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Renal-clearable Molecular Semiconductor for Second Near-Infrared Fluorescence Imaging of Kidney Dysfunction

Jiaguo Huang, Chen Xie, Xiaodong Zhang, Yuyan Jiang, Jingchao Li, Quli Fan* and Kanyi Pu*

Abstract: Real-time imaging of kidney function is important to assess the nephrotoxicity of drugs and monitor the progression of renal diseases; however, it remains challenging because of the lack of optical agents with high renal clearance and high signal-to-background ratio (SBR). Herein, a second near-infrared (NIR-II) fluorescent molecular semiconductor (CDIR2) is synthesized for real-time imaging of kidney dysfunction in living mice. CDIR2 not only has a high renal clearance efficiency (~90% injection dosage at 24 h post-injection), but also solely undergoes glomerular filtration into urine without being reabsorbed and secreted in renal tubules. Such a unidirectional renal clearance pathway of CDIR2 permits real-time monitoring of kidney dysfunction in living mice upon nephrotoxic exposure. Thus, this study not only introduces a molecular renal probe but also provides useful molecular guidelines to increase the renal clearance efficiency of NIR-II fluorescent agents.

Introduction

Kidney plays a crucial role in removing xenobiotics and metabolic wastes from blood into urine and maintaining body fluid and electrolyte homeostasis.^[1] Such an intense metabolic activity makes kidney highly vulnerable to systemic insults. Thus, renal impairment (RI) often occurs for patients with ischaemia, sepsis or nephrotoxin exposure.^[2] With a high morbidity and mortality, RI places an increasing economic burden on healthcare system.^[3] Thus, real-time monitoring of kidney dysfunction is imperative not only to preclude nephrotoxic drugs from entering the market but also to timely conduct renoprotective interventions to prevent the progression into severe complications such as end stage kidney disease (ESKD) in hospital.^[4] In clinic, measurement of blood urea nitrogen (BUN) and serum creatinine (sCr) is the routine to assess kidney dysfunction; however, they are delayed biomarkers and have poor sensitivity due to the effect of age, gender, muscle mass and many other anthropometric variables.^[5] Renal biopsy is a definitive way to identify kidney dysfunction, but it is

unfortunately invasive and carries the risk of potential internal damage to the targeted or nearby organs.^[6]

Noninvasive imaging techniques such as single-photon emission computed tomography (SPECT),^[7a] positron emission tomography (PET),^[7b] magnetic resonance imaging (MRI),^[8] and computed tomography (CT)^[9] have been used to assess kidney dysfunction by real-time monitoring of the kidney clearance kinetics of imaging agents. However, these methods are suffered from high cost, low accessibility, and potential risk of exposure to radiation and nephrotoxicity caused by contrast agents.^[10-12] Although optical imaging is a relatively inexpensive, sensitive, and common method for preclinical studies,^[13-17] it has been rarely exploited for kidney imaging.

The key challenges in real-time optical imaging of kidney function consists in the development of optical agents with high renal clearance and high signal-to-background ratio (SBR). However, only a few optical agents can undergo renal clearance, which include cysteine-coated CdSe/ZnS core-shell quantum dots with the size below 5.5 nm,^[18] silica Cornell dots,^[19] the zwitterionic fluorophore (ZW800-CDPL),^[20] and porphyrin-polyethylene glycol (PEG).^[21] Based on the passive retention of nanoparticles in blocked kidneys, Zheng et al. developed gold nanoclusters for real-time fluorescence imaging of kidneys in a unilateral ureteral obstruction model.^[22-23] We recently developed a series of fluorescent molecular renal probes that change their signals in response to the biomarkers related to kidney injury.^[24] However, all these agents can only emit light in first near-infrared window (NIR-I, 650-900 nm), which has limited penetration depth and high tissue autofluorescence.^[25] In contrast, fluorophores in the second near-infrared window (NIR-II, 1000-1700 nm) shows superior resolution and sensitivity over NIR-I, thus allowing investigation of deep anatomical features in vivo.^[26-28] However, few optical NIR-II imaging agents including CH1055-PEG^[29], Nd-DOTA and **lipoic acid-based sulfobetaine coated gold nanoclusters**^[30] have been reported to excrete through renal clearance, and they have not been used for imaging of RI.

Herein, we report a highly renal-clearable molecular semiconductor (CDIR2) for real-time noninvasive NIR-II fluorescent imaging of kidney dysfunction in murine model. CDIR2 contains two key functional components (Figure 1a): NIR-II fluorophore moiety and renal-clearance-enabling moiety ((2-hydroxypropyl)- β -cyclodextrin, HP β CD). The NIR-II fluorophore possesses a typical shielding unit-donor-acceptor-donor-shielding unit structure with benzo[1,2-c:4,5-c'] bis ([1,2,5] thiadiazole) (BBTD), 3,4-ethoxylene dioxothiophene (EDOT), and dialkyl fluorene as the acceptor, donor and shielding units, respectively. After systemic administration of CDIR2 into living mice, it can go through glomerular filtration into urine without being reabsorbed and secreted in renal tubules. Such a unidirectional renal clearance pathway of CDIR2 allows real-time, noninvasive monitoring of kidney dysfunction in living mice during progression of RI.

