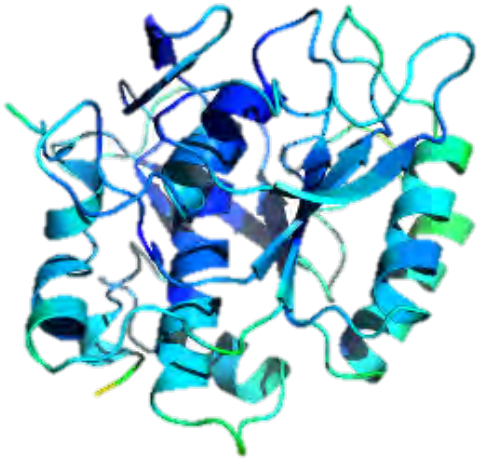
Formulation of Enzymes for Industrial Applications



Grit Baier, Sonja Kübelbeck, Rute André, Katrin Tücking, Jan-Philip Merkl, Ping Li, Anna Hoschek

BASF SE, Formulation Research Platform

Advanced Materials and Systems Research

Formulation of Biological Actives

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Outline

- BASF - we create Chemistry
- Process chain for enzyme development
- Analytical tools – enzyme shelf life characterization
- Encapsulation via droplet formation
- Solid formulation of enzymes
- Cooperation with Universities

BASF – We create Chemistry

- BASF SE is one of the largest chemical producers in the world
- BASF Group comprises subsidiaries and joint ventures in more than 80 countries
- BASF operates six integrated production sites and 390 other production sites in Europe, Asia, Australia, America and Africa
- 2018 BASF employed >120,000 people, sales €62.7 billion, EBIT €6.3 billion

Research & Development - Major Growth Driver for BASF

- Over €9 billion annual sales with innovations
- Around 3,000 running research projects
- 10,000 employees worldwide in R&D

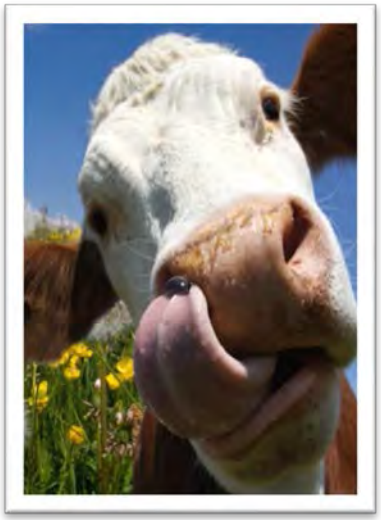
Enzymes are a Growth Field for BASF

Targeting different fields (detergents, cleaners, animal and human nutrition, plant health, crop protection, cosmetics)

Home care

Protease
Amylase
Lipase
Mannanase
Cellulase
Pectate lyase

Animal & human nutrition

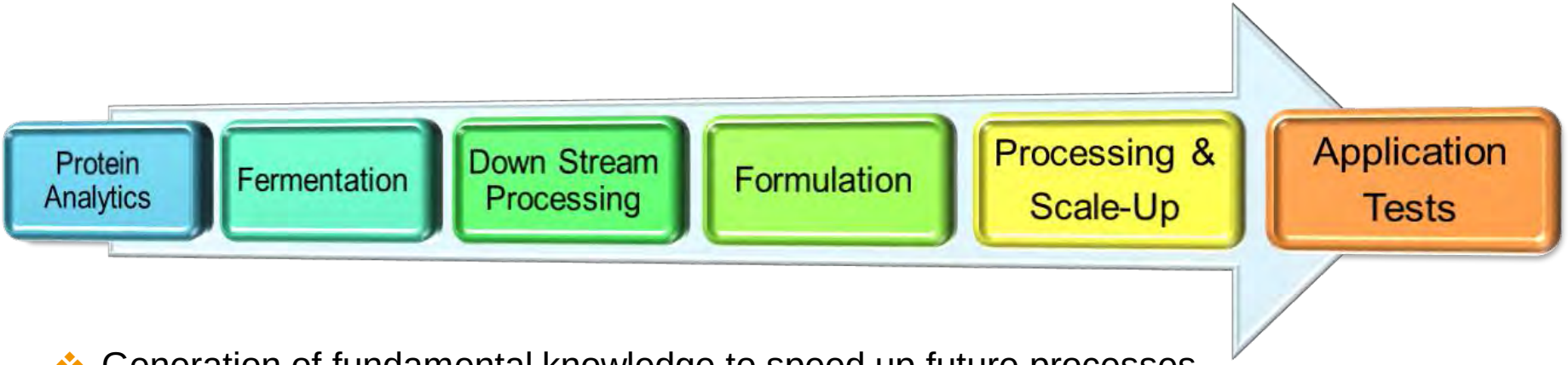


Phytase
Amylase
Cellulase
Xylanase
Galactosidase
Mannanase
Lipase
Protease

Agricultural applications

Hydrolase
Esterase
Phosphatase
Dehydrogenase

Process Chain for Enzyme Development



- Generation of fundamental knowledge to speed up future processes
- Working on formulation concepts - Combination of technology and formulation
- Each enzyme is different (pH stability, pI …..)
- Each formulation needs to be adapted to a new enzyme
- Biological actives require special knowledge - Specific analytical tests
- Formulation & handling properties (e.g. solid formulations: flowability, dispersibility, dust)
- Usage of modelling tools to speed up formulation development (DoE, Modde, JMP)

