

- ChitoBioEngineering -

**“Metabolic and Enzyme Engineering
for the Biotechnological Production
of Partially Acetylated Chitosans”**

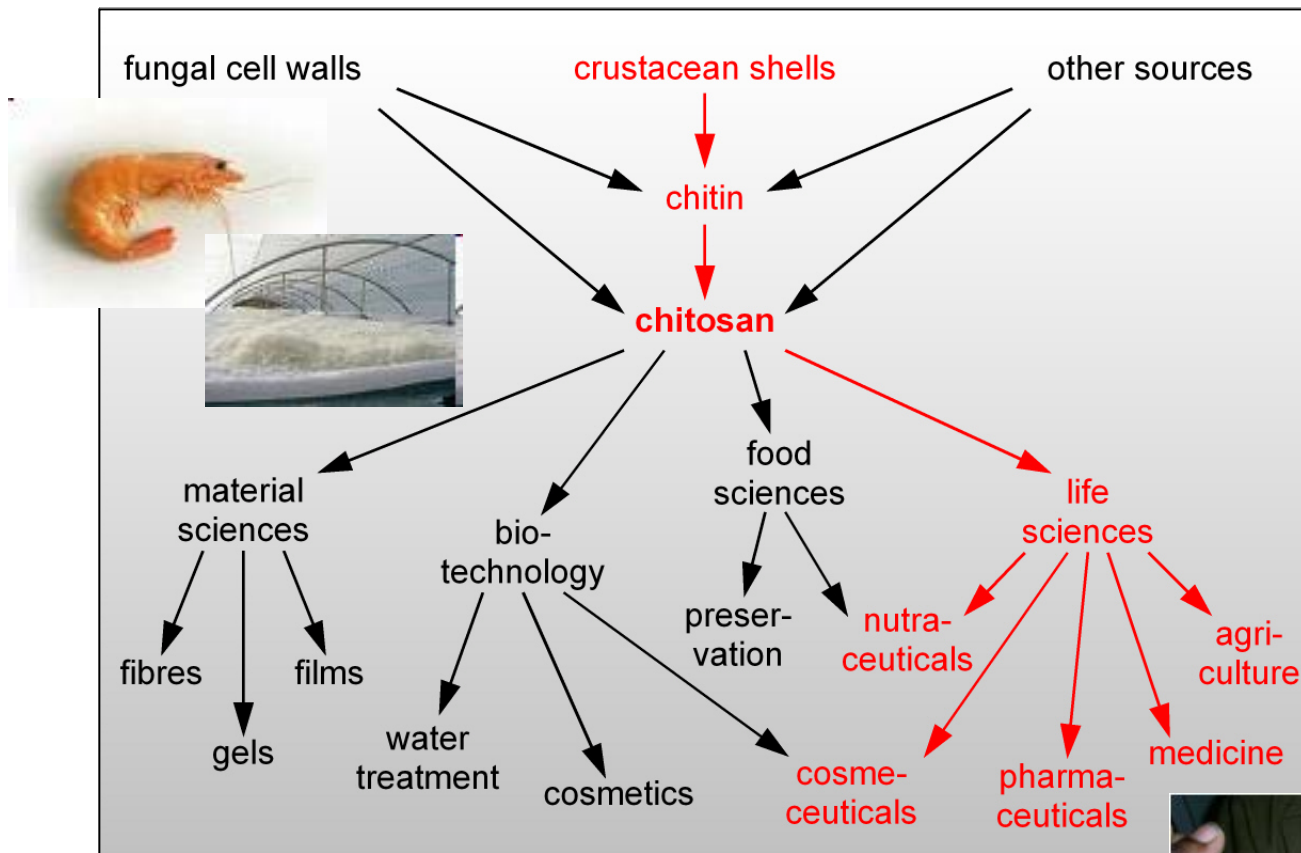
Pre-Final Report



Bruno Moerschbacher . moersch@uni-muenster.de
Westfälische Wilhelms-Universität . Münster . Germany



