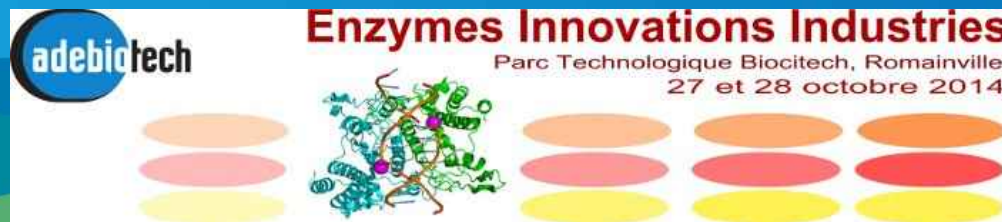# Novel enzyme development:

Screening & design

Noël van Peij - Principal Scientist Strain Development & Screening

DSM Biotechnology Center, Delft, the Netherlands

                       HEALTH · NUTRITION · MATERIALS

Mission

Our purpose is to create brighter lives for people today and generations to come

We connect our unique competences in Life Sciences and Materials Sciences to create solutions that nourish, protect and improve performance

Dow Jones Sustainability Indexes
Member 2010/11

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Building on an impressive history

Hoffman La Roche's
Vitamins (1930s)

Chemical synthesis & biotechnology

Life Sciences

Gist-Brocades (1869)

Biotechnology

Materials Sciences

DSM (1902)

Energy, chemistry & polymer technology

Vitamins
Omega's
Carotenoids
Premixes for food & feed
Enzymes
Minerals
Cultures & Yeasts
Nutraceuticals
Pharmaceuticals
Cellulosic bioethanol
Biomedical materials
Bio-plastics
High Performance Plastics
Polyamides and precursors
Resins for coatings and composites
Functional Materials
Solar – advanced surfaces

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DSM – key activity areas

Health
Advanced, cost-effective health and medical innovations, and healthier food and beverages, to meet the needs of a growing and ageing global population

Nutrition
World's leading producer of vitamins and nutritional ingredients meeting the growing need for more nutritious and more sustainable food and animal feed

Materials
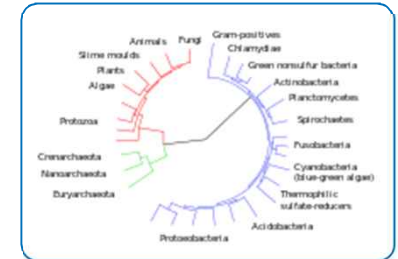Enabling lighter, stronger, more advanced and more sustainable performance materials

DSM's 24,500 employees deliver annual net sales of about € 10 billion

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Industrial enzyme/strain development @ DSM

Biodiversity Platform
Enabling the identification of novel enzymes and pathways (*in-vivo*, *in-vitro* & *in-silico*), and HT (in-application) screening

Systems Biology Platform
Enabling the modeling and design of DNA, proteins, pathways (= metabolites) being part of the cell factory in connection with experimentation
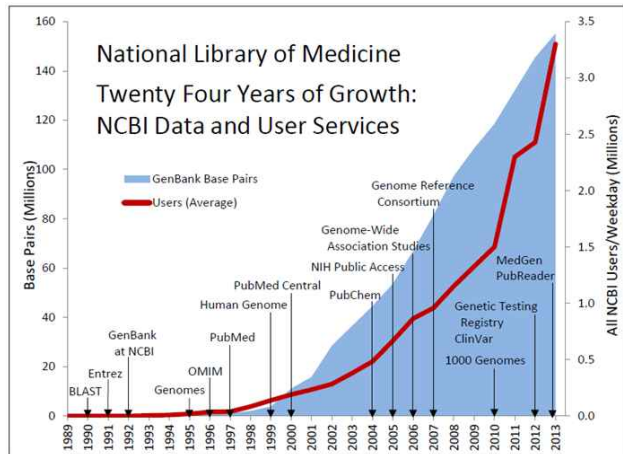

Synthetic Biology Platform
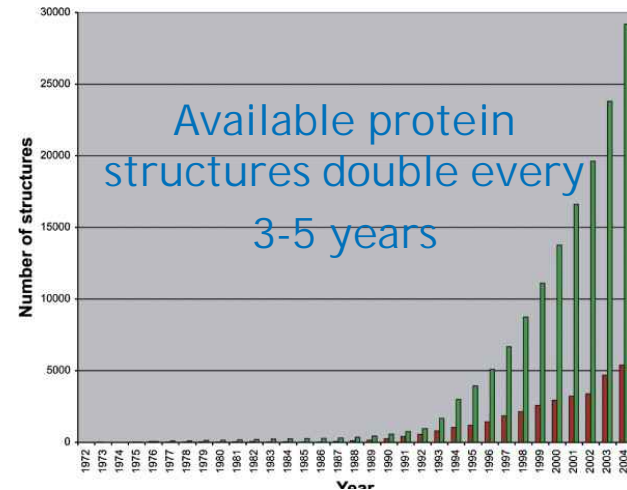Enabling the rapid engineering & characterization of microbes, yeast, fungi and algae

… for the production of enzymes, metabolites and biomass

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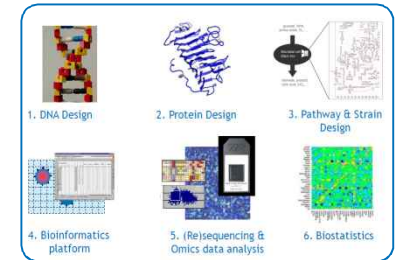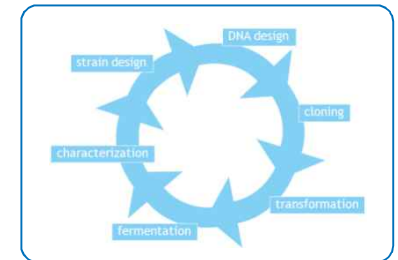
Screening and discovery: *Trends*

Unlimited sequence availability

Available protein structures double every 3-5 years

Reading and writing DNA will become a commodity
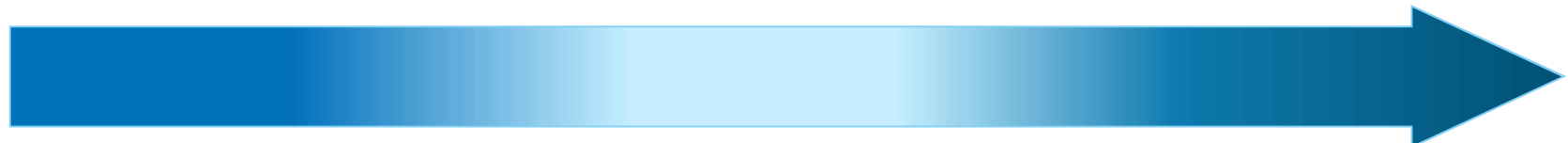
Need for standardization, parallelization & automation in strain engineering

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A brief history of screening & protein engineering

1970	1980	1990	2000	2010	2020

Site-directed mutagenesis

Cassette/sat. mutagenesis

Error-prone PCR

DNA shuffling

smart library design

computational design?

