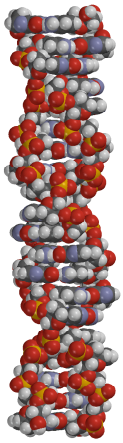
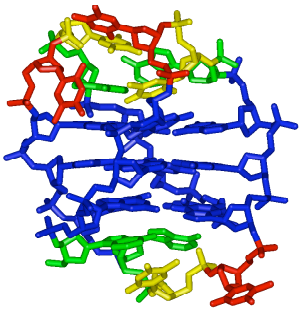
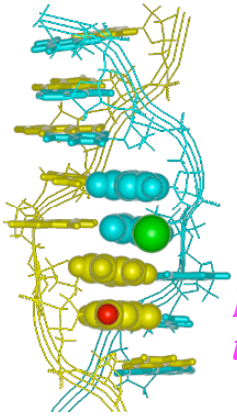


Structure

Function



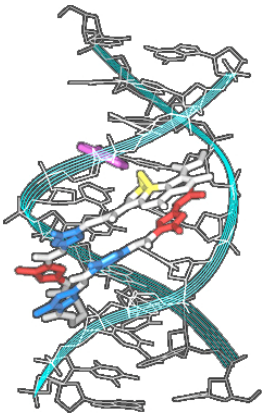
DNAの多様な構造とその反応性



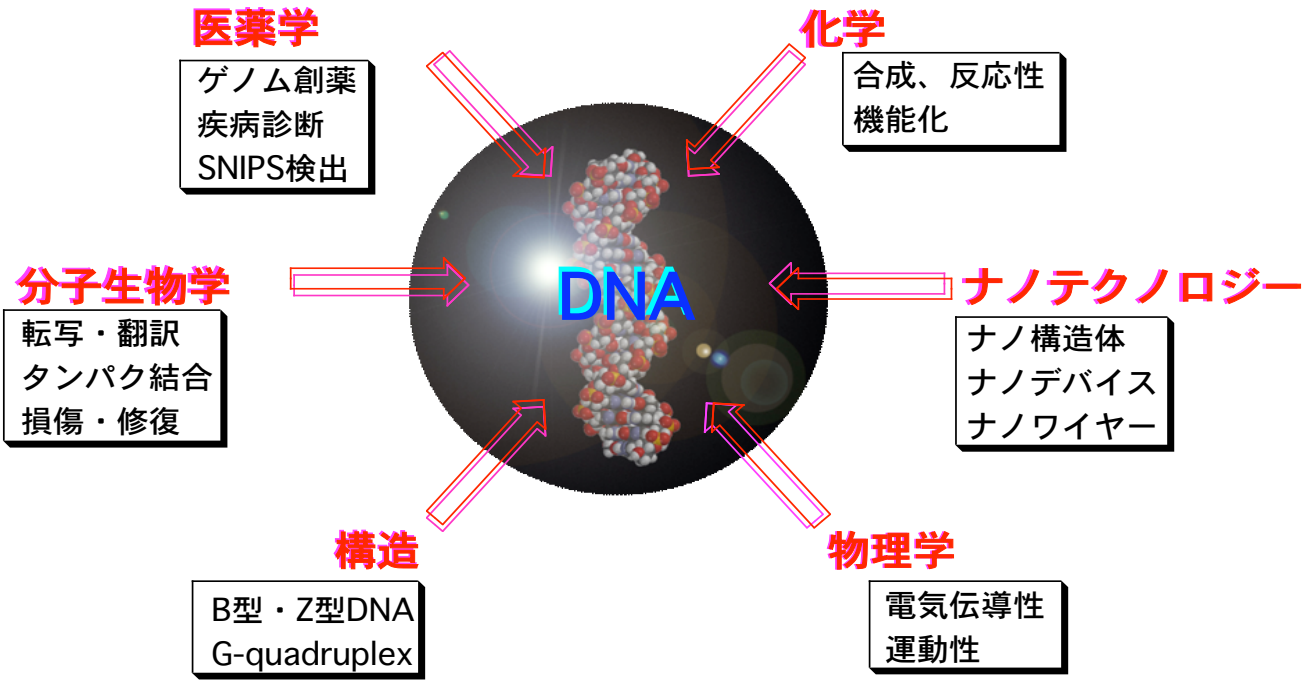
Electron transfer

京大院理　杉山　弘

Therapy



DNAは様々な学問分野から研究されている



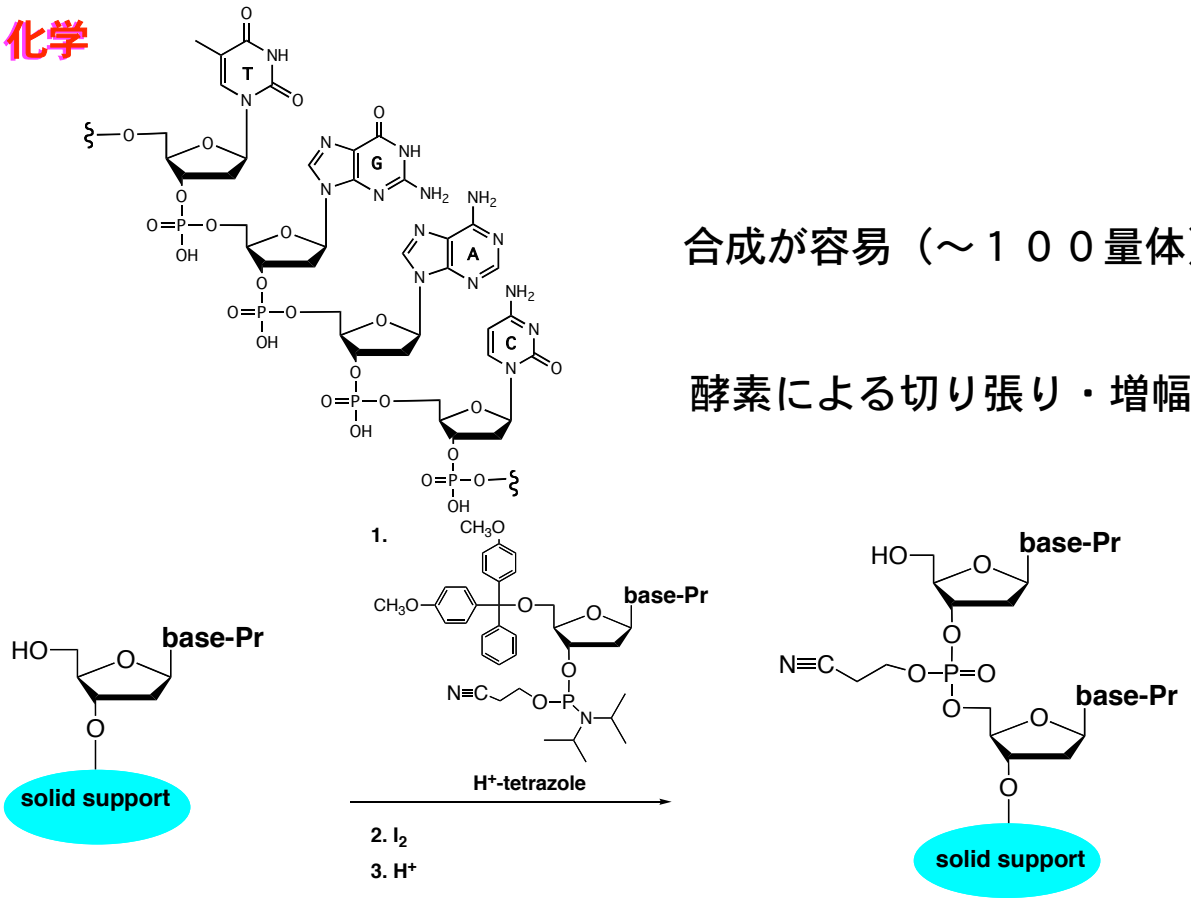
しかし相互のつながりは少なく一面的

例えば、DNAデバイスにおいて電荷移動のしやすさなどは考慮されていない。

タンパクーDNA相互作用で軌道の重なりなどは考慮されていない。

DNAは分子材料として研究すべき生物物質である

化学

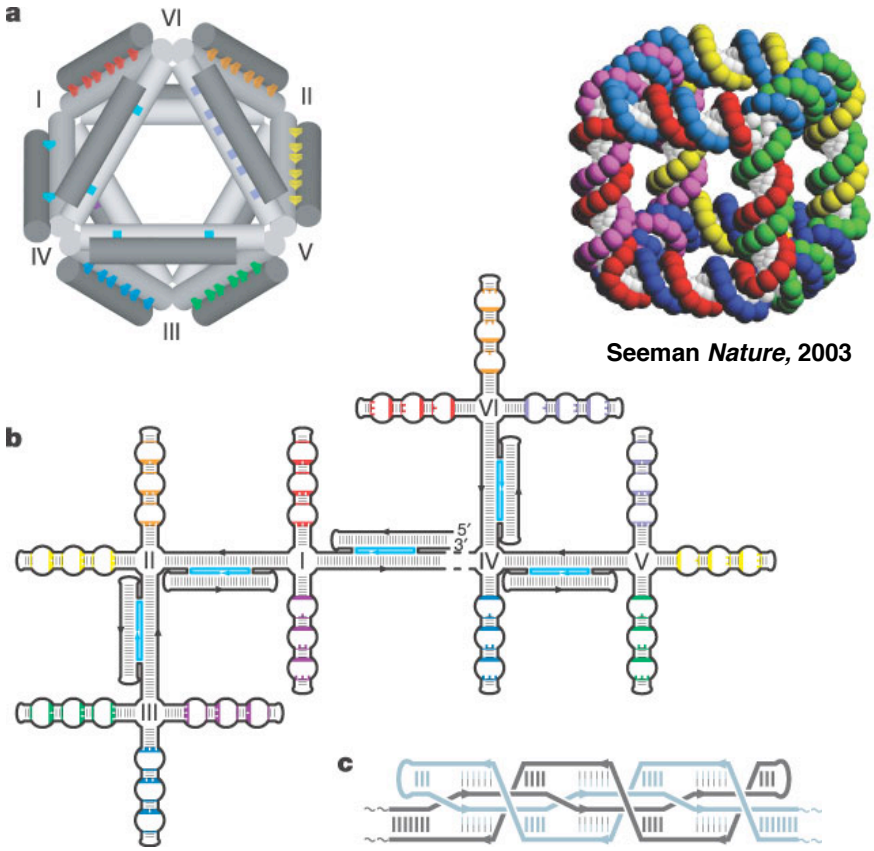


合成が容易（～１００量体）

酵素による切り張り・増幅が可能

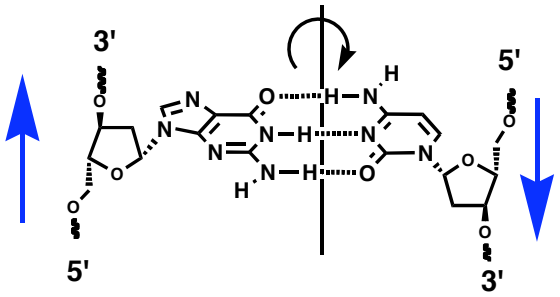
Joice 2004

Rothemund 2006

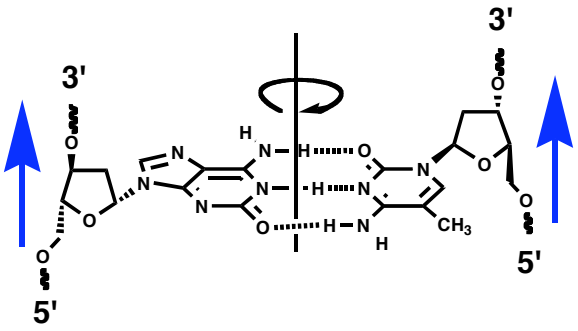


