

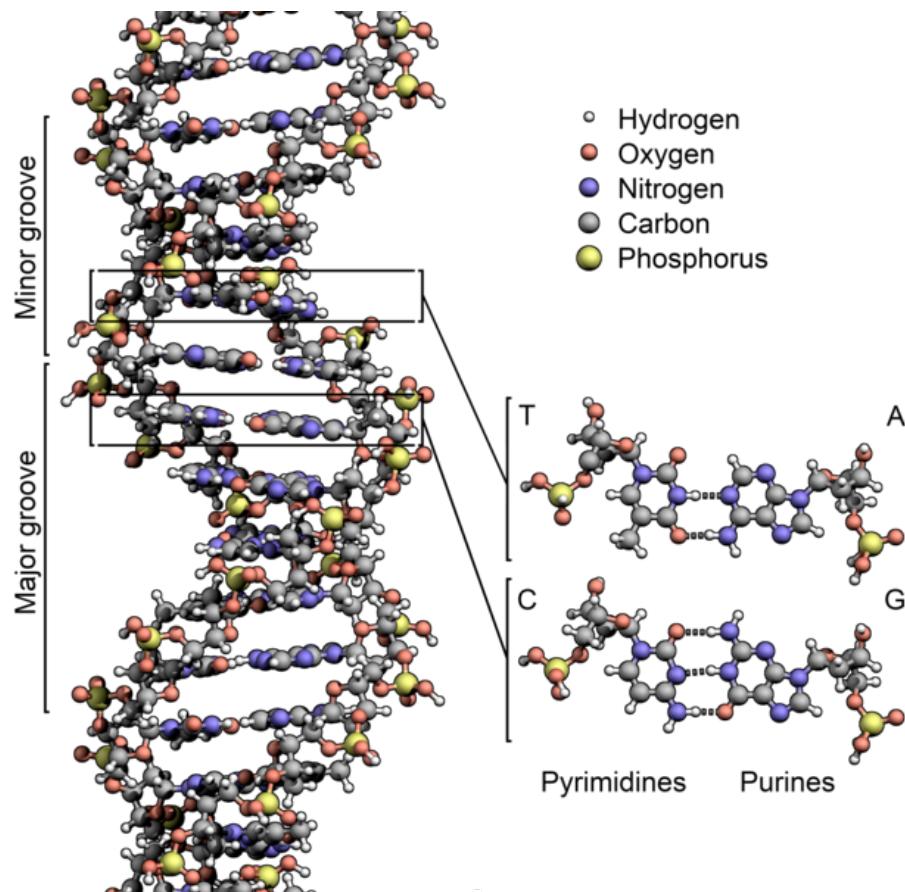
# DNA baserolling

A Friday afternoon project

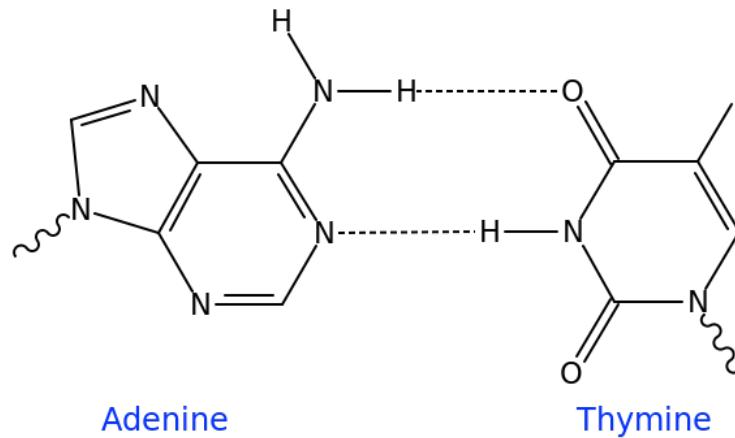
by

Jocelyne Vreede and David W.H. Swenson

# DNA

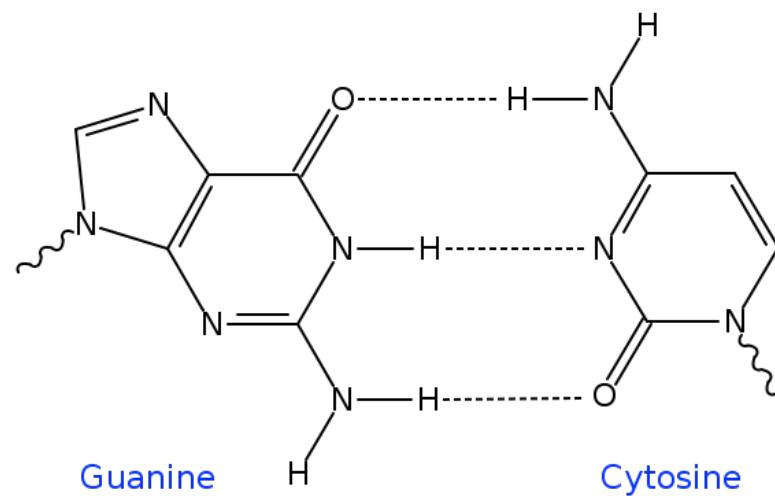


# Watson-Crick pairing



Adenine

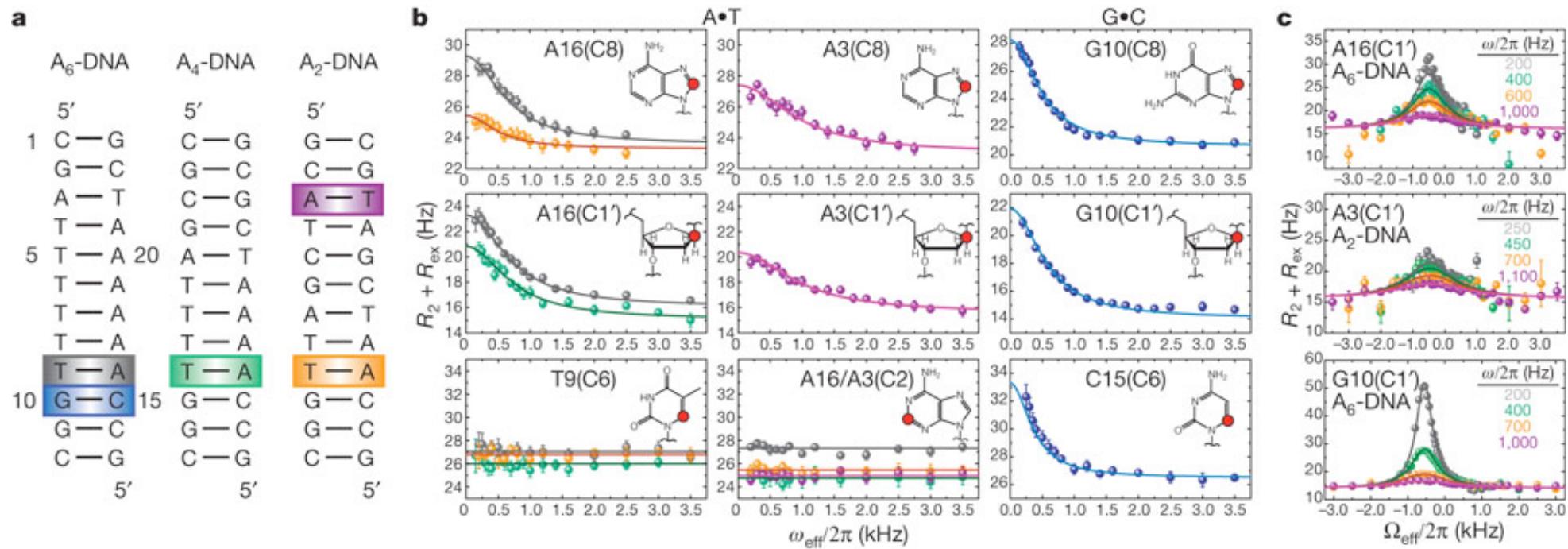
Thymine



Guanine

Cytosine

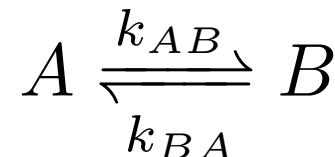
# Probing excited states in DNA with carbon relaxation dispersion NMR



Chemical exchange processes around the glycosidic bond.

# NMR

ground state



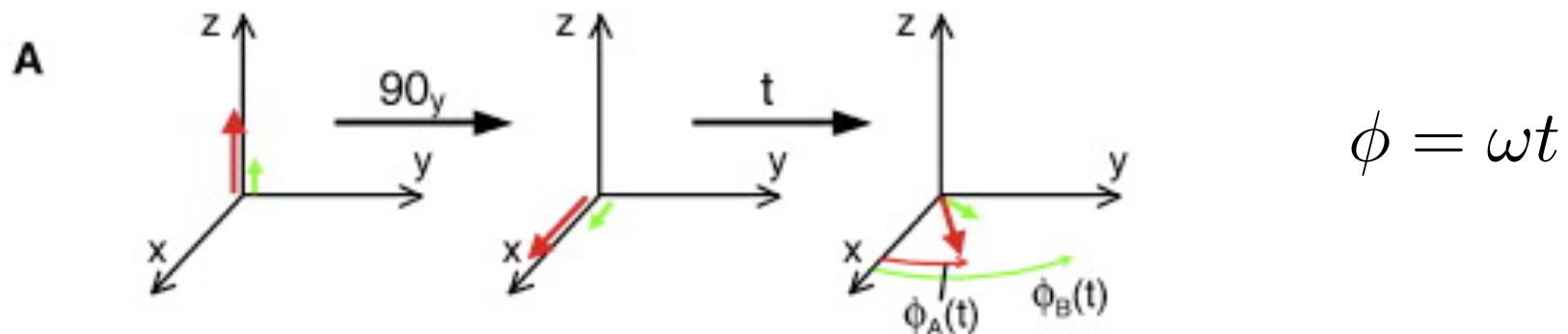
excited state

exchange rate

$$k_{ex,AB} = k_{AB} + k_{BA}$$

assuming TST

$$k_{AB} = \kappa \frac{k_B T}{h} \exp\left(\frac{\Delta G_{A \rightarrow TS(AB)}}{RT}\right)$$



$$\phi = \omega t$$

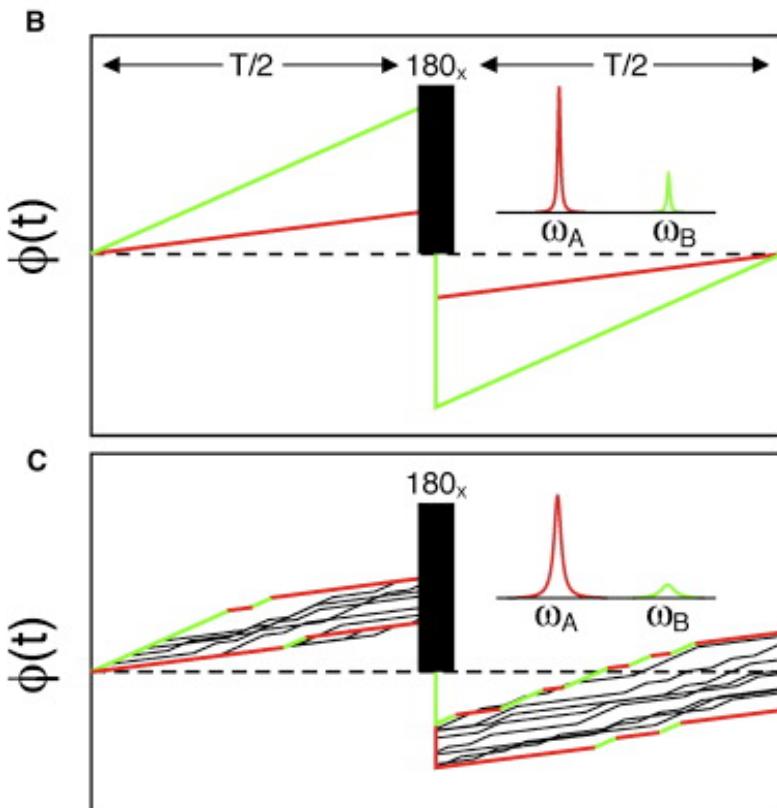
# Chemical exchange

ground state



exchange rate

$$k_{ex,AB} = k_{AB} + k_{BA}$$



180<sub>x</sub>: apply pulse to return magnetization to x-axis

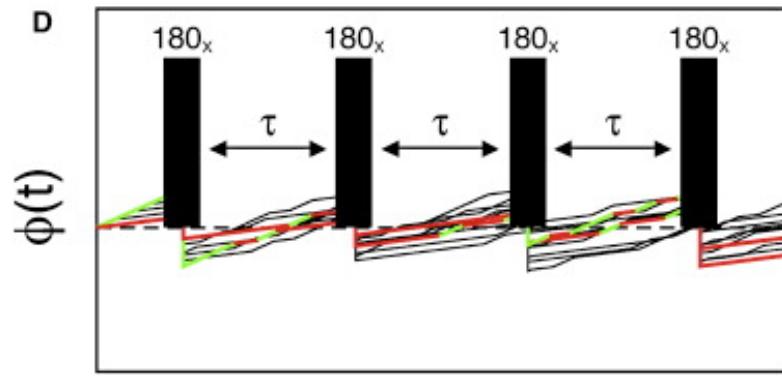
$$k_{ex,AB} \ll \Delta\omega_{AB}$$

well-separated resonances

$$k_{ex,AB} \approx \Delta\omega_{AB}$$

line-broadening

# Relaxation dispersion NMR



$180_x$ : apply pulse to return magnetization to x-axis  
succession of pulses at time interval tau

