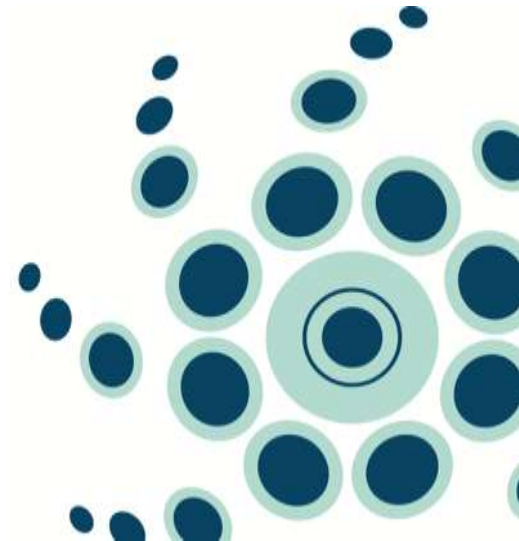


# Bioengineered Protein-Polymer Beads (PolyBind™) for Affinity Purification

Andy Herbert, Ph.D.

VP Development, PolyBatics Ltd

[a.herbert@polybatics.com](mailto:a.herbert@polybatics.com)



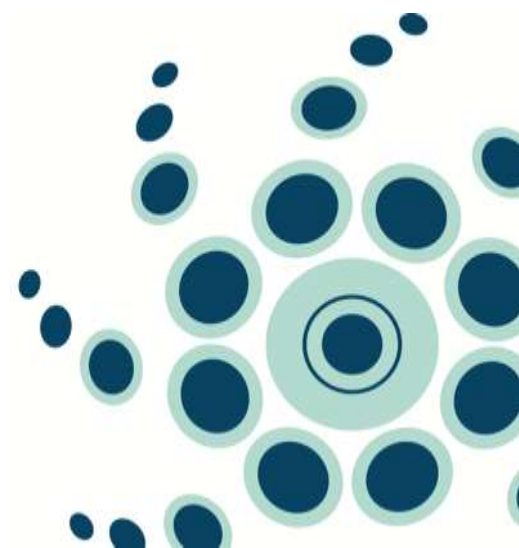
# For what you are about to receive ....

- Introduction to PolyBatics
- Bacterial polyester beads
- Engineering the bead surface
- PolyBind™ affinity purification materials
- How PolyBind™ offers a paradigm shift in protein purification





**PolyBatics Ltd**  
**Palmerston North**

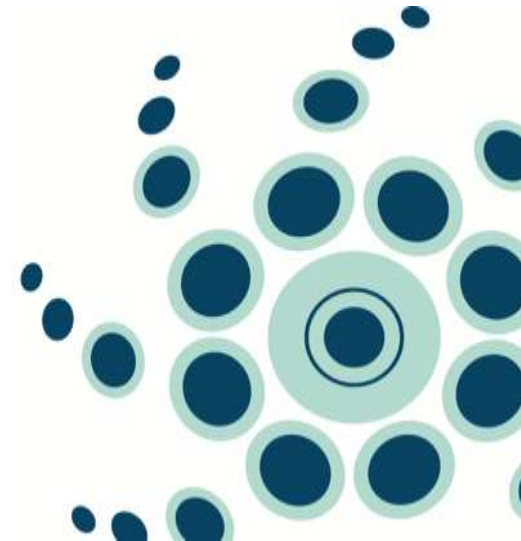


# PolyBatics Ltd

- Spin-out company of Massey University
- Founded in 2009
- 14 Employees
- Technology:  
A disruptive platform technology for cost-effective production of highly functionalized biobeads

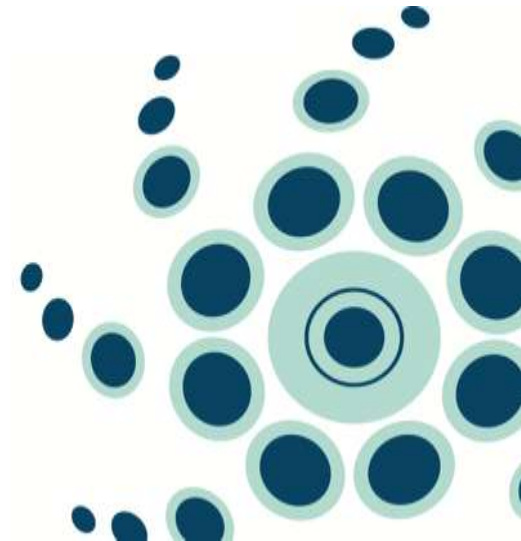
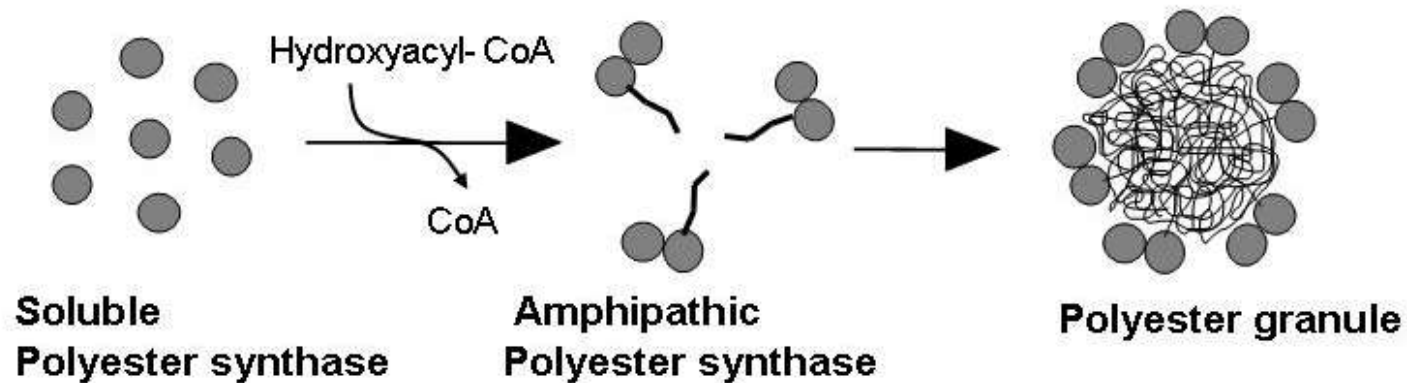
## *Our Vision*

*To help create a cleaner, healthier world through the application of our technology.*

