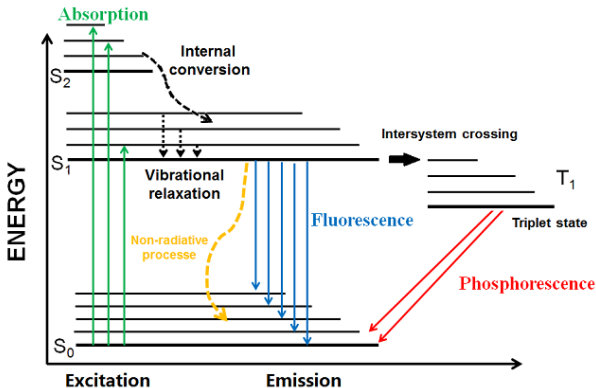# Ringvorlesung Biophysik 3
Theoretische Aspekte der Photobiologie

AK Burghardt (Institut für Physikalische & Theoretische Chemie)

Theoretical Chemistry of Complex Systems

AK Burghardt

chemical + biological + material processes

chemistry "bottom-up":

electrons + nuclei

Theoretical Chemistry of Complex Systems

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quantum density $\qquad \varrho \longleftrightarrow \psi^*\psi \qquad$ wave function

$\updownarrow$

classical probability distribution $\qquad P$

Theoretical Chemistry
of Complex Systems

AK Burghardt

Teaching:

Theoretische Chemie I (Bachelor) – Burghardt
Theoretische Chemie II + Praktikum (Bachelor/Master) – Burghardt
Theoretical Photochemistry (Master) – Burghardt

Mathematische Methoden I (Bachelor) – Hegger
Mathematische Methoden II (Bachelor) – Hegger
Mathematische Methoden III (Bachelor, Master) – Hegger
+ MD-QC Praktikum (Bachelor[1]) – Burghardt, Hegger

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[1]Wahlpflichtmodul "Computational Chemistry"

Topics

1 Photochemistry
  Quantum Effects in Biological Systems
  Photochemistry: Quantum Mechanics at Work
  Quantum (QM) or Quantum/Classical (QM/MM-MD)?

Topics

❶ Photochemistry
Quantum Effects in Biological Systems
Photochemistry: Quantum Mechanics at Work
Quantum (QM) or Quantum/Classical (QM/MM-MD)?

❷ Modeling of Photoinduced Uncaging @CLiC
Can Theory Predict the Best Cages?
Spectroscopy: What's the Best Way to Prepare your System?
How to Tackle Multiple Time Scales?

Topics

1 Photochemistry
Quantum Effects in Biological Systems
Photochemistry: Quantum Mechanics at Work
Quantum (QM) or Quantum/Classical (QM/MM-MD)?

2 Modeling of Photoinduced Uncaging @CLiC
Can Theory Predict the Best Cages?
Spectroscopy: What's the Best Way to Prepare your System?
How to Tackle Multiple Time Scales?

3 Photoregulation of DNA & RNA
Conformational Control using Covalently Attached Photoswitches
QM/MM Simulations
MM-MD Simulations

Photochemistry
Modeling of Photoinduced Uncaging
Photoregulation of DNA & RNA

Quantum Effects in Biological Systems
Photochemistry: Quantum Mechanics at Work
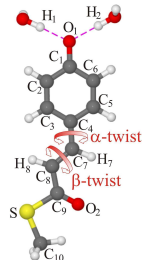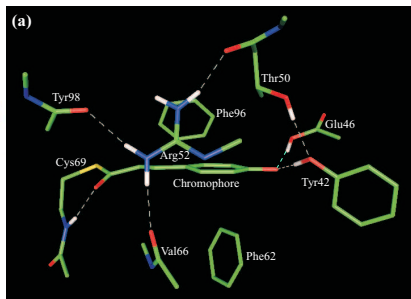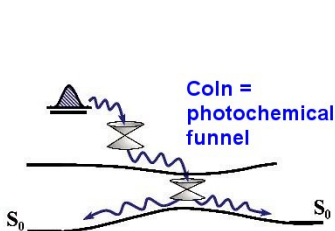Quantum (QM) or Quantum/Classical (QM/MM-MD)?

Why Worry about Quantum Effects?

- Most properties/processes in biological & material systems can be taken to belong to the classical limit ($\lambda_{dB} \ll L$, classical Wigner limit, . . .)

- Hence, MD (= Molecular Dynamics) simulations are extensively used: classical-mechanical evolution of all nuclear degrees of freedom on the lowest (electronic ground state) Born-Oppenheimer (BO) surface

- However, some quantum effects are important:
  - tunneling (proton transfer, electron transfer)
  - light-induced processes: coherent superpositions
  - nonadiabatic ("non-BO") dynamics

- Methods are needed for quantum dynamics in many dimensions
  - approximate wavefunction and density operator methods
  - semiclassical approaches
  - mixed quantum-classical hybrid approaches

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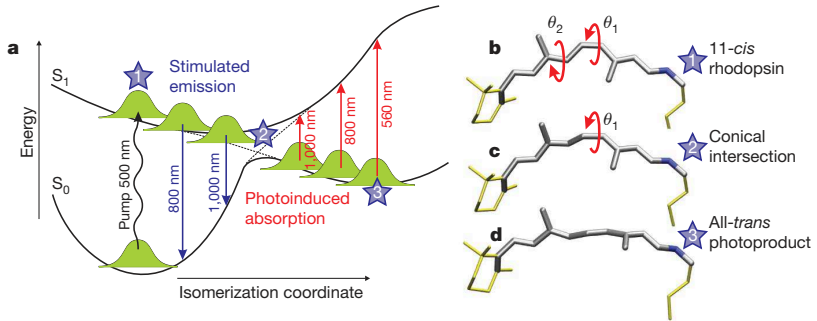
Photoactive Proteins: Bio-Photochemistry
Example PYP = Photoactive Yellow Protein

Gromov, Burghardt, Köppel, Cederbaum, J. Phys. Chem. A **115**, 9237 (2011), JACS **129**, 6798 (2007)

- photochemistry determined by conical intersection (CoIn) topology
- local environment significantly influences α vs. β twist
- interplay of steric effects / electrostatics / H-bonds / fluctuations
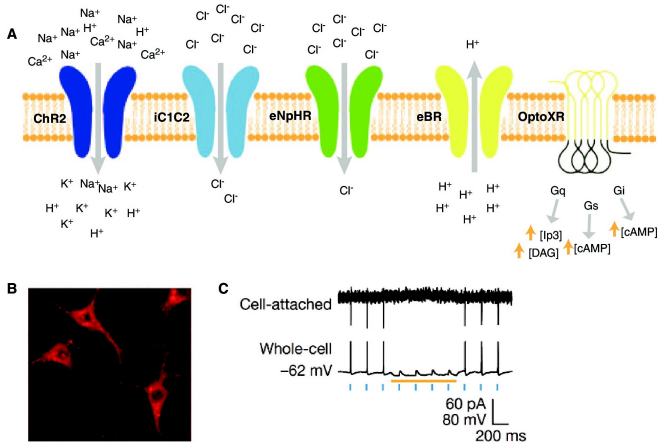- excited-state lifetime: ~ 700 fs (in solution: ~ 10 ps)

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Quantum (QM) or Quantum/Classical (QM/MM-MD)?

