Thermophoresis

Like charged molecules move in electrical fields, temperature gradients also induce a directed motion of molecules. The effect is known as thermophoresis, thermodiffusion or Soret effect.

Basics of thermophoresis

- When an aqueous solution is locally heated particles start to move in the given temperature gradient. The resulting movement is described by a linear thermophoretic drift $v = - D_T \nabla T$ which is then counteracted by a backdiffusive flux $j = -D \nabla c$ with diffusion coefficient D. These two fluxes lead to a steady state molecule distribution which is characterized by the Soret coefficient. The Soret coefficient is defined as the ratio $S_T = D_T/D$ and determines the magnitude of the change in concentration in steady state. In steady state, a temperature difference ΔT results in a change in concentration Δc, which can be derived from the following equation $c/c_0 = \exp(-S\Delta T)$. The concentration in the heated region c is normalized with respect the initial concentration 0 *c* before applying the temperature gradient.
- But the theoretical models predicting thermophoresis in aqueous solutions are still subject of ongoing debate and experiments. There are various approaches ranging from hydrodynamic flow theories and thermo-electrophoresis (i.e. a Seebeck effect) to thermodynamic models. In case of the Seebeck effect, ions in the buffer move along a thermal gradient and give rise to an electric field, which in turn moves the molecules by electrophoresis. But it was found that for buffer systems based on NaCl which are used for many biotechnological applications, the resulting Seebeck contribution is suggested to be small and cannot be responsible for the measured concentration gradients. To give an estimate if hydrodynamic flows can account for the particle movement an effective Peclet number P_e is calculated:

$$P_e = a\, v/D = a\, (D_T/D)\, \nabla T = a\, S_T\, \nabla T$$

Here, a denotes the particle radius, v the particle drift velocity, D the diffusion coefficient, D_T the thermophoretic mobility, S_T the Soret coefficient and ∇T the temperature gradient. For microscopic particles in moderate temperature gradients this Peclet number is much smaller than 1. Such a system is diffusion dominated and hydrodynamic flows play a minor role. Only provided that $P_e >> 1$, which is experimentally challenging to achieve even for micrometer-sized particles, a local fluid flow of solvent around the molecules could experimentally be confirmed.

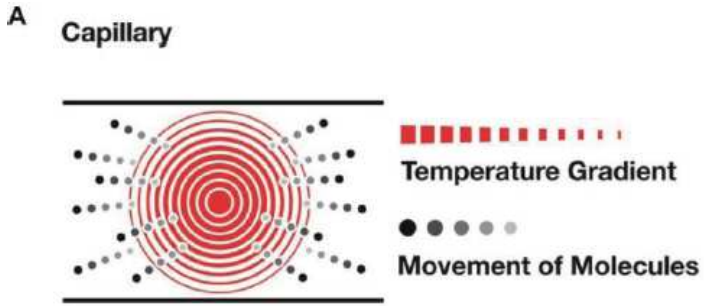
- In the limit of moderate temperature gradients, diluted molecules and under interface-free conditions, the steady state of thermophoresis can be described by a local Boltzmann distribution of the particle density. The diffusive movement of single molecules allows the reversible equilibration between particle positions due to temperature differences in the Gibbs free enthalpy of the molecule-solvent complex. Assuming a local equilibrium the Soret coefficient *ST* is given by the temperature derivative of the Gibbs free energy *G* of the molecule-solvent complex:

$$S_T = (1/k_B T) \cdot (\partial G/\partial T) = - S/k_B T.$$

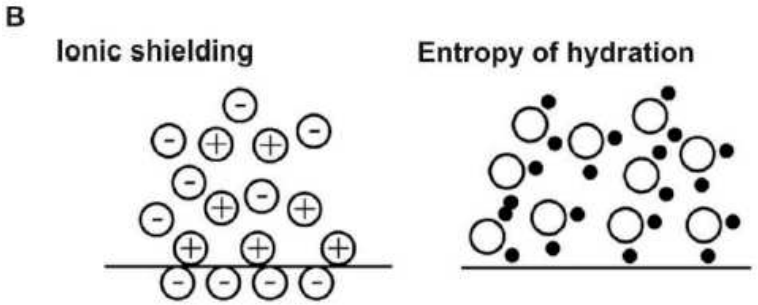
The second part of the equation is derived by locally applying the thermodynamic relation $dG=-SdT+Vdp+\mu dN$. For single particles at constant pressure the Soret coefficient equals the negative entropy S of the particle-solvent system. In water, two contributions dominate the particle entropy: the entropy of ionic shielding and the entropy of hydration (Figure 1).

Duhr,S. and Braun,D. (2006) Why molecules move along a temperature gradient. *Proc. Nat. Acad Sci. USA*, **103**, 19678–19682.
Duhr,S. and Braun,D. (2006) Thermophoretic Depletion Follows Boltzmann Distribution, *Phys. Rev. Lett.*, **96**, 168301.

➢ Effects of particle-particle interactions can be neglected as we normally work in highly diluted systems. The entropy of ionic shielding can be calculated from the temperature derivative of Gibbs free enthalpy. This enthalpy can be interpreted as the electric energy stored in a capacitor build of the molecule's surface and the surrounding ion cloud.



Molecules move in a temperature gradient, an effect termed thermophoresis. Typically, in aqueous solutions the movement is directed away from regions of elevated temperature.



