

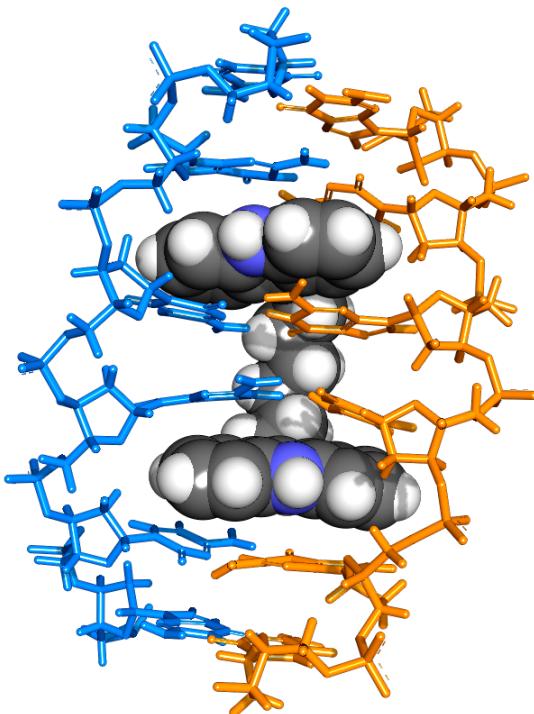
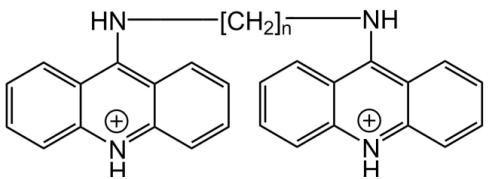
Computational Investigation of Neighbour Exclusion in DNA-Intercalator Complexes

Never Stand Still

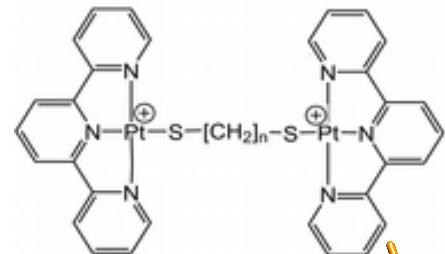
Science

School of Chemistry

Diacridine (**C-n**)



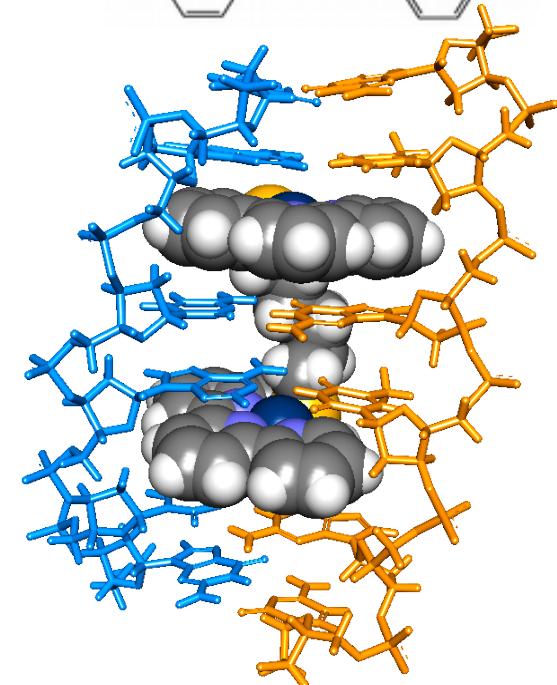
Di-(terpy)Pt(II) thiolate (**D-n**)



Keiran Rowell
(z3374843)

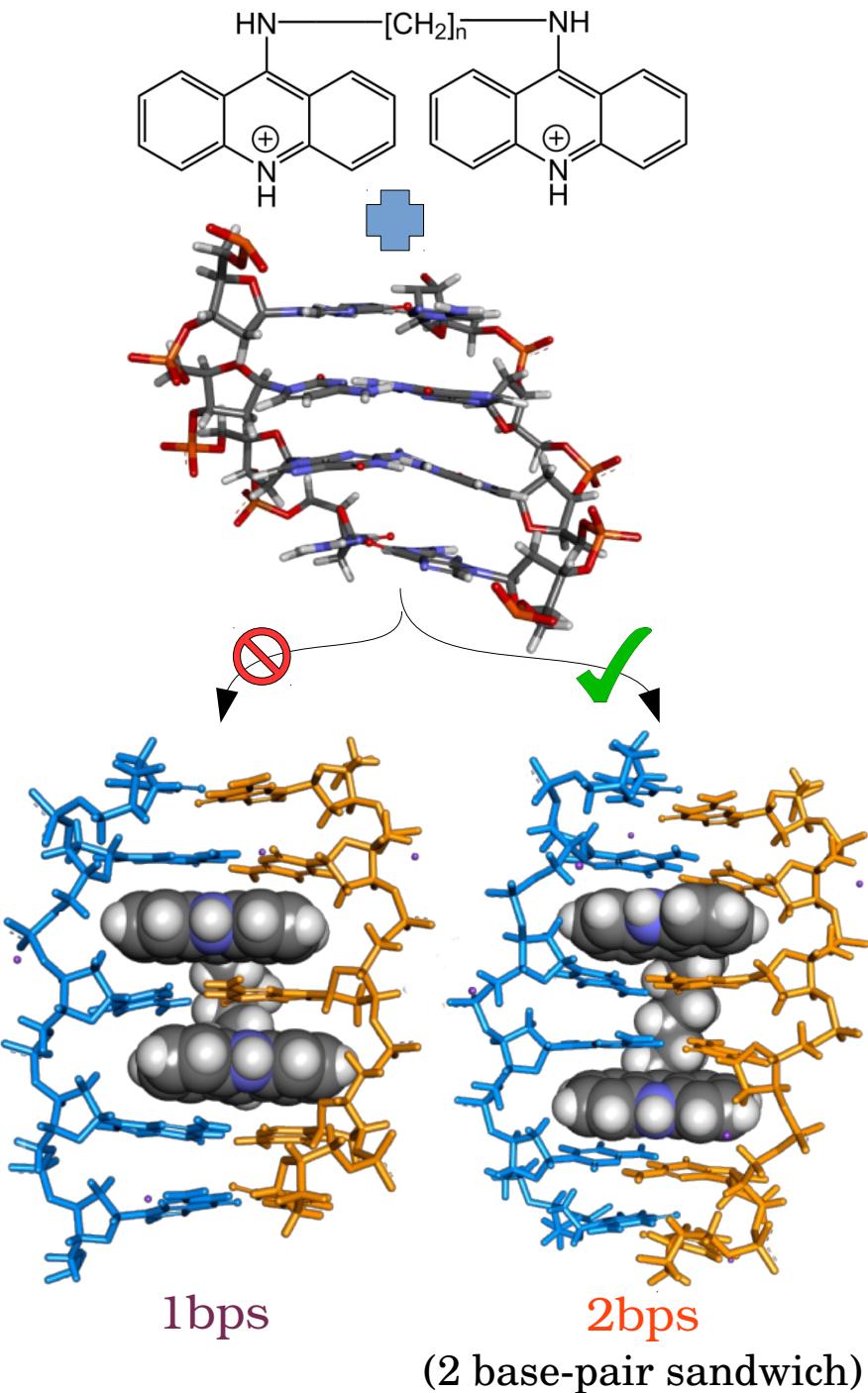
BSc. Chem. (Adv.)

Supervisor: Dr. Graham Ball
Co-Supervisors: A/Prof. Larry Wakelin
Dr. Don Thomas



Background

- **Intercalation:**
 - Chromophores insert between base-pairs
 - Causes: electrostatics, stacking & hydrophobics
- **Neighbour exclusion:**
 - Chromophores cannot occupy adjacent sites
 - Cause(s) not definitely settled
 - Indications that linked dimers can violate it¹⁻³
- **Biological significance**
 - Nucleic acid staining (e.g. ethidium bromide)
 - Transcription factor inhibitors
 - Chemotherapeutic drugs
- **Understanding structure aids design**
 - These ligands are parent compounds, good testbed



1. Wakelin, L. P. G.; Romanos, M.; Chen, T. K.; Glaubiger, D.; Canellakis, E. S.; Waring, M. J. *Biochemistry* **1978**, 17, 5057–5063.

2. McFadyen, W. D.; Wakelin, L. P. G.; Roos, I. A.; Hillcoat, B. L. *Biochem. J.* **1987**, 242, 177–183.

3. Atwell, G.; Stewart, G.; Leupin, W.; Denny, W. J. *Am. Chem. Soc.* **1985**, 107, 4335–4337.

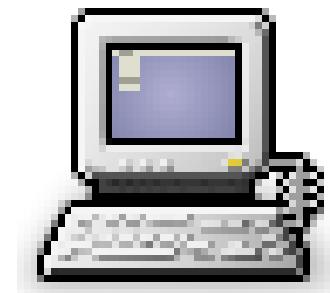
Rationale and Theories

- Why computational?

