

Molecular architecture of synaptic vesicles 99

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Synaptic vesicles

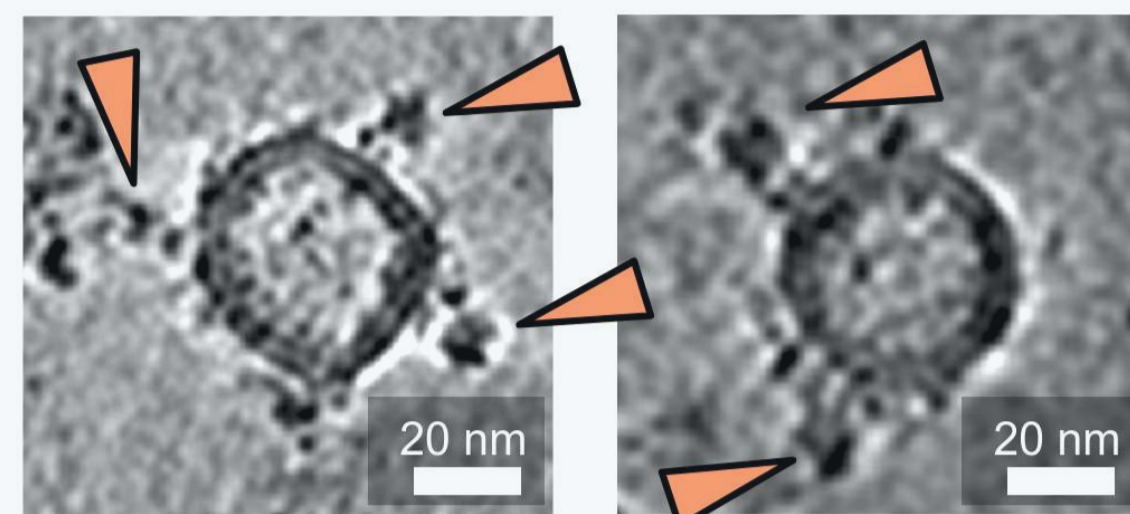
Introduction

Synaptic vesicles (SVs) store and transport neurotransmitters to the presynaptic active zone for release by exocytosis. An **average SV** model was proposed based on proteomic analysis. In our study, we define the structural details of the **individual SVs** both **purified from mouse brain** and from **on-grids-grown hippocampal neurons**, and examine the structural heterogeneity of their molecular architecture by using cryo electron tomography (cryo-ET).

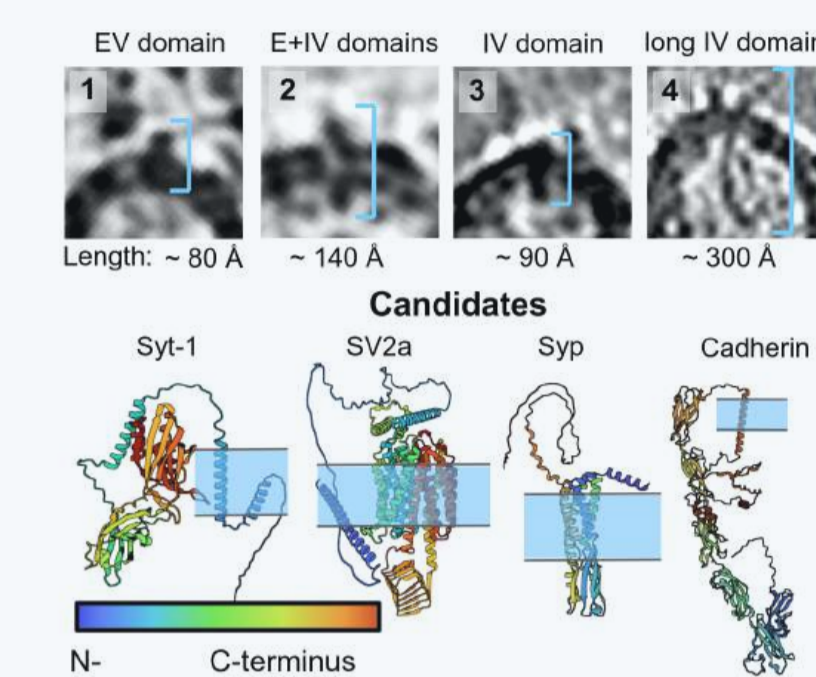
V-ATPase-Syp interaction

We used an **integrative approach** combining mass spectrometry, AlphaFold3 complex prediction, and Bioluminescence Resonance Energy Transfer (BRET) to identify the protein density near the V-ATPase in the SV membrane, which we observed using cryo-ET. The density corresponds to **Synaptophysin (Syp)**, a 38 kDa protein crucial for SV biogenesis.

