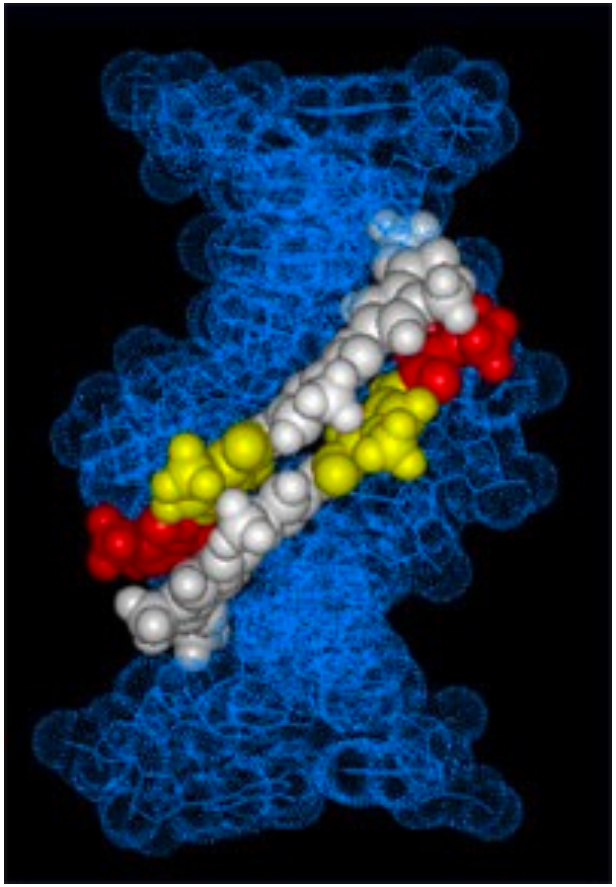


Academic Career of Peter B. Dervan: From Molecular Recognition of DNA to Transcription Regulator

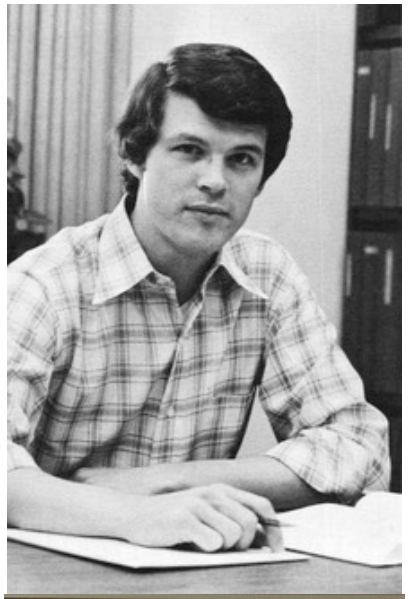
Nick Y. Shin
The Knowles Group
Princeton University

Group Meeting
July 2, 2021

Outline

1. Biosketch of Peter Dervan
2. Early projects on molecular recognition of DNA
3. Affinity Cleaving Method
4. Major groove recognition by triple helix formation
5. Minor groove recognition by Py-Im polyamides
6. Gene regulation and translational research

Peter B. Dervan



Bren Professor of Chemistry
California Institute of Technology

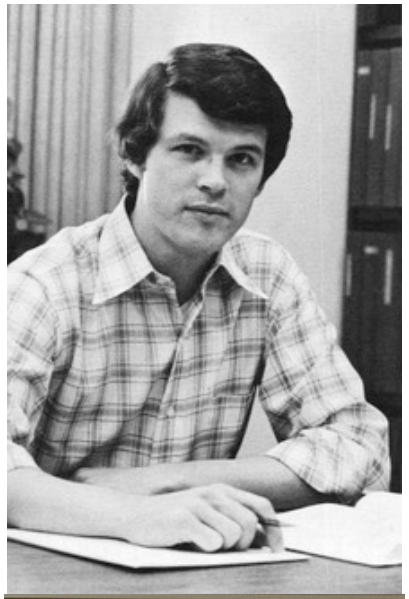
Research Focus

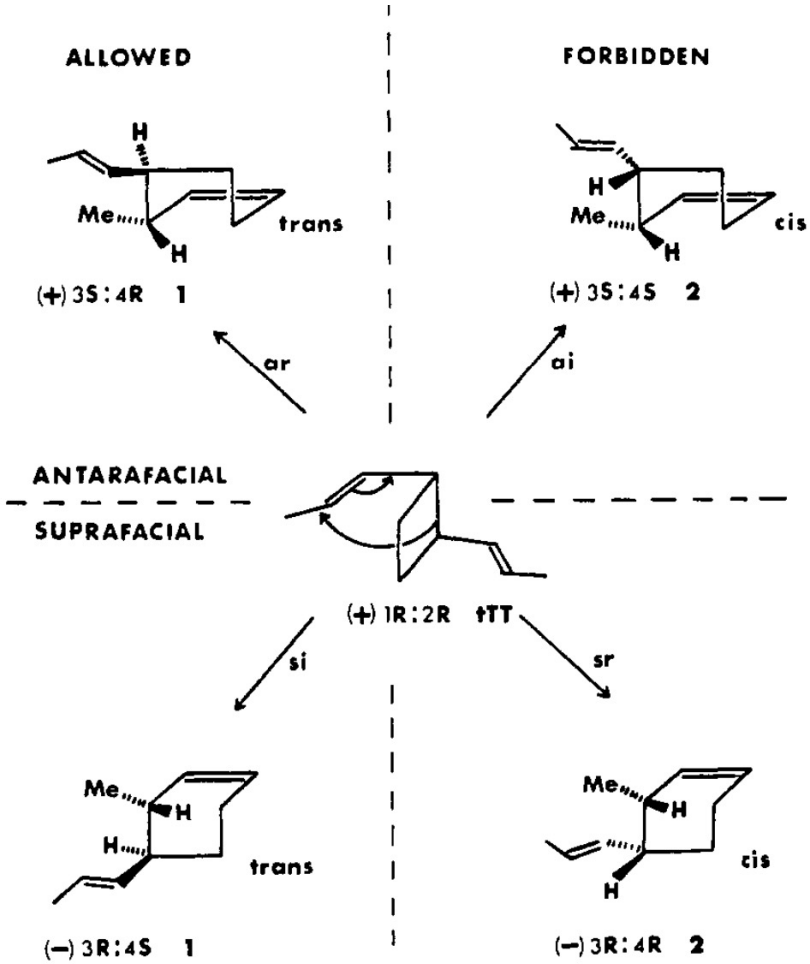
- Sequence-specific recognition of DNA
- Transcription factor antagonist and oncology

Career

- 1945: Born in Boston, MA.
- 1967: B.S. from Boston College
- 1967-1972: Ph.D. at University of Wisconsin and Yale
  Advisor: Jerome A. Berson
  Thesis: The stereochemistry of the thermal rearrangements of trans- and cis-1,2-dialkenylcyclubutanes
- 1973: 6-Month NIH Postdoc with Eugene Van Tamelen at Stanford
- 1973: Assistant Professor at Caltech
- 1979: Associate Professor
- 1982: Professor of Chemistry
- 1988-present: Bren Professor of Chemistry
- 1994-1999: Chair, Division of Chemistry and Chemical Engineering
- 2020: Bren Professor of Chemistry, Emeritus

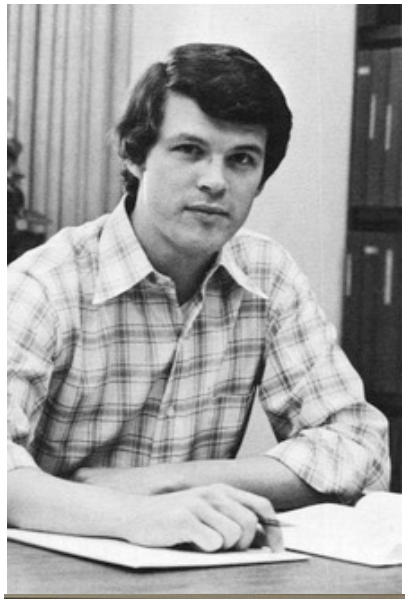
Peter B. Dervan





Berson, J. A.; Dervan, P. B. *J. Am. Chem. Soc.* **1973**, *95* , 269–270.

Peter B. Dervan



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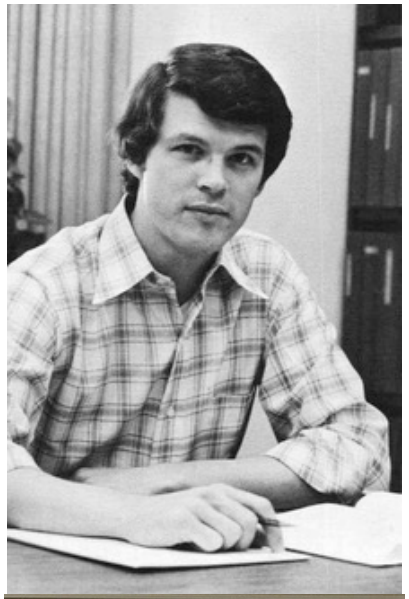


Awards

- Elected member of
  - National Academy of Sciences
  - American Academy of Arts and Sciences
  - National Academy of Medicine
  - National Academy of Inventors
  - French Academy of Sciences
  - American Philosophical Society
  - German National Academy of Sciences Leopoldina
- Arthur C. Cope Award, Linus Pauling Medal, Tetrahedron Prize, Ronald Brelow Award in Biomimetic Chemistry, National Medal of Science, Prelog Medal, Priestley Medal

Foundation

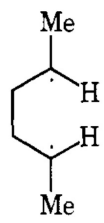
- 1987-2013: Founding member, Scientific Advisory Board, Gilead Sciences
- 1994-96: Chair, Scientific Advisory Council, Abbott Laboratories
- 2015-21: Chair, Scientific Advisory Board, Robert Al. Welch Foundation
- Former member of advisory boards at IGEN, Beckman Coulter, Inc., GeneSoft Pharmaceuticals, Yale University Council, The Scripps Research institute, Science History Institute

Notable Alumni

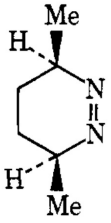
- Peter Schultz (Scripps), Sam Gellman (UW Madison), Alana Schepartz (UC Berkeley), Eric Kool (Stanford), Laura Kiessling (MIT), Eric Carreira (ETH-Züric), Scott Strobel (Yale), and more

Transition to the Interface of Chemistry and Biology

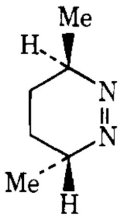
- First two years (1973-75) focused on the mechanistic studies of 1,4-biradicals.