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Results and Discussion

Synthesis and characterization. BrIR2 was synthesized according to the previous study,^[31] followed by substituting bromide with azide on the side chains to afford N3IR2 (Scheme S1). At last, HP β CD was conjugated to the alkyl chains of N3IR2 via click reaction to afford CDIR2. Nuclear magnetic resonance (¹H-NMR) spectrum confirmed the chemical structures and purity of alkyne-functionalized HP β CD, BrIR2, N3IR2 and CDIR2 (Figure S1-4). MALDI-TOF-MASS assay further validated that CDIR2 had an average molecular weight of ~4500 Da, proving the conjugation of two HP β CD moieties per fluorophore (Figure S5). To synthesize the control fluorophore with the optical profile in NIR-I window, CyOH was synthesized according to our previous studies^[24] and conjugated with the alkyne-functionalized HP β CD to afford CCD (Scheme S1), which had an average molecular weight of ~2200 Da (Figure S6&7), showing one HP β CD moiety per NIR-I fluorophore.

CDIR2 had an absorption maximum at ~770 nm in phosphate buffered saline (PBS). While its fluorescence spectrum exhibited an emission peak at 1050 nm under 808 nm excitation (Figure 1b). The fluorescence quantum yield of CDIR2 was determined to be ~2.2% in PBS (Table S1), which was higher than carbon nanotubes (0.4%)^[32] and the reported NIR-II molecular dye CH1055-PEG (0.3%)^[29] and PEG coated IR1061 nanoparticles (0.5%)^[33]. In contrast, CCD had the absorption and emission maxima at 695 and 720 nm, respectively, showing a fluorescent quantum yield of 21% in PBS solution (Figure 1c and Table S1). CDIR2 had a high photostability in PBS with negligible fluorescence decay under a continuous 808 nm laser at a power density of 0.30 W cm⁻² for 10 min (Figure S8). In contrast, a continuous decrease in fluorescence intensities was observed for Indocyanine green (ICG), which had the absorption and emission maximum at 790 and 820 nm (Figure S9), respectively.

To compare the penetrating ability of ICG (NIR-I) and CDIR2 (NIR-II), the fluorescence signals were acquired through the chicken breast tissues. The fluorescence signals for both ICG and CDIR2 decreased with the increasing thickness of chicken tissue (Figure 1d). The NIR-I fluorescence signal of ICG was close to the background noise at a 2 cm thickness of chicken tissues, whereas the NIR-II fluorescence signal for CDIR2 was still visible even at the chicken tissue thickness of 3 cm. In addition, the SBR for CDIR2 (51.8, 33.1, 19.6, 5.47, and 4.0) were 1.3, 6.6, 8.5, 5.0 and 4.0-fold higher than that for ICG (39.9, 5.0, 2.3, 1.1, and 1.0) at a chicken tissue thickness of 0.5, 1.0, 2.0, 3.0, and 4.0 cm, respectively (Figure 1e). These data clearly proved that CDIR2 had not only deeper tissue penetration but also higher sensitivity than ICG, which was ascribed to the minimized background signals due to reduced light scattering and tissue absorption in the NIR-II window. No obvious cytotoxicity was observed for CDIR2 using 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) assay (Figure S10), showing its potential for biological applications.

Renal clearance and in vivo stability studies. To investigate the pharmacokinetics of CDIR2, the concentration of CDIR2 in the

blood was analyzed by high-performance liquid chromatography (HPLC) after a single intravenous (i.v.) injection into living mice. Blood concentration curves implied that CDIR2 had a two-compartment profile of in vivo kinetics (Figure 3c). The CDIR2 concentration in blood was close to 0 % injected doses (ID) g⁻¹ at 95 min post-injection and its elimination half-life ($t_{1/2\beta}$) was estimated to be 20±3.1 min, indicating its rapid elimination from the body. To study its renal clearance, CDIR2 in the urine of living mice was quantified as a function of time after i.v. injection. Based on HPLC, renal clearance efficiency was determined to be 65±3.3% ID at 2 h post-injection and 87±2.3% ID at 24 h post-injection (Figure 2b&S11), which is slightly lower than CCD (94.5±1.7% ID at 24 h post-injection, Figure S12). In contrast, the renal clearance efficiency of N3IR2 (NIR-II fluorophore without HP β CD conjugation) only reached 3.8±0.7% ID at 24 h post-injection (Figure S13). After 24 h urinary recovery, the mice were dissected and the residual CDIR2 in the body was analyzed after homogenization of major organs in PBS. The results revealed that the residual CDIR2 (~10%) was mainly accumulated in liver and intestine (Figure 2c), negligible CDIR2 was found in other organs. Nevertheless, the renal clearance efficiency of CDIR2 was higher than Nd-DOTA (~50% ID at 3 h post-injection),^[30] and similar to the CH1055-PEG (~90% ID at 24 post-injection).^[29a,b] Such a high renal clearance feature was attributed to its high hydrophilicity and