Thermodiffusion in water is dominated by ionic shielding (left) and water hydration (right)

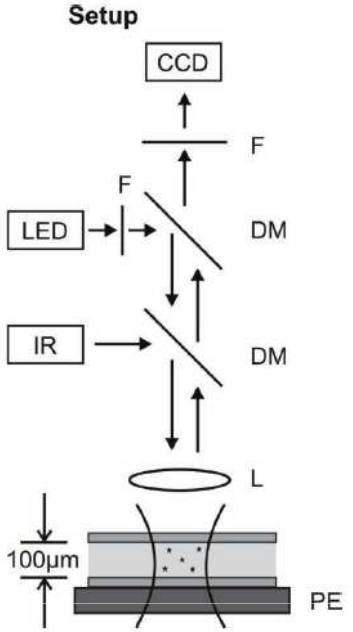
➢ With these contributions the Soret coefficient can be written as:

$$S_T = A/k_BT\ [\ -s_{hyd} + (\beta\sigma^2_{eff}/4\ \varepsilon\varepsilon_o\ T)\lambda_{DH}]$$

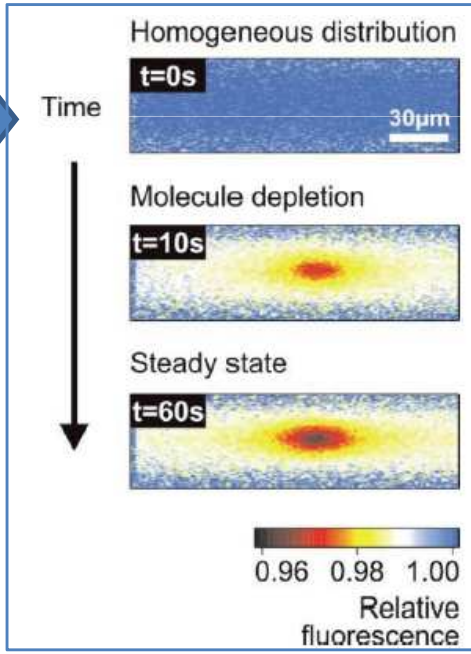
Here, s_{hyd} is the hydration entropy per molecule surface area A, k_BT the thermal energy, σ_{eff} the effective charge per surface area and ε and ε_0 the permittivity of water and free space, respectively. DH represents the Debye length corresponding to the salt concentration in use. The parameter $ß$ contains the temperature dependence of both permittivity of water and Debye length: $ß=1 - (T/\varepsilon)\ (d\ \varepsilon/dT)$. s_{hyd} accounts for the change in water structure due to the presence of the molecules, including for example the creation of the water cavity for the molecule and the hydrophobic interactions at its interface. The direct contribution from Brownian motion is typically small (ST = 0.0034 K^{-1}). However it can make a contribution for small molecules measured at large salt concentrations and thus add a small error to a derived value for the entropy of hydration. The approach was tested with polystyrene beads with varying particle size, salt concentration and temperature and for the case of double stranded DNA with varying length. The theory could characterize the Soret coefficient quantitatively without fitting parameters. Further confirmation of the approach resulted from the study of quartz beads. Subsequent experiments on polystyrene beads came to different conclusions concerning the size dependence. However, the thermodynamical description of thermophoresis depicts the movement of small particle in solutions quite well and indicates that the Soret effect might be able to monitor changes on a molecular level as they occur for example along binding reactions.

Hill,N.E. (1969) Temperature dependence of the dielectric properties of water, *J. Phys C*, **3**, 238-239.
Dhont,J.K.G. (2004) Thermodiffusion of interacting colloids. I. A statistical thermodynamics approach, *J. Chem. Phys.*, **120**, 1632.
Dhont,J.K.G. (2004) Thermodiffusion of interacting colloids. II. A microscopic approach, *J. of Chem. Phys.*, **120**, 1642.

Experimental Setup and Thermophoresis Measurements.



*An epi-fluorescence microscope is extended by an additional dichroic mirror (DM) that couples an infrared laser (IR) into the beam of light. The laser is focused into an aqueous solution inside a **capillary**. Due to absorption of IR radiation by the water molecules it creates a local temperature profile that allows quantifying the Soret coefficient of particles in the solution. The capillary is placed on top of a peltier stage (PE) to adjust the overall sample temperature. L, lens; LED, light emitting diode; F, filter; CCD, CCD camera.*



Fluorescence images are acquired over time. Initially, fluorescently labeled molecules are distributed evenly. After switching on the heating with a focused infrared-laser, the molecules experience the thermophoretic force in the temperature gradient and typically move out of the heated spot. In the steady state, this molecule flow is counterbalanced by ordinary mass diffusion and a steady-state concentration profile is established. From the concentration profile the Soret coefficient is inferred.

Baaske,P., Duhr,S. and Braun,D. (2007) Melting curve analysis in a snapshot,*Applied Physics Letters*, **91**, 133901.
Cordero,M.L., Verneuil,E., Gallaire,F. and Baroud,C.N. (2009) Time-resolved temperature rise in a thin liquid film due to laser absorption, *Physical Review E*, **79**, 011201.