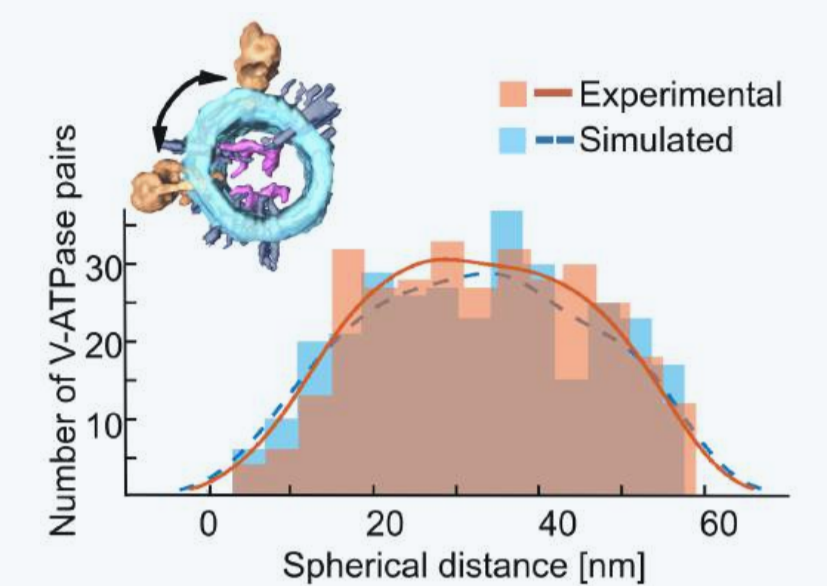
SVs in a cryo-electron tomogram



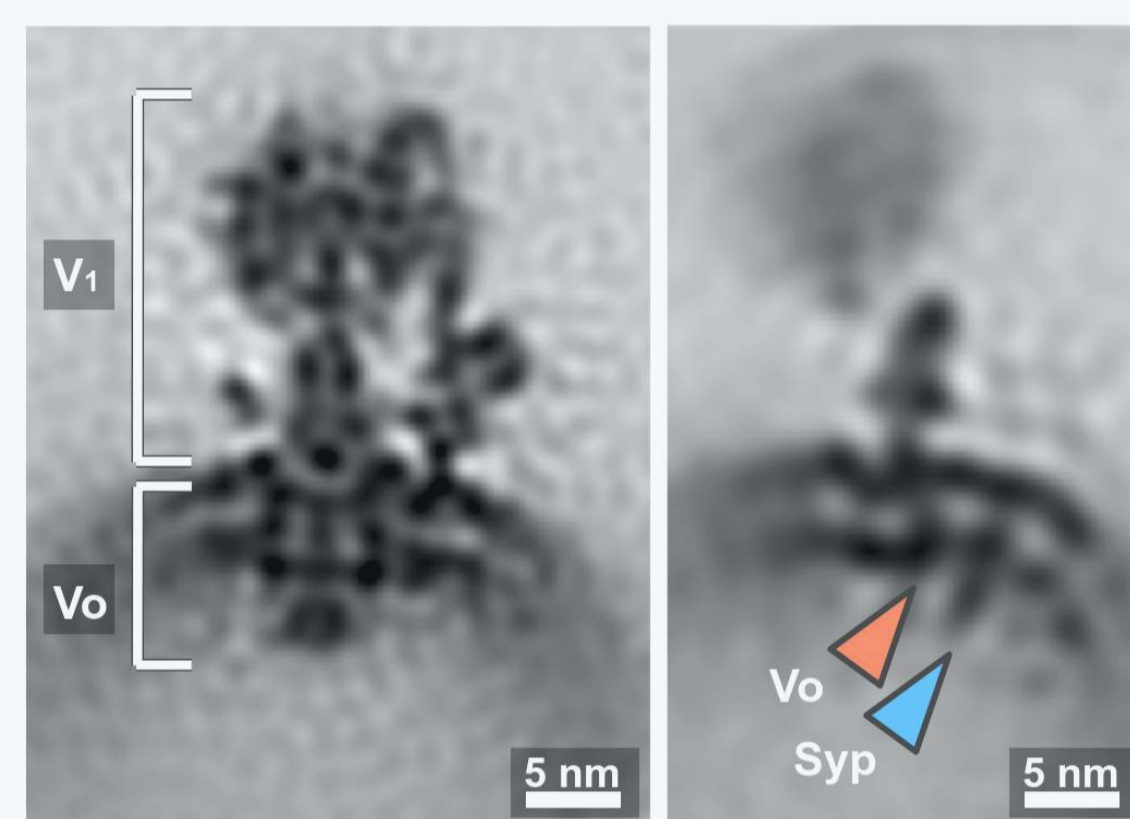
Proteins on SV surface



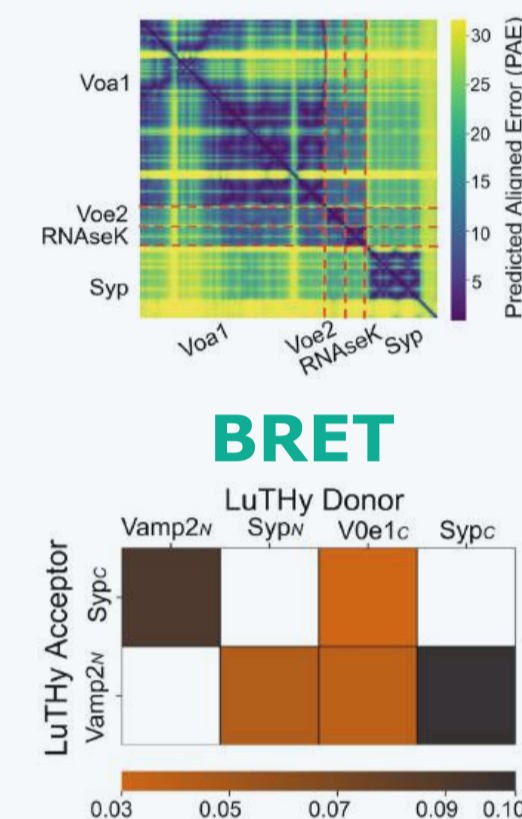
V-ATPases are randomly distributed on SV surfaces