1



2



3

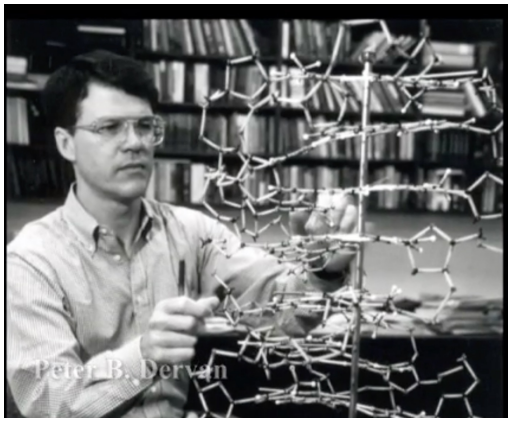
Table I. Percent Yields[a]

Reactant	Conditions	2			
cis-2	*b*	74.7	8.5	16.3	0.5
	c	72.9	9.7	16.3	1.1
trans-3	*b*	80.5	12.7	5.7	1.1
	c	74.4	14.9	8.9	1.8

[a] Percent yield based on total hydrocarbon product. Typical absolute yields of hydrocarbon products from **2** and **3** were 50 and 80% at 306° and 439°, respectively. VPC analysis using 20 ft. × 1/8 in. 10% dibutyl tetrachlorophthalate; flame ionization detector. [b] Chamber pyrolysis (30 s at 306 ± 2°, est pressure >25 mm). [c] Chamber pyrolysis (5 s at 439 ± 2°, est pressure >31 mm).

Dervan, P. B.; Uyehara, T. *J. Am. Chem. Soc.* **1976**, *98*, 1262–1264.

- In 1975, the Dervan lab begins their studies on the chemical biology of DNA.



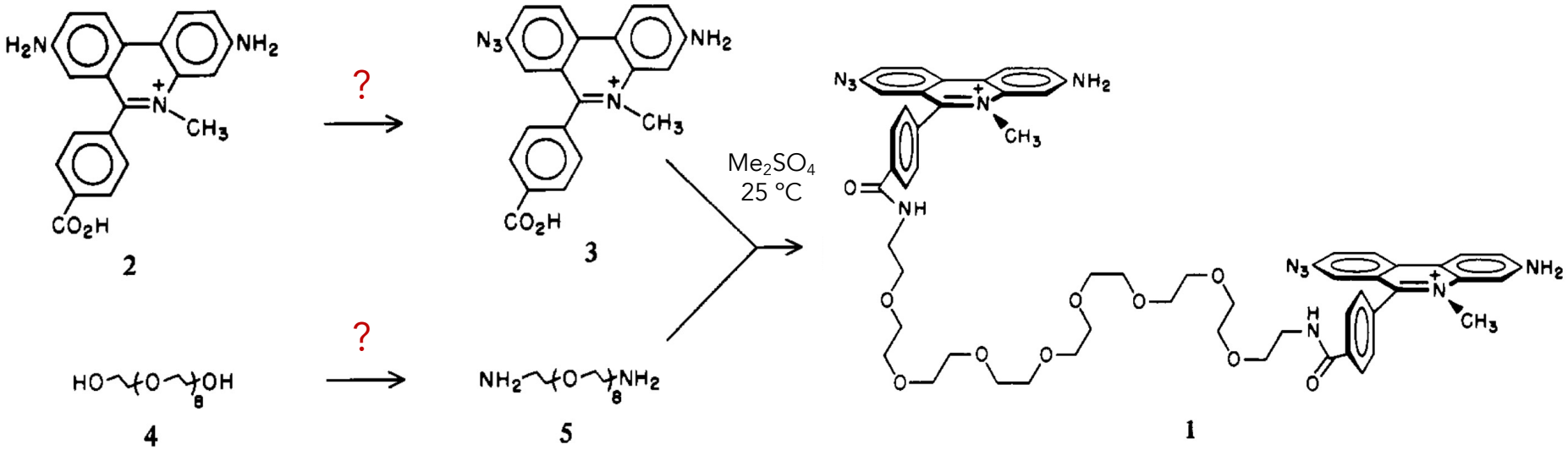
"… much of the breakthrough papers in physical organic had been written in the preceding 20 years (1952–72). With forty years of research in front of me, I needed to move in a new direction! … In 1975 our research group at Caltech decided to focus on **double helical DNA and molecular recognition in water.** This was before DNA sequencing or reliable methods for the synthesis of DNA were available. An x-ray crystal structure of the right handed double helix would be published in 1981."

Dervan, P. B. A Personal Perspective on Chemical Biology: Before the Beginning. *Isr. J. Chem.* **2019**, *59*, 71–83.

Interhelical DNA-DNA Cross-Linking

- Question: What is the 3D structure of condensed DNA? Ball of yarn, coaxial spool or chain-folded?

Design principle: interhelical cross-linkers that are bifunctional, nucleic acid-specific, water soluble, chemically stable, photoactivated, and efficient



Bis(monoazidomethidium)octaoxahexacosanediamine
(**BAMO**)

Mitchell, M. A.; Dervan, P. B. Interhelical *J. Am. Chem. Soc.* **1982**, *104*, 4265–4266.

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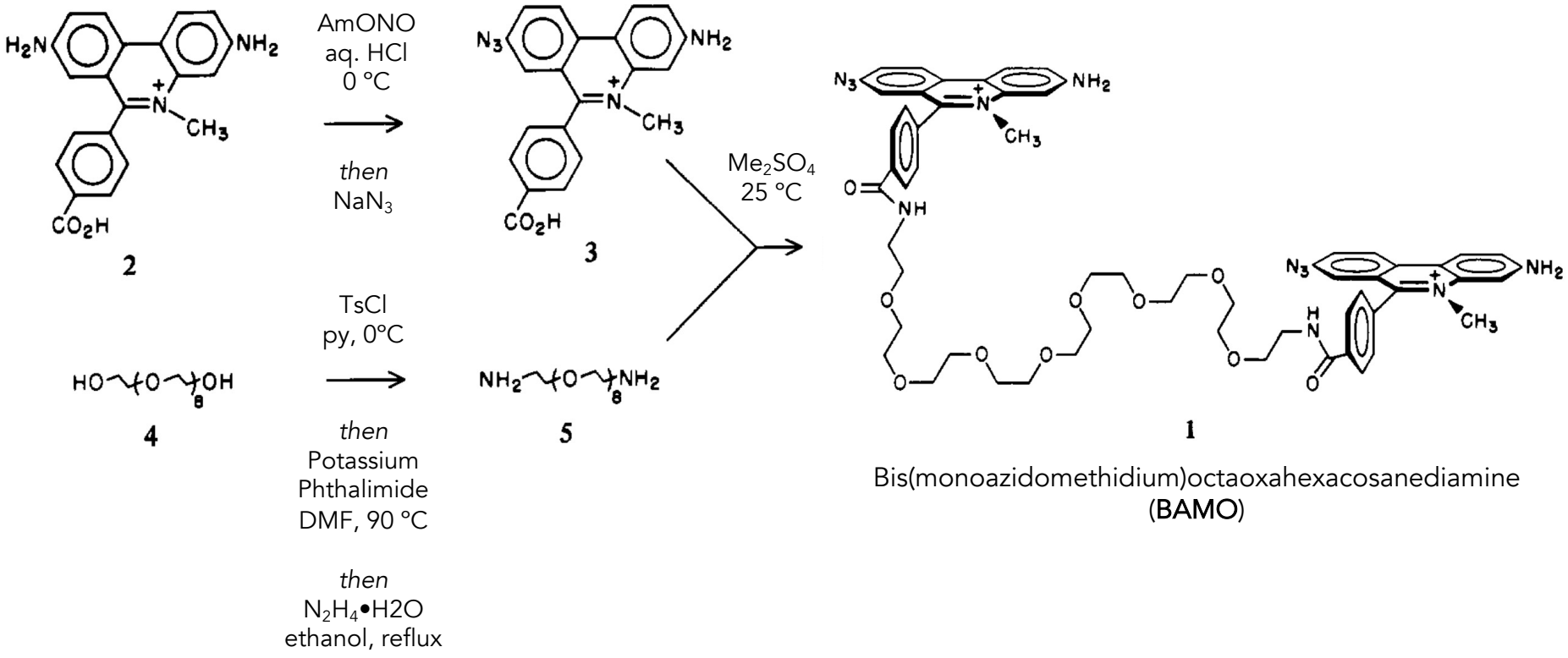

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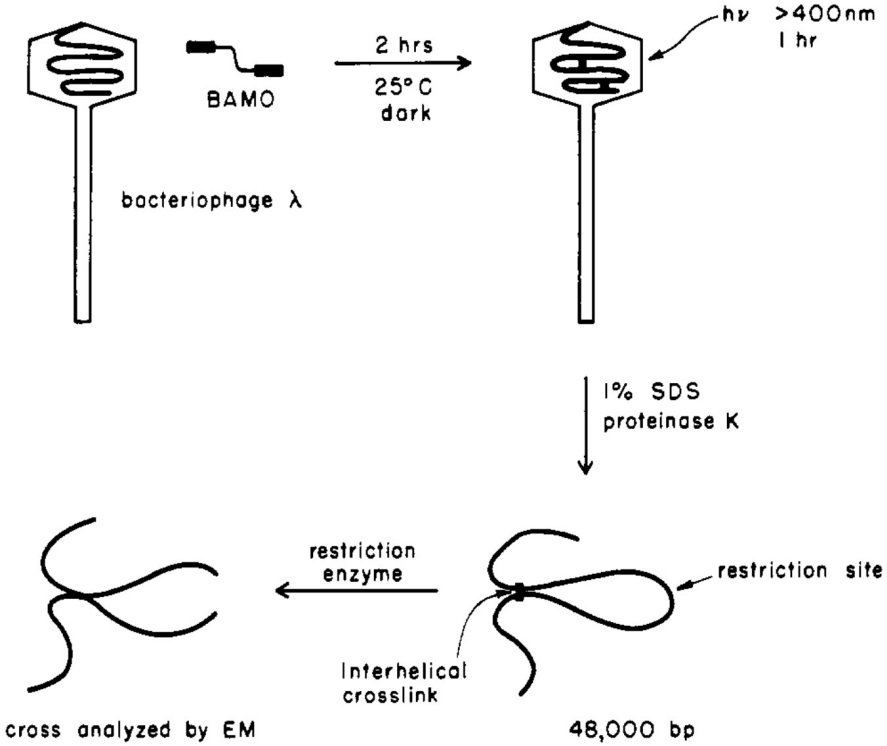
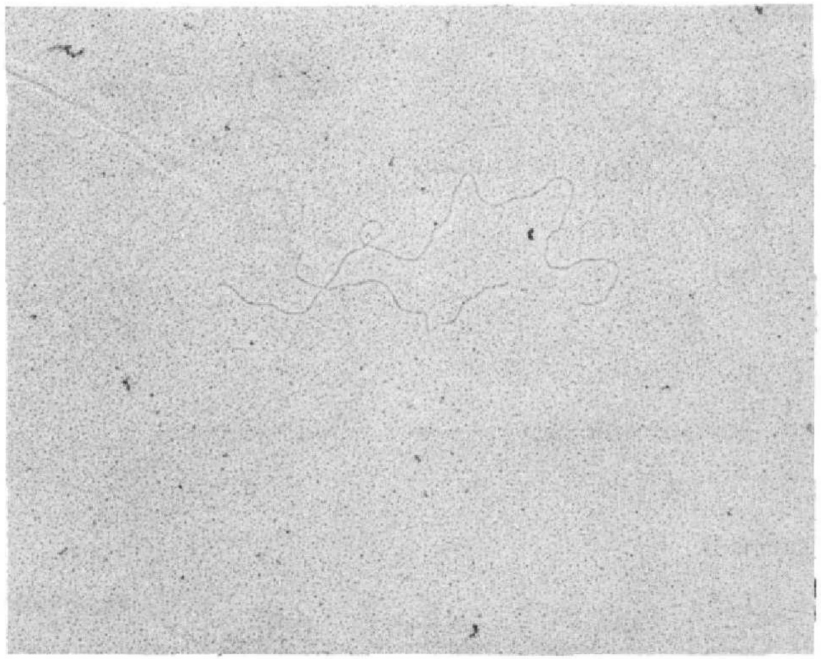




