Molecular Interaction of European Lyme Disease Spirochetes probed by AFM

Lisa Hain[1], Martin Strnad[2,3], Marie Vancová[2,3], Ryan O. M. Rego[2,3], Peter Hinterdorfer[1], Yoo Jin Oh[1]

[1]Institute of Biophysics, Johannes Kepler University Linz, Gruberstrasse 40, 4020 Linz, Austria

[2]Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Branišovská 31, CZ-37005 České Budějovice, Czech Republic

[3]Faculty of Science, University of South Bohemia, Branišovská 1760, CZ-37005 České Budějovice, Czech Republic

Abstract

Lyme disease is the most common vector borne disease in the Northern hemisphere. Adherence of its causative agent, i.e. bacteria belonging to the Borrelia genus, to cells or the extracellular matrix (ECM) of tissues is a key step to promote dissemination and colonization leading to further development of the illness. Bacterial adhesins are essential factors for the interaction between the bacteria and ECM components.[1,2] Despite its high relevance, many details remain to be elucidated regarding their physiological functions, such as characterizing the main molecular interactions between bacterial adhesins and ECM during the initial adhesion stage. In this study, the interaction forces between borrelial surface proteins from infectious European *Borrelia* genospecies and different ECM proteins are probed. Using single molecular force spectroscopy (SMFS), binding activity and strength for the interactions are determined. Interaction specificities are validated by blocking experiments from adding adhesins into the measurement solution. Dynamic force Spectroscopy (DFS) is used to further characterize the energy landscape of the interaction, determining binding modes and kiniteic bininding constants.
Our results elucidate the complex adhesive properties of borrelial surface proteins to ECM in pathogen adherence during the infection process.

Borrelia

- causative agent of **Lyme disease**
- transmitted by **hematophagous athropods**
- main vector in Europe: *Ixodes ricinus*
- **60 000 cases per year**[3]
- multiple infectious genospecies
- different symptoms depending on genospecies[4]:
  - dermatological: *B. afzelii*
  - neurotropic: *B. garinii, B. bavariensis*

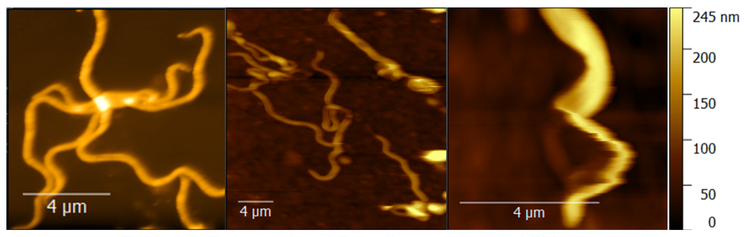


Figure 1 - Topography of *Borrelia* recorded by AFM

Expression of Borrelial Adhesins

Recombinant borrelial adhesins were expressed in *E. coli* cells and purified by affinity column chromatogrpahy.
Successful purification was determiend by SDS-page electrophoesis and Coomassie Blue Staining of the gel.
Protein concentration was determined using the Bradford assay.

Borrelial adhesins studied:
BBK32 *B. afzelii*: fibronectin binding protein, 40 kDa
DbpA *B. bavariensis*: decorin binding protein A, 17kDa

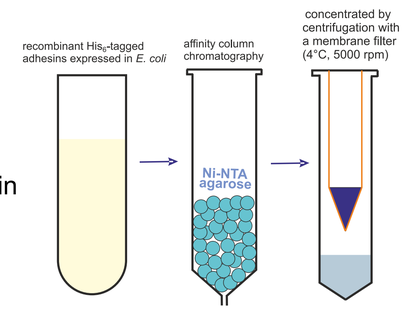


Figure 2 - Preparation of borrelial adhesin samples

Tip/Surface Functionalization

AFM tips (MSCT, Bruker, USA) and silicon surface were functionalized with amino groups via APTES coating in gas phase. Furthermore different types of PEG-linkers were used to fix the purified proteins on the AFM tips and surfaces.

PEG-maleimide linker and tris-NTA were used for the borrelial adhesins and PEG-acetal or PEG-aldehyde for coupling the lysine residues of the ECM components to the tip or surface.[5]

