Immobilization of MUC-1 peptides on Supported Lipid Bilayers for Early Pancreatic Cancer Detection

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Pancreatic cancer is highly lethal due to the detection of the tumor at an advanced stage, in most cases after it has already metastasized. This is because it presents few symptoms in its early stages [1]. Currently, the main biomarker used for diagnosis in symptomatic patients is carbohydrate antigen CA19-9, with a sensitivity of 72%.[2] However, it has a much worse predictive value [3]. Hence, there is a need to develop new biomarkers for earlier detection and integrate them in diagnostic platforms that can be used to improve patient prognosis .

MUC-1 is a transmembrane protein that is overexpressed and aberrantly glycosylated in tumoral cells [4]. Circulating antibodies against tumoral MUC-1 could potentially be used for early cancer detection [5]. In this project, we focused on immobilizing different MUC-1 variants that resemble tumoral MUC-1 on Supported Lipid Bilayers (SLBs). SLBs have highly superb anti-fouling properties, and their components can be tuned, including for example the addition of lipids that carry functional groups for click chemistry [6]. Specifically, we incorporated a DBCO-phospholipid that is suitable for Strain-Promoted Azide-Alkyne Click Chemistry (SPAAC) with azide-functionalized MUC-1 variants. Once MUC-1 was immobilized, human serum samples from healthy and sick patients could be introduced to verify the specific and selective recognition of healthy and sick individuals.

Different MUC-1 variants were synthesized to study their binding to the SLB. Initially, with an azide group on the peptide and a DBCO-modified phospholipid in the SLB, the SPAAC reaction was monitored using Quartz Cristal Microbalance with Dissipation (QCM-D). The results showed that the reactivity of the click chemistry varied depending on the amino acid closest to the azide group, with the presence of cysteine leading to higher efficiency (Figure 1B) . However, when the functional groups were reversed—DBCO on the peptide and azide on the SLB—the amino acid had no influence on binding (Figure 1C). Once MUC-1 was immobilized on the SLB, its recognition by the anti-MUC-1 antibody SM3 was studied. In both cases, MUC-1 was specifically recognized by the antibody (Figure 2). These results demonstrate that MUC-1 can be immobilized on an SLB without losing its recognition properties, making it a promising system for a diagnostic tool.