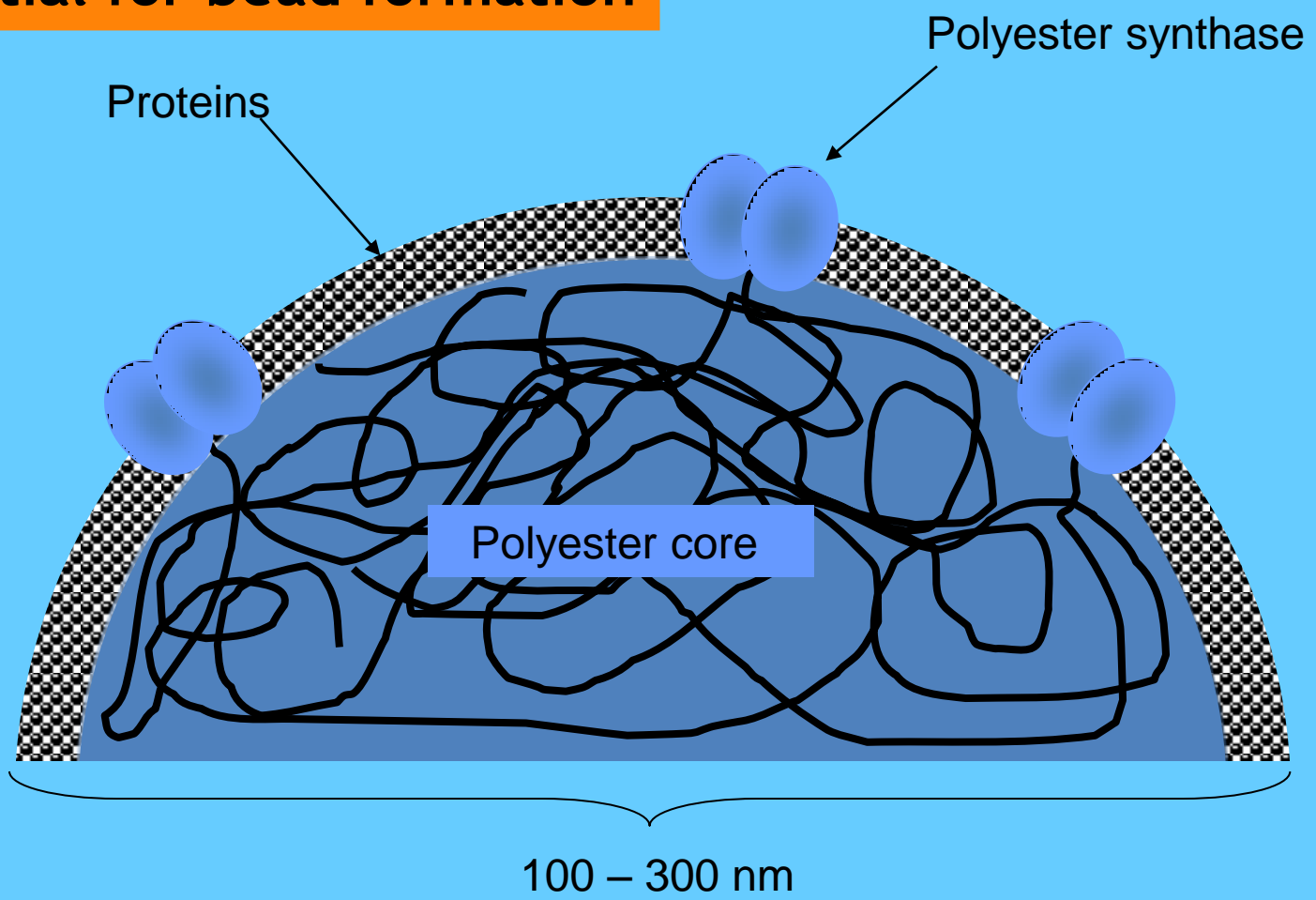




# Biopolyester beads self-assemble within cells

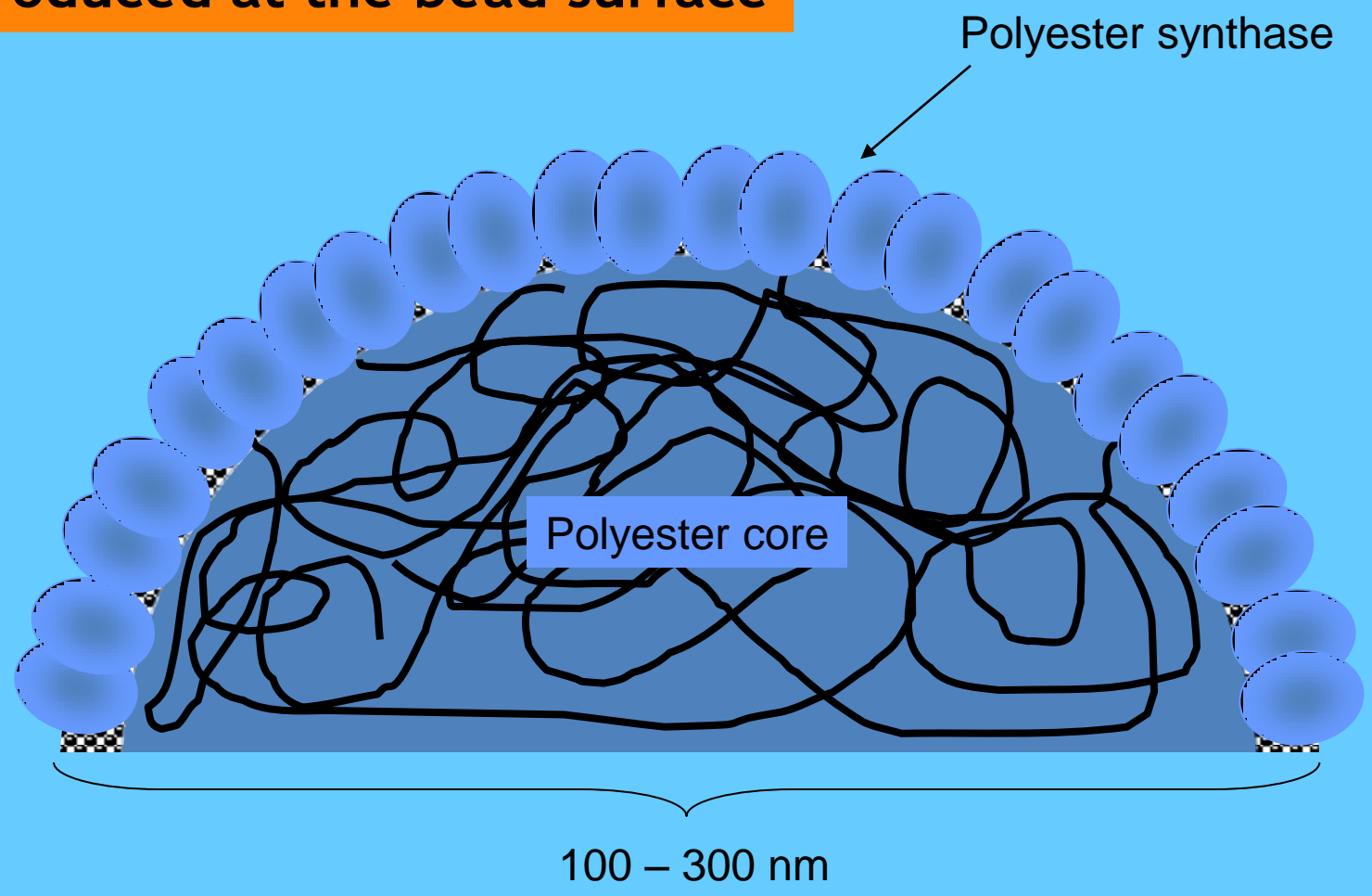


Only the polyester synthase is essential for bead formation



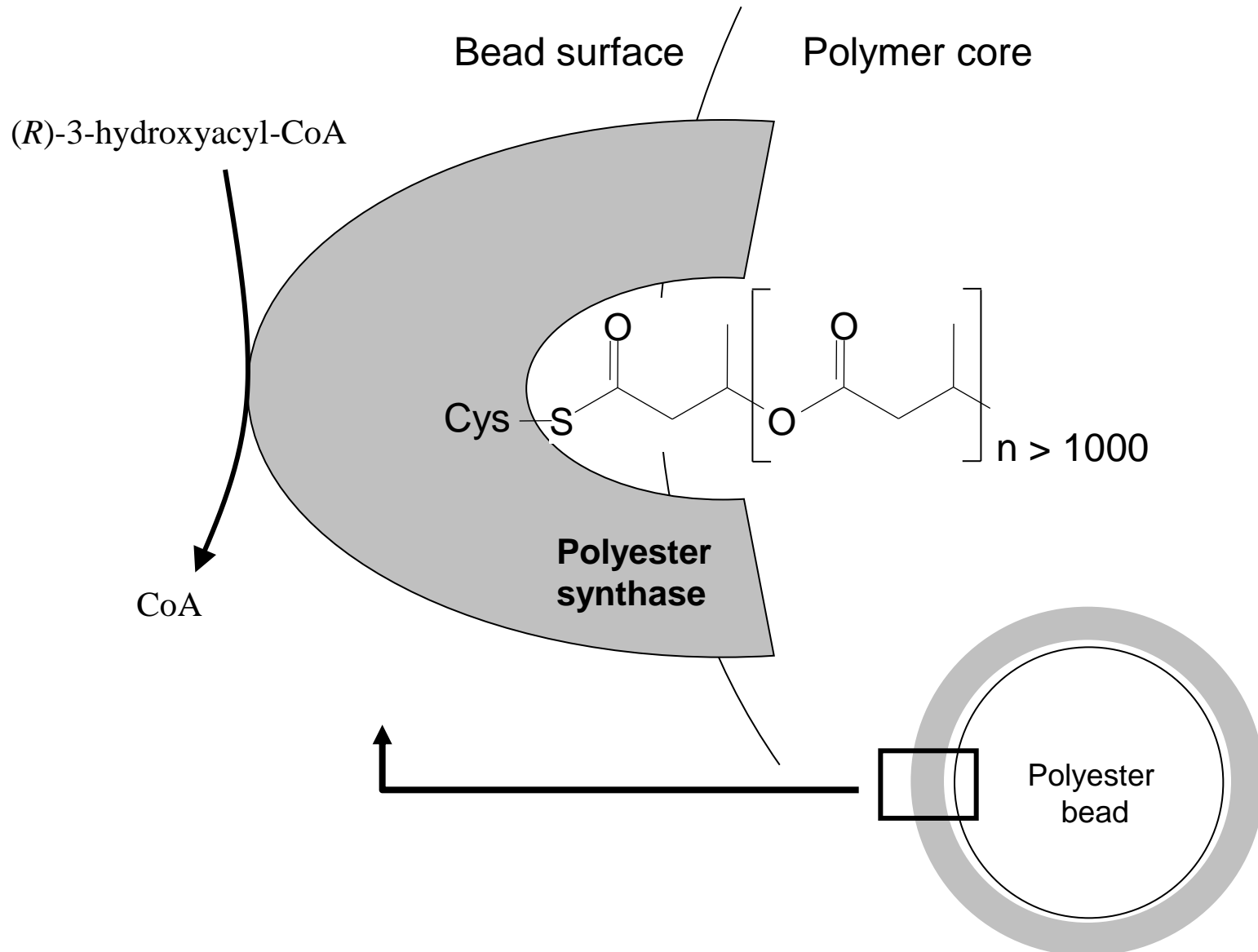
**Polyester bead**

The polyester synthase can be overproduced at the bead surface

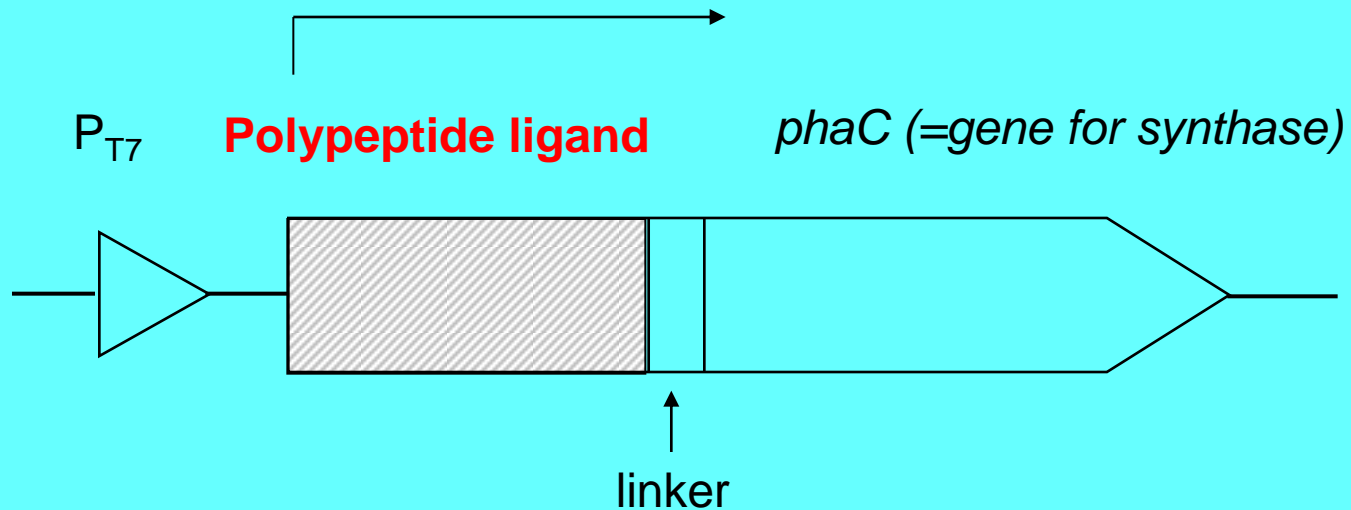


**Polyester bead**

# The synthase has a natural chemical cross-link to the polymer support



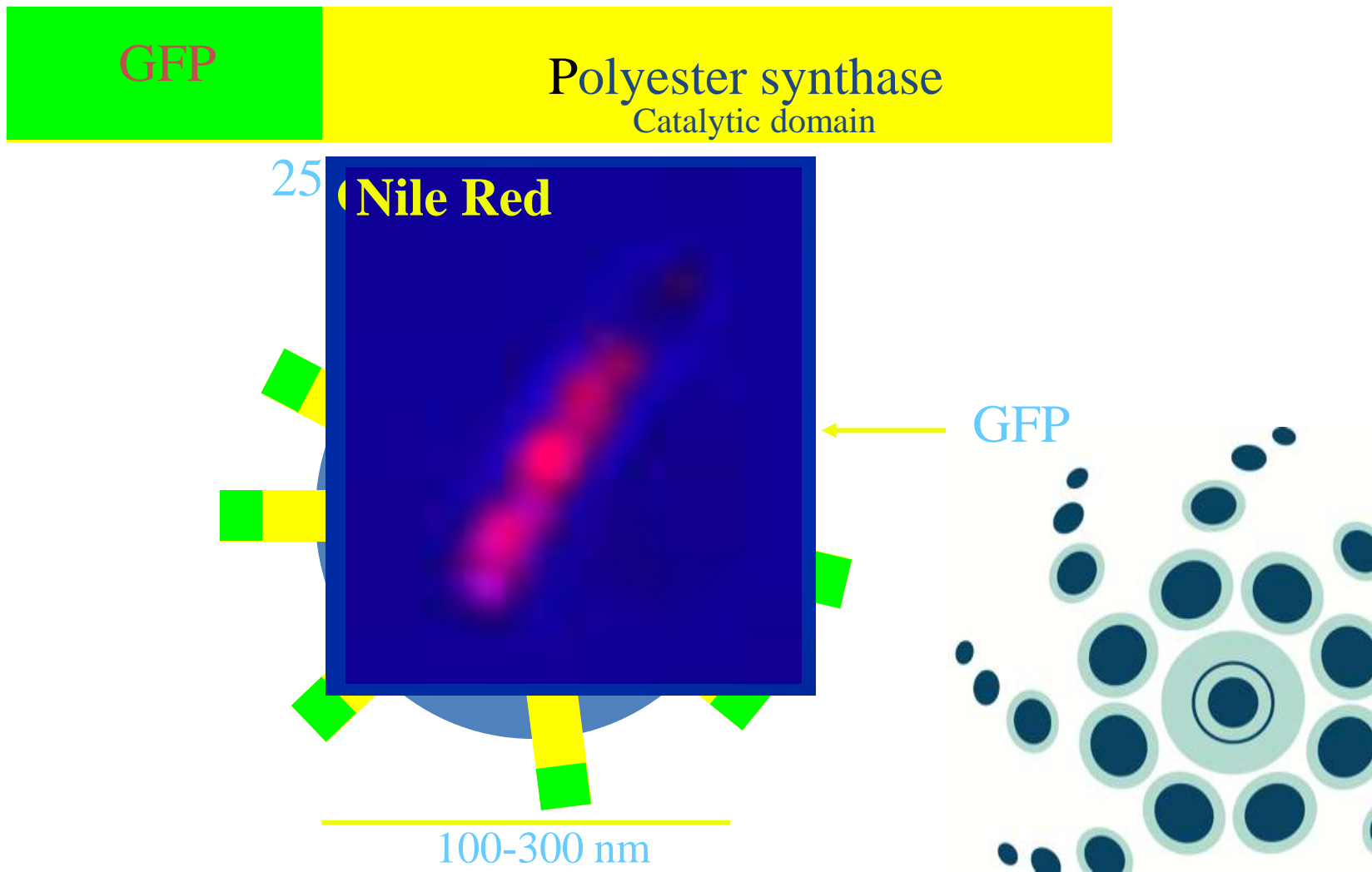
# Hybrid gene technology



Cloning and expression of hybrid gene



A hybrid gene encoding a green fluorescent protein (GFP) -polyester synthase fusion enabled display of GFP at the polyester bead surface

