Revealing secrets of material specific peptide binding: From advanced NMR analysis to computational process modeling

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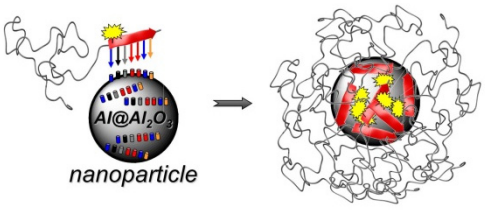
Complex of topics: B3 Analysis tools for dynamic interfaces to understand nanostructures

Short version (~150 words)



The adhesive system of marine mussels stimulated intensive interdisciplinary research.[*Science* **1981**, *Annu. Rev. Mater. Res.* **2011**] Mussels adhere onto virtually any hard substrate in hostile environments and outperform technical wet-glues. Besides these universal and complex protein based adhesives, 12*mer* peptides can be selected by biocombinatorial means to exhibit highly specific adhesion onto material surfaces.[*Nature* **2000**] Those material specific adhesives promise a scope of next generation applications from nanoengineering of nanoparticle surfaces, to specific glues for nerve surgery.[Börner et al. *JACS* **2014**, *ACS Macro Lett.* **2012***; JACS* **2012**] The effects on the molecular scale, which originate the material specific adhesion of peptides are, however, by far not understood. Soft multipoint interactions, surface related docking, locking of peptide conformations and surface induced folding processes are discussed.[*JACS* **2011**]

Objectives of the present project are the development of analytical tools and their companion with computational methods to gain insight into material specific adhesion. Advanced NMR analytics such as solution-based nuclear Overhauser and saturation transfer difference (STD) spectroscopies under transient binding conditions as well as surface enhanced local Raman spectroscopy (SERS) probing will be combined with modern molecular simulation to investigate dynamic interactions of peptides with material surfaces and thus elucidating mechanisms of recognition.

Focus will be set on the understanding of three recently selected peptides, showing specific adhesion onto surfaces of (i.) gold, (ii.) aircraft aluminum and (iii.) magnesium fluoride. The adhesive processes will be analyzed by advanced NMR techniques revealing insight into peptide surface contacts and contact developments throughout the adhesion process. The results will be accompanied with SERS-probing to analyze changes of vibrational dynamics of the surface adhered peptides. Based on those analytical data, molecular modeling of peptide conformation on the surface and conformational pathways during peptide adhesion process will be performed and reveal the origin of material specific adhesion on the molecular level. The discussed opinions about specific peptide adhesion should be modeled and validated. With the aid of atom-based models of surfaces and peptides (parametrization inside the software package GROMACS) and by using a sophisticated statistical thermodynamics software platform ZIBMolPy with domain decomposition and free-energy estimation [SIAM JMMS **2007**, Lecture Notes **2013**], adhesion scenarios can be analyzed thermodynamically. Moreover, kinetic effects have to be simulated and recent non-thermodynamic effects such as "rebinding effects" [JCP **2012**] will be taken into account requiring new computational method developments.

Overall goal of the project (~ 1/3 page):

The project aims to establish analytical tools and companion those with computational methods to understand material specific adhesion of peptides onto 2D and 3D surfaces. While several peptide sequences have been described, which were selected via biocombinatorial means to strongly or even highly specifically adhere onto surfaces of interest, the molecular origin for specific adhesion still needs to be revealed.

Major challenges in Analytical Sciences result from the fact that adhesion processes occur at rather ill-defined interfaces and binding is heterogeneous in nature. Analytical methodologies established for investigating defined peptide-receptor interactions have to be transferred to heterogeneous systems with less defined binding events. Saturation transfer difference (STD) NMR spectroscopy and solution-based nuclear Overhauser spectroscopy experiments (NOESY) will be used to elucidate transient peptide-surface contacts and contact developments during binding processes. SERS sensors should be used to probe the different states of peptide binding via changes of vibrational states of the peptide while adhering on the surface of interest. Curvature dependency of adhesion modes and interaction strengths should be analyzed, as well.

The experimental studies should go along with theoretical investigations of the adhesion process. Especially, the suggested, existing opinions about specific peptide adhesion should be modeled and compared with experimental data for validation/falsification. Soft multipoint interactions, surface related docking, and locking of peptide conformations are connected to a thermodynamics point of view. Surface induced folding processes could be analyzed thermodynamically, too, but they are also connected to a kinetics point of view. In this context, the "rebinding effect" should be discussed. The adhesion processes can be analyzed by new computational methods developed (and still developing) in the working group of Marcus Weber at the Zuse Institute Berlin.

This project meets the challenges of “complex B3”, where analysis tools for dynamic interfaces are developed with the aim to understand nanostructured materials.

