Scaffold design

General

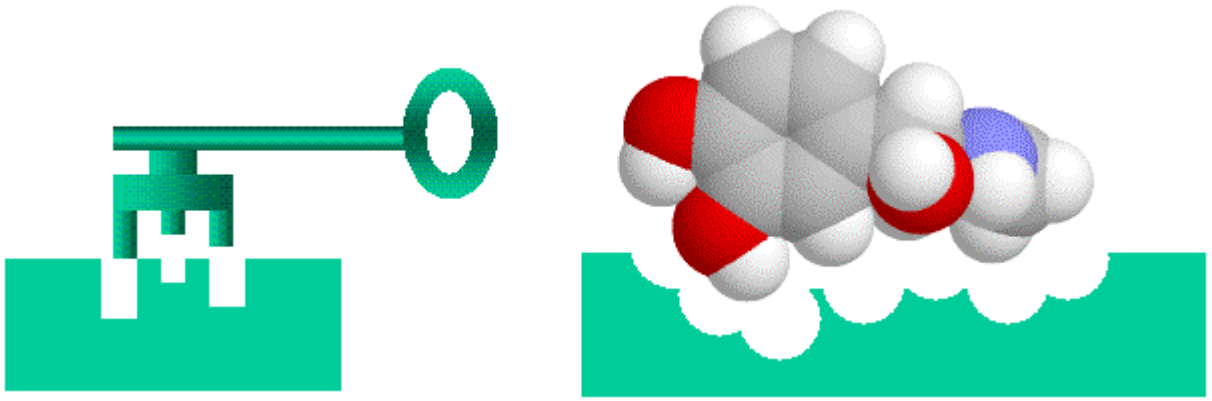
- Structurally well-defined host with atoms in an arranged array
- Receptors with special binding moieties
- Binding is governed by molecular recognition forces

General

- Complementarity - the greater the degree of guest envelopement the greater the selectivity
- Sometimes high selectivity is not sought (a group af analyte is to be recognized)
- Amphipilicity (hydrophobic interor, hydrophilic exterior)
- Preorganization
- Convergence

Complementarity

- Pairwise interactions between host and guest
- Adopts Fischer's *key-and lock* principle



Preorganization

- Binding by flexible unorganized scaffolds is entropically **not** favored
- Preorganization overcomes the entropy factor
- Structurally restricted scaffolds
- Dispute on too much rigidity / optimal angles etc.
- Induced fit (guest induced organization of best geometries)
- Strong interaction (high ΔH) vs. entropy
- Loose and flexible interaction not strong
- Balance between preorganization and induced fit

Convergence

- Creation of *concave* cavity

- Macrocycles and clefts

Design strategies

- *de novo* synthesis
  - Former examples / decoration with new functions
  - Intuition
  - Modeling (computational)
  - Gut instinct
- Combinatorial search
  - Impart some minimal level of design
  - Make a library of receptors and screen them
- Molecular imprinting
  - Eliminates the need for choosing a scaffold
  - Polimerization around the chosen analyte

de novo synthesis of ligands

Computational tools to model host-guest interactions

- Evaluate complementarity
- Predicting affinity / selectivity
- May reveal distances / geometries / strain

Mining Minima algorithm (M2)

- Computes free-energy of binding
- Configuration energy is the sum of contribution of low enegy conformations
- In conjunction with ConCept program to rank potential receptor structures

Mode Integration Algorithm (MINTA)

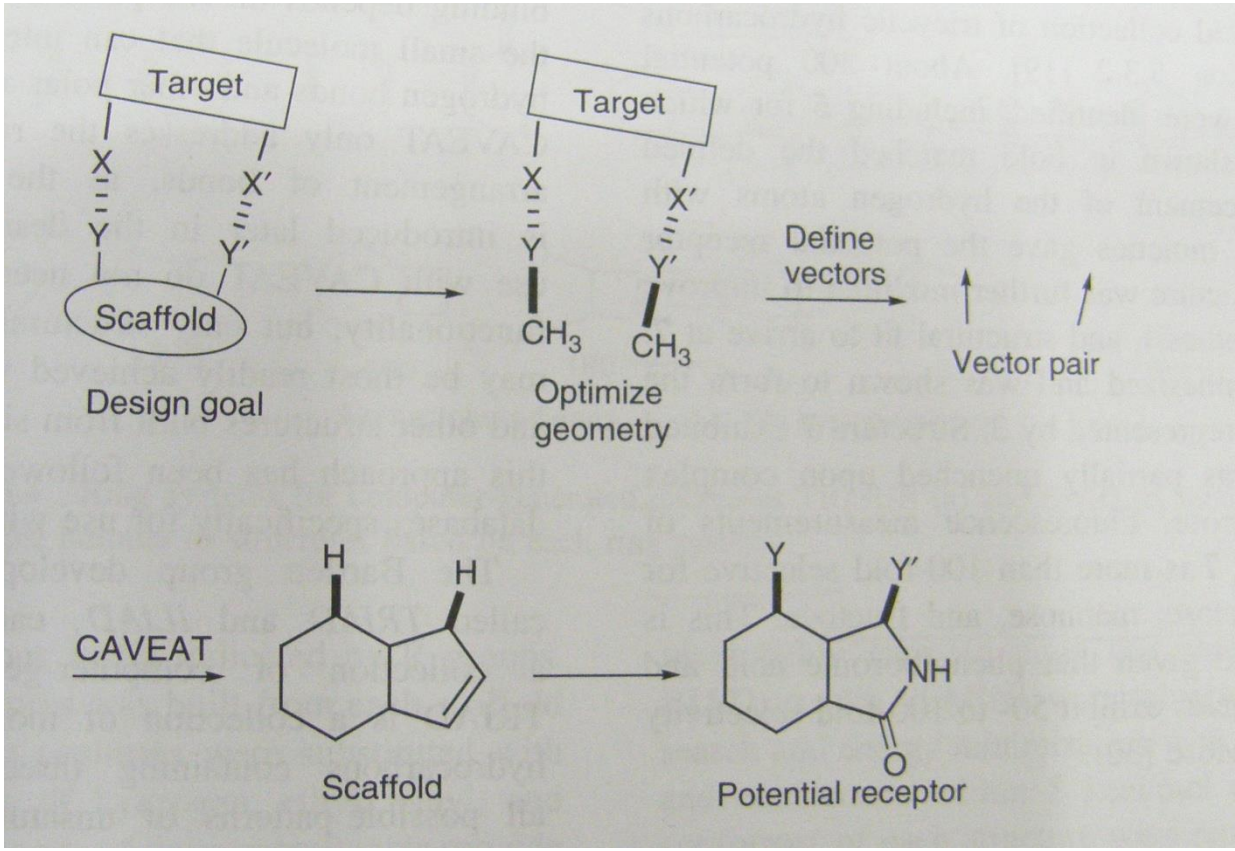
- Includes exhaustive conformational search to identify low-energy conformers
- Calculates binding-free-energy
- Used for virtual screning of libraries