?

Key driver:
- Activity-based screening

Key driver:
- Sequence-based screening
- HT screening robotics
- HT DNA sequencing

Key drivers:
- Advanced bioinformatics & modeling
- Progressively cheaper DNA synthesis

Importance of structure information:

low   high

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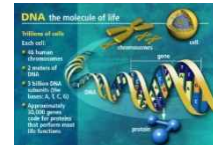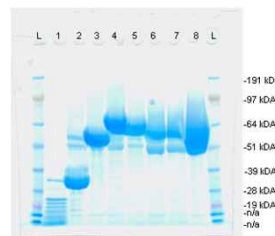
Enzyme discovery screening tools

- Strain with GFP reporter for secretion stress

- Genomic library 20.000 clones

- High GFP strains selected by FACS

| Reporter strain | Genomic libraries | Mixed population | FACS Cell sorting |
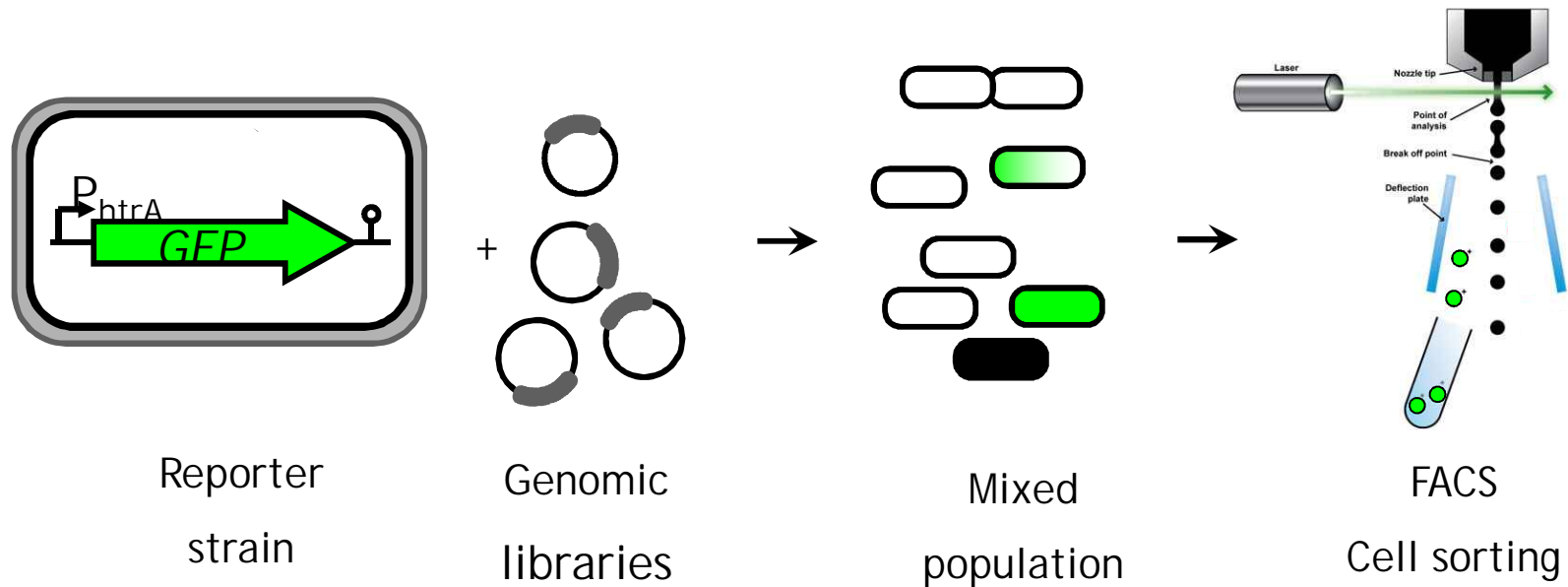
Trip (2011) Microb. Biotechnol. 4(5):673-682.

Protein engineering
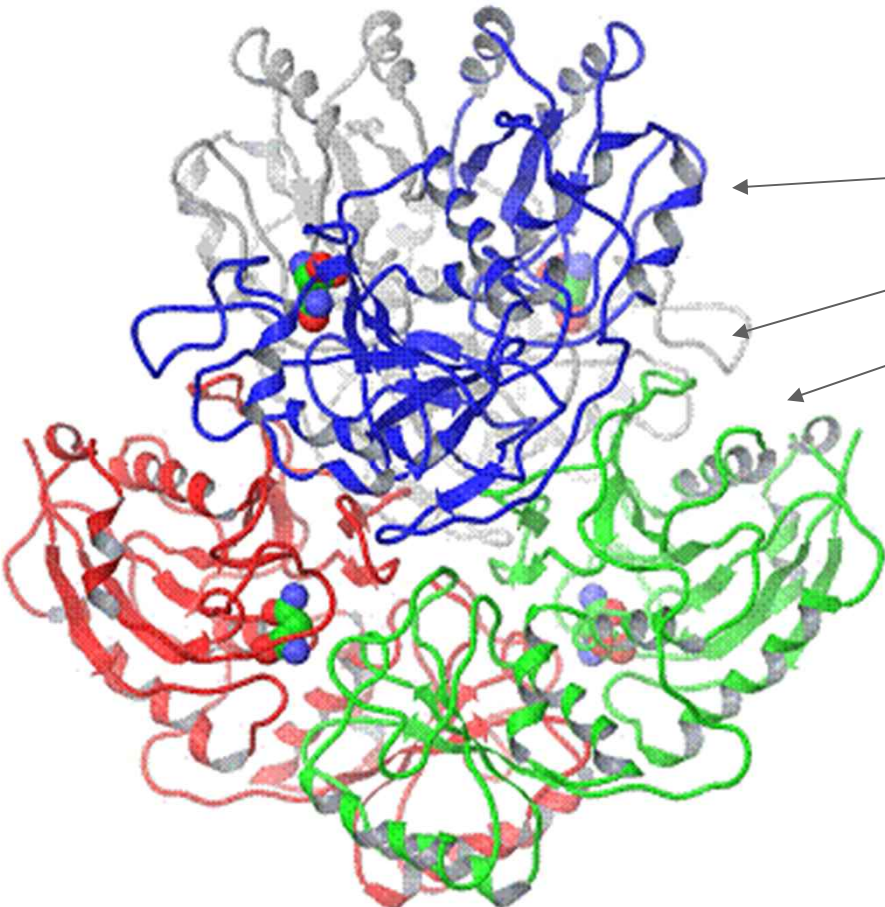
Baking enzyme example

