Bioinspired nanomaterials

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nanoSeminar
Institute of Materials Sciences
11.11.2010

Forschungszentrum Dresden Rossendorf

Institute of Radiochemistry • www.fzd.de • Member of the Leibniz Association

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

Forschungszentrum Dresden-Rossendorf e.V. (FZD)

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

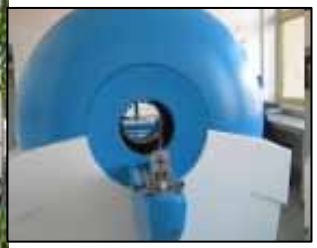
Institute of Radiochemistry

Dresden

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

Radiochemical lab building 8b

Leipzig

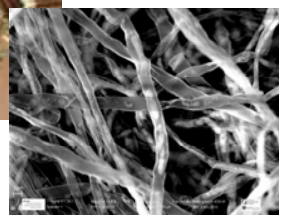


Radiochemical laborytories
FZD research site Leipzig, Division Reactive Transport

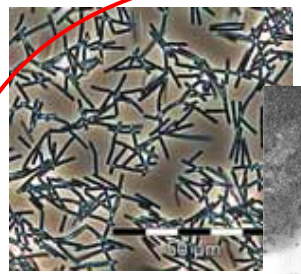
Grenoble

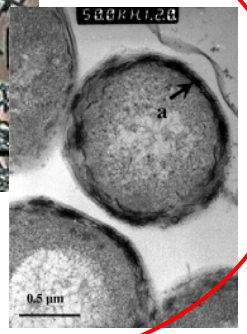
ESRF Beamline ROBL, France
Division Molecular Structures

Radio-ecological research on the behaviour of actinides in nature

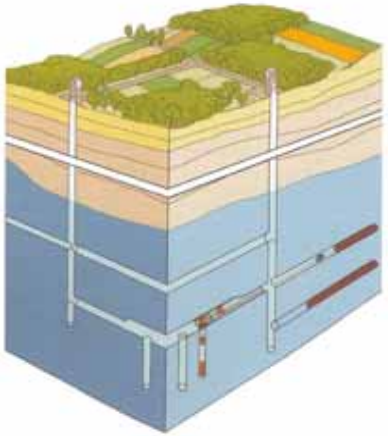


Fungi

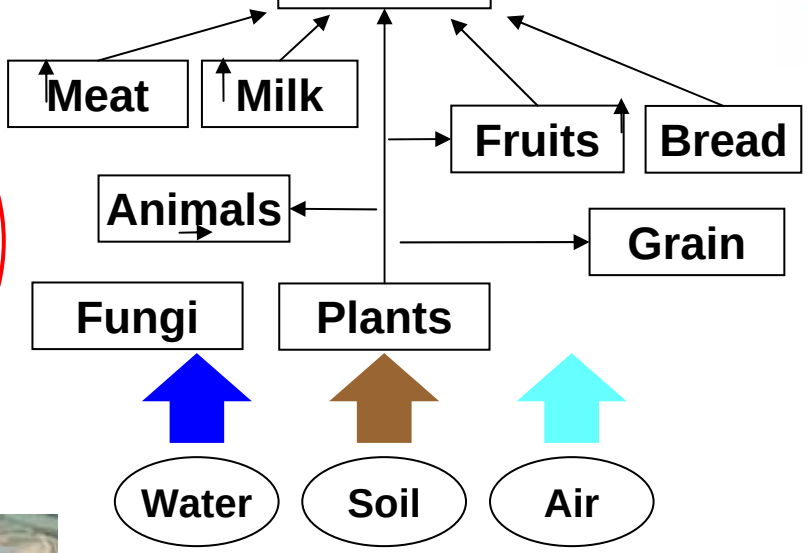




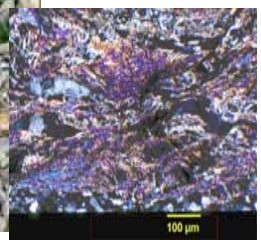
Bacteria



Long term disposal of radioactive waste



Water



Soil

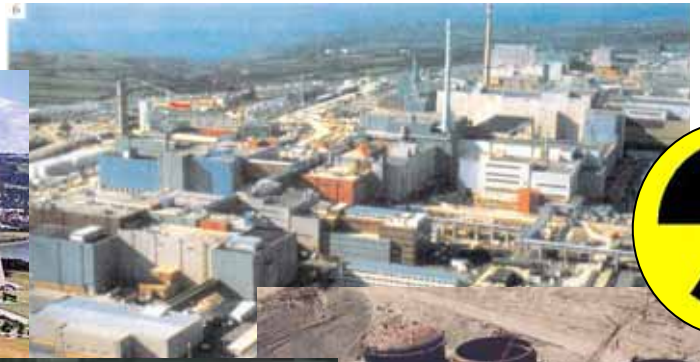
Algae/plants

Natural and anthropogen sources of actinides and radionuclides

Uranium deposits and minerals

Nuclear power plants

Reprocessing plants



Storage tanks

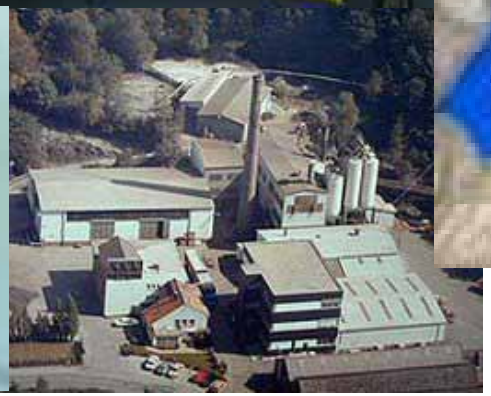
Uranium mining waste piles

Nuclear explosions

Nuclear warhead, USA

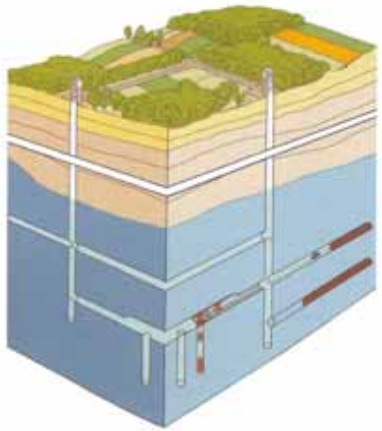
Phosphate fertilizer

Uranium ammunition



Cement production

Storage

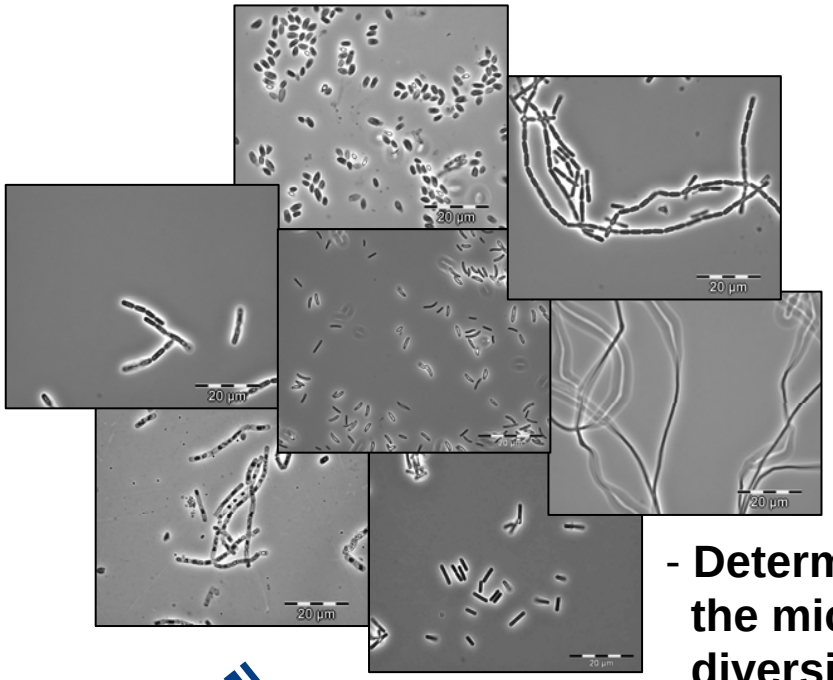


➔Our aim: protect people and environment from being affected by actinides

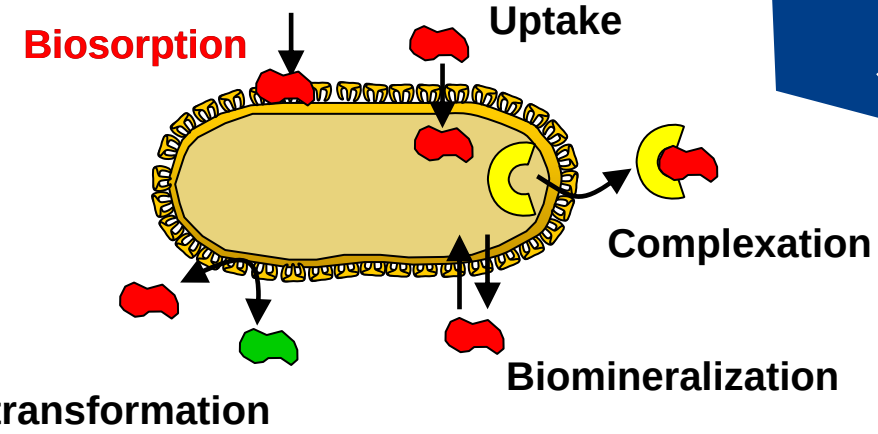
Fundamental research

Microbe radionuclide interaction

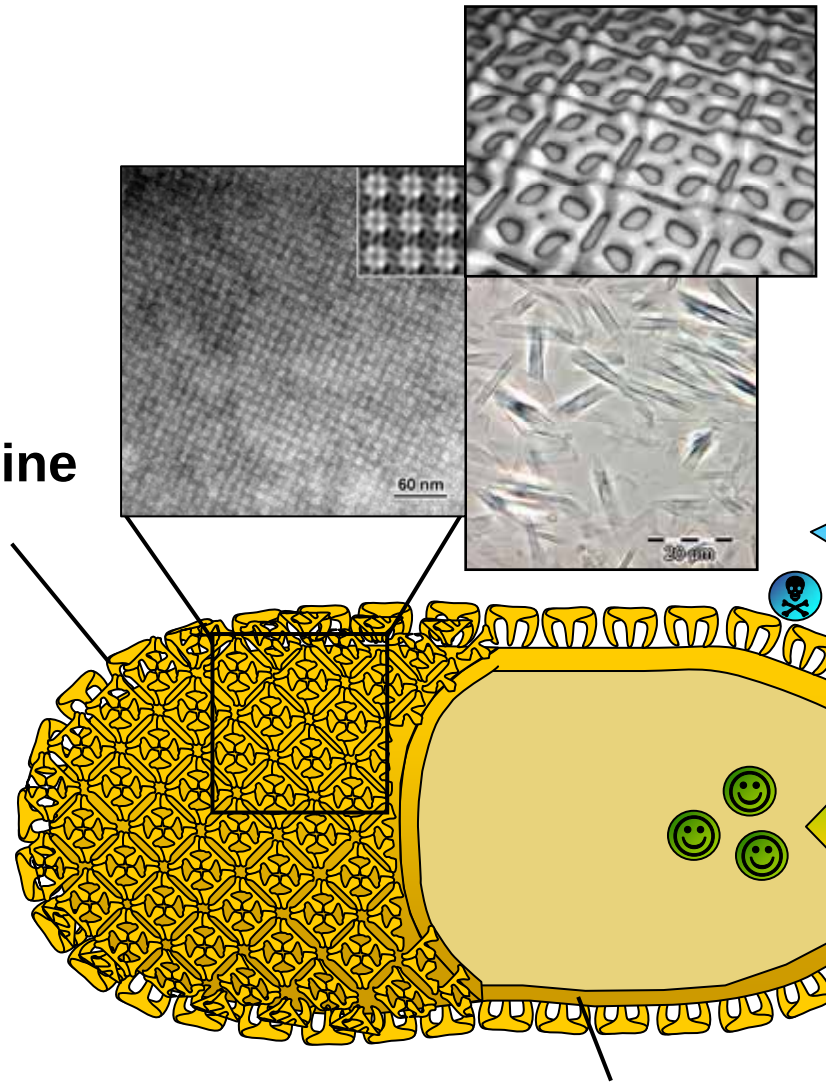
Uranium mining waste pile “Haberland” nearby Johann-georgenstadt, Saxony



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



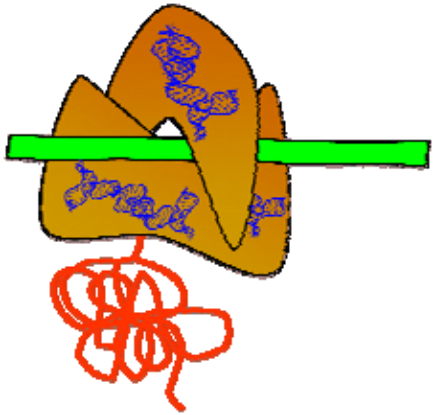
Research on the interaction of actinides with microorganisms



Interesting properties of S-layer

- Self assembling proteins
- Multifunctional
- Intelligent interface to the bacterial environment
- Metall binding
- Some with high stability

I Identified isolates with S-layer proteins (16S rRNA gene analyses)



JG-B7

Lysinibacillus fusiformis SW-B9 (AY907676)

Lysinibacillus sphaericus DSM28^{T} (AJ310084)

Lysinibacillus sphaericus JG-A12

Fundamental research

JG-B53

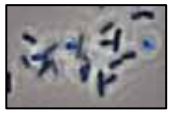
Application oriented research

JG-B62

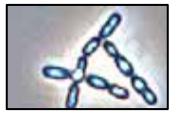
JG-B58

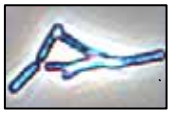
Bacillus psychroviridis 433-D9 (AY266991)

JG-B5T

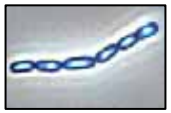


Bacillus pumilus Tbl (AB195283)





JG-B12
JG-B35
JG-B37
JG-B41



Bacillus cereus AH 521 (AF290554)

Bacillus mycoides ATCC6462 (AB021192)

Bacillus weihenstephanensis DSM118221 (AB021199)

