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Probing Protein Dynamics on Microsecond and Nanometer Scales with X-ray Photon Correlation Spectroscopy

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Protein dynamics in crowded and hydrated environments span nanometer length scales and microsecond time windows that remain difficult to access with established techniques such as neutron spin echo or NMR relaxometry. Recent advances in coherent X-ray sources have made X-ray Photon Correlation Spectroscopy (XPCS) [1] a powerful addition to this toolbox, enabling direct access to collective protein motion at molecular length scales. In this talk, I will discuss how XPCS has been used to resolve anomalous diffusion and cage effects in ferritin solutions[2], as well as nanoscale stress relaxation in hydrated lysozyme systems [3]. A central theme will be radiation damage not only as a limitation, but as a dynamic process that unfolds on comparable time and length scales as the intrinsic protein motion [4]. By exploiting megahertz repetition rates at XFELs, we demonstrate how protein dynamics can be captured before the onset of beam-induced aggregation, a strategy termed "correlation before aggregation". These results highlight both the opportunities and limits of using coherent X-rays to study soft biological matter, and illustrate how controlled X-ray illumination can be used to disentangle intrinsic collective dynamics from beam-induced responses. [1] F. Perakis and C. Gutt, Towards molecular movies with X-ray photon correlation spectroscopy, Phys. Chem. Chem. Phys. 22, 19443 (2020). [2] A. Girelli et al., Coherent X-rays reveal anomalous molecular diffusion and cage effects in crowded protein solutions, Nat. Commun. 16, 10814 (2025). [3] M. Bin et al., Coherent X-ray Scattering Reveals Nanoscale Fluctuations in Hydrated Proteins, J. Phys. Chem. B 127, 4922 (2023). [4] M. Reiser et al., Resolving molecular diffusion and aggregation of antibody proteins with megahertz X-ray free-electron laser pulses, Nat. Commun. 13, 5528 (2022).

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