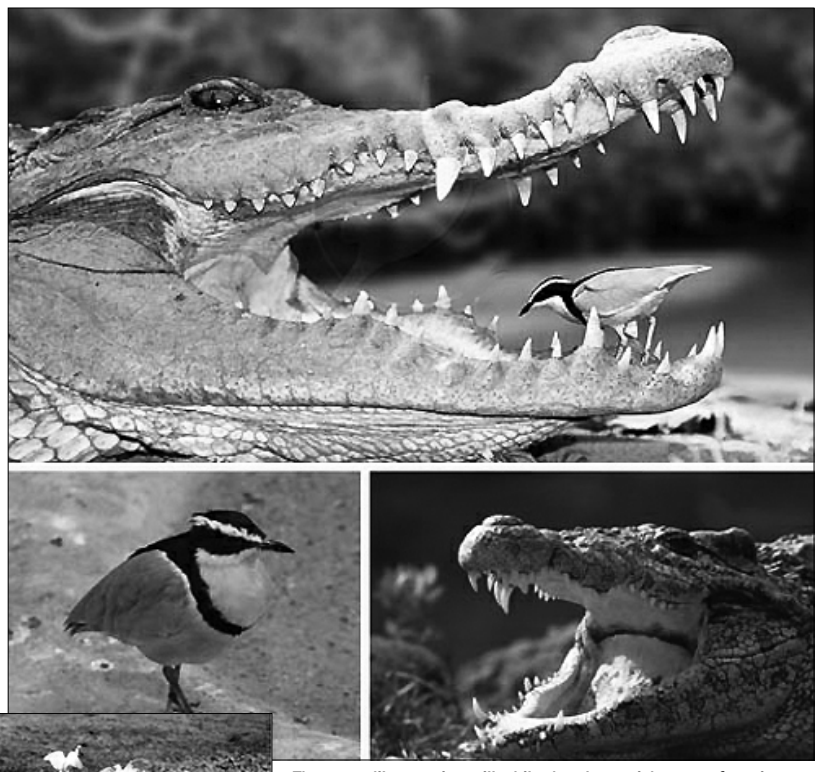
Bacteria give comrades a leg up

Cooperation and team work are basic in the natural world, says s ananthanarayanan



The crocodile remains still while the plover picks meat from its mouth. This cleans the crocodile's teeth and prevents infection while providing a somewhat scary meal for the hungry bird. Egrets (left) perform much the same service for hippos and buffalos.

THERE are many examples of species competing for resources and they modify their behaviour so that all of them derive the optimum benefit of the environment. The importance of biodiversity itself is based on interdependence and the need for one species to support another, even if only to ensure its own survival.

But a paper by Henry Lee, Michael Molla, Charles Cantor and James Collins, from Boston, in the journal *Nature* describes a case of bacteria which have become notorious for finding ways to evade antibiotics, showing altruism and fellowship by enabling other, non-resistant strains of bacteria to survive an antibiotic attack. The remarkable thing is that the helping bacteria act in this way despite a definite cost to themselves in the process.

Symbiosis

Organisms often need the machinery of other organisms to maintain their basic needs. And where the other organism provides some of the first organism's needs in return, we have direct symbiosis. An example is that of the Egyptian plover and the crocodile. A number of parasites, insects, or bits of decaying meat, too small for the crocodile to deal with, infest the reptile's teeth. In the interest of dental hygiene, the crocodile patiently allows the plover free access to its fearsome mouth and the bird feeds on the insects and other tidbits. The result is that the crocodile avoids toxins that develop in its mouth, and the bird finds ample food, always content that no predator dare approach its feeding area.

A less dramatic example is that of the countless bacteria that live in our digestive tract. They find a safe and well-provided dwelling and they pay the rent by myriad roles in the digestion of food, beyond the capacity of the juices and enzymes that the body itself produces.

Antibiotic resistance

Apart from the useful kind that live in the intestines and aid digestion, there are thousands of bacteria which play the opposite role — of causing dangerous disease. Bacteria themselves are single-celled creatures and are found in practically every habitat on earth. In the human body, bacteria are there in greater numbers than body cells! The vast majority is rendered harmless by the immune system, but those that are not neutralised cause diseases like cholera, syphilis, leprosy and respiratory ailments like tuberculosis. And as bacteria are easily transported, the diseases are infectious.

The battle against bacteria has been a losing one till the discovery and wide use of antibiotics. Antibiotics are relatively small-sized molecules that are able to render bacteria harmless by interfering with the bacteria's own life processes. As single-celled organisms, bacteria depend on the chemical structure of their envelope to absorb and process nutrients or to enable essential metabolism within themselves.