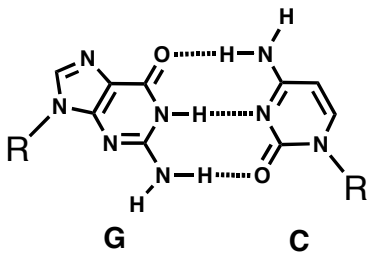
Seeman *Nature*, 2003

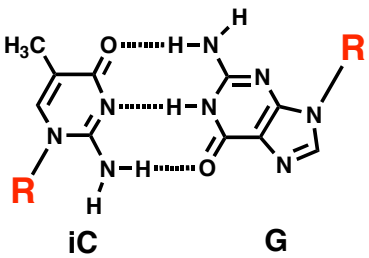
逆平行

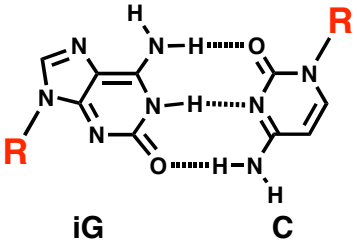


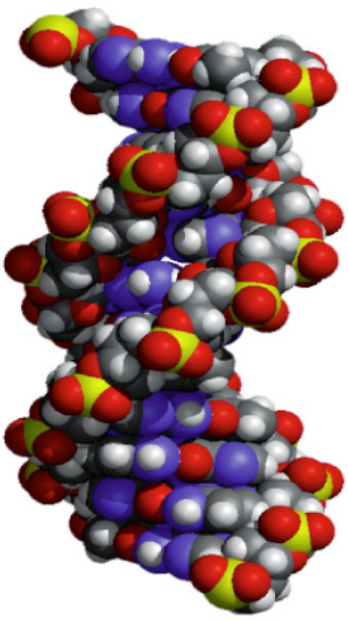
平行



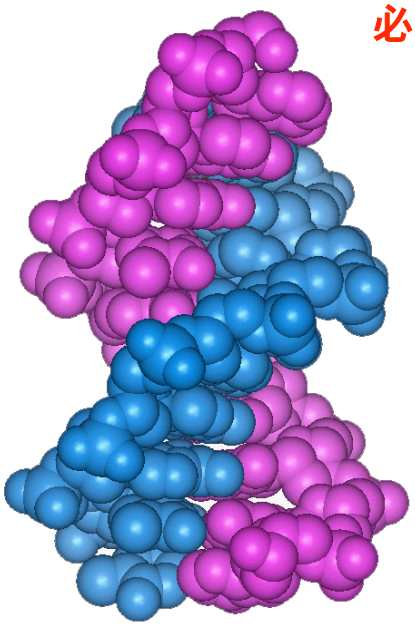






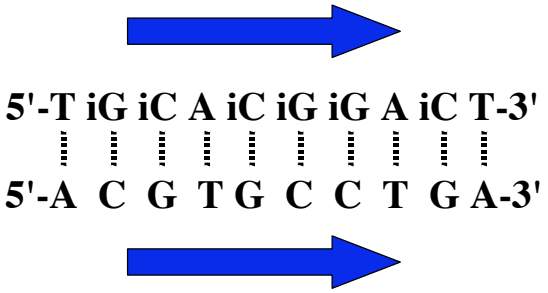


B型（右巻き）



平行DNA

必ずしも逆平行でなくてもよい！

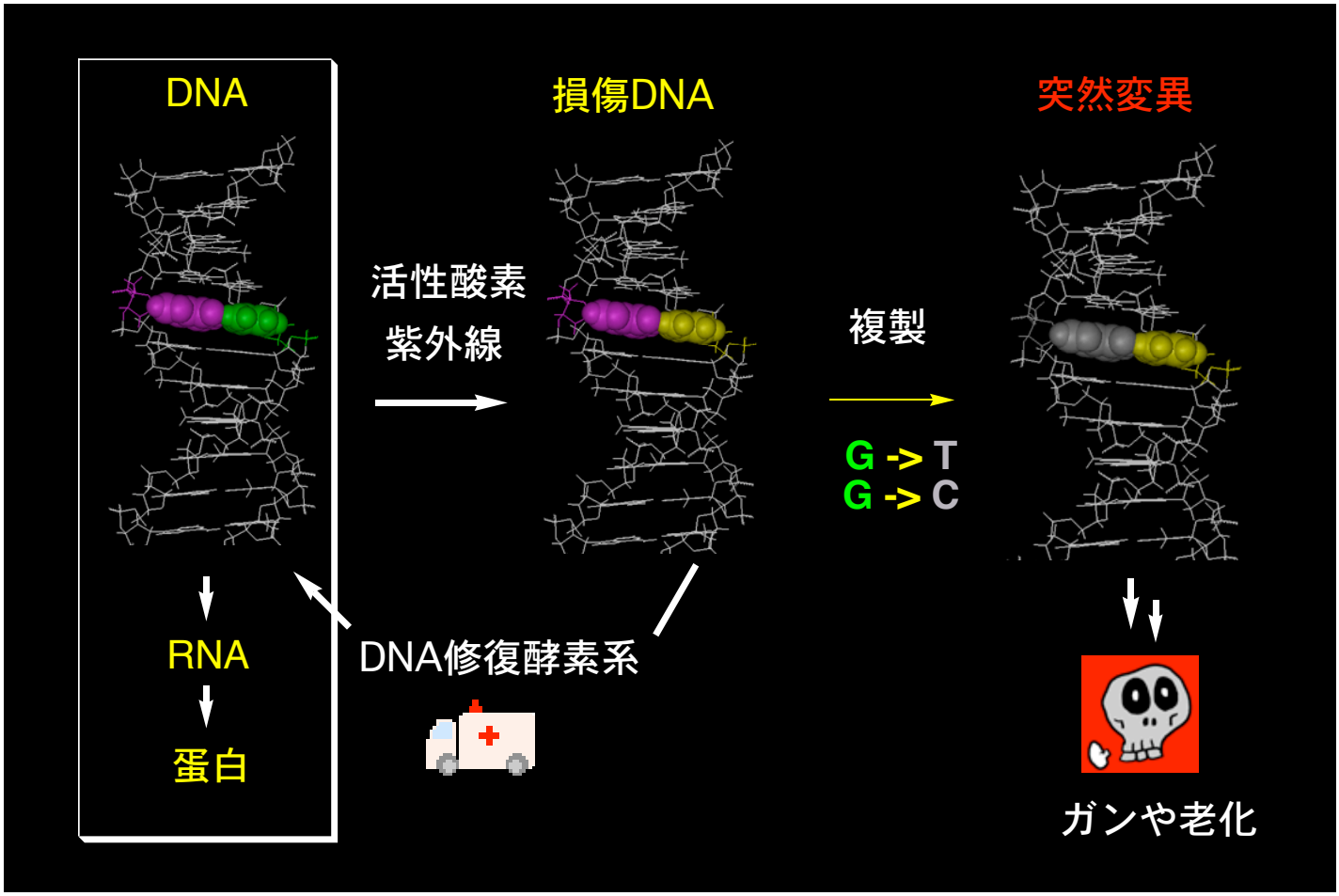


J. Am. Chem. Soc. 1996, 118, 9994

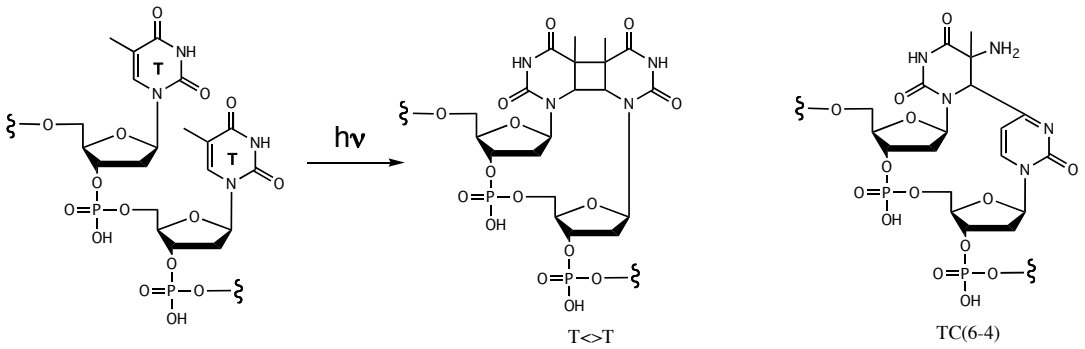
Biophys. J. 1998, 75, 1163

分子系の構造と電子状態ー「生物物質科学」を目指して

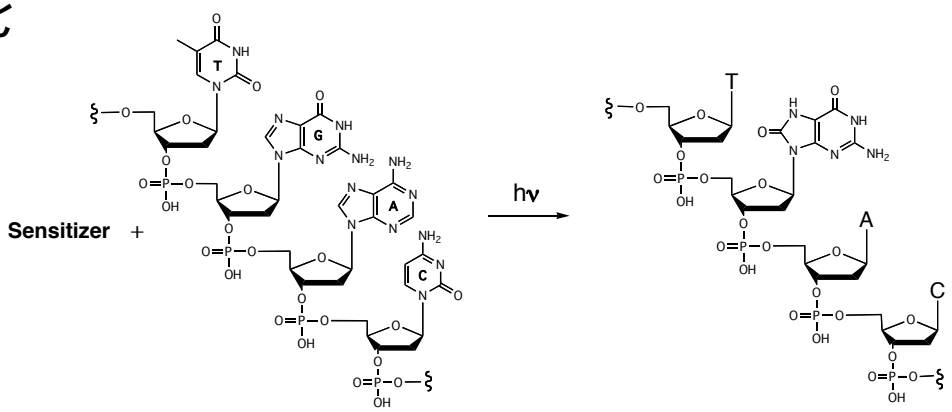
DNAの構造と損傷は密接に関連している


DNA
損傷DNA
突然変異
活性酸素
紫外線
複製
G -> T
G -> C
RNA
蛋白
DNA修復酵素系
ガンや老化

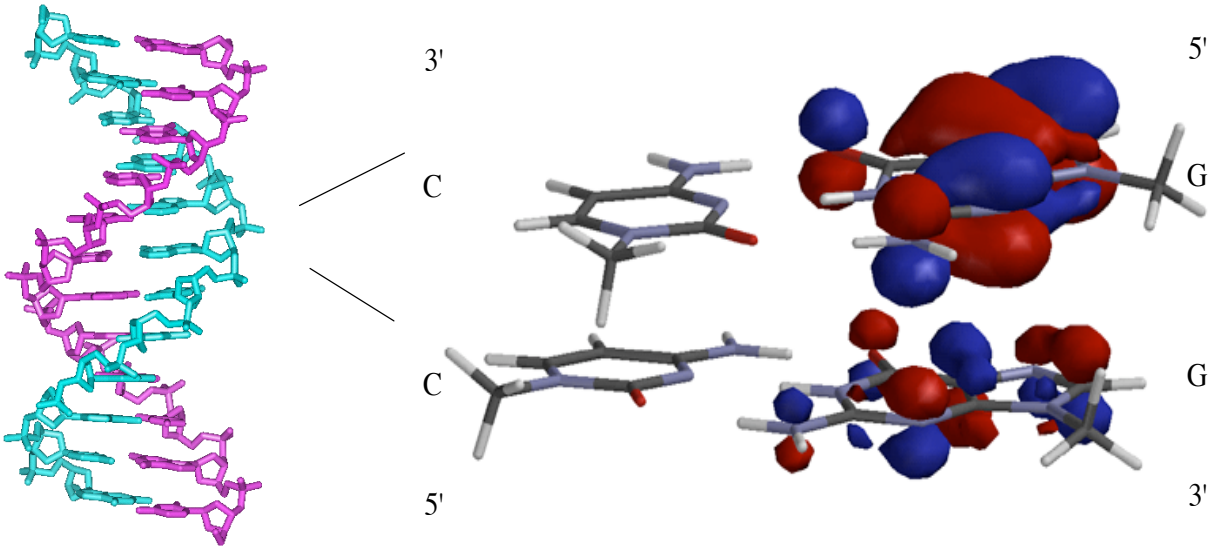
紫外線照射


hν
T<>T
TC(6-4)