Analytical Tools

Assess formulation stability

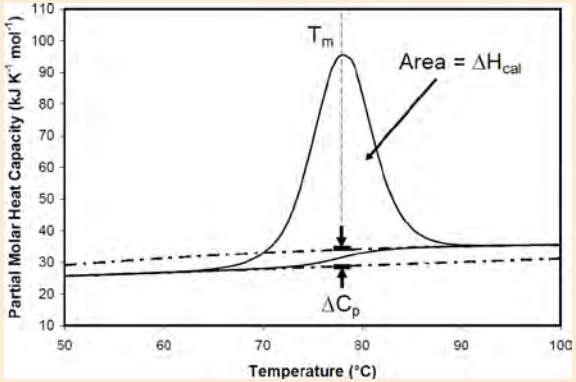
- Development of analytical tools to assess physico-chemical stability and enzyme shelf life in formulations

Analytical Tool	Application
Chromatography (HPLC, GC, SEC, IEC)	Studying degradation
Spectroscopy (UV-Vis, Fluorescence)	Enzyme activity (shelf life)
Dynamic light scattering	Size distribution, precipitation, agglomeration
Electrokinetic potential	Overall charge, coagulation, flocculation
Thermal analysis (ITC, DSC, DSF)	Binding affinity, folding, defolding, denaturation
Microscopy (SEM, TEM)	Morphology, size and size distribution
Titration (colorimetric, redox)	Functional group

Enzyme Shelf Life Characterization

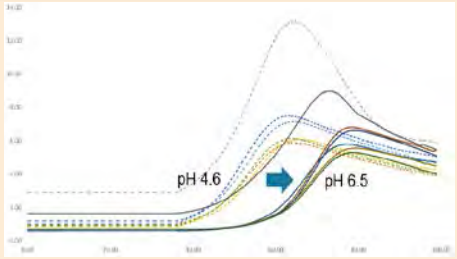
Enzyme conformational stability

nanoDSC



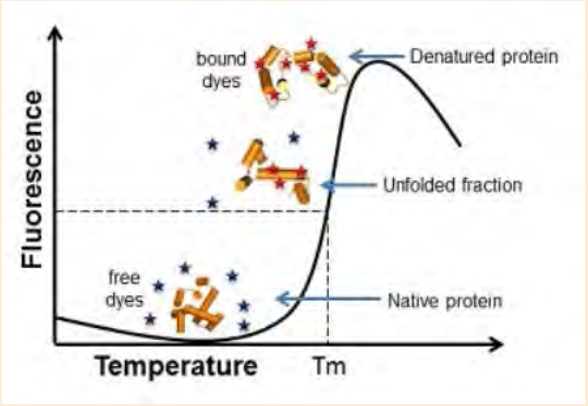
T_m Thermal transition temperature: 50% of the molecules are in their native state and 50% are in a denatured, unfolded state.

Evaluation of influences on enzyme stability e.g. pH, salts, builder or buffers, surfactants, polymers



Lightcycler

Differential Scanning Fluorimetry



Thermal shift assay quantifies the change in thermal denaturation temperature of a protein.

Enzyme shelf life

Gallery™
Automated photometric analyzer
Capacity of up to 350 tests/hr
Small sample volume (60 µL)

Plate reader

"Pre-screening" of enzyme formulation (absorption & fluorescent based assays)

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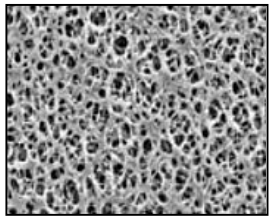
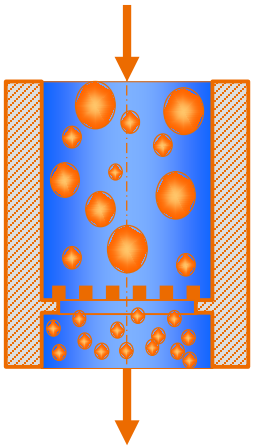
Encapsulation via Droplet Formation

