Published in final edited form as:
Acc Chem Res. 2013 March 19; 46(3): 681–691. doi:10.1021/ar3000647.

The role of surface functionality in determining nanoparticle cytotoxicity

Sung Tae Kim, **Krishnendu Saha**, **Chaekyu Kim**, and **Vincent M. Rotello***
Department of Chemistry, University of Massachusetts-Amherst, 710 North Pleasant St., Amherst, MA 01003 (USA)

CONSPECTUS

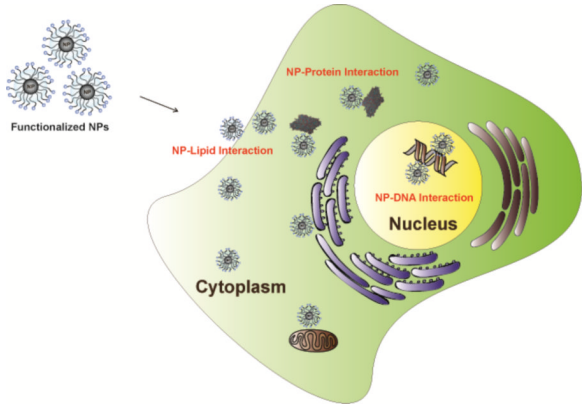
Surface properties dictate the behavior of nanomaterials *in vitro*, *in vivo* and in the environment. Such properties include surface charge and hydrophobicity. Also key are more complex supramolecular interactions like aromatic stacking and hydrogen bonding, and even surface topology from the structural to the atomic level. Surface functionalization of nanoparticles (NPs) provides an effective way to control the interface between nanomaterials and the biological systems they are designed to interact with. In medicine, for instance, proper control of surface properties can maximize therapeutic or imaging efficacy while minimizing unfavorable side effects. Meanwhile, in environmental science, thoughtful choice of particle coating can minimize the impact of manufactured nanomaterials on the environment.

A thorough knowledge of how NP surfaces with various properties effect biological systems is essential for creating NPs with such useful therapeutic and imaging properties as low toxicity, stability, biocompatibility, favorable distribution throughout cells or tissues, and favorable pharmacokinetic profiles--and for reducing the potential environmental impact of manufactured nanomaterials, which are becoming increasingly prominent in the marketplace.

In this Account, we discuss our research and that of others into how NP surface properties control interactions with biomolecules and cells at many scales, including the role the particle surface plays in determining *in vivo* behavior of nanomaterials. These interactions can be benign, beneficial, or lead to dysfunction in proteins, genes and cells, resulting in cytotoxic and genotoxic responses. Understanding these interactions and their consequences helps us to design minimally invasive imaging and delivery agents.

We also highlight in this Account how we have fabricated nanoparticles to act as therapeutic agents via tailored interactions with biomacromolecules. These particles offer new therapeutic directions from traditional small molecule therapies, and with potentially greater versatility than is possible with proteins and nucleic acids.

*To whom correspondence should be addressed. rotello@chem.umass.edu.



INTRODUCTION

Nanoparticles (NPs) are promising scaffolds for applications such as imaging,[1] diagnostics,[2] drug delivery,[3] catalysis,[4] solar cells,[5] and sensors.[6] NPs with a variety of core materials (metal, semiconductor, and organic) can be applied to biological systems.[7] The surface functionalization of NPs introduces an additional dimension in regulating NP interfacial properties[8] that can be used to dictate interactions with biosystems.[9,10] These interactions play a predominant role in determining the efficacy and toxicity[11] of NPs in biological and environmental systems.[12,13]

Biological systems respond strongly to NP surfaces.[14] NPs can interact with cellular components including DNA, proteins, and lipids, as well as with cells or tissues. For example, NPs can cause structural reconstruction and phase transition of the cell membrane.[15] After internalization into the cells, they can also interact with cytosolic components (e.g. proteins and enzymes)[16,17] as well as nuclear components (e.g. DNA)[18] leading to disturbance of electron/ion transport through membranes,[19] the production of endogenous reactive oxygen species (ROS),[20] and genotoxicity.[21] Beyond the cellular level, NP-cell interactions can cause adverse physiological effects such as inflammation[22] and immunological responses[23] that lead to dysfunction of the tissues and organs.[24]

In this Accounts, we focus on our research and others in the field into the role of NP surface functionality in governing their toxicity. We will also discuss how we have harnessed our current understanding of NP interfaces to engineer tunable NPs for improved therapeutics.

Surface Functionalization of Nanoparticles

Surface functionalization of NPs is an important aspect in tailoring NPs for specific therapeutic/diagnostic purposes. A wide variety of synthetic and natural ligands have been attached to NP surfaces, improving the stability/solubility of the NPs[25] as well as incorporating targeting ligands and/or therapeutic agents.[26] Characterization of these particles is challenging, but essential for their use. Several analytical tools are available to characterize the NP surface composition and the purity of NPs. Chemists rely on multiple characterization techniques such as nuclear magnetic resonance (NMR), thermogravimetric analysis (TGA), dynamic light scattering (DLS), mass spectrometry (MS) and transmission electron microscopy (TEM) to ascertain NP structure and purity.[27] In brief, the initial characterization using ^{1}H NMR confirms the success of place exchange reaction by checking for the absence of any sharp peaks arising from free ligands. MS measurements further confirs the ligand purity and coverage on the NP surface. In addition, DLS measurements provide additional information about NP properties such as the size and the

surface charge of NPs, thereby confirming their colloidal stability. TEM analysis provides crucial information on the size and homogeneity of NPs. This extensive surface characterization is mandatory for understanding the behavior of these systems for fundamental studies as well for quality control in their applications.

INTERACTIONS OF NANOPARTICLES WITH BIOLOGICAL SYSTEMS

Surface Functionality of Nanoparticles and Membrane Perturbation

NPs can cause structural reconstruction and phase transition of the cell membrane's lipid bilayer.[28] Direct interaction of surface functionalized NPs with cells can damage the integrity of membrane structure, with the extent of leakage depending on the NP surface chemistry.[29] In early studies on NP toxicity, Rotello *et al.* demonstrated that a cationic mixed monolayer protected cluster (MMPC1) showed higher cytotoxicity than its anionic analogue (MMPC2), demonstrating the key role of surface charge on NP cytotoxicity (Figure 1a).[30] Likewise, MMPC1 disrupted anionic phosphatidylcholine/phosphatidylserine vesicles more efficiently than MMPC2 due to strong electrostatic interactions with the negatively charged lipid bilayer (Figure 1b). Similarly, alkylamine functionalized NPs with 2 nm gold cores were shown by Holl to disrupt supported lipid bilayers (SLB).[31] These NPs expanded pre-existing defects within the SLB and aggregated on the anionic mica substrate. Zhu *et al.* reported that the degree of SLB disruption depends on the surface chemistry with carboxylate functionalized polystyrene NPs of varying diameters (d=28, 62, and 140 nm).[32] These findings were verified by simulation/modeling studies using a mesoscale thermodynamic model.[33]

Mukherjee and Rotello *et al.* further investigated the role of the NP surface charge on cell membrane potential. Four gold NPs with varying surface charges (e.g. cationic, anionic, zwitterionic, and neutral) were incubated with cells (Figure 2a).[34] Positively charged gold NPs depolarized the membrane potential in a dose dependent manner across different cell types compared to other NPs (Figure 2b). Furthermore, cationic NPs rapidly increased the intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$ by stimulating plasma membrane Ca^{2+} influx as well as Ca^{2+} release from the endoplasmic reticulum, with concomitant inhibition of proliferation of human bronchial epithelial cells (BEC) and human airway smooth muscle cells (ASM) (Figure 2c).[35] Taken together, positively charged NPs lead to perturbation of the cell membrane by structural reconstruction and phase transition of the lipid bilayer. Furthermore, they induce cytotoxicity and/or cell death due to intracellular signaling by changing the membrane potential and increasing $[Ca^{2+}]_i$.

These findings demonstrate that positively charged NPs can modulate cell-membrane potential, ultimately disrupting the lipid bilayer during cellular uptake. Such depolarization of cell membrane enhances the cellular uptake of cationic NPs, but inhibits cell proliferation, and ultimately induces cell death. Therefore, like two sides of the same coin, these opposing actions require attention and understanding in designing NP surface functionality.

Surface Functionality of Nanoparticles and Genotoxicity

NPs can affect gene regulation and genotoxicity through the direct interaction with genetic materials or by promoting endogenous oxidative stress and inflammation.[21] Rotello *et al.* investigated mixed monolayer protected gold clusters (MMPCs) functionalized with tetraalkylammonium ligands that can interact with the DNA backbone (Figure 3a).[36] This complimentary electrostatic interaction with the DNA (37 mer) inhibited T7RNA polymerase *in vitro* (Figure 3b). A further study observed that MMPC-DNA interaction could be influenced by the levels of glutathione (GSH) that controls the intracellular redox environment.[37] Although the interaction between MMPC and DNA varies from the choice of monolayer coverage, up- or down-regulated transcription derived by NP interaction can

cause cellular DNA damage and genotoxicity. For example, El-Sayed *et al.* reported 30 nm gold NPs featuring arginine-glycine-aspartic acid (RGD) and nuclear localization signal (NLS) peptides cause DNA damage and cytokine arrest in human oral squamous cell carcinoma cells (HSC).[38] Chen *et al.* also demonstrated cationic amine-modified polystyrene NPs retarded the G0/G1 phase in the cell cycle with concomitant decrease in the expression level of cyclin D and cyclin E.[39] Hussain *et al.* likewise reported gene expressions related to apoptosis, cell cycle, and DNA repair were up-or down-regulated due to gold NP surface charge and functionality.[40]

In addition to surface charge, surface hydrophobicity of NPs also plays an important role in cytotoxicity and consequent DNA damage. Rotello *et al.* synthesized gold NPs featuring quaternary ammonium functionality with a systematically varied hydrophobic alkyl chain (Figure 4a).[41] Increasing hydrophobicity on the NP surface resulted in higher cytotoxicity (Figure 4b) with concomitant ROS production in HeLa cells (Figure 4c). However, comet assays using NP-HEX showed relatively lower % Tail DNA and Tail length, signifying decreased DNA damage with increasing particle hydrophobicity (Figure 4d), probably due to the up-regulation of autophagic processes under oxidative stress.[42] Therefore, surface functionality plays an important role in DNA damage as well as ROS production, with potential toxic consequences.

