Biomicrofluidics REVIEW scitation.org/journal/bmf

Nanocalorimeters for biomolecular analysis and cell metabolism monitoring

Cite as: Biomicrofluidics 14, 011503 (2020); doi: 10.1063/1.5134870
Submitted: 11 November 2019 · Accepted: 10 January 2020 ·
Published Online: 31 January 2020

Shuyu Wang,[1] Xiaopeng Sha,[1] Shifeng Yu,[2] and Yuliang Zhao[1,a)]

AFFILIATIONS

[1]Department of Control Engineering, Northeastern University, Qinhuangdao, Hebei 066001, People's Republic of China
[2]Department of Mechanical Engineering, Columbia University, New York, New York 10027, USA

[a)]**Author to whom correspondence should be addressed:** zhaoyuliang@neuq.edu.cn

ABSTRACT

Nanocalorimeters, or microfabricated calorimeters, provide a promising way to characterize the thermal process of biological processes, such as biomolecule interactions and cellular metabolic activities. They enabled miniaturized heat measurement onto a chip device with potential benefits including low sample consumption, low cost, portability, and high throughput. Over the past few decades, researchers have tried to improve nanocalorimeters' performance, in terms of sensitivity, accuracy, and detection resolution, by exploring different sensing methods, thermal insulation techniques, and liquid handling methods. The enhanced devices resulted in new applications in recent years, and here we have summarized the performance parameters and applications based on categories. Finally, we have listed the current technical difficulties in nanocalorimeter research and hope for future solutions to overcome them.

I. INTRODUCTION

A. Calorimetry for biology

Calorimetry is a technique that measures the thermal properties of the material. It is universally used in physical and chemical reactions involving the release or absorption of heat and provides significant insights into the reaction process. Therefore, it has been widely used as a power tool in scientific discovery.

Calorimetry and biology are doomed to have a relationship, as virtually all biological processes are accompanied with heat absorption or generation. Ever since a long time ago, heat is deemed as a symbol of life, while cold is a synonym for death. Thermodynamic studies using calorimeters provide a wealth of information that is not only of fundamental scientific interest but also of immense practical utility in biotechnology.

Most commercial calorimeters measure the power differential between the sample and reference to maintain a zero temperature between the cells [Fig. 1(a)]. They are categorized in two types based on the operation mode: isothermal titration calorimeters (ITCs) and differential scanning calorimeters (DSCs). ITC measures the energy associated with a chemical reaction triggered by the mixing of two components at a constant temperature.[1] DSC measures the heat thermally induced by phase transitions at rising temperatures.

One of the calorimeter's biosensing application areas is on biomolecule interactions, for example, protein,[2] nucleic acid,[3] lipids,[4] carbohydrates,[5] etc. To gain an insight into the energetic–function–structure correlations of the biomolecules,[6] calorimeters are used in several steps in the early stages of the small-molecule drug discovery process. They unveil the nature of intra- and intermolecular forces that stabilize the native state as well as binding interactions. To be more specific, it can characterize the underlying physicochemical properties that stabilize native molecular structures and elucidate the thermodynamic driving forces that control and modulate receptor recognition within macromolecules. This provides detailed and quantitative information for decision making in lead discovery and optimization.[7]

One type of calorimeter, ITC, can yield abundant information on the reaction process, including binding affinity, stoichiometry, and the energetic forces driving the intermolecular association, by interpreting the equilibrium constant (K_a), Gibbs free energy (G), enthalpy (H), and entropy (S). The ITC's thermogram is shown in Fig. 1(c). We have seen ITCs' application on enzyme catalyzed reactions, protein–protein,[8] protein–peptide, protein–DNA,[9] protein–drug interactions, etc. These binding process could be related to cell signaling,[10] Alzheimer's disease,[11] and muscle contraction.[12,13]

The other type of calorimeter, DSC, measures excess heat capacity changes (ΔC_p) associated with temperature dependent phase

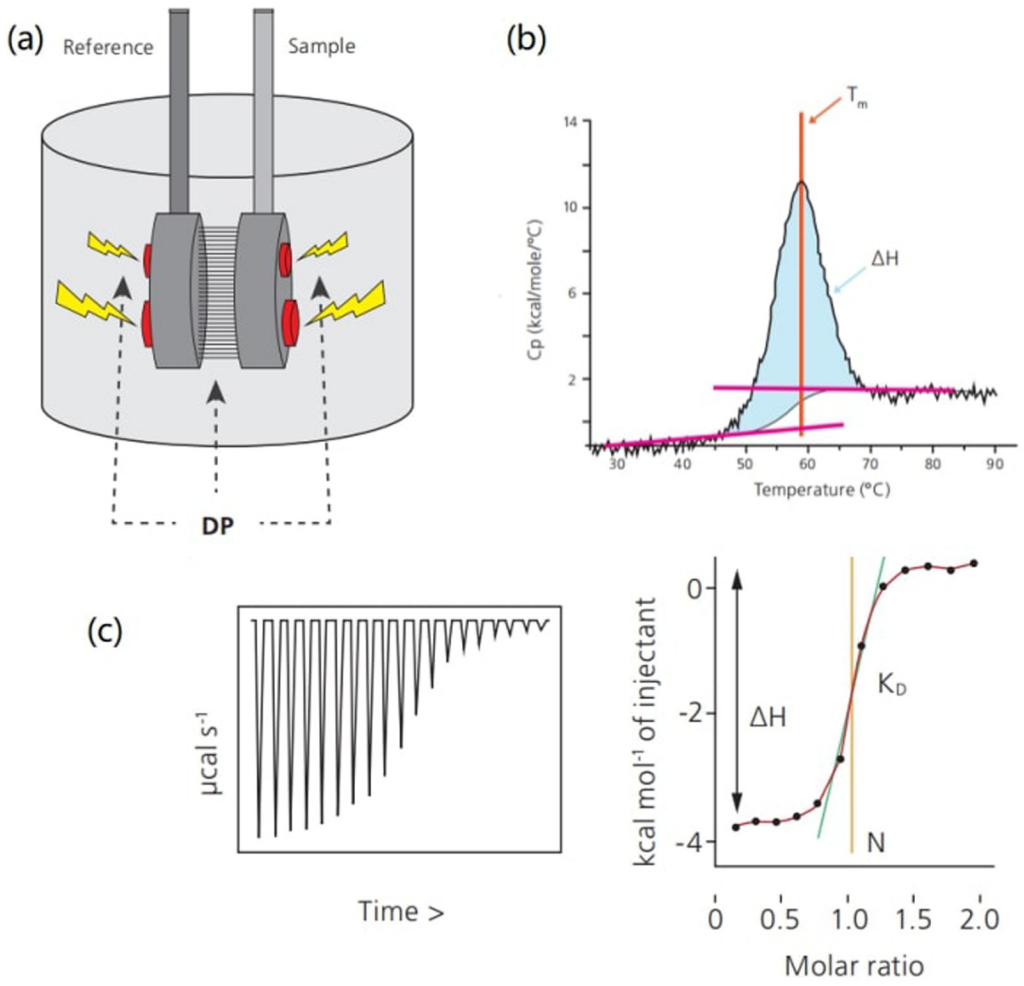

FIG. 1. (a) Commercial calorimeters' operating principle. It operates by measuring the power differential between the reference and sample side to maintain zero temperature difference between the two cells. (b) A typical DSC thermogram of protein unfolding showing the relationship between heat capacity and temperature. The transition temperature (T_m) and enthalpy (ΔH) can be acquired through data interpretation. (c) a typical ITC thermogram to illustrate power vs time. Heats are used to extract affinity (K_D), stoichiometry (N), and binding enthalpy (ΔH) using an appropriate binding model.

