DNA hybridization in solution is a second order process

ssDNA

dsDNA

A

A

P
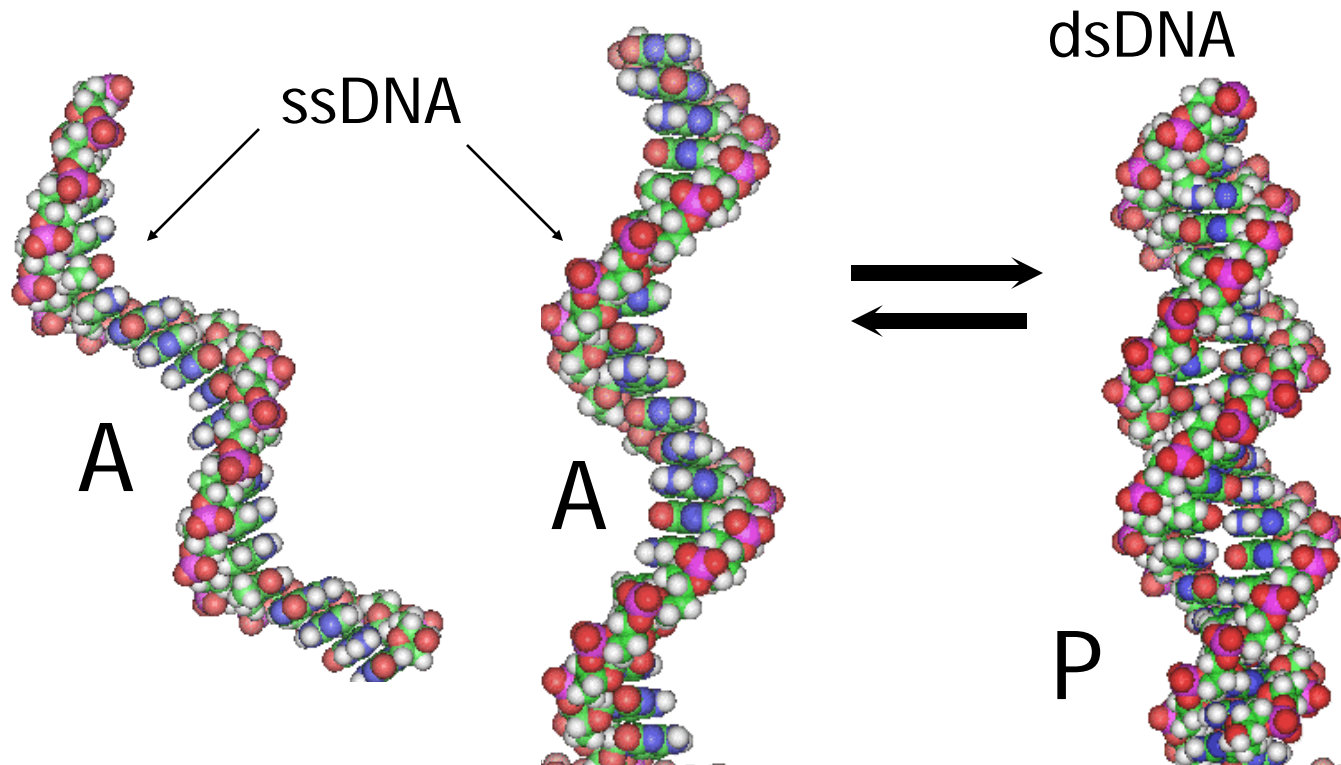
DNA hybridization on a surface is a first order process

