Chemo-enzymatic synthesis routes for the production of bio-based chemicals from sugar and waste residues

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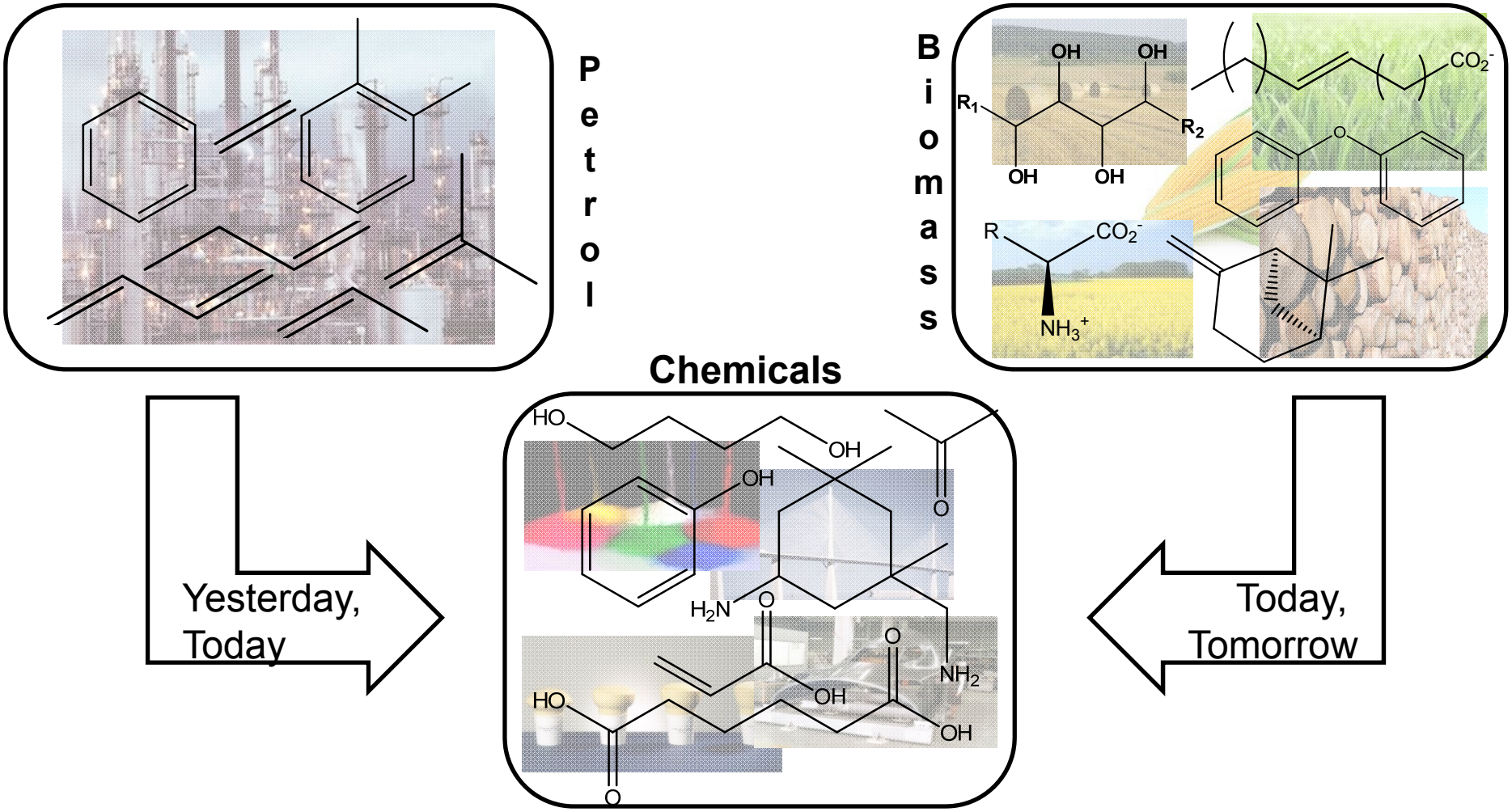
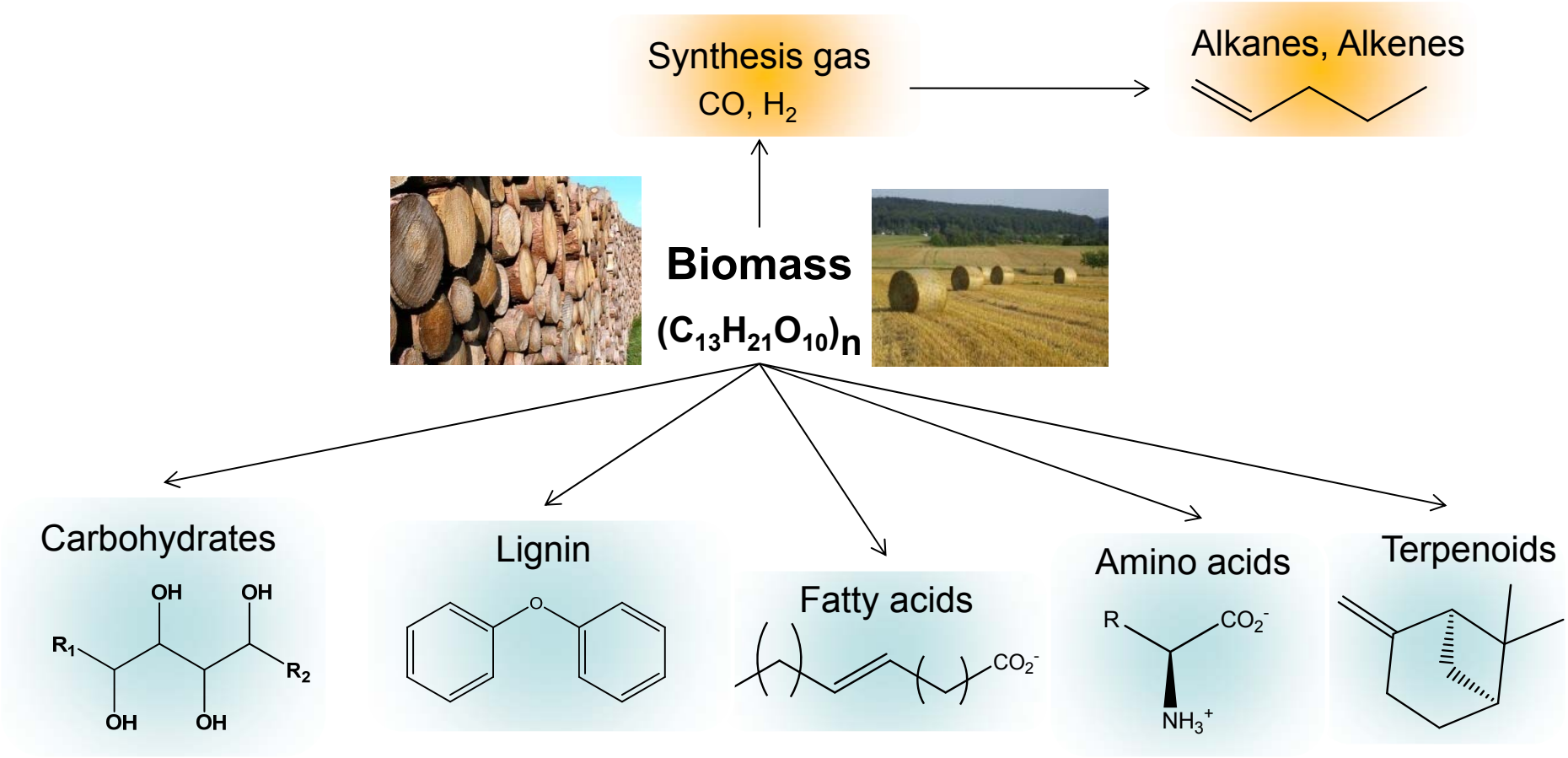
São Paulo, 09.11.2017

