

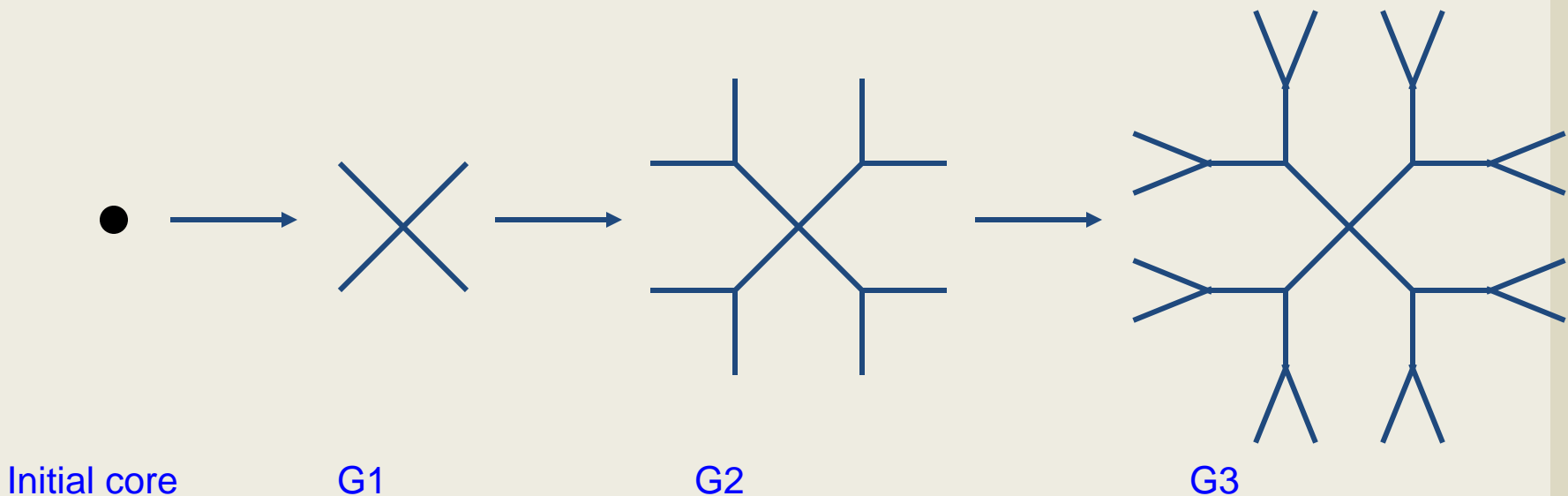
2.1.2.2. Dendrimer, DNA

1. **What Are Dendrimers?**
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 - 1.2. The Structure of Dendrimers
 - 1.3. Examples of Dendrimers
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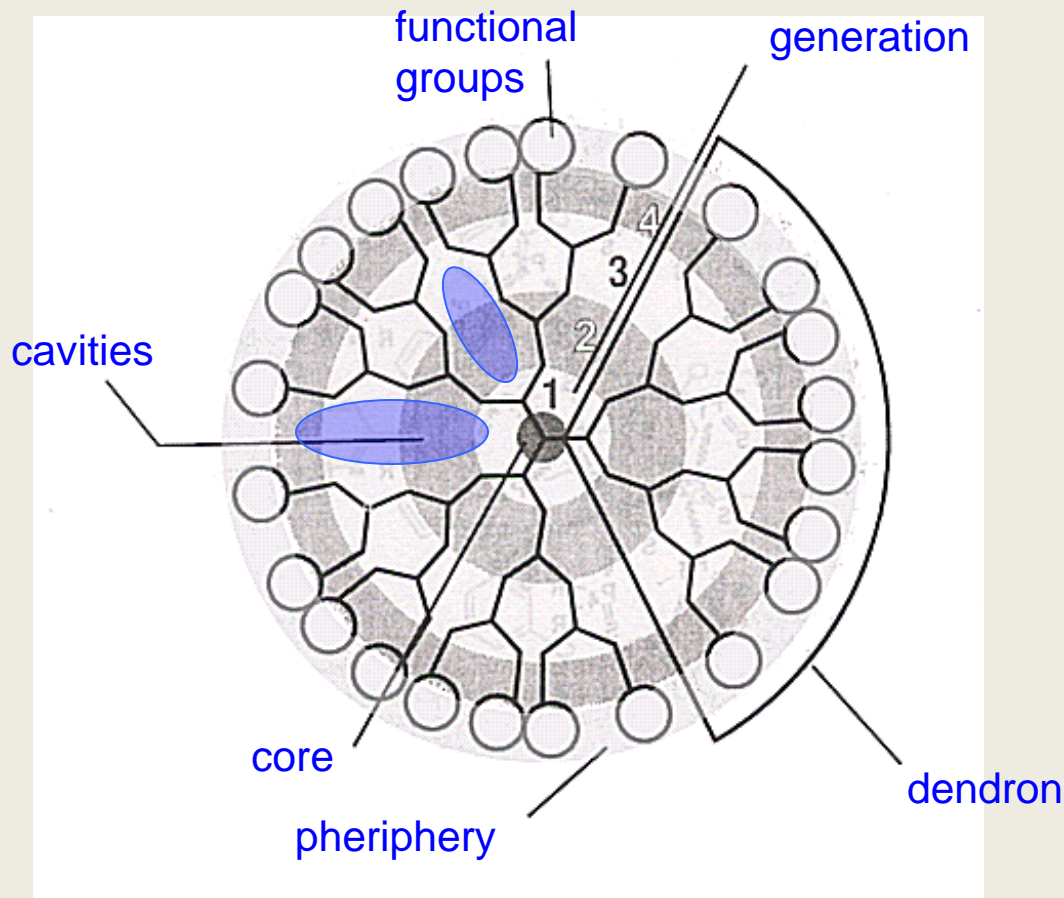


1.1 Definition of Dendrimers

; Evolving around a core atom or molecule, they possess repeating “generations” of branch again and again until an almost globular shape with a dense surface is reached.

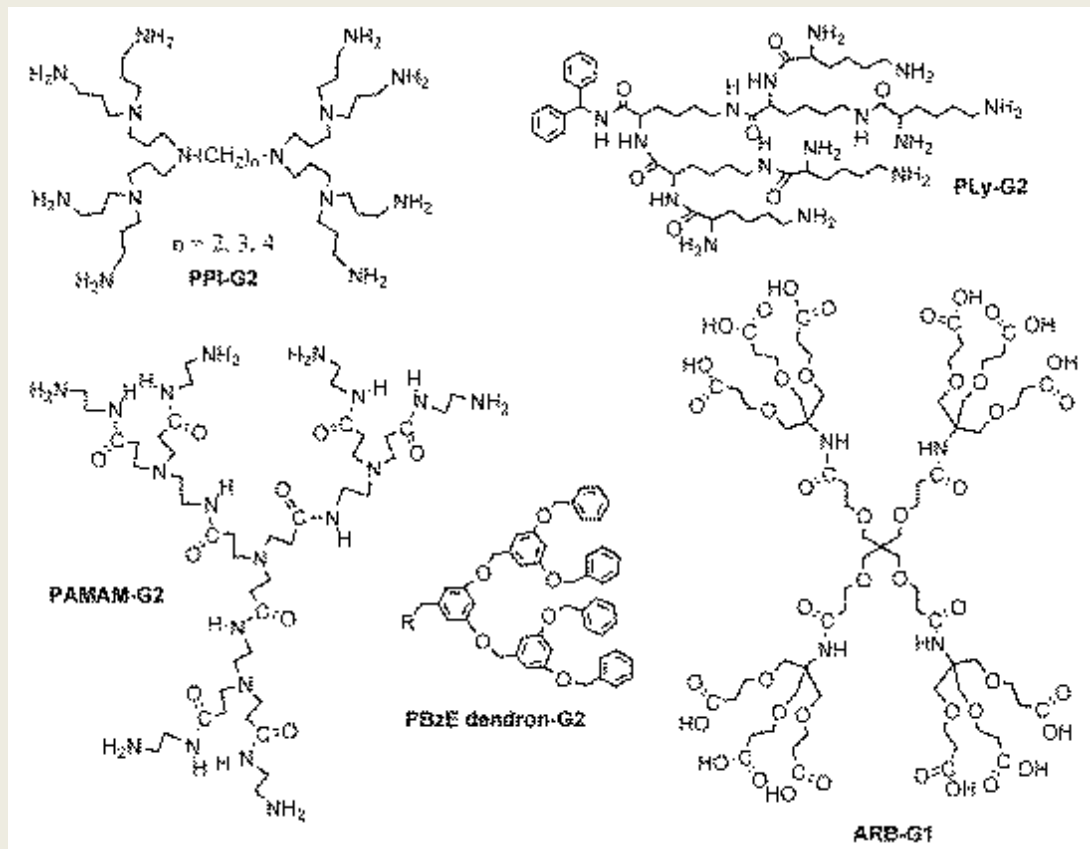


1.2 The Structure of Dendrimers



- ; Nano-sized spherical shape
- ; Low polydispersity
- ; Periphery - polyfunctionality
- ; Cavity (vacant space)
- possible to use as drug carrier

1.3 Examples of dendrimers



PPI, Poly(propyleneimine)

Ply, Polylysine

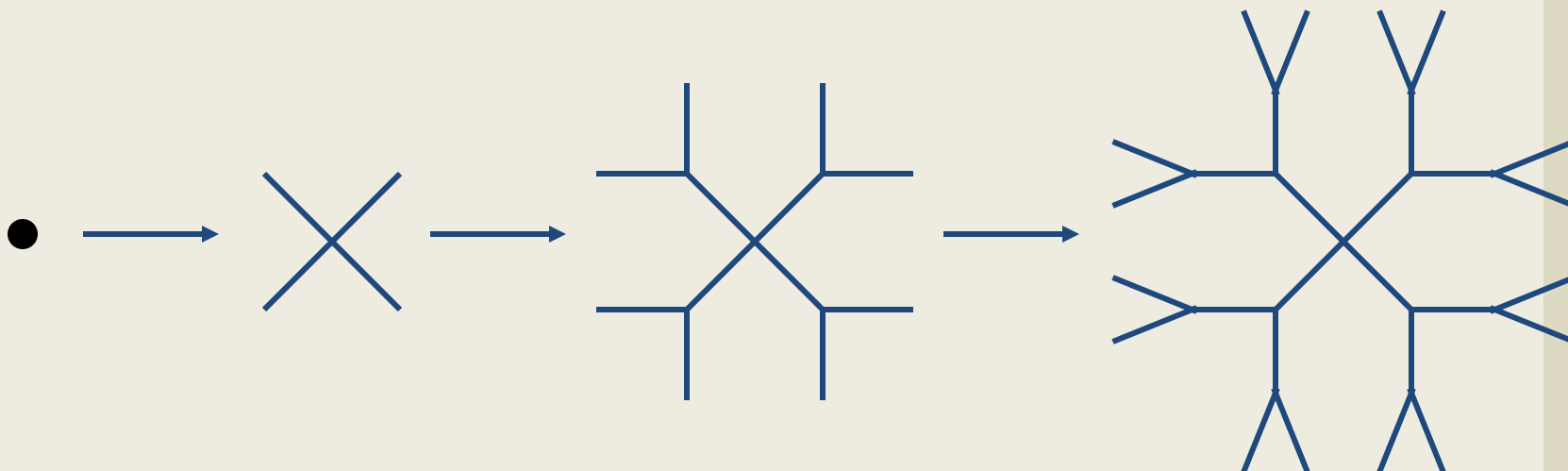
PAMAM, poly(amidoamine)

ARB, arborol

PBzE, poly(benzyl ether)

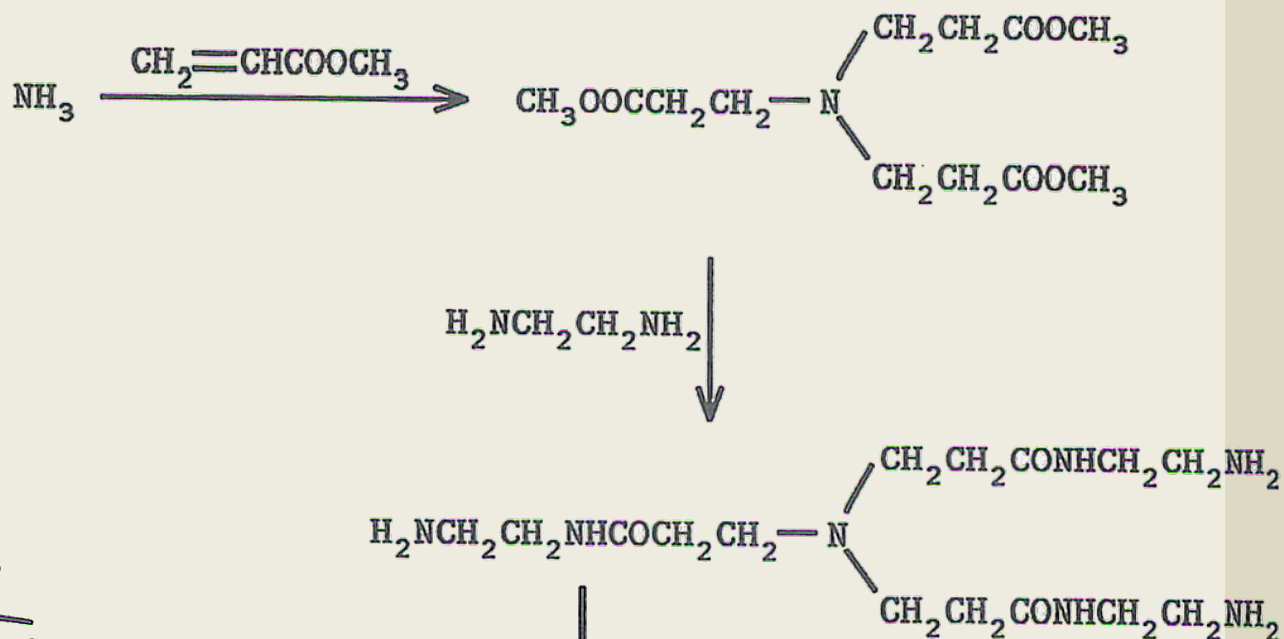
2.1 Divergent Approach (core to periphery)

; The divergent method starts with a core molecule and builds outward by attaching to the core successive layers (or generations) of monomer units one at a time.



