

Reducing the need for animal use for scientific purposes: replacing antibodies with aptamers

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Antibodies, including polyclonal and monoclonal antibodies, are reagents that are used extensively for a wide range of applications in biological research, clinical diagnostics and therapeutics. The production of antibodies, by necessity, requires the active immunization of animals such as mice, rats, rabbits, guinea pigs, horses, sheep, goats, chickens or other animal species. Immunization involves treating (by injection) animals with an antigen (a foreign substance), along with a range of adjuvants which are pharmacological agents that assist the promotion of an immune response. This manipulation not only has the potential to cause animals pain, distress, decreased appetite and/or weight loss, but can sometimes also induce a tissue reaction at the injection site resulting in an inflammatory response, abscesses, or even tissue necrosis or ulceration. When producing monoclonal antibodies, the immunisation step is followed by a "scale up" procedure. Such a procedure can include the production of ascites (also known as abdominal fluid). Extrapolated from the human experience, ascites can have adverse

effects including abdominal discomfort, indigestion, and breathing difficulty. Although the ascites fluid method of monoclonal antibody production is a very efficient method of producing a good antibody response, it is increasingly being replaced by an alternative cell culture procedure because of the potential pain thresh-holds experienced by the experimental animals. Nevertheless, the primary immunization step requiring the extensive use of experimental animals remains.

The concepts of replacement, reduction and refinement are regarded as foundation principles with respect to the ethical use of animals for scientific procedures. Recently, a new technology to replace the use of animals in the production of "antibodies" has been developed. The new technology involves the use of aptamers which can be used instead of antibodies for all the scientific purposes listed above. Aptamers are single-chain nucleic acid molecules, typically 15-40 nucleotides long. The term "aptamer" derives from the Latin aptus meaning "to fit". When in a solution, the chain of nucleotides folds itself up into

a certain three-dimensional shape. The specific shape of the aptamer allows it to bind tightly with its target molecule. The recognition between aptamer and its target is based on the relationship of "lock-and-key", just like an antibody specifically binds to its antigen. Now the question is how can we produce aptamers to replace antibodies?

A new technique called Systematic Evolution of Ligands by EXponential enrichment (SELEX) has made it possible to select highly specific nucleic acid-based ligands against target molecules. Importantly, SELEX is entirely an in vitro laboratory-based process that does not require the use of animals. The challenge to finding an aptamer to a chosen target requires identifying the aptamer in an extremely large pool of chemically-synthesized nucleic acids. However, in the past a potential problem that has limited the use of this technology has been the ability to produce an ideal aptamer that possesses a high affinity (i.e. one that binds tightly to its target) with high specificity (i.e. one that binds to that particular target only). A New Zealand research team, involving a collaboration between AgResearch (a Crown Research Institute) and Victoria University of Wellington have recently developed a technique to produce aptamers with both high affinity and specificity. This novel technique was named the "In Vitro Directed Evolutionary Process". The generation of aptamers using this process is a New Zealand first in research and has been recently published in a highly-rated international journal, *Clinical Chemistry* [1].

Aptamers against both protein and non-protein target molecules, including steroid hormones [2], have now been generated successfully using this novel in vitro process. Based on their binding specificity and sensitivity, the aptamers were demonstrated to detect their target molecules in a variety of applications typically used by antibodies. These include detection of proteins bound to a solid support (i.e. "Western blotting"), in solution (at picomolar concentrations) and the purification of target molecules. Given the ability to develop aptamers for both protein and non-protein targets, as well as their suitability for use in multiple assays, this methodology has immense potential to significantly reduce animal use for the generation of

antibodies. Details of this method have been presented at the Reproductive Biology Satellite Meeting of the Queenstown Molecular Biology Meeting in September 2009 [3]. Aptamers are being internationally recognized as an emerging technology for replacing antibodies and Dr. Lin has been invited to be a speaker at the forthcoming International Congress of Antibodies-2010, in Beijing, China [4].

