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Supplementary Information for

Solute movement in the t-tubule system of rabbit and mouse cardiomyocytes

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Extended methods and 2 Supplementary figures

Methods

Myocyte Preparation. All procedures carried full ethical approval and complied with relevant UK legislation and in accord with the Guide for the Care and Use of Laboratory Animals (NIH). Ventricular cardiomyocytes were enzymatically isolated from the hearts of male mice (C57BL/6, 25 g) or rabbits (New Zealand White, 2.5 kg), as described previously (1, 2). Briefly, mice were heparin injected (500 IU, *i.p.*) for 5 min, killed by cervical dislocation, heart rapidly removed and washed in a standard physiological saline solution (see below) that also contained 0.1 $mmolL^{-1}$ $CaCl_2$ and 10 $IUmL^{-1}$ heparin. The heart was mounted on a Langendorff perfusion system (37 °C) and enzymatic dissociated using collagenase II (Worthington Biochemical Corp., Lakewood, NJ, USA) and protease XIV (Sigma-Aldrich Co. Ltd., St. Louis, MO, USA).

Solutions. The standard solution used for mouse cell isolations contained (in $mmolL^{-1}$): 130 NaCl, 5.4 KCl, 1.4 $MgCl_2$, 0.4 NaH_2PO_4, 10 D-glucose, 4.2 HEPES, 20 taurine and 10 creatine, pH=7.4. For rabbit cells, the solution was similar, except that it contained 4.5 KCl, 3.5 $MgCl_2$ and 5 HEPES. Cell storage solutions were either the standard solution that also contained 0.1mM $CaCl_2$, or Kraftbruhe (KB), which contained (in $mmolL^{-1}$): 100 L-isomer glutamic acid, 30 KCl, 10 HEPES, 1 EGTA, 5 Na pyruvate, 20 taurine, 20 glucose, 5 $MgCl_2$, 5 succinic acid, 5 creatine, 2 Na_2ATP, 5 ß-OH butyric acid. All experiments were performed at room temperature in KB medium.

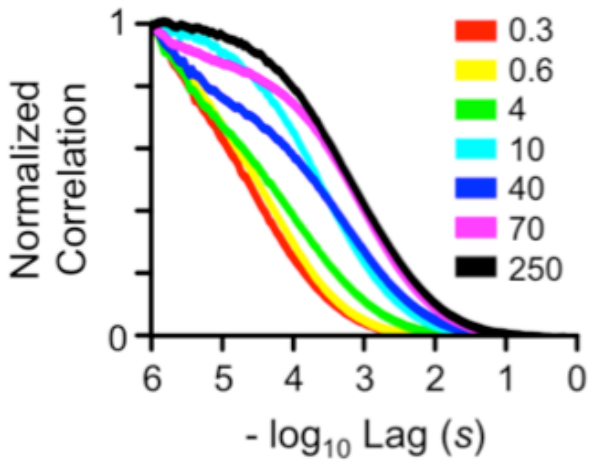

Fig. S1. Exemplar correlograms calculated from Fluorescence Correlation Spectroscopy (FCS) measurements for solutes with molecular weights (MWs) of 0.3-250 kDa (indicated at right).

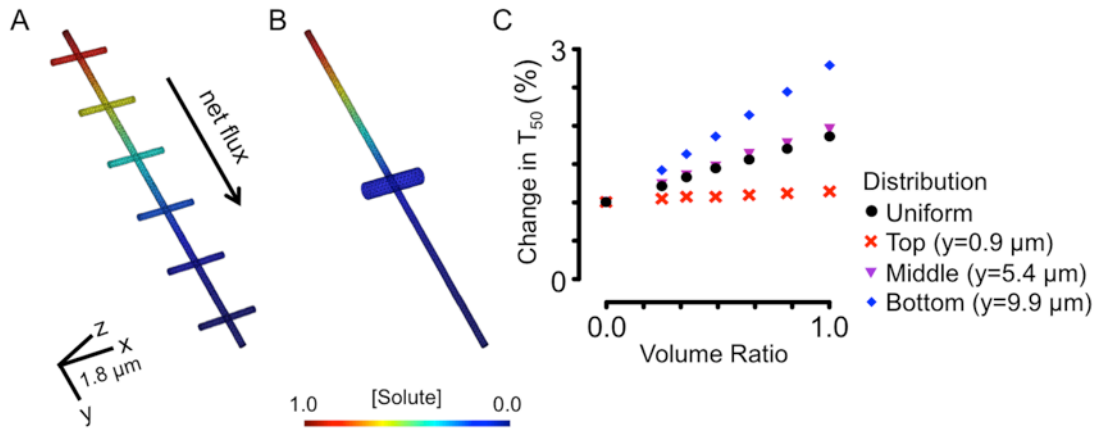

Fig. S2. Computer simulations showing the effect of longitudinal or axial (*x*) tubules on net diffusion along a linear transverse or radial (*y*) tubule. (A) Model geometry with uniform distribution of axial elements. The t-tubule has a radius of 100 nm and length of 10.8 µm, reflecting the typical half width of cardiomyocytes. (B) Uneven distribution of axial t-tubules with equivalent volume to (A). (C) Summary of simulation results. For uniformly distributed axial t-tubules (black circles), FRAP half time (T_{50}) increases linearly with the axial:transverse tubule volume ratio. For unevenly distributed axial t-tubules the radial position changes their effect on T_{50}. In rabbit myocytes, 70.9 ± 3% (n=11) tubules are transversely-oriented, while in mouse this value is 45.4 ± 1% (n=12), which correspond to axial:transverse tubule volume ratios of 0.41 and 1.2, respectively**.**

Movie S3. Simulation the effect of solute superfusion in a FRAP experiment on a myocyte. Frames show the cell from Fig. 2 on a coverslip-bottomed chamber. The superfusion flow rate is 1 mms^{-1} and solute concentration is indicated by a rainbow color table scaled between 0.7 and 1.0. The movie should play in real time, simulation duration was 12s.

References

1. Bryant SM, et al. (2015) Altered distribution of ICa impairs Ca release at the t-tubules of ventricular myocytes from failing hearts. *J Mol Cell Cardiol* 86:23–31.

2. Hancox JC, Levi AJ, Lee CO, Heap P (1993) A method for isolating rabbit atrioventricular node myocytes which retain normal morphology and function. *Am J Physiol Heart Circ Physiol* 265(2):H755–H766.