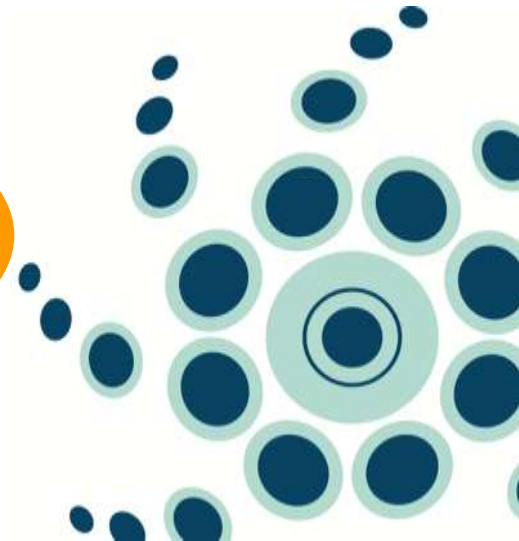


# Bacterial polyester inclusions as a platform technology for the production of tailor-made polymer beads

Protein engineering of polyester synthase provides surface functionalisation of polyester beads



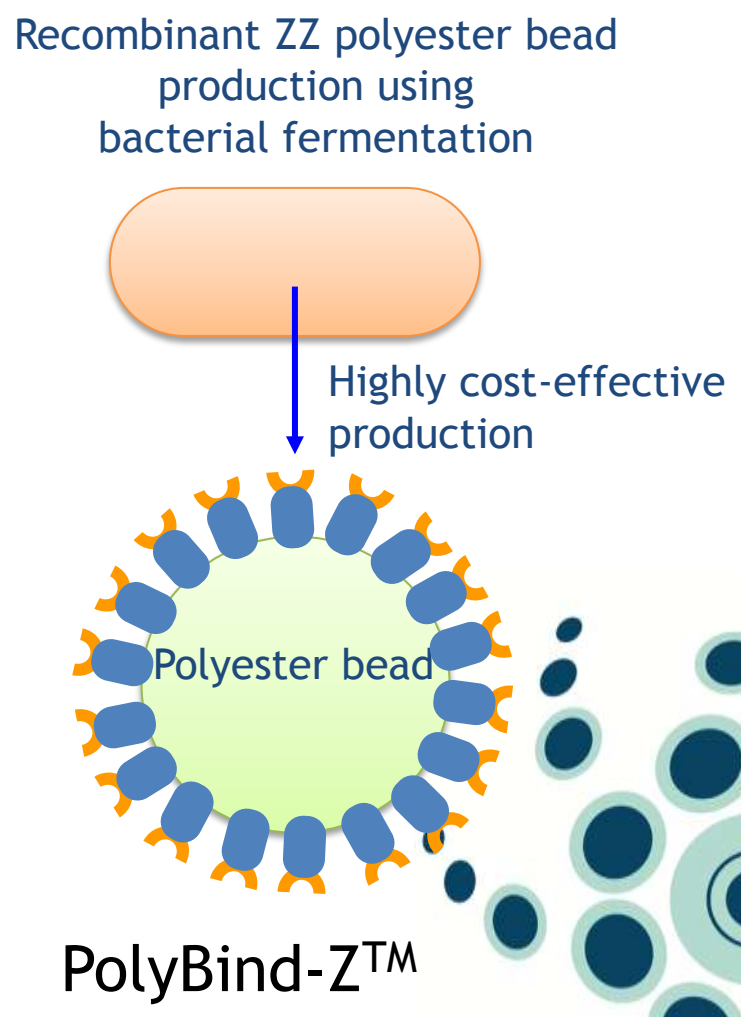
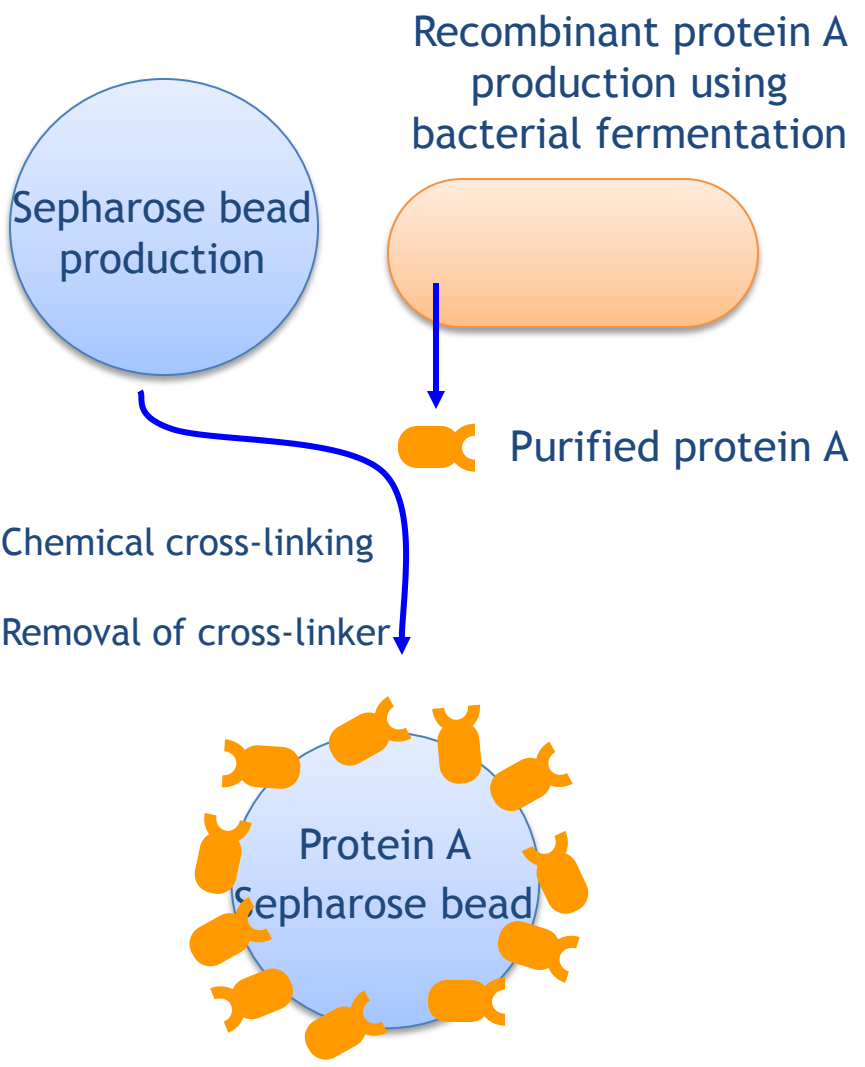
Highly efficient,  
high density,  
surface display  
of binding domains



