Supporting Information

to

Quantitative bioimaging by LA–ICP-MS: a methodological study on the distribution of Pt and Ru in viscera originating from cisplatin- and KP1339-treated mice

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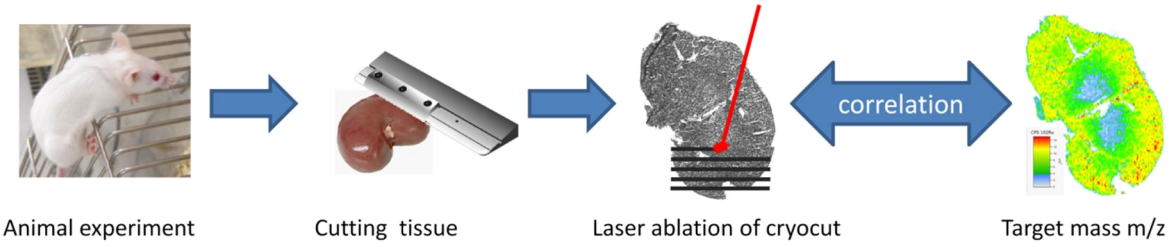

Scheme S1: Work-flow in mass spectrometry-based bioimaging experiments. The frozen animal tissue is cryocut into slices and ablated by a high energetic laser beam. The generated aerosol is transferred into the ICP-MS, recorded, and visualized in an intensity-dependent manner reflecting the shape of the ablated area.

Table S1: Conditions used for microwave-assisted acid digestion of samples and standards.

Step	t [min]	Max. E [W]	T [°C]
1	2	700	85
2	5	700	135
3	4	1000	180
4	12	1000	180
Ventilation	10	0	

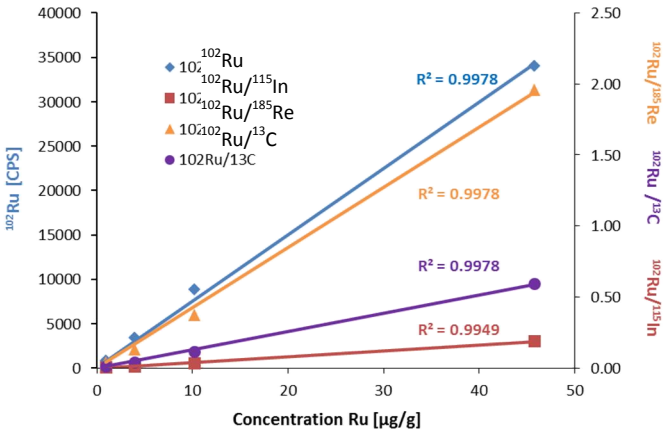

Figure S1: Comparison of the calibration curves for the quantification of Ru in tissue homogenates. The calculation is based on counts per second (CPS) of the registered isotope ^{102}Ru (♦, left ordinate) and compared with ^{102}Ru/^{195}Re (▲), ^{102}Ru/^{13}C (●) and ^{102}Ru/^{115}In (■), right ordinate.

Table S2: Comparison between theoretical Ru and Pt concentrations in spiked liver homogenates and experimentally determined values upon microwave digestion/ICP-MS.

	Concentration / [µg/g]		
Standard	**Pt, Ru (theoretical)**	**Pt (experimental)**	**Ru (experimental)**
1	0.98	0.79 ± 0.05	0.96 ± 0.03
5	4.96	3.17 ± 0.70	3.96 ± 0.08
10	9.70	8.93 ± 0.10	10.25 ± 0.77
50	45.53	39.24 ± 0.92	45.72 ± 1.01

Table S3: Precision and recovery of Ru and Pt, determined by LA-ICP-MS in two samples of homogenized liver (Homo1 and Homo2) originating from mice treated either with KP1339 or cisplatin, respectively. Recovery is referenced to microwave digestion of the sample followed by ICP-MS analysis. LA-ICP-MS data is based on averaged line scans. The range for the observed accuracy and precision for each element is given in bold numbers.

