



Recent Advances in Large-Scale Purification of Biotherapeutics

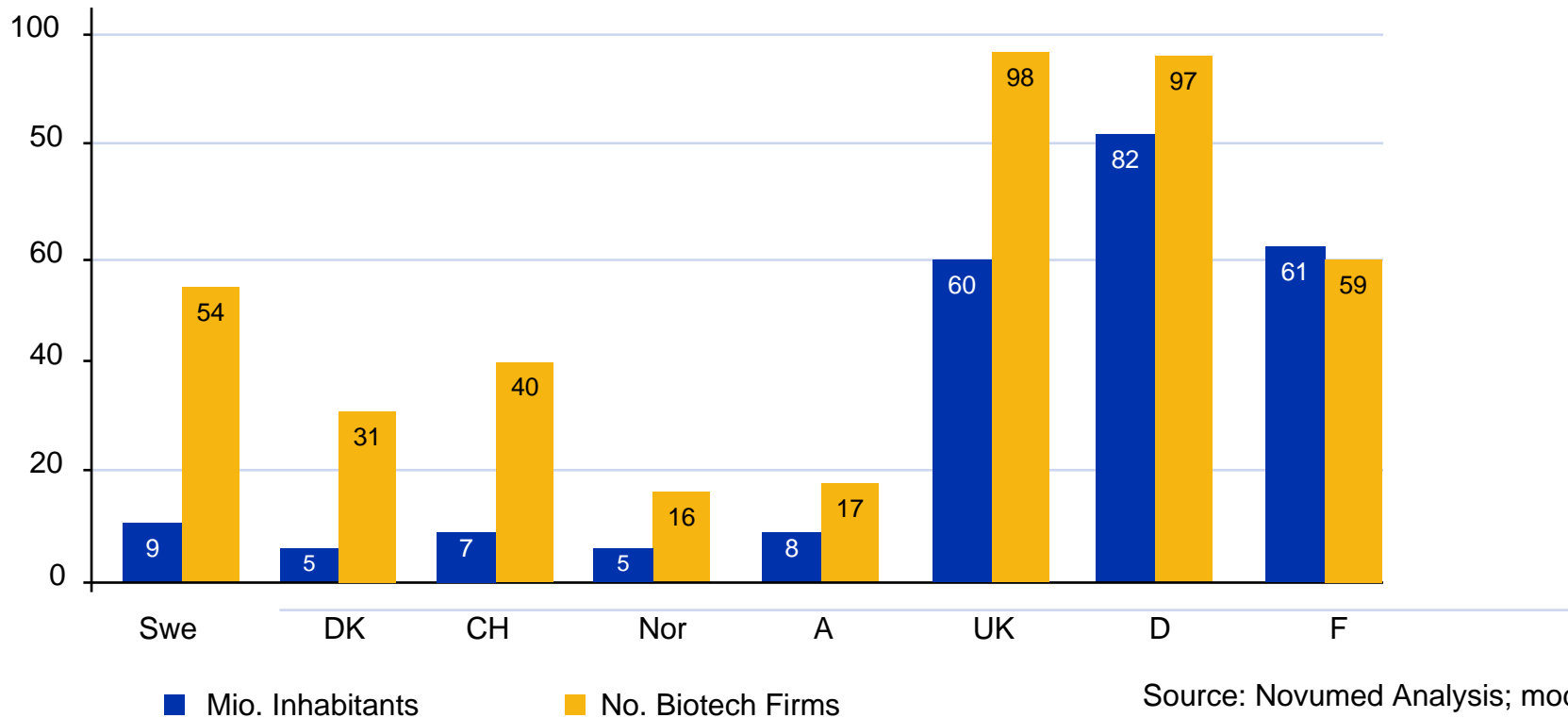
Dialogue Biopharm 2010

- How to Speed up Biopharm Development

Dr. Lothar Jacob

Number of biotechs developing new drugs* and number of inhabitants per country

*clinical and pre-clinical phases of development



Source: Novumed Analysis; modified acc. to Biotechnol. J. 2009, 4, 172–173

Manufacturing process

- The manufacturing process is an important part of the bio-therapeutic drug
- The product defines the manufacturing process
- The process is part of the final product
- Individual process steps must be optimized to achieve best results (e.g. platform strategies for mAbs, adenoviruses, plasmids!)

Alternatives in dsp

- Alternative tools (AbC)
 - Precipitation
 - Crystallization
- Chromatographic alternatives
 - Membrane adsorption techniques
 - Different column design
 - Expanded bed adsorption
 - Bio-SMB/ MCSGP*
 - Radial flow columns
 - Ready to use columns/disposables
 - Liquid/liquid partitioning
 - Aqueous two phase systems
- Media design

„New“ separation technologies??

**Multi-column Countercurrent Solvent Gradient Purification (MCSGP):*

Alternatives in dsp, comments

▪ Alternative tools (AbC)

– Precipitation

(AS, PEG, EtOH etc.)

– Crystallization

time consuming

▪ Chromatographic alternatives

– Membrane adsorption techniques

Since late 1980ies

– Different column design

• Expanded bed adsorption

Launched 1990ies, not widely accepted

• Bio-SMB/ MCSGP*

Relatively new, not enough experience

• Radial flow columns

Since late 1980ies, Sepragen + Proxys

• Ready to use columns/disposables

– Liquid/liquid partitioning

Discontinued mid 1990ies

– Aqueous two phase systems

Works for small molecules

▪ Media design

Target related methods

Target	Specific chromatographic step	Market size [\$bn]
mAb	Protein A Affinity	~34
DNA	Q/TMAE; strong AEX	?
Adenoviruses	DEAE; weak AEX	~22*
Prions (plasma derived products)	Synthetic Affi-ligand	9
rProt	-	29
Ab-fragments	-	?
insulins	HP polishing	~13

* Vaccine market

Where do we come from and where to go?

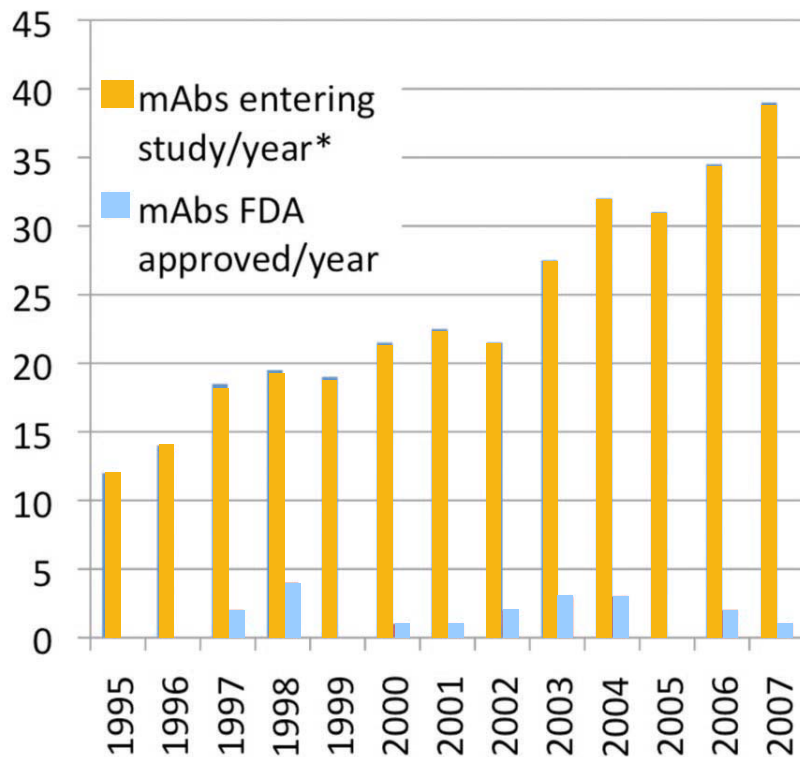
- “In the past, we’ve been operating more on the low end of resin [capacities](#), [loading densities](#), and [filtration capacities](#).
- [Metric-ton](#) kind of perspective, is this realistic?
- “The main separation technologies like [chromatography](#) and [filtration](#) methods are good technologies and [cycling](#) columns is becoming easier with automated systems.
- New class of molecules? (Fab, new scaffolds, RNA, oligos)
- Always tried to increase [yield](#)

