

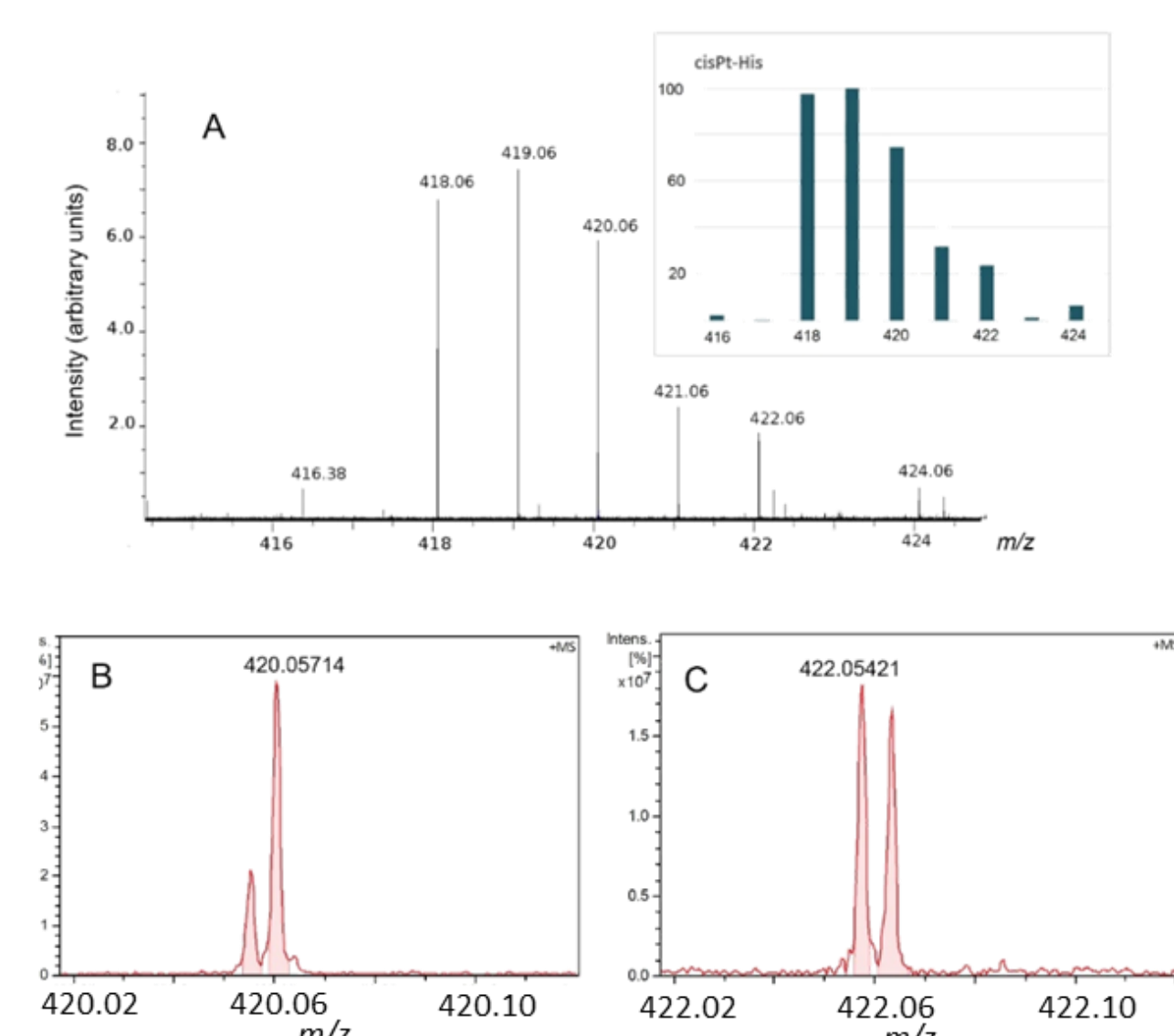


# Elusive monofunctional adducts of cisplatin with natural aminoacids revealed by IRMPD spectroscopy and photofragmentation kinetics

