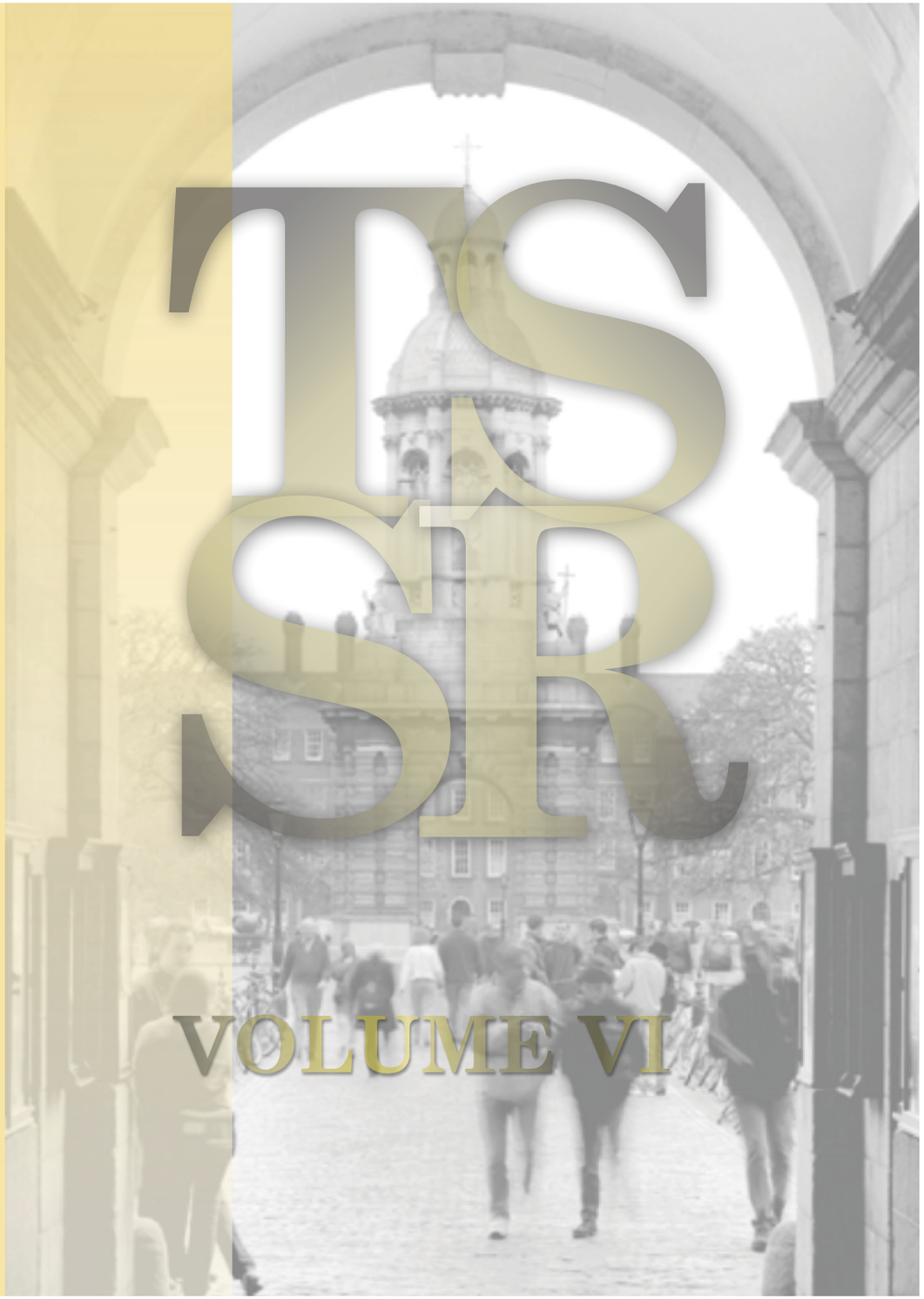
TSSR

TSSR Volume VI 2020



This book is dedicated to:

Every curious mind who asks not only what more we can know about the world around us, but how we can use that knowledge to uplift the world with us as we progress.

Trinity Student Scientific Review

Volume VI

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Without their long standing support and guidance, Volume VI would not have been possible.

Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

The board of TSSR would like to extend our thanks to the Student Open Access Project (SOAPBOX) and the Office of the Dean of Research, Prof. Linda Doyle.

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Editorial Note

Welcome

When the incumbent Editorial Team of the Trinity Student Scientific Review one a rainy afternoon in November 2019, no one could have foreseen the new world in which this book would ultimately be published. But, against all odds, here in your hands is the long-awaited Volume VI.

In the midst of a global pandemic, we have seen first hand how dependent our world is on the scientific community, how it slows to a halt when science cannot provide the answers. We are reminded doubly of the importance of publications like TSSR, encouraging the pursuit of scientific learning at all levels of academia — and accessible, sound scientific communication shared across all levels of society.

In coming months, understanding our changing world through science will be vital to rebuilding our spirit of survival, and recovering our trust to function cohesively as communities once more, addressing different challenges around the world. Even in times of rapid, radical change, science remains a stable regenerating body, as with each new generation we further build on the learning passed down to us, adding to it learning from changes we experience in our own time.

The TSSR echoes that iterative scientific process, evolving and adapting as a reflection of, and response to the changing world around it. Each year new students with a wide wealth of insight contribute to this journal, mentored by reviewers, editors, and advisors who refine these insights through a prescriptive methodology into rigorously analysed, well-presented research reviews. Mentors, who were once at the selfsame stage of their journeys in the scientific field. Never has it been more crucial to work together as a scientific community, to meet each other at the intersections of our disciplines; to collaborate, and learn from our established ideas while still making space for the new. The Trinity Student Scientific Review makes true progress towards these goals.

Our direction this year has focused on amplifying the brightest young scientific minds in Trinity, from all walks of life. We have forged relationships with new disciplines across the University, and wider partnerships beyond it allowing us to explore the variety of ways in which science can be understood and communicated; be that refining how we contribute to ethically open-access academia, or learning about scientific ethics in journalism and public interest spaces.

We made a commitment, in the face of mounting fears, “social distancing” demanding the isolation of ideas as much as people, and barriers to fulfilling collaboration, to not only reflect passively, but actively support excellence across the diverse minds of our student scientific body — finding new avenues to expand the enlightenment science can bring not just to scientists, but to everyone — in times of uncertainty, misinformation and fear of ‘the other’.

This volume was created in line with the TSSR’s annual tradition, of highlighting the diligence of scientific enquiry that has produced these reviews — and we have been fortunate, despite the challenges of our unique time, to set a new precedent for TSSR after its fifth year, in further studying the purpose and ethics of scientific enquiry and development. We hope it provokes you to ask new questions, and offers talented new voices of young Irish science to help you answer them.

We would both like to thank each member of this year's outstanding editorial board: Naoise Irwin, Cathal Keane, Uju Obilor Anyanwu, Kate Kleinle, Alan O'Doherty, and Pierce Sinnott. Your efforts have made this volume of TSSR into something we, and our authors, can be sincerely proud of.

On behalf of the editorial board, we would also like to express our thanks to the Faculty of Engineering, Maths, and Science, for their continued support of the TSSR. Thank you also to Danielle Olavario and Tigran Simonian of TSSR Volume V for their resources and advice.

Finally, thank you to our authors for your countless hours of research and revisions. We hope that the review writing process was a rewarding one, and the skills it has offered you will stand to you in your futures.

And so, it is our pleasure to present to you the Trinity Student Scientific Review Volume VI.

Shubhangi Karmakar
Editor-In-Chief

Lucy Fitzsimmons
Deputy Editor-In-Chief

Trinity Student Scientific Review Vol. VI

Life Sciences

Letter from the Editors

In this year's edition, the articles selected for the Life Sciences section illustrate the diverse range of moderatorships studied here in Trinity College. The link between scientific research and our daily lives has become fundamental. Hence, this diversity in life science is vital in improving human living standards.

The authors featured in this paper have produced enlightening discussions in areas of cancer research, neurochemistry, genetics, and more. It is evident that the complexity of their respective fields encourages the use of critical thinking and communication in each paper.

The keen interest and enthusiasm for life sciences are well reflected in the quality of submissions received this year. It is this natural curiosity in authors and readers alike, that keeps scientific journals such as ours alive and running.

As with any publication, TSSR is an excellent platform for the sharing of knowledge. These reviews are incredibly informative and we can learn a lot from them. Moreover, they are an excellent example of collaboration. In the process of completing a review, editors, peer reviewers and authors alike have the opportunity to share ideas and in doing so, learn a lot from each other.

The final product is therefore a compendium of well-rounded articles, highlighting the importance of collaboration in scientific writing and introducing students to learn about the publication process before they start their careers in science.

The importance of research in the field of life sciences has undoubtedly been highlighted by the ongoing pandemic. Over the past couple of months, researchers all around the world have harnessed their knowledge and resources to further understand and

solve the COVID-19 crisis.

Trinity has been at the forefront of such investigation, advancing research on tackling the pandemic with the acclaimed Trinity COVID-19 Immunology Project, along with various other vital research projects. The commitment of the experts at Trinity to the health and well-being of our nation and beyond is hugely inspiring for undergraduate researchers, which has been reflected in the perseverance of those involved in this year's Life Sciences section of TSSR.

The authors and peer-reviewers alike must be commended for committing to their roles in these uncertain times and coming together to give us TSSR Vol. VI, truly embodying the ethos of scientific research at Trinity. There is no doubt that the authors featured in this year's volume hold great potential as the major contributors to their fields of research in years to come.

It has been an honour to be involved in this year's edition of the Trinity Student Scientific Review as the life sciences editorial board. We have taken great enjoyment in reading all of the reviews submitted by students who make this journal possible and thank them for their incredible work. We proudly introduce to you the reviews that comprise the life sciences section and hope you enjoy reading them as much as we did.

Naoise Irwin,
Cathal Keane,
Uju Obilor Anyanwu

Life Sciences Editors

Trinity Student
Scientific Review
Volume VI

Current and Emerging Treatment Strategies in Multiple Myeloma

Joyce Barry
Junior Sophister
Biochemistry

Multiple myeloma (MM) is a haematological malignancy characterised by the neoplastic proliferation of antibody producing clonal plasma cells in the bone marrow. 'Myeloma' refers to the malignancy of the bone marrow, and the prefix 'multiple' is used because the disease manifestation incorporates many organs. MM could therefore be thought of as a "a combination of numerous diseases with a common clinical phenotype".[1] This malignancy is currently treatable, but incurable, and the cause is still unknown. Current prognosis is 4-5 years if treated. The advent of novel drugs such as proteasome inhibitors, immunomodulatory drugs and monoclonal antibodies has significantly transformed the therapeutic landscape. When combined with autologous stem cell transplantation, these drugs have vastly improved outcomes and response rates for patients. New agents are continuously coming to light, the most recent and notable example being histone deacetylase inhibitors. Experimental therapies such as vaccines are also showing promise, with the potential to decrease incidences of multiple myeloma in the first instance. Ongoing research is aiming to improve the management of multiple myeloma in patients, while also working towards a cure.

Introduction

Multiple myeloma accounts for 1% of all cancers globally and approximately 10% of haematological malignancies with the median age at diagnosis being 72 years. The vast majority of cases of MM have evolved from monoclonal gammopathy of undetermined significance (MGUS), a premalignant stage with the rate of progression at 1% per year. Some patients go through a more advanced premalignant stage called smouldering multiple myeloma (SMM). Progression rates from this stage are 10% per year for the first 5 years, 3% per year for the next

5 years, and 1.5% per life year thereafter. [2-4]

Patients who have yet to exhibit symptoms indicating end organ damage do not require treatment, and studies have indicated that treatment at this early stage has no benefit. These patients should instead be monitored for disease progression.[5] Patients presenting with end-organ damage and diagnosed with active MM can be treated with conventional chemotherapy to halt progression and reduce disease-related symptoms.[6] However, in recent years, stem cell transplantation and novel drugs have more commonly been used in first line treatment as they typically produce a more favourable response.

Stem Cell Transplantation

Myeloablative high-dose therapy with autologous stem cell transplantation has become a staple of MM treatment, demonstrating superior survival rates compared to conventional cytostatic treatments. [6] However, due to the advanced age of many MM patients, care must be taken in determining eligibility for transplant. Kumar et al.[7] conducted a systematic review into the benefits of tandem versus single autologous hematopoietic stem cell transplant and found no difference in overall survival (OS), however they did note a superior event-free survival and response rate with tandem transplantation. A more recent study did however report a difference in OS when tandem vs. single transplantation was compared after bortezomib was utilised in induction and maintenance therapy. [2, 6, 7]

Allogeneic stem cell transplantation has been used in the treatment of MM for many years. Despite improvements to the tolerability of the conditioning regimen, the antitumor benefits of the treatment are often partly offset by the side effects arising from graft vs. host disease. While allogeneic stem cell transplantation is not recommended as an upfront therapy, some patients in the age range 30-40 years, may choose to undergo this treatment after myeloablative conditioning therapy, as the prognosis of 5-10 years survival with conventional treatments is not satisfactory. [6]

Novel and Emerging Drugs

The emergence of novel classes of drugs has drastically altered the treatment strategies available and significantly improved outcomes for patients. There are three main classes of novel drugs to date: proteasome inhibitors (PIs), immunomodulatory drugs (IMiDs) and monoclonal antibodies (mAbs). More recently, histone deacetylase inhibitors (HDACIs) have come to light as having potential for use in treatment of MM.

Proteasome Inhibitors

The proteasome was identified as a therapeutic target in MM in the early 2000s, with the first proteasome inhibitor (PI), bortezomib following shortly thereafter. MM cells produce large amount of protein (called M-protein) and rely on proteasome-

controlled pathways to degrade this protein. Inhibition of the proteasome, leading to accumulation of protein aggregates, is highly toxic to the MM cells, leading to cell cycle arrest, inhibition of proliferation and the induction of apoptosis. The 20S catalytic core is the site of action of proteasome inhibitors. Both of the inner β-rings contain 3 proteolytic sites, the chymotrypsin-like (β5), trypsin-like (β2), or caspase-like (β1) sites. The chymotrypsin-site is the most important for inhibition, however inhibition of all 3 leads to optimal inhibition of the proteasome. Figure 1 outlines the binding of the most common proteasome inhibitors. [8]

Bortezomib is a boronic acid PI that is approved both as a first line treatment and in patients with relapsed/refractory MM (RRMM). Bortezomib causes reversible inhibition of the proteasome and mainly binds the β5 site. Its usefulness in treatment is limited due to adverse side effects, most significantly peripheral neuropathy, and the development of resistance. [8]

Carfilzomib is a relatively new PI that is approved by the US Food and Drug Administration for use in patients who have received "at least two prior therapies, including bortezomib and an immunomodulatory drug, and have shown disease progression on or within 60 days of the completion of the last therapy". [9] Carfilzomib has an epoxyketone active moiety and binds irreversibly, therefore providing sustained proteasome inactivation. Carfilzomib also binds preferentially to the chymotrypsin-like (β5) site. [8, 10] Carfilzomib displays activity in heavily pre-treated patients, hence its importance in treating relapsed patients. Carfilzomib is also less neurotoxic than bortezomib, with the most frequently reported side effects being fatigue, anaemia, nausea, and thrombocytopenia. [11, 12]

Ixazomib is the newest PI to be approved, and is the first to have oral bioavailability, meaning it can be administered in capsule form. Ixazomib, like bortezomib, has a boronic acid active moiety, and binds the β5-subunit. [8]

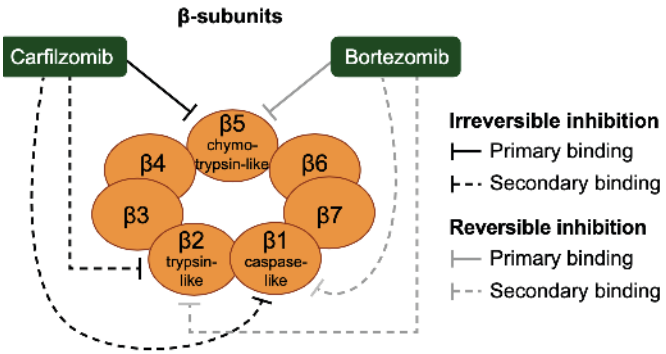

Figure 1: *Binding of proteasome inhibitors to the β subunits of the 20S catalytic core. Taken from.*[8]

Immunomodulatory Drugs (IMiDs)

Immunomodulatory drugs (IMiDs), including thalidomide, lenalidomide and pomalidomide, have become commonplace in the treatment of MM and are mainly used in combination with other novel agents such as proteasome inhibitors and monoclonal antibodies. The primary target of IMiDs is the protein cereblon (CRBN). IMiDs alter the substrate specificity of this protein's E3 ubiquitin ligase complex, which results in the breakdown of downstream proteins and the negative regulation of certain transcription factors, including MYC. [13]

Thalidomide was the first IMiD to be approved. It is less commonly used nowadays, although it is still useful in the treatment of frail and/or renally impaired patients as it has low myelotoxicity. The anti-angiogenesis effect of thalidomide is thought to be a major contributor to its efficacy. Lenalidomide was the second IMiD to receive approval and works well in combination with other novel agents. While the mechanism of action is not fully understood, lenalidomide can induce apoptosis directly and indirectly by inhibition of the functions of bone marrow stromal cells (BMSCs) that support the MM cells. Both agents have undergone clinical trials in maintenance therapy after autologous stem cell transplantation, with neither delivering promising results. [13]

Pomalidomide is a third-generation immunomodulatory drug that is less toxic than thalidomide and lenalidomide. It is approved for use in patients exhibiting disease progression who have received at least two previous therapies, including lenalidomide and bortezomib and is given in combination with low-dose dexamethasone. Pomalidomide works by inhibiting the cell cycle and inducing apoptosis of MM cells. [14]

Monoclonal Antibodies (mAbs)

Monoclonal antibodies are the newest class of novel agents developed for the treatment of MM and include such drugs as elotuzumab and daratumumab. This class of drugs targets specific antigens that are highly expressed on myeloma cells. [15]

Daratumumab was awarded breakthrough therapy drug status and received approval for use in patients who had received three previous therapies by the US FDA in 2015. Daratumumab has since been approved for use in a wider variety of cases, including newly diagnosed patients who are ineligible for autologous stem cell transplant. Daratumumab binds CD38, a 46-kDa type II transmembrane glycoprotein which is overexpressed by MM cells. Daratumumab can induce cell death through a variety of mechanisms including complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), induction of apoptosis, and modulation of CD38 enzyme activity. [16] Daratumumab also has an immunomodulatory role by depleting CD38+ immune regulatory cells, in turn stimulating clonal expansion of helper and cytotoxic T cells. No such functionality

has been demonstrated with elotuzumab. Daratumumab combines well with proteasome inhibitors and immunomodulators, and is important in the treatment of relapsed and/or refractory MM. [15, 17, 18]

Elotuzumab is another monoclonal antibody used in the treatment of relapsed and refractory MM. The cell surface glycoprotein SLAMF7 is the primary target of elotuzumab. SLAMF7 expression is seen in all subgroups of MM patients, regardless of cytogenetics. There is little to no expression of SLAMF7 in normal tissues, while over 95% of bone marrow myeloma cells express the protein, meaning elotuzumab can deliver highly targeted treatment with minimal side effects. The primary mechanism of action of elotuzumab is NK-cell mediated ADCC. [18]

There have been no studies to date investigating the efficacy of retreatment with a monoclonal antibody in patients refractory to another monoclonal antibody. Elotuzumab and daratumumab have distinct targets, making combination therapy in patients refractory to anti-CD38 monoclonal antibodies theoretically possible. [18]

Histone Deacetylase Inhibitors (HDACI)

Histone deacetylase inhibitors (HDACIs) have exhibited potential for use in treatment of relapsed/refractory MM with panobinostat, ricolinostat and vorinostat being the most prevalent. [19] Panobinostat remains the only HDCAI to have received approval. It is not effective as a monotherapy, but it has been tested in combination with many other drugs including bortezomib, dexamethasone, carfilzomib and lenalidomide. Such trials have shown panobinostat to be an effective therapy, even in heavily pre-treated patients. [20]

Factors to Consider When Devising Treatment Plans for Both Transplant Eligible and Ineligable Patients

Due to the older age of the majority of MM patients, high dose therapy is not possible in many cases. To improve tolerability, drugs may be given in reduced doses and on altered schedules. Due to the incurable nature of MM, maintaining quality of life remains a major concern. Standard treatment options for transplant ineligible patients combine traditional drugs such as melphalan and prednisone with novel treatments such as immunomodulatory drugs e.g. thalidomide and proteasome inhibitors e.g. bortezomib. Lenalidomide and dexamethasone have been shown to be effective in combination for the treatment of MM in elderly patients. The toxic effects of treatment should be monitored and mitigated via dose adaptation, if necessary, to ensure adherence to treatment, leading to optimal response rates. [6]

Younger and fitter patients with MM tend to be able to tolerate high dose therapy. Many studies have indicated a positive correlation between depth of response and survival, and for this reason, more effective and intensive treatments are employed. [6] The combination of novel drugs such as thalidomide, bortezomib, or lenalidomide with standard drugs has produced higher response rates when compared to those of the standard of vincristine, doxorubicin plus dexamethasone. It is therefore

advisable that novel drugs be used in primary treatment.[6] Studies have shown that a combination of three drugs, including one or two novel drugs, produces better responses than combinations of two drugs e.g. bortezomib plus dexamethasone plus thalidomide. The addition of a fourth drug to the primary treatment has not demonstrated any positive impact on response rates. [6, 21]

Maintenance

Continuous treatment following autologous stem cell transplantation, known as maintenance, has been made feasible though the emergence of novel drugs with lower levels of toxic side effects, such as thalidomide and lenalidomide. Thalidomide was one of the first novel drugs to be used continuously, however long-term use has not proved feasible, mainly due to peripheral polyneuropathy. Some studies suggest there may be some increase in overall survival, however the efficacy of thalidomide as a maintenance drug is not clear. Lenalidomide has also been studied as a long-term maintenance drug and is feasible, with side effects being mainly haematological. Bortezomib has also shown some favourable results as a maintenance drug. The use of maintenance itself is still a matter of debate and overall survival rates remain inconclusive. Many argue the benefits of a treatment-free interval for patients' quality of life, an especially important consideration due to the incurable nature of MM. [6]

Vaccine Therapy

Vaccine therapy for MM is an area of ongoing research. While the precise factors are unknown, there is significant evidence to suggest that the immune system plays an important role in the progression of smouldering multiple myeloma (SMM) to symptomatic or active MM. A 2018 trial assessed the possibility of preventing or slowing this progression through boosting the immune system via vaccination during the asymptomatic SMM stage. The PVX-410 vaccine, a human leukocyte antigen A2-restricted multi-peptide cancer vaccine, was assessed both as monotherapy and in combination with lenalidomide. An immune response was detected in 95% of patients, and inclusion of lenalidomide increased the magnitude of this response. The study found the vaccine to be safe and immunogenic, warranting further studies for use in patients with SMM. [22]

Treatment at Relapse or Progression

Treatments options for a patient presenting with relapsed or progressed MM must carefully balance efficacy and the risk to the patient's quality of life. Relapse can be either clinical or biochemical and both are managed differently. A clinical relapse is characterised by the presence of end organ damage and should be treated immediately.

Biochemical relapses are characterised by increases in protein markers in blood or urine, without end organ damage, and should be observed for kinetics before treating.[6] Proliferative relapses should be treated rapidly. A

2012 review concluded "if the doubling time of the monoclonal protein is ≤ 2 months, treatment is indicated even in the absence of [end organ damage]". [23]

If the relapse has occurred after a prolonged treatment-free interval, retreatment using a specific drug is feasible with the possibility of a meaningful response being achieved. The interpretation of 'treatment-free interval' is variable, defined in the USA as more than 6 months, and in Europe as more than 12 months. Conventional cytostatic agents such as melphalan, cyclophosphamide, bendamustine, as well as novel drugs, including thalidomide, bortezomib, and lenalidomide can be used in the treatment of relapses, with a combination of the above drugs highly recommended. Sophisticated drug combinations and participation in clinical trials utilising experimental drugs should be considered in the case of patients who are refractory to novel drugs. A second autologous stem cell transplant may be considered in the case of young patients who achieved a progression-free survival (PFS) of 24 months after initial transplant. [2, 6]

Multi-Drug Resistance in Multiple Myeloma

Multi-drug resistance (MDR) is a major problem in treatment of many cancers, including MM. Patients are typically MDR negative at diagnosis, and develop MDR upon exposure to chemotherapeutic agents, making treatment at relapse more difficult. Multi-drug resistant cancers exhibit resistance to a broad spectrum of unrelated drugs. This resistance can be either intrinsic or acquired. In order to treat MDR, many mechanisms of resistance must be combated at once. If just one is treated, it is very likely the cancer will adapt to resist via a different mechanism. [24] Currently, treatment options are extremely limited, and prognosis is very poor when patients develop triple-refractory MM, that is, MM that is resistant to the three major classes of novel agents: PIs, IMiDs and mAbs. Currently, the only options for treating these patients are conventional chemotherapy and salvage autologous stem cell transplantation. Finding new treatment options in the case of triple refractory MM is a major research priority. [25]

Conclusion and Future Perspectives

Despite the recent significant advances, the molecular biology of multiple myeloma is still not fully understood and important questions remain unanswered. Ongoing studies aim to advance the understanding of the pathogenesis of multiple myeloma, and as a result uncover new drug targets and improve prognosis for patients.

