AUC Analysis of Membrane Proteins
Lethbridge, Canada
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Sedimentation Equilibrium of Membrane Proteins: Determination of Molar Mass and Equilibrating Status

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Why Sedimentation Equilibrium?

- Purified protein system
- Want to know the mass and/or stoichiometry
- Want to know the stability of a membrane protein oligomer

Outline

- Some fundamentals
- Strategies for how to density match
- Distinguish between Reversible *versus* Frozen systems
- Units for a reversible system

The Buoyant Molecular Weight is the Experimentally Determined Parameter in an SE Experiment

$$M_B = M_P\left(1-\phi'\rho\right)$$

The Buoyant Molar Mass can be Approximated as a Sum of the Component Terms

$$M_B = M_P\left(1 - \bar{v}_P\rho\right)$$

Protein Contribution

Another Way to Write this Equation

$$M_B = M_P\left[\left(1-\bar{v}_P\rho\right)+\delta_{cs}\left(1-\bar{v}_{cs}\rho\right)+\delta_{H_2O}\left(1-\bar{v}_{H_2O}\rho\right)\right]$$

$$\delta \equiv \frac{\text{grams of a particular component bound}}{\text{grams of protein}}$$

Note that δ is unitless

Under "normal" solvent conditions, the water term is negligible

$$\delta_{H_2O}\left(1-\bar{V}_{H_2O}\rho\right)-->0$$

Consider a soluble protein in 50 mM Tris, 200 mM NaCl: $\rho = 1.0056$

Consider the case where an "average" amount of water bound: $\delta = 0.3$

$$\delta_{H_2O}\left(1-\bar{v}_{H_2O}\rho\right) = 0.3\left(1-(0.99)(1.0056)\right) = 0.00133$$

The water term is normally ignored

$$M_B = M_P\left[\left(1-\bar{v}_P\rho\right)+\delta_{cs}\left(1-\bar{v}_{cs}\rho\right)+\delta_{H_2O}\left(1-\bar{v}_{H_2O}\rho\right)\right]$$

$$M_B \cong M_P\left[\left(1-\bar{v}_P\rho\right)+\delta_{cs}\left(1-\bar{v}_{cs}\rho\right)\right]$$

Then you only have the co-solvent term to worry about

One strategy for SE is to *Density Match* the co-solvent term

Want: $\delta_{CS}\left(1-\bar{v}_{CS}\rho\right)-->0$

When this is the case: $M_B \cong M_P\left[\left(1-\bar{v}_P\rho\right)\right]$

Do this by adjusting the solvent density such that:

$$\bar{v}_{CS}\rho-->1$$

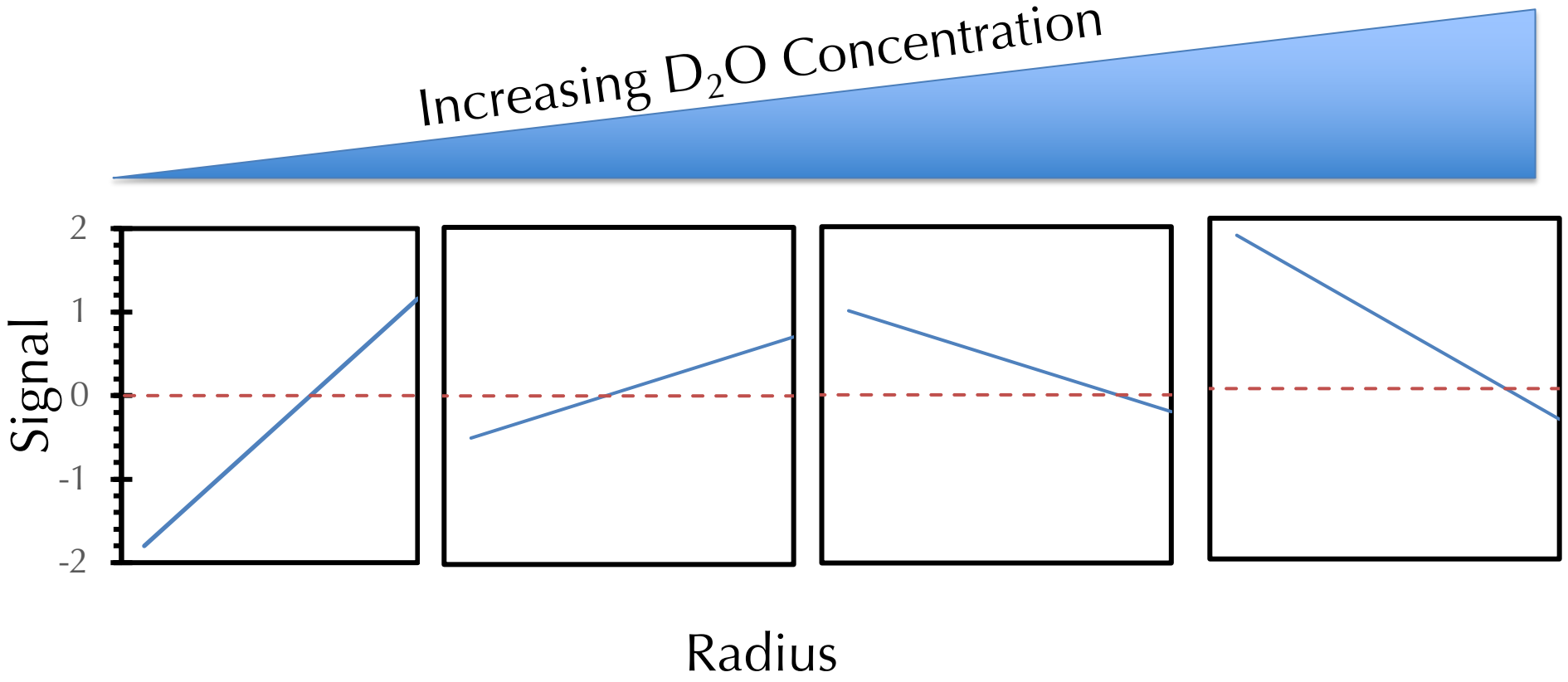
Remember you are already "density matching" the water term…
Ideally you do not want to mess that up.

Heavy water is best for density matching co-solvent

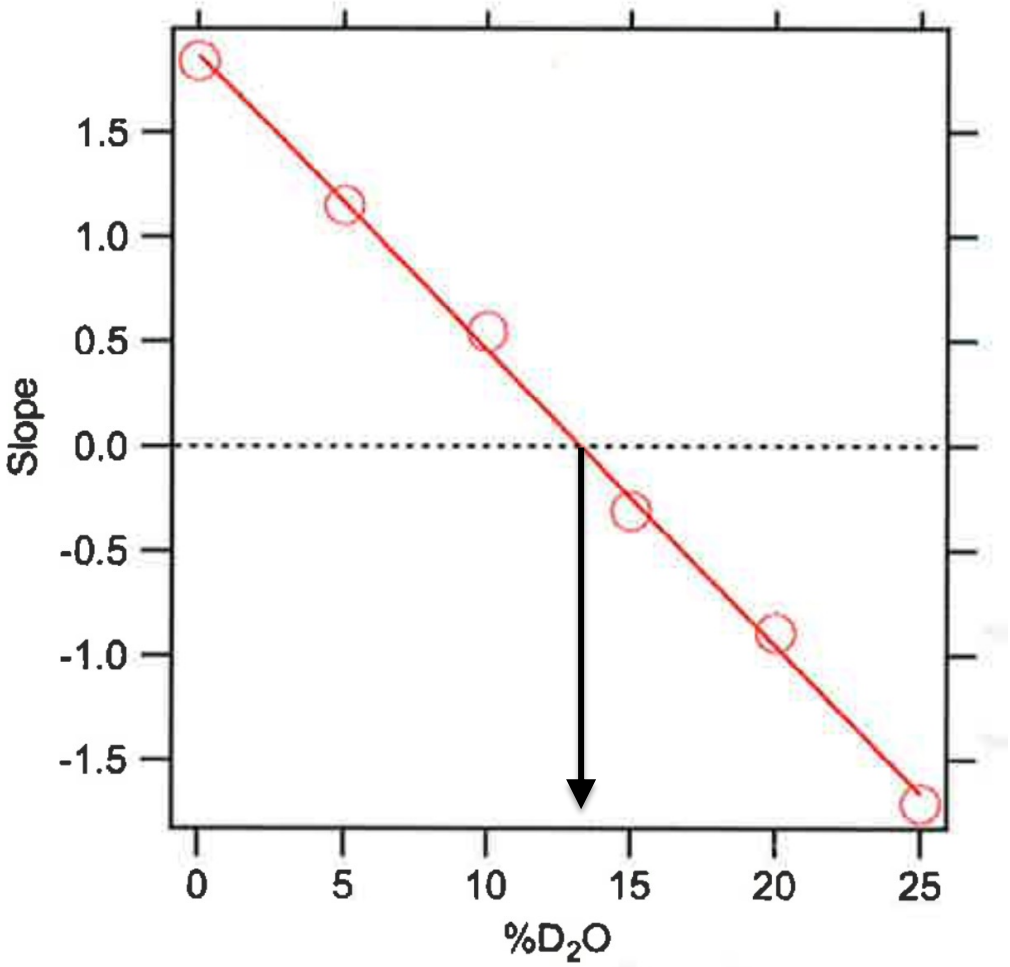
When using either D_2O or $D_2{}^{18}O$, the water term(s) will still have negligible contributions.

This assumes all water is distributed around the protein in proportion to its mole fraction in the solvent.

Find the percent heavy water required by centrifuging detergent-alone overnight at 50K rpm at different D_2O concentrations



Plot the slope as a function of D_2O (Still no protein in the sample)



Once you find your "density match" point, you just do SE as normal with the $\%D_2O$ you need.

Practical tips on detergents

Many detergent vbars are (mostly) known or can be (mostly) calculated using Traub's rule (see also Durschlag)

Favorable vbar values that can be matched with heavy water fall between 0.9 and 1.0 ml g^{-1}. (Closer to 1 is better)

- Polyoxyethylenes, C_8E_5, $C_{12}E_8$
- Sulfobetaines, $C_{12}SB$, $C_{14}SB$
- Dodecyphosphocholine (DPC)
- Dodecylmaltoside (among the most dense)

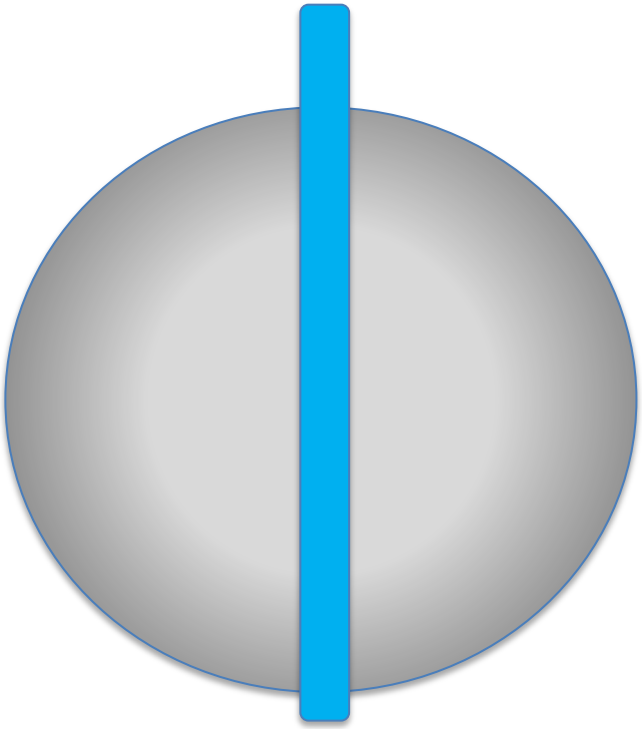
- Other buffer components can affect detergent vbar values

If you use something besides water to density match, two things can happen:

$$M_B = M_P\left(1-\bar{v}_P\rho\right)$$

Protein Contribution (Stays the Same)

If you density match and observe a single species with a molar mass you expect, you are done!



