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NPTEL ONLINE CERTIFICATION COURSE

# Lecture 2: Sensor Fabrication and Characterization

## Functionalization Methods and Performance Parameters

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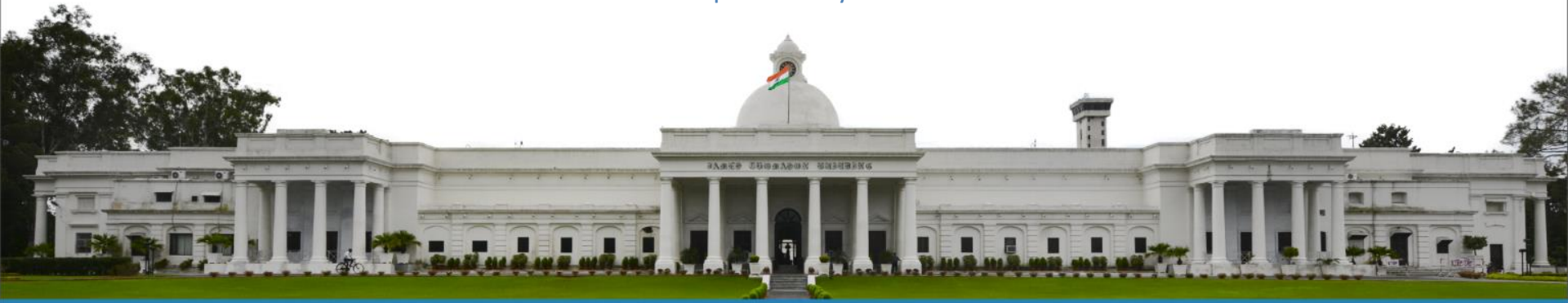


# Outline

- Functionalization/ Immobilization methods
- Performance parameters

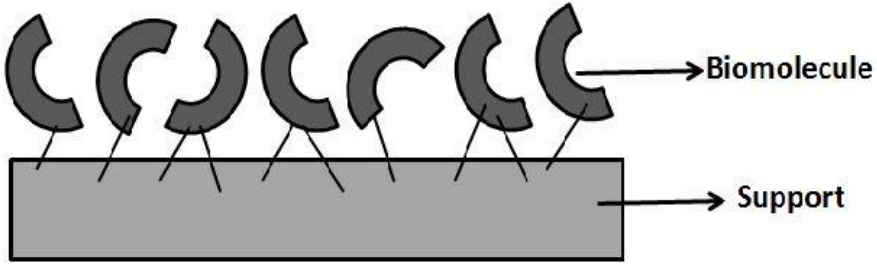
# Criterion for Functionalization Protocols/ Supports

- For the immobilization/functionalization of biomolecules on a particular support, the very first step is the selection of a suitable protocol or support/substrate.
- Immobilization process is different for different applications.

- Support should be homogeneous and has chemical, physical and biological stability.
- The protocol and the support both should be nontoxic and immune to environmental conditions i.e. pH, temperature, humidity.
- The support should be nonreactive to immobilized molecule and product of the final reaction.
- The support should have several functional groups to immobilize molecule and the protocol shall have good capability to hold them for long time.
- Sometimes support selection demands the application specific characteristics of it

# Immobilization Techniques

***Covalent binding***




- Amide bond formation
- Amidation reaction
- Schiff base formation
- EDC-NHS chemistry
- Thiol-disulfide interchange
- Diazotization

1. Resistant to the changes in the temperature, ionic strength, pH, etc. of the ambient
2. Can be reused and recycled
3. May suffer from potential loss of activity of the immobilized molecule

# Covalent Bonding

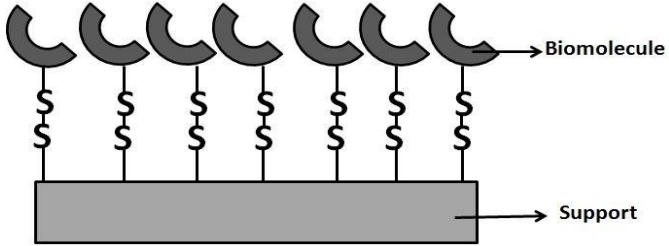
## *Thiol binding*

Thiols are organo-sulphur compounds that contain carbon bonded sulphydryl group (-SH)

Thiols make coordinate covalent bonds (dative bonds) with metal ions to form metal thiolate complexes. The lone pair of electrons forming this dative bond comes from sulphur. The most common thiol compound used in SPR based sensors utilizing covalent bonding is 4-Aminothiophenol (4-ATP).