0.01

Total 144 *Bacillus/Lysinibacillus* isolates

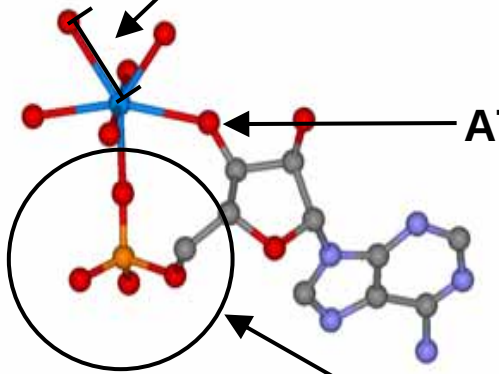
Metal binding by bacterial S-layers

Elements

Molecular biology

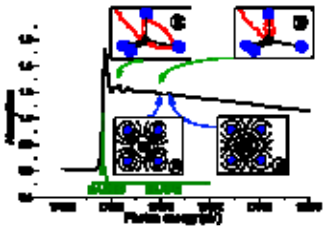
ICP-MS

Microscopy



Spectroscopy

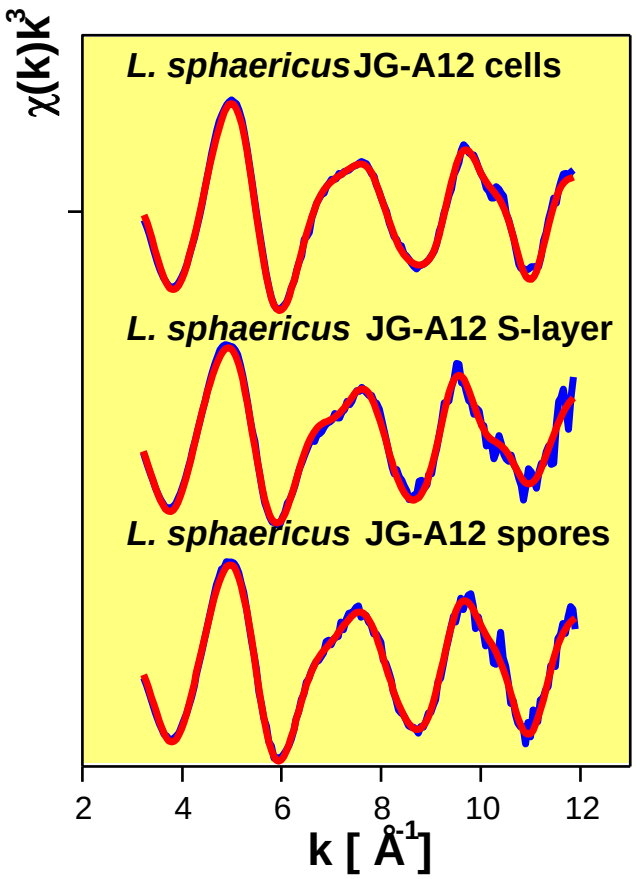
XAS

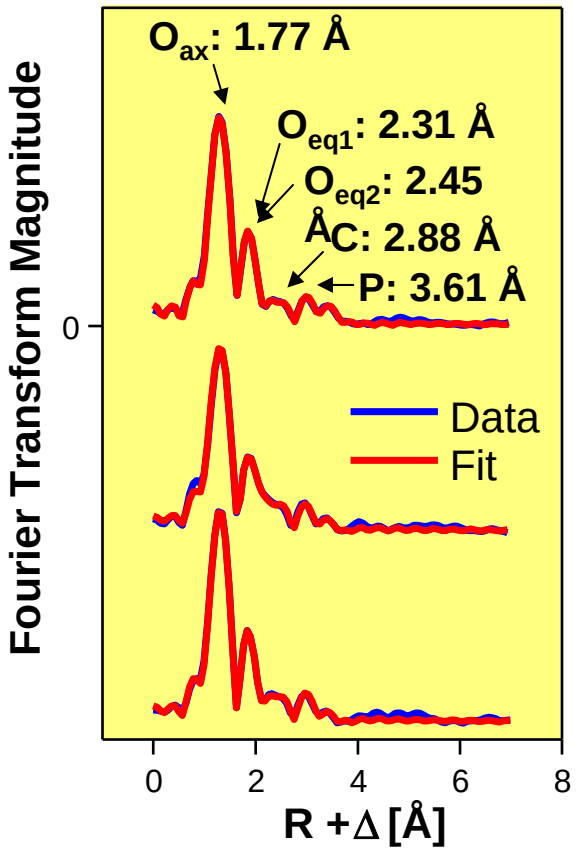


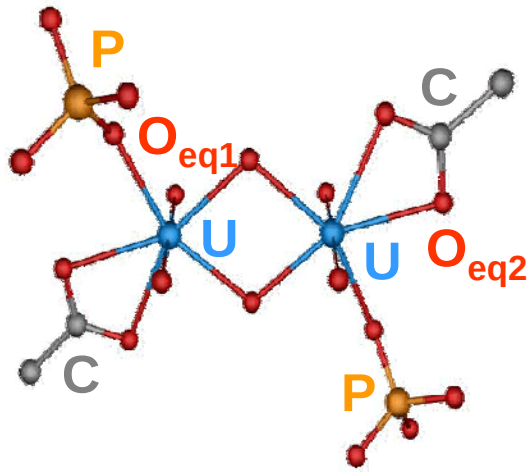
ATR-FTIR

TRLFS

I EXAFS investigations of formed uranium protein complexes at pH 4,5





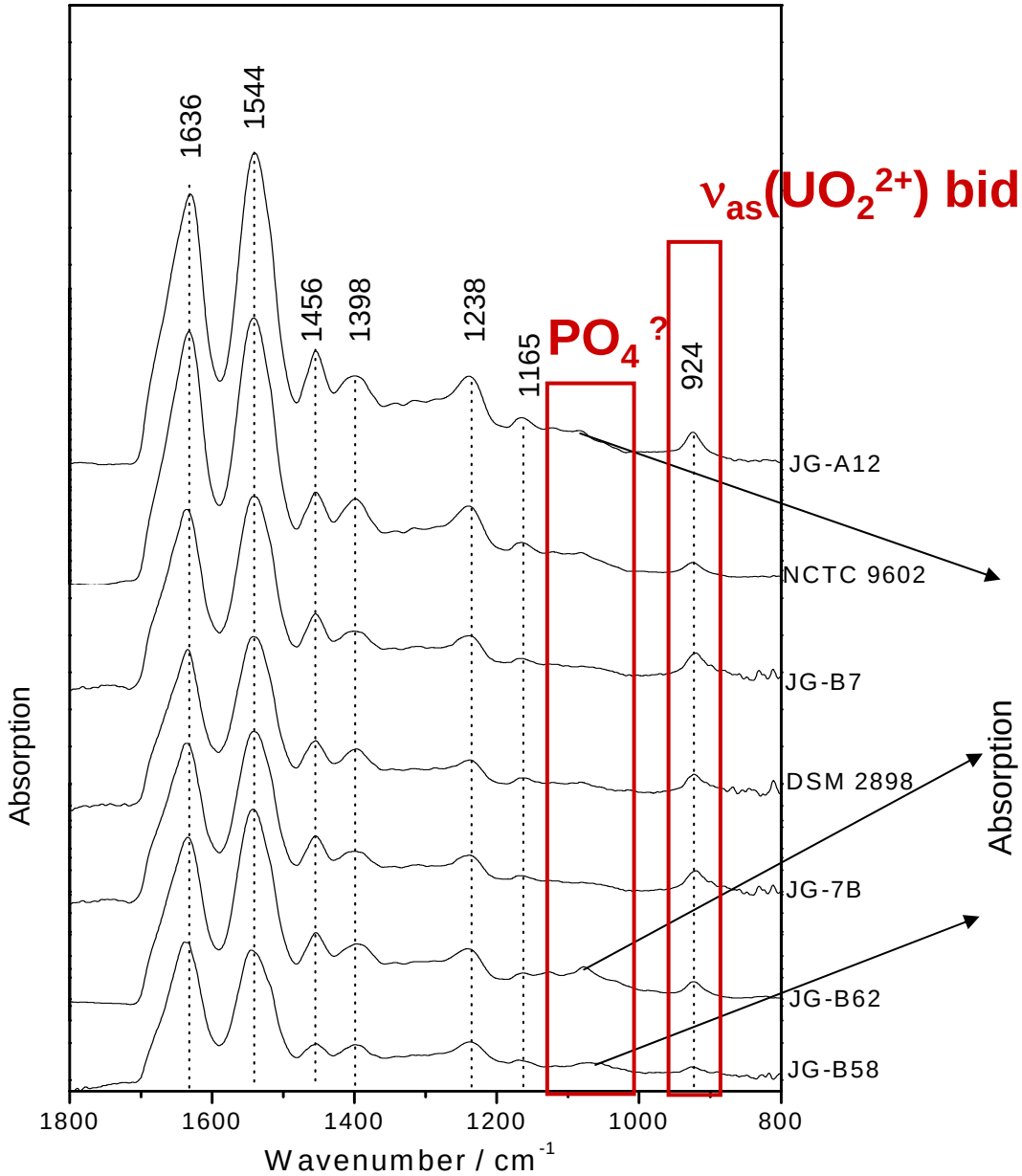


Used model for the fit of the uranium spectra (parts of two molecules, meta-autunite and uranyltriacetate).

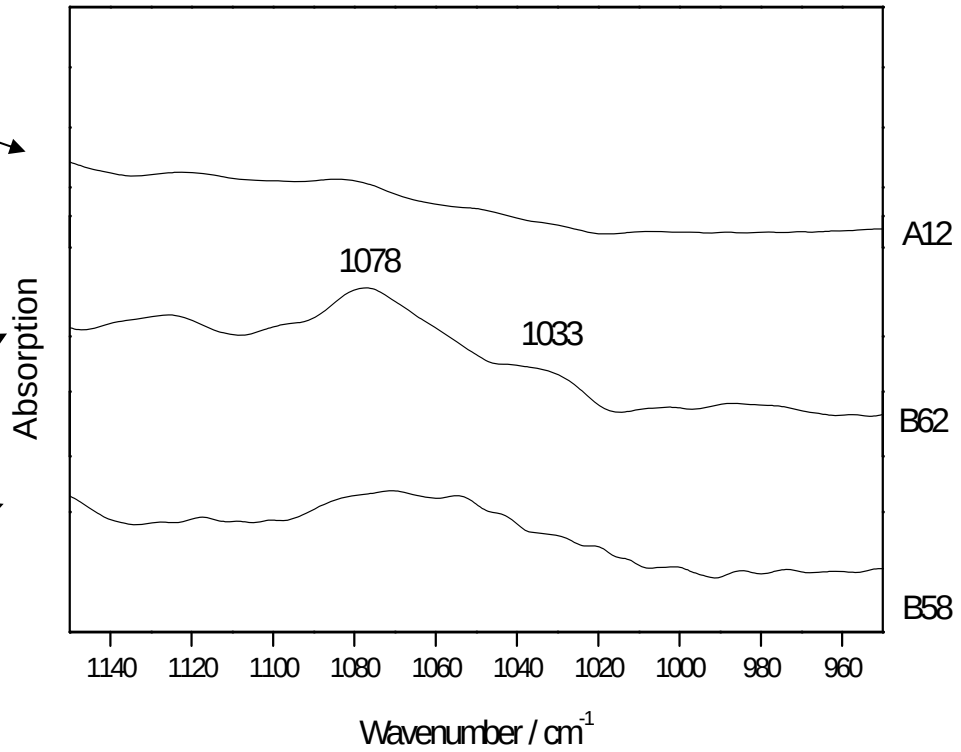
Merroun, M.et al.(2005) Appl. Environ. Microbiol. 71(9): 5532-5543

Raff, J. et al. (2004) In Water-Rock Interaction, Richard B. Wanty und Robert R. Seal II, A.A. Balkema Publishers, New York, p 97-701

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

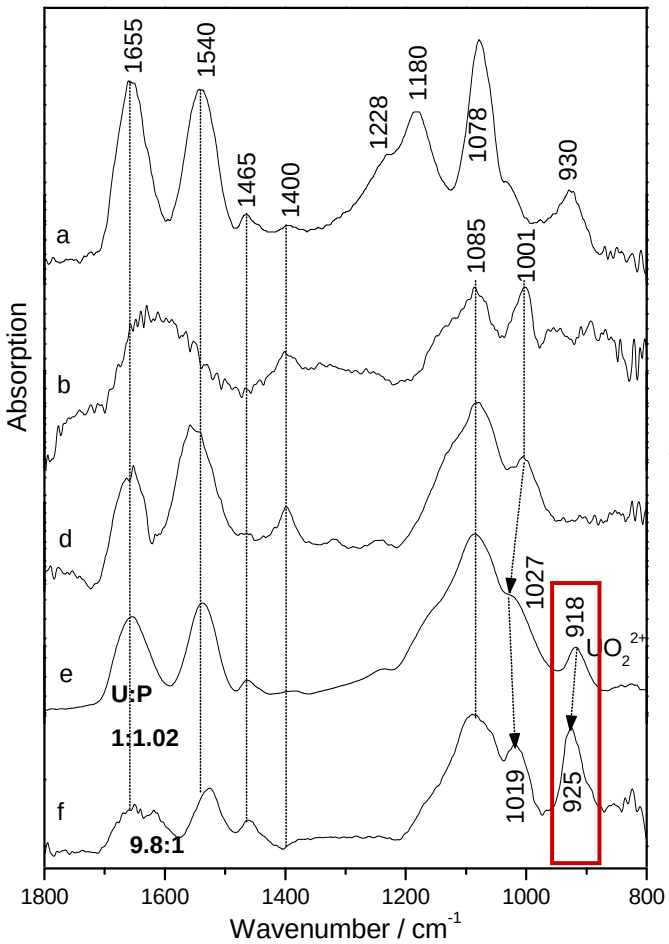


$\nu_{as}(UO_2^{2+})$ bidentate binding by COOH



III Supporting EXAFS/IR investigations using poly-Glu and Phosvitin, pH 4

Phosvitin



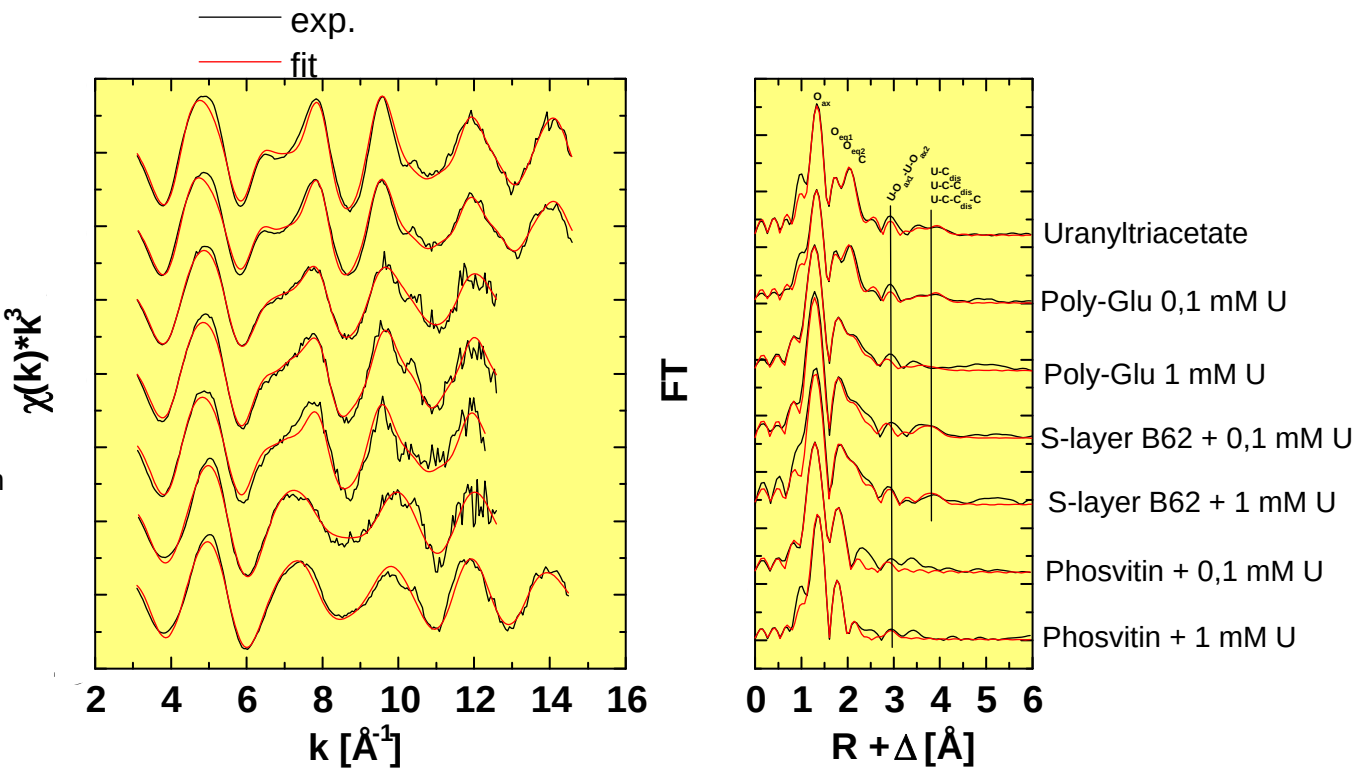
Phosvitin Solution

Denatured phosvitin from saturation

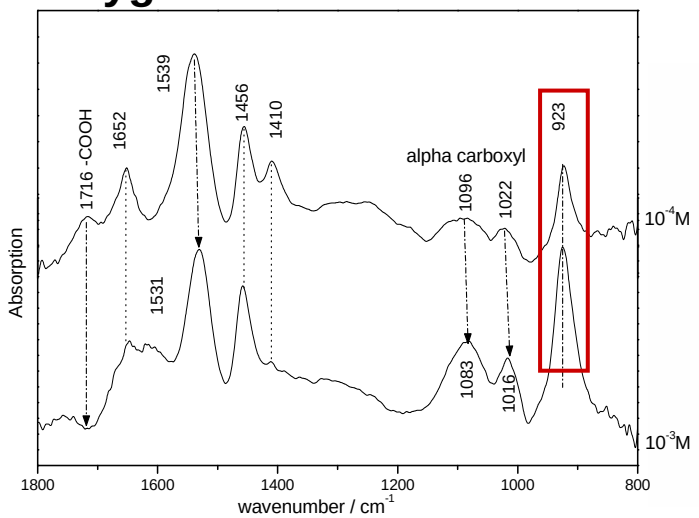
Denatured phosvitin with PbCl

Denatured phosvitin with 10^{-4} M U(VI)

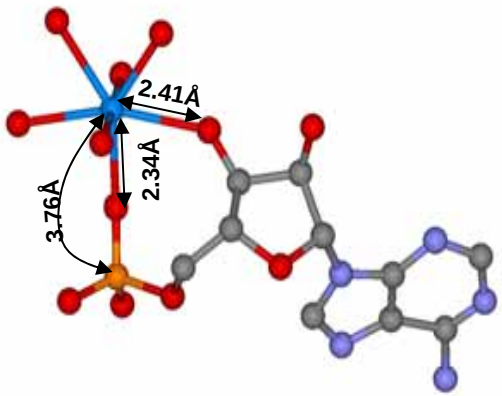
Denatured phosvitin with 10^{-3} M U(VI)



