COMBINING COMPUTATIONAL METHODS AND EPR SPECTROSCOPY FOR PROTEIN–LIGAND BINDING SITE ANALYSIS

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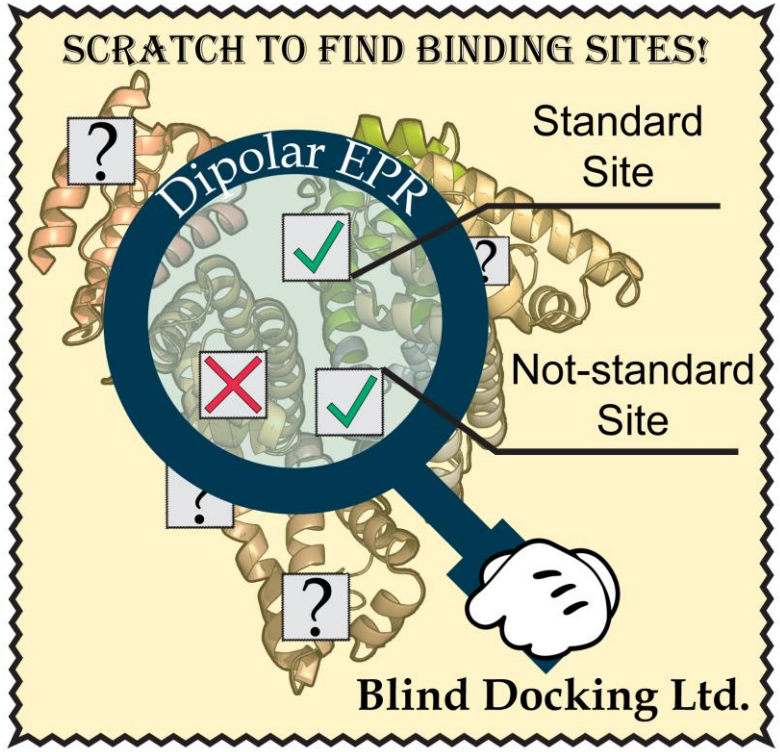
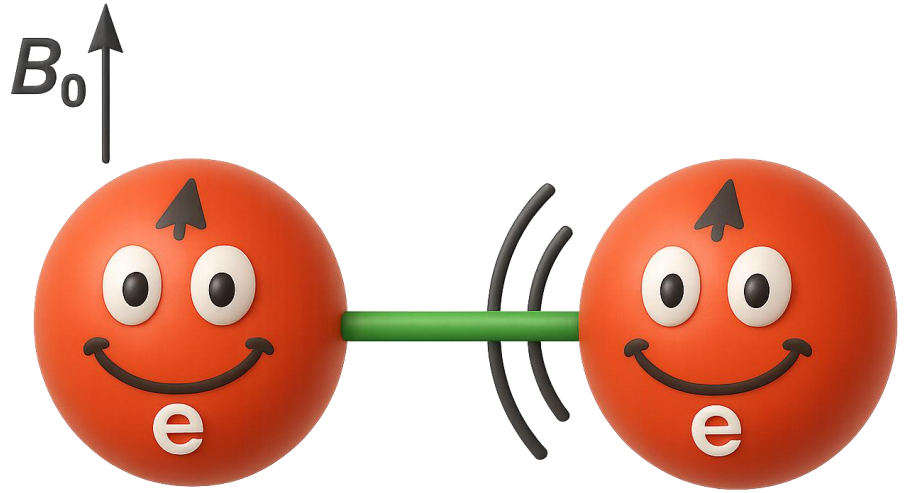


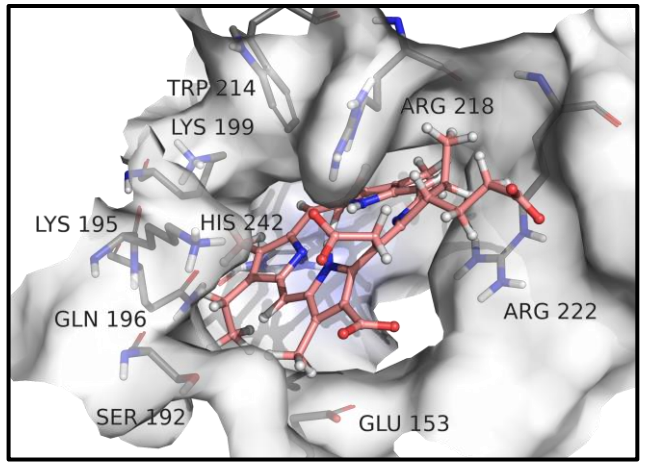


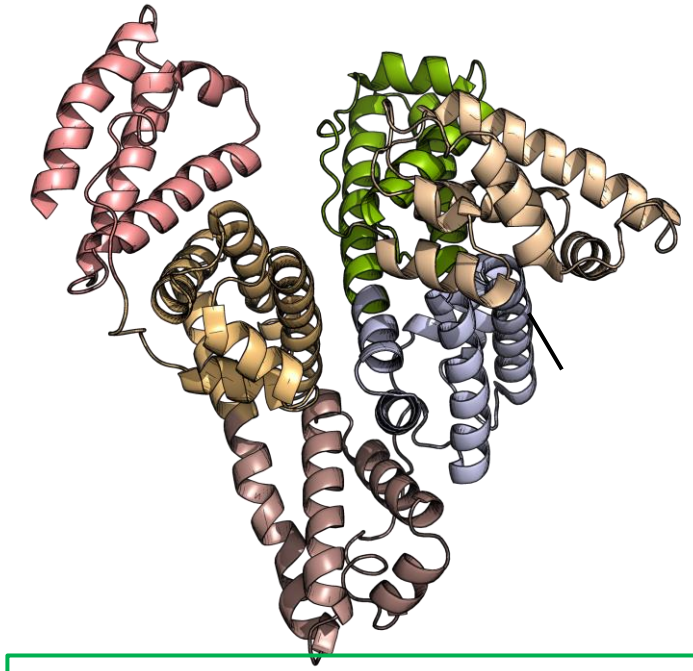
Determining binding sites

Understanding the **binding sites** of drug molecules with biomolecules is essential for understanding their function, improving their properties, and predicting off-target interactions.

However, the use of standard structural biology methods is often complicated:

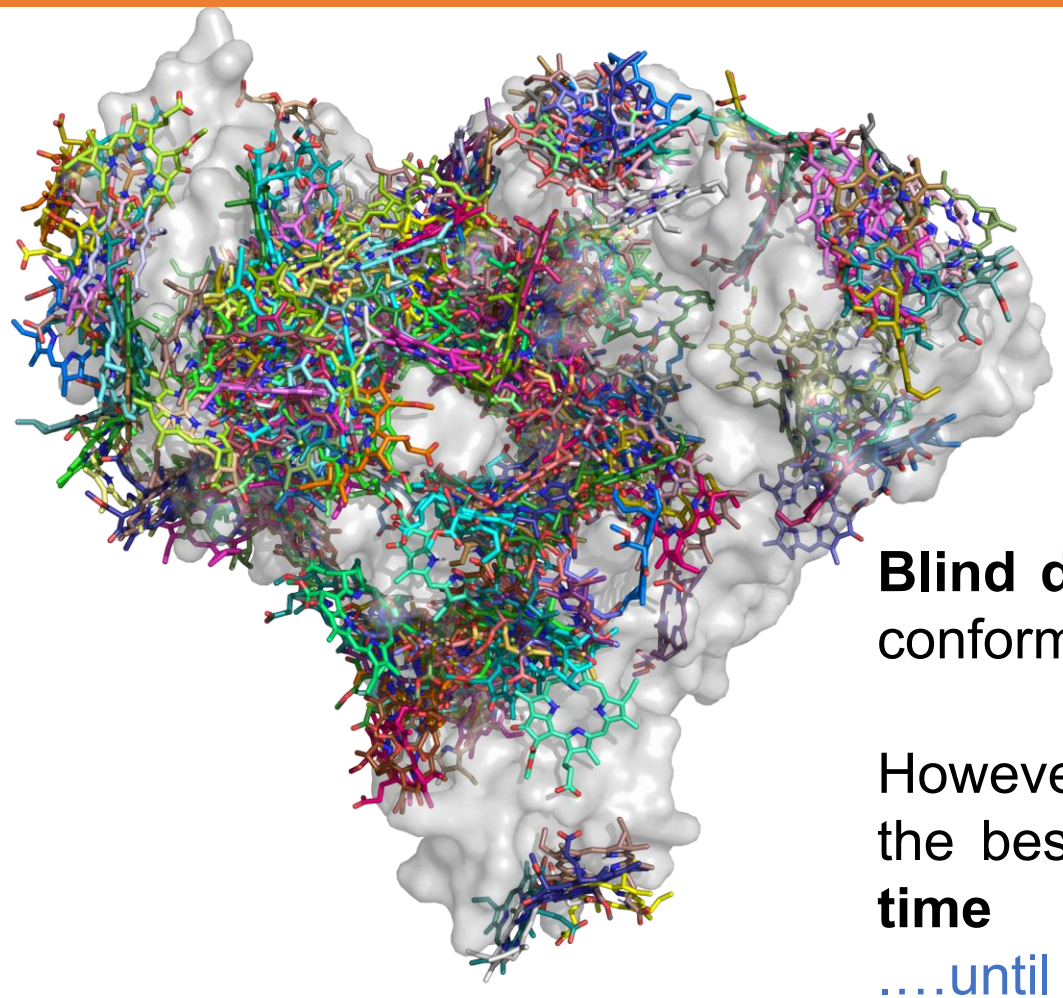
X-Ray – difficult to crystallize labile complexes with ligands bound on the surface.
NMR – difficult to measure large proteins and long distances
Cryo-EM – limited by a minimum protein size and expensive.
Fluorescence – ambiguous when several binding sites are present





Because of these experimental challenges, **computational modeling** methods are often used.

Blind docking



Blind Docking with GPU

Efficient exploration of the conformational space

Reduction of required experimental data

Blind docking allows for rapid exploration of the protein–ligand conformational space.

However, it often struggles to find the correct binding site —even the best algorithms guess it correctly **only about 50 % of the time**

….until we add experimental data!

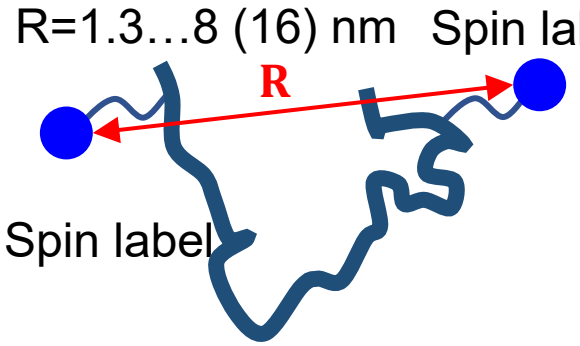
Chen, Y.-C. Beware of Docking! Trends Pharmacol. Sci. 2015
Huang, Y: A Blind Docking Strategy Accelerated by GPUs. J. Chem. Inf. Model. 2023

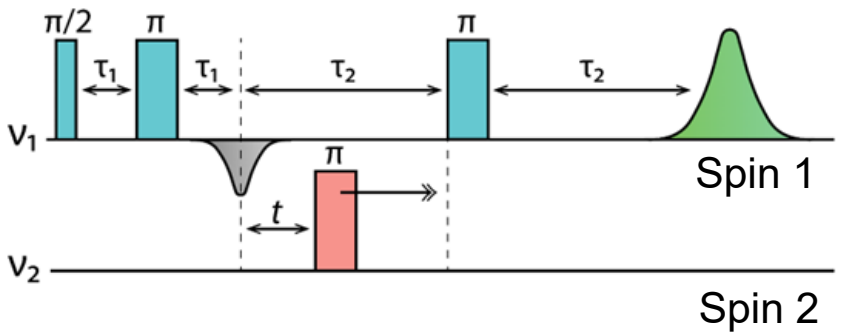
This study:

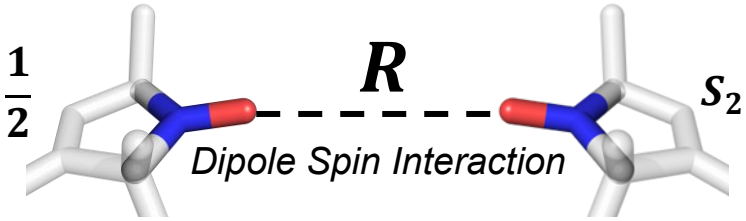
Using dipolar EPR data as a filtering and validation tool to refine complex configurations from molecular modeling , especially blind docking.

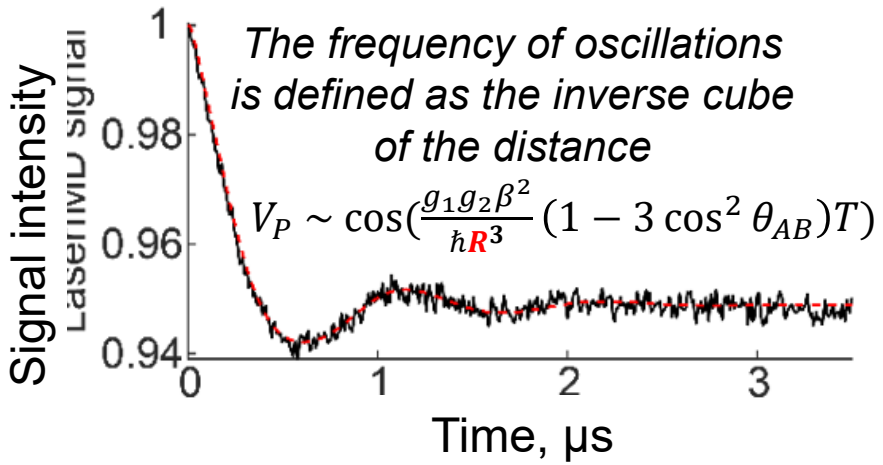
EPR in biology

Electron Paramagnetic Resonance is a method that allows you to study the interactions between unpaired electrons



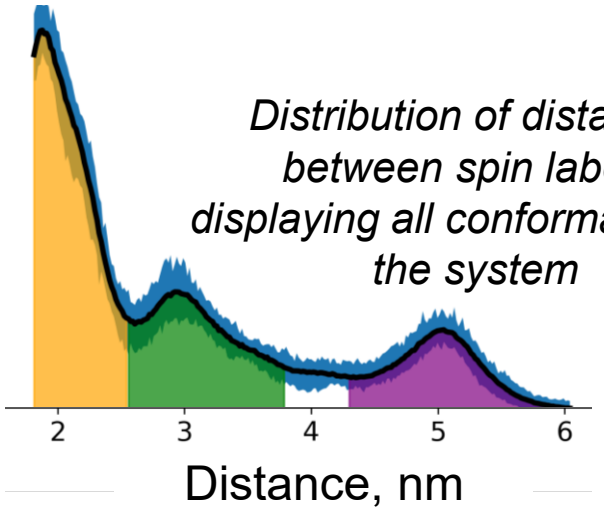


$S_1 = \frac{1}{2}$  $S_2 = \frac{1}{2}$



The frequency of oscillations is defined as the inverse cube of the distance

$$V_P \sim \cos\left(\frac{g_1 g_2 \beta^2}{\hbar R^3}(1 - 3\cos^2\theta_{AB})T\right)$$