Figure 1. Chemical structure and optical characterization of CDIR2 and CCD. (a) Chemical structure of CDIR2 and CCD. ($R_2 = \text{H}$ or $\text{CH}_2\text{CHOHCH}_3$). (b) Absorption and fluorescence spectra of CDIR2 (25 μ M) in PBS (10 mM, pH 7.4) at 37 $^{\circ}$ C. (c) Absorption and fluorescence spectra of CCD (25 μ M) in PBS (10 mM, pH 7.4) at 37 $^{\circ}$ C. (d) Representative fluorescence images of ICG (100 μ M) and CDIR2 (100 μ M) solutions upon overlaying chicken tissues with the desired thickness on top of the samples. The representative fluorescence images acquired at 815 nm upon excitation at 775 nm for ICG and at 1000-1100 nm upon excitation at 808 nm for CDIR2, respectively. (e) SBRs for fluorescence imaging of ICG and CDIR2 as a function of chicken tissue depth in panel of d. Data are the mean $\pm$ SD. $n = 3$ independent measurements.

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low molecular weight (~4.5 kDa) relative to the glomerular filtration molecular weight cutoff (50 kDa).^[29c]

To determine in vivo stability of CDIR2, the optical and chemical profiles of CDIR2 recovered from urine were measured and compared with its pure form in PBS. CDIR2 in the urine and PBS had the same characteristic peak with a retention time of 3.4 min in HPLC spectra (Figure 2d). The absorption (Figure 2e), fluorescence (Figure 2f) and mass spectra (Figure S5) of excreted CDIR2 in the urine were almost identical to those of CDIR2 in PBS, suggesting that no obvious metabolism occurred after 24 h circulation in living mice. Moreover, histological hematoxylin and eosin (H&E) staining revealed that CDIR2 did not cause any tissue damages after 24 h injection, confirming its good

biocompatibility (Figure S14).

To compare the SBRs of CCD (NIR-I) and CDIR2 (NIR-II) in kidney imaging, the fluorescence signals were acquired for whole-body fluorescence imaging of living mice. A strong fluorescent signal was observed in the kidneys at 1 min post-injection of CDIR2 in mice (Figure 2g&S15). However, the signals of CCD in kidneys were only observed after 20 min injection. The SBR for CDIR2 (3.2, 8.8, 6.7, 6.2 and 4.8) were 2.5, 5.2, 3.7, 2.8, and 1.7-fold higher than that for CCD (1.3, 1.7, 1.8, 2.2 and 2.8) at 1, 4, 12, 20 and 60 min post-injection, respectively (Figure 2h&S15). Thus, CDIR2 had higher sensitivity than CCD, confirming the advantages of NIR-II fluorescence imaging of kidneys in living mice.

Figure 2. Renal clearance and in vivo stability studies of CDIR2. (a) Schematic illustration of the renal excretion of CDIR2 through the urinary tract. (b) Renal clearance efficiency as a function of time post-injection of CDIR2 ($1 \mu\text{mol kg}^{-1}$ body weight) in living mice, three lines represent the measurements in three independent mice. (c) HPLC analysis of the amount of CDIR2 excreted from kidneys into urine (red bar) and residual CDIR2 in major organs of mice (black bar) after 24 h injection of CDIR2. Major organs were homogenized in PBS and centrifuged to remove insoluble components. The supernatant containing extracted CDIR2 were analyzed by HPLC assay. Heart (He), liver (Li), spleen (Sp), intestine (In), muscle (Mu), lung (Lu), skin (Sk), kidneys (Ki), bladder (Bl). (d) In vivo stability studies of CDIR2 through HPLC analysis of urine samples after i.v. injection of CDIR2. HPLC traces of the pure compound and blank urine are also indicated for comparison. In vivo stability studies of CDIR2 using optical characterization. Absorption (e) and fluorescence (f) spectra of CDIR2 (25 μM) in PBS and the urine samples after 24 h i.v. injection of CDIR2 and excretion from living mice. (g) Representative NIR-I and NIR-II fluorescence images of living mice after i.v. injection of CCD ($8 \mu\text{mol kg}^{-1}$ body weight) and CDIR2 ($1 \mu\text{mol kg}^{-1}$ body weight), respectively. The red circles indicate the kidneys in the dorsal side. (h) SBRs for fluorescence imaging of CCD and CDIR2 at different post-injection time in panel of g, CDIR2 versus CCD groups, $n=3$, mean $\pm$ s.d., * $p<0.05$, ** $p<0.01$.

In vivo renal clearance pathways of CDIR2. Renal clearance involves three main pathways including glomerular filtration, tubular secretion and tubular reabsorption (Figure 3a).^[34-35] Glomerular filtration is the first step in renal clearance, followed by tubular secretion (from peritubular capillaries to tubular lumen) and reabsorption (from tubular lumen to the circulatory system), which are mediated by the organic anion transporters (OAT) and the organic cation transporters (OCT) in renal tubular cells.^[35] To gain insight into the clearance pathway of CDIR2 in the kidneys, real-time NIR-II fluorescence imaging of kidneys and bladder was conducted in three groups of living mice treated with saline, probenecid or cimetidine at a dosage of 150 mg kg^{-1} .