Today's Challenges

increase purification throughput by reducing the operational requirements

- such as buffer preparation times,
- CIP times and utilities,
- reduction of buffer hold tank volumes and
- labor reduction

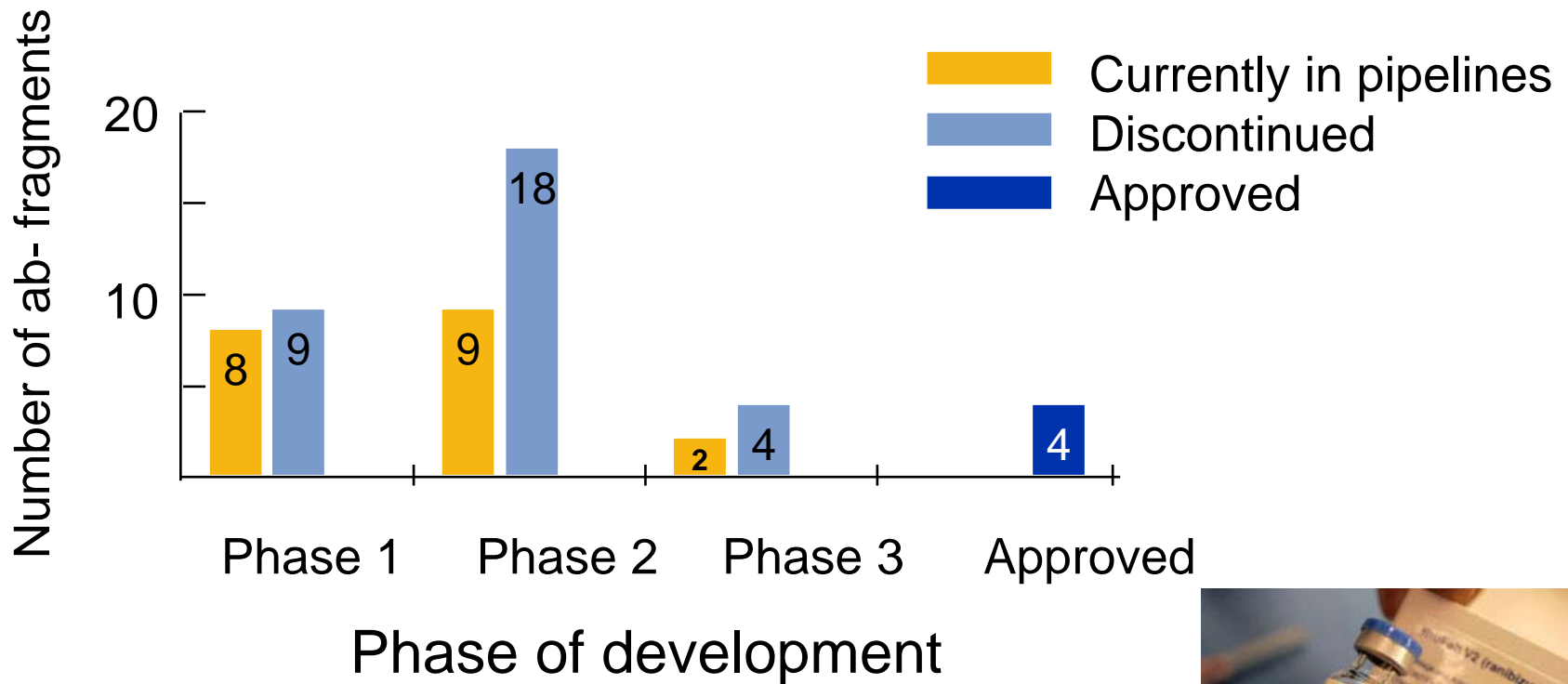
Therapeutic monoclonal antibodies entering clinical study or approved



- Data from 1995 to 2007 presented as two-year moving average

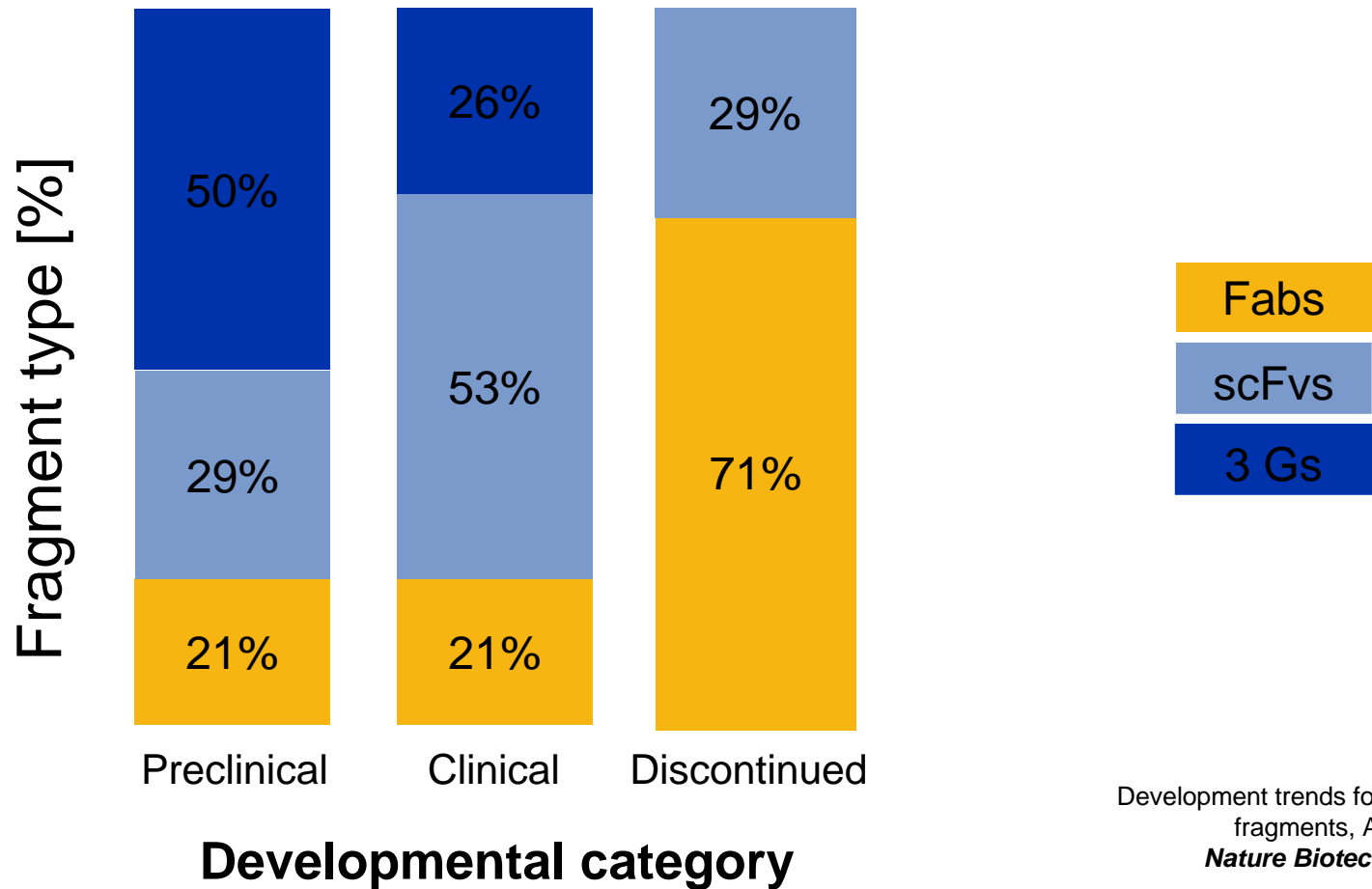
- Source: Tufts Center for the Study of Drug Development, taken from mAbs 1:1, 86-87; January/February 2009]; Janice M. Reichert: Global antibody development trends