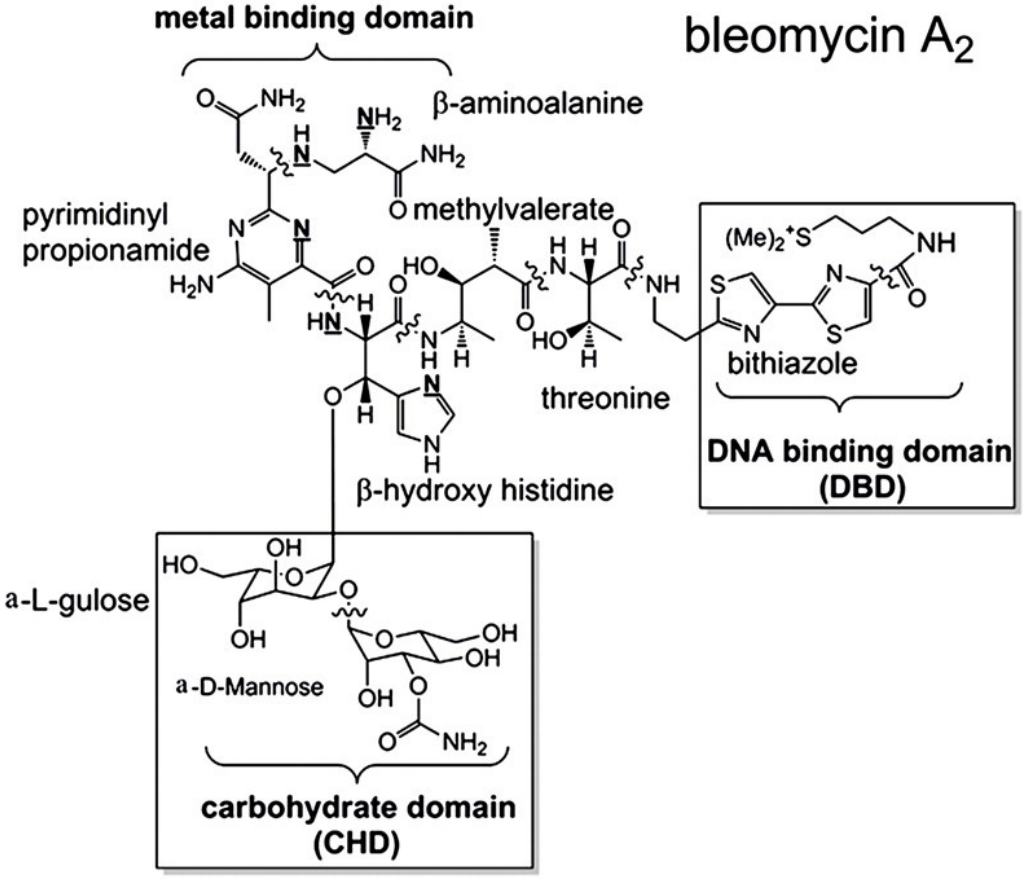
In the absence of cross-linker: 98% linear restriction fragments, 2% crossed structures
BAMO treatment: 73% linear restriction fragments, 17% crossed structures

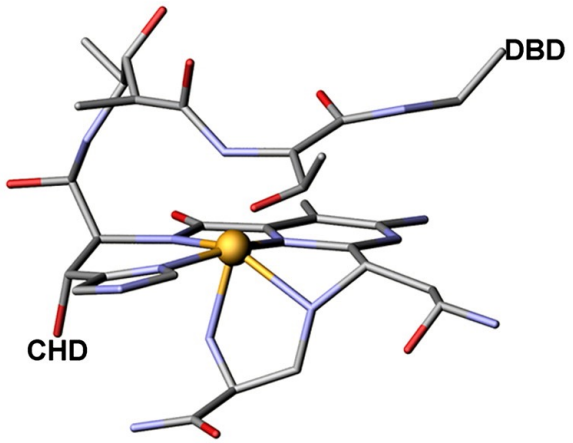
Each cross-link reflects "interhelical nearest neighbors," but at the time DNA sequencing was impossible.

Mitchell, M. A.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 4265–4266.

DNA Recognition and Reaction

- Motivation: Bleomycin





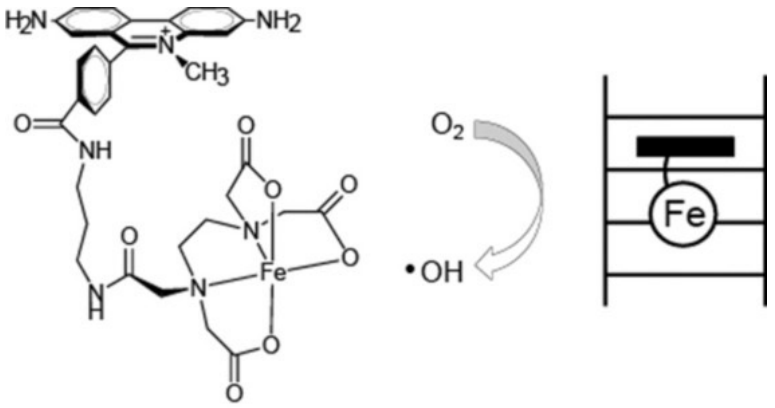
Bleomycin-Fe(II)

- A glycopeptide antibiotic/antitumor
- Binds to and cleaves DNA in the presence of Fe(II) and O_2, presumably through hydroxyl radical.

Solomon, E. I. *et al. Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 22419–22424.
Hertzberg, R. P.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 313–315.

DNA Recognition and Reaction

- Goal: Synthetic mimic of Bleomycin for the recognition and cleavage of DNA



Methidiumpropyl-EDTA
(MPE)

Table I. Cleavage of pBR-322 Plasmid[a]

reagent	concn, M	% form I	% form II	% form III	#Scission/DNA S^b
Fe(II)	10^{-4}	92	8	0	0.08
EDTA-Fe^{II} [c]	10^{-4}	94	6	0	0.06
EDTA-Fe^{II} [c]	5×10^{-4}	38	62	0	0.97
MPE-Fe^{II}	10^{-6}	72	28	0	0.33
MPE-Fe^{II}	5×10^{-6}	40	60	0	0.92
bleomycin-Fe^{II}	10^{-7}	65	29	6	
bleomycin-Fe^{II}	10^{-6}	0	49	51	

Table II. Cleavage of pBR-322 Plasmid in the Presence of DTT[a]

reagent	concn, M	% form I	% form II	% form III	#Scission/DNA S
MPE-Fe^{II}	10^{-8}	82	18	0	0.20
	10^{-7}	43	57	0	0.84
	10^{-6}	0	85	15	9.2
bleomycin-Fe^{II}	10^{-8}	67	29	4	
	10^{-7}	0	79	21	
	10^{-6}	0	54	46	
Fe(II)	10^{-6}	90	10	0	0.11

[a] All reactions contain 1 mM DTT. Reaction conditions and analyses are as in Table I.

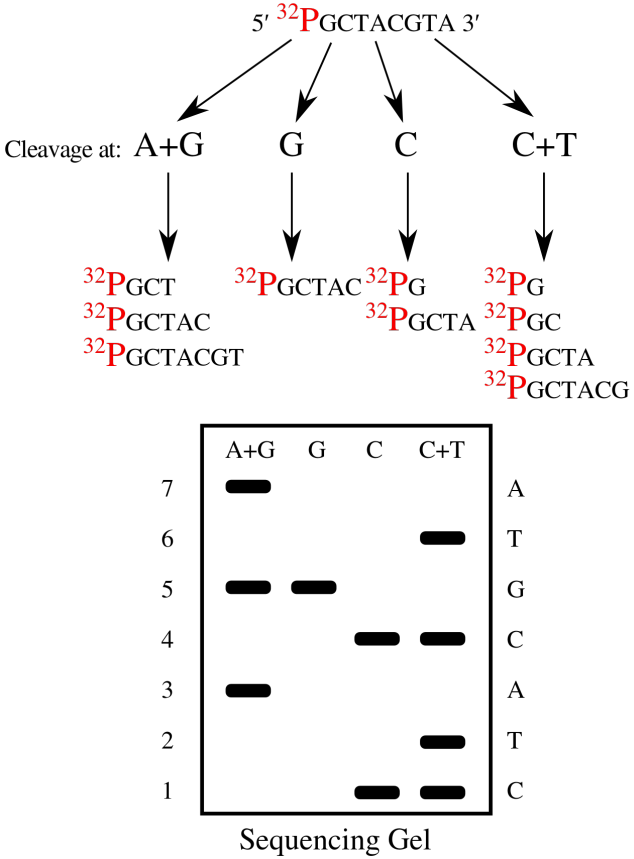
Solomon, E. I. *et al. Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 22419–22424.
Hertzberg, R. P.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 313–315.

DNA Footprinting Method for Small Molecule Binders

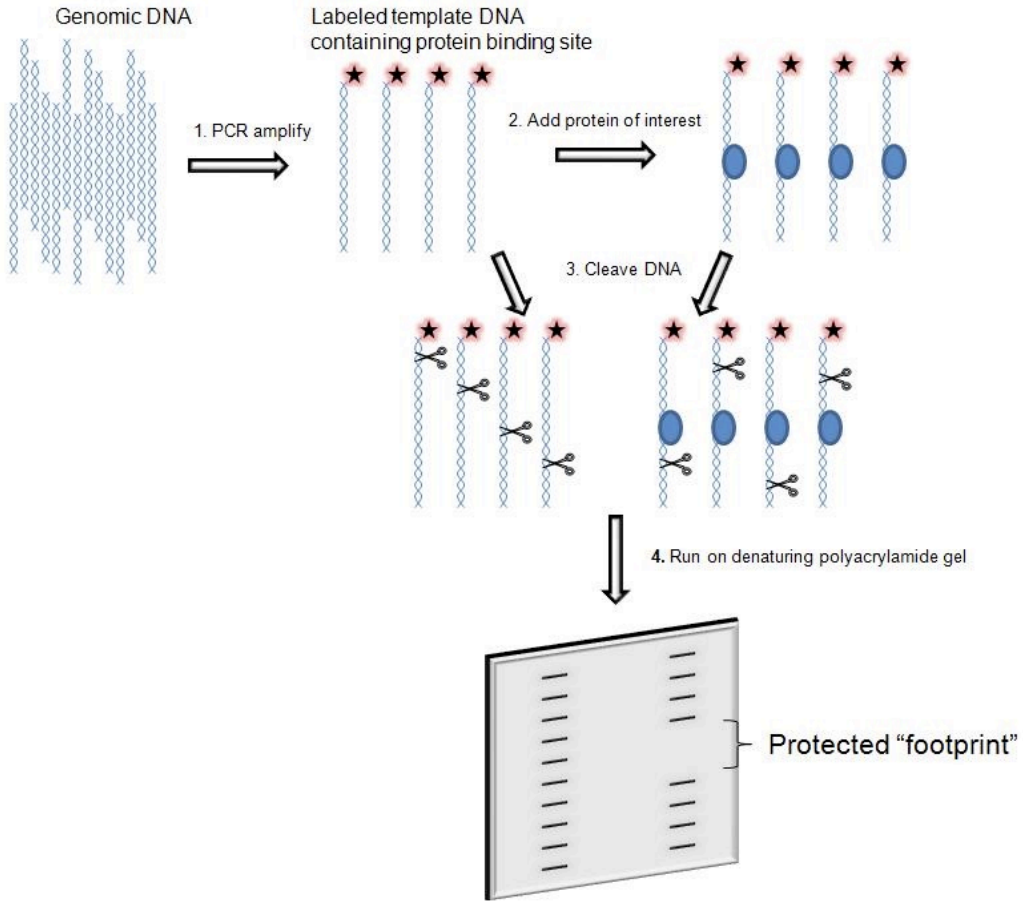
- Previous work from Galas and Schmitz: DNAase footpriting for the detection of protein-DNA binding (1978)