		^{102}Ru				^{195}Pt			
	Internal Standard	none	^{115}In	^{185}Re	^{13}C	none	^{115}In	^{185}Re	^{13}C
Homo1	**Recovery [%]**	93	**116**	106	97	119	81	105	91
	RSD [%]	4.9	3.3	**7.8**	4.7	9.4	12.1	10.7	9.8
Homo2	**Recovery [%]**	108	114	**92**	102	**120**	**70**	91	86
	RSD [%]	2.9	**2.8**	6.9	2.5	**14.6**	10.1	**7.6**	8.8

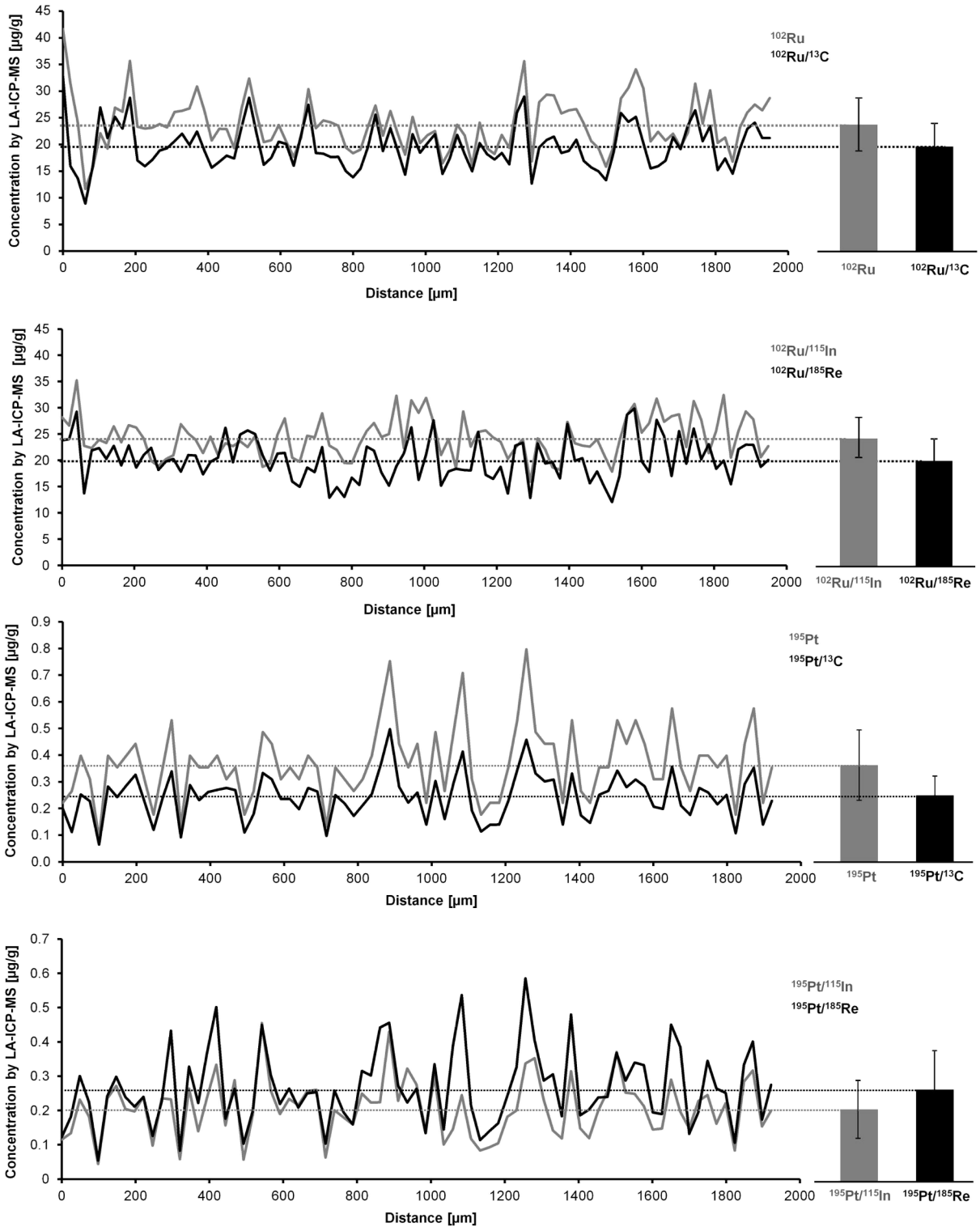

Figure S2: Concentrations of Ru and Pt along a single line-scan (1950 µm) as determined by LA–ICP-MS in homogenized mouse liver originating from mice treated with either KP1339 or cisplatin, respectively. The bar charts on the right represent the average and standard deviation of the line-scan. The target concentrations are 22.0 µg/g for Ru and 0.257 µg/g for Pt.

