PLNT 2530
2024

Unit 2
NUCLEIC ACIDS

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Review

1) Avagadro's number as the number of molecules (6.02×10^{23}) which make up 1 mole.

2) The molecular weight of a mole of any type of molecule

1 mole = 1 gram equivalent weight

eg. 1 molecule of H_2O = 18 atomic mass units

1 mole of H_2O = 18 grams

6.02×10^{23} molecules of H_2O = 18 grams

Review

3) Metric prefix units and how to interconvert between them.

Prefix	Unit	Example
	10^{0}	gm (gram)
milli	10^{-3}	mg
micro	10^{-6}	µg
nano	10^{-9}	ng
pico	10^{-12}	pg
femto	10^{-15}	fg

Properties of Nucleic Acids

Nucleic acids (review)

A Structure

B Directionality and Conventions in Notation

C Double Strandedness

D Detection and Quantitation

A Nucleic Acid Structure

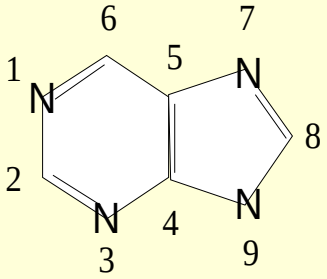
The monomeric unit of DNA/RNA is the nucleotide

consists of-

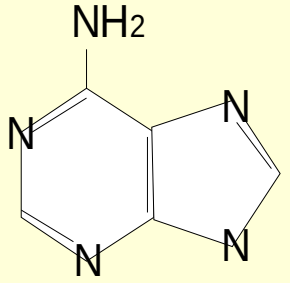
1) nitrogenous base unit
2) sugar unit
3) phosphate group

1) nitrogenous base unit

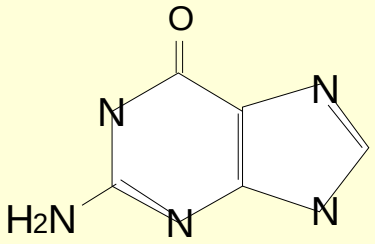
a. Purine bases *basic structure*



Adenine A
(6-aminopurine) RNA & DNA

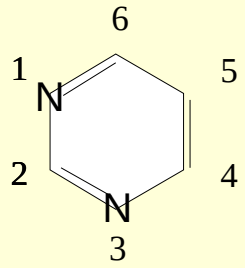


Guanine G
(2-amino-6-oxopurine) RNA & DNA

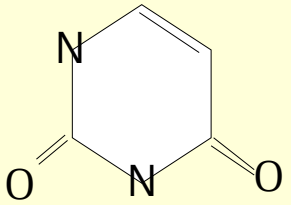


1) nitrogenous base unit

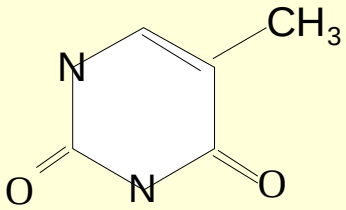
b. Pyrimidine bases *basic structure*



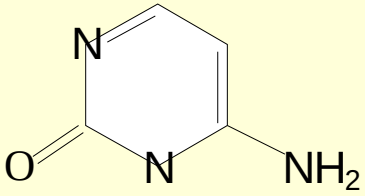
Uracil U
(2,4-dioxopyrimidine) RNA



Thymine T
(5-methyl-2,4-dioxopyrimidine) DNA



Cytosine C
(4-amino-2-oxopyrimidine) RNA & DNA

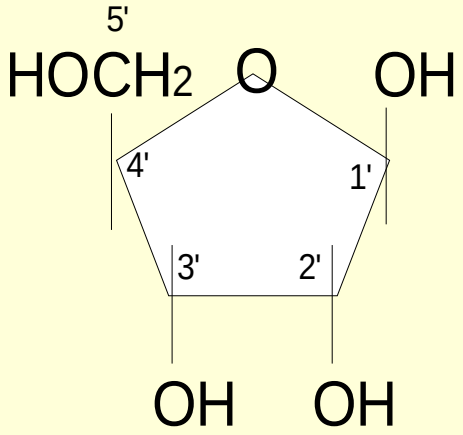


Features: Non-polar, non-ionic, planar

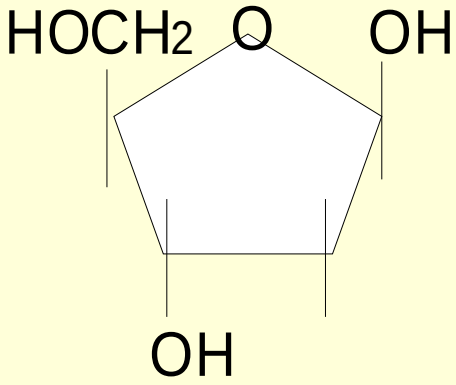
2) sugar unit

a. ribose

ring numbering



a. 2-deoxyribose

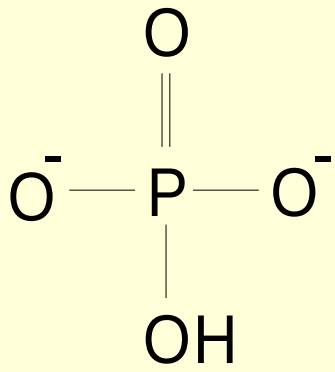


Features: Polar, non-ionic

3) phosphate group

$pK_1 = 1$

$pK_2 = 6.5\text{-}7.5$



Features: ionic

The assembled nucleotide

Eg. deoxyadenosyl 5' monophosphate

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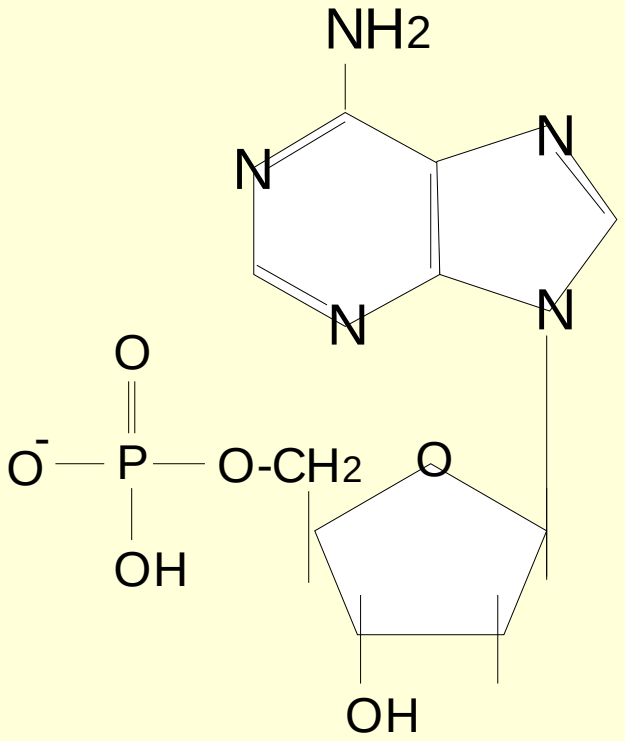
Phosphate is the only unit ionized between pH 3 and 11.



