Some Biophysical And Biochemcial Interactions Of Nanomaterials On Protein Oxidation In Biological System

Y K Lahir*, A V Chitre And P M Dongre

Department of Biophysics, University of Mumbai, Vidyanagari, Santa Cruz (E), Mumbai-400098, India
*Corresponding author: lahiryk@gmail.com

Abstract: Oxidation and reduction is a ubiquitous metabolic phenomenon that reflects on the structural and functional well being of the cell, tissue, and organ, in a biological system. It symbolizes the state of functional equilibrium and organismal integrity in totality. The homeostatic state is the result of combined and coordinated interactions between prooxidants, oxidants, and antioxidants, inactivated and activated free radicals at cellular level. Proteins, alone and in conjugated form constitute a group of biomolecules that is devoted to the cellular structural and functional aspects of inter and intracellular communication. Mostly damage to proteins is non-reparable. This state of proteins results in fluctuation in enzyme affectivity, structural and functioning of protein molecules, this is likely to affect transportation and signaling mechanism within a cell and with its ambient environment. There have been reports on the cellular modifications produced due to nanomaterials; these include changes in cell membrane, cytoskeleton, conformational changes in biomolecules etc, reflecting on the changes in cellular elasticity, motility, degree of adherence, invasion etc. The physicochemical properties of nanomaterials are the basis of their varied effectiveness; there have been reports suggesting a huge influx of nanomaterials in biosystem. There appears to be a need to evaluate their impact and understand the probable mechanism involved. This may help to reduce the derogative effects. Thus it was thought imperative to over view the role of nanomaterials in protein oxidation encompassing physiological and specifically biophysical aspects in a biological system.
[Y K Lahir, A V Chitre And P M Dongre. **Some Biophysical And Biochemcial Interactions Of Nanomaterials On Protein Oxidation In Biological System.** *Rep Opinion* 2016;8(7):66-71]. ISSN 1553-9873 (print); ISSN 2375-7205 (online). http://www.sciencepub.net/report. 11. doi:10.7537/marsroj080716.11.

Key words: Protein-oxidation; nanomaterials; kininogen; plasma-prekallikrein; free radicals; premature-senescence; antioxidants; cellular response;

Background

Recently multidimensional impact of nanomaterials has become very obvious on the human lives and industries; these impacts may be good or adverse. It is very essential to restore a balance between the harmful and beneficial aspects. Proteins are basically responsible for the cellular structural and functional aspects in totality. These varied biomolecules having wide potentials undergo change during metabolic interactions, oxidation-reduction is one of the phenomena that influence proteins in many aspects and impacts protein metabolism. Nanomaterials are present in biota and abiota the components of the environment either naturally and/or due to anthropogenic activities. Thus, nanomaterials obviously exist in and around biosystem. Nanomaterials have wide range of size, shape, physicochemical nature and these include metals, metal oxide, quantum dots, carbon derived, engineered nanomaterials target dependent for specific purposes. When proteins are subjected to metal catalyzed oxidation very less amino acid residues undergo modifications and comparatively less number of peptide bonds exhibit cleavage; the products formed react with metal-binding sites located on the protein resulting in the formation of reactive oxygen species, Stadtman, (1990). Electrochemical aspects of oxidation reduction is also applicable to the oxidation of proteins, these involve location of the peroxidant functional group on the reactive surface of nanomaterials, occurrence of the reactive redox-cycle on the surface of nanoparticles made from transitional metals, the inter reactive ability of nanoparticles with cell and cell organelles and the physicochemical parameters of nanomaterials and their reactive affinity towards the cell, Fubini and Hubbard, (2003); Manke et al, (2013).

