

Supplementary material for the article:

Nišavić, M.; Janjić, G. V.; Hozić, A.; Petković, M.; Milčić, M. K.; Vujčić, Z.; Cindrić, M.
Positive and Negative Nano-Electrospray Mass Spectrometry of Ruthenated Serum Albumin
Supported by Docking Studies: An Integrated Approach towards Defining Metallo drug
Binding Sites on Proteins. *Metallomics* **2018**, *10* (4), 587–594.
<https://doi.org/10.1039/c7mt00330g>

Supporting Information

Positive and negative nano-electrospray mass spectrometry of ruthenated serum albumin supported by docking studies: an integrated approach towards defining metallodrug binding sites on proteins

Marija Nišavić^a, Goran Janjić^b, Amela Hozić^c, Marijana Petković^a, Zoran Vujčić^d, Miloš Milčić^d, and Mario Cindrić^{c*}

^aDepartment of Physical Chemistry, “Vinča” Institute of Nuclear Sciences, University of Belgrade, Mike Petrovića Alasa 12-14, 11000 Belgrade, Serbia

^bInstitute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoseva 12, 11000 Belgrade, Serbia

^cCentre for Proteomics and Mass Spectrometry, Division of Molecular Medicine, Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia

^dFaculty of Chemistry, University of Belgrade, Studentski trg 12-14, 11000 Belgrade, Serbia

**Correspondence to: mario.cindric@irb.hr*

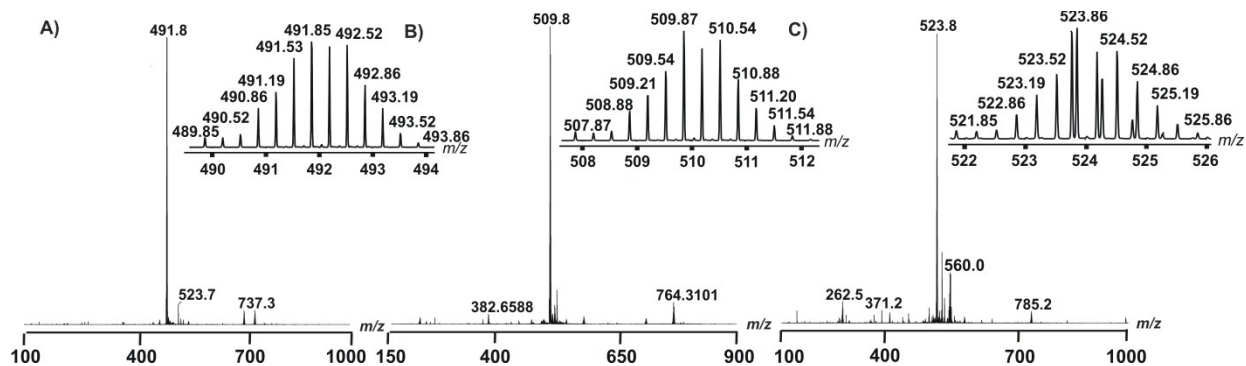


Fig. S1. Positive ion mode ESI MS spectra of angiotensin II adducts with compounds $[\text{Ru}(\text{Cl-tpy})(\text{en})\text{Cl}]^+$ (A), $[\text{Ru}(\text{Cl-tpy})(\text{dach})\text{Cl}]^+$ (B) and $[\text{Ru}(\text{Cl-tpy})(\text{bipy})\text{Cl}]^+$ (C). Inset in each spectrum shows isotopic distribution of triply charged ruthenated peptide. Additional peaks at m/z 737.3, 764.3 and 785.2 for compounds en, dach and bipy, respectively, correspond to doubly charged ruthenated angiotensin II.

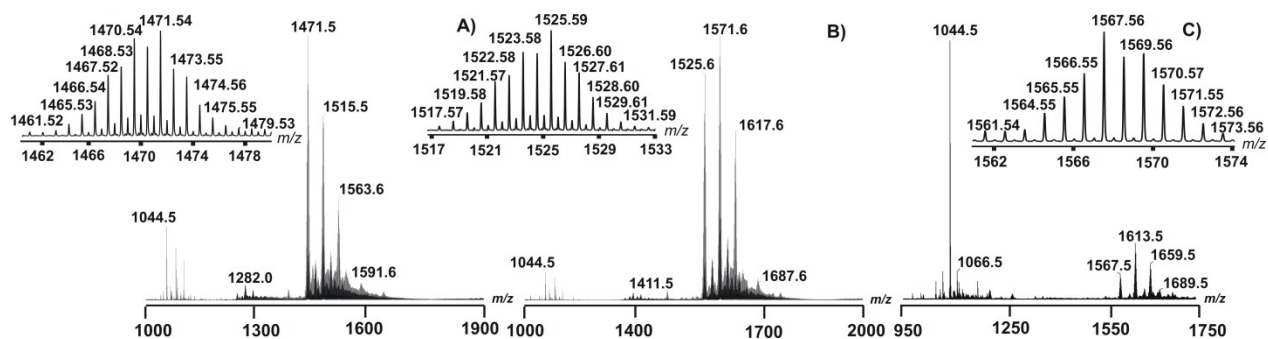


Fig. S2. Negative ion mode ESI MS spectra of angiotensin II adducts with compounds $[\text{Ru}(\text{Cl-tpy})(\text{en})\text{Cl}]^+$ (A), $[\text{Ru}(\text{Cl-tpy})(\text{dach})\text{Cl}]^+$ (B) and $[\text{Ru}(\text{Cl-tpy})(\text{bipy})\text{Cl}]^+$ (C). Inset in each spectrum shows isotopic distribution of singly charged ruthenated peptide. Two subsequent peaks in each spectrum (at m/z 1515.5 and 1563.6 for en compound, 1571.6 and 1617.6 for dach and 1613.5 and 1659.5 for bipy compound) represent mono- and di-formic acid adducts. Signal at 1044.5 in each spectrum correspond to free angiotensin II.