"simple conjoining of the Maxam-Gilbert DNA sequencing method and … DNAase-protected fragment isolation"

- Maxam-Gilbert Sequencing (1976)



- DNAase Footprinting (1978)

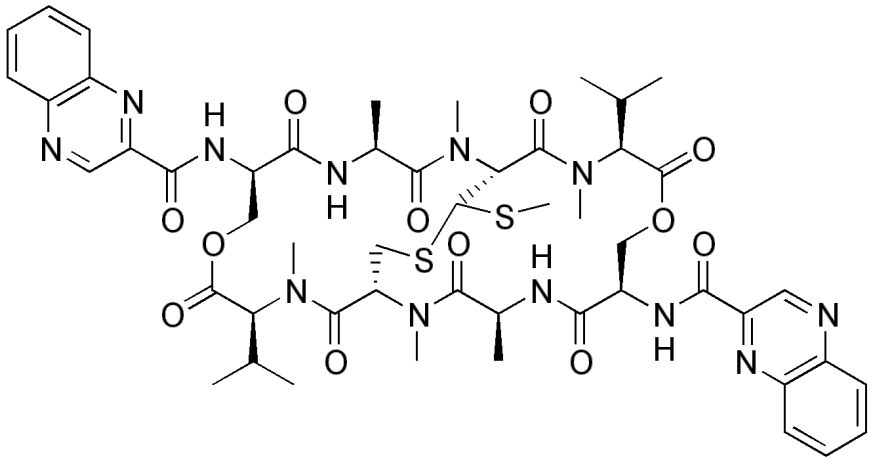


Galas, D. J.; Schmitz, A. *Nucleic Acids Res.* **1978**, *5*, 3157–3170.

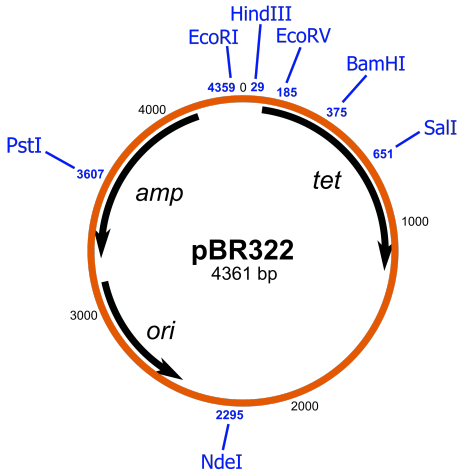
DNA Footprinting Method for Small Molecule Binders

- Goal: High-resolution DNA Footprinting method for small molecule DNA binders

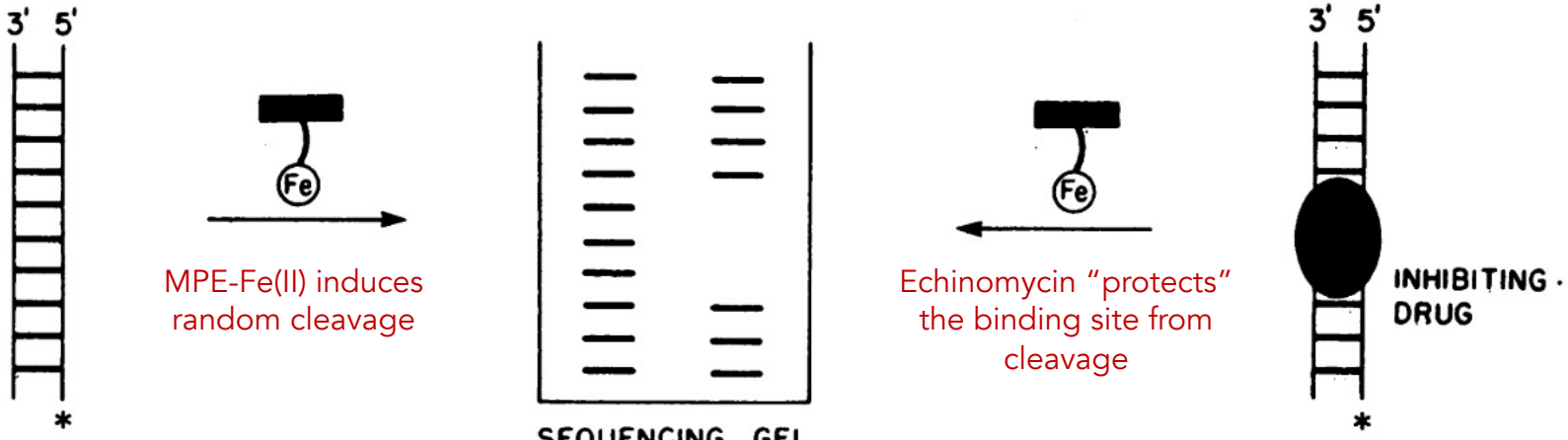
Target: Binding sequence of Echinomycin (Antibiotic)



Model System: Restriction fragments of pBR-322 Vector

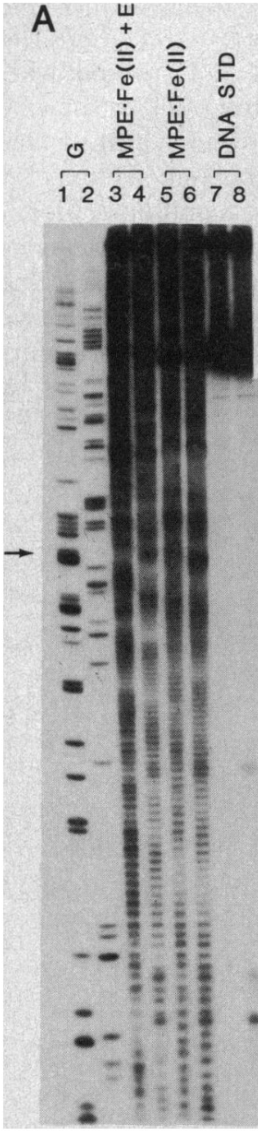


Method Design:

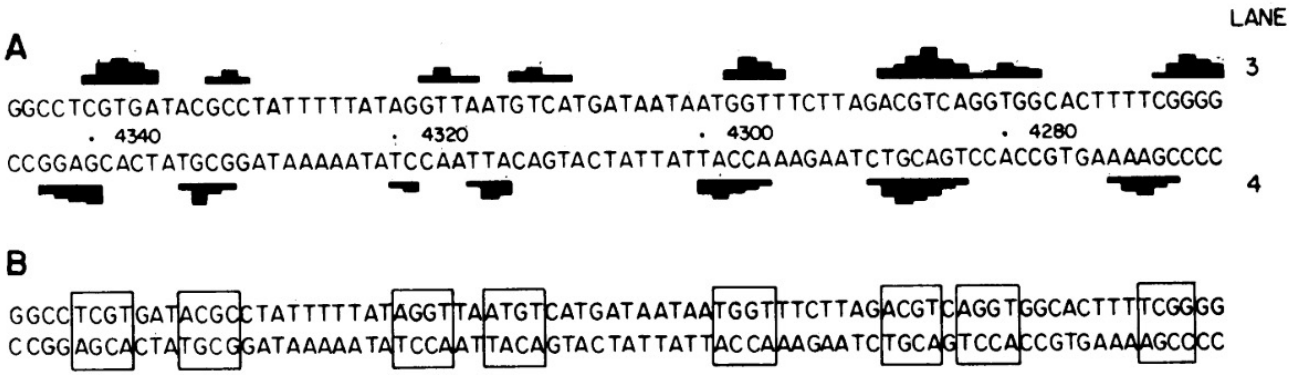


Van Dyke, M. M.; Dervan, P. B. *Science*. **1984**, *225*, 1122–1127.

DNA Footprinting Method for Small Molecule Binders



Autoradiogram of ^{32}P end-labeled DNA resriction fragments



MPE-Fe(II) footprints of echinomycin determined by densitometry

Fragment	Site (5′–3′)	Location	Binding
517	TCGT	4343–4340	s
517	ACGC	4336–4333	w
517	AGGT	5322–4319	w
517	ATGT	4317–4314	w
517	TGGT	4301–4298	m
517	ACGT	4290–4287	s
517	AGGT	4285–4282	w
517	TCGG	4273–4270	m
167	TCGA	24–27	s
167	GCGG	38–42	s
167	CAGT	53–56	w
167	ACGC	67–70	w
167	CCGT	79–82	w
280	CCGG	410–413	w
280	TCGG	469–472	m

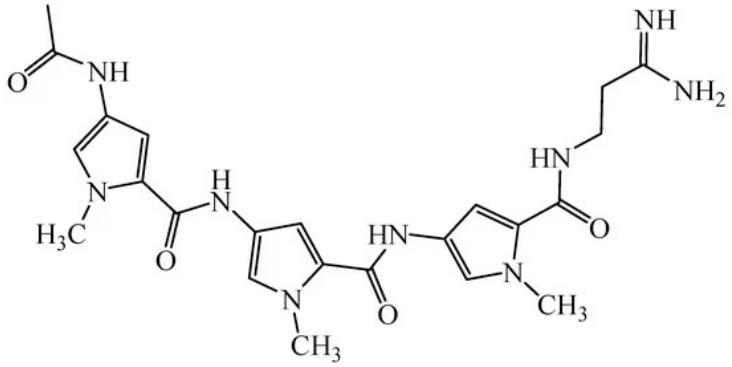
Four-base-pair binding sites of echinomycin

Van Dyke, M. M.; Dervan, P. B. *Science*. **1984**, *225*, 1122–1127.

