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Renal Clearable Noble Metal Nanoparticles: Photoluminescence, Elimination and Biomedical Applications

Jing Xu¹, Chuanqi Peng¹, Mengxiao Yu¹, and Jie Zheng¹

¹Department of Chemistry and Biochemistry, The University of Texas at Dallas, Richardson, TX 75080, USA

Abstract

Metal nanoparticles have demonstrated broad and promising biomedical applications in research laboratories, but how to fulfil their promises in the clinical practices demands extensive effort to minimize their nonspecific accumulation in the body. In the past six years, we have developed a class of renal clearable noble metal nanoparticles with tunable visible and near-infrared emission, which can behave like small molecular contrast agents to be effectively eliminated through the kidneys. By taking advantage of the unique clearance pathway, we were able to gain some fundamental understanding of how engineering nanoparticles cleared out of the body through urinary system. Moreover, they also provided unique opportunities in early cancer detection and kidney functional imaging that were often challenging to be achieved with non-renal clearable nanoparticles and small molecular probes. In this review, we summarize key factors that govern in the renal clearance of luminescent noble metal nanoparticles and their strengths in cancer targeting and kidney functional imaging. At the end, we also outline several key challenges that need to be addressed before they can be considered in the clinical practices.

Graphical abstract

Correspondence to: Jie Zheng.

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Introduction

Noble metal nanoparticles (NPs), especially gold and silver NPs, have found diverse functionalities and broad applications in preclinical cancer detection and treatment.^{1–5} For instance, gold NPs (AuNPs) have strong and tunable optical absorption due to surface plasmon resonance, which can serve as contrast agents for photoacoustic mapping of cancer site;^{6, 7} In addition, plasmonic AuNPs can effectively convert light into heat with an efficiency of 4–5 orders of magnitude higher than Rhodamine 6G dye, hold great promise in photothermal therapy.^{8–10} By taking advantage of surface enhanced Raman scattering or integrating of fluorescence modality, AuNPs and silver NPs (AgNPs) exhibited great potential as contrast agents for imaging-guided surgery with improved tumor accumulation compared to organic dyes.^{11–13} While these preclinical studies have show great potential of noble metal NPs in the disease diagnosis and therapy, severe uptake of these nanoparticles by mononuclear phagocyte system (MPS, including liver, spleen, bone marrow, etc.) has been hampering their clinical translation. Significant accumulation of nanoparticles in liver and extremely slow hepatic excretion raises serious concern on their long-term health hazard. Not limited to metal nanoparticles, many other inorganic engineering NPs also experience the same challenge in their clinical translation.^{14–16} To address this common challenge, many groups have been working on expediting elimination of engineering NPs from the body by altering their clearance pathways. After nearly one decade, a variety of renal clearable engineering nanoparticles have been developed,^{17–19} which have been thoroughly discussed in many review papers. In this review, we will only focus on ultrasmall renal clearable luminescent noble metal nanoparticles that our group have been developing in the past six years. We will briefly discuss synthetic strategy and photoluminescence mechanism of ultrasmall luminescent noble metal NPs. Some fundamental understanding of effects of particle structural properties such as size, surface chemistry and density, on some *in vivo* behaviours of these renal clearable NPs will be presented. In addition, their applications on early cancer detection, kidney functional imaging and labelling will also be discussed.

PHOTOLUMINESCENCE MECHANISM

In the past 15 years, we have been focused on ultrasmall luminescent metal nanoparticles, which can give tunable and intrinsic luminescence without conjugation of organic dyes. These luminescent metal nanoparticles are different from conventional non-luminescent metal nanoparticles, which are not luminescent due to their large density of states. We developed a variety of ultrasmall noble metal nanoparticles with intrinsic emissions by tuning their particle size, surface chemistry, valence states as well as crystallinity.²⁰ Among all of the known luminescent metal nanoparticles, AuNPs of few-nm sizes with thiolated coating are particularly interesting because their unique emission is size-independent but can be tuned by altering the coverage density and conformation of surface coating ligands. Since these luminescent thiolated gold nanoparticles of few nanometers are a major nanoprobe we used to explore nano-bio interactions *in vivo*, we will discuss their synthesis, characterizations and photoluminescence mechanisms in details as follows.

