

The Sweetness of Thermal Stability: Using DSC to Probe Increases in Thermal Stability from the Addition of Polyols

David Sek

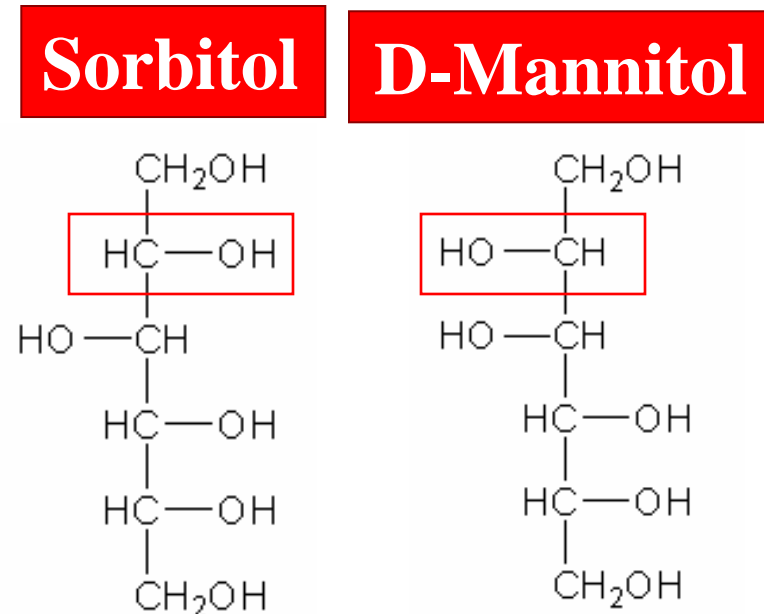
Scientist II

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Wyeth
BioPharma

Is Mannitol A Better Stabilizer Than Sorbitol?

- Mannitol is used 3x more often than sorbitol in liquid formulations
- Both excipients stabilize proteins in the liquid state through preferential exclusion
- Both excipients are GRAS listed/parenteral
- The excipients are stereoisomers of one another
- Free rotation around C-C bond
- Is mannitol more stabilizing than sorbitol?
- Can DSC be used to study any differences between them?

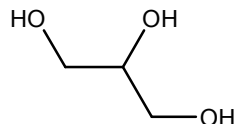


History of Polyols Studies and DSC

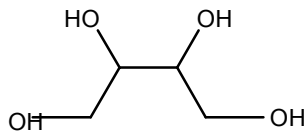
- **Back, Oakenfull and Smith - 1979**
 - ▶ Ovalbumin, lysozyme, conalbumin, chymotrypsinogen A
 - ▶ Glycerol, Sorbitol, (Glucose and Sucrose)
- **Gekko and Timasheff - 1981**
 - ▶ Ribonuclease A, chymotrypsinogen A, insulin, BSA
 - ▶ Glycerol
- **Singh and Singh - 2003**
 - ▶ Lysozyme
 - ▶ Propylene glycol, Glycerol, Mannitol, (Sucrose and Lactose)
- **Mishra, Seckler and Bhat - 2005**
 - ▶ Citrate Synthase
 - ▶ Ethylene glycol, Glycerol, Erythritol, Xylitol and Sorbitol
- **What's Missing?**

Polyol Structures

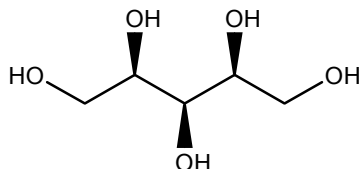
Glycerol



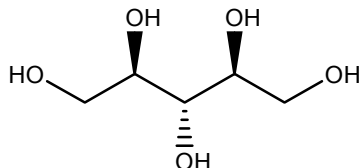
Erythritol



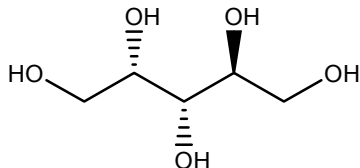
Xylitol



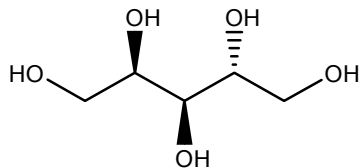
Ribitol



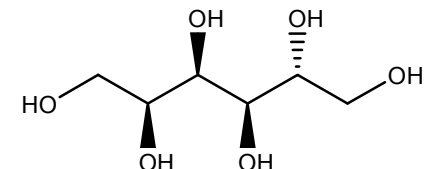
D-Arabitol



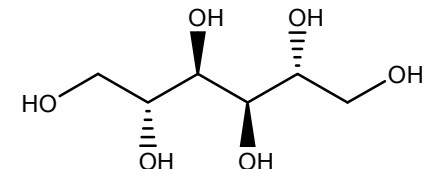
L- Arabitol



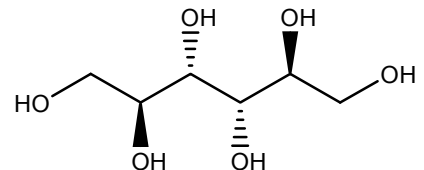
Sorbitol



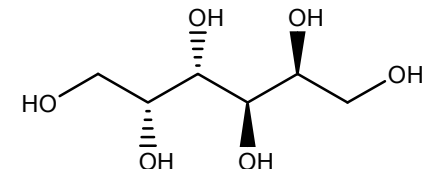
D-Mannitol



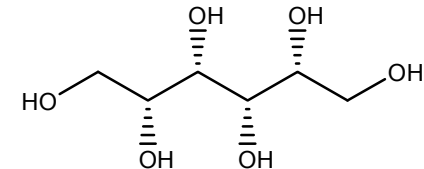
L-Mannitol



Galactitol



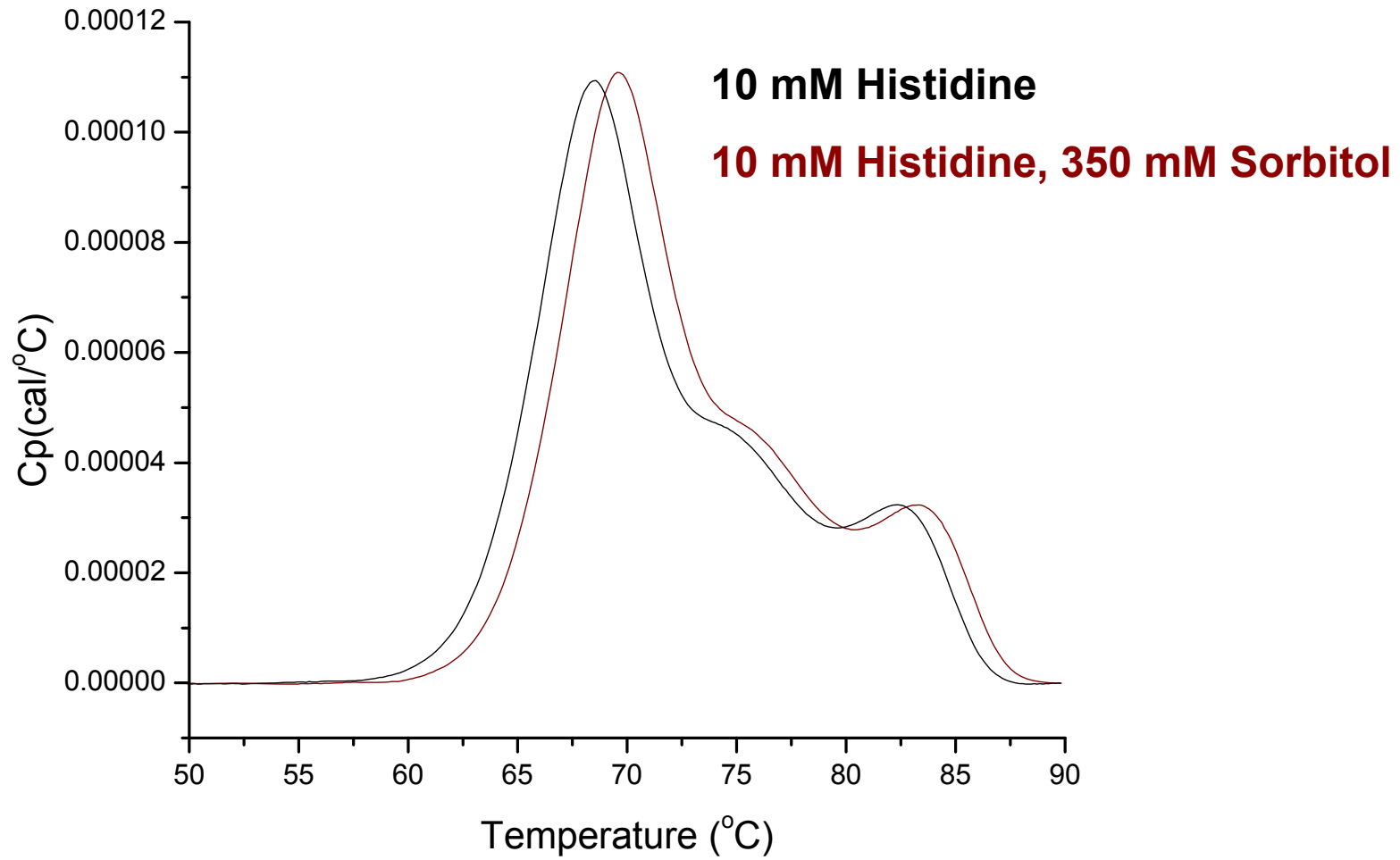
Iditol



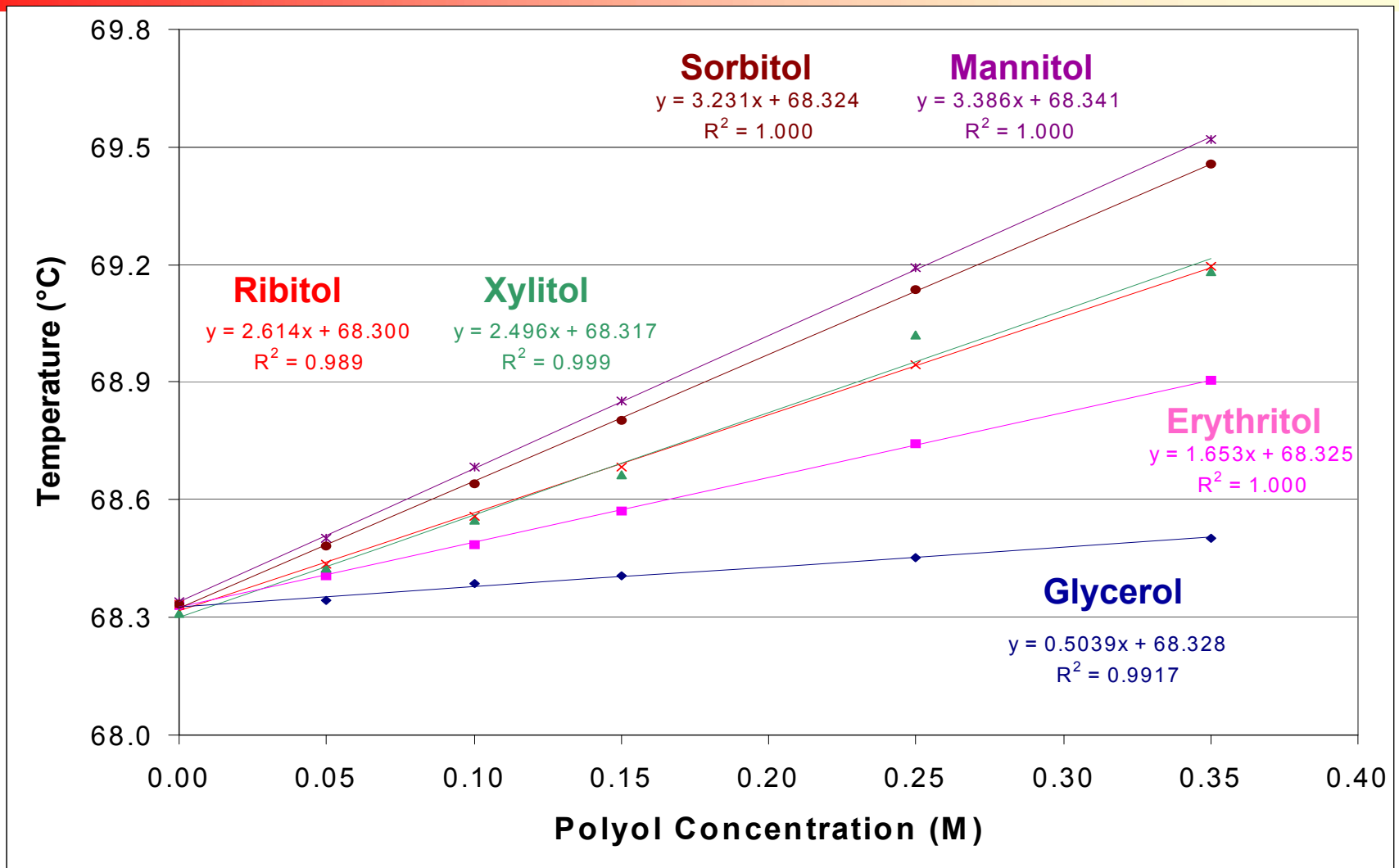
Polyol Titrations

- **Dialyzed 7 mAbs into water and diluted to 6 mg/mL**
- **Made stock concentration of Histidine of 175 mM, pH 6**
- **Made stock concentrations of polyols of 500 mM**
 - ▶ Glycerol, Erythritol, Xylitol, Ribitol, Mannitol, and Sorbitol
- **Programmed Tecan to Spike in Final Concentrations of:**
 - ▶ 10 mM Histidine
 - ▶ 1.25 mg/mL Protein
 - ▶ 0 – 350 mM of Polyol
- **Proteins:**
 - ▶ κ IgG₁: K1A, K1B
 - ▶ λ IgG₁: L1A, **L1B**
 - ▶ κ IgG₂: K2A, K2B
 - ▶ κ IgG₄: K4