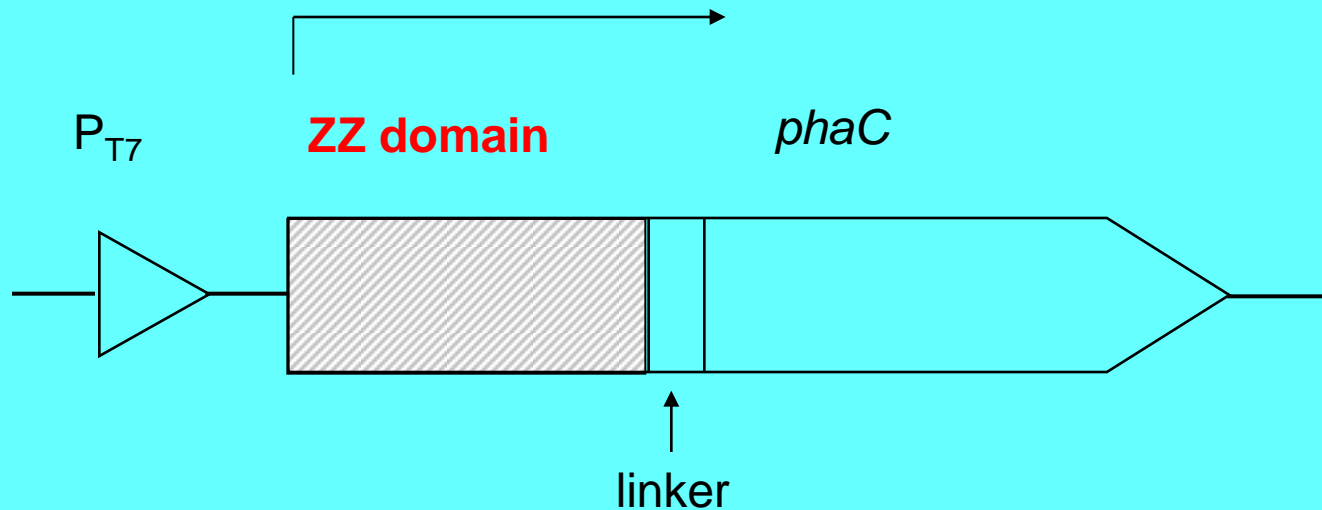
# Manufacture of IgG binding beads

## Conventional

## PolyBatics approach



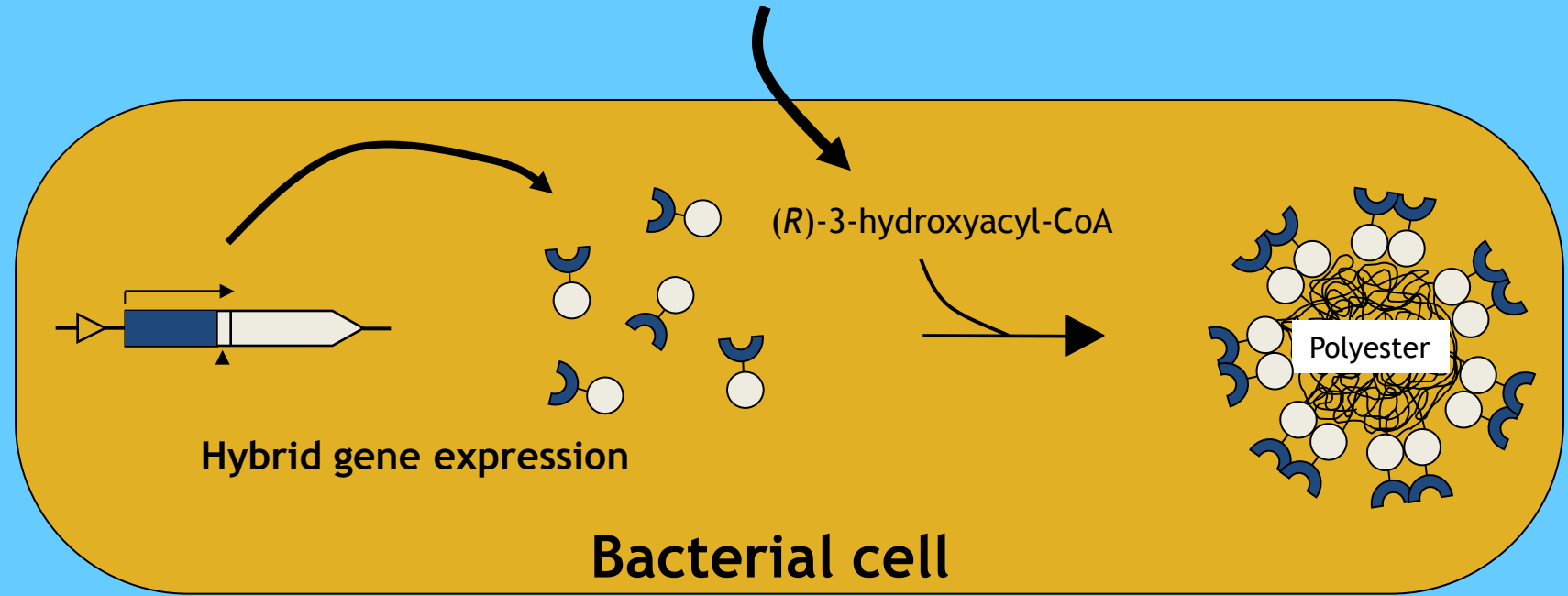
# Hybrid gene technology



Cloning and expression of hybrid gene

# Production in the bacterial cell

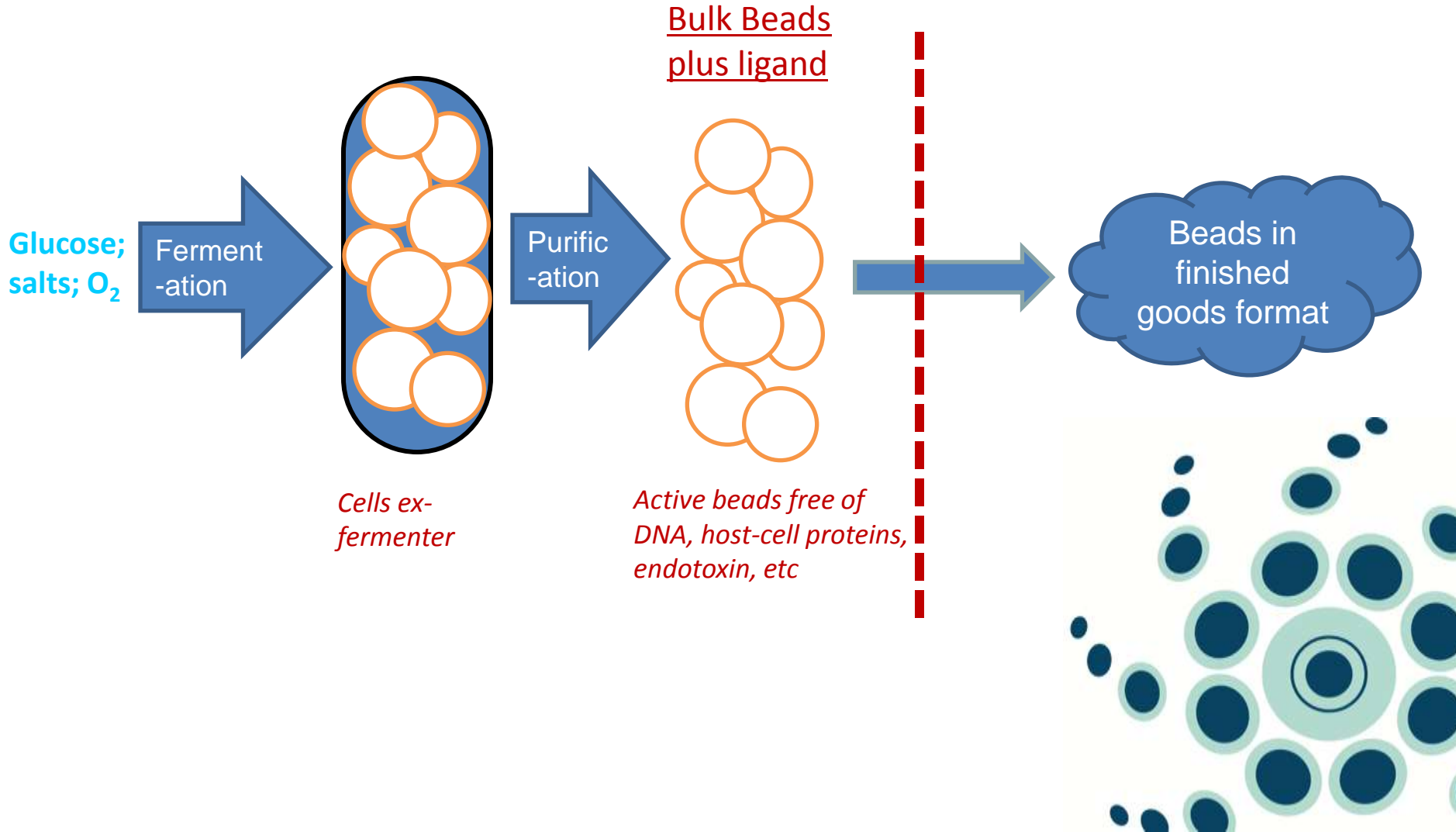
Glucose/Carbon source



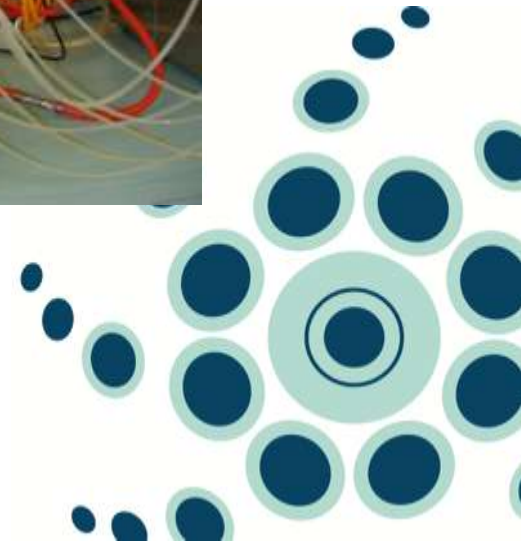
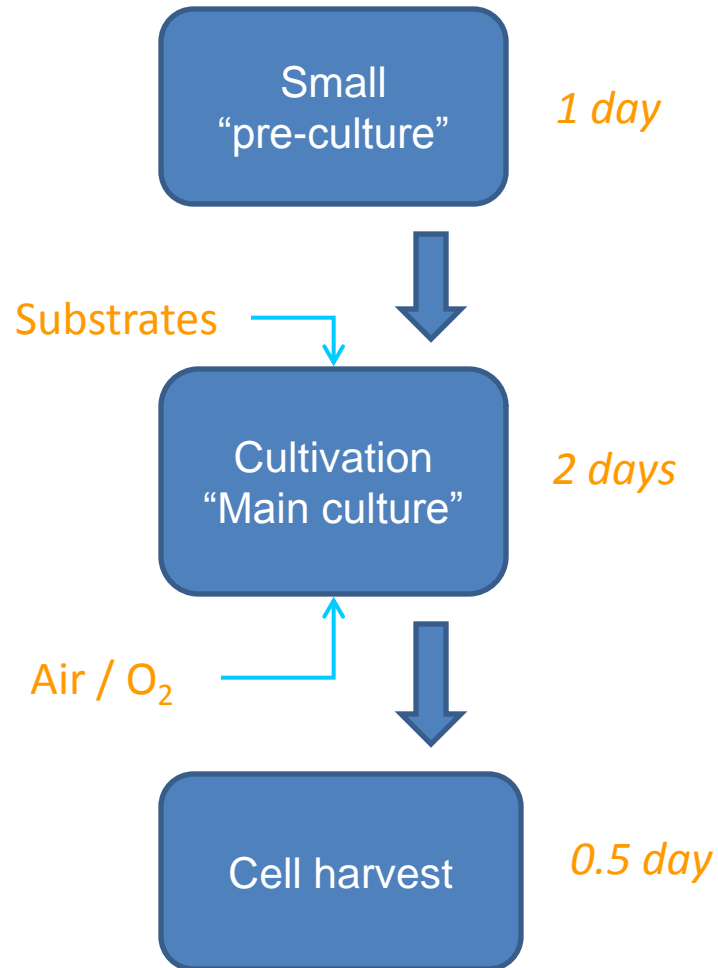
ZZ domain displayed at polyester granule