$$\tau \geq k_{ex,AB}$$

$$\nu_{CPMG} = \frac{1}{2\tau}$$

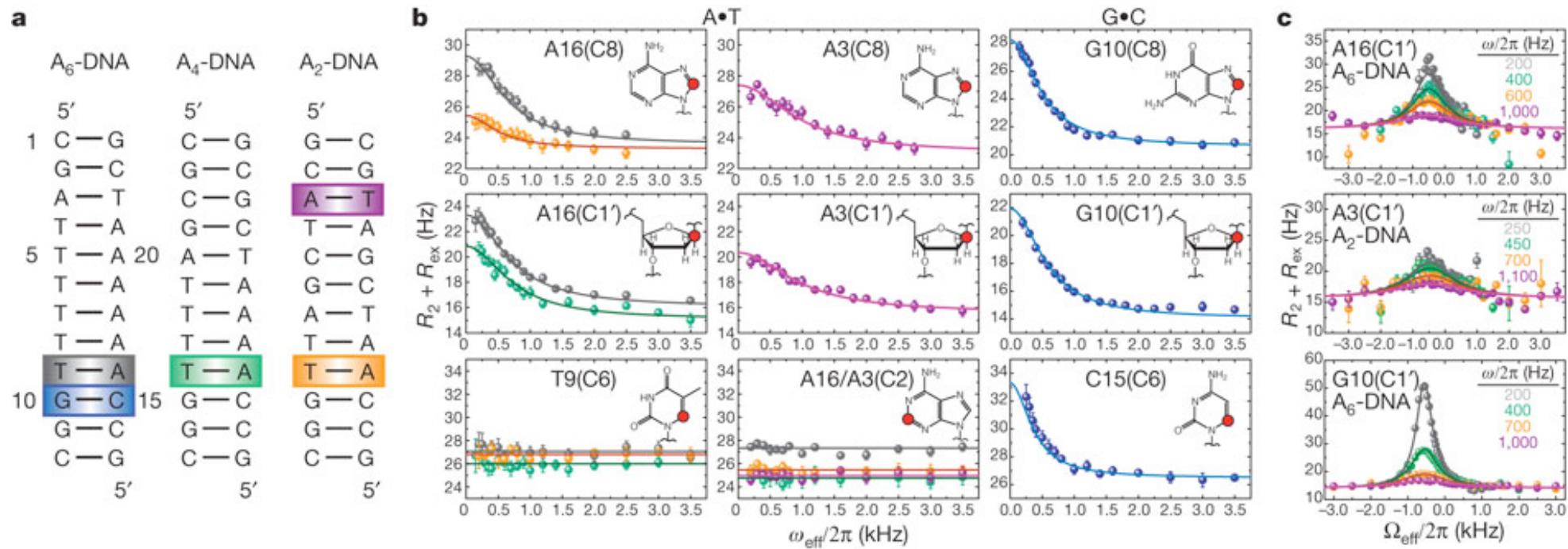
$$R_{eff}(\nu_{CPMG}) = -\ln(I(\nu_{CPMG})/I_0)/T$$

I: Intensity of ground state

RD-NMR can detect excited states populated as little as 0.5 %, if

- exchange at ms time scale
- sizable chemical shift change

# Probing excited states in DNA with carbon relaxation dispersion NMR



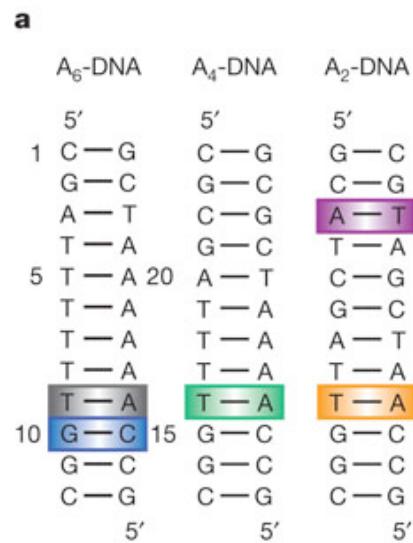
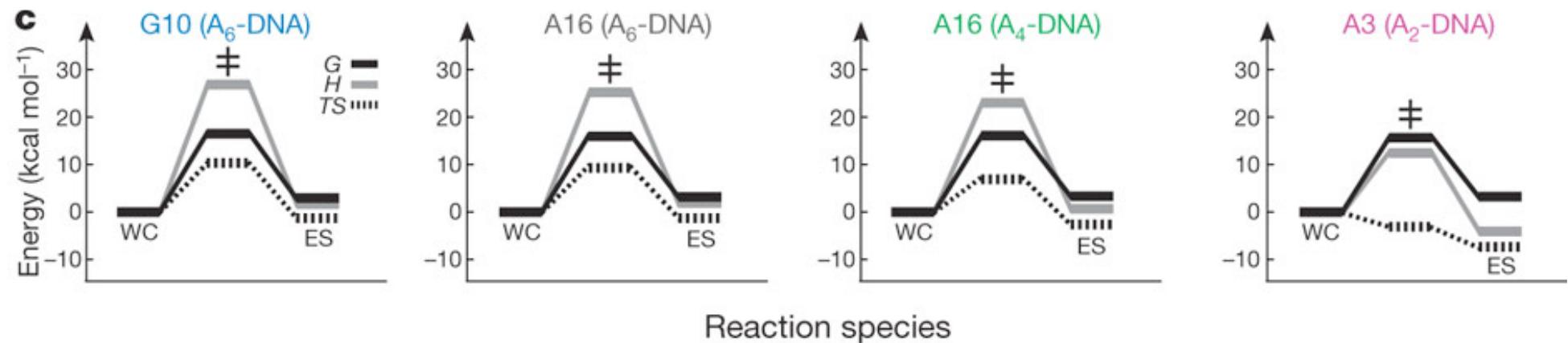
Chemical exchange processes around the glycosidic bond.

# Thermodynamic analysis

A16 (C8/C1') A <sub>6</sub> -DNA <sup>b</sup>	$p_{B,C8}$ (%)	0.378±0.018	0.386±0.019	0.359±0.018	0.384±0.021	0.393±0.072	0.859±0.491
	$p_{B,C1'}$ (%)				0.345±0.036	0.418±0.032	0.353±0.048
	$k_{ex,C8}$ (s <sup>-1</sup> )	944±78	3680±200	6080±390	936±98	3290±390	5930±420
	$k_{ex,C1'}$ (s <sup>-1</sup> )				1020±160	3780±240	6170±430
	$k_{A,C8}$ (s <sup>-1</sup> )				3.60±0.42	12.9±2.9	50.9±30.3
	$k_{A,C1'}$ (s <sup>-1</sup> )	3.57±0.34	14.2±1.03	21.8±2.3	3.53±0.67	15.8±1.6	21.8±3.33
	$k_{B,C8}$ (s <sup>-1</sup> )				936±98	3280±390	5880±930
	$k_{B,C1'}$ (s <sup>-1</sup> )	941±78	3670±200	6060±390	1020±160	3760±230	6150±430
	$R_{1,C8}$ (Hz)	1.70±0.02	2.41±0.04	2.67±0.04	1.70±0.02	2.41±0.04	2.67±0.04
	$R_{2,C8}$ (Hz)	29.1±0.1	23.0±0.2	20.4±0.3	29.1±0.1	23.0±0.2	20.4±0.3
	$R_{1,C1'}$ (Hz)	1.64±0.05	1.97±0.04	1.98±0.04	1.64±0.05	1.99±0.04	1.98±0.04
	$R_{2,C1'}$ (Hz)	19.5±0.2	15.6±0.2	14.3±0.1	19.5±0.2	15.5±0.2	14.5±0.1
	$\Delta\omega_{AB,C8}$ (ppm)		2.31±0.05		2.30±0.07	2.21±0.22	1.45±0.45
	$\Delta\omega_{AB,C1'}$ (ppm)		3.35±0.08		3.58±0.15	3.20±0.15	3.41±0.22

$$\ln \left( \frac{k_i(T)}{T} \right) = \ln \left( \frac{k_B \kappa}{h} \right) - \frac{\Delta G_i^T}{RT_{hm}} - \frac{\Delta H_i^T}{R} \left( \frac{1}{T} - \frac{1}{T_{hm}} \right)$$

# relaxation dispersion as function of temperature

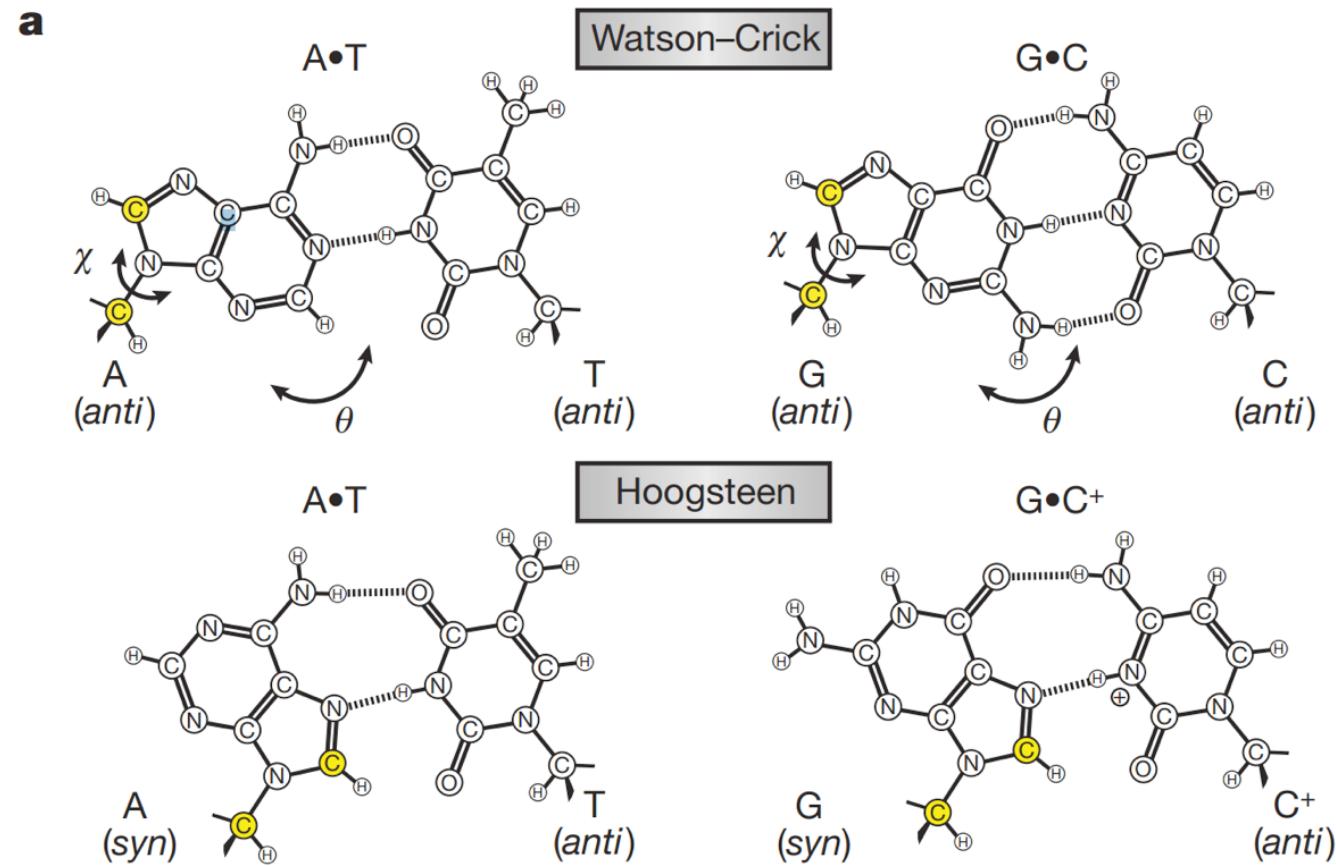


Activation free energy

~16 kcal/mol

Small free energy difference between  
WC and excited state

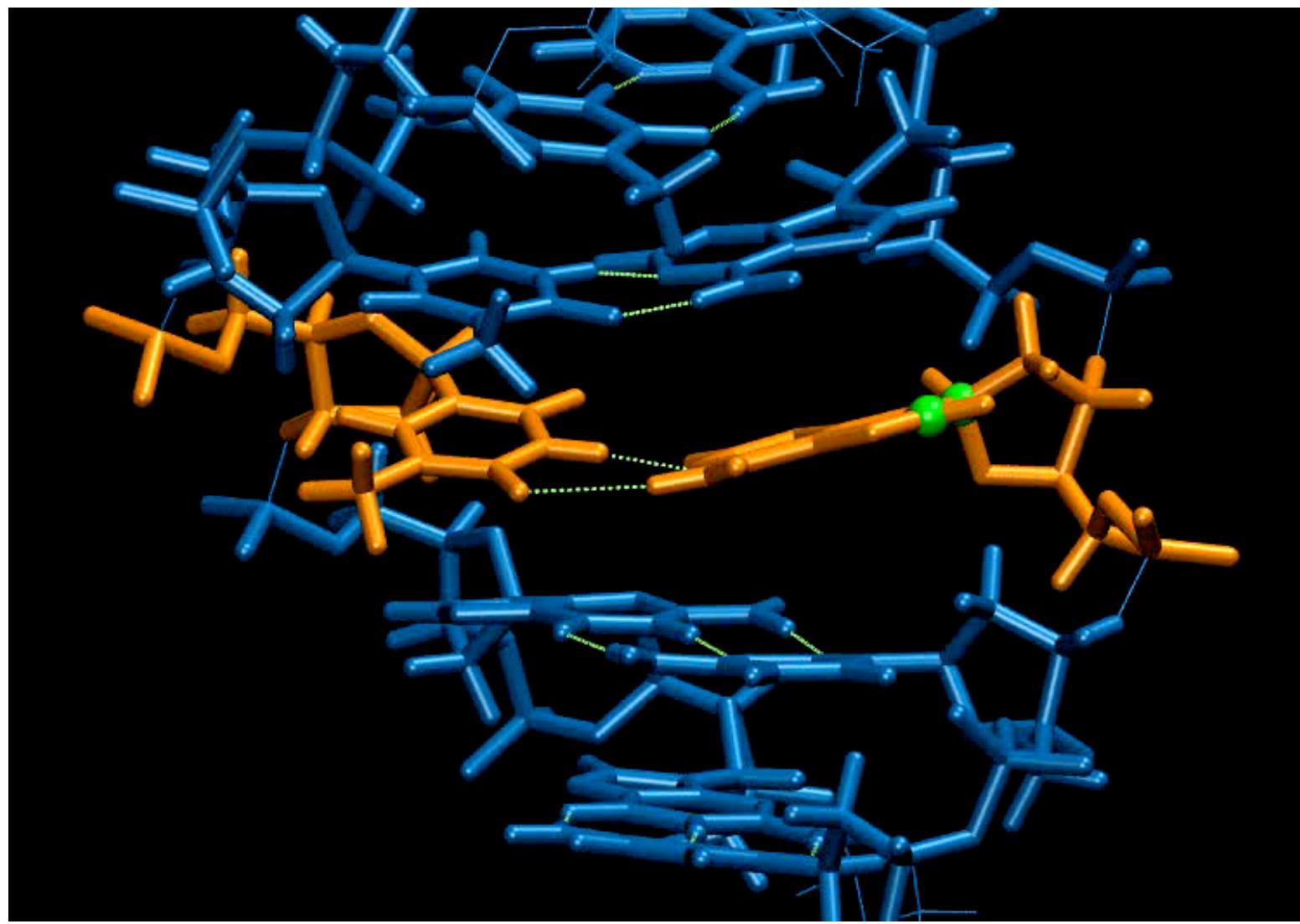
# Hoogsteen basepairing



# CPR

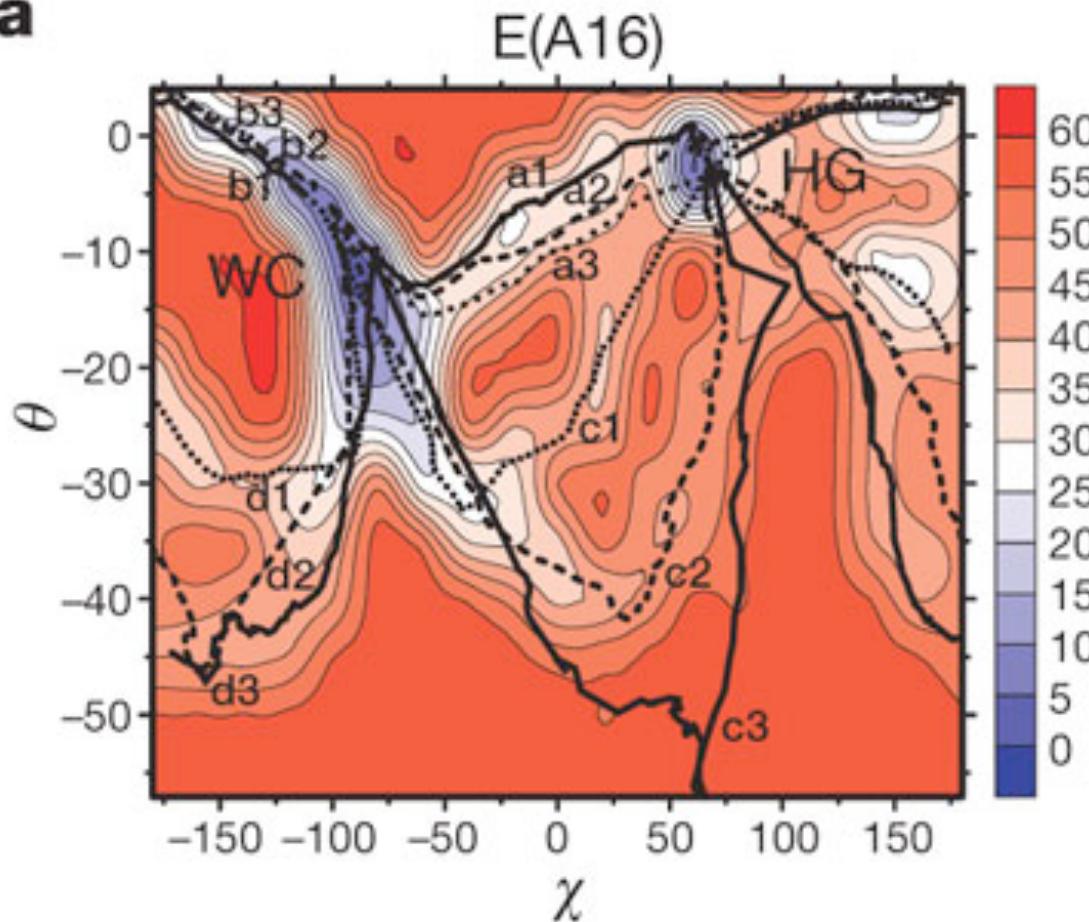
## conjugate peak refinement

- method aimed at finding a series of true saddle points on a potential energy surface.
- requires reactant and product structures as input, and reaction coordinates along which intermediate conformations are constructed.
- results in a string of points in conformation space that follows adiabatic valleys.

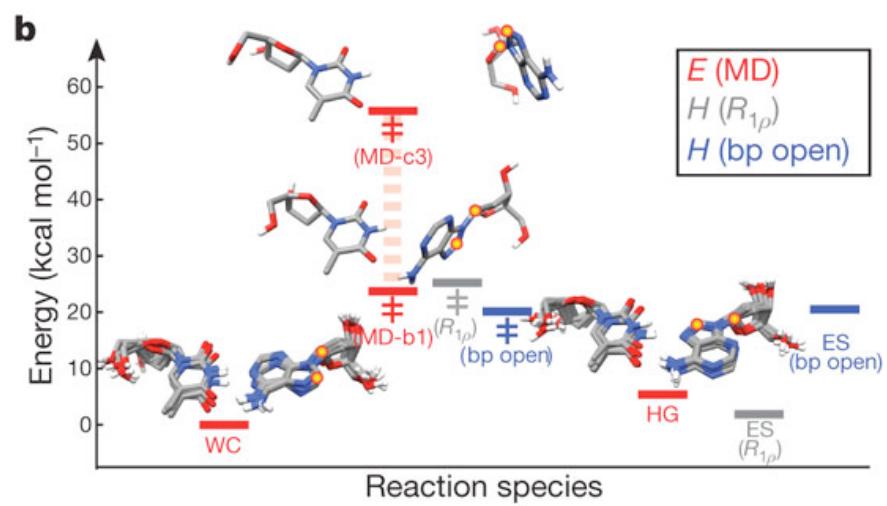


# Transition

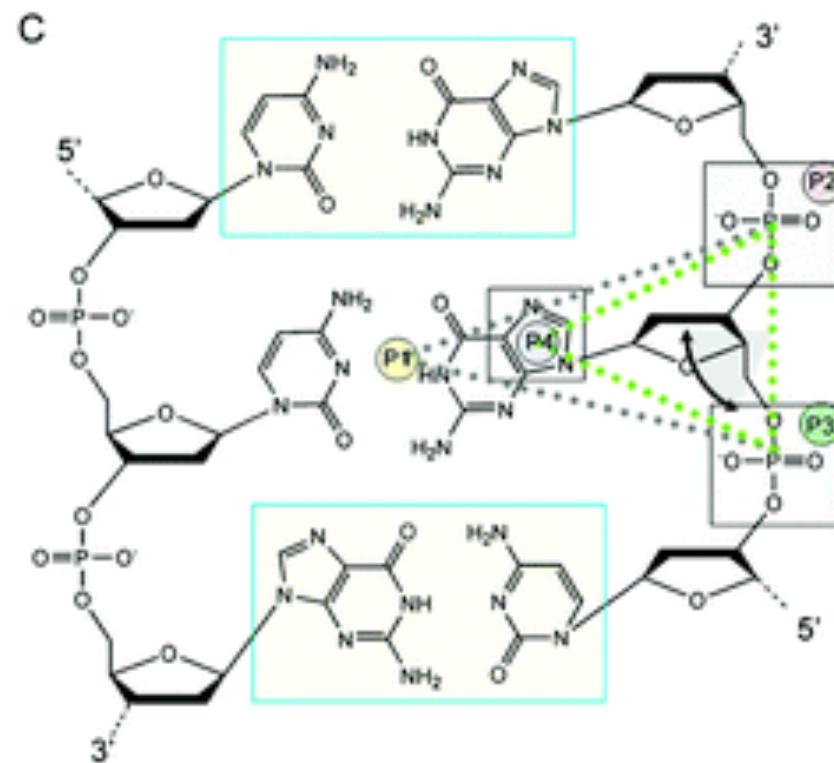
a



b



# base opening angle



# The system

a

A<sub>6</sub>-DNA

5'

1 C—G

G—C

A—T

T—A

5 T—A 20

T—A

T—A

T—A

10 T—A 15

G—C

C—G

5'

DNA in ideal B-DNA conformation

5' –GGATTTTTGGC–3'

