Experimental report

29/08/2022

Proposal: 8-04-916 **Council:** 4/2021

Title: The Relevance of Conformational Entropy for Protein-Ligand Interactions in Citrate Synthase

Research area: Biology

This proposal is a resubmission of 8-04-904

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Samples: Citrat Synthase

Instrument	Requested days	Allocated days	From	To
IN16B Si 111 BATS	3	3	12/05/2021	15/05/2021

Abstract:

Molecular dynamics plays an important role for the biological function of enzymes. For protein ligand interactions, changes of conformational entropy of protein and hydration layer are relevant for the binding process. We suggest to investigate differences in protein dynamics and conformational entropy of citrate synthase (CS) in the ligand-free and in two different ligand-bound states by QENS on IN16B BATS mode. We would like to determine the change of conformational entropy Sconf of CS upon ligand binding from mean square displacements. Isothermal titration calorimetry (ITC) data will inform us about the entropy change S during ligand binding that includes both protein and hydration water components. By combining QENS and ITC results we will separate the contributions of protein Sconf and hydration water Shydr and how both of them regulate ligand binding in CS. We suggest to use the BATS mode to probe a large energy transfer range at high energy resolution for detailed line-shape analysis of the QENS broadening. Therefore, our experiment is not feasible on standard neutron backscattering spectrometers located at neutron reactors due to their restricted energy transfer range.

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.