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The Long Road to Micro-Dynamic Presynaptic FRET Measurements

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A, USA.

I will review the history and lead-up to our attempt to measure changes in FRET in assembled SNARE complexes in a very small space and a very short time, where only a tiny fraction of each of the labeled SNARE proteins is part of a SNARE complex. After recounting the saga of grant support and technical development, I will summarize our main findings: Using donor dequenching by receptor bleach as well as FLIM, we find a resting FRET indicating a number of assembled SNARE complexes exceeding the expected fraction of synaptobrevins (or VAMPs) that are in assembled SNAREs of docked and primed vesicles, likely reflecting the existence of still-assembled ‘‘orphan SNAREs’’ left over from prior bouts of secretion. We can detect the dispersion of all three SNARE proteins – VAMP, SNAP-25, and syntaxin – as well as the FRETting complex of assembled SNAREs itself, from the center of the presynaptic active zone to the periphery during and after a train of action potentials. Using sensitized acceptor emission on donor excitation, we also detect the disassembly of N-terminally labeled SNAREs prior to endocytosis of vesicle membrane and proteins, and the assembly of new SNAREs as replacement vesicles dock and prime, and we occasionally see a transient FRET increase while vesicles fuse during the train. By donor quenching, we also detect the rearrangement of C-terminally labeled SNAREs when vesicles fuse with the plasma membrane, and their subsequent dispersion, disassembly, and re-assembly. A variety of control experiments and statistical tests rule out most sources of artefact and alternative interpretations. I will also speculate on why SNARES disperse and are disassembled in the periphery, rather than in the central active zone where exocytosis occurs, as we had originally expected.