Phase of clinical development for 54 antibody fragment therapeutics



Modified according to Aaron L Nelson & Janice M Reichert: Development trends for therapeutic antibody fragments; Nature Biotechnology 27, 331 - 337 (2009)

Distribution of fragment type across developmental categories



Development trends for therapeutic antibody fragments, A Nelson & J Reichert, *Nature Biotech* 27, 331 – 337, 2009

Biologics & Biosimilars

2010: ~ 1/3 of all newly approved drugs will be biologics

Biosimilars: The market for generic versions of biologics is becoming more and more interesting as the patents of first-generation products like EPO, G-CSF, human growth hormone, insulin and interferon are expiring.

Total sales of off-patent biologics: ~ \$20 billion

This market will considerably increase over the next few years.







Improvements of stationary phases

- Matrix related
 - Monolithic devices
 - Synthetic co-polymers
 - Stabilized carbohydrate based resins
 - Magnetic beads

- Ligands
 - Affinity tags
 - Mimetic (synthetic) ligands
 - Mixed mode/dual mode

- Coupling chemistry/surface modification
 - Tentacles
 - Dextrane enhancers...

Various strategies by different suppliers

Manufacturer	Base BeadTechnology	Modification Technology
<p>GE Healthcare</p> 	Sepharose	IEX, HIC, Protein A
 <p>TOSOH</p>	Glycidyl-methacrylate (GM)	IEX, HIC
	New polymeric resins	Tentacle-IEX, HIC
	Controlled Pore Glass (CPG)	IEX, Protein A
	Polystyrene (PST)	Hydrophilisation, IEX, HIC, Protein A
	Glycidyl-methacrylate (GM) Hydroxyapatite	IEX, HIC, Protein A

Role of Base Matrix & Surface Chemistry

- **Base Matrix** defines parameters such as:
 - **Mechanical stability**
 - **Porosity** (influenced by surface chemistry)
 - **Biocompatibility** (influenced by surface chemistry)
 - **Chemical stability**
 - **Ease of modification**

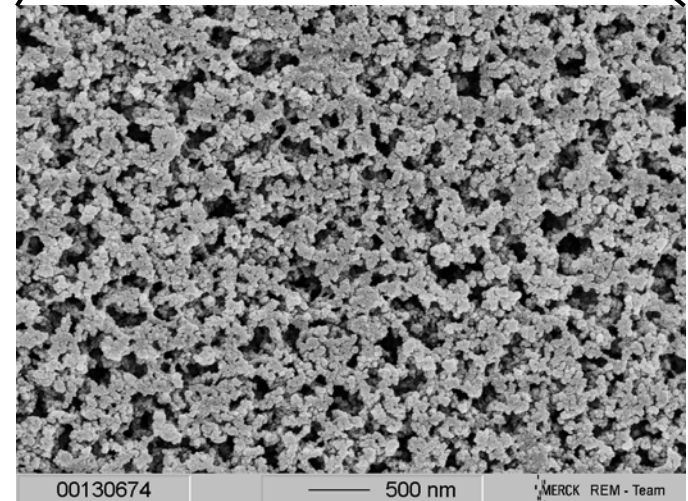
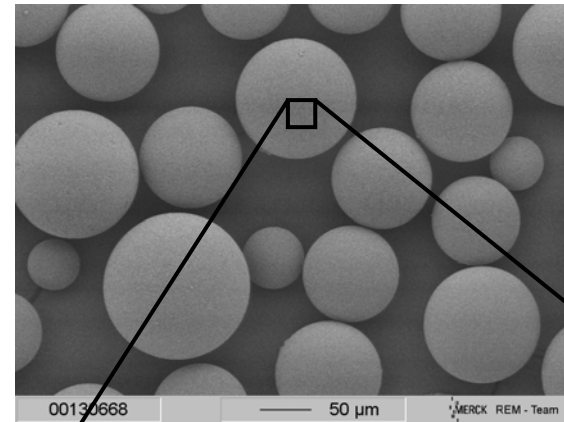
- **Surface Chemistry** defines parameters such as:
 - **Capacity** (together with porosity)
 - **Selectivity** (influenced by base matrix)
 - **Biocompatibility**
 - **Chemical stability**

These parameters enable end-users state-of-the-art processes ensuring

- Reliability
- Efficiency
- Robustness
 - Scalability
 - Prediction
 - Capability to do scale down experiments
- GMP compliance
- Product quality
- (*Workplace safety*)

