Experimental report

13/09/2019

Proposal: 8-05-442 **Council:** 10/2018

Title: Regulation of Myc-Max DNA binding in cancer: The Max homodimer

Research area: Biology

This proposal is a new proposal

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Samples: hMax
DMax
E-box DNA

Instrument	Requested days	Allocated days	From	To
D22	2	2	13/07/2019	15/07/2019

Abstract:

The Myc-Max heterodimer assembly functions as a central hub in cellular growth control, by regulating a wealth of biological functions including proliferation, apoptosis, differentiation, and transformation. Uncontrolled Myc expression disturbs the carefully tuned balance of cell growth regulation, which turns the Myc-Max heterodimer into an oncoprotein multimodular platform and a key contributor to the development of many human cancers. To bind DNA, the c-terminal region of Myc must form a heterodimer with the protein Max. Crystal structures describing the Myc-Max and Max-Max dimers have so far only included the core DNA-binding motif, including the bHLHzip region. By circular dichroism spectroscopy, we have shown that regions flanking the Max bHLHzip core add significant helical propensity, which does not agree with a bHLHzip core flanked by disordered regions. Furthermore, we found that the full-length Max-Max homodimer is more stable than the Max-bHLHzip homodimer. Here, we will use SANS to describe the full Max-Max-DNA envelope. Neutron scattering envelopes in this project will be critical to proceed towards modelling of the full Myc-Max-DNA heterodimer structures.

Regulation of Myc-Max DNA binding in cancer.
Part 1: The Max homodimer

Scientific background The Myc-Max heterodimer assembly functions as a central hub in cellular growth control, by regulating a wealth of biological functions including proliferation, apoptosis, differentiation and transformation[1,2]. Increased Myc levels in the cell are caused by mutations disrupting ubiquitination and/or translocation and lead to increased Myc-Max heterodimer formation over the otherwise prevailing Max heterodimer. Uncontrolled Myc expression disturbs the carefully tuned balance of cell growth regulation[3], which turns the Myc-Max heterodimer into an oncoprotein multimodular platform and a key contributor to the development of many, if not most, human cancers (reviewed in [4]). To bind DNA, the c-terminal region of Myc must form a heterodimer with the protein Max (Fig. 1). Crystal structures describing the Myc-Max and Max-Max dimers have so far only included the core DNA-binding motif, including the bHLHzip region[9,10,11]. In combined circular dichroism spectroscopy and limited proteolysis approach, we have shown that regions flanking the Max bHLHzip core add helical propensity to the fold[12], which does not agree with a Max bHLHzip dimer motif flanked by disordered regions. Furthermore, we found that the full-length Max heterodimer, comprising Max residues 1-132, is significantly more stable both in the absence and presence of DNA, compared to the $Max_{18\text{-}106}$ fragment comprising only the bHLHzip region[12]. However, since X-Ray crystallography and NMR have both failed to describe entire Max-Max or Myc-Max protein assemblies, structural contributions by regions flanking the core DNA binding motif of the Max-Max or Myc-Max dimers remain unknown (Fig. 1).

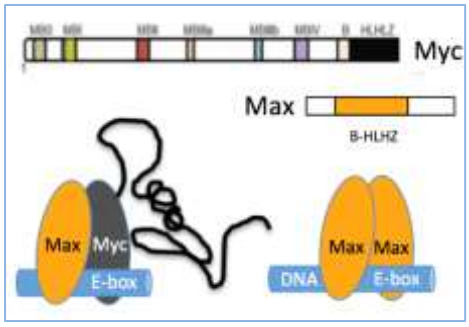

Figure 1 *Myc and Max sequences and DNA binding*

Proposed experiment In this project we have used neutron scattering to investigate the structured envelope of DNA-bound complexes of Max-Max. We have used contrast variation of solvent and proteins to investigate the structural role of Max flanking regions in the Max-Myc heterodimer when bound to the classical "E-box" DNA motif.

