ESF short visit report: New Approaches to Biochemical Sensing with Plasmonic Nanobiophotonics

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Host: Dr Volker Deckert, The University of Jena

A TERS Investigation of Insulin Fibrillation

The purpose of the visit to Volker Deckert's lab was to use tip enhanced Raman spectroscopy (TERS) to investigate fibrils formed from insulin. A number of proteins can misfold and form fibrils through the process of fibrillogenesis, which is the causative factor in a number of diseases, including: Alzheimer's disease, Type II diabetes mellitus, Parkinson's and Huntington's disease and Creutzfeldt-Jakob disease (CJD). While studies using conventional Raman techniques have been informative in studying fibril structure and fibril forming kinetics, these techniques have their own limitations. Specifically the lack of spatial resolution, which TERS is not limited by. The direct coupling of topological information from AFM and structural information from Raman spectroscopy provides a powerful means for investigating fibril organization.

Insulin is a small peptide hormone consisting of two peptide chains connected by two disulphide bridges (Fig.1). Insulin has previously been used as a model system for studying fibril formation due to its propensity to form fibrils under a variety of conditions [1-3].

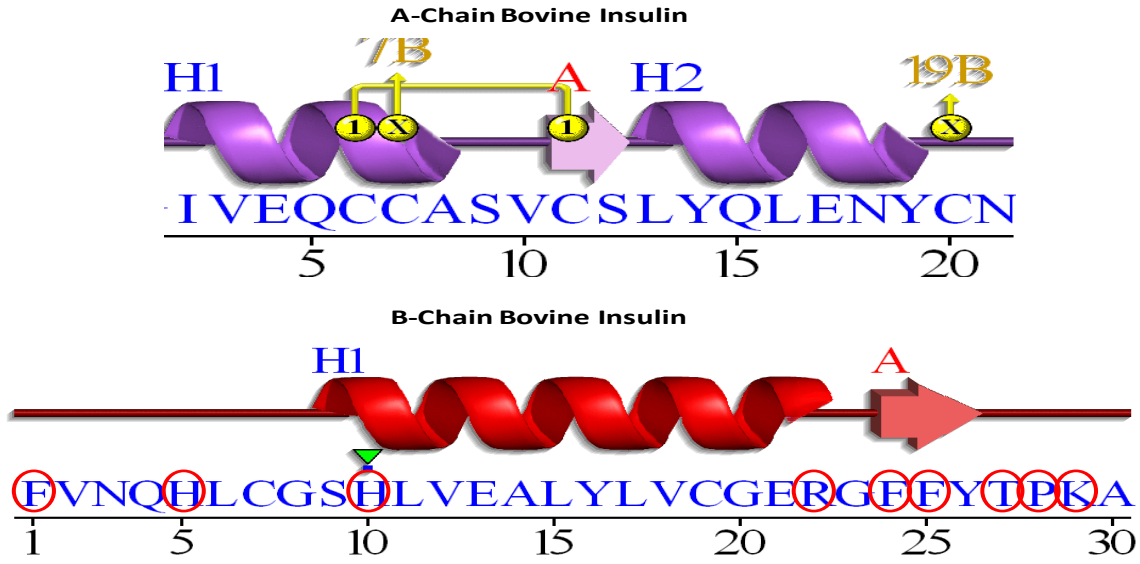

Fig.1 - Schematic representation of Bovine insulin from PDB ID 2pz6, with the A chain shown in purple and the B chain shown in red. Amino acids unique to the B chain are highlighted with circles.

To further investigate the fibrils formed by insulin, fibrils produced from the independent A and B chains, which are no longer linked via disulphide bridges, were studied using TERS. Before the visit to Jena (financed by this travel grant), the structures of fibrils formed by A-chain, B-chain and intact insulin had not been compared. Intact insulin fibrils have been recently studied by employing TERS by the Deckert group at Jena [4], and characterization of the structures of independent A- and B-chain insulin fibrils is an important step in understanding their properties and the process of fibrillation.

The primary work carried out at Jena began with the formation of fibrils from the independent A and B chains of insulin. Once the fibrils were formed they were prepared for examination with AFM (JPK) and TERS on a previously described system that utilises AFM (JPK) on an inverted Raman microscope (Horiba) [5]. A simple schematic (Fig.2) conveys the principal experimental setup used in the TERS experiment.