Synthetic Strategy

These ultrasmall gold nanoparticles with zwitterionic glutathione coating (GS-AuNPs) have been found to luminesce either in visible (emission center at 600 nm) or near-infrared (NIR, emission center at 810 nm) range.^{21, 22} The synthesis of luminescent GS-AuNPs with different emissions is based on the reduction of chloroauric acid (HAuCl_4) by glutathione (GSH, a tri-peptide existing in cytoplasm abundantly with weak reducing ability) with different stoichiometry and temperature. The gradual dissociation process of polymeric gold thiolates ($[\text{Au-SR}]$) (Figure 1) takes place during the reaction instead of instant Au-S bond breakage and reduction by strong reducing agents (as NaBH_4), which is distinct from many non-luminescent AuNPs.^{23, 24} Not limited to glutathione, this synthetic strategy was applied well to other thiol ligands, for instance, amphiphilic ligands as poly(ethylene glycol) thiol (PEG-SH), other thiol ligands like cysteamine, etc. The dual-ligand surface coating has also been achieved to prepare luminescent glutathione/cysteamine-coated gold nanoparticles (GC-AuNPs), which have pH-dependent interaction with live cells.^{25, 26} The synthetic method also can be applied to preparation of luminescent glutathione-coated silver and copper NPs (GS-AgNPs, GS-CuNPs) with minor modification in reaction conditions.^{27, 28}

Size-Independent Emission of Thiolated Metal Nanoparticles

Both 600 nm-emitting and 810 nm-emitting GS-AuNPs exhibit very large Stokes shifts due to the charge transfer between surface gold atoms and thiol group of surface ligands on the AuNPs. X-ray photoelectron spectroscopic studies show that a number of gold atoms in the luminescent AuNPs are in their Au^{I} state, distinct from valence states of gold atoms (in Au^0 state) of fully reduced non-luminescent AuNPs. Because of this difference in valence states of gold atoms and strong charge-transfer between surface gold atom and sulphur atom of the ligands, quantized single-electron transitions become available, which results in the discrete excitation and emissions from these few-nm gold nanoparticles. Interestingly, unlike the observation from quantum dots, the emission of luminescent AuNPs is size-independent. By investigating the structure-emission relationship in the same sized GS-AuNPs but with 600 nm and 810 nm emissions, we have found that the density and conformation of surface ligands on gold nanoparticles play a much more important role in emission wavelength (Figure 2). For example, the amount of glutathione ligands on 600 nm-emitting AuNPs are nearly 2 times higher than that on 810 nm-emitting GS-AuNPs. As a result, the Au-S coordination number in 600 nm-emitting GS-AuNPs is significantly higher than that in 810 nm-emitting GS-AuNPs: higher degree of Au-S bonding induces the 600 nm emission center with stronger Au-S charge transfer and the lower degree of Au-S bonding leads to 810 nm emission center with weaker Au-S charge transfer.²²

RENAL CLEARANCE AND PARTICLE STRUCTURAL FACTORS

The administration of ultrasmall noble metal NPs results in a rapid elimination of these NPs through the renal system with minimized accumulation in the normal tissues and organs, which is distinct from non-renal clearable inorganic NPs of large sizes. The hepatic excretion of non-renal clearable nanoparticles is an extremely slow and inefficient process that potentially induces long-term toxicity concerns. On the other hand, the kidney serves as a more efficient filter for the removal of any foreign materials; thus fundamental

understanding of how nanomaterials are eliminated through the urinary system is critical to expediting their clinical translation.

Kidney Filtration and Excretion

Kidney is a major organ to rapidly remove excessive small molecules and waste products of metabolism efficiently through urinary system. The elimination process takes place in its basic structural and functional unit, nephron, a spherical epithelium structure enveloping a capillary tuft, where blood filtration and urine excretion are completed. The wall of tuft is primary filtration barrier, consisting of endothelium layer with fenestrae (70–90 nm), outer foot processes (podocytes) with slits (4–11 nm) and sandwiched basement membrane with holes (2–8 nm). The combination of these structural barriers sets a filtration threshold of 6–8 nm for effective renal clearance (Figure 3A).¹⁶ For nanoparticles with hydrodynamic diameter above this size threshold, their renal clearance is exponentially reduced (Figure 3B).²⁹ The urinary filtration is not just size-dependent but also depends on the charge of the nanoparticles because the basement membrane is negatively charged. Direct comparison of filtration of nano-sized structure with opposite charges has been studied using charge-tunable dextran. It was noted that for dextran with same molecular weight, glomerular filtration was more efficient for positively charged ones than neutral dextran. Negatively charged dextran exhibited slowest filtration due to electrostatic repulsion from basement membrane.³⁰