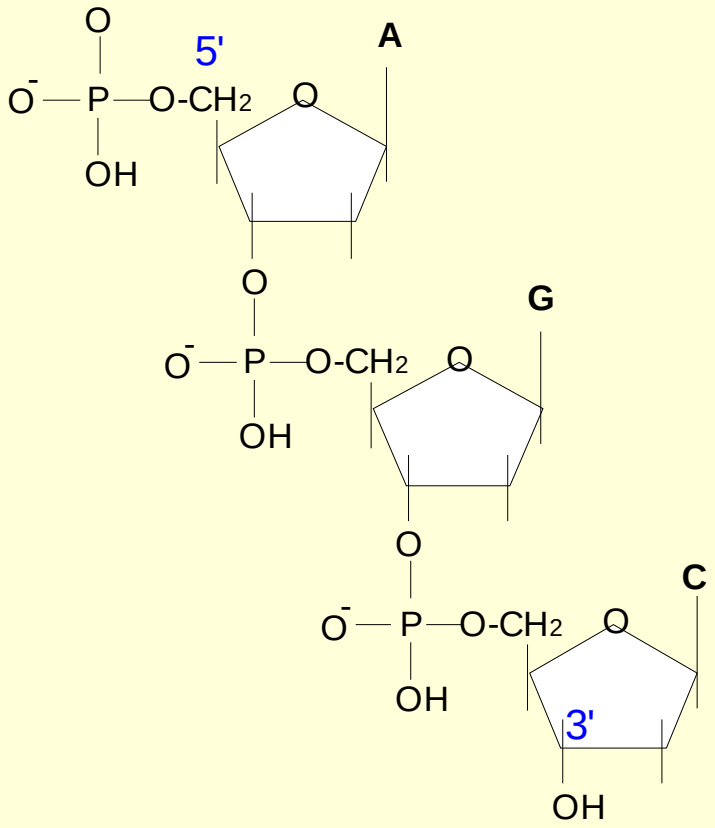
B Directionality and Conventions in Notation

Backbone is a repeating alternate of phosphate and sugar groups.

Because the only difference is in the nitrogenous base a simplified description of the sequence is written as just the base sequence ie AGC

By accepted convention base sequence is read (and written) in the 5'-3' direction unless indicated otherwise.

AGC not CGA



Since DNA is a three-dimensional structure,
you really need to visualize it in three dimensions.

https://youtu.be/TrNeuH6JOVc

Conventions

- if a sequence is not labelled, the left end of the sequence is assumed to be the 5' end in single stranded sequence.
  - ATCGG is the same as $^{3'}$GGCTA$^{5'}$ but different from GGCTA
- ATCGG from natural DNA will have a phosphate group on the A end (5‘) and a free OH group on the G end (3’).
- For double stranded sequences, in which the individual stands are the reverse complements of each other, the upper strand is assumed to be written in the 5' to 3' direction if no designation is included.

ACTTGC	is	$^{5'}$ACTTGC$^{3'}$
TGAACG		$^{3'}$TGAACG$^{5'}$

C Double Strandedness

1) Types of double-stranded nucleic acid molecules

2) Factors affect the stability of the duplex

1) Types of double-stranded (ds) nucleic acid molecules

DNA-DNA homoduplex

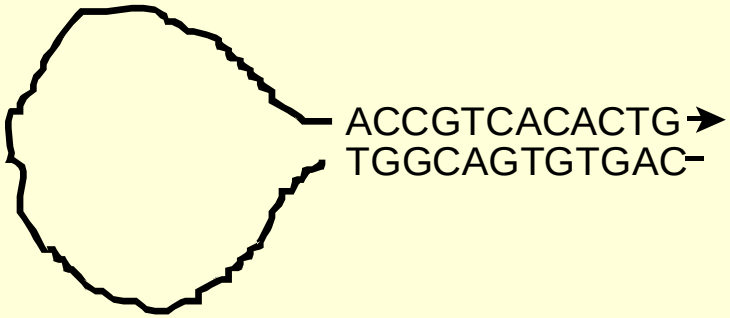
DNA-RNA heteroduplex

RNA-RNA homoduplex

The ds association depends on **complementary base pairing** of strands arranged in an **anti-paralle**l manner.

Intermolecular (between)

Intramolecular (within)



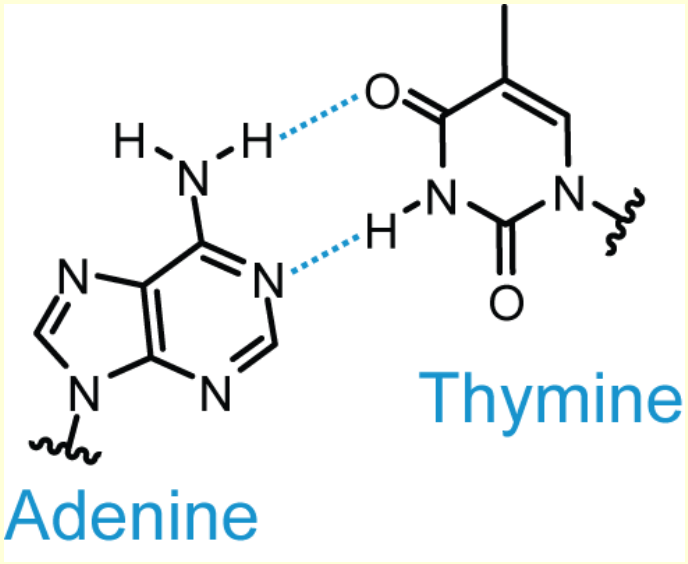
Double strandedness occurs as a result of several **types of interactions** – consider two

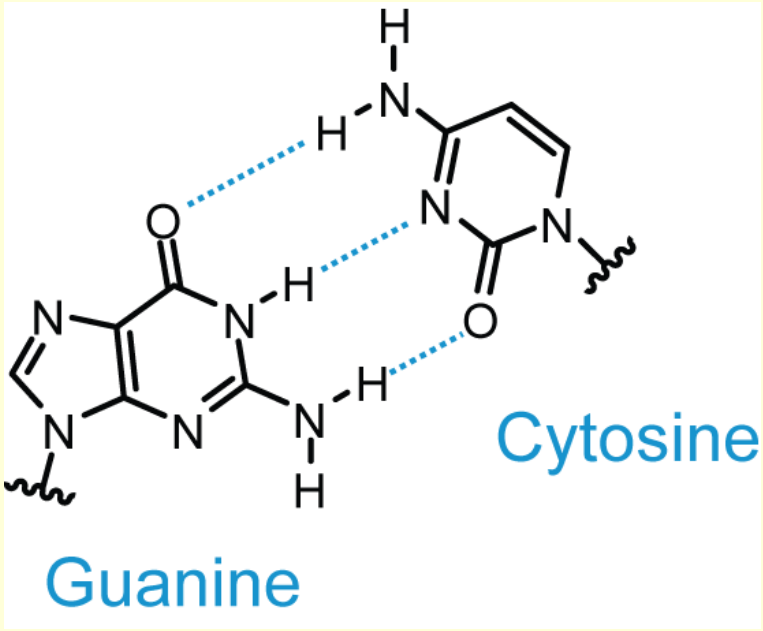
a) **Hydrophobic interactions** are driven by the tendency of water molecules to maximize interactions with other water molecules and other polar molecules and to minimize contact with non-polar groups.

