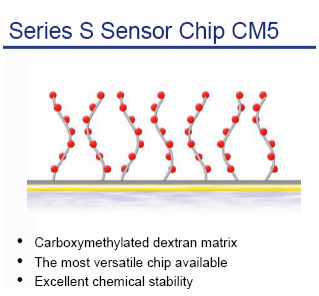
Designing a Biacore Experiment: Common Questions and Answers

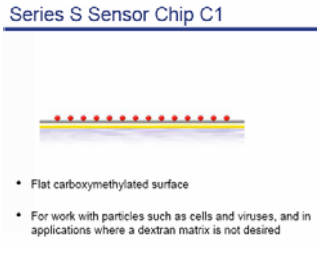
Biacore T100 Applications:What type of experiment do you want to do?

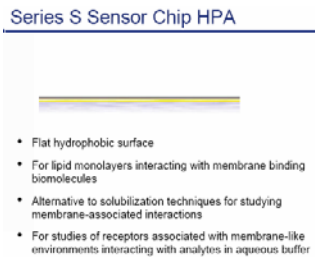
- Specificity - Yes/No binding, Ligand fishing
- Kinetics - Kinetic rate analysis, k_a and k_d
- Affinity - K_A and K_D
- Concentration - How much active molecule present
- Multiple Interactions - More than one molecule binding
- Thermodynamics - How temperature affects binding (4-45ºC)

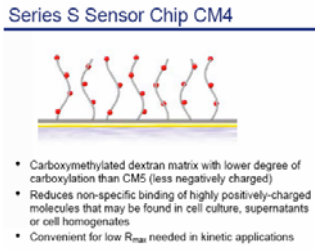
Which Chip to Use?

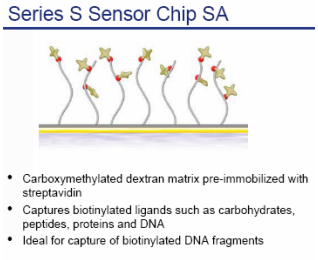
- CM5 - Carboxymethylated dextran matrix, most versatile chip
- CM4 - Lower degree of carboxymethylation than CM5 (less negatively chardged) reduced nonspecific binding
- CM3 - Shorter carboxymethylated dextran matrix than CM5, works well with whole cells
- C1 - Flat carboxymethylated surface, no dextran matrix, works for attaching cells and viruses
- SA - CM3 chip with streptavidin
- HPA - Flat hydrophobic surface, for lipid monolayers interacting with membrane binding biomolecules
- L1 - Carboxymethylated dextran matrix with lipophilic substaces, capture of liposomes
- NTA - Carboxymethylated dextran matrix with NTA, capture of His-tagged ligands

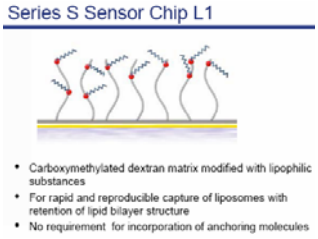


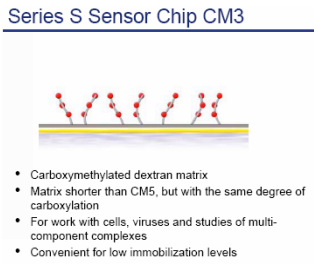


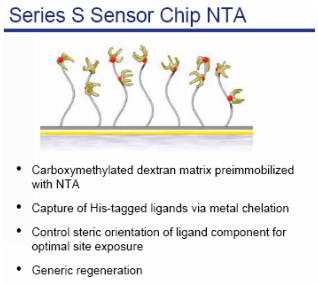












What buffer should I have my samples in?

Recommended Buffers:

HBS-N - 0.1 M HEPES, 1.5 M NaCl

HBS-P - 0.1 M HEPES, 1.5 M NaCl and 0.05% v/v Surfactant P20

HBS-EP - 0.1 M HEPES, 1.5 M NaCl, 30mM EDTA and 0.05% v/v Surfactant P20

Which molecule to immobilize (ligand)?