If you density match and observe a molar mass is intermediate between masses of oligomers, you may decide to distinguish between equilibrating system and frozen mixtures

Equilibrating

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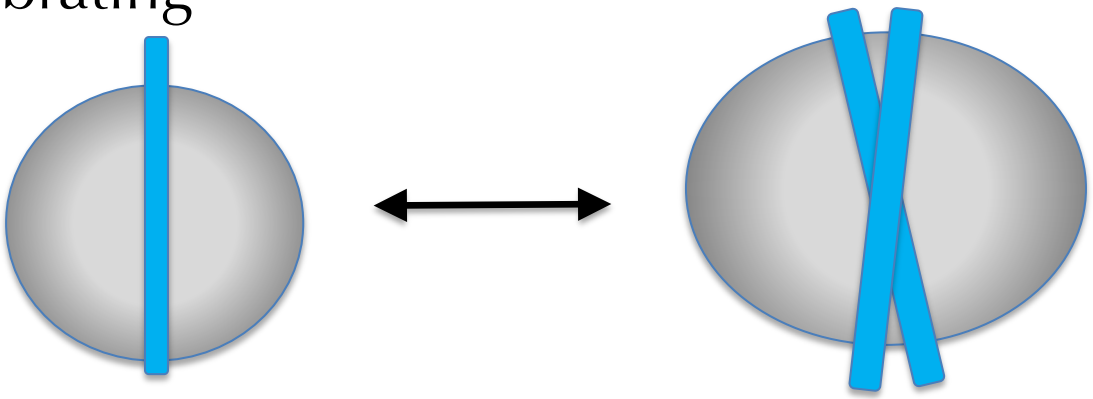
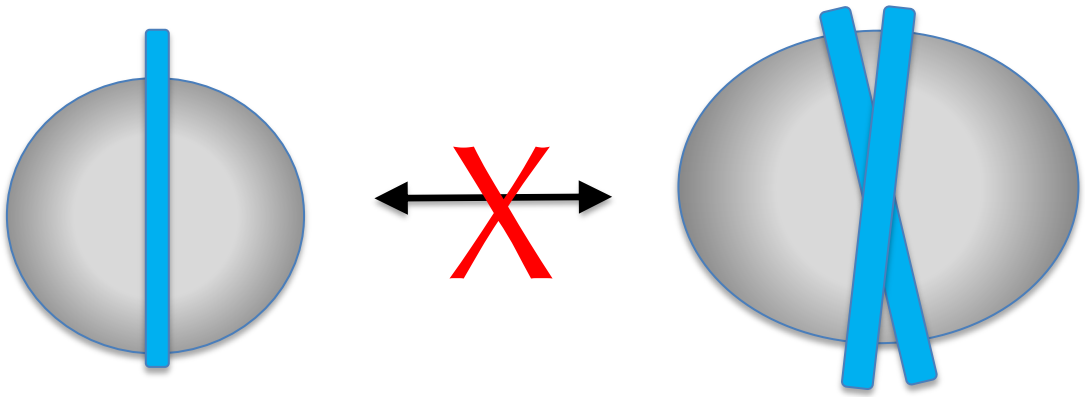
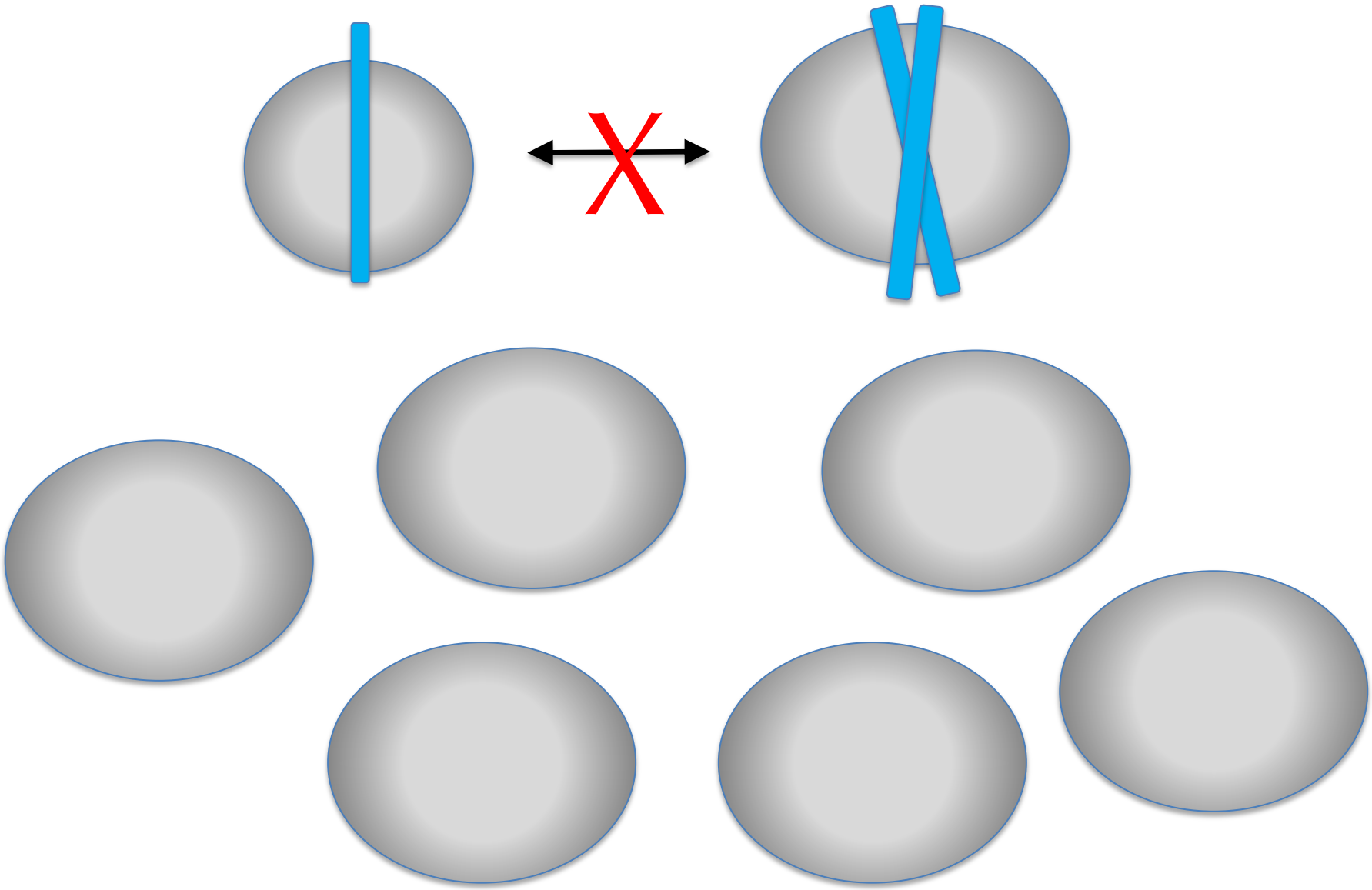


Frozen



Nothing happens to the protein oligomeric state upon dilution of a frozen system



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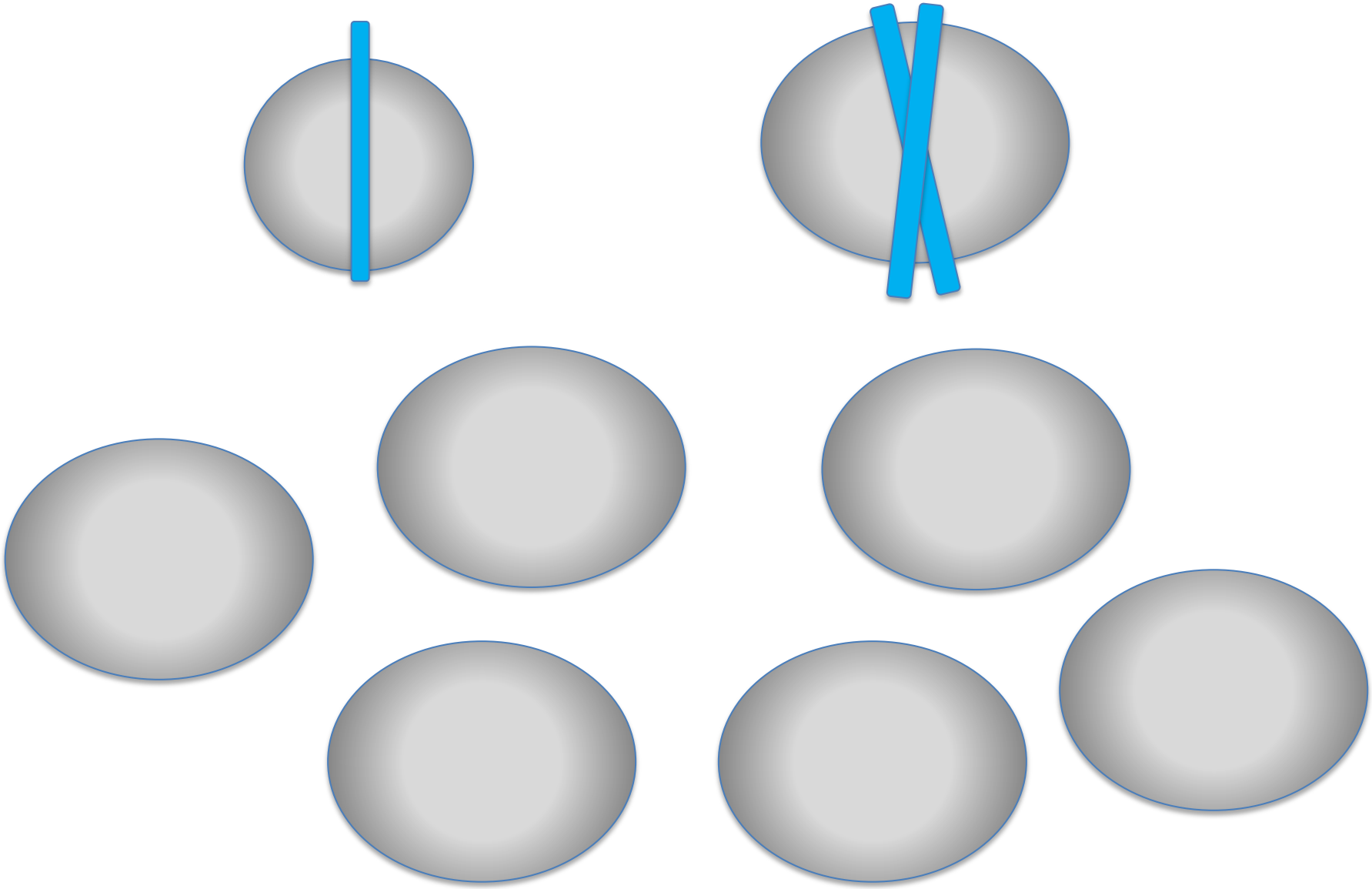
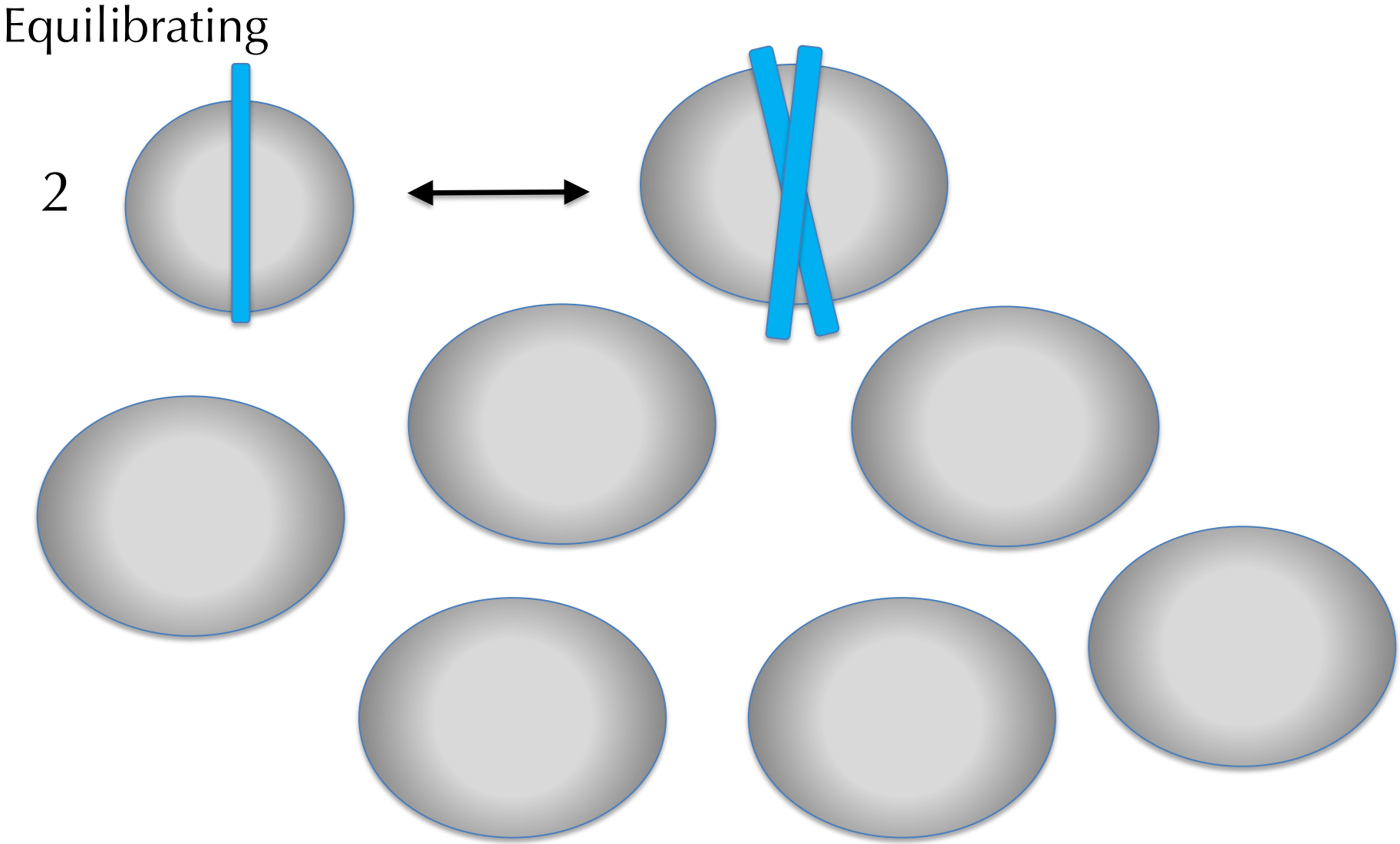