Bio-Photochemistry: Retinal/Rhodopsin

Polli et al., Nature 467, 440 (2010)
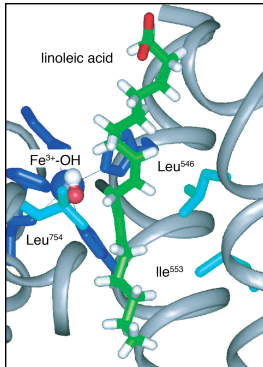
- isomerisation within ∼200 femtoseconds (but 5 picoseconds in solution)
- isomerisation happens in a volume conserving fashion

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From Bio-Photochemistry to Optogenetics

Guru et al., Int. J. Neuropsychopharmacology 1-8 (2015)

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Quantum (QM) or Quantum/Classical (QM/MM-MD)?

Quantum Tunneling in Enzymes
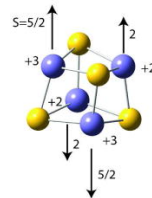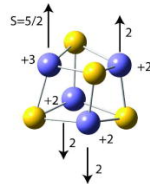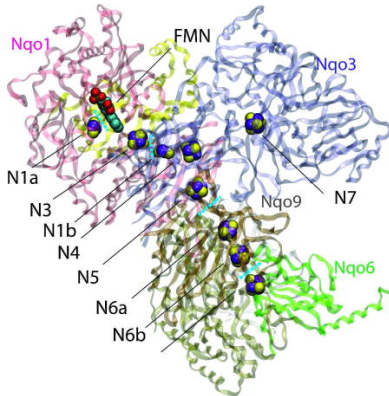

Meyer, PNAS 105, 1146 (2008)
"Enzyme structure and dynamics affect hydrogen tunneling:
The impact of a remote side chain (I553) in soybean lipoxygenase-1"
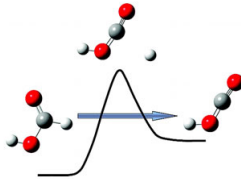
- "Did enzymes evolve to capitalize on quantum tunneling?" The Scientist, 2005

- "Our present findings on hydrogen transfer under physiological conditions cannot be explained without invoking both quantum mechanics and enzyme dynamics" Klinman, Nature 1999

- "Taking Ockham's razor to enzyme dynamics and catalysis": no need to go beyond Transition State Theory (TST) + tunneling corrections

  Glowacki et al., Nature Chem. 2012

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Electron Tunneling: Respiratory Chains

"Quantum Electron Tunneling in Respiratory Complex I1", Hayashi, Stuchebrukhov, J. Phys. Chem. B 115, 5354 (2011)

- "electronically wired" iron-sulfide (FeS) clusters
- quantum interference resulting from multiple tunneling pathways

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Coherent Photoinduced Energy and Charge Transport in Biological and Material Light-Harvesting Systems
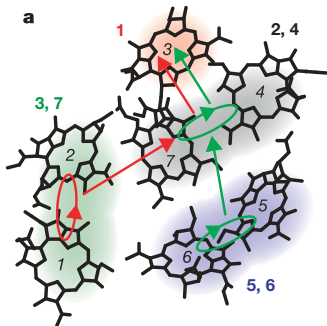
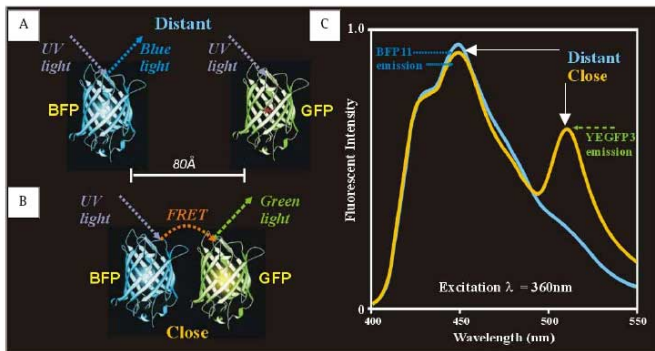Lee, Cheng, Fleming, Science 316, 1462 (2007)
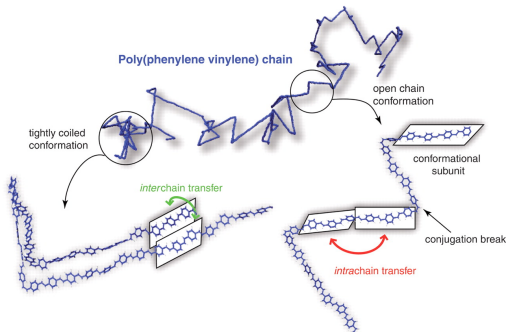
Collini, Scholes, Science 323, 369 (2009)

- one would expect an extremely rapid dephasing (decoherence): $\tau_{\mathrm{dec}} < 50$ fs
- but observed coherence lifetimes are ~ 300 fs to 1-2 ps (or more)

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Quantum Effects in Biological Systems
Photochemistry: Quantum Mechanics at Work
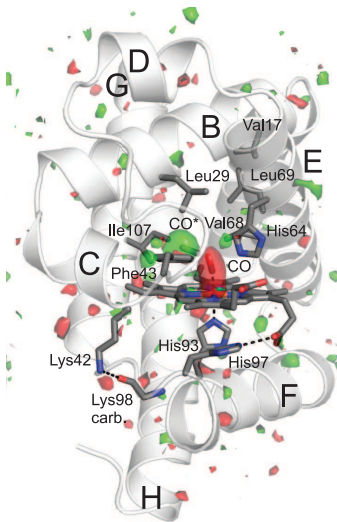Quantum (QM) or Quantum/Classical (QM/MM-MD)?

Fluorescence Resonance Energy Transfer (FRET)

E.g., Fluorescence Resonance Energy Transfer (FRET) between fluorescent proteins: calculate FRET rates $\propto r_{DA}^{-6}$

Figure 1. The use of GFP variants to produce FRET. In A, Distant; UV light excites BFP (donor fluorophore) to emit blue light (peak emission = 450nm), but GFP (acceptor) is not close enough to draw energy from the excited donor. In B, Close; proximity of GFP to BFP allows non-radiative energy transfer, the stimulated BFP exciting GFP to fluoresce green (peak emission = 510nm). Figure 1C shows the expected emission spectra when the GFP fluorophores are either close or distant.

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Quantum (QM) or Quantum/Classical (QM/MM-MD)?

