FM-Sim: Protocol Definition, Simulation and Rate Inference for Neuroscience Assays

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Abstract. Synaptic vesicle recycling at the presynaptic terminal of neurons is essential for the maintenance of neurotransmission at central synapses. Among the tools used to visualise the mechanics of this process is time-series fluorescence microscopy. Fluorescent dyes such as FM1-43, or engineered fluorescent versions of synaptic vesicle proteins such as pHluorins, have been employed to reveal different steps of this key process [3, 7]. Predictive *in silico* modelling of potential experimental outcomes would be highly informative for these time consuming and expensive studies.

We present FM-Sim [9], user-friendly software for defining and simulating fluorescence microscopy experimental assays, with the following features: intuitive user definition of experimental protocols; automatic conversion of protocol definitions into time series rate value changes; domain-specific simulation model of a synaptic terminal; experimental data used for model parameter value inference; automatic Bayesian inference of parameter values [1, 5] and reduction of inferred parameter set size for Bayesian inference.

1 The Synaptic Vesicle Cycle

Within chemical synapses of central nervous system (CNS) neurons, neurotransmitter is released from the presynaptic terminal to propagate the neural signal to the postsynaptic terminal of the following neuron. This neurotransmitter is stored in vesicles within the presynaptic terminal. These vesicles are exocytosed in response to an incoming action potential (Figure 1). To prevent vesicle depletion, compensatory endocytosis of plasma membrane allows regeneration of these vesicles. Two forms are studied within CNS nerve terminals:

- **Clathrin Mediated Endocytosis (CME)** [6]. Individual vesicles are reconstructed directly from the plasma membrane. Following reacidification of the vesicle contents and refilling with neurotransmitter, these vesicles rejoin the vesicle pools.

– **Activity Dependent Bulk Endocytosis (ADBE)** [4] is a second endocytosis mechanism triggered by periods of high stimulation. Here, large areas of plasma membrane are endocytosed as endosomes, which are later broken down into individual vesicles for reuse.

FM-Sim uses a hybrid stochastic model with delays of the vesicle cycle for simulation and inference. The model supports the behaviour of different fluorescent probes. The kinetic rates and associated time delays of state transitions are the parameters of the model.

2 Fluorescence Microscopy Imaging

Time-series fluorescent microscopy is one of the tools used to study the mechanisms of the synaptic vesicle cycle. Fluorescent probes added to nerve terminals allow us to obtain time-series images of nerve terminal behaviour under stimulation. The two commonly used forms of fluorescent probes are FM dyes (such as FM1-43 and FM2-10), and engineered pH-sensitive fluorescent synaptic vesicle proteins (pHluorins).

The change in fluorescence of a nerve terminal as a whole over time and under changing stimuli gives insight into internal behaviour. By using either FM dyes or pHluorins in combination with chemical inhibitors, or on various knockdown animal models, different aspects of the synaptic vesicle cycle can be isolated and studied. It is this variety of potential experiments which makes FM-Sim useful at the design phase. New experiments can be simulated based upon rate parameters obtained from prior similar experiments.

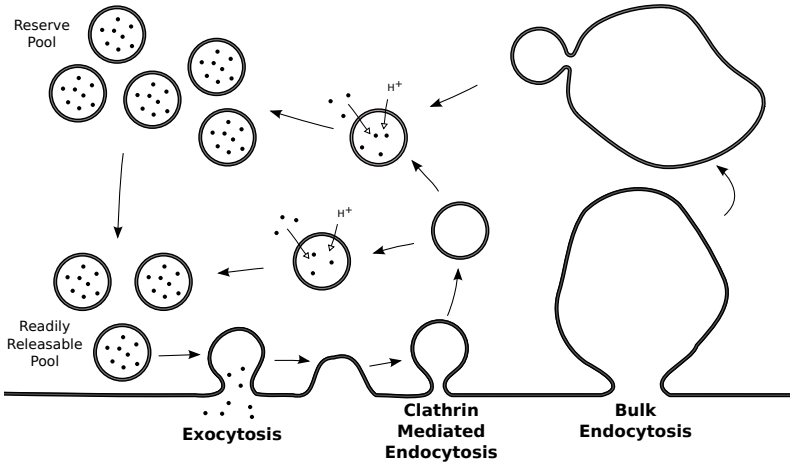

Fig. 1. The synaptic vesicle cycle, showing vesicle exocytosis and endocytosis.