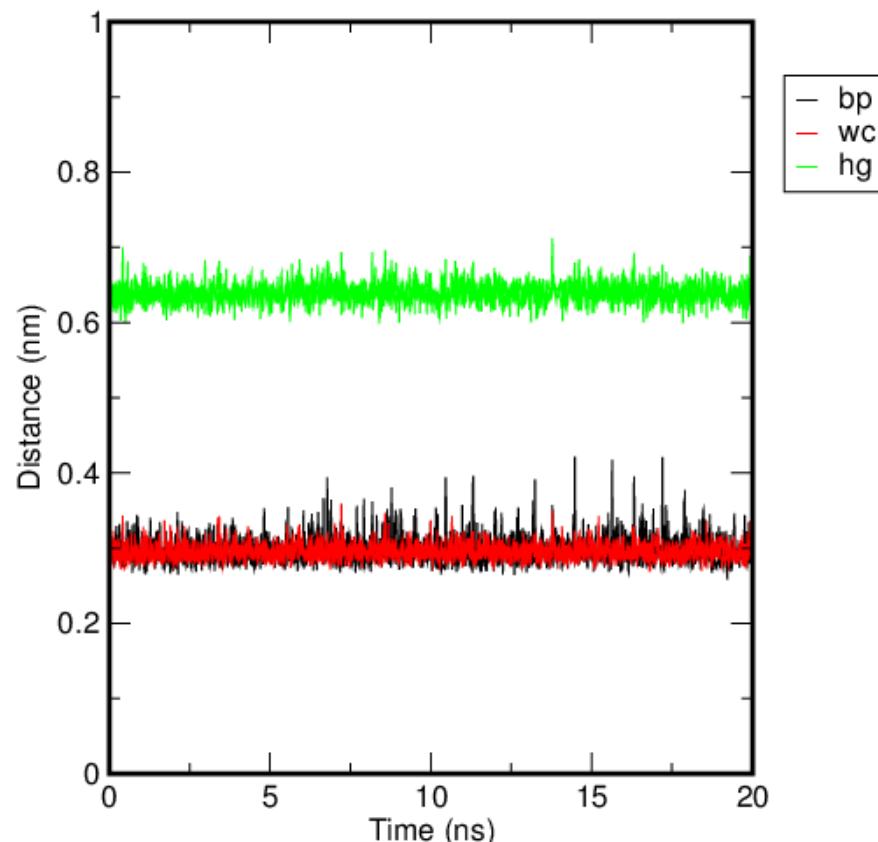
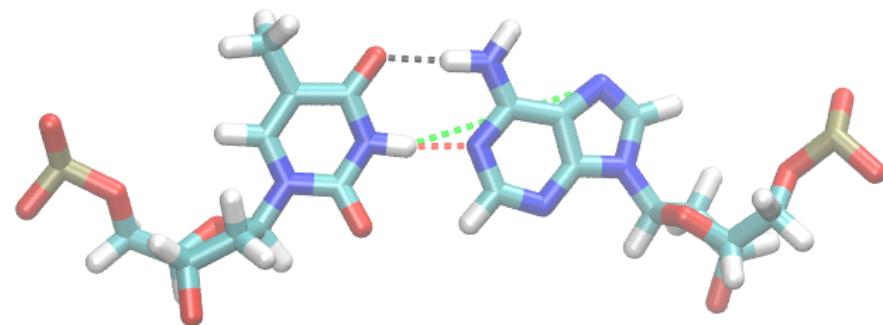
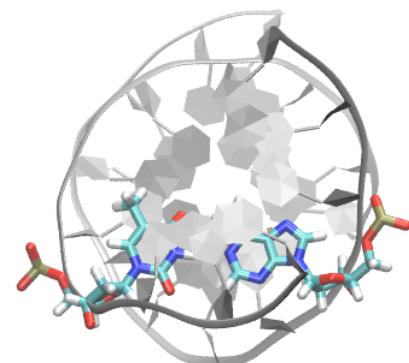
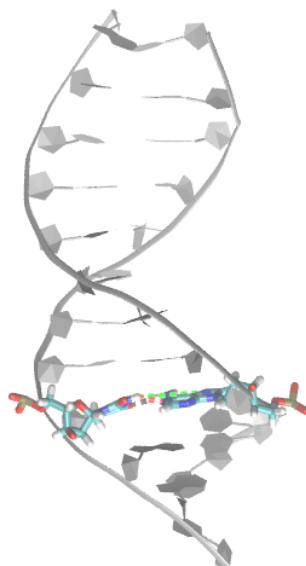
3' –CCTAAAAAAACCG–5'

AMBER03

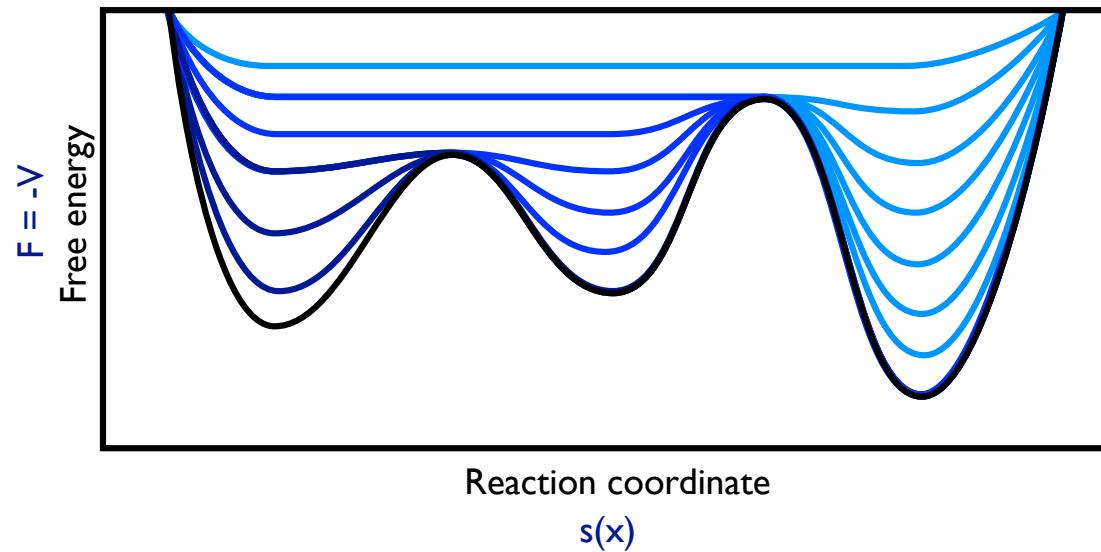
TIP3P water

NpT ensemble

20868 atoms



# Metadynamics



$$V_{bias}(s(x), t) = \sum_{t' < t} H \exp \left( \frac{-|s(x) - s(x_G(t'))|^2}{2w^2} \right)$$

$$F(s) = - \lim_{t \rightarrow \infty} V_{bias}(s, t)$$

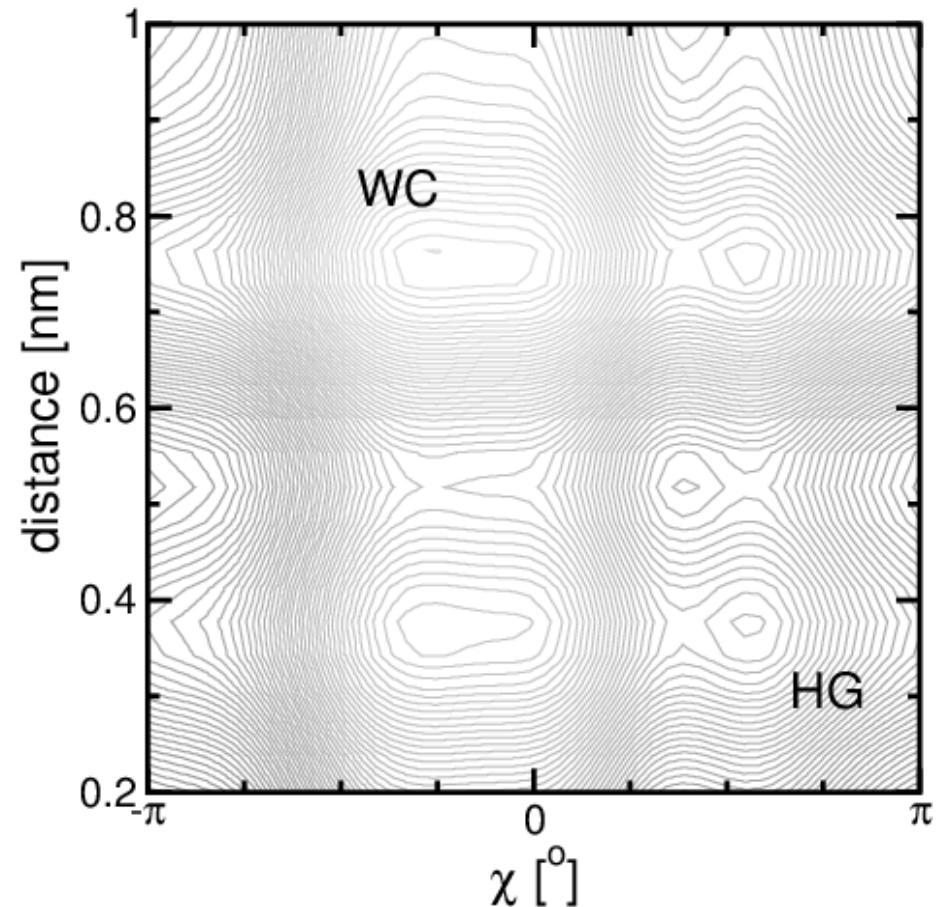
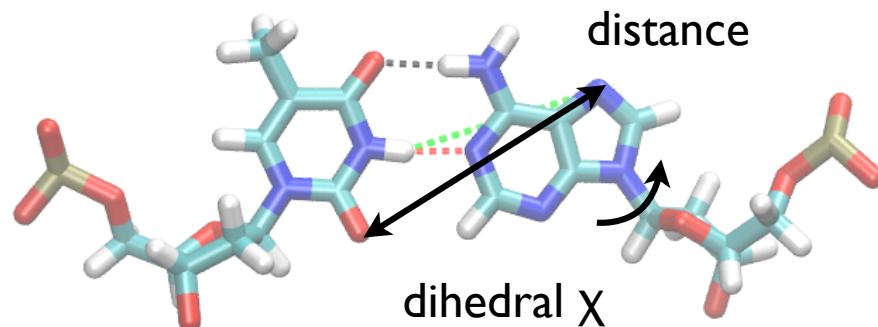
# Biased transition

2-D metadynamics

width ( $w$ ): 0.1 nm; 0.35 rad

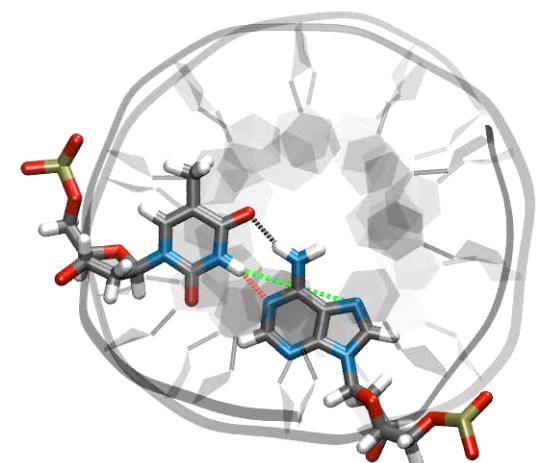
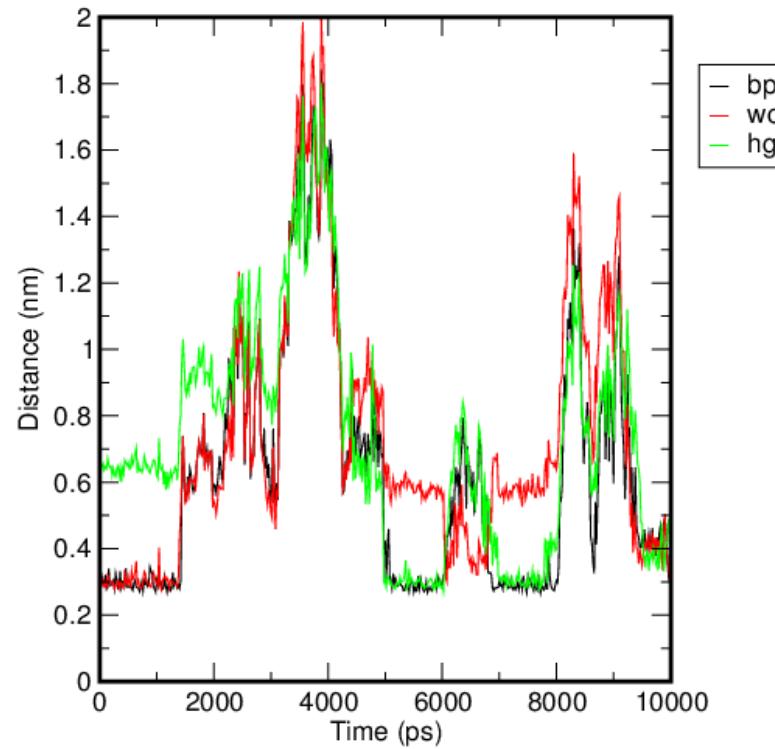
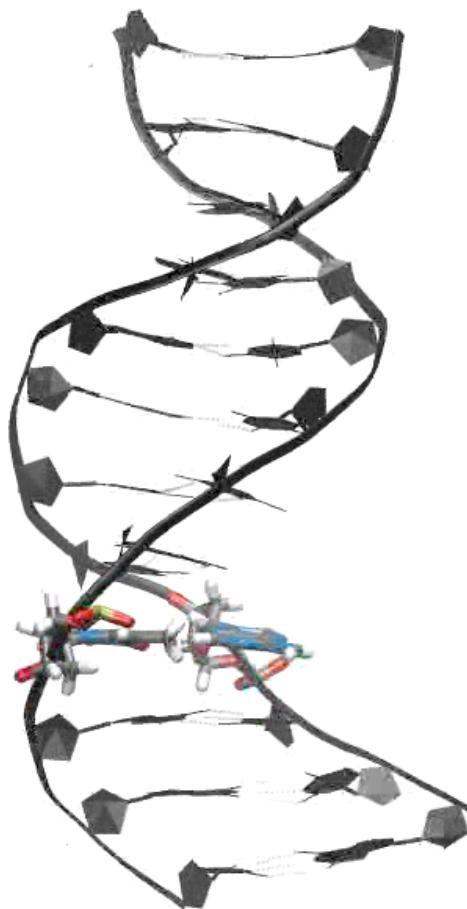
height ( $H$ ): 0.25 kJ/mol

