# Experimental report

06/09/2022

**Proposal:** 8-02-902 **Council:** 10/2019

**Title:** The role of charge regulation on the interaction of Histatin 5 with cell membranes

**Research area:** Chemistry

**This proposal is a continuation of 9-13-851**


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**Samples:** Hst5
Syntethic lipids
Hst5 variants

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| D17 | 3 | 0 | | |
| FIGARO | 3 | 3 | 18/05/2021 | 21/05/2021 |


## Abstract:

A number of salivary proteins act as the first line of defense against bacterial and fungal overgrowth. Among these, Histatin 5 (Hst5) is a salivary gland secreted cationic peptide, characterized as an intrinsically disordered protein. Hst5 is a histidine-rich, 24 amino acids protein, which activity of inhibition of growth and viability of Candida albicans has been evaluated using a variety of techniques. However, the underlying mechanism is still not very well known. The aim of this project is to understand the underlying mechanism and the effect of histidines. For this purpose five variants of histidines have been designed and the goal is to understand how the lipid environment influences the activity of the proteins, utilizing neutron and X-ray scattering in combination with computer simulations.

# Experimental report for 8-02-902 on FIGARO

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17-5-2021 to 21-5-2021

During the experiment 9-13-851 performed on FIGARO we measured the interaction of a salivary protein, Histadin 5 (Hst5), with model lipid membranes. In previous experiments (confirmed by data collected during some tests on the SuperAdam instrument in September 2019), we have observed that Hst5 penetrates the bilayer and cumulates in the proximity of the solid substrate (silica in this case) (see report 9-13-656). The aim of the current experiment was to investigate the importance of the histidines in the sequence of Hst5 for the ability of the peptide to penetrate the bilayer and form a cushion.

Four variants of Hst5 were designed with a varying number of histidines in the sequence (0-4), in which histidine was replaced by glutamine. As expected, the number of histidines was indeed of importance, and at a low (10mM NaCl) ionic strength, reflectivity curves different from that obtained when interacting Hst5 with the model membrane were obtained as shown in Figure 1.

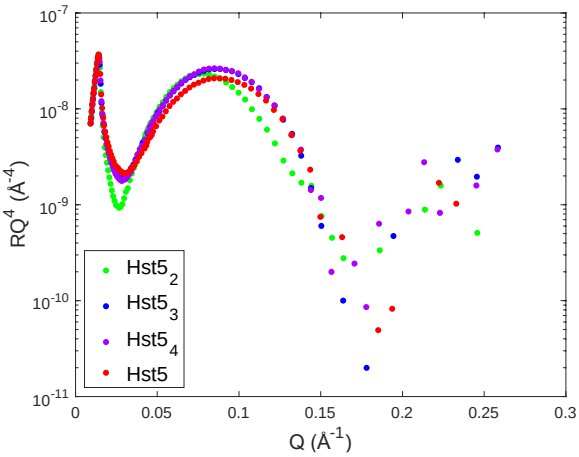


***Figure 1**. Reflectivity curves for a silicon-supported POPC:POPS after interaction with Hst5 and variants in D-buffer. The analysis of the data is still in progress.*

Initial analysis suggests a peptide-lipid interaction in all cases except Hst5, hence, the variants are not able to penetrate the bilayer and form a cushion without disrupting the integrity of the bilayer.

The kinetics of interaction was also measured for one variant (4 histidines), but data suggests that the interaction was too fast to be captured during the acquisition of NR data.