

***Computational prediction and
experimental analysis
of RNA structures***

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Double-stranded RNA

dsRNA appears to be a fundamental component of life:

Since long time it is known that the genome of retroviruses, such as the hepatitis C and human immunodeficiency viruses, consists of dsRNA.

Many cases of hairpins formed by local base-pairing of dsRNA have been analyzed.

dsRNA has been demonstrated to interact specifically with a wide spectrum of proteins such as nucleases, helicases, and polymerases.

The recent discovery of the role of dsRNA in the mechanism of RNA interference has renewed interest in the determination of RNA secondary structure.

RNA secondary structure prediction tools

In 1986 Turner reported 11 thermodynamic parameters for prediction of RNA duplex stability.

In 2003 Zuker developed a dynamic programming algorithm to predict nucleic acid folding and hybridization, using Turner free energy parameters and constructed the package mfold.

In 2003 Knudsen introduced the tool Pfold RNA secondary structure prediction using stochastic context-free grammars.

In 2009 Lu et al. improved RNA secondary structure prediction by maximizing expected pair accuracy.

In 2010 Bellaousov and Mathews introduced Probknot for prediction of RNA secondary structure including pseudoknots.

In 2010 Lou and Clot used Wang-Landau sampling to calculate thermodynamics of RNA structure.

mfold

The portal for the mfold web server is:
<http://mfold.rna.albany.edu/?q=mfold>.

The server provides access to both RNA and DNA folding and hybridization software.

The length limit is currently 8000 nt.

The folding temperature ranges between 0 and 100 ° C.

Na⁺ concentration can vary between 0.01 and 1 M, Mg⁺⁺ concentration between 0 and 0.1 M.

It is possible to introduce constraints to force or prohibit a string of consecutive base to pair.

A parameter controls how many foldings will be computed and how different they will be from one another.

The thermodynamic outputs include estimated free energy, enthalpy, entropy and T_m .

Single-molecule techniques

Confocal microscopy: a laser excites, through the objective of a microscope, only a small area.

Total internal reflection fluorescence microscopy (TIRFM): the exciting laser is reflected through a prism, and the emission goes through the objective.

Optical tweezers experiments: a laser provides an attractive or repulsive force depending on the refractive index.

Fluorescence resonance energy transfer (FRET): a donor chromophore transfers energy to an acceptor chromophore through non-radiative dipole-dipole coupling.

Atomic force microscopy (AFM): a cantilever is used to scan a surface to produce an image.

Atomic Force Microscopy

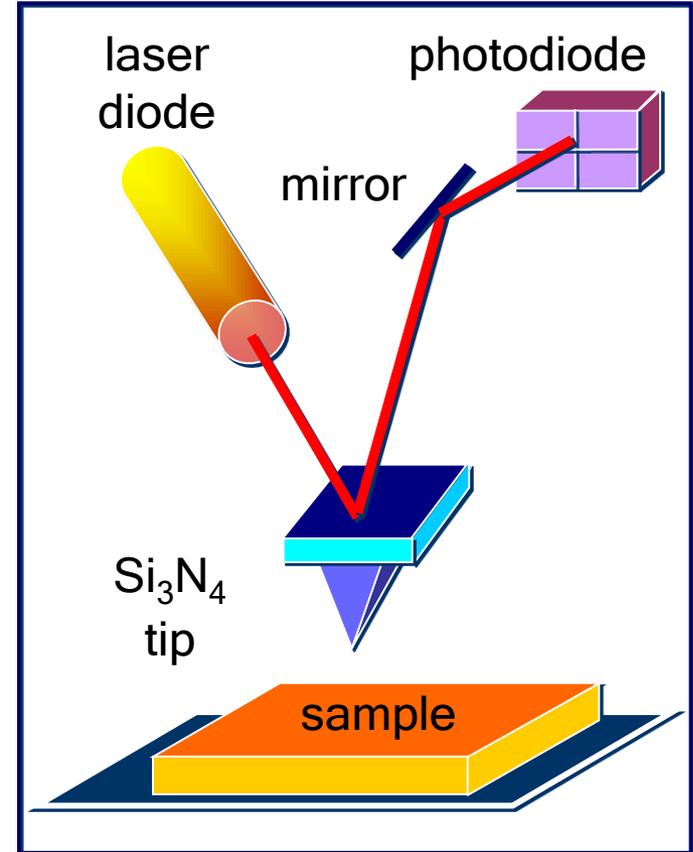
AFM is a very high-resolution type of scanning probe microscopy, with demonstrated resolution in the order of fractions of nanometer.