Photoinduced CO Ligand Dissociation from Myoglobin

Direct observation of ultrafast collective motions in CO myoglobin upon ligand dissociation

Thomas R. M. Barends,[1]* Lutz Foucar,[1] Albert Ardevol,[2] Karol Nass,[1] Andrew Aquila,[3]
Sabine Botha,[1] R. Bruce Doak,[1] Konstantin Falahati,[4] Elisabeth Hartmann,[1]
Mario Hilpert,[1] Marcel Heinz,[2,4] Matthias C. Hoffmann,[5] Jürgen Köfinger,[2]
Jason E. Koglin,[5] Gabriela Kovacsova,[1] Mengning Liang,[5] Despina Milathianaki,[5]
Henrik T. Lemke,[5] Jochen Reinstein,[1] Christopher M. Roome,[1] Robert L. Shoeman,[1]
Garth J. Williams,[5] Irene Burghardt,[4] Gerhard Hummer,[2]
Sébastien Boutet,[5] Ilme Schlichting[1]*

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Modeling of Photoinduced Uncaging
Photoregulation of DNA & RNA

Quantum Effects in Biological Systems
Photochemistry: Quantum Mechanics at Work
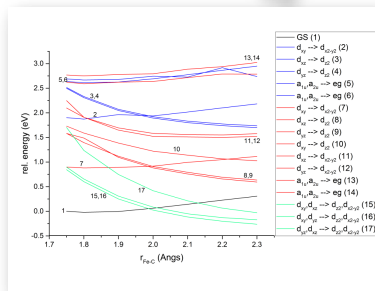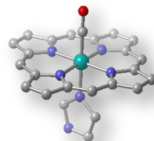Quantum (QM) or Quantum/Classical (QM/MM-MD)?

PHOTODISSOCIATION OF MYOGLOBIN

carboxy-hemoproteins are among the most
studied systems to evaluate (collective)
protein dynamics that determine structure
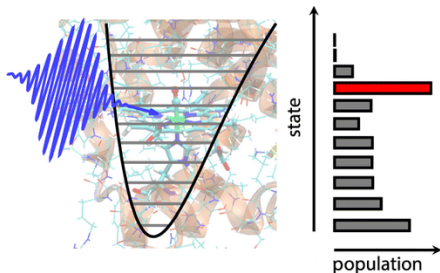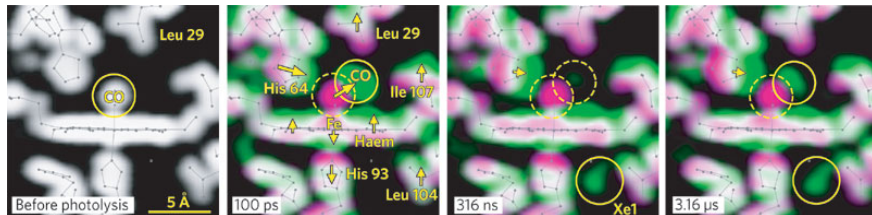and reactivity

quantum mechanical *ab initio* calculations
at TD-DFT and CASSCF/CASPT2 level reveal
complex setting of excited states

large manifolds of singlet, triplet and quintet
states within range of experimental excitation

interaction based on vibronic coupling
and/or spin-orbit coupling may cause overall
ultrafast dissociation within < 100 fs

Photochemistry
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Quantum Effects in Biological Systems
Photochemistry: Quantum Mechanics at Work
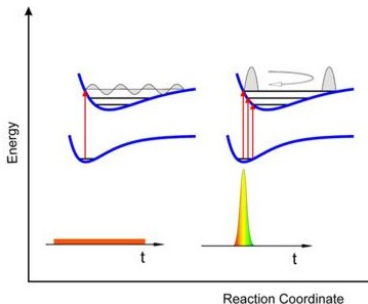Quantum (QM) or Quantum/Classical (QM/MM-MD)?

Quantum Oscillators in Highly Specific Environments

- CO-myoglobin complex
- photoinduced CO dissociation from myoglobin
- state-specific excitation feasible
- relaxation/decoherence depend critically on local environment

Debnath, Falvo, Meier, J. Phys. Chem. A, 117, 12884 (2013)

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Light Pulses Create Excited-State Wavepackets

left: CW excitation,
right: pulsed excitation

http://www.uni-heidelberg.de/fakultaeten
/chemgeo/pci/motzkus/research/wavepackets.html

- wavepacket $=$ superposition of eigenstates

$$\psi(x,t) \quad = \quad \sum_n c_n \varphi_n(x) e^{-iE_n t/\hbar}$$

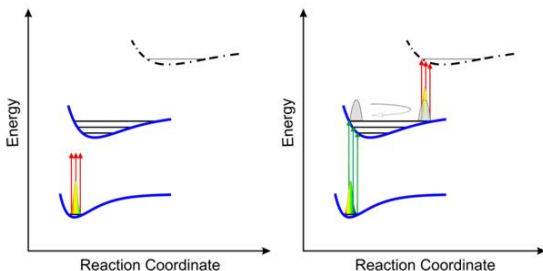non-stationary state (in contrast to a single eigenstate)

- solve the time-dependent Schrödinger Equation:

$$i\hbar \frac{\partial \psi}{\partial t} = \left(\hat{H}_{\mathrm{mol}} - E(r,t)\hat{\mu} \right) \psi$$

where $\hat{H}_{\mathrm{mol}}$ is the molecular Hamiltonian and $\hat{\mu}$ is the dipole operator

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Femtochemistry: Watching Wavepackets in Real Time
Nobel Prize Ahmed Zewail 1999

http://www.uni-heidelberg.de/fakultaeten
/chemgeo/pci/motzkus/research/wavepackets.html

Pump-Probe Spectroscopy

- nonlinear optical spectroscopy
- wavepacket dynamics in electronically excited states: quantum coherence
- theoretical interpretation: explicit simulation needed!

Theory: electronic structure ($\longrightarrow$ potential surfaces) $+$ quantum dynamics

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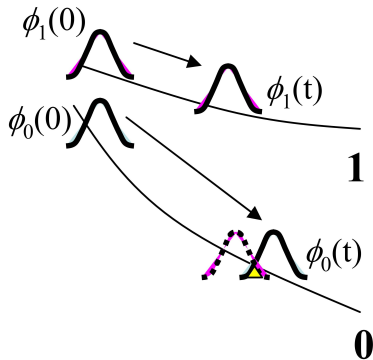Quantum (QM) or Quantum/Classical (QM/MM-MD)?

