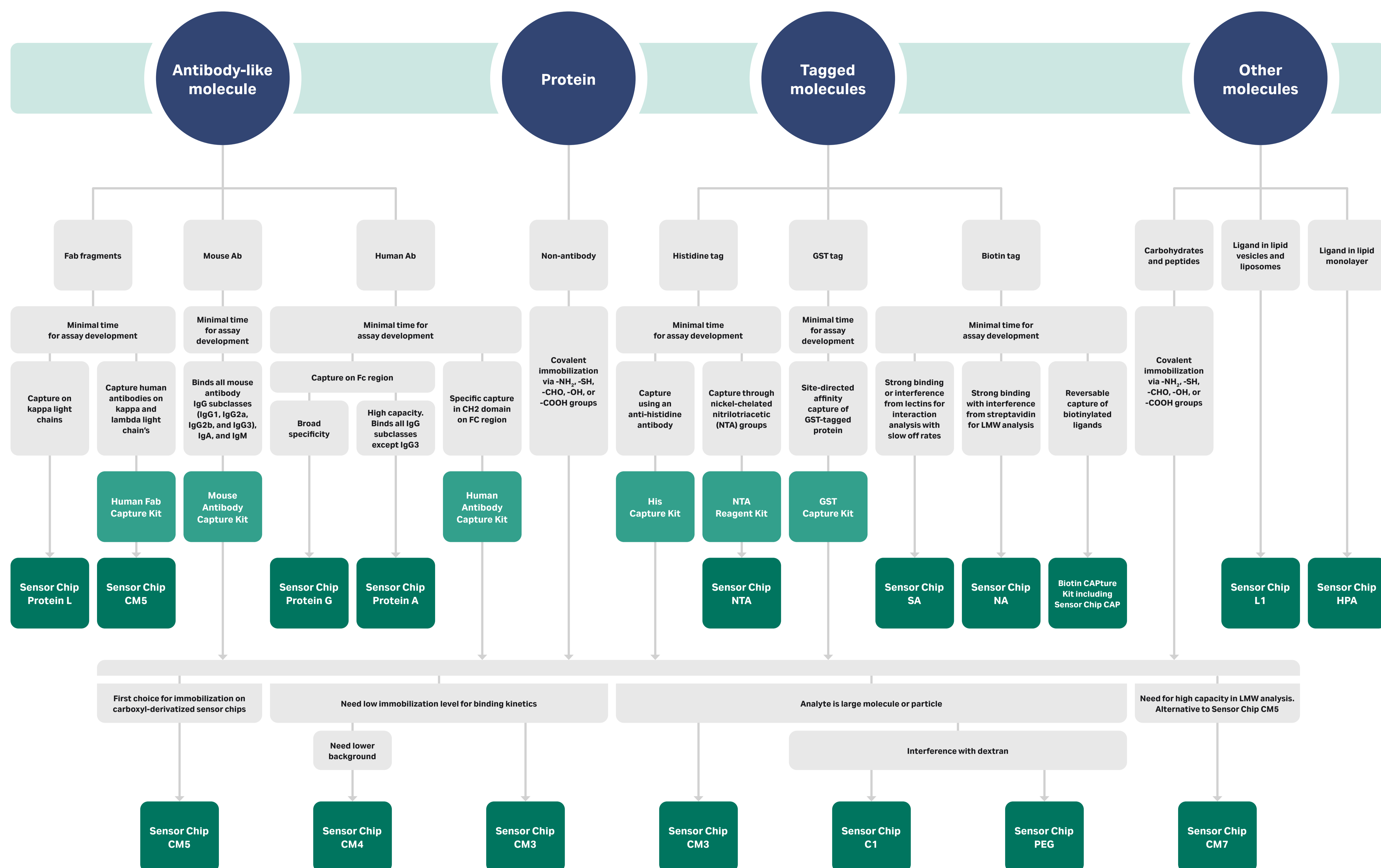


How to choose a suitable attachment approach for your ligand

Save time and effort for your Biacore™ SPR interaction analysis study



Terminology

- **Ligand:** the interaction partner attached to the surface.
- **Analyte:** the interaction partner in solution passed over the attached ligand.
- **Capture molecule:** the binding partner attached to the surface for easy attachment of ligand of interest.
- **Prefunctionalized surface:** the binding partner immobilized to the surface for easy assessment of analyte binding.