The information is gathered by "feeling" the surface with a mechanical probe. Piezoelectric elements that facilitate tiny but accurate and precise movements on (electronic) command enable the very precise scanning.

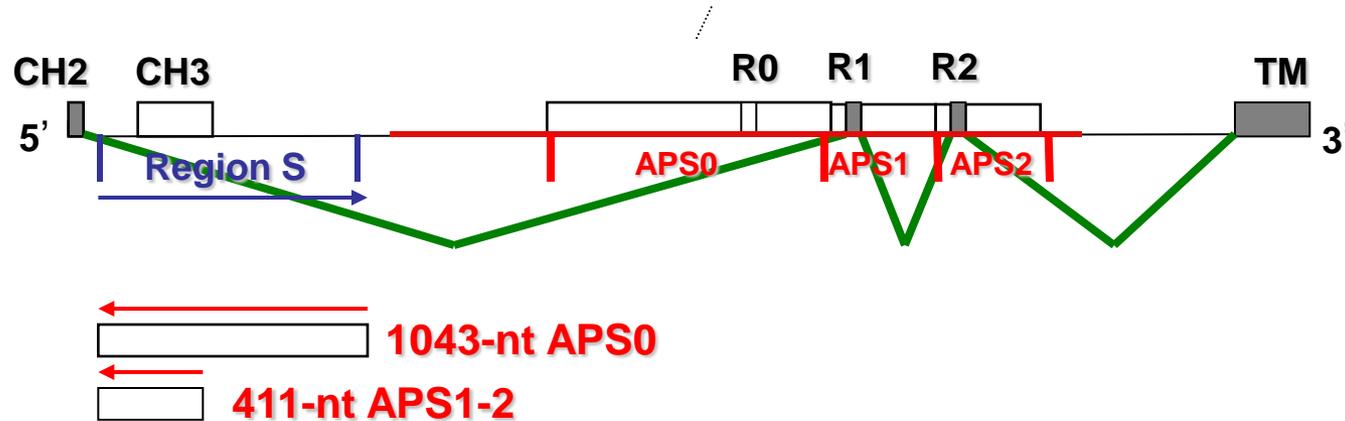
The AFM provides a three-dimensional surface profile.

Samples observed by AFM do not require any special treatments.

Most AFM modes can work perfectly well either in ambient air or even in liquid environment. This allows us to study biological macromolecules and also living organisms.



Splicing of pre-mRNA encoding the membrane-bound form of Immunoglobulin heavy chain in Antarctic teleost



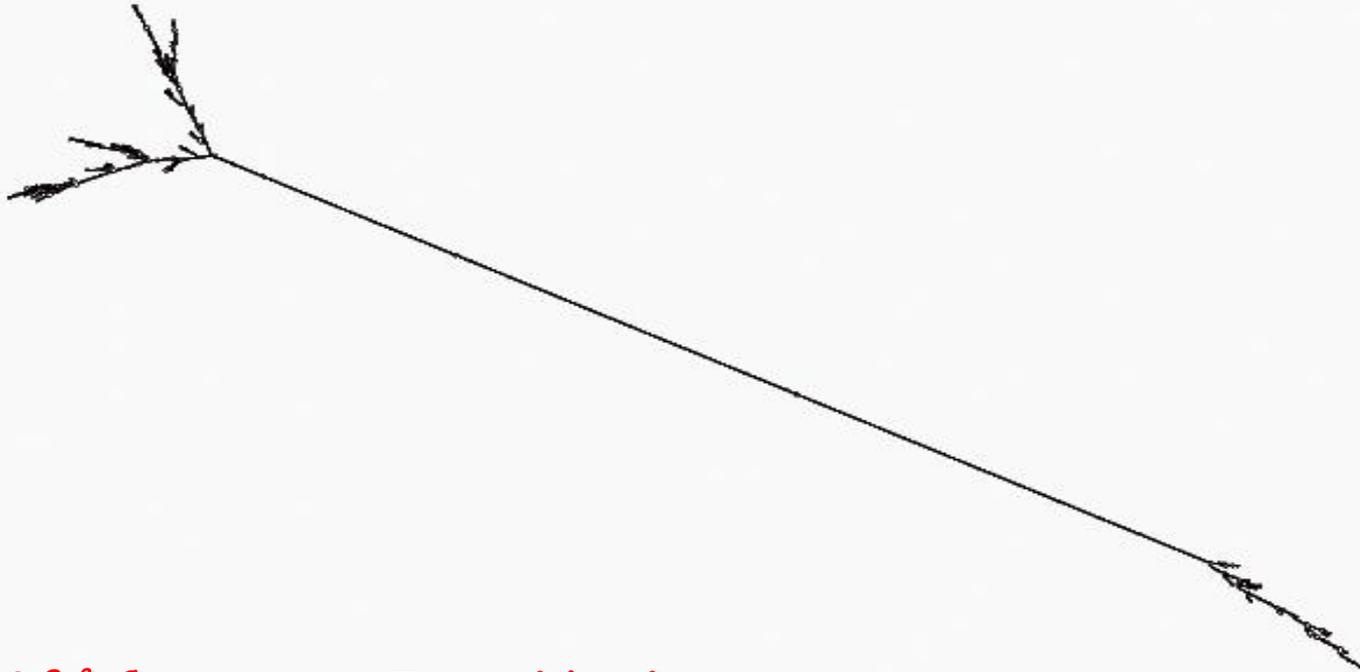
Synthesis of an RNA molecule corresponding to the genomic region

