A high pressure cell for SANS up to 5kbar in combination with light scattering to investigate liquid samples

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Abstract

We report on a high pressure cell to use with SANS in a pressure range up to 5kbar. The cell offers the new possibility to investigate liquid samples by a specially designed sample chamber, which allows changing of samples relatively easily. Since the cell construction uses fused sapphire as window material, also light scattering investigations can be performed simultaneously to the SANS measurements. In this paper we describe the construction of a high pressure cell and we demonstrate the applicability of the construction for SANS in combination with dynamic light scattering showing data on the biological molecule lysozyme.

PACS numbers: 07.35.+k High-pressure apparatus; shock tubes; diamond anvil cells, 61.05.fg Neutron scattering (including small-angle scattering), 78.35.+c Brillouin and Rayleigh scattering; other light scattering

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I. INTRODUCTION

The use of high pressure in natural sciences has gained more and more attention since the pioneering work of Bridgeman [1]. The solid-solid phase transitions, phase equilibria in general or the use of high pressure to elucidate reaction kinetics via the concept of activation volumes are examples to name only a few. The use of small angle neutron scattering, SANS, together with high pressure is a rapidly growing field of research because neutron scattering yields the proper q-range for studying conformational changes of matter. It further offers the possibility of using contrast variation techniques which has just recently gained much attention through a variety of biological/physical problems that have been tackled by high pressure techniques and SANS [2–6]. Recent developments in this area report on time resolved measurements using pressure jump techniques to explore the kinetics of phase behaviour and structure formation in complex matter. However, this has so far only been reported with X-rays and laser light scattering [7, 8]. We present in this paper a setup which combines SANS and dynamic light scattering up to 5kbar at the same time. An option for performing pressure jumps is also implemented. Technically one of the most prominent problems is the so called media separation, which is needed to separate the pressure transmitting liquid, mostly hydraulic oil, from the liquid samples. Early attempts on a high pressure cell for liquid, viscous polymeric samples have been reported [9] in a pressure range up to 2.5kbar. There an O-ring separates the sample from the pressure transmitting oil. However, no container was used and therefore sample change was difficult and time consuming. Other cells have further in common that they use either aluminium or niobium as window materials, which are transparent for neutrons but not for visible light and therefore no light scattering investigations can be undertaken at the same time [10–13]. Another technical drawback is the usually relatively large beam diameter in neutron scattering compared to X-rays or light. Therefore a rather big sample size is needed and thus the high pressure device has to take care about that constrain. We will present in this paper a method by which a relatively easy change of liquid samples can be achieved. This method uses a small container which carries the liquid sample (the so called inner container method). It is sealed against the pressure transmitting oil by an O-ring, which at the same time allows the pressure to be transmitted and performs separation of media. A further advantage of our construction is its defined sample thickness which does not change during the experiment. The thickness is controlled by the dimensions of the inner container and can be varied according to transmission requirements by changing the container window thickness. In this paper we will introduce our new SANS high pressure cell design with relatively easy sample change and the online possibility to do dynamic light scattering. In order to prove its functioning we will present light scattering data on egg-white lysozyme up to 5kbar and SANS data up to 3kbar.

II. THE HIGH PRESSURE CELL FOR SANS

The basic ideas behind the SANS high pressure cell are the following: Easy change of sample, reliable media separation which means that the pressure transmitting hydraulic oil does not mix with the sample, use of window material which is also optically transparent and last but not least a relatively large pressure range accessible with the possibility to change temperature and perform pressure jump experiments. We have constructed a pressure jump generator on the basis of a moving piston that allows changing pressure in a range of ΔP about maximally 1000bar in about 1s. Also a repetitive mode is possible as the whole setup is computer controlled via self-written software. First results from that system will be published elsewhere. Our cell design allows a maximum pressure of 5kbar pressure which has been tested and approved by a finite element calculation. Irreversible damage of the cell occurs at pressures exceeding 8kbar. The cell can be operated in a temperature range between 4 and 80°C by means of a surrounding jacket in which a cooling liquid circulates. The temperature accuracy is within 1K. In our experiments we have used pressures up to 4.5kbar which were achieved by a screw-type positive displacement pressure generator (Sitec) and were measured by a pressure transducer (Burster) with an accuracy of 5bar. The cell is shown in Figs. 1 and 2. It is equipped with two sapphire windows which are mounted vis-à-vis on a highly polished inner cell holder leaving in its middle an empty inner space where the sample container, called "pill", is placed. This inner space is sealed against the windows by silver rings. For pressures up to 2.5kbar also a viton O-ring is good enough. The pressure transmitting oil can access the inner space via holes normal to the polished surfaces. This detail is shown more explicitly in Figs. 3 and 4. The inner space is filled with the pill. It consists of a brass ring which has on its periphery a number of small holes. Prior to the high pressure experiment this pill has to be filled with the sample. To do so, a sapphire plate is pressed into one side of the brass ring by means of a special tool. Then a viton O-ring is placed into the brass ring, and the sample is filled in very carefully in order to avoid air bubbles. This assembly is then closed by another sapphire plate which is pressed in the other side of the brass ring using the same tool as before. The dimensions are chosen such that the plates now squeeze the O-ring which in turn will seal the holes on the periphery of the brass ring. The plates have typically a thickness of 3mm which leaves an open space of about 1.3ml volume and about 2mm thickness for the sample. In principle also other thinner plates can be used. By that a larger volume and hence larger path-lengths for the neutrons can be achieved. That might be useful for highly deuterated samples, where usually the transmission is high. Placing this pill now in the inner space of the high pressure cell will allow the hydraulic oil to transmit the pressure on to the sample via the holes on the periphery of the brass ring. The thickness of the pill is chosen such that the sapphire windows are in contact with the sapphire plates when the cell is ready to use. Due to that contact against the sapphire windows the sample thickness is fixed and does not change with pressure (less than 10μm, which corresponds to a 1% variation in path length and thus in transmission). The inner part of the cell which contains the pill is held in place via two rather massive stainless steel parts which are connected by eight steel bolts.

