

A biosensor-based concept for antibody-GPCR interaction studies using immobilized lipoparticles

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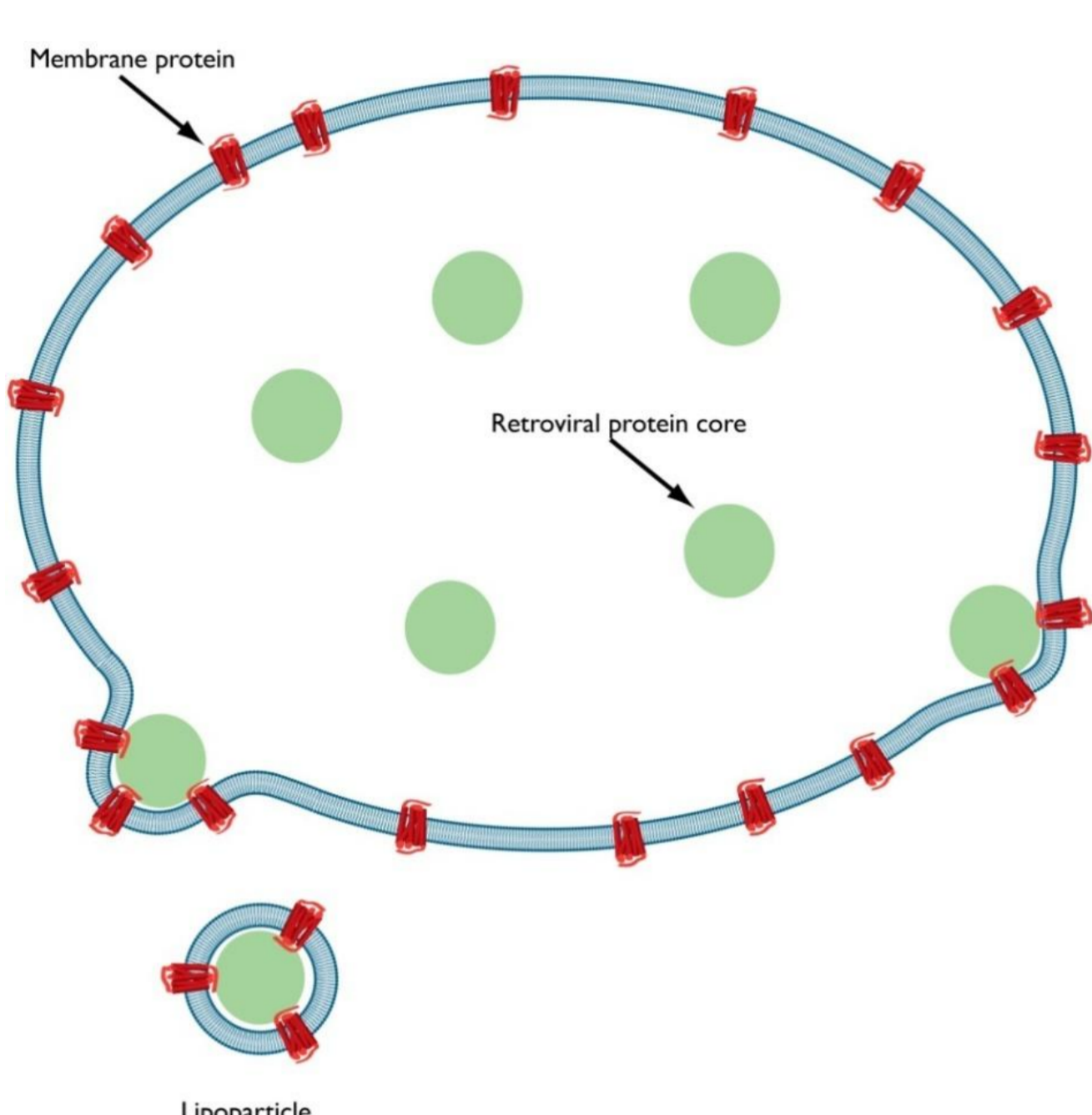
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INTRODUCTION

In this study lipoparticles containing the CXCR4 chemokine receptor were immobilized using memLAYER on the Attana LNB carboxyl sensor chip. The applicability of lipoparticles and memLAYER for functional studies of GPCRs on the Attana A200 biosensor are demonstrated as well as the benefits of the memLAYER-immobilization in terms of functionality of the immobilized receptor and the enhancement in the antibody binding response.