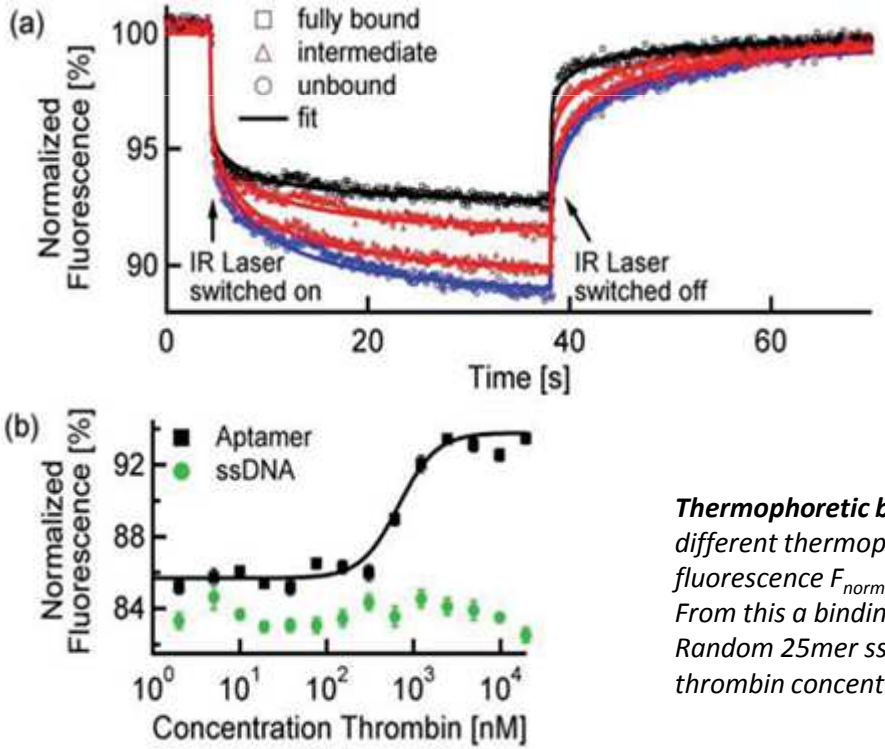
Thermophoresis –binding studies

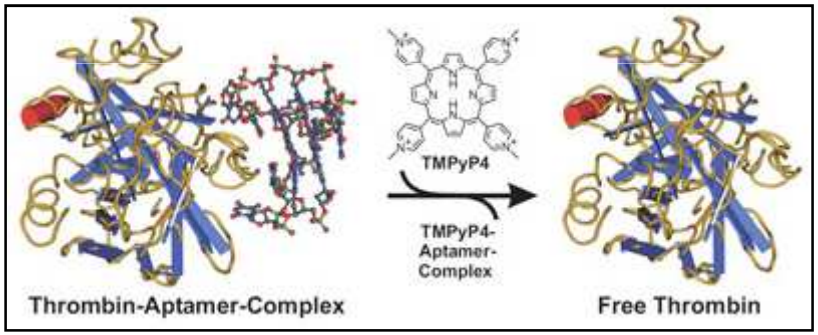
* Biomolecular interactions

To analyze binding events, the measurement is performed at various concentration ratios of the binding partners. Typically, the fluorescent binding partner is kept at a constant concentration and the unlabeled molecule is titrated until a saturation of all binding sites is obtained. Due to linearity of the fluorescence intensity and the thermophoretic depletion, the normalized fluorescence from the unbound molecule $F_{norm}(A)$ and the bound complex $F_{norm}(AT)$ superpose linearly. By denoting x the fraction of molecules bound to targets, the changing fluorescence signal during the titration of target T is given by:

$$F_{norm} = (1-x)F_{norm}(A) + xF_{norm}(AT)$$

The analysis of the interaction of a 5.6 kDa aptamer to thrombin (MW = 37 kDa) is used to exemplify the principle of thermophoretic binding analysis. The thermophoresis of 100 nM thrombin-aptamer was measured in 10% human serum with various concentrations of thrombin (0 nM to 19500 nM). The aptamer was labeled with a Cy5 dye at the 5' end. The observed time traces of the pure aptamer differ significantly from the traces of aptamers bound to thrombin (Figure a). Plotting the normalized fluorescence F_{norm} at a given time t against the varying thrombin concentration results in a binding curve (Figure b) with an EC_{50}=680 nM and a Hill coefficient of 2. Control experiments with a randomly chosen sequence ssDNA show no binding signal (Figure b).





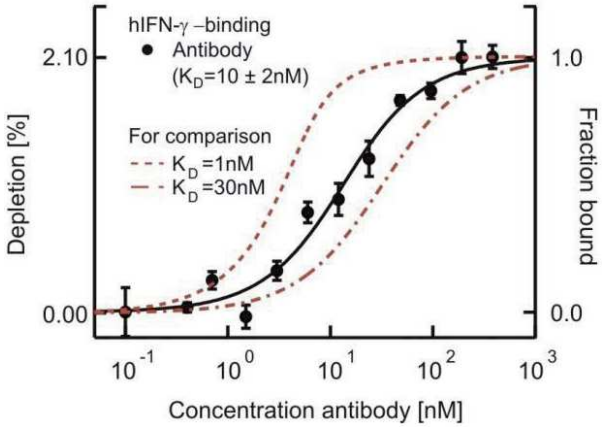
Thermophoretic binding studies. (a) *Time series of a DNA thrombin aptamer show different thermophoresis for the unbound and bound state.* ***(b)*** *The normalized fluorescence F_{norm} at t=30s is plotted for different concentrations of thrombin (black). From this a binding curve is inferred and the affinity of the interaction can be calculated. Random 25mer ssDNA (green) shows no change in thermophoresis with increasing thrombin concentration.*

L. C. Bock, L. C. Griffin, J. A. Latham, E. H. Vermaas, J. J. Toole, (1992) Selection of single-stranded-DNA molecules that bind and inhibit human thrombin. *Nature*, **355**, 564.

P.Baaske, C.J. Wienken, P. Reineck, S. Duhr, D. Braun (2010) Optical thermophoresis for quantifying the buffer dependence of aptamer binding. Angew. Chem. Int. Ed, 49, 1.

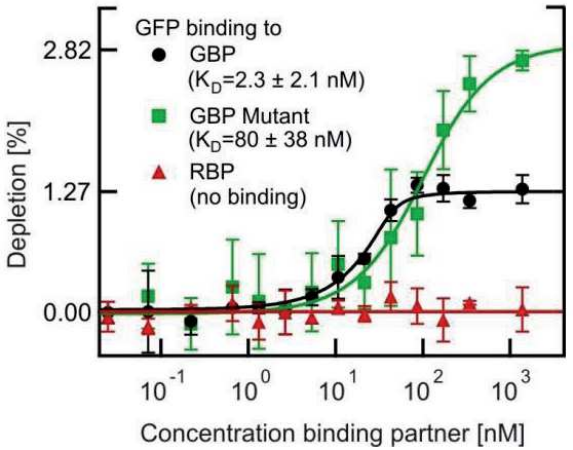
Thermophoresis –binding studies

* Protein-protein interactions



The interaction of an anti-Interferongamma antibody (Anti-IFN-γ, 120 kDa) with its antigen, human interferon gamma (hIFN-γ, 17 kDa). The interaction showed that also protein thermophoresis leads to sufficient thermophoresis signals which allow for a discrimination of different binding states. For comparison, the expected binding behavior for K_D = 1 nM and K_D = 30 nM is also plotted.

