Title

Protocol to record and quantify the intracellular pH in contracting cardiomyocytes

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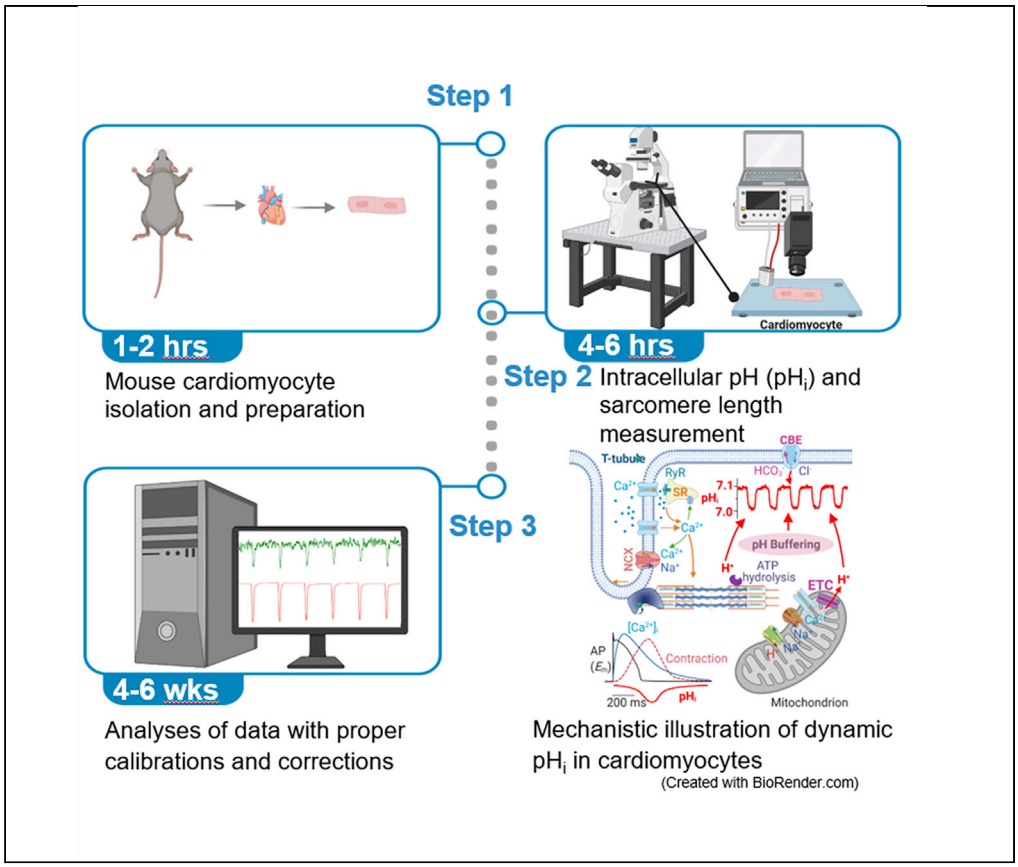

Intracellular pH (pH_i) plays critical roles in the regulation of cardiac function. Methods and techniques for cardiac pH_i measurement have continued to evolve since early 1960s. Fluorescent microscopy is the most recently developed technique with several advantages over other techniques including higher spatial and temporal resolutions, and feasibility for contracting cell measurement. Here, we describe detailed methods for mouse cardiomyocyte isolation, and simultaneous measurement and quantification of pH_i and sarcomere length in contracting cardiomyocytes.

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Highlights

Cardiomyocytes exhibit beat-to-beat cellular acidifications, termed "pH_i transients"

pH_i transients are coupled to cardiomyocyte contractions

Techniques for simultaneous measuring of cardiomyocyte contractions and pH_i

Protocol for the recording and quantification of pH_i transients in cardiomyocytes

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Protocol to record and quantify the intracellular pH in contracting cardiomyocytes

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SUMMARY

Intracellular pH (pH_i) plays critical roles in the regulation of cardiac function. Methods and techniques for cardiac pH_i measurement have continued to evolve since early 1960s. Fluorescent microscopy is the most recently developed technique with several advantages over other techniques including higher spatial and temporal resolutions, and feasibility for contracting cell measurement. Here, we describe detailed methods for mouse cardiomyocyte isolation, and simultaneous measurement and quantification of pH_i and sarcomere length in contracting cardiomyocytes.

For complete details on the use and execution of this protocol, please refer to Lyu et al. (2022).

BEFORE YOU BEGIN

The protocol describes the specific steps for simultaneous pH_i and sarcomere length recordings in contracting mouse ventricular and atrial myocytes. However, the pH_i and sarcomere length recording protocol can also be applied to cardiomyocytes from other species.

Methods for cardiac pH_i measurement have continued to evolve since early 1960s ranging from the use of open-tipped glass micropipette electrodes, 5,5 dimethyl-2,4-oxazolidinedione (DMO), isotopes, pH-sensitive microelectrodes, nuclear magnetic resonance, and fluorescent dyes (Bers and Ellis, 1982; Blank et al., 1992; Ellis and Thomas, 1976a; b; Hunjan et al., 1998; Kirschenlohr et al., 1988; Lavallee, 1964; Schroeder et al., 2010; Valkovic et al., 2019; Waddell and Bates, 1969). Fluorescent microscopy is the most recently developed technique with several advantages over other techniques including higher spatial and temporal resolutions, and feasibility for contracting cell measurement.

We designed and developed a method to measure the pH_i and sarcomere contractions in contracting cardiomyocytes based on the IonOptix contractility system. A xenon lamp was used as an excitation light source. For SNARF-1 pH dye, a selected wavelength of 550 $\pm$ 10 nm excitation filter was installed in the filter wheel of the Zeiss Observer A1 inverted microscope. The emitted fluorescent light was split by a 605 nm long-pass dichroic mirror with the shorter wavelengths through a band-pass filter of 585 $\pm$ 10 nm, and the longer wavelengths through a band-pass filter of 630 $\pm$ 15 nm to two photomultipliers, respectively. For pHrodo Green dye, a selected wavelength of 500 $\pm$ 10 nm excitation filter was installed in the filter wheel. The emitted fluorescent light was

filtered using a band-pass filter of 535 ± 15 nm and collected by a photomultiplier. The contraction was measured using a high-speed camera (MyoCam-S, 240–1,000 frames/s) to record the sarcomere movement.