- NMR difficult. Rapid, non-specific kinetics
- Previous ambiguous data was interpreted *via* space-filling models^{1,2}
- New computational methods allow explicit break-down of interactions

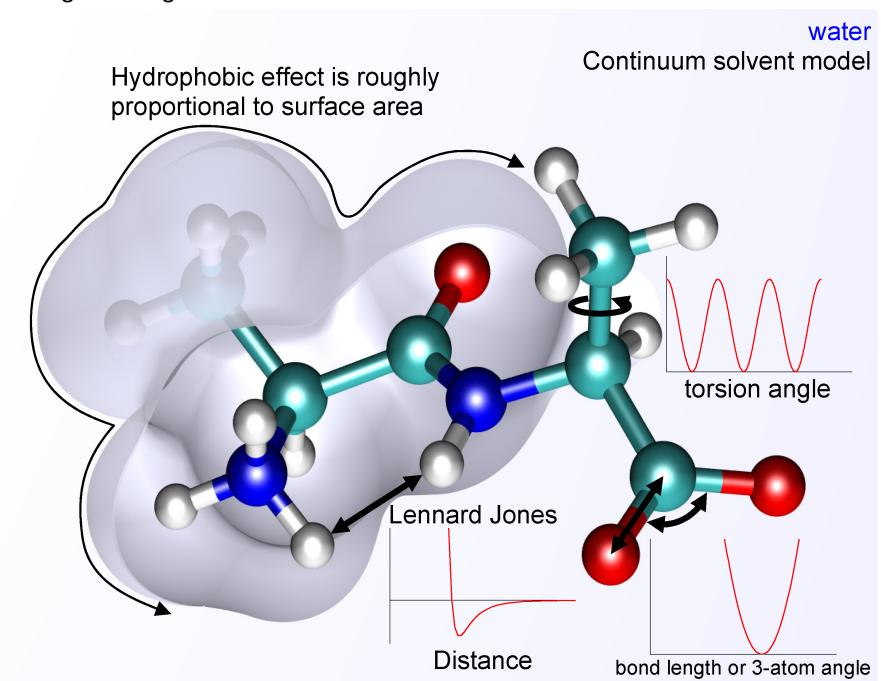


Source: King's College London archive



- Open question:

- DNA sterics^{3,4}
- Specific sugar pucker requirements⁵
- Helical unwinding stability⁶
- Inter-chromophore repulsion
- Vibrational entropy losses⁷
- Polyelectrolyte effects⁸



Source: Boas F.E., Harbury P.B. *Curr. Opin. Struct. Biol.*, 2007, 17, 199-204.

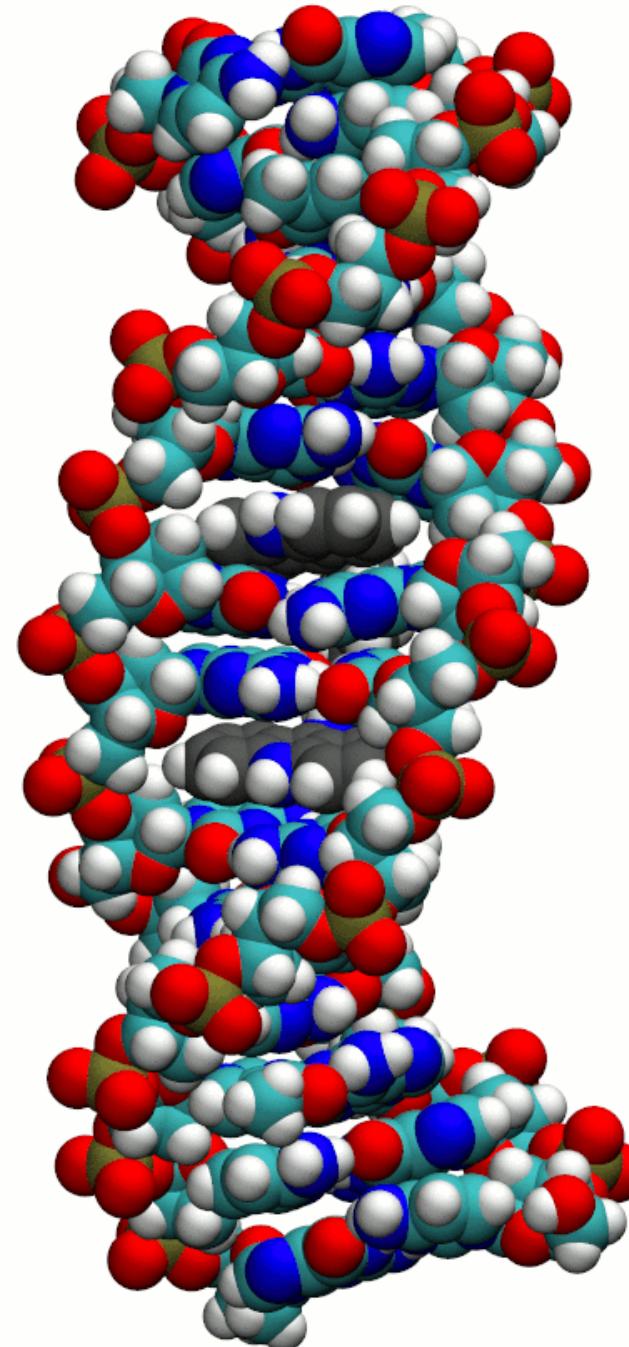
1. Wakelin, L. P. G.; et al. *Biochemistry* 1978, 17, 5057–5063.
2. McFadyen, W. D.; et al. *Biochem. J.* 1986, 238, 757–763.
3. Ihmels, H.; Otto, D. *Top. Curr. Chem.* 2005, 258, 161–204.
4. Rescifina, A. et al. *Eur. J. Med. Chem.* 2014, 74, 95–115.

5. Miller, K. J.; Pycior, J. F. *Biopolymers* 1979, 18, 2683–2719.
6. Williams, L.; et al. In *Structure & Function Volume 1*; 1992; pp. 107–124.
7. Rao, S. N.; Kollman, P. A. *PNAS*. 1987, 84, 5735–5739.
- 8 Friedman, R. A.; Manning, G. S. *Biopolymers* 1984, 23, 2671–2714.

Molecular Dynamics (MD)

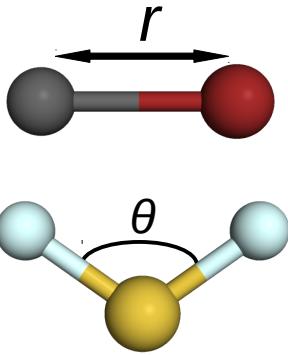
- **Choice in complexes**
 - Pyrimidine-purine (pyrimide: C/T, purine: G/A) steps chosen (**1bps**: CAC, CGC, TAT, TGT + equivalent **2bps**: CACA, CGCG, TATA)
 - Various linkers, major/minor groove
 - **151** unique complexes in total
- **Structural dynamics of intercalation**
 - MD using AMBER suite (ff12SB)
 - 10 ns simulation times on each complex
 - Explicit solvent and counter-ions
 - 14 Å water-box (TIP3P) 'tiled' to produce bulk
- **MD fully parameterised -**

Need appropriate force-field!

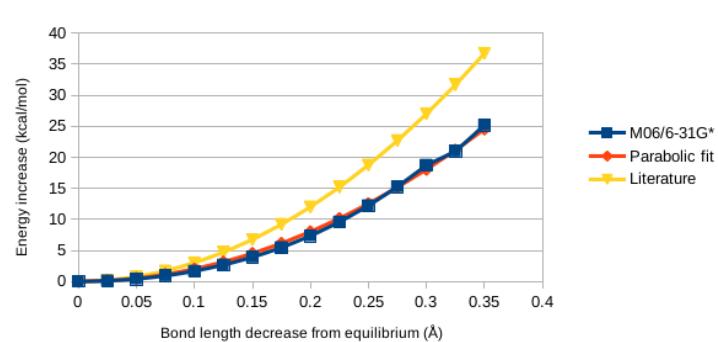


The Components of a Force-Field

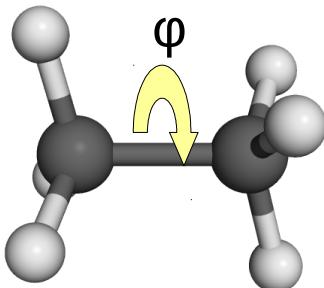
$$\sum_{bonds} K_r (r - r_0)^2 + \sum_{angles} K_\theta (\theta - \theta_0)^2$$