RNA was synthesized from a recombinant 4567nt-DNA template of the Antarctic teleost *Chionodraco hamatus* using the RiboMAX Large Scale RNA Production System (Promega).

The DNA template was linearized by digestion with *Hind* III prior to *in vitro* transcription.

The transcription reaction was carried out under the control of the T7 RNA polymerase promoter.

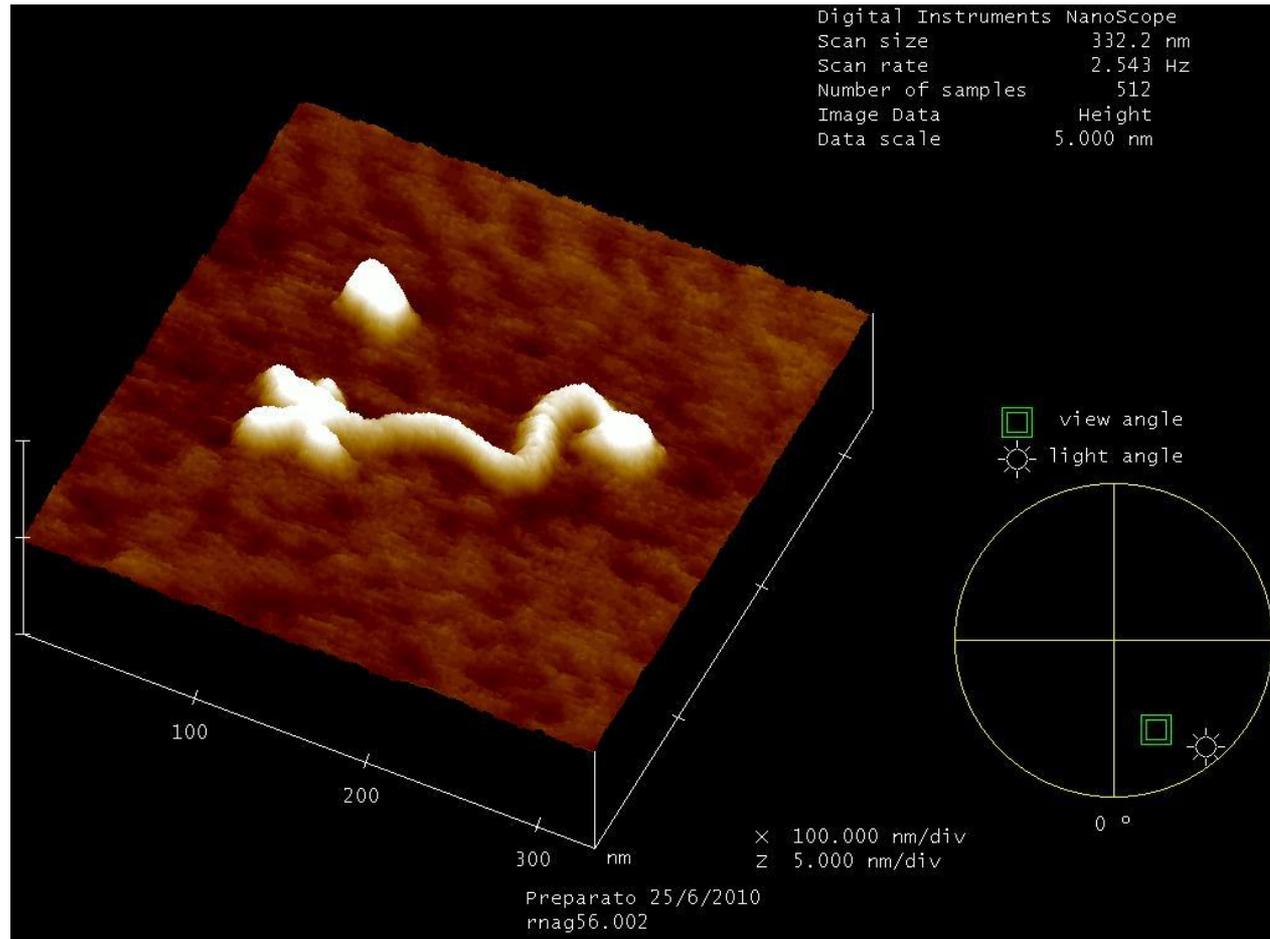
mfold structure prediction of the synthetic RNA at 0 ° C