In summary, once NPs are internalized into cells the surface functionality of NPs dictates genotoxicity both directly and indirectly. Notably, hydrophobicity of NP's surface is as important as the surface charge in dictating genotoxicity. Based on our findings, modulation of hydrophobicity suggests an opportunity to regulate genotoxicity Overall, these studies clearly indicate that by modulating the simple chemistry of the NP surface, an optimum monolayer can be found to minimize genotoxicity and other detrimental subcellular events.

Surface Functionality of Nanoparticles and Protein Function

Interaction of surface functionalized NPs with proteins can lead to conformational changes perturbing protein function. The properties of surface monolayers control the extent of protein denaturation on NP surfaces.[43,44] In early studies, Rotello *et al.* investigated NP-protein interactions using anionic mercaptoundecanoic acid (MUA)-functionalized gold NPs (2 nm core).[45] MUA-gold NPs interacted selectively with the protease chymotrypsin (ChT), resulting in an inhibition of enzymatic activity (apparent inhibition constant, K_i=10.4±1.3 nM). This inhibition is a two-step process featuring a rapid reversible inhibition step driven by electrostatic interaction, followed by a slower irreversible denaturation process. In addition, anionic functionalized NPs irreversibly inhibit the activity of ChT, resulting in protein denaturation (Figure 5a).[46] Cationic surfactants (S1, S2 and S3), however, allow the release of ChT from anionic NPs (Figure 5b). For example surfactant 1 (S1) dissociated ChT through the formation of bilayer structures, whereas cationic thiol-terminated surfactant (S2) and alcohol-terminated surfactant (S3) were incorporated into the anionic monolayer of the gold NP, without increase of the hydrodynamic radius of the gold NPs (Figure 5b).

Rotello *et al.* demonstrated that amino acid-functionalized gold NPs could be used to control the stability of adsorbed ChT. Hydrophilic amino acids on the NP surfaces destabilized the proteins due to competitive hydrogen bonding as well as disruption of salt bridges inside the protein.[47] Furthermore, short oligo(ethylene glycol) (mono, di, and tri(ethylene glycol)) tethers increase the rate of protein denaturation,[48] while tetra(ethylene glycol) chains improved the stability of ChT at the NP surface.[49] In similar fashion, Hamad-Schifferli *et al.* reported gold NPs with polyethylene(glycol) ligands were appended to a specific cysteine (Cys102) of *S. cerevisiae* Cyt c resulting in denaturation of Cyt c.[50] Denaturation of Cyt c can lead to malfunction of electron transfer between Coenzyme Q-Cyt c reductase and Cyt c oxidase, and reduction of detoxifying ROS.[51,52] Likewise, when uptaken into cells, surface

functionalized NPs are able to interfere with cell signaling molecules or proteins,[53] either through a chaperone-like-activity[54] or by changing a molecular structure due to the aggregation and fibrillation.[55]

Based on these findings, after systemic administration, surface functionalized NPs can interact and denature proteins present in the serum as well as intracellular enzymes following uptake, causing toxicity. Importantly, charged NPs can inhibit enzyme activity to varying degrees and can lead to the denaturation of enzymes proteins. Practically, however, it is difficult to predict *a priori* the effects of NPs on specific enzymes due to the complexity and diversity of interactions available.

Immunological Impact of Surface Functionalized Nanoparticles

Surface functionalized NPs can cause immune responses[56] and/or immunotoxicity through several mechanisms.[57] For example, Jang *et al.* reported cationic silica-titania NPs functionalized with amine groups were immunotoxic to macrophage cells (J774A.1).[58] Peer *et al.* reported cationic lipid-based NPs induced T helper cell 1 (Th1) cytokines and activated the Toll-like receptor-4 (TLR-4)[59] at a rate of at least 10 times higher than neutral or anionic NPs. In addition to surface charge, Rotello *et al.* have recently demonstrated that the surface hydrophobicity of NPs dictates the immune response of splenocytes (Figure 6).[60] NPs (NP1-8) with different hydrophobicities (Figure 6a) showed a direct, quantitative correlation between hydrophobicity and immune activation related to the gene expression of cytokines (e.g. interferon (IFN)-α, tumor necrosis factor (TNF)-γ, interleukin (IL)-2, 6, and 10). In particular, increasing the hydrophobicity of the NP surface elicited increased the expression of TNF-α, a pro-inflammatory cytokine (Figure 6b), and the expression of IL-10, an anti-inflammatory cytokine (Figure 6c). Likewise, Deng *et al.* have also reported anionic polyacrylic NPs can induce pro-inflammation through the interaction between NPs and fibrinogen, resulting in activation of the Mac-1 receptor of the monocytes.[61]

Our recent research demonstrates that NP surface properties including surface charge and hydrophobicity dictate immune responses. In addition to the surface charge, the hydrophobicity of surface ligands elicits different cytokine expressions and provides different molecular and cellular changes in immune cells. Therefore, surface hydrophobicity as well as charge must be taken into account when designing nanomaterials designed to elicit or avoid immune responses. Finding the key surface elements responsible for of generating immune responses would provide NPs with improved biocompatibility and minimal immunotoxicity.

Biodistribution of Nanoparticles: Organ Toxicity

Beyond the cellular level, NPs accumulate in tissue and organs after topical or systemic administration *in vivo*, with their biodistribution and pharmacokinetics strongly dependent on NP surface properties,[62] and the type and amount of absorbed macromolecules (e.g. serum proteins).[63]

Recently, Rotello *et al.* reported the uptake, distribution, excretion, and toxicity of positively charged ~2 nm core gold NPs with different surface functionality in Japanese medaka fish (Oryzias latipes) (Figure 7).[64] They showed that hydrophilic surface functionality on NPs (Figure 7a) facilitates clearance, potentially minimizing environmental impact. Conversely, hydrophobic NPs penetrated into the circulatory system of the fish, leading to a widespread distribution of particles into the organs of the fish and ultimately leading to fish mortality in less than 1 day. (Figure 7b).

In a murine model, Rotello and Mukherjee *et al.* systemically investigated how surface charge of the gold NPs affects accumulation in organs (Figure 8).[65] After intravenous (IV)

(Figure 8a) or intraperitoneal (IP) administration (Figure 8b), neutral (TEGOH) and zwitterionic (TZwit) NPs demonstrated reasonable (hours) circulation times, whereas cationic (TTMA) and anionic (TCOOH) NPs possessed relatively short half-lives. Cationic TTMA-NPs were cleared 4.3 times faster than anionic TCOOH-NPs after IV administration. However, both TCOOH-NPs and TTMA-NPs were poorly retained in circulation after IP administration. All four gold NPs accumulated in the liver and spleen mainly by resident macrophage cells (e.g. Kupffer cell) after IV injection whereas they accumulated in the pancreas after IP administration. Besides organs, NPs accumulated differently in solid tumors in a murine model. TEGOH and TZwit NPs accumulated in tumors more than cationic TTMA NPs after IV injection. Therefore, different patterns of NP accumulation can be generated by their surface functionality, with concomitant organ-level effects.

Based on the *in vivo* data, surface functionalization of NPs should be considered when designing NPs because the surface charge of NPs alters their pharmacokinetics, tumor uptake and biodistribution. Our data showed that neutral and zwitterionic NPs demonstrated a higher area under the curve (AUC), lower clearance and a longer circulation time than charged NPs via IP and IV administration of NPs into mice. Thus, we expect that neutral and zwitterionic ligands of NPs can reduce acute organ toxicity, resulting from low amount of NP accumulation. Unlike the organ distribution, however, neutral/zwitterionic NPs are accumulated in higher amount in tumor than charged NPs. Therefore, careful design of the NP surface can improve pharmacokinetic profiles as well as increase tumor uptake.

Tailoring Nanoparticle Surface Functionality for Therapeutic Applications

Surface Functionality of Nanoparticles in Delivery Strategies—As described above, surface properties dictate the cytotoxic responses caused by NPs. As such, surface functionalization can help in the creation of NPs with improved therapeutic efficacy. Moreover, the functional versatility of NP monolayers provides an excellent platform for delivery vehicles. Rotello *et al.* have used a gold NP functionalized with photocleavable *o*-nitrobenzyl ester moieties for photoregulated release of the anticancer drug 5-fluorouracil in cancer cells.[66] In this work, the zwitterionic ligand on the surface of the NP aided solubility while limiting intracellular uptake.