Particle Size Effect

Owing to the structural features of filtration barriers, the renal clearance of nanoparticles is primarily size-determining: rapid and efficient renal elimination happens only to the nanomaterials with HD below the KFT; whereas, the nanoparticles with size above KFT retain in the blood and are eventually eliminated through the hepatic excretion, regardless of their surface functionalization and opsonization. GS-AuNPs with HD of 2.1 nm were found $50.5 \pm 7.1\%$ ID (percentage injection dose) in urine and $3.7 \pm 1.9\%$ ID in the liver 24 h p.i. (post-injection) while only 4.0 ± 0.6 and $0.5 \pm 0.1\%$ ID of 6 and 13 nmGS-AuNPs were renally excreted and their liver uptakes were 27.1 ± 2.3 and $40.5 \pm 6.2\%$ ID, respectively.²⁹ Furthermore, for ultrasmall metal NPs with HD below the KFT, high renal clearance efficiencies have been achieved. For instance, GS-AuNPs with HD of 3.3 nm exhibit efficient clearance of ~45% at 24 h p.i., comparable to that of 2.1 nm GS-AuNPs.^{21, 29, 31} In addition, clearance kinetics of both NPs follow the logarithmic growth with time and the first 2 h clearance contributes significantly to the total amount of the NPs cleared out of the body.

Surface Chemistry Effect

Aside from the particle size, the surface chemistry of nanoparticles also plays important roles on their elimination route. The charge state of the coating ligands is critical to the adsorption of serum proteins (~6 nm) as well as overall size in physiological environment. It has shown that zwitterionic nanoparticles, as compared to positively and negatively charged, exhibit effective resistance to serum protein adsorption and retain solubility and stability with desirable size; neutral PEGylated surface modification of nanomaterials has also been commonly applied to prevent serum absorption and prolong circulation time in blood.³²

However, these two different surface chemistries can lead to distinct renal clearance kinetics of ultrasmall AuNPs. For example, even though renal clearance efficiencies for both NPs (GS-AuNPs, ~45%; PEG-AuNPs, ~50%) were comparable at 24 h p.i., GS-AuNPs were rapidly cleared out from blood, while elimination of PEG-AuNPs was a gradual and slow process. For zwitterionic GS-AuNPs, the AuNP signal in bladder followed logarithmic increase within 10 min after injection; whereas, fluorescence from PEG-AuNPs was rarely detected until after 1 h p.i. (Figure 4A). Pharmacokinetics study further confirmed that PEG-AuNPs have longer distribution half-lives than GS-AuNPs (Figure 4B).²⁵ While different surface ligands can induce significant difference in the renal clearance kinetics, the ligand density has little effect on renal clearable AuNPs. For example, the 600 nm-emitting GS-AuNPs showed twice the surface GSH coverage as 810 nm-emitting GS-AuNPs, however, the renal clearance efficiencies of these two AuNPs were quite comparable (45.6% ID (not published) and ~45% ID at 24 h p.i. for the 600 nm-emitting and 800 nm-emitting GS-AuNPs, respectively).^{21, 31}

Particle Density Effect

Particle density (in terms of metal core density) has rarely been considered a factor of nanoparticle's blood circulation and renal clearance, compared with other particle structural features like size and surface chemistry. However, our recent study unravelled the particle density-dependent *in vivo* behaviours for the ultrasmall metal nanoparticles. As shown in Figure 5, renal clearance of metal nanoparticles within first 2 h post-injection exponentially decreases as density increases from 8.96 to 19.32 g/cm³. This observation is consistent with previous theoretical modelling of large NPs in blood vessels. Such intriguing density-dependent renal clearance likely originates from margination of these metal nanoparticles, which is strongly dependent of particle density. Due to hemodynamic force, the high-density NPs can reach the blood vessel walls more quickly. As a result, in laminar blood flow, the heavy NPs move slowly than those low-density ones because the low-density ones are close to the central blood flow. As a result, the low-density NPs can reach the kidney more quickly and are eliminated from the body much faster.²⁸