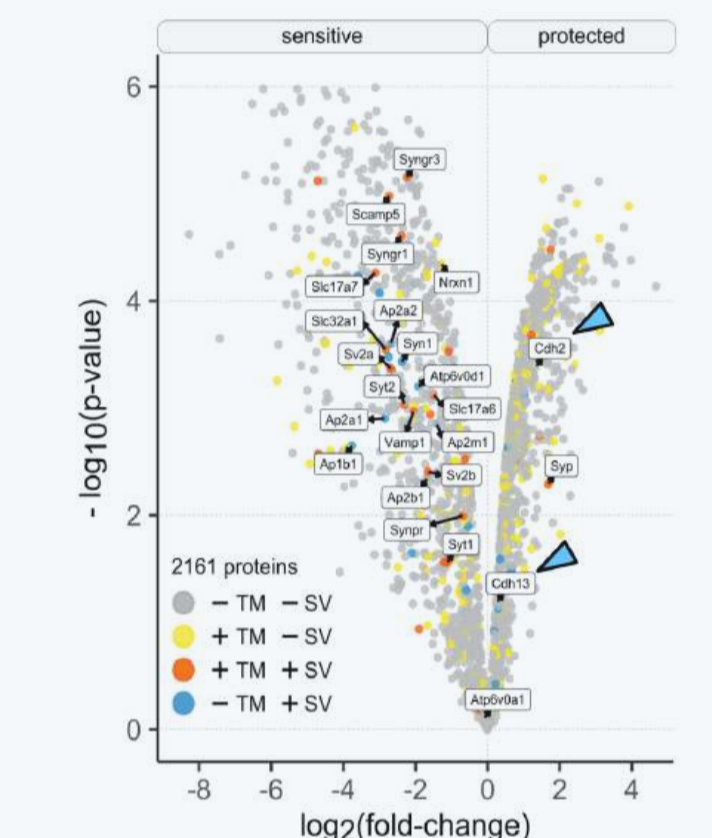
V-ATPase interacts with Syp



AlphaFold3



Proteinase K assay



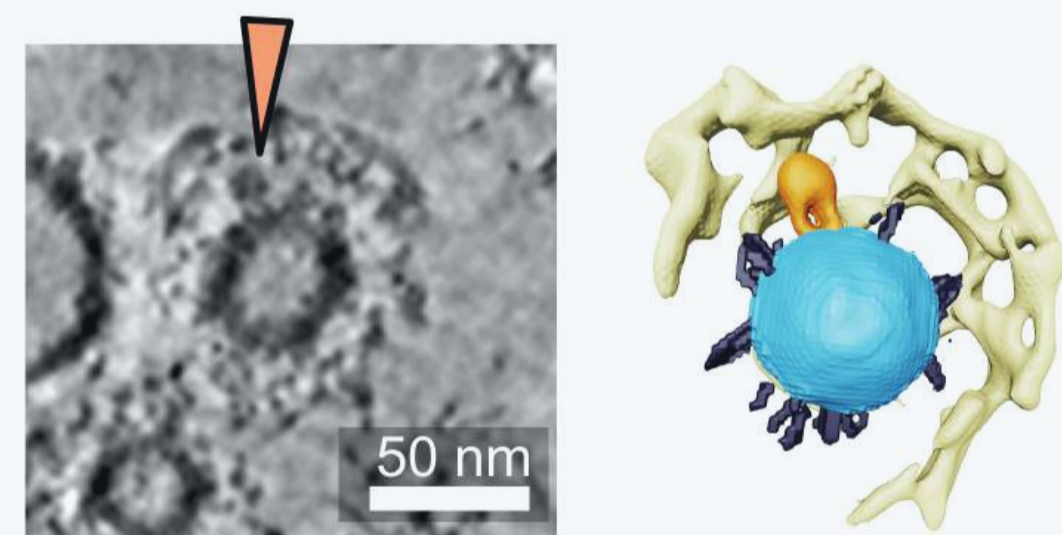
Clathrin-coated vesicles

We found clathrin-coated vesicles (CCVs) in both preparations, with partially coated vesicles observed in isolated samples (45%) and neurons (37%). Some isolated CCVs had a V-ATPase under the cage, suggesting early V1-Vo reassembly after SV fusion.