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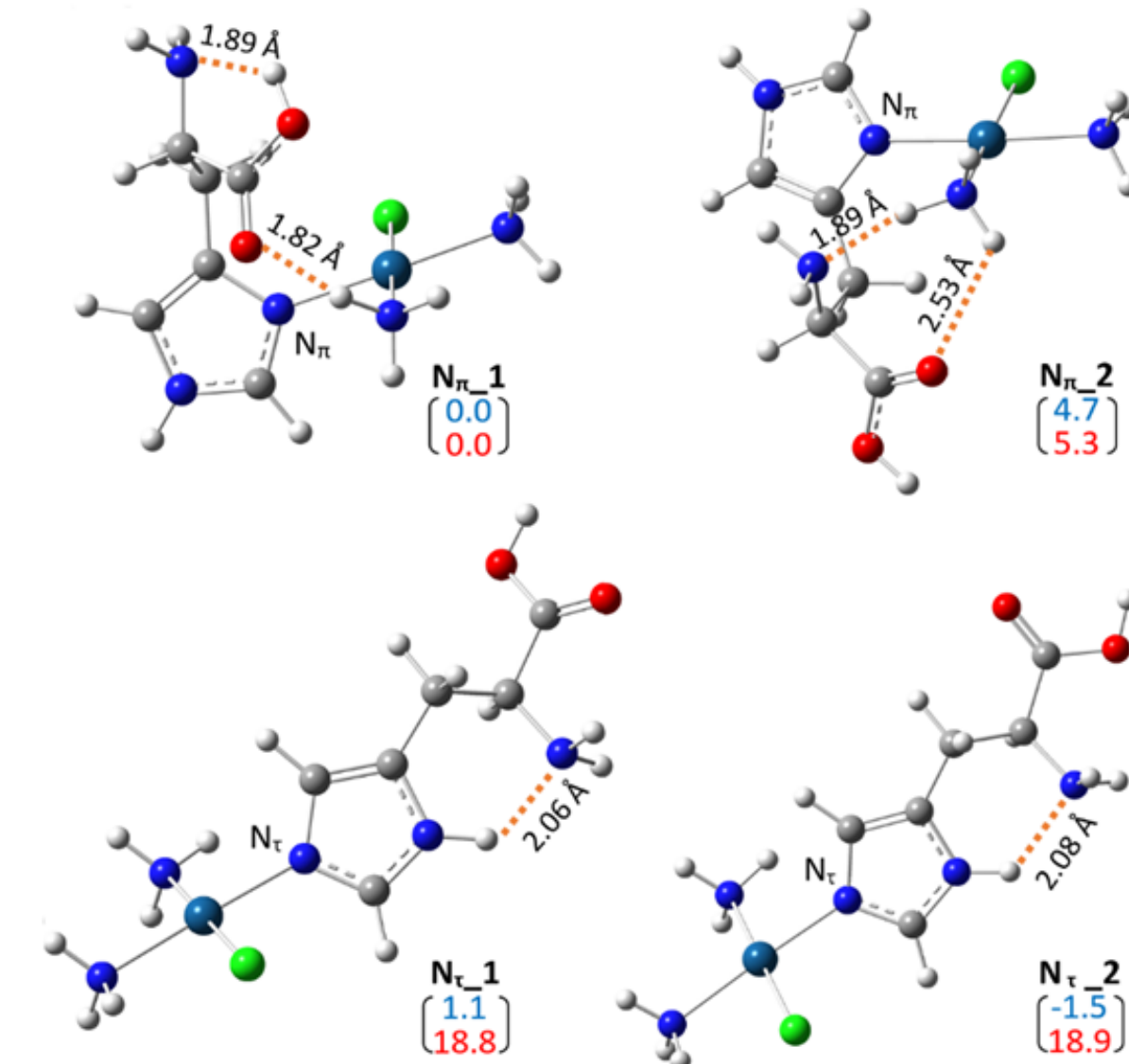
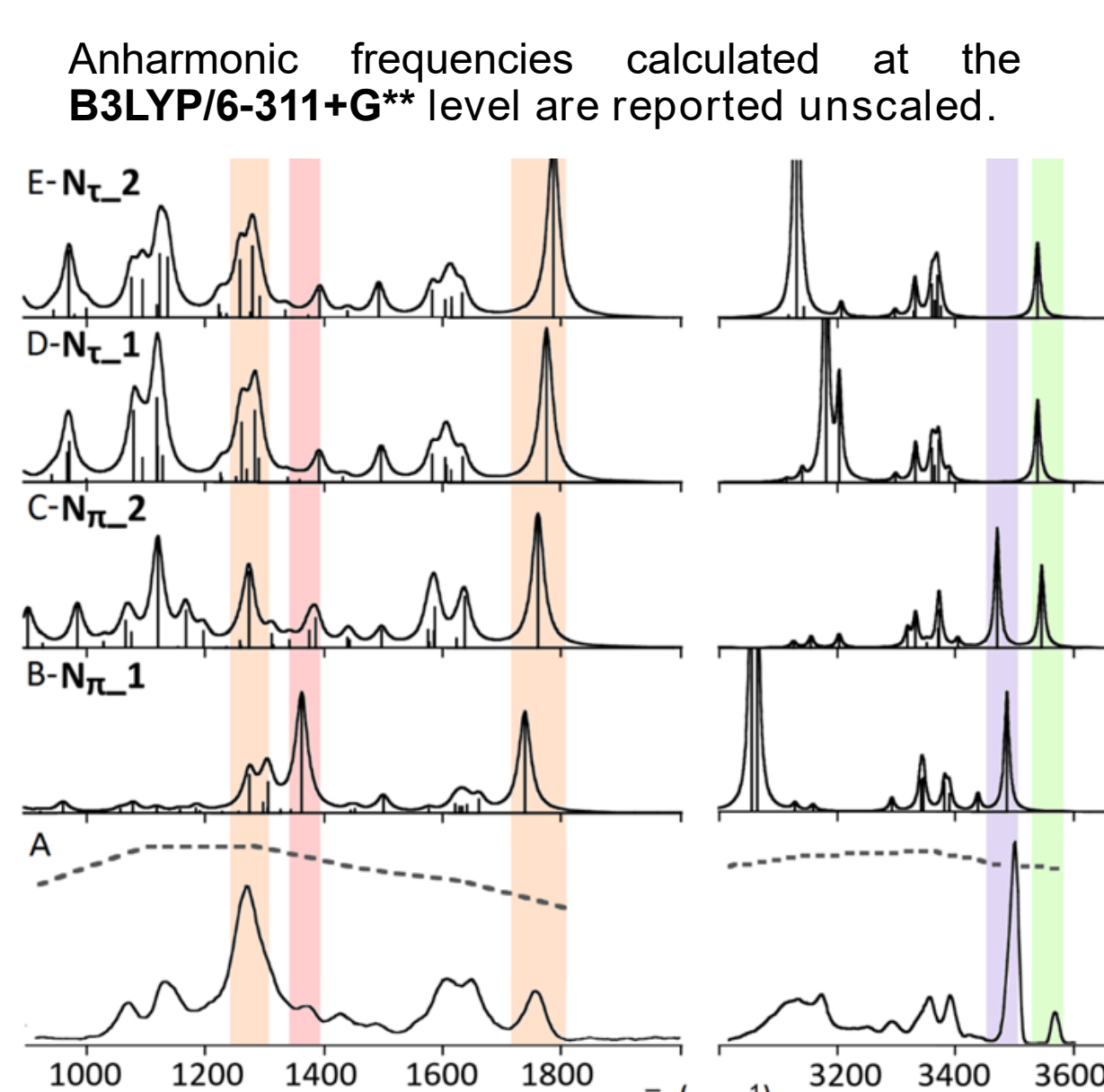
## High Resolution mass analysis



Excerpt of the mass spectrum of a solution of cisplatin and His recorded with a 7 T FT-ICR instrument showing the cluster of  $cis-[PtCl(NH_3)_2His]^+$  at  $m/z$  418 (panel A).