$$\Delta G^{0^{\circ}C} = - 3835 \text{ Kcal/mol}$$

$$\Delta G^{0^{\circ}C} (\text{S/APS0}) = - 2498 \text{ Kcal/mol (65\%)}$$

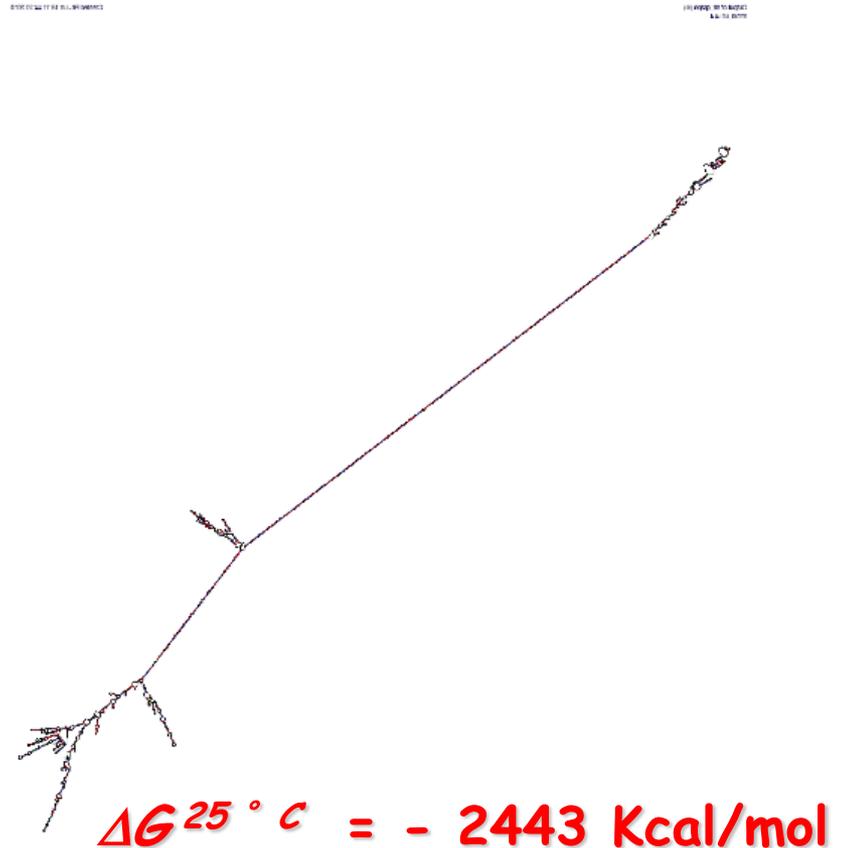
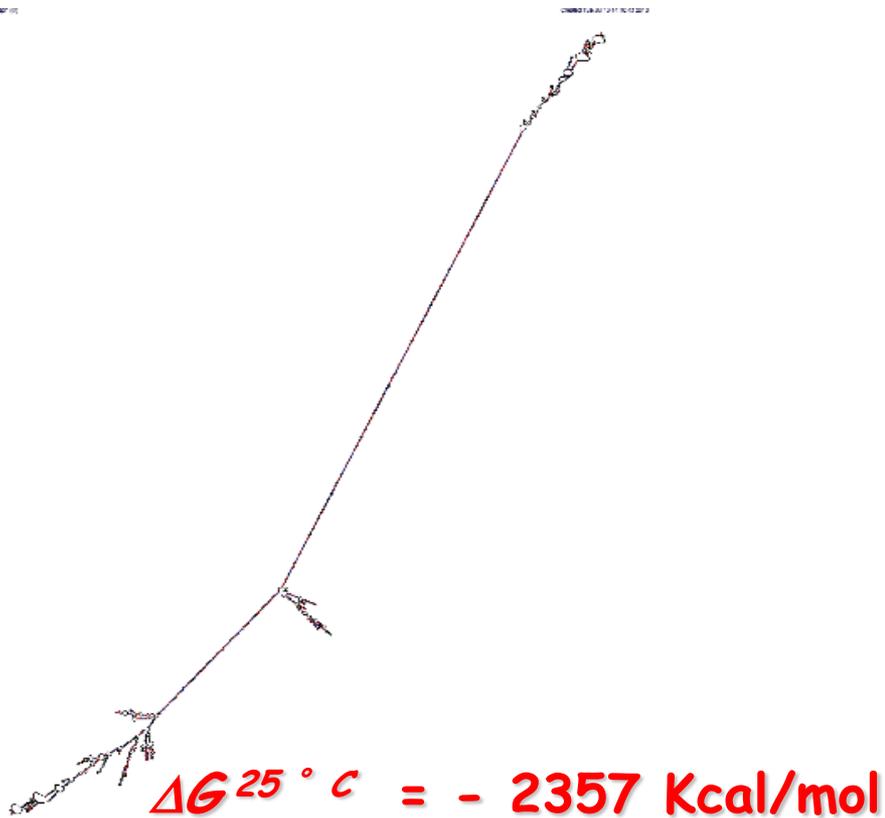
AFM image of the synthetic RNA folded on ice