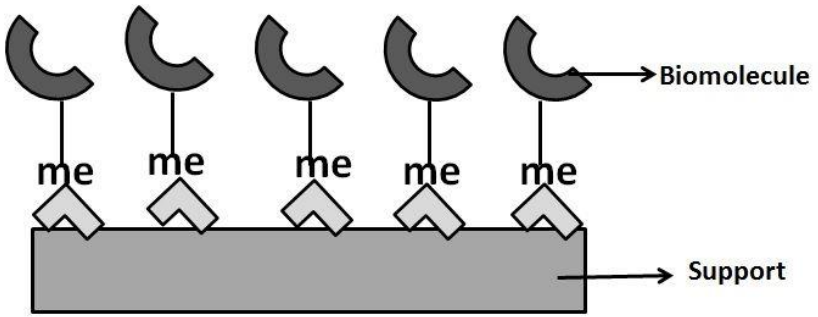
## *Disulfide binding*

The activity of this method can be changed by changing the pH of the used thiol group which is its extra advantage.



## *Metal binding*

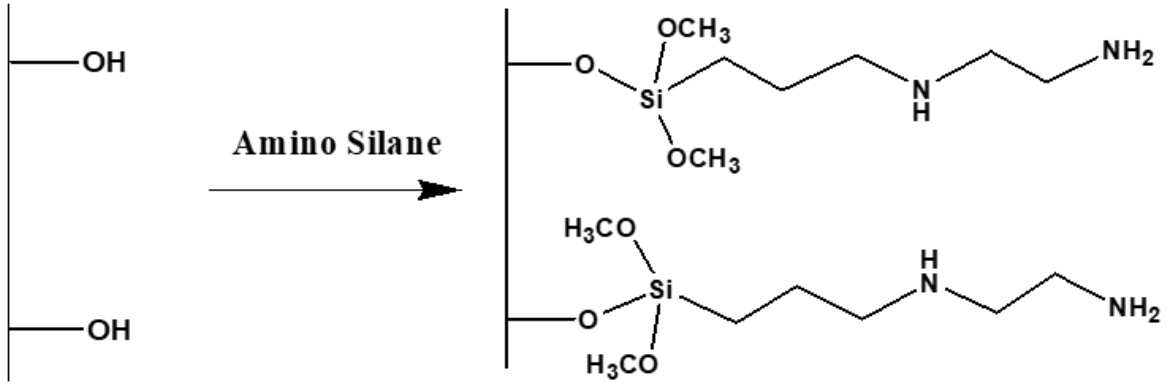
- Ligands are weakly bonded with metal ions so that some specific amino acids can be used as alternative for this.
- Easy regeneration of the support matrix is possible without reducing the immobilization product.



# Silanization

Silanization is the process of covalent bonding of various kinds of silanes over the surface of hydroxyls or oxides such as silica

In the case of silica, it is first treated with piranha solution (70:30, $H_2SO_4$: $H_2O_2$) to hydoxylate the surface

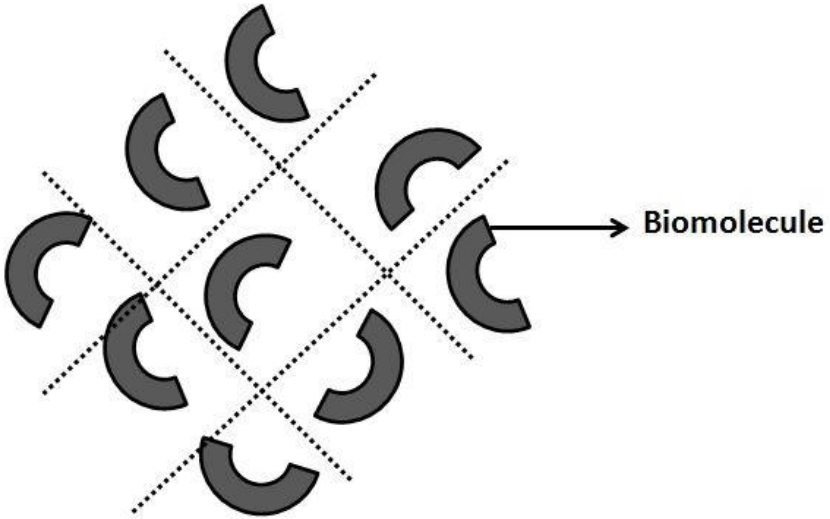


# Entrapment

- An irreversible method where biomolecule is immobilized in the lattice structure or in polymer membrane.