Separation of formally isobaric ions, for example the ion at nominal  $m/z$  420 is resolved into distinct peaks for  $[^{194}Pt^{37}Cl(NH_3)_2His]^+$  and  $[^{196}Pt^{35}Cl(NH_3)_2His]^+$  is shown in panel B. Another example is shown in the enlarged excerpt of panel C with baseline resolved peaks for the two isotopomers at  $m/z$  422, namely  $[^{196}Pt^{35}Cl(NH_3)_2His]^+$  and  $[^{196}Pt^{37}Cl(NH_3)_2His]^+$ .

## IRMPD spectroscopy



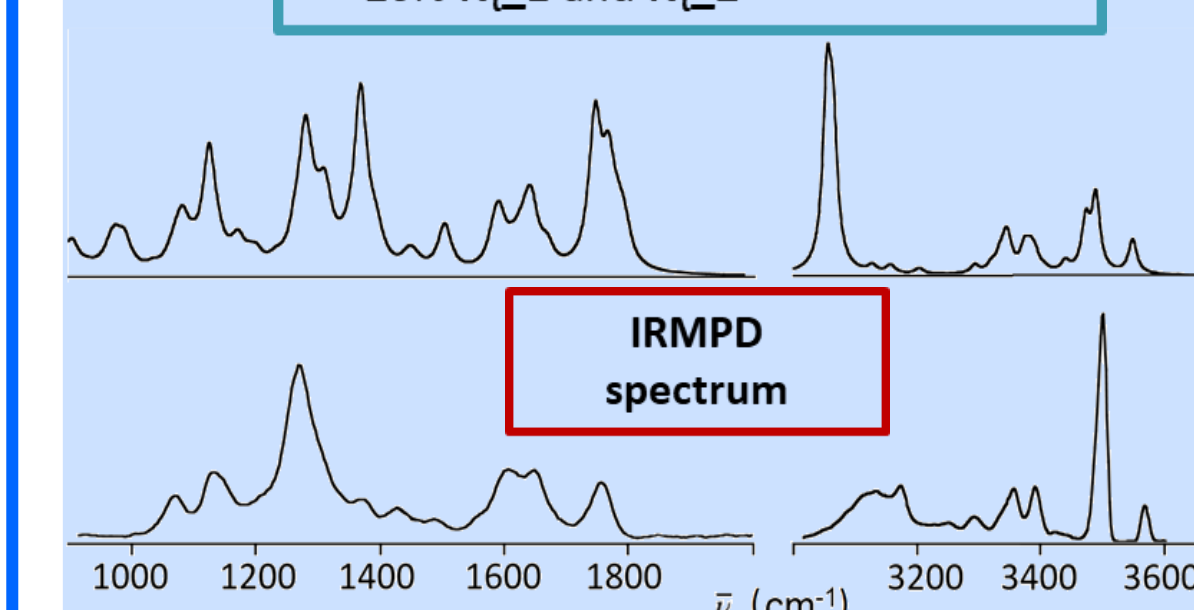
Geometries optimized at the B3LYP/6-311+G\*\* (Pt = LanL2TZ) level, relative free energies at 298 K are reported in blue for the B3LYP/6-311+G\*\* and in red for the  $\omega$ B97-XD/6-311+G\*\* levels

IRMPD spectroscopy showed the presence of multiple isomers and conformers in the gas-phase population.

