Nanophysics research will revolutionise medicine and biological science

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Abstract

The impact of nanophysics research on the biological sciences is discussed. Particular attention is paid to the techniques of imaging biological structures aided by the use of nanoscale particles. These include the use of semiconducting quantum dots and metal clusters. Conclusions regarding the bearing of such techniques on bioscience and medicine as a whole are discussed.
1. Introduction

1.1 Issues to resolve – problem

In this essay I will outline the potential applications of nanotechnology to medicine and the biosciences. As I will show this is a particularly active area of research, with many exciting applications due in both the near and also more distant future. Applications are too numerous to list but include novel drug delivery systems, nano-engineered prosthetics and artificial blood cells [1,2]. I will concentrate on the role that nanoscale particles play in biological research, showing that nanoparticles are already playing an important role in cell imaging, an area in bioscience research probing cell structures and interactions. This will eventually lead to a greater understanding of biological processes, such as those which cause disease allowing progress to be made towards cures. Through the discussion I shall emphasise the contribution of physics research to the various areas and evaluate its influence to date and possible future roles.

1.2 How can physics research contribute to medicine and the bioscience?

Throughout the last century and earlier there has been a tendency for fundamental physics research to find important applications in medicine and biological science. One of the most widely appreciated applications of physics research is that of X-rays to medical imaging. Since their discovery in 1895 by Röntgen, X-rays have become the most widely used form of medical imaging in hospitals and with the later introduction of computerised axial tomography (CAT) they have become a much more powerful technique [3]. Following chronologically another important discovery was the identification of the structure of DNA by Watson and Crick via the means of Bragg diffraction [4]. The 1970’s and 80’s saw huge advances in medical imaging techniques with the application of the phenomena of nuclear magnetic resonance (NMR) to medical techniques with the nuclear prefix being dropped. Also substantial advances were made in the application of fundamental nuclear physics research to commercial applications. These applications were widespread amongst industry and particularly useful were the resulting applications in medicine, both in imaging and diagnostics were radioisotopes can be used to image internal structures and processes. Particularly notable is the technique of positron emission tomography (PET).

In the early to mid part of the twentieth century it would have been quite unimaginable for basic quantum processes such as NMR and nuclear decay to find such widely used applications. This highlights the fact that fundamental physics research is vitally important, not only to those seeking to find out how nature works from a purely academic viewpoint but to potentially groundbreaking applications totally un-envisaged at the time.

1.3 What is Nanophysics?

Nanophysics is part of the wider area of scientific research which exists under the title of nanoscale research or nanotechnology. “Nano” is an increasingly overused prefix for many areas of research and much hype surrounds the subject, mainly generated by the media through science fiction stories involving “nano-bots” and the “grey-goo” scenario [5]. Nanophysics research is primarily concerned with the manipulation of matter on the molecular and atomic level. This incorporates the fabrication, modification and characterisation of systems on the length scale of the nanometre; \(10^{-9}\text{m}\) with the general consensus among the field that the characteristic length scale of nanoscience extends from 1nm to 100nm. Nanoscale systems are especially interesting to the physicist as they often take on novel physical properties due to their small scale. For example a macroscopic mass of gold exhibits profoundly different properties to a nanocluster consisting of say a dozen gold atoms [6]. It is due to these special characteristics shown at the nanometre scale that the field is widely accepted to yield important and wide ranging applications in the future. Nanoscience is an intrinsically interdisciplinary area of research encompassing physics,
chemistry and bioscience to name but a few areas. Nanoscale particles have always existed, however our window in the nano world only really opened following the invention of scanning probe microscopy (SPM) in the early 1980’s. Scanning tunnelling microscopy (STM) [7] and the similar technique of atomic force microscopy (AFM) [8] provided the perfect tool to allow the exploration of matter on the nanometre scale which previously had only been open to more complex and financially costly methods such as electron spectroscopy and transmission electron microscopy (TEM).

