Tips and tricks of the trade

How dense are membrane protein complexes?

Ariel Lustig, former analytical ultracentrifugation wizard at the Biozentrum of the University of Basel, knows how to circumvent pitfalls associated with molecular weight determinations of membrane proteins.

Lab Hint

Dear Editor,

The "Clear Solutions"-article in your bench philosophy series (Lab Times issue 1, pg 48) is well written and describes clearly the problems of membrane proteins in solution.

In addition to centrifugation I would like to mention that, due to preferential solvation, the addition of densifiers such as sucrose and Nycodenz to, for example, octyl-glucoside (OG) detergent solutions, leads to micelles and micelle-MP-complexes that show a much lower density than expected (1.08 g/ml with the densifier (hydrated) instead of 1.16 g/ml without the densifier [unhydrated]). Note that the density of a solubilized membrane-protein-detergent complex (MPDC) depends on the detergent and the solvent used.

In 1976, Charles Tanford and his staff were the first to measure the molecular weight (MW) of a membrane protein using analytical ultracentrifugation (AU) (PNAS, 73 (1976) 4467-4470). Subsequently, several different methods were developed. Tanford used a clever trick. He added deuterium (with a density of 1.1 g/ml) to the solvent until the solvent density became equal to the detergent micelles-only (det-m) density. Such density matching is also called gravitational transparency (GT). Detergent micelles are always assembled from a solution of detergent molecules that exceeds a critical micelle concentration (CMC). At gravitational transparency the molecular weight of the membrane proteins alone can be determined via sedimentation equilibrium in the same way as it would be for soluble proteins.

The Tanford approach can be used for all systems as long as det-m does not exceed the density of deuterium. Unfortunately this is not the case for several commonly used detergents such as octyl-glucoside (OG) and dodecylmaltoside (DDM), which are often used by crystallographers. Hence, this method is not applicable in most cases.

In 2001 Jürg Rosenbusch and colleagues at the Biozentrum of the University Basel introduced a slightly different method (Micron, 32 (2001) 75-90). They performed analytical ultracentrifugation of membrane proteins with the detergent octyl-tetraoxyethylene (C8E4,5). Since the density of octyl-tetraoxyethylene (~ 1.005 g/ml) is similar to normal buffers a densifier is not needed. Unfortunately, Rosenbusch's method is only applicable to a small number of membrane proteins that do not aggregate in C8E4,5.

Ariel Lustig looks back on more than 30 years of experience with analytical ultracentrifugation.

We have, therefore, searched for a method that works with detergents having det-m densities exceeding 1.1 g/ml (Lustig et al., BBA, 1464 (2000) 199-206). To this end, we measured the density of OG or DDM-micelles in sucrose, Nycodenz and Metrizamide. Our search was primarily for detergents in which the membrane proteins remain biologically active, as is the case for OG and DDM. First we ran detergent micelles in sucrose and buffer by sedimentation equilibrium, determining their density graphically by using the positive and negative slopes ($dlnc/dr^2$) derived from the sedimentation equilibrium equation. To our surprise, those values were as much as 50% lower than their published values. This anomaly can be easily explained as a water layer that is formed by the preferential solvation of det-m in the sucrose-solvent reduces the total detergent density.

We have called this effect "hydrated density". This enabled us to execute runs of our membrane-protein-detergent complex (Mpdc) at the hydrated densities of the detergent alone, thus achieving gravitational transparency and the Mw-only of the membrane protein. We have also described an additional correction for the density of the membrane proteins alone when only partly hydrated.

Knowledge of the gravitational transparency density effect can be applied to the preparative centrifugation of hydrated detergents micelles/membrane proteins in three systems:

- *in solutions with a densifier of a lower density than gravitational transparency, they can be pelleted,*
- *in solutions with a densifier of higher density than at gravitational transparency, they can be recovered in the supernatant.*
- *at gravitational transparency they will be immobile; a situation that may be suitable for a comparison of different particles and conditions.*

The diameter of tubes (approx. 10 mm) and the radial distance of preparative vertical rotors (about 6.5 cm) are similar to analytical ultracentrifuge rotors. In certain cases this may be of help to achieve floatation or sedimentation gradients.

