Immobilisation of Proteins

• Absorption

• Entrapment

• Covalent attachment
Immobilisation of Proteins

Adsorption

• Proteins readily adsorb onto surfaces
• May denature on doing so
• May be sterically hindered
• Leads to non-specific binding, i.e. an interference in a sensor
Immobilisation of Proteins

Entrapment

• Hydogels – polymer networks in which the pendant groups on the polymer are hydrophylic leading to trapped water in which other molecules, e.g. proteins may also be entrapped

• Good if a monolayer is not an integral part of the sensor mechanism as thicker layer may lead to a larger response

• Diffusion limitations may cause a slower response.
Photoinitiator

initiator

monomer

cross-linker
Exposing a Window in Silicon Oxide

iii. Illuminate, e.g. with UV light, through a mask

Mask

Photoresist

Silicon oxide

Si Substrate
A hope for the future: Immobilisation with conducting polymers
Immobilisation of Proteins

Covalent attachment

- Probably the most common immobilisation procedure
Derivatising silica or glass surfaces

Glass or silica surface

\[
\begin{align*}
\text{Si-OH} & + \text{C}_2\text{H}_6\text{O-Si-R} \rightarrow \text{Si-O-Si-R} + 3 \text{C}_2\text{H}_6\text{OH} \\
\text{Si-OH} & + \text{C}_2\text{H}_6\text{O-Si-R} \rightarrow \text{Si-O-Si-R} + 3 \text{C}_2\text{H}_6\text{OH}
\end{align*}
\]

Volatile as is the silane derivative solvent, e.g. chloroform

\[R\] varied depending on the application

e.g. \( R = -\text{CH}_3 \) and a hydrophilic surface is converted to a hydrophobic surface (used in some liquid crystal device fabrications)

\( R = -\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2 \) used in protein immobilisation as follows
Linking to derivatised surfaces

Glass or silica surface

\[
\text{Si} : \text{O} \quad \text{Si} : \text{O} \quad \text{Si} : \text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2 \\
\text{Si} : \text{O} \\
\text{Si} : \text{OH}
\]

+ linking chemicals

\[
\text{NH}_3^+ + \text{OOC}
\]

Protein

Protein
IMMOBILIZATION OF CAPTURE ANTIBODY

A.
- 0-Si-(CH₂)ₙ-CO.NH
- CAPTURE IgG

B.
- 0-Si-(CH₂)ₙ-CO.NH
- AVIDIN
- HN HN
- S(CH₂)ₙ-CO.NH
- BIOTINYL-IgG
ORIENTING THE CAPTURE ANTIBODY

Random immobilisation links, e.g. via any surface amino group, leads to some ineffective orientations.
Immobilisation of Proteins

Photo-activated covalent attachment

Photonic activation of disulfide bridges achieving oriented protein immobilization on biosensor surfaces

Maria Teresa Neves-Petersen, Torben Snabe, Søren Klitgaard, Meg Duroux and Steffen B. Petersen

Protein Sci. 2006 15: 343-351
Exposing a Window in Silicon Oxide

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Photo-immobilisation of the Fab fragment of IgG
Fab fragments of IgG

- **Fragment antigen binding (Fab)**
- **Papain**: An enzyme that splits a specific peptide bond

Fabs are the antigen binding fragments of antibodies, and they can be isolated from antibodies using enzymes like papain. Papain is a proteolytic enzyme that selectively cleaves peptide bonds in certain parts of proteins, allowing the isolation of Fabs and Fc fragments.
Derivatising silica or glass surfaces to give a thiol rich surface
Photo-immobilisation of the Fab fragment of IgG
Amino Acids: Fluorescent

- **Tryptophan** (Trp, or W)
  
  ![Tryptophan structure](image)

- **Tyrosine** (Tyr, or Y)
  
  ![Tyrosine structure](image)

- **Phenylalanine** (Phe, or F)
  
  ![Phenylalanine structure](image)
Energy transfer from the excited state tryptophan to an adjacent disulphide link —splitting the link to give to reactive sulphurs that readily react with the thiols on the derivatised sensor surface.
Photo-immobilisation of the Fab fragment of IgG
ORIENTED CAPTURE ANTIBODY

SUBSTRATE (e.g. glass or plastic)