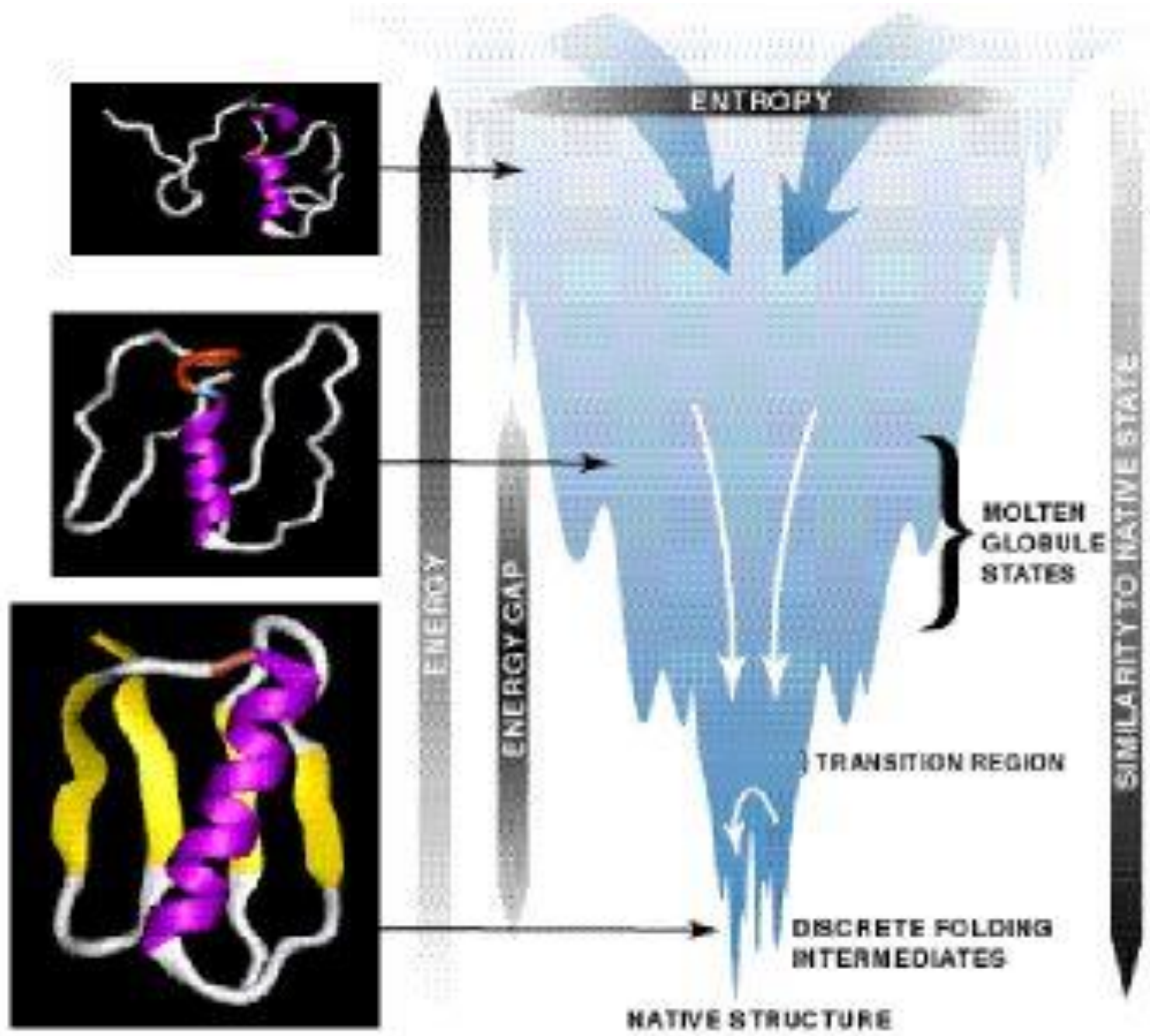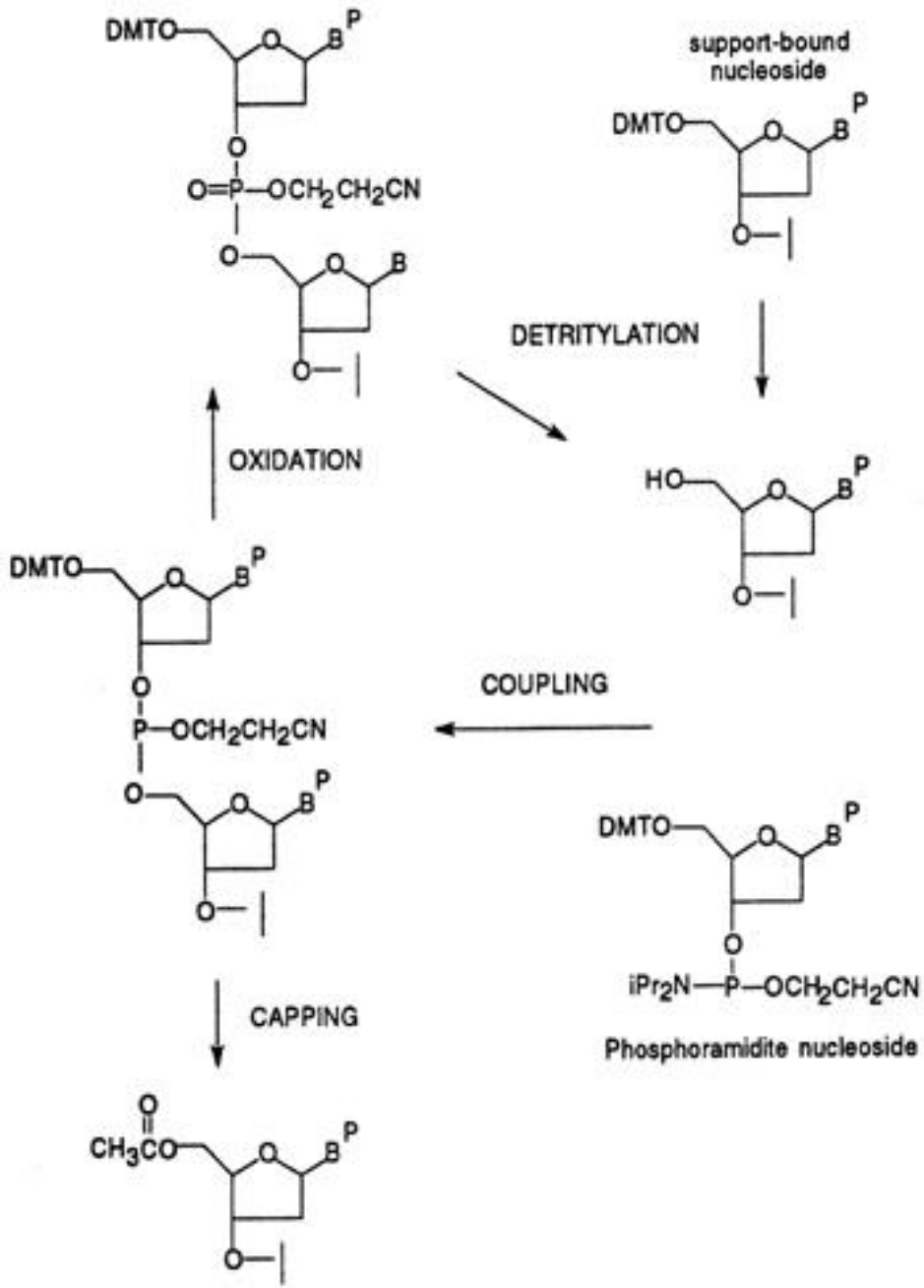- Ionic interactions
  - $F = (q_1 q_2)/(d^2 \epsilon)$
  - ϵ the dialectric (water 85)
  - Weak in water << -kcal/mol
- Van der Waales
  - Lennard-Jones potential
