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# Diversity in Gold Finger Structure Elucidated by Traveling-Wave Ion Mobility Mass Spectrometry

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*In Memory of C. Frank Shaw III*

***Abstract:*** *Traveling wave ion mobility (TWIM) mass spectrometry (MS) is a powerful method for the structural and conformational analysis of proteins and peptides, enabling the differentiation of isomeric peptides (or proteins) that have the same sequence but are modified at different residues. In this study, the TWIM-MS technique was used to separate isomeric $Au^{I}$ metallopeptide ions that were formed by $Zn^{II}$ displacement from the parent zinc fingers (ZFs). The synthetic gold finger peptides were derived from the C-terminus of the HIV nucleocapsid p7 protein (NCp7-F2) and finger 3 of the Sp1 transcription factor (Sp1-F3). TWIM-MS enabled the acquisition of distinct product ion spectra for each isomer, clearly indicating the binding sites for the major conformers in the presence of multiple coordination possibilities. Collision cross-section measurements showed that the aurated peptide has a slightly more compact structure than the parent zinc compound NCp7-F2, which showed only one conformation.*

The identification of metal-binding sites in metalloproteins has relied predominantly on a combination of solid-state X-ray crystallography and solution NMR spectroscopy techniques. With the development of soft ionization techniques such as ESI and MALDI, mass spectrometry has emerged as an important technique for studying metallodrugs in complex biological samples and for characterizing their interactions with biomolecules and potential targets on the molecular level.[1] In all cases, structural elucidation assumes one preferred binding site for the metal ion although in many cases, multiple binding possibilities exist. An example is the displacement of $Zn^{2+}$ in zinc finger (ZF) peptides by $Au^{3+}$ and $Au^{1+}$ producing "gold fingers". These displacements have important biological consequences and potential therapeutic applications through the inhibition of the intrinsic nucleic acid interactions of the parent zinc fingers.[2] Mass spectrometry has identified multiple $\{Au_nF\}$ ($n = 1$–3) species following $Zn^{2+}$ displacement in both the C-terminal finger of the HIV NCp7 nucleocapsid protein (NCp7-F2, F2) and finger 3 of the Sp1 transcription factor (Sp1-F3, F3), a feature easily explained by the presence of multiple cysteine residues in the zinc finger template.[3] Similar results have been obtained using poly(ADP-ribose) polymerase 1 (PARP-1; ADP = adenosine diphosphate).[4] The $Zn^{2+}$ ion in the "full" two-finger nucleocapsid (NC) protein is also displaced by $Au^{III}$ (from $[Au(dien)(9\text{-EtGuanine})]^{3+}$) producing $\{Au_nF\}$.[5] The gold complex shows micromolar efficacy in inhibiting viral infectivity, consistent with the ability to alter the native structure.[5] The auration of ZF peptides represents a problem of wide interest in bioinorganic chemistry as well as a fundamental problem for inorganic chemistry as we are evaluating the replacement of a tetrahedrally coordinated $Zn^{II}$ ion by a linearly coordinated $Au^{I}$ ion. Herein, we demonstrate that the use of traveling-wave ion mobility mass spectrometry (TWIM-MS) coupled with collision induced dissociation (CID) techniques on isolated conformers enables the unequivocal elucidation of specific binding sites and modes of gold-substituted NCp7-F2 and Sp1-F3. While linear Cys–Au–Cys coordination is indicated for NCp7-F2, a Cys–Au–His coordination mode is observed for Sp1.

Owing to the thiophilicity and soft Lewis acidity of gold(I), sulfur-rich biomolecules are logical cellular targets. In this respect, interference with thioredoxin–thioredoxin reductase constitutes a general consensus as a potential mechanism for the biological activity of gold drugs.[6] Early studies on $Au^{I}$ compounds such as aurothiomalate suggested that possible zinc finger inactivation might contribute to their antiarthritic activity.[7] Using $[AuCl(PPh_3)]$, both $\{(PPh_3)AuF\}$ and $\{Au_nF\}$ species are formed with HIVNCp7-F2 and Sp1-F3, and the transcription factor tends to show a higher propensity for $\{Au_nF\}$ over the gold phosphine adduct.[8] The properties of $[AuCl(PR_3)]$ make it an ideal system for probing cysteine nucleophilicity in biomolecules, directly analogous to "organic" electrophiles such as maleimide and iodoacetamide.[9] The distal Cys49 residue is indicated as the initial site of attack of *N*-ethylmaleimide (NEM), with a near-complete reduction in reaction rate when the modified NC was complexed with nucleic acids.[9c]

