Photoregulation of DNA and RNA by Azobenzene

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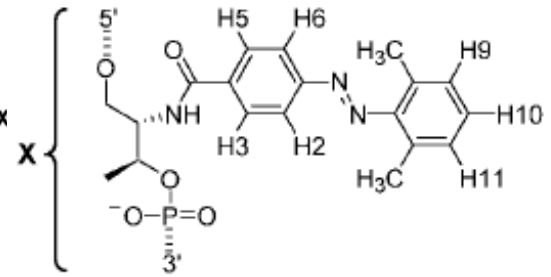
Theoretical Photochemistry 2019

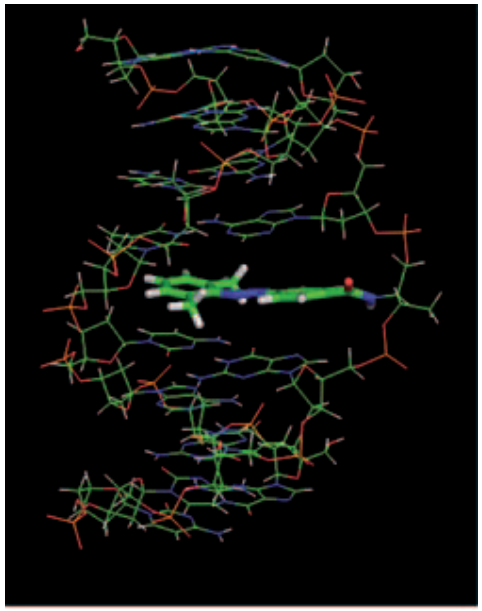
Azobenzene + DNA

- experiments by Asanuma & co: light-triggered conformational changes
- cis/trans melting temperatures are a sensitive function of substituents:
  - 2',6'-dimethyl-*trans*-azobenzene slightly stabilizing
  - 2',6'-dimethyl-*cis*-azobenzene destabilizing
  - p-^{i}Pr-*trans*-azobenzene destabilizing
  - p-^{i}Pr-*cis*-azobenzene slightly destabilizing

Duplex	Temp. [C]		ΔT
CXG/GC	Trans	Cis	
Native	47,7	47,7	
Azo	48,9	43,2	5,7
2',6'-Me-Azo	50,9	36,3	14,6

a) 5′-G^{1} C^{2} G^{3} A^{4}XG^{5} T^{6} C^{7} C^{8}-3′
3′-C^{16}G^{15}C^{14}T^{13} C^{12}A^{11}G^{10}G^{9}-5′

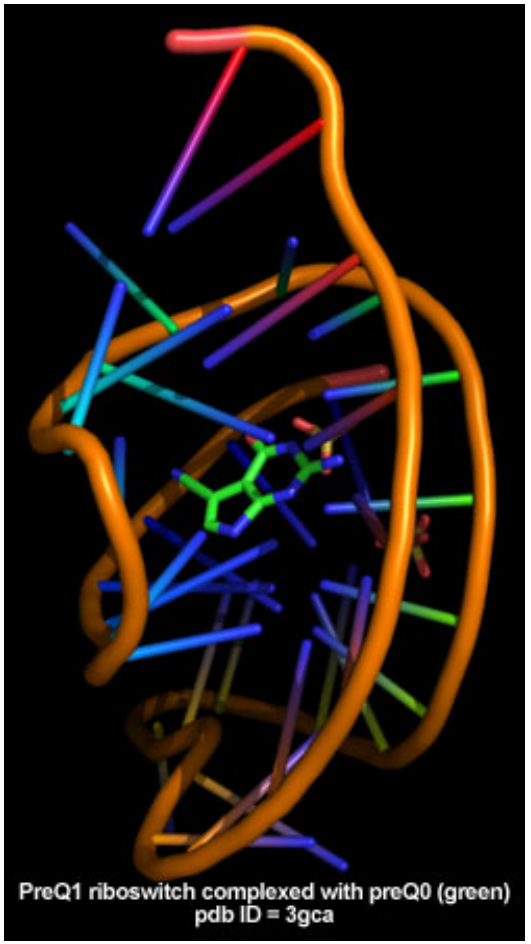




Asanuma and collaborators, Chem. Eur. J. 2009, Nucleic Acids Symposium Series 2007

RNA: e.g., Riboswitch-Ligand Complexes

- controlled riboswitch (de-)activation by small ligand species (e.g., metal ions, amino acids)
- recent works by Asanuma/Heckel: photoinduced activation by photoswitches (azobenzene, spiropyrane)
- characterization of RNA folding intermediates
- SFB 902 "Molecular mechanisms of RNA based regulation"



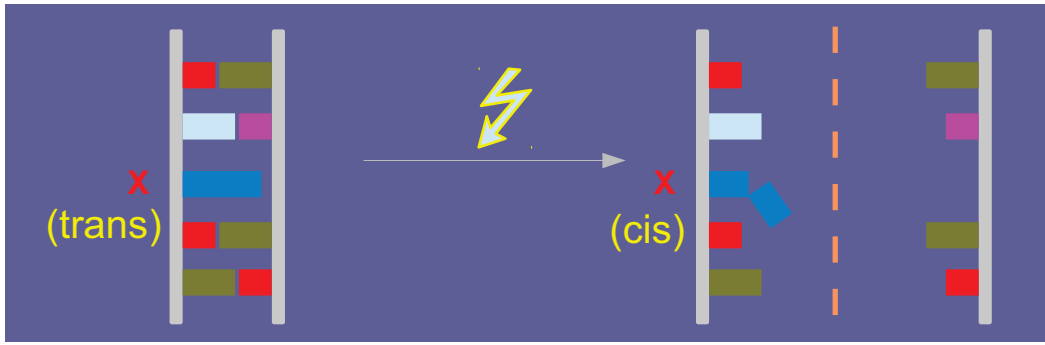
Spitale et al., J. Biol. Chem. 284, 11012 (2009)

How Does a Photoswitch Control DNA / RNA Unfolding?