3 FM-Sim: Protocol Definition and Simulation

FM-Sim allows the definition of experimental protocols (Figure 2). These are timed sequences of events, including reagent addition, and electrical or chemical stimulation of neurons. Each protocol event can have rate parameter values set manually, inferred from observations, or inherited from protocol events already active.

Once defined, the set of protocol events are converted into a sequence of rate change events for simulation (Figure 3). At each rate change event, the set of rate values in effect are calculated, accounting for value inheritance. A single value is used for each inferred protocol event parameter when generating Bayesian inference proposals, ensuring consistency if that parameter value is used in multiple rate change events. This simplification of protocol definition entry and automatic rate event generation with inherited rate values is a feature not found in many of the general purpose simulators currently available, such as VCell [8].

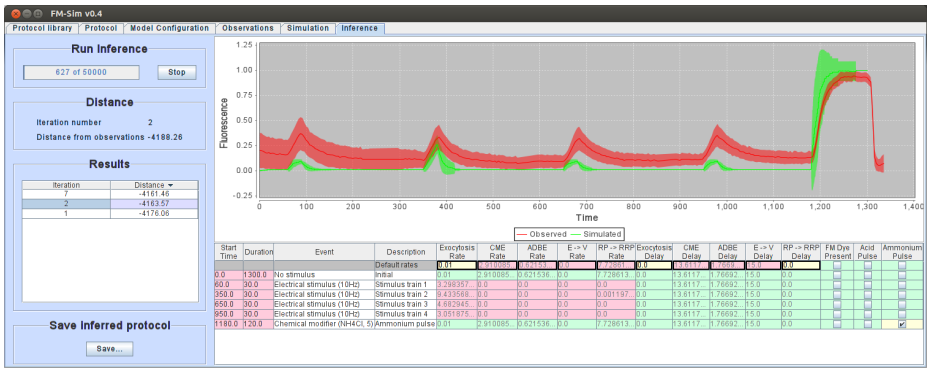

Fig. 2. Example parameter inference of a defined protocol, the parameter values in red are inferred from observed experimental data. The graph shows a sample simulation using these parameter values compared against the supplied experimental data.

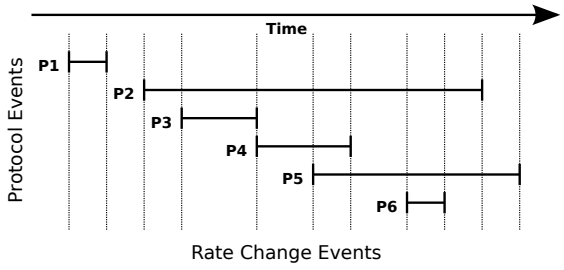

Fig. 3. Example rate change event generation. Protocol events P1,. . .,P6 are defined with start times and durations. These protocol events are then used to generate a sequence of events where rate values may change.

Protocols are simulated stochastically using the Delayed Stochastic Simulation Algorithm (DSSA) [2] with hybrid extensions, and the results of multiple simulation runs aggregated to provide mean and variance of the simulated model results. These results show both the expected fluorescence level, and the numbers of vesicles and endosomes at each stage of the synaptic vesicle cycle.

Rate parameters for a experimental protocol that have not been fixed by the user can be inferred from attempts to match a set of observed experimental data. A Bayesian approach to parameter inference is used, based on a Particle Marginal Metropolis-Hastings scheme using Sequential Monte Carlo estimates of marginal likelihoods [5, 1].

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FM-Sim is available at http://homepages.inf.ed.ac.uk/s9269200/software/.

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