  - $F = D_0[(R_{eq}/R)^{12} - 2(R_{eq}/R)^6]$
  - 1.3 kcal/mol/CH_2
- Hydrogen bond
  - Vapor phase about -6 kcal/mol
  - Water about -0.5 to -1.5 kcal/mol
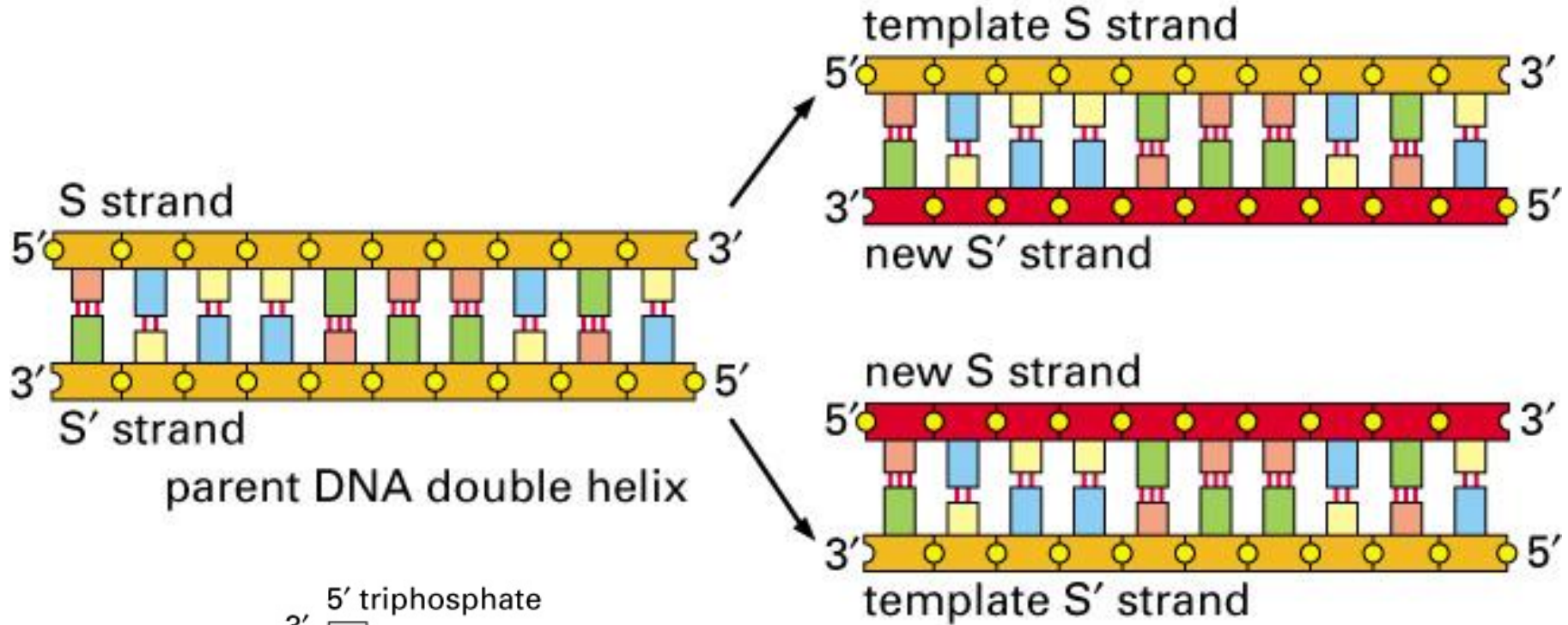 - $F = D_0[5(R_{eq}/R)^{12} - 6(R_{eq}/R)^6]\cos^2(DHA)$