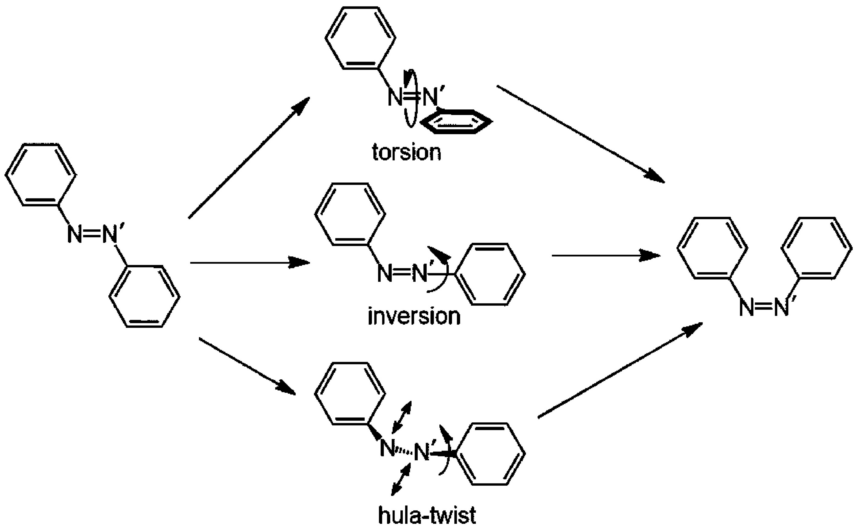
Combine four approaches:

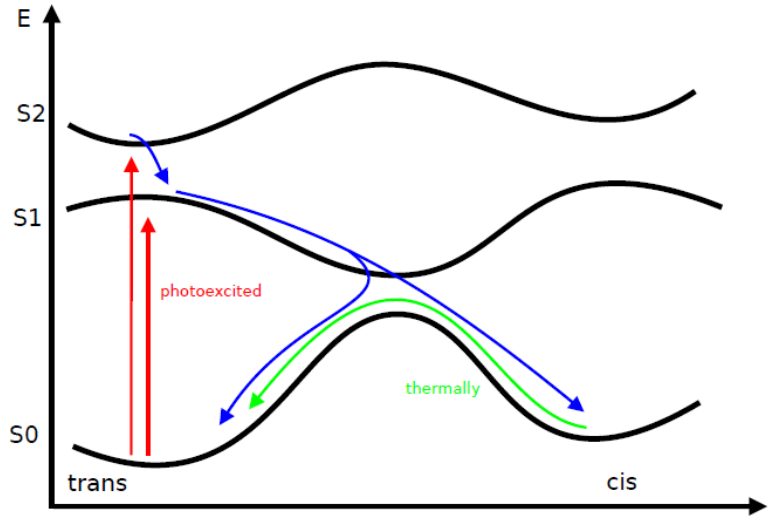
1. characterization of chromophore: QM (electronic structure)
2. ultrafast photoswitch dynamics (fs-ps scale): QM/MM + MD simulations including RNA
3. ps-ns time scale: MD
4. μs-s time scale: kinetic modeling: e.g., Markov State Models

QM = Quantum Mechanics
MM = Molecular Mechanics
MD = Molecular Dynamics



Azobenzene Switch





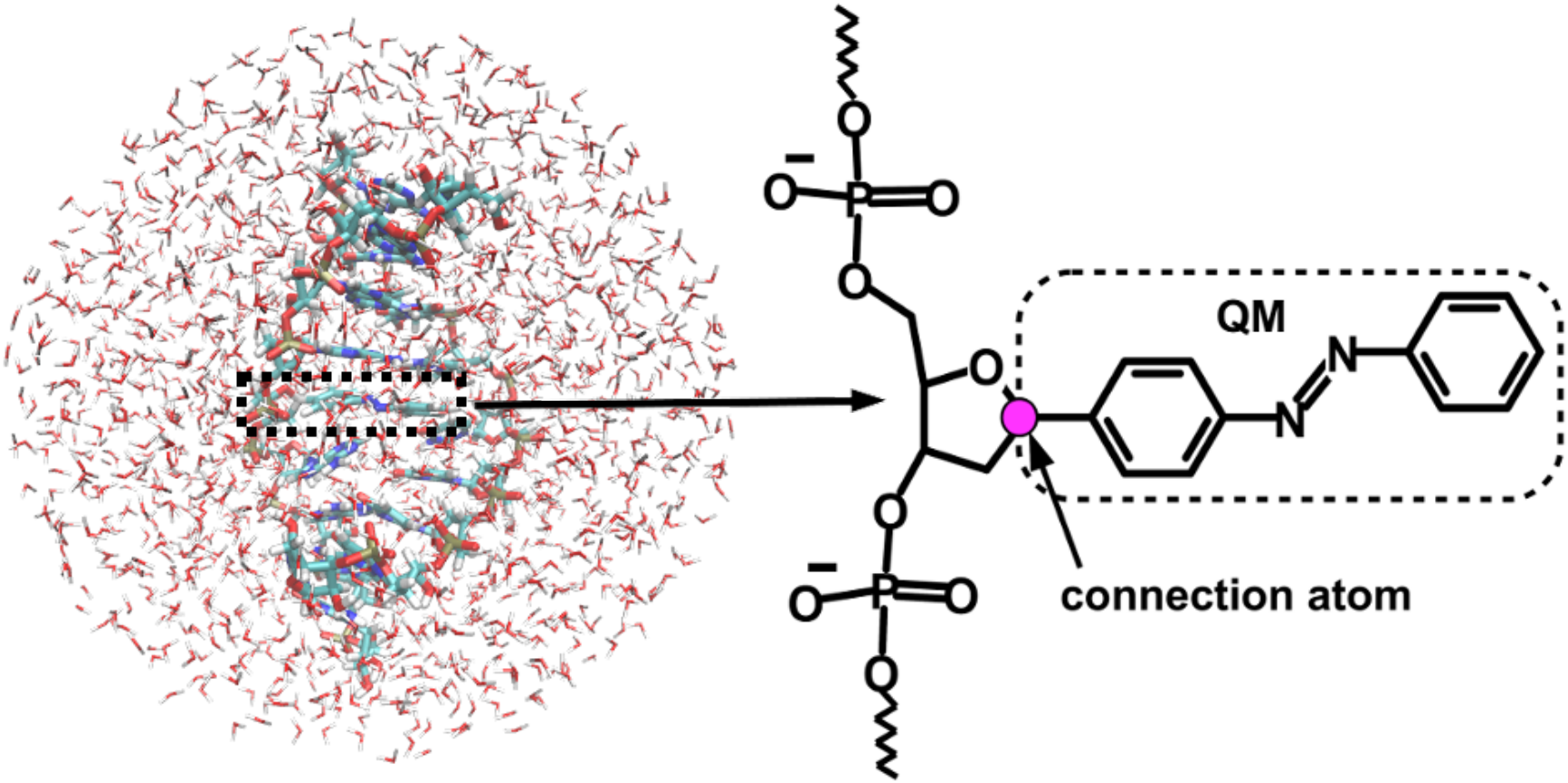
Quick et al., J. Phys. Chem. B, 2014, 118, 8756

