

Figure 2 A model for the fusion of yeast vacuoles, as proposed by Peters *et al.*<sup>1</sup>. First, the two fusing membranes are pinned together by SNARE proteins (not shown). a, A dimer of proteolipid rings (V0) forms. Yellow indicates hydrophilic and green, hydrophobic surfaces. Lines mark the borders between individual proteolipid monomers. The central cavity of each ring is closed off. b, The ring opens as the proteolipid subunits separate laterally. An aqueous channel — the fusion pore — forms in the centre. At the same time, lipids invade the spaces between the subunits. c, The fusion pore expands, ultimately causing the proteolipids to separate vertically. One problem with this model, however, is that the faces of the cavity in an end up facing lipid. This would be energetically unfavourable if the cavity were hydrophilic, as expected.

between two hydrophilic stripes. Such surfaces mimic lipid bilayers and allow lipids to invade the proteolipid ring without leaving their bilayer configuration. This model is attractive because it explains fusion without invoking temporary lipid intermediates<sup>9</sup>.

Do the proteolipid complexes dimerize directly, or indirectly through their associated subunits (Fig. 1a)? If the former, how are these subunits accommodated in the complex? Do proteolipids indeed separate laterally to form an aqueous channel? And what is the significance of the lone t-SNARE that Peters *et al.* found in the dimer of proteolipid complexes? Does it signal the break-up of the *trans*-SNARE complex before fusion, and does this break-up require NSF? Wickner, Mayer and colleagues<sup>6,7</sup> have developed a powerful system that seems well suited for addressing such questions.

From a physiological viewpoint, why do vacuoles wait for the formation of a V0 dimer when SNAREs alone can cause fusion of liposomes? A similar question is why protein-based water channels exist in membranes, when lipid bilayers alone pass water. The answer to this question — increased speed and tighter control — may also apply to fusion. We don't yet know whether fusion events other than vacuole fusion rely on the proteolipid rings. But increased speed would be particularly useful in the case of neurotransmitter release — neurotransmitter-containing synaptic vesicles fuse with the plasma membrane less than 1 millisecond after neurons are stimulated. By contrast, one round of SNARE-mediated fusion of recon-

stituted liposomes takes 30 to 40 minutes<sup>4</sup>.

The presence of a ring of proteolipids at the fusion site may also make it easier for a vesicle to close its fusion pore soon after releasing its contents, preventing the membrane constituents from mixing with the target membrane. Closing fusion pores have been observed during most kinds of secre-

tion. After closing its fusion pore, the vesicle would be free to disconnect from the plasma membrane and prepare for re-use elsewhere. A proteolipid-based ring may also allow the flux of contents through fusion pores to be controlled, as proposed for hippocampal neurons<sup>10</sup>. And it may add an element of safety: fusion intermediates lacking sufficient protein lead to hemifusion<sup>5</sup>, an unproductive association between membranes that fails to allow the release of cargo.

It will be interesting to see further work on the role of proteolipids in fusion, and in particular whether proteolipids are also involved in the fusion of secretory vesicles with the plasma membrane. Many years ago, Israel and colleagues<sup>8</sup> proposed that neurotransmitter is released not from vesicles but through a V0 proteolipid complex, which they called the mediatoaphore. It would be poetic if they were now proven partially correct. ■

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#### Fluid dynamics

## That sinking feeling

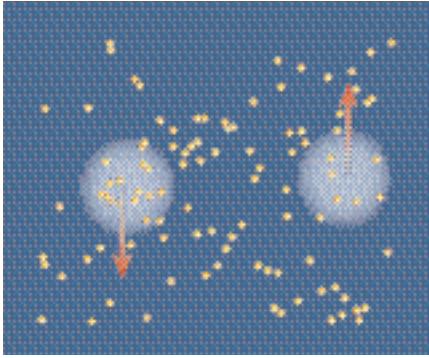
Michael P. Brenner and Peter J. Mucha

The physics of slowly falling particles in a fluid remains surprisingly enigmatic. Luckily, laws that work for dilute suspensions also appear to apply to higher — and more useful — particle concentrations.

Particles moving through a viscous fluid interact with each other, because each individual particle drags fluid along with it, which then drags on other particles. These hydrodynamic interactions are important in many processes, from the deposition of silt in a river to the motion of dust in the air and the centrifugation of proteins. But despite a century of research, the character of these motions is still hotly debated, even for dilute suspensions in which the particles are well separated. On page 594 of this issue, Segrè *et al.*<sup>1</sup> provide experimental evidence that the laws governing particles settling under gravity at high concentrations are quantitatively similar to those at very low concentrations. This result is unexpected, because it has long been assumed

that particle motion in a dense suspension is qualitatively different from the dilute case.