Quantum Coherence – and Decoherence

$$|\psi(t)\rangle = c_0(t)|0\rangle|\phi_0(t)\rangle + c_1(t)|1\rangle|\phi_1(t)\rangle$$
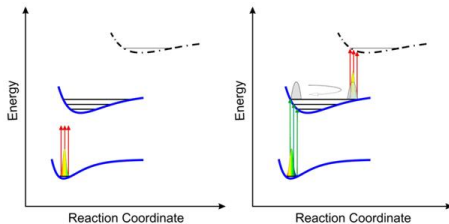
electronic coherence:

$$\rho_{01}(t) = \text{Tr}[|0\rangle\langle 1|\hat{\rho}(t)]$$
$$= \langle 1|\hat{\rho}(t)|0\rangle = c_1^*(t)c_0(t)\langle\phi_1(t)|\phi_0(t)\rangle$$

- coherence $\propto$ overlap of nuclear wavefunctions
- typical decoherence times: ~ 30 fs
- loss of coherence cannot be captured by a classical trajectory picture


picture: P. Rossky et al.

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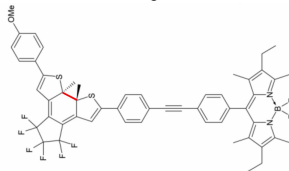## Vibrational Coherence & Coherence Transfer

Pump-Probe spectroscopy

http://www.uni-heidelberg.de/fakultaeten
/chemgeo/pci/motzkus/research/wavepackets.html

- vibrational coherence
  ↔ wavepacket motion
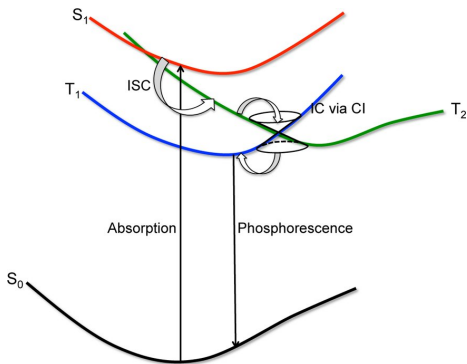- coherence transfer due to
  coupled vibrations
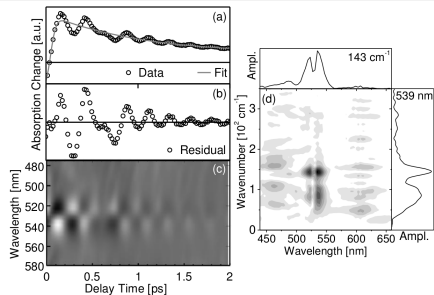
Vibrational coherence transfer in an
electronically decoupled molecular dyad

Schweighöfer et al., Scientific Reports (2015)

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Quantum Effects in Biological Systems
Photochemistry: Quantum Mechanics at Work
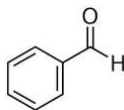Quantum (QM) or Quantum/Classical (QM/MM-MD)?

Even in Small Systems, Excited-State Dynamics is Complex

Ou, Subotnik, J. Phys. Chem. C. 117, 19839 (2013)

- Example: benzaldehyde

- ISC = intersystem crossing
- IC = internal conversion
- CI = conical intersection

- ISC time scale:
  $\sim 10^{-9}$ s – "untypically" fast!
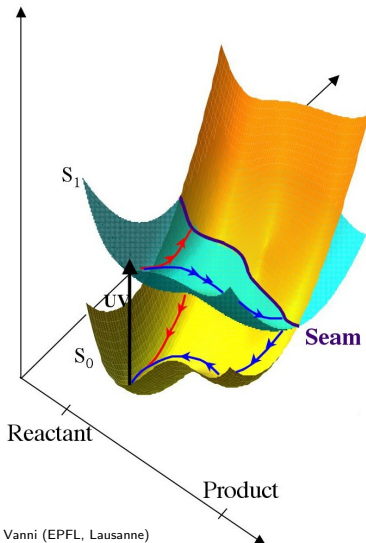- phosphoresence lifetime
  $\sim 2 \times 10^{-3}$ s

- state-to-state couplings needed
- characterize special topologies: conical intersections
- identify most relevant coordinates

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Beyond Born-Oppenheimer

- "non-adiabatic" dynamics
- quantum dynamical description required
- mean-field (Ehrenfest) and surface-hopping approaches often insufficient

graphics: Stefano Vanni (EPFL, Lausanne)

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What Do We Need To Calculate?

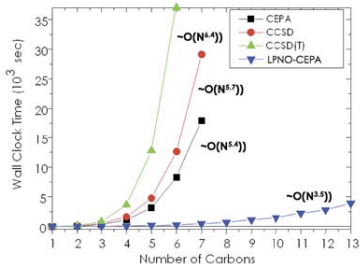electronic structure : $\boxed{H_{\mathrm{el}}\psi_n^{\mathrm{el}}(r,R) = E_n(R)\,\psi_n^{\mathrm{el}}(r,R)}$

- high-level methods (CC2, ADC(2),CASSCF/CASPT2,MRCI)
- density functional methods (DFT, TDDFT)
- quantum mechanics / molecular mechanics (QM/MM) hybrid methods
- linear scaling methods

quantum dynamics : $\boxed{i\hbar\dot{\psi}_n^{\mathrm{nuc}}(R) = (T_{\mathrm{nuc}} + E_n(R))\,\psi_n^{\mathrm{nuc}}(R)}$

- multiconfigurational methods (MCTDH)
- mixed quantum-classical methods
- reduced-dimensional descriptions (effective modes, mesoscopic descriptions)
- Markovian and non-Markovian master equations
  $\longrightarrow$ method development!

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Exponential Scaling Problem

Standard methods: exponential scaling
⟶ limit reached already for 6 carbon atoms
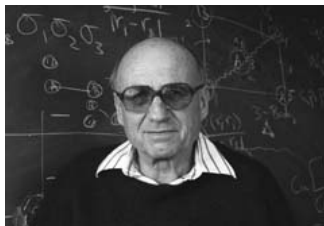
"I cannot foresee any technological progress which should make such calculations feasible."
Walter Kohn, Nobel Lecture 1998

- here, electronic structure example – but same problem for quantum dynamics!
- development of approximate methods is of key importance

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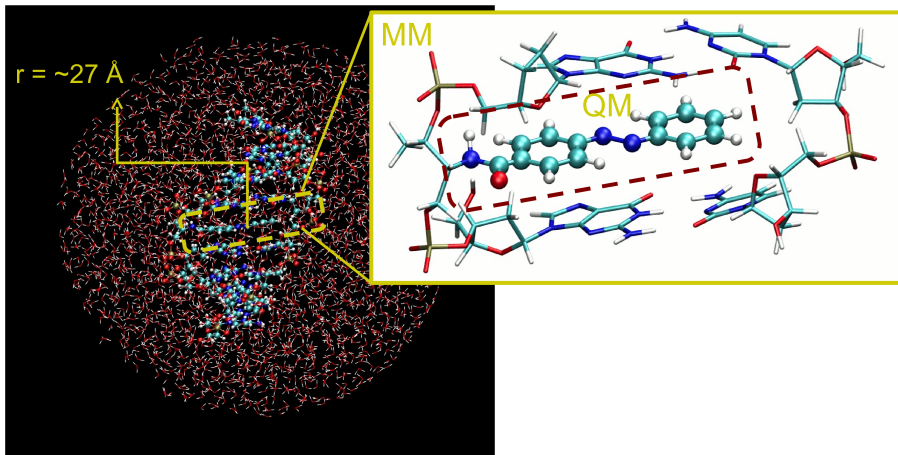
Various Approximate Approaches