Drug resistance is an area of concern, especially for patients who are multi-refractory to novel agents. Although advances in therapeutic management of multiple myeloma have led to incremental increases in overall survival for patients, the disease is still considered incurable.

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Metabolomics and its Applications to Personalized Medicine

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The primary concern of this review is the role of metabolomics and clinically useful biomarkers for disease diagnoses. Demonstrating the variations between individuals in therapeutic outcome and disease susceptibility is a common challenge in clinical practice "due to complicated interactions between genetic and environmental factors". [1] The concept of personalised medicine is of great interest as it is a therapeutic approach involving the use of genetic and epigenetic information to tailor drug therapy and preventative care. This review highlights important aspects of the innovative field of metabolomics. Namely, measurement methods of the metabolome, the contributions that metabolomics has made, and could potentially make regarding personalizing medicine. Furthermore, current advancements in statistical analysis and methodologies that are enhancing the field of metabolomics to personalize medicine.

Introduction

The objective of personalized medicine is to grant health-care practitioners the tools to prescribe medicine tailored to the needs of the patient in an adequate time frame in an attempt to maximize efficacy and minimize side-effects. This will allow the prediction of possible diseases in the near or distant future, assessing susceptibility among populations. Metabolic variation could hold the key to personalized medicine. A variation of the genome, proteome, transcriptome and metabolome could lead to variation in therapeutic outcome or disease susceptibility of a patient.

With this concept in mind it is necessary for the combination of fields such as; metabolomics, genomics and proteomics as it could yield an improved

understanding of functional changes that accompany a specific disease or abnormality in the biological system. This progressive approach will be the driving force for providing personalised medicine for the population.

The endeavor of metabolomics is to record all metabolites within a biological sample, the primary ambition being to create a global understanding of said system. Metabolites are understood to be by-products of cellular metabolism with a weight of 1 kDa or less.[3] Water-soluble metabolites can communicate with the environment and the microbiome due to the mobility around the open biological system.[3] Consequently, metabolomics is essential for "systems biology" due to its scope analogous to field such as genomics and proteomics. [4] Hence, where proteomics identify what could happen, metabolomics identifies what is currently happening in a system.

"This realization demands a different perspective and requires the measurement of transcriptional, proteomic and metabolomics data in order to obtain a complete picture of the systems response to environmental or genetic stress". [4] Various research has shown the relative downregulation and upregulation of genes or proteins in order to interpret fluctuations in a biological function. Likewise, regular metabolic pathways such as gluconeogenesis and glycolysis vary in terms of the cellular concentration of an enzyme over time, but this doesn't automatically lead to a proportional alteration in metabolic flux.[5,6]

Metabolomics encompasses the complete analysis of low molecular weight molecules (metabolites) in biological systems and such an investigation is done, typically, through techniques such as nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry (MS) in combination with multivariate statistical analysis.[7] The objective is to observe fluctuations in biomarker concentration and to identify a coherent relationship among fluctuations and specific disease states or external perturbations such as diet or therapeutic intervention.

This is typically based on the knowledge that an infection is expected to alter homeostasis ultimately leading to variability in biomarker concentrations and/or profiles. Therefore, metabolomics has the potential ability to both diagnose and monitor various diseases, especially if based on samples which may be collected non-invasively such as blood and urine. Since 2008 more than 140 papers have been published on disease research using biofluids metabolomics, mainly; blood plasma, serum, urine and other biofluids more specific of the conditions under study [7] (See Fig. 1 for a brief timeline).

In 2012, it was apparent that productivity was exponential by comparison, awareness of metabolomics and enticing new strategies which can advance our understanding of diseases and management to the scientific and medical communities.

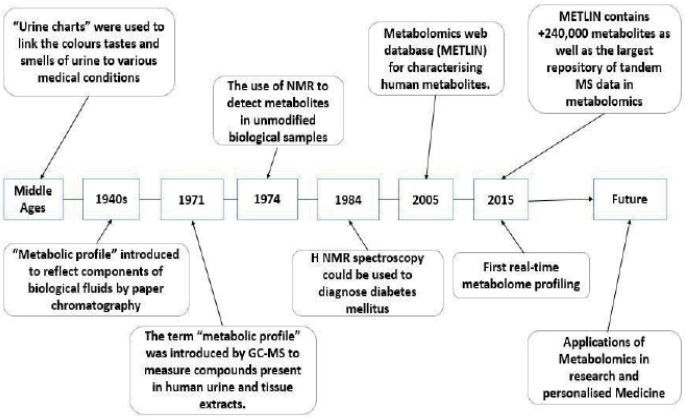

Figure 1: *A brief timeline of metabolomics development and applications (adapted from [8]).*

Metabolomics: Current Outlook

As metabolomics is growing in popularity as a discipline it must look to the future and begin to anticipate how the field may develop in order to transition into the modern-day world. Work is ongoing to improve technology and computational methods. This article highlights that whilst many biomarkers have been described in the academic literature; few have made it into the clinic.

Dipstick tests are currently in use for rapidly quantitating metabolite levels in urine, this method lacks real-time prolonged monitoring the health of a patient.[9] Metabolomics will achieve the ultimate goal of "disease-prediction" by working in conjunction with genomics and proteomics establishing a "metabolic profile". This approach would eradicate trial and error regimes, thereby lowering clinical mortality rates by approximately 70%.[10]

It should be noted that patient availability can be quite diverse depending on the disease in question and obtaining samples that are of value can prove an even more demanding task. Trivedi et al. has shown that over the past 100 years more than 1600 publications "claimed" to discover a new biomarker applying the metabolomics technique.[25] The majority of the publications lack statistical power, the sole problem being finite sample size, and in turn the biomarkers lack applicability.

The general consensus is that this is one of the fundamental barriers that is hindering the transition of metabolomics into a clinical setting. This statement is appropriate when applied to the top five causes of death in the United Kingdom (heart disease, dementia/Alzheimer's disease, malignant neo-plasms of the trachea, lung cancer and chronic lower respiratory disease) as there is a lack to identify

biomarkers associated with such diseases [11, 12] (Fig. 2). For proof of principle, the top three causes of death across Europe are malignant neo-plasms of the trachea, chronic lower respiratory disease and heart disease [13], and recruiting candidates into a study should not be too impossible due to the abundance of patients, suggesting that the statement by Broadhurst et al. is valid. [14]

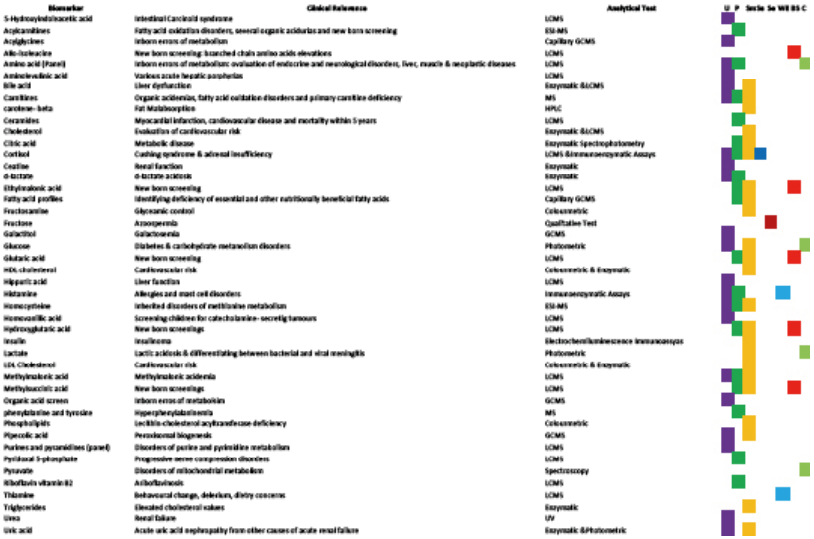

Figure 2: *Clinically useful biomarkers. Originally sourced from the Mayo Clinic, US [11]. U: urine, P: plasma, Sm: saliva, WB: whole blood, BS:dried blood spot, C: CSF (cerebrospinal fluid).*

Personalized Medicine and Disease Profiling

Metabolomics can aid the response profile of medical intervention by monitoring fluctuations in metabolites from fluids such as blood, sweat and urine. Metabolite profiling is quite advantageous as it allows for the observation of multiple metabolites at once., Aafter further examination the field could directly correlate fluctuations to a specific disease and response to therapeutic intervention. Profiling numerous metabolites rather than a single biomarker is likely to yield a higher sensitivity and selectivity.

A prime example is the plasma baseline, the levels of xanthine, 2-hydroxyvaleric acid, succinic acid, stearic acid and fructose prior to simvastatin treatment was observed to reliably predict the response mechanism in reducing lipoprotein cholesterol. [15] Models such as “optimized potential for liquid simulations” (OPLS) have a significantly high yield of 70(%) and 78(%) specificity.[4]

Metabolomics may be used to identify metabolic flux during the course of treatment demonstrating its monitoring capabilities. One such study analysed urine samples of Tuberculosis (TB) patients compared to a control group. The 6-month long therapeutic intervention used isoniazid, ethambutol, or pyrazinamide, and found that afflicted patients begun to display a metabolic profile similar to that of the control group.[4] A success story of metabolomics is its use to identify metabolic fluctuations associated with psoriasis [16].

Studies suggest that an increased demand for glutamine in association with psoriasis, this biomarker had previously not been identified as an indicator.[17] Glutamine in excess demand has also been associated with diseases characterized by increased cellular proliferation, such as in cancers. The same metabolomics study also identified β-sitsosterol which is a commonly employed herbal remedy, therefore suggesting that metabolomics may also be used for the identification of external treatments outside of the knowledge or recommendation of the physician. "More importantly, the metabolomics results were consistent with trends previously observed in genomics and proteomics studies" [17](Fig.2). In this manner, metabolomics may assist in determining whether co-administration of a complementary treatment was beneficial or detrimental to a therapeutic outcome of the patient. [4]

Metabolomics for the Masses

Personalized medicine may not be able to access its full potential until biomarkers provide enough insight to transition them successfully into wearable technology that is readily available to the wider population. Implanting biosensors into technologies such as smart phones, smart watches for monitoring heart conditions, necklaces and glucose monitoring contact lenses are all helpful innovations with the ability to make biomarker discovery a more personalised task.

Technology involved in translating biochemical alterations into data and signals is not a new process. The share quantity of biochemical reactions occurring in a system at any given time means that metabolomics rarely lack a sufficient quantity of data, it is often the case that a sizable population is lacking. For this reason alone the ability to translate metabolic profiles onto wearable technologies would provide addition information to the research that acts as a compliment to their own. [11]

Biosensors being integrated with wearable technology in an attempt to personalize metabolomics is a colossal task and would require immense computing power and data storage which demands an advanced cloud-computing environment. [18] Wearable technology would give researchers the edge as they could be uploaded onto intelligent cloud services such as Microsoft Azure or Google Cloud. [19]The data collection of an individual over time via portable devices has the potential of producing copious amounts of data which could be interpreted via the cloud in turn producing predictions for future health risks. Furthermore, researchers have used

survey telemetry data using smart phone apps in attempt to monitor occurrence, presentation and management of mobile Parkinson's Disease to aid in prediction.[19] A smart-phone based app can be used to monitor and further understand the association between pain and the weather conditions for people suffering from rheumatoid arthritis. [16] Innovations such as these may sound inaccessible, however, the counterargument to this is the moon-shot project by Alphabet. An ambitious research project that is attempting to advance biosensors in wearable technology and enhanced detection of cancer by understanding the inherent variability of the metabolome. [20] Although it is underdeveloped it yet has extraordinary potential in detecting early onset of diseases by examining bio-fluids.

Metabolomics will soon become common practice when tackling issues such as: diagnosis, analysis, monitoring and progression of various disease. In order for this to become a reality we must first enhance our understanding of pathogen-host mechanisms and how they can impact on the chemical profile of bio-fluids [4] i.e. metabolites. Assuming the technology required for this process becomes a conventional process and affordable [10], the metabolic profiling machinery could be operated at a local general practitioner's office. The rationale behind this would be to allow metabolite screening for a patient at regular intervals. This successfully allows for efficient disease diagnosis and monitoring, therefore improving overall health status.

The Future of Healthcare and Personalized Medicine

"The main use of metabolomics is for biomarker discovery". [21] The detection of biomarkers in a large populations and conversion of the data into cheap, quick, reliable methods that allow public access, making it "personal". Personalised medicine has been practiced within an evidence-based framework, in this method an individual is treated for diseases based on the most popular medicine. Post-drug consumption assessments are made in order to evaluate whether this has relieved symptoms. After assessing the patient they may: 1) stay on the drug, 2) be prescribed an alternate medicine, or 3) be given treatment to relieve side effects of the first drug. This is clearly a slow process and is possibly dangerous to the patient.

Precision medicine can aid in combating the increased mortality globally caused by microorganisms, the most noticeable risks are human immunodeficiency viruses (HIV) and Mycobacterium tuberculosis. In the previous decade antimicrobial resistance (AMR) has become more of a concern as previously harmless pathogens become cause for further investigation. Neill et al. emphasized that this growing threat that is bacterial infection will kill more humans than heart disease by the year 2050. [13] Due to the ability of metabolomics to detect metabolite fluctuations when a pathogen is present, it is likely that the field will contribute to understanding of AMR and host-pathogen interaction.[11] Tasks such as these could be catapulted into success with the launch of "precision medicine initiative" which was announced during the State of the Union address by former President Obama. [22]

Personalized medicine must consider both the genotype and phenotype of the individual before they undergo medical treatment, , tthus, becoming dependent on analytical methods in order to assess risk and to present healthcare options [11], not unlike the AI approved by the U.S. Food and Drug Administration (FDA). This system will be dependent on a fundamental understanding of biomarkers, if the foundation is not solid then researchers would struggle to accurately identify underlying pathology, making precision medicine impossible. [23]

It is common knowledge that diseases are the cause of alterations in human metabolism. Therefore, the driving force behind metabolomics is it usage of biomarker discovery to enhanced portrayal of a disease phenotype. Furthermore, allowing for progressive methods that ultimately grant a "cure". [24] Dhanasekaran et al. highlights the importance of screening a patient prior to infection by using the example of prostate specific antigen (PSA) biomarker. An increased concentration of the PSA does not necessarily correlate to prostate cancer but a gradually increasing PSA level occurring in conjunction with age is a characteristic of an enlarged prostate[25].

A metabolic profiling scheme that is designed well and reasonably priced may not be possible or even accessible due to multiple challenges, some of which include: labour and consumer costs, ethical, legal and social issues.[11] In order for personalised screenings to be successful the risk-benefit ratio needs to be defined clearly per disease. [26] The discovery of biomarkers is encouraged as research displays its potential in dramatically reducing fatal health conditions such as: cancer, congenital disease, heart and respiratory diseases.

Conclusion

This review has highlighted the most recent improvements in the field of metabolomics emphasizing the benefits it could potentially have in areas such as personalised medicine and general research. The process of metabolic profiling has proven itself frequently with regard to drug and surgical intervention, decreasing risk and increasing efficiency.[4]

Many studies have been published and awarded public investment, but the field requires reorganization. The progression of metabolomics is still hindered by the constitutional limitations in experimental design, the anomalous technology expense, variability amongst samples and complex data analysis.[27]

The problems that burden metabolomics and biomarker discovery reside as much in the culture and organization of academic research as in deficiencies in analytic technology [27], this review would be interested in studies that concentrate on public perception of this cloud data base. Security and marketing of this approach would need to be prioritised as a misunderstanding or the fear of hacking the database may cause for a restless public that could delay progress.[28]

Science and many of its key thinkers have been subjected to this type of public scrutiny, Darwin being a primary example. In other words, the field must drive forward towards undertaking a large cohort multi-centre studies to enhance the discovery process and obviously market the research in a manner that is well understood by the public to not hinder the progression of the field.

Recently, the U.S. National Aeronautics and Space Administration (NASA) studies have given a glimpse of how powerful and useful it is to have an understanding of the human metabolome.[30] Although there is a lot of ground to cover in this field, as highlighted above, the applications of metabolomics in preventative medicine in conjunction with screening leads to limitless opportunities. Indeed, one may even say that the field of metabolomics has the potential to serve an integral and essential role in the survival of humanity.

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Natural Sciences

Letter from the Editor

This year's selections for the Natural Sciences section each pertain to very different aspects of natural science, but share a common thread: they describe an evolution.

"Restoring the Mesopotamian Marshes" is an overview of the history of the damage done to marshes across Iraq and Iran in recent decades, and provides an analysis of the attempts made to rectify this damage as well as a summary of the challenges inhibiting those attempts. "Towards Establishing the Genomic Trends of Coevolving Mutualisms" discusses research into the process of coevolution, providing a fascinating and in-depth look at observable genetic changes which occur as a result of mutualistic interactions between species.

While the latter references evolution in a stricter, technical sense, both papers describe changes in our world which stem from relationships between organisms, their environments, and one another. Unfortunately, in a modern world defined by unprecedented interconnectedness, such relationships increasingly result in harm. This is particularly true when anthropogenic activity changes the nature of existing relationships, destroys habitat to which species have perfectly evolved, or forces species to either adapt rapidly or face extinction.

The often-virulent relationship between humanity and the rest of our planet is one of the most pressing issues of our generation. It is my hope that deepening our understanding of the natural sciences will contribute to a holistic understanding of the systems that maintain life as we know it- potentially enabling us to preserve these complex and delicate relationships before it is too late.

Importantly, these lessons would not have been imparted so accurately or eloquently were it not for input from academic advisor Dr. Jeremy Piggott, as well as peer-reviewers Dr. Mathias

Kuemmerlen and Dr. Marcin Penk. Their comprehensive, detailed edits contributed strongly to the quality of this final publication, and I cannot thank them enough for their scrutiny and dedication.

Thanks as well to all who contributed their work this year, even amidst the pandemic — now more than ever, it's critical that we have authors like you who are willing to speak up for science.

Kate Kleinle

Natural Sciences Editor

Trinity Student Scientific Review Volume VI

Restoring the Mesopotamian Marshes: Iraq's Garden of Eden

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Senior Sophister
Botany

The Mesopotamian Marshes, sometimes known as the Garden of Eden, were once twice the size of the Everglades and of unique ecological and cultural significance in Iraq. This review aims to investigate why the wetland is receding and to assess the potential restoration of the Mesopotamian Marshes. Solutions to restoring the marshes are based around restoring ecosystem functionality by improving water flow, quality, quantity and distribution, as well as improving soil health. Prior research has established that there is potential for partial restoration of the wetlands under current conditions, but efforts to reach this potential have displayed mixed results, and anthropogenic activities continue to slow restoration efforts. The findings suggest that more research needs to be done on local ecosystem functionality, which might better influence governmental initiatives and restoration programmes.

Introduction

Wetlands are areas of land inundated by water, in which water defines or influences area biogeochemistry1. Wetlands provide many important ecosystem functions such as flood prevention, improved water quality, and preservation of biodiversity, and thus are important in wildlife and vegetation conservation.[1,2] Wetlands also provide pollutant filtration and water retention services which decrease the cost of water treatment[2,3] and may act as a carbon sink.[4,5]

The freshwater Mesopotamian Marshes are an important ecological haven for vegetation and wildlife. Located near the confluence of the Euphrates and Tigris before the rivers flow into the Persian Gulf, the marshlands consist of three main marshes: Al-Hammar, Central and Al-Hawizeh.[7] Combined, the main marshes span an area of 15-20,000km.[6] The marshes are also essential to the way of life of the Marsh Arabs, also known as the Ma'dan.[6]

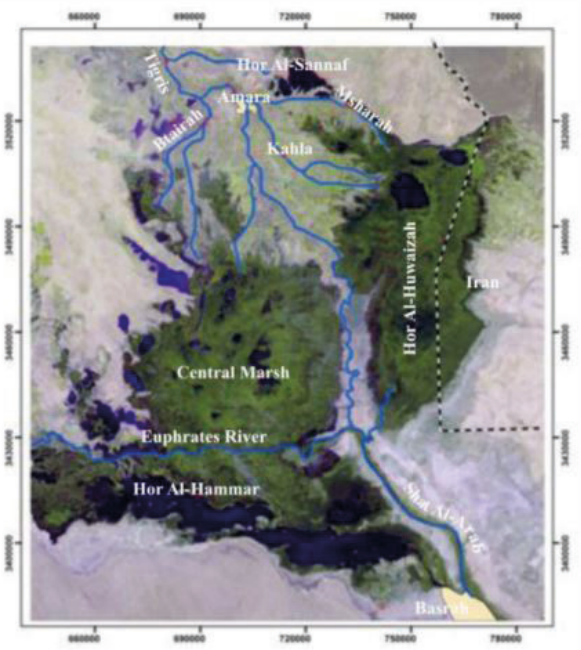

Figure 1: *An aerial image of the area covered by the marsh in 1973 before large-scale draining. The largest marshes were the Al-Hawizeh and Central marshes; Al-Hammar was the smallest marsh. The Tigris splits into the Kahlah, Msharah, Btairah and Amara rivers. The other marshes were in Iraq, while part of the Al-Hawizeh marsh stretched into Iran.*[8]

The ecological significance, conservation value, and functional importance of the marshes were undervalued by both British engineers and the postcolonial Iraqi government,[6] partially due to pressure to be seen as "modern" and "developed" by the Western world.[9] Draining the marshes and changing the traditional lifestyle of the Marsh Arabs all fed into the idea of "modernism".[10]

Water flow has been altered since the 1950s, and under Saddam Hussein's regime, a mass draining began in the 1990s.[6] The original project on which Saddam's plan was based came from British ideas in the 1950s proposing that a series of canals, embankments and sluices be used to reclaim the marshes. [6,9,] Saddam's plan was implemented under the justification of washing away salt from millions of hectares of over-irrigated farmland and the proposition to reclaim land for food production.[6] The other justification was related to capturing Iraqi outlaws and dissidents of the regime who he believed were hiding in the marshes. [10]

Both are worth considering when reviewing context for the initial draining of the marshlands. By 2003, 93% of the historic marshes had been destroyed.[11] The extensive reduction of this ecosystem drastically altered the landscape and had huge consequences for local industry such as the fishing and basket making industry.[12] The Marsh Arabs were driven to leave the marshes in huge numbers, as the draining of the marshes profoundly impacted their culture and way of life.

Connectivity between wetlands was greatly reduced after the draining, which created isolated islands of marsh habitat.[13] The decrease in habitat connectivity led to localised species extinctions and reduced species diversity,[14] particularly because the marshlands had been a permanent and migratory habitat for millions of birds from Siberia and Africa[15,16] and once contained up to 80 avian species.