2. Dendrimer Construction Strategies

✓ Divergent synthesis of PAMAM dendrimer



G5

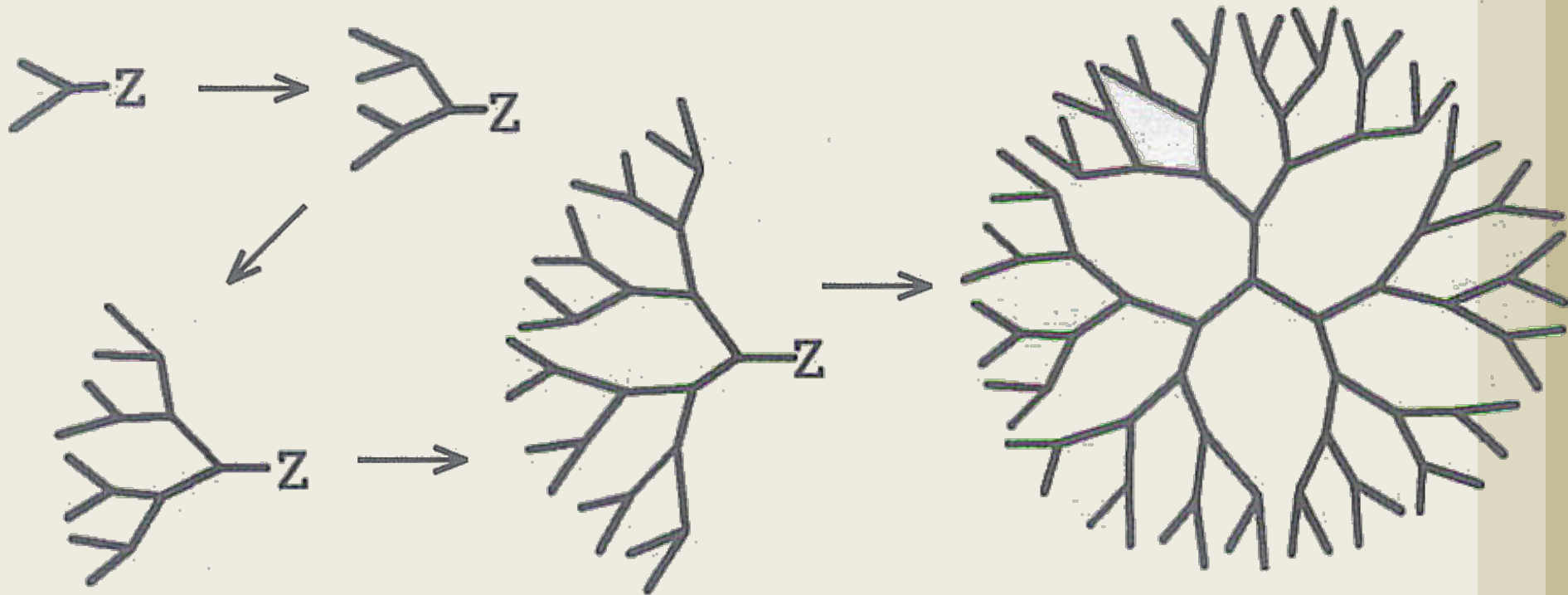
Repetitive sequencing
of MA and EDA

Ref. "Principle of Polymerization" 4th Ed, Hoboken, N.J. : Wiley, 2004



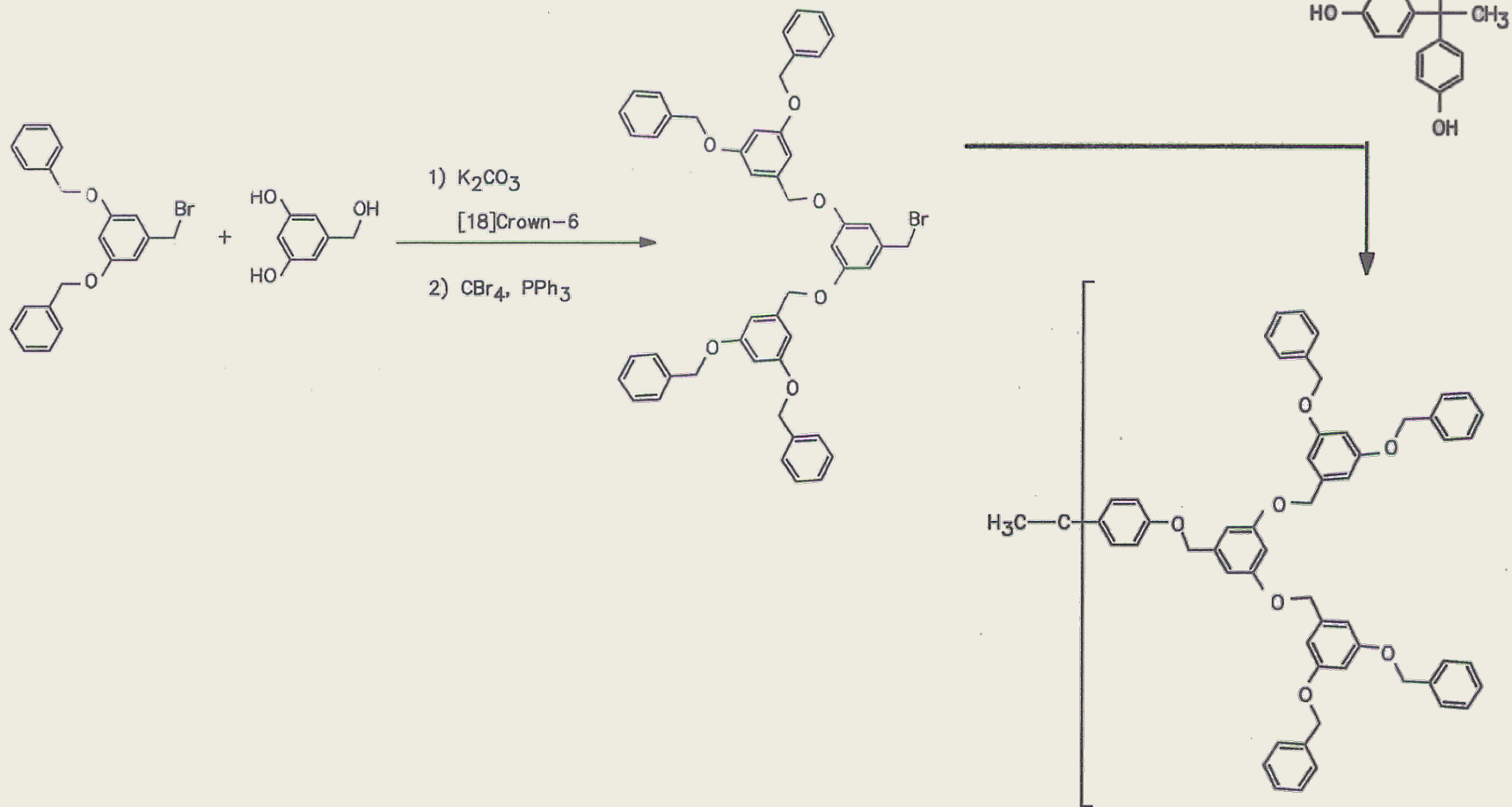
2.2 Convergent Approach

; Dendrimeric fragments are synthesized by repetitive reactions, and then several are joined together in the last step by using a central core molecule to form the dendrimer.



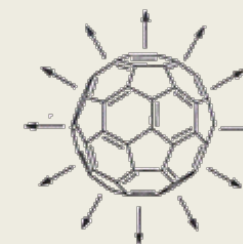
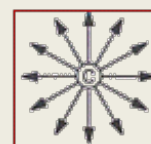
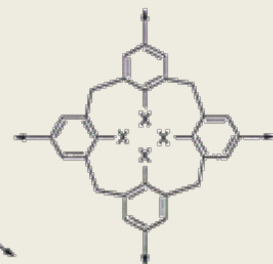
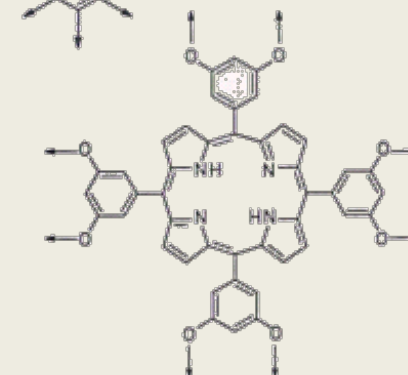
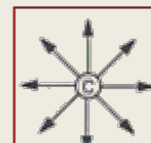
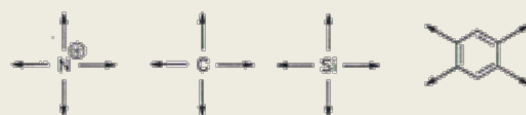
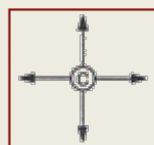
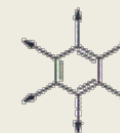
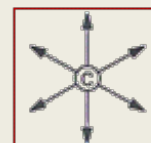
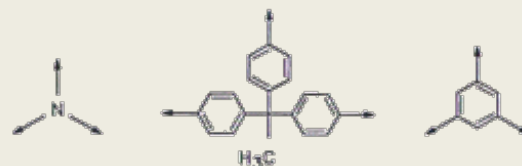
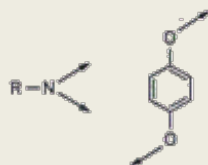
2. Dendrimer Construction Strategies

✓ Convergent routine by building up poly(phenyl ether) dendrons



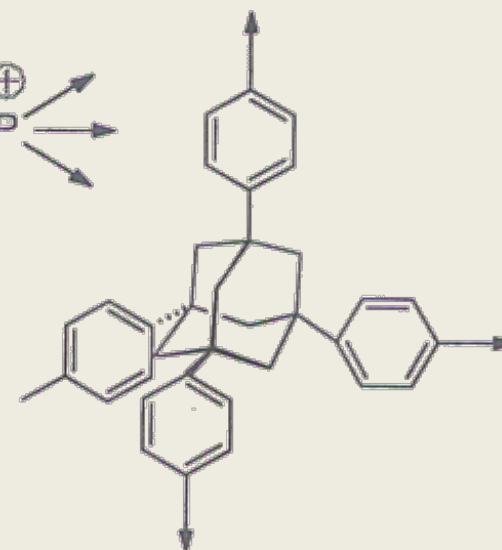
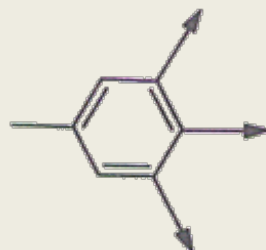
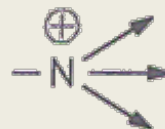
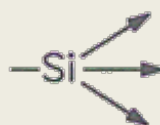
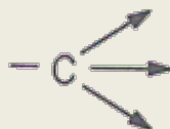
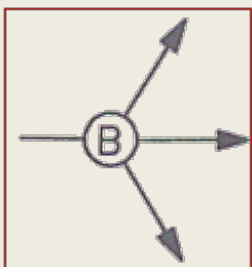
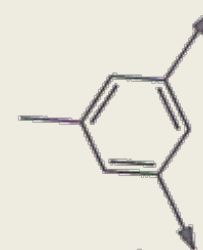
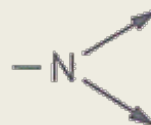
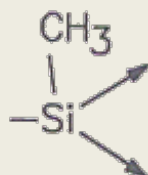
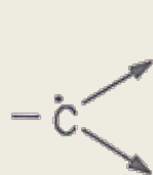
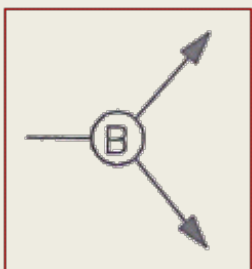
2.3 Cores & Branching units for building up the dendrimer