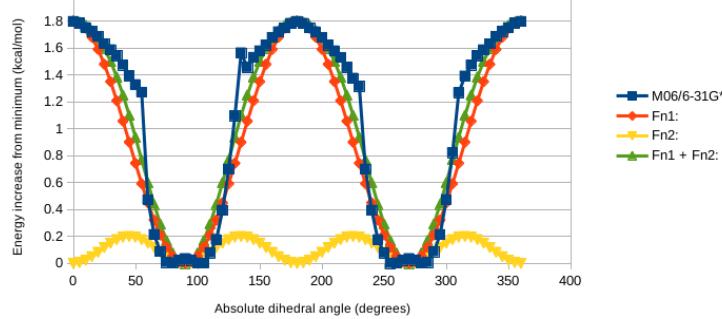
Pt-S bond energies



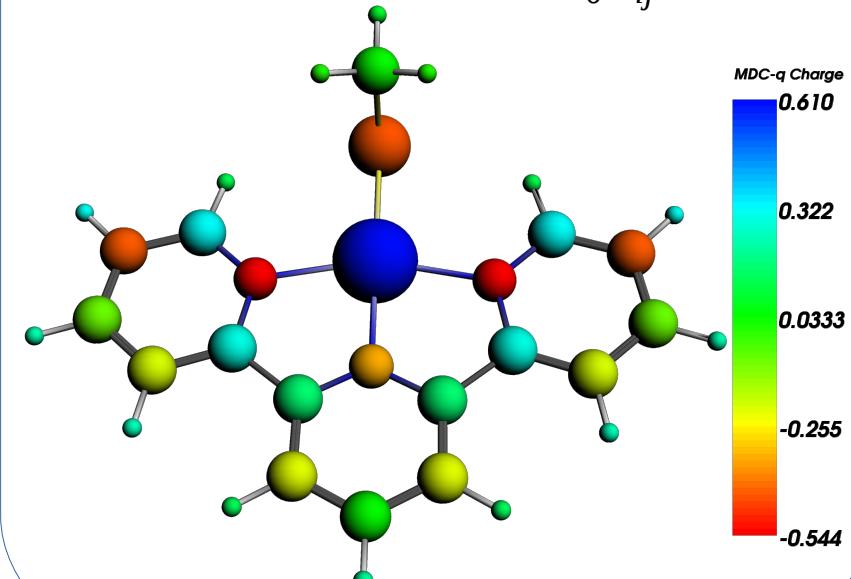
$$+ \sum_{dihedrals} \frac{V_n}{2} [1 + \cos(n\varphi - \gamma)]$$



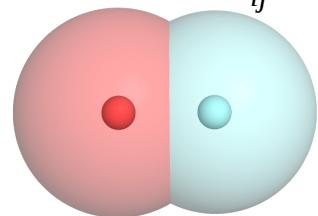
na-Pt-ss-c3 dihedral energies



$$+ \sum_{charges} \frac{q_i q_j}{4 \pi \epsilon_0 r_{ij}}$$



$$+ \sum_{van\;der\;Waals} 4 \epsilon_{ij} \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^6 \right]$$

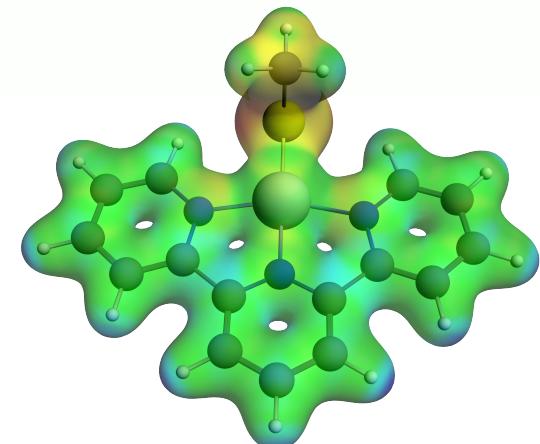
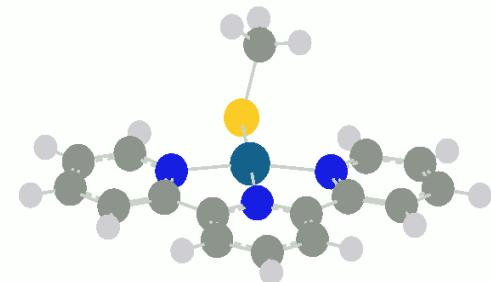
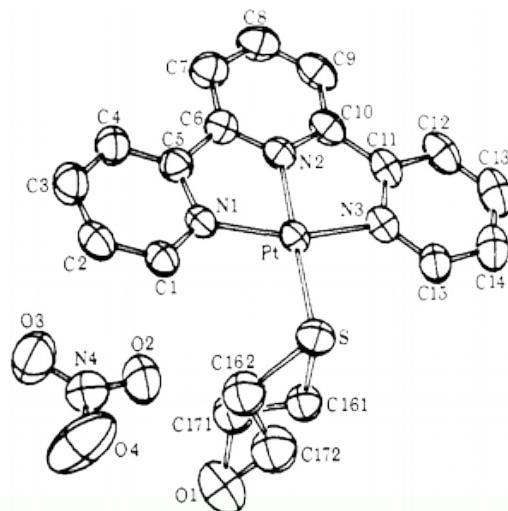


Wang, J.; Wolf, R. J. *Comput. Chem.* 2004, 25, 1157–1174.

1. Hambley, T. W. *Inorg. Chem.* 1998, 37, 3767–3774.

Parameterisation Methodology

- DFT used to determine moiety's properties
 - Previous parameters lacking/unsuitable¹
- Geometry optimisation
 - M06/TZP + relativistic corrections
 - Error to crystal structure²: Bonds: 0.013 Å, Angles: 0.72°
- Relaxed scans
 - Energy profile as bonds/angle/etc. change
 - M06/6-31G** + LANL2DZ (Pt)
- Partial charges
 - All partial charge methods arbitrary
 - MDC-q: Partial charges from molecular quadrupole



1. Juranic, N.; Likic, V.; Kostic, N. M.; Macurat, S. *Inorg. Chem.* **1995**, 34, 938–944.

2. Jennette, K. W.; Gill, T.; Sadownick, A.; Lippard, S. J. *J. Am. Chem. Soc.* **1975**, 213, 6159–6168.

Intercalation Site Stability: Diacridines

	C-4 major	C-4 minor	C-5 major	C-5 minor	C-6 major	C-6 minor	C-7 major	C-7 minor	C-8 major	C-8 minor
TATA (2bps)										
CACA (2bps)										
CGCG (2bps)										
TAT (1bps)										
TGT (1bps)										
CAC (1bps)										
CGC (1bps)										

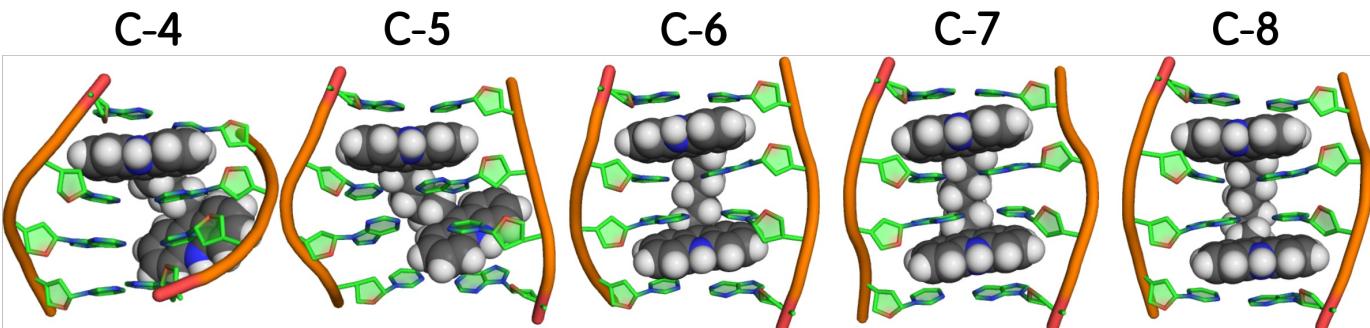


 Stable over 10ns
 Distorted intercalation
 1 chromophore unintercalates

- **Obeying structures fit data**

- Functionality
- Chromophore roll

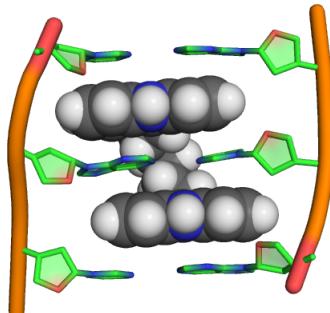
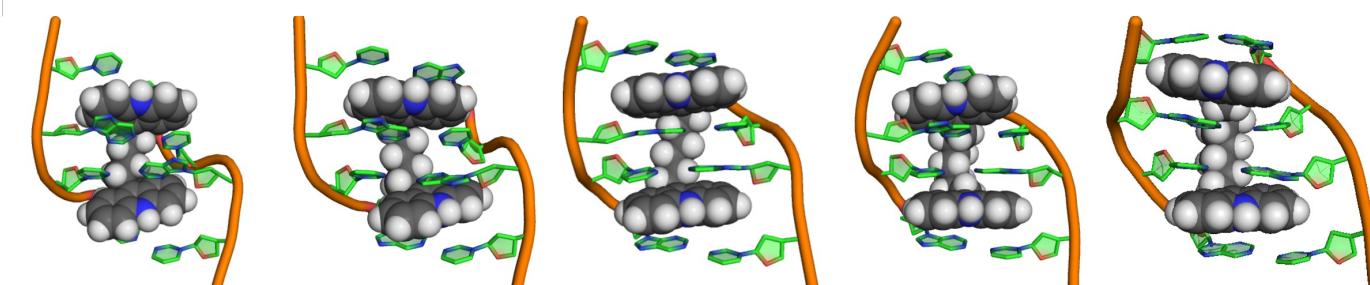
Major