Non-covalent conjugation of drugs onto the NP surface monolayer provides an alternative approach to covalent conjugation, potentially overcoming prodrug related issues. Rotello and coworkers demonstrated an efficient way to encapsulate anticancer drugs inside the hydrophobic monolayer of gold NPs allowing subsequent release in cancer cells.[67]

Surface properties of NPs can control NP penetration in tissues as well as drug delivery/release. Cationic NPs improved delivery of drug payload to the majority of cells in a tumor model, whereas anionic NPs, perform better at delivering drugs deep into the tumor model.[68]

Beyond Carriers: Nanoparticles as Therapeutics—NPs provide delivery vehicles featuring high drug loading efficiency, low toxicity, improved pharmacokinetic profile, and high cellular uptake.[69,70] However, NPs can be engineered to be cytotoxic for use as potential therapeutics in their own right.[71] For example, Rotello *et al.* reported the use of cationic gold NPs as therapeutic agents by controlling their cytotoxicity (Figure 9).[72] The cationic NPs functionalized with a terminal diaminohexane moiety strongly interact with cell membranes and subcellular compartments, resulting in membrane disruption and cytotoxicity. However, the complexation of NP-NH_2 with cucurbit[7]uril (CB[7]) reduces the ability of the particles to disrupt endosomal membranes, lowering toxicity. The host–guest complex on the particles can be intracellularly disassembled by adding the orthogonal guest molecule 1-adamantylamine (ADA) that has a very high affinity for CB[7].

Intracellular displacement of CB[7] from the nanoparticle results in endosomal escape of the gold NP-NH_2, activating the cytotoxicity of gold NP-NH_2 and inducing cell death. This supramolecular approach provides a new strategy for triggering therapeutic systems.

SUMMARY AND FUTURE PERSPECTIVES

The appropriate engineering of surface functionality is crucial for controlling the subcellular and cellular transport of NPs as well as their overall biodistribution and pharmacokinetics. In this review, we summarized efforts to determine the effect of surface functionality on NP cytotoxicity. The interaction of NPs with biosystems plays an important role in triggering toxicity through a range of mechanisms, including membrane perturbation, oxidative stress and DNA/macromolecular damage.

Understanding of nanomaterial toxicity is central to predicting the potential environmental implications of nanomaterials. This knowledge will also play a central role in the development of new nanotherapeutics. While there are many ways to approach this issue, it is clear that nanotoxicology is a truly multiscale endeavor, integrating molecular, cellular, and organismic insights. Coupling of these investigations with the tools provided through organic, polymer, and materials synthesis will provide a fruitful field for both fundamental and applied bionanotechnology.

Acknowledgments

Support from the NIH (R01 EB014277 and GM077173) is gratefully acknowledged.

Biography

SUNG TAE KIM received his Ph.D in Pharmaceutical Science from Seoul National University in South Korea. He worked as a postdoctoral researcher at Seoul National University and as a research assistant professor at Korea University. Currently, he is a postdoctoral researcher at the University of Massachusetts at Amherst under the guidance of Professor Rotello. His research interests focus on nano-bioscience and drug delivery applications.

KRISHNENDU SAHA received his B.Sc. in Chemistry from Jadavpur University, India and M.Sc. in Chemistry from Indian Institute of Technology-Madras, India. He is pursuing his Ph.D at the University of Massachusetts at Amherst under the guidance of Professor Rotello. His research interest involves the investigation of gold nanoparticle monolayer properties for cell-surface recognition and delivery applications.

CHAEKYU KIM obtained his B.E. in Chemical Engineering in 2002 and M.E. in Polymer science and engineering in 2005 from Inha University in South Korea. He received his Ph.D in Chemistry from the University of Massachusetts at Amherst under the guidance of Professor Rotello. His research interests focus on bionanotechnological applications via engineering the interface between biomacromolecules and nanomaterials.

VINCENT M. ROTELLO is the Charles A. Goessmann Professor of Chemistry at the University of Massachusetts at Amherst. He has been the recipient of the NSF CAREER and Cottrell Scholar awards, as well as the Camille Dreyfus Teacher-Scholar, the Sloan Fellowships, and the Langmuir Lectureship, and is a Fellow of the American Association for the Advancement of Science (AAAS) and of the Royal Society of Chemistry (U.K.). His research program focuses on using synthetic organic chemistry to engineer the interface between hard and soft materials, and spans the areas of devices, polymers, and nanotechnology/bionanotechnology.

REFERENCES

1. Smith AM, Duan HW, Mohs AM, Nie SM. Bioconjugated quantum dots for in vivo molecular and cellular imaging. Adv. Drug Delivery Rev. 2008; 60:1226–1240.
2. De M, Rana S, Akpinar H, Miranda OR, Arvizo RR, Bunz UHF, Rotello VM. Sensing of proteins in human serum using conjugates of nanoparticles and green fluorescent protein. Nat. Chem. 2009; 1:461–465. [PubMed: 20161380]
3. Davis ME, Chen Z, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. Nat. Rev. Drug Discov. 2008; 7:771–782. [PubMed: 18758474]
4. Gates BC. Catalysis: Individual nanoparticles in action. Nat. Nanotechnol. 2008; 3:583–584. [PubMed: 18838991]
5. Weickert J, Dunbar RB, Hesse HC, Wiedemann W, Schmidt-Mende L. Nanostructured organic and hybrid solar cells. Adv. Mater. 2011; 23:1810–1828. [PubMed: 21509826]
6. Zhang J, Song SP, Wang LH, Pan D, Fan CH. A gold nanoparticle-based chronocoulometric DNA sensor for amplified detection of DNA. Nat. Protocols. 2007; 2:2888–2895.
7. De M, Ghosh PS, Rotello VM. Applications of nanoparticles in biology. Adv. Mater. 2008; 20:4225–4241.
8. Nel AE, Madler L, Velegol D, Xia T, Hoek EMV, Somasundaran P, Klaessig F, Castranova V, Thompson M. Understanding biophysicochemical interactions at the nano-bio interface. Nat. Mater. 2009; 8:543–557. [PubMed: 19525947]
9. Saha K, Bajaj A, Duncan B, Rotello VM. Beauty is skin deep: a surface monolayer perspective on nanoparticle interactions with cells and biomacromolecules. Small. 2011; 7:1903–1918. [PubMed: 21671432]
10. Stark WJ. Nanoparticles in biological systems. Angew. Chem. Int. Ed. Engl. 2011; 50:1242–1258. [PubMed: 21290491]
11. Handy RD, von der Kammer F, Lead JR, Hassellov M, Owen R, Crane M. The ecotoxicology and chemistry of manufactured nanoparticles. Ecotoxicology. 2008; 17:287–314. [PubMed: 18351458]
12. Xia T, Li N, Nel AE. Potential health impact of nanoparticles. Annu. Rev. Public Health. 2009; 30:137–150. [PubMed: 19705557]
13. Handy RD, Owen R, Valsami-Jones E. The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs. Ecotoxicology. 2008; 17:315–325. [PubMed: 18408994]
14. Moyano DF, Rotello VM. Nano meets biology: Structure and function at the nanoparticle interface. Langmuir. 2011; 27:10376–10385. [PubMed: 21476507]
15. Li Y, Gu N. Thermodynamics of charged nanoparticle adsorption on charge-neutral membranes: a simulation study. J. Phys. Chem. B. 2010; 114:2749–2754. [PubMed: 20146444]
16. Mahmoudi M, Lynch I, Ejtehadi MR, Monopoli MP, Bombelli FB, Laurent S. Protein-nanoparticle interactions: opportunities and challenges. Chem. Rev. 2011; 111:5610–5637. [PubMed: 21688848]
17. Aggarwal P, Hall JB, McLeland CB, Dobrovolskaia MA, McNeil SE. Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy. Adv. Drug Deliv. Rev. 2009; 61:428–437. [PubMed: 19376175]
18. AshaRani PV, Low Kah Mun G, Hande MP, Valiyaveettil S. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano. 2009; 3:279–290. [PubMed: 19236062]
19. Teodoro JS, Simoes AM, Duarte FV, Rolo AP, Murdoch RC, Hussain SM, Palmeira CM. Assessment of the toxicity of silver nanoparticles in vitro: A mitochondrial perspective. Toxicol. in Vitro. 2011; 25:664–670. [PubMed: 21232593]
20. Celardo I, Pedersen JZ, Traversa E, Ghibelli L. Pharmacological potential of cerium oxide nanoparticles. Nanoscale. 2011; 3:1411–1420. [PubMed: 21369578]
21. Singh N, Manshian B, Jenkins GJS, Griffiths SM, Williams PM, Maffeis TGG, Wright CJ, Doak SH. NanoGenotoxicology: The DNA damaging potential of engineered nanomaterials. Biomaterials. 2009; 30:3891–3914. [PubMed: 19427031]
22. Park EJ, Park K. Oxidative stress and pro-inflammatory responses induced by silica nanoparticles in vivo and in vitro. Toxicol. Lett. 2009; 184:18–25. [PubMed: 19022359]

