sarbolouki, A.A.Moosavi-Movahedi, M.A. Shokrgozar and S.Z. Moosavi-Nejad " Interference of arsenic trioxide on magnesium dependent polymerization of microtubule proteins" *Journal of Iran. Chem. Soc.*6(4), 715-721(2009).
- 33- H. Heli, A. Jabbari, M. Hajjizadeh, A. A. Moosavi-Movahedi "Copper nanoparticles-modified carbon paste transducer as a biosensor for determination of acetylcholine" *Biosensors and Bioelectronics* 24, 2328-2333 (2009).
- 34- E. Sharifi, N. Sattarahmady, M. Habibi-Rezaei, M. Farhadi, N. Sheibani, F. Ahmad and A. A. Moosavi-Movahedi "Inhibitory effects of  $\beta$ -cyclodextrin



- and trehalose on nanofibril and AGE formation during glycation of human serum albumin" *Protein Peptide Letters* 16(6), 653-9 (2009).
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- 38- H. Heli, M. Hajjizadeh, A. Jabbari, A. A. Moosavi-Movahedi "Fine steps of electrocatalytic oxidation and sensitive detection of some amino acids on copper nanoparticles" *Analytical Biochemistry* 388, 81-90 (2009).
- 39- H. Heli, A. Jabbari, A.A. Moosavi-Movahedi and M. Tabeshnia "Electrooxidation and determination of mannitol and saccharose on a cobalt hydroxide nanoparticles-modified glassy carbon electrode" *Chem. Anal. (Warsaw)*, 54, 619-628 (2009).
- 40- P. Daneshgar, P. Norouzi, M. R. Ganjali and A. A. Moosavi-Movahedi " Dysprosium hydroxide nanowires modified electrode for determination of rifampicin drug in human urine and capsules by adsorptive square wave voltammetry " *Current Analytical Chemistry* 5, 246-255 (2009).
- 41- R. Yousefi, Y. Shtchutskaya, J. Zimny, Jean-Charles Gaudin, A. A. Moosavi-Movahedi, V. I. Muronetz, Y. Zuev, Jean-Marc Chobert and T. Haertlé "Chaperone-like activities of different molecular forms of  $\beta$ -casein. Importance of polarity of N-terminal hydrophilic domain." *Biopolymers* 91(8), 623-632 (2009).
- 42- P. Daneshgar, P. Norouzi, A. A. Moosavi-Movahedi, M. R. Ganjali, E. Haghshenas, F. Dousty and M. Farhadi " Fabrication of Carbon nanotube and Dysprosium nanowire modified electrodes as a sensor for determination of Curcumin" *Journal of Applied Electrochemistry* 39, 1983-1992 (2009).



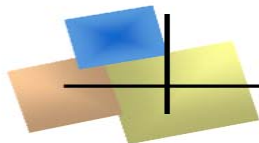
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- 43- H. Heli , M. Zarghan , A. Jabbari , A. A. Moosavi-Movahedi  
"Copper nanoparticles-carbon microparticles nanocomposite for electrooxidation and sensitive detection of sotalol" *Sensors & Actuators: B. Chemical* 140,245-251(2009).
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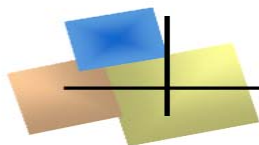
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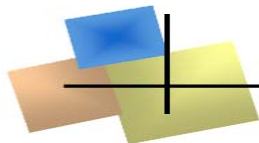
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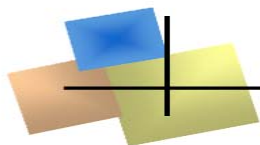
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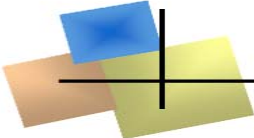
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## Kinetic analysis for suicide-substrate inactivation of microperoxidase-11: A modified model for bisubstrate enzymes in the presence of reversible inhibitors

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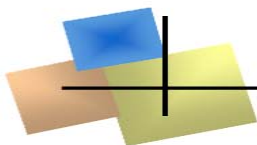
### ABSTRACT

Kinetics of microperoxidase-11 (MP-11) as a heme-peptide enzyme model in oxidation reaction of guaiacol (AH) by hydrogen peroxide was studied in the presence of amino acids, taking into account the inactivation of MP-11 during reaction by its suicide substrate, H<sub>2</sub>O<sub>2</sub>. Reliability of the kinetic equation was evaluated by non-linear mathematical fitting. Fitting of experimental data into a new integrated kinetic relation showed a close match between the kinetic model and the experimental data. Indeed, it was found that the mechanism of suicide-peroxide inactivation of MP-11 in the presence of amino acids is different from MP-11 and/or horseradish peroxidase. In this mechanism, amino acids compete with hydrogen peroxide for the sixth co-ordination position of iron atom in the heme group through a competitive inhibition mechanism.