Probenecid^[36] and cimetidine^[37-38] are the OAT and OCT inhibitor with known inhibition efficacy for blocking tubular secretion and reabsorption, respectively. All the groups were then intravenously injected with CDIR2. Whole-body longitudinal NIR-II fluorescence imaging were conducted at different post-injection timepoints. At 4 min post-injection of CDIR2 in saline-treated mice, a strong fluorescent signal was observed in the kidneys and bladder (Figure 3b). The signal in the kidneys reached maximum at 4 min post-injection of CDIR2 and then decreased with time (Figure 3d), while the signals of CDIR2 in the bladder increased with post-injection time (Figure 3e). The ratios of bladder-to-kidney intensity in saline-treated mice derived from the NIR-II fluorescence

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images were calculated to be 0.20 ± 0.05 and 2.81 ± 0.12 at 4 and 100 min post-injection of CDIR2, respectively, clearly showing the rapid excretion of CDIR2.

Similar trends in the revolution of signals were observed for probenecid and cimetidine-treated mice in both kidneys and bladder (Figure 3b, d&e), suggesting the clearance of CDIR2 was through glomerular filtration because tubular secretion and reabsorption were blocked. The ratios of bladder-to-kidney intensity were 0.22 ± 0.02 and 2.71 ± 0.17 in probenecid-treated mice and 0.29 ± 0.04 and 3.01 ± 0.13 in cimetidine-treated mice at 4 and 100 min post-injection of CDIR2, implying CDIR2 has almost the same clearance rate in inhibitors-treated mice relative

to the saline-treated mice. This was again validated by the observation of similar NIR-II fluorescence signals in the resected kidneys from mice treated with saline, probenecid and cimetidine (Figure S16). Therefore, CDIR2 was exclusively cleared via glomerular filtration without secretion and reabsorption in renal tubules. In comparison, N3IR2 was undetectable in the kidneys and bladder but was mainly accumulated in the liver at 60 min post-injection (Figure S17).

To further investigate the clearance pathway of CDIR2, the pharmacokinetics of CDIR2 in living mice treated with probenecid or cimetidine were studied. Blood concentration curves implied that CDIR2 had a two-compartment profile of in vivo kinetics

Figure 3. In vivo renal clearance pathways of CDIR2. (a) Schematic illustration of a nephron and renal clearance pathways: glomerular filtration, tubular secretion and tubular reabsorption. Probenecid (an OAT inhibitor) or cimetidine (an OCT inhibitor) was intraperitoneally injected (150 mg kg^{-1}) into living mice to block the tubular reabsorption and secretion, followed by i.v. injection of CDIR2 ($1 \mu\text{mol kg}^{-1}$ body weight). (b) Representative NIR-II fluorescence images of living mice at $t = 1, 4, 12, 20, 60$ or 100 min after injection of CDIR2 in mice treated with saline, probenecid or cimetidine. The red circles indicate the kidneys and bladder in the dorsal and ventral side, respectively. NIR-II fluorescence images acquired at $1000\text{--}1100 \text{ nm}$ upon excitation at 808 nm at a power density of 0.30 W cm^{-2} . (c) Blood concentration ($\% \text{ ID g}^{-1}$) decay of CDIR2 in living mice treated with saline, probenecid or cimetidine. (d) NIR-II fluorescence intensities of kidneys and (e) bladder as a function of time post-injection of CDIR2 in living mice treated with saline, probenecid or cimetidine. (f) The ratios of bladder-to-kidney intensity of CDIR2 as a function of time post-injection of CDIR2 in living mice treated with saline, probenecid or cimetidine. Data in (c–f) are the mean $\pm$ SD. $n = 3$ independent mice.

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either in the presence or absence of inhibitors (Figure 3c). The CDIR2 concentrations in blood in all treated mice were close to 0 % injected doses (ID) g^{-1} at 95 min post-injection, indicating neither acceleration in CDIR2 clearance through secretion pathway nor deceleration in CDIR2 clearance via reabsorption pathway. The elimination half-lives ($t_{1/2\beta}$) were estimated to be 22 ± 2.5 and 21.4 ± 3.6 min for probenecid- and cimetidine-treated mice, respectively. These data showed no significant difference relative to that of saline-treated mice, which further proved that CDIR2 has no tubular reabsorption and secretion in the kidneys

probably due to no charge profile of the chemical structure of CDIR2.