KEY RESOURCES TABLE

REAGENT OR RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Carboxy SNARF®-1 AM	Thermo Fisher Scientific	Cat# C1272
pHrodo™ Green AM	Thermo Fisher Scientific	Cat# P35373
HEPES	Sigma-Aldrich, USA	Cat# H4034
MES	Sigma-Aldrich, USA	Cat# M3671
2,3-Butanedione Monoxime (BDM)	Sigma-Aldrich, USA	Cat# B0753
(±)-Blebbistatin	Abcam, USA	Cat# Ab120425
(-)-Isoproterenol hydrochloride (ISO)	Sigma-Aldrich	Cat# I6504
5-(N-Ethyl-N-isopropyl) amiloride (EIPA)	Sigma-Aldrich	Cat# A3085
Rotenone	Sigma-Aldrich	Cat# R8875
Antimycin A	Sigma-Aldrich	Cat# A8674
2-[2-[4-(trifluoromethoxy) phenyl] hydrazinylidene]-propanedinitrile (FCCP)	Sigma-Aldrich	Cat# C2920
Verapamil hydrochloride	Sigma-Aldrich	Cat# V4629
Nigericin Sodium Salt	Sigma-Aldrich	Cat# N7143
Collagenase type II	Worthington, USA	Cat# LS004176
Ketamine hydrochloride injection (100 mg/mL)	Vedco, Inc., USA	NDC 50989-161-06
Xylazine Sterile Solution (20 mg/mL)	Akorn Inc., USA	NDC59399-110-20
Heparin (1000 USP units/mL)	Fresenius Kabi USA, LLC	NDC63323-540-57
Bovine serum albumin (BSA)	Sigma-Aldrich	Cat# A9418
Fetal bovine serum (FBS)	Thermo Fisher Scientific	Cat# 10437028
Poly-L-Lysine (0.1%) solution	Sigma-Aldrich	Cat# P-8920
Tetramethylrhodamine, methyl ester (TMRM)	Thermo Fisher Scientific	Cat# T668
Experimental models: Organisms/Strains		
C57Bl/6J mice (wild type, male and female, 10–16 weeks old)	The Jackson Laboratory	https://www.jax.org/
Software and algorithms		
GraphPad Prism	GraphPad Software	https://www.graphpad.com
Origin Pro 2021	OriginLab Corp., Northampton, MA	https://www.originlab.com
BioRender	BioRender	https://biorender.com
IonWizard (64-bit) (7.2.7.138)	IonOptix	https://www.ionoptix.com
Other		
Inverted Microscope	ZEISS, Jena, Germany	Observer A1
Calcium and Contractility System	IonOptix LLC. Westwood, MA, USA	https://www.ionoptix.com/products/systems/calcium-and-contractility-system
Myopacer Cell Stimulator	IonOptix LLC. Westwood, MA, USA	https://www.ionoptix.com/products/components/stimulators/myopacer-cell-stimulator
FHD Microscope Chamber	IonOptix LLC. Westwood, MA, USA	https://www.ionoptix.com/products/components/cell-tissue-chambers/fhd-microscope-chamber-system
25 mm × 25 mm Micro Cover glass, No. 1	VWR International, Radnor, PA, USA	https://us.vwr.com/store/product/4645789/vwr-micro-cover-glasses-square-no-1
Osmometer	Wescor, Inc. USA	Vapro 5600
pH meter	Thermo Scientific, USA	Orion Star A111
Dual-Channel Temperature Controller	Warner Instruments, Holliston, MA, USA	https://www.warneronline.com/dual-channel-temperature-controller-tc-344c
Perfusion pump	World Precision Instruments, USA	PERIPRO-4HS
Pressure monitor	World Precision Instruments, USA	BP-1
Water bath temperature controller	Fisher Scientific, USA	Model 9105
Confocal microscope	ZEISS	LSM700

(*Continued on next page*)

Continued

REAGENT OR RESOURCE	SOURCE	IDENTIFIER
ELGA Veolia water purification system	Veolia Water Systems LTD, USA	Purelab Chorus PC1LSCXM2
0.22 μm filter	EMD Millipore Corporation, USA	Cat# SCGP00525
Langendorff apparatus	Harvard Apparatus	EasyCell System for Cell Isolation, 73-4430
Nylon mesh cell strainer	Corning	Cat# 431752

MATERIALS AND EQUIPMENT

Timing: 2–4 h

Solution preparations:

Note: Prepare all solutions using the ELGA Veolia water purification system (Purelab Chorus PC1LSCXM2) with a resistivity of 18.2 MΩ.cm. Other water purification systems with a resistivity of 18.2 MΩ.cm could also be used.

Note: Prepare at a temperature between 21°C and 22°C.

Note: Filtration with a 0.22 μm filter.

Note: Store solutions at 4°C, and the maximum time for storage is 7 days.

Note: Before use, warm the solution to a temperature between 21°C and 22°C.

- Blebbistatin stock solution: 100 mM blebbistatin stock solution is made by adding 855 μL of dimethyl sulfoxide (DMSO) to 25 mg of blebbistatin.
- 5-(N-Ethyl-N-isopropyl) amiloride (EIPA) stock solution: 50 mM EIPA stock solution is made by adding 1.668 mL of DMSO to 25 mg of EIPA.
- Isoproterenol (ISO) hydrochloride stock solution: 1 mM ISO stock solution is diluted from 100 mM ISO stock solution by purified water. 100 mM ISO stock solution is made by adding 4.037 mL of purified water to 100 mg ISO.
- Verapamil hydrochloride stock solution: 10 mM verapamil hydrochloride is made by adding 10 mL of 100% ethanol to 50 mg of verapamil hydrochloride.
- Rotenone stock solution: 1 mM rotenone stock solution is made by adding 20 mL 100% ethanol to 7.9 mg of rotenone.
- Antimycin A stock solution: 40 mM antimycin A stock solution is made by adding 1.2 mL 100% ethanol to 25 mg of antimycin A.
- FCCP stock solution: 100 mM FCCP stock solution is made by adding 393.4 μL of DMSO to 10 mg of FCCP.
- Nigericin stock solution: 10 mM nigericin stock solution is made by adding 1.34 mL of 100% ethanol to 10 mg of nigericin.
- $CaCl_2$ stock solution: 1 M $CaCl_2$ stock solution is made by adding 100 mL purified water to 14.70 g $CaCl_2 \cdot 2H_2O$.
- $MgCl_2$ stock solution: 1 M $MgCl_2$ stock solution is made by adding 100 mL purified water to 20.33 g $MgCl_2 \cdot 6H_2O$.
- NaH_2PO_4 stock solution: 1 M NaH_2PO_4 stock solution is made by adding 100 mL purified water to 11.99 g NaH_2PO_4.

Refer to the key resources table "Reagent or Resource" for the list of materials and equipment.

- **Perfusion solution for cardiomyocyte isolation** (osmolarity: 316 ± 2 mOsmol/kg, n=7).

Reagent	Final concentration (mM)	Amount / 1 L
NaCl	113	6.60 g
KCl	4.7	0.35 g
$MgSO_4 \cdot 7H_2O$	1.2	0.30 g
$Na_2HPO_4 \cdot 7H_2O$	0.6	0.16 g
KH_2PO_4	0.6	0.08 g
$NaHCO_3$	12	1.01 g
$KHCO_3$	10	1.00 g
D-Glucose	10	1.80 g
Taurine	30	3.75 g
HEPES	10	2.38 g
pH 7.4 with NaOH		

- Perfusion solution containing collagenase type II.

Add 50 mg collagenase type II to 50 mL perfusion solution to a final concentration of 1 mg/mL. The collagenase activity for digestion solution is ~280 U/mL based on the collagenase activity of ~280 U/mg.

- Stop solution for cardiomyocyte isolation.

Add 2 mL FBS to 18 mL of perfusion solution.

- **Ca^{2+} restoration solution containing 0.2 mM Ca^{2+}** (osmolarity: 312 ± 2 mOsmol/kg, n=7). Supplemented with BSA (1 mg/mL).

Reagent	Final concentration (mM)	Amount / 1 L
NaCl	133.5	7.80 g
KCl	4	0.30 g
$MgSO_4 \cdot 7H_2O$	1.2	0.30 g
$Na_2HPO_4 \cdot 7H_2O$	1.2	0.32 g
$CaCl_2 \cdot 2H_2O$	0.2	0.03 g (or 0. 2 mL of 1 M stock solution)
D-Glucose	10	1.80 g
Taurine	20	2.50 g
HEPES	10	2.38 g
pH 7.4 with NaOH		

- **Ca^{2+} restoration solution containing 0.5 mM Ca^{2+}** (osmolarity: 317 ± 2 mOsmol/kg, n=7). Supplemented with BSA (1 mg/mL).