Coastal fisheries used the marshlands for spawning migrations and nursery grounds for penaeid shrimp (Penaeidae) and the populations have drastically declined.[15.] The marsh contained many species of plant such as the common reed (Phragmites australis) and aquatic fern (Salvinia natans). Many plant populations have drastically declined because of habitat destruction. Ironically, in an effort to create industries, Saddam's plan has damaged others.[10]

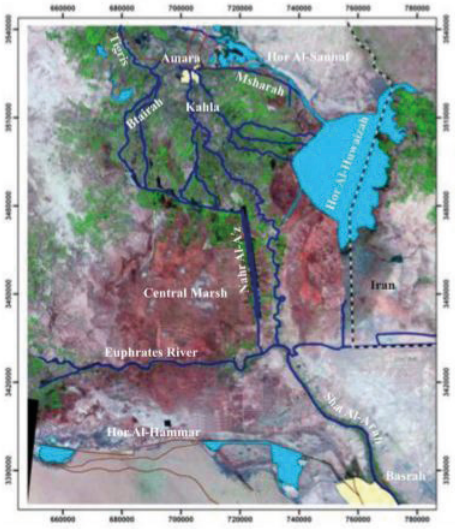

Figure 2: *An aerial image of the marsh after the draining in 2000. The Central and Al-Hammar marshes have nearly completely disappeared. Al-Hawizeh has mostly receded from Iran. The added canals can be seen e.g. Nahr Al-Az, which divert the water away from the marshes.*[8]

This review will explore the potential restoration of the Mesopotamian Marshes and reestablishment of core ecosystem functions. Restoration of core ecosystem

functions has the potential to increase the ecosystem's resistance to disturbance, allowing it to be self-sustaining. [3]

The goal of the restoration project as stated by the Iraqi government is to restore the marshes to 75% of the original state of the marshes in 197317. Challenges facing this restoration project will be discussed, as well as the likelihood of the Iraqi government reaching their restoration goal.

Key Functions and Disturbances in the Mesopotamian Marshes

Hydrological Function	Importance in the Ecosystem
Water Supply	Important source of water, contains much of Iraq's inland fresh water.
Water Storage	The marsh is an effective store of mostly rain and ground water.
Water Quality	The marsh acts as a natural filter of pollutants and maintains the salinity concentrations
Erosion Control	Mediates soil erosion in an arid environment which impact's loss of land, may play a role in controlling dust storms.

Biological Function	Importance in the Ecosystem
Primary Productivity	Maintains a high level of productivity; decomposing plants play a key role in nutrient cycling, reduces speed of water flow.
Carbon Sequestration	Wetlands act as a carbon sink and absorbs large amounts of carbon while producing low GHG emissions.
Food chains	Provides nutrients to animals which support certain industries, such as fishing
Habitat	Provides much needed habitat for a wide variety of flora and fauna both terrestrial and aquatic.
Biodiversity	Contains a rich diversity of plants, diatoms, phyto-plankton algae and animals such as fish and birds.

Table 1a and 1b: *Hydrological and biological ecosystem functions relevant to the case study* [1,3-6,11-14,16-18,22,23,25,26,33].

Key Disturbance	Effect
Water flow	Damming has affected water flow which is key to establishing many functions in the ecosystem.
Salinity	Impacts water quality and increase toxicity to plants and animals.
Agricultural run-off	Exacerbates the marshes salinity concentrations and toxicity level, increases chance of eutrophication.
Drought	Increase in evapotranspiration will reduce water availability

Table 2: *The key disturbances in the marsh, the effect of these disturbances on the marsh, and the synergistic effect of the disturbances together* [1,4-6,11-14,17-23,25,26,29,34].

Unforeseen Challenges and Mixed Restoration Success

The restoration of the marshland has had mixed success, and restoration of ecosystem functionality largely depends upon how Iraq and surrounding countries manage the water flow of the Tigris and Euphrates.[14,18] Since the 1960's, 32 dams have been constructed on the Tigris and Euphrates,[14,17] including the Atatürk dam built in Turkey, which has a reservoir capacity greater than the annual flow of the Euphrates.[19] Salts are introduced in a number of ways to the marshes. Some salts come from the water of the freshwater Tigris and Euphrates.

Increasing levels of salts from these rivers are flowing into the marsh due to increased levels of pollutants and agricultural run-off into these river's waters.[18] The reduced water flow to the marshes downstream may exacerbate many disturbances including salinity.[14] Increased damming and drought reduce water flow and increase rates of evapotranspiration, which also increases salinity of the water (see Table 2). These issues will occur regardless of conservation efforts local to the Mesopotamian marshes, potentially stymying restoration success.

Other factors, such as rainfall, also play a natural role in maintaining marsh water levels. Though rainfall is typically reliable,[20] recent reports indicate that Iraq is receiving inconsistent and less frequent rainfall from year to year, which may be linked to climate change.[21] The measurement of restoration is the degree of flooding observed and distribution of vegetation which may not be enough for successful restoration. The measurement does not take into account soil quality or salinity, and overlooks other ecosystem functions such as rate of decomposition. Increase in flooded areas did not bring about the success expected, indicating that there are other factors limiting the restoration of the marshland.[22]

In 2003, in an attempt to restore the marshland,[11] dikes and canal diversions were hacked and destroyed, allowing water from the Tigris and Euphrates to flow back into the marsh11. These releases of water were generally uncontrolled. The flooding seems to have restored some parts of the marsh, rehabilitating core ecosystem functionality in some areas [22] (see Table 1). However, the damaged state of the marsh has proven more resistant to restoration than previously anticipated.[23] Restoration is failing in other parts of the marsh due to high soil and water salinities, indicating that certain yet-unidentified disturbances are limiting ecosystem function restoration, reducing ecological resilience, and entrenching damage[24] (see Table 2).

Furthermore, though it can be a very fast means of restoring habitat,[13] uncontrolled release of water may not be the best way to yield consistent levels of restoration. Since dikes were breached in 2003, increase in salt water flow from the Persian Gulf to the river Shatt Al-Arab and the eastern Al-Hammar marsh may be preventing the re-establishment of freshwater marsh species. This may be a potentially permanent change due to scale of the devastation of the marsh.[14,25] In contrast, the reflooding of the Abu Zarag section of the marsh with Tigris water has resulted in better water quality, higher algal productivity, and increased biodiversity of plant and bird species.[11]

Threats to Water and Soil Quality

The Al-Hawizeh marsh, located on the Iranian border,[13] is the best-quality marsh and the only one remaining in its natural state. The marsh contains high-quality water, non-saline soils, and dense native vegetation.[26] There has been rapid re-establishment and high productivity of native vegetation and wildlife in parts of the marsh which could be assessed as having high potential for restoration given local ecological conditions.[27] Re-colonisation of P. australis suggests that a good soil seed bank will be important for re-establishing the reed beds that were once an iconic feature of the marshlands.[9]

Due to the large area of the marshes, the presence of a viable seed bank will make restoration of vegetation easier rather than large-scale re-planting of native vegetation. Once established, vegetation and wildlife will naturally migrate to ecologically suitable habitats.[11,28] The key to restoration seems to be water flow; it is crucial that the volume of water allows sufficient movement of non-contaminated water and flushes salts through the ecosystems.[25] With increased water flow, connectivity between habitats will improve, helping to maintain species populations (see Table 1). It will be important to take into account seasonal changes and the effects of ecosystem functionality to determine how they will affect water quality, and therefore vegetation and wildlife- all of which contribute to ecosystem resilience.[29]

Soil health and composition also constitute key components of the success of marshland restoration efforts. Soil microbial content, as well as nitrogen and

phosphorus content, are highest in undisturbed marsh. All these components are important for restoring plant productivity. [22,30] One element found in soil which is particularly detrimental to marsh health is Selenium, which can accumulate in multiple organisms along the food chain and negatively affect the reproduction capacity of fish and birds.[22] Selenium is present in high amounts in diked areas of the marshland, so release of these dikes may have disastrous consequences for wildlife. The source of the Selenium is agricultural water drainage which is then concentrated by evapotranspiration in these diked areas.

Unfortunately, increasing water flow alone will not be enough to restore soil quality.[31] Because some damage to the soil has been incurred by loss of vegetation, it may be worth considering the introduction of new types of vegetation that can grow in the new system- possibly those with similar functions to native vegetation. The physiological niche of introduced vegetation would need to be monitored to assess the state of plant populations under local environmental conditions.[32] In the Al-Hammar marsh it is still possible to grow salt-tolerant native species such as saltbush (Atriplex sp.) and Athel tree (Tamarix aphylla).[30] However, the presence of these halophytic species indicates highly saline conditions which would need to be further reduced before more freshwater tolerant species could be introduced.

This is an international problem with the construction of 32 dams on the Tigris and Euphrates since the 1960's [14,17] such as the Atatürk dam built in Turkey in 1998 which has a reservoir capacity greater than the annual flow of the Euphrates.[19] These dams are not being built with the restoration of the marshes in mind. The reduced water flow may exacerbate many disturbances including salinity.[14] Increased damming and drought reduce water flow and increase rates of evapotranspiration, which increases salinity of the water. If pollutants continue to flow into the marsh these pollutants could continue to have an effect on the filtering process[1.4,25]

Other possible obstacles to marsh restoration come from threats to water quality introduced upstream. These include water-quality degradation due to agricultural pollutants and other runoff from multiple countries flowing from these rivers33. Impacts on hydrological functions from stressors external to the ecosystem need to be assessed upstream, as disturbances caused there impact the functionality of the marsh and therefore the resilience of the ecosystem[33] (see Table 1).

Marsh filtration is an ecosystem service with significant downstream benefits which spread beyond Iraq[34] : the clear waters of the Persian Gulf are home to many important fish populations maintained to some extent by the marshes' filtering process.[14] Waters have been observed to be degraded along the coasts of Kuwait, associated with the reduced function of the marsh ecosystem.[12,27.] The decimation of the Mesopotamian marshes have far-reaching impacts on water quality, and the ecological ramifications are not just limited to Iraq.

Altogether, reduced water flow, agriculturally-driven damage, and other primary threats to marsh health are all anthropogenically sourced. Unless human activity changes, it is unclear whether water or soil quality will improve, or whether the

Iraqi government will be able to reach its 75% goal.[35] Indeed, current agricultural practices and the magnitude of disturbance across the marsh indicate that it may continue to suffer for the foreseeable future.[17] Restoration of key functions such as water flow and soil quality of the marsh ecosystem can improve other aspects of the system such as biodiversity, which will contribute to long-term ecosystem stability.[10] Restoration success should be measured by the degree of ecosystem functionality in multiple facets in order to assess the ability of the marsh to be self-sustaining.

Conclusion

A primary obstacle to restoring the marsh is river water quantity and flow. It is clear that restoring the function of water flow alone is not enough to save the marsh. Soil health is also crucial, but the process to restore this function successfully on such a large scale could be slow. Focusing on restoring ecosystem functions may be the best strategy in conservation programmes. Restoring of ecosystem functions gradually and observably allows species to return of their own accord.

The way in which restoration success is assessed may need to incorporate other ecosystem functions and ability to cope with disturbances. If anthropogenic effects, such as runoff from agricultural operations, are not mitigated, then it will be difficult for the marsh to maintain a steady degree of restoration- leading to long-term ecological harm for both Iraq and neighbouring countries.

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Can we Establish Genomic Trends for Coevolving Mutualisms?

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Although work has steadily begun in characterising the genetic basis of competitive multispecies interactions, such as predator-prey and host-parasite relationships, it is only in the past two decades that methods based on modern genome sequencing have been brought to bear on mutualistic interactions. This review draws together some of these disparate studies to roughly sketch out the diversity of genome level evolution seen in coevolving mutualisms, such as increased substitution rates, degradation in the genetic quality of mutualism due to human actions, extreme genome reduction in insect endosymbionts and the horizontal gene transfer from organelles that forged eukaryotic life. Some of the current problems confounding genetic research on mutualisms are mentioned and suggestions for further studies are put forth. By bringing together ecology, genetics, cellular biology and evolutionary theory, this type of under-explored multidisciplinary research has the potential to robustly contribute to our understanding of the function and evolution of highly integrated biological systems. Although it is currently premature to define anything more than very general trends that are specific to coevolving mutualisms not also seen in other coevolving biological systems, the research is only beginning and has already contributed a wealth of fundamental explanations to some of biology's core questions.

Introduction

Coevolution, the process whereby adaptations in one species elicit adaptations in another and vice versa, can be categorised on many levels.[1] This review is concerned with two broad kinds, mutualisms and arms races. Considering bipartite (two species) interactions, in the case of arms races, adaptations in one species are detrimental to the other, such as a parasitic species evolving access to a new host.

This in turn creates a new selection pressure on the host, such as for the immune system to evolve a new receptor that allows detection of the parasite.[2] This antagonistic coevolutionary process was first formalised by Leigh van Valen as the 'Red Queen hypothesis,'[3] and the advent of genome sequencing has brought evidence at the sequence level in the form of faster rates of nucleotide substitution—a marker for an increased rate of evolution—in genes coding for immune system proteins, for example. [4,5]This kind of increased molecular evolution is not limited to animals and their parasites, but can be found in parasitic plants,[6] bacteriophage infection genes[7] and bacterial populations coevolving with phages.[8]

A mutualism, on the other hand, refers to interactions whereby an adaptation in either species benefits both. They range from facultative (one or both species are not dependent on the other for survival) to obligate (one or both cannot survive without the other).[9] Classical examples include flowering plants and their insect pollinators,[10] the fungi and algae or cyanobacteria that form lichen, [11]and the endosymbioses that drove the evolution of eukaryotes and plants.[12]

An important clarification is needed before continuing. Symbiosis refers to close interactions between individuals for most of their lives, and therefore includes both parasitisms and mutualisms.[9] Thus, not all mutualisms are symbiotic, and not all symbioses are mutualistic. Endosymbiosis is a specific case of symbiosis whereby one organism, the endosymbiont, lives within the host (e.g. within its cells or tissues).[13] By comparing recent genetic studies of bipartite mutualisms, we can assess whether or not it is appropriate to begin ascribing trends at the genomic level that are characteristic of mutualistic coevolution.

Increased Genome-Wide Sequence Evolution in Mutualisms

An early study found mutualistic fungi in lichens had increased rates of nucleotide substitutions (single base pair mutations) relative to non-mutualist fungal species,[14] but more precise characteristics were not forthcoming as their analysis was limited to rDNA. While observing more nucleotide substitutions than would be expected under models of baseline or 'neutral' evolutionary rates can be indicative of positive selection on these sequences (due to the new mutations affording an adaptation to an environmental pressure), other processes can produce similar effects.

For example, new mutations can accumulate in previously essential sequences due to relaxed negative selection (changes in environmental circumstances may mean mutations at previously damaging locations can now persist in a population). Similarly, the 'nearly neutral' theory of molecular evolution predicts genome-wide increases in mutations under reduced population sizes,[15] such as population bottlenecks, a constraint that can be found in some symbiotic mutualisms.[16] It was therefore unknown whether this trend in rDNA genes extended across the genome or if it more specific.

Once more sequence data became available, a subsequent study of various bacterial and fungal endosymbionts found the endosymbionts had consistently higher rates of nucleotide substitution, and their observations were consistent with both reduced negative selection and reduced population size.[17]

Moving to less symbiotic mutualisms, ant-plant mutualisms are a well-studied example of a mutualism that has evolved many times during the evolution of flowering plants.[18] A recent study sequenced the entire genomes of three mutualist ant species and by comparing their sequences to those of their closest non-mutualist relatives, discovered a genome wide increase in the rate of nucleotide substitutions.[19]

Importantly, the increased substitution rates here were not due to factors like reduced population size (as discussed for endosymbionts). They speculated the increased rate seen in mutualists in general could be due to both relaxed purifying selection and or reduced population size, depending on the type of mutualism. Thus, further research should attempt to discover if there are indeed selection pressures specific to different mutualism types that yield the same increased substitution rates, as this would be valuable information when it comes to conservation of endangered species with mutualistic partners.

A key difference between these discoveries and those in antagonistically coevolving systems is that the increased rates of sequence evolution in antagonistic pairs was observed specifically at loci involved in outcompeting the other species. Thus, the advent of whole genome sequencing has tentatively revealed different trends at the genetic level between these two types of coevolution. If the trend is for increases in genome-wide mutations in all mutualisms, this would confound attempts to pinpoint precisely which genetic changes are those that allowed the mutualism to occur. This problem may be attenuated if studies were to focus on newly evolved mutualisms (in geological time), as more varied statistical tests are available when the adaptations are recently evolved.

Importance of Plasmids in Bacteria-Plant Mutualisms

Studies of rhizobia genomes, the proteobacteria that inhabit the nodules of nitrogen (N) fixing plants like legumes (e.g. peas, lentils, beans), have presented how dynamic genome evolution in endosymbiotic mutualisms can be. A 2007 review outlined how soil-dwelling species of proteobacteria have larger genomes than their endosymbiont counterparts, and that genes involved in the symbiosis are frequently clustered on plasmids, often as transposable elements,[20] meaning acquisition of a plasmid via horizontal gene transfer may turn a previously non-symbiotic species into a symbiotic one.

Human activity is not exempt from changing the selection pressures on such multipartite systems. For example, the use of N-fertilisers for crop production can cause decline of legume-rhizobia mutualisms by removing the need of the plant to maintain cooperation with the rhizobia. This is a core principle in the

theory of mutualisms—they are maintained only as each species does something in exchange for something else in return.[21]

Here, the bacteria fix atmospheric nitrogen into a biologically useable form for the plant, who in turn supplies the bacteria with key metabolites. Adding industrial N-fertilisers lessens the need for maintaining N-fixing bacteria and results in reductions in plant biomass due to reduced genetic quality of their rhizobia (although other related mechanisms for mutualism loss are proposed).[22] Another study found increased differentiation and loss of genes on the symbiotic plasmids of N-fertilised legume rhizobia, compared to those of non N-fertilised legumes.[23]

Thus, the role of plasmids cannot be overlooked when it comes to studying mutualisms involving bacteria. However, this kind of ecological genomics research is new and so studies such as those discussed here and in the previous section are sparse.[24] Understanding the reasons for mutualism decline at the genetic level therefore presents an important direction for further research, as it may help inform agricultural practices for restoration of reduced quality crops, especially in the case of legumes.

More generally, it underscores the need to shift thinking away from the category of a 'species' and towards a complex systems view of life when it comes to thinking of the food we grow.

Trends Towards Gene Loss in Endosymbionts

Gene loss in endosymbionts is perhaps the most well characterised feature of genome evolution in coevolving mutualisms. In order to uncover details concerning the genes involved, comparing endosymbiont genomes to those of related non-endosymbiotic species, or studies comparing host genomes with and without endosymbionts, are key.

The ciliate Paramecium bursaria and its algal Chlorella endosymbionts are becoming a model system for exploring how endosymbiont acquisition can occur.[25] By comparing the genome of P. bursaria to its closest relatives, researchers discovered it encodes more genes for N-metabolism (directly beneficial for the Chlorella) but fewer for oxygen binding, due to oxygen provided by Chlorella. Comparative studies can also reveal why supposedly essential proteins appear to be lost. One recent study compared the genomes of lichenised fungi to non-mutualist fungi and discovered atp9 was lost from the mitochondria of ten species of lichenised fungi.[26]

However, in these cases they found functional copies of the gene in either their algal partner or an additional fungal endosymbiotic species in the lichen. Although going beyond bipartite mutualisms requires more intensive research, this is a direction that should be pursued. While loss of genes deemed essential appears to shake conventional biological wisdom, when genomic studies include all mutualistic partners possible, a more nuanced view materialises of how

coevolving organisms can become integrated in order to survive and flourish in varied environments.

It may be hypothesised that as studies incorporate more actors, the concept of a 'genome' as a singular thing possessed by singular organisms may become less useful and even counterproductive, replaced instead by a view of integrated systems comprised of multiple delocalised genomes.

McCutcheon and Moran[27] highlighted how genome reduction can become extreme, taking the example of insect bacterial endosymbionts. In some cases bacterial genomes can become streamlined to encode as little as 100-200 proteins, comparable to a reasonably sized viral genome.[28] However, all these genomes retain the genes needed for supplying the host with nutrients, show increased sequence evolution and constitutively express heat shock proteins. What can drive such convergent trends?

Large reductions in bacterial population size (such as population bottlenecks that occur in bacterial endosymbiont populations due to the spatial constraints of living within a host cell), if frequent, are known to result in 'jettisoning' of bacterial genes.[29] When coupled with an inherent genetic deletional bias in bacteria,[30] this helps explain genome reduction.

Less recombination due to lower population size also amplifies genetic drift, giving higher rates of sequence evolution, but it also increases secondary structure errors in proteins that would explain the need for upregulated heat shock proteins.[27] It should also be noted that genome reduction can be a feature of some parasite genomes and is not specific to mutualistic coevolution.[31]

Notably, the extent of genome degradation appears to correlate with the evolutionary age of the endosymbiotic mutualism (the oldest known being the bacteria that likely became mitochondria). Endosymbionts recently acquired can have higher numbers of pseudogenes, mobile elements and chromosomal rearrangements, features of genome degradation that are absent in older endosymbiont genomes, such as in the well-studied Buchnera aphidicola.[27] These suspected ex-proteobacteria live obligately within aphids (e.g. greenflies) and have lost genes involved in defence and phospholipid biosynthesis.[32]

The known age of the Buchnera-Aphid mutualism and Buchnera's genome size was also used as corroboration for a model that predicts the ages of endosymbiotic mutualisms in general, based on the level of genome decay of the endosymbiont (see Figure 1). [33]Thus, seemingly specific research into endosymbionts can serve in developing general evolutionary models. Continued genomics research into these systems may help complete the spectrum of evolutionary models of mutualisms, adding to well-established examples such as those explaining colour-warnings to predators and their associated mimicry.

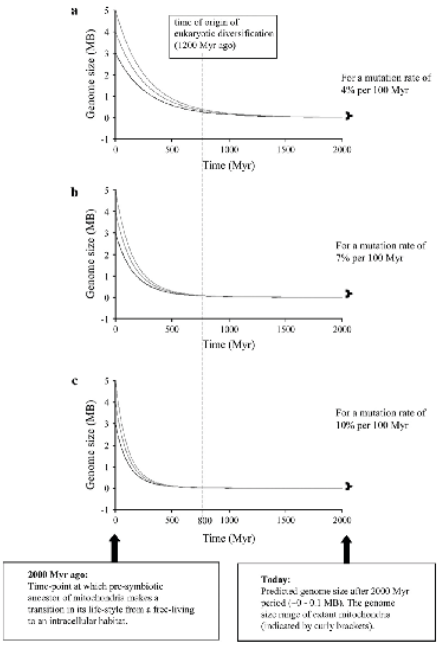

Figure 1: *Three predictions of the genome size of the extant mitochondria. Adapted from* [33]

Horizontal Gene Transfer (HGT) and Eukaryote Evolution

From the point of view of evolution, we must ask whether it makes functional sense to refer to these as endosymbionts or organelles. Here it is worth clarifying the terms used. Mitochondria and plastids are termed organelles and were once endosymbionts.[34] The latter are usually defined by their ability to encode all of their own essential proteins relative to organelles, who instead import most of their proteins encoded by genes in the nuclear genome.[35] It therefore seems logical to think of the transition from mutualistic symbiont to organelle as a gradual and multilevel process. Since the bacterial endosymbionts discussed above (such as Buchnera) are obligate mutualists with highly streamlined genomes, we may regard them as occupying a 'middle ground' between endosymbiont and organelle, given they cannot survive independently. Though there is no reason to assume becoming an organelle represents an evolutionary endpoint,[36] once the mutualism becomes obligate and extensive gene loss occurs, reacquiring autonomy seems unlikely.

In their same review of insect bacterial endosymbionts, McCutcheon and Moran highlighted that there was no evidence of nuclear-encoded protein import into any insect endosymbiont structure nor HGT to the host genome,[27] and so they cannot yet be considered organelles.

But are there any known cases where the beginning of this transition to organelle can be observed? Paulinella chromatophora was previously believed to host cyanobacteria-like endosymbionts,[37] but the loss of the photosynthetic psaE gene from the endosymbiont to the nuclear genome would seem to qualify the endosymbionts as organelles instead.[38]

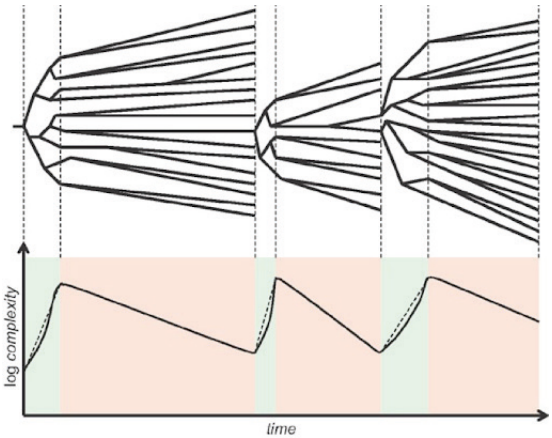

Figure 2: *The biphasic model of punctuated evolutaion of genomes. Periods of compressed cladogenesis punctuating long phases of quasi-stasis in the history of a particular lineage. Adapted from* [42]

While out of context these multidisciplinary studies may appear obscure, they bring us to the heart of some of biology's core questions. What does it mean for a gene to be essential? When do two vastly different species, from different domains of life, become merged into something wholly new? Is this a process life anywhere in the universe must undergo to reach complexity? Understanding the processes of endosymbiont acquisition and gene loss matters because this is currently how we believe eukaryotes arose and evolved.

