PHARMASEL-PROSPERITY NEWSLETTER

PharmaSEL Prosperity

A bimonthly newsletter

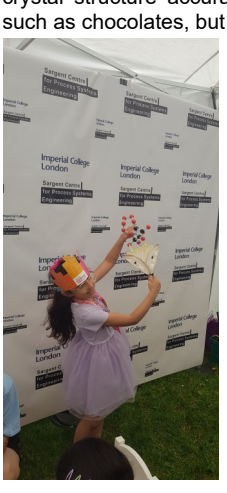
ISSUE 7 NOVEMBER 2022

In this issue:

- *Angela Lonergan and Jana Pierron* share their experiences of the administrative and community outreach aspects of the project.
- *Research from the Heng group* on the usage of crystallisation as a potential purification process for longer-chain peptides.
- *Kostas Katsoulas*, a PhD student in Work Package 3, shares his background and perspective on the partnership.
- A glance at a *comprehensive review* on strategies for improving peptide stability and delivery
- A highlight of our most *recent publications*.

Project Manager Perspective: Angela Lonergan & Jana Pierron

Like other research projects, the PharmaSEL-Prosperity Programme involves lots of moving parts — including planning, budgeting, coordinating, managing resources, and much more — that all need to be managed to make sure that the project stays on track. One of the events that we helped co-ordinate in June this year was the project's participation in the annual Great Exhibition Road Festival. This annual celebration of science and the arts is a collaboration between cultural and research institutions based around South Kensington including Imperial College London, the Royal Albert Hall, Science Museum, V&A, Natural History Museum. It is an incredible opportunity for researchers to engage people with their research in fun and creative ways. Via an interactive hands on display, PharmaSEL-Prosperity researchers demonstrated the importance of crystal structure prediction by comparing the crystal structure of tempered vs non-tempered chocolate. Festival visitors were shown how melted chocolate is made up of a network of unstable crystals, while tempered chocolate is composed of a network of stable crystals thereby affecting both the appearance and texture of the chocolate. Young visitors were then encouraged to play with computer programs and hands on activities which demonstrated the importance of modelling in predicting crystal structure accurately for producing products with desirable qualities which contain crystals such as chocolates, but also medicines.



Angela Lonergan (Project Manager) & Jana Pierron (Sargent Centre Manager)
Department of Chemical Engineering, Imperial College London
j.pierron@imperial.ac.uk
a.lonergan@imperial.ac.uk

Research Feature: Towards crystallisation as a purification process for peptides (Hamish Mitchell, WP2)

Aims and Methodologies:

Within WP2, I work alongside Dr Mingxia Guo and Mr Enshu Liang (as well as others in the Heng Research Group) primarily on experimental solubility determination and the crystallisation of peptides, varying from 2-40 amino acids in length. My work focuses specifically on the application of crystallisation to longer chain peptides. Due to the level of accuracy and repetitive nature of these crystallisations, part of my research focuses on the usage of automated liquid handling robots to expedite the process.

The main challenge for the crystallisation of long-chain peptides is their low crystallisability – due to the length of the peptide chain, the molecules are not only large but also very flexible. Peptides also occupy a unique space in the physicochemical space between small molecules and proteins, and incur distinct phenomena such as gelation, which is undesirable from a process development perspective. As such, the first step in peptide crystallisation is the establishment of solution conditions (involving factors such as peptide concentration, pH, precipitant concentration, and temperature) that lead to the formation of crystals and limit gelation. From here, the aim is then to scale-up crystallisation to process-relevant volumes.

Sample Case Study:

My research is primarily focused on a model peptide provided by Lilly, which contains 39 amino acid residues. Many of these amino acids are non-proteinogenic (i.e. non-standard) and the chain is also acylated to improve bioavailability. Due to the high number of uncommon residues, initial searches using the Protein Data Bank did not yield any similar results which had been crystallised. As such, initial work focused on using commercially available sparse matrix screening methods at the 200 nL scale. Sparse matrices aim to cover as many crystallisation conditions as possible, and as such multiple vapour diffusion screens were tested over a range of peptide concentrations. After a few weeks, one of these screens led to a successful 'hit' with the appearance of crystals alongside gelation. These conditions were then optimised via fine-tuning of the crystallisation parameters, namely the buffer pH, precipitant concentration, and salt concentration, and were also scaled from 200 nL to 2 µL. This further optimisation led to much higher nucleation rates for one of the conditions tested and reduced degrees of gelation. These crystals were subsequently harvested and have been sent off for single crystal X-ray diffraction to verify that they are indeed composed of peptide.