deposition frequency: 0.5 ps<sup>-1</sup>

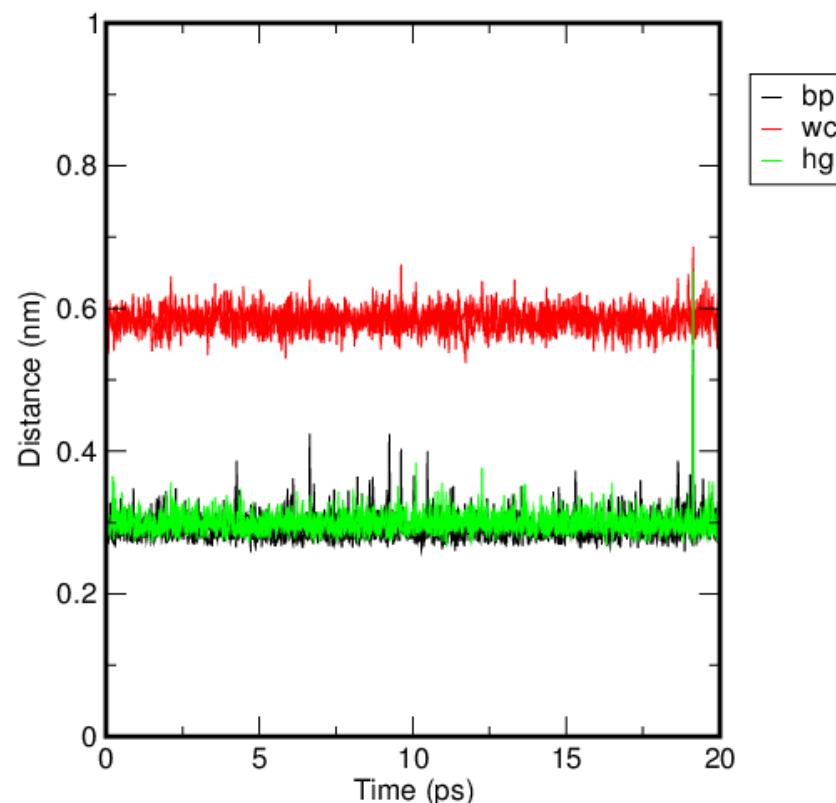
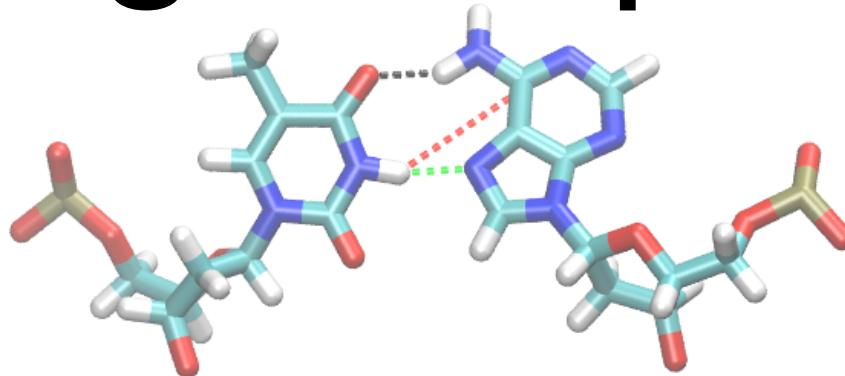


contoured at 5  $k_B T$

# Only one transition

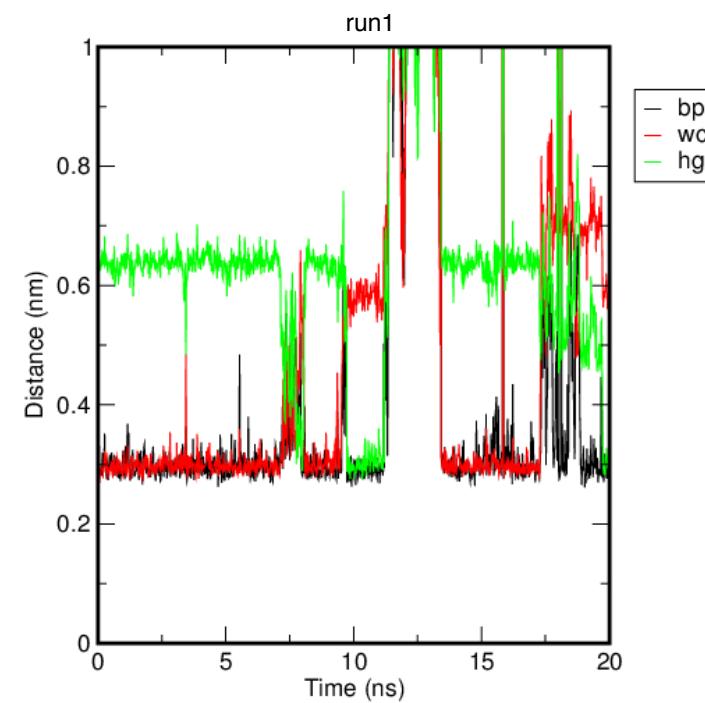
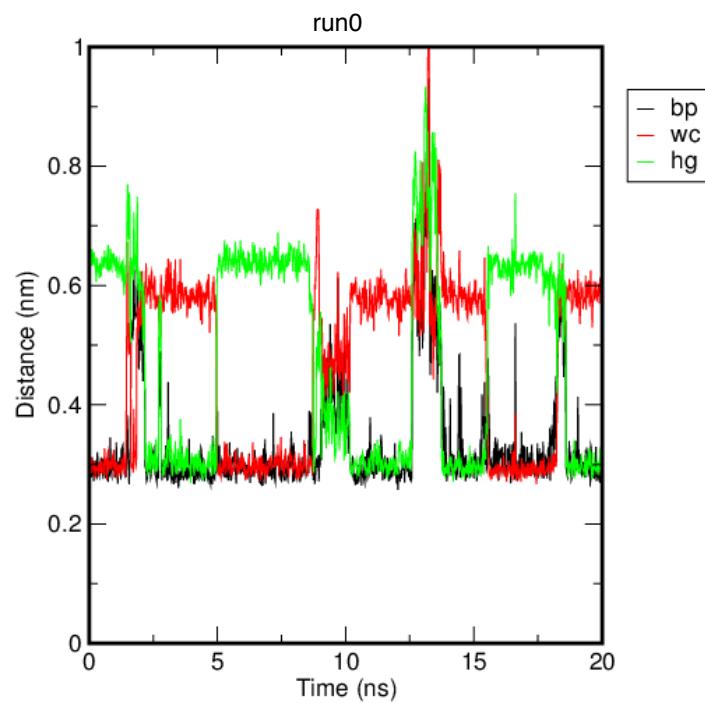


# Hoogsteen pairing

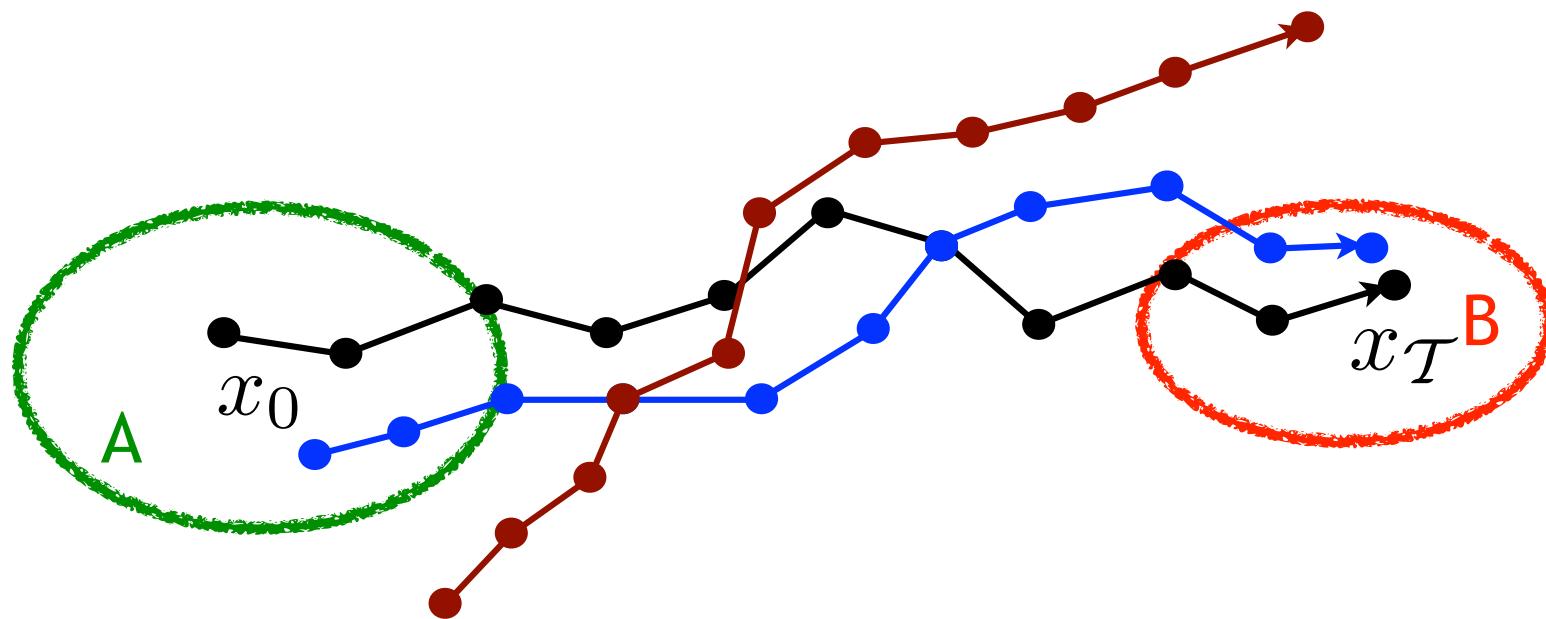


# Stable state check

5x200ns, started from final  
configuration 20ns MD run



# Transition path sampling



$$P_{acc}[x^{(o)}(\mathcal{T}) \rightarrow x^{(n)}(\mathcal{T})] = h_A[x_0^{(n)}]h_B[x_{\mathcal{T}}^{(n)}]$$