Increased stability of the encapsulated enzyme against environmental influences

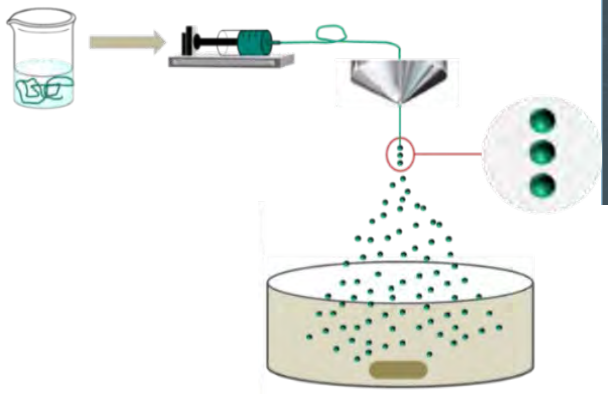
Membranes





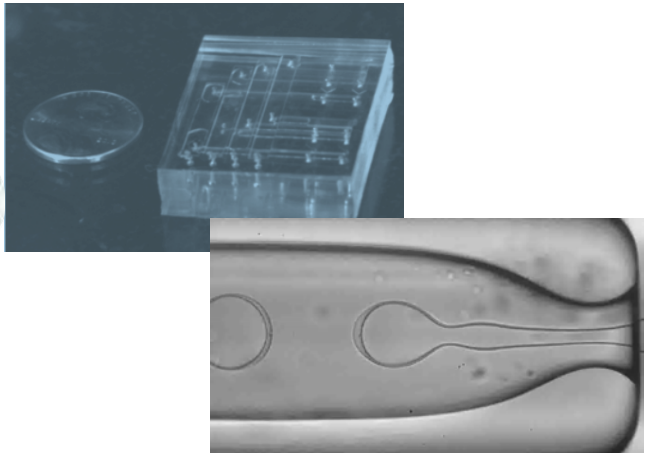
Droplet sizes < 80 µm

Vibrating nozzle



Droplet sizes > 80 µm

Microfluidics



Droplet sizes < 30 µm

- Smart and mild procedures (low energy dissipation rates)
- No chemical reaction - no enzyme destruction
- Membranes & VN: (Narrow) particle size distribution
- µF: monomodal particle size distribution
- Scalable technologies
- Tunable properties (shell thickness, particle size/shape)
- Adjustable release characteristics

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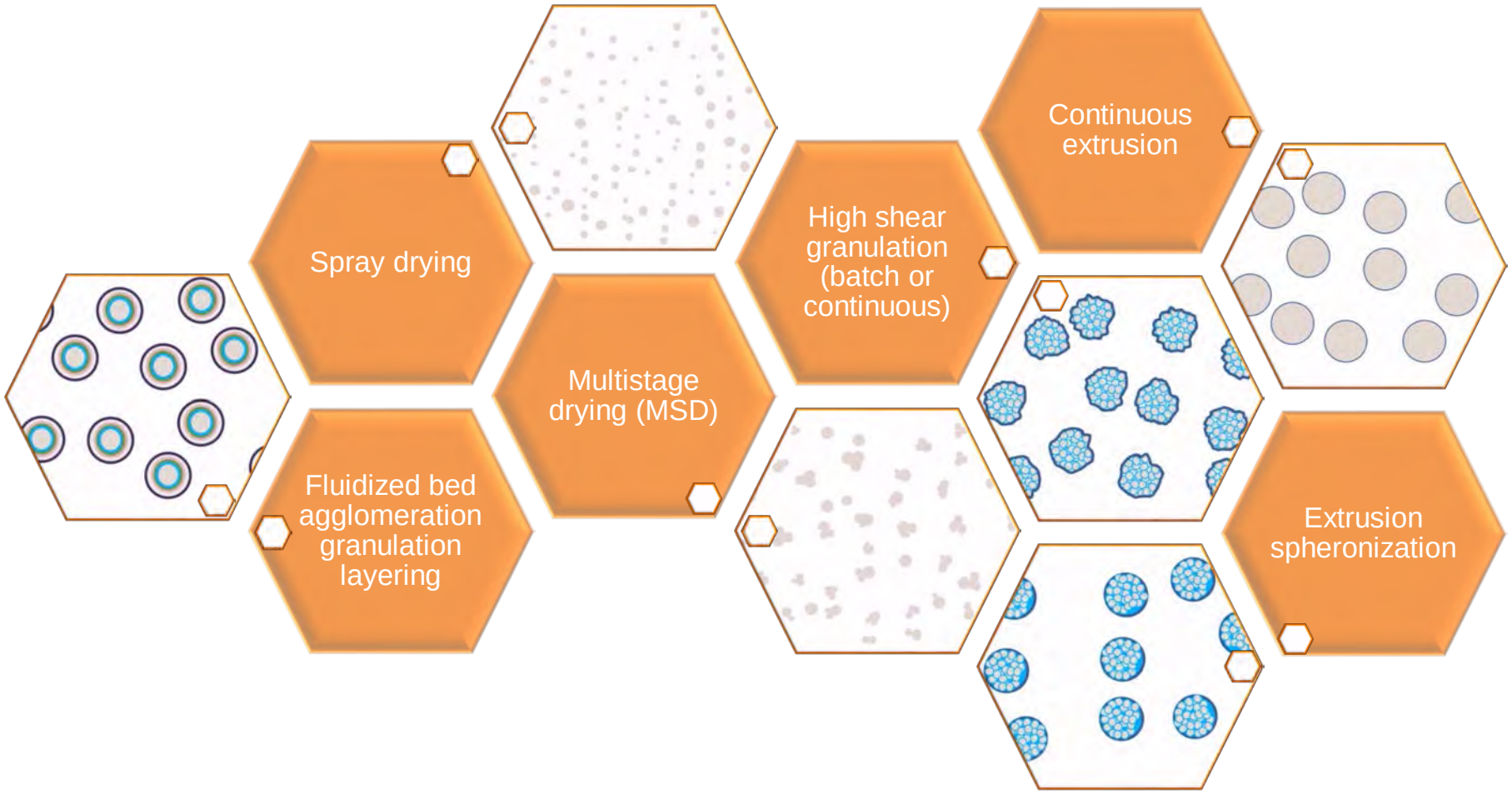
Solid Formulation of Enzymes

