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nanoDSF analysis of *Halobacteria* proteome

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Key-words

Halophile, salinity, nanoDSF, protein stability, protein solubility, *Haloarcula marismortui*

Abstract

Adaption to environmental conditions is reflected by protein adaptation. In particular, proteins of extremophiles display distinctive traits ensuring functional, structural and dynamical properties under permanently extreme physical and chemical conditions. While it has mostly been studied with approaches focusing on specific proteins, biophysical approaches have also confirmed this link between environmental and protein adaptation at the more complex and diverse scale of the proteome. However, studies of this type remain challenging and often require large amounts of biological material.

We report here the use of nanoDSF as a tool to study proteome stability and solubility in cell lysates of the model halophilic archaeon *Haloarcula marismortui*. Notably, our results show that, as with single halophilic protein studies, proteome stability was correlated to the concentration of NaCl or KCl under which the cells were lysed and hence the proteome exposed. This work highlights that adaptation to environmental conditions can be experimentally observed at the scale of the proteome. Still, we show that the biochemical properties of single halophilic proteins can only be partially extrapolated to the whole proteome.

Introduction

In cellulo biochemical and biophysical properties of proteins result from a complex interplay of factors: temperature, pH, ionic force, water activity, interactions with other biomolecules, crowding, etc. While the cell may control some of these factors to preserve protein functions, physical and chemical conditions of the environment can drastically alter protein behaviours. In particular, in extreme environments, organisms thrive under permanent extreme conditions and their proteins face considerable physical and chemical challenge. However, it is now clear that protein adaptation is tightly associated with environmental adaptation, especially for extremophiles (Reed et al. 2013; Singh et al. 2020; Ando et al. 2021). Notably, comparative studies between homologous proteins of organisms isolated from various environments are useful for assessing the degree of adaptation of an organism to various conditions (Coquelle et al. 2010; Dick et al. 2016). However, protein traits associated with adaptation to extreme conditions and their consequences on function, stability, solubility or dynamics have mostly been shown with model enzymes which do not reflect the complexity and the diversity of the proteome.

While *in silico* approaches highlight global sequence biases in extremophiles associated with protein adaptation as revealed by *in vitro* studies of specific proteins (Kozlowski 2017; Avagyan et al. 2020; Panja et al. 2020), only few approaches have been used to experimentally assess protein adaptation at the scale of the proteome. In particular, *in vivo* neutron scattering have allowed quantitative observation of the intracellular dynamics of proteins (Zaccai 2020), further stressing the link between proteome properties, environmental adaptation and cellular fitness (Zaccai 2013) as exemplified with thermophiles and psychrophiles (Tehei et al. 2004), piezophiles (Martinez et al. 2016) and halophiles (Vauclare et al. 2015, 2020). However, this technique remains complex and requires large amounts of biological material.

Here we show that differential scanning fluorimetry (DSF), and especially label-free nanoDSF, represents a trackable approach to assess protein adaptation to environmental conditions at the scale of the proteome. This biophysical technique measures intrinsic fluorescence of tryptophan and light scattering as a proxy for, respectively, protein stability and solubility over a temperature gradient and allows experimental determination of apparent protein melting temperature (T_m). Among the many applications of nanoDSF, it has been extensively used for screening of chemical conditions (buffer and ligands) favoring protein stability or solubility, making it useful for structural studies of specific proteins (Niesen et al. 2007; Phillips and de la Peña 2011). In contrast, system-wide uses of DSF, such as thermal proteome profiling (TPP) (Savitski et al. 2014; Mateus et al. 2016; Sridharan et al. 2019), thermal proximity coaggregation (TPCA) (Tan et al. 2018) or Cellular Thermal Shift Assay (CETSA) (Tan et al. 2015; Dai et al. 2019) use full or lysed cells rather than purified proteins or complexes. However, to our knowledge, these approaches have mostly been limited to drug interaction screening and have never been used to measure general biophysical properties of a proteome. In this study, we used the model halophilic archaea *Haloarcula marismortui* to prove the usefulness of nanoDSF to highlight the link between proteome and environmental adaptations.

Halophilic microorganisms require high concentrations of salt in their environments to achieve optimal growth and thrive in hypersaline environments such as the Dead Sea, the Great Salt lake or the Lake Natron. Among them, halophilic archaea from the Halobacteria class often display the highest salt-tolerance and salt-dependency. *H. marismortui* was

isolated from the Dead Sea (Ventosa et al. 2015) and grows in media containing 1.7-5.1M NaCl and achieve optimal growth at 3.4-3.9M (Ventosa et al. 2015). In these organisms, the outside osmolarity of Na^+ is mainly countered by a high intracellular accumulation of K^+, which may reach concentrations of 2 to above 4M while intracellular concentration of Na^+ generally remains below 2M. Depending on the growth conditions and on the technique used, it has been shown that *H. marismortui* can intracellularly accumulate 1.4-5.5M K^+ (Ginzburg et al. 1970; Jensen et al. 2015). While K^+ is slightly more kosmotropic than Na^+, according to Hofmeister series, cytoplasm of these halophiles remains an extreme environment itself.

To cope with these extreme intracellular conditions, halobacterial proteins display various traits which have been extensively studied with model proteins such as the malate dehydrogenase (MDH) of *H. marismortui*. In comparison to their non-halophilic homologs, halophilic proteins are overall highly negatively charged and less hydrophobic, especially at the surface (Coquelle et al. 2010). The associated multilayered (Britton et al. 2006) solvation shell associates water and salt ions such as Na^+, K^+, Cl^- (Bonneté et al. 1993; Madern et al. 2000b) and displays a disruption of pentagonal water networks (Talon et al. 2014), in which fewer water molecules bind salt ions more tightly. As a result, halophilic proteins present enhanced solubility and stability in hypersaline environments (Madern et al. 2000a; Tadeo et al. 2009). These traits make them highly unstable in low salinity environments: most unfold under 2-3M KCl (Eisenberg 1995; Coquelle et al. 2010). However, many proteins in Halobacteria show a combination of properties of both halophilic and non-halophilic proteins (Coquelle et al. 2010). In particular, DNA-binding, membrane and ribosomal proteins present less halophilic traits (Becker et al. 2014). Therefore, one should be extremely careful when extrapolating behavior of a single protein to all elements of the proteome.

