# BioThaw: An Eco-Friendly Biological Deicer

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## Market Need

Traditional deicers cause significant road erosion, contaminate waterways, harm household pets, and damage plants and aquatic life

- North America holds **43% of the global deicer market**, using **300 million metric tons** annually
- **42% of the US population** is willing to spend more than a **25% premium** on sustainable products
- Sustainable alternatives like propylene glycol have begun entering the market, but most have some negative environmental effects or simply dilute traditional chloride products
- There is currently no biological deicing product on the market, which is an opportunity to **disrupt the traditional market**

## BioThaw Specifications

Concentrated AFP liquid solution that:
- Is safe for infrastructure, pets, and environment
- Is presented as a stable protein emulsion
- Completely inhibits ice growth up to -7.1°C (~19.2°F) when ~62.5 mL/m² of 1 mg/mL AFP solution is applied to the roads

**BIOTHAW** eco-friendly liquid deicer

## Antifreeze Protein

Plants, insects, and fish contain antifreeze proteins that promote thermal hysteresis so that the organisms can stay alive under harsh conditions.
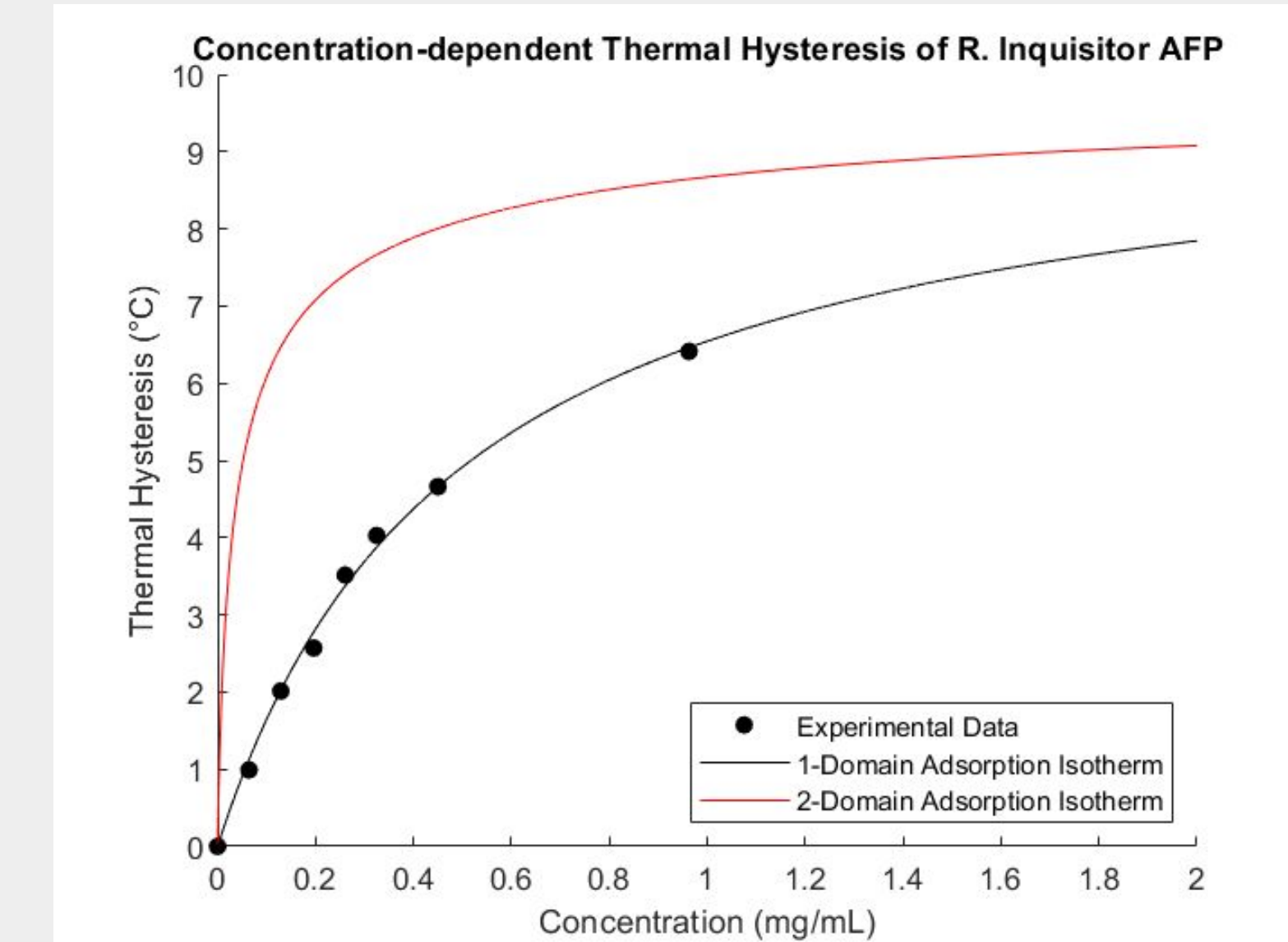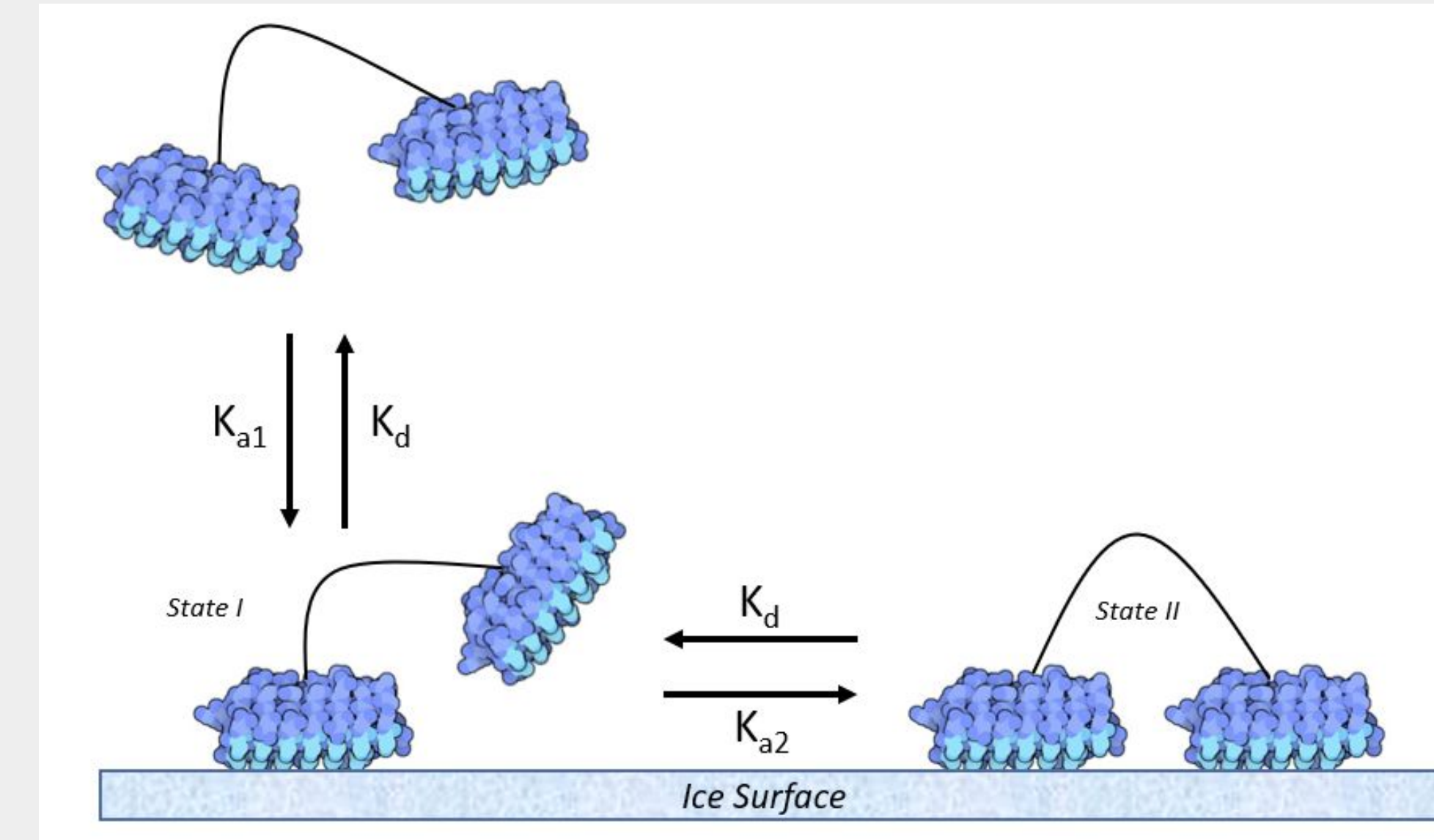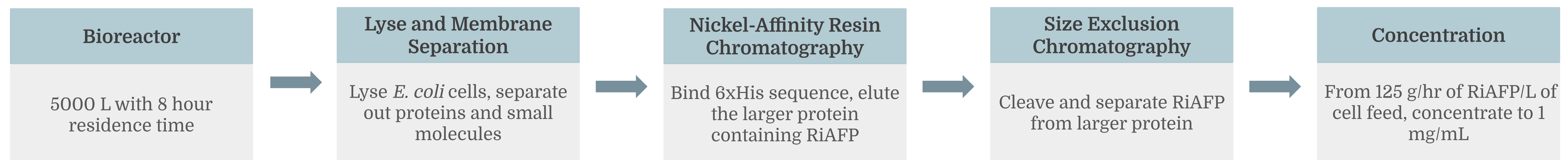


