

# EVALUATION OF INTERACTION KINETICS BETWEEN FUSED CYTOKINES AND RECEPTORS USING TOTAL INTERNAL REFLECTION ELLIPSOMETRY

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Granulocyte-colony stimulating factor (GCSF) and stem cell factor (SCF) are cytokines that are both involved in proliferation, differentiation and survival of hematopoietic cells. It has been shown that these proteins combined can work synergistically enhancing granulopoiesis [1]. Fusion proteins are being developed to create multifunctional proteins. In addition, their therapeutic properties are improved such as increased biological activity and extended circulation half-life [2, 3]. Spectroscopic total internal reflection ellipsometry (TIRE) can be applied to measure the interaction of proteins with their receptors to evaluate their activity. By registering the analytical signal, it is possible to eventually calculate the association constant of a protein which shows how fast it binds to the immobilized receptor [4].

The aim of this study is to measure the interaction between recombinant cytokines GCSF-L $\alpha$ -SCF, SCF-L $\alpha$ -GCSF and their receptors using TIRE and calculate the association constants.

In this work a self-assembled monolayer (SAM) was formed by 11-mercaptoundecanoic acid (11-MUA) and activated on BK7-glass/Cr-Au slide which later was mounted inside of flow-through-cell. On top of it the layer of Protein G was created and GCSF or c-kit receptor covalently immobilized. The measurements of ligand binding to the receptor were performed in the spectral range between 300 nm and 1000 nm. The interaction kinetics of GCSF-L $\alpha$ -SCF and SCF-L $\alpha$ -GCSF were measured with both GCSF and c-kit receptors. Also, SCF monomer and c-kit receptor interaction was measured.

Association constants  $k_a$  were calculated for each interaction which were quite similar for SCF-L $\alpha$ -GCSF and c-kit receptor, SCF-L $\alpha$ -GCSF and GCSF receptor, SCF and c-kit receptor, whereas  $k_a$  for GCSF-L $\alpha$ -SCF and c-kit receptor was slightly higher. Also, the association constant of GCSF-L $\alpha$ -SCF and GCSF receptor could not be calculated because there was no interaction between them.

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