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Photoacoustic Imaging of Nanoparticle Transport in the Kidneys at High Temporal Resolution

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Abstract

Noninvasive monitoring of kidney elimination of engineered nanoparticles at high temporal and spatial resolution will not only significantly advance our fundamental understandings of nephrology at nano scale but also render engineered nanoparticles new functionalities in early detection of kidney disease, which is influencing more than 10% of population worldwide. Taking advantage of strong NIR absorption of the well-defined $\text{Au}_{25}(\text{SG})_{18}$ nanocluster, we successfully used photoacoustic (PA) imaging to in-situ visualize its transport through the aorta to the renal parenchyma and its subsequent filtration into the renal pelvis at a temporal resolution down to 1s. High temporal and spatial resolution imaging of $\text{Au}_{25}(\text{SG})_{18}$ kidney elimination allows us for the first time to accurately quantify glomerular filtration rate (GFR) of individual kidneys in normal and pathological conditions using PA method, broadening biomedical applications of engineered nanoparticles in preclinical kidney research as a new tool for interrogating kidney functions at high temporal resolution and the anatomic level.

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Keywords

Biophysics; nanoparticles; photoacoustic imaging; renal function; glomerular filtration rate

Unraveling nanoparticle transport in live animals is expected to greatly advance our fundamental understandings of *in vivo* nano-bio interactions and physiology at the nano scale, which is extremely important to translate nanomedicines into the clinics.^[1] For instance, Chan et al. quantitatively investigated how blood flow dynamics impacted blood clearance mechanism of different sized gold nanoparticles (AuNPs) in liver and observed that more than 1,000-fold reduction of particle velocities in liver sinusoids is responsible for 7.5 times more interaction between blood-borne nanoparticles and hepatic cells as compared to peripheral cells.^[2] Not limited to liver clearance, kidney elimination of quantum dots has also been firstly examined by Choi et al., leading to the identification of a key size threshold for effective glomerular filtration of engineered nanoparticles.^[3] Using ultrasmall AuNPs as model, our group has also advanced the fundamental understandings of nephrology at the nano scale by developing a class of renal clearable AuNPs and discovered an inverse size-scaling law in glomerular filtration of engineered nanoparticles in the sub-nm regime, which opens up new pathways to evaluating kidney dysfunctions.^[4]

While significant progression has been made in fundamental understanding of physiology at the nano scale, one big challenge in the field is to continue improving temporal resolution in the noninvasive imaging of *in vivo* transport of engineered nanoparticles at anatomic level. In the past years, *in vivo* fluorescence^[5], PET^[6] and SPECT^[7] imaging have been adopted to unravel kidney clearance kinetics of AuNPs but these techniques did not offer anatomic information as well as clearance kinetics in different kidney compartments. Meanwhile, planar X-ray^[8] and CT^[9] imaging can provide anatomic information of AuNP clearance but the temporal resolution is relatively low, making it hard to directly visualize nanoparticle transport coupled with the blood flow dynamics. To address these challenges, here, we report that using photoacoustic (PA) imaging, we can directly image the *in vivo* transport of Au₂₅(SG)₁₈ nanocluster through the aorta to the renal parenchyma and its subsequent filtration into the renal pelvis at a temporal resolution of 1s. The high-temporal and spatial information derived from *in vivo* transport of Au₂₅(SG)₁₈ in the different kidney compartments allows us to noninvasively quantify GFR of individual normal and injured kidneys within very first minute, which offers a new imaging tool for not only interrogating kidney dysfunction in the preclinical settings but also advancing our understanding of nephrology at nano scale with higher temporal and spatial resolution.

PA imaging that combines the advantages of optical and ultrasound imaging, is becoming an increasingly important tool in the preclinical and even clinical biomedical research.^[10] Owing to the over 20% cardiac blood output received in kidneys, PA imaging has been used for noninvasive imaging of renal vasculatures, blood perfusion and injuries.^[11] However, this strong blood PA signal has also been a road barrier for visualizing *in vivo* transport of engineered nanoparticles in the kidneys. To address this challenge, we synthesized the well-defined glutathione coated Au₂₅ nanoclusters (Au₂₅(SG)₁₈, Figure S1), which is known to be highly renal clearable and has strong absorption in the near infrared (NIR) region (Figure

