# When membranes get crowded: interfacial constraints on protein binding and remodeling

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Cellular membranes regulate protein binding, remodeling, and signaling through complex interfacial interactions. Amphipathic proteins such as α-synuclein (αSyn) bind lipid membranes cooperatively, forming heterogeneous domains rather than uniform distributions. While this clustering is essential for membrane remodeling, it may also promote pathological aggregation associated with neurodegenerative diseases. However, the molecular determinants governing this behavior remain unclear.

Here, we investigate αSyn binding to model membranes and extracellular vesicles using circular dichroism, microscopy, cryo-TEM, and single-vesicle TIRF imaging. We show that macromolecular membrane crowding, introduced via polymer-grafted lipids, shifts the binding equilibrium without altering αSyn's α-helical structure. Larger polymers enhance membrane-induced folding and promote earlier binding saturation, consistent with stabilization of the membrane-bound state via excluded-volume effects.

At the vesicle level, binding is strongly cooperative and heterogeneous. Membrane crowding reduces accessible surface area, limits protein packing density, and suppresses curvature generation and remodeling, while regulating post-binding accumulation and spatial organization. Under physiological conditions and in biologically derived vesicles, binding affinity is reduced but heterogeneity persists, highlighting the role of lipid complexity and electrostatic screening.

Together, our results show that cooperative protein binding is governed not only by lipid composition but also by interfacial crowding and membrane accessibility, linking membrane organization to protein clustering and remodeling, with implications for cellular function and disease.

**Keywords:**
Cooperative binding, Interfacial crowding, α-synuclein, Protein–lipid interactions, Extracellular vesicles