transitions that could lead to unfolding/dissociation enthalpy (ΔH) and thereby enables elucidation of the forces that maintain macromolecular stability. The DSC's thermogram is shown in Fig. 1(b). Higher T_m values are a typical representation of stability. Furthermore, recent studies showed that DSC tests on protein were potentially used in disease diagnosis. For example, diagnosing diabetes could be potentially enabled based on a plasma proteome DSC test.[14]

The other promising field calorimeter application in biosensing is cell metabolic activity monitoring, including mammalian and micro-organism cells. These studies can be crucial in many ways, such as drug development, toxicology studies, clinical investigation, and environmental studies.[6] For instance, tumor cells are reported to show a higher metabolic rate,[15] and calorimeters could be the potential tool to diagnose them.[16] Therefore, it would have profound scientific impacts to unveil single cell thermal activities in real time. Also, there is a growing interest to couple the biomass formation with catabolic processes. Another example is to identify potential infections by measuring the excess heat when bacteria growth exceeds the threshold with the calorimeter.[6] In addition to research purposes, monitoring microbial growth is also useful in industrial processes such as food production[17] or biomedical applications.[18]

Overall, calorimetric techniques with high sensitivity exemplified some representative applications, such as macromolecular recognition and cell bioprocess monitoring. The thermogram measured with calorimeters can yield abundant information about structural data and the reaction mechanisms, impacting fields such as drug discovery, disease diagnosis, etc.

B. Advent of nanocalorimeters

Conventional commercial calorimeters for biosensing in aqueous environment, though reach high resolution (10 nW level)

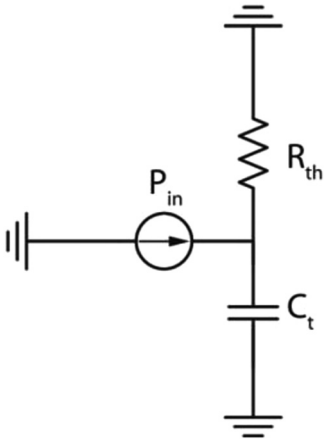

FIG. 2. A nanocalorimeter system's equivalent electrical circuit. The system's thermal mass can be deemed as the capacitor (C_t) and the thermal resistance corresponds to resistor R_{th}.

and precision, typically use a larger volume of sample (>500 μl) and the throughput is relatively low (cycle time >1 h). Those instruments require a long thermal relaxation time to establish equilibrium and homogeneity. Heating the scanning calorimeter is also time consuming, as it may need 1 h to reach 90 °C from room temperature. Moreover, bulk calorimeter could hardly be tailored for some specific research tasks. For instance, it is impossible to cultivate cells within the chamber with good visibility.[19] Therefore, researchers are calling for new types of calorimeters that have high throughput and can meet some special requirements.

The advent of microfabrication technology made nanocalorimeters possible. Some people name it microcalorimeters or nanocalorimeters. A nanocalorimeter typically consists of a thin membrane, a small chamber for holding samples and thermal sensing elements. The system's equivalent electrical circuit is shown in Fig. 2. The thermal resistance of the device corresponds to the resistor (R_{th}), and the calorimeter's thermal mass can be denoted as the capacitor (C_t). This structure leads to higher thermal insulation (<1 mW/K), lower sample amount (<10 μL),[20] and high sensitivity (1–100 V/W).[21] While upscaling reduces the influence of environmental fluctuations, smaller size shortened the thermal diffusion process and measurement time. Moreover, the tiny scale could result in more uniform thermal distribution,[22] lower addenda mass, and potentially enable parallel operations.[23]

II. NANOCALORIMETERS' DESIGN AND PERFORMANCE

A. Thermal sensing methods

Most nanocalorimeters measure the heat based on temperature change. Thermal sensing methods can be divided in three categories based on thermal contact: invasive, semi-invasive, and noninvasive thermometry.

In an invasive temperature measurement, the thermometer is directly in contact with the medium of interest. Commonly seen examples are thermopiles,[24,25] thermistors,[26,27] resistive temperature detectors (RTDs),[28] resonant thermal sensors,[29] and bimaterial microcantilevers.[30,31] Semi-invasive types measure the temperature change indirectly by temperature sensitive materials based on fluorescence,[32] liquid crystal,[33] or quantum dot.[34,35] They are advantageous to obtain a high spatial resolution (<1 μm), yet they suffering from low temperature resolution. Noninvasive thermometers can remotely measure the temperature change based on techniques such as IR thermography.[36,37] Until now, invasive thermometers are still dominant in calorimetry and we will dive deeper into these invasive thermometry methods in the following paragraphs.

Thermocouples or thermopiles employ the Seebeck effect of thermal electric materials to measure the temperature difference. They are fabricated on thin films and integrated with nanocalorimeters. Thermopiles require no extra power, meaning no additional heat is introduced.[24] To enhance the temperature sensing ability, series connections of the junctions were needed. Yet, this could impair the thermal insulation and cause electronic noise in the signal, which impairs the detection limit. The theoretical detection limit for a thermocouple could be on the order of 10–100 μK, depending on the bandwidth.

Thermistors utilize the temperature dependent resistance to measure the temperature as calibrated, so they can measure absolute temperatures. Semiconductor thermistors normally have a negative temperature coefficient (NTC), and the temperature response could be one order more sensitive than metal ones.[38] One advantage of thermistors is they do not need to be connected in series for high sensitivity, which saves space for small region patterning. Similar to thermopiles, the ultimate detection limit is the material's noise. With appropriate instrumentation, the temperature detecting resolution could reach 10–100 μK at 0.5–20 Hz.[39] Yet, temperature sensing with a thermistor required electrical currents to pass through it, which might introduce additional heat. One strategy is to bring in a reference side to the calorimeter to form a Wheatstone bridge, so that the heat from sensing current could be counteractive.

Bimaterial microcantilevers exhibit high sensitivity in biosensing.[30,31,40,41] The materials in the two layers of microcantilevers have different coefficients of thermal expansion, which cause the device to bend in response to changes in the temperature. Temperature changes are calculated from the amount of bending. The sample of interest can be placed adjacent to the tip of the cantilever (Fig. 3) or trapped in the cavity formed by the beam

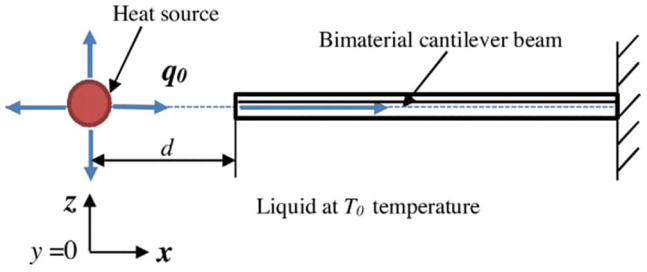

FIG. 3. Schematic of the bimaterial cantilever beam temperature sensor. The microheater is positioned at the distance *d* from the free end of the cantilever beam. Reproduced with permission from Voiculescu *et al.*, Sens. Actuators A Phys. **242**, 58–66 (2016). Copyright 2016 Elsevier.

(Fig. 12).[31] This method was reported to reach 1 nW power resolution in aqueous environments.