Here we present the first proteome thermal denaturation analysis in Halobacteria using nanoDSF.

Materials and methods

Cultivation of *H. marismortui* cells

H. marismortui was cultivated aerobically at 37°C under mild agitation. Initial stocks were purchased at the DSMZ-German Collection of Microorganisms. Composition of medium was 208g.L^{-1} NaCl, 78g.L^{-1} $MgCl_2 \cdot 6H_2O$, 500mg.L^{-1} $CaCl_2 \cdot 2H_2O$, 125mg.L^{-1} $MnCl_2$, 5g.L^{-1}, NaBr 580mg.L^{-1}, 10g.L^{-1} yeast extract, pH adjusted to 7.5 with addition of 10M NaOH after dissolution.

E. coli was cultivated was cultivated aerobically at 37°C under stronger agitation and cells were pelleted in exponential phase.

Preparation of proteome extracts

2mL of a culture in mid or late exponential phase was centrifugated at 20,000 g for 15 minutes. Supernatant was discarded by aspiration and pellet was resuspend in 1mL of solutions of various KCl or NaCl concentrations. Cells were then lysed by sonication or by making them go back and forth several times through a 0.6mm diameter needle using a syringe, taking advantage of the inner fragility of halobacterial cells.

MDH protein production and purification

H. marismortui malate dehydrogenase (MDH) enzyme was produced and purified as previously described (Bonneté et al. 1994).

Characterization of *H. marismortui* proteome and MDH by differential scanning fluorimetry

Cell lysates extracts were centrifugated at 20,000 g for 15 minutes and the supernatant (soluble fraction of cell lysate) was loaded in Prometheus™ NT.48 capillaries. Thermal denaturation curves were determined by measurements of protein intrinsic fluorescence. This analysis was performed using label-free, native differential scanning fluorimetry (nanoDSF; apparatus: Prometheus NT.48, NanoTemper). The tryptophan residues of the proteins in proteome extracts were excited at 280 nm and the fluorescence intensity was recorded at 330 and 350 nm. Excitation power was set at 80 or 100%. The temperature of the measurement compartment increased from 20 to 95 °C at a rate of 2°C per minute. Light scattering was also measured as a way to detect aggregation events during heating. For renaturation assays, after final temperature reached it immediately decreased back to 20°C at the same rate of 2°C per minute.

H. marismortui MDH was diluted in various salt solutions to the same final protein concentration (5ng.mL^{-1}).

Results

To investigate proteome dependency on salt in an extreme halophilic microorganism, we used nanoDSF to measure the stability and aggregation state of proteins contained in soluble fraction of *H. marismortui* cells lysed in various NaCl and KCl solutions. After centrifugation, the supernatant was loaded in the capillaries, hence limiting the sample to the fraction of the proteome that remains soluble in the corresponding salt conditions.

Detailed data measured by the Prometheus NT.48 (NanoTemper) are given in Fig. S1. Like single protein studies with nanoDSF, as described in application notes of the manufacturer (Martin et al. 2017), first derivative of ratio of 350/330nm fluorescence and first derivative of light scattering were easier to interpretate and compare between salt conditions than any of the other measurements (350 and 330 fluorescence respective signals, derivatives or not, 350/330nm ratio, light scattering). Thus, thermal denaturation and aggregation curves for the proteins contained in the capillaries were obtained. In spite of the fact that the sample contained numerous types of proteins, peaks of fluorescence ratio and light scattering derivatives, respectively associated with main unfolding and aggregation events, could be reproducibly observed. In particular, harvesting cells at various OD_{600} during the exponential phase had little effect on denaturation curve shape and peak position, which was linearly correlated to salt concentration, either NaCl or KCl (Fig. S2).

To assess dependency of peak position on biological material abundancy, the lysates were diluted by various factors, while remaining in 4M KCl, and and thermal denaturation and aggregation curves were compared (Fig. S3). Height of aggregation peaks was very sensitive to dilution factor as no clear peak could be observed when lysates were diluted 10 times. Denaturation peaks were less sensitive to dilution factor as they could still be recognized at higher dilutions but with decreasing sharpness and resolution. Moreover, dilution slightly shifted the horizontal position of the peak but to a lower extent than changing salt concentration did in the previous experiment. Nonetheless, little difference was observed in the thermal denaturation profile when with a dilution factor of 2.

To compare behaviour of *H. marismortui* proteome to a single halophilic protein used as control, thermal denaturation and aggregation curves were obtained with both cell lysates and purified MDH enzyme from the same organism (Fig. 1a and Fig. 1b). For both samples, increasing concentration of NaCl or KCl delayed thermal denaturation. However, while MDH of *H. marismortui* reached its peak stability at 4.0M NaCl, proteome stability continued to be stabilized by higher concentrations of NaCl. In contrast, no denaturation peak could be obtained with *E. coli* lysates in similar conditions, even at lowest salt concentrations (Fig. S4).

Unlike denaturation curves, aggregation curves presented one or two peaks of first derivative of light scattering at more constant temperatures, around 70-75°C for cell lysed in most conditions and always at temperatures higher than the ones associated with peak denaturation event. Sharpest and most-recognizable peaks happened at high concentrations of NaCl or KCl, depending on the experiment (Fig. S2) Aggregation peak at concentration of salt below 3M was observed only once.

As with denaturation, no aggregation peak could be observed when the capillaries were loaded with *E. coli* lysates under any salt concentration (Fig. S4), showing that the peaks could not be a product of the salt only. Even less conditions allowed observation of aggregation peaks with the purified *H. marismortui* MDH as they could only be clearly observed with NaCl and at concentrations above 4.0M.