In addition to not requiring animals in their generation, Aptamers have numerous competitive advantages over antibodies as listed below:

- 1). Aptamers can be generated by a simple in vitro process for virtually any targets, even those that have high toxicity or low immunogenicity. Antibodies with these characteristics would be extremely difficult, if not impossible, to produce in animals.
- 2). Aptamers are stable under a wide range of buffer conditions and are resistant to harsh treatments such as physical or chemical denaturation, exhibiting no loss of activity.
- 3). Aptamers characteristically have better tissue penetration, because of their smaller size compared to antibodies.
- 4). Aptamers tend to bind to grooves and clefts on target proteins and can recognize binding pockets in ways resembling that of small molecules. An aptamer with this property is very useful as a tool for drug development.
- 5). Aptamers have not been observed to be immunogenic, because nucleic acids are not typically recognized by the human immune system as foreign agents. An aptamer with this property is very useful as a drug candidate.
- 6). Aptamers are specific drug-like antagonists of protein function. Aptamers have a high inhibitory potential compared to that of antibodies.
- 7). Aptamers can be chemically synthesized, offering an unlimited source and a wide variety of targeted modifications such as fluorescent reporters.

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Omelas revisited: The terrible paradox of animal suffering

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This paper has been derived from an oral presentation, which was given to a combined meeting of New Zealand's National Animal Welfare Advisory Committee and National Animal Ethics Advisory Committee on 11 March 2009.

In this article, I will stand back from technical, hands-on details of legislation, codes, committees, funding and the like, to place these in their wider ethical and philosophical context. I would like to remind you of the basic problem which focuses us all on animal welfare – the very nature of the human-animal relationship itself. I will approach my subject by recounting to you a short but powerful story by the writer Ursula Le Guin, then unpacking its relevance for the human-animal relationship generally, and for your role as the official custodians of that relationship.

The story in question is 'The Ones Who Walk Away From Omelas', written about forty years ago. On the face of it, it has nothing to do with animals and Le Guin herself was probably not thinking of their condition as she wrote it. However, it is intended as an open-ended fable, parable or allegory, its central theme adaptable to many different situations. For me, the moral dilemma at the core of the story – the 'terrible paradox' in my title – perfectly encapsulates the central problem underlying the animal welfare debate.

Omelas is an imaginary city state whose inhabitants live

metaphorically in perpetual sunlight, but whose lives are haunted by a profoundly disturbing shadow. Le Guin introduces us to the people at their most joyous, on a festival marking the first day of summer, but she stresses that their joy on this day is simply an intense expression of the general happiness that typifies Omelas the whole year round. For the purposes of the fable, she wants us to imagine 'The Good Society'. She spends most of the story evoking her personal vision of such a society – no monarchs, no slaves, no militarism, sexual pleasure without prurience, healthy bodies, wise minds, gentle relationships. She doesn't want to impose her own personal preferences however, so invites her readers to delete or add details in line with their own vision of what would constitute communal wellbeing. The only premise on which she insists is that Omelas, no matter how hard it may be to imagine, is a land where everyone is happy and all its inhabitants are good, decent people.

This happiness does not mean the citizens of Omelas are naïve and childlike. It is not a fairy tale land of dulcet shepherds, noble savages or grand utopians. Like us, the people of Omelas are mature, complex, intelligent and passionate adults, their scholars wise, their science profound. Le Guin concedes it is hard to imagine, but for the sake of what is to follow Omelas is the good and happy society, where arts, sciences and harmonious relationships flourish on an infrastructure of economic sufficiency and good health.

Now Le Guin almost casually adds one small detail she has previously omitted. In one of the buildings there is a dark cellar containing a cupboard. It has a locked door and no window. Only a little dusty light comes in through the cracks between the boards. In that cupboard crouches a small naked child. The child appears simple-minded. Perhaps it was born defective, or perhaps it has become imbecilic through fear and neglect. It occasionally picks its nose or fumbles vaguely with its toes or genitals. Its buttocks and thighs are a mass of festering sores, as it sits constantly in its own excrement.

Occasionally the locked door rattles terribly and people are there. One of them hastily fills the food and water buckets; the others do not come close but stare at the child with frightened, disgusted eyes. Then the eyes disappear and the door is locked again.