Antibiotics act through their chemical structure, which binds to some part of the bacteria, or the products of the bacteria, so that an essential function of the bacteria or their product is blocked. Antibiotics can be developed to affect only categories of harmful bacteria but leave other cells unharmed, hence their value in medicine.

But in an environment with antibiotics, there are some individuals or strains of bacteria that are able to survive. If this number is viable, it would survive and multiply to increase the fraction of bacteria that is resistant. It is this process that leads to widespread resistance and one of the causes is casual or incomplete treatment with antibiotics, which leaves a viable school of the resistant strain to multiply unhindered.

Chance mutation

Bacteria develop resistance through a chance mutation in the DNA of a few cells, which usually leads to a change in its envelope, which prevents the antibiotic from carrying out its attack. This feature gives these few individuals such an advantage that they are able to pump out the antibiotic faster than it can kill them. The mutant bacteria pass on the modified gene to their daughters and it is not long before the whole population is of modified bacteria and resistant to the antibiotic. The resistant strain spreads fast and there could soon be an epidemic against which the traditional antibiotic response is useless.

The work of Henry Lee and others at Boston has studied afresh the collective response of bacteria exposed to antibiotics. The work showed that when a population of bacteria, which had developed no resistance to antibiotics, was exposed to a moderate, that is, not fully lethal concentration of the antibiotic *norfloxacin*, the growth rate of bacteria initially dropped, but soon as resistance developed it began to rise. Raising the norflaxacin level again led to a drop and recovery in the growth rate. Continuing this process for 10 days has led to a population five times more powerful in resisting norflaxacin than at the start.

But the more detailed result of the study was that when there was a balanced mix of resistant and non-resistant bacteria, the collective resistance to the antibiotic was actually stronger than when the bacteria were almost all of the *resistant* kind. And still, when there were just a few resistant bacteria, these few did resist the antibiotic more effectively than the rest of the population.

This kind of behaviour becomes explicable if we imagine that the resistant bacteria elute some substance that helps the non-resistant bacteria to stave off the antibiotic. Collective resistance is then most powerful when the substance is sufficient to prime all non-resistant bacteria. This kind of behaviour is useful in helping the whole bacteria population to avoid extinction without having to wait for the initially mutant variety to become the dominant component. The result is then directly favourable to the mutant variety, both to survive the antibiotic attack as well as to maintain genetic biodiversity.

The Boston group has found that resistant bacteria actually produce a small molecule called *indole*, which diffuses into neighbouring cells and sets off a molecular defence against norfloxacin. The noteworthy thing is that indole production is normal for bacteria, but this when antibiotics are *not* present, the indole production falling off when the antibiotics level rises. Resistant bacteria, on the other hand, have evolved to continue producing indole in high antibiotic conditions, albeit at the cost of their own fitness.

Such behaviour, to suffer a cost to produce a substance that is not required for their own survival but is useful for others, may be thus be called a form of *altruism*. That it is finally survival-promoting, by boosting the collective response, may explain the selection for altruistic behaviour, but the "cooperative" response is unmistakable.

Lee and others repeated the study with another antibiotic, *gentamycin*, and found identical results. The behaviour is thus not antibiotic-specific and more study of detailed population dynamics of bacteria under antibiotic dosing could enable a more effective and safer use of antibiotics.

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On the tip of an iceberg

In Kolkata alone, almost 90 of every 100,000 individuals suffer from Alzheimer's, writes rhishav n choudhury**. Hopefully, one of the techniques currently being researched will lead to treatment and allow senior citizens to live normally in the sunset years**

Once there is even minimal cognitive impairment, the brain is damaged, inflamed, burning like an inferno.
— Dr Caleb Finch, director of the Gerontology Research Institute, University of Southern California.

YOU'VE walked out of your house, started your car, and leave for work. On the way you realise you've forgotten something — could be your lunch, checking your kids' homework or paying the phone bill. Memory is a strange thing; sometimes even after racking our brains for hours we just can't seem to get the word, experience or fact that we want. But that's neither abnormal nor suggestive of any disease, since we don't catalogue and file every piece of information flowing into our minds.

On the other hand, in the past century several diseases associated with degradation of memory and cognitive functioning have been identified. Before the 20th century, most humans didn't live beyond 50, today the average lifespan is about 70. Though a boon, the downside is several previously unknown age-related medical problems, such as Alzheimer's.