1A). We chose a single laser wavelength of 800 nm to excite Au₂₅(SG)₁₈ as it is close to the isosbestic point of the major endogenous chromophores deoxyhaemoglobin (Hb) and oxyhaemoglobin (HbO₂) for minimized breath interference (Figure 1A). In addition, single-wavelength excitation enabled us to acquire images at a high temporal resolution (~ 1s), which is essential for imaging-based techniques to quantify GFR. Prior to *in vivo* PA imaging, we tested the PA signal of a series of different concentration Au₂₅(SG)₁₈ solutions in tissue-mimicking phantoms (Figure S2) and the signal increase was found to linearly ($R^2=0.99$) correlate with the increase of Au₂₅(SG)₁₈ concentration (Figure 1B), making it straightforward to convert PA signal to concentration when analyzing PA images. Since high power pulsed laser was used to generate PA signals *in vivo*, the photochemical stability of Au₂₅(SG)₁₈ was also evaluated. While conventional organic dyes decreased by 27%–72% in their NIR absorption after 3 min exposure to 808nm diode laser (0.5W/cm² power density), Au₂₅(SG)₁₈ showed almost unchanged NIR absorption after the same laser irradiation (Figure S3), due to high chemical stability of Au₂₅ nanocluster. Different concentrations of Au₂₅(SG)₁₈ exhibited fairly stable PA signals during the exact same pulsed laser excitation used for *in vivo* imaging (Figure S4). Moreover, the absorption and molar extinction coefficient of the excreted Au₂₅(SG)₁₈ after *in vivo* PA imaging experiments were also measured and found identical to the injected ones (Figure S4). This high photochemical and physiological stability further ensures that PA signal of Au₂₅(SG)₁₈ can be accurately correlated to its concentration *in vivo*.

In vivo PA imaging was conducted with a multispectral optoacoustic tomography (MSOT) imaging system where BALB/c mice were positioned perpendicular to the ultrasound transducer array to achieve cross-sectional imaging of the kidneys (Figure 1C). With a temporal resolution of ~ 1s, a bolus of intravenously administered Au₂₅(SG)₁₈ (20mg Au/kg body weight) could be clearly visualized and was transported through the aorta to renal parenchyma, followed by being rapidly filtered into the renal pelvis for elimination (Figure 1D and movie S1), even though strong blood absorption presented in the kidneys. By quantifying the time interval (t) between the signal onset of renal parenchyma and that of renal pelvis (Figure 1E and Figure S5), we found that Au₂₅(SG)₁₈ only took an average of ~18.5s (18.5±2.5s) to be filtered and transported to the pelvis after entering the mouse kidney, which is unattainable by our previous *in vivo* fluorescence^[5] and planar X-ray^[8] based kidney imaging because of a lack of tomographic information and insufficient temporal resolution. Moreover, analysis of the time-signal curves reveals that Au₂₅(SG)₁₈ has a decay half-life of 133.8±34.8s in renal parenchyma and 233.9±38.8s in renal pelvis, much shorter than the decay half-lives (8.6±5.2min and 8.9±3.6min for parenchyma and pelvis, respectively) of our previously reported 2.5nm renal clearable GS-AuNPs^[8], suggesting that Au₂₅(SG)₁₈ has even weaker interactions with different compartments of the kidney. Such minimal kidney interaction also echoed the notably fast renal clearance kinetics of Au₂₅(SG)₁₈ with over 60% ID being eliminated within just 30min post injection, accounting for more than 90% of total renal clearance in the first 24 hours (Figure S6) and considerably faster than that of GS-AuNPs^[9] (< 35% ID at 2h p.i.). This rapid decay of Au₂₅(SG)₁₈ kinetics in renal parenchyma was distinct from that of large non-renal clearable gold nanorods (AuNRs), which cannot be filtered by the kidney and had a parenchyma kinetics that hardly decayed in the initial time period (Figure S7). Interestingly, with such

high temporal resolution, we were able to differentiate a slight but statistically significant difference in Au₂₅(SG)₁₈ decay half-life between parenchyma and pelvis (Figure 1F), which was not observed in our previous X-ray imaging of GS-AuNPs elimination in kidney^[8] due to inadequate temporal resolution. The ability to image the transport process of Au₂₅(SG)₁₈ at high temporal resolution with detailed anatomical information further made it possible to use PA imaging to directly measure single-kidney GFR, which is considered the key overall index of kidney function and routinely used to monitor the progression of renal diseases.^[12]

