

Technical Bulletin



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Reference for Reichert Carboxyl Surface Catalog number 13206061 – 10% Alkane thiol (PEG)₆-COOH , 90% Alkane Thiol (PEG)₃-OH: Lahiri, Joydeep, Lyle Isaacs, Joe Tien and George M. Whitesides. 1999. A Strategy for the Generation of Surfaces Presenting Ligands for Studies of Binding Based on an Active Ester as a Common Reactive Intermediate: A Surface Plasmon Resonance Study. *Analytical Chemistry*, 71, 777-790.

General Principles:

- ? Amount of protein or peptide immobilized depends on: nature of protein, e.g. size, hydrophobic, hydrophilic, contaminants present (Tris is usually bad)
- ? pH and ionic strength of immobilisation buffer – generally 1 pH unit below the P_i of the protein
- ? This bulletin is intended as an overview of available coupling methods. Contact Reichert Inc. for more detailed information.

1) Slides Presenting a Carboxyl Surface

- a) Amine Coupling – Carboxyl is converted to NHS ester by exposing surface to 40 mg/mL EDC, 5 mg/mL NHS for 8 to 10 minutes. An amine on e.g. protein reacts with the NHS ester to form an amide bond between the surface and protein
- b) Streptavidin/ Neutravidin Surface – Streptavidin or Neutravidin is amine coupled to COOH as described in a).
- c) Protein G / Protein A Surface - Protein G or Protein A is amine coupled to COOH as described.
- d) Nickel NTA Surface: Amine Couple N⁺, N⁺ -Bis(carboxymethyl)-L-lysine Hydrate, (Fluka 14580) in 20 mM Sodium Acetate pH 5.2. Inject 40 mM NiSO₄ to form Ni chelate. Inject 200 mM Imidazole pH 7.4 to regenerate. For an alternate surface and general methodology see: Sigal, George B. et.al. 1996. A Self-Assembled monolayer for the Binding and Study of Histidine Tagged Proteins by Surface Plasmon Resonance. *Analytical Chemistry*. 68: 490-497.

Technical Bulletin

- 2) Creating an Amine Surface: Start with the Reichert COOH surface and amine couple ethylene diamine (0.5 M pH 8.5 with HCl). Compounds containing a carboxyl can then be immobilized by injecting the compound mixed with an equal concentration of EDC.
- 3) Maleimide Coupling using BMPH – Immobilization via a –SH group on a protein.
 - a) Start with Reichert COOH slide
 - b) Activate with EDC/NHS
 - c) inject 1 M ethanolamine-HCL, pH 7.0 in 100 mM NaPO₄ buffer
 - d) Inject 50 mM BMPH N-[β-maleimidopropionic acid]hydrazide in 10 mM Na borate buffer, 1 M NaCl pH 8.5
 - e) Inject Ligand
 - f) Deactivate with 50 mM Cysteine, 1 M NaCL, 100 mM Na Acetate pH 4.
- 4) Thiol Coupling
 - a) Start with Reichert COOH slide
 - b) Activate with EDC/NHS
 - c) inject 80 mM PDEA (2-(2-pyridinyldithio)ethaneamine) in 100 mM Na Borate pH 8.5 immediately after solution preparation
 - d) inject ligand
 - e) Deactivate with 50 mM Cysteine, 1 M NaCL, 100 mM Na Acetate pH 4.
- 5) DNA immobilisation. Traditionally 5' biotinylated DNA is immobilized on a strepavidin surface. Alternately, 5' SH-DNA can be used to form a self-assembled monolayer or mixed self-assembled monolayer directly on bare gold. see papers by Rosina M. Georgiadis, Boston Univ. Dept of Chemistry. E.g. Wolf, Lauren K. Yang Gao, and Rosina M. Georgiadis. 2004. Sequence-Dependant DNA immobilization: Specific versus Nonspecific Contributions. *Langmuir*, 20, 3357-3361.
- 6) Hydrophobic Surface for creating a lipid monolayer
 - a) A self-assembled monolayer of e.g. mercaptoundecane can be created on a plain gold slide by immersing the gold slide in a 1 mM thiol in 100% ethanol for 24 hours. The gold should be cleaned with Pirhana solution prior to forming the self-assembled monolayer. Pirhana solution is 30% of 30% H₂O₂ and 70% H₂SO₄.
- 7) Hydrophobic surface for capturing Vesicles
 - a) Start with Reichert COOH slide
 - b) Activate with EDC/NHS
 - c) Dissolve 5 mg phytosphingosine in 0.5 mL DMSO. Add 0.5 mL 40 mM Na Acetate pH 5.2. Solution will become slightly cloudy. Spin to remove cloudiness. Inject over activated surface for 45 minutes. Deactivate surface with 1 M ethanolamine pH 8.5.

Technical Bulletin

- d) Inject 1 mM lipid vesicles. Time for immobilization depends on lipid. Inject 50 mM NaOH to remove loosely bound vesicles. Vesicles can be removed with a short injection of 20 mM CHAPS.