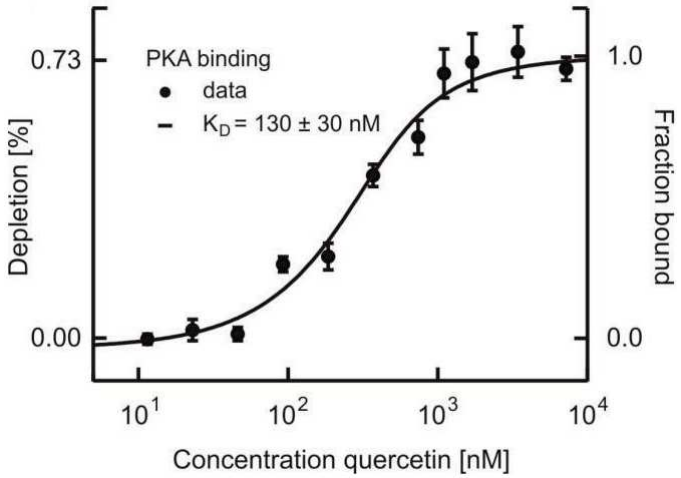
The interaction of the intrinsic fluorescent protein GFP with binders of varying affinities is measured. A reduced binding affinity from 2.3 ± 2.1 nM for GBP to 80± 38 nM for GBP mutante was detected. The depletion also changed from 1.27% to 2.82% for GBP and GBP mutnat (R37A) respectively. This indicates that **thermophoretic amplitude not only depends on the size of the complex but also contains contributions form both charge and structure**. GFP: a green fluorescent protein; GBP: a small antibody fragment known as GFP-binding protein; GBP mutant (R37A): single point mutantion at position 37 an arginine was replaced by an alanine.



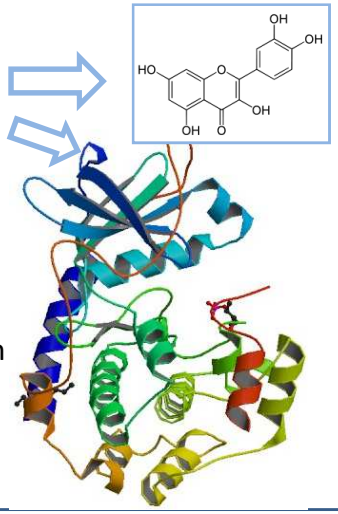
Wienken,C.J., Baaske,P., Rothbauer,U., Braun,D., Duhr,S. (2010) Protein Binding Assays in Biological Liquids using Microscale Thermophoresis. *Nature Communications*, **1**:100.

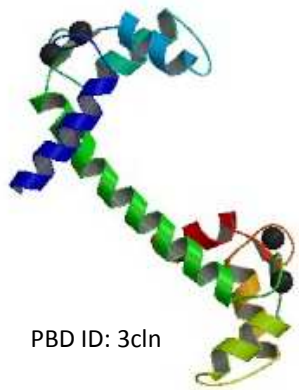
Thermophoresis –binding studies

* Small binding partner -protein interactions



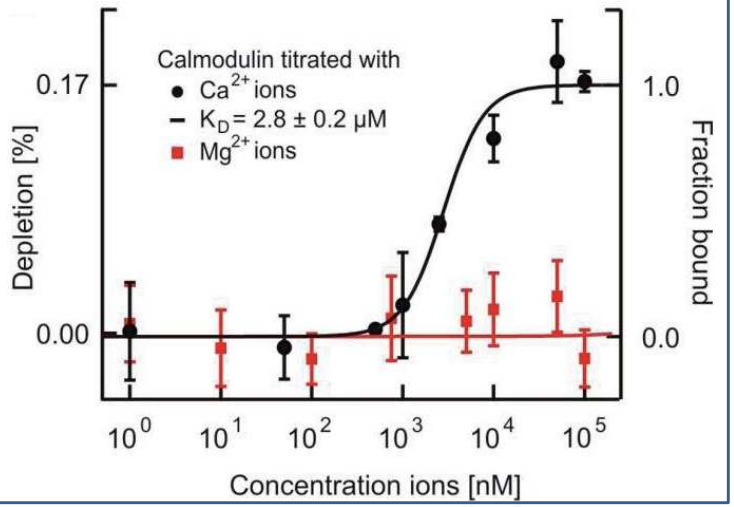
The binding of the small, uncharged inhibitor **quercetin** (0.34 kDa) to the **cAMP-dependent kinase** (PKA, 38kDa) is measured. The thermophoretic concentration signal changed from 3.8% for the unbound state to 4.53% for the bound state, showing a 19% relative change upon binding of quercetin (left). Despite the small changes in the molecule's mass (only 1%), a signal-to-noise ratio of 12.5 allows an accurate determination of the interaction strength. The determined affinity (130 ± 35 nM) is in good agreement with reported values.





PBD ID: 3cln

The binding of Ca^{2+}-ions to fluorescently labeled calmodulin (CaM, 16.7 kDa) is measured. CaM regulates a multitude of protein targets and is the primary receptor for calcium ions in human cells . Upon binding to calcium, CaM undergoes a conformational change, rearranging more than 35 water molecules per CaM which allows regulating a multitude of protein targets. This **conformational change** and **the rearranged water molecules** lead to a significant change in the thermophoretic properties. The inferred dissociation constant of 2.8 ± 0.2 µM and a cooperativity of 1.97 ± 0.2 are in good agreement with the reported K_D values for CaM-Ca^{2+} which in the range from 1 to 10 µM.