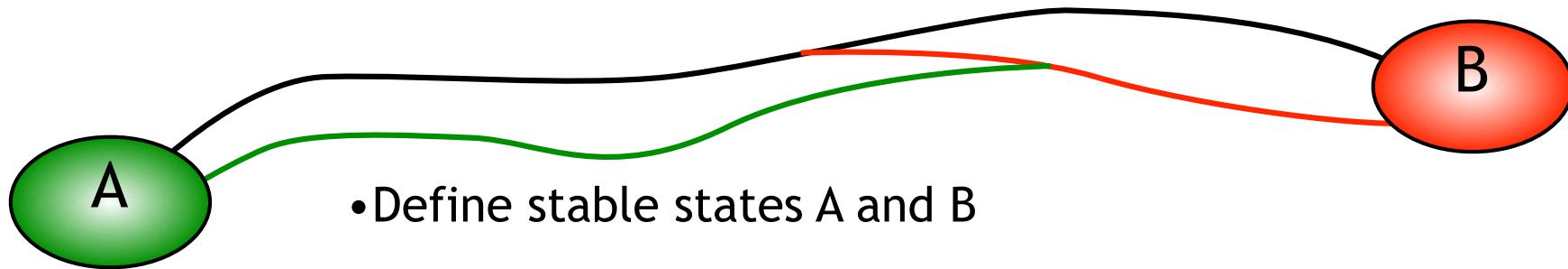
Rejection:

Shooting point too far from the barrier

Intermediate state traps the path

$$h_A(t) = \begin{cases} 1 & x_t \in A \\ 0 & x_t \notin A \end{cases}$$

# Optimal TPS algorithm for protein conformational changes

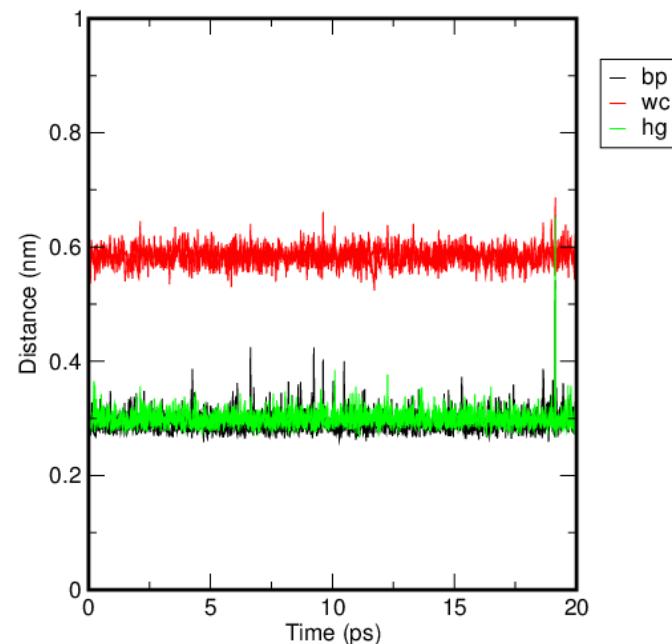
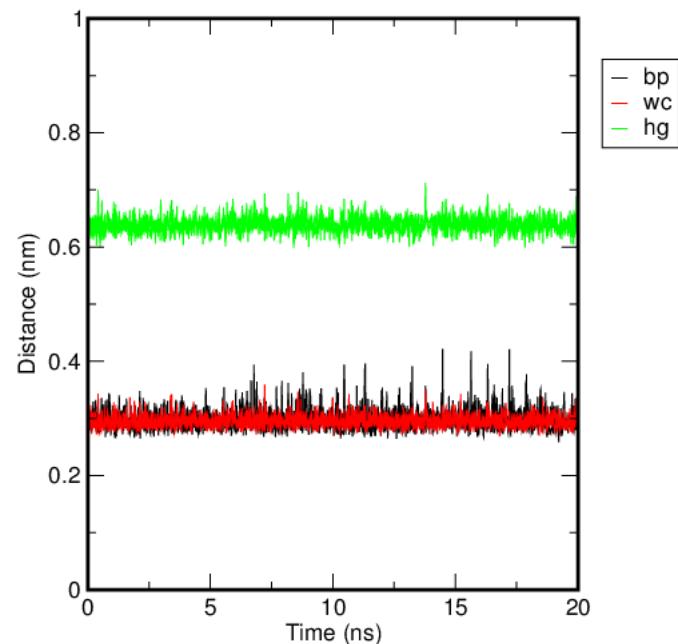
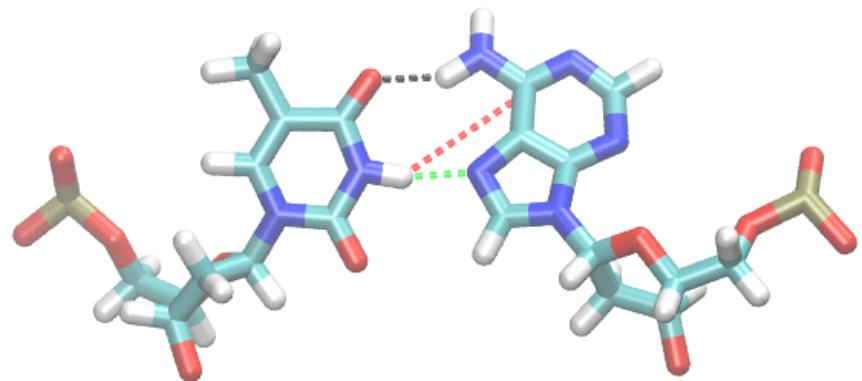
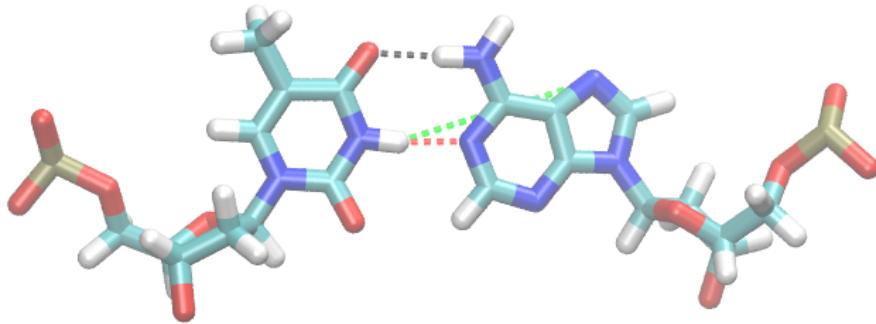


- Define stable states A and B
- Start from initial path
- Draw random number  $\alpha_2$  in  $(0,1]$ .  
If  $\alpha_2 > 0.5$ , integrate forward in time,  
else integrate backward in time: reverse momenta.
- Propagate system with MD and stochastic thermostat until reaching
  - maximum path length
  - a stable state
- Concatenate new trajectory with relevant part of old trajectory.
- If resulting pathway connects A and B accept, else reject.
- Repeat

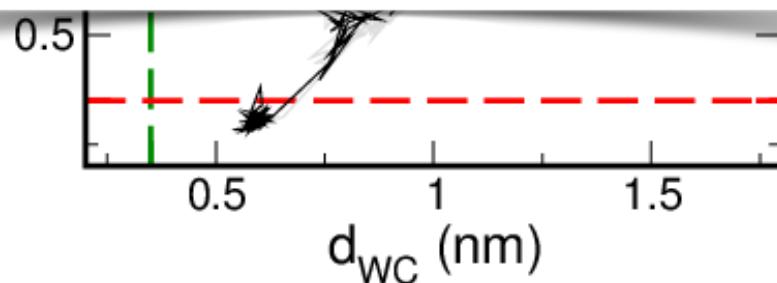
A

basepair (bp) hydrogen bond  
WC hydrogen bond  
HG hydrogen bond

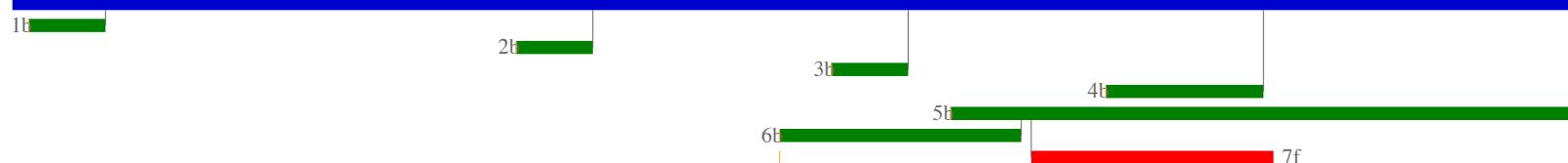
B

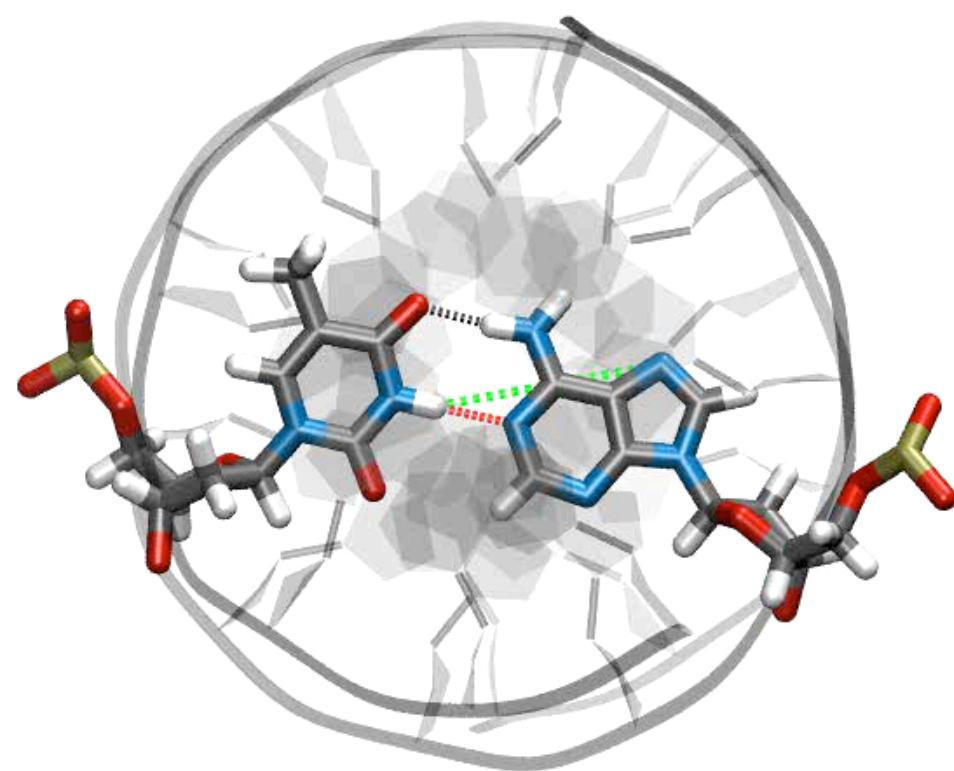
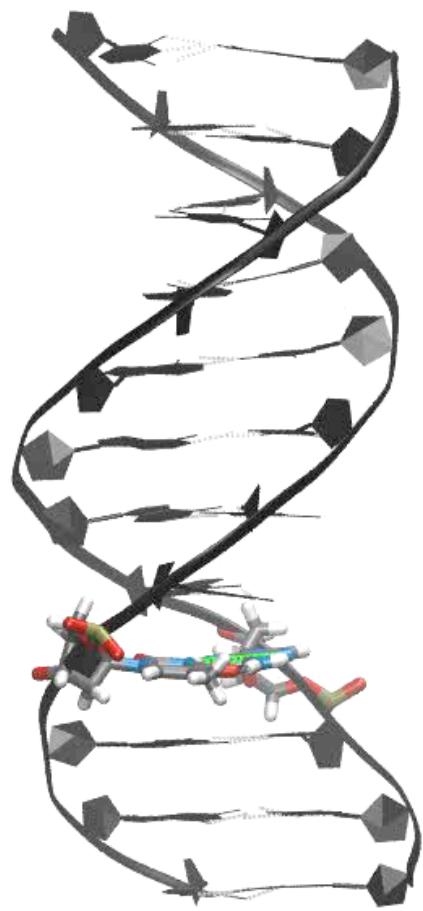