TEV site        6xHis

eGFP        RiAFP
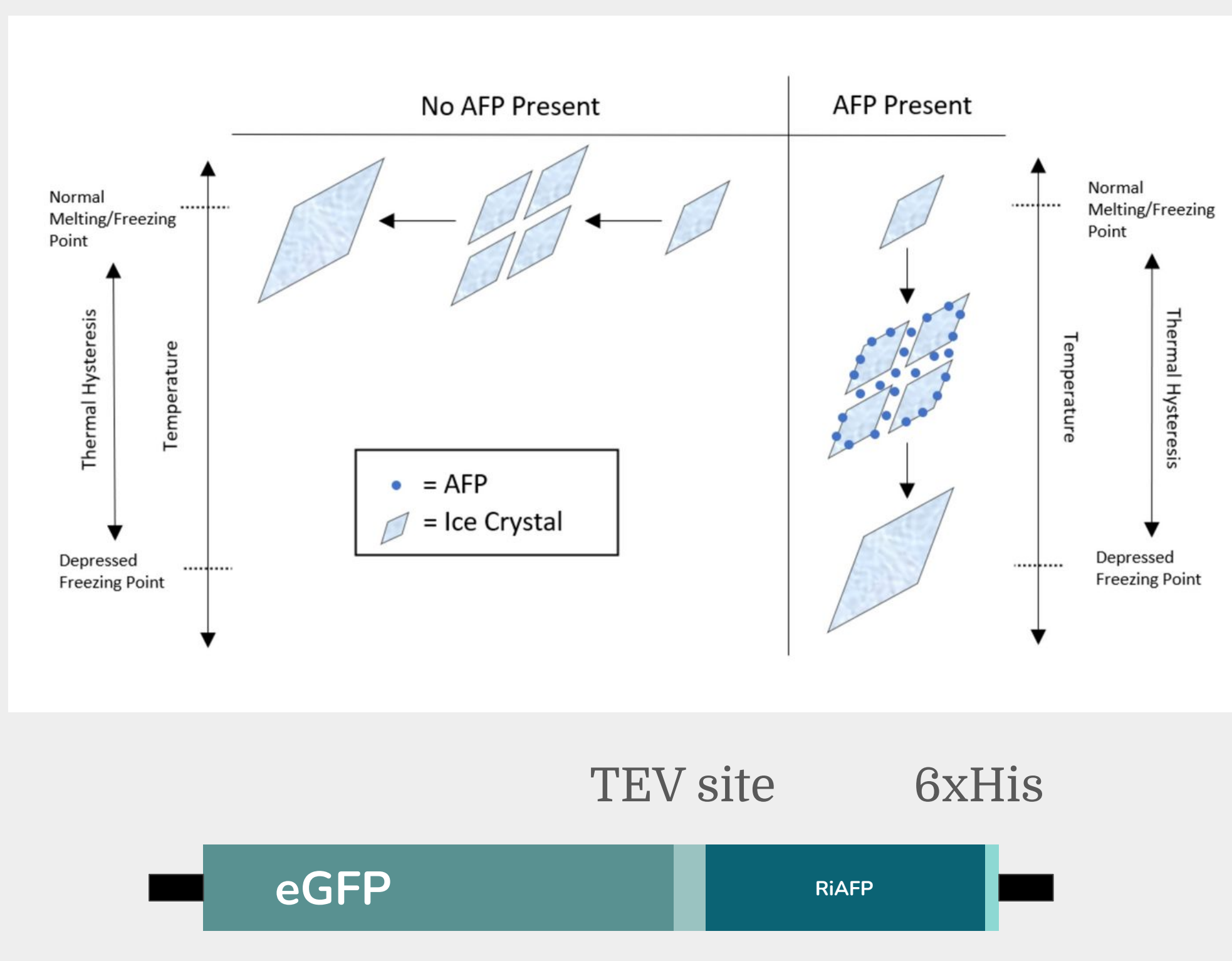
## Thermal Hysteresis Modelling