- more precise picture: conical intersection ("photochemical funnel")
- ultrafast (fs-ps scale) decay of electronically excited states
- several competing pathways: isomerization, rotation, "hula-twist"
- picture can be modified by substituent effects, environmental constraints

"Femtosecond spectroscopy reveals huge differences in the photoisomerization dynamics between azobenzenes linked to polymers and azobenzenes in solution", Bahrenburg et al., PCCP 16, 11549 (2014)

QM/MM simulations

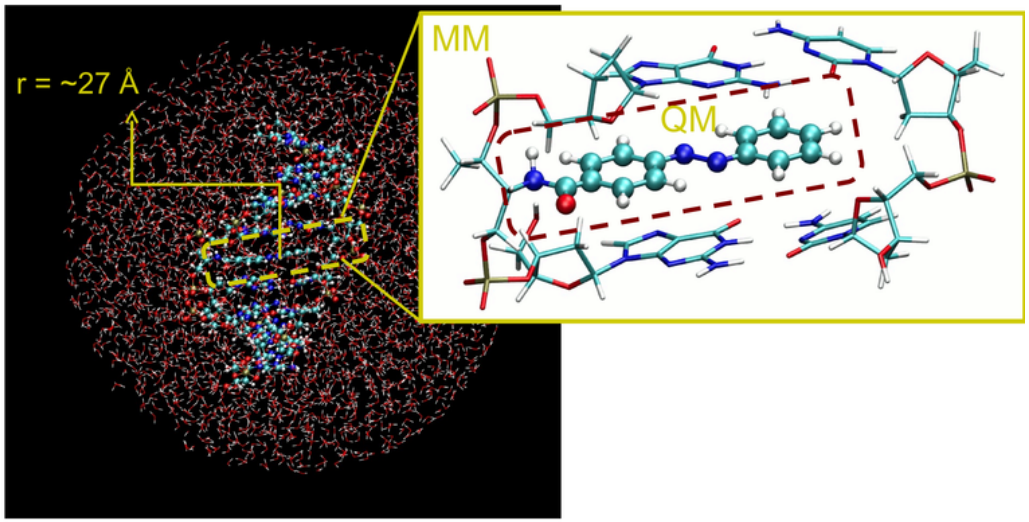
Padmabati Mondal; collaboration with M. Persico & co (Pisa)

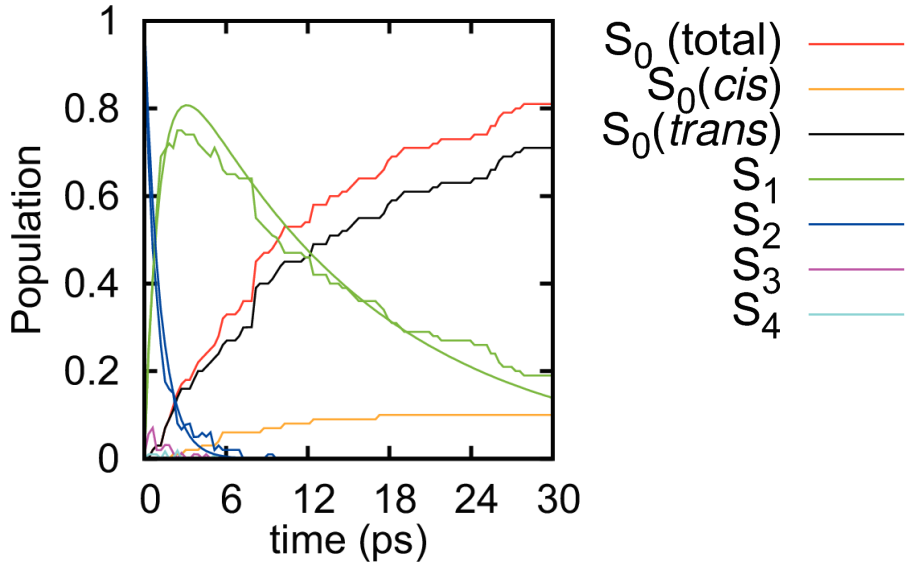


- QM part: MOPAC / MM part: TINKER for RNA + ions + water
- electrostatic embedding / link atom scheme for covalent bonding

