Introduction to Chromatide

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Business focus

Custom purification

Custom peptide synthesis

Custom oligonucleotide synthesis

Custom polymer synthesis

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Chromatide IP

Two patents

Polymer encapsulation (PCT filed 2007)

Within a rigid support skeleton

Micro reactors (PCT filed 2008)

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Applications

Affinity chromatography – initial focus

Enzyme immobilisation – via R&D Grant

Peptides - ongoing

Oligonucleotides - ongoing

Chiral chromatography

Transition metal catalysis

Cell culture - via collaboration with SCC

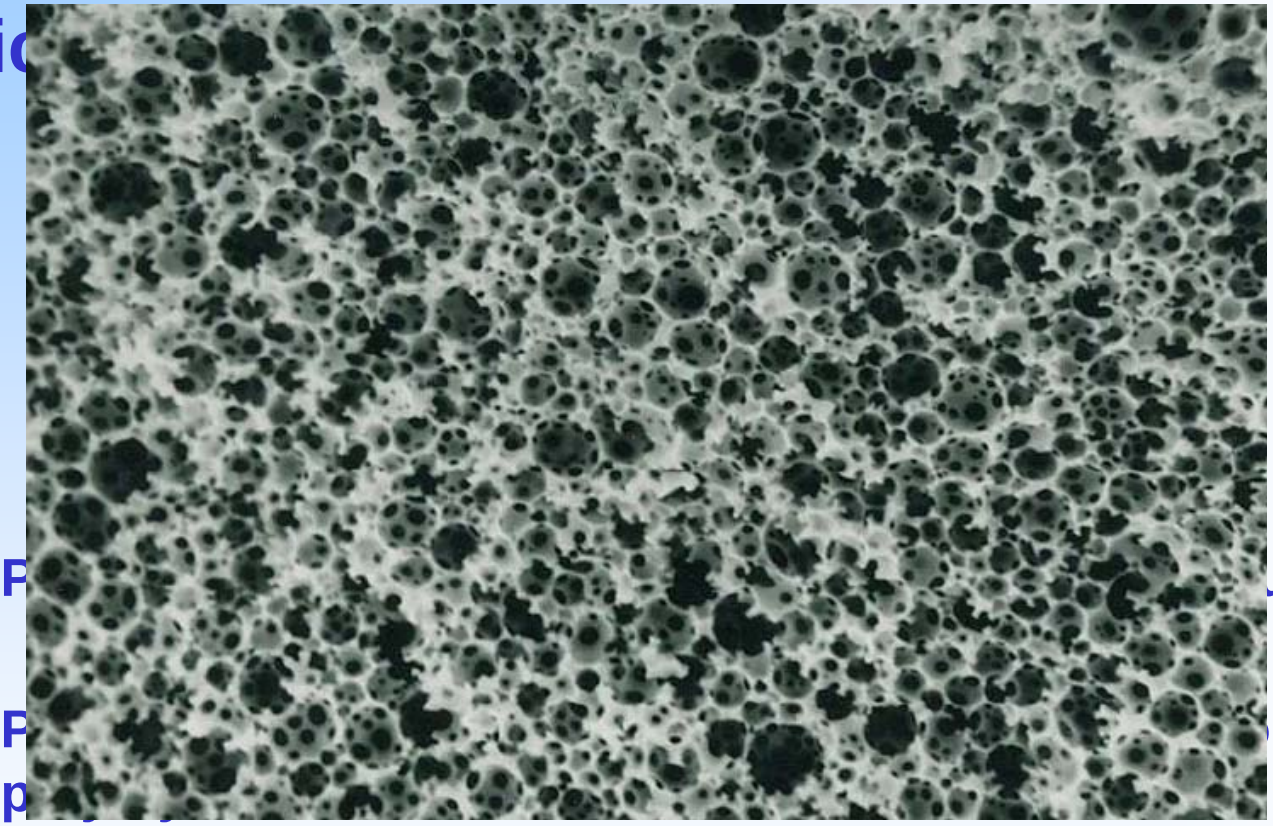
Slow release

Benefits of Polymer Encapsulation

Feature	Benefit
Much softer polymers can be encapsulated in rigid skeleton	Combines benefits of highly cross linked rigid polymers and low cross-linked polymer gels Mechanical robustness
Access to active sites in the polymer is dramatically improved	Presenting the polymer backbone in a quasi-homogeneous state improves process efficiency
Cross-linking can be reduced to minimum levels sufficient to hold polymer matrix together	Access to active sites is unrestricted which improves efficiency Full capacity of polymer backbone is possible
Uniform bead size	Lowers costs of production by eliminating need to classify according to particle size Regular column bed formation
Lower cost, mass produced stationary phase components	Reduces production and processing costs
Can be used in expanded bed, fluidized bed and traditional column based systems	Enables faster processing times, easy scale up and improved dynamic binding capacity

Solid supports

Mic



P uhr

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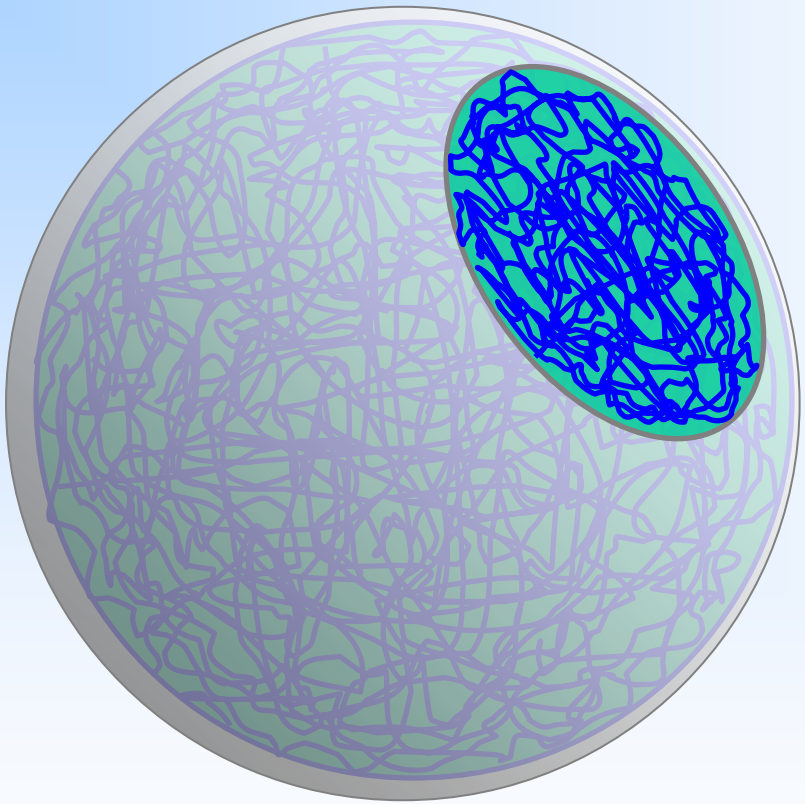
Chromatide technology



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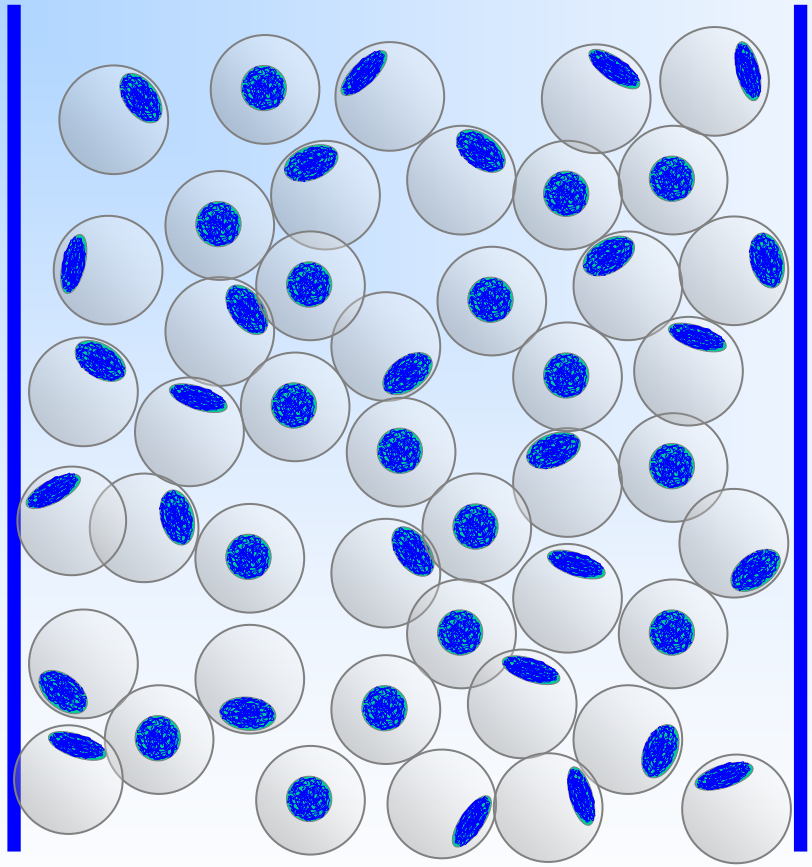
Chromatide technology



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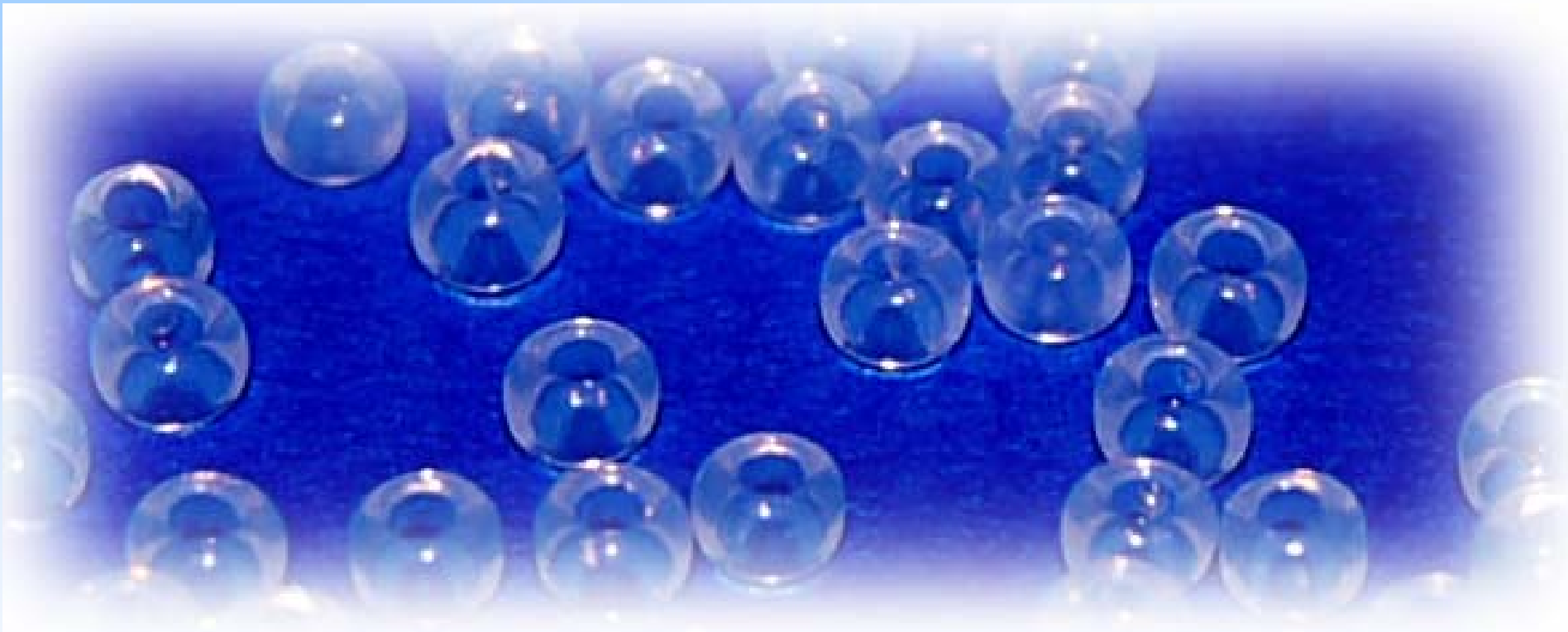
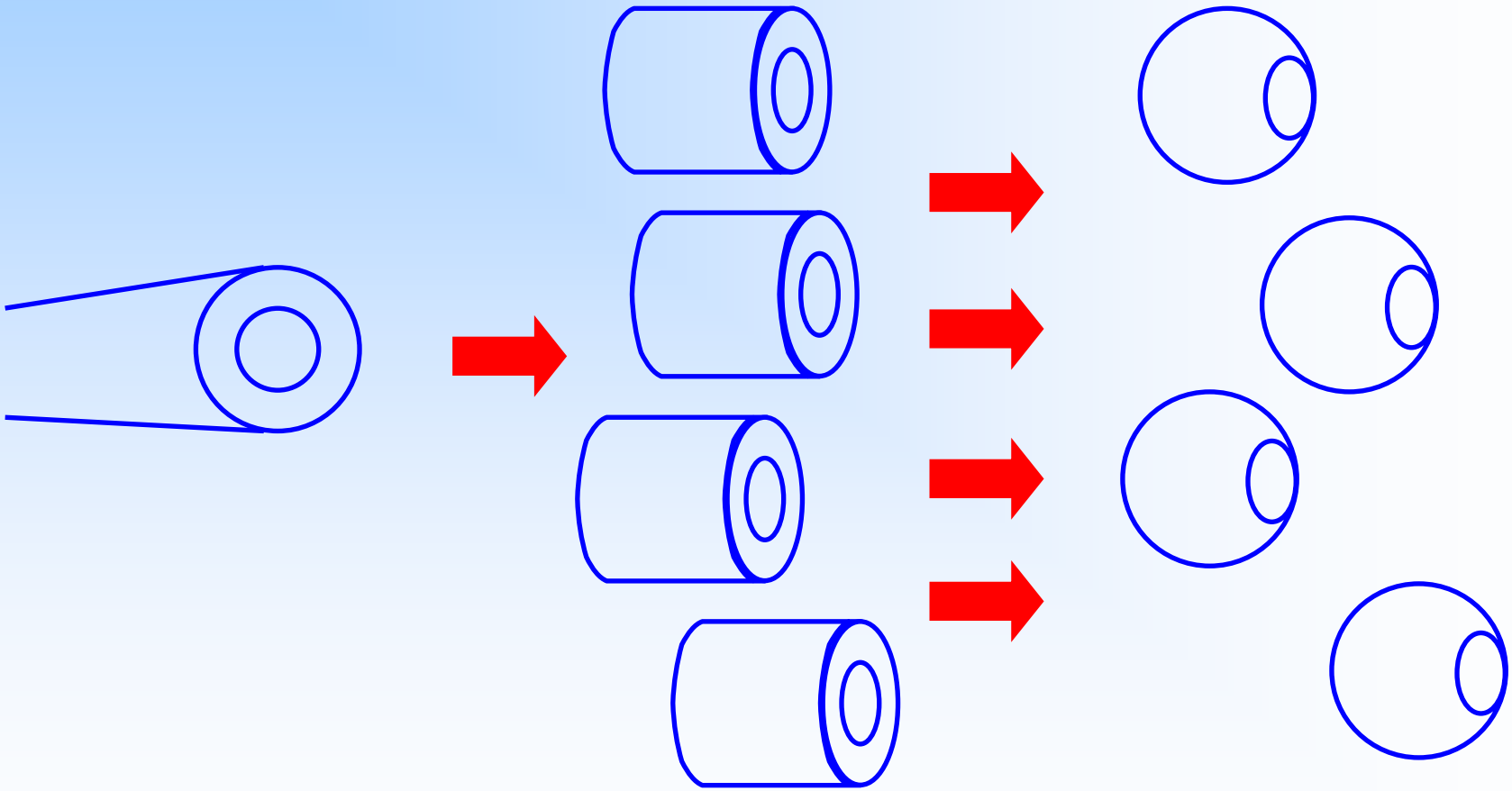
Seed beads



Used in textile and jewellery trade

How are seed beads made?



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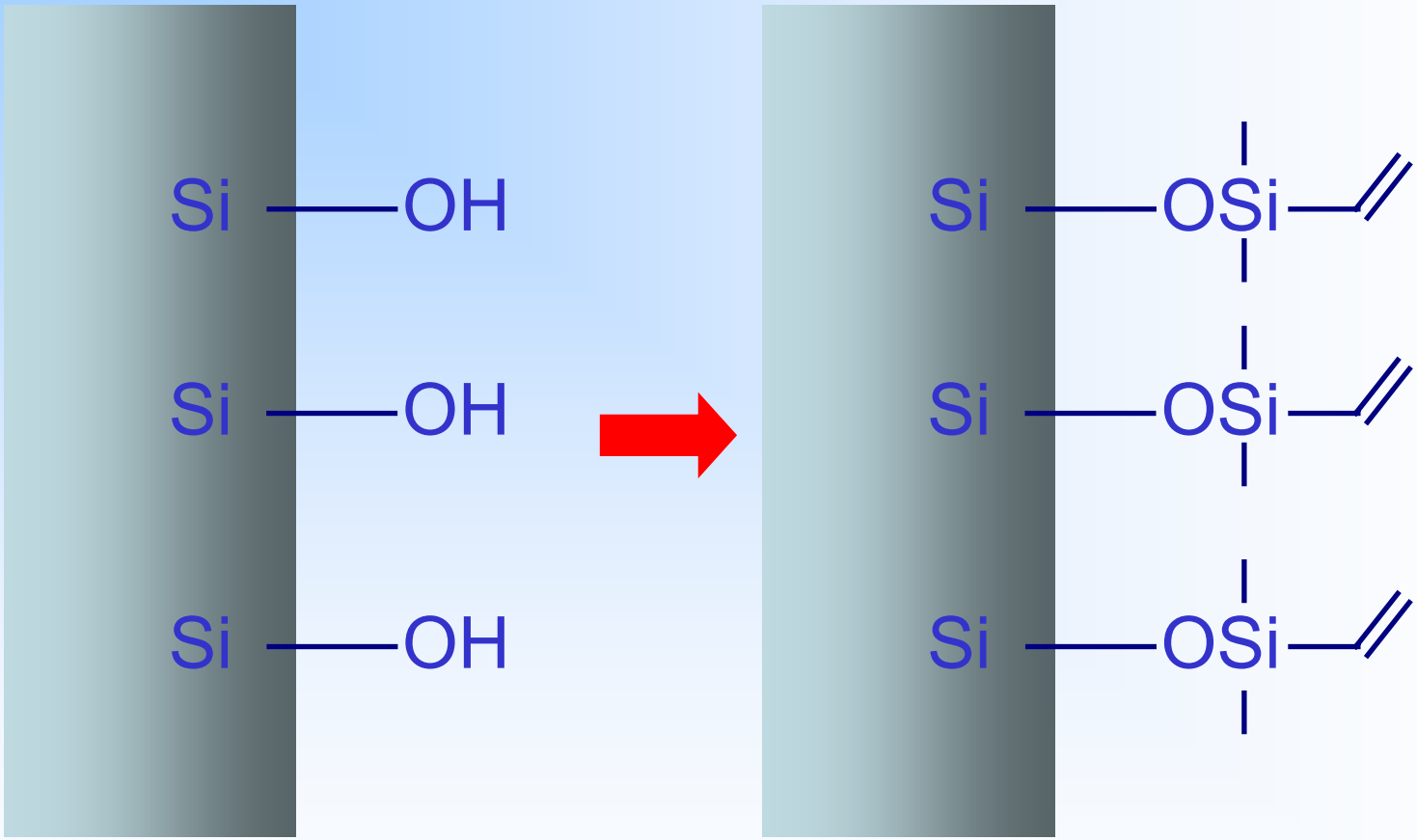
Commercial sources?

