The Optimized Quantum Dots Mediated Thermometry Reveals the Efficiency of Myosin Extracted from Muscle Mini Bundles

Meishan Li (meishan.li@ki.se)
Karolinska Institutet https://orcid.org/0000-0002-1351-9325
Lucia Coppo
Karolinska Institutet
Bhanu P. Jena
Wayne State University School of Medicine
Lars Larsson
Karolinska Institutet

Methodology

Keywords: Thermometry, Quantum dots, Myosin, Efficiency, ATPase

Posted Date: September 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-903082/v1

Abstract

Background: The quantum dots (QD) has been investigated as thermometrical sensor in biological microenvironment and applied to measure the muscle efficiency with underlying mechanisms, i.e., a reduction in fluorescent intensity of QD reflects an increase in temperature caused by heat release during ATP hydrolysis, denoting the efficiency of the motor protein myosin. The aim of this study is to optimize the QD mediated thermometry for measuring the efficiency of freshly extracted myosin from muscle mini bundles rather than pre-purified myosin and test this approach in preparations with different myosin isoform.

Methods: The protocol of myosin extraction used in the single muscle fiber *in vitro* motility assay was modified slightly for extracting myosin from the muscle mini bundles. Moreover, the quantitation of extracted myosin was calculated from the total extracted protein since the ratio of myosin to total protein was constant, performing through spectrophotometric measurement of UV absorbance at 280 nm. The change in fluorescence intensity of QD thermometry measurement of myosin ATPase enzymatic reaction was plotted over time, and the slope of the linear negative regression between time course and relatively decreased fluorescence intensity was used to reflect the efficiency of extracted myosin.

Results: The optimized QD mediated thermometry is established for evaluating the efficiency of myosin extracted from muscle mini bundles. Moreover, myosin isoform specific differences in the myosin efficiency were observed in comparison between slow myosin and fast myosin, i.e., the low myosin has lower efficiency than fast myosin, evidenced by a higher heat release.

Conclusions: The optimized QD mediated thermometric measure of myosin efficiency in muscle mini bundles provides a nanoscale approach to evaluate myosin function based on a minimal amount of muscle, which is essentially required in the muscle research.

Background

The motor protein myosin is a mechanoenzyme converting chemical energy from ATP hydrolysis to mechanical work during muscle contraction and is expressed in different isoforms determining the contractile properties of the muscle. To date, nine distinct myosin heavy chains (MyHC) isoforms have been identified in mammalian striated muscle, and four of them (type I, IIa, IIx and IIb MyHCs) are expressed in normal adult limb and trunk mammalian skeletal muscle [1]. There is a close relationship between the maximum velocity of unloading shortening (Vo), the actin-activated ATPase activity of myosin, and the myosin isoform expression of single muscle fibres, which means different isoforms of myosin confer distinct contractile properties to muscle fibres [2–9]. Correspondingly, the changes in isoform expression, specific mutations or post-translational modifications of myosin will influence muscle function [10–14]. So far, several miniature approaches, such as single fibre or myofibril contractile measurement or single fibre *in vitro* motility assays, have been applied to evaluate muscle function at cellular, subcellular or molecular levels [9, 15, 16]. However, the biochemical properties of

myosin from these preparations remain incomplete and the introduction of cadmium telluride quantum dots (CdTe QD) mediated thermometry to measure myosin efficiency at the protein level has complemented the understanding of the myosin function *in vitro* [12, 17]. The underlying mechanism of the approach is that the heat loss during ATP hydrolysis by myosin ATPase enzymatic reaction will increase the temperature in the microenvironment, and QD fluorescence intensity will accordingly decrease with thermal resolution of ~ 1 mK. In addition, small amounts of muscle tissue are routinely collected in investigation of pathophysiological mechanisms or diagnosis of neuromuscular disorders and there is a compelling need to use a minimum muscle tissue. In this study, we have verified and quantified the extracted myosin content from muscle mini bundles, optimized the procedures of QD mediated thermometry for measuring the myosin efficiency and observed isoform specific differences in myosin efficiency.

Methods

Animals, muscle tissues and bundle preparations

Fast- and slow- twitch skeletal muscles were collected from the hindlimbs of young Sprague Dawley rats, i.e., the fast-twitch extensor digitorum longus (EDL) muscle and the slow-twitch soleus (SOL) muscle. The animal experiments were carried out according to the guidelines of the Swedish Board of Agriculture and approved by the ethical committees at Karolinska Institutet. The EDL and SOL muscle tissues were dissected into bundles of approximately 50 fibers (-5 mm long) in relaxing solution containing 50% (vol/vol) glycerol at 4 °C and tied to glass capillaries, stretched to about 110% of their resting slack length. Afterwards the bundles were chemically skinned by treatment for 24 hours at 4 °C in a relaxing solution, and then stored at -20 °C. Within one week after above skinning treatment, the bundles were cryo-protected by transferring them to relaxing solutions containing increasing concentrations of sucrose (0, 0.5, 1.0, 1.5, and 2.0 M) at 30-minute intervals, and then frozen in liquid propane chilled by liquid nitrogen. The frozen bundles were stored at -140 °C. Before the experiment, the bundle was incubated in sucrose solutions with decreasing concentrations (2.0, 1.5, 1.0 and 0.5M) sequentially at 30-minute intervals and then kept in the skinning solution at -20 °C for 2 weeks or shorter prior to usage. [9, 18] The methods are presented in a schematic diagram (Figure 1)

The extraction of myosin and the spectrophotometric quantitation

A muscle mini bundle consisting of 10-20 fibers (that may be adjusted according to the measured concentration of extracted protein) was separated gently and incubated in the microtube with 20 µl a high-salt buffer (0.5 m KCl, 25 mm Hepes, 4 mm MgCl2, 4 mm EGTA, pH adjusted to 7.6 before adding 2 mm ATP and 1% β-mercaptoethanol) at 4 °C for 30 minutes. The solution containing extracted myosin and other minor proteins was kept on ice for the application to the different sections of the experiment, i.e., the spectrophotometric quantification of the concentration, QD-mediated thermometry and 12% SDS-

PAGE to determine the relative content of myosin in the extracted proteins. The concentration of extracted protein was quantified by conventional spectrophotometry using absorbance at 280 nm (Nanodrop, Thermo Scientific), which does not require any standard curve, but the blank control (contains high-salt buffer only) and internal control (different concentration of BSA, such as 1, 2, 3 and 4 mg/ml) were applied for quality control. Every sample and control for A280 measurement have been briefly and sufficiently vortexed and the quantification was repeated at least 3 times for consistency. The extracted myosin was proportional to the extracted total protein (see the section of results). The A280 value of the extracted total protein was therefore used to represent the concentration of the extracted myosin in most situations unless specified otherwise.