The CAVEAT program

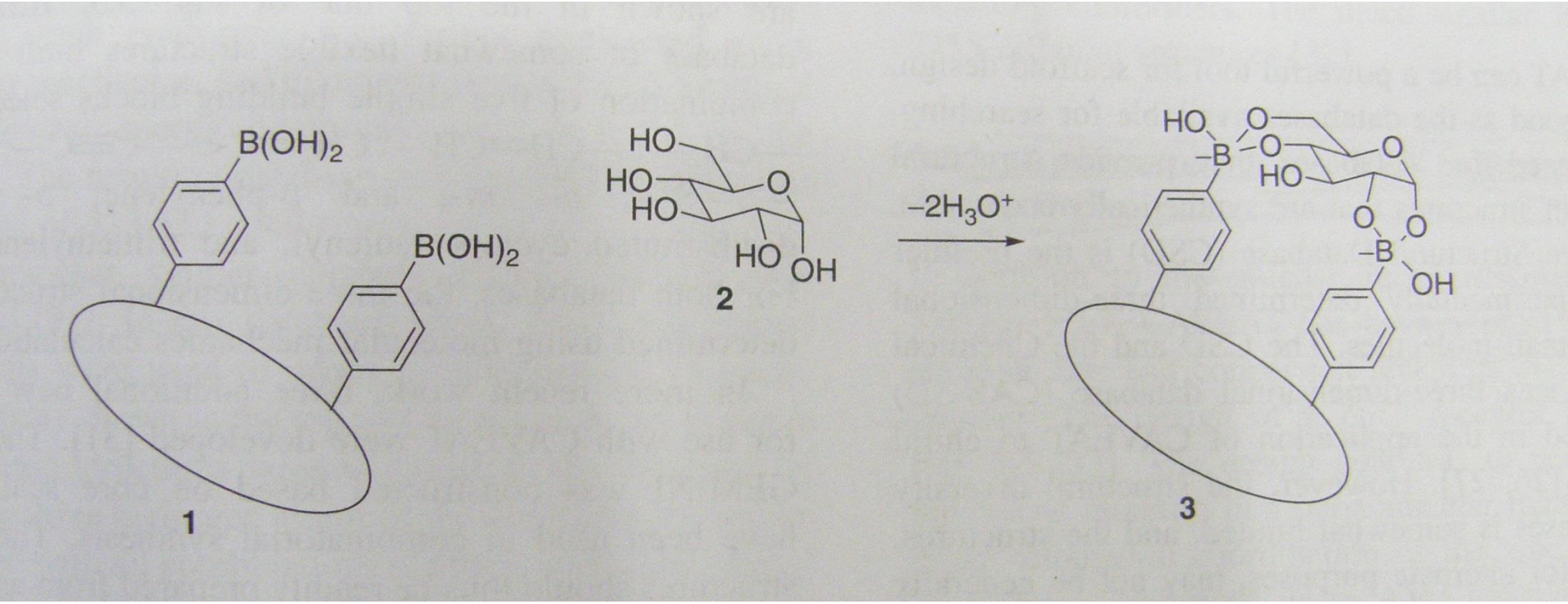
- Based on a vector relationship among bonds
- Searches 3D databases for templates
- Sorts the hits into group of structures with same parent framework
- Need for searchable databases
  - Cambridge Structural database (CSD)
  - Chemical Abstract Services 3D database (CAS-3D) – for chiral ligand searches