Sequence-Specific Affinity Cleaving

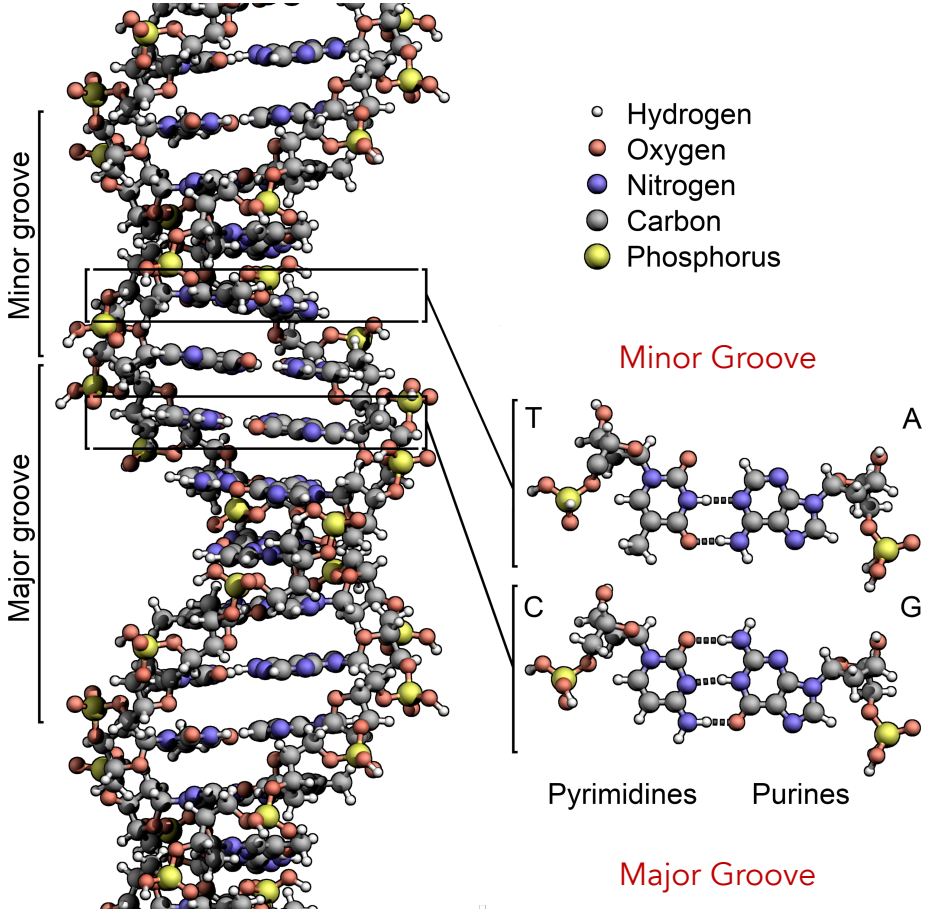
Next Step: Sequence-specific artificial restriction endonuclease

- Motivation: Distamycin (Antibiotic)



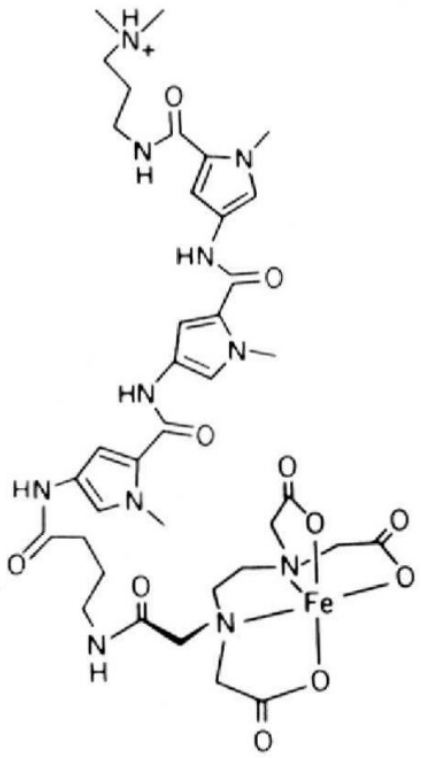
- Binds to minor groove of DNA
- Preference for A/T rich biopolymers

- Primer: Groovy DNA



Schultz, P. G.; Taylor, J. S.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 6861–6863.
Taylor, J. S.; Schultz, P. G.; Dervan, P. B. *Tetrahedron* **1984**, *40*, 457–465.

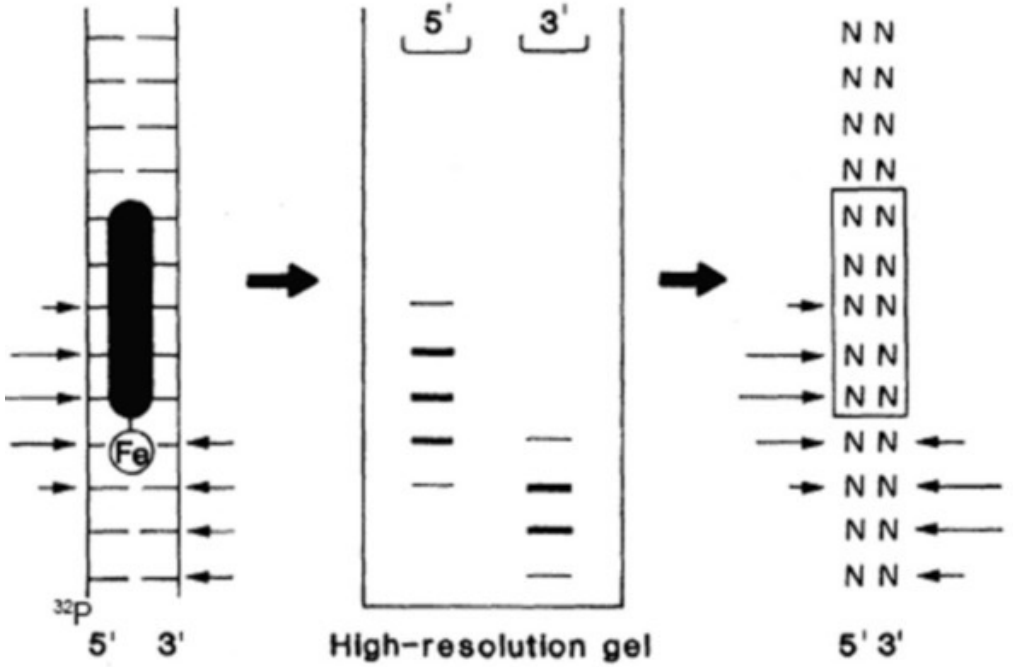
Sequence-Specific Affinity Cleaving



Distamycin-EDTA
(DE)

DNA Cleavage with
^{32}P-end labeled
pBR-322 fragments

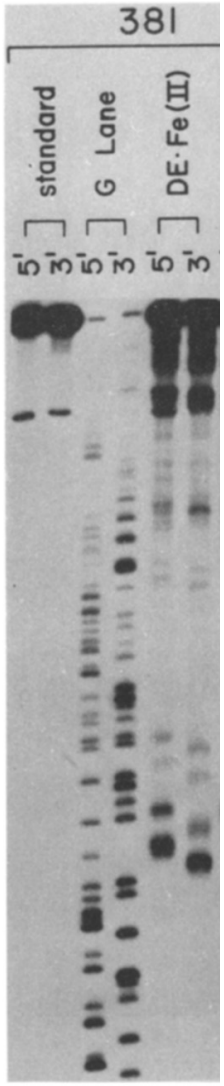
DE-Fe(II)
DTT
O_2



A single experiment can provide the binding site sequences and the groove location.

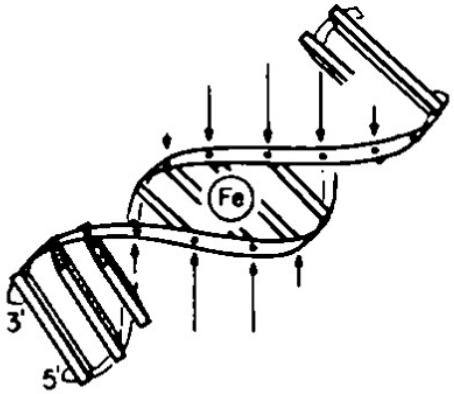
Schultz, P. G.; Taylor, J. S.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 6861–6863.
Taylor, J. S.; Schultz, P. G.; Dervan, P. B. *Tetrahedron* **1984**, *40*, 457–465.

Sequence-Specific Affinity Cleaving

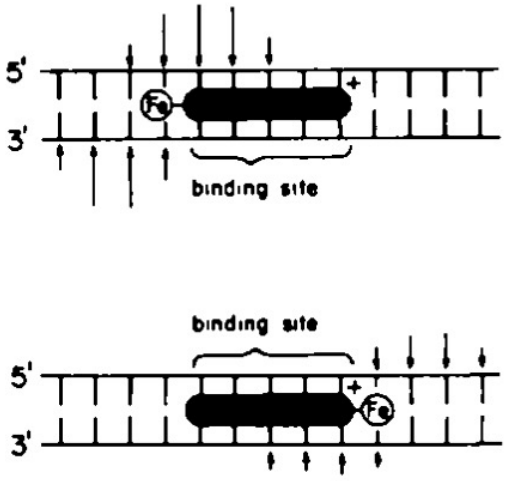


Autoradiogram of Maxam-gilbert sequencing gels

Histogram of the DNA cleavage patterns



Cleavage patterns asymmetric to 3′ side reveal its binding to minor groove

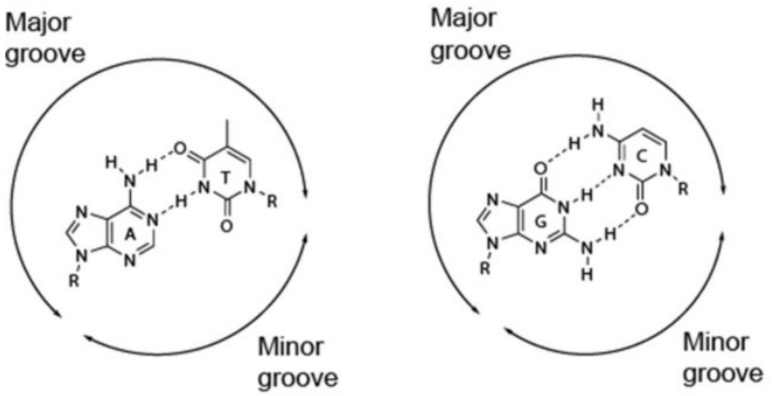


Major target sequence: 5′-AAATT-3′

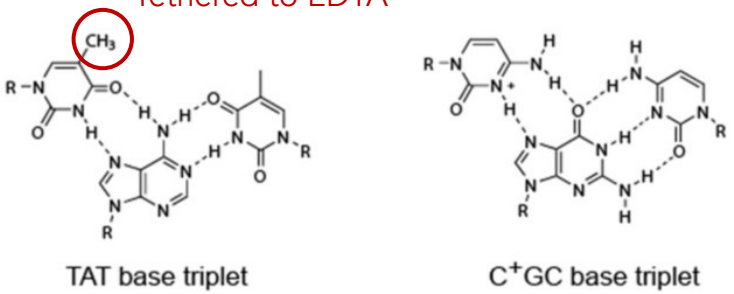
Schultz, P. G.; Taylor, J. S.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 6861–6863.
Taylor, J. S.; Schultz, P. G.; Dervan, P. B. *Tetrahedron* **1984**, *40*, 457–465.