The specific features present on the surface of nanoparticles such as hydrophobicity, Moyano et al, (2012), polarizability, Hansch et al, (2003), polar surface area, Palm et al, (1997), Vander Waal surface area, Nel et al, (2009), solvent surface accessible area, Wang (2012), refractivity, Crippen (1999) etc, induce their reactivity and/or oxidation while some nanoparticles enhance the oxidative system present in the cell to generate reactive species of oxygen and nitrogen related to mitochondria, NAD (P) H-oxidase enzymes, and primary inflammation, Knaapen et al, (2004). Nano-sized nanoparticles exhibit alteration in their electronic properties of their surface; these alterations create reactive groups on the surface of nanoparticles, Buzea et al (2007). This fact is

supported by the interaction between similar sized Zn and Si NPs with cells resulting in varied degree of cytotoxicity; Zn nanoparticles being more active than SiO_2 NPs produce more of O_2^{*-}. The free radicals get bound to the surface of NPs or form free entities when suspended in water, Buzea et al (2007).

Signaling system of cell is affected when metals like iron, copper, chromium, cadmium, mercury, lead and nickel deplete glutathione sulfhydryl groups attached to proteins and this in turn produces reactive oxygen species and hydroxyl radical, Genestra (2007). Signaling pathways such as H1F-1, NF-KkB, P13k, mitogen activated protein kinase (MAPK) are disturbed whenever the redox homeostasis is changed; as a result cellular functions like cell division, inflammation, growth, apoptosis, survival, metastasis etc. are adversely affected, Li et al, (2010) and Pojlak-Blazi et al, (2010). Wagner et al (2015) proposed that although α-helical coiled coil folding motif decides the design of the peptide but parameters like pH can influence the changes, in such cases β-sheet formation takes place depending on the pH. CuO, Fe_3O_4, Fe_2O_3, TiO_2, Ag nanoparticles cause oxidative stress, Song et al, (2012).

The interactions related to free radicals have been regulated by inducible antioxidant system at cellular level. When these radicals are present in low concentration these are not hazardous and participate in the signaling and homeostasis at physiological level but when these are in higher concentration cause oxidative stress (one of the causative factor), this in turn enhances the damage of biomolecules and their accumulation in cell, tissue/biosystem, Song et al, (2012). During aging process and pathogenesis, an increased extracellular and intracellular status of oxidative stress has been observed. This disturbed balance could act as a parameter to study the responses of cells in normal conditions, oxidative stressed condition and repair mechanism. This status either arrests the growth (transient), leads to premature senescence or death. Some of the responses that may be affected are tumor suppressing apoptosis, ability to proliferate, arresting transient growth, cause inflammation, senescence, and necrosis etc, Fabiola et al, (2012). The investigations indicate metal oxide like TiO_2 and MgO produced reactive oxygen species and these caused protein oxidation, Wagner et al (2010). Carbon based NMs like fullerene, CNT, C-black and cerium oxide have ability to scavenge the free radicals in cellular environment, thus, may be considered as antioxidants, Ghiazza et al, (2013). NPs exhibit pro-oxidative effects and these in turn stimulate signaling pathways, functioning of transcription factors and cytokine cascade, thereby influencing the varied range of cellular responses, Manke et al, (2013). Engineered nanoparticles/materials induce derogative structural and functional modifications in a cells/tissues; these materials when interact with endogenous macromolecules or because of their surface properties, produce ROS, Ghiazza et al, (2013).

Nanomaterials are among the causative agents for carbonyl stress in biosystem. When commercial ceria (5% crystalline ceria dispersion in water with particle size 31±4nm) administered intravenously in rat resulted in protein carbonylation and this was assessed by estimating protein bound 4-hydroxy 2-trans-nonenal (HNN), protein bound 3-nitrotyrosine (3-NT) and protein carbonyls were formed after 1 and 2 h post administration, Yokel et al, (2009). Topical dermal exposure to unpurified SWCNT (40μg, 80μg, 160μg for 5 days) caused elevated oxidation of protein, thiol and carbonyl stress in mice, Murray et al, (2009). Raman active Au NPs induced protein oxidation and it increased protein carbonyl contents; the increase was found to be in proportion to the concentration of NPs used, resulting in carbonyl stress in the experimental animal, Thakor et al, (2011).