The optimized QD-mediated thermometry of myosin ATPase enzymatic reaction

The assay for determining the saturated ATP concentration was performed firstly. Commercial control myosin protein (1 mg, Cytoskeleton, Inc., Denver, CO) was dissolved in 100 μL of myosin resuspension buffer (15 mM Tris HCl of pH 7.5, 0.2 M KCl and 1 mM MgCl2) then diluted to the concentration of 0.2 μM. For each measurement, 1 μL of the control myosin was pipetted to a well containing 30 μL low-salt buffers (25mM KCl, 25 mm Hepes, 4 mm MgCl2, 1 mm EGTA, pH adjusted to 7.6 before adding 1% β-mercaptoethanol) on a black 384-well microtiter plate, followed by the addition of 1 μL Cadmium telluride core-type QD (1 mg/mL) (Sigma-Aldrich) and 30 μL of the low-salt buffers containing different ATP concentrations (0, 3.5, 4.5, 5, and 6 mM ATP in low-salt buffers) respectively. After the quantitation, the extracted myosin was optimally diluted for actual measurement, then 1 μL of extracted myosin was pipetted to a well containing 30 μL low-salt buffer, followed by the addition of 1 μL QD (1 mg/mL) and 30 μL of the blank control (0mM ATP) or ATP solutions (5mM ATP), respectively. The addition of blank control/ATP solutions and the detection of fluorescence signal were performed at the same time to avoid any inconsistency with time course due to the instantaneous enzymatic reaction. The QD fluorescence signal was recorded every 15 seconds for 5 minutes by a fluorescence spectrophotometer (TECAN, Infinite M200, Switzerland), while the excitation and emission wavelengths were fixed at 310 and 530 nm, respectively. The measurements for each preparation were performed in sextuplicate with both the blank control and ATP solution at 25°C. The low salt buffer and QD were kept at room temperature (22°C) and the remaining solutions were kept on ice.

Myosin isoform expression and relative quantitation

After myosin extraction, the mini bundle was placed in SDS sample buffer in a microfuge tube and stored at −80 °C. The composition of MyHC isoforms was determined by 6% SDS-PAGE. The acrylamide concentration was 4% (wt/vol) in the stacking gel and 6% in the running gel, and the gel matrix included 30% glycerol. Sample loads were kept small to improve the resolution of the MyHC bands (type I, IIa, IIx and IIb). Electrophoresis was performed at 120 V for 22 h with a Tris-glycine electrode buffer (pH 8.3) at

10 °C (SE 600 vertical slab gel unit; Hoefer Scientific Instruments, Holliston, MA, USA). The gels were silver-stained and subsequently scanned in a GS-900 Calibrated Densitometer (Bio-Rad). The volume integration function (Image Lab software 6.0, Bio-Rad) was used to quantify the relative amount of each MyHC isoform when more than one isoform was expressed. After the QD-mediated thermometry assay, the remaining myosin preparations were kept in urea buffer in a microfuge tube and stored at −80 °C. The relative quantitation of MyHC contents in total extracted protein was determined by 12% SDS-PAGE. After centrifugation and heating (90°C for 2 minutes) a volume of 4 μl was loaded on 12% SDS-PAGE. The total acrylamide and Bis concentrations were 4% (wt/vol) in the stacking gel and 12% in the running gel. The gel matrix included 10% glycerol. Electrophoresis was performed for 5 h with a Tris–glycine electrode buffer (pH 8.3) at 15°C (SE 600 vertical slab gel unit, Hoefer Scientific Instruments). The gels were stained with Coomassie blue (SimplyBlue SafeStain, Invitrogen), as this staining shows high reproducibility and the ability to penetrate the gel and stain all proteins present, i.e., allowing accurate quantitative protein analyses. The gels were subsequently scanned to determine the relative contents of myosin heavy chain in total extracted protein [9, 19, 20].

Data analysis and statistic

QD fluorescence signals were detected and normalized to the starting fluorescent value and the corresponding relative fluorescence intensity formed a negative hyperbolic regression plotted over time. The linear part of the curve corresponding to the initial 60 seconds, was used to calculate the slope of the relative fluorescence intensity over time. The slope of the blank control subtracted from the slope of the “real” reaction then normalized to the concentration of the extracted myosin, indicating myosin efficiency. For the sextuplicate measurement of each preparation, the subtracted and normalized slope values were evaluated individually according to the criterion, i.e., calculated slopes which fell outside one standard deviation were excluded and the remaining qualified slopes were included, and the slopes in negative values (indicating null reactions) were excluded (Figure 2). Statistical analyses were performed by SigmaPlot software version14. The data were presented as mean ± standard deviation and analyzed by the Student's unpaired t test.

Results

Quantitation of extracted myosin and QD-mediated thermometry

The ratio of myosin to total extracted protein was determined by 12% SDS-PAGE. The relative contents of total myosin in both the commercially purified myosin preparation and the extracted myosin from mini bundles were 87±3 and 86±3% (Fig. 3a), respectively, i.e., the ratio of myosin to total extracted proteins was stable and similar to the commercially purified control myosin, indicating that the A280 measure of the total extracted proteins can be used as an estimate of the extracted myosin concentration. The blank control (the high salt buffer) and the internal control (BSA in different concentrations in the high salt

buffer) were measured by spectrophotometric A280 method prior to quantifying the total protein extracted from mini bundles (Fig. 3b).