Change of resources of the ORGANO-chemical industry



Nature‘s Chemical Diversity for the Diversity of Chemical Products

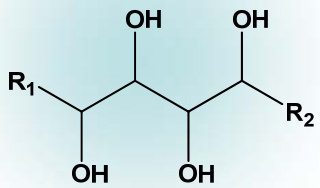


Nature‘s Chemical Diversity for the Diversity of Chemical Products

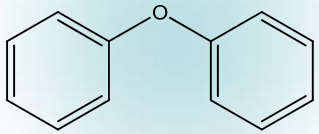
Biomass

$(C_{13}H_{21}O_{10})_n$

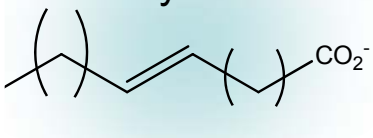
Carbohydrates



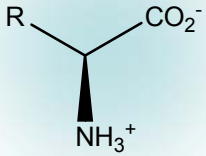
Lignin



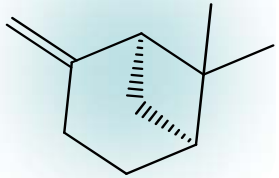
Fatty acids

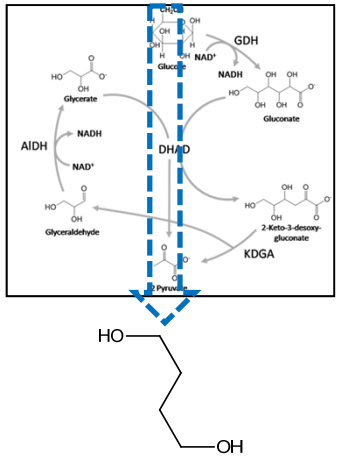


Amino acids

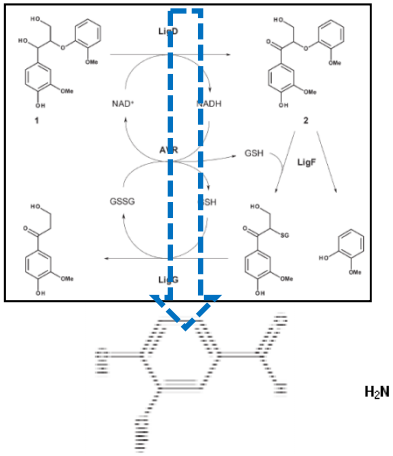


Terpene

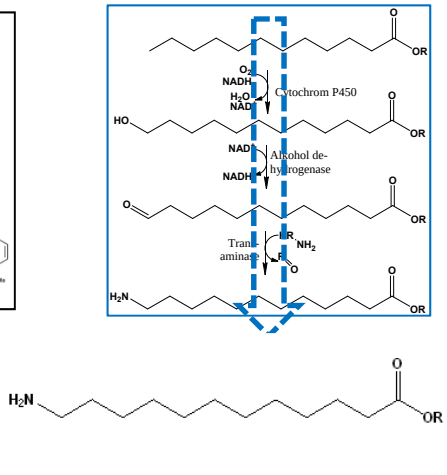




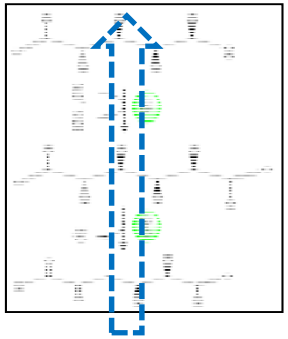
Diols

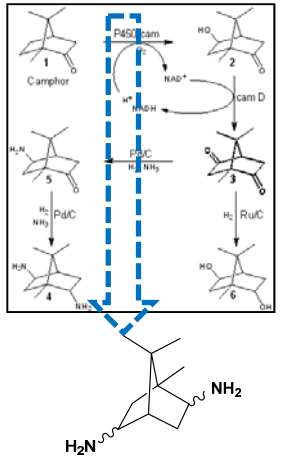


Phenols



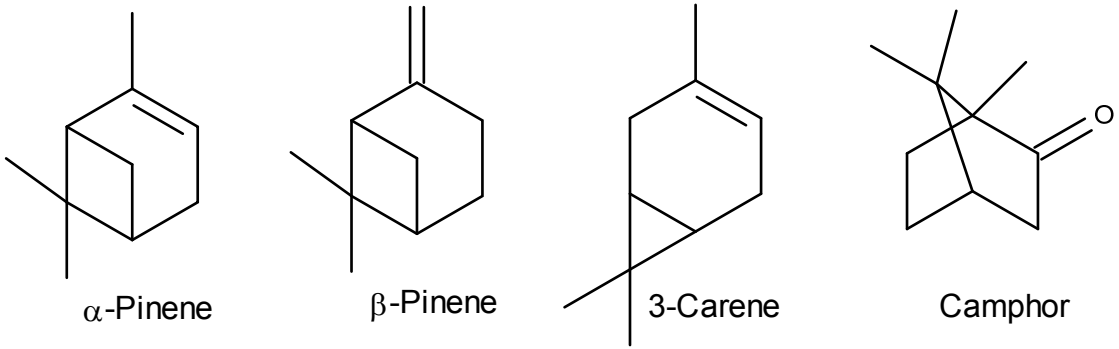
Bifunctional alkanes





Cycles

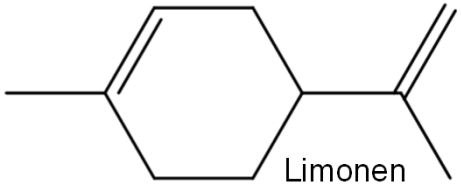
Monoterpene based building blocks



α-Pinene β-Pinene 3-Carene Camphor

Accumulation of terpenes in the cellulose production

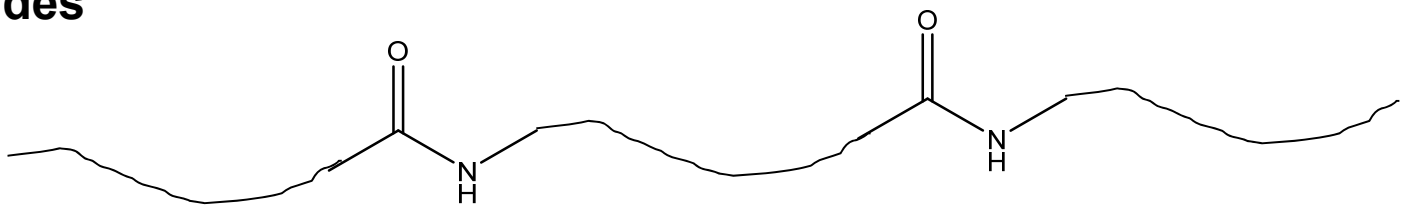
Cellulose and wood pulp production	Amount of cellulose [t/a]	Amount of terpenes [t/a]	Amount of α-Pinen [t/a]
Zellstoff Stendal	670.000	3.000*	1.800
Germany	2.700.000	11.000	6.600
Europe	31.000.000	155.000	93.000
World	130.000.000	650.000	390.000



Limonen

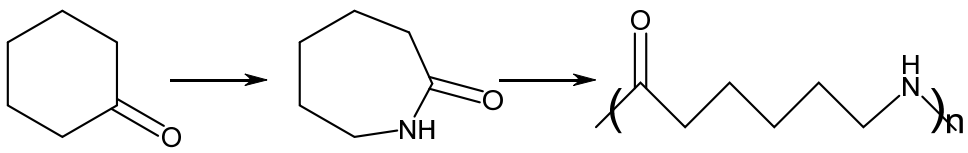
Terpenes for cyclic Co-Monomers in High Performance Polymers

=> Polyamides



- Linear chains, made of Diamines and Dicarboxylic acids or amino-carboxylic acids
- World market 25 Billion USD, annual growth rate 4 % (2017)
- Generally high durability and strength
- Application as automotive applications, carpets, shoes, textiles etc.

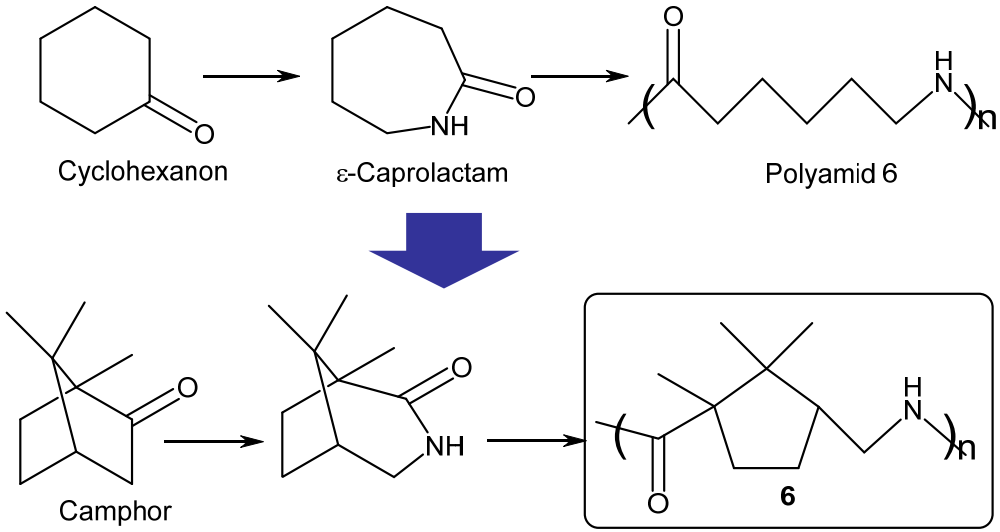
Typical production using fossil based cyclohexanon:

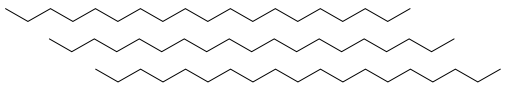


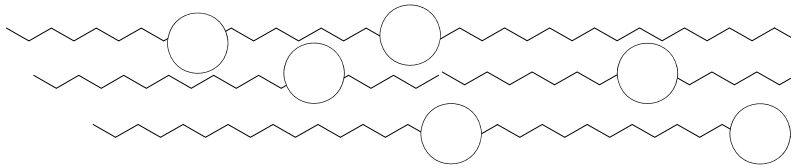
Cyclohexanon ε-Caprolactam Polyamid 6

Adjustment of properties using plasticers!

