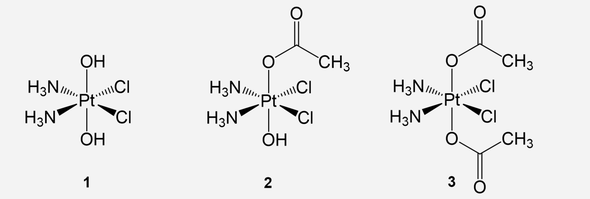
Dissociation of mass-isolated encounter complexes of platinum(IV) prodrugs and ascorbic acid elucidates details on their bioactivation

Davide Corinti,[1] Maria Elisa Crestoni,[1] Simonetta Fornarini,[1] Eslam Dabbish,[2] Emilia Sicilia,[2] Elisabetta Gabano,[3] Elena Perin,[3] and Domenico Osella[3]

[1] Dipartimento di Chimica e Tecnologie del Farmaco, Università di Roma "La Sapienza", Roma, Italy. [2] Department of Chemistry and Chemical Technologies Università della Calabria, Arcavacata di Rende (CS), Italy. [3] Dipartimento di Scienze e Innovazione Tecnologica, Università del Piemonte Orientale, Alessandria, Italy.

A multimethodological approach involving reduction kinetics determination, electrochemistry, tandem mass spectrometry and IR ion spectroscopy in association with quantum-mechanical DFT calculations was exploited to gain insights on the reduction mechanism of cisplatin-based Pt(IV) derivatives.

Three cisplatin-based complexes having two hydroxido (**1**) or one hydroxido and one acetato (**2**) or two acetato ligands (**3**) in axial position were studied in their interaction with glutathione and ascorbic acid, species involved in the reductive bioactivation of Pt(IV) prodrugs.



• The issue we are facing:

The trend of reduction kinetics in the presence of ascorbic acid in solution (**1** > **2** > **3**) does not reflect the respective reduction peak potentials following the inverse order **1** < **2** < **3**.

Kinetics[a] and electrochemical data[b] for the reduction of 1-3.

Compound	$t_{1/2}$ (s × 10^2)[a]	E_p (V)[b]
1	5.2	- 0.815
2	12.3	- 0.618
3	320	- 0.486

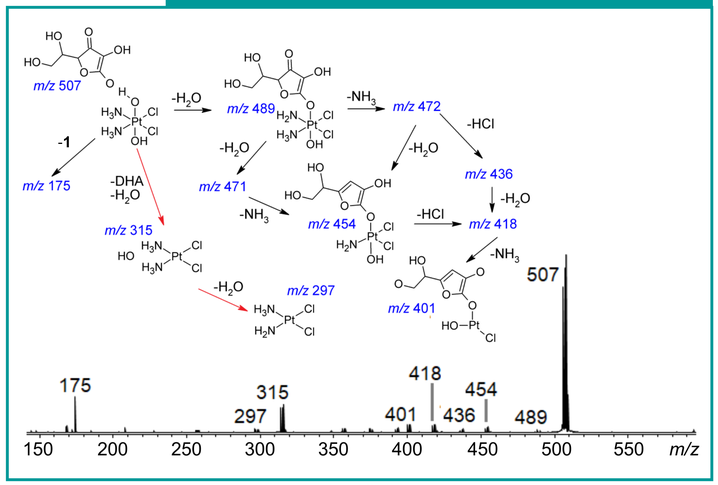
[a] Half-life (s) at 37°C in the presence of a 10 fold excess $HAsc^-$.
[b] Reduction peak potential (V vs Ag/AgCl), GC electrode, scan rate 0.2 V s^{-1}.

• What can we do about it?

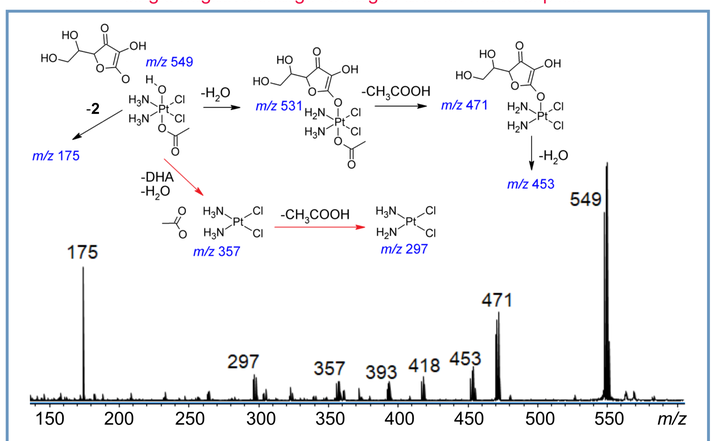
To explain this dichotomy at a molecular level we turned to a study in the **simplified gas-phase environment**.

The **Pt(IV) complexes associated with a single reductant molecule** (corresponding to the **encounter complex** occurring along the reaction coordinate in the bimolecular reaction in solution) were successfully delivered in the gas-phase to be characterized by IR ion spectroscopy. They were sampled for their reactivity behavior both under photo-activation and by collision induced dissociation (CID). The complexes display a reduction reactivity ordering comparable to the one observed in solution.

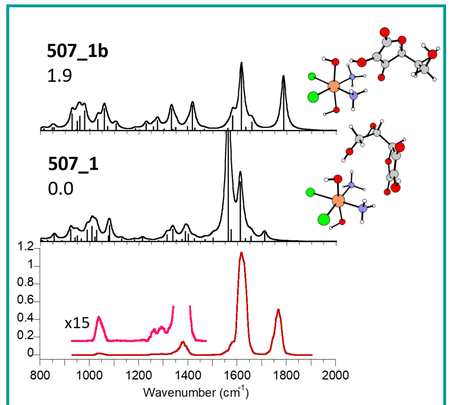
Mass spectrum of [**1**+HAsc]$^-$ activated by resonant IR photons at 1780 cm^{-1}.
The dissociation channel leading to the reduced fragments at *m/z* 315 and 297 is highlighted in red.



