Institution: University of Leicester		
Unit of Assessment: 8		
Title of case study: NanoMIPs: Transforming Diagnostics with Molecularly Imprinted Polymer Nanoparticles		
Period when the underpinning research was undertaken: 2013–2019		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s): 1) Prof Sergey Piletsky 2) Dr Elena Piletska 3) Dr Kal Karim	**Role(s) (e.g. job title):** 1) Professor in Bioanalytical Chemistry 2) Lecturer in Bioanalytical Chemistry 3) Senior Lecturer in Organic and Computational Chemistry	**Period(s) employed by submitting HEI:** 1) 2013–Present 2) 2013–Present 3) 2013–Present
Period when the claimed impact occurred: 2013–2020		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact

Conventional diagnostics and drug development are costly, complex and time consuming, often requiring the use of animals. Antibodies are vital in this process but are frequently unreliable, fragile and costly to manufacture. Research at the University of Leicester (UoL), led by Prof. Sergey Piletsky, has addressed these issues with the use of Molecularly Imprinted Polymer nanoparticles (NanoMIPs).

NanoMIPs are a versatile, reliable and cost-effective alternative to antibodies, do not require animals for their production, and have improved development time by up to 600%. This research has been commercialised via a spinout company, MIP Diagnostics Limited, which provides a range of custom synthetics to world-leading pharmaceutical companies. Patents and licences have been issued to advance a range of gene therapies to treat rare diseases and development of new diagnostic tools. Impact from this research is both **economic** (GBP5,100,000 investment and secured employment in high tech area for 17 researchers and engineers), and **increased capability** (development of manufacturing facilities for addressing needs of the diagnostic industry in new materials and reagents as a replacement for antibodies).

2. Underpinning research

Traditional antibodies are vital tools for drug discovery and point-of care diagnostics, but they are expensive and time consuming to produce and can be unreliable due to their fragility and propensity to biodegrade. NanoMIPs solve these challenges as they are robust and created by a highly predictable and reproducible process using the target molecule as a template **[P1, P2]**. A MIP binder with affinity and specificity similar to the best monoclonal antibodies can be produced cost-effectively in under two weeks compared to several months for a traditional antibody.

Since 2014, UoL researchers have advanced the practical applications of nanoMIPs, produced using a solid-phase approach, across diverse fields including: molecular diagnostics; drug discovery, development and delivery; petrochemicals; and law enforcement. Their research can be broadly categorised in two distinct areas:

The Use of MIP Nanoparticles in Diagnostics

Prior to Leicester Biotechnology Group research at UoL, nanoMIP utility was limited by the high binding site heterogeneity, the presence of residual template and the need for complex production

methods. These issues were solved by the introduction of a solid-phase approach to synthesis **[R1]**. The resulting nanoMIPs were virtually free of template and showed a high ("monoclonal") affinity for the target molecule. This result enabled the first successful use of nanoMIPs in assays and sensors without a cold chain supply **[R2, P3]**, providing similar affinity and selectivity to monoclonal antibodies **[R3]**. Being able to utilise nanoMIPs in assays represented a significant step-change in the field and within three years the UoL team developed novel nanoMIP-based assays for biomarkers, drugs and proteins with demonstrable superiority to existing products (e.g. ELISA) in terms of user-friendliness of the protocol, and its speed **[R3]**.

The discovery was also significant in that it provided a method of production that was not reliant on animals because the use of synthetics negates the requirement for an active immune response.

Targeting Cells Using nanoMIPs

UoL research provides significant advances in the potential use of nanoMIPs in both *in vitro* and *in vivo* therapeutic applications, as well as in bioimaging and drug discovery.