References

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Figures

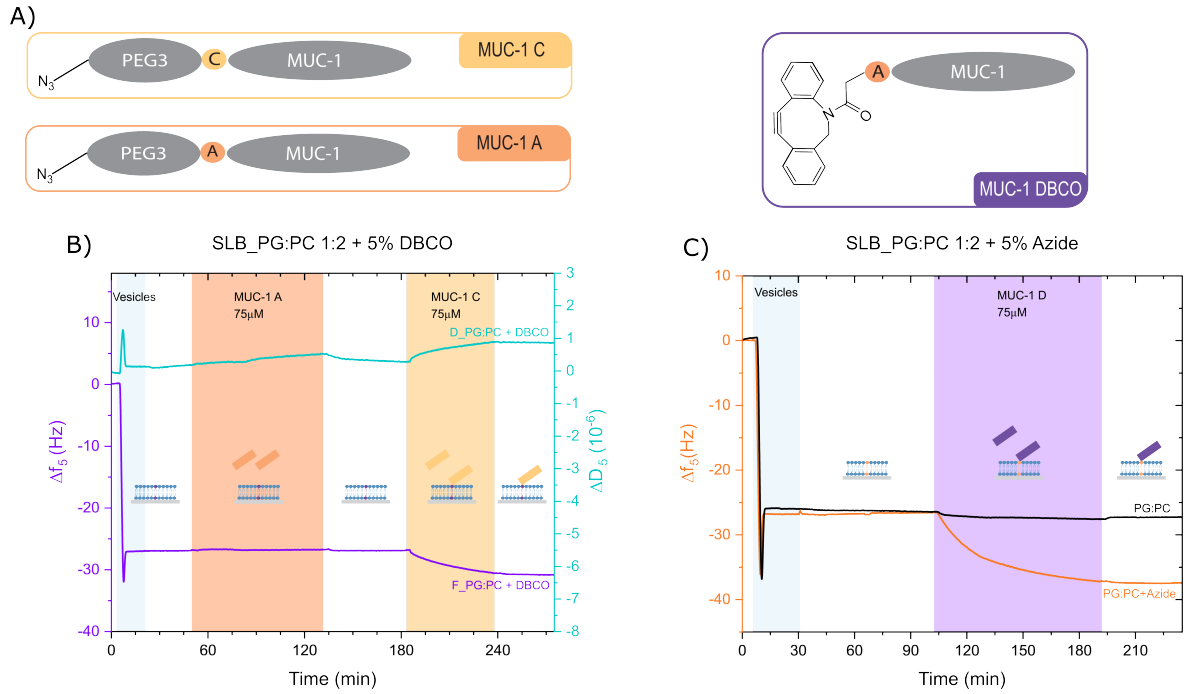

Figure 1. **A)** Scheme of the different MUC-1 peptides. **B)** MUC-1 A does not bind to the DBCO-SLB (orange area) since there is no frequency change instead, MUC-1 C binds successfully (yellow area). **C)** MUC-1 DBCO specific coupling to the Azide functionalised SLB. QCM of Azide functionalised SLB (orange) versus control SLB (black) analysing the binding of MUC-1 D (purple area), where there is a change in frequency only in the functionalised SLB.

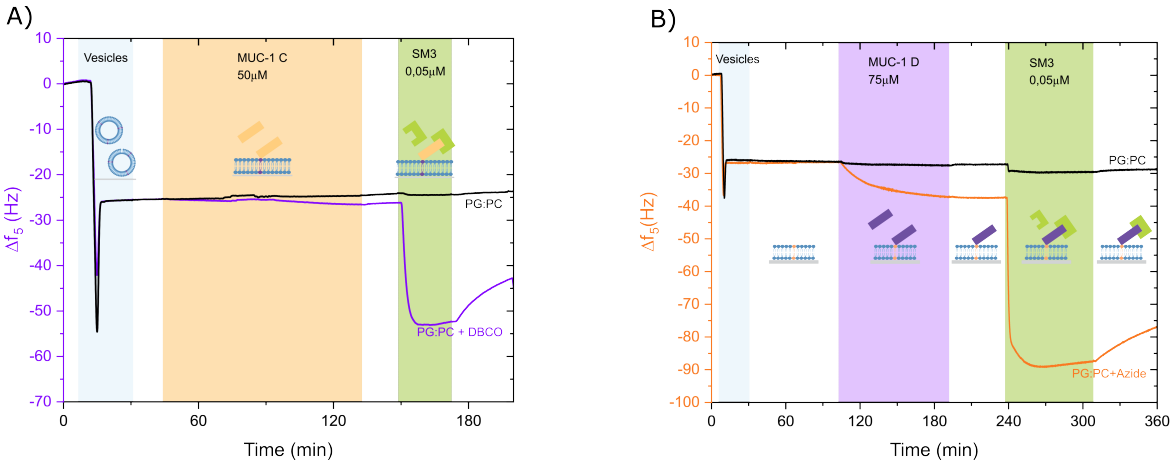

Figure 2. **A)** Antibody recognition of bound MUC-1 C to the DBCO-SLB. QCM of functionalised (purple) and control (black) SLB, in the yellow area the MUC-1 C was flushed and it just bound to the DBCO-SLB. After, in the green area the antibody binds specifically to the MUC-1 C. **B)** QCM of the azide functionalised SLB (orange) and control SLB (black) with MUC-D (purple area). Specific binding of the SM3 antibody (green area) to the MUC-D + Azide SLB (orange).