- **Violating structures don't**

- No steric strain

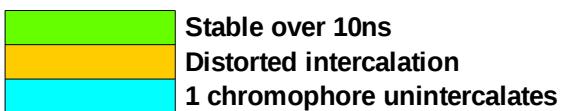
Minor



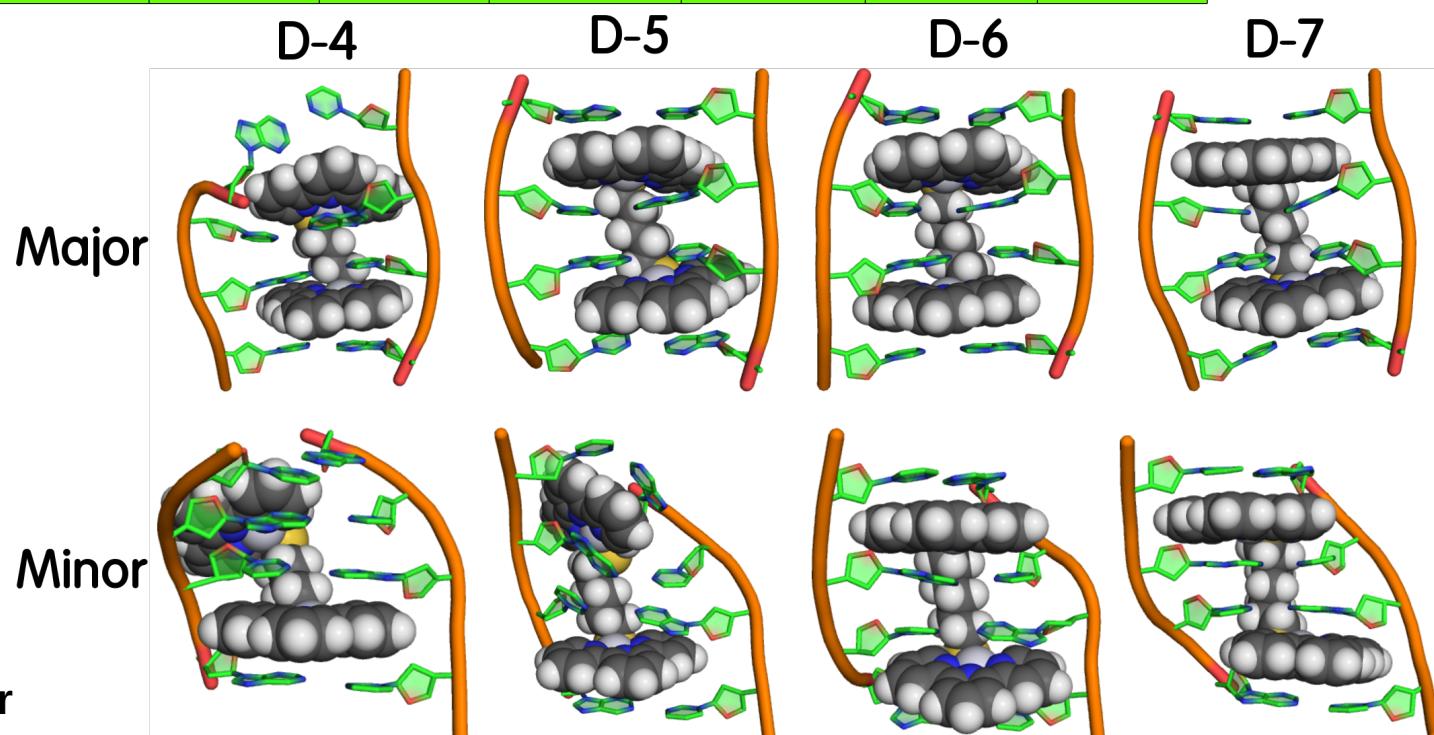
Intercalation Site Stability: (terpy)Pt(II) Dimers

	D-4 major	D-4 minor	D-5 major	D-5 minor	D-6 major	D-6 minor	D-7 major	D-7 minor
TATA (2bps)	Green	Cyan	Green	Yellow	Green	Green	Green	Green
CACA (2bps)	Green	Yellow	Green	Yellow	Green	Green	Green	Green
CGCG (2bps)	Green	Yellow	Green	Yellow	Green	Green	Green	Green

TAT (1bps)	Green							
TGT (1bps)	Green							
CAC (1bps)	Green							
CGC (1bps)	Green							



- **2bps structures viable**
- **Major groove preference**
 - Pt above guanine's O6
- **MD indicates intercalators are not violating neighbour exclusion principle**



Free Energy/Entropy Methods (MMPBSA)