TECHNOLOGIES

GPCR PRODUCTION



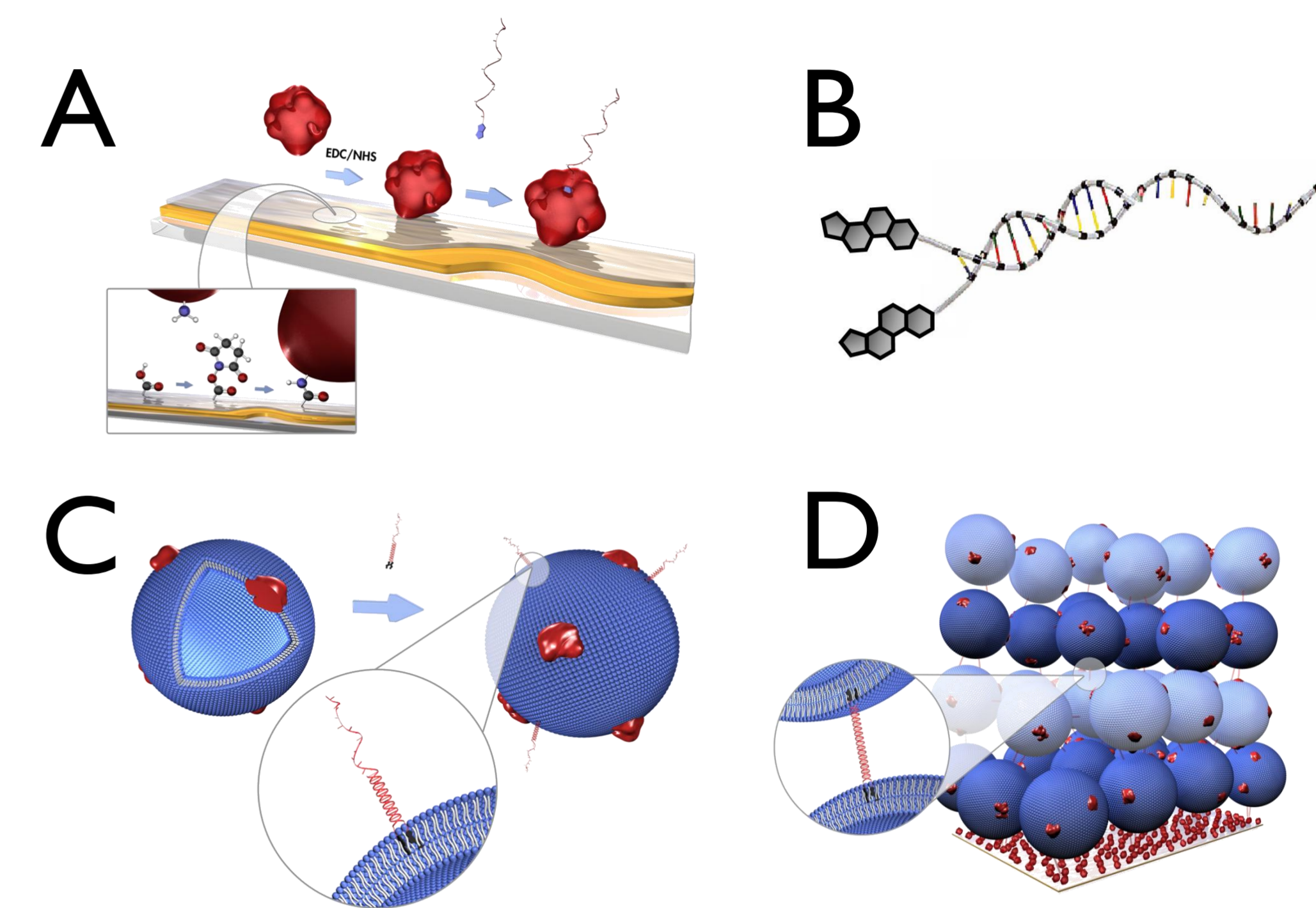
The Lipoparticle is a lipid vesicle model system from Integral Molecular that is produced by cells that highly overexpress a specific membrane protein. These cells also contain retroviruses. When the retroviral core proteins bud off from the cells they become enveloped by the cell membrane to produce lipoparticles containing the over expressed membrane protein.

