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Structural analyses of metallodrug/β-lactoglobulin adducts for rational design of new biomaterials

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Abstract:
β-lactoglobulin (BLG) is a good system for the preparation of micro- or nanoparticles for pharmaceutical industry. It has been demonstrated that β-lactoglobulin–pectin nanoparticles can transfer cytotoxic Pt compounds to cancer cells. With the aim to unveil the molecular basis of the metallodrug recognition by BLG, we are analyzing the interactions between this protein and a number of metallodrugs. The interaction between cisplatin (CDDP), the most used Pt-based anticancer agent, and BLG has been investigated both in solution and at solid state. The results reveal that cisplatin interacts with the protein without affecting the overall protein 3D structure. Oxaliplatin (OXA) is preferred to cisplatin because of a lower toxicity and an increased reactivity against cisplatin resistant tumors. The adduct formed upon reaction of BLG with the metal complex has been synthetized and structurally characterized by X-ray crystallography and electrospray ionization mass spectrometry. Structural analyses demonstrate that OXA binds BLG *via* coordination to Met7 side chain upon releasing oxalate ligand. In vitro cytotoxicity data reveal that cisplatin and oxaliplatin exert higher cytotoxicity in their adduct with BLG than the free drugs. These results suggest that BLG could act as a carrier for anticancer metallodrugs.

Keywords: Metallodrugs; metal-protein interactions; anticancer agents.

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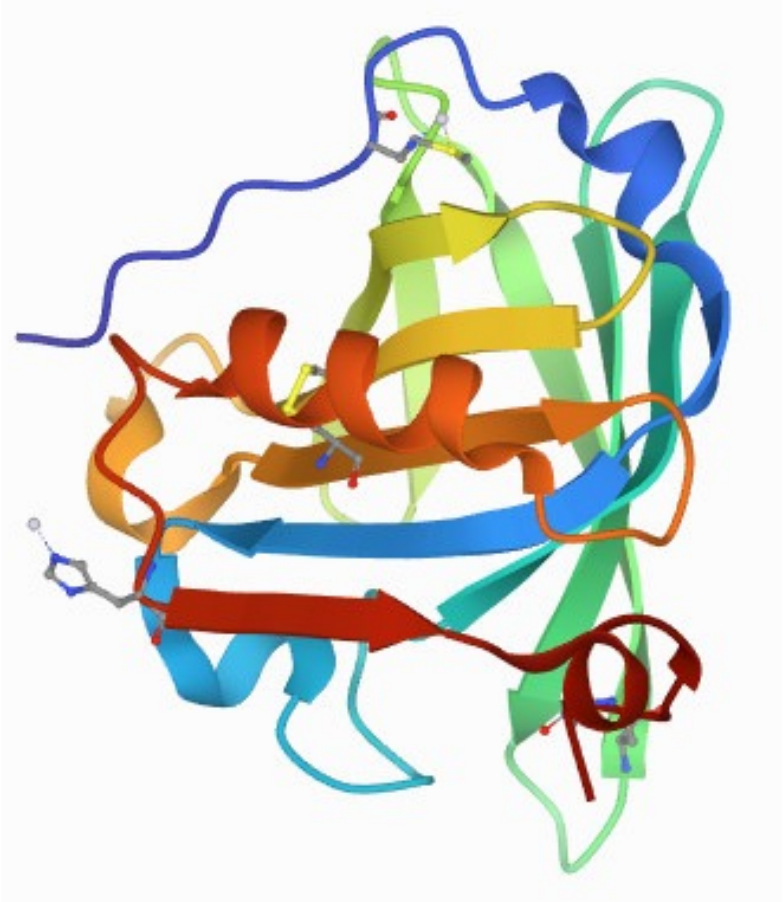
Introduction



- **β-lactoglobulin** is the major whey protein in cow's milk (18.4 kDa, 162 amino acids)
- It is a globular protein composed by **8-stranded antiparallel β-barrel** with a **3-turn α-helix** on the outer surface.

β-lactoglobulin is a good system for production of delivering vehicles for orally administrated bioactive molecules

E. Dufour *et al. Biochimica et Biophysica Acta*, 1994, **1205**(1), 105.
Z. Teng *et al. Food Chemistry*, 2016, **204**, 391. Z. Teng *et al. Food Chemistry*, 2014, **159**, 333.
Z. Shafaei *et al. Int. J. Biol. Macromol.*, 2017, 13, 1685