2. Nanoscale particles probing biological systems: Quantum Dots

2.1 Introduction

Physicists seek to discover the inner workings of nature at an ever finer and more detailed level. The atom, which had been thought of as a fundamental entity as early in the time as that of the Greeks, was discovered to be composite following the discovery of the electron and then the nucleus. However the nucleus itself was quickly discovered to be composite with the discovery of the neutron and the proton. Our current level of understanding rests with the quarks and leptons as the fundamental constituents along with the bosons to mediate forces. I like to think of this sort of model when considering how those who work in the field of biology envisage living organisms. A creature for example is composed of a collection of organs (neglecting the most basic organisms). Then each organ comprises of many cells, each of which perform a certain task and contain many smaller structures fabricated from molecules such as proteins, themselves formed from amino acids. It is as this level, the molecular level that our need to further break down matter for the purposes of biological understanding stops, however it is at the level of proteins that important information concerned with biological processes can be gained. Such knowledge is desirable for example in the search for cures to diseases as changes in cell structure and growth are often a cause and also an effect of disease.

Recent research has shown that certain nanoscale particles have an important application in the field of biological cell imaging, enabling biologists to probe the internal structure of living cells with many advantages over previously used techniques. These nanoparticles are called quantum dots or nanocrystals.

2.2 What are quantum dots and why are they interesting?

One of the key areas of research in nanoscale physics is to investigate how the properties of materials are changed when we manipulate them on an atomic level. It has long been know that the properties of a cluster of a few atoms are very different to the bulk material [6]. Semiconducting materials give rise to particularly interesting effects when their extent is limited to a relatively small number of atoms. Consisting of only a few hundred to a few thousand atoms at most, quantum dots, also sometimes referred to as nanocrystals bridge the gap between the bulk solid state and that of single atoms. Therefore it comes as no surprise that such materials exhibit a mix of properties seen in both conventional solid state physics and also physics usually associated with quantum particles such as atoms.

In solid state physics semiconducting materials have the defining feature that an energy gap separates the energy bands of the conductance and valence electrons [9]. In bulk semiconducting material such as the silicon used in microchips the gap width is a fixed parameter defined by the particular material. When used in light emitting diodes (LED’s) this is evident in the fact that when light is emitted from the semiconductor, as the result of deexcitation of an electron from the conduction to the valence band, it is always emitted at a fixed wavelength, representative of the semiconductor energy gap. Due to their small size, semiconducting quantum dots are governed by the principles of quantum mechanics and as a result their properties differ greatly from bulk semiconducting material. A phenomenon
known as the quantum-size effect determines the width of the band gap in quantum dots and also breaks down the continuous energy bands of bulk semiconductor leading to the formation of discrete energy levels, analogous to atomic energy levels. These effects are due to quantum confinement, where the spatial extent of the electron wavefunctions become comparable to the physical dimensions of the entire quantum dot. As a result quantum dots may be modelled, to a first approximation by the “quantum box” model [10], a three dimensional spherical infinite potential well in which the electrons are confined. As with other similar potentials in quantum mechanics the electrons may exist in a number of states each with a set energy, thus giving rise to the energy levels of the quantum dot as shown in figure one.

![Energy levels of a semiconducting quantum dot.](image)

The energy gap of the quantum dot, $E_{g(QD)}$, is the difference in energy between that of the highest level in the valence band and the lowest level in the conduction band. It can be shown through the quantum box model that energy gap varies with the size of the quantum dot. In fact the relationship between $E_{g(QD)}$ and the quantum dot radius goes as $1/R^2$; the energy gap increasing as the dimensions of the quantum dot decreases. This property naturally means that semiconducting quantum dots of different sizes emit light at differing wavelengths following excitation of electron-hole pairs. This property has lead to much research with many potential applications ranging from multicolour lasing [11], a new generation of LED’s [12], single electron transistors [13] and as I will describe here optical tags for biological experiments.