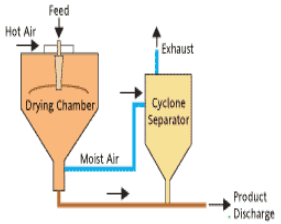
Technologies Main Categories



Solid Formulation of Enzymes

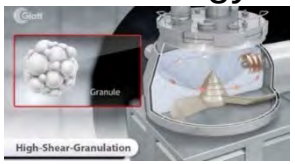
Spray Drying & Multistage Drying

- Product is dried during atomization
- Product is atomized in a spray dryer, dried and agglomerated in a fluidized bed



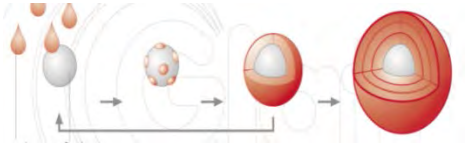
Batch & continuous high shear granulation/agglomeration

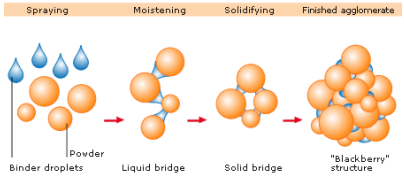
- Granulates are formed during mixing with liquid additives
- Cont. high shear agglomeration: similar mechanism, but with lower shear energy input due to short processing time



Fluidized bed agglomeration & granulation-layering

- Enzyme solution and additives are sprayed to form liquid bridges
- Enzyme solution is sprayed on a carrier and further coated with multiple layers





Extrusion spheronization & continuous extrusion

- Mixing enzyme with matrix materials, extrusion
- Spheronization
- Drying & coating

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Cooperation with Universities

We leverage a global network of PostDoc Centers

CARA
California Research Alliance

NORA
North American Center for Research on Advanced Materials

JONAS
Joint Research Network on Advanced Materials and Systems

NAO
Network for Advanced Materials Open Research

University of California, Berkeley
Biosciences & -engineering
Sensors and Systems,
Materials, Electronics,
Catalysis

University of California, Los Angeles
Materials for Energy and Electronics

Stanford University
Electronics,
Materials,
Computer Science & Modelling, Catalysis

University of California, San Francisco
Biosciences & -engineering,
Computational Biosciences

University of California, Davis
Agricultural Sciences
Food Science and Nutrition
Formulation

University of Massachusetts, Amherst
Polymer chemistry,
- physics & -
engineering, food
science, analytics

Harvard University, Cambridge
Micro/nano-structured materials, soft matter science, bioengineering

Massachusetts Institute of Technology, Cambridge
Materials science & engineering, integrated systems, bioengineering, computer science

I.S.I.S.- Université de Strasbourg
Supramolecular chemistry and physics, nano-structured materials

University of Freiburg
Polymer chemistry and - processing, micro-systems and surface modification

ETH Zurich
Material science, colloidal engineering, biosystems & – surfaces, construction

Tsinghua University
Colloids

Beijing University of Chemical Technology
Composites

Beijing Institute of Technology
Isocyanate Chemistry

Changchun Institute of Applied Chemistry
Polymer Physics

Fudan University
Colloids; Living Polymerization

Kyoto University
Living Polymerization

Hanyang University
Composites

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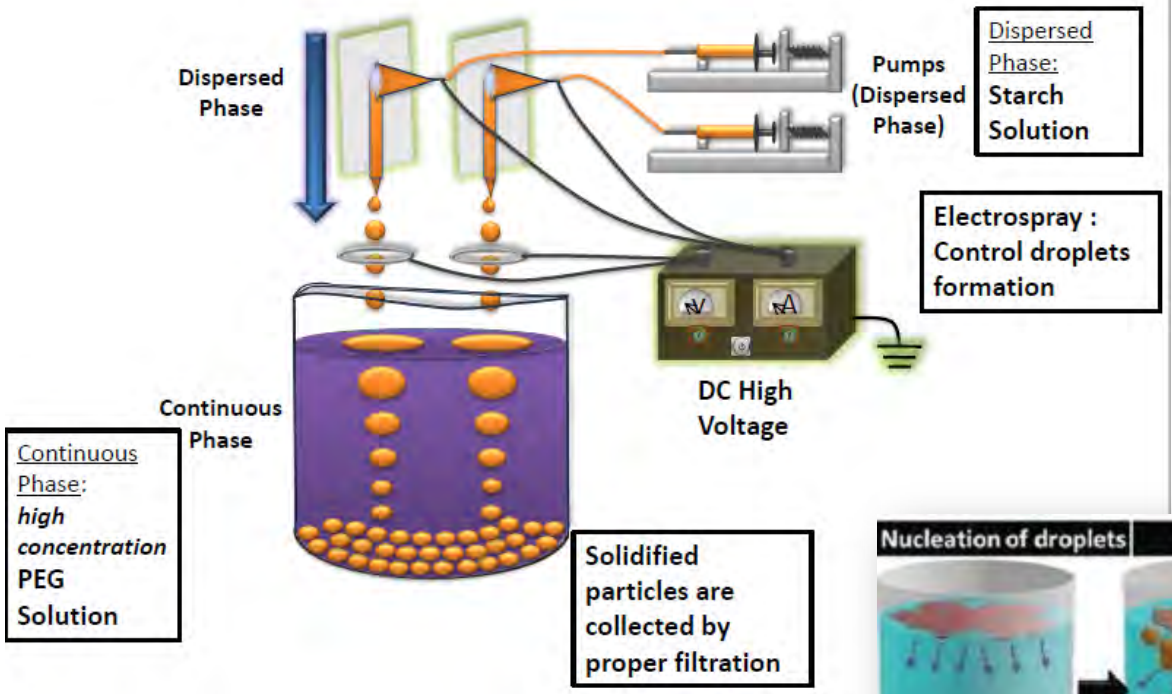
Cooperation with Universities