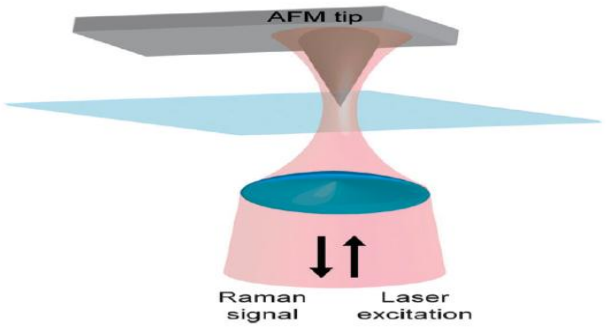

Fig.2 Schematic representation of TERS setup [6]

Data

The AFM data (Fig.2) highlights the morphological differences between fibrils formed from the A- and B-chains of insulin respectively. Specifically, the mature A-chain fibril shows a periodic repeating unit indicated in the height profile, which is not present in the B-chain fibril. Further analysis of the AFM data is being performed to determine the dimensions of the height and width of the fibrils in order to classify the fibril morphologies.

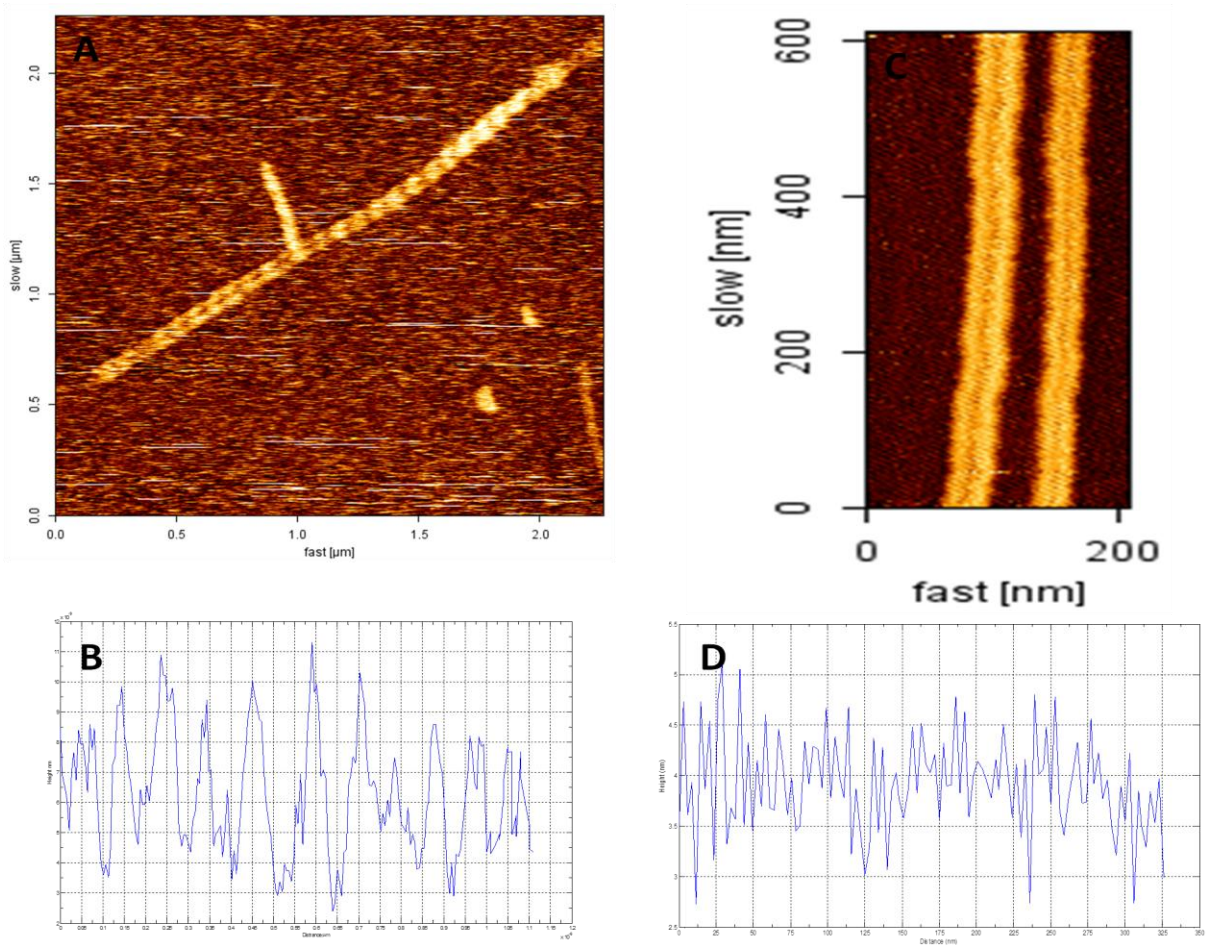

Fig.3 AFM images of the A- and B-chain insulin fibrils with corresponding height profiles along the length of the fibril. A, AFM image of A-chain fibril and B its height profile; C, AFM image of B-chain fibrils and D, the corresponding height profile of the B-chain fibril.