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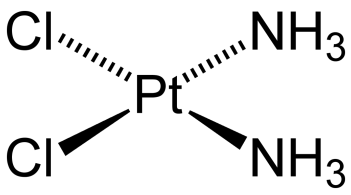
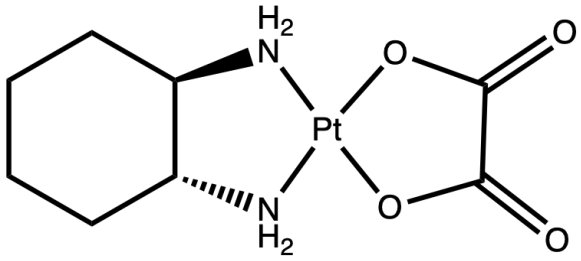
Introduction





Cisplatin

- The most used platinum-based anticancer agent in clinics
- Several side effects

Oxaliplatin

Preferred to cisplatin for treatment of colorectal cancer due to its lower toxicity and activity against CDDP-resistant cells

The interactions between β-lactoglobulin and these Pt-based metallodrugs have been analyzed

Z. Izadi *et al. Chem Biol and Drug Design* 2016, **88**(2), 209. Z.H. Siddik**,** *Oncogene* 2003, **22**, 7265.
L. Kelland, *Nature Reviews Cancer* 2007, **7**, 573.
S. Bogliolo *et al. Expert Opin Investig Drugs* 2015, **24(9)**, 1275

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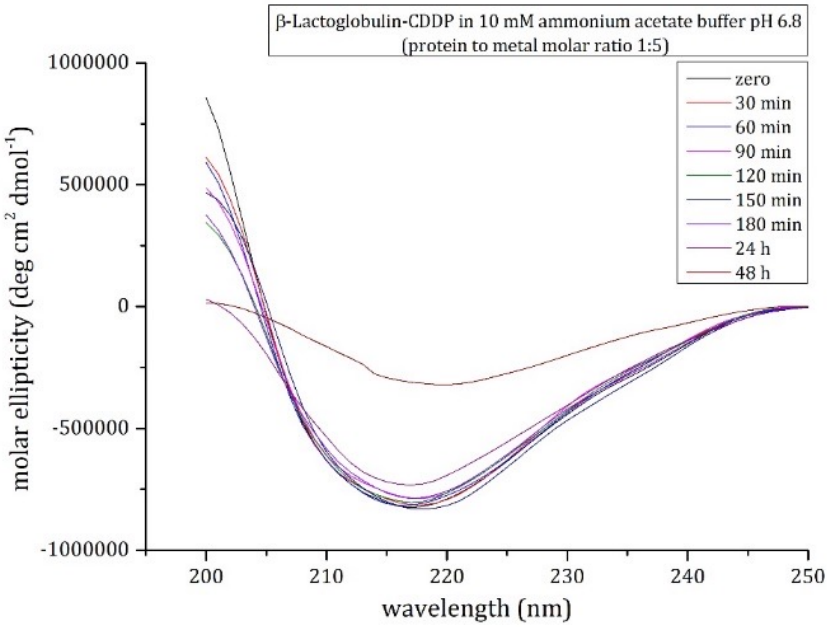
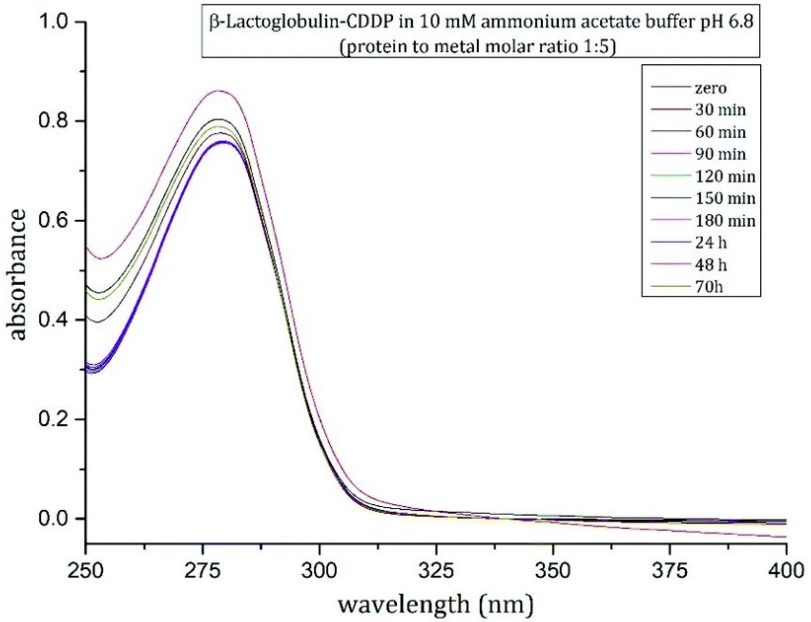
Results and Discussion: interaction with Cisplatin
In solution characterization

- Absorption intensity decreases in the first two hours
- After two hours, an increase of absorption intensity occurs, due to CDDP binding





β-lactoglobulin conserves its secondary structure in the presence of the metal compound