Azobenzene & RNA: QM/MM + Surface Hopping

QM/MM = Quantum Mechanics/Molecular Mechanics

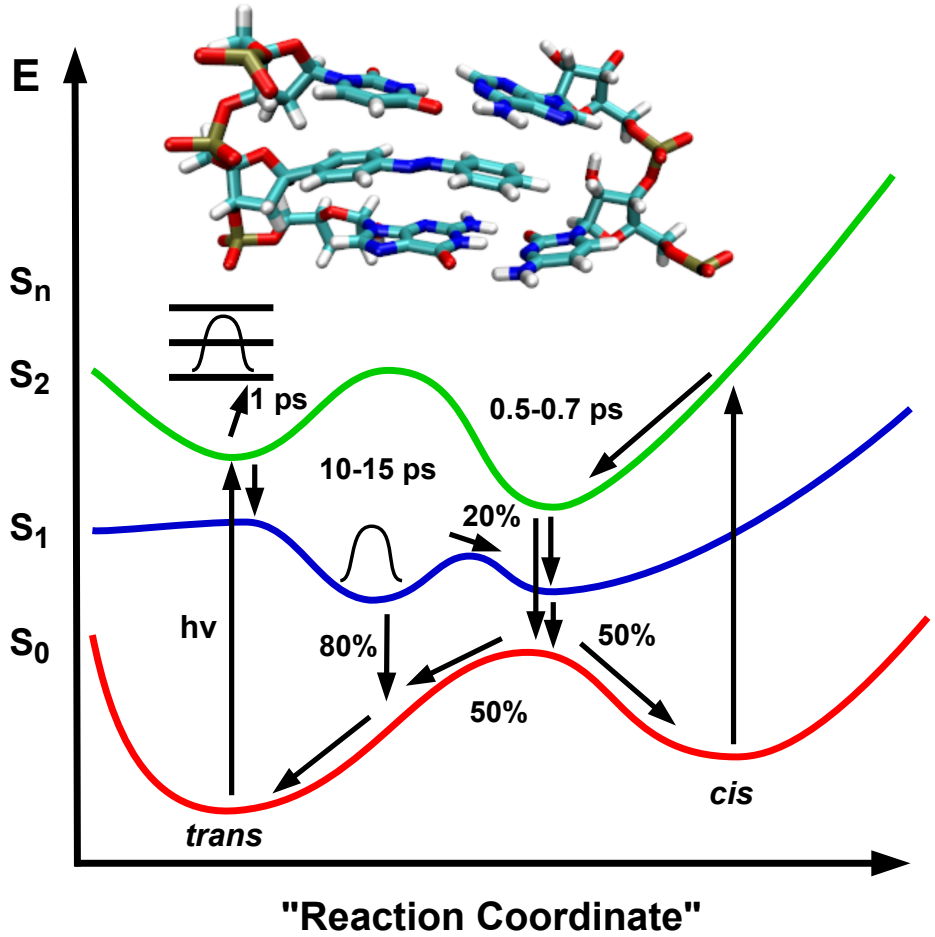




- *trans-cis* isomerization of azobenzene photoswitch triggers unfolding of RNA
- QM part: FOMO-SCF-CI (FOMO = Floating Occupation Molecular Orbital)
- MM part: TINKER (interfaced with MOPAC), Amber ff99sb force field
- QM/MM electrostatic embedding; connection atom scheme for covalent linkage
- initial excitation to $S_2(\pi - \pi^*)$
- quantum yield $\Phi_{\text{trans}-\text{cis}} = 10\%$, $\Phi_{\text{cis}-\text{trans}} = 50\%$
- comparatively slow *trans-cis* isomerization: ca. 20 ps instead of 300 fs (gas phase)

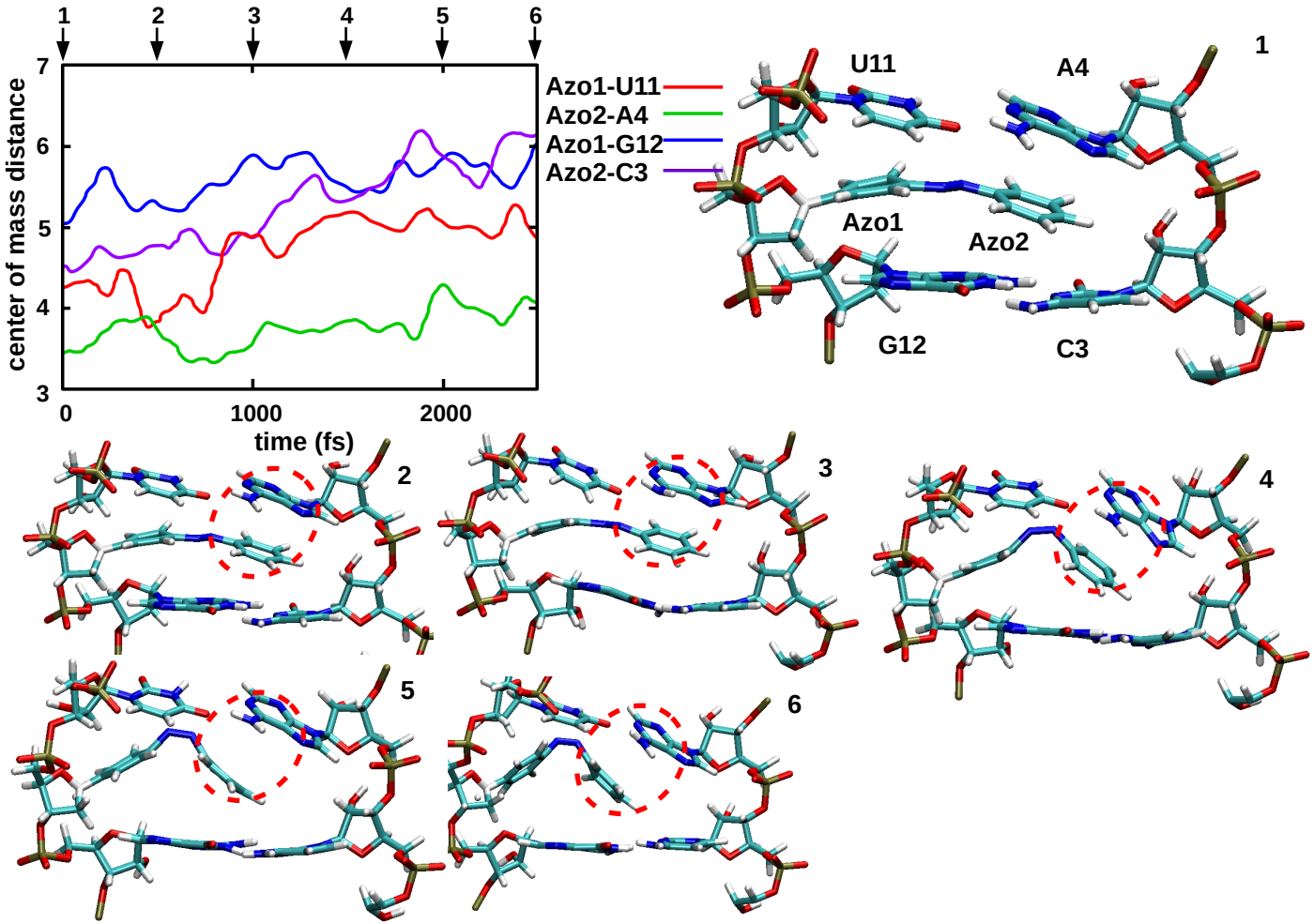
Mondal, Granucci, Rastädter, Persico, Burghardt, Chem. Sci. 9, 4671 (2018)

Modified dynamics in RNA environment



- rapid decay from "bright" S_2 state to S_1 state
- long S_1 lifetime, presumably due to stacking interactions