Reagent	Final concentration (mM)	Amount / 1 L
NaCl	133.5	7.80 g
KCl	4	0.30 g
MgSO4·7H2O	1.2	0.30 g
$Na_2HPO_4 \cdot 7H_2O$	1.2	0.32 g
$CaCl_2 \cdot 2H_2O$	0.5	0.07 g (or 0.5 mL of 1 M stock solution)
D-Glucose	10	1.80 g
Taurine	20	2.50 g
HEPES	10	2.38 g
pH 7.4 with NaOH		

- **Ca^{2+} restoration solution containing 1 mM Ca^{2+}** (osmolarity: 323 ± 1 mOsmol/kg, n=7). Supplemented with BSA (1 mg/mL).

Reagent	Final concentration (mM)	Amount / 1 L
NaCl	133.5	7.80 g
KCl	4	0.30 g
MgSO4·7H2O	1.2	0.30 g
$Na_2HPO_4·7H_2O$	1.2	0.32 g
$CaCl_2•2H_2O$	1	0.15 g (or 1 mL of 1 M stock solution)
D-Glucose	10	1.80 g
Taurine	20	2.50 g
HEPES	10	2.38 g
pH 7.4 with NaOH		

- **HEPES-buffered Tyrode's solution** (osmolarity: 324 ± 2 mOsmol/kg, n=7).

Reagent	Final concentration (mM)	Amount / 1 L
NaCl	120	7.01 g
Na-Glutamate	24	4.06 g
KCl	4	0.30 g
$MgCl_2•6H_2O$	1	0.20 g (or 1 mL of 1 M stock solution)
NaH_2PO_4	0.33	0.04 g (or 0.33 mL of 1 M stock solution)
$CaCl_2•2H_2O$	2	0.29 g (or 2 mL of 1 M stock solution)
D-Glucose	10	1.80 g
HEPES	10	2.38 g
pH 7.4 with NaOH		

- **HEPES-buffered Ca^{2+}-free Tyrode's solution** (osmolarity: 329 ± 4 mOsmol/kg, n=8).

Reagent	Final concentration (mM)	Amount / 1 L
NaCl	120	7.01 g
Na-Glutamate	24	4.06 g
KCl	4	0.30 g
$MgCl_2•6H_2O$	1	0.20 g (or 1 mL of 1 M stock solution)
NaH_2PO_4	0.33	0.04 g (or 0.33 mL of 1 M stock solution)
D-Glucose	10	1.80 g
HEPES	10	2.38 g
pH 7.4 with NaOH		

- **HCO_3^--buffered Tyrode's solution** (osmolarity: 317 ± 3 mOsmol/kg, n=16).

Reagent	Final concentration (mM)	Amount / 1 L
NaCl	120	7.01 g
NaHCO3	24	2.02 g
KCl	4	0.30 g
$MgCl_2•6H_2O$	1	0.20 g (or 1 mL of 1 M stock solution)
NaH_2PO_4	0.33	0.04 g (or 0.33 mL of 1 M stock solution)
$CaCl_2•2H_2O$	2	0.29 g (or 2 mL of 1 M stock solution)
D-Glucose	10	1.80 g
Gassed by 5% CO_2 and 95% O_2 before use.		

- **HCO_3^--buffered Tyrode's solution containing 10 mM BDM** (osmolarity: 316 ± 6 mOsmol/kg, n=7).

Reagent	Final concentration (mM)	Amount / 1 L
NaCl	120	7.01 g
NaHCO3	24	2.02 g
KCl	4	0.30 g
$MgCl_2 \cdot 6H_2O$	1	0.20 g (or 1 mL of 1 M stock solution)
NaH_2PO_4	0.33	0.04 g (or 0.33 mL of 1 M stock solution)
$CaCl_2 \cdot 2H_2O$	2	0.29 g (or 2 mL of 1 M stock solution)
D-Glucose	10	1.80 g
2,3-Butanedione Monoxime	10	1.0 g
Gassed by 5% CO_2 and 95% O_2 before use.		

- **HCO_3^--buffered Tyrode's solution containing 25 μM blebbistatin** (osmolarity: 312 ± 2 mOsmol/kg, n=6).

Reagent	Final concentration (mM)	Amount / 1 L
NaCl	120	7.01 g
NaHCO3	24	2.02 g
KCl	4	0.30 g
$MgCl_2 \cdot 6H_2O$	1	0.20 g (or 1 mL of 1 M stock solution)
NaH_2PO_4	0.33	0.04 g (or 0.33 mL of 1 M stock solution)
$CaCl_2 \cdot 2H_2O$	2	0.29 g (2 mL of 1 M stock solution)
D-Glucose	10	1.80 g
Blebbistatin	0.025	0.25 mL of 100 mM stock solution
Gassed by 5% CO_2 and 95% O_2 before use.		

- **HCO_3^--buffered Tyrode's solution containing 10 mM NH_4Cl** (osmolarity: 329 ± 2 mOsmol/kg, n=10).

Reagent	Final concentration (mM)	Amount / 1 L
NaCl	120	7.01 g
NaHCO3	24	2.02 g
KCl	4	0.30 g
$MgCl_2 \cdot 6H_2O$	1	0.20 g (or 1 mL of 1 M stock solution)
NaH_2PO_4	0.33	0.04 g (or 0.33 mL of 1 M stock solution)
$CaCl_2 \cdot 2H_2O$	2	0.29 g (or 2 mL of 1 M stock solution)
D-Glucose	10	1.80 g
NH_4Cl	10	0.53 g
Gassed by 5% CO_2 and 95% O_2 before use.		

- **HCO_3^--buffered Tyrode's solution containing 20 mM sodium acetate** (osmolarity: 359 ± 5 mOsmol/kg, n=10).

Reagent	Final concentration (mM)	Amount / 1 L
NaCl	120	7.01 g
NaHCO3	24	2.02 g
KCl	4	0.30 g

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Continued		
Reagent	Final concentration (mM)	Amount / 1 L
$MgCl_2 \cdot 6H_2O$	1	0.20 g (or 1 mL of 1 M stock solution)
NaH_2PO_4	0.33	0.04 g (or 0.33 mL of 1 M stock solution)
$CaCl_2 \cdot 2H_2O$	2	0.29 g (or 2 mL of 1 M stock solution)
D-Glucose	10	1.80 g
Sodium Acetate	20	2.70 g
Gassed by 5% CO_2 and 95% O_2 before use.		

- **HCO_3^--buffered Tyrode's solution containing 10 μM EIPA** (osmolarity: 329 ± 1 mOsmol/kg, n=6).

Reagent	Final concentration (mM)	Amount / 1 L
NaCl	120	7.01 g
NaHCO3	24	2.02 g
KCl	4	0.30 g
$MgCl_2 \cdot 6H_2O$	1	0.20 g (or 1 mL of 1 M stock solution)
NaH_2PO_4	0.33	0.04 g (or 0.33 mL of 1 M stock solution)
$CaCl_2 \cdot 2H_2O$	2	0.29 g (or 2 mL of 1 M stock solution)
D-Glucose	10	1.80 g
5-(N-Ethyl-N-isopropyl) amiloride	0.01	0.2 mL of 50 mM stock solution
Gassed by 5% CO_2 and 95% O_2 before use.		

- **HCO_3^--buffered Tyrode's solution containing 0.05 μM ISO** (osmolarity: 313 ± 1 mOsmol/kg, n=6).