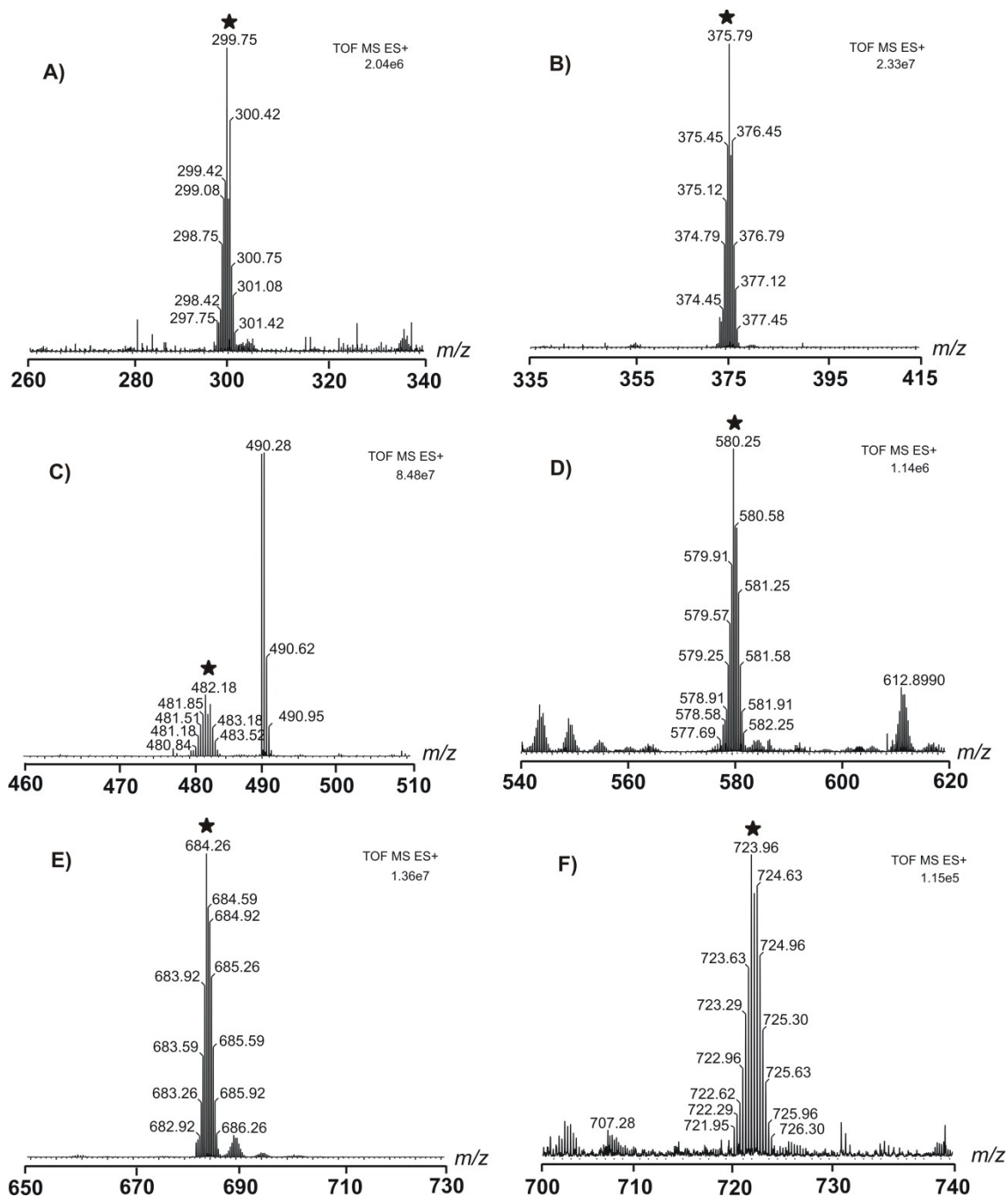


Fig. S3. Enlarged positive ion mode LE MS^E spectra showing [Ru(Cl-tpy)(en)]-bound HSA sequences: DAHK (A), SEVAHR (B), SLHTLFGDK (C), HPDYSVLLLLR (D), DVFLGMFLYEYAR (E) and HPHYAPELLFFAK (F). Target sequences are marked with a black asterisk in each spectrum.