- ZZ domain
- Linker
- synthase

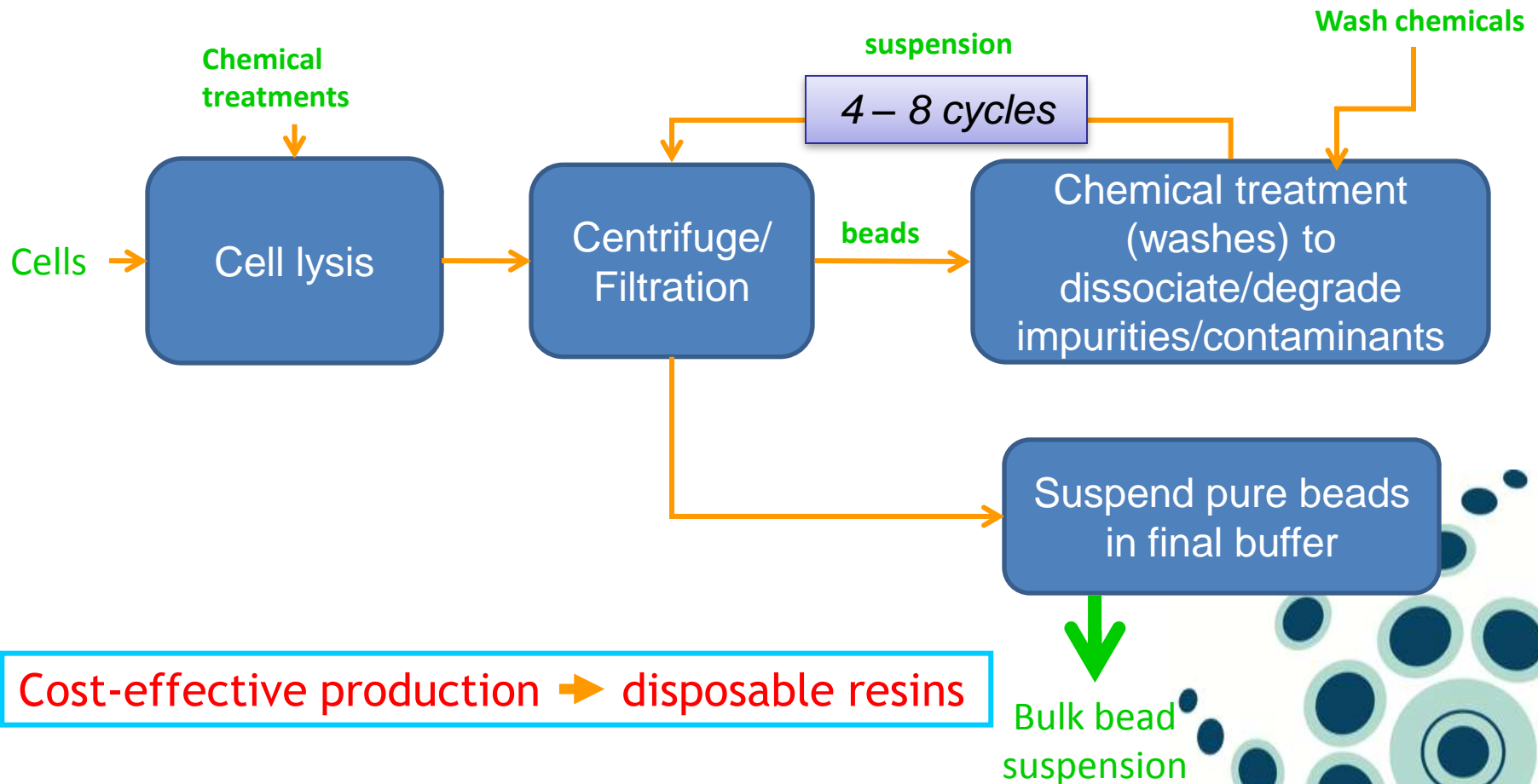
# Polybatics Manufacturing Process



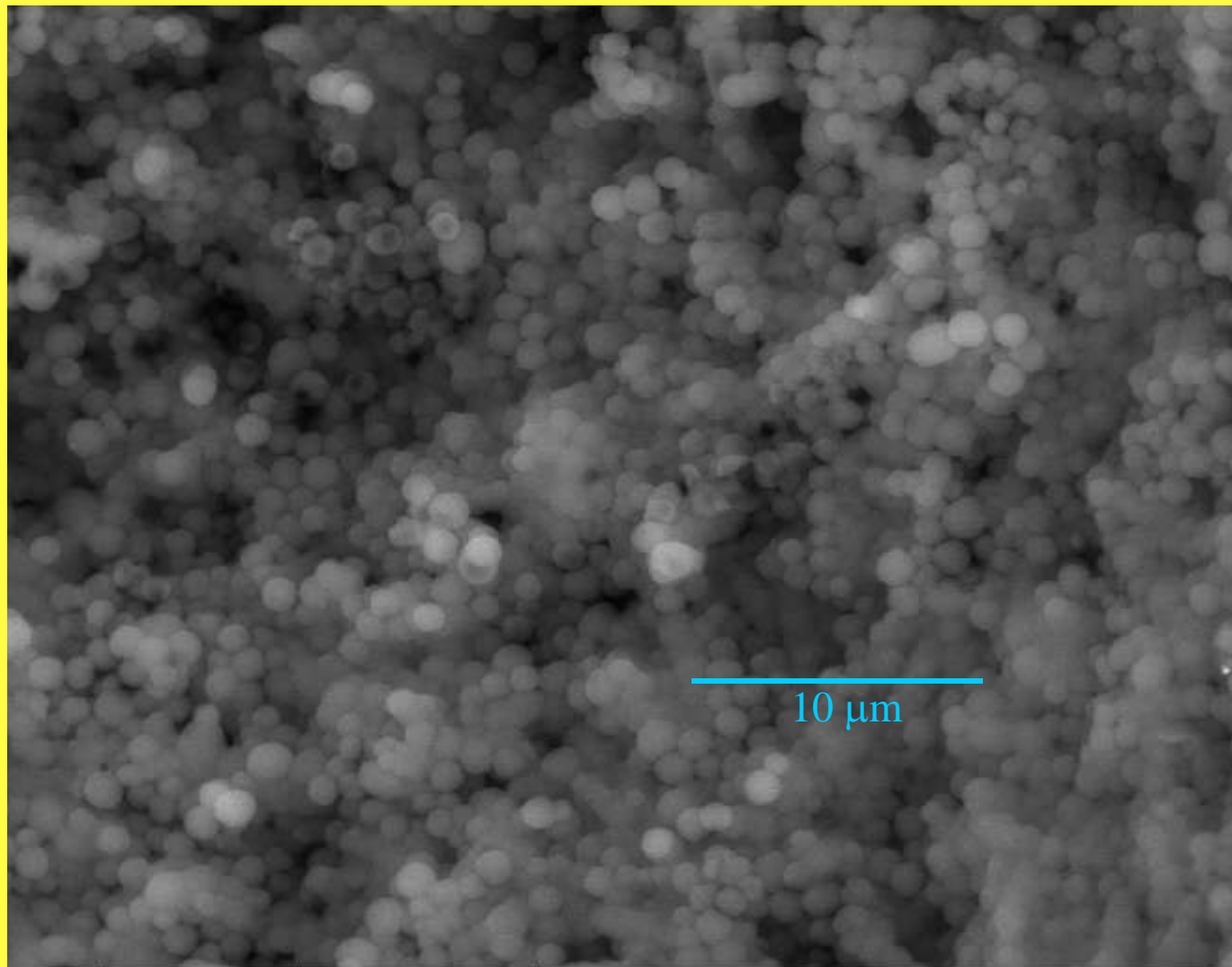
# Fermentation Process



# Purification Process

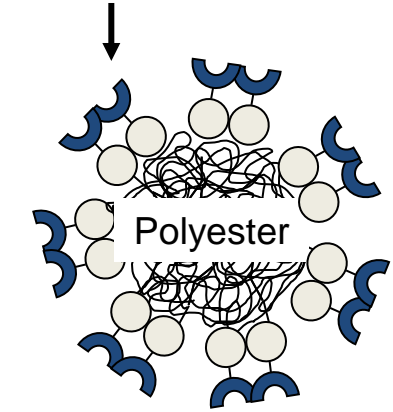


# Electron microscopy image of dried PolyBind-Z™

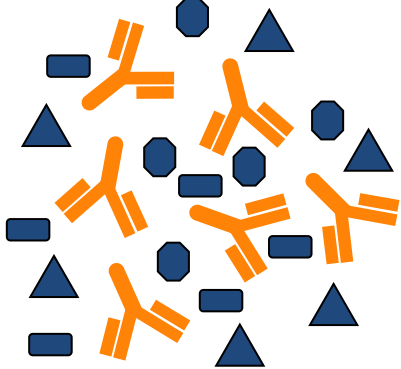


# Application: PolyBind-Z™

ZZ domain ligand



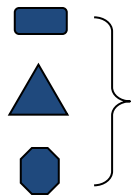
pH 7.0



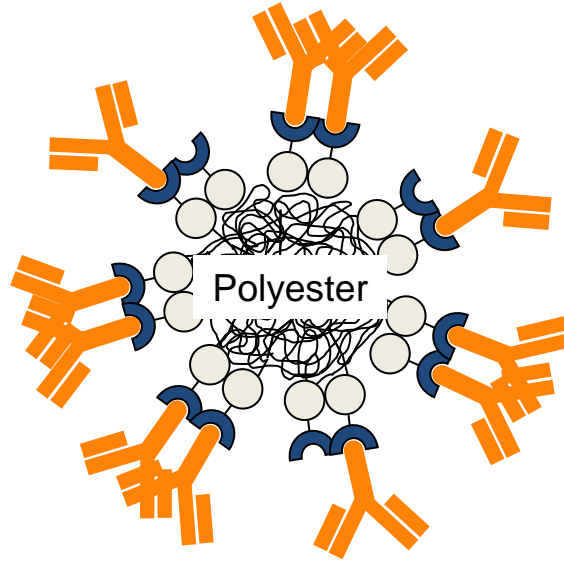
Crude antibody solution



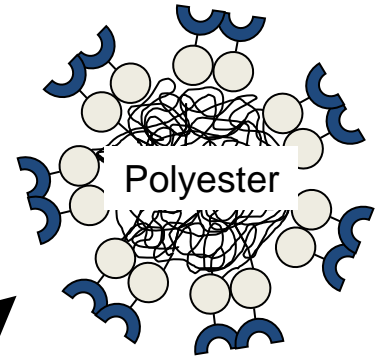
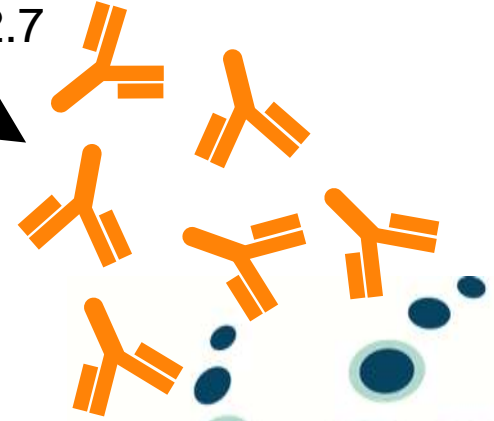
IgG



Other species



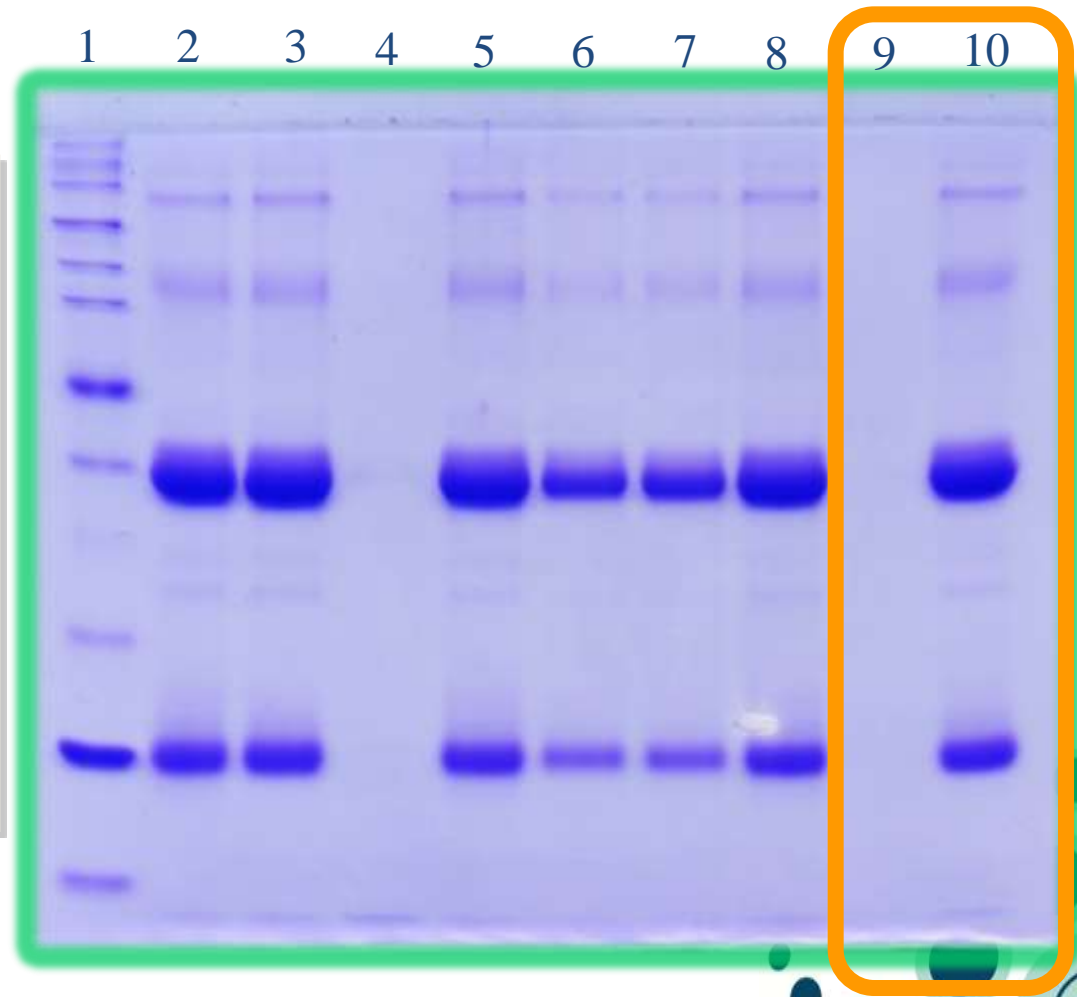
pH 2.7



# Human IgG binding and recovery

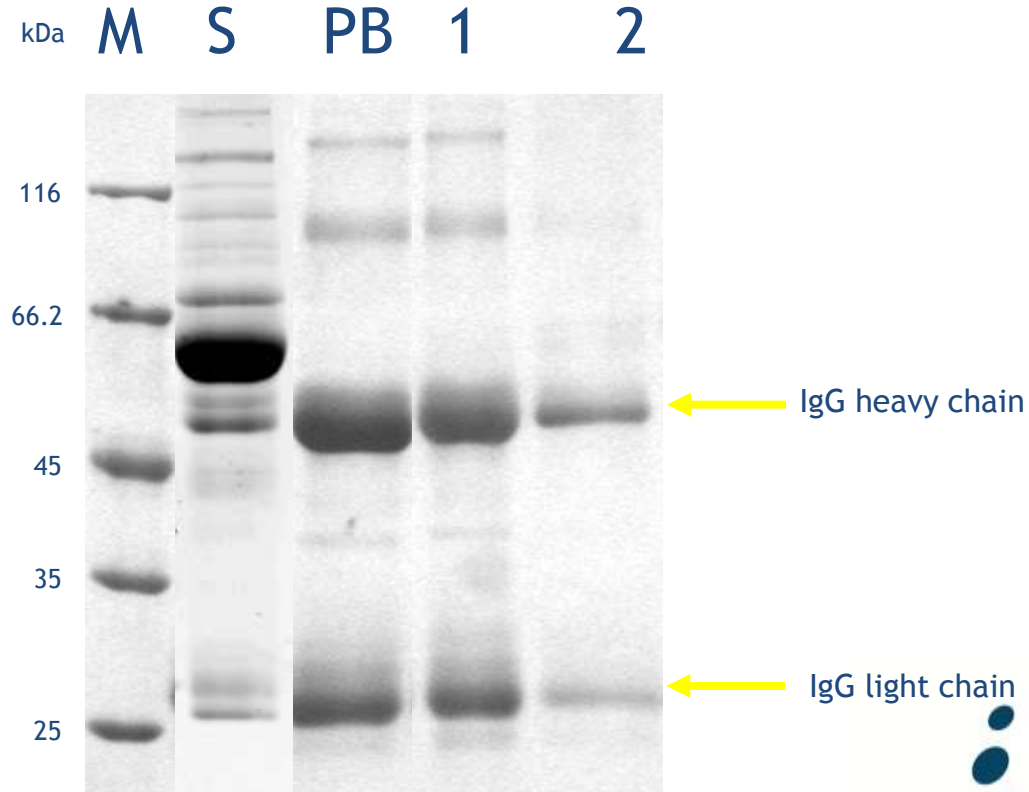
- 1: BioLabs Ladder
- 2: Pure IgG (5 g/L)
- 3: PB WT Unbound
- 4: PB WT Elution
- 5: Pure IgG (5 g/L)
- 6: Protein A Sepharose Unbound
- 7: Protein A Sepharose Elution
- 8: Pure IgG (5 g/L)
- 9: PolyBind-Z™ Unbound
- 10: PolyBind-Z™ Elution