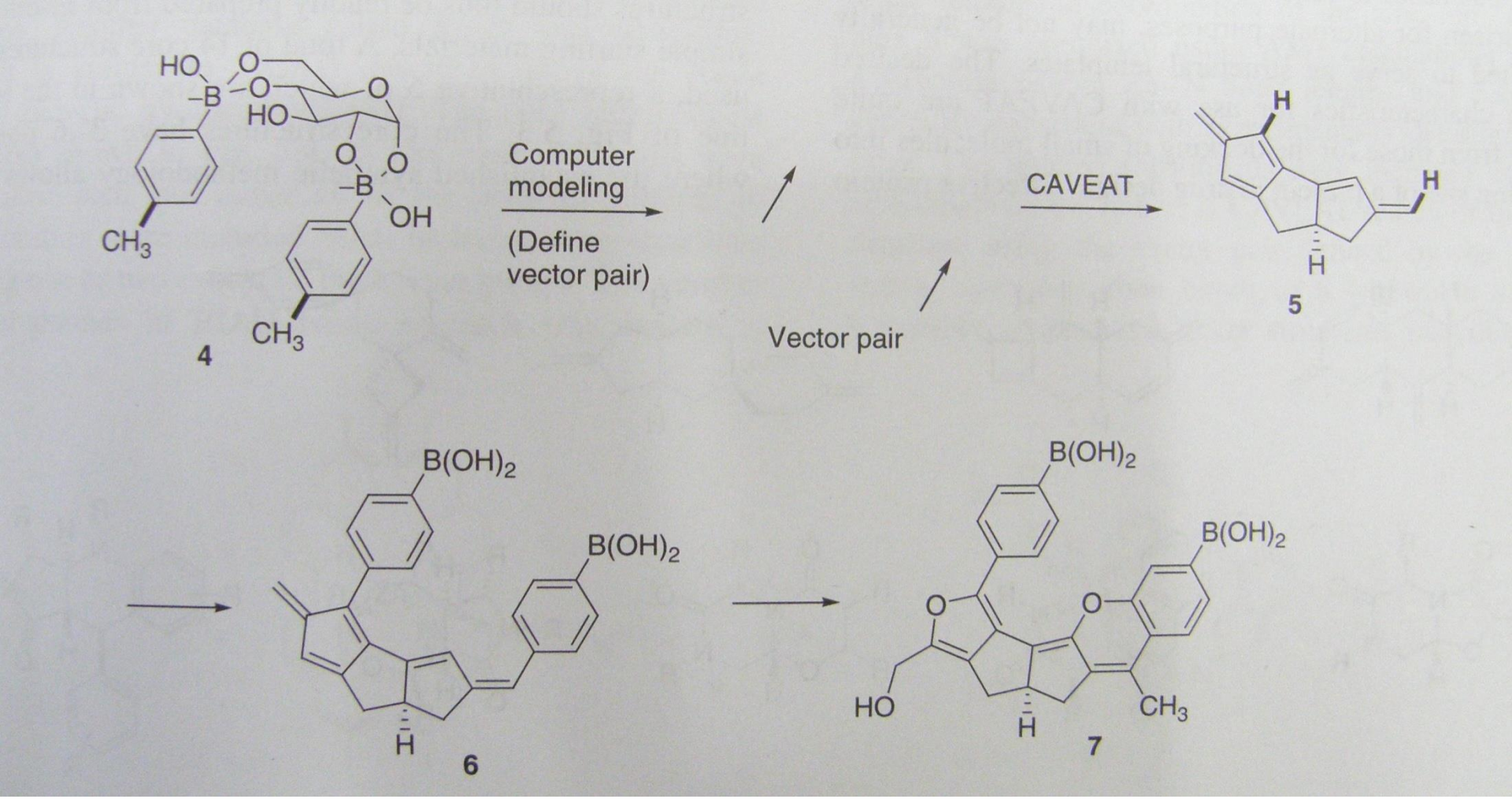
Example for scaffold design with CAVEAT

- Scaffold is simplified to Me
- Me-Y(Y') define vectors
- Identifies potential structures displaying groups in desired orientation
- Result is modified for acessibility, synthesis etc



Example for Glucose sensor design





- Me-Ar is defined as vectors
- Structure of complex 4 was minimized (Hartree-Fock)
- A database for tricyclic CH's was searched → **5 (6)**
- Structure was modified for stability, synthesis

- Receptor was connected to a signaling unit

- 7 was found to be 100 fold selective for glucose vs mannose, galactose etc.

- There are many other programs applying similar strategies

- HostDesigner, OVERLAY, LINKER

ConCept a receptor building program

- CONstruct reCEPTor
- Significantly different approach
- Relies less on user defined Host-Guest interactions
- Probes favorable interactions between defined guests and building components selected from a library
- Defines non-polar and H-bonding interactions

Summary of *de novo* receptor design

- Intuitive design is greatly facilitated by computing programs and searches from defined databases
- Identifies possible scaffolds
- Requires pencil and paper for final structure design

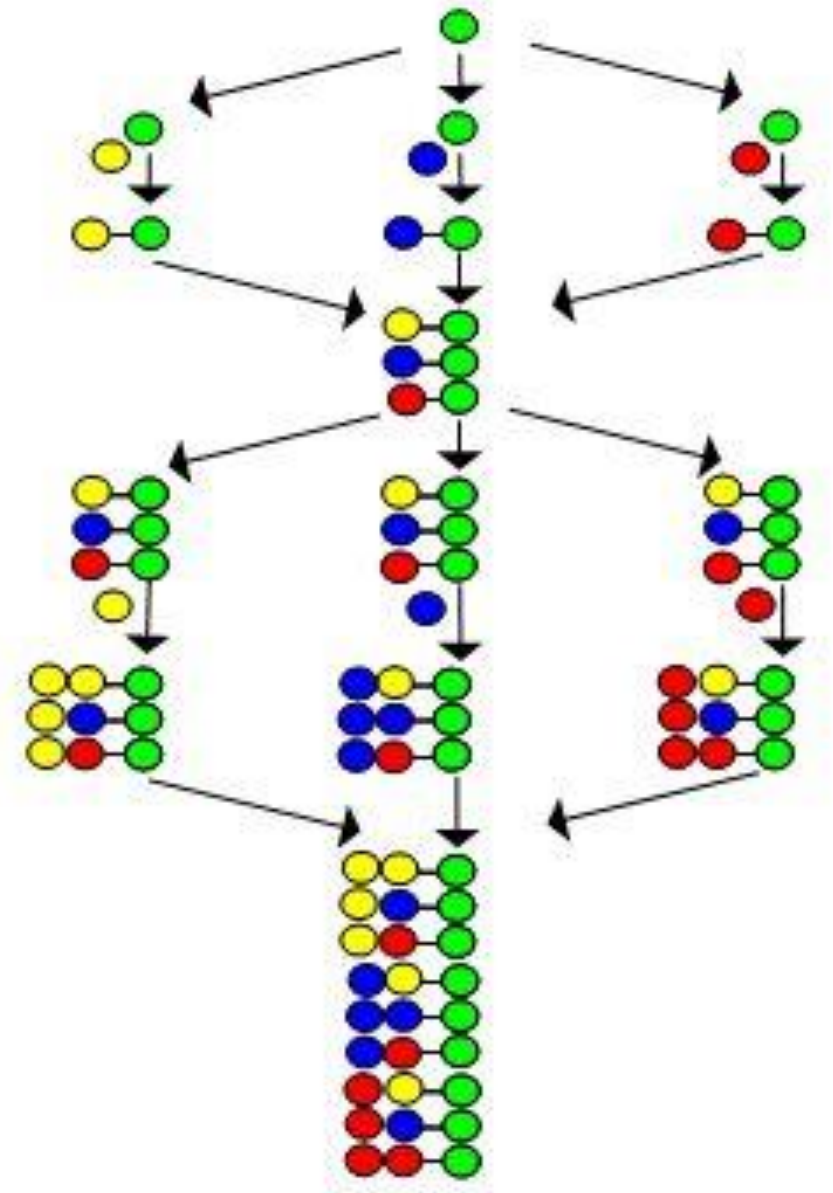
Combinatorial search for ligands

- Versatile tool originally used for drug discovery
- A large number of structurally related compounds
- Library can be
  - A mixture of compounds (split and mix method)
  - Individual compounds synthesized paralelly (e.g. in 96 well plate each well contains one single compound)
- High throughput screening methods are needed

Library of mixtures – Split and mix method

- Árpád Furka (Hruby, Lam, Houghten)
- Originally developed for peptide libraries (solid phase synthesis) but can be extended to systems where properties can be added modularly (subunits)
- One bead one peptide principle
- Number of library members = N^b (N = number of monomers, b = number of splitting cycles)