The people never say anything to the child, who has

not always lived in a cupboard and can remember the sunlight and its mother's voice, but the child itself would sometimes speak: "I will be good," it would say. "Please let me out. I will be good." It was never answered. It speaks less and less often now. It used to scream for help and cry a good deal, but now it only makes a kind of whining noise.

Some citizens of Omelas have come to see the child, others are content to know it is there, but they all understand it has to be there. They understand that their happiness, the beauty of their city, the tenderness of their friendships, the health of their children, the wisdom of their scholars depend wholly on this child's abominable misery. They know it would be a good thing indeed to bring it up into the sunlight out of that vile place and to comfort it. But if it were done, in that day and hour all the prosperity and beauty and delight of Omelas would wither and be destroyed. The terms are strict: not even a kind word may be spoken to the child.

This stern edict is explained to children when they are capable of understanding and most of those who come to see the child are young people. No matter how well the matter has been explained to them, they are always shocked and sickened at the sight. They feel disgust, anger, outrage, impotence – emotions to which they had thought themselves superior. They would like to do something for the child, but there is nothing they can do.

Often the young people go home in tears, or in a tearless rage, when they have seen the child. They may brood over it for weeks or years. Finally, however, their tears at the bitter injustice dry when they begin to perceive and accept the terrible justice of reality that underlies that apparent injustice. They comfort themselves that even if the child could be released, it would not get much good from its freedom: a little vague pleasure no doubt, but little more. It is too degraded and fearful to know any real happiness. Indeed, after so long it would probably be wretched without walls about it to protect it, and darkness for its eyes, and its own excrement to sit in.

There is more involved, though, than speculations about whether the child would be happier free and befriended than imprisoned and uncomforted. The young people feel guilt at the sufferings of the child, but they would be far guiltier if they exchanged all the goodness and

grace of every life in Omelas for one single, small improvement - if they threw away the happiness of thousands for the chance of the happiness of one.

However, there is something more profound than naked self-interest involved. They know that it is the acceptance of their helplessness which is perhaps the true source of the moral splendour of their lives. Theirs is no vapid, irresponsible happiness. They learn that they, like the child, are not free. But above all, it is from the existence of the wretched one, snivelling there in the dark and their knowledge of its existence, that they learn compassion. It is their capacity to face and accept the terrible paradox of Omelas without losing their compassion that makes them triumphantly human.

There is only one more thing to tell. At times one of the adolescent girls or boys who go to see the child does not return home to weep or rage, does not in fact, go home at all. Sometimes also a much older man or woman falls silent for a day or two and then leaves home. These people go out into the street at dusk. They walk down the street alone. They keep walking straight on, out of the city of Omelas. They walk ahead into the darkness and they do not come back. The place they go towards is a place even less imaginable to most of us than the city of happiness. But they seem to know where they are going, the ones who walk away from Omelas.

Well, that's Le Guin's fable. On the surface, it's obviously not about animals, but equally obviously it can be taken out of the domain of open-ended allegory and applied with perturbing relevance to the human-animal relationship. You've undoubtedly already done some translating, but at the risk of being over-obvious, I will spell out a few changes that are required to adapt it to our present purposes.

The first and most glaringly obvious bit of translation involves substituting the word 'animal' for 'child' throughout the story. We should conjure up the mental image of one specific animal in a concrete situation:

- a pregnant sow trapped in a gestation stall so small she cannot even turn round
- a late winter lamb, dying of exposure alone and without succour
- a duck unable to fly, its wing shattered by an inexperienced marksman
- a calf taken prematurely from its mother and

- packed into a lorry
- a chicken plunged alive on a conveyer belt into scalding water
- a laboratory mouse racked by the effects of poison
- a tiger imprisoned in a zoo
- a bewildered and frightened puppy in an over-crowded animal shelter...

Of course one could multiply the images, but the point is obvious: for the metaphorical child of Omelas, let's substitute the misery of a specific and very real case of animal pain or distress.

Now for a second and equally obvious substitution. In Omelas, the scales were heavily weighted: just one child in a pan on one side of the scales, an entire nation on the other. In the real world of human-animal relations, the balance is quite different: we are talking not of one but of an astronomical number of animals whose well-being is sacrificed for human happiness. For example, in New Zealand, around 150 million farm creatures are processed each year for the benefit of four million human New Zealanders.

