Thermogravitational Trapping

Lab Course AG Braun

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Introduction

This lab course is designed to introduce you to some of the basic aspects of working in a biophysics lab, mainly fluorescence microscopy and handling biochemicals, and the focus of the Braun lab in particular, i.e. thermogravitational trapping. Throughout the course, you will learn how to build a chamber that can be used to locally accumulate molecules in solution. You will build the chamber and connect it to the experimental setup that is provided, fill it with a fluorescent dye and run the experiment. In the end, you will analyze your results and compare it to your theoretical predictions.

To prepare for this lab course, read the following publications thoroughly:

- "**Extreme accumulation of nucleotides in simulated hydrothermal pore systems**", Baaske et al., doi: 10.1073/pnas.0609592104

- "**Heat flux across an open pore enables the continuous replication and selection of oligonucleotides towards increasing length**", Kreysing et al., Nature Chemistry 7, 203–208 (2015) doi:10.1038/nchem.2155

The lab course will start with a short discussion of the theoretical background. You should be able to answer all the questions below (not necessarily in written form, you can bring notes if you want).

Theoretical Background

The phenomenon of *thermophoresis* is highly complex and still not fully understood, but it can be incorporated into the *convection-diffusion equation* as a phenomenological model. The ordinary convection-diffusion equation for a species *c* is as follows:

$$\frac{\partial c}{\partial t} = \nabla \cdot (D\nabla c) - \nabla \cdot (vc)$$

where *c* is the concentration of the species, *D* is the diffusion coefficient, and$v$is the convective velocity of the fluid medium with $v = v_{NS}$(NS= Navier Stokes). Vector quantities are indicated by bold characters.

The thermophoretic force can be incorporated by adding an additional term that is coupled to the temperature gradient:

$$\frac{\partial c}{\partial t} = \nabla \cdot (D\nabla c + D_T c\nabla T) - \nabla \cdot (vc)$$

where$\nabla T$is the temperature gradient,$v = v_{NS} + v_T$ where $v_T = D_T \cdot \nabla T$the velocity due to thermophoresis and$D_T$the *thermodiffusion coefficient*, which measures the strength of the thermophoretic force on this particular species. Here, *c* is a nondimensionalized quantity normalized to unity.

In addition, we typically assume that the diffusion constant *D*, thermodiffusion constantD_T, and temperature gradient$\nabla T$are constant, in addition to assuming that the fluid is incompressible:$\nabla \cdot v = 0$. We thus get:

$$\frac{\partial c}{\partial t} = D\nabla^2 c + D_T(\nabla T \cdot \nabla c) - v \cdot \nabla c$$

The thermophoretic force is often described with the *Soret coefficient* S_T:

$$S_T = D_T / D$$

The Soret coefficient has units of [1 / K].

Mathematically, the thermal trap described in the papers works like this. The applied temperature gradient produces a ∇T throughout the system. This not only induces a thermophoretic force, but creates a flow profile *v* via buoyant convection as well. The form of this profile is nontrivial, but an analytical approximation can be found in *Mast et. al. (2013)* for the interested reader. In the steady state, we thus have the following differential equation:

$$\nabla^2 c = \frac{1}{D} v \cdot \nabla c - S_T(\nabla T \cdot \nabla c)$$

The boundary conditions then depend on the specific parameters of the thermal trap. In general, there is no analytical solution because of the complex nature of the trap geometry and flow profile. Nevertheless, the linearity of the differential equation makes it relatively simple to simulate in finite-element solvers such as COMSOL.

Questions

1. Imagine that scientists on the international space station are trying to replicate the thermal trap experiment. Will it still work, and why or why not?

2. Does the direction of convection in the thermal trap influence the accumulation? If so, how (what would happen if you changed the direction)?

3. We assumed earlier that the temperature gradient ∇T across the thermal trap was constant in the steady state, implying that the profile is linear. Justify this assumption.

   Hint: The equation governing heat transfer in a fluid is mathematically equivalent to the convection-diffusion equation for concentrations, and is given by:

   $$\frac{\partial T}{\partial t} = \nabla \cdot \left(\frac{k}{c_p \rho} \nabla T\right) - \nabla \cdot (vT)$$

   where T is the temperature (in this case of water), k is the thermal conductivity of water [W / (m K)], c_p *is the specific heat [kJ / (kg K)],* ρ *is the density [kg / m*^{3}*], and the water is incompressible, 150*μ*m is L the length scale of the trap over which the temperature gradient decays. You may assume material parameters to be constant. The order of magnitude of velocity due to buoyant convection is around 1* μ*m/s.*

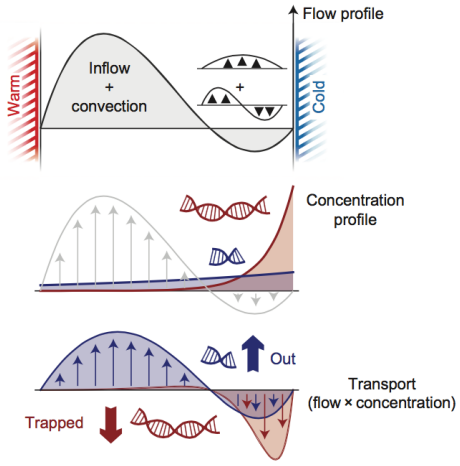
4. Diffusion is a macroscopic manifestation of microscopic equilibrium thermodynamics – that is, random motion of particles at the microscale combines to form diffusive motion at the macroscale, resulting in a smearing out of concentration and an overall increase in entropy. In the thermal trap, however, thermophoresis and convection oppose diffusion and produce a low entropy state, where molecules are accumulated rather than diffuse. This suggests that the accumulation process is a nonequilibrium system being driven by some external energy. Where does this energy come from?