After the maximum temperature (95°C) was reached, the measurement continued along a temperature decrease to the initial temperature (20°C) by selecting the refolding ramp option with the aim to investigate the reversibility of the denaturation and aggregation events observed during the heating process. The associated curves are given in Fig. 2a and Fig. 2b. During the cooling ramp, peaks of first derivative of ratio of 350/330nm fluorescence, putatively associated with renaturation events, were observed with both *H. marismortui* cell lysate and MDH but only in the presence of 2.5 to 3.5M of either NaCl or KCl. In addition, these peaks were fainter and less sharp than those observed in the denaturation curves. Moreover, no peak of first derivative of light scattering was observed at all.

Since denaturation curves of cell lysates were more complex than those of the MDH, we chose thereafter to compare only the proteome melting temperatures (T_{Pm}), namely temperatures associated with dominant denaturation event and peak of first derivative of ratio of 350/330nm fluorescence. To assess reproducibility of relation between salt concentration and T_{Pm} value, we retrieved them from the 4 independent experiments shown in Fig. S1, obtained with *H. marismortui* cells pelleted from independent cultures at various OD_{600} values. T_{Pm} was plotted as a function of NaCl and KCl concentration (Fig. 3a and Fig. 3b) and slopes of the lines fitting the data were calculated (Fig 3c). Comparing slopes rather than T_{Pm}, allowed direct comparison of dependence of thermostability on NaCl and KCl of proteins contained in the soluble fraction of cell lysates. Results showed that T_{Pm} was statistically and significantly more dependent on KCl than NaCl and higher, at equal molarity, with KCl than with NaCl.

Discussion

By using nanoDSF to analyze cell lysates of *H. marismortui* in various salt conditions, we could successfully and reproducibly analyze the KCl and NaCl dependencies of stability and solubility of the proteome. Since lysates were centrifugated prior to loading the capillaries, the representative character of the proteome of the samples remains questionable. In particular, proteins which were not soluble in the salt solutions, such as membrane proteins, probably pelleted and did not contributed to the denaturation and aggregation curves. Yet, the complexity of the curves and the linear response to the salt concentration suggest that the capillaries contained a large portion of similar proteins of the soluble fraction the proteome. Still, the observations could either be representative of the global protein compositions or dominated by one or few specific proteins which would be more abundant or would have more DSF-relevant tryptophan residues.

Since cytosol ionic composition in Halobacteria is dominated by K^+ rather than Na^+, most NaCl conditions did not reflect physiological intracellular environment. However, various studies have shown that *H. marismortui* can accumulate Na^+ at concentrations as high as 2.6-3.0M, depending on the growth conditions and stage (Ginzburg and Ginzburg 1976; Thombre et al. 2016). Nonetheless, independently from the physiological likeliness of the conditions, studying biochemical and biophysical properties of halophilic proteins in high concentrations of ions less abundant in cytosols of Halobacteria such as Na^+ and Mg^{2+} have revealed intriguing effects of salt type and concentration on protein stability and solubility (Ebel et al. 2002).

Overall, our data showed that increasing salinity delayed thermal denaturation of the *H. marismortui* proteome, which was interpretated as an expected stabilizing effect of both

NaCl and KCl. Consistent with previous studies on this single protein (Calmettes et al. 1987; Madern and Zaccai 1997; Coquelle et al. 2010), MDH was also similarly stabilized by increasing concentration of both NaCl and KCl. However, as indicated by the shift of the melting temperature, MDH was more sensitive than the proteome to the increase of NaCl or KCl concentration. Moreover, the purified enzyme reached peak stability at 4M NaCl whereas the proteome of *H. marismortui* continued to be stabilized to the highest concentrations. In addition, a denaturation peak could be observed when cells were lysed in deionized water (0M salt condition) whereas the MDH showed no denaturation peak at concentrations lower than 1M KCl or NaCl. Since MDH of *H. marismortui* has been shown to almost entirely precipitate at such low salinities (Coquelle et al. 2010), the capillaries in the corresponding conditions may have been loaded with less to no protein. While the cells may have released some intracellular ions during the lysis, their dilution was too high to explain the persistence of a proteome denaturation peak in pure water. Other explanations include the presence of other cellular factors, such as chaperonins or small organic molecules ensuring proteome stability in low salt conditions, or the possibility that proteins from high molecular weight assemblies, which retain activity and hence structure in low salt conditions (Shevack et al. 1985) showing a lower sensitivity to low salinity conditions, such as ribosomes, which are less sensitive to low salinity conditions, contributed more to the peak than in the other salt conditions. In addition, other enzymes from *H. marismortui* are functional below 2M KCl, such as a catalase-peroxidase (Cendrin et al. 1994), an esterase (Camacho et al. 2009), an AMP-forming acetyl-CoA synthetase (Bräsen and Schönheit 2005) and an alkaline phosphatase (Goldman et al. 1990).

The denaturation curves of *E. coli* lysates showed an opposite behaviour with an initial decrease of first derivative of ratio of 350/330nm fluorescence which was followed by an increase, even when cells were lysed in pure water (0M salt condition). However, the signal over noise ratio was considered to be too low to propose proper explanations for such profile, especially in comparison to the experiments with *H. marismortui*. Absence of both denaturation and aggregation clear peaks with lysates of *E. coli* may be caused by the fact that its proteins could denature on a wider range of temperatures, limiting the possibility of observing define peaks.

Interpretation of the absence of aggregation peak at low salinities was even less trivial. Indeed, aggregation curves of MDH showed no peaks below 4.0M of NaCl or at any KCl conditions. Since proteins of halophiles are exceptionally soluble (Talon et al. 2014) and since denaturation peaks were observed at the same time, an explanation for the absence of aggregation peak could be that proteins, pure MDH and those in cell extract as well, remained soluble after denaturation in low salinity conditions. However, as aggregation was dependent on dilution factor, such explanation should be taken with caution.