The result: Non-polar nitrogenous bases in nucleic acids are forced together, while the polar sugars and phosphate groups are pulled into interactions with water.

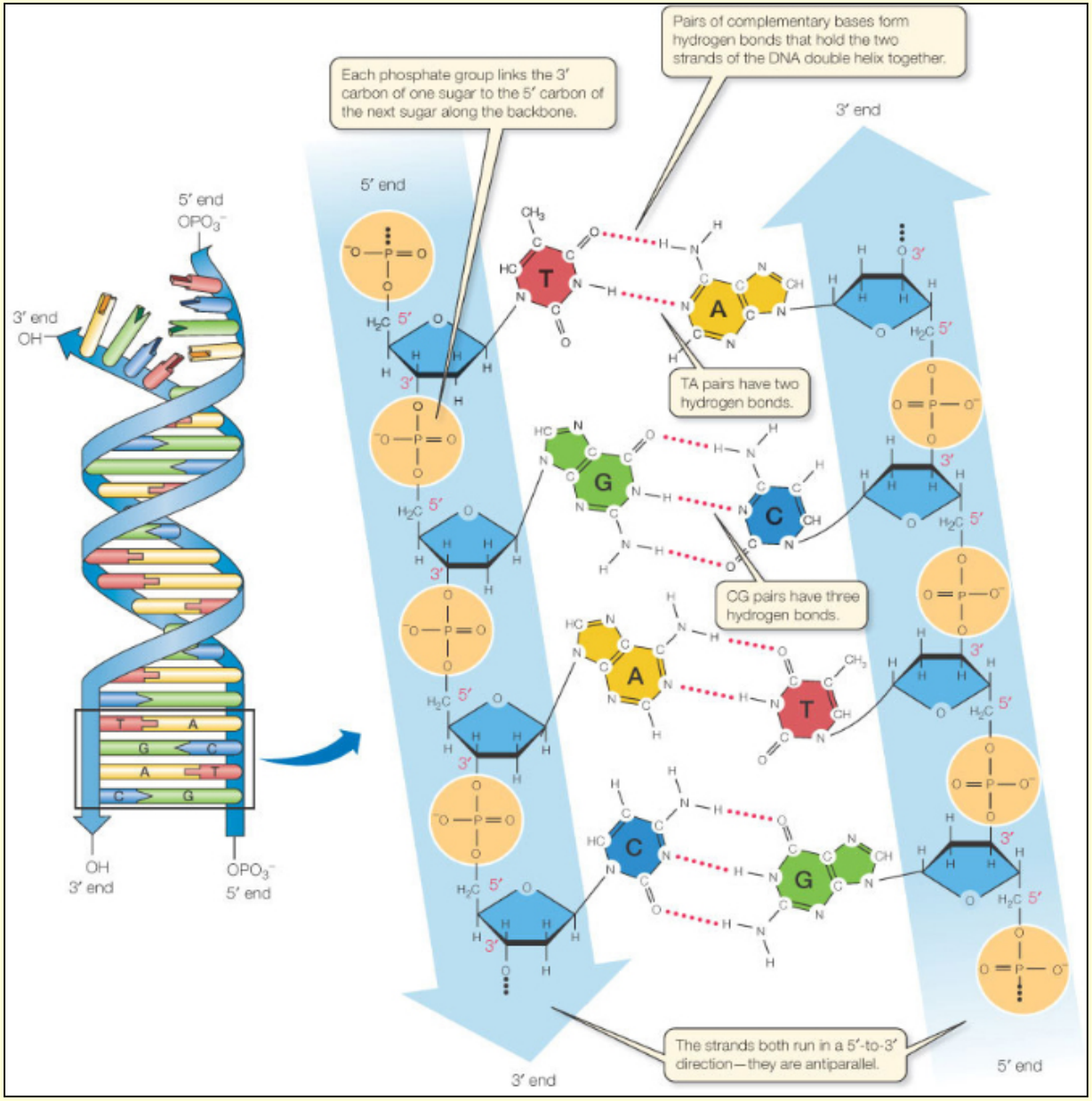
Concentration of the nitrogenous bases in a common region makes it easier for them to find complementary base pairs, and excludes water as a H-bonding competitor.

b) **H-Bonding** These are weak non-covalent bonds where a H is shared between two electron donating groups.





Complementary bases (A-T, A-U, G-C) are stabilized by hydrogen bonds. The stability of the interaction is proportional to the number of H-bonds.



http://www.nature.com/scitable/topicpage/discovery-of-dna-structure-and-function-watson-397

In base pairing

G-C forms 3 H-bonds

G
|||
C

will not exist at room temp in water

A-T(U) forms 2 H-bonds per nucleotide

G A T C G C G T A G
||| || || ||| ||| ||| ||| || || |||
C T A G C G C A T C

will exist as duplex at 30C 50% of the time

If the adjacent bases in a sequence cannot pair as well, the original interaction between 2 bases will be disrupted by the simple kinetic energy of the molecules.

Double strandedness is based on the cooperative interaction of many consecutive bases in a sequence.