ENTROPY

ENERGY

ENERGY GAP

MOLTEN GLOBULE STATES

TRANSITION REGION

DISCRETE FOLDING INTERMEDIATES

NATIVE STRUCTURE

SIMILARITY TO NATIVE STATE

DMTO — 5' B^P

support-bound nucleoside

DMTO — 5' B^P
O— |

DETRITYLATION

OXIDATION

O=P—OCH₂CH₂CN
O— 5' B
O— |

HO — 5' B^P
O— |

DMTO — 5' B^P
O
|
P—OCH₂CH₂CN
O— 5' B^P
O— |

COUPLING

DMTO — 5' B^P
O
|
iPr₂N—P—OCH₂CH₂CN

Phosphoramidite nucleoside

CAPPING

CH₃CO — 5' B^P
O— |

AGA									

AGA
AGG

GCA	CGA						GGA		
GCC	CGC						GGC		AUA
GCG	CGG	GAC	AAC	UGC	GAA	CAA	GGG	CAC	AUC
GCU	CGU	GAU	AAU	UGU	GAG	CAG	GGU	CAU	AUU

Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile
A	R	D	N	C	E	Q	G	H	I

UUA
UUG   AGC
                                 AGU

UUA					AGC					
UUG					AGU					
CUA				CCA	UCA	ACA			GUA	
CUC				CCC	UCC	ACC			GUC	UAA
CUG	AAA		UUC	CCG	UCG	ACG		UAC	GUG	UAG
CUU	AAG	AUG	UUU	CCU	UCU	ACU	UGG	UAU	GUU	UGA

Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
L	K	M	F	P	S	T	W	Y	V	

Figure 6–50. Molecular Biology of the Cell, 4th Edition.

Pro CCA to GAC is 3 mutations but CCC to GAC is only 2 Mutations

S strand

5′ 3′

S′ strand

parent DNA double helix

template S strand

5′ 3′

new S′ strand

new S strand

5′ 3′

template S′ strand

4th Edition.

5′ triphosphate

3′

HO—

incoming
deoxyribonucleoside
triphosphate

3′   5′

HO—   primer
                     strand

                     template
                     strand

5′   3′

pyrophosphate

5′-to-3′
direction of
chain growth

3′   5′

HO—

5′   3′
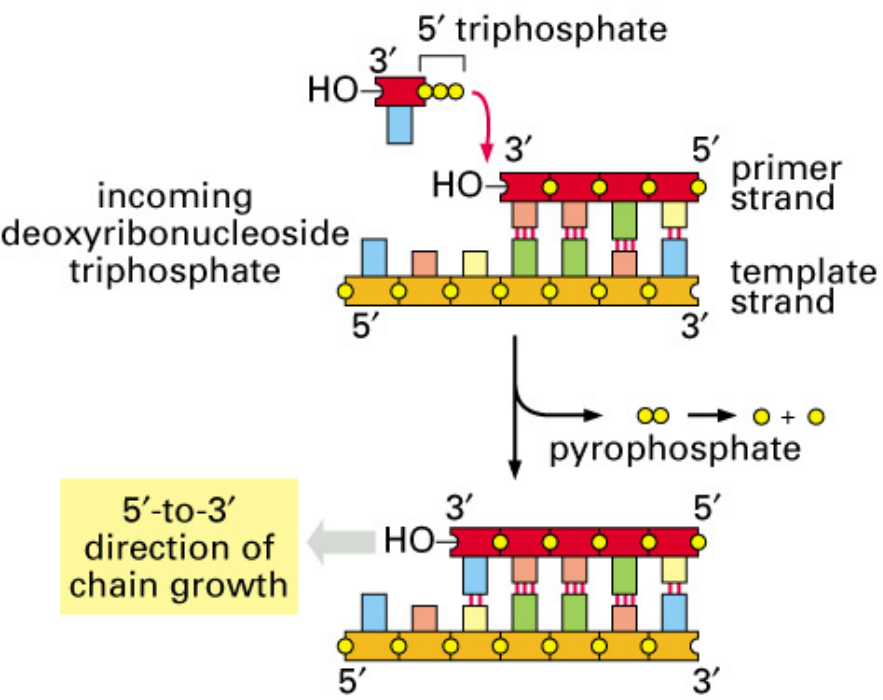
(A)

PCR mutagenesis example 1

The ends of PCR products can be easily manipulated. For example we can add sites for restriction endonucleases, making it easier to clone the products

Target for amplification:

```
5'-GTTTAGAGACCTAGACTA...........ATATTACGCGAGTAGCT-3'
    ::::::::::::::::::          ::::::::::::::::
3'-CAAATCTCTGGATCTGAT...........TATAATGCGCTCATCGA-5'
```

Primers are designed with extra sequences at their 5' ends

```
5'-GTTTAGAGACCTAGACTA...........ATATTACGCGAGTAGCT-3'
                                 ::::::::::::::
                            3'-TATAATGCGCTTAAG-5'

     5'-GGATCCTAGACTA-3'
        :::::::::::
3'-CAAATCTCTGGATCTGAT...........TATAATGCGCTCATCGA-5'
```