Detailed description of the experiment We recorded SANS small and large angle data to establish the respective inter-component distances and topology of Max homodimer binding to its corresponding E-box DNA. To resolve the complex components, we exploited the intrinsic DNA-protein scattering contrast by adjusting the D_2O content in an H_2O buffer. We routinely produce Max protein from overexpression in *Escherichia coli*, including labelling with ^2H. Uniformly 68%-deuterated d_{68}Max, uniformly protonated hMax. The Max-Myc heterodimer has a molecular weight of 70.5 kDa, the ds-DNA targeted by Max will comprise 49 base pairs, with a molecular weight of 30.3 kDa[4,8]. The molecular weight of the entire complex, Max dimer and the DNA, will thus amount to 100.8 kDa. The DNA oligos (2x49 bp) will be purchased as chemically synthesized with the ^1H isotope.

Instrument, beamtime and sample environment We proposed to measure on D22 using instrumental setups of 1.5 and 8 meters that enable to cover both the Guinier-range (radius of

gyration) and the wide-angle range thoroughly (solvent-subtraction, medium-range information) for this relatively small complex (MW 100 kDa): 0.01 $Å^{-1}$ to 0.6 $Å^{-1}$.

In the current application we intended to measure the following complexes (we measured at 10°C):

- hMax dimers in complex with 49-mer DNA duplex at 0%, 40%, 60% and 100% D_2O;
- $d_{68\%}$Max dimer in complex with 49-mer DNA duplex at 0%, 40% 60% (Match Point of 49-mer dsDNA), 80% and 100% D_2O (Match Point of $d_{68\%}$Max);
- The corresponding reference samples (boron, empty quartz cuvette, water, buffer) at the instrumental configuration.