Table S4: Overview of studies dealing with LA-ICP-MS imaging of kidneys originating from cisplatin treated animals

Study	Animal	treatment	Sample preparation	LA-ICP-MS [µg/g]	verified
Zoriy [1]	NMRI mice	3 mg/kg (i.p.) 60 min	Cryocut, quantification via matrix matched standards	Cortex: 6-14 Medulla: ~26	No
Moreno-Gordaliza [2]	Wistar rats	5 mg/kg (i.p.) 5 d	FFPE, quantification by spiking standard onto control tissue slice	average: 14 medulla << cortex	No
		16 mg/kg (i.p.) 3 d		average: 144 medulla << cortex	
Reifschneider [3]	C57BL/6N mice	15 mg/kg 1 h	Bouin's fixative, embedded in polymerizing resin Technovit 7100, Standards prepared in Technovit 7100	Cortex: 85 Medulla << cortex	No
		15 mg/kg 4 d		Cortex: 2 Medulla << cortex	
this paper	Balb/C mice	15 mg/kg (i.p.) 24 h	Cryocut, quantification via matrix matched standards	Cortex: ~9 Medulla: <0.8	MW/ICP-MS: 11.2±0.6

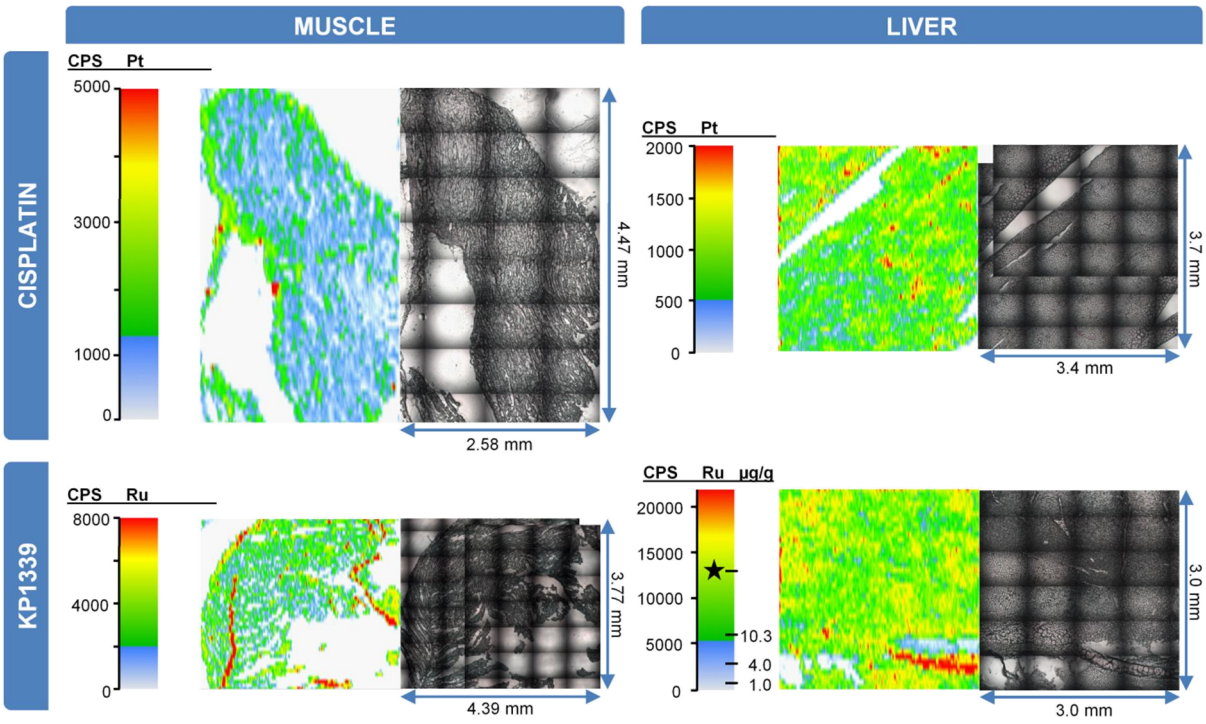

Figure S3: Distribution of ^{195}Pt and ^{102}Ru in liver and muscle of mice treated either with cisplatin or KP1339. Visualization was based on 44 and 48 parallel line scans (scan direction left to right, alignment of the lines