ESRF	**Experiment title:** Thermal denaturing of Bacteriorhodopsin	**Experiment number**: SC-511
Beamline: BM05	**Date of experiment**: from: 23. November 98 to: 30. November 98	**Date of report**: 14.12.1999
Shifts:	**Local contact(s)**: A. Souvorov	*Received at ESRF:*

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Report:

We have carried out a temperature dependent x-ray diffraction study of thin films of purple membranes (PM) with the native membrane protein bacteriorhodopsin (BR). The high degree of alignment with respect to the silicon subtrates allows for the application of modern interface sensitive scattering techniques. In the present study, we have focused on the structural changes of BR in PM at the thermal denaturing transition. A partial unfolding of the helices is observed rather than the complete unfolding process known from helix to coil transitions. While BR remains threaded into the lipid bilayer in the denatured state, changes in the short range lateral structures are associated with the partial unfolding of the trans-membrane helices.

For the investigation, PM was spead in a film of about $10^1\mu m$ thickness on Si (100) (mosaitity less than 0,025°) and kept in a humidiy and temperature controlled chamber on the diffractometer at BM5. Measurements of the reflectivity, diffuse scattering and the lattice diffraction peaks at grazing incidence (GID) were carried out at 20keV . It is well known that the bacteriorhodopsin trimers assemble on a hexagonal lattice below the melting temperature of about $T_m \approx 80°$ (T_m depends on the re.hum.) [1]. Above T_m the protein lattice melts to a quasi two-dimensional liquid, as evidenced from the lateral form factor . Increasing the temperature above T=95°C thermal denaturing of BR was studied, both by monitoring the

helix scattering and the lateral form factor of the trimers. In the GID scans, a new peak at q_{II}=0.12Å^{-1} appears, probably due to a deformation of the trimer structure (see Fig.1).

In the study we have shown that thermal denaturing of BR is associated only with a partial loss of the secondary structure, and that the bulk of the protein remains organized within the membrane, most probably still in a trimer configuration [2]. Upon denaturing, the 1.28 Å^{-1} helix peak broadens irreversibly, see Fig.2. The final width corresponds to only about two positionally correlated helical pitches, indicating strong distortions of the helices. The thermal expansion coefficient of the helix (in the folded state) was found to be κ=(1.1 ±0.2)10^{-4} K^{-1}. By application of interface sensitive scattering techniques on highly aligned PM, the helical peaks can be mapped in two dimensions. With the aid of corresponding numerical modelling, more details of the partial unfolding process could be revealed in future, eventually including specific information on individual helices. Ultimately, the modelled molecular structure in such a study must simultaneously explain the two-dimensional high angle data of the helical interference pattern, and the two-dimensional mappings in the low angle region, reflectingthe form factor of the tertiary structure. Finally, such studies could help to reveal the temperature dependent structure of many other protein rich membrane systems, which are lacking 3D or 2D cystallinity and therefore are not amenable to x-ray cystallography.

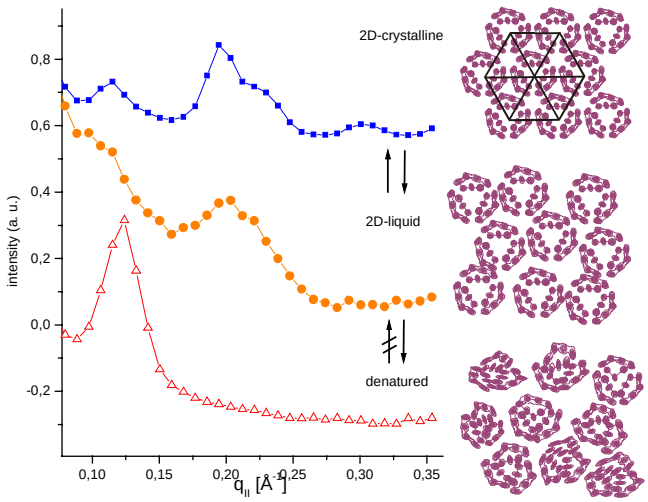

Fig.1: GID-Scans intersecting the truncation rods of the lattice and schematics of the trimer structure.

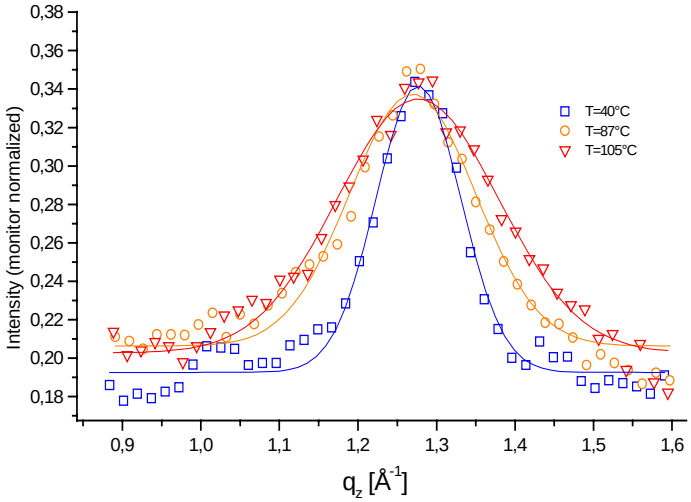

Fig.2: Broadening of the helix peak at the irreversible denaturing transition.

References:

[1] I. Koltover, J.O. Rädler, T. Salditt, C.R. Safinya, Phys.Rev.Lett. **82**, 3185 (1999); I. Koltover, T. Salditt, J. Rigaud, C.R. Safinya, Phys. Rev.Lett.. **81**, 2494 (1998).

[2] J.Müller, C. Münster, T.Salditt, sent to Biophysical Journal.