酸化

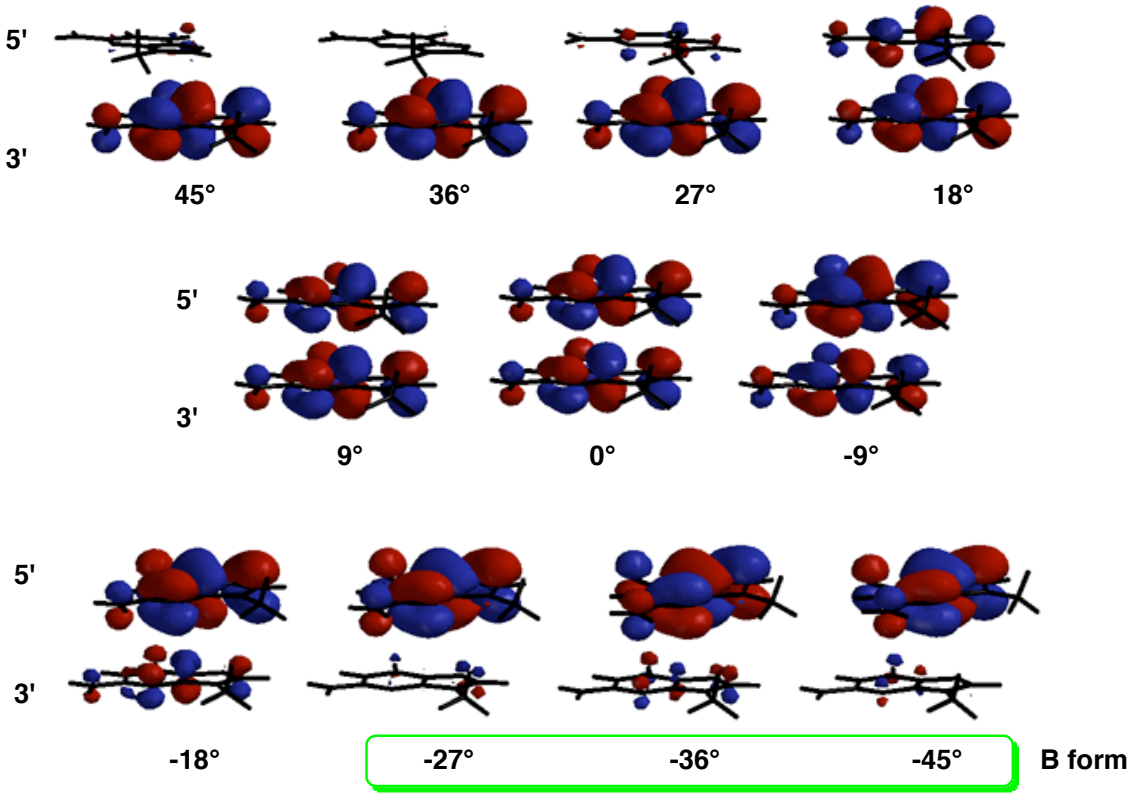

Sensitizer +
hν

酸化的損傷はGG配列の5’側のGで起こる



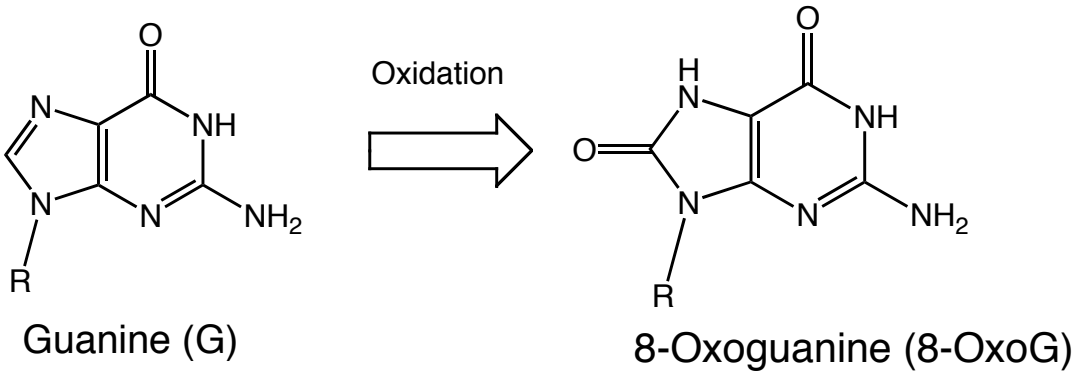
HOMO of DNA is localized at 5’G of GG sequence
Cation radical (Hole) is stabilized at GG sequence

J. Am. Chem. Soc. 1996, 118, 7063; 1995, 117, 6406 被引用回数670回

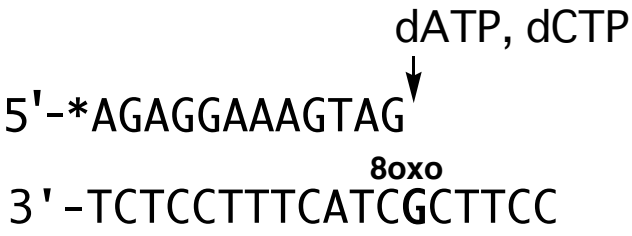


グアニンの重なりのずれが反応性を支配している！

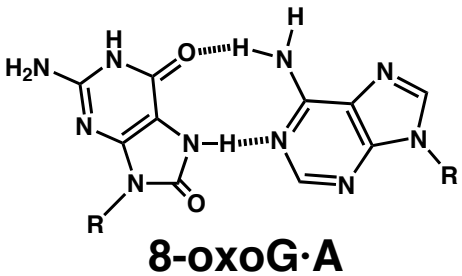
GGサイトの主生成物は8-OxoGである



8-oxoGの相手側にAがとりこまれる

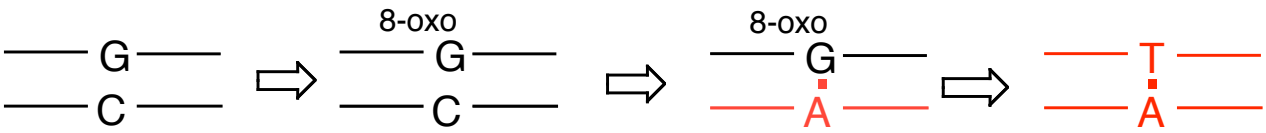


Nature 1991, 349, 431-434



8-oxoG·A

Proc. Natl. Acad. Sci. USA 1995, 92, 719-723

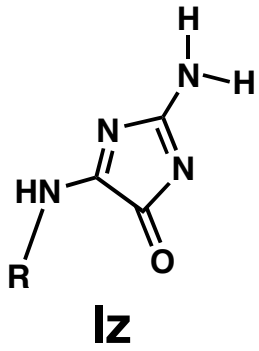


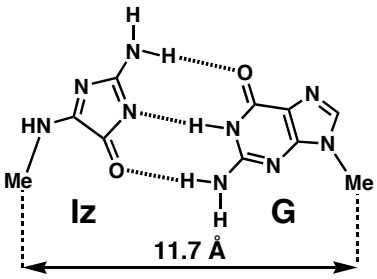
8-oxoGによるG->T 突然変異

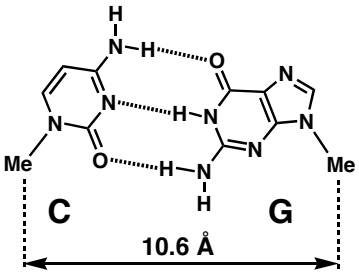
しかし8-oxoGでは高頻度で起こるG->Cへの変異は説明できない

イミダゾロン(Iz)が生成することを見いだした

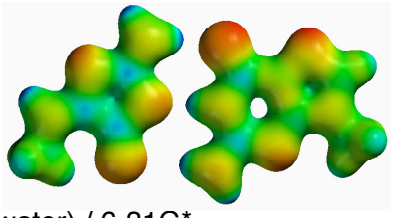
Kino et al. J. Am. Chem. Soc. 1998, 120, 7373

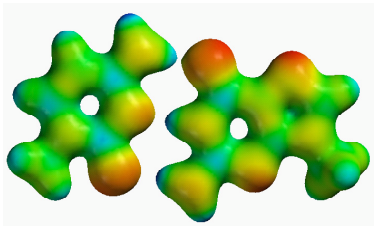






stabilization energy　-12.2 kcal/mol　　-13.5 kcal/mol



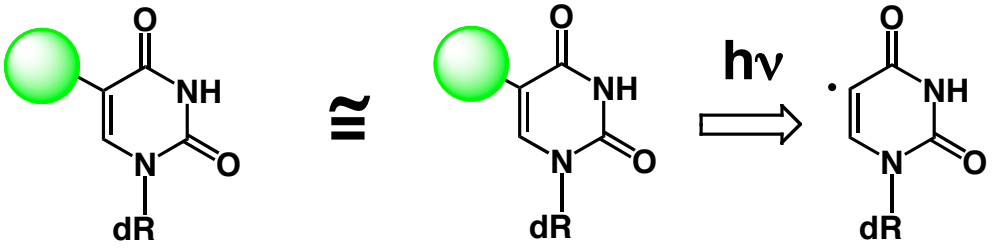


SCRF (ε = water) / 6-31G*

Gの相手側にIzが選択的に取り込まれる　Kino et al. Chem & Biol 2001, 8, 369

5－ブロモウラシルでDNAの電子状態を観る

Photochemistry of 5-Halouracil in DNA



Me (1.95 Å)

Thymine

X = Br (2.0 Å)
= I (2.15 Å)