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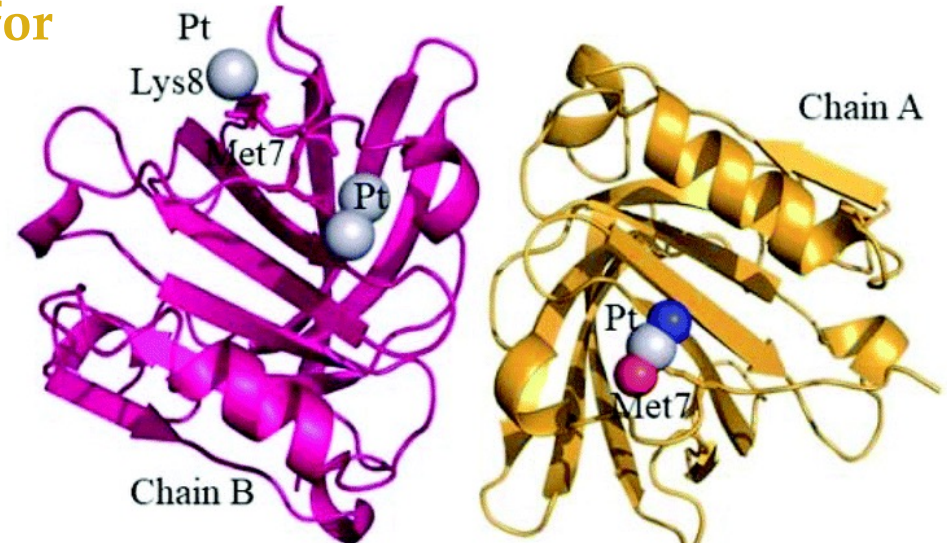
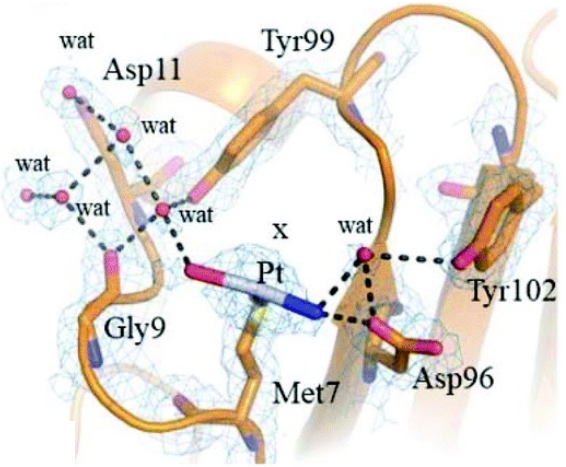
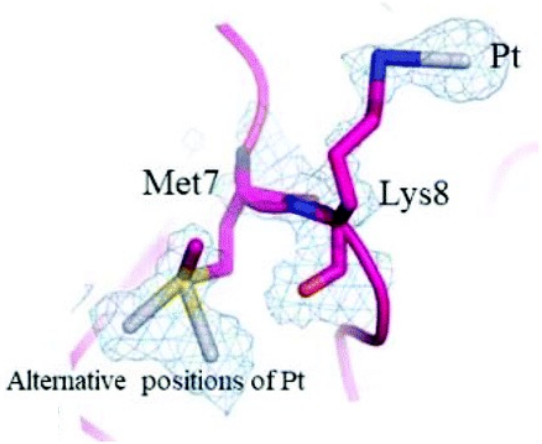
Results and Discussion: crystallographic data

CDDP soaking for 18h





A **Pt** fragment is found close to the side chain of **Met7** both A and B chains