We chose to study the products from the reaction of $[AuCl(PEt_3)]$ (see the Supporting Information, Figure S1), which is structurally similar to auranofin,[10] with two zinc finger sequences representing the C-terminal finger of the $Cys_2HisCys$ ($Cys_3His$) HIV nucleocapsid NCp7 protein (NCp7-F2) and finger 3 of the $Cys_2His_2$ Sp1 transcription factor (Sp1-F3; Figure 1; see the Supporting Information for experimental details). In both cases, $\{(PEt_3)AuF\}$ and $\{AuF\}$ species were formed while the gold finger $\{AuF\}$ species was formed in higher intensity (Figure S2).[8] We chose the $\{AuF\}$ species in the 4+ charge state for detailed studies, and the corresponding spectra along with that of a control NCp7-ZF2

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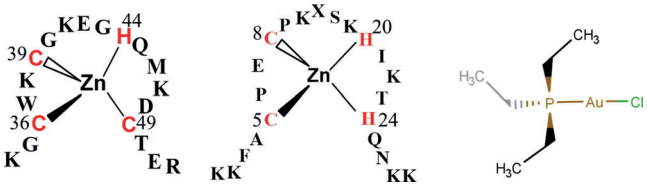

***Figure 1.*** Structures of NCp7-ZF2, Sp1-ZF3, and [AuCl(PEt$_3$)]. X = RFMSDHL. The numbering for ZF2 follows that for the full NC for clarity of discussion.

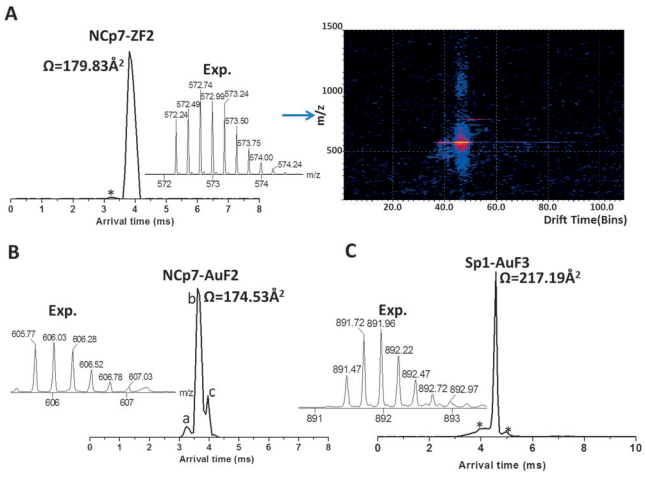


***Figure 2.*** ATDs as determined by TWIM-MS and ESI-MS spectra of A) NCp7-ZF2, B) NCp7-AuF2, and C) Sp1-AuF3 in the 4+ charge state. Isomers can be differentiated from each other by their respective ATDs following ion mobility separation. Small, non-dominant peaks in the spectra arise from noise and less abundant overlapping ions (*). A plot of *m*/*z* versus the drift time (bins) for the separated (NCp7-ZF2) 4+ charge state is shown in the top right.

sample are shown in Figure 2. The peaks were then isolated for further IM analysis. The arrival time distributions (ATDs) of these isolated species are also shown in Figure 2. As expected, the "native" NCp7 C-terminal zinc finger peptide existed as a single conformer (Figure 2A). For the gold(I) finger formed from C-terminal NCp7-ZF2, three peaks were observed in the ATD, indicating that the {AuF} adduct exists as three isomeric species (labelled a, b, and c) in the gas phase, with one conformer dominating (Figure 2B). The ATD for the equivalent {AuF} adduct from the C-terminal Sp1-ZF3 is simpler as it exhibits only one peak, indicating the existence of a single conformer in the gas phase (Figure 2C). The difference in ATD suggests differing reactivity preferences of the gold(I) drug towards cysteine within zinc finger peptides.