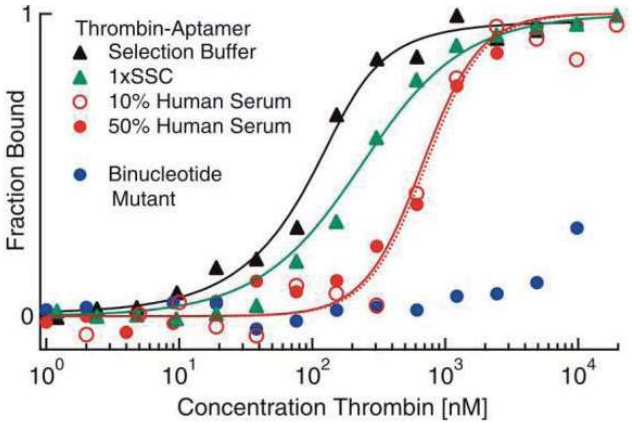


Project, E., Friedman, R., Nachliel, E., Gutman, M. (2006) A molecular dynamics study of the effect of Ca2+ removal on calmodulin structure. *Biophys. J.*, **90**, 3842- 3850.

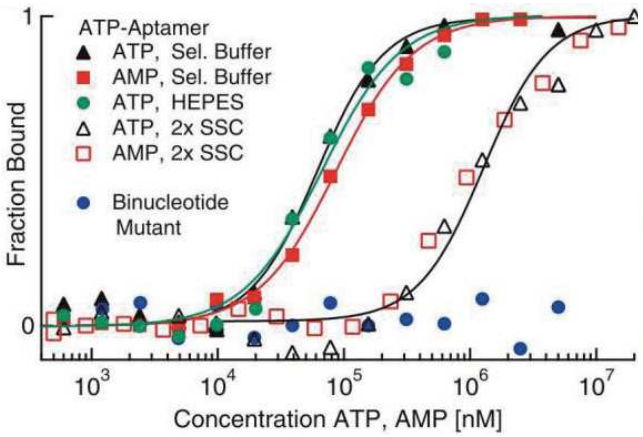
Tsuruta, H., Sano, T. (1990) A fluorescence temperature-jump study on Ca2+- induced conformational changes in calmodulin. *Biophys. Chem.,* **35**, 75-84.

Thermophoresis –binding studies

* Buffer dependence



Aptamer-thrombin binding in selection buffer, SSC (saline-sodium citrate) and 10% and 50% untreated human serum. a control experiment with a binucleotide mutant was performed that showed no binding. The experiments revealed a high dependence of the binding affinity on the biological matrix (i.e. solution in which the interaction takes place). While the dissociation constant for the aptamer-thrombin binding in selection buffer (K_D=30 nM) is in good agreement with literature, the affinity decreases to K_D=190 nM in 1xSSC. In 50% (10%) human serum the binding was best fitted with the Hill equation, yielding an EC_{50}=720 nM (670 nM).



ATP BINDING RNA APTAMER IN COMPLEX WITH AMP PDB ID: 1RAW

Aptamer binding to ATP and AMP in selection buffer, HEPES and 2x SSC. A control experiment with a binucleotide mutant was performed that showed no binding. A cooperative binding was found . Interestingly, in 2x SSC buffer, the EC_{50} values of the ATP/AMP-aptamer binding were both strongly shifted to lower affinities, resulting in an almost 20 times lower EC_{50}. For the ATP-aptamer the unexpected big shift to lower affinities in the SSC buffer as compared to the TRIS and HEPES buffers can be attributed to a competing interaction of the strongly negative $Citrate^{3-}$ with the Mg^{2+}-ions as the latter are essential for the aptamer-ATP/AMP binding.

P Reineck, C.J. Wienken, D Braun (2010) Thermophoresis of single stranded DNA. Electrophoresis 31, 279.

Advantages – Binding study

- High sensitivity,
  Not depend on the size ratio of the two binding partners (protein-protein affinities and low molecular weight binders).
- The use of a fluorescence label
  - Determine binding affinities directly in biological liquids, blood serum/ crude cell extract
  - Determine affinities of unpurified, expressed antibodies directly in the supernatant of a cell suspension --- fast antibody selection
  - Quantify the binding reaction near *in vivo* conditions --- dose response relations.
- Small sampling

Thermophoresis –Melting Studies

Fluorescence melting analysis usually is performed using intercalating fluorescent dyes, which fluoresce strongly in the presence of double-stranded DNA and show a considerable decreased fluorescence without double stranded DNA. The most familiar of these is ethidium bromide, but asymmetric cyanine dyes, such as SYBR Green I are even brighter and are dyes of choice in fluorescence melting analysis and RT-PCR. For protein melting curves, fluorescent dyes like SYPRO Orange are applied which bind to the hydrophobic regions of a protein and increase their fluorescence upon binding. When a protein unfolds more hydrophobic region become accessible for the dye and the fluorescence increases. **A major drawback of this technique** is that the intercalating dye may affect the stability of the protein. As thermophoresis also monitors surfaces properties of molecules it is possible to use this effect as a read-out for a melting curve analysis. Especially, the high sensitivity even to minute changes in the molecules surface will allow for a very detailed melting profile and provide deeper insight into the mechanisms upon unfolding.

Background

The temperature dependence of the Soret effect shows a characteristic dependence. They measured the thermophoretic properties of various biomolecules, which is described well with an empirical fitting function:

$$S_{T,i}(T) = S_{T,i}^{\infty}\left[1-\exp\left(\frac{T^{*}-T}{T_{0,i}}\right)\right]$$

In this equation S_T represents the thermophoretic limit for high temperatures T, T^* is the temperature where S_T switches its sign and T_0 is a rate of exponential growth that embodies the strength of the temperature effect. This high temperature limit of the Soret coefficient is like the Soret coefficient S_T at room temperature a unique characteristic for molecules and therefore also differs for double- and single-stranded molecules. This is reflected in the index i.