✓ Cores

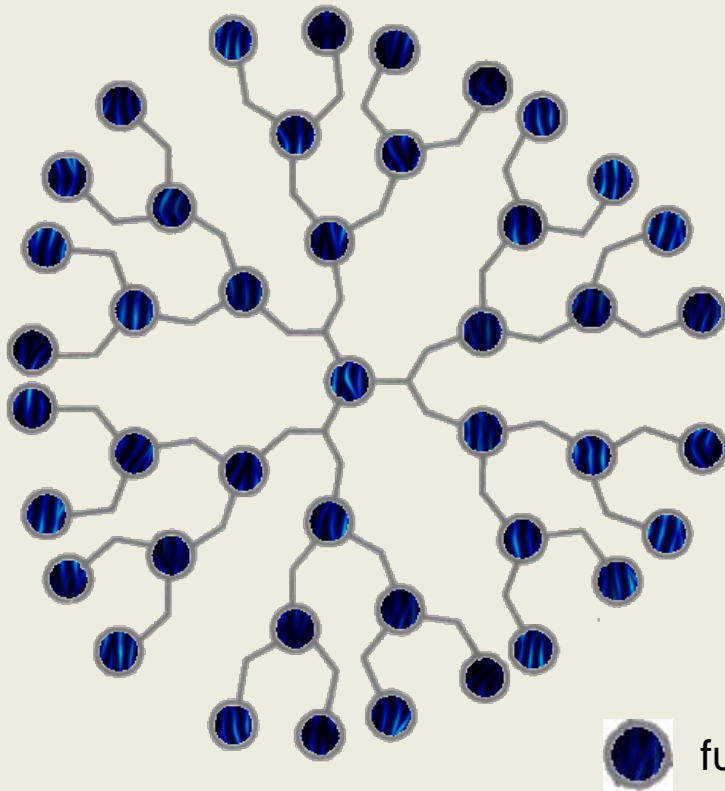


2. Dendrimer Construction Strategies

✓ Branching units



3.1 Functionalized Dendrimers

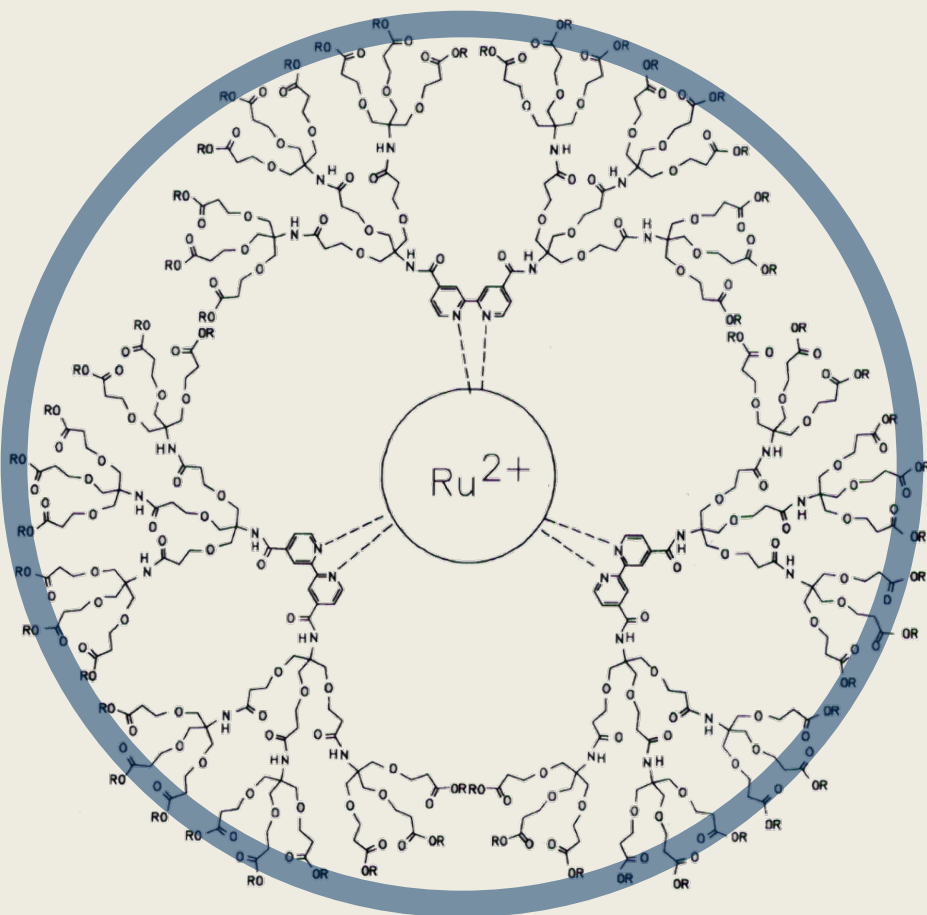


Core, branches, periphery

- ; Introduction of functional group to dendrimers
- ; The kind of sites in dendrimer molecules can be functionalized.
→ core, branch, periphery
- ; A lot of functional groups can be introduced to dendrimer molecules.

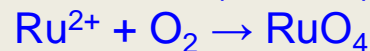
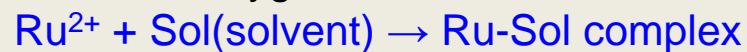
3. The Ideas for Finding Applications of Dendrimers

✓ Metal-functionalized dendrimers



; Ru²⁺ can be applied in medical diagnostics.

; Ru²⁺ can be quenched by solvent or with dissolved oxygen.



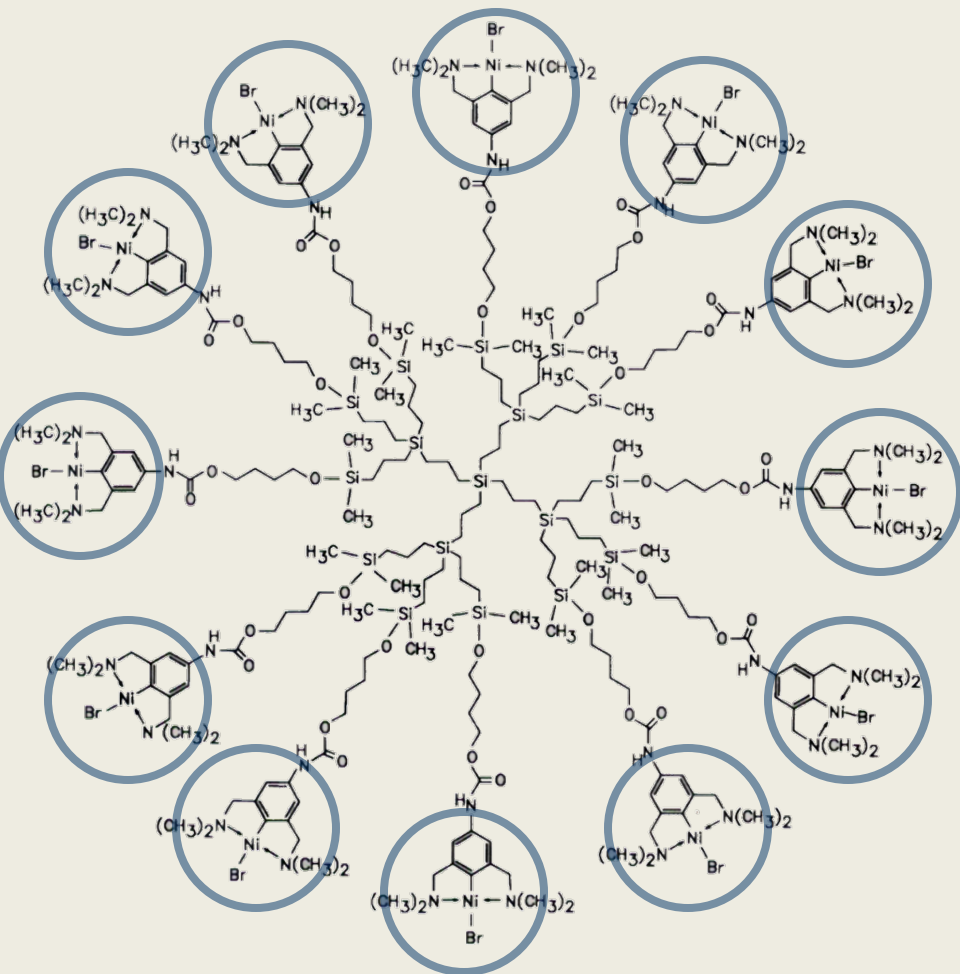
; Dendrons can serve as shields for Ru²⁺ (Shielding effect)

→ Prevent quenching processes with the solvent or with dissolved oxygen

→ Increase of lifetime of the excited state of Ru²⁺

3. The Ideas for Finding Applications of Dendrimers

✓ Metal-functionalized dendrimers



; periphery-functionalized dendrimer by aryl nickel (catalyst)

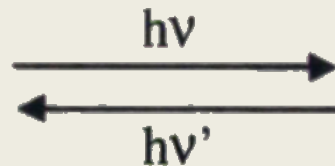
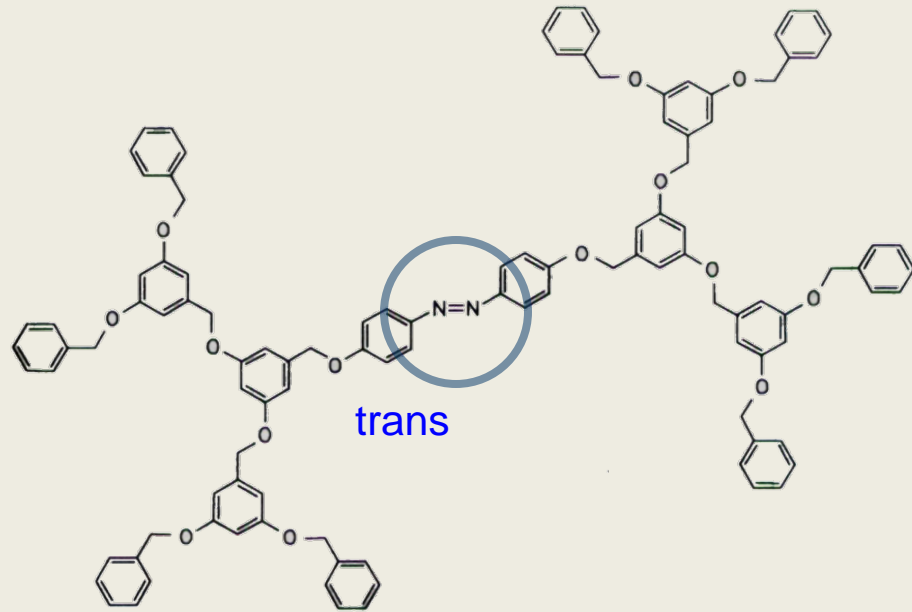
; In a molecule, a lot of aryl nickels are contained.

→ can be used for new kind of catalyst
→ “Dendralyst”

; As varied the kind of metal, different electrochemical properties can be introduced to dendrimers.

3. The Ideas for Finding Applications of Dendrimers

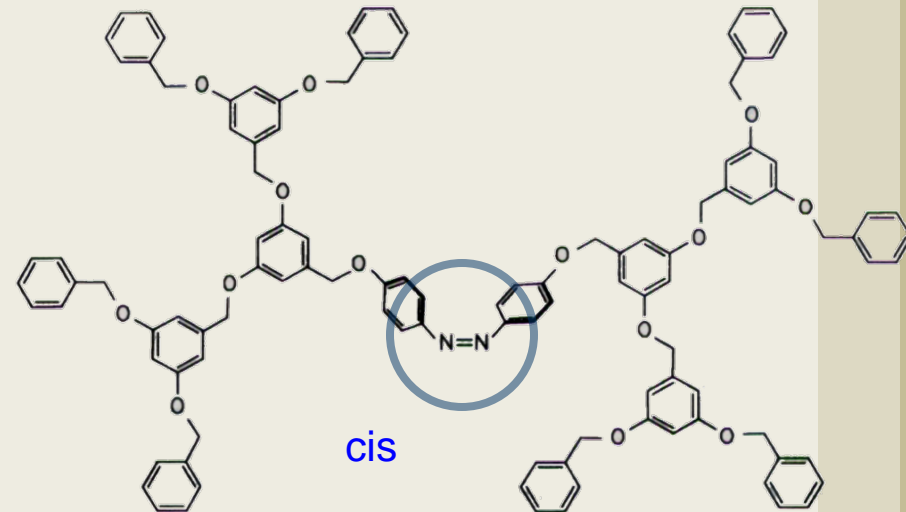
✓ Photoactive azobenzene dendrimers



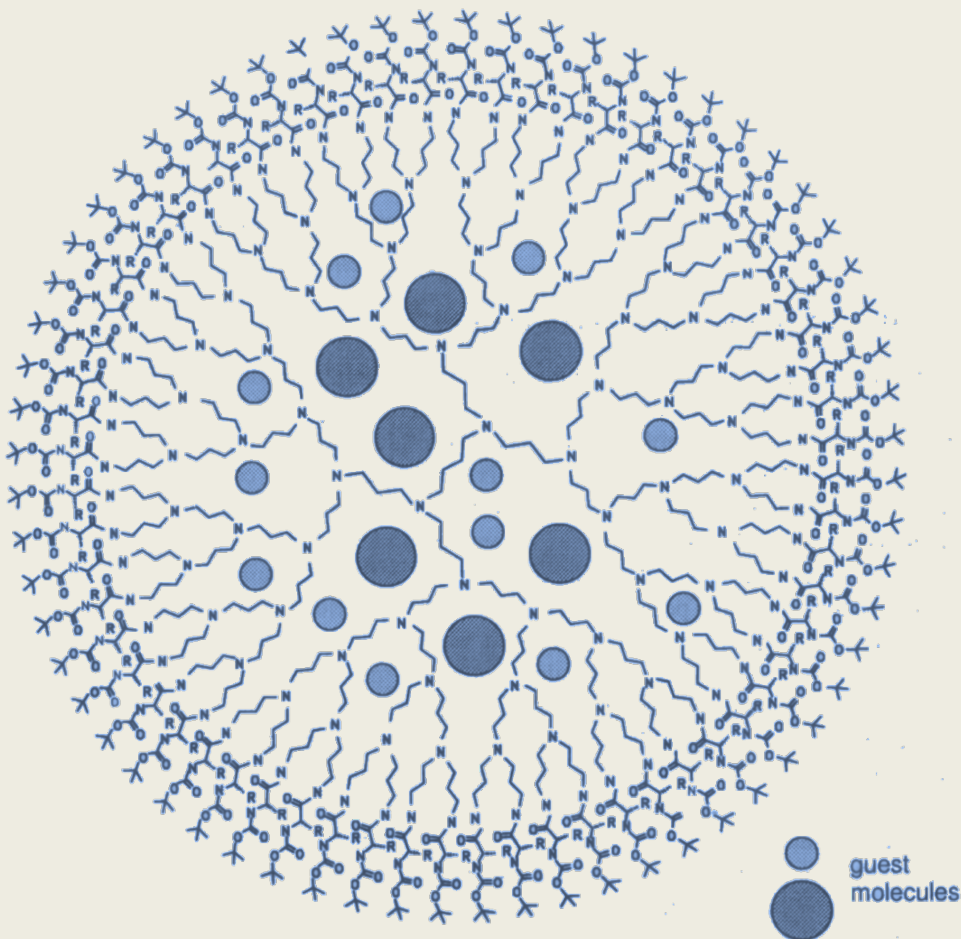
; core-functionalized by azo compound

; Azo-type compounds can be easily and reversibly photoisomerized.