### 2.3 Quantum dots as biological tags

To understand the processes occurring in biological interactions it is desirable to monitor the interactions of multiple proteins or cells within an organism [14]. Existing imaging techniques available to biological research are based on fluorescent dyes [15, 16], fabricated from naturally occurring proteins. A prime example of this is that of the pHAT-GFP protein which is a naturally fluorescent protein and is extracted from jellyfish and similar marine creatures. A recent article in the journal Nature gives an introduction to quantum dots as a biological tool and draws comparisons with the conventional dye techniques [17]. The article summarises the drawbacks of conventional dyes which I outline here.
One of the key drawbacks of conventional dyes is that they emit light over a range of wavelengths. This does not prove a serious problem until the researcher attempts to use two different dyes simultaneously, for example to tag two separate proteins inside a cell. The range in wavelength emission is such that the emission spectra of the two dyes more often than not overlap, making subsequent analysis very difficult. The article states that the opinion of Carolyn Larabell, a cell biologist working at the University of California, is that “tagging with two colours is possible if you are careful” and that “some people claim to be able to use three separate colours, however this is very tricky.” This emphasises a key limitation in the existing tagging procedure, limiting experiments to the tagging of proteins to a maximum of three different proteins. In this respect quantum dots show a significant advantage, with chemist Shuming Nie, working at Indiana University stating that he has produced a collection of dots large enough to give “a range of over a dozen different emission colours”, with the property of narrow, non overlapping emission spectra.

A further disadvantage of conventional dyes is that fluorescence can only be induced via illumination with light of a particular frequency. Hence if a number of dyes are used within a localised area, such as a single living cell, the cell must be irradiated with a series of lasers at differing frequencies. The resulting amount of power dissipated within a living cell is far from desirable. However due to the band structure of the quantum dots, it is possible to excite them at any wavelength as long as it is shorter than the wavelength of emission. Hence many different sized dots, corresponding to many tagging colours may be used within a localised area but only a single laser need to be used to excite the entire series of dots. The article states that, as would be expected this is potentially much less damaging to the biological system.

The third important advantage that quantum dots hold over traditional dyes is that quantum dots are extremely stable and continue to fluoresce for many hours. This compares to the characteristic emission lifetime of conventional dyes which is in the range of a few minutes as described in the article. In addition to this the structures of dyes are degraded following repeated cycles of excitation and emission, causing their optical emissions to fade rapidly. Paul Alivisatos of the University of California states that quantum dots have enabled experimentalists to observe cellular interactions for much longer time periods, with the possibility of making movies of long term interactions of biological molecules, each tagged with a different colour dot. He states that “experiments are no longer restricted to five or ten minute periods before the dye fades.”

In terms of further novel applications the article suggests that quantum dots may find uses in medical imaging, but adds that this would require the emission of the dots to lie within the infrared wavelength range due to human tissue being reasonably transparent to infrared. Also another potential application outlined is that of quantum dots being used for the trial and evaluation of new drugs. Drugs operate by binding with several different types of molecule within the body, however any undesired binding with certain molecules would be responsible for side effects of the drug. Introducing a set of quantum dots into the body that selectively bind to different molecules could according to the article, provide a test as to whether the drug is binding to the correct sites with multiple colour tagging making the task more straightforward. These last two points are clear examples of how in the future quantum dots could directly influence medical research.

The Nature article mentions one of the main problems with quantum dots in biological applications at the moment which is considered in more detail in a very recent article in the journal, Science [18] and also an article from Physics Web [19]. The key disadvantage of quantum dots when used in biological experiments is that the semiconducting materials used generally do not react well to water, severely limiting their usefulness in biological applications. Also it was found that dots in solution tended to aggregate into larger clusters, presumable via electrostatic interactions. The Science article outlines ongoing research at Rockerfeller University in New York were quantum dots have been given new coatings enabling them to function correctly in aqueous environments, selectively bind to various targets inside cells and to limit aggregation [20]. The process employed is essentially a chemical one where an individuating semiconducting quantum dot which is hydrophobic in
nature is encapsulated within a shell known as a micelle, which has a hydrophobic centre but a hydrophilic outer shell shown schematically in figure two. This makes the quantum dot compatible for use inside live cells. The quantum dots described in the article were used for \textit{in vivo} imaging using time lapse photography and were found to fluoresce for over four days with no detrimental effect upon the live organism.

