fully characterized all possible Deadpan loop deletion and substitution variants (some 42 proteins in all) without purifying a single protein. Furthermore, the use of chemical synthetic methods permits the incorporation of amino acids not found in nature. As all of the steps involved in combinatorial synthesis and characterization can be automated, high-throughput functional screening of any synthetic protein domain is within reach. The availability of gene products from any open reading frame from the many sequenced genomes has spawned high-throughput crystallography for identifying new structural domains; the techniques used by Winston and Gottesfeld give us a glimpse of ways to characterize these new domains functionally as well. The era of functional genomics is upon us. ■

Satish K. Nair and Stephen K. Burley are in the Laboratories of Molecular Biophysics and the Howard Hughes Medical Institute, The Rockefeller University, 1230 York Avenue, New York, New York 10021, USA.
e-mail: nairs@rockvax.rockefeller.edu

1. Littlewood, T. & Evan, G. I. *Helix–Loop–Helix Transcription Factors* (Oxford Univ. Press, New York, 1998).
2. Winston, R. L. & Gottesfeld, J. M. *Chem. Biol.* **7,** 245–251 (2000).
3. Murre, C., McCaw, P. S. & Baltimore, D. *Cell* **56,** 777–783 (1989).
4. Ferre-D'Amare, A. R., Prendergast, G. C., Ziff, E. B. & Burley, S. K. *Nature* **363,** 38–45 (1993).
5. Ma, P. C., Rould, M. A., Weintraub, H. & Pabo, C. O. *Cell* **77,** 451–459 (1994).
6. Grandori, C. & Eisenman, R. N. *Trends Biochem. Sci.* **22,** 177–181 (1997).
7. Grandori, C., Mac, J., Siebelt, F., Ayer, D. E. & Eisenman, R. N. *EMBO J.* **15,** 4344–4357 (1996).

Non-equilibrium physics

Freezing by heating

H. Eugene Stanley

Everyone has noticed that cooperation among individuals can become less efficient when the individuals are nervous or lack a clear direction. Why is this? Writing in *Physical Review Letters*, Dirk Helbing, Illés Farkas and Tamás Vicsek[1] show that increasing the erratic motions of interacting entities can lead to the breakdown of an efficient pattern of interactions and finally produce a lasting deadlock. This work may be relevant to the movement of light (rising) and heavy (sinking) particles in a vertical column of fluid, and in a wider context to the movement of pedestrians or vehicles in constrained spaces and under heavy traffic conditions.

Transitions induced by random fluctuations or noise have always fascinated physicists[2–5]. For example, stochastic resonance is a noise-related effect whereby fluctuations can vastly improve the detection of a signal. Helbing *et al.*[1] study a system consisting of a relatively small number of entities, about 100, driven in opposite directions under the influence of noise and interacting through a simple, repulsive force. Such driven interactions take place under non-equilibrium conditions, which means they do not have to follow the expected behaviour patterns of classical equilibrium systems[6,7]. In their model, Helbing *et al.* discover a new transition that they call 'freezing by heating'. To order a system by heating violates the thermodynamic principles that govern macroscopic equilibrium systems.

Helbing *et al.*[1] report a transition from a fluid state to a frozen state on increasing the noise amplitude — effectively the 'temperature' of the system (Fig. 1). Whereas the fluid state consists of self-organized lanes of uniform directions of motion (Fig. 1a), which can be disturbed by sufficiently strong fluctuations, the frozen state corresponds to a crystallized configuration of the entities (Fig. 1c). This crystallized state can be destroyed by extreme fluctuations, giving rise to a third, disordered (or 'gaseous') state with randomly distributed entities. Thus, with increasing 'temperature', the authors observe an atypical sequence of transitions from fluid to solid to gas.

Freezing by heating is the opposite of melting, in which increasing the temperature increases the energy, and order is destroyed. It is also different from noise-induced ordering in glasses or granular media. In that case, increasing the 'temperature' drives the system from a disordered metastable state (corresponding to a local energy minimum) to an ordered stable state (corresponding to the global energy minimum). Instead, freezing by heating shows an increase in order with increasing temperature, although the total energy increases at the same time. Because the entropy (amount of disorder) in the ordered crystallized state is smaller than in the less ordered fluid state, the thermodynamic principle of maximization of free energy does not apply. For similar reasons, the apparently related ordering phenomenon seen in experiments with colloidal particles in confined spaces[8] does not correspond to freezing by heating, but future experiments may reveal their exact relationship.

Such unusual behaviour is possible only because of the far-from-equilibrium nature of the transition. The organized fluid state (Fig. 1a), characterized by lanes, is usually more stable than the crystallized structure, because the total energy is lower in this state. The crystallized state is therefore metastable — it is sensitive to structural perturbations, such as the interchange of a few entities. If the interface is rough, as in most spontaneously formed jammed states (Fig. 1b), some entities may break through and form 'channels' so the jam eventually collapses. But if the interface between oppositely moving entities is, by chance, flat enough, a stationary crystal is formed. This ordered state, created with the help of large fluctuations, remains frozen even when the noise is reduced again (Fig. 1c). According to Helbing *et al.*, the formation of ordered states requires both frictional and driving forces acting on each entity. Without friction, both lanes and the crystallized state will eventually give way to a disordered 'gaseous' state.