1. *On-the-fly* Quantum-Mechanics/Molecular Mechanics (QM/MM) + Surface Hopping (SH) – Example: Photocontrol of DNA and RNA
2. Parametrized Hamiltonians + Quantum Dynamics (QD) – Example: Elementary Events in Organic Photovoltaics
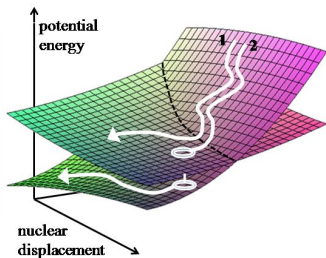
Surface Hopping (SH):
stochastic trajectories mimic wavepacket motion on coupled potential surfaces

M. Barbatti, http://www.newtonx.org/

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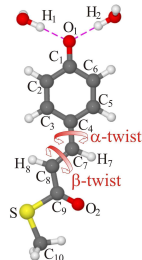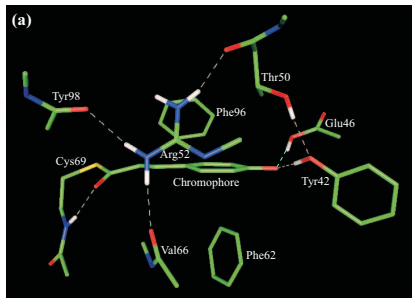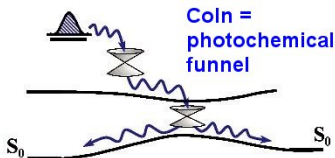**Quantum (QM) or Quantum/Classical (QM/MM-MD)?**

QM/MM simulations

2013 Nobel Prize for Martin Karplus/Michael Levitt/Arieh Warshel

various approaches: NEWTON-X, MNDO/Chemshell, MOPAC (Pisa version), ...

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Photoactive Proteins: Bio-Photochemistry
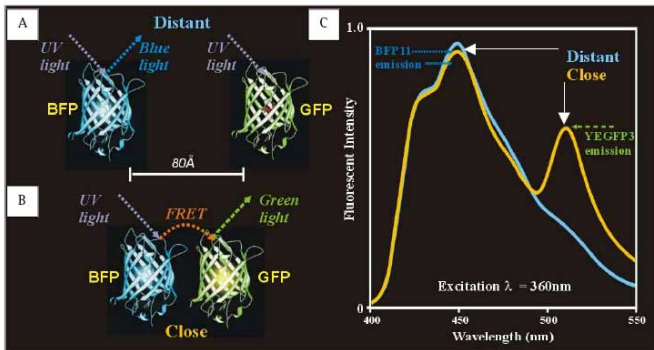**Example PYP = Photoactive Yellow Protein**

Gromov, Burghardt, Köppel, Cederbaum, J. Phys. Chem. A **115**, 9237 (2011), JACS **129**, 6798 (2007)

- photochemistry determined by conical intersection (CoIn) topology
- local environment significantly influences α vs. β twist
- interplay of steric effects / electrostatics / H-bonds / fluctuations
- excited-state lifetime: ~ 700 fs (in solution: ~ 10 ps)

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Sometimes Rate Theories are Sufficient . . .

E.g., Fluorescence Resonance Energy Transfer (FRET) between fluorescent proteins: calculate FRET rates $\propto r_{DA}^{-6}$

Figure 1. The use of GFP variants to produce FRET. In A, Distant; UV light excites BFP (donor fluorophore) to emit blue light (peak emission = 450nm), but GFP (acceptor) is not close enough to draw energy from the excited donor. In B, Close; proximity of GFP to BFP allows non-radiative energy transfer, the stimulated BFP exciting GFP to fluoresce green (peak emission = 510nm). Figure 1C shows the expected emission spectra when the GFP fluorophores are either close or distant.

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Can Theory Predict the Best Cages?
Spectroscopy: What's the Best Way to Prepare your System?
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Photocontrol by Uncaging
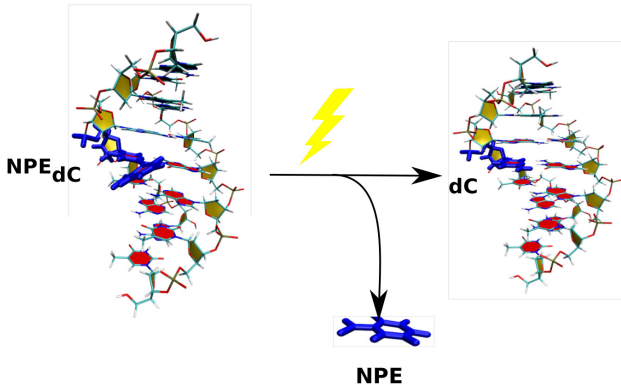

- Graduate School CLiC = Complex scenarios of Light Control

Photochemistry
Modeling of Photoinduced Uncaging
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Can Theory Predict the Best Cages?
Spectroscopy: What's the Best Way to Prepare your System?
How to Tackle Multiple Time Scales?

Spectroscopy: What's the Best Way to Prepare Your System?
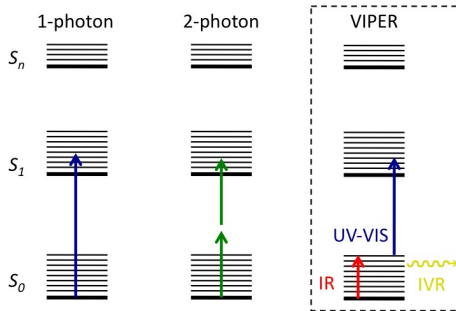
Criteria:

- absorption efficiency (e.g., single-photon vs. two-photon)
- spatial resolution: two-photon induced fluorescence decrease as the fourth power of the distance from the focal plane ("emission from a single point")
- frequency-domain selectivity: e.g., higher selectivity in the infrared domain as used in the VIPER experiment
- design of sequential (two-color) uncaging systems

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Calculation/Prediction of Uncaging Efficiencies
(e.g., o-Nitrobenzyl Caging Groups)
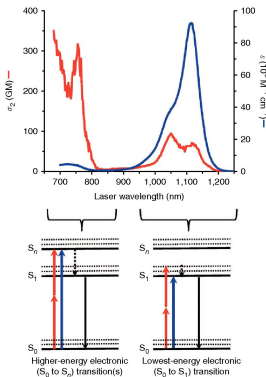
Drobizhev et al., Nat. Meth. 8, 393 (2011)

