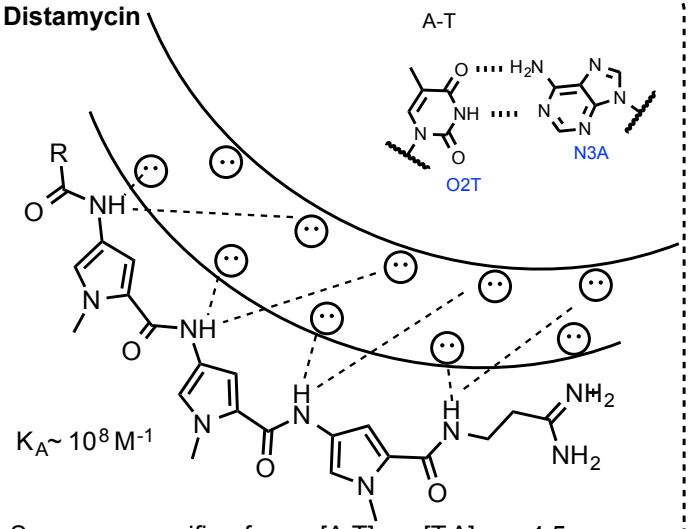
Lecture 3 - Binding of small molecules to DNA

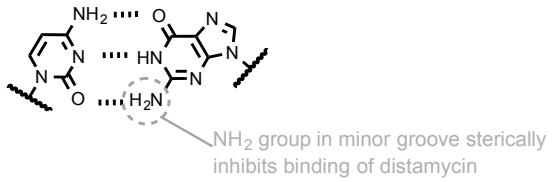
Binding of small molecules to DNA
- Electrostatics
- Intercalation
- Groove binding through Van der Waals and hydrogen bonding

Distamycin



-Sequence specific - favors $[A:T]_n$ or $[T:A]_n$, n=4,5.
-Binds and displaces the spine of hydration in the minor groove.
-Bifurcated hydrogen bonding of one NH to two lone pairs
-Entropic driving force due to displacement of H2O.
-Enthalpic driving through Van der Waals and hydrogen bonding.
-Can also have 2:1 binding mode (2 distamycin/site)
-No major change in DNA upon binding

Why not G:C?

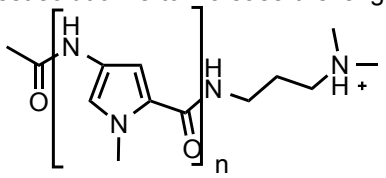


Distamycin-Increasing selectivity

-Selectivity is not great i.e. $(1/2)^5$- one in every 32 sites is an AT rich run of 5 bases

How do you increase selectivity?

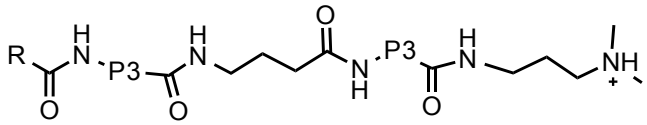
-Simplest solution is to increase the length of polypyrrole



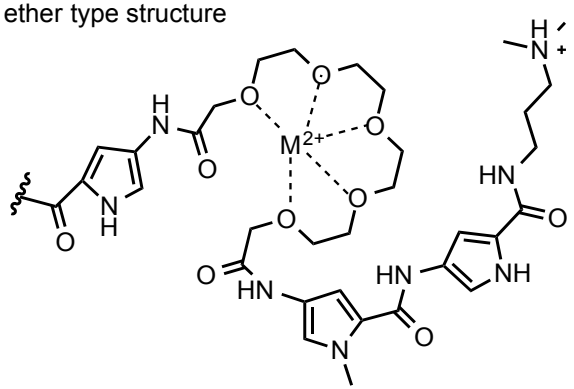
Distamycin = P3 (n=3)

This works for n≤5, but for n>5 the curvature of the polypyrrole backbone doesnt match the curvature of DNA.