23. Dobrovolskaia MA, Germolec DR, Weaver JL. Evaluation of nanoparticle immunotoxicity. Nat. Nanotechnol. 2009; 4:411–414. [PubMed: 19581891]
24. Lasagna-Reeves C, Gonzalez-Romero D, Barria MA, Olmedo I, Clos A, Ramanujam VMS, Urayama A, Vergara L, Kogan MJ, Soto C. Bioaccumulation and toxicity of gold nanoparticles after repeated administration in mice. Biochem. Biophys. Res. Commun. 2010; 393:649–655. [PubMed: 20153731]
25. Kanaras AG, Kamounah FS, Schaumburg K, Kiely CJ, Brust M. Thioalkylated tetraethylene glycol: a new ligand for water soluble monolayer protected gold clusters. Chem. Commun. 2002:2294–2295.
26. Wang F, Wang YC, Dou S, Xiong MH, Sun TM, Wang J. Doxorubicin-Tethered Responsive Gold Nanoparticles Facilitate Intracellular Drug Delivery for Overcoming Multidrug Resistance in Cancer Cells. ACS Nano. 2011; 5:3679–3692. [PubMed: 21462992]
27. Miranda OR, Chen HT, You CC, Mortenson DE, Yang XC, Bunz UHF, Rotello VM. Enzyme-Amplified Array Sensing of Proteins in Solution and in Biofluids. J. Am. Chem. Soc. 2010; 132:5285–5289. [PubMed: 20329726]
28. Jing BX, Zhu YX. Disruption of supported lipid bilayers by semihydrophobic nanoparticles. J. Am. Chem. Soc. 2011; 133:10983–10989. [PubMed: 21631111]
29. Lin JQ, Zhang HW, Chen Z, Zheng YG. Penetration of lipid membranes by gold nanoparticles: Insights into cellular uptake, cytotoxicity, and their relationship. ACS Nano. 2010; 4:5421–5429. [PubMed: 20799717]
30. Goodman CM, McCusker CD, Yilmaz T, Rotello VM. Toxicity of gold nanoparticles functionalized with cationic and anionic side chains. Bioconjugate Chem. 2004; 15:897–900.
31. Leroueil PR, Berry SA, Duthie K, Han G, Rotello VM, McNerny DQ, Baker JR, Orr BG, Holl MMB. Wide varieties of cationic nanoparticles induce defects in supported lipid bilayers. Nano Lett. 2008; 8:420–424. [PubMed: 18217783]
32. Jing BX, Zhu YX. Disruption of supported Lipid bilayers by semihydrophobic nanoparticles. J. Am. Chem. Soc. 2011; 133:10983–10989. [PubMed: 21631111]
33. Ginzburg VV, Balijepailli S. Modeling the thermodynamics of the interaction of nanoparticles with cell membranes. Nano Lett. 2007; 7:3716–3722. [PubMed: 17983249]
34. Arvizo RR, Miranda OR, Thompson MA, Pabelick CM, Bhattacharya R, Robertson JD, Rotello VM, Prakash YS, Mukherjee P. Effect of nanoparticle surface charge at the plasma membrane and beyond. Nano Lett. 2010; 10:2543–2548. [PubMed: 20533851]
35. Monteith GR, McAndrew D, Faddy HM, Roberts-Thomson SJ. Calcium and cancer: targeting Ca^{2+} transport. Nat. Rev. Cancer. 2007; 7:519–530. [PubMed: 17585332]
36. McIntosh CM, Esposito EA, Boal AK, Simard JM, Martin CT, Rotello VM. Inhibition of DNA transcription using cationic mixed monolayer protected gold clusters. J. Am. Chem. Soc. 2001; 123:7626–7629. [PubMed: 11480984]
37. Han G, Chari NS, Verma A, Hong R, Martin CT, Rotello VM. Controlled recovery of the transcription of nanoparticle-bound DNA by intracellular concentrations of glutathione. Bioconjugate Chem. 2005; 16:1356–1359.
38. Kang B, Mackey MA, El-Sayed MA. Nuclear targeting of gold nanoparticles in cancer cells induces DNA damage, causing cytokinesis arrest and apoptosis. J. Am. Chem. Soc. 2010; 132:1517–1519. [PubMed: 20085324]
39. Liu YX, Li W, Lao F, Liu Y, Wang LM, Bai R, Zhao YL, Chen CY. Intracellular dynamics of cationic and anionic polystyrene nanoparticles without direct interaction with mitotic spindle and chromosomes. Biomaterials. 2011; 32:8291–8303. [PubMed: 21810539]
40. Schaeublin NM, Braydich-Stolle LK, Schrand AM, Miller JM, Hutchison J, Schlager JJ, Hussain SM. Surface charge of gold nanoparticles mediates mechanism of toxicity. Nanoscale. 2011; 3:410–420. [PubMed: 21229159]
41. Chompoosor A, Saha K, Ghosh PS, Macarthy DJ, Miranda OR, Zhu ZJ, Arcaro KF, Rotello VM. The role of surface functionality on acute cytotoxicity, ROS generation and DNA damage by cationic gold nanoparticles. Small. 2010; 6:2246–2249. [PubMed: 20818619]

42. Scherz-Shouval R, Shvets E, Fass E, Shorer H, Gil L, Elazar Z. Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. Embo J. 2007; 26:1749–1760. [PubMed: 17347651]
43. Lynch I, Salvati A, Dawson KA. Protein-nanoparticle interactions what does the cell see? Nat. Nanotechnol. 2009; 4:546–547. [PubMed: 19734922]
44. Verma A, Rotello VM. Surface recognition of biomacromolecules using nanoparticle receptors. Chem. Commun. 2005; 3:303–312.
45. Fischer NO, McIntosh CM, Simard JM, Rotello VM. Inhibition of chymotrypsin through surface binding using nanoparticle-based receptors. Proc. Natl. Acad. Sci. U.S.A. 2002; 99:5018–5023. [PubMed: 11929986]
46. Fischer NO, Verma A, Goodman CM, Simard JM, Rotello VM. Reversible "irreversible" inhibition of chymotrypsin using nanoparticle receptors. J. Am. Chem. Soc. 2003; 125:13387–13391. [PubMed: 14583034]
47. You CC, De M, Han G, Rotello VM. Tunable inhibition and denaturation of alpha-chymotrypsin with amino acid-functionalized gold nanoparticles. J. Am. Chem. Soc. 2005; 127:12873–12881. [PubMed: 16159281]
48. You CC, De M, Rotello VM. Contrasting effects of exterior and interior hydrophobic moieties in the complexation of amino acid functionalized gold clusters with alpha-chymotrypsin. Org. Lett. 2005; 7:5685–5688. [PubMed: 16321022]
49. Hong R, Fischer NO, Verma A, Goodman CM, Emrick T, Rotello VM. Control of protein structure and function through surface recognition by tailored nanoparticle scaffolds. J. Am. Chem. Soc. 2004; 126:739–743. [PubMed: 14733547]
50. Aubin-Tam ME, Hamad-Schifferli K. Gold nanoparticle cytochrome c complexes: The effect of nanoparticle ligand charge on protein structure. Langmuir. 2005; 21:12080–12084. [PubMed: 16342975]
51. Min L, Jian-xing X. Detoxifying function of cytochrome c against oxygen toxicity. Mitochondrion. 2007; 7:13–16. [PubMed: 17276741]
52. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of aging. Nature. 2000; 408:239–247. [PubMed: 11089981]
53. Shang W, Nuffer JH, Dordick JS, Siegel RW. Unfolding of ribonuclease A on silica nanoparticle surfaces. Nano Lett. 2007; 7:1991–1995. [PubMed: 17559285]
54. Ishii D, Kinbara K, Ishida Y, Ishii N, Okochi M, Yohda M, Aida T. Chaperonin-mediated stabilization and ATP-triggered release of semiconductor nanoparticles. Nature. 2003; 423:628–632. [PubMed: 12789335]
55. Wagner SC, Roskamp M, Pallerla M, Araghi RR, Schlecht S, Koksch B. Nanoparticle-induced folding and fibril formation of coiled-coil-based model peptides. Small. 2010; 6:1321–1328. [PubMed: 20517875]
56. Zolnik BS, Gonzalez-Fernandez A, Sadrieh N, Dobrovolskaia MA. Minireview: Nanoparticles and the Immune System. Endocrinology. 2010; 151:458–465. [PubMed: 20016026]
57. Chang C. The immune effects of naturally occurring and synthetic nanoparticles. J. Autoimmun. 2010; 34:J234–J246. [PubMed: 19995678]
58. Oh WK, Kim S, Choi M, Kim C, Jeong YS, Cho BR, Hahn JS, Jang J. Cellular Uptake, Cytotoxicity, and Innate Immune Response of Silica - Titania Hollow Nanoparticles Based on Size and Surface Functionality. ACS Nano. 2010; 4:5301–5313. [PubMed: 20698555]
59. Kedmi R, Ben-Arie N, Peer D. The systemic toxicity of positively charged lipid nanoparticles and the role of Toll-like receptor 4 in immune activation. Biomaterials. 2010; 31:6867–6875. [PubMed: 20541799]
60. Moyano DF, Goldsmith M, Solfiell DJ, Landesman-Milo D, Miranda OR, Peer D, Rotello VM. Nanoparticle hydrophobicity dictates immune response. J. Am. Chem. Soc. 2012
61. Deng ZJ, Liang MT, Monteiro M, Toth I, Minchin RF. Nanoparticle-induced unfolding of fibrinogen promotes Mac-1 receptor activation and inflammation. Nat. Nanotechnol. 2011; 6:39–44. [PubMed: 21170037]

62. Dobrovolskaia MA, Aggarwal P, Hall JB, McNeil SE. Preclinical studies to understand nanoparticle interaction with the immune system and its potential effects on nanoparticle biodistribution. Mol. Pharm. 2008; 5:487–495. [PubMed: 18510338]

63. Joshi P, Chakraborty S, Dey S, Shanker V, Ansari ZA, Singh SP, Chakrabarti P. Binding of chloroquine-conjugated gold nanoparticles with bovine serum albumin. J. Colloid Interface Sci. 2011; 355:402–409. [PubMed: 21216410]