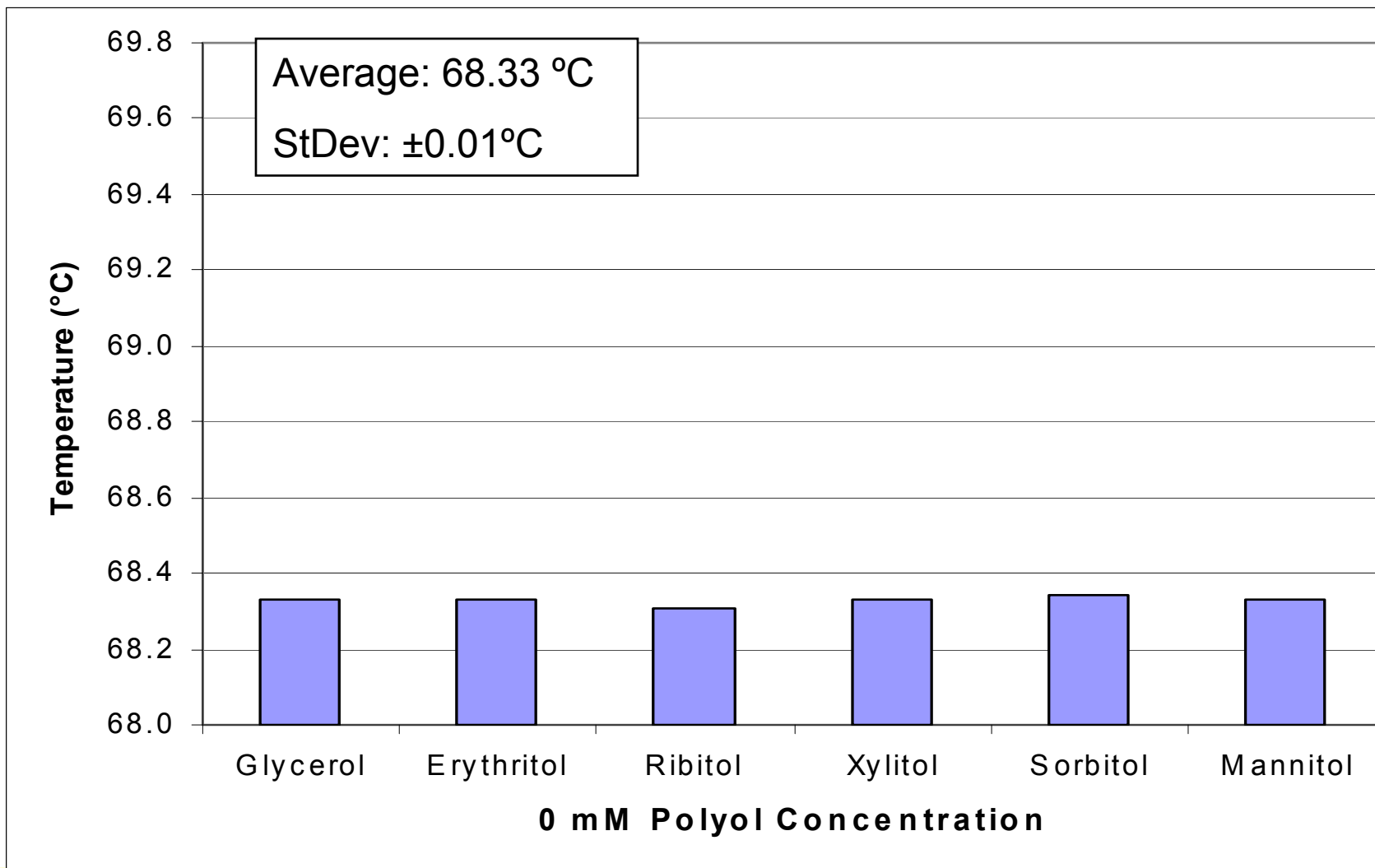
Polyol Titration: L1B Thermogram



Polyol Titration: L1B T_m 1



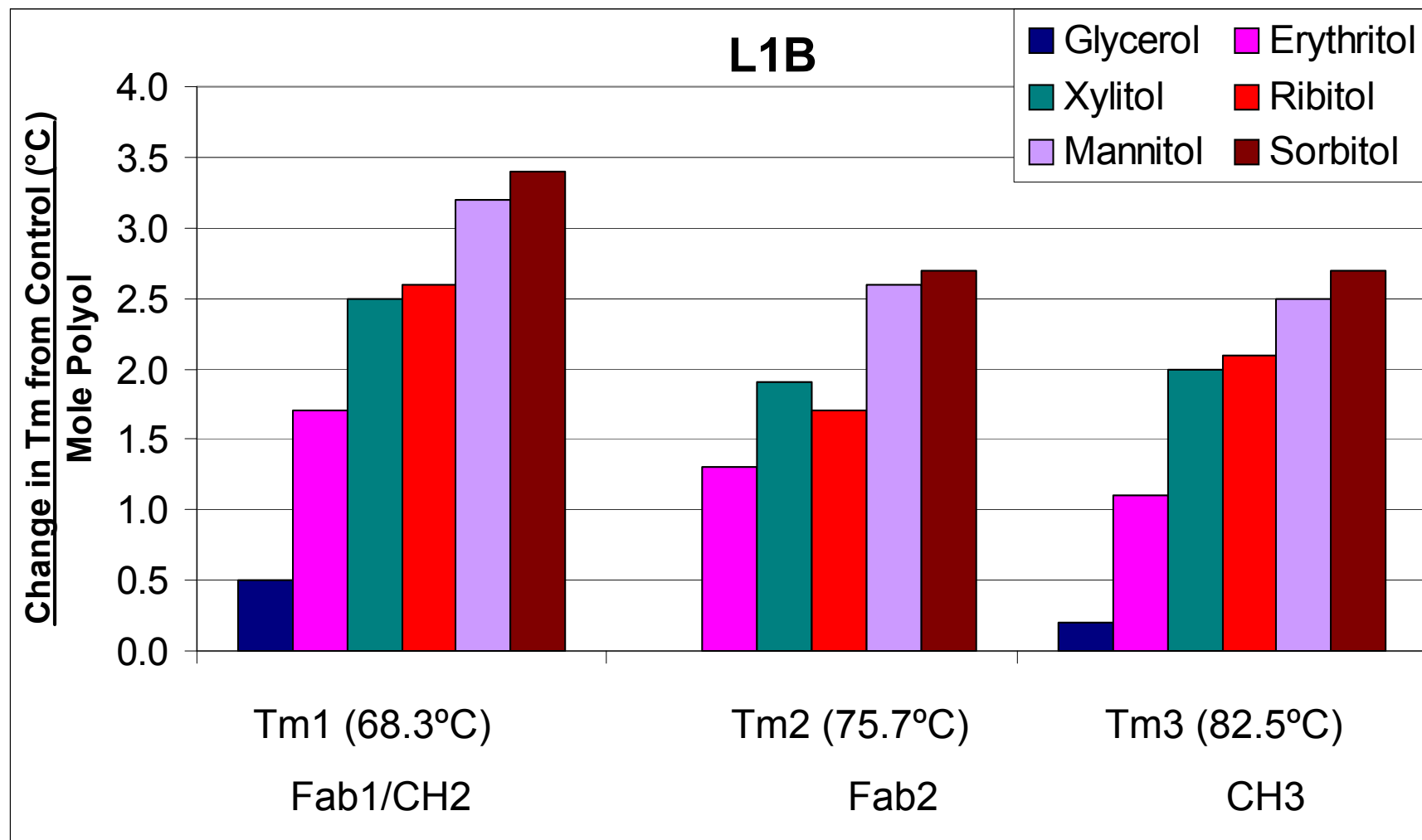
Polyol Titration: L1B T_m1



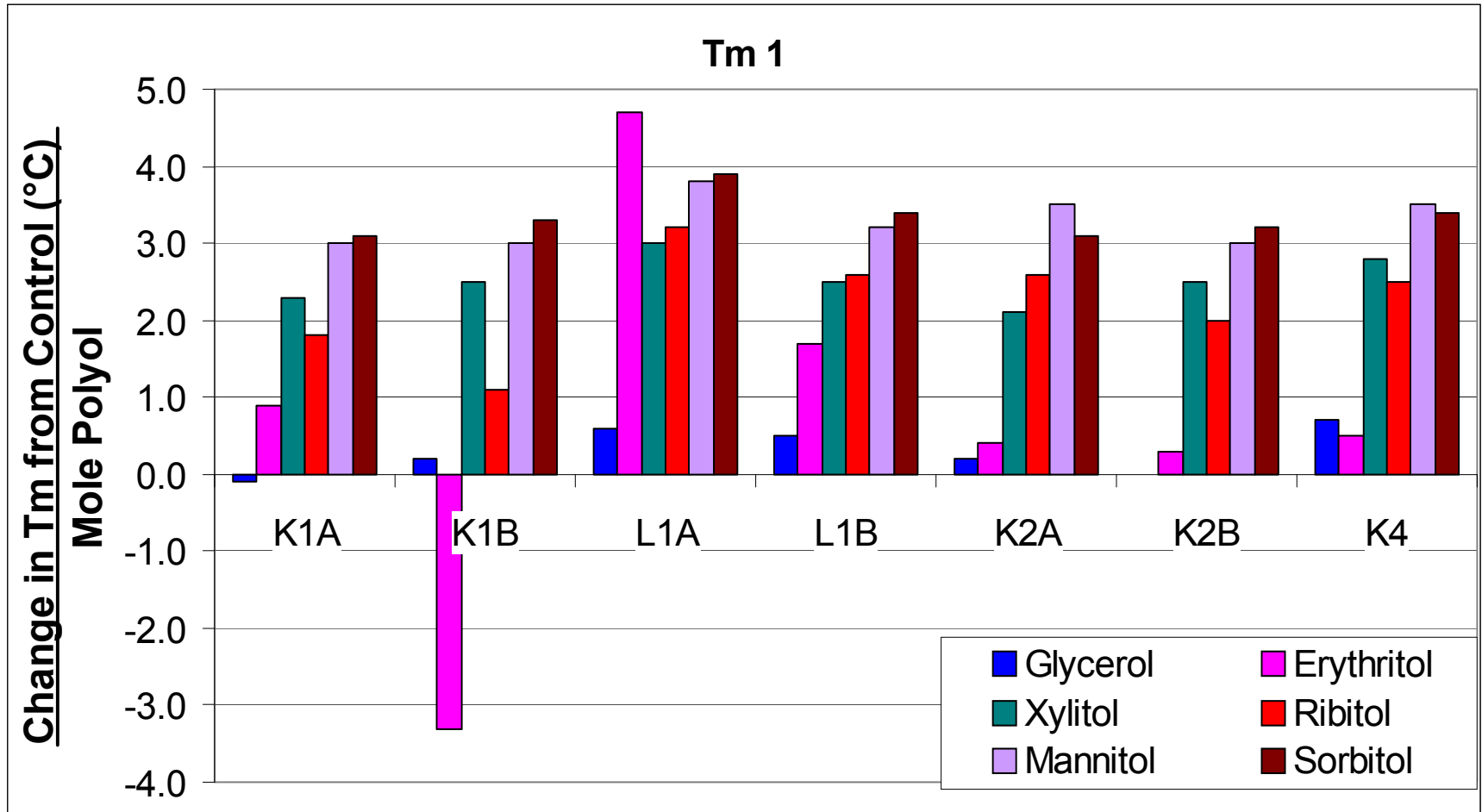
Areas of Focus

- **Subunit**
- **Isotype**
- **Transition Number**

Increase in Thermal Stability: Subunit

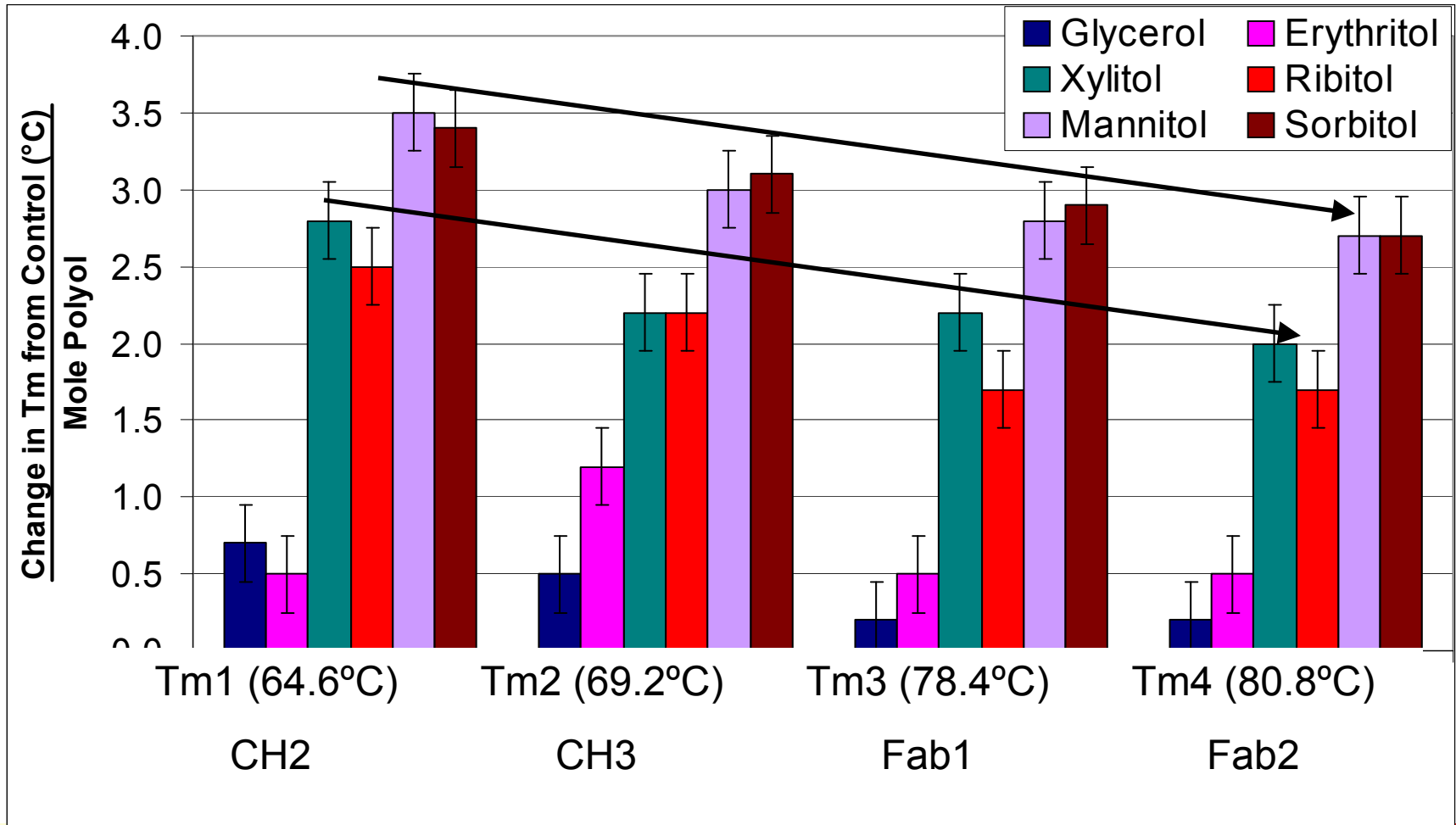


Increase in Thermal Stability: Isotype

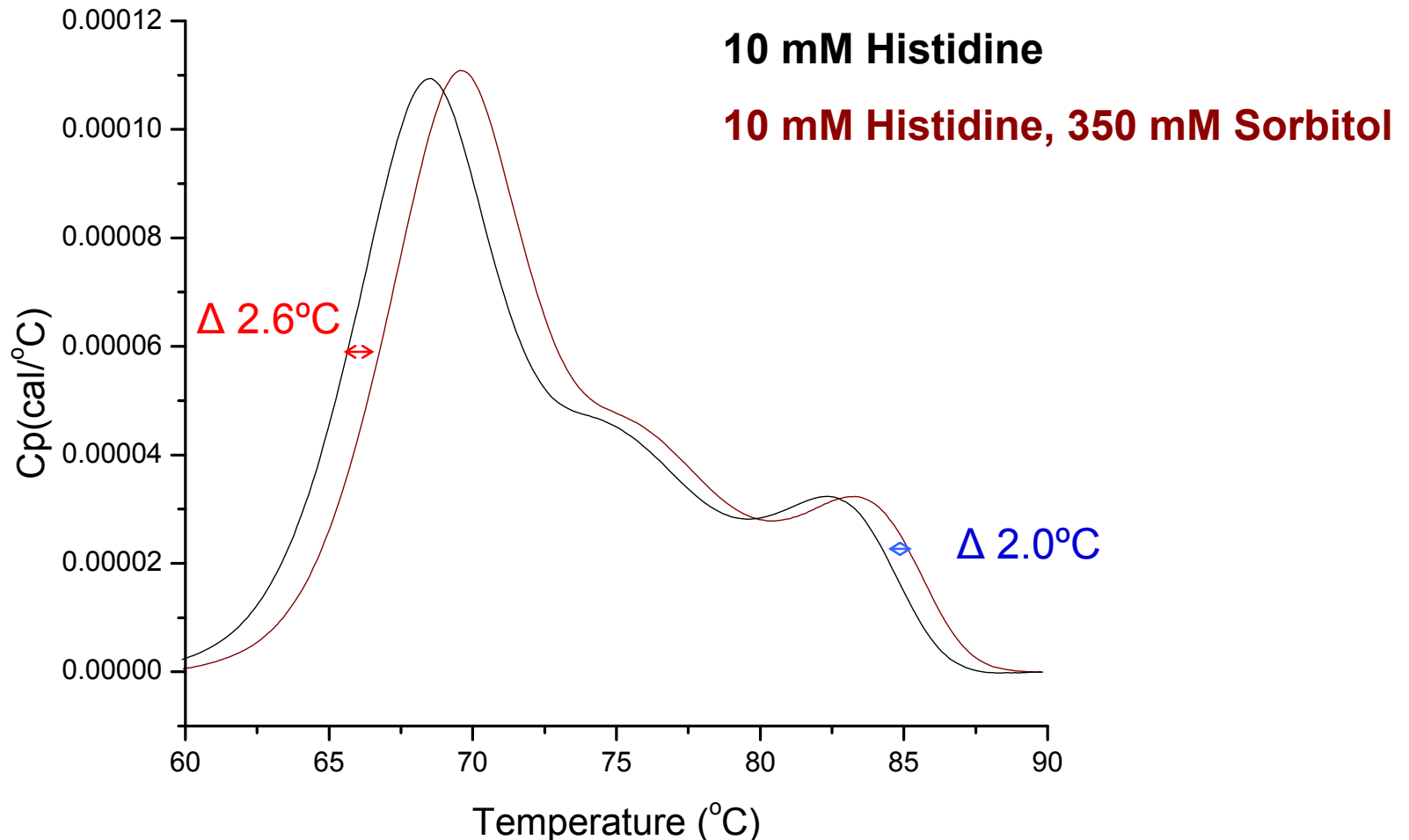


Increase in Thermal Stability: Tm