Resonant thermal sensor would alter the resonant frequency responding to temperature change. The vibration is considered a simple spring with an effective mass connected to the free end. To apply this technique on a liquid sample, heat needs to be introduced to the sensing beam.[29] The method could lead to 10 μK level of temperature resolution and 1 nW level of power resolution.[42]

B. Liquid delivery methods and chamber configurations

Nanocalorimeters for biosensing can be classified into two types based on the configuration: open-type and closed-type (Fig. 4). Open-type calorimeter utilizes air's thermal insulation property and required a micropipette to deliver samples to the sensing region. To reduce the convective heat loss and evaporation, a cavity with minimum air space is needed.[43] Such devices can generate remarkable signal responsivity,[43] especially when an ultralow amount of sample is used. However, the testing consistency can be impacted by the undefined liquid sample shape, which is determined by the surface wetting, and it varies from time to time. Also, the evaporation heat loss and thermal fluctuation during sample injection will be reflected as the noise in measurements, which will further hamper the signal to noise ratio.[44]

Closed-type calorimeters can partly resolve the problems mentioned above with a reaction chamber and microfluidics system. They help to prevent evaporation and the liquid can form a predefined shape, reducing the chance of random errors. But it also brings in additional challenges. For example, the microfluidic system might be sophisticated, with valves, mixers, syringes, etc. The dead volume could form consuming more sample than expected. Furthermore, the reaction chamber is adding addendum mass to the calorimeter sensor, affecting the measurement result. Commonly used materials for the closed-type microfluidics are polydimethylsiloxane (PDMS),[45,46] poly(methyl methacrylate) (PMMA),[47] parylene,[27] and silicon nitride.[48]

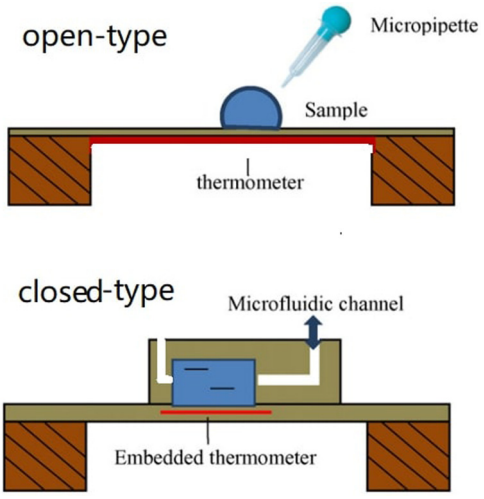

FIG. 4. Open-type and closed-type calorimeters. The open-type calorimeter normally dispenses the liquid droplet with a micropipette, and the closed-type calorimeter delivers the liquid with an enclosed microfluidic chamber.[91] Reproduced with permission from Khaw *et al.*, Microelectron. Eng. **158** (2016). Copyright 2016 Elsevier.

Based on the motion of the liquid during calorimetric sensing, calorimeters can also be categorized into flow-through and static types. The flow-through type calorimeter performs measurement while the liquid sample is flowing through the detection region,[49,50] while the static type measures the samples in the chamber when the liquid is still. Although the reported static type generally has higher sensitivity than the flow-through type, to the authors' knowledge, there is no clear evidence to compare the performance of the two types.

C. Thermal insulation methods

Thermal insulation is vital to improve the sensitivity of nanocalorimeters. Lower thermal conductance means the measured thermal event can lead to a higher temperature change. An inherent strategy of nanocalorimeter is to use a two-dimensional substrate to reduce the thermal loss. Although a one-dimensional structure can lead to even higher thermal insulation and higher detection resolution,[51] it is hard to be applied on liquid samples.

Other strategies to further enhance the thermal insulation were explored. The closed-type calorimeters can form thinner and complicated 3D wall structures to isolate the system with minimum addendum mass.[48] Vacuum could also be used to increase the thermal insulation significantly when well-designed with parylene nonpermeable microfluidics to withstand the pressure.[24,27]

D. Sensing configurations

Calorimeters can sense the sample's heat by referencing the surrounding thermal bath. The method was already seen in calorimeters with a high detection resolution.[24,43] Another type of calorimeters use a reference side almost identical to the sample side to improve the performance and solve the problems in a single measurement. A schematic illustration is shown in Fig. 5. The reference side should

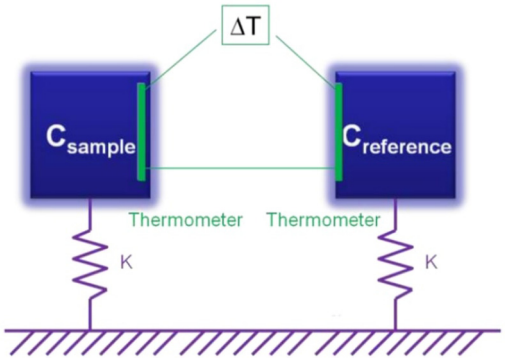

FIG. 5. Thermal scheme of a calorimeter sensor with reference. The temperature difference ΔT is the detection of interest. *k* is the thermal conductance of the sample and reference cell.

TABLE I. Nanocalorimeters' classification methods

Classification methods	Classification types
Measure principle	Heat conduction
	Power compensation
Configuration	Open-type
	Close-type
Operation mode	Isothermal titration calorimeter (ITC)
	Differential scanning calorimeter (DSC)
Sensing configuration	Single measurement
	Twin measurement with a reference
Heating current	AC
	DC
Liquid motion	Static and flow-through

be highly symmetric to the sample side to achieve the common mode rejection, so that the unwanted noise signal could be partly counteracted and the baseline performance could be improved.

E. Heating currents

DC heating current is often used in calorimetric sensing. They can serve to calibrate the sensor or raise the temperature for scanning calorimeters. An AC voltage is applied to the heater, and by the Joule effect, an AC heat wave with twice the input frequency is produced. The heat wave propagates through the liquid sample resulting in amplitude decay and phase delay and is detected by the thermopile placed on the membrane.[48,52,53] The nanocalorimeter classification methods are listed in Table I. Meanwhile, the author's have summarized detailed information about nanocalorimeters that are applied in biomolecular interactions in Table II.

III. NANOCALORIMETERS' APPLICATIONS IN BIOSENSING

A. Biomolecular interaction

1. Isothermal type

Nanocalorimeters' application in compound binding tests was pioneered by the Palo Alto research center. Open-type calorimeters were more frequently used by then due to the ease of construction and high sensitivity. The liquid was either merged by electrostatic force[8,23] or added by micropipettes.[54] The enthalpy arrays were successfully applied on three types of biochemical reactions: protein–ligand binding reactions, with the examples of RNase A (61μM) and cytidine 2′-monophosphate, streptavidin (36μM, 1.25 mg/ml), and biotin; enzymatic reactions, such as the phosphorylation of glucose (500μM) by hexokinase; and organelle reactions, such as mitochondria suspension exposure to 2,4-dinitrophenol (DNP) (1.2mM).