The observed specific proteome behaviour could result from the fact that it contains proteins which are less acidified than model halophilic enzymes such as the MDH. In particular, it has been shown that protein acidification occurs to a lesser extent in various protein types: membrane-associated proteins, subunits of large molecular weight assemblies and nucleic acid-binding proteins, which are either excluded from the cytosolic hypersaline solvent or interact with DNA or RNA, which are negatively charged, hence requiring positively charged residues (Becker et al. 2014). As such, proteasome (Franzetti et al. 2002) and ribosome (Shevack et al. 1985) of *H. marismortui* are less sensitive to low KCl concentrations than MDH. Contribution of these proteins, especially ribosomal proteins which are among the most abundant in many cell types (Liebermeister et al. 2014), may hence explain the differences

observed between *H. marismortui* proteome and MDH. Nonetheless, this stresses that the behaviour of the proteome can only partially be extrapolated from that of a given enzyme.

Our experimental setup allowed little to no observation of peaks of first derivative of ratio of 350/330nm fluorescence during the cooling step following the heating. When observed, these renaturation peaks were less distinctive than those associated with denaturation. Moreover, no peak of first derivative of light scattering could be observed at all, showing that aggregation process was also mostly irreversible. Since aggregation peaks happened after denaturation peaks during the heating step, irreversible aggregation may be the reason why renaturation was very limited, even with the pure MDH. In the case of the proteome, the limited renaturation could be associated with a specific subset of proteins whose denaturation was reversible, rather than a partial renaturation of the whole proteome. This was unlike the ß-lactamase from the moderately halophilic bacterium *Chromohalobacter* sp. 560 which has been shown to refold spontaneously after thermal denaturation in 0.2M NaCl (Tokunaga et al. 2004). However, after being expressed in non-halophilic *Escherichia coli* cells where it has undergone low salt denaturation, *H. marismortui* MDH can be refolded *in vitro* by increasing the salinity (Cendrin et al. 1993; Franzetti et al. 2001). Similarly, a nucleoside diphosphate kinase from the extremely halophilic archaea *H. salinarum*, which has been expressed in *E. coli*, has also been shown to require high salt concentration for refolding while being stable and active in low salinity conditions (Ishibashi et al. 2001), stressing the possibility of renaturation after low salt denaturation.

However, it should be kept in mind that renaturation may be enhanced by changing the maximum temperature reached during the cooling step. Nonetheless, salt plugs were occasionally observed at the ends of the capillaries, presumably due to water evaporation, hence potentially changing the salt concentrations which the samples were exposed to. Hence, nanoDSF may not allow the proper monitoring of renaturation processes as well as denaturation and aggregation processes for halophilic systems.

As observed with T_{Pm} values and dependencies on salts, KCl was significantly more efficient at stabilizing *H. marismortui* proteome than NaCl. While Na^+ is closer to the salting-in and denaturing side of the Hofmeister series (Okur et al. 2017), several studies suggest instead that K^+ is more chaotropic (Collins 1997) or less kosmotropic (Cray et al. 2013). Nonetheless, studies on single proteins have shown contrasted effects of these salts on their biophysical and biochemical properties.

Interestingly, neutron scattering experiments have shown that MDH of *H. marismortui* is more resilient in NaCl than in KCl (Ebel et al. 2002), contributing to a higher stability in NaCl than in KCl (Tehei et al. 2001, 2004). While Na^+ is more stabilizing at low salt concentrations, little difference between the two cations on stabilitiy of MDH of *H. marismortui* was reported at high concentrations (Zaccai et al. 1986b; Madern and Zaccai 1997). In addition, it has been shown that this enzyme interacts with slightly more salt ions and slightly less water molecules in NaCl than in KCl solutions (Bonneté et al. 1993). However, the requirement for salt on stability is higher at low temperatures (Zaccai et al. 1986a; Bonneté et al. 1994). Nonetheless, stabilisation of this enzyme in hypersaline solvents is of enthalpic nature, in contrast to the entropic stabilization of non-halophilic BSA by salts (Tehei et al. 2001).

Moreover, it has been observed that unfolding of this enzyme was faster in low concentrations (1M) of KCl than in NaCl (Pundak et al. 1981). Similarly, two dihydrofolate

reductases from *Haloferax volcanii* have been shown to be more stabilized by NaCl than by KCl (Wright et al. 2002).

Concerning the enzymatic activity, various intracellular enzymes of *Halaorchaea*, such as 3-hydroxy-3-methylglutaryl-coenzyme A reductase (Bischoff and Rodwell 1996) or NAD^+-dependent DNA ligase (Poidevin and MacNeill 2006), both from of *H. volcanii*, were shown to be more active in KCl. In contrast, it has been shown that extracellular proteinases of *Halobacterium salinarum*, which are exposed to a NaCl-rich extracellular environment, display enhanced activity with NaCl rather than KCl (Norberg and Hofsten 1969).

Nevertheless, our results show that soluble fraction of proteome in cell lysate of the extremely halophilic archaea *H. marismortui* does not behave identically to one of its purified proteins and is more efficiently stabilized by KCl rather than NaCl. In contrast to the enthalpy-dominated stabilization of its MDH, proteome of *H. marismortui* could be for example stabilized by a combination of entropic and enthalpic effects. Because of the specificities of each particular enzyme, the link between protein adaptation and environment adaptation should be highlighted with global, proteome-wide approaches rather than single protein studies.

Moreover, this work confirms that reduced proteome resilience, and presumably lowered protein stability (Tehei et al. 2004), observed with cells of *H. salinarum* stressed with low salt conditions (Vauclare et al. 2020) can be directly explained by the effect of lower intracellular accumulation of KCl on proteome behaviour rather than accumulation of small molecular weight compounds or by a change in protein expression.