Building on their successful demonstration of the ability of nanoMIPs to safely penetrate cell membranes and thus deliver a targeted drug dosage to the patient **[R4]**, the team were able to accelerate their development for a wide range of medical conditions including:

- New nanoMIPs to target antitumor activity in both kidney and cardiovascular systems **[R5]**.
- New nanoMIPs targeting the quorum sensing system in Gram-positive and Gram-negative bacteria, which for the first time demonstrated that nanoMIPs can prevent streptococcus-induced pneumonia **[R6]**.
- Original protocols for the identification of protein epitopes that can be used to generate antibodies, aptamers and MIPs in addition to enabling snapshot imprinting of cell membranes **[P4]**.
- Novel approach for preventing the inactivation of viral vectors by Adeno-Associated Viruses and enhancement of efficiency of gene therapy **[P5]**.

3. References to the research

R1. Canfarotta F., Poma A., Guerreiro A., **Piletsky S**. (2016). *Solid-phase synthesis of molecularly imprinted nanoparticles (MIP NPs) for diagnostic applications*. Nature Protocols, 11, 443-455.

R2. Garcia-Cruz A., Cowen T., Voorhaar A., Piletska E., **Piletsky S. A.** (2020). *Molecularly imprinted nanoparticles-based assay (MINA) - detection of leukotrienes and insulin*. Analyst, 145, 4224-4232.

R3. Smolinska-Kempisty K., Guerreiro A., Canfarotta F., Caceres C., Whitcombe M., **Piletsky S**. (2016). *A comparison of the performance of molecularly imprinted polymer nanoparticles for small molecule targets and antibodies in the ELISA format*. Sci. Reports, 6, 37638.

R4. Canfarotta F., Waters A., Sadler R., McGill P., Guerreiro A., Papkovsky D., Haupt K., **Piletsky S**. (2016). *Biocompatibility and internalization of molecularly imprinted nanoparticles*. Nano Research, 9, 3463–3477.

R5. Cecchini A., Raffa V., Canfarotta F., Signore G., **Piletsky S**., MacDonald M., Cuschieri A. (2017). *In vivo recognition of human vascular endothelial growth factor by molecularly imprinted polymers*. NanoLetters, 17, 2307-2312.

R6. Motib A., Guerreiro A., Al-Bayati F., **Piletska E**., Manzoor I., Shafeeq S., Kadam A., Kuipers O., Hiller L., Cowen T., **Piletsky S**., Andrew P. W., Yesilkaya H. (2017). *Modulation of quorum*

sensing in a gram positive pathogen by linear imprinted copolymers with anti-infective properties. Angew. Chemie, Int. Ed., 56, 6555-16558.

Patent applications:
P1. Piletsky S.A., Guerreiro A., **Piletska E.V.,** Chianella I., **Karim K.,** Turner A.P.F. Preparation of soluble and colloidal imprinted polymers by living polymerization. **PCT/GB06/001986 (granted).**
P2. Piletsky S. A., Guerreiro A., Whitcombe M. J. Preparation of molecularly imprinted polymers. **UK 0921025.3, EP 2507278 A1 (granted).**
P3. Piletsky S., O. S. Ahmad, A. Garcia Cruz. Electrochemical Sensors. **GB1809276.7**.
P4. Piletsky S., Piletska E., Canfarotta F., **Karim K.,** Jones D., Norman R., Guerreiro A. Methods and kits for determining binding sites. **PCT/GB2018/050707, US20200033356 A1.**
P5. Genethon and University of Leicester Tools and method for preventing AAV neutralization by antibodies **PCT/FR2020/000173.**
P6. Piletsky S.A., Piletska O., Guerreiro A., Poma A., **Karim K., Piletsky S.** Microplates with enhanced immobilisation capabilities controlled by magnetic field. **US20150119274A1**.