Dilution of an equilibrating system should result in dissociation of oligomers



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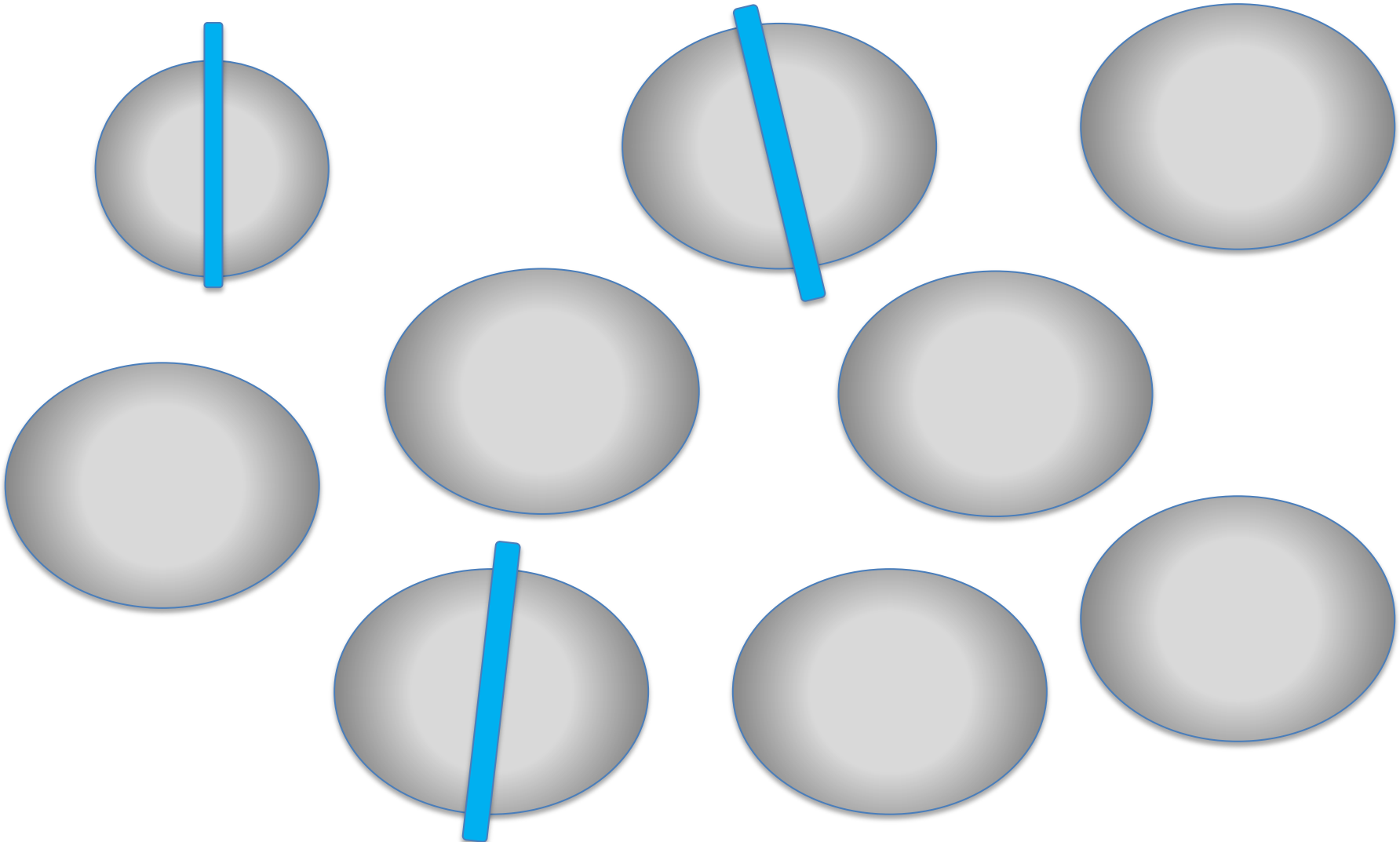
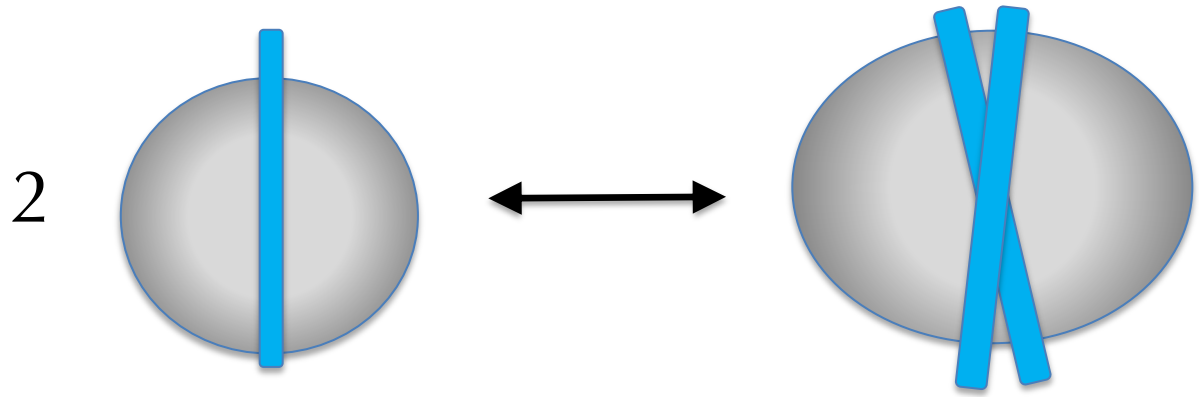


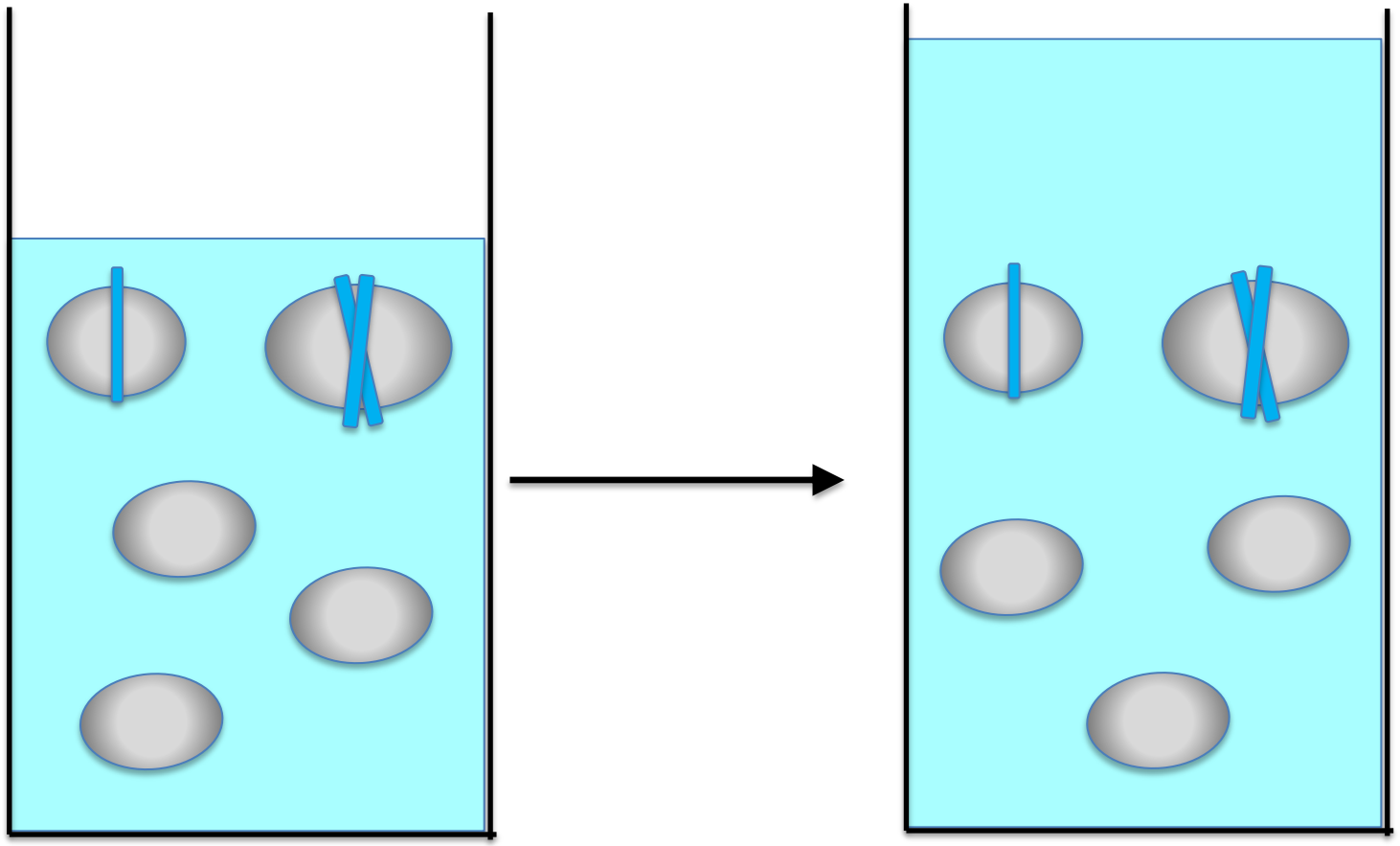
Equilibrating membrane protein oligomers will show a mass dependence on the micellar detergent concentration



Membrane Proteins are not soluble in water.

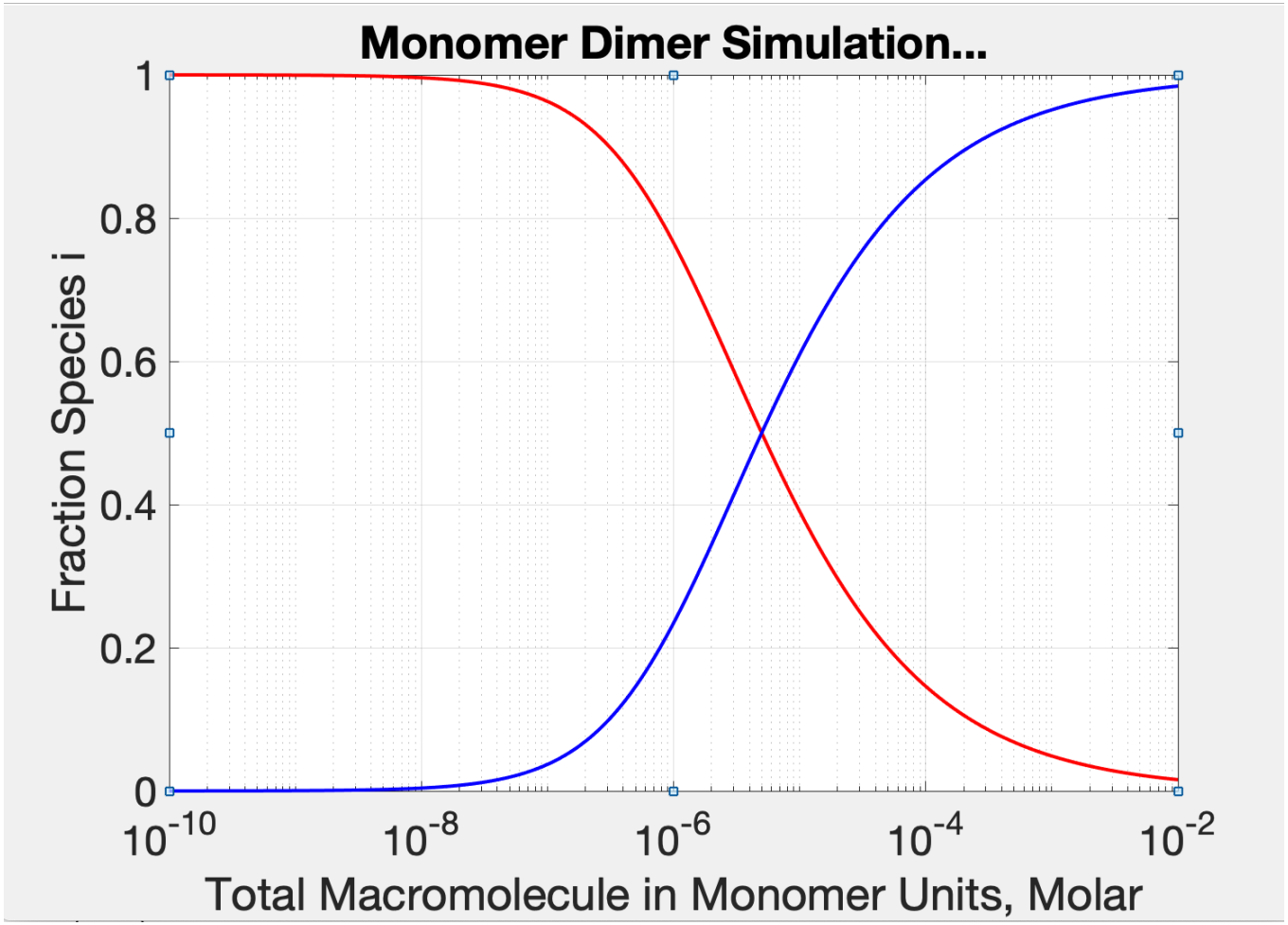
Fleming, KG (2002) "Standardizing the free energy change of transmembrane helix-helix interactions" *J. Mol. Biol.* **323**: 563-571.

