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B003 Innovative analytical system for screening on lectins

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Lectins are proteins or glycoproteins from plants or animals, which are able to bind specifically sugar-residues of cell walls or membranes. This reaction changes the physiology of the cell wall and influences the metabolism of the cell. Some lectins of plants stimulate the immune system by unspecific activation of T cells or influence cell division; others cause agglutination of cells (e.g., erythrocytes) and are therefore from therapeutic interest (1).

In a new approach, biomolecular interaction analysis (BIA) was utilized for a screening program on lectins. In a first step, a lectin-binding sugar was covalently immobilized on a surface of thin glass plate (100 µm). Then, the test solution was divided in several parts and different mono-saccharides were added to each part. Individual samples were analysed by BIA and characteristics of the binding-domains were specified. Alternatively, glasses coated with different types of sugars may be used. In dependence of the added monosaccharide, a more or less stable binding to the sugar-surface of the glass-support was monitored. Additionally, the method can be used for a bio-guided fractionation of nature-derived extracts. As an example, BIA analysis was tested for the production of a recombinant lectin in *E. coli*.

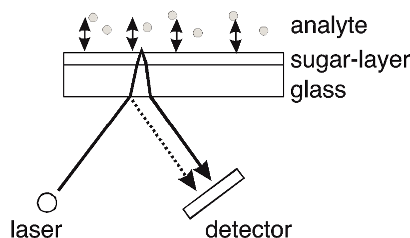

Figure. Scheme of the interferometric detector (2) for the analysis of sugar-lectin interactions. The reflected light of the sugar-layer is analysed by a CCD-array.

References: 1. Tsokos M. et al. (2002) Virchows Archiv-An International Journal of Pathology 440 (2): 181-186. **2.** Nikitin P.I. et al. (2000) Quantum Electronics 30 (12): 1099-1104.

B004 Recombinant fluorescent proteins for testing of affinity modules in phytochemical analysis

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To examine the quality and content of bioactive compounds in herbal medical products, modern affinity techniques, e. g. those based on affinity modules, can be used. These modules consist of polymeric material coated with biomolecules (e.g., antibodies) which specifically interact with compounds of interest. To characterise the quality of modules, a powerful tool for testing based on recombinant DsRed was developed. DsRed is a recently discovered flourescent protein from a corallimorpharian of the *Discosoma* genus exhibiting an intrin-sic and unique red fluorescence. Further on, DsRed is of impressive brightness and stability against pH changes, de-naturants, photobleaching, and does not require any cofactors for fluorescence (1).

After functionalising the polymer-surfaces of modules by plasma-treatment, a spacer was introduced which displayed a metal-chelating group at its outer end (Figure 1). In the next step, Ni-ions were trapped and bound to recombinant His-tagged DsRed. The modified DsRed was overexpressed in *E. coli* for the first time. A nearly quantitative immobilisation of this model protein was obtained for solutions containing 5 - 30 µg of His-DsRed, related to one affinity module with a weight of 50 mg. Immobilisation of His-DsRed could be performed within 120 min. Affinity modules allowing coupling of proteins as monolayers as well as those carrying an polypropylene-glycol gel structure at the inner surface were tested. The latter one showed a significantly better binding capacity and shorter immobilization times.

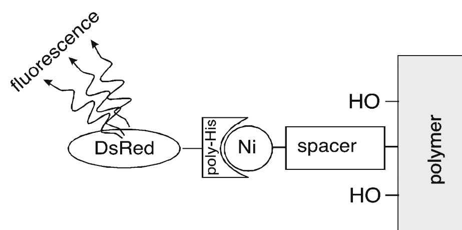

Figure 1. Immobilization of DsRed on polymeric surfaces.

References: 1. Baird, G.S. and Tsien, R.Y. (2000) Biochem. 97, 11984-11989.