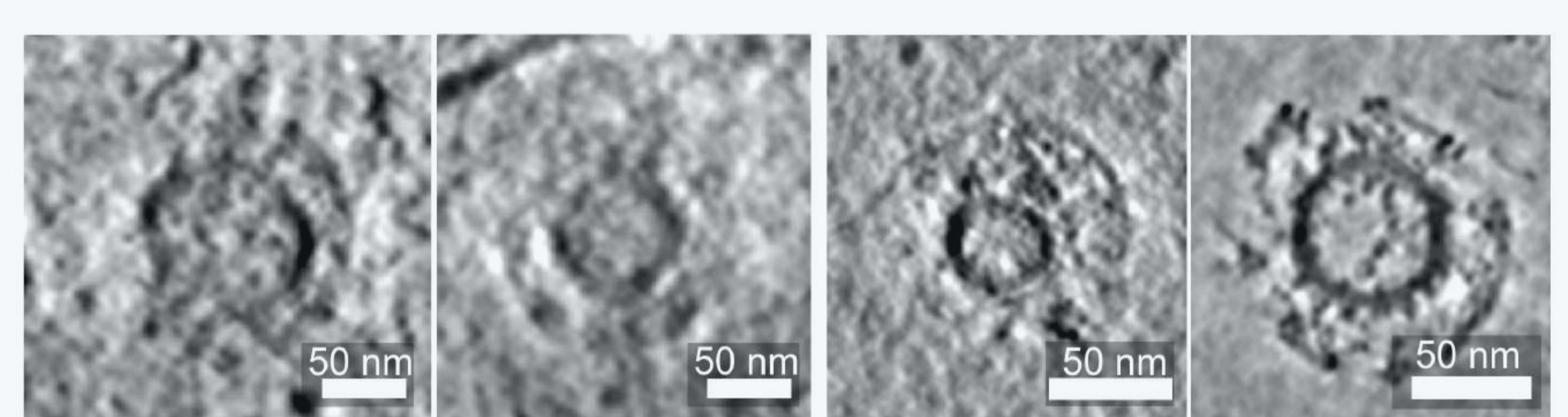
Empty clathrin baskets

Both sample preparations contained empty clathrin baskets without vesicles inside. In neurons, empty baskets and CCVs were preferentially located ~100 nm from the cell membrane. We hypothesize that these may serve as clathrin reservoirs for rapid CCV reassembly.