III. LIGHT SCATTERING AT HIGH PRESSURE

It is possible to perform DLS on a sample in the pressure cell. As the pressure cell was designed for SANS, several drawbacks for DLS had to be overcome. The thick sapphire windows induce a parallel offset of non-perpendicular beams and slightly scramble the polarisation of the incoming and outgoing photons. The latter can be overcome by placing an accordingly oriented polariser in front of the detector system, filtering out the scrambled part of the scattered light. The parallel offset in combination with the comparably small illuminated part of the relatively thin sample simply causes a more delicate alignment procedure. Another optical problem arises from the reflections from all the surfaces in this stack of parallel flat windows. Multiple reflections are scattered back and forth and superpose with the scattered light from the sample. By orienting the incident beam non-perpendicularly to the window surfaces and by tilting the whole scattering plane against them, the scattering volume can be accessed by the detection system. Due to the rather complicated beam path through the pressure cell windows, an additional calibration measurement has to be performed to properly determine the precise scattering angle θ. To do so we have measured the diffusion coefficient of our sample in a standard commercial ALV light scattering goniometer. From the so obtained exact relation between relaxation rate and scattering angle for our sample, it is then possible to calculate the scattering angle of the DLS setup at the SANS instrument. Thus the scattering angle in the high pressure experiment presented in this paper could be determined to be θ=138 °. The definition of the scattering vector q is $q = |\vec{q}| = \frac{4\pi \mathrm{n}}{\lambda}\sin(\frac{\theta}{2})$, where n is the refractive index, λ is the excitation wavelength and θ is the scattering angle. Performing high pressure light scattering experiments one has to take into account that due to measurements at a fixed scattering angle the value of q is pressure dependent because the refractive index n is pressure dependent. In a recent publication we have demonstrated how data have to be treated in order to account for this effect [14]. In our DLS setup in the SANS sample environment, a vertically polarised beam from a 17mW He-Ne laser (Melles-Griot) with a wavelength of 632.8nm illuminates the sample. The scattered light passes a vertical polariser, is collimated into a mono mode fibre beam-splitter and is then fed into two Avalanche Photo Diodes (APD, Perkin Elmer). The output signal of both APDs is then processed with an external two channel correlator (correlator.com Flex99OEM-12) in order to perform a pseudo cross correlation which eliminates undesired detector side effects (after pulsing). The used setup is sketched schematically in Fig. 5.

From the experimental setup it is rather difficult to determine the exact scattering angle. Please note that with a few modifications, the DLS setup described beforehand can also be used for Diffusing Wave Spectroscopy (DWS) to characterise the dynamics of turbid samples. We have already successfully measured turbid samples by using an enhanced DWS setup which allows for the distinction between ergodic and non-ergodic samples (the so-called double-cell DWS setup).

IV. PERFORMANCE TEST OF THE HIGH PRESSURE CELL USING A LYSOZYME STANDARD SAMPLE

To test the performance of the high pressure cell we have prepared a solution of egg-white lysozyme at a concentration of 50mg/cm^3 in 50mM acetate buffer in D2O (that corresponds to about 1.3% added salt to the D2O). The experiments have been performed at the temperature T=24 °C.

A. Dynamic light scattering (DLS) under high pressure

For the homodyne case the experimentally measured intensity autocorrelation function $G^{(2)}(q,t) = \langle I(q,t)I(q,0)\rangle$ is related to the normalized autocorrelation function $g^{(1)}(q,t)$ via the Siegert relation [15]

$$G^{(2)}(q,t) = \langle I\rangle^2 \left(1 + f|g^{(1)}(q,t)|^2\right) \tag{1}$$

where f is an experimental factor and $\langle I\rangle$ is the mean intensity. The field autocorrelation function $g^{(1)}(q,t)$ is related to an apparent diffusion coefficient D_{app} of the system with equilibrium concentration fluctuations via $g^{(1)}(q,t) = \exp(-D_{app}q^2t)$. This is usually written as $g^{(1)}(q,t) = \exp(-\Gamma t) = \exp\left(-\frac{t}{\tau}\right)$, where Γ is the relaxation rate and τ is the relaxation time. To model the field autocorrelation function $g^{(1)}(q,t)$ for a size distribution of diffusing particles we used the cumulant expansion for $g^{(1)}(q,t)$,

