# Experimental report

14/02/2017

**Proposal:** 8-03-870 **Council:** 4/2016

**Title:** Assembly of the ternary Brat-Pum-hb mRNA protein-RNA complex during embryonal development

**Research area:** Biology

**This proposal is a new proposal**


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**Samples:** Protein-RNA complex Brat-Pum-NRE1/2-NRE2 mRNA
Protein-RNA complex Brat-Pum-NRE2 mRNA
Protein-RNA complex Brat-Pum-NRE1-NRE2 mRNA

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| D22 | 3 | 1 | 10/07/2016 | 11/07/2016 |


## Abstract:

RNAs and their interaction with RNA-binding proteins (RBPs) play a central role in the regulation of gene expression. Thus far the focus has mainly been on single RNA binding domains (RBDs) and the mechanism of interaction with their RNA target. However, specificity and affinity is often low for single RBDs to explain their biological function. Therefore, it is essential to explore how general but distinct RBPs bind together cooperatively to increase affinity and specificity to their cognate mRNA targets. Recently, we successfully combined X-ray crystallography, NMR and small-angle X-ray and neutron scattering to determine the structure of a ternary multi-protein RNA complex. In this new example here, we aim to determine the structure of another ternary complex consisting of two distinct RNA binding proteins. The NHL domain of Brat and the Pum-HD domain of Pumilio bind to hunchback mRNA to ensure proper embryonal development in flies. Both proteins have orthologs in humans and mutations in Brat orthologs TRIM2 and TRIM3 are linked developmental disorder in vertebrates. A detailed picture of the binding mechanism would extend our understanding of the protein-RNA recognition code.

Experimental report for experiment 8-03-870:

# Assembly of the quaternary Brat-Pum-Nanos-*hb* mRNA protein-RNA complex during embryonal development

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The aim of the experiment was to obtain small-angle neutron scattering data to employ contrast matching of subunit-selectively perdeuterated protein-RNA complex samples. The complex of interest is the Brain tumour (Brat) NHL domain bound to Pumilio homology domain of Pumilio and *hunchback* mRNA, which forms a ternary complex. Each protein's structure is known, even while bound to its cognate RNA sequence derived from the *hunchback* mRNA. The binding of both proteins to mRNA is essential during early development of *Drosophila* embryos, and the understanding of complex assembly on a molecular level will shed light on orthologous protein samples in humans, involved in neuronal development (TRIM-NHL proteins TRIM2 and TRIM3).

Nothing is known about how both proteins recognize the RNA in concert. Due to the size of the complex, nuclear magnetic resonance spectroscopy (NMR) can only provide very sparse restraints and therefore it has to be complemented with small angle scattering data. Also, crystallization of this complex was so far not possible. Contrast matching of subunit-selectively perdeuterated samples by measuring in varying $D_2O$ concentrations (0 %, 42 %, and 70 %) will enable localization of each component within the overall complex.

The data acquired at ILL in summer 2016 was of excellent quality, and we were able already without NMR to derive a three-dimensional model of this ternary complex (Figure 1).

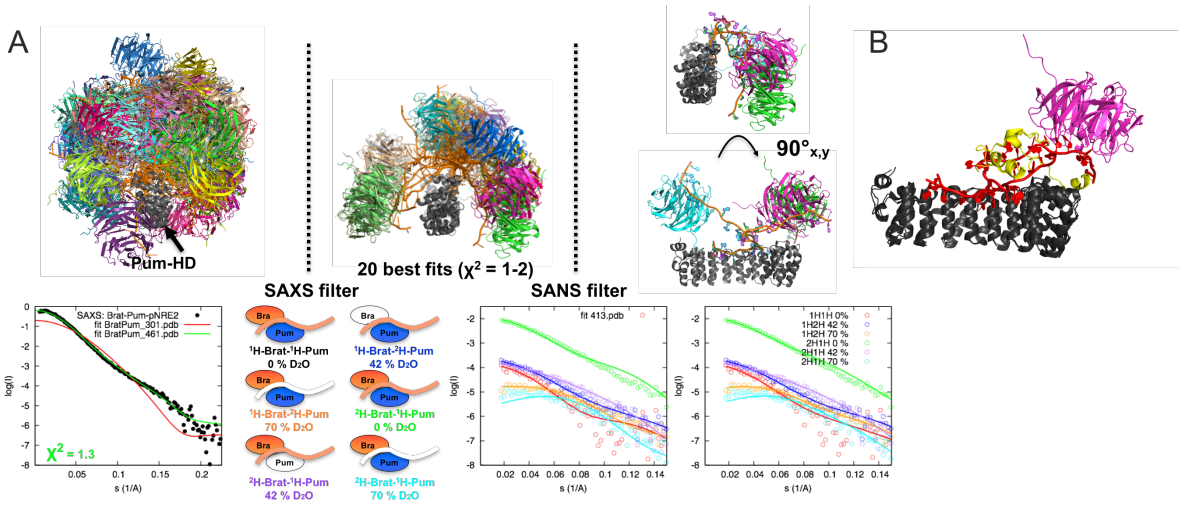


Figure 1: A. Based on input structure of single Brat-NHL and Pum-HD bound to RNA, 1000 structures were calculated with fixed RNA-protein restraints. The unbound RNA nucleotides were randomized, resulting in a large conformational space all structures can occupy. A SAXS filter was applied to filter out all structures with a $\chi^2 > 2$. The conformational space was reduced and structures with a more compact domain arrangement remained. After applying a similar SANS filter, where remaining structures needed to fit the SANS data satisfactorily ($\chi^2 < 1.5$

$\chi^2_{min}$) , only three structure are left. B. Superimposition of our best Brat-Pum-RNA model with the Pum-Nanos-RNA structure from Weidmann et al. Nanos seems to bridge the gap between Pum and Brat.

The aim is now to obtain paramagnetic relaxation enhancements by NMR to validate the orientation of each component with respect to each other. Furthermore, we plan to acquire more SANS data to extend the complex with Nanos and with longer RNA to assemble a 2:2:2:1 complex (Brat-Pum-Nanos-RNA). For this, we plan to submit another proposal for 2017.