Combination of advanced tentacle technology and new base beads: Eshmuno™ S

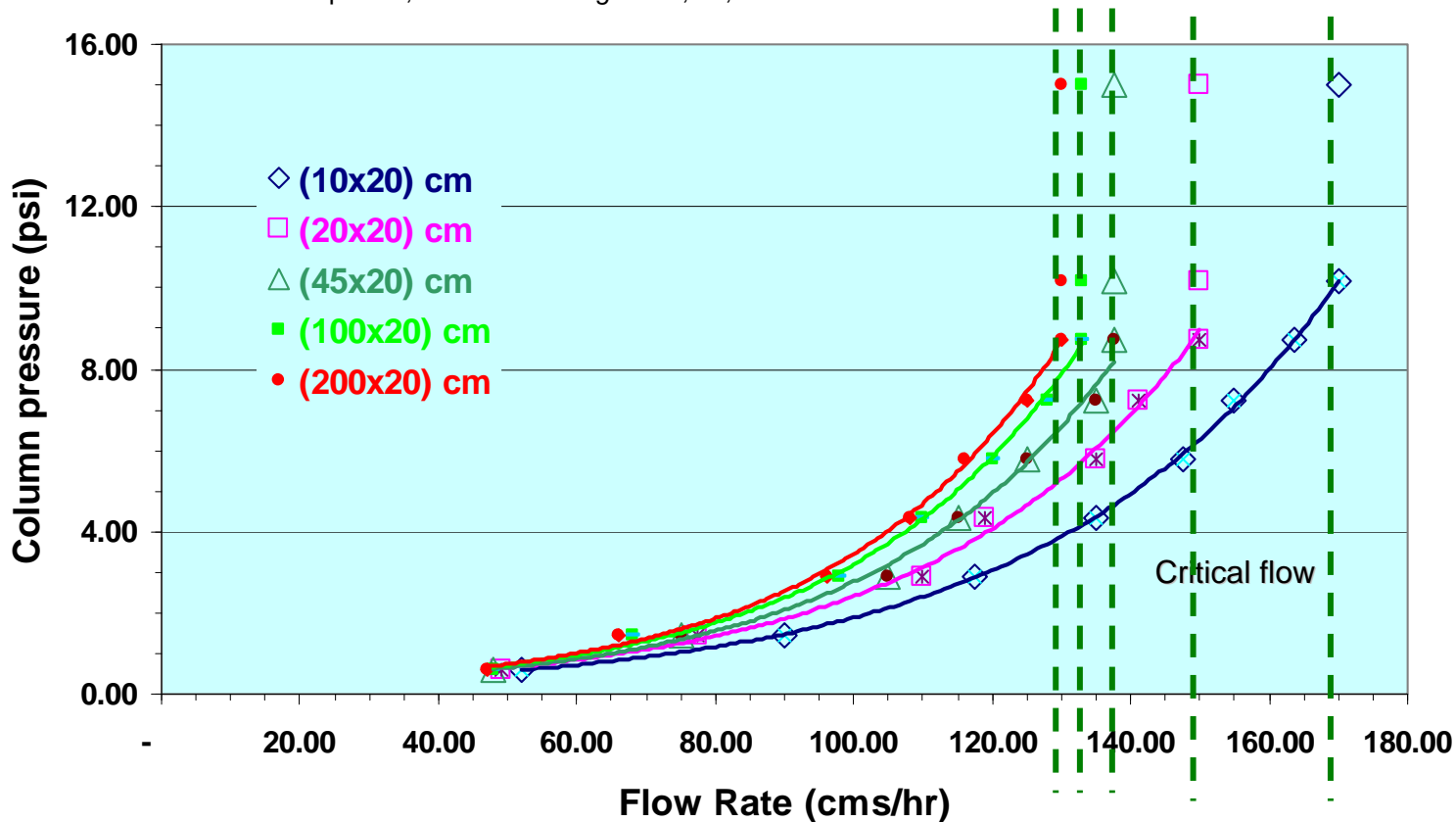
- very hydrophilic polyvinyl derivative beads
- no aggregates
- mean particle size: 75-95 μm
- no shell formation
- good pore accessibility
- mean pore size: 80 nm
- high rigidity due to high cross-linking level



Pressure Flow Curve Non-rigid materials

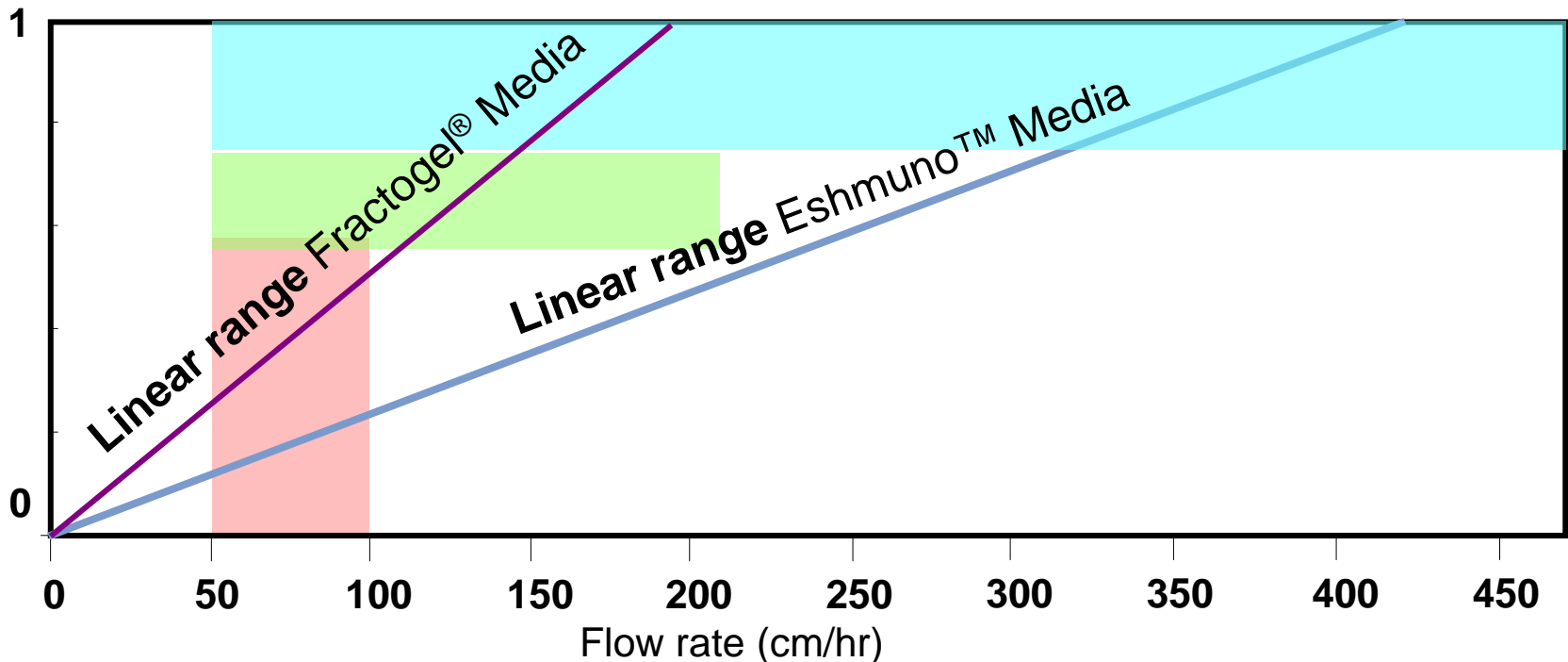
Phenyl Sepharose 6FF 20cms bed height flow packed in PBS

Stickel and Fotopoulos, *Biotechnol. Prog.* 2001, 17, 744-751



Scalability of new Eshmuno™ media

Column backpressure (bar)



Operating range
Agarose

Operating range
Fractogel®

Operating range
Eshmuno™

Features of different cation exchangers

	Competitor CEX	Fractogel SO ₃ ⁻	Eshmuno S
DBC ¹ [mg/mL settled resin]	64	70	96
HCP clearance factor (capture)	121	52	150
	28	108	77
HCP clearance factor (post Protein A)	n.d.	3.7 ²	5 ²

mAb03 data

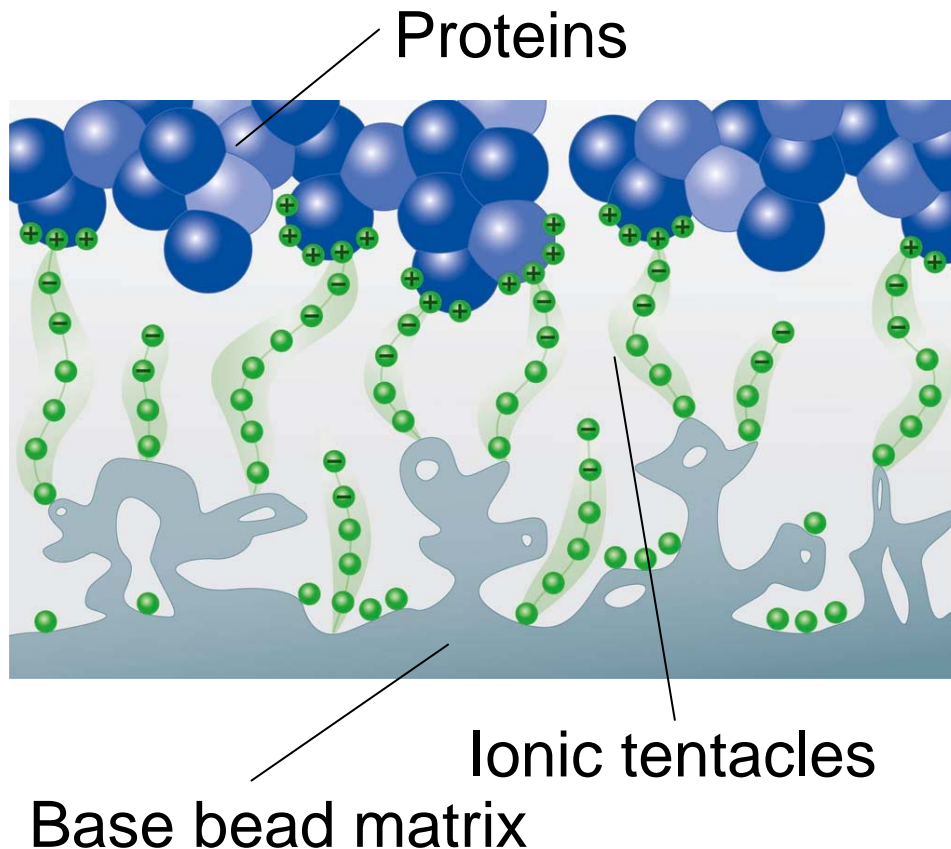
¹Capacities were determined at 5 % breakthrough with buffer pH 6 containing 20 mM phosphate and 20 mM NaCl (conductivity 4.3 mS/cm). Experiments were performed at a residence time of 5 min with 1 mL Scout columns.

²The mAb concentration was 0.62 mg/mL.

Purified vs diluted mAb capacity

	Mab02 capacity from diluted CCS	Purified Mab02
Fractogel EMD SO3 (M)	70	75
Eshmuno S	96	93
Resin C	64	114

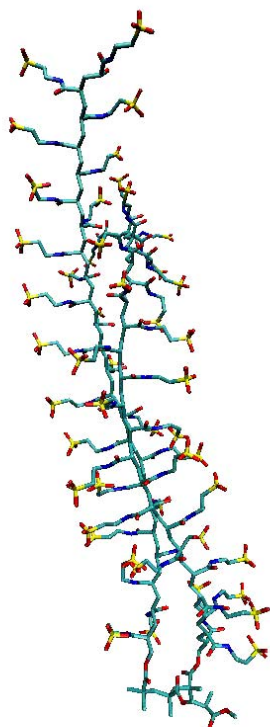
Tentacle-type Ion Exchange Sorbents give Flexibility for multi-point Interactions



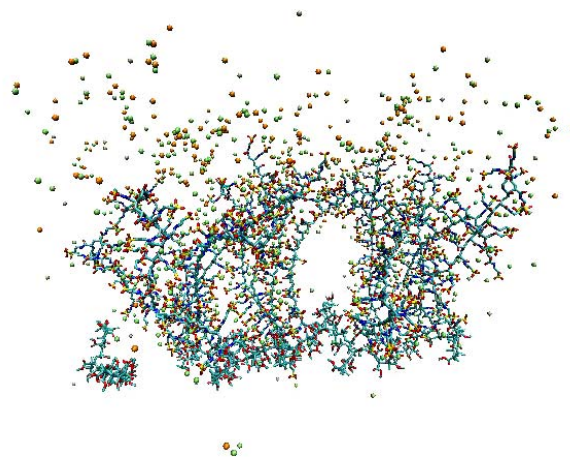
- Ligands have to be presented in a flexible and easy accessible way
- Tentacles IEX present the ionic group along the tentacle polymer
- The tentacle IEX-groups are forming a three-dimensional ion exchange network
- 20 years of experience in tentacle grafting technologies for biochrom resins