Change Amino acid

Changes performance
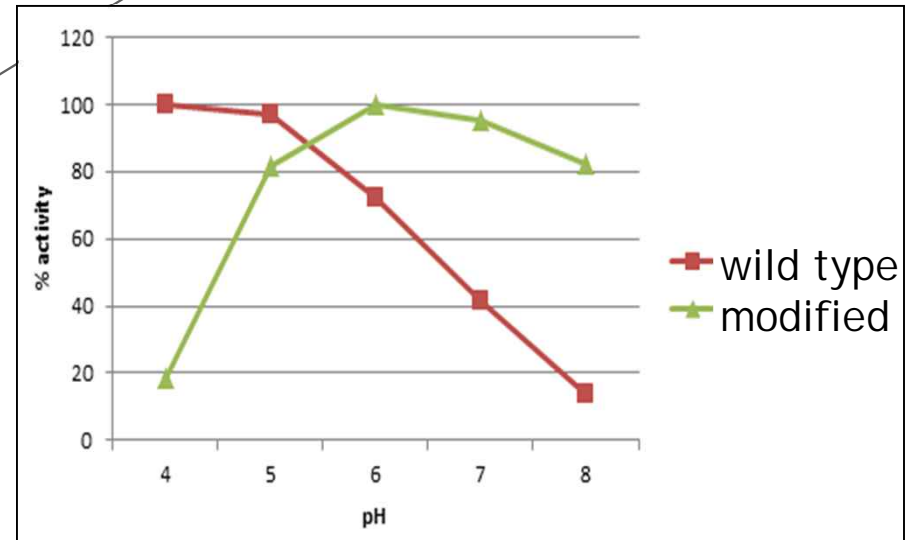
Industrial strain development @ DSM

Biodiversity Platform
Enabling the identification of novel enzymes and pathways (*in-vivo*, *in-vitro* & *in-silico*), and HT (in-application) screening

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Enabling the modeling and design of DNA, proteins, pathways (= metabolites) being part of the cell factory in connection with experimentation

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Enabling the rapid engineering & characterization of microbes, yeast, fungi and algae

… for the production of enzymes, metabolites and biomass

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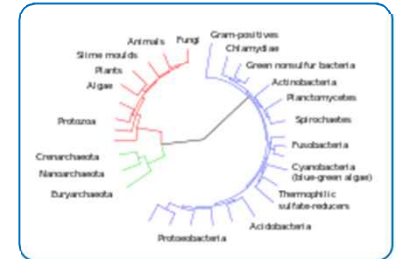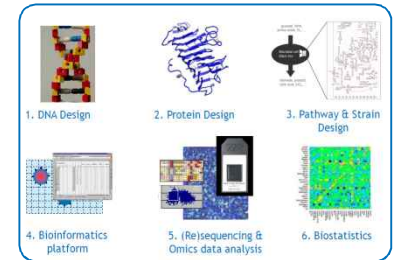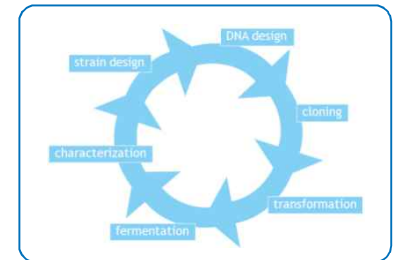
Design of cell factory components

DNA → mRNA → Protein

Cell Factory

Product

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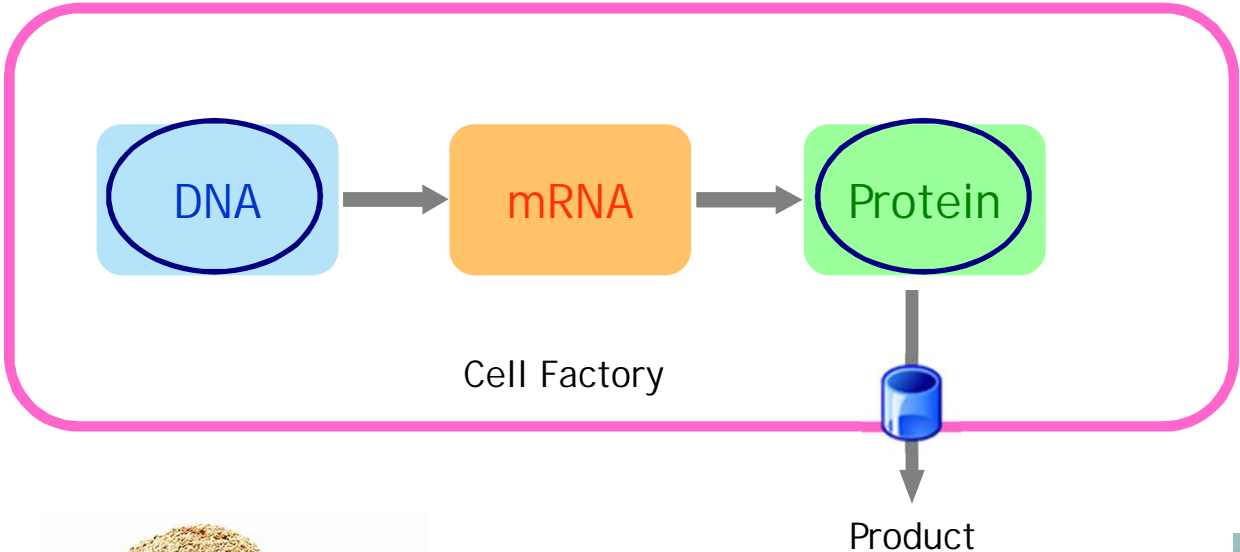
DNA sequence design to increase protein production

