PHARMASEL-PROSPERITY NEWSLETTER

A bimonthly newsletter

ISSