Bioinspired nanomaterials

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nanoSeminar
Institute of Materials Sciences
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Outline

- Forschungszentrum Dresden-Rossendorf
- Institute of Radiochemistry

- Microbe radionuclide interaction
- Bacterial S-layers
- Metall binding by bacterial S-layers

- Application potential of bacterial S-layers
- S-layer based materials
- Biomass productions

- Summary
- Future cooperation IRC-FZD with IfWW-TUD
Ion-Beam Physics and Materials Research
Radiation Physics
Radiochemistry
Radiopharmacy
Safety Research
High Magnetic Field Laboratory

Advanced Materials
Cancer Research
Nuclear Safety Research

Ion-Beam Center
Radiation Source ELBE
Rossendorf Beamline (ESRF, Grenoble)
PET-Center
TOPFLOW-Facility
Dresden High Magnetic Field Laboratory
• **Member of the Leibniz-Association**

  Change to the **Helmholtz Association**
  as of 01.01.2011, as **HZDR**
  (Result of the evaluation 2007)

• **Foundation:** 01.01.1992 (e.V.)

• **Employees:** ~ 800 total (incl. PhD, Annex, projects, trainees)
  ~ 400 basic funding
  ~ 370 scientists

• **Budget 2009:** ~ 60 Mio € basic funding
  ~ 20 Mio € third party funding
Institute of Radiochemistry

- Office and lab building 8a
- Micobiological and radiochemical labs,
  Divisions Biogeochemistry, Surface Processes, Biophysics

- Radiochemical lab building 8b
- Radiochemical laboratories
  FZD research site Leipzig, Division Reactive Transport
- ESRF Beamline ROBL, France
  Division Molecular Structures
Radio-ecological research on the behaviour of actinides in nature

Long term disposal of radioactive waste

Humans

Meat, Milk

Fruits, Bread

Animals

Grain

Fungi

Plants

Water, Soil, Air

Fungi, Bacteria

Water, Soil, Algae/plants
II Sources of radioactive heavy metals (uranium)

Natural and anthropogenic sources of actinides and radionuclides

- Uranium deposits and minerals
- Nuclear power plants
- Reprocessing plants
- Uranium mining waste piles
- Nuclear warhead, USA
- Nuclear explosions
- Uranium ammunition
- Cement production
- Phosphate fertilizer
- Storage tanks
- Storage

Our aim: protect people and environment from being affected by actinides
Fundamental research
Microbe radionuclide interaction

Uranium mining waste pile “Haberland” nearby Johanngeorgenstadt, Saxony

- Determination of the microbial diversity via gen analysis
- Isolation of bacteria

Research on the interaction of actinides with microorganisms

Biosorption

Uptake

Complexation

Biotransformation

Biominalization
Bacterial S-layers

Paracrystalline protein envelope (S-layer)

Cell wall

Interesting properties of S-layer
- Self assembling proteins
- Multifunctional
- Intelligent interface to the bacterial environment
- Metall binding
- Some with high stability

Heavy metals

Trace elements
I identified isolates with S-layer proteins (16S rRNA gene analyses)

- **Lysinibacillus fusiformis** SW-B9 (AY907676)
- **Lysinibacillus sphaericus** DSM28T (AJ310084)
- **Lysinibacillus sphaericus** JG-A12
- **Lysinibacillus sphaericus** JG-B53
- **Lysinibacillus sphaericus** JG-B62
- **Bacillus psychroviridis** 433-D9 (AY266991)
- **Bacillus fusiformis** SW-B9 (AY907676)
- **Bacillus sphaericus** DSM28T (AJ310084)
- **Bacillus mycoides** ATCC6462 (AB021192)
- **Bacillus weihenstephanensis** DSM118221 (AB021199)
- **Bacillus cereus** AH 521 (AF290554)
- **Bacillus pumilus** Tbl (AB195283)

Total 144 Bacillus/Lysinibacillus isolates
Metal binding by bacterial S-layers

Molecular biology

ICP-MS

Spectroscopy

XAS

ATR-FTIR

Microscopy

TRLFS

Elements

Uranium

Pd

Platinum

As
EXAFS investigations of formed uranium protein complexes at pH 4.5

L. sphaericus JG-A12 cells
L. sphaericus JG-A12 S-layer
L. sphaericus JG-A12 spores

Fourier Transform Magnitude

O$_{ax}$: 1.77 Å
O$_{eq1}$: 2.31 Å
O$_{eq2}$: 2.45 Å
C: 2.88 Å
P: 3.61 Å

Used model for the fit of the uranium spectra (parts of two molecules, meta-autunite and uranyltriacetate).