Molecular dynamic simulations of tentacle structures

Prof. Carlo Cavalotti, Politecnico di Milano, Work of EU-project



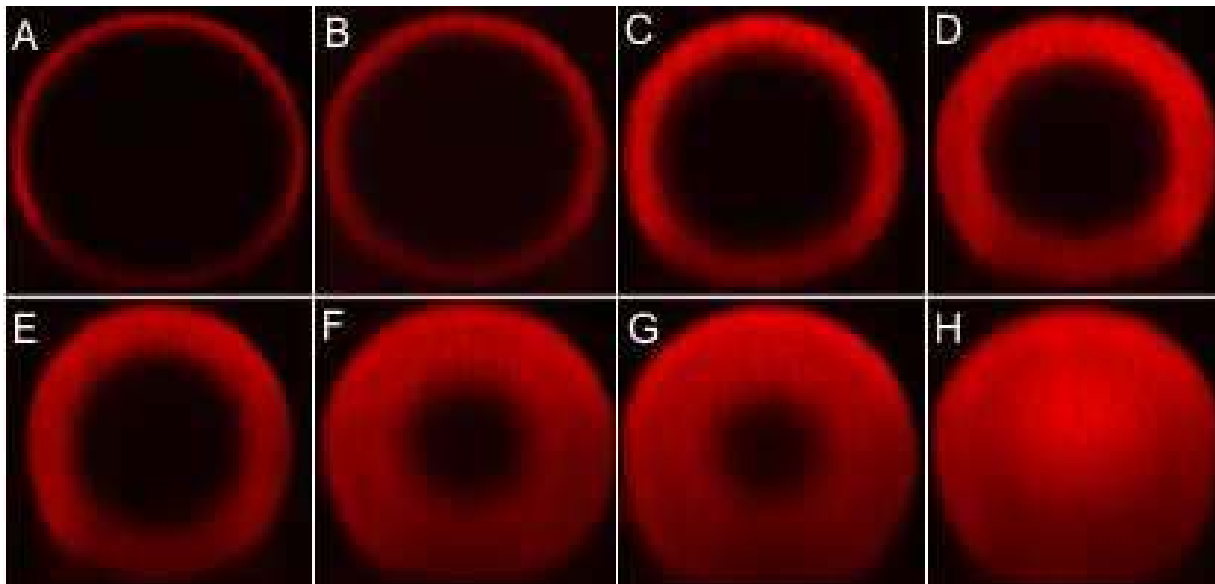
Tentacle dimer



High flexibility of tentacle IEX

Batch adsorption at different times of mAb02

Fractogel® EMD SO3 (M)