Split and mix method

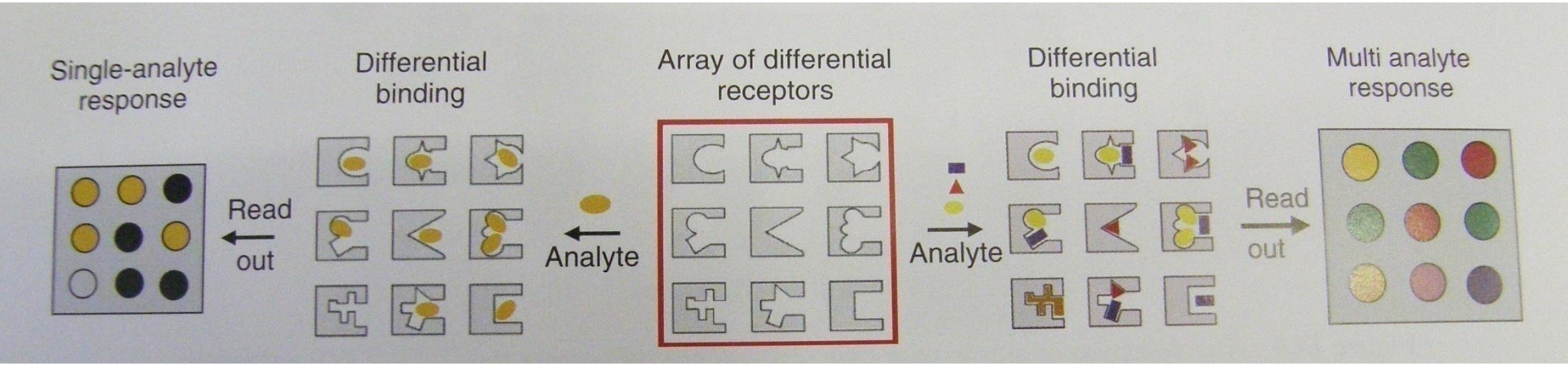


Combinatorial strategies

- Target oriented approach
  - Target is fixed
  - Members of the library contain derivatives of recognition motifs
- Diversity oriented approach
  - Library contains multipurpose collections of potential ligands
  - Several analytes are tested

Arrays of ligands

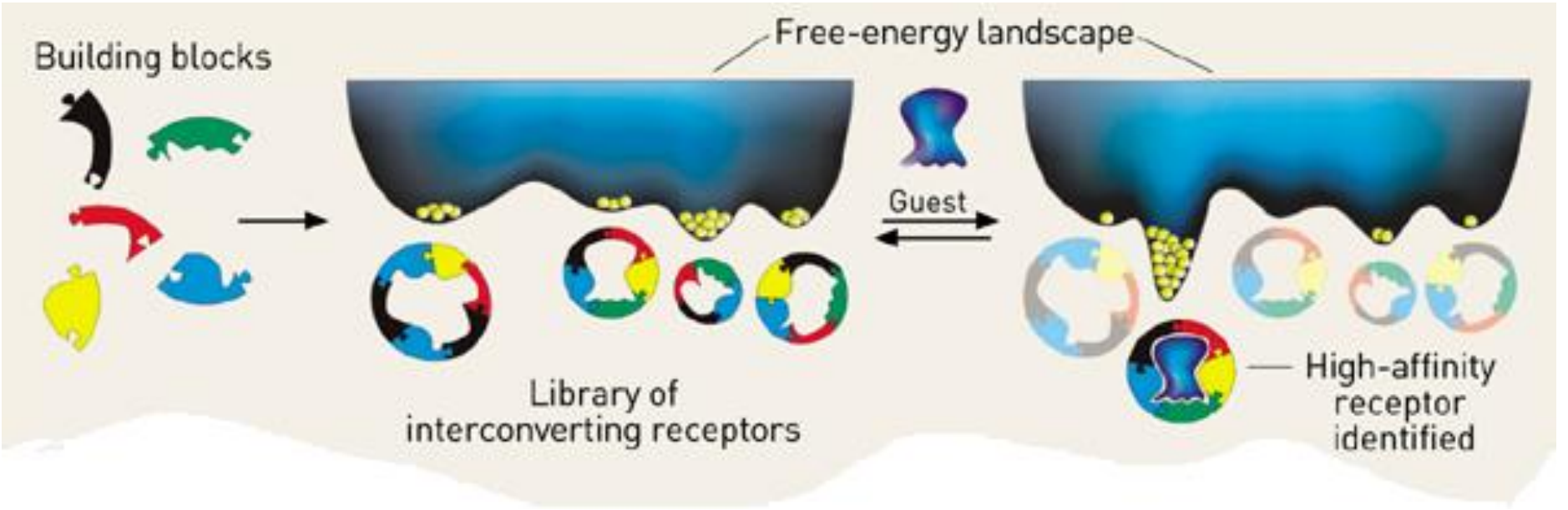
- Employs a collection of ligands
- Designed on the basis of certain recognition motifs
- Each leaves a fingerprint that can be combined



Dynamic libraries

- Dynamic combinatorial chemistry (DCC)
- Connecting the building blocks using reversible reactions
- Reversibility allows continuous interchange of subunits (thermodinamic control)
- Analyte affects the equilibrium by shifting it
- Ligands with the highest affinity binding constant will accumulate