While endosymbiotic theory is now widely established,[39] it is only with the recent availability of DNA sequences that we have begun to discover the extent of gene loss and HGT during eukaryogenesis.[40] For example, protection from endosymbiont transposable elements—uncontrolled HGT—has been suggested as the selective force that drove the evolution of the eukaryotic nucleus and spliceosome,[41] and at the very least dramatically changed the make-up of eukaryotic chromosomes, providing new raw material for genome evolution.[34]

Although we sometimes assume evolution tends towards greater complexity, the evolution of eukaryotes from archaea in general involved extensive reductive evolution,[42] whereby an increase in genome complexity (e.g. due to HGT) is followed by periods of deletional refining and streamlining (Figure 2).

Going forward will likely require even more collaboration across previously specialist areas of study. For example, bioenergetic studies may be core to explaining why some genes are lost and others persist, especially in the case of enzymes lost in key metabolic pathways. At least in the case of mitochondria, hypotheses for why their organellar genomes have been retained include the challenge of translocating hydrophobic membrane proteins all the way from the nucleus,[43] or that the genes are regulated by products of the electron transport chain itself and therefore must reside in close proximity.[44]

Similar research may help explain the extent of gene loss in insect bacterial endosymbionts discussed in the previous section, while ecological studies of the genetic basis of mutualism breakdown may tell us whether or not all endosymbionts, given enough evolutionary time, transition to organelles or if there are functional reasons for maintaining levels of cellular autonomy.

Concluding Remarks

Can we ascribe any general genomic trends that are characteristic and perhaps predictive of mutualistic coevolution? The emerging picture is that mutualisms show different genomic trends depending on the type of mutualism. So far, we can say endosymbiotic mutualisms can involve extensive genome reduction and horizontal gene transfer, and that both endosymbionts and free-living mutualists show increased sequence evolution relative to their non-mutualistic relatives.

As these genomic trends are not wholly unique to mutualisms in general, a reverse genetics approach, where analysis of sequence data alone can indicate whether or not two species are coevolving mutualistically, is not yet possible. At the very least, genomic studies of coevolving mutualisms have made important contributions to our understanding of the fundamental processes that have shaped and continue to shape life on Earth, in turn shaping the commonly accepted notions we use to understand it.

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Chemical Sciences

Letter from the Editor

The Trinity Student Scientific Review is an example of explaining scientific concepts to people unfamiliar with the subject. This is the main objective of accessible science communication. Especially with the rise of post-truth politics, it is vital that reliable and evidence based science is communicated to the public in an understandable manner.

The aim of TSSR is to bridge the gap between undergraduates and scientific research, an opportunity for students to familiarise themselves with an area of research that is of interest to them outside of their course of study. This year's chemistry submissions are great examples of the research being conducted in Trinity and shows the high level of knowledge, dedication and imagination by students here.

We begin with Ireland's biggest killer and the second largest cause of death globally, cancer. A notorious collection of diseases that have negatively impacted many people's lives. Ríona Devereux's review details work done on the inhibition of Tyrosine Kinases (TKs). Due to overexpression and deregulation, TKs are often the cause of cancer and this review how this can be prevented.

Justynne Joy Fabian's review describes the benefits of lanthanide (Ln) incorporation into Metal-Organic Frameworks (MOFs) compared to their transition metal counterparts. Their interesting photophysical properties are presented and how Ln-MOFs can be used as small molecule detectors is illustrated.

Finally, Maeve Ward's review teaches us about the growing importance of Boronic Acids in the biomedical field. Their ability to detect saccharides and their exciting potential as glucose sensors for diseases such as diabetes is discussed.

Contributions to the field of Chemistry in TSSR Volume VI would have not been possible without the continued support of the staff and students in the School of Chemistry, who were instrumental in this success. A big thank you to Professor Mike Southern, academic supervisor for the Chemistry section for the past 5 years. Despite this turbulent time, he was always available to provide feedback and guidance when needed.

I am also grateful to the peer-reviewers Helene Mihigo, Éadaoin Whelan and Prof. Mike Southern who generously gave some of their spare time to ensure that this year's reviews continued the excellent standard of reviews seen in previous years. I must also thank everyone who submitted a review this year. Without you TSSR wouldn't exist, and it demonstrates a passion for the subject which I hope you carry through in your future.

This year's Chemistry section showcases the depth and breath of the subject. It gives a glimpse into the different types of research being conducted in the School of Chemistry. This section along with the rest of the TSSR Volume VI will be available for anyone to read, in the spirit of sharing open-access knowledge, with the hope of lighting the spark of scientific curiosity.

Alan O'Doherty

Chemical Sciences Editor

Trinity Student Scientific Review
Volume VI

Tyrosine Kinase Inhibitors as Anti-Cancer Therapeutics

Ríona Devereaux
Senior Sophister
Medicinal Chemistry

Tyrosine kinases play a crucial role in the cellular processes of proliferation, growth, survival and migration and their overexpression and deregulation, due to mutations, can lead to oncogenesis and metastases. The research into the novel avenue of tyrosine kinase inhibitors (TKIs) has been revolutionary, as they are more specific and less toxic when compared to conventional cancer therapies. To date, many novel TKIs have been discovered and are increasingly used in the treatment of cancer. This review briefly summarises the design and mechanism of action of some approved TKIs and probes some of the future prospects for these small molecule therapeutics.

Introduction

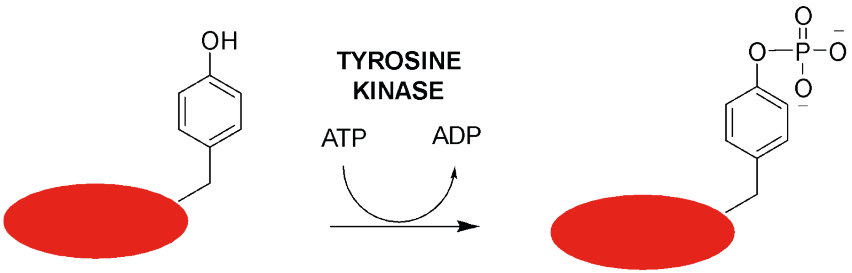
Cancer is the second leading cause of death globally, with approximately 18.1 million cases and 9.6 million deaths per year.[1] In Ireland, cancer is the biggest killer, with someone receiving a cancer diagnosis every three minutes and a death caused by cancer every hour.[2] This burden of cancer continues to grow nationally and globally, creating a clinical need for anti-cancer therapies.

Drugs currently developed for cancer include alkylating agents, antimetabolites, plant alkaloids and anti-tumour antibiotics. In the treatment of some types of cancer these conventional therapies can be found to be ineffective and harmful. These common therapies non-specifically target important cellular metabolism and division processes in both cancerous and non-cancerous cells.[3] This has led to the search for novel and specific molecular targets and signalling pathways within cancer cells to find more effective and selective therapies with less toxicity.[4]

One such novel targets are protein kinases. Approximately 2% of eukaryotic genes code for approximately 500 protein kinases, with these enzymes modulating their activity through reversible phosphorylation of specific amino acid residues, serine,

threonine or tyrosine, on the protein substrate.[5] Phosphorylation is regarded as one the most important post-translational modifications, playing a crucial role in intercellular communication, homeostasis, responses to external stimuli and to the correct function of the immune and nervous systems.[6] This phosphorylation reaction involves the binding of the enzyme cofactor, adenosine triphosphate (ATP), and the substrate protein to the catalytic site of the protein kinase.

Upon binding, the gamma-phosphate of ATP is transferred to the hydroxyl group of a specific amino acid residue on the substrate (Scheme 1). The phosphorylated substrate and adenosine diphosphate (ADP) are released from the catalytic site. Phosphorylation causes an alteration in the activity of the protein substrate by acting as an on/off switch for substrate function.[7] Protein kinases can then be classified according to their residue-specificity into serine/threonine-specific kinases (STPKs) and tyrosine-specific kinases (TPKs).[8] For the purpose of this review only TSKs will be considered.



Scheme 1: *Phosphorylation of a tyrosine residue on a protein substrate (red) by a tyrosine kinase enzyme with ATP as a cofactor.*[7]

The crucial role played by protein kinases in cell signalling leads to their involvement in oncogenesis and metastases of different types of cancer. The constitutive overactivation of protein kinases can lead to oncogenesis. Over the past few decades multiple malignancies in humans have been associated with the dysfunction of protein kinases. This abnormal activity of protein kinases can act as either a driver or a consequence of the disease.

Kinase signalling pathways determine many of the phenotypes of tumour biology, such as; proliferation, survival, metabolism and evasion of tumour suppression via immune responses.[9] This abnormal oncogenic activation of kinases is caused by multiple mutations in genetic and epigenetic material, resulting in increased activity, in overexpression or in the loss of negative regulation of the kinases. Kinases act as important targets for the development of novel cancer therapies, with small molecule kinase inhibitors being one of the main components in the pipeline of anti-cancer drug development.[10]

BCR-ABL tyrosine kinase inhibitors

Leukaemia is a group of blood cancers that begin in bone marrow, resulting in abnormal blood cell formation and is the most prevalent type of cancer in children. Specifically, chronic myelogenous leukaemia (CML) accounts for 14% of all leukaemia worldwide.[1] Imatinib mesylate (Gleevec or Glivic, Novartis) (Figure 1) was the first FDA-approved small-molecule protein TKI and is used in the treatment of CML.[11]

Imatinib has a 2-phenyl-aminopyrimidine structure and is an inhibitor of several tyrosine kinases, including the Abelson proto oncogene (ABL) tyrosine kinase. In over 90% of adults with CML, a consistent abnormality is present in a chromosome and is referred to as the Philadelphia (Ph) chromosome.[12] This chromosome is generated by a translocation and fusion between the ABL tyrosine kinase on chromosome 9 and a break-point cluster (BCR) gene on chromosome 22.[13] This results in a BCR-ABL fusion protein, which causes the tyrosine kinase to become constitutively active, playing a significant role in CML.[14] It is this constitutively active kinase that is the therapeutic target for Imatinib.[15]

This target is ideal as it is present in all CML cancer cells and absent in non-malignant cells. Imatinib exerts its therapeutic effect by binding close to the ATP-binding site of the inactive conformation of the kinase, stabilising this non-ATP bound form of the kinase, inhibiting kinase activity and "switching off" signalling pathways that promote leukogenesis.[16,17] The main components of Imatinib that interact with the ATP-binding site of the BCR-ABL kinase are the amide nitrogen as a hydrogen bond donor, the protonated nitrogen of the piperazine ring as a hydrogen bond donor, the nitrogen linker as a hydrogen bond donor and the pyridinyl nitrogen acts as a hydrogen bond acceptor (Figure 2). The protonated piperazine ring can also part-take in charge-charge interactions to further increase the binding affinity for the binding site.[18, 19]

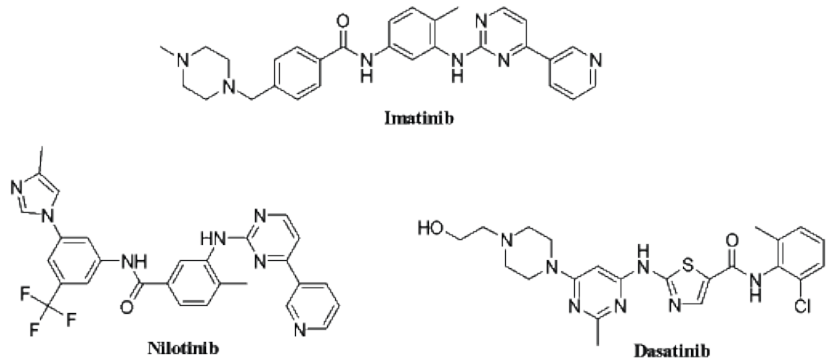


Figure 1: *Chemical structures of the BCR-ABL kinase inhibitors: Imatinib, Nilotinib and Dasatinib.*

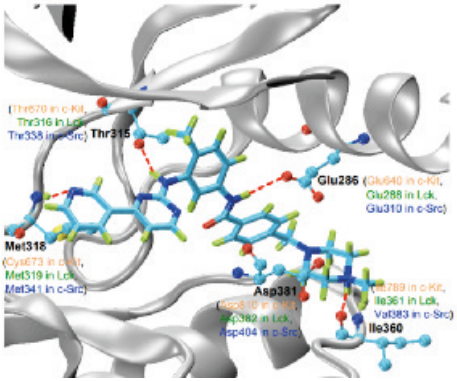


Figure 2: *Main Interactions between Imatinib (thick sticks) and BCR-ABL tyrosine kinase (Grey/Ball and Stick).*[19]

Nilotinib (Tasigna, Norvartis) (Figure 1) is an Imatinib analogue and is designed to have a higher specificity and affinity for the BCR-ABL tyrosine kinase. Nilotinib has a higher potency against BCR-ABL and is also active against imatinib-resistant BCR-ABL.[20] The development of Nilotinib began with the crystal structure of imatinib-BCR-ABL complex and it was concluded that the selectivity could be maintained by binding to the inactive ABL kinase domain, incorporating different binding groups to the N-methylpiperazine moiety, whilst retaining the amide pharmacophore.[21]

The N-methylpiperazine was replaced by a 3-methylimidazole and a trifluoromethyl group was added to the anilinocarbonyl substituent, increasing the Van der Waals interactions (Figure 3). The other main components of Nilotinib involved in interacting with the binding site are the pyridinyl nitrogen as a hydrogen bond acceptor, the pyrimidine ring in a π-cation interaction, the nitrogen linker as a hydrogen bond donor, the phenyl ring in a π-π stacking interaction, the amide oxygen as a hydrogen bond acceptor and the amide nitrogen as a hydrogen bond donor.[22]

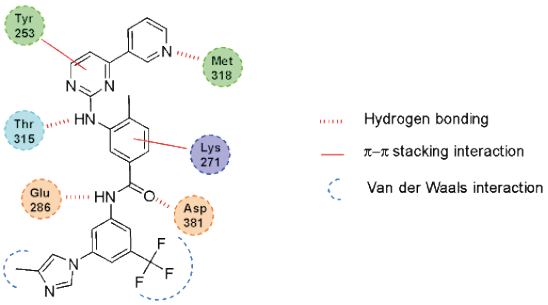


Figure 3: *Main interactions between Nilotinib and the c ABL kinase (Adapted).*[23]

Dasatinib (Sprycel, Bristol-Myers Squibb) (Figure 1) is another orally available ABL kinase targeted therapy used to treat cases of CML and acute lymphoblastic leukaemia (ALL). In contrast to Imatinib, Dasatinib binds to both the open and closed conformations of the BCR-ABL kinase and because of this Dasatinib inhibits both the mutant-type and imatinib-resistant BCR-ABL kinases.[24] Dasatinib was discovered through testing of a series of thiazole-based compounds with activity in ABL kinases.[25]

In Dasatinib, the core phenyl moiety has been replaced by an aminothiazole that occupies the adenine site in the ATP-binding pocket. The pyridine group has been replaced by a hydroxyethyl piperazine moiety, which is solvent exposed upon binding.[26]

EGFR-tyrosine kinase inhibitors

The development of specific TKIs began with the synthesis of hydroxyphenyl mimics of tyrosine (Figure 4). One such mimic was the natural product Erbstatin, a non-selective inhibitor of epithelial growth factor receptor (EGFR) and other kinases. Optimisation of this mimic lead to the development of the first potent tyrosine kinase inhibitor, Tyrophostin and from this conformational restriction by cycle formation led to the discovery of the quinazoline family of TKIs.[2]

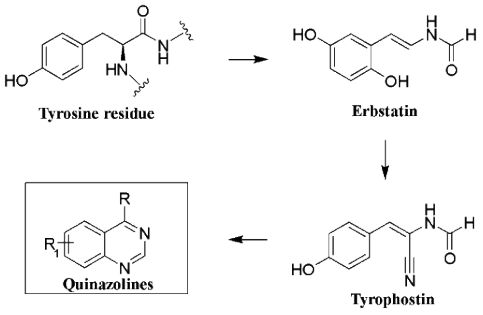


Figure 4 : *Discovery pathway of quinazolines as TKIs.*[27]

Lung cancer still remains one of the main causes of deaths by cancer in the world, with non-small cell lung cancer (NSCLC) accounting for approximately 80% of lung cancers.[1] In an aim to improve NSCLC prognosis and treatment, investigations have been carried out into the inhibition of certain specific cellular growth pathways, such as epidermal growth factor receptor (EGFR). EGFR is a receptor involved in the signal transduction pathways involved in cellular survival and growth and increased expression of this receptor is found in 40-80% of NSCLC cases.[28]

The EGFR is a transmembrane receptor tyrosine kinase activated by the binding of growth factors, such as epidermal growth factor (EGF), to the extracellular domain of the receptor. Signal transduction occurs, activating gene transcription and

inducing cellular responses such as cell proliferation.[29] Two types of EGFR are the HER1 and HER2.

Various methods of inhibition of EGFR have been developed, including small molecule inhibitors of intracellular EGFR tyrosine-kinase activity (Gefitinib and Erlotinib) and competitive binding of the extracellular receptor by monoclonal antibodies (Cetuximab and Trastuzumab). Gefitinib (Iressa, AstraZeneca) (Figure 5) was the first EGFR small molecule inhibitor approved for the treatment of NSCLC following failure of platinum and docetaxel treatments.[30]

It is an orally available anilinoquinazoline that inhibits EGFR activity by competitively binding to the intracellular ATP-binding domain of the EGFR tyrosine kinase.[31] The discovery of Gefitinib was based on studies carried out to characterize the catalytic mechanism of EGFR tyrosine kinase inhibition, which resulted in the structure-activity relationship discovery, shown in Figure 6 below, of 4-anilinoquinazoline class as a promising series of inhibitors. Several compounds from this series were synthesized and tested and Gefitinib was identified to be a potential effective inhibitor.[32]

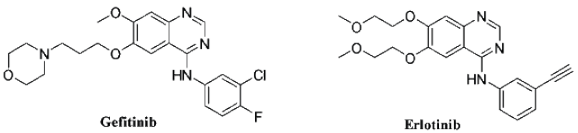


Figure 5: *Chemical structures of the EGFR kinase inhibitors: Gefitinib and Erlotinib.*

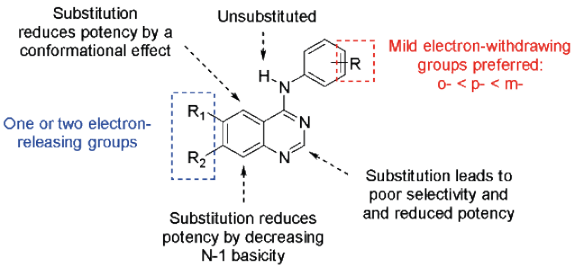


Figure 6: *Structure-activity relationship study in 4-anilinoquinazolines as EGFR inhibitors*[32]

Studies have found that Gefitinib has no survival advantage in NSCLC and has been reported to cause lethal pulmonary toxicity.[33] These findings lead to the replacement of Gefitinib with a closely related small molecule inhibitor, Erlotinib (Tarceva, Genentech) (Figure 5). Erlotinib is used in the treatment of advanced or metastatic NSCLC, again following failure of prior chemotherapies. Both compounds similarly bind to the EGFR tyrosine kinase ATP-binding cleft.

The N-1 of the quinazoline ring acts as a hydrogen bond acceptor and interacts with a methionine residue and the N-3 of the ring interacts with a threonine residue through a bridging water molecule. The electron deficient ring found in the two compounds occupies an empty hydrophobic pocket.[34]

Despite the efficacy of Gefitinib and Erlotinib as EGFR TKIs nearly all patients develop resistance after treatment for 3 to 7 months. This resistance is due to point mutations, the most common of which is the T790M (substitution of the threonine on codon 790 for a methionine). This mutation introduces a bulky methionine across the ATP-binding pocket, sterically blocking access to the EGFR TKIs but not to ATP.[35]

VEGFR tyrosine kinase inhibitors

Sunitinib (Sutent, Pfizer) (Figure 7) is an oral, small-molecule multi-targeted receptor tyrosine kinase inhibitor, approved by the FDA for the treatment of renal cell carcinoma (RCC) and imatinib-resistant gastrointestinal stromal tumour (GIST). Sunitinib was the first cancer drug approved for the simultaneous inhibition of two different targets. These two targets are platelet-derived growth factor receptors (PDGFRs) and vascular endothelial growth factor receptors (VEGFRs), both of which play an important role in tumour cell proliferation and angiogenesis.

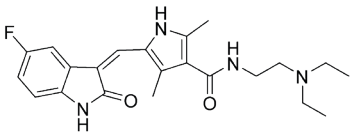


Figure 7: *Chemical structure of Sunitinib*

The simultaneous inhibition of these targets reduces vascularization of tumours and triggers apoptosis of cancer cells, resulting in tumour shrinkage. Vascular endothelial growth factors (VEGFs) regulate vascular development and angiogenesis upon binding to VEGFRs, of which there are three types: VEGFR-1, VEGFR-2 and VEGFR-3. VEGFR-2 is important in the regulation of vascular endothelial function.[36] Platelet-derived growth factors (PDGFs) regulate cell growth, division and are particularly important in the formation of blood vessel and there are many types, including, PDGF-A, -B, -C and -D, which can form either homo or heterodimers.

There are two isoforms of the PDGF receptor (PDGFR), the PDGFR-α and PDGFR-β, with PDGFR-β the target receptor for Sunitinib.[37] Simultaneous inhibition of VEGFR-2 and the ATP-binding site of the PDGFR-β induces greater anti-tumour activity than separate inhibition.[38] The pyrrolidinone ring provides a hydrogen bond acceptor and donor for polar residues and the remainder of the structure

provides hydrophobic interactions for non-polar residues in the binding site of VEGFR-2.[39]

Ruxolitinib (Jakafi or Jakavi, Incyte) (Figure 8) is an oral small molecule tyrosine kinase inhibitor for the treatment of a group of myeloproliferative neoplasms (MPNs), including: primary myelofibrosis, polycythemia vera (PV) and essential thrombocythaemia (ET).[40, 41] Ruxolitinib is a janus kinase (JAK) inhibitor and is selective for JAK subtypes JAK1 and JAK2 over JAK3 and TYK2. The JAK-STAT (signal transducer and activator of transcription factors) signalling pathway is important in controlling cellular processes such as differentiation, growth, development and survival.[42]

Dysregulated JAK-STAT signalling can lead to oncogenesis and tumour formation. In 2005, it was discovered that an activating mutation in the JAK2 gene resulted in the substitution of a valine at codon 617 by a phenylalanine and leads to a constitutively active JAK2.[43] This mutation was found to be present in patients with various MPNs.[44] Ruxolitinib inhibits JAK activity by competitively binding to the ATP-binding site of the kinase domain and is specific for the mutated form of JAK2. This inhibition prevents activation of STAT and downstream effects on cellular processes.[45]

The structure (Figure 8.) includes a pyrrolopyrimidine ring and acts as a hydrogen bond donor and acceptor and the rest of the structure, including the cyclopentane ring provide hydrophobic interactions to non-polar residues within the binding site.

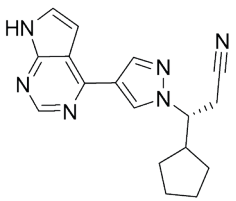


Figure 8: *Chemical Structure of Ruxolitinib*

Future Prospects

The development of resistance has been a major problem in the use of TKIs.[47] The emergence of resistance to current TKIs has been a driver for the search for alternative drug targets. Resistance develops indiscriminately to the various types and generations of TKIs. Another problem faced by TKIs is the complexity of tumour pathways. Multiple signalling pathways and regulatory factors are found within a tumour, with each one having a separate role in tumorigenesis. Therefore, inhibiting one pathway or one factor will not be sufficient to inhibit the growth of tumours.

One way to overcome the high rate of acquired resistance and complexity is the development of multitargeted TKIs. The partial inhibition of multiple targets can give rise to a greater effect when compared with the complete inhibition of one target.[48] When inhibiting a single target, the cancer cells have time to adapt and avoid the hindered pathway by activation of alternative pathways.