Conclusion

In spite of the fact that nanoDSF was developed for simpler systems, salt-dependency of *H. marismortui* could successfully be established and compared to those of a purified enzyme from the same organism. While both were stabilized by increasing salt concentration, *H. marismortui* proteome, unlike its MDH, was more efficiently stabilized by KCl, the physiological intracellular salt of Halobacteria, than by NaCl, the dominant salt in most hypersaline environments. Since properties of single proteins may not reflect those of the global proteome from which they originate, biophysical approaches like the one proposed here may help understanding the link between protein adaptation and adaptation to an environment.

This work represents, to our knowledge, the first attempt to assess stability of large fractions of the proteome of extreme halophiles using nanoDSF. This technique shows enough sensitivity to observe both denaturation and aggregation peaks in such complex samples in various conditions. Comparative analysis using this technique may support interpretation of other biophysical measurements such as neutron scattering. Moreover, this technique could be used with cell lysates from other organisms to compare their biophysical and biochemical traits in relation to the physical and chemical conditions of their relative environments. In particular, it may unravel specific traits at the proteome level unseen with single-protein studies in thermophiles, psychrophiles, acidophiles or alkaliphiles.

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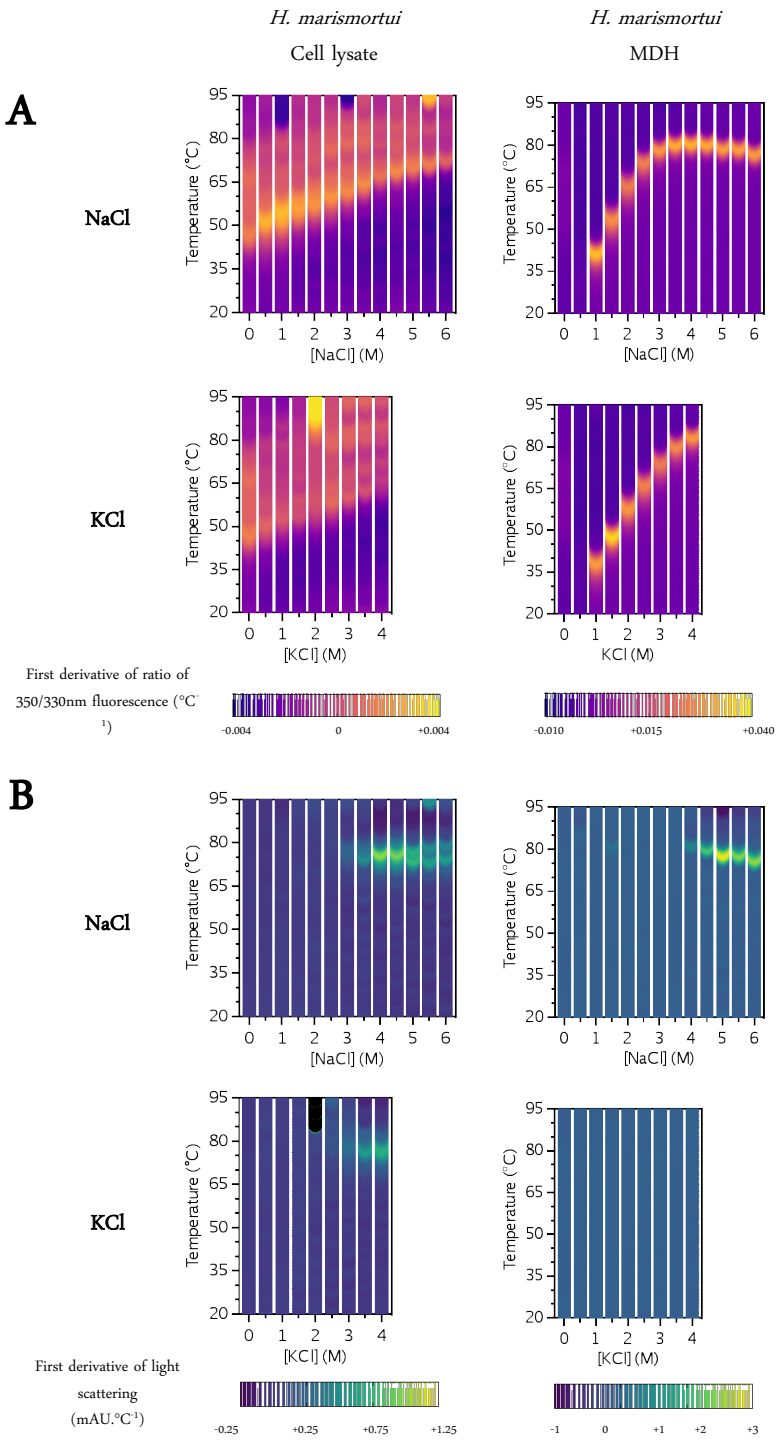
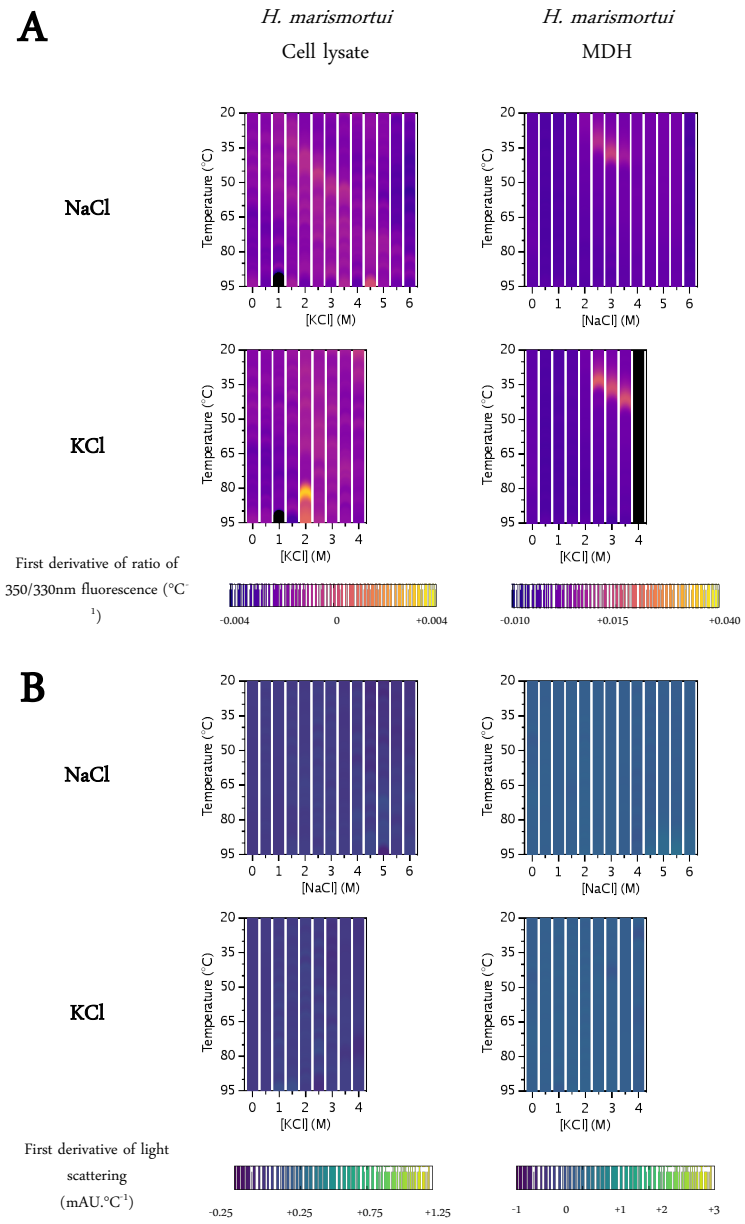