- **Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA)**

- Post-processing of MD trajectories
 - Hess's law like relation

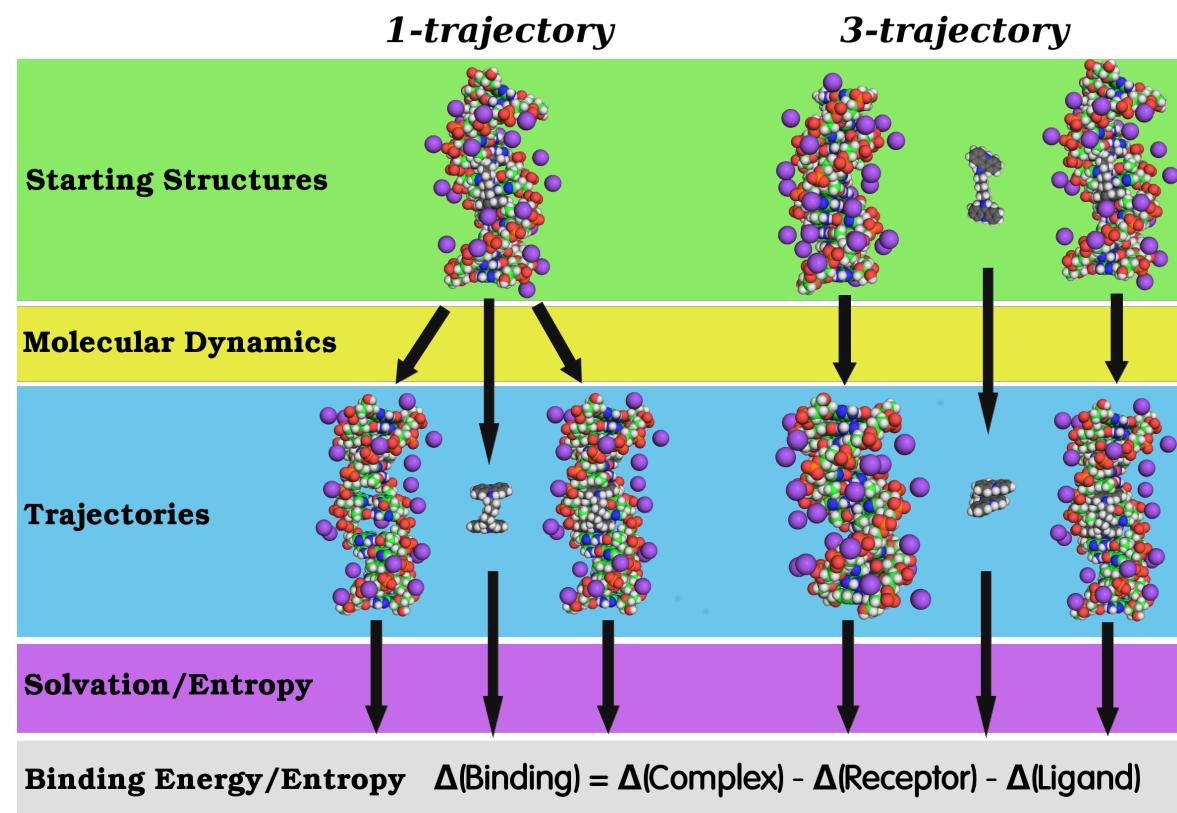
- **Binding Energies**

- 3-trajectory approach
 - Non-linear Poisson-Boltzmann

- **Entropy Calculations**

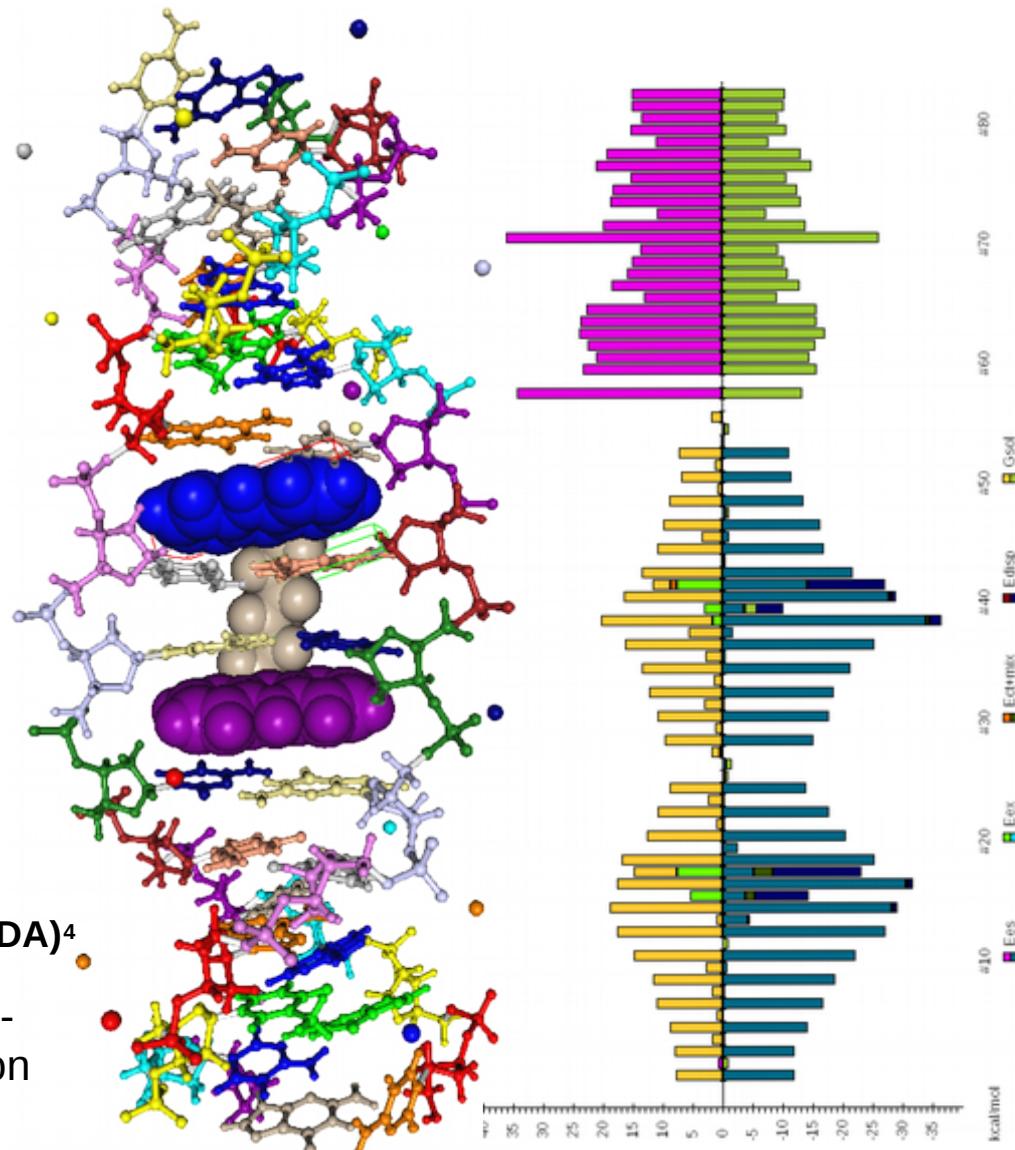
- Harmonic normal-mode analysis

- **Gave poor results; limitations of method**



Fragment Molecular Orbital Method (FMO)

- Computer time scaling programs
 - QM methods, typically scale N^{4+}
 - Fragment methods circumvent scaling
 - Splits jobs over many CPUs
- Fragment Molecular Orbital (FMO)¹
 - Fragment along selected sp³ bonds
 - Solve electron density in 'Coulomb bath'
 - Pair interaction between each fragment
 - RI-SCS-MP2/6-31G* + PCM[1(2)] suitable^{2,3}
- Pair Interaction Energy Decomposition Analysis (PIEDA)⁴
 - Intuitive components: electrostatic, exchange-repulsion, dispersion charge-transfer, solvation



1. Kitaura, K.; Ikeo, E.; Asada, T. *Chem. Phys. Lett.* **1999**, 701–706.

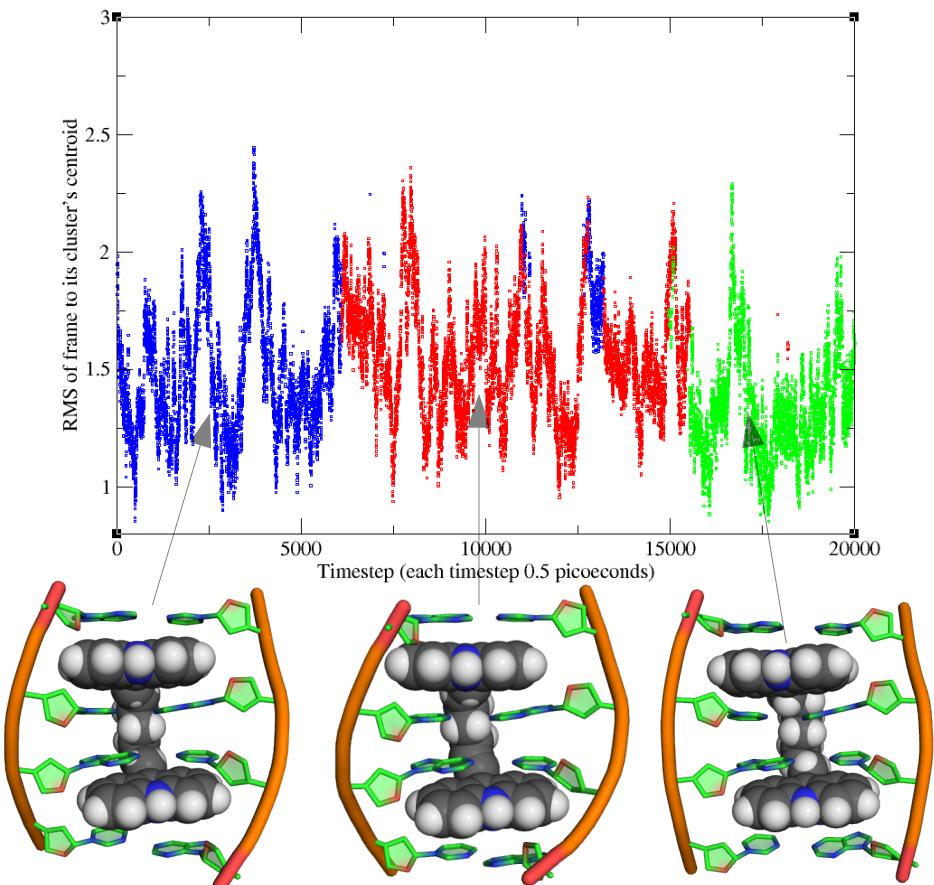
2. Fedorov, D. G.; Nagata, T.; Kitaura, K. *Phys. Chem. Chem. Phys.* **2012**, 14, 7562–7577.

3. Fukuzawa, K.; et al. *Comput. Theor. Chem.* **2014**, 1034, 7–16.

4. Fedorov, D.; Kitaura, K. *J. Comput. Chem.* **2007**, 28, 222–237.

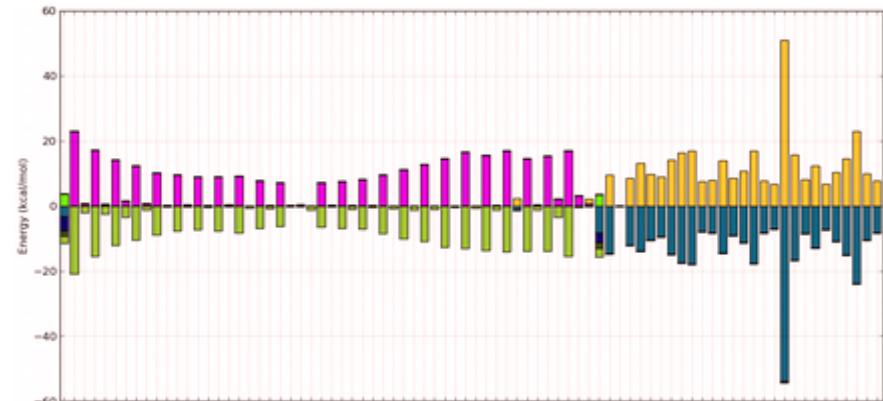
Clustering Analysis, FMO Energy Analysis

- Clustering analysis of trajectories
 - Sort like conformations into 'clusters'
 - Use 'centroid' of most populous cluster
- Querying FMO energies
 - Fragment pairs means $\sim N^2$ pair energies
 - Python query and search code written



```
for complex in complexes_list:  
    if complex['ligand'] == 'C3NC3':  
        for fragment_a in complex['fragments']:  
            if fragment_a.frag_type == 'LNK':  
                frag_a = fragment_a  
            for fragment_b in complex['fragments']:  
                if fragment_b.frag_type == 'G':  
                    frag_b = fragment_b  
                print_IFIE(frag_a, frag_b, Ees+Eex+Edisp+Ect+Gsol', complexes_list)
```