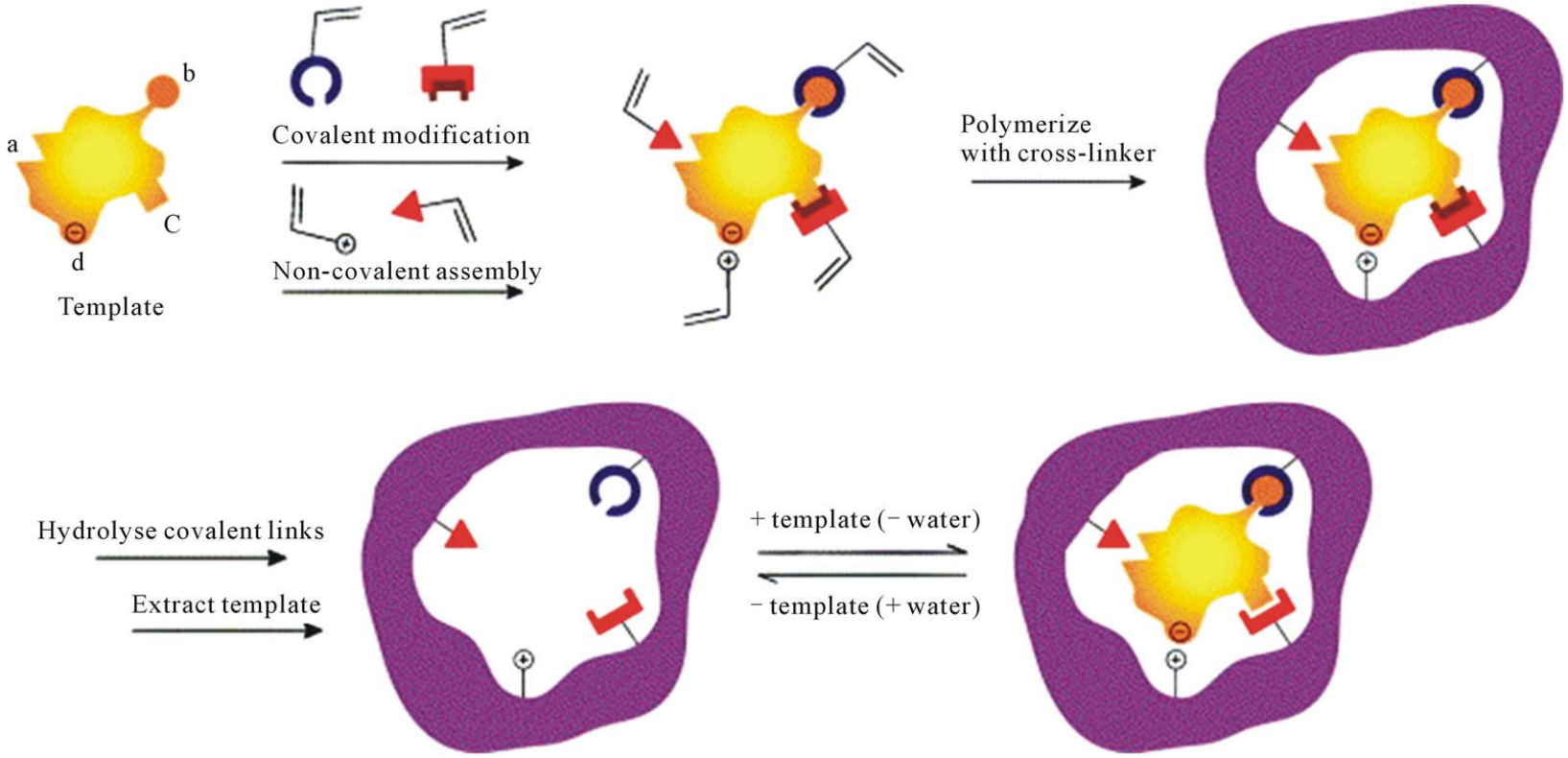
Dynamic library screening



Molecular imprinting in ligand design

- Polimerization in the presence of a template
- Removal of the template leaves the binding site complementary with the template
- Non-covalent, metal-ligand and covalent interactions

Molecular imprinting in ligand design



Advantages

- Rationally tailorable properties (vs. Modelling)
- Easy and cheap access (vs. antibodies)
- Excellent chemical, thermal and physical properties

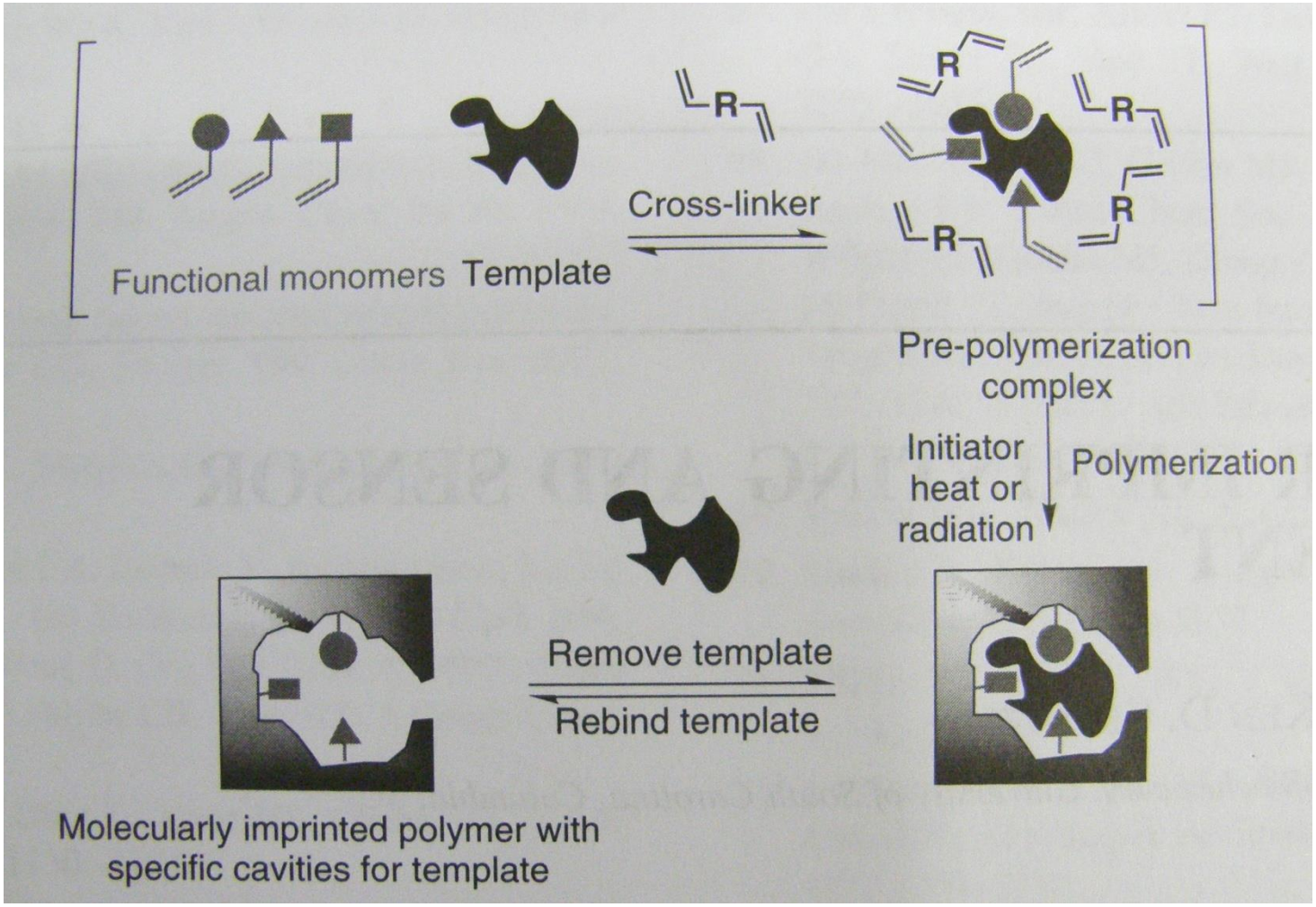
Disadvantages

- Poor or moderate selectivities
  - imprinting generates different types of binding sites
  - Binding site heterogeneity results in much lower capacity than expected after the number of template molecules
  - less than 10 % of binding sites have high affinities (works better at lower guest concentrations)
  - works better under non-aqueous media, but water reduces non-specific binding (more selective)