In vivo NIR-II fluorescence imaging of kidney dysfunction. As the renal clearance of CDIR2 was unidirectionally via glomerular filtration without tubular secretion or reabsorption, its fluorescence signal changes in kidneys could reflect impairments in glomeruli or tubules. Thus, CDIR2 has a potential for real-time imaging kidney dysfunction. To do so, real-time NIR-II fluorescence imaging was conducted in living mice treated with cisplatin, which

Figure 4. Real-time detection of kidney function via NIR-II fluorescence imaging of CDIR2 in living mice. (a) Schematic illustration of living mice treated with cisplatin (20 mg kg^{-1}) and NIR-II fluorescence imaging at different post-treatment timepoints. (b) Representative NIR-II fluorescence images of living mice after i.v injection of CDIR2 ($1 \mu\text{mol kg}^{-1}$ body weight) at different post-treatment timepoints (24, 48 or 72 h). The control groups were treated with saline. (c) Blood concentration (%ID g^{-1}) decay of CDIR2 in living mice treated with saline or at $t = 24, 48$ or 72 h post-treatment with cisplatin. (d) NIR-II fluorescence intensities of kidneys and (e) bladder as a function of time post-injection of CDIR2 in living mice treated with saline or at $t = 24, 48$ or 72 h post-treatment with cisplatin. (f) The ratios of bladder-to-kidney intensity of CDIR2 as a function of time post-injection of CDIR2 in living mice treated with saline or at $t = 24, 48$ or 72 h post-treatment with cisplatin. Data in (c-f) are the mean $\pm$ SD. $n = 3$ independent mice.

was an antineoplastic drug with known nephrotoxicity. Cisplatin was intraperitoneally injected into living mice at a nephrotoxic dosage (20 mg kg^{-1} body weight),^[39] followed by i.v. injection of CDIR2 at different time points post-treatment with cisplatin (24, 48 and 72 h, Figure 4a). The control groups were treated with saline. Whole-body longitudinal NIR-II fluorescence imaging were conducted at different cisplatin post-treatment timepoints. The profile for the signal either in kidneys or bladder as a function of imaging time remained similar to saline-treated control group when cisplatin post-treatment time was no more than 48 h (Figure 4d&e). However, at 72 h post-treatment of cisplatin, the signal in the kidneys remained stable instead of gradually decreasing at 4 min post-injection of CDIR2 (Figure 4d), indicating CDIR2 retention in the kidneys. The signal in bladder was observed at 4 min post-injection of CDIR2 in control group but delayed after 20 min injection of CDIR2 in mice at 72 h post-treatment of cisplatin (Figure 4b) and the signals increased much slower (Figure 4e). The ratios of bladder-to-kidney intensity in mice after 72 h treatment of cisplatin were calculated to be 0.44 ± 0.04 and 1.10 ± 0.05 at 20 and 100 min post-injection of CDIR2, respectively, which were 2.0-fold and 2.45-fold lower relative to the control mice (0.87 ± 0.04 and 2.68 ± 0.12 at 20 and 100 min post-injection). Similarly, NIR-II fluorescence signals in the resected kidneys from mice at 72 h post-treatment of cisplatin was 2-fold higher than that of control mice (Figure S18). In addition, the blood elimination half-lives ($t_{1/2\beta}$) of CDIR2 in the mice treated with saline and the mice 24 or 48 h cisplatin treatment were similar (23 ± 3.3 min), but it prolonged to 41.4 ± 5.2 min for the mice 72 h cisplatin treatment (Figure 4c), confirming the decelerated clearance of CDIR2. This was due to the fact that tubules were damaged after 72 h treatment of cisplatin, leading to a significantly decrease in kidney function that slow down the glomerular filtration of CDIR2.

Figure 5. In vitro detection of kidney function with other assays. (a–b) Change in sCr and BUN in living mice after treated with saline or at $t = 24, 48$, or 72 h post-treatment of cisplatin (20 mg kg^{-1} body weight). (c) Determination of GFR and (d) the ratios of bladder-to-kidney intensity of CDIR2 in living mice after treated with saline or at $t = 24, 48$, or 72 h post-treatment of cisplatin. GFR was measured by the standard FITC-Inulin assay. Statistical analysis in (a–d): two-tailed student's *t*-test. Saline versus cisplatin-treated groups, $n=3$, mean $\pm$ s.d. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (e) Representative photomicrographs of H&E

staining in paraffin embedded kidney sections from mice after treatment of saline or after treatment of cisplatin for 24, 48, or 72 h. Green arrows and triangle indicate hyaline casts and loss of the brush border, respectively. (Scale bar = $85 \mu\text{m}$). Confocal fluorescence microscopy images of kidney slices from mice after treatment of saline or after treatment of cisplatin for 24, 48, or 72 h. The blue and green signals come from DAPI and caspase-3 antibody staining, respectively (Scale bar = $85 \mu\text{m}$).