Polyglutamate



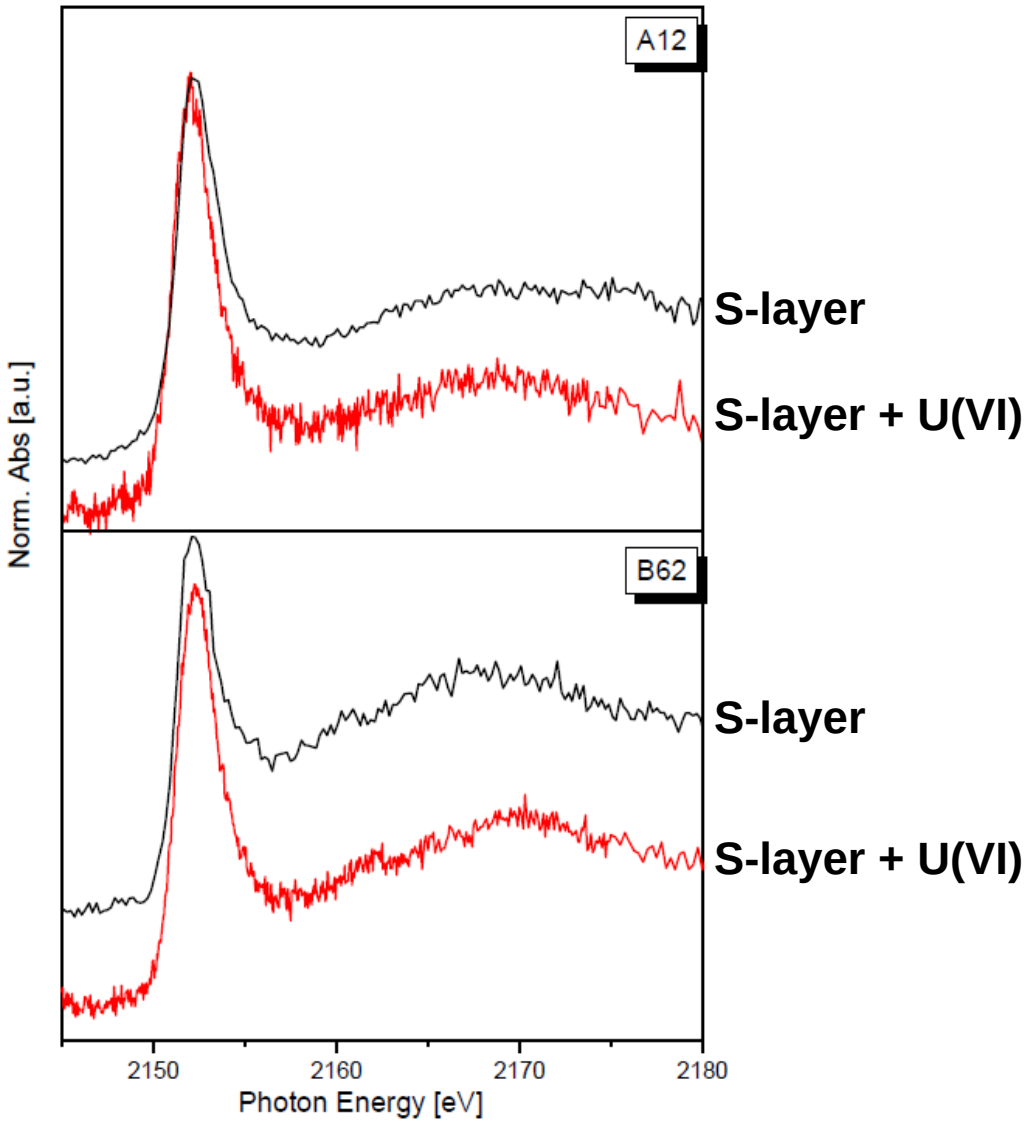
Feldman-Complex



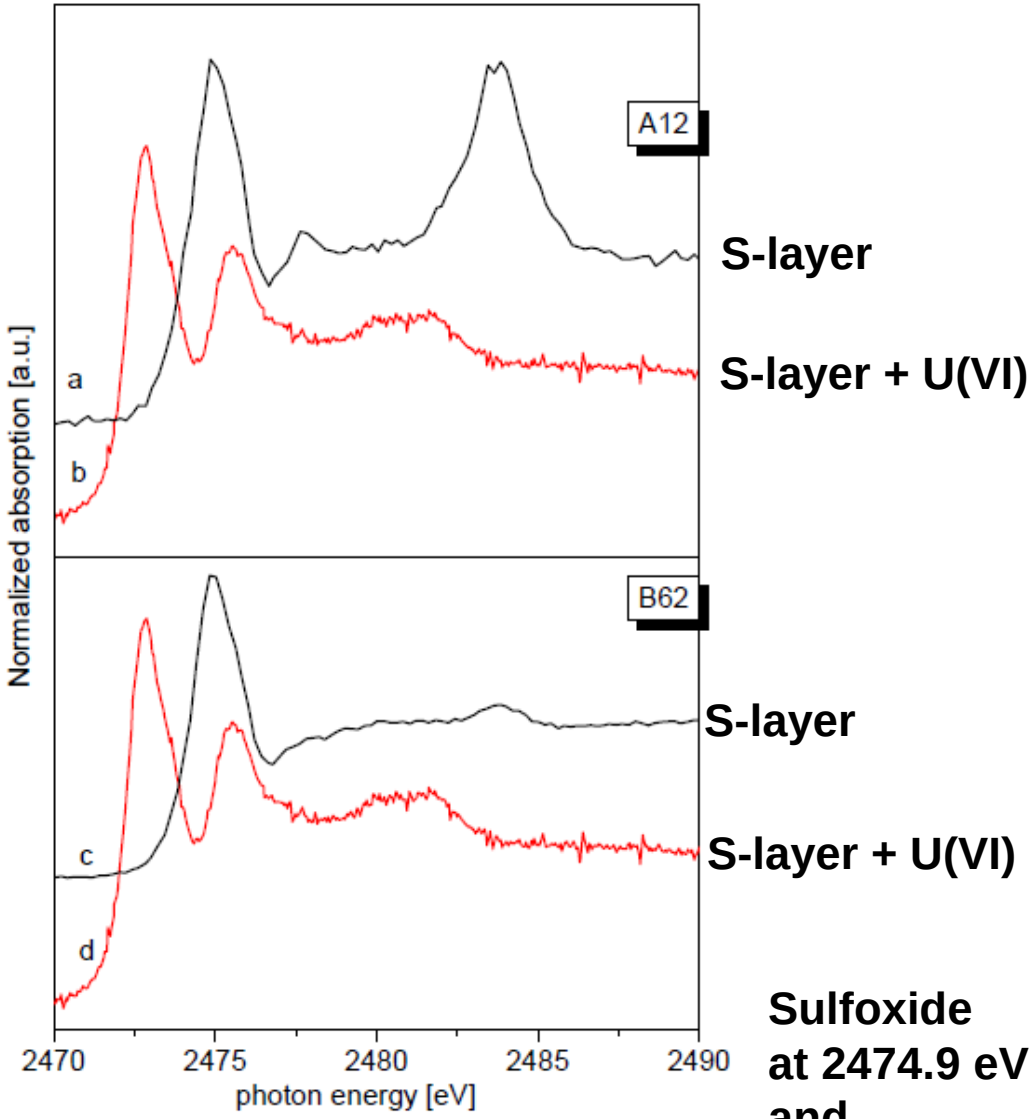
Li, B. et al.(2010) J Inorg Biochem 104(7), 718-725

IV P/S-K edge XANES measurements

P K-edge XANES



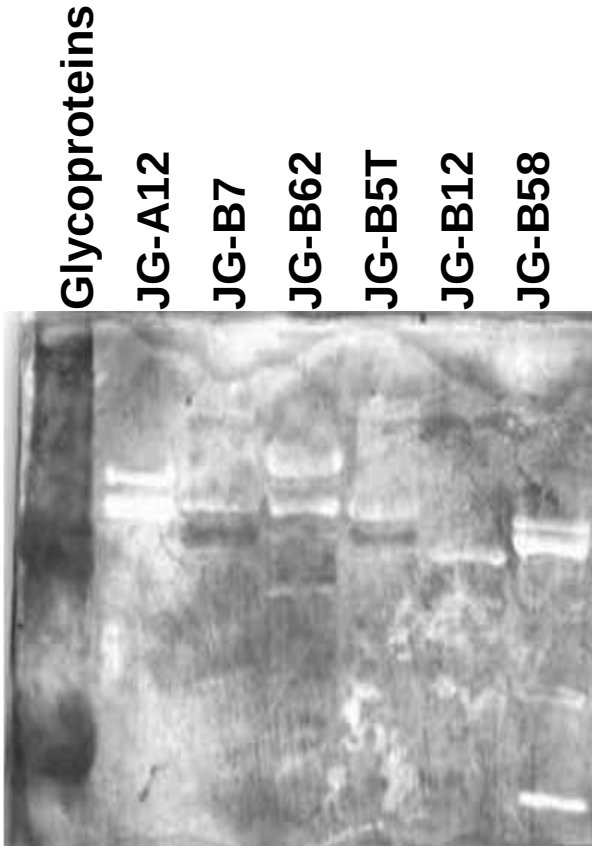
S K-edge XANES



Sulfoxide at 2474.9 eV and sulfate at 2483.8 eV

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

V Posttranslational modifications of the S-layer proteins: glycosylation



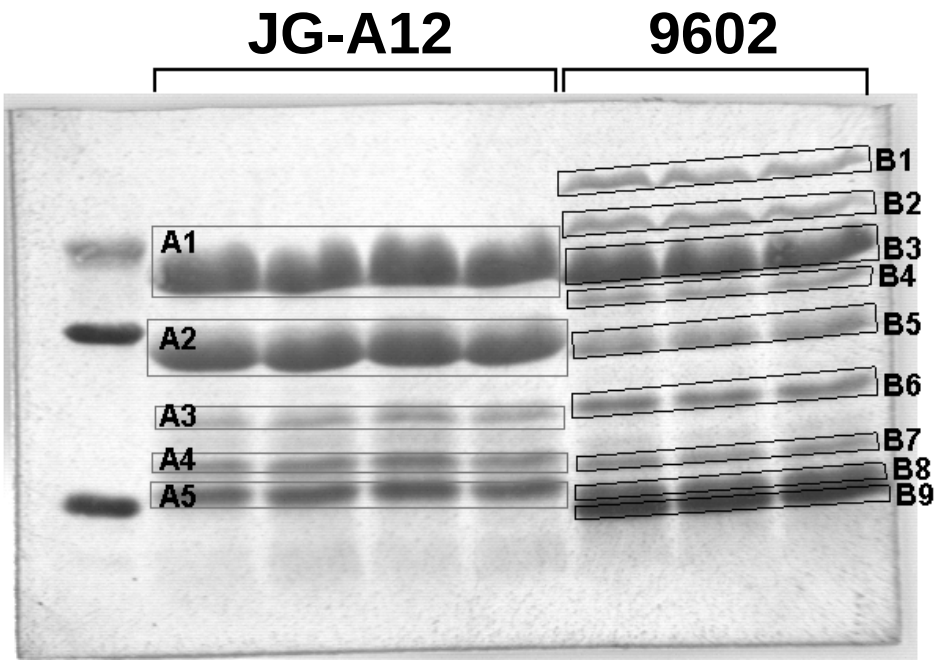
Detection of glycosylated proteins by means of the "DIG glycan detection kit". Dark bands indicate glyco-sylation.

S-layer	Absorption maxima (nm)	Sugar residue (mol/mol)
JG-A12	-	0,03
JG-B5T	477	2,46
JG-B7	482	1,93
JG-B12	482	4,74
JG-B58	477	2,33
JG-B62	482-485	3,79

Modified colorimetric determination of sugars after Dubois.

Weinert, U. et al.(2010) Anal Biochem (in preparation)

Proteolytic digestion of native S-layers from *L. sphaericus* JG-A12 and NCTC 9602 using the endoproteinase Glu-C (–Glu-P_1´– and –Asp-P1 – in Phosphate buffer)



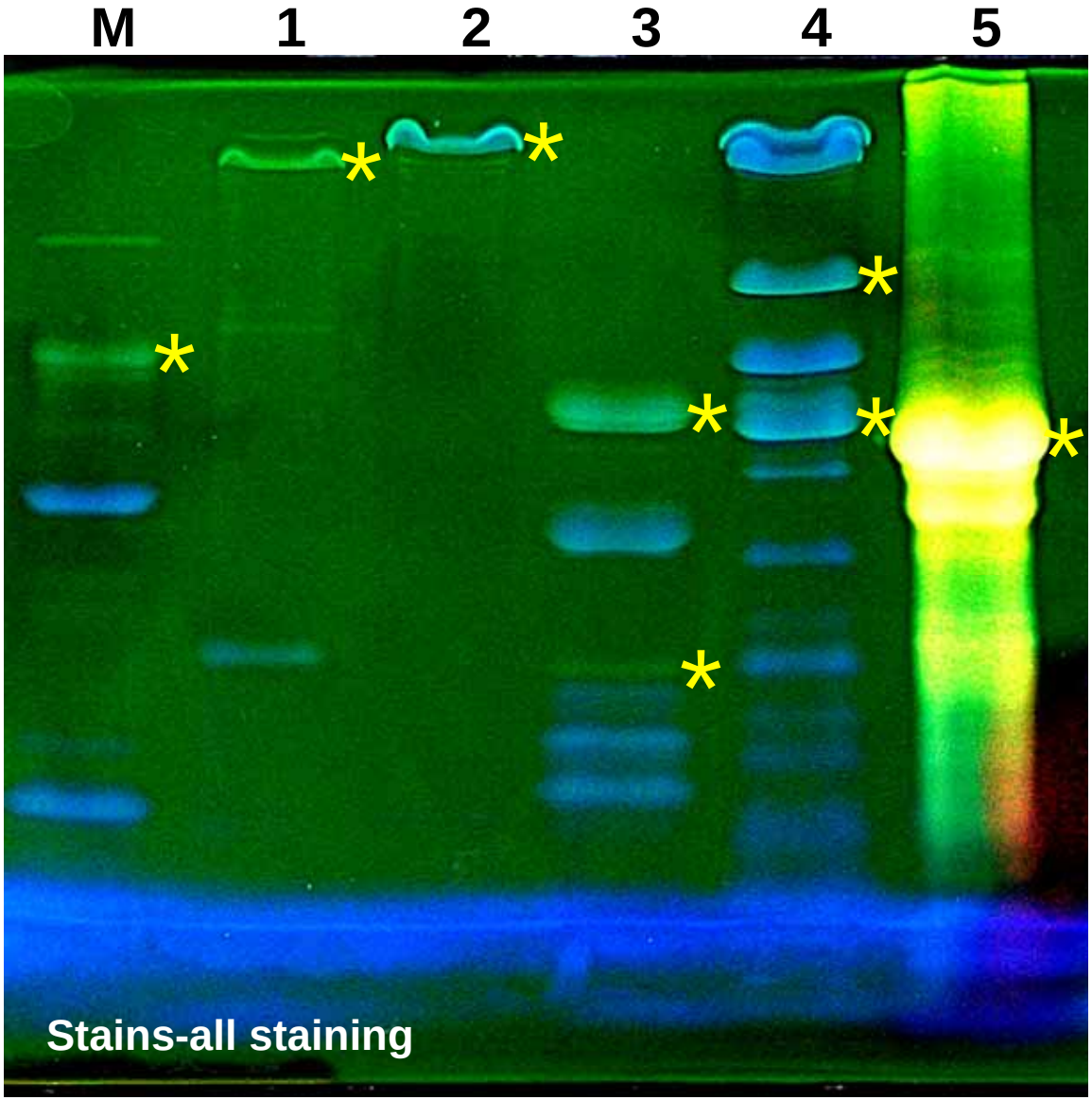
JG-A12

MANQPKKYKKFVATAATATLVASAIVPVASAAGFSDVAGNDHEVAINALADAGIINGYADGSFKPNQTIN
RGQVVKLLGRYLEAQGQEIPADWNSKQRFNDLPVTAEEELVKYAALAKDAGVFNGSNGNLNASQTMQRQQ
MAVVLVRAIKEIAGVDLVAEYKKANFVTEIGDLDKAYSAEQRTAIVALEYAGITNVAHFNPGNSVTRGQF
ASFLYRTIENVVNNPEAGVAAVKAINNTTVEVTFDEEVDNVQALNFLISDLEVKNAAVKLTNKKVVVLTT
AAQTADKEYTVSLGEEKIGTFKGIAAVVPTKVDLVEKSVQGKLGQQVTLKAQVTVAEGQTKAGIPVTFFI
PGSANGVKTPVTVEAVTNEEGIATYSYTRYAATNDTVTVYANGDRSKFSTGYVFWAVDQTLEITEVTTGA
TINNGANKTYKVTYKHPETGKPVSGKVLNVSVKENIDVTVDKLQNVTVNGIAVVQTSDNNTRAAQITTDS
KGEATFTVSGSNAEVTPVVFEATPVQVTNTNGAVTTTSYTQKYTADILQASAAKVTFGAVQAAYTLEVTR
DGGEVAATGVENGRKYNLVVKDKDGKLAANETVNVAFNEDIDGVISTVTSAEFVKVENDKQVRYEGKKIT
VKTNSKGEASFVISSEAVNTYATPIAWIDINNQSAKDANLDKGEPSAVAPISYFQAEYLDGSKLVSYKDT
TETDKFTGSETATFKVQLTNQSGKVVKNSGYTTPNVSYTVYNTGANNVKVDGVEIAPNRVHTVVAKDGVI
NVTTVDNKSSSVKVLATGVAKQTNGNKEFAFTSKEATATFTATNEVSNPYTGAVKHYNTDKKTITFDNKD
AIKYAGVKGKTYKYFGLGSTPIANADAFIATLSGQGAGNQVTVTYKEEDDVVSFYIISVVNGGIGNPTTD
PALANGVTGTIAFTNTSFNASANQTITVKDADLNVNATVADTTTVTITDSAKTTFNITLTETGVNTGEFT
GTLTSAQLALLKDGVITATYNDAKNANNVAATATATATLNTTVGAVNFAAKATTEYSEGVGTAAKVEGST
VLTSKVDTGDLVLVVDGVQFTTVVTAIDPGTSATASLTAVVAEINAAAKKVGFTADVATVDADKIVLTSP
TKGTSSSVEVKDLGTTTGLGLTKAPEVKGTAGAAVATQWKFTVTTTPAVGETITVKVGTKEASHKVVAGN
DLAAIATALNTAIGADYNIALVTGSNVITVTQATPAKTTDELSVTVTK

9602

MANQPKKYKKFVATAATATLVASAIVPVASAAGFSDVAGNDHEVAINALADAGIINGYADGSFKPNQTIN
RGQVVKLLGRYLEAQGQEIPADWNSKQRFNDLPVTAEEELVKYAALAKDAGVFNGSNGNLNASQTMPRQQ
MAVVLVRAIKEIAGVDLVAEYKKANFVTEIGDLDKAYSAEQRTAIVALEYAGITNVAHFNPGNSVTRGQF
ASFLYRTIENVVNAPEAGAAAVKAVNNTTVEVTFDEEVDNVQALNFLISDLEVKNAAVKQTNKKVVVLTT
APQTADKEYTVSLGEEKIGTFKGIAAVVPTKVDLVEKSVQGKLGQQVTLKAQVTVAEGQTKAGIPVTFFI
PGSANGVKSPVTVEAVTDENGVASYTYTRYAATNDTVTVYANGDRSKFSTGYVFWAVDTTLEITEVTTGD
IINNGANKTYKVAYKDPETGKPVSGKVLNVSVKENIDVTVDKLQNVTINGIKVAQTSTSNTTAAQVTTDS
KGEATFTITGANAEVTPVVFAGEAVTGQTLPSQKYNADALQTTAKKVKFGALQADYILEVTRDGGETAAT
GYDNGRKYKLVVKDKNGKLAANETVNVAFNEDIDGVISTTTDAKFVKLDNEDNQVGWDTTVDKDAKKITV
KTDSKGEASFIIGNTKENTYYTPIAWIDVNYQSGKVGTLDQGEPSATAPISYFQSPTIDGSKLVAENKDG
KKPSDFEDKDAIFKVVLVNQSGKEMKNHNYKTEKVSYTVNNTGSNEAVVEYTYNGKVESETLAPNRATSV
VADNGTITVKPNGKTTSVKVLATGVAKEDKTNGKEYAFTAKEATAKFTATKEISNPYTGAIKYYDTDKKT
ITFDGKDAIKYAGESGKKYKYYSGNIEVATEKAFIDLLANASGKVTVTYKVEDDTKSFHIINVGTDVANK
PVEKKDDIKPNNAPVAKQVADQVIASNGSKLTFTANDVASDADKDTLKIVTAYTTNSTVATATTDANQL
GFSVEPKGEGSTTVTVVVTDGKDNINVSFKVNVSTASYTSVVNSGNPVKDFAPAVPTAATAKLPEFTSVA
KAGTIKVQGFDIPLTLGSTQAQVLETLNNFITDYKINASAKLVDKAIVISSTETGNAATLTVEGDSTLEL
VAGTVLGTVSTTPGSVGTATPAKYAVKVLNGVSNGTKVDFKFTIGAKTVTVTVDATAGAKAVSDVVDTLA
AATLDGYSITKNGDVIELTQTTPATTTDKLVVETKKAN

Proteolytic digestions of native S-layer endoproteinase Glu-C



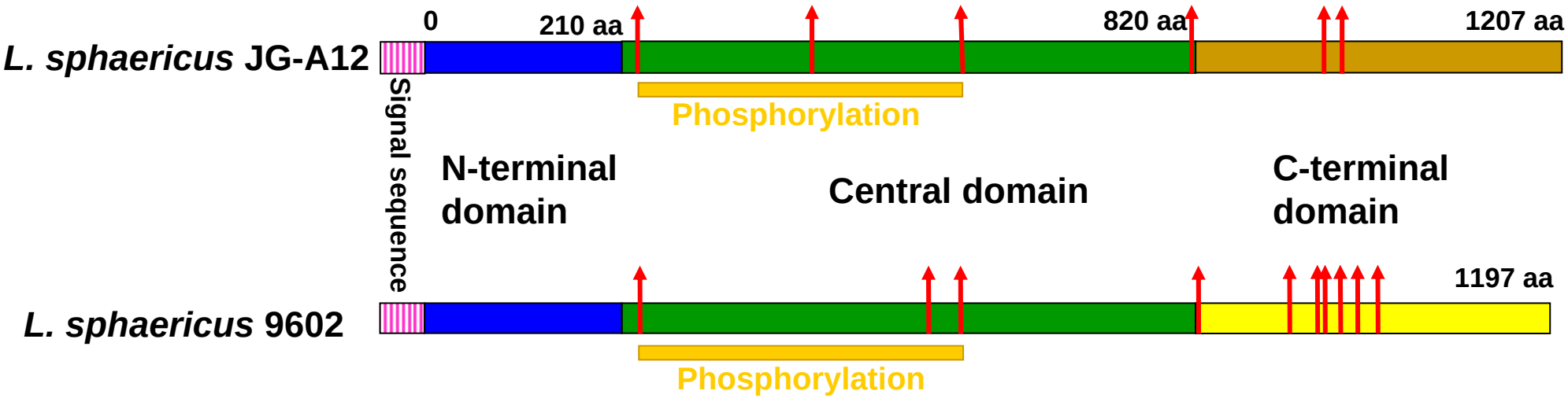
Samples
M: Marker
1: S-layer JG-A12
2: S-layer NCTC 9602
3: Glu-C digested JG-A12 SL
4: Glu-C digested 9602 SL
5: Phosvitin