$$\ln g^{(1)}(q,t) = -\Gamma t + \mu_2 t^2 \tag{2}$$

with μ_2 being the second cumulant. The ratio μ_2/Γ^2 is a measure of the size polydispersity of the system. If the system is mono-disperse, then μ_2 is zero and the cumulant expansion recovers a simple single exponential fit to $g^{(1)}(q,t)$. From the concentration dependence of the obtained diffusion coefficient D_{app} we can calculate the translational diffusion coefficient at zero concentration D_0 according to [16]

$$D_{app}(c) = D_0(1 + k_d c) \tag{3}$$

where k_d is a parameter describing the particle interactions and c is the concentration. The so obtained D_0 is related to the particle size by the Stokes-Einstein relation:

$$D_0 = \frac{kT}{6\pi\eta r} \tag{4}$$

Here kT has the usual meaning, η is the solution viscosity and r is the hydrodynamic radius of the diffusing particle. The pressure dependence of k_d has been reported by Banachowicz and was found to be a monotonically decreasing function with pressure [17]. It seems as if k_d approaches a constant value at pressures beyond 1kbar of about 7x10^{-4}cm^3/g. We have performed DLS measurements in the high pressure cell configuration as shown schematically in Fig. 5. The obtained normalized intensity autocorrelation functions from the sample in our SANS cell are shown as a function of pressure in Fig. 6. From the cumulant analysis of the data at P=1bar at a q-value of q=1.85x10^{5}cm^{-1} we find according to eq. 2, an apparent diffusion coefficient of D_{app}=1.16x10^{-6}cm^2/s in good agreement with the data of Banachowicz [17]. Since the product of $k_d c$ is in the order of 10^{-5}, we can safely approximate, following eq.3, the pressure dependence of D_0 by the one of D_{app}. The latter pressure dependence is plotted as $\Gamma = D^2_{app}$ in the inset of Fig. 6. Obviously there is no or at least a very small variation of the apparent relaxation rate with P. Intuitively one would expect that the rate is slowed down with increasing P. However, there are two effects which tend to cancel each other and we rationalize it in the following way: Eq. 4 relates the rate with viscosity and hence we find $\Gamma_{corr} \approx q^2(P)/\eta(P)$, where the P-dependence of q is given by the P-dependence of n. $\eta(P)$ can be found in [18], and to calculate the respective changes in n according to the recipe given in [14] we used literature values [27] for the density of light and heavy water. Contrary to η and n, the pressure dependence of radius r is negligible as is later rationalized by SANS from the shape invariant scattering curves with varying pressure. So for the pressure dependence of the viscosity and index of refraction corrected relaxation rates, also shown in the inset of Fig. 6, show no dramatic P-dependence and are up to 1.4kbar in agreement with Banachowicz [17]. This finding supports the assumption that pressure is usually not able to change the conformation of lysoyzme unless pressure exceeds a certain critical pressure threshold value. Then typically an irreversible rearrangement of structure occurs. The structural stability of lysoyzme against pressure is probably buried in the dense structure stabilized by interplay between hydration and electrostatic interaction. For pressures up to 2kbar for example volume changes in the order of 2.4% have been reported [19]. This small variation is in agreement with our findings. We find by dynamic light scattering lysozyme to be structurally stable up to 4.5kbar and this is also supported by SANS monitoring the structure directly as described in the following section. We should note here that at P=5kbar we find a different behaviour by performing light scattering experiments, namely exactly this beforehand mentioned structural changes. This is indicated by a loss of the apparent P-independence of the correlation function (in Fig. 6 the curve for 5kbar does not superimpose with all other pressures) and the unusual time dependent long-time tail of the correlation function. Moreover this effect is irreversible upon release of pressure. Hence we conclude that structure has changed. Unfortunately we have no SANS data for this case since we ran short with beam time, but since the effect has been established we will study it in future work.

B. SANS under high pressure

We have performed the SANS experiments at the SANS I machine at the SINQ spallation source at the PSI [20]. We used thermal neutrons of wavelength λ=0.5nm featuring a wavelength spread $\Delta\lambda/\lambda$ of about 0.1. The data analysis was performed using the BerSANS software package [21], which accounts for all necessary corrections due to background, transmission, count rate/dead time ratio, possible multiple scattering, masking and radial averaging of the raw data. A standard water sample was used for calibration to absolute scattering intensities and also to account for non-uniform detector efficiency [22]. We have measured at 2 m detector distance, which gives access to a q-range of $0.5 < q/\mathrm{nm}^{-1} < 3.5$, where q is the scattering vector, defined as $q = |\vec{q}| = \frac{4\pi}{\lambda}\sin(\frac{\theta}{2})$, with λ being the incident neutron wavelength and θ the scattering angle. Note that light scattering q's are at least one order of magnitude smaller than those from SANS, however, its variation is smaller.

The results of the SANS measurements are shown in Fig. 7 as a function of pressure up to 3kbar. Basically one notices an increase of scattering intensity with pressure which results in a change of contrast due to the compression of the solvent, similar as in the case of glucose isomerase. As in that case, lysozyme seems to possess a stable conformation with regard to the exertion of pressure since the curves can be scaled on top of each other without any noticeable change of shape. Without doing any analysis on the q-dependence of $I(q)$ of the lysozyme, we process only the values of the forward scattering for q=0.