Specific aims and how they may be reached (~ max. 1 page):

The major aim in this study is to develop analytical tools to explore the origin of material specific adhesion of oligopeptides onto material surfaces. First due to ease of preparation peptide binding on gold and alumina particles (aircraft aluminum) and subsequently after methodologies were installed peptides for magnesium fluoride binding will be investigated. The following questions should be addressed:

- What are the molecular interactions of peptides and surfaces constituting (i.) strong binding and (ii.) specific recognition of certain surfaces (gold, aluminum oxid and magnesium fluoride) by peptides?
- How do these molecular interactions depend on the curvature of the surface?
- How does the interaction develop during the adhesion process?

▪ <u>Objectives</u>

- Synthesis of a peptide adhesive domain (AD_{Au}) to target gold surfaces. Synthesis of a set of sequence derivatives (Ala or Ser-scans) as control compounds to elucidate important binding motifs.
- Synthesis of a peptide adhesive domain (AD_{alu}) to target aluminum surfaces. Synthesis of a set of sequence derivatives (Ala or Ser-scans) as control compounds to elucidate important binding motifs. Synthesis of structurally constrained peptides

e. g. cyclic analogues of the linear AD_{alu} peptides to investigate changes in specificity and binding strength.
- Initial characterization of the peptides (MS, NMR, CD, FT-IR)
- Synthesizing gold colloids and alumina colloids (50 nm) and characterization of those by DLS, XRD, elemental analysis, XPS, SERS, NMR.
- Incubation/elution assays and Langmuir-binding isotherms will provide a picture of strength and dynamics of binding as well as revealing important residues, subsequences or motifs for binding.
- Synthesizing a set of gold and alumina (nano)particles with different dimensions (5 nm-1 µm) to address the issue of curvature depending binding effects. Characterization of particles and peptide binding study (incubation/elution assays & Langmuir-Binding Isotherms).
- SERS-probing of peptide adhesion onto gold particles to identify changes in vibrational freedom.
- NMR spectroscopy under different conditions to probe transient states of peptide/surface interactions, identifying important residues for binding, structurally locked conformations and peptide orientation on the surfaces.
- SERS-probing of peptide coatings for “back imaging” of solvent exposed peptide sides.
- Time dependent analysis of adhesive processes by correlation of several different adhesive states (condition sweep).
- Development of computational methods to describe (i) strong and (ii) specific binding:
    (1.) parametrization of atom-based models of the surfaces (with different curvature) and parametrization of the corresponding peptides
    (2.) applying computational statistical thermodynamics, development of corresponding adhesion models
    (3.) developing and applying computational molecular kinetics methods, development of corresponding adhesion models and models to describe the binding process
- Synthesis of a peptide adhesive domain (AD_{MF}) and a set of sequence derivatives to target magnesium fluoride surfaces. Initial characterization of the peptides (MS, NMR, CD, FT-IR).
- Synthesizing magnesium fluoride particles (20 nm) and characterization of those by DLS, XRD, elemental analysis, XPS, SERS, NMR.
- Incubation/elution assays and Langmuir-binding isotherms.
- NMR spectroscopy and SERS-probing to gain insight into peptide surface binding.
- The outlook would be the generation of sequence-function relationship, which might be mimicked by synthetic, non-peptidic polymers.
- Development of computational methods to describe (i) strong and (ii) specific binding on heterogeneous, partially crystalline surfaces of MgF_2-particles.

- <u>Experimental approach</u>

The synthesis parts will be performed in the laboratory at HU Berlin (AG Börner) as outlined above. Synthesis of peptides and inorganic particles will require 15% of the overall project. For characterization and development of analytical tools 40% and for computational approaches about 45% can be estimated. Assistance during peptide and particle synthesis might be provided by a technician, if larger amounts of materials will be required for analysis purposes. While analysis of the peptide can be performed in Börner Laboratory, particle analysis will be carried out with assistance in the inorganic department (AG Kemnitz).
After proving the chemical identity of the peptides, careful assignment of the 1H-NMR resonances requiring 2D NMR-measurements will be performed (NMR facility of the department of chemistry). Additionally RAMAN spectra are recorded in aqueous solution and surface enhanced RAMAN spectroscopy (SERS) will be measured in collaboration with AG Kneipp to assign interacting amino acid residues in solution.