- A Langmuir isotherm model relates **fractional coverage** ($\theta_T$) to **AFP protein concentration**
- An extended Langmuir adsorption model predicts the thermal hysteresis activity of the dimerized protein.

$$\theta_T = \frac{1}{2K_1 K_2 \cdot C} + \frac{1}{2K_1} + 1 - \sqrt{\left(\frac{1}{2K_1 \cdot K_2 \cdot C} + \frac{1}{2K_1}\right)^2 + \frac{1}{K_1 K_2 \cdot C}}$$

$$K_i = \frac{k_{ai}}{k_d}$$





Concentration-dependent Thermal Hysteresis of R. Inquisitor AFP

Legend: Experimental Data; 1-Domain Adsorption Isotherm; 2-Domain Adsorption Isotherm

## Manufacturing

| Bioreactor | Lyse and Membrane Separation | Nickel-Affinity Resin Chromatography | Size Exclusion Chromatography | Concentration |
|---|---|---|---|---|
| 5000 L with 8 hour residence time | Lyse *E. coli* cells, separate out proteins and small molecules | Bind 6xHis sequence, elute the larger protein containing RiAFP | Cleave and separate RiAFP from larger protein | From 125 g/hr of RiAFP/L of cell feed, concentrate to 1 mg/mL |

## Manufacturing Equipment

**Bioreactor**
- 5000L Bioreactor
- DI Water System
- Autoclaves

**Separation**
- High Pressure Homogenizer
- 2000L/h Membrane

**Purification**
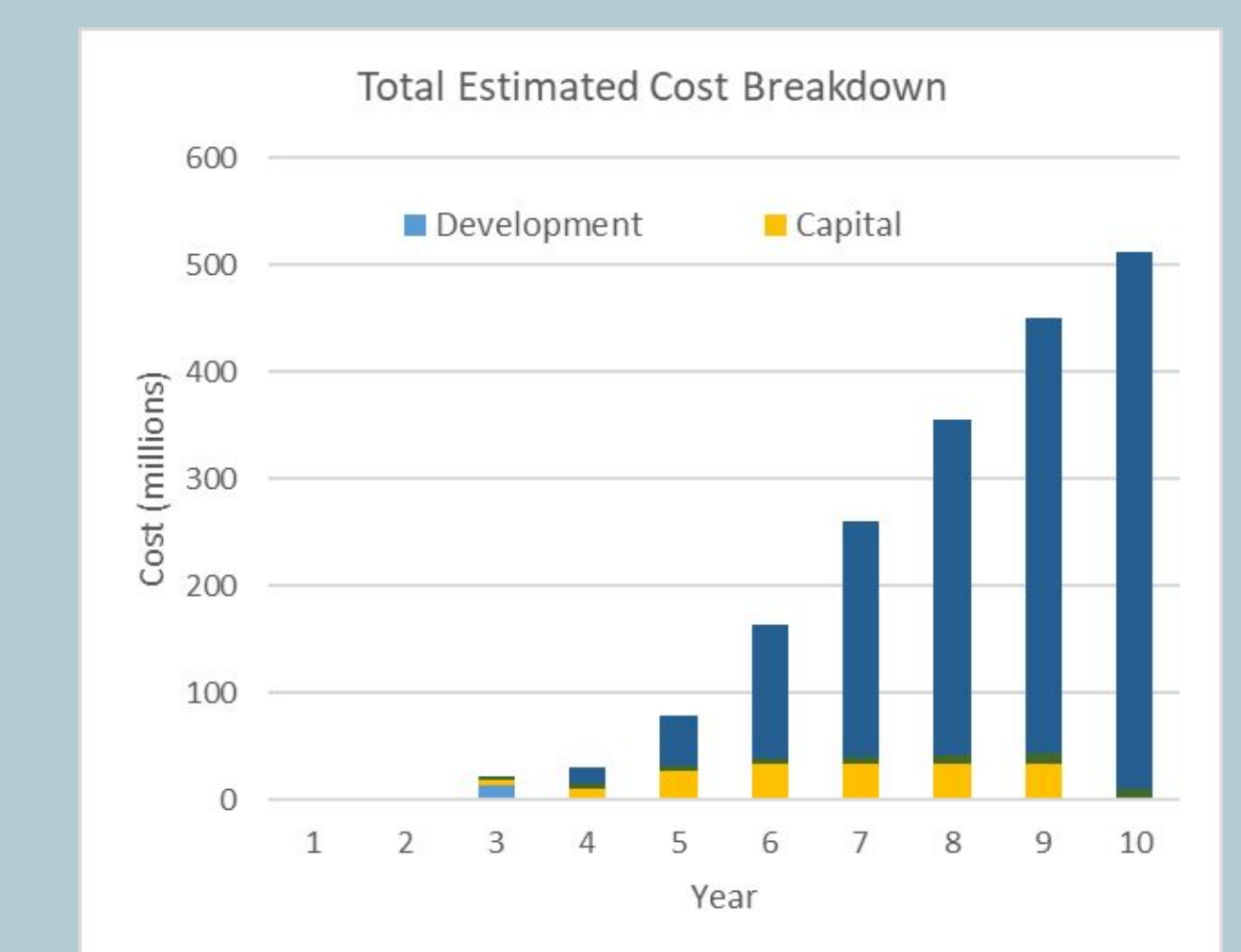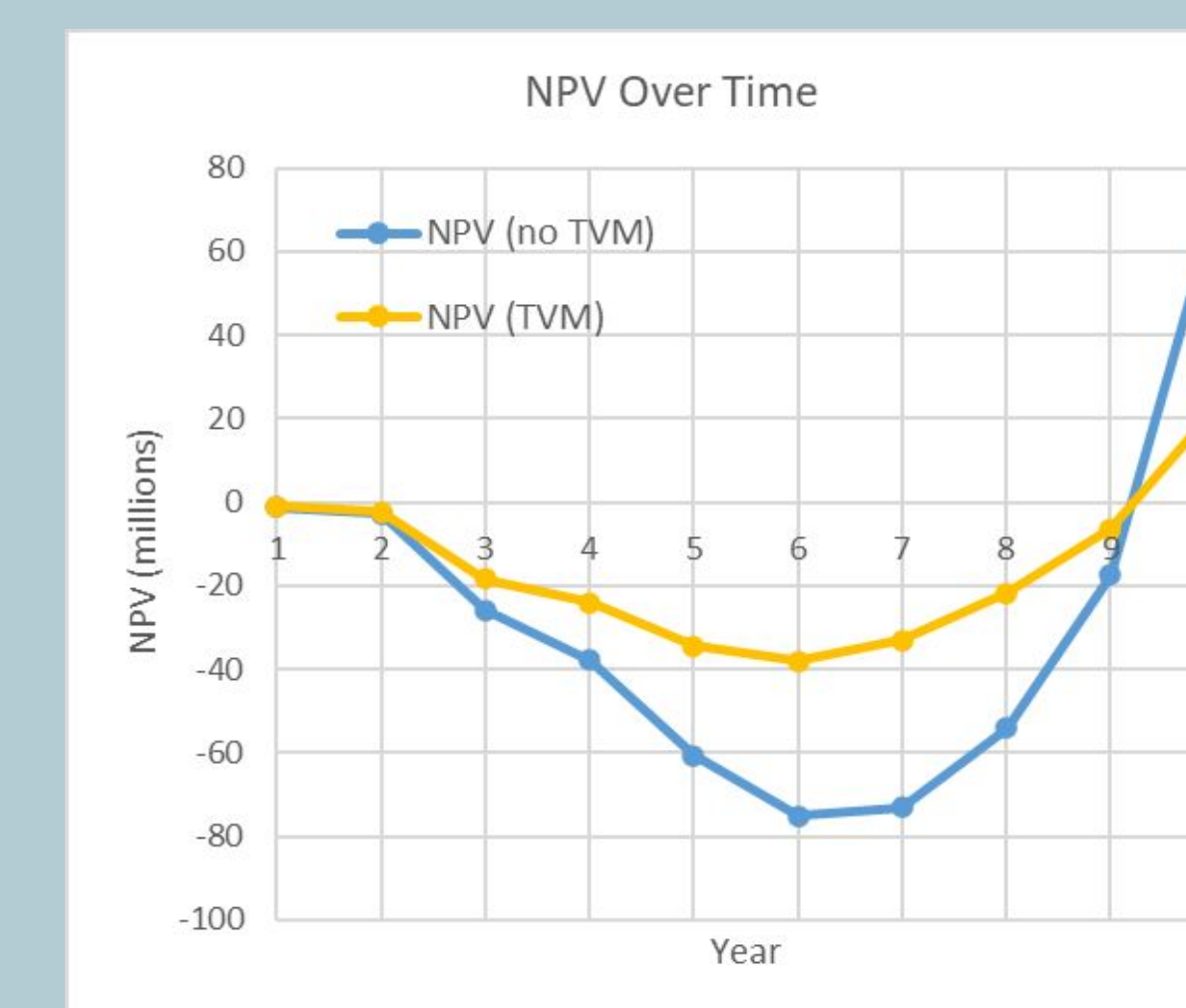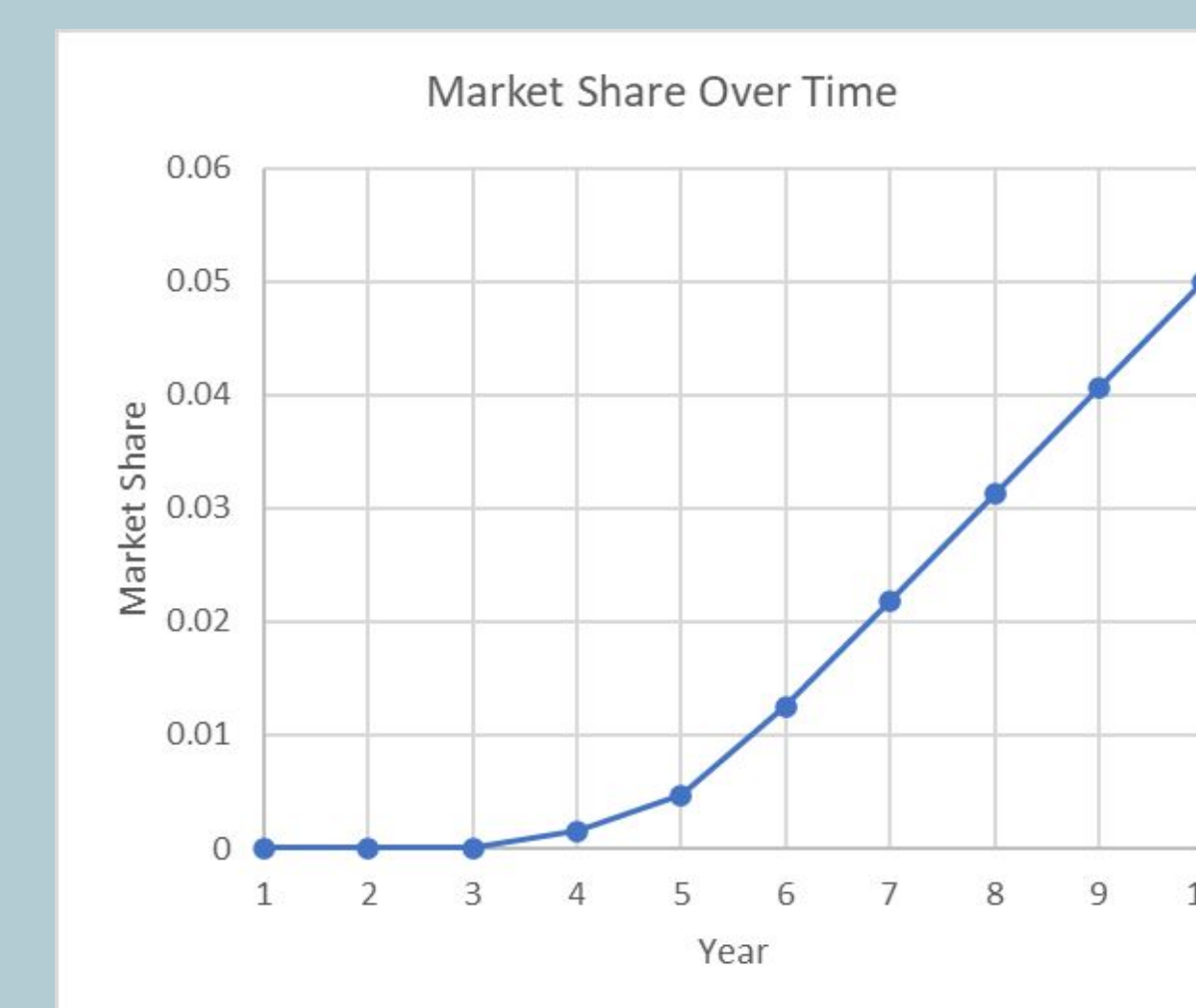- IMAC Column
- SEC Column
- Centrifuge



