# Bio-material recognition under a virtual microscope

## Towards an atomistic picture of phage display: the case of $TiO_2$ binding peptides

***M. Delle Piane[1], M. Michaelis[1,2], C. Perry[1,2],***
*[1]Hybrid Materials Interfaces Group, Faculty of Production Engineering and Bremen Center of Computational Materials Science, University of Bremen, [2]Biomolecular and Materials Interface Research Group, Interdisciplinary Biomedical Research Centre, School of Science and Technology, Nottingham Trent University (UK)*

### In Short

- Combinatorial biological protocols, i.e. phage display, are applied to identify peptide sequences specifically binding inorganic materials, such as $TiO_2$ (Titania).
- Titania is an attractive material used in medical and environmental applications.
- Peptide-based approaches to the generation and functionalization of Titania are an important research perspective.
- A fundamental understanding of the atomistic aspects of peptide-materials recognition during phage display is required.
- We will tackle this problem, by performing large scale enhanced sampling molecular dynamics simulations.

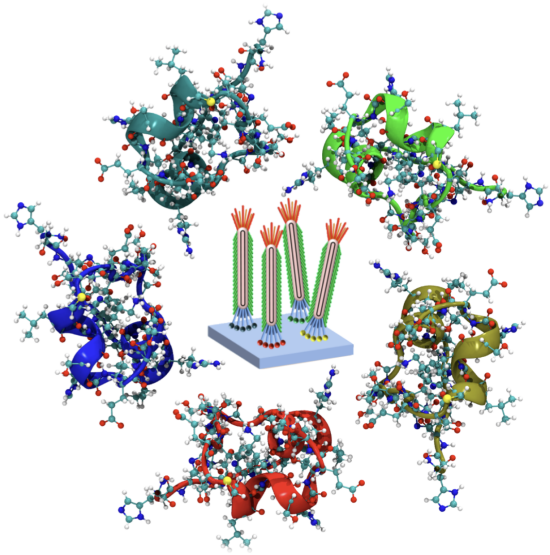

***Figure 1:*** *Schematic view of the phage-material interaction surrounded by the displayed peptides in our "minimal phage" model.*

The identification of the primary structure (amino acid sequence) of a peptide which possesses the property to bind selectively and/or specifically to a substrate of interest is referred to as genetically engineered polypeptides for inorganics (GEPI).[1] This idea is based on the concept of molecular recognition, which was exported to materials science following the seminal research of Stanley Brown in the 1990s.[2] Within this framework, combinatorial biological protocols like for instance phage display (PD), are applied to identify peptide sequences to allow for faster, rational design.[1] Additionally, these display techniques enable the identification of the genotype of the peptide that binds to the targeted material without requiring a detailed knowledge of the system.[1] Using phage as a display host is the most commonly applied technique. The filamentous bacteriophage M13 was the first phage used in a combinatorial biological protocol and is still used most frequently nowadays in research areas ranging from drug discovery to the identification of materials-specific binding peptides. For this purpose, randomized peptide libraries with up to $10^9$ variations of sequences encoding peptides of fixed lengths are inserted into the capsid genome of the phage. An unrivaled property of these viruses is the direct relation of its genotype and its phenotype as, for example, chimeric peptides are directly expressed on the surface of the coat protein pIII via a linking sequence displayed at one end of the bacteriophage (Figure 1). Another outstanding property of the bacteriophages is their ability to infect bacteria and replicate within them, which leads to their quick reproduction.

Since the adaptation of phage display for the identification of specifically binding peptides to materials, a huge variety of sequences for different materials could be identified. However, it has to be noted that combinatorial biology protocols possess some drawbacks and biases associated with their use.[1] These constraints include, but are not limited to the effect of the C-terminal attachment to the phage and its subsequent impact on their conformational propensity, non-specific interactions of the linking sequence and the phage body with the materials surface and the tethered peptide as well as the reduced incorporation of Cysteine residues due to the inherent biology of the M13 Phages. Accordingly, the peptides identified might not be the strongest achievable binders for a specific substrate. The limited understanding of how to exploit the relationship between peptide sequences identified during phage display and their

corresponding materials-binding affinity as well as their specificity of interaction is so far a significant obstacle towards reliable peptide-based strategies for the generation and organization/activation of (nanostructured) inorganic materials.