Further Disadvantages

- Limited polimer formats
- Lack of inherent signaling mechanism

Synthesis of MIPs



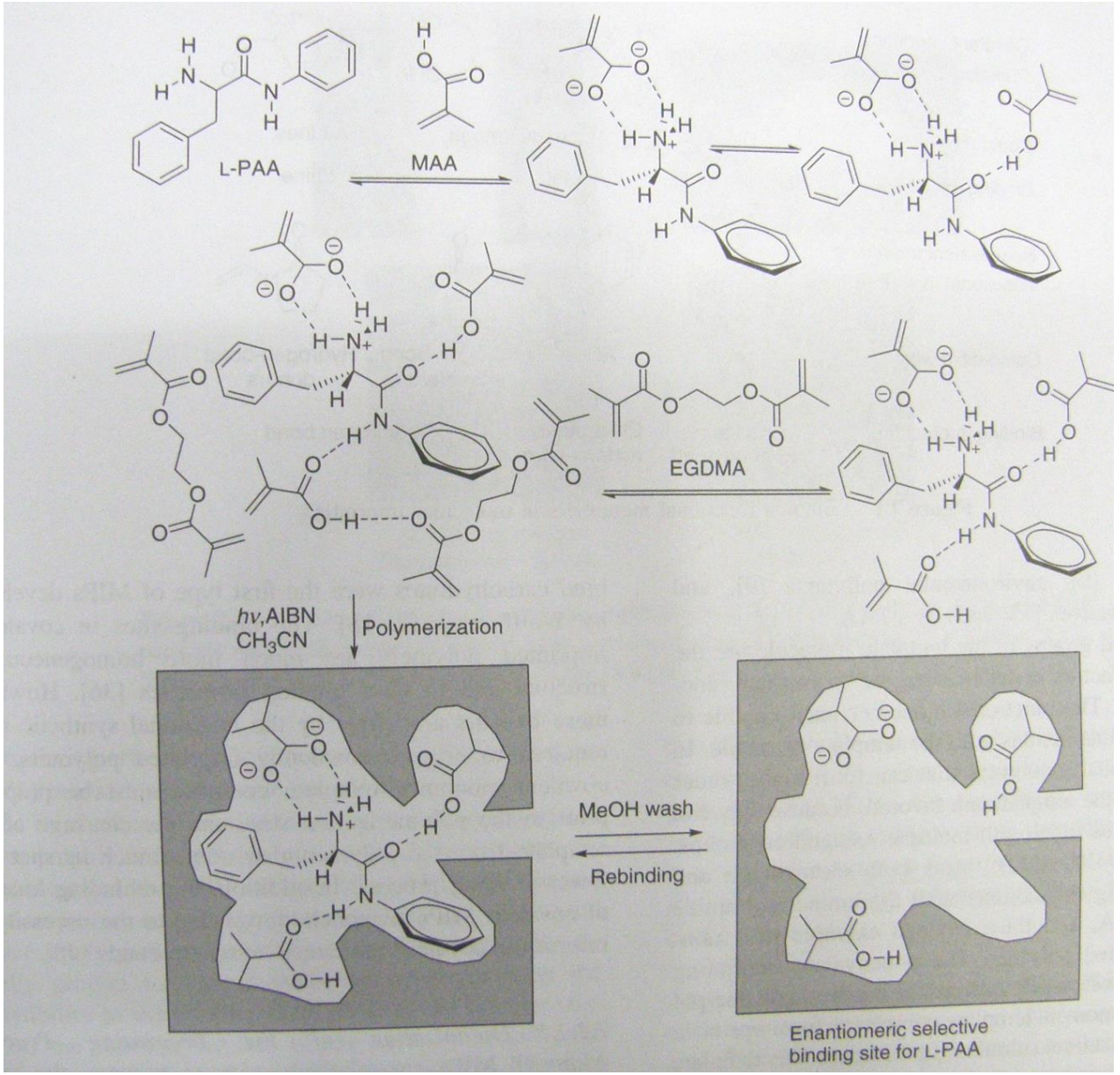
Monomer : with appropriate binding function; cross linker forms rigid matrix that preserves shape; iniciator often a radical that induces reaction