K4

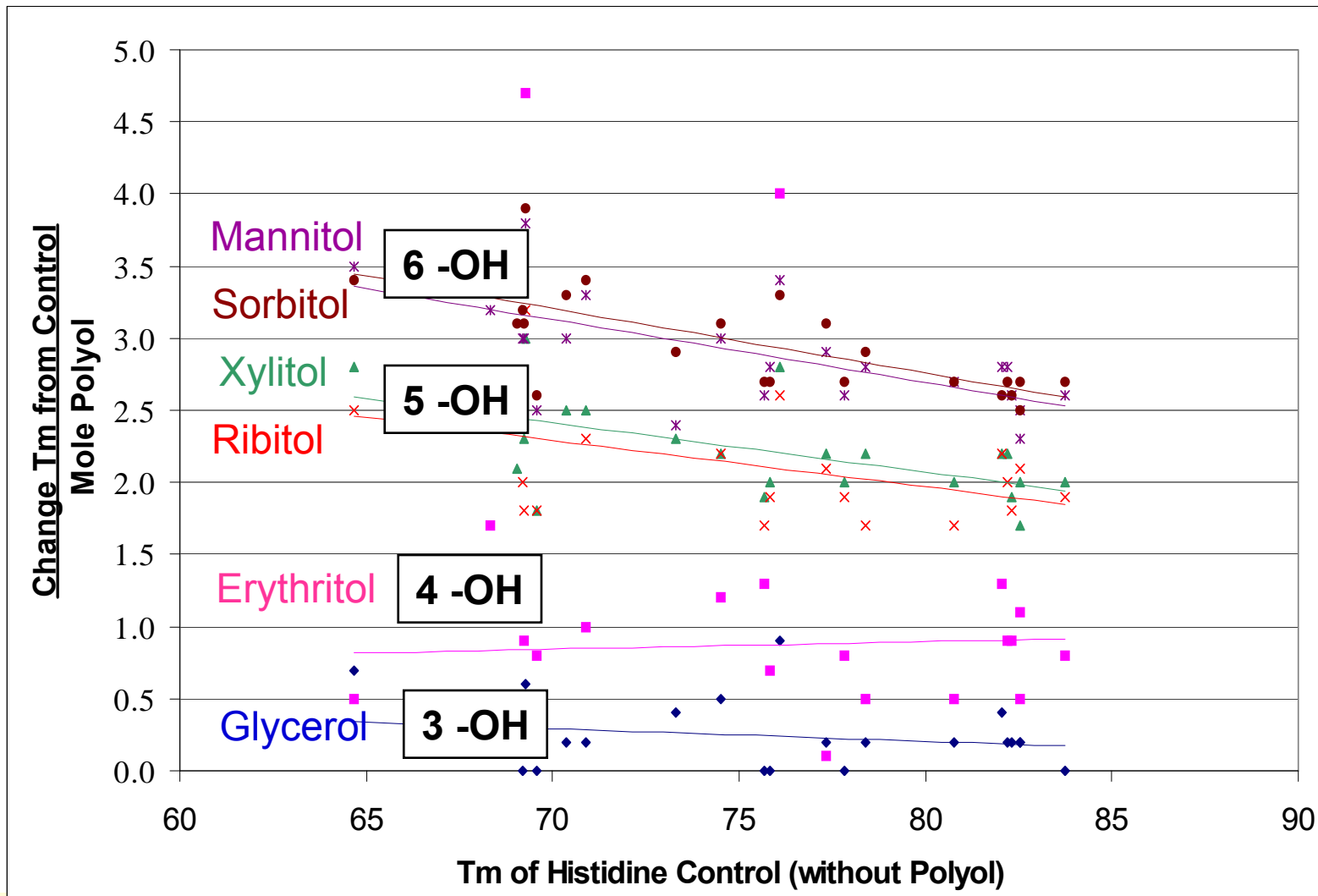


Polyol Titration: L1B

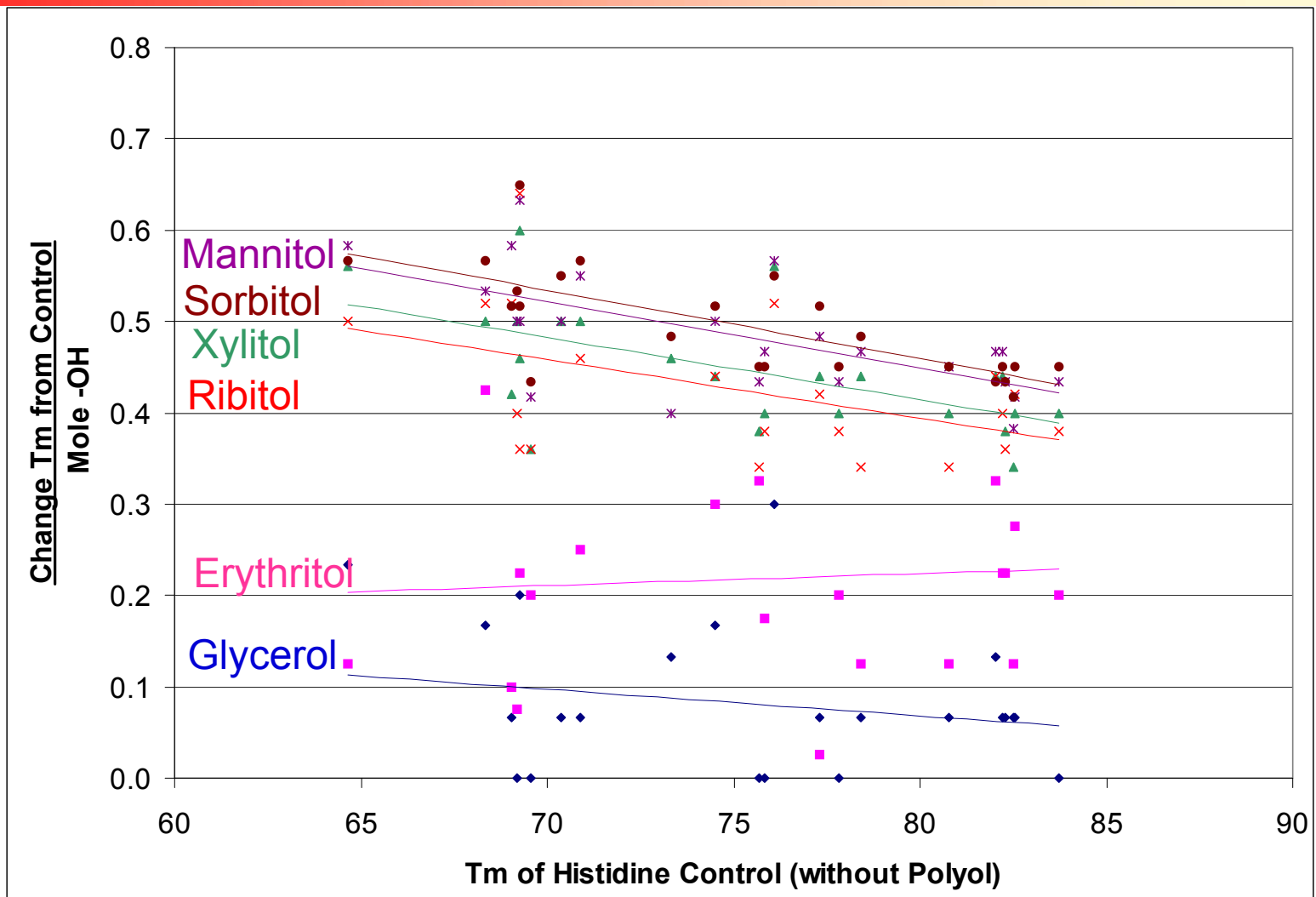


Difference in T_m at lower temperatures is greater than the difference at higher temperatures

Change in Tm/Mole Polyol



Change in Tm/Mole Alcohol



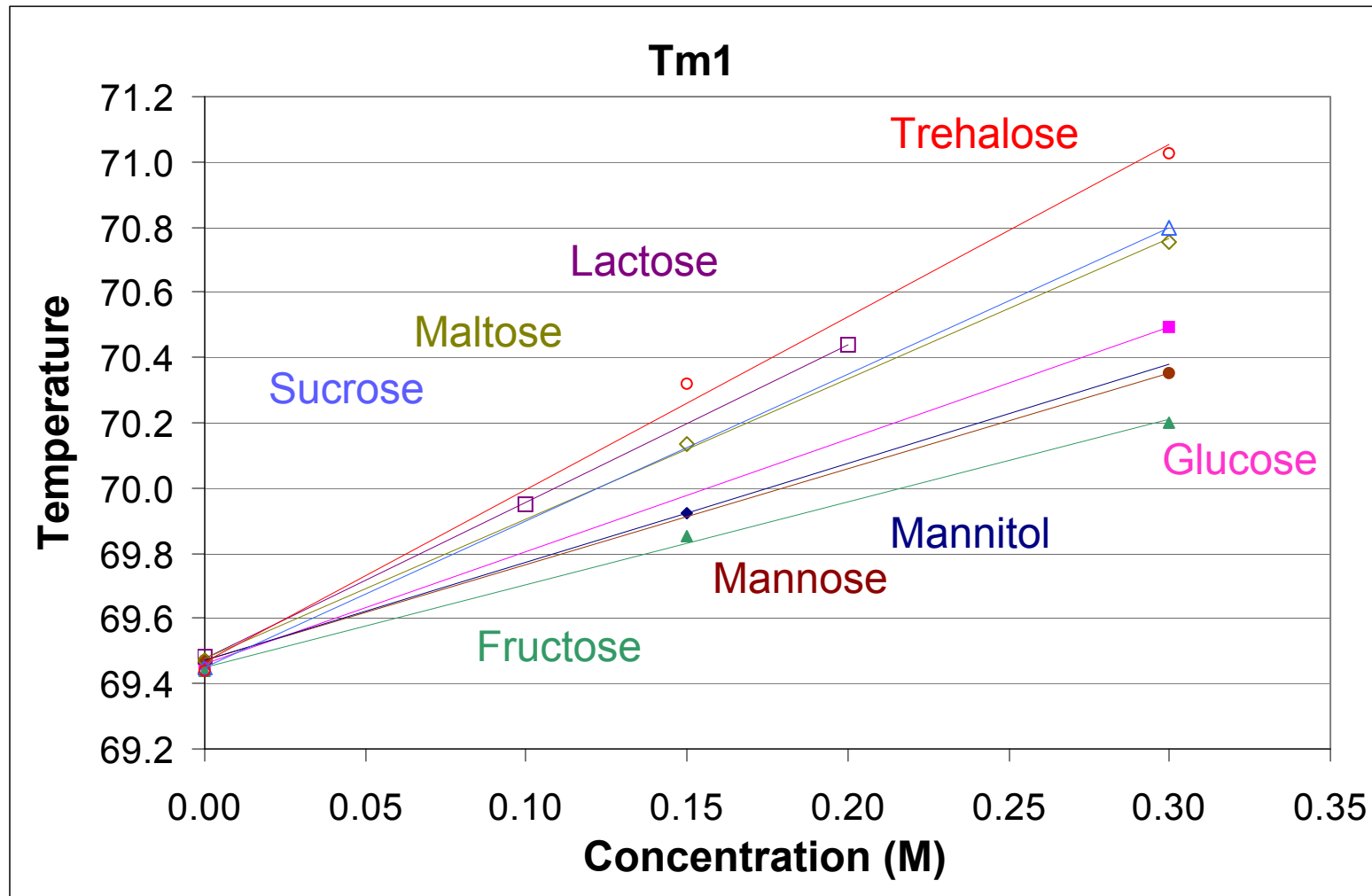
Further Questions

- **Is the number of alcohols still the dominating factor for carbohydrates?**
- **Would the combination of two polyols have an additive or synergistic effect?**