To quantify single-kidney GFR, we utilized the Patlak-Rutland method, a plot technique widely applied in the field of nuclear medicine^[13], CT^[14] and MRI^[15] for renal function assessment, to analyze the PA renal images. The kidney was simplified as a two-compartment model where Au₂₅(SG)₁₈ was one-way filtered from the vascular compartment to tubular compartment with a constant proportionality α (Figure 2A). Within a kidney volume of V , the total glomerular filtrate, $F(t)$, at time t could be expressed as $F(t) = \alpha \int b(t) dt$, where $b(t)$ represents the average concentration of Au₂₅(SG)₁₈ in kidney vasculatures. Let V_B be the intrarenal blood volume within the kidney volume V , therefore the total amount of Au₂₅(SG)₁₈ in kidney vasculatures, $B(t)$, equals to $V_B \times b(t)$. If $C(t)$ represents the total amount of Au₂₅(SG)₁₈ in the kidney volume V and $c(t)$ is the average concentration of Au₂₅(SG)₁₈ within it, then $C(t) = V \times c(t)$. During the initial kidney elimination period in which the outflow of Au₂₅(SG)₁₈ from tubular compartment is negligible, clearly $C(t) = B(t) + F(t)$ and dividing this equation by $V \times b(t)$ yields the following equation:

$$\frac{c(t)}{b(t)} = \frac{V_B}{V} + \frac{\alpha}{V} \frac{\int b(t) dt}{b(t)}$$

Thus, if we plot a curve with $c(t)/b(t)$ as the y-axis and $\int b(t) dt/b(t)$ as the x-axis, then ideally the curve would be linear with a slope of α/V representing the blood clearance rate per unit volume of kidney (GFR per kidney volume) and a y-axis intercept of V_B/V , which denotes the fractional vascular volume (fvv). Since kidney receives blood supply directly from abdominal aorta, the concentration of Au₂₅(SG)₁₈ in kidney vasculatures ($b(t)$) can be approximated by that in aorta.

By placing region of interest (ROI) over the aorta as well as renal parenchyma of the two kidneys (Figure 2B), the PA signal kinetics curve of each part could be obtained, which was further converted to Au₂₅(SG)₁₈ concentration kinetics based on the *in vitro* phantom study-derived standard curve. To ensure the “negligible tubular outflow” condition, only the time points before significant amount of Au₂₅(SG)₁₈ reached renal pelvis (when parenchyma signal is still ascending) were adopted for analysis. A typical aorta kinetics shows two peaks, corresponding to the first pass and the recirculation of a bonus injection of Au₂₅(SG)₁₈ (Figure 2C). The renal parenchyma kinetics of Au₂₅(SG)₁₈ in both kidneys are comparable to that of gadolinium (Gd)-chelates used in the dynamic contrast-enhanced MRI (DCE-MRI) for GFR measurement^[16], with peaks reflecting bonus contrast perfusion by the blood (Figure 2D). Based on the described method, we were able to plot the Patlak-Rutland graph (Figure 2E) of each kidney and the data points exhibited excellent linearity ($R^2 > 0.96$ for all the mice). Less than 10% difference in the derived fvv between left and right kidneys

(Figure 2F and Figure S8) was observed, which is consistent with the fvv (~8%) obtained by the micro-CT analysis of kidney vasculature in mice^[17]. GFR per kidney volume was calculated from the slope of each fitting and converted to the clearance of plasma volume (corrected for hematocrit) per minute (Figure 2G and Figure S8). Both left and right kidneys shared a GFR value of $\sim 0.6 \text{ mL} \cdot \text{min}^{-1} \cdot \text{mL}^{-1}$ that was also very close to the reported single kidney GFR measured by DCE-MRI in mice recently^[18]. To further validate the current $\text{Au}_{25}(\text{SG})_{18}$ PA imaging-based method, we compared the calculated global GFR (sum of individual kidney GFR) with the global GFR measured by the blood clearance of FITC labeled inulin (FITC-inulin). The obtained global GFR values are comparable (Figure 2H) (241.0 ± 23.3 vs $211.6 \pm 51.3 \text{ } \mu\text{L}/\text{min}$, $p=0.36$, see supporting information and Figure S9 for detailed calculation), which are also consistent with the mice GFR values in literatures^[19], further indicating that PA imaging could serve as a powerful tool to quantify GFR of individual kidneys.