The concentrations of extracted total proteins in the EDL and SOL group were 0.95±0,33 and 1.50±0,62 mg/ml (assuming 1Abs=1mg/ml), respectively. The optimally diluted concentrations were 0.42±0.07 and 0.34±0.04 mg/ml, respectively, were applied to the myosin ATPase reaction by QD-mediated thermometry. The fluorescence signal of QD itself is stable in the time course, which was verified by applying H_2O as substrate in the reaction. However, QD fluorescence intensity decayed significantly when exposed to low salt buffer and control myosin (Fig. 3c). Therefore, the interference from self-decaying QD fluorescence was corrected in every measurement by the introduction of blank control measurements. To determine the concentration of ATP corresponding to the saturated reaction, different concentrations of ATP were applied, and finally 5 mM was chosen with reference to the previous study (Fig. 3,c) [12].

QDs thermometric measurement of the myosin ATPase reaction

According to the selection criterion, 77% of the measurements had at least three out of sextuplicate measurements in both blank and 5mM ATP reactions and were enrolled for statistical analysis. The other 23% had only two out of sextuplicate measurement and were omitted (Table 1 and Fig. 4). The correlation coefficients of the linear regression during 60 and 90seconds time courses were significantly different, and the plots in the 60 seconds course had a stronger linear relationship and were chosen in the following analysis (Table 2).

Table 1

The number of preparations with sextuplicate measurements that meet the selection criterion, and only preparations containing at least 3 qualified measurement were included in the statistics

	II/VI	III/VI	IV/VI	V/VI
EDL (n=19)	6	8	5	0
SOL (n=21)	3	9	8	1
Total (n=40)	9	17	13	1
(%)	23	17	13	3

Table 2

The correlation coefficients of the linear regression in 60 and 90seconds courses

	60 seconds		90 seconds	
	EDL	SOL	EDL	SOL
	ATP 0 / 5 mM	ATP 0 /5 mM	ATP 0 / 5 mM	ATP 0 /5 mM
Mean	0,945 / 0,944	0,944 / 0,939	0,913 / 0,908	0,913 / 0,905
SD	0,008 / 0,009	0,008 / 0,009	0,013 / 0,010	0,012 / 0,011

The calculated slopes, i.e., the subtracted and normalized slopes for EDL (12.9±3.9) and SOL (19.2±2.7) were significantly different (p=0.006) while the SOL presented a steeper decay of the QD fluorescence intensity than EDL (Fig. 4a-d). The average MyHC isoform composition in the EDL was 55 ± 17% IIb, 37 ± 10 % IIx, 6 ± 9 % IIa, and 1 ± 3 % I, in the SOL was 89 ± 9 % I and 11 ± 9 % IIa (Fig. 4e).

In all measurements of myosin ATPase reactions, the experimental temperature was set at 25℃. Considering the temperature of the blank control measurement as the baseline, a large majority of corresponding temperatures of the ATPase reaction fluctuated in the range of 24.9 to 25.1℃. However, a small number of preparations exceeded this range but were within 25±0.2℃, such as 11% (6/57) and 3% (2/70) of individual measurements in EDL and SOL groups, respectively (Fig. 4f).

Discussion

Myosin, the dominant motor protein in skeletal muscle, is a mechanoenzyme converting chemical energy from ATP hydrolysis into mechanical work. Myosin is expressed in different isoforms that determines the contractile properties of skeletal muscle. At the skeletal muscle fiber level, unloaded shortening velocity is directly related to, and dependent on, the specific activity of myosin ATPase that is predominantly determined by the myosin isoform [21, 22]. However, our understanding of myosin ATPase properties at the muscle fiber level remains incomplete. As a semiconductor nanocrystal, quantum dots were selected as a nanoscale thermometer [12, 17] because of their superior fluorophores and thermal sensitivity compared to organic dyes or other fluorescent proteins. Furthermore, QD have been investigated to measure altered temperature of the ATPase reaction by purified myosin to define myosin efficiency [12, 17]. The decreased fluorescence intensity of QD reflects higher amount of heat production in the process of ATP hydrolysis, signifying lower work efficiency of the myosin motor protein [12, 17, 23]. In this study, we optimized this approach for QD-mediated thermometry measurement [12] to evaluate the efficiency of myosin freshly extracted from muscle mini bundles rather than pre-purified myosin and observed the myosin isoform effect on the myosin efficiency.

It must be noted that the concept of efficiency in the present and the founding study [12] was defined from the perspective of thermometry rather than enzymology where the Michaelis constant (Km) and the catalytic constant for the conversion of substrate to product (kcat) are used to evaluate the enzymatic reaction and even sometimes the ratio kcat/Km has been used to represent the catalytic efficiency of the enzyme [24, 25]. Theoretically, the total amount of energy released during ATP hydrolysis is the same, but

the fraction that is converted into chemical or mechanical energy or heat seems to be modulated by the ATPase [26]. From this point of view, the present study reveals that the SOL myosin ATPase causes more heat production but less mechanical energy than EDL myosin ATPase. As a crucial factor, the ambient temperature affects both QD fluorescence and enzyme activity, therefore, the reaction temperature was carefully controlled and kept stable to minimize the error between measurements. The reaction rate is expected to vary between 4 and 8% per degree centigrade [24]. Thus, the 0.1℃ temperature fluctuations will result in a negligible ± 0.6% error.

