Imparting Biological Functions to Artificial Materials: From Active Membranes to Self-Replication


Fraunhofer-Institute for Applied Polymer Research IAP, Potsdam-Golm
Chair for Polymer Materials and Technology, Universität Potsdam, Germany
Biological transformation of materials

Beyond Bionics: Biologisation of Technology

Substitution of parts /elements by bio-materials /organisms

Addition /concatenation/ implementation of bio-materials /organisms to form a conjoint or coherent technology

Enhancement by Addition Interaction Bio ↔ Tech

Compensation and Enhancement by Prohetics

“Biofacts” by change or newly creation

Taken from: Prof. K. Kornwachs, Ulm University: „BIOLOGICAL TRANSFORMATION OF MANUFACTURING, Futura in Res Discussion Meeting, Berlin, 28.-29. June 2018“
Why incorporate biological functions into artificial materials?

- huge diversity of functionality in nature
  - e.g. antimicrobial, (chemically & physically) active, self-assembling (finite growth), programmable, information storage, etc.

- functions extremely well developed
  - difficult/impossible to mimic with artificial materials

- challenge: maintain function upon transfer to artificial environment
  - approach: polymer-protein conjugates as building blocks
The Concept: Protein-Polymer Conjugates & Self-Assembly

Protein-polymer colloidal particle → Use interfacial self-assembly to form thin layer (e.g. Pickering) → Stabilize by matrix cross-linking

✓ Protein imparts functionality to polymer material

✓ Self-assembly processes to form larger entities
Outline

A. Active Membranes from:
   i) channel protein-polymer conjugates
   ii) enzyme-polymer conjugates

B. Pickering Emulsions for Enzymatic (Cascade) Reactions

C. Patchy Particles
Artificial membranes based on protein-polymer conjugates – Characteristics of ideal membranes

- Precise control of pore size in nanometer range
  ⇒ precise size exclusion

- Low thickness
  ⇒ short diffusion pathway, high flux

- Possibility to functionalize pores
  ⇒ high chemical selectivity

⇒ for membrane-guided processes: fast separation of educt from product

Incorporation of FhuA channel protein from *E. coli*

Uli Glebe, Himanshu Charan, Maria Mathieu *et al.*, Cooperation Partner: Prof. Uli Schwaneberg, RWTH Aachen
FhuA Variants – Position of Amino Groups

- Design of different Amino variants
- Control of position of lysine residues within the variants
- Chiral protein channels: separation of D- and L-amino acids
Artificial membranes based on protein-polymer conjugates – Overview Conjugate Synthesis

Artificial membranes based on protein-polymer conjugates

FhuA-Polymer Conjugate

interfacial self-assembly

POROUS SUPPORT

UV cross-linking

Characterization of protein-polymer conjugates – SFM

Dried assembly

Crosslinked assembly

Aggregates

20 nm membrane

5 nm membrane

FhuA ΔCVF\textsuperscript{tev} K\textsubscript{11}\textsuperscript{up}-PNIPAAm-PDMMIBA

2.6 x 10^{-2} \text{ mg/ml}

FhuA WT-PDMAEMA-PDMMIBMA

2.5 x 10^{-3} \text{ mg/ml}
Membrane at water-toluene interface – high stability!

Toluene

MPD Buffer
(USM exposed)

FhuA ΔCVF$^{tev}K_6^{mid}$ Conjugate
with DMIAAm
(UV cross-linked)

Water

Characterization of protein-polymer conjugates – water flux measurements

- Water flux, size exclusion, enantiomeric separation
- Increase in temperature ensures water flux through pores only

At transmembrane pressure 10 mbar
Characterization of protein-polymer conjugates – water flux measurements

Flux \(\text{L} \cdot \text{m}^{-2} \cdot \text{h}^{-1}\) vs. Hydrostatic Pressure mbar

- **Support**
- **20 Equivalents**
- **50 Equivalents**
- **1 Layer**

*FhuA wt-PDMAEMA-PDMMIBMA*
Incorporation of 2-Desoxy-D-ribose-5-phosphate aldolase (DERA)

Stefan Reinicke, Shuhao Wang et al.,
Cooperation Partner:
Prof. Jörg Pietruszka, FZ Jülich
Enzymes integrated into Membranes