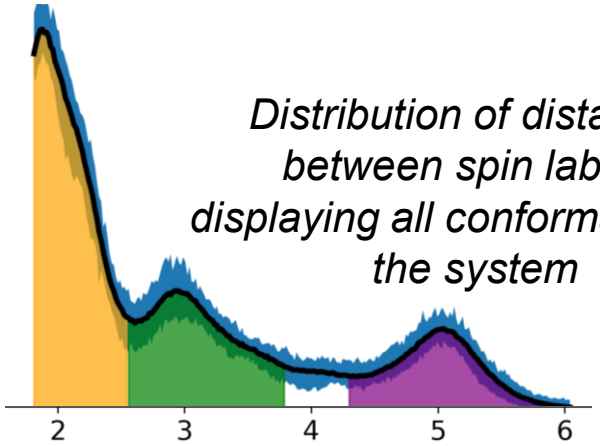


Distribution of distances between spin labels, displaying all conformations of the system

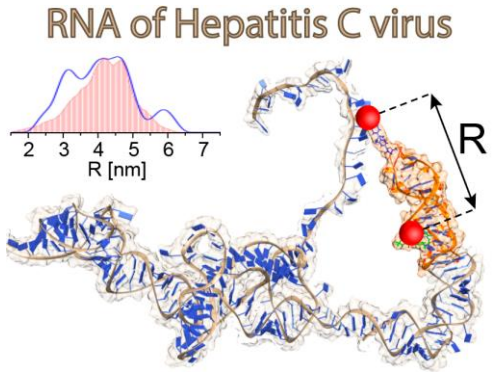
EPR in biology

Advantages of dipolar EPR techniques:

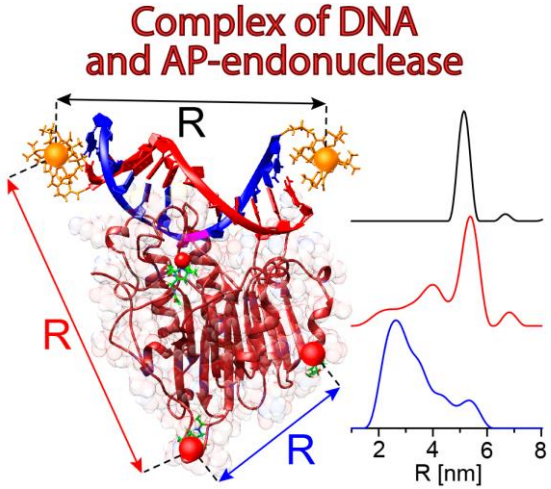
High sensitivity — down to micromolar concentrations.
No limitations on the size or complexity of the system.
Provides information on multiple coexisting conformations.



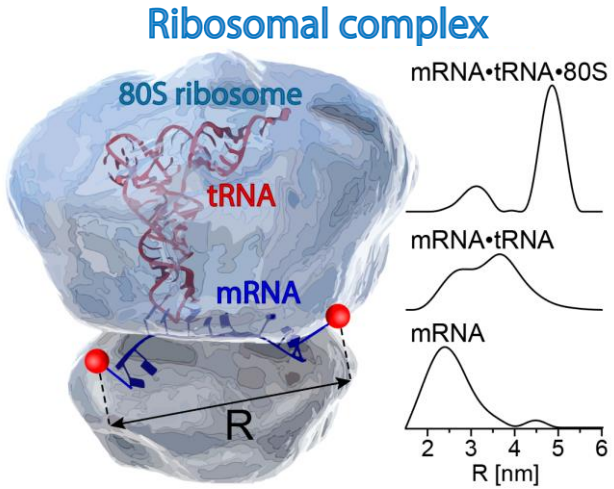
Distribution of distances between spin labels, displaying all conformations of the system



Nucleic acids research, 44(16), 7935-7943



Nucleic Acids Research 47.15 (2019): 7767-7780



Nucleic acids research, 47(22), 11850-11860

However, a single distance measurement is not enough to reconstruct the full structure of a complex.!

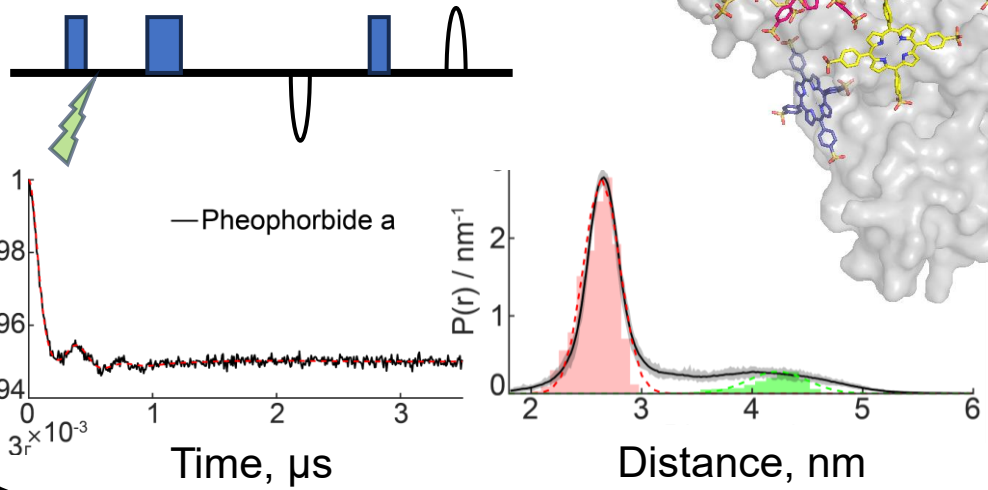
Integrative approach

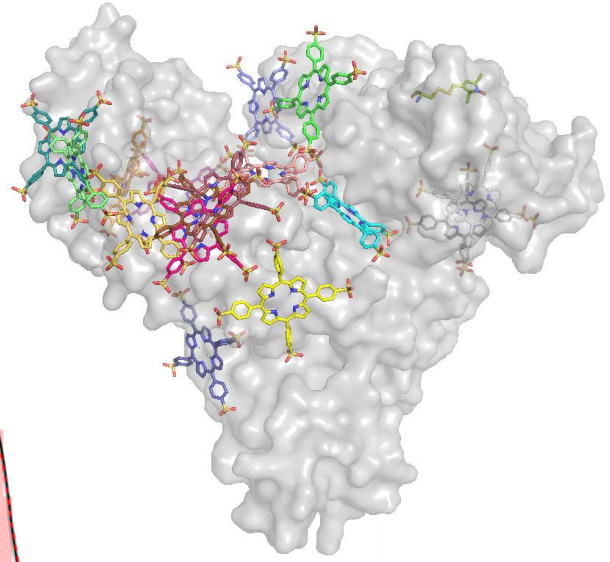
GPU-accelerated blind docking

explores all possible interaction modes

Dipolar EPR spectroscopy

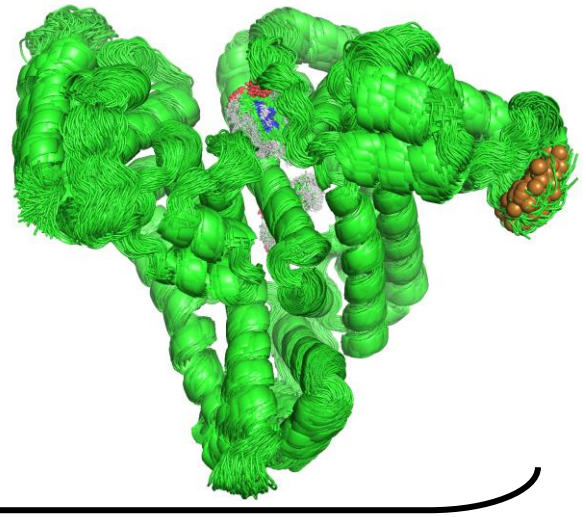
provides experimental distance distributions between the ligand and the spin label on the protein.