The proposed model can successfully determine the kinetic parameters including inactivation by hydrogen peroxide as well as the inhibitory rate constants by the amino acid inhibitor.

Kinetic parameters of inactivation including the initial activity of MP-11,  $\alpha_0$ , the apparent inactivation rate constant,  $k_i$  and the apparent inhibition rate constant for cysteine,  $k_1$  were obtained  $0.282 \pm 0.006 \text{ min}^{-1}$ ,  $0.497 \pm 0.013 \text{ min}^{-1}$  and  $1.374 \pm 0.007 \text{ min}^{-1}$  at  $[\text{H}_2\text{O}_2] = 1.0 \text{ mM}$ ,  $27^\circ\text{C}$ , phosphate buffer  $5.0 \text{ mM}$ , pH 7.0. Results showed that inactivation and inhibition of microperoxidase as a peroxidase model enzyme occurred simultaneously even at low concentrations of hydrogen peroxide (0.4 mM). This kinetic analysis based on the suicide-substrate inactivation of microperoxidase-11, provides a tool and model for studying peroxidase models in the presence of reversible inhibitors. The introduced inhibition procedure can be used in designing activity tunable and specific protected enzyme models in the hidden and reversibly inhibited forms, which do not undergo inactivation.

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## Biosensors and Bioelectronics

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# Copper nanoparticles-modified carbon paste transducer as a biosensor for determination of acetylcholine

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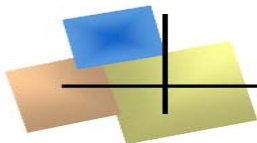
Nanobiosensor

Electrocatalysis

### ABSTRACT

The electrocatalytic oxidation of acetylcholine (ACh) on two different copper-based transducers, copper microparticles-modified carbon paste electrode (m-CPE) and copper nanoparticles-modified carbon paste electrode (n-CPE), was investigated. In the voltammograms recorded using m-CPE, a single anodic oxidation peak related to the oxidation of ACh was appeared which was related to the electrocatalytic oxidation of ACh via the electrogenerated Cu(III) species in an EC' mechanism. Using n-CPE, however, two overlapped anodic peaks appeared which were related to two fine tunable steps of oxidation. ACh oxidized on n-CPE with higher rates at low potentials with respect to m-CPE. The kinetic of the reaction was formulated and the charge-transfer resistance of the system was obtained both theoretically and experimentally. The catalytic rate constant, the transfer coefficient for the electrocatalytic oxidation and the diffusion coefficients for ACh were reported using chronoamperometry, pseudo-steady-state polarization measurement and electrochemical impedance spectroscopy. Sensitive and time-saving sensing procedures in both batch and flow systems were developed for the analysis of ACh, and the corresponding analytical parameters were reported.

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## International Journal of Biological Macromolecules

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### Effects of mobile phone radiofrequency on the structure and function of the normal human hemoglobin

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Naghmeh Sattarahmady<sup>a,f</sup>, Ahmad Sharifzadeh<sup>a</sup>, Shahrokh Safarian<sup>c</sup>,  
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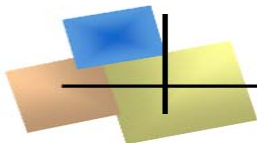
Oxygen affinity

#### ABSTRACT

Widespread use of mobile phones has increased the human exposure to electromagnetic fields (EMFs). It is required to investigate the effect of EMFs on the biological systems. In this paper the effect of mobile phone RF (910 MHz and 940 MHz) on structure and function of HbA was investigated. Oxygen affinity was measured by sodium dithionite with UV–vis spectrophotometer. Structural changes were studied by circular dichroism and fluorescence spectroscopy. The results indicated that mobile phone EMFs altered oxygen affinity and tertiary structure of HbA. Furthermore, the decrease of oxygen affinity of HbA corresponded to the EMFs intensity and time of exposure.