In the actin-myosin interaction, ATP binding firstly induces a conformational change in myosin and causes myosin to detach from actin, followed by ATP being hydrolyzed to ADP and inorganic phosphate (Pi), then myosin rebinds to actin and the force generating power-stroke accompanies subsequent Pi release, consequently, ADP is released, and the cycle repeats upon ATP binding [27]. Real time acquisition of reaction with millisecond time resolution is needed to measure the myosin ATPase cycle rate constants. Accordingly, the stopped-flow apparatus provides satisfactory and reproducible results and the related studies showed that myosin isoforms possess different enzymatic activity and different efficiency for the conversion of chemical into mechanical energy [21]. For example, muscle fibers expressing the IIb MyHC isoform have the highest rate of ATPase activity, followed by IIx, IIa and I fibers [22]. In the absence of actin, most myosin binds ATP rapidly and irreversibly, and release phosphate very slowly [21], the present QD-mediated thermometry is sensitive to detect the changing temperature in this process.

Like the single fiber *in vitro* motility assay, myosin can also be extracted from muscle mini bundles successfully, which has been verified by 12% SDS-PAGE. Moreover, there was no significant difference in the ratio of myosin to total protein among the extracted myosin preparations themselves or when comparing with commercially purified myosin preparations, i.e., the quantitation of total extracted proteins is considered a reliable measure of myosin quantity. The extraction of myosin from mini bundles needs the addition of ATP, which means the myosin ATPase reaction has been initiated prior to the QD measurement, which indicates the necessity to assess blank control in all measurements. The advantage of using A280 measurement is that it can be accomplished faster, avoiding any delay that may cause myosin inactivation prior to the QD-mediated thermometry. The spectra of high salt buffer (blank control), BSA and extracted myosin in the same buffer exhibit significant, compatible, and reproducible absorbances, supporting the reliability of this quantitation method.

Even though QD has super fluorescence, its photoluminescence signal in a highly crowded bioenvironment incline to be more sensitive to the presence of a variety of ions and molecules [28]. This feature may explain the phenomenon that the fluorescent intensity of QD decayed significantly when incubated in salt solution. Since both the high or low salt solution are critical and necessary for the extraction and function of myosin *in vitro*, it is imperative to have the blank control introduced during the QD-mediated thermometry measurement to correct the self-decaying interference. All preparations of extracted myosin were assayed, paired with controls, in sextuplicate replicates to minimize error, and over 75% of all preparations have at least three qualified measurements, being sufficient to produce reliable results.

Conclusions

In this study, we have extracted the myosin from muscle mini bundles and verified the constant ration of the extracted myosin to total protein, indicating the quantitation of extracted myosin can be represented by quantitating total myosin through spectrophotometric measurement with reproducible output. Moreover, we have optimized the protocol of the QD-mediated thermometry by introducing blank control, stabilizing reaction temperature and screening disqualified measurement, and demonstrated that slow myosin isoform exhibits a lower myosin efficiency that fast myosin. The optimized QD-mediated thermometry provides a new conceptual perspective to evaluate isoform-dependent function of *in vitro* myosin extracted from minimal amounts of muscle tissue that is essentially required in basic, translational, and clinical muscle research.

Abbreviations

QD: quantum dots; MyHC: myosin heavy chains; Vo: maximum velocity of unloading shortening; CdTe: cadmium telluride; EDL: extensor digitorum longus; SOL: soleus; Pi: inorganic phosphate

Declarations

Ethics approval and consent to participate

All procedures were approved by approved by the ethical committees at Karolinska Institutet.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Swedish Research Council (grant nos. 8651, 7154 and 1001), Stockholm City Council (grant nos. Alf 20150423, 20170133), Centrum För Idrottsforskning (grant nos. 2020-0014,

118-2021) and Karolinska Institutet to L.L.

Authors' contributions

ML designed the study, performed the experiments, and analyzed the data with assistance of LC. The manuscript was written by ML, LC, BPJ and LL and the final version was approved by all authors.

Acknowledgements

We thank Drs. Qing Cheng and Alex B. Addinsall for their support and consultation, as well as Yvette Hedström for performing SDS-PAGE.

Authors' information (optional)

Not applicable.

References

1. Schiaffino S, Reggiani C. Myosin isoforms in mammalian skeletal muscle. J Appl Physiol (1985). 1994;77(2):493–501.
2. Barany M. ATPase activity of myosin correlated with speed of muscle shortening. J Gen Physiol. 1967;50(6):uppl:197–218.
3. Bottinelli R, Betto R, Schiaffino S, Reggiani C. Unloaded shortening velocity and myosin heavy chain and alkali light chain isoform composition in rat skeletal muscle fibres. J Physiol. 1994;478(Pt 2):341–9.
4. Greaser ML, Moss RL, Reiser PJ. Variations in contractile properties of rabbit single muscle fibres in relation to troponin T isoforms and myosin light chains. J Physiol. 1988;406:85–98.
5. Larsson L, Moss RL. Maximum velocity of shortening in relation to myosin isoform composition in single fibres from human skeletal muscles. J Physiol. 1993;472:595–614.
6. Li X, Larsson L. Maximum shortening velocity and myosin isoforms in single muscle fibers from young and old rats. Am J Physiol. 1996;270(1 Pt 1):C352–60.
7. Reggiani C, Potma EJ, Bottinelli R, Canepari M, Pellegrino MA, Stienen GJ. Chemo-mechanical energy transduction in relation to myosin isoform composition in skeletal muscle fibres of the rat. J Physiol. 1997;502(Pt 2):449–60.
8. Schluter JM, Fitts RH. Shortening velocity and ATPase activity of rat skeletal muscle fibers: effects of endurance exercise training. Am J Physiol. 1994;266(6 Pt 1):C1699–713.
9. Li M, Larsson L. Force-generating capacity of human myosin isoforms extracted from single muscle fibre segments. J Physiol. 2010;588(Pt 24):5105–14.

