Experimental Report

Proposal: **8-04-687** **Council:** 10/2012

Title: RNA Dynamics-Function Relations inViroids and Ribosomes

This proposal is continuation of: 8-04-680

Researh Area: Biology

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Samples: ribosomes from H. marismortui and T. thermophilus, plant viroid

Instrument	Req. Days	All. Days	From	To
IN5	4	2		
IN16	6	4	03/06/2013	07/06/2013
IN13	8	6	25/06/2013	01/07/2013

Abstract:

RNA displays a rich diversity of properties, which also led to the formulation of the RNA World hypothesis in origin of Life studies, and the nucleic acid field is flourishing with the discovery and characterisation of more and more vital functions involving RNA molecules. In the context of the dynamics-function relation, the proposal is to characterise by neutron scattering the dynamics of RNA molecules with different structures, adapted to different biological function and activity under different environmental conditions: a viroid that may represent a vestige of the RNA world and ribosomal subunits from thermophiles and halophiles.

Report on experiment 8-04-687 on IN16 and IN13 (June 2013) and on experiment CRG 2027 on IN13 (July 2013)

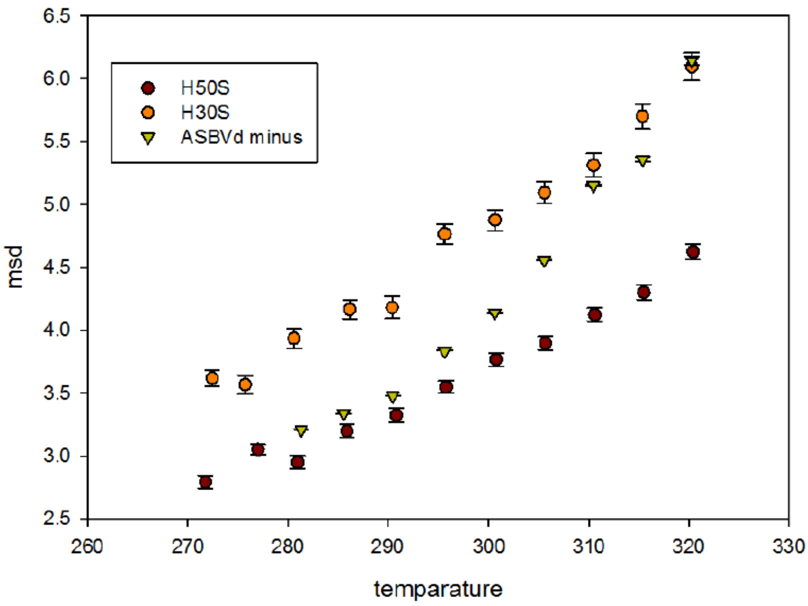
RNA Dynamics-Function Relations in Viroids and Ribosomes

Background. RNA displays a rich diversity of properties and the nucleic acid field is flourishing with the discovery and characterization of more and more vital functions involving RNA molecules. In the context of the dynamics-function relation, the proposal is to continue the characterization by neutron scattering of RNA dynamics in different systems with various structures, adapted to different biological function and activity under several environmental conditions: a viroid that may represent a vestige of the RNA world and ribosomal subunits from thermophiles and halophiles. Our driving hypothesis is that further to structure being adapted to function, appropriate molecular motions are also necessary. Dynamics is very sensitive to environment, which explains why under certain conditions a well-structured system is not active. Dynamics refers to the forces that stabilize structure and define motions. In this sense, we proposed that adaptation occurred *via* evolutionary selection of dynamics [1].

Previous results. Initial experiments were performed on positive and negative strand viroid under different Mg++ concentrations and ribosomal subunits from *Thermus thermophilus* and *Haloarcula marismortui*. First results were reported in the experimental report CRG 1864. Mean square fluctuations and corresponding force constants revealed significant differences in dynamics between the various ribosomal subunits, which are now being analyzed in the context of their activity profiles and extremophile adaptation. In the case of the viroid, data showed the dry state to be very resilient as expected, while the hydrated state was found to be significantly 'softer' in comparison with measurements on other RNA samples or the ribosomal subunits.
In the experimental report 8-04-680 we reported on investigations on the Avocado Sunblotch Viroid (ASBVd), which was studied on the instruments IN5, IN6 and IN13. Samples were examined for both polarities of ASBVd, and different sample states: dried pellet, pellet hydrated by same amount of water (w(H_2O)=w(pellet)), and hydrated powder containing half the amount of water (wH_2O)=1/2w(pellet)), without added magnesium ion and with, in the concentration $^1Mg^{2+}$ per 2 nucleotides.

Current experiments. The investigation was so far concentrated on ribosomal subunits under standard buffer conditions for each species and only viroid dynamics was measured as a function of solvent environment. However, a wide variety of solvent conditions have already been characterized for ribosomal subunits and associated with structural and functional integrity [2], on top of their adaptive characteristics that make them fit to their physiological environment. We now measured 30S and 50S subunits from *H. marismortui* (in which salt effects are the most extreme) under two solvent conditions each, chosen for their functional effects, with the aim to correlate dynamics and function. For viroid: Differences in dynamics were found in the initial experiments between plus and minus RNA strands and with respect to Mg++ concentration. Viroids travel from outside (NaCl environment) to inside (KCl) cells, where they are active. To compare with ribosomal RNA, which is active in KCl and not in NaCl, we wanted to measure the respective effects of NaCl and KCl solvents on viroid plus, minus strand dynamics. However, due to a lack of enough beam time, we only measured the viroid minus strand so far.

The figure shows an example of mean square displacements extracted from elastic incoherent neutron scattering data taken on IN16. All three samples display similar slopes (resilience) at the lower temperatures. The apparent transition in the viroid to a softer structure at 290K should be confirmed. Also, the significantly higher MSD for the ribosomal 50S subunit should be investigated further by calculating for instance the force constant.



To complete this thesis work with reliable function/dynamics results, we need to do further measurements on samples under complementary salt conditions (in KCl and NaCl under high concentration with careful hydration control) on the two time scales of IN13 and IN16B, respectively.

References

[1] Tehei M, Franzetti B, Madern D, Ginzburg M, Ginzburg BZ, Giudici-Orticoni MT, Bruschi M, Zaccai G (2004), Adaptation to extreme environments: macromolecular dynamics in bacteria compared in vivo by neutron scattering. EMBO Rep 5:66-70.

[2] Yonath, A. (2002). THE SEARCH AND ITS OUTCOME: High-Resolution Structures of Ribosomal Particles from Mesophilic, Thermophilic, and Halophilic Bacteria at Various Functional States. Annu. Rev. Biophys. Biomol. Struct. 31, 257-73.