SIZING NANOMATERIALS IN BIOLOGICAL FLUIDS BY CFRAP: FROM CHARACTERIZING PROTEIN AGGREGATION TO DIAGNOSING EPITHELIAL AND VASCULAR PERMEABILITY

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Measuring the size of nanosized materials in complex biological fluids, such as blood or cerebrospinal fluid, is of great importance in a wide range of applications in the life sciences. In drug delivery, for instance, the effective size of nanomaterials in biofluids is important because it directly influences the biodistribution in the body. Indeed, even though nanomedicine formulations may be stable under normal storage conditions, they may very well aggregate after administration into a biological fluid such as blood. Similarly, there is a growing appreciation that the colloidal stability of therapeutic proteins needs to be tested in blood as protein aggregation after intravenous administration will alter their functionality and may induce immunogenic responses. Yet, methods to investigate submicron protein aggregates in serum are virtually non-existent. Being able to size nanomaterials in biofluids is of interest to medical diagnosis as well, for instance to determine intestinal or vascular barrier permeability which is related to several pathologies, such as sepsis, liver disease, inflammatory bowel disease and neurodegenerative diseases. Barrier permeability can be assessed by administering inert size probes, e.g. orally or intravenously, followed by quantification of the size and amount of probes that have leaked through the barrier.

Here we report on the use of fluorescence recovery after photobleaching (FRAP) to measure size distributions of nanomaterials in biological fluids [1]. We propose an improved FRAP methodology that enables the measurement of continuous distributions of diffusion coefficients (cFRAP), which can be easily converted to equivalent size distributions. A rectangle is photobleached and the full tempo-spatial information available in the confocal recovery images is exploited using a dedicated theoretical recovery model to extract a continuous distribution of diffusion coefficients. Following detailed validation of the cFRAP-sizing approach, we demonstrate its strength and versatility in a number of challenging sizing applications. First we demonstrate that cFRAP-sizing enables accurate determination of protein aggregation in undiluted blood serum. Next, in combination with the administration of a broad range of inert size probes, we show that cFRAP-sizing allows to characterize in great detail the intestinal and vascular permeability in mice. Importantly, since a single measurement is sufficient to determine the full size distribution of probes that have leaked through the barrier, we find that the number of animals needed to assess the barrier permeability is reduced up to five times compared to classic approaches where probes of different size are administered and analyzed separately.

Reference

[1] R. H. Xiong, R. E. Vandenbroucke, K. Broos, T. Brans, E. Van Wonterghem, C. Libert, J. Demeester, S. C. De Smedt, and K. Braeckmans, 'Sizing Nanomaterials in Bio-Fluids by Cfrap Enables Protein Aggregation Measurements and Diagnosis of Bio-Barrier Permeability'. **Nature Communications** 09/2016; 7, 12982.