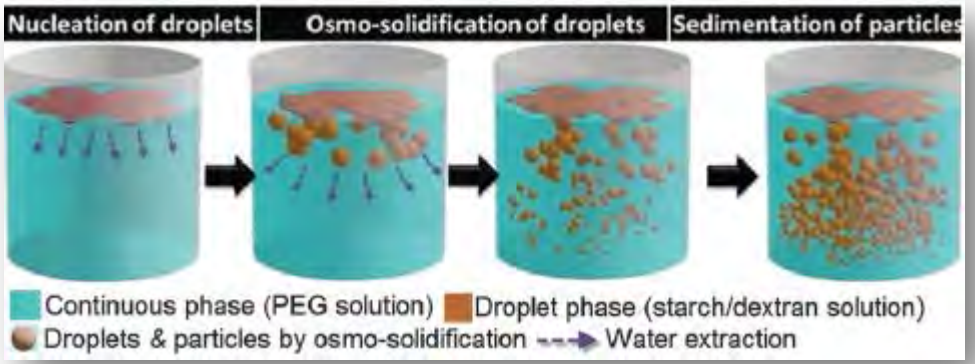
Osmo-solidification of all-aqeous emulsion

Cooperation with University Hong Kong



Advantages of all-aqueous emulsions

- Non-toxicity
- Cost-effectiveness
- Simplicity in fabrication process



Osmo-solidification of all-aqueous emulsion with enhanced preservation of protein activity†

Qingming Ma,[ab] Yang Song,[ab] Grit Baier,[c] Christian Holtze[c] and Ho Cheung Shum*[ab]

J. Mater. Chem. B, 2016, **4**, 1213–1218

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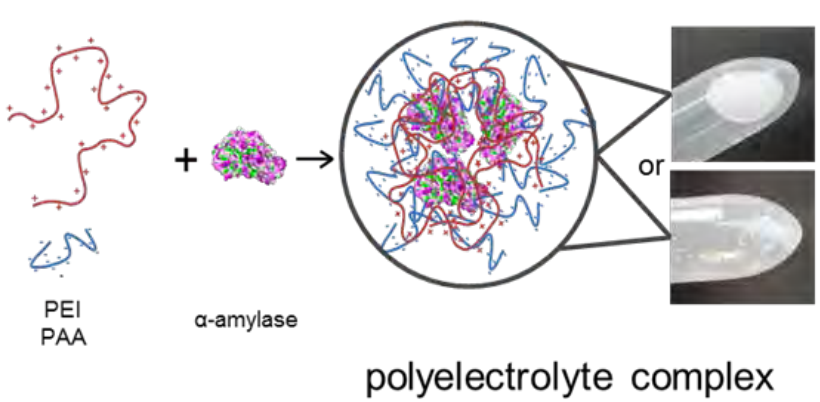
Cooperation with Universities

Liquid Formulation of Enzymes – TU Darmstadt, Prof Andrieu-Brunsen

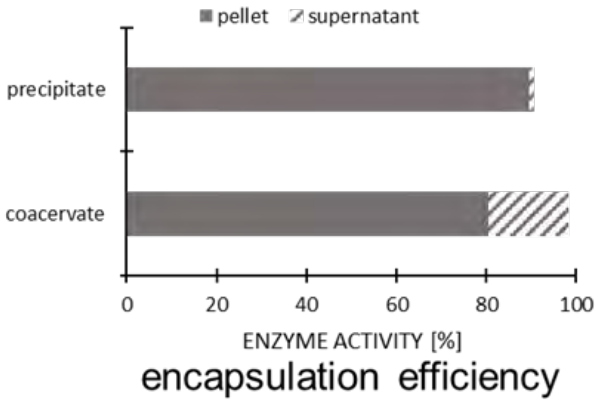
Immobilization of α-amylase in Polyelectrolyte Complexes