Molecular dynamics simulation

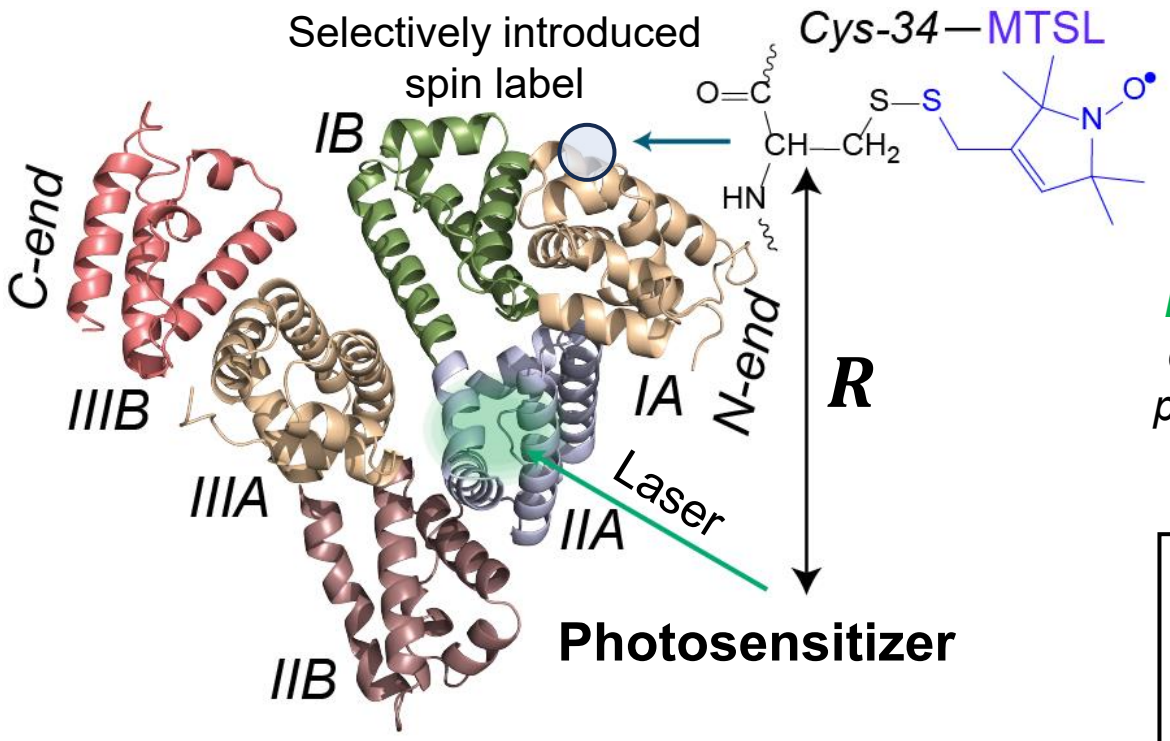
checks the structural stability over time



experimentally guided modeling, where every computational step is validated by real data.

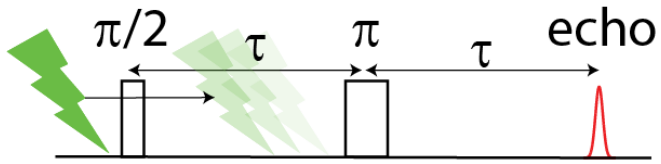
Experimental EPR setup

Study of human serum albumin binding with photosensitizers used in photodynamic therapy.

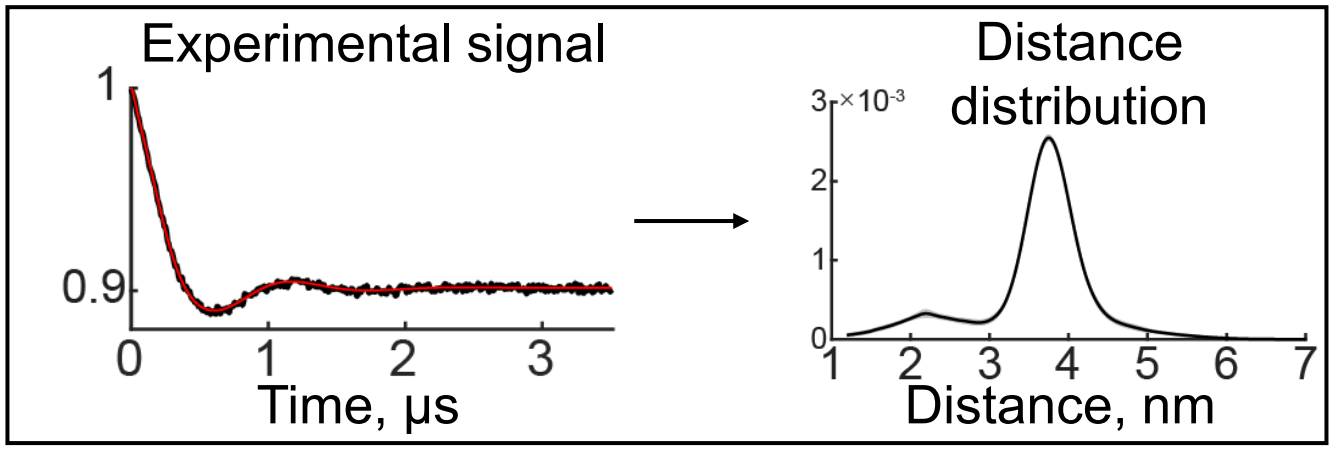


EPR experiments make it possible to obtain the distribution of distances to bound ligands in the range of 1-8 nm