5-Halouracil

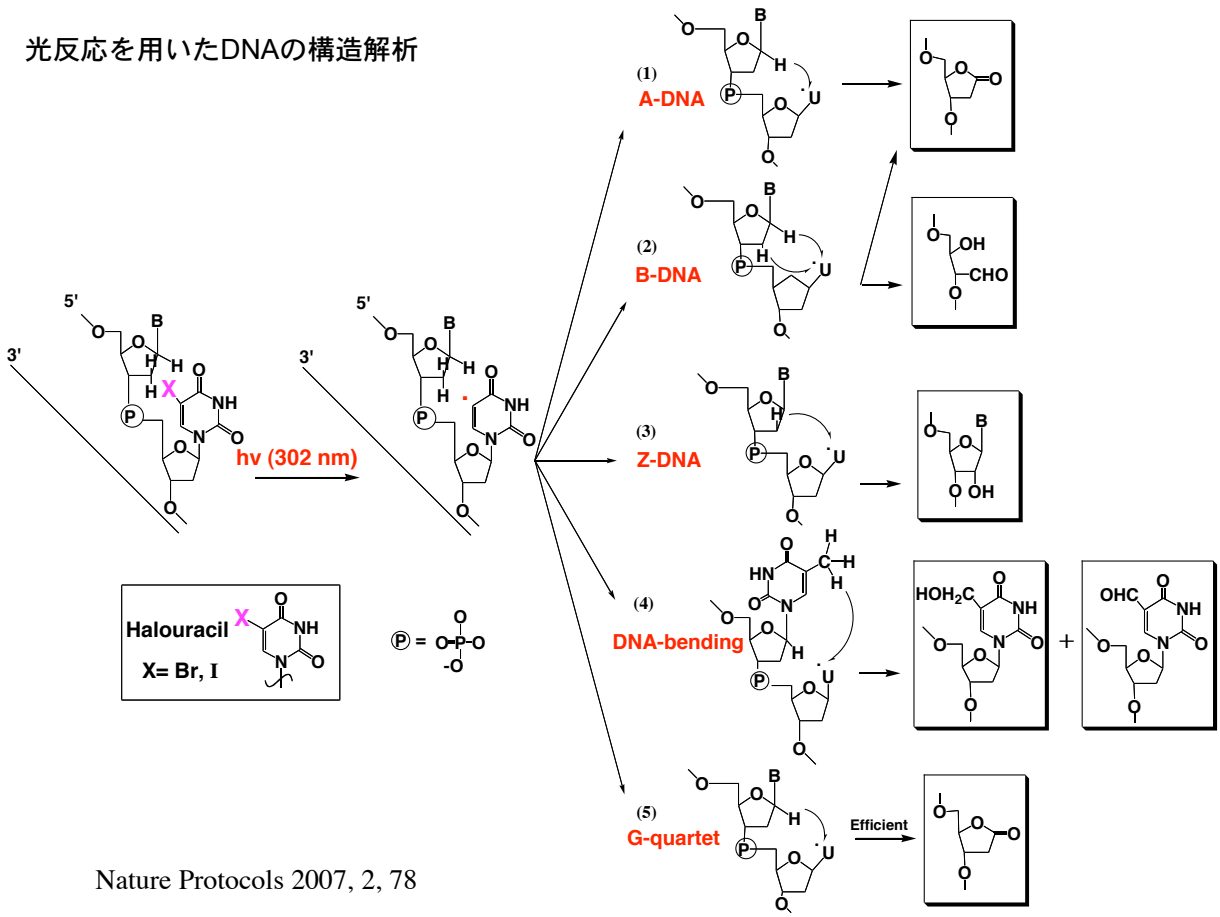
Uracilyl-5-yl radical

ファンデアワールス半径 ⟶ 構造は変化しない

5-halouracil は細胞中においてTの代わりに取り込まれる

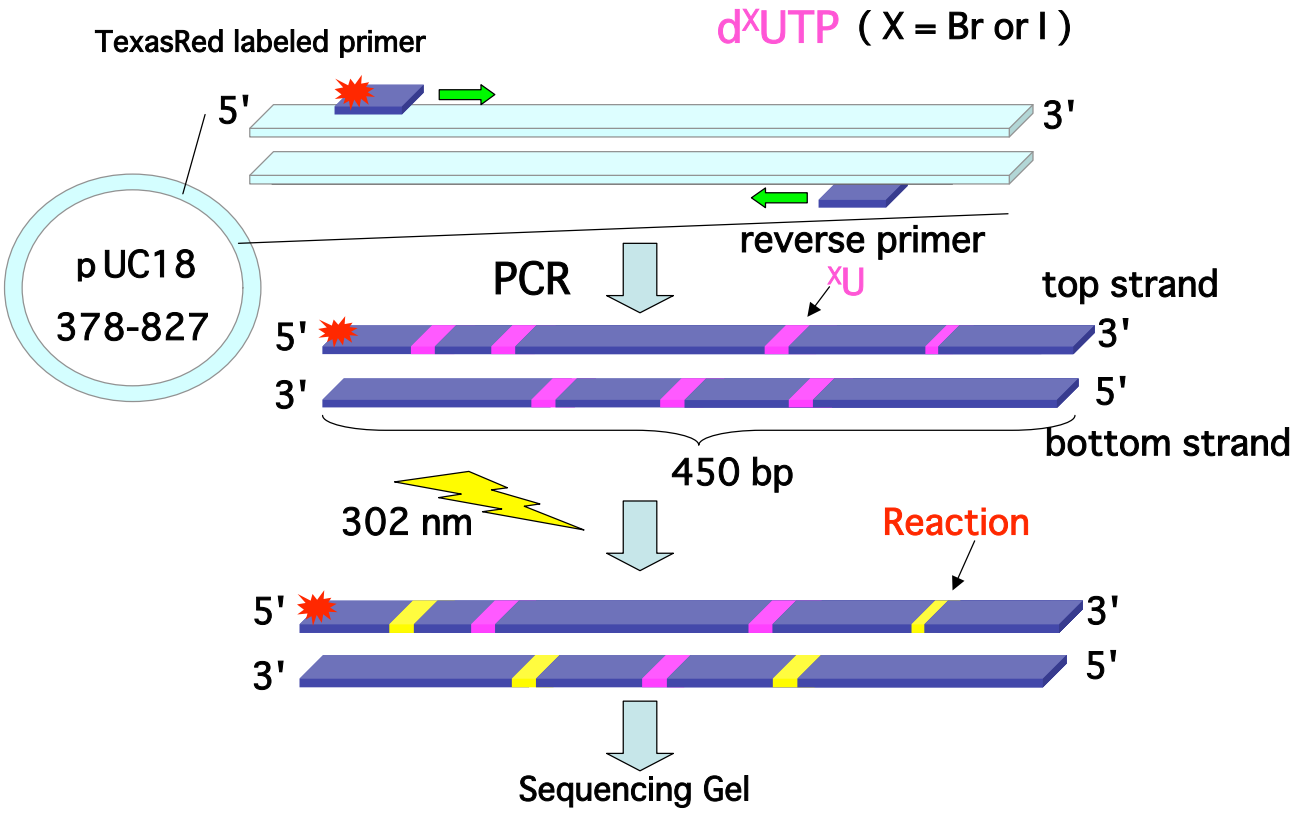
光照射によって、5-halouracil からウラシルラジカルが生成し水素を引き抜く

光反応を用いたDNAの構造解析

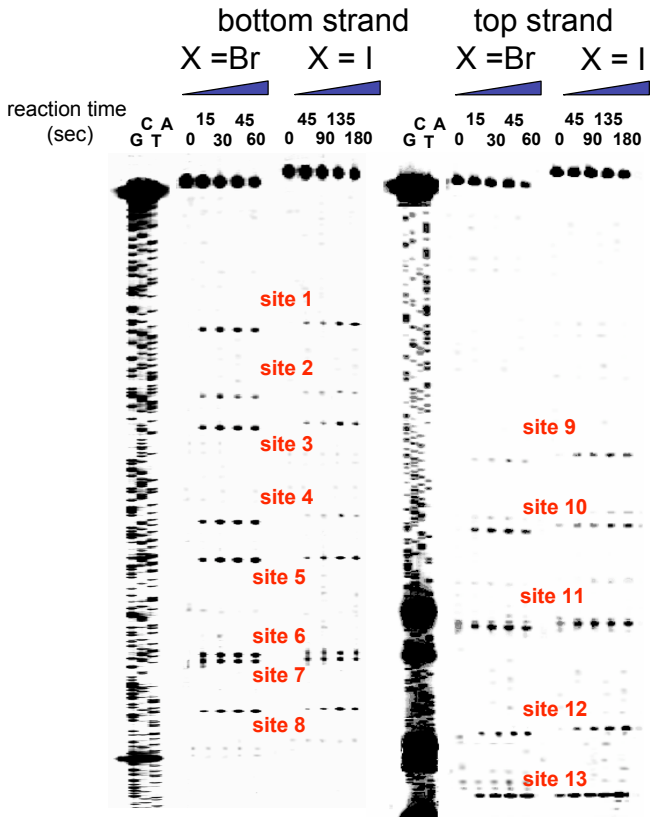


Nature Protocols 2007, 2, 78

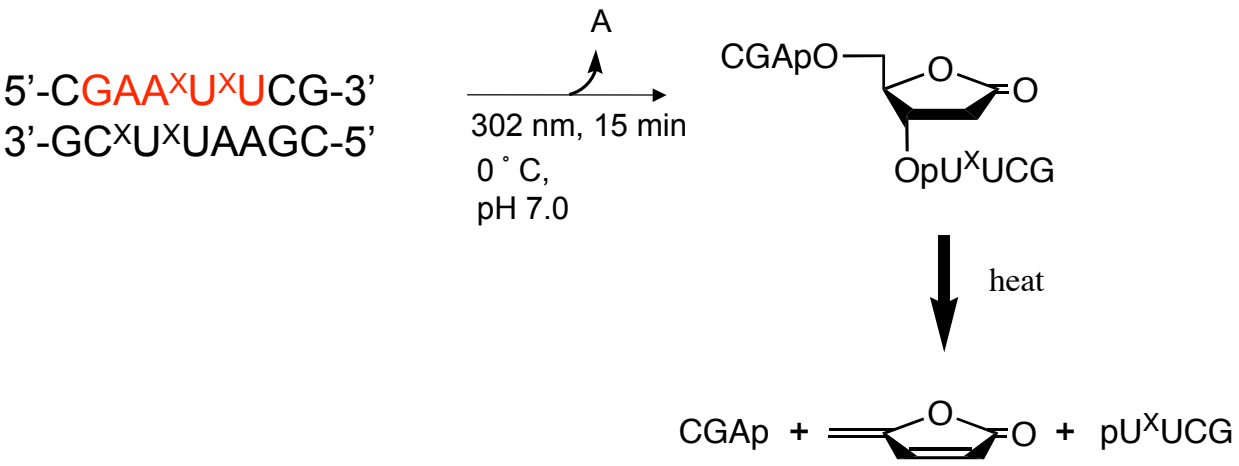
Photoreaction of 5-Halouracil-Containing DNA Fragment



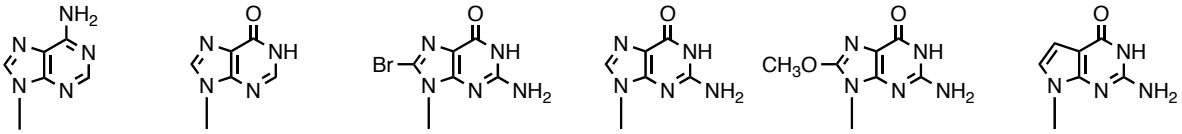
Specific Cleavage at (G/C)AAXUXU and (G/C)AXUXU