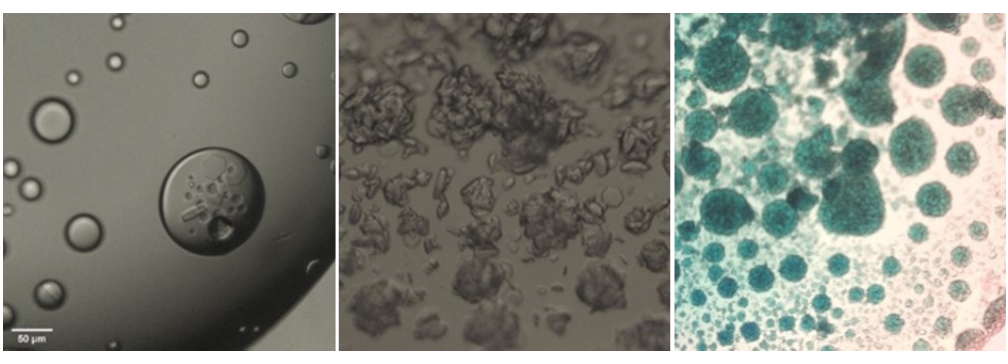


Figure 1: Left to right: Initial crystals obtained from sparse matrix screening; crystals generated from optimisation of conditions and scaleup; Crystals stained with methylene blue dye to rule out the presence of salt crystals.

Relevance to Lilly:

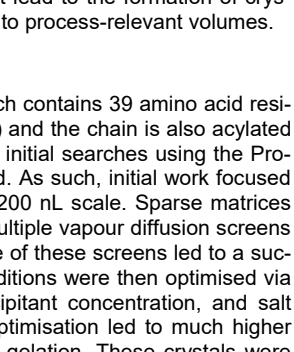
Crystallisation is of interest to the partnership as it may offer a viable alternative to industrially standard chromatography-based purification steps, which suffers from expensive resin costs and high solvent usage. Crystallisation has the potential to be both more economically and environmentally efficient, requiring both milder process conditions and lower solvent volumes. As such, the successful crystallisation of longer peptides at process-relevant timescales and volumes will be beneficial for the adoption of crystallisation as a purification or polishing step during peptide drug development.

Future Work:

Beyond the immediate future (which will hopefully be focused on structure determination for the peptide via single crystal X-ray diffraction), my future work will focus on continuing the optimisation of process conditions, followed by the scale-up from microlitre to millilitre scales. Initial studies will focus on the transition from vapour diffusion experiments, which are commonplace in crystallography studies but very difficult to scale-up, to microbatch experiments. These microbatches will offer a more process-relevant system of study, from which further scale-up can be performed. There is also the potential for the data generated from these optimisations (in the form of images) to be used to train a classifying neural network, which will expedite experimental studies by assisting with time-consuming drop classification, which is currently done manually.

Hamish Mitchell
Research Postgraduate
Department of Chemical Engineering
hamish.mitchell16@imperial.ac.uk

Researcher Spotlight: Konstantinos (Kostas) Katsoulas, WP3



Kostas holds an MEng in Chemical Engineering from the Aristotle University of Thessaloniki, Greece. His dissertation thesis focused on the modelling and optimisation of the Lyophilisation (freeze drying) process. He was also involved in projects with the Laboratory of Food Industry Technology of the same institution.

In the endeavour of expanding the areas of his expertise as well as contributing to the field of Process Systems Engineering, Kostas is currently pursuing a PhD at UCL. Specifically, he conducts research on the feasibility and in-silico optimisation of High Performance Liquid Chromatography (HPLC) for WP3 under the supervision of Prof. Eva Sorensen, Prof. Luca Mazzei and Dr Max Besenhard.

The ultimate goal of the project is the development of a framework for improved decision-making during process development in an industrial scale. This framework will be constructed capitalising on first-principle models as well as data-driven methods. Currently, the work conducted involves the in-silico investigation of the simultaneous manipulation of operating conditions such as temperature, pH, and solvent composition for the purification of small ionisable molecules. Studying the interaction between these effects and ionisable molecules retention will provide insight on how separation performance can be improved. The insight from small ionisable molecules will contribute to modelling these interactions for peptides. Thus, future work will involve modelling chromatographic separations of peptides at varying conditions of temperature, pH, and solvent composition, and consequently optimisation of the design and operation of peptide purifications.