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You know a clever trick too? Feel free to contact us at:

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Product Survey: Pipettes and pipetting robots

Torture or pleasure? Ergonomics makes all the difference

Pipettes are the most essential and most frequently used tools in any clinical or research laboratory. So take your time to choose an ergonomic model that meets your needs.

Usually manual pipetting involves five steps: depressing the pipette's plunger, holding the plunger down, aspiring the liquid, dispensing it and expelling it. After the last step the tip is ejected and a new one applied. Pretty mundane stuff I hear you say, but have you ever wondered how often your thumb depresses the plunger or tip ejector and how much cumulative force is used in carrying out a normal day's lab work? The plunger force of a traditional manual pipette is equivalent to between three and four kilograms. This means that if you press the plunger, say, 150 times a day, your thumb has to move a total mass of nearly half a ton.

Tingling hands

Small wonder that many pipette users complain of thumb pain or experience tingling in their hands, fingers and shoulders. According to a study published in 1994 by Marianne G. Bjornsten and collegues from the University Hospital in Uppsala, Sweden, lab workers who wield their pipettes for more than six hours a week attract a significantly higher risk of repetitive strain injuries. Indeed, an astonishingly high risk of strain and muscle injuries is associated with heavy pipetting. One of the most well known hazards is carpal tunnel syndrome, which causes severe pain in the hand and fingers, especially the thumb.

Health problems can be avoided by choosing pipettes not only according to their precision and accuracy but also by taking a closer look at their ergonomics. The plunger force of modern ergonomic manual pipettes is much lower than that of traditional pipettes. Usually it does not exceed 0.5 to 1 kg in the aspiring and dispensing step. However, you should check the force required to eject tips, noting that ejection forces can be much higher than plunger forces.

Some ergonomic pipette models are equipped with an additional trigger that controls the aspiring step. You pull this trigger with your forefinger to aspirate a liquid before dispensing it by depressing the plunger with the thumb. Forces that could overstrain your fingers are thus significantly reduced.

Push the button

The easiest way to get rid of the plunger force associated with manual pipettes, however, is to buy an electronic pipette. Instead of pushing the plunger with your thumb, you simply touch a button. Though the plunger force is reduced to zero, electronic pipettes have some disadvantages. Prices are approximately two times higher than that of manual pipettes and some models are rather heavy.

The typical weight of an electronic single-channel pipette ranges between 150 and 300 g, in contrast to weights of between 90 and 140 g in manual pipettes. Nevertheless, an electronic pipette may be the right choice, especially if you are working with 96-multiwell plates requiring a multichannel pipette.

Pipetting slaves made of steel

If you are planning high throughput applications in your lab, such as screening assays that rely on 96- or 384-well plates, consider buying a pipetting robot. Pipetting slaves made of steel and plastic free you from mundane and time-consuming pipetting. They never grumble about repetitive pipetting for hours, work faster than the best skilled technician and make almost no errors so long as they are properly programmed.

Sounds too good to be true? There is indeed one small drawback: pipetting robots are expensive. You can easily spend 20.000 to 50.000 euros on a liquid handling robot. But they do almost anything for you, including RNA, DNA and protein purification, SNP-genotyping, sample preparation for MALDI-TOF mass spectrometry, powder dispension, real time PCR sample preparation and so on. Most liquid handling robots can be integrated into bigger workstations or can serve as an interface to other equipment such as plate readers and washers.

Over the next few pages you will find more detailed information about the pipettes and pipetting robots that are currently on the market.

HARALD ZÄHRINGER

Should raise a smile on the face, no grimace with pain: working with modern, state of the art pipettes.