The concentration dependence a membrane protein oligomer in an equilibrating system does not depend on the total aqueous volume



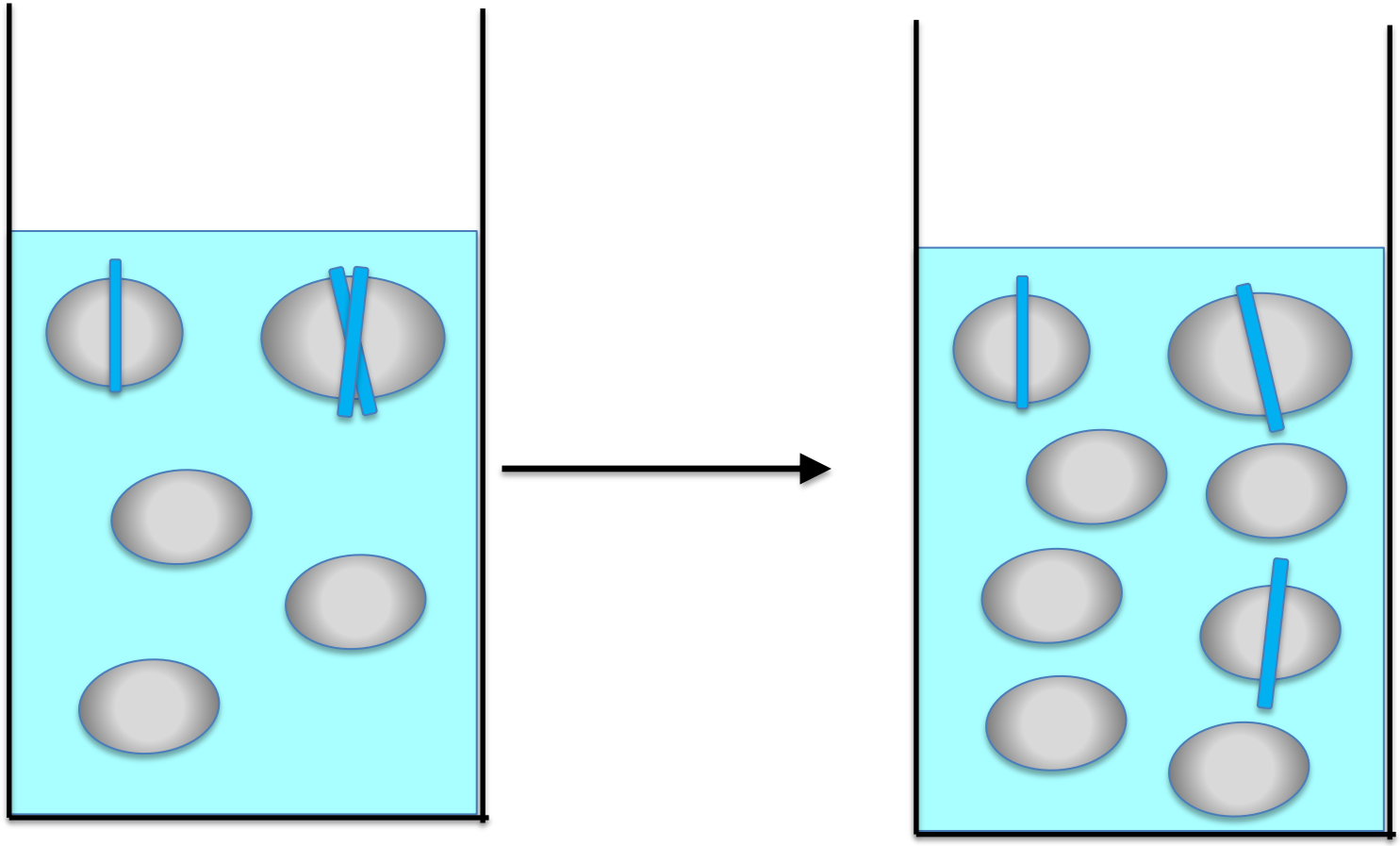
Fleming, KG (2002) "Standardizing the free energy change of transmembrane helix-helix interactions" *J. Mol. Biol.* **323**: 563-571.

Let's pause and consider a familiar equilibrating system: Monomer-dimer rxn of soluble proteins



This unit convention does not apply to membrane proteins

The concentration dependence a membrane protein oligomer in an equilibrating system depends on the volume of *hydrophobic* solvent available



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Quandry: The equilibrium constant we normally measure is in bulk aqueous units

$$K_A \quad \frac{\text{liters}}{\text{mole}} \longrightarrow K_{A,\,aq}$$

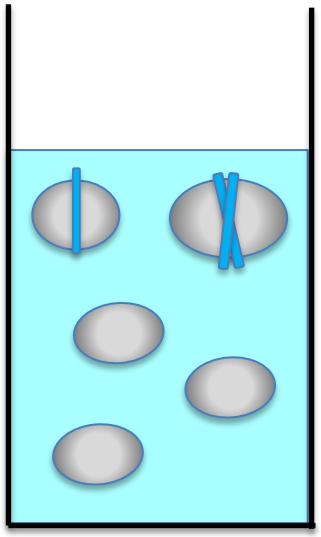
The detergent concentration we normally think about is in bulk aqueous units

$$[\text{Det}] \quad \frac{\text{moles detergent}}{\text{liters water}} \longrightarrow [\text{Det}]_{aq}$$

Fleming, KG (2002) "Standardizing the free energy change of transmembrane helix-helix interactions" *J. Mol. Biol.* **323**: 563-571.

Only the detergent in the micelles can serve as solvent

$$[micellar\ detergent]_{aq} = [total\ detergent]_{aq} - cmc_{aq}$$



This allows us to define a mole fraction equilibrium constant

$$K_x = \left[K_{A,aq}\left(\frac{liters}{mole}\right)\right]\left[[micellar\ detergent]_{aq}\left(\frac{moles}{liter}\right)\right]$$

Fleming, KG (2002) "Standardizing the free energy change of transmembrane helix-helix interactions" *J. Mol. Biol.* **323**: 563-571.

Putting this altogether allows us to define a mole fraction equilibrium constant, Kx

$$K_x = \left[K_{A,aq}\left(\frac{liters}{mole}\right)\right]\left[[micellar\ detergent]_{aq}\left(\frac{moles}{liter}\right)\right]$$

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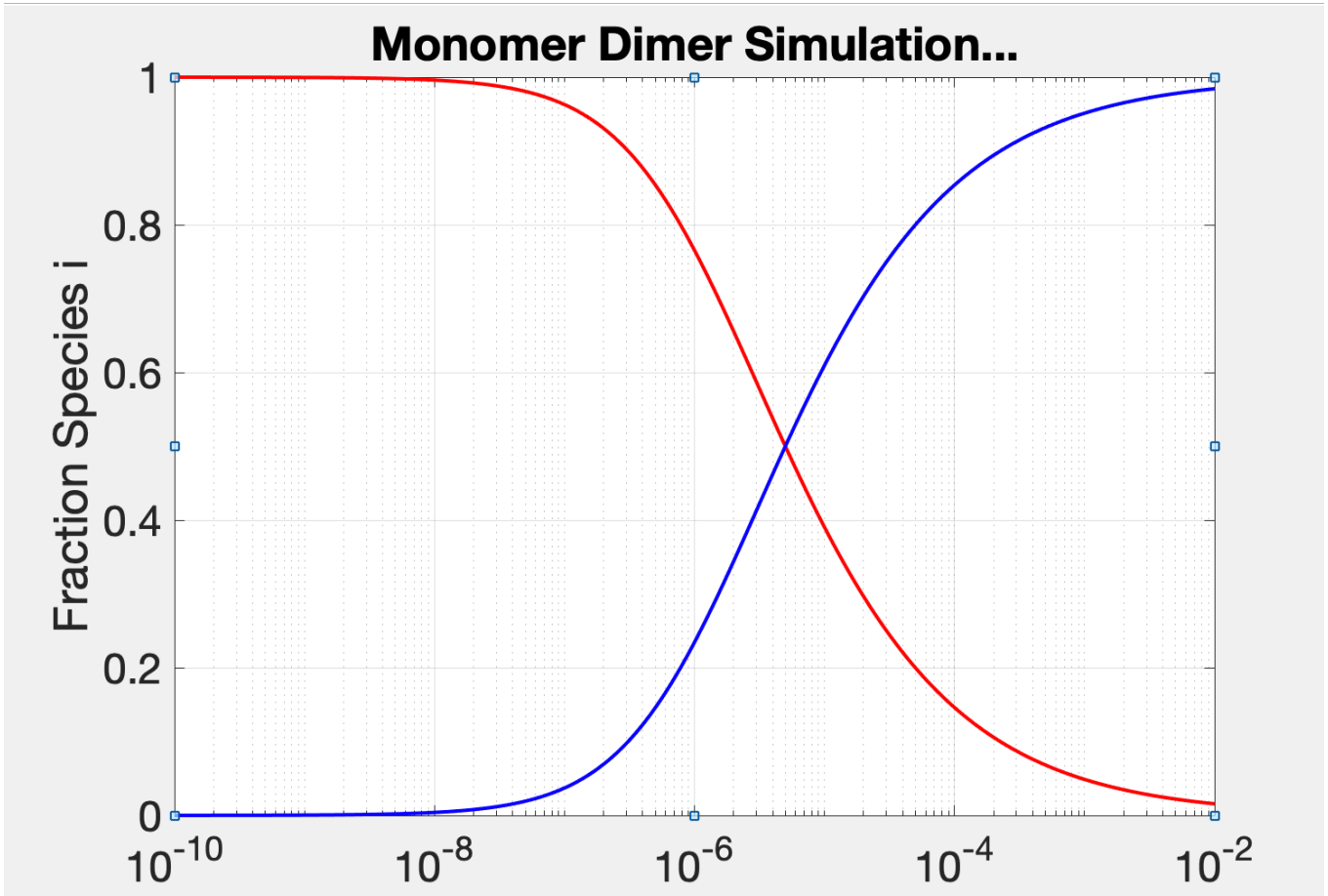
The x-axis for Membrane Protein Equilibration is Mole Fraction



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Mole fraction K_x shows an interesting property

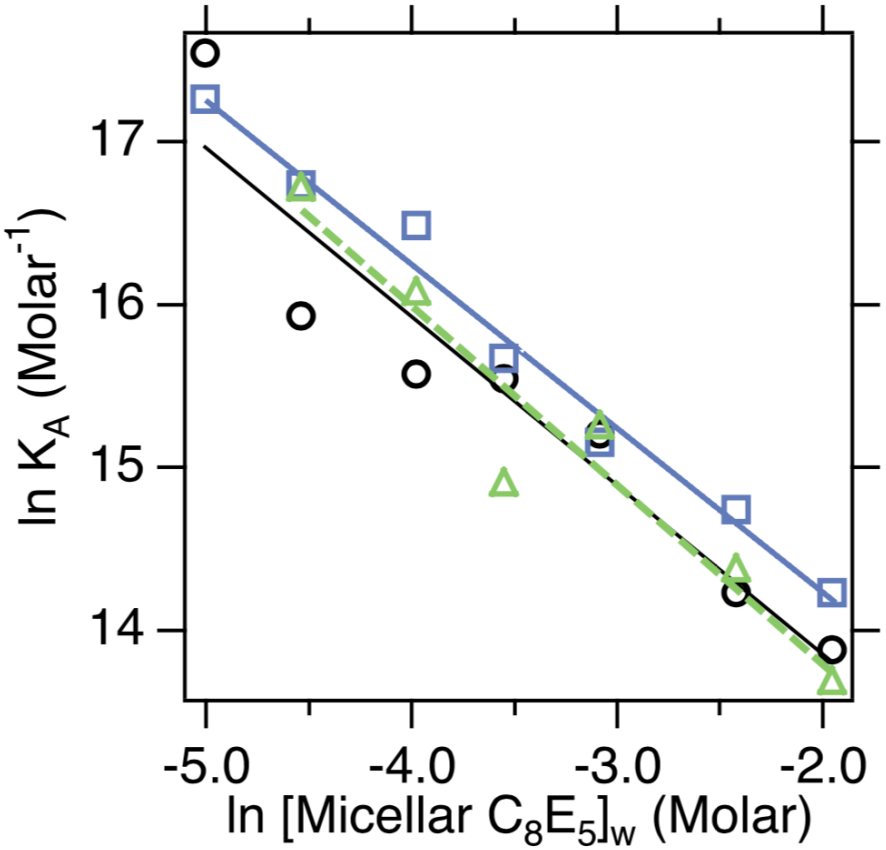
$$K_x = \left[K_{A,aq}\left(\frac{liters}{mole}\right)\right]\left[[micellar\ detergent]_{aq}\left(\frac{moles}{liter}\right)\right]$$

Rearrange, take the natural log and the derivative:

$$\frac{\partial ln K_{A,aq}}{\partial ln[miceller\ Det]_{aq}} \equiv 1$$

Fleming, KG (2002) "Standardizing the free energy change of transmembrane helix-helix interactions" *J. Mol. Biol.* **323**: 563-571.