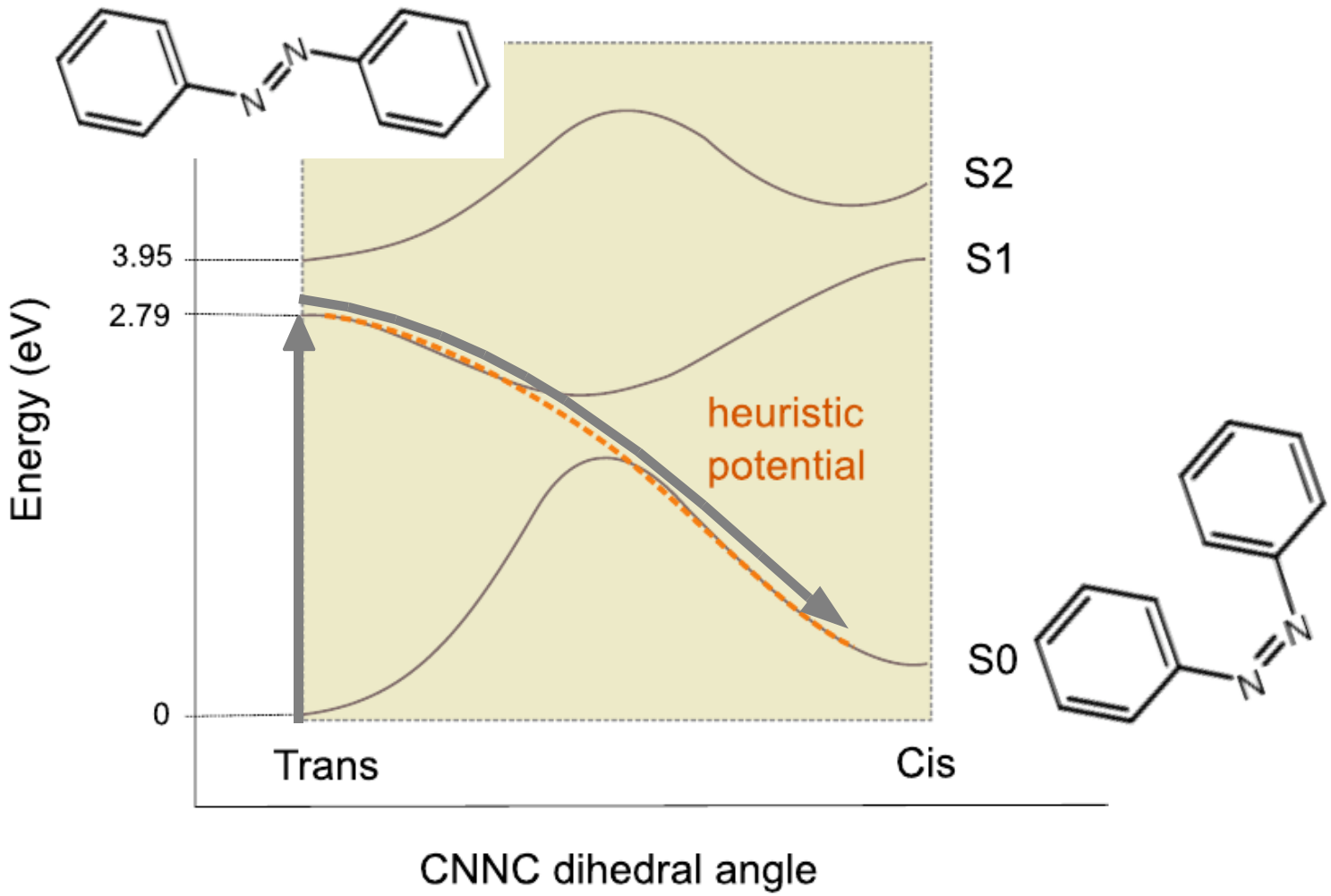
How exactly does the dynamics happen?



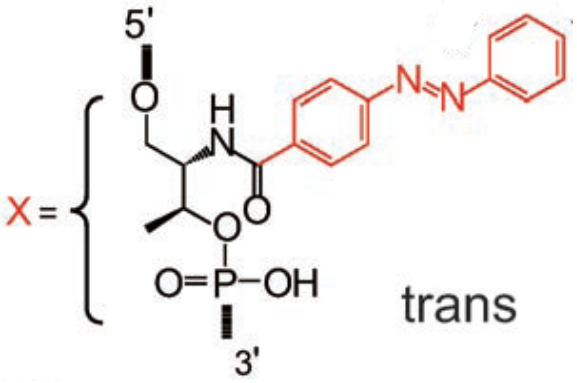
- stacking interactions remain conserved as much as possible
- trajectory switches between S_1 and S_0 around $t \sim$1.5 ps

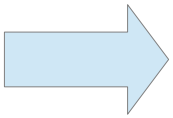
Drastic simplification: isomerisation via effective potential

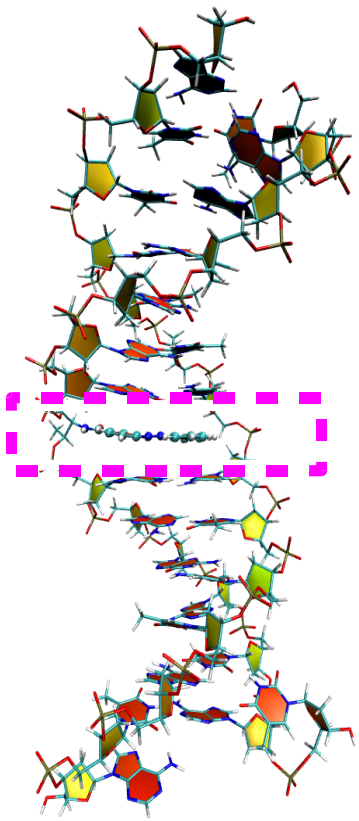
Biswas, Burghardt, Biophys. J., 107, 932 (2014)

