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Project :	Oriented antibodies on tailor-made Cu surfaces
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Fields of interest:	Surface modification, organic monolayers, micro and nanofabrication, biosensing, antibody engineering, molecular farming
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Introduction

Biosensors are important for many medical and bioanalytical applications. Therefore, more and more scientists bring (bio-) functionalized surfaces and subsequent immobilization of biomolecules into focus. To realize such a detector the biomolecules have to be immobilized covalently to the surface in an oriented way. Also gold is, currently, the 'number one'-material for the production of biosensors, the chip industry shows a great interest in copper. Copper provides the possibility of direct integration with currently existing CMOS (Complementary Metal Oxide Semiconductor) technology.

Goal

We aim to couple antibody fragments in an oriented way onto functionalized copper surfaces. Llama antibody fragment, also known as VHH, possess the same high binding specificity than conventional antibodies by being smaller, highly stable and able to properly refold after denaturation. These properties, especially the unique binding to a particular target and the ability to respond to substances at a very low level make them the optimal choice to function as a sensor element in a biosensor. In our laboratory we study the functionalization of Llama antibodies with non-natural groups that enable the specific immobilization on a copper surface, representing the biosensor surface.
To specifically functionalize the antibody fragments with an azide group we use the amino acid homolog azidohomoalanine. This homolog is incorporated in the llama antibody fragments by *E.coli* during the production. The strain promoted 1,3-diploar cycloaddition (SPAAC) or the copper(I) catalyzed 1,3-diploar cycloaddition (CuAAC) between an azide and an alkyne can then be used to immobilize the proteins (Fig. 1).

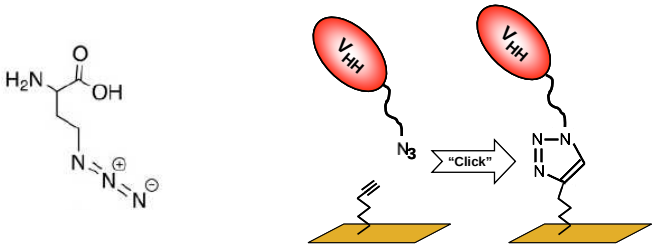

Fig.1 *Left: methionine analog azidohomoalanine (AHA). Right: once incorporated in the protein the azide group of AHA is accessible for click reaction.*

Progress achieved

To couple the molecules to a copper chip an alkyne terminated surface is needed. Research at our laboratory showed that molecules with different functionalized end groups form dense self-assembled monolayers (SAMs) on copper. The formation of monolayers on copper surfaces is studied and the quality of the SAMs is characterized using XPS, IRRAS and contact angle measurements.
Recently our group has succeeded in preparing oxide free NHS-ester terminated monolayers on Cu substrate (Fig 2). Such monolayers can serve as an amine reactive platform for further functionalization to yield alkyne terminated monolayers on Cu.

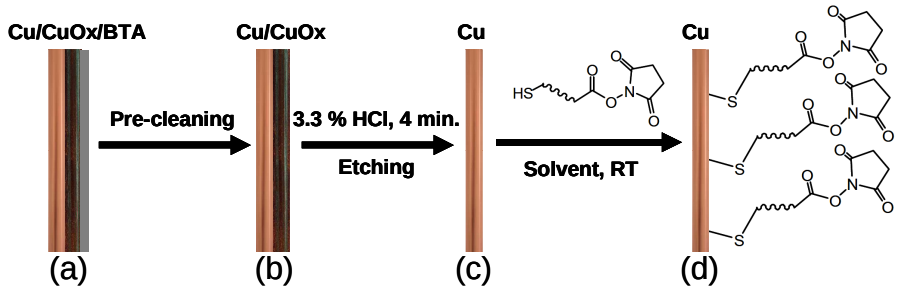

Fig.2 *Formation of monolayers on copper. A chemomechanically polished copper wafer was coated with benzotriazole (BTA) as a corrosion inhibitor (a). After pre-cleaning treatments to remove BTA layer, the cleaned copper (b) was etched to remove copper oxide from the surface. The etched copper (c) was immersed in thiol solutions to form NHS-ester-terminated SAMs (d).*

Further research

We are currently concentrating our research on the characterization of the azidohomoalanine modified llama antibody fragments. Successful incorporation and accessibility of AHA in the VHH will be investigated.

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