For the forward scattering I(q=0) we can write [23]

$$\begin{aligned} I(q=0)K &= c\frac{N_A}{M_W}\left(\frac{b}{V}-\rho_0\right)^2 V^2 S(q=0) \\ &= c\frac{N_A}{M_W}\left(b-\rho_0 V\right)^2 S(q=0) \end{aligned} \tag{5}$$

where K is a calibration constant and $\Delta\rho = \frac{b}{V} - \rho_0$ is the scattering contrast of the lysozyme, b=4.50x10^{-10}cm is the scattering length, V=17.8nm^3 the volume [24] and b/V

the scattering length density of the lysozyme and ρ_0 is the scattering length density of the solvent (heavy water with a scattering length density of ρ_0=6.36x10^{10}cm^{-2}). Clearly b does not depend on P since it is entirely given by the atomic composition; also V does not change much with P since the scattering curves show no change in shape. This finding is supported by recent SANS results on glucose isomerase, where also the shape of the scattering curves did not change with P [25]. Therefore b/V is considered as being not pressure dependent, and likewise the structure factor at q=0 which was calculated using a RMSA calculation as $S(q=0)=0.68$ [26]. What differs is mainly the scattering length density ρ_0 of the D_2O, because the number density of scatterers depends on P. Following Eq. (5), a plot of $I(q=0)$ as a function of pressure should follow the pressure dependence of the density for heavy water [18]. This relation is shown in Fig. 8 and shows an excellent agreement between experiment and literature values.

V. CONCLUSIONS

We report on a SANS high pressure cell which uses fused sapphire as window material and thus allows simultaneously to perform light scattering investigations beneath the SANS measurements. The cell works for pressures up to 5kbar in a temperature range between 4-80 °C and uses a special container technique that allows separating sample and pressure transmitting oil easily. SANS and simultaneous DLS data on an egg-white lysozyme solution under high pressure are presented and show the functioning of the setup.

Acknowledgments

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VI. CAPTIONS

Figure 1: Middle cut through the high pressure cell.

Figure 2: Details of high pressure cell after one half of the steel body was removed. What is seen is the other half of the steel body on which the inner cell holder lies. Mounted on that is one big sapphire which in the completely mounted state is hidden within the other half of the steel body. The sample pill is located on the bottom of the sapphire in the middle of the inner cell holder. The silver ring which seals the inner volume filled with oil (and pill) against the sapphire lies around the sample pill. Also a Teflon sealing foil is seen between sapphire and inner cell holder.

Figure 3: Shown is an inner circular section of the cell as in Fig. 1 with details of the sample container (pill). The cut is along the holes of the brass ring to illustrate where the hydraulic oil can transmit the pressure via the viton O-ring.

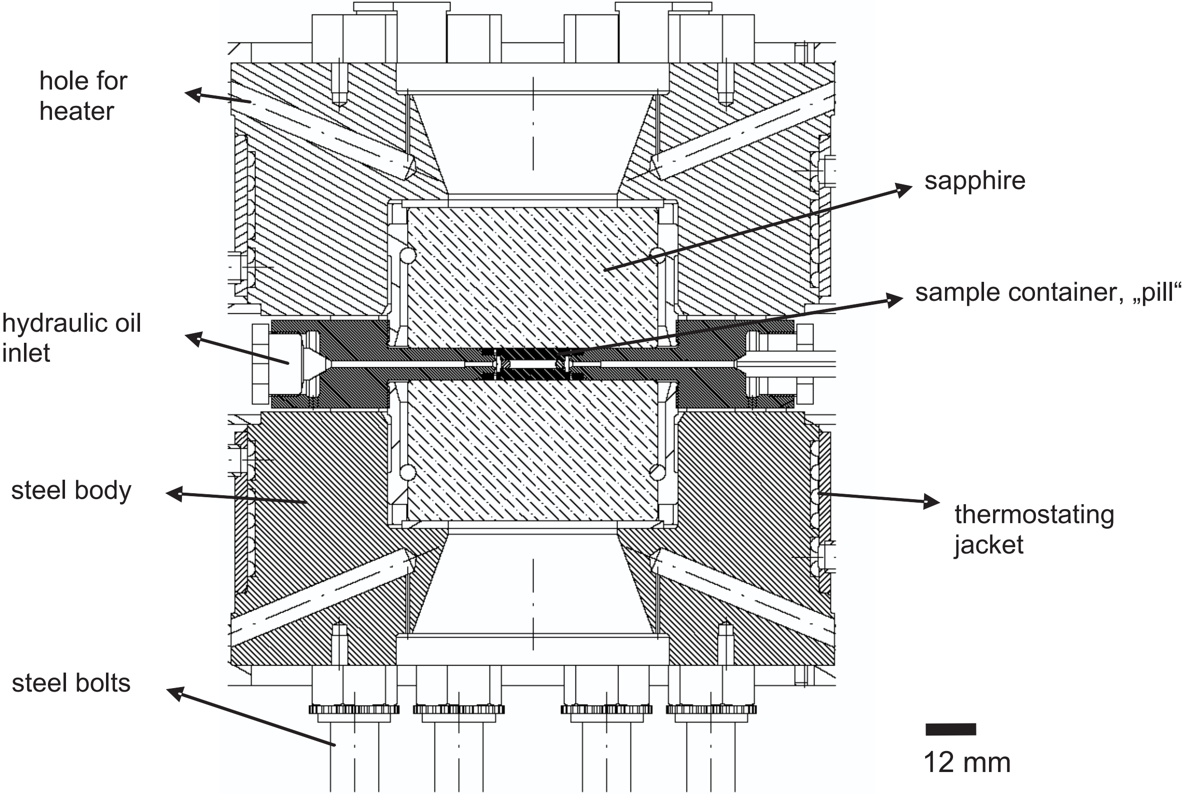
Figure 4: Components of the pill. The brass ring with holes on the periphery, the two sapphire plates and the O-ring. The sample in the pill is sealed against the hydraulic oil when the two sapphire plates press the O-ring against the brass ring holes from inside.