from top to bottom) for Ru-containing viscera and 46 and 56 for Pt. The corresponding greyscale images were recorded with the built-in camera of the laser ablation system prior to ablation of the sample. The color scales represent the recorded counts per second (CPS) of the registered metal ion isotopes by LA–ICP-MS. Red colored areas of apparent Ru enrichment in liver and muscle are due to tissue duplication according to the histological image and the fact that their intensity is twice as high compared to neighbouring tissue. Quantitative amounts of Ru in liver, was obtained by ablating matrix matched standards within the same run. In this case, the concentration obtained by MW/ICP-MS was appropriately assigned to the color scale bar with an asterisk. Its corresponding color according to the scale is in good accordance with the color available in the picture obtained by LA–ICP-MS, proving validity of the method in real samples. Analysis of muscle was performed without simultaneous ablation of standards.

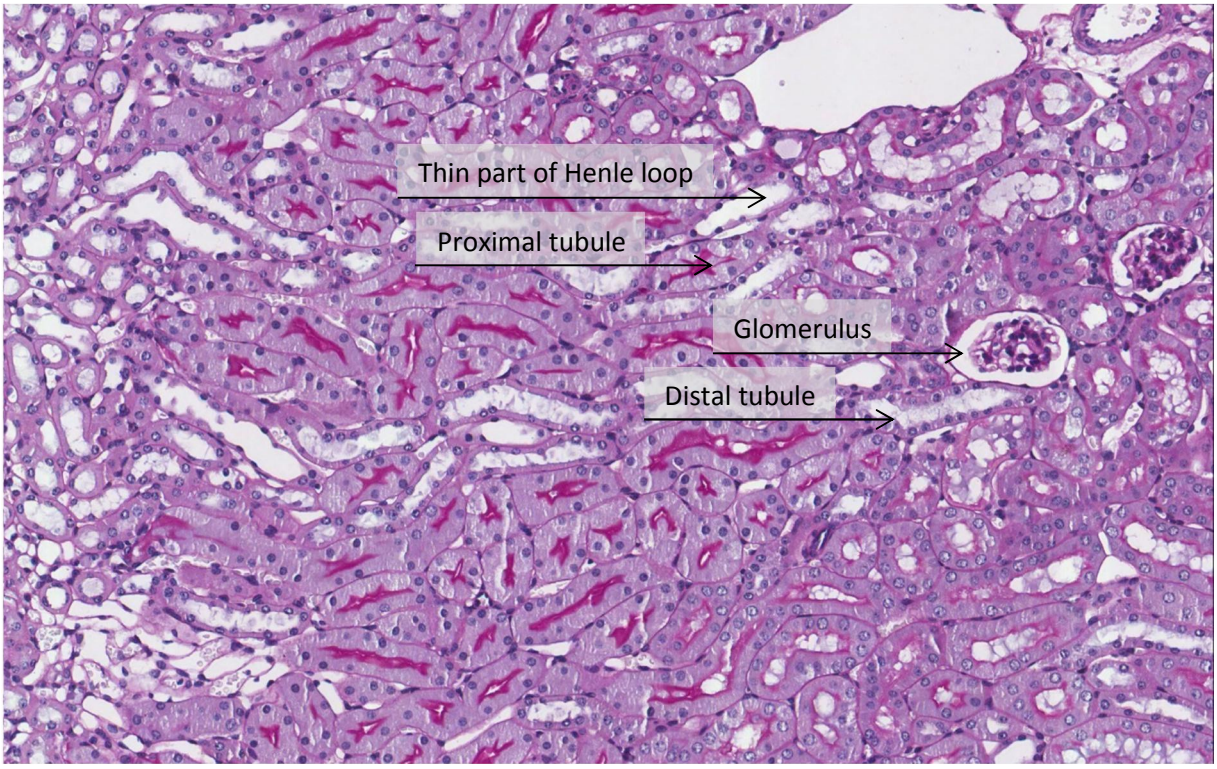

Figure S4: Kidney section (PAS staining) of a KP1339-treated mouse (50 mg/kg, i.v., 18 h).

