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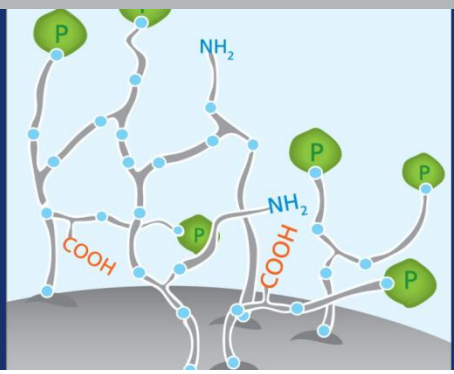

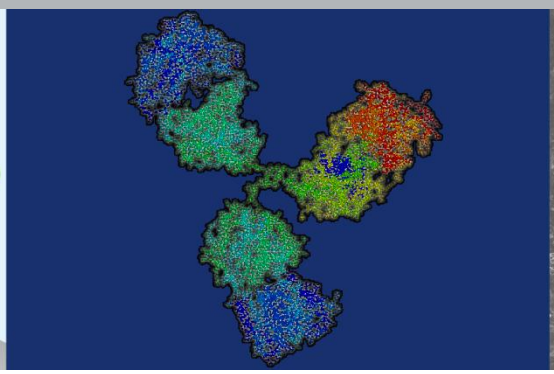

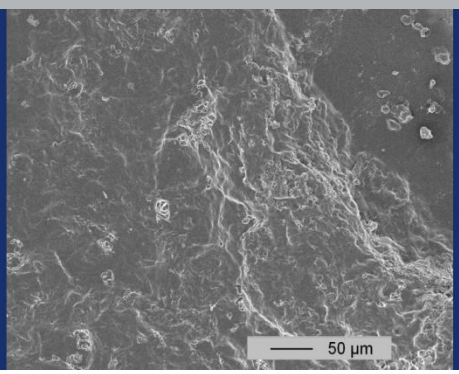

1 Scheme of nano-hydrogel film

2 Immunoglobulin molecule

3 REM-micrograph of a swollen and freeze-dried gel

Immobilizing active Proteins

Surface Bonded Nanohydrogel Films host Proteins

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Background

Many applications in the field of biotechnology and biomedical diagnostics rely on immobilized bioactive molecules. They catalyze reactions and they help to detect medical conditions. For some of these molecules the immobilization is straight-forward. Others tend to lose their function upon being immobilized. In particular proteins can alter their ternary structure due to molecular interactions which then influence their biochemical properties.

In order to prevent this kind of undesired alterations the immobilization site has to provide a certain environment.

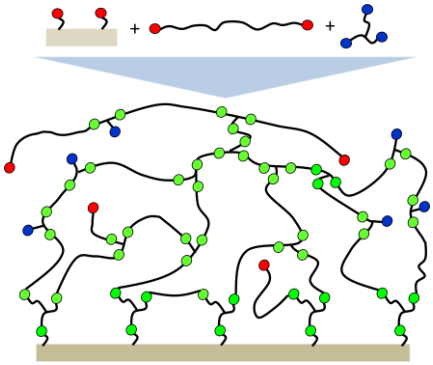
Concept

Proteins work in aqueous solutions. For a good immobilization hydrogels seem to be an appropriate environment.

These materials consist mainly of water and the gel-forming network molecules are usually concealed with surrounded water molecules. The actual coupling site is required to be present in an appropriate concentration. Last but not least, the hydrogel network must be sufficiently "loose" to allow the diffusion of the proteins.

A new concept for the preparation of surface bonded networks was developed in order to meet these demands. Bi-functional molecules react with tri-functional molecules and form a network. The former ones are incorporated as branches while the latter ones form the nodes or junctions. The mesh size is largely determined by the size of the branch molecules. The basic physical and chemical properties are determined by the properties of these molecules.

Since the reactions are more or less incomplete some functional groups remain and can be used for the immobilization and for further reactions.



Bi- and trifunctional molecules form a network on a surface

Poly(ethylene glycol) (PEG) was used as a branch molecule because it is known for its good compatibility with proteins. PEG is available with a variety of functional groups at the end of the chains. Bisamino-PEG reacts readily with triepoxide linkers or with triacid chloride linkers.

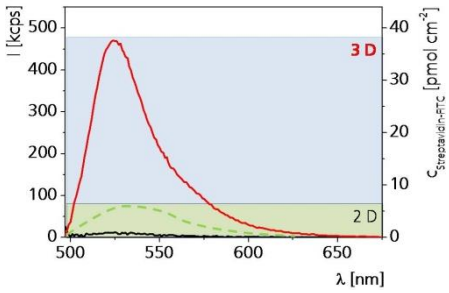
Adding a third component to the reaction system provides new opportunities for the modification of the basic network properties. For example, an amino acid is coupled to the network via the amino group and introduces carboxylic acid groups.

The reaction scheme resembles a tool box. According to particular demands, networks with a well defined structure and tailored properties can be prepared. The parameters are:

- layer thickness: 5 nm .. 500 nm
- mesh size (branch length): <5 nm .. >200 nm
- basic network chemistry
- functional groups: 5 pmol cm^{-2}.. ~800 pmol cm^{-2}

Examples

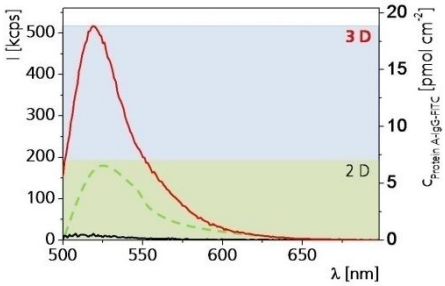
Aminated biotin was be added to the reaction mixture for the preparation of biotin-containing networks. Using the protein streptavidin which was labelled with fluoresceine isothiocyanate (FITC) the amount of biotin in the network can be quantified by the fluorescence intensity.



Fluorescence spectra of FITC-labelled streptavidin immobilized at a biotin-containing network. (red: immobilized in a 3D network; green: immobilized on a flat surface; black: background from interactions with a biotin-free network)

In the case of a network with a thickness of about 30 nm streptavidin can be coupled with a concentration which is about six times higher than after the immobilisation on a flat surface. The streptavidin shows only very few interactions with biotin-free hydrogels which indicates a low unspecific binding.

In other experiments label-free streptavidin was used and biotinylated protein A was coupled. Then FITC-labelled immunoglobulin G recognized the protein A. All the complex of immobilized molecules has a molecular mass of about 260 kDalton. The fluorescence intensity was three times higher than after an immobilization on a flat surface.



Fluorescence spectra of FITC- labelled IgG bonded to protein A which was immobilized via streptavidin inside a biotin-containing network (red) compared with a flat surface (green) and the background signal from a biotin-free hydrogel (black)

Development status

Together with partner biologists successful application tests were run with microarrays for the immobilization of proteins and RNA. The interactions were detected with fluorescence readers and with MALDI-TOF. The coating on SPR-prisms gives rise to more intense signals. The networks were prepared on polymers (PE, COC), glass, and metals.

We are able to produce the networks on slides in a batch size of 50 pieces. The synthesis of the network in a highly efficient roll-to-roll process is under development.

Partners for the application of the hydrogels and for further developments are welcome.