Consideration of self-driving forces in simple systems of interacting entities has opened a fascinating new area of research[6]. Such work is relevant to understanding the flocking of birds, which can be viewed as a non-equilibrium version of the conventional equilibrium transition to ferromagnetism in systems of interacting spins. It also applies to the collective patterns of motion formed

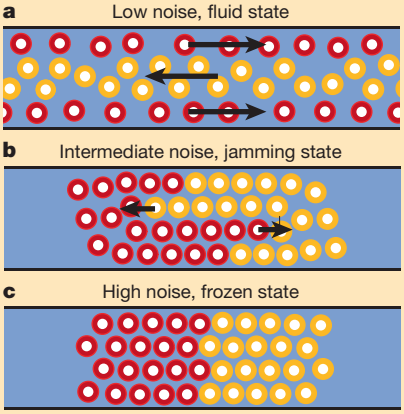

Figure 1 **Freezing by heating according to a study by Helbing *et al.*[1].** a, **For small fluctuation (noise) levels, the oppositely moving entities separate into freely moving lanes. Such a pattern minimizes the interactions between entities, if their density is not too high, and is stable with respect to small disturbances.** b, **When the noise (or 'temperature') is increased beyond a critical intensity, the fluctuations prevent lane formation. As a consequence, some of the oppositely moving entities block one another locally from time to time, which usually leads to unstable jams with rough interfaces, as new entities arrive at the boundaries of the blocked area.** c, **If, by chance, the jam develops a 'flat' interface perpendicular to the desired direction of motion and it exists long enough, all the entities arrange themselves in a hexagonal 'crystal' structure in which the driving forces are balanced.**

by pedestrians, or to transitions to inefficient, congested traffic states, which can be triggered by fluctuations in the traffic flow. The latter resembles freezing by heating because there is a metastable regime of traffic flow at medium vehicle densities, in which flow is stable under small fluctuations (giving rise to free flow), whereas the build-up of traffic jams (akin to a frozen state) occurs with larger fluctuations. These ideas are essential when trying to prevent traffic breakdowns by suppressing fluctuations in the flow. Industrial estimates of the potential market for traffic-controlling measures, such as intelligent speed limits, on-ramp controls or driver-assistance systems, exceed US$1 billion a year.

Freezing by heating is one of the most intriguing phenomena found in self-driven systems. A deeper understanding of the role of confined spaces and fluctuations in the transport and jamming of entities is likely to be helpful in developing more efficient designs for granular hoppers and pedestrian facilities. In particular, the freezing-by-heating transition may apply to the behaviour of pedestrians in panic situations, such as in a smoke-filled room, in which jammed states tend to build up. So perhaps this remarkable phenomenon will interest builders of emergency exits and escape routes[9]. ■

H. Eugene Stanley is at the Center for Polymer Studies, Department of Physics, Boston University, 590 Commonwealth Avenue, Boston, Massachusetts 02215, USA.

e-mail: hes@bu.edu

1. Helbing, D., Farkas, I. J. & Vicsek, T. *Phys. Rev. Lett.* **84,** 1240–1243 (2000).
2. Gammaitoni, L., Hänggi, P., Jung, P. & Marchesoni, F. *Rev. Mod. Phys.* **70,** 223–288 (1998).
3. Reimann, P., Bartussek, R., Häußler, R. & Hänggi, P. *Phys. Lett. A* **215,** 26–31 (1996).
4. Nicolis, G. & Prigogine, I. *Self-Organization in Nonequilibrium Systems: From Dissipative Structures to Order through Fluctuations* (Wiley, New York, 1977).
5. Horsthemke, W. & Lefever, R. *Noise-Induced Transitions* (Springer, Berlin, 1984).
6. Helbing, D., Herrmann, H. J., Schreckenberg, M. & Wolf, D. E. *Traffic and Granular Flow '99* (Springer, Berlin, 2000).
7. Laughlin, R. B. *et al. Proc. Natl Acad. Sci. USA* **97,** 32–37 (2000).
8. Rudhardt, D., Bechinger, C. & Leiderer, P. *Phys. Rev. Lett.* **81,** 1330–1333 (1998).
9. Wei, Q.-H., Bechinger, C., Rudhardt, D. & Leiderer, P. *Phys. Rev. Lett.* **81,** 2606–2609 (1998).