64. Zhu ZJ, Carboni R, Quercio MJ, Yan B, Miranda OR, Anderton DL, Arcaro KF, Rotello VM, Vachet RW. Surface properties dictate uptake, distribution, excretion, and toxicity of nanoparticles in Fish. Small. 2010; 6:2261–2265. [PubMed: 20842664]

65. Arvizo RR, Miranda OR, Moyano DF, Walden CA, Giri K, Bhattacharya R, Robertson JD, Rotello VM, Reid JM, Mukherjee P. Modulating pharmacokinetics, tumor uptake and biodistribution by engineered nanoparticles. PLos One. 2011; 6:e24374. [PubMed: 21931696]

66. Agasti SS, Chompoosor A, You CC, Ghosh P, Kim CK, Rotello VM. Photoregulated release of caged anticancer drugs from gold nanoparticles. J. Am. Chem. Soc. 2009; 131:5728–5729. [PubMed: 19351115]

67. Kim CK, Ghosh P, Pagliuca C, Zhu ZJ, Menichetti S, Rotello VM. Entrapment of hydrophobic drugs in nanoparticle monolayers with efficient release into cancer cells. J. Am. Chem. Soc. 2009; 131:1360–1361. [PubMed: 19133720]

68. Kim B, Han G, Toley BJ, Kim CK, Rotello VM, Forbes NS. Tuning payload delivery in tumour cylindroids using gold nanoparticles. Nat. Nanotechnol. 2010; 5:465–472. [PubMed: 20383126]

69. Peer D, Karp JM, Hong S, FaroKHzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. Nat. Nanotechnol. 2007; 2:751–760. [PubMed: 18654426]

70. Petros RA, DeSimone JM. Strategies in the design of nanoparticles for therapeutic applications. Nat. Rev. Drug Discov. 2010; 9:615–627. [PubMed: 20616808]

71. Ferrari M. "Nanogeometry: beyond drug delivery". Nat. Nanotechnol. 2008; 3:131–132. [PubMed: 18654480]

72. Kim C, Agasti SS, Zhu ZJ, Isaacs L, Rotello VM. Recognition-mediated activation of therapeutic gold nanoparticles inside living cells. Nat. Chem. 2010; 2:962–966. [PubMed: 20966953]

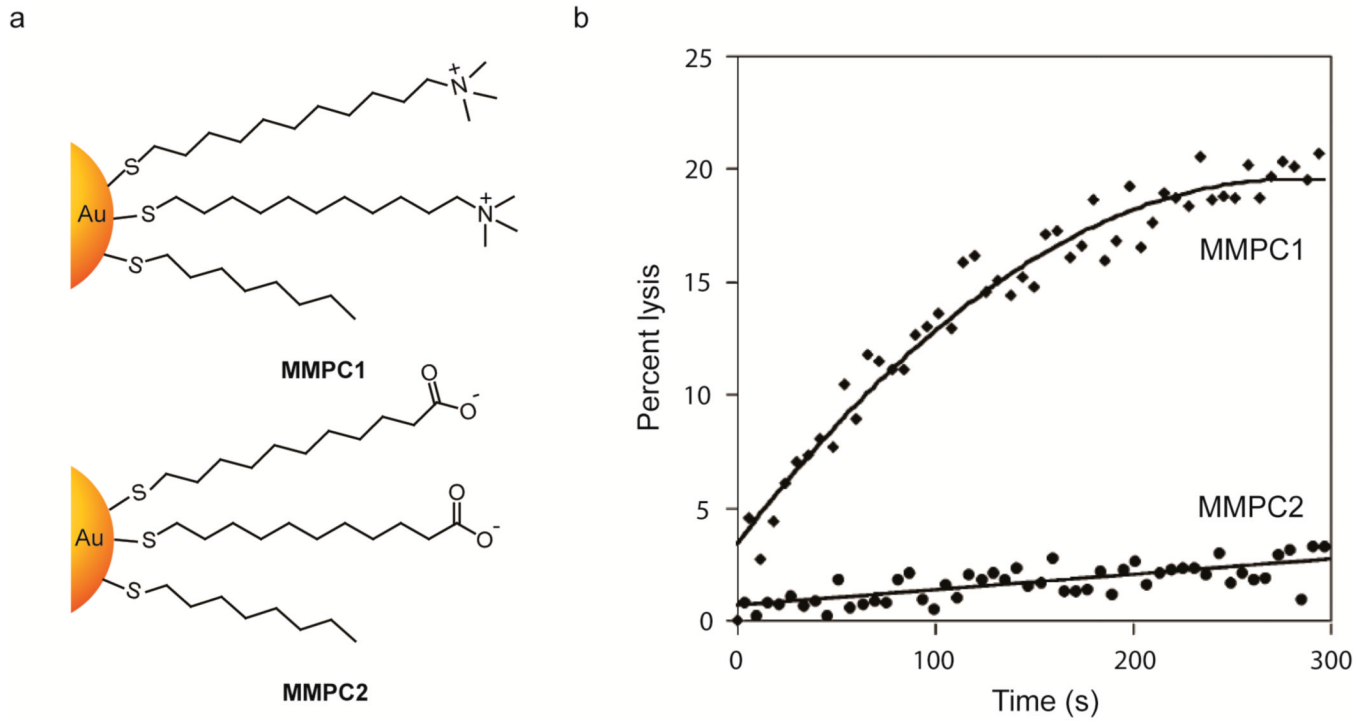


Figure 1.

Effect of functionalized NPs on the disruption of lipid bilayers. (a) Surface functionalized MMPC1 and MMPC2 and (b) Comparison of cationic MMPC1 and anionic MMPC2 (220 nM) in disrupting vesicles with an overall negative charge (SOPC/SOPC, L-R-stearoyloleoyl-phosphatidylcholine/L-R-stearoyl-oleoyl-phosphatidylserine). Reprinted with permission from *ref.*30. Copyright 2004 American Chemical Society.

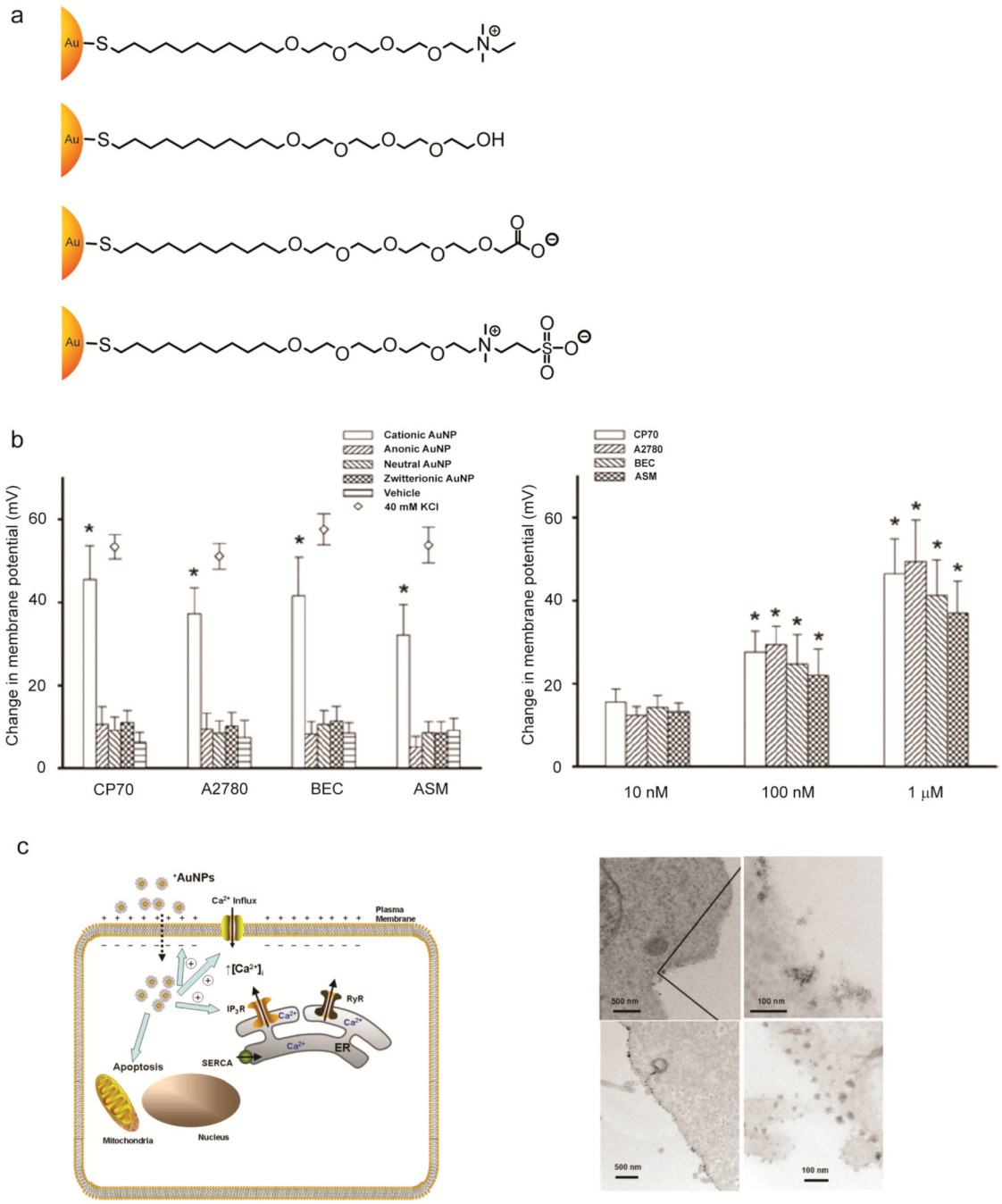


Figure 2.