→ Photoresponsive molecule



3.2 Host-Guest Chemistry with Dendrimers

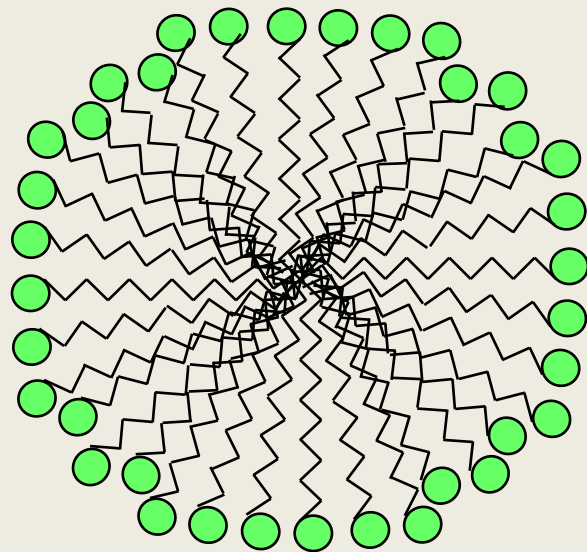
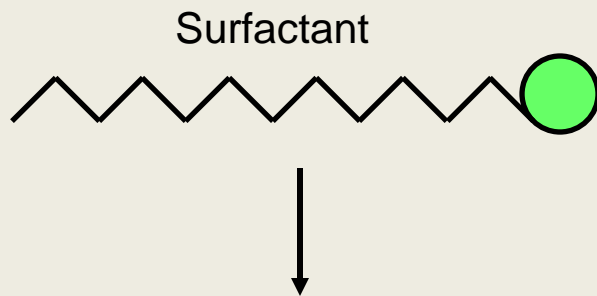


15th-generation polyamine dendrimer

; Dendrimers have large cavities.

- possible to contain a lot of guest molecules
- release guest molecules size selectively upon hydrolysis of the dense outer shell

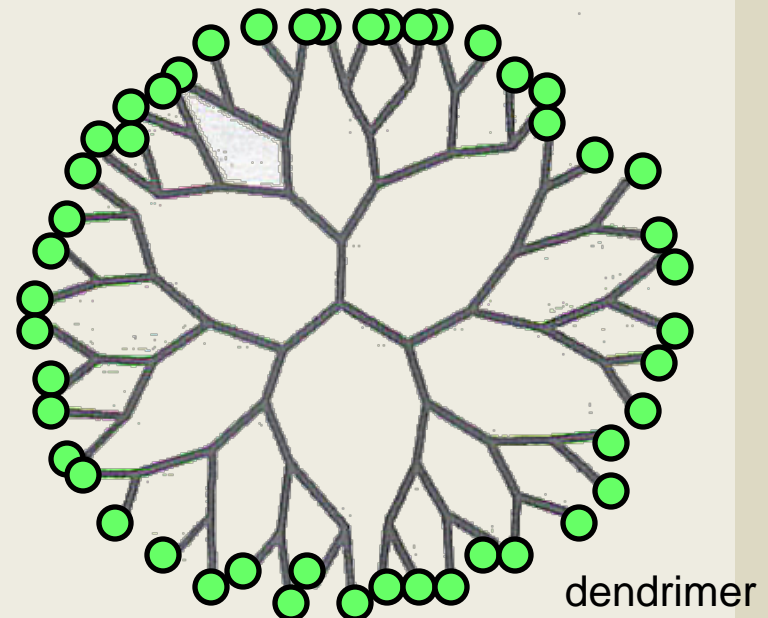
3.3 Dendritic Micelles



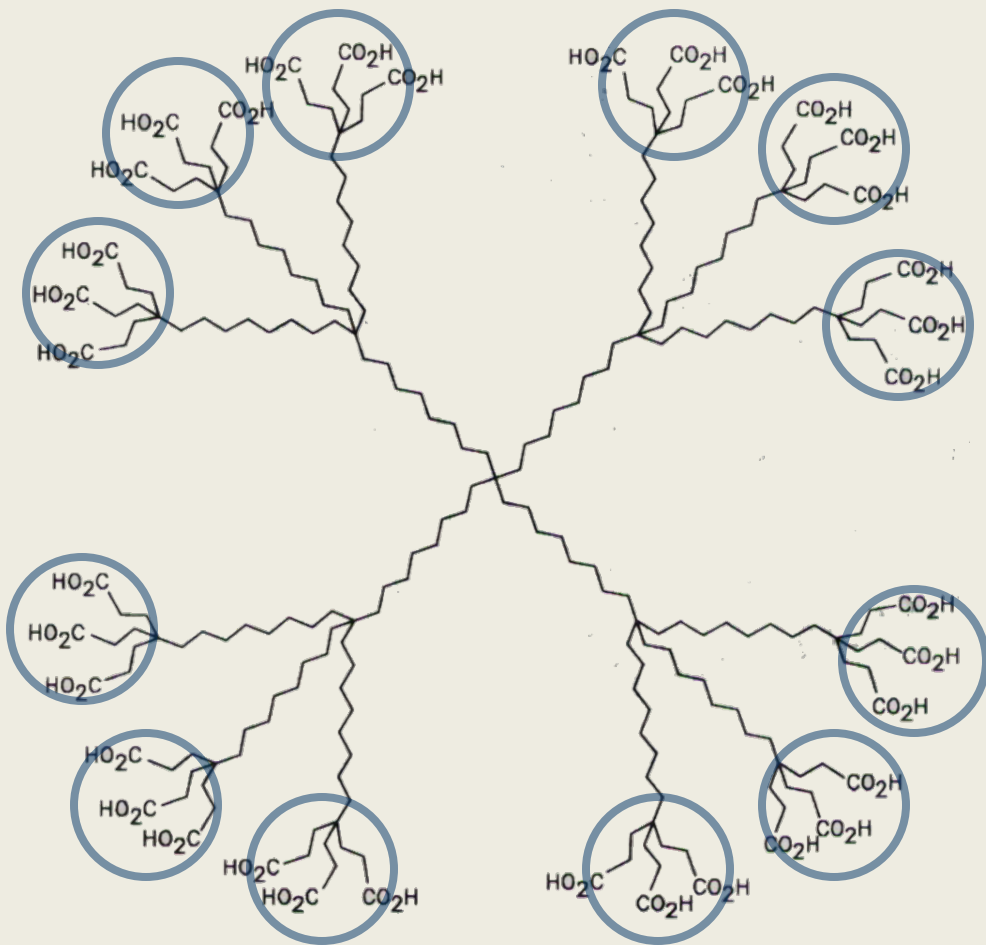
; The molecular structure of dendrimer are similar to micelle formed by surfactant.

; Dendrimers can be a “unimolecular micelle”
→ Not to require micellization condition

≡



3. The Ideas for Finding Applications of Dendrimers

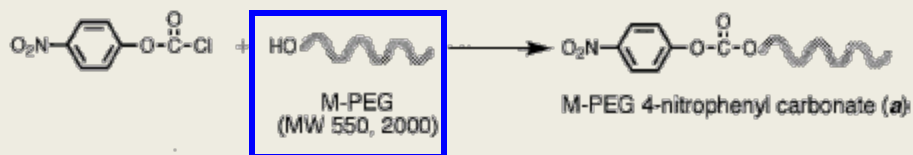


; branching unit – aliphatic chain
(hydrophobic)
terminal groups – carboxylic acid
(hydrophilic)

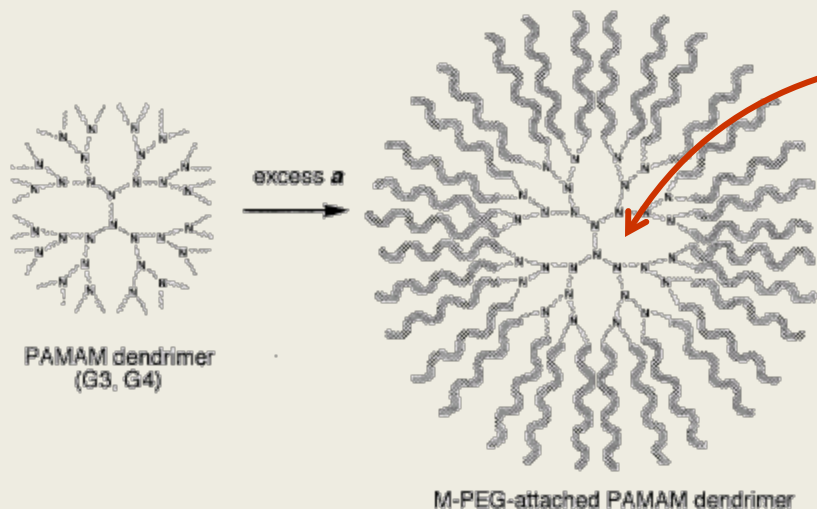
→ “unimolecular micelles”

3.4 Dendrimers in Drug Delivery Systems

; The studies on DDS by using dendrimers are based on host-guest chemistry and dendritic micelle concept.



Biocompatible



(A) ADR (Adriamycin)

CC12C(=O)C3=C(O)C(=O)C=C(C3)C(O)C1O[C@H]2[C@@H](O)C[C@@H](O)CO

(B) MTX (Methotrexate)

NC1=CC=C(C=C1)N2C=NC(=C2)C=C(N)C=C2C(=O)N(C)C2=CC=C(C=C2)C(=O)N[C@@H](C)C(=O)O

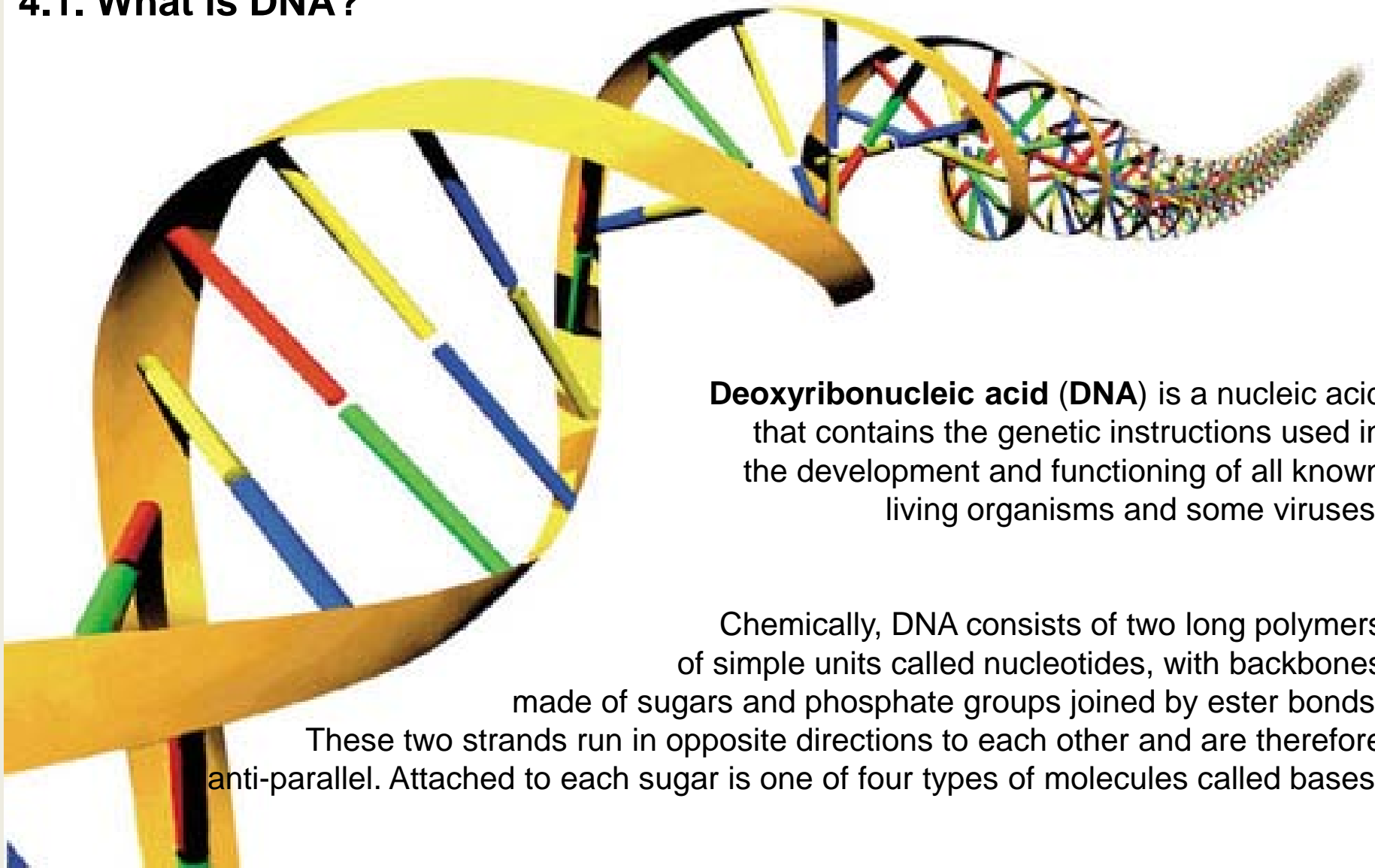
Anticancer drugs

→ These Dendrimers having an interior for encapsulation of drugs and a biocompatible surface

Ref. Kojima et al. *Bioconjugate Chem*, **11** (2000) 910



4.1. What is DNA?



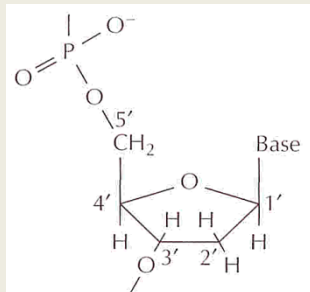
Deoxyribonucleic acid (DNA) is a nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms and some viruses.

