Experimental report

21/03/2024

Proposal:	**7-05-562**	**Council:**	10/2022
Title:	The dynamics of adenine and thymine on carbon surfaces		
Research area:	Chemistry		

This proposal is a new proposal

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Samples: $C_5D_6N_2O_2$/graphite
$C_5D_5N_5$/graphite

Instrument	Requested days	Allocated days	From	To
WASP	9	6	05/04/2023	11/04/2023

Abstract:

We propose to measure the dynamics of the nucleobases adsorbed on exfoliated graphite, focussing on the diffusion and dynamics of adenine, thymine and the adenine-thymine (AT) dimer. Measured diffusion rates and mass transfer will help to understand the rate of bond formation and proton transfer in a system that is critical for biology and medicine, paving the way to a better understanding of hydrogen bond formation in adsorbed DNA base pairs as well as in tautomerisation rates and proton tunnelling in AT dimers. Moreover, despite a few structural studies, no experimental information regarding the diffusion of DNA bases adsorbed at carbon interfaces is available up to now, while an understanding of the interaction and dynamics of biomolecules at carbon materials is essential for designing novel biomedical and catalytic applications.

Report on experiment 7-05-562:
“The dynamics of adenine and thymine on carbon surfaces”

Scientific background and aims of the experiment:

Our proposal aimed to measure the dynamics of nucleobases on graphite, and in the long-term perspective hydrogen bond formation and proton transfer in adsorbed DNA base pairs, focusing initially on the adenine-thymine dimer (AT, see Figure 1).

Within the blueprint of life, spontaneous “spelling mistakes” can occur, leading to potentially catastrophic errors in genetic replication and transmission. Among the causes for these spontaneous mutations is the quantum mechanical tunnelling of hydrogen atoms within the bonds binding the DNA duplex together [1,2]. The energetics of the canonical and tautomeric forms of the Adenine-Thymine (AT) and Guanine-Cytosine (GC) base pairs have been extensively modelled with *ab initio* quantum- mechanical techniques such as density functional theory (DFT) [3-5]. There have also been numerous experimental studies attempting to ascertain whether such tautomerism can lead to non-trivial populations of mutagenic nucleotides, but a b=road consensus is still lacking due to the difficulties in measuring proton transfer in single base pairs within a DNA.

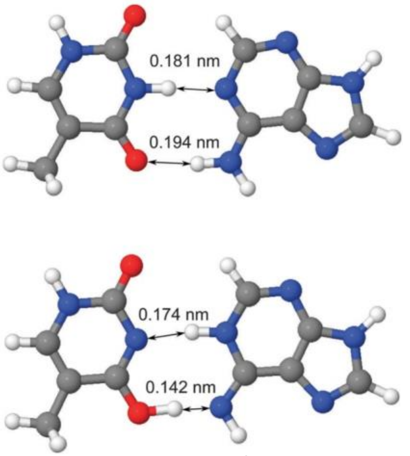

Figure 1: Formation of an Adenine-Thymine (AT) dimer and the corresponding proton transfer

Following our previous spin-echo studies of H_2O dynamics on 2D materials [6,7] and neutron scattering measurements considering the structure and dynamics of small molecules on graphite [8-10], we planned to measure as a first step the surface dynamics and diffusion of adenine and thymine adsorbed on exfoliated graphite. While the structure of nucleobases such as adenine on graphite has been studied experimentally [11,12], information about the dynamics is only available from theoretical studies up to now [13].

Sample Preparation:

The first part of the sample preparation was similar to the procedure of our prior neutron scattering experiments for hydrocarbons on graphite [9,10]. Circular disks of exfoliated graphite where cut with a cork cutter from a sheet of high purity exfoliated graphite for use as our substrate surface. Due to the low vapour pressure of adenine and thymine we then chose a different approach for the addition of the molecules. The sample cell was fitted with a high-temperature vacuum valve which could be connected to a pumping system. The cell was filled with the required graphite and the corresponding amount of molecular adsorbates before being pumped down and sealed. The system was then placed in a cryofurnace at 250 °C and baked overnight, ensuring the complete sublimation and distribution of adsorbates across the substrate surface, which was verified by visual confirmation of the ‘disappearance’ of the adsorbate crystals the following day.