Side chain of **Lys8** coordiantes a **Pt** fragment in chain B

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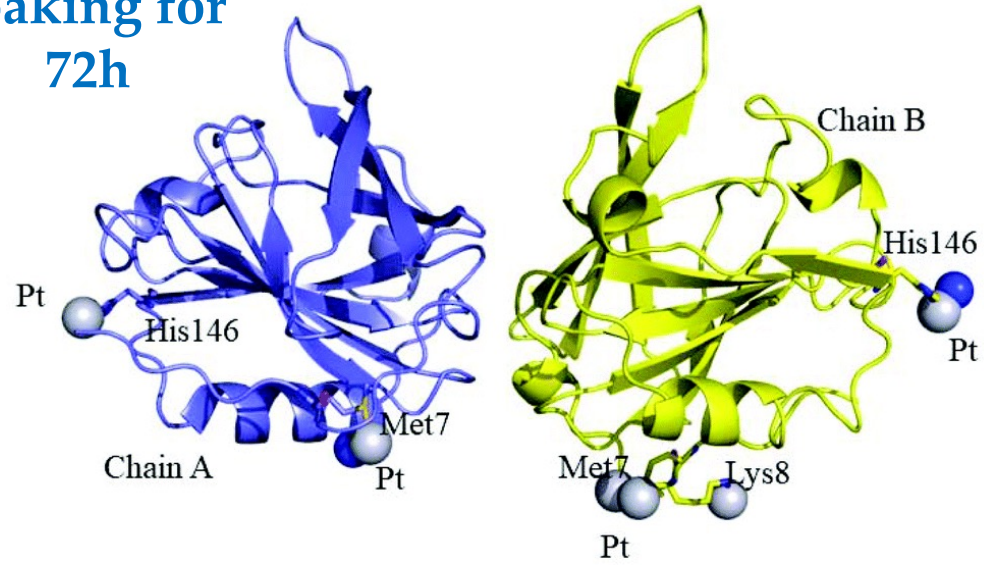
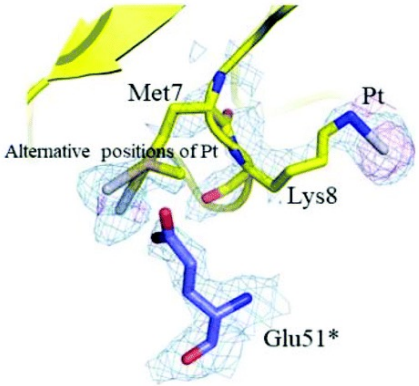
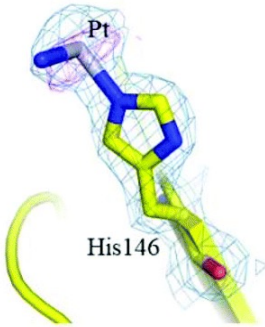
Results and Discussion: crystallographic data

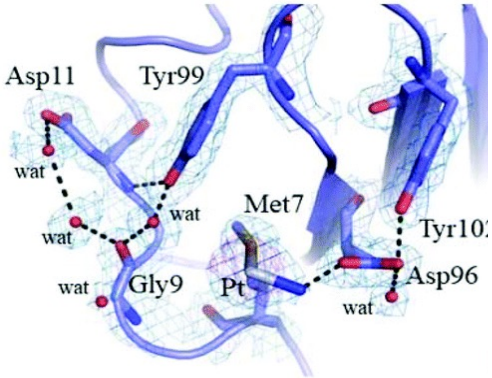
CDDP soaking for 72h

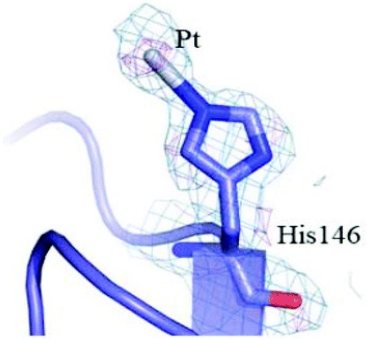






An additional **Pt** binding site is found close to the side chain of **H146** in both A and B chains





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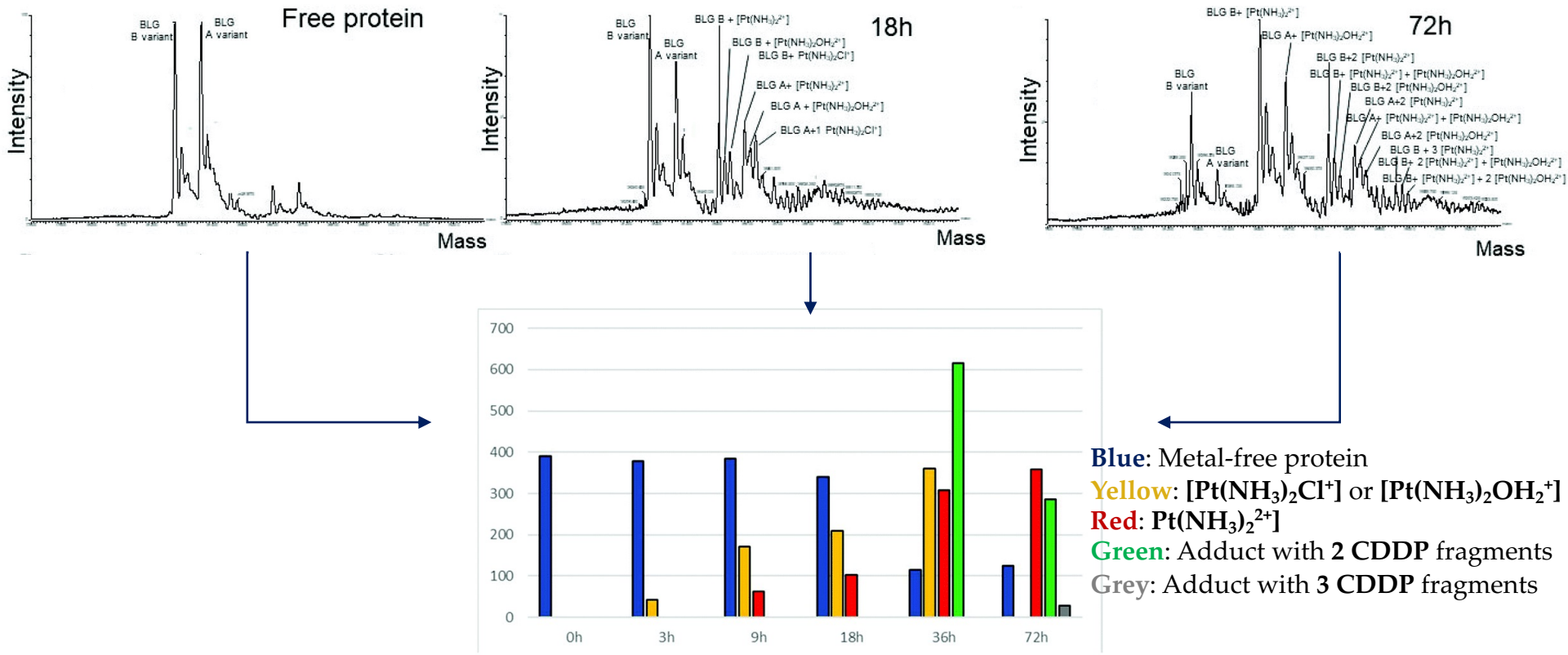
Results and Discussion: Mass spectrometry analysis