2 min

4 min

8 min

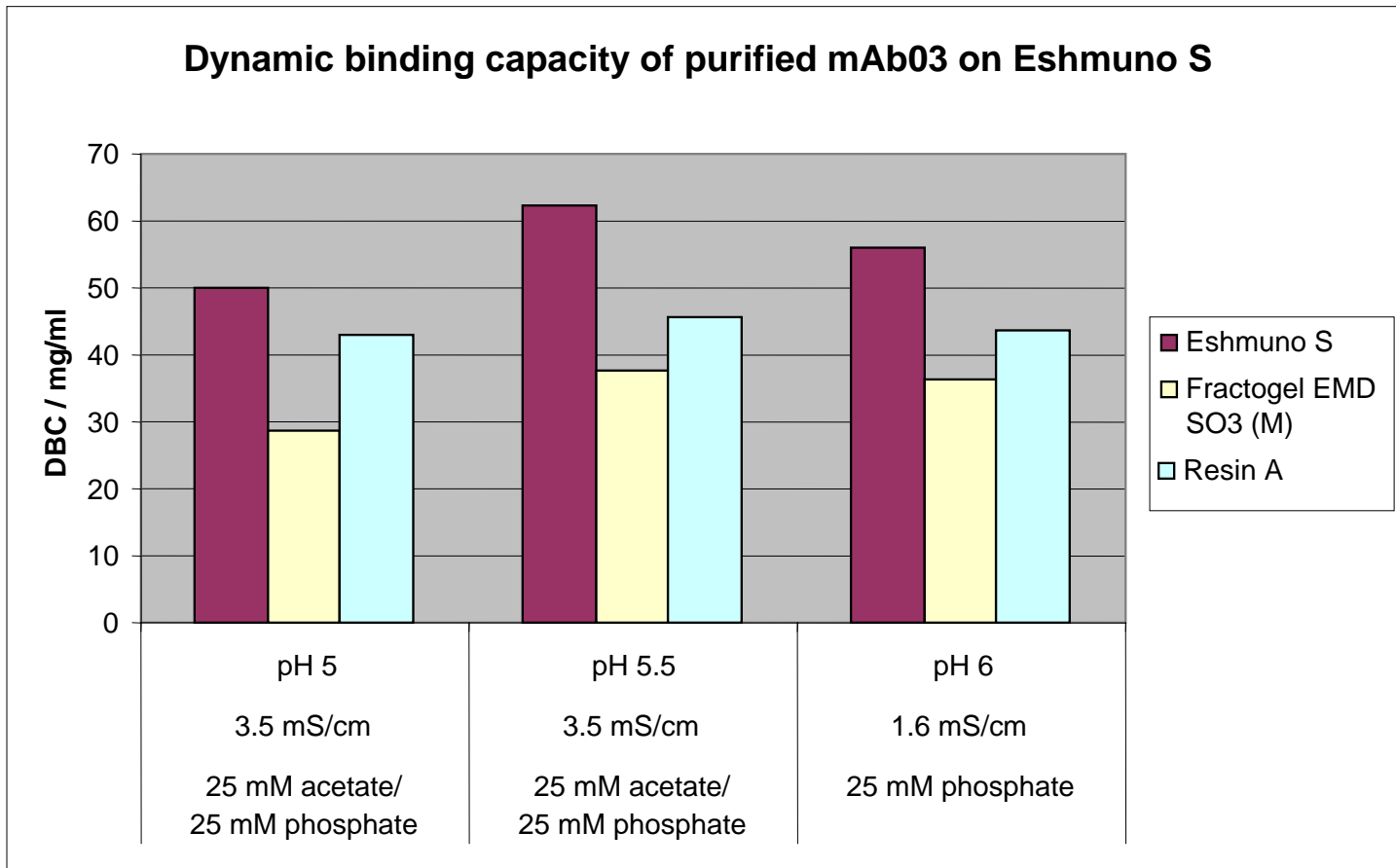
16 min

Eshmuno™ S

mAb02 labeled with fluorescent dye CLSM at pH 5, cond. 4.0 mS/cm

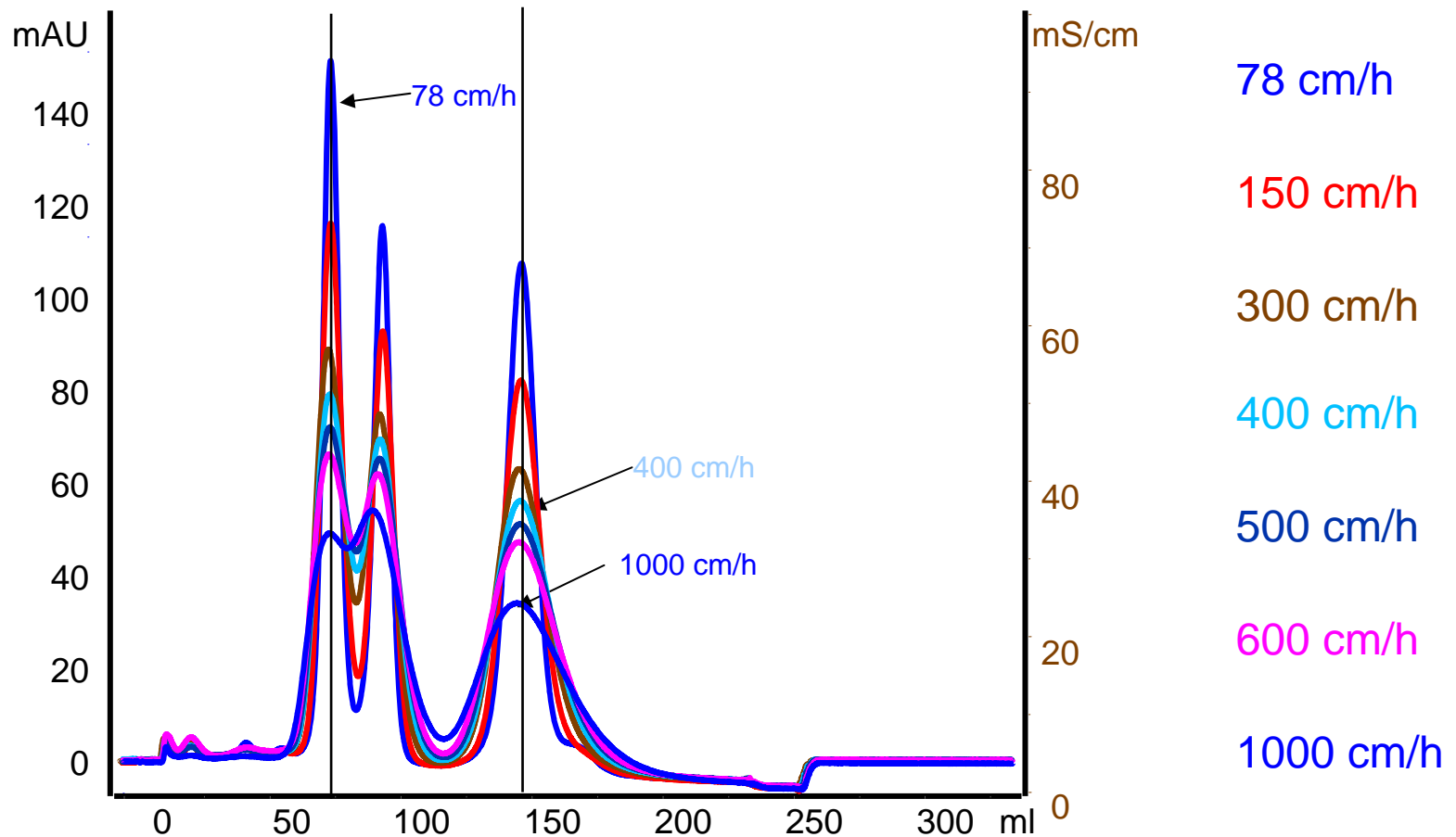
Higher binding capacity for pure mAb

1 ml scout columns, residence time 2 min, mAb conc 5 mg/ml

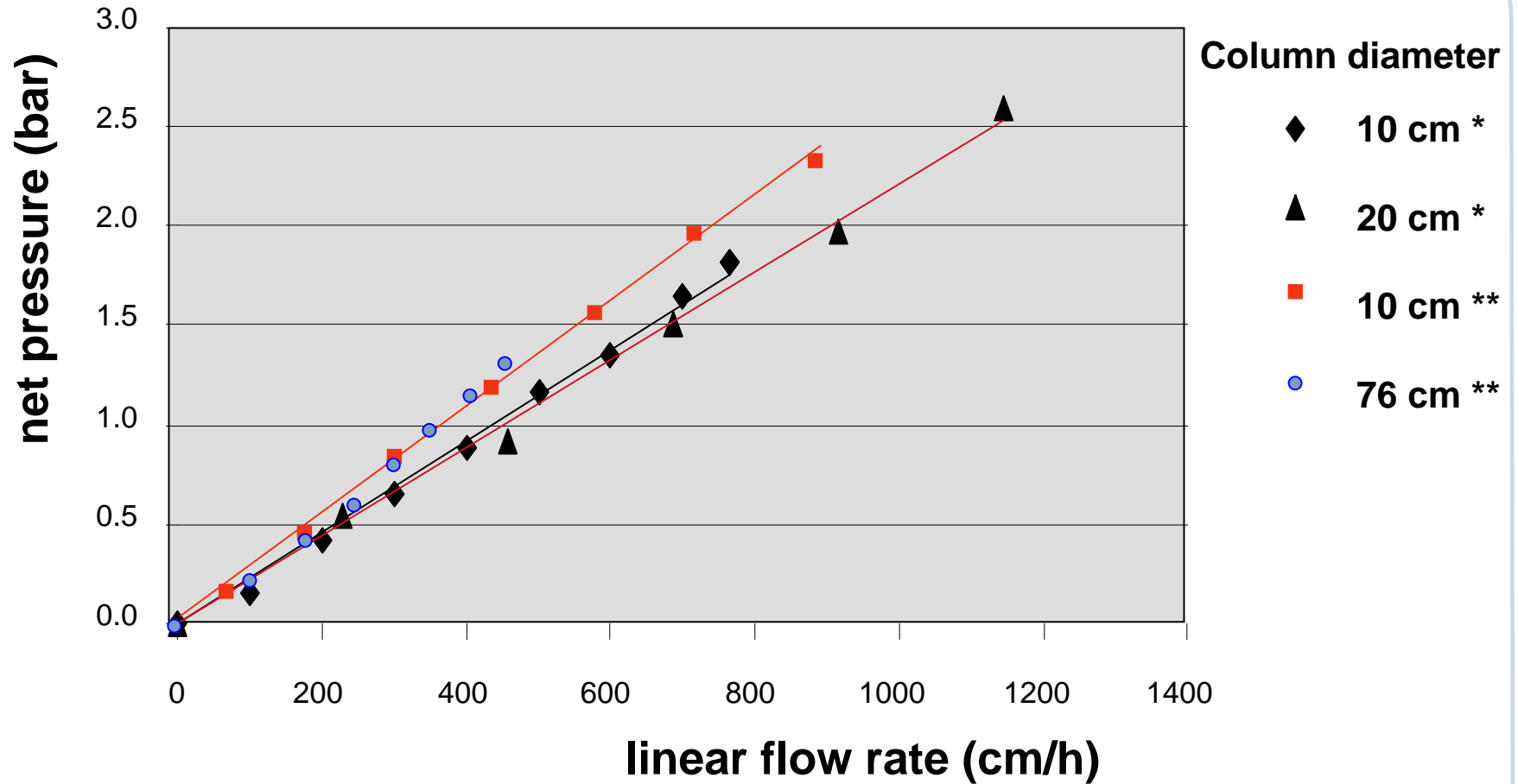


Resolution versus flow rate

Eshmuno™ S: High resolution even at high flow rates



Pressure flow curves

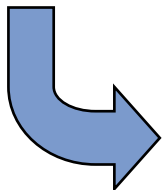


* flow packed in 0.15 M NaCl, 20 cm bed height, 8 % compression

** flow packed in 0.01 M NaOH, 20.5 cm bed height, 10.5 % compression

What to do?