Chemically, DNA consists of two long polymers of simple units called nucleotides, with backbones made of sugars and phosphate groups joined by ester bonds. These two strands run in opposite directions to each other and are therefore anti-parallel. Attached to each sugar is one of four types of molecules called bases.

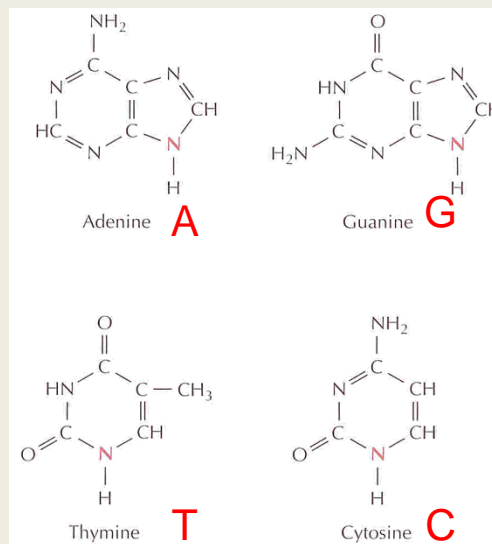


4.2. Structure of DNA

- Nucleotide (deoxyribonucleotide)
 - the basic unit of a DNA strand
 - A deoxyribonucleotide has three components: a pentose sugar or deoxyribose, a phosphate group, and one of four nitrogen-containing bases.
 - An organic base attached to the 1' carbon of the doxyribose sugar of the nucleotide.
 - A phosphate group attached to the 5' carbon of the sugar moiety.

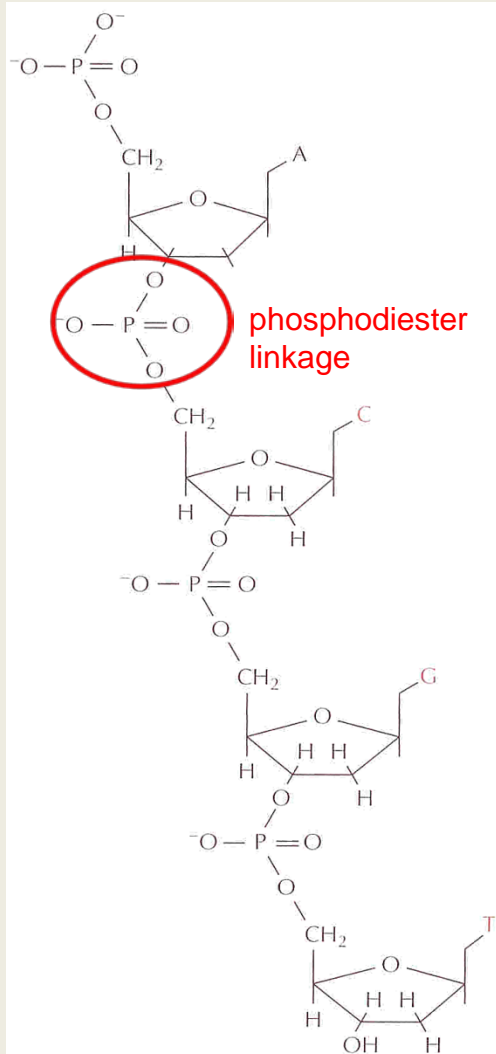


Nucleotide



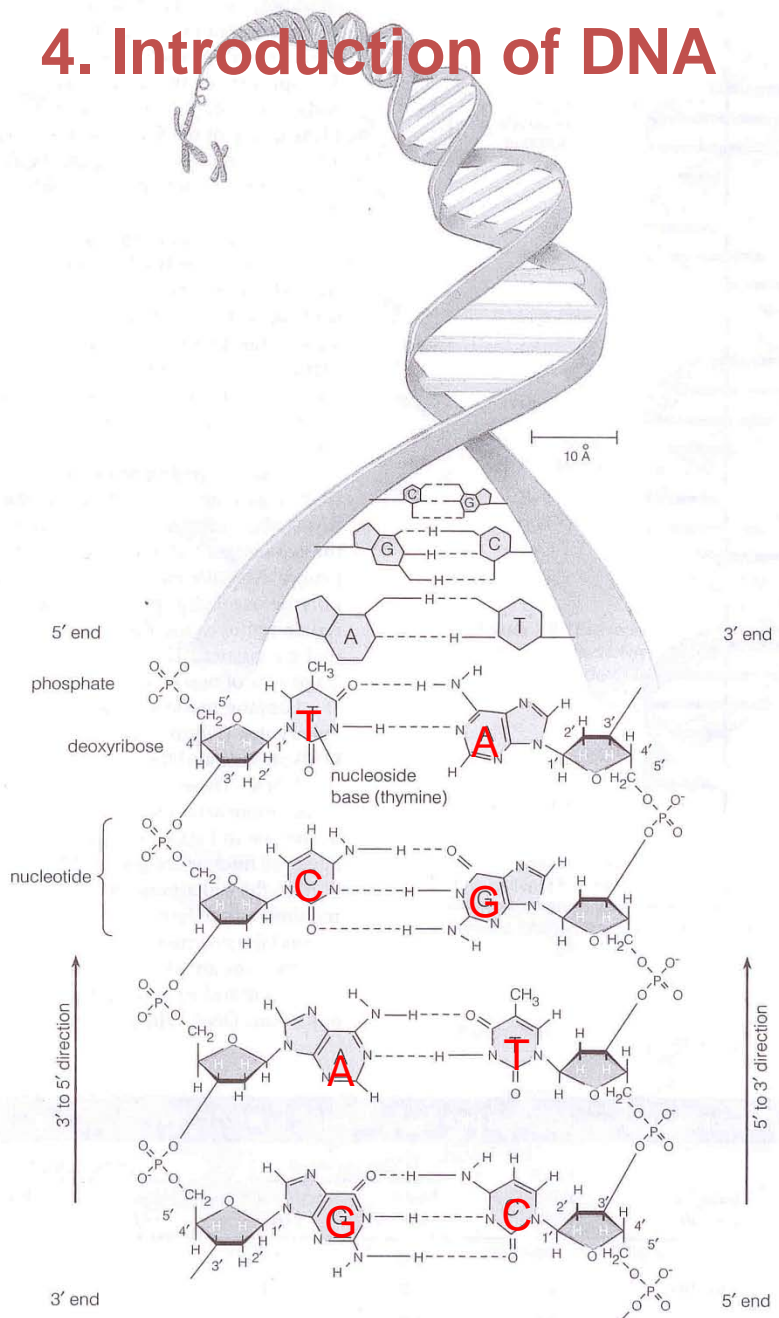
Four bases of a DNA molecules

4.2. Structure of DNA

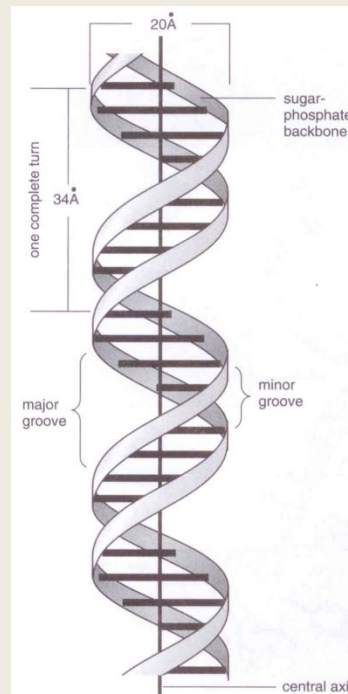


- DNA (Deoxyribonucleic acid)
 - a long polymer consisting of repeating units called deoxyribonucleotides
 - The nucleotide of a DNA chain are linked by phosphodiester bonds between the 5'-phosphate group of one nucleotide and the 3'-hydroxyl group of another nucleotide.
 - A DNA strand may be made up of many thousands of nucleotides.

4. Introduction of DNA



- Double-stranded molecule (Double helix)
 - Hydrogen bonding between opposite bases holds the two strands of the DNA molecule together.
(A ↔ T, G ↔ C)
 - For hydrogen bonding of the bases, the strands must be antiparallel to one

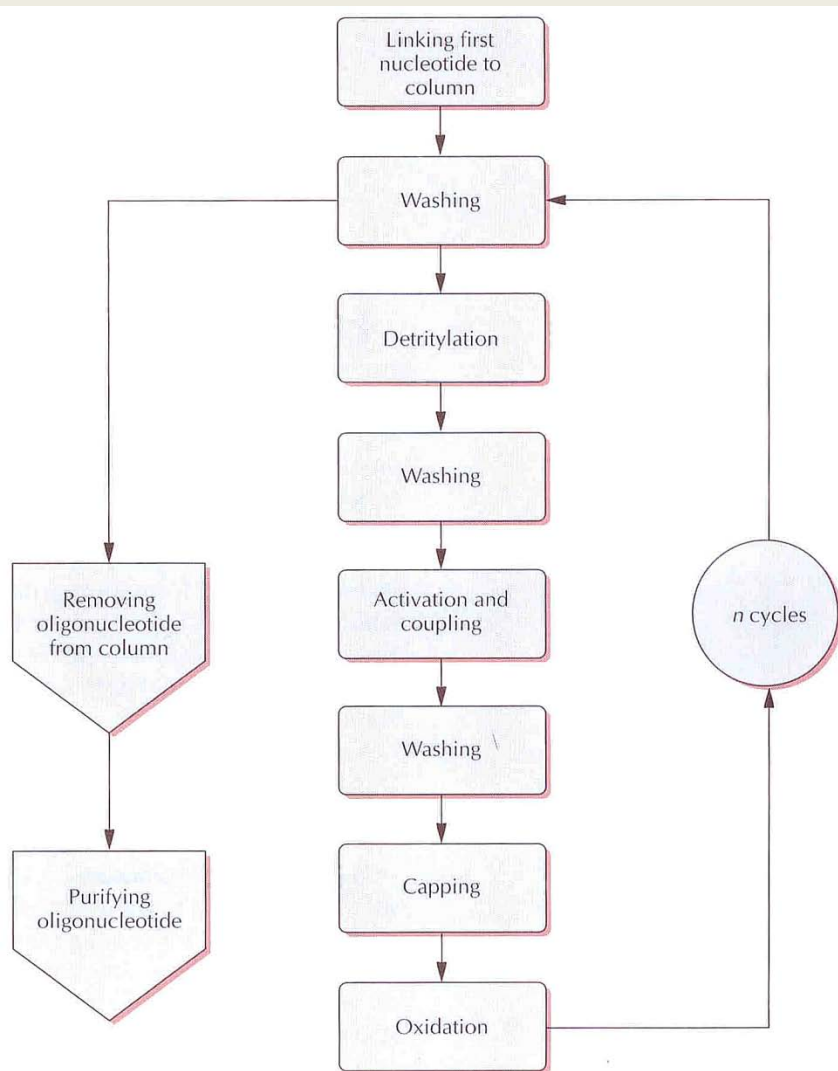


another so that one strand goes from 5' to 3' and the other 3' to 5'.

- The two strands do not spontaneously separate under physiological conditions because the many hydrogen bonds keep the base pairs together.