Immunology

Commit ye helpers

Anne O'Garra

Different types of immune cell defend the body against different pathogens. For example, immune cells known as type 1 helper T cells (T_H1 cells) are important for protection against intracellular pathogens such as bacteria, and are also implicated in organ-specific autoimmune diseases[1–5]. T_H2 cells, by contrast, are needed to eradicate flatworms, roundworms and other extracellular parasites, and are also involved in allergic reactions[1–4]. Part of what makes T_H1 and T_H2 cells different is that they express different types of secreted proteins known as cytokines[1–5], and, conversely, different cytokines also have a say in whether a specific progenitor cell becomes a T_H1 or a T_H2 cell[3,4]. But other mechanisms are also likely to be involved in the commitment of a progenitor cell to a particular differentiation pathway[4–6].

Now, Szabo and colleagues[7], writing in *Cell*, identify such a mechanism. They report the isolation of T-bet, a new T_H1-specific factor that, remarkably, not only controls the expression of interferon-γ — the hallmark T_H1 cytokine — but also represses T_H2-specific cytokines.

What did we already know about the differentiation of T-helper cells? Have a look at Fig. 1 (overleaf). A particular type of naive T cell can develop into a T_H1 or a T_H2 cell. Development of T_H1 cells is driven by a cytokine called interleukin-12, which is produced by immune cells known as macrophages and dendritic cells. Interleukin-12 induces the naive T cell to produce the signature T_H1-cell cytokine, interferon-γ. Commitment to the T_H1 lineage is, in turn, enhanced by interferon-γ, which upregulates expression of the interleukin-12 receptor while inhibiting the growth of T_H2 cells[4,5]. Another protein that is important in T_H1 differentiation is Stat4 (for signal transducer and activator of transcription-4), a transcription factor found in the cell's cytoplasm[5].

On the other hand, the cytokine interleukin-4 induces T_H2 development and the production of interleukins 4, 5 and 13, through activation of the transcription factor Stat6 (refs 3–5). Interleukin-4 also downregulates expression of the interleukin-12 receptor on developing cells, helping them to commit to the T_H2 lineage[3–5]. So, a specific genetic programme results in differentiation towards a particular T-helper lineage.

As well as Stat4 and Stat6, other lineage-specific transcription factors are needed for the differentiation of T-helper cells. So far, we know of two more T_H2-specific transcription factors — c-Maf and GATA-3 (refs 8–10). c-Maf increases the expression of interleukin-4 (ref. 8), while GATA-3 can induce the expression of a broad spectrum of T_H2-specific cytokines in developing[9–14] and committed[14] T_H1 cells, and inhibit the production of interferon-γ[11–14].

In contrast, we are still lacking crucial details about the molecular basis of T_H1 differentiation. We do know that a protein called ERM is induced by interleukin-12 in a Stat4-dependent manner and is specific to T_H1 cells, but this protein does not affect the production of T_H1-specific cytokines[15]. Now, however, Szabo *et al.*[7] have filled in the gaps by identifying a previously unknown protein, T-bet, as being key to T_H1 differentiation.

T-bet belongs to the so-called T-box family of transcription factors. These proteins are important in a myriad of developmental processes, but have never cropped up in the immune system before. Szabo *et al.* found that T-bet is rapidly and selectively induced in developing T_H1, but not T_H2, cells. When the authors introduced T-bet DNA into a mouse T-cell line, they found significant activation of an interferon-γ gene construct that was introduced at the same time. In contrast, T-bet expression repressed the activation of the interleukin-2 gene, in keeping with previous observations that, although T_H1 cells express interleukin-2 to start with, they downregulate its expression as they differentiate. However, T-bet had no effect on transactivation of the interleukin-4 promoter.

So, T-bet induces expression of the key T_H1 cytokine, interferon-γ, in this T-cell line. Interestingly, Szabo *et al.* also showed that mixed populations of B cells and natural killer cells (yet more immune cells) showed increased T-bet expression after being cultured with, among other proteins, interleukin-12. Only small amounts of interferon-γ appeared in these cells, but these levels increased after addition of interleukin-18, as did the expression of T-bet. Perhaps interleukin-18 induces another factor required for interferon-γ production[4], or perhaps T-bet must be expressed above a certain threshold level to achieve significant induction of interferon-γ[7]. Indeed, other transcription factors affect the determination of other lineages in a dose-dependent way[16]. In any case, it seems that interleukin-12 induces expression of T-bet, and that this is enhanced by interleukin-18. In turn, T-bet, possibly with other factors, increases expression of interferon-γ. This theory is supported by other recent findings[17].

Szabo *et al.*[7] also found that T-bet diverts naive T cells into the T_H1 pathway, and, remarkably, can even convert committed T_H2 cells into T_H1 cells. First, the authors expressed T-bet in T cells that had been cultured for a short time together with stimuli important for T_H2 differentiation. Expression of T-bet induced a large number of these cells to produce interferon-γ, and led to a marked reduction in the number of interleukin-4-producing cells, independently of interferon-γ.

Stable commitment to a particular helper