Guidelines for producing data and using models associated with

Patchy fibrosis promotes trigger–substrate interactions that both generate and maintain atrial fibrillation

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Follow the installation guidelines in the documentation (Basic_use.txt or Full_documentation.pdf)

Please download the folder containing all geometry files (tissue and cell structures) and state files. These can be found here:
https://github.com/michaelcolman/MSCSF_state_and_geometry_files

Therefore, ensure your PATH.txt is properly setup to point to this directory.

You will need to compile for this project:
model_single_0D
model_tissue_0D_nework

List of tissue models that are used in this study:
2D_human_atria_fibrosis_300x300_OY
2D_human_atria_fibrosis_300x300_field_control
2D_human_atria_fibrosis_300x300_field_remodelled

Human_atria_AKL_heart_1_cleaned
Human_atria_AKL_heart_2_cleaned
Human_atria_AKL_heart_3_cleaned

Each with associated map files. Please see "Tissue_model_list.txt" in the documentation for more details.

Regarding fibrosis maps:

We have two different types of map associated with the tissue models.

The first is a binary fibrosis map – this is 1 where there is fibrosis and 0 elsewhere. It is of the size of Ntissue.

For the 2D tissue models, these have the form:
"2D_human_atria_fibrosis_map_ln_X_perc_Y.txt"
Where X is the length-scale used to create the map (10, 25, 37, 50) and Y is the total fibrosis coverage percentage (25, 50).

For the 3D models we just have one map per geometry:
"Human_atria_AKL_heart_1_fibrosis_map.dat"
Note that "reflected" should be used if the corrected geometry is used (R-L swapped compared to data). However, connection maps have not been made for these corrected geometries, and so if you want to use premade connection maps, use the cleaned geometry not the reflected/corrected.

The second maps are the connection maps used by the network model that are generated from these fibrosis maps. Here, within the area determined by the fibrosis map, a set percentage of axial and transverse connections were removed. Outside the map, they were left alone. These maps depend on myocyte orientation (which itself depends on tissue model in 2D) and fibrosis state, and are of size Njunctions (> Ntissue).

For the 2D models they have the form:

"2D_human_atria_fibrosis_300x300_field_remodelled_JNmap_10_25.txt"

Note that because the orientation depends on the tissue model, the maps are individual to the tissue model chosen. The last two numbers are the length-scale and percentage coverage, corresponding to the original fibrosis map.

For the 3D models they have the form:

"Human_atria_AKL_heart_1_cleaned_Junction_discrete_map_0.200000_0.800000.dat"

Where now the numbers at the end refer to the percentage of axial and transverse connections that were removed.

We will go through the process of how these data were created in this study, using the minimal cell model.

This has also been provided in bash script form to make it easy for you to implement yourself and perform your own simulations. Please see Interface_focus_2023_Patchy_Fibrosis_data_script.sh.

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Part 1: create spontaneous release function settings files

First, we want to create the SRF settings files, such that we can control precisely the SCRE dynamics. These correspond to the functions and distributions shown in **Fig. 2** of the manuscript.

CaSR-dependence

Run the "model_single_0D" code with the following options (you may adjust the SRF parameters however you see fit).

```
./model_single_0D Total_time 1 SRF_mode Dynamic SRF_model General SRF_Pset User_control SRF_Dyn_PSCR_threshold 1.05 SRF_Dyn_CaSR_max 1.8 SRF_Dyn_CaSR_Prange 0.175 SRF_Dyn_ti_sep_max 1800 SRF_Dyn_ti_sep_min 950 SRF_Dyn_ti_width_max 1250 SRF_Dyn_ti_width_min 250 SRF_Dyn_MD_max 800 SRF_Dyn_MD_min 100 SRF_Dyn_duration_width_max 500 SRF_Dyn_duration_width_min 25 SRF_Dyn_H 1.5 Write_SRF_settings On reference SRF_write
```

This will create settings files for each CaSR (in steps of 1um) which contain the SRF parameters given by the above distribution:

Outputs_0Dcell_SRF_write/Parameters/SRF_distributions/SRF_Settings_file_x.txt

where x is the CaSR in uM (e.g., 1100 for 1.1 mM)

Copy or move this folder to store somewhere the simulations will be able to see it.