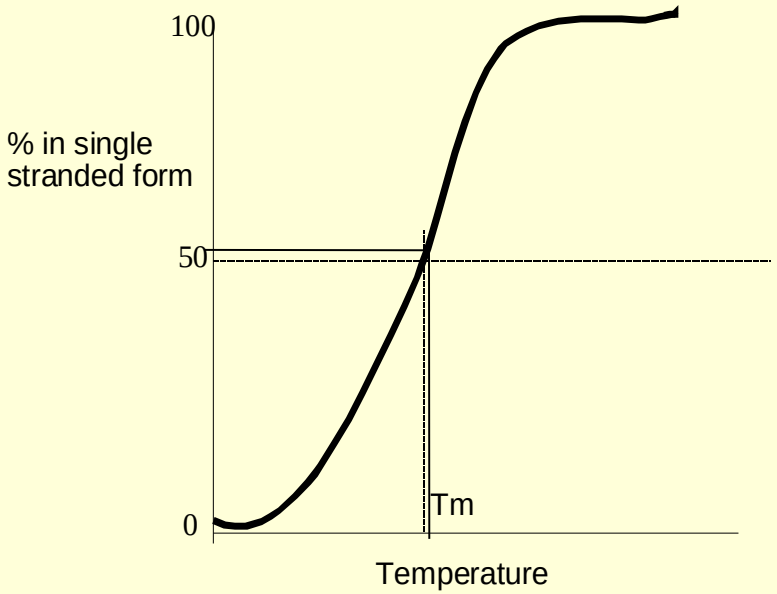
3) Factors affecting the stability of the duplex

a) **Temperature**

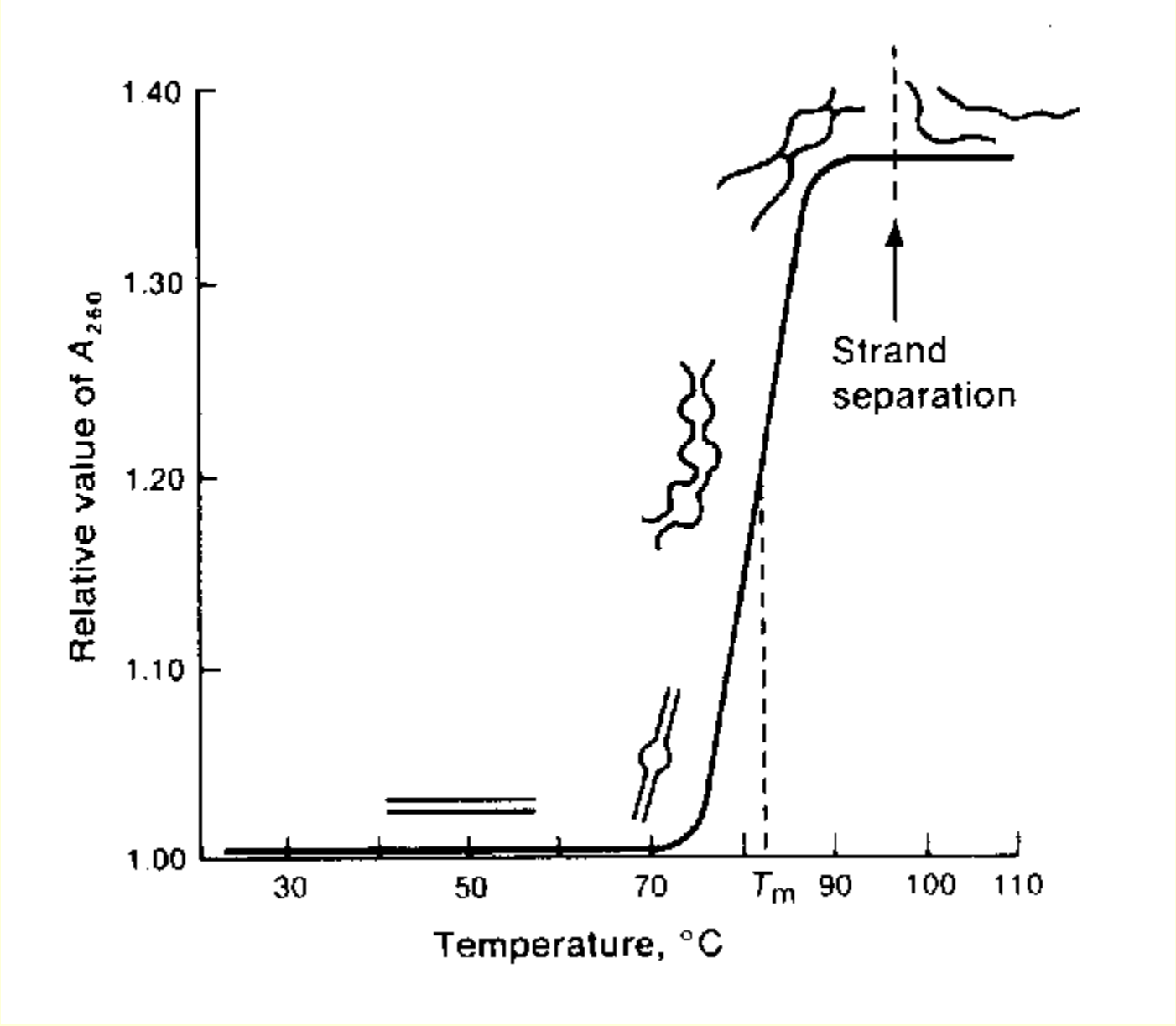
- affects the kinetic energy of solvent and solute molecules

Higher temperatures reduce stability of helix structure

Tm= melting temperature, is the 50% dissociation point in the ss-ds transition



Helix to random coil transition curve



from http://bioscience.egloos.com/5308111

b) **Stability of duplexes (cond.)**

Stability increases

i) as G-C to A-T ratio increases

ii) with length of duplex (up to 500 nt)

iii) degree of complementarity.

Perfect matching between strands yield more H-bonds and greater stability than imperfectly matched strands.

AGCTAGGATCAT	TAGGCTAGTAC
TCGATCCTAGTA	ATCTGATCCTG
Perfect match	Imperfect match

c) **The solvent conditions**

i) Chemicals which disrupt solvent structure, destabilize the double helix structure

-Formamide, urea, alkali and certain cations (Hg^{2+})

ii) Chemicals which increase solution structure, stabilize the helical structure

-Na+ and K+ increase solvent structure and the hydrophobic interaction

Conditions to disrupt DNA duplex structure in solution normally involve

i) elevated temperatures in combination with ii) low (Na^+ / K^+) salt and

iii) if necessary the addition of formamide.

Conditions to encourage DNA duplex structure normally involve

i) lower temperature and ii) high salt.