A
B
H. marismortui
Cell lysate
H. marismortui
MDH
NaCl
KCl
Temperature (°C)
[NaCl] (M)
[KCl] (M)
KCl (M)
First derivative of ratio of 350/330nm fluorescence (°C-1)
-0.004
0
+0.004
-0.010
+0.015
+0.040
First derivative of light scattering (mAU.°C-1)
-0.25
+0.25
+0.75
+1.25
-1
0
+1
+2
+3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First derivative of ratio of 350/330nm fluorescence (°C-1)
-0.004
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+0.004
-0.010
+0.015
+0.040
B
NaCl
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First derivative of light scattering (mAU.°C-1)
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+0.75
+1.25
-1
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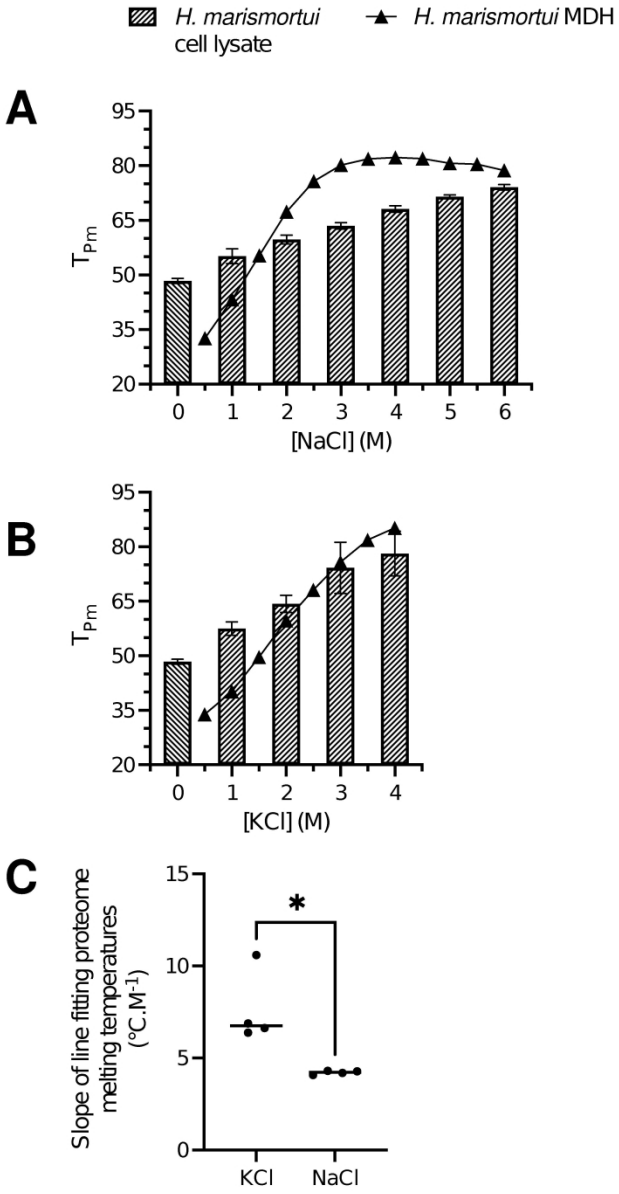
+1
+2
+3



Average values TPm defined as the temperature associated with maximum first derivative of 350/330nm fluorescence ratio measured with H. marismortui cell lysates under various concentrations of NaCl (A) and KCl (B) in 4 independent experiments. Abnormal high values at the end of the heating were excluded. Errors bars represent standard deviation. Melting temperatures of H. marismortui MDH, which were measured identically, are also given. For each independent experiment, linear regression of TPm as a function of NaCl and KCl concentration was made, as shown in Fig. S2b. Slopes of lines fitting the values are given and their difference is significative (*) according to a Mann-Whitney test (P = 0.086) (C).

118x219mm (600 x 600 DPI)

Fig. S1 Details of nanoDSF analysis of *H. marismortui* cell lysates

Values of 350 and 330nm fluorescence and their first derivatives, of 350/330 ratio and light scattering measured on soluble fractions of *H. marismortui* cells lysates upon a 20-95°C temperature increase at various NaCl and KCl concentrations. Thes data correspond to those presented in Fig. 1 (OD_{600} = 1.104).