A similar open-type calorimeter, named the thermometric enzyme linked immunosorbent assays (ELISA) system,[55] was used for a clinically relevant assay (Fig. 6). It measured an anticancer monoclonal antibody drug at therapeutic concentrations.[56] The activity of catalase was measured by spectrographically monitoring the consumption of H_2O. Closed-type calorimeters' liquid manipulation is operated by injecting the liquid in either continuous flow[57] or droplets.[45] The schematic view of a closed-type calorimeter's operation is shown in Fig. 7. They are used for biochemical reactions potentially applied in clinical analysis, bioprocess control, and environment control.[58] Similar to enthalpy arrays, the device performance was often demonstrated by biochemical reactions like the oxidation of glucose catalyzed by glucose oxidase[45,47] or protein ligand binding like the reaction of streptavidin solution with biotin solution.[59] Other enzyme reactions could be used are like urea hydrolysis catalyzed by urease[24] and alkaline phosphatase (AP) to convert para-nitrophenyl phosphate (PNPP) into para-nitrophenol (PNP) and phosphate.[60]

To expand the power and applications of the closed-type chip calorimetry, other approaches could be integrated with the system. One example is the nanohole arrays (NHAs). It operates in a microchannel coflow to determine the change in concentration while detecting energy released in a chemical reaction.[57,62] This method provides more insights into the reaction in real time. Another strategy is to integrate magnetic beads[63] with a calorimeter to increase the reaction surface area and extend the reaction duration beyond the nanocalorimeter time constant. As a result, fast hybridization reaction of two strands of DNA was demonstrated with the technique, with one immobilized on the magnetic beads.

Aside from the heat detection of macromolecular interaction, thermal conductivity and diffusivity of aqueous protein bovine serum albumin (BSA) sample [20% (w/v)] could be measured by an AC nanocalorimeter.[52] The heater's modulated heat propagates through the liquid sample and was sensed by the thermometers on the other side (Fig. 8). This method could further be used to calculate the heat capacity without DSCs.

2. Temperature scanning type

Temperature scanning type nanocalorimeters are less reported due to technical difficulties. Two scanning modes were explored in the literature: slow scan mode (~5 K/min) and fast scan mode (>100 K/s). The slow scan calorimeters[26,64,65] can use external heating sources for scanning. 10–100 mg/ml of lysozyme denatured and IgG samples were successfully demonstrated by a group from Virginia Tech.[66]

Fast scan mode can effectively magnify the power signal, since the power change is proportional to the scanning speed ($\Delta P = S \cdot \Delta C_p$) at a given heat capacity change. A research group from Xensor Integration[67,68] combined a commercial nanocalorimeter with a microfluidic cover to prevent evaporation (shown in Fig. 9). The chip was powered by a commercial flash DSC instrument and was scanned at a maximum rate of 1000 K/s. Lysozyme (1% concentration), bovine serum, and olive oil were characterized by the method. A more recent study applied on human blood serum showed differences between cancer patients and normal patients indicating the fast DSC as a potential cancer diagnostic tool. The fast scanning rate was further increased to 8000 K/s to study the lysozyme denaturation. Due to the fast rate, the protein samples were dissolved in glycerol to prevent evaporation.[69]

B. Cell metabolic activity monitoring

1. Thermopile methods

Calorimetric sensing with the thermopile method in both the static mode and the flow-through mode can determine the heat

TABLE II. A summary of nanocalorimeters based on thermopiles or thermistors that were applied in biomolecular interactions. Information includes publication year, institutions, methods, characterization results, and applications

Year	Institution	Sensing method	Thermal sensitivity	Construction material	Sample amount	Operation mode and configuration	Responsivity, time constant, and thermal insulation	Resolution and performance	Biology application
2004–2008[5,24]	Palo Alto Research Center	Amorphous silicon thermistor/ vanadium oxide	TCR = −2% to 2.8%/K	Polyimide	500 nl	Isothermal, droplet merge, open-type	1000 μW/K, 1.3 s	50 μK, 100 nW, 0.2 W/L	Protein–ligand binding interactions
2008–2019[43,54–56]	Vanderbilt University	20 bismuth/ antimony (Bi/Sb) thermopiles	24–410 μV/K per junction, total 3.6 mV/K	SiO_2/Si_3N_4	2.5–50 nl	Isothermal, titration, capillary, open-type	3–80 V/W 65–220 μW/K (2.5–50 nL)	1.4–132 nJ, 1.5 nW/Hz1/ 2, 22 nW, 0.44 W/L	IgG1, trastuzum ab binding
2008–2019[45,64]	Columbia University	50 antimony–bismuth (Sb–Bi) thermopile	125 μV/K, Total 13 mV/K	Polyimide/ PDMS	1–1.2 μl	Temperature scan/ isothermal, closed-type	4–8 V/W, 1000 μW/K	20 nW, 0.02 W/L	Lysozyme protein unfolding
2009–2019[25,28]	Cal Tech University/ KAIST	Au/Ni thermopile/ vanadium oxide	110 μV/K, TCR −2.2%/K	Parylene, SU8/PDMS	3.5 nl	Isothermal injection, closed-type	7.1 V/W, 1.3 s, 6.7–12 μW/K,	4.2–10 nW, 6 nJ, 270 μK, 0.83 W/L	urea hydrolysis catalyzed by urease
2014[48]	Marquette University	Nickel RTD	0.258%/K	SixNy	200 nl	isothermal titration, closed- type	1.33 s, 58.87 K/W	NA	Thermal diffusivity and specific heat measurement
2016–2018[26,65,66]	Virginia Tech	Vanadium oxide	TCR −2% to 2.8%/K	PDMS, Polyimide	1 μl	Temperature scan, closed-type	6 V/W, 800 μW/K	35 nW, 0.035 W/L	Lysozyme, IgG denaturation
2005–2019[19,49,50,60,63,65,70–73,75,76]	TU Bergakademie Freiberg	118 BiSb/Sb thin-film thermopile	>1 mV/K	PMMA, SixNy, SU8	10 μl	Isothermal, closed-type, flow-through	4–6 V/W	10–50 nW 0.01 W/L	Biofilm, bacterial, embryo, human erythrocytes metabolic monitoring
2012–2016[61,62]	Xensor Integration	Silicon thermopile	2.4 mV/K	TOPAS cover, silicon nitride/oxide	15 nl	Fast scanning, close-type	5.5 V/W, 2 kK/W	1 μW, 2.5 mK	Lysozyme, bovine serum, olive oil, human blood serum
2018–2019[86–90]	Tohoku University	Silicon thermopile, vanadium oxide	TCR −1.4%/K, 4.9 mV/K	Silicon (bridge) SU8		Isothermal close-type	0.44 V/W, 20 ms	0.5 mK/ $\sqrt{\text{Hz}}$	Enzymatic reaction

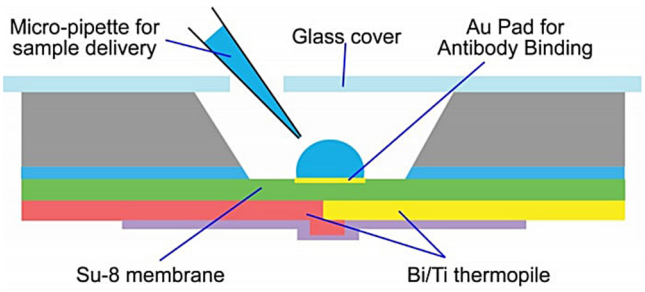

FIG. 6. Nanocalorimeter device cross section layout. The device is open-type and liquid is delivered through a micropipette.

production in micro-organisms and animal cells. *Escherichia coli* activity (>1 nW) under different conditions was measured in the static mode with a microfluidic system.[70] The thermal profiles matched the exponential growth kinetics yielding the sample's growth rate.