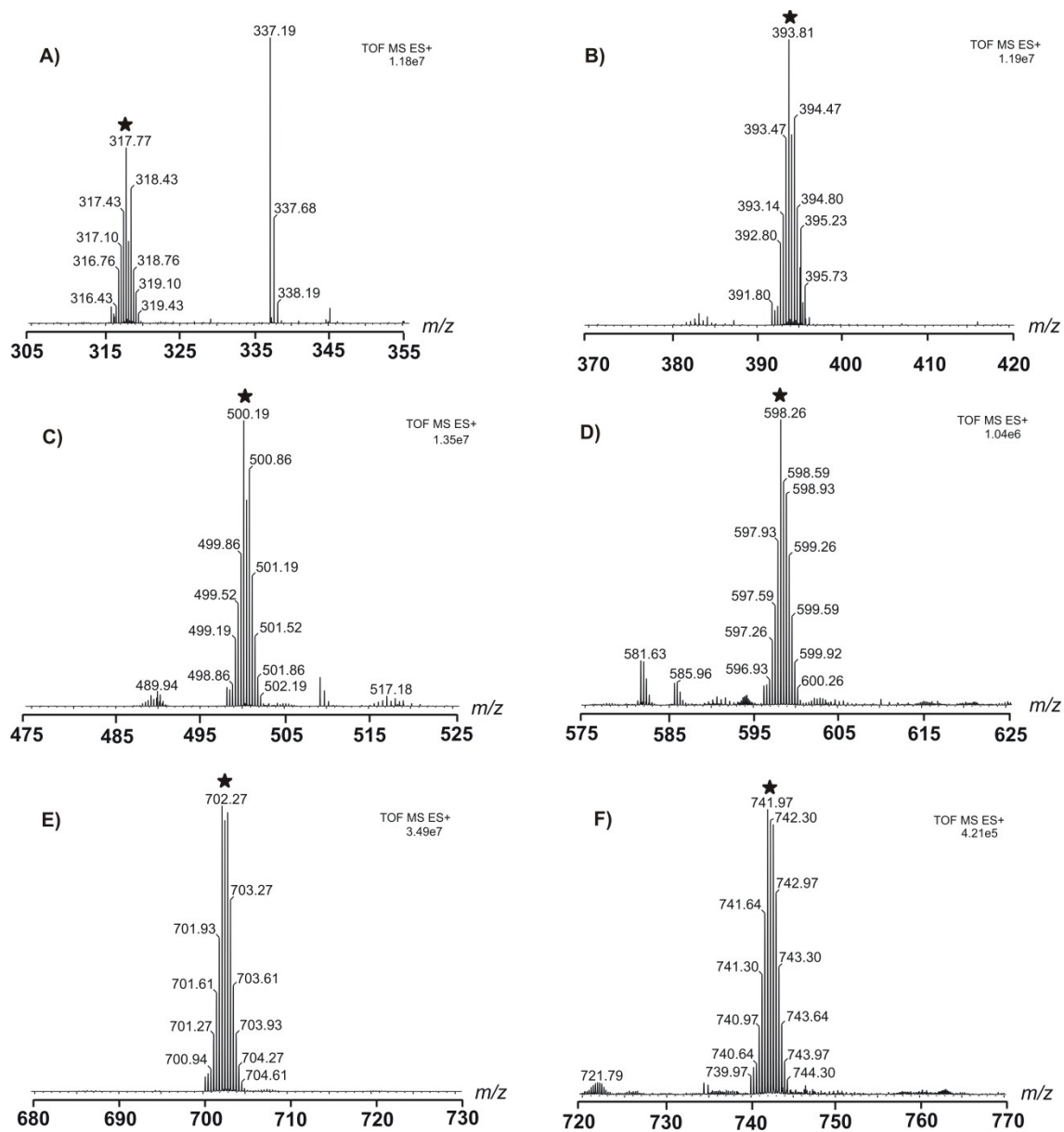


Fig. S4. Enlarged positive ion mode LE MS^E spectra showing [Ru(Cl-tpy)(dach)]-bound HSA sequences: DAHK (A), SEVAHR (B), SLHTLFGDK (C), HPDYSVLLLR (D), DVFLGMFLYEYAR (E) and HPYFYAPELLFFAK (F). Target sequences are marked with a black asterisk in each spectrum.