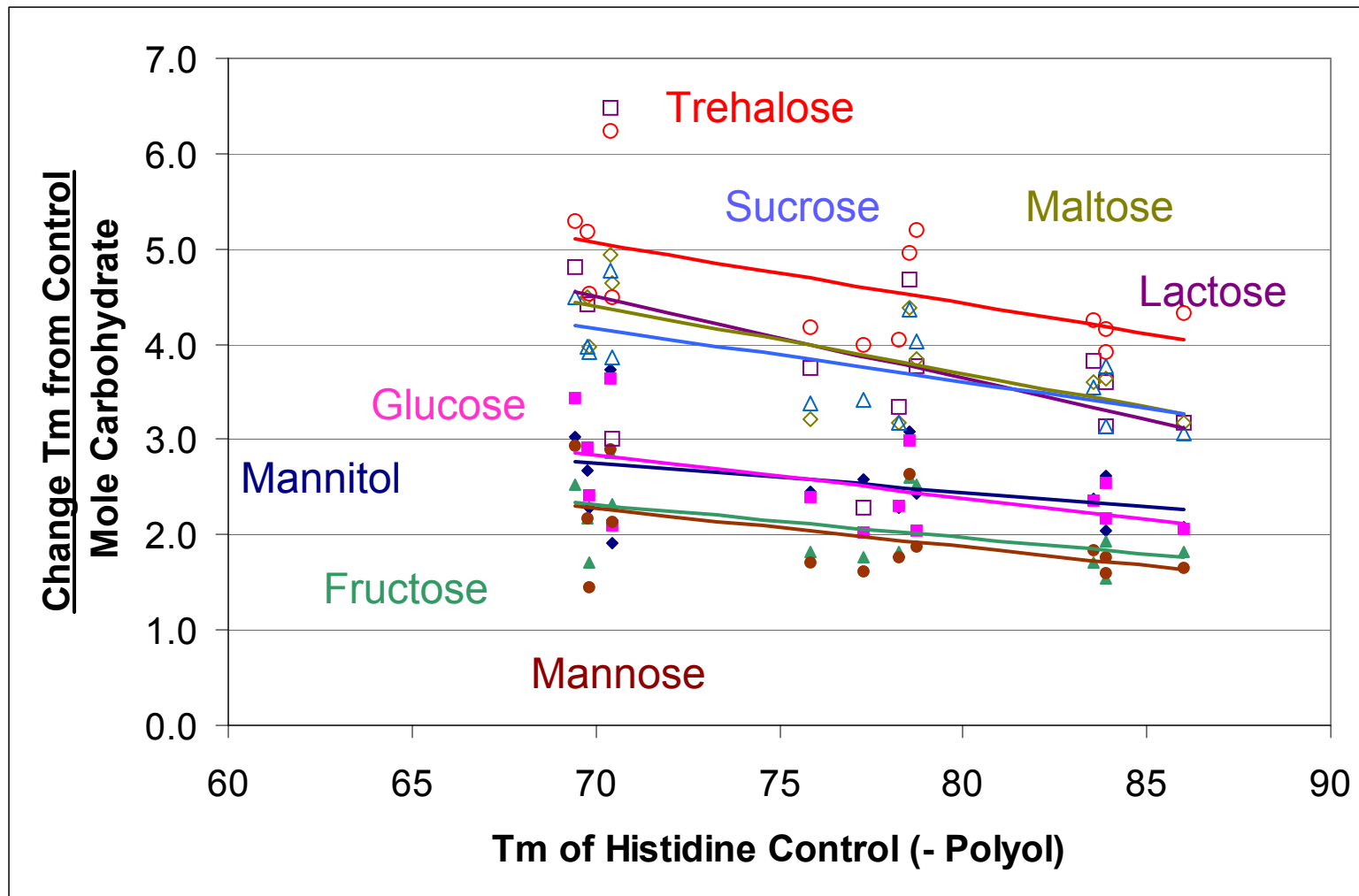
Carbohydrate Titrations

- **Dialyzed 5 mAbs into water and diluted to 6 mg/mL**
- **Made stock concentration of Histidine of 175 mM, pH 6**
- **Made stock concentrations of carbohydrates of 600 mM**
 - ▶ Control: Mannitol
 - ▶ Monosaccharides: Fructose, Glucose, Mannose
 - ▶ Disaccharides: Lactose, Maltose, Sucrose, Trehalose

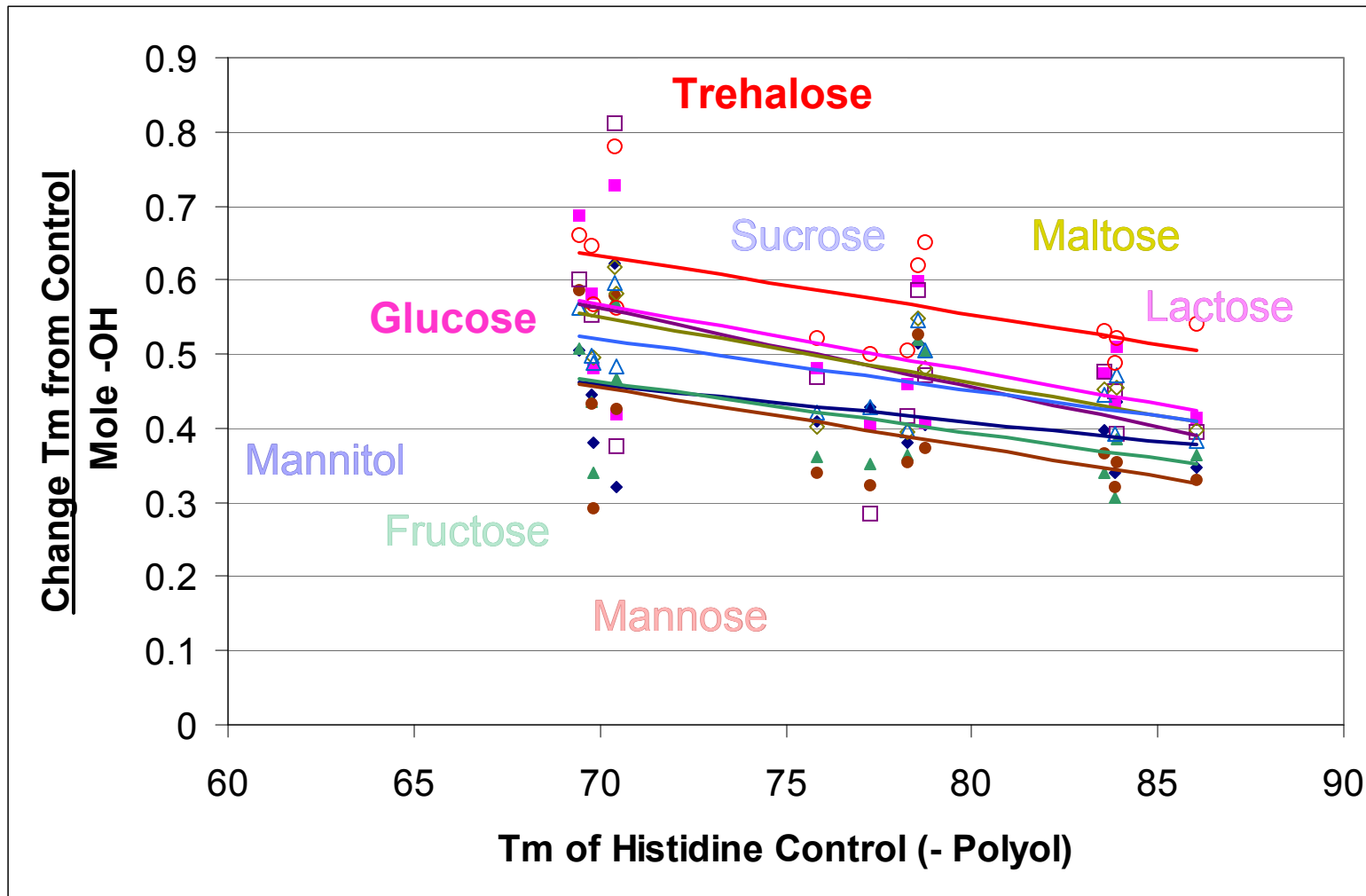
Carbohydrate Titration: L1B T_m1



Change in Tm/Mole Carbohydrate

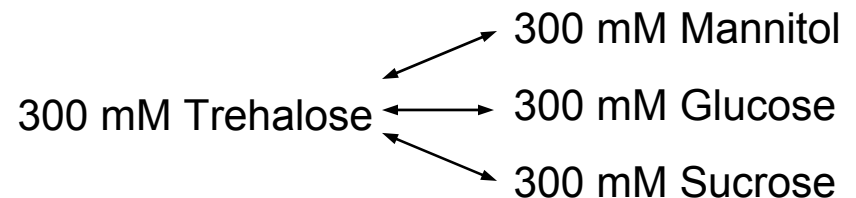
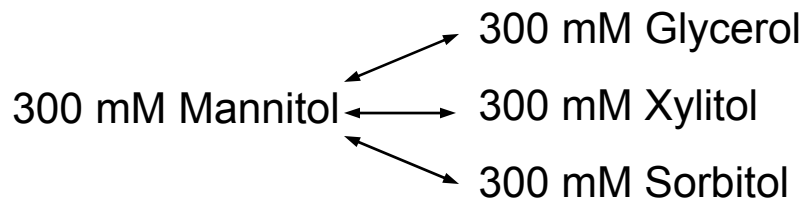


Change in Tm/Mole Alcohol

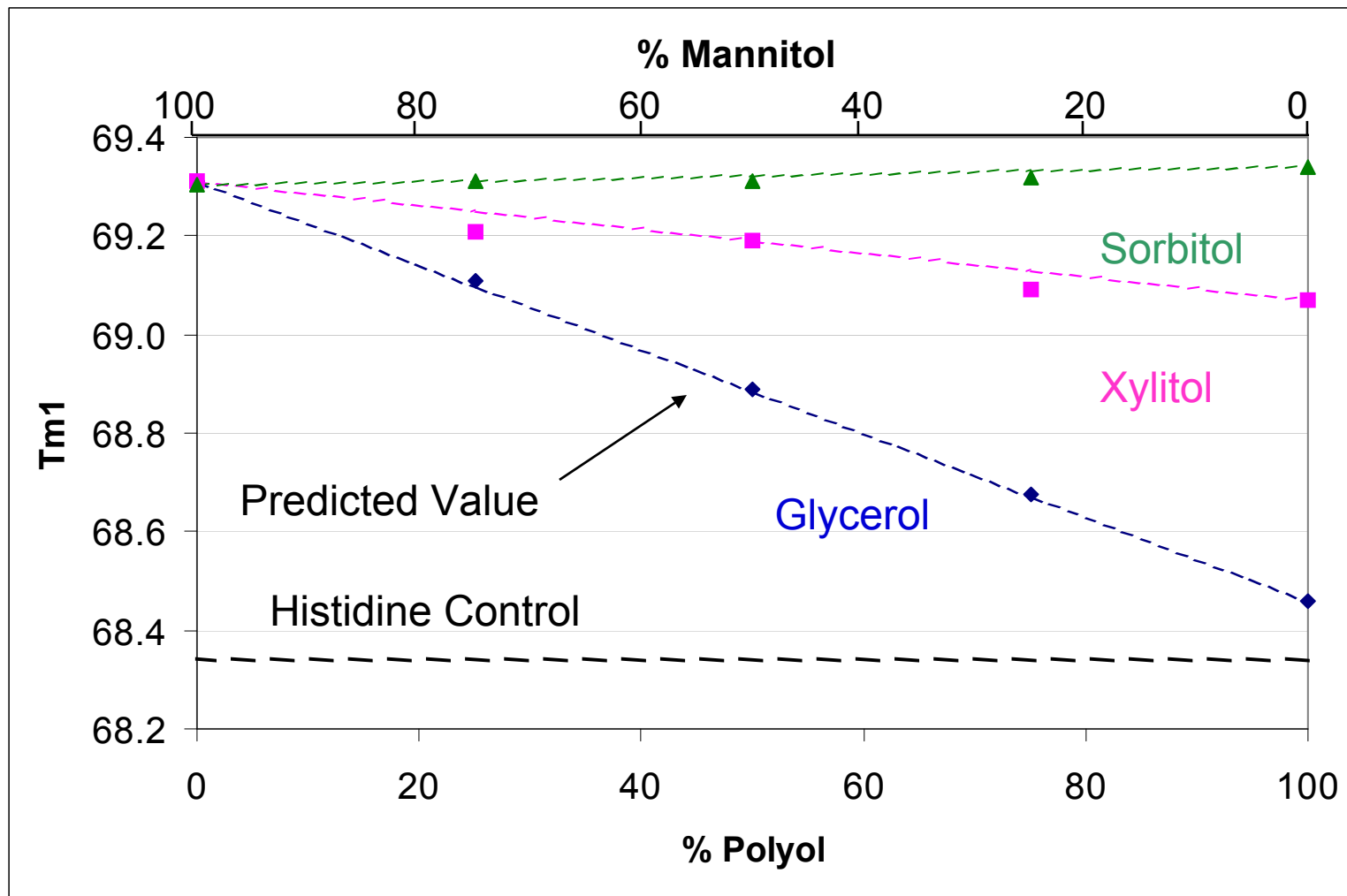


Combinations of Polyols

- Formulated L1B into 10 mM histidine, pH 6.0
- Spiked in 300 mM polyol or carbohydrate
- Titrated the one solution against the three other solutions
- Maintained a final polyol or carbohydrate concentration of 300 mM