BIOSENSOR TECHNOLOGY



The Attana 200 QCM-based biosensor can be used to determine specificity, kinetics and affinity, amongst other binding characteristics of biomolecules and macrostructures such as cells, antibodies, proteins, lipoparticles, viruses and bacteria. The Attana 200 enables study of interactions under temperatures and crude conditions (up to 100% serum), matching in vivo environment. Eliminating the need to purify, label or pre-treat the samples, the Attana 200 provides biologically relevant data and saves time by speeding up molecular screening, selection and characterization.

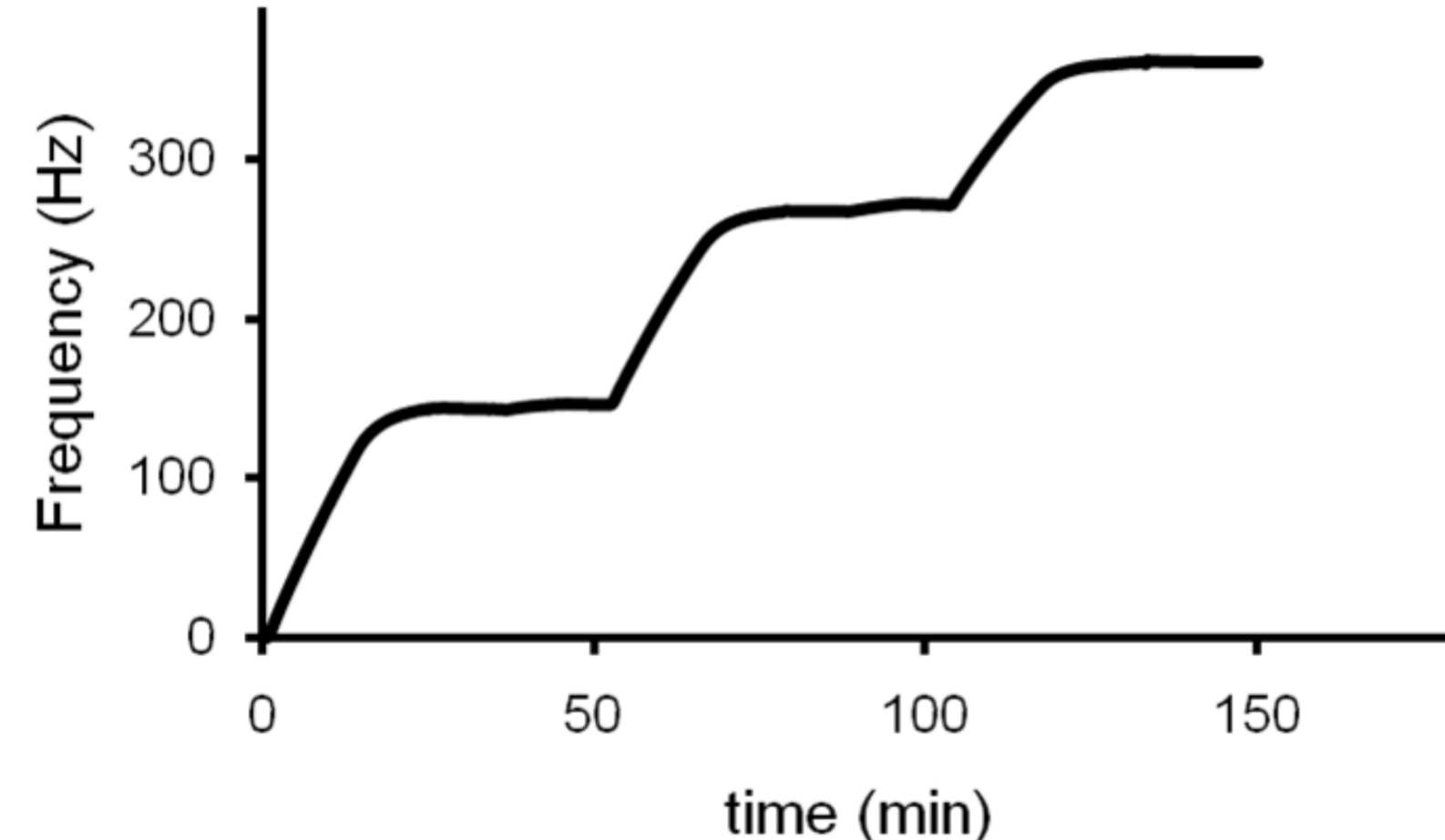
MEMBRANE PROTEIN BIOSENSOR ANALYSIS



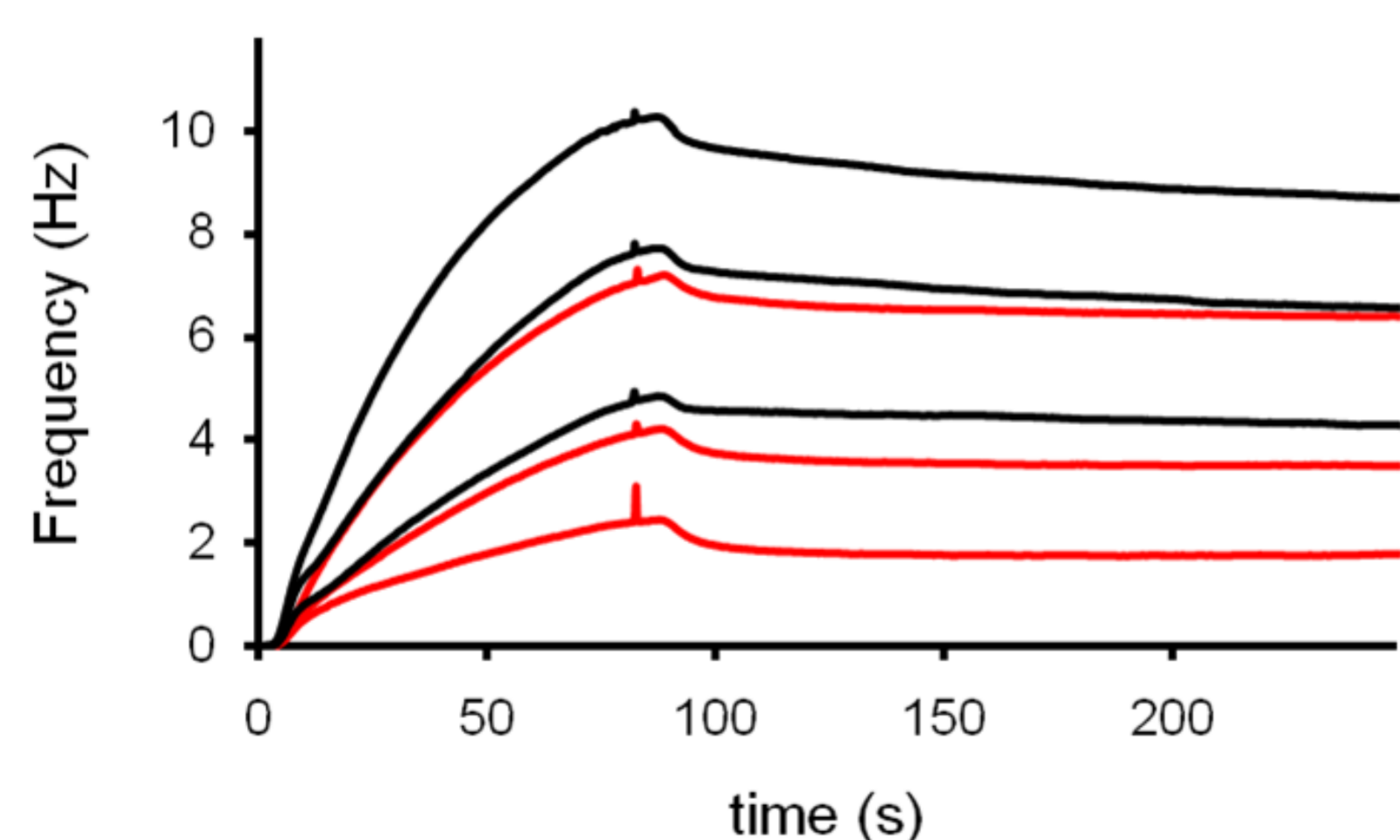
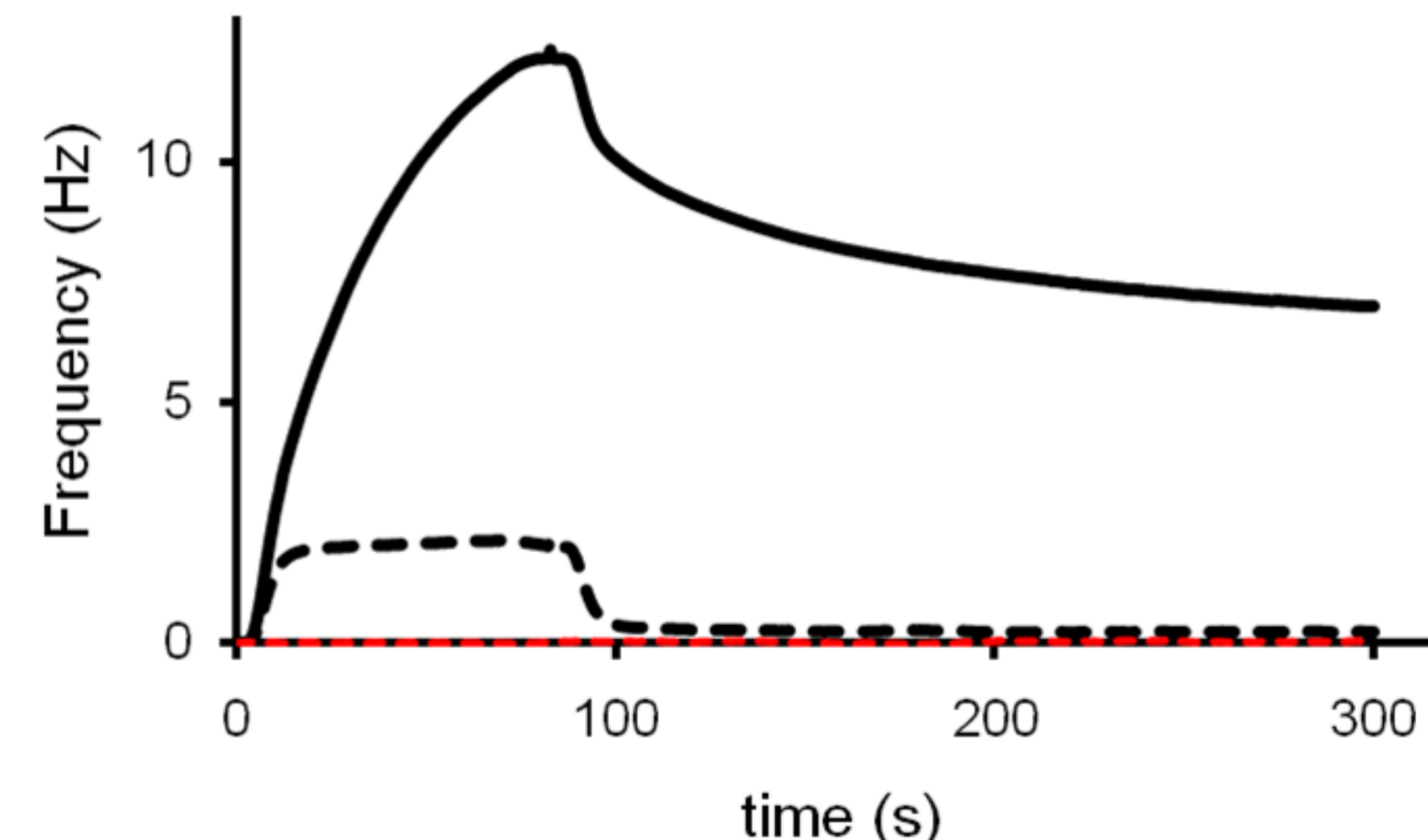
The methodology behind the memLAYER chemistry kit, using which lipid vesicles can be immobilized to biosensor surface via DNA-hybridization. (A) The sensor surface is functionalized with NeutrAvidin in complex with a biotinylated DNA. (B) The proprietary cholesterol/DNA tag is (C) spontaneously incorporated into the lipid bilayer. The lipid vesicles can then be anchored to the sensor surface via hybridization between the DNA at the sensor surface and the DNA exposed at the lipid vesicle surface. (D) Using the same principle of DNA-hybridization, multiple layers of lipid vesicle can be attached.