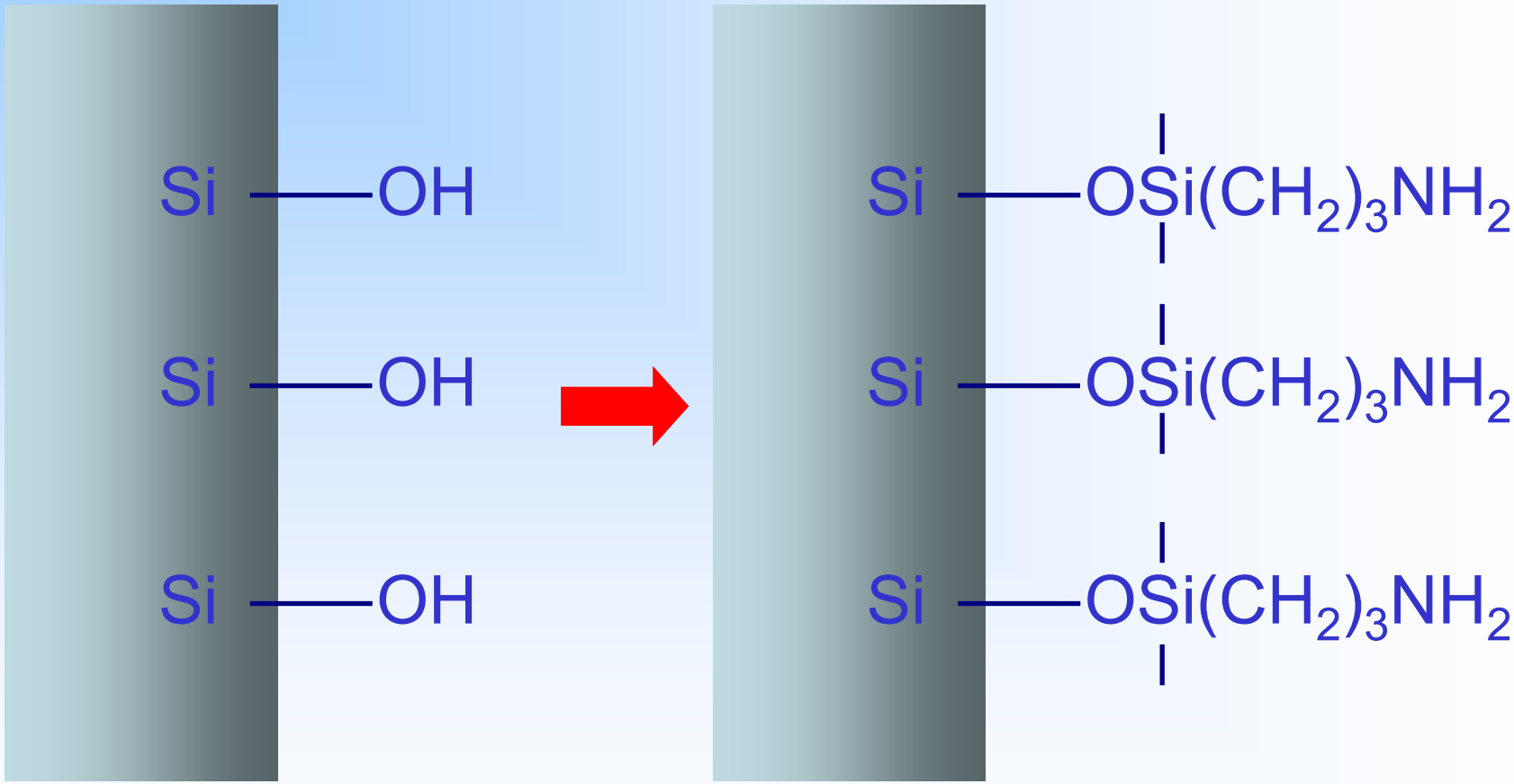
Mass produced

- 30-40 tons per day
- Japan, China, India, Poland
- Smallest commercially available cost from €1-6/kg
- Japanese beads have very precise dimensions

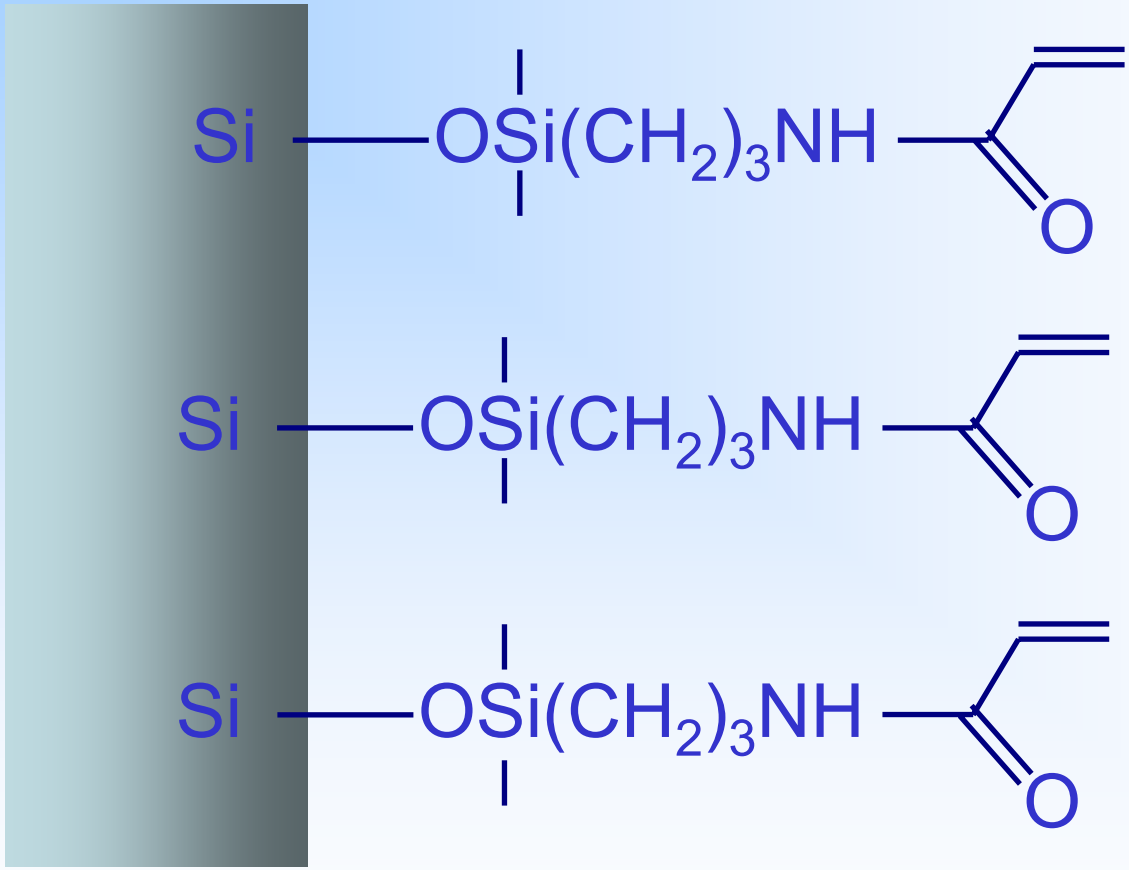
Surface Chemistry



Surface Chemistry



Surface Chemistry



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How do we get the polymer in the hole?