![Semiconducting quantum dot surrounded by a hydrophilic outer shell.](image1)

Another research paper detailing the results of imaging of live cells using quantum dots lead to promising conclusions [14]. The aim of the research was to address the concern that quantum dots could possibly interfere with the normal physiology of living cells, particularly over extended imaging periods. The work concluded that over the twelve day period of investigation, cells were labelled “without affecting cell growth or development”. This is a particularly satisfying finding as it shows that one of the key advantages of quantum dots. That is to say, their stability and long emission periods can be employed without any detrimental affect on the living tissue. The paper also shows results for the multicolour imaging of cells, where five different sized quantum dot were used to emit at five discrete wavelengths ranging from blue at 510nm up to red at 613nm. The results are a clear demonstration of two of the key advantages of quantum dots over conventional dyes, with apparently with no adverse affects on the living cells.

The use of quantum dots to label cells associated with cancer cells was reported in another paper [21]. The paper reports all of the usual advantages over conventional dyes and compared the intensity of emission of the quantum dot’s with that of the Alexa 488-streptavidin a typical organic dye. Figure three clearly shows the continued high intensity of the quantum dot emission compared to the exponential type fall of in intensity from the conventional dye.

![Comparison of the emission from CdSe/ZnS nanocrystals with Alexa 488-streptavidin organic fluorescent dye. The quantum dots clearly out perform the conventional dye [21].](image2)
Hence from the literature it seems that the use of semiconducting quantum dots is a very active area of research and although in the early stages of application, the technique holds several advantages over existing methods of cell tagging and subsequent imaging.

2.4 Conclusions regarding quantum dots.

As the title of this essay suggests I am looking for candidate research activities to fit the title of “Nanophysics Research will revolutionise medicine”. At first sight quantum dots seem to fit this statement well. However despite the many advantages quantum dots may bring to biological research, questions still remain over their suitability in many situations and as to whether they will truly revolutionise or just merely contribute to the field. Also I pose the question of actually how much of the research into the biological application of quantum dots can be called nanophysics and not just simply chemistry?

The key advantages of quantum dots over existing techniques have been described above, however I briefly summarise these benefits here. Namely quantum dots enable experiments to be done where cells or individual internal components of cells are tagged with different size dots, which fluoresce in different colours that are more easily resolved than the emissions of multiple organic dyes. Also a group of dots may be excited by laser light of a single frequency, reducing the potential for damage of the living tissue under investigation. In addition to this quantum dots also outperform traditional fluorescent dyes in terms of durability, emitting light for hours or days at a time compared to time periods of only a few minutes for conventional dyes. However it is my impression that useful these advances may be to biological research, advances are in fact all they are and do not constitute a revolution in the field. The same structures and processes can be observed in cells as had previously been with organic dyes. It appears to be a refinement in the procedure rather than a significant advancement. Having said that, quantum dots do enable scientists to extend experiments over longer periods and so can observe how systems evolve over time scales previously unobservable and this is the only major scientific benefit. It is my view that quantum dots enable scientists to perform more complex experiments such as tracking several proteins simultaneously, allowing the production of colourful images which are attractive to publish in journals. However it seems that often this provides little in the way of advancement in the understanding of the processes occurring since the processes could have been observed with traditional organic dye methods albeit over a longer period of time due to the need to tag one protein species at time, hence making the process more labour intensive with less striking images. To draw an analogy to physics the situation resembles the advancement in particle physics experiments where certain decays may be observed in a collider but when someone builds another collider operating at higher energies many more of these decays are observed. This allows for elaborate experiments and impressive statistics but the fundamental physics are not revolutionary.