Magnetic nanoparticles are formulated suitably for clinical applications and are preferably water based colloids to enhance their utility. This calls for prolonged stability of formulated colloid against precipitation and agglomeration of the magnetic nanoparticles. It is essential to maintain heating efficiency of the engineered ferro-colloidal fluid for effective longer duration, Ghiazza et al, (2013). If agglomeration of the constituents occurs it changes the heating efficiency due to the clusters formation of magnetic nanoparticles; these clusters modify magnetic dipole interaction between the particles. Precipitation of the contents are likely to change heat flow dynamics affecting specific power absorption of single domain; ultimately specific power absorption is related to average size of the particle and size distribution width, Sanze et al (2015). The response of proteins during oxidation could be well illustrated by studying the oxidation of methionine and its interaction with aromatic group. When methionine present in a protein is oxidized a range of proteins get modified and their structural and functional aspects also undergo a change. Experimentation on methionine-aromatic interaction has shown that the primary forces related to folding of protein and protein-protein interaction get altered; oxidation of methionine enhances the effectiveness of "methionine-aromatic-interaction-motif", Lewis et al, (2015); this effectiveness can be established based on parameters like critical spreading dispersion (CSD), prevalence of interaction between dimethyl sulfoxide (DMSO) and aromatic groups, interaction between tyrosine, tryptophan and dimethyl sulfoxide (DMSO), these are able to donate or form H-bond to sulfonyl oxygen. The relative bond energies approximately correlate with

relative abundance of phenylalanine-tyrosine-tryptophan-DMSO interaction. The oxidation of methionine exhibited protein stability within distinct structure and design of secondary structure of stable protein. Oxidation of methionine and its relevance to the binding with/to lymphotoxin-α TNFR-1 (LT-α) and structure and dynamics of calmodulin; the distinct feature (motif) of methionine can play a key role in understanding the sensing ability of protein and its mode of responding to stress during oxidation, Lewis et al, (2015). Protein binding occurs when nC60 interacts with BSA; it indicates theoretical possibility of entrance on NPs in cell without damaging it. The membrane potential changed because of the exposure of proteins involved in maintaining membrane potential. Possibly nC60 affected the membrane integrity and/or nC60 interfered in electron transport proteins, Lyon and Alvaez, (2008). Metal containing NMs like Ag NPs and quantum dots are able to release toxic ions while some NMs physically puncture the cell membrane. There is an interaction between proteins and nanoparticles having same size and magnitude to protein either through chaperon like activity or via changing the conformation of protein, Wagner et al (2010). Also major protein modifications are likely to occur due to the interaction with nitric oxide which caused reactive stress. There can be three modes of modifications namely, binding between N-/iron-heme binding, S-nitrosylation of reduced cysteine residue and C-nitration of tyrosine and tryptophan residue, with exception to nitric oxide binding heme iron protein, the other two modifications needed secondary reactions of NO and formation of nitrogen oxide, Ichiropoulos, (2003).

Biophysical Impact Of Nanomaterials On Protein Oxidation

When nanomaterials are up taken in a biosystem, biomolecules and cell organelles like DNA, proteins, cell membrane and cytoskeleton undergo conformational changes. Modifications in the cell membrane and cytoskeleton bring changes in cellular elasticity, motility, ability of adherence and invasion, Wu et al, (2013). The exposure of cells to carbon based nanomaterials, metals, metal oxides, semiconductor nanomaterials affect cells, and the affected cells give responses; these responses are expressed in biophysical aspects reflecting on the dysfunctioning, pathogenesis or toxicity. Security of nanomaterials and the biosystem is related to the bio-nano-interface; this state is the indication of various interactions. Bayat et al suggested that classical integrated behavior with integrated nanoimpact index is the probable parameter that can acts as an early warning signals to assess risk boundaries, Bayat et al (2015).

The functionalized CNTs interact with bovine serum albumin via diimide activated amidation; these are highly soluble in water and albumin protein links intimately with CNTs, the product formed exhibits good bioactivity, Huang et al, (2002). Non-specific binding or adsorption of proteins is less effective with nucleic acids. The probable cause of it may be the globular structure of protein while the linear and flexible back bone of ssDNA and/or RNA molecule prevents its seamless binding to tubular SWNT as a result higher surface area gets exposed to water. Larger protein like fibrinogen (MW 340kDa) did not show enough binding to SWNT in comparison to smaller proteins like streptavidin (MW 60kDa). In practice, protein binding to SWNT is mostly used to recognize and sensing biomolecular investigation and not for solubilizing SWNT, Huang et al, (2002). The forces which participate in the interaction between SWNT and proteins include electrostatic, hydrogen bonding, hydrophobic or entropic in nature. These forces act depending on the conformation of proteins, surface, charge of substrate, pH, ionic strength, temperature of the ambient solution etc, Ke and Qiao (2007).