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

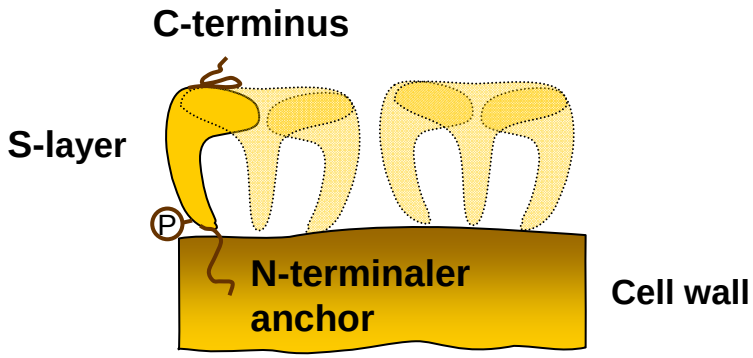
P-positive bands are indicated by an asterix

N-terminal sequencing of the fragments

VIII Sequence analyses and localisation of the modified amino acids

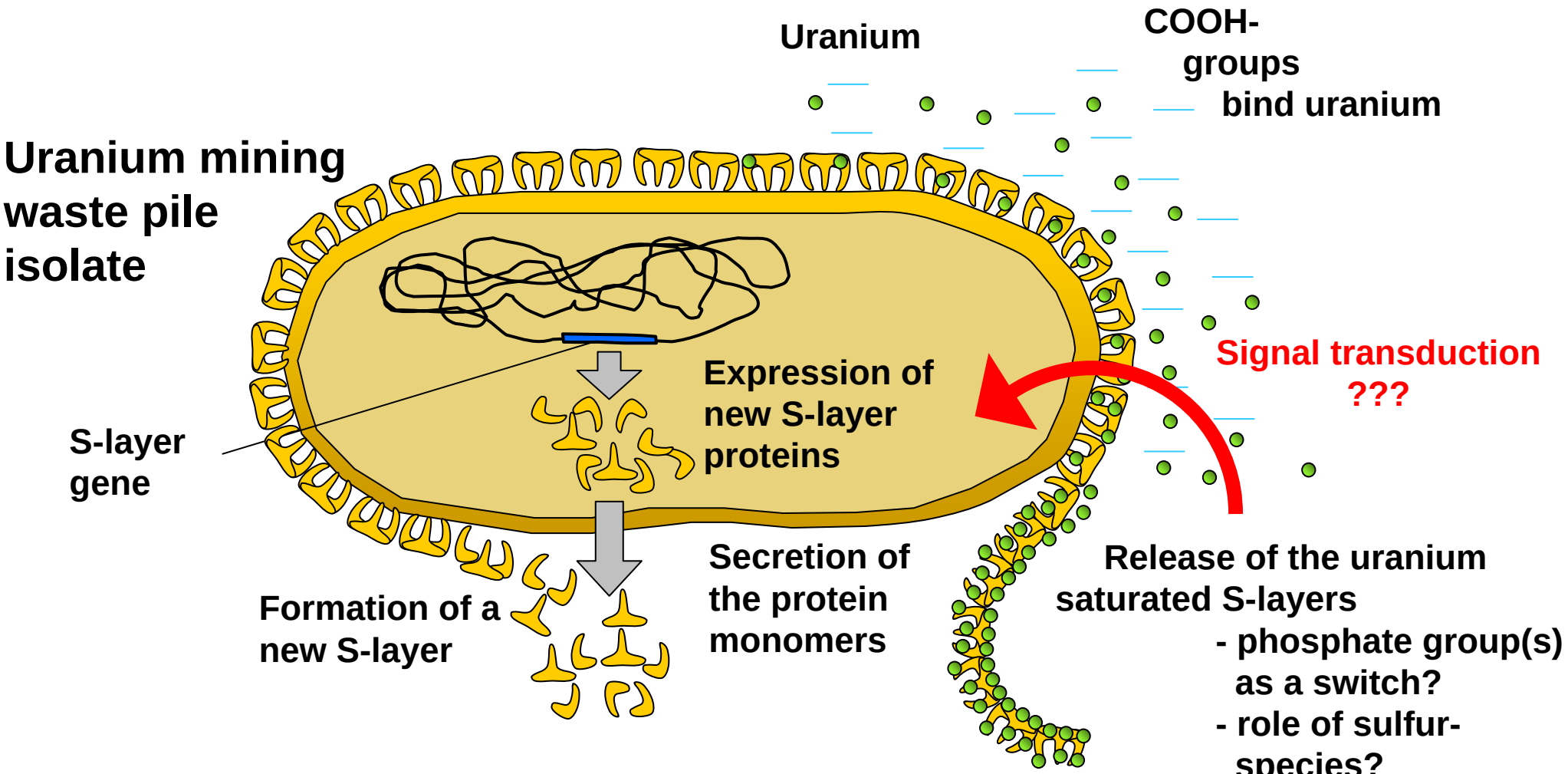


Glu-C cleaving sites in the native protein; theoretical 125 (JG-A12) and 141 (9602) Possible sites.



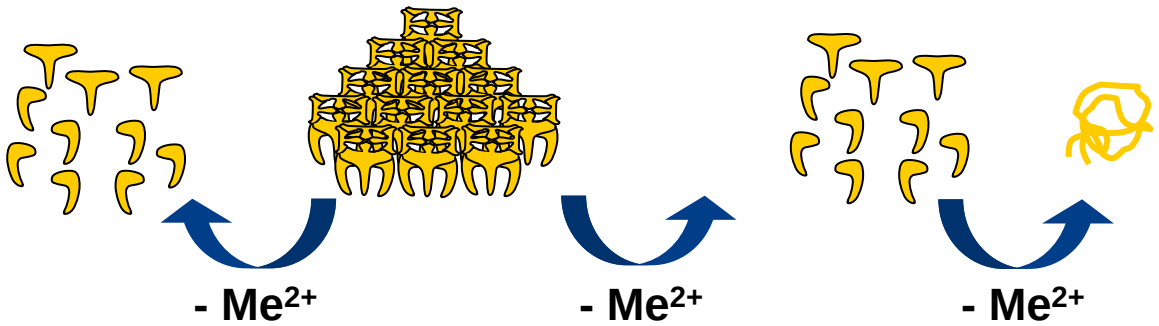
Pollmann, K. et al. (2005) Microbiology, 151(9): 2961-2973

IX Hypothesis: phosphorylated amiono acids mediate the release of S-layers

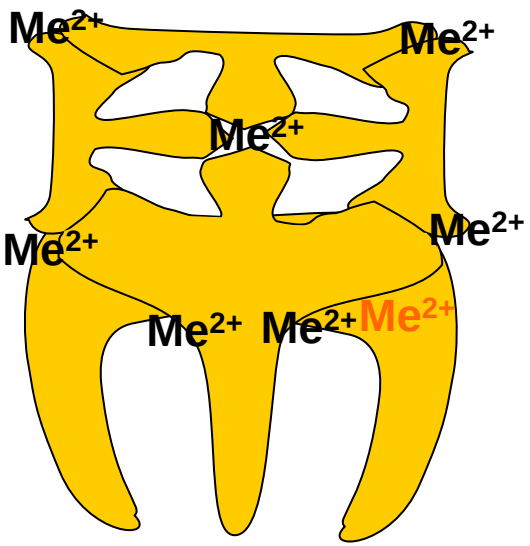


pH 9 8 7 6 5 4 3

Monomers (%) 25 9 9 16 17 60 100 (5 mg/ml S-layer JG-A12, 0.1 M NaCl)

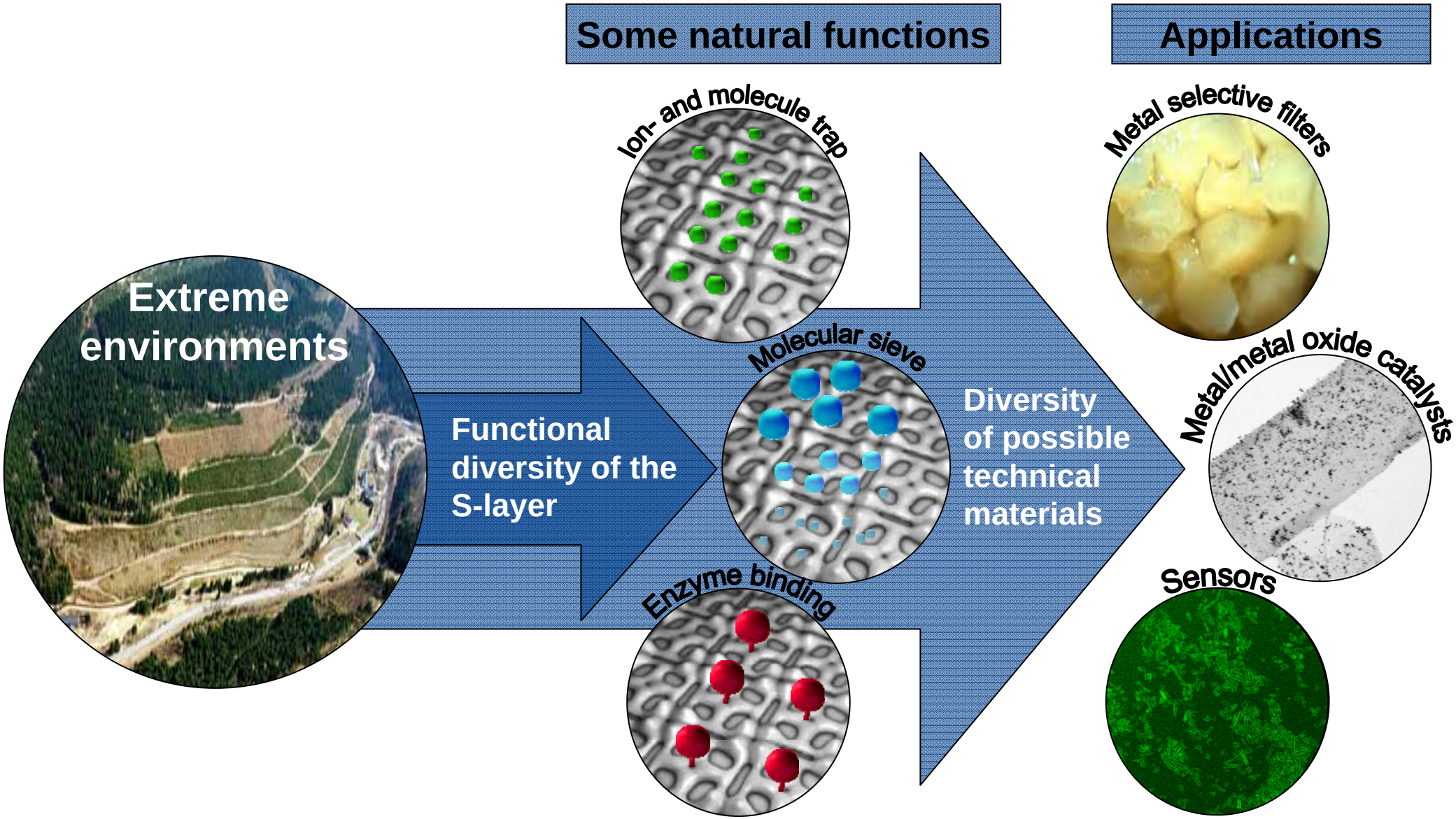


Hypothesis:



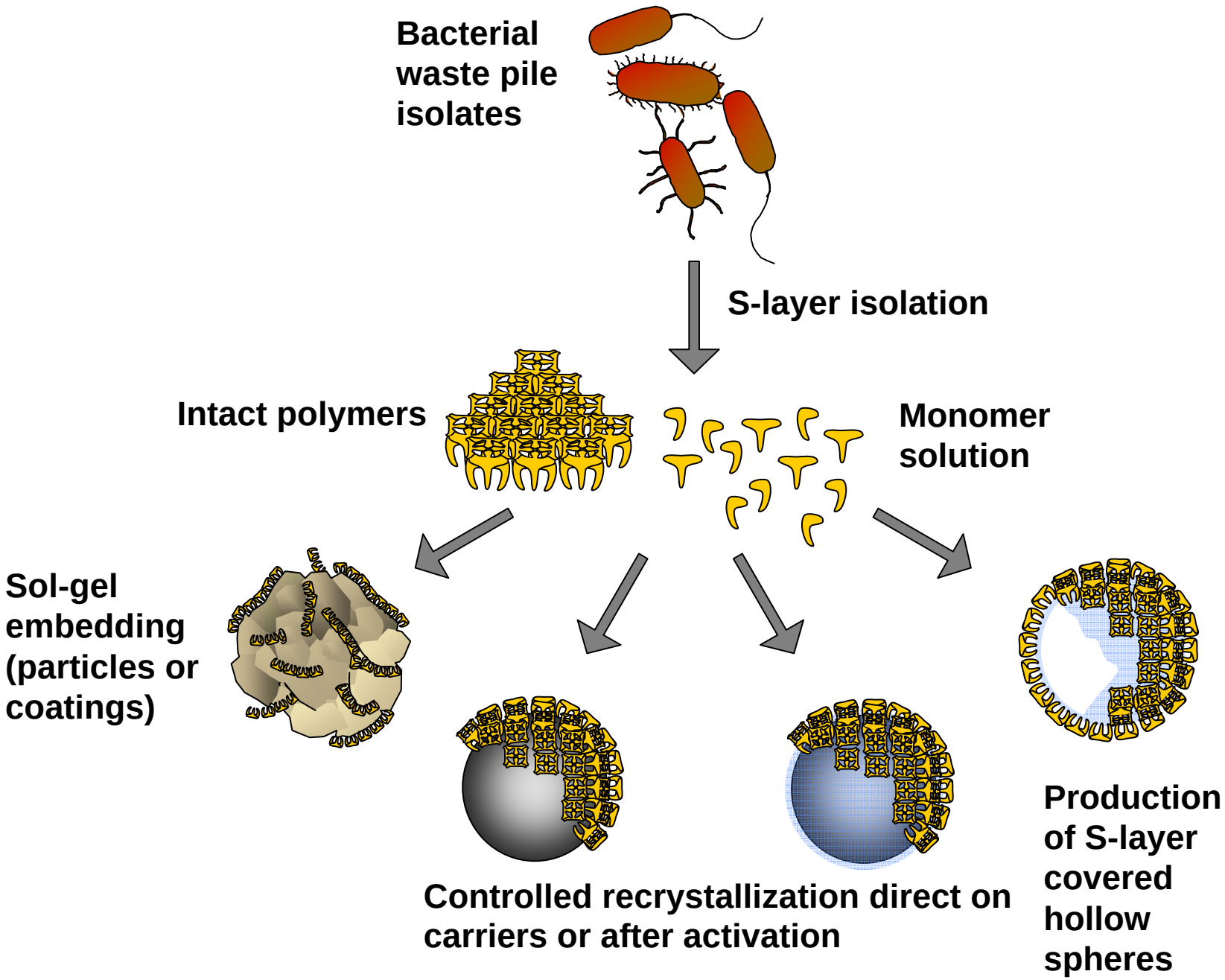
Application oriented research

Application potential of bacterial S-layers

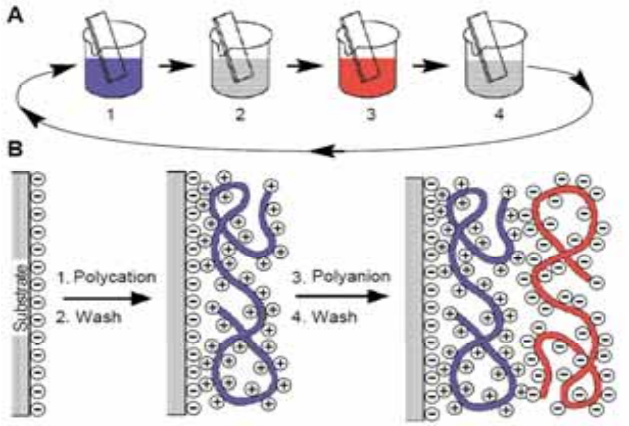


Pollmann, K. et al. (2006) Biotechnology Advances 24 (1), 58-68

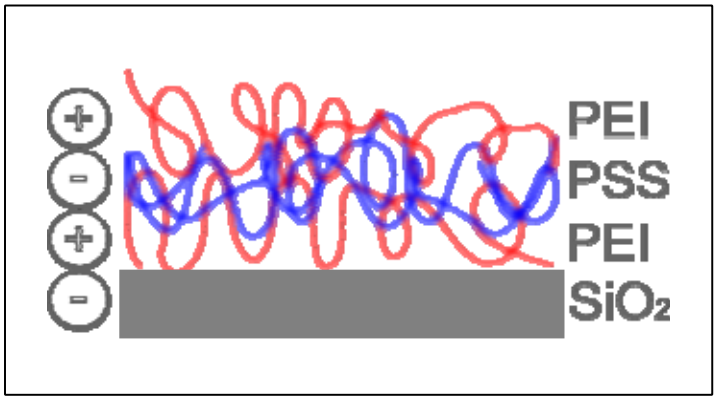
S-layer based materials



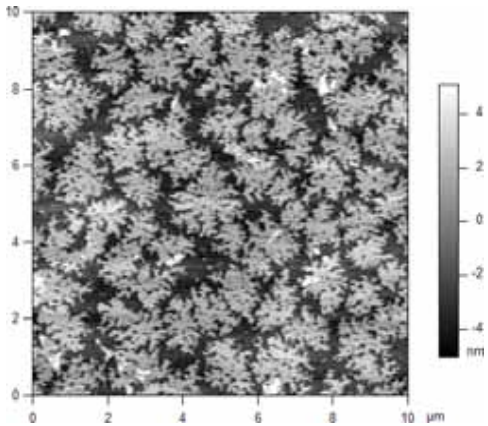
- **To improve the surface properties for S-layer recrystallization a polyelectrolyte interface can be introduced**
- **Simple LbL-coating**
- **Layer composition influences the stability of the protein coating**



