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Quantifying the Dynamic Interactions Between a Clathrin-Coated Pit and Cargo Molecules[1] AUBREY WEIGEL, National Institutes of Health, Bethesda MD, MICHAEL TAMKUN, DIEGO KRAPF, Colorado State University, Fort Collins CO — Clathrin-mediated endocytosis is a major pathway of internalization of cargo in eukaryotic cells. This process involves the recruitment of cargo molecules into a growing clathrin-coated pit (CCP). However, cargo-CCP interactions are difficult to study because CCPs display a large degree of lifetime heterogeneity and the interactions with cargo molecules evolve over time. We use single-molecule total internal reflection fluorescence (TIRF) microscopy, in combination with automatic detection and tracking algorithms, to directly visualize the recruitment of individual voltage-gated potassium channels into forming CCPs in living cells. Contrary to widespread ideas, cargo often escapes from a pit before abortive CCP termination or endocytic vesicle production. By measuring tens of thousands of capturing events, we build the distribution of capture times and the times that cargo remains confined to a CCP. An analytical stochastic model is developed and compared to the measured distributions. Due to the dynamic nature of the pit, the model is non-Markovian and it displays long-tail power law statistics. Our findings identify one source of the large heterogeneities observed in CCP maturation and provide a mechanism for the anomalous diffusion of proteins in the plasma membrane.

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