Blue: Metal-free protein
Yellow: **$[Pt(NH_3)_2Cl^+]$** or **$[Pt(NH_3)_2OH_2^+]$**
Red: **$Pt(NH_3)_2^{2+}]$**
Green: Adduct with **2 CDDP** fragments
Grey: Adduct with **3 CDDP** fragments

Bar graph summarizes the results of mass spectrometry course experiments. Two forms of native protein are observed for A and B variants of the protein. After three hours the first Pt/β-lactoglobulin is formed. Longer reaction times (9h) give rise to another protein-metal fragment. Only after 36h the presence of two binding Pt sites is observed, while 72h are needed for observing adducts of the protein with three CDDP molecules

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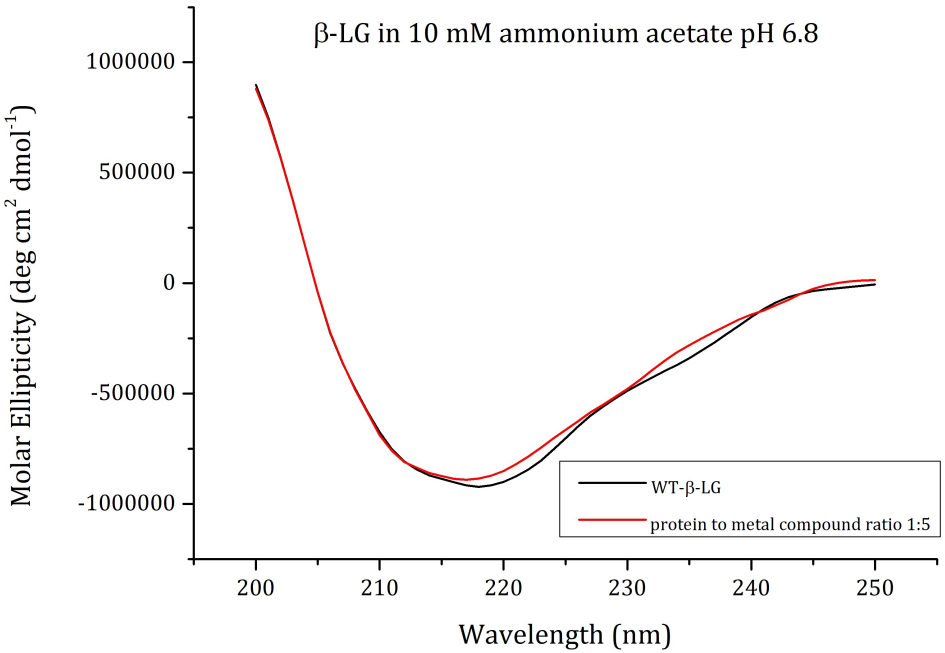
Results and Discussion: interaction with Oxaliplatin
In solution characterization