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

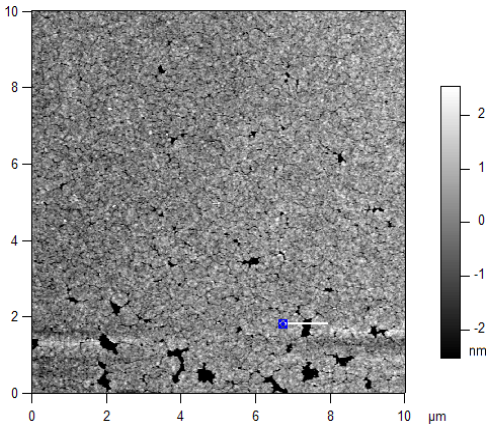


Without polyelectrolyte support



- Recrystallization on SiO_2
- 57 % coverage
- 12 h incubation

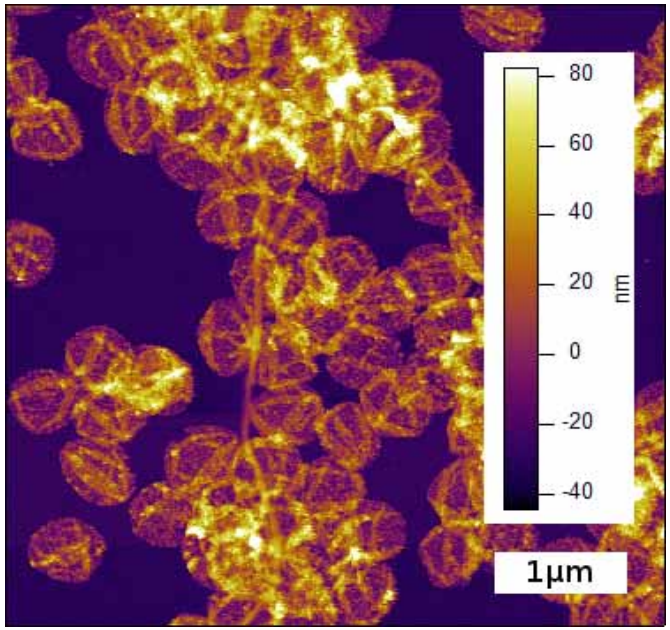
with polyelectrolyte support

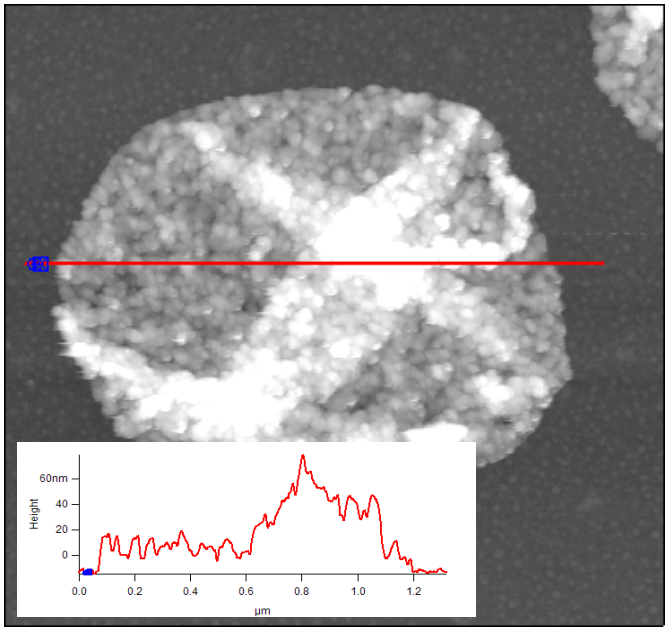


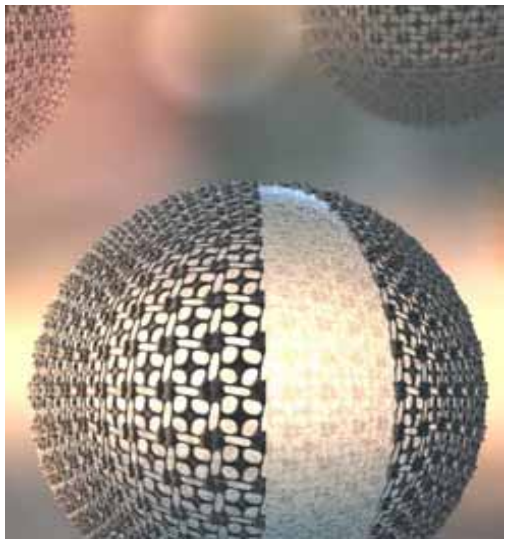
- Recrystallization on PE-coated Si
- >90 % coverage
- < 30 min incubation
- Monolayer

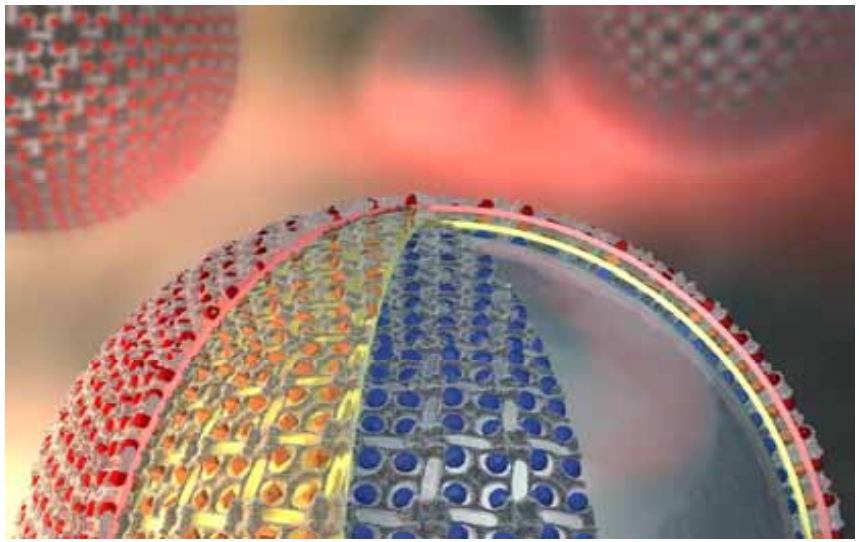
II S-layer coated polyelectrolyte hollow sheres

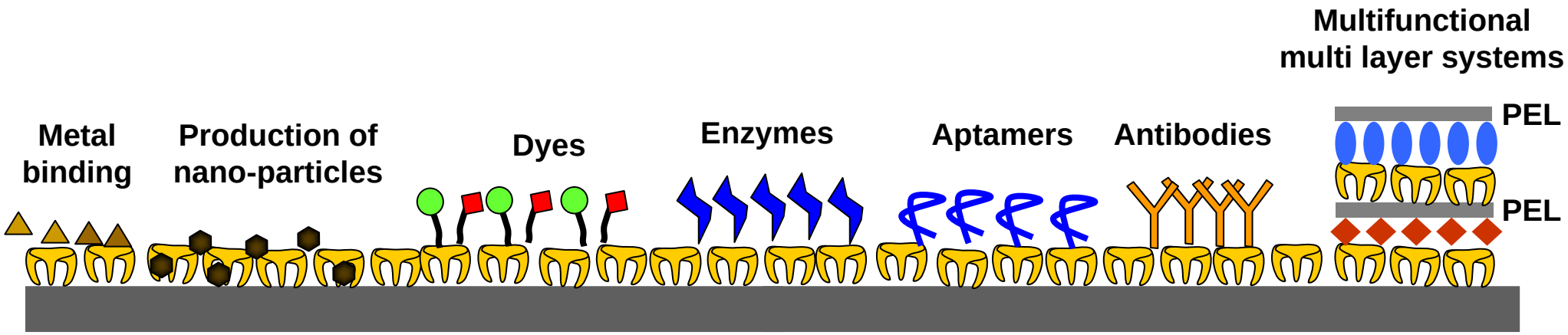
- Coating of spheric templates ($CaCO_3$, CaP) with multiple layers (4-10-…) of polyelectro-lytes (PSS, PAH)
- Dissolution of the core material by HCl
- Coating of the spheres with S-layer(s) via recrystallisation and LbL-techniques
- Mono- or multifunctional transparent speres
  - Magnetic “beads”
  - Combination of binding molecules and catalysts
  - …





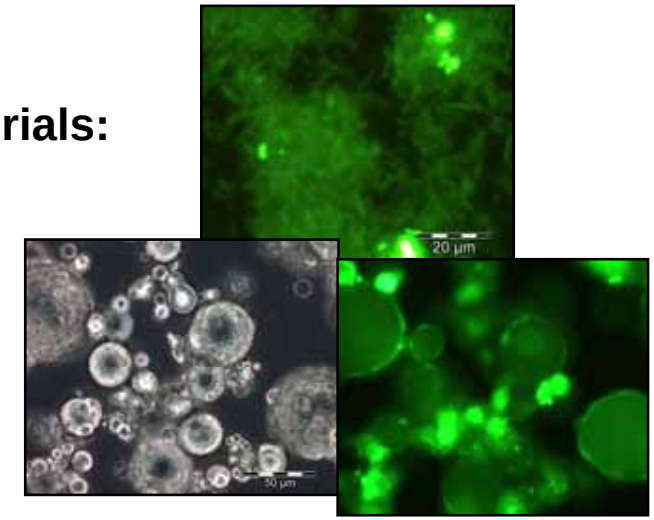


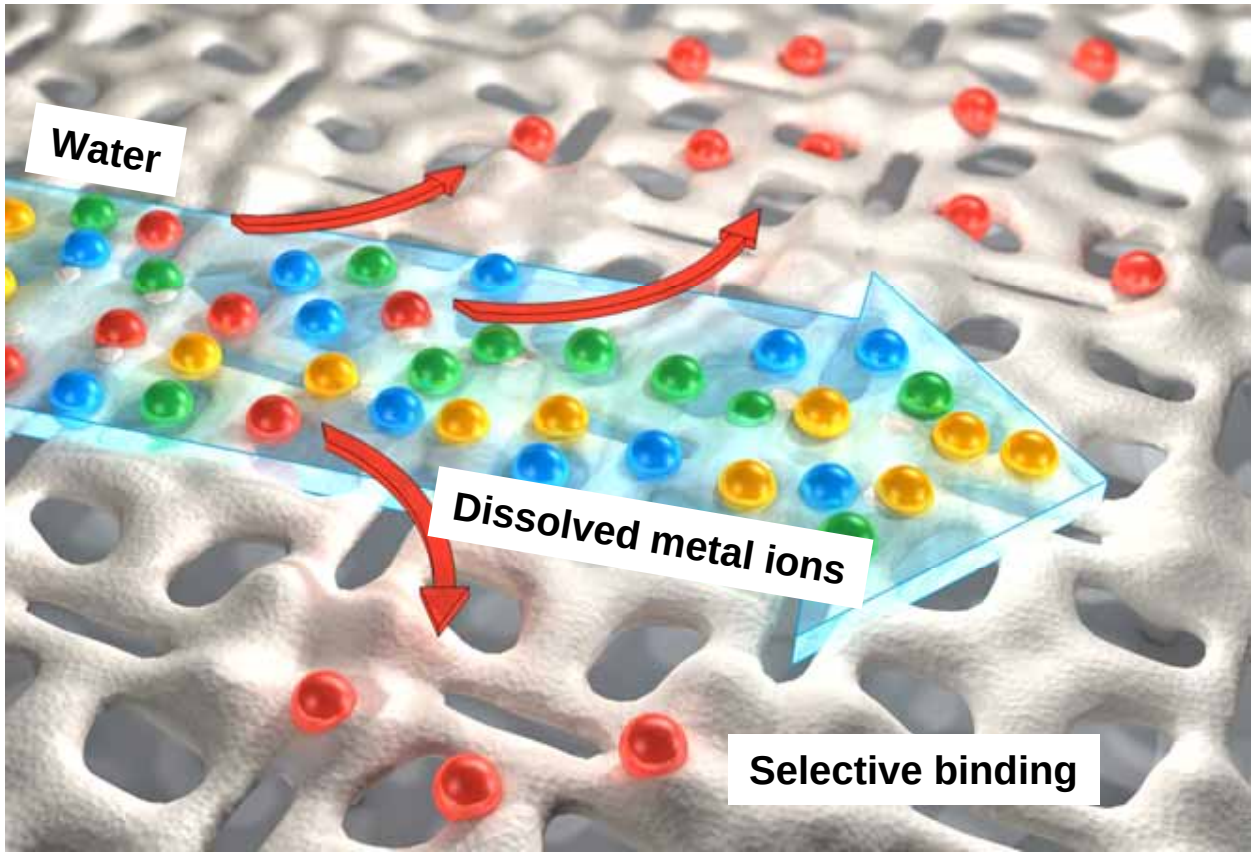


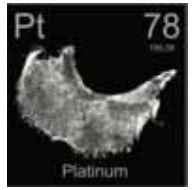


Possible carrier materials:

- Glas
- Ceramics
- Metal
- Plastics







Advantages compared to previous approaches:

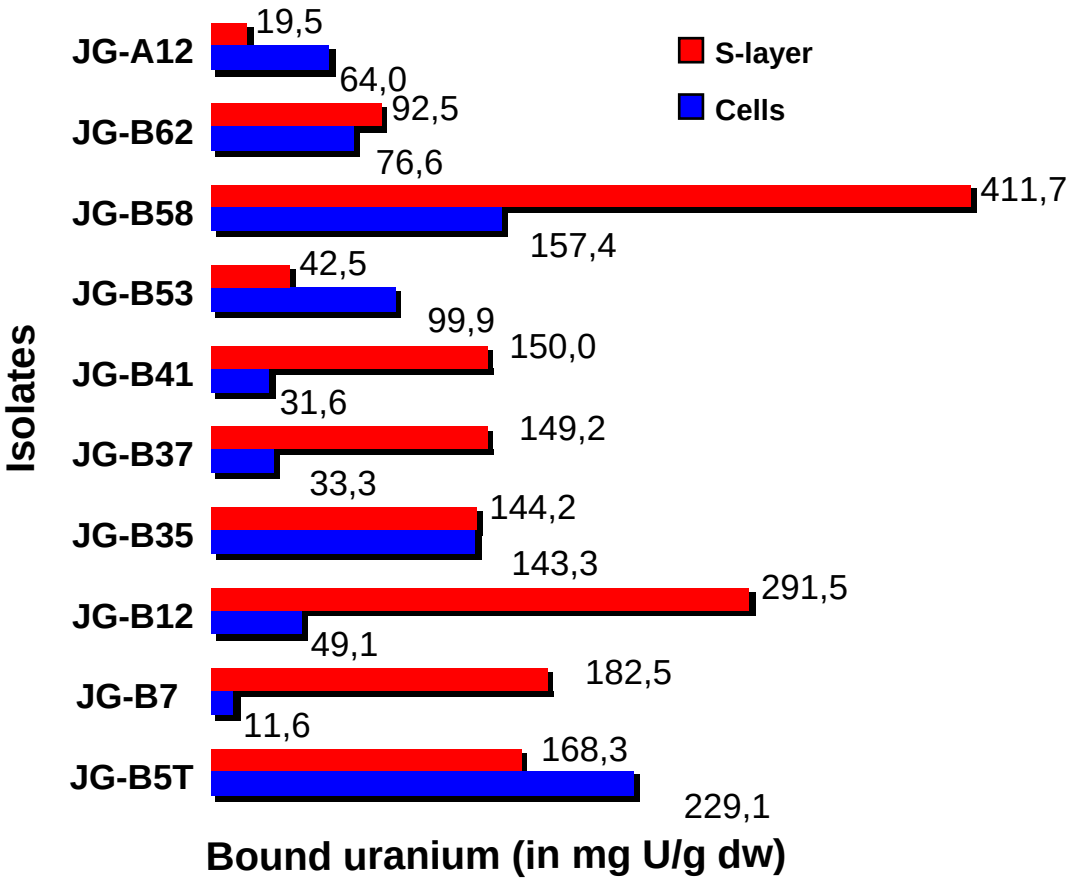
- **Binding properties**
- **Environmental-friendly disposal**
- **Selectivity ?**

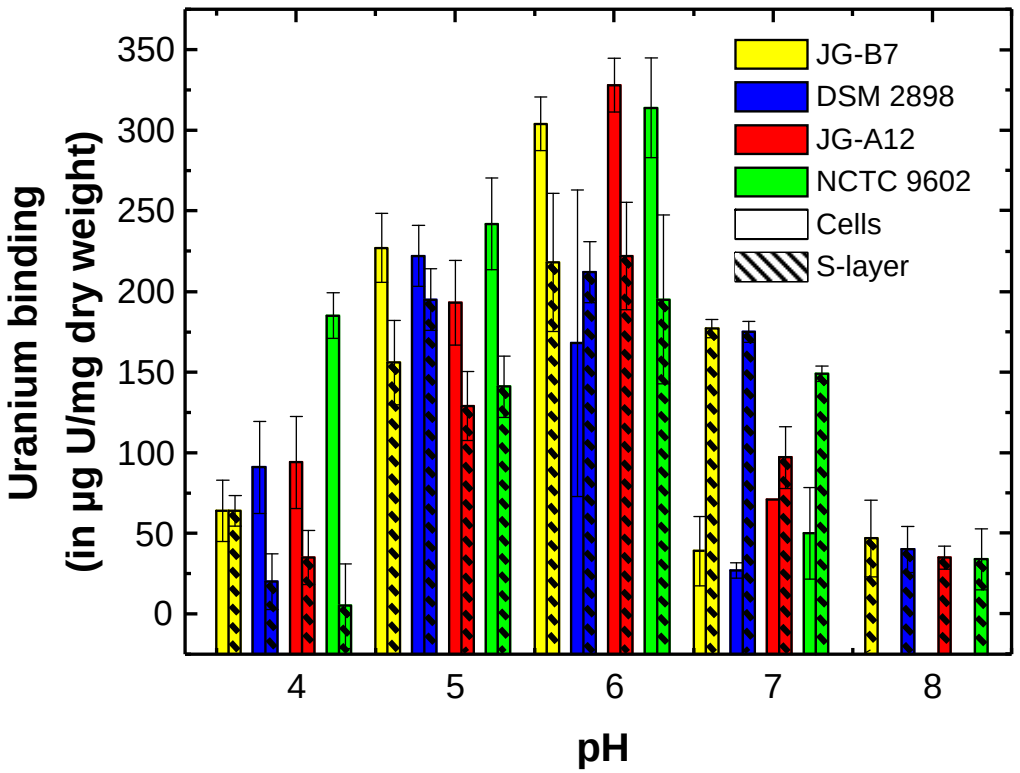
http://www.periodictable.com

Raff, J. et al. (2003) Chem Mater 15(1): 240-244

Patent DE 101 46 375

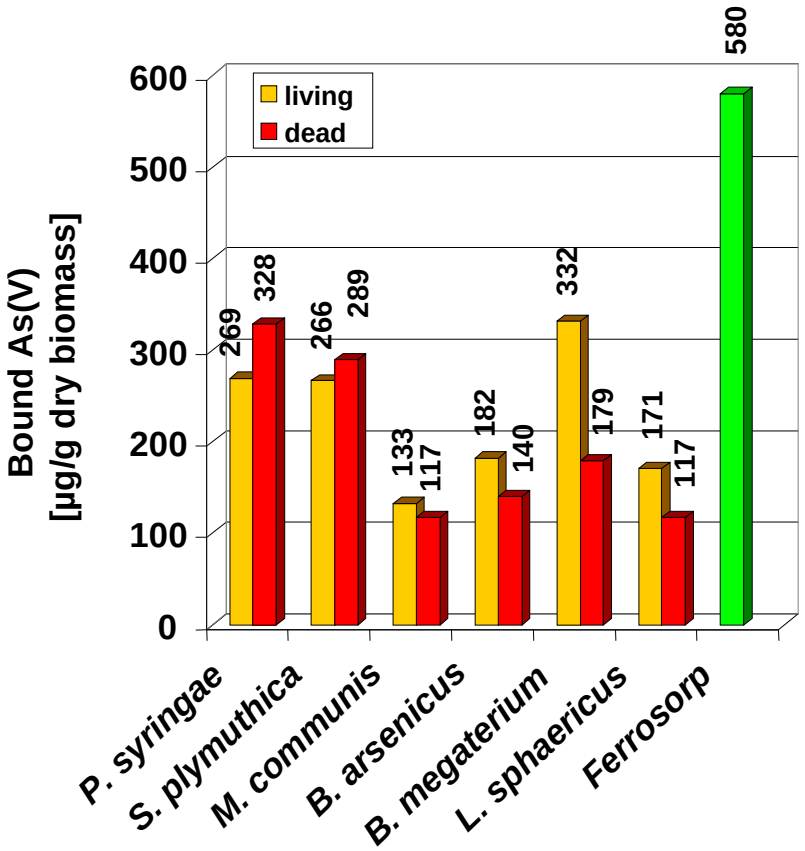
V Uranium binding by cells and S-layer proteins