Mix monomer solution with beads

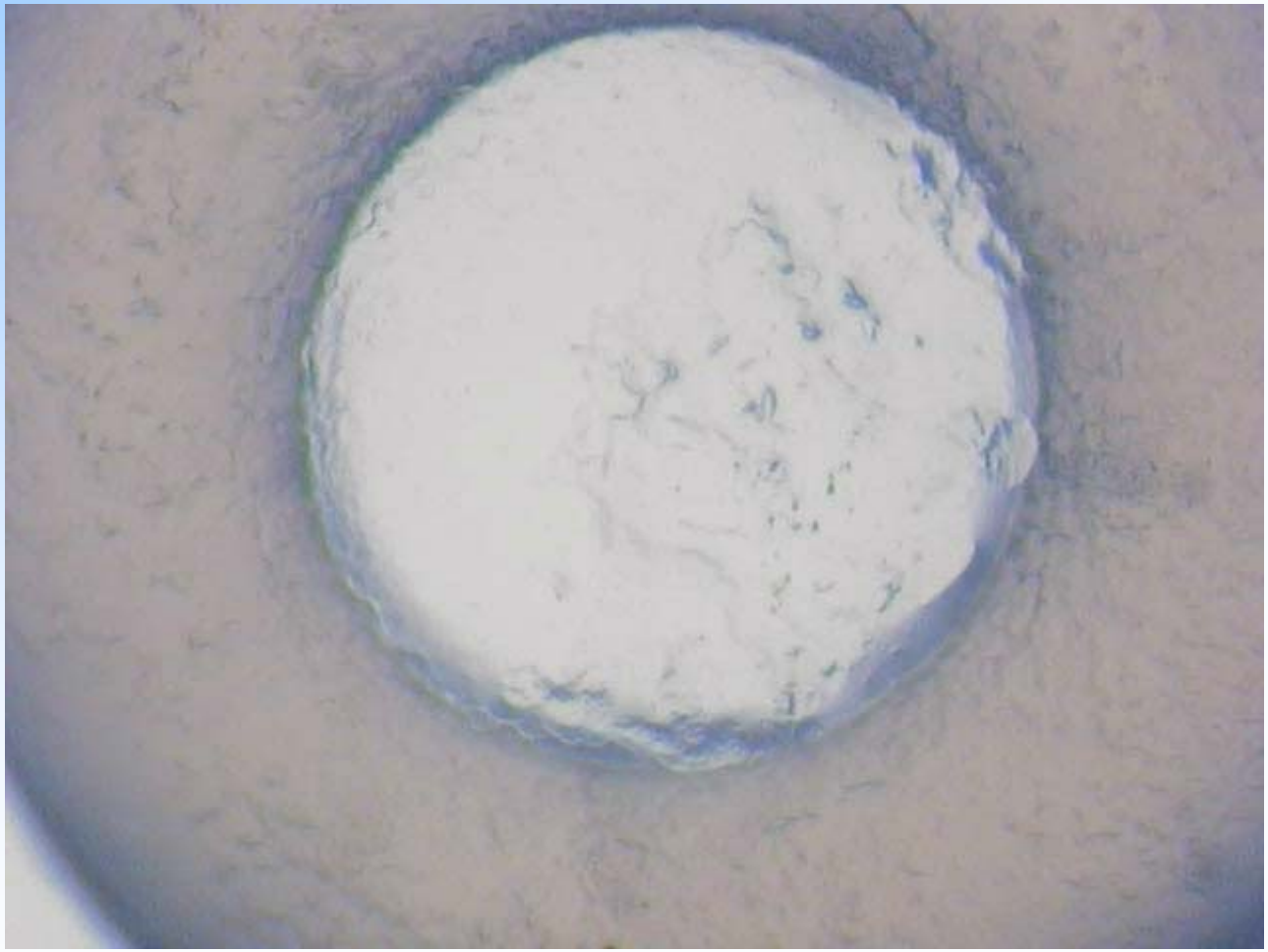
Drain off excess monomer solution

Monomer solution is retained by capillary action

Initiate polymerisation

Remove small particles of polymer from outside of beads by abrasion

Polydimethylacrylamide



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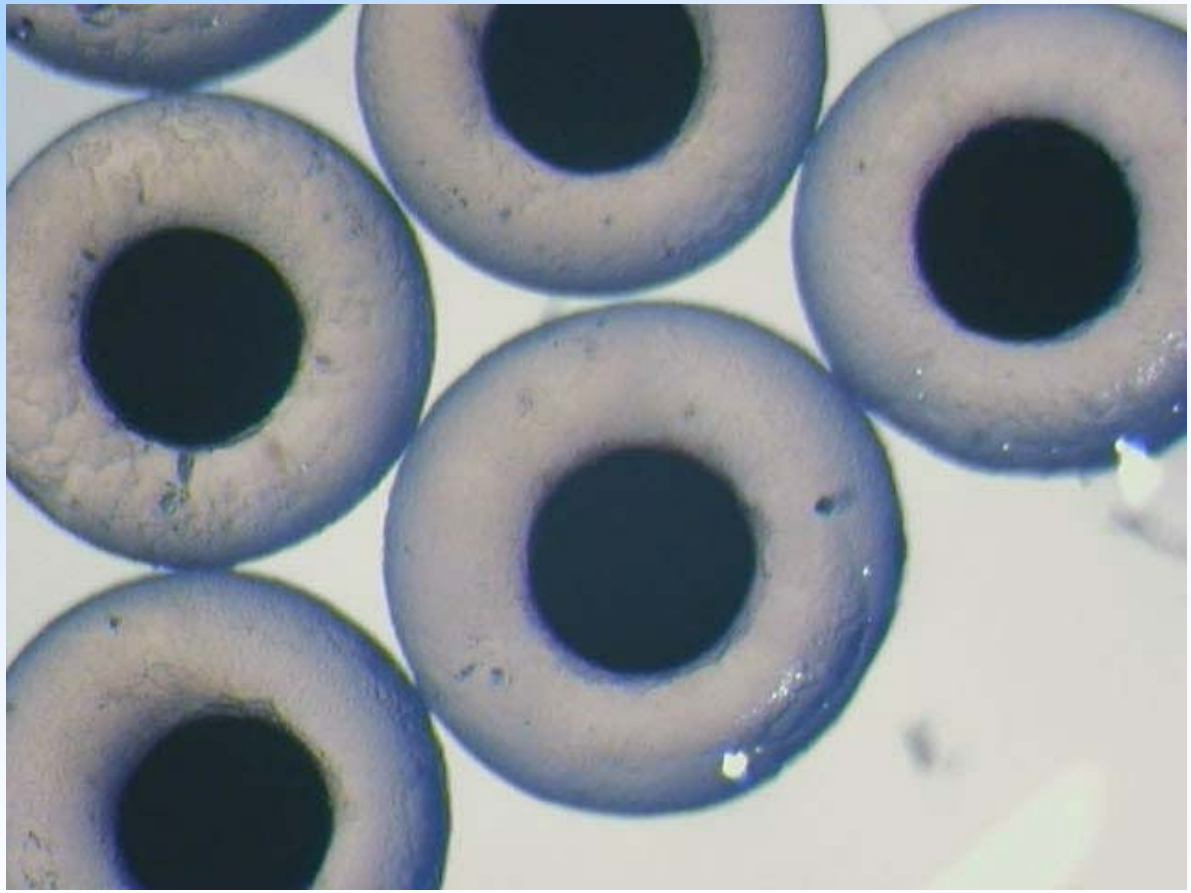
Polydimethylacrylamide (Ninhydrin)