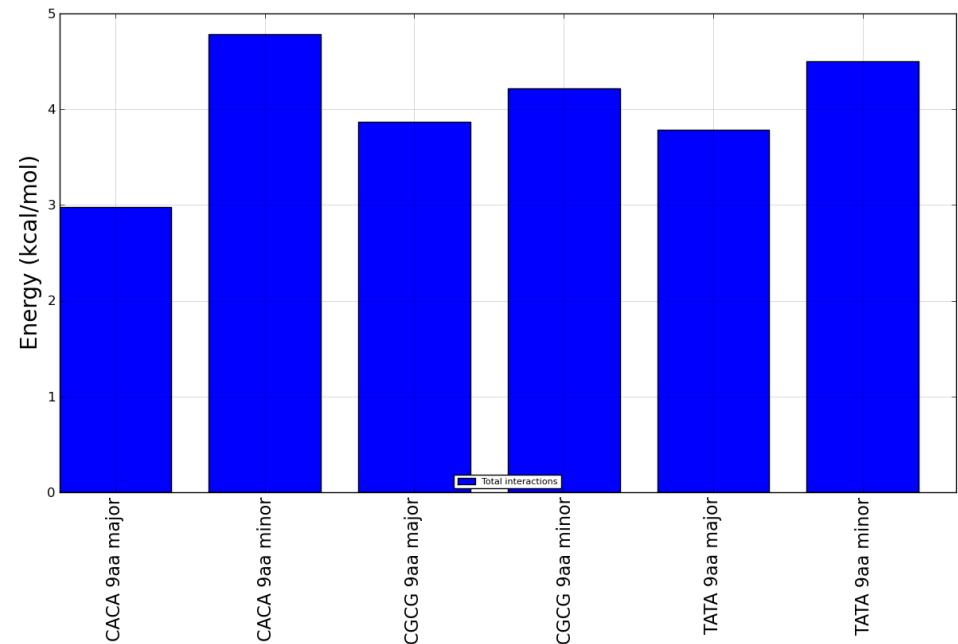
- Plotting FMO energies
 - Matplotlib¹ plotting code written



1. Hunter, J. D. *Comput. Sci. Eng.* 2007, 9, 90–95.

Inter-chromophore Repulsion

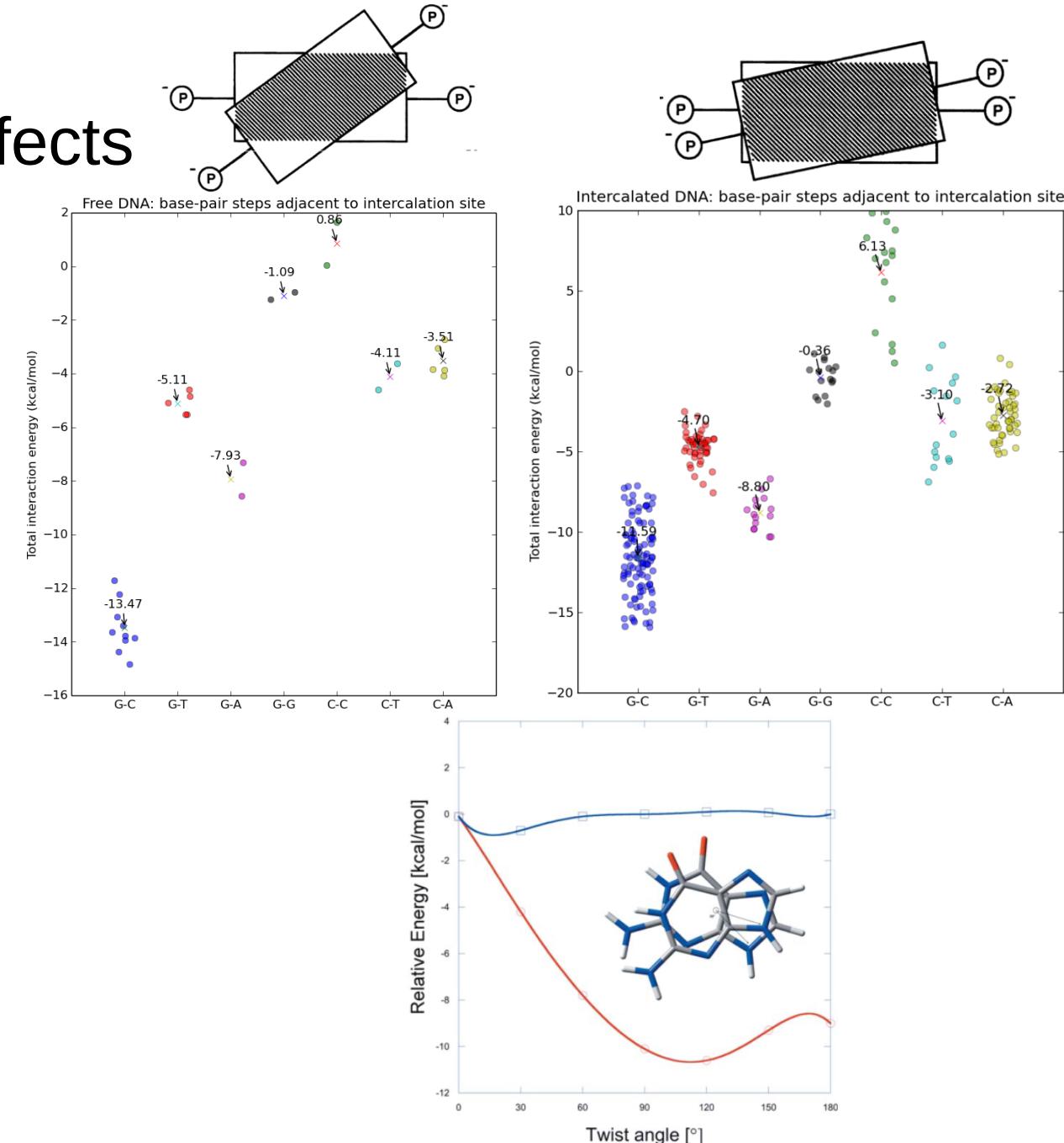
- **2bps → 1bps increase repulsion ~4 kcal/mol**
 - ~10 kcal/mol ES repulsion
 - Total reduced by solvation
- **Not sole cause of exclusion**
 - Doesn't account for uncharged chromophores



*Difference in inter-chromophore repulsion between 2bps and their equivalent 1bps complexes.
(Only 2bps listed, ligand: 9-aminoacridine)*

Helical Unwinding Effects

- **Intercalating DNA unwinds helix**
 - Increase vdW overlap
 - Should be harder to insert into¹
- **FMO energies show little change**
- **Agree with high level QM**
 - Solvation nullifies twist²
- **Unwinding doesn't cause neighbour exclusion**



1. Williams, L.; Egli, M.; Gau, Q. In *Structure & Function Volume 1*; 1992; pp. 107–124.

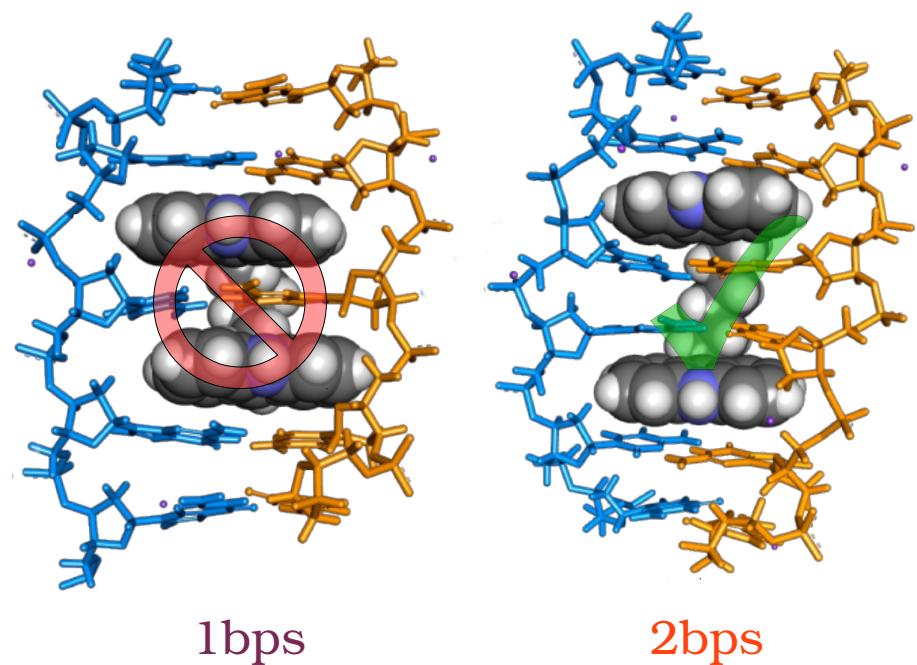
2. Sponer, J.; Sponer, J. E.; Mládek, A.; Jurečka, P.; Banáš, P.; Otyepka, M. *Biopolymers* **2013**, 99, 978–988.

Conclusions

- Modelling indicates intercalator not violating neighbour exclusion

- Causes?