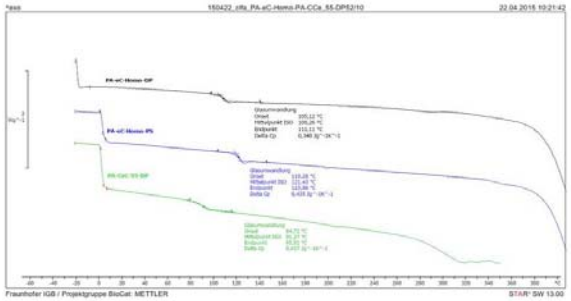
Cyclic Co-Monomers for High Performance Polymers

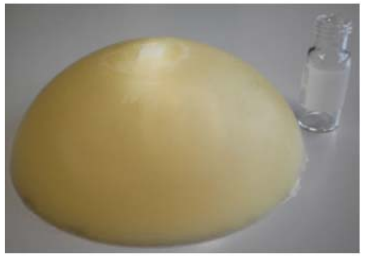


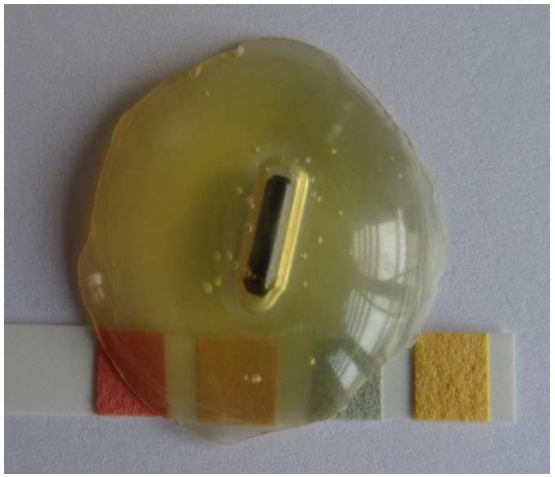




- M_N = 22 000 g/mol; M_W = 38 000 g/mol; Tg: ca. 110 -120°C; no melting point up to 350°C
- Light weight and highly stable
- Highly shock-resistant (even after cooling with liquid nitrogen)
- Transparent





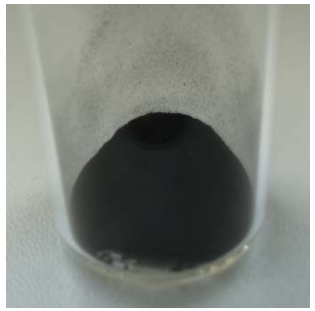


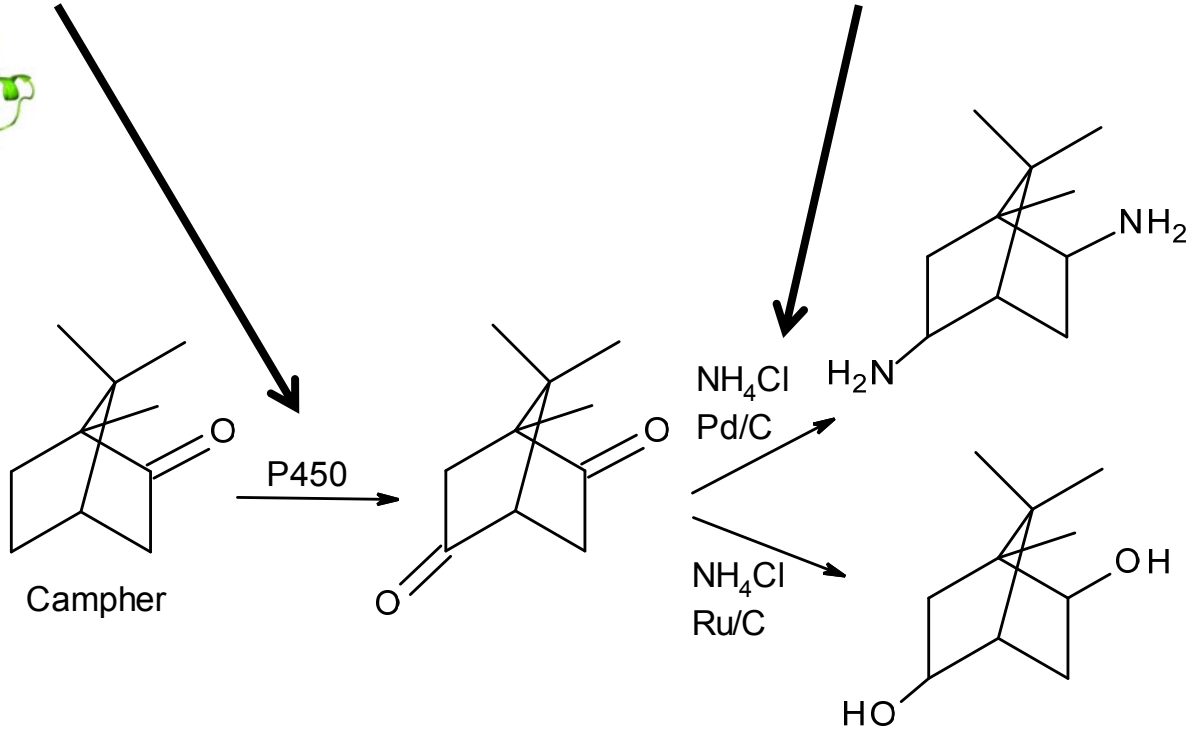
DPAM **10 2014 221 061.5**

Combination of Biotechnology and Chemistry for alternative monomers

1. High selectivity of enzyme catalysis

2. High activity and robustness of chemical catalysis

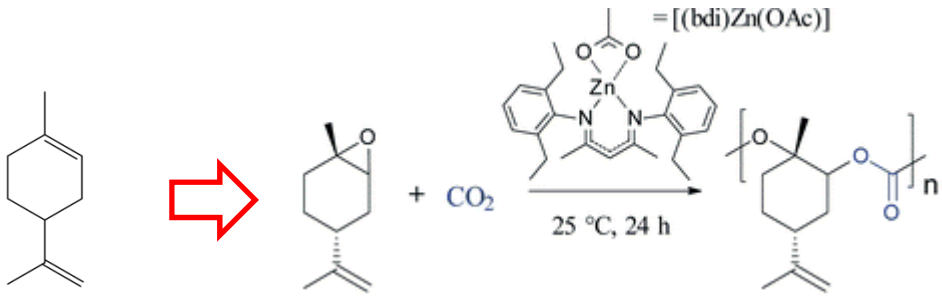


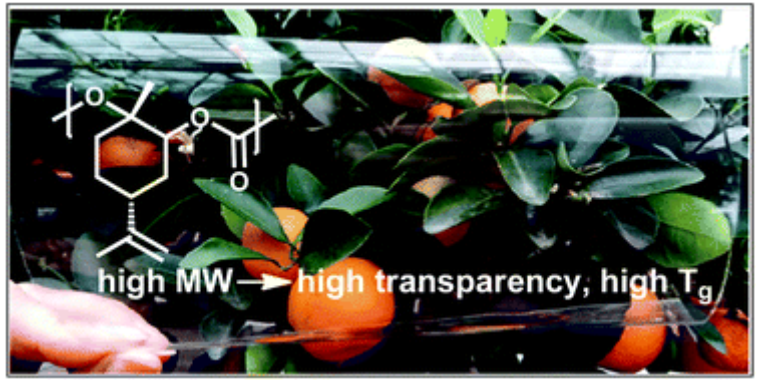


M. Hofer, H. Strittmatter, V. Sieber *ChemCatChem* **2013**, 3351
http://faculty.sites.uci.edu/poulos/cytochrome-p450/

Terpene based Epoxides - Sugars as green solvents

=> Epoxyresins & Polycarbonates





Green Chem., 2016, 18, 760–770

Challenge: Sustainable process => sustainable solvents

Actions:

(i) substitution of hazardous solvents by ones with better environmental, health & safety properties

(ii) use of "bio-solvents", i.e. solvents produced with renewable resources

(iii) substitution of organic solvents with supercritical fluids

(iv) substitution of organic solvents with ionic liquids that show low vapour pressure

=> Sugar based Deep Eutectic Solvents (DES)

Sugar based solvents

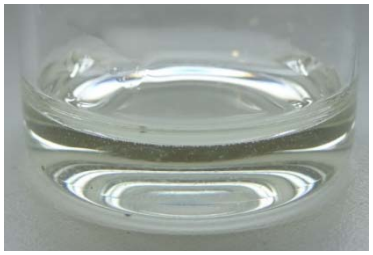
=> Deep Eutectic Solvents (DES)

→ a class of ionic liquids

→ prepared by mixing two components:

→ quaternary ammonium salt (such as choline chloride ChCl)