II ATR-FT-IR investigations of formed uranium protein complexes at pH 4

$\nu_{as}(UO_2^{2+})$ bidentate binding by COOH

$\text{PO}_4$ ?
III Supporting EXAFS/IR investigations using poly-Glu and Phosvitin, pH 4

Phosvitin

- Absorption spectrum of Phosvitin in different conditions:
  - Solution
  - Denatured phosvitin from saturation
  - Denatured phosvitin with PbCl
  - Denatured phosvitin with $10^{-4} \text{M U(VI)}$
  - Denatured phosvitin with $10^{-3} \text{M U(VI)}$

- Wavenumber / cm$^{-1}$:
  - 1655, 1540, 1465, 1400, 1228, 1180, 1086, 1001, 930

Polyglutamate

- Absorption spectrum of Poly-Glu in different conditions:
  - Solution
  - Denatured phosvitin with $10^{-4} \text{M U(VI)}$
  - Denatured phosvitin with $10^{-3} \text{M U(VI)}$

Feldman-Complex

- Absorption spectrum of Feldman-Complex:
  - $1716 \text{ cm}^{-1}$ (CON)
  - $1631 \text{ cm}^{-1}$ (CON)
  - $1606 \text{ cm}^{-1}$ (CON)

Li, B. et al. (2010) J Inorg Biochem 104(7), 718-725
IV P/S-K edge XANES measurements

S-layer
S-layer + U(VI)

P K-edge XANES

- No evidence, that Phosphate groups are not involved in the U(VI) binding, but sulfur-species interact also.

S-layer
S-layer + U(VI)

S K-edge XANES

Sulfoxide at 2474.9 eV and sulfate at 2483.8 eV
Posttranslational modifications of the S-layer proteins: glycosylation

Detection of glycosylated proteins by means of the “DIG glycan detection kit”. Dark bands indicate glycosylation.

<table>
<thead>
<tr>
<th>S-layer</th>
<th>Absorption maxima (nm)</th>
<th>Sugar residue (mol/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JG-A12</td>
<td>–</td>
<td>0,03</td>
</tr>
<tr>
<td>JG-B5T</td>
<td>477</td>
<td>2,46</td>
</tr>
<tr>
<td>JG-B7</td>
<td>482</td>
<td>1,93</td>
</tr>
<tr>
<td>JG-B12</td>
<td>482</td>
<td>4,74</td>
</tr>
<tr>
<td>JG-B58</td>
<td>477</td>
<td>2,33</td>
</tr>
<tr>
<td>JG-B62</td>
<td>482-485</td>
<td>3,79</td>
</tr>
</tbody>
</table>

Modified colorimetric determination of sugars after Dubois.

Proteolytic digestion of native S-layers from \textit{L. sphaericus} JG-A12 and NCTC 9602 using the endoproteinase Glu-C (–Glu-P$_1$´ – and –Asp-P$1$ – in Phosphate buffer)
VII Posttranslational modifications of S-layer proteins: phosphorylation

Proteolytic digestions of native S-layer endoproteinase Glu-C

Samples
M: Marker
1: S-layer JG-A12
2: S-layer NCTC 9602
3: Glu-C digested JG-A12 SL
4: Glu-C digested 9602 SL
5: Phosvitin

P-positive references in lane...
M: Ovalbumine 1,73 P:1 (0.12 %)
5: Phosvitin 100 P:1 (10 %)

P-positive bands are indicated by an asterix

Stains-all staining

N-terminal sequencing of the fragments
VIII Sequence analyses and localisation of the modified amino acids

Institute of Radiochemistry
Dr. Johannes Raff
www.fzd.de
Member of the Leibniz Association

Pollmann, K. et al. (2005) Microbiology, 151(9): 2961-2973
IX Hypothesis: phosphorylated amino acids mediate the release of S-layers

Uranium mining waste pile isolate

S-layer gene

Expression of new S-layer proteins

Formation of a new S-layer

Secretion of the protein monomers

Release of the uranium saturated S-layers
- phosphate group(s) as a switch?
- role of sulfur-species?