The independent control functions

The other way is that we want to control the synchronisation and duration distributions independently of each-other.

We will do this with a simple bit of C code that contorls the parameters using simple linear relationships, and writes a settings file.

You can find that bit of code in the "Tools" folder: "Create_SRF_settings_linear_variation.c". What it does will be summarised here:

By defining:

X = synchronisation width factor (0 = unsynchronised, 1 = fully synchronised)

Y = duration width factor (0 = wide distirbution, 1 = tight distribution)

We can define the distribution parameters as such:

```
width_F1  = ti_width_max - (ti_width_max - ti_width_min)*X;
k_ti_F1   = width_F1;
k_ti_F2   = 1.5 * k_ti_F1;

ti_sep = 500+ti_width_max; // constant -> ensure that any ti is >500 ms, i.e. 500+max width = ti sep

duration_width = duration_width_max - (duration_width_max - duration_width_min)*Y;

MD = (150 - 125*Y) + duration_width; // shifts median according to width, so that tighter distributions have a shorter median
```

And then write a settings file in the same format as the CaSR ones, with X and Y in the filename:

```
sprintf(string1, "SRF_settings_files_DC/SRF_settings_%0.2f_%0.2f.txt", X, Y);
out = fopen(string1, "wt");
fprintf(out, "9\n");
fprintf(out, "SRF_mode Direct_Control\n");
fprintf(out, "SRF_Pset User_control\n");
```

```
fprintf(out, "SRF_DC_PSCRE 1.000000\n");
fprintf(out, "SRF_DC_CF %f\n", CF_ti_sep);
fprintf(out, "SRF_DC_ti_sep %f\n", ti_sep);
fprintf(out, "SRF_DC_ti_W1 %f\n", k_ti_F1);
fprintf(out, "SRF_DC_ti_W2 %f\n", k_ti_F2);
fprintf(out, "SRF_DC_MD %f\n", MD);
fprintf(out, "SRF_DC_duration_W %f\n", duration_width);
fclose(out);
```

Part 2: Create model state files

The purpose of these is that we will need to run many, many simulations with the same starting conditions, in order to calculate probability of focal excitation. We therefore want to minimise the time needed for each individual results simulation. We can do this by pacing the tissue models to stable-state and saving the state file, allowing us to then start simulations from this state. We will do this by first pacing single cell models to stable state, and then inputting these into tissue to help the tissue get to stability more quickly.

Single cell:
First we want to create single cell and tissue state files corresponding to different conditions (AF, ISO etc) and pacing rates.
This will write state files into "MSCSF_state_and_geometry_files" folder, so ensure you have that and the PATH.txt points towards it

First, we do single cell across a range of parameters to keep things flexible. We will pace for 250 beats to reach stable state.

loop over [cycle_length] e.g. 200-1000 in steps of 100:

[BCL] = [cycle_length]
[Total_time] = [cycle_length*250]

./model_single_0D Model minimal Celltype RA Beats 250 BCL [BCL] Total_time [Total_time] Write_state On Remodelling none ISO 0.0 Reference Control_control Results_Reference [BCL]

./model_single_0D Model minimal Celltype RA Beats 250 BCL [BCL] Total_time [Total_time] Write_state On Remodelling AF ISO 0.0 Reference AF_control Results_Reference [BCL]

./model_single_0D Model minimal Celltype RA Beats 250 BCL [BCL] Total_time [Total_time] Write_state On Remodelling none ISO 1.0 Reference Control_ISO Results_Reference [BCL]

./model_single_0D Model minimal Celltype RA Beats 250 BCL [BCL] Total_time [Total_time] Write_state On Remodelling AF ISO 1.0 Reference AF_ISO Results_Reference [BCL]

The state files (in the MSCSF_state_and_geometry_files folder) should look something like:

Integrated_0D_model_minimal_BCL_600_region_RA_ISO_0.00_ACh_0.00_remodelling_AF_drug_none_mut_none_env_intact_ref_none_state.dat

We can see that BCL, ISO, remodelling etc are all automatically isolated in the filename, so that we have separate state files.
For this reason, we didn't need to pass in a state reference.

