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Comprehensive analysis of photosensitizer-biomolecule interactions using hybrid EPR and computational methods

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Motivation and Aim: Photodynamic therapy (PDT) is a promising non-invasive therapeutic approach valued for its selectivity. PDT relies on photosensitizers (PSs) that accumulate at tumor sites and interact with oxygen and light to produce reactive oxygen species. These reactive species induce controlled cell death and enhance immune responses. PSs are non-toxic until activated by light, distinguishing PDT from other cancer treatments. However, the effectiveness of PDT is influenced by the biodistribution and pharmacodynamics of the PSs.

The intracellular localization of PSs directly impacts their mechanism of action and overall efficacy in PDT. Localization within target tissues and cell organelles depends on the chemical structure of PSs, including charge, lipophilicity, size, and asymmetry. Most clinically used PSs are macrocyclic tetrapyrrole structures (such as porphyrins, chlorines, and phthalocyanines) administered intravenously. These compounds are chosen for their high absorption in the visible light spectrum and their efficiency in generating singlet oxygen. However, their tendency to aggregate and their poor tumor-targeting capabilities limit their potential in PDT.

One strategy to enhance PDT effectiveness is using transport proteins to improve PS solubility and distribution. Human serum albumin (HSA), the most abundant protein in blood plasma, serves as a natural drug carrier due to its biocompatibility, water solubility, and ability to bind various compounds. HSA's hydrophobic pockets can stabilize PSs, preventing aggregation and extending their half-life. The affinity of PSs for HSA affects their plasma concentration, distribution, and therapeutic efficacy. However, the exact binding sites and interactions between PSs and HSA remain poorly understood, complicating efforts to optimize PSs for clinical use.

Traditional methods like fluorescence-based techniques provide limited and often ambiguous information about PS binding sites on HSA. These methods struggle to distinguish between multiple binding sites. Pulsed dipolar Electron Paramagnetic Resonance (EPR) spectroscopy provides nanometer-scale structural information and captures conformationally distinct states of the system under study without limitations on biomolecule size. Unlike other methods, pulsed dipolar EPR spectroscopy provides the whole distance distribution between labels.

Recently, LaserIMD (laser-induced magnetic dipole) spectroscopy has been proposed to measure the distances between the photoexcited state of PS and a stabilized nitroxide label. Ongoing methodological work on light-induced PDS using photoexcited triplet states opens new opportunities for structural studies of biological complexes with photoactive molecules, including those used in PDT [1]. LaserIMD EPR spectroscopy

offers precise measurements of distances between PSs and nitroxide spin labels on protein, capturing the full distance distribution between labels. This technique overcomes the limitations of other methods by discriminating between different binding sites. However, even with precise distance measurements, identifying exact binding sites can be challenging due to complex structural dynamics.

The aim of this study [2] is to develop and apply this integrative approach to investigate the interactions between various PSs (anionic, neutral, and cationic) and HSA. By combining high-resolution EPR spectroscopy with sophisticated computational models, we aim to provide a detailed, experimentally validated map of PS binding sites on HSA. This will enhance our understanding of PS-HSA interactions and support the development of more effective PDT agents.

Methods and Algorithms: In this study, we employed a novel integrative approach combining experimental laser-induced EPR spectroscopy with advanced computational techniques, including molecular docking and molecular dynamics simulations, to investigate the structures of human serum albumin with photosensitizers.

The following steps outline our methodology:

1. Laser-Induced Dipolar EPR Spectroscopy (LaserIMD). The experimental investigation began with LaserIMD EPR spectroscopy conducted at 30 K using a Q-band Bruker Elexsys E580 spectrometer equipped with a cryogenic system from Oxford Instruments. Samples were photoexcited using a Nd:YAG laser at 532 nm. This method provided high-resolution distance measurements between nitroxide spin labels and photoexcited porphyrins, revealing intricate details of the binding sites and conformational dynamics of the complexes.
2. Molecular Docking. Initial blind docking was performed to identify potential binding sites of photosensitizers on HSA. Utilizing AutoDock-GPU, we conducted extensive docking simulations with 1000 LGA runs on a population of 2048 individuals, leveraging GPU acceleration to manage computation times efficiently. The use of GPU acceleration allowed for the generation of up to 1000,000 poses, significantly enhancing the thoroughness of the search.
3. Cluster Analysis and Filtering. After the initial docking, the generated poses were subjected to k-means clustering to group similar binding poses. Clusters were filtered by comparing the potential binding sites with experimental distance distributions obtained from LaserIMD. This rough comparison helped eliminate clusters that were clearly inconsistent with experimental data, ensuring that only plausible binding sites were considered for further analysis.
4. Focused Docking. The filtered clusters from the blind docking phase were subjected to focused docking. This step involved refining the predictions by concentrating on specific regions of interest identified during the initial docking phase. Smaller grid boxes tailored for each type of photosensitizer were employed, enhancing the accuracy of the binding site predictions.
5. Molecular Dynamics (MD) Simulations. The docked complexes underwent molecular dynamics simulations using GROMACS with the Gromos54a7 force field. Ligand topologies were generated by the Automated Topology Builder (ATB). The system was subjected to energy minimization, followed by heating and equilibration, before a 100 ns production run. MD simulations accounted for the mobility and dynamic behavior of the complexes, ensuring that the modeled distances reflected realistic conformational changes.

6. Spin Label Conformation Modeling. To accurately model the distance distributions between spin labels and photosensitizers, we used the ChiLife software to predict the possible conformations of spin labels attached to the Cys34 residue.
7. Comparison and Validation. The experimentally obtained distance distributions from LaserIMD were compared with those predicted by molecular dynamics simulations. This detailed comparison ensured that the computational models accurately reflected the experimental data. The integration of experimental EPR measurements with computational docking and dynamics provided a robust framework for identifying and validating binding sites.

Results: The integrative approach combining LaserIMD EPR spectroscopy and molecular modeling provides clear structural information, including precise distances between spin labels at Cys34 of HSA and porphyrins. This approach allows for accurate filtering and comparison of molecular modeling results with experimental data, enabling the construction of a comprehensive, experimentally validated picture of photosensitizer binding sites and their distribution.

Our findings reveal that binding does not always occur in the standard sites of albumin and often involves multiple sites, which is crucial for interpreting fluorescence data commonly used for characterizing binding. Additionally, comparing docking results with experimental data highlights the limitations of blind docking without experimental validation, necessitating a reevaluation of previous results.

We identified the influence of substituent charge and the presence of metal ions on the localization of binding sites. The consistency between calculated and experimental distance distributions validates our approach.

This methodology is not limited to proteins and can be applied to other systems of interest. For instance, similar techniques were used to investigate the binding of quadruplex DNA with the porphyrin TmPyP4 [3].

Conclusion: In summary, the results demonstrate the potential of our developed approach for investigating biological complexes with photoactive ligands. Combining direct observation of photoactive centers via EPR with the predictive power of computational modeling offers a powerful tool for addressing challenges in photodynamic therapy and other biological applications.

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