Lecture 5. Functionalization of Metal, Semiconductor, or Quantum Dot Nanoparticles

The purpose of the lecture: to familiarize students with functionalization of metal, semiconductor, or quantum dot nanoparticles.

Expected results: students getting information about functionalization of metal, semiconductor, or quantum dot nanoparticles.

FUNCTIONALIZATION FOR IMPROVED DISPERSION AND DISSOLUTION

Bare metal nanoparticles agglomerate rapidly, possibly due to attractive van der Waals (VDW) forces, low zeta (z) potential, and/or the pH (close to the nanoparticles' isoelectric points for "zero charge") of the dispersion liquid. The z values greater than ю25 mV or less than _25 mV typically indicate a stable colloid because of charge repulsion between two nanoparticles. An increase in z values stabilizes the nanoparticles, resulting in greater dispersion. One of the procedures commonly used to stabilize colloidal nanoparticles is coupling the surface with a thiol group either during or after synthesis. Thiols form a noncovalent but stable bond with gold nanoparticles.

Studies have shown that mono-thiol ligands form relatively weak bonds with gold nanoparticles, especially at high temperatures, in the presence of competing thiols or oxidizing agents and in solvents where the ligand is extremely well solvated. A multivalent interaction between ligands with multiple thiol groups and the surface of gold nanoparticles may prevent ligand dissociation under extreme conditions. The coupling of the nanoparticle surface with an organic layer consisting of poly-L-lysine (PLL), poly-L-glycone (PLG), or polyethylene glycol (PEG) reduces the surface reactivity and improves dispersion in solution. Functionalized nanoparticles exhibit high bioavailability, evade the immune system, selectively release active ingredients on-site, and perform special functions such as biological switches. PEG, being water soluble and nontoxic, has an advantage over PLL,which exhibits high cytotoxicity, and PLG, which exhibits poor water solubility. PEG functionalization (PEGylation) stabilizes the nanoparticles by adjusting their hydrophilic and hydrophobic properties and increasing their zeta potential and steric hindrance. PEGfunctionalized nanoparticles, due to their ability to bind the cell membranes, can serve as excellent drug carriers. PEGs coupled with biomolecules such as lectin, lactose, and biotin have been used in cellular and intracellular targeting of biological materials. The stability and internalization of PEGylated nanoparticles may be affected by factors such as the molecular weight of PEG, the attached functional groups, the ligand, and the size of the nanoparticles used. In general, PEGylation has following advantages:

• High-molecular-weight PEGs may remain in the blood circulation for a longer period than low-molecular-weight PEGs (t1/2 increased from 18 min to 1 day as the PEGs' molecular weight increased from 6000 to 190,000).

• PEGylated nanoparticles accumulate in the tissues/organs, such as muscle, skin, bone, and the liver, irrespective of the molecular weight.

• Small PEG, but not large PEG, tended to freely translocate from the circulation to extravascular tissues and vice versa.

• Urinary clearance decreased with increasing PEG molecular weight. Liver clearance increased with the increasing PEG molecular weight. PEG uptake by Kupffer cells was enhanced as the molecular weight became greater than 50,000.

• Attachment of large PEG moieties often reduces activity of the drug, and higher concentrations of the conjugate are necessary to achieve the required biological activity. Permanent PEGylation is generally not applicable to small-molecule drugs because the bulky carrier usually prevents their binding to targets and cell penetration.

The beneficial effects of PEG (e[CH2eCH2eOe]ne) are attributed to its unusual structure and properties. PEG is water soluble but also exhibits hydrophobic properties. Poly-(butylene glycol), e[CH2eCH2eCH2eOe]ne, having one more methylene (CH2) group and poly(methylene

oxide), e[CH2eOe]ne, which has one less methyl group, are both hydrophobic and insoluble in water. Thus, there is something unique about PEG's eCH2eCH2eOe unit. Possibly, the ethyl group yields sufficient hydrophobic properties to this hydrophilic molecule. PEG forms thin monolayers at the airewater interface that have distinct hydrophilic and hydrophobic regions. Water molecules bind to the ethylene oxide group via H-bonds to the eOe group of polyethylene oxide. Another unique property of PEG is that its solubility in water depends on its molecular weight and, to a lesser extent, on the concentration. This is one of the least understood aspects of PEG's properties.

