# Experimental report

12/09/2023

**Proposal:** 8-04-957 **Council:** 4/2023

**Title:** Probing drug pharmacokinetics by QENS. The effect of cisplatin-like anticancer agents on HSA blood protein.

**Research area:** Biology

**This proposal is a resubmission of 8-04-933**


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**Samples:**
H2O-hydrated HSA protein
H2O-hydrated Pd2Spm-HSA
D2O-hydrated Pd2Spm-HSA
Lyophilised HSA
Lyophilised Pd2Spm-HSA protein
Lyophilised cisplatin-HSA protein
D2O-hydrated HSA
H2O-hydrated cisplatin-HSA
D2O-hydrated cisplatin-HSA
HSA in D2O solution
cisplatin-HSA in D2O solution
Pd2Spm-HSA in D2O solution

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| IN5 | 2 | 2 | 01/09/2023 | 04/09/2023 |
| IN16B Si 111 BATS | 6 | 2 | 09/09/2023 | 11/09/2023 |


## Abstract:

Inelastic neutron scattering will be applied to probe the interaction between cisplatin-like anticancer drugs and human serum albumin (HSA), based on their effect on the dynamical behaviour of the protein. This drug-HSA interaction is associated with drug transport/bioavailability and with antitumour efficacy. These are major limiting factors in cancer treatment, that can severely undermine chemotherapy. A dinuclear palladium compound developed by the team will be tested, in addition to the clinically used drug cisplatin. In combination with extensive QENS data gathered by the applicants on the impact of these drugs on human cancer cells and DNA, as well as with synchrotron-based THz results on drug-HSA interplay, this study is expected to contribute to a better understanding of the mode of action of metallodrugs, with a view to improve their therapeutic efficiency. This is a re-submission of proposal 88056 after revision according to reviewers comments (marked as underlined text).

# Experimental Report – 8-04-957

**1 PRINCIPLE INVESTIGATOR**

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**2 EXPERIMENT DETAILS**

Experiment Number: **8-04-957**
Title: PROBING DRUG PHARMACOKINETICS BY QENS
THE EFFECT OF CISPLATIN-LIKE ANTICANCER AGENTS ON HUMAN SERUM ALBUMIN
Equipment/Facility Used: **IN5** & **IN16B**
Dates: 1-4 Sept 2023/IN5 & 5-7 Sept 2023/IN16B-BATS

**3 ABSTRACT/OBJECTIVES**

Drug binding drastically affects the internal diffusive dynamics of proteins – either through direct metal coordination or by interplay with the protein´s hydration layer – and this effect can only be assessed by quasi-elastic neutron scattering. QENS was applied to probe the interaction between cisplatin-like anticancer drugs and human serum albumin (HSA). This drug-HSA interplay is associated with drug transport and bioavailability, which are major limiting factors in cancer treatment that can severely undermine chemotherapy success. A dinuclear palladium compound newly developed by the team was tested, in addition to the clinically used drug cisplatin. In combination with extensive QENS data gathered by the applicants on the impact of these drugs on human cancer cells and DNA, as well as with synchrotron-based THz results on drug-HSA adducts, the present study is expected to contribute to a better understanding of the mode of action of metallodrugs, with a view to improve their therapeutic efficacy.

**4 REPORT**

QENS data was collected for aducts of human serum albumin with the spermine complex $Pd_2Spm$ (Spm=spermine, $H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$), and with the conventional platinum drug cisplatin (*cis*-$(NH_3)_2PtCl_2$). Measurements were also performed for the free protein and drugs, for comparison purposes. Solid samples – both hydrated (in $H_2O$ and $D_2O$) and lyophilised adducts – and $D_2O$ solutions were analysed. IN5 (low resolution 5A) was used, at a resolution of *ca.* 80 μeV FWHM. Due to a chopper failure, the measurements at IN16B in BATS mode (BATS *ca.* 150 μeV energy transfer at 3.5 μeV resolution) could not be carried out. When performed, these will allow access to overlapping energy axes, on a similar Q-range, which will enable a simultaneous fit of the spectra from both instruments. The drugs´ influence on the protein was evaluated at two levels: on the side chain fluctuations (IN5, ps timescale) and on the backbone dynamics (BATS, ns timescale). The results from hydrated powders (at 100 and 310 K) yielded information on the diffusive dynamical processes of the side chains, as well as on the hydration water dynamics. In turn, the experiments in solution (at 280 and 310 K) allowed to obtain data under conditions closer to the physiological ones.

**Results from IN5**

The QENS profiles obtained for free HSA evidenced an expected behaviour – faster dynamics for the $H_2O$-hydrated system (revealing the fast motions of the labile H´s from the protein side chains), and progressively lower flexibility for the $D_2O$-hydrated and lyophilised HSA (Fig. 1 (A)). Upon drug exposure a change in the protein´s diffusive properties was observed, revealing a drug impact which is associated with the *in vivo* cytotoxicity (anticancer effect). The palladium agent appears to have a more significant effect on protein dynamics than cisplatin, mainly on the labile hydrogens (fast dynamics, in the ps timescale) – since some variation of the QENS profile was detected for the $H_2O$-hydrated $Pd_2Spm$-HSA adduct (Fig. 1 (B)), but hardly for $D_2O$-hydrated $Pd_2Spm$-HSA. Regarding the measurements in $D_2O$-solution at physiological temperature (50 mg/ml for HSA/maximum solubility and saturated solutions for the drug-HSA samples), the QENS profiles clearly revealed a drug-prompted increase in flexibility as compared to the free protein (Fig. 1 (C)), which is probably due to conformational rearrangements (loss of the native structure) upon drug interaction/binding – cisplatin displaying a slightly higher effect.