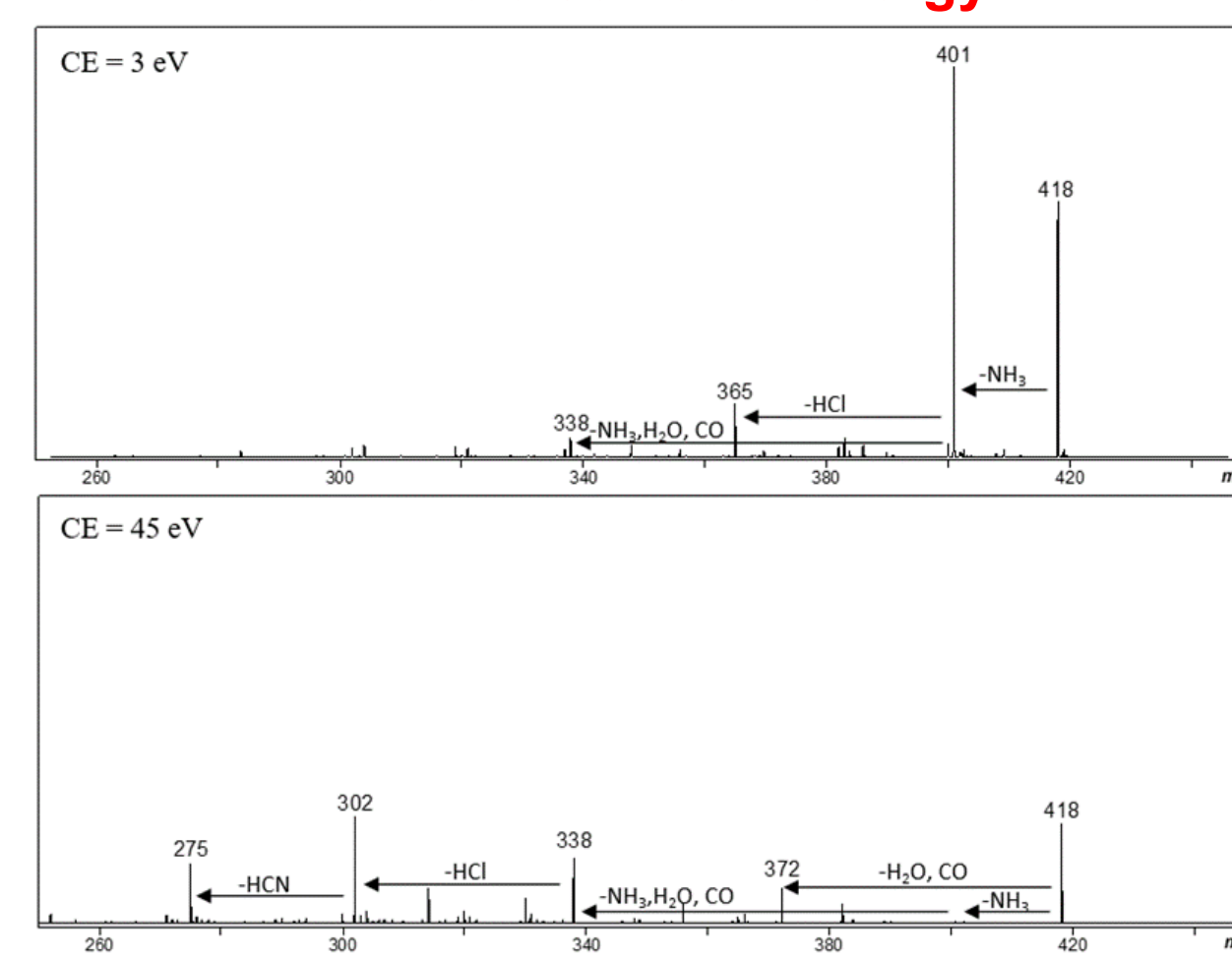
Photofragmentation kinetics on conformer-specific vibrational modes were employed to evaluate their contribution. The so-obtained conformer ratio was used to produce an experimentally determined averaged theoretical spectrum finally obtaining good agreement with the IRMPD data.

Averaged theoretical spectrum from experimental obtained conformer percentages:

- 45%  $N_{n-1}$
- 30%  $N_{n-2}$
- 20%  $N_{n-1}$  and  $N_{n-2}$

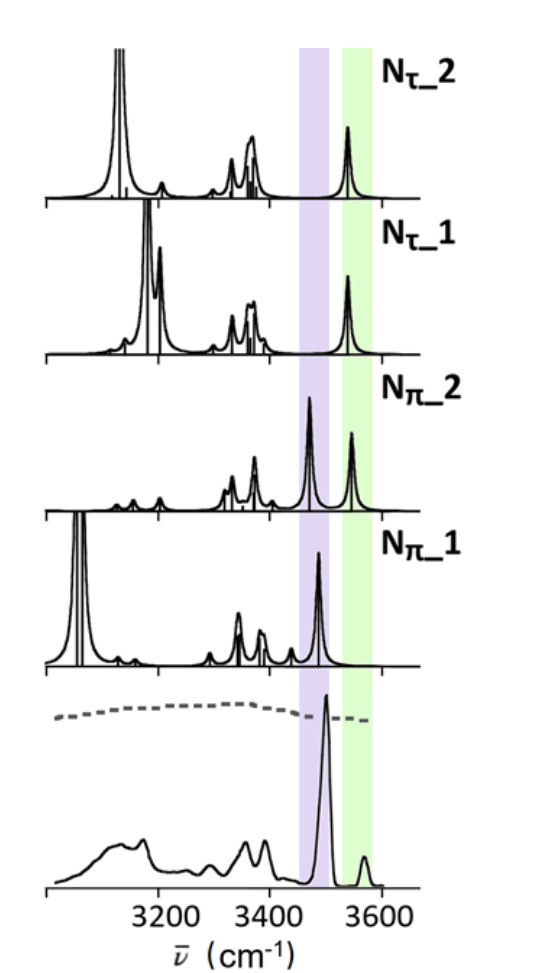
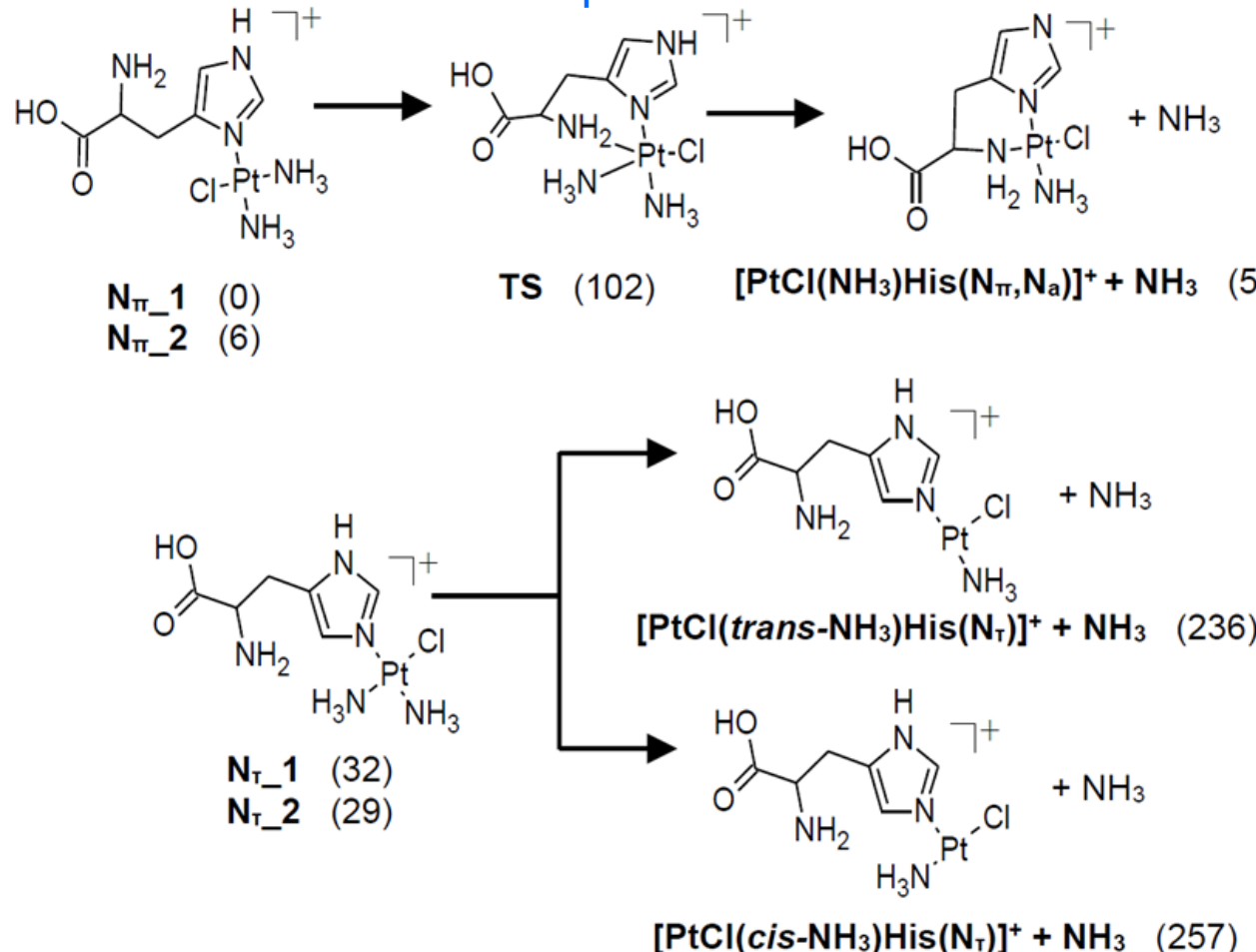