4. Details of the impact

The market for research antibodies and reagents is estimated to be worth USD10,100,000,000 **[E1]**. Progress has been limited by inherent challenges including antibody fragility, expense, complexity and time required to produce **[E2]**. Recent developments in the computational design and automated synthesis of nanoMIPs by the UoL team has meant that a reliable supply of soluble nanoparticles with predetermined molecular recognition properties, sub-nanomolar affinities, defined size and surface chemistry, is available for industrial use for the first time **[R1, P1, P2],** which is essential for **increasing industrial capabilities**. This represents significant progress, ensuring that diagnostic applications, drug discovery and the subsequent benefits to patient health outcomes can be accelerated, with creation of nanoMIPs using LBG technology taking less than two weeks on average in comparison to several months required for the development of antibodies **[R1, E2]**. These technological achievements were supported by several new patents enabling large-scale diagnostic applications **[P1–P6]**.

The emergence of nanoMIPs as an alternative to antibodies in diagnostics and in vivo applications is due primarily to the research of the UoL team **[E2]**. As NittoDenko writes:
"*The group was able to hit targets that several experienced researchers working in parallel were unable to achieve. Their use of in-silico modelling in combination with a simple, low cost, solid-phase extraction protocol was able to achieve the desired results in rapid time"* **[E3]**.

The diagnostic applications of nanoMIPs have been further supported by two new inventions:
(i) Development of a novel MIP-based assay performed in magnetic microplates (culminating in translation and adoption of assays by AstraZeneca, Thermo Fisher and other companies for the analysis of blood antigens, leukotrienes, insulin and other proteins) **[R2]**.
(ii) Discovery of a method for transforming binding events into detectable signals using redox-active nanoMIPs **[P3]**. This process enables integration of nanoMIPs into a broad range of sensor devices, using advanced manufacturing tools such as screen and ink-jet printers. This technology is licensed to MIP Diagnostics Limited (a UoL spinout company) with the intention of developing commercial products for a leading diagnostic company and tapping into the huge global biosensor market valued at USD18,600,000,000 **[E1]**.

Commercialisation of nanoMIP technology developed by the UoL team has been accomplished primarily via MIP Diagnostics Limited **[E2, E4]**. MIP Diagnostics Limited, founded in 2013 with the intention to commercialise technology developed by the UoL team, has continued to grow over the last seven years. The original GBP1,500,000 funding came from Mercia Fund Managers. In making their decision to support the company, Mercia concluded that *"recent research has highlighted the benefits of MIPs and blue-chip companies are starting to recognise their potential, in particular in applications where antibodies cannot be used. This funding will allow MIP Diagnostics to make a step change in the business, by ... developing its own products to take advantage of the huge global market"* **[E2]**.

At present, the company employs a team of 17 highly skilled scientists and engineers, currently generates over GBP750,000 in revenue per year, and has secured repeat business with a range of leading global blue-chip clients **[E4]**. In 2018, MIP Diagnostics Limited was recognised as the *"Best MIP Commercialisation Specialists in the UK"* **[E5]**. In July 2020, MIP Diagnostics Limited completed a GBP5,100,000 funding round to accelerate its global expansion. The co-investment has come from Mercia Asset Management, a founding investor and the largest shareholder in the company, and was led by Downing Ventures, BGF and Calculus Capital. This development enabled MIP Diagnostics Limited to grow, expand and diversify its product portfolio and capability as a result of the improved cost-efficiency provided by the use of synthetic binders rather than natural antibodies. *"To date, 5 licenses have been issued, primarily to the in vitro diagnostics industry but also to some bioprocessing companies"* **[E4]**. This is the second time that Mercia chose to invest in MIP Diagnostics, indicating their trust and confidence in the technology, and also in the future commercial success of the company.

The collaboration with the UoL team and MIP Diagnostics has continued in recent years, diversifying from diagnostics to other areas, as recognised by the company: *"The research from Leicester has also led to the company building further expertise in epitope mapping* [experimentally identifying the binding site of an antibody on its target antigen (usually, on a protein)]*, and sensor technology which has increased our business capabilities to pursue our strategic objectives*" **[E5]**.