Effect of gold NPs with different surface charges on cellular membrane potential. (a) Cationic, anionic, neutral, and zwitterionic NPs. (b) Membrane potential changes following the exposure to NPs for ovarian cancer cells (CP70 and A2780), human bronchial epithelial cells (BEC), and human airway smooth muscle cells (ASM) using cell permeable fluorescent membrane potential indicator RH414 and real-time fluorescence microscopy. In addition, the extent of membrane potential change was analyzed in a cationic NP concentration dependent manner. (*$p < 0.05$) (c) Scheme of NP effects on cell and TEM of cationic NP interactions with plasma membrane. Reprinted with permission from *ref.*34. Copyright 2010 American Chemical Society.

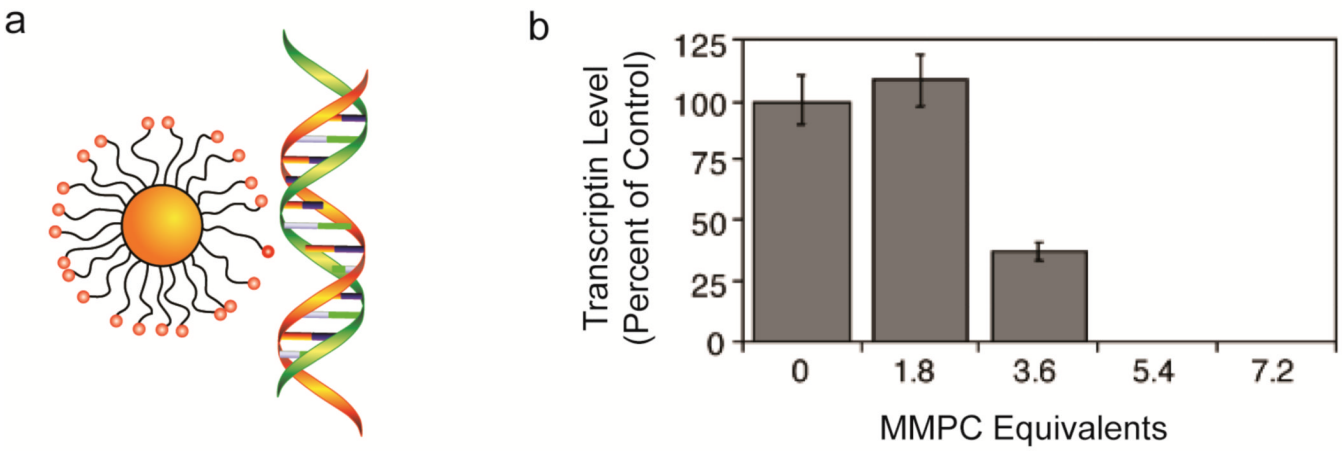


Figure 3.

Interaction between positively charged NPs and DNA. (a) Mixed monolayer protected gold clusters (MMPCs) and double stranded DNA (37mer). (b) The amount of RNA detected relative to levels produced in the absence of MMPCs. Reprinted with permission from *ref.* 36. Copyright 2001, American Chemical Society.

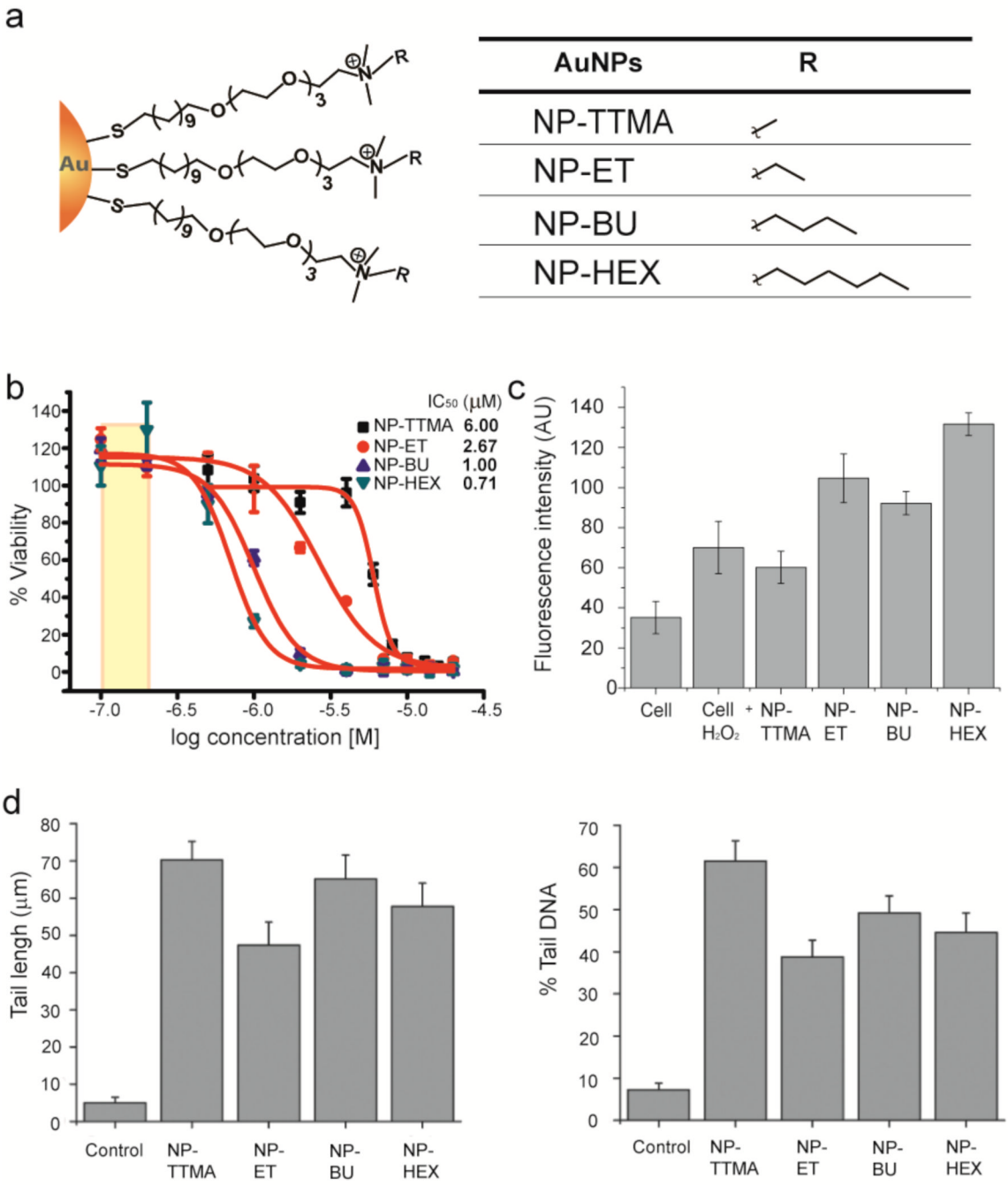


Figure 4.

Cytotoxicity and genotoxicity of gold NPs with different hydrophobicities. (a) Gold NPs (e.g. NP-TTMA, -ET, -BU and -HEX) (b) IC_{50} values of these NPs were determined by alamarBlue® assay. (c) ROS was quantitatively determined by the oxidation of 2′,7′-dichlorodihydrofluorescein diacetate dye. (d) Tail length and % Tail DNA were measured by the Comet assay. Reprinted with permission from *ref.*41. Copyright 2010 Wiley-VCH Verlag & Co. KGaA.