partially acetylated chitosans



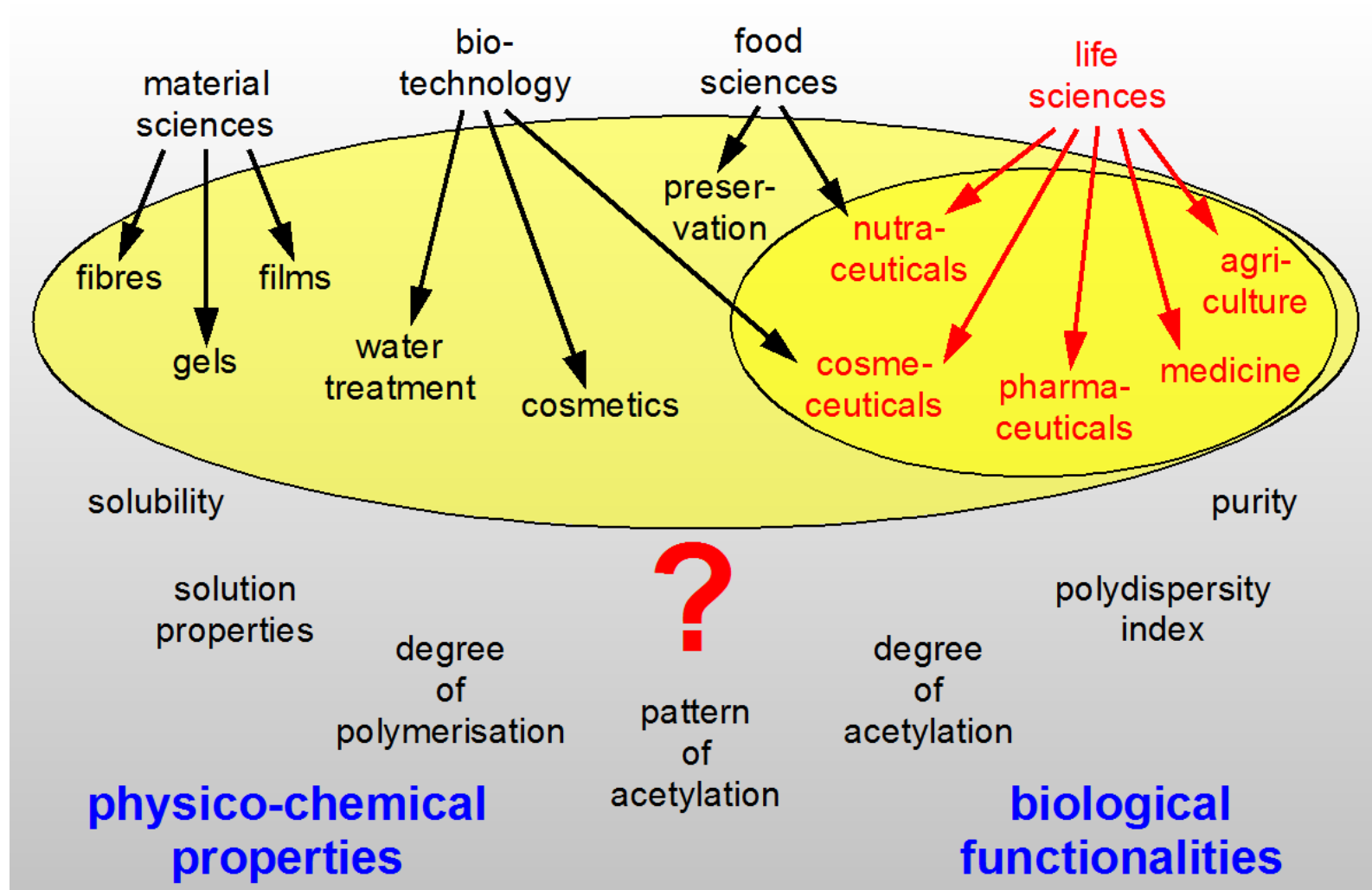
plant protection



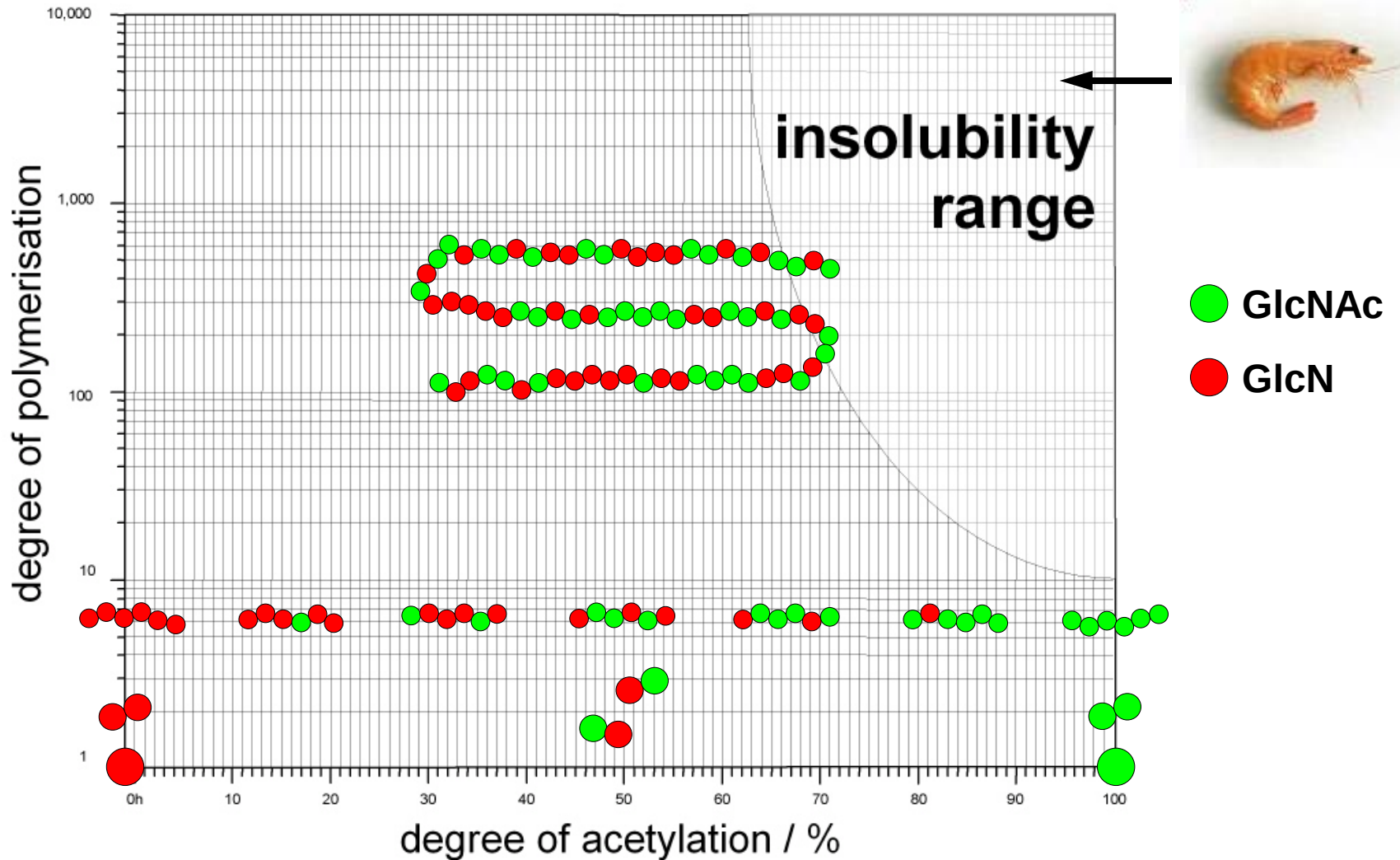
scar-free
wound healing



structure-function relationships of chitosans



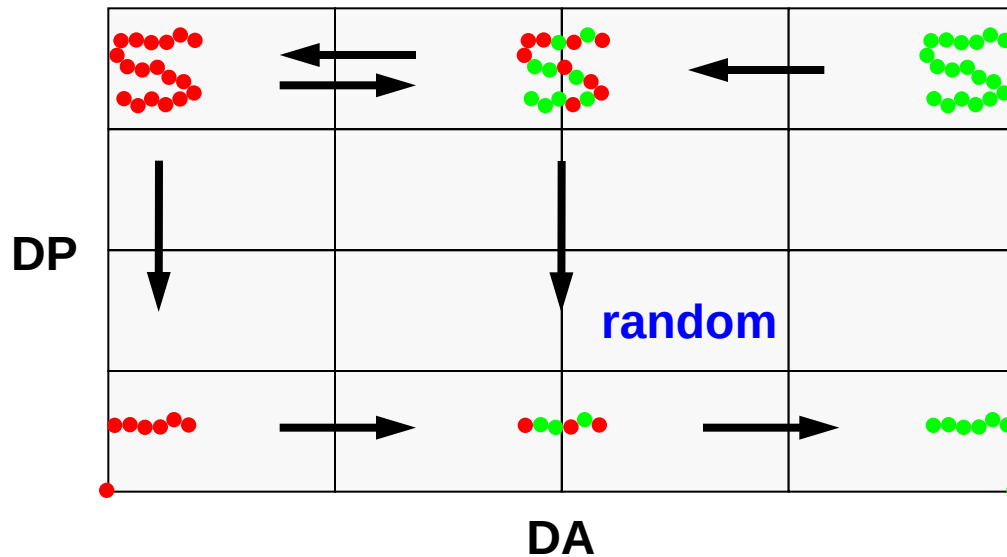
chitosan matrix



chemical generation of chitosans

● GlcNAc

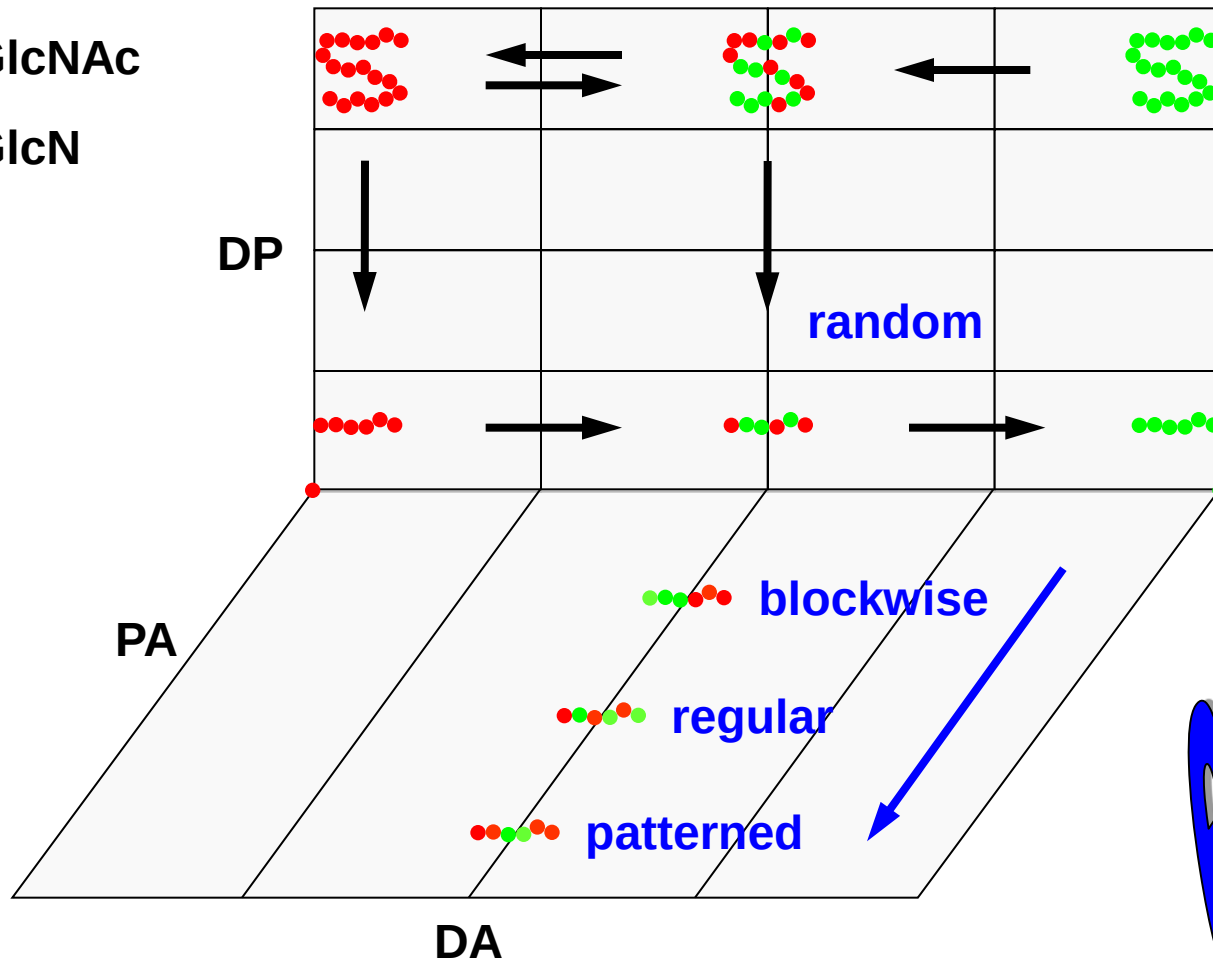
● GlcN



enzymatic modification of chitosans

● GlcNAc

● GlcN



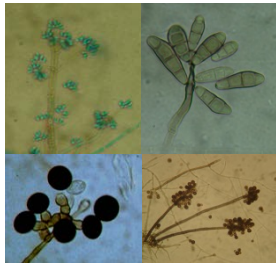
ENZYMES



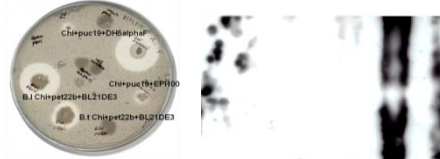
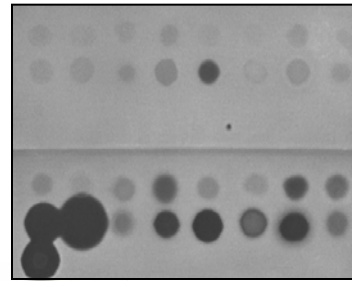
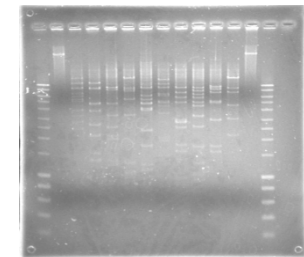
knowledge-based and un-biased discovery programs



endophytic fungi
from tropical trees

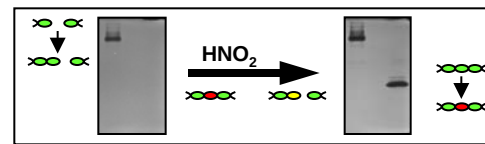


metagenomics
from soil samples

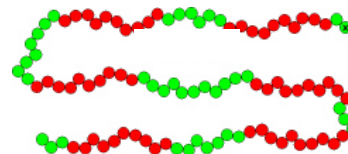


screening assays

**novel chitosan
modifying enzymes**



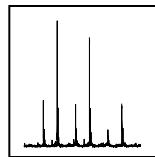
chitosans
with non-random PA



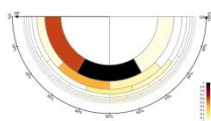
enzymatic
fingerprinting



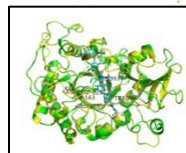
mass-spectrometric
sequencing



bioinformatic
modeling



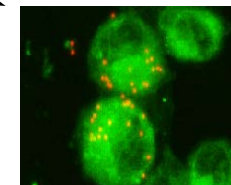
optimisation by
protein engineering



plant disease
protection



scar-free
wound healing



nanoparticles for
drug delivery

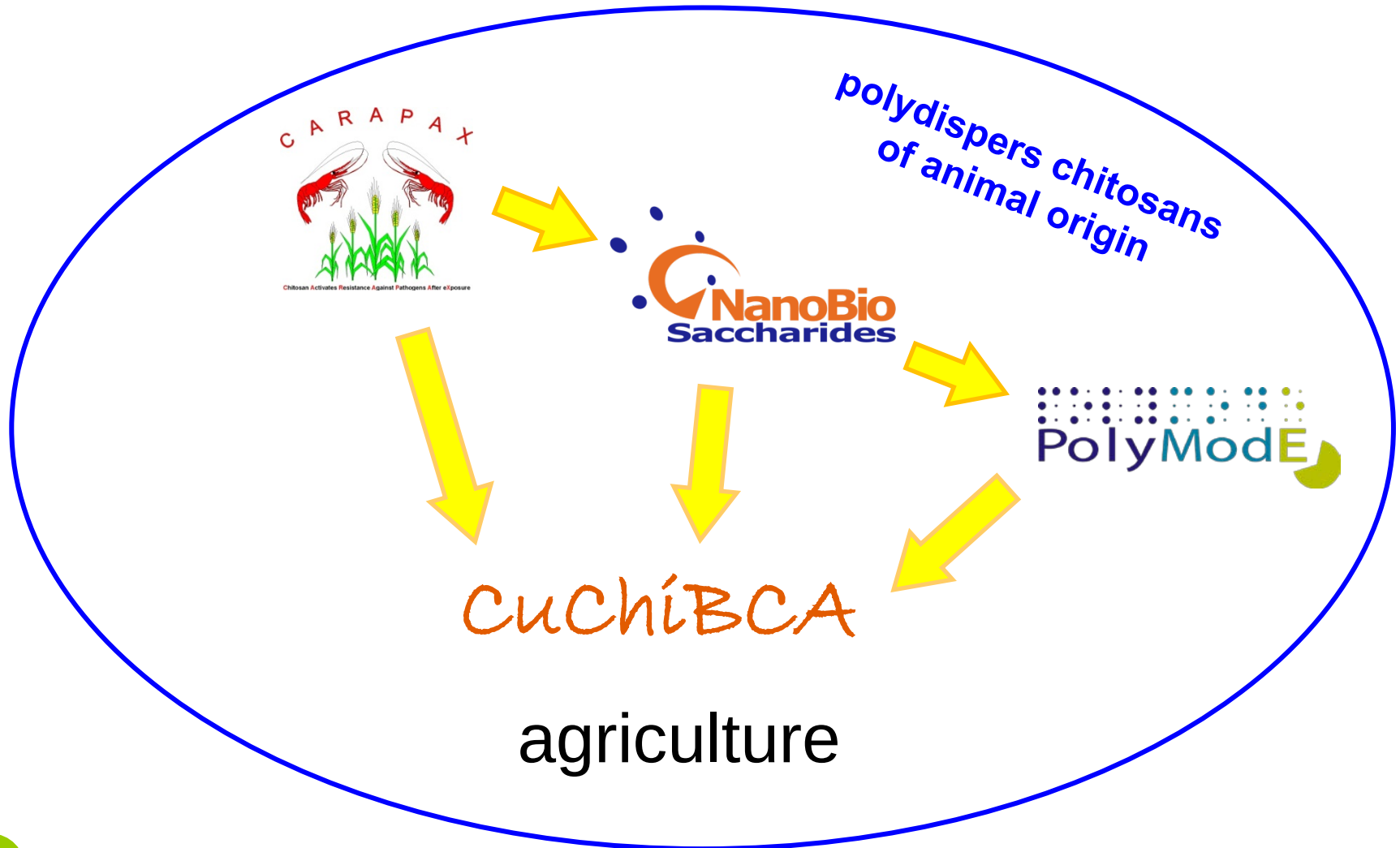
**seed coating
(CaraSeed)
&
foliar spray
(CaraPlant)**