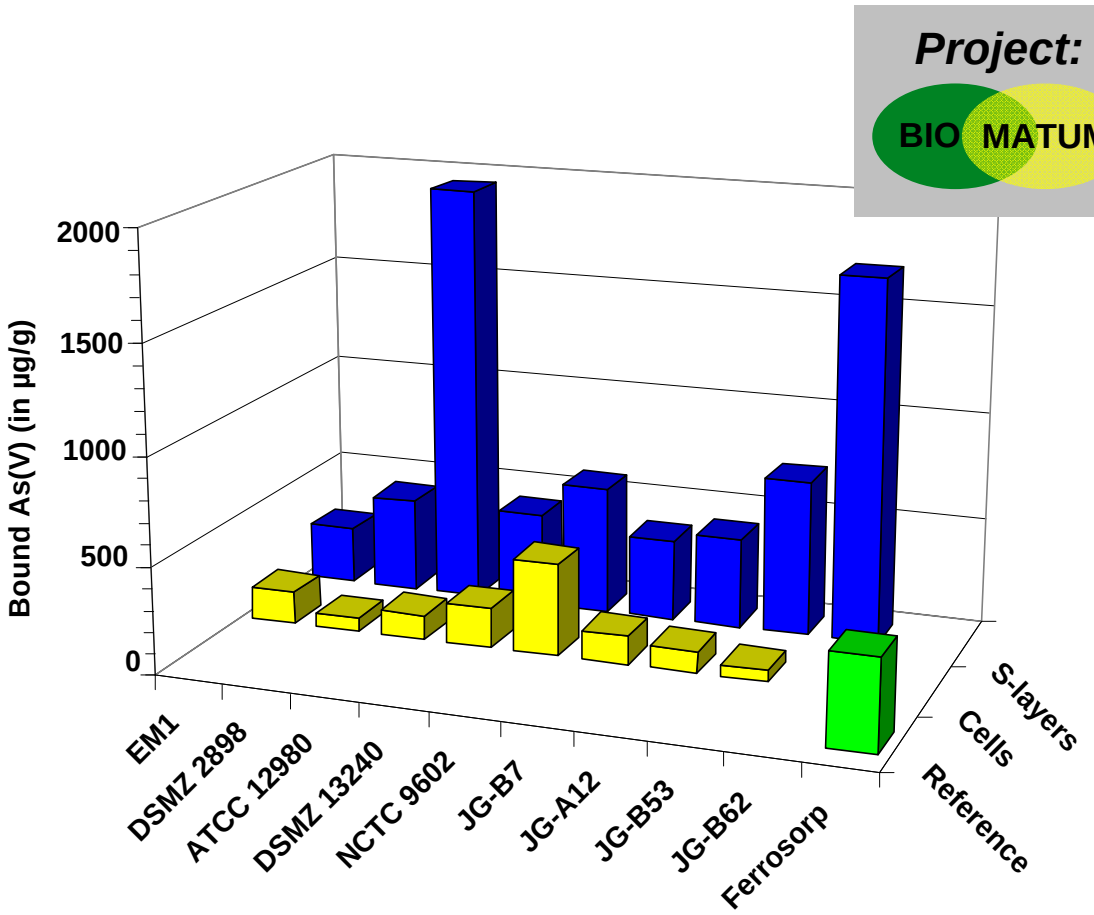


Raff, J., Selenska-Pobell, S. (2006) NEI 51 (619) 34-36

VI Arsenic binding by cells and S-layer proteins



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

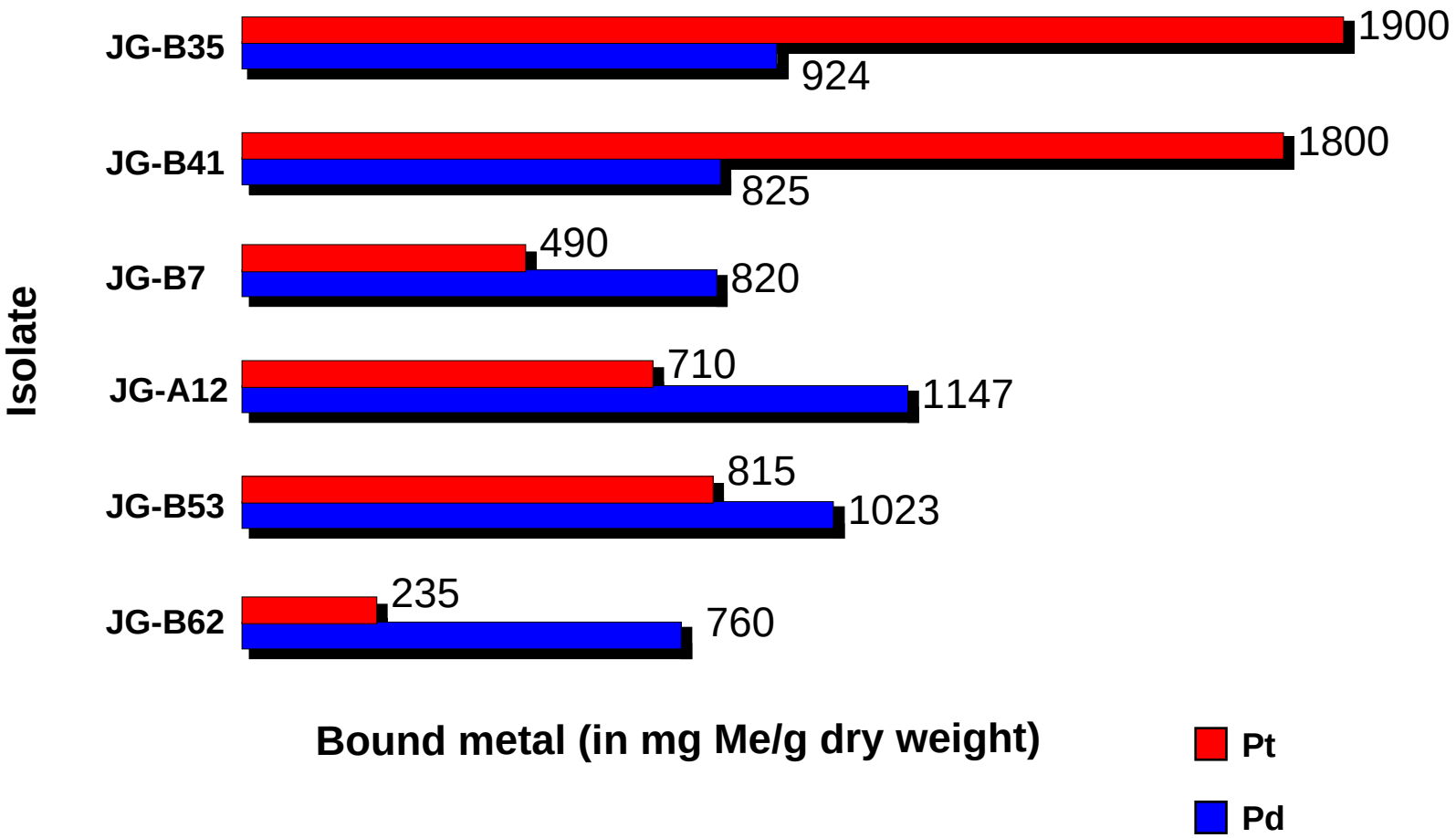


As(V) bindung by cells and S-layers of reference strains and uranium mining waste pile isolates, using a As(V) solution with [As] = 10 mg/l

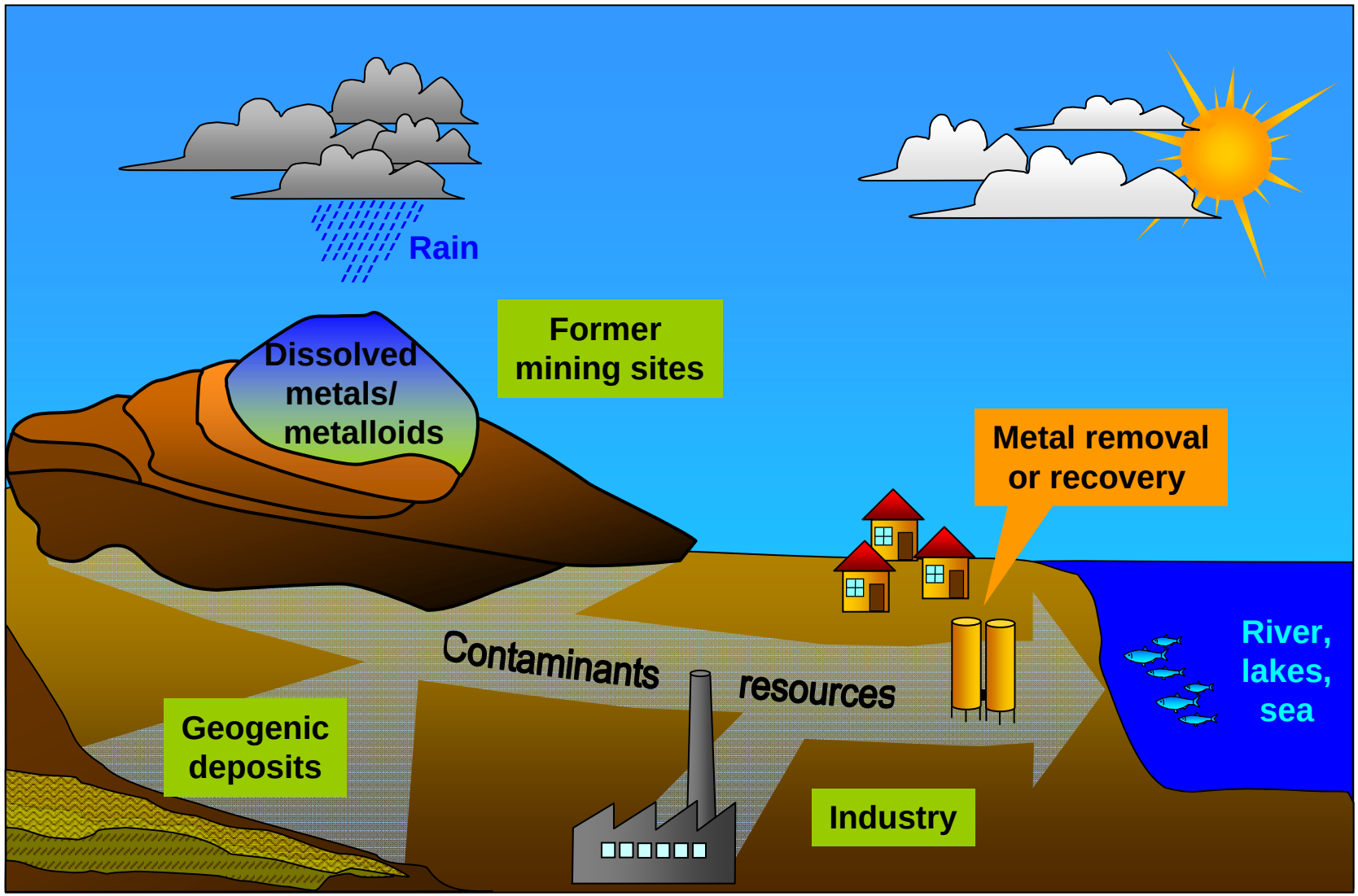
Reference material: Ferrosorp (granulated ferric hydroxide)

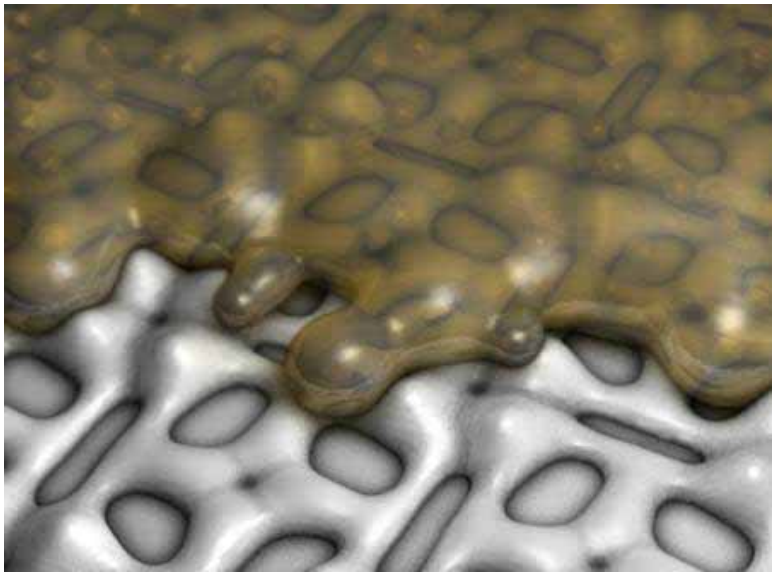
Matys, S. et al. (2010) FZD-Report 530

VII Pt and Pd binding by S-layer



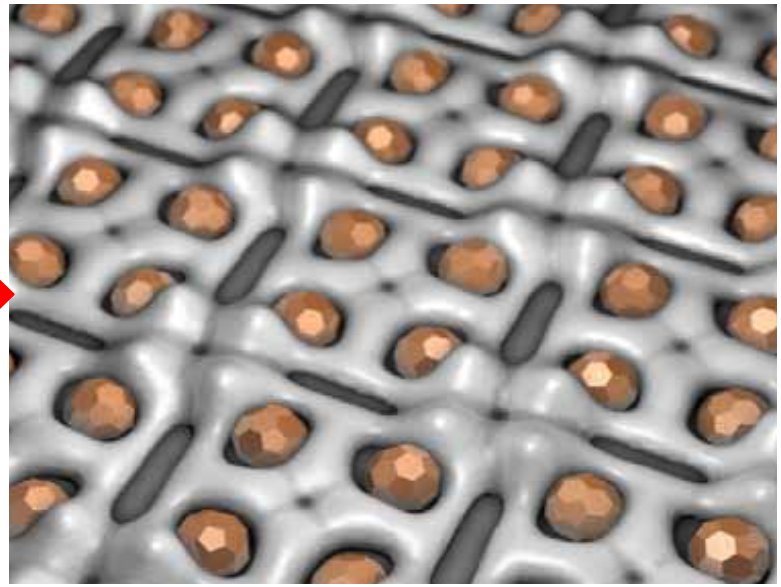
Fahmy, K. et al. (2006) Biophysical Journal 91 (3), 996-1007


Rain
Former mining sites
Dissolved metals/ metalloids
Metal removal or recovery
River, lakes, sea
Contaminants
resources
Geogenic deposits
Industry



Incubation with a metal salt soultion

+ Addition of reducing / precipitation agents



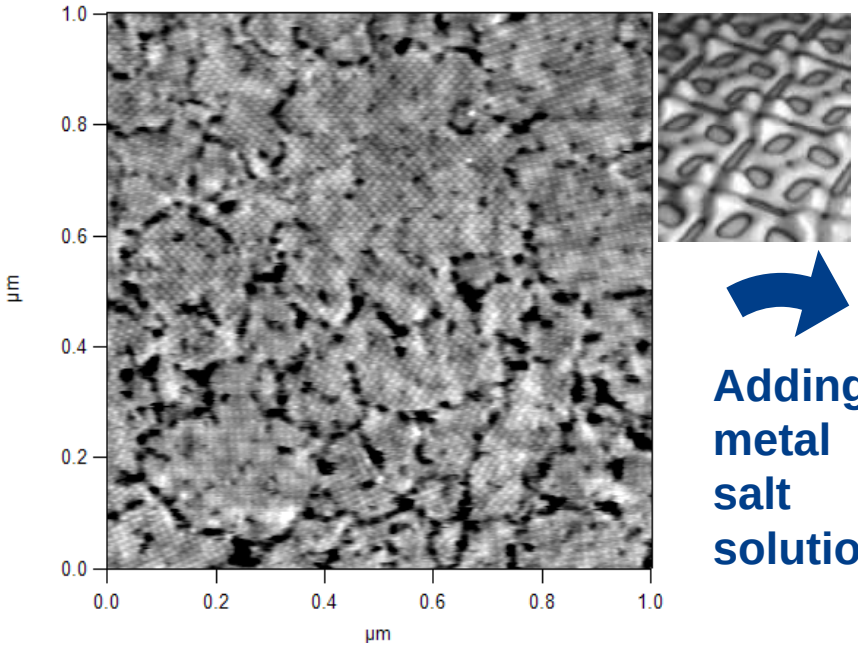
Defined catalytic active nano-particles e.g.

