# Experimental report

14/02/2017

**Proposal:** 9-13-645 **Council:** 4/2016

**Title:** Peptide-loaded dendrimers as degradable drug delivery materials; a study of the peptide/dendrimer association using neutron reflection

**Research area:** Soft condensed matter

**This proposal is a new proposal**


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**Samples:** LL-37 peptide
PEG (10k) - G4-dendrimer (10xCOOH; 6xalkene)

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| FIGARO | 2 | 3 | 18/11/2016 | 21/11/2016 |
| D17 | 2 | 0 | | |


## Abstract:

The use of peptide and protein drugs is quickly increasing; however these drugs are often sensitive to degradation. It is therefore essential to develop new drug delivery systems that protect the peptides from degradation whilst also slowing release rates. Within FORMAMP (EU grant no. 604 182) we have developed a library of biodegradable linear- dendritic (LD) structures as drug delivery carriers for antimicrobial peptides (AMPs). These LDs can form well defined bilayer coatings on plasters and biomaterials that degrade over time and release incorporated AMPs. To evaluate the functionality of these new materials we propose to study different peptide loading techniques (pre- and post- loading) as well as bilayer degradation process.

# Experimental report 9-13-645: Peptide-loaded dendrimers as degradable drug delivery materials – a study of the peptide/dendrimer association using neutron reflection

The aim of the experiment was to study linear-dendritic (LD) structures as degradable drug delivery systems for antimicrobial peptides. We aimed to study both the formation of LD layer and its interaction with an antimicrobial peptide, LL-37.

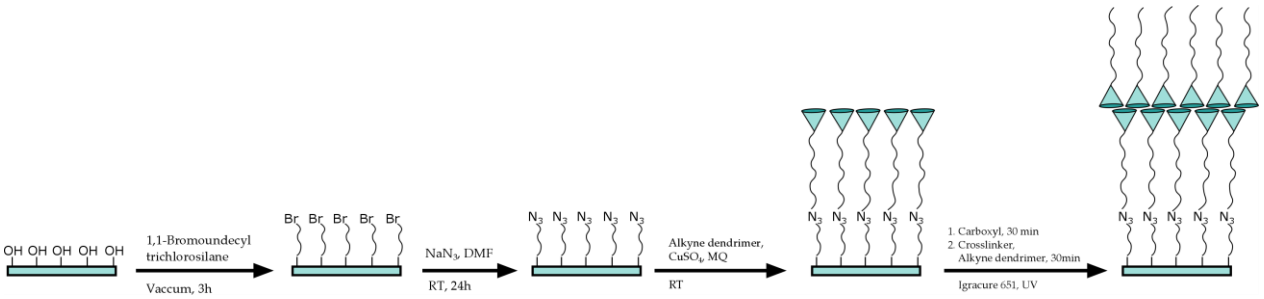


**Figure 1. Scheme of the proposed experiment, chemical modifications before and during experiment included.**

Before the experiment, silicon surfaces were pre-treated with a bromosilane (figure 1) and subsequently the bromide was exchanged for an azide. The treated surface (green markers in figure 2a) was significantly different from a bare silicon surface (black markers in figure 2a). The change corresponds to a 17 nm thick layer of silane with 40 % coverage (figure 2b) and 6.3 Å roughness. This is an interesting finding showing that it is possible to make covalent surface modifications of silica crystals and still have a low enough roughness to see details in the reflectometry curve. If the surface coverage could be increased even more (to 90 %) whilst maintaining the low roughness, there would be more reflectivity at high Q. This would increase the chances to see small changes in this region, cyan simulation in figure 2b.