The protein retains its secondary structure in presence of **oxaliplatin**

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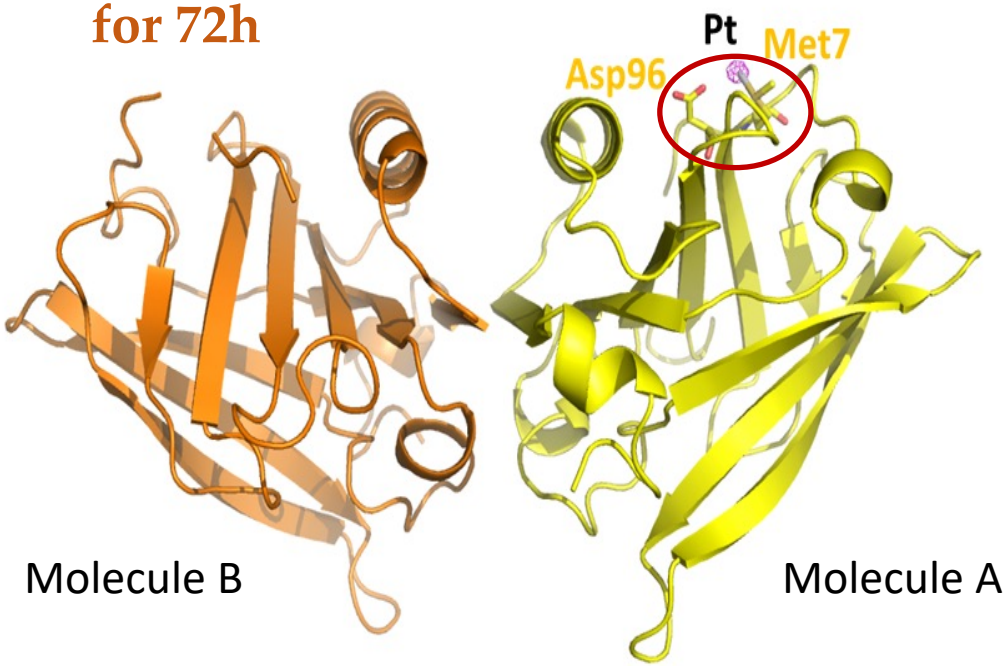
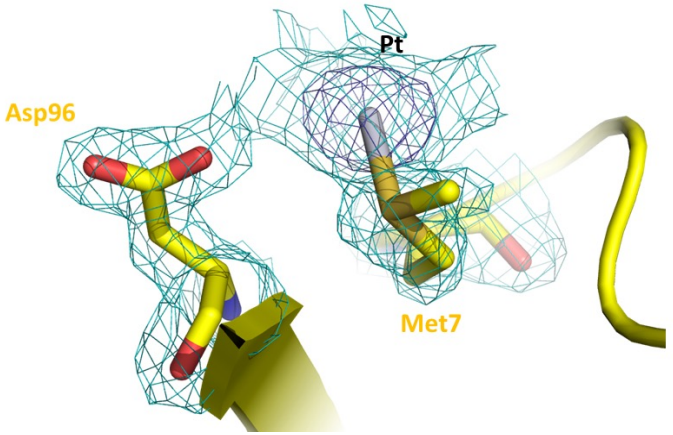
Results and Discussion: crystallographic data

OXA soaking for 72h





- As in the adduct with cisplatin, a **Pt** binding site is observed in proximity of **Met7** side chain of molecule A
- Access to the side chain of **Met7** of molecule B is hampered by crystal lattice