Tissue:
Next, we want to create the tissue state files. This will be a two-step process: (1) Read in single cell state files and write the average tissue state file; (2) Read in the ave coupled state file and write a full tissue state file.

Using just one tissue geometry as an example we will write the state files for each condition. We will pick BCL of 400 to keep it simple.

Ave tissue state write:
./model_tissue_0D_network Tissue_order geo Tissue_model 2D_human_atria_fibrosis_300x300_OY Model minimal Celltype RA BCL 400 Remodelling none

ISO 0.0 Read_state single_cell Write_state ave Tissue_type homogeneous Total_time 4000 Beats 10 Spatial_output_interval_data 0 Spatial_output_interval_vtk 0 D1 0.4 D_AR 7 reference Tissue_ave_state_write

Full tissue write:

We now change Read_state from single_cell to ave, and Write_state from ave to On

./model_tissue_0D_network Tissue_order geo Tissue_model 2D_human_atria_fibrosis_300x300_OY Model minimal Celltype RA BCL 400 Remodelling none ISO 0.0 Read_state ave Write_state On Tissue_type homogeneous Total_time 4000 Beats 10 Spatial_output_interval_data 0 Spatial_output_interval_vtk 0 D1 0.4 D_AR 7 reference Tissue_state_write_Control State_Reference_write Control

A couple of notes:

a. We will be ensuring tissue_type is "homogeneous"
b. We have set the spatial data to not output as we don't need spatial snapshots of voltage.
c. We will also explicitly set our diffusion properties of D1 and D_AR
d. For the full tissue write, we also pass in a "State_Reference_write" to append the name of the state file, as we must differentiate between the whole tissue state of control and fibrosis (as these maps are not automatically in the tissue state file name). AF, ISO condition etc are still automatically in the state file name – it is just differentiating the fibrosis state that is needed.

To do this in full, we want to perform a nested loop over the three 2D tissue models and the different fibrosis maps, and ± AF remodelling and ± ISO

Loop over:
[tissue] = OY, field_control, field_remodelled
  [map] = 10_25, 10_50, 25_25, 25_50, 37_25, 37_50, 50_25, 50_50

./model_tissue_0D_network Tissue_order geo Tissue_model 2D_human_atria_fibrosis_300x300_[tissue] Model minimal Celltype RA BCL 400 Remodelling [none/AF] ISO [0.0/1.0] Read_state ave Write_state On Tissue_type homogeneous Total_time 4000 Beats 10 Spatial_output_interval_data 0 Spatial_output_interval_vtk 0 D1 0.4 D_AR 7 Junction_modulation_map On Junction_modulation_map_file 2D_human_atria_fibrosis_300x300_[tissue]_JNmap_[map].txt reference Tissue_[tissue]_state_write_Fib_[map] State_Reference_write fib_[map]

The state files will look something like:

Integrated_net_model_minimal_BCL_400_ISO_0.00_ACh_0.00_remodelling_none_drug_none_mut_none_geo_2D_human_atria_fibrosis_300x300_OY_homogeneous_anisotropic_ref_fib_10_25_state.dat

Part 3: The arrhythmia substrate

Here we will perform simulations to assess wave breakdown during rapid pacing. No SRF or SCRE will be imposed in these simulations. This corresponds to **Fig. 9** of the manuscript.

NOTE: To run this, please repeat the creation of tissue state files at the BCLs that you want (as we only did so for BCL=400 previously).

We will loop over all tissue models and a couple of fast cycle lengths, applying five beats and then leaving for a quiescent duration.