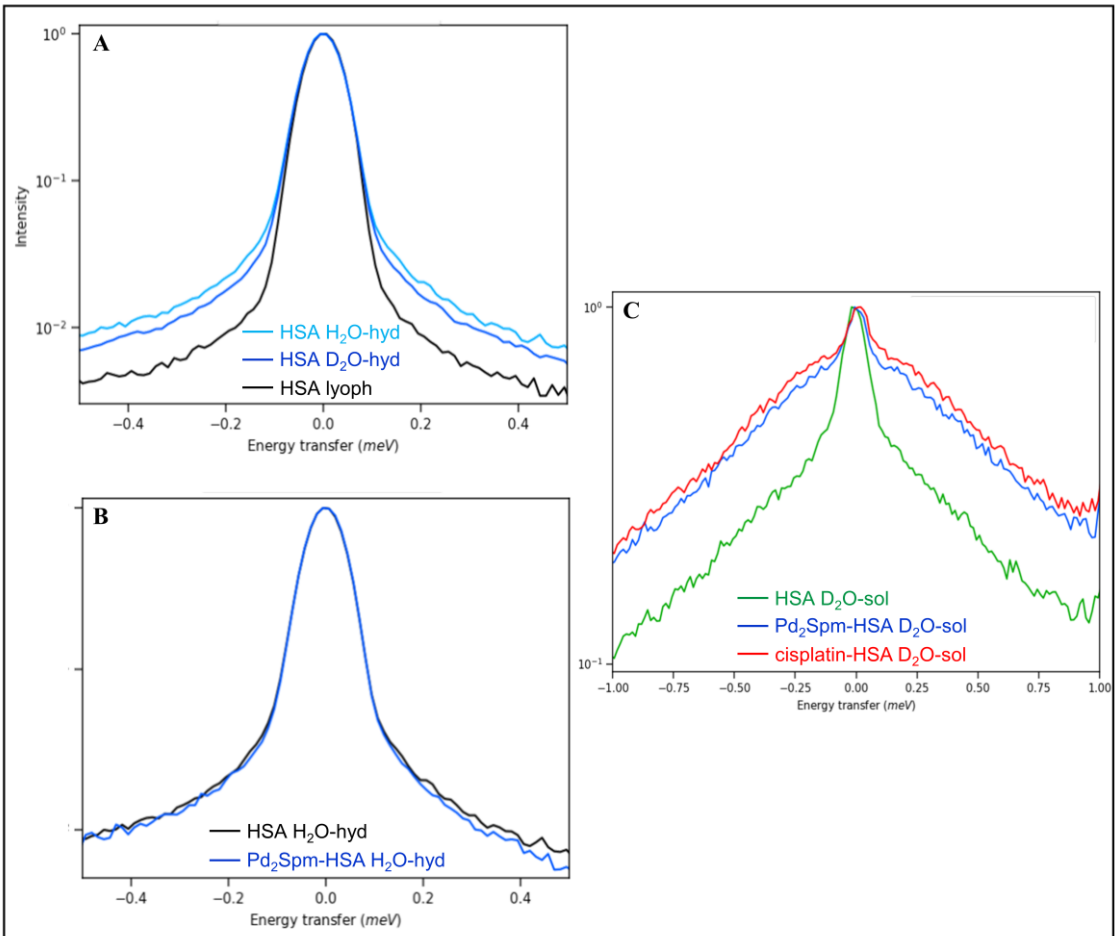


**Figure 1** – QENS profiles (310 K, averaged over the entire Q range, log scale) for: **(A)** human serum albumin (HSA), lyophilised and $H_2O$- or $D_2O$-hydrated; **(B)** untreated and $Pd_2Spm$-exposed HSA; **(C)** $D_2O$-solutions of HSA, untreated and drug-treated.

**Results from IN16B/BATS**

Due to a chopper failure, the experiment at IN16 had to be postponed.

Data will be fitted to existing models (formerly optimised by the team [1-3] and the local contact [4]), to obtain diffusion coefficients and relaxation times. Interpretation of the results will be assisted by previous studies on drug effects on bovine serum albumin [5], as well as on DNA [2,6-8], cells [8] and tissues [3]. A cisplatin predominant impact has been found on DNA's spine of hydration, while $Pd_2Spm$ has showed a major impact on DNA's backbone dynamics [2]. Additionally, former THz and INS/QENS spectroscopy data obtained by the team (at Diamond and ISIS) have evidenced drug-triggered conformational changes and increased protein flexibility for cisplatin-HSA and $Pd_2Spm$-HSA [9]. Coupled to the present results, relevant information on the metallodrugs´ impact on protein dynamics should be achieved. This type of molecular level studies on protein metalation by cisplatin-like drugs are still scarce, despite their relevance for unveiling the mode of action of metallodrugs aiming at an enhanced therapeutic effect coupled to a better patient compliance (*i.e.* increased bioavailability and lower side-effects).

[1] M.P.M. Marques *et al. PCCP* 19 (2017) 2702.
[2] M.P.M. Marques *et al.* Molecules 25 (2020) 246.
[3] M.P.M. Marques *et al.* PCCP 24 (2022) 15406.
[4] Grimaldo *et al.* Quart.Rev.Biophys. 52 (2019) 1.
[5] Grimaldo *et al. PCCP* 17 (2015) 4645.
[6] A.L.M. Batista de Carvalho *et al.* Faraday Disc. 187 (2016) 273.
[7] I. Lamego *et al.* J.Proteome Res. 16 (2017) 1773.
[8] A.L.M. Batista de Carvalho *et al.* PCCP 21 (2019) 4162.
[9] L.A.E. Batista de Carvalho *et al.* Biophys.J. 120 (2021) 3070.