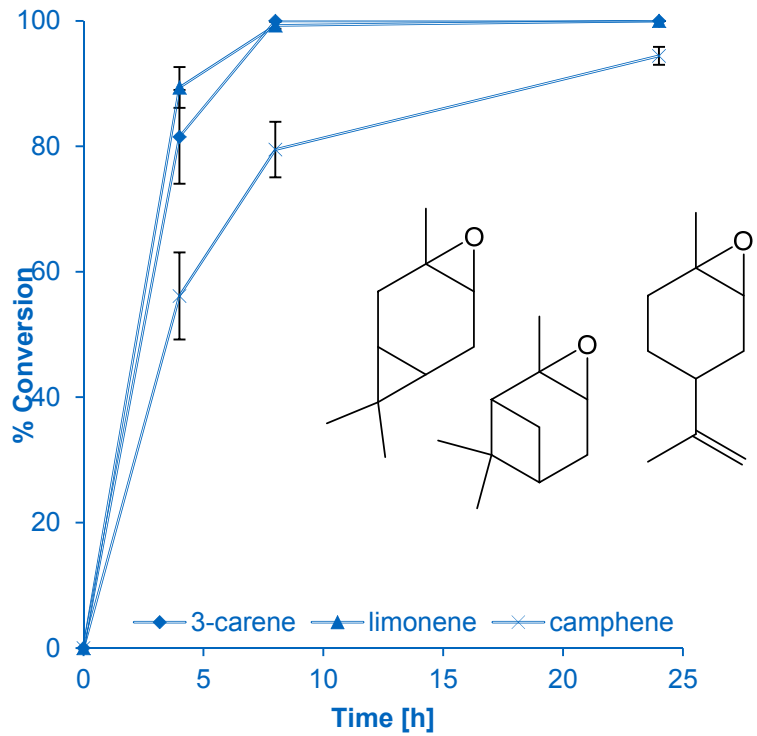
→ hydrogen bond donor (HBD) (alcohol, amide, carboxylic acid)



- **Sorbitol**
- **Glucose**
- **Fructose**

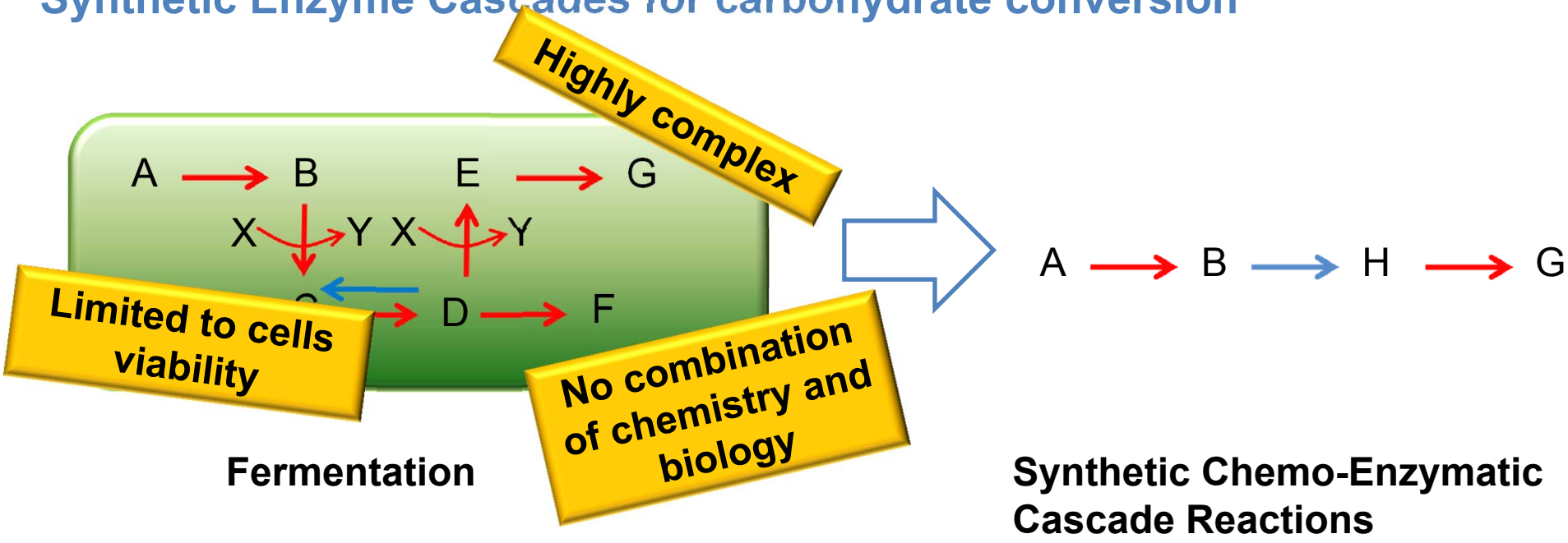
Liquid at
60 °C or below

→ polar and non-volatile
→ of natural origin
→ capable of biological degradation
→ easy product removal (dissolution in water)



Ranganathan *et al.*, 2017, *Green Chemistry*, 2017, 19, 2576–2586

Synthetic Enzyme Cascades for carbohydrate conversion



Fermentation

Synthetic Chemo-Enzymatic Cascade Reactions

Challenges:

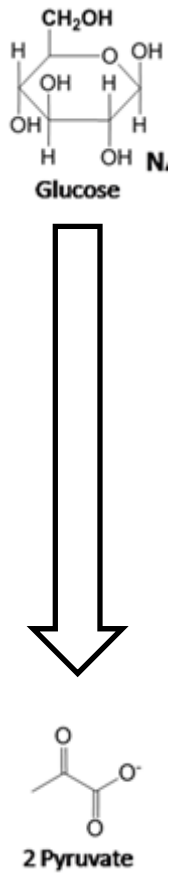
- Compatible Enzymes/Catalysts
- Promiscuity for reduced number of enzymes
- Thermodynamics: All the way downhill
- Cheap and stable cofactors