Bioreactor 17%
Separation 14%
Purification 69%
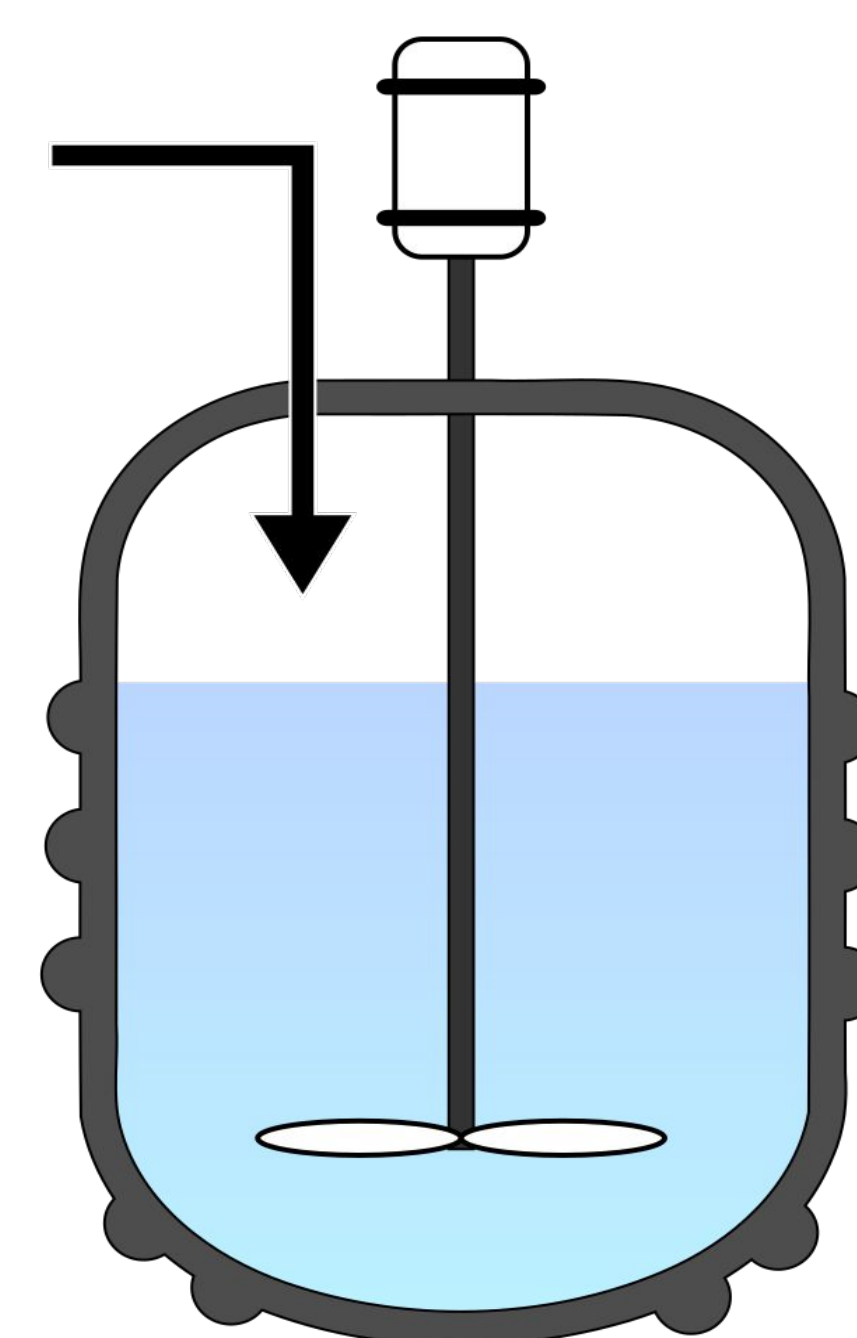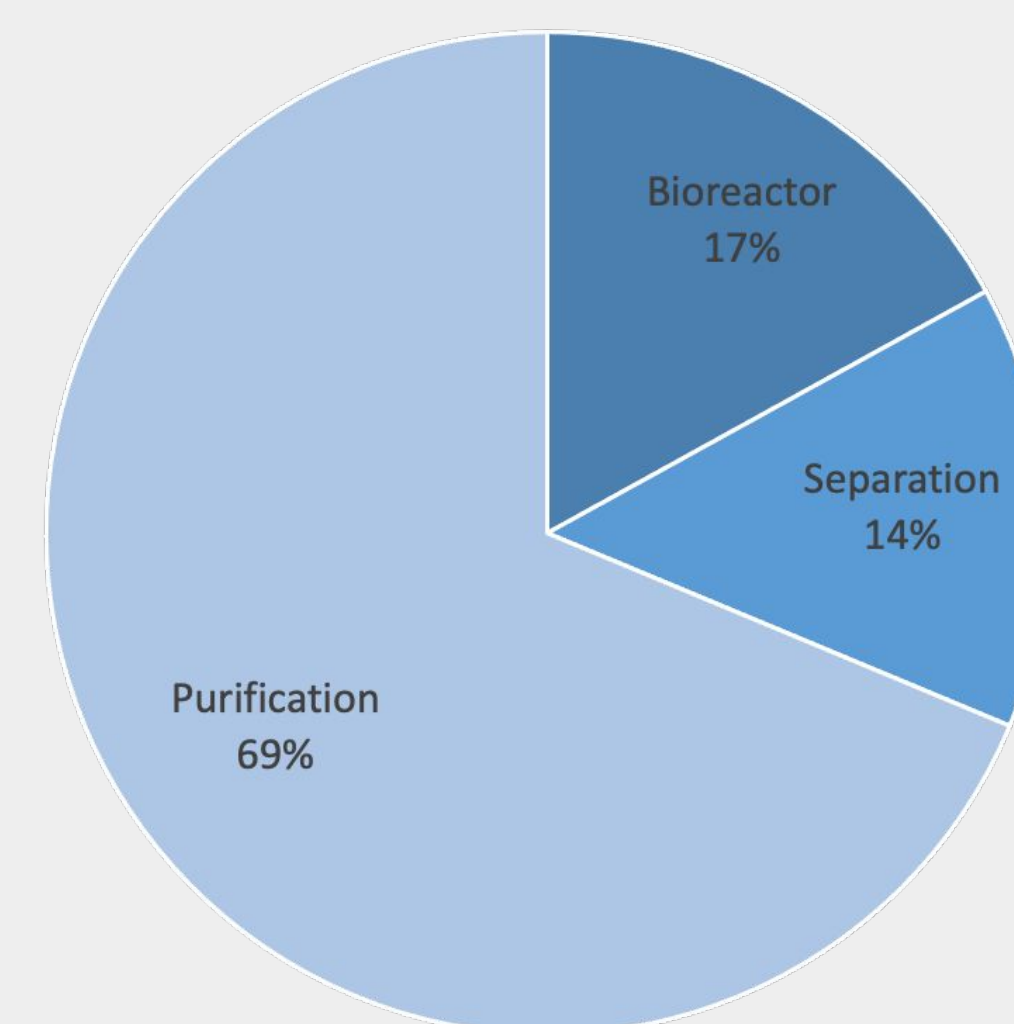
## Product Timeline