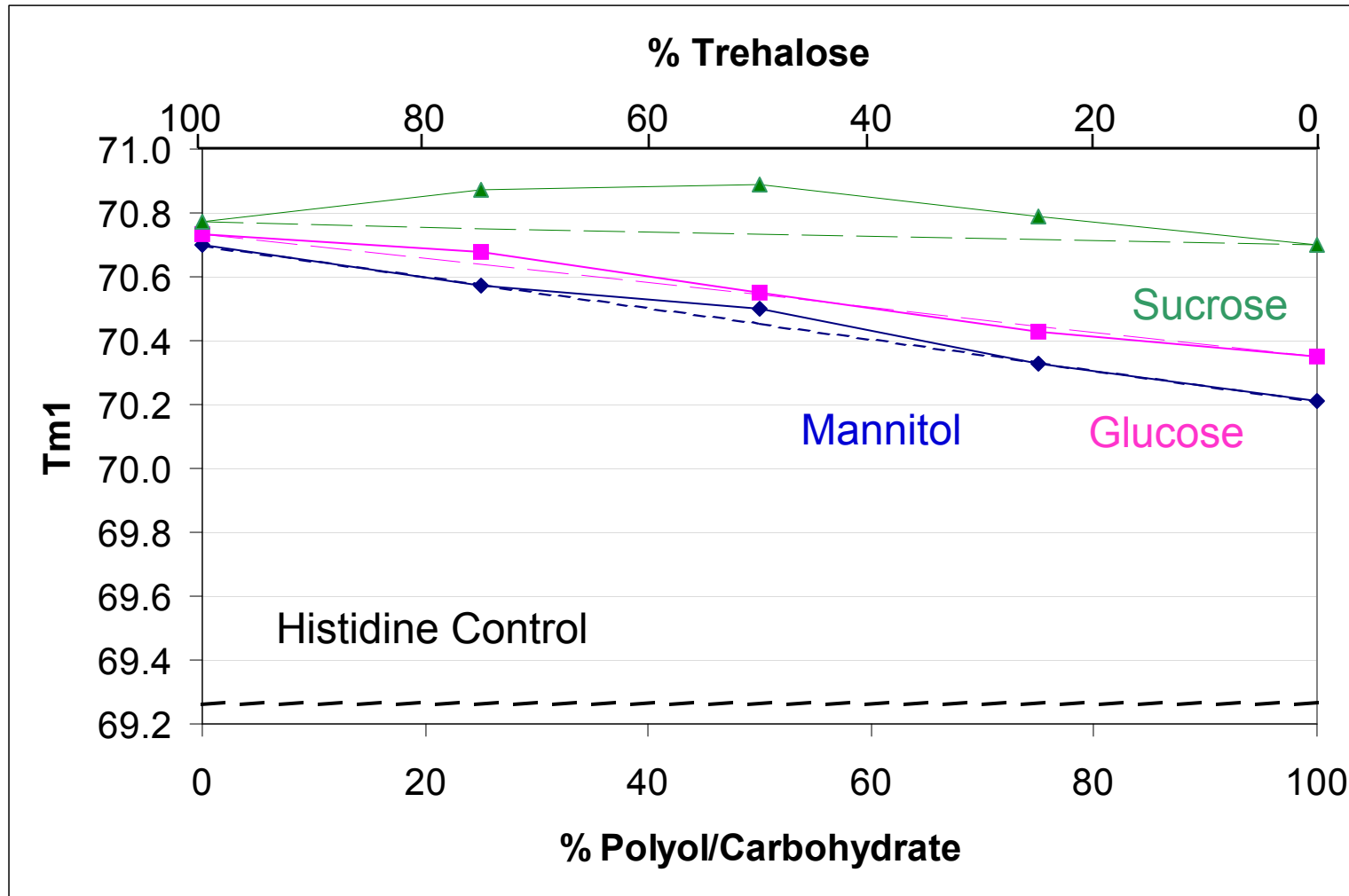


Combination of Polyols: L1B



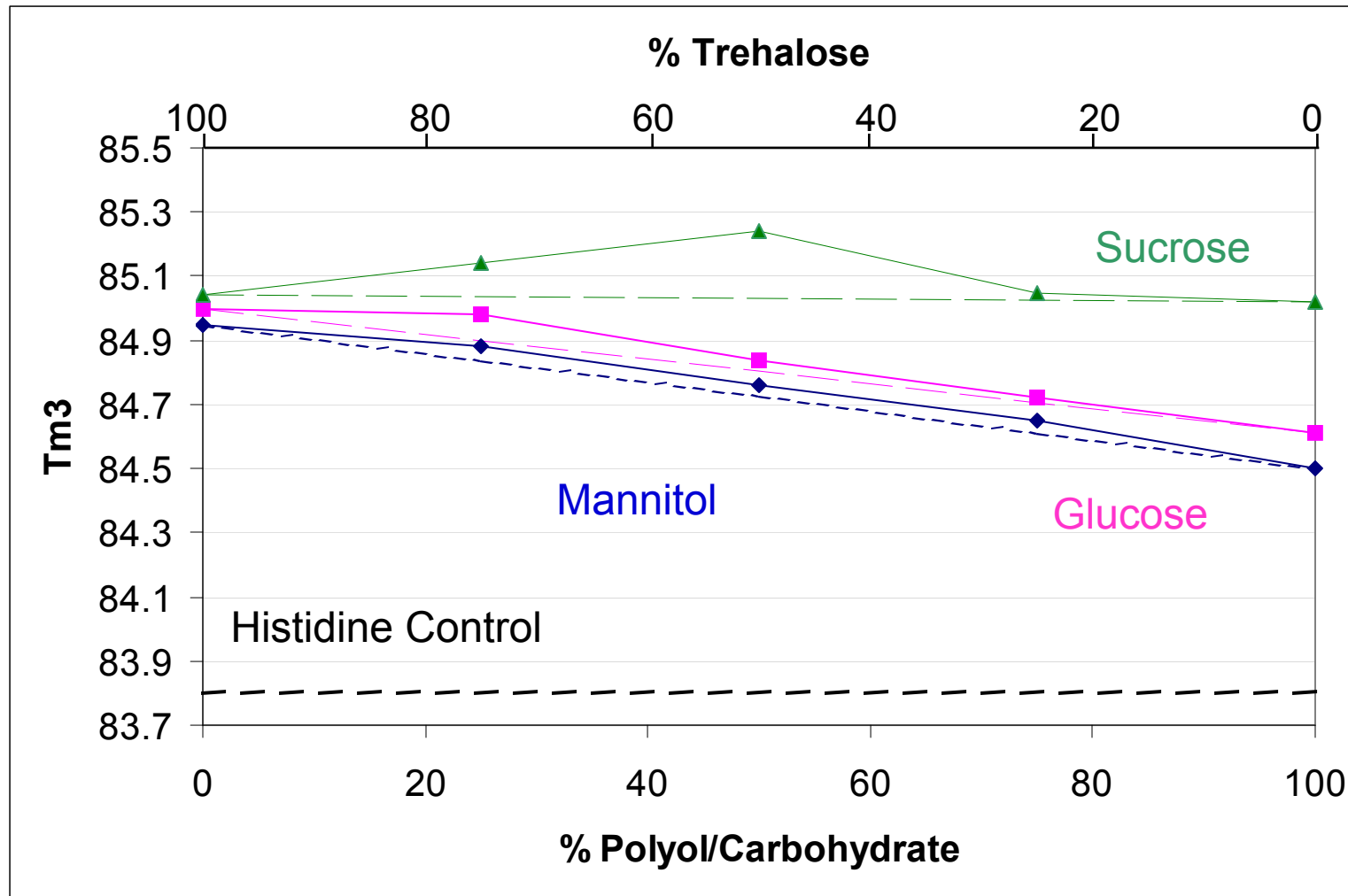
Titration of two polyols results with an additive effect

Combination of Carbohydrates: L1B



Titration of two carbohydrates results with an synergistic effect

Combination of Carbohydrates: L1B

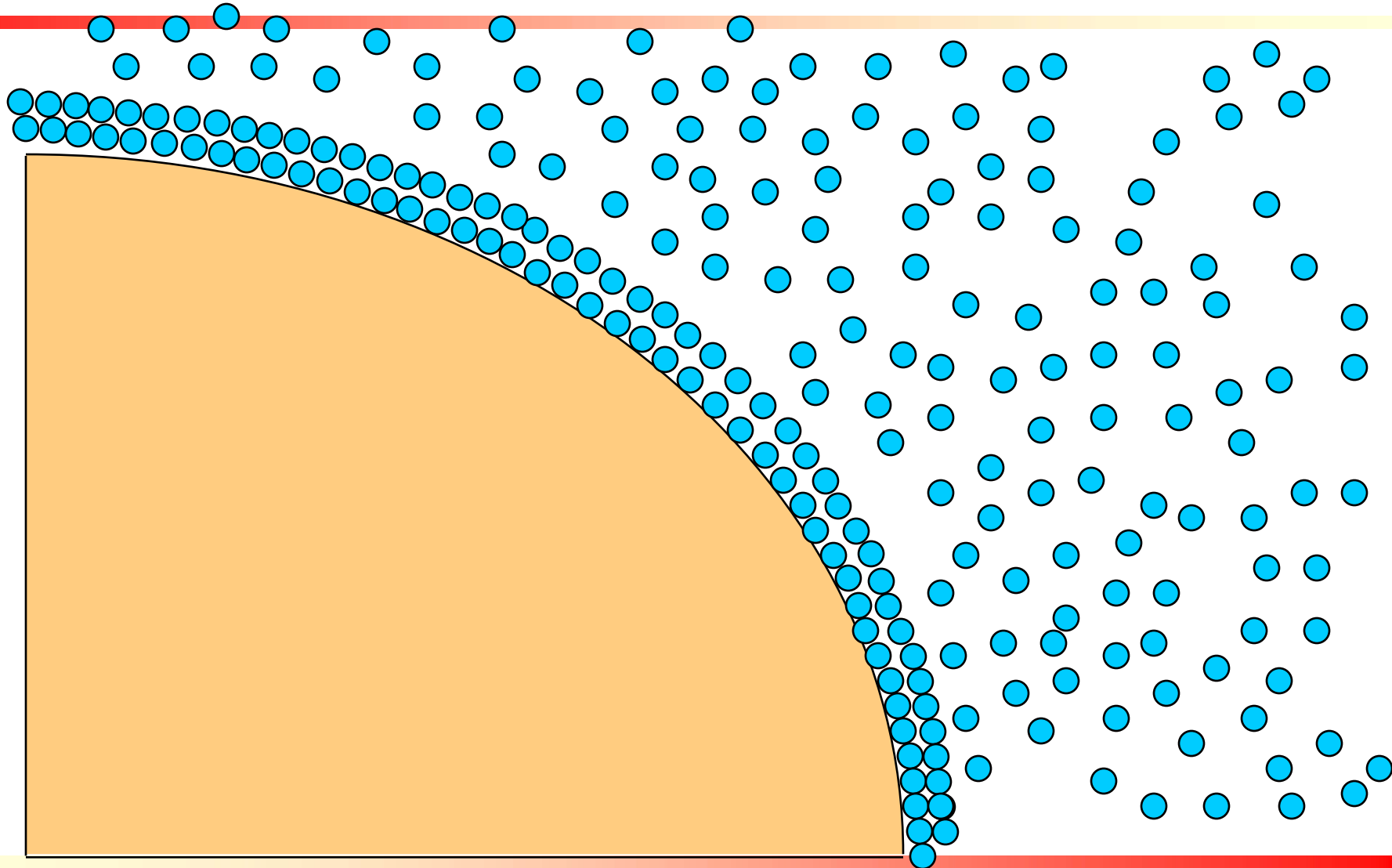


Titration of two carbohydrates results with an synergistic effect

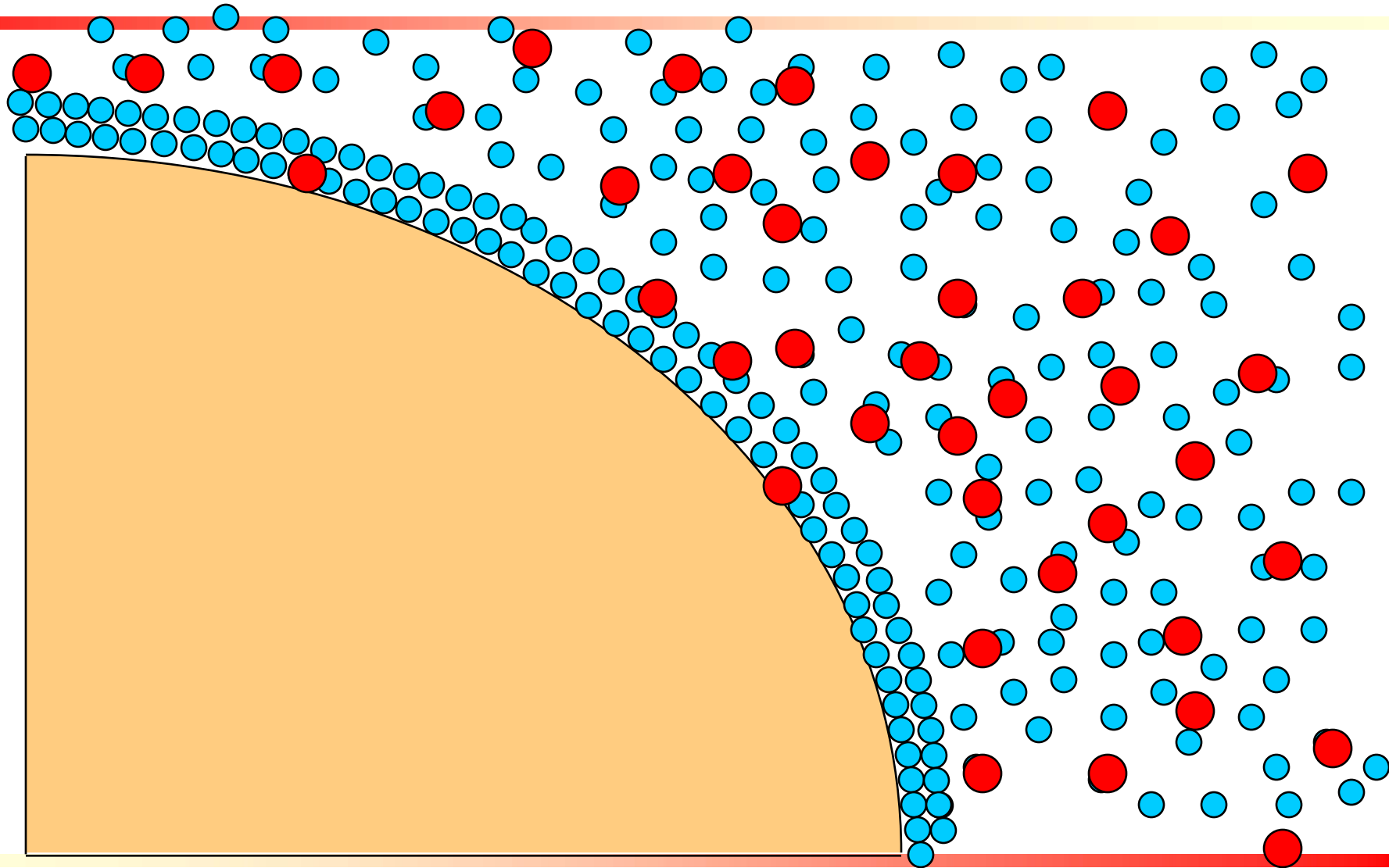
Mechanisms of Action

- **Why do glycerol and erythritol not stabilize as well as the larger polyols?**
- **Why are the number of alcohols no longer the dominating factor for carbohydrates?**