MIP Diagnostics is now fulfilling an increasing number of requests from major diagnostic and pharmaceutical companies for custom nanoMIP synthesis to be used in a diverse range of biomedical applications including biomedical monitoring and diagnostics. NanoMIP technology is now being applied to numerous diagnostic fields including cardiac disease, cancer and autoimmune conditions, providing high temperature resistance and excellent selectivity over traditional bioreceptors in a biosensor platform **[P3]**. Since its establishment, MIP Diagnostics Limited have successfully developed over 50 publicly available technologies with targets stretching from cocaine, salbutamol and copper to haemoglobin and insulin. Other examples involve development of a troponin I assay based on nanoMIP suitable for cardiovascular disease monitoring and an interference suppression solution for Veravas **[E6]**.

This considerable success is set to continue, with planned expansion into the Oil and Gas industries as well as Security/Law Enforcement where the value and utility of nanoMIP technology is emerging. The influence on national and international law enforcement practice is via the *BorderSens* project for the European Union which, by 2023, will result in the creation of a portable, wireless device with the capacity to quickly test for illegal drugs and be utilised by European Border Control in the fight against organised crime. The creation of this device is dependent upon field-leading UoL research into nanoMIPs which are critical in its operation **[E8]**.

The non-diagnostic applications of MIP technology that have an impact on healthcare evolved from collaboration with Genethon. Genethon is a non-profit biotherapy R&D organization created and funded by the Association Française Contre les Myopathies. Its mission is to design gene therapy products for rare diseases. Its work with the UoL MIPs team has been supported as part of a GBP6,000,000 EU funding (CureCN grant) **[E9]**. The technology developed by UoL in partnership with Genethon **[P3]** is being used within the Immunology and Liver disease team at Genethon to address pre-existing humoral immunity against Adeno-Associated Virus Vector, by removing circulating anti-AAV neutralizing antibodies. Genethon has acknowledged that this technology is "*a very useful tool in the gene therapy field*" **[E10]**.

The COVID-19 global pandemic has led to an unprecedented demand for rapid and accurate diagnostic solutions, to enable mass testing and screening, antigen-detecting rapid diagnostics tests are quickly being developed. In April 2020, MIP Diagnostics announced a new venture with Stream Bio, "*a company that develops and manufactures a range of transformative bioimaging molecular probes, to develop a COVID-19 antigen reagent for assays, a lateral flow Rapid Diagnostic Test and an 'ELISA' type assay for high throughput screening or mass testing*" **[E7]**. MIP Diagnostics has developed a SARS-COV-2 nanoMIP demonstrating a high sensitivity and high affinity to the COVID-19 spike protein and cultured virus. MIP Diagnostics have partnered with Stream Bio to bring a ready conjugated SARS-COV-2 nanoMIP for fluorescent-based assays to market **[E11]**.

5. Sources to corroborate the impact

E1. https://www.marketsandmarkets.com/Market-Reports/research-antibodies-reagents-market-94212793.html, Ugalmugale, S., Swain, R. (Global Market insights Inc., 2018).
E2. Mercia Investments Press Releases:
2016: https://www.mercia.co.uk/plastic-antibody-developer-secures-customers-after/
2017: https://www.mercia.co.uk/news-media/news/2017/jun/28/plastic-antibody-developer-strengthens-board-follo/
2018: https://www.mercia.co.uk/synthetic-antibody-firm-secures-series-investment/
E3. NittoDenko Testimony.
E4. Testimony from MIP Diagnostics.
E5. Biotechnology Awards 2018: Best MIP Commercialisation Specialists in the UK
E6. List of Developed Technologies Linked to LG Publications from MIP Diagnostics: https://www.mip-dx.com/our-publications
E7. MIP Diagnostics News: https://www.mip-dx.com/news
E8. Grant agreement ID 833787: https://cordis.europa.eu/project/id/833787, and https://bordersens.eu/
E9. Grant agreement ID 755225: https://www.curecn.eu/
E10. Genethon testimonial.
E11. MIP Diagnostic Covid19: https://www.mip-dx.com/covid19-nanomip