- The lattice structure should be in right porosity/ softness so that immobilized molecule be stable and there is no leaching of its properties

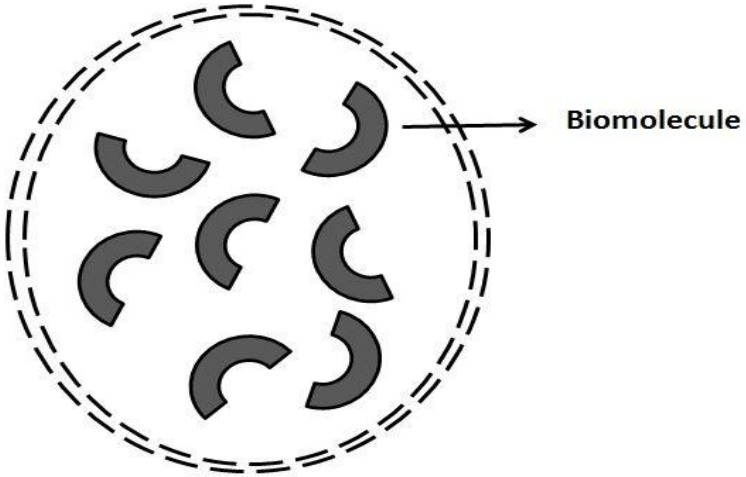
- Good activity and stability to immobilized enzyme/molecule because there is no need of modification of the functional groups




- High loading capacity but costly and has diffusion limitations

# Encapsulation

- An irreversible process in which biomolecules are immobilized in semi permeable membrane.
- Molecular recognition element molecules freely float inside this semi-permeable membrane
- Analyte molecules can undergo bidirectional diffusion



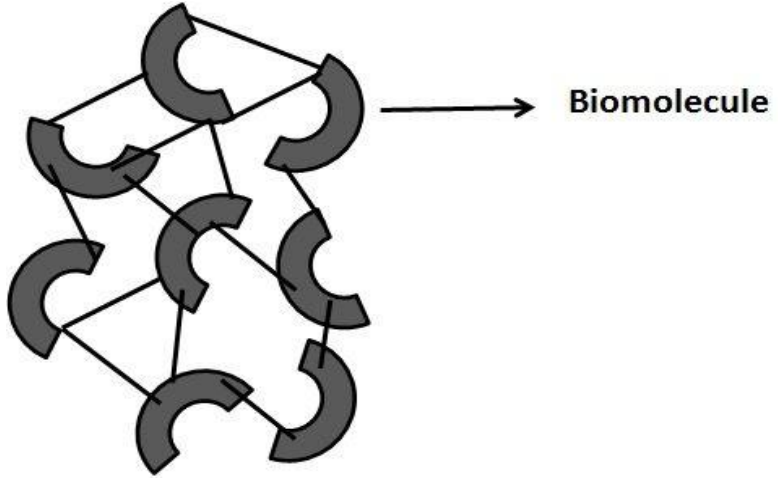

less possibility of the contamination of biomolecules from bad environmental conditions

biopolymers such as cellulose, nylon, alginate, chitosan, polyvinyl acetate, gelatine, boric acid

Susceptible to contamination because the immobilization is performed within the membrane

# Cross Linking

- Also an irreversible method.
- Does not need any support mechanism for immobilization- cost effective
- Cross-linking method is stable but the utilization of the harsh chemicals makes it less applicable
- high purification of the biomolecules is needed for cross-linking; so it is time consuming and demands labor.



# Adsorption

A reversible immobilization based on the interaction between molecule and support matrix

Based on weak interactions.
(Vander Waal forces, hydrogen bonding, ionic interaction, affinity binding, etc.)

Simple, low cost, reversible, retentive and fast technique, because it works in very modest conditions.

Due to weak interactions, the rate of leakage of biomolecules is high, loading capability of the support is random and the binding is unstable => Poor repeatability

Prone to non-specific bindings => Leads to fouling

# Molecular Imprinting

Molecular imprinting (MIP) is a technique for the creation of binding sites with the memory of size, shape and functional group of imprinted molecules (target molecules).