We will focus here on $TiO_2$ (Titania), which is an attractive material used in medical and environmental applications based on its optical, adsorbent, and catalytic properties as well as its use in biomedical implant materials. Peptide-based approaches to the generation and functionalization of Titania are therefore an important research perspective. We choose two 12mer peptides for our investigation, Ti-1 (QPYLFATDSLIK) and Ti-2 (GHTHYHAVRTQT), which were originally identified via Phage display against 100 nm titanium nanoparticles.[3] It was revealed that although these peptides have very similar binding affinities towards Titania, their binding shows profoundly different modes of surface interaction.[4]

A fundamental understanding of the atomistic aspects of peptide-materials recognition during phage display in comparison to peptide binding in their intended application is absolutely necessary to advance peptide-based generation and organization of nanostructured inorganic materials.

Therefore, the general question we aim at answering during the proposed project is:

**How does the attachment to a phage, as happens in widespread phage display binding assays, influence the conformation and biomaterial interaction behavior of titania binding peptides?**

We plan to tackle this question by:

1. Identifying, via a validated mixed computational-experimental approach,[5,6] employing enhanced sampling molecular dynamics simulations and experimental circular dichroism spectroscopy, the conformational landscape of titania peptide binders when free in solution and when attached to the phage body.

2. Measuring, comparing our QCM-D experimental results with adsorption free energies computed by metadynamics coupled with an hamiltonian replica exchange method, offering a structure-activity relationship, explaining the bio-material interaction at the atomistic level.

3. Comparing the interaction of the peptides with titania as free in solution as opposed to linked to a phage, understanding how the additional structural constraint influences the way these biomolecules approach and interact with the inorganic material.

We believe that the obtained results, although obtained for titania binders, will be of general interest within the larger (bio)material community, adding caveats to the extrapolation of results from phage display assays and providing a better atomistic understanding of the biomolecular recognition of inorganic material surfaces.

## WWW

http://www.hmi.uni-bremen.de

## More Information


[1] Sarikaya, M.; Tamerler, C.; Jen, A. K. Y.; Schulten, K.; Baneyx, F. *Nat. Mater.* **2003**, 964, 577-585. doi:10.1038/nmat964

[2] Brown, S. *Nat. Biotechnol.* **1997**, 15, 269-272. doi:10.1038/nbt0397-269

[3] Puddu, V.; Slocik, J. M.; Naik, R. R.; Perry, C. C. *Langmuir* **2013**, 29, 9464-9472. doi:0.1021/la401777x

[4] Sultan, A. M.; Westcott, Z. C.; Hughes, Z. E.; Palafox-Hernandez, J. P.; Giesa, T.; Puddu, V.; Buehler, M. J.; Perry, C. C.; Walsh, T. R. *ACS Appl. Mater. Interfaces* **2016**, 8, 18620-18630 doi:10.1021/acsami.6b05200

[5] Michaelis, M.; Hildebrand, N.; Meissner, R. H.; Wurzler, N.; Li, Z.; Hirst, J. D.; Micsonai, A.; Kardos, J.; Delle Piane, M.; Colombi Ciacchi, L. *J. Phys. Chem. B* **2019**, 123, 6694-6704 doi:10.1021/acs.jpcb.9b03932

[6] Hildebrand, N.; Michaelis, M.; Wurzler, N.; Li, Z.; Hirst, J. D.; Micsonai, A.; Kardos, J.; Gil-Ley, A.; Bussi, G.; Koeppen, S.; Delle Piane, M.; Colombi Ciacchi, L. *ACS Biomater. Sci. Eng.* **2018**, 4, 4036-4050 doi:10.1021/acsbiomaterials.8b00819