# Initial path



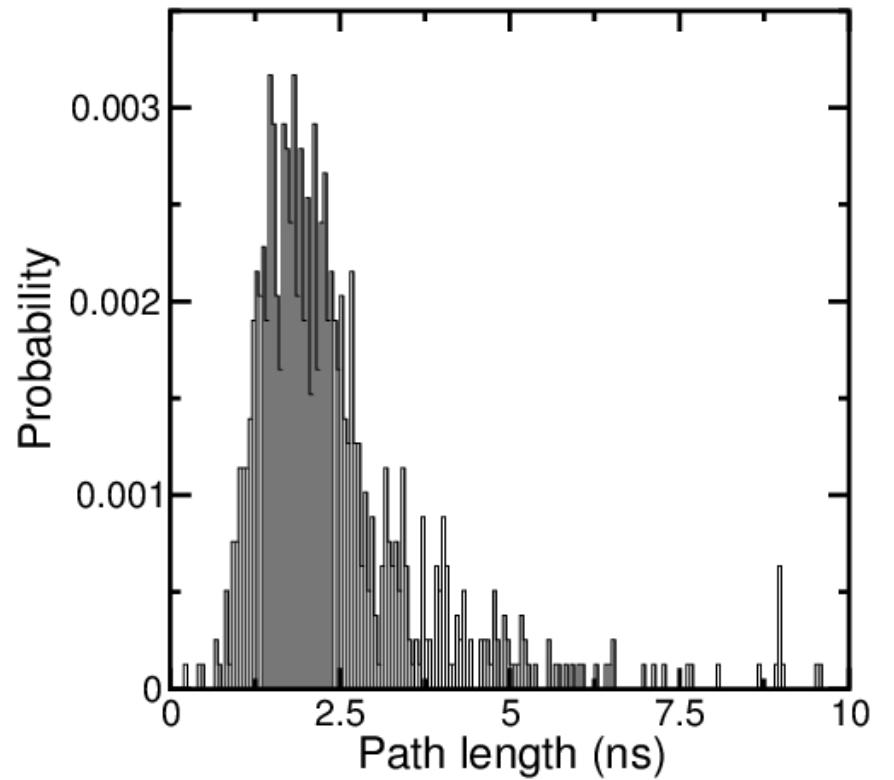
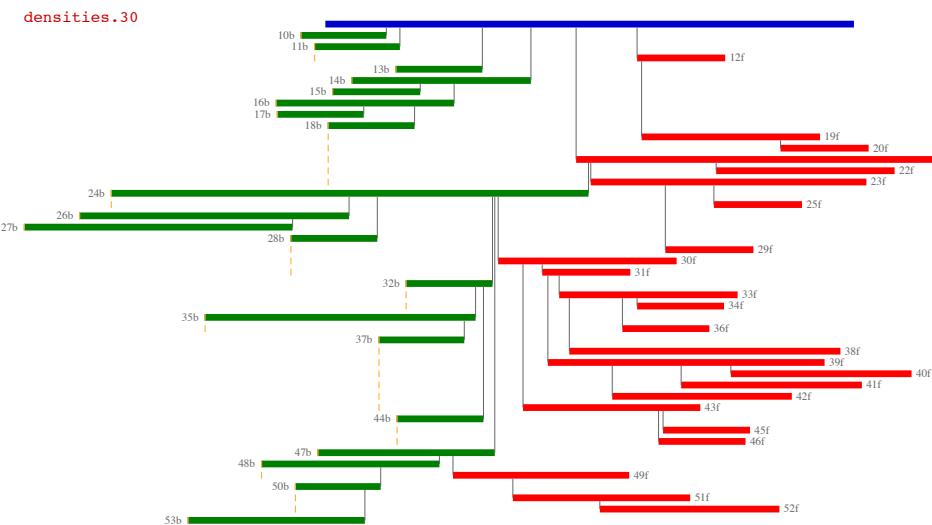
prokin01.14



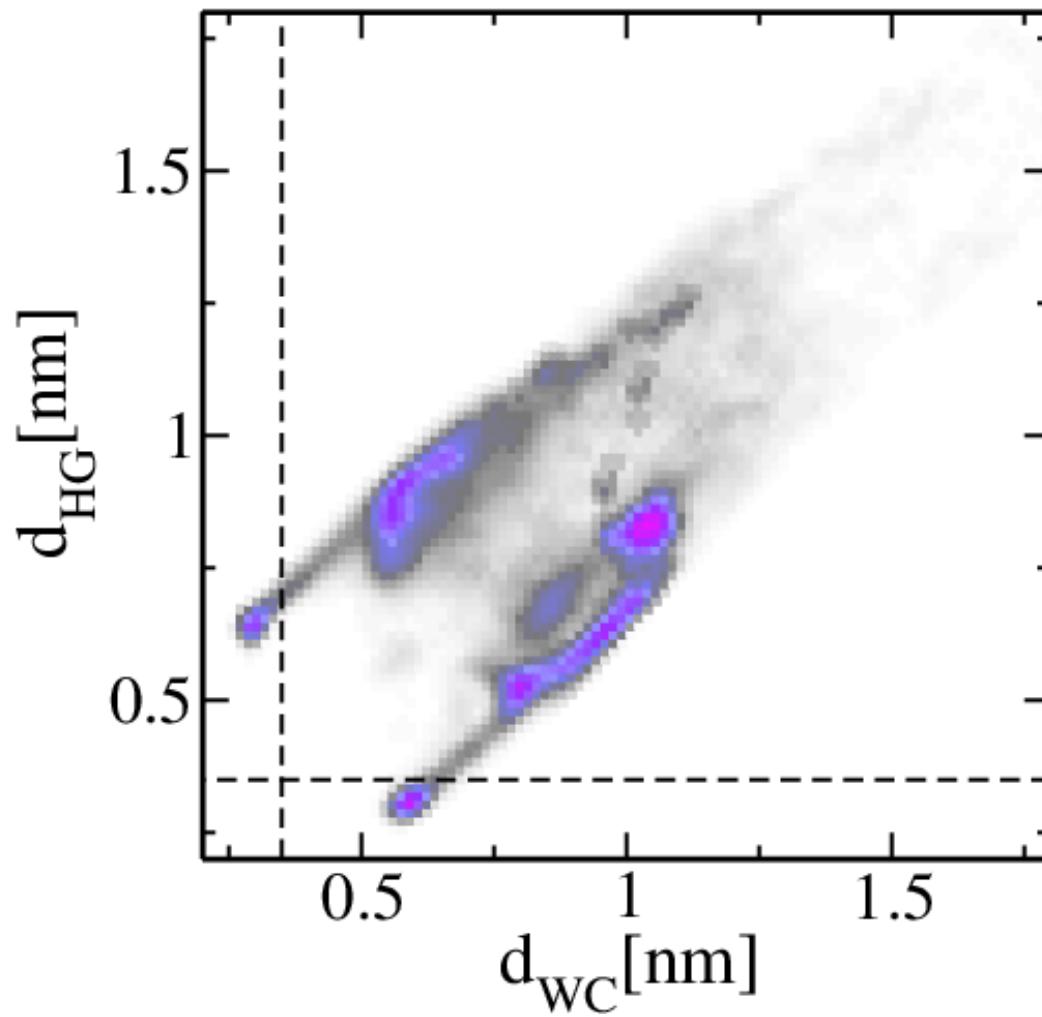


# TPS results

- 854 accepted paths
- acceptance ratio ~50 %
- average path length 2 ns
- ~50 decorrelated paths

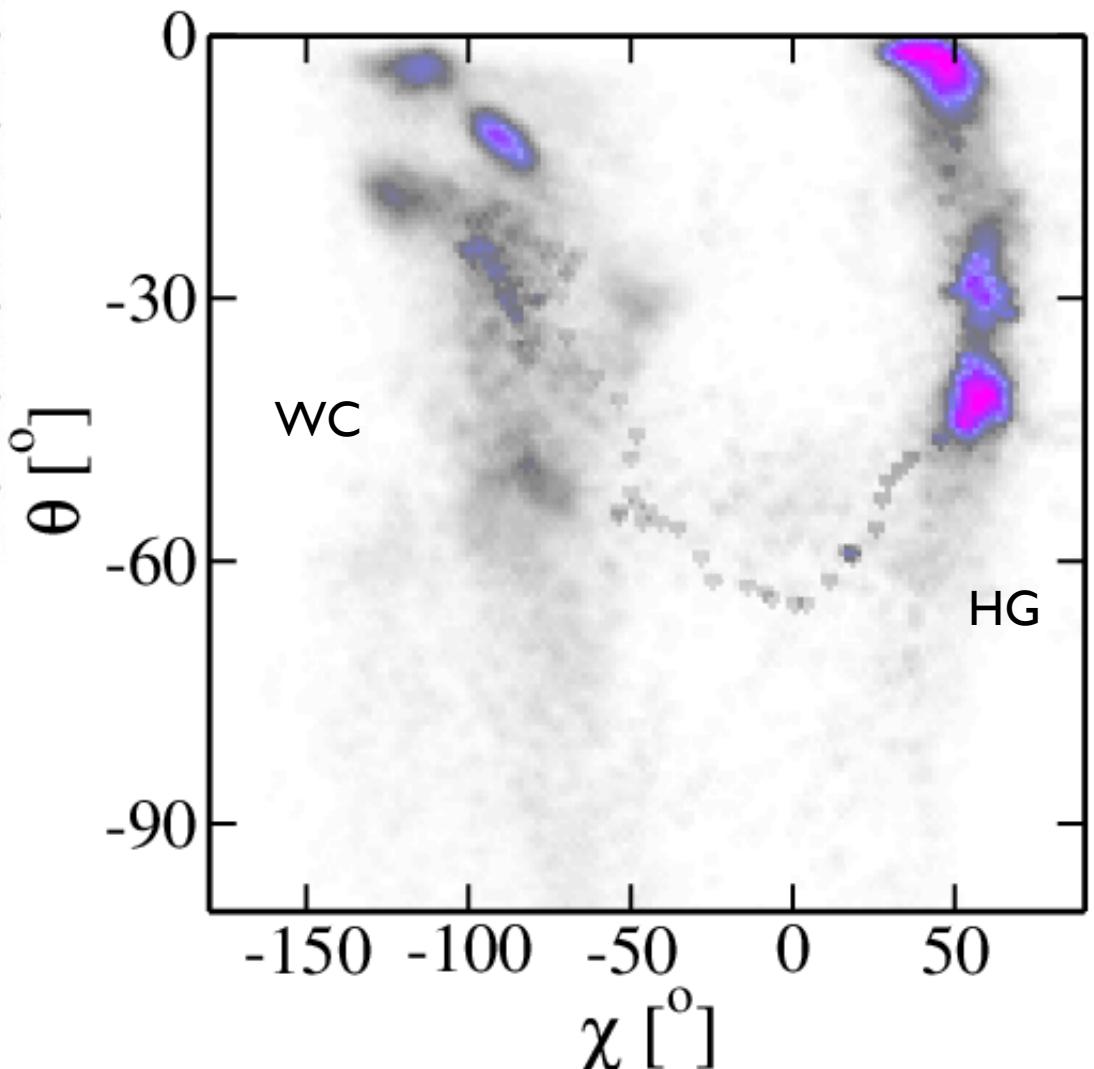
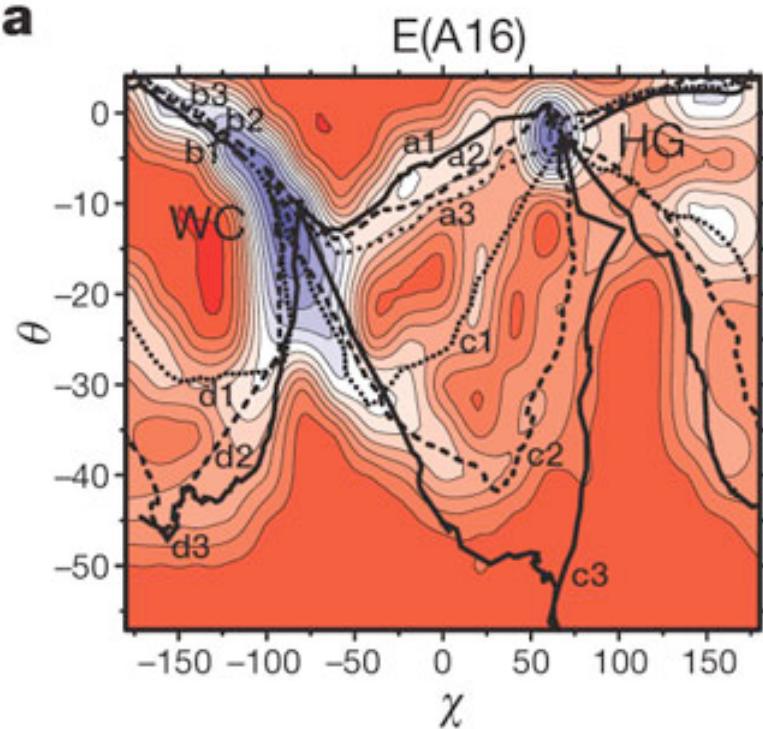