Green: Higher than expected based on codon statistics
Red: Lower than expected based on codon statistics
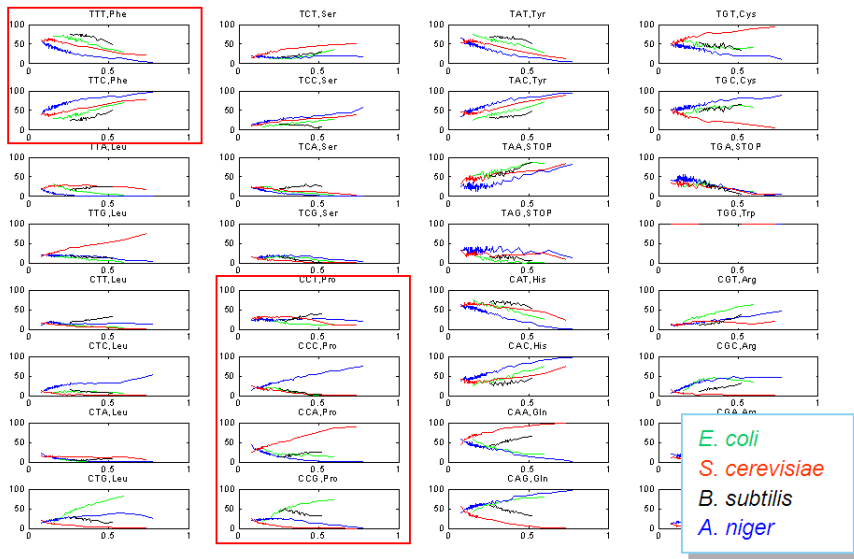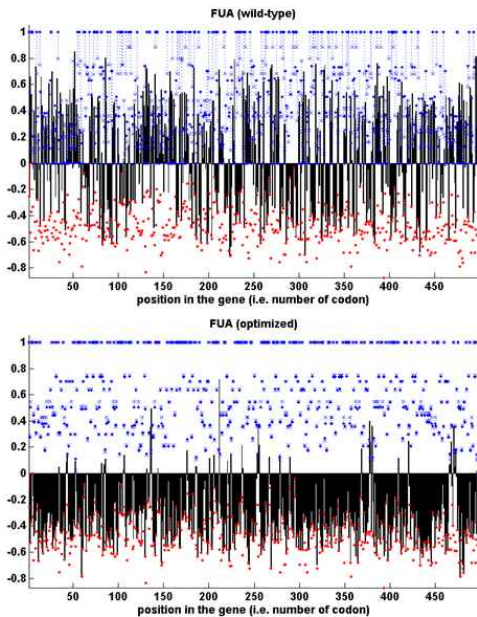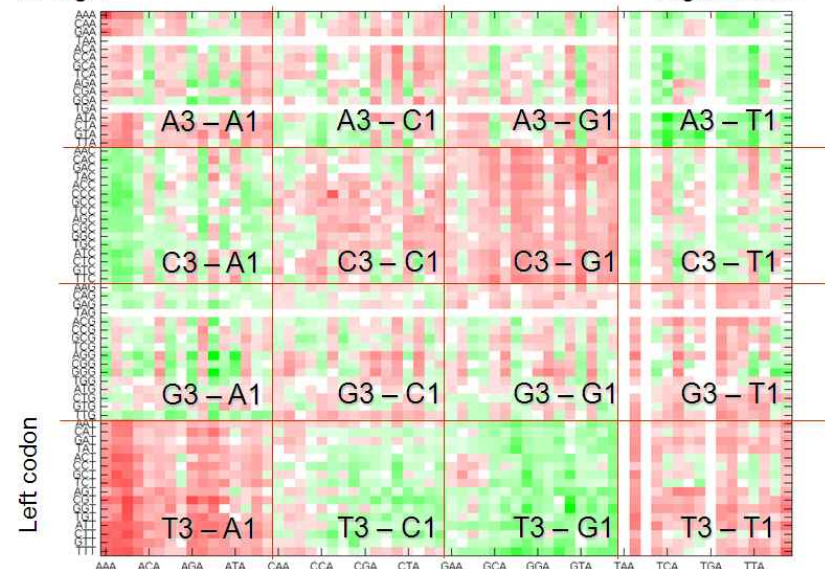
- Statistically significant bias in codon pair usage found
- Method for optimizing genes through codon pair optimization invented and implemented
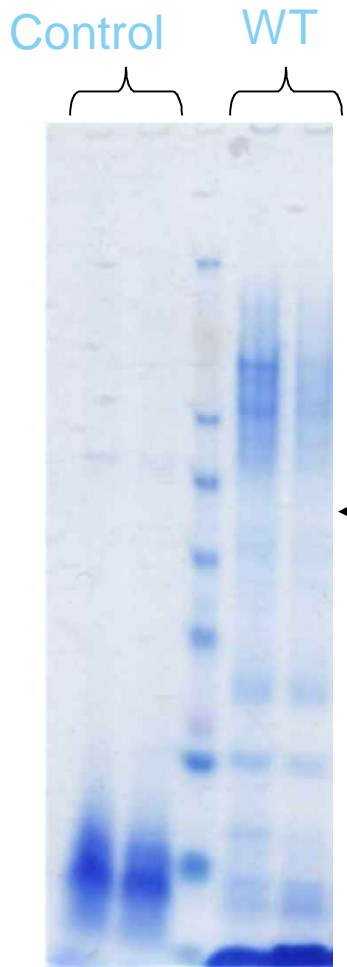- Several optimized genes created (e.g. FUA in *A. niger*)
- WO2008/000632

Broad application…

Wild-type   Codon-optimized

E. coli

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Protein*: A. niger* chitinase

