ESRF	**Experiment title:** Ultrafast protein denaturation by nanoheaters	**Experiment number**: SC2440
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Report:

Laser-excited gold nanoparticles in aqueous suspension act as nanoscale heat sources to the surrounding within ultrashort times [1, 2]. By employing pulsed small-angle x-ray scattering the structural dynamics of an absorbed protein layer has been temporally resolved. The scattering data reveals a layer expulsion from the surface.

We have employed a pump-probe technique using femtosecond laser pulses as heat pulses and x-rays as the probe for the nanoscale structure relaxations. By a combination of x-ray techniques it was possible to measure the particle temperature, pressure transients in the water phase, vapor bubble morphology and particle shape [3-5]. Briefly, the femtosecond amplifier system (Dragon, KMLabs) at ID09b was synchronized to the pulses of x-rays and used for a stroboscopic pump-probe experiment. The bandwidth of the x-ray beam was relaxed to 2.6 % in order to increase the flux and maximize the signal-to-noise ratio. The flowing particle suspension was excited at 390 nm, within the interband (IB) absorption of gold. The IB absorption is hardly sensitive to size and shape. Therefore the average absorbed energy per unit cell is well defined. A suspension of protein conjugated gold nanoparticles was pumped through the interaction area of the focused x-ray and laser pulses. While the particles had been produced in advance of the experiment by an adaptation of the Turkevich method [6], they had been conjugated just prior to the experiment with bovine serum albumin (BSA; Roth, protease free). Mercaptosuccinic acid was used as linker. It adsorbs covalently to gold and BSA is attached to the carboxylic groups. By this linker the protein denaturation due to strong adsorption is reduced. For a suspension of gold particles (from a 2 mM monomer solution) a maximum coverage is achieved at a 3 µM BSA concentration, which is close to the calculated full coverage in dense packing at 2-2.5 µM. The BSA is visible in small angle scattering, as the gold particles themselves deliver a strong scattering contrast, which is modified by the protein layer.

The mechanism of thermal laser excitation of gold nanoparticles is well understood with a rapid heating of the lattice and a subsequent cooling step in the order of 250 ps for the 16 nm particles. The laser fluence is precisely tuned and can serve as temperature scale. The scattered x-rays are analyzed in small angle scattering (SAXS) [7] and wide angle scattering geometry (WAXS) [3]. The measurement of the gold (111) peak position allows to determine the particle temperature and the threshold fluence for particle melting. In SAXS one can determine the mesoscale changes of density around the particles. By the simultaneous solution of the heat transfer equations the temperature profile within a region in the aqueous phase close to the particle surface is derived.

In the present case the laser pulses were stretched to 4 ps. In this case irreversible structural modifications of the particles can be ignored [8] and the particle dynamics are completely reversible. A comparison of the small angle scattering signal between bare particles and the protein coated particles shows, that strong modifications of the scattering patterns are induced for the coated particles, while no changes are observed at the same time for bare particles. The laser fluence in fig. 1 has been set to just below the threshold for vapor bubble formation around the particles [5]. Consequently only a faint structural change may be expected, while the particles have a high temperature. This is indeed the case for the bare particles, as seen for the Porod invariant in fig. 1. The Porod invariant P

$$P = \int_0^\infty I(q)q^2dq = 2\pi^2\Delta\rho^2 r_e^2\Phi(1-\Phi) \qquad (1)$$

is a measure for the global scattering length density contrast $r_e\Delta\rho$ change after laser excitation, Φ the filling fraction. The scattering contrast can arise from the protein shell around the gold particles with an average protein density exceeding the water density. Reported values for the (hydrated) density of BSA are in the range of 1.35 g/cm^3 [9]. A transient scattering contrast arises also from the appearance of a vapour bubble around the heated particle. The coated particles show a large signal, with a sub-nanosecond transient and a constant signal towards long time delays. The transient is caused by the onset of bubble formation.

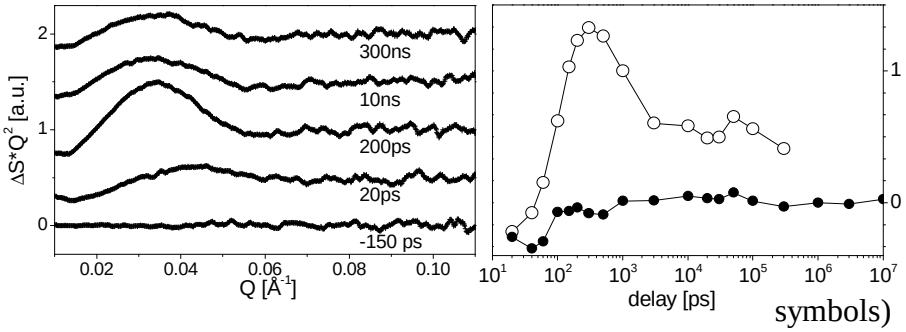

Fig. 1. Difference SAXS of a laser excited gold nanoparticle suspension (left) with albumin coating. The curves have been scaled for clarity. The Porod invariant at a laser fluence of 93 J/m^2 is shown for the bare (filled symbols) and the bioconjugated particles (open symbols).

After the vapour bubbles have vanished after 10-100 nanoseconds, the remaining scattering distribution is a clear indication of the complete expulsion of the protein layer from the surface. The laser excitation shows that the protein is detached due to the fast heating. A similar observation has been described for DNA conjugated with gold particles and a disruption of the thiol bond [10]. More interestingly this phenomenon was initiated at a local temperature at the outside of the particle far above 100 °C, just below the spinodal decomposition point of the water phase. We find that for small laser fluence these irreversible structural modifications are completely absent. This observation points towards an unexpectedly large tolerance of the protein layer to very short heating stimuli, not in agreement with the temperature of static denaturation [11].

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