The flow-through mode was more frequently applied on cell metabolic activity measurement. Freiberg's device[47,71] was applied to monitor the bacterial growth in real time, highlighting its applicability for bioprocess monitoring.[19,72] Its schematic composition is shown in Fig. 10. Furthermore, extensive studies were done on biofilms to investigate the bacterial resistance mechanism, which has become a particular concern for antibiotic treatment.[73]

Apart from micro-organism studies, research studies on animal cells were also reported by closed-type flow-through nanocalorimeters. The study by Freiberg's group on zebrafish embryos[49] allowed the calculation of heat power values of a few hundred nanowatts per embryo. Such a system could be combined with the segmented flow technology, which consisted in repetitive sequence of sample segments separated by immiscible inert segments.[74,75] Using the technique, the metabolic activity changes of human erythrocytes caused by cell sickling was studied for the first time.[76] In addition, nanocalorimetry combined with respirometry enabled the direct investigation of anaerobic metabolic process of *Trypanosoma cruzi* cells by switching the oxygen concentration.[77] Another calorimetry sensing technique applied on worms confined the worm populations in a microfluidic chamber and close to the sensor surface (Fig. 11). The technique was used to measure the

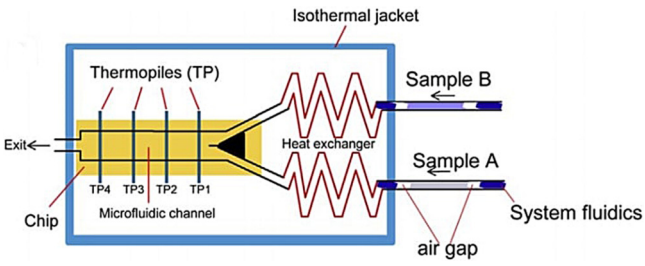

FIG. 7. Schematic representation of a closed-type microfluidic nanocalorimeter.[60,61]

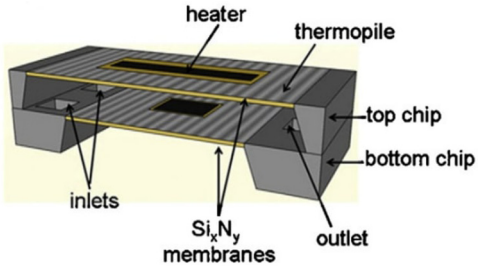

FIG. 8. Fluidic AC chip calorimeter composed of two stacked chips with silicon nitride membranes that define the liquid chamber. Reproduced with permission from Adrega and Herwaarden, Sens. Actuators A Phys. **167**(2), 354–358 (2011). Copyright 2011 Elsevier.

metabolic heat production of *Caenorhabditis elegans* larval populations (60–220 organisms).[78] However, due to insufficient sensitivity, thermopile sensors, in overall, can hardly detect metabolic heat at a very low level of cells (<10).

2. Beam methods

A bimaterial microcantilever placed adjacent to the cell was reported to measure the heat of four to seven murine brown adipocytes cells stimulated with norepinephrine based on deflection.[79] The measurement method was noninvasive and provided plenty of oxygen for the cells at its native state, allowing researchers to observe them for several hours and to discover their gradual, long-term increase in temperature. Bimaterial cantilevers can also form very small close-chambers. They measure the heat (Fig. 12) by introducing liquid samples into the cantilever microchannel and detecting the beam's deflection.[80] To date, the device was reported to measure the heat capacity of organic compounds.[31] It will be very promising to apply on cell thermal characterization, as it has shown mass measurement at a single cell level.

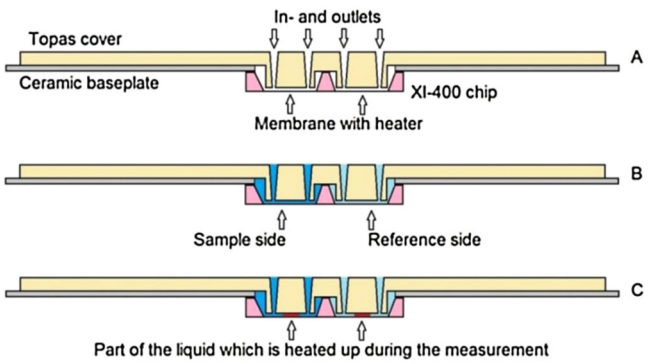

FIG. 9. Fast scan DSC chip cross-sectional view. (a) An empty device showing the configuration. (b) A device filled with a sample and reference liquid. (c) The red part indicates the area directly heated up during the measurement.[68] Reproduced with permission from Wolf *et al.*, Thermochim. Acta **603**, 172 (2015). Copyright 2015 Elsevier.

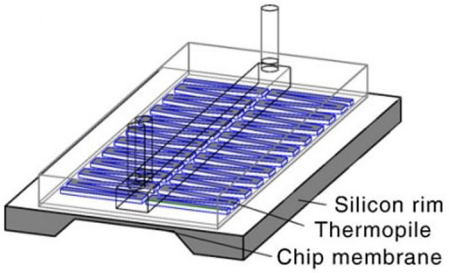

FIG. 10. Scheme of Freiberg's chip calorimeter with integrated thin-film thermopiles.

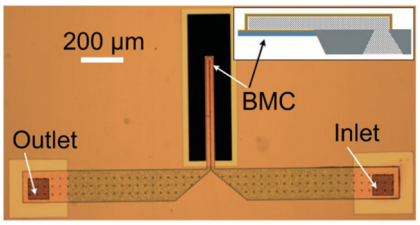

FIG. 12. Top view of a chip containing the BMC (bimetallic microchannel cantilever), sample delivery channels, and inlet/outlet. Liquid reagents are confined within the channel. Reproduced with permission from Faheem Khan *et al.*, Lab Chip, **14**(7), 1302–1307 (2014). Copyright 2014 Royal Society of Chemistry.

Resonator calorimeter with nanowatt resolutions has been demonstrated to detect metabolic heat emission of a single brown fat cell (BFC).[42,81] The resonator is placed in the vacuum, and the heat is conducted from the sample in the microfluidic channel via a heat guide.[29,82] Tohuku University's double-supported resonator (Fig. 13) showed a temperature and power resolution of 79 μK and 1.90 nW. Such a level of measurement could be of a significant impact on disease diagnosis.

IV. TECHNICAL DIFFICULTIES

A. Temperature control strategies

Commercial bulk calorimeters for biosensing show high performance in feedback control. They are working by monitoring the differential power and applying to the cell heaters to maintain zero temperature difference between the reference and sample cells. The minimized temperature difference is primarily achieved by advanced control. The cell chamber is made of bulk metal, with higher thermal conductance than the nanocalorimeter. The thermal insulation is only 8.4 W/K (calculated by 200 μl of the sample amount and 10 s of response time). Yet, the device showed a noise level of only 0.84 nW with the control technology.

Most nanocalorimeters' power resolution or detection limit heavily relies on high thermal insulation, while temperature feedback control is rarely integrated on the chip due to technical difficulty. This could be related to the nature of the lab made device: hardly a standard sensor could be fabricated at a low manufacturing volume. We might expect nanocalorimeters with more improvements when implemented with more advanced control.

B. Liquid induced issues

Normally, biological samples are in aqueous environments. This could cause issues not anticipated in solid samples. One of the difficulties is the liquid solution has orders of magnitude higher heat storage capacity than the sample of interest, so it dampens the impulse heat signal during fast biochemical reactions. Also, a liquid sample can hardly be adapted to one-dimensional structures for thermal isolation,[83,84] and therefore the detection resolution could hardly be compared to those applied on solid materials. Meanwhile, liquid evaporation causes heat loss and mass loss, which could affect the detection accuracy. Last, liquids could potentially cause device failure when permeated to the sensing element.