One point that most of the scientific literature I have been able to review has avoided is that of the possible toxicity of quantum dots and their effects on living organisms. As mentioned quantum dots are produced from semiconductor materials with popular materials being Cadmium Selenide (CdSe), and Indium Arsenide (InAs) to name but two. These materials are generally thought of to be highly toxic and their ingestion into the human body is avoided. Therefore I believe the use of such semiconducting nanocrystals in applications involving humans will be severely limited until much more research has been done. The only concerns regarding potential detrimental effects of quantum dots and nanoparticles in general on humans and the environment as a whole were voiced in a report by the Greenpeace Environmental Trust [22]. Here it was mentioned that as nanoparticles are small enough to penetrate living cells (entirely the intended purpose in many diagnostic applications) they could accumulate in organs with unknown effects. Also one contributor to the report states that proteins in the bloodstream could attach to nanoparticles, changing their physical shape or surface chemistry and this could lead to unforeseen consequences such as blood clotting [23].
Another point made by the report was that “quantum dots, nanoparticles and other throwaway nanodevices may constitute whole new classes of non-biodegradable pollutants that scientists have very little understanding of.” This comment was made following the statement by V.Colvin that the large and active surface area of nanoparticles (a particularly useful aspect in applications such as catalysis) would prove a problem if nanoparticles infiltrated the water courses. In such an environment they could adsorb a relatively large amount of pollutants which could then be transported over large distances in water. I believe these points on possible health and environmental concerns to be valid however it is unsurprising that such a review would focus heavily on this and it seems that much of the comment is purely speculation and lacks solid scientific backing. It is easy to say nanoparticles could present serious environmental problems however much more research has to be done.

The prospect of IR emitting quantum dots taking a role in medical imaging seems far off due to the problems associated with aggregation as mentioned in the literature. Here I imagine a situation where if enough dots were to aggregate, a structure could be formed large enough to become trapped inside the body, too large to pass through the kidneys, causing poisoning to the body. Hence until more research is done into the effects of quantum dots on living organisms, the stability of the coatings which protect the hydrophobic semiconducting core form the biological structures and vice versa, and the problems associated with nanoparticles accumulating within the body, the future of quantum dots in medical imaging remains uncertain. As this is a current topic of research new material is being published on a weekly basis. A news article in the latest edition of Physics World [24] outlines the current research of Paul O’Brien of Manchester University and Nanoco, a company that produces quantum dots [25]. The article explains that the company uses a novel method of production where the chemistry involved is much safer than other methods. The highly toxic Cd and Se are transformed into a stable substance known as a “precursor molecule”. O’Brien states that as the handling of hazardous chemicals is minimised the production process is more suitable for scaling up, with multigram batches of quantum dots already being produced. Also it seems this process allows better control of the size of the core and shells, hence allowing a better control over the physical properties. This current work emphasises the need for further research and the advances that it brings to the subject.

Another point to consider is the how well quantum dot research fits the context of nanophysics research. The fundamental mechanism of fluorescence at discrete wavelengths, and variation of wavelength with dot radius is clearly described by fundamental physics. However current research into the application of quantum dots to biology centres of the synthesis of coatings that enable efficient selective binding to molecules and also coatings that improve the durability of the dots whilst not compromising the emission characteristics. This type of research as I found in the literature is more suitably described as chemistry or biochemistry. It seems that although physics enabled the initial understanding of quantum dots its role in the refinement of these nanoparticles into potentially useful biological tools is limited. Therefore to summarise quantum dots are a useful tool however I would not regard them as revolutionary.
3. Immobilisation of proteins: Metal Nanoclusters