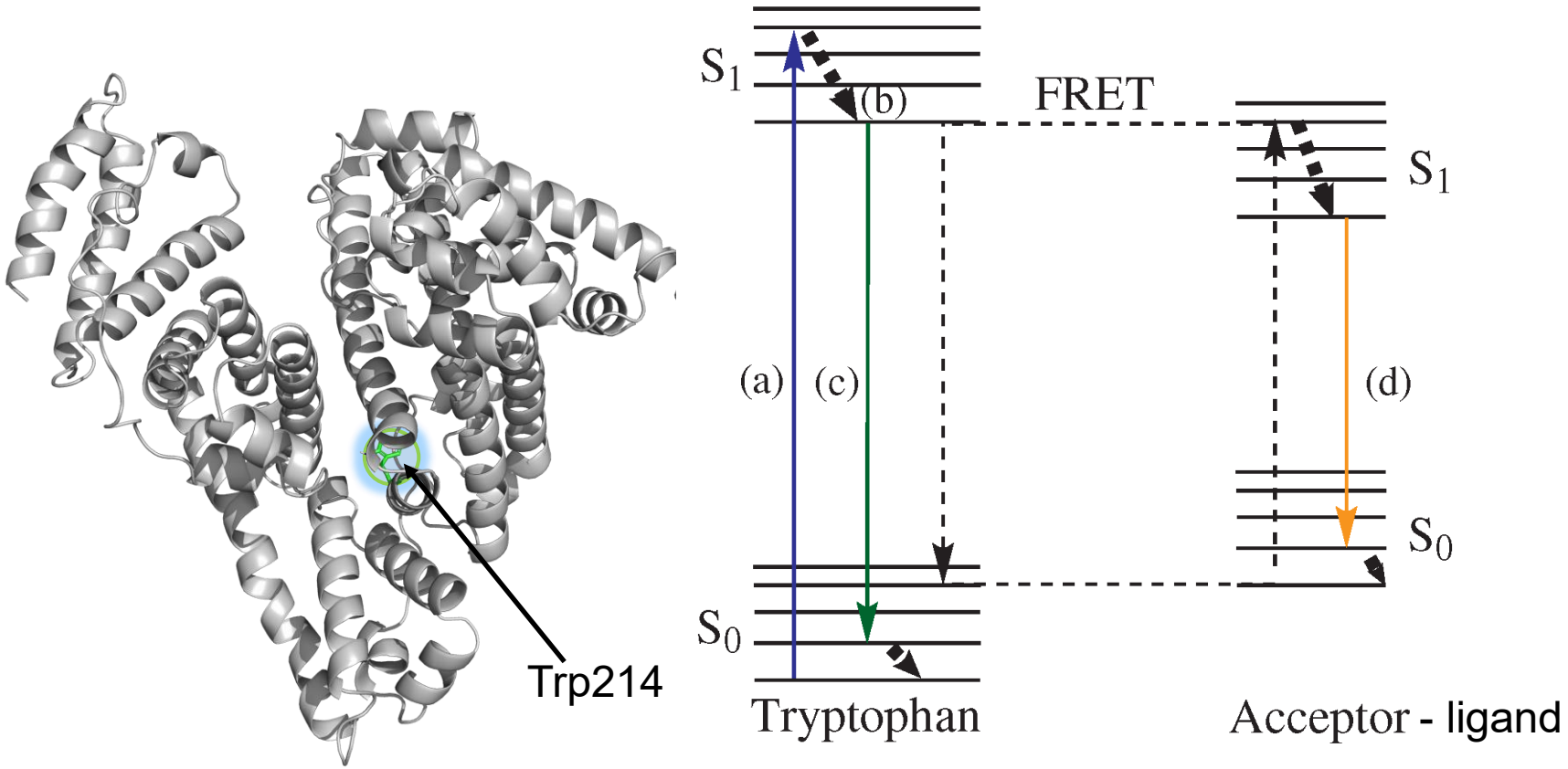
LaserIMD



Laser pulses excite the triplet state of the photosensitizer at different delays,modulating dipolar interaction between the spin label and the photoactive molecule.The time-dependent signal contains information about distances in the system.



Limitations of FRET

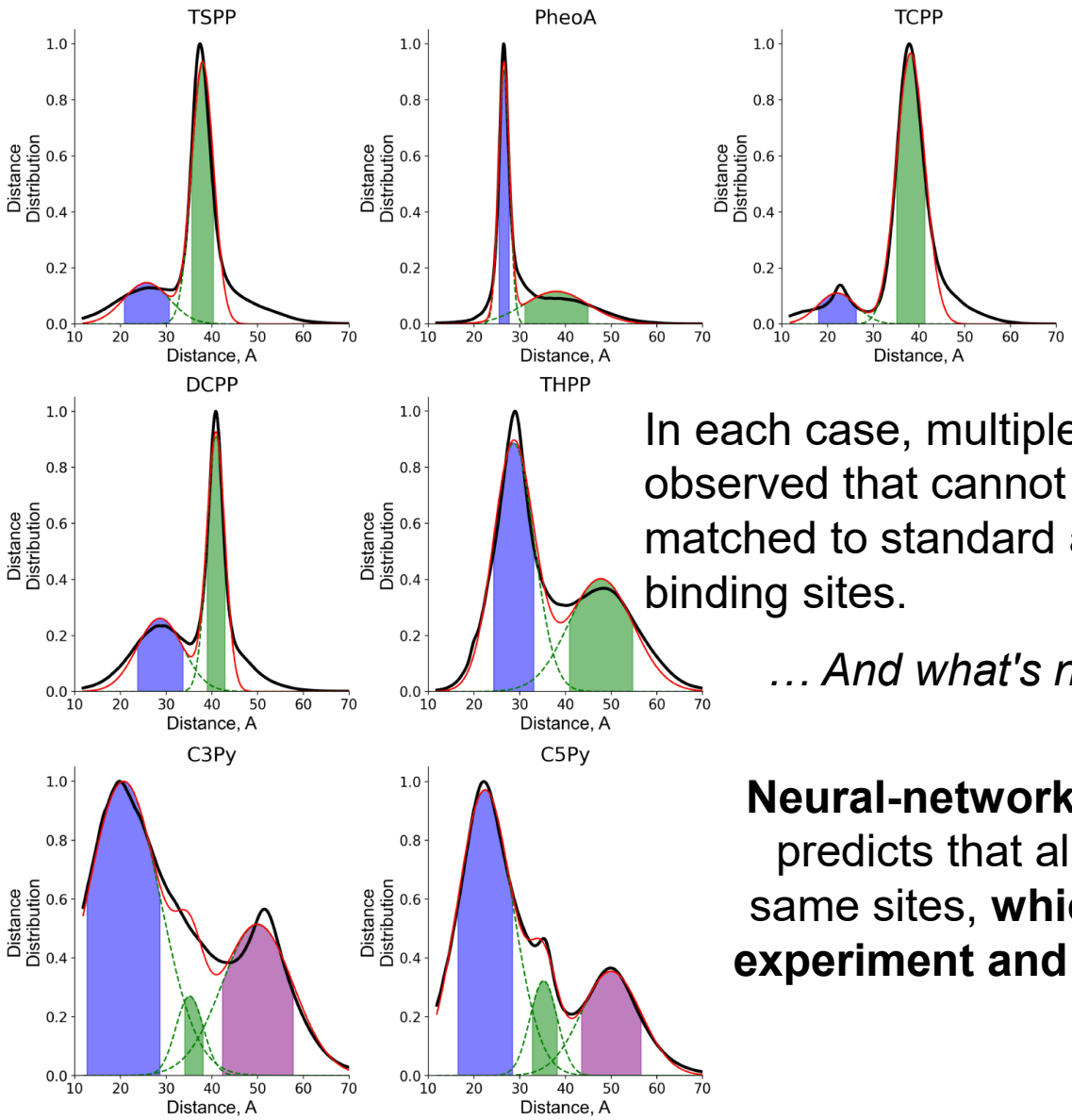


Povinelli, A. P. R. *et al., Journal of Photochemistry and Photobiology B: Biology* **2023**, *242*, 112693:

Most published results using the FRET technique to estimate distances in albumin fail to align with crystallographic data

Reason: The absence of a simple one-to-one donor-acceptor relationship, especially when more than one binding site is occupied