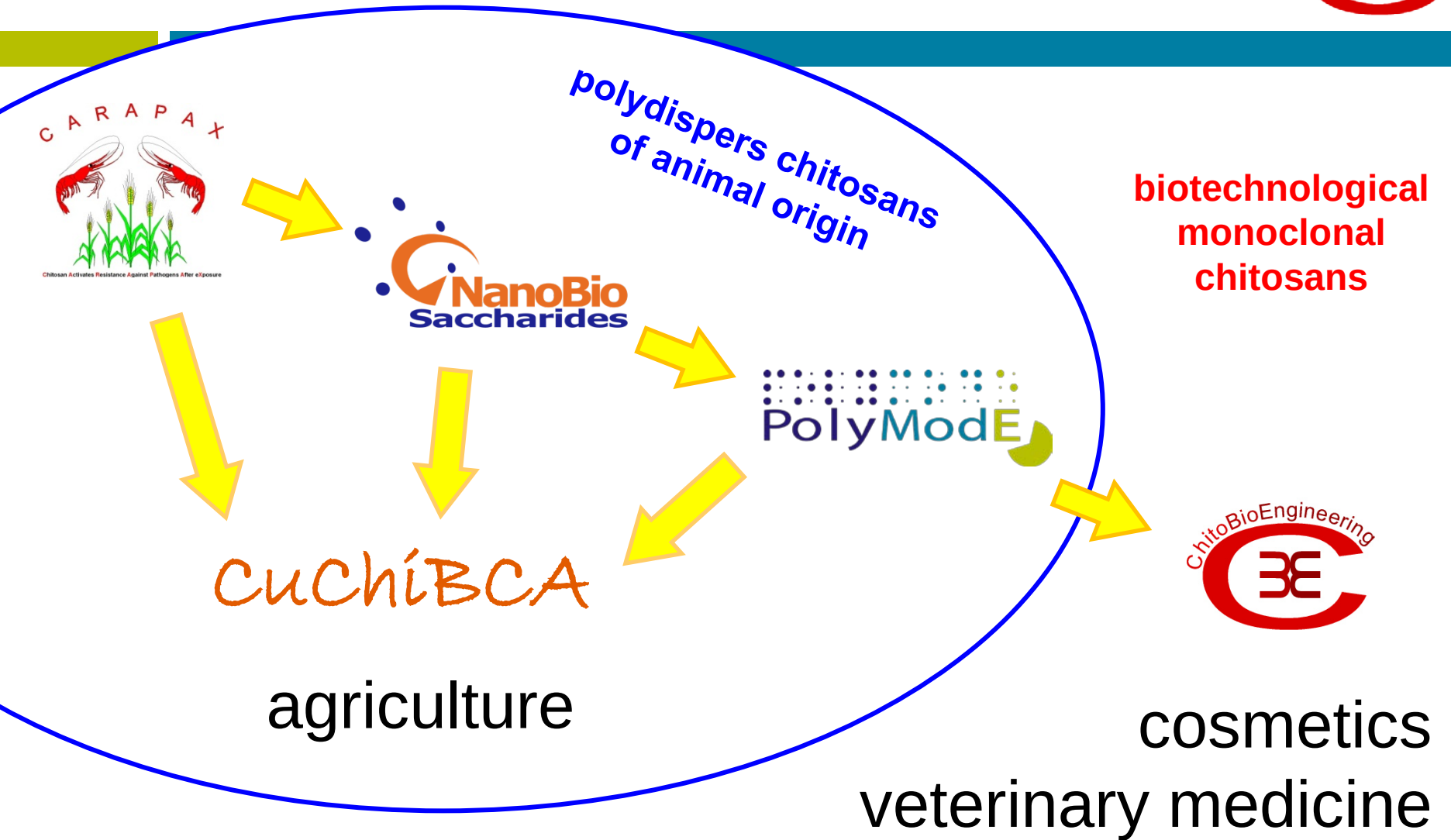
**disease
resistance
&
stress
tolerance
&
yield
increase**



next generation chitosans



next generation chitosans



project aim

to biotechnologically produce partially acetylated chitosan oligomers with fully defined architecture,

- i.e.
- known degree of polymerisation (DP)
 - known degree of acetylation (DA)
 - known pattern of acetylation (PA)

for detailed analysis of structure-function relationships and, hence, reliable bio-activities and applications

general project approach

by expressing and optimising known and novel chitin synthase and chitin deacetylase genes in suitable host strains, metabolically engineered for maximum yield



role of academic partners



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genetic engineering of suitable host strains over-expressing known and novel chitin synthases and chitin deacetylases



PERSONA CIENCIA EMPRESA
Universitat Ramon Llull

protein engineering of chitin synthases and chitin deacetylases to produce fully defined chitosan oligomers



metabolic engineering of host strains for maximum yield of fully defined chitosan oligomers



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role of industrial partners

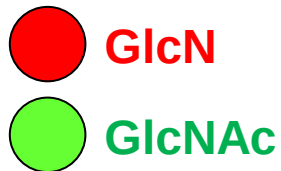
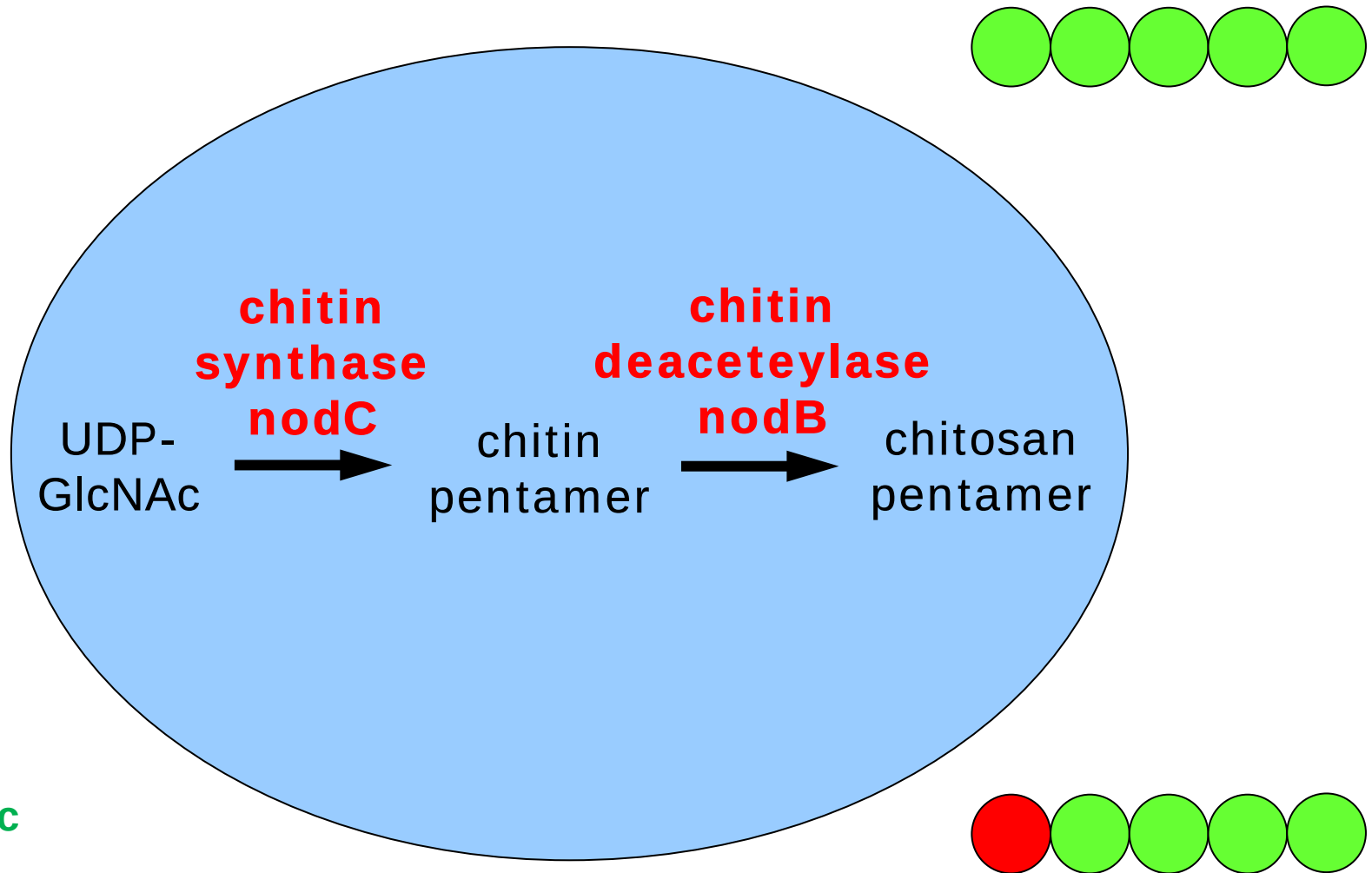