-To solve this add in an aminobuteric linker to reorient the pyrrole units



-One can also make a distamycin who's binding dependent on metal ion binding
-Add in a metal binding sequence i.e. a PEG spacer which will bind to a metal ion to form a crown-ether type structure



-Sr^{2+} and Ba^{2+} show high binding affinity
-Unfortunately this doesn't work with Ca^{2+}

-Another solution would be to space distamycin with an interchelator

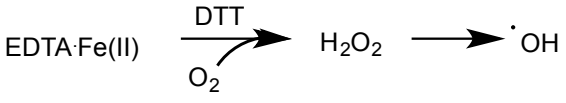
P3 ~~~ EtBr ~~~ P3

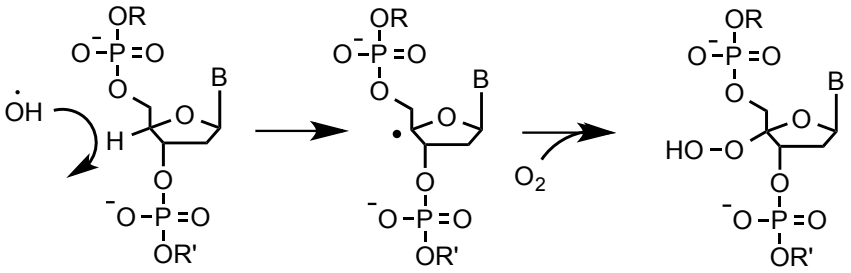
$(A/T)_n$ GC/CG $(A/T)_2$

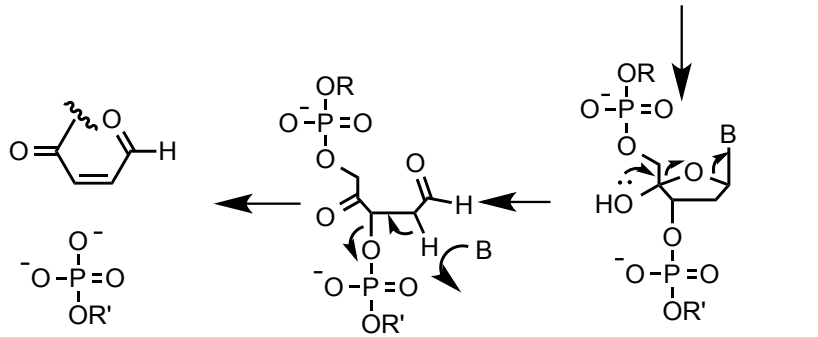
Sequence Specific Binder

- How do you determine where small molecules bind?

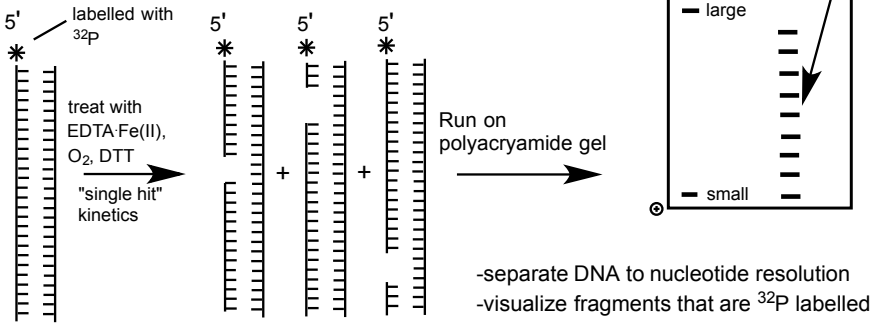
-Take advantage of the following reaction:







DNA backbone is cleaved

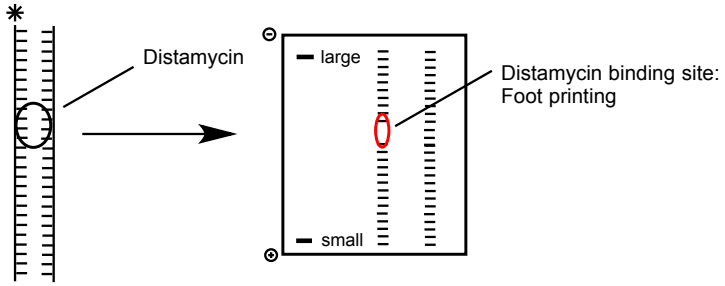


-separate DNA to nucleotide resolution

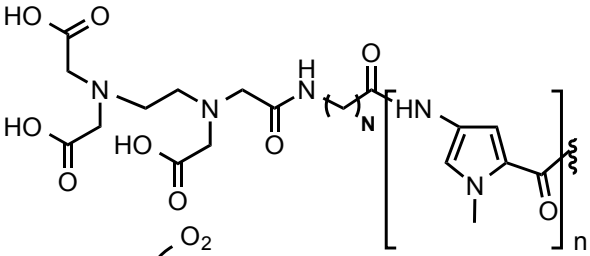
-visualize fragments that are ^{32}P labelled