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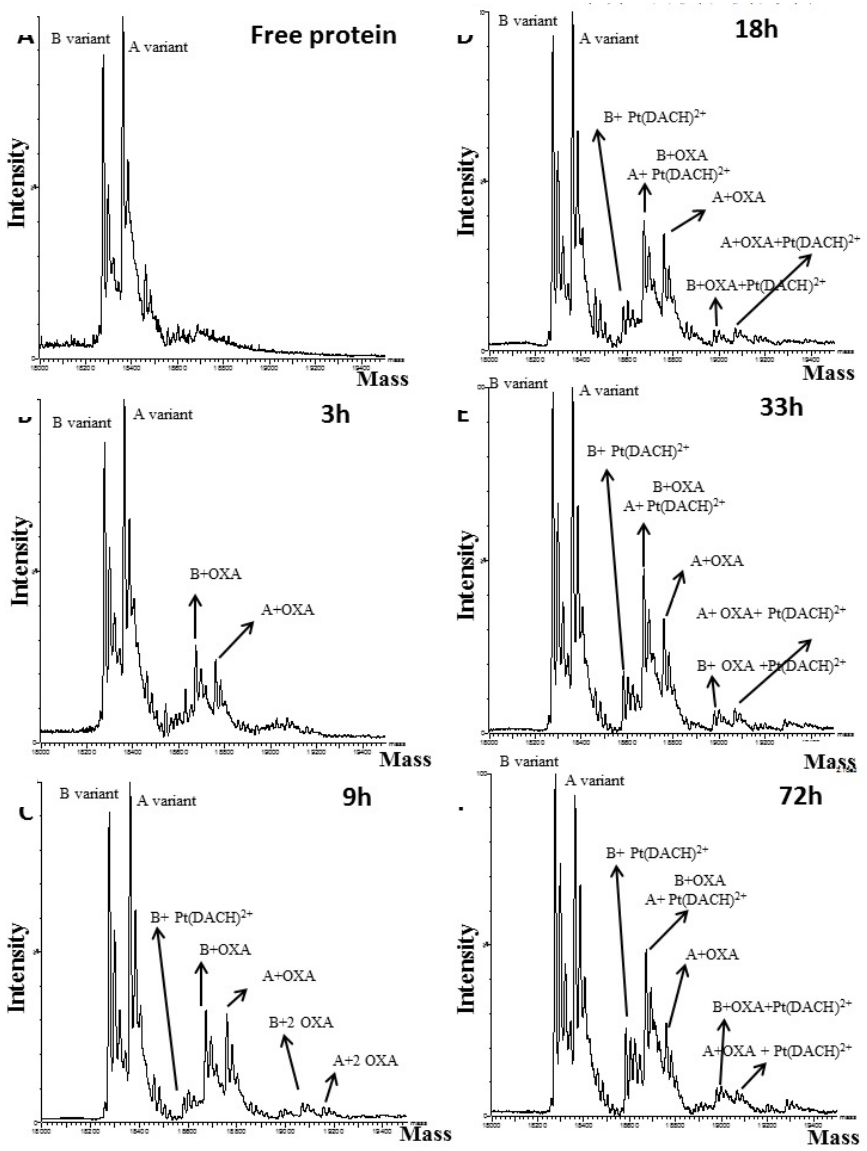
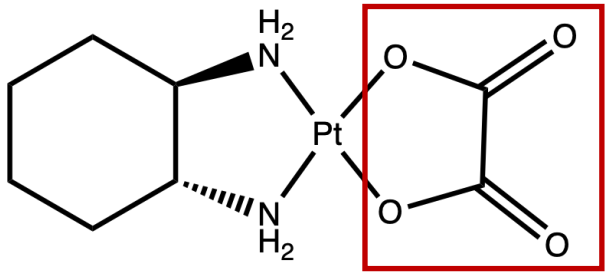
Results and Discussion: Mass spectrometry analysis



Oxaliplatin first binds ***β*-lactoglobulin** non-covalently (3h) and successively it coordinates to protein residue side chains (9h) upon relasing of oxalate moiety (72h)



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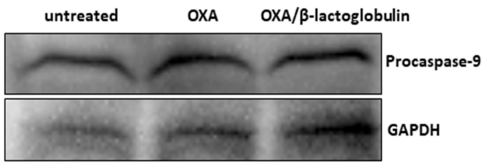
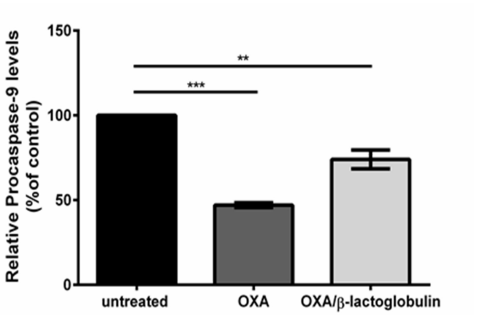
Results and Discussion: In vitro cytotoxicity analyses

MTT assay reveals IC_{50} values much lower for metal/protein adducts than the those of free drugs

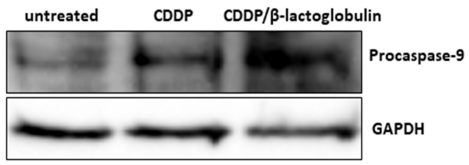
	IC_{50} μM (48h)			
	A431	SVT2	HaCaT	BalbC 3T3
OXA	90.7 ± 16.2	4.9 ± 1.2	10.0 ± 1.0	89.1 ± 2.2
OXA/BLG	17.1 ± 2.4	1.5 ± 0.07	12.0 ± 2.0	11.0 ± 2.3
CDDP	39 ± 12*	195 ± 7*	6.6 ± 0.3*	240 ± 47*
CDDP/BLG	8.7 ± 0.5	3.5 ± 0.9	4.4 ± 0.1	12.0 ± 0.9

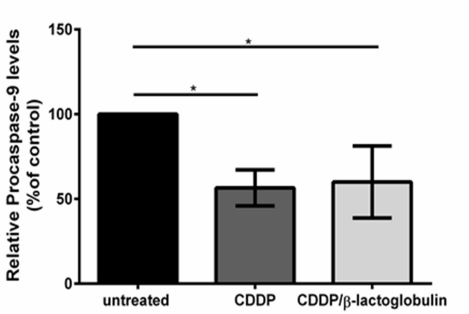
oxaliplaltin





cisplatin





A decrease in pro-caspase 9 indicates that metal/protein adducts are able to induce apoptosis