Optimum conditions are normally chosen to maximize hybrid selectivity (the most perfect combination of complementary DNA/RNA)

Hybridization: Use of a probe to identify a specific DNA sequence from a population of many different sequences

There are many different hybridization procedures (eg. Southern blots, Northern blots), but they all have the following in common:

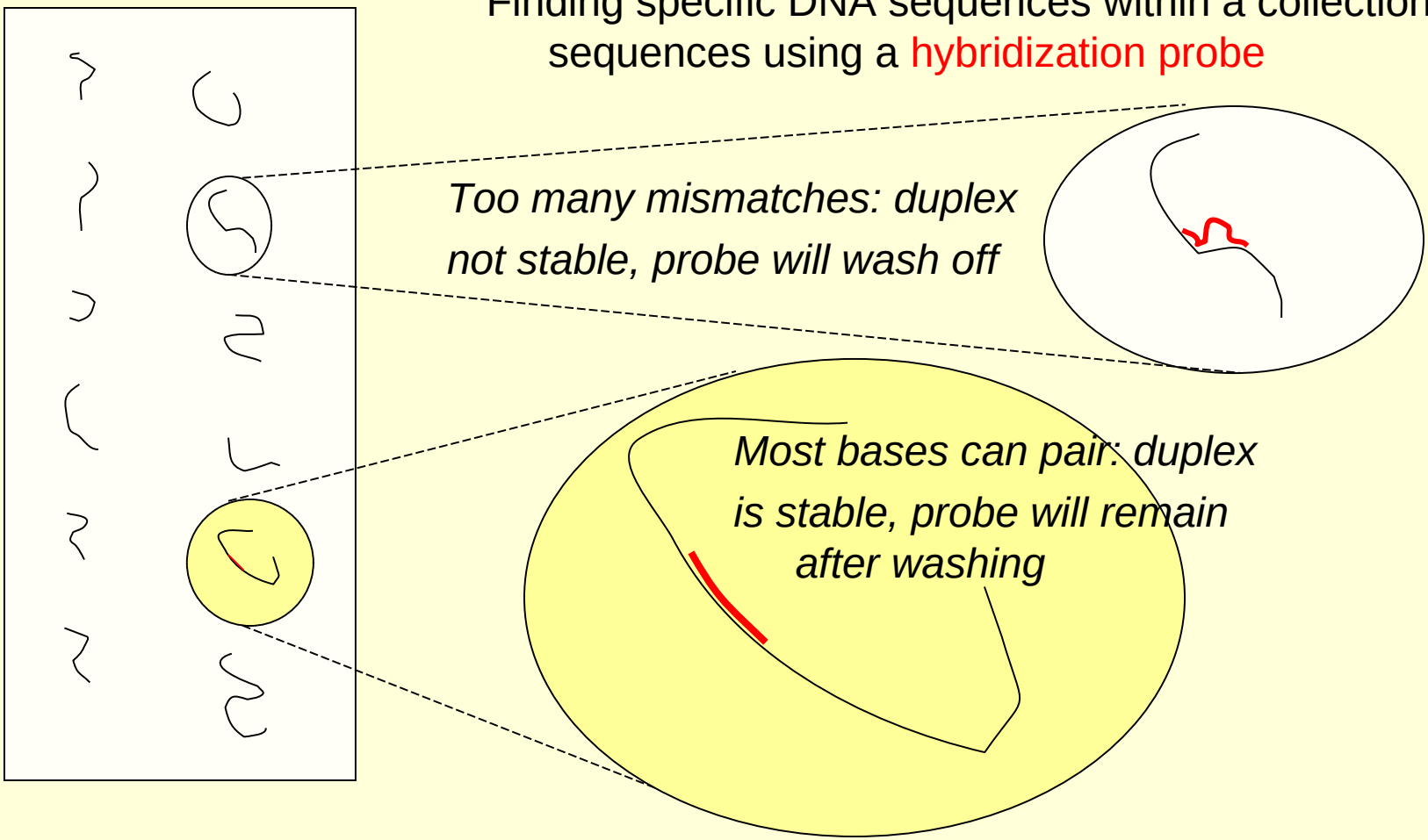
target - An unlabled DNA or RNA, usually bound to a physical substrate such as a filter.

probe - A DNA or RNA that carries a label (eg. flourescent tag).

The probe is incubated with the substrate. If one of the targets has a sequence that is complementary to the probe, the probe will form a stable hybrid with the target. We can see where the probe has bound by visualizing the label.

Application: Selecting an optimum temperature for probe hybridization

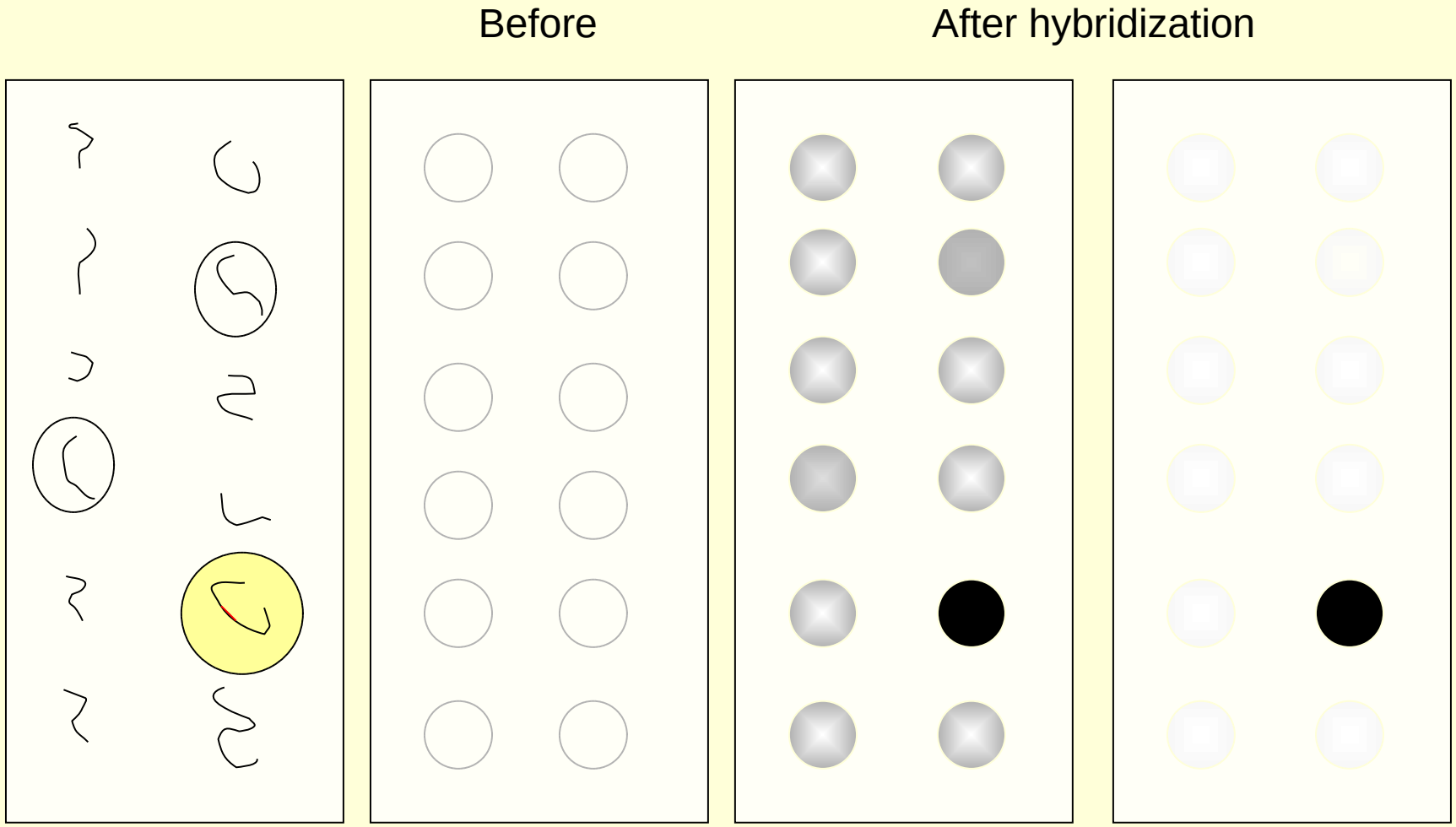
Finding specific DNA sequences within a collection of sequences using a hybridization probe