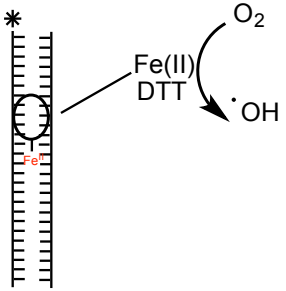
-If you bind a molecule (distamycin) to the DNA, it will provide either steric blockage or quench the cleavage reaction

-The resulting gel will have a "foot-printed" binding site



To increase resolution add an Fe(II) binding motif to generate site specific ·OH adjacent to ligand binding site.





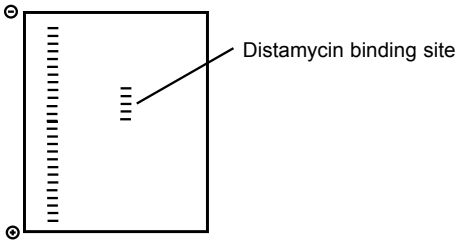
-generates OH at the site of binding

-reacts at a diffusion controlled rate constant

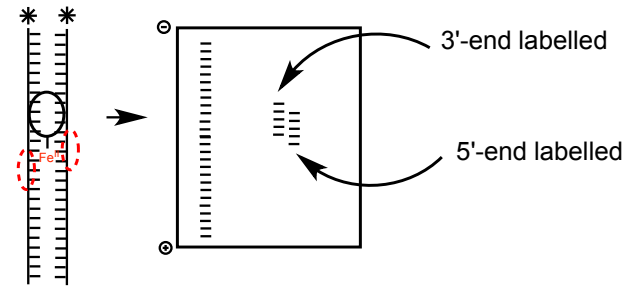
-in water small molecules diffuse at 10^9-$10^{10}M^{-1}s^{-1}$

-large molecules 10^6-$10^7M^{-1}s^{-1}$

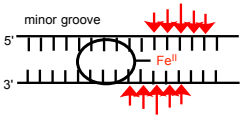
-end up with local cleavage of distamycin binding site



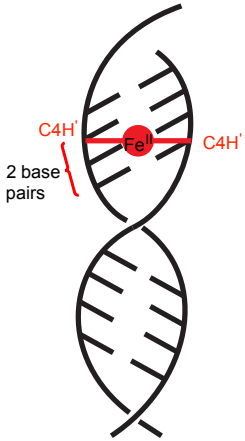
-If you label both strands you can track cleavage on both strands



-observed that cleavage on the 5' strand was shifted in the 3' direction

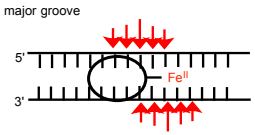


-this is due to the helicity of right-handed DNA

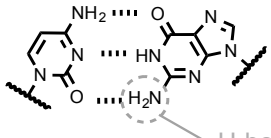


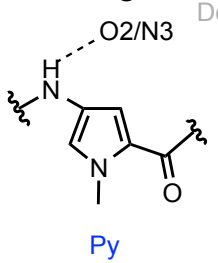
-Fe(II) cleaves 2 base pairs away on the other strand due to helicity of the strands

-But helicity in the major groove is different, so Fe(II) cleavage of major groove binders results in a shift in the 5' direction
-used to distinguish between major or minor groove binders