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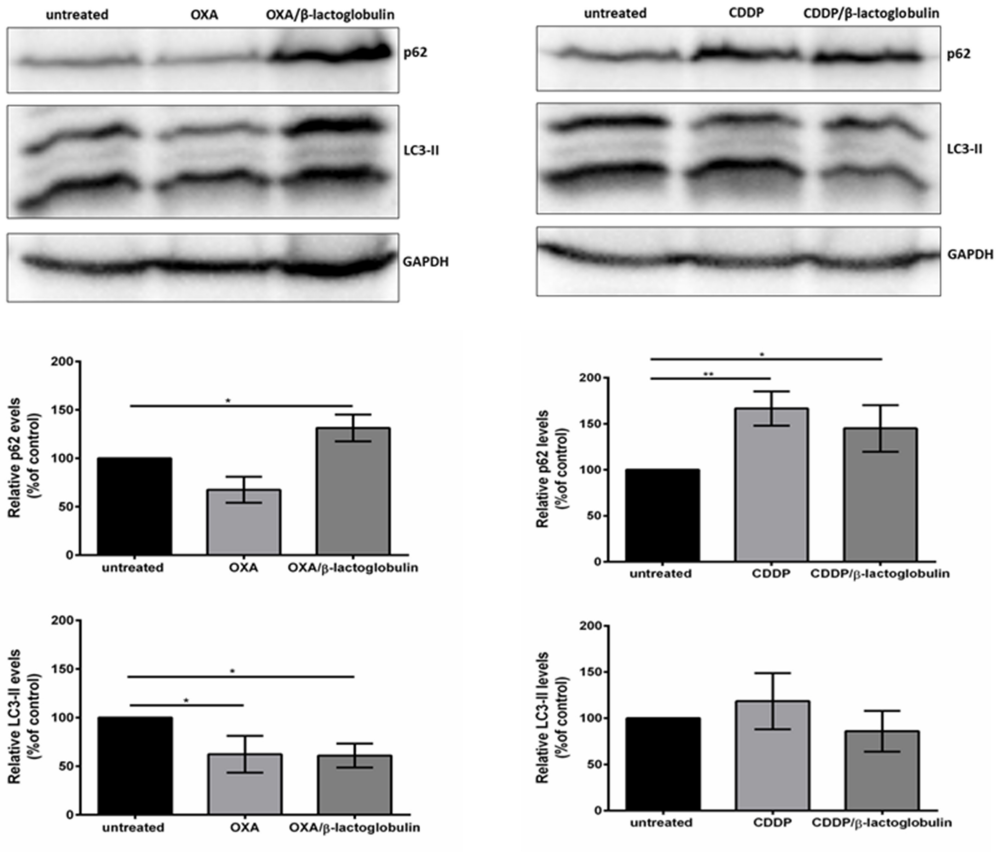
Results and Discussion: In vitro cytotoxicity analyses



An alteration of autophagic markers p62 and LC3 was observed for the metal protein adducts

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Conclusions

- The interactions between ***β*-lactoglobulin** and Pt-based anticancer metallodrugs **cisplatin** and **oxaliplatin** have been investigated both in solution and at solid state.

- Spectroscopic analyses reveal that ***β*-lactoglobulin** binds **cisplatin** or **oxaliplatin** without altering its overall conformation.

- The structure of the adduct formed upon reaction of cisplatin with *β*-lactoglobulin has been solved by X-ray crystallography. The results reveal that the number of **cisplatin** fragments bound to the protein increases with the incubation time. Pt binding sites are observed close to the side chain of **Met7**, **Lys8** and **His146**.

- The structure of the adduct formed upon reaction of oxaliplatin with *β*-lactoglobulin reveal the presence of a single Pt binding site close to **Met7**.

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Conclusions

- ESI-MS analyses reveal that **BLG** binds **CDDP** in a monodentate mode and then in a bidentate fashion. Up to 3 **CDDP** molecules can bind the protein.

- ESI-MS data suggest that **OXA** rapidly binds the protein non-covalently and then via coordination of a $[Pt(DACH)]^{2+}$ fragment to a **β-lactoglobulin** residue side chain.

- Since the Pt binding to **Met** side chain could be reversible, our data suggest that **β-lactoglobulin** could be used as Pt-based drug delivery system.

- Cytotoxicity data reveal that CDDP/BLG and OXA/BLG adducts exert higher cytotoxicity than free drugs and that the mechanism of action involves apoptosis.

These results open the way for a rational design and development of new biomaterials based on metallodrug/β-lactoglobulin adducts potentially useful as oral drugs

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Acknowledgments

Prof Antonello Merlino
Dr Giarita Ferraro

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