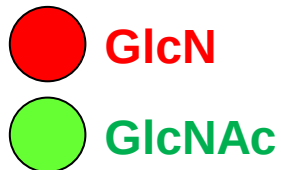
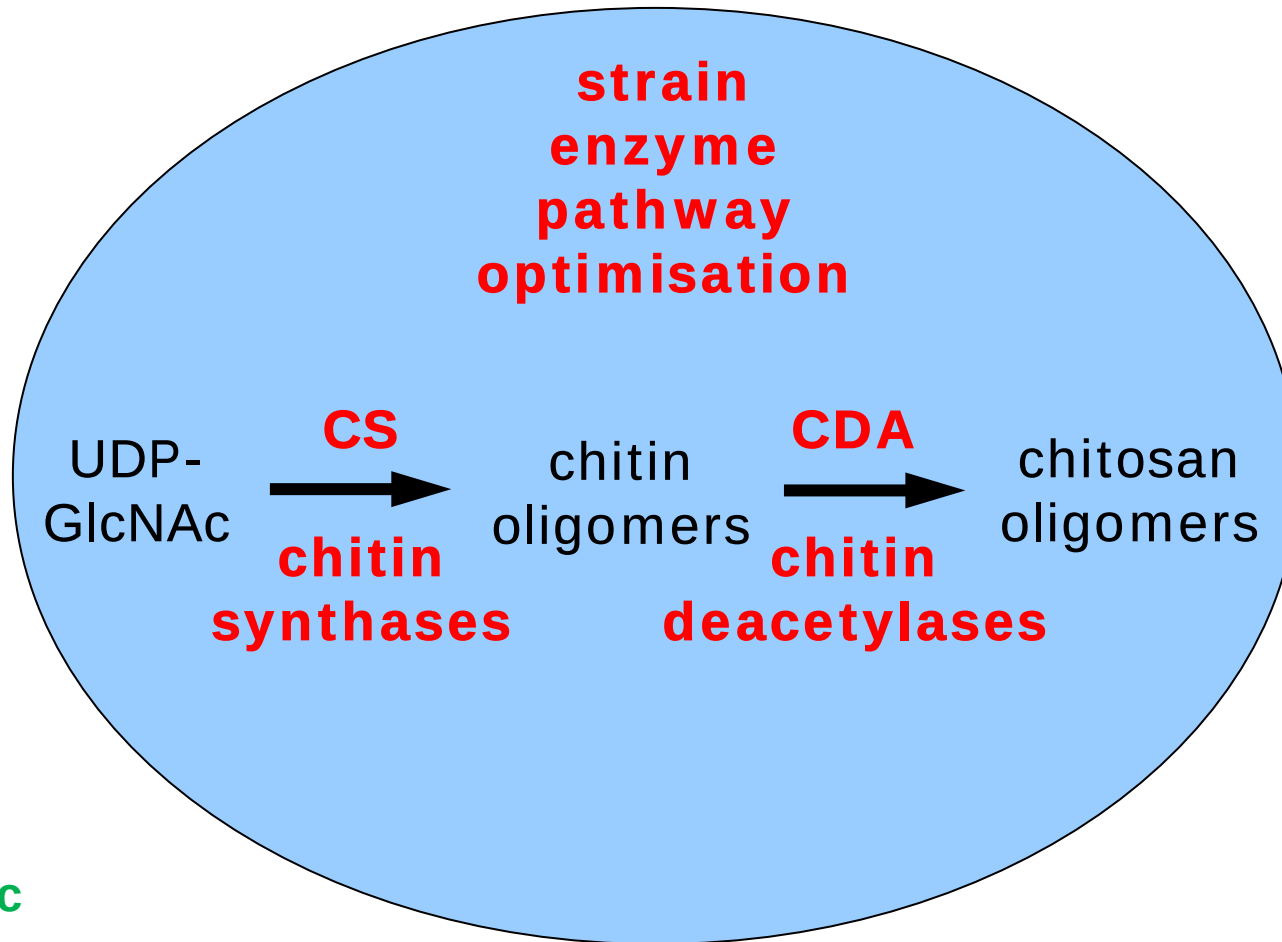
technology transfer and **up-scaling**
of fermentation from the lab scale
to the pilot scale

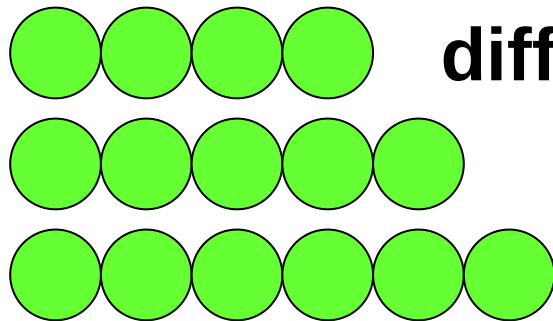


technology transfer and co-ordination of
trial applications with partners in the
cosmetics & veterinary medicine industry

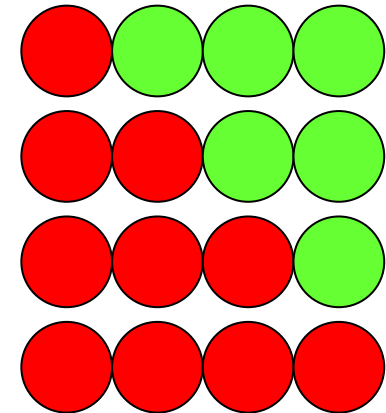








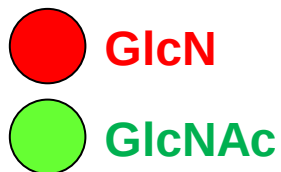
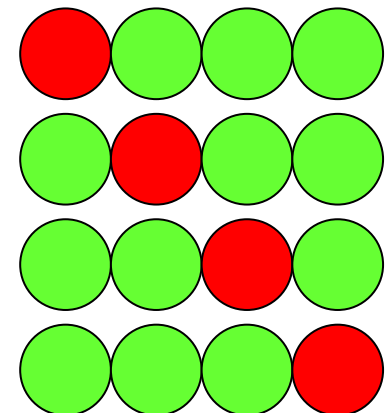
different DPs