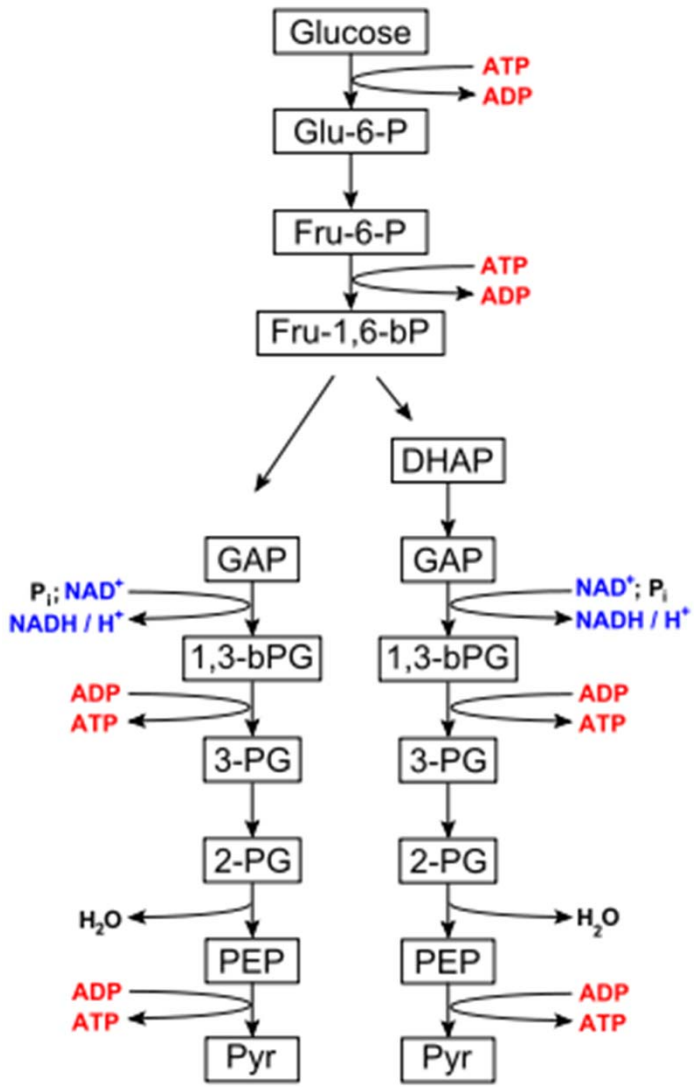
Example: Ethanol, Isobutanol, Butandiol

A novel and artificial glycolytic pathway…

Glycolysis

⇒ **10 Enzymes**
⇒ **2 Cofactors**



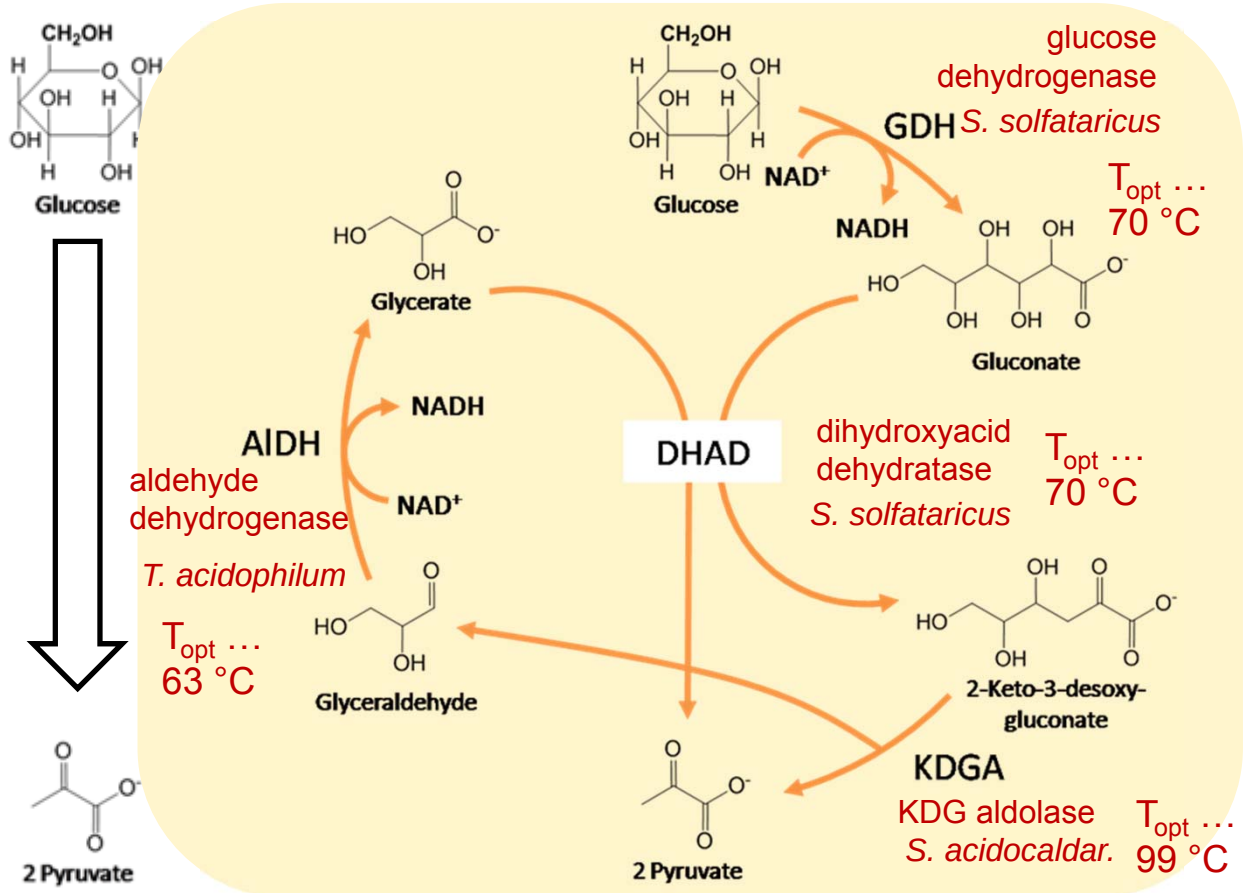


A novel and artificial glycolytic pathway…

„Promiscuity for enzyme reduction“

Artificial Glycolysis

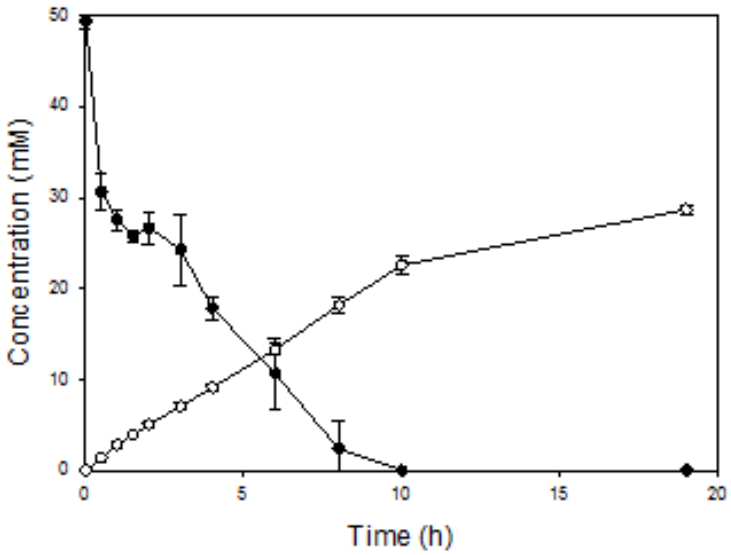
To
⇒ 4 Enzymes
⇒ 1 cofactor



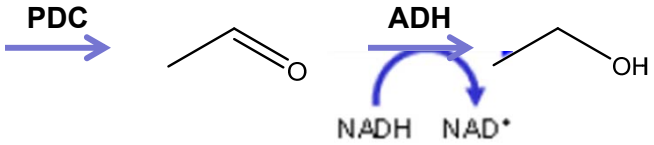
Guterl JK *et al.*, **2012** *ChemSusChem* **5**(11), 2165

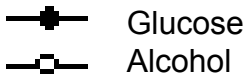
In vitro production of alcohols

Ethanol – 6 Enzymes

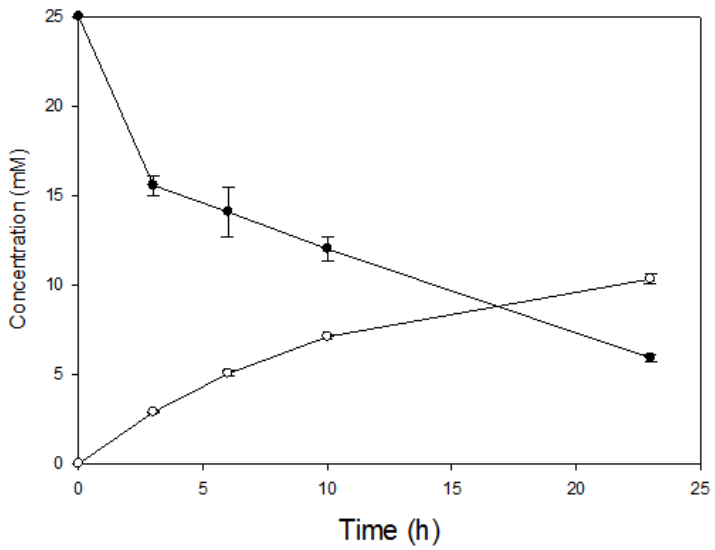


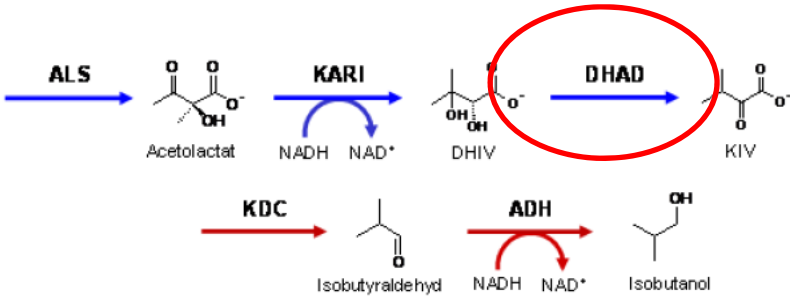
Glucose concentration was doubled to allow for direct comparison.





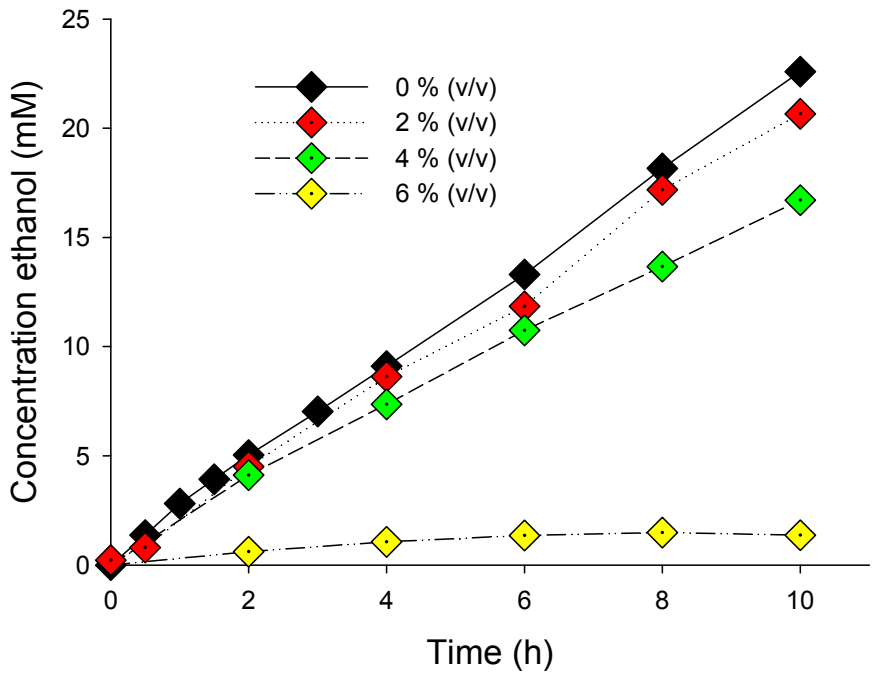
Isobutanol – 8 Enzymes





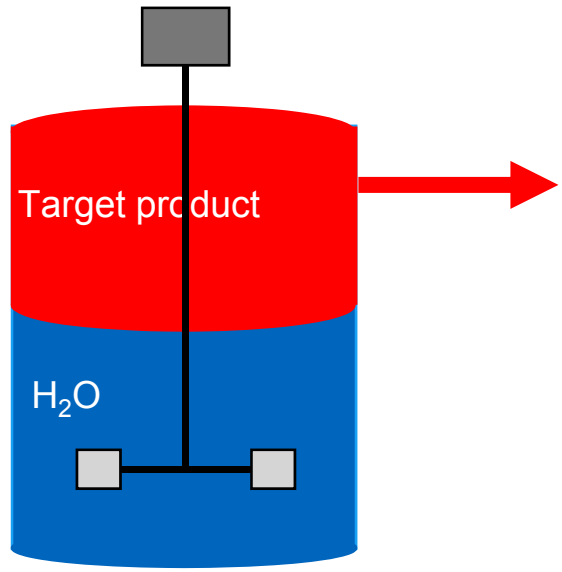
Guterl JK *et al.*, *ChemSusChem* **2012**

Advantage of *in vitro* production for product isloation



Product formation in the presence of isobutanol

Water insoluble products



Reactor

Guterl JK *et al.*, **2012** *ChemSusChem* **5**(11), 2165

Profitability– Enzyme costs for chemicals production

		Currently	Slight optimization
Assumption	Number of enzymes	8	7
	Cost of enzymes	1000 €/kg	200 €/kg
	Average activity per enzyme	8 u/mg	35 u/mg
	Activity for all enzymes	1 u/mg	5 u/mg
	Process life time for enzyme	10 h	100 h
Calculation	Amount Isobutanol/ Enzyme	54 kg/kg	2,7 to/kg
	Amount Enzyme / Isobutanol	18.5 g/kg	0.37 g/kg
	Cost of enzyme for Isobutanol	18.5 €/kg	0.07 €/kg

Enzyme Engineering for improved Enzymes

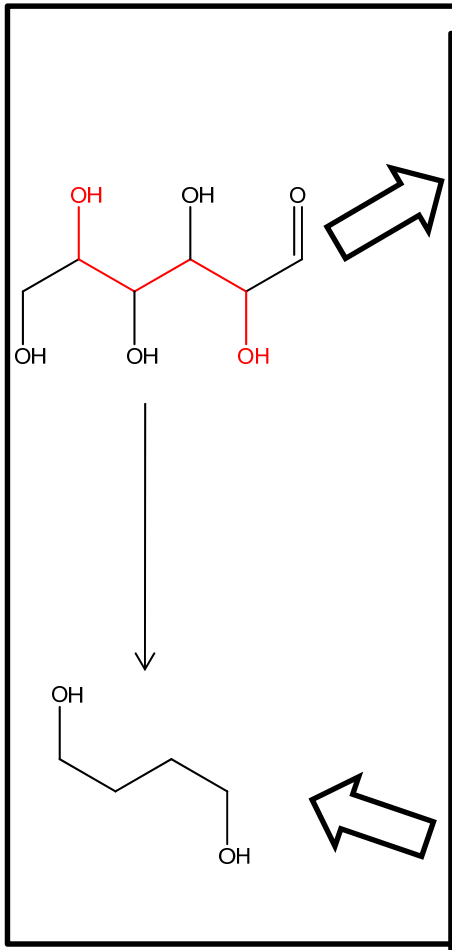
⇒ **Promiscous**

⇒ **Easy production**

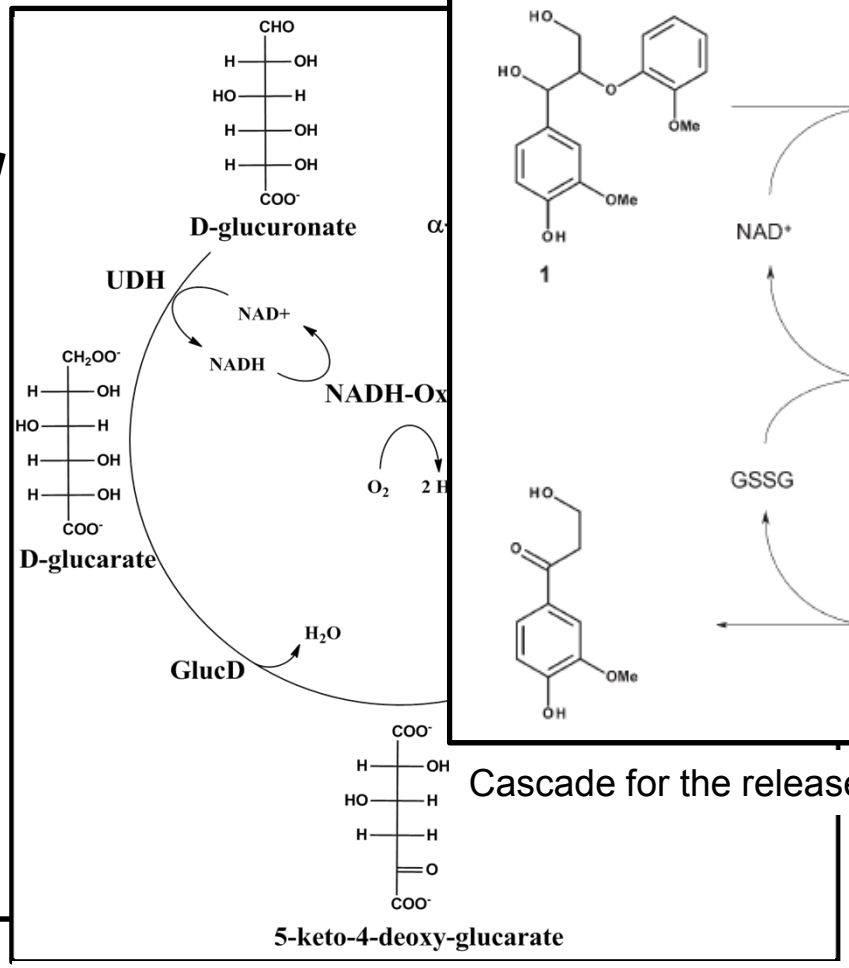
⇒ **High Activity**

⇒ **High Stability**

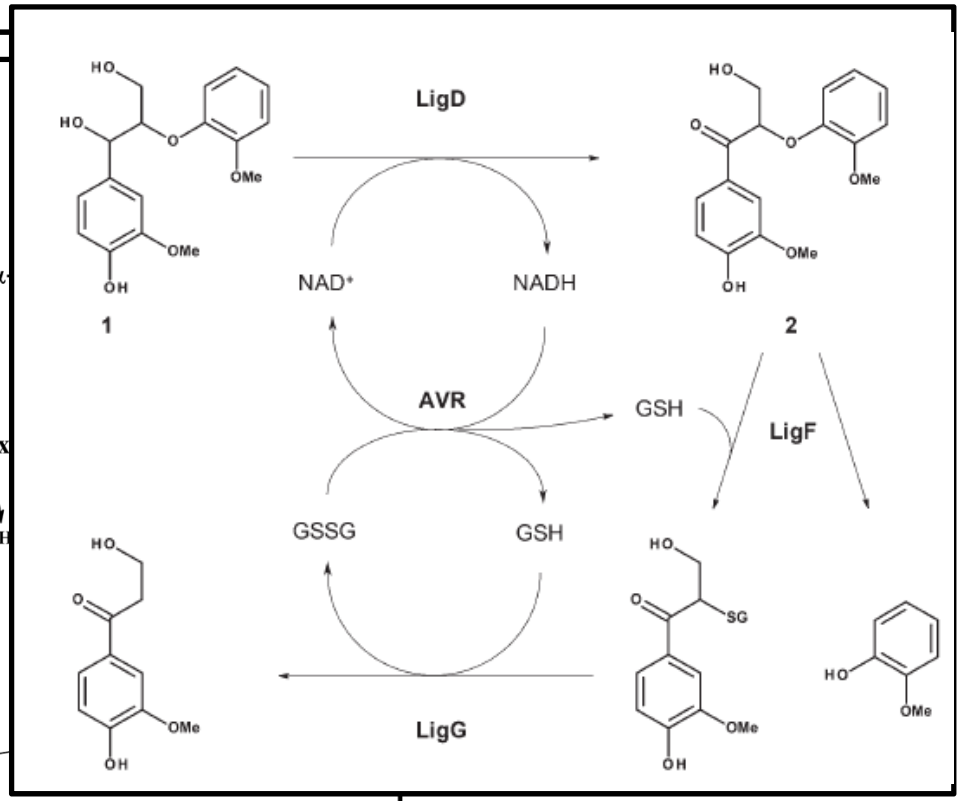
The power of synthetic cascades



4-step formation of 1,4 butandiol from glucose



5 enzymes for the formation of α–ketoglutatarate from uronic acids



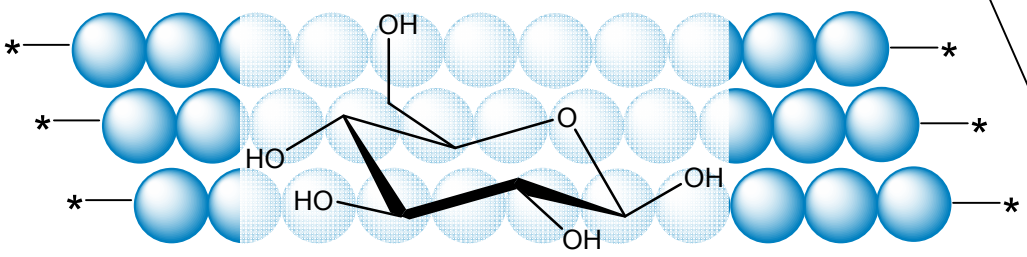
Cascade for the release of monomers from lignin

