

## Electronic Supplementary Information

Establishment of two complementary *in vitro* assays for radiocopper complexes achieving reliable and comparable evaluation of *in vivo* stability

Kristof Zarschler, Manja Kubeil, Holger Stephan

Helmholtz-Zentrum Dresden-Rossendorf, Institute of Radiopharmaceutical Cancer Research, Dresden, Germany

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## Supplemental materials

iTLC-SA and iTLC-SG plates were purchased from Agilent Technologies. Sodium chloride (Cat. # S7653), acrylamide/bis-acrylamide solution (Cat. # A3699), glycine (Cat. # G8898), sodium dodecyl sulphate (SDS; Cat. # L3771), *N,N,N',N'*-tetramethylethylenediamine (TEMED; Cat. # T9281), and ammonium persulfate (APS; Cat. # A3678) were supplied by Sigma-Aldrich. 2-Amino-2-hydroxymethyl-propane-1,3-diol (TRIS; Cat. # A2264) was purchased by AppliChem. Formic acid (Cat. # 100264) was gained from Merck KGaA.

## Supplemental methods

To investigate the linearity of the described serum assay, serum aliquots were thawed on ice and filtered using syringe filters with a pore size of 0.45 µm. Filtered serum (220 µL) was diluted with 1 M HEPES/NaOH buffer pH 7.4 (45 µL). Increasing initial activities of [<sup>64</sup>Cu]CuCl<sub>2</sub> in 100 mM MES/NaOH buffer pH 5.5 (100 µL, 2-14 MBq) were mixed with 1 M HEPES/NaOH buffer pH 8.0 (50 µL) to adjust the pH to 7.6. This solution (135 µL) was added to the diluted serum (265 µL) and incubated for 1 h at 37°C. Afterwards, 2×Laemmli sample buffer (400 µL) was added and the remaining activities were determined using ISOMED 2010. Importantly, no reducing agent was added to the Laemmli buffer and the samples were not heated. The mixtures were separated using non-reducing SDS-polyacrylamide gel electrophoresis (SDS-PAGE) with acrylamide concentrations of 5% in the stacking and 10% in the resolving gel. Two µL of each sample were loaded into each well of the gel. The SDS-PAGE was run at r.t. and 80 V until the dye front reached the resolving gel and then increased up to 140-160 V. After electrophoresis, the gel was washed for 1 min in H<sub>2</sub>O and exposed for 10 min to a reusable imaging plate (Fujifilm). Following electronic autoradiography using a radioluminography laser scanner, quantitative analysis of average band intensities was performed with the Advanced Image Data Analysis (AIDA) program.

**Table S1.** Radio-TLC conditions

[ <sup>64</sup> Cu]Cu-complex	R <sub>f</sub>	mobile Phase	stationary phase
TETA	0.9	0.9 % NaCl (w/v)	iTLC-SG
DOTA	0.9	0.9 % NaCl (w/v)	iTLC-SG
NOTA	1	0.9 % NaCl (w/v)	iTLC-SG
Cyclam	0	H <sub>2</sub> O + 0.1 % HCOOH	iTLC-SA
diamSar	0	H <sub>2</sub> O + 0.1 % HCOOH	iTLC-SA
EDTA	1	0.9 % NaCl (w/v)	iTLC-SG
NOTA-sdAb	0.2	0.9 % NaCl (w/v)	iTLC-SA

**Table S2.** Composition of the native polyacrylamide gel used for SOD challenge

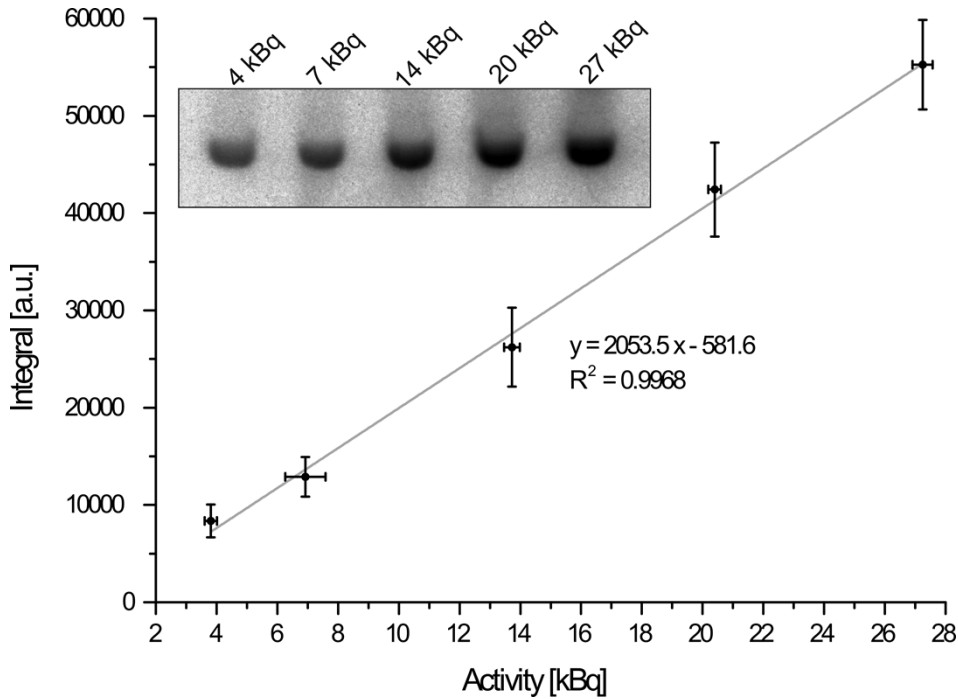
Ingredients	15 % resolving gel [mL]	5 % stacking gel [mL]
H <sub>2</sub> O	1.1	3.4
30 % acrylamide/bis-acrylamide solution (v/v)	2.5	0.83
1.5 M TRIS/HCl buffer (pH 8.8)	1.3	-
1.0 M TRIS/HCl buffer (pH 6.8)	-	0.63
10 % APS (w/v)	0.05	0.05
TEMED	0.002	0.002

**Table S3.** Composition of the SDS polyacrylamide gel used for serum assay

Ingredients	resolving gel [mL]		5 % stacking gel [mL]
	10 %	15 %	
H <sub>2</sub> O	1.9	1.1	3.4
30 % acrylamide/bis-acrylamide solution (v/v)	1.7	2.5	0.83
1.5 M TRIS/HCl buffer (pH 8.8)	1.3	1.3	-
1.0 M TRIS/HCl buffer (pH 6.8)	-	-	0.63
10 % APS (w/v)	0.05	0.05	0.05
TEMED	0.002	0.002	0.002
10 % SDS (w/v)	0.05	0.05	0.05

**Table S4.** Composition of the native and SDS PAGE running buffer

Ingredients	native running buffer	SDS running buffer
H <sub>2</sub> O	1 L	1 L
TRIS	15.1 g	15.1 g
Glycine	72 g	72 g
SDS	-	5 g



**Figure S1.** Linear dependence of radioluminographic blackening intensities (integral) and [<sup>64</sup>Cu]CuCl<sub>2</sub> activities. Increasing initial activities of [<sup>64</sup>Cu]CuCl<sub>2</sub> ranging from 2-14 MBq were subjected to serum assay and the experiment was performed as described in the supplemental methods section. The remaining activities of serum samples were determined before SDS-PAGE and converted to the effectively loaded sample volume. Each point represents the mean ± SD of three samples.