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Spectroscopic decoding of genetic adaptions in the fatty acid binding behavior of Serum Albumin

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The amphiphilic transport protein Serum Albumin may carry the understanding of its evolutionary pathway within species-dependent fatty acid binding properties. In a large genetic-spectroscopic study, we applied a spin-probing approach on the seven binding pockets of Albumin and performed advanced double electron-electron resonance (DEER) and classic continuous-wave (CW) electron paramagnetic resonance (EPR) spectroscopy to investigate this protein class. By utilizing the spin-labeled fatty acids 5- and 16-DOXYL stearic acid (DSA) as model ligands, we were able to compare dynamic protein structures, internal environments of the binding pockets and the binding dynamics of fatty acids to Serum Albumins from up to seven different species. The species of interest include herbivores, carnivores, humans and crab-eating macaques. We discovered a highly complex, challenging biophysical system with various contributing components such as protein oligomers and distinct ligand binding states. The combination of different EPR experiments, frequencies and time regimes in DEER enabled an innovative deep view into the ligand binding mechanism of Albumin while we could filter the molecular distances between bound ligands depending on the desired perspective and accuracy. We found similarities but also surprising differences in the genetic-spectroscopic binding profiles of the species and correlated the EPR data with genetic and bioinformatic analyses, dynamic light scattering (DLS) and Zeta potential

measurements, as well as native mass spectrometry (MS) experiments. We propose that nutrition specializations, sequence similarities and different oligomerizations could play decisive roles for the observed binding adaptions. This interdisciplinary study could significantly extend our knowledge on transport proteins in general and reflects some of the main aspects of collaborative research beyond amphiphilicity.