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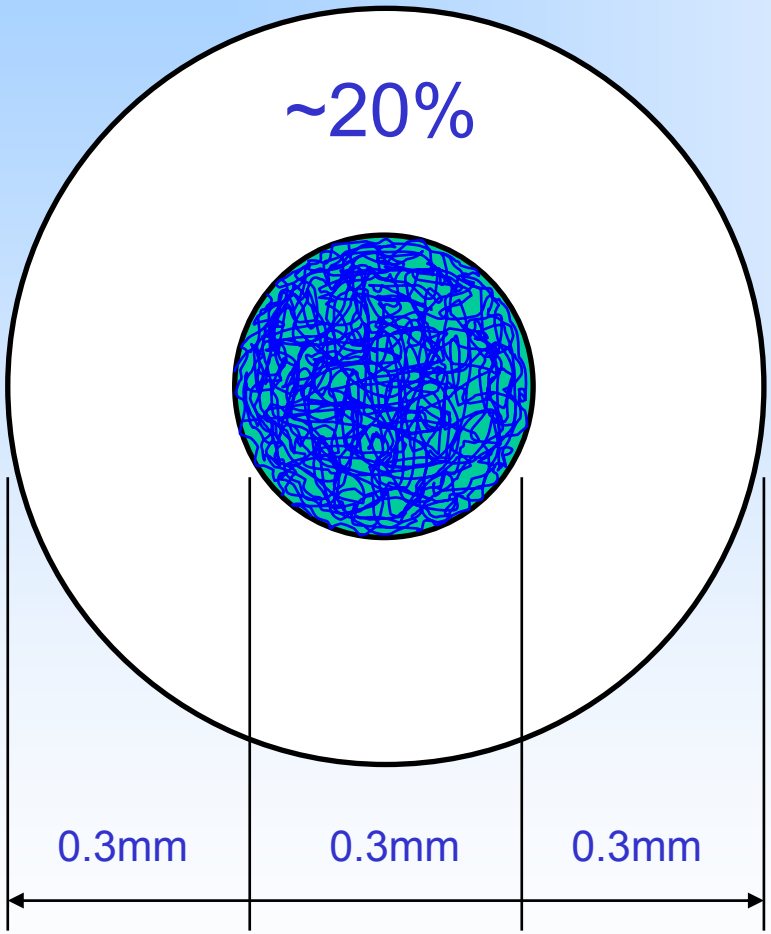
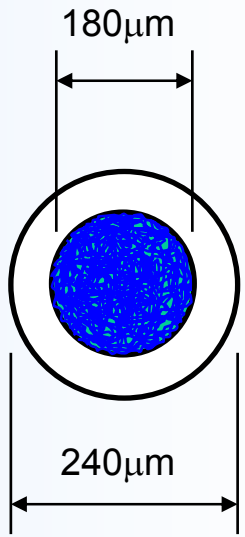
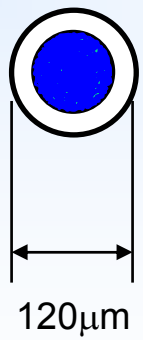
Bead capacity and size



60%





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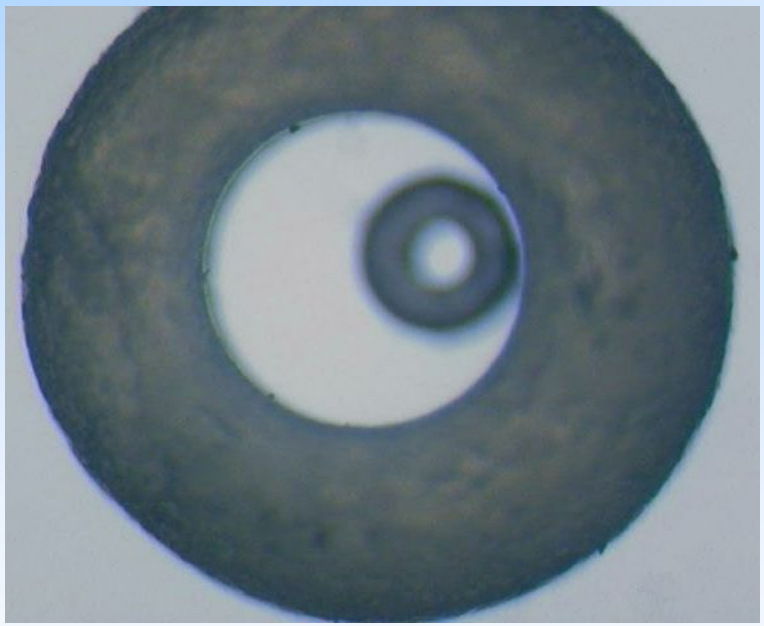
Smaller beads!