C. Power per volume resolution and signal to noise ratio improvement

Although some nanocalorimeters' power resolutions have reached the nanowatt level, the power per volume might become a problem, since there is a trend of worse power per volume performance at a smaller amount as reported by some researchers.[71] The shrunk volume of the sample generated less heat at the same concentration, which makes it especially important to consider the

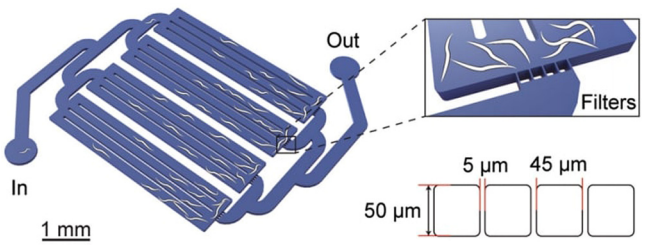

FIG. 11. Microfluidic chip for *C. elegans* nanocalorimetric assays. Reproduced with permission from Krenger *et al.*, Lab Chip **18**(11), 1641 (2018). Copyright 2018 Royal Society of Chemistry.

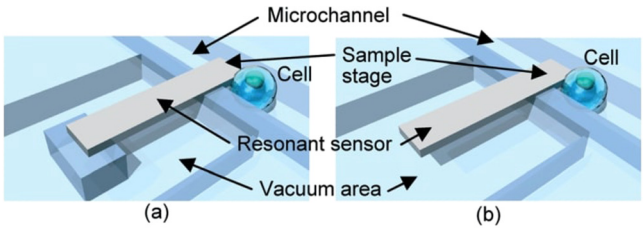

FIG. 13. Schematic of the resonator calorimeter. (a) The double-supported type. (b) The cantilevered type. Reproduced with permission from Inomata *et al.*, Lab Chip, **16**(18), 3597–3603 (2016). Copyright 2016 Royal Society of Chemistry.

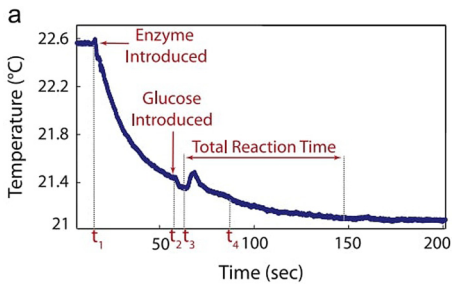

FIG. 14. A nanocalorimeter's baseline drift as liquid flows through the sensing area. The signal of interest becomes harder to distinguish due to the baseline performance. Reproduced with permission from Davaji and Lee, Biosens. Bioelectron. **59**, 120–126 (2014). Copyright 2014 Elsevier.

volume specific power. A high surface to volume ratio might be accountable for this phenomenon, as the smaller sample is more susceptible to outside perturbation. This might eventually limit the application at an extremely low volume.

In addition, the theoretical resolution obtained from thermal insulation and temperature resolution could not be easily guaranteed in real applications. Thermal signal could arise from anywhere, and any disturbance could hamper the resolution. For example, introducing an additional sample could deviate the temperature from its original, and thus the ability to monitor the minimum temperature change could deteriorate as the measurement range is enlarged (Fig. 14). Moreover, baseline drift resulted from the gradual change in temperature or asymmetry between sample and reference could dramatically limit the detection ability. They could be deemed as unwanted energy flow added to the theoretical noise additionally.

D. Integration for industrial usage

Nanocalorimeters for macromolecular interaction sensing have the potential to enable high throughput drug screening.[23,26] Yet, as of now, it is almost impractical to apply nanocalorimeters for industrial applications. Real industrial usage would consider device reliability, ease of operation, and readiness for automation. These were still the shortcomings of nanocalorimeters compared to conventional ones, and additional efforts are needed to overcome the technical difficulties.

V. CONCLUSION AND FUTURE PERSPECTIVES

The power of calorimeters in biosensing has been extensively demonstrated in macromolecule interaction detection and celluar activity monitoring. Nanocalorimeters used low volume samples in thermal measurement with high throughput. Potentially, this could propel the drug discovery process and enable disease diagnosis.

The need for high performance nanocalorimeters in the biosensing field has stimulated its development in the past few decades. Different thermal sensing, thermal insulation, liquid delivery methods, and sensing configurations were explored, and we categorized them in detail in this paper. In recent years, they demonstrated many successful applications, as we listed some of them to inspire future extensions. Despite the exploding research papers showing latest progress, some technical difficulties were still hampering the nanocalorimeters' development progress. This review pointed them out for future design improvement.

Future prospects of biocalorimetry is highly promising, especially when the following aspects were taken care of. The bioprocess monitoring should be at even lower cost to meet the economic incentives. Similarly, there is a strong requirement for high robustness and easy manipulation devices. In this way, nanocalorimeters would have even higher impact applications.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (NNSFC) (Grant No. 61873307), the Fundamental Research Funds for the Central Universities (Grant No. N172304035), and the Scientific Research Project of Colleges and Universities in Hebei Province (Grant No. ZD2019305).

REFERENCES

[1]S. Leavitt and E. Freire, "Direct measurement of protein binding energetics by isothermal titration calorimetry," Curr. Opin. Struct. Biol. **11**(5), 560–566 (2001).
[2]K. P. Ritchie *et al.*, "Hyperthermia (heat shock)-induced protein denaturation in liver, muscle and lens tissue as determined by differential scanning calorimetry," Int. J. Hyperthermia **10**(5), 605–618 (2009).
[3]P. J. Mikulecky and A. L. Feig, "Heat capacity changes associated with nucleic acid folding," Biopolymers **82**(1), 38–58 (2006).
[4]C. Demetzos, "Differential scanning calorimetry (DSC): A tool to study the thermal behavior of lipid bilayers and liposomal stability," J. Liposome Res. **18**(3), 159–173 (2008).
[5]M. Wootton and A. Bamunuarachchi, "Application of differential scanning calorimetry to starch gelatinization. III. Effect of sucrose and sodium chloride," Starch **32**(4), 126–129 (1980).
[6]O. Braissant, A. Bachmann, and G. Bonkat, "Microcalorimetric assays for measuring cell growth and metabolic activity: Methodology and applications," Methods **76**, 27–34 (2015).
[7]J. E. Ladbury, G. Klebe, and E. Freire, "Adding calorimetric data to decision making in lead discovery: A hot tip," Nat. Rev. Drug Discov. **9**, 23 (2009).
[8]M. I. Recht *et al.*, "Enthalpy array analysis of enzymatic and binding reactions," Anal. Biochem. **377**(1), 33–39 (2008).
[9]C. J. Morton and J. E. Ladbury, "Water-mediated protein-DNA interactions: The relationship of thermodynamics to structural detail," Protein Sci. **5**(10), 2115–2118 (1996).
[10]S. Gelkop *et al.*, "T cell activation-induced CrkII binding to the Zap70 protein tyrosine kinase is mediated by Lck-dependent phosphorylation of Zap70 tyrosine 315," J. Immunol. **175**(12), 8123 (2005).
[11]H. Chen *et al.*, "Apolipoprotein E4 domain interaction mediates detrimental effects on mitochondria and is a potential therapeutic target for Alzheimer disease," J. Biol. Chem. **286**(7), 5215–5221 (2011).
[12]C. Joseph *et al.*, "A structural characterization of the interactions between titin Z-repeats and the α-actinin C-terminal domain," Biochemistry **40**(16), 4957–4965 (2001).
[13]W. H. J. Ward and G. A. Holdgate, "Isothermal titration calorimetry in drug discovery," Prog. Med. Chem. **38**, 309–376 (2001).
[14]N. C. Garbett *et al.*, "Calorimetric analysis of the plasma proteome: Identification of type 1 diabetes patients with early renal function decline," Biochim. Biophys. Acta **1830**(10), 4675–4680 (2013).