- Re-use and increased stability
- Easier product work-up
- Barrier properties (membrane)
  → Continuous removal of product (avoids product inhibition)
  → Increased productivity
  → **Conversion and separation at the same time**

2-Desoxy-\(\alpha\)-ribose-5-phosphate aldolase (DERA)
Enzyme immobilization
Film formation at the interface

Route A:
Accomodation of the enzyme within a pre-formed polymer layer

Route B:
Self-assembly of enzyme/polymer conjugates

- Mild immobilization conditions
- Polymer forms film matrix, but also brings in stabilizing effects

Enzyme immobilization
DERA containing thin films via self-assembly of conjugates

Conjugate synthesis via grafting-to

- 3 of 4 cysteines are addressed

The conjugation has various advantages:

(i) Increased tolerance towards acetaldehyde

(ii) Film forming ability upon self-assembly:

(iii) Limitation of activity loss during immobilization:

Pickering Emulsions for Enzymatic (Cascade) Reactions

Uli Glebe et al.,
Cooperation Partner:
Prof. Changzhu Wu, University of Southern Denmark, Odense
Pickering Emulsions for Enzymatic (Cascade) Reactions

- **Pickering vs. 2-Layer System**
  - Pickering interfacial biocatalysis (PIB)

- Improvement by using enzyme-polymer conjugates
  - Stability
  - Interfacial activity

- **Carrier-free PIB**

- **Cascade reactions**
Benzaldehyde lyase (BAL)-PNIPAAm Conjugates

- MALDI-TOF MS (a) → Macroinitiator
- SDS-PAGE (b) → Modification (1: marker; 2: native BAL; 3: BAL MI; 4-6: BAL-DP75, BAL-DP100, BAL-DP200)
- CD spectra (c) → Secondary structure – α-helix

Conjugates with different polymer chain length: BAL-DP75, BAL-DP100, BAL-DP200

Benzaldehyde lyase (BAL)-PNIPAAm Conjugates

Thermoresponsivity:

Thermal stability @50°C improved:

Enzymatic activity retained:

Stable o/w-Pickering Emulsions:

**Pickering Interfacial Biocatalysis with BAL-PNIPAAm**

- Optimized conditions: water to oil ratio 5:5 and 30 mg/ml
- 270-fold higher efficiency of BAL conjugates than unemulsified BAL system (**D17: BAL on silica NPs**)
  - Reuse of BAL-DP75 conjugates after precipitation possible without loss of efficiency

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Pickering Interfacial Biocatalysis for Cascade Reactions

Glucose oxidase (GOx)-PNIPAAm conjugates + chloroperoxidase (CPO) with Thioanisol

Glucose oxidase (GOx)-PNIPAAm Conjugates + Lipase B *Candida Antarctica* (CalB) with cyclohexene

Patchy Particles

D. John, M. Zimmermann, S. Mehr, N. Puretskiy, M. Reifarth, M. Sperling, D. Grigoriev
Why Patchy Particles? Self-Assembly Concept

- **Impart ability to self-replicate to artificial material**
  - in analogy to polymerase chain reaction of DNA

- **pre-requisite: multi-directional control of interactions between building blocks – tri-valency**

![Diagram showing tri-valent colloidal particle](image)

- **Nucleobase** (reversible)
- **Phosphodiester backbone** (irreversible)
- **Tri-valent colloidal particle** (nm to micron)
2D Modification and 3D Structure Printing

Particle Release in Ethanol

Particle Release in Acetone

2D Patches

3D Patches

2 μm

μCP – Single Patch Particles

1. Ink Deposition (PEI via Spin coating)
2. Monolayer Creation
3. Printing Step
4. Particle Release using Ultrasound
µCP – Single Patch Particles

1. Ink Deposition (PEI via Spin coating)
2. Monolayer Creation
3. Printing Step
4. Particle Release using Ultrasound

**Particle Size**

- 5µm
- 4µm
- 2µm
- 1µm
μCP – Double Patch Particles

µCP – Double Patch Particles

Ink concentration

µCP – Double Patch Particles

Particle Size

5µm 4µm 2µm 1µm

Intaglio Printing using Structured PDMS Stamps: PEI in Ethanol on Silica

Intaglio Printing using Structured PDMS Stamps: PEI in Ethanol on Silica


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Intaglio Sandwich Printing using Structured PDMS Stamps