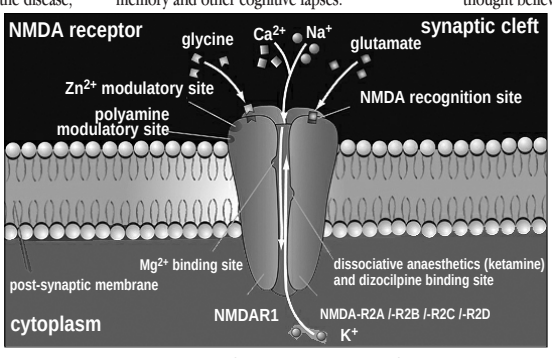
First described by German psychiatrist Alois Alzheimer in 1906, the disease is characterised by extracellular plaques and tangles in neurons, the specialised cells that process and transmit signals in the brain. This leads to diminished working of the nervous system and eventual loss of memory and cognitive functions. In the 1980s and '90s, research unravelled the fundamental biochemistry that leads to this formation of plaques and tangles, along with the discovery that several genetic factors underlay the disease and led to the introduction of the first drugs. Rapid progress has taken place in the last decade; now imaging and spinal fluid samples enable tracking the disease, and research indicates that preventive treatment may help.

"It's a devastating disease, a devastating medical condition, not just for the person suffering, but it destroys the entire family, sucking the happiness out of life like a black hole. We are sitting on the tip of an iceberg; a large number of our fellow citizens are going to be struck down by this terrible disease in the future. For this reason until the possibility of a cure comes along, it is essential to develop awareness and a support network both for those suffering and their families who are have to live with the consequences of this disease" says leading geriatrics expert of Kolkata, Dr PK Pooviah.

With new tools like brain imaging and spinal fluid tests, Alzheimer's related biomarkers can be monitored for signs of biological changes such as mounting levels of toxic proteins. The three main indicators of Alzheimer's now detected by these tools is amyloid accretion, tau build-up and brain shrinkage. Amyloid accretion is the build up of the protein fragment *amyloid-beta* that is linked with the formation of new memories.

This accretion results in damage to synapses, the interface or gap connecting neurons. The damage to the synapses blocks chemical signals (neurotransmitters) from reaching receptors on receiving neurons which disturbs the transmission of signals along the nervous system. The second indication, build-up of the protein *tau*, critical to the functioning of neurons, begins to behave oddly. These proteins begin to have an accumulation of phosphate groups causing detachment from the microtubules, which lead to the production of more tau proteins in an attempt to hold the microtubules together. This results in the formation of tangles that interfere with cellular functions.

The last indicator, brain shrinkage, is due to the death of nerve cells as the disease advances, thus shrinking the brain in areas involving memory and high level brain functions. The shrinkage accelerates over time and ultimately involves many more areas of the brain, leading to noticeable memory and other cognitive lapses.



The NMDA receptor is one of the main mediators of excitatory neuro-transmission. The binding of both glutamate and glycine activates this receptor — a ligand gated ion channel that permits the movement of calcium, sodium and potassium across the post-synaptic membrane. The NMDA receptor is composed of the main NMDAR1 sub-unit and the NMDA-R2A, NMDA-R2B, NMDA-R2C and NMDA-R2D sub-units.

Is Alzheimer's curable? At the moment, the answer, unfortunately, is no. Current drugs treat cognitive symptoms only, not the underlying disease processes, and only work for a limited time, from months to a few years. Of these drugs, acetylcholinesterase inhibitors (donepezil, galantamine) and NMDA receptor antagonists (memantine) having some of the most promising results. The former blocks the action of the enzyme acetylcholinesterase, initiating an increase of the chemical acetylcholine in the brain. This, in turn, improves cognition, mood, and behaviour and so promotes better daily functioning. The latter helps to subdue over-activity by a signalling chemical, *glutamate*, that can lead to the death of neurons. As far as the genetic causes behind Alzheimer's goes, it has been found that the gene APOE4 may increase susceptibility to Alzheimer's, though people who are carriers may not get the disease.

Ongoing research holds the promise of major changes in Alzheimer's treatment in the next decade. Some of the drugs under study include inhibitors of the enzymes that produce amyloid-beta, vaccines that clear amyloid-beta, amyloid-beta aggregation blockers, anti-tau compounds, and neuroprotective agents. An alternative school of thought believes that some form of exercise, a good diet, and cognitive exercises like reading, puzzles, chess, etc, has a positive effect in combating Alzheimer's. However, clinical trials still need to confirm that these activities actually halt the progression of Alzheimer's.

"I was surprised and, at the same time, very sad, (about the lack of evidence of the effects of exercise, diet, etc, on Alzheimer's)," said Dr Martha L Daviglus, Professor of Preventive Medicine, Feinberg School of Medicine at Northwestern University. "This is something that could happen to any of us, and yet we are at such a primitive state of research."

Within our very own city, almost 90 of every 100,000 individuals suffer from the disease.

That's roughly 15,000, and the numbers recorded are steadily rising as more families become aware of the symptoms and seek medical help. The majority of sufferers are over 65 years, but 20 per cent range from 30-50 years. And this is in our very own backyard. I'm not taking statistics from any other city in India or abroad, this is happening right here in Kolkata.