## CID experiments at variable collision energy

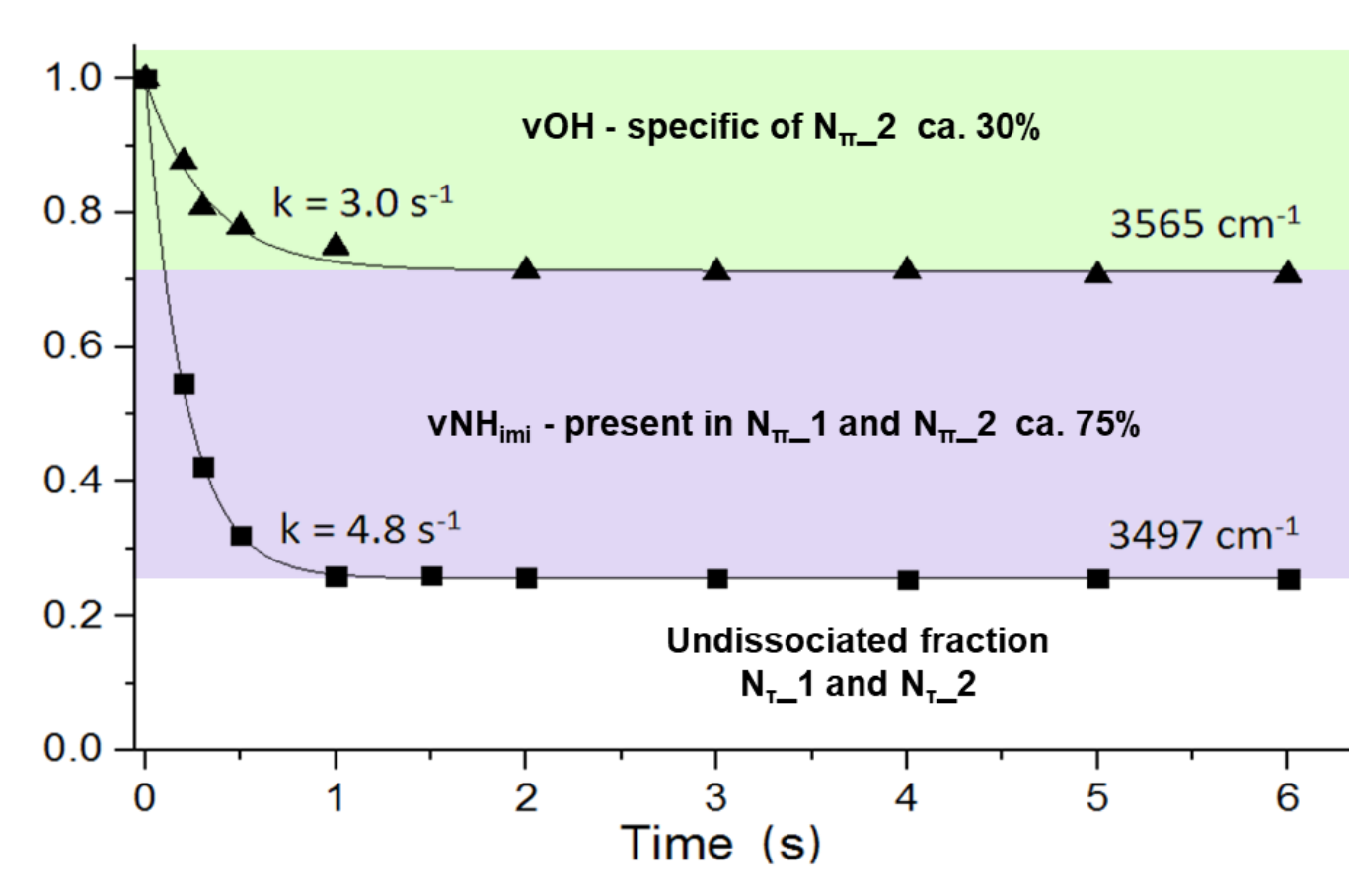


Despite the huge amount of water loss already observable at low CE, the presence of a fraction of precursor ion resistant to dissociation at high CE suggests two species are sampled by ESI, arguably the two isomers  $N_{n-1}$  and  $N_{n-2}$ .