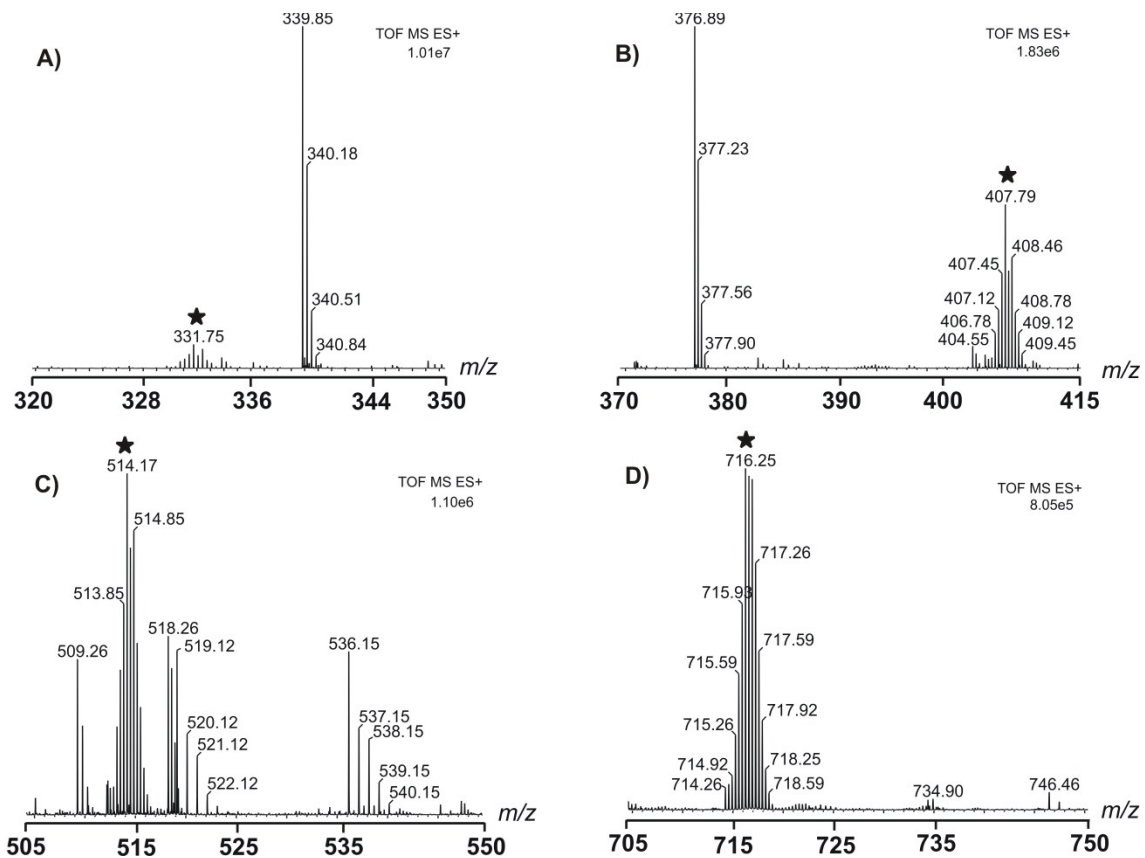


Fig. S5. Enlarged positive ion mode LE MS^E spectra showing [Ru(Cl-tpy)(bipy)]-bound HSA sequences: DAHK (A), SEVAHR (B), SLHTLFGDK (C) and DVFLGMFLYEYAR (D). Target sequences are marked with a black asterisk in each spectrum.

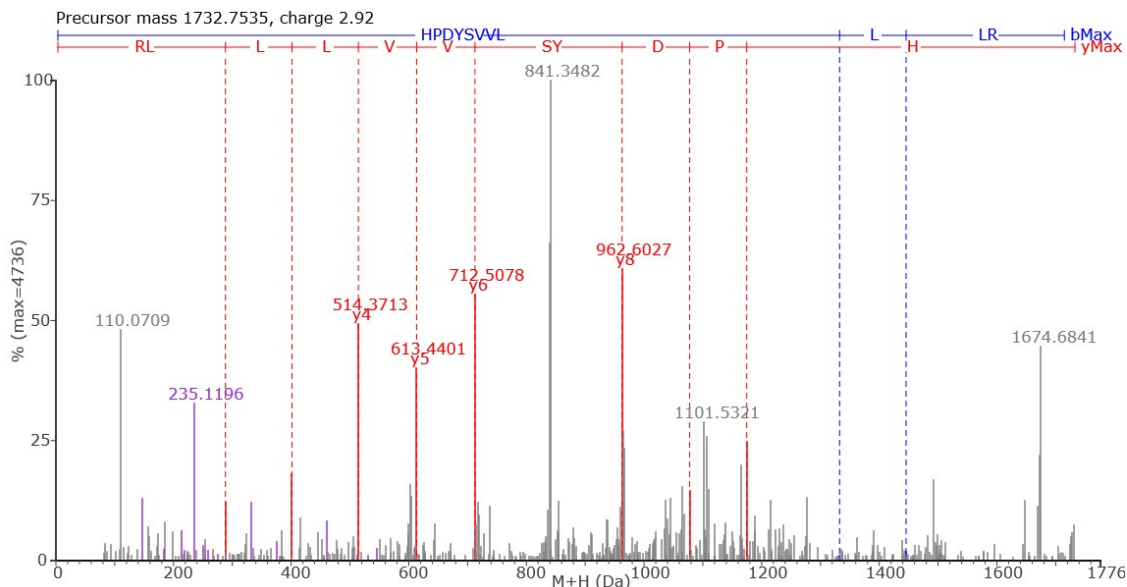


Fig. S6. PLGS generated HE MS^E spectrum of ³³⁸HPDYSVLLLR³⁴⁸ HSA sequence adduct with compound [Ru(Cl-tpy)(en)Cl]⁺. The identified precursor is triply positively charged ion with a mass of 1732 Da. The identified mass corresponds to the peptide adduct with compound [Ru(Cl-tpy)(en)Cl]⁺, after Cl ligand hydrolysis.

Table S1. PLGS software identified HE MS^E peptide fragment ions. marks neutral loss (H₂O, NH₃).