In an ideal monomer-dimer system, the apparent association constant shows a linear dependence on ln[micellar detergent] with a slope of unity



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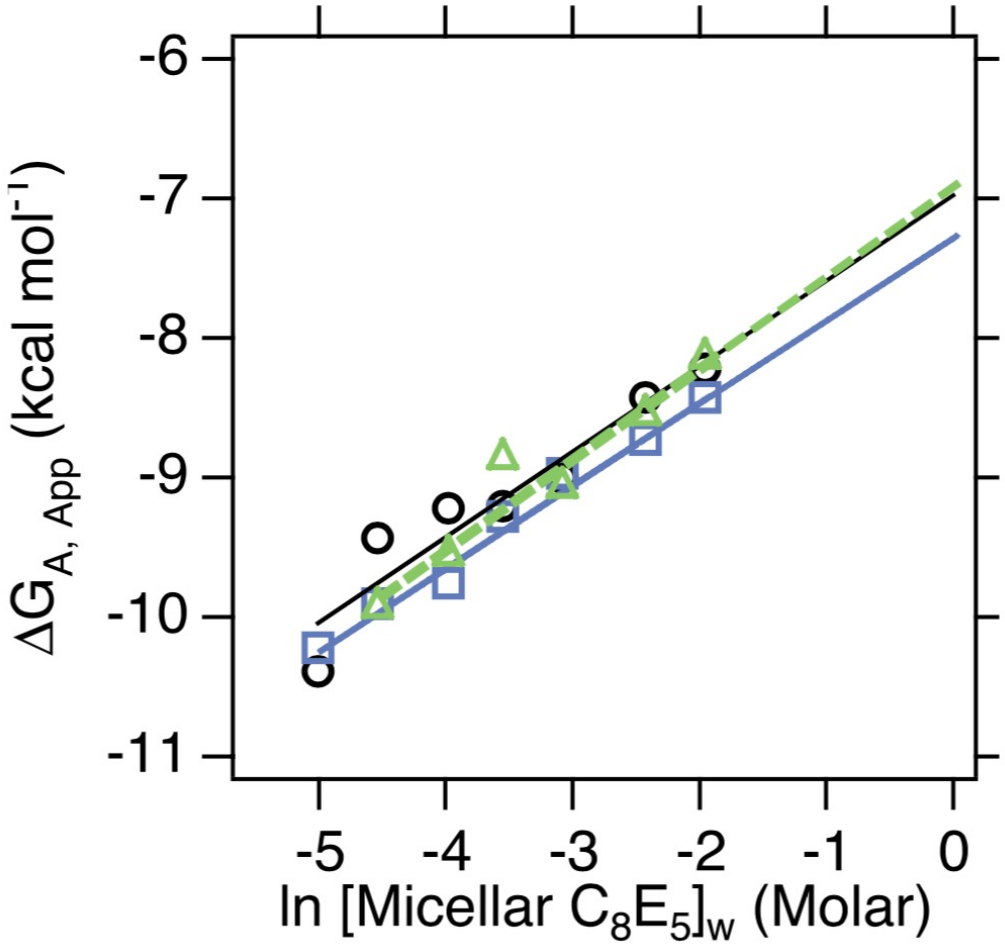
The Slope is *RT* in ΔG Space

$$\Delta G_{app} = \Delta G^{\circ}_{X} + RT \ln[\text{micellar Det}]_w$$



Fleming, KG (2002) "Standardizing the free energy change of transmembrane helix-helix interactions" *J. Mol. Biol.* **323**: 563-571.

Forced Co-Habitation: when two proteins occupy the same micelle without a thermodynamically-meaningful interaction

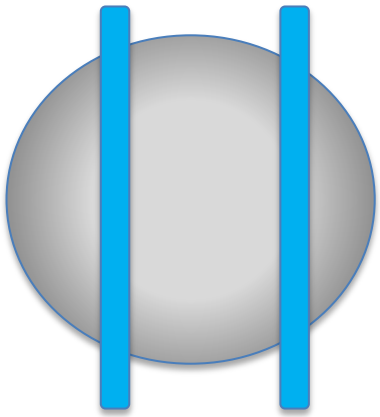
Think about it as “crowding”

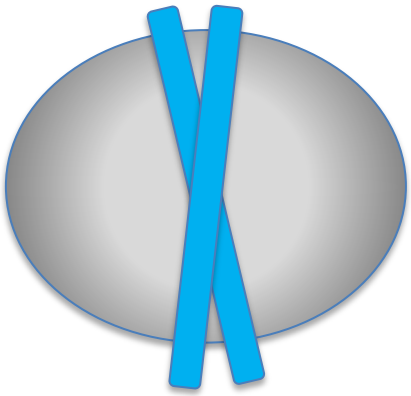
Also called “artifactual togetherness”

Kobus, FJ and KG Fleming (2005) “The GxxxG-containing transmembrane domain of the CCK4 oncogene does not encode preferential self-interactions. *Biochemistry* 44: 1464-1470.

Also true for lipid vesicles…
Fleming, KG (2018) “Taking Deterministic Control of Membrane Protein Monomer-Dimer Measurements” *Journal of General Physiology* 150: 181-183. DOI: 10.1085/jgp.201711913 PubMed PMID: 29343502; PubMed Central PMCID: PMC5805552

Forced Co-Habitation shows up as the same molar mass in SE under density-matching conditions





It can be described as the random distribution of proteins within micelles (and vesicles) is given by a Poisson equation

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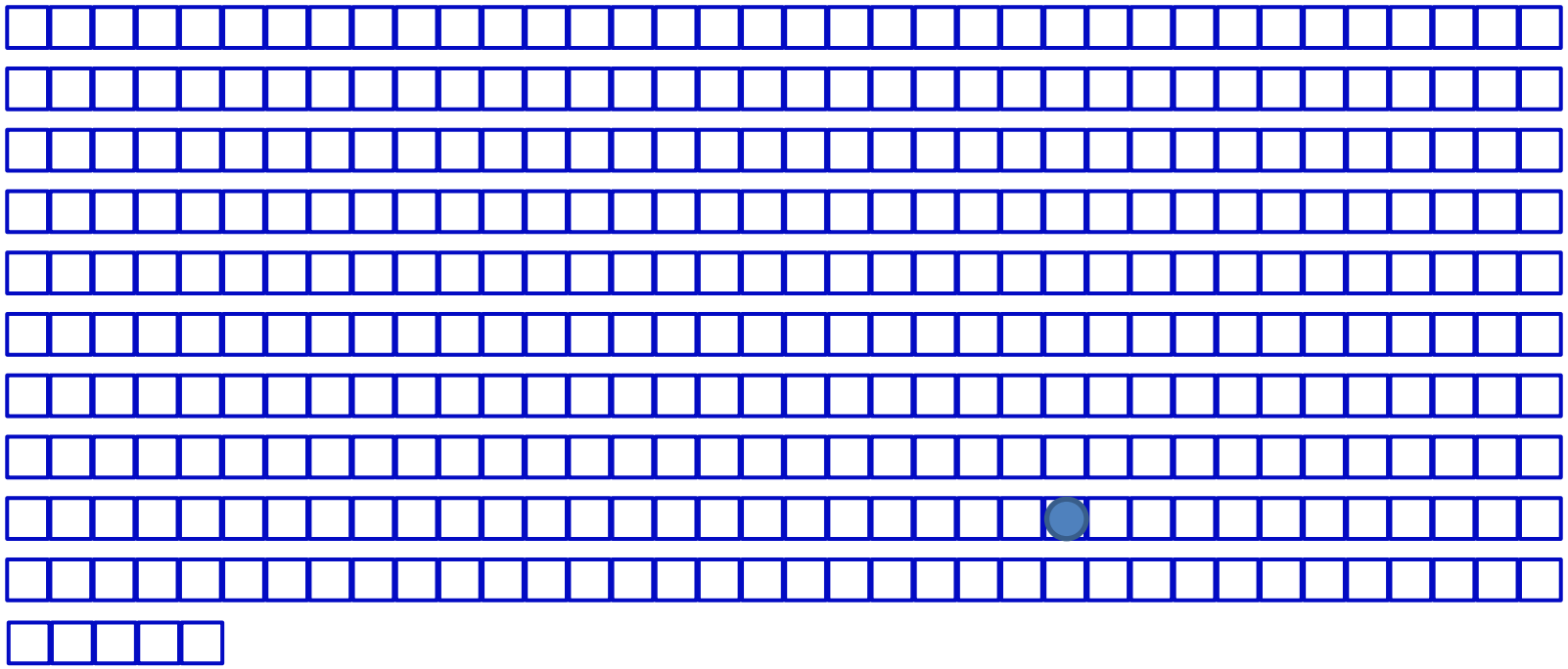
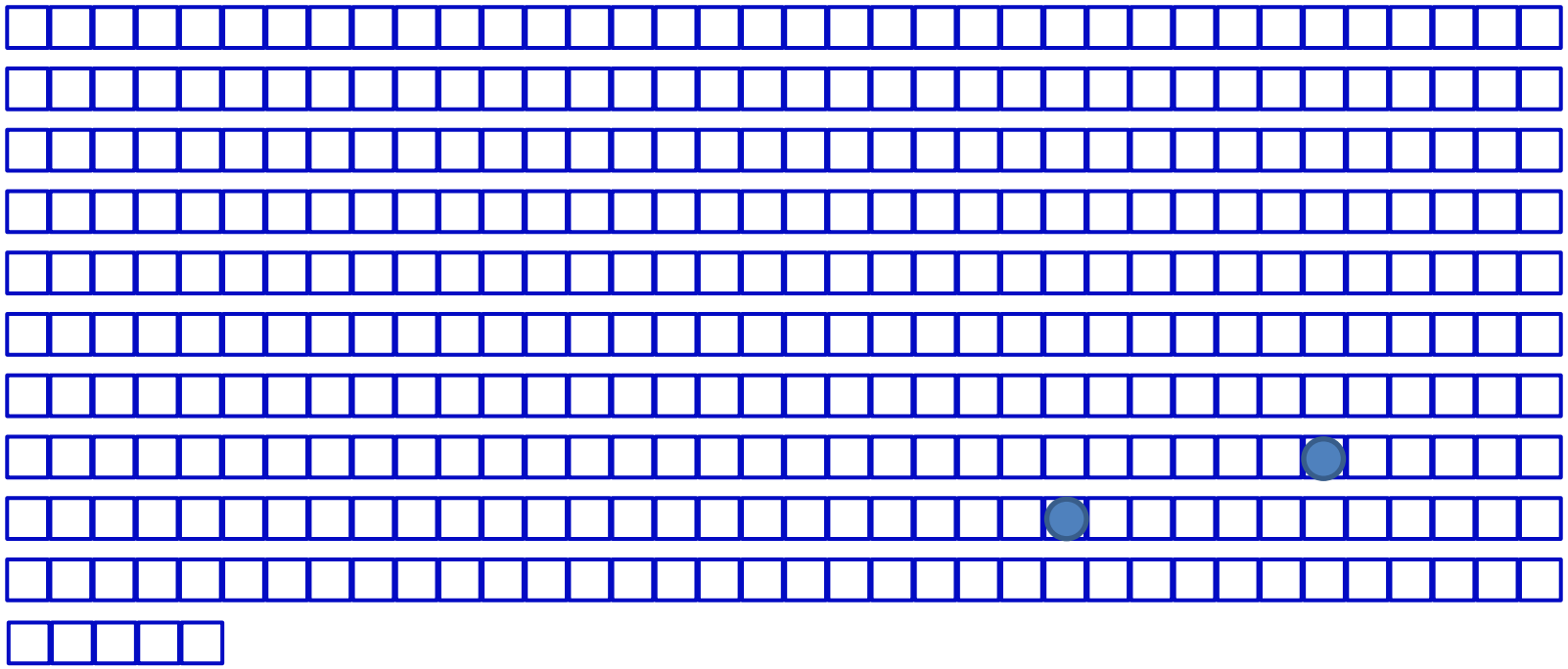
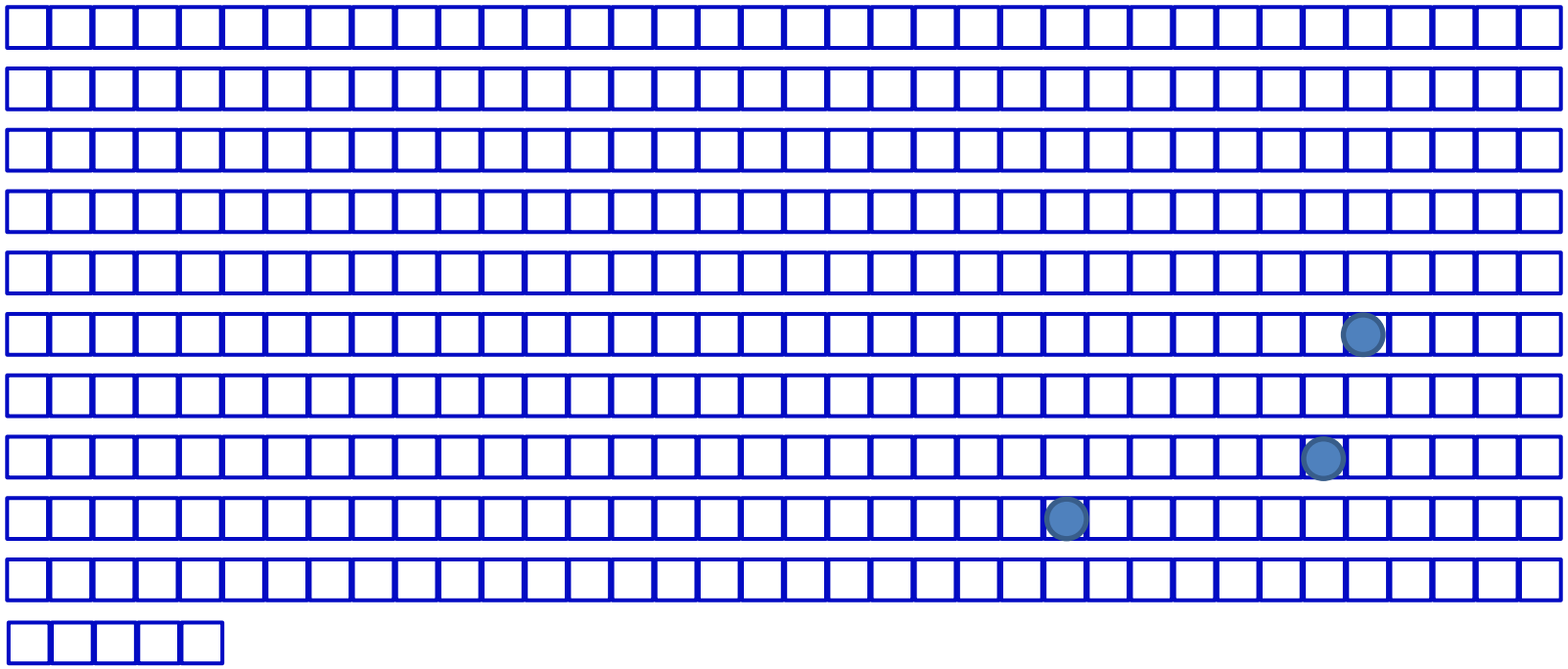
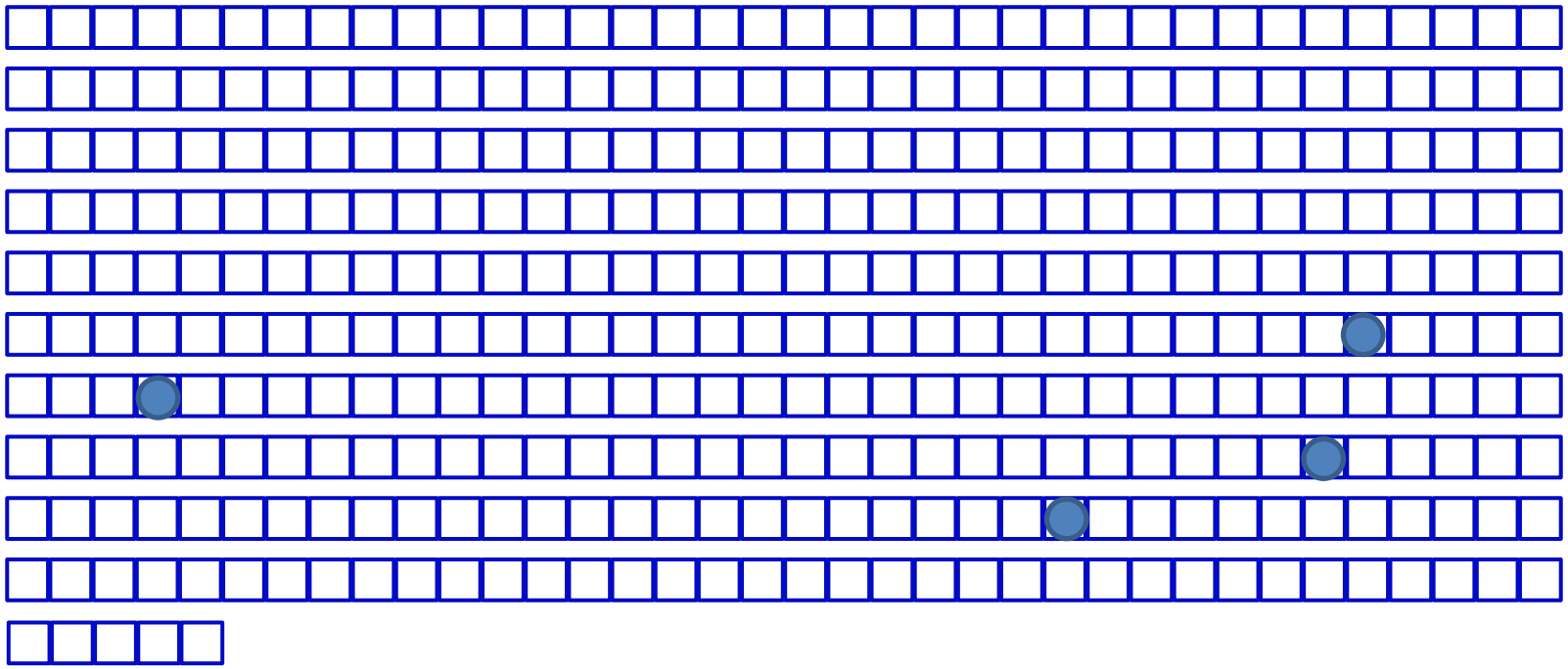
Also Known as the Birthday Problem