A third bit of tweaking concerns the kinds of suffering involved. With the child, it is shut up and left to suffer from the related trinity of confinement, isolation and neglect. The citizens of Omelas passively acquiesce to this neglect, but they do not actively plan and practise ways of inflicting pain upon the child, of plunging it into states of chronic depression or panic terror, or of violating its biological integrity.

Of course a huge number of animals also experience such passive suffering: a layer hen in a cramped and barren cage; a dog left tied up alone round the clock; cattle in a parching, treeless pasture... it could be a very long list. However, those animals are not the victims of active, planned callousness; they do not suffer from physical pain or emotional distress over and above their confined, neglected and/or lonely situation.

Much other animal suffering however, is actively, consciously and systematically inflicted. In the real world, unlike in Omelas, humans are agents of suffering. If a lab mouse is dying of a painful cancer, it's because somebody deliberately inflicted it; if a deer stumbles through the bush with a

crossbow bolt in its innards, somebody shot it; if a cow is worn out, crippled and prematurely aged, it is because somebody figured out and implemented a science-based regime of over-production of milk. In all such cases, suffering occurs not because humans have turned their backs on it, but because they have leaned forward and deliberately inflicted it.

A fourth way in which Omelas differs from the real world is that in Omelas there is no rational causal link between the snivelling creature in the dark and the general well-being of the citizenry at large. It's just a 'given' of the fable, a stern edict whose source is unknown. It's a magical link, if you like. In the case of animal suffering in the real world and its human benefits, the causal chain is clear, rational, material and explicable. Indeed, it is regularly explained to the squeamish precisely why a specific form of animal distress is absolutely essential:

- that elephant has to be a zoo exhibit because children like riding on its back
- that boar has to be hunted by dogs and have its throat slit because wild life safaris are good for our tourist trade
- that dairy cow has to be shipped for weeks through the tropics because a farmer must make money out of it
- that mouse has to be given cancer or an academic will not be able to continue obtaining grants and publishing papers
- that family cat has to be handed over to the SPCA for euthanasia, since its owners must enjoy the legal right to dispose of their own private property as they see convenient...

These explanations of why animal suffering is unavoidable have hardened into sacrosanct dogmas, almost as magical as the law of Omelas. For each form of animal suffering, there is a human pressure group claiming its vested interests would be damaged if that suffering were to cease. Such groups direct the full force of their moral outrage at anyone who speaks up on behalf of animals – moral outrage one might expect to be directed against those who cause suffering, not those who protest against it. Thus, although the causal link between suffering and well-being differs between Omelas and our own world, one being magical and impersonal, the other rational and personal, the social upshot is the same. Those whom the link distresses are accused of undermining the foundations of 'The Good Society'.

Now for one final tweak to adjust Omelas to the real world, then I'll start unpacking the allegory.

In Omelas, those who cannot accept the stern decree physically walk away from the city. In the real world, the walking away is metaphorical. Those who can no longer in good conscience bear the animal misery on which it is founded renounce all collusion with the system. They do not consume animal based foods, do not sport clothes or adornments made from dead animals, they avoid animal-based entertainments, spectacles and recreation, they refuse medicines derived from animal research, and probably do not own their own companion animals. This is a quietist, personal solution to the terrible paradox of Omelas: those who adopt it may not live easily with Omelas, but they can at least live with themselves.

Up till now, I have simply been demonstrating that with a few necessary tweaks, the Le Guin fable can be transferred from a human child in an imaginary world to non-human animals in the real world and showing that when we do so, it emerges that the plight of the latter is far worse than that of the former. However, I have not yet reached the real core of the fable. The story is obviously not about animals, but neither is it about an abused child. It is an allegorical representation of the most vexing philosophical problem in the modern world. This problem relates to the ethical issues generated by the moral philosophy known as Utilitarianism.