Pick et al., *Microbial Biotechnology,* **8**, 633–643
Beer et al., *Metabolic Engineering* 40 (2017) 5–13
Reiter et al. *Green Chem.*, 2013, **15**, 1373

Polysaccharides as Biogenic Polymers

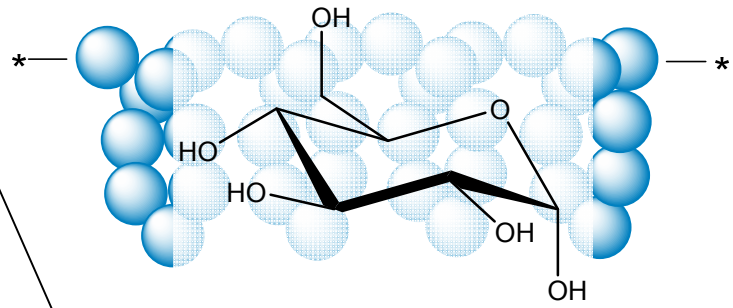
Linear homopolymers

Cellulose



β-Glucose

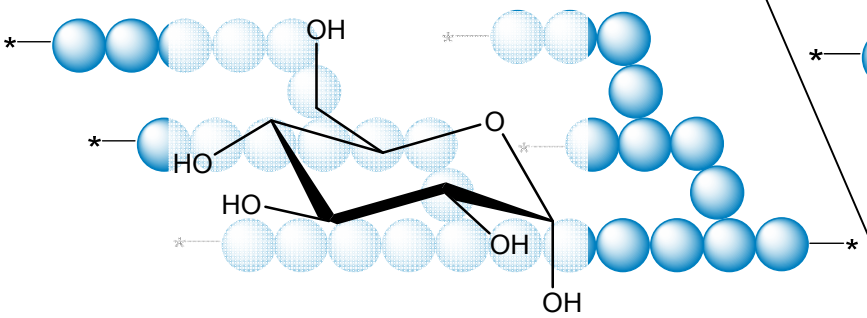
Amylose



α-Glucose

Branched homopolymers

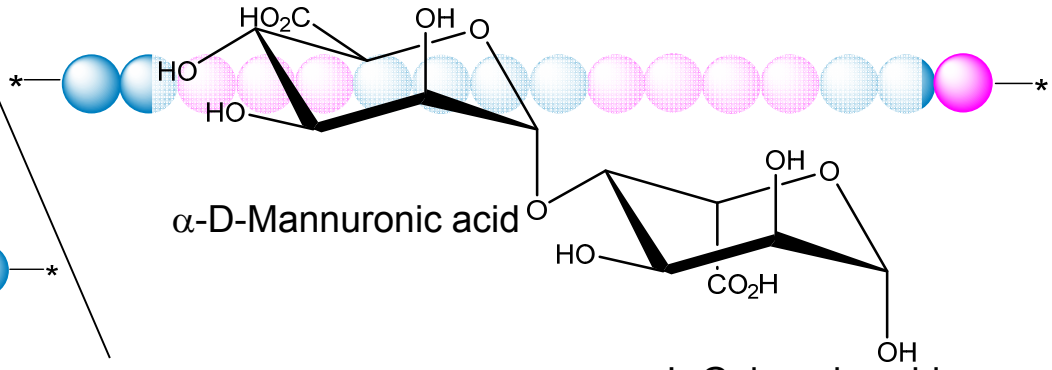
Amylopektin



α-Glucose

Linear block polymers

Alginat

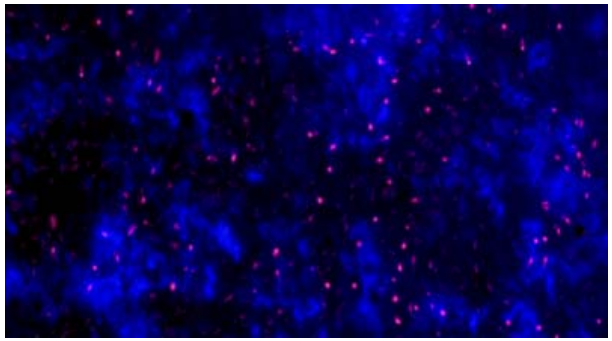


α-D-Mannuronic acid

α-L-Guluronin acid

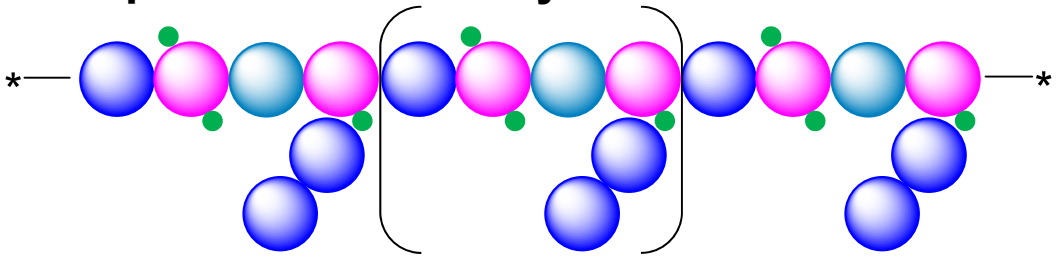
Exopolysaccharides as Biogenic Polymers

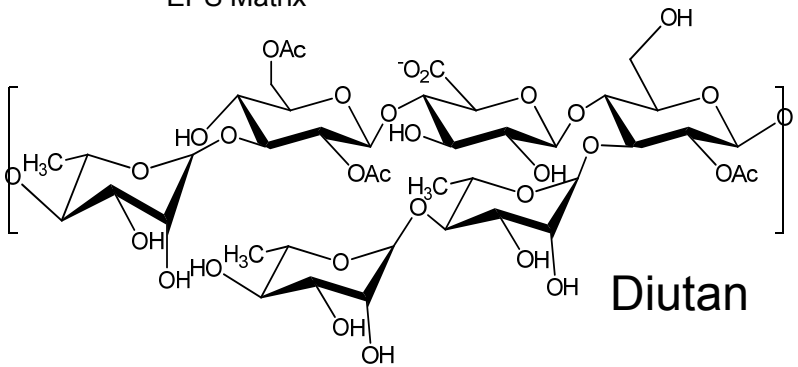
- Secreted by the microorganisms
  - Good & Bad Biofilms



Pseudomonas syringae (rot) embedded in EPS Matrix

Complex mikrobial Polymers





- Ca. 6000 Polysaccharide forming microorganisms have been described*

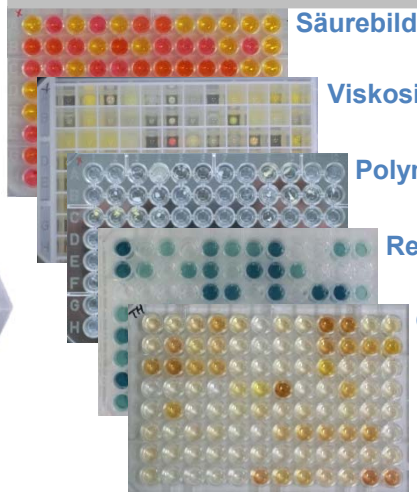
- Ca. 15 - 20 (Exo)polysaccharides are used commercially

* Bacterial Carbohydrate Structure DataBase

Automated High-Throughput Analysis
=> from sample to monomer composition within one day

Strain collection

Part1: Automated Screenin

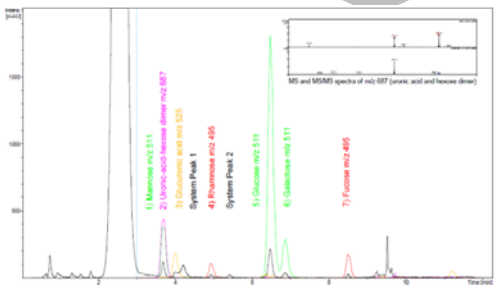


- Säurebildung
- Viskosität
- Polymerbildung
- Restglukosegehalt
- Gesamtzuckergehalt
- …
- …
- Weitere Module

Part 2: Carbohydrate Fingerprint

- Pyruvat Substituenten

Monomerzusammensetzung:
- Pentosen, Hexosen
- Deoxy-, amino-Zucker
- Uronsäuren
- Di- und Trimere
- Zuckermodifikationen: z.B. Pyruvateketal
- Seltene Zucker
- ….