Figure 5: Schematic sketch of the setup which allows simultaneous SANS and DLS measurements.

Figure 6: Normalized intensity autocorrelation functions for lysozyme (50mg/ml) in 50mM acetate buffer as a function of pressure as indicated. In the inset, the values for the pressure dependence of the bare relaxation rates Γ and Γ_{corr} are shown (the latter is corrected for the pressure dependencies of η and n).

Figure 7: SANS results for lysozyme in D2O as a function of pressure as indicated. Also shown for comparison is the background of the high pressure cell. It shows no q-dependence and amounts to about 0.12cm^{-1}.

Figure 8: Comparison between the pressure variation of the forward scattering and the density change for heavy water. Density data from [18].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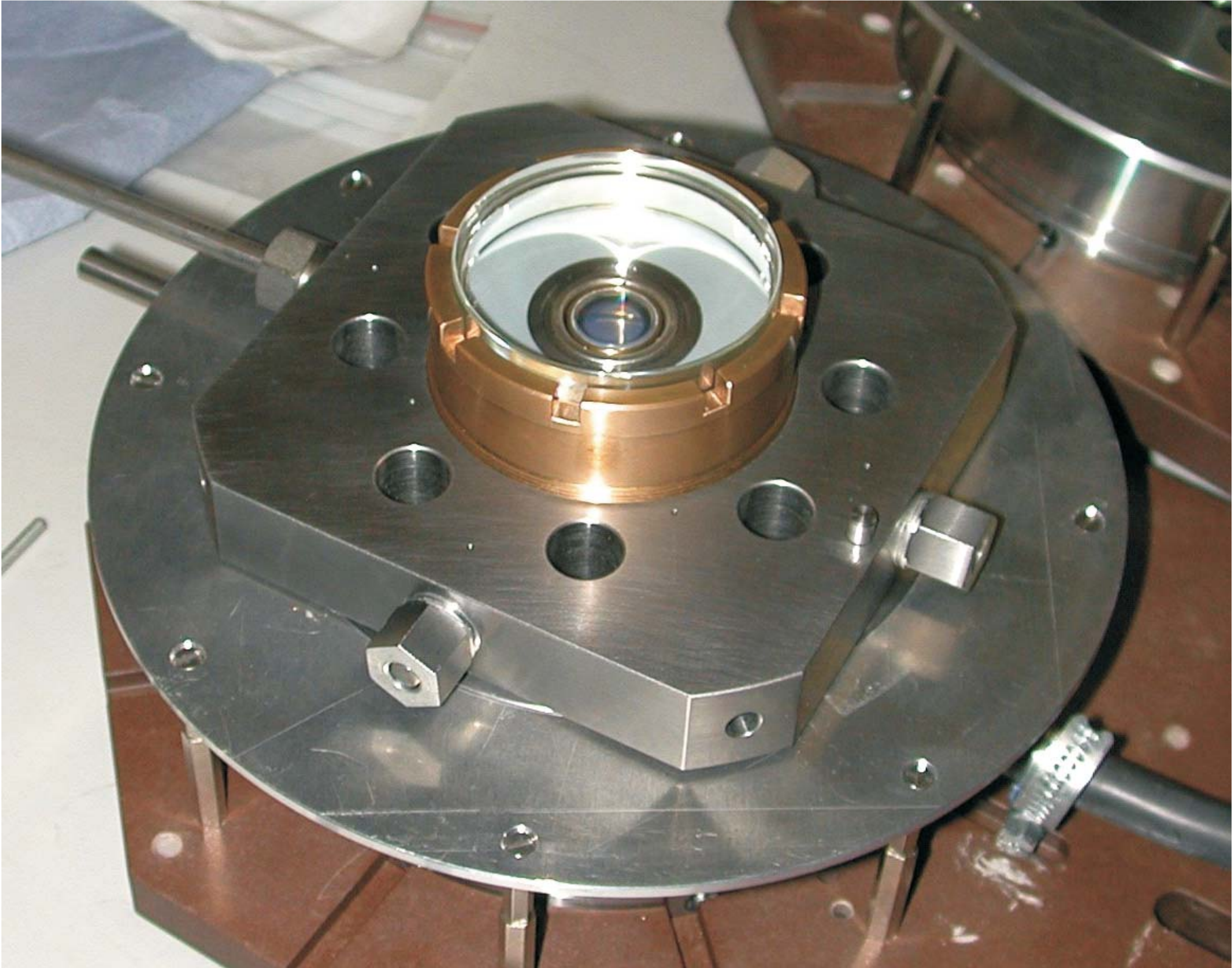
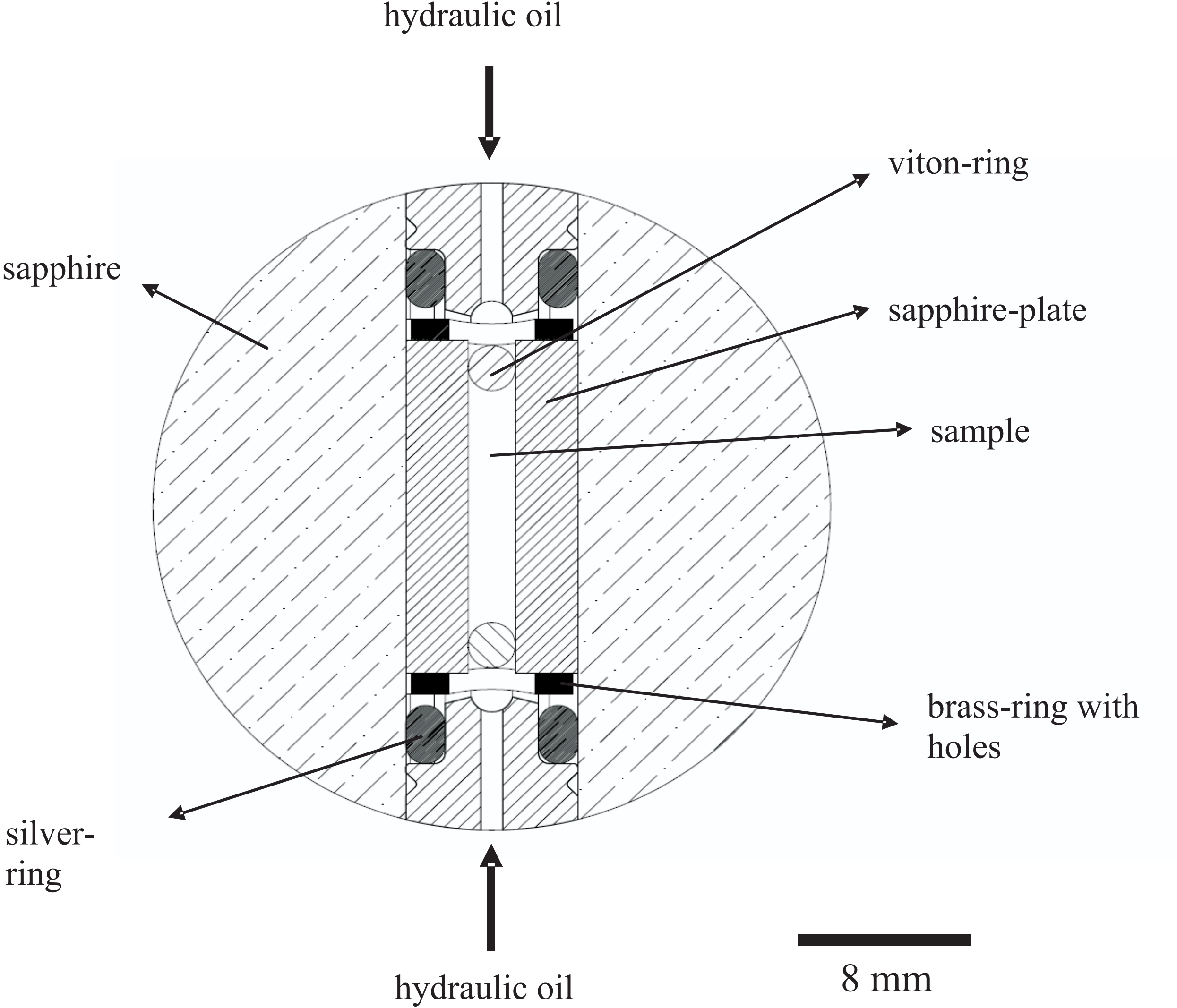
sapphire
sample container, „pill“
hydraulic oil inlet
steel body
thermostating jacket
steel bolts
12 mm