Utilitarianism was founded by Jeremy Bentham in the eighteenth century, and made a major come-back in the works of contemporary philosophers like Peter Singer. You are, of course, very familiar with it. It involves what might alternatively be called a cost/benefit or harm/benefit analysis, or else a means/end calculation. Policy makers figure out what social benefits they wish to generate, the top three being wealth, health and happiness, calculate the 'collateral damage' or sacrifice that would be required to gain such benefits, and then balance the two, aiming for maximum 'utility' at the minimum cost.

In terms of means/ends calculations, they define their goals – again wealth, health and happiness are the top three – and figure out what means can be employed to achieve those ends. This is often a purely technical matter of devising efficient means to achieve pre-established goals, but opens up the disturbing moral question: when

do the ends justify the means? It is quite clear that this is what Le Guin's fable is about: what is the cost of 'The Good Society'? What means are justified to achieve it?

Applying this to the human-animal relationship, the odd thing is that Utilitarianism has been used in quite different and sometimes mutually incompatible ways. On the one hand, Peter Singer in his 1975 book *Animal Liberation*, employed it as a weapon in the cause of total animal emancipation from human dominion. On the other hand, it is explicitly and unapologetically coded into our own Animal Welfare Act and the regulations that flow from it to legitimise the on-going ownership and exploitation of animals by humans. It is on the latter interpretation of the Utilitarian calculation that I will focus.

Let's suppose I am one of those young people mentioned by Le Guin, not yet sufficiently mature to have accepted that a 'terrible justice' underlies the apparent 'bitter injustice' of animal suffering. I have four questions to put to the custodians of Omelas.

First, in their application of the Utilitarian harm/benefit equation, the benefits all accrue to humans; the harm is only done to animals. Is it they alone who pay the costs of on-going human well-being. Is this just?

Putting that another way, in their means/ends technical calculations, humans and their well-being are always regarded as ends in themselves, non-human animals merely as means or instruments to achieve those ends. Humans are regarded as having intrinsic value, animals possess only instrumental value. We know, or should know, that they are living organisms like ourselves, that their experiences matter to them as much as ours do to us, that they, like us, suffer physical pain and emotional distress and that they, like us, are capable of happiness. In short, their lives have an inherent value for them as ours do to us. Is it just then, to treat animals as unfeeling 'things', existing only as instrumental means to achieve human ends: to regard ourselves as subjects, animals as objects?

Third, the Utilitarian calculation assumes that in every and all cases, human good trumps animal suffering. It is sufficient for an individual or group to want something, to justify tormenting an animal. But

why should human wishes, even whims, always be accorded priority? Many alleged forms of human good may, on even casual inspection, prove to be ignoble, trivial, achievable by other means, or mistaken.

Let's take those one by one. 'Ignoble': why should the unsavoury joy of a human hunter outweigh the distress of his non-human victim? 'Trivial': why should deer have their antlers cut off to pander to limp but horny Asian gentlemen who have not heard of Viagra? 'Achievable by other means': why should laboratory animals have poisons or diseases forced upon them, when the safety of shellfish and vaccines can be tested in other, non-lethal ways. Or again, why should humans devour animal food products when their nutritional needs could be met more economically and with less damage to the environment, by a vegetable diet. 'Mistaken': people quite simply do not need nearly as much animal based food or medication as they currently consume; indeed, often that over-consumption can be downright harmful. Why then, is it considered just, that any expression of human need, no matter how unsavoury, trivial or misguided, should always trump the harm to animals it requires?

My emotional adolescent has one last question for the custodians of Omelas which restates the first three by distilling their philosophical essence. The Utilitarian equation manifestly implies the unquestioned assumption that the human species, which appeared on this planet around 100,000 years ago is qualitatively different from and superior to all other animal species. It assumes a Great Divide between the moral community constituted by human society, a community within which we respect one another's rights and empathise with one another's misfortunes, and all other non-human animals. The latter do not belong to our moral community, so there is no binding obligation upon us to treat them with consideration. They are different in kind from us and that difference in itself legitimises our subordination of them.