- DNA sterics
- Specific sugar pucker requirements
- Helical unwinding stability
- Inter-chromophore repulsion
- Vibrational entropy losses
- Polyelectrolyte effect



1bps

2bps

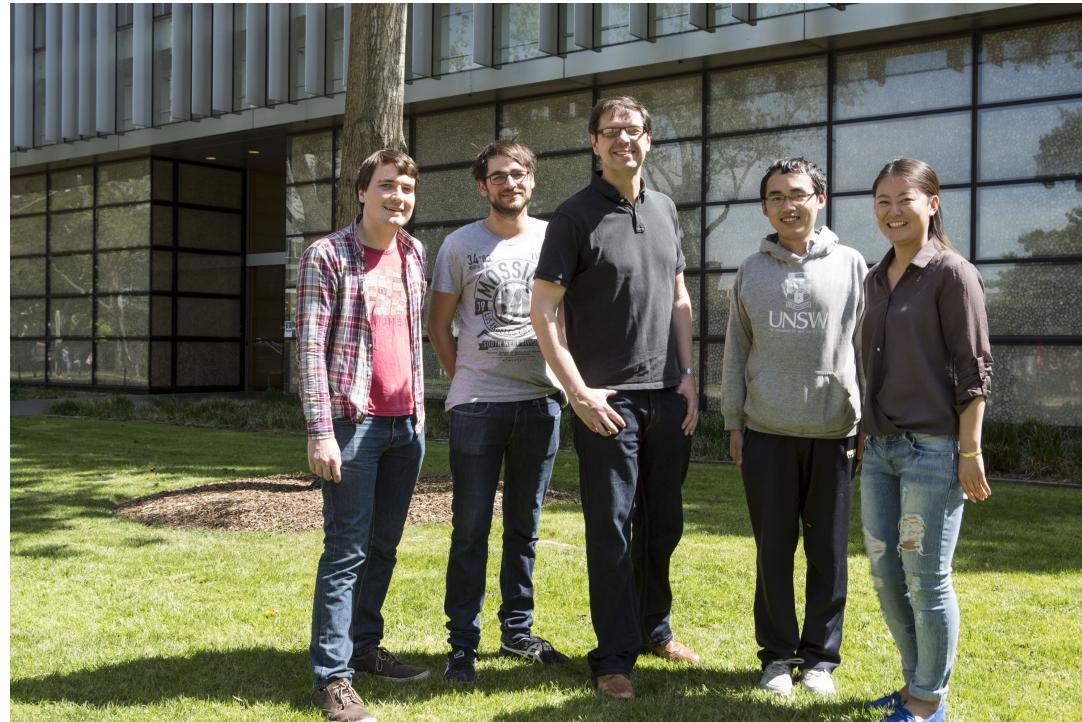
Acknowledgements

Supervisors:

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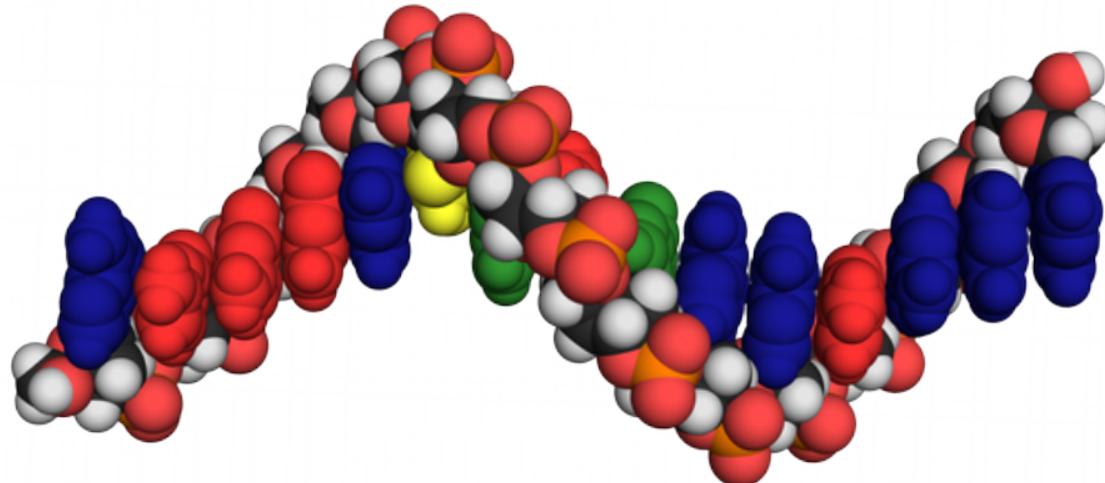


Colleagues:

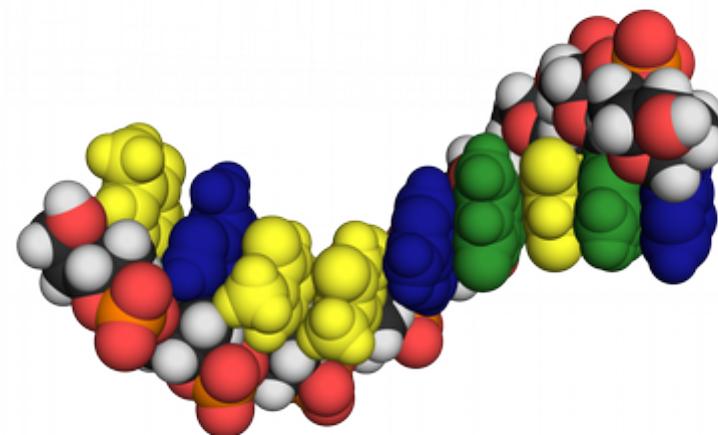
Chris Pracey

The rest of the Ball group

Friends and family



THANK

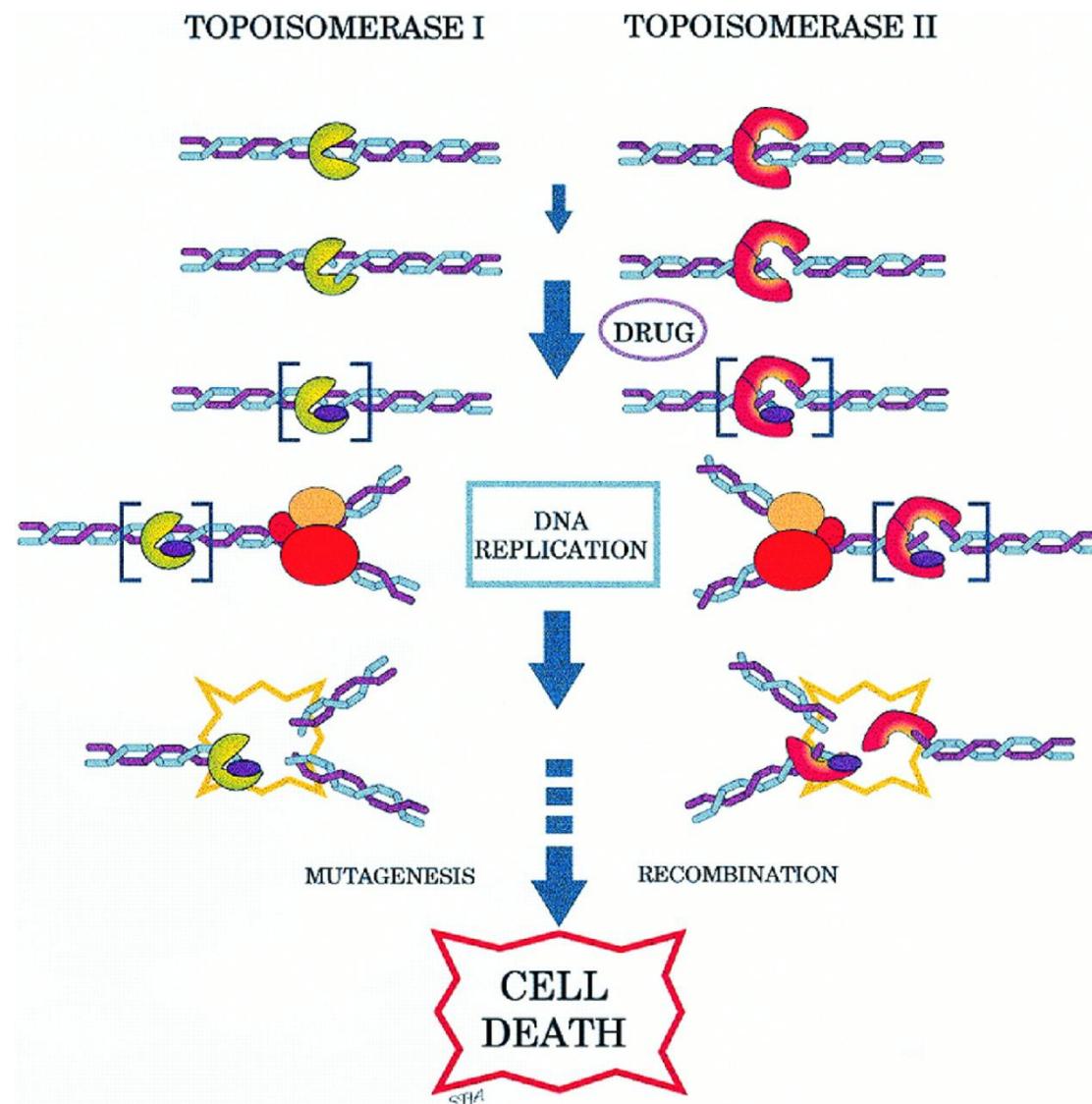


YOU



UNSW
AUSTRALIA

Topoisomerase Inhibition



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Ab initio Methods Details

- **Resoultion of Identity (RI)¹**

$$(\nu \mu | \lambda \sigma) \approx \sum_{PQ} (\mu \nu | P) V_{PQ}^{-1} (Q | \lambda \sigma) \quad V_{PQ} = (P | Q)$$

- **Spin-Component Scaling (SCS)²**

$$E^{corr}(SCS - MP2) = p_S E_{\uparrow\downarrow} + p_T E_{\uparrow\uparrow+\downarrow\downarrow} \quad p_s = (6/5) \quad p_T = (1/3)$$

