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SUPPLEMENTARY MATERIAL TO

Physicochemical characterisation of dihydro-alpha-lipoic acid interaction with human serum albumin by multi-spectroscopic and molecular modelling approaches

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Molecular docking analysis

Docking simulations were carried out with the Schrodinger Maestro Suite (Schrödinger, LLC, New York, NY, 2018) using the crystal structure of HSA complexed with warfarin,[1] (PDB code: 2BXD, obtained from the RCSB PDB database (https://www.rcsb.org/)). The DHLA structure was drawn in the ChemDraw program (PerkinElmer Informatics, 2017). All structures were prepared in Maestro software, using default procedures. Up to 20 different docked structures were generated with the Induced fit docking protocol.[2] The obtained docking structures were examined and the best structure was selected based on the number of receptor–ligand interactions and docking score.

Molecular dynamics (MD) simulations

MD simulations were performed in the Schrodinger Desmond software package.[3] The docked structure selected for MD was solvated with TIP3P explicit water model and neutralised *via* counter ions. A 0.15 M KCl salt solution was added. To calculate the interactions between all atoms, OPLS 2003 force field was used. For the calculation of the long-range Coulombic interactions, the particle-mesh Ewald (PME) method was used, with a cut-off radius of 0.9 nm for the short-range van der Waals (vdW) and electrostatic interactions.

During the course of the simulation, a constant temperature of 310 K and a pressure of 1.01235 bar were maintained, using a Nose–Hoover thermostat, and the Martyna Tobias Klein method. MD simulation of 50 ns with a 2.0 fs step was

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performed and the collected trajectory used in the MD analysis to assess the docking pose and the stability of the protein–ligand interactions.

Molecular docking and dynamics results

The binding site on subdomain IIA consists of a binding pocket placed deeply in the core of the subdomain and is formed from all six helices of the subdomain and the loop-helix residues 148–154 of subdomain IB.[16] The interior of the pocket is mostly hydrophobic, apart from the two clusters of polar amino acid residues (Tyr150, His242, Arg257 and Lys195; Lys199, Arg218 and Arg222).

The results from the induced fit docking simulation showed that HSA is able to bind DHLA at subdomain IIA binding site (Fig. S-1A). The energetically most favourable conformation of the docked pose demonstrated that the main interactions are salt bridges formed by the carboxyl group of DHLA with Arg218 and Arg222 residues of HSA, followed by hydrogen bonds formed between the sulfhydryl group of DHLA and Arg257, Ser287 residues of HSA (Figs. S-1B and S-1C). Molecular docking suggested that DHLA binds at the subdomain IIA binding site in a defined conformation, thus favouring interactions with specific amino acid residues. Considering the high torsional flexibility of DHLA due to nine dihedral angles that give many possible rotamers of DHLA,[4] the recorded change in the UV-absorption spectrum (Fig. 3B, main manuscript) could point to a DHLA-conformational shift towards rotamers that have the highest probability of binding to HSA.

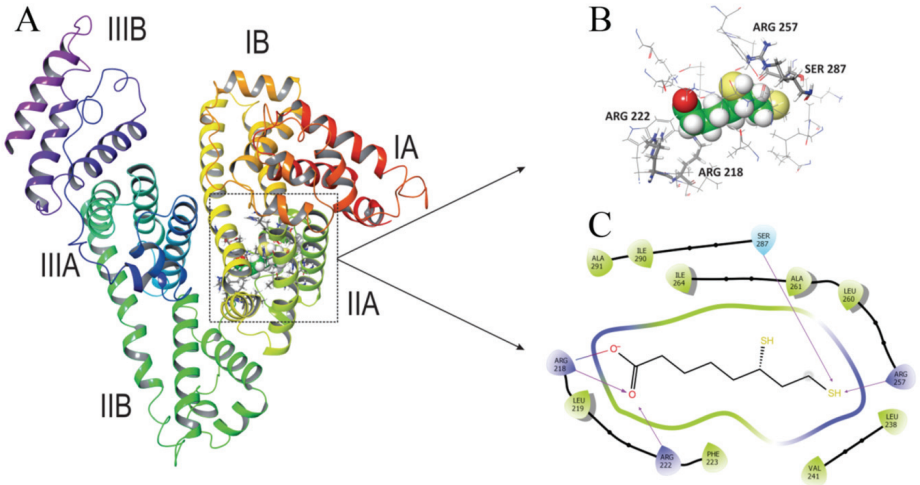

Fig. S-1. An overview of HSA with DHLA bound into subdomain IIA (A and B). The domains are colour coded and represented as secondary structure ribbons. Subdomain IIA composition and key interactions diagram (C). All amino acid residues in close contact with DHLA are displayed, with key amino acid residues marked.

For verification of docking simulation results, HSA–DHLA interactions were monitored throughout a 50 ns molecular dynamic simulation. The best conformation obtained in the molecular docking was set as the starting point for MD. The obtained MD trajectory was used to analyse both complex stability and the persistence of the main HSA–DHLA interactions over the simulation time used.

