ESRF	**Experiment title:** X-ray photon-correlation spectroscopy of nanoparticles in complex biological environments	**Experiment number**: SC-5173
Beamline:	**Date of experiment**: from: 01.10.21 to: 04.10.21	**Date of report**: 12/13/2021
Shifts:	**Local contact(s)**: Zinn, Thomas	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Felix Lehmkühler, DESY, Hamburg, Germany

Dr. Fabian Westermeier*, DESY, Hamburg, Germany

Dr. Francesco Dallari*, DESY, Hamburg, Germany

Prof. Dr. Wolfgang Parak, Center for Hybrid Nanostructures (CHyN), Universität Hamburg, Hamburg, Germany

Dr. Florian Schulz*, Center for Hybrid Nanostructures (CHyN), Universität Hamburg, Hamburg, Germany

Ferdinand Otto*, Center for Hybrid Nanostructures (CHyN), Universität Hamburg, Hamburg, Germany

Report:

The unique tunable properties of nanoparticle-based drugs enabled the development of new promising therapeutical and diagnostical concepts regarding drug-delivery, contrast agents and cancer treatments. Despite the high potential that these concepts offer, only a small amount of nanoparticle-based medicines have passed clinical trials and were officially approved. Upon in vivo application, nanoparticles (NPs) undergo various interactions with the complex and often crowded environment, which may result in altered functionality, degradation or agglomeration. Hence, understanding the fundamental bio-nano interactions in physiological entities is inevitable to ensure their application in therapeutic and diagnostic medicine.
We performed X-ray photon correlation spectroscopy (XPCS) experiements at ID02 to study NP dynamics in biological systems. Dynamic behavior of Au NPs of different sizes and with different surface modifications were investigated in the presence of proteins, in bovine serum, bovine whole blood and in HeLa cells.

Au NPs with different surface coating and sizes ranging from 12-50 nm were measured in various conditions. Before the measurements, all samples were thoroughly mixed in Eppendorf tubes to ensure a homogeneous distribution. For samples with distinct incubation times, a strict preparation protocol was followed, where the samples were prepared temporally delayed to guarantee equivalent conditions. The samples were transferred to quartz capillaries (length of 80 mm, diameter of 1 mm and wall thickness of 0.01 mm) and sealed with glass beads and wax. For all samples, control measurements without AuNPs were carried out corresponding to the investigated environment.

Data were acquired by USAXS and XPCS measurements over a total of 12 shifts. The detector distance was set to 30.7 m. Measurements were carried out at an energy of 12.223 keV. The beam size was set to 40 x 15 µm² (v x h). Static USAXS scattering patterns were recorded by an Eiger2 4M detector. For studying dynamics by XPCS an Eiger 500k detector with a maximum framing-rate of 23 kHz was used. In regard to the biological nature of the samples, the general measurement procedure consisted of three USAXS measurements: without

photon flux attenuation, with Molybdenum sheet absorber and Zirconium sheet absorber to investigate potential radiation damage. Subsequently, 10 XPCS measurements (for each attenuation) were performed. Conclusively, static USAXS patterns were acquired again to probe beam-induced sample alteration over time. In between measurements, the investigated position on the capillary was adjusted to prevent multiple beam exposures on the same location.

Considering the improved coherence properties at ESRF by the next-generation upgrade and the fast relaxation rates of the latest Eiger 500k detector available at ID02, first the detection limit for aqueous Au NPs with different sizes (12, 20, 40 and 50 nm) was examined.

Second, protein interaction with the NP surface was investigated. Au NPs were exposed to varying bovine serum albumin (BSA) concentrations. In-depth evaluation of the influence on the hydrodynamic diameter of the particles was done by applying Au NPs with identical physicochemical properties except for the ligand shell thickness of the α-methoxypoly(ethylene glycol)-ω-(11-mercaptoundecanoate) (PEGMUA) coating. The formation of a complex protein corona consisting of proteins with different affinities was probed by exposing the particles to bovine serum and bovine plasma, which was extracted from bovine whole blood.

Third, XPCS measurements on pure bovine whole blood and on Au NPs exposed to bovine whole blood were performed at room temperature and at 37 °C using the thermoregulated sample holder at ID02. Dynamic behavior of Au NPs in blood were investigated for different incubation times in respect to bio-nano interactions on a longer time-scale and the influence of cell sedimentation during measurements.

Fourth, the feasibility of XPCS measurements of NPs incorporated in cells was evaluated by exposing Au NPs of 20 and 40 nm to HeLa cells for 24 h.
Currently, data analysis and interpretation is ongoing, we foresee to obtain first results within the next 6 months.