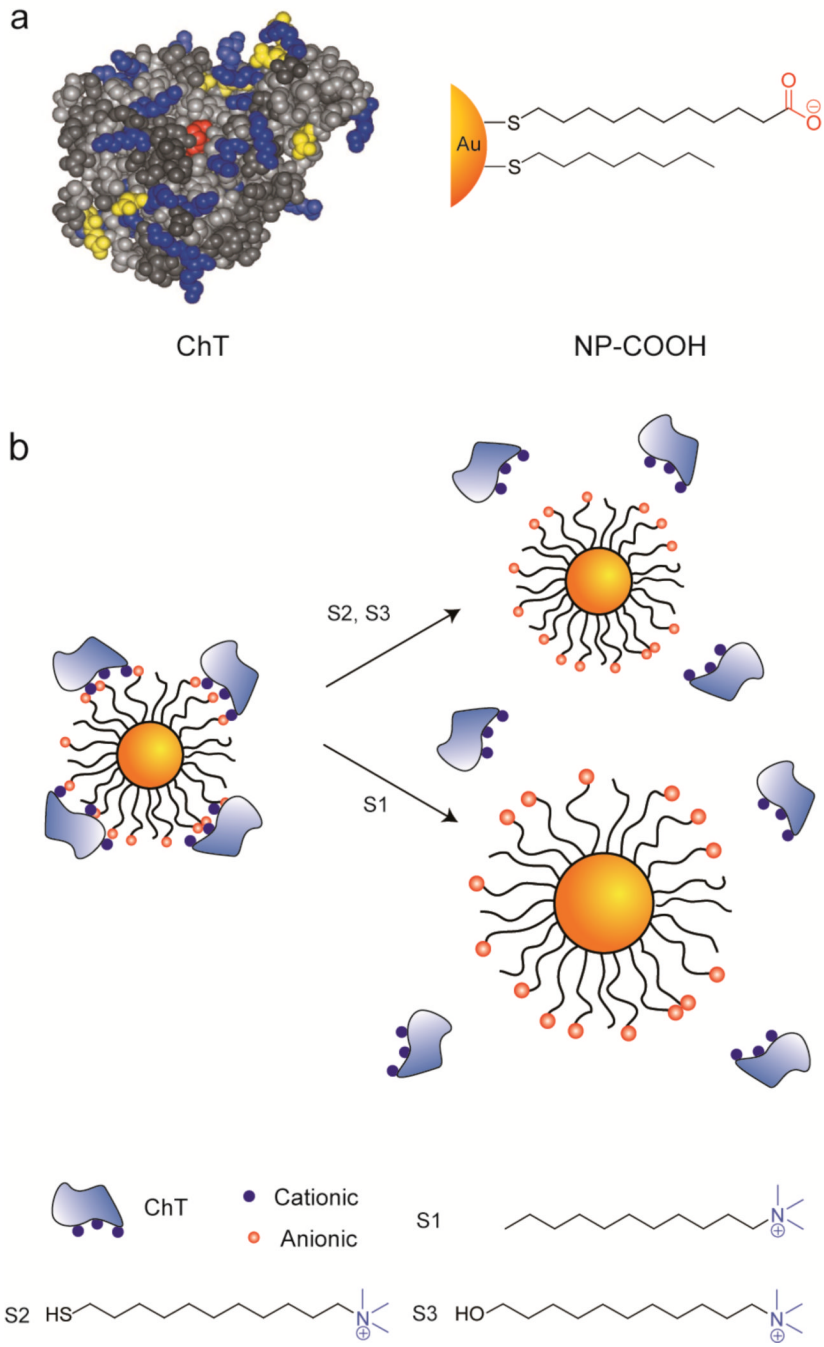


Figure 5.
Reversible/irreversible interaction of ChT with surface functionality of gold NP. (a) Space-filling model of ChT and structure of anionic MUA-gold NP (MUA-NP) (b) ChT released from the surface of NP by the addition of different trimethylamine-functionalized surfactants (S1, S2 and S3). Reprinted with permission from *ref.*46. Copyright 2003, American Chemical Society.

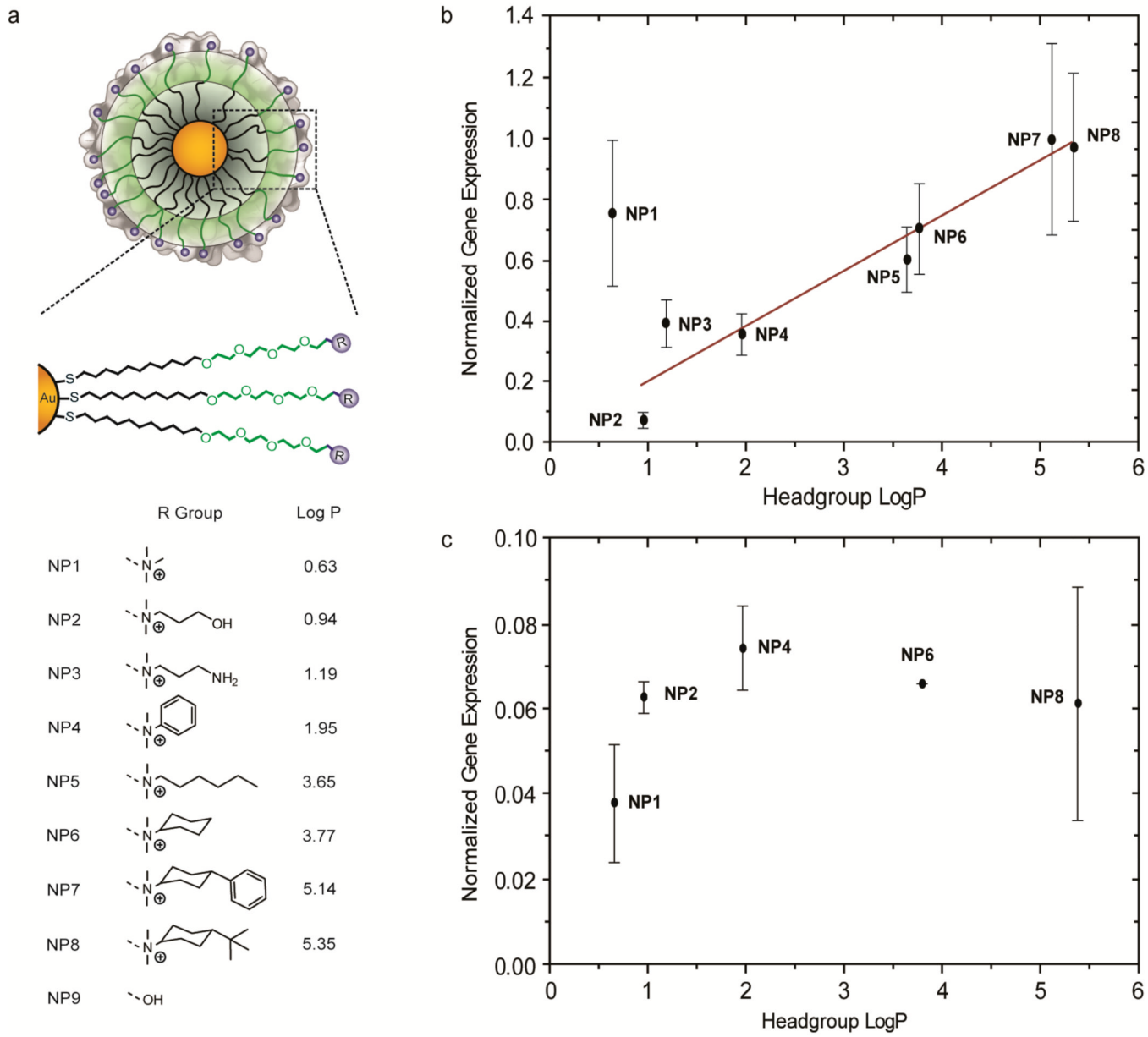


Figure 6.

Effect of NP surface hydrophobicity on gene expression related to immune response. (a) Surface functionalized gold NPs controlling the surface hydrophobicity and cytokine gene expression of (b) TNF-α *in vitro* and (c) IL-10 *in vivo* as function of NP headgroup LogP. LogP represents the calculated hydrophobic values of the head group. Reprinted with permission from *ref.*60. Copyright 2012, American Chemical Society.

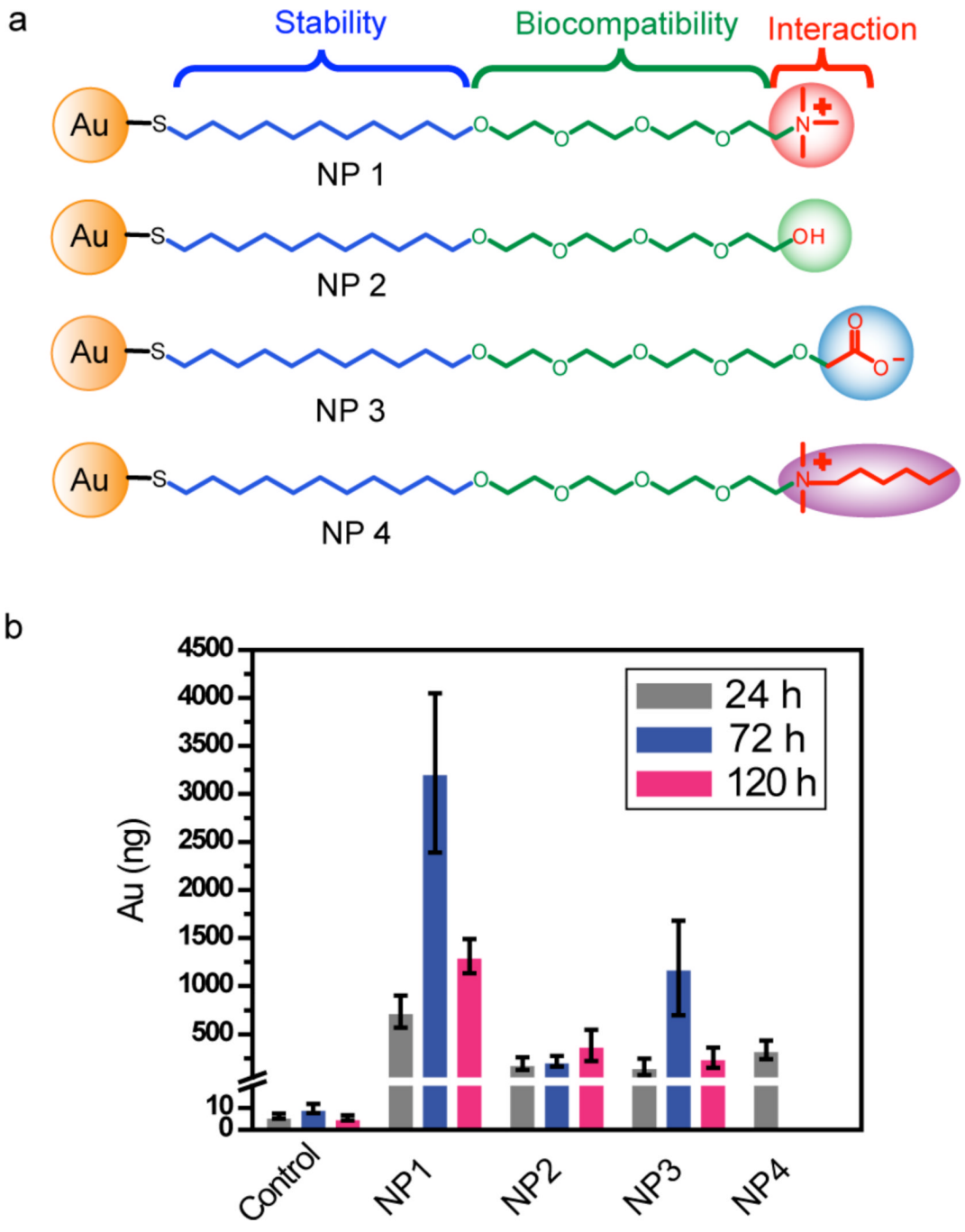


Figure 7.