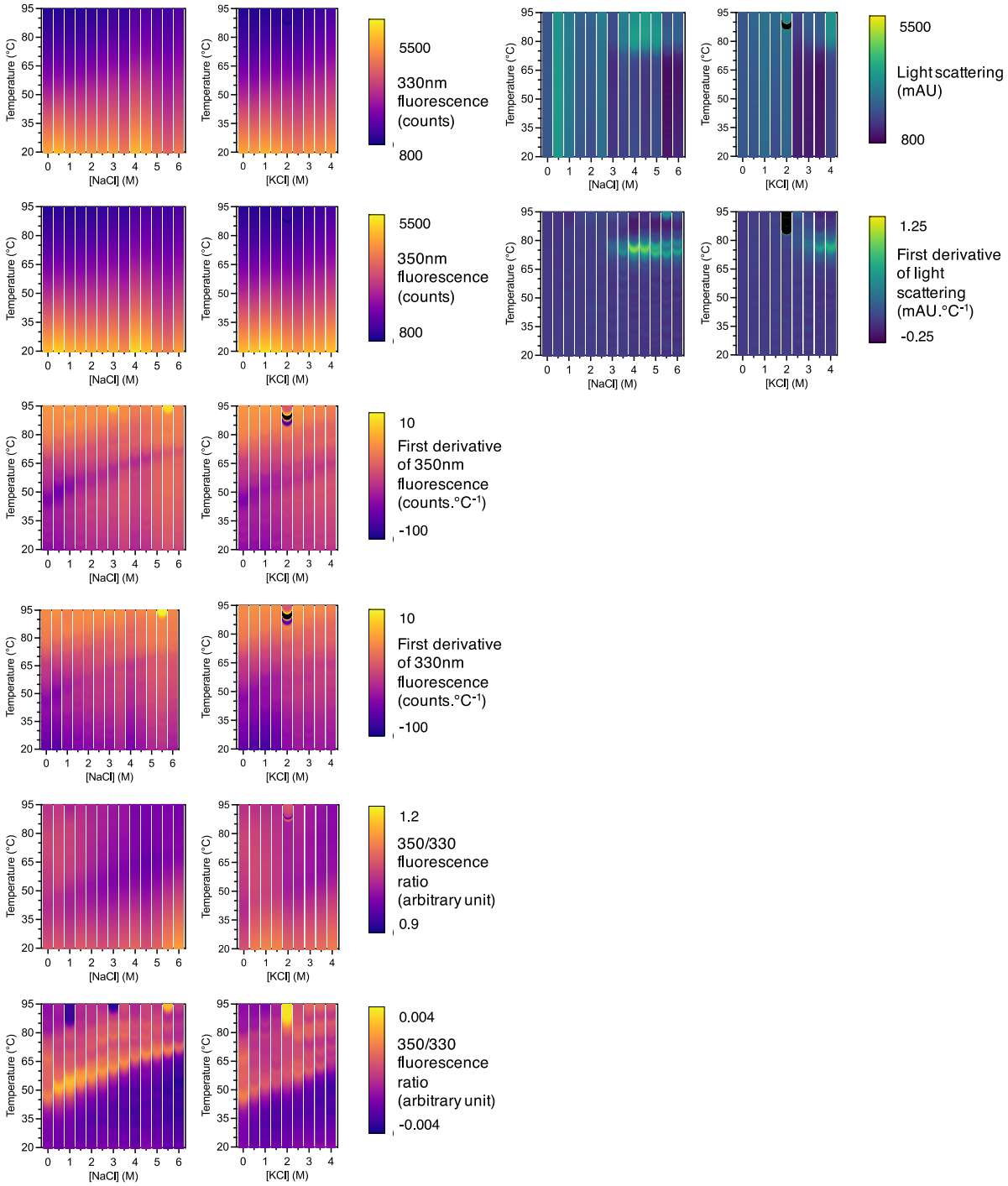


Fig. S2 Reproducibility of nanoDSF analysis of *H. marismortui* cell extracts

NanoDSF analysis of *H. marismortui* cells harvested at various OD_{600} values (A) and values of proteome melting temperature (T_{Pm}), defined as the temperature associated with the peak of first derivative of ratio of 350/330nm fluorescence (B). Abnormal values occasionally happening close to the end of the experiment were excluded. Relation between salt concentration and T_{Pm} was fitted for each experiment with a linear regression. r^2 values are indicated. Data was obtained with larger steps in salt concentrations than in Fig. 1, except for the first column (DO 1.104) which was derived from this figure.