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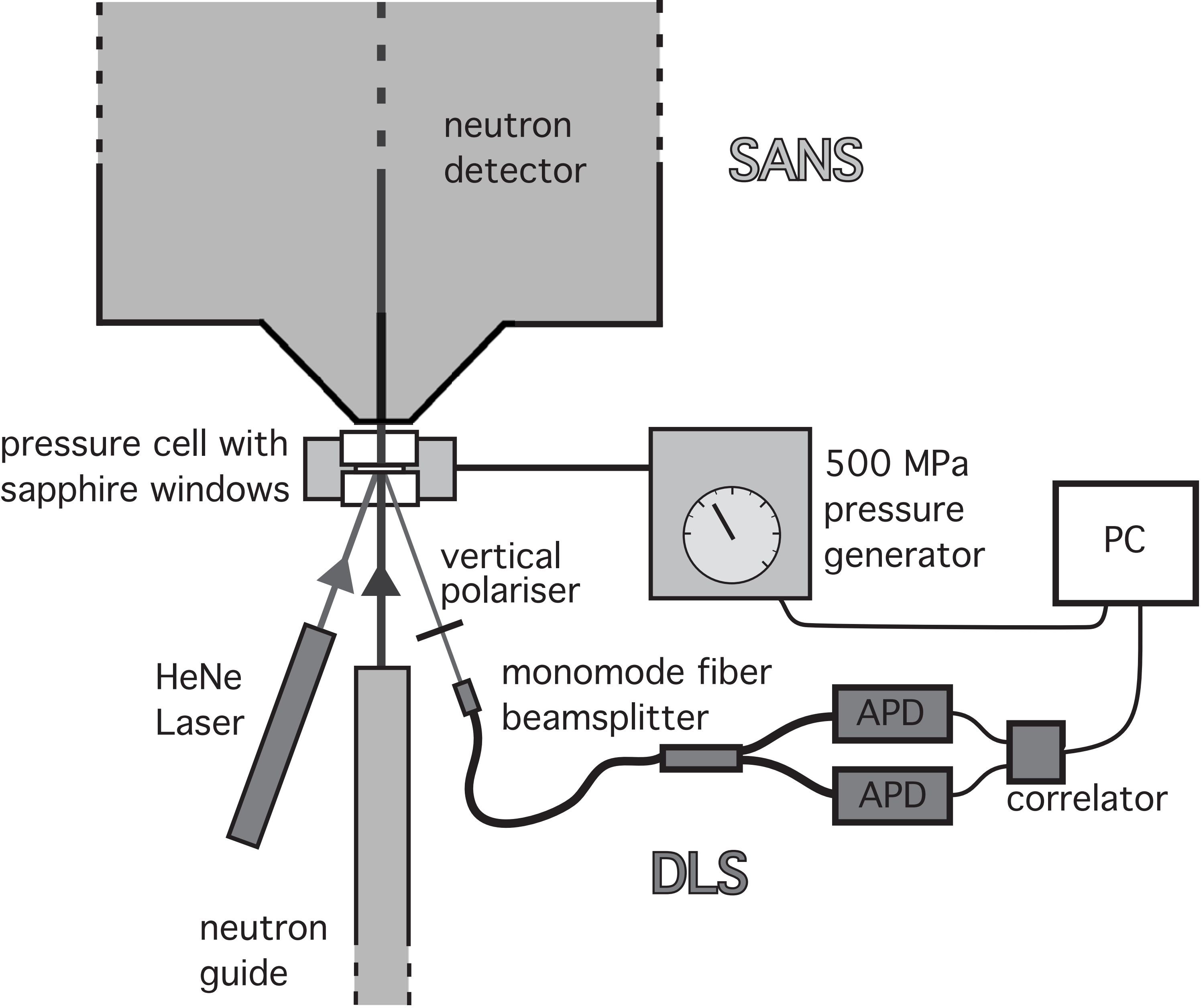
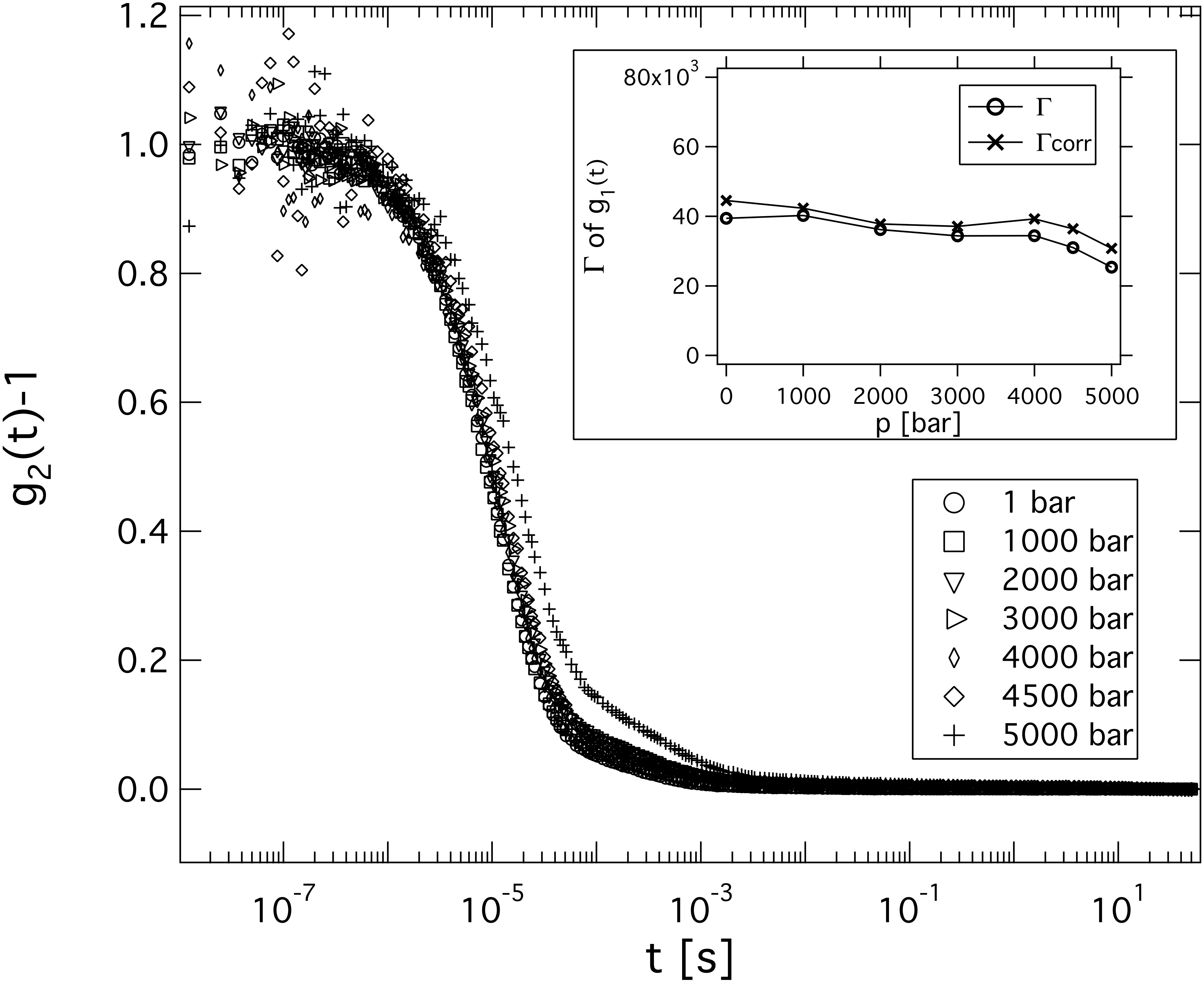