EPR on protein–photosensitizer complexes

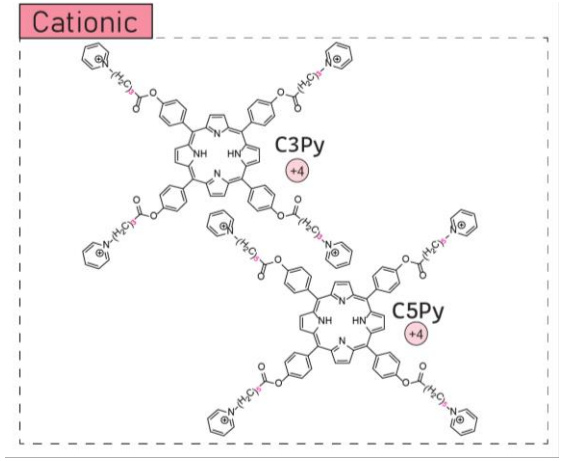


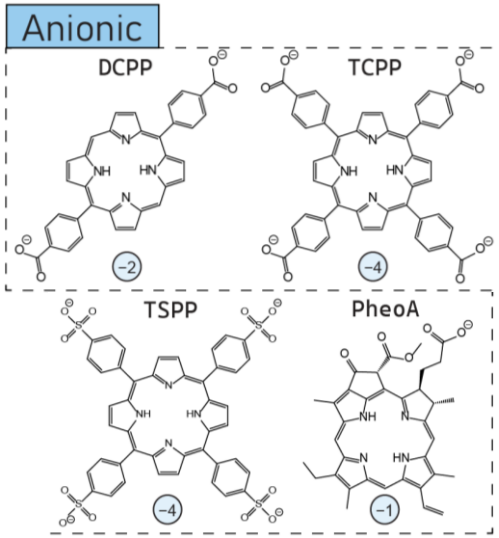
In each case, multiple peaks are observed that cannot be fully matched to standard albumin binding sites.

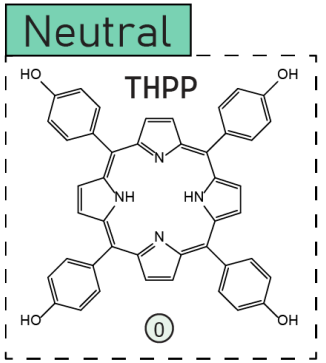
… And what's next?

Neural-network docking (DiffDock) predicts that all ligands bind at the same sites, **which contradicts both experiment and chemical reasoning**

Anions, Cations and Neutral Photosensitizers with Different Charges and Side Groups