Sensor chip formats

- Sensor chip classic format are designed for Biacore X100, Biacore 3000, and Biacore C SPR systems.
- Series S Sensor Chip are for use with Biacore 8K+, Biacore 8K, Biacore S200, Biacore T200, and Biacore 4000 SPR systems.



Which molecule to attach to the surface?

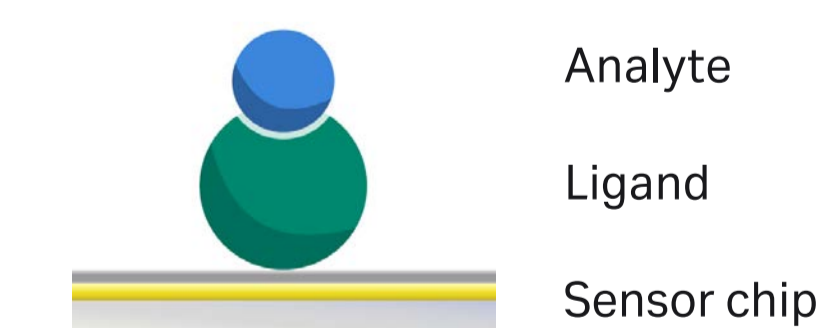
- **Take advantage of affinity tags** that are present on your molecule.
- **Valency:** attach the interactant with most binding sites to avoid avidity problems.
- **Molecule amount:** attach the interaction partner with lowest amount to save reagents.
- **Molecule purity:**
 - Prefunctionalized/capture surfaces: no specific purity consideration needed.
 - Direct coupling: attach the purest interactant to ensure specific binding interactions.
Note: cross-reactivity examination /consideration is always needed in interaction analysis experiments
- **Molecular weight:** avoid attachment of very large (relative molecular mass, $M_r > 1\,000\,000$) or small ($M_r < 500$) molecules. If significant difference in molecular weight of interacting proteins, attach the smaller protein molecule.

Direct immobilization or capture approach?

Covalent direct attachment advantages

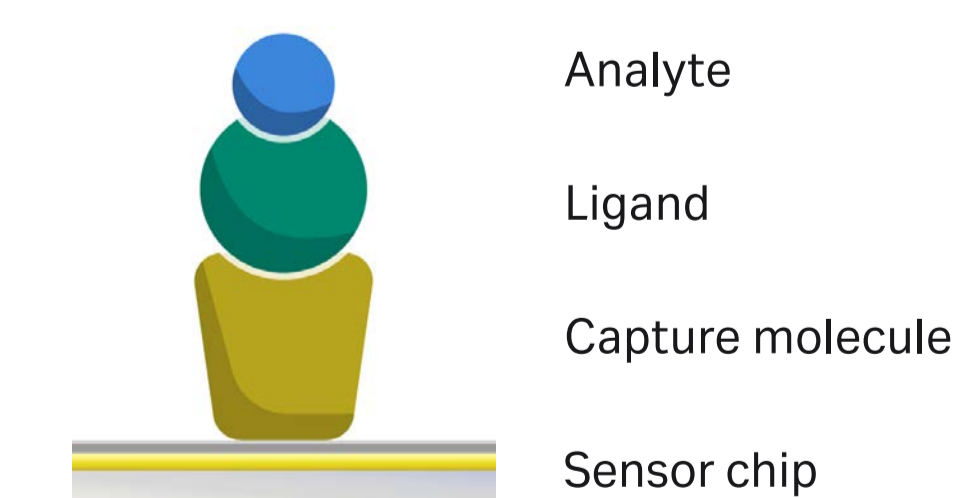
- Consumes less ligand
- Higher binding capacity
- Very stable immobilization with no leakage
- Shorter cycle times

Examples: Amine coupling, Ligand-thiol coupling, Surface-thiol coupling, Maleimide coupling, Aldehyde coupling



Capture-based attachment advantages

- Orientation-specific attachment
- Selective capture of ligand from crude samples
- Simpler assay development (regeneration is independent of the captured molecule¹)
- Interactant on the surface is easily changed¹
- For ligands that are difficult to immobilize or regenerate
- For unstable ligands



Examples: see selection guide above.

¹ Except for streptavidin/biotin due to the extremely high affinity

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