Control   WT

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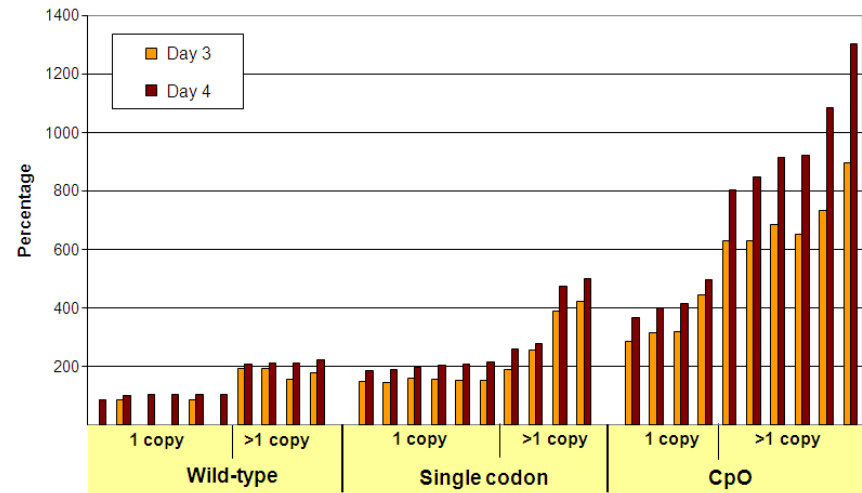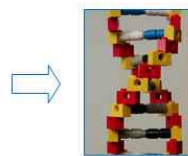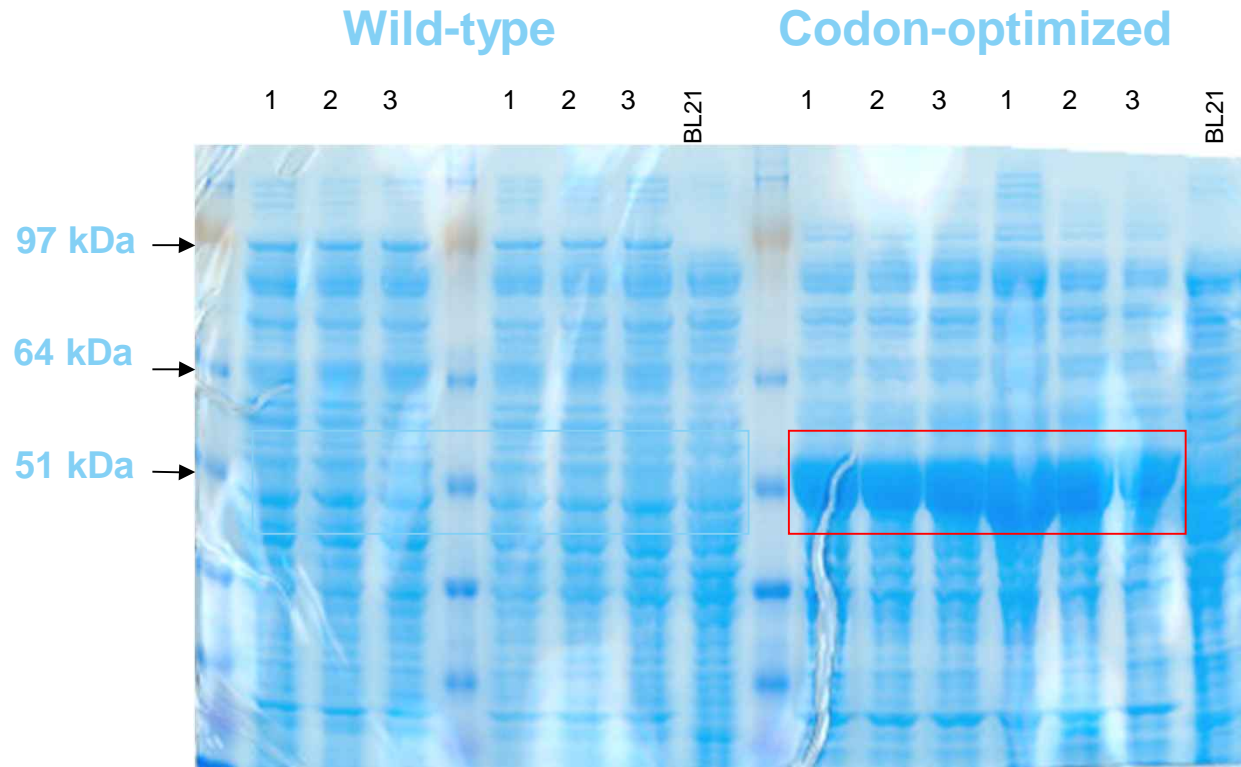
Protein: Classifier approach

- Approach:
  - Dataset of 638 overexpression attempts in *A. niger*
    - 268 successful
    - 370 not successful
  - Statistical classification to select protein features that distinguish between secreted proteins and non-secreted proteins in a dataset
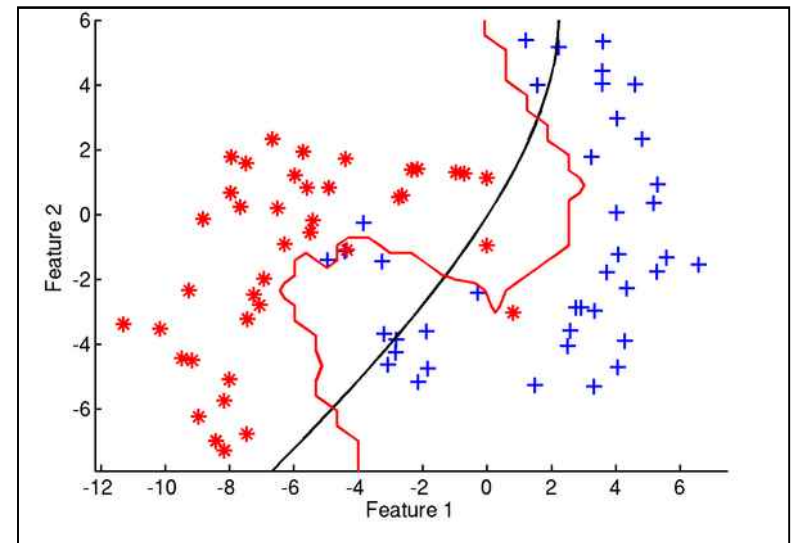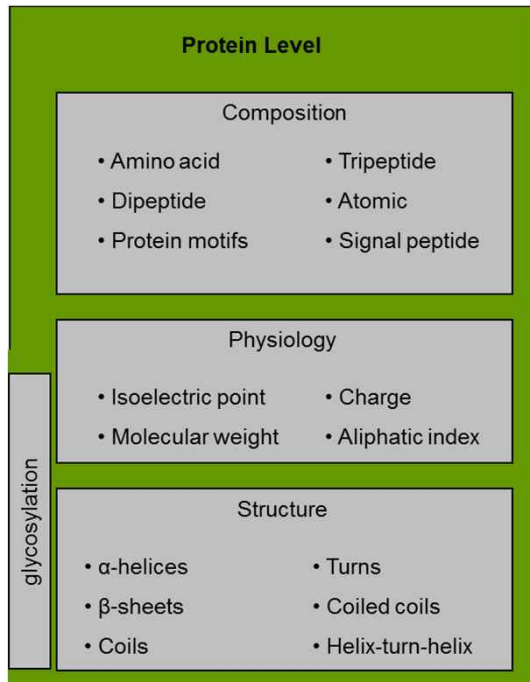
Protein: *A. niger* chitinase optimized
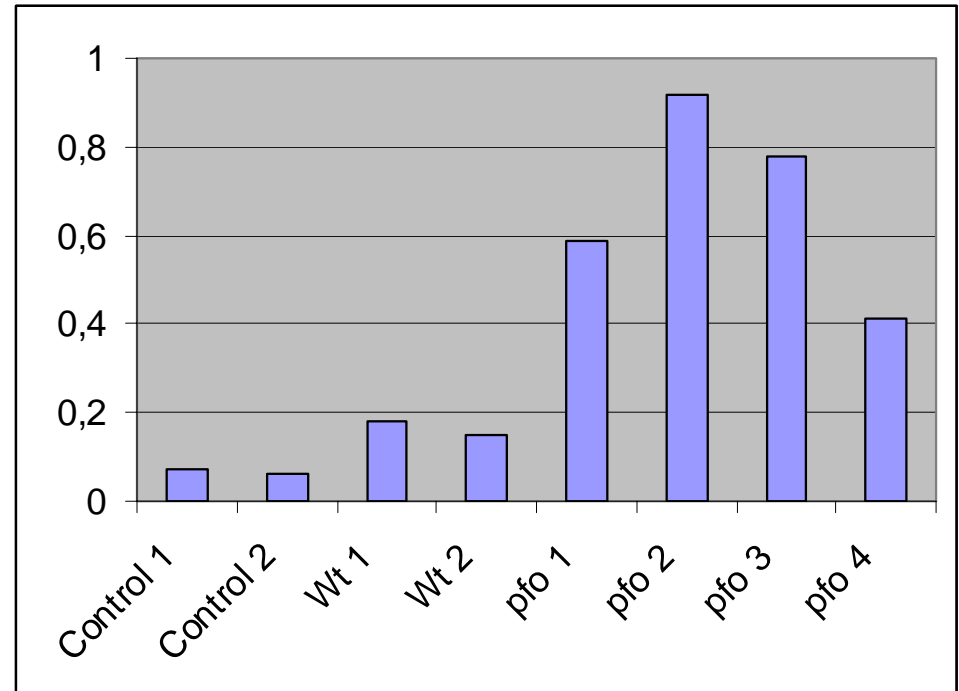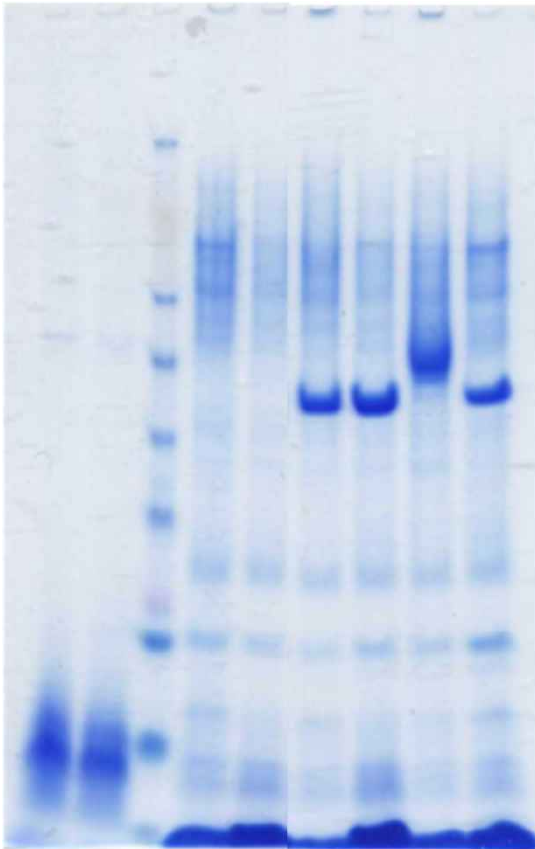
WO2010/102982

