Experimental Report

Proposal: **9-13-497** **Council:** 10/2012

Title: Specific Protein Adsorption to PEGPolymer Brushes

This proposal is a new proposal

Researh Area: Soft condensed matter

Main proposer: **SCHNECK EMANUEL**

Experimental Team: SCHNECK EMANUEL
BERTS Ida

Local Contact: FRAGNETO Giovanna

Samples:
Hepes
NaCl
DSPC (phospholipids)
PEGlipid2000, PEGlipid5000
antiPEG proteins

Instrument	Req. Days	All. Days	From	To
D17	4	3	12/07/2013	15/07/2013

Abstract:

Protein adsorption to material surfaces causes problems in numerous medical applications. A favoured approach in order to prevent
protein adsoprtion is to decorate surfaces with brushes of terminally anchored, neutral water soluble polymers (NWSP). But despite
the great importance of NWSP-functionalization, the interaction of proteins with NWSP is not fully understood.
In particular, little is known about the role of specific protein adsorption in the regularly observed "brush failure", where protein
adsorption occurs despite NWSP functionalization. Here, we propose a systematic investigation of this phenomenon on a detailed
structural level. Anti-PEG proteins specifically binding to the terminus od PEG polymers will be localized using neutron reflectometry (NR) with contrast variation. The results will provide a valuable basis for
the "rational design" of protein-repellent surface functionalization.

EXPERIMENTAL REPORT

EXPERIMENT N° **9-13-497** INSTRUMENT D17

DATES OF EXPERIMENT 12/07/2013 to 15/07/2013

TITLE Specific Protein Adsorption to PEG Polymer Brushes

EXPERIMENTAL TEAM
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Date of report 14/01/2014

Protein adsorption to material surfaces causes problems in numerous medical applications [1, 2], such as implanted biomedical devices (e.g., stents). A favored approach in order to prevent protein adsorption is to decorate surfaces with brushes of terminally anchored, neutral water soluble polymers (NWSP) [3, 4]. However, the interaction of proteins with these polymers is not fully understood. In particular, little is known about the mechanisms responsible for regularly observed "brush failure", where protein adsorption occurs despite NWSP-functionalization. Three fundamentally different modes of protein adsorption (Fig. 1) have been identified [5, 6]: (i) Primary adsorption at the substrate surface due to surface-protein attraction. (ii) Secondary adsorption at the outer edge of the polymer brush due to van der Waals attraction between the substrate surface and the proteins. (iii) Ternary protein adsorption into the polymer brush itself. Moreover, adsorption can be non-specific or specific like in case of anti-NWSP proteins. For a predictive “rational design” of protein-repellent functionalization it is essential to quantify the contribution of each mechanism to undesired protein-adsorption depending on the properties of the polymer layer (i.e., polymer length and lateral grafting density).

During the experiment 9-13-497 we structurally characterized the specific adsorption of PEG antibodies (ABs) onto PEG brushes of defined polymerization degree ($N = 114$) and lateral density using neutron reflectometry with contrast variation (Fig. 1 left). The PEG grafting density was accurately adjusted by Langmuir-Schaefer transfer of water-insoluble lipid monolayers incorporating defined fractions of lipid-anchored PEG (1 – 10 mol%) onto silicon substrates hydrophobically functionalized with octadecyltrichlorosilane (OTS). The antibodies used bind specifically to PEG end segments. The experiment revealed that adsorption of the antibodies occurs in dense layers at the brush peripery [7]. Primary and secondary adsorption are experimentally ruled out and the adsorbed protein amount systematically increases with the grafting density, which is in contrast to our recent results on primary protein adsorption, where the opposite is observed [8]. The brush conformation is not significantly altered by AB binding within the experimental accuracy.