The inhibition of multiple targets decreases the chance that the cancer cell will adapt and allows for faster elimination of these cells. The use of combination therapy could also be a solution and has already been used in the treatment of cancer. Studies have shown that the use of TKIs along with a conventional therapy results in a better outcome for patients.[49,50,51]

The current approved therapeutics focus on a small subset of the human genome, neglecting many other possible kinase targets. This highlights the need for development of tools and selective probing techniques to determine the function of these unknown kinase targets.[52] Further work needs to be carried out to discover new pharmacophores that could act as a skeleton for more diverse kinase inhibitors. The current method for kinase inhibitor discovery is high throughput screening (HTS), however this is of less benefit as the most useful pharmacophores have already been retrieved by this method.[53]

Conclusion

An analysis of design and mechanism of action of some approved TKIs is presented here to provide an overview of the current understanding of this area and to demonstrate some of the limitations and challenges faced in future research. Future work may lead to the discovery and design of structurally-novel, small-molecule TKIs that will bypass resistance mechanisms and inhibit multiple targets simultaneously. Such TKIs will provide anti-cancer therapeutics that are superior to the state-of-the-art therapies.

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Lanthanide-based Metal-Organic Frameworks as Luminescence Sensors

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Metal-organic frameworks (MOFs) are three-dimensional crystalline materials composed of metal ions linked by organic ligands. Due to their highly porous nature and tunable framework structures, MOFs have been used for numerous applications, ranging from gas storage and separation, catalysis, sensing of metal ions and small molecules, and as photoactive materials. The incorporation of lanthanides as metal nodes within MOF structures has become a popular strategy. Unlike their transition metal analogues, lanthanide metal atoms are much larger, giving rise to higher coordination numbers, resulting in more available metal sites for coordinating ligands and solvents. In addition to high thermal and water stability, lanthanide-based MOFs (Ln-MOFs) have been used for their attractive photophysical properties. Photoluminescence occurs following excitation of photosensitizing organic ligands. Ligand to metal charge transfer (LMCT) is a process that enhances the luminescence of lanthanide ions within a coordination complex. The organic ligands act as photosensitizers which absorb light and transfer this energy to the lanthanide ion, which emits light at a specific wavelength and intensity. This phenomenon can be used in a variety of applications, including the detection of nitroaromatic compounds, metal ions, small molecules, and solvents.

Introduction

Metal-organic frameworks (MOFs) are crystalline compounds which consist of metal units that are linked by organic ligands. [1–6] They are a class of coordination polymers that are distinguished by their potential possession of voids.[7] The structure of these materials can be modified by varying the inorganic and/or organic units, thus opening up opportunities for the variation and optimization of the properties available.[5, 8–10]

A secondary building unit (SBU) is a metal cluster or complex in which modification of the ligand and metal coordination environments can lead to the formation of extended networks using linker molecules.[11] For MOFs, there are two types of SBUs.[12] Inorganic SBUs involve metal ions or metal clusters assembled by the coordination of organic ligands to metal ions. Organic SBUs involve synthesis of the organic ligands prior to MOF synthesis itself.[6,12,13]

The utilization of SBUs to synthesize extended porous frameworks via the assembly of inorganic SBUs with a variety of organic SBUs is referred to as reticular chemistry.[6,12,13] The tunability of the SBUs and linkers relating to their inherent geometric and chemical properties leads to the prediction of the geometry of the overall framework.[6,11,13]

When synthesizing MOFs, the general procedure is to react the metal salts with the pre-assembled bridging ligands.[2] There are many parameters to consider in MOF synthesis. These include the concentration of the reactants,[14] pH,[15] nature of the solvent,[16] temperature,[17] counterion of the metal salt,[18] time,[19] and the ratio of metal to ligand.[20] From these conditions, a multitude of reaction methods have been developed and is summarized below. [13]

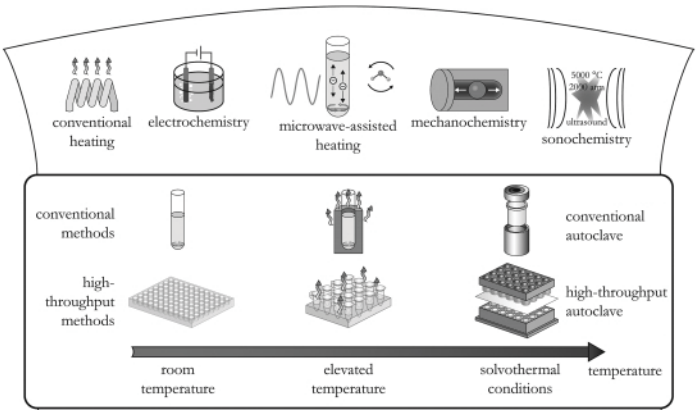

Figure 1: *Overview of strategies used in the synthesis of MOFs.*[13]

The design of MOF syntheses methods involves taking into account the chemical and geometrical properties of the organic and inorganic SBUs towards the overall MOF structure, and allows for the MOF to be selective for specific guest molecules.[21–23] This flexibility afforded by the synthesized ligand, coupled with the appropriate selection of the metal center, results in materials with tunable structures that lead to a variety of highly desirable properties. Such properties originating from this structural diversity include unprecedented surface areas which implicate promising applications in gas storage,[21,24] catalysis,[5,25] and as fluorescence sensors.[22,26–31]

Aluminosilicate zeolites are a separate class of porous materials that are widely used as heterogeneous catalysts and compete in certain aspects with MOFs.[32] Zeolites have properties similar to MOFs such as large surface areas, channel sizes and uniformity of the pores.[33] Zeolites differ from MOFs in that they are purely inorganic, and so do not have the added tunability afforded by the organic ligand as with MOFs.[32]

However, MOFs are not considered suitable in gas-phase catalysis as the more robust zeolites. This is due to the tendency of MOFs to collapse after solvent removal, leading to loss of micro-porosity.[32] Here, MOFs cannot compete with zeolites in the gas-phase, although, MOFs are more suitable for use in liquid-phase catalysis.[32,34]

Another consideration is in the thermal stability of MOFs. MOFs would not be ideally selected for certain reactions that require harsh conditions, while zeolites have the robustness required for such reactions. On the other hand, MOFs can be used for high-value-added reactions, including the synthesis of fine chemicals or individual enantiomers.[32,34]

A number of criteria must be considered when designing a MOF-based chemical sensor. One of such criteria is the selectivity of the MOF for the guest molecule.[35,36] Due to the structural diversity afforded by the possible variation in the organic and inorganic SBUs of the MOF, the MOF can be modified to accurately bind to the target. Structural diversity of this nature can be attributed by a change in the pore and aperture sizes of the MOF. Size selectivity is an example of this modification, where the aperture sizes govern whether the analyte atoms adsorb onto the MOF.[36]

Additionally, the interactions between a functional group and the analyte can tailor the type of response desired. For example, as reported by Wang et al., metal-ion sensing was made possible by the preferential binding of the sulfur atoms of a thienothiophene-based ligand towards Cu^{2+} ions, resulting in an observable response in the form of a decreased emission as was obtained for the Eu^{3+} and Tb^{3+} -based MOFs.[22] The open-metal sites of MOFs are just as essential in sensing also. For example, the use of the open-metal sites of the Eu^{3+} -based MOF, Eu(BTC) where BTC=benzene-1,3,5-tricarboxylate, demonstrated potential in small molecule sensing.[37] This MOF was selective for dimethylformamide (DMF) and acetone via the enhanced fluorescence and quenching effects observed in the presence of these solvents.[37]

Why the Lanthanides?

The lanthanides are normally trivalent ions with unfilled or partially filled 4f valence orbitals. The ground state of these lanthanide trivalent ions have the electronic configuration [Xe]4f n (n = 0-14)[38] The lanthanides comprise the first row of the f-block elements starting from lanthanum (4f 0) to lutetium (4f 14).[38]

Lanthanide ions are considered in this review as they have displayed desirable properties that make them suitable in their role as inorganic nodes within MOFs. Some of the properties included in their suitability for such porous materials include possessing high coordination numbers,38 water and thermal stability,[39] and characteristic photophysical behaviour.[38] The possession of high coordination numbers can result in a flexibility in Ln-MOF geometry and an improved accessibility to unusual topologies.[40,41]

The luminescent properties of lanthanides feature sharp line emission spectra and characteristic colour of emitted light. The line emission spectra observed for the lanthanides is due to the release of a photon of light following excitation of electrons from a lower 4f energy level to a higher 4f energy level. The sharpness of the line emission spectra is also attributed to the shielding effect of the 4f electrons by the filled 5s and 5p orbitals.[38,42,43]

When the lanthanide ion is directly excited, the excitation energy is typically inefficiently absorbed.[44] As the f-f transitions are within the 4f shell, these transitions are Laporte forbidden. This leads to a low molar absorption coefficient, of approximately less than 10 M-1 cm^{-1},[42,44] yielding a weakly intense absorption spectrum and poor luminescence.[38,44,45]

This drawback when synthesizing lanthanide coordination complexes can be overcome by the use of ligand-centered or charge transfer bands.[46] The excitation of the lanthanide species is done either by the direct excitation of the lanthanide, or by an indirect mechanism known as ligand to metal charge transfer (LMCT). Light is absorbed more efficiently by the chelated organic ligand which transfers some charge density to the metal center and causes enhanced luminescence via LMCT.[38,42]

Photosensitization or the "antenna effect" is typically applied in these instances, where the ligand acts as the photosensitizer and funnels the excitation energy to the lanthanide ion. Factors affecting the intensity of luminescence observed include the intensity at which the ligand absorbs light; the efficiency at which LMCT occurs; and the efficiency of the emission of light by the metal.[22,38,42,46–50]

Supposing there is an efficient transfer from the organic ligand to the metal ion (such that competing processes such as ligand phosphorescence of thermal dissipation is negligible) then it emphasizes the fact that the emission line-like spectra is due to these intraconfigurational f-f transitions.[51]

The 4f orbital shielding implies that interaction of the 4f electrons with their environment is limited and bonding between the lanthanide ion and the ligand is mainly non-covalent.[38,44,51,52] This leads to one of the attractive features of lanthanides in that the emitted light is of a specific wavelength which is characteristic of the lanthanide ion itself and independent of its chemical environment.[44]

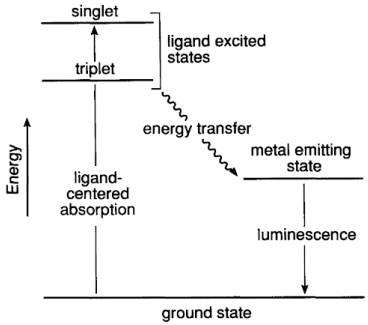

Figure 2: *Jablonski diagram demonstrating the sensitization of lanthanide complexes.*[46]

Photoluminescence

Photoluminescence is a type of luminescence and is a process that typically occurs by the excitation of an organic ligand such that the absorption of a photon of UV light leads to n-π* or π-π* transitions.[38,42,46,47,49,53,54] These transitions occur by the promotion of an electron from the ground state to the first excited singlet state, S1. Here, the electron could return to the ground state via a radiative emission process known as fluorescence, which occurs by vibrational relaxation.

Alternatively, intersystem crossing could occur.[38,42,46,47,49,53,54] This is the transfer of the electron from the singlet state, where the electron is spin-paired, to an available excited triplet state. In the triplet state, the electron changes its spin orientation so that its spin matches the other electron in the ground state. From here, phosphorescence can occur, which can be considered as delayed luminescence. This results from the radiative emissive relaxation of the electron in the triplet state back to the ground state of the ligand.[38,42,46,47,49,53,54]

As demonstrated by the work of Weissmann et al., the Laporte forbidden 4f-4f transition resulting in the typically low intensity emission of lanthanide ions can be overcome by the suitable selection of the organic ligands coordinated to the lanthanide ion.48 If the organic ligand is a good light harvester, brought about by the possession of a π-conjugated system, photosensitization of the lanthanide occurs and the antenna effect can be employed.[22,38,42,47–50,55–57] This involves the transfer of energy from the excited triplet state of the ligand to cause the excitation of the lanthanide ion that the ligand is bonded to. The eventual relaxation of the electron from its higher vibrational emissive state back to its 7f ground state results in narrow spectral emission bands.[38,49]

Relating to efficient energy transfer from ligand to the metal ion, it is noted that this process results in luminescence only when the triplet state of the ligand is equal or above the resonance level of the metal ion.[44,51] This is another factor

affecting the efficiency of LMCT, as well as minimizing ligand phosphorescence and thermal dissipation following ligand excitation as previously mentioned.[51] This phenomenon can be adjusted by considering the substituent effects of the organic ligand, as the substituent groups can affect the energy of the triplet state. Modifying these groups should be targeted towards an efficient coupling between the triplet state of the ligand and the emissive resonance level of the lanthanide ion.[51]

This photosensitization of the lanthanide ion via a light harvesting ligand has led to numerous applications in luminescence sensing of small molecules,[31,58] nitroaromatic explosives,[30] anions,[59] DNA strands,[30] as well as the synthesis of lanthanide-based dyes and assays.[47]

Luminescence sensors

An important application of these increasingly popular Ln-MOFs for their characteristic luminescence behaviors is their use as sensors for nitroaromatic compounds and solvent molecules.[27,60,61] The following applications involve Tb^{3+} and Eu^{3+}-based MOFs. Upon irradiation of these lanthanide ions with UV radiation, emission occurs in the visible region, which is one of the many reasons for their extensive use in luminescence sensing.[44]

The implications of the sensing abilities of Ln-MOFs provide some solutions into the detection of nitroaromatic compounds as such compounds are typically utilized in chemical explosives, including compounds 2,4,6-trinitrotoluene (TNT) and picric acid (PA).[62] Work done by Wang et al. involved the synthesis of a Tb^{3+} -based MOF, which was termed UPC-11, to provide a material with sensitivity to nitroaromatic compounds.[61] This MOF, $[Tb_3(NO_3)(BPTA)_2(H2O)_6]\cdot$ $3Diox{\cdot}8H_2O$ (H_4BPTA = [1,1'-biphenyl]-2,2',5,5'- tetracarboxylic acid, Diox = 1,4-dioxane), features a 3D open framework with three crystallographically independent Tb^{3+} ions which are each eight coordinated.[61]

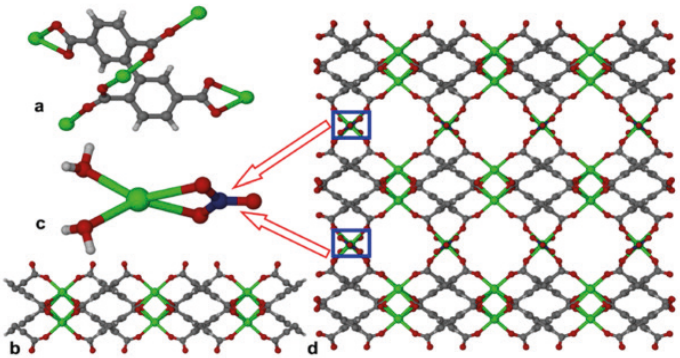

Figure 3: *UPC-11; $[Tb_3(NO_3)(BPTA)_2(H_2O)_6]\cdot$ $3Diox{\cdot}8H_2O$ (H_4BPTA = [1,1'-biphenyl]-2,2',5,5'-tetracarboxylic acid, Diox = 1,4-dioxane).*[61]

When UPC-11 was tested for its sensing capabilities, a suspension with dimethyl sulfoxide (DMSO) was made and the photoluminescence was tested in the presence of benzene, toluene, chlorobenzene and nitrobenzene. Among these compounds, nitrobenzene exhibited a quenching behavior, which was attributed to the photo-induced electron transfer from the excited UPC-11 to the electron deficient nitrobenzene analyte.[61]

More analogues of nitroaromatic compounds were then tested similarly for the aromatic compounds. The results showed a notably significant luminescence quenching, with preference for the 4-nitrophenol analyte. However, it was found that the response time when the MOF was exposed to 4-nitrophenol vapor at room temperature was 7 days until a notable quenching was observed.[61]

A more rapid response time was reported for another Ln-MOF involving the detection of nitrobenzene using two MOFs based on Eu^{3+} and Tb^{3+} with H_2DMTDC = 3,4 -dimethylthieno[2,3-b]-thiophene -2,5 - dicarboxylic acid as the ligand.[60] The two isostructural MOFs, $[Eu_2(DMTDC)_3(DEF)_4]\cdot DEF\cdot 6H_2O$ and $[Tb_2(DMTDC)_3(DEF)_4]\cdot DEF\cdot 6H_2O$, consisted of an asymmetric unit of two lanthanide ions that were both eight-coordinated to two oxygen atoms from N, N-diethylformamide (DEF) molecules and six oxygen atoms from five DMTDC ligands. The sensing properties of these MOFs were explored by testing a variety of analytes, of which nitrobenzene showed the most notable quenching of the MOFs.[60]

The response time was much quicker than UPC-11, in which immersion of the MOFs in nitrobenzene took less than 25 seconds to exhibit a complete diminishing of luminescence. The crystallinity retention after five cycles of washing and reusing the MOFs in nitrobenzene exhibits a practical high recyclability for use as sensors.[60] Such materials have the potential to solve real-world problems with the detection of chemical explosives for environmental cleaning, military issues, land mine detection and forensic research.[62] One of the main limitations is the scaling up to sufficient quantities of these MOFs and a general optimization of current synthesis methods.[63]

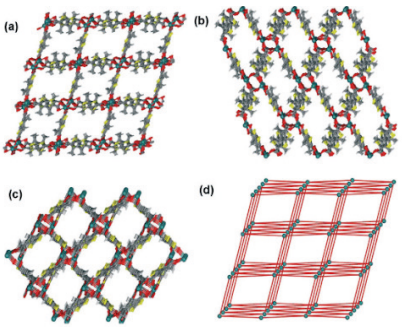

Figure 4: *The two isostructural MOFs, $[Eu_2(DMTDC)_3(DEF)_4]\cdot DEF\cdot 6H2O$ and $[Tb_2(DMTDC)_3(DEF)_4]\cdot DEF\cdot 6H2O$.*[60]

Furthermore, Ln-MOFs have been employed in their potential use as reliable DMF vapor photosensors.27 The toxicity of DMF is well-reported, affecting both humans and animals.[64–66] In a study by Y. Li. et al., a Eu^{3+} -based MOF was found to exhibit luminescence effects that correspond to sensitivity to DMF vapor.[27] In the Eu^{3+} -based MOF; $[Eu_2L_3(H_2O)_4]\cdot 3DMF$ (L=2′,5′-bis(methoxymethyl)-[1,1′:4′,1′′-terphenyl]-4,4′′-dicarboxylate), the Eu^{3+} ions are linked by carboxylate bridges, where its trigonal-prismatic geometry affords eight coordination sites accommodating six oxygen donor atoms from these carboxylates and two sites for the water molecules.

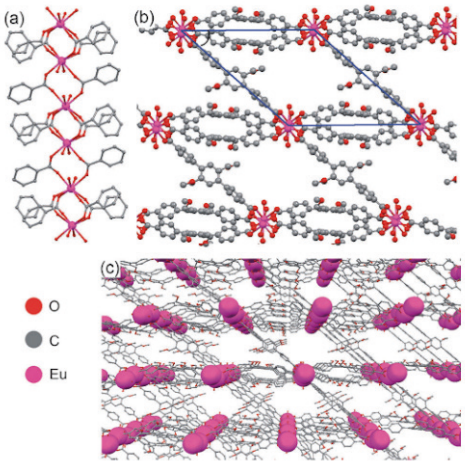

Figure 5: *$[Eu_2L_3(H_2O)_4]\cdot 3DMF$ (L=2′,5′-bis(methoxymethyl)-[1,1′:4′,1′′-terphenyl]-4,4′′-dicarboxylate).*[27]

Along the crystallographic a-axis, DMF molecules occupied the 1D channels, and it was found that they could be exchanged with water molecules. After various organic solvents were tested for sensing applications, it was concluded that the ligand-DMF interaction was important for selective sensing towards DMF.[27] This conclusion was based on the possibility that this interaction affected the excited energy levels of the ligand due to the presence of DMF. This would lead to the accommodation of efficient LMCT, resulting in strong luminescence.[27] This is another example of using Ln-MOFs to provide a solution to detection of toxic compounds that pose a risk to human health.

Conclusion

The issues in this review relating to detection of harmful compounds such as chemical explosives, solvents, toxic metal ions and small molecules, were outlined. The use of MOFs, in particular Ln-MOFs, were explored based on their applicability to provide practical solutions to these real-world issues.

Along with the well-established research in the utilization of these crystalline materials to tackle challenges in gas storage and separation, catalysis, and in molecular sensing of a variety of pollutants, Ln-MOFs have a variety of attributes that highlight promising applications.

Lanthanides in general possess an inherent ability that make them attractive for their integration into these framework structures; their characteristic luminescence that can be sensitized for their intense and readily observable photoemission with rapid response times and high reusability. Their tolerance to exposure to air and moisture, accessibility to complex topologies, and photophysical behavior, are some of the many advantages that lanthanide ions provide in their incorporation into MOF structures.

However, there remains a limitation in the utilization of MOFs and Ln-MOFs, in that the current synthetic methods available make it difficult to scale-up these materials in sufficient quantities. Further work in improving the yield of these luminescent MOFs may involve advancement and optimization of the synthetic strategies currently in use.[63]

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TS
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Boronic Acid based polymers for Saccharide Sensing

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Within biomedical applications the smart design of responsive polymers, specifically designed to interact with their surrounding environment, is resulting in many novel microscale devices. At their most fundamental, these have encompassed materials which convert biological changes into a modulation of optical, electrical, or mechanical signals. In this regard, Boronic acid (BA) derivatives are becoming an ever more popular topic of research in the biomaterials field owing to their ability to reversibly bind biologically relevant diols. This review will detail the design of stimuli responsive BA polymers capable of detecting saccharides in biological media such as ocular fluid and blood. Such sensors are of biomedical interest due to their potential as novel glucose sensors for the treatment of diabetes.

Introduction

Diabetes mellitus is a condition caused by an auto-immune response to insulin-producing beta cells of the pancreas which results in the body's inability to efficiently produce and use insulin. This leads to altered blood-glucose levels and can result in complications that can include blindness, renal failure, peripheral nerve damage of the extremities resulting in limb amputation, cardiovascular disease and cancer.[1] Many of these complications can prove fatal if not sufficiently treated. Imperative to an improved prognosis, is appropriate medication, such as synthetic insulin injections, and the careful monitoring of blood glucose levels.

Currently glucose monitoring is generally performed by the patient several times daily via finger-prick blood sampling, a method considered by patients to be invasive and inconvenient. At present, this condition affects approximately 425 million people worldwide and is a leading cause of death.[2] Unfortunately, this

number continues to rise annually and as such there is a growing need for non-/minimally- invasive glucose sensors, in order to increase the low rate of patient compliance with the current method, improve patient satisfaction and ultimately minimise the risk of long-term complications of hypo-/hyperglycaemia and of diabetes-related fatalities.

Many of the current methods of glucose sensing, such as finger-prick blood sampling, are achieved by electrochemical means, through the use of the glucose oxidase (GOx) enzyme. When subjected to glucose, the immobilised GOx catalyses the oxidation of glucose to gluconolactone in the presence of molecular oxygen, thereby producing hydrogen peroxide (H_2O_2) and water.[3] Gluconolactone is further oxidised to gluconic acid in the presence of the redox cofactor flavin adenine dinucleotide (FAD^+), an electron acceptor, which is consequently reduced to $FADH_2$ (1). $FADH_2$ reacts with oxygen to generate H_2O_2 and regenerate FAD^+. When these reactions occur at the anode in a sensor, the amount of generated H_2O_2 can be correlated to the number of transferred electrons, and ultimately the concentration of glucose present in solution can be deduced.[4]

Whilst enzymatic approaches such as these are commonly employed in glucose sensing for diabetics, with great sensitivity and specificity, there are several shortcomings to these approaches. As these measurements are taken intermittently, the lack of a continuous measurement can increase the risk of undetected periods of hypo-/hyperglycaemia. Additionally, the incompatibility of enzymes in certain conditions such as adverse pH and temperature, can result in a limited shelf-life of the sensor and the need for periodic recalibration. Therefore, there is a drive for development of non-invasive solid-state continuous sensors that do not rely on enzymatic means of detection.

Chemical sensors have been proposed as an attractive alternative to the enzymatic approaches outlined above. One such class of chemical sensors relies on the use of boronic acid (BA) derivatives for the detection of saccharides. Such sensors will be the focus of this review.