Plasma proteins like histidine-proline rich glycoprotein (high MW), kininogen and plasma prekallikrein show strong affinity with Supra paramagnetic iron oxide nanoparticles (SPION) and dextran coated SPION, Ke and Qiao (2007) and Simberg et al, (2009). Further, these proteins have histidine–rich domain and are found to be bounded to the negatively charged iron oxide core not to the neutral dextran coating. The weakly bounded proteins are found to be mannose-binding lectin, mannose associated serine proteases, apolipoprotein, β-2-glycoprotein and clotting factors FXI and FXII; albumin and transferrine do not exhibit significant bonding with SPION indicating the bonding of protein with NPs is a selective process, Simberg et al, (2009) and Singh et al (2010). It has been observed that plasma opsonin did not participate in the removal of SPION from circulation by macrophages present in the liver; there seemed no interaction between SPION and receptors present on the macrophages, scavenger receptors are involved in the removal of NPs, Simberg et al, (2009) and Singh et al (2010). SPION coated with human serum albumin are taken up by macrophages due to the interaction with surface receptor such as glycoprotein (gp60) receptor and cysteine–receptor which are mostly present on the larger range of cells, Xie et al, (2010).

High solubility of SWNT and MWNT in water can be obtained easily by organic functionalization, derivatization with N-protected glycine. Geogarkilas et al, have suggested that SWNT and MWNT exhibit specific mechanical, thermal, chemical and electronic

properties and these help to show tremendous combining affinity, thus, these are very useful materials for nano-technological applications. Further, their findings show that the covalent bonding of SWNT with amino acids is brought about by functionalization of side walls of SWNTs/MWNTs with N-protected amino acids on 1.3-dipolar-cycloaddition reaction, Gerogaklias et al (2002). SWNTs are toxic to skin, this toxicity might be dependent upon the metal–particularly iron contents of SWNT via the ability of metal to react with skin, initiate the oxidative stress and induces redox sensitive transcription factor there by leading to inflammation (in the skin cell culture). The doses of SWNT 40µg/mouse, 80µg/mouse and 160µg/mouse resulted in increase in the dermal cell number and skin thickening due to the accumulation of polymorphonuclear leukocytes and mast cells, Murray et al, (2009).

The cell membrane acts as the prime bio-nano interface during the interaction with nanomaterials, Murray et al, (2009). Nanomaterials caused either ion-channel blockage or generation of holes in the membrane or change the alignment of the membrane by inducing rapid strain transition, Wu et al, ((2013). Nanomaterials cause change in the permeability of the membrane and this leads to leakage of enzyme and other contents of the affected cells and similar effect has been observed in the affected cells where the holes have been made in cell membrane by nanomaterials. Verma et al, reported that nanoscaled mater enter cell involving membrane bound endosomes but do not reach cellular functional system; whereas the biomolecules having similar size penetrate cell membrane without disrupting it. The cationic nanosized particles are able to move across the cell membrane by making transient holes resulting cytotoxic conditions, Rosi et al, (2006); Leroueil et al, (2007) and Verma et al, (2008). The positive surface charge on the nanoparticles is responsible for the change in the fluid phase of lipid quantum dots in membrane while negative and neutral charges on the surface of nanoparticles did not bring any toxicity. Size of the nanomaterials is an important factor; nanoparticles having size less than 1.2 nm and bigger than 20nm could not cause such effects on membrane. Nanoparticles with larger size than 20nm have caused sub-nanometer striations of alternating anionic and hydrophobic groups or same moieties has been observed to be located randomly on the cell membrane, Rosi et al, (2006), Leroueil et al, (2007) and Verma et al, (2008).