To further investigate the nature of these conformational changes upon auration of the zinc finger peptides, we measured the average collision cross-sections (CCSs) of the zinc finger and {AuF} peptides in the 4+ charge state (Figure 2). Under the same conditions, the major {AuF} conformer derived from NCp7-F2 appeared to be slightly more compact ($\Omega = 174.53$ Å$^2$) than the zinc finger peptide itself ($\Omega = 179.83$ Å$^2$) according to the smaller CCS and shorter drift time. On the other hand, the Sp1-derived {AuF} has a slightly longer ATD and a significantly higher CCS of $\Omega = 217.19$ Å$^2$, implying a significantly less compact structure.

To understand these differences in CCS and to identify the auration sites of the various {AuF} conformers, we selected a narrow arrival time range for each conformer and subjected the peaks to CID fragmentation. Crosslinking is destroyed upon CID fragmentation, producing a discrete set of shorter, but still gold-bound fragments from which the original binding sites can be deduced, as observed in MS/MS studies of a platinum-crosslinked peptide.[11] The selected regions are shown in Figure 3. The MS/MS spectra of the major and

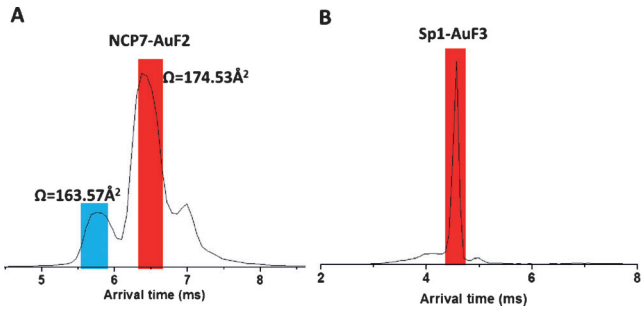


***Figure 3.*** ATDs of A) NCp7-AuF2 and B) Sp1-AuF3 in the 4+ charge state determined with a Synapt G2-Si instrument. For the conformer-selective CID experiment, the CID fragment spectra of the compact (blue) and more elongated (red) conformers are from the arrival time ranges indicated in blue and red. The overlap with the third and major conformer precluded its isolation.

minor conformers of the {AuF} species derived from NCp7-F2 in the 4+ charge state are shown in Figure 4. The identified gold-binding fragments from the MS/MS spectrum of the major conformer are shown in Table S1. Analysis of the CID spectrum of the major conformer revealed that the $Au^+$ ion was mainly bound to the $Cys^{36}$ and $Cys^{49}$ residues as we observed aurated $a_3$-$NH_3$, $y_4$, $y_6$, $y_7$, and $y_8$ fragments. The observation of aurated $y_{11}$ to $y_{15}$ cannot give definite assignment of the auration site but is consistent with the auration of $Cys^{49}$. Considering the tendency of gold(I) to form two-coordinate, linear complexes with thiolates, it is reasonable to propose that $Au^I$ coordinates the CCHC zinc finger through $Cys^{36}$–$Cys^{49}$ crosslinking (Figure 4a). The extracted arrival time distributions of the aurated $y_4$-$NH_3$ and $a_3$-$NH_3$ product ions are shown in Figure S3, and are consistent with the profile from Figure 3. The observation of aurated $y_4$-$NH_3$ in both the "red" and "blue" conformers shows that $Cys^{49}$ was metalated in both cases. The aurated $a_3$-$NH_3$ is mainly located in the dominant major conformer although some overlap with the minor conformer is observed.

The gold-binding fragments identified from the MS/MS spectrum of the minor conformer are shown in Table S2. The minor conformer is more compact ($\Omega = 163.57$ Å$^2$) and is well separated from the major conformer. Aside from $Cys^{49}$ auration, we observed aurated $a_8$-$NH_3$, $y_7$, and $y_8$ in the MS/MS, in total indicative of auration at either $Cys^{36}$ or $Cys^{39}$. Considering the above assignment of the major conformer, the minor conformer should be aurated at $Cys^{39}$ and $Cys^{49}$ (Figure 4b).