Scheme 4: Revised Mechanism for the Thermal Reactions of the Primary Photochemical aci-Transients A Formed from 1 in Aqueous Solution.[a]
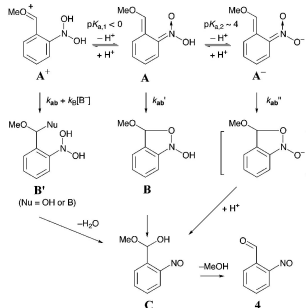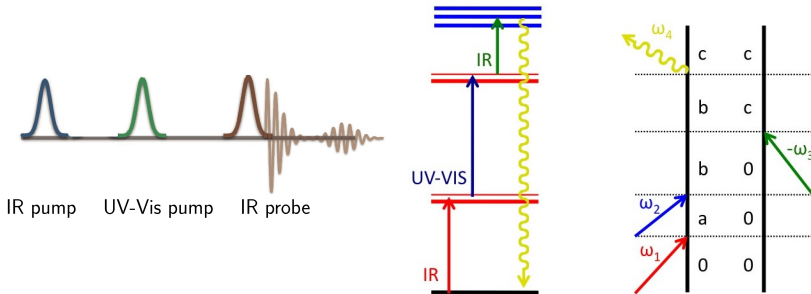
Il'ichev et al., J. Am. Chem. Soc. 126, 4581 (2004)

- efficiency $=$ $\boxed{\varepsilon \cdot \Phi}$ (1-photon) or $\boxed{\delta \cdot \Phi}$ (2-photon)
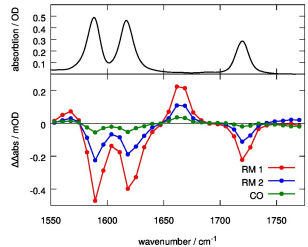- intrinsic quantum yields (Φ): reaction paths, intermediates required

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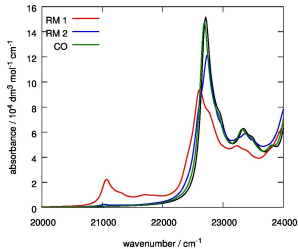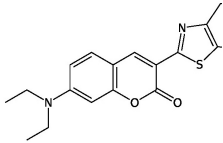**Spectroscopy: What's the Best Way to Prepare your System?**
How to Tackle Multiple Time Scales?

Spectroscopies can be Sophisticated . . .

A4: Vibrationally Promoted Electronic Resonance (VIPER) 2D spectroscopy

IR pump   UV-Vis pump   IR probe

- mixed IR/UV-Vis pulse sequence
- shaped femtosecond IR pulses
- calculate optical response functions

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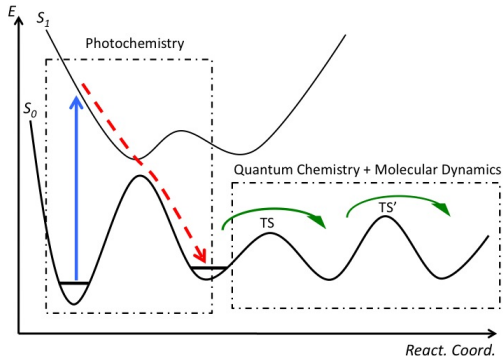
Simulation of VIPER experiment

- first step: calculate absorption spectrum with vibrational pre-excitation

$$\alpha(\omega) \;=\; \frac{4\pi^2\omega_I}{3\hbar c}\sum_n |\langle\psi_n^E|\hat{\mu}|\psi_0^G\rangle|^2\delta(\omega_I-\omega_n) = \frac{2\pi\omega_I}{3\hbar c}\int_{-\infty}^{\infty} dt\langle\phi_E(0)|\phi_E(t)\rangle e^{i\omega t}$$

- complementary time-independent and time-dependent approaches
  (full quantum approach needed, for hundreds of normal modes!)
- identify the modes that are best suited for VIPER effect

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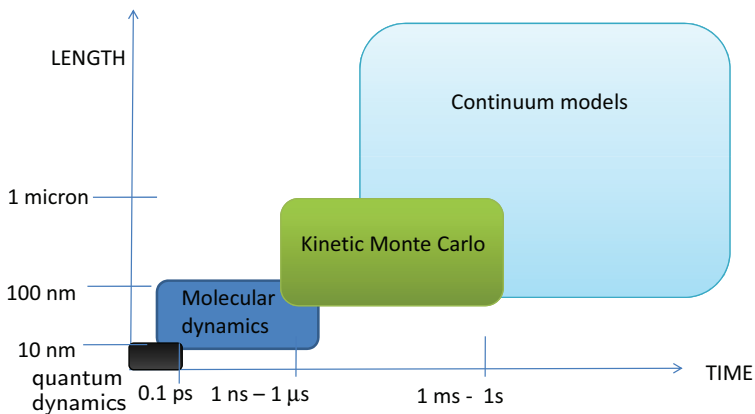**How to Tackle Multiple Time Scales?**

How to Tackle Multiple Time Scales?

Hierarchy of methods:

- quantum dynamics: femtoseconds to picoseconds
- classical molecular dynamics (MD): picoseconds to nano-/microseconds
- kinetic models: microseconds to seconds (and longer)

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Hierarchy of Time and Length Scales

- Coarse-graining techniques can bridge length scales

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QM/MM Simulations
MM-MD Simulations

Topics

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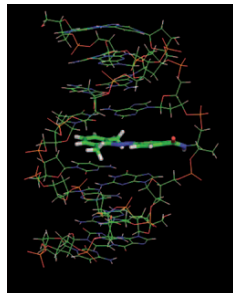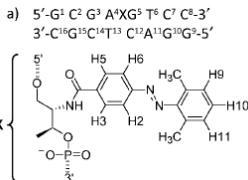Conformational Control using Covalently Attached Photoswitches
QM/MM Simulations
MM-MD Simulations

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

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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"

PreQ1 riboswitch complexed with preQ0 (green)
pdb ID = 3gca
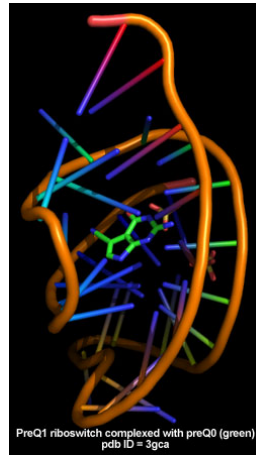
Spitale et al., J. Biol. Chem. 284, 11012 (2009)

Photochemistry
Modeling of Photoinduced Uncaging
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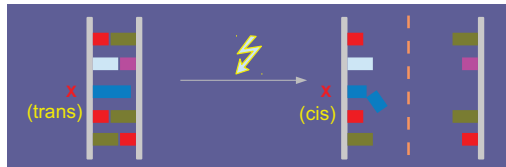
How Does a Photoswitch Control DNA / RNA Unfolding?