10. Husom AD, Peters EA, Kolling EA, Fugere NA, Thompson LV, Ferrington DA. Altered proteasome function and subunit composition in aged muscle. Arch Biochem Biophys. 2004;421(1):67–76.
11. Salah H, Li M, Cacciani N, Gastaldello S, Ogilvie H, Akkad H, Namuduri AV, Morbidoni V, Artemenko KA, Balogh G, et al. The chaperone co-inducer BGP-15 alleviates ventilation-induced diaphragm dysfunction. Sci Transl Med. 2016;8(350):350ra103.
12. Laha SS, Naik AR, Kuhn ER, Alvarez M, Sujkowski A, Wessells RJ, Jena BP. Nanothermometry Measure of Muscle Efficiency. Nano Lett. 2017;17(2):1262–8.
13. Maruta S, Homma K, Ohki T. Conformational changes at the highly reactive cystein and lysine regions of skeletal muscle myosin induced by formation of transition state analogues. J Biochem. 1998;124(3):578–84.
14. Prochniewicz E, Lowe DA, Spakowicz DJ, Higgins L, O'Conor K, Thompson LV, Ferrington DA, Thomas DD. Functional, structural, and chemical changes in myosin associated with hydrogen peroxide treatment of skeletal muscle fibers. Am J Physiol Cell Physiol. 2008;294(2):C613–26.
15. Larsson L, Li X, Frontera WR. Effects of aging on shortening velocity and myosin isoform composition in single human skeletal muscle cells. Am J Physiol. 1997;272(2 Pt 1):C638–49.
16. Harrington WF, Karr T, Busa WB, Lovell SJ. Contraction of myofibrils in the presence of antibodies to myosin subfragment 2. Proc Natl Acad Sci U S A. 1990;87(19):7453–6.
17. Kuhn ER, Naik AR, Lewis BE, Kokotovich KM, Li M, Stemmler TL, Larsson L, Jena BP. Nanothermometry Reveals Calcium-Induced Remodeling of Myosin. Nano Lett. 2018;18(11):7021–9.
18. Li M, Deguchi T, Nareoja T, Jena BP, Hanninen P, Larsson L. Nanometric features of myosin filaments extracted from a single muscle fiber to uncover the mechanisms underlying organized motility. Arch Biochem Biophys. 2015;583:1–8.
19. Cacciani N, Salah H, Li M, Akkad H, Backeus A, Hedstrom Y, Jena BP, Bergquist J, Larsson L. Chaperone co-inducer BGP-15 mitigates early contractile dysfunction of the soleus muscle in a rat ICU model. Acta Physiol (Oxf). 2020;229(1):e13425.
20. Akkad H, Corpeno R, Larsson L. Masseter muscle myofibrillar protein synthesis and degradation in an experimental critical illness myopathy model. PLoS One. 2014;9(4):e92622.
21. Bottinelli R, Canepari M, Reggiani C, Stienen GJ. Myofibrillar ATPase activity during isometric contraction and isomyosin composition in rat single skinned muscle fibres. J Physiol. 1994;481(Pt 3):663–75.
22. Weiss S, Rossi R, Pellegrino MA, Bottinelli R, Geeves MA. Differing ADP release rates from myosin heavy chain isoforms define the shortening velocity of skeletal muscle fibers. J Biol Chem. 2001;276(49):45902–8.
23. Chern M, Kays JC, Bhuckory S, Dennis AM. Sensing with photoluminescent semiconductor quantum dots. Methods Appl Fluoresc. 2019;7(1):012005.
24. Scopes RK. Enzyme Activity and Assays. In: *eLS.* edn.

25. Eisenthal R, Danson MJ, Hough DW. Catalytic efficiency and kcat/KM: a useful comparator? Trends Biotechnol. 2007;25(6):247–9.
26. de Meis L. Uncoupled ATPase activity and heat production by the sarcoplasmic reticulum Ca2+-ATPase. Regulation by ADP. J Biol Chem. 2001;276(27):25078–87.
27. De La Cruz EM, Ostap EM. Kinetic and equilibrium analysis of the myosin ATPase. Method Enzymol. 2009;455:157–92.
28. Martynenko IV, Litvin AP, Purcell-Milton F, Baranov AV, Fedorov AV. Gun'ko YK: Application of semiconductor quantum dots in bioimaging and biosensing. J Mater Chem B. 2017;5(33):6701–27.