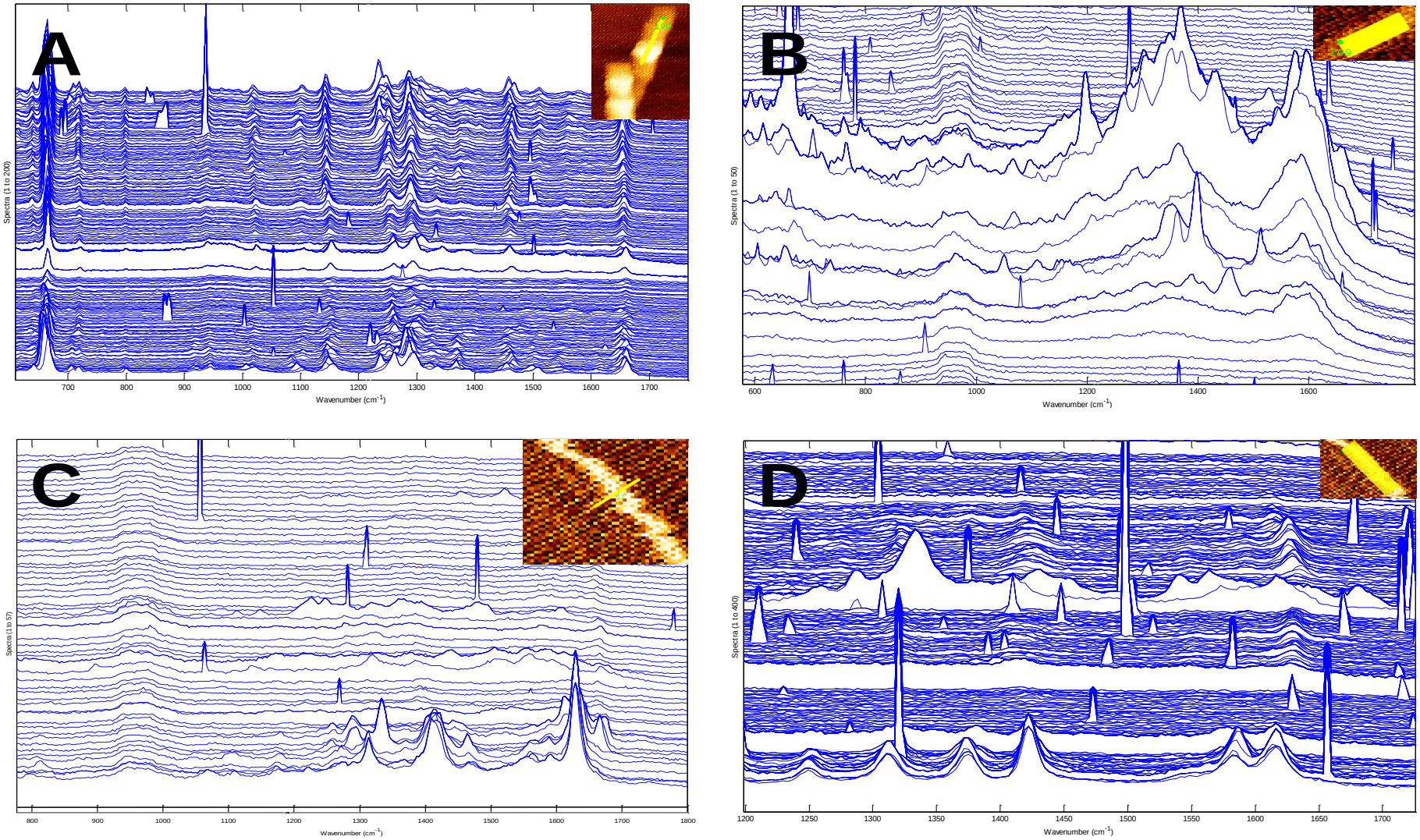

Fig.4 TERS experiments along Fibrils of the A- and B-chains of bovine Insulin. A & B, TERS spectra of A-chain fibrils and their corresponding topological AFM map. C & D, TERS spectra of B-chain fibrils and their corresponding topological AFM maps.

The data presented (Fig.4) is a representation of the high quality TERS spectra that were gathered for both fibril species. Grids of spectra were then checked for characteristic marker bands, which include secondary structure indicators e.g. α–helix and β–sheet, and side chains. Moreover, the spectra will be further investigated using chemometric approaches, however this analysis is still ongoing and not included in this report.

Secondary structure was assessed through the Amide-I and Amide-III regions. Amide I bands between 1660-1678 cm^{-1} indicate the presence of β-sheet structure, whilst bands from 1630-1659cm^{-1} are indicative of α-helix. In TERS the amide I response is sometimes suppressed, in these cases the amide III region (1225-1300cm^{-1}) was used to assess α-helix and β-sheet content. While full analysis of the many data collected will take some months to complete, preliminary analyses already highlight several striking results, see Table 1.

Table 1. Summary of secondary structure markers for A & B chain insulin fibrils

	β-sheet from amide I	β-sheet from amide III	α-helix from amide I	α-helix from amide III	Mixed α/β	mixed from amide III	suppressed amide I
A-Chain fibrils	30	3	158	88	180	8	111
	8 %		**43 %**		**49 %**		
B-Chain fibrils	8	2	266	59	130	26	87
	2%		**66%**		**32%**		

*A-chain fibrils: 479 spectra from 16 grids on 11 different fibrils. **B-chain fibrils: 491 spectra from 13 grids on 6 different fibrils.