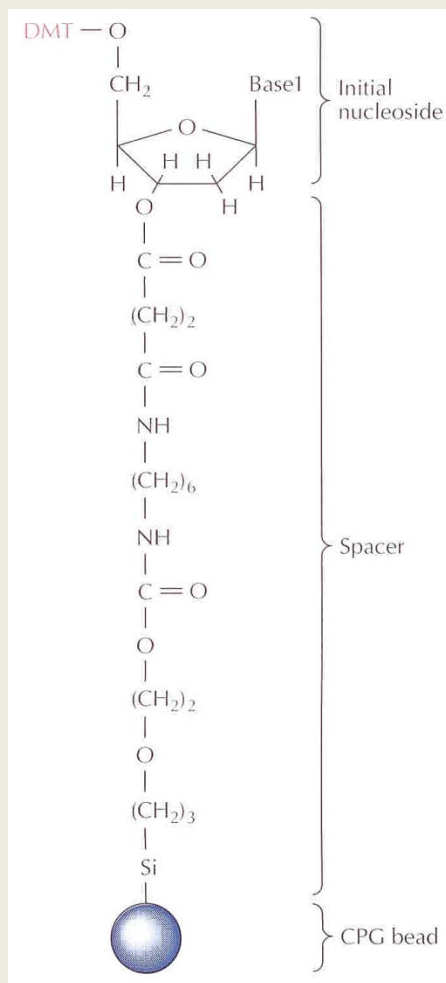


4.3. Chemical synthesis of DNA



Flow chart for phosphoramidite method

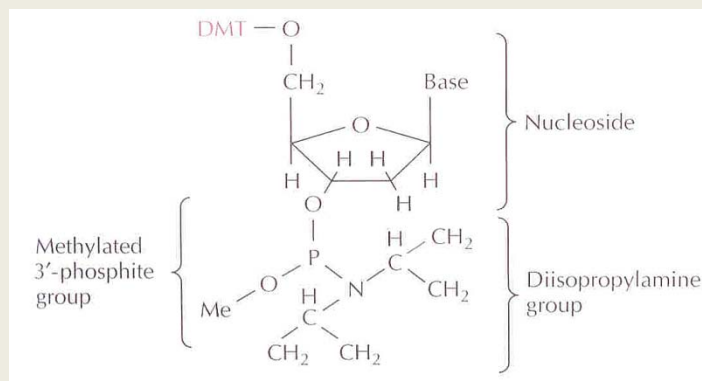
- ❑ Phosphoramidite method
 - ❑ Chemical synthesis of DNA oligonucleotides
 - ❑ Solid-phase synthesis; attachment of the growing group DNA strand to a solid support
 - ❑ The multistep synthesis can be conducted in one reaction vessel.

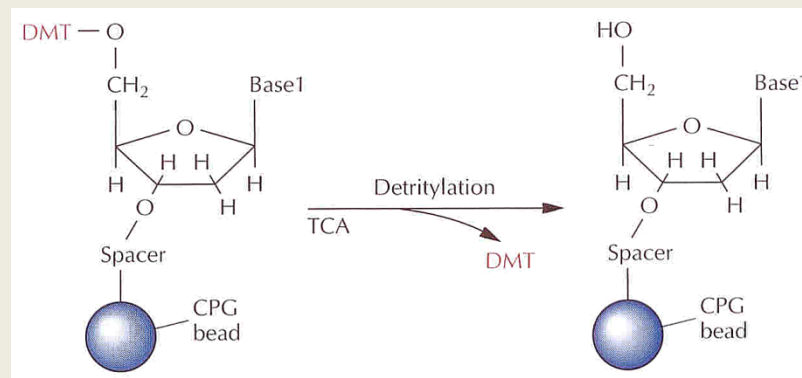


- Linking first nucleotide to column (1st step)
 - The initial nucleoside is attached to an inert solid support (controlled pore glass, CPG).
 - A dimethoxytrityl (DMT) group has been attached to the 5'-terminus of the initial nucleoside to prevent 5'-hydroxyl group from reacting nonspecifically, prior to the addition of the second nucleotide.

※ Phosphoramidite

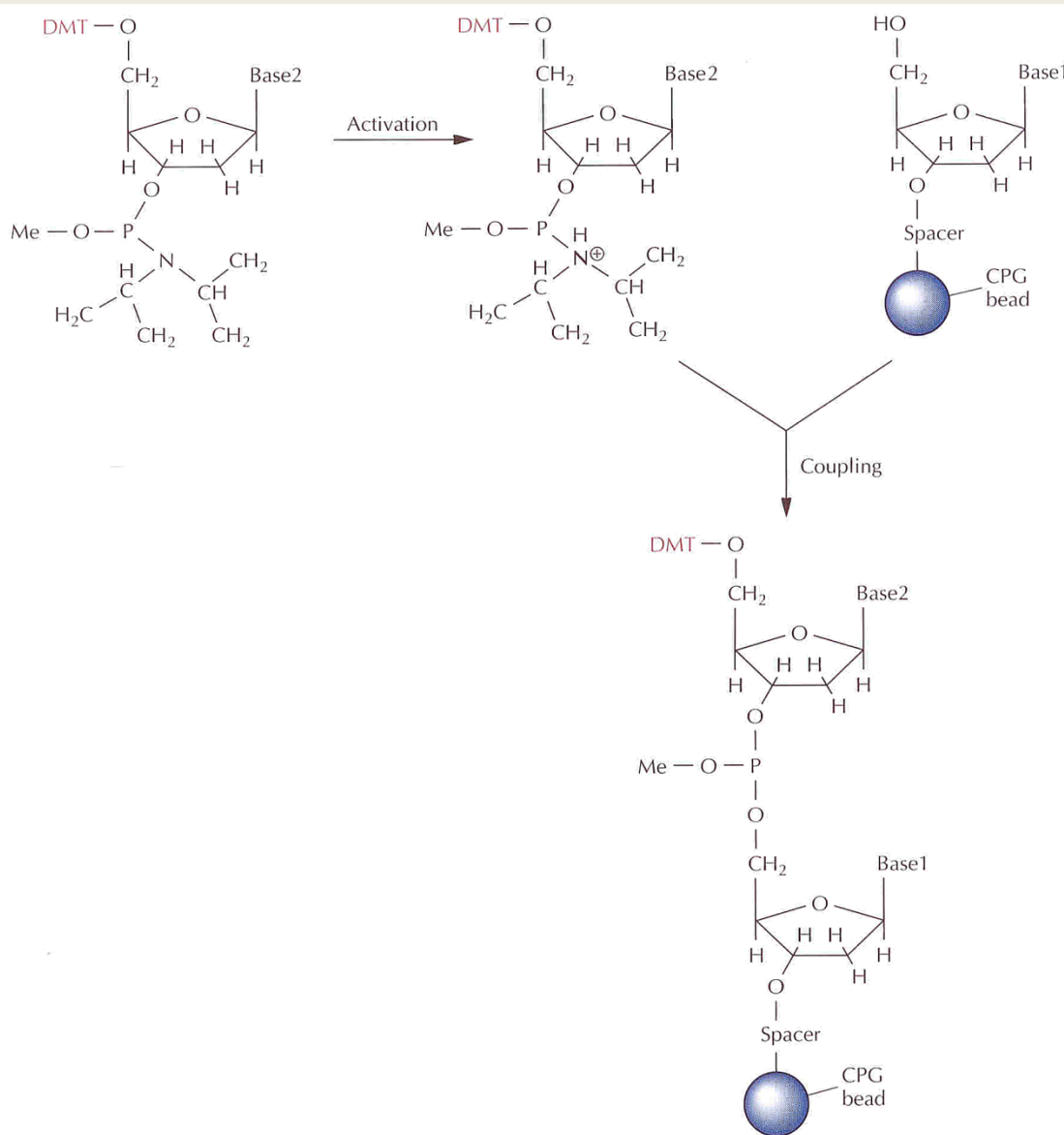
- A methyl group protects the 3'-phosphite, and DMT group is on 5'-hydroxyl group of the deoxyribose sugar.





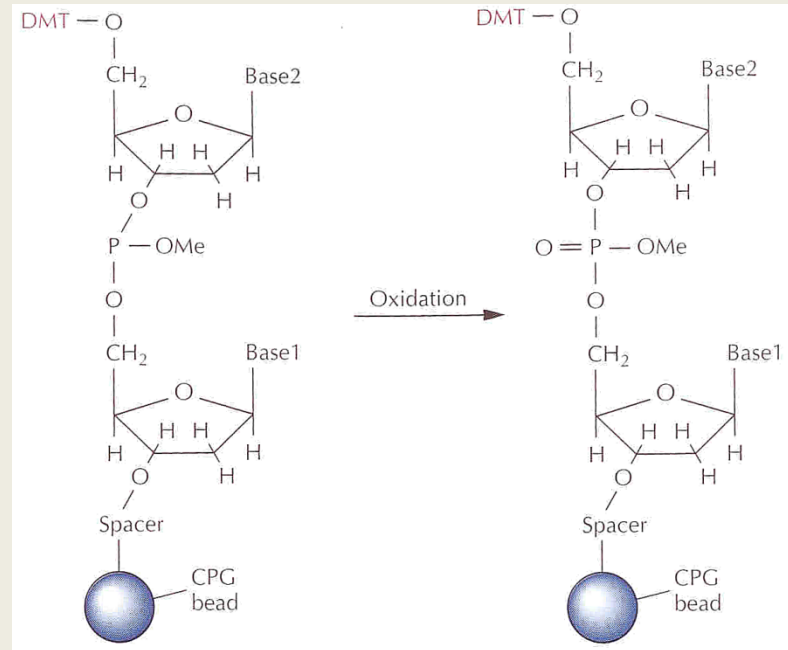
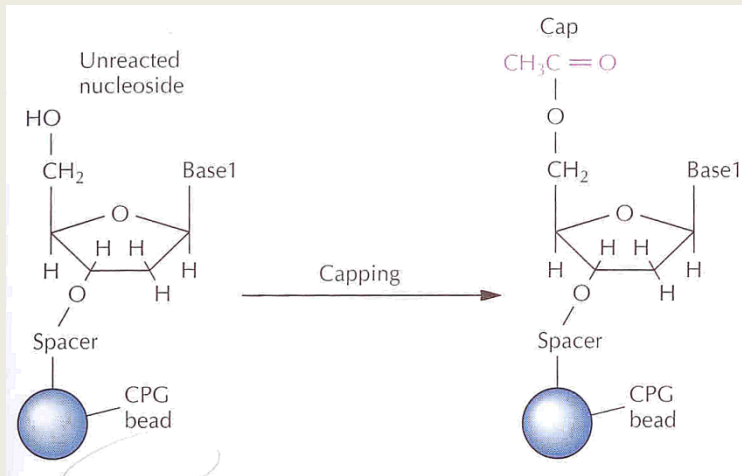
- ❑ Washing (After all steps)
 - ❑ Washing with anhydrous reagent to remove water and any nucleophiles
 - ❑ Flushing with Ar to purge anhydrous reagent
- ❑ Detritylation (2nd step)
 - ❑ 5'-DMT group is removed from the attached nucleoside by treatment with trichloroacetic acid(TCA) to yield a reactive 5'-hydroxyl group

4. Introduction of DNA



- Activation and Coupling (3rd step)
 - Addition of tetrazole: activation of a phosphoramidite
 - 3'-phosphite forms covalent bond with the 5'-hydroxyl group of initial nucleoside.

4. Introduction of DNA



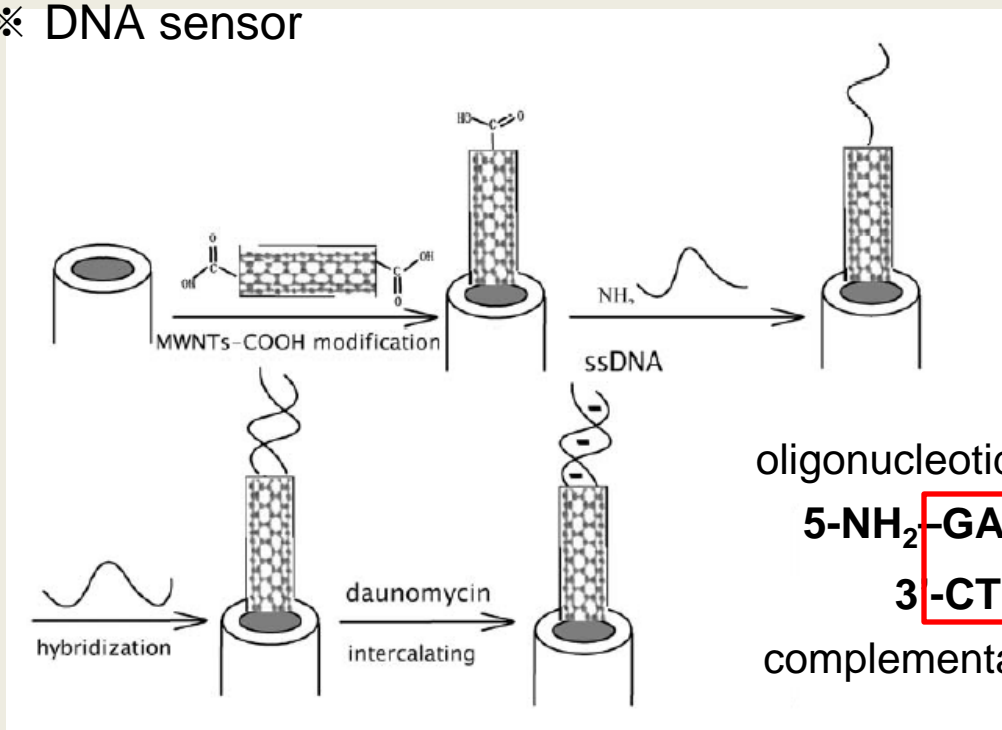
- ❑ Capping (4th step)
 - ❑ The unlinked residues must be prevented from linking to the next nucleotide during the following cycle.
 - ❑ Acetylation of the unreacted 5'-hydroxyl groups.
- ❑ Oxidation (5th step)
 - ❑ The internucleotide linkage is in the form of a phosphite triester bond, which is unstable and prone to breakage in presence of either acid or base.
 - ❑ The phosphite triester is oxidized with an iodine mixture to form the more stable pentavalent phosphate triester.



4.4. DNA hybridization

- DNA hybridization
 - The pairing of two DNA molecules often from different sources
 - Driving force: hydrogen bonding between nucleotides.