- **Møller–Plesset 2nd order perturbation theory (MP2)**

$$\hat{H} = \hat{H}_0 + \lambda \hat{V}$$

$$E_1 = \langle \psi^{(0)} | \hat{V} | \psi^{(0)} \rangle$$

$$E = \lambda^0 E^{(0)} + \lambda^1 E^{(1)} + \dots + \lambda^n E^{(n)}$$

$$E_2 = \langle \psi^{(0)} | \hat{V} | \psi^{(1)} \rangle$$

1. Ishikawa, T.; Kuwata, K. *Chem. Phys. Lett.* **2009**, 474, 195–198.

2. Grimme, S. *J. Comput. Chem.* **2004**, 25, 1463–1473.

Leach, A. R., *Molecular Modelling: Principles and Applications*; 2nd Ed., Pearson, London, UK, 2001; pp.114-115

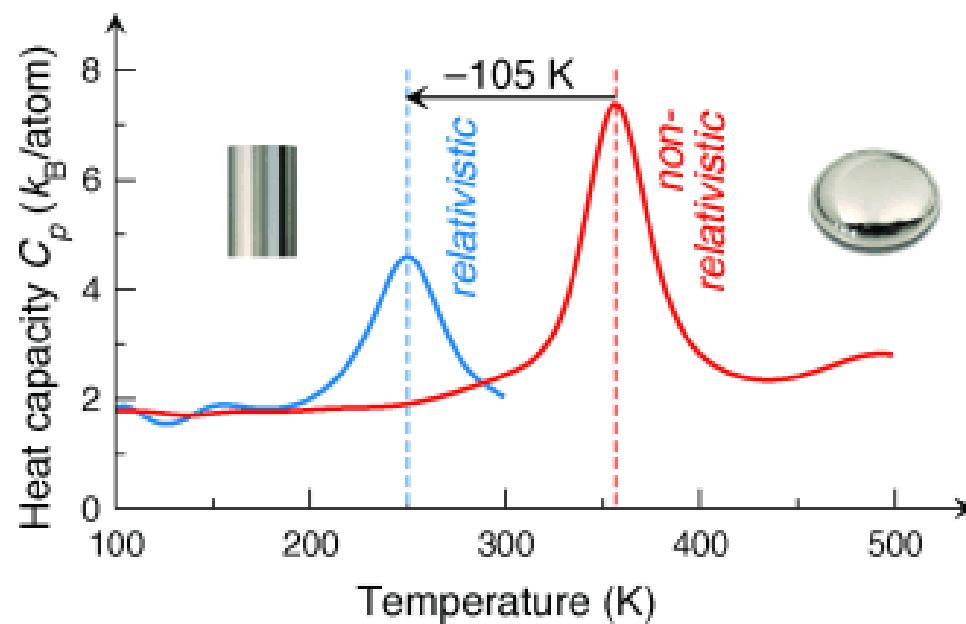
Relativistic Effects in Chemistry

Relativistic Mass

$$m_{rel} = \frac{m_e}{\sqrt{1 - (v_e/c)^2}}$$

Bohr Radius

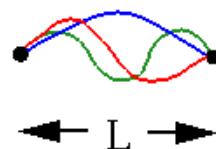
$$a_0 = \frac{\hbar}{m_e c \alpha}$$



Normal Mode Analysis

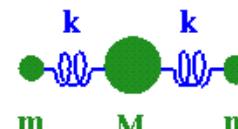
Examples of Normal Modes

Guitar string
fixed at both ends



$$\lambda = 2L / n$$
$$n = 1, 2, 3$$

Balls & Springs
in 1D

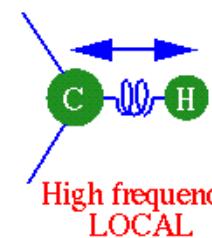


ω	mode
0	(Pure Translation)
$(k/m)^{1/2}$	(Center at Rest)
$[(k/m)(1+2m/M)]^{1/2}$	

Proteins

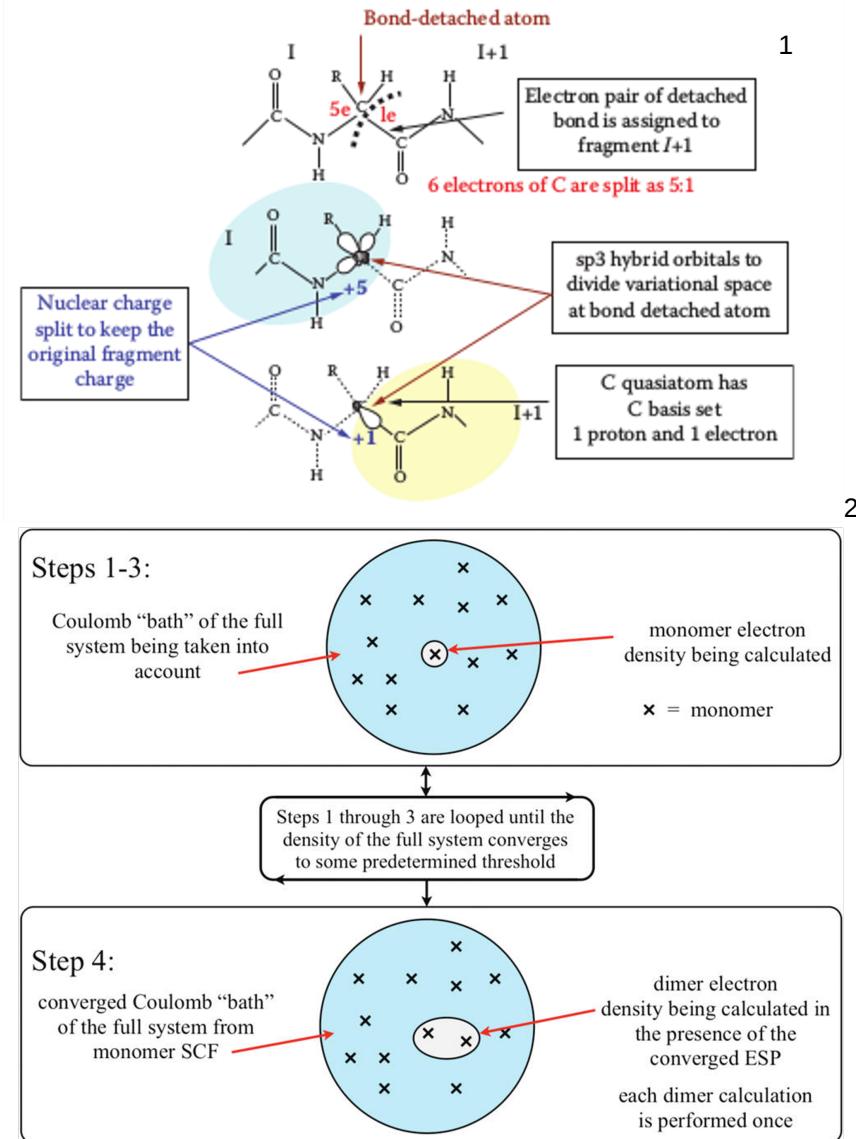
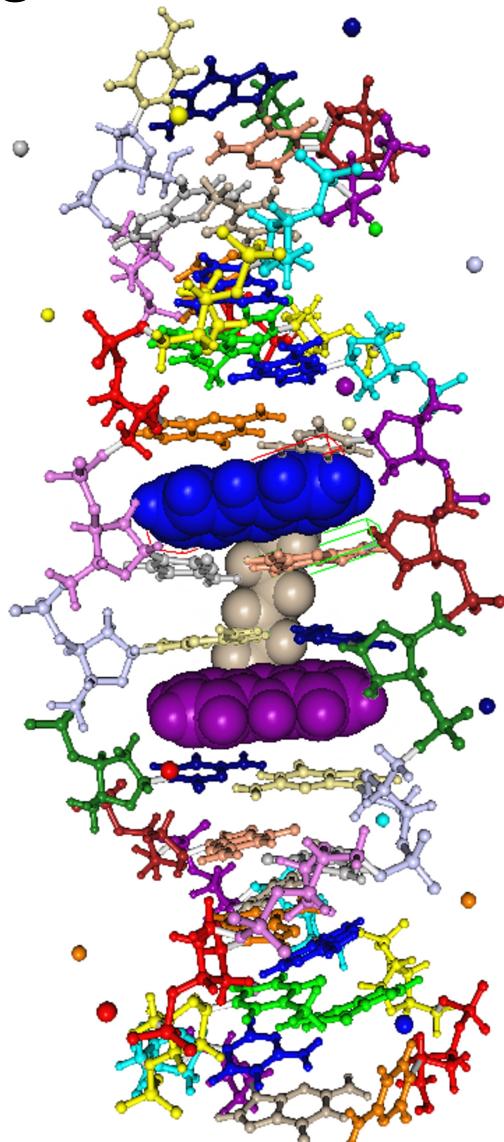


Low frequency
GLOBAL



High frequency
LOCAL

FMO Fragmentation



1. Fedorov, D.; Kitaura, K. *The Fragment Molecular Orbital Method: Practical Applications to Large Molecular Systems*; CRC Press: Boca Raton, Florida, 2009; p. 2888.

2. Gordon, M. S.; Fedorov, D. G.; Pruitt, S. R.; Slipchenko, L. V. *Chem. Rev.* **2012**, *112*, 632–672.