- Chose right methods & conditions
- Select qualified raw materials
- Fit everything into
 - existing facilities
 - or into a profitable model to justify investments for a new plant
- Spend efforts in the



adaption of dsp strategies

What will be in the future

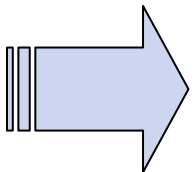
- Increasing or decreasing demand of mAbs is not clear
- Leading position of mAbs will be changed (Fab, 3Gs..., rProt)
- Less block busters in the future
- Biosimilars with reduced COGs
- Accumulated knowledge facilitates process development
- Shift to microbial systems
- Use of extended screening systems or platform approaches



Adaption of dsp strategies

Conclusions

- Molecular Modelling and CSLM show potential for optimisation of classical tentacle resins
- Synthesis strategy in combination with automatic screening is helpful
- Successful transfer of tentacle technique to a new base bead resulted in the first product Eshmuno S
- Eshmuno Q will follow soon



Significant improvement in dynamic IgG binding capacity was possible for CEX

Don't forget:

The quality of raw materials is a key success factor for manufacturing safe drugs

The Recovery and Purification track offers timely, state-of-the-art approaches to current issues of concern to the biopharm industry

Experience Biopharm`10



15th – 17th September 2010
Purification of Antibodies
Mannheim University
of Applied Sciences

Experience Biopharm`10



20th – 22nd September 2010
Purification of Biomolecules
Mannheim University
of Applied Sciences



hochschule mannheim



hochschule mannheim



Thank you for your attention



conclusion Today's Challenges

- In conclusion, there are many opportunities for improvements as we face the challenges in downstream processing, but there are relatively few prospects for
- major technical advances to solve our problems. As new biopharmaceutical products move through the pipeline,
- and cost pressures mount, downstream processing is clearly going to advance. We will, however, continue to face technical and regulatory challenges as we work to improve recovery yields and process throughput while decreasing operating costs.

Experience drives innovation

Milestones ...

Tswett reports on his results of separating chlorophyll

1903

First commercial product for chromatographic adsorption

1904

Standardized adsorbents according to Brockmann

1934

First commercial ready-to-use TLC plates

1956

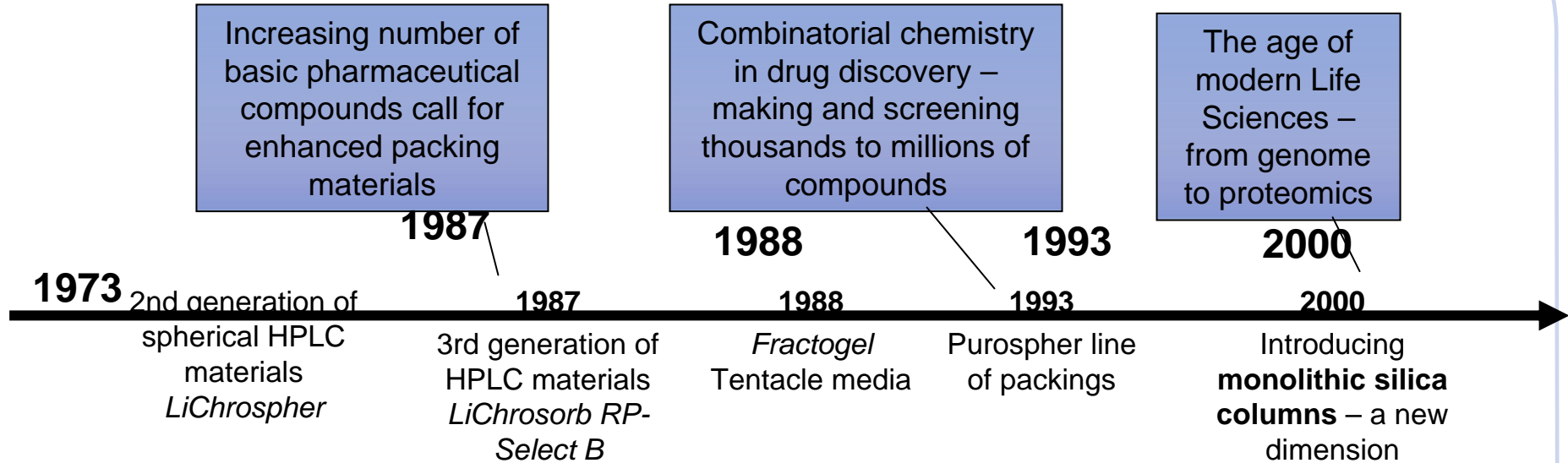
HPLC comes to life... Instruments to operate at high pressures

1966

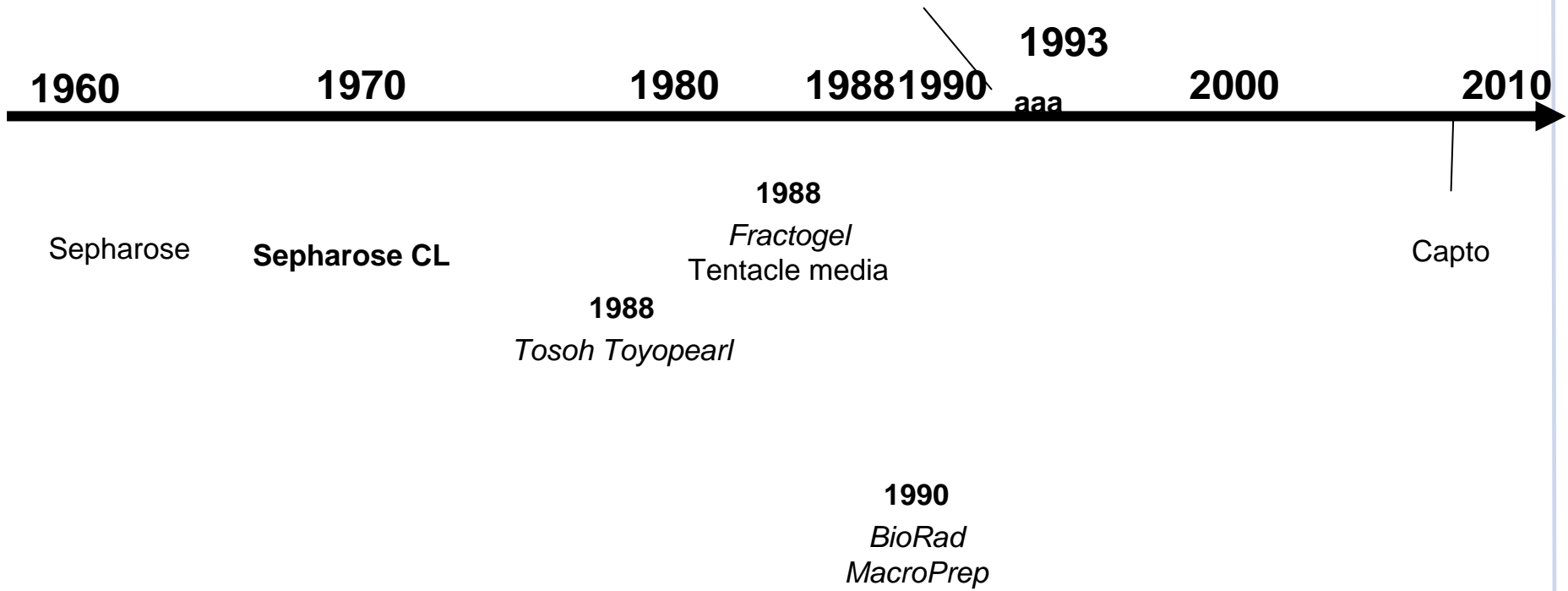
1st generation Merck HPLC Sorbents *Merckosorb*

1969

Experience drives innovation ...milestones



Experience drives innovation ...milestones



The latest platform from GEHC