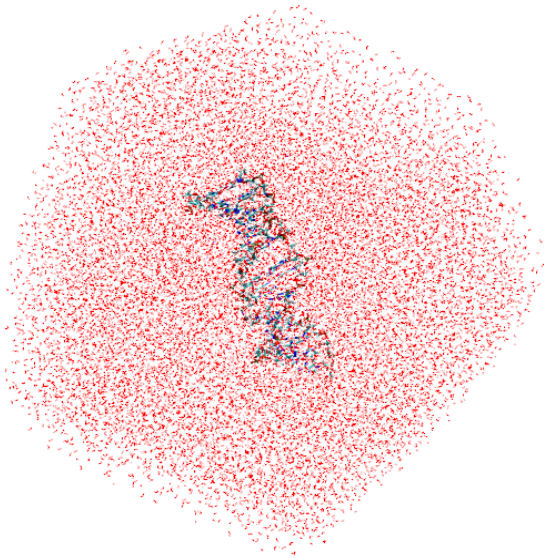


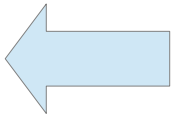
Simulation Set-Up







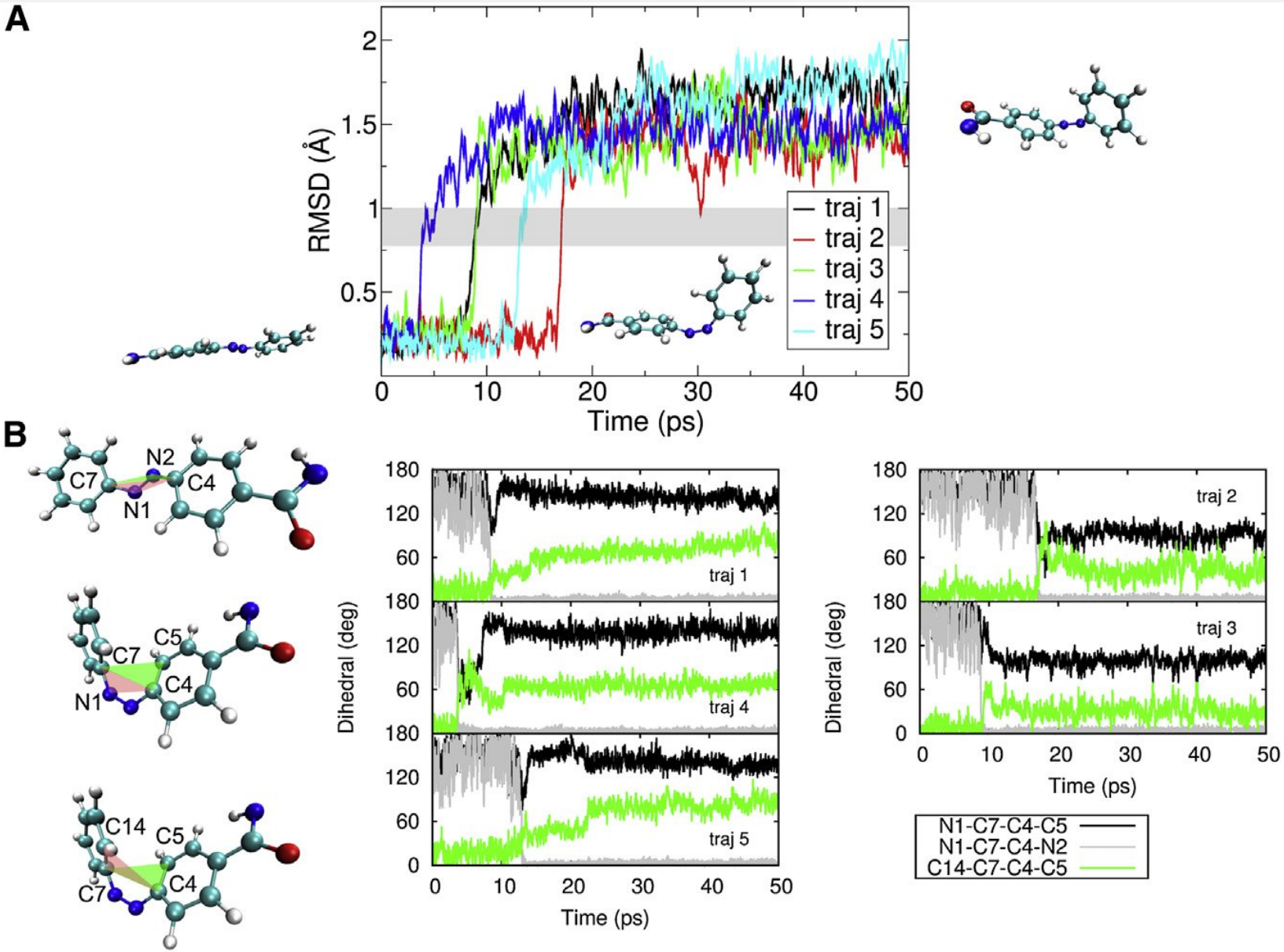




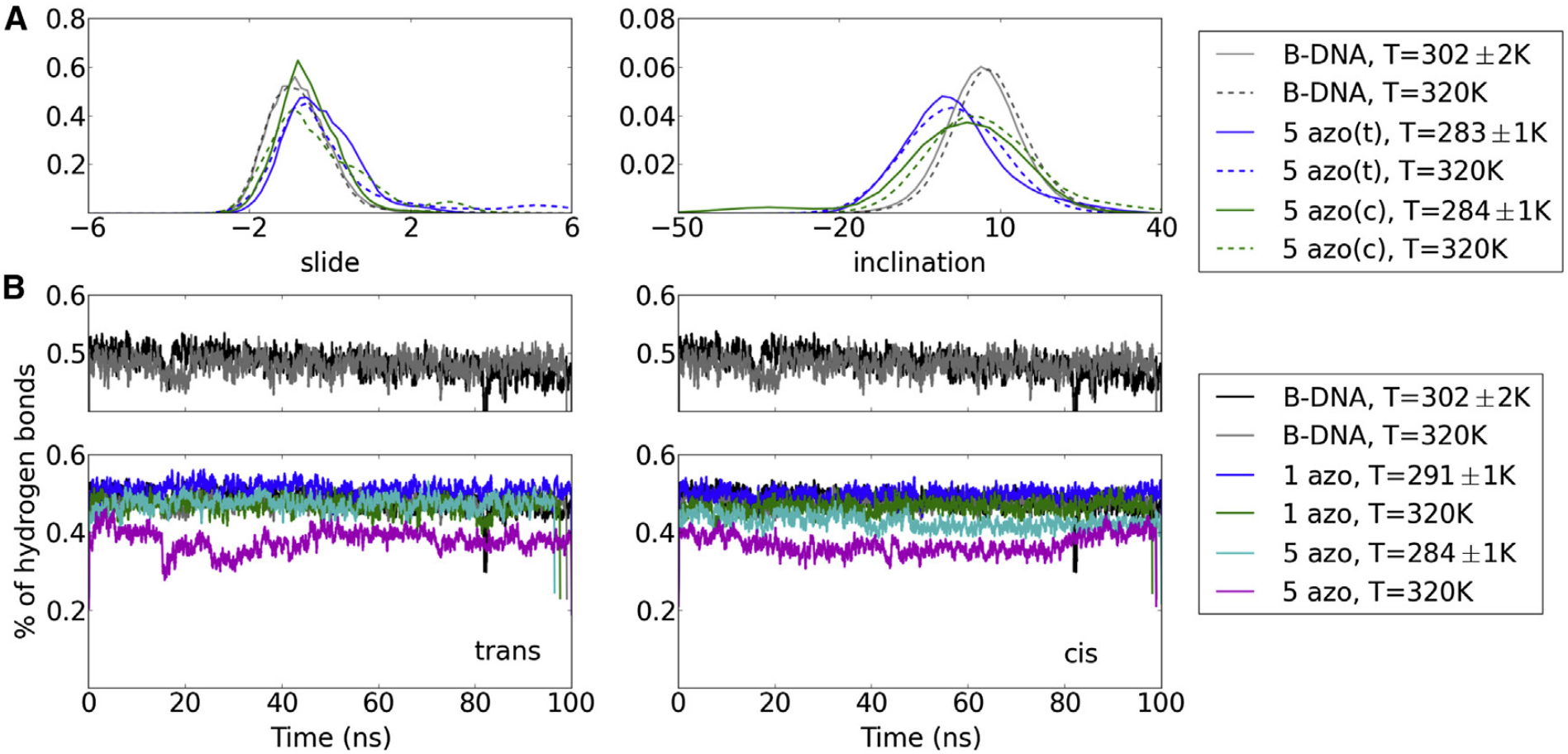
- 5'TAAGAAGXGAGATAT-3' sequence
- azobenzene + 14 mer DNA 29357 atoms
- L-threoninol linkage
- AMBER99 force field with parambsc0 correction
- NAMD program
- 26 Na+ ions
- 2 trans, 2 cis trajectories of duration 50 ns each

