The input parameters (those that you might want to vary) that are used in the stirred case are:

INITIAL\_COUNT

NDAYS

MEDIUM\_VOLUME

OXYGEN\_BDRY\_CONC

The drug parameters

The treatment protocol

Here is an example of a PROTOCOL section for a single drug dosing event:

PROTOCOL

1

DRUG

PR104A

2 hour at which the drug is added to the medium

100 hours before the drug is flushed out – a large value here indicates no flushing

0.005 volume of added drug solution (mL)

0.18 O2 concentration in added solution

0.18 O2 concentration in flushing volume (irrelevant if no flushing)

0.2 drug concentration of added drug solution

In response to your specific questions:

* You mentioned that the stirred model would have the potential to model drug uptake in a single cell which could then be scaled to the density of the cell suspension. Is this scaling integrated into the variability of the ‘INITIAL COUNT’ and VCELL\_PL (i.e. cell volume fraction)?

VCELL\_PL is the cell volume in pL. I doubt that you want to change this. In the model the drug uptake (and releases) of a single cell is scaled by multiplying by the number of cells (always INITIAL\_COUNT when there is no cell division or death). There is no reason to do it in this case, but you could, for example simulate 1/10 the number of cells in 1/10 of the medium volume.

* The model assumes that there is no cell growth or proliferation during the experiment duration, so does this mean that the lines from DIVIDE\_TIME to RANDOMISE\_INITIAL\_V (lines 5 to 10) are ignored.

Yes, as are all the lines from 17 to 42 and 44 to 122.

* As far as I can tell, the duration of the output is based on ‘NDAYS’ (line 11). I presume that for my simulations, I could leave it at any arbitrary value as the time step for the output is in 30 minute intervals. I would only need to extract the first six time steps to model my data set (experiment duration of 180 mins).

Yes, and NDAYS does not need to be an integer. I have been testing with NDAYS = 0.3.

* Is the WELL\_AREA and MEDIUM\_VOLUME (lines 15-16) based on the volume of the glass vials used for stirred cell suspension experiments? Or are these parameters ignored as the constant stirring would lead to almost immediate dispersal and equilibration of the added drug volume (indicated by FULLY\_MIXED = 1 (line 17))?

The WELL\_AREA is unused in the stirred case, only MEDIUM VOLUME is used. The volume of the added drug volume is added to the medium volume in calculating the subsequent concentrations.

* As the cells are pre-equilibrated and maintained with the appropriate gas phase (basically 100% oxygen or 0% oxygen), and it is assumed that there is no cell growth, proliferation or death during the experiment duration, lines 18 – 123 relating to the diffusion, consumption and metabolism of oxygen, glucose and lactate (along with additional tracers and radiation) are ignored for simulations.

Yes.

* What does ‘DNB’ refer to in line 125 (CLASS\_NAME)?

DNB is from the .drugdata file, and it refers to the class of the drug (don’t ask me what that means.)

* Are the medium diffusion coefficient (MEDIUM\_DIFF) and multicellular layer diffusion coefficients (DIFF\_COEF) ignored in the stirred model given you are dealing with a single cell suspension without diffusional limitations?

Yes, these diffusion coefficients are irrelevant, the first because the medium is stirred, the second because we are not simulating a spheroid.

* If I was wanting to parameterize CELL\_DIFF\_IN and CELL\_DIFF\_OUT based on the cellular uptake of the prodrug under supraoxic cultures, I presume that the parameters in lines 133 to 138 (i.e. KMET0, C2, KO2, VMAX and KM) would be ignored if I were to set the O2 concentration in the experimental protocol to a high value. If the C2 was set to 1 (proportion of prodrug metabolism that is oxygen-dependent) and the KO2 (O2 conc for half maximal prodrug metabolism) was set to an arbitrary low value (i.e. 0.01 uM O2), it would be assumed that prodrug metabolism would be dependent entirely on severe hypoxia. The metabolite would be effectively ignored under supraoxic conditions.

Yes. The metabolism parameters are never ignored, but when CO2 is very high and C2 = 1 the rate of metabolism goes to zero.

* Parameterization of the prodrug (Kmet0) and metabolite (CELL\_DIFF\_IN and CELL\_DIFF\_OUT, Kmet0 etc.) should be relatively straight forward when the O2 concentration in the experimental protocol is set to a low value (i.e. 0 uM).

I can’t be much help with guesses for the parameter values. If you have some idea of which of the drug parameters are likely to be similar to those of drugs you’ve worked with previously that will suggest which ones can possibly be fixed. In general parameter estimation is not very straightforward. See how you get on.