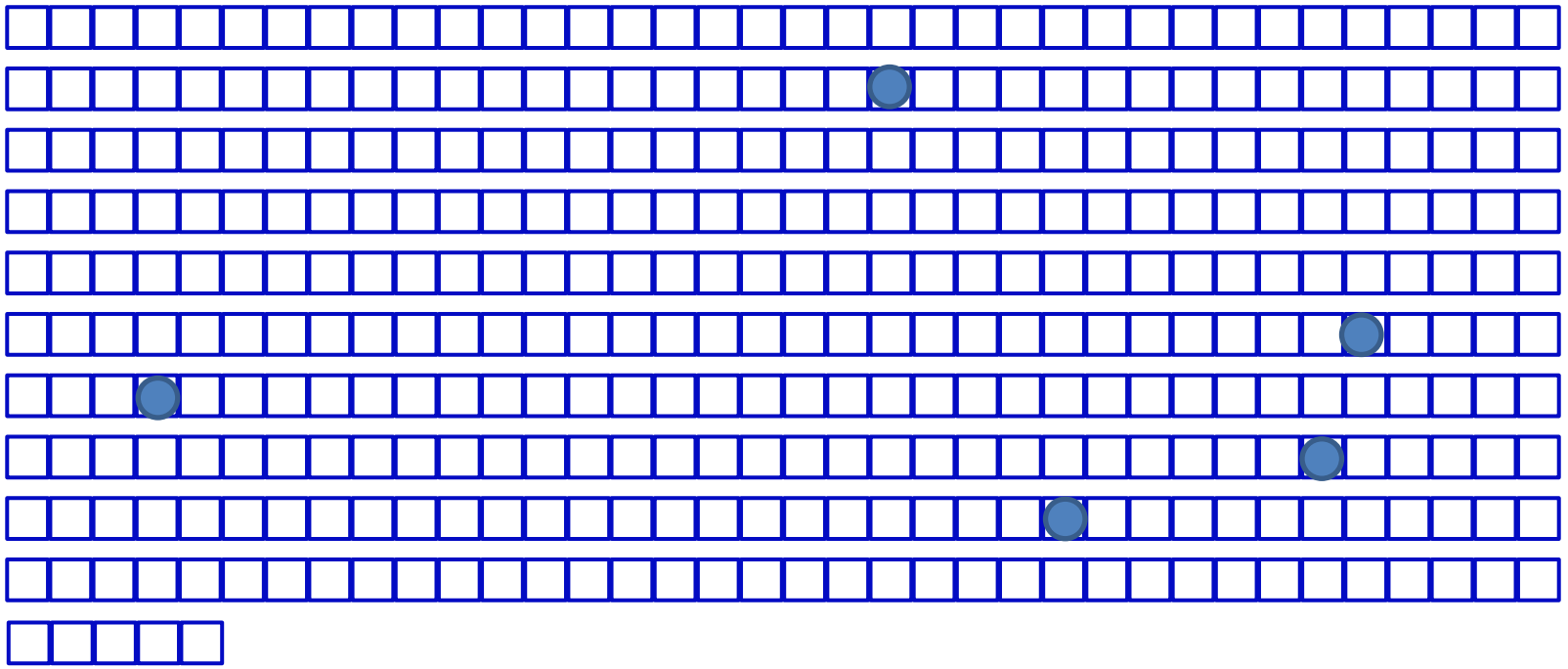
Assume 365 Days in the Year…

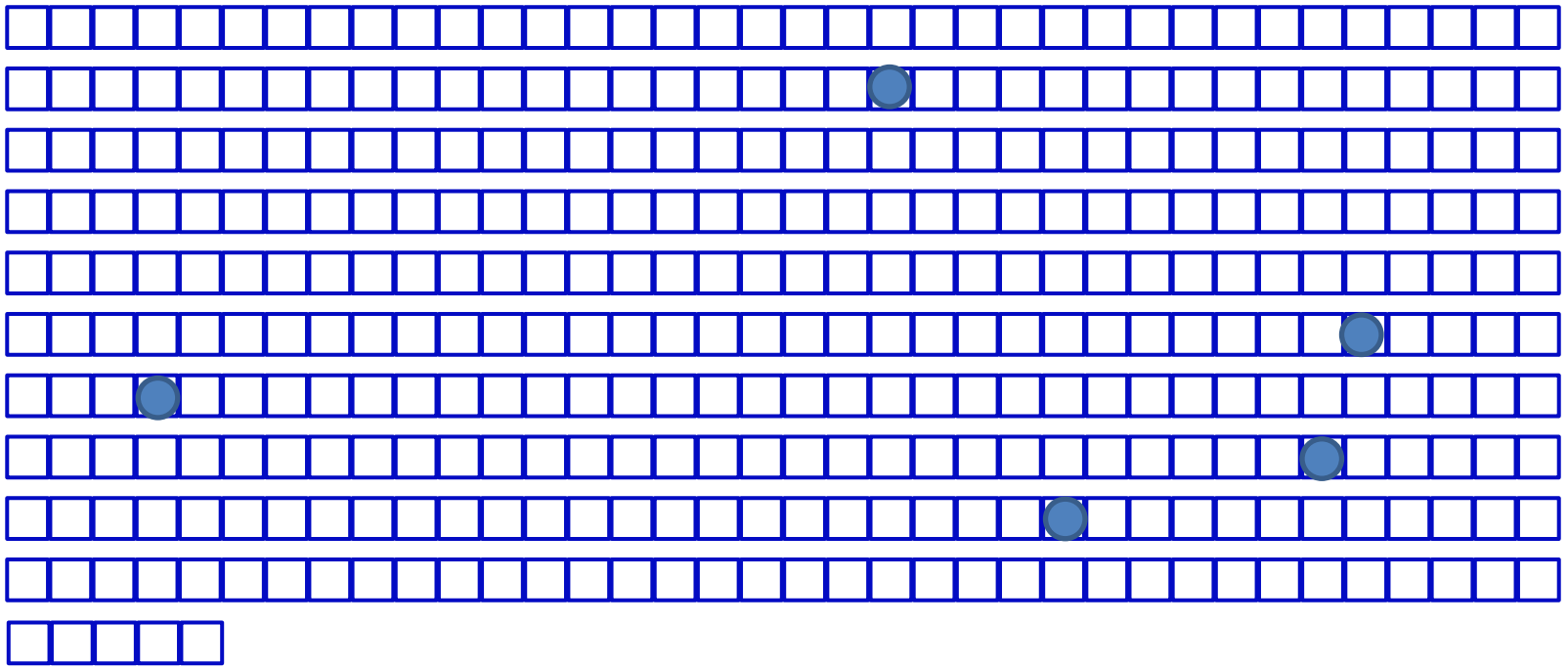


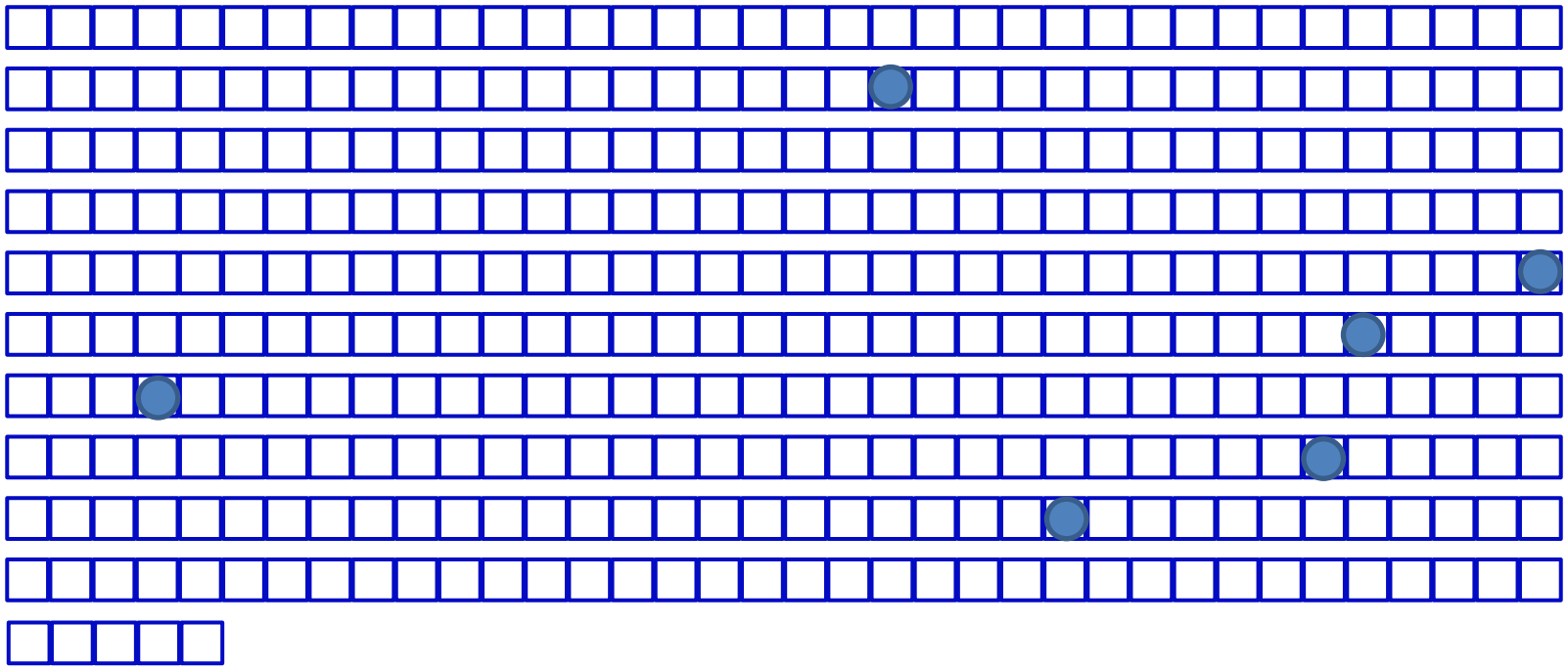


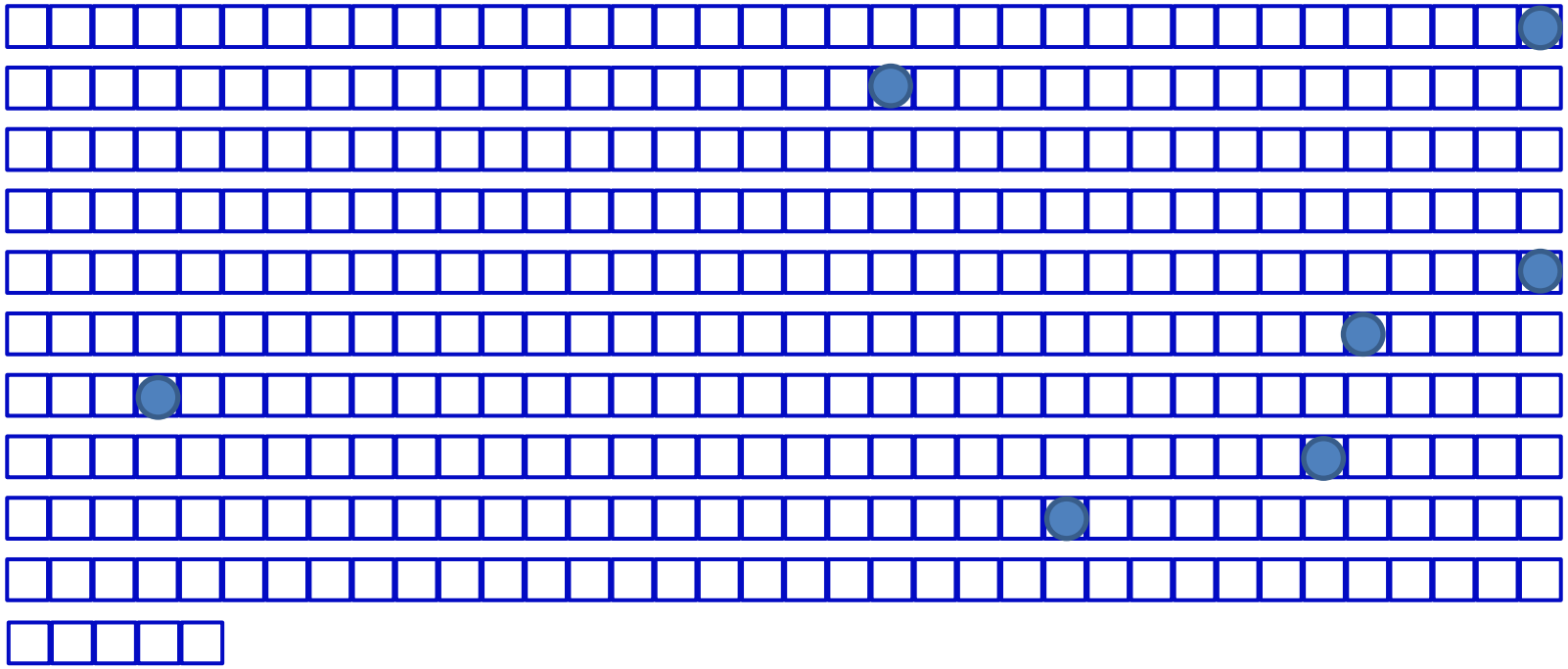


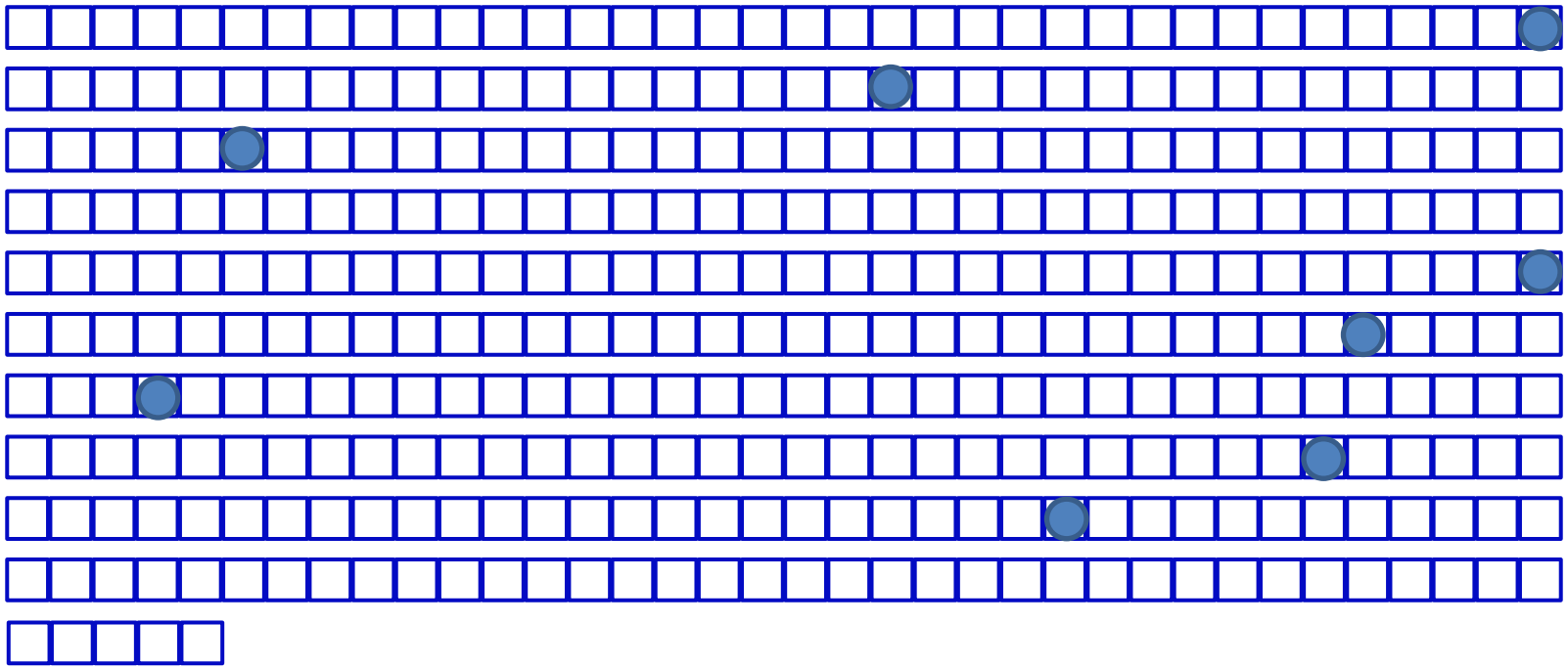


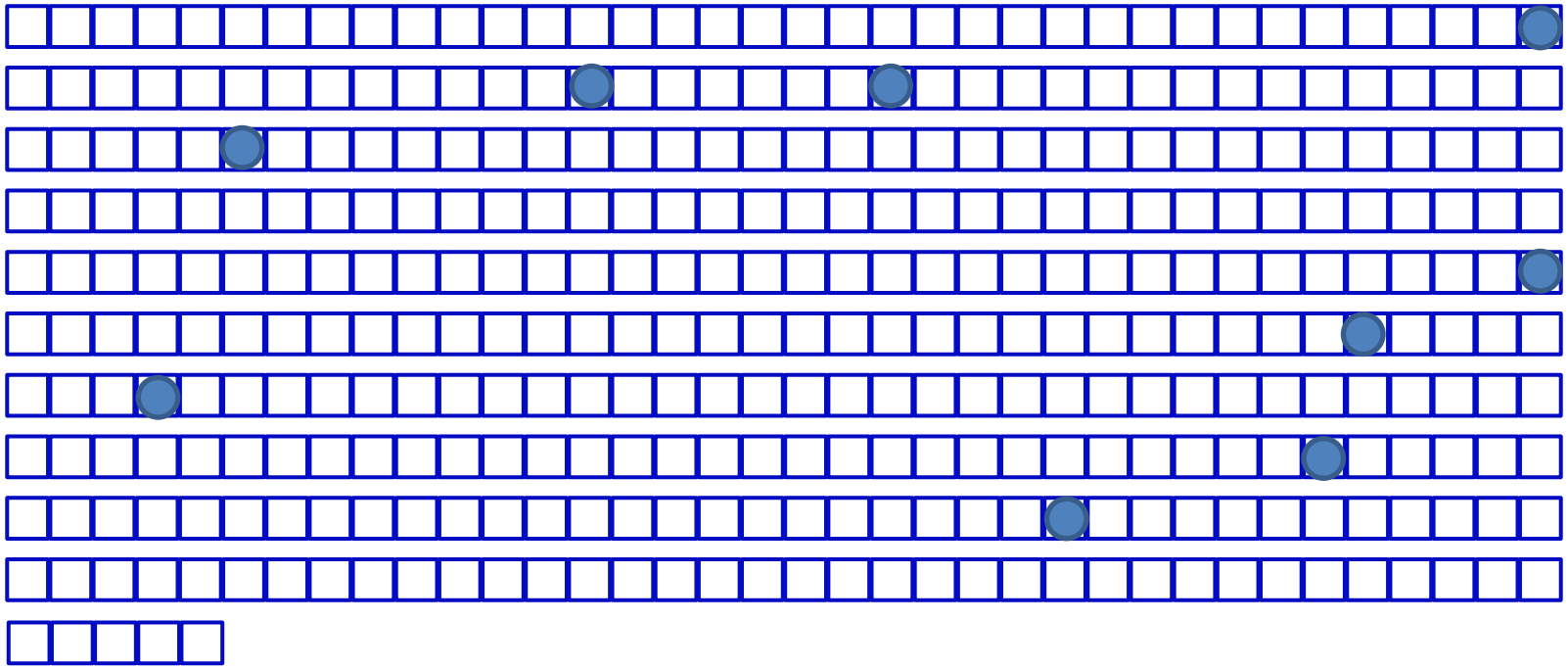


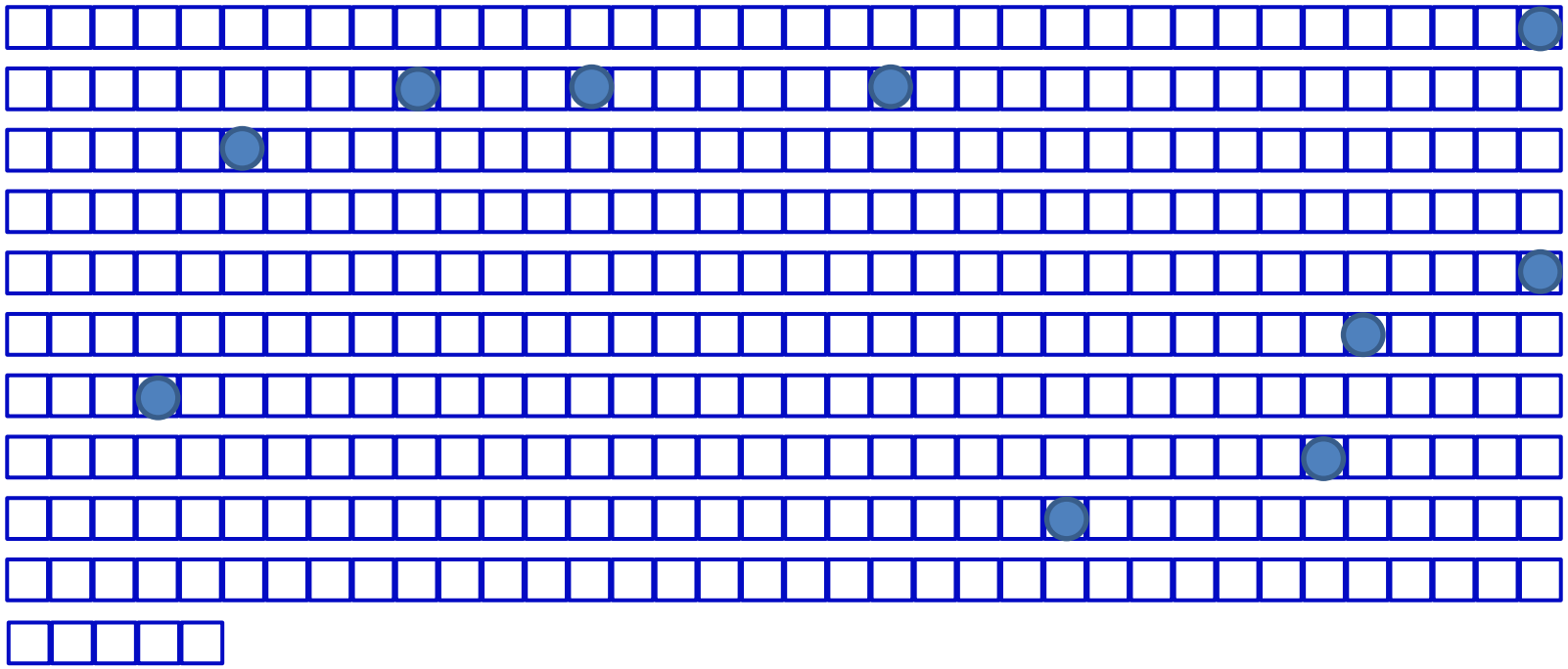


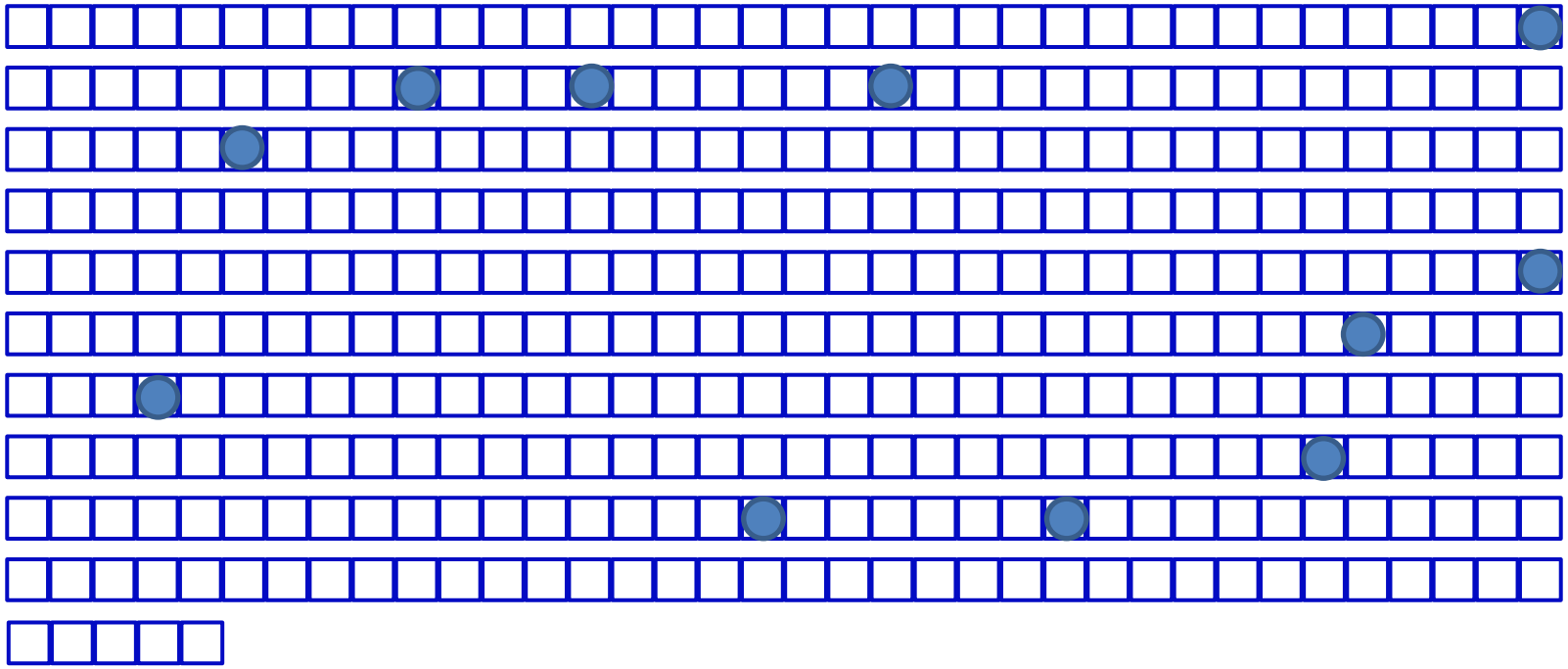