RESULTS

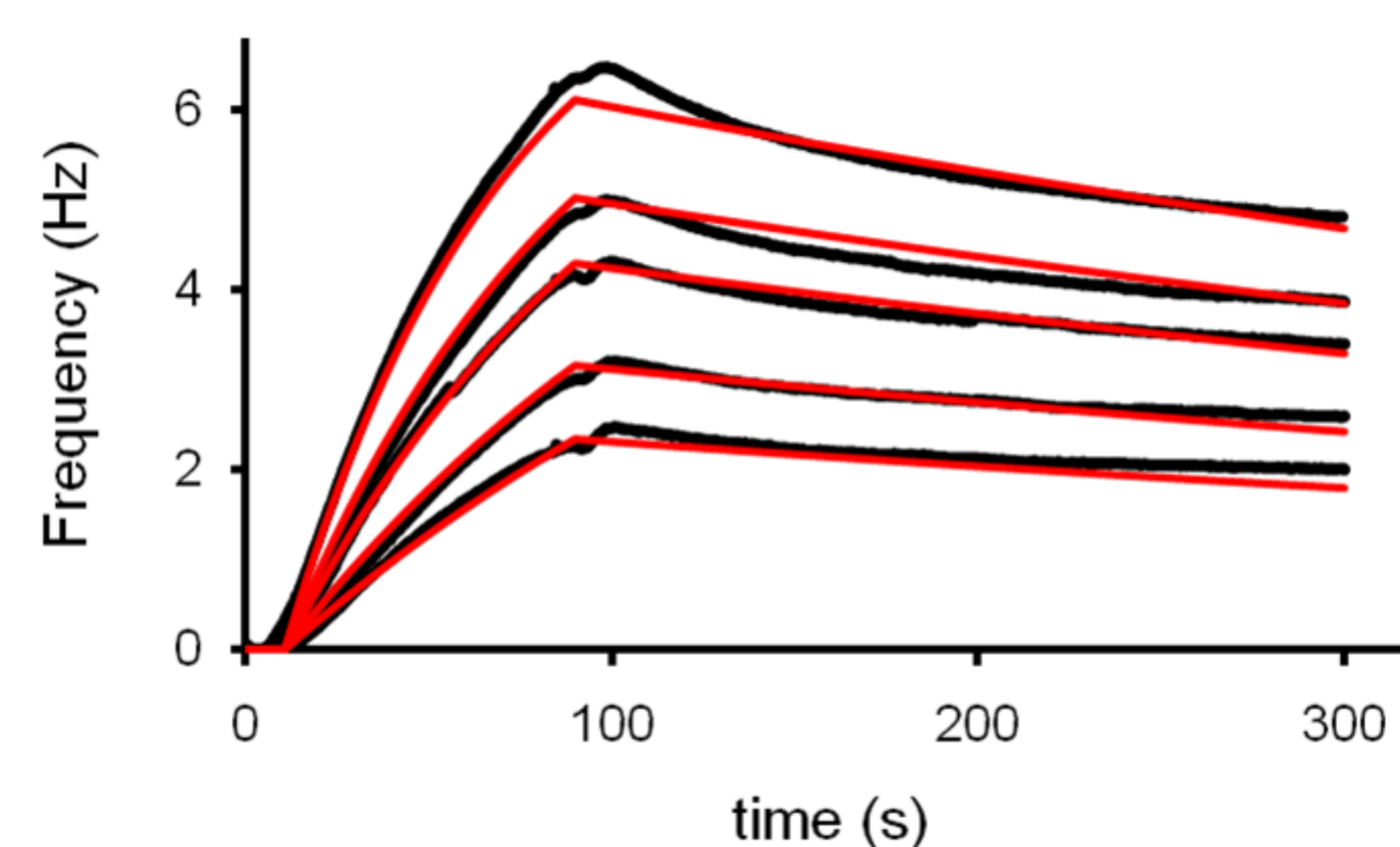
The graph to the right shows the response from a typical immobilization of a multilayer of lipoparticles on the Attana LNB carboxyl sensor chip. Here 3 layers of CXCR4-containing lipoparticles were immobilized using memLAYER on a LNB carboxyl sensor chip that had been pre-functionalized with NeutrAvidin-biotinDNA.



The graph to the right shows that the antibody binding is specific. No unspecific binding occurs to neither the lipoparticle nor the sensor surface demonstrating the benefits of the Attana Low Nonspecific Binding (LNB) sensor surface. Ab-binding to a LNB sensor surface without (---) and with lipoparticle containing (—) and not containing (---) the CXCR4 receptor.



The graph to the left demonstrates the benefits of immobilizing a multilayer of lipoparticles with respect to the increase in response that is obtained in the subsequent ligand binding analysis. Here the interactions of Ab (15, 10 and 5 nM) to the CXCR4 receptor in a multilayer (—) and a single layer (---) of immobilized lipoparticles were measured



The left graph shows fits (—) of the kinetic traces (—) from interaction of the 5, 10, 15, 20 and 25 nM of antibody to the CXCR4 receptor. The fits give a binding constant of 1 nM, in good agreement with literature values obtained from interactions studies using whole cells. This shows that the GPCRs in the lipoparticles, immobilized using memLAYER onto the Attana LNB carboxyl sensor chip, are fully functional.

CONCLUSIONS

This study demonstrates the applicability of the Attana A200 biosensor in combination with memLAYER immobilization for functional characterization of membrane proteins, such as GPCRs. Using the lipoparticle system the often time-consuming and expensive process of i) purifying and ii) reconstituting the membrane protein of interest into liposomes for functional studies of membrane proteins are circumvented. The results also illustrate the benefits of immobilizing multiple layers of lipoparticles using memLAYER, which increases the sensor surface density of the receptor and the response in subsequent ligand-receptor binding events.

ACKNOWLEDGEMENT: We thank Integral Molecular for providing the lipoparticles used in the study.