BIOMEDICAL APPLICATIONS

Tumor Targeting

EPR effect of renal clearable metal NPs—Functionalized nanomaterials have shown great promise in cancer detection and therapy, which is partially because NPs can accumulate specifically in cancer site through the enhanced permeability and retention (EPR) effect. EPR effect derives from abnormal tumor vasculature, poorly aligned and defective endothelium of cancer vascular structure as well as diminished lymphatic drainage in tumor environment. Compared to small molecules that are easily cleared out from the leaky tumor blood vessel, NPs achieve extravasation from bloodstream and accumulation in cancer site at much higher concentration for much longer period of time.^{33, 34} However, EPR effect used to be believed only working for those non-renal clearable nanoparticles because they can escape from the kidney filtration and retain in the blood much longer than small molecules. With the emergence of renal clearable NPs, whether they can still retain EPR effect, a big strength of nanomedicines over small molecules to be addressed. To answer this

fundamental question, we investigated the tumor targeting of renal clearable metal NPs and organic dye (IRDye CW800, m.w. of ~ 1100 Da). We found that targeting efficiency of GS-AuNPs (2.3 %ID/g, percentage injection dose per gram) was 10 times higher than that of IRDye CW800 at 12 h p.i.. Furthermore, the distinct tumor targeting efficiency of GS-AuNPs from IRDye CW800 was attributed to the elimination half-life of GS-AuNPs (8.5 h), which was nearly 9 times longer than that of IRDye CW800 (0.98 h). This study clearly indicated that renal clearable noble metal NPs could still passively target tumors through EPR effect.³¹

EPR effect can be further enhanced without sacrificing renal clearance. As what we discussed in the previous section, switching surface ligand of gold nanoparticles from zwitterionic glutathione to PEGylated ligand can significantly increase the blood retention time of gold nanoparticles, which can further enhance tumor targeting efficiency of renal clearable gold nanoparticles. As shown in Figure 6A–C, renal clearable PEG-AuNPs exhibited the targeting efficiency of 8.0 ± 1.5 and 8.3 ± 0.9 %ID/g at 1 h and 12 h p.i., which was significantly higher than GS-AuNPs (2.3 %ID/g at 12 h p.i.) as well as many other non-renal clearable passive or active targeting agents (Figure 6D).³³ Pharmacokinetics analysis showed that such high tumor targeting of PEG-AuNPs originated from elongation of distribution half-life, which was 10 times than that of GS-AuNPs with same core size.²⁵

Particle density and tumor targeting—Our most recent study has demonstrated that particle density can also significantly affect tumor targeting of renal clearable metal NPs because particle density strongly influences the circulation of nanoparticles in the laminar blood flow and their retention in the blood. As previously discussed, increased renal clearance efficiency of NPs has been found with decreased particle density due to reduced tendency for particle margination in blood vessels. Pharmacokinetics study showed that difference in clearance rate between GS-AgNPs and GS-AuNPs induced the significant difference in blood AUC (area under curve) within 24 h and the AUC of GS-AuNPs with higher particle density (19.32 g/cm^3) was 5 times larger than GS-AgNPs with lower particle density (10.49 g/cm^3). Since EPR effect is strongly dependent of the blood retention of the NPs, GS-AuNPs exhibited tumor targeting efficiency of 2.3 %ID/g, 3.1 times higher than GS-AgNPs (0.75 %ID/g) with identical size and surface chemistry. By analysing targeting efficiencies of different density metal NPs, we found that tumor targeting efficiency was linearly dependent on particle density, which provided a complementary strategy for tuning targeting of nanomedicines in addition to size, shape and surface chemistry.²⁸

Noninvasive Kidney Functional Imaging

As a “silent killer”, kidney disease affects nearly 10% of all adults worldwide, which is often asymptomatic at early stage but causes severe kidney failure later on without early awareness and treatment. Noninvasive *in vivo* imaging techniques, as magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT) and computed tomography (CT), have been widely used clinically to address this challenge by monitoring the kidney clearance kinetics (KCK) of renal clearable probes.^{35, 36} However, these techniques are often of high cost, low accessibility and potential risk of exposure to radiation; meanwhile, the molecular fluorescence imaging commonly used in preclinical

practice cannot achieve high contrast due to the long-term non-specific tissue accumulation. With renal clearable NIR luminescent GS-AuNPs, we were able to successfully develop high-contrast noninvasive fluorescence kidney imaging technique, which could be used to accurately stage kidney dysfunction.