To allow a precise analysis of the melting temperature all thermophoretic melting curves are corrected for a standard temperature dependence as described above. This reduces the signal to transitions which deviate from the standard temperature dependence and allows monitoring changes in the molecular structure. Presenting the data in the thermophoretic limit for high temperatures S_T^{∞} provides information about the thermophoretic properties of a molecule like the molecular size and conformation and can be used to distinguish different kinds of molecules. This $S_{T,i}^{\infty}$, is constant for each conformational state i ={*ss*,*ds*} of the nucleic acid and due to the linearity of thermophoretic depletion, the experimentally measured apparent S_T^{∞} is a linear superposition of the closed and melted state, showing a transition from $S_{T,ds}^{\infty}$, to $S_{T,ss}^{\infty}$, given by: $S_T^{\infty}(x) = x \cdot S_{T,ss}^{\infty} + (1-x) S_{T,ds}^{\infty}$

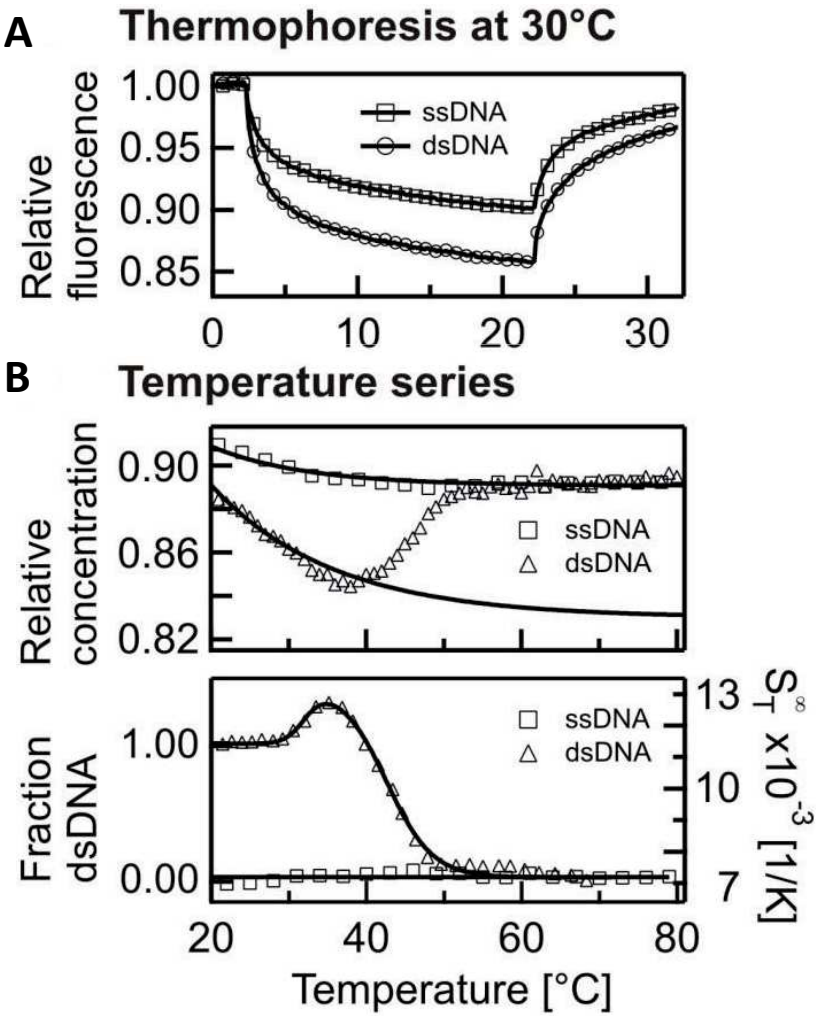
The parameter x represents the fraction of molecules in the single-stranded state. $S_{T,ss}^{\infty}$, is the high temperature limit S_T^{∞} for single-stranded DNA, $S_{T,ds}^{\infty}$, for double-stranded DNA. The melting temperature Tm represents the temperature at which x=0.5 holds. For most melting experiments the melting process is interpreted as a two state process between an open and denatured state of the molecule. Such an interpretation ignores intermediate states (e.g. due to the unfolding of tertiary structures) but still is a good approximation for many DNA melting curves. Nevertheless, $S_T^{\infty}(x) = x \cdot S_{T,ss}^{\infty} + (1-x) S_{T,ds}^{\infty}$ can also be expanded to a more complex unfolding behavior by introducing an additional intermediate state.

Iacopini,S., Rusconi,R., Piazza,R. (2004) The “macromolecular tourist”: Universal temperature dependence of thermal diffusion in aqueous colloidal suspensions. *Phys. J. E*, **19**, 59-67.
Mergny,J. and Lacroix,L. (2003) Analysis of thermal melting curves. *Oligonucleotides*, **13**, 515-537.

Thermophoretic melting curve. The overall sample temperature is increased stepwise typically between 15°C and 95°C depending on the expected thermal transitions of the sample. At every temperature the Soret coefficient is analyzed (Figure A). The Soret coefficient is then plotted over the sample temperature yielding a distinct melting profile for the sample molecule (Figure 13B, top). To obtain the exact melting profile the additional heating by the IR laser must also be taken into account. By correcting this temperature series with the temperature dependence of the Soret coefficient according to

$$S_{T,i}(T) = S_{T,i}^{\infty}\left[1-\exp\left(\frac{T^{*}-T}{T_{0,i}}\right)\right]$$

a melting profile of the high temperature limit S_T^{∞} (Figure B, bottom) is obtained.
From this the stability of the molecule can be derived and also possible additional intermediate states are observable.



(a) Single- and double-stranded DNA show different time series of the normalized fluorescence in the heated laser spot which is then converted to a relative concentration value.