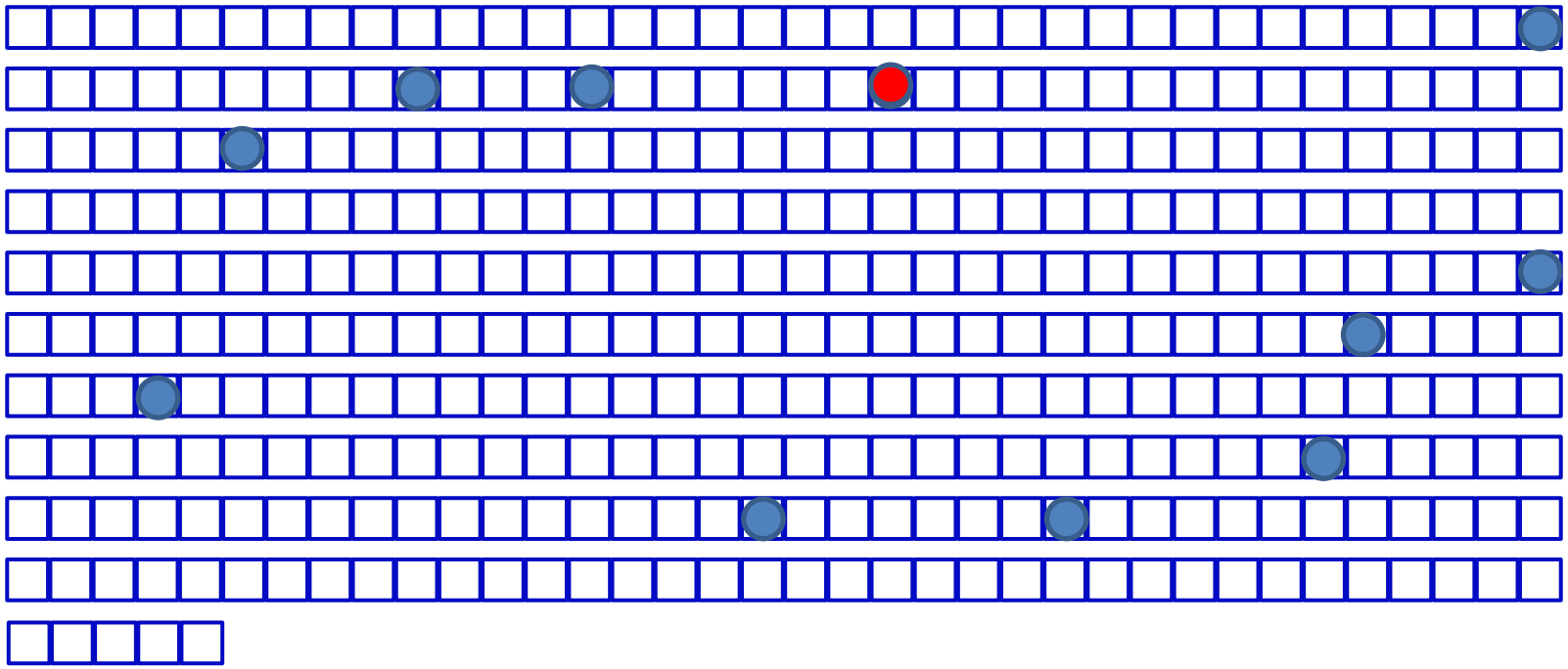




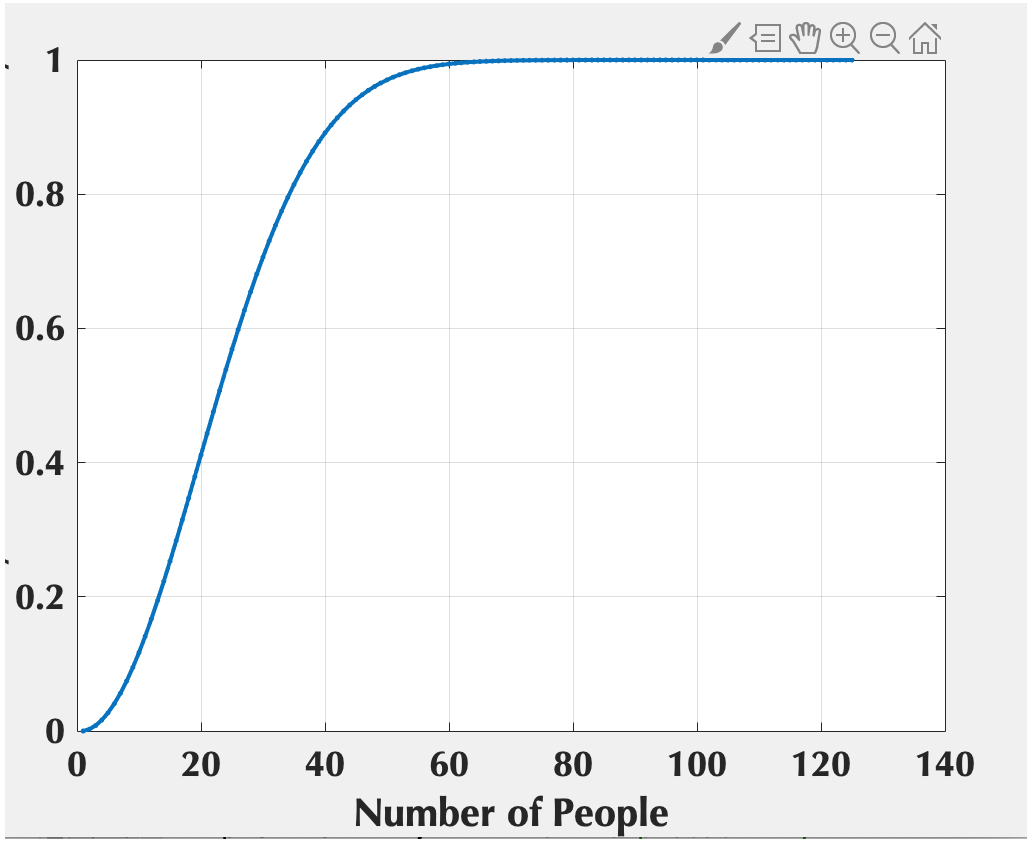




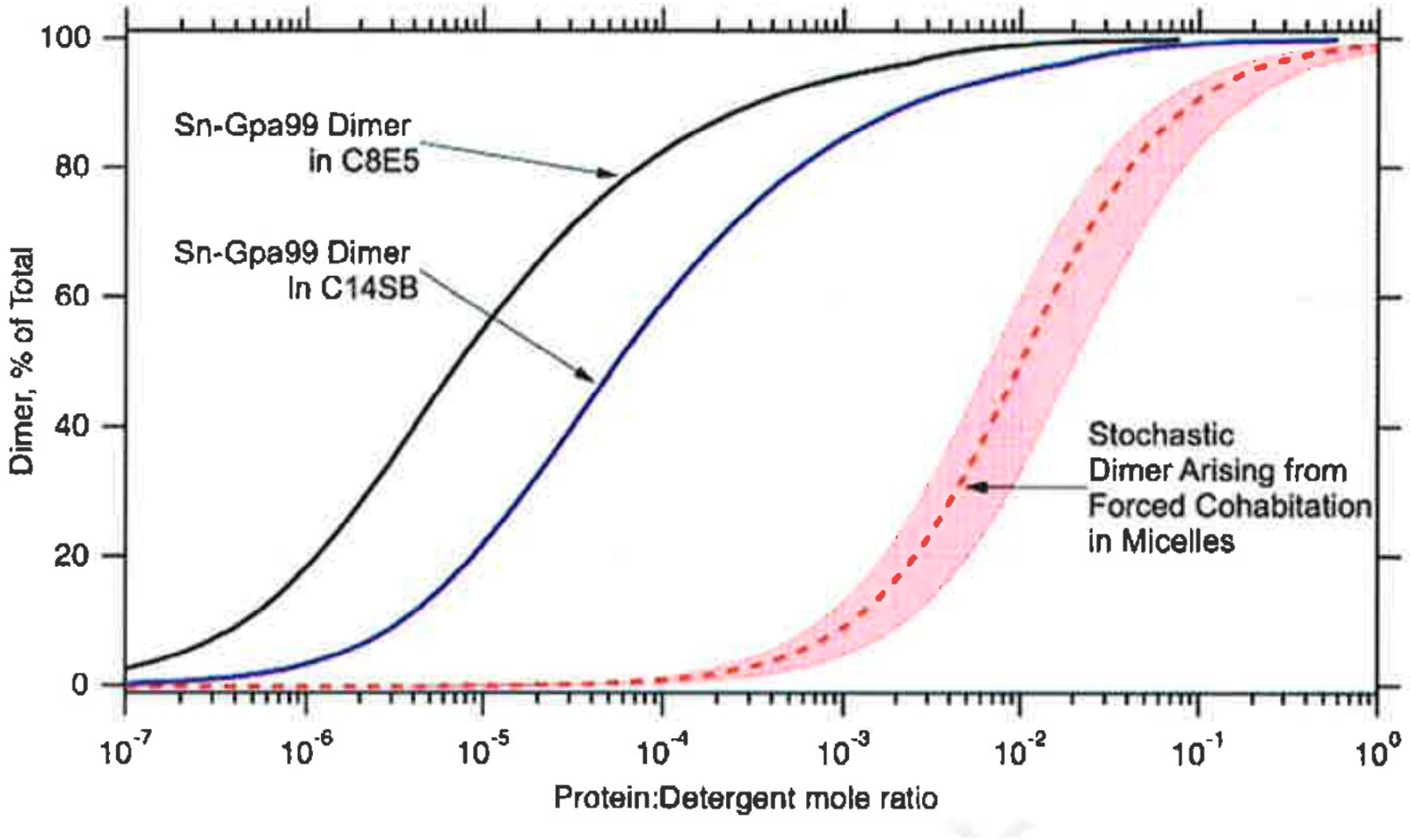




Baseline Probability for Birthday Co-Occupancy is a Poisson



The Random Distribution of Proteins Within Micelles is Given By a Poisson Equation



Workflow for Equilibrium Constant Determination

1. Conduct SE normally (3 speeds + 3 protein concentrations) in the background of a constant detergent concentration
    - Make sure you are not too close to the cmc

2. Perform a global fit of this SE data to assess whether the protein is equilibrating through out the entire hydrophobic phase. If equilibrating, measure the $K_{A,aq}$

3. Do this over several different detergent concentrations

4. Plot $\ln KApp_{Aq}$ as a function of $\ln[\text{Miceller Det}]_{Aq}$