If T too high - no hybrid formation

too low - non-specific binding of probe

We only see signal where the probe binds.
The rest is blank.



Application: Selecting an optimum temperature T_m for probe hybridization

polynucleotide probes (50 -1000+ nt)

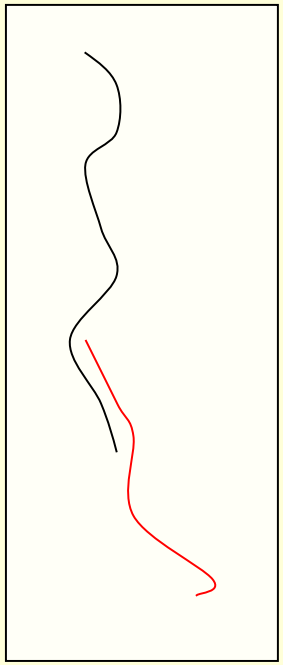
T_m affected by

1) salt concentration (M in moles/L)

2) base composition (%G+C)

3) length of the shortest chain in the duplex (n) and

4) the amount of the helix destabilizing agent present - such as formamide (%).



Note: In many cases only part of two strands will overlap, or hybridize. The other sequences on each strand may not be complementary. We only need to consider the part of the two strands which overlap.

Application: hybridization of long polynucleotides

Formula for optimal hybridization temperature

$T_d = 81.5\ C + 16.6 \log [M] + 0.41(\%G+C) - 500/n - 0.61(\%\ formamide)$

where M is [NaCl]
n is the length of the sequence in nucleotides

-from Anal. Biochem. 138 (267-284) 1984

Example:
DNA duplex 100 nt, 40% G+C, in 0.3M NaCl, 20% formamide

Td = 81.5 +16.6 (-0.52) + 0.41(40) - 500/100 - 0.61(20) = 72 °C

- It is usually best to perform hybridizations at about 5 °C below T_d.
- In this case, Hybridization temp = 67 °C (72-5)

Application: Selecting an optimum temperature for probe hybridization

Small nucleotides - Oligonucleotides (10-25 nt)

Formula $T_m = 4(G+C) + 2(A+T)$ assumes 0.9 M NaCl

-a temperature 5^0C below the T_m is frequently used for hybridization of near perfectly matched sequences.

- example

Polymerase chain reaction (PCR) also needs optimum hybridization, but based temperature, rather than on high salt conditions

D Detection and Quantitation

Detection (visualization) of nucleic acids
eg. after electrophoretic separation

Quantitation (amount) - techniques use small amounts of DNA/RNA but required a good estimate of the amount of DNA/RNA

Fluoresence Detection of DNA (RNA):

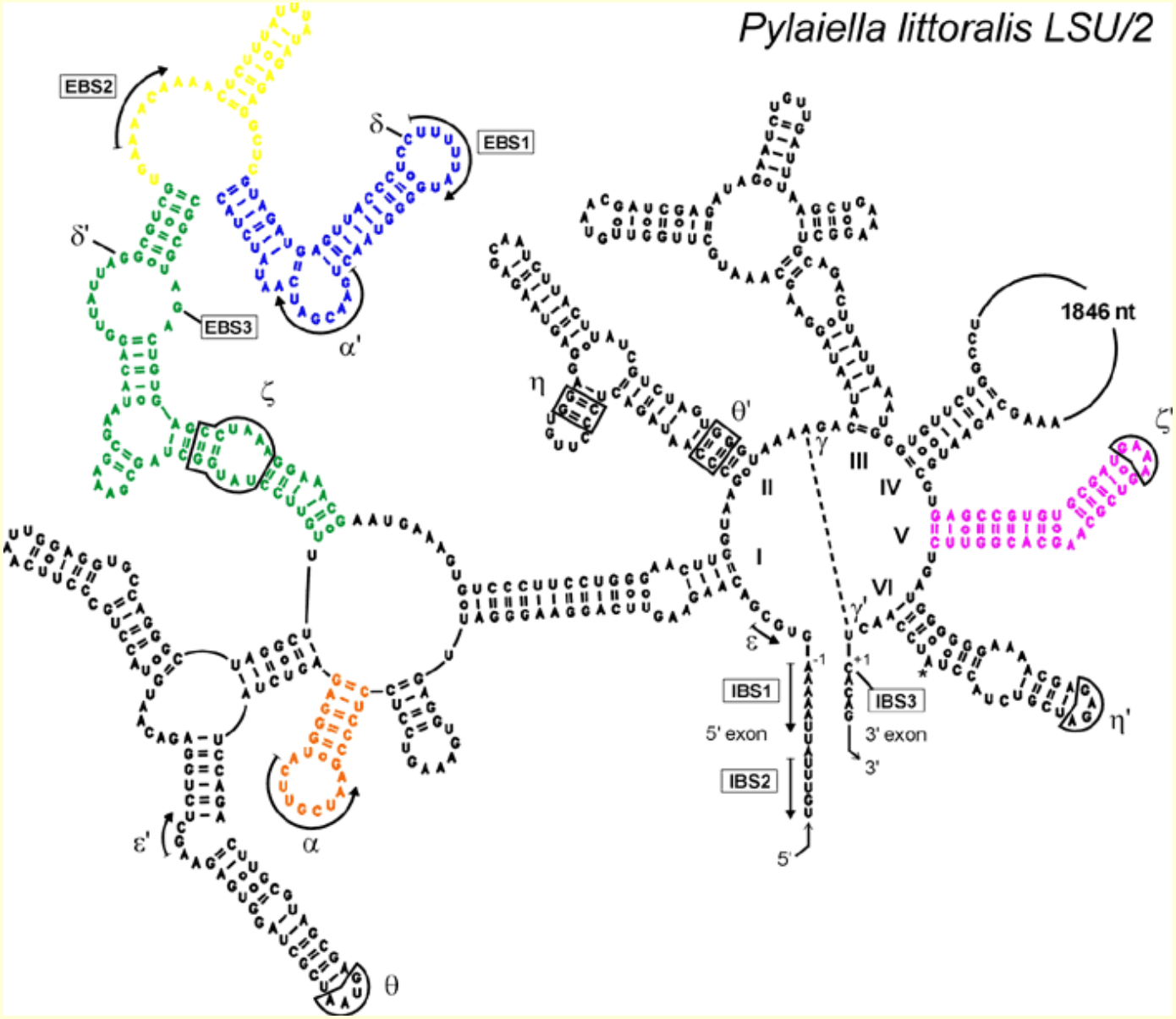
DNA and RNA do not fluoresce but can complex with fluorescent cmpds