Chemistry of Boronic Acids

Boronic acids have found application in both organic synthesis and drug development. In the lab they are often used as protective agents for diols, as nucleophiles in Mannich reactions [5] and as intermediates in coupling reactions. They exhibit relatively low toxicity, thus making them attractive lead compounds in drug discovery and are currently used in antiseptics, antibiotics and chemotherapeutics.[6] However, more recently aromatic boronic acids have found use as potential receptors for biologically relevant molecules such as saccharides. [7]

An important feature of boronic acids is their reactivity. In neutral form, boronic acids exist in a trigonal planar conformation with a sp2 hybridised boron centre and a generic R-B(OH)2 structure. As a result of a vacant pz-orbital on the boron centre, these compounds act as Lewis acids, and thus can form strong, reversible Lewis

acid-base interactions with a number of molecules such as carbohydrates, amino acids and neurotransmitters.[8] This property allows for molecular recognition of certain analytes and is frequently exploited in the search for new minimally invasive sensing technologies and synthetic receptors.

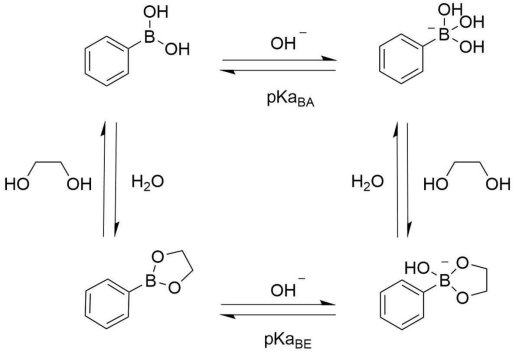
Aryl-boronic acids are particularly popular in such applications, as the electron withdrawing phenyl ring causes charge delocalisation, increasing the stability of the boronic acid. As well as interacting with Lewis bases, these acid compounds form stable cyclic esters upon reacting with 1,2- and 1,3-diols. This ester formation is highly reversible and thus can be probed for the development of novel saccharide sensors.

Boronic Acids for Saccharide Sensing

The formation of a boronate ester from a boronic acid and a diol (Scheme 1) is one of the strongest reversible interactions in modern synthetic chemistry, and thus can be readily used for the construction of molecular receptors.[9] The strength behind this reversible interaction lies within the chemical nature of the tri-valent boron centre. Monosubstituted boronic acids have a pKa ranging from 7 to 9,[9] although a more accurate value for specific phenylboronic acids based on substituent effect can be predicted using a Hammett-plot.

pKa plays an integral role in the ability of boronic acids to reversibly bind to 1,2- or 1,3- diols, such as saccharides or other Lewis bases, forming 5- or 6- membered cyclic esters in which the boronate ion - a charged, tetrahedral species is present. Subsequent to the addition of these reactive diols to BAs, is a shift in equilibrium towards the anionic- or tetrahedral charged species.

Ultimately, when used in sensing applications, the binding affinity of diols to BAs depends heavily on the pH of the environment, with the optimal pH for sensing applications falling below the pKa of the boronic acid and above the pKa of the boronate ester, where pKa is defined as the pH at which 50 % of the boronic acid exists as the hydroxy boronate anion species.[10]



Scheme 1 : *Scheme depicting the interaction of Phenyl Boronic Acids with diols. Adapted from* [11].

One potential way of exploiting the reactivity of boronic acids for sensing applications is to incorporate these molecules into solid state systems such as polymers. Polymers are materials which have a molecular structure built from a large number of 'building blocks' or monomer units bonded together via crosslinkers. Such materials have found uses in many popular products including 'plastics' and are used frequently in a vast array of biomedical applications. One such polymeric structure that is of particular interest in the design of sensors is hydrogels.

Hydrogels are 3-dimensional cross-linked hydrophilic polymer networks capable of holding large volumes of water. Hydrogel structures can be chemically or physically crosslinked, with the former consisting of covalent networks that do not dissolve in water without breakage of covalent bonds,[12] while physical hydrogels are those which are held together by ionic, hydrogen or hydrophobic forces.

The properties of hydrogels are dependent on their bulk structures,[13] which allows for the potential to design stimuli-responsive hydrogels that can be synthesised to include specific functional groups essential for the detection of certain analytes. Stimuli-responsive hydrogels include, among others, pH-responsive hydrogels, which consist of charged polymer chains which could swell/shrink in response to changes in environmental pH [14] and thermo-responsive hydrogels [15] which could give similar responses in response to temperature changes due to the effect of temperature on the hydrophobic forces within the polymer.

Another application of stimuli responsive hydrogel structures is their potential to be used as biosensors. Inherent to the structure of these polymeric networks is a porous, hydrophilic environment which makes them suitable for use in detection of analytes [16] present in aqueous environments as molecules can pass into the network and bind to certain functional groups, resulting in a detectable response. Examples of such hydrogel structures used in sensing applications include boronic acid hydrogels.

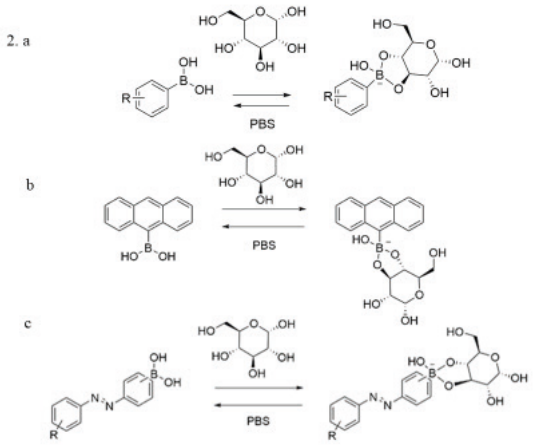
Boronic Acid Hydrogel Structures for Saccharide Sensing

Boronic Acid (BA) hydrogel structures have great potential for use in saccharide sensing. A BA monomer, when incorporated into a polymer such as a hydrogel, yields a responsive polymer and thus the BA mechanism of binding to diols (Scheme 2) can be exploited for sensing applications in the solid state.

Many sensing mechanisms probe the Lewis acidity of BA monomers and thus function based on the knowledge of binding affinities of BAs to diols in aqueous media. Such binding affinities are generally governed by factors including the pKa of the BA moiety and the pH and composition of the aqueous medium.[17] In BA functionalised hydrogels, changes in pH will increase the number of charged boronate species present. This leads to an increase in electrostatic repulsion of the fixed ions, and consequently a volumetric change in the hydrogel as a result of the Donnan effect.[18]

As the pKa of the boronate ester is lower than the pKa of the neutral BA and there are reactive diols present in solution, the equilibrium shifts in favour of the boronate species, and thus the hydrophilicity of the hydrogel is increased. These combined events increase the mixing free energy of the system which is accompanied by a change in osmotic pressure, allowing saccharides to diffuse out of the aqueous solution.

They become immobilised in the hydrogel, thus causing the hydrogel to swell.[18] Such saccharide binding induced swelling is illustrated below (Figure 1) , a response which is possible due to the elastic stretching of the polymer chain fragments.[19]



Scheme 2 : *Schematic of reversible saccharide binding at OH sites in various BA moieties. A is a phenyl boronic acid, B is an azobenzene boronic acid and C is an anthryl substituted boronic acid.*

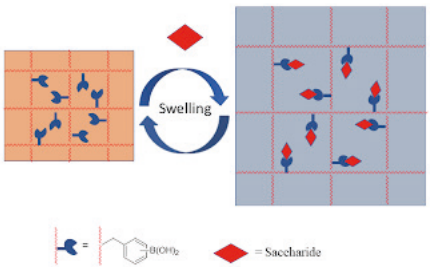


Figure 1: *Illustration of such reversible reactions, as shown in scheme 2, that results in a volumetric change in the BA hydrogel upon glucose intake or depletion. Adapted from [20].*

Detection Methods Employed in Boronic Acid Saccharide Sensing

When considering the development of sensors, it is important that one considers signal transduction mechanisms - how will the signal be transmitted in such a way as to deliver a detectable response? Signal transduction mechanisms for BA-sensors, rely heavily on a conformational change (accompanied by a change in charge) of the boron atom upon sugar binding. Popular signal transduction mechanisms for the detection of saccharides in BA-based sensors include electrochemical, optical and fluorescent methods.

Signal transduction mechanisms based on fluorescence work by incorporating molecules with fluorescent properties into these sensors. Such fluorophores, commonly aromatic rings or conjugated pi-bond systems, work by absorbing incident light or electromagnetic radiation. This absorbance corresponds to the excitation of an electron from its ground energy state (S_0) to a higher energy state (S_1 or S_2).

The electron is inherently unstable in the higher energy state and therefore begins to lose energy by emitting light. This energy can be emitted via radiative and non-radiative pathways including phosphorescence, fluorescence and internal conversion. Fluorescence occurs when the energy of the photon emitted is equal to the difference between the eigenstates of the transition, however, some of the energy is lost in internal conversion and vibrational relaxation meaning that the energy of fluorescent photons is always less than that of exciting photons.

The incorporation of fluorophores into boronic acid materials poses an attractive mechanism for the detection of saccharides. Photoinduced Electron Transfer (PET) is one such mechanism and involves saccharide detection based on fluorescence quenching. This quenching results from the transfer of excited electrons from a donor to an acceptor as outlined in Figure 2. In short, PET occurs if the oxidation potential of the receptor is smaller in magnitude than that of the fluorophore. [21]

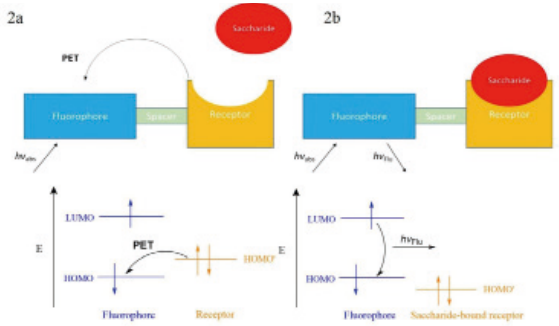


Figure 2 *A) A molecular orbital diagram shows the energetic depositions of the corresponding frontier orbitals, in PET from a saccharide-free receptor to a photo-excited fluorophore.*

B) The electron transfer from the saccharide bound receptor is blocked resulting in fluorescence or the 'on' state, with a corresponding MO diagram. Adapted from [21].

BAs commonly used in PET systems include azobenzene and anthryl-substituted boronic acids seen in scheme 2.[7,22] Saccharide binding to such systems suppresses PET and consequently increases fluorescence, resulting in a quantifiable response.

Intramolecular Charge Transfer (ICT) is a similar mechanism based on fluorescence where a charge transfer complex (CT complex) is present in the synthetic receptor. This CT complex consists of both an electron donor and acceptor present in the same fluorophore,[23] where, in the case of boronic acids in saccharide sensing, the boronic acid acts as an electron acceptor in its neutral form. Upon binding to a diol at a certain pH, the boronic acid group changes to its anionic form and no longer functions as an electron acceptor, resulting in visible spectral changes, [23] a response that correlates to the perturbation of the charge transfer nature of the excited state. [24] Such signal transduction mechanisms are often readily employed in the development of biosensors.

Recent Developments in Saccharide Sensing

The development of novel biosensors based on boronic acid polymers is of particular relevance in the treatment of diabetes, and naturally many research groups have explored this area with the aim of creating sensors to improve the care and compliance of patients suffering from such diseases.

Such interests have inspired the development of biosensors designed for at-home, real-time testing by the patient. One such example by Wolfbeis and co-workers, involves the measurement of glucose concentrations in ocular fluid via the incorporation of copolymerised aniline and 3-aminophenylboronic acid into a polymer film similar to a contact lens.[25] This polymer film has an absorption spectrum between 500 and 800 nm thus, any changes upon binding of saccharides in the ocular fluid result in marked differences in the corresponding spectrum, therefore allowing glucose concentration to be monitored by absorption spectroscopy.

Similar work has been done by the Michaels group at California Institute of Technology, focusing on the development of a novel conductimetric sensor for the detection and continuous concentration monitoring of specific biomolecules, such as glucose, in aqueous solutions (e.g., blood or plasma).[26] The sensor, based on a BA hydrogel, liberates a hydrogen ion upon glucose binding, with the corresponding change in ionic concentration correlating to the overall glucose concentration in solution, and thus poses an attractive potential sensor.

Whilst there are many more examples similar to these in literature, (a 2017 review by Breun and Florea [26] provides an insight into other developments in wearable sensors), none have thus far managed to reach market. However, interest from companies such as Google and Microsoft suggests that there is a serious market

potential for novel approaches to self-monitoring of diseases such as diabetes. [26]

Conclusion

Polymers based on phenyl boronic acids have been shown to respond, with great selectivity and sensitivity, to the presence of biomolecules such as saccharides in biologically relevant media. When appropriate signal transduction methods are employed, these stimuli responsive BA polymers have the potential to be developed as wearable biosensors capable of delivering continuous, real-time data relevant to disease and disease markers. These sensors provide a non-invasive, continuous means of detecting glucose concentrations, and thus are a promising alternative to current enzymatic based glucose testing methods.

Therefore, wearable sensors such as the examples outlined above, have the potential to play a critical role in the detection and treatment of diseases such as diabetes and ultimately improve patient compliance and satisfaction. Unfortunately, many of these examples still have further clinical reviews and evaluations to undergo before they can be approved and marketed for medical use.

However, despite their current status as unapproved, these wearable sensors bear promise in the field of medical technology. If one considers the ever-rising popularity of wearable fitness devices such as smartwatches and fitness trackers for tracking heart rate and blood pressure, it is possible to see a future where, incorporated into these devices, we have biosensors capable of monitoring disease markers and detecting relevant biomolecules such as glucose.

Thus, one can expect to see interest in such biosensors increase in future years, not only as we aim to improve the treatment and management of diseases such as diabetes, but as we move into an ever-more health-conscious age.

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TS
SR

Physical Sciences

Letter from the Editor

The study of physics covers the entire timeline of our universe, from origin to predicted ending. Physicists attempt to peek into the distant past, interrogate the mysteries of the present and foretell the future. As such, it seems appropriate that our reviews delve into the past with historical issues of contention within quantum mechanics as well as cutting-edge developments in nanotechnology in the present.

In our first review, the author pays tribute to the process of discovery and deduction in physics as they discuss the Einstein-Podolsky-Rosen paradox. This addresses a significant controversy in our early understanding of quantum mechanics, with recent giants of the field such as Einstein and Bohr clashing, and Irish scientist John Stewart Bell also making significant contributions. The concept of quantum entanglement demonstrated in this paradox remains relevant, with applications to quantum computing and quantum teleportation.

Nano-antennae are the focus of the second review: a promising technology focusing on light harvesting, which have developed within the burgeoning, multidisciplinary field of nanoscience especially since the turn of the millennium. Nanotechnology, heralded by Feynman in 1959 and indeed unknowingly applied throughout history has seen massive growth since the development of scanning tunnelling microscopy in 1981, alongside other fabrication and microscopy techniques essential to the field.

This research is especially relevant to Trinity, which boasts Ireland leading nanoscience institute, CRANN, the Centre for Research on Adaptive Nanostructures and Nanodevices. Nano-antennae are a fascinating facet of this paradigm shift in technology, with promising applications as bio-sensors, photocatalysts and nano-emitters.

These reviews also highlight the tools available to a physicist, with the Einstein-Podolsky-Rosen paradox first explored through Gendankenspiel, or thought experiments, and empirical experiments later developed to test Bell's Inequalities. The practicality of physics is demonstrated when discussing nano-antennae, with the effects of nano-antenna material, media, structure and geometry upon its properties explored.

I would particularly like to thank our authors for these insightful reviews, as well as our academic advisor, Professor Mauro Ferreira for his guidance throughout the process of preparing the review. Further thanks go to our reviewers for their commitment and assistance, embodying the rigor of the peer review process. This process reminds us that science is by no means monolithic but always evolving, and that we must always retain a certain scepticism; that even a reasonable explanation, especially one which we personally prescribe to, may be proven lacking with further study.

Pierce Sinnott

Physical Sciences Editor

Trinity Student Scientific Review Volume VI

Nanoantennae : Big Leaps for Small Detections

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Senior Sophister
Physics

The development of nanoantennae in the early 21st century fundamentally changed the view on light harvesting techniques prior to its inception. The ability of a particle to simultaneously absorb more electromagnetic radiation than is incident to it, concentrate this energy below diffraction limited volume and therefore create substantial field enhancement inspired a new wave of photonic research. Such research enabled the discovery and utilisation of plasmons which seamlessly integrated into the development of hot carrier-chemistry. This in turn, revolutionised current practices of photocatalysis and photochemistry through the use of antenna- coupled redox reactions. Current studies into the parameters affecting this plasmon-assisted absorption have yielded optimisations in morphology, dielectric tuning and dimer-coupling with future workings directed towards biological applications. This review explains the key aspects concerning nanoantenna performance, namely, their size, surrounding environment and material composition. Moreover, the on-going research into nanoantenna viability as sensors, photocatalysts and nanoemitters will be discussed.

Introduction

Harvesting energy from light has been utilised for millenia, with photosynthetic life evolving from this function alone. It is only in the last half century that humans have been able to benefit from this resource to aid in modern technologies such as photocatalysis,[4] optical sensors [5] and optoelectronics. [8] Photonics research has seen greater interest in recent years which brought with it a paradigm shift towards the development of more efficient light harvesting technologies.

One such avenue is the use of nanoantennae (nA)s for light trapping and utilisation. These devices are nanoscale metallic structures that are able to efficiently capture and utilise light in a very small volume of space. Inspired by radio frequency antennas, these nAs allow for receiving and transmitting of signals from UV to IR wavelength ranges.[1-3]

A voltage is induced across a receiving antenna via incident radiation which causes a charge separation. As the voltage fluctuates the charge oscillates between the poles of the metal. This movement of charge is accompanied by an electromagnetic (EM) field, which can re-radiate in a process known as 'scattering'.

This scattered field can interact with other receiver antennas thus transmitting an electrical signal from one point to another. The main difference between radio frequency antenna and nanoantenna is of course, their size. This size difference is a direct consequence of the wavelength of the EM radiation they detect. Radio waves typically have a wavelength of 290 m and according to Radio Theory, its antenna should be of the length corresponding to half the incident wavelength ($\lambda/2$).[1] One visible consequence of this is that radio towers must be between 100-200 m in height to receive signals, much in the same way wavelengths of 400 nm are received by antennas 200 nm in size.

Interactions between oscillating EM radiation and metallic structures at the nanoscale result in interesting phenomena that are not seen at the macroscale. Such phenomena can and has been used to optimise the light-trapping capabilities of nAs.[7,18] The key principle behind these capabilities is enabling the antennas to resonate with incident light. For sub-wavelength optical antennas, localised surface plasmon resonances (LSPR)s are the causation of the observed EM absorption. These LSPRs are generated upon excitation of a curved metallic nanoparticle with plane wave light, an accumulation of oscillating polarised charge occurs due to the restoring force arising from the particles curvature.

This oscillation is of the same frequency as the incident light and results in a resonant mode, which is confined to the structure in more than one direction. Furthermore, the decay of these modes can excite electrons to a higher energy state (e^-). This leaves a positive hole (h^+) residing in its ground state. These charge carriers can be further utilised for chemical reactions at the nanostructure surface (Fig 1).

Plasmon resonance and hot-carrier genesis enable the versatility of these devices. Many parameters influence the mode of resonance and population of charge carriers in the nA, however, for the purposes of this review, three essential components will be explored; geometry, dielectric media and material of the structure.

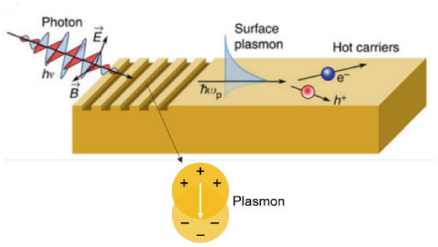

Figure 1 :*Schematic of plasmon-induced hot carrier generation upon irradiation of metal surface. Adapted from* [13]

Influence of geometry

The dimensions and geometry of the nanostructures play an important role in enhancement of near-fields on nA. Many studies have investigated different topologies to optimise the field-enhancing properties of plasmonic nanoparticles: different shapes, sizes and arrays have been fabricated.[10,11,20] These studies all aim to capture light in the most efficient way possible and concentrate it into sub-wavelength volumes for a variety of different purposes.[5,9,15]

nAs have a unique ability to enhance light capture by possessing a scattering cross section that is greater than the physical size of the nanostructure. This is a resonant effect where aspect ratios and particle densities are crucial, due to the criterion that 'ideally' the antenna should be half the size of the wavelength incident to it. [1] Eustis et al, demonstrated the wavelength shifting that can occur upon varying the aspect ratio of nanospheres and nanorods (Fig 2 Left). It can be seen that upon increasing the aspect ratio of the nanorod from 2.5 to 7.5, a red shift of absorbance is seen from 700 to 1000 nm. This spectral shift is described by Mie's theory which states that with increasing particle sizes, latent extrinsic effects become more pronounced leading to excitations of more multipole modes.[33]

Also illustrated by Eustis' work is the change in form of spheres to rods. This impacts the absorbance modes of the particles as spheres show only one absorbance peak and rods show two peaks (two different resonant modes and polarisation selectivity). In contrast to this resonant effect there exists a non-resonant effect that can be utilised to yield further field enhancement, namely, the lightening-rod effect. This phenomenon arises from the electric field lines that concentrate around a highly curved surface (Fig 2 Right).

It has been shown, theoretically and experimentally, that this non-resonant component can amplify near-fields without the conjunction of any resonant components.[13]

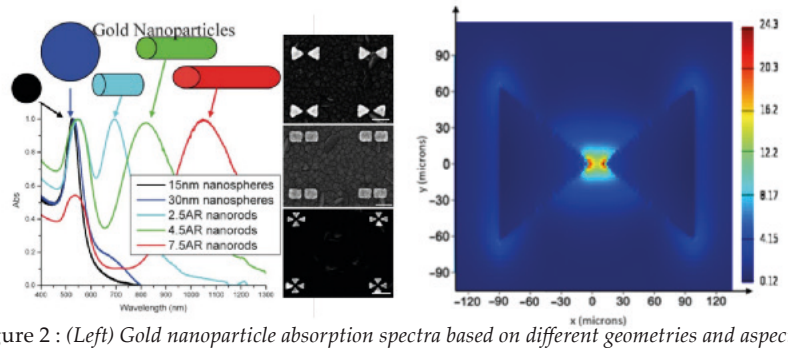

Figure 2 : *(Left) Gold nanoparticle absorption spectra based on different geometries and aspect ratios, (inset) SEM images of gold-fabricated nA design. Scale bar is 100 nm. Taken from* [7]*. (Right) Aluminium bowtie nano-antenna dimer, illustrating concentrated field enhancement due to lightning-rod effect at pointed corner.*

Influence of dielectric media

The premise of LSPR is that its resonance is bound to the surface of a sub-wavelength sized nanostructure at the interface between the metal and dielectric. It has been shown above that the LSPR mechanism can enhance EM fields, the intensity of which, is the sum of the squares of their amplitudes. The surrounding environment of nanoantennae has seen much work as of late to make up for such short comings as; thermal instability, energy loss and narrow non-linarities.[34]

For the sake of simplicity the Drude model can be used to incorporate a dielectric influence on LSPRs.[35] Although the model is used primarily for electron scattering in bulk metals its quantitative terms are useful guides for the purposes of this analysis. The model yields values for plasma frequency ωp and relaxation time τ of the mean free path for specific plasmonic metals which enable the resonant frequency LSPR, ω_{LSPR} to be calculated as shown:

$$\omega_{\mathrm{LSPR}} = \omega_{\mathrm{p}} \sqrt{\frac{\mathbf{1}}{\mathbf{1} + \mathbf{2}\varepsilon_{\mathrm{m}} + \chi} - \frac{\mathbf{1}}{\omega_{\mathrm{p}}^2 \tau^2}} \tag{1}$$

where

$$\omega_{\mathrm{p}} = \sqrt{\frac{\mathrm{n_e e^2}}{\varepsilon_0 \mathrm{m}^*}} \tag{2}$$

ε_0 is the permittivity of free space, n_e denotes the number density of electrons, e is the elementary charge, m* is the effective mass of the electrons and χ is the interband susceptibility of the metal, which is a measure of the likelihood of

interband transitions to take place. The product term $\omega_p^2\tau^2$ for common dialectrics such as air or water is larger than the $1 + 2\varepsilon_m + \chi$ term, this can yield the term negligible in the above equation.[37] Doing so yields only one variable ε_m, the dielectric constant of the surrounding media.