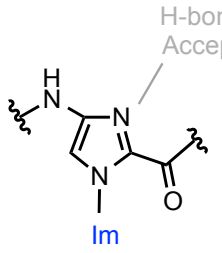


-but still limited to AT base pairs

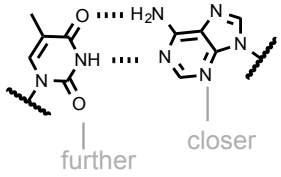


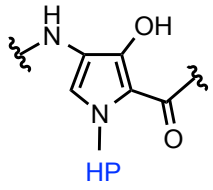


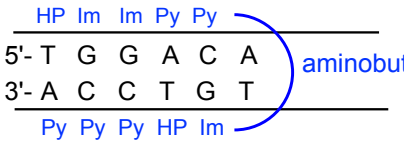
Modify to:



-TA vs. AT





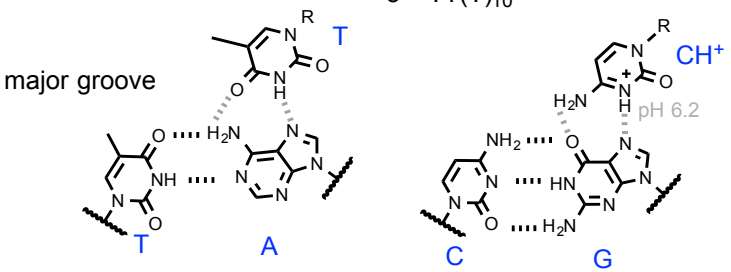


Im/Py	GC
Py/Im	CG
HP/Py	TA
Py/HP	AT

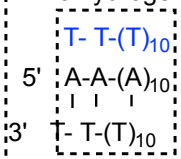
Typically bind with $K_D<10^{-9}\ M^{-1}$

Binding of longer sequences

5' $AA(A)_{10}$
3' $TT(T)_{10}$

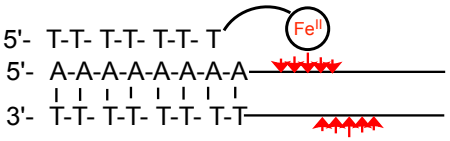


-Use Hoogsteen base pairing in the major groove
-Two hydrogen-bonds from protonated CH^+ (pH 6.2) binding to GC
-Two hydrogen-bonds from T binding to AT



CH^+- T - T -CH^+- T
5' - G - A - A - G - A -
3' - C - T - T - C - T -

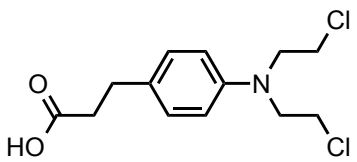
-This only works for -$(Py)_n$- or -$(Pu)_n$ - sequences (i.e. GATGA does not work).



-cleavage pattern shifted in the 5' direction
-indicates major groove binder

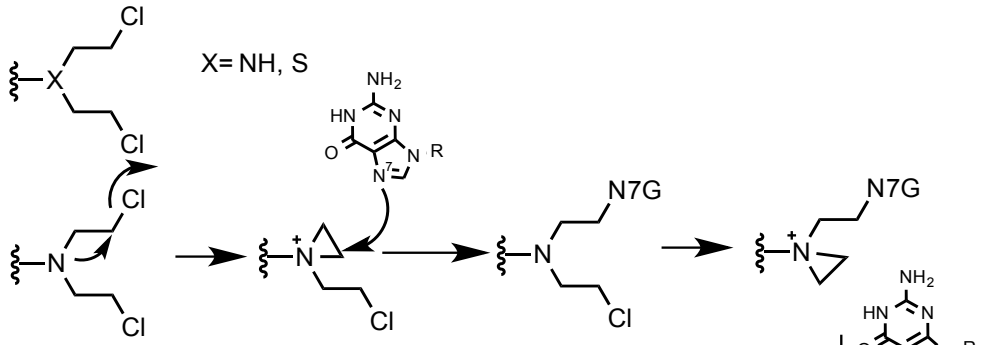
-Hoogsteen is parallel Py/Pu versus antiparallel Py/Pu

Chemical Modification of DNA

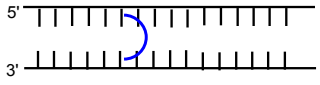


-Chlorambucil - anti-cancer drug
-Alkylates the N7 on G

Mustards



anchimeric effect



-Crosslinks the DNA
-Interstrand crosslinking negatively impacts replication and transcription

G N7 Alkylation leads to tautomerization and mispairing