

Monitoring RNA Folding Intermediates using the DynaPro Plate Reader Plus

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RNA Folding

- Structures of folding intermediates of RNA are often poorly defined;
- Stable intermediates can often be isolated;
- Folding states change as a function of Mg^{2+} concentration;
- General perception is that folding intermediates are rich in secondary structure, but lack extensive amounts of tertiary contacts;
- Partially folded structures are dynamic and are unlikely to be crystallized;
- Small-angle X-ray scattering (SAXS), circular dichroism (CD) spectroscopy and molecular modeling has been employed to probe structural changes during RNA folding.

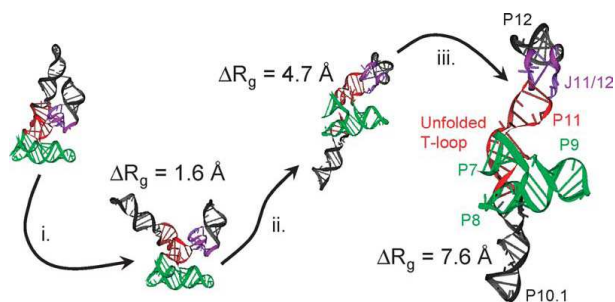
Goal of this Study

Can Dynamic Light Scattering probe changes in molecular size of RNA intermediates and yield similar information as SAXS?

Comparison of DLS to SAXS:

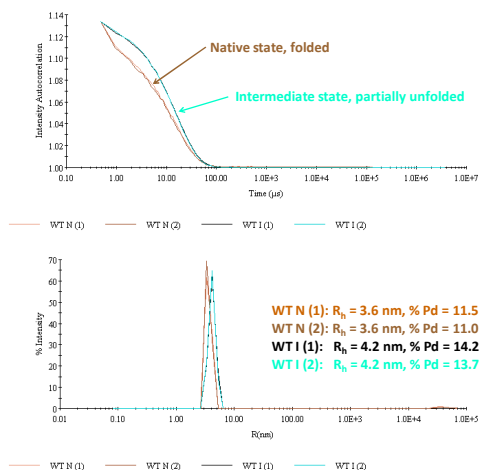
- SAXS measures the rms radius (Radius of gyration, R_g) while DLS measures the hydrodynamic radius (R_h);
- DLS is a non-invasive method (no structural damage due to high X-ray radiation);
- DLS is easy to use, readily available benchtop instrument vs. use of the beamline at the Advanced Photon Source at the Argonne National Laboratories;
- With the DLS Plate Reader, fast and automated screening of a wide variety of RNA mutants and buffer conditions is possible.

Modeling of RNA Unfolding



ΔR_g indicates the change in the rms radius observed by SAXS for the different folding states.

DLS Results for Wild Type (WT)

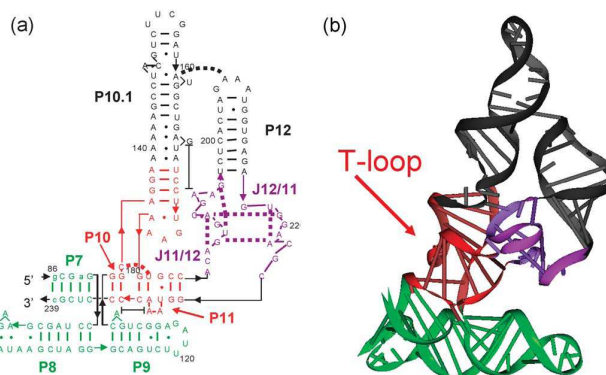


Comparison of DLS and SAXS Results

Sample	R_h (nm) DLS	R_g (nm) SAXS	R_g/R_h
WT N	3.6	3.3	0.9
WT I	4.2	4.2	1.0
$\Delta(I-N)$	0.6	0.9	-

Sample	R_h (nm) DLS	R_g (nm) SAXS	R_g/R_h
Double N	3.8	3.5	0.9
Double I	4.3	4.4	1.0
Double U	5.7	6.7	1.2
$\Delta(I-N)$	0.5	0.9	-
$\Delta(U-I)$	1.4	2.3	-

Crystal Structure of *Bacillus Subtilis* RNase P RNA

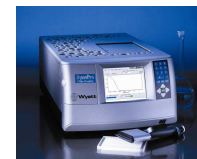


154 nucleotides, 50 kDa molar mass,
30-50 Å molecular size depending on folding state

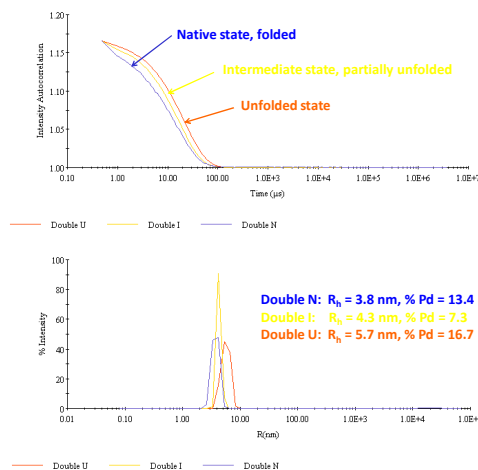
Experimental Conditions

Samples:

- Double Mutation, 0.3 mg/mL:**
 - unfolded state (U): 20 mM Tris, 0 mM $MgCl_2$
 - intermediate state (I): 20 mM Tris, 0.4 mM $MgCl_2$
 - native state (N): in 20 mM Tris, 10 mM $MgCl_2$
- Wild Type (WT), 0.36 mg/mL:**
 - intermediate state (I): 20 mM Tris, 0.4 mM $MgCl_2$
 - unfolded state (U): 20 mM Tris, 0 mM $MgCl_2$
- Sample Preparation:** All samples were filtered with a 0.02 μm Anotop syringe tip filter to remove large aggregates
- Sample volume:** 50 μL , 384 well microplate
- Measurement temperature:** 37°C.
- Measurement time:** 50-500 sec per well



DLS Results for Double Mutation (Double)



Conclusions

- Pronounced differences in R_h are observed for the different folding states of Wild Type and Double Mutation RNA.
- DLS results are consistent with results obtained by SAXS.
- The increasing R_g/R_h corroborates the reduced "compactness" of the RNA molecule during the unfolding process.
- The DynaPro Plate Reader is a fast and reliable screening tool to monitor RNA folding.