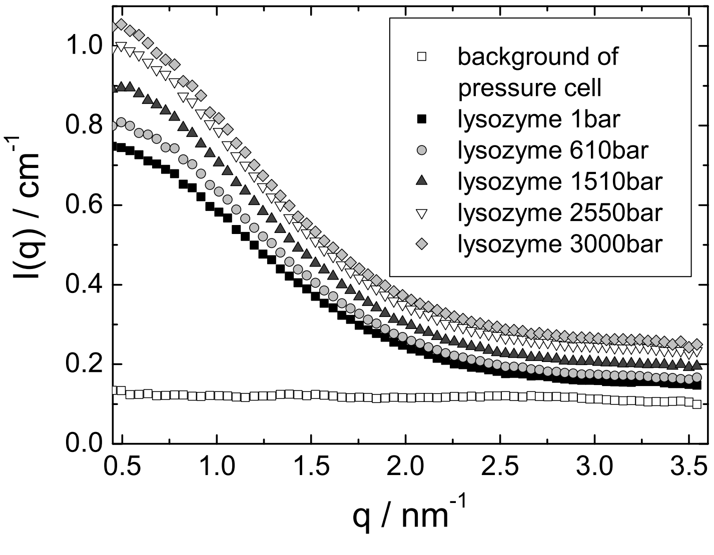
viton-ring
sapphire
sapphire-plate
sample
brass-ring with holes
silver-ring
hydraulic oil
8 mm