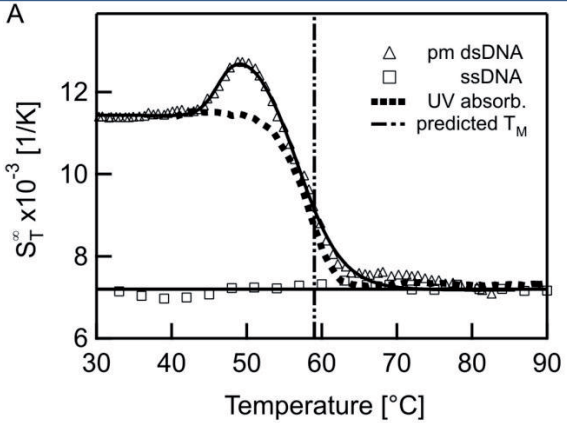
(b) When repeated for different temperatures, single-stranded DNA (ssDNA) as well as double-stranded DNA (dsDNA) shows a pronounced temperature dependence, which was fitted using

$$S_{T,i}(T) = S_{T,i}^{\infty}\left[1-\exp\left(\frac{T^{*}-T}{T_{0,i}}\right)\right]$$

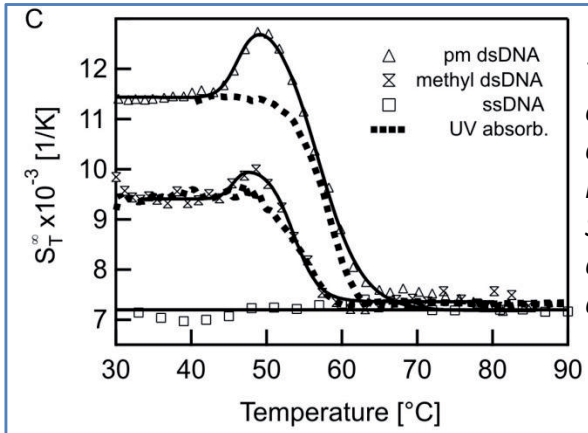
correcting the data for this temperature dependence, a typical DNA melting curve appears.

Thermophoretic melting curves quantify the conformation and stability of RNA and DNA. (2011) Nucleic acids research 39, e52.

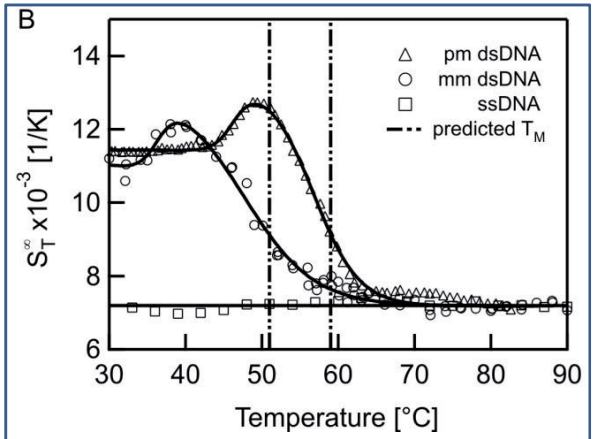
Nucleic Acids melting curves



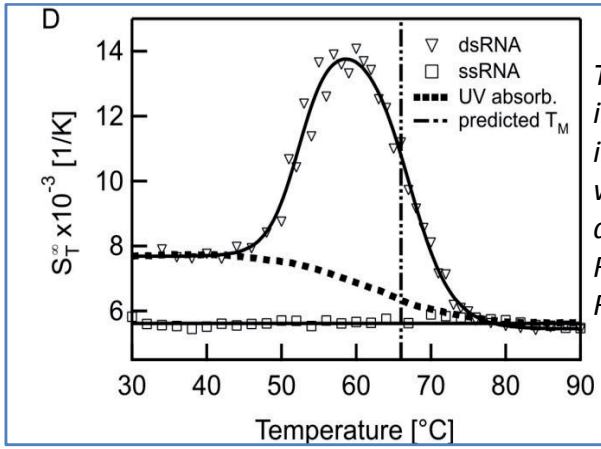
The melting of a 22 bp long DNA oligonucleotide was analyzed with thermophoresis and UV absorbance and compared to thermodynamic calculations.



The effect of methylated bases on the stability of DNA was analyzed with thermophoresis and compared to UV absorbance. Besides monitoring the melting of the two strands, the Soret coefficient at low temperatures showed a significant reduction in the thermophoretic amplitude resulting from the modified bases.



DNA containing a single base mismatch was compared to perfectly matching DNA. The mismatch showed a changed melting temperature Tm due to the lower thermal stability by the base mismatch.

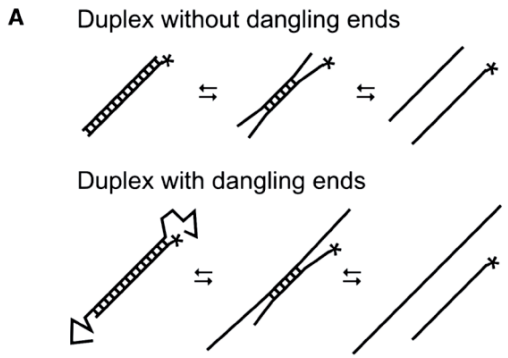


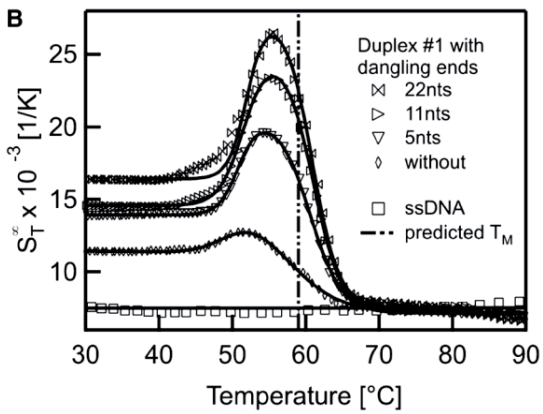
The melting of RNA showed a strong change in S_T^∞ before the actual melting occurs. This increase in S_T^∞ prior to melting for dsRNA was attributed to a partial melting of the double strand beginning at the ends of the RNA duplex. It was significantly larger for RNA as compared to DNA.

Thermophoretic melting curves quantify the conformation and stability of RNA and DNA. (2011) Nucleic acids research 39, e52.

Effects of dangling ends_ The hypothesis was tested with DNA strands with dangling ends added to both sides of the duplex. Such modification should increase the thermophoretic amplitude as the surface of the DNA strand increases upon unfolding.