# path density



# Comparison

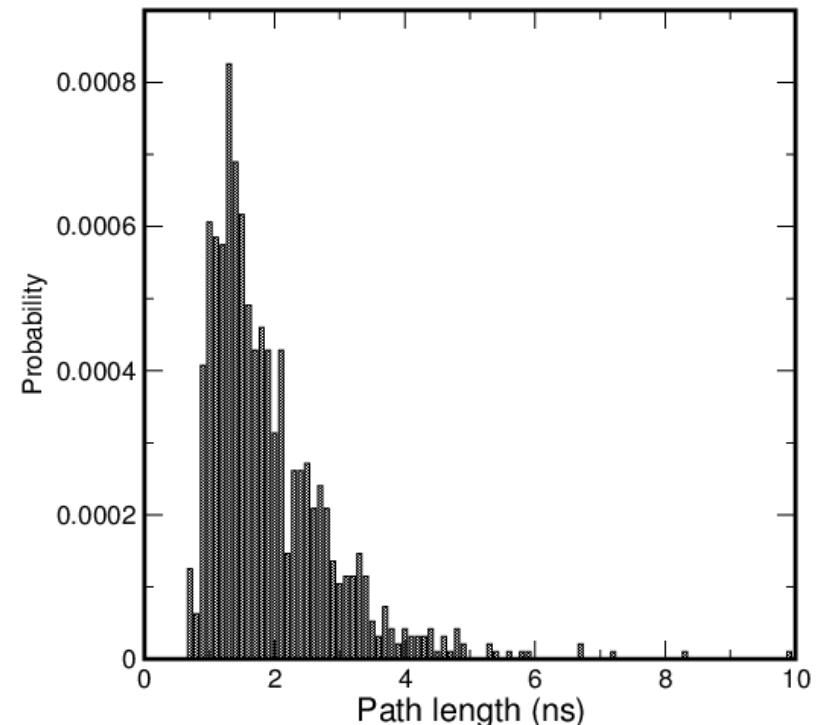
a



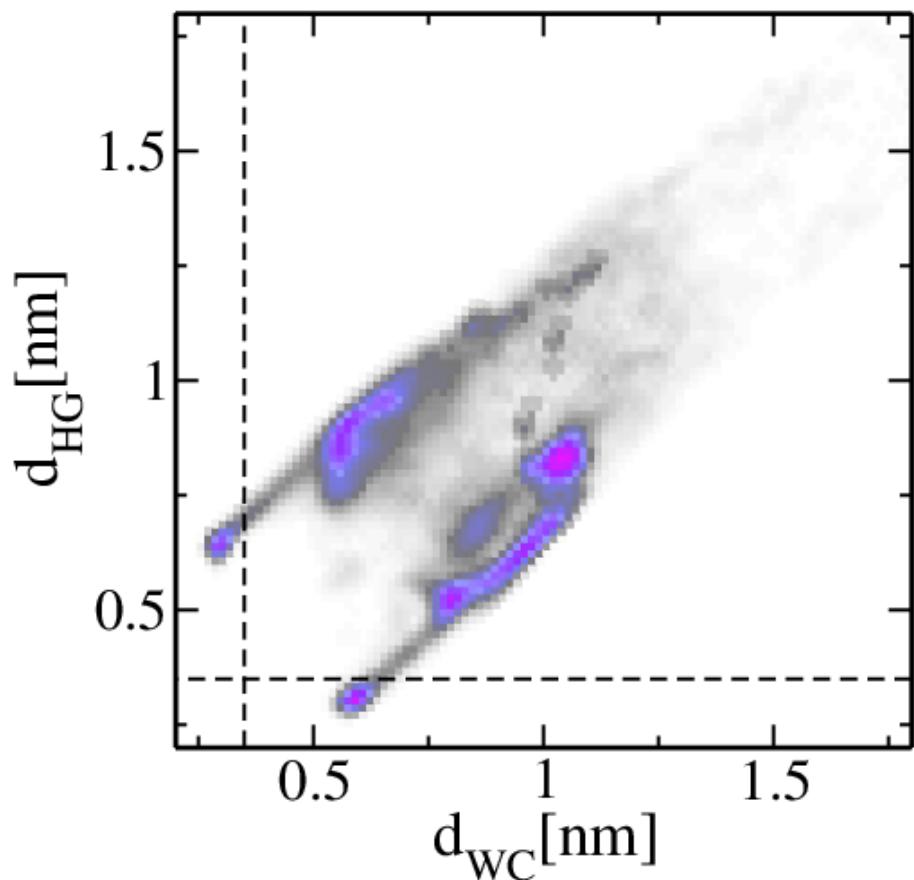
# Different force field AMBER99sb-parmbsc0

Pérez, Marchán, Szovil, Cheatham, Laughton and Orozco  
Biophys. J. 2007 v. 92, p. 3817

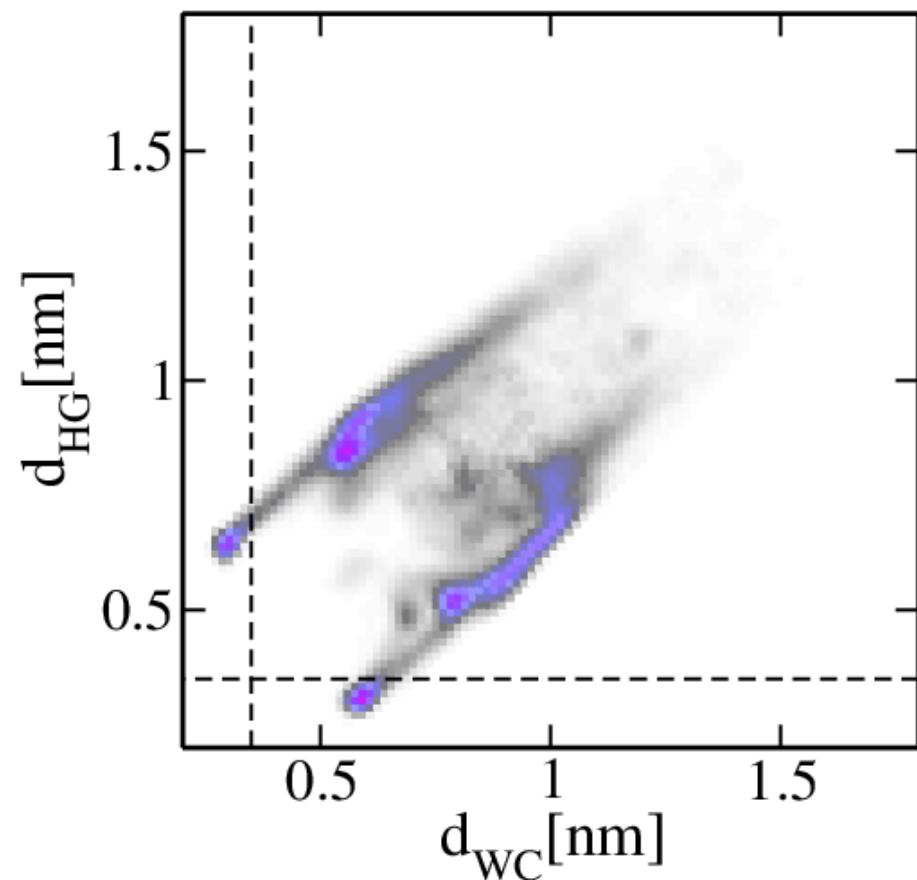
- Using one of the AMBER03 paths resulted in crashes
- New path generated with metadynamics,  
equilibrated mtd path
- Results TPS simulations:
  - 1020 accepted paths
  - acceptance ratio ~50 %
  - average path length 1.7 ns
  - ~20 decorrelated paths



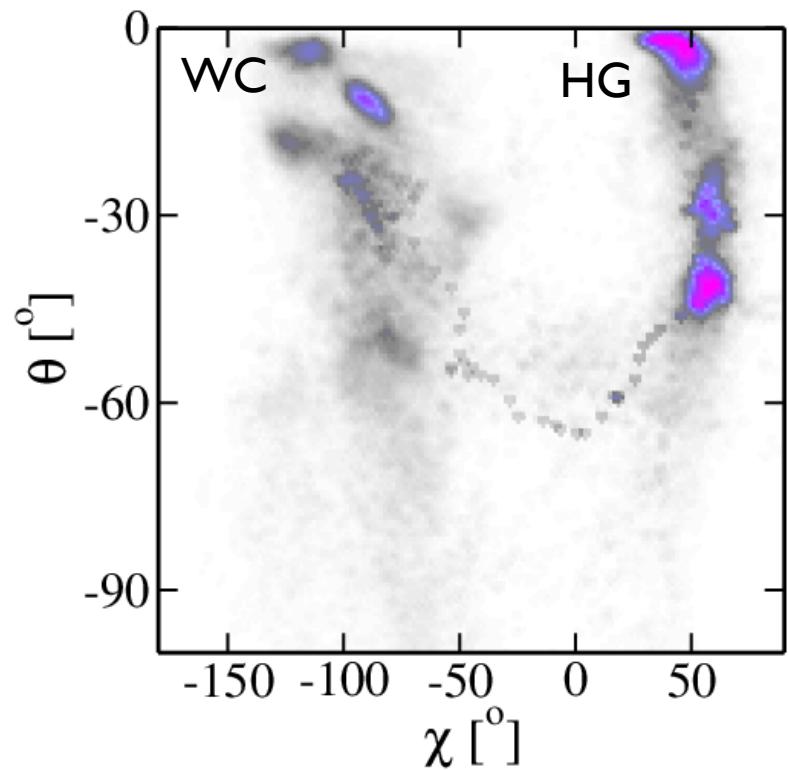
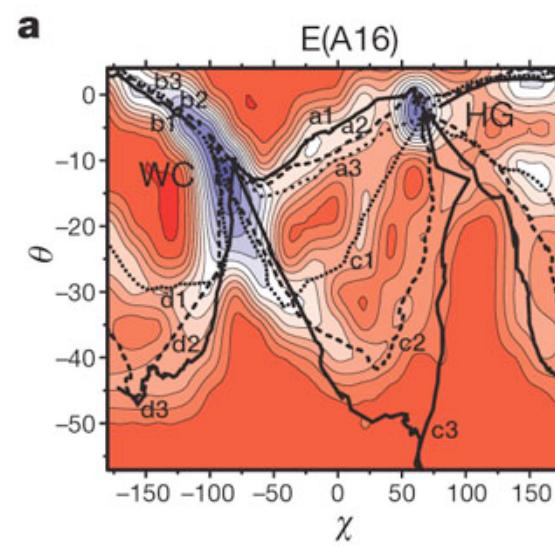
# path density



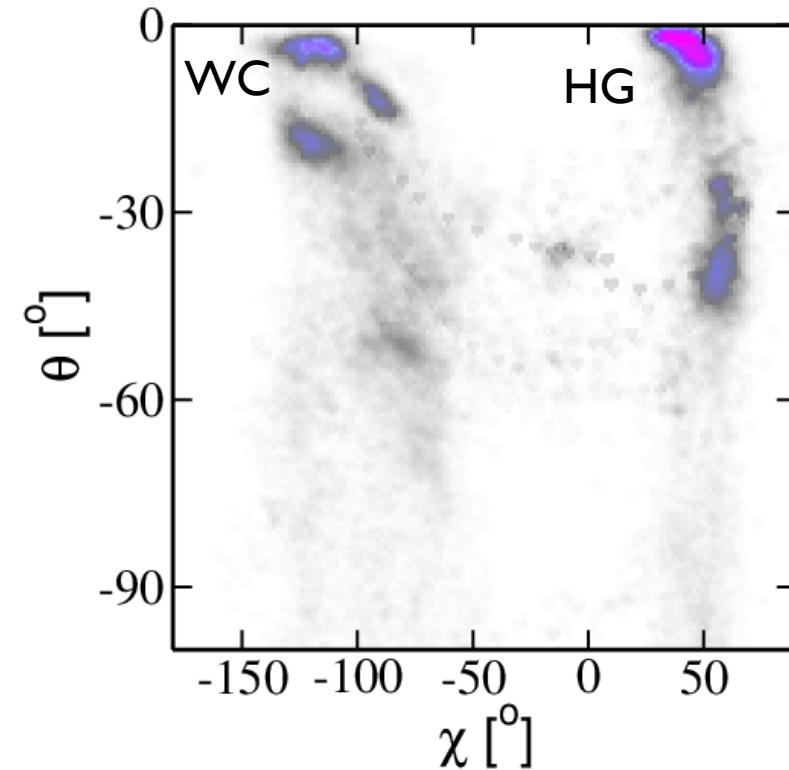
Amber03



Amber99sd-ildn-parmbsc0



Amber03



Amber99sd-ildn-parmbsc0