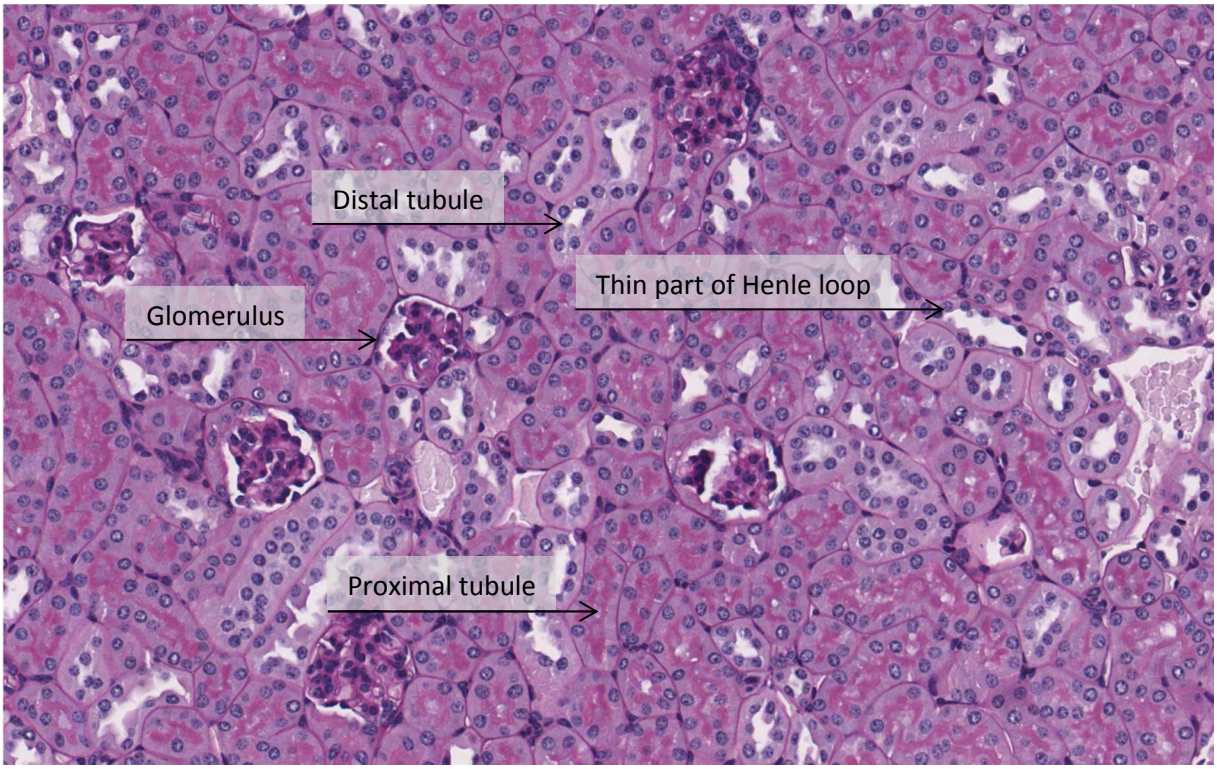

Figure S5: Kidney section (PAS staining) of a cisplatin-treated mouse (15 mg/kg, i.p., 24 h).

References

1. M. Zoriy, A. Matusch, T. Spruss and J. S. Becker, *Int. J. Mass Spectrom.*, 2007, **260**, 102-106.
2. E. Moreno-Gordaliza, C. Giesen, A. Lázaro, D. Esteban-Fernández, B. Humanes, B. Cañas, U. Panne, A. Tejedor, N. Jakubowski and M. M. Gómez-Gómez, *Anal. Chem.*, 2011, **83**, 7933-7940.
3. O. Reifschneider, C. A. Wehe, K. Diebold, C. Becker, M. Sperling and U. Karst, *J. Anal. At. Spectrom.*, 2013, **28**, 989-993.