The Wonders of Biomolecular Recognition: What’s in it for Chemists?
Complementary Pairing Holds the Two Strands of DNA Together
The Double-Helical Structure of DNA

“Complementary” strands held together by noncovalent interactions
Hydrogen Bonding Stabilizes the Double-Helical Structure of DNA

Guanine

Adenine

Cytosine

Thymine

Guanine-Cytosine

Adenine-Thymine
Lack of complementary strand allows RNA to fold into variety of 3-D structures

DNA versus RNA

DNA (R = H)
RNA (R = OH)

A A
A G
T - A
A - T
G - C
C - G

GCGATAAAGATCG

Primary Structure

Secondary Structure

Pseudoknot

Tertiary Structure
Amplification of Genetic Information During Gene Expression

(1): Transcription: Many messenger RNA copies from one DNA gene
(2): Translation: Many protein copies from one messenger RNA

- Traditional medicinal chemistry targets protein
- More potent drugs target RNA or DNA instead of protein
- RNA easier to target sequence specifically
Peptide Nucleic Acid (PNA): A Structural Mimic of DNA

Synthesized by early organisms millions of years ago

Synthesized by Danish chemists in 1991

- Same number of single bonds in backbone (6) and between base/backbone (3)
- Lack of negative charges on PNA eliminates Coulombic repulsion: stronger binding
- “Hybridization” with complementary DNA/RNA strand follows Watson-Crick rules
**Antisense Applications of PNA**

- **PNA binds to complementary RNA with high affinity**

- **If PNA-RNA hybrid blocks ribosome binding or translation, protein synthesis can be inhibited.**

- **Target drug-resistant bacteria: new antibiotics**
Antisense PNAs Targeted Against E. coli

- PNAs targeted against *lacZ* (β-galactosidase) and *bla* (β-lactamase) genes.

- In vitro experiments: gene-encoding plasmids, cell extracts with RNA polymerase, ribosomes

- Assess antisense effect by monitoring enzyme activity

- Enzyme activities significantly reduced by antisense PNA, but not control PNA.

- mRNA levels unaffected by antisense PNA.

- Transcription is not inhibited, but protein synthesis is.

### lacZ target

5' GAAAGACGUAGACCAU

CTTGTGATACCTGG (N)

*lacZ* mRNA

(anti-*lacZ* PNA #1284)

### bla target

5' GGAAGAGUUGAAGUUC

TCTCATACTCATAAG (N)

*bla* mRNA

(anti-*bla* PNA #1438)

CCTCTCATACTCAT (N)

(anti-*bla* PNA #1439)

### no target

AGGTGTCAGCGAAGC (N)

(control PNA #1176)
Bactericidal Properties of Antisense PNAs

- Bacteria with β-lactamase gene are resistant to ampicillin.
- Anti-β-lactamase PNA blocks translation of mRNA.
- Ampicillin-resistant bacteria killed by ampicillin (triangles).

Antisense PNAs cause ampicillin-resistant E. coli to look like ampicillin-sensitive bacteria
Curing a Bacterial Infection Using an Antisense PNA

- HeLa cells: cancer cell line
- Infected with *E. coli* or *E. coli* with plasmid for mutant *Acp* gene. Infection causes cells to shrink, die.
- Anti-acp PNA-peptide added at different concentrations.

- No effect of PNA on uninfected HeLa cells
- PNA cures infection by normal *E. coli*
- PNA does not cure infection by *E. coli* with mutant plasmid.
Antisense PNAs Targeted to Ribosomal RNA

**Ribosome:** Responsible for all protein synthesis

*PNA targeted to rRNA could block expression of all genes*

- 3 PNAs targeted to ss regions of Peptidyl transferase center.
- 1 PNA targeted to α-sarcin loop and mRNA binding region.
Antisense Activities of Anti-rRNA PNAs

- PNAs targeted to peptidyl transferase center, α-sarcin loop block expression of β-galactosidase (triangles in A)

- Effect comparable to antibiotic drug tetracycline (open circles in A)

- Protein synthesis blocked (solid lines in B) but not transcription (dashed lines in B)
**Bactericidal Activity of Anti-rRNA PNAs**

Concentration (μM)

Antisense PNAs prevent growth of bacteria (spot).

Effect comparable to tetracycline
PNA Can Recognize Mutated Sequences
Improving the Affinity of PNA