5µL loaded



100 mg human IgG/g drained beads

# Comparison of protein profiles of elution fractions using human serum

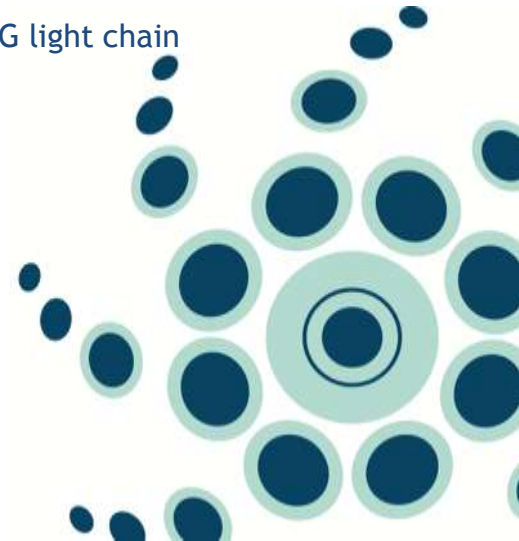


S, Human serum

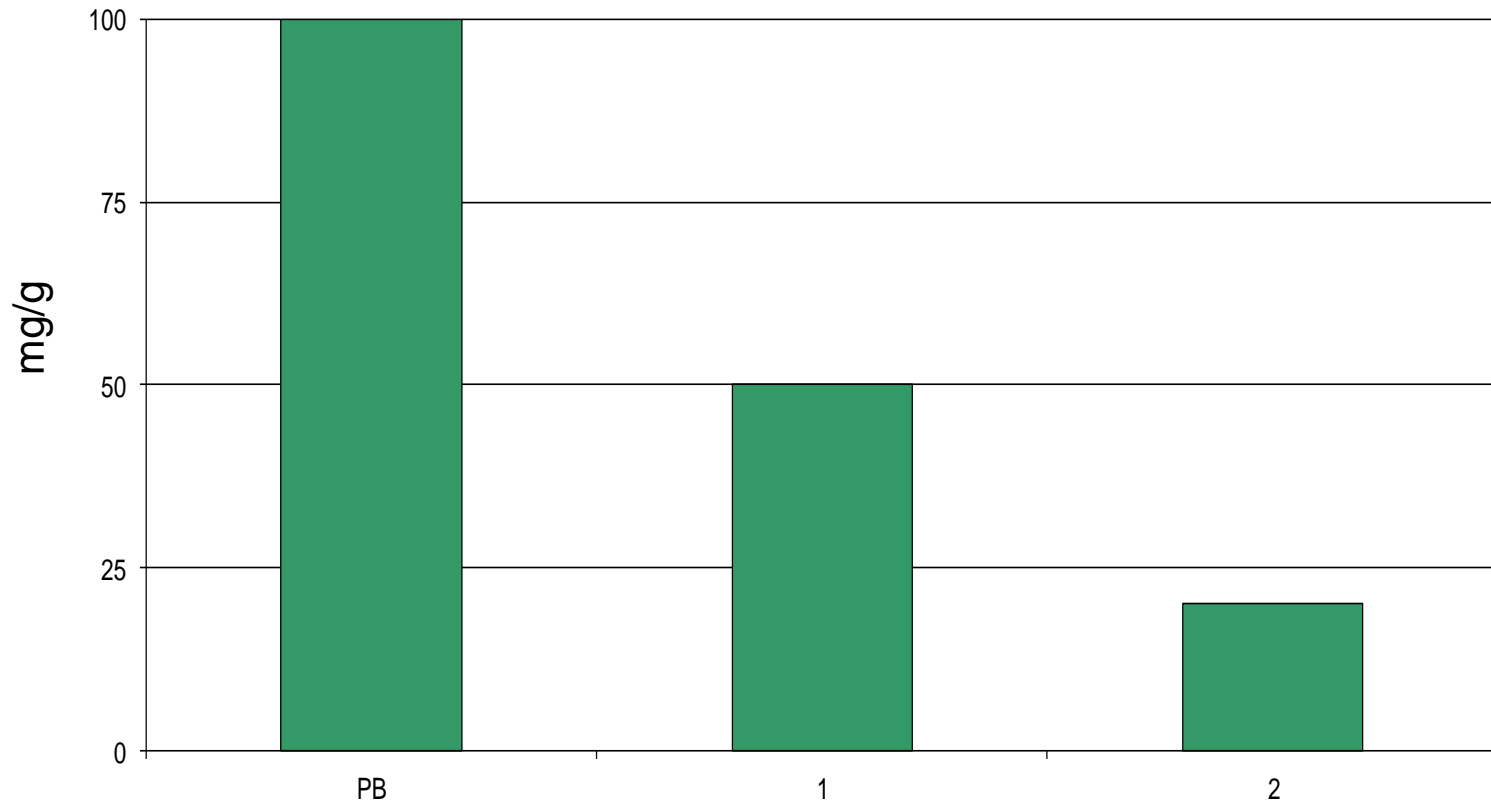
PB, Elution fraction/PolyBatics PolyBind-Z™

1, Elution fraction/commercial protein A sepharose beads

2, Elution fraction/commercial protein A sepharose beads



# Static IgG binding capacity (Eluted IgG in mg/g drained bead mass)

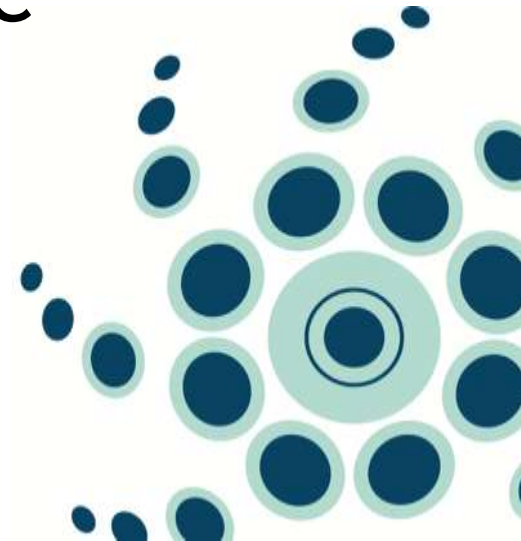


**PB: PolyBind-Z™**

**1, 2: Commercial protein A sepharose beads**

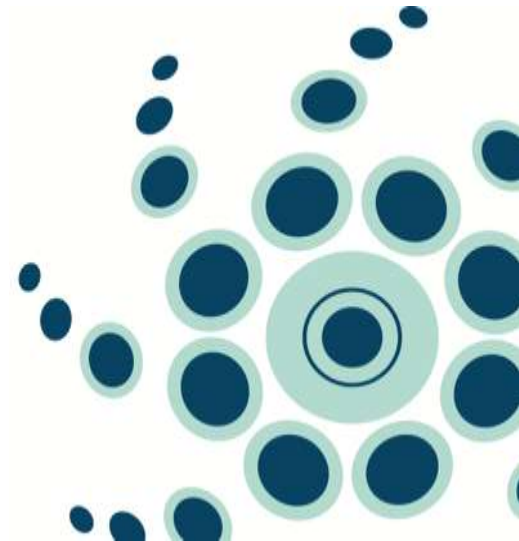
# Bead properties

- Protein ligand shell/biopolyester core
- Size range of 100-300 nm
- Nonporous; convective interaction
- Bead structure stable at pH 2-11
- Bead structure stable up to 95°C
- Long shelf life



“... the polished bodywork of bioprocessing hides an engine of despair that groans under the strain of current demands ....”

Uve Gottschalk  
Biopharm International  
Sept 2011

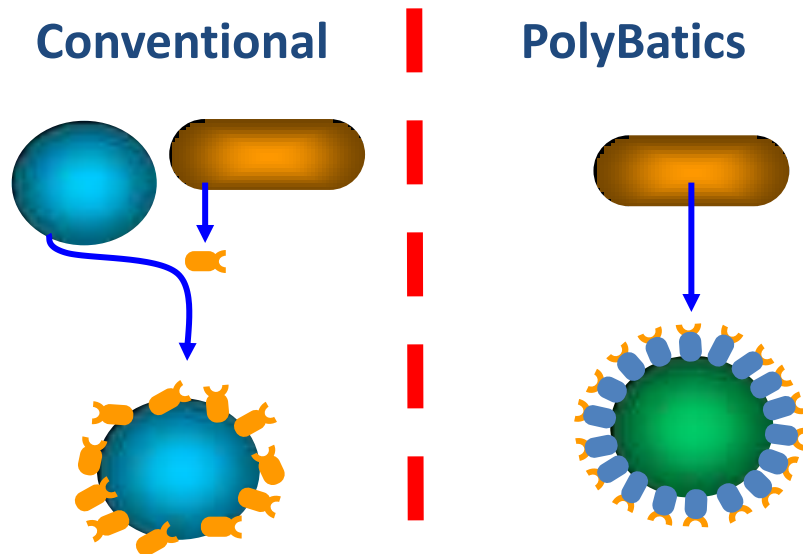


# PolyBind™ - a Game-Changer for Protein Purification

**Specific** (affinity, custom) binding;

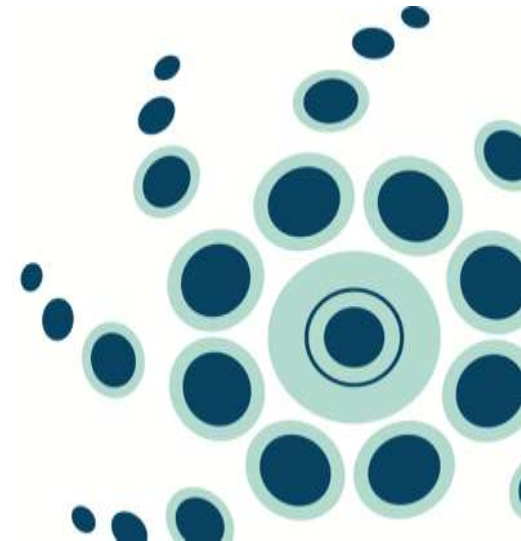
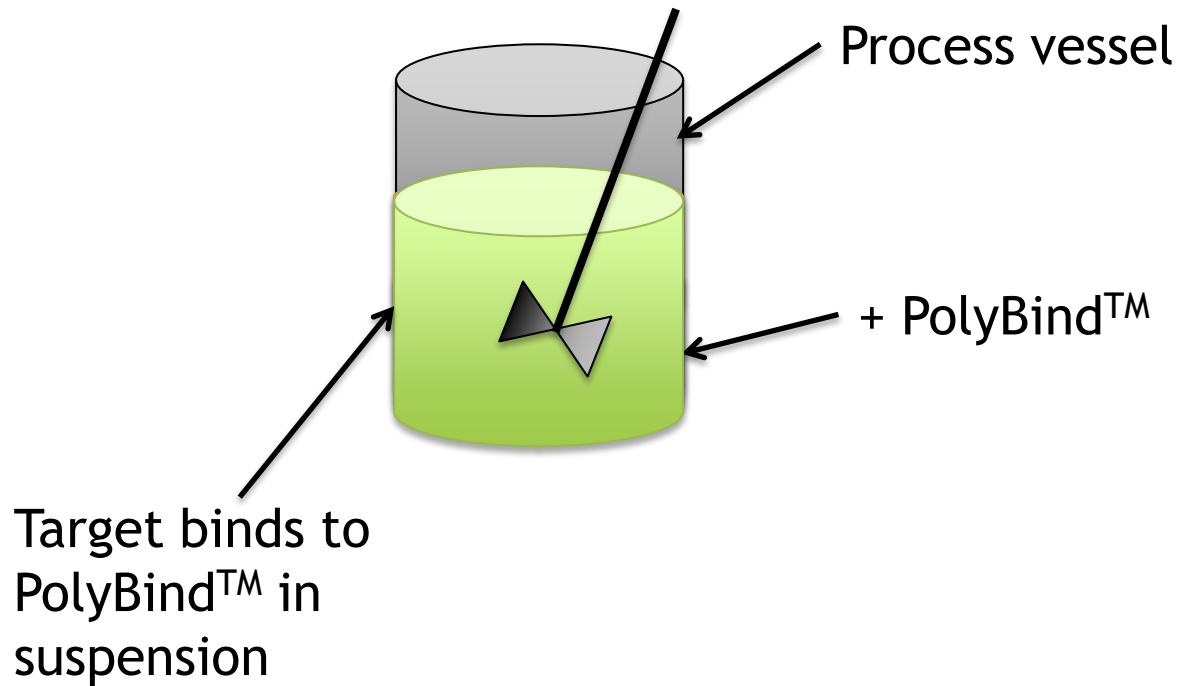
**Cheap** enough for **single-use**;