Adsorbtion behavior will be studied via established incubation/elution assays, Langmuir-binding isotherms and quartz crystal balance/ellipsometry-coupling provide initial insight into adhesion behavior and identifies important residues/motifs for binding of the peptide towards gold and aluminum surfaces (binding occurs to alumina oxide layer of passivized aluminum) and in the second phase of the project also to magnesium fluoride. Protocols for binding assays are established in the AG Börner and can be adapted to the system of interest.
Advanced NMR analytics of peptide/surface interactions will be used to determine the structures and orientation of peptides bound to the inorganic nanoparticle surfaces. Under conditions leading to rapid peptide exchange at the surface high-resolution solution-based nuclear Overhauser spectroscopy experiments (NOESY) can be used to determine the three-dimensional structure of the bound peptide. Saturation-transfer difference (STD) provide structure and orientation of peptides bound to surfaces. The NOESY cross-peaks will arise predominantly from the bound conformation, so the structure can be determined using the standard methods for NMR protein structure determination. Magnetization transfer from the particle surface can be used to determine the binding orientation of the peptides. NMR experiments will be performed in the NMR core facility of the Department of Chemistry under supervision of (AG Börner with AG Mügge HU-Berlin). 700 MHz measuring time might be available with Prof. Meyer (University Hamburg), where collaboration could be initiated if required.
Probing the surface adhered peptides under different conditions SERS Ag^0-particle probes might provide insight into solvent exposed residues of peptides bound to the inorganic surfaces. Moreover, changes of vibrational modes could be followed for gold particle binding under transient binding conditions. SERS will be performed in collaboration with AG Kneipp (HU-Berlin).
The effects of curvatures on specific peptide binding are of interest (Figure. 2). For that purpose, a set of particles (i.-iii. gold, alumina, and MgF_2) with different dimensions (5 nm-5 µm) will be synthesized and analyzed with the established NMR and SERS tools.

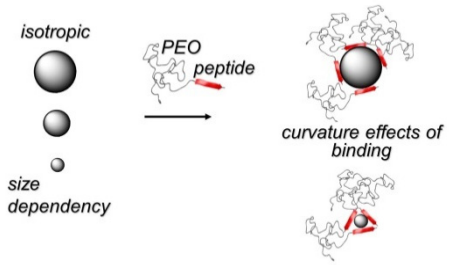


Figure 2. Size specificity and size effects of specific binding of peptides onto inorganic particle surfaces.

- Computational approach

The computational methods and the modeling part will be performed in the Computational Molecular Design Group of M. Weber at the Zuse Institute Berlin (ZIB). The peptides and surfaces will be modeled using the open source software package GROMACS (available at http://www.gromacs.org/). The simulation will be done by domain decomposition tools using the open source software platform ZIBMolPy (available at https://github.com/CMD-at-ZIB/ZIBMolPy). Statistical thermodynamics data will be analyzed by free-energy estimators included in the ZIBMolPy-package. For the analysis of binding processes, Markov State Models are used [Methods **2010**], which have to be implemented in Matlab (licensed by the ZIB and available at http://www.mathworks.de/products/matlab/). For the fast computation of statistical data, ZIB has a limited in-house access to the HRLN-supercomputer (see https://www.hlrn.de/home/view).

Collaboration (~ 1/3 page):

The aspect focused on here relates on the specific recognition of inorganic surfaces peptides. The synthesis and initial characterization is performed in the laboratory of Prof. Börner (HU-Berlin) and Prof. Kemnitz (HU-Berlin).
The spectroscopic methodologies will be investigated in close collaboration with Prof. Kneipp (HU-Berlin) and the core NMR-facility of the Department of Chemistry. The computational approaches will be guided by PD Dr. Weber (ZIB). During the entire period of the PhD thesis, the student closely interacts with both the HU-Berlin and the ZIB.
The estimated project time assignment will be approximately 15% project time used for synthesis and characterization, 40% time for establishing spectroscopy methodologies and binding assays (binding analysis, NMR, SERS) and 45% time for computational method development and application of those.

Connection with other projects

The PhD project addresses an aspect of one of the major project lines of the SALSA case based studies (cf. Fig. 3). This ultimately targets the analysis of complex composites or biomaterials in external fields and develops new analytical tools to provide insights into complex materials.

The proposed project addresses the line of research (B3 “Analysis tools for dynamic interfaces to understand nanostructures”) from the side of development of analytical tools for interface characterization. A different project with Kemnitz, Kneipp (Börner), will start independently to investigate SERS sensors to obtain insights into the interfaces in compatibilized composites. While the topics are related and the projects synergistically profit both from the outcome of each project, it is important that they do not critically rely on each other.

The modeling of the binding process is connected to the "rebinding effect" theory developed in Sfb-765 ("Multivalency", R. Haag). Intensive cooperations are possible. The rebinding model will be published (and, therefore available) soon inside the doctoral thesis of A. Bujotzek which is under review now.

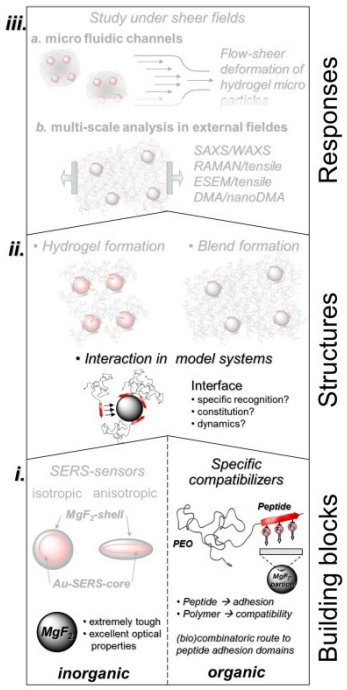


Figure 3. Project outline and connection plan for the SALSA research line (“Structural and functional analysis of hierarchical materials” (The covered sections are subjects of other Ph.D.-projects).

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