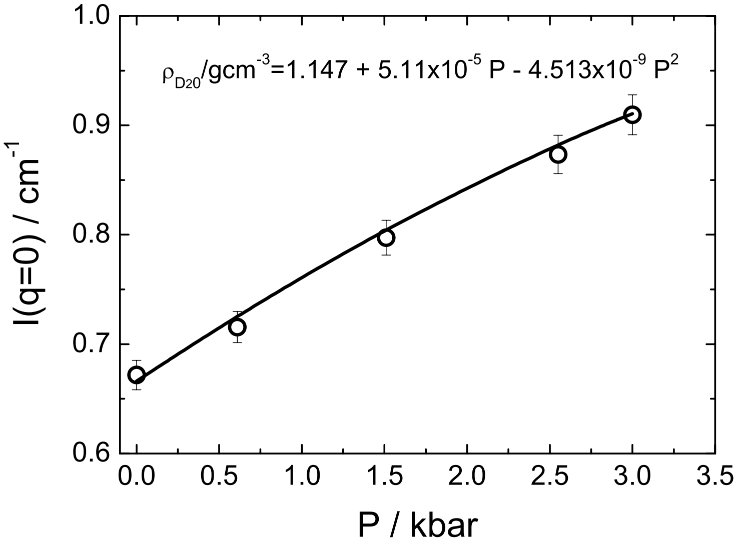

neutron
detector
SANS
pressure cell with
sapphire windows
500 MPa
pressure
generator
PC
vertical
polariser
HeNe
Laser
monomode fiber
beamsplitter
APD
APD
correlator
DLS
neutron
guide



g$_2$(t)-1
1.2
1.0
0.8
0.6
0.4
0.2
0.0
10^{-7}
10^{-5}
10^{-3}
10^{-1}
10^{1}
t [s]
1 bar
1000 bar
2000 bar
3000 bar
4000 bar
4500 bar
5000 bar
Γ of g$_1$(t)
80x10^3
60
40
20
0
0
1000
2000
3000
4000
5000
p [bar]
Γ
Γcorr



I(q) / cm^{-1}
q / nm^{-1}
0.0
0.2
0.4
0.6
0.8
1.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
□ background of pressure cell
■ lysozyme 1bar
● lysozyme 610bar
▲ lysozyme 1510bar
▽ lysozyme 2550bar
◆ lysozyme 3000bar



$\rho_{D20}/gcm^{-3}=1.147 + 5.11x10^{-5} P - 4.513x10^{-9} P^2$
I(q=0) / cm^{-1}
P / kbar
1.0
0.9
0.8
0.7
0.6
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5