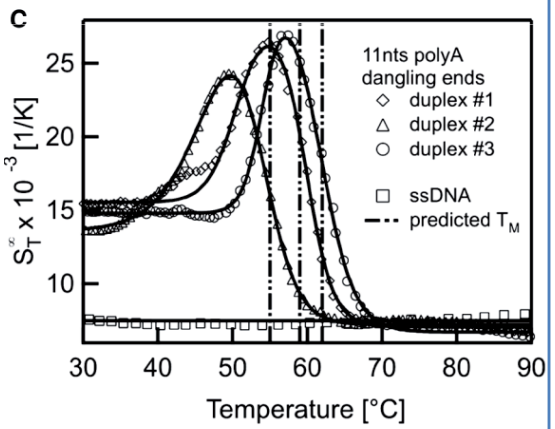
As a hypothesis for the transition before the actual melting of the two DNA strands, an unbinding of the duplex-ends in a pre-melting transition was proposed. This 'end effects' should increase when adding overlapping DNA strands to both sides of one duplex strand.

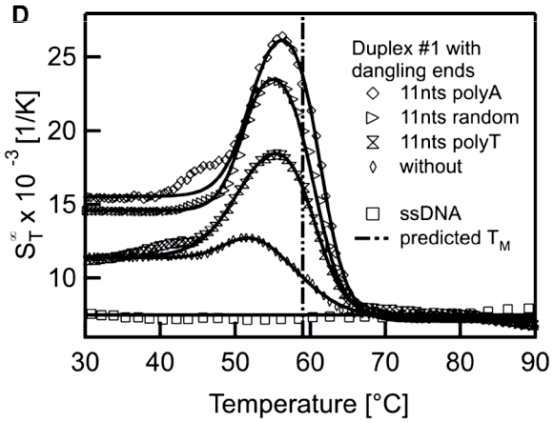




The data for dangling ends of various lengths showed an increase of the Soret coefficient due to a change in the conformation before the actual melting transition. The final melting temperature matches the situation without dangling ends.

When changing the composition of the duplex forming sequence a shift in the melting temperature but also in the first transition was observed.





To rule out any potential structures of the dangling ends, duplex #1 was analyzed with 11 nt (nucleotide) overhangs of random sequence and compared to dangling ends with polyA and polyT sequence. Irrespective of the dangling ends, the melting temperature remained constant.

- Increasing length of the dangling ends, the amplitude of the first transition increased as.
- The duplex sequence has no influence on the amplitude of the initial transition whereby tertiary structures of the duplex part could be ruled out.
- The initial transition was present in all duplex melting experiments giving further hints that the effect might be a general part of the unfolding process of double stranded DNA.

Thermophoretic melting curves quantify the conformation and stability of RNA and DNA. (2011) Nucleic acids research 39, e52.

Advantages-Melting study

- Beside the standard melting temperature T_m, the data showed that additional information about conformational states is accessible.
- Due to low sample volumes per measurement at nanomolar concentrations expensive or rare samples can be used sparingly.
- No FRET pairs are necessary reducing the cost and complexity of mutation-specific analysis.
- It is possible using intercalating fluorophores and that thermophoresis can be employed to quantify the length of PCR products. This indicates that also a thermophoretic thermal stability analysis is possible using intercalating dyes which would further decrease the costs and complexity of a melting experiment. Such an approach would combine a typical fluorescence melting curve with the advantage of thermophoresis to monitor the unfolding of tertiary structures.
- Its sensitivity for conformational states thermophoresis provides a promising experimental approach for testing biological models and allows for a more detailed understanding of the melting behavior of biologically relevant nucleic acids.

Company who’s in the business

NANO TEMPER technologies – Microscal thermophresis

- Measure affinities without surface immobilization, in free solution
- Measure label-free
- Measure with excellent selectivity using a fluorescent dye or fluorescent protein
- Measure with low sample consumption unmatched by other techniques
- Study membrane proteins directly in liposomes or detergent solution
- Measure affinities (K_D, dissociation constant) between any (bio)molecules in 10 minutes
- Measure sub-nM to mM range of dissociation constants
- Measure at a temperature of choice
- Measure in a broad range of solution conditions where measurements are possible in any buffer, including complex detergent mixtures needed for membrane proteins
- Crude sample compatibility - measure under close to native, physiological assay conditions, since measurements are possible in serum or cell lysate
- Study multi-component reactions like ternary complex formation, the order of assembly, interfering factors, cooperativity and the like
- Discriminate between different binding sites on a target of interest
- Determine the number of binding sites of biomolecules
- Measure oligomerization of biomolecules
- Study the binding energetics ΔG (free energy), ΔH (enthalpy) and ΔS (entropy)
- Study protein folding and stability

Current interaction analysis tools	Disadvantages
Surface Plasmon Resonance (SPR)	This technique requires the immobilization of one binding partner on a sensor surface, raising issues of steric hindrance or molecular activity. These surface based methods are very sensitive in detecting minimal changes in the mass of sensor, but high affinity interactions are often not accessible for measure due to mass transport effects and transient binders are in most cases difficult to quantify .
Back-scattering interferometry (BSI)	A label-free technology and do not need a label or surface coupling. It typically suffers from a refractive index background arising from nonreacted ligand, particularly at high concentrations. In addition, temperature-induced changes of the refractive index may interfere with the binding signal and render the interpretation difficult.
Isothermal titration calorimetry (ITC)	A label-free technolog y and do not need a label or surface coupling. It can conduct measurements at a relatively low throughput and with exceptionally high sample consumption.
Fluorescence correlation spectroscopy (FCS)	FCS relies on a significant change in the diffusion constant upon binding and is only applicable to a limited number of biomolecular interactions .

Questions

Still additional work is required to understand the intricacies of thermophoresis in more detail.

- Exact relations between the amplitude of the thermophoresis signal and changes of the protein conformation or in its hydration shell (e.g. release of water molecules from binding sites)
- A better understanding of thermophoresis and of the influences of molecular properties is strongly needed.
- More information about the entropy of hydration and hydrophobic and hydrophilic effects is still needed. Especially in the case of interaction studies such details would allow deeper insights in molecular processes and, given a precise molecular dynamics calculation, would allow correlating the signal strength with binding to specific sites of the protein.