Compounds used are intercalating dyes
eg. RedSafe™, propidium iodide and SYBR green 1

- RedSafe can visualize 50 ng of DNA when exposed to UV light

RNA can bind these dyes wherever double-strandedness occurs

Large ribosomal RNA precursor



(Detection and Quantitation)

- Semi-quantitative estimation of unknown sample of DNA by comparing to fluorescence of known amounts of DNA
- fluorescence is dependent on the amount of RedSafe bound
- fluorescence for equi-molar amounts of 1000 bp long and 500 bp long DNA should be ~ 2:1
- equal weights of these two sizes of DNA would have similar fluorescence

(Detection and Quantitation)

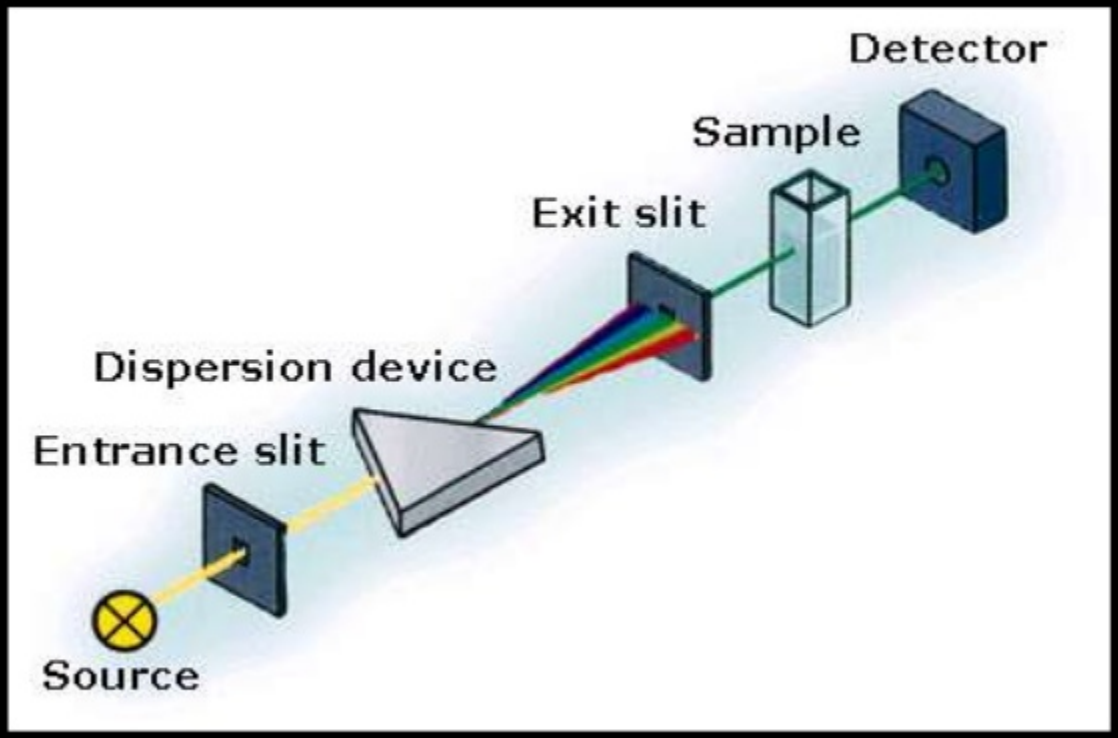
UV Absorbance Quantitation based on sample absorbance of light at a wavelength 260 nm (A_{260})

A_{260} is due to the aromatic nitrogen bases in the nucleic acids

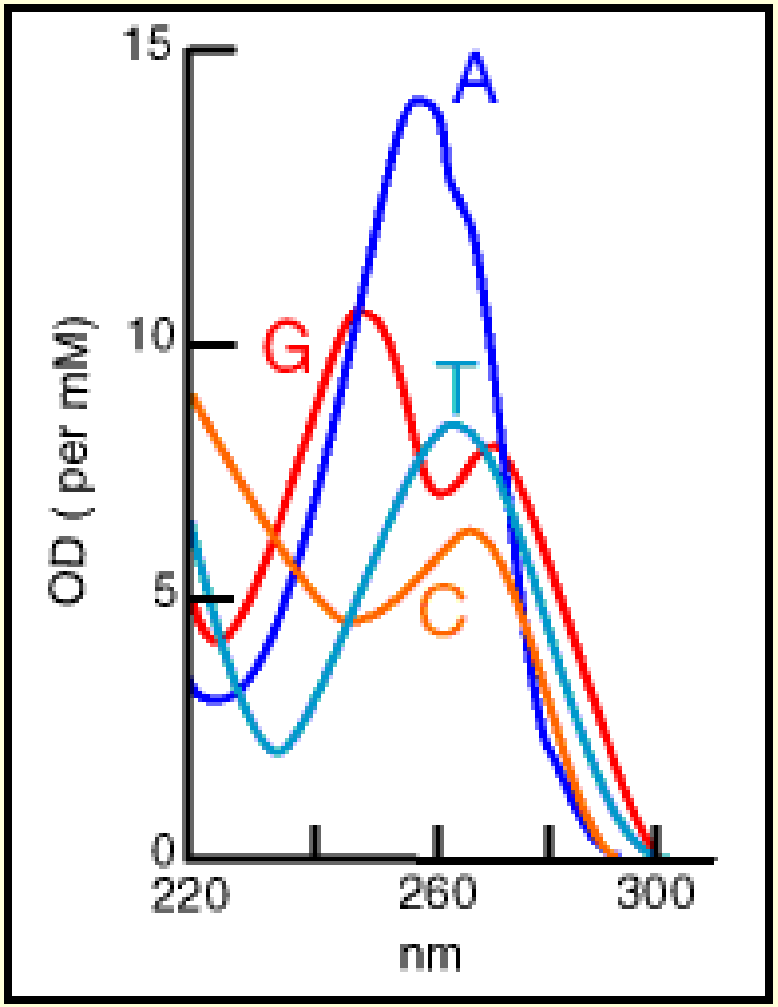
The A_{260} of a nucleic acid solution at this wavelength is assumed to be related to the concentration of nucleic acids in the solution.