With the unique molecular-like clearance profile, renal clearable GS-AuNPs demonstrated great promise in noninvasive assessment of kidney clearance kinetics at a high contrast, compared with organic fluorescent probes. With quantitative comparison on imaging contrast of 810 nm-emitting GS-AuNPs and NIR-emitting organic probe, IRDye 800CW, we demonstrated that the contrast of kidney-to-background generated by 810 nm-emitting GS-AuNPs could be up to $240.0 \pm 55.3\%$ (left kidney) or $223.2 \pm 41.5\%$ (right kidney) at 60 min p.i., which was 50 times higher than that of IRDye 800CW. Besides, the detection window was 10 h for 810 nm-emitting GS-AuNPs as compared with 0.6 min for IRDye 800CW, which was achieved by the efficient clearance of NIR GS-AuNPs from normal tissues.³⁷

High contrast and long detection window of noninvasive imaging of kidney clearance kinetics offer exciting opportunities to evaluate kidney dysfunction. Using unilateral ureteral obstruction (UUO) mouse model, we found that kidney clearance kinetics of 810 nm-emitting GS-AuNPs were extremely sensitive to kidney function impairments (Figure 8A), which were hardly detected by clinical kidney function markers as blood urea nitrogen (BUN) and serum creatinine (CREA). Through the analysis of time fluorescence intensity curves (TFICs), we found that these ultrasmall nanoprobe not only identified the kidneys with impaired function directly, but also differentiate well between different dysfunctional stages as mild, severe dysfunction and even adaptive function. As shown in Figure 8B–C, TFIC profile of normal kidney was characterized as a sharp peak followed by a deep platform representing rapid clearance, while obstructed kidney displayed a mild and delayed increase and a tiny peak, suggesting retarded function. The high-contrast imaging of renal clearable near-infrared-emitting GS-AuNPs further provided rich information on kidney function, like blood perfusion, relative functional status of impaired kidneys as well as clearance capability. Moreover, further pathological analysis of kidneys showed no structural change after the administration of GS-AuNPs, which confirmed the bioavailability and non-observable toxicity.³⁸

Ultrasmall Luminescent AuNPs as Stealthy Probes for Labelling

In addition to the applications in cancer targeting and kidney imaging, we recently found that ultrasmall luminescent GS-AuNPs could serve as imaging probes to label other nanoparticles. For instance, GS-AuNPs of ~2nm can be used to coat ~9 nm iron oxide NPs, which not only rendered stable iron oxide NPs an additional fluorescence function but resulted in significantly minimized serum protein adsorption on iron oxide NPs. This discovery shows novel application of ultrasmall GS-AuNPs as a functional surface ligand for improving physiological stability of large inorganic NPs in addition to rendering them new functionalities.³⁹

Not limited to label large inorganic nanoparticles, these ultrasmall luminescent AuNPs can also be used to label small proteins such as insulin. In contrast to large inorganic NPs that

often failed to maintain function of labelled protein due to nonspecific serum protein adsorption, monomeric insulin after being labelled with GS-AuNPs exhibited comparable bioactivity. This result indicated ultrasmall GS-AuNPs could also behave like small dye molecule in some specific protein labelling, offering opportunity for multimodal imaging of functional proteins in physiological environment.⁴⁰

Conclusion

Fundamental understanding of nano-bio interactions *in vivo* is key to successful clinical translation of engineering nanoparticles. In the past six years, we developed a class of renal clearable luminescent noble metal nanoparticles and investigated their interactions with serum protein *in vitro*, quantified their renal clearance *in vivo* and explored their biomedical applications. Our studies show that size, surface chemistry and density all have significant impact on renal clearance kinetics and passive tumor targeting. By taking advantage of effective renal clearance and efficient tumor targeting of these renal clearable metal nanoparticles, we were able to enhance tumor targeting specificity and minimize their toxicity. Not limited to cancer detection, these metal nanoparticles also enabled noninvasive *in vivo* fluorescence imaging to serve as a low-cost but high-sensitivity tool for diagnosing and staging kidney dysfunction.