Konstantinos (Kostas) Katsoulas
PhD Student
Department of Chemical Engineering
konstantinos.katsoulas.20@ucl.ac.uk

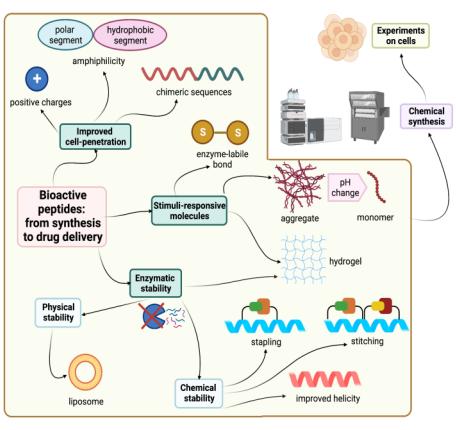
Featured Publication

Strategies for Improving Peptide Stability and Delivery

Othman Al Musaimi, Lucia Lombardi, Daryl R. Williams, Fernando Albericio

DOI: 10.3390/ph15101283

Abstract



Peptides play an important role in many fields, including immunology, medical diagnostics, and drug discovery, due to their high specificity and positive safety profile. However, for their delivery as active pharmaceutical ingredients, delivery vectors, or diagnostic imaging molecules, they suffer from two serious shortcomings: their poor metabolic stability and short half-life. Major research efforts are being invested to tackle those drawbacks, where structural modifications and novel delivery tactics have been developed to boost their ability to reach their targets as fully functional species. The benefit of selected technologies for enhancing the resistance of peptides against enzymatic degradation pathways and maximizing their therapeutic impact are also reviewed. Special note of cell-penetrating peptides as delivery vectors, as well as stapled modified peptides, which have demonstrated superior stability from their parent peptides, are reported.

Publications

1. Muhieddine, M.H., S. Jonuzaj, S.K. Viswanath, A. Armstrong, A. Galindo, and C.S. Adjiman, *Model-based solvent selection for integrated synthesis, crystallisation and isolation processes.* Comput. Aided Chem. Eng., 2022. **51**: p. 601-606, 10.1016/B978-0-323-95879-0.50101-6
2. Al Musaimi, O., O.M.M. Valenzo, and D.R. Williams, *Prediction of peptides retention behavior in reversed-phase liquid chromatography based on their hydrophobicity*. *J. Sep. Sci.* **2022**. 10.1002/jssc.202200743
3. Al Musaimi, O., L. Lombardi, D.R. Williams, and F. Albericio, *Strategies for Improving Peptide Stability and Delivery*. *Pharmaceuticals*. **2022**; 15(10): p. 1283. 10.3390/ph15101283 2
4. Wehbe, M., A.J. Haslam, G. Jackson, and A. Galindo, *Phase behaviour and pH-solubility profile prediction of aqueous buffered solutions of ibuprofen and ketoprofen.* Fluid Ph. Equilibria, 2022. **560**: p. 113504, 10.1016/j.fluid.2022.113504
5. Sugden, I.J., D.E. Braun, D.H. Bowskill, C.S. Adjiman, and C.C. Pantelides, *Efficient Screening of Coformers for Active Pharmaceutical Ingredient Cocrystallization*. Cryst. Growth Des., 2022. **22**(7): p. 4513-4527, 10.1021/acs.cgd.2c00433

Co-Editor-in-Chief
Hamish Mitchell
PhD Student
Imperial College London
hamish.mitchell16@imperial.ac.uk

Co-Editor-in-Chief
Othman Almusaimi
Research Associate
Imperial College London
o.al-musaimi@imperial.ac.uk

Disclaimer: This research program is funded by Eli Lilly and Company, Imperial College of London, University College London and from EPSRC Grant Ref: EP/T518207/1. The contents of this newsletter are chosen and edited by researchers and students of Imperial College London. Their editorial work is independent of Eli Lilly and Company and EPSRC.

Imperial College London · UCL · Queen Mary University of London · Lilly · UKRI Engineering and Physical Sciences Research Council · Royal Academy of Engineering