The adsorption of protein on magnetic nanoparticles showed that surface charge on the magnetic nanomaterials influence the amount and rate of adsorption of proteins; positively charged surface (positive polyethyleneimine-PEI-MNPs) exhibit higher rate of adsorption in comparison with negatively charged surface (negative polyacrylic acid-PAA-MNPs) on magnetic nanoparticles, although both of these particles show similar negative zeta potential within culture, Calatayud et al, (2014). Visualization techniques are likely to provide relatively better understanding of the mechanism involved in the interaction of nanomaterials being investigated, Ostrowski et al (2015). These techniques facilitate visualization of nanomaterials and evaluation of cell/tissues and the changes occurring due to these analytes. This might be helpful in, over all better evaluation and meaningful understanding of the mechanism involved.

Conclusion

Under normal conditions, oxidative process in a biological system helps to sustain the structural and functional integrity of cell and its organelles. Mostly, protein oxidation may be understood or investigated by tyrosine nitration, cysteine oxidation and carbonylation processes. The damage to protein or biological system can be non-specific and wide spread or both. In non-specific oxidation, hydroperoxides are involved that enhance oxidation and chain reaction affecting proteins and related molecules. These proteins can be used as biomarkers to evaluate protein oxidation both *in vivo* and *in vitro* depending on the oxidant used or available. In most cases the damage caused to proteins is likely to be non-repairable and results are derogative such as loss of enzymatic affectivity, structural and signaling inability (partial or total). Engineered iron oxide nanoparticles are cytotoxic in nature; these can be exploited to treat cancer cells because such nanoparticles have ability to increase permeability and deeper drug penetrating efficiency. Magnetic nanoparticles (iron oxide) are likely to play better role in the treatment of cancer in comparison to the traditional therapy; these are formulated suitably for clinical applications and are preferably water based colloids to enhance their utility. This calls for prolonged stability of formulated colloid against precipitation and agglomeration of the magnetic nanoparticles. It is envisaged that studies on bio-nano-interface may direct the methodology of synthesis of nanomaterials so that size, shape, physicochemical aspects of surface of nanomaterials should match the appropriate intrinsic physicochemical parameters leading to ideal biomedical appliances. Nanomaterials when get lodged in a biosystem, biomolecules like DNA, proteins, cell membrane and cytoskeleton undergo conformational changes. Modifications in the cell membrane and cytoskeleton reflect the changes in cellular elasticity, motility, ability of adherence and

invasion, Visualization techniques are likely to provide coherent picture for better understanding of the nanomaterials being investigated. Even though lot of investigations have been done but still there seems to be a greater need for better understanding of overall mechanism of various aspects of protein oxidation with reference nanomaterials.

Conflict Of Interest

The authors declare no conflict of interest.

Contribution Of Authors

Authors contributed equally.