So, it can be shown that the LSPR of a metal is very much dependent on the media that surrounds it, such that the higher the dielectric constant of the surrounding material the lower the ω_{LSPR} and the stronger the resulting field confinement. It is possible to extrapolate from this the corresponding wavelength of the LSPR, λ_{LSPR}, following the equation $\lambda = c/\omega$, however it is important to implement a phase correction coefficient, as we are dealing with sine waves a correction of 2π is used to account for periodicity, which yields:

$$\lambda_{\mathrm{LSPR}} = \frac{2\pi c}{\omega_{\mathrm{LSPR}}} \tag{3}$$

For an overall depiction of the factors that influence λLSPR the below equation holds:

$$\lambda_{\mathrm{LSPR}} = \frac{2\pi c}{\omega_{\mathrm{p}}\sqrt{\frac{1}{1 + 2\varepsilon_{\mathrm{m}} + \chi}}} \tag{4}$$

Therefore, it can be seen that changes in the dielectric constant of surrounding media can increase or decrease (red-shift or blue-shift) the wavelength of the LSPR. 2019 work by Mitsai et al, led to fabricated 'all-dielectric' nAs which work off the basis of dielectric influence on plasmonic resonance.

Their research with $Si_{1-X}Ge_X$ nanostructures enabled tunable resonances as a function of Ge concentration for the purposes of light-to-heat conversion. [36] Future workings in all-dielectric nAs can allow for emission feedback of adsorbed analytes ranging from biomolecules to pollutants to test their composition and nA-anyalyte thermal response. [36]

Material of Nano-antenna

The selection of plasmonic materials to be used has proven to be vital in recent years. [31] Thus far, eight metals with plasmonic characteristics have been successful as viable nAs: Mg, Al, Ni, Cu, Pd, Ag, Pt and Au. The commonality between these materials is their band structures. These band structures are a composition of energy states with variations in crystal momentum (k-vector) in the Brillouin zone. The band gaps present in these structures can be separated into two broad terms, direct and indirect. The former meaning that the minimum energy state of the conduction band and the maximum energy of the valence band are at the same point in k-space. The latter infers that the conduction band minimum (CBM) and

the valence band maximum (VBM) do not possess the same point in k-space. (Fig 3). For electronic transitions to take place, the energy gap between the VBM and VCM needs to be overcome. Photonic absorption of energies greater than that of the band gap energy ($E_{photon} > E_{gap}$) will yield electron transition.

These transitions are very sensitive to band structures due to the selection rule that requires the initial and final electronic states to possess no change in crystal momentum. For an indirect band gap species photonic absorption isn't sufficient, a momentum shift is required to reach the CBM. This is granted in the form of phononic energy (lattice vibration). The combination of photonic and phononic energy yields a VBM to CBM electronic transition, illustrated by Fig(3).

The occupation of these energy states becomes important when dealing with electronic transitions within these structures. In metals the occupation of states is denoted by Fermi level (E_f) where all states below this level are occupied. The Fermi level cuts through the conduction band in a metal rendering it partially filled with many accessible states. In a semiconductor, it can be found approximately half-way between the valence and conduction band yielding a band gap. These band gaps and the transitions that are allowed between them characterise which materials are appropriate to use as nAs.

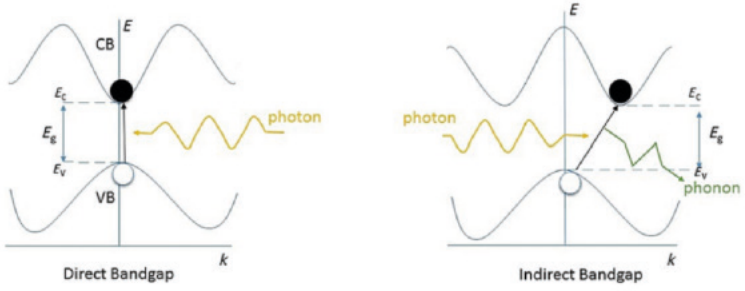

Figure 3 : *Band gap diagram of direct (left) and indirect (right) modes. Valence band (VB), Conduction band (CB), Valence band maximum (E_v), Conduction band minimum (E_C) and Band gap energy (E_g).*

Additionally, other criteria such as, chemical and thermal stability, bulk plasma frequency, nonlinear response, and fabrication constraints[31] have shown that Al, Ag and Au have proven to be of notable quality. [19,20] The reason for this lies within the interband susceptibility (χ) of the material used. This parameter manifests itself in the bandwidth of plasmonic modes (Γ) which is given by:

$$\Gamma = \gamma\sqrt{\frac{2\kappa\Omega}{\gamma}} \tag{5}$$

where

$$\kappa = \frac{\chi 2}{1 + \chi_1 + 2\epsilon_m} \qquad \Omega = \frac{\omega_p}{\sqrt{1 + \chi_1 + 2\epsilon_m}}$$

Here, γ is the damping constant and χ is split into a real part (χ') which accounts for the average likelihood of an interband transition to take place and an imaginary part (χ'') which accounts for the interband transition contribution to the bulk dielectric function. Since $\chi_{Au} > \chi_{Ag}$ it can be expected that the band width for plasmonic modes in gold will be larger and occur at higher wavelengths than that of silver, which has been proven experimentally.[20,21]

Due to silvers high intensity, easily-tuned plasmonic modes and third harmonic generation (THG) it has been held in high regard as a viable material for nanoantenna fabrications. [32] In which, THG is a non-linear process that allows for multi-photon absorption on the surface of the metal. However, in a practical sense gold is used more frequently due to the degradation of silver nanoparticles by its reaction with atmospheric sulphur despite its enhanced plasmonic properties.

Applications and Developments

The versatility of nanoantennae became apparent when semiconductor-based emitters became outclassed. These type of emitters have slow spontaneous emission with lifetimes lasting anywhere between 1-10 ns, this yields discrepancies with typical high-speed nanoscale optoelectronics such as LEDs and lasers.[38] The introduction of single plasmonic nanopatch antennae enabled ultrafast and efficient spontaneous emission with a rate greater than 90 GHz.[38]

Moreover, advancements in electron-beam lithograhy and other such sub-micron fabrication tools enabled enhanced resolution at the nanoscale. The following highlight a small section of the current work in nA technology. In 2013, Mubeen et al, demonstrated that a nanoscale autonomous photosynthetic device could be fabricated using TiO2 to cap an array of aligned gold nanorods, whose exposed parts were coated with platinum nanoparticles and a cobalt-based oxygen evolution catalyst (Co-OEC).

When this nanostructure array was placed in water and exposed to visible light (λ > 410 nm) it produced 0.25 ± 0.025 μmol h^{-1} of H_2 with no signs of degradation in photosynthetic activity.[30] This work took advantage of plasmon-induced hot carrier generation in gold that allowed an electron to become available to the platinum nanoparticle reduction catalyst and a hole to become available to Co-OEC. This yielded the photolysis of water with visible light (Fig 4).

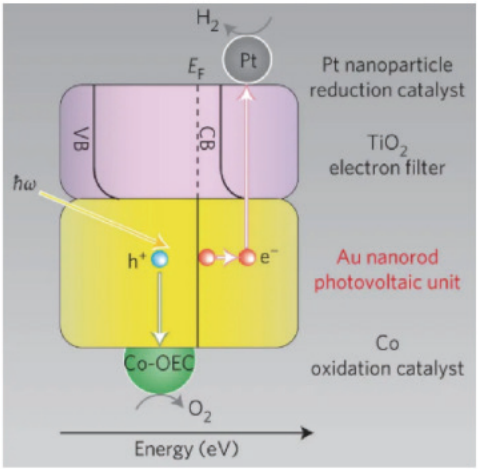

Figure 4 : *Schematic of artificial photosynthetic nanostructure by Mubeen et al.* [30]

In 2017 Cortés et al, showed that nano-localised chemical reactions can be driven at certain sites on nAs. Their work demonstrated the reduction of 4-nitrothiophenol (4-NTP) to 4-aminothiophenol (4-ATP) groups when exposed to light corresponding to the resonant frequency of the nanostructure. This yielded further reactions that could be observed under SEM to map the location-specific 'hot-spots'.[3] The hot-spots being regions on the nA with direct field-enhancement as a consequence of highly-curved surface area.

The hot carrier-induced chemical reactions illustrate that charge transfer reactions are obtainable with the use of nanostructures in the presence of an EM field without the need for expensive reducing agents and reaction conditions. Lastly, nAs have proved invaluable in the domain of sensors due to their sensitivity to wavelength and polarisation of incident radiation (Fig 5).

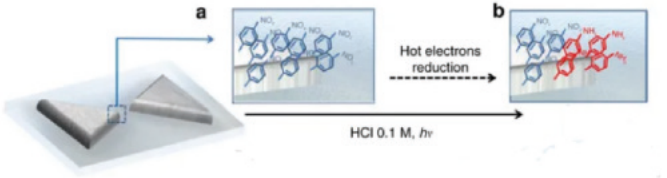

Figure 5 : *Cortés' work on hot electron reduction of 4-NTP to 4-ATP.*[3]

In a 2018 study conducted by Mubarak et al, nanoantennae were coupled to IR detectors via a bolometer so to improve response times and uptake of incident radiation. This yielded an increased energy transfer and decreased production cost due to lack of cooling and polarising apparatus required. Many morphologies and coupling regimes were tested, each demonstrating different uptakes, energy losses and signal transfers.[22]

It was concluded that the utilisation of nA-coupling resulted in an overall increase of IR detectivity. Primarily due to the decrease in selectivity of EM absorption and increase in energy transfer based on the nanostructure operating in the THz regime (Fig 6).

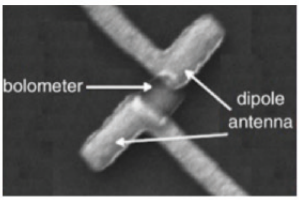

Figure 6 : *Nanoantenna-coupled IR detector by Mubarak et al.*[22]

Conclusions

A brief insight into the current workings of nanoantennae has been given in this review. Many avenues have been explored with many more to come, including their uses in biological and bio-medical fields (23-25). It has been emphasised that the topology of nAs is indeed the cornerstone for current research with the only limiting factor being that of sub-micron resolution of fabrication tools. However, the scalability of nA-based sensors and electronics remains an issue with many fabrications and performances requiring specific high-cost tools and light sources (26).

Nano-localised photochemistry, on the other hand, has shown proven success using nAs for redox and catalytic reactions (27-29). Lastly, Di Martino et al, showed that germanium nanowire growth on gold nanoparticles can be contolled by light alone. Uniform conductive wires were synthesised from gold plasmonic seeds with their growth rate being controlled by thermal-feedback mechanism induced by light (9).

Overall, the many components encapsulated by nanoantennae allows for more efficient sensors, faster energy transfer in signalling systems and greener photochemistry with the aspirations of biological sensing and therapies in years to come.

Acknowledgements

I would sincerely like to thank the Hobbs group for their continued support on the project.

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Quantum Entanglement & The Legacy of The EPR Paradox

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The development of quantum mechanics in the early 20th century fundamentally challenged the deterministic view of reality that had been universally held prior to its inception. The indeterminacy of the properties of physical systems until observation was a particular point of philosophical contention. In this review a point of contention famously pioneered by Einstein, Podolsky and Rosen in their 1935 paper challenging Quantum Mechanics as a complete theory of nature is examined. Various inequalities were subsequently formulated that distinguish quantum mechanical predictions from the predictions of local hidden variable theories which might restore determinism. The experimental confirmation of the failure of local hidden variable theories to account for quantum mechanical correlations led to the discovery of quantum teleportation as a novel and unintuitive method of communication.

Introduction: Commutation Relations and Uncertainty

Within the context of quantum mechanics, a physical observable can be thought of as any dynamical variable can be measured. Commutation relations between physical observables of a system are a fundamental aspect of the study of quantum mechanics. The commutator of physical observables, can be understood to represent the possibility or lack thereof, of simultaneous measurement of the observables in question. For physical variables there exist corresponding operators which act on states and map these states onto physical space. Two hermitian operators ^A and ^B commute if;

$$[\hat{A}, \hat{B}] \equiv \hat{A}\hat{B} - \hat{B}\hat{A} = 0 \tag{1}$$

A fundamental relation of this class is the non-commutative nature of the position (ˆx) and momen- tum (ˆp) operators given by;

$$[\hat{x}, \hat{p}] = i\hbar \quad where \quad \hbar \approx 1.05 \times 10^{-34} m^2 \cdot kg \cdot s^{-1} \tag{2}$$

This non-commutative relationship is the basis of Heisenberg's Uncertainty Principle[1] which states that there is a fundamental uncertainty associated with the simultaneous measurement of both position and momentum, therefore they cannot both be known to arbitrary precision. This relationship is represented by the lower bound;

$$\Delta\hat{x}\Delta\hat{p} \geqslant \frac{\hbar}{2} \tag{3}$$

where Δ^x and Δ^p represent the uncertainty associated with the position and momentum operators respectively.

The Einstein-Podolsky-Rosen Paradox

The indeterminacy of values of non-commuting physical observables until measurement and the apparent loss of knowledge were points of particular contention within the nascent field of quan- tum theory. The premise for rejection of quantum theory in the Einstein-Podolsky-Rosen (EPR) paradox paper is a proof by contradiction. The primary philosophical prerequisites for a tenable physical theory were defined to be;

1. Completeness in which every element of physical reality must have have a counterpart in physical theory.

2. Reality the result of the measurement of physical observables exists independently of whether or not a measurement is made.[2] Classically, bodies can be said to have properties such as position and velocity that exist independently of measurement. These philosophical pre- requisites disallowed non commuting observables in quantum theories from sharing the same reality.

While the effect of the momentum operator $\hat{p}(\frac{\hbar}{i}\frac{\partial\psi}{\partial x})$ on a state vector may yield a definite real value, the expectation value of the position operator operator on a wavefunction is given by the equation;

$$\langle\psi|\hat{x}|\psi\rangle = \iint_{-\infty}^{+\infty} \psi^*(x)x'\delta(x-x')\psi(x')\,dx\,dx' = \int_{-\infty}^{+\infty} \psi^*(x)x\psi(x)\,dx \tag{4}$$

Where $\int_{-\infty}^{+\infty} \psi^*(x)\psi(x)\,dx$ is the product of the wavefunction and its complex conjugate. The expectation value of the position operator is therefore undefined until measurement of position occurs. In a gedanken experiment, a system of two particles described by the joint wavefuntion $\Psi(x_1,x_2)$, interact during some interval $[0 < t < T]$. The wavefunction $\Psi(x_1,x_2)$ was postulated to be of the form;

$$\Psi(x_1, x_2) = \int_{-\infty}^{+\infty} e^{(\frac{i}{\hbar})(x_1 - x_2)p}\, dp \tag{5}$$

After the described time interval (T < t), the action of the momentum operator ˆp on the individual components of the system is taken into consideration. The action of the momentum operator on the eigenfunction for first particle can be represented by the eigenvalue equation;

$$\frac{\hbar}{i}\frac{\partial \Psi(x_1)}{\partial x} = \frac{\hbar}{i} \times \frac{i}{\hbar} \times p \int_{-\infty}^{+\infty} e^{(\frac{i}{\hbar})(x_1 - x_2)p}\, dp = p\Psi(x_1) \tag{6}$$

The action of the momentum operator on the eigenfunction for the second particle yields the relation;

$$\frac{\hbar}{i}\frac{\partial \Psi(x_2)}{\partial x} = \frac{\hbar}{i} \times \frac{i}{\hbar} \times -p \int_{-\infty}^{+\infty} e^{(\frac{i}{\hbar})(x_1 - x_2)p}\, dp = -p\Psi(x_2) \tag{7}$$

Similarly, in order to obtain the position eigenvalues associated with the individual particles, one can take the expectation value of the position operator with respect to the position basis states. For the 1st particle, a relation similar to Eq.4 can be deduced;

$$\langle \psi | \hat{x} | \psi \rangle = x_1 \int_{-\infty}^{+\infty} e^{(\frac{-i}{\hbar})(x_1 - x_2)p(\frac{i}{\hbar})(x_1 - x_2)p}\, dx = x_1 \int_{-\infty}^{+\infty} dx \tag{8}$$

with x_1 being the expectation value for the first particle in the two particle system. The same analysis for the 2nd particle yields the expectation value of x_2. The respective positions and mo- menta of the two particles had been resolved without disturbing the other particle in the system. Therefore they were deemed to share the same reality. However, position and momentum are non- commutative variables in quantum theory as defined by the Uncertainty Principle. If this is true, the particles within the two system wavefunction cannot share the same reality. From this contra- diction, it was concluded by EPR that quantum theory cannot be a complete theory of nature by violation of the completeness definition given.

Hidden Variables and Bell Inequalities

The existence of Hidden Variables was postulated as a counter explanation to the inherent indeterminacy of the results of observation until measurement. The nature of such hidden variables is unspecified as they, by nature cannot be

directly obtained from experiment. Despite being theoret- ically unobtainable, these variables would determine results of measurement on quantum correlated systems. would determine In the example the interaction of the of system the measurement consisting of apparatus two spin-1/2 with particles, the particle these leading hidden variables to a pre- determined observable result.

The primary assumption inherent in the the EPR proof by contradiction is locality. This assumption can be stated as;

1. Locality: measurements on individual particles that were initially correlated do not affect the results of measurement on other particle when these particles are space-like separated.

An Observer A may measure the spin of one particle along an axis ω_1 and Observer B to measure the spin of the other particle along ω_2. Assuming that the results A and B for each observer are dependent on hidden variables, the locality assumption can be formulated as;

$$A(\omega_1, \lambda) = \pm 1 \quad B(\omega_2, \lambda) = \pm 1$$

The feasibility of hidden variables as an explanation for the results of measurement can be determined by comparison of the expectation values of hidden variable theories and experimental observations of quantum correlated systems. The correlation between outcomes A and B of measurements along the ω_1 and ω_2 axes given a hidden variable distribution ϱ (λ) is given by the integral;

$$P(\omega_1, \omega_2) = \int \rho(\lambda) A(\omega_1, \lambda) B(\omega_2, \lambda)\, d\lambda \tag{9}$$

Bell[3] was able to derive certain inequalities for the expectation values of spin measurements for hidden variable models using correlation functions of the same class (Eq.9). These inequalities can then be compared to the statistical predictions of quantum mechanical experiments. The first experimentally relevant formulation of Bell Inequality was derived by Clauser, Horne, Shimony Holt (CHSH)[4]. In the CHSH reformulation, it is assumed that Observer A measure along another axis ω_1' in addition to the original ω_1 axis. Similarly, Observer B may measure along ω_2 and ω_2'. A trivial extension of the locality of assumptions for the two axis case yields;

$$A(\omega_1, \lambda) = \pm 1 \quad A(\omega_1', \lambda) = \pm 1 \quad B(\omega_2, \lambda) = \pm 1 \quad B(\omega_2', \lambda) = \pm 1$$

Using this, CHSH focused on correlations between the measurements of Observers A and B along the different axes subject to the locality conditions above. Of particular interest was the the correlation;

$$B_{CHSH} = P(\omega_1, \omega_2) + P(\omega_1', \omega_2') + P(\omega_1', \omega_2) - P(\omega_1, \omega_2') \tag{10}$$

As the result of the measurements along the axes and subsequent

between measurements being determined by hidden variables λ derived from the hidden variable distribution $\varrho(\lambda)$, the correlation above can be rewritten in terms of the integral given by Eq.9;

$$B_{CHSH} = \int \rho(\lambda)[A(\omega_1,\lambda)B(\omega_2,\lambda) + A(\omega_1',\lambda)B(\omega_2',\lambda) + A(\omega_1',\lambda)B(\omega_2,\lambda) - A(\omega_1,\lambda)B(\omega_2',\lambda)]d\lambda \quad (11)$$

Grouping terms with common factors in Eq.11 allows the primary consequence of

$$\int \rho(\lambda)[A(\omega_1,\lambda) + A(\omega_1',\lambda)]B(\omega_2,\lambda) + [A(\omega_1',\lambda) - A(\omega_1,\lambda)]B(\omega_2',\lambda)d\lambda \quad (12)$$

Due to the condition that the results A and B of measurements must be ±1, the correlation given in Eq.10 must obey the CHSH inequality;

$$-2 \leqslant B_{CHSH} \leqslant 2 \quad \text{or} \quad |B_{CHSH}| \leqslant 2 \quad (13)$$

It can be concluded that theories in which hidden variables are responsible for the results of measurements by Observers A and B along the four axes are bounded above by a total correlation value of 2.

The Non Separability of EPR States

Within the context of quantum satisfy corresponding ω_1 , ω_1' , ω_2 , and ω_2' can be defined as observables which satisfy $\omega_1{}^2 = \omega_1'^2 = \omega_2{}^2 = \omega_2'^2$. The eigenvalues of these Hermitian operators therefore must be ±1 corresponding to the outcomes of spin measurements along axes. Using the language of commutators as described in the Introduction: Commutation Relations & Uncertainty section, as the four observables are well defined, then simultaneous measurements of these observables can occur;

$$[\omega_1,\omega_2] = [\omega_1,\omega_2'] = [\omega_1',\omega_2] = [\omega_1',\omega_2'] = 0 \quad (14)$$

Similar to the case of hidden variables correlations, correlations between the results of measurement of observables can be analysed. The quantum mechanical case takes a similar form to Eq.10 where the correlation is given by;[5,6]

$$[\omega_1,\omega_2] = [\omega_1,\omega_2'] = [\omega_1',\omega_2] = [\omega_1',\omega_2'] = 0 \quad (15)$$

Taking the square of this correlation yields the Khlafin-Tirelson-Landau relation;[7]

$$B_{QM}^2 = 4I + [\omega_1,\omega_1'][\omega_2,\omega_2'] \quad (16)$$

The second form of the quantum mechanical formulation BQM shown in Eq.15 can be understood as two dot product operations with the first term being the dot product of two vectors, ω_1 and (ω_2 - ω_2'). The second term can similarly be

considered as the dot product of two vectors. Given that the dot product is related to angle between two vectors, this correlation is maximised when the terms in the dot product are anti-parallel to each other as shown in Fig.1.

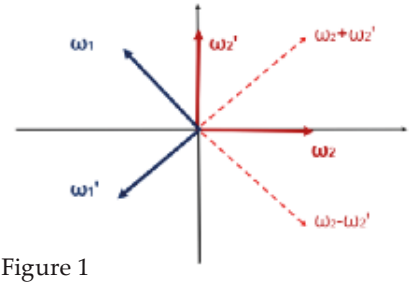

Figure 1

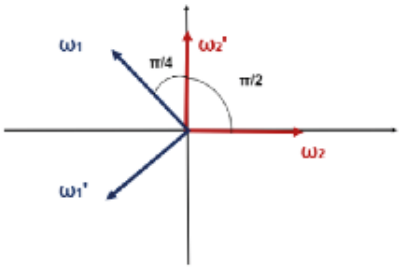

Figure 2

Figure 1: *The ω_2 and ω_2' axes are initially defined measurement axes. From this the vectors (ω_2 - ω_2') and (ω_2 + ω_2') can be represented. To maximise B_{QM} these vectors must be antiparallel to to ω_1 and ω_1' respectively.*

Figure 2: *The BQM correlation can also be described in terms of the angles between the axes along which observables are measured if the terms in the first expression of Eq.15 are taken as dot products.*

As the vectors depicted in Fig.1 are composed of the unit vectors ω_2 and ω_2', the length of these vectors is $\sqrt{2}$. The sum of these gives $2\sqrt{2}$ which is B_{QM}. Similarly, as the dot product of the axes of observable measurement is related to the the cosine of the angle between the axes, the correlation can be written as;

$$B_{QM} = |cos\left(\frac{3\pi}{4}\right) + cos\left(\frac{3\pi}{4}\right) + cos\left(\frac{3\pi}{4}\right) - cos\left(\frac{\pi}{4}\right)| = 2\sqrt{2}$$

As the quantum correlation BQM is greater than the Bell Inequality shown in Eq.13, it can be concluded that quantum mechanics as a framework does not obey local realism.

The CHSH formulation of the Bell Inequalities was the first that could be related to experiment. In quantum correlation experiments the polarization of pairs of photons was investigated rather than the spin states of spin-1/2 particles. The two emitted photons originate from the changes in angular momentum of a Calcium-40 atom.[8,9] Two channel variable polarizers were employed which may change the polarization of photons during transmission as shown in Fig.2. The four fold coincidence rates for vertical and horizontal polaisation along axes $\vec{a}$ and $\vec{b}$ can therefore be counted. Using such experimental apparatuses, the experiments of Aspect yielded correlation coefficients consistent with the violation of Bell Inequalities for local hidden variable theories.