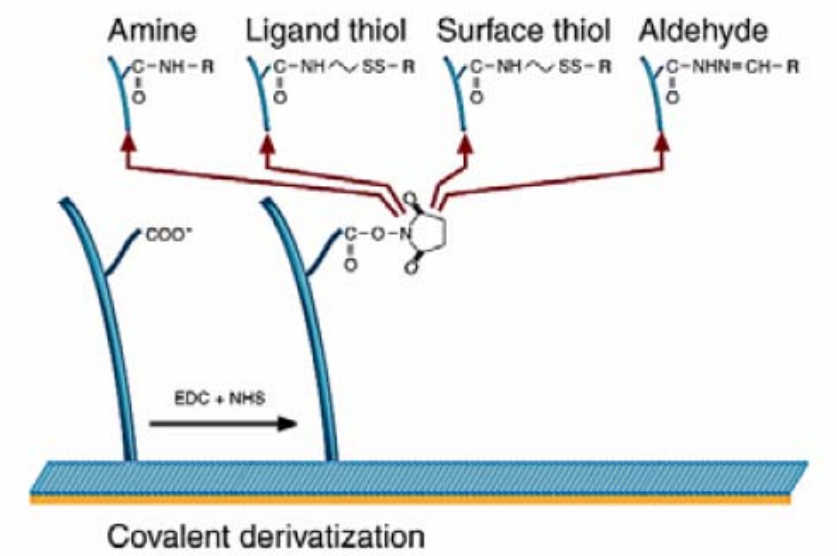
1. Immobilized molecule must maintain it's functionality
2. Valency – number of binding sites (if known)
3. Charge – the molecule with the higher isoelectric point should be immobilized
4. Concentration – immobilize more "precious" sample if possible, 5-50ug/ml

5. Purity – at least 95% pure for direct coupling method
6. Tagged – tagged molecules can bind to specific chips. Ex. biotinylated ligand binding to a strepavidin (SA) chip.
7. Size of molecule - 100Da minimum to immobilize; if possible, immobilize the smaller molecule for larger signal intensities when binding the larger analyte.

Which immobilization method to use?

Two Methods: Direct Immobilization and Capture Approach

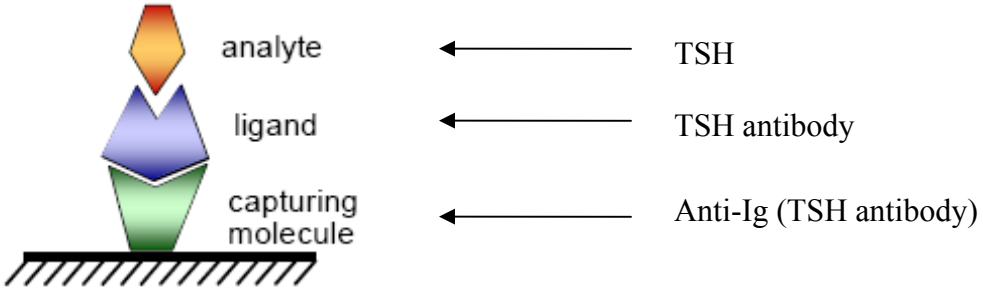
Direct Immobilization



Direct Immobilization chemistries:

- Amine
- Ligand thiol
- Surface thiol
- Aldehyde

Capture Approach



Other capture molecules:

- Streptavidin : Biotin (ligand)
- anti-Biotin : Biotin (ligand)
- anti-GST : GST (ligand)
- Ni2+·NTA : 6xHis (ligand)

Advantages of capture approach:

- Can regenerate down to capture molecule so there is always a fresh surface
- Homogenous presentation of ligand
- Purification is not needed

What do you need to know about your analyte?

- Concentration
- Purity
- Solubility
- Appropriate buffer conditions
- Valencies