Figures

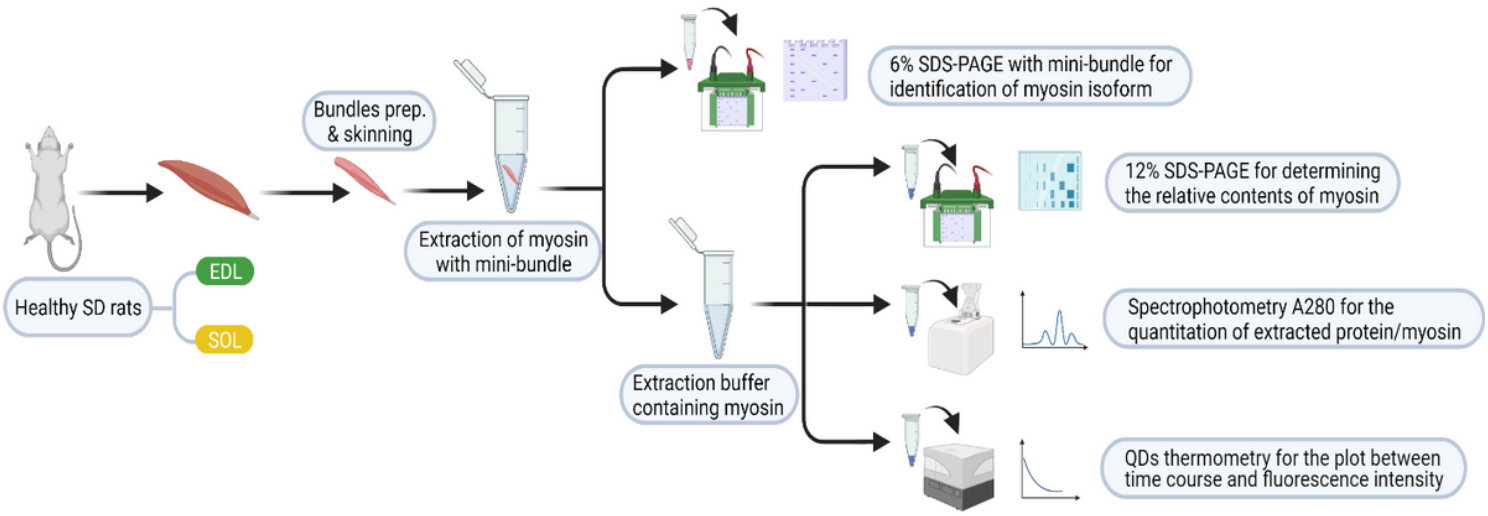
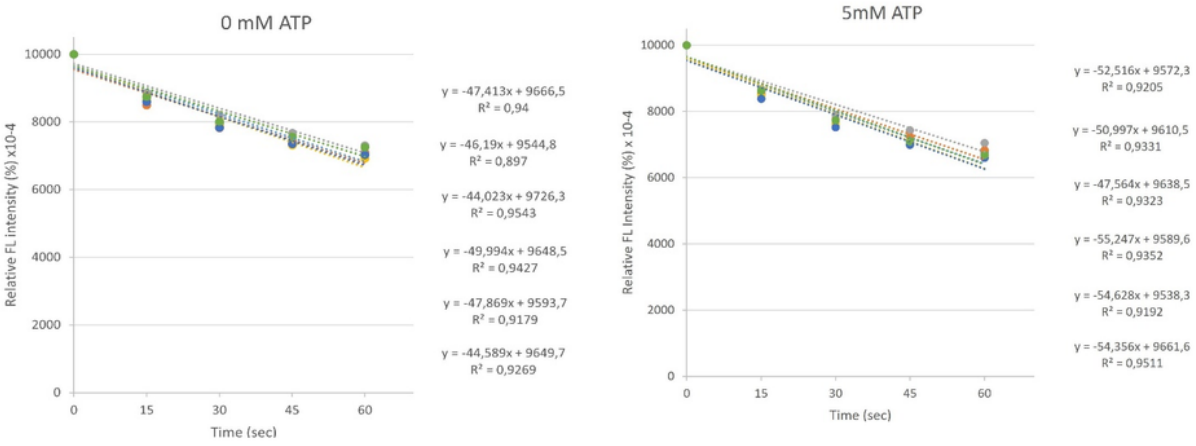


Figure 1

The workflow chart of the QD-mediated thermometry experiments.

Cycle Nr.	Time [s]	E1	E3	E5	F1	F3	F5	E1	E3	E5	F1	F3	F5
1	0	37139	36914	38554	37322	34724	38262	100,0	100,0	100,0	100,0	100,0	100,0
2	15	32421	31359	34177	32237	29810	33450	87,3	85,0	88,6	86,4	85,8	87,4
3	30	29582	28844	31607	29421	27179	30668	79,7	78,1	82,0	78,8	78,3	80,2
4	45	27608	27339	29566	27273	25515	28893	74,3	74,1	76,7	73,1	73,5	75,5
5	60	26339	26136	28130	25810	24405	27745	70,9	70,8	73,0	69,2	70,3	72,5
6	75	25449	25224	27121	24944	23450	26786	68,5	68,3	70,3	66,8	67,5	70,0
7	90	24444	24441	26189	24206	22718	26139	65,8	66,2	67,9	64,9	65,4	68,3
8	105	23849	23696	25674	23372	22073	25546	64,2	64,2	66,6	62,6	63,6	66,8
9	120	23351	23014	25323	23006	21660	24999	62,9	62,3	65,7	61,6	62,4	65,3
10	135	22895	22741	24886	22588	21269	24651	61,6	61,6	64,5	60,5	61,3	64,4
11	150	22569	22386	24393	22055	20979	24487	60,8	60,6	63,3	59,1	60,4	64,0
12	165	22227	21842	24115	21613	20947	24115	59,8	59,2	62,5	57,9	60,3	63,0
13	180	21780	21534	23767	21231	20606	23945	58,6	58,3	61,6	56,9	59,3	62,6
14	195	21576	21198	23667	20912	20383	23704	58,1	57,4	61,4	56,0	58,7	62,0
15	210	21185	20799	23345	20639	20162	23362	57,0	56,3	60,6	55,3	58,1	61,1
16	225	21069	20415	23052	20286	20018	23331	56,7	55,3	59,8	54,4	57,6	61,0
17	240	20792	20092	22856	20203	19893	23084	56,0	54,4	59,3	54,1	57,3	60,3
18	255	20461	20011	22668	19766	19739	22883	55,1	54,2	58,8	53,0	56,8	59,8
19	270	20343	19775	22432	19663	19640	22733	54,8	53,6	58,2	52,7	56,6	59,4
20	285	19991	19641	22439	19401	19473	22549	53,8	53,2	58,2	52,0	56,1	58,9
21	300	19918	19391	22116	19098	19251	22272	53,6	52,5	57,4	51,2	55,4	58,2
Cycle Nr.	Time [s]	E2	E4	E6	F2	F4	F6	E2	E4	E6	F2	F4	F6
1	0	36234	37044	37555	36732	35298	35800	100,0	100,0	100,0	100,0	100,0	100,0
2	15	30808	31719	32581	31110	29595	30885	85,0	85,6	86,8	84,7	83,8	86,3
3	30	27628	28759	29650	27933	26557	27711	76,2	77,6	79,0	76,0	75,2	77,4
4	45	25685	26824	27915	25682	24667	25420	70,9	72,4	74,3	69,9	69,9	71,0
5	60	24524	25323	26491	24226	23300	23938	67,7	68,4	70,5	66,0	66,0	66,9
6	75	23624	24264	25219	22992	22420	22756	65,2	65,5	67,2	62,6	63,5	63,6
7	90	23145	23508	24398	22342	21713	21786	63,9	63,5	65,0	60,8	61,5	60,9
8	105	22492	23122	23636	21658	21078	21084	62,1	62,4	62,9	59,0	59,7	58,9
9	120	21926	22660	23123	21213	20591	20430	60,5	61,2	61,6	57,8	58,3	57,1
10	135	21591	22389	22712	20766	20027	20061	59,6	60,4	60,5	56,5	56,7	56,0
11	150	21290	22066	22173	20283	19544	19594	58,8	59,6	59,0	55,2	55,4	54,7
12	165	20901	21884	21751	19955	19349	19180	57,7	59,1	57,9	54,3	54,8	53,6
13	180	20622	21651	21430	19615	19035	18879	56,9	58,4	57,1	53,4	53,9	52,7
14	195	20407	21446	21032	19325	18800	18681	56,3	57,9	56,0	52,6	53,3	52,2
15	210	20085	21173	20681	19139	18523	18403	55,4	57,2	55,1	52,1	52,5	51,4
16	225	19970	21029	20502	18787	18167	18010	55,1	56,8	54,6	51,1	51,5	50,3
17	240	19697	20835	20251	18584	17965	17821	54,4	56,2	53,9	50,6	50,9	49,8
18	255	19540	20741	19933	18431	17711	17419	53,9	56,0	53,1	50,2	50,2	48,7
19	270	19268	20432	19785	18222	17498	17167	53,2	55,2	52,7	49,6	49,6	48,0
20	285,1	19178	20327	19562	18051	17202	16983	52,9	54,9	52,1	49,1	48,7	47,4
21	300,1	18986	20147	19450	17786	17025	16733	52,4	54,4	51,8	48,4	48,2	46,7