Sequence-Specific Cleavage by Triple Helix Formation

Watson-Crick Base Pairs



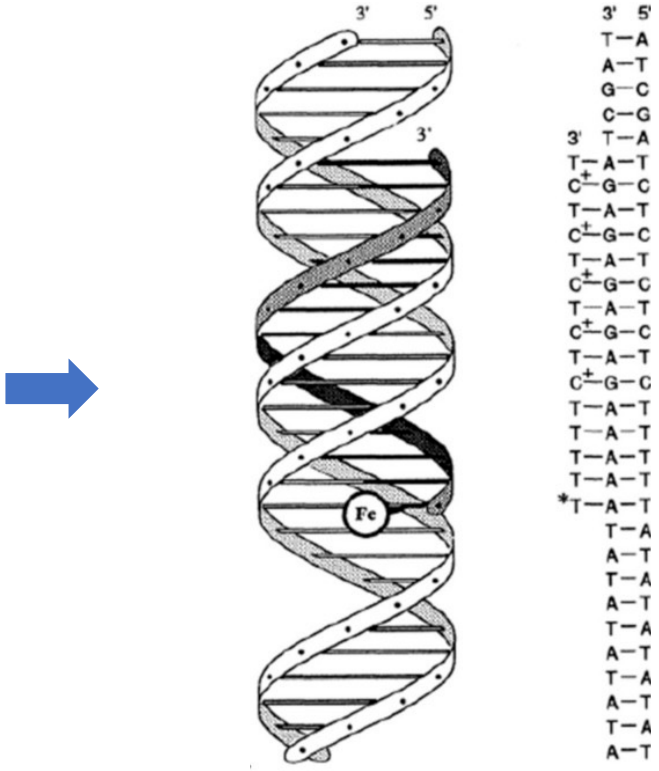
Tethered to EDTA



TAT base triplet

C^+GC base triplet

Hoogsteen Base Pairs



DNA-EDTA-Fe(II)
Sequence-specific cleavage by triplex formation in the major groove?

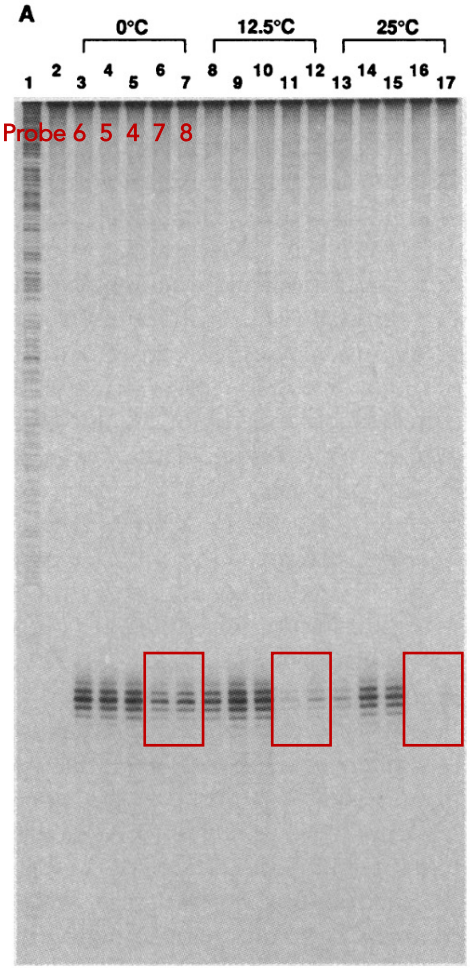
Moser, H. E.; Dervan, P. B. *Science* **1987**, *238*, 645–650.

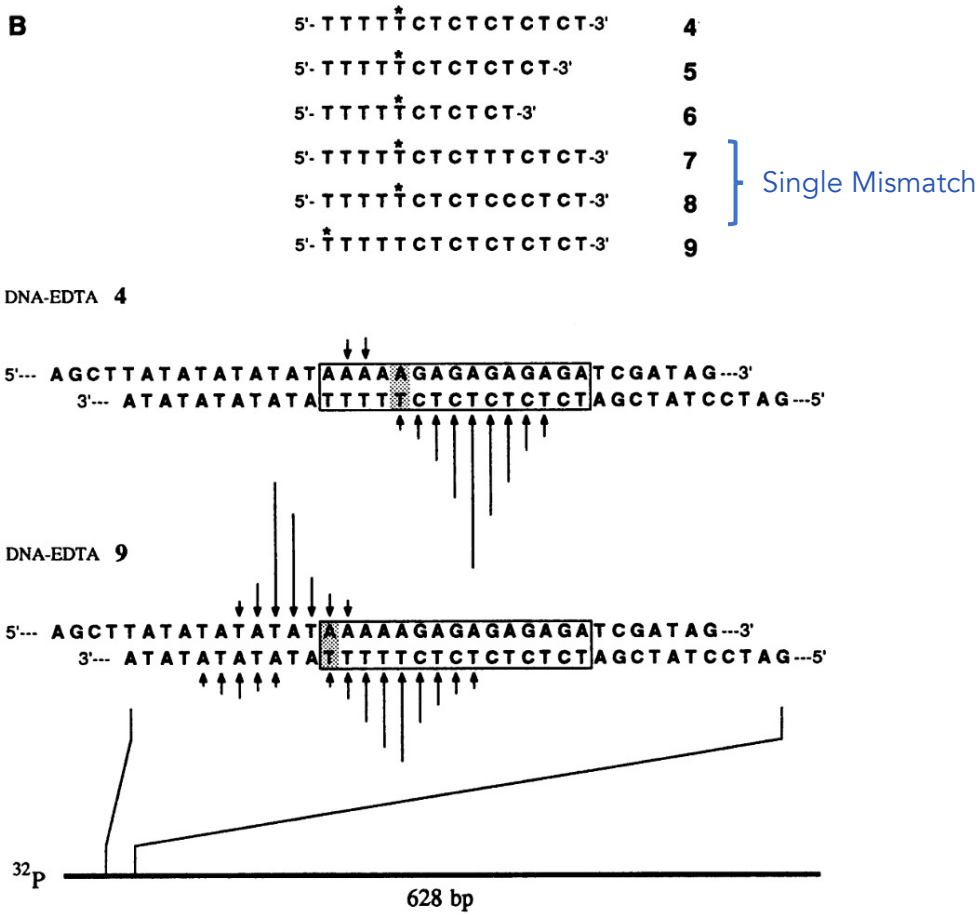
Sequence-Specific Cleavage by Triple Helix Formation

Autoradiogram of Maxam-gilbert sequencing gel.

628 bp fragment

(Lane 1) G+A
(Lane 2) (-)



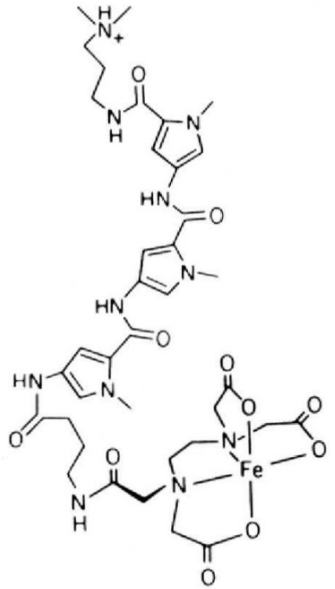


- Sequence-specific (single target site, mismatch not tolerated)
- Cleavage pattern asymmetric to 5′ – Major groove binding
- Later expanded to yeast and human chromosome *in vitro*
- Limitation: Poor cellular uptake, conditional requirements for triplex formation

Moser, H. E.; Dervan, P. B. *Science* **1987**, *238*, 645–650.

Minor Groove Recognition and Pairing Rules

- Pyrrole-based polyamides evolved over the course of 20 years (1982-2002)
- In-house methods of footprinting and affinity cleaving allowed for screening new heterocyclic amino acids.
- **Goal**: Side-by-side antiparallel polyamide pairs to distinguish the four Watson-Crick base pairs!

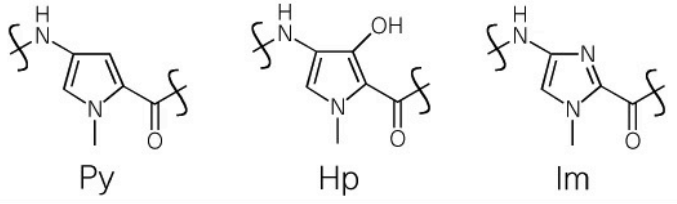


Humble (?) Beginning (1982):
Distamycin-EDTA-Fe(II)

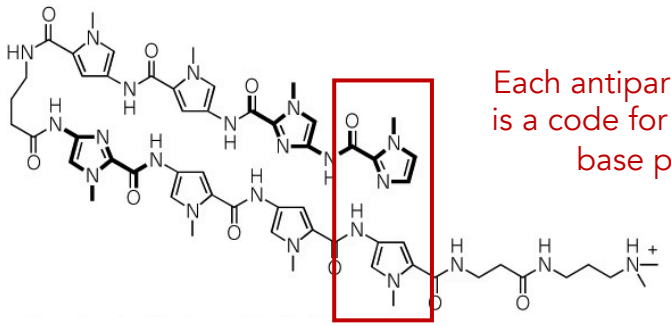
16 years of optimization

- New heterocycles
  - Footprinting
- Affinity analysis (K_d)
  - XRD
  - …

1. Three monomer amino acids:



2. Hairpin motif for antiparallel binding



Each antiparallel pair is a code for one WC base pair

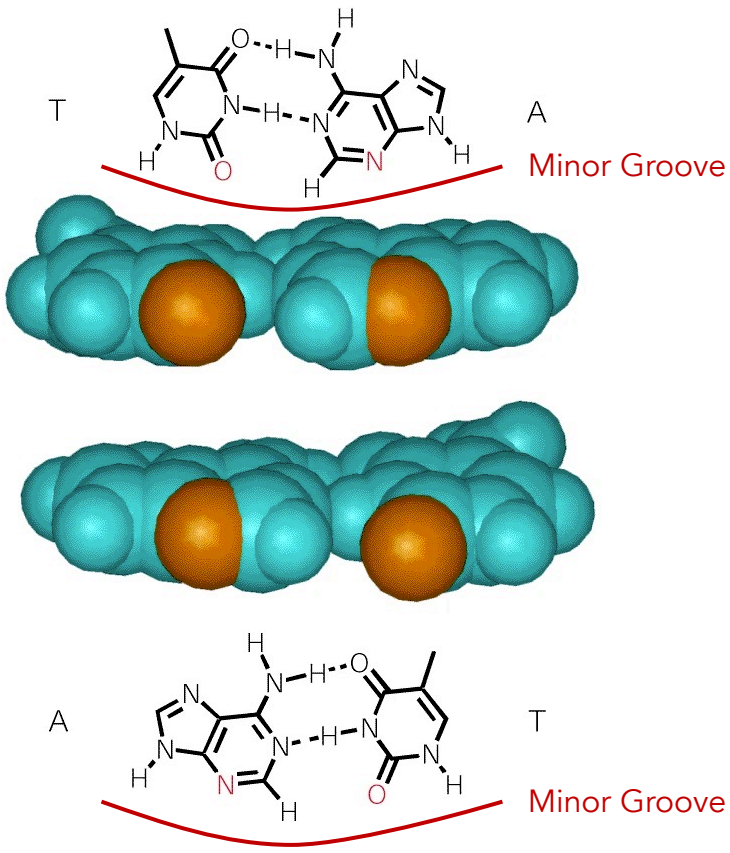
3. One-to-one paring code for all Watson-Crick base pairs

Pair	G·C	C·G	T·A	A·T
Im/Py	+	−	−	−
Py/Im	−	+	−	−
Hp/Py	−	−	+	−
Py/Hp	−	−	−	+

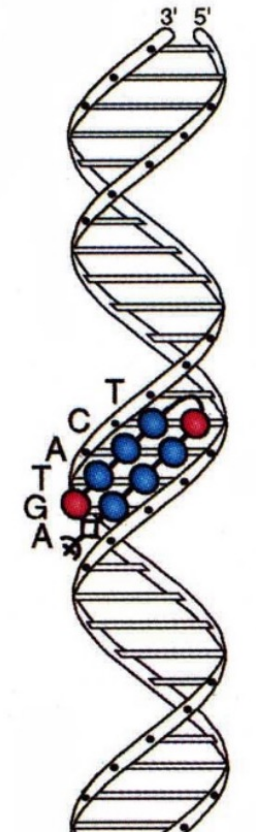
White, S.; Szewczyk, J. W.; Turner, J. M.; Baird, E. E.; Dervan, P. B. *Nature* **1998**, *391*, 468–471.