MIP for L-Phenylalanine

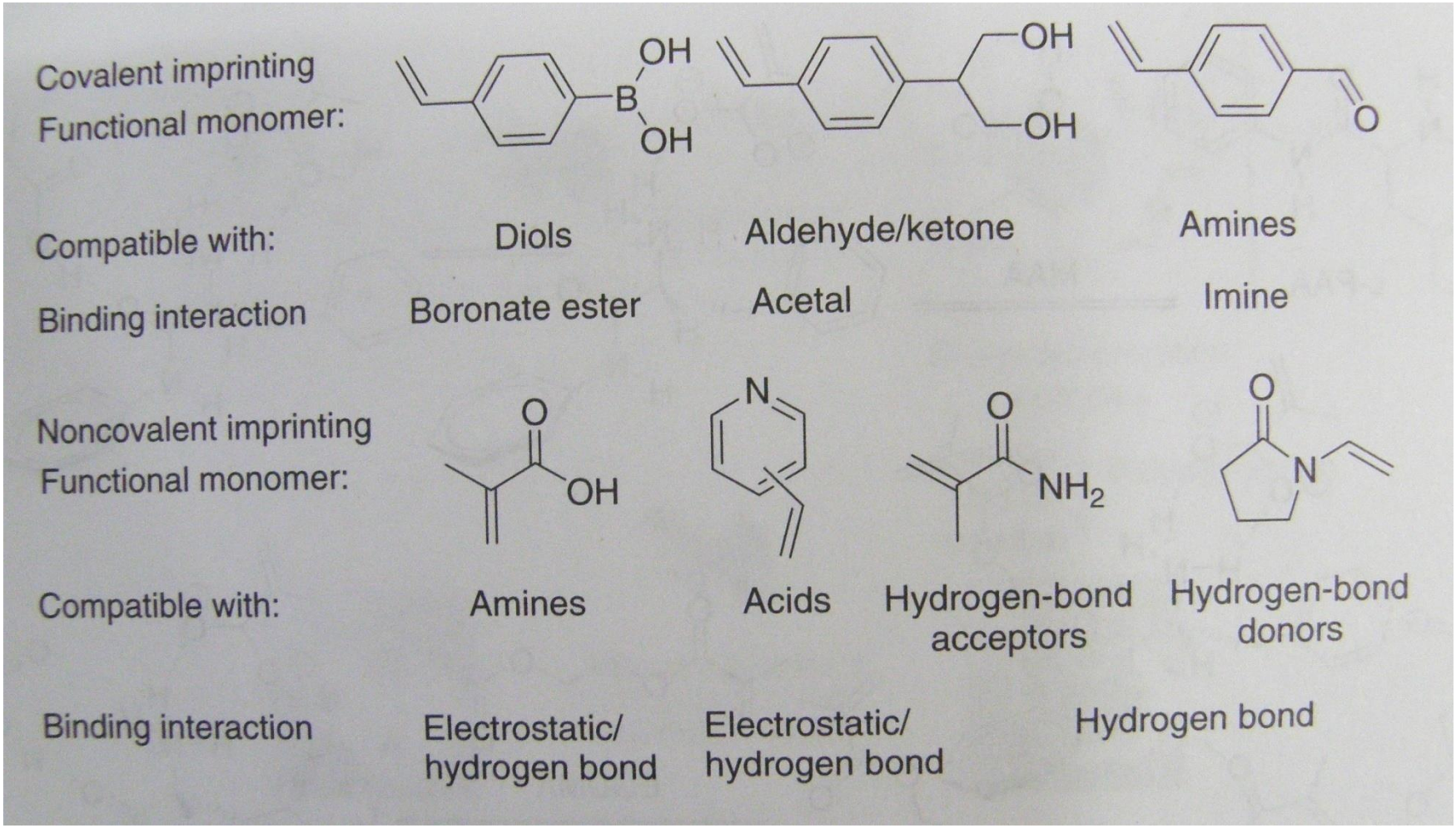
MAA = methacrylic acid

EGDMA = ethylene glycol dimethacrylate

AIBN (iniciator) = azobisisobutironitrile



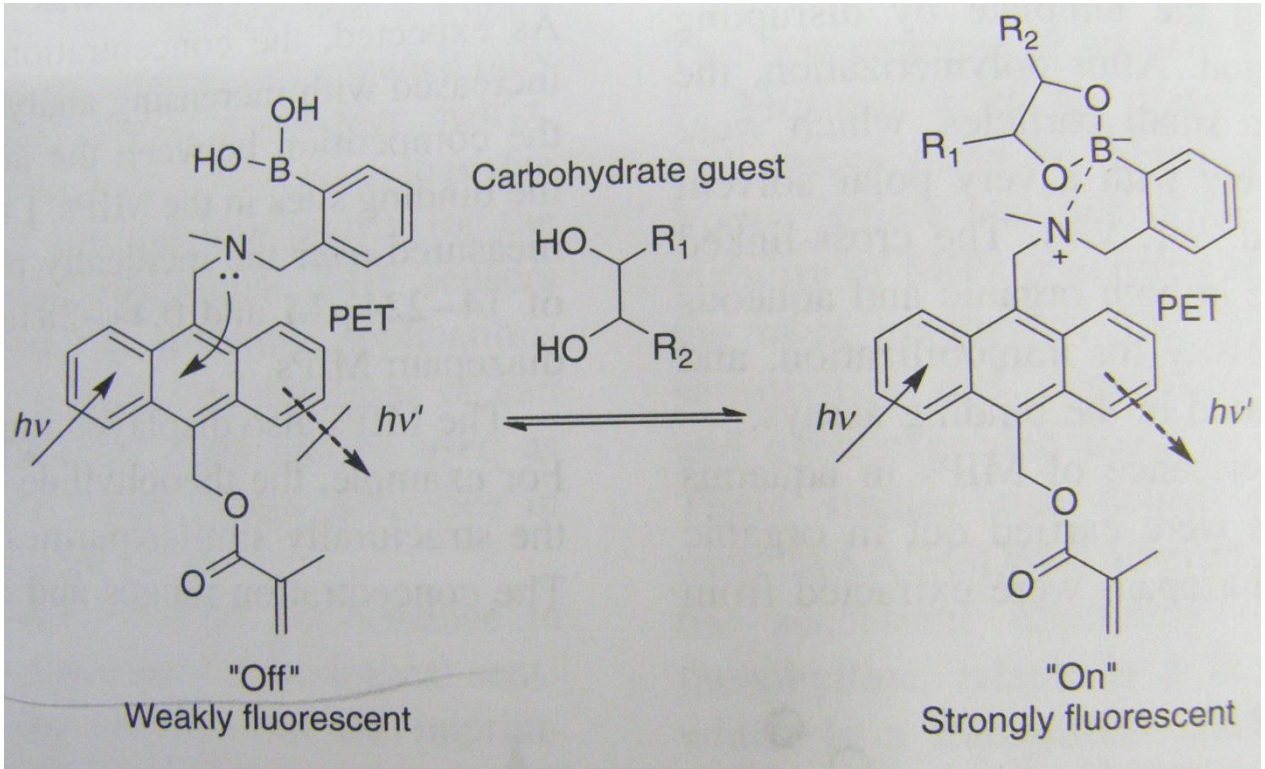
Functional Monomers for MIPs



Signaling with MIPs

- Indirect method
  - Radio / fluorescently labeled guest

- Direct method
  - e.g. with special monomer



Stoichiometry of complexes

- Method of continuous variation (Job's method, Job plot)
- Direct titration (at high affinities)

Job's method

- Different samples are made where H and G are present in different molar fractions (e.g. X_H changes from 0 to 1, while X_G changes the opposite) – the total molar concentartion of H and G is held constant

- A property change characteristic of binding is measured (e.g. fluorescence, absorbance, heat etc.) – system must obey Beer's law