	Slope ATP 0 mM		Slope ATP 5 mM	Substraction	Subs./A280	Qualified
E1	47,4	E2	52,5	5,1	12,9	12,9
E3	46,2	E4	51,0	4,8	12,2	12,2
E5	44,0	E6	47,6	3,5	*9,0	
F1	50,0	F2	55,2	5,3	13,3	13,3
F3	47,9	F4	54,6	6,8	17,1	17,1
F5	44,6	F6	54,4	9,8	*24,8	
				Mean	14,9	**13,9**
A280 original and averaged 1.58				SD	5,5	**2,2**
A280 diluted and applied 0,39				M+SD	20,4	
				M-SD	9,4	

Figure 2

An example of data analysis of the sextuplicate assays for an extracted myosin preparation. In this case, 4 values of 6 subtracted and normalized slope were evaluated to be qualified and rest 2 values (marked with star) were unqualified because they fell outside of the range of mean ± SD according to the criterion.

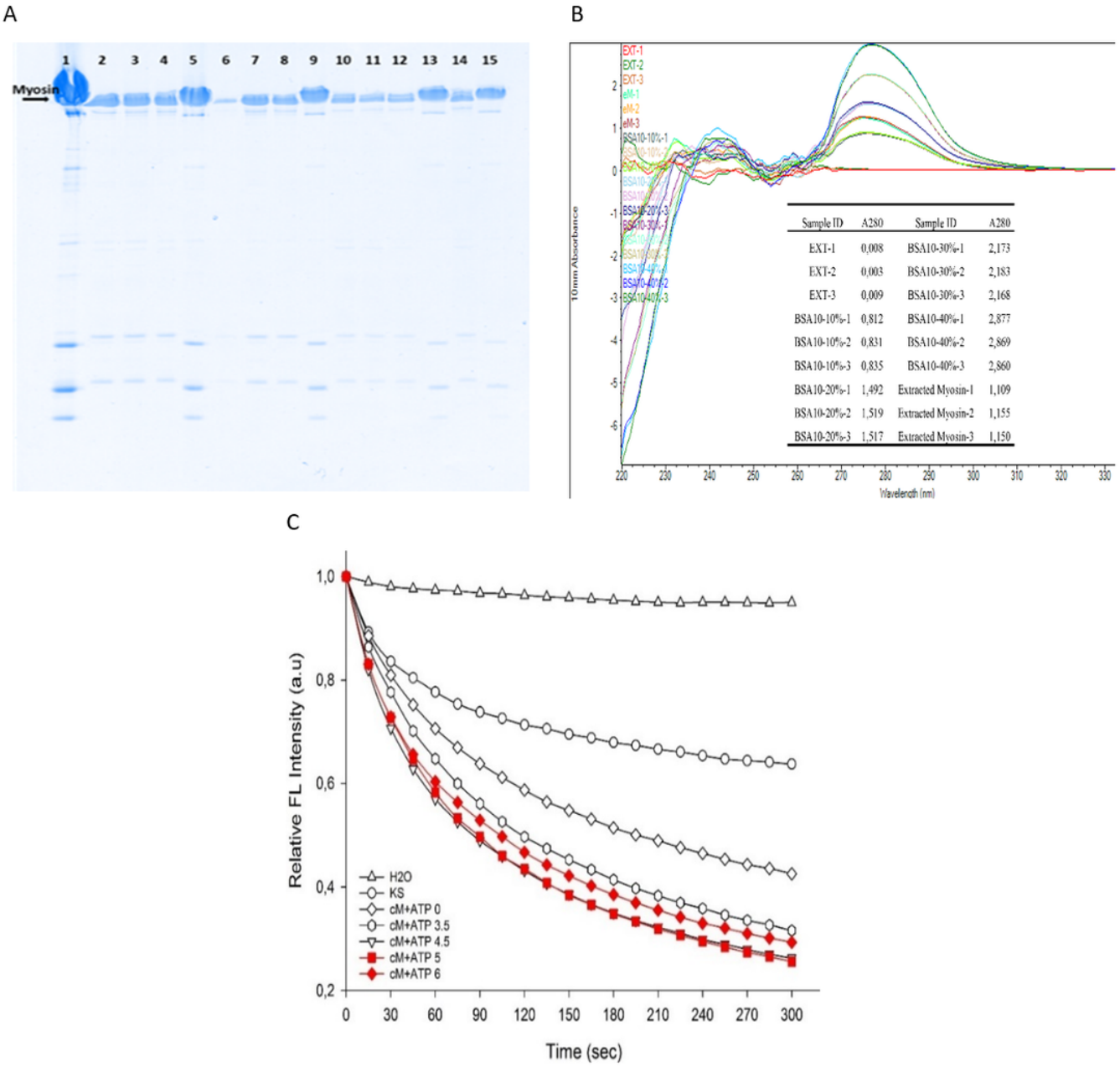


Figure 3

a The image of 12% SDS-PAGE of the commercially control myosin in different concentrations (lanes 1, 5, 9 and 13) and the extracted myosin from different preparations (the remaining lanes). b An example of the spectra and values of protein quantitation in the blank control, internal control and extracted total protein (contains proportional myosin) by spectrophotometric A280 method. c Time-dependent decay in fluorescence intensity in