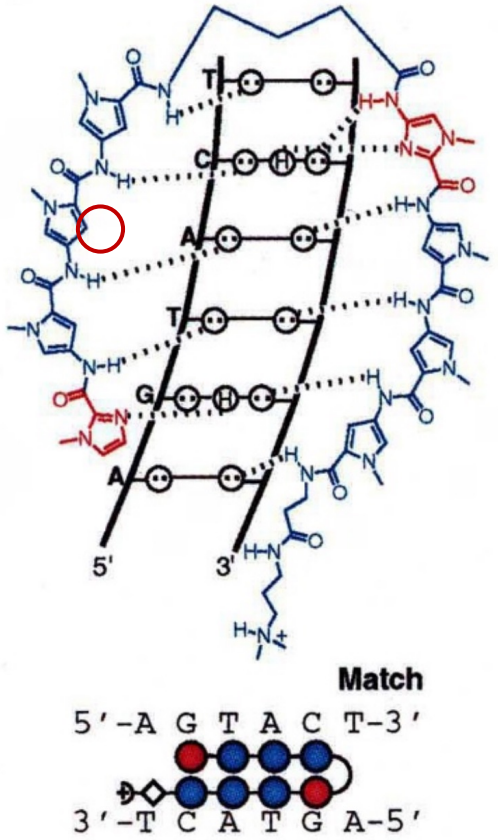
Recognition of A•T and T•A Base Pairs

- Distinguishing the minor grooves of A•T vs. T•A is challenging



- Previous binding model:

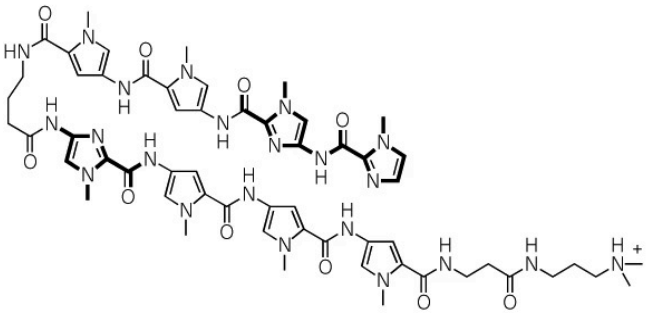




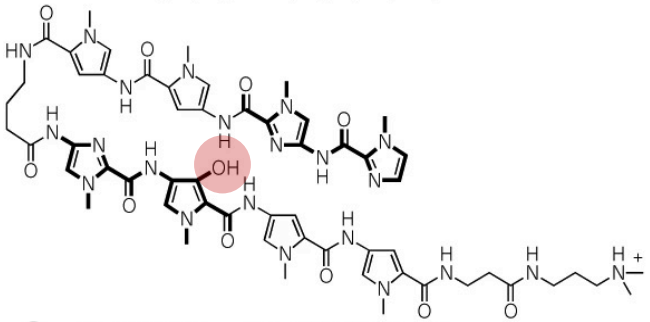
White, S.; Szewczyk, J. W.; Turner, J. M.; Baird, E. E.; Dervan, P. B. *Nature* **1998**, *391*, 468–471.
Kielkopf, C. L.; White, S.; Szewczyk, J. W.; Turner, J. M.; Baird, E. E.; Dervan, P. B.; Rees, D. C. *Science* **1998**, *282*, 111–115.

Recognition of A•T and T•A Base Pairs

• Installation of 3-hydroxypyrrole for distinguishing A•T from T•A



1 ImImPyPy-γ-ImPyPyPy- β-Dp

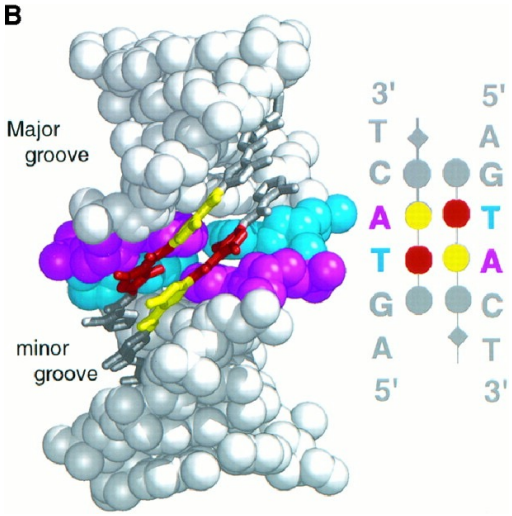


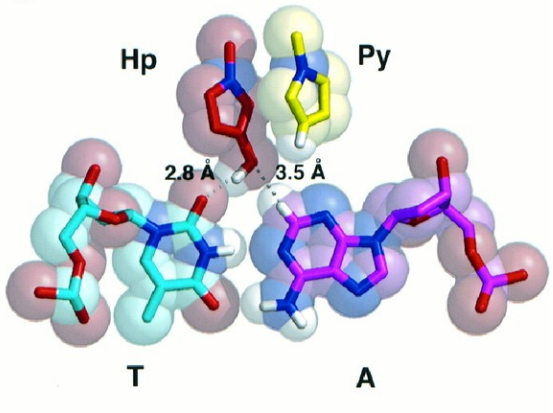
2 ImImPyPy-γ-ImHpPyPy- β-Dp

• Hp/Py pair can distinguish A•T from T•A by 77-fold

	K_D (nM)		
Polyamide*	5′-TGGTCA-3′	5′-TGGACA-3′	K_{rel}†
1 Py/Py	0.077	0.15	2.0
2 Py/Hp	15	0.83	0.06
3 Hp/Py	0.48	37	77

• Crystal structure shows minor groove binding with key hydrogen bonding interactions



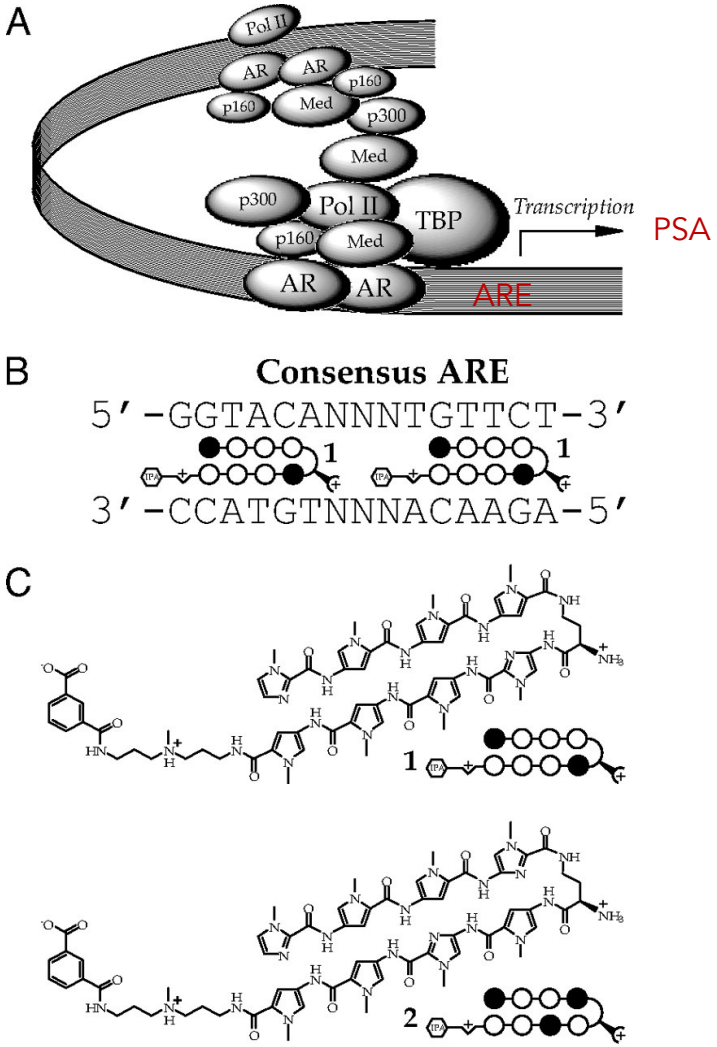


* Every protein DNA factor binds to the major groove.

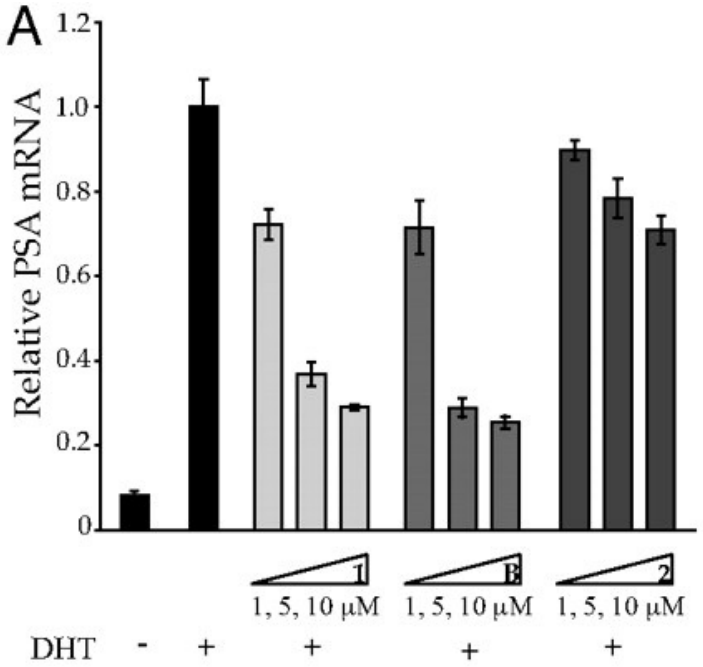
White, S.; Szewczyk, J. W.; Turner, J. M.; Baird, E. E.; Dervan, P. B. *Nature* **1998**, *391*, 468–471.
Kielkopf, C. L.; White, S.; Szewczyk, J. W.; Turner, J. M.; Baird, E. E.; Dervan, P. B.; Rees, D. C. *Science* **1998**, *282*, 111–115.

Gene Regulation through Androgen Receptor Antagonist

• Androgen receptor (AR) is a transcription factor that binds to androgen response element (ARE) to induce prostate-specific antigen (PSA) – **important in prostate cancer.**



• Polyamide antagonists bind to ARE and downregulate the transcription level of PSA.