Trajectory dynamics

Biswas, Burghardt, Biophys. J., 107, 932 (2014)



DNA Destabilization

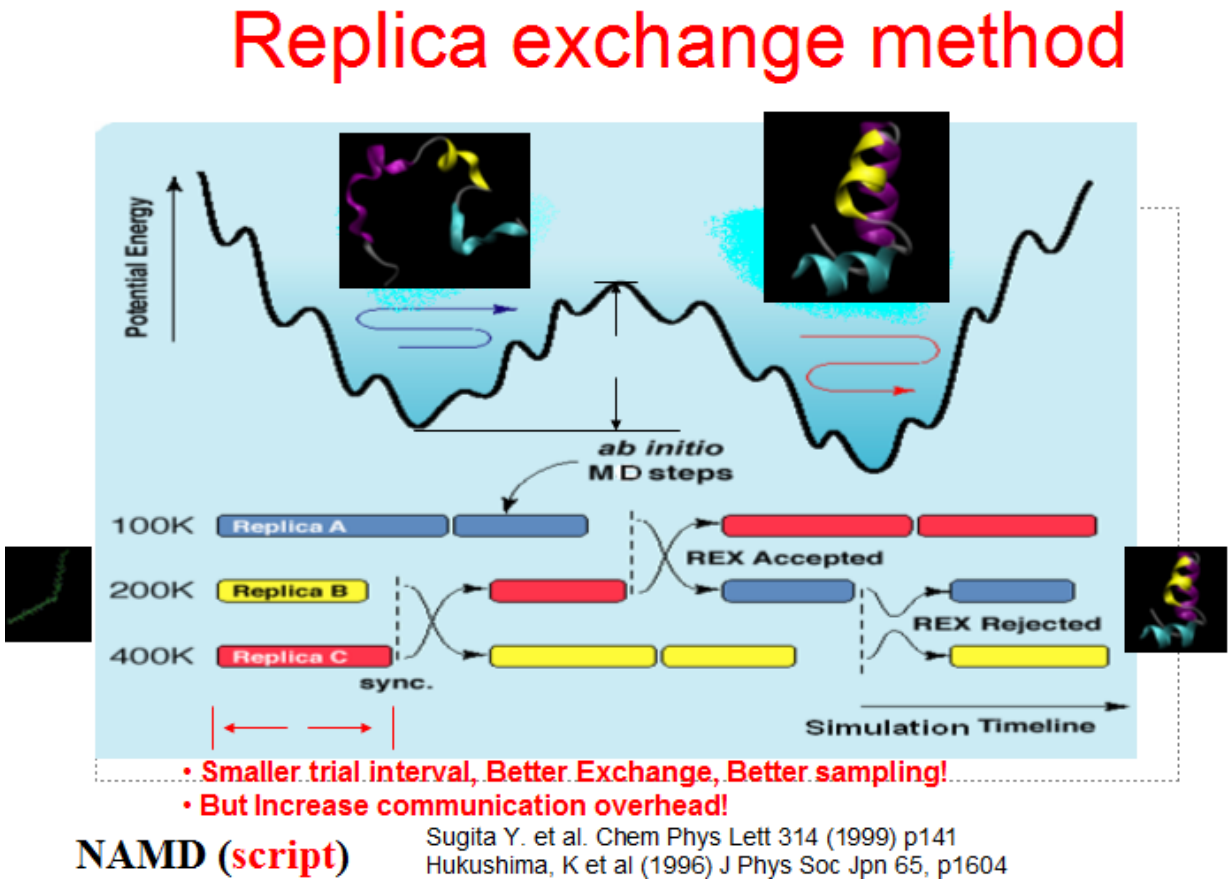


- distribution of helical parameters (slide, inclination) changes noticeably as a function of azobenzene attachment
- in this case, cis and trans forms do not show a significant difference