But what are the ultimate philosophical grounds for that assumption of absolute difference? It used to have a theological legitimation, as found for instance in the Book of Genesis, where the Hebraic god Jehovah gave first Adam then Noah indisputable dominion over all living things. As God disposes of humans, so we may dispose of animals. Today, most Westerners

outside the American Bible belt have discarded supernatural authority for their dominion over their fellow passengers on Noah's ark. Where else can we turn? Religion's great rival Science offers no answer. Not Darwin, who postulated continuity and overlap between homo sapiens and other animals, rather than a fundamental difference in kind. Not microbiology, which has discovered that humans and round worms are built basically from the same genetic alphabet. Not contemporary ethology, which shows that other animals have the same or analogous emotional capacities, social bonds, communicative abilities and even, if in their own terms, cognitive faculties.

Is the Great Divide, which we must assume if we wish to justify our exploitation of animals, based on no ethical principle at all? Does it simply stem from the fact that in the course of our biological and cultural evolution, humans gained power over other animals through our superior technology and our language-based capacity for social organisation and planning? Is it not our monopoly of power and that alone, which enables us to operate the Utilitarian equation always and everywhere in our own favour? We exploit animals simply because we can.

However, in the human world over the last 200 years or so, the maxim that 'might is right' has been fundamentally questioned. Humans have challenged the power monopolies of absolute monarchs over subjects, slave-owners over slaves, men over women, whites over blacks, imperialists over colonised people. Why should the same power monopoly practised by the owners or exploiters of animals not be similarly challenged? Since we can find no ethical, no philosophical, no religious and no scientific justification for assuming a difference in kind between humans and the animals in their power, then surely it is as legitimate to demand that moral concern should be practised towards those animals as it has been to demand concern for exploited, oppressed, stigmatised or marginalised humans. Even the non-human environment and the preservation of the endangered species it contains, has come from virtually nowhere on the social and political agenda and now been placed firmly near its top.

Surely it is time, in fact long overdue, for human-inflicted animal suffering to be placed equally high

on that agenda. There is nothing trivial, nothing to be sneered at about avoidable pain and emotional anguish. We all get upset if a child has these inflicted on it. There is no reason to be less upset when they are inflicted on animals. There are not many people in this country actively engaged on behalf of animals. A generous calculation puts it at no more than about 3% of the population. Unfortunately, there is a tendency for them to occupy different camps and to pull in different, sometimes even mutually hostile directions. The plea with which I will end this paper is for all people who actively care about animal well-being to sink their differences, sit around the same table and figure out a campaign to get politicians, judges, the media and the general public equally concerned. This has been done with other unlikely causes, like rights for people with disabilities, so it could be done for animals.

There is an alternative to walking away from Omelas. That is to stay within its walls and actively crusade for The Good Society where happiness is not just a human prerogative, bought at the expense of the wretched one, snivelling in the dark. To achieve that however, routine legislation and regulation, though necessary, are not in themselves sufficient. Although we are mature adults, we need to remain as emotionally distressed as Le Guin's young people by the terrible paradox of human-inflicted animal suffering. As with all other progressive movements, from the abolition of slavery to the protection of the environment, it is only this emotional charge which will convert passive acquiescence into active transformation.

2010 ANZCCART Conference

Download the Abstract Book from the recent ANZCCART Conference [here](#).

Recent articles of interest:

Unpublished negative results may explain limited translation of promising treatments to the clinic

There was a recent report in Nature News (published on line 30 March 2010) which highlighted the importance of publishing negative results from animal studies and suggest that our current failure to do so is possibly a significant factor in explaining why so few animal studies translate into clinical healthcare outcomes. The full report is available at: www.nature.com/news/2010/100330/full/news.2010.158.html

The Gender Bias in Animal Models

Serious questions have been raised in an article published in the News Focus section of the Magazine Science (25 March 2010) about the importance of using both male and female rodents in many laboratory studies. This article suggests that the exclusion of female animals from studies that are particularly based around neurological or immunological factors may give biased results. This article may be viewed on line at: www.sciencemag.org/cgi/reprint/327/5973/1571.pdf

Updated ANZCCART Publications

The following fact sheets have been updated and are now available at www.adelaide.edu.au/ANZCCART/publications/facts.html

The Domestic Chicken
Frogs and Toads as Experimental Animals
The Sheep

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