Fragmentation paths for  $N_{n-1}$  and  $N_{n-2}$  isomers. Relative enthalpies at 298 K in kJ mol<sup>-1</sup> calculated at  $\omega$ B97X-D/6-311+G\*\* level are in parentheses.



## IRMPD kinetics



Both OH and imidazole NH stretching modes have been assayed at fixed photon energy, but varying the irradiation time. Both decaying curves show a mono-exponential trend followed by a plateau. The undissociated fractions were correlated to the percentage of isomers which don't present the vibrational mode activated at the assayed frequency.

## $cis-[PtCl(NH_3)_2(histidine)]^+$

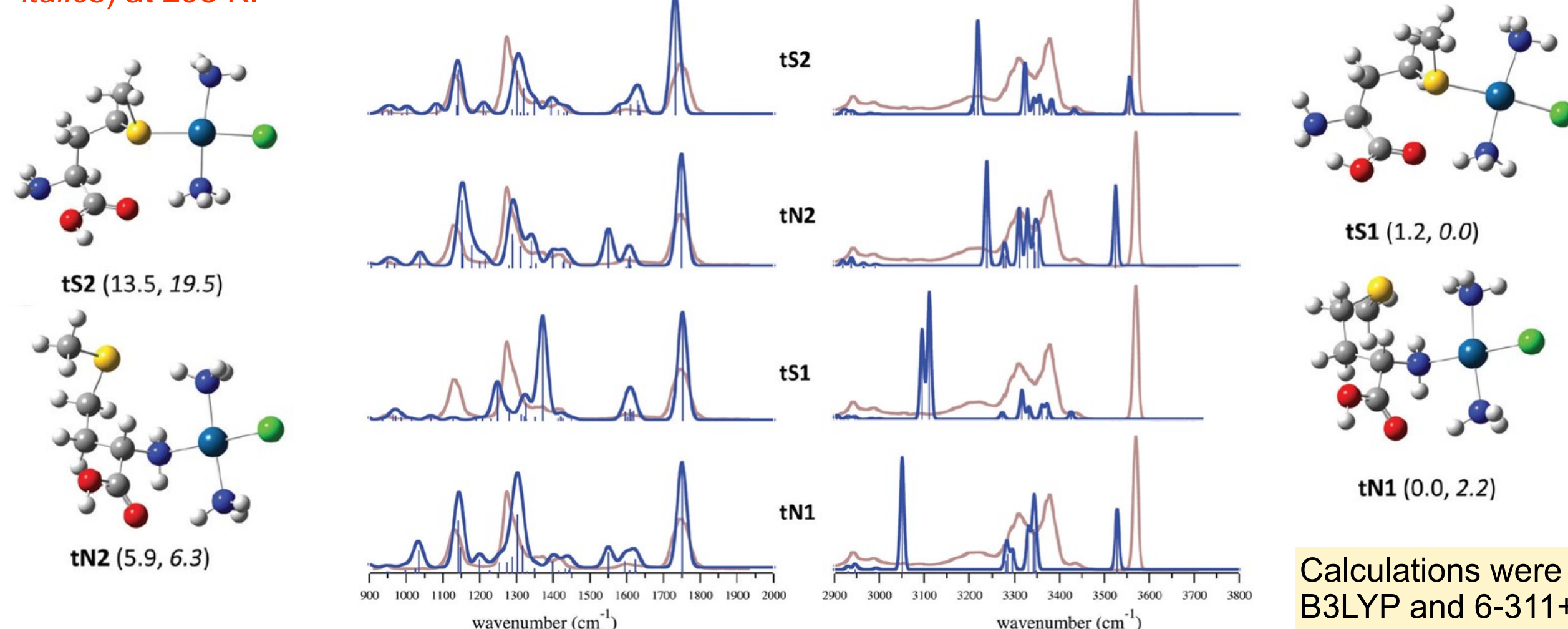
Cisplatin  $cis-[PtCl_2(NH_3)_2]$  is a widely used antineoplastic drug whose activity is related to interaction with DNA leading to disruption of transcription and finally cell death.<sup>1</sup> The interaction of the drug with peptides and proteins is recognized as an overlooked topic<sup>2</sup> considering the scarce effort devoted in understanding it despite its importance in cisplatin metabolism and distribution. Cisplatin has to be activated by aquation, the substitution of a chloride with water, leading to a positively charged species which undergoes subsequent substitution with biological nucleophiles and the release of water. We report the first molecular characterization of the primary complexes generated with aminoacids like histidine<sup>3</sup> and methionine<sup>4</sup> using MS and MS-based techniques. The combination of IR multiple photon dissociation spectroscopy, theoretical calculations and finally IRMPD kinetics has allowed us to obtain a qualitative representation of the conformational variability of the sampled ions.