Journal of Applied Polymer Science

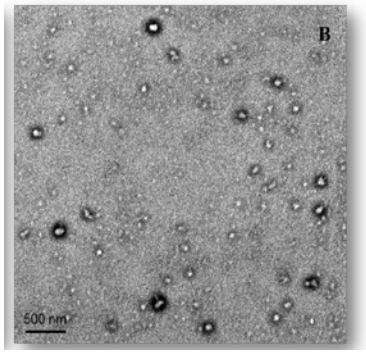
*Sonja Kübelbeck, Jules Mikhael, Sebastian Schoof, Annette Andrieu-Brunsen, Grit Baier**



polyelectrolyte complex

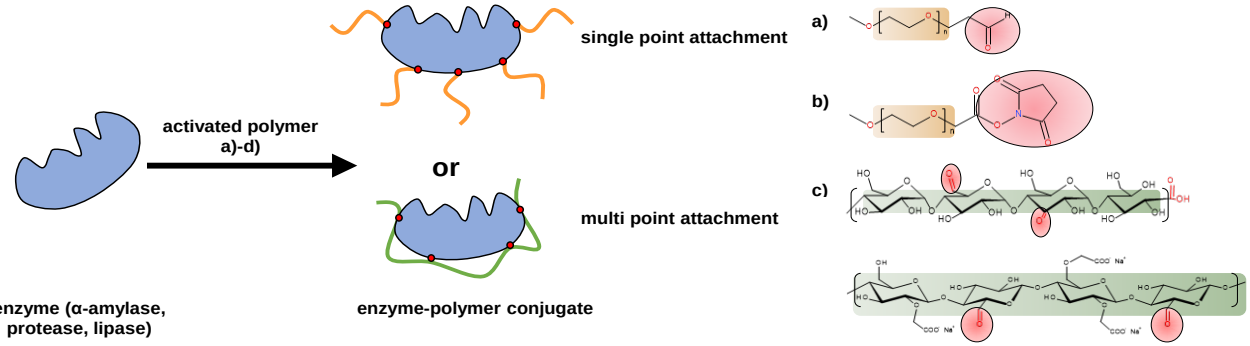


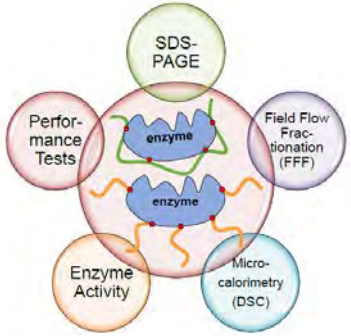
encapsulation efficiency



Enzyme-Polymer Conjugates to enhance enzyme shelf life in a liquid detergent formulation *(Macromolecular Bioscience, 18 (7), 2018)*

Sonja Kübelbeck, Jules Mikhael, Harald Keller, Rupert Konradi, Annette Andrieu-Brunsen, Grit Baier**





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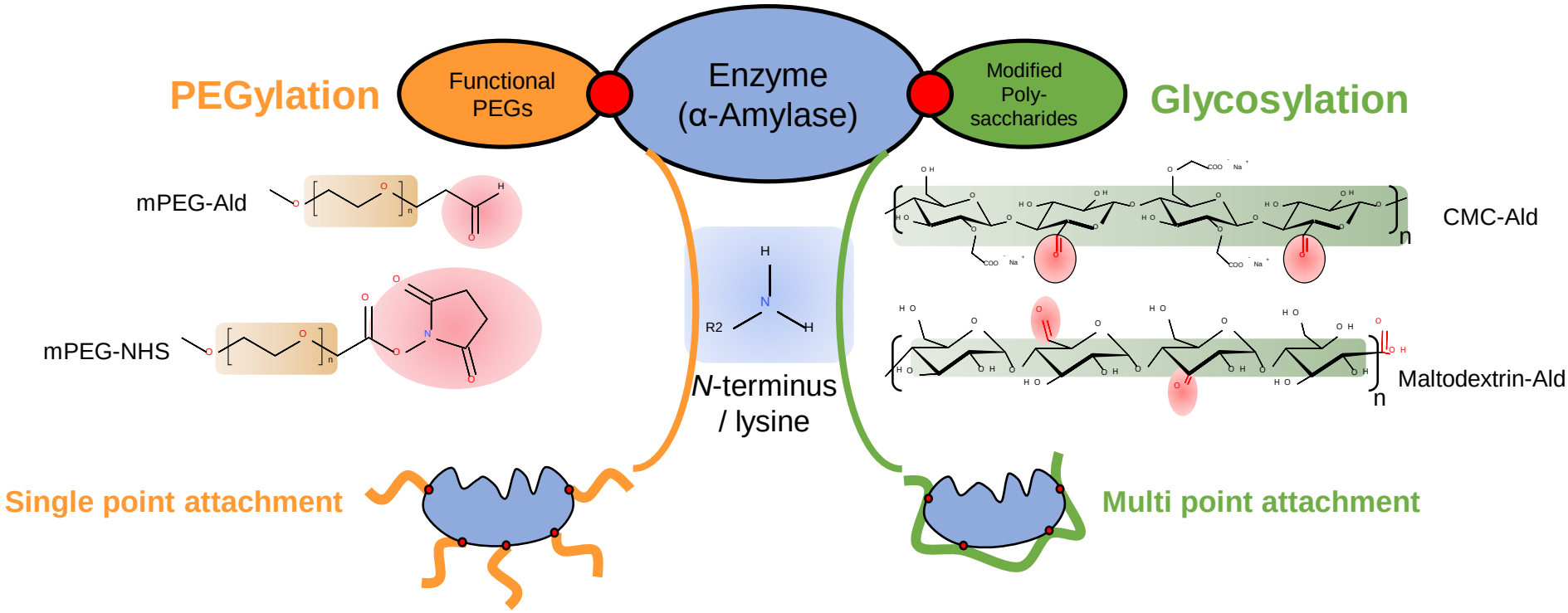
Enzyme-Polymer Conjugates