References

1. Bayat N, Lopes VR, Sancheze-Dominiquez M, Lakshmanan R, Rajarao G, Cristabol S, Assessment of functional iron oxide NPs in vitro: introduction to integrated nanoimpact index, Environmental Sci: Nano, 2015(2):380-394; doi: 10.1039/C5EN00016E.
2. Buzzea C, Pacheco II, Robbie K, Nanomaterials and nanoparticles: sources and toxicity, Biointerphases, 2, MR17, 2007: http://dx.doi.org/10.1116/1-2815690.
3. Calatayud MR, Sanz B, Raffla V, Riggio C, Ibarra R, Goya G F, The effect of surface charge of functionalized Fe_3O_4 NPs on protein adsorption and cell up take, Biomaterials, 2014, 35(24): 6389-6399.
4. Crippen GM, Variable resolution invariants: 3D quantitative structure-active relations, (QSAR), at variable resolutions, J Computational Chem, 1999, 20(14):1577-1585.
5. Fabiola S, Ourania ET, Athanassios K, Ioannis PT, Oxidative stress-mediated biomolecular damage and inflammation in tumor genesis, In vivo, 2012, 26(3):395-402.
6. Fubini B, Hubbard A, ROS and RNS generation by silica inflammation and fibrosis: Free Radical Biology and Medicine, 2003, 34(12): 1507-1516, Doi: 10.1002/(SICI) 1096-987X(19991115)20:14<1577:AID-JCC11>3.0.CO:2-1.
7. Genestra M, Oxyl radicals, redox-sensitive signaling cascades and antioxidants, Cellular Signaling, 2007, 19:1807-1819.
8. Georgakilas, V, Tagmatarchis N, Pantarotto D, Bianco A, Briand JB, Prato M Amino acid functionalization of water soluble carbon nanotubes, Chem Commun, 2002, 24:3050-3051, doi: 101039/B209843A.
9. Ghiazza M, Carella E, Simnetta Oliaro-Basso, Corazzari I, Viola F, Fenoglia I, Predictive tools to evaluate oxidative potential of engineered NMs, J Physics: Conference Series, 2023, 429 (2013) 012024, doi: 101088/1742-6596/429/1/012024/ Nanosafe 2012.
10. Hansch C, Steinermetz WE, Leo AJ, Makepati SB, Kurup A, Hockman D, On the role The role of polarizability in chemical biological interaction, J Chem Inf Comput Sc, 2003, 43(1): 120-125; doi: 10, 1021/ci02038b.
11. Huang W, Taylor S, Fu K, Lin Y, Zhang D, Hanks TW, Rao AM and Sun YP, Attaching proteins, to C-nanotubes via diimide-activated amidation, Nano Letters, 2002, 2: 311.
12. Ichiropoulos H, Biological selectivity and functional aspects of protein tyrosine nitration, Biochem Biophys Res Comm, 2003, 305(3): 776-783.
13. Knaapen AM, Borm PJA, Albrecht C, Schins RPF, Inhaled particles and lung cancer, Part A: Mechanism, interaction, J Cancer, 2004, 109(60: 799-809.
14. Ke PC, Qiao R, Carbon nanomaterials in biological system, J Physics, 2007, 19.373101; Doi: 10.1088/0953-8984/19/373101.
15. Leroueil PR, Hong SY, Mecke A, Baker JR, Orr BJ, Holl MMB, Nanoparticles interaction with biological membrane: does nanotechnology present a janus face? Acc Chem Res, 2007, 40:335-342.
16. Lewis AK, Cembran A, Senkow TL, Perrell G, McCarthy M, Her C, Horn B, Valley C, Gao J, Pomerantz WC, Thomas DD, Hinderliter A, Sachs JN, Oxidation increases strength of methionine-aromatic interaction, Biophysics J, 2015, 108(2): supplement 1.p 17 a, 27Jan 2015, DOI: dx.doi.org/10.1016/j.bpj.2014.11.117.
17. Li JJ, Muralikrishnan S, Ng CT, Yung LY, Bay BH, Nanoparticles induced pulmonary toxicity, Exp Biol Med, 2010, 235(9): 1025-1033.
18. Lyon DY, Alvaez PJJ, Fullerene water suspension (nC60) exert antibacterial effects via ROS dependant protein oxidation, Environ Sci Technol, 2008, 42:8127-8132.
19. Manke A, Wang L, Rajanasakul, Mechanism of nanoparticles induced oxidative stress and toxicity, BioMed Res Int, 2013: Article ID 942916, 15 p; http://dx.doi.org/10.1055/2013/942916d.
20. Moyano DF, Goldsmith M, Solfiell D J, Landsmann-Milo D, Oscar RM, Peer D, Rotello V M, (2012), Nanoparticles hydrophobicity dictates immune responses, J Am Chem Soc, 2012, 134(9): 3965-3967; doi: 10.1021/ja2108905.
21. Murray AR, Kisin E, Leonard SS, Yong SH, Komminein C, Kagan VE, Castronova V, Shevdova AA, Oxidative stress and

