Experimental Report

Proposal: **8-03-782** **Council:** 10/2012

Title: SANS characterization of the transcription factors Pbx1 and Prep 1 in complex with DNA

This proposal is continuation of: 8-03-705

Researh Area: Biology

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Samples: pbx1
Prep1
DNA oligo

Instrument	Req. Days	All. Days	From	To
D11	2	1	01/07/2013	02/07/2013

Abstract:

Homeodomain (HD) transcription factors include the Pbx, Prep and Hox families, which are involved in human cancer (1). The aim of this project is to characterize the structure of the tumor suppressor protein Prep1 (2) in complex with its binding partner Pbx1, and to clarify their interaction with DNA. The mechanism of interactions between Pbx1 and Prep1 will be uncovered and the structure of the heterodimer bound to DNA will allow us to gain insights into the DNA target specificity and binding properties of the Prep1–Pbx1 heterodimer. We have done SANS measurements on December 2011 at ILL but data were inconclusive, due to the too low concentration of the complex. HD proteins are difficult to crystallize in their full length, and then we believe that SANS data of the Prep1-Pbx1 complex are essential to unravel their binding mode to DNA. Furthermore we are proposing to investigate the Prep1-Pbx1-HoxB1 HDs all assembled on DNA. By selective deuteration of Prep1 HD, we are actually willing to solvent match all

Proposal 8-03-782

SAXS/SANS characterization of the transcription factors Pbx1 and Prep1 in complex with DNA

Proposers: BRUCKMANN Chiara, MARIANI Paolo, ORTORE Maria Grazia, SPINOZZI Francesco, and MACCARINI Marco

Instrument: D11; local contact: SCHWEINS Ralf

Background

Homeodomain transcription factors include the Pbx and the Meis/Prep families involved in human cancer (Wong, P. et al., Genes Dev 21, 2762-74 (2007), Longobardi, E. et al. Mol Oncol 4, 126-34 (2010)). The protein Prep1 in complex with its binding partner Pbx1 acts as tumor suppressor by interacting with DNA. We intend to clarify the structure of the Prep1 and Pbx1 alone and in complex with DNA, in order to elucidate the functions of this class of transcription factors during cancer development. Small angle scattering experiments will elucidate the binding architecture to DNA of these transcription factors in solution. SAXS and SANS techniques will help us to understand the structural rearrangement of the complexes upon DNA binding.

Samples

Purified Prep1-Pbx1 (Prep1 $_{mW}$~35 kDa Pbx1 $_{mW}$ ~30 kDa) complex was brought to ILL, both alone and bound with specific 21 base pair DNA double strand oligo (mW ~12 kDa). Before SANS experiment, the sample was checked by SDS PAGE, gel filtration, and SAXS and resulted to be pure, highly homogeneous and monodisperse.

Prep1-Pbx1 complex concentration was ~4.4 mg/ml, and Prep1-Pbx1-DNA complex was 2.2 mg/ml

Methodology

Neutron scattering with the support of contrast variation can distinguish between the different elements involved in the protein-DNA complexes. The scattering length density of the DNA at natural contrast (equivalent to a mixture D2O:H2O of about 0.7) differs remarkably from that of the proteins (~D2O:H2O ~40%).

In order to observe specifically each component of the protein-DNA complex we wanted to use contrast variation. We decided to change the solvent composition by modifying the H_2O/D_2O ratio. Just before SANS data collection, Prep1-Pbx1 and

Prep1-Pbx1-DNA complexes were buffer exchanged against buffers containing respectively 20%, 42%, 66% or 96% D_2O.

Data collection

During the 24 hours of SANS experiment, we have measured the following samples, at different H2O:D2O ratios, in order to match out protein and DNA with contrast variation:

1- Prep1-Pbx1complex in 96% D2O

2- Prep1-Pbx1-DNA complex in 96% D2O

3- Prep1-Pbx1-DNA complex in 66% D2O

4- Prep1-Pbx1-DNA complex in 42% D2O

5- Prep1-Pbx1-DNA complex in 20% D2O

6- Prep1-Pbx1-DNA complex in 0% D2O

7- DNA

SANS measurements were done on D11 in the q range 0.01 Å-1-0.7 at λ=4.5Å at room temperature. The samples were contained in quartz cuvettes of 2mm path length. Scattering from buffer was subtracted from scattering from the solutions of protein-DNA particles.

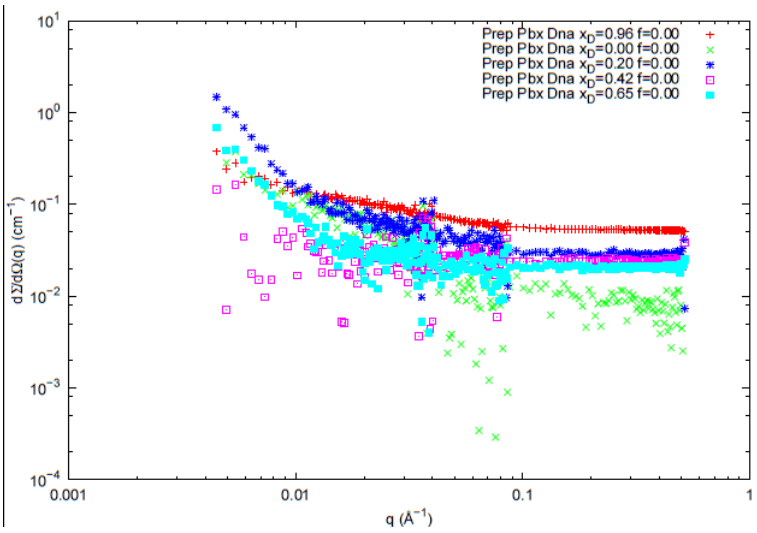

Figure 1: Subtraction curves Q (Å^{-1}) against I(Q), of Prep1-Pbx1-DNA in: (a) H_20, (b) 20% D_2O, (c) 42% D_2O, (d) 66% D_2O, and (e) 96% D_2O

Problems encountered

The presence of D2O in the buffer at any concentration influences the solubility of the sample. Unfortunately it was not possible to process the data, because at low angle all the samples in D2O seemed aggregated.