Liquid Formulation of Enzymes

Enzyme-Polymer Conjugates

= covalent coupling of an enzyme to an inert biocompatible, water-soluble polymer



- ❑ Reductive Amination with aldehyde → secondary amine
- ❑ Esterification with NHS active ester → amide

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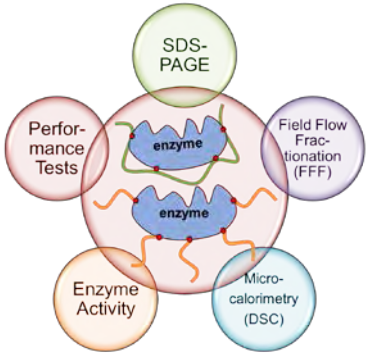
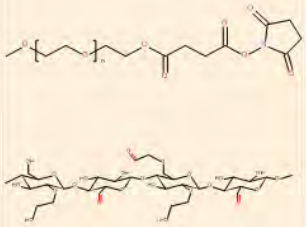
Enzyme-Polymer Conjugates

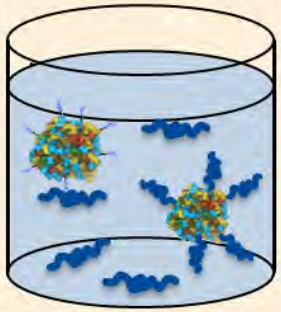
Systematic Approach



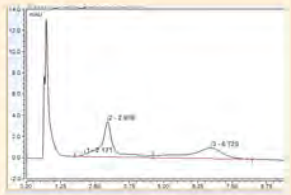
Conjugation



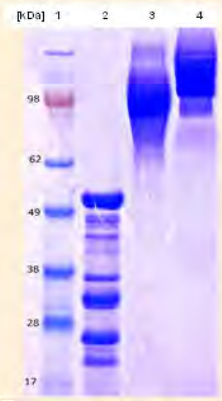
Enzyme + Polymer



Reaction Monitoring



HPLC SDS-PAGE



Purification

Dialysis
yield = 100%

or

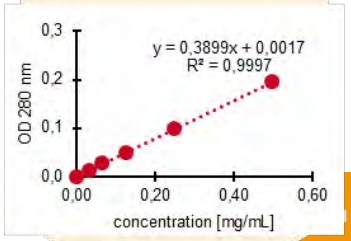
FFF
yield <100%

Concentration + Activity

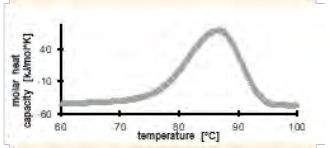
UV Assay

BCA Assay

Enzyme Activity Assays



Characterization, Storage + Performance Tests



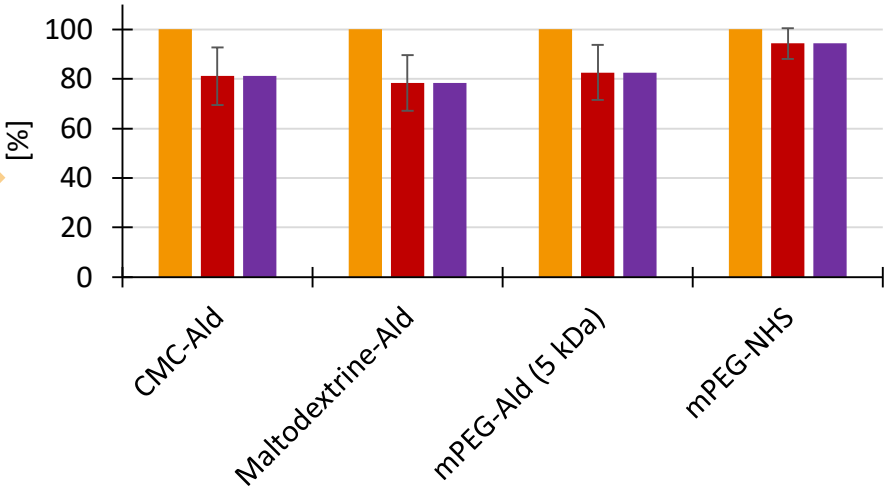
nanoDSC

Storage & Performance Tests

Enzyme-Polymer Conjugates

Results

α-amylase
+
CMC-Ald
Maltodextrin-Ald
mPEG-Ald
mPEG-NHS



Conversion Rate

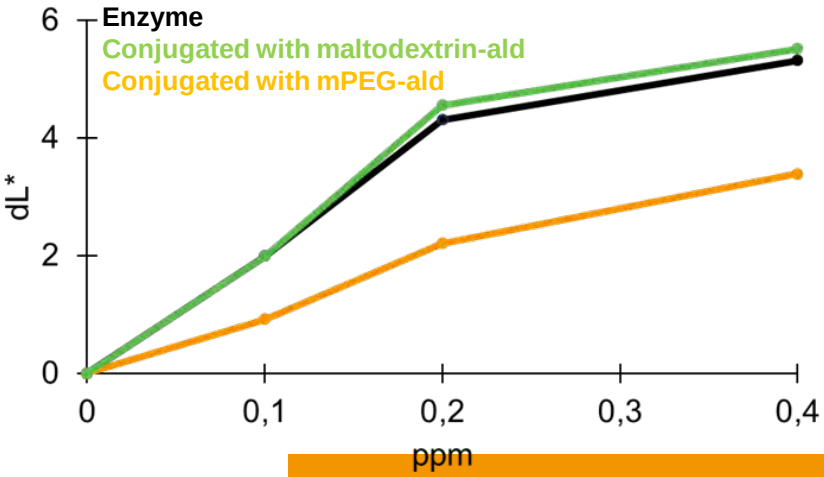
Enzyme Activity

Yield = Conversion Rate x Enzyme Activity

Very efficient conversion rate (100%) with at least 80% enzyme activity & overall yield.

Launder-O-meter

Stain (rice starch)
Detergent Formulation (Persil) @ 40 °C