Effect of NPs with different functionalities on total accumulation of gold in Japanese medaka fish. (a) Gold NPs with different functionalities and (b) total amount of gold detected in fish after exposure of 20 nM gold NP concentrations. The gold amounts are the sum total of gold found in various organs (brain, heart, liver, gonads, gills, intestines and dorsal fin) and on appendage. Reprinted with permission from *ref.*64. Copyrights 2010 Wiley-VCH Verlag & Co. KGaA.

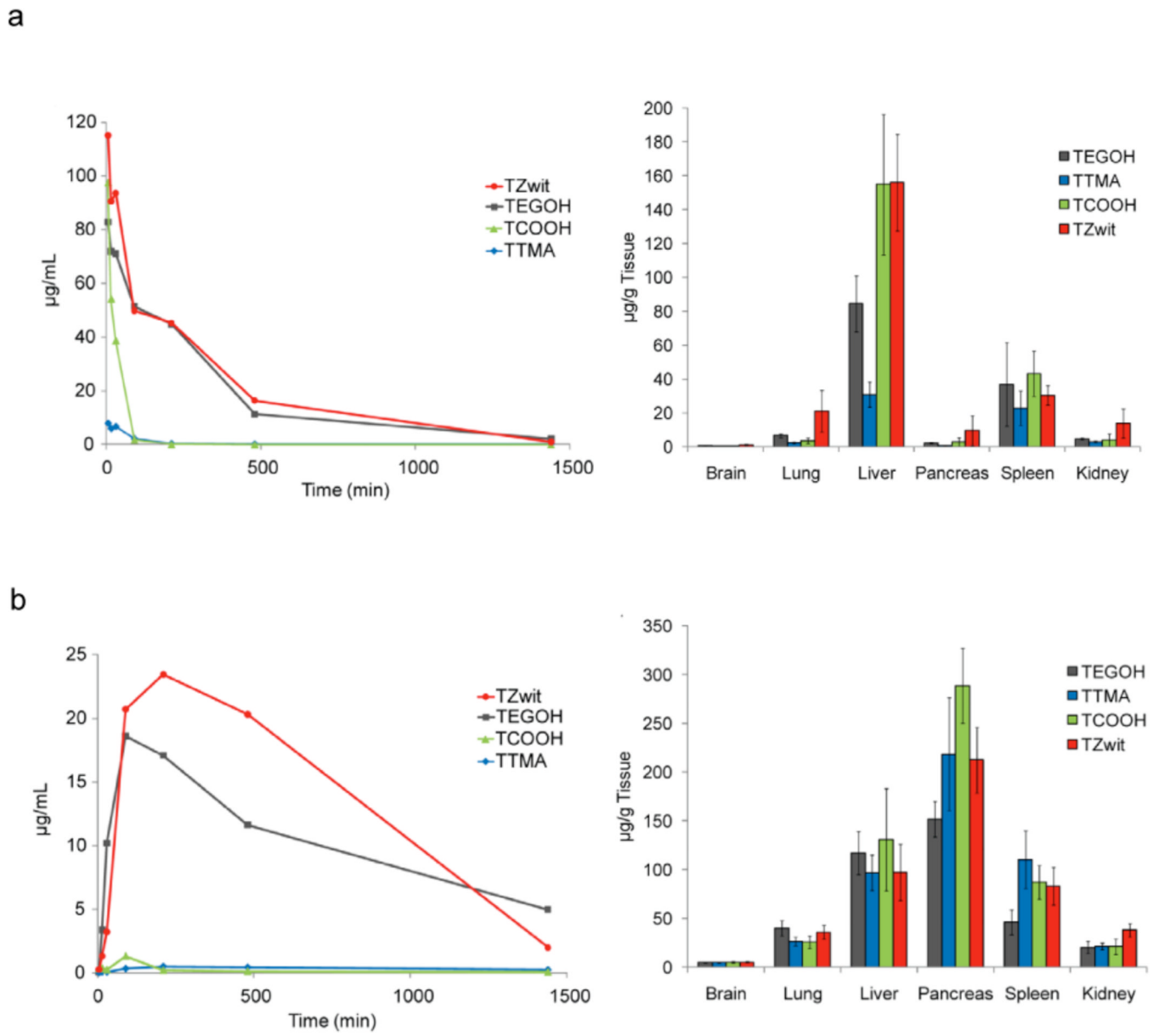


Figure 8.

Pharmacokinetic profiles (left panel) and biodistribution (right panel) of gold NPs *in vivo*. Four kinds of NPs (TEGOH, TTMA, TCOOH and TZwit) were administered into mice via (a) intravenous or (b) intraperitoneal route. Pharmacokinetic studies were performed for 1 day in normal male CD1 mice and organs were collected 1 day after administrations from ovarian cancer cell (CP-70) transplanted HEJ/C3H mice. Adapted from *ref.*65.

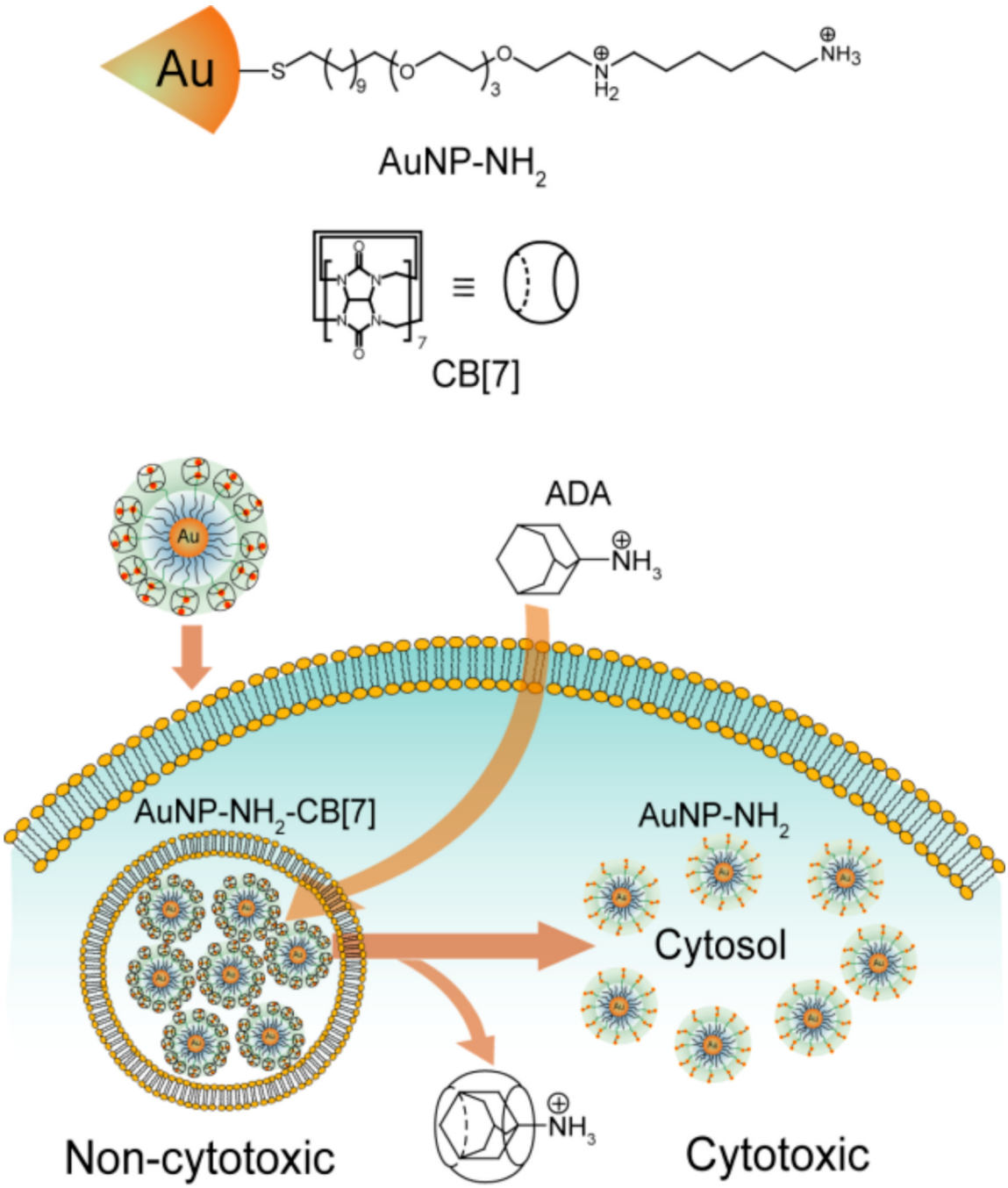


Figure 9.

Schematic description of the use of intracellular host–guest complexation to trigger gold nanoparticle cytotoxicity. Cytotoxicity of gold NP-NH_2-CB[7] is activated by the dethreading of CB[7] from the nanoparticle surface by ADA. Reprinted with permission from *ref.*72. Copyright 2010 Nature Publishing Group.