The PCR product now has sites for BamHI and EcoRI at its ends

```
5'-GGATCCTAGACTA...........ATATTACGCGAATTC-3'
   ::::::::::::::           ::::::::::::::::
3'-CCTAGGATCTGAT...........TATAATGCGCTTAAG-5'
```

6

Background: SELEX isolates activities from vast pools of (10^{12}-10^{15}) randomized molecules

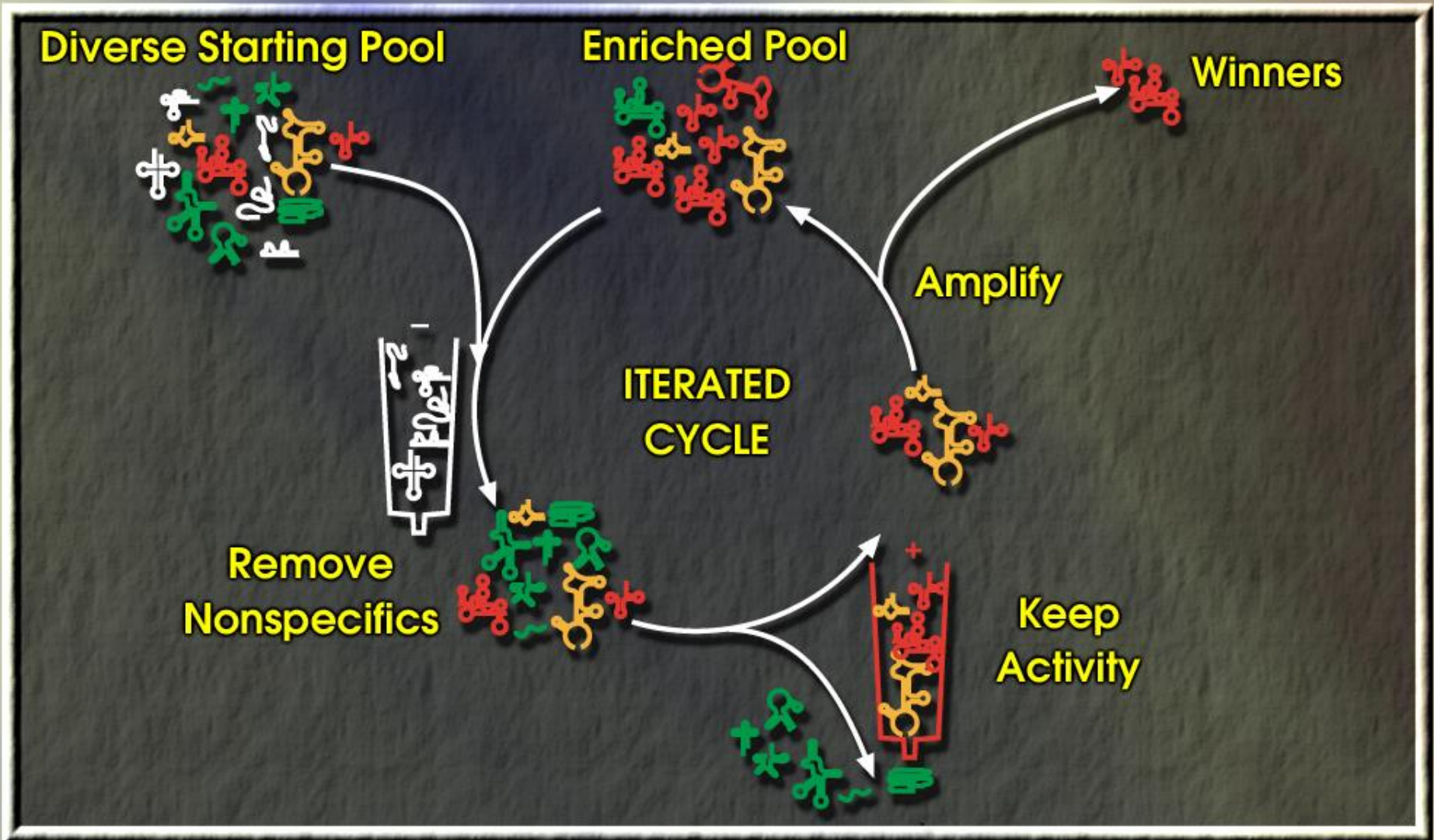Diverse Starting Pool

Enriched Pool
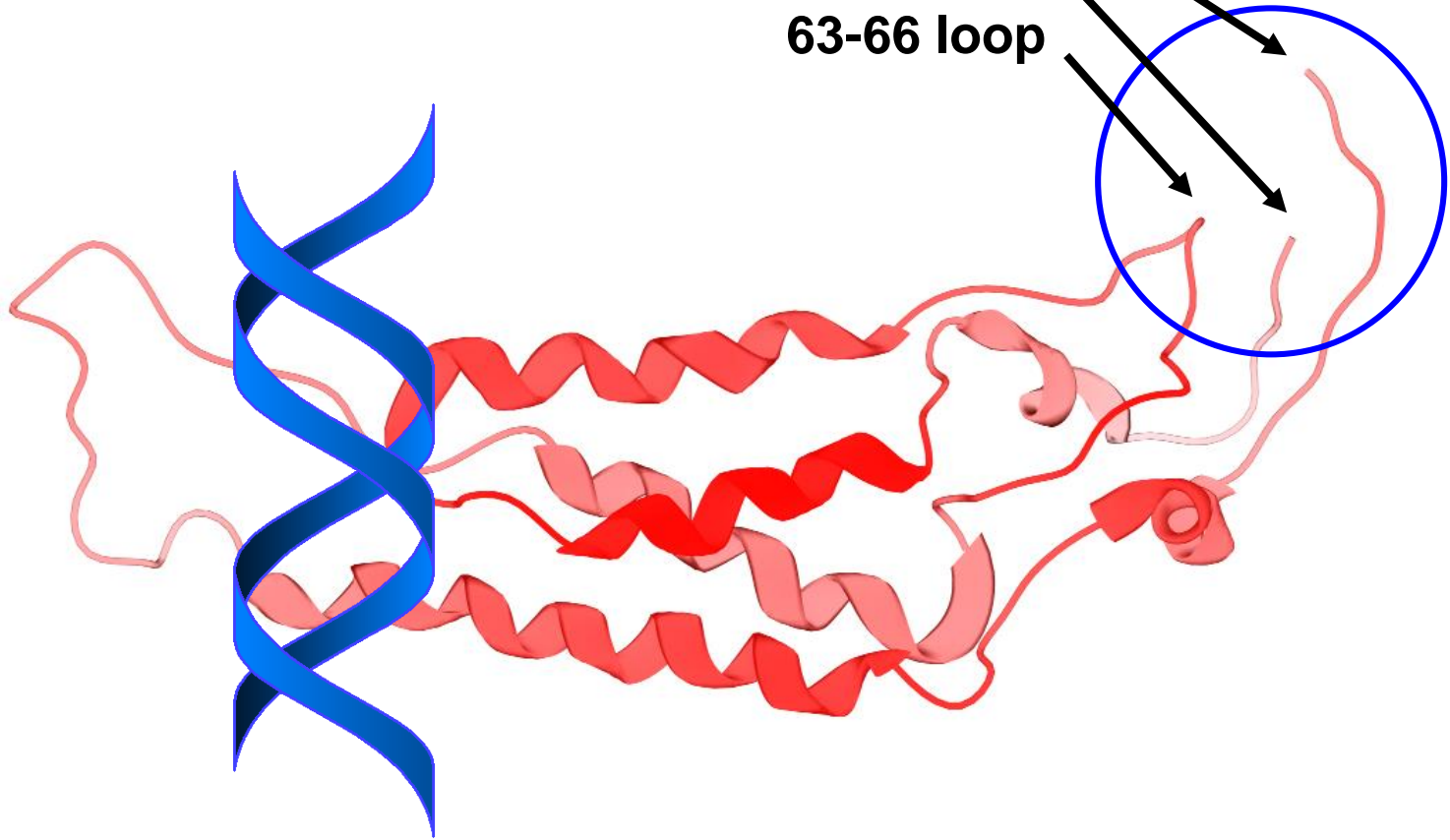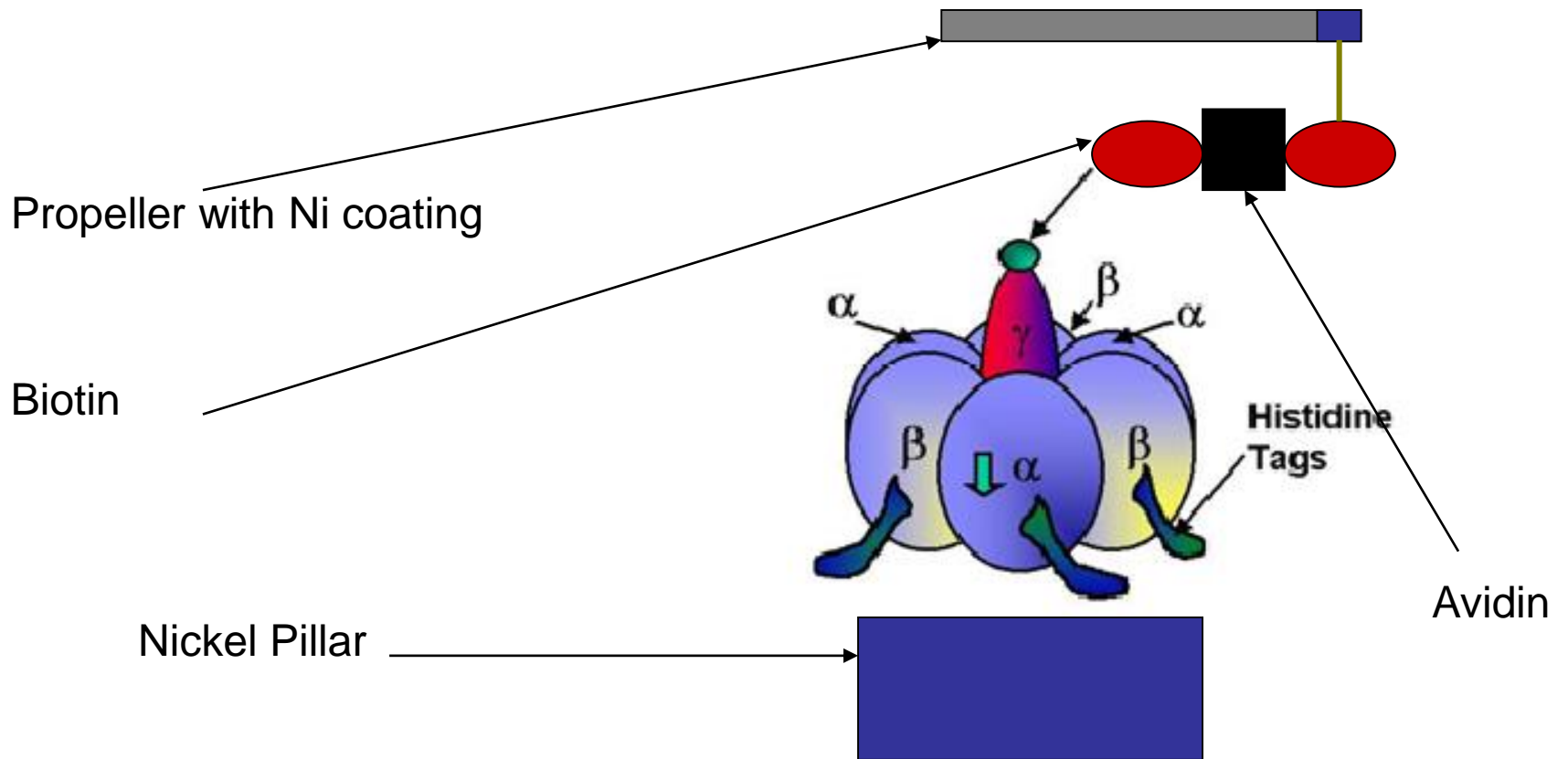
Winners

Amplify

ITERATED CYCLE

Remove Nonspecifics

Keep Activity

Propeller with Ni coating

Biotin

Nickel Pillar

Histidine Tags

Avidin

Peptide synthesis

Understand coupling yields

Example of 20 mer

     1. Starting with 2 micromole of beads

     2. coupling efficiency 97%

Yield = $2*(.97)^{19}$ = 1.12 micromoles