Signal transduction ???

Uranium

COOH-groups bind uranium

Uranium COOH-groups bind uranium
## Investigation of the hierarchic organisation of S-layer proteins

<table>
<thead>
<tr>
<th>pH</th>
<th>9</th>
<th>8</th>
<th>7</th>
<th>6</th>
<th>5</th>
<th>4</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomers (%)</td>
<td>25</td>
<td>9</td>
<td>9</td>
<td>16</td>
<td>17</td>
<td>60</td>
<td>100</td>
</tr>
</tbody>
</table>

(5 mg/ml S-layer JG-A12, 0.1 M NaCl)

**Hypothesis:**

![Diagram of S-layer protein organisation with Me²⁺ binding at different pH values]
Application oriented research
Application potential of bacterial S-layers

Some natural functions

- Ion and molecule trap
- Molecular sieve
- Enzyme binding

Applications

- Metal selective filters
- Metal/metal oxide catalysts
- Sensors

Pollmann, K. et al. (2006) Biotechnology Advances 24 (1), 58-68
S-layer based materials

Bacterial waste pile isolates

S-layer isolation

Intact polymers

Monomer solution

Sol-gel embedding (particles or coatings)

Controlled recrystallization direct on carriers or after activation

Production of S-layer covered hollow spheres
I S-layer coatings with polyelectrolyte support

- To improve the surface properties for S-layer recrystallization a polyelectrolyte interface can be introduced
- Simple LbL-coating
- Layer composition influences the stability of the protein coating

Modified after Decher, G. SCIENCE 277(5330), 1232-1237

Without polyelectrolyte support
- Recrystallization on SiO₂
- 57 % coverage
- 12 h incubation

With polyelectrolyte support
- Recrystallization on PE-coated Si
- >90 % coverage
- < 30 min incubation
- Monolayer
II S-layer coated polyelectrolyte hollow spheres

- Coating of spheric templates (CaCO$_3$, CaP) with multiple layers (4-10-...) of polyelectrolytes (PSS, PAH)
- Dissolution of the core material by HCl
- Coating of the spheres with S-layer(s) via recrystallisation and LbL-techniques
- Mono- or multifunctional transparent spheres
  - Magnetic “beads”
  - Combination of binding molecules and catalysts
  - …
III Functionalization of S-layer coatings

Possible carrier materials:
- Glas
- Ceramics
- Metal
- Plastics

Metal binding
Production of nano-particles
Dyes
Enzymes
Aptamers
Antibodies

Multifunctional multi layer systems
Advantages compared to previous approaches:
- Binding properties
- Environmental-friendly disposal
- Selectivity ?
V Uranium binding by cells and S-layer proteins

Bound uranium (in mg U/g dw)

Isolates

- JG-A12
- JG-B62
- JG-B58
- JG-B53
- JG-B41
- JG-B37
- JG-B35
- JG-B12
- JG-B7
- JG-B5T

**Uranium binding (in µg U/mg dry weight)**

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>19,5</td>
<td></td>
<td>64,0</td>
<td>42,5</td>
<td>99,9</td>
<td>31,6</td>
<td>33,3</td>
<td>149,2</td>
<td>33,3</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td>76,6</td>
<td></td>
<td>42,5</td>
<td>99,9</td>
<td>150,0</td>
<td>33,3</td>
<td>33,3</td>
<td>149,2</td>
<td>33,3</td>
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<tr>
<td>6</td>
<td></td>
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<td>157,4</td>
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<td>76,6</td>
<td>157,4</td>
<td>149,2</td>
<td>143,3</td>
<td>143,3</td>
<td>291,5</td>
<td>182,5</td>
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<tr>
<td>7</td>
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<td>229,1</td>
<td></td>
<td>157,4</td>
<td>229,1</td>
<td>291,5</td>
<td>291,5</td>
<td>411,7</td>
<td>411,7</td>
<td>411,7</td>
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<td>8</td>
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</tr>
</tbody>
</table>

VI Arsenic binding by cells and S-layer proteins

As(V) binding by living and dead biomass, using a As(V) solution with [As] = 10 mg/l

As(V) binding by cells and S-layers of reference strains and uranium mining waste pile isolates, using a As(V) solution with [As] = 10 mg/l

Reference material: Ferrosorp (granulated ferric hydroxide)