Smoluchowski carried out an early study of particle interactions in viscous fluids, described in a 1912 paper<sup>2</sup>, “On the practical applicability of Stokes’ law ...”. Smoluchowski wanted to calculate the average sedimentation velocity of identical solid particles, each of which is heavier than the underlying fluid, as they slowly sink through the fluid. The calculation of the average velocity has been revised every 30 years since — by Burgers<sup>3</sup> in 1942 and Batchelor<sup>4</sup> in 1972. Batchelor’s calculation works at low particle concentrations, and assumes that the particle positions are sufficiently random. Experiments have generally shown that Batchelor’s prediction



**Figure 1** The physical mechanism underlying velocity fluctuations of particles in a sediment. A random distribution of particles has particle-rich regions, which fall faster than the average, and particle-depleted regions, which fall more slowly and so rise relative to the average. A fundamental question in sedimentation research is what sets the size of the particle-rich and particle-depleted regions. (Adapted from an argument by E. J. Hinch.)

is consistent with the data at low particle concentrations (for examples, see ref. 5). But at higher concentrations, strong interactions between groups of particles produce significant complications, which can be understood using sophisticated computer simulations<sup>6</sup>.

However, the average velocity does not completely characterize the sedimentation process — individual particle motions fluctuate about the average. Rather curiously, little effort appears to have been directed at understanding these fluctuations until 1985, when Caflisch and Luke<sup>7</sup> pointed out that Batchelor's assumptions implied that the variance of particle velocities about the mean scaled linearly with the size of the box containing the sediment — the bigger the box, the bigger the velocity fluctuations. This result is counterintuitive, flying in the face of common experience with normal materials — it implies that the diffusivity of the sedimenting particles depends on the size of the container. In contrast, we know that the diffusion of dye molecules moving in a cup of liquid is controlled by the temperature, the viscosity of the liquid, and the size of the dye molecules, all of which are local quantities and independent of the size of the cup.

Although numerical simulations<sup>8</sup> support the Caflisch–Luke idea, experiments<sup>9,10</sup> have indicated that the velocity fluctuations are independent of the box size. In particular, experiments with dilute solutions<sup>11</sup> appear to indicate that ‘universal’ concentration-dependent laws govern the velocity fluctuations, independent of the container shape. In these experiments the volume fraction of solid particles varies between 0.01 and 5%. The interpretation of these experiments, and the mechanisms determining the size of the velocity fluctuations, has generated a lot of controversy.

Segrè *et al.*<sup>1</sup> now take these experiments a

step further, by demonstrating that the sedimentation laws described in ref. 11 also apply to much higher volume fractions (between 5 and 50%), as long as three well-studied physical effects are taken into account. These are the dependence of the average sedimentation velocity on concentration; the dependence of the effective viscosity on concentration; and the structure factor, which describes the limitations imposed on otherwise random particle positions as the volume fraction increases and the particles become both more closely packed and more ordered. Segrè *et al.* show that, by correcting the dilute sedimentation laws<sup>11</sup> for these effects, they can predict velocity fluctuations across the full range of volume fractions.

Their result contradicts the conventional wisdom that dense suspensions involve very different physics from their dilute counterparts. At high volume fractions the particles are closer together, so particle jamming and other subtle hydrodynamic effects should be more important. In dilute suspensions, well-separated particles interact most strongly through long-range flows generated by each moving particle; but in dense suspensions, particle motion is inhibited by particles that are almost touching. Because most practical applications occur at high concentrations<sup>5</sup>, research into dilute sedimentation has sometimes been dismissed as the study of ‘contaminated water’ — of intellectual interest, but irrelevant for practical problems. The work of Segrè *et al.* shows that the physics of the two regimes are fundamentally connected.

Segrè and colleagues have raised the stakes for figuring out what controls the size of the velocity fluctuations in sedimentation, as we now know that the same theory will apply to all particle concentrations. The physical mechanism that sets the size of these velocity fluctuations is still very much an open question (Fig. 1). In their paper, Segrè *et al.* correct the dilute scaling laws of ref. 11 to obtain predictions of how velocity fluctuations vary with volume fraction — but a similar correction to the Caflisch–Luke argument could also be fit to their data. This brings us back to the fundamental diffusion confusion: is the diffusivity of a sediment a local quantity, as in the case of a dye molecule moving in water, or does it depend on the size of the container?