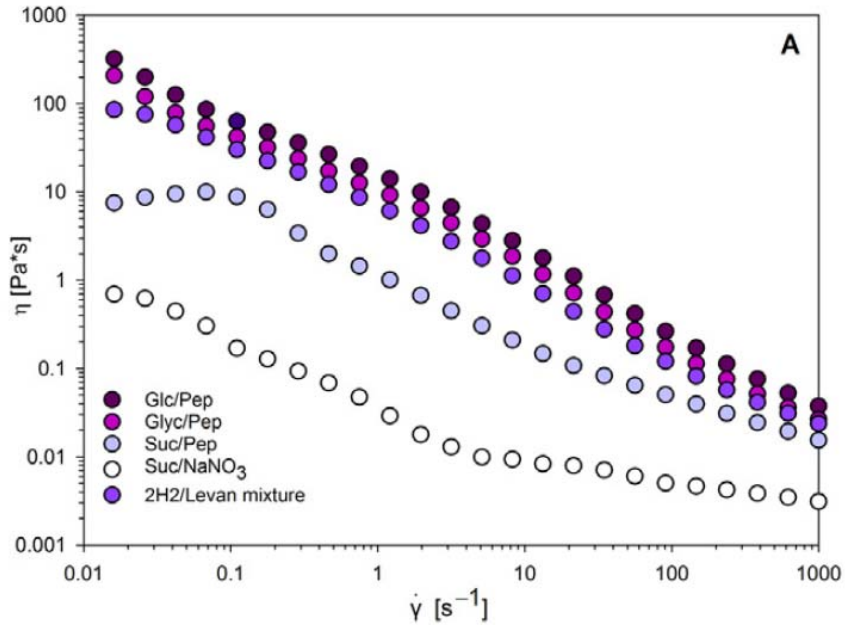
Ruehmann et al. 2014, J. Chromatogr. A, Rühmann et al. (2016) JoVE10(e53249)

Example: New Polymer for Cosmetics

Polymer eines *Paenibacillus* Isolats

- High productivity (0.25 g L^{-1} h^{-1})
- High viscosity (35 Pa · s, shear-rate of 1 s^{-1}, Conc. 1 % w/w of Polymer)
- Good film formation
- Further application e.g. in lubrication
- Properties can be tailored by fermentation conditions:

Components:
Glucose, mannose, galactose, glucuronic acid, pyruvate

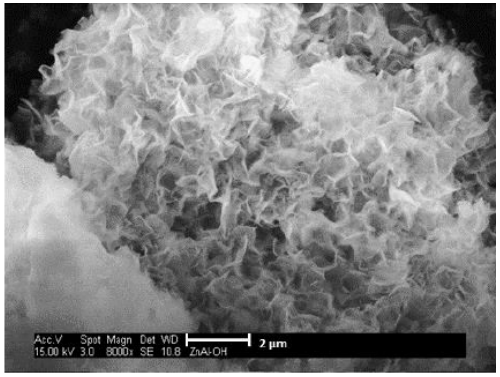


Ruetering et al . 2016 , Carbohydrate Polymers 148 (2016) 326–334.

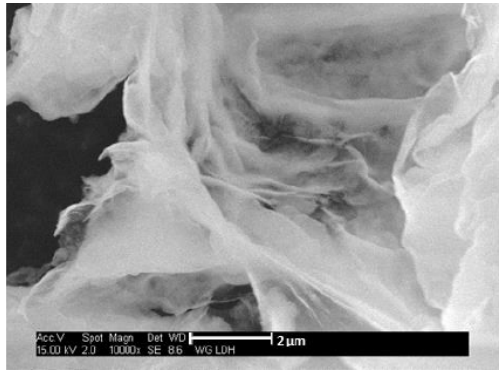
Example: Incorporation of Polysaccharides into mortar

Aluminum mortar forms Layered Double Hydroxide (LDH)

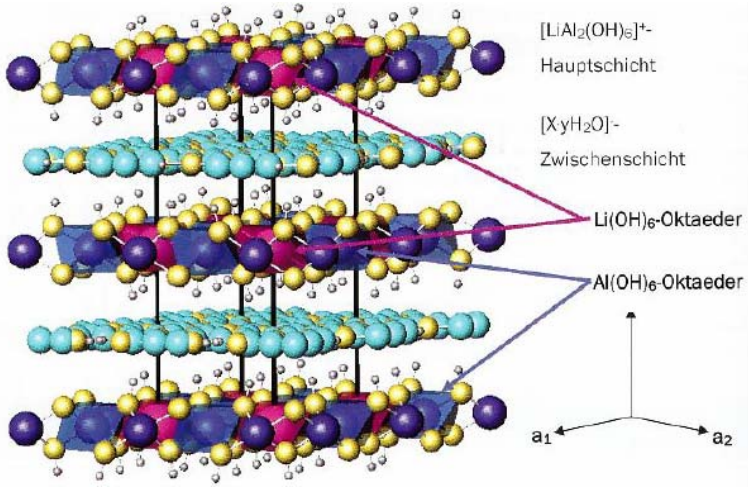
Incorporation in Zn-Al-LDH layers

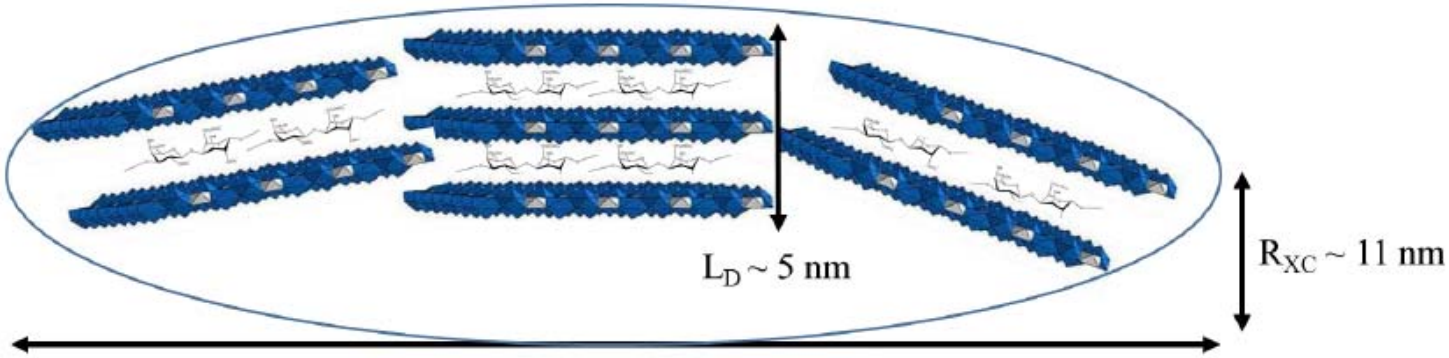


Control



With Polysaccharides





de Reese J., Sperl N., Schmid J., Sieber V., Plank J.; *Effect of biotechnologically modified alginates on LDH structures*, Bioinspired, Biomimetic and Nanobiomaterials, 4(3), **2015,** 174-186 , de Reese, Dissertation 2016

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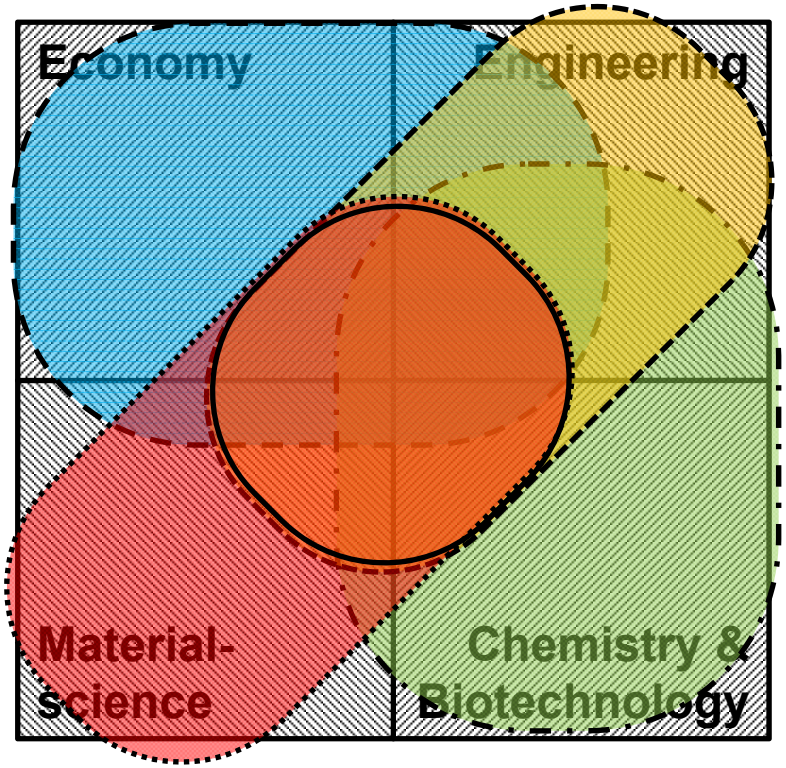
Going for a new generation of scientists to enable bioeconomy

Interdisciplinary study programs with elements of
natural sciences, engineering and economics



Bachelor- and Master study courses (year of start)

- Chemical Biotechnology (2017/2019)
- Energy- and Process Engineering (2019/2019)
- Bioeconomy (2018/2020)
- Biogenic Materials (2019/2019)
- Renewable Resources (2013/2009)

TU München, Campus Straubing for Biotechnology and Sustainability

Straubing as Center of biogenic resources

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