Peptide sequence	Number of ions	Fragment ion identity
⁵ SEVAHR ¹⁰	12	b ₂ , b ₃ , b ₃ , b ₄ , b ₄ , b ₅ , Y ₂ , Y ₃ , Y ₄ , Y ₅ , Y ₅ , Y ₆
⁶⁵ SLHTLFGDK ⁷³	9	Y ₂ , Y ₂ , Y ₃ , Y ₃ , Y ₄ , Y ₅ , Y ₆ , Y ₇ , Y ₇
¹⁴⁶ H <u>P</u> YFYAPELLFFAK ¹⁵⁹	10	b ₁₀ , Y ₂ , Y ₃ , Y ₄ , Y ₆ , Y ₇ , Y ₈ , Y ₁₂ , Y ₁₃ , Y ₁₄
³²⁴ D <u>V</u> FLGMFLYEYAR ³³⁶	7	b ₁ , b ₁₁ , Y ₈ , Y ₉ , Y ₁₀ , Y ₁₁ , Y ₁₂
³³⁸ H <u>P</u> DYSVLLLR ³⁴⁸	13	b ₂ , b ₅ , Y ₂ , Y ₃ , Y ₄ , Y ₅ , Y ₆ , Y ₇ , Y ₈ , Y ₈ , Y ₉ , Y ₁₀ , Y ₁₁

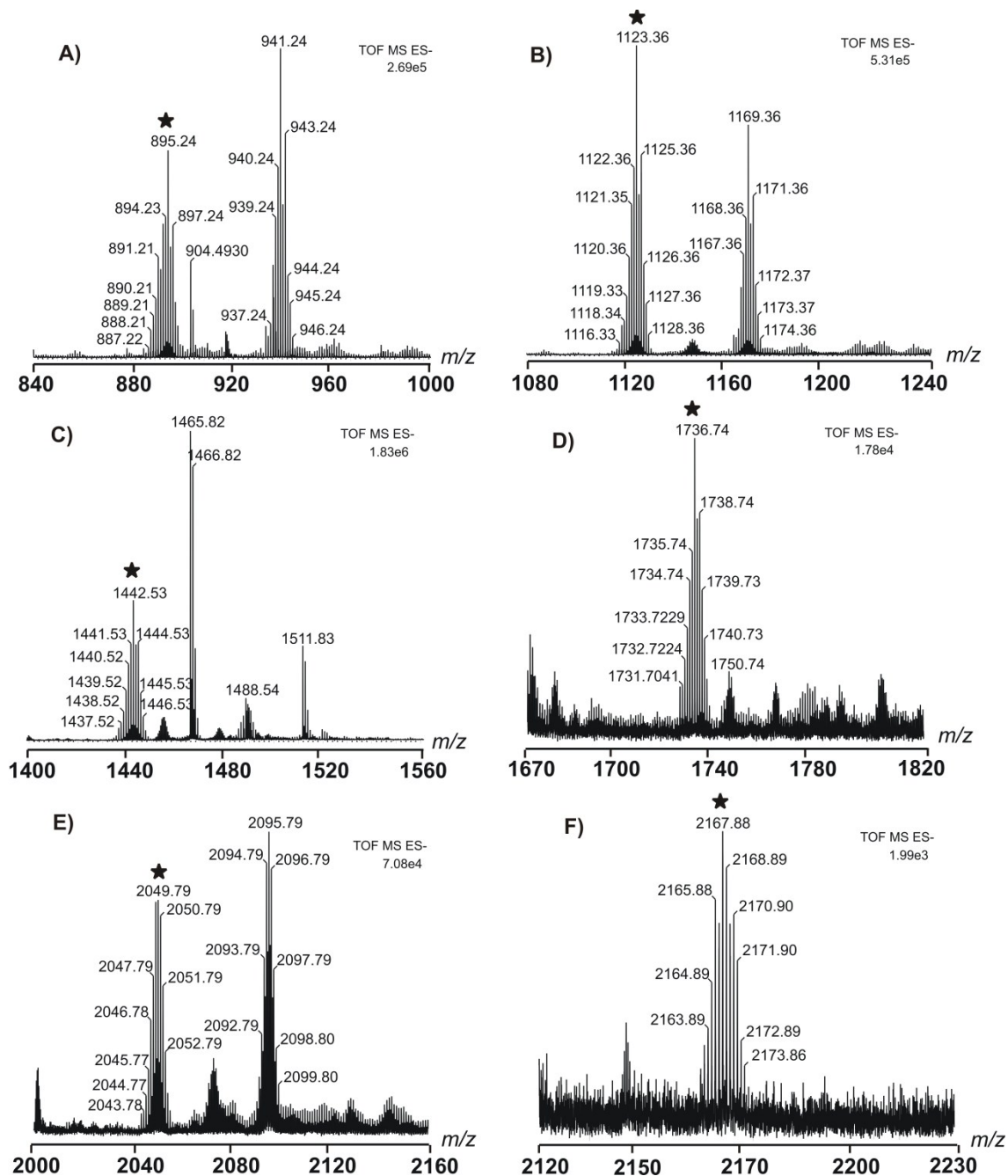


Fig. S7. Enlarged negative ion mode LE MS^E spectra showing [Ru(Cl-tpy)(en)]-bound HSA sequences: DAHK (A), SEVAHR (B), SLHTLFGDK (C), HPDYSVLLLR (D), DVFLGMFLYEYAR (E) and HPYFYAPELLFFAK (F). Target sequences are marked with a black asterisk in each spectrum.