[BCL] = 150, 200
[tissue] = OY, field_control, field_remodelled
[rem] = none, AF
[ISO] = 0, 1.0

#First, for control tissue model:

./model_tissue_0D_network Tissue_order geo Tissue_model 2D_human_atria_fibrosis_300x300_[tissue] Tissue_type homogeneous Model minimal Celltype RA BCL [BCL] NBeats 5 Total_time 3000 Remodelling [rem] ISO [ISO] Read_state On state_reference_read Control reference Rapid_pace_Control_[tissue] results_reference rem_[rem]_IS0_[ISO]_BCL_[BCL]

#Now, loop over fibrosis maps:
[map] = 10_25, 10_50, 25_25, 25_50, 37_25, 37_50, 50_25, 50_50

./model_tissue_0D_network Tissue_order geo Tissue_model 2D_human_atria_fibrosis_300x300_[tissue] Tissue_type homogeneous Model minimal Celltype RA BCL [BCL] NBeats 5 Total_time 3000 Remodelling [rem] ISO [ISO] Read_state On state_reference_read fib_[map] Junction_modulation_map On Junction_modulation_map_file 2D_human_atria_fibrosis_300x300_[tissue]_JNmap_[map].txt reference Rapid_pace_Fib_[map]_[tissue] results_reference rem_[rem]_IS0_[ISO]_ BCL_[BCL]

Part 4: Determine the focal excitation probabilities given different SCRE dynamics

First, we will do this in 2D. We will do this versus CaSR for illustrative purposes. This corresponds to the type of data presented in **Figs. 6-8** of the manuscript.

Let's assume we placed the SRF settings files, created in part 1, in a folder called "Settings_files" in the present directory.

We will illustrate how to do this for the CaSR-dependent case (looping over one variable to control SRF), but it is exactly the same process if using the independently controlled settings files.

- Note that settings files must always be the first argument passed.
- We will only apply a single paced beat, and set the total time to 2000 ms to leave that quiescent period during which SCRE may occur.
- We will also pass "Delayed_CaSR_IC" to impose an exact CaSR and Cai at a defined time (500 ms) that corresponds to the input CaSR. For the independent distributions, just use a consistent value e.g. 1000 uM.
- Ensure to read in the correct state file for the tissue status.
- We will name our parent output folder by the tissue geometry and fibrosis condition, and the results folder by the CaSR and remodelling/ISO condition

Loop over:
[run] = 1-X in steps of 1
[CaSR] = 900-1300 in any sensible step (2-25)
[tissue] = OY, field_control, field_remodelled
[rem] = none, AF
[ISO] = 0, 1.0

#First, for control tissue model:

./model_tissue_0D_network Settings_file Settings_files/SRF_Settings_file_[CaSR].txt Tissue_order geo Tissue_model 2D_human_atria_fibrosis_300x300_[tissue] Tissue_type homogeneous Model minimal Celltype RA BCL 400 NBeats 1 Total_time 2000 Remodelling [rem] ISO [ISO] Read_state On state_reference_read Control Delayed_CaSR_IC On CaSR_IC_delay 500 CaSR 1000 Cai 0.1 reference PTA_Control_[tissue] results_reference rem_[rem]_IS0_[ISO]_CaSR_[CaSR]_run_[run]

#Now, loop over fibrosis maps:
[map] = 10_25, 10_50, 25_25, 25_50, 37_25, 37_50, 50_25, 50_50

./model_tissue_0D_network Settings_file Settings_files/SRF_Settings_file_[CaSR].txt Tissue_order geo Tissue_model 2D_human_atria_fibrosis_300x300_[tissue] Tissue_type homogeneous Model minimal Celltype RA BCL 400 NBeats 1 Total_time 2000 Remodelling [rem] ISO [ISO] Read_state On state_reference_read fib_[map] Delayed_CaSR_IC On CaSR_IC_delay 500 CaSR 1000 Cai 0.1 Junction_modulation_map On Junction_modulation_map_file 2D_human_atria_fibrosis_300x300_[tissue]_JNmap_[map].txt reference PTA_Fib_[map]_[tissue] results_reference rem_[rem]_IS0_[ISO]_CaSR_[CaSR]_run_[run]

Then do the equivalent across the independent control variables (instead of CaSR) and/or using the 3D models (now there is only one fibrosis map, at least!)