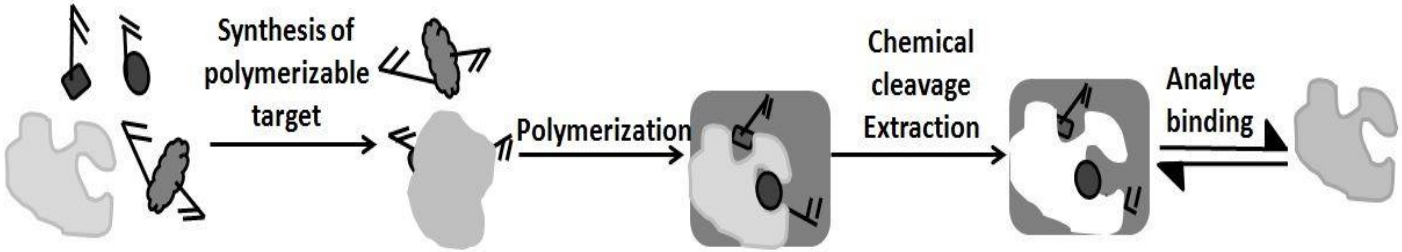
- Molecular imprints are synthesized by copolymerization of monomer carrying certain functional group and cross-linker with template molecules.

- Monomers are arranged around the template through polymerization process with high degree of cross-linking

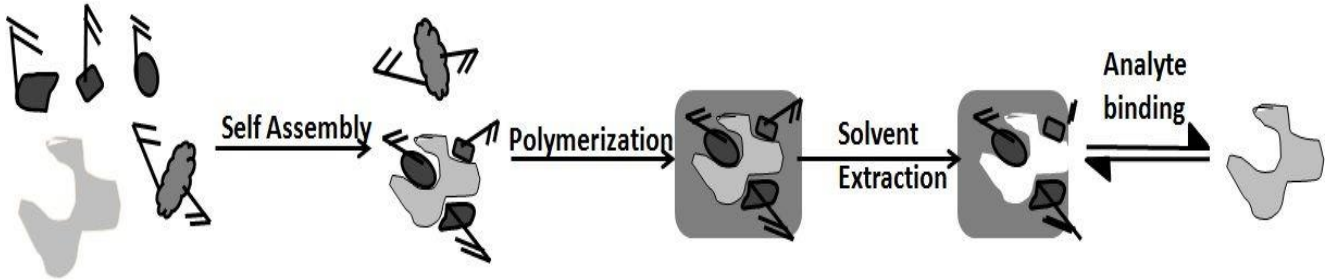
- Removal of these template molecules, which leaves certain recognition binding sites

- Polymerization process depend on temperature, polymerization medium i.e. type of monomer and cross linker

## Covalent Imprinting




## Non- Covalent Imprinting



# Performance Parameters of a Sensor

1. **SENSITIVITY:** Value of the response per analyte concentration.

2. **DETECTION ACCURACY:** How accurate the measurement is?

3. **LIMIT OF DETECTION:** What is the minimum concentration/amount of the analyte which can be sensed?

4. **SELECTIVITY/SPECIFICITY:** Chemicals Interference must be minimized for obtaining the correct results

5. **RESPONSE TIME:** Time necessary for having about 95% of the response.

6. **RESOLUTION:** Capability of the sensor to monitor the smallest difference in the analyte parameter. Actually the resolution is not the characteristic of the sensing probe but that of the detector.

7. **REPEATABILITY:** How many times a sensor can give the similar result in similar conditions of operation

8. **REPRODUCABILITY:** The number of sensors manufactured with the same principle have similar response

9. **NOISE:** Random variation of the O/P signal which has no information

10. **DYNAMIC RANGE:** Range between LOD and maximum concentration which can be measured with confidence

11. **LINEARITY:** A good sensor is expected to have linear calibration curve. It is linear when the output signal is directly proportional to analyte concentration throughout the range of the sensor

12. **DRIFT:** A small undesirable change in the characteristic curve with time.

13. **REUSABILITY:** Can the sensor be used again?

14. **SHELF LIFE:** What happens to the performance when kept for long time

15. **FIGURE OF MERIT (FOM):** Ratio of the sensitivity of the sensor and the width of the spectral curve. A good sensor always finds the minimum full width at half maximum (FWHM) of the spectrum and high sensitivity so that the FOM is high.

# Summary

1. Various functionalization methods for sensor fabrication were presented.
2. Performance parameters for sensors were discussed in detail.

**Thank You**

# Thank You