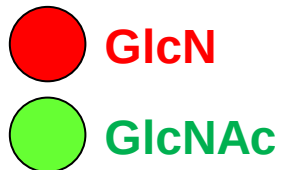
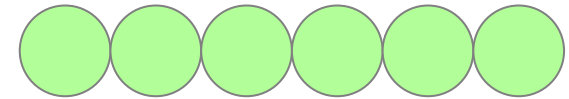
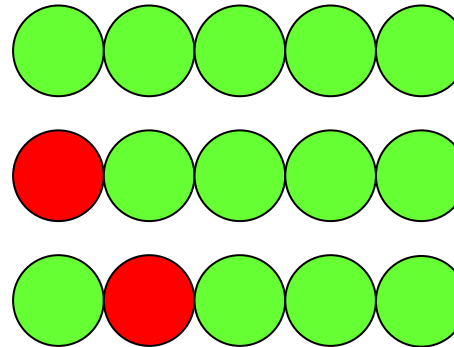
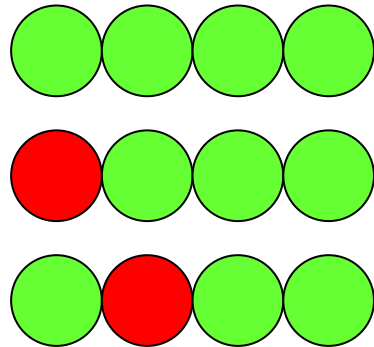
different DAs



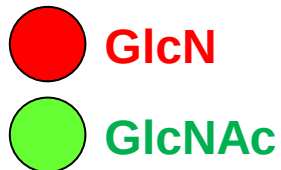
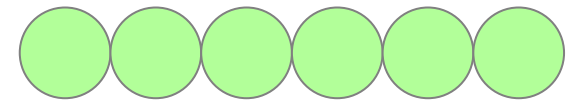
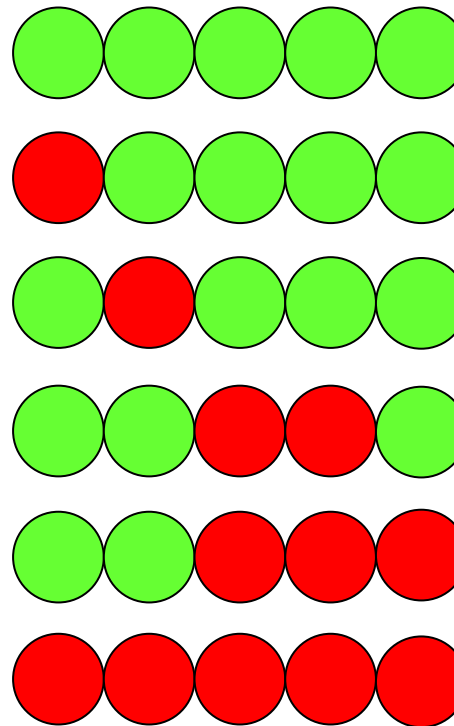
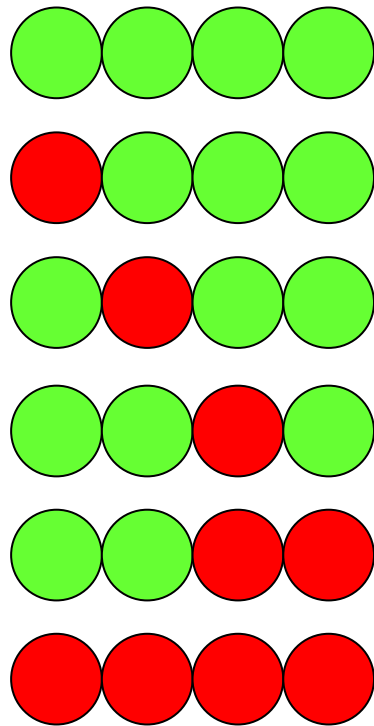
different PAs



targets reached at mid-term (in vivo)



targets reached at mid-term (in vivo & in vitro)

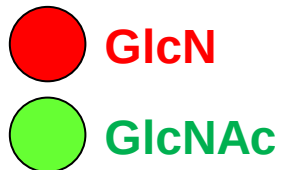
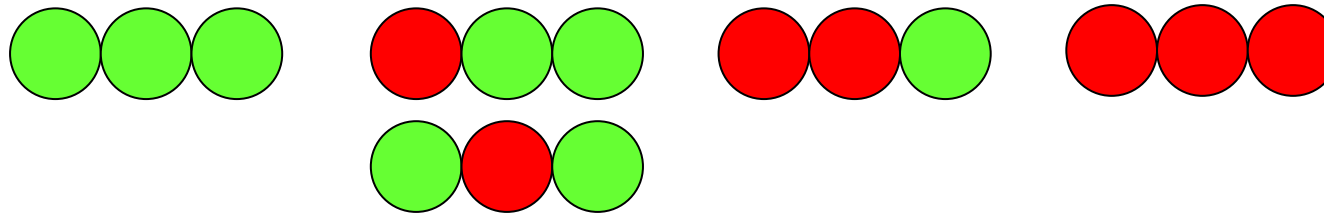


targets reached pre-final (in vivo & in vitro)

dimers: 4 of 4

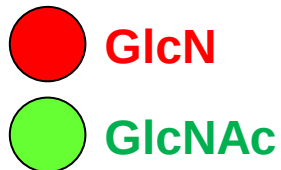
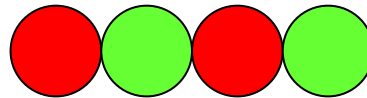
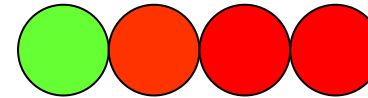
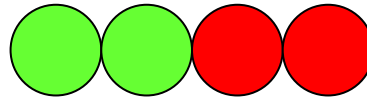
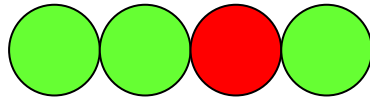
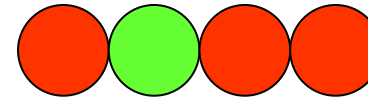
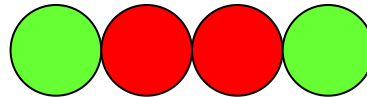
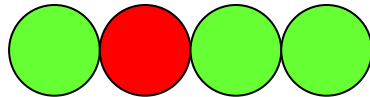
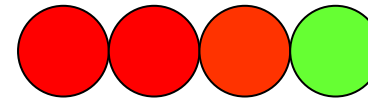
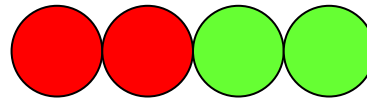
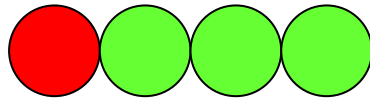
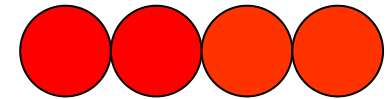
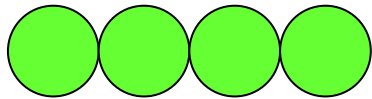


trimers: 5 of 8



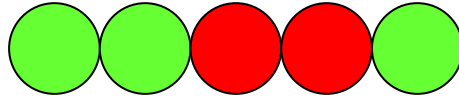
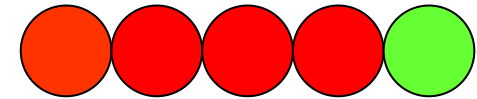
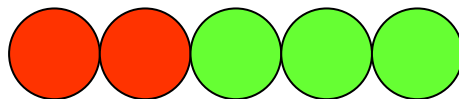
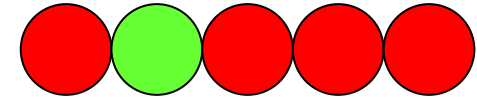
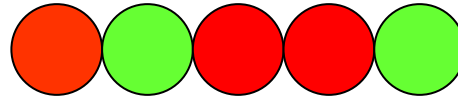
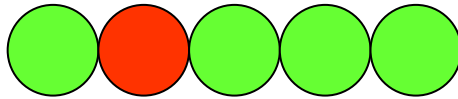
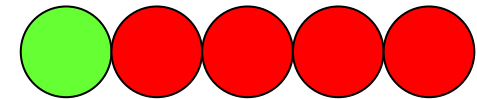
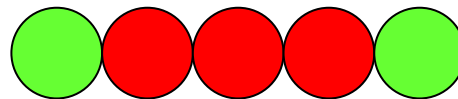
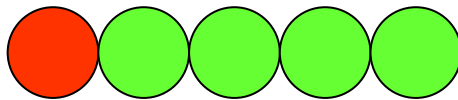
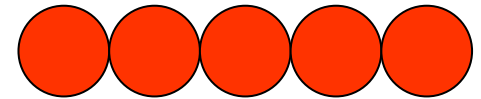
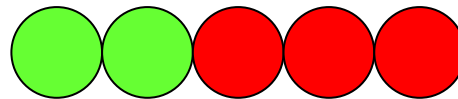
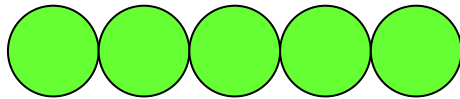
targets reached pre-final (in vivo & in vitro)

tetramers: 12 of 16



targets reached pre-final (in vivo & in vitro)

pentamers



GlcN

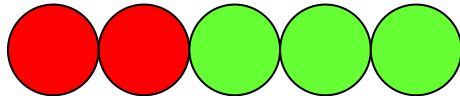
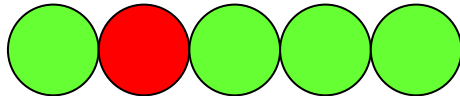
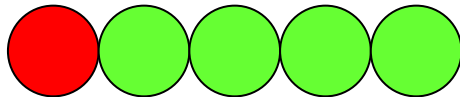
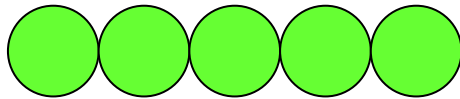


GlcNAc



yields reached pre-final (in vivo & in vitro)

pentamers



GlcN



GlcNAc

100 L fermenter

ca. 1 kg

ca. 99 % pure



Megazyme >ca. 95 % pure

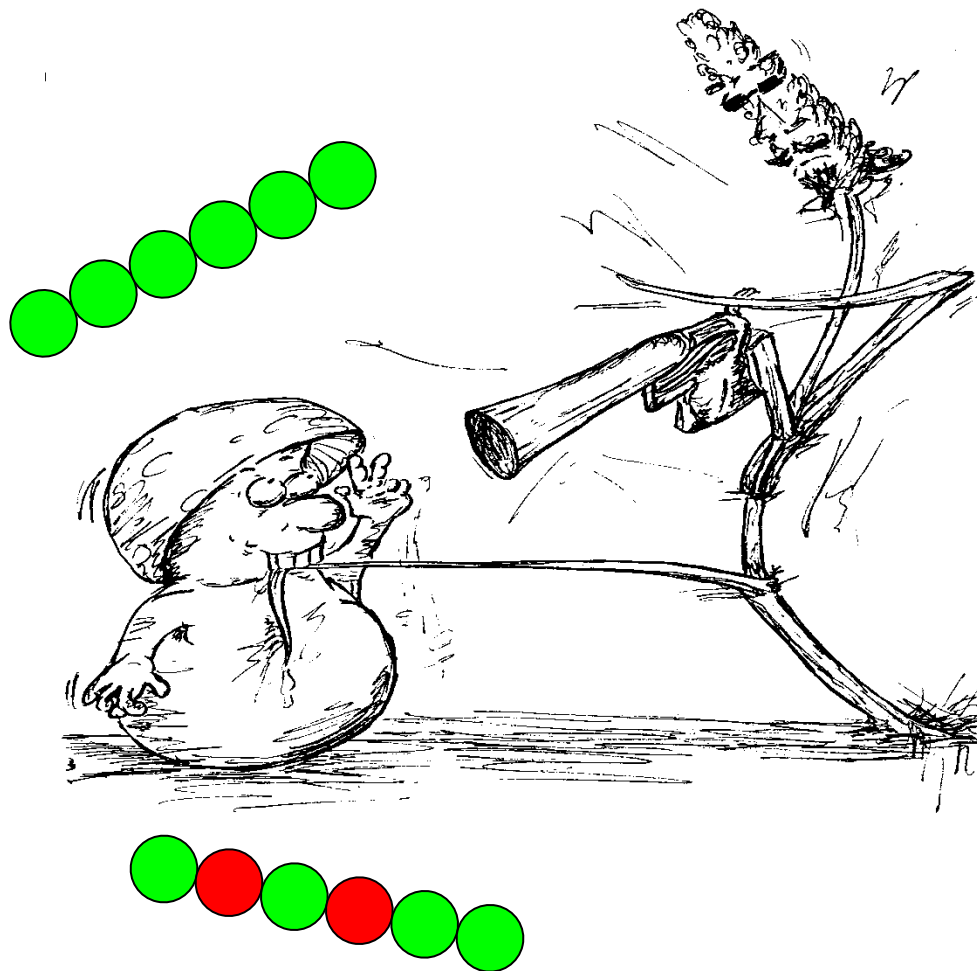
20 mg = 200 €

in vitro deacetylation

ca. 50 mg each

ca. 99 % pure



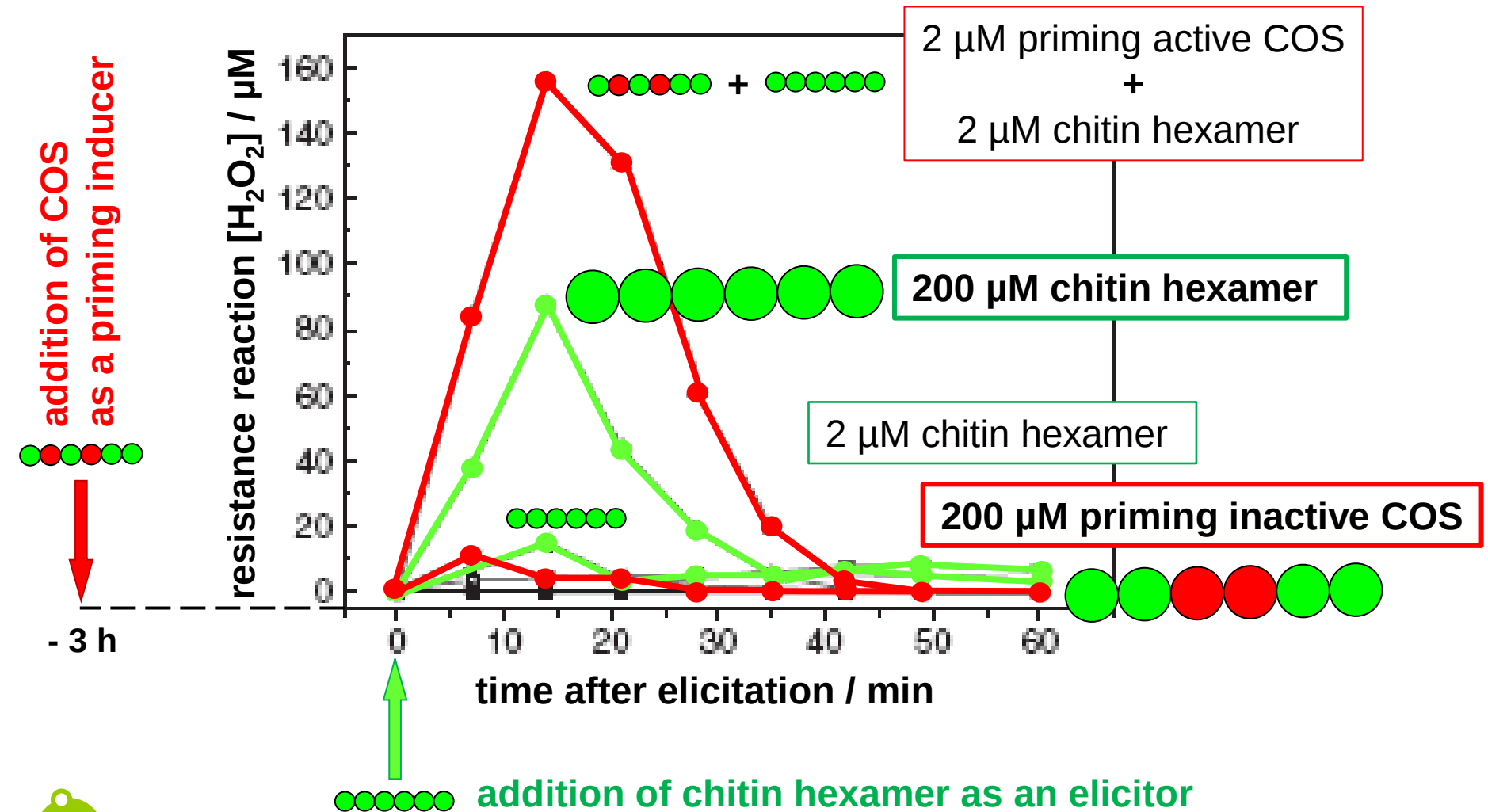


„pathogen !“
(= „stopp all activities,
and defend yourself“)

„pathogen ?“
(= „keep going, but
prepare for defense“)



bio-assays for cellular priming

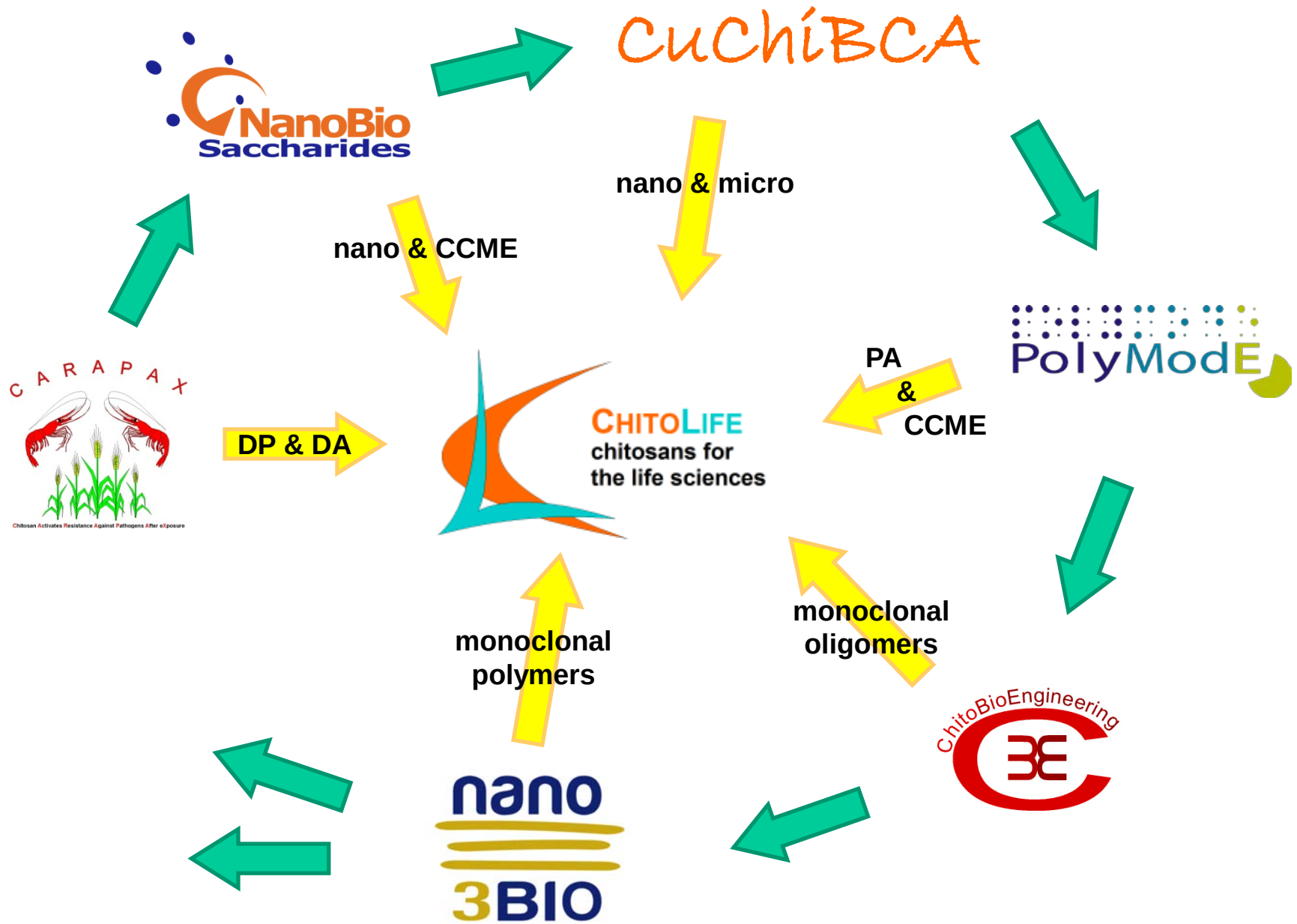




tasks for the cost-neutral extension

- Belgium** down-stream processing of partially acetylated oligomers
- Spain** more oligomer diversity from deacetylase chimeras
- Germany** more bio-assays and application tests





thank you



Bundesministerium
für Bildung
und Forschung



agentschap voor Innovatie
door Wetenschap en Technologie

University Münster

Prof Dr. Bruno Moerschbacher
Dr. Stephan Kolkenbrock
Shoa Naqvi
Stefanie Hamer
Stefan Cord-Landwehr

Heppe Medical Chitosan

Katja Richter
Torsten Richter



University Ghent

Prof Dr. Wim Soetaert
Prof. Dr. Marjan De Mey
Dr. Jo Maertens
Dr. Joeri Beauprez
Pieter Coussement
Wouter Van Bellegem

BioBase Europe Pilot Plant

Prof. Dr. Wim Soetaert
Dr. Hendrik Waegeman
Kristof Callemin
Diedrik Snoeck
Georges Moureau
An Vermeulen
Jeroen De Smet



University Ramon Llull Barcelona

Prof. Dr. Antoni Planas
Dr. Eduardo Andrés
Dr. Xevi Biarnés
Xavi Pérez

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Bruno Moerschbacher . WWU-IBBP
Westfälische Wilhelms-Universität . Münster . Germany

