Experimental Report

Proposal: **8-04-708** **Council:** 10/2012

Title: Molecular dynamics of a pair of enantiomers in complex with acetylcholinesterase

This proposal is a new proposal

Researh Area: Chemistry

Main proposer: **LINUSSON Anna**

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Samples: mus musculus acetylcholinesterase (mAChE)
C5685 (R): (R)-4-(dimethylamino)-N-((1-ethylpyrrolidin-2-yl)methyl)-2-methoxy-5-nitrobenzamide
C5685(S): (S)-4-(dimethylamino)-N-((1-ethylpyrrolidin-2-yl)methyl)-2-methoxy-5-nitrobenzamide

Instrument	Req. Days	All. Days	From	To
IN16	7	6	16/05/2013	22/05/2013
IN13	7	5	06/05/2013	11/05/2013
IN6	7	4	13/05/2013	17/05/2013

Abstract:

The enzyme acetylcholinesterase (AChE) is an essential component of the nervous system, where it catalyses the hydrolysis of the neurotransmitter acetylcholine. In our research, we use this system to investigate fundamental aspects of molecular recognition, mechanism of catalysis/inhibition and rational drug design. In this proposal we would like to investigate the molecular dynamics of AChE in the presence or absence of ligands (inhibitors). After successful investigation of the enzyme with and without inhibitors, it should shed lights on a new aspect newer tested before by neutron scattering. The results obtained by neutron scattering will be related to data from other computational and experimental techniques applied by us to this particular system.

Report 8-04-708 – IN13, IN6 and IN16 and CRG 2031 – IN13

Molecular dynamics of a pair of enantiomers in complex with acetylcholinesterase

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The enzyme acetylcholinesterase (AChE) is an essential component of the nervous system, where it catalyses the hydrolysis of the neurotransmitter acetylcholine. In our research, we use this system to investigate fundamental aspects of molecular recognition, mechanism of catalysis/inhibition and rational drug design. In this proposal we wanted to investigate the molecular dynamics of AChE in the presence or absence of ligands (inhibitors). More specifically, we studied *Mus musculus* AChE (*m*AChE) upon complexation with two of enantiomeric ligands (inhibitors) at a pico- to nanosecond time range using elastic incoherent neutron scattering. The objective was to investigate the effect of the inhibitors on the dynamics of *m*AChE and to link the flexibility to thermodynamic properties determined with isothermal titration calorimetry (ITC).

F. Gabel and co-workers [1] and J. Peters and co-workers [2, 3] have previously successfully used elastic, quasi-elastic and inelastic incoherent neutron scattering to investigate the temperature dependence of the dynamics of AChE and butyrylcholinesterase, in complex or not with an inhibitor. The measurements were carried out on the scattering spectrometers IN6, IN13 and IN16 (ILL). The results showed a strong correlation between activity and dynamics; e.g. increased flexibility led to increased enzyme activity. Here, we wanted to build on that knowledge and used neutron scattering to explore the temperature dependent dynamics of *m*AChE, C5685(*R*)· *m*AChE and C5685(*S*)· *m*AChE in order to investigate changes in dynamics induced by ligand binding. Again we used the same spectrometers IN6, IN13 and IN16 for a better comparison with the previous results and to have a complete dynamical picture within the time window up to 1 ns.

The differences between the samples' dynamics are extremely small and a very careful data analysis is needed to see them. To reduce the standard deviation, we calculated the intensities summed over the whole Q-range of the instrument and not only the mean-square-displacements.

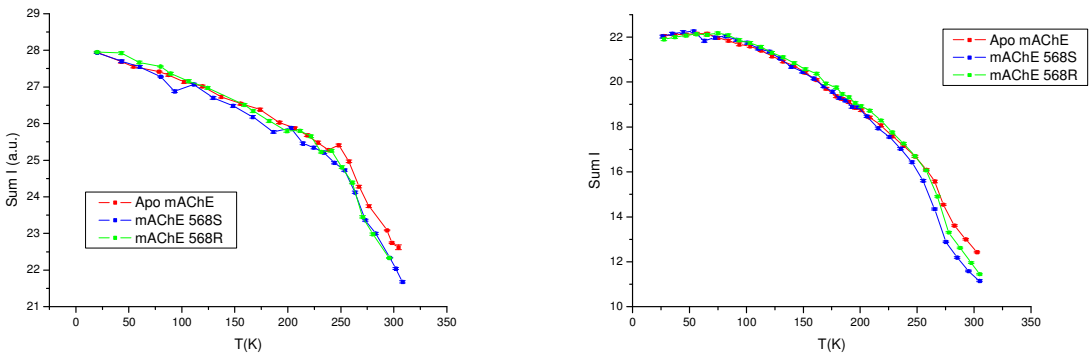

Figure 1: Intensities summed over the accessible Q-ranges on IN6 and IN16.

We show first results collected on IN6 (energy resolution of 90 µeV and Q-range of 0.33 – 2.05 $Å^{-1}$) and IN16 (energy resolution of 1 µeV and Q-range 0.19 – 1.93 $Å^{-1}$) in Figure 1. The summed intensities are inversely proportional to the atomic mean-square-displacements. Therefore it appears that on both instruments the complexed forms of the enzyme are slightly more flexible than the wild type sample, but it remains very difficult to establish a hierarchy between the two different complexes. Further statistical data analyses are under progress.

On IN6, we also measured the density of states of the three samples (see Figure 2), but the results were almost identical for them.

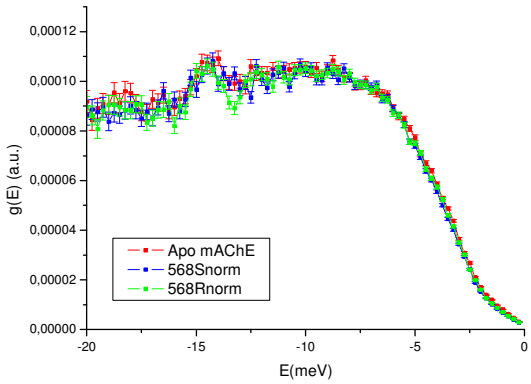

Figure 2: Normalized densities of states of the three samples taken at 80 K.

References

[1] Gabel, F.; Weik, M.; Masson, P.; Renault, F.; Fournier, D.; Brochier, L.; Doctor, B. P.; Saxena, A.; Silman I.; and Zaccai, G., *Biophys. J.,* 2005, 89, 3303–3311.
[2] Peters, J.; Trovaslet, M.; Trapp, M.; Nachon, F.; Hill, F.; Royer, E.; Gabel, F.; van Eijck, L.; Masson, P.; Tehei, M., *Phys. Chem. Chem. Phys.* (2012), 14, 6764–6770.
[3] Trovaslet, M.; Trapp, M.; Weik, M.; Nachon, F.; Masson, P.; Tehei, M.; Peters, J., *Chem Biol Int* 203 (2013) 14 – 18.