  

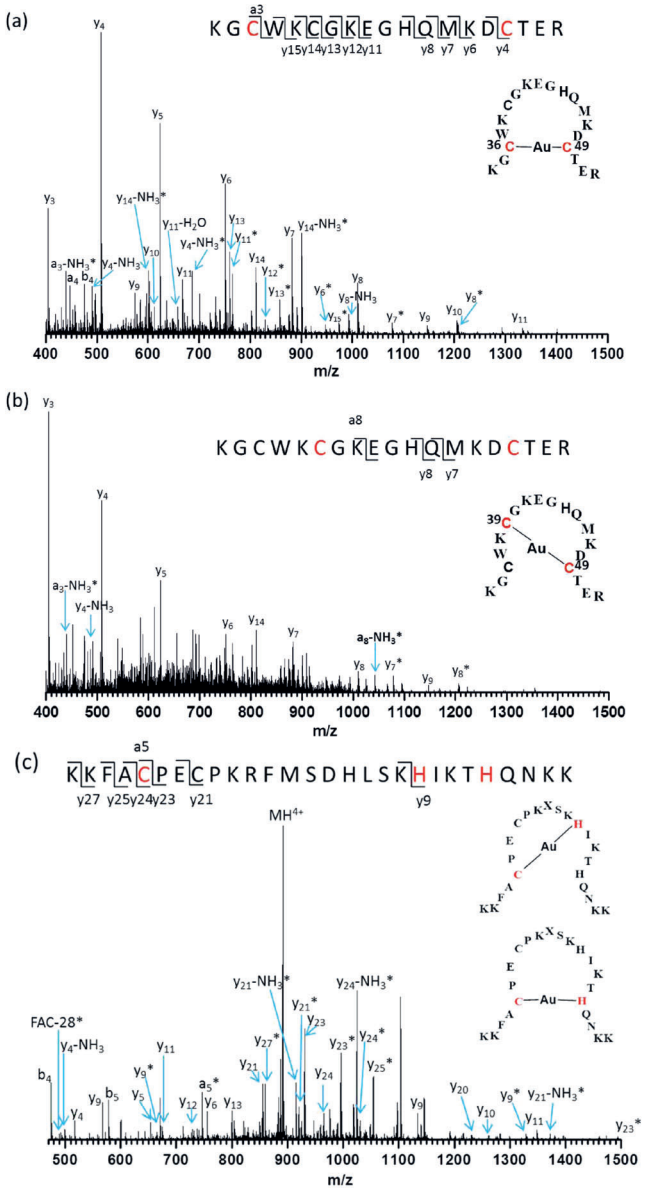


***Figure 4.*** Collision-induced dissociation fragment spectra of the peaks of the a) major and b) minor conformers of NCp7-AuF2 in the 4+ charge state and c) the only conformer of Sp1-AuF3 in the 4+ charge state (Figure 3). The structure proposed for each conformer is shown in the corresponding spectrum; a ion = b ion − carbonyl (C=O).[16]

$Cys_3His$ zinc fingers are particularly susceptible to electrophilic attack, and the C-terminal NCp7-F2 has been identified as one of the most reactive with $Cys^{49}$ being the most labile site.[12] The auration of $Cys^{49}$ in both conformers confirmed its enhanced reactivity. As stated, $Cys^{49}$ is also the site of attack of NEM, and treatment of infectious virus with NEM eliminated retroviral infectivity in a manner proportional to the NEM concentration.[13] Replacement of $Zn^{2+}$ in the full zinc finger NC eliminated the nucleic acid binding abilities of the peptide, which is crucial to its biological function in the HIV replication cycle.[15] These results strengthen our analogy between "organic" and Lewis acid electrophiles and suggest that in addition to being useful probes of NC topography, the Au-based agents could provide a rich source of targeted anti-HIV agents based on a thiolate modification strategy.[5,14]

For {AuF} derived from Sp1-F3, only one conformation was observed (for its MS/MS spectrum, see Figure 4c). The observation of aurated $a_5$ showed that $Cys^5$ is a gold-binding site while the aurated $y_9$ is indicative of auration at either $His^{20}$ or $His^{24}$. The observation of aurated $y_{21}$, $y_{23-25}$, and $y_{27}$ is also consistent with the binding at $His^{20}$/$His^{24}$ and $Cys^5$ (Figure 4c). The identified gold-binding fragments from the MS/MS spectrum are shown in Table S3. A linear Cys–Au–Cys motif is impossible from the MS/MS assignment, which was confirmed by the collision cross-section of oxidized apo-Sp1F3 with a disulfide bond formed between the two Cys ligands (Figure S4). The oxidized apo-Sp1F3 (189 $Å^2$) is much more compact than the gold finger (217 $Å^2$). Thus the gold finger with the proposed Cys–Au–His structure has a more open structure than oxidized apoF3 (Figure 4C and Figure S4a).