The observed *RMSD* values for HSA alone and HSA–DHLA complex show that the simulation equilibrated with the fluctuations falling within the 0.1 – 0.25 nm range. This suggests that minor conformational changes occurred during the simulation (Fig. S-2A).

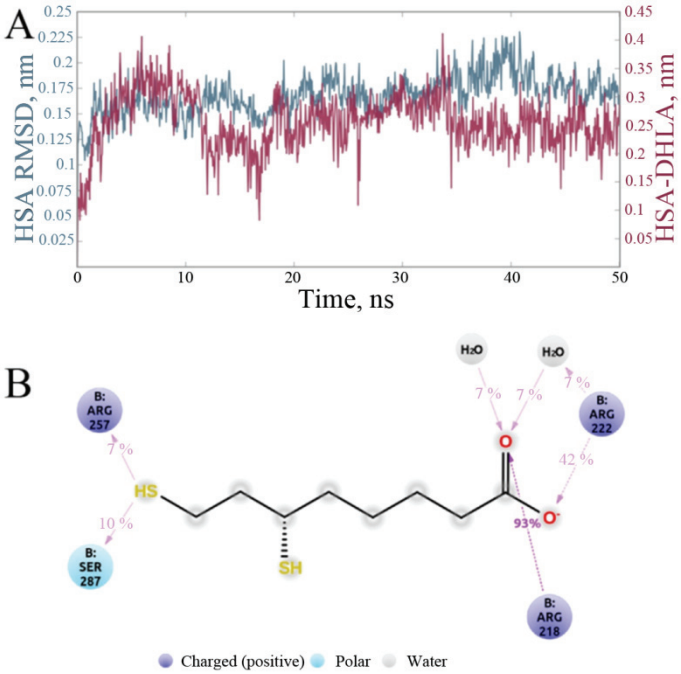

Fig. S-2. HSA and DHLA *RMSD* plot (A) and the observed key interactions during 50 ns simulation time (B).

The monitored HSA–DHLA interactions in the formed complex showed that the most important interaction is a salt bridge formed between the DHLA carboxyl group and Arg218. This interaction was present over 93 % of the simulation time, making it a key interaction for DHLA binding to HSA and it also indicates the correct orientation of DHLA inside the subdomain II binding site. The salt bridge with Arg218 is further reinforced by interaction with Arg222 (42 % of simulation time). Once DHLA is in the correct orientation in the subdomain II binding site, additional hydrogen bonds between sulfhydryl group and Ser287 and Arg257 are formed. These hydrogen bonds are maintained for 10

% (Ser287) and 7 % (Arg257) of the total simulation time (Fig. S-2B). All other observed interactions were present for less than 5 % of total simulation time (Fig. S-3).

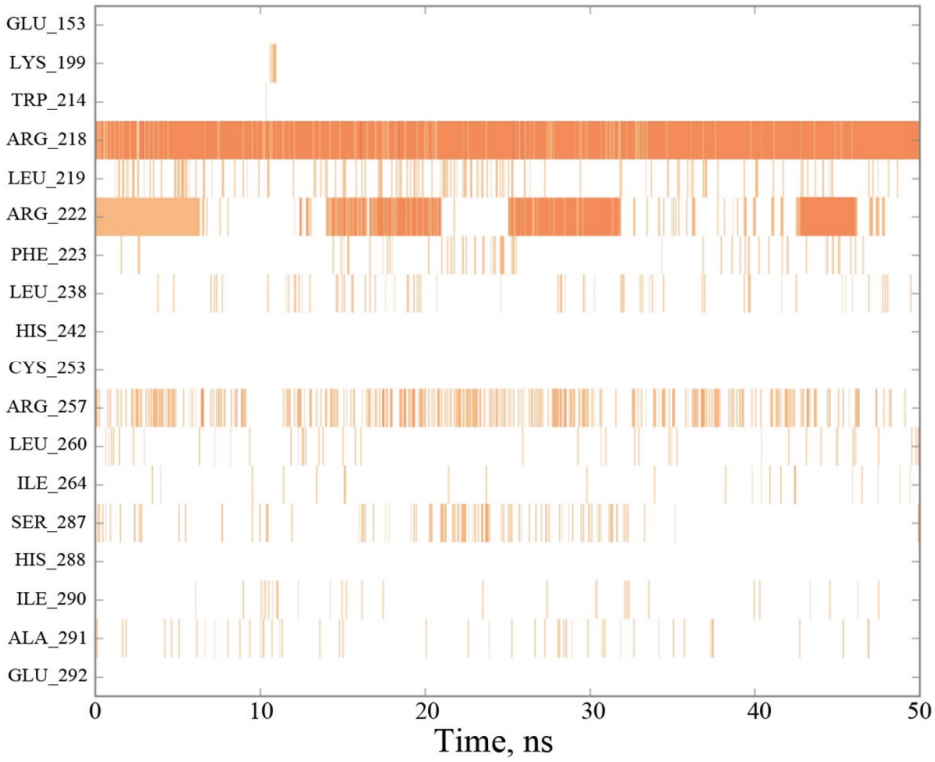

Fig. S-3. Summary of DHLA–HSA interactions observed during 50 ns simulation time. Each orange line represents one established interaction during a 1 ns time frame.

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