ssDNA

dsDNA

A

B

P
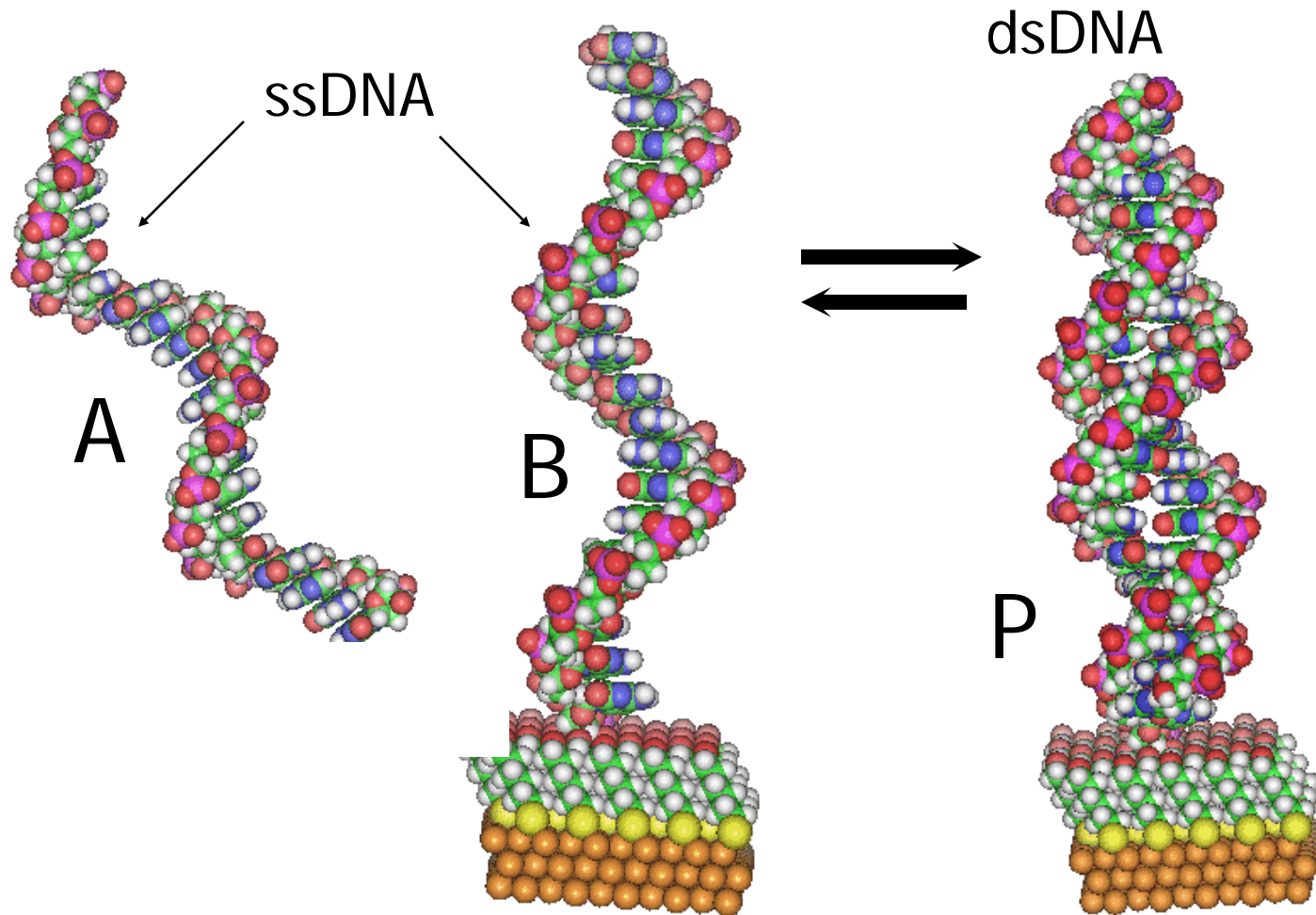
DNA hybridization kinetics
The rate constant

Hybridization association rates are strongly dependent on salt concentration, temperature, and lengths of the two strands, but only minimally dependent on G-C content, mismatching, formamide, and pH. Under standard conditions (0.18 M monovalent cation and $T_m = 25°$ C), the association rate constant is:

k (liters x moles nucleotide^{-1} x sec^{-1}) = 5 x 10^4 x $L^{1/2}$,

L = the length of the probe [L = number of bases]

DNA hybridization kinetics relationship to melt temperature

For DNA hybridization, this rate constant calculated for standard conditions of 0.18 M monovalent cation concentration. The rate is increased sevenfold at 1 M and decreased tenfold at 0.07 M monovalent cation concentation.

For strand lengths of 50 to 5000 nucleotides, the rate is directly proportional to the square root of the length of the shorter of the two strands. For DNA/DNA association, the maximum rate occurs 25 ° C below the T_m with only minimal variation in rate from 15 to 35 ° C below the T_m.
The rate of association approaches zero near the T_m.

DNA hybridization kinetics surface vs. solution

For hybridization of a single-stranded target in excess to a probe (eg, polynucleotide immobilized on a filter), the reaction has pseudo first-order kinetics. The time to reach half-maximal hybridization $\tau_{1/2} = \ln(2)/k$.

Where both strands are at equivalent concentrations (eg, double-stranded DNA), the reaction has second-order kinetics and the $\tau_{1/2} = I/k[A]$. There is evidence that the rate of hybridization in solution is significantly faster than the rate of hybridization of a target to an immobilized probe, in part due to decreased probe accessibility and decreased diffusion rates of target diffusion to the probe.

Concentration Over Time Analysis

Treating the hydridization of two strands of a 100mer in solution as a second order process we have the following time dependence.

$$\frac{[A]}{[A]_o} = \frac{1}{1 + k[A]_o t}$$

$k = 5 \times 10^5 \ M^{-1}s^{-1}$

Legend:
- 10^{-8} M
- 10^{-7} M
- 10^{-6} M
- 10^{-5} M

Y-axis: C/C_o

X-axis: Time (seconds)