While we have achieved significant progress in development of renal clearable noble metal nanoparticles and demonstration of their preclinical applications, there are still many challenges as well as fundamental questions that need to be addressed before they can be really used in clinical practices. For example, whether these renal clearable metal nanoparticles are biocompatible enough for their future applications in the clinics. While cellular toxicity of Au₅₅ clusters has been observed due to their strong interaction with chromosomes,⁴¹ our recent studies show that the maximum tolerated dose of renal clearable GS-AuNPs after intravenous injection was over 1 g/kg body weight in murine model, implying that these gold nanoparticles are fairly biocompatible to the body. Such low toxicity might be mainly due to their fast clearance and extremely low accumulation in the body. Another important question is whether the rapid renal clearance observed in the mouse model can also be really translated to large animals, which is critical to their future applications in human. Thus, the detailed studies on clearance and toxicity of these renal clearable NPs in the large animals such as dogs or monkeys also need thorough investigation. In addition, considering that noble metal NPs have also found promising therapeutic applications such as drug or gene delivery and photothermal therapy,^{8, 9, 42–47} how to further broaden biomedical applications of these renal clearable metal NPs in diseases curing is another important direction to explore. For example, compared to large noble metal NPs, these ultrasmall renal clearable ones have much larger surface-to-volume ratios; as a result, they should be able to carry more drug molecules in total than the large NPs even though each small NPs might carry fewer drug molecules than the big ones. Additionally, since these renal clearable metal NPs have clearance pathways different from the large ones, whether the drug molecules will have distinct biodistribution is also interesting to study. Nevertheless, these promising results demonstrated in the mouse model lay down a solid foundation for their future clinical translation and the fundamental

understandings we gained from these studies will not be limited to metal NPs but many other types of engineering NPs, which can also expedite their clinical translation.

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Figure 1. Schematic illustration of the formation of ~2 nm luminescent GS-AuNPs from dissociation process of polymeric gold thiolates ([Au-SR]).

Figure 2.

A) Hypothesized two distinct local Au bonding environments of 600 nm-emitting and 810 nm-emitting GS-AuNPs. B–C) Absorption, excitation and emission spectra of 600 nm-emitting and 810 nm-emitting GS-AuNPs. D–E) Transmission electron microscopy (TEM) images and size analysis of 600 nm-emitting and 810 nm-emitting GS-AuNPs.²² (Reprinted with permission from Ref 22. Copyright 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim)

Figure 3.

A) Glomerular filtration is a nanoscale phenomenon. The glomerular capillary wall is made of three specialized layers: fenestrated endothelium, glomerular basement membrane, and podocyte extensions of glomerular epithelial cells.¹⁶ (Reprinted with permission from Ref 16. Copyright 2013 Elsevier) B) Fitted exponential ($R^2 = 0.995$) relationship between the HD of different GS-AuNPs and the amount ratio accumulated in urine/liver.²⁹ (Reprinted with permission from Ref 29. Copyright 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim).

Figure 4.

A) Renal clearance profiles (inset: PEG-AuNPs in urine measured by inductively coupled plasma mass spectrometry at 12 h and 24 h p.i.) and B) pharmacokinetics of PEG-AuNPs and GS-AuNPs after intravenous injection.²⁵ (Reprinted with permission from Ref 25. Copyright 2013 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim).

Figure 5.

A) Renal clearance profiles of four metal NPs within 48 h after intravenous injection. B) The exponential decay of 2-h renal clearance as particle core density decreases.²⁸ (Reprinted with permission from Ref 28. Copyright 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim).

Figure 6.

A) *In vivo* NIR fluorescence images of the mouse intravenously injected (i.v.) with PEG-AuNPs at 5, 12, 18, 24, and 48 h p.i.. B) time-dependent contrast index of the tumor area after i.v. of the PEG-AuNPs or GS-AuNPs. C) Accumulation and retention kinetics of the PEG-AuNPs in tumor and normal tissues.²⁵ (Reprinted with permission from Ref 25. Copyright 2013 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim). D) Tumor targeting efficiencies of representative non-renal clearable inorganic NPs and renalclearable inorganic NPs following i.v..³³ (Reprinted with permission from Ref 33. Copyright 2015 American Chemical Society)

Figure 7.

A) The relationship between area under the curve (AUC) and particle core density. B) Fitted linear relationship between tumor accumulation and particle core density.²⁸ (Reprinted with permission from Ref 28. Copyright 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim).

Figure 8.

A) Noninvasive fluorescence images of mice at different time points post-injection of GS-AuNPs. B–C) Time-fluorescence intensity curves (TFICs) of kidneys in the sham control group and the UUO model. *percentage of relative renal function (%RRF=[peak value of LK or RK/(peak value of LK + peak value of RK)]×100%), **clearance percentage at 60 min = [(peak value-intensity at 60 min)/peak value]×100%.³⁸ (Reprinted with permission from Ref 38. Copyright 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim).

Figure 9. Schematic illustration of GS-AuNPs conjugated with insulin.⁴⁰ (Reprinted with permission from Ref 40. Copyright 2015 American Chemical Society).