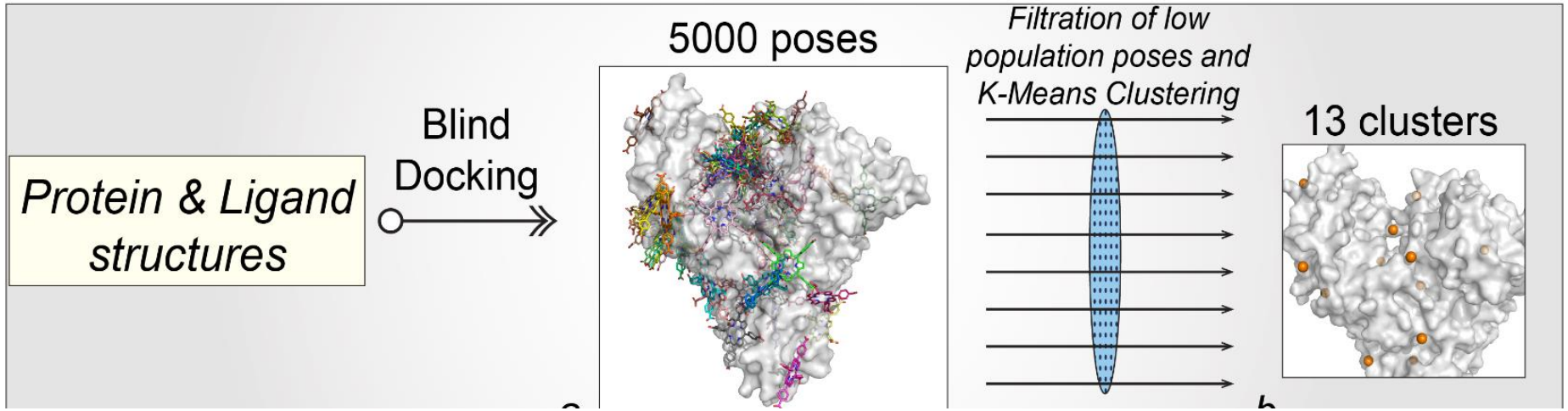




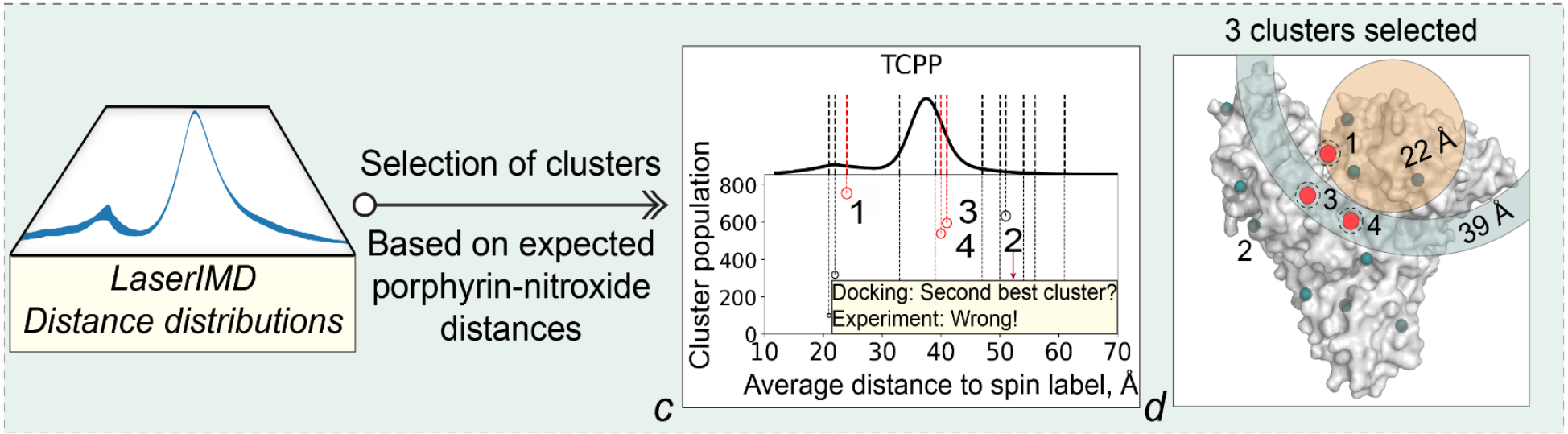
Algorithm for identifying binding sites

Example for TCPP

1. Initial Docking



2. Filtration of potential sites corresponding to the EPR data



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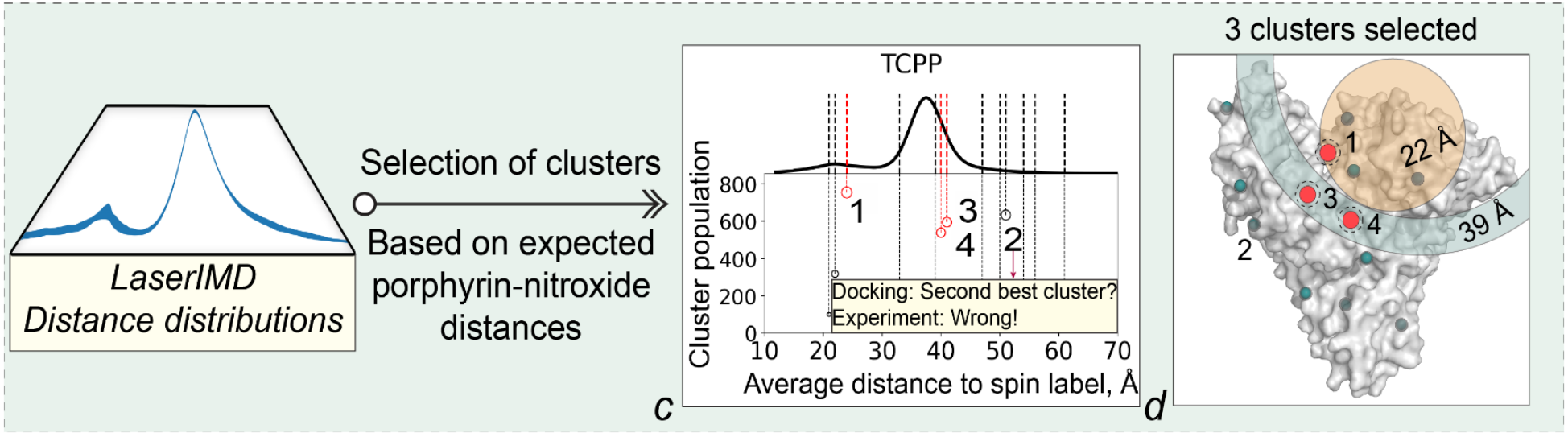
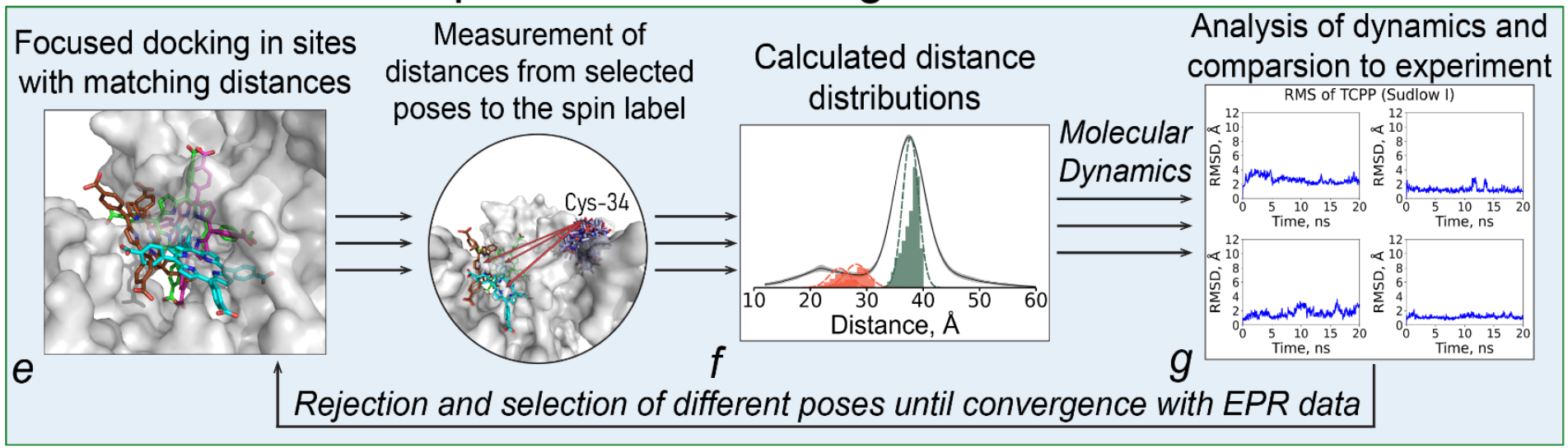
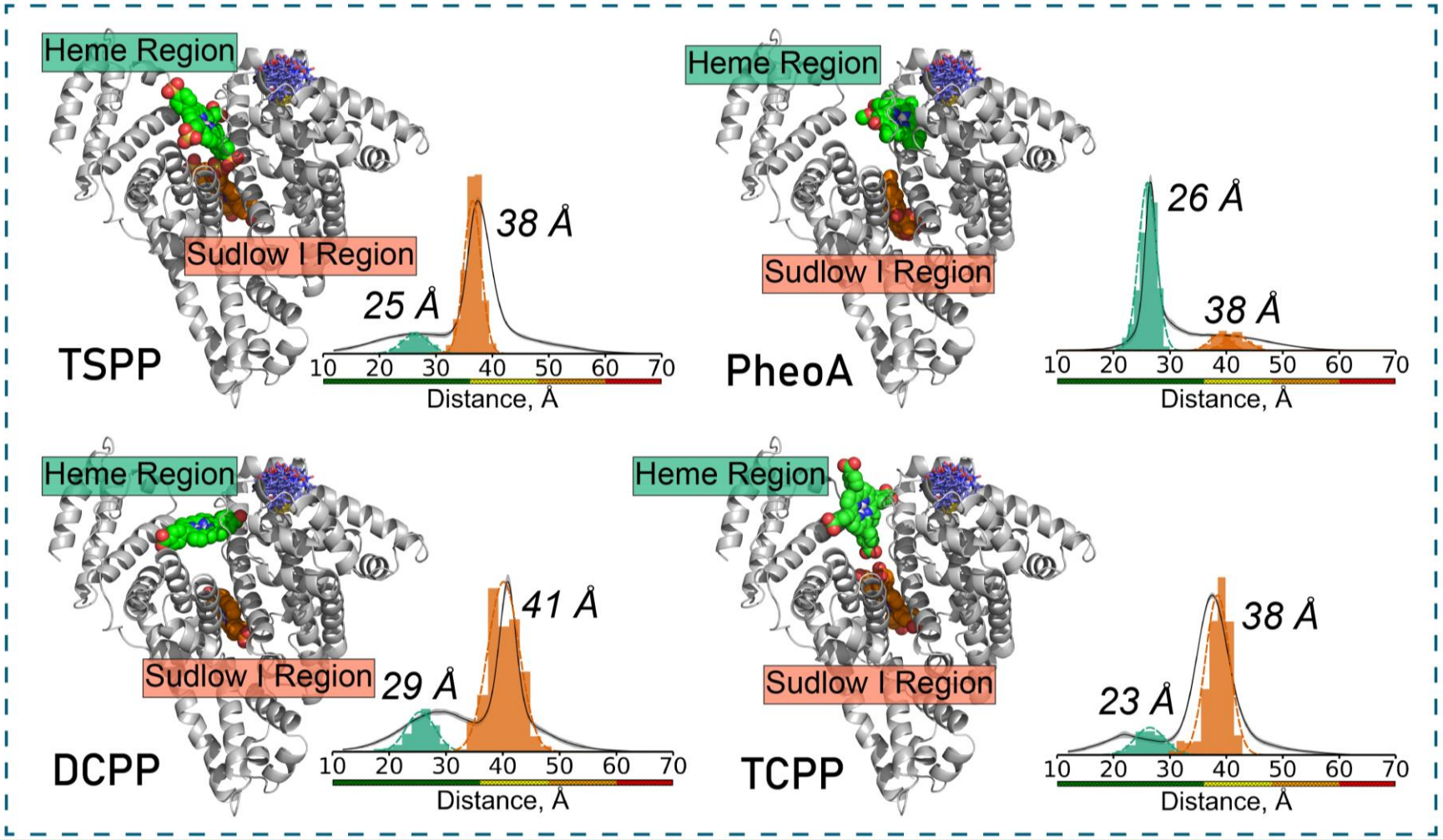