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## International Dairy Journal

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### Enzymatic digestion and antioxidant activity of the native and molten globule states of camel $\alpha$ -lactalbumin: Possible significance for use in infant formula

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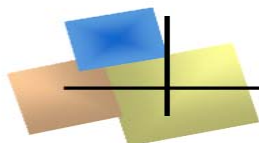
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#### ABSTRACT

Native- and molten globule states of  $\alpha$ -lactalbumin ( $\alpha$ -La) from camel and bovine milk were used for comparative assessment of digestibility and antioxidant activity. The proteolysis assessments were performed in the presence of gastrointestinal enzymes, using the *o*-phthaldialdehyde assay, and the antioxidant activity was carried out using a 2,20-azinobis(3-ethylenebenzothiazoline-6-sulfonic acid based method. Camel and bovine  $\alpha$ -La revealed similar sensitivity to proteolysis by pepsin. The degree of hydrolysis (DH) of camel  $\alpha$ -La by either trypsin or chymotrypsin was noticeably higher than that of the bovine protein counterpart. This can be explained by the different conformational and structural features of these proteins, as shown by studies of intrinsic- and 8-anilino-naphthalene-1-sulfonic acid fluorescence. The greater antioxidant activity of camel  $\alpha$ -La could be explained by the higher content of antioxidant amino acid residues and different conformational features between bovine and camel  $\alpha$ -La. The results may suggest that  $\alpha$ -La produced from camel milk may be used for infant formulae as an alternative to that produced from bovine milk.

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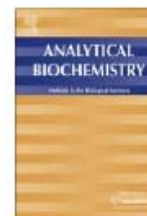


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Analytical Biochemistry

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## Fine steps of electrocatalytic oxidation and sensitive detection of some amino acids on copper nanoparticles

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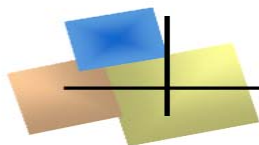
Nanosize effect

Electrocatalysis

### ABSTRACT

The electrocatalytic oxidation of five amino acids—glycine, aspartic acid, cysteine, glutamic acid, and tyrosine—on two copper-based electrodes comprising copper microparticle-modified carbon paste electrode (m-CPE) and copper nanoparticle-modified CPE (n-CPE) was investigated. In the voltammograms recorded using m-CPE, a single anodic peak related to the oxidation of amino acids appeared and was related to the electrocatalytic oxidation of the amino acids via the electrogenerated Cu(III) species. Using n-CPE, however, two overlapped anodic peaks in the voltammograms appeared and were related to two fine tunable steps of the oxidation process. The currents of the two peaks were controlled by diffusion and were confirmed by chronoamperometric measurements. The amino acids were oxidized on n-CPE at higher rates and at lower potentials compared with m-CPE. This was attributed to the nanosize of copper nanoparticles. Some primary linear-chain amines and primary branched-chain amines were oxidized on the copper-based electrodes as markers. The catalytic rate constants, the transfer coefficients, and the diffusion coefficients for the amino acids are reported. Simple, sensitive, and time-saving sensing procedures in both batch and flow systems were developed for the analysis of the amino acids, and the corresponding analytical parameters are reported.

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## Interaction between silver nanoparticle and bovine hemoglobin at different temperatures

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A. Divsalar · S. Rezaei-Zarchi ·  
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**Abstract** The binding of silver nanoparticles to bovine hemoglobin (Bhb) was studied by fluorescence, UV–Visible, and circular dichroism (CD) spectroscopic techniques at different temperatures of 20, 37, and 42 °C. The absorption spectrum of sorlet band, in the presence of silver nanoparticle, showed a significant spectral change, which indicated the heme groups of Bhb were directly attacked and degraded by silver nanoparticle. The fluorescence data explained that the nanoparticle binding to Bhb occurred at a single binding site, which demonstrated a dynamic quenching procedure. Nanoparticles could reduce the fluorescence of tryptophanyl residues of Bhb to a lesser extent. Circular dichroism studies demonstrated a conformational change of Bhb in the presence of silver nanoparticles. The helicity of Bhb was reduced by increasing silver nanoparticle concentration at different temperatures. Thermodynamic analysis of the protein interaction by silver

nanoparticles suggested that the binding process is only entropy driven.