inflammatory response in dermal toxicity of SWCNT, Toxicology, 2009, 257(3): 161-171
22. Nel AE, Medler L, Velegol D, Xia T, Hoek EM, Samsundaran P, Klaessig F, Castronova V, Thompson N, Understanding biophysicochemical interactions at nano-bio-interface, Nature material, 2009, 8:543-557: doi: 10.1038/nmat2442.
23. Ostrowski A, Nordmayer D, Boreham A, Hoizhanson C, Mendhenk L, Graf C, Meinke M C, Vogt A, Hadam S, Lademan J, Ruhl E, Alexier, Gruber AD (2015) Overview on the localization of nanoparticles in tissues and cellular contents by different imaging techniques, (Review), Beilstein J Nanotechnology, 2015, 6:263-280, Doi: 10.3762/bjnano6.25.
24. Palm K, Stienberg P, Luthman K, Arturson P, Polar molecular surface properties predict the intestinal absorption of drugs in humans, Pharmaceutical Res, 1997, 14(5):568-571, Doi: 101023/A:10.2188/625088.
25. Pojlak-Blazi M, Jaganjac M, Zarkovic N, Cell oxidative stress: risk of metal NPs, In Handbook of Nanophysics: Nanomedicine and Nanorobotics, CRC Press, New York, pp 1-17 (Ispravak podataka Bio Tax).
26. Rosi NL, Giljorhann DA, Thaxton CS, Lytton-Jean AKR, Han MS, Mirkin CA, Oligonucleotide-modified Au NPs for intracellular gene regulation, Science, 2006, 312, 1027-1030.
27. Sanze B, Calatayud M, Cassinelli N, Ibarra M, Goya G, Long term stability and reproducibility of magnetic colloids are key issues for steady values of specific power absorption over time, European J Inorganic Chem, 2015, 16.02:22; DOI: 10.1002/ejic.201500303.
28. Simberg D, Park J H, Karmali P P, Zhang W M, Mc Crek et al, Differential proteomics analysis of the surface heterogeneity of dextran iron oxide NPs and the implications for their *in vivo* clearance, Biomaterials,2009, 30:3926-3933.
29. Singh N, Jenkins GJS, Asodi R, Doak SH, Potential toxicity of supra paramagnetic iron oxide nanoparticles, SPION, Nano Reviews, 2010, 1: 5358; doi: 10.3402/nano.v1io.5358.
30. Song MF, Li YS, Kasai H, Kawai K, Metal nanoparticles induced-micronuclei and oxidation of DNA damage in mice, J Clinical Biochem and Nut, 2012, 50(3):211-216; Doi: 10.3164/jcbn.11.70.
31. Stadtman E R, Metal ion-catalyzed oxidation of proteins: Biochemical mechanism and biological consequences, Free Radical Biology and Medicine, 1990, 9(4): 315-325.
32. Thakor AS, Paulmurugan R, Kempen P, Zavaleta C, Sinclair R, Massaud TE, Gambhir SS, Oxidative stress mediates the effects of Raman-Activate gold NPs in human cells, Small, 2011, 7(1): 126-136, doi: 10.1002/small.20100/small.201001466.
33. Verma A, Uzun O, Hu YH, Watson N, Achen SC, Irvin DJ, Stellacci F Surface structure regulated cell membrane, penetration by monolayer-protected NPs, Nat Mater, 2008, 7(7): 588-595.
34. Wagner SC, Roskam PM, Pallerla M, Araghi RR, Schlect S, Koksch B, Nanoparticles induced folding and fibril formation of coiled-coil-base model peptide, Small, 2010, 6:1321-1328.
35. Wang J, Hou T, Develop and test a solvent accessible surface area based model in conformational entropy calculation, J Chem Inf Modl, 2012, 52(5):1199-1212.
36. Wu YL, Putcha N, Ng KW, Leong DT, Lim CT, Loo JSC, Chen X, Biophysical responses upon the interaction of nanomaterials with cellular interface, Account of Chemical Res, 2013, 46(3):782-791 doi: 10.1021/ar3000464.
37. Xie J, Wang J, Nin G, Huang J, Chen K, Li X et al, Human serum albumin coated iron oxide NPs for efficient cell labeling, Chemical Commun, (Camb), 2010, 46: 433-435.
38. Yokel RA, Florence RL, Unrine JM, Tseng MT, Graham UM, Wu P, Grulke EA, Sultana R, Hardas SS, Butterfield DA, Biodistribution and oxidative stress effects of a systematically-introduced commercial ceria engineered nanomaterial, Nanotoxicity, 2009, 3(3):234-248: doi: 10.1080/17435390902974496.

7/25/2016