Here, Segrè *et al.* identify a natural ‘effective temperature’ for sedimentation, which depends on the change in gravitational energy caused by the movement of particle-rich and particle-depleted regions. This is not a real temperature, but a concept borrowed from statistical mechanics, in which a ‘characteristic temperature’ describes the random behaviour in the system. The physical mechanisms setting the size of the fluctuations in particle number — and therefore the effective temperature, velocity fluctuations, and diffusion constant — are due for another revision. Perhaps 30 years after



#### 100 YEARS AGO

The current number of the *Proceedings of the Royal Society* contains a paper of much interest to all who are devoted to the canine race. It describes Dr. Copeman's successful endeavours to isolate the micro-organism responsible for distemper in dogs... Dr. Copeman has now isolated a small coccobacillus, growing readily on most of the ordinary culture media at the body temperature, from the exudations from the lungs, the tracheal mucus, and from the nasal secretion of dogs suffering from distemper. A cubic centimetre of a broth-culture of this microbe, injected beneath the skin of the abdomen in a dog weighing 7 kilograms, is sufficient to induce an attack of distemper terminating fatally in about a week from the date of inoculation. A vaccine has also been prepared which Dr. Copeman states can protect dogs against attacks of distemper... An injection of 2 cubic centimetres of such vaccine was apparently sufficient to protect fox-terrier pups weighing about 1½ kilograms when exposed to distemper infection. From *Nature* 31 January 1901.

#### 50 YEARS AGO

Attempts have been made to determine whether pure tones of equal loudness but different pitch are also different in quality, and whether it is possible to find frequency differential thresholds for the quality differences. Following the usual practice, the terms ‘loudness’, ‘pitch’ and ‘quality’ are used to refer specifically to auditory sensations, while ‘intensity’ and ‘frequency’ refer to characteristics of the sound wave stimulus. In this preliminary work, the experimenter was the subject for the comparisons, which were made monaurally with an earphone. It was found possible not only to observe quality differences, but also to describe them. The descriptions were always metaphorical and never altogether satisfactory but appeared to be fairly stable, that is, they seemed equally apt even after long periods of practice in listening and long rest periods over the course of several months. The following are typical of the listener's remarks: 500 c.p.s. sounds ‘purer, clearer, more bell-like’ than 125 c.p.s.; 250 c.p.s. sounds ‘somewhat narrower, thinner and not so fuzzy’ as 125 c.p.s.; 2,000 c.p.s. sounds ‘sharp and hard’ when compared with the ‘soft, blunt, more comfortable’ 125 c.p.s.; 1,500 c.p.s. has a ‘reedy’ quality not observed in 1,000 c.p.s. From *Nature* 3 February 1951.

Batchelor's calculations, we can hope for a new theory in 2002.

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Functional genomics

# Silent genes given voice

Athel Cornish-Bowden and María Luz Cárdenas

Many genes have little apparent influence on growth rates or metabolic fluxes in an organism. But their roles can be revealed by comparing the effects of mutations on two or more metabolite concentrations.

Most mutations have no noticeable impact on an organism. This implies that changing the activity of some enzyme or other by a substantial factor has little effect; even complete deletion of a gene may not be easily detectable if there are appropriate fail-safe features in the design of the organism. Many genes, up to 85% of those in yeast, do not appear to be required for survival, and a high proportion of these seem to have no detectable effects on metabolic fluxes — the chemical processes that result in energy production or growth. This presents a major barrier to functional studies of a genome. How can we hope to deduce the function of a gene that has no apparent effect?

Writing in *Nature Biotechnology*, Léonie Raamsdonk and colleagues argue that examining metabolite concentrations, which in total are known as the ‘metabolome’, rather than fluxes, is much more likely to reveal such ‘silent’ genes (*Nature Biotechnol.* **19**, 45–50; 2001). The authors call their method FANCY, which comes from ‘functional analysis by co-responses in yeast’ (in this case brewer’s yeast, *Saccharomyces cerevisiae*). It uses the fact, long known but often ignored, that typical effects of changes in enzyme activity on metabolite concentrations are much larger than their effects on metabolic fluxes. The authors looked at two mutations affecting 6-phosphofructo-2-kinase, an enzyme whose

product fructose 2,6-bisphosphate acts as a signal to regulate energy production; two mutations in the cytochrome oxidase complex, which catalyses a different energy-related process; and a mutation not related to energy metabolism that was used as a control.