Intaglio Sandwich Printing using Structured PDMS Stamps

Poly(methyl vinyl ether-alt-maleic acid)

Polyethylenimine

Isoelectric point (IEP) of MF particles at pH ~ 7

- IEP of Poly(methyl vinyl ether-alt-maleic acid): pH < 7
- IEP of Polyethylenimine: pH > 7
pH-dependent interactions between mono-patchy particles

The statistics of particle dimer formation

<table>
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<tr>
<th>pH</th>
<th>n_{particle-patch}</th>
<th>n_{patch-patch}</th>
<th>n_{particle-particle}</th>
<th>n_{tot}</th>
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</thead>
<tbody>
<tr>
<td>6.8 (ethanol-water)</td>
<td>65 % 222</td>
<td>5 % 18</td>
<td>30 % 105</td>
<td>100 % 345</td>
</tr>
<tr>
<td>9.2 (ethanol-PBS buffer)</td>
<td>86 % 300</td>
<td>3 % 10</td>
<td>11 % 38</td>
<td>100 % 348</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>pH</th>
<th>n_{particle-patch}</th>
<th>n_{patch-patch}</th>
<th>n_{particle-particle}</th>
<th>n_{tot}</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.8 (ethanol-water)</td>
<td>53 % 112</td>
<td>5 % 11</td>
<td>42 % 87</td>
<td>100 % 210</td>
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<tr>
<td>6.1 (ethanol-PBS buffer)</td>
<td>81 % 249</td>
<td>3 % 10</td>
<td>15 % 47</td>
<td>100 % 306</td>
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Self-Assembly Systems

Electrostatic Coupling

Amphiphilic Silica-Particles

Oppositely charged patches

Alignment induced by Solvent Polarity

Self-Assembly – Avidin & Biotin Coupling

Coupling of two distinct particle species functionalized with avidin (labelled with quantum dots) and biotin

Photo-responsive Supramolecular Coupling (with B.-J. Ravoo, WWU Münster)

Two particle species functionalized with cyclodextrin and arylpyrazole coupled into heterodimers.
Summary

- Successful incorporation of channel proteins into artificial matrix yields ultrathin stable nanoporous membrane

- Incorporation of enzymes in polymer matrices yields efficient way to create active membranes
  - immobilized enzymes retain almost full initial activity

- Micro-contact printing is versatile tool to create multi-patch particles with control of patch geometry and positions
  - allows programmed particle assembly
Collaborations

Prof. Ulrich Schwaneberg (FhuA, RWTH Aachen)
Prof. Jörg Pietruszka (DERA, Forschungszentrum Jülich)
Prof. Changzhu Wu, (Pickering, University of Southern Denmark)

Funding

Patchy Particles  FhuA- & Enzyme Membranes & Capsules
Thank you very much for your attention!
Pickering Emulsions Revisited

Channel Protein FhuA – Engineering of Size and Channel Interior

- FhuA Synthesis and Engineering performed by Group of Prof. U. Schwaneberg, RWTH Aachen
- e.g. creation of a larger pore in the channel

![Diagram showing FhuA wild type and FhuA Δ1-159 with a difference in pore size and the removal of the cork domain.](image)
Characterization of protein-polymer conjugates – CD spectroscopy

α-helix
β-sheet
Random coil
Enzyme immobilization
DERA containing thin films via Langmuir-Schaefer

Thiolactone:
- hydrophoblizes polymer
- Reactive towards lysines
- Hydrolyzable
  → hydrophilization and crosslinking

P(NIPAAm-co-TlaAm)

Langmuir balance

Enzyme immobilization
DERA containing thin films via Langmuir-Schaefer

a. Covalent binding of the enzyme

b. Hydrophilization and crosslinking of the polymer matrix

c. Covalent attachment to the support

Enzyme immobilization
DERA containing thin films via Langmuir-Schaefer

- Multilayer deposition controls membrane thickness
- Enzyme immobilization without activity loss!

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Reinicke et al. ACS Appl. Mater. Interf. 2017, 9, 8317-8326
Untreated PDMS

- more oligomers $\rightarrow$ more hydrophobic $\rightarrow$ adhesion to PDMS $<$ cohesion in the ink layer
  $\rightarrow$ Higher density but lower surface charge of patches
PDMS treatment with ethanol

Reduced thickness of PEI patches generated by treated PDMS