**Quick** to create & develop.



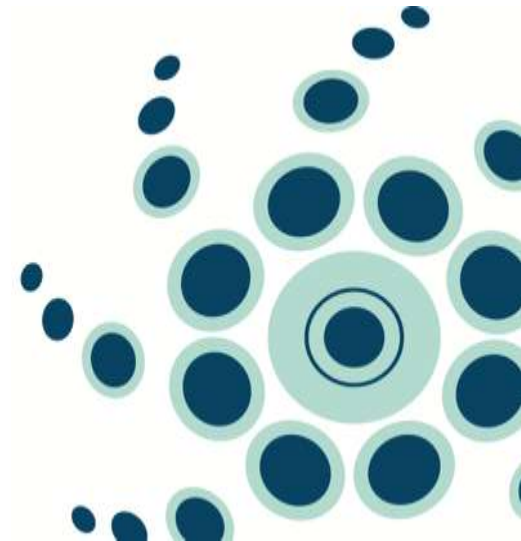
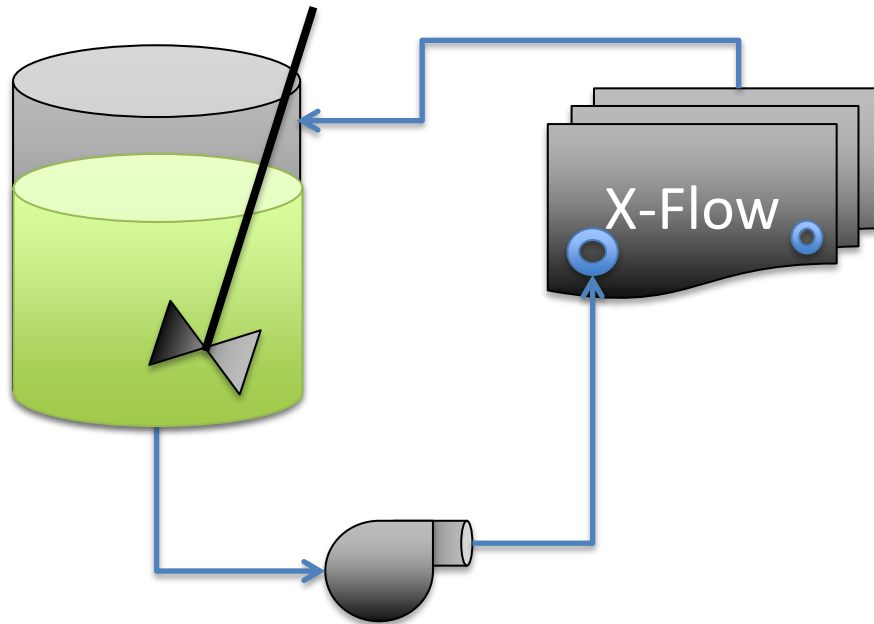
# PolyBind™: Single-Use Concept

## 1. *Binding target species (equilibrate with PolyBind™ in suspension)*



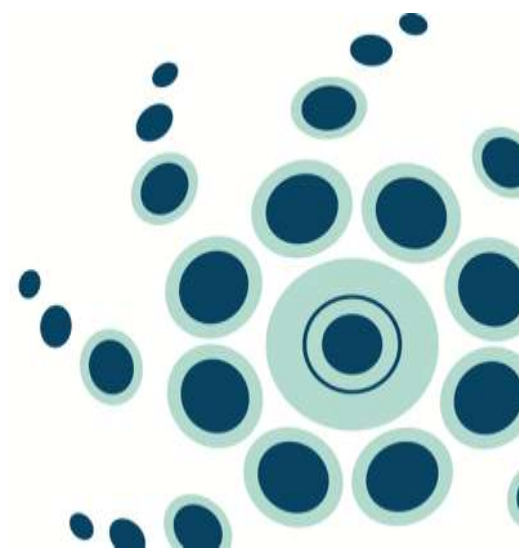
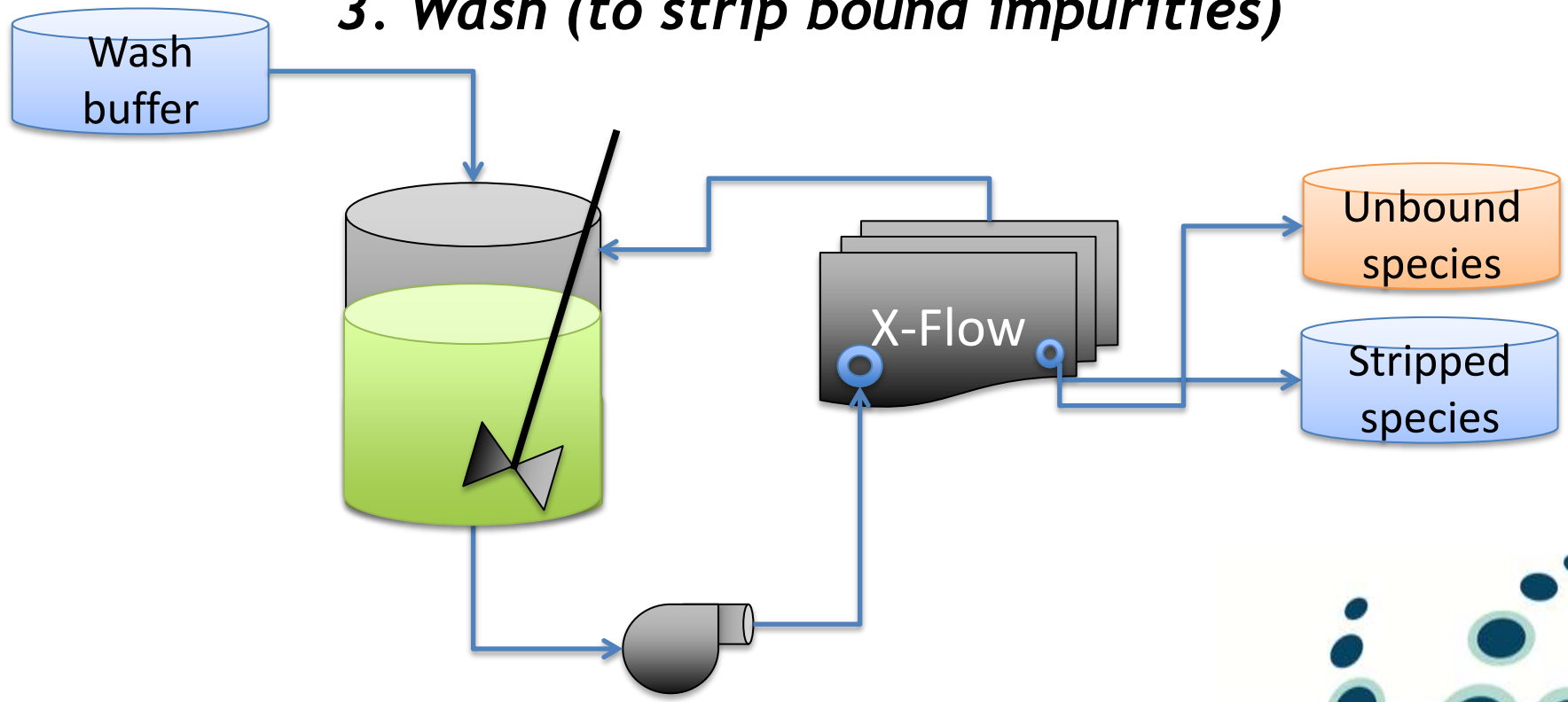
# PolyBind™: Single-Use Concept

*Use X-flow technology to retain PolyBind™ and allow flow/washing*



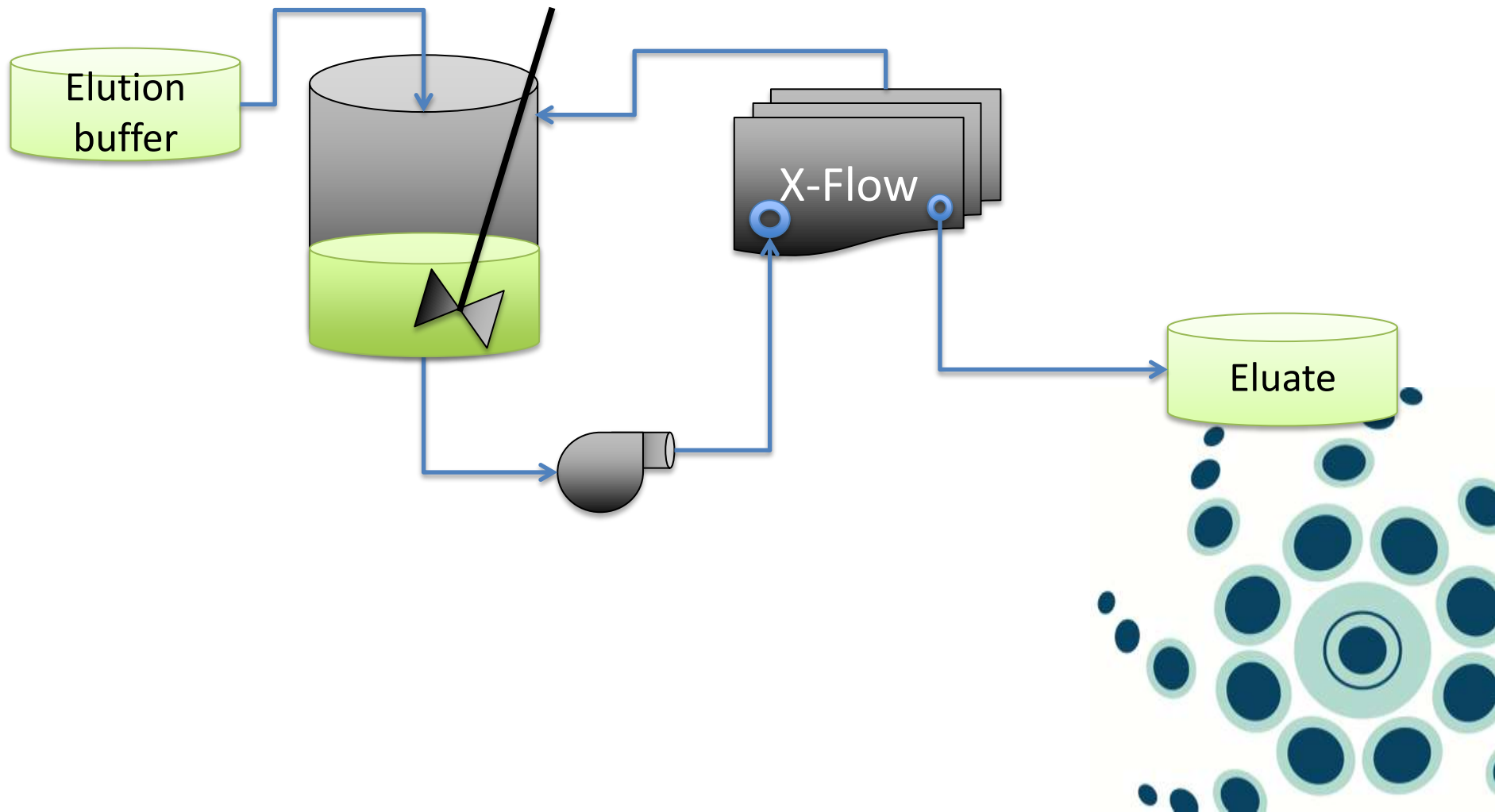
PolyBind™: Single-Use Concept

- 2. Concentrate (to remove load impurities)
- 3. Wash (to strip bound impurities)