Part 5: The simple fibroblast coupling model

Now repeat using a simple model of fibroblast coupling that involves a shift of the resting potential. This will be achieved through adding "IK1_Erev_shift 10". This corresponds to data as presented in **Figs. 11-13** of the manuscript.

It is important that we fully understand the distinction between the fibrosis map and the junction map, as we will need both for this. We will use the junction map to disturb the inter-cellular coupling in fibrosis regions, as we have previously, but also the fibrosis map to tell the simulation where to apply the IK1 shift. You must therefore ensure that the parameters of each map are the same (unless you want the fibroblast region to be different to the loss of inter-cellular coupling region).

By passing in

Direct_modulation_map On Direct_modulation_map_file 2D_human_atria_fibrosis_map_In_[x]_perc_[y].txt IK1_Erev_shift 10

We are telling the model to apply "IK1_Erev_shift 10" (a "direct modulation" of the parameters) only to regions defined by the map.

We will need to create proper tissue state files for this condition. Now, we will need more beats for stable state due to the mismatch between the fibroblast coupling zone and elsewhere.

As before, loop over all variables that you want to combine with fibroblast coupling.

./model_tissue_0D_network Tissue_order geo Tissue_model 2D_human_atria_fibrosis_300x300_[tissue] Tissue_type homogeneous Model minimal Celltype RA BCL 400 Remodelling [none/AF] ISO [0.0/1.0] Read_state ave Write_state On Tissue_type homogeneous Total_time 20000 Beats 50 Spatial_output_interval_data 0 Spatial_output_interval_vtk 0 D1 0.4 D_AR 7 Junction_modulation_map On Junction_modulation_map_file 2D_human_atria_fibrosis_300x300_[tissue]_JNmap_[map].txt Direct_modulation_map On Direct_modulation_map_file 2D_human_atria_fibrosis_map_In_[x]_perc_[y].txt IK1_Erev_shift 10 reference Tissue_[tissue]_state_write_Fib_[map]_fibroblast State_Reference_write fib_[map]_fibroblast

And now repeat as before with different SRF parameters, ensuring you read in the new state files and add fibroblast coupling in some way as a note to your reference to keep the data separate. Some further examples are show in Interface_focus_2023_Patchy_Fibrosis_data_script.sh.

Part 6: Pacing interrupt

Here, simply vary the pacing rate and number of beats to explore how SCRE may interact during pacing. Such that you can read in your BCL-dependent state files while also varying the pacing rate in this model, you can use the "S2" argument. Set your BCL to the one you want for the state file, and set NBeats to 1. Now, add the additional arguments "S2 [x] NS2 [y]" and it will apply y beats at an interval of x after the single beat applied at the first BCL (for which, as it is just one beat, the BCL itself does not affect).

Corresponds to **Fig. 10** of the manuscript.

Part 7: Spontaneous arrhythmia

This is a combination of the fibroblast model with rapid SCRE and different pacing approaches, corresponding to **Fig. 14** of the manuscript.

Please explore and experiment to find interesting dynamics yourself - I'm sure there is plenty of potential new data!

The key aspects here are with and without fibroblast coupling, having relatively rapid SCRE, and varying the number of applied stimuli and pacing rate.

Try a few different SRF settings for rapid and synchronised pacing. Example ranges:

SRF_mode Direct_Control
SRF_Pset User_control
SRF_DC_PSCRE 1.0
SRF_DC_CF 0.4
SRF_DC_ti_sep [between 150 and 500]
SRF_DC_ti_W1 [50-100]
SRF_DC_ti_W2 [50 - 100]
SRF_DC_MD [80 - 300]
SRF_DC_duration_W [50 - 150]

Just ensure that ti_sep - ti_W1 > 0 and MD - duration_W > 50

And combine with a varying small number of applied stimuli at different rates (tail interaction matters).