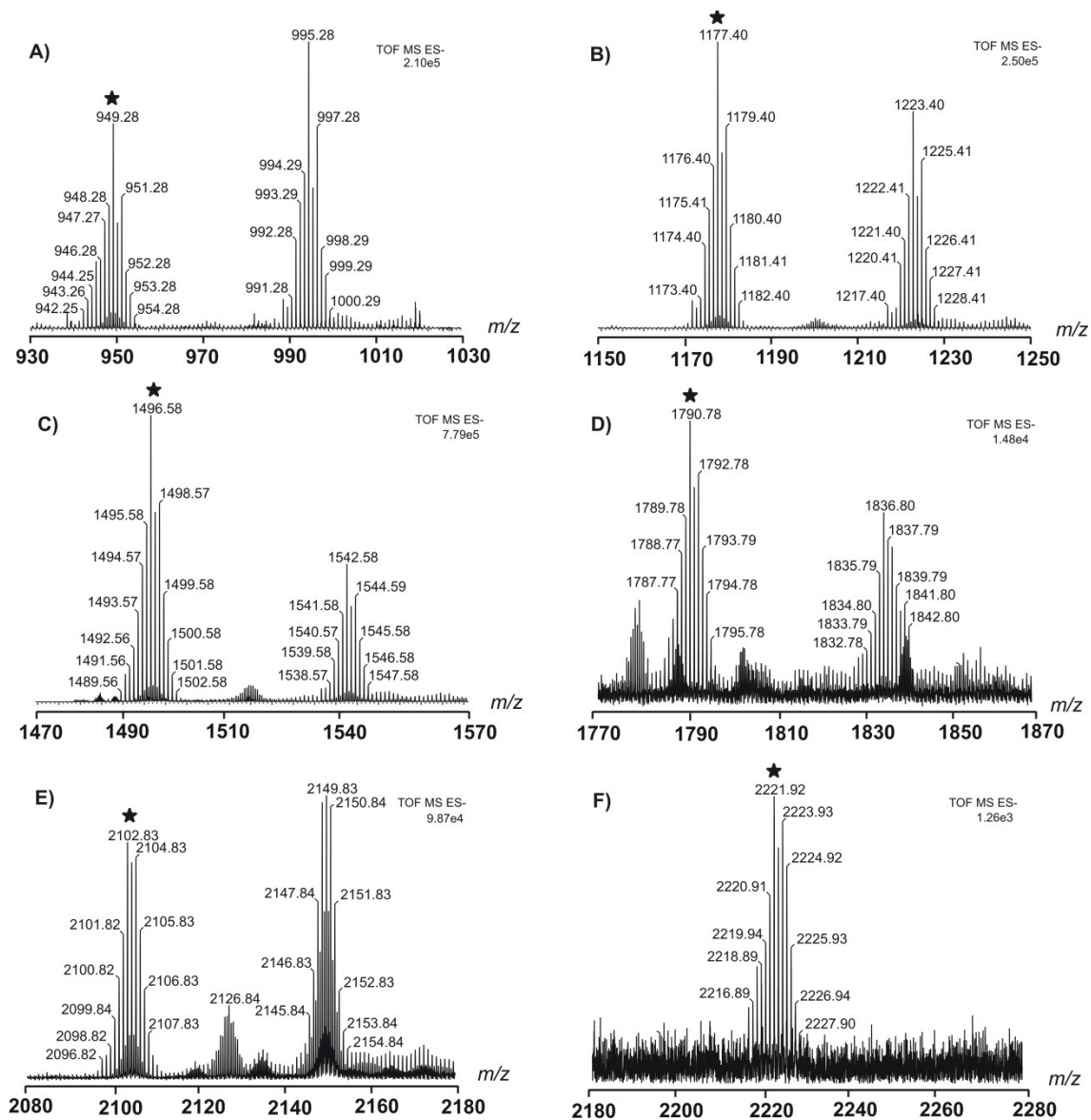


Fig. S8. Enlarged negative ion mode LE MS^E spectra showing [Ru(Cl-tpy)(dach)]-bound HSA sequences: DAHK (A), SEVAHR (B), SLHTLFGDK (C), HPDYSVLLLR (D), DVFLGMFLYEYAR (E) and HPYFYAPELLFFAK (F). Target sequences are marked with a black asterisk in each spectrum.

