Experimental report

29/08/2022

Proposal: 8-04-917 **Council:** 4/2021

Title: Protein Flexibility and Conformational Entropy in Ligand Binding Targeting the Carbohydrate Recognition Domain of Galectin-3

Research area: Biology

This proposal is a new proposal

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Local contacts: Tilo SEYDEL
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Samples: acetyllactosamine
lactose
Galectin

Instrument	Requested days	Allocated days	From	To
IN16B Si 111 BATS	4	2	15/05/2021	17/05/2021

Abstract:

Drug design is predicated on knowledge of the three-dimensional structure of the protein-ligand complex and the thermodynamics of ligand binding. In the proposal for IN16B BATS mode we suggest to investigate the effect of ligand binding on the molecular dynamics of galectin-3 (Gal3C) using QENS. We propose to investigate the effects of three ligands on the dynamics of Gal3C: lactose (lac) and two synthetic high affinity inhibitors L2 and L3. Based on NMR changes of conformational entropy of the protein backbone DeltaS_bb and protein side-chains DeltaS_ss have been estimated for the three ligands. We suggest to investigate using QENS perturbation of internal relaxation rates and amplitudes of motion of Gal3C by ligand binding on the ps to ns time scale. From the determined amplitudes of motion, we will calculate conformational entropy changes of Gal3C upon ligand binding. We would like to make a quantitative comparison between QENS and already available NMR, ITC and X-ray crystallography data to obtain a better picture of the role of ligand binding for the dynamics of Gal3C, and to establish QENS as a quantitative tool for the study of protein-ligand binding.

Experiments 8-04-916 “The Relevance of Conformational Entropy for Protein-Ligand Interactions in Citrate Synthase” and 8-04-917 “Protein Flexibility and Conformational Entropy in Ligand Binding Targeting the Carbohydrate Recognition Domain of Galectin-3” have been permed sequentially as remote experiments because of travel restrictions from Germany to France in 2020 and 2021. We are very thankful for the help of the entire IN16B team and in particular for the help provided by Dr. Tilo Seydel and Dr. Markus Appel without whom the experiments wouldn’t have been feasible at all.

Experiment 8-04-916 “The Relevance of Conformational Entropy for Protein-Ligand Interactions in Citrate Synthase”

Originally it has been planned to investigate the effect of the binding of the ligand oxaloacetate on the dynamics of the enzyme citrate synthase. The protein and the chemicals have been ordered by Sigma Aldrich well in advance of the experiment on IN16B, but they arrived only 6 months after the experiment took place. As a backup plan we have measured instead the dynamics of the enzyme phosphoglycerate kinase (PGK) in presence and absence of the ligands ATP and glycerate (13mM MgATP and 41mM 3PG at PGK concentration of 50 mg/mL). This ensures a protein-ligand saturation of more than 90% in the bound-state. These conditions are the same as in the original SANS & NSE publication by Inoue et al. Large Domain Fluctuations on 50-ns Timescale Enable Catalytic Activity in Phosphoglycerate Kinase, Biophysical Journal 2010.

In the study of Inoue et al. 2010, large scale domain motions have been observed in PGK. The amplitude and frequency of those domain motions are suppressed by ligand binding. The aim of the experiment on IN16B BATS mode was to investigate whether or not ligand binding influences fast ps to ns motions in PGK as well. QENS was measured of 50 mg/mL PGK, 50 mg/mL PGK with bound ligands and the corresponding buffers. The instrument reference was determined by a vanadium measurement. QENS data have been Fourier transformed into the time domain and are currently being analysis within the mathematical model of the fractional Brownian oscillator. Analysis of the data is part of the PhD thesis of Ms Abir Hassani.

8-04-917 "Protein Flexibility and Conformational Entropy in Ligand Binding Targeting the Carbohydrate Recognition Domain of Galectin-3"

Carbohydrate recognition domain of galectin-3(Gal3C) in the apo-form and with the ligands lactose (lac) and the first-generation low-micromolar inhibitors 3'-benzamido-Nacetyllactosamine (L2) and 3'-(4-methoxy-2,3,5,6-tetrafluorobenzamido)-N-acetyllactosamine (L3) have been provided by our cooperation partner Prof. Mikael Akke from Lund experiment. Samples have been measured on IN16B using the BATS option. Unfortunately, the corresponding background solutions could be measured only later on IN5.

QENS data are currently still under evaluation. Experimental data will allow us to determine whether or not QENS will provides similar values for the difference of conformational entropy due to ligand binding in Gal3C as it has been observed originally by NMR (Diehl et al. J. Am. Chem. Soc. 2010, 132, 41, 14577–14589; Verteramo et al. J. Am. Chem. Soc. 2019, 141, 5, 2012–2026).

Analysis of the properties of Gal3C is a ongoing cooperation within the LINXS theme INTEGRATIVE PHARMACOLOGY AND DRUG DISCOVERY.