※ DNA sensor



DNA hybridization
(Hydrogen bonding: A ↔ T, G ↔ C)

oligonucleotide probe

5-NH₂-GAGCGGCGCAACATTTTCAGGTCGA-3'

3'-CTCGCCGCGTTGTAAAGTCCAGCT-5'

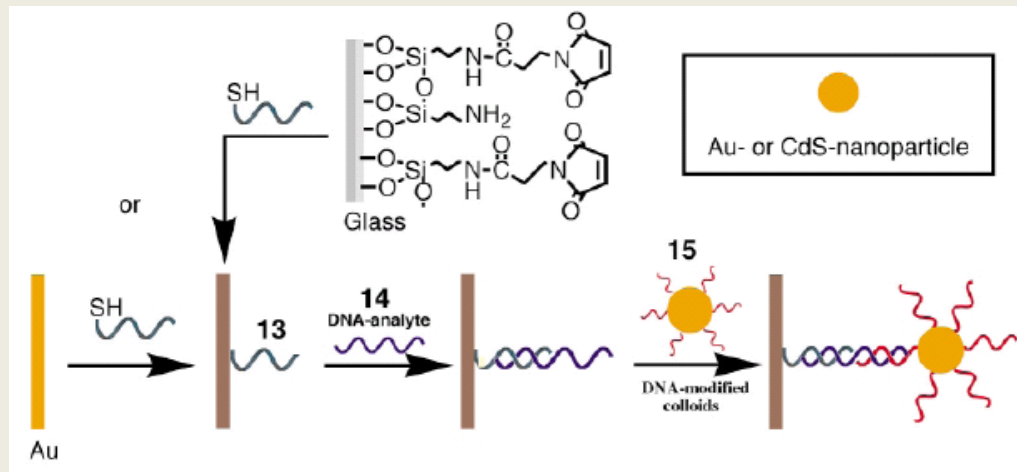
complementary target oligonucleotides

Anal. Bioanal. Chem. 2003, 375, 287-293

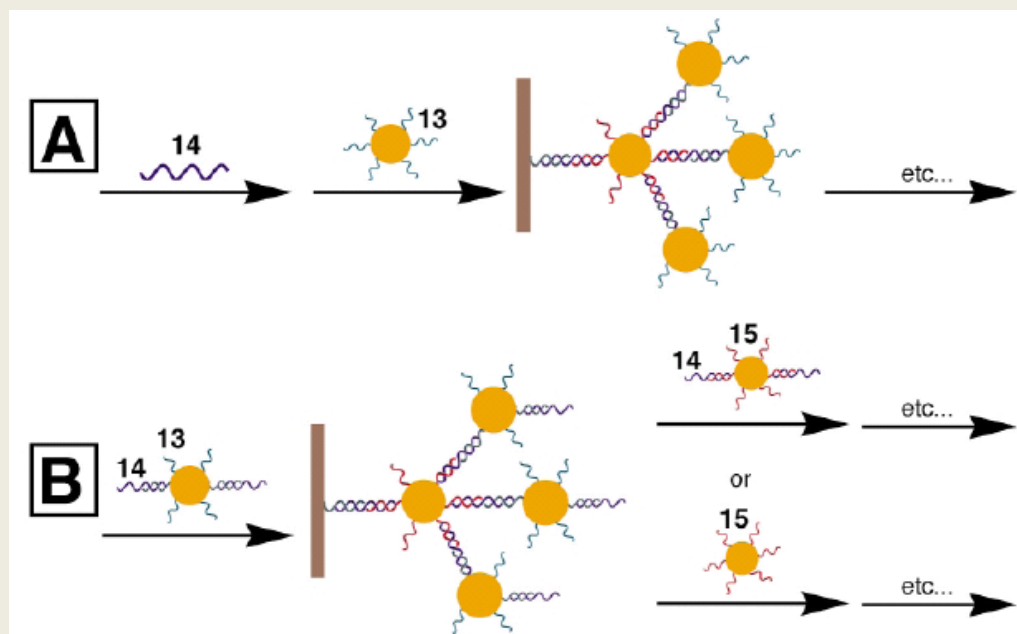


4. Introduction of DNA

※ DNA-linked nanoparticle superstructure



- DNA is a promising construction material for artificial nano-structured devices.
- One of attractive features of DNA is the great mechanical rigidity of short double helices, so that they behave effectively like a rigid rod spacer.



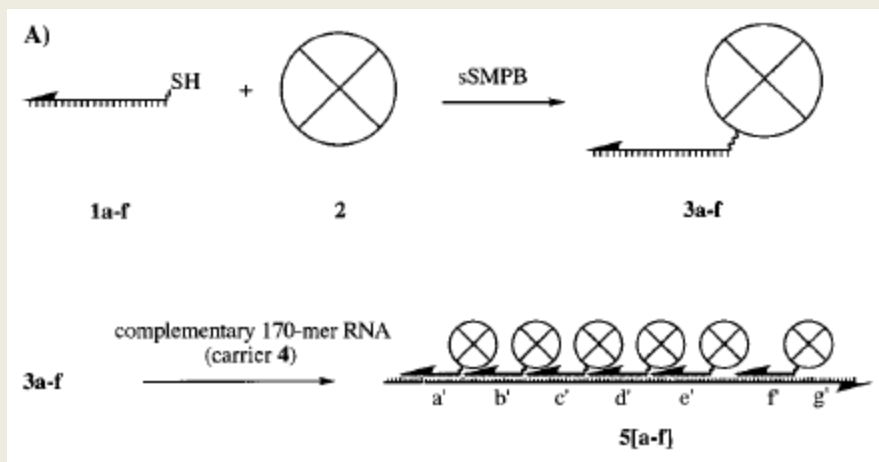
Example oligonucleotides:
13 = 5'-TCTATCCTACGCT-(CH₂)₆-SH-3'
14 = 5'-AGCGTAGGATAGATATACGGTTCCGCGC-3'
15 = 5'-HS-(CH₂)₆-GCGCGAACCGTATA-3'

Chem. Commun. 2001, 2035-2045



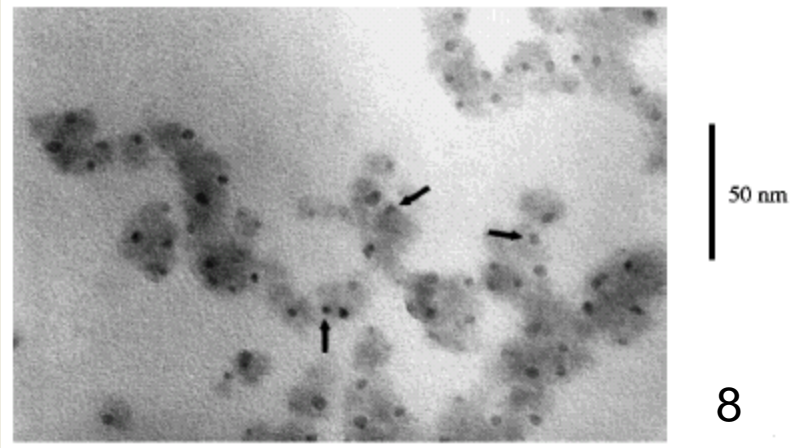
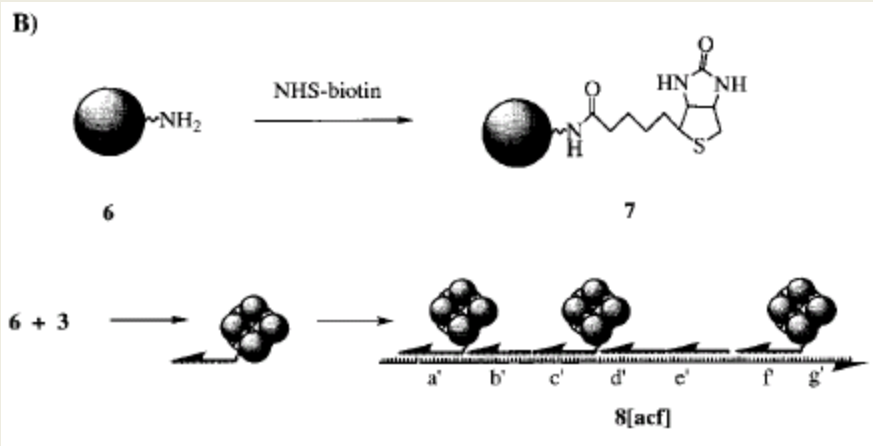
5.1. DNA-directed assembly of proteins

- ❑ Covalent DNA-Streptavidin(STV) Conjugates
 - ❑ STV's native binding capacity for four biotin molecules is supplemented by a highly specific binding site for complementary nucleic acids.
 - ❑ The conjugates can be utilized as biomolecular adapters for positioning biotinylated components along a nucleic acid backbone.



- 1: 5'-thiol-modified oligonucleotides
- 2: streptavidin(STV)
- 3: DNA-STV conjugates
- 4: RNA(5'-a'-b'-c'-d'-e'-f'-g'-3')
- 5: supramolecular aggregates

5. Self-assembled nanostructures based on DNA



6: Monoamino-modified gold cluster

7: biotinylated cluster

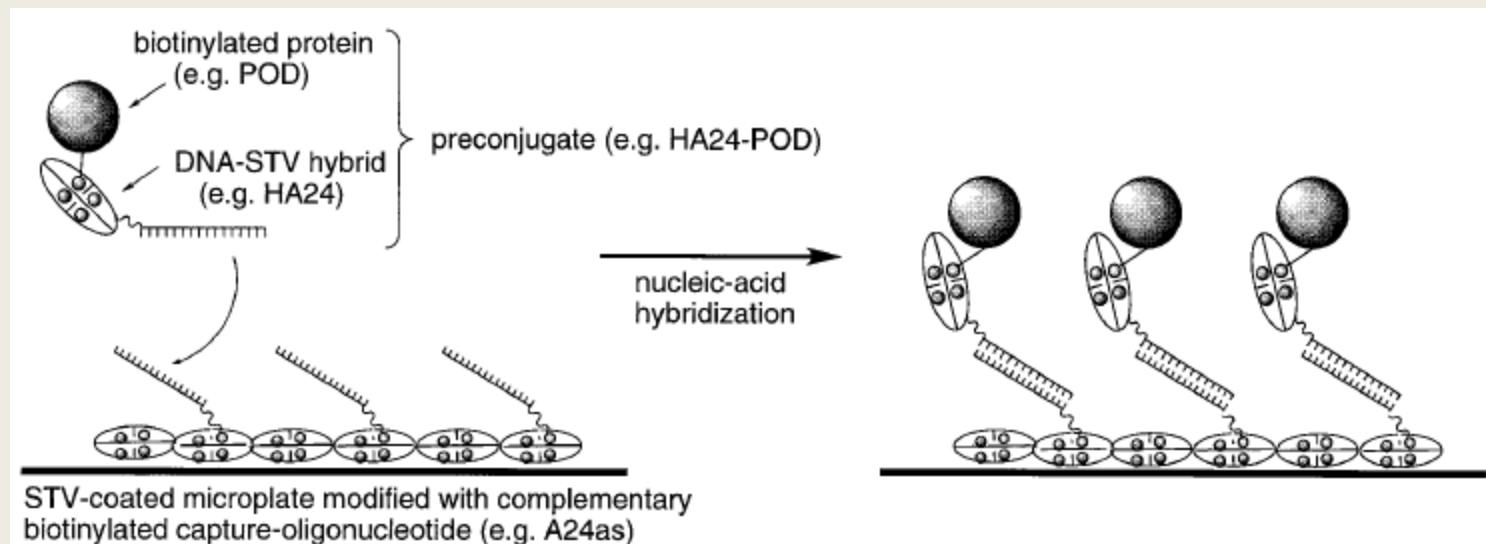
8: supramolecular aggregates

Angew. Chem. Int. Ed. 1998, 37, 2265-2268



5. Self-assembled nanostructures based on DNA

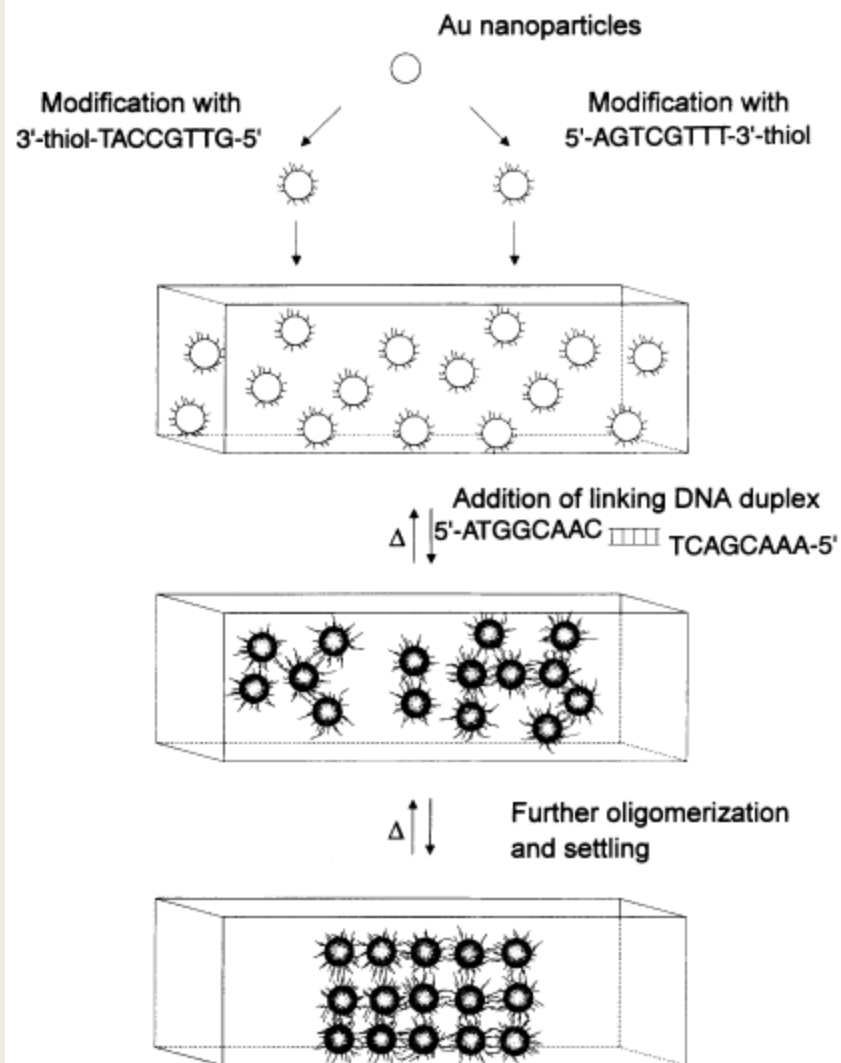
- DNA-Directed Immobilization
 - The noncovalent attachment of oligonucleotide fragments to various biotinylated biomaterials
 - The single-stranded DNA-tagged proteins were immobilized to complementary surface-bound capture oligonucleotides by means of specific nucleic acid hybridization.