With the number of people susceptible to Alzheimer's increasing in proportion to greater longevity, a cure or preventive drug has become vital. Hopefully, one of the techniques currently being researched will lead to treatment that will allow senior citizens to live normally in the sunset years.

The writer is a freelance contributor

Creative chaperones

tapan kumar maitra **explains the protein-folding problem and its different characteristics**

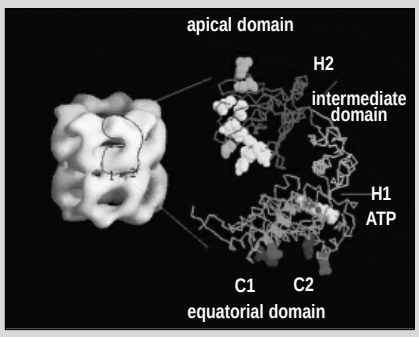
SINCE biochemist Christian Anfinsen won the Nobel Prize in 1972 for showing that the enzyme *ribonuclease* refolds to its original shape after denaturation *in vitro*, scientists have believed that the final protein shape — the secondary and tertiary structures — forms spontaneously.

Recently it has been shown, however, that many proteins do not normally form their final active shape vividly without the help of proteins called *chaperones* or *molecular chaperones*. These chaperones do not provide the three dimensional structure of the proteins they help but, rather, bind to a protein in the early stages of folding to prevent unproductive folding or to allow denatured proteins to refold correctly.

Like human chaperones, they prevent or undo "incorrect interactions", according to J Ellis. That is, many proteins have a large number of different structures they fold into. Many of these structures wou1d have no enzymatic activity or would form functionless aggregates with other proteins. Molecular chaperones allow proteins to fold into a thermodynamically stable and functional configuration. Each cycle of refolding requires ATP energy.

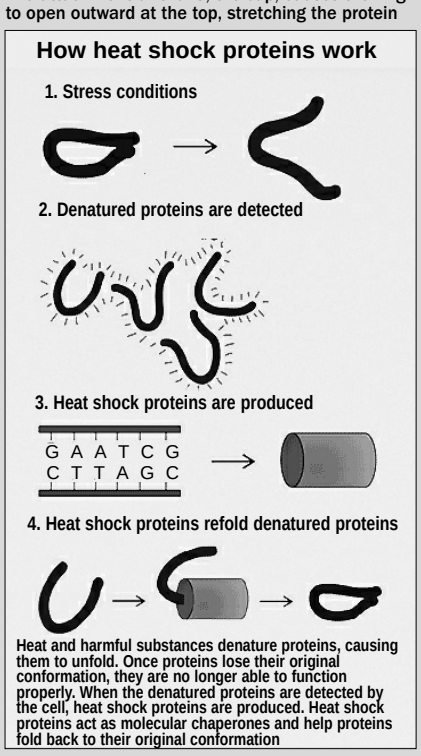
A well-studied class of chaperones is known as *chaperonins* — Hsp60 proteins — because they are heat shock proteins. About 60 kilodaltons (60,000 deltons) in size, they occur in bacteria, chloroplasts and mitochondria. One of the best studied of these chaperonins is the protein GroE of E. coli, which in its active form is composed of two components — GroEL and GroEs.

GroEL (Hsp60) is made up of disks, each composed of seven copies of a polypeptide. GroES (Hsp10) is a smaller component composed of



The structure of GroEL (Hsp60).

seven copies of a small subunit. GroEL forms a barrel in which protein folding takes place. The barrel is shaped in such a way that entering proteins of a certain size make contact at interior points in either the upper or lower ring of GroEL. The attachment of GroES, the cap, causes the ring to open outward at the top, stretching the protein



Heat and harmful substances denature proteins, causing them to unfold. Once proteins lose their original conformation, they are no longer able to function properly. When the denatured proteins are detected by the cell, heat shock proteins are produced. Heat shock proteins act as molecular chaperones and help proteins fold back to their original conformation

inside. This stretching takes energy from the hydrolysis or ATP molecules located inside the rings. When GroES dissociates, the protein can fold into a new, more functional configuration. If it doesn't, the cycle repeats.

There are several classes of molecular chaperones, proteins of different sizes and shapes that recognise different groups of proteins or protein conformations. GroEL recognises about 300 different proteins, small enough to fit into the barrel (20-60 kilodaltons) and having hydrophobic surfaces. These include many proteins in the transcription and translation machinery of the cell. Hsp90, another heat shock protein, recognises proteins involved in signal transduction. Hsp70 recognises hydrophobic regions in polypeptide side chains, many of which extend across membranes.

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