Matys, S. et al. (2010) FZD-Report 530
VII Pt and Pd binding by S-layer

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Bound metal (in mg Me/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JG-B35</td>
<td>Pt 1900, Pd 924</td>
</tr>
<tr>
<td>JG-B41</td>
<td>Pt 1800, Pd 825</td>
</tr>
<tr>
<td>JG-B7</td>
<td>Pt 490, Pd 820</td>
</tr>
<tr>
<td>JG-A12</td>
<td>Pt 710, Pd 1147</td>
</tr>
<tr>
<td>JG-B53</td>
<td>Pt 815, Pd 1023</td>
</tr>
<tr>
<td>JG-B62</td>
<td>Pt 235, Pd 760</td>
</tr>
</tbody>
</table>

VIII Removal of contaminants or recovery of resources

- Rain
- Dissolved metals/metalloids
- Former mining sites
- Metal removal or recovery
- Geogenic deposits
- Contaminants
- Resources
- Industry
- River, lakes, sea
IX Metal/metal oxide catalysts

Defined catalytic active nanoparticles e.g.
- Pt, Pd, Fe/Pd, Fe/Pt
- ZnO, TiO₂
with sizes of 1-26 nm

Incubation with a metal salt solution

+ Addition of reducing / precipitation agents
X S-layer based synthesis of defined and regular arranged nanoparticles

1. Adding metal salt solution

2. Adding reducing reagents

3. Protein removal via UV
XI Synthesis of Pt nanoparticles on different coatings

Protein removed by UV

Nanoparticles produced on PE without S-layer

Nanoparticles produced on PE + S-layer
→ Possibly migration and agglomeration of the particles
→ Further improvement necessary
Advantages compared to previous approaches:
- Environmental-friendly (disposal, immobilization of nano-particles)
- Higher efficiency (required energy, day light sensitivity)

Patent pending
XIV ZnO based photocatalysts for the elimination of pharmazeutica

Top: XRD-pattern of S-layer supported ZnO (red graph) and equally prepared ZnO without protein (green graph). S-layer prepared ZnO-particles have a size of about 14 nm.

Right: Diclofenac elimination by S-layer supported ZnO in suspension (A) and immobilized on a carrier (B).
XV Photocatalytic elimination of pharmaceuticals in water
Advantages compared to previous approaches:
- Transferability
- Chip-based quantification of several analytes
- lower unspecific binding

Patent pending
XVII S-layer bound fluorescence dyes and aptamers

FRET-pair

a) b) c)

S-layer aptamer composite
d) e) f)

Thrombin binding by the S-layer bound aptamers (IAsys)

100 µg
### Biomass production

Possible strategies:
- High density cultivation
- Heterologous expression

<table>
<thead>
<tr>
<th>Process</th>
<th>Yield in g/l (z.B. JG A12)</th>
<th>Manpower requirements</th>
<th>€/g S-layer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biomass</td>
<td>S-layer</td>
<td></td>
</tr>
<tr>
<td>Conventional</td>
<td>3</td>
<td>0.03</td>
<td>6.9</td>
</tr>
<tr>
<td>Pilot plant</td>
<td>6</td>
<td>0.16</td>
<td>1.4</td>
</tr>
<tr>
<td>Optimisation</td>
<td>12</td>
<td>0.85</td>
<td>1.4</td>
</tr>
<tr>
<td>Aim</td>
<td></td>
<td></td>
<td>&lt;10</td>
</tr>
</tbody>
</table>
Summary

• S-layers are essential for many bacteria to survive in extreme environments.

• S-layer proteins are highly complex proteins with many different functional groups and modifications, directly affecting their molecular behavior (conformation, hierarchic organization, metal binding).

• Their metal binding and self-assembly properties make S-layers very worthwhile for the development of new materials for
  • environmental techniques
  • biotechnology
  • nanotechnology,
  but for their application we need to know more about them.
Future cooperation IRC-FZD with IfWW-TUD

• Submitted EU-project “ActiveBrane”
  Development of reactive nanomembranes with low biofouling for decomposition of organic pollutants in water streams by applying a novel sandwich technology

• Arsenic binding by S-layers
  → Planned DFG project
  → Arsenic binding aptamers

• Aptamer based sensors for different pollutants
  → Planned project
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Thank you for your attention...

...questions, remarks?