3 different concentrations [ppm]
Measuring of L* before and after washing
→ brightness



Higher stability of conjugated enzymes within standard detergent formulations & wash performance as good as native amylase

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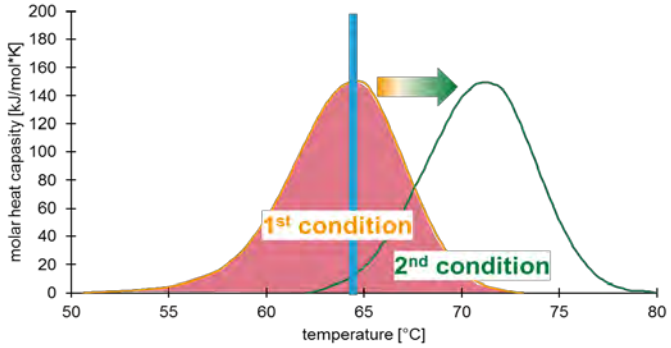
Enzyme-Polymer Conjugates

Results – Thermal Stability

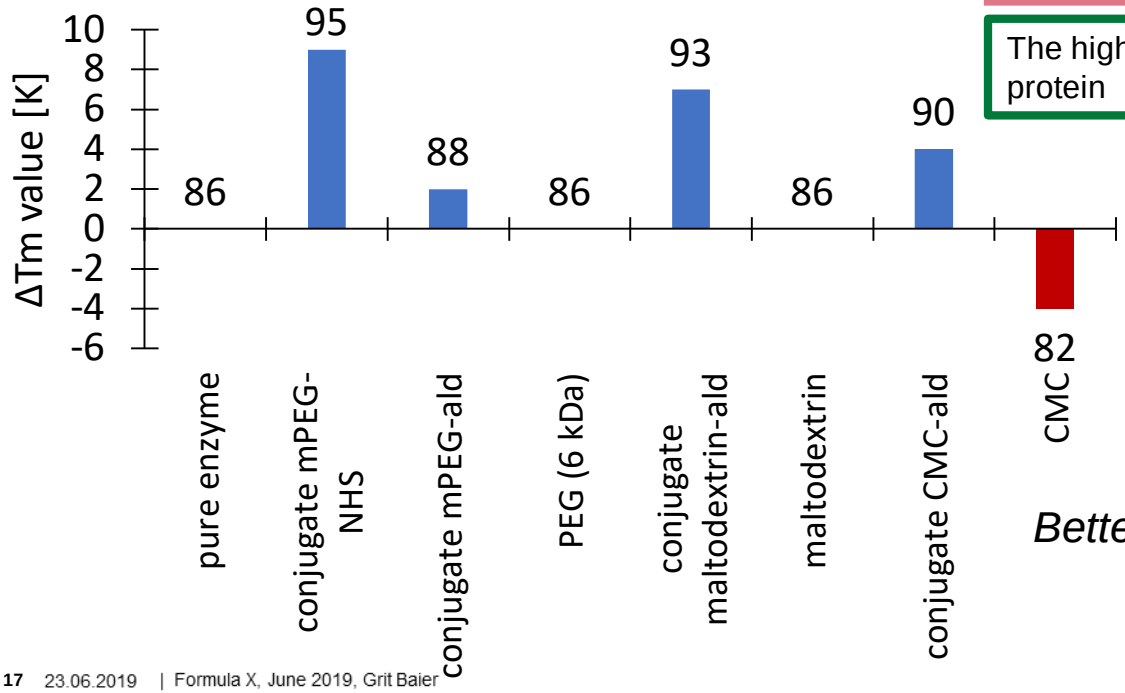
Differential Scanning Calorimetry (nanoDSC)



T_m: 50% of the protein are in their native and 50% are in a denatured state

ΔH_{cal} (area under the curve):
Sum of all enthalpy changes due to protein unfolding

The higher T_m value, the more stable the protein



Better results with conjugated enzymes

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Summary

- Identification of the best enzyme formulation concept to improve stability
- Understanding the factors/ingredients which contribute to enzyme stabilization
- Screening of new materials, procedures and smooth technologies
- Development of analytical methods to check physico-chemical, colloidal & biological properties in formulated systems
- Choosing a proper formulation/encapsulation technology helps to increase enzyme survivability
- External partners help us to be more successful

Thank you very much for your attention !

We create chemistry