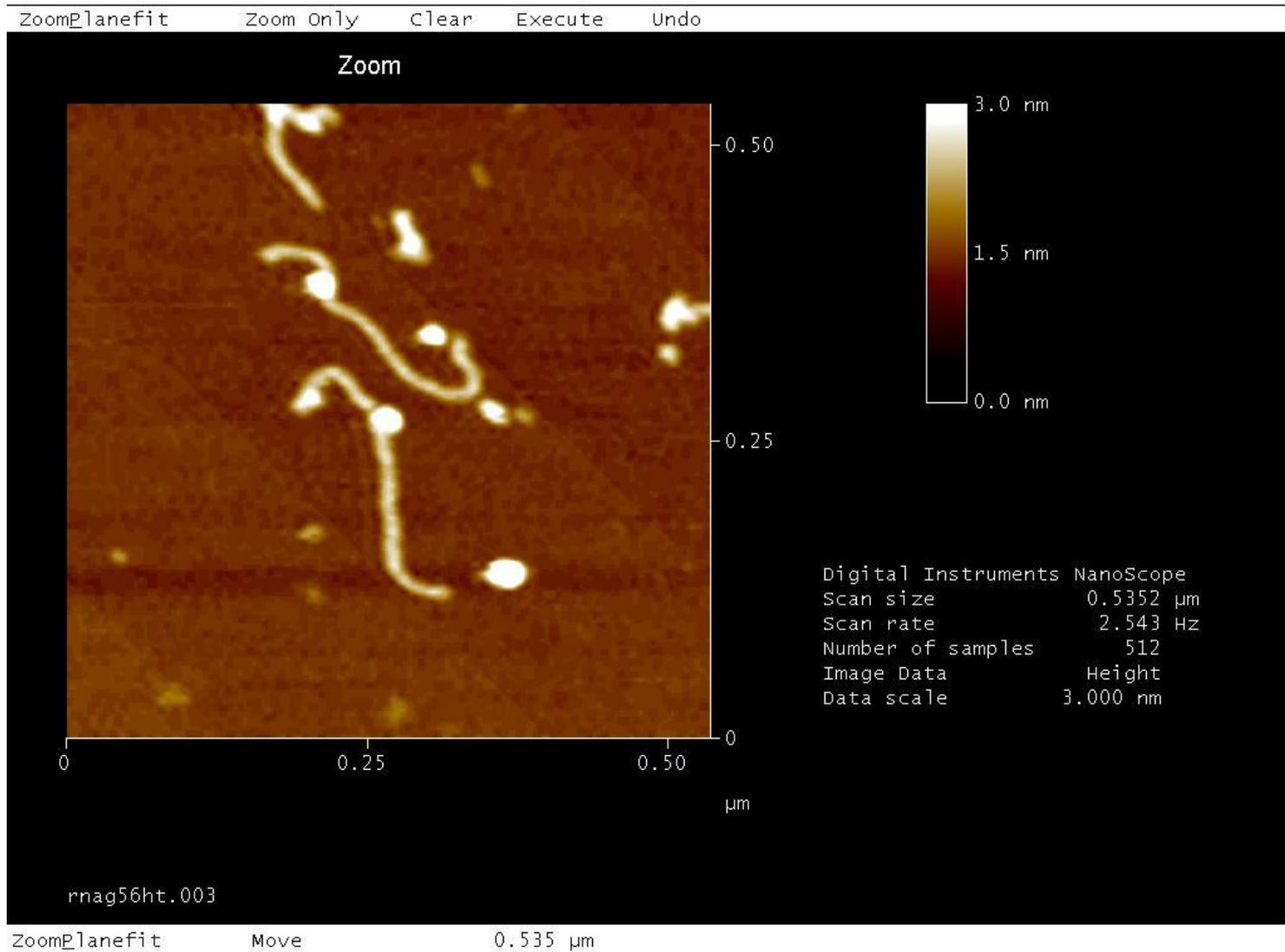
Length: 244 ± 16 nm

Calculated bp number: 841 ± 55

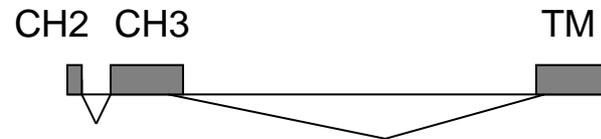