Product of Photoirradiated d(CGAA^{X}U^{X}UCG)$_2$



Efficiency Linearly Correlates with IP of Base



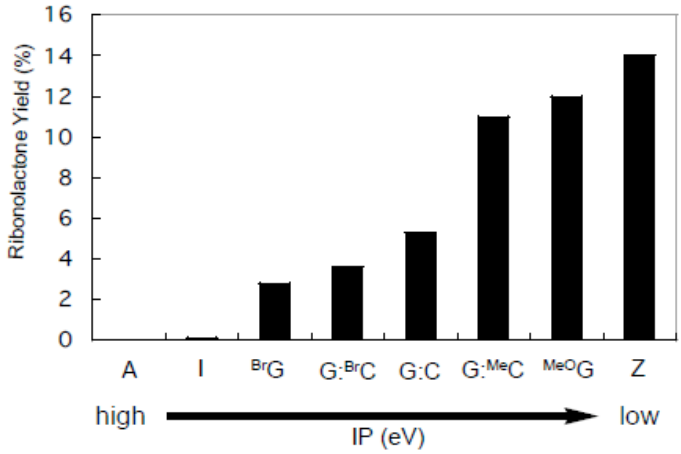
A *I* *^{Br}G* *G* *^{MeO}G* *Z*

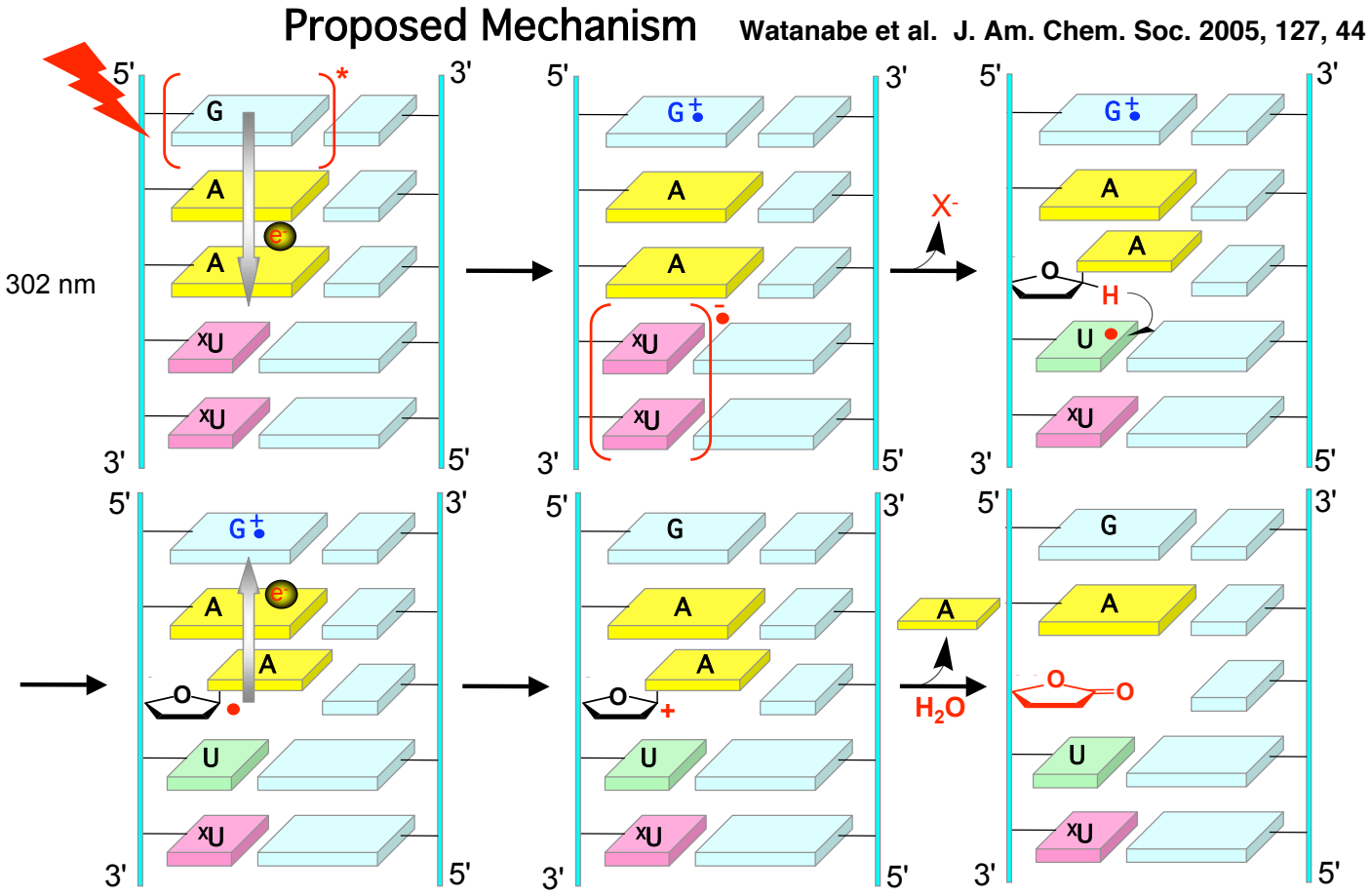
electron deficient ⟺ **electron rich**

5'-CXAA^{Br}UTGC-3'
3'-GCTT A ACG-5'

hν

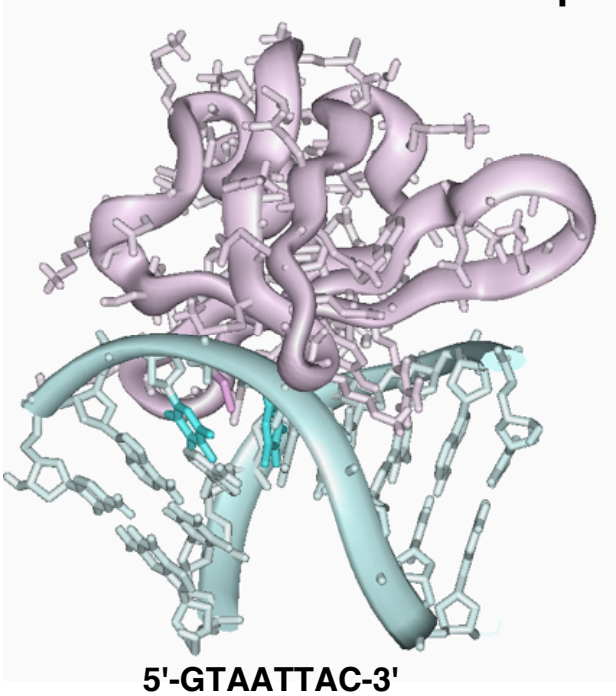
5'-CXALUTGC-3'





蛋白からDNAへの光電子移動

Structures of Sso7d-DNA Complex

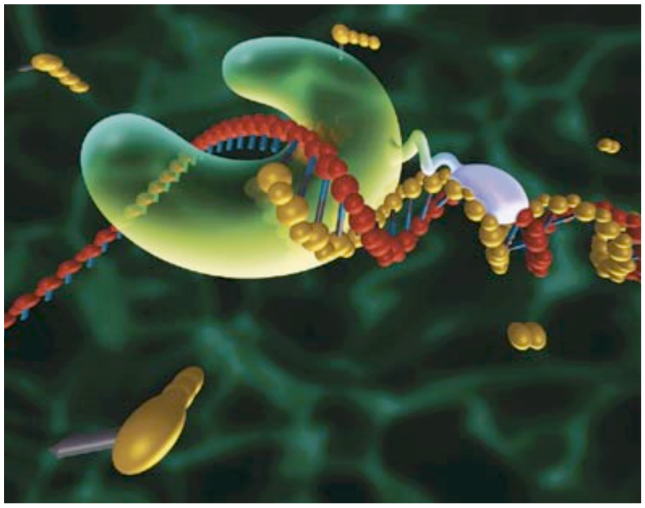


5'-GTAATTAC-3'
3'-CATTAATG-5'

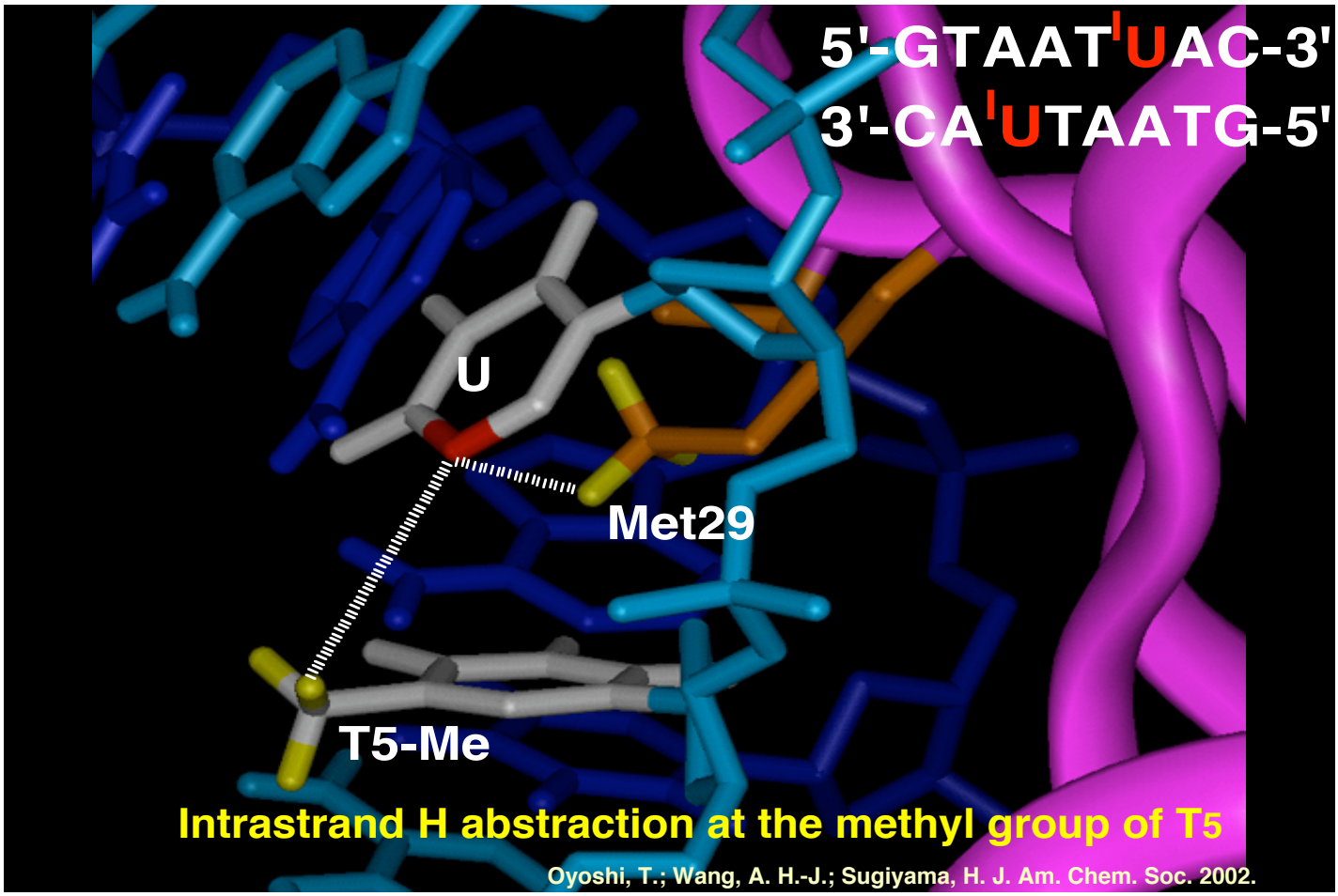
Sso7d-DNA Complex

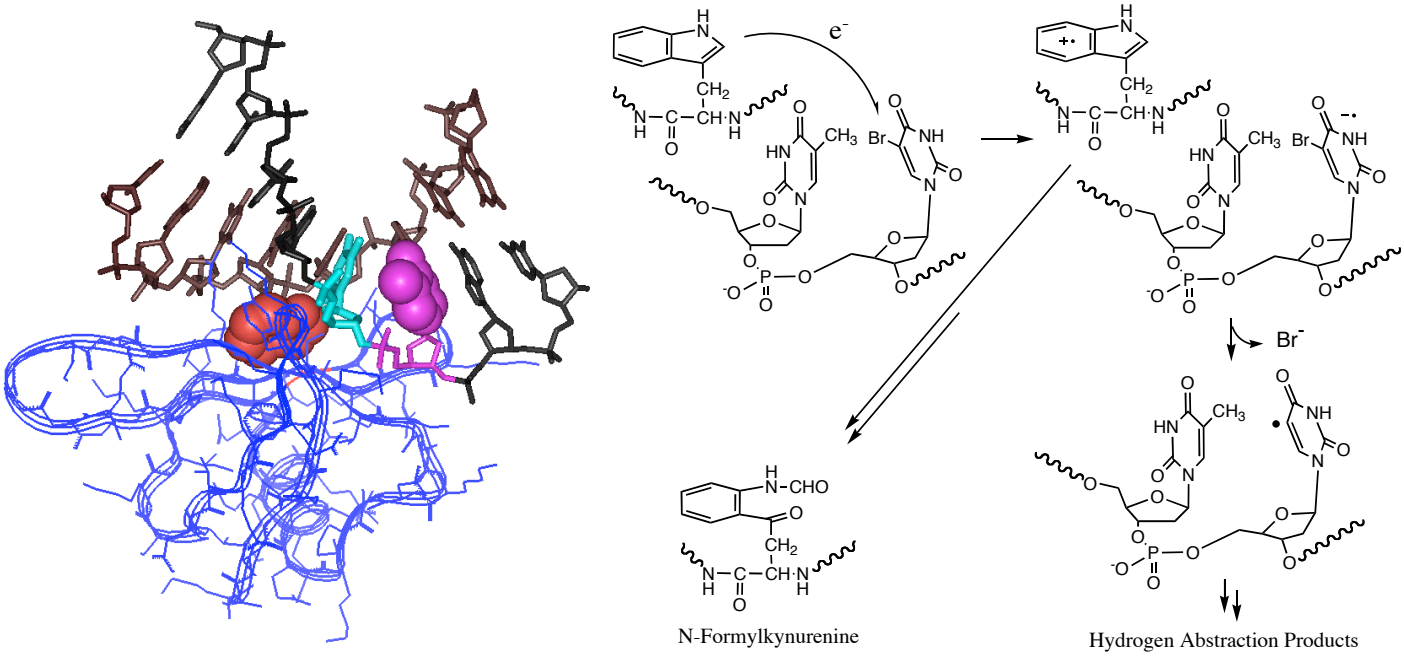
Wang, A. H.-J.et al., *Nature Structural Biology 1998.*