# PolyBind™: Single-Use Concept

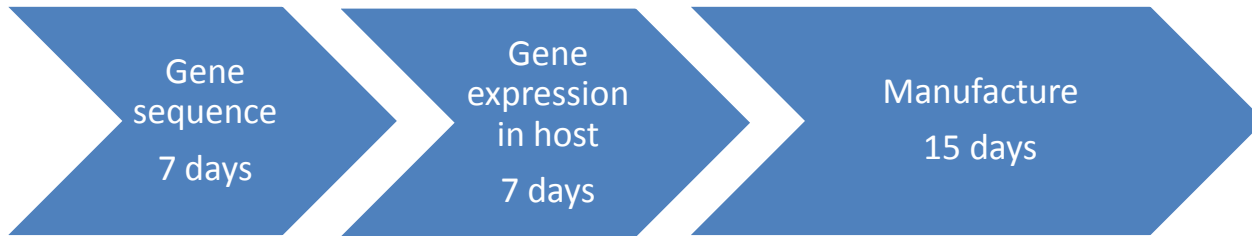
## 4. Elute (product, bound species)



# How long to Create & Supply a New, Custom PolyBind™ Product?

- Separation (target) identified
- Polypeptide binding ligand identified

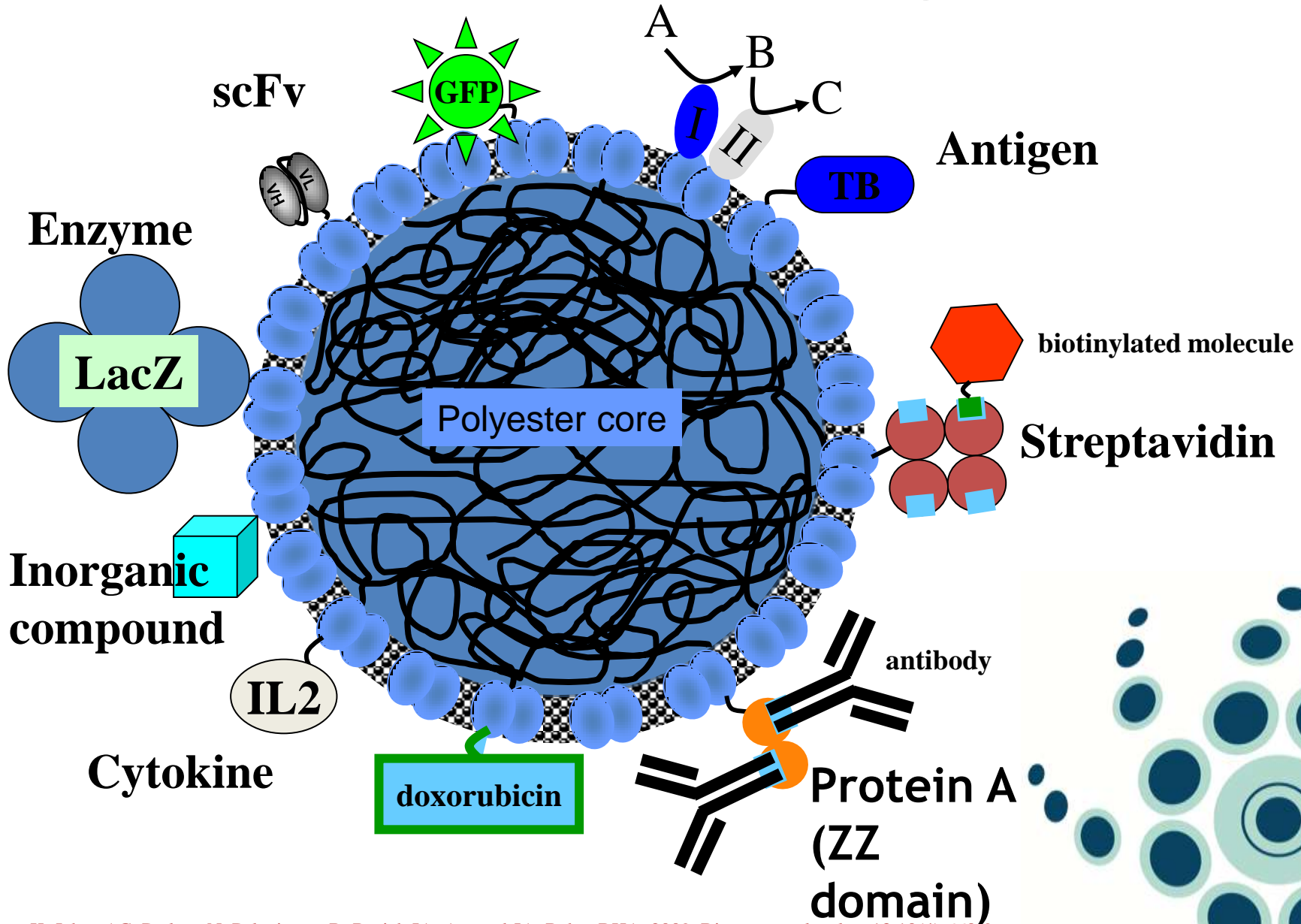
Start!



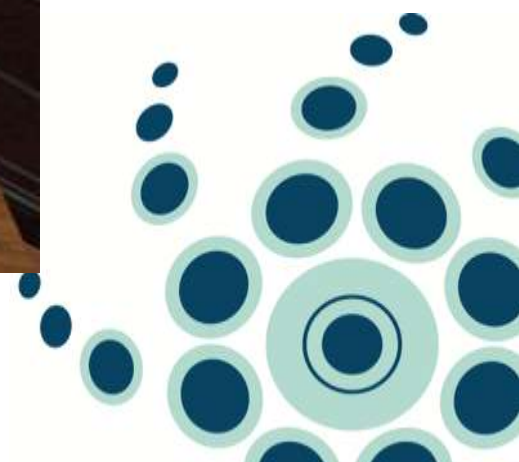
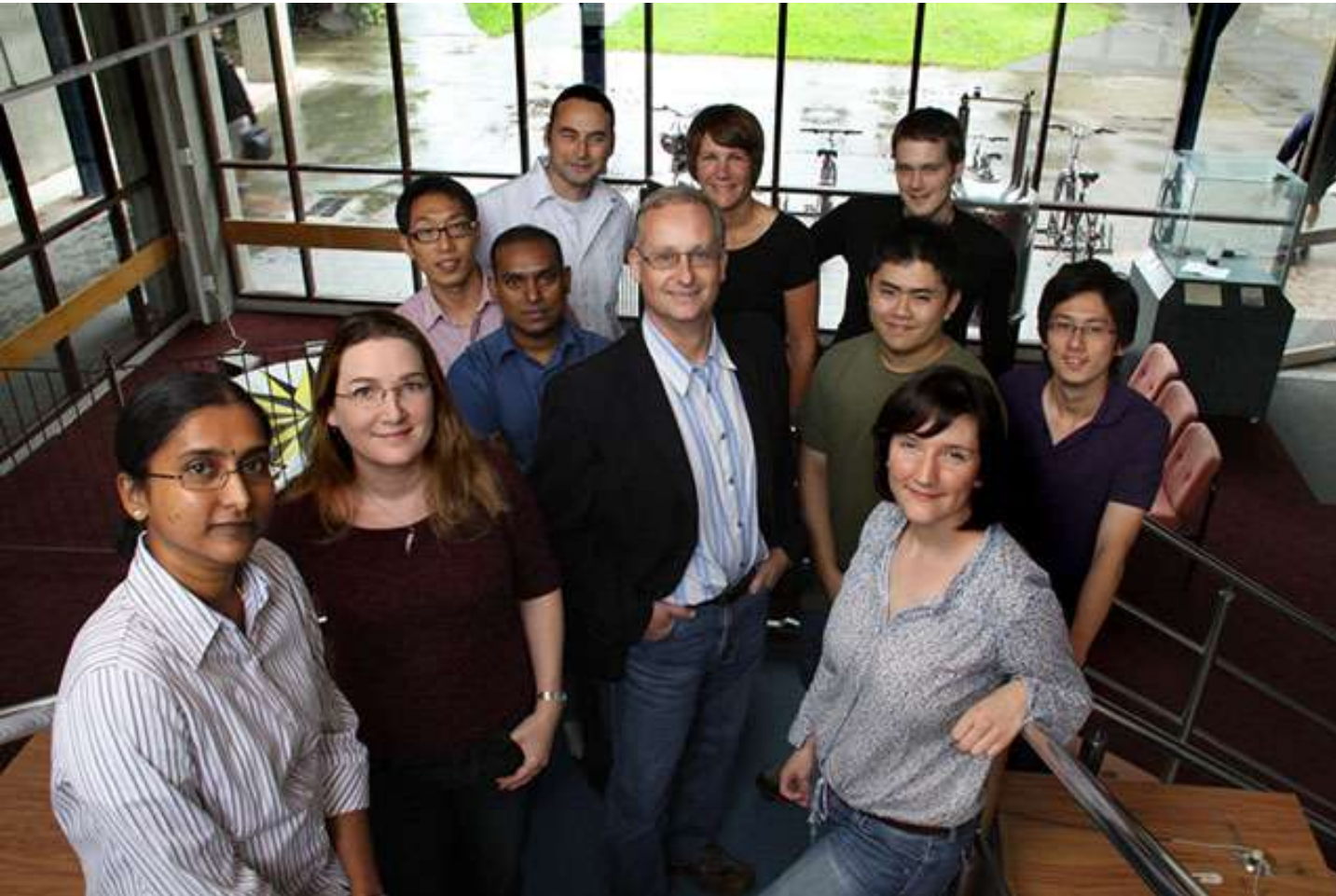
29 Days



# A platform technology



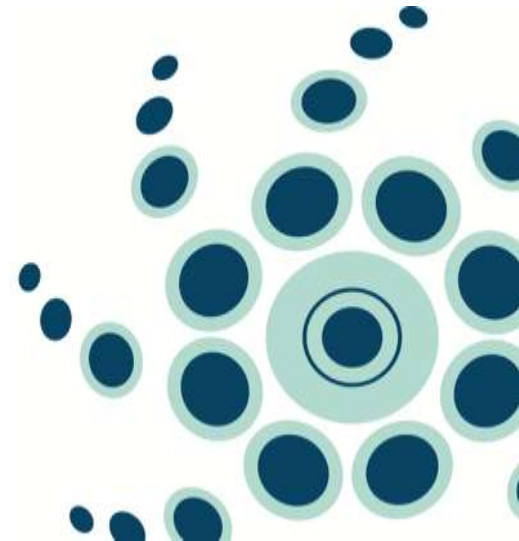
# Acknowledgments



# But wait, there's more.....

We are seeking new members to join our team:

- **Research & Development Scientist**  
Fermentation
- **Assay Development Team Leader**
- Applications welcome!



- Come and talk to us about your bioseparation challenges.
- Thanks for your attention!