Novel enzyme development: Screening & design

Target activity & matrix effect

Diversity sources

Filters: class specific, BioIT, smart design, etc

Assay strategy

Enzyme leads to PMCs

Industry needs

Enzyme class selection

DSM Enzyme Collection

1000's

100's

10's

BioIT-driven selection and design of candidate enzymes

Historical approach

Enzyme screening → better focus, less screening
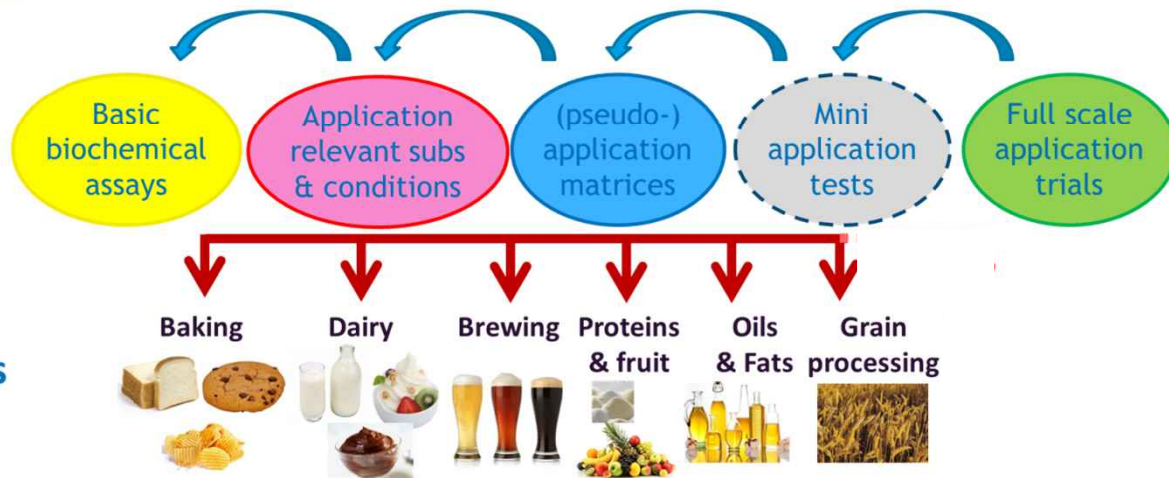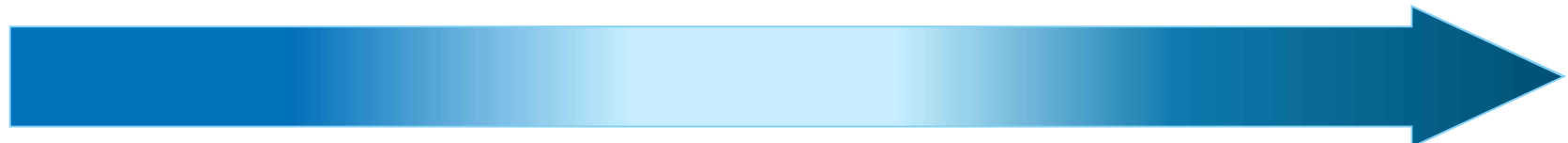
Basic biochemical assays

Application relevant subs & conditions

(pseudo-) application matrices

Mini application tests

Full scale application trials

Baking   Dairy   Brewing   Proteins & fruit   Oils & Fats   Grain processing

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A brief history of screening & protein engineering

1970   1980   1990   2000   2010   2020   **?**

| Site-directed mutagenesis | Cassette/sat. mutagenesis | Error-prone PCR | DNA shuffling | smart library design | *computational design?* |

Key driver:
- Activity-based screening

Key driver:
- Sequence-based screening
- HT screening robotics
- HT DNA sequencing

Key drivers:
- Advanced bioinformatics & modeling
- Progressively cheaper DNA synthesis

Importance of structure information:

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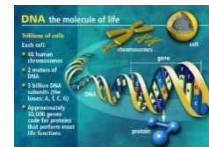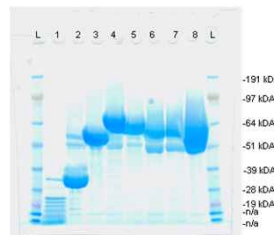
IMPROVE: Smart Library Design – Less = More