While low-molecular-weight PEG generally induces cells or vesicles to adhere, highmolecularweight PEG causes them to repel. Bare metal nanoparticles, in addition to undergoing aggregation, also bind to biological proteins, especially plasma proteins. Protein adsorption on nanoparticles involves bond formation between proteins and surfaces, lateral diffusion on the surface, and conformational changes or rearrangements of adsorbed proteins. Driving forces for protein adsorption are hydrophobic interaction, electrostatic attraction, VDW, and hydrogen bonding. Aggregation and protein binding both facilitate their binding and subsequent engulfment into the macrophages, and ensuing nanoparticles' hepatic clearance. This results in considerably lower residence time of bare nanoparticles in systemic blood, resulting in lower bioavailability. PEGs block the binding of proteins onto the nanoparticles' surface by forming hydrogen bonds with water molecules, thus generating large repulsive forces. Blocking the adsorption of proteins on to nanoparticles also blocks their phagocytosis by macrophages, resulting in an increase in their circulation time. PEG chain length, conformation, and number density on the surface are important factors for resisting protein adsorption. However, the hydrophobic sites in the PEG may produce attractive forces on proteins.

Macrophages are one of the first responders against foreign particles including the nanoparticles (others being bacteria, toxins, etc.). Studies have shown that intratumoral complement activation increases the concentration of complements 3 and 5 (C3 and C5, respectively) that may participate in cancer pathogenesis. Therefore, nanoparticle PEGylation may, in addition to suppressing phagocytosis, also suppress the complement concentrations, thus enhancing the potency of anticancer drugs. Contrary to the above observations, several studies have shown that the steric hindrance of the PEG chains (or other nonionic surfactants) on nanoparticles could not prevent activation of the complements. More research may be needed to resolve this issue of the beneficial and harmful effects of PEGylated nanoparticles.

FUNCTIONALIZATION OF PEG FOR MEDICINAL/SCREENING APPLICATIONS

PEGylated metal nanoparticles, for performing specific functions, require specific end groups (eNH2, eCOOH, eOH, eN3 and/or eSH) that interact, via either covalent or noncovalent bonding, with drugs, imaging dyes, or antibodies of specific proteins. For metal nanoparticles, PEGs bi-functionalized with eSH groups at one end and eNH2, eCOOH, eOH or eSH groups at the other can be synthesized.

ACID OR ENZYME CLEAVABLE LINKERS

Acid cleavable linkers remain stable at physiological pH but disintegrate at pH less than 6.0. The enzyme-cleavable linkers contain an enzyme-selective substrate as a linker that is hydrolyzed by the enzyme. For cancer treatment, the cathepsin B substrates, such as Gly-Phe-Leu-Gly (GFLG), are used as the linker. Because cathepsin B is highly overexpressed in cancer cells, the cathepsin B substrate-linkers are disintegrated, resulting in an onsite release of drugs and/or dyes. An important advantage of the acid-cleavable linker is that the released drug has full activity and is unencumbered by the bulky macromolecular carrier. Studies have shown that the plasma and the extracellular fluid of normal tissues/cells have pHof around 7.4,while normal lysosomes and the extracellular fluid of cancer cells have pH of around <6. The pH-sensitive probes remain intact at normal physiological pH but disintegrate at acidic pH, thus allowing onsite release of the drugs. One of the commonly used acid-cleavable linkers is hydrazine linker. This approach allows the macromolecular carrier. Studies have shown that the plasma and the extracellular fluid of normal

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METAL NANOPARTICLES FUNCTIONALIZED WITH TUNABLE SWITCHES

Traditionally, metal nanoparticles are functionalized using thiolated ligands in a reducing environment. This procedure, although effective, may not be compatible with a wide range of intelligent functional groups, such as tunable switches, including a bifunctional stable electron donoreacceptor functionalization. Klajn et al. (2010a,b) devised novel functionalization of nanoparticles in which multiple components are not chemically bonded but cannot dissociate because of their topological linkage.

There is much freedom of mechanical movement in the catenane rings to produce various co-conformers. These functional groups can play a key role in acting as switches to detect oxidative stress or other disorders. Klajn et al. (2009a,b,c) functionalized metal (Au, Pt, Pd) nanoparticles using weakly protected "precursors" and dithiolanes into a redox-active mechanically interlocked molecules that retained their "switching" activity when attached to the nanoparticles. The oxidation potentials of the switches can be modulated by the properties of the metal nanoparticle surfaces. Many studies have reported DNA catenane systems with potential use in DNA topological labeling (Hua et al., 2013; Johann et al., 2013). To synthesize single-strand DNA catenanes, two DNA strands are synthesized and then cyclized using either enzymatic template-directed ligations or photocrosslinking.

Sannones and Sugiyama (2010, 2012) have developed a G-quadruplex method for the cyclization to avoid the introduction of modified bases. The DNA G-quadruplex is of great interest because of its roles in key biological processes, such as the maintenance of telomeres and regulation of gene transcription.

NONCOVALENT FUNCTIONALIZATION

Functional groups bind to nanoparticles via covalent bonds (shared electrons, single bondesigma, double bondseone sigma one p, triple bondeone sigma and two p) and relatively weak noncovalent interactions arising due to formation of hydrogen bonds, ionic interactions, and VDW interactions, such as London dispersion, dipole-related interactions, and p-electronmediated interactions.