In addition to normal kidneys, we also investigated whether combination of PA imaging and renal clearable $\text{Au}_{25}(\text{SG})_{18}$ can be used to evaluate GFR changes of individual kidneys in unilateral ureteral obstruction (UUO) mice since UUO is known to reduce GFR of the diseased kidney. UUO model was established by surgically ligating the left ureter of mice (Figure 3A) and PA imaging was conducted three days afterwards. Enlarged renal pelvis (hydronephrosis) of the left UUO kidney could be clearly seen in PA tomography due to the build-up of fluid, confirming the success of this renal disease model (Figure 3B). Unlike that in the unobstructed right kidney where strong $\text{Au}_{25}(\text{SG})_{18}$ signal was observed during its transporting from renal parenchyma to the pelvis, the left UUO kidney only exhibited faint signal increase in the parenchyma and even weaker signal in the pelvis at the same time (Figure 3B and movie S2) as a result of reduced blood perfusion and GFR, leading to a drastic difference in their renal parenchyma kinetics (Figure 3C). The same plot technique was then used to generate the Patlak-Rutland graph for both the left UUO kidney and the contralateral right kidney (Figure 3D). The derived fvv of left UUO kidneys were $\sim 65\%$ lower than that of contralateral right kidneys ($3.3 \pm 0.95\%$ vs $9.3 \pm 1.9\%$) (Figure 3E and Figure S10), which was consistent with the results from previous studies^[20] measuring renal arterial blood flow that found acute reduction ($>50\%$) of renal blood perfusion in UUO kidneys. GFR values deduced from the slopes showed that the left UUO kidneys ($0.26 \pm 0.02 \text{ mL} \cdot \text{min}^{-1} \cdot \text{mL}^{-1}$) had only $\sim 45\%$ GFR of those contralateral right kidneys ($0.57 \pm 0.04 \text{ mL} \cdot \text{min}^{-1} \cdot \text{mL}^{-1}$) (Figure 3F and Figure S9), which was slightly higher than the GFR values measured previously for UUO kidneys (5%–25% that of the contralateral kidneys) through the bilateral ureter catheterization method^[20c, 21]. This discrepancy may result from our recently reported fact that transport of probes in different segments (cortex-to-medulla-to-pelvis) of UUO kidney was significantly slowed down together with increased renal cellular uptake as compared to that of the contralateral unobstructed kidney^[8], rendering ureter catheterization a technique that tends to underestimate the GFR of UUO kidneys.

Successful quantification of GFR with $\text{Au}_{25}(\text{SG})_{18}$ also implies that $\text{Au}_{25}(\text{SG})_{18}$ nanoclusters are eliminated *in vivo* through freely glomerular filtration with minimal renal tubular secretion or reabsorption, which was confirmed by histological analysis of $\text{Au}_{25}(\text{SG})_{18}$ distribution in different kidney compartments (Figure 4A). With silver enhancement, $\text{Au}_{25}(\text{SG})_{18}$ nanoclusters were visualized locating mainly in the glomeruli and

tubular lumens as well as peritubular capillaries, whereas few Au₂₅(SG)₁₈ appeared inside tubular cells. Moreover, after a thorough perfusion of the kidney to remove blood in vasculatures, Au₂₅(SG)₁₈ was no longer presented in glomeruli or peritubular capillaries and much fewer Au₂₅(SG)₁₈ could be found in tubular lumens as well, clearly indicating that renal tubular secretion or reabsorption of Au₂₅(SG)₁₈ was minimal. Comparison of the pharmacokinetics of Au₂₅(SG)₁₈ and FITC-inulin shows that they share nearly identical blood clearance kinetics in the early elimination phase (Figure 4B), consistent with the freely glomerular filtration nature of Au₂₅(SG)₁₈. The divergence in the later elimination phase may result from that Au₂₅(SG)₁₈ has stronger margination effect^[22] than that of FITC-inulin in blood vessels, which decreases the circulating rate of Au₂₅(SG)₁₈ in laminar blood flow during general circulation and increases its extravasation, thus slowing down kidney elimination of Au₂₅(SG)₁₈ especially when its concentration in blood is greatly reduced in the later elimination phase^[23].

In summary, the *in vivo* transport of Au₂₅(SG)₁₈ nanoclusters from aorta to renal parenchyma and subsequent elimination to renal pelvis was noninvasively imaged at high temporal- and spatial- resolution with photoacoustic imaging. Such high-temporal-resolution imaging coupled with detailed anatomical information enabled us for the first time to accurately quantify single-kidney GFR of normal as well as diseased kidneys using an engineered nanoparticle. Analysis of the Au₂₅(SG)₁₈ *in vivo* transport and behavior profiles further suggests that Au₂₅(SG)₁₈ could serve as an excellent exogenous glomerular filtration marker. Considering the clinical significance of GFR in evaluating renal function and the advantages of gold nanoparticles in multimodality imaging^[22], our findings not only advance the fundamental understandings of nanoparticle-kidney interaction but also provide preclinical research new practical tools to measure renal function in a clinically relevant gauge.