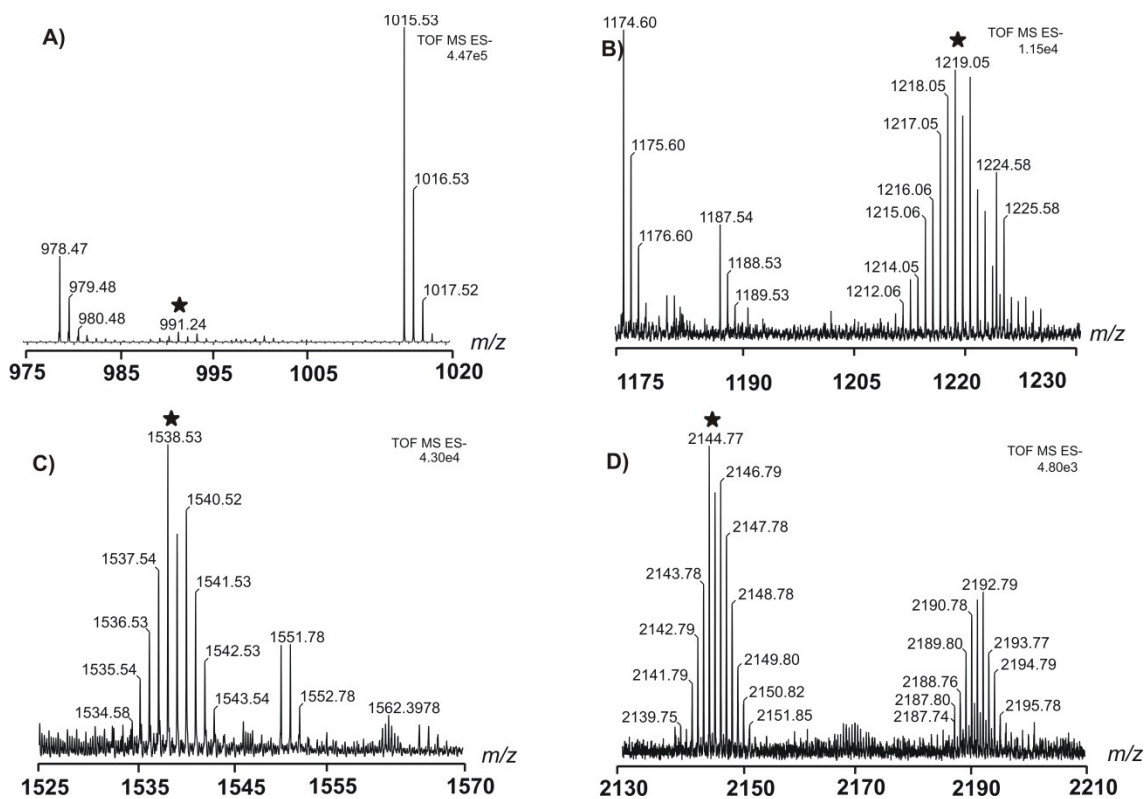


Fig. S9. Enlarged negative ion mode LE MS^E spectra showing [Ru(Cl-tpy)(dach)]-bound HSA sequences: DAHK (A), SEVAHR (B), SLHTLFGDK (C) and DVFLGMFLYEYAR (D). Target sequences are marked with a black asterisk in each spectrum.

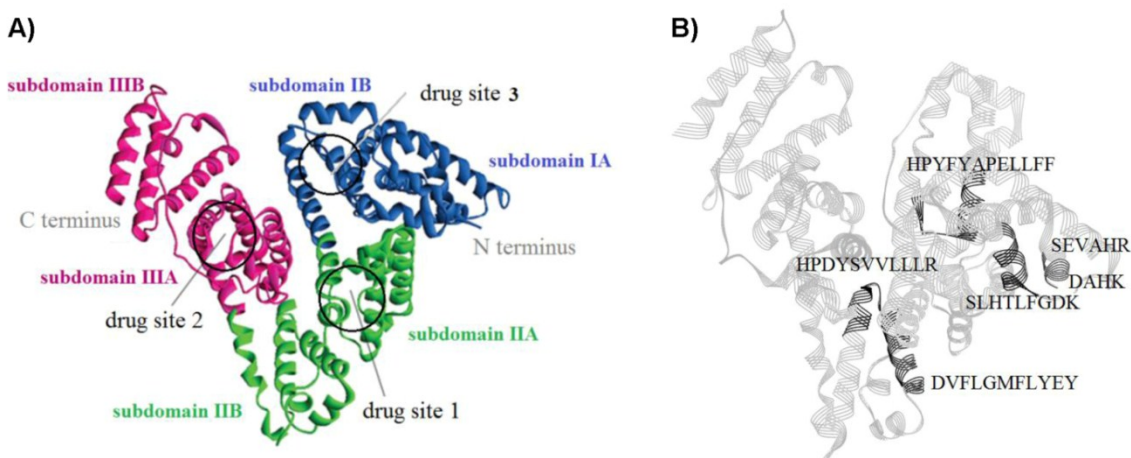


Fig. S10. HSA structure with major drug binding sites (A) and spatial localisation of MS-identified sequences for the binding of compounds [Ru(Cl-tpy)(en)Cl]⁺, [Ru(Cl-tpy)(dach)Cl]⁺ and [Ru(Cl-tpy)(bipy)Cl]⁺.