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

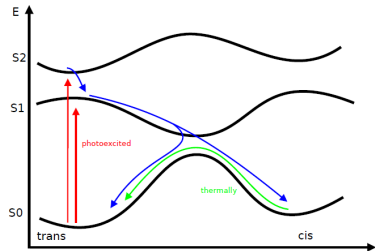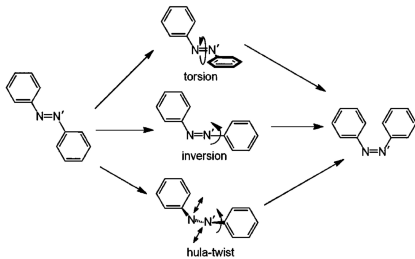QM = Quantum Mechanics
MM = Molecular Mechanics
MD = Molecular Dynamics

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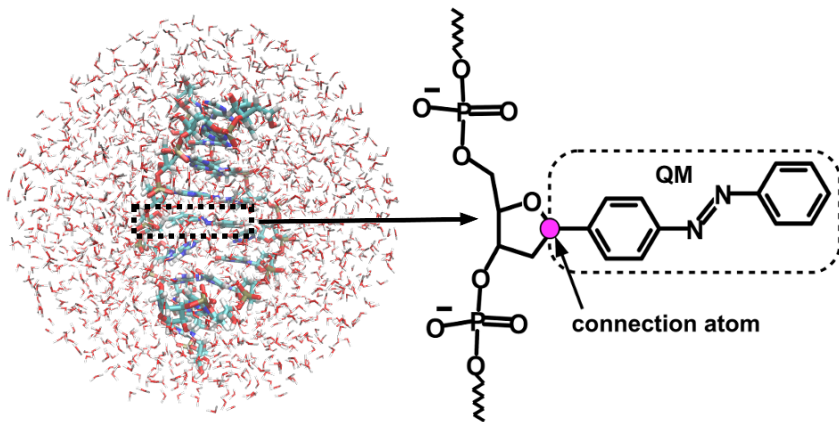QM/MM Simulations
MM-MD Simulations

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)

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Photoregulation of DNA & RNA

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QM/MM Simulations
MM-MD Simulations
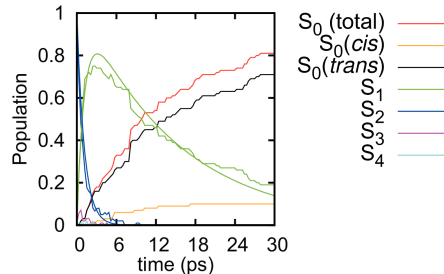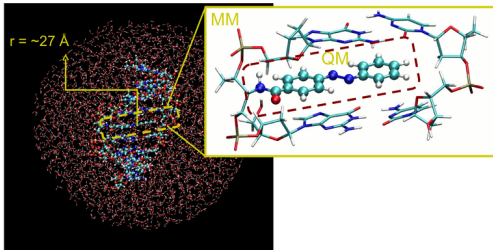
QM/MM simulations

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

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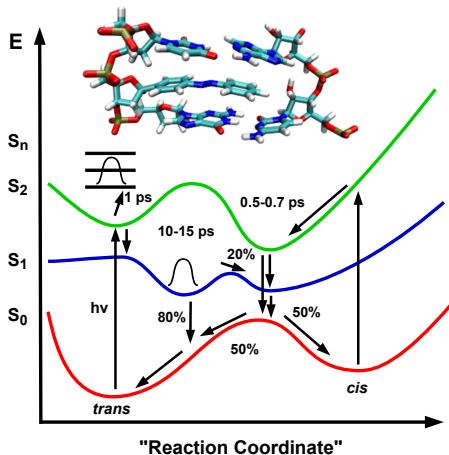**QM/MM Simulations**
MM-MD Simulations

Azobenzene & RNA: QM/MM + Surface Hopping

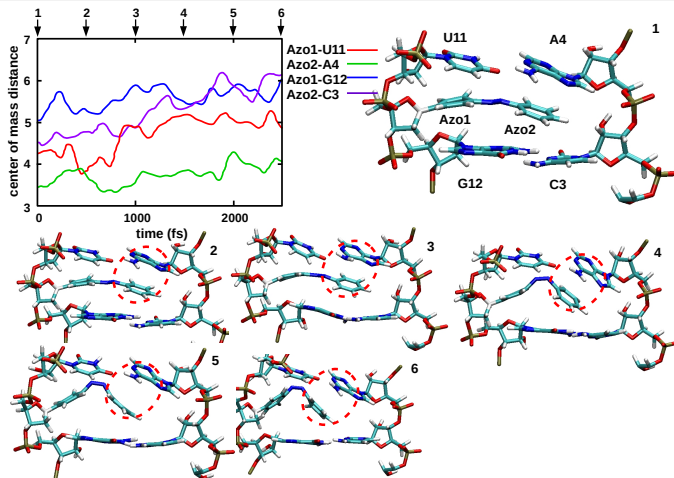QM/MM = Quantum Mechanics/Molecular Mechanics

- *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 $\Phi_{\text{trans-cis}} = 10\%$, $\Phi_{\text{cis-trans}} = 50\%$
- comparatively slow *trans-cis* isomerization: ca. 20 ps instead of 300 fs (gas phase)

Mondal, Granucci, Ortiz-Sanchez, Persico, Burghardt, to be published

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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

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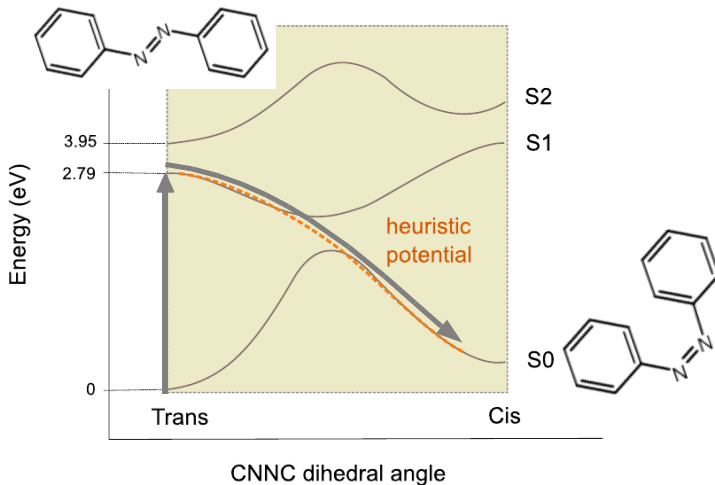**QM/MM Simulations**
MM-MD Simulations

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

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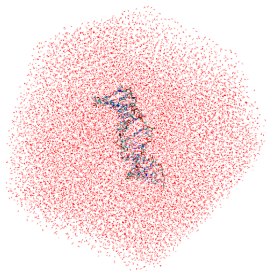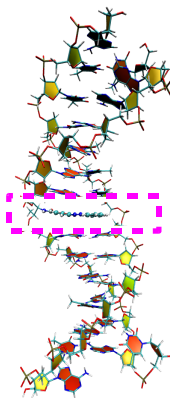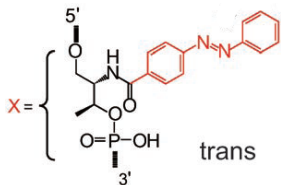QM/MM Simulations
MM-MD Simulations