Sso7d:
Chromosomal protein from
hyperthermophilic archaeabacteria
Sulfolobus solfataricus



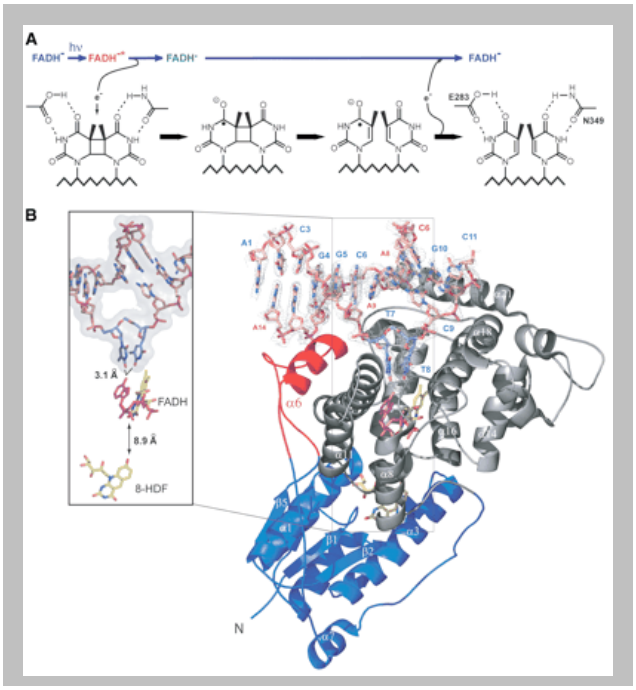
Phusion High-Fidelity DNA Polymerase





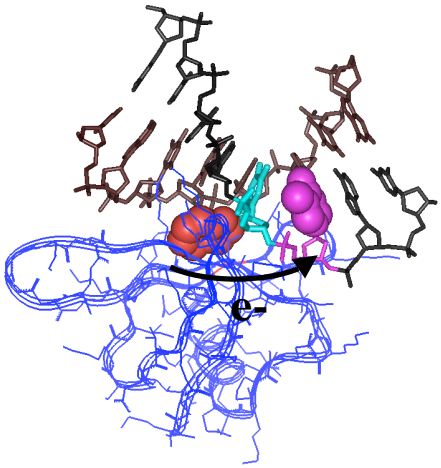
Trp残基からの電子移動で反応が進んでいる

Photolyase



(Carell and Essen. Science 2004)

Sso7d



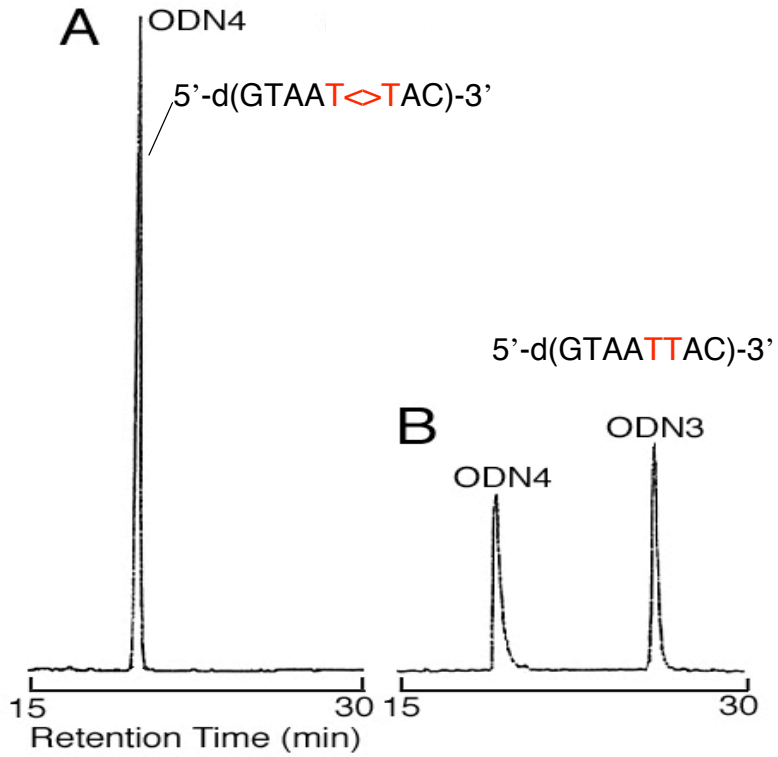
T<>T

Sso7d ?

T T

Can Sso7d repair T<>T dimer?

Photoreactivation of Thymine dimer by Sso7d



5’-d(GTAAT<>TAC)-3’

hν

Sso7d

5’-d(GTAATTAC)-3’

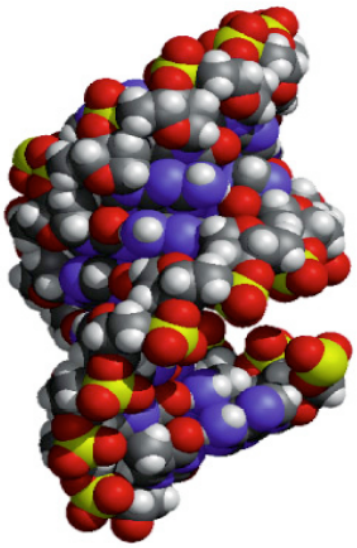
Sso7dに光回復能がある

蛋白からDNAへの電子移動が起こる！

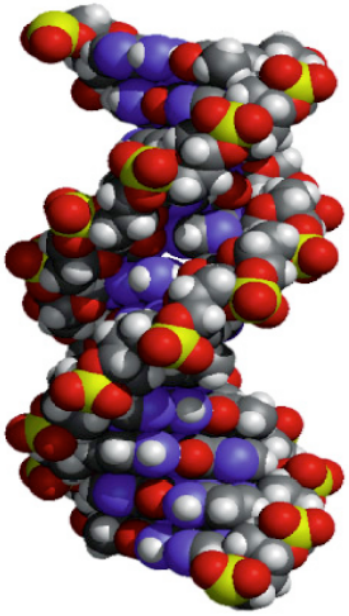
PNAS, 2006, 103, 16655

電子移動はDNAの構造に依存する

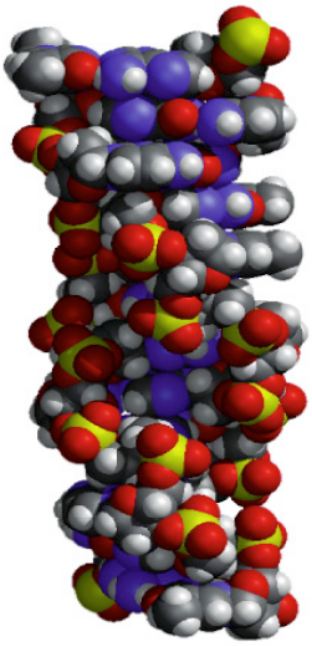
The Three Helical Forms of DNA



A型（右巻き）
エタノール添加



B型（右巻き）

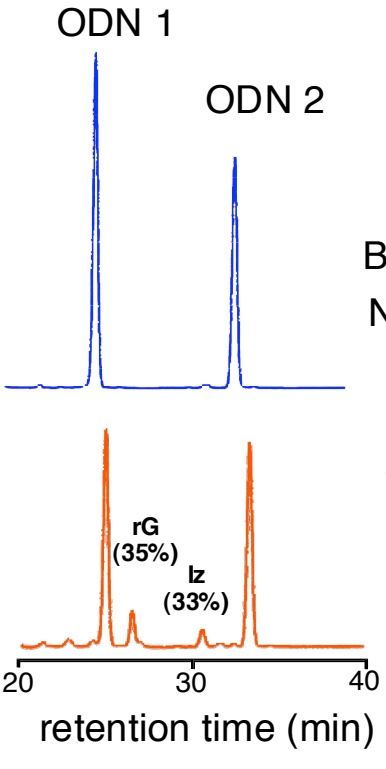


Z型（左巻き）
塩添加

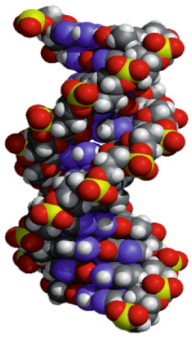
5'-d(CG CG^{Br}UG CG)-3' ODN 1
3'-d(GC^{m}GC AC^{m}GC)-5' ODN 2

302 nm
30 min
0 °C

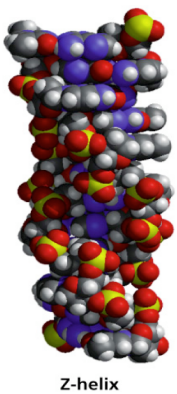
HPLC analysis



B-DNA
No reaction

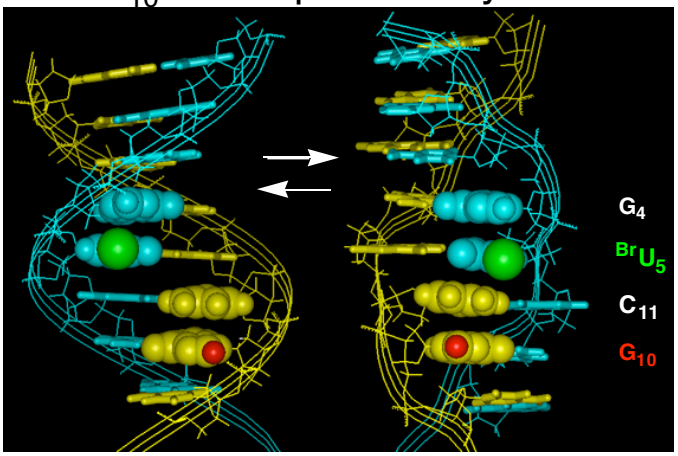


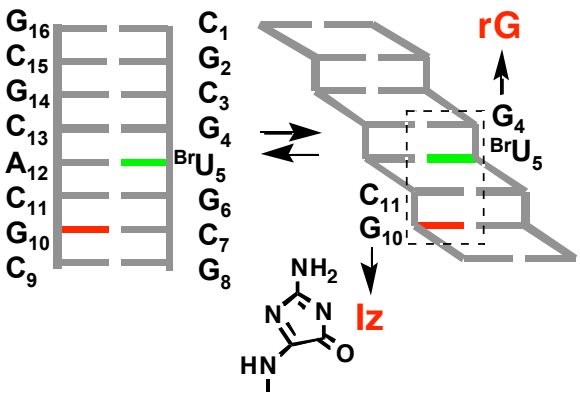
Z-DNA



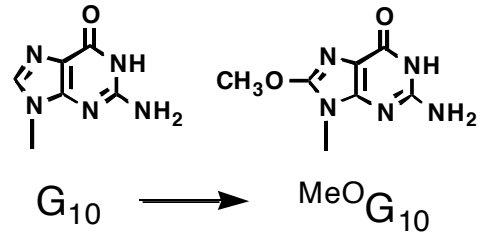
B-Z transition dramatically increased the photoreactivity

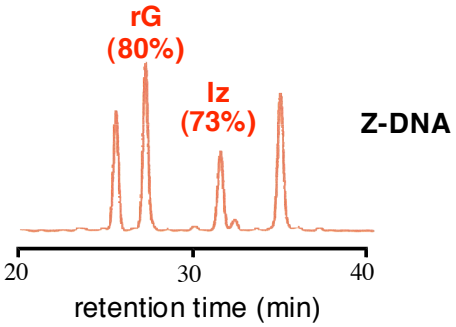
G_{10} in complementary strand was simultaneously oxidized.