Mass spectrometry and tandem MS have been used successfully to characterize gold–peptide/protein interactions but cannot assign specific binding sites in multireceptor systems nor suggest the existence of conformers.[17] Circular dichroism spectra of C-terminal NCp7 in the presence of [AuCl($PEt_3$)] (1 equiv) showed the clear time-dependent formation of conformations distinct from that of the parent zinc finger (Figure S5). The presence of multiple conformers can also be inferred from the initial $^{31}P$ NMR spectra where two distinct $^{31}P$ NMR peaks indicate at least two different environments for the P (and hence Au) atoms (Figure S6). The IM spectra add significantly to our ability to designate specific structures, which may not even be possible from some solution studies. To the best of our knowledge, this is the first demonstration that {AuF} species can exist in structurally distinct conformations, representing a novel use of IM in protein structure analysis complementary to other spectroscopic and X-ray techniques.[3–4,17–18]

Ion mobility has been used to study changes in the tertiary structure of ubiquitin upon metalation with cisplatin and revealed the presence of up to three different conformations for the Ub–{$Pt(NH_3)_2$} monoadduct.[19,20] Ruthenium(II)–peptide interactions studied by IM highlight the important role played by the ligand in determining the shape of the adduct formed.[21] Furthermore, the conformational diversity of cancer-associated mutations in the zinc-bound p53 tumor suppressor protein has been probed by ion-mobility mass spectrometry.[22] A further relevant example is the assignment of Cu coordination sites on a methanobactin model peptide with potential $Cys_2His_2$ binding sites analogous to the Sp1-F3 case.[23]

In summary, we have shown the utility of ion mobility measurements combined with MS/MS for the study of zinc finger proteins/metal–drug interactions. The ion mobility experiments are rapid (ms timescale) and, when combined with MS/MS, will provide information not only on the shape and conformer profile, but also on metalation selectivity and reactivity.[24] IMS enables the separation of peptide species of the same mass-to-charge ($m/z$) ratio that exhibit multiple conformations.[25] Therefore, upon coupling to mass spec-

  

trometry, we can obtain very specific and complementary information about analytes.[26] In this particular case, the ability to identify precisely the nature of the auration and zinc displacement on the protein elucidates the details whereby DNA/RNA recognition is abrogated, which is considered essential in the inhibition of antiviral activity by these drugs. The screening of new metal-based anticancer or antiviral agents would benefit from quantitative information on the shape changes induced upon zinc finger protein metalation and the selectivity and reactivity of metalation.

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## Conflict of interest

The authors declare no conflict of interest.

**Keywords:** conformers · gold fingers · mass spectrometry · protein structures · zinc fingers

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# Communications

## Mass Spectrometry

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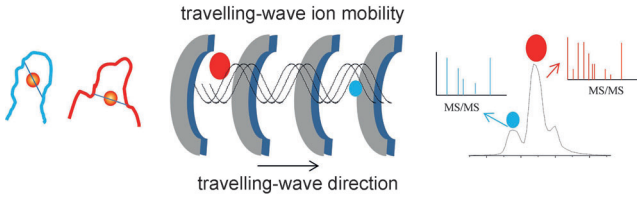
Diversity in Gold Finger Structure Elucidated by Traveling-Wave Ion Mobility Mass Spectrometry




**A golden opportunity**: Traveling wave ion mobility (TWIM) mass spectrometry (MS) was used to separate isomeric metalated peptide ions derived from gold(I) complexes, enabling the acquisition of distinct product ion spectra for each isomer (blue and red). Measurements of the collision cross-sections showed that the aurated peptide has a slightly more compact structure than the parent zinc finger.