Reagent	Final concentration (mM)	Amount / 1 L
NaCl	120	7.01 g
NaHCO3	24	2.02 g
KCl	4	0.30 g
$MgCl_2 \cdot 6H_2O$	1	0.20 g (or 1 mL of 1 M stock solution)
NaH_2PO_4	0.33	0.04 g (or 0.33 mL of 1 M stock solution)
$CaCl_2 \cdot 2H_2O$	2	0.29 g (or 2 mL of 1 M stock solution)
D-Glucose	10	1.80 g
Isoproterenol hydrochloride	0.00005	0.05 mL of 1 mM stock solution
Gassed by 5% CO_2 and 95% O_2 before use.		

- **HCO_3^--buffered Tyrode's solution containing 2.5 μM verapamil** (osmolarity: 325 ± 1 mOsmol/kg, n=6).

Reagent	Final concentration (mM)	Amount / 1 L
NaCl	120	7.01 g
NaHCO3	24	2.02 g
KCl	4	0.30 g
$MgCl_2 \cdot 6H_2O$	1	0.20 g (or 1 mL of 1 M stock solution)
NaH_2PO_4	0.33	0.04 g (or 0.33 mL of 1 M stock solution)
$CaCl_2 \cdot 2H_2O$	2	0.29 g (or 2 mL of 1 M stock solution)
D-Glucose	10	1.80 g
Verapamil hydrochloride	0.0025	0.25 mL of 10 mM stock solution
Gassed by 5% CO_2 and 95% O_2 before use.		

- **HCO_3^--buffered Tyrode's solution containing 2 μM rotenone** (osmolarity: 323 ± 1 mOsmol/kg, n=7).

Reagent	Final concentration (mM)	Amount / 1 L
NaCl	120	7.01 g
NaHCO3	24	2.02 g
KCl	4	0.30 g
$MgCl_2 \cdot 6H_2O$	1	0.20 g (or 1 mL of 1 M stock solution)
NaH_2PO_4	0.33	0.04 g (or 0.33 mL of 1 M stock solution)
$CaCl_2 \cdot 2H_2O$	2	0.29 g (or 2 mL of 1 M stock solution)
D-Glucose	10	1.80 g
Rotenone	0.002	2 mL of 1 mM stock solution
Gassed by 5% CO_2 and 95% O_2 before use.		

- **HCO_3^--buffered Tyrode's solution containing 4 μM antimycin A** (osmolarity: 321 ± 1 mOsmol/kg, n=6).

Reagent	Final concentration (mM)	Amount / 1 L
NaCl	120	7.01 g
NaHCO3	24	2.02 g
KCl	4	0.30 g
$MgCl_2 \cdot 6H_2O$	1	0.20 g (or 1 mL of 1 M stock solution)
NaH_2PO_4	0.33	0.04 g (or 0.33 mL of 1 M stock solution)
$CaCl_2 \cdot 2H_2O$	2	0.29 g (or 2 mL of 1 M stock solution)
D-Glucose	10	1.80 g
Antimycin A	0.004	0.1 mL of 40 mM stock solution
Gassed by 5% CO_2 and 95% O_2 before use.		

- **HCO_3^--buffered Tyrode's solution containing 4 μM FCCP** (osmolarity: 325 ± 1 mOsmol/kg, n=5).

Reagent	Final concentration (mM)	Amount / 1 L
NaCl	120	7.01 g
NaHCO3	24	2.02 g
KCl	4	0.30 g
$MgCl_2 \cdot 6H_2O$	1	0.20 g (or 1 mL of 1 M stock solution)
NaH_2PO_4	0.33	0.04 g (or 0.33 mL of 1 M stock solution)
$CaCl_2 \cdot 2H_2O$	2	0.29 g (or 2 mL of 1 M stock solution)
D-Glucose	10	1.80 g
FCCP	0.004	0.04 mL of 100 mM stock solution
Gassed by 5% CO_2 and 95% O_2 before use.		

- **HCO_3^--buffered Tyrode's solution with 10 mM chloride ions** (osmolarity: 298 ± 1 mOsmol/kg, n=15).

Reagent	Final concentration (mM)	Amount / 1 L
Na-Glutamate	120	20.29 g
NaHCO3	24	2.02 g
KCl	4	0.30 g

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Reagent	Final concentration (mM)	Amount / 1 L
$MgCl_2 \cdot 6H_2O$	1	0.20 g (or 1 mL of 1 M stock solution)
NaH_2PO_4	0.33	0.04 g (or 0.33 mL of 1 M stock solution)
$CaCl_2 \cdot 2H_2O$	2	0.29 g (or 2 mL of 1 M stock solution)
D-Glucose	10	1.80 g
Gassed by 5% CO_2 and 95% O_2 before use.		

- **pH calibration solutions with pH 6.5, 7.5 and 8.5** (osmolarity: 321 ± 1 mOsmol/kg, 316 ± 0.9 mOsmol/kg, and 322 ± 1 mOsmol/kg, n=6).

Reagent	Final concentration (mM)	Amount / 1 L
KCl	140	10.36 g
$MgCl_2 \cdot 6H_2O$	1	0.20 g (or 1 mL of 1 M stock solution)
HEPES	20	4.77 g
Nigericin	0.01	1 mL of 10 mM stock solution
pH 6.5, 7.5 or 8.5 with NaOH		

- **pH calibration solution with pH 5.5** (osmolarity: 314 ± 1.4 mOsmol/kg, n=6).

Reagent	Final concentration (mM)	Amount / 1 L
KCl	140	10.36 g
$MgCl_2 \cdot 6H_2O$	1	0.20 g (or 1 mL of 1 M stock solution)
MES	20	3.90 g
Nigericin	0.01	1 mL of 10 mM stock solution
pH 5.5 with NaOH		

STEP-BY-STEP METHOD DETAILS

Mouse cardiomyocyte isolation and preparation

Timing: 1–2 h

Note: Preheat the water bath (Model 9105, Fisher Scientific, USA) of the Langendorff apparatus to 37°C. Start the Perfusion solution flow to be sure the perfusion system of the Langendorff apparatus is prefilled by Perfusion solution, and avoid any air trapped in the perfusion system.

1. Mouse anesthesia. Inject 80 mg/kg of ketamine and 5 mg/kg of xylazine to adult mice (both male and female, 12–16 weeks) intraperitoneally. Assess level of anesthesia by firm toe pinch, and proceed when the surgical anesthesia plane is reached.
2. Inject 300 USP unit sodium heparin intraperitoneally.
3. Perform a midline thoracotomy and heart excision.
4. Transfer the heart to a petri dish containing Perfusion solution.
5. Trim the connective tissues using a fine scissor.
6. Cannulate the heart through aorta (approximately 5 mm length) onto a Langendorff apparatus prefilled with Perfusion solution, and avoid aortic valve damage during cannulation.
   a. Start the electrical perfusion pump (Peri-Star Pro, World Precision Instruments, USA) with lower flow rate to start the perfusion with the Perfusion solution at 37°C.
   b. Read the pressure value on the pressure monitor (BP-1, World Precision Instruments, USA) which is connected with the Langendorff apparatus. Figure 1A shows the experimental setup.

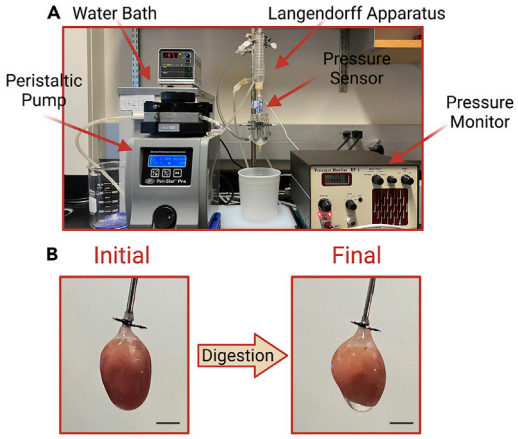

Figure 1. Langendorff perfusion system for cardiomyocyte isolation and the perfused heart

(A) Langendorff apparatus and experimental setup used for heart perfusion and digestion.