B-DNA **Z-DNA**

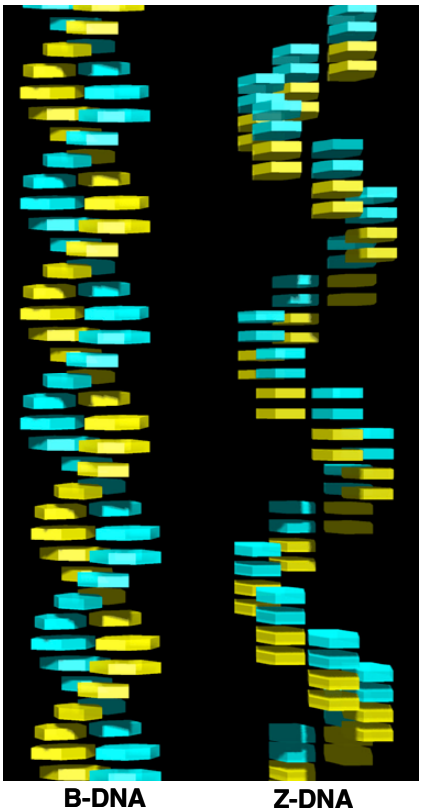


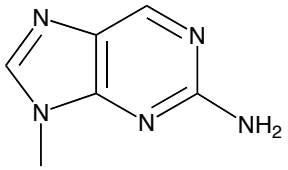


G_{10} at the end of four-base π-stacks act as an electron donor !

Tashiro Sugiyama J. Am. Chem. Soc. 2003, 125, 15282

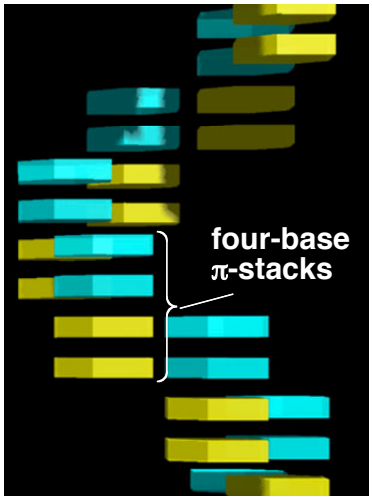
電子状態はZ型とB型で大きく異なる





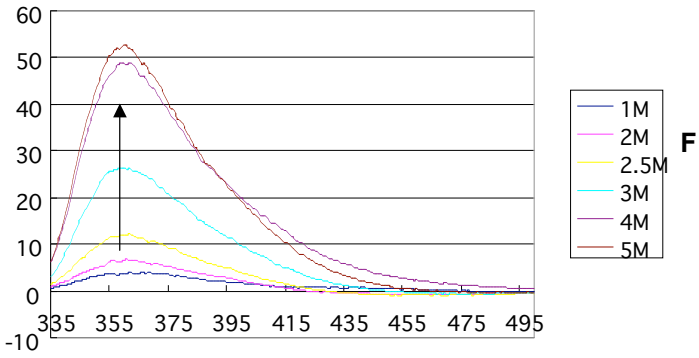
アミノプリン(Ap)

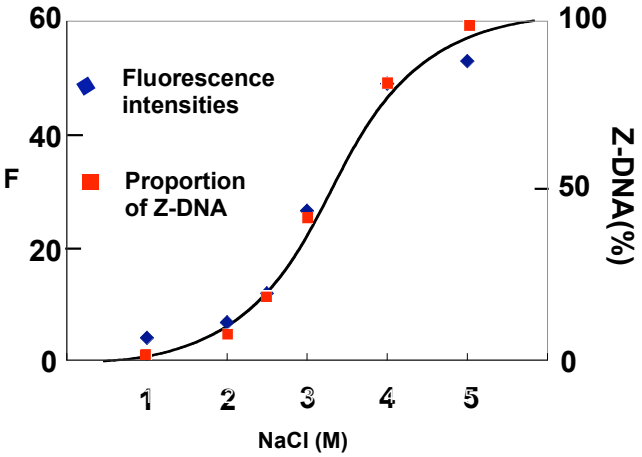
典型的な蛍光プローブであるが
B型構造ではGによる電子移動で蛍光は弱い



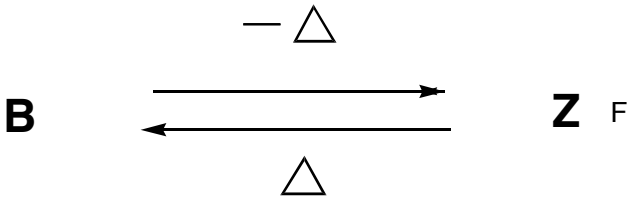
Apの蛍光はZ型の中では増大する

5’-d(CGCApCGCGCG)-3’
3’-d(GCGT GCGCGC)-5’