Comparison with clinical diagnostic assays. To compare the kidney dysfunction detection ability of CDIR2 with the clinical methods, sCr and BUN in the blood of living mice as well as glomerular filtration rate (GFR) was measured using the commercial assays in control and cisplatin-treated mice. The first statistically significant increase in sCr and BUN was observed at 72 h post-treatment of cisplatin (Figure 5a&b), which was 2.2-fold and 2.0-fold higher than the control group, respectively. Such increased sCr and BUN levels were attributed to the decline in kidney function. This was further confirmed by the observation of $\sim 55\%$ decreases in GFR (Figure 5c) and $\sim 60\%$ decreases in the ratio of bladder-to-kidney intensity of CDIR2 (Figure 5d) at 72 h post-treatment of cisplatin. H&E staining showed normal tubular morphology when cisplatin post-treatment time was no more than 48 h, but the loss of the brush border and hyaline casts at 72 h post-treatment of cisplatin (Figure 5e). Moreover, immunofluorescence caspase-3 staining revealed that cell apoptosis was only observed for the kidneys at 72 h post-treatment of cisplatin (Figure 5e). These data were consistent with the above imaging results, suggesting CDIR2 is capable of noninvasively monitoring kidney dysfunction by NIR-II fluorescence imaging.

Conclusion

In summary, we have synthesized a NIR-II fluorescent molecular semiconductor (CDIR2) and demonstrated its application in noninvasive imaging of kidney dysfunction in murine model. CDIR2 not only had higher SBR than NIR-I molecules (ICG&CCD), but also possessed a high renal clearance efficiency ($\sim 90\%$ ID after 24 h injection), minimal in vivo metabolism and high biocompatibility. After systemic administration of CDIR2 into living mice, it is primarily cleared via glomerular filtration into urine without being reabsorbed and secreted in renal tubules. Such a unidirectional renal clearance pathway of CDIR2 permitted real-time, noninvasive monitoring of kidney dysfunction in living mice during progression of cisplatin-induced RI. The kidney dysfunction detection ability of CDIR2 is consistent with clinical diagnostic assays including sCr, BUN and GFR assays. Thus, CDIR2 not only holds great promise for noninvasive monitoring of kidney dysfunction in preclinical settings, but also permits investigation of nephrotoxicity and high-throughput drug screening in living animals. To the best of our knowledge, CDIR2 represents the first NIR-II fluorophore valid for real-time noninvasive assessment of kidney function in living animals.

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Keywords: kidney dysfunction • second near-infrared window • renal clearable • fluorescence imaging • molecular semiconductor