3. Iterative multi-step search of binding sites based on EPR data



Results — Anionic ligands

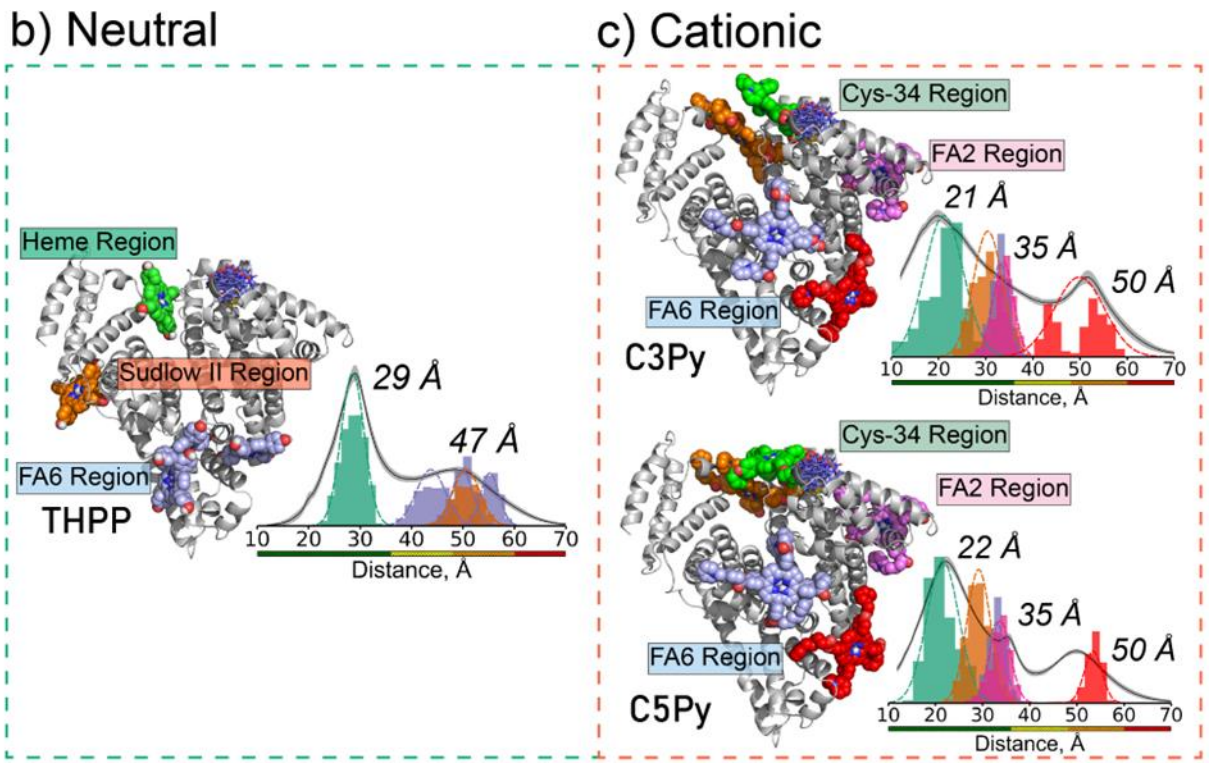


Two binding sites are identified for anionic photosensitizers:
Heme site and **Sudlow I.**
The relative site populations strongly depend on the ligand size
— something that docking alone does not capture

Results — Neutral and cationic ligands

Binding does not always occur in the standard albumin sites and often several sites are occupied, which highlights the limitations of fluorescence methods and the importance of complementary approaches.

FRET failed to detect the main site near the Heme region,



no FRET data exist

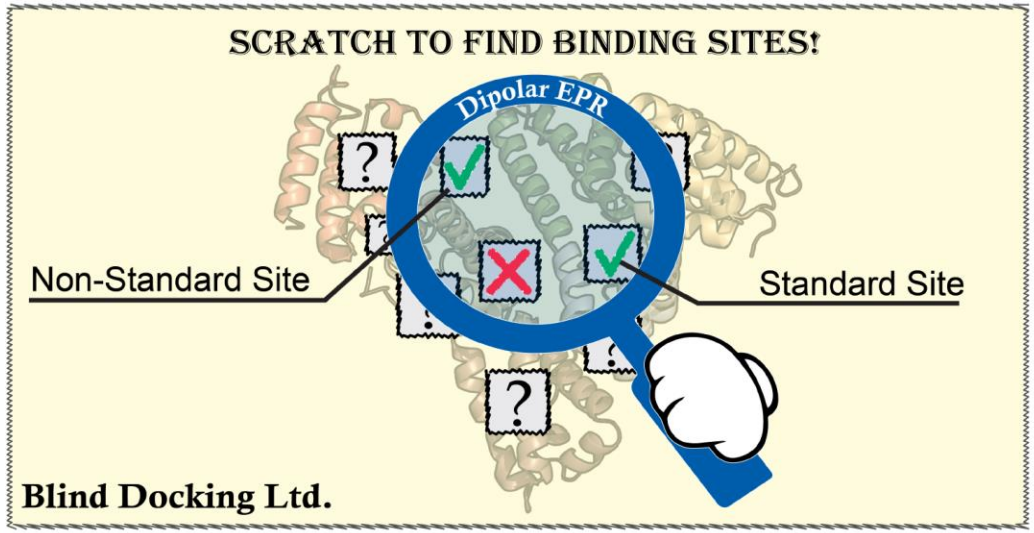
- bind at several sites,mostly on the negatively charged protein surface.
This makes them more mobile and harder to detect by other methods.
Docking predicts similar probabilities for all sites, even for those inconsistent with experiment.

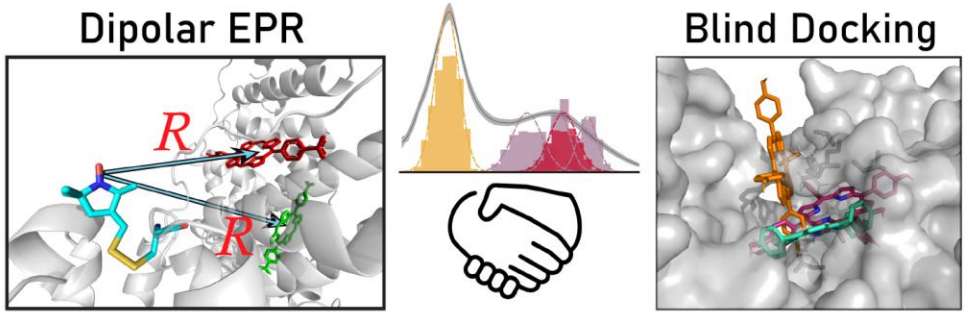
There is no perfect method — but by combining imperfect ones, we can get closer to the truth

EPR spectroscopy combined with molecular modeling **allows us to** obtain **experimentally validated binding sites** and explain the **distribution of ligands** among different regions.

The approach is fast, reliable, and applicable to flexible and non-crystallizable systems.

It can be extended to other targets — including proteins, nucleic acids.





An efficient experimentally-validated determination of ligand binding sites

Acknowledgments

International Tomography Center SB RAS

Mikhail Kolokolov

Natalya Sannikova

Tamara Khlynova

Roman Podaron

Sergey Demetev

N.N.Vorozhtsov Novosibirsk Institute of Organic Chemistry SB RAS

Dr. Igor A. Kirilyuk
Dr. Yuliya F. Polienko,
Prof. Elena Bagryanskaya

Institute of Chemical Biology and Fundamental Medicine SB RAS

Dr. Alexey S. Chubarov

MIREA–Russian Technological University

Dr. Zhdanova K.A.

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ARTICLE | April 11, 2025

Enhanced Binding Site Identification in Protein–Ligand Complexes with a Combined Blind Docking and Dipolar Electron Paramagnetic Resonance Approach

Mikhail Kolokolov, Natalya Sannikova, Sergei Dementev, Roman Podarov, Kseniya Zhdanova, Natal'ya Bragina, Alexey Chubarov, Matvey Fedin*, and Olesya Krumkacheva*

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