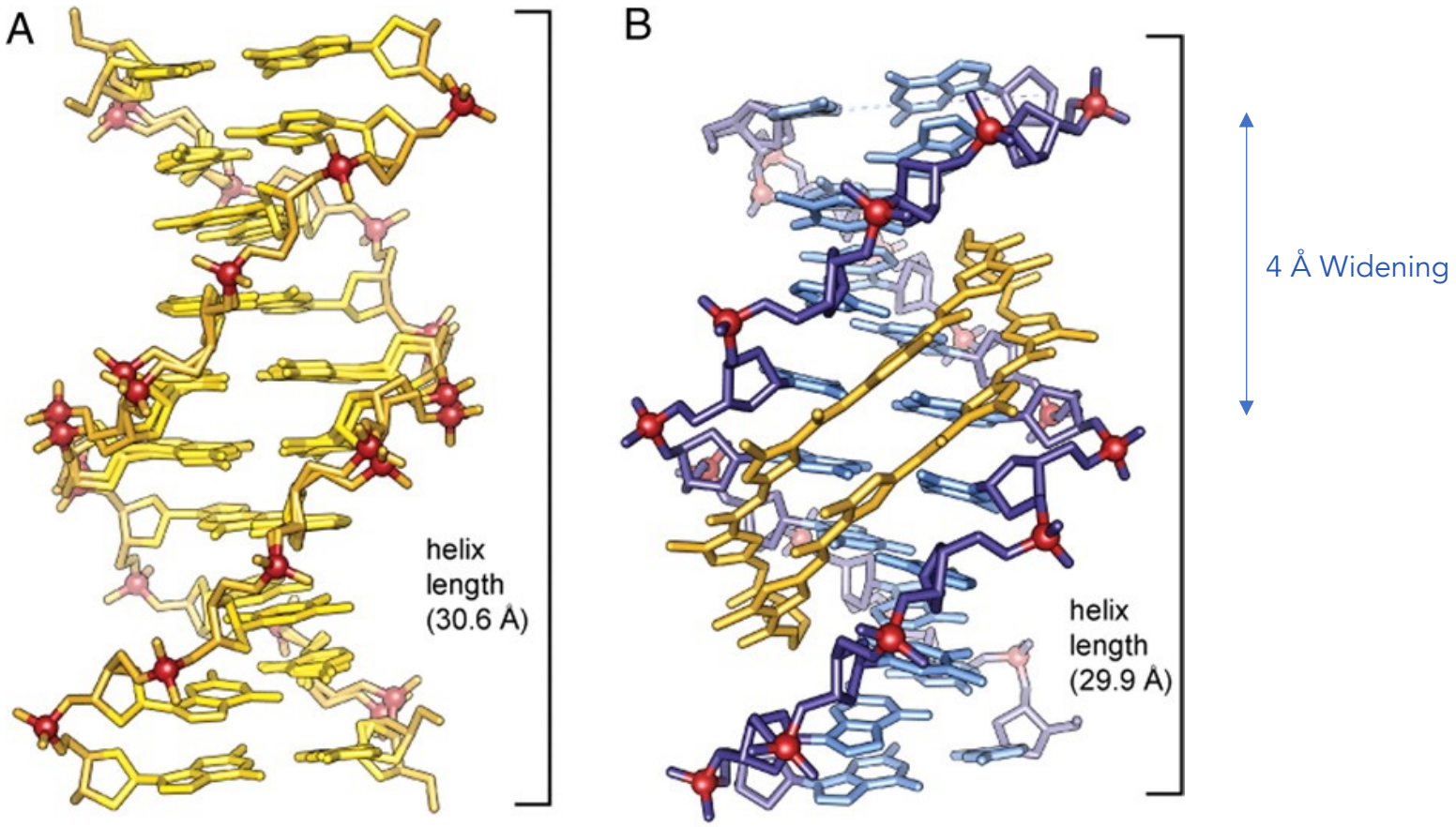
Dihydrotestosterone
(Necessary inducer for AR)

Bicalutamide
(Synthetic antiadrogen)

Nickols, N. G.; Dervan, P. B. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 10418–10423.

Allosteric Modulation of DNA by Polyamide Binders

Wait…
Transcription factors are major groove binders.
How would polyamide minor groove binders downregulate them?

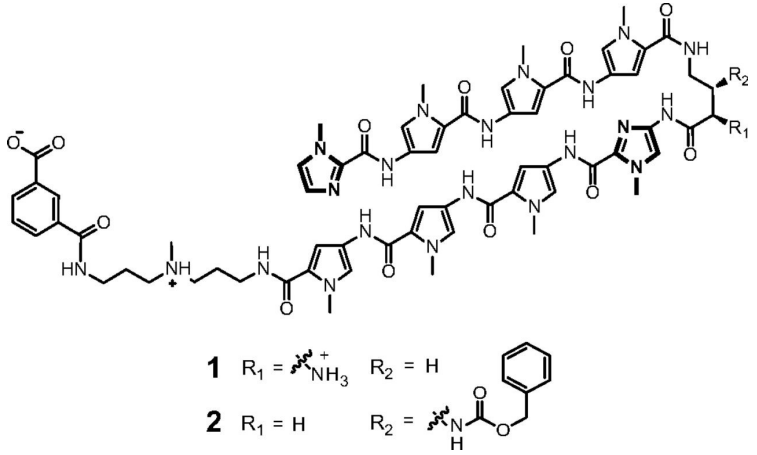


Allosteric mechanism for competitive inhibition of DNA-protein interaction

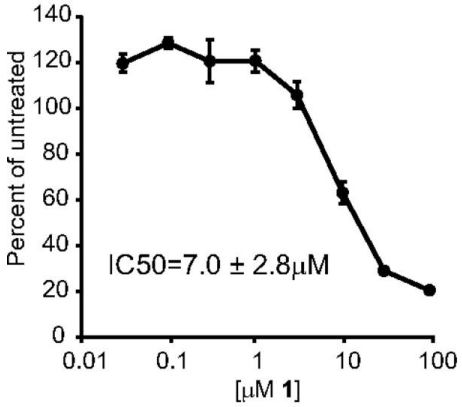
Chenoweth, D. M.; Dervan, P. B. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 13175–13179.

Antitumor Activity of Polyamides

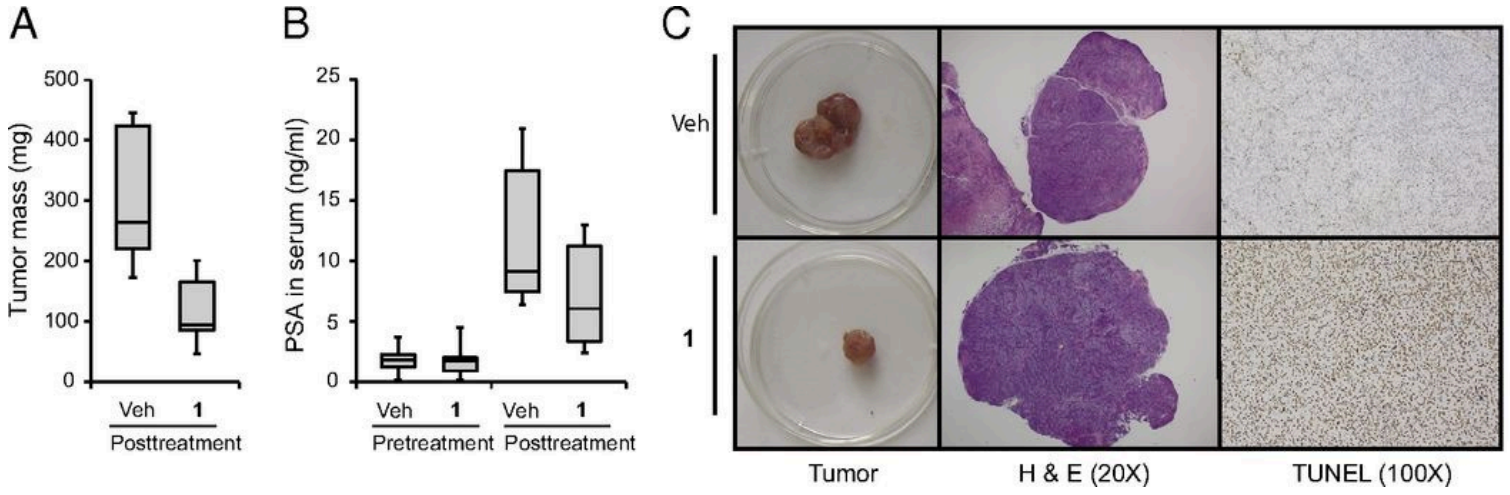
- Designed a polyamide antagonist for RNA polymerase II.



- Cytotoxicity in LNCaP cells after 72 h (Prostate cancer cell line)

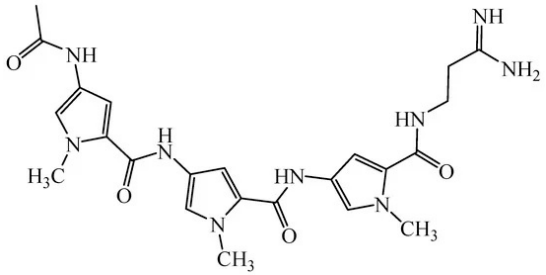


- Antitumor activity in prostate cancer xenografts. (LNCaP cells)

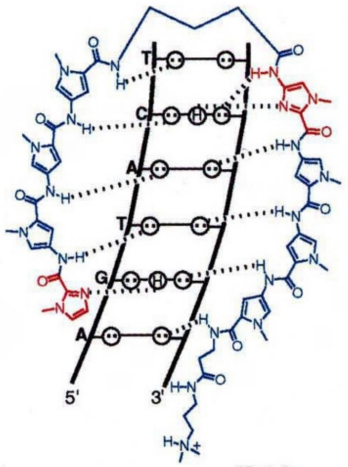


Yang, F.; Nickols, N. G.; Li, B. C.; Marinov, G. K.; Said, J. W.; Dervan, P. B. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 1863–1868.

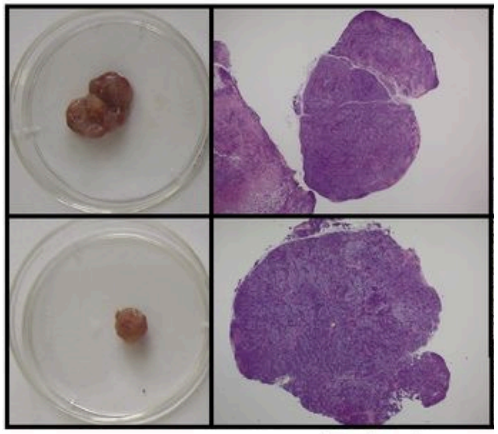
Summary



Natural Product



Non-Covalent Interaction



Oncology

"I am grateful for the contributions by the 192 graduate students and postdoctoral fellows who passed through my laboratory at Caltech 1973–2018. It was difficult for them to have an advisor who was always the learner and never the expert in the room as we migrated from biophysical chemistry to cell biology to xenograft mouse experiments. There was risk and many projects failed but I believe the experience fortified my former coworkers for success later in their careers."

Dervan, P. B. A Personal Perspective on Chemical Biology: Before the Beginning. *Isr. J. Chem.* **2019**, *59*, 71–83.