[15]T. D. Vreugdenburg *et al.*, "A systematic review of elastography, electrical impedance scanning, and digital infrared thermography for breast cancer screening and diagnosis," Breast Cancer Res. Treat. **137**(3), 665–676 (2013).
[16]M. Monti *et al.*, "Microcalorimetric investigation of cell metabolism in tumour cells from patients with non-Hodgkin lymphoma (NHL)," Scand. J. Haematol. **36**(4), 353–357 (1986).
[17]M. M. Schuler *et al.*, "Investigation of the potential of biocalorimetry as a process analytical technology (PAT) tool for monitoring and control of Crabtree-negative yeast cultures," Appl. Microbiol. Biotechnol. **93**(2), 575–584 (2012).
[18]M. M. Schuler and I. W. Marison, "Real-time monitoring and control of microbial bioprocesses with focus on the specific growth rate: Current state and perspectives," Appl. Microbiol. Biotechnol. **94**(6), 1469–1482 (2012).
[19]T. Maskow *et al.*, "Potentials and limitations of miniaturized calorimeters for bioprocess monitoring," Appl. Microbiol. Biotechnol. **92**(1), 55–66 (2011).
[20]S. Yu *et al.*, "Review of MEMS differential scanning calorimetry for biomolecular study," Front. Mech. Eng. **12**(4), 526–538 (2017).
[21]A. W. van Herwaarden, "Overview of calorimeter chips for various applications," Thermochim. Acta **432**(2), 192–201 (2005).
[22]S. Yu *et al.*, "A novel polyimide based micro heater with high temperature uniformity," Sens. Actuators A Phys. **257**, 58–64 (2017).
[23]F. E. Torres *et al.*, "Enthalpy arrays," Proc. Natl. Acad. Sci. U.S.A. **101**(26), 9517–9522 (2004).
[24]L. Wonhee *et al.*, "High-sensitivity microfluidic calorimeters for biological and chemical applications," Proc. Natl. Acad. Sci. U.S.A. **106**(42), 18040–18040 (2009).
[25]E. B. Chancellor *et al.*, "Heat conduction calorimeter for massively parallel high throughput measurements with picoliter sample volumes," Appl. Phys. Lett. **85**(12), 2408–2410 (2004).
[26]S. Wang *et al.*, "Micro-differential scanning calorimeter for liquid biological samples," Rev. Sci. Instrum. **87**(10), 105005 (2016).
[27]J. Koh, W. Lee, and J. H. Shin, "High-sensitivity chip calorimeter platform for sub-nano watt thermal measurement," Sens. Actuators A Phys. **241**, 60–65 (2016).
[28]J. Wu *et al.*, "Polydimethylsiloxane microfluidic chip with integrated microheater and thermal sensor," Biomicrofluidics **3**(1), 012005 (2009).
[29]N. Inomata *et al.*, "Pico calorimeter for detection of heat produced in an individual brown fat cell," Appl. Phys. Lett. **100**(15), 154104 (2012).
[30]I. Voiculescu *et al.*, "Investigation of bimaterial cantilever beam for heat sensing in liquid," Sens. Actuators A Phys. **242**, 58–66 (2016).
[31]M. F. Khan *et al.*, "Heat capacity measurements of sub-nanoliter volumes of liquids using bimaterial microchannel cantilevers," Appl. Phys. Lett. **108**(21), 36–52 (2016).
[32]T. Hayashi *et al.*, "A cell-permeable fluorescent polymeric thermometer for intracellular temperature mapping in mammalian cell lines," Plos One **10**(2), e0117677 (2015).
[33]V. N. Hoang, G. V. Kaigala, and C. J. Backhouse, "Dynamic temperature measurement in microfluidic devices using thermochromic liquid crystals," Lab Chip **8**(3), 484 (2008).
[34]J. Yang, H. Yang, and L. Lin, "Quantum dot nano thermometers reveal heterogeneous local thermogenesis in living cells," ACS Nano **5**(6), 5067–5071 (2011).
[35]G. Kucsko *et al.*, "Nanometre-scale thermometry in a living cell," Nature **500**(7460), 54–58 (2013).
[36]A. Lochbaum *et al.*, "On-chip narrowband thermal emitter for mid-IR optical gas sensing," ACS Photonics **4**(6), 1371–1380 (2017).
[37]A. Minakov *et al.*, "High-speed dynamics of temperature distribution in ultrafast (up to 10^8 K/s) chip-nanocalorimeters, measured by infrared thermography of high resolution," J. Appl. Phys. **125**(5), 054501 (2019).
[38]S. Wang *et al.*, "Atomic layer-deposited titanium-doped vanadium oxide thin films and their thermistor applications," J. Electron. Mater. **46**(4), 2153–2157 (2017).
[39]S. Sadat, E. Meyhofer, and P. Reddy, "High resolution resistive thermometry for micro/nanoscale measurements," Rev. Sci. Instrum. **83**(8), 084902 (2012).
[40]K. Amemiya, D. Fukuda, and T. Zama, "Very low-noise large-area calorimeter based on bimetallic micromechanical transduction: Toward sub-nanowatt resolution in air at room temperature," Appl. Phys. Express **9**(11), 117201 (2016).
[41]B. G. Burke and D. A. LaVan, "Characterization of a 10-MHz quadrant APD for measuring frequency oscillations and tip displacements of microcantilevers," Appl. Phys. B **109**(1), 127–132 (2012).
[42]N. Inomata, M. Toda, and T. Ono, "Highly sensitive thermometer using a vacuum-packed Si resonator in a microfluidic chip for the thermal measurement of single cells," Lab Chip **16**(18), 3597–3603 (2016).
[43]B. Lubbers and F. Baudenbacher, "Isothermal titration calorimetry in nanoliter droplets with subsecond time constants," Anal. Chem. **83**(20), 7955–7961 (2011).
[44]F. Yi and D. A. La Van, "Nanoscale thermal analysis for nanomedicine by nanocalorimetry," Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. **4**(1), 31–41 (2012).
[45]B. Wang, Y. Jia, and Q. Lin, "A microfabrication-based approach to quantitative isothermal titration calorimetry," Biosens. Bioelectron. **78**, 438–446 (2016).
[46]S. Wang *et al.*, "Microfabrication of plastic-PDMS microfluidic devices using polyimide release layer and selective adhesive bonding," J. Micromech. Microeng. **27**(5), 055015 (2017).
[47]Y. Zhang and S. Tadigadapa, "Calorimetric biosensors with integrated microfluidic channels," Biosens. Bioelectron. **19**(12), 1733–1743 (2004).
[48]B. Davaji *et al.*, "A novel on-chip three-dimensional micromachined calorimeter with fully enclosed and suspended thin-film chamber for thermal characterization of liquid samples," Biomicrofluidics **8**(3), 034101 (2014).
[49]J. Lerchner *et al.*, "Nano-calorimetry of small-sized biological samples," Thermochim. Acta **477**(1–2), 48–53 (2008).
[50]T. Maskow *et al.*, "Chip calorimetry for the monitoring of whole cell biotransformation," J. Biotechnol. **122**(4), 431–442 (2006).
[51]S. Sadat *et al.*, "Room temperature picowatt-resolution calorimetry," Appl. Phys. Lett. **99**(4), 043106 (2011).
[52]T. Adrega and A. W. van Herwaarden, "Chip calorimeter for thermal characterization of bio-chemical solutions," Sens. Actuators A Phys. **167**(2), 354–358 (2011).
[53]E. Iervolino, A. W. van Herwaarden, and P. M. Sarro, "Calorimeter chip calibration for thermal characterization of liquid samples," Thermochim. Acta **492**(1), 95–100 (2009).
[54]J. Xu *et al.*, "A microfabricated nanocalorimeter: Design, characterization, and chemical calibration," Anal. Chem. **80**(8), 2728–2733 (2008).
[55]B. Lubbers *et al.*, "Microfabricated calorimeters for thermometric enzyme linked immunosorbent assay in one-nanoliter droplets," Biomed. Microdevices **21**(4), 85 (2019).
[56]E. Kazura *et al.*, "Nano-calorimetry based point of care biosensor for metabolic disease management," Biomed. Microdevices **19**(3), 50 (2017).
[57]M. Modaresifar and G. J. Kowalski, "Microscale calorimetric device for determining reaction parameters," Thermochim. Acta **655**, 137–144 (2017).
[58]P. Bataillard, "Calorimetric sensing in bioanalytical chemistry: Principles, applications and trends," TrAC Trends Anal. Chem. **12**(10), 387–394 (1993).
[59]S. Yoon, S. Park, and Y. Kim, "A micromachined microcalorimeter with split-flow microchannel for biochemical sensing applications," Sens. Actuators B Chem. **134**(1), 158–165 (2008).
[60]M. M. C. H. van Schie *et al.*, "Fast and accurate enzyme activity measurements using a chip-based microfluidic calorimeter," Anal. Biochem. **544**, 57–63 (2018).
[61]V. Baier *et al.*, "Highly sensitive thermopile heat power sensor for micro-fluid calorimetry of biochemical processes," Sens. Actuators A Phys. **123–124**, 354–359 (2005).
[62]M. A. Sen *et al.*, "A continuous flow microfluidic calorimeter: 3-D numerical modeling with aqueous reactants," Thermochim. Acta **603**, 184–196 (2015).
[63]L. M. Ahmad *et al.*, "Binding event measurement using a chip calorimeter coupled to magnetic beads," Sens. Actuators B Chem. **145**(1), 239–245 (2010).
[64]L. Wang, B. Wang, and Q. Lin, "Demonstration of MEMS-based differential scanning calorimetry for determining thermodynamic properties of biomolecules," Sens. Actuators B Chem. **134**(2), 953–958 (2008).