(B) The heart images before digestion (Initial) and after digestion (Final). Scale bar: 2 mm.

c. Monitor the perfusion pressure increasing to a steady value. At the same time, monitor the dripping of the Perfusion solution from the apex of the heart. If the pressure keeps increasing and no dripping of the solution from the heart, there must be aortic valve damages suggesting the failure of the retrograde perfusion.

7. Washout the blood by perfusion for 3 min, adjust the perfusion speed at a flow rate about 4–6 mL/min and control the initial pressure at ≈60 mmHg using pressure monitor. Switch the perfusion solution to Perfusion solution containing collagenase type II (1 mg/mL) for digestion of the tissue.
8. Continuous monitoring of the declining perfusion pressure for 15–20 min during tissue digestion by collagenase type II, until the pressure is below 30 mmHg. Check the color of the heart surface, and the softness of heart tissue to determine the digestion level. The digested heart becomes soft and dilated (Methods video S1-Before digestion and Methods video S2-After digestion), and the surface color tone changes to yellowish as shown in Figure 1B.
9. Remove the heart from the perfusion apparatus and transfer to a petri dish containing Stop solution. Dissect and separate ventricles and atria, mince and pipette in the solution to mechanically dissociate the tissue and liberate individual ventricular and atrial myocytes.
10. Harvest single isolated cardiomyocytes.
    a. Filter the solution with cells through nylon mesh cell strainer (100 μm), and collect the cells in 15 mL tubes waiting for the settling of the cells to the bottom of tubes (5–10 min).
    b. Remove the supernatant carefully not disturbing the cell pellets.
    c. Restore concentration of Ca^{2+} stepwise by adding 5 mL Ca^{2+} restoration solution containing 0.2, 0.5, or 1 mM Ca^{2+}.
    d. Resuspend the cell pellet after adding the restoration solution, and incubate the cell at 37°C water bath for 10–15 min.
    e. Repeat step c followed by removing of the supernatant.
11. Store the cell in Ca^{2+} restoration solution containing 1 mM Ca^{2+} at a temperature between 21°C and 22°C for recording.

Note: The cardiomyocytes in the solution can be stored for 4–6 h. Assess the cell viability by the morphological changes and the contractility of the cell in response to electrical stimulations. Figure 2 showed the images of isolated cells. The healthy cells show clear striations with rod shape, sharp-edged instead of blunt-edged, and contract with normal fractional shortening (6%–12% at a temperature between 21°C and 22°C with a pacing rate of 0.5 Hz) in response to electrical stimulations.

Intracellular pH and sarcomere length measurement in cardiomyocytes

Timing: 4–6 h

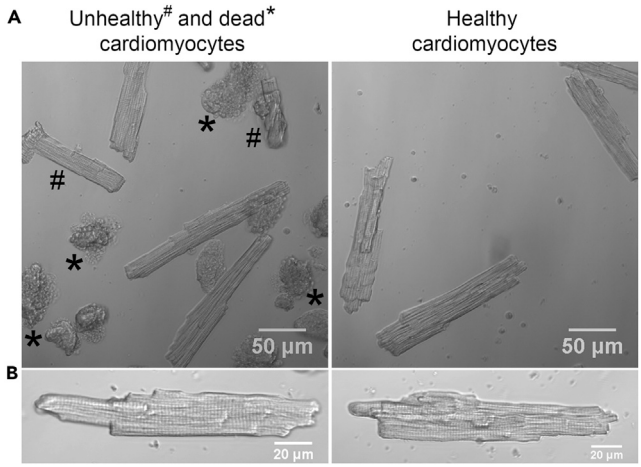

Figure 2. Microscopic images of the isolated ventricular myocytes

(A) Representative images of isolated ventricular myocytes. The unhealthy and dead ventricular myocytes in the left panel were indicated, and the right panel showed the healthy ventricular myocytes.

(B) Representative images of a single ventricular myocyte selected for experiment with rod shape, sharp edges, and clear striations.

12. Recording system setup. A diagram of the recording system is shown in Figure 3.
    a. Install a piece of 25 × 25 mm No. 1 micro cover glass (VWR International, PA, USA) to the FHD Microscope Chamber (IonOptix, MA, USA), and place and clip the chamber on the stage of the inverted microscope. Glass-bottom dishes are not appropriate for the recording because of the lack of the stimulation electrodes, and the large solution volume in the dish which reduces solution exchange speed.
    b. Connect the temperature-controlled gravity-driven perfusion system and the Myopacer Cell Stimulator (IonOptix, MA, USA) to the recording chamber. Set and test the solution flow rate at 4–5 mL/min. Test and monitor the temperature of the recording solution in the chamber (36°C–37°C for physiological temperature or 21°C–22°C for room temperature) during the perfusion by pre-setting the temperature for the in-line solution heater.
    c. Install the emission filter cube for pHrodo Green or SNARF-1 imaging on the Cell Framing Adapter, and choose the excitation filters by rotating and positioning the filter wheel on the microscope.
    d. Turn on the power of the microscope, the IonOptix system, Myopacer Cell Stimulator and the computer. Run IonWizard software (IonOptix, MA, USA).
13. Dye loading into cardiomyocytes.
    a. **Method A**: Incubate cardiomyocytes in a 1.5 or 2 mL Eppendorf tube containing HEPES-buffered Ca^{2+}-free Tyrode's solution with addition of either 5 μM SNARF-1 AM or 5 μM pHrodo Green for 5 min at a temperature between 21°C and 22°C. After removing the loading solution, cardiomyocytes are washed twice using HEPES-buffered Ca^{2+}-free Tyrode's solution and resuspended the cells.
    b. **Method B**: Seed cardiomyocytes in the recording chamber with an appropriate density to identify single cells, and wait for 5 min for the cells to settle and attach to the cover glass. The cell yield from each isolation may vary depending on the isolation. Add the cells to recording chamber by an incremental step for achieving the appropriate density as shown in Figure 2A right panel. Wash-in Ca^{2+}-free Tyrode's solution containing either 5 μM SNARF-1 AM or 5 μM pHrodo Green, and incubate for 2–3 min, and wash-out the remaining solution using HCO_3^--buffered Tyrode's solution gassed by 5% CO_2 and 95% O_2. **Method A** and **Method B** both work well.

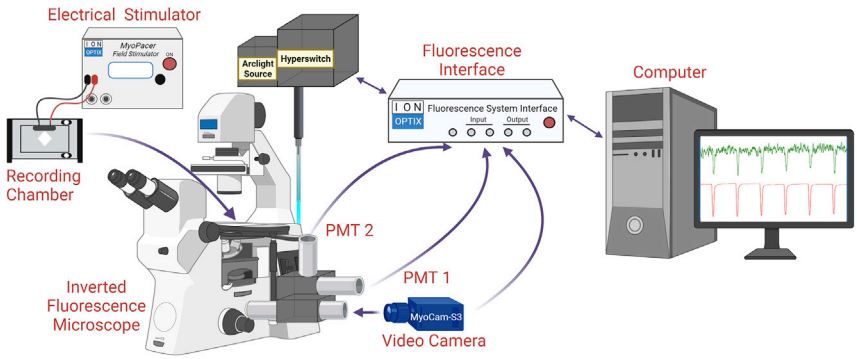

Figure 3. Diagram of the IonOptix recording system configured for measuring the sarcomere contraction and intracellular pH

Created with Biorender.com.