mfold structure prediction of the synthetic RNA at 25 ° C



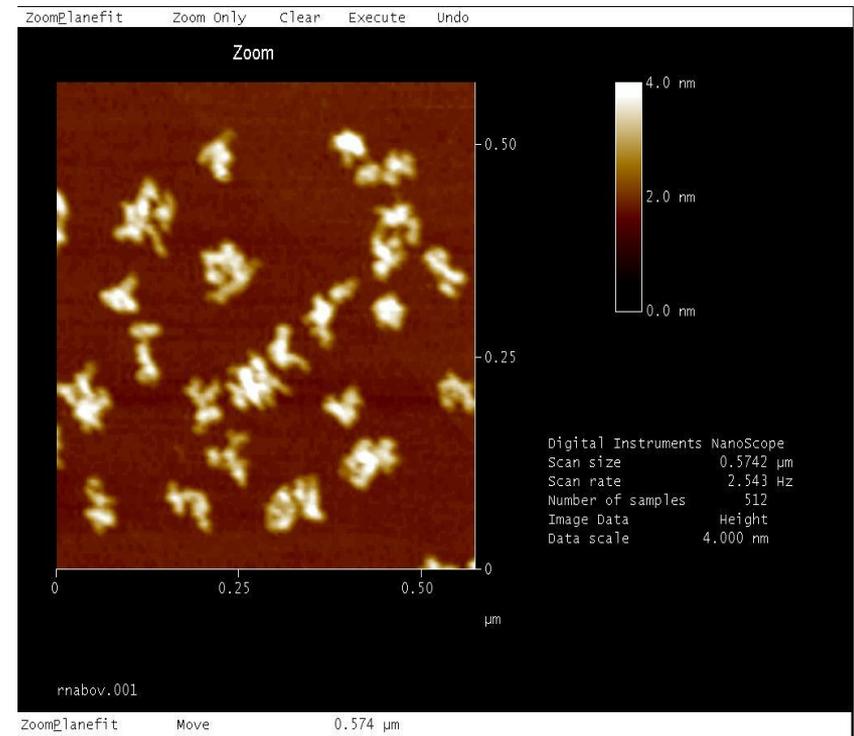
AFM image of the synthetic RNA folded at 25 ° C