- **1st year** — Lab-scale research, patent filing
- Begin scale up — **2nd-3rd year**
- **4th year** — Hit the market
- Break even — **9th year**

## Financial Analysis

| (in million dollars) | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | Year 6 | Year 7 | Year 8 | Year 9 | Year 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Development | -1.10 | -1.79 | -13.45 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Capital | 0.00 | 0.00 | -5.51 | -11.02 | -27.55 | -33.06 | -33.06 | -33.06 | -33.06 | 0.00 |
| Business | 0.00 | 0.00 | -4.00 | -4.10 | -4.30 | -6.06 | -7.57 | -9.13 | -9.97 | -11.14 |
| Operating | 0.00 | 0.00 | 0.00 | -15.65 | -46.95 | -125.20 | -219.10 | -313.00 | -406.90 | -500.80 |
| Revenue | 0.00 | 0.00 | 0.00 | 18.71 | 56.13 | 149.68 | 261.94 | 374.20 | 486.46 | 598.72 |
| Cash Flow | -1.10 | -1.79 | -22.96 | -12.06 | -22.67 | -14.64 | 2.21 | 19.01 | 36.53 | 86.78 |
| NPV (no TVM) | -1.10 | -2.89 | -25.85 | -37.91 | -60.58 | -75.22 | -73.01 | -54.00 | -17.47 | 69.31 |
| NPV (TVM) | -0.98 | -2.30 | -18.40 | -24.09 | -34.37 | -38.11 | -33.03 | -21.81 | -6.30 | 22.32 |



Market Share Over Time



NPV Over Time — NPV (no TVM); NPV (TVM)



Total Estimated Cost Breakdown — Development; Capital

## References

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4. Yue C, Zhang ZY. Cloning and expression of Tenebrio molitor antifreeze protein in Escherichia coli. Mol Biol Rep (2009) 36:529–536.