Experimental report

09/12/2020

Proposal: 8-03-1003 **Council:** 10/2019

Title: Regulation of MYC-MAX DNA binding in cancer. Part 2: The MYC-MAX heterodimer

Research area: Biology

This proposal is a continuation of 8-05-442

Main proposer: Francesca CAPORALETTI

Experimental team: Francesca CAPORALETTI

Local contacts: Anne MARTEL

Samples: hMYC-dMAX
dMYC-hMAX

Instrument	Requested days	Allocated days	From	To
D22	4	3	18/09/2020	21/09/2020

Abstract:

The MYC protein is a central hub in cellular growth control and a key regulator of gene expression. To bind DNA, the c-terminal region of MYC must form a heterodimer with the protein MAX. Our recent proteomic mapping of the MYC interactome using mass spectrometry suggests that the conserved MYC regions MBIIIb and MBIV functionally collaborate with the bHLHZ region in regulating tumorigenicity. Crystal and NMR structures of MYC-MAX have to date only included the core DNA-binding bHLHzip rmotif, thus structural contributions by flanking regions remain unknown. As a first step towards investigating the full MYC-MAX-DNA assembly we have studied MAX-MAX-DNA by SANS, where our data suggest that flanking MAX regions co-fold with its bHLHZ both alone and on DNA. We now aim to investigate the DNA-bound MYC-MAX heterodimer, including regulatory regions N-terminal to the MYC bHLHZ region. By strategic deuteration we aim to derive the separate structured envelopes of MYC and MAX to elucidate the degree of co-folding. Better understanding of the MYC-MAX structure will significantly enhance development of cancer therapeutics targeting this complex as well as its regulatory interactors.

Regulation of Myc-Max DNA binding in cancer. Part 2: The Max-Myc heterodimer

Francesca Caporaletti, Vamsi K. Moparthi, Bjorn Wallner. Anne Martel, Maria Sunnerhagen

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).

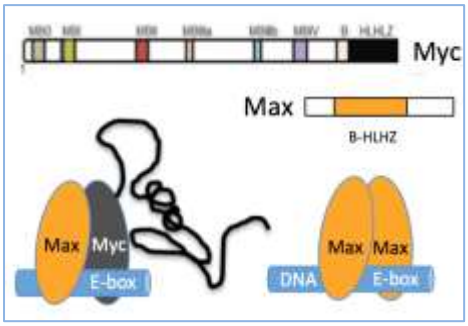


Figure 1 *Myc and Maxsequences and DNA binding*

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

A detailed description of the experiment We recorded SANS small and large angle data to establish the respective inter-component distances and topology of Max-Myc binding to its corresponding E-box DNA. To resolve the complex components, we will exploit 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 and uniformly protonated hMyc. 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.

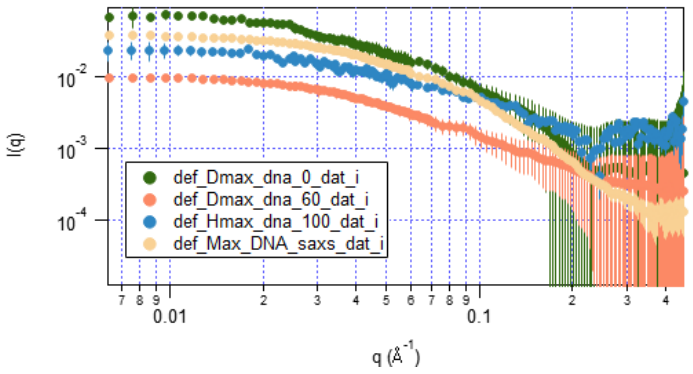
The instrument, beamtime and sample environment We measured on D22 using instrumental setups of 2 and 8 meters that enable to cover both the Guinier-range (radius of gyration) and the wide-angle range thoroughly (solvent-subtraction, medium-range information) for this relatively small complex (MW 100 kDa). We measured at 10°C.
In the current application we measured the following complexes with the SEC-SANS:

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

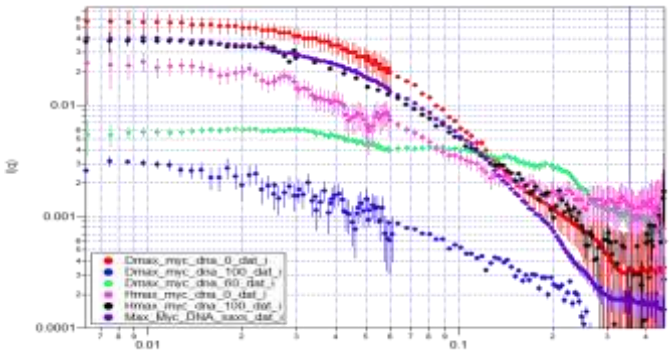
- d_{68}Max-hMyc dimer in complex with 49-mer DNA duplex at 0%, 60% (Match Point of 49-mer dsDNA), and 100% D_2O (Match Point of $d_{68\%}$Max);
- hMax-hMyc dimer in complex with 49-mer DNA duplex at 0% and 100% D_2O;
- The corresponding reference samples (boron, empty quartz cuvette, water, buffer)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

Furthermore, we have also acquired in SEC-SAXS at BM-29 both the complexes.

Data Analysis: As a first step we did the data reduction using GRASP and the IGOR macro NCNR. The reduced data for the two different complexes are in figure 2 and 3.



2 Reduced data od Max homodimer with the DNA. the data are acquired at D22 at ILL and BM29 at ESFR.



3 Reduced data of Myc-Max homodimer with the DNA. The data are acquired at D22 at ILL and BM29 at ESFR

From a preliminary data analysis, we have found the dimension and the shape of the two complexes. Both of them presents a cylindrical shape and both of them presents similar dimensions: a radius of 16 A and a length of 140 A. But for the different data set in particular for the heterodimer Myc-max the length can change significantly, that could mean that the flanking regions are flexible and they tend to have different shapes, but we haven't noticed any change in the radius so probably the flanking regions stay under the DNA.

We will refine the data analysis, using mainly the ATSAS suite of software (DAMMIN and MONSA for *ab initio* shape reconstruction) and Rosetta and Modeller to describe the dynamic of the system by a structural ensemble.

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.