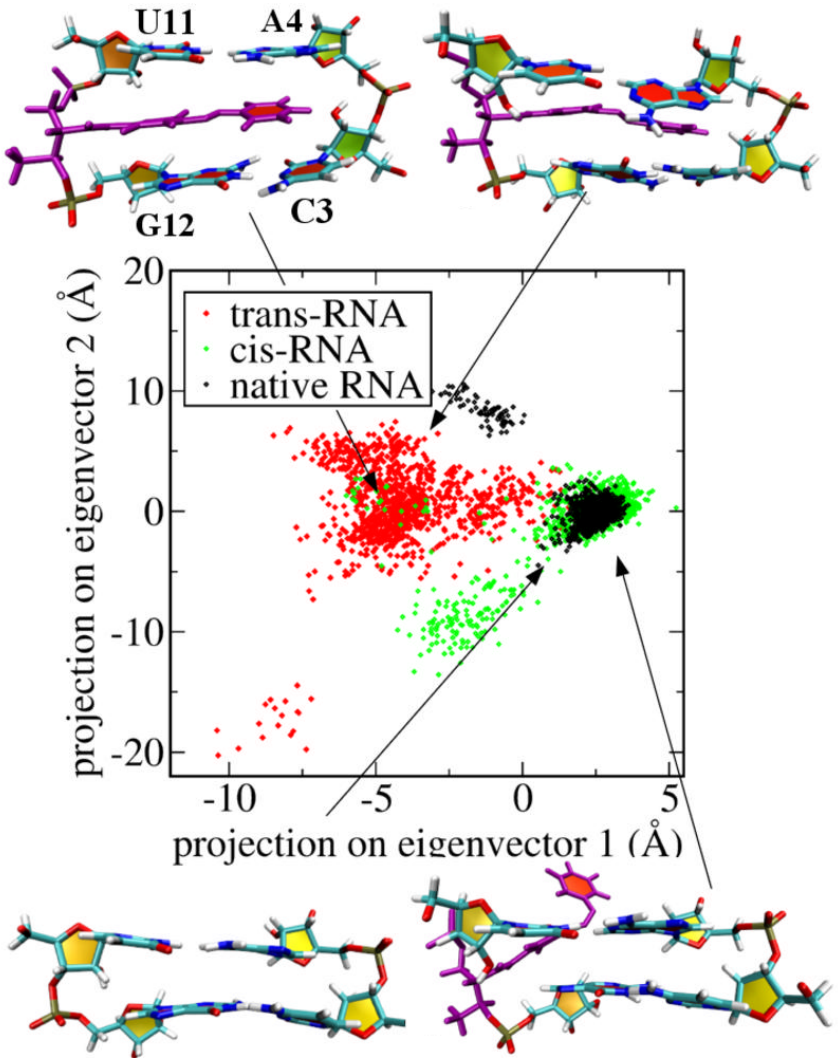
Replica Exchange MD (REMD) simulations

- enhanced sampling technique: "watch" the unfolding of the double helix!
- REMD: run multiple isothermal MD simulations in parallel at a sequence of increasing temperatures and intermittently swap between temperatures

[Sugita, Okamoto, 1999]



Destabilization of Double Strand Structure



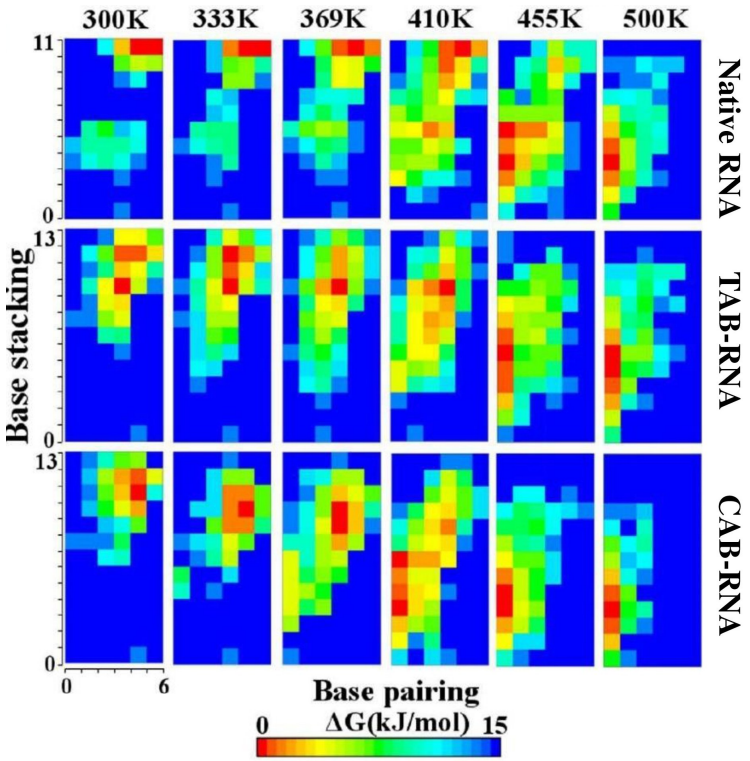
Rastaedter, Biswas, Burghardt, J. Phys. Chem. B, 118, 8478-8488 (2014)

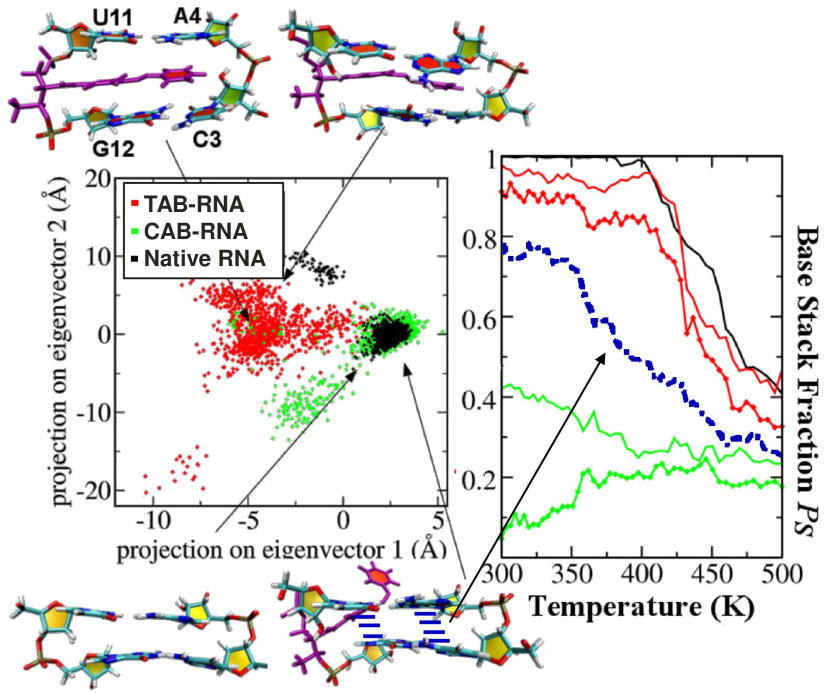
- several conformational ensembles for *trans* form (red)
- single conformational ensemble for *cis* form (black), close to native RNA ⟶ *cis* azobenzene has flipped sideways out of the double strand!

- degree of destabilization depends on several factors:
  - stacking interactions
  - hydrogen bonding
  - properties of the linker
  - presence of abasic site

Free Energy Landscape and RNA Melting

(RNA tetraloop hairpin, D-threoninol linker)





Rastaedter, Biswas, Burghardt, J. Phys. Chem. B, 118, 8478-8488 (2014)

- unfolding more rapid for the trans/cis substituted species than for native RNA
- folding parameter $Q(t) = \frac{1}{2}(P_H(T) + P_S(T))$
- estimated melting temperatures T_m: cis/trans/native: $382^\circ/418^\circ/427^\circ$
- scaled values T_m^{scaled}: cis/trans/native: $305^\circ/334^\circ/342^\circ$

Bottom line: multiscale methods needed!