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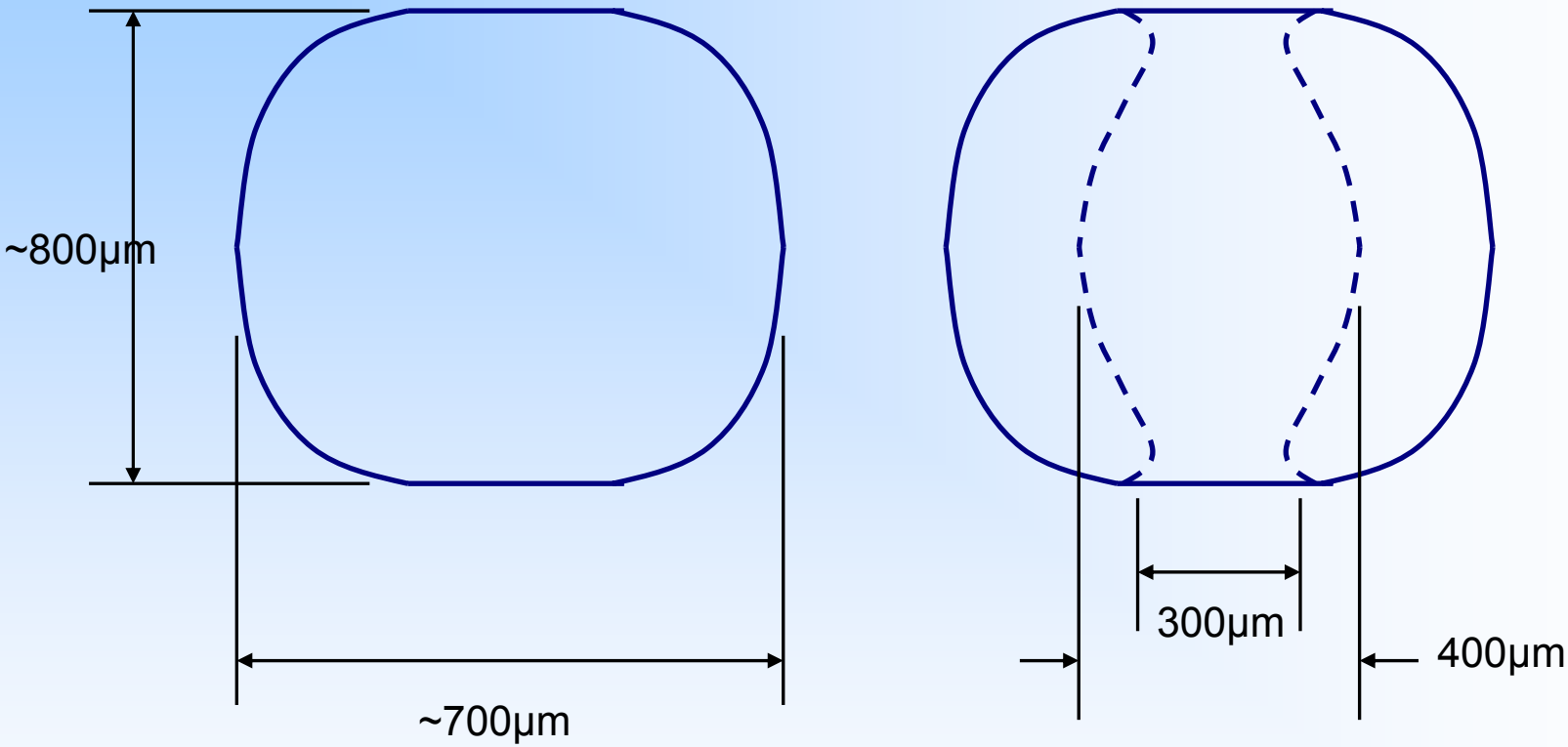
Custom made seed beads!



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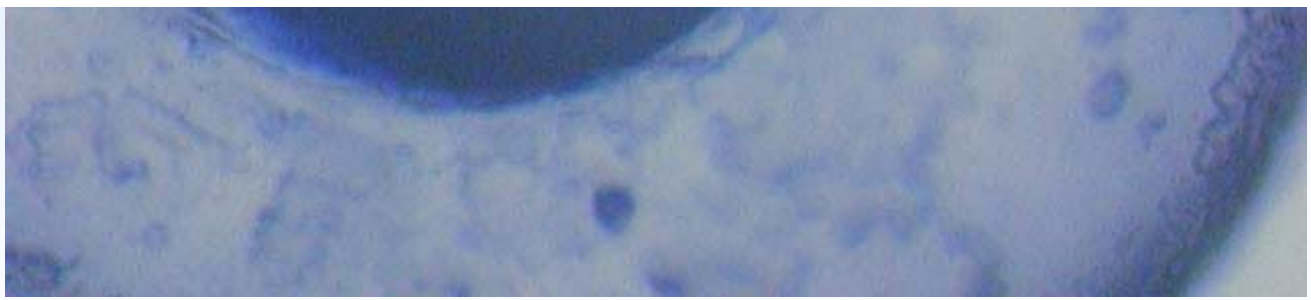
Custom made seed beads!

~40% of bead volume is polymer
(determined by Fmoc analysis)



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Enzyme immobilisation

Polymer/beads

Types
Linkers

Enzyme immobilisation

Penicillin G amidase
Catalase
Glucose oxidase

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Polymerisations

Polydimethyl acrylamide

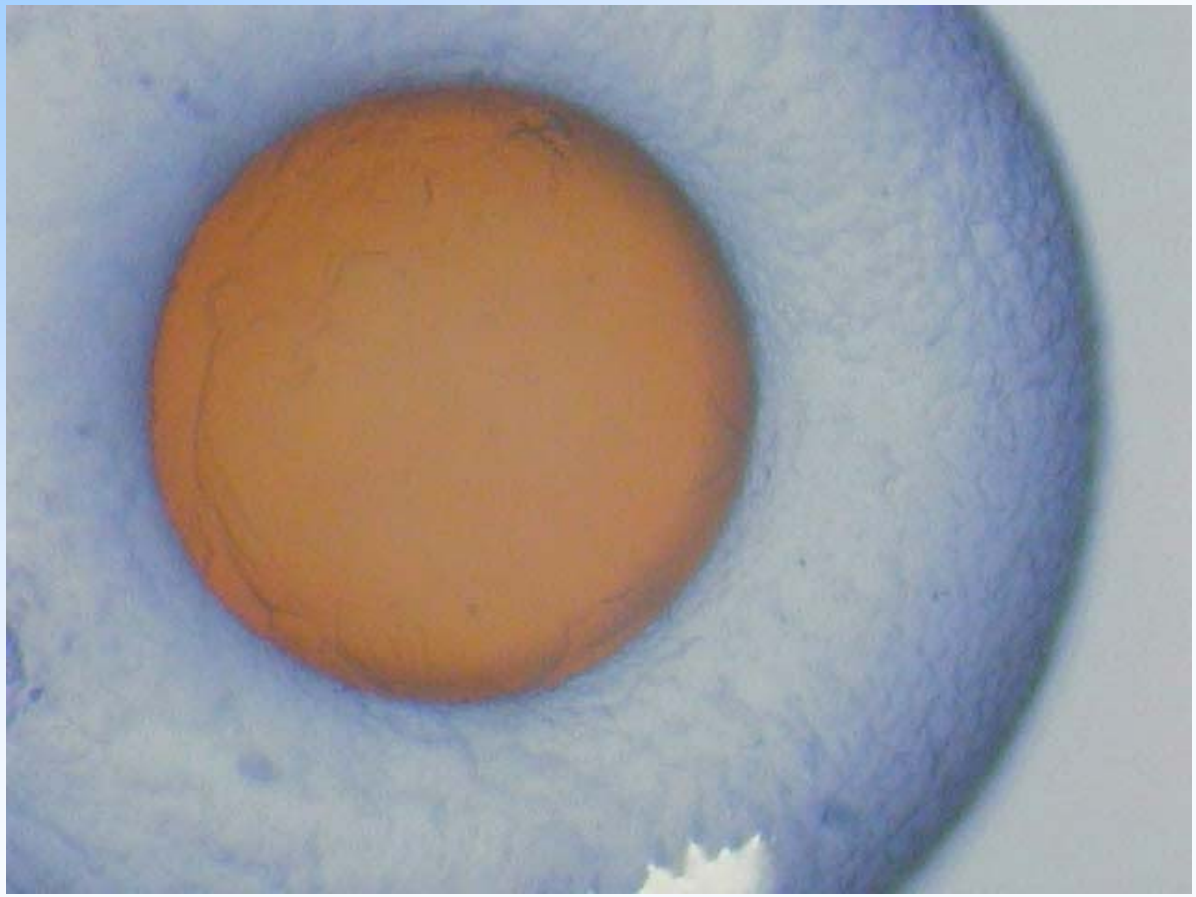
2-Aminoethylmethacrylate hydrochloride

Acryloylsarcosine methyl ester
(followed by treatment with 1,2-diaminoethane)