**Keywords** Silver nanoparticle · Bhb · Circular dichroism · Fluorescence · Nanobiotechnology

### Introduction

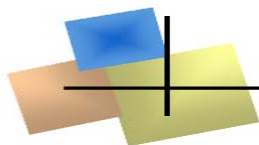
There has been considerable progress in the synthesis of nanomaterials with precise dimensions, geometries, and surface properties (Peng et al. 2000; Strano et al. 2003). Now, there is an increasing interest in understanding and controlling the interactions of nanomaterials with biological molecules such as proteins (Luk et al. 2003; Roach et al. 2006; Hong et al. 2004; Karajanagi et al. 2004). Proteins have been used to functionalize nanomaterials and to influence their properties for applications ranging from sensing (Barone et al. 2005; Besteman et al. 2003; Patolsky and Lieber 2005) and diagnostics (Loo et al. 2005; Medintz et al. 2005) to delivery (Pantarotto et al. 2004; Shi Kam et al. 2004), and for the design of nanocomposites (Luckarift et al. 2004; Pender et al. 2006; Park et al. 2001; Niemeyer 2001). Nanomaterial properties in turn have a strong influence on the structure and function of proteins and there has been increasing emphasis on obtaining a fundamental understanding of these effects.

Bovine hemoglobin (Bhb) is well known for its function in the vascular system of animals, being a

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### Effect of salts and sodium dodecyl sulfate on chaperone activity of camel $\alpha S_1$ -CN: Insulin as the target protein

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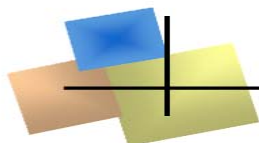
Aggregation

#### ABSTRACT

In this study camel  $\alpha S_1$ -casein ( $\alpha S_1$ -CN) was purified, using a two-step purification procedure. The anti-aggregation (chaperone-like) ability of the purified protein sample was examined in a wide range of experimental conditions and at different concentrations of camel  $\alpha S_1$ -CN, in the presence of salts and sodium dodecyl sulfate (SDS). To examine chaperone-like activity of camel  $\alpha S_1$ -CN, bovine pancreatic insulin was used as the target protein. Insulin aggregation performed chemically in the presence of 20 mM dithiothreitol (DTT) and was studied at 360 nm wavelength by UV-vis spectrophotometer. Camel  $\alpha S_1$ -CN exhibited a dose-dependent chaperone-like activity as the molar ratios of chaperone/target protein varied between 0 and 0.07. The presence of salts or surfactants changing the protein properties had an influence on chaperone capacity of camel  $\alpha S_1$ -CN. The results of UV-visible and fluorimetric measurements indicated that the salts neutralize the chaperone-like activity of casein due to dehydration effect and the increased association and aggregation of proteins, while SDS plays a role as chaperone and chaperone-like properties of camel  $\alpha S_1$ -CN enhanced in the presence of SDS due to the binding of the hydrophobic tail of SDS and  $\alpha S_1$ -CN to the exposed hydrophobic sites of insulin strongly preventing aggregation of insulin.

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## Chaperone-Like Activities of Different Molecular Forms of $\beta$ -casein. Importance of Polarity of N-Terminal Hydrophilic Domain

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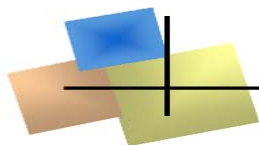
### ABSTRACT:

As a member of intrinsically unstructured protein family,  $\beta$ -casein ( $\beta$ -CN) contains relatively high amount of prolyl residues, adopts noncompact and flexible structure and exhibits chaperone-like activity *in vitro*. Like many chaperones, native  $\beta$ -CN does not contain cysteinyl residues and exhibits strong tendencies for self-association. The chaperone-like activities of three recombinant  $\beta$ -CNs wild type (WT)  $\beta$ -CN, C4  $\beta$ -CN (with cysteinyl residue in position 4) and C208  $\beta$ -CN (with cysteinyl residue in position 208), expressed and purified from *E. coli*, which, consequently, lack the phosphorylated residues, were examined and compared with that of native  $\beta$ -CN using insulin and alcohol dehydrogenase as target/substrate proteins. The dimers ( $\beta$ -CND) of C4- $\beta$ -CN and C208  $\beta$ -CN were also studied and their chaperone-like activities were compared with those of their monomeric forms.

Lacking phosphorylation, WT  $\beta$ -CN, C208  $\beta$ -CN, C4  $\beta$ -CN and C4  $\beta$ -CND exhibited significantly lower chaperone-like activities than native  $\beta$ -CN. Dimerization of C208  $\beta$ -CN with two distal hydrophilic domains considerably improved its chaperone-like activity in comparison with its monomeric form. The obtained results demonstrate the significant role played by the polar contributions of phosphorylated residues and N-terminal hydrophilic domain as important functional elements in enhancing the chaperone-like activity of native  $\beta$ -CN.  
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Keywords: chaperone-like activity; aggregation; alcohol dehydrogenase (ADH); insulin

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## Comparative thermostability of mesophilic and thermophilic alcohol dehydrogenases: Stability-determining roles of proline residues and loop conformations