For high PEG grafting densities the AB layer at the brush periphery becomes crowded and the molecules assume elongated upright conformations that minimize the required area per molecule (Fig. 1 right). After terminal adsorption, the dense AB layers cover the PEG brushes, so that the brushes are no longer functional as passivation layers suppressing immune response by the organism.

The results demonstrate that neutron reflectometry with contrast variation allows the determination of protein density profiles in polymer brushes in an unambiguous manner [7, 8].

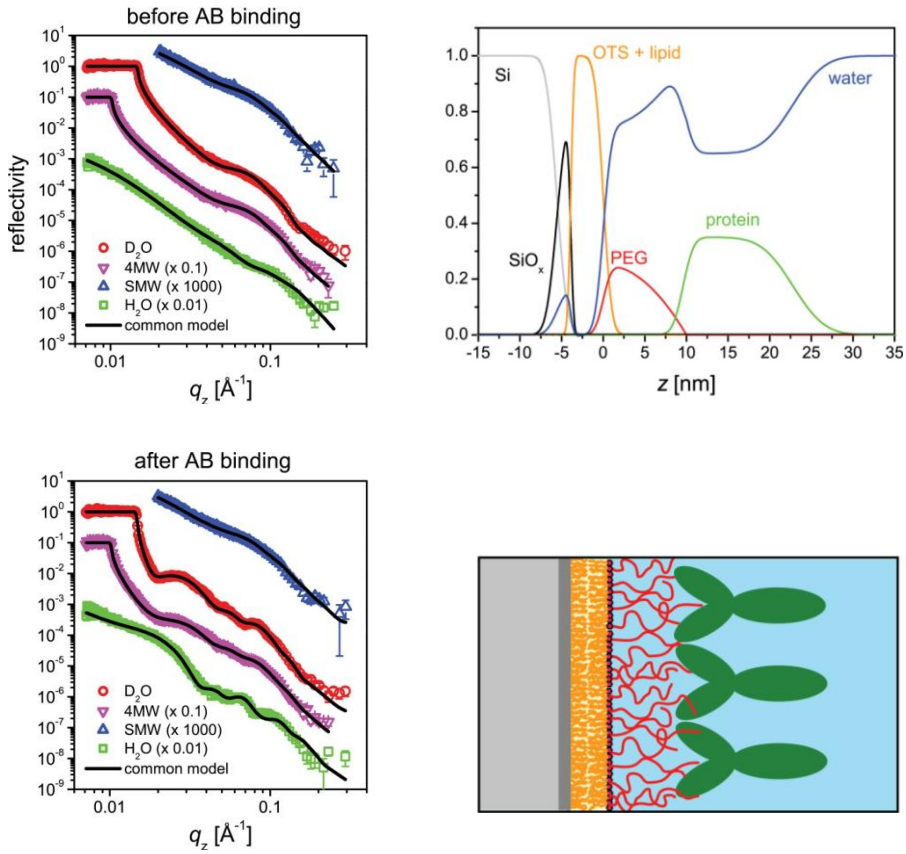

Figure 1: (left) Reflectivity curves of a dense PEG brush (10 mol% lipid-anchored PEG) before (top) and after (bottom) incubation with PEG antibody proteins. (right top) Reconstructed density profiles of silicon (Si), silicon oxide (SiOx), OTS, lipid, PEG, protein, and water. (right bottom) Cartoon of a crowded layer of oriented and elongated ABs bound to the brush periphery.

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[2] Hlady & Buijs, J. Curr. Opin. Biotechnology 7, 72 (1996)

[3] Elbert & Hubbell, Annu. Rev. Mater. Sci. 26, 365 (1996)

[4] Goddard & Hotchkiss, Prog. Polym. Sci. 32, 698 (2007)

[5] Halperin, Langmuir 15, 2525 (1999)

[6] Currie, Van der Gucht, Borisov, Cohen Stuart , Pure Appl. Chem. 71, 1227 (1999)

[7] Schneck, Berts, Halperin, Daillant, Fragneto, in preparation

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