- The maximum of the plot indicates the stoichiometry of the complex

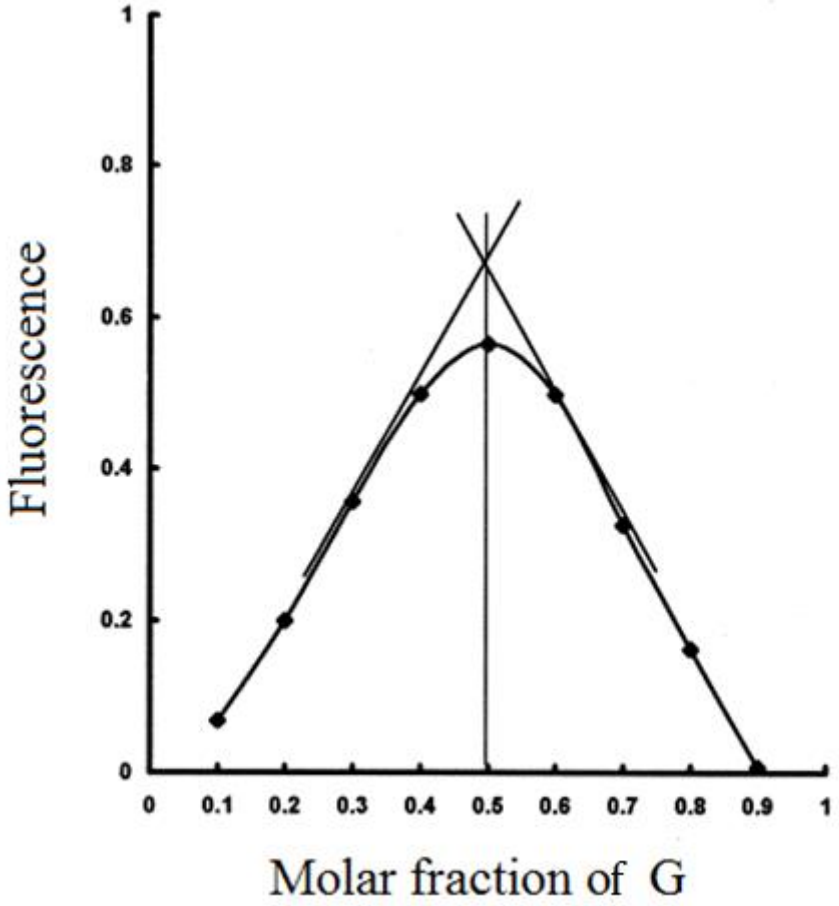
Job's plot

$H + G \leftrightarrow HG$

H and G are not fluorescent, HG is highly fluorescent

Fluorescence peaks where the most HG is present

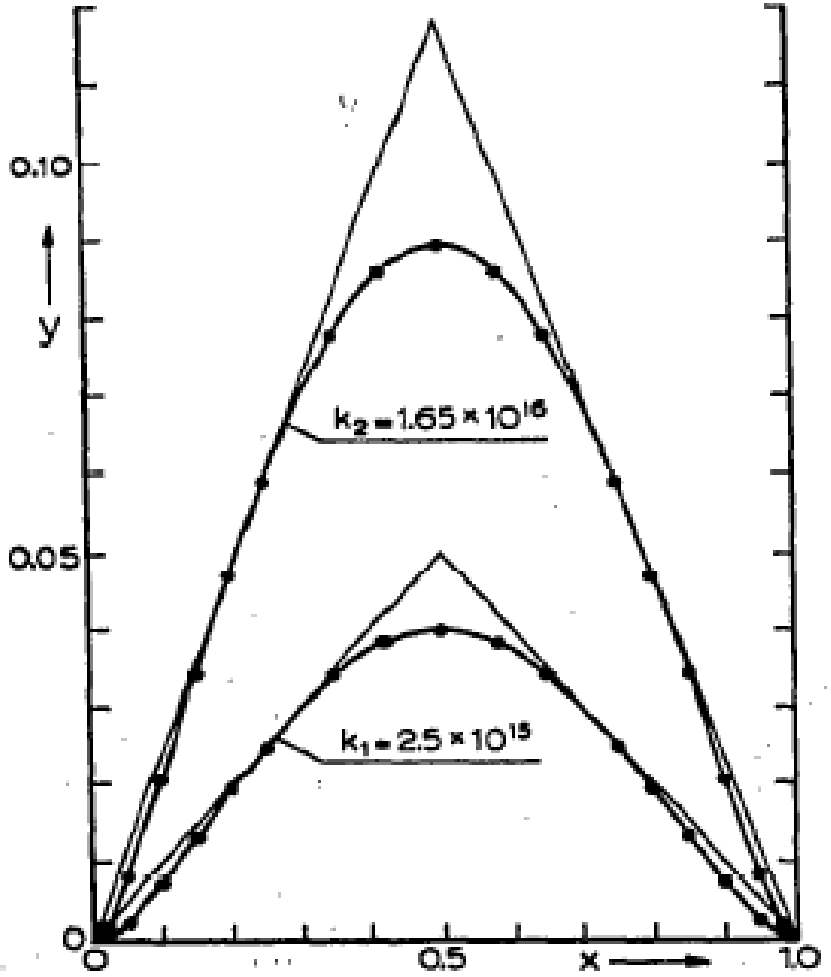
0.5 indicates 1:1 binding stoichiometry



Job's plot

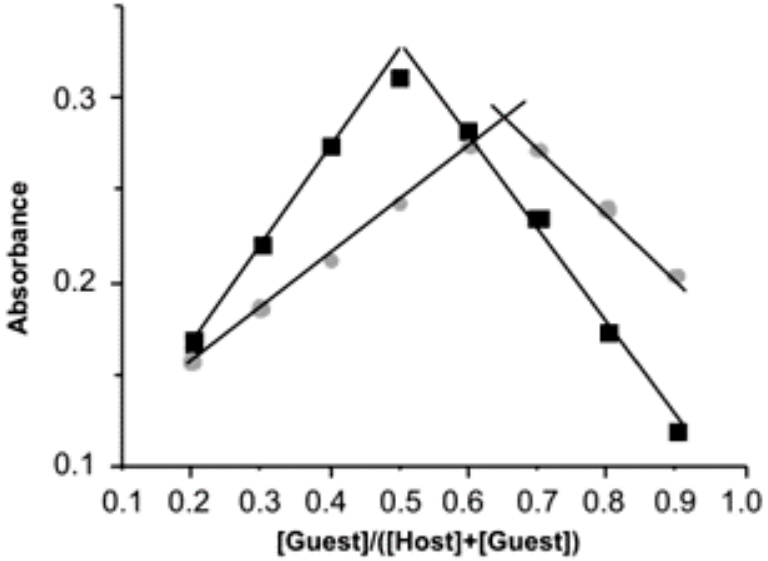
Shape of the Job plot indicates the measure of binding constant

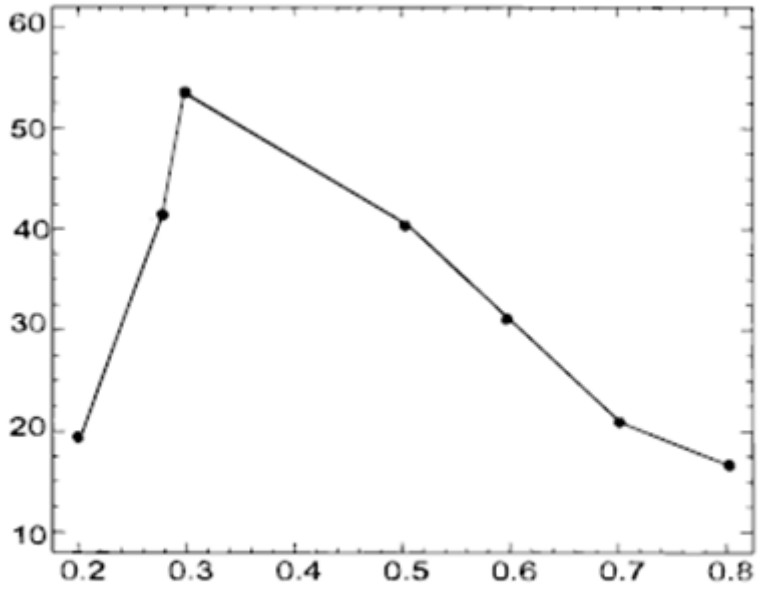
The sharper the higher



Job's plot

Other stoichiometries…





Molar fraction of Guest

High affinity binders – titration method

Titrating H with G

Solution of G contains the same concentration of H as H solution (dilution factor)

For high Ka's the curve is saturation type with sharp break

For Ka > 10^6 M^{-1}