Pt, Pd, Fe/Pd, Fe/Pt

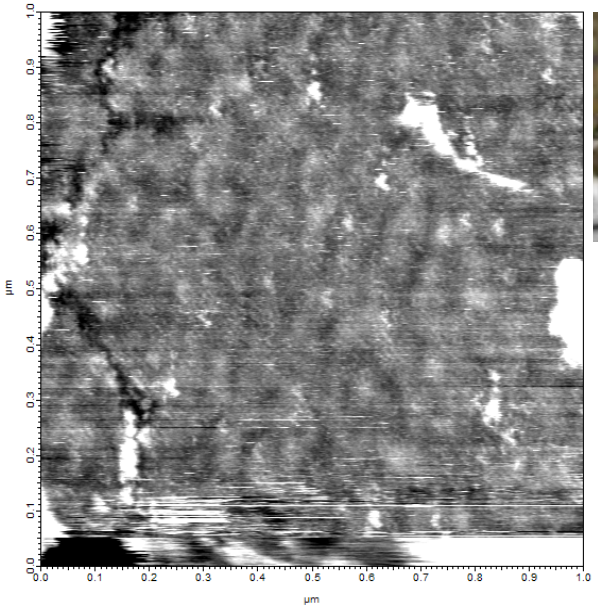
ZnO, TiO_2

with sizes of 1-26 nm

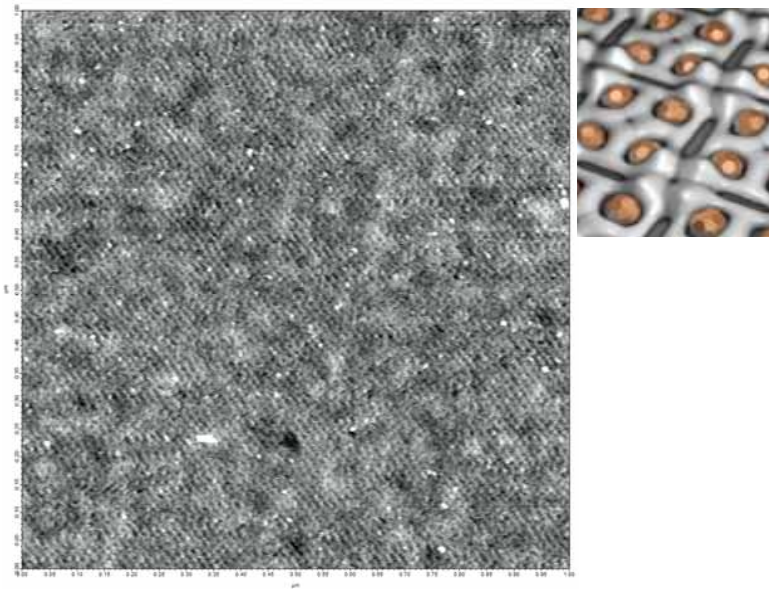
X S-layer based synthesis of defined and regular arranged nanoparticles



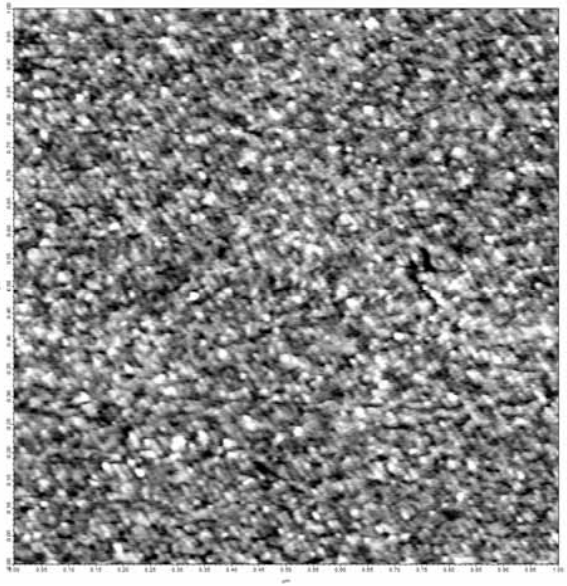
Adding metal salt solution

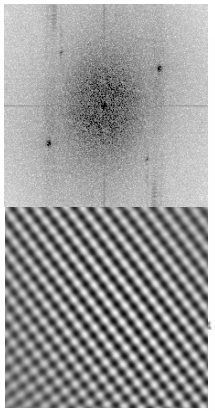


Adding reducing reagents

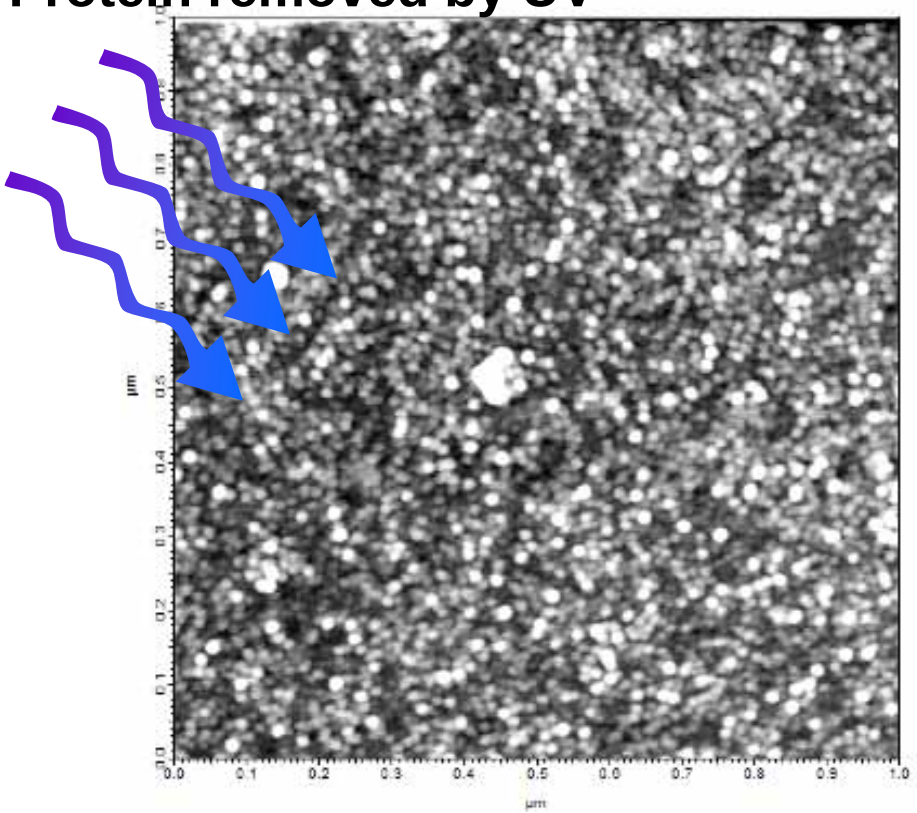


Protein removal via UV

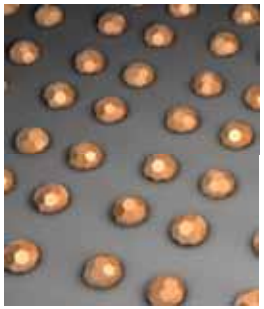




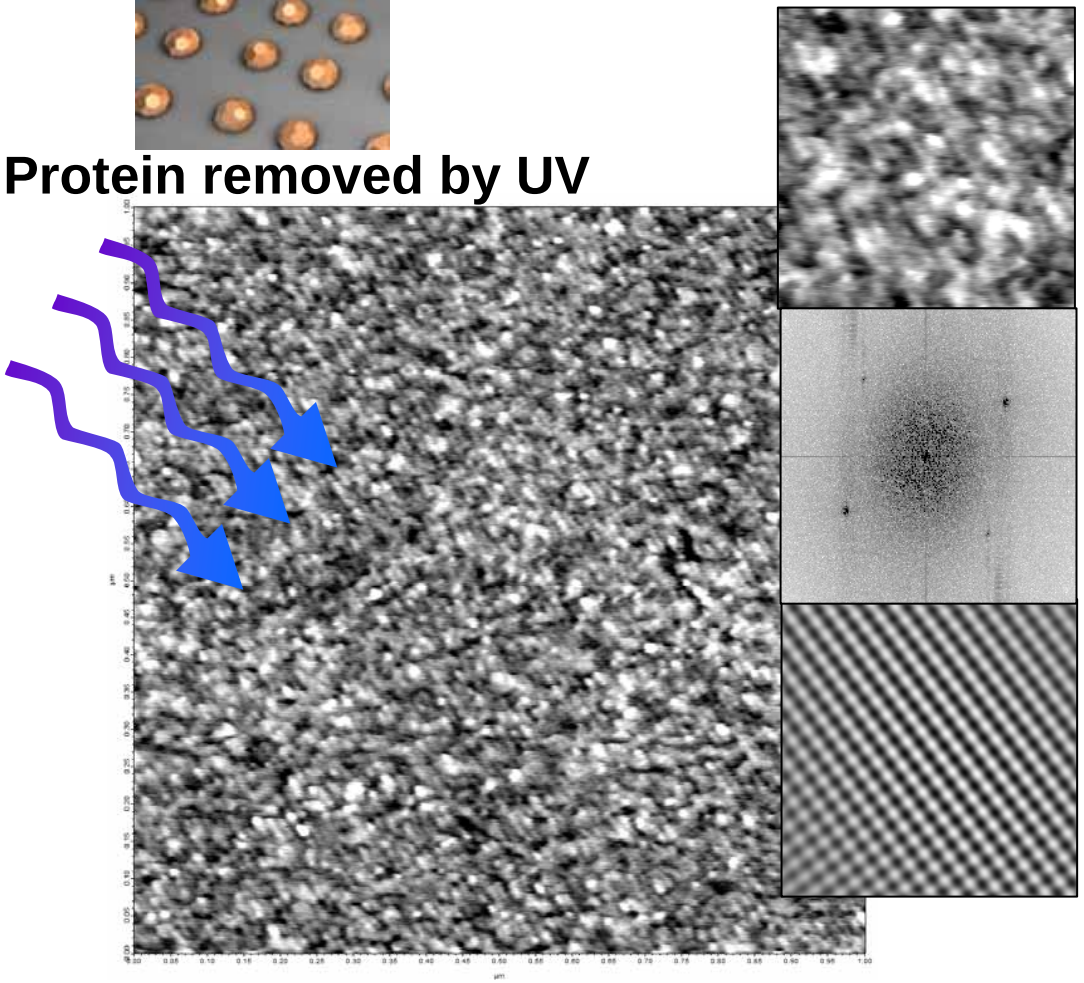
Protein removed by UV



Nanoparticles produced on PE without S-layer

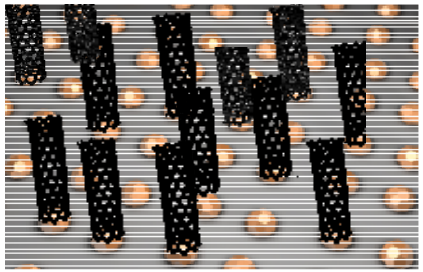


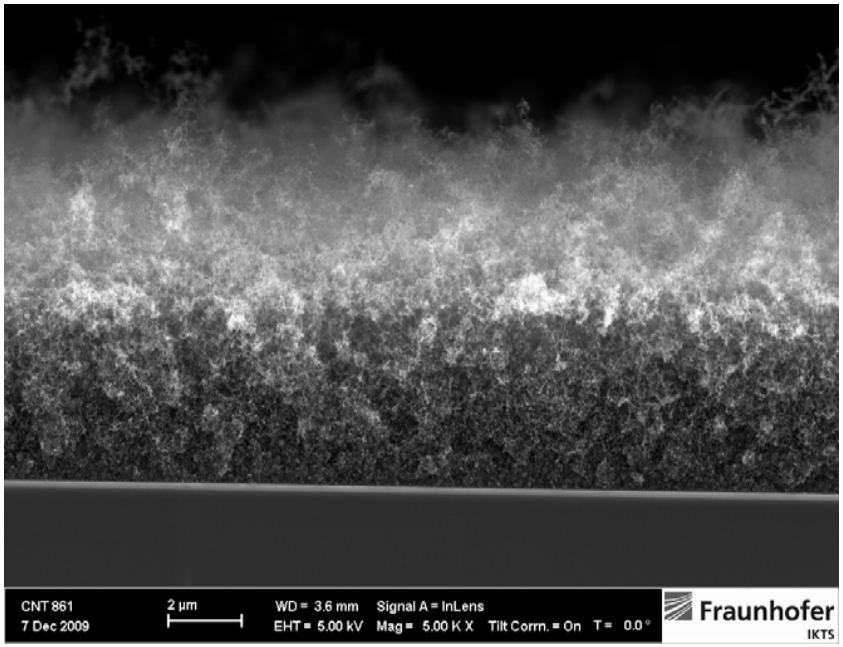
Protein removed by UV

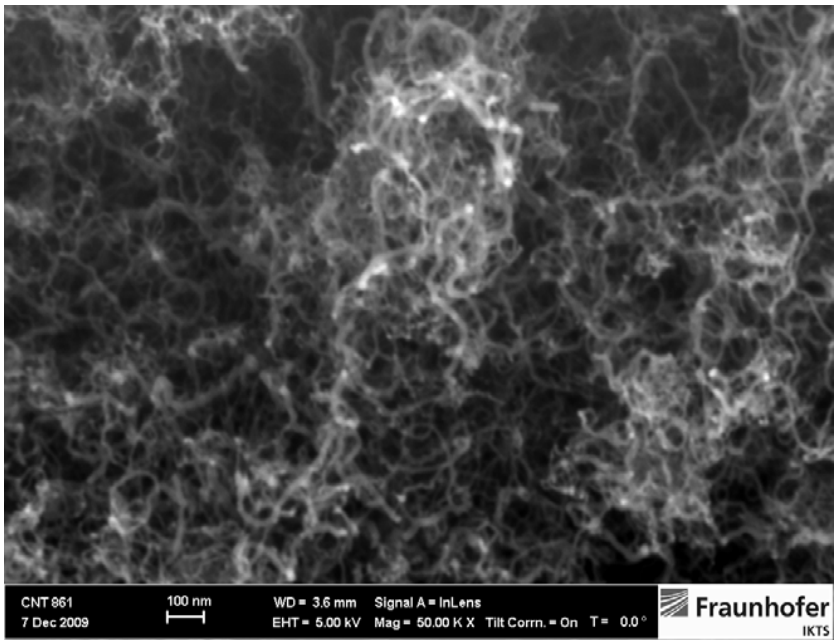


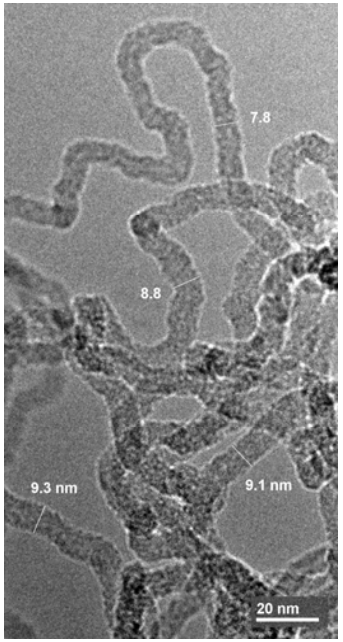
Nanoparticles produced on PE + S-layer

XII Precious metal catalysts for the synthesis of defined CNT´s





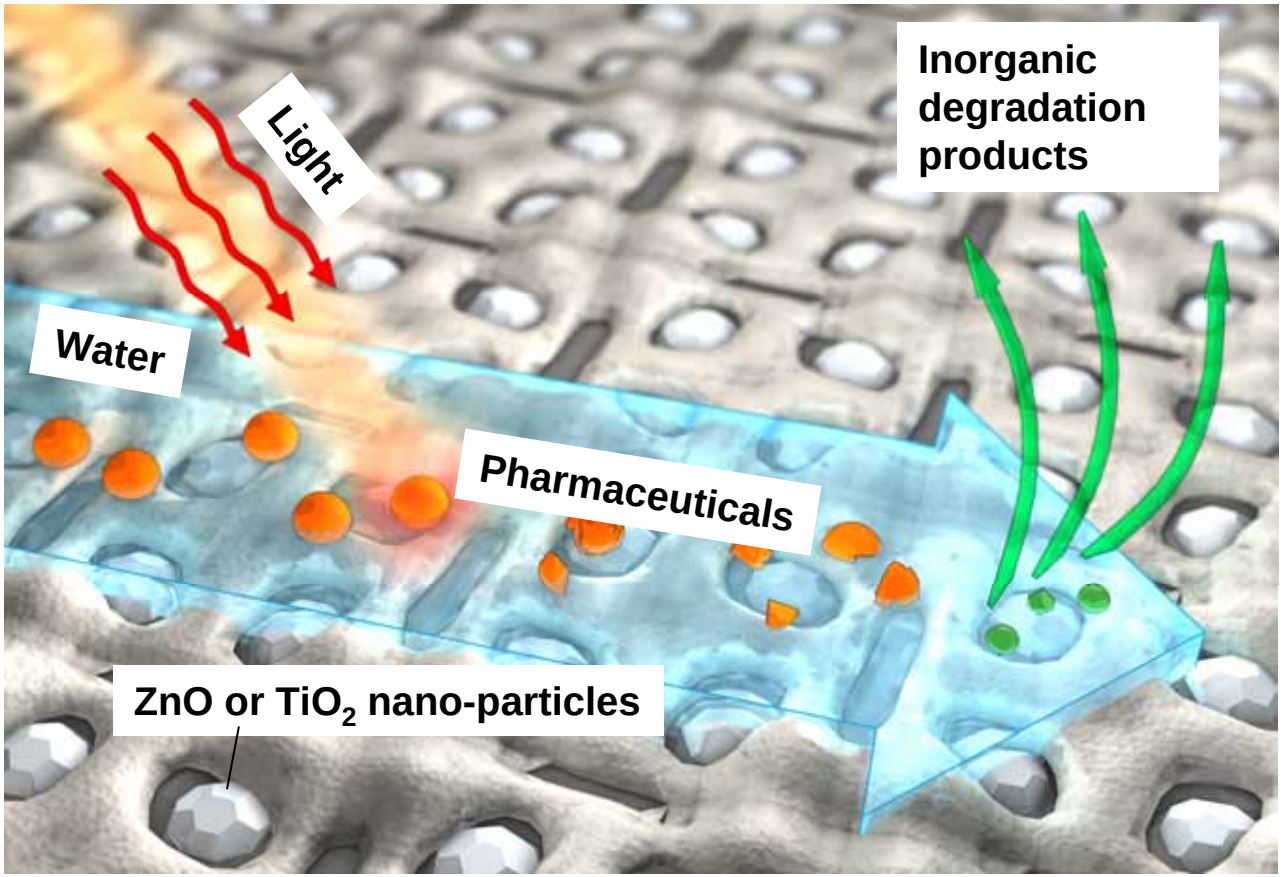




→ **Possibly migration and agglomeration of the particles**
→ **Further improvement necessary**

Projects:

NanoAqua

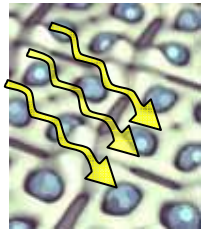


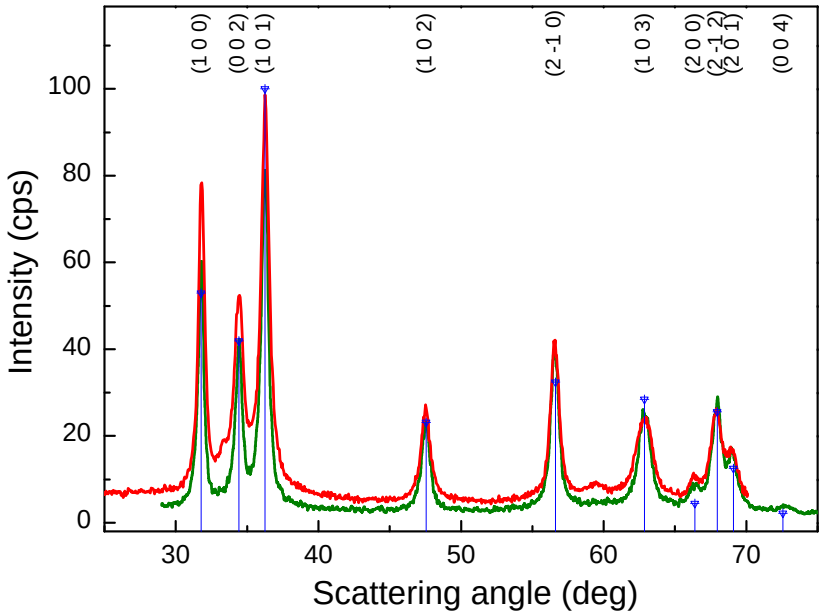
Advantages compared to previous approaches:

- **Environmental-friendly (disposal, immobilization of nano-particles)**
- **Higher efficency (required energy, day light sensitivity) ?**

Patent pending

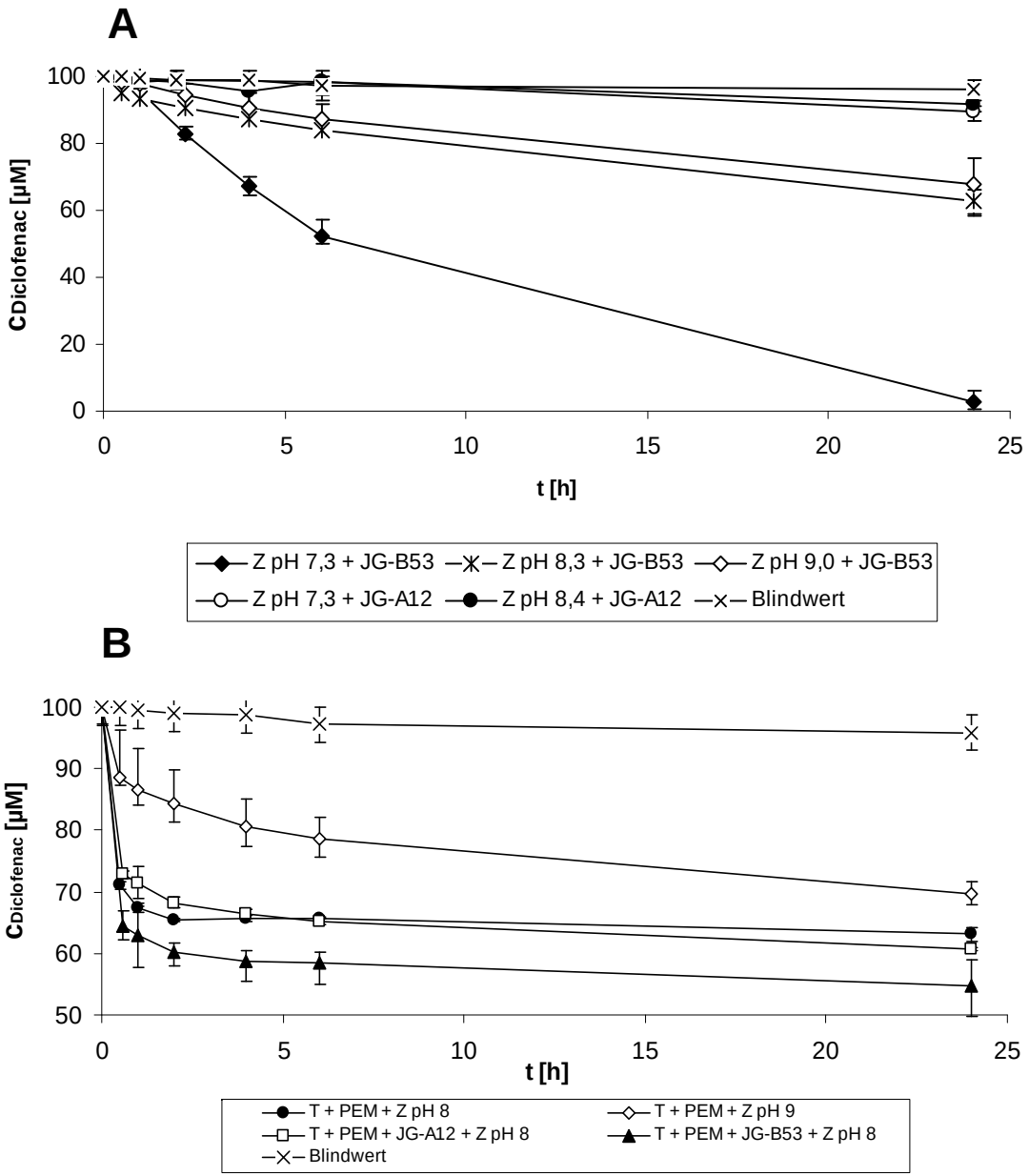
XIV ZnO based photocatalysts for the elimination of pharmazeutica

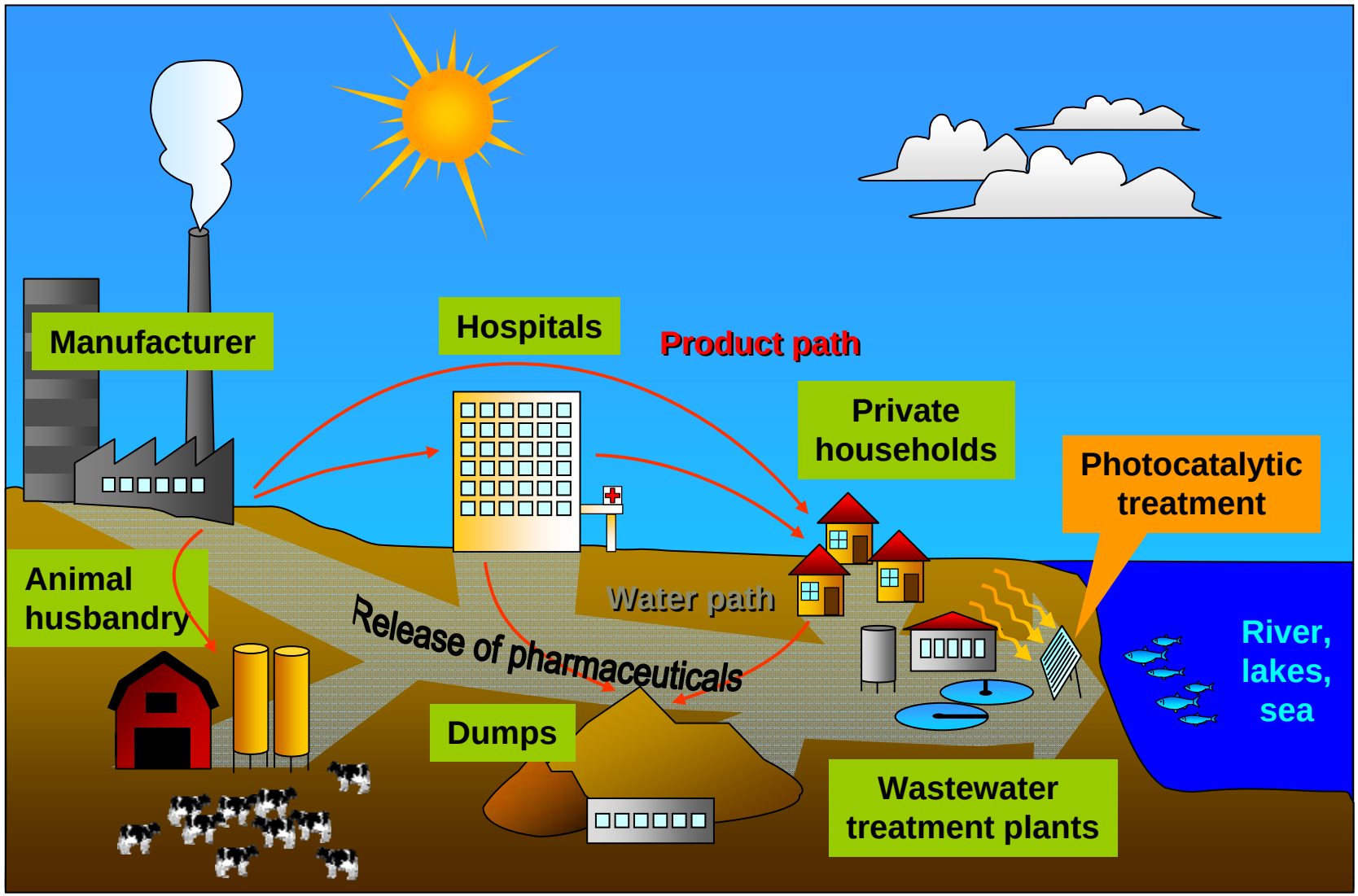




Top: XRD-pattern of S-layer supported ZnO (red graph) and equally prepared ZnO without protein (green graph). S-layer prepared ZnO-particles have a size of about 14 nm.

Right: Diclofenac elimination by S-layer supported ZnO in suspension (A) and immobilized on a carrier (B).

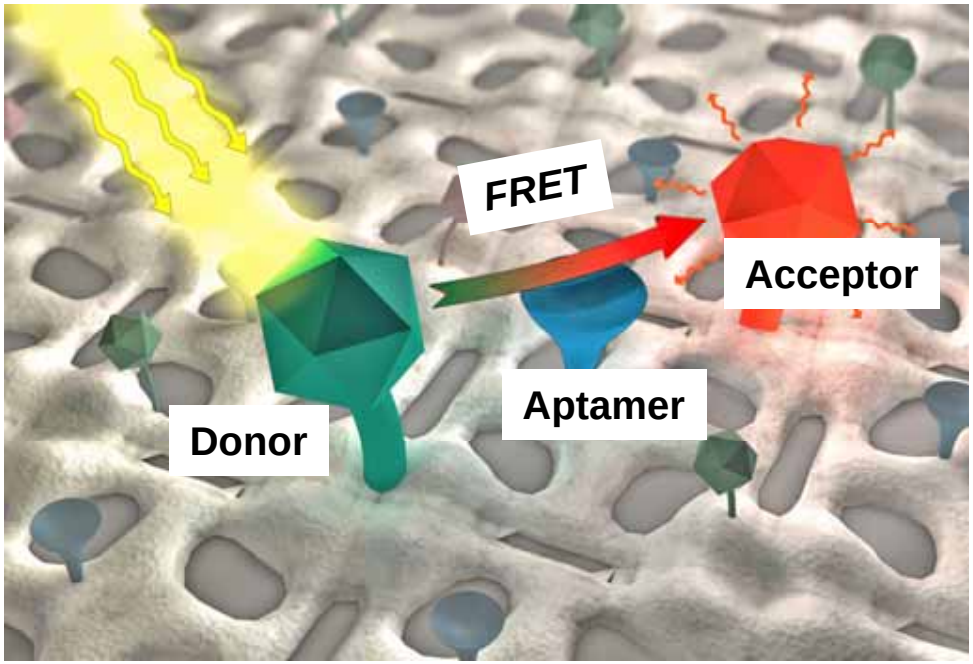


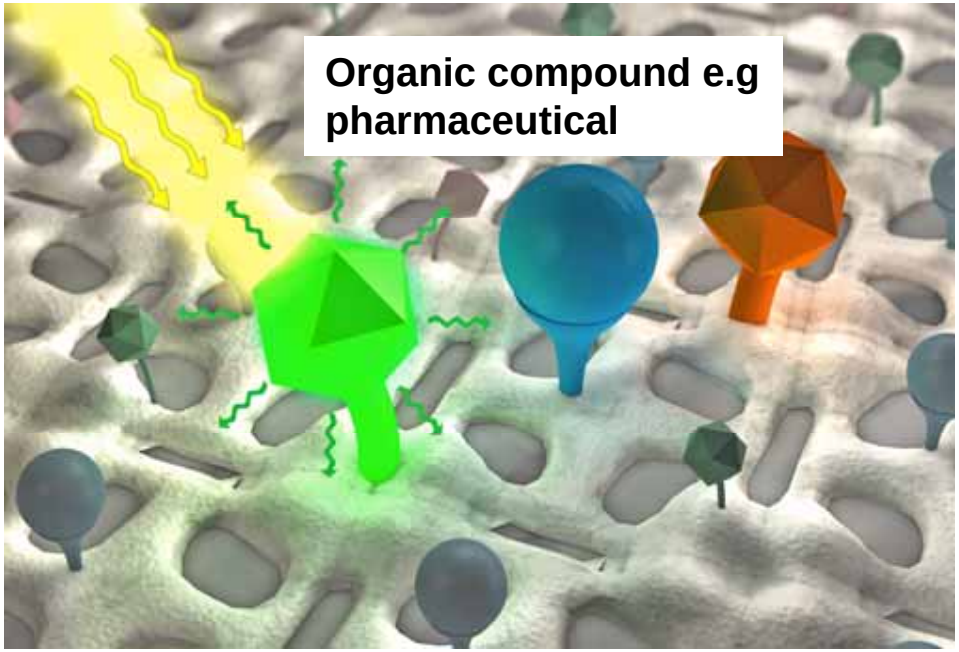

Manufacturer
Hospitals
Product path
Private households
Photocatalytic treatment
Animal husbandry
Water path
Release of pharmaceuticals
River, lakes, sea
Dumps
Wastewater treatment plants

Project:

BIONA

AptaSens



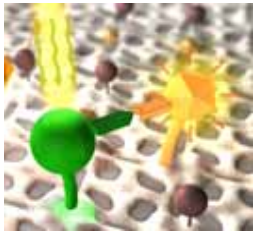


Advantages compared to previous approaches:

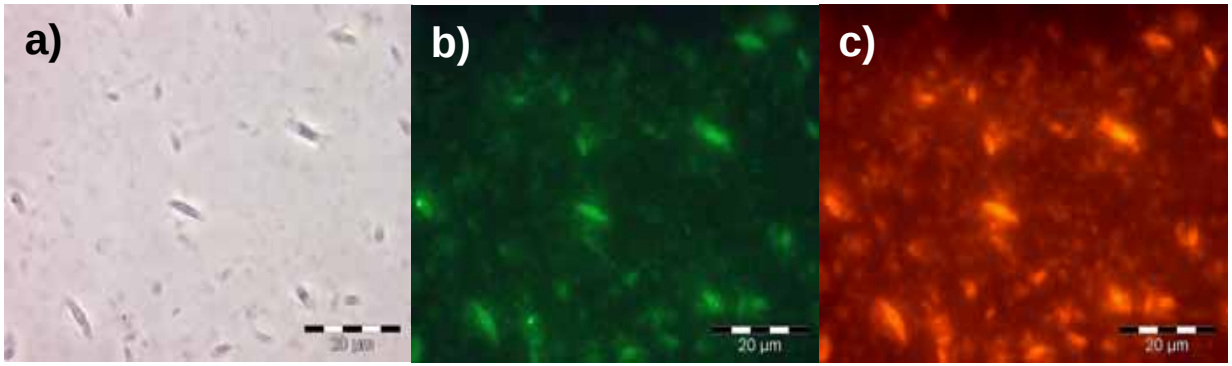
- **Transferability**
- **Chip-based quantification of several analytes ?**
- **lower unspecific binding ?**

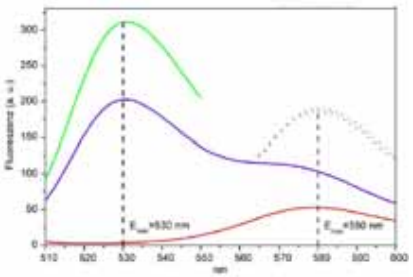
Patent pending

XVII S-layer bound fluorescence dyes and aptamers

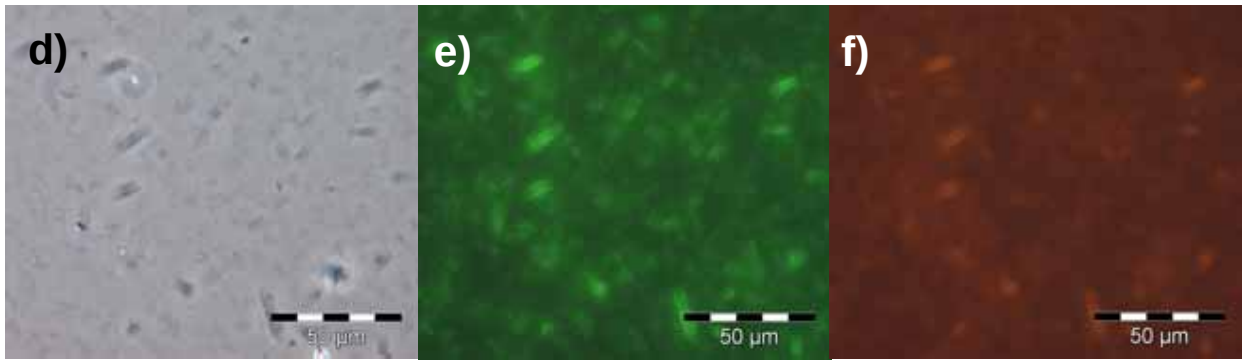


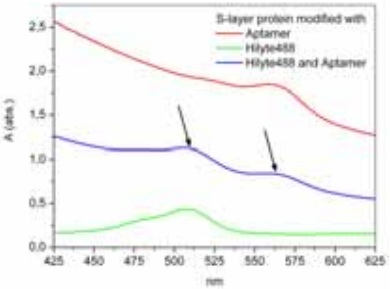
FRET-pair



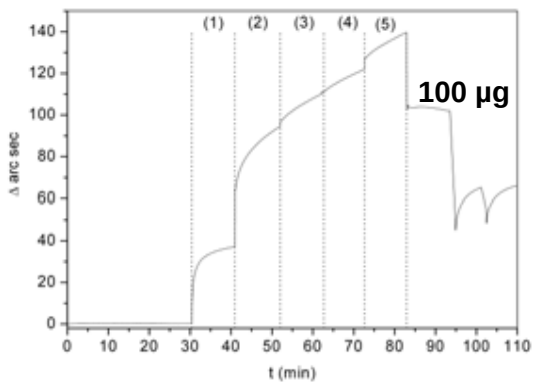


S-layer aptamer composite





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



Biomass production

Process	Yield in g/l (z.B. JG A12)		Manpower requirements	€/g S-layer
	Bio-mass	S-layer		
Conven-tional	3	0,03	6,9	19000
Pilot plant	6	0,16	1,4	730
Optimisation	12	0,85	1,4	100
Aim				<10

Possible strategies:

- **High density cultivation**
- **Heterologous expression**

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

Acknowledgement

Research:

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… questions, remarks?