Supplementary Material

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Acknowledgements

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Figure 1.

A) Molar extinction coefficient of $\text{Au}_{25}(\text{SG})_{18}$ as compared to that of Hb and HbO_2 in the NIR range. Dotted line indicates the laser wavelength (800 nm) used to excite $\text{Au}_{25}(\text{SG})_{18}$ in in-vitro and in-vivo experiments. B) PA signal intensity increase as a function of $\text{Au}_{25}(\text{SG})_{18}$ concentration measured by in-vitro phantom studies. C) Illustration of in-vivo PA imaging experiment where mice were positioned perpendicular to the scanning plane so that cross-sectional imaging of both kidneys could be achieved. D) Representative pre-injection PA images of normal mice and post-injection PA images of different time points showing the transport of $\text{Au}_{25}(\text{SG})_{18}$ from abdominal aorta to renal parenchyma and eventually to the renal pelvis in both kidneys. E) Representative PA signal kinetics in renal parenchyma and renal pelvis of a normal kidney. The figure on the right shows the time interval between the

signal onset of the two kinetics curves. F) Decay half-lives of the PA signal in renal parenchyma and renal pelvis (n=3). RK, right kidney; LK, left kidney. Statistical significance is evaluated by two-sample (Welch's) t-test (**p< 0.001).

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Figure 2.

A) Scheme of the two-compartment kidney model for GFR assessment. B) ROIs chosen for the analysis of PA signal kinetics in aorta and renal parenchyma. C) Typical aorta $Au_{25}(SG)_{18}$ concentration kinetics curve. D) Typical parenchyma $Au_{25}(SG)_{18}$ concentration kinetics of both kidneys in normal mice. E) Typical Patlak-Rutland plot of both kidneys in normal mice. F) Derived fractional vascular volume (fvv) of right and left kidneys in normal mice (n=3). G) Derived GFR per kidney volume of right and left kidneys in normal mice (n=3). H) Comparison of the global GFR in normal mice obtained by the current PA imaging method and the standard FITC-inulin clearance method (n=3 for $Au_{25}(SG)_{18}$ and n=4 for FITC-inulin). Statistical significance is evaluated by two-sample (Welch's) t-test ("n.s.", $p > 0.05$, no significant difference). RK, right kidney; LK, left kidney.

Figure 3.

A) Scheme of the URO mice model that was established by complete ligating of the left ureter. B) Representative PA images of pre-injection and post-injection of $\text{Au}_{25}(\text{SG})_{18}$ in URO mice at multiple time points. The transport and clearance of $\text{Au}_{25}(\text{SG})_{18}$ in the left URO kidney was clearly compromised comparing to that of the contralateral right kidney. Dotted line in yellow outlines the renal parenchyma of right and left kidneys. C) Typical parenchyma $\text{Au}_{25}(\text{SG})_{18}$ concentration kinetics of left URO kidney and contralateral right kidney. D) Typical Patlak-Rutland plot of left URO kidney and contralateral right kidney. E) Derived fractional vascular volume (fvv) of left URO kidneys and contralateral right kidneys (n=4). F) Derived GFR per kidney volume of left URO kidneys and contralateral right kidneys (n=4). Statistical significance is evaluated by two-sample (Welch's) t-test (**p<0.005). RK, right kidney; LK, left kidney.

Figure 4.

A) H&E and silver stained kidney sections of Au₂₅(SG)₁₈ injected mice with or without kidney perfusion. Kidneys from PBS injected mice without perfusion that underwent the same silver staining process were served as control. Arrows in blue color indicate Au₂₅(SG)₁₈ found in glomeruli whereas arrows in white indicate Au₂₅(SG)₁₈ found in renal tubules as well as peritubular capillaries. Scale bar, 15 μm. B) Pharmacokinetics of intravenously administered Au₂₅(SG)₁₈ and FITC-inulin. Inserted is the pharmacokinetics-derived clearance parameters (CL) of both probes in the initial 20 min p.i. Statistical significance is evaluated by two-way repeated measures ANOVA (pharmacokinetics) and two-sample (Welch's) t-test (CL) ("n.s.", $p > 0.05$, no significant difference).