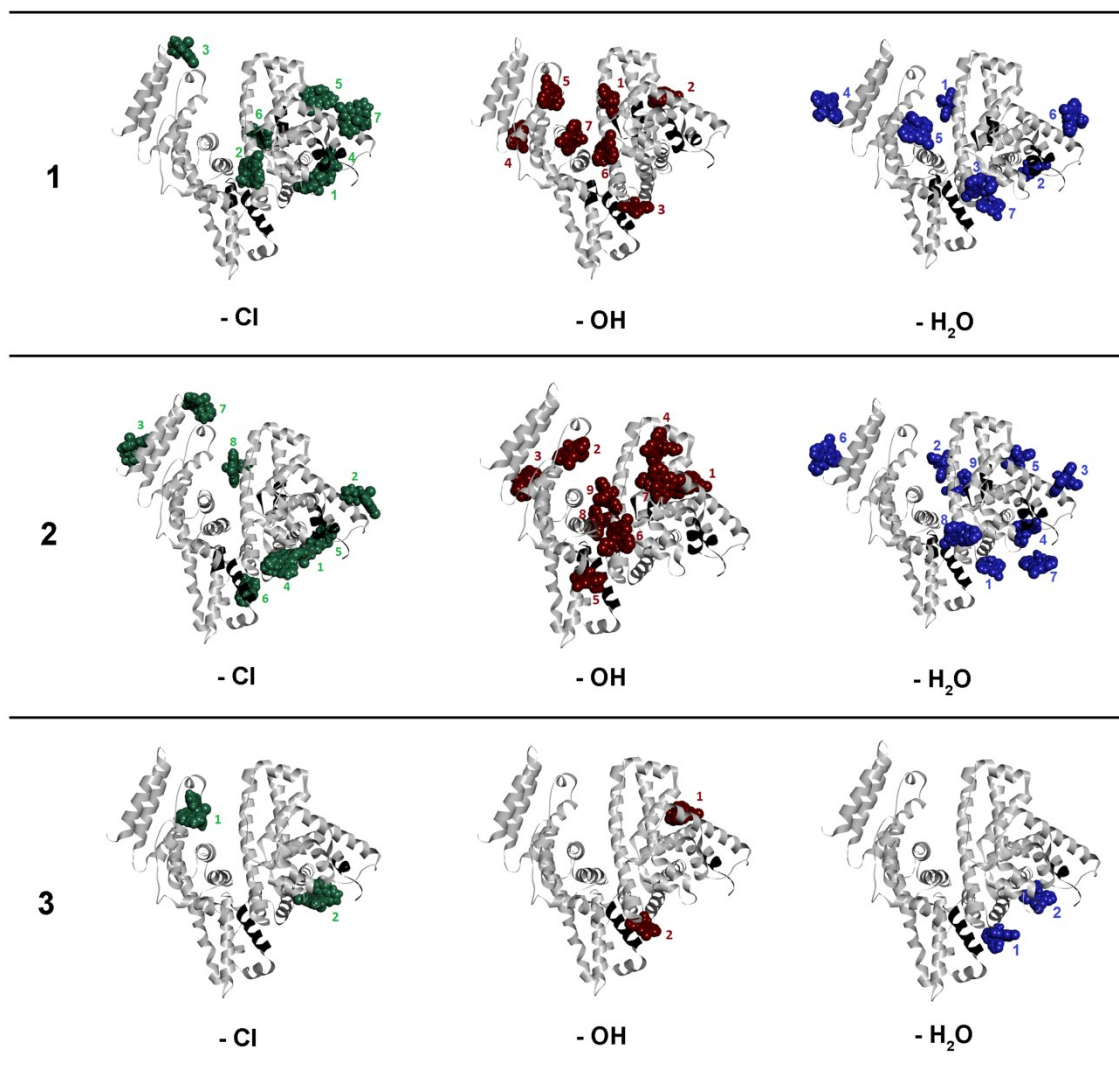


Fig. S11. HSA binding sites for chloro, hydroxo and aqua forms of compounds $[\text{Ru}(\text{Cl-tpy})(\text{en})\text{Cl}]^+$, $[\text{Ru}(\text{Cl-tpy})(\text{dach})\text{Cl}]^+$ and $[\text{Ru}(\text{Cl-tpy})(\text{bipy})\text{Cl}]^+$. Chloro forms are shown green, hydroxo red and aqua forms are shown blue. Target MS-identified HSA sequences are highlighted black.

Table S3. Binding energies of chloro, hydroxo and aqua forms of compounds $[\text{Ru}(\text{Cl-tpy})(\text{en})\text{Cl}]^+$, $[\text{Ru}(\text{Cl-tpy})(\text{dach})\text{Cl}]^+$ and $[\text{Ru}(\text{Cl-tpy})(\text{bipy})\text{Cl}]^+$, for each HSA binding site. Binding energies of chloro complexes that correspond to MS-identified sequences are highlighted green, while hydroxo complexes are marked red. The remaining binding energy values that most probably correspond to non-covalent interactions are black.

Ru(II) compound	Binding site No	Binding energy (kcal/mol)		
		-Cl	-OH	-H ₂ O
1 <i>[RuL(4'-Cl-tpy)(en)]</i>	1	-8,12	-7,80	-7,90
	2	-7,27	-6,32	-7,83
	3	-7,22	-5,84	-7,40
	4	-7,09	-5,77	-
	5	-6,99	-5,42	-
	6	-6,83	-5,34	-
	7	-6,74	-5,22	-
2 <i>[RuL(4'-Cl-tpy)(dach)]</i>	1	-9,14	-8,56	-3,02
	2	-8,67	-7,75	-2,95
	3	-8,53	-7,08	-2,73
	4	-8,53	-6,93	-2,66
	5	-8,38	-6,82	-
	6	-8,37	-6,77	-
	7	-7,46	-6,55	-
	8	-7,18	-6,48	-
	9	-	-6,47	-
3 <i>[RuL(4'-Cl-tpy)(bipy)]</i>	1	-7,97	-6,33	-1,78
	2	-6,98	-5,84	-1,44