Why, though, should changes in enzyme activity have a larger effect on metabolite concentrations than on fluxes? The reason can be seen by looking at what happens when a rock falls into a river. Any transient interruption of the flow of water is rapidly nullified by the increasing level of water just above the rock and the decreasing level just below: as soon as the required pressure is reached, the flow returns to just what it was before. So the rock has no steady-state effect on the flow, although it does have a long-term effect on the water levels, which remain different from their original values as long as the obstacle remains in place. An observer with access only to the steady-state value of the flow can learn nothing about the existence of an obstacle, let alone its location. But an observer with access to the water levels in different places can both detect that an obstacle is present and find out where it is by comparing its effects on the levels above and below it.

As with rivers, so with the genome of an organism such as yeast. Gross properties such as growth rate that depend on fluxes may suggest that most genes are silent. For instance, chemostat culture allows microorganisms to be maintained indefinitely in the phase of exponential growth in a medium of constant composition, the slower-growing strains gradually being eliminated by dilution. Although this approach can in principle reveal very slight differences in growth rates, Raamsdonk *et al.* found that it failed to reveal the deletion of one or other of the two yeast genes that code for 6-phosphofructo-2-kinase. The mutants achieve growth rates equal to those of the wild type by increasing the concentrations of metabolites upstream from the impediment and decreasing those of downstream metabolites (just as for the rock in the river). The effects on a metabolite such as fructose 6-phosphate are not only easily measurable but also detectably different for the two mutants.

Measurements of relatively few metabolite concentrations can thus give voice to apparently silent genes. But individual concentrations are often less informative than one might wish, because quite different mutations may affect the same concentration to a similar extent; in any case, with a completely unknown gene there is no prior knowledge of which concentrations to examine. What is needed is a comprehensive way of studying many metabolites together. The FANCY method provides this, and can reveal quite subtle effects of changes in genotype. The C in FANCY stands for ‘co-response analysis’ (Hofmeyr, J.-H. S. & Cornish-Bowden, A. *J. Theor. Biol.* **182**, 371–

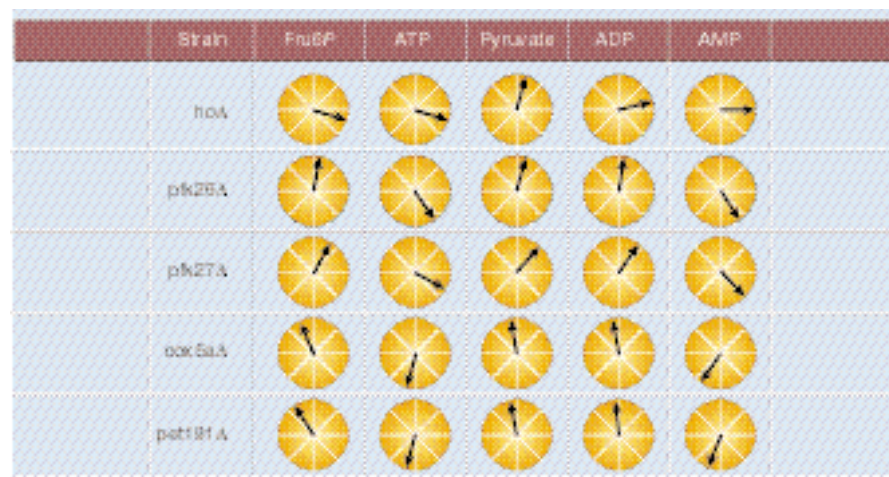


Figure 1 Study of silent genes using co-response angles, from the experiments described by Raamsdonk *et al.* Five yeast strains are shown ( $\Delta$  indicates a mutant in which a gene has been deleted). The *pfk26\Delta* and *pfk27\Delta* mutants are defective in 6-phosphofructo-2-kinase activity; *cox5a\Delta* and *pet191\Delta* are mutants of the cytochrome oxidase complex; *ho\Delta*, included as a control, is defective in a part of metabolism not concerned with energy production. Changes in all of the metabolite concentrations shown are relative to changes in the glucose 6-phosphate concentration, which corresponds to the horizontal axis in all cases. The other metabolites are fructose 6-phosphate (Fru6P); pyruvate; and the tri-, di- and monophosphate forms of adenosine phosphate. ATP is the primary energy carrier in the cell, and all the metabolites shown are involved in energy production. Note that the different classes of mutants are characterized by different angles. For simplicity, only the angles are illustrated here, but a full analysis would take account of the magnitude as well as the direction of each vector.