SURFACE MODIFICATION BY HYDROPHOBIN PROTEINS

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Introduction: Hydrophobins are small fungal proteins that can self-assemble into amphipathic monolayers at hydrophobic:hydrophilic interfaces. The monolayers formed by class I hydrophobins are composed of fibrillar structures called rodlets. The monolayers are very robust and can also reverse the wettability of the surface that it binds to. Previous work has shown that hydrophobins can be applied to modify various materials, such as carbon nanotubes and silicon chips. The layer not only increases the biocompatibility of the materials, but also provides a suitable platform for protein immobilization. However, this has involved non-covalent attachment of functional groups to the hydrophobin monolayer. Here, we have used the well studied hydrophobin EAS to show the potential of using hydrophobin proteins for surface modification and protein immobilization via covalent crosslinking. This has the potential to produce very stable and biocompatible surfaces with tailored functionalities.

Methods: *EAS production* The proteins were expressed recombinantly and purified. *Fluorescein isothiocyanate (FITC) labelling* Ten-fold excess of FITC (Sigma, MO) was used for labelling and the products were purified using reverse phase high pressure liquid chromatography (RP-HPLC). *Enzyme-linked immunosorbent assay (ELISA)* Monolayers were formed by FITC-labelled EAS on Teflon® coated glass slides. Mouse anti-FITC antibody (Sigma, MO) and goat-anti-mouse HRP conjugated antibody (Santa Cruz Biotechnology, CA) were used for detection of immobilised FITC. *Contact angle measurement* A hydrophobin monolayer was formed on OTS-coated silicon chips and the contact angle formed by water drops placed on top of the protein layer was measured using a Kruss DSA 10MK2 drop shape analyser. *ThioflavinT (ThT) binding assay* EAS proteins were prepared in a solution of 40 µM ThT (Sigma, MO) and vortexed to induce assembly of hydrophobin rodlets, which bind to ThT, allowing the process to be monitored with fluorescence. *Electron microscopy* Rodlets were formed on the surface of a drop of protein solution and transferred to carbon coated copper grids (ProSciTech, QLD). Samples were then stained with 2% uranyl acetate (BDH Chemicals, UK) and examined using a Philips CM12 EM. *N-terminal modification of EAS* The EAS mutant 3R (which contains only one primary amino group) was modified with the crosslinker sulfo-SDAD (Thermo Scientific, IL). The product 3RC was purified using RP-HPLC. *Glucose oxidase (GOD) immobilisation* OTS-silicon chips with different coatings were incubated in a solution of GOD (Sigma, MO). UV light was applied to activate the crosslinker, allowing GOD to be covalently linked to the chip. The activity of immobilised GOD was detected with Amplex Red® (Sigma, MO).

Results:

N-terminus is available for modification

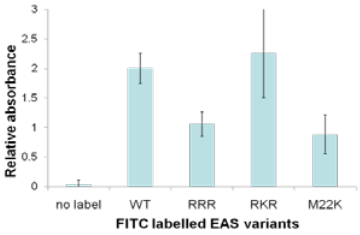

Figure 1. Signal detected by ELISA from FITC-labelled surfaces. The amino group on the N-terminus and the middle lysine were available for modification and the immobilized groups were solvent accessible.

N-terminal modification alters EAS self-assembly

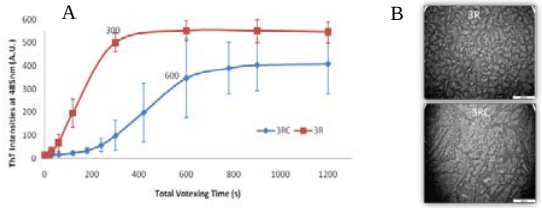

Figure 3. (A) ThT binding assay and (B) Electron microscope images for 3R and 3RC indicate modified EAS can form rodlets but at a slower rate.

Modified hydrophobin was still highly surface active

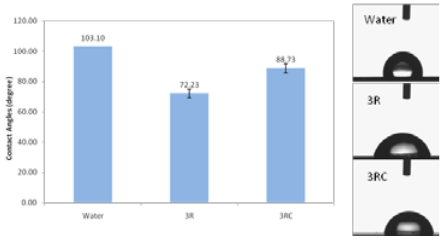

Figure 4. Contact angles of OTS-silicon chips with different coatings.

GOD was bound tightly without crosslinking

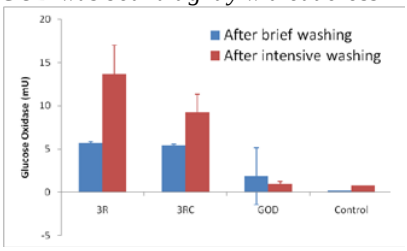

Figure 5. GOD activity detected on different coated OTS-silicon chips.

Conclusions: Here we show the possibility of using hydrophobin proteins as a platform for covalent attachment of proteins to substrate materials via N-terminal modification. Although results have shown GOD binds tightly and non-covalently to rodlets and maintains its activity, crosslinking would be preferable for protein immobilisation as it is more stable over time and allows control of the orientation of the functional groups.

References: A.H.Y.Kwan, PNAS, 2006:103:3621-3626