- [1] V. Jha, G. Garcia-Garcia, K. Iseki, Z. Li, S. Naicker, B. Plattner, R. Saran, A. Y. Wang, C. W. Yang, *Lancet* **2013**, *382*, 260-272.
- [2] L. S. Chawla, P. W. Eggers, R. A. Star, P. L. Kimmel, *N. Engl. J. Med.* **2014**, *371*, 58-66.
- [3] M. A. Perazella, S. G. Coca, *Nat. Rev. Nephrol.* **2013**, *9*, 484-490.
- [4] P. E. De Jong, D. de Zeeuw, *Lancet* **2005**, *365*, 913-914.
- [5] W. H. Beierwaltes, L. M. Harrison-Bernard, J. C. Sullivan, D. L. Mattson, *Compr. Physiol.* **2013**, *3*, 165-200.
- [6] S. Cui, H. T. Heller, S. S. Waikar, G. M. McMahon, *Kidney Int. Rep.* **2016**, *1*, 324-326.
- [7] a) F. Joutet, S. Walrand, K. S. Parreira, P. J. Courtoy, S. Pauwels, O. Devuyst, F. Jamar, *Am. J. Physiol. Renal. Physiol.* **2010**, *298*, F454-F460; b) D. Jiang, Z. Ge, H. J. Im, C. G. England, D. Ni, J. Hou, L. Zhang, C. J. Kuttyreff, Y. Yan, Y. Liu, S. Y. Cho, J. W. Engle, J. Shi, P. Huang, C. Fan, H. Yan, W. Cai, *Nat. Biomed. Eng.* **2018**, *2*, 865-877.
- [8] H. Kobayashi, S. Kawamoto, S. K. Jo, N. Sato, T. Saga, A. Hiraga, J. Konishi, S. Hu, K. Togashi, M. W. Brechbiel, R. A. Star, *Kidney Int.* **2002**, *61*, 1980-1985.
- [9] L. O. Lerman, M. Rodriguez-Porcel, J. C. Romero, *Kidney Int.* **1999**, *55*, 400-416.
- [10] J. P. Laissy, J. M. Idée, P. Fernandez, M. Floquet, F. Vrtovsniak, E. Schouman-Claeys, *Nephron Clin. Pract.* **2006**, *103*, 50-57.
- [11] K. Kalantarina, *Curr. Drug Targets* **2009**, *10*, 1184-1189.
- [12] P. H. Vivier, P. Storey, H. Rusinek, J. L. Zhang, A. Yamamoto, K. Tantiello, U. Khan, R. P. Lim, J. S. Babb, D. John, L. W. Teperman, H. Chandarana, K. Friedman, J. A. Benstein, E. Y. Skolnik, V. S. Lee, *Radiology* **2011**, *259*, 462-470.
- [13] a) J. K. Willmann, N. Van Bruggen, L. M. Dinkelborg, S. S. Gambhir, *Nat. Rev. Drug Discov.* **2008**, *7*, 591-607; b) S. Ye, J. J. Hu, D. Yang, *Angew. Chem. Int. Ed.* **2018**, *57*, 10173-10177; *Angew. Chem.* **2018**, *130*, 10330-10334.
- [14] a) Q. Miao, C. Xie, X. Zhen, Y. Lyu, H. Duan, X. Liu, J. V. Jokerst, K. Pu, *Nat. Biotechnol.* **2017**, *35*, 1102-1110; b) J. Li, K. Pu, *Chem. Soc. Rev.* **2019**, *48*, 38-71; c) P. Cheng, Q. Miao, J. Li, J. Huang, C. Xie, K. Pu, *J. Am. Chem. Soc.* **2019**, *141*, 10581-10584; d) X. Zhen, J. Zhang, J. Huang, C. Xie, Q. Miao, K. Pu, *Angew. Chem. Int. Ed.* **2018**, *57*, 7804-7808; *Angew. Chem.* **2018**, *130*, 7930-7934.
- [15] a) J. F. Lovell, C. S. Jin, E. Huynh, H. Jin, C. Kim, J. L. Rubinstein, W. C. Chan, W. Cao, L. V. Wang, G. Zheng, *Nat. Mater.* **2011**, *10*, 324-332; b) Q. Miao, D. C. Yeo, C. Wiraja, J. Zhang, X. Ning, C. Xu, K. Pu, *Angew. Chem. Int. Ed.* **2018**, *57*, 1256-1260; *Angew. Chem.* **2018**, *130*, 1270-1274.
- [16] a) G. Hong, A. L. Antaris, H. Dai, *Nat. Biomed. Eng.* **2017**, *1*, 0010; b) P. Cheng, J. Zhang, J. Huang, Q. Miao, C. Xu, K. Pu, *Chem. Sci.* **2018**, *9*, 6340-6347.
- [17] a) D. T. Quang, J. S. Kim, *Chem. Rev.* **2010**, *110*, 6280-6301; b) H. Li, X. Li, W. Shi, Y. Xu, H. Ma, *Angew. Chem. Int. Ed.* **2018**, *57*, 12830-12834; *Angew. Chem.* **2018**, *130*, 13012-13016.
- [18] H. S. Choi, W. Liu, P. Misra, E. Tanaka, J. P. Zimmer, B. I. Ipe, M. G. Bawendi, J. V. Frangioni, *Nat. Biotechnol.* **2007**, *25*, 1165-1170.
- [19] A. A. Burns, J. Vider, H. Ow, E. Herz, O. Penate-Medina, M. Baumgart, S. M. Larson, U. Wiesner, M. Bradbury, *Nano Lett.* **2008**, *9*, 442-448.
- [20] H. Kang, J. Gravier, K. Bao, H. Wada, J. H. Lee, Y. Baek, G. El Fakhri, S. Gioux, B. P. Rubin, J. L. Coll, H. S. Choi, *Adv. Mater.* **2016**, *28*, 8162-8168.
- [21] H. Huang, R. Hernandez, J. Geng, H. Sun, W. Song, F. Chen, S. A. Graves, R. J. Nickles, C. Cheng, W. Cai, J. F. Lovell, *Biomaterials* **2016**, *76*, 25-32.
- [22] B. Du, X. Jiang, A. Das, Q. Zhou, M. Yu, R. Jin, J. Zheng, *Nat. Nanotechnol.* **2017**, *12*, 1096-1102.
- [23] M. Yu, J. Zhou, B. Du, X. Ning, C. Authement, L. Gandee, P. Kapur, J. T. Hsieh, J. Zheng, *Angew. Chem. Int. Ed.* **2016**, *55*, 2787-2791; *Angew. Chem.* **2016**, *128*, 2837-2841.