V-ATPase under the clathrin cage

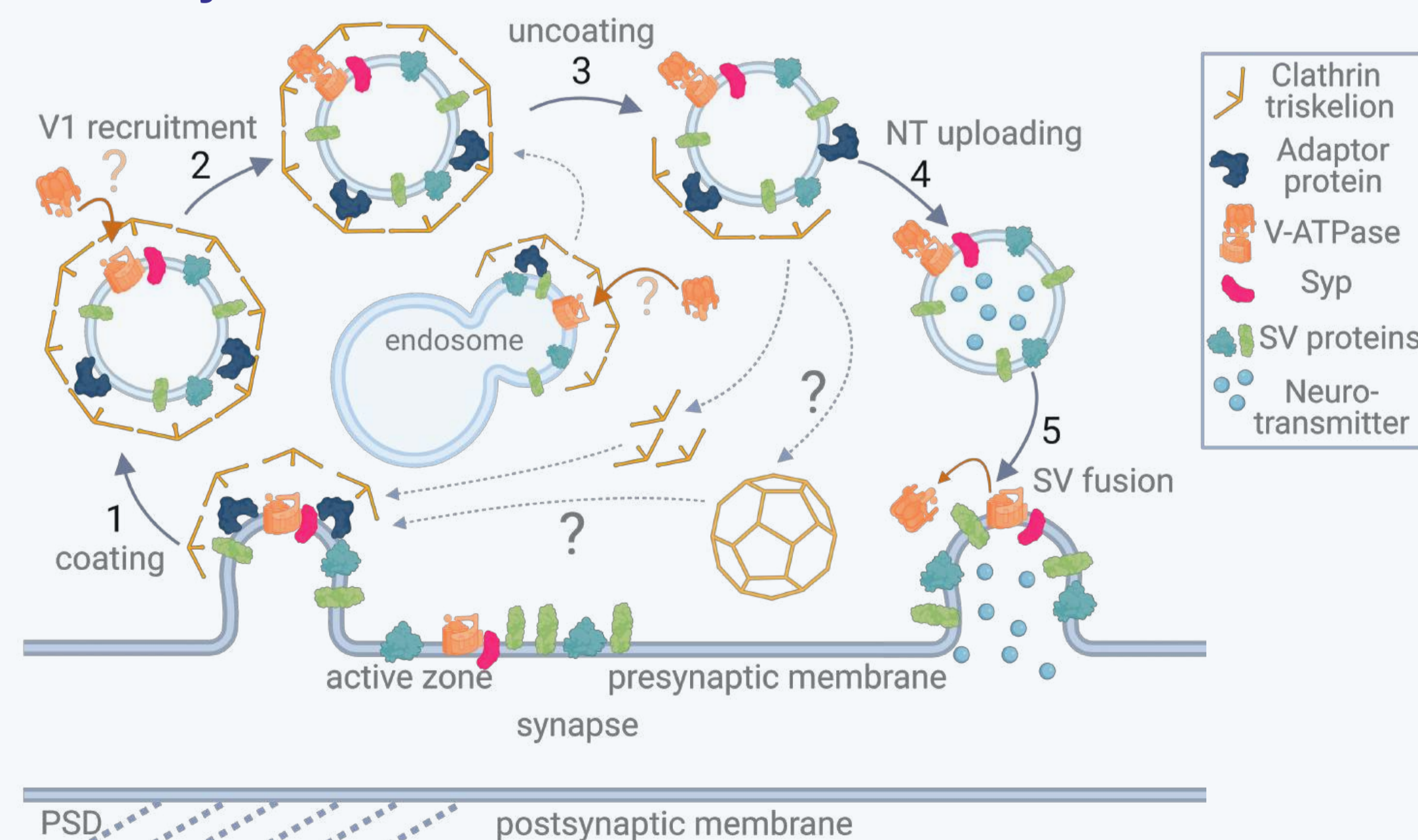


CCVs in neurons

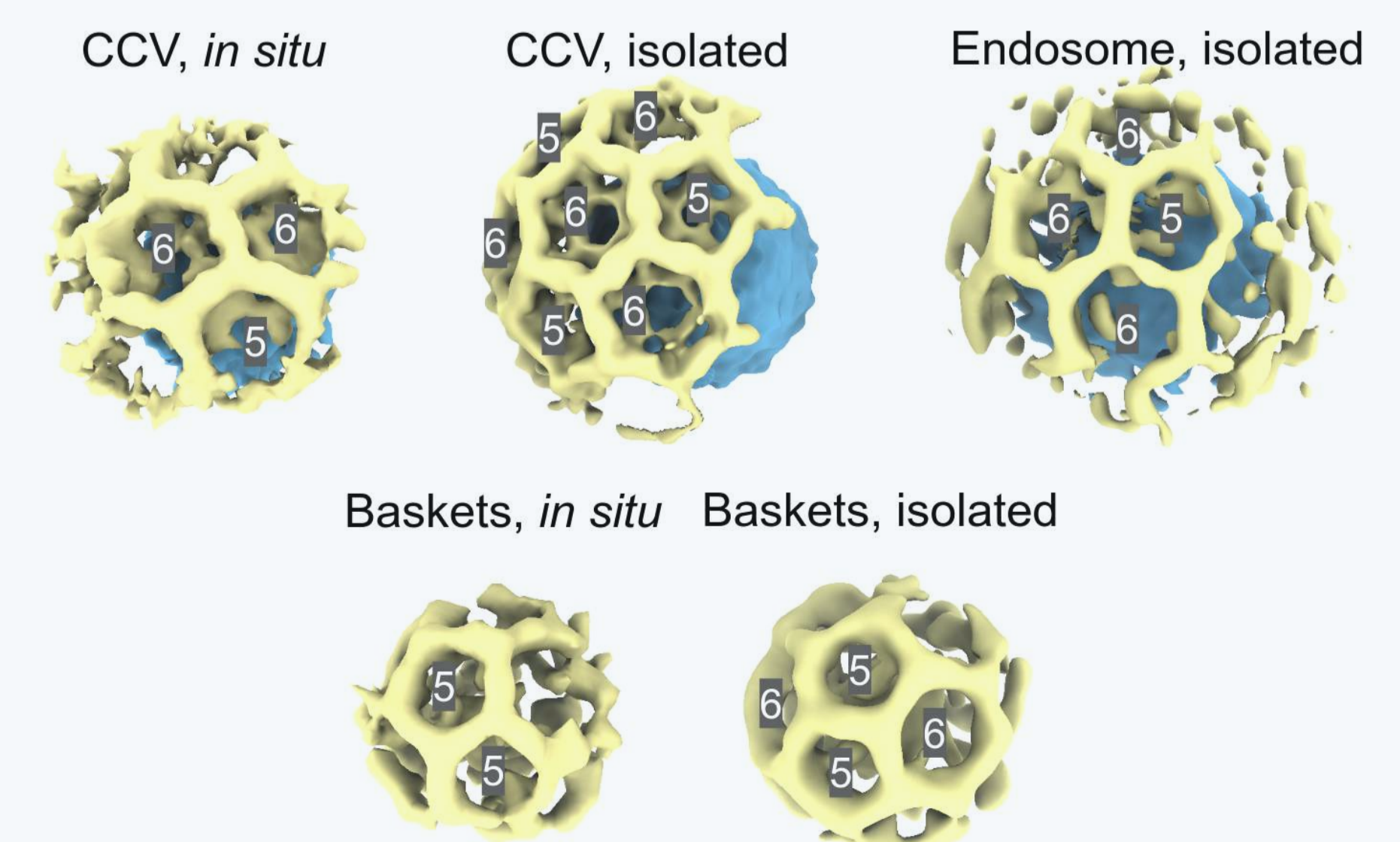


Isolated CCVs

Summary scheme



Empty baskets are smaller than CCVs, StA



We thank the Core Facility for cryo-Electron Microscopy of the Charité-Universitätsmedizin Berlin for support in the data acquisition. We thank Dr. Thiemo Sprink, Dr. Christoph Diebold, and Metaxia Stavroulaki for their help with grid preparation and data collection. We thank Der Fonds der Chemischen Industrie | FCI for personal fellowship for UK. This data was published: U. Kravčenko et al, Molecular architecture of synaptic vesicles, Proc. Natl. Acad. Sci. U.S.A. 121 (49) e2407375121, <https://doi.org/10.1073/pnas.2407375121> (2024).