Note: Timings of dye loading by **Method A** need to be well controlled due to removing and wash-out of the dye solution may take some extra time, but this method needs less volume of dye loading solution which is cost-effective. Dye loading by **Method B** takes less time, but needs large amount of dye solution which is costly, and the perfusion tube of the dye solution needs to be washed very well to keep the tube clean for next usage.

⚠ CRITICAL: The loading time should be carefully controlled to ensure the dye is loaded in the cytosol without overloading into the intracellular organelles. The loading time should be brief, usually, 5 min are enough. Reduction of the dye concentrations could also help to limit dye loading into the intracellular organelles. Figure 4 shows the time-dependent dye loading process. Because mitochondria occupy at least 30% of cell volume, we compare the dye loading to the cytosol and mitochondria. 5 min loading of pHrodo Green shows the dye signal mostly in cytosol, while 30 min loading of pHrodo Green shows both cytosol and mitochondrial dye distribution. To quantitatively estimate the cytosol dye loading, use saponin solution (50 μg/mL) to perfuse the cell for monitoring the dye intensity changes and evaluating the percentage of the dye loaded in the cytosol. Cardiomyocytes' attachment to the cover glass is difficult to achieve depending on the quality control of the cardiomyocyte isolation and post-isolation incubation time as we showed in Figure 2. If cardiomyocytes do not adhere well to the cover glass surface due to the cover glass surface quality alterations from different brands, the cover glass will be pre-treated with 0.1% (w/v) poly-L-Lysine solution for 30 min. Poly-D-Lysine could also be used for cover glass coating, and the difference is that poly-L-Lysine is the biologically active form of lysine, and can be degraded by protease in cell culture. Other reagents such as laminin and fibronectin could also be used for coating the cover glass.

14. If the cells are loaded by **Method A**, seed the dye-loaded cardiomyocytes on the cover glass of the recording chamber, and be sure the cell density is appropriate to identify individual cells as shown in Figure 2A right panel. If the cells are loaded by **Method B**, skip the cell seeding procedure. Perfuse the cells with a gravity-driven solution perfusion system. The perfusion speed is kept at 4–5 mL/min.

⚠ CRITICAL: To control the temperature and solution exchange, the perfusion tube diameters and the flow speed need to be well controlled and pre-tested.

15. Cell selection. Illuminate the cells using bright field light, and check the cell quality under the microscope. Select 63× oil or 40× oil lens for imaging. Apply a continuous square bipolar pulse stimulation with a 2 ms pulse-width and 10 V amplitude at a frequency of 0.5 Hz using Myopacer

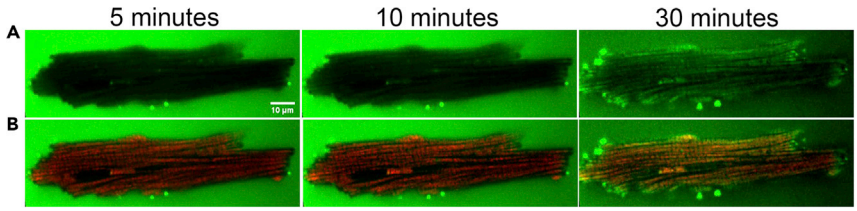

Figure 4. Time-dependent pHrodo Green loading into cardiomyocytes

For labeling of mitochondria, incubate cardiomyocytes in Tetramethylrhodamine, methyl ester (TMRM) solution (5 nM) for 30 min at 37°C, and the TMRM solution is made by HEPES-buffered Ca^{2+}-free Tyrode's solution. TMRM (red color) accumulates in active mitochondria with intact membrane potentials. After washout of TMRM loading solution by HEPES-buffered Ca^{2+}-free Tyrode's solution, incubate the cells in pHrodo Green (green color) loading solution for 5, 10 and 30 min. The images were taken by using Zeiss LSM 700 confocal microscope at the time point as indicated on the top of the images.

(A) pHrodo Green images from a TMRM- and pHrodo Green-loaded cardiomyocyte. The strong background green color results from the pHrodo Green dye in the loading solution. For testing the time-dependent dye loading on the same cardiomyocyte, we did not washout the dye solution during the imaging, which generated strong background green dye signals.

(B) Merged images of TMRM and pHrodo Green from the cardiomyocyte loaded with both dyes.

At 5 min, pHrodo Green dye is loaded into cytosol (A) indicated by the relatively weaker green signal (comparing to strong background signal from pHrodo dye loading solution and the green signal from 30 min loading), but the integrated weak pHrodo Green signal from the whole cell can be easily detected and recorded using PMT of IonOptix system in the recording solutions without any dyes. The merged image (B) at 5 min shows strong red TMRM signal further demonstrating the pHrodo Green dye is loaded into cytosol instead of mitochondria. With time going, more pHrodo Green dye is loaded into the cytosol and mitochondria. The yellow color regions in merged image at 30 min indicate the significant loading of pHrodo Green into the mitochondria.

Cell Stimulator, check the beating status of cardiomyocytes and identify beating and healthy cardiomyocytes for pH recording. Stop the stimulation and pacing.

16. Cell positioning. Select a single cardiomyocyte, move to the center of the illumination field and rotate the handle on the Cell Framing Adapter to align the cell in a horizontal position. Move the cell to the imaging frame. Adjust the four edge positions of the frame to include the whole relaxed cell completely in the frame for imaging. Switch the bright field illumination filter to the red filter position, and switch the light path to the camera, via the side port of the microscope.
17. Recording using the IonWizard software by starting a new experiment. Choose the experimental setting for pH measurement which uses the hardware configured in the task manager of the software including the excitation wavelength and the calibration parameters for the lens. Finely adjust the focus on the microscope to see clearly the striation structure of the cardiomyocyte on the screen as shown in Figure 2B.
18. pH calibration. Perfuse the cardiomyocyte by pH calibration solutions with pH 5.5, 6.5, 7.5 or 8.5 sequentially. For each step, recording the fluorescence signal of pHrodo green or SNARF-1 for 3–5 min till the signal reaches steady state. Repeat the calibration by reversing the solution sequence, i.e., perfuse the cardiomyocyte by pH calibration solutions with pH 8.5, 7.5, 6.5 or 5.5 sequentially. Repeat the calibration process by randomly choosing the sequence of calibration solutions. At the final step, move the cell out of the imaging frame and record the background fluorescent signal. Combined and average all the data from different cells.
19. Test the photobleaching and dye leakage. Record fluorescent signals of pHrodo Green or SNARF-1 continuously from single cardiomyocyte without any interventions for ~10 min with pacing and without pacing. At the final step, move the cell out of the imaging frame and record the background fluorescent signal.
20. Test the dye loading efficiency and specificity. Record fluorescent signals of pHrodo Green or SNARF-1 continuously from single cardiomyocyte for 2–3 min with continuous perfusion of HCO_3^--buffered Tyrode's solution, and switch the perfusion solution to HCO_3^--buffered Tyrode's solution containing 50 μg/mL saponin. Monitor the fluorescent signal changes after saponin treatment.