Assumption valid provided the sample is pure or the solution does not contain contaminants which also absorb at this wavelength

Spectrophotometer



Absorbance spectra of principal bases of DNA and RNA



from http://www.scienceisart.com/A_DNA/UVspectrum_2.html

UV Absorbance Quantitation

a) For simple mononucleotides

eg. Adenosine monophosphate, AMP

Beer's Law $A_{260} = \epsilon^{AMP}_{260}\, cl$

where A = the absorbance measured at 260 nm

ϵ^{AMP}_{260} = molar extinction coefficient of AMP at 260 nm (Liter mol^{-1} cm^{-1})

c = molar concentration (moles/L)

l = path length of the light through the sample in cm (normally 1.0 cm)

UV Absorbance Quantitation

Nucleotide	ϵ_{260}
A	13,400
G	8,100
C	6,100
U	8,200
T	7,900

The Beer's Law relationship cannot be used in polynucleotides because

1) the proportion of different bases varies from one DNA sample to the next and thus no generally applicable Є exists

2) a hypochromic effect occurs with double stranded molecules.

Hypochromic effect

-refers to the decrease in molar absorptivity (Є) of free nucleotides when they are arranged in single stranded DNA and the further decrease in Є when the DNA is increasingly double stranded. When the base pairs are stacked they cannot absorb as much energy

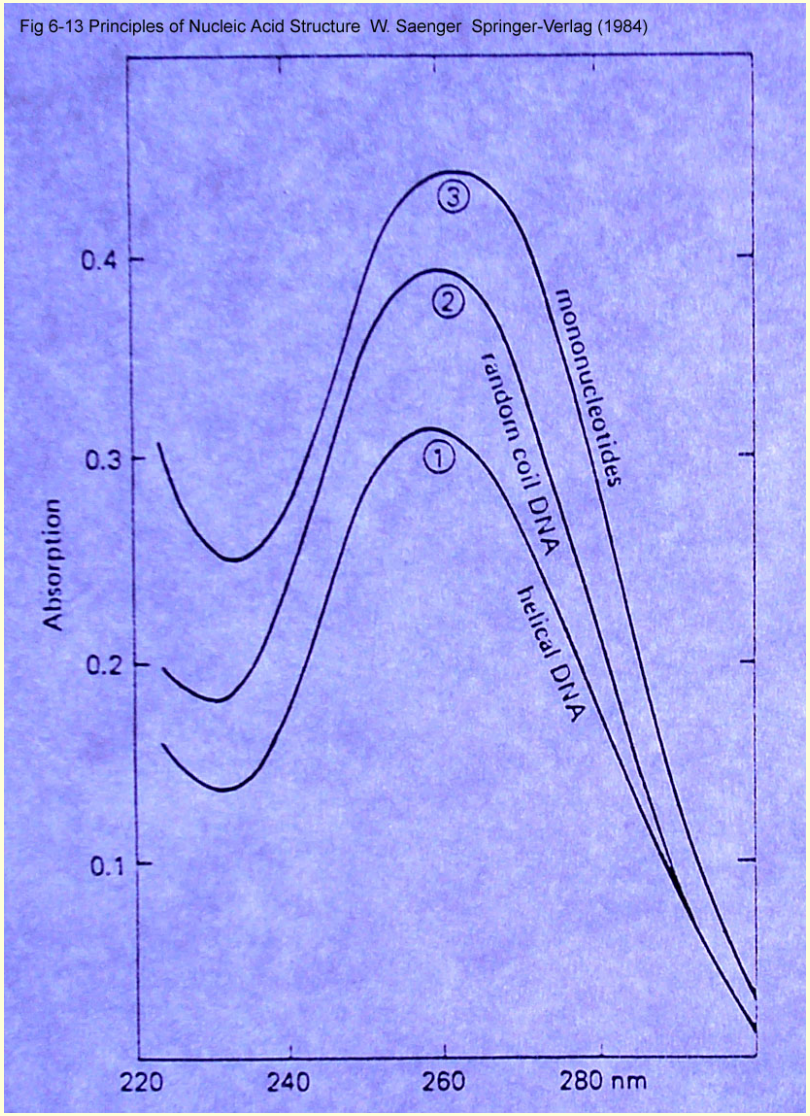


Fig 6-13 from Principles of Nucleic Acid Structure W. Saenger

b) with polynucleotides

Because of these problems with the direct application of Beer's law

General approximations are used to relate absorbance to a weight concentration based on the nature of the DNA/RNA being examined.

It is these estimates which are commonly used.

If A_{260} =1	for dsDNA	then conc = 50 µg/ml
	for ssDNA	then conc = 40 µg/ml
	for oligonucleotides	then conc = 30 µg/ml

Purity of DNA/RNA from UV measurements

The ratio of two UV absorbance measurements can be used to estimate the **purity** of a sample of nucleic acids.

The ratio (A_{260}/A_{280}) of pure DNA or RNA should be between 1.8-2.0.

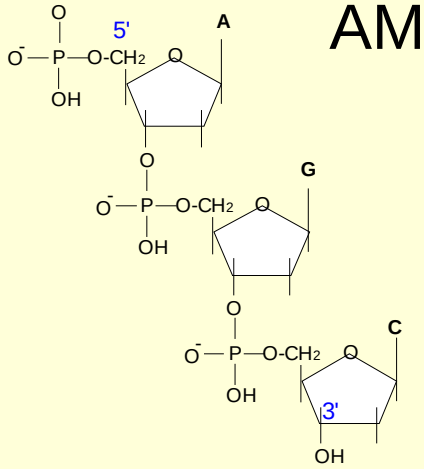
(see Fig 6-13)

Major contaminants in DNA and RNA preparations are typically protein and phenolics. Both of these absorb UV light more strongly at 280 nm than 260 nm.

Effect of contaminants on A_{260}/A_{280} ratio

Average nucleotide (nt) and base pair (bp) weights

(with deoxyribose)			(with ribose)	
dAMP	312		AMP	328
dCMP	288			
dGMP	328			
dTMP	289			
Avg	304			



If Na (23 g/mole) is counter ion to ionized PO_4, then the average mass per nucleotide is 327 (rounded to 330 for most estimates)

Therefore 330 g/mole nt or 660 g/mole bp are the average masses frequently used

Take home problem

You have been given a 2.0 ml sample of DNA. The DNA in the sample is composed of molecules which are all of one size, 1000 base pairs (ie. double stranded). Measurement of the absorbance at 260 nm gives you a value of A_{260} = 0.5 units.

a) Determine the mass (µg) of DNA present.

b) Determine the molar concentration of the DNA present in picomoles per microliter (pmol/µl).