[65]S. Yu *et al.*, "Thermodynamic analysis of a MEMS based differential scanning calorimeter model," Sens. Actuators A Phys. **291**, 150–155 (2019).
[66]S. Wang *et al.*, "A power compensated differential scanning calorimeter for protein stability characterization," Sens. Actuators B Chem. **256**, 946–952 (2018).
[67]R. Splinter, "Fast differential scanning calorimetry of liquid samples with chips," Thermochim. Acta **603**, 162–171 (2015).
[68]R. Splinter *et al.*, "Measuring human blood serum with chip based fast liquid differential scanning calorimetry," Thermochim. Acta **639**, 76–83 (2016).
[69]T. A. Mukhametzyanov *et al.*, "Fast scanning calorimetry of lysozyme unfolding at scanning rates from 5 K/min to 500,000 K/min," Biochim. Biophys. Acta **1862**(9), 2024 (2018).
[70]J. L. Garden *et al.*, "Thermodynamics of small systems by nanocalorimetry: From physical to biological nano-objects," Thermochim. Acta **492**(1–2), 16–28 (2009).
[71]J. Lerchner, T. Maskow, and G. Wolf, "Chip calorimetry and its use for biochemical and cell biological investigations," Chem. Eng. Process. **47**, 991–999 (2008).
[72]F. Buchholz *et al.*, "Chip-calorimetry provides realtime insights into the inactivation of biofilms by predatory bacterial," Biofouling **3**(28), 351–362 (2012).
[73]F. Mariana *et al.*, "Chip-calorimetric monitoring of biofilm eradication with antibiotics provides mechanistic information," Int. J. Med. Microbiol. **303**(3), 158–165 (2013).
[74]T. Hartmann *et al.*, "Thermopile chip based calorimeter for the study of aggregated biological samples in segmented flow," Sens. Actuators B Chem. **201**, 460–468 (2014).
[75]A. Wolf *et al.*, "Toward high-throughput chip calorimetry by use of segmented-flow technology," Thermochim. Acta **603**, 172 (2014).
[76]J. Lerchner *et al.*, "A chip calorimetry-based method for the real-time investigation of metabolic activity changes in human erythrocytes caused by cell sickling," J. Therm. Anal. Calorim. **136**(2), 771–781 (2019).
[77]J. Lerchner *et al.*, "Direct determination of anaerobe contributions to the energy metabolism of Trypanosoma cruzi by chip calorimetry," Anal. Bioanal. Chem. **411**(17), 3763–3768 (2019).
[78]R. Krenger, T. Lehnert, and M. A. M. Gijs, "Dynamic microfluidic nanocalorimetry system for measuring Caenorhabditis elegans metabolic heat," Lab Chip **18**(11), 1641–1651 (2018).
[79]M. K. Sato *et al.*, "Temperature changes in brown adipocytes detected with a bimaterial microcantilever," Biophys. J. **106**, 2458–2464 (2014).
[80]M. Faheem Khan *et al.*, "Nanomechanical identification of liquid reagents in a microfluidic channel," Lab Chip **14**(7), 1302–1307 (2014).
[81]T. Yamada, N. Inomata, and T. Ono, "Sensitive thermal microsensor with pn junction for heat measurement of a single cell," Jpn. J. Appl. Phys. **55**(2), 027001 (2016).
[82]I. Voiculescu *et al.*, "Nano and microsensors for mammalian cell studies," Micromachines (Basel) **9**, 439 (2018).
[83]E. Dechaumphai and R. Chen, "Sub-picowatt resolution calorimetry with niobium nitride thin-film thermometer," Rev. Sci. Instrum. **85**(9), 094903 (2014).
[84]J. Zheng *et al.*, "Sub-picowatt/kelvin resistive thermometry for probing nanoscale thermal transport," Rev. Sci. Instrum. **84**(11), 114901 (2013).
[85]B. Davaji and C. H. Lee, "A paper-based calorimetric microfluidics platform for bio-chemical sensing," Biosens. Bioelectron. **59**, 120–126 (2014).
[86]Z. Wang, M. Kimura, and T. Ono, "Manufacturing and characterization of simple cantilever thermal biosensor with Si-metal thermocouple structure for enzymatic reaction detection," Thermochim. Acta **668**, 110–115 (2018).
[87]N. Inomata, M. Toda, and T. Ono, "Microfabricated temperature-sensing devices using a microfluidic chip for biological applications," Int. J. Autom. Technol. **12**(1), 15–23 (2018).
[88]Z. Wang *et al.*, "Enzyme immobilization in completely packaged freestanding SU-8 microfluidic channel by electro click chemistry for compact thermal biosensor," Process Biochem. **79**, 57–64 (2019).
[89]Z. Wang *et al.*, "Silicon-based micro calorimeter with single thermocouple structure for thermal characterization," IEEE Electron Device Lett. **40**(7), 1198–1200 (2019).
[90]N. Inomata *et al.*, "Vanadium oxide thermal microsensor integrated in a microfluidic chip for detecting cholesterol and glucose concentrations," Microsyst. Technol. **23**(7), 2873–2879 (2017).
[91]M. K. Khaw, F. Mohd-Yasin, and N. T. Nguyen, Microelectron. Eng. **158**, 107–117 (2016).