Regarding secondary structure, the main observation between the two fibril species is that the B-chain appears to have a higher presence of α-helix on the surface of the fibrils. What makes this information of particular interest is bulk Raman measurements of fibrils are completely dominated by β-sheet, while TERS highlights that native α-helical structure appears to be preserved on the surfaces of the fibrils, which is consistent with previous work investigating insulin from the Jena group.

Table 2. Summary of side chain markers for the A- and B-chain insulin fibrils

A-Chain	TERS	Amino Acid contribution	B-Chain	TERS	Amino Acid contribution
Tyr	10%	10%	Tyr	6%	7%
CYS	23%	20%	CYS	8%	7%
Disulphide	7%	N/A	HIS	6%	7%
			PHE	**3%**	10%
			PRO	2%	3%

Whilst both peptide chains consist of a number of amino acids, some of which are unique to one chain or the other, not all provide a strong Raman cross section in TERS. Therefore Table 2 only includes prominent bands which can be easily identified / confirmed. When the presence of side chain bands is normalised to their contribution to the polypeptide chain, most occur at a frequency expected to observe, except for phenylalanine. This would indicate that while most side chains are exposed on the surface of the fibrils for both chains, whilst phenylalanine is either buried in the core of the fibril, or involved in an interaction which obscures it from the TERS tip.

While the TERS data presented in this report was measured in air, which is the standard experimental setup for TERS, measuring TERS while in solution, which has not been previously reported, was also attempted, and successful measurements were recorded. This is a great development for TERS as it opens up the ability to measure applicable samples in an aqueous environment, which for protein studies, such as the one reported here, is of great importance.

References

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2. Ivanova, M.I., et al., *Molecular basis for insulin fibril assembly.* Proc Natl Acad Sci U S A, 2009. **106**(45): p. 18990-5.
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