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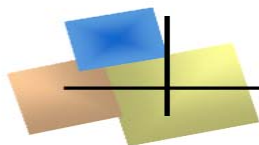
Proline

Multimeric proteins

### ABSTRACT

The present study demonstrates the comparative thermal, conformational and kinetic stabilities of the three closely related enzymes; the mesophilic yeast alcohol dehydrogenase (YADH), horse liver alcohol dehydrogenase (HLADH), and the extreme-thermophilic *Thermoanaerobacter brockii* alcohol dehydrogenase (TBADH). The mid-point unfolding temperatures for TBADH and HLADH were at least 10 °C and 6 °C higher, respectively, than that of YADH. When YADH was completely inactivated by thermal stress, the residual activities of HLADH and TBADH were 70% and 100%, respectively. The optimum temperature ( $T_{opt}$ ) activities of HLADH and TBADH were at least 40 °C and 55 °C higher, respectively, than that of YADH. Due to the higher rigidity of HLADH and TBADH, the enzymatic activation energies of HLADH and TBADH were higher than that of YADH. Geometric X-ray analysis indicated a comparatively higher coil (turn and loop) percentage in TBADH and HLADH than in YADH. Pairwise alignment for TBADH/HLADH exhibited a similarity score approximately 2.5-fold greater than that of the TBADH/YADH pair. Multiple alignments made with ClustalW revealed a higher number of conserved proline residues in the two most stable enzymes (HLADH/TBADH). These extra prolines tend to occur in surface loops and are likely to be responsible for the increased stability of TBADH and HLADH, by loop rigidification.

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## Investigation on the interaction of newly designed anticancer Pd(II) complexes with different aliphatic tails and human serum albumin.

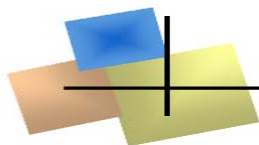
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### Abstract

The pharmacokinetics and pharmacodynamics of any drug will depend, largely, on the interaction that it has with human serum albumin (HSA), the most abundant plasma protein. The interaction between newly synthesized Pd(II) complexes, 2,2'-bipyridin octyl dithiocarbamate Pd(II) nitrate (Octpd), 2,2'-bipyridin butyl dithiocarbamate Pd(II) nitrate (ButPd), 2,2'-bipyridin ethyl dithiocarbamate Pd(II) nitrate (EIPd), antitumor components, with human serum albumin, a carrier protein, were studied at different temperatures of 27 and 37 degrees C by fluorescence spectroscopy, far UV circular dichroism (CD), and spectrophotometric and differential scanning calorimetry (DSC) techniques. By the analysis of fluorescence intensity, it was observed that Pd(II) complexes have strong abilities to quench the intrinsic fluorescence of HSA through a dynamic quenching procedure. The binding parameters were evaluated by the fluorescence quenching method. The thermodynamic parameters, including  $\Delta H$  degrees,  $\Delta S$  degrees, and  $\Delta G$  degrees, were calculated by the fluorescence quenching method and indicated that hydrophobic forces play a major role in the interaction of Pd(II) complexes with HSA. Far-UV-CD results represented that Pd(II) complexes induced a decrease in content of the alpha helical structure of protein. The binding of newly designed drugs (Pd(II) complexes) on the blood carrier protein of HSA resulted in significant alterations on the structure and conformation of protein via decreasing stability of HSA by decreasing the  $T(m)$ , a red shift in maximum fluorescence intensity, a decrease in content of the alpha-helical structure, and the increase of the nonpolar or accessible hydrophobic surface of HSA to solvent.

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## Enzyme Inhibition and Activation: A General Theory

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The rate of an enzymatic reaction may be changed by a moderator. Usually, the effect is to reduce the rate, and this is called inhibition. Sometimes the rate of enzyme reaction is raised, and this is called activation. Not only enzyme activation is subject of a less detailed presentation, but also enzyme inhibition and activation are very often discussed independently in enzymology. I attempt to introduce a general model of enzyme inhibition and activation to allow one to interpret inhibition and activation from a mechanistic or physical perspective using the significance of cooperativity as a new approach. The magnitude of interaction between substrate and inhibitor binding sites is given by the  $\alpha$  parameter and the magnitude of increasing catalytic reaction constant is given by the  $\beta$  parameter, which both parameter values characterize the type of inhibition and activation. The moderation of mushroom tyrosinase is described by application of the model as a typical.

**Keyword:** Inhibition, Activation, Cooperativity, Enzyme kinetics

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