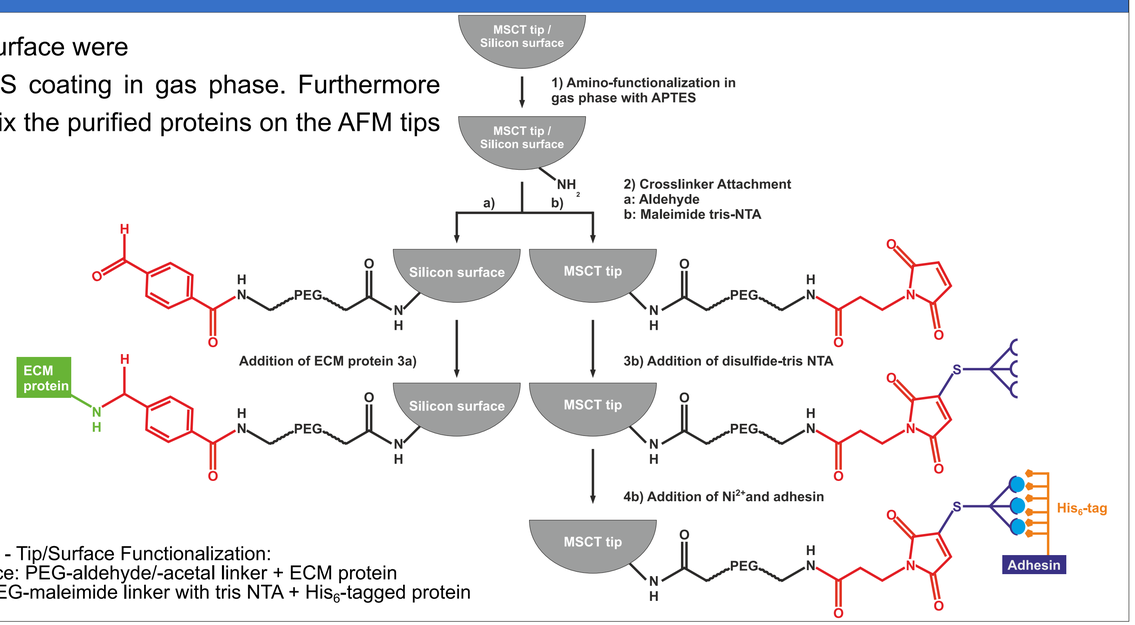
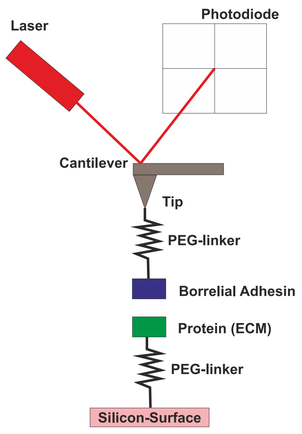
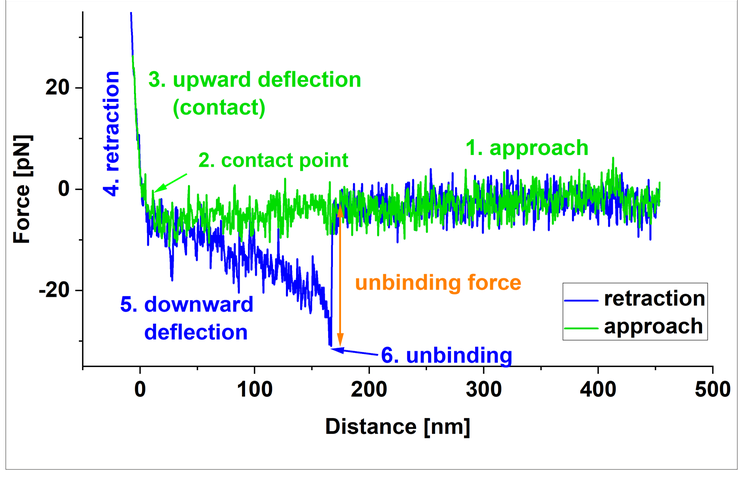


Figure 3 - Tip/Surface Functionalization:
a) surface: PEG-aldehyde/-acetal linker + ECM protein
b) tip: PEG-maleimide linker with tris NTA + His_6-tagged protein

Atomic Force Microscopy

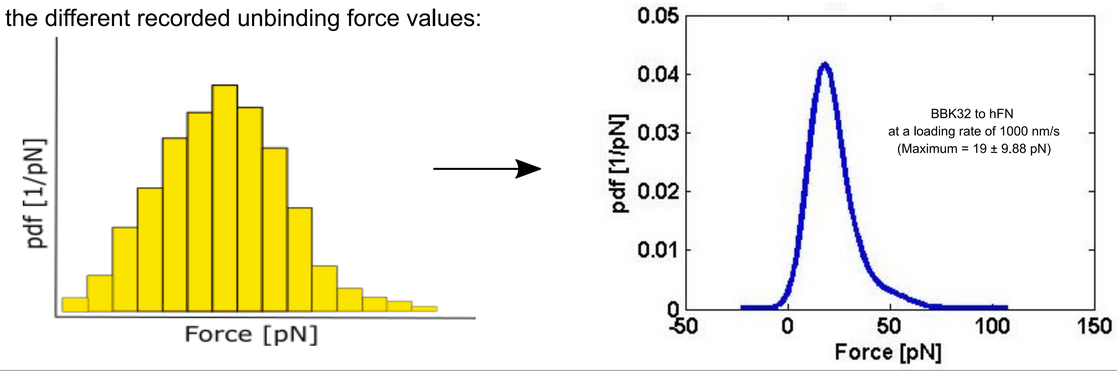


Force-Distance Curve

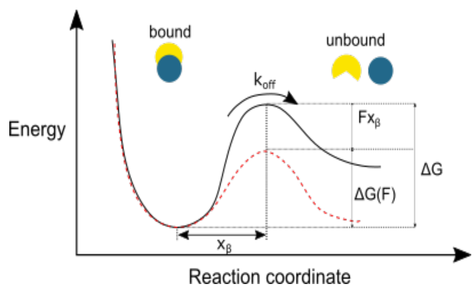


Probability Density Function

For each tip and each loading rate approximaetly 1000 force-disctance curves are recorded and the unbinding events in each curve are determined. The probabiltiy density function is derived from histograms of the different recorded unbinding force values:



Bell Evans Model

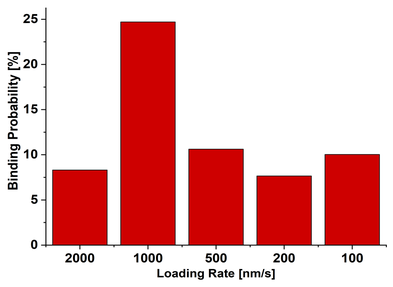


$$F^*(r) = \frac{k_B T}{x_\beta} \ln \frac{r x_\beta}{k_{off} k_B T}$$

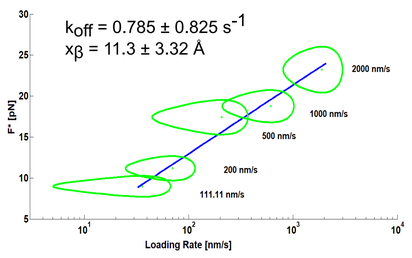
k_B ... Boltzmann's constant
T ... Temperature
x_β ... length scale of the interaction energy potential
r ... loading rate
k_{off} ... kinetic off rate constant

BBK32 from *B. afzelii*

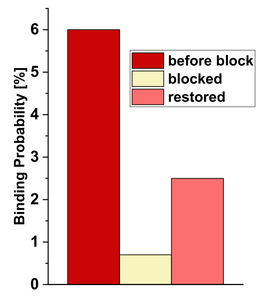
Probability of BBK32 binding to human fibronectin (hFN)



Bell-Evans model for the interaction between BBK32 and hFN:

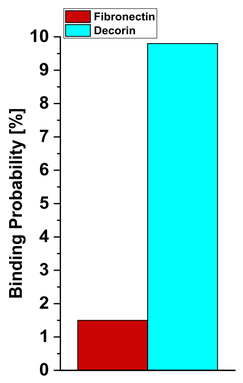


Block experiment (BBK32/hFN)



DbpA from *B. bavariensis*

Binding probability for DbpA/hFN (red) and DbpA/decorin (blue) at 500 nm/s



Results

BBK32 from *B. afzelii*

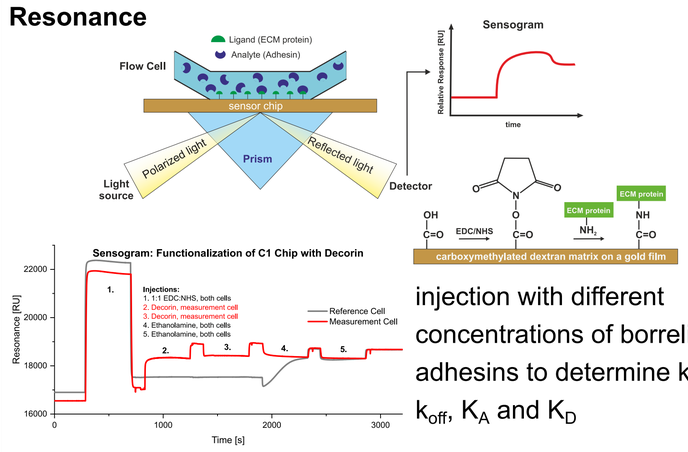
- **specific interaction to hFN** as shown by the block experiment (binding probability decreases significantly upon addition of free adhesin to experimental chamber)
- Dynamic Force Spectroscopy: dissociation force increases with the loading rate, use **Bell-Evans model**:
  - length scale of the interaction energy potential, x_β **= 11.3 ± 3.32 Å**
  - kinetic off rate, k_{off} **= 0.785 ± 0.825 s^{-1}**

DbpA from B. bavariensis

- significantly higher binding probability to decorin (10%) than to hFN (1.3%)

Ensemble vs Single molecule measurements

Complementation of DFS with Surface Plasmon Resonance



injection with different concentrations of borrelial adhesins to determine k_{on}, k_{off}, K_A and K_D

Outlook

BBK32 from *B. afzelii*

- additional measurements for BBK32/hFN
- interaction to other ECM proteins

DbpA from *B. bavariensis*

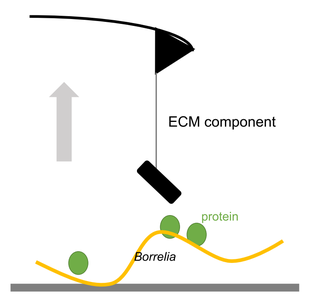
- DFS for DbpA-decorin
- interaction to other ECM proteins

Establishment of an **immobalization strategy** for *Borrelia* cells on silicon surfaces via

- mechnical trapping
- concanavalin A
- gelatine

SMFS of ECM components on *Borrelia*

investigation of collective bond behaviour and dissocation energy



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Contact: Lisa Hain, lisa.hain@jku.at, Gruberstrasse 40, 4020 Linz, Austria