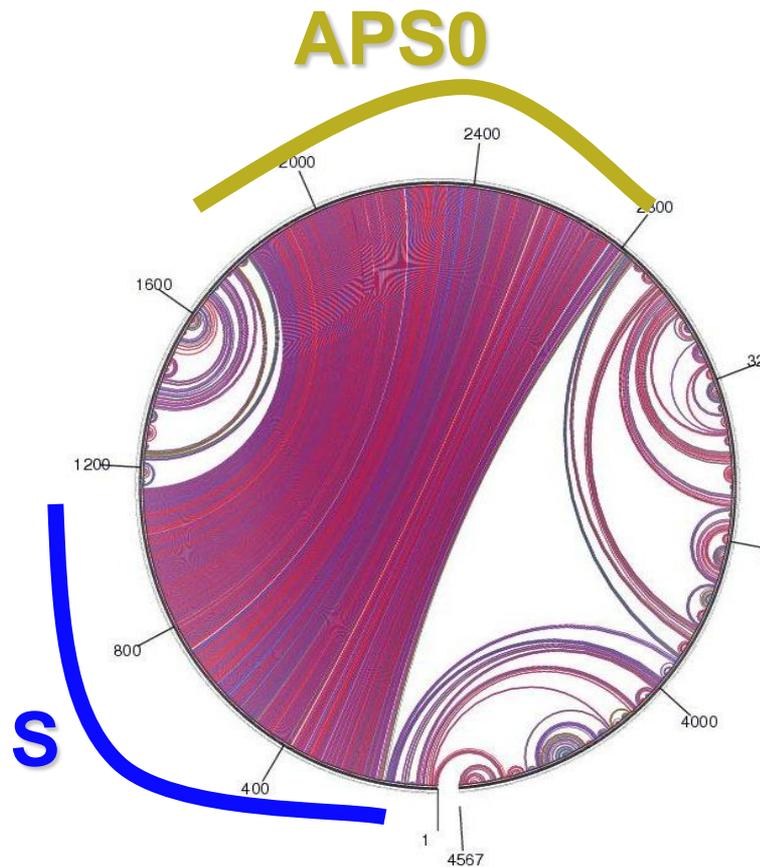
Splicing of pre-mRNA encoding the membrane-bound form of Immunoglobulin heavy chain in non-Antarctic teleost



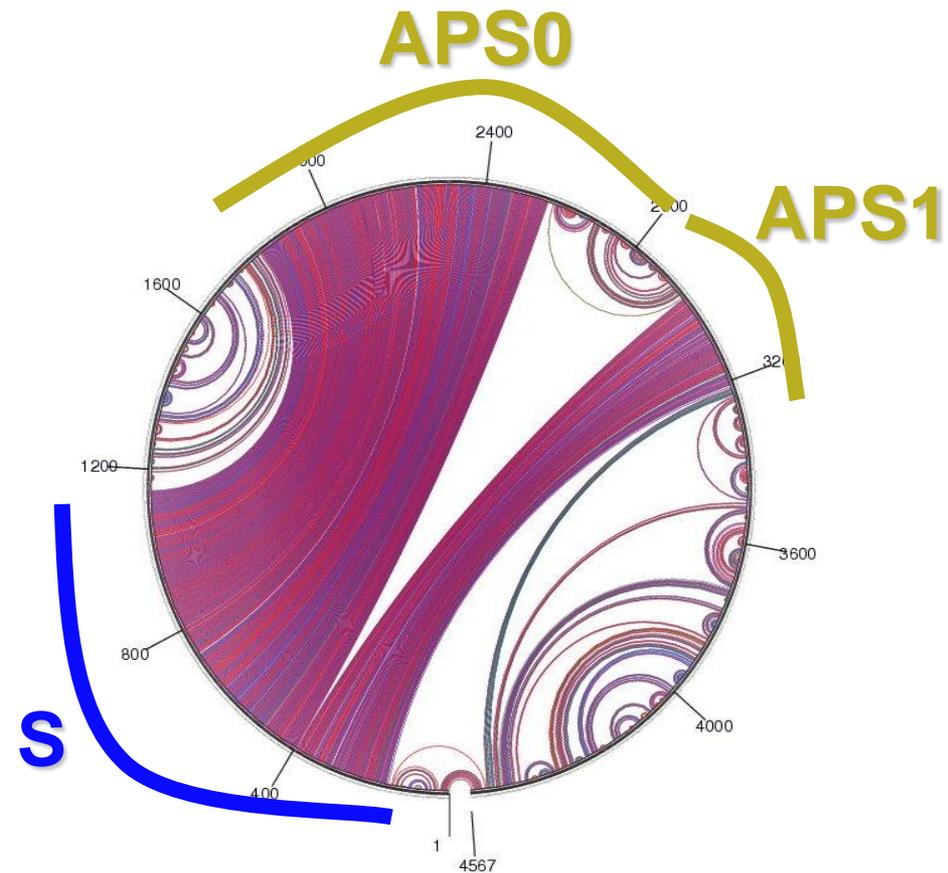
$$\Delta G^{25^\circ C} = -1073 \text{ Kcal/mol}$$



Different pairing of anti-parallel regions of Antarctic teleost pre-mRNA at different temperatures