3.1 Introduction

Another area of current research where nanophysics is finding an application in the biosciences is in the use of metal nanoclusters for the immobilisation of proteins, allowing imaging by scanning probe microscopy. Nanoclusters are similar to quantum dots, although generally comprising of less than $10^2$ atoms. The application to biological imaging is being pioneered by those working in the Nanoscale Physics Research Laboratory (NPRL) at the University of Birmingham. Here metal clusters are produced by a complex method which I shall not discuss in detail but is well described in the literature [26,27]. To summarise, the end result is a beam of ionised clusters each consisting of a known number of atoms. These clusters are deposited onto a suitable substrate such as graphite. The cluster impact energy may be controlled such that the clusters implant into the graphite surface and remain stable in that location at room temperatures. The resulting clusters are then used as binding sites for proteins. Gold is the current metal of choice due to its mildly hydrophobic nature which attracts hydrated proteins, its relative inertness and capability to induce binding with certain chemical groups called thiols that exist within the surface structure of many proteins. As the gold clusters are implanted within the graphite substrate the gold and any proteins bound to it are stable at room temperature in both air and aqueous environments. This allows a range of experiments to be carried out including scanning probe microscopy in an aqueous environment, mimicking as closely as possible the conditions proteins experience within live organisms. Scanning tunnelling microscopy (STM) and atomic force microscopy (AFM) [7,8] were in my opinion one of the key developments in 1980’s science because as I have stated earlier they are a perfect tool for observing micro and nanoscale systems. This research is a particularly clear example of how nanophysics research can directly contribute to bioscience. The work carried out, although not directly related to medicine, is of the same type of that of the quantum dots. Namely it provides a tool for biologists to use to gain more understanding of the processes occurring within proteins and their interactions. However this research is not yet as well advanced as the quantum dot research.

3.2 Biological scanning probe microscopy

AFM has recently attracted considerable attention from biologists seeking to investigate the structure and topography of biomolecular structures. The aspects of AFM that make it a particularly attractive technique for use with biological structures is its capability of high resolution imaging while operating in aqueous environments and also the possibility of imaging protein interactions in real time [28]. The basic physics that lies behind the operation of the AFM is the measurement of the force interaction between the surface being scanned and a very fine tip which is raster-scanned across the sample as shown in figure four. More detailed discussions of the construction and operation of the AFM may be found in text books [29].
A particularly interesting review of the techniques of imaging biological samples using AFM is given in a paper by Peter Wagner, an expert in the field [30]. The paper states that although much progress has been made in the imaging of biomolecules one key problem yet to be resolved is that of lateral resolution of hydrated proteins. Accurate measurements of dimensions of structures are obviously desirable along with the observation of the overall form of individual bio molecules, however hydrated molecules pose particular problems due to the softness of the samples. Due to the atomic dimensions of the AFM tip, it is clear that significant pressure is often applied to the soft specimen under investigation, causing them to deform. Therefore Wagner states that protocols have to be developed to optimise the imaging conditions while at the same time maintaining the natural structure and operation of the biological samples. From this he concludes that the imaging of bio molecules requires “appropriate substrates” and progress in AFM imaging is “most likely to come from surface science.”

A suitable substrate for immobilisation must in the words of Wagner “preserve the activity and integrity of the specimen while firmly anchoring the specimen to the substrate.” Although this initially sounds rather an undemanding request research has proved it to be rather a complex problem with a number of possible solutions each exhibiting positive and negative aspects. The key differences in immobilisation strategy can be traced back to the type of interaction that occurs between the sample and the substrate. Namely does this interaction result from a chemical bond, formed via the transfer of electronic charge or merely from physisorption of the specimen onto the surface where a net attractive force, composed from van der Waals, electrostatic and hydration forces amongst others, hold the sample onto the substrate.