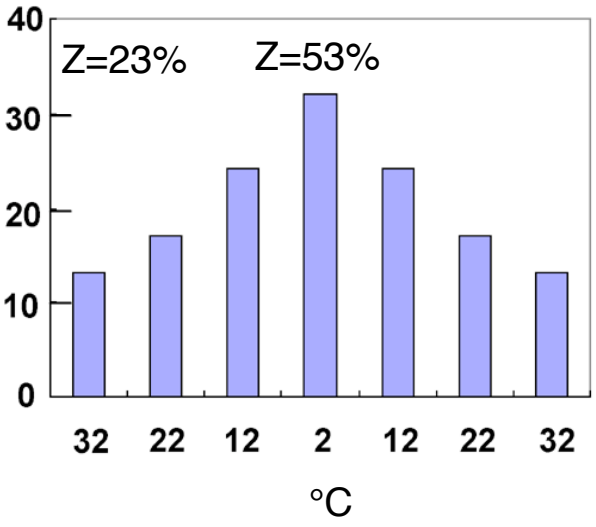


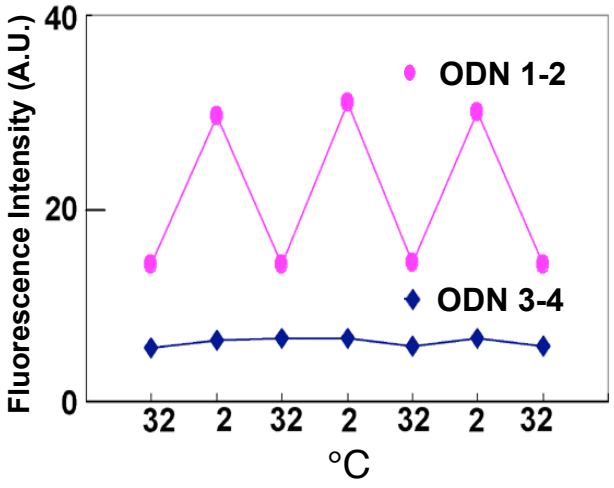


蛍光を温度でコントロールする

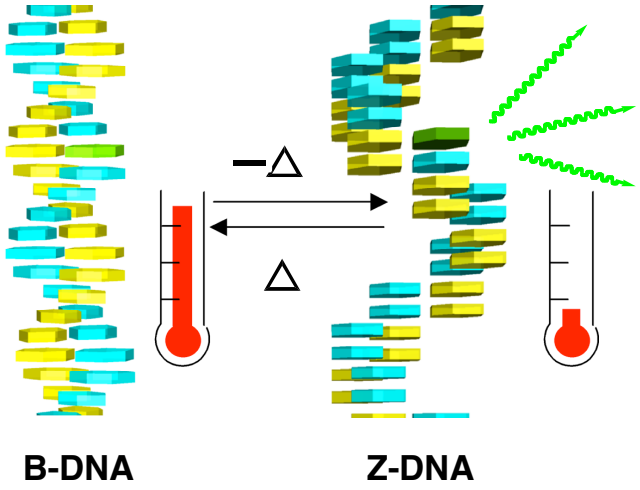


Z型DNAは低温下で安定である



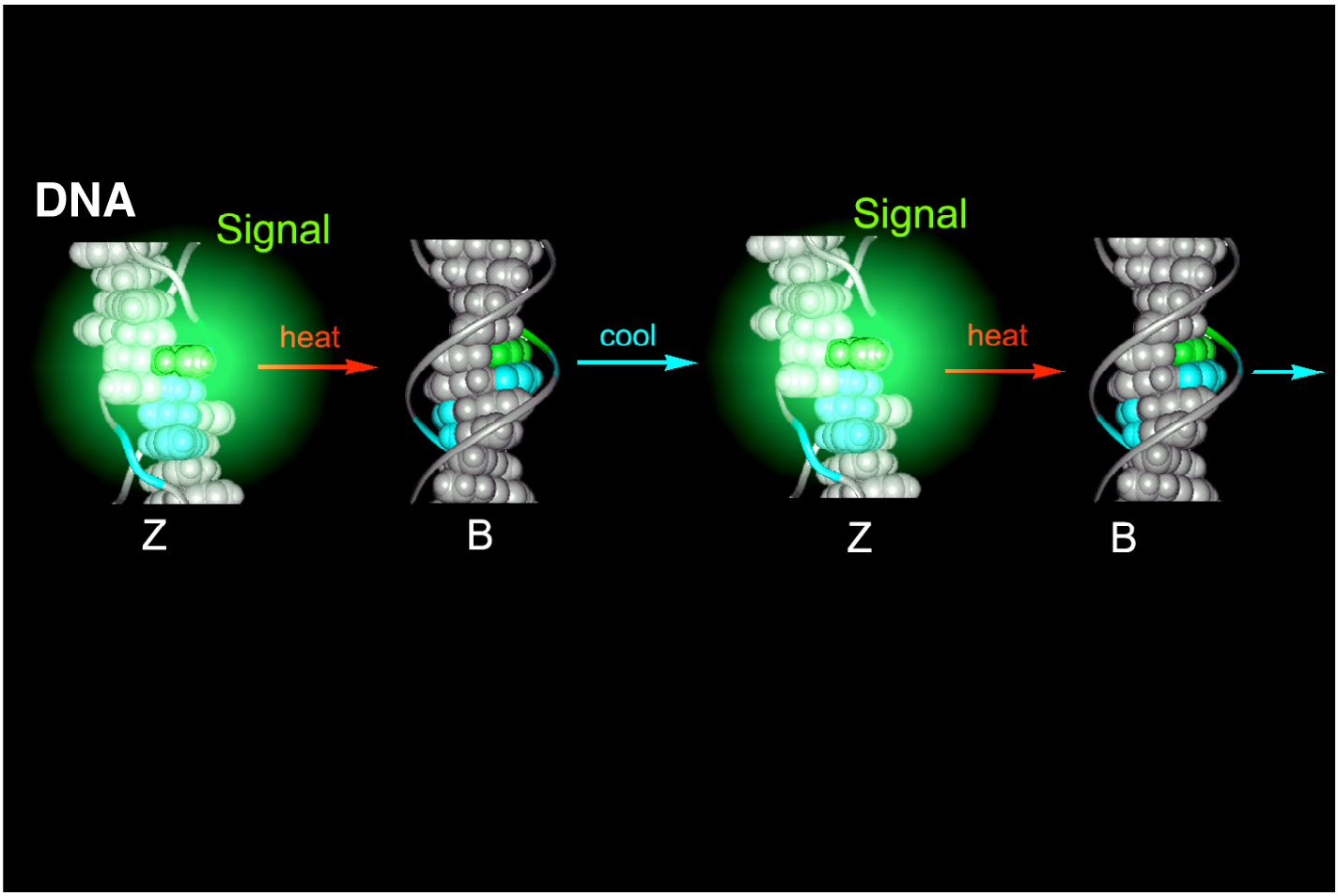


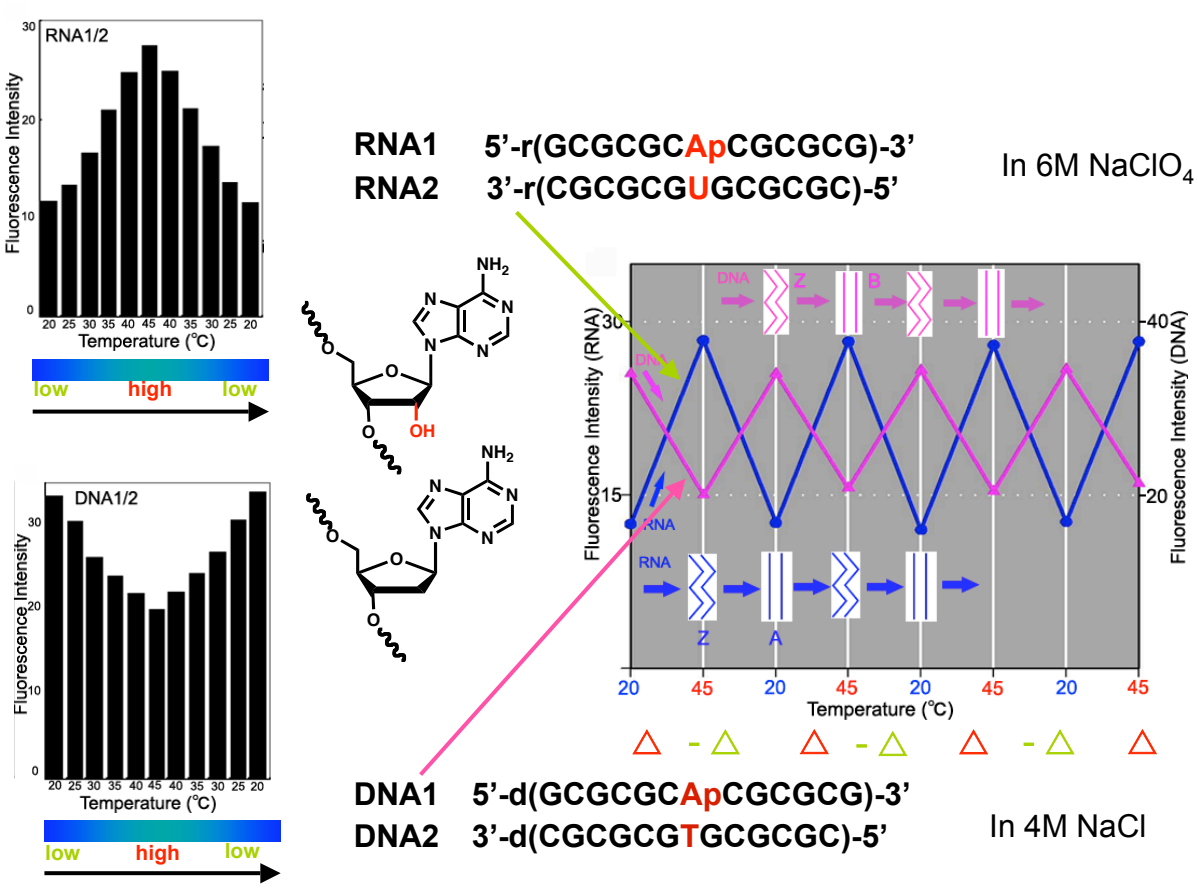
Change of fluorescene is fully reversible !

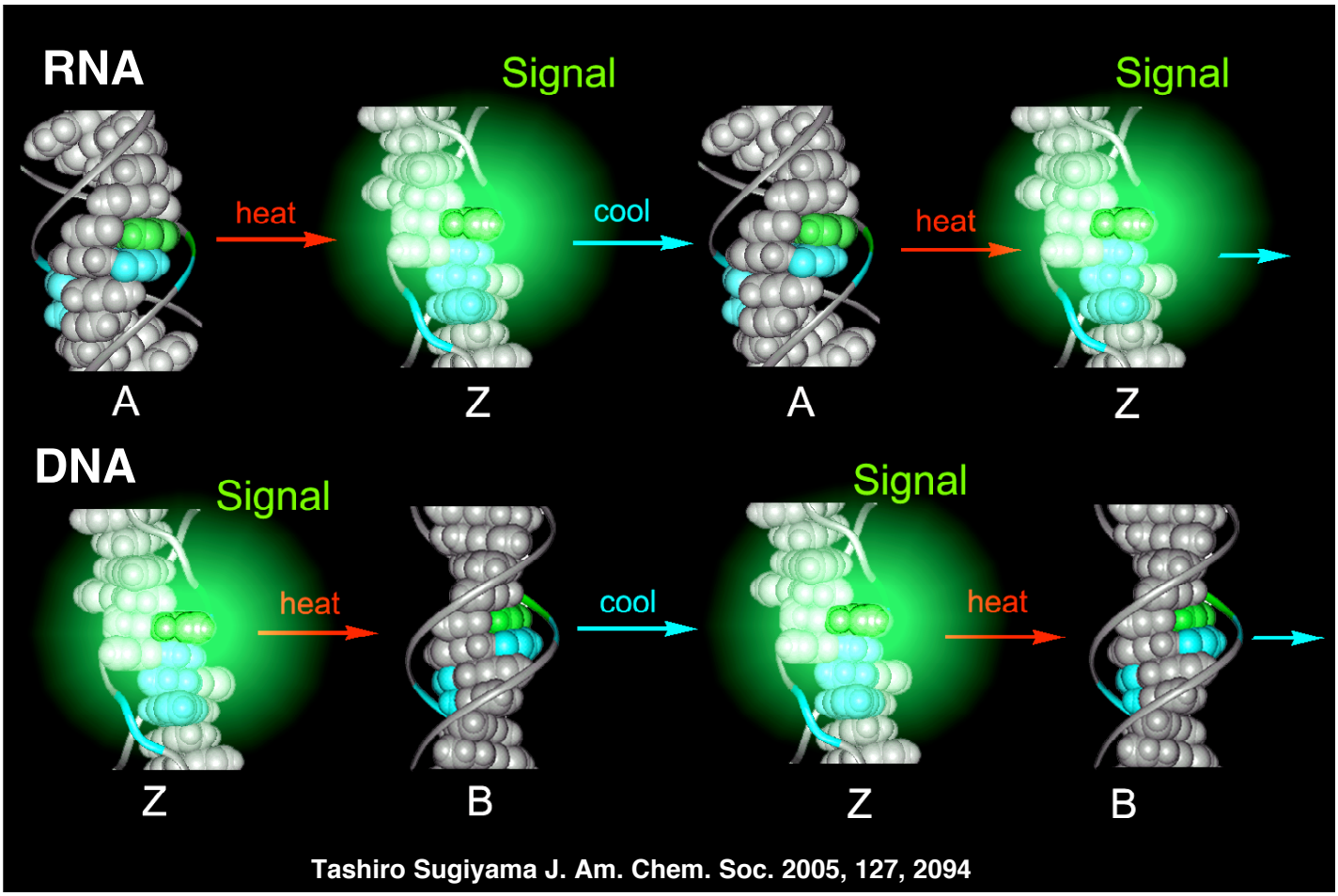


Molecular thermometer based on different π-stack of B and Z-DNA

Tashiro Sugiyama Angew. Chem. Int. Ed. 2003, 42, 6018




RNA1/2
Fluorescence Intensity
Temperature (℃)
low high low
DNA1/2
Fluorescence Intensity
Temperature (℃)
low high low
RNA1 5'-r(GCGCGCApCGCGCG)-3'
RNA2 3'-r(CGCGCGUGCGCGC)-5'
In 6M NaClO4
NH2
OH
DNA
Z
B
RNA
A
Fluorescence Intensity (RNA)
Fluorescence Intensity (DNA)
Temperature (℃)
DNA1 5'-d(GCGCGCApCGCGCG)-3'
DNA2 3'-d(CGCGCGTGCGCGC)-5'
In 4M NaCl


RNA
Signal
Signal
heat
cool
heat
A
Z
A
Z
DNA
Signal
Signal
heat
cool
heat
Z
B
Z
B
Tashiro Sugiyama J. Am. Chem. Soc. 2005, 127, 2094

DNAは分子材料として研究すべき生物物質である

分子系の構造と電子状態ー「生物物質科学」を目指して

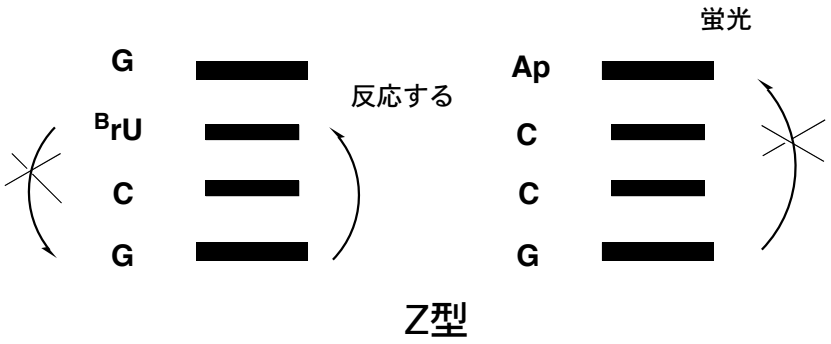
Acknowledgments

Kyoto University
Department of Chemistry
Dr. Toshikazu Bando
Dr. Yan Xu
Dr. Kennichi Shinohara
Dr. Ryu Tashiro

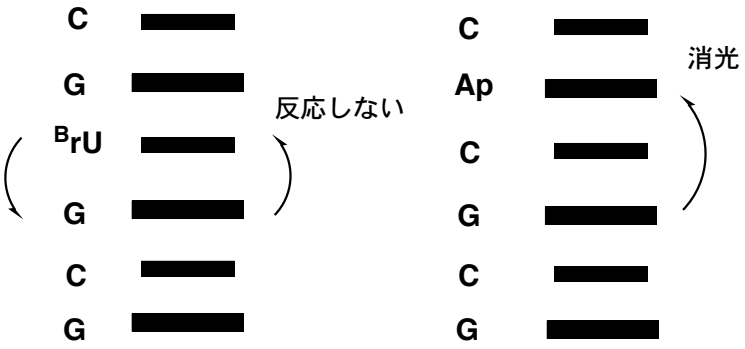
Academia Sinica
Institute of Biol. Chem.
Dr. Andrew Wang

Tokyo Medical & Dental University
Institute of Biomaterials and Bioengineering
Dr. Kiyohiko Kawai
Dr. Shuji Ikeda
Dr. Katsuhito Kino
Dr. Takanori Oyoshi
Mr. Takayoshi Watanabe
Ms. Reiko Ikeda

The work was supported by a grant-in-aid for scientific research from the Ministry of Education, Science, Sports and Culture, Japan and SORST of Japan Science and Technology Corporation (JST).



Z型



B型