## $cis/trans-[PtCl(NH_3)_2(methionine)]^+$

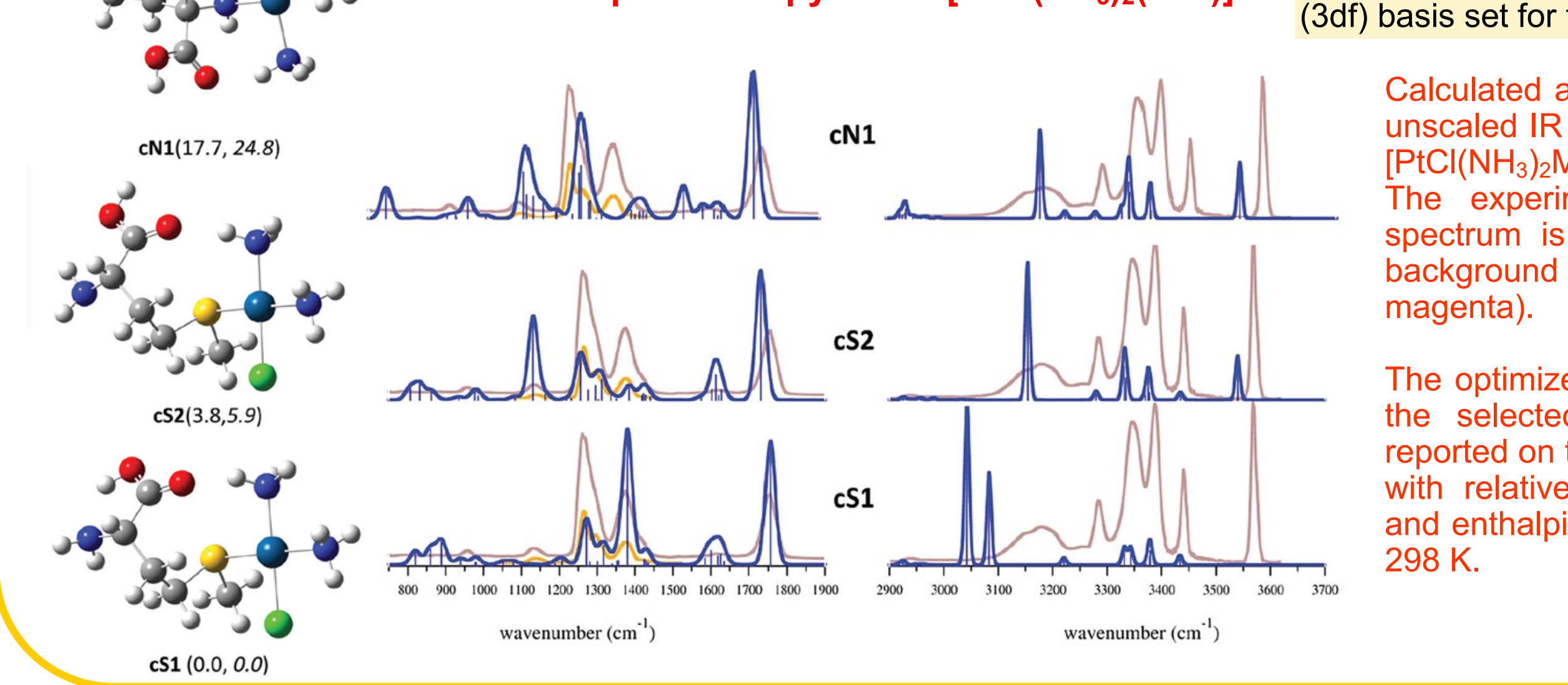
### IRMPD spectroscopy of $trans-[PtCl(NH_3)_2(Met)]^+$

Calculated anharmonic and unscaled IR spectra for  $trans-[PtCl(NH_3)_2Met]^+$  isomers. The experimental IRMPD spectrum is plotted in the background (in pale magenta).

The optimized structures of the selected isomers are also reported, together with relative free energies and enthalpies (in *italics*) at 298 K.



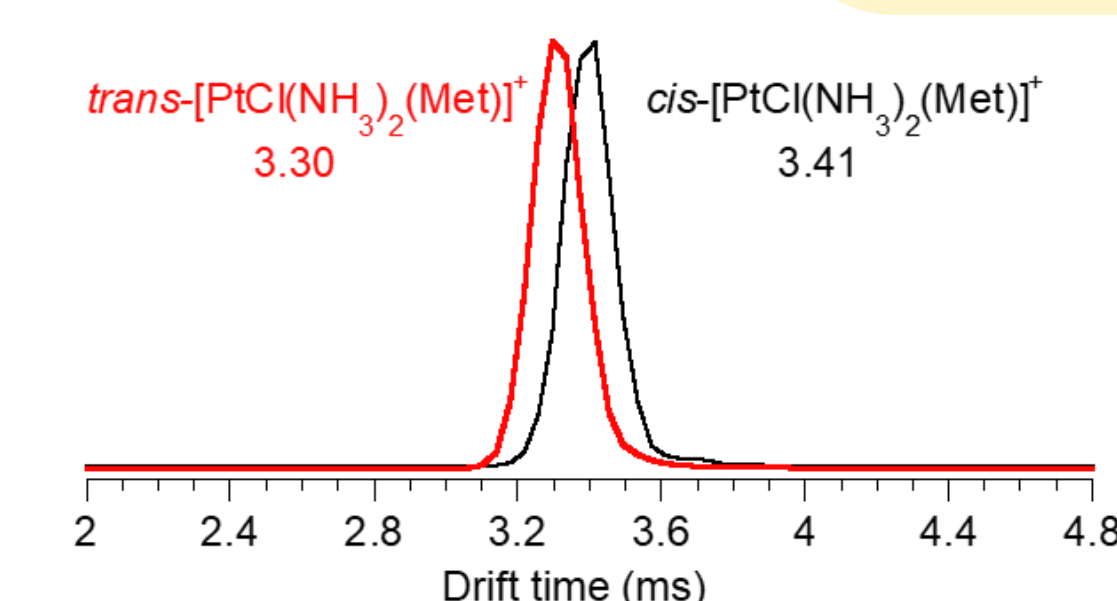
### IRMPD spectroscopy of $cis-[PtCl(NH_3)_2(Met)]^+$



Calculations were made using B3LYP and 6-311++G(2df,pd) for the light atoms. The pseudopotential LANL2TZ-f was adopted for Pt and the 6-311++G (3df) basis set for the S atom.