Drastic simplification: isomerisation via effective potential

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

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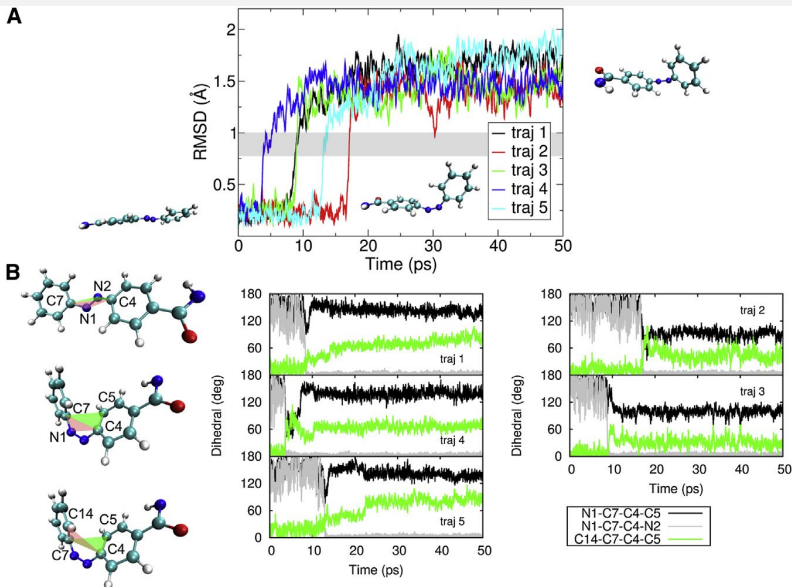QM/MM Simulations
MM-MD Simulations

Simulation Set-Up

- 5'TAAGAAG**X**GAGATAT-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

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QM/MM Simulations
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Trajectory dynamics   Biswas, Burghardt, Biophys. J., 107, 932 (2014)

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Modeling of Photoinduced Uncaging
Photoregulation of DNA & RNA
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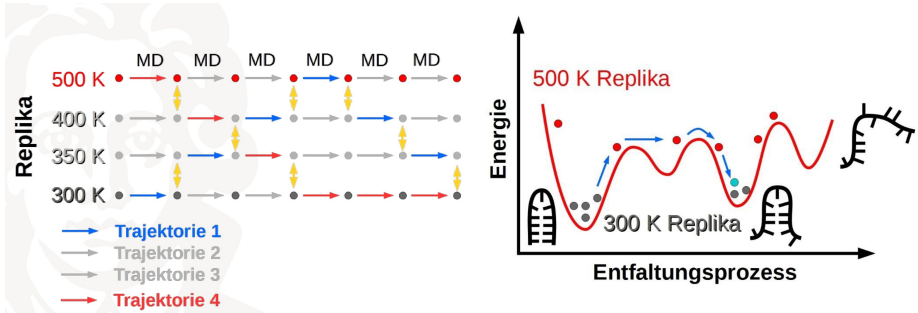QM/MM Simulations
MM-MD Simulations

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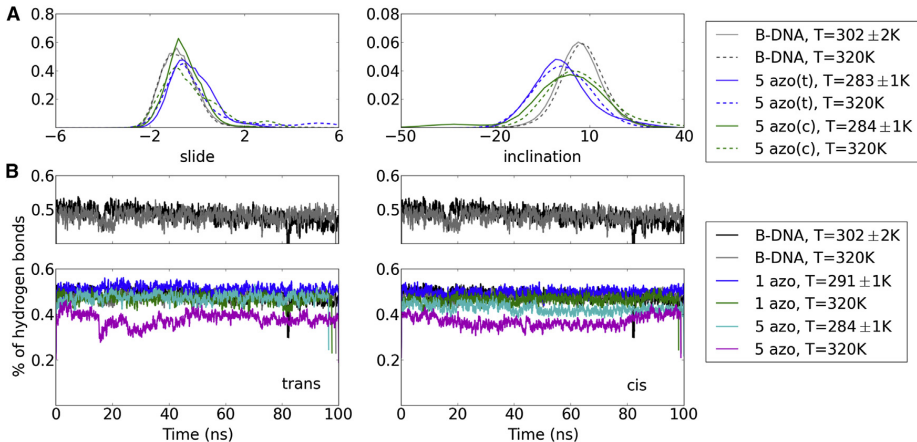
- distribution of helical parameters (slide, inclination) changes noticeably as a function of azobenzene attachment
- in this case, cis and trans forms do not show a significant difference

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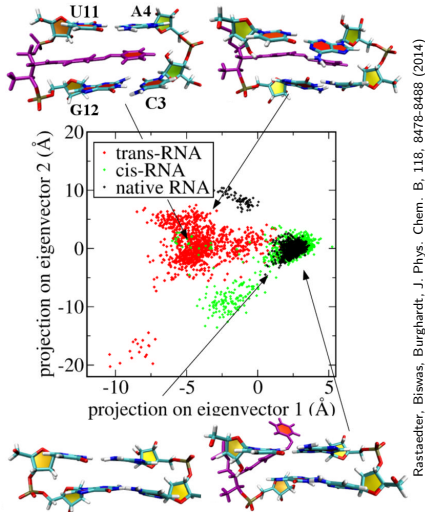QM/MM Simulations
MM-MD Simulations

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
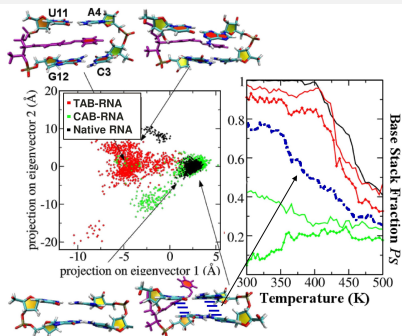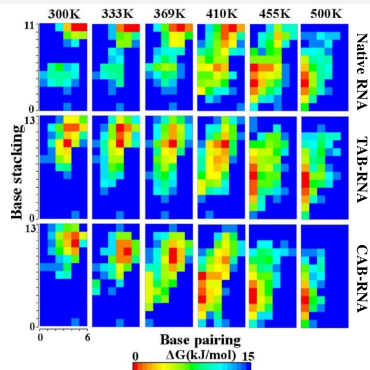
[Sugita, Okamoto, 1999]

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Destabilization of Double Strand Structure

U11   A4

G12   C3

projection on eigenvector 2 (Å)

- trans-RNA
- cis-RNA
- native RNA

projection on eigenvector 1 (Å)

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

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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: $382°/418°/427°$
- scaled values T_m^{scaled}: cis/trans/native: $305°/334°/342°$

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Bottom line: multiscale methods needed!