Anal. Biochem. 1999, 268, 54-63



5.2. Organization of inorganic nanoclusters



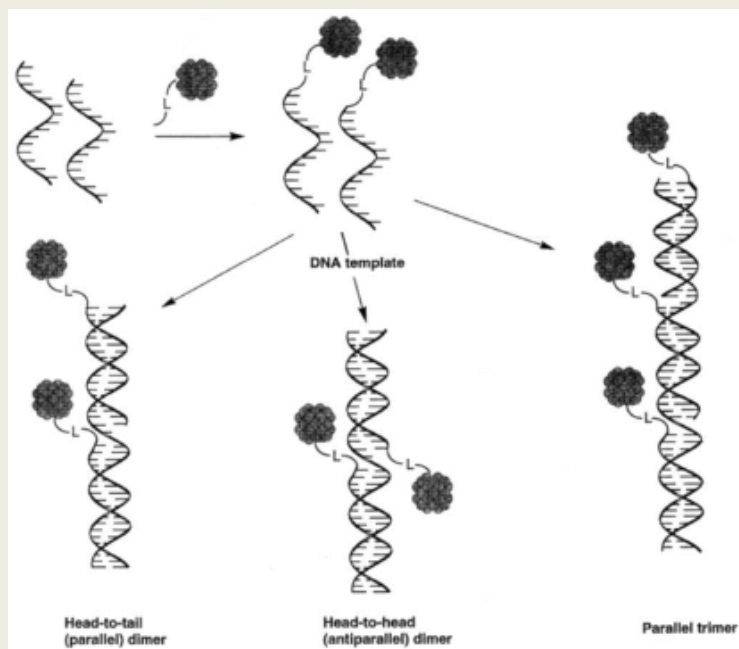
- A DNA-based method for assembling nanoparticles into macroscopic materials
 - Many methods have been developed for assembling colloidal particles into useful aggregates and materials.
 - Colloidal Au nanoparticles are reversibly assembled into macroscopic aggregates through a DNA-based method.
 - This assembly process can be reversed by thermal denaturation.

Nature. 1996, 382, 607-609



5. Self-assembled nanostructures based on DNA

- ❑ Organization of Nanocrystal molecules using DNA
 - ❑ Au nanocrystals are organized into spatially defined structures based on DNA hybridization.
 - ❑ Individual Au nanocrystals are attached to single stranded DNA oligonucleotides of defined length and sequence.
 - ❑ DNA-nanocrystal conjugates assemble into dimers and trimers on addition of complementary single-stranded DNA template.



oligo1) 5'-HS-CAGTCAGGCAGTCAGTCA-3'

oligo3) 5'-HS-CTTGCAGTCTGCTTGGAG-3'

oligo4) 5'-CAGTCAGGCAGTCAGTCA-SH-3'

template2) 5'-TGACTGACTGCCTGACTGTTGACTGACTGC
CTGACTG-3'

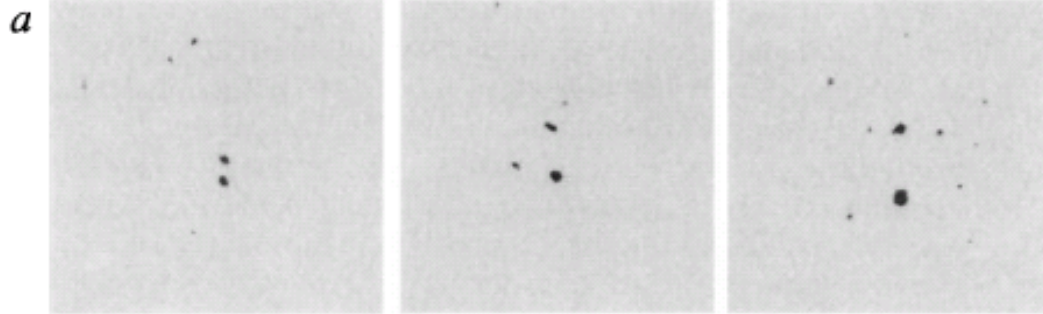
template5) 5'-CTCAAGGACTAGTGCAAGTTGACTGACTGC
CTGACTG-3'

template6) 5'-TGACTGACTGCCTGACTGTTGACTGACTGC
CTGACTGTTGACTGACTGCCTGACTG-3'

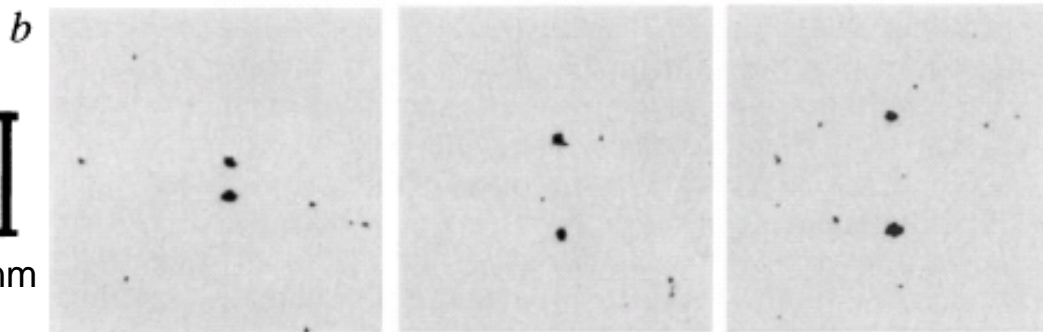
Nature. 1996, 382, 609-611



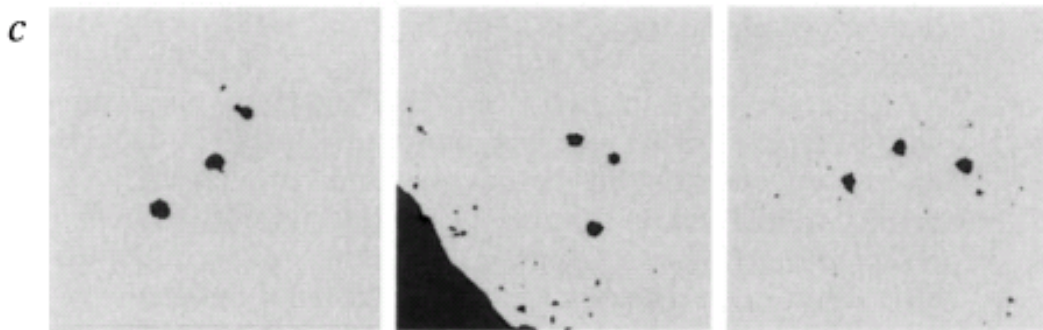
5. Self-assembled nanostructures based on DNA



Head-to-head
oligo1 + template2



Head-to-tail
oligo3 + oligo4 + template 5

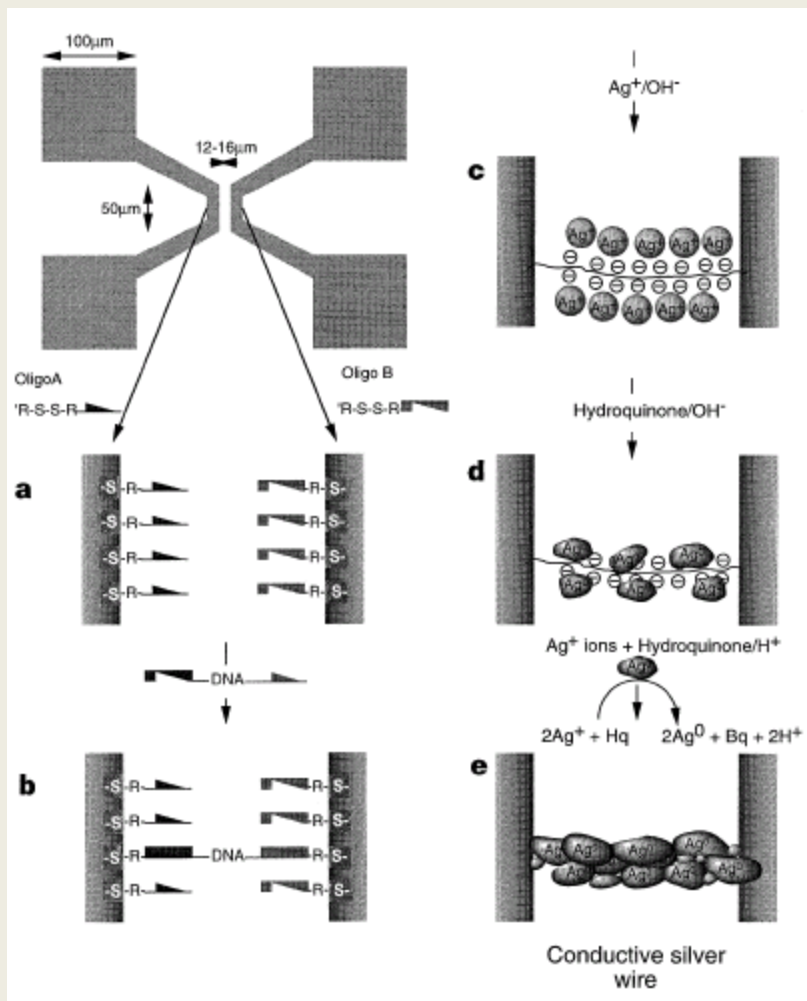


Trimer
oligo1 + template6

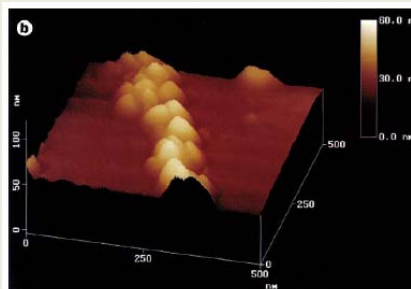
Nature. 1996, 382, 609-611



5.3. DNA-templated synthesis



- The electrostatic and topographic properties of the DNA molecule is utilized as template to synthesize nanostructures.
- Silver metals is victorially deposited along the DNA molecule(phosphate backbone).
- Three-step chemical deposition:
 1. selective localization of silver ion (Ag^+/Na^+ ion exchange)
 2. formation of complexes between the silver and the DNA molecules
 3. reduction of silver ion-exchanged DNA

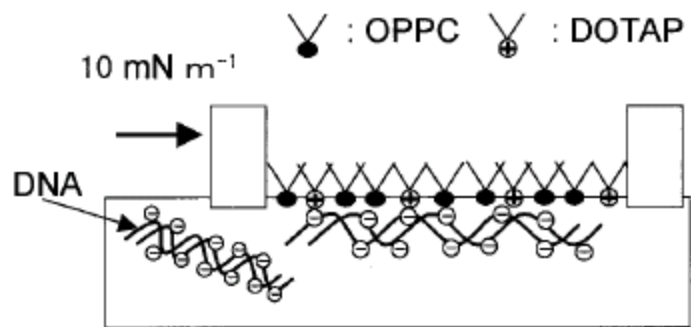


12 μm long, 100 nm wide conductive silver wire

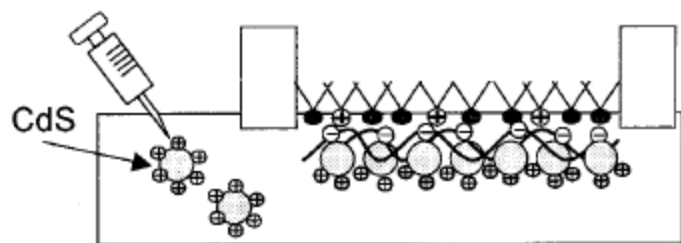
Nature. 1998, 391, 775-778



5. Self-assembled nanostructures based on DNA

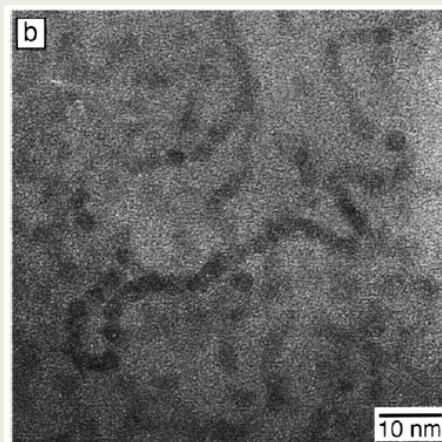


(1) Deposition of DNA on a Monolayer



(2) Addition of CdS Nanoparticles

- Preformed, positively charged 3 nm CdS nanoparticles are deposited on DNA template.
- The particles are arranged in a dense quasi-one-dimensional packing.
- Using the electrostatic interaction between the cationic surface modifiers on the CdS nanoparticles and the phosphate groups in DNA double strands as a template.



J. Phys. Chem. B 1999, 103, 8799-8803