- [24] J. Huang, J. Li, Y. Lyu, Q. Miao, K. Pu, *Nat. Mater.* **2019**, DOI: 10.1038/s41563-019-0378-4.
- [25] S. Wang, Y. Fan, D. Li, C. Sun, Z. Lei, L. Lu, T. Wang, F. Zhang, *Nat. Comm.* **2019**, *10*, 1058.
- [26] a) B. Li, L. Lu, M. Zhao, Z. Lei, F. Zhang, *Angew. Chem. Int. Ed.* **2018**, *57*, 7483-7487; *Angew. Chem.* **2018**, *130*, 7605-7609; b) L. Liu, S. Wang, B. Zhao, P. Pei, Y. Fan, X. Li, F. Zhang, *Angew. Chem. Int. Ed.* **2018**, *57*, 7518-7522; *Angew. Chem.* **2018**, *130*, 7640-7644; c) M. Zhao, R. Wang, B. Li, Y. Fan, Y. Wu, X. Zhu, F. Zhang, *Angew. Chem. Int. Ed.* **2019**, *58*, 2050-2054; *Angew. Chem.* **2019**, *131*, 2072-2076;
- [27] a) X. Hao, C. Li, Y. Zhang, H. Wang, G. Chen, M. Wang, Q. Wang, *Adv. Mater.* **2018**, *30*, 1804437; b) D. Huang, S. Lin, Q. Wang, Y. Zhang, C. Li, R. Ji, M. Wang, G. Chen, Q. Wang, *Adv. Funct. Mater.* **2019**, *29*, 1806546.
- [28] X. Jiang, C. Cao, W. Feng, F. Li, *J. Mater. Chem. B* **2016**, *4*, 87-95.
- [29] a) A. L. Antaris, H. Chen, K. Cheng, Y. Sun, G. Hong, C. Qu, S. Diao, Z. Deng, X. Hu, B. Zhang, X. Zhang, O. K. Yaghi, Z. R. Alamparambil, X. Hong, Z. Cheng, H. Dai, *Nat. Mater.* **2016**, *15*, 235-242; b) W. Wang, Z. Ma, S. Zhu, H. Wan, J. Yue, H. Ma, R. Ma, Q. Yang, Z. Wang, Q. Li, Y. Qian, C. Yue, Y. Wang, L. Fan, Y. Zhong, Y. Zhou, H. Gao, J. Ruan, Z. Hu, Y. Liang, H. Dai, *Adv. Mater.* **2018**, *30*, 1800106; c) A. Ruggiero, C. H. Villa, E. Bander, D. A. Rey, M. Bergkvist, C. A. Batt, K. Manova-Todorova, W. M. Deen, D. A. Scheinberg, M. R. McDevitt, *Proc. Natl. Acad. Sci. U S A* **2010**, *107*, 12369-12374.
- [30] a) Y. Yang, P. Wang, L. Lu, Y. Fan, C. Sun, L. Fan, C. Xu, A. M. El-Toni, M. Alhoshan, F. Zhang, *Anal. Chem.* **2018**, *90*, 7946-7952; b) Y. Li, X. Li, Z. Xue, M. Jiang, S. Zeng, J. Hao, *Biomaterials* **2018**, *169*, 35-44; c) Y. Chen, D. M. Montana, H. Wei, J. M. Cordero, M. Schneider, X. Le Guével, O. Chen, O. T. Bruns, M. G. Bawendi, *Nano Lett.* **2017**, *17*, 6330-6334.
- [31] Q. Yang, Z. Ma, H. Wang, B. Zhou, S. Zhu, Y. Zhong, J. Wang, H. Wan, A. Antaris, R. Ma, X. Zhang, J. Yang, X. Zhang, H. Sun, W. Liu, Y. Liang, H. Dai, *Adv. Mater.* **2017**, *29*, 1605497.
- [32] L. J. Carlson, S. E. Maccagnano, M. Zheng, J. Silcox, T. D. Krauss, *Nano Lett.* **2007**, *7*, 3698-3703.
- [33] X. D. Zhang, H. Wang, A. L. Antaris, L. Li, S. Diao, R. Ma, A. Nguyen, G. Hong, Z. Ma, J. Wang, S. Zhu, J. M. Castellano, T. Wyss-Coray, Y. Liang, J. Luo, H. Dai, *Adv. Mater.* **2016**, *28*, 6872-6879.
- [34] R. E. Tolls, J. M. Dille, *J. Urol.* **1955**, *74*, 197-201.
- [35] a) J. Huang, N. Gretz, S. Weinfurter, *Eur. J. Pharmacol.* **2016**, *790*, 92-98; b) J. Huang, S. Weinfurter, C. Daniele, R. Pieriacca, R. Federica, L. Della Ciana, J. Pill, N. Gretz, *Chem. Sci.* **2017**, *8*, 2652-2660.
- [36] a) W. Lee, R. B. Kim, *Annu. Rev. Pharmacol. Toxicol.* **2004**, *44*, 137-166; b) X. Xue, L. K. Gong, K. Maeda, Y. Luan, X. M. Qi, Y. Sugiyama, J. Ren, *Mol. Pharm.* **2011**, *8*, 2183-2192.
- [37] H. Ehrsson, I. Wallin, *Am. J. Pathol.* **2010**, *177*, 1573-1574.
- [38] S. H. Wright, W. H. Dantzler, *Physiol. Rev.* **2004**, *84*, 987-1049.
- [39] R. Galgamuwa, K. Hardy, J. E. Dahlstrom, A. C. Blackburn, E. Wium, M. Rooke, J. Y. Cappello, P. Tummala, H. R. Patel, A. Chuah, L. Tian, L. McMorrow, P. G. Board, A. Theodoratos, *J. Am. Soc. Nephrol.* **2016**, *27*, 3331-3344.

Entry for the Table of Contents

RESEARCH ARTICLE

A second near-infrared (NIR-II) fluorescent molecular semiconductor is synthesized. The fluorophore not only has a high renal clearance efficiency, but also undergoes unidirectional glomerular filtration into urine, enabling real-time NIR-II fluorescence imaging of kidney dysfunction.

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Pu*

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**Renal-clearable Molecular
Semiconductor For Second
Near-Infrared Fluorescence
Imaging of Kidney Dysfunction**