Mass spectra are in negative ion mode. All the species in the schemes present a single negative charge. Charges are not made explicit.

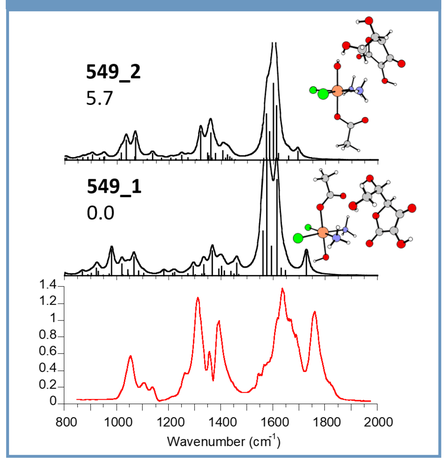


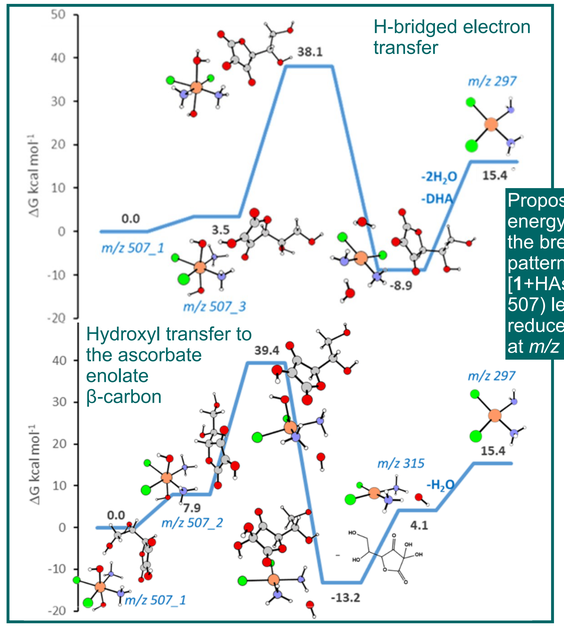
Mass spectrum of [**2**+HAsc]$^-$ activated by resonant IR photons at 1420 cm^{-1}.
The dissociation channel leading to the reduced fragments at *m/z* 357 and 297 is highlighted in red.



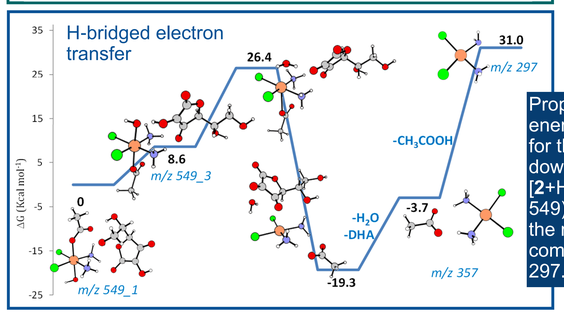
IRMPD spectrum of [**1**+HAsc]$^-$ (red) compared with the calculated IR spectra of **507_1** and **507_1b** at the B3LYP-D3 level. Relative free energies (298 K) are reported in kcal mol^{-1}.

IRMPD spectrum of [**2**+HAsc]$^-$ (red) and calculated IR spectra of **549_1** and **549_2** at the B3LYP-D3 level. Relative free energies (298 K) are reported in kcal mol^{-1}.





Proposed free energy profiles for the breakdown pattern of [**1**+HAsc]$^-$ (*m/z* 507) leading to the reduced complex at *m/z* 297.



Proposed free energy profile for the break-down pattern of [**2**+HAsc]$^-$ (*m/z* 549) leading to the reduced complex at *m/z* 297.

Conclusions

- **Encounter complexes** for the reaction of **1-3** with either ascorbate or glutathione have been isolated and assayed
- The **adducts** of **1** and **2** with ascorbate are prone to **reduction when activated**, releasing Pt(II) species as major products.
- The experimental evidence compared to DFT calculations highlight the **role of an axially coordinated hydroxido ligand** in promoting the **reduction** of Pt(IV) prodrugs.
- The **trend in reduction peak potentials** (E_p values: **1** < **2** < **3**) is **not** in this case **a reliable indicator** for the corresponding chemical reductions

References: • D. Corinti, M.E. Crestoni, S. Fornarini, F. Ponte, N. Russo, E. Sicilia, E. Gabano, D. Osella, Elusive **Intermediates in the Breakdown Reactivity Patterns of Prodrug Platinum(IV) Complexes,** *J. Am. Soc. Mass Spectrom.* 30 (2019) 1881–1894. doi:10.1007/s13361-019-02186-7. • D. Corinti, C. Coletti, N. Re, R. Paciotti, P. Maître, B. Chiavarino, M.E. Crestoni, S. Fornarini, **Short-lived intermediates (encounter complexes) in cisplatin ligand exchange elucidated by infrared ion spectroscopy**, *Int. J. Mass Spectrom.* 435 (2019) 7–17. doi:10.1016/j.ijms.2018.10.012. • D. Corinti, M.E. Crestoni, S. Fornarini, E. Dabbish, E. Sicilia, E. Gabano, E. Perin, D. Osella, **A multi-methodological inquiry of the behavior of cisplatin-based Pt(IV) derivatives in the presence of bioreductants with a focus on the isolated encounter complexes**, *JBIC J. Biol. Inorg. Chem.* (2020). doi:10.1007/s00775-020-01789-w.