Much of the research in biological AFM imaging is performed on muscovite mica substrates. This material is a glassy aluminosilicate mineral that cleaves easily allowing thin sheets suitable for substrates to be fabricated. Here the adsorption of specimen molecules occurs via the physisorption process mainly from electrostatic interaction and also due to the hydrophilic nature of the substrate when using hydrated molecules. Wagner summarises the advantages of mica to be that very high lateral resolution is regularly achieved. He accounts for this due to the “close packing or constraining” of specimen molecules on the surface of the mica. This occurs due to the very flat nature of the substrate and also because of its hydrophilic surface properties. Such close packing improves lateral resolution greatly by means of reducing the contact pressure of the AFM tip on any one molecule, by spreading the force more uniformly through the adsorbed molecules. An example of an image formed through use of such techniques is shown in figure five.
Figure 5: GroEL chaperonin molecules on a mica substrate. The close packing of the molecules is clearly visible, allowing the ring structure of many of the molecules to be resolved [31].

The advantages of mica substrates with regard to reduced specimen deformation and subsequent improved lateral resolution are well documented in the article of Wagner however his discussion omits a key disadvantage with the technique. This is to say that the weak physisorption occurring between the substrate and adsorbate is the origin both of the strengths and weaknesses of the mica technique. This weakness is that structures are easily moved around the substrate by the AFM tip itself especially if the number density of individual molecules is low enough to inhibit the formation of large scale close packed lattice structures. Therefore the technique is not particularly appropriate for the imaging of single bio molecules such as single proteins. This being particularly important for the topological analysis of individual molecules and observation of any time dependent properties. As Wagner does mention, experiments done at cryogenic temperatures would certainly improve resolution due to reduced thermal motion of the adsorbate. However this line of enquiry is generally not considered useful by biologists as the conditions used are obviously far removed from those seen in living organisms. Therefore I question the potential of conventional mica substrates in terms of imaging small quantities or even single proteins and advances in imaging of real time protein interactions.

The other main class of substrate is that where a chemical bond forms between the substrate and adsorbed molecules. In this case the bonding between the adsorbate and substrate is known as chemisorption. Typical substrates used often consist of gold [32] or other transition metals. However the arrangement of molecules over these extended substrates is often similar to that over the mica substrate making the analysis of small groups or even single molecules difficult. Often a chemical fixative is employed to aid the immobilisation of proteins onto the surface however this is far from satisfactory as the integrity of the physiological conditions is undermined [23,34].

This is where the nanocluster approach to immobilisation displays significant advantages. The graphite substrate used is known to be hydrophobic in nature, hence the mildly hydrophilic gold islands prove natural binding sites for the proteins underinvestigation in the research [35] as shown schematically in figure six. The main author of the initial work, John Collins suggests that the “cluster surface chemistry can be tuned to allow selective immobilisation of proteins in a reproducible manner”. By this he means the current work on the immobilisation of molecules containing the thiol (SH) chemical group, could easily be extended to molecules containing other active sites by means of adding different chemical
functional groups to the gold clusters. This highlights a clear advantage of the method, selective binding of essentially any protein could be achieved after surface chemistry consideration of the gold surface had been made. The work of Collins et al. included the AFM imaging of two proteins, namely pHAT-GFP and Human Oncostatin M, both binding with the gold clusters to form islands, stable at room temperature and under repeated AFM imaging.

![Diagram](image)

**Figure 6:** Immobilisation of proteins onto metal clusters.

A paper by C. Leung et al. [36] gave details of an extension to the work by means of the immobilisation of another protein called groEL, and it was also demonstrated that another protein called HRP which does not include a thiol group could bind to the gold clusters. Further work lead to the experimentation with more dilute protein solutions and cluster films, yielding more well separated protein islands as shown in figure seven. The aim of this was partly to obtain a significant amount of clusters with only one bound protein, enabling high resolution imaging of the topography of the protein and providing a basis for further work in the area.