Calculated anharmonic and unscaled IR spectra for  $cis-[PtCl(NH_3)_2Met]^+$  isomers. The experimental IRMPD spectrum is plotted in the background (in pale magenta).

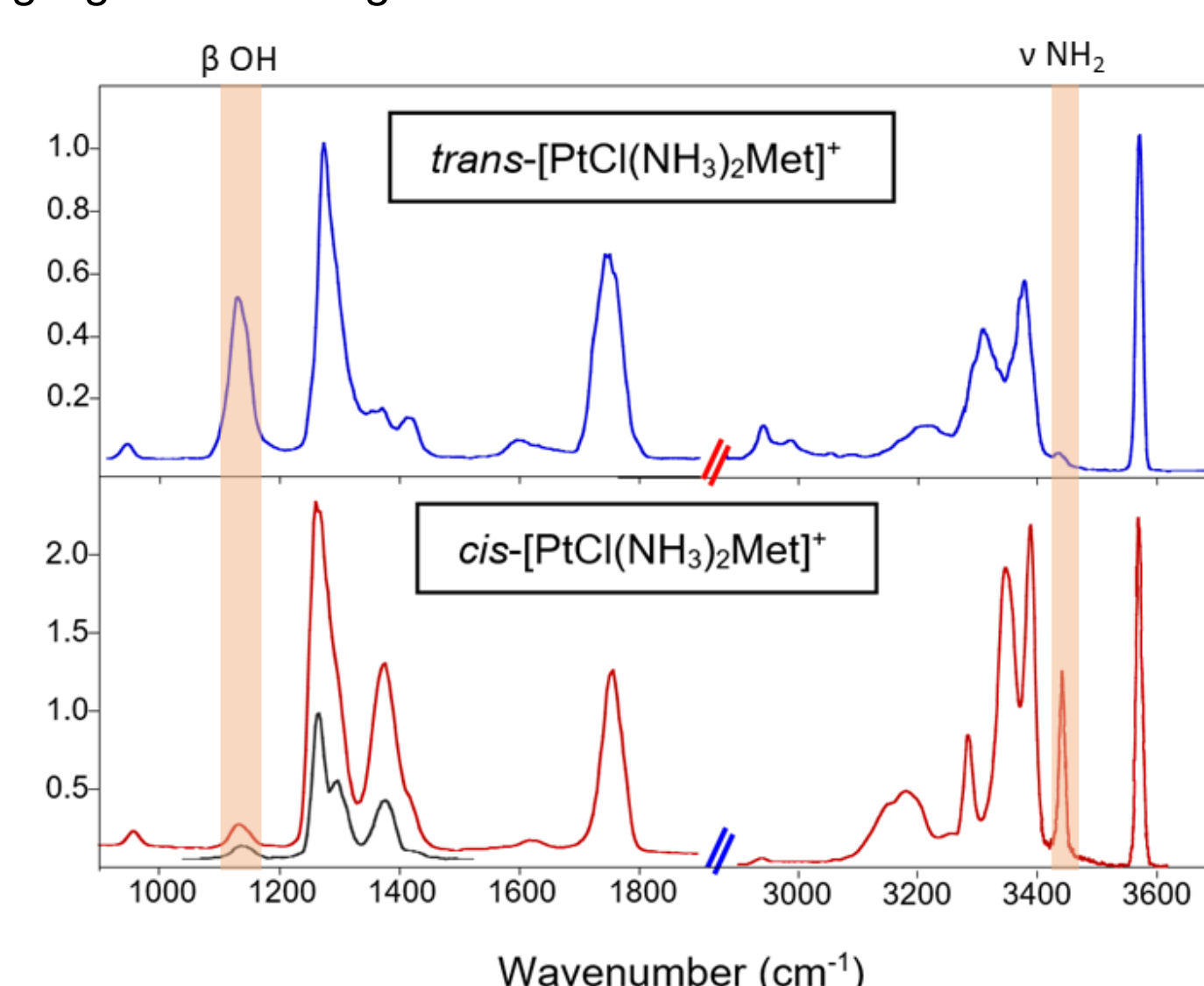
The optimized structures of the selected isomers are reported on the left together with relative free energies and enthalpies (in *italics*) at 298 K.



Ion mobility profiles (top figure) and IRMPD spectra (bottom figure) are reported for both  $cis$ - and  $trans$ - $[PtCl(NH_3)_2(Met)]^+$ .

Despite the expected high structural similarity between the two assayed species, the drift time of the trans isomer is slightly shorter than the cis isomer one suggesting the former to have a somewhat tighter structure.

In addition, the IRMPD spectra show a few notable differences in particular regarding the intensity of the bands attributable to the asymmetric stretching of the  $\alpha$  NH<sub>2</sub> group and the OH bending. Both are highlighted in the figure below.



**References:** (1) Klein A. V., Hambley T. W., *Chem. Rev.* **2009**, 109, 4911; (2) Casini A., Reedijk J. *Chem. Sci.* **2012**, 3, 3135; (3) Corinti D., De Petris A., Coletti C., Re N., Chiavarino B., Crestoni M. E., Fornarini S., *ChemPhysChem* **2017**, 16, 318; (4) Paciotti R., Corinti D., De Petris A., Ciavardini A., Piccirillo S., Coletti C., Re N., Maitre P., Bellina B., Barran P., Chiavarino B., Crestoni M. E., Fornarini S., *Phys. Chem. Chem. Phys.* **2017**, 19, 26697.



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