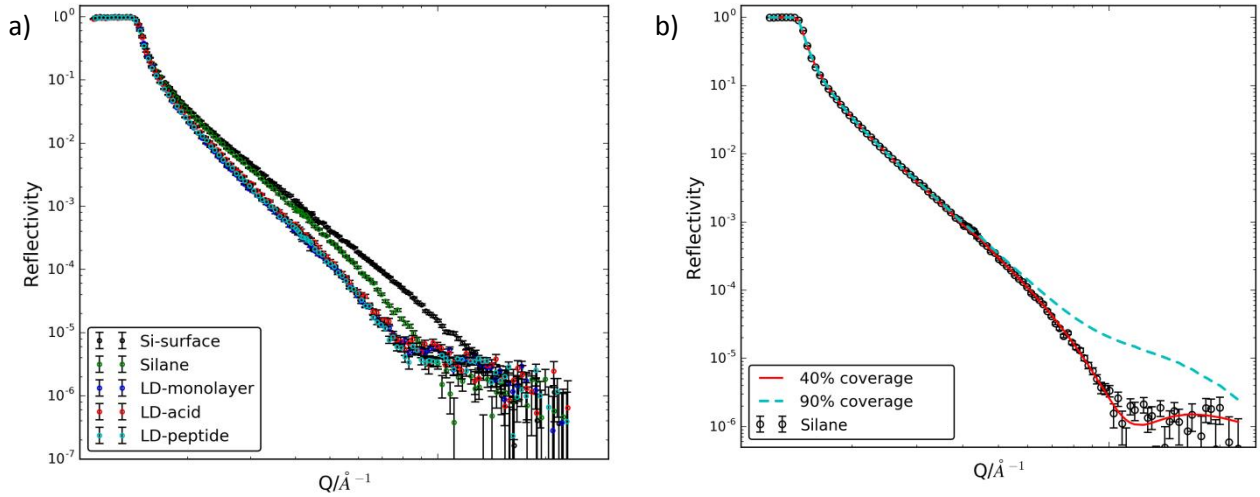


**Figure 2. a) Reflectometry data ($D_2O$) of bara silica surface compared to silane layer, LD-adsorption and LL-37 addition. b) fitted data of the silane-treated Si-crytals, a 17 nm thick layer with 6.3 Å roughness and 40% coverage was observed, also compared to a simulation of a 90% silane coverage.**

Upon addition of allyl-terminated LD molecules, an adsorption can be seen on the surface (blue markers in figure 2a). A small change in reflectivity can also be seen when the allyl-terminated LD-structures are converted into carboxylic acids seen as a roughening from extension into solution (red markers, figure 2a) as well as a minor change when the antimicrobial peptide LL-37 (cyan markers, figure 2a) is added.

Unexpectedly, rinsing with EtOH removed the monolayer, leaving the silane-functionalized surface (red and green dots in figure 3). Due to this, the intended LD-bilayer (figure 1) was not reached in the experiment.

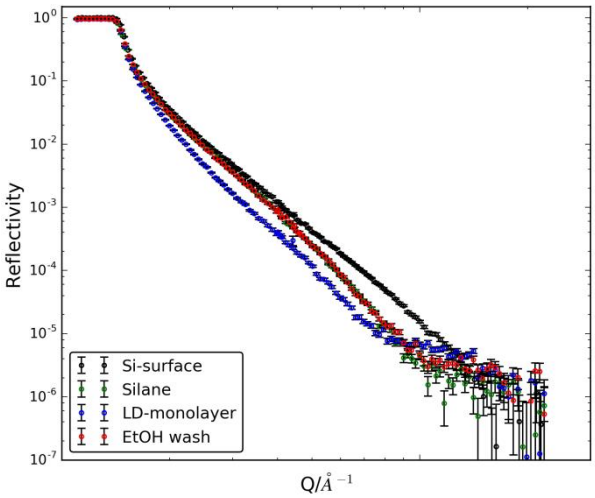


**Figure 3. Experimental data before surface modification (black), after silanisation (green), after LD-adsorption onto the silane surface (blue) and after EtOH rinsing of the LD-surface (green).**

From the lack of stability in EtOH, we conclude that the monolayer was physisorbed and the *in situ* click reaction did not occur in the neutron cell as intended. The reaction relies upon on a UV-initiated reaction, in the experiment initiated through a plastic tube before pumping the solution into the neutron cell. It is possible that the initiation was too short to get sufficient amount of radicals, or that the transport into the neutron cell needs to be faster to ensure reactive radicals to the surface.

Alkyne-azide click chemistry is usually a sufficiently quick reaction and usually gives very high yields, however, this reaction may not be the best choice for a neutron cell due to the issue of reaction initiation. Therefore, we are currently investigating other reactions that are as quick and efficient as an alkyne-azide click-reaction, but do not require the same initiation. Heat-initiated reactions may fit this brief.

To summarize: An LD-covered surface was obtained by physisorption rather than by click chemistry. Furthermore, small changes in this layer could be observed upon chemical modification and LL-37 addition. The pre-treatment of the silicon crystals was successful and gave a thin layer of low roughness terminated with a carboxylic acid moiety.