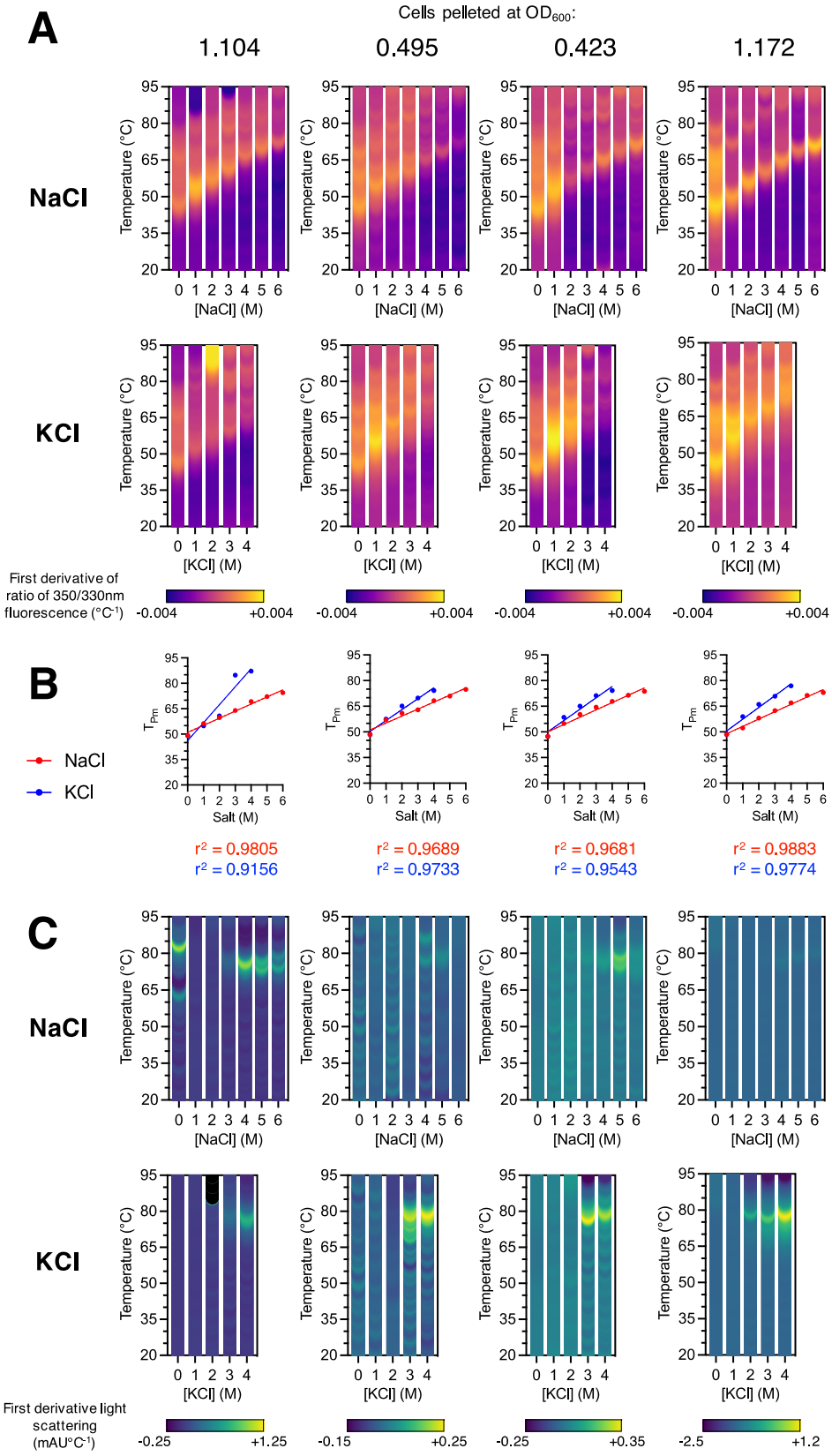


Fig. S2 Effect of dilution on thermal denaturation and aggregation curves of *H. marismortui* lysates

Final concentration of KCl was always 4M, only lysates were diluted.

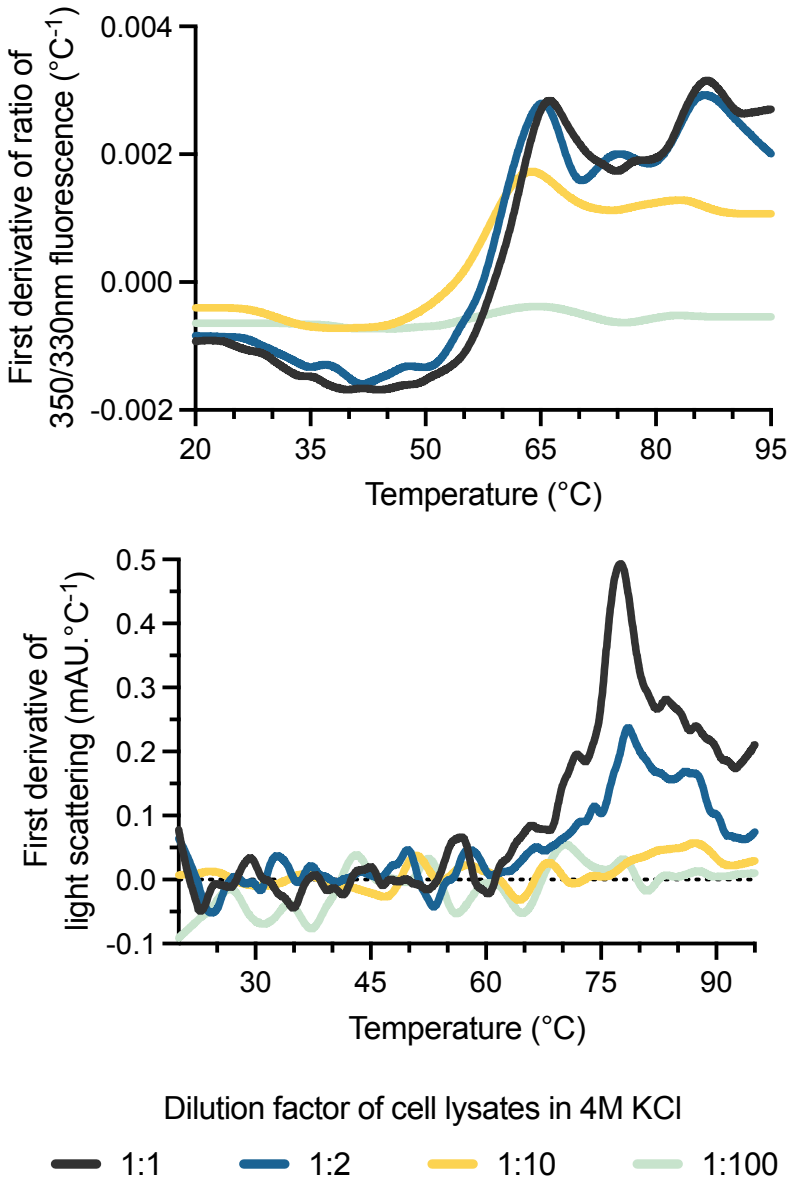


Fig. S4 nanoDSF analysis of *E. coli* cell lysates upon heating

NanoDSF analysis of cell lysates of non-halophilic *Escherichia coli* in various salt conditions. On the left, the color scales are the same than those used in Fig. 1 and Fig. 2. On the right a different color scale was used to improve contrast.