![Image](image)

**Figure 7:** GroEL chaperonin immobilised onto gold clusters [37]
In my opinion the immobilisation of protein molecules by size selected metal clusters will prove a substantial step forward in the imaging of proteins and other biological molecules. Advantages of the technique are that proteins can be strongly bound to the substrate via chemical bonds. This allows the imaging of the proteins to be carried out easily with AFM techniques without the problems of the probe tip moving proteins around as is often experienced on other surfaces such as mica. This is achieved in a more desirable way to that of flat extended gold substrates where the adsorbed molecules are distributed across the surface, generally forming large close packed aggregates, the cluster method confines the immobilisation to specific sites. The deposition of the clusters can be done with a high degree of control and dispersed arrays of clusters can be produced, maximising the separation of the proteins and hence reducing the interactions between them. This allows the investigation of the topology of proteins without the interactions normally associated with the close packed structures formed for example on mica. The situation where a single protein molecule adsorbed on to each cluster can be envisaged to provide a significant leap forward in the understanding of protein structures by elimination any interactions with other proteins at all. I believe that this result will be achieved before long following further advances in the control of cluster deposition and the production of well dispersed arrays. Also advances in the protein handling procedure and the use of very dilute solutions, containing the bare minimum of proteins to enable a single protein to bond with each cluster will prove useful. This would prove particularly useful in real time imaging of protein-protein interactions where AFM would be used to observe structural changes over time. I also believe that the immobilisation of proteins using metal clusters provides an ideal opportunity for tagging experiments using semiconducting quantum dots, where the advantages of both methods could be combined in a single novel experiment.

Combined with advances in AFM probe technology such as the use of carbon nanotubes as AFM probes [38], the possible use of electrosatically balanced buffer solutions [39] and also low temperature imaging in certain situations, the resolution of AFM imaging could be greatly improved with the cluster method providing a highly suitable basis for such work.

However there are also disadvantages with the cluster deposition method. The preparation of substrates is very much more involved that that of the preparation of say mica substrates of which many can be cleaved from one piece of mica within minutes. The deposition of clusters requires the use of a highly technical apparatus which obviously requires a significant financial input as well as the time and expertise to operate. Therefore a single cluster substrate takes much more time to fabricate that a mica substrate and is many times more expensive. Therefore it seems unlikely that apparatus for the production of such cluster films will become widespread and may well remain confined to physics laboratories where cluster films are used in much more wider research, making the costs justifiable. This problem was noted by J.A. Collins and the possible re-use of cluster films was mentioned in his paper. Experiments were performed with acid being used to remove bound proteins without damaging the clusters themselves [35]. The work continues and the integrity of the clusters after protein removal is still an unknown factor. It is my opinion that the re-use of cluster films may well be achievable and other methods of dislodging bound proteins such as agitation with ultrasound or by passing electrical current though the sample could prove preferable to treatment with acid. This could avoid issues with possible contamination of subsequent experiments due to reactions occurring at the gold clusters when using such a strong acid.

To summarise the use of nanoscale particles implanted in graphite surfaces seems a very attractive method of protein immobilisation for AFM experiments and I believe the method although in its infancy, will become well documented in the future following further research. The contribution of physics to the method cannot be questioned since physicists
have been producing cluster films for many years in experiments to investigate their physical properties. However I am sceptic to whether the method will prove truly revolutionary. The fact remains that many experiments have been performed and are more easily performed with flat substrates such as mica or extended gold surfaces. Also transmission electron microscopy (TEM), and X-ray diffraction are the leading techniques in the structural analysis of proteins and their resolution is difficult to match with scanning probe techniques [40]. However I believe AFM techniques combined with the selective binding sites of cluster films can find a niche area of research particularly with proteins in the liquid phase where information regarding real time protein-protein interactions is sought.

Therefore from this discussion of only two applications of nanoscale particles to experiments in biological science it can be seen that there is a tremendous contribution that can be made. It is my view that the majority of such applications will contribute greatly to the refinement of experimental techniques however as I have explained I doubt that many will be seen as revolutionary. However there are many more potential applications which may be years away but have more potential to revolutionise medicine and the biological sciences. Our exploration of the “nano-world” has only really taken off within the last twenty years at most, twenty years from now there could be developments which are quite un-imaginable at the present.
4. References