21. Measurement of the intrinsic fluorescent signal of cardiomyocytes. Seed the non-loaded dye-free cardiomyocytes on the cover glass and choose single live cardiomyocyte for 3 min recording using the optics for pHrodo Green and SNARF-1. Repeat the measurement across cardiomyocytes with different sizes and shapes. At the final step, move the cell out of the imaging frame and record the background fluorescent signal. For dye-loaded cells, repeat this step to obtain the dye-induced fluorescent signal. The measurement will be repeated on 30–50 cells for calculation of the averaged values.
22. Dynamic pH_i recording.
    a. Choose single healthy dye-loaded cardiomyocyte for pH_i imaging and measurement. Repeat steps 15–17. Finely adjust the focus on the microscope to see clear striation structures of the cardiomyocyte on the screen.
    b. Adjust the size of the sarcomere selection window to cover at least 10 sarcomeres and move the window to the center of the cell. Set the solution temperature control, start pacing the cell, and record the fluorescent signals of pHrodo Green or SNARF-1 and the sarcomere length simultaneously. For each pHrodo Green loaded cardiomyocyte, complete pH_i recordings by perfusion using pH 5.5 calibration solution to acquire the peak fluorescence intensity value for normalization and proper calibration.
    c. Change the pacing rates if necessary using Myopacer Cell Stimulator.
    d. For each recording, at the final step, move the cell out of the imaging frame and record the background fluorescent signal.
23. Pharmacological interventions. To test the effects of drugs and chemicals on the pH_i, we use bath solutions containing different drugs or chemicals to perfuse the cardiomyocyte for 3–5 min, record the pH_i till steady state is achieved and switch to control solution. For example, we test the effects of blebbistatin by switching the perfusion solution from HCO_3^--buffered Tyrode's solution to HCO_3^--buffered Tyrode's solution containing 25 μM blebbistatin, and we test the FCCP effects by switching the perfusion solution from HCO_3^--buffered Tyrode's solution to HCO_3^--buffered Tyrode's solution containing 4 μM FCCP. After the recordings achieve steady state, we switch the perfusion solution back to HCO_3^--buffered Tyrode's solution to observe the washout effects. After the drug test, remove the cells which have been exposed to the drug in the recording chamber and add fresh cells for loading and start another new test.

Analyses of data with proper calibrations and corrections

Timing: 4–6 weeks

24. Use IonWizard, Origin Pro 2021 (OriginLab, Northampton MA, USA) and GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA) to analyze the pH_i data.
25. Estimate and eliminate the intrinsic fluorescent signal of cardiomyocytes.
    a. Obtain the intrinsic fluorescent signal from each cell by subtracting the background fluorescent signal from the recorded raw intrinsic fluorescence signal. Average the intrinsic fluorescent signals from 30-50 different cells as an index of the intrinsic fluorescence intensity.
    b. Obtain the dye-induced fluorescent signal from 30-50 dye-loaded cells by subtracting the background fluorescent signal from the raw fluorescent signal. Average the fluorescent signals from dye-loaded cells as an index of the dye-induced fluorescence intensity.
    c. Calculate the ratios between the intrinsic fluorescence intensity and the dye-induced fluorescence intensity. Use the ratios to calculate the component of the intrinsic fluorescent signal in the total fluorescent signal recorded in dye-loaded cells and subtract this component from the total fluorescent signal in dye-loaded cells in all other experiments.
26. Corrections of the photobleaching and dye leakage.
    a. Subtract the background fluorescent signal and eliminate the intrinsic fluorescent signal.
    b. Fit the photobleaching and dye leakage process of the fluorescent signal using a linear equation.

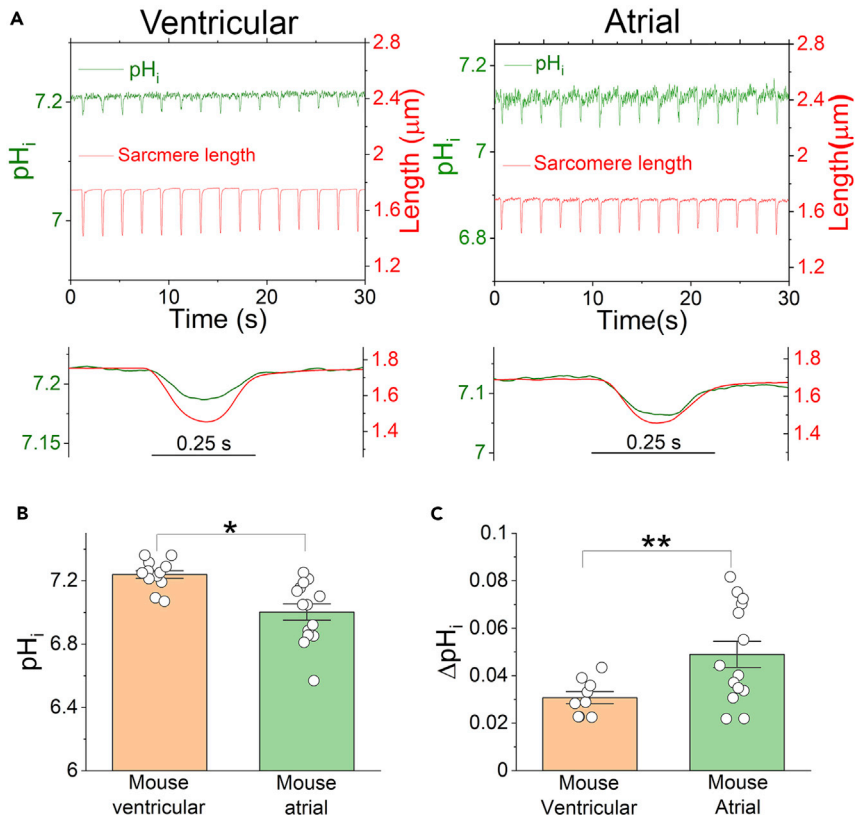

Figure 5. Beat-to-beat dynamic pH_i in mouse ventricular and atrial myocytes

(A) Representative traces of pH_i in mouse ventricular (left) and atrial (right) myocytes in parallel with the sarcomere length measurement at a temperature between 21°C and 22°C. Cardiomyocytes were paced at 0.5 Hz with pHrodo Green loading.

(B and C) Comparisons of baseline pH_i (B) and amplitude of pH_i transients (ΔpH_i) (C) between mouse atrial and ventricular myocytes. *p=0.00043, n=13 for ventricular myocytes and n=14 for atrial myocytes; **p=0.020, n=9 for ventricular myocytes and n=14 for atrial myocytes (two-sample *t*-test). Data are represented as mean ± SEM (Lyu et al., 2022).

c. Using the slope resulting from the linear fitting to correct all the pH measurements to minimize the effects of photobleaching and dye leakage by subtracting the slope factor.

Note: For cardiomyocytes with pacing and without pacing, perform the analysis independently. Compare the slope factors to rule out the effects of electrical and mechanical activities of cardiomyocytes on the photobleaching and dye leakage process. Temperature is another important factor in photobleaching and dye leakage, therefore, correct the experimental data at 21°C–22°C and 36°C using the slope factors from 21°C–22°C and 36°C, respectively.

27. pH calibrations.
    a. Subtract the background fluorescent signal, eliminate the intrinsic fluorescent signal, and correct the photobleaching and dye leakage.
    b. Convert the SNARF-1 emission ratio (F_{580}/F_{640}) to a pH_i value using the standard calibration method (Blank et al., 1992; Buckler and Vaughan-Jones, 1990; Niederer et al., 2008; Sirish et al., 2017) to obtain the calibration curve.
    c. For pHrodo Green, normalize the fluorescence intensity signals by the peak intensity at pH 5.5, and fit the normalized intensities using a sigmoidal function to obtain the standard calibration curve for pHrodo Green.