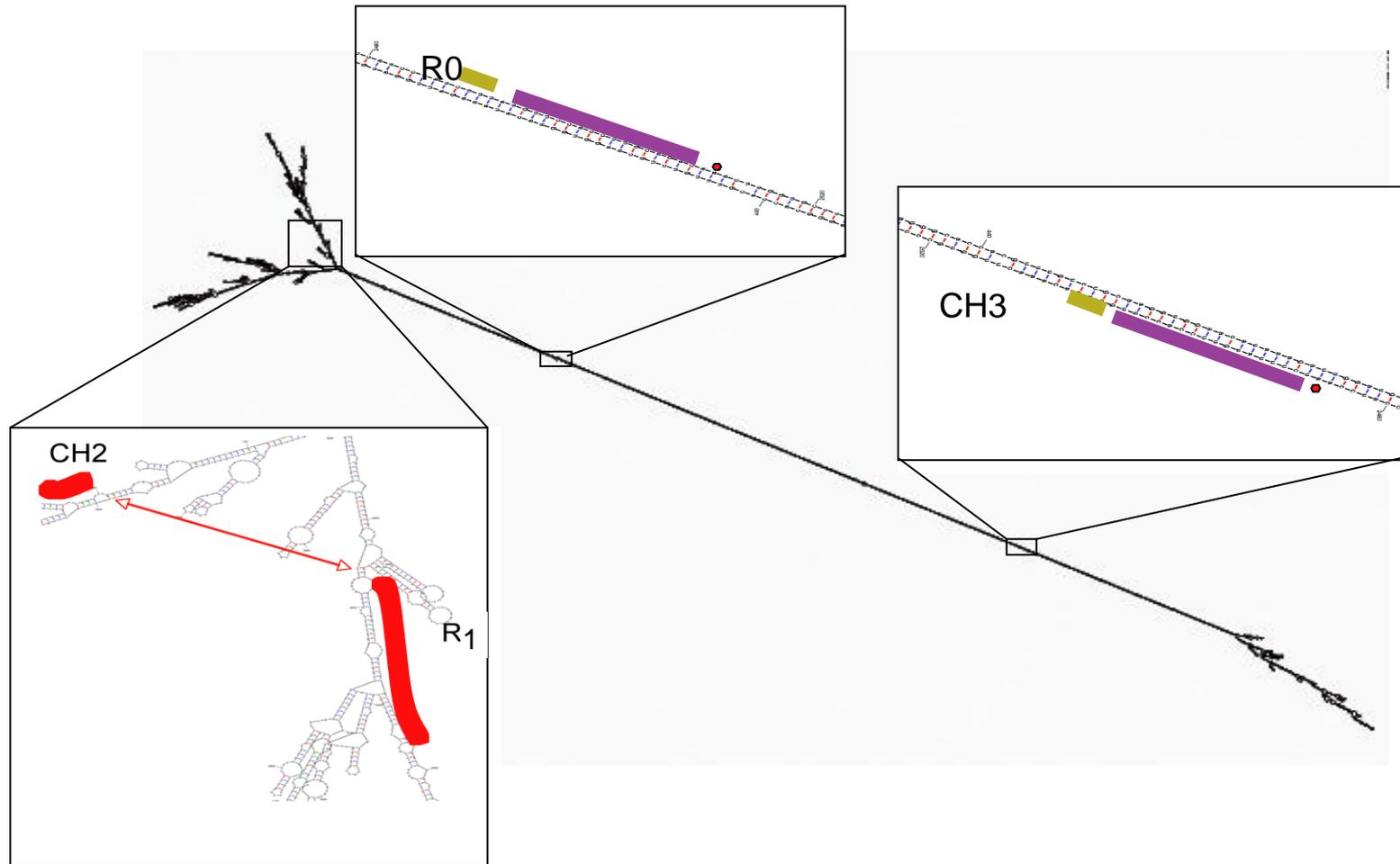


$$\Delta G^{0^{\circ}C} = -3835 \text{ Kcal/mol}$$



$$\Delta G^{25^{\circ}C} = -2443 \text{ Kcal/mol}$$

The Antarctic teleost Ig splicing is driven by the temperature dependent pre-mRNA secondary structure



Conclusions

The structures of the Ig pre-mRNA computationally predicted, are in good agreement with those experimentally observed.

The predicted RNA structures as well as those experimentally observed show a strict dependence on the temperature.

The atypical Antarctic teleost Ig splicing is driven by the physiological temperature.