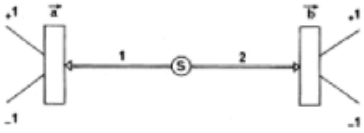

Figure 3

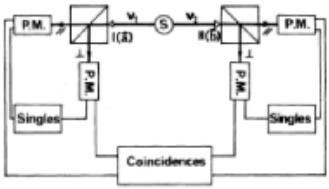

Figure 4

Figure 3: *An idealised experimental setup for the EPR experiment. Two spin-1/2 particles in the singlet state originate from a source. These particles travel in the* $\vec{a}$ and $\vec{b}$ *directions before being measured along these axes. Adapted from.*[8]

Figure 4: *The experimental setup of the 1st Aspect experiment. Horizontally and vertically polarized photons originate from a Calcium-40 source. These photons encounter a polarizer with rates of coincidence between polarized photons being measured. Adapted from.*[8]

Experimental evidence for quantum entanglement of correlated photons led to the confirmation of the existence of more general bipartite states in superposition represented by the ket vectors;

$$|\phi^{\pm}\rangle = \frac{1}{\sqrt{2}}(|00\rangle_{AB} \pm |11\rangle_{AB}) \quad \text{and} \quad |\psi^{\pm}\rangle = \frac{1}{\sqrt{2}}(|01\rangle_{AB} \pm |10\rangle_{AB}) \qquad (17)$$

The quantum bit or qubit is the fundamental unit of quantum information analogous to the bit of classical information theory.[12] The eigenstates given by Eq.17 correspond to observers A and B being in possession of one qubit each. These measurements can either be correlated $(|00\rangle_{AB} \pm |11\rangle_{AB})$ or anti-correlated $(|01\rangle_{AB} \pm |10\rangle_{AB})$. For example, the term be in Eq.17 correlated may represent both observers measuring 0 or both observers measuring 1. The second term represents one observer measuring 0 whilst the other measure 1 and vice versa. The two level quantum mechanical system described by these eigenstates can be applied to a variety of states such as the vertical and horizontal or vertical polarization of photons[13], the singlet and triplet spin states of electrons[14] as well as alkali metals of spin 3/2.[15] The inherent non separability of qubits in these maximally entangled states is the basis of many applications in the transmission of information that are exclusive to quantum information theory. Perhaps the most unintuitive application of the maximal entanglement of states is the use of these eigenstates for the quantum teleportation of qubits.

Quantum Teleportation

The specific relationship between the violation of Bell's inequalities and quantum teleportation is currently a matter of contention.[10] Despite this, it is generally agreed that the non-separability of entangled states is responsible for the quantum teleportation of qubits.[11] The teleportation of a state from observer A to observer B is a phenomenon in quantum theory with no classical analog. Teleportation in this sense is not analogous to representations in science fiction as the transmission

of information cannot occur instantaneously[18] as well as the perfect measurement of a physical system being forbidden by Heisenberg's Uncertainty Principle.[19] Observer A may send observer B information concerning an unknown state denoted by $|\phi\rangle_C$ both classically and quantum mechanically. [20] Assuming that observers A and B are in possession of qubits comprising the maximally entangled state $|\phi^+\rangle_{AB}$ the resulting tripartite product state can be represented by;

$$|\phi_C\rangle\,|\phi^+\rangle_{AB} = (|0\rangle_C + |1\rangle_C)\frac{1}{\sqrt{2}}(|00\rangle_{AB} + |11\rangle_{AB}) \tag{18}$$

This pure product state contains no classical correlation or quantum entanglement with the EPR pair.[20] Observer A can perform a Bell measurement on the unknown qubit C, uniting it with their half of the entangled pair to the state of the qubit C, uniting it with their half of the entangled pair to form $|\phi^+\rangle_{AC}$. Observer A's qubit loses its well defined state as the state of the qubit $|\phi\rangle_C$ is unknown. [19]

$$\frac{1}{\sqrt{2}}(a\,|000\rangle_{CAB} + a\,|011\rangle_{CAB} + b\,|100\rangle_{CAB} + b\,|111\rangle_{CAB} \tag{19}$$

The resultant tripartite state can be separated into constituent parts consisting of linear combinations of the four maximally entangled states $|\phi^\pm\rangle_{AC}$, $|\psi^\pm\rangle_{AC}$ and the qubit $|\phi\rangle_B$.

$$\frac{1}{2}a(|\phi^+\rangle_{CA} + |\phi^-\rangle_{CA})\,|0\rangle_B + \frac{1}{2}b(|\psi^+\rangle_{CA} + |\psi^-\rangle_{CA})\,|1\rangle_B \tag{20}$$

$$\frac{1}{2}a(|\phi^+\rangle_{CA} - |\phi^-\rangle_{CA})\,|0\rangle_B + \frac{1}{2}b(|\psi^+\rangle_{CA} - |\psi^-\rangle_{CA})\,|1\rangle_B \tag{21}$$

These equations can be factored into quantum and classical components. The quantum component is comprised of the maximally entangled states and the qubit state being teleported. The classical components can be regarded as the 2 bit strings that correspond to the action of quantum gates on the original pure product state $|\phi\rangle_C\,|\psi^+\rangle_{AB}$. The four tensor product bases states can be seen as information transmitted classically when the four terms given above are algebraically expanded;

$$\frac{1}{2}\,|\phi^+\rangle_{CA}\,(a\,|0\rangle_B + b\,|1\rangle_B) \longrightarrow |00\rangle, \frac{1}{2}\,|\psi^-\rangle_{CA}\,(a\,|1\rangle_B + b\,|0\rangle_B) \longrightarrow |01\rangle,$$

$$\frac{1}{2}\,|\phi^-\rangle_{CA}\,(a\,|1\rangle_B - b\,|0\rangle_B) \longrightarrow |11\rangle, \frac{1}{2}\,|\psi^-\rangle_{CA}\,(a\,|0\rangle_B - b\,|1\rangle_B) \longrightarrow |10\rangle,$$

Upon observer A performing the bell measurement, observer B receives 1 of 4 classical 2 bit string and a pure state represented $QG\,|\phi\rangle_B$ where QG is the operation of 1 of 4 Quantum Gate operators on the quibit of observer B.

$$\frac{1}{2}\left|\phi^{+}\right\rangle_{CA}\begin{vmatrix}1 & 0\\ 0 & 1\end{vmatrix}\left|\phi\right\rangle_{B}+\frac{1}{2}\left|\psi^{+}\right\rangle_{CA}\begin{vmatrix}0 & 1\\ 1 & 0\end{vmatrix}\left|\phi\right\rangle_{B}+ \tag{22}$$

$$\frac{1}{2}\left|\phi^{-}\right\rangle_{CA}\begin{vmatrix}1 & 0\\ 0 & -1\end{vmatrix}\left|\phi\right\rangle_{B}+\frac{1}{2}\left|\psi^{-}\right\rangle_{CA}\begin{vmatrix}0 & 1\\ -1 & 0\end{vmatrix}\left|\phi\right\rangle_{B} \tag{23}$$

These local unitary operators correspond to Pauli Operators or "Quantum Gates" which transform one of the four maximally entangled eigenstates into the one of the other three eigenstates given by;

$$I=\begin{vmatrix}1 & 0\\ 0 & 1\end{vmatrix}\qquad Z=\begin{vmatrix}1 & 0\\ 0 & -1\end{vmatrix}\qquad X=\begin{vmatrix}0 & 1\\ 1 & 0\end{vmatrix}\qquad iY=\begin{vmatrix}0 & 1\\ -1 & 0\end{vmatrix} \tag{24}$$

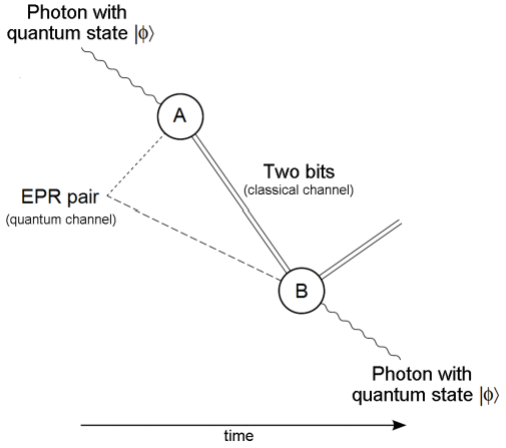

Figure 5: *A modified version of the quantum state teleportation diagram originally found in.*[20] *The transmission of 2 bit strings through the classical channel is a necessary prerequisite for the teleportation of the unknown state* $|\phi\rangle$. *The reliable teleportation of an N-state particle requires a classical channel with a capacity of* $2\log_2(N)$*bits. A channel capacity lower than this necessitates the use of superluminal transmission*[20] *which is not possible relativistically.*

These quantum gates correspond to the Quantum Phase Flip Gate(Z), the Quantum NOT(Bit Flip Gate)(X) and the Identity Gate.[1] Geometrically, these gates correspond to 180° rotations around the X, Y and Z axes[16] with the I quantum gate corresponding to the same eigenstate. Observer B can therefore perform the appropriate local unitary Quantum Gate operation to obtain the unknown state $|\phi\rangle_B$. However, due to the no cloning principle [2], $|\phi\rangle_B$ cannot be interpreted as the original unknown state $|\phi\rangle_C$ before the Bell measurement by observer A.

Rather the original qubit state $|\phi\rangle_C$ is destroyed with observer B obtaining a suitable replacement $|\phi\rangle_B$ at a later period.[20] While the measurements by different observers are not simultaneous, the possibility of observer B obtaining a qubit state is possible due to the commutation of the quantum gate matrices for different observers on the projected states. The teleportation of states can occur over arbitrarily long distances with the only prerequisite being that the classical information derived from the process must be broadcast to the possible locations of observer B[20] as seen in Fig.5.

More recently, the practical application of the teleportation of states over arbitrary distances has become apparent with the realization of teleportation across metropolitan fibre networks[22]. By employing the use of superconducting nanowire photon detectors (SNSPDs), research groups in Calgary[23] and Heifei[24] teleported photons across several kilometres of optical fibre networks with a Fidelity(F) of (78 ± 1)% [23] and (77 ± 1)% [24] respectively with F ≥ 2/3 corresponding to transmission of information by entanglement. However, a maximum of 2 eigenstates out of a possible 4 were detected in the Heifei experiment leading to an upper bound on the probability of success of 50% at a low rate of 2 photons per hour[22,24]. The parameters of the Calgary experiment were such that the Bell state corresponding to no correction (i.e, corresponding to the action of the Identity Quantum Gate by Observer B) was set. This lead to an upper bound on the probability of success being at most 25% at a faster teleportation rate of 17 photons per hour[22,23].

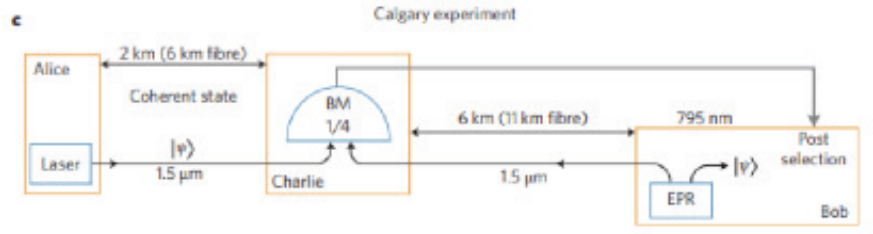

Figure 6: *A partial schematic of the Calgary quantum teleportation experiment. In this case BM 1/4 represents Bell Measurement equipment distinguishing 1 of 4 Bell states with the maximum probability of success being 25%. The curved arrows represent the transmission of photons.The grey lines represent the communication of the performance of a local unitary operation to observer B (Bob). The state* $|\psi\rangle$ *represents photon states. Adapted from* [22]

The realization of a Quantum Internet[25] is dependent on the extension on the range of teleportation. Ground-to-satellite uplink employs the use of satellite platforms and space based links which can connect remote points as well as reduce losses through quantum channels as the propagation medium of teleportation is empty space. Teleportation of a state representing the polarization state of a single photon

$|\phi\rangle_1 = \alpha|H\rangle_1 + \beta|V\rangle_1$ from an observatory in Ngari, Tibet (altitude of 5047m) to the Micius Satellite with the ground to satellite distance varying from 500km to 1400km[26] has been achieved with a average fidelity of (80 ± 1) which is also greater than the classical bound of 66% suggesting that teleportation occurred via quantum entanglement.

Conclusion

Despite the technological advances that allow the detection of entangled states today, the ideal four-state Bell measurement remains technologically impossible today for the detection of single polarization states[22]. In addition to this, the mechanisms of the Bell measurement that observer A makes on their half of a maximally entangled eigenstate and the qubit to be transported is not completely understood[27]. Nevertheless, there has been great degree of progress in quantum information theory as a result of the manipulation of entanglement. The harnessing of the information-theoretical properties of non separable EPR pairs may vary well be vital to the transition into the quantum information age. As such, the EPR Paradox and the transmission of quantum information are forever entangled.

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Karmakar Medal for Scientific Ethics

Letter from the Editor

"The key challenge is working out whom you are talking to.
Whom do you want to be moved by what you are writing?

That is whom you need to write for. If you do that,
you can work out how they want to receive a message,
not just how you want to say it."

Emmet Ryan
Guest Judge
Connected Editor, *Business Post*

It seemed fitting after five years, for the Trinity Student Scientific Review to explore new avenues for young scientific minds to make an impact on the landscape of ethical, accessible scientific communication in Volume VI.

As our authors have undergone the editorial process with their reviews, the future direction of the journal has undergone a similar iterative process in parallel. Our intention for Volume VI was not just to recruit the best contributors in our customary tradition, but optimise sharing our principles of reasoned analysis, critical thinking and scientific inquiry that underpin every review in this book, with a broader audience than ever before.

We were most fortunate to benefit from SOAPBox, broadening our horizons with regards to the power of democratising academic knowledge through open-access publication. From our first meeting onwards, we were also determined to look inwards, and harness the variety of lived experience on our editorial team to solicit contributions to the journal from students who for a plethora of reasons, may otherwise less readily see themselves represented in the identity of the scientific academic, certainly less so at an early stage of their education and career.

While scientific writing contains a plurality of objective facts, our experiences this year through the TSSR as well as learning from scientific

journalism, and public engagement through Science Gallery Dublin, fed directly into more structured editorial adjustments to our TSSR peer-review process from Volume VI. They made us increasingly cognisant of how language saturates the gaps between figures and formulae with subjectivity; how interpretations affirmed by historically biased citations may further entrench old biases in the guise of new scientific arguments and persuasion, and how despite being openly accessible, jargon makes academia forbidding and othering to engage with for many curious minds.

In concert with assessing sound logical consistency, mathematical reasoning or statistical analysis, we began evaluating contributions from the outset with greater author and reviewer guidance on inclusive language and citation quality — emphasising the importance of 'science for more than science's sake', acknowledging the globally interlinked cultural, social and commercial contexts amid which scientific inquiry is undertaken — to situate the outcomes of our critical reasoning further out into the 'real world', just a little beyond the university gates.

There remained one avenue that the TSSR had thus far left unaddressed, beyond basing our writing, review and dissemination of all fields of science in more rigorous principles of ethics and accessibility. It felt timely faced with the imminent danger of a 'post-truth' world, where insidious agendas are conveniently arrogated an air of intrinsic superiority to fracture society along the lines of 'public versus experts', to instil in TSSR a precedent of encouraging excellence among those curious to examine the discipline of scientific ethics in itself.

And so, I extend immense gratitude to every author, and particularly to inaugural winner Albert Yee, who submitted essays for consideration towards our inaugural Medal in Scientific Ethics — a space for 1,500-word contributions on matters ethical, pertaining to the application of science and technology to challenges faced by every person, in the everyday world.

This space functions differently to other TSSR review sections, in that the format is designed to be briefer and more narrative, deriving its bases of reference both within and outside of scientific and ethical theory, and evidencing its arguments with real-world applications and developments in science and technology.

This Medal exists to celebrate those who ponder beyond the core scientific research they undertake, examine what the purpose of science is in augmenting social good — be that accelerating innovation, improving communication, ensuring greater cohesion or wellbeing — and highlight where science risks falling short of its possible sustainable, ethical impact.

Our inaugural submissions focused primarily on the contentious growing role of Artificial Intelligence in Medicine, where I would like to thank our guest judge Emmet Ryan for the generosity of his time, insight and attention, towards defining how every essay of high-caliber was evaluated in the first year of this Medal.

His experience has helped model this Medal as an opportunity for authors who appreciate the need for ethical scientific understanding to percolate through all levels of academia, and become accessible to all levels of society. This will be vital to shaping and reshaping submission guidelines in future iterations of the Medal, as it seeks to mentor, solicit and reward the development of rigorous inter-disciplinary thought in Scientific Ethics within the student body of Trinity College.

In doing so the TSSR affirms that it is not just necessary to make our scientific endeavours ethical and accessible by today's standards; it is possible to encourage curiosity among those who seek to improve the ethical foundations and accessibility of scientific development and application, so they may set the standards of tomorrow.

Shubhangi Karmakar

Editor-in-Chief

Trinity Student Scientific Review, Volume VI

Artificial Intelligence- The Future of Medicine or a Dangerous, Overhyped Idea?

Albert Yee
Fourth Year
Medicine

Ever since AI-powered Watson beat 74-time—winning Ken Jennings on Jeopardy in 2011, artificial intelligence has been envisioned as a viable technology of the imminent future. AI has attracted interest in the healthcare industry because of its potential to reduce error in clinical decision-making and of allowing hospitals to handle larger patient volumes.

AI differs from available clinical decision support tools in that it can amend its diagnostic and treatment algorithms with experience; currently, CDS tools are programmed on static coding. Since the US Food and Drug Administration has approved its first AI medical device for use in 2018,[1] several questions warrant answers:

1. Could the development of AI for healthcare violate patient confidentiality?

2. Can AI adapt to new treatment guidelines?

3. Can AI exacerbate health disparities?

4. Is AI safe for healthcare? Will it require supervision?

5. Who is responsible for the decisions made by AI?

Arguments will be made in favour of continuing to develop AI for healthcare and of delaying the deployment of AI in healthcare for high-risk decisions until there are satisfactory solutions to the listed questions.

Can AI Keep the Identity of Patients in Training Sets Anonymous?

Generous amounts of patient data are supplied to technology firms for the training of AI systems[2] which is ethically permissible if the identity of patients whose data is used can be kept anonymous. However, anonymising patient data does not necessarily ensure the anonymity of patients whose data is used to train AI systems. For example, faces can be reconstructed from MRI brain scans with software that can reverse-engineer these images and be matched to photos of real people in the age of facial recognition technology.[3] That is not to say that the development of AI systems inherently violates patient confidentiality.

AI can be ethically permissible for healthcare if access to patient data offered to develop AI systems is restricted to technology firms whose use of training sets can be monitored. For instance, access to a centralised cloud where training sets can be stored would only be granted to AI systems that have been screened for the ability to deanonymize data.

Does AI Need Real Patient Data In Its Training Set?

Does the patient data in training sets need to be factual? If fictitious data can be used to train AI systems, there would be no need to monitor how technology firms protect the anonymity of patients whose data are used in training sets. Unfortunately, AI systems require real patient data to be trained. In 2018, the US Food and Drug Administration approved larotrectinib, a cancer drug purposed for solid tumours exhibiting NTRK gene fusions,[4] based on a study of 55 patients (four of whom have lung cancer). Meanwhile, IBM, the firm behind Watson, faced the hurdle of training its AI system on when it is to recommend this new drug to oncologists who treat lung cancer with data from only four patients.

Since AI systems require onerous amounts of data to formulate robust treatment algorithms, experts at Memorial Sloan Kettering Cancer Center created fictitious patients so Watson could incorporate larotrectinib in its decision-making tree. The results were disappointing when IBM learned that Watson was making "unsafe and incorrect" cancer treatment recommendations.[5,6] AI can be ethical for diseases in which treatment guidelines can be amended with studies that employ small sample sizes if AI systems can be updated with patches that help it incorporate new treatment guidelines in its decision-making process.

Can AI Discriminate?

"An AI-derived algorithm is only as good as the data with which it works". [7] AI can be poor at diagnosing melanomas in Black patients if data is not collected from a diverse population of patients during the construction of training sets [8] or underestimate the needs of Black patients if the algorithm uses a variable that can be influenced by racial disparity (e.g. healthcare spending) in calculating health

risk.[9] AI has the potential to marginalisehistorically disadvantaged populations when used for triaging and prioritising referrals. [2] However, AI can be ethical for use in healthcare if it makes decisions based on scientific variables and if successfully tested on historically disadvantaged populations.

Is AI Safe to Use Without Supervision?

AI has the potential to titrate the dose of drugs given to patients by the bedside (e.g. computerised IV pumps) [10] and in the outpatient setting (e.g. insulin pumps)[11], scenarios that are impossible for doctors to constantly supervise. The healthcare industry should learn from the death of Elaine Herzberg who was fatally struck by a self-driving car [12]and realise that AI is not ready for use without supervision. Hence the scope of AI in healthcare should be limited to circumstances in which physicians can supervise its use unless medical device makers are willing to take liability over their AI products.

Is AI Accurate?

The angle at which a photo of a skin lesion is taken can influence whether AI decides it is benign or malignant.[13] Changing word choice on a clinical note with synonyms can alter the diagnosis that AI makes or the risk of opioid abuse that AI calculates for a patient. [14] AI should give the same result regardless of the multiple ways the same input can be entered before it can be considered ethical for use in healthcare.

AI can potentially lose accuracy if there is a mismatch between training and operational data.[2] For instance, when disease patterns changed, an AI system that predicts the risk of acute kidney injury was more likely to issue false positives. [15]AI can be ethical for use in healthcare if it can detect changes in epidemiological trends and place more weight on recent data so it can adjust its decision-making tree as necessary.

As AI learns from experience, its decision-making tree can change over time. Diagnoses made by an AI system in its early days of use should be reproducible for a similar case that the AI system could see in its later days of use if AI is to be ethical for use in healthcare.

Doctors should know how AI reaches its conclusions to have confidence to accept the decision made by AI. AI systems that analyse medical imaging already show doctors how they reach their decision by outlining the lesion from which the diagnosis was derived. [16] However, outside medical imaging, AI systems are less transparent in showing doctors how conclusions are reached because technology firms fear that would require that they publicise proprietary source-code. Despite that, it is wise for technology firms to show the gist of how their AI systems reach conclusions in order to garner consumer confidence when marketing these products.

Who is Responsible for Clinical Decisions Made by AI?

Doctors are liable for any errors that stem from the use of AI under the policy recently unveiled by the AMA [17] since it is intended to complement, not replace, doctor expertise. [18] Yet doctors risk becoming complacent with the use of AI by not questioning its accuracy when required. One study proposes that AI systems indicate its degree of confidence in uncertain clinical scenarios so doctors can be alerted to question the accuracy of the AI system being used.[19] A group of authors recommend that AI systems be able to err on the side of caution when faced with ambiguous cases that could carry poor prognoses if left untreated. [2]

However, there is a lack of measures to safeguard against complacency when false negative diagnoses are given by AI systems [20] or when doctors ignore evidence that contradicts the diagnosis given by AI systems because it confirms what they suspected. [21] Doctors are prone to automation bias regardless of their level of experience [22]; ideally, AI should be highly sensitive and highly specific for its ethical use in healthcare because of the enormous amount of trust that doctors will place in it.

Conclusion

Thanks to advances in deep learning, AI has re-entered popular discourse and is being hailed in the medical community as a clinical decision support tool that can reduce error and help hospitals handle larger patient volumes. AI should not be banned from use in healthcare, rather it should pass the following six tests before being authorised for use:

1. AI be used under doctor supervision. Moreover, doctors will place a lot of trust on AI when it comes to fruition. AI has the potential to augment doctors' ability to diagnose but let it not replace the doctor's intelligence as competence is required to supervise the use of this technology.

2. AI give accurate recommendations (regardless of epidemiological trends or the ways that identical inputs can be entered), its degree of confidence in its recommendations and be able to err on the side of caution in ambiguous cases

3. AI not sacrifice the privacy of patients used in its training sets

4. AI explain its diagnoses

5. AI not lose accuracy on historically disadvantaged populations

6. The doctor or the medical device maker be responsible for the decisions rendered by AI

These tests form the requirements laid out by the European Commission last April for the ethical use of AI.[23]

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