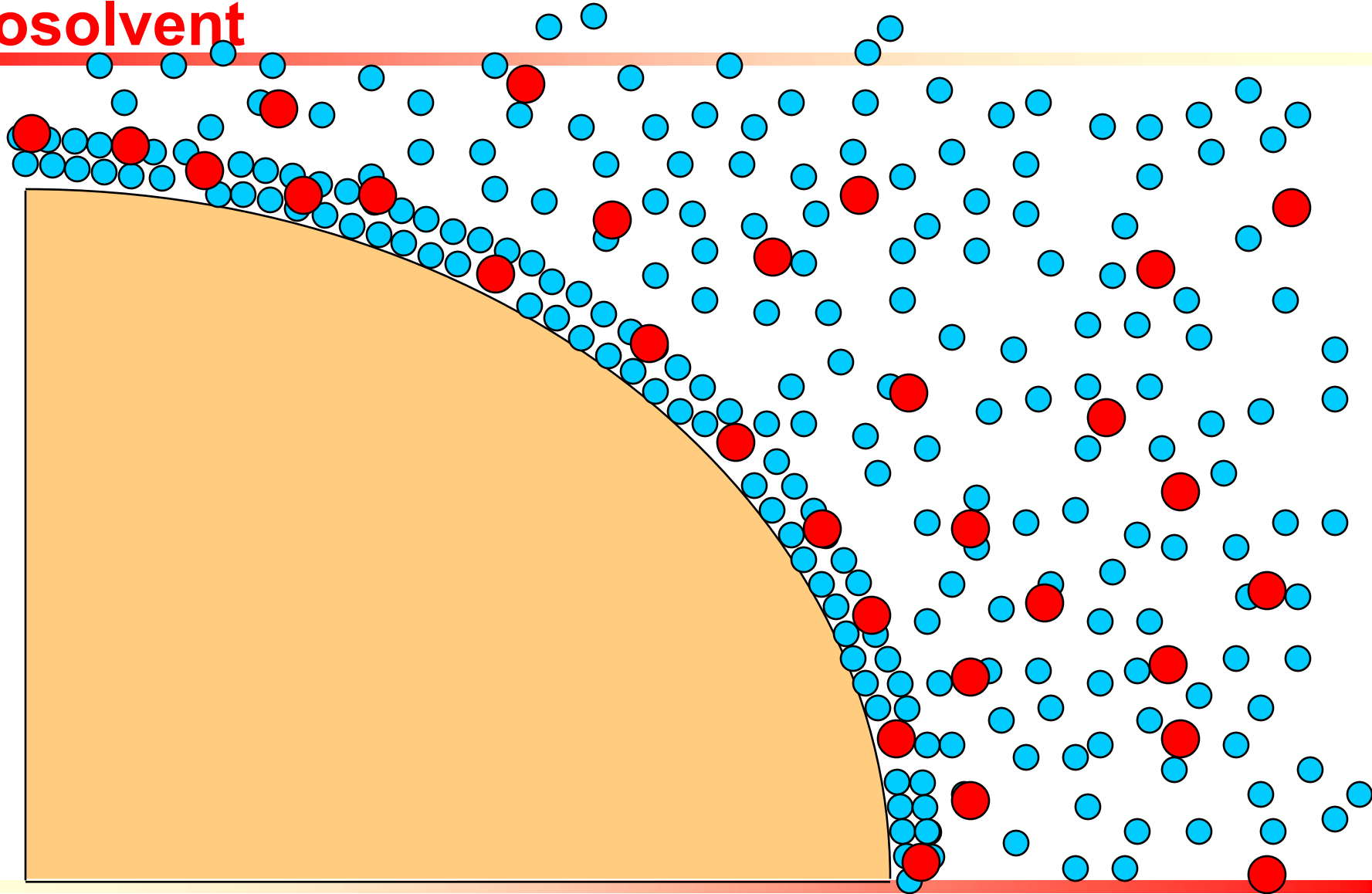
Protein Hydration



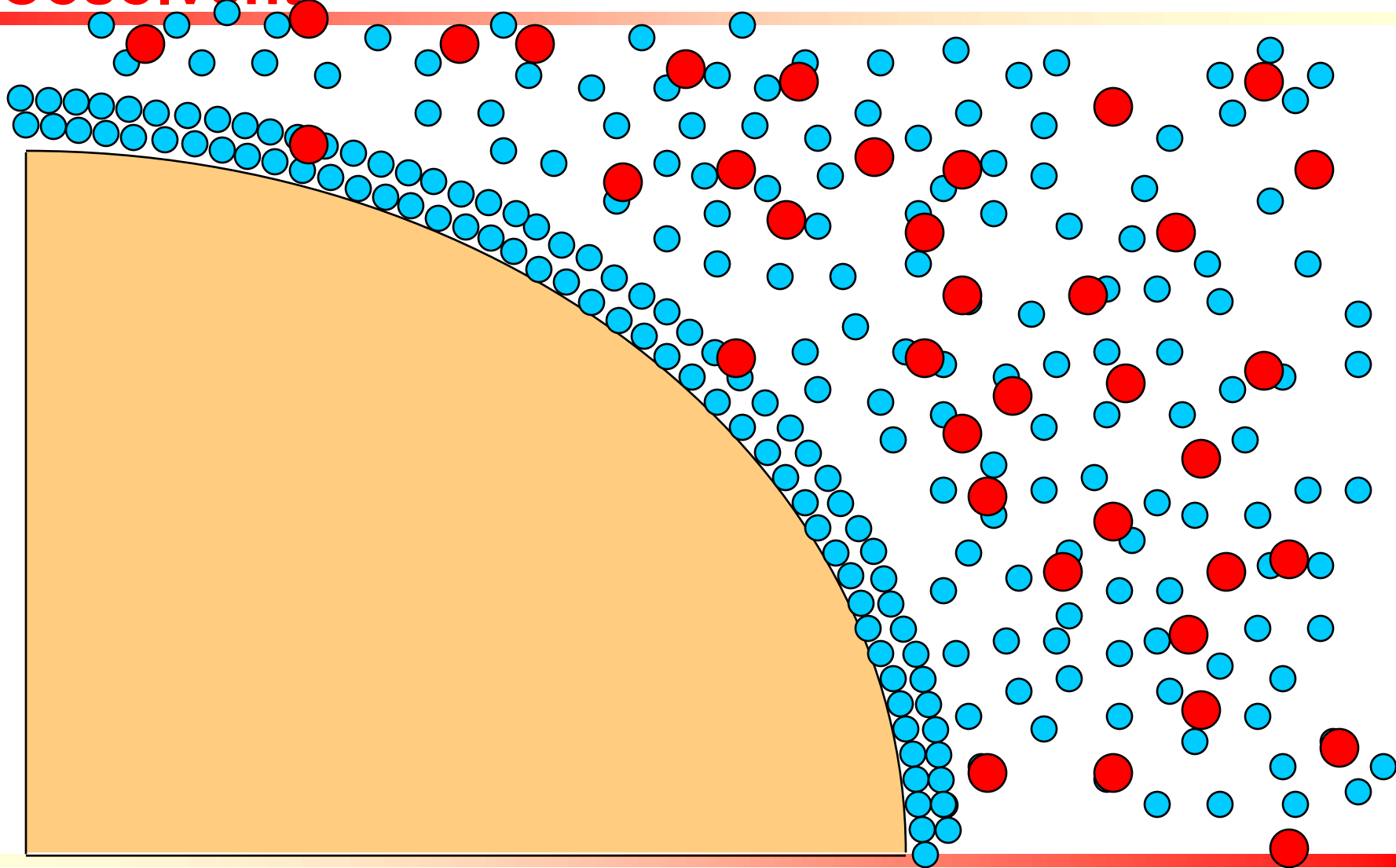
Protein Hydration – Addition of a Cosolvent



Protein Hydration – Preferential Binding of a Cosolvent

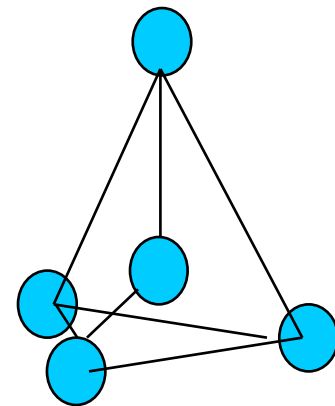


Protein Hydration – Preferential Exclusion of a Cosolvent

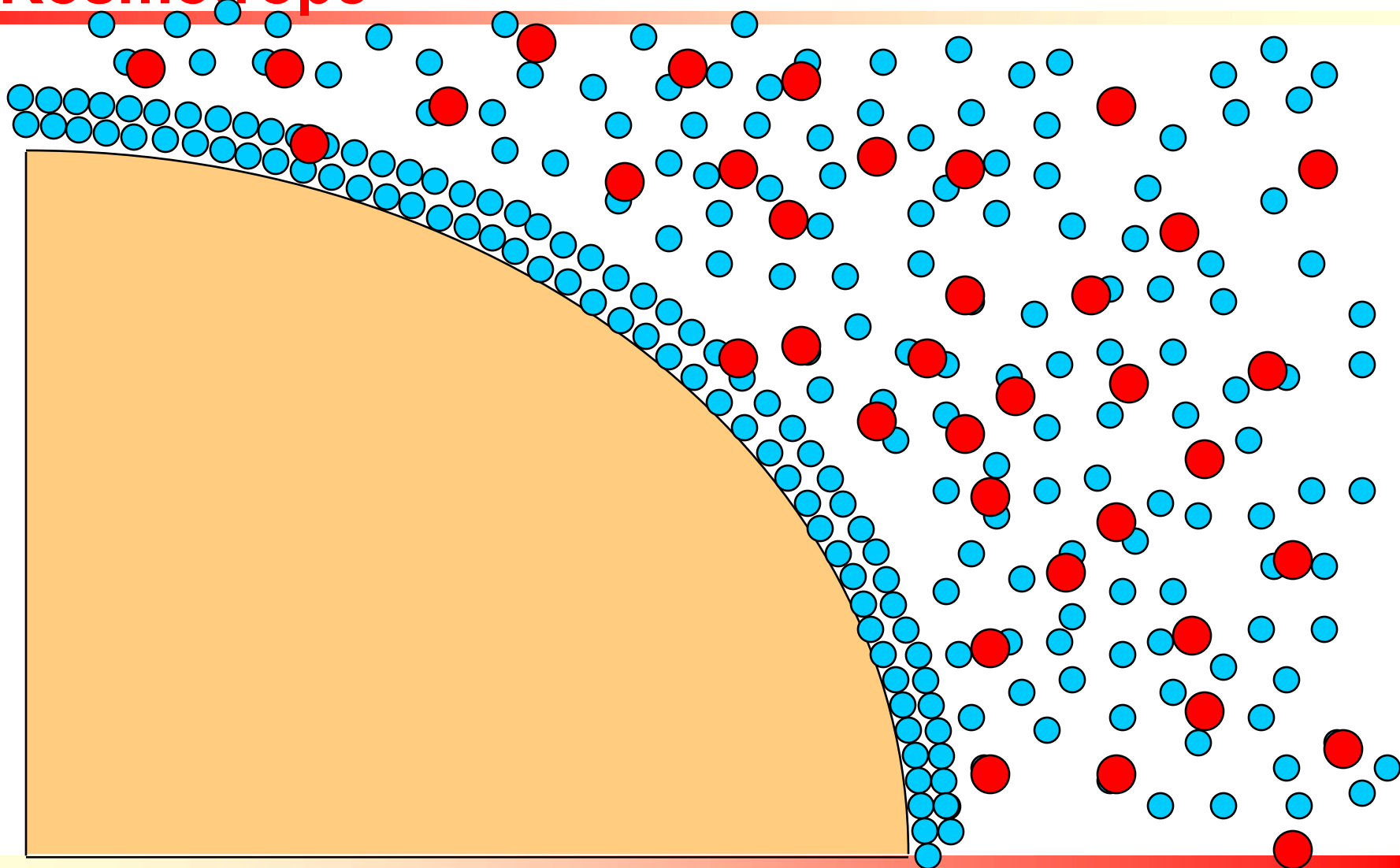


Effect of Cosolvents on Water

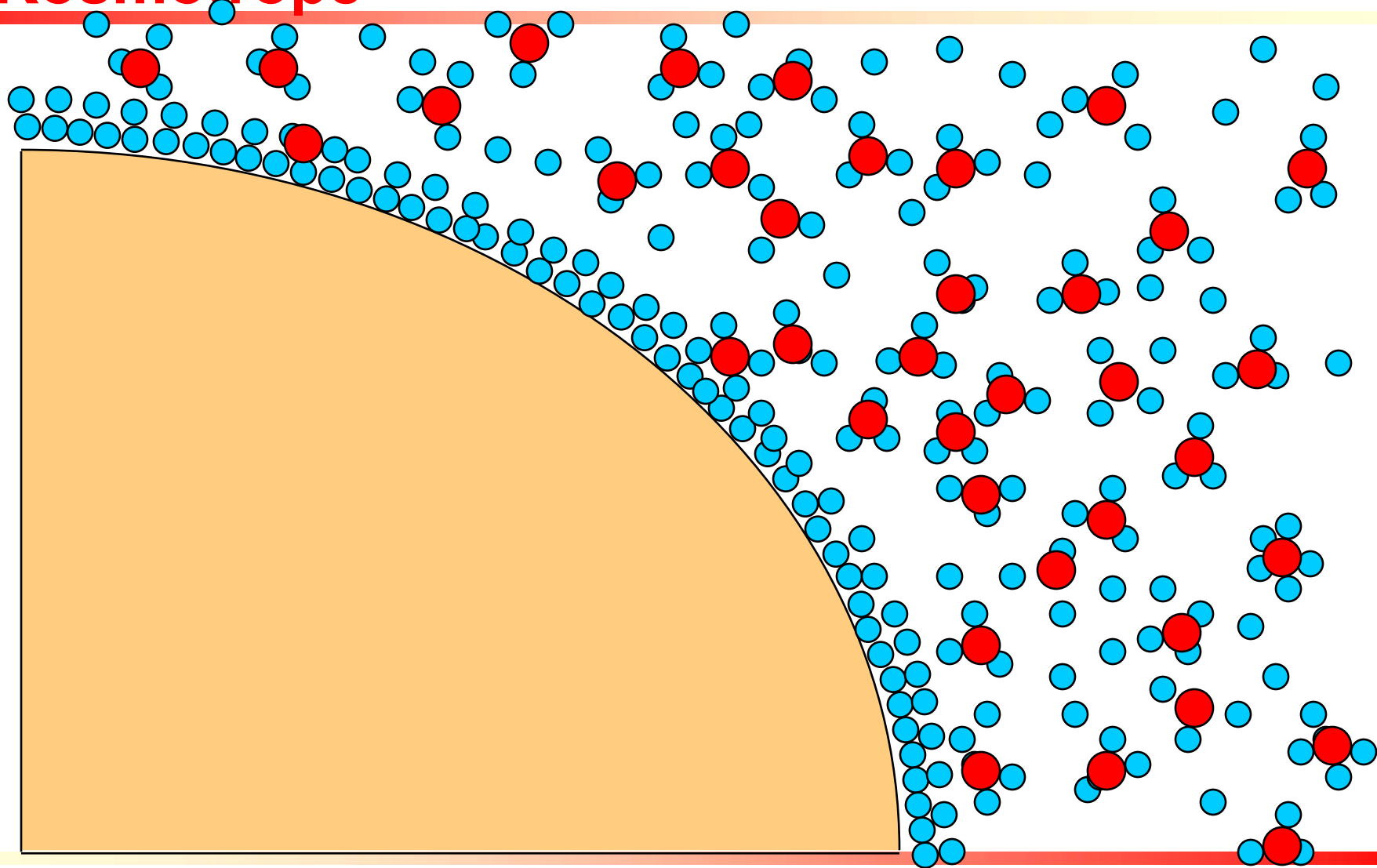
- **Water naturally forms structure in solution**
- **This structure can either be**
 - ▶ Strengthen by kosmotropes (e.g. sugars and polyols)
 - ▶ Weakened by chaotropes (e.g. guanidine)