28. Convert the fluorescent signals to pH values.
    a. Subtract the background fluorescent signal, eliminate the intrinsic fluorescent signal, and correct the photobleaching and dye leakage.
    b. Perform the curve fitting to obtain the kinetic parameters using the "Operations/Monotonic Transient Analysis…" menu in IonWizard software.
    c. Average 10 cycles of the signals and convert the fluorescent signals to pH using the calibration curves obtained in step 27. Figure 5 shows the representative pH_i traces and summary data from mouse ventricular and atrial myocytes.

EXPECTED OUTCOMES

We record the pH_i and sarcomere length on contracting cardiomyocytes, and find the beat-to-beat intracellular acidifications in synchrony with cardiomyocyte contractions, termed "pH_i transients", revealing a beat-to-beat dynamic pH_i regulation system in cardiomyocytes (Lyu et al., 2022). We further test the underpinning mechanisms of pH_i transients by using myofilament ATPase inhibitors, changing the pH_i buffering capacity, altering sarcolemma Cl^-/HCO_3^- transporter activities, changing pacing rates, activating and inhibiting β-adrenergic signaling, and inhibiting mitochondrial electron transport chain activities. The strategy could be expanded to many other applications by controlling the solution perfusion. Using this protocol, we have identified and demonstrated a dynamic pH_i regulatory system in cardiomyocytes in addition to the well-studied dynamic electrical (action potentials), Ca^{2+} (Ca^{2+} transients), and mechanical regulatory systems (contractions). The results show that a cardiac cycle is sculpted not only by action potentials, Ca^{2+} transients, and contractions but also by cyclical changes in pH_i. Our findings may reveal broader features of pH_i handling in excitable cells and open the gate of discovery into cellular proton regulation.

QUANTIFICATION AND STATISTICAL ANALYSIS

We estimate a sample size of 5 per experiment to detect at least 15% difference before and after the change of the conditions with alpha=0.05 for a two-tailed test to give the power of the study >0.95, assuming the standard deviation of the differences to be 5% (SigmaStat, Systat Software Inc.). No data were excluded. Data are presented as mean ± S.E.M. Shapiro -Wilk test was used for normality test. Statistical comparisons were achieved by one-way ANOVA combined with Tukey's post hoc analyses among three or more groups, or paired sample *t*-test (two-tailed) or two-sample *t*-test when the data follow a normal distribution if comparing the effects before and after the application of drugs on the same cell. If the data did not follow a normal distribution or with a small sample number, a non-parametric paired Wilcoxon signed-rank test was used. Statistical significance was set at $p<0.05$. The statistical analyses were performed using Origin Pro 2021 (OriginLab, Northampton MA, USA) and GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA).

LIMITATIONS

We optimized the experimental condition to load the dye to the cytosol. Due to the dyes' intrinsic properties, we could not rule out the effects of the dye loading into intracellular organelles which may affect the results. To this end, saponin treatment was performed to quantify the dye loading, demonstrating specific cytosol loading in our experiments. Secondly, the fluorescent dye may be subjected to photobleaching and leakage, which may cause the baseline pH drift and affect the quantification of the experimental results. To circumvent this effect, we have conducted control experiments to record the baseline signal changes resulting from the photobleaching and dye leakage, which have been used for the correction of the pH measurement. Alternatively, in our future study, we will use pH biosensors for cytosolic or organelle targeting to minimize the dye loading limitations for a precise spatial control and pH measurement either using *in vivo* or *in vitro* transgene delivery techniques.

TROUBLESHOOTING

Problem 1

Cardiomyocyte quality control is critical to the pH_i and contraction measurement. The isolation procedures of mouse cardiomyocytes need to be optimized during the experiments (Alam et al., 2020; Jian et al., 2016; Li et al., 2020) (steps 1–11).

Potential solution

Heart excision, trimming and cannulation need to be rapid and precise. Heart excision should be performed appropriately to ensure the remaining ascending aorta section is long enough (approximately 5 mm) for cannulation. The aorta should be securely cannulated to ensure there is no leak for the maintenance of proper retrograde perfusion. The concentrations of collagenase type II may need to be adjusted and optimized with each new batch of collagenase. The Ca^{2+} restoration step is also critical to the quality control of the cardiomyocyte, which needs to be performed carefully and consistently.

Problem 2

Cardiomyocytes may not seed well on the cover glass of the chamber depending on the cell quality, glass type, and surface quality. The perfusion may wash away the cells during recordings (steps 14–23).

Potential solution

Choose cover glass from different manufacturers (for example Corning, VWR) may help. Another solution is to coat the cover glass with 0.1% (w/v) Poly-L-Lysine for 30 min before seeding the cells.

Problem 3

Cell selection and framing. Choosing the high-quality cardiomyocytes with the normal contraction and positioning the cells entirely in the imaging frame are essential for the pH_i and contraction measurement. Inappropriate positioning may introduce motion artifacts into the measurement (steps 15–16).

Potential solution

Pre-screening the cardiomyocytes using the pacing protocol is required to determine high-quality cells. The quality of the cells could be determined by their morphology, striation pattern, and contractility. Choose the rod shaped cells with sharp intercalated discs and clear striations as shown in Figure 2B, and the cells exhibiting regular contractions only with pacing without alternans or arrhythmic activities. In addition, high-quality cardiomyocytes usually attach well to the cover glass. Cardiomyocytes need to be entirely positioned in the imaging frame. Choosing the appropriate size of the cells is required for a 63× oil lens or switching to a 40× oil lens to accommodate the large cells. Adjust the focus properly to see the clear striation pattern of the cell before recordings.

Problem 4

Dye loading may not be specific to cytosol due to the intrinsic properties of the dye (step 13).

Potential solution

The loading time should be carefully controlled to ensure that the dye was loaded only in the cytosol without overloading into the intracellular organelles. The loading time and temperature need to be controlled and optimized for each batch of the cells. The loading time should be brief, usually, 5 min is enough. Using lower concentrations of the dye could also help limiting off-target labeling. To quantitatively estimate the cytosol dye loading, wash-in 50 μg/mL saponin solution is necessary to monitor the dye intensity changes and evaluate the percentage of the dye loaded in the cytosol.

Problem 5

Photobleaching and leakage of the dyes have significant effects on the quantification of pH_i when the recordings last more than 3 min (step 19).

Potential solution

Record fluorescent signals continuously from dye-loaded single cardiomyocyte without any interventions for ~10 min with pacing and without pacing. The photo bleaching and leakage of the dyes will be monitored and quantified by fitting the time course. Using the parameters extracted from the curve fitting to correct the time course of the pH_i recordings with a duration longer than 3 min.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Xiao-Dong Zhang (xdzhang@ucdavis.edu).

Materials availability

This study did not generate new unique reagents or animal models.

Data and code availability

The data supporting the current study have not been deposited in a public repository because they are only used for the publication of this manuscript but are available from the corresponding author on request.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2022.101301.

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AUTHOR CONTRIBUTIONS

X.D.Z. proposed and developed the concept, designed the study, established the method, initially tested the concept by experiments, analyzed the data, made figures, and wrote the manuscript. N.C. helped on experiment design, data analysis and interpretation, and revised the manuscript; Y.L. performed the intracellular pH and contraction measurement, data analysis and organization, and revised the manuscript; V.T. isolated the mouse cardiomyocytes, and contributed to the cardiomyocyte isolation protocol writing; J.O. helped on cardiomyocyte isolations; P.N.T. helped on cardiomyocyte isolations; E.N.Y. revised the manuscript and helped on the data acquisition and analysis.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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