Experimental report

19/08/2022

Proposal: 8-03-1044 **Council:** 10/2020

Title: Simultaneous SANS and DLS: a powerful combination for elucidating themolecular mechanisms of Wilson's disease

Research area: Biology

This proposal is a new proposal

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Samples: Human hemoglobin (Sigma Aldrich)
human erythrocytes
Zinc chloride
Penicillamine
Glutathione
phosphate buffer in D2O

Instrument	Requested days	Allocated days	From	To
D11	2	2	28/05/2021	30/05/2021

Abstract:

Wilson's disease is a condition caused by impaired copper metabolism, ultimately leading to severe consequences such as aggregation of hemoglobin (Hb) and the destruction of red blood cells (erythrocytes). In this experiment, the kinetics, molecular mechanisms and structural changes accompanying these pathologicial processes will be investigated. In addition, the effects of two commonly used treatments for Wilson's disease will be studied on a molecular level.
Importantly, the combination of real-time SANS and in-situ DLS will allow to obtain additional dynamic information on Hb aggregation and structural evolution of erythrocyte membranes. The synergy between SANS and DLS will thus enable a comprehensive, quantitative characterisation of the pathological mechanisms underlying Wilson's disease, paving the way towards enhanced treatment options.

Experimental report - 8-03-1044 (D11, 25/05-28/05/2021)

Scientific background. In the human body, copper is an essential element for a variety of metabolic processes such as the respiratory chain, and its metabolism is thus tightly regulated. In the case of a condition named Wilson's disease, however, copper metabolism is impaired, which leads to an accumulation of copper in the liver. The strong oxidative effects of free copper lead to the formation of reactive oxygen species, thereby destroying liver cells and releasing copper ions into the bloodstream. In addition to severe liver damage, neurological and psychiatric symptoms can ensue. A potentially lethal consequence of Wilson's disease is a destruction of red blood cells (RBCs). This is, inter alia, due to oxidative damage and a resulting denaturation and aggregation of hemoglobin (Hb) inside the RBCs. So far, the understanding of the mechanism behind Wilson's disease is rather phenomenological. Therefore, the aim of this proposal is to apply an interdisciplinary, quantitative approach including small-angle scattering to obtain a comprehensive characterisation of the kinetics as well as Hb form and structure changes throughout copper-mediated Hb/RBC damage.

Experimental results. In this experimental report, SANS (D11) data characterising human RBCs, hemoglobin (Hb) and empty RBCs (Hb-depleted RBC "ghosts") are shown. Furthermore, Cu-induced changes in Hb are featured (data from ID02, ESRF).

Hb and RBCs were purified by the proposers from healthy donor blood obtained from the French Blood Bank (EFS). RBC ghosts were obtained by depleting purified RBCs of Hb. Fig. 1 shows example SANS data of RBCs, Hb and RBC ghosts. The oscillations of the Hb form factor are well visible at $q > 0.1$ Å^{-1} and also dominate the high-q scattering of RBCs and RBC ghosts. The oscillations are slightly smeared out in the case of the RBC ghosts, indicating the intended loss of hemoglobin. Both RBCs and ghosts show strong scattering at lower q than hemoglobin, which is traced back to their overall larger structures. Both low q regions follow $I \sim q^{-4}$, indicating a sharp interface between the scatterers and the solvent matrix. It is furthermore possible that surface-fractal structures are present.

The fast kinetics of Hb aggregation upon Cu addition were assessed during an inhouse beamtime at ID02 (ESRF, data shown in Fig. 2). A Hb solution with the same concentration as the one used on D11 was measured in the presence and in the absence of 10 µM $CuCl_2$ (added in an aqueous solution to mimic pathological copper concentrations similar to those found in the bodies of Wilson's disease patients). Without $CuCl_2$, a small degree of aggregation is observed around 0.1 nm^{-1} after 1 min (Fig. 2, top). Since specific tests were conducted and the experimentall setup was optimised to to eliminate radiation damage inasmuch possible, this observation may be due to mild drying effects in the measurement capillary. In the presence of $CuCl_2$, however, the aggregation is much more pronounced, indicating an actual effect of the added $CuCl_2$ on Hb.

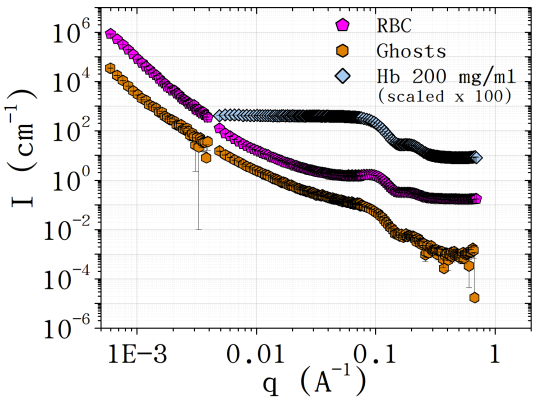


Figure 1: SANS data (D11) on RBCs, RBC ghosts and hemoglobin in phosphate buffer (see text for details).

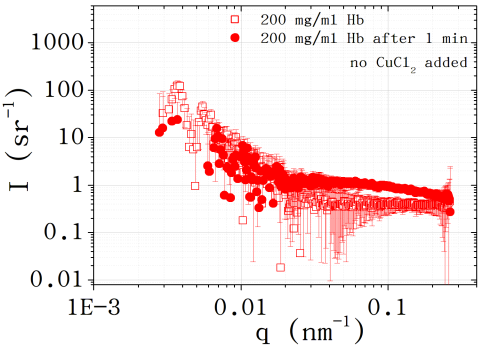
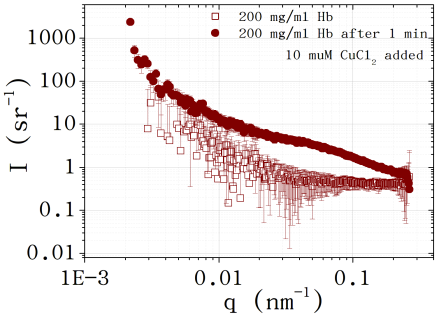


Figure 2: SAXS data (ID02) on Hb in the absence (top) and presence (bottom) of $CuCl_2$. See text for details.



Conclusions. The SANS data presented here provide a detailed characterisation of RBCs and Hb purified from donor blood. Fast aggregation kinetics as a response to $CuCl_2$ were captured using complementary SAXS data. The data presented here provide a solid basis for the characterisation of blood samples and their usage for the detection of pathological mechanisms, such as Wilson's disease. The proposers intend to publish these data, along with a follow-up collaborative project (proposal to be submitted in Sept. 2022) for the investigation of virus-RBC interactions.