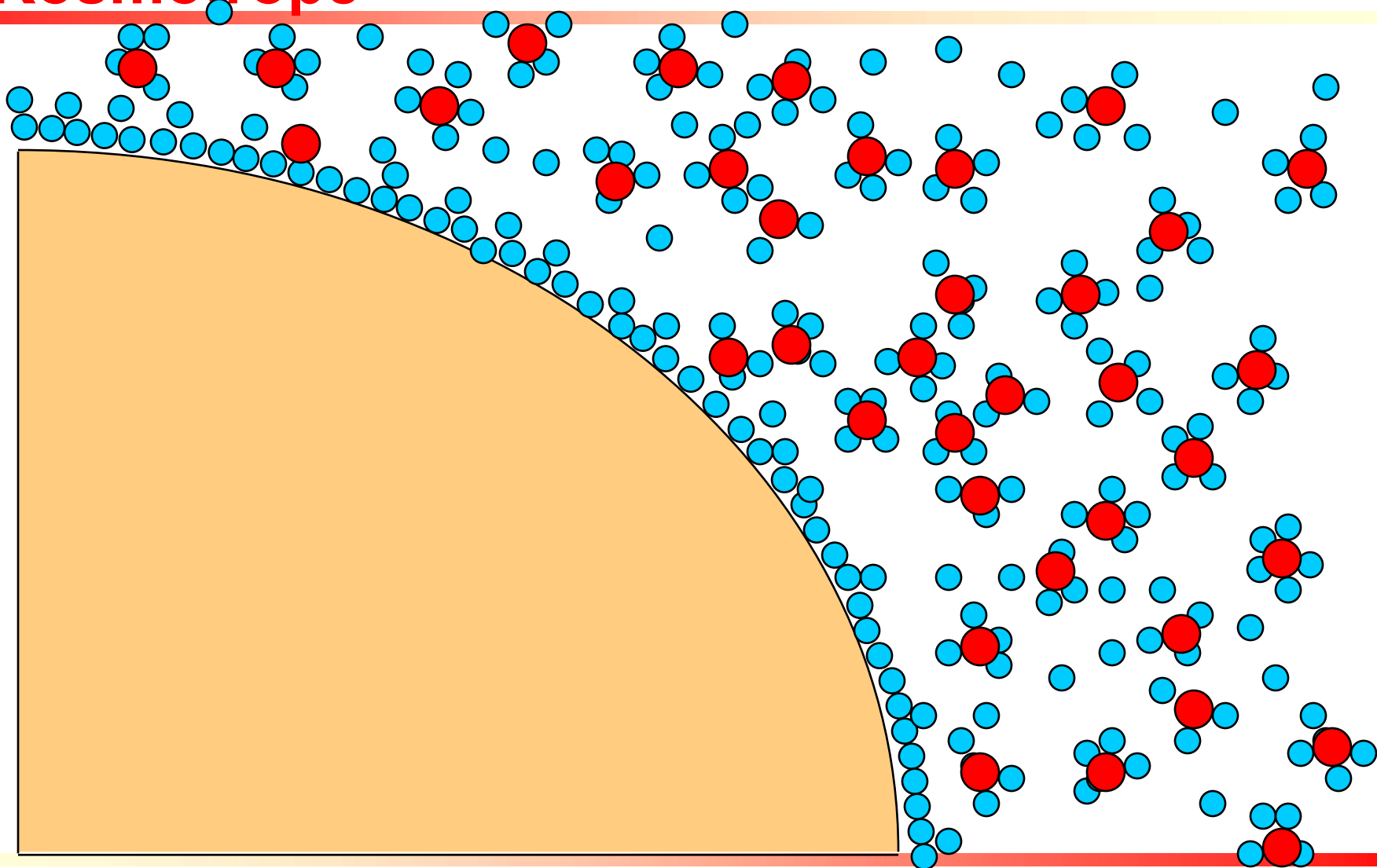
Protein Hydration – Preferential Exclusion of a Kosmotrope



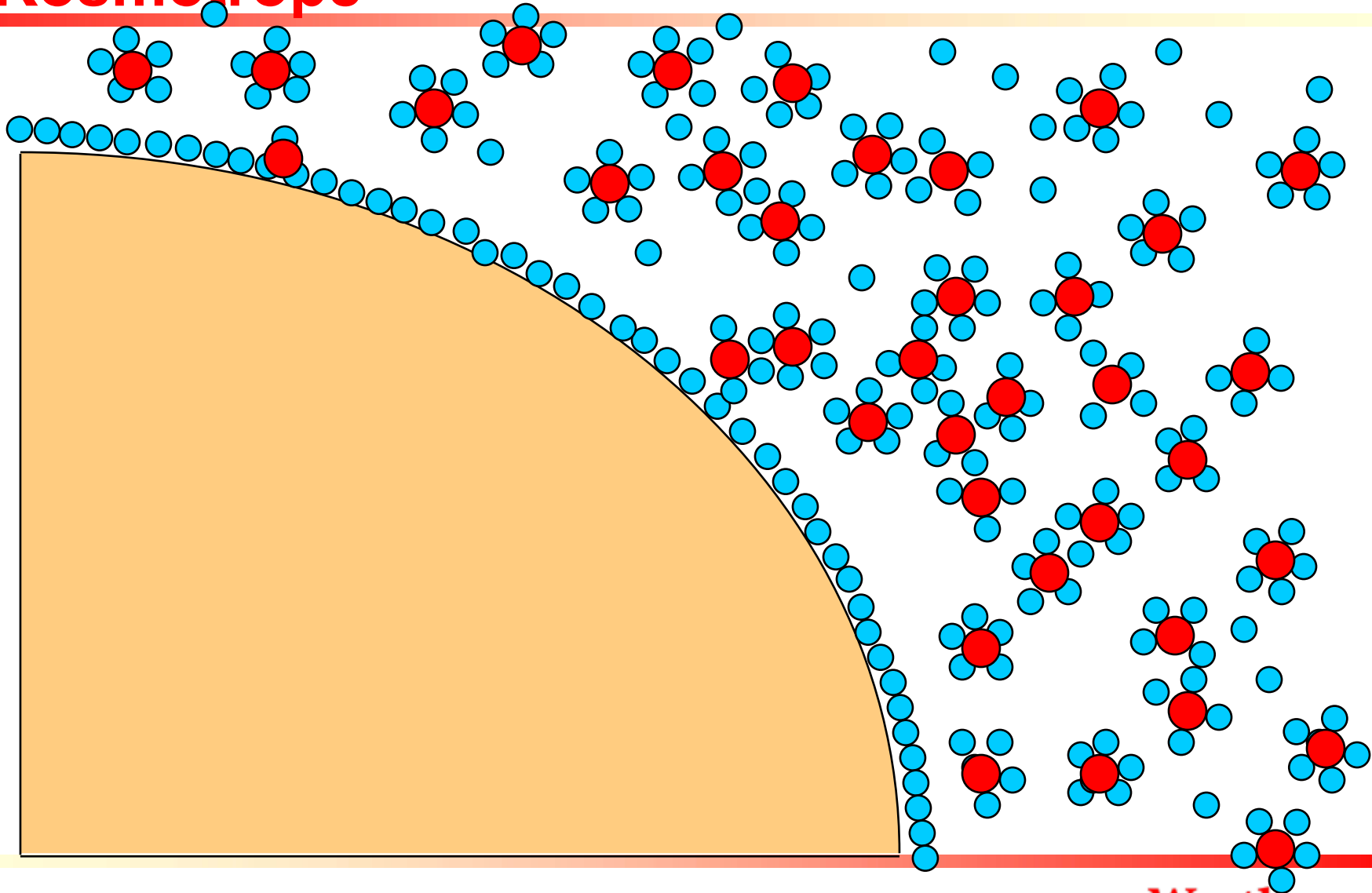
Protein Hydration – Preferential Exclusion of a Kosmotrope



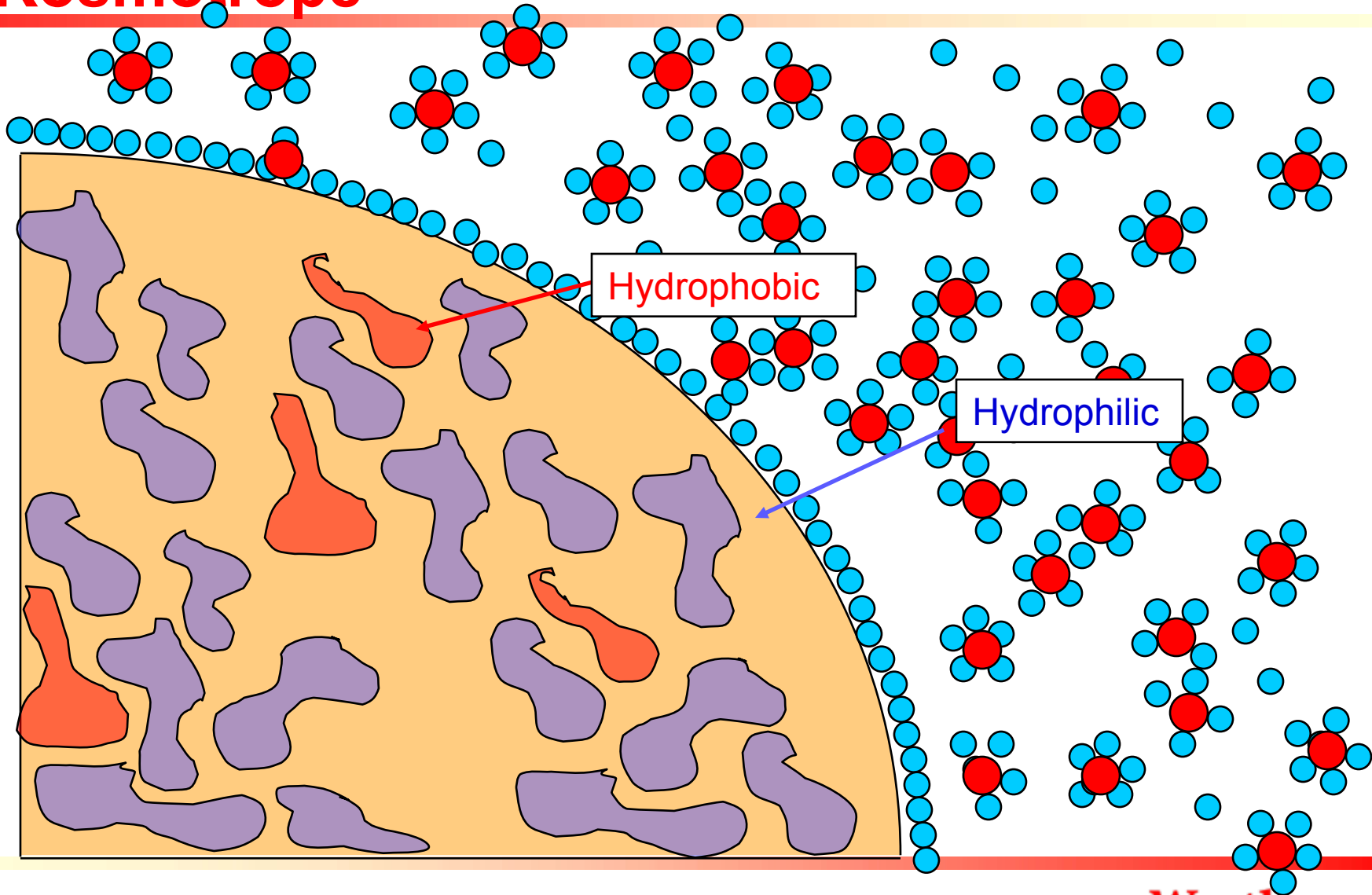
Protein Hydration – Preferential Exclusion of a Kosmotrope



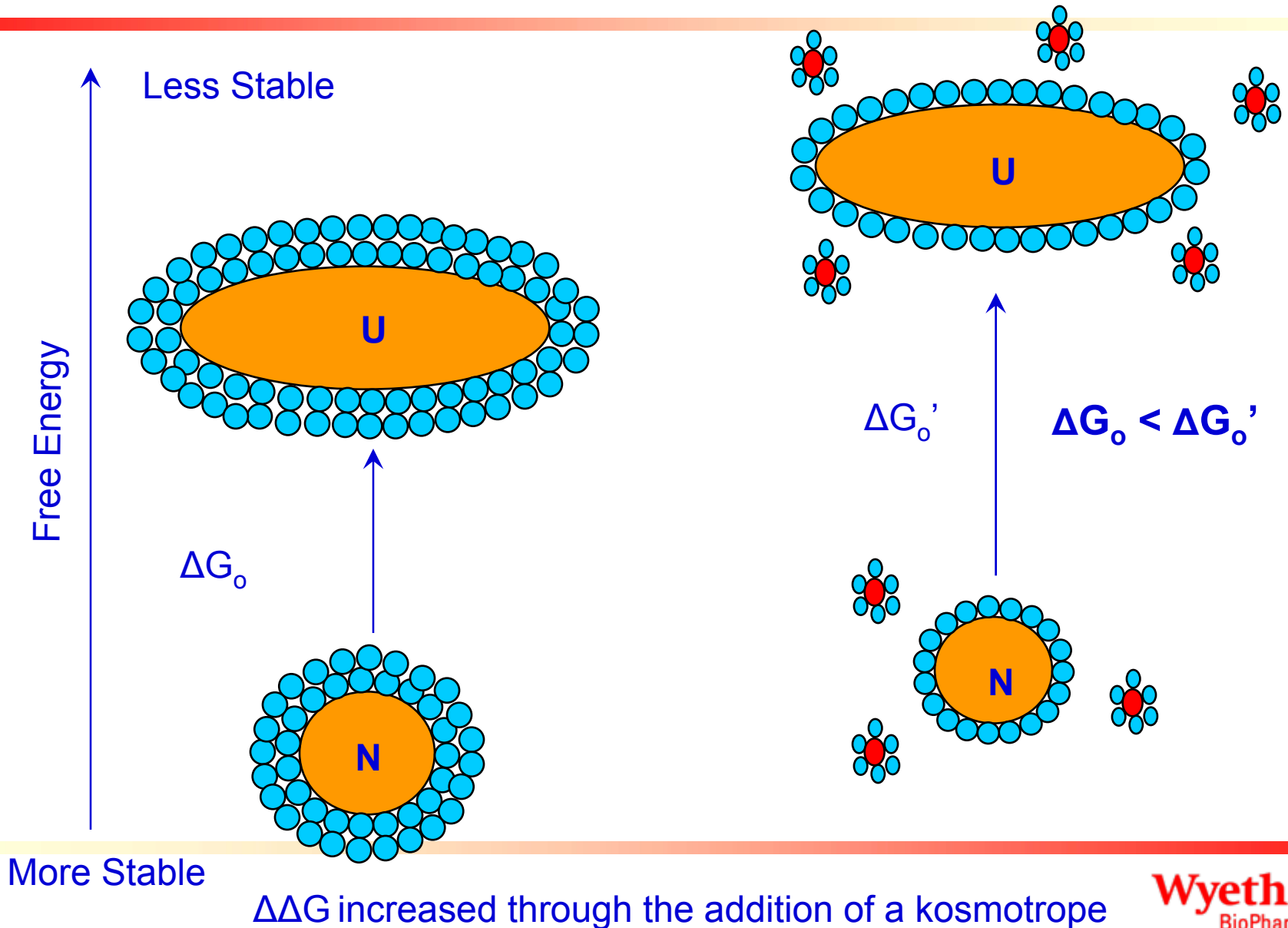
Protein Hydration – Preferential Exclusion of a Kosmotrope



