Electronic Supporting Information

Gold finger formation studied by high-resolution mass spectrometry and *in silico* methods

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Experimental Section

Materials

The gold compound Auphen was prepared according to previously reported procedures (B. P. Block and J. C. Bailar, *J. Am. Chem. Soc.*, 1951, 73, 4722-4725). Auranofin was purchased from Alexis Biochemicals (San Diego, CA, USA).

High-resolution Mass Spectrometry

The peptidePYKCPECGKSFSQKSDLVKHQRTHTG (ZF2) was purchased from Peptide Specialty Laboratories GmbH (Heidelberg, Germany). The apo-zinc-finger peptide was dissolved in 25 mM $(NH_4)_2CO_3$ buffer (pH 7.4) to a stock concentration of 500 μ M. The disulfide bonds were reduced using 3 molar equivalents of dithiothreitol (DTT) for 2 hours at room temperature. Auphen (10 mM) and Zn acetate (100 mM) stock solutions were prepared using MilliQ water and stored at -20°C, while auranofin stock solution (10 mM) was freshly prepared in ethanol prior to use. Thus, apo-ZF2-AuPhen and apo-ZF2-auranofin adducts were prepared by diluting 5 μ L of the ZF2 stock solution with 44.3 μ L of 25 mM (NH₄)₂CO₃ buffer (pH 7.4) and adding 0.75 μ L of Auphen or auranofin stock solution, respectively. This allowed reaching a metal compound:peptide ratio of 3:1 in each sample. Afterwards, samples were incubated for 5 min at room temperature prior analysis.

ZF2-Zn was prepared by mixing 5 μ L of ZF2 stock, 7.5 μ L of 1 mM Zn²⁺ and 37.5 μ L of 25 mM (NH₄)₂CO₃ buffer (pH 7.4). Reactions with gold coordination complexes were performed by adding 5 μ L of ZF2 stock, 7.5 μ L of 1 mM Zn²⁺, 0.75 μ L of 10 mM Auphen or auranofin, to 36.8 μ L of 25 mM (NH₄)₂CO₃ buffer (pH 7.4). The mixtures were incubated for at least 15 min at room temperature.

After incubation, samples were diluted 100-fold with MilliQ water and ionized using a TriVersaNanomatenanospray system (Advion Biosciences, Ithaca, NY), with a spraying potential of 1.6 kV and 1.5 psi gas pressure. The resulting ions were analyzed using an Orbitrap Elite ETD FT mass spectrometer (Thermo Scientific, Bremen, Germany) at a resolution setting of 60,000 (at m/z 400).

Computational studies

Calculations on metal-core models (Figure S5) were performed employing the Gaussian 09 (G09) program¹ together with the DFT method. The B3LYP²/SDD³/6-31+G**⁴ combination was chosen and optimization were run in the gas phase and the nature of all stationary points was confirmed by normal mode analysis.

Geometry optimizations on the Zn- and Au-fingers were performed using the two-layer ONIOM method⁵⁻ ⁷ implemented in G09. The quantum mechanical (QM) part of the system was modeled with the DFT method, employing the B3LYP² functional in conjunction with the SDD³ effective core potential basis set for the Au and Zn atoms and the 6-31G^{*4} basis set for the remaining atoms. The molecular mechanical (MM) part was treated with the AMBER force field.⁸ The boundary between QM and MM regions was treated using link hydrogen atoms. The metal ions were represented by a bonded model. Equilibrium angles and bond distances involving Zn and Au were taken from the QM calculation performed on metalcore models (Table S1). The force constants for bond and angle for the atoms bonded to Zn were taken from the zinc AMBER force field.⁹ The Au-ligand parameters (bond stretching and angle bending) were estimated analyzing the frequencies obtained via QM calculation performed on the corresponding metalcore models. The remaining amino acids were treated with standard amber ff99 parameters.¹⁰ The starting coordinates for the Cys₂His₂ protein models were derived from the structure (PDB accession code 1ZNF) of the synthetic peptide corresponding to the 31st zinc finger from the Xenopus protein Xfin (Xfin-31), solved by solution NMR structure determination.¹¹ This peptide has comparable dimensions and similar sequence (Ace-YKCGLCERSFVEKSALSRHQRVHKD-NH₂) to the ZF2 peptide used for experimental work (PYKCPECGKSFSQKSDLVKHQRTHTG). The Cys₂HisCys models where obtained by substituting the His23 with the Cys23 in the corresponding Cys₂His₂ structures.

The different stages of the QM/MM study (file preparation, job monitoring and results analysis) were performed using the TAO package.¹²

Molecular graphics images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR001081).¹³

Figures



Figure S1. ESI Orbitrap FTMS mass spectrum of the reduced ZF2 peptide in the m/z region of the 3+ charge state. The inset shows the isotopic envelope of the experimentally observed peptide and the simulated pattern of the reduced form with elemental composition $(C_{127}H_{204}N_{39}O_{39}S_2)^{3+}$.



Figure S2. ESI Orbitrap FTMS mass spectrum of the ZF2-Auphen complex obtained after 5 minutes incubation time. The inset shows the experimental isotopic pattern (top panel), and the simulated isotopic patterns of the $(C_{127}H_{201}N_{39}O_{39}S_2Au)^{3+}$ (bottom panel) and $(C_{127}H_{203}N_{39}O_{39}S_2Au)^{3+}$ (middle panel) compositions, corresponding to metal charge states of Au(III) and Au(I), respectively.