QD-mediated thermometry experiments in controls and in ATP solutions at different concentrations. The red curves represent saturated (5mM) and oversaturated (6 mM) ATP concentrations.

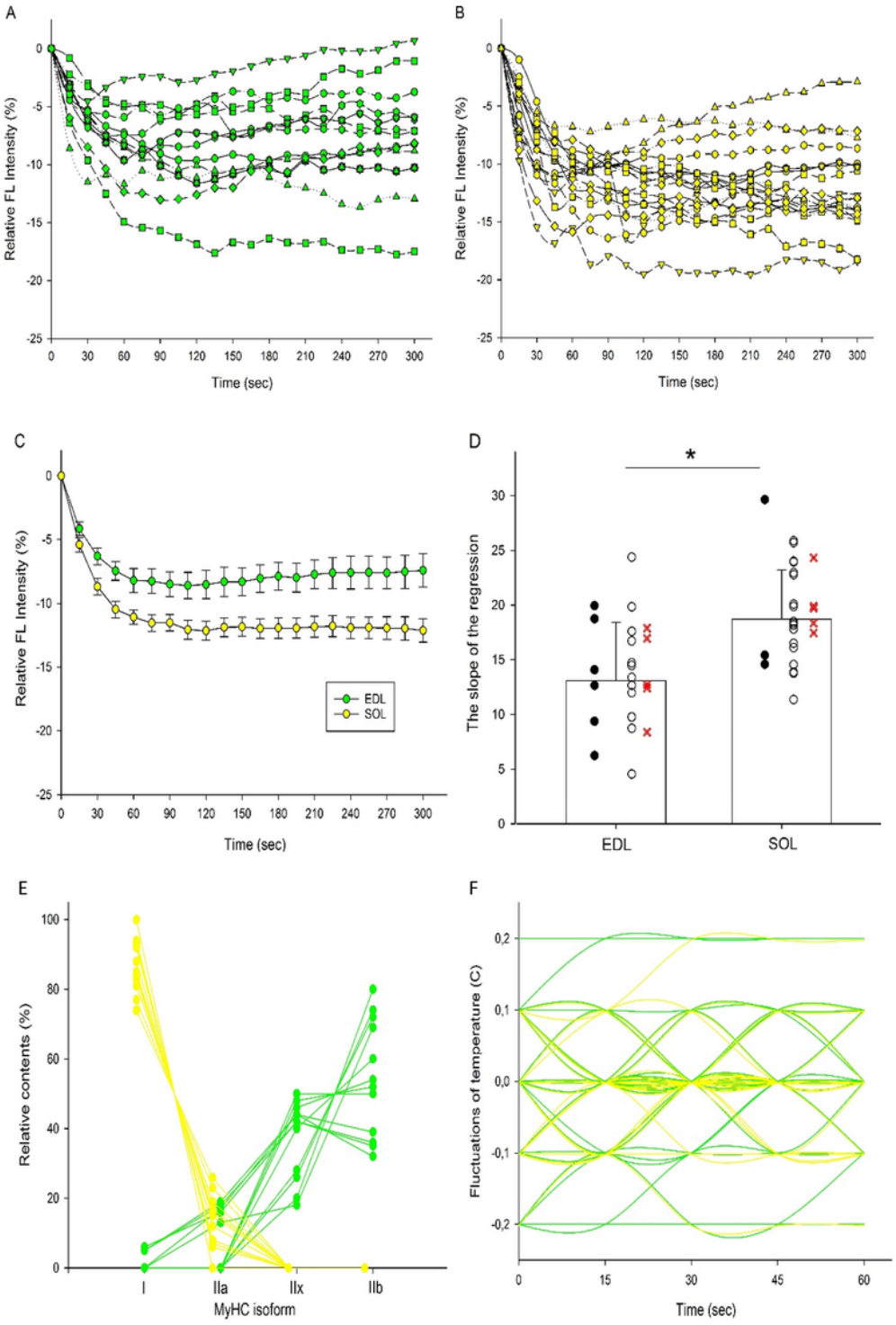


Figure 4

The individual plot of QDs relative fluorescence intensity over time in EDL a and SOL b groups, respectively, demonstrate higher efficiency of EDL. c The averaged plot of relative fluorescence intensity over the time. The values are presented as means ± SEM. d The distribution of the slopes (-60seconds) in EDL and SOL groups, respectively. The values are presented as means ± SD and scatter plot. The open circles dots represent the preparations contain at least 3 qualified measurement, and the black circles

represent those that contain only 2 qualified measurements (not included in statistical analyses), and the red cross represent mean values per individual rat. (*P<0.05). e The individually relative contents of MyHC isoforms in EDL (green lines) and SOL (yellow lines), respectively. f The fluctuations of temperature in QD-mediated thermometry of EDL (green lines) and SOL (yellow lines) experiments (25℃ is considered as zero in y axis).