CLEAR (PEG based)

Polymer loading	up to 2 mmole/g
Cross linking	3 mole% of monomers

Enzyme immobilisation



Penicillin amidase

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Penicillin G Amidase

Used in immobilised form in the manufacture of Amoxycillin

Converts Penicillin G to 6-amino penicinillic acid

Penicillin G Amidase

Activation of support bound amino groups and amino groups on enzyme with glutaraldehyde

Potassium phosphate buffer, pH 7

Activity assay:

Penicillin G conversion to 6-amino penicillanic acid (Dimethylaminobenzaldehyde at 37°C, 415nm, 1 unit is amount of enzyme converting 1µmole in 1 minute under assay conditions)

Preliminary Results

Ref.	Enzyme	Polymer function	Enzyme linkage	Activity (U/g)
66	Penicillin G Amidase	amino ethyl methacrylate:HCl	glutaraldehyde	10.1
76	Penicillin G Amidase	acryloyl sarcosine methyl ester/1,2-diaminoethane	glutaraldehyde	10.1
82	Catalase	amino ethyl methacrylate:HCl	glutaraldehyde	5.3
83	Catalase	amino ethyl methacrylate:HCl	glutaraldehyde	8.1

Activity Comparisons

Activity of "wet" beads	9 U/cm^3
Activity of “dry" beads	13 U/cm^3
Activity of “dry" polymer in beads	420 U/cm^3

Penicillin G amidase on Eupergit has an activity of ~120U/g

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Latest Results

Activity now at 27U/cm^3 of immobilised enzyme

Activity of immobilised enzyme per gram of polymer is now more than 10 times greater than Eupergit

>50% of enzymatic activity is retained upon immobilisation

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Current Focus in Biocatalysis

Immobilisation of Lipases

Immobilisation of whole cells

Yeast and Bacteria

Ketoreductase immobilisation

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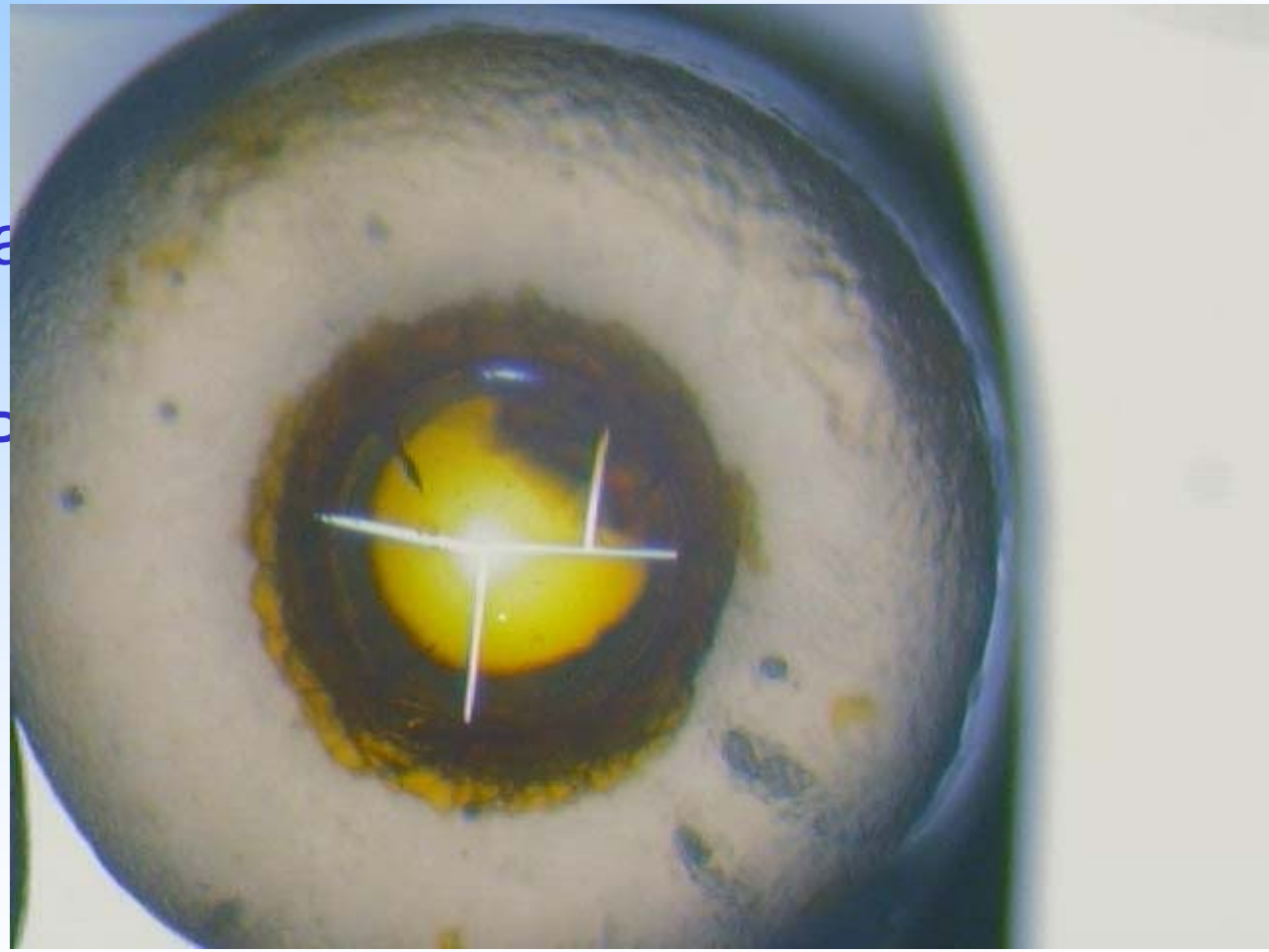
PdEncat™



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The Future for Immobilised Biocatalysts

More efficient use of catalyst and polymer

Move towards continuous flow systems

Multi-column processes in series

Environmentally friendly chemistry

Acknowledgements

Dean Simpkin

Our industrial and academic collaborators

Matthew Tidmarsh

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Close

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