Figure S3. ESI Orbitrap FTMS mass spectrum of the ZF2-auranofin complex obtained after 5 min incubation time. The inset shows the experimental isotopic pattern (top panel), and the simulated isotopic patterns of the $(C_{127}H_{201}N_{39}O_{39}S_2Au)^{3+}$ (bottom panel) and $(C_{127}H_{203}N_{39}O_{39}S_2Au)^{3+}$ (middle panel) compositions of cations, corresponding to metal charge states of Au(III) and Au(I), respectively.



Figure S4. ESI Orbitrap FTMS mass spectrum of the ZF2-Zn complex obtained after 5 minutes incubation time. The inset shows the experimental (top panel) and simulated (bottom panel) isotopic patterns of the $(C_{127}H_{202}N_{39}O_{39}S_2Zn(II))^{3+}$ composition peptide cation.



Figure S5. Metal-core models optimized by DFT method at the B3LYP/SDD/6-31+G** level (Cym = deprotonated cysteine (anionic thiolate)).



Figure S6. Overlap between the calculated ZF structure **2a** (light blue) and the experimental one obtained by NMR (**1**) (brown).



Figure S7. Highlight of the weak van der Waals contacts between the Au^+ and the uncoordinated His23 (3.733 Å) and the Cys3 (4.669 Å) in **3a** and **3b**.



Figure S8. Calculated structures of Au(I)-GFs **3c** where the Au(I) center (yellow sphere) is coordinated by the Cys3 and Cys6 residues.



Figure S9. Overlap between the calculated structures of the ZF **2a** (brown) and the Au(I)-GF **3c** (light blue). Zn(II) is represented as grey sphere and Au(III) as yellow sphere.



Figure S10. Overlap between the calculated structures of the Au(I)-GF **3a** (brown) and the Au(I)-GF **3c** (light blue). Au(I) ions are represented as yellow spheres.



Figure S11. Calculated structures of Au(I)-GFs 5a (left) and 5b (right) bearing a protonated Cys23.



Figure S12. Overlap between the calculated structures of the ZF **2b** (brown) and the Au(III)-GF **4b** (light blue). Zn(II) is represented as grey sphere and Au(III) as yellow sphere.

	M–N _{His19}	M–N _{His23}	M-S _{Cys3}	M-S _{Cys6}	N_{His19} – M – N_{His23}	S _{Cys3} -M-S _{Cys6}	N _{His19} -M-S _{Cys6}	N _{His23} -M-S _{Cys3}
2a	2.112	2.167	2.458	2.278	97.3	116.2	130.9	93.1
3a	2.143	-	-	2.307	-	-	177.8	-
4 a	2.175	2.272	2.473	2.396	92.2	82.9	173.7	154.2
5a	2.131	-	-	2.335	-	-	165.2	-
	MN	N I NI	MS	MS	NM.N.a.	SaM-Sa	N MS	NM_S
	IVI-INHis19	IVI-IN _{Cys23}	IVI-S _{Cys3}	WI-SCys6	THis19-IVI-I Cys23	SCys3 IVI SCys6	His19-IVI-SCys6	Cys23-IVI-SCys3
2b	2.107	2.482	2.400	2.324	84.6	120.7	119.7	83.4
2b 3b	2.107 2.122	2.482 -	2.400	2.324 2.319	84.6	120.7	119.7 165.4	83.4
2b 3b 4b	2.107 2.122 2.166	2.482 - 2.428	2.400 - 2.508	2.324 2.319 2.406	84.6 - 88.6	120.7 - 81.9	119.7 165.4 101.8	83.4 - 89.2

Table S1. Selected bond distances (Å) and angles (degree) obtained from the QM/MM calculation.

Table S2. Selected bond distances (Å) and angles (degree) obtained from the QM calculation performed on metal-core models.*

	M–N1	M–N2	M–S3	M–S4	N1-M-N2	S3-M-S4	N1-M-S4	N2-M-S3					
[M(Cym) ₂ (His) ₂]													
[Zn(Cym) ₂ (His) ₂]	2.149	2.118	2.352	2.299	102.2	127.2	103.5	101.1					
[Au(Cym)(His)]	2.125	-	2.305	-	-	-	177.5	-					
[Au(Cym) ₂]⁻	-	-	2.364	2.365	-	178.4	-	-					
[Au(Cym) ₂ (His) ₂] ⁺	2.172	2.169	2.357	2.359	91.7	90.7	176.4	177.7					
[M(Cys) ₂ (His) ₂]													
$[Zn(Cys)_2(His)_2]^{2+}$	1.991	2.007	2.507	2.451	116.2	97.7	116.0	103.9					
[Au(His) ₂] ⁺	2.050	2.046	-	-	179.6	-	-	-					
[Au(Cys) ₂ His) ₂] ³⁺	2.106	2.108	2.462	2.453	91.1	90.3	176.8	174.9					
	M–N1	M–S2	M–S3	M–S4	N1-M-S2	S3-M-S4	N1-M-S4	S2-M-S3					
[M(Cym) ₃ (His)]													
[Zn(Cym) ₃ (His)] ⁻	2.196	2.349	2.469	2.340	95.9	113.1	100.6	120.7					
[Au(Cym) ₃ (His)]	2.160	2.428	2.361	2.429	85.9	90.2	176.1	174.4					

* Cym = deprotonated cysteine(anionic thiolate)

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