Protein Hydration – Preferential Exclusion of a Kosmotrope



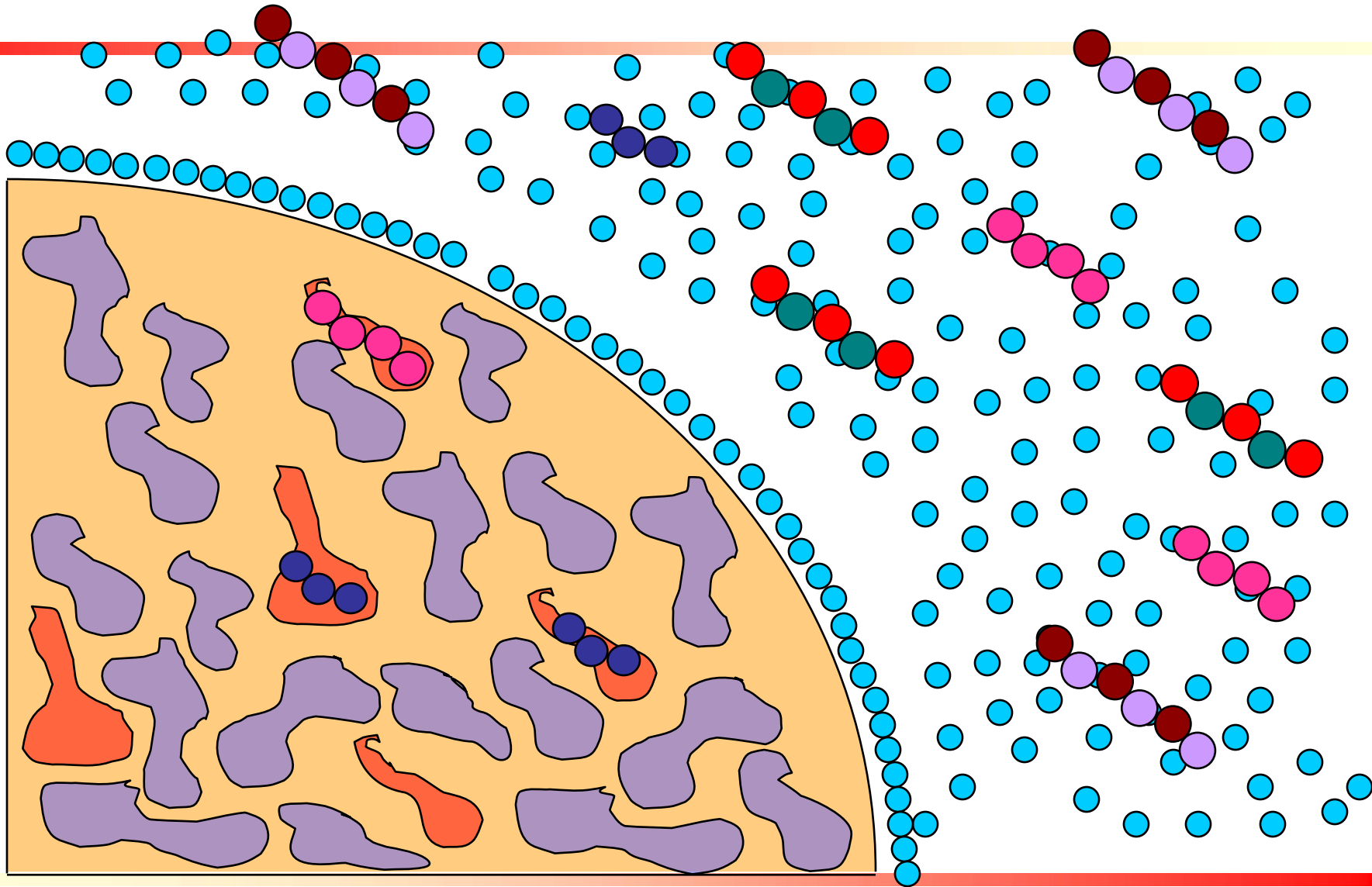
Polyols Destabilize the U more than the N



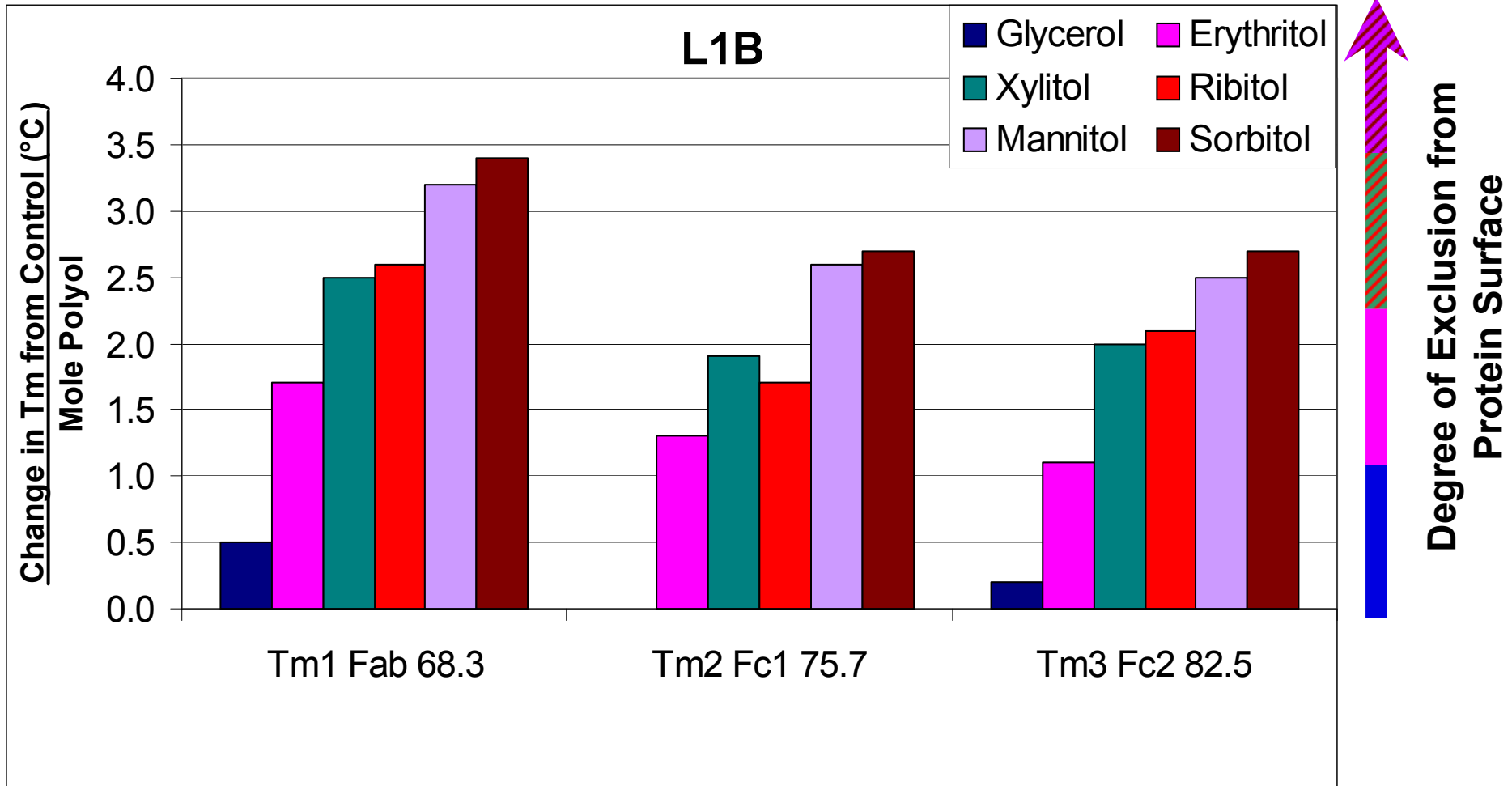
Properties of a Good Kosmotrope/stabilizer

- Needs to have the ability to order water
- Needs to be excluded from the surface of the protein

Glycerol and Erythritol: Size Does Matter

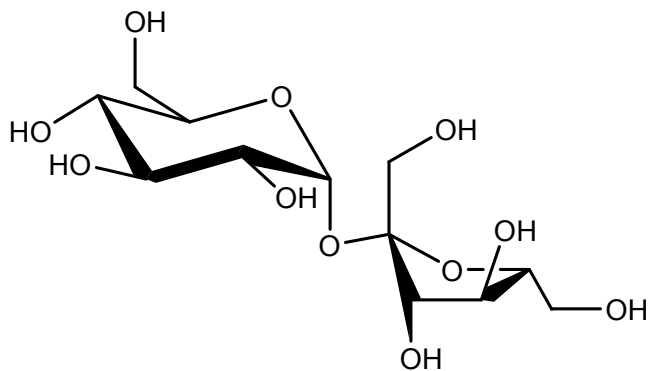


Polyol Titration: L1B T_m 1



Locking in the Hydrogen Bonds

Sucrose

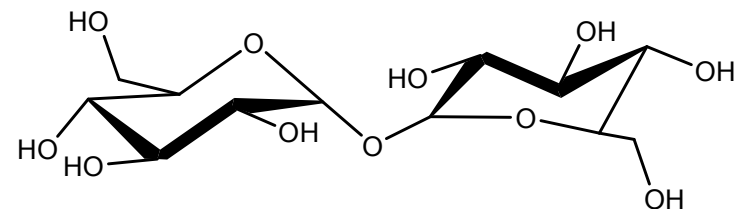


2.5 x Greater

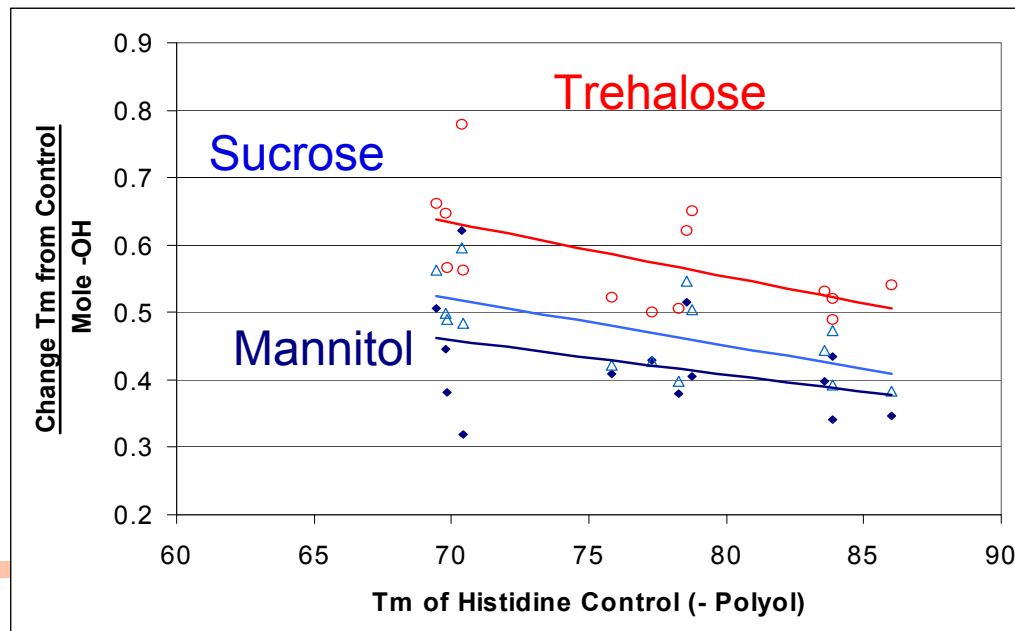
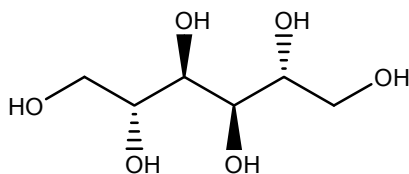


Water Shell

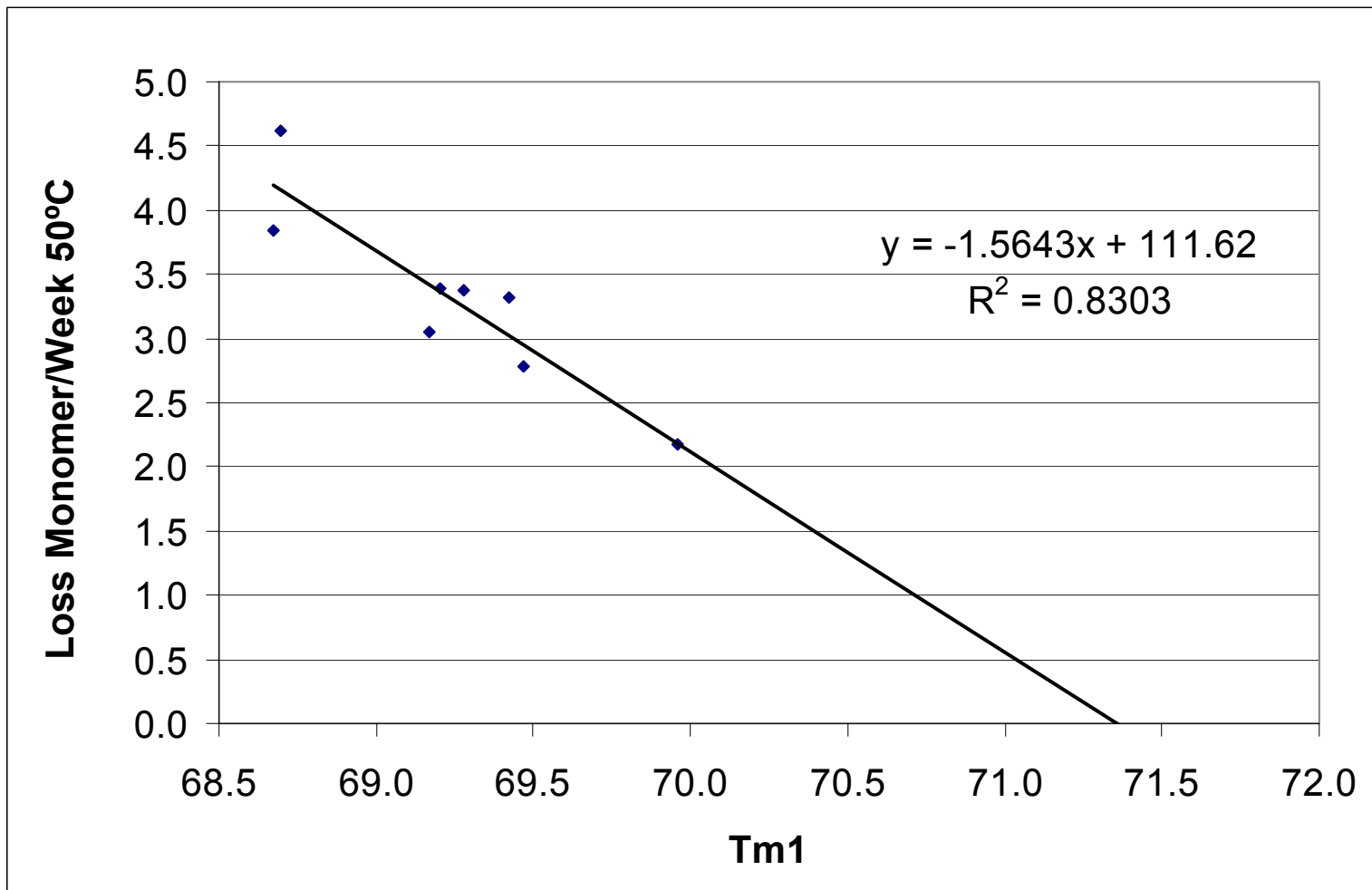
Trehalose



Mannitol



Real Time Stability of L1B Formulated in Polyols/Sugars



Conclusions

- **Larger polyols exhibit greater thermal stability due to the increased number of hydroxyl groups**
- **There is no significant preference for a protein between stereoisomers of a polyol**
- **The larger the starting T_m , the less thermal stability can be induced by the addition of polyols**
- **Smaller polyols may associate with the surface of a protein and therefore not increase the T_m as greatly**
- **Carbohydrates can induce greater thermal stability because their more rigid ring structure is better at ordering water**

Acknowledgements

- **Wyeth - DPD**

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- ▶ Verna Frasca

References

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