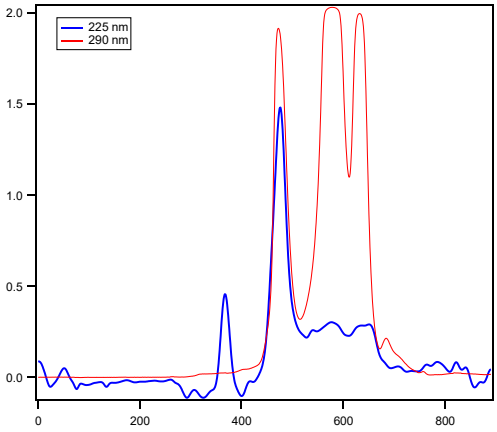

Figure 2 *Example of Gel filtration*

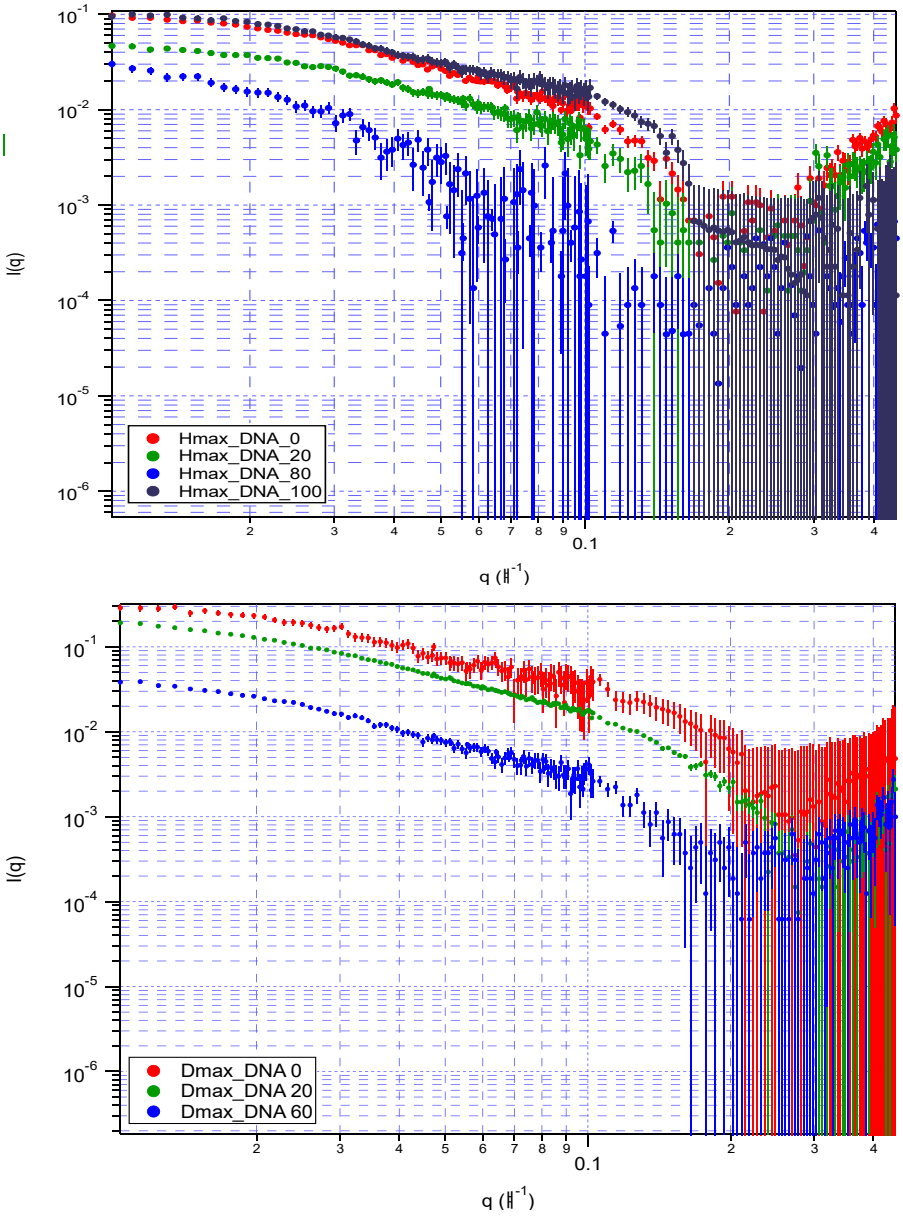

Figure 3 *Data of Max dimer and DNA at the two condition of protein (hydrogenated and partially deuterated) in different %D2O into buffer, those were acquired in D22@ILL*

However, the complex was unstable, and it was creating aggregates in particular at high concentration of D2O into the buffer, consequently we performed a Gel filtration before for each sample (Fig.2), and we took the partition where the complex was well formed and stable. Therefore, we measured, as it is shown in Fig.3 :

- hMax dimers in complex with 49-mer DNA duplex at 0%, 20%, 80% and 100% D_2O;
- $d_{68\%}$Max dimer in complex with 49-mer DNA duplex at 0%, 20% 60% (Match Point of 49-mer dsDNA)

References

1.Nie, Z. et al. Cell **151,** 68–79 (2012). **2.** Meyer, N. & Penn, L. Z. Nat. Rev. Cancer **8,** 976–990 (2008). **3.** Lin, C. Y. et al. Cell **151,** 56–67 (2012). **4.** Albihn et al. Adv. Cancer Res. **107,** 163–224 (2010). **5.** Soucek, L. et al. Genes Dev. **27,** 504–513 (2013). **6.** Zirath, H. et al. Proc. Natl. Acad. Sci. U.S.A. **110,** 10258–10263 (2013). **7.** Jung et al. Oncogene **36**: 1911-1924 (2017). **8.** Castell et al. (2018) Sci Rep. 8(1):10064. **9.** Nair & Burley (2003). Cell 112:193-205. **10.** Ferre-D'Amare et al (1993) Nature 363, 38-45. **11.** Wang et al (2017). Nucleic Acids Res 45: 2396-2407. **12.** Pursglove et al, BBRC 2004, 323(3:750-9. **13.** Kalkat et al (2018) Molecular Cell, *in press*. **14.** Anandapadamanaban et al (2016). Structure 24(8): 1311-1321.