Due to unforeseen circumstances, the samples initially planned for use, fully deuterated adenine and thymine, were not available. Some new samples produced by collaborators at the University of Surrey where however only partially deuterated due to the time constraints: (approximately 59%) for adenine while the thymine had a better deuteration (approximately 90%) but there was an additional unknown chemical component left within the sample. Thus, our experiments focused on the hydrogenated versions of adenine and thymine.

Experiments:

We had been allocated 9 days of beamtime to complete our experiments on WASP performing neutron spin-echo measurements. However, due to a shortage in one of the correction coils a day prior to our experiments were due to start, we were limited to 8 days. Due to a failure in our first attempt at sample preparation, likely due to an improper vacuum seal, the first measurements were made were of an old sample, 0.2 ML hydrogenated triazine, $C_3H_3N_3$, adsorbed on exfoliated graphite, at temperatures ranging from 100 K to 300 K, with a 2 K resolution scan. The sample showed dynamics from 300 K.

The following day we were able to confirm the success of the sample preparation method and start measurements on our new samples, beginning with 0.5 ML hydrogenated thymine, $C_5H_6N_2O_2$, at temperatures ranging from 100 K to 500 K. We then performed the same experiments with 0.5 ML hydrogenated adenine, $C_5H_5N_5$, this time starting from 200 K since there were minimal dynamics seen at 100 K. An example measurement from this sample is shown in Figure 1. It is interesting to note that these results suggest that the dynamics are rather slow (0.1-0.2 ns at 500 K) with an energy broadening of just 3-8 μeV. It is also interesting to see (Figure 1) that the dynamics sets in at about 200 K, but from 300 to 500 K there is hardly any change in the timescale. We thus hope to follow these measurements with TOF or at a backscattering geometry.

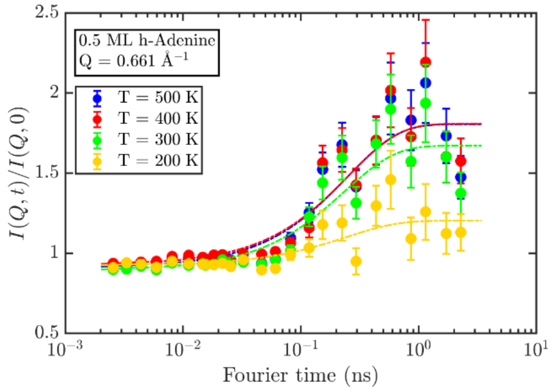

Figure 1. The intermediate scattering functions of hydrogenated adenine, as function of temperature. Increasing temperature shows an increasing scattering function demonstrating more active dynamics.

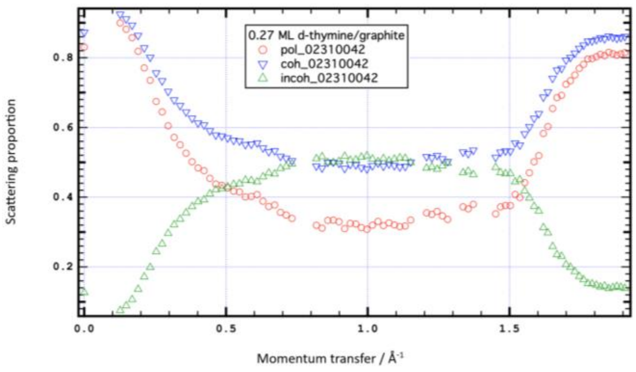

Figure 2. Polarisation analysis of the deuterated thymine sample. The high incoherent scattering signal suggests a deuteration of approximately 59%. The coherent signal is too low for reasonable measurements.

In an attempt to make use of the deuterated samples we did have, we performed similar experiments on 0.28 ML deuterated thymine, $C_5D_6N_2O_2$, with temperatures ranging from 100 K to 400 K, however the limited deuteration meant there was a large proportion of incoherent scattering. A comparison of the coherent and incoherent scattering results is shown in Figure 2. Finally, we attempted to perform these experiments with the deuterated adenine, $C_5D_5N_5$, however due to a loose screw the sample became misaligned, and measurements could not be made.

Alongside Van de Waals DFT calculations being performed by collaborators and possible additional measurements on a backscattering, or TOF instrument, we hope to publish these results.

References

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