   The accumulation, however, results in a steady state – the concentration does not change over time once steady state is reached and the walls of the trap are fixed at constant temperature. In this case, how can you reconcile the thermodynamic requirement of a nonequilibrium driving force with the obviously time-independent steady state of the accumulation?

5. In *Kreysing et. al. (2015)*, the authors demonstrate size-selective trapping – under the right conditions, longer DNA is preferentially trapped over shorter DNA. What does this imply about the Soret coefficient of DNA?

Bonus Question:

This size-selection is implemented by adding an inflow to the system (see figure below copied from paper). The authors claim that the size selection can be understood by superimposing the convective flow profile with the inflow profile to create a new combined fluid velocity, which is asymmetric and can select for size through trapping.



Kreysing et al. (2015)

In other words, they argue that one can use the principle of superposition (https://en.wikipedia.org/wiki/Superposition_principle) to simply add the two separate velocities together and the combined solution will approximately be the real velocity of the modified system. However, fluid flow is described by the Navier-Stokes equation, which is *nonlinear* in general:

$$\frac{du}{dt} + (u \cdot \nabla)u - \nu\nabla^2 u = -\nabla p + g$$

where u is the fluid velocity, ν is the kinematic viscosity of water [m^2 / s], ∇p is the gradient in fluid pressure and g is the acceleration due to gravity.

How can the superposition of the two flow velocities be justified, then? Explain your reasoning.

Hint #1: Since you're not actually solving the Navier-Stokes equations, the heart of the argument can be found in the steady 1-dimensional case, neglecting gravity and pressure:

$$u\frac{du}{dx} - \nu\frac{d^2u}{dx^2} = 0$$

where x is the vertical length dimension (the long axis of the trap).

Hint #2: It may be useful to non-dimensionalize the above equation, using the following:

$$\bar{u} = \frac{u}{u_c}, \bar{x} = \frac{x}{L}$$

where u_c is a characteristic flow velocity and L is the characteristic length scale (in this case, the vertical size of the trap since that is the direction of the flow).

Hint #3: It may be helpful to consider the Reynolds number:

$$\mathrm{Re} = \frac{u_c L}{\nu}$$

The Reynolds number gives the relative strength of the inertial forces of the fluid compared to

the viscous forces. It is a highly useful nondimensional parameter that governs behaviors of fluid systems, and regimes of flow are often described by the Reynolds number (i.e. low Reynolds number flow, high Reynolds number flow).

Experimental Part

Aim:

Manufacture a Thermogravitational trap and investigate the trapping efficiency of the organic fluorescent molecule Cy5.

Safety:

You will be working with elevated temperature (about 150 °C), be careful and use tweezers where possible.

You will not be working with hazardous chemical substances during this lab course, however it is advisable to wear gloves as to not contaminate your samples or the setup.

Please wear closed shoes and long trousers (also in summer!).

Trap manufacturing:

Your instructor will show you how the traps are made and will build one with you.

Experimentation & Evaluation

You will use fluorescent Cy5 to characterize the accumulation chamber.

Cy5 Accumulation

Fill your chamber with water and choose a position at which you want to analyze the accumulation. Take a dark image of the water-filled chamber while the LED is turned on and the chamber is protected from outside light. Save the image.

Next, fill your chamber with 1 μM Cy5 and close off your in- and outlets. Start the image acquisition. You will now get images of your cold trap. Turn on the temperature gradient and set it to the temperatures given below.

You will screen through three different hot side temperatures: 60 °C, 70 °C, and 80 °C (cold side 20°C)

During the accumulation, set the program to take an image every 10s and an exposure time of 3000ms.

You can check your accumulation by loading the images into ImageJ. Once it has reached a steady state, turn off the temperature gradient and end the image acquisition, flush your chamber with fresh Cy5 and repeat the procedure with a higher resistor temperature. Make sure to save your data into a new folder.

Evaluation

You should now have the following data:

- Accumulation profile of Cy5 for three different temperature gradients

In your evaluation, present a complete set of your data and results. Use the questions below as a guideline. If your results are not as expected, explain why you think that happened.

S_T = ~0.01is the Soret coefficient for Cy5).

What type of accumulation would you expect from thermophoresis alone? How does that compare with the accumulation you see in your experiments? Should there be a difference, why, why not?

Fit your data to Eq. 1 from Baaske et al. (PNAS 2007) and determine the calculated temperature gradient for each run. Compare it to the temperature gradients you measured. Do they match? If not, what error sources do you see?