Computational Design

Rational Design
(phylogeny, structure, docking)

Smart Library Design

Random
(error-prone, saturation, recombination)

Statistics
(library statistics & simulation, data analysis)

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The enzyme optimization cycle
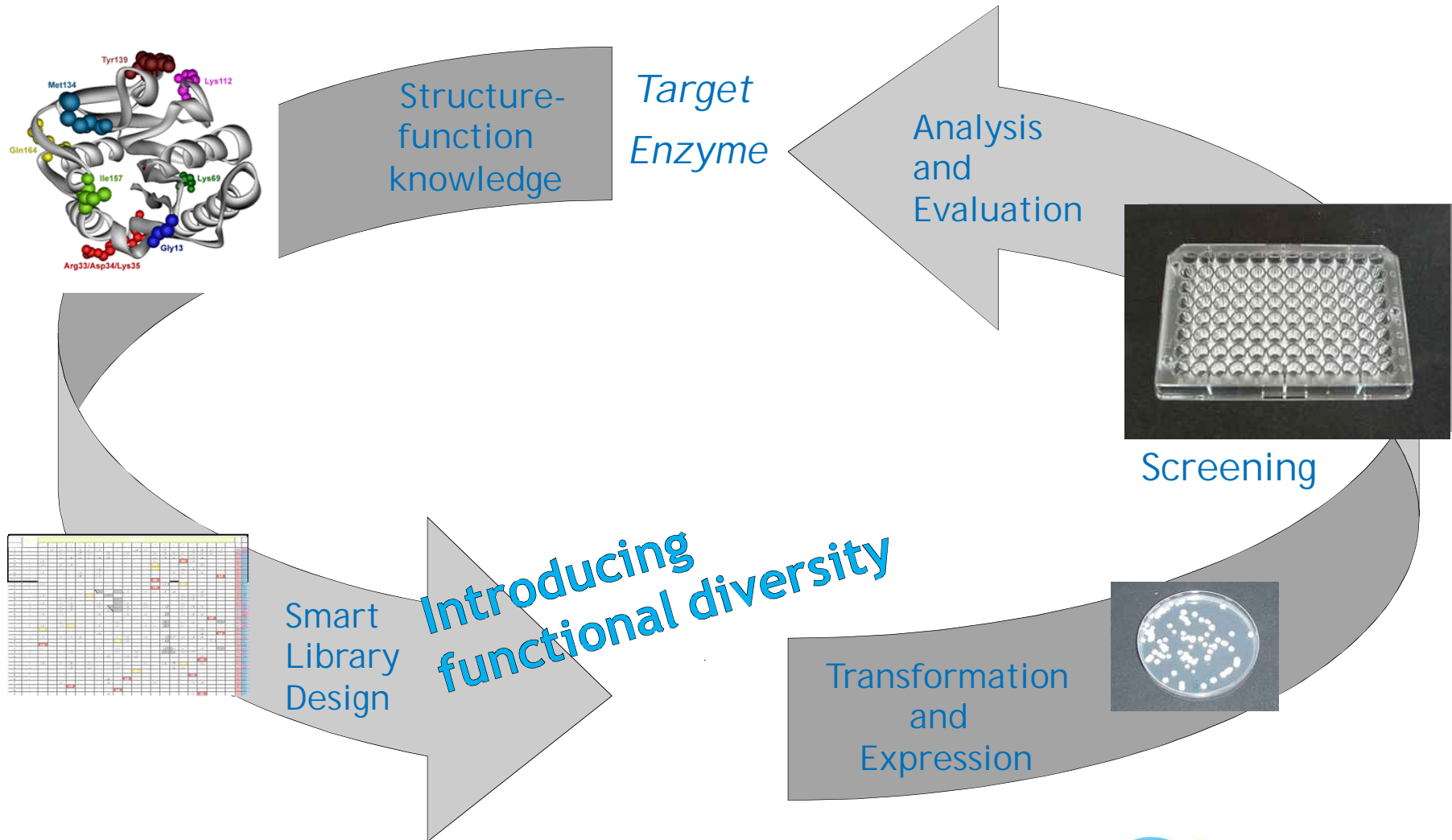
The protease action of chymosin in milk

whey

casein marcopeptide

κ-casein

κ-casein cleavage site

Casein particle

Chymosin

coagulation

+

curd

Chymosin

α-casein

glycosylation
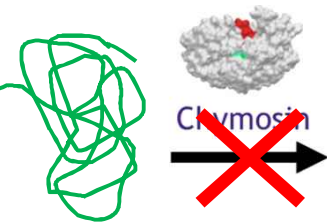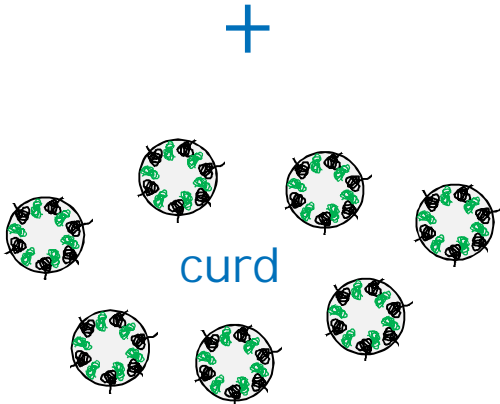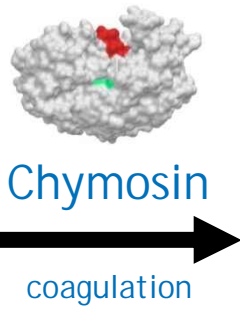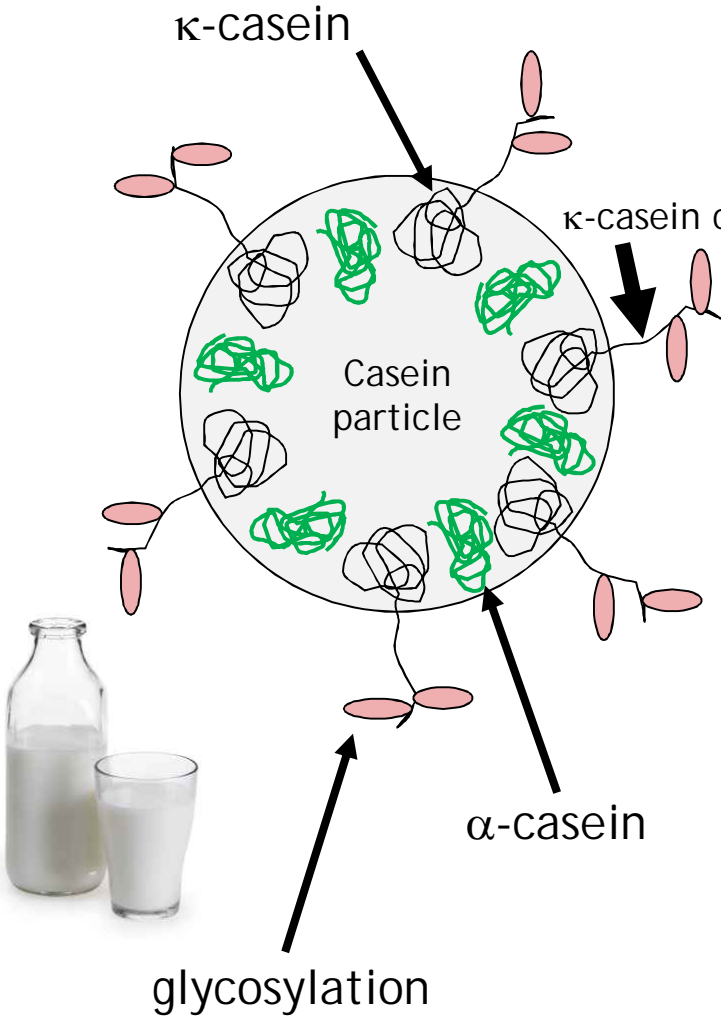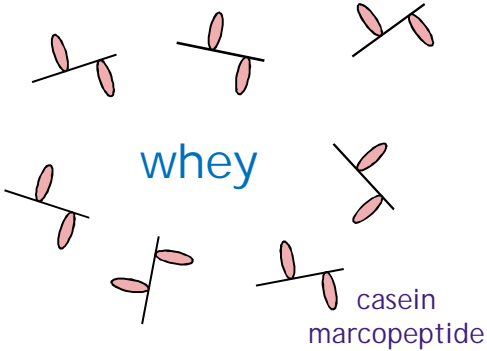
Target: limit proteolysis of α-casein (high C/P: coagulation/proteolysis ratio)
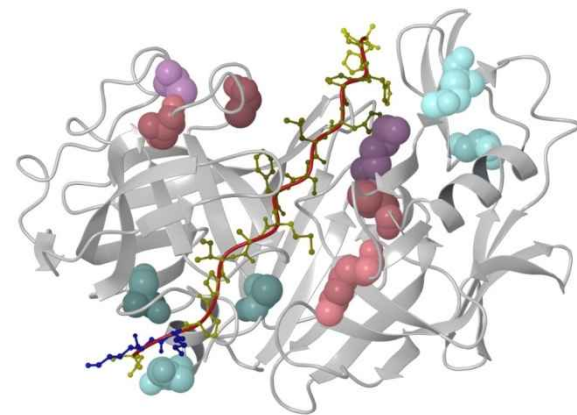
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Development of high specificity chymosin

Structural analysis of mammalian chymosin sequence variations
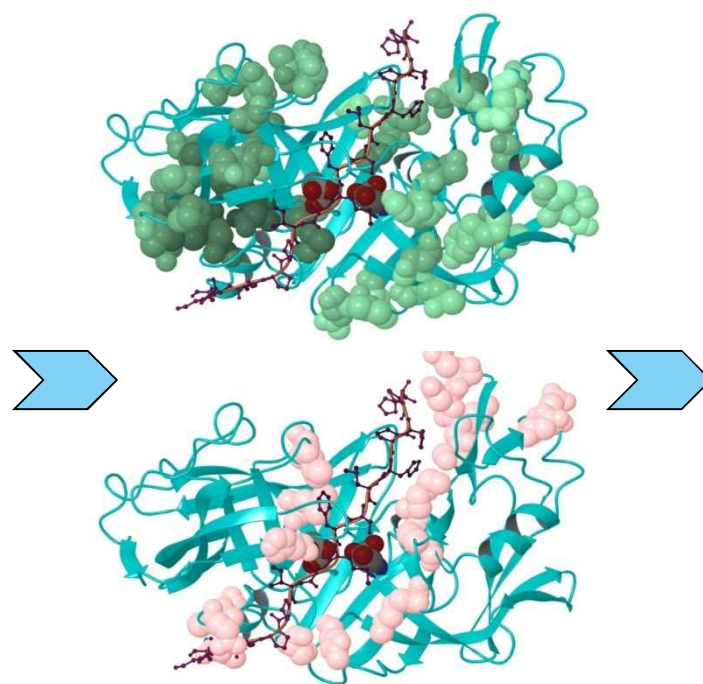
Chymosin variants

Hits with improved Specificity / activity

Unprecedented hit rates using Smart Library Design

Library type	# screened	Enzyme	Target improvement	Hit rate
random	6000	A	specific activity	0.4%
saturation	4000	A	specific activity	2.5%
saturation (sequenced)	1000	B	substrate selectivity	2.5%
Combinatorial	7000	B	substrate diversity	3%
Smart Library	85 (70% active)	Chymosin	Specificity Activity	>10%

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Can we predict novel specificity sequence space *in silico*?
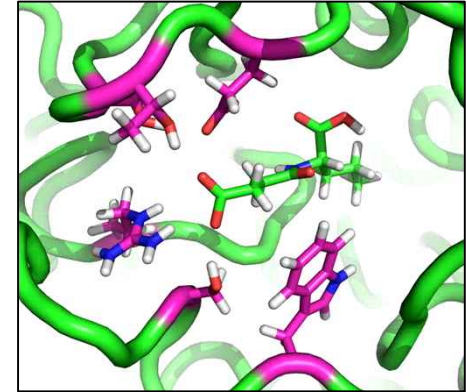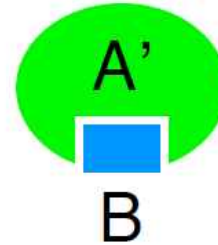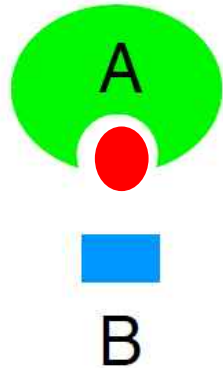
Input: Wild-type enzyme and desired substrate

Output: Optimal sequences for binding desired substrate

Advantages:

- Drastic reduction of sequence space (wrt full saturation mutagenesis)
- Reduced screening efforts (# clones & rounds)
- Combinatorial libraries allow screening for synergistic mutations

Ollikainen, de Jong, Kortemme (2014), *in preparation*

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Conclusions

- Biodiversity rapidly exploding through sequencing
- Rationalization of
  - Protein expression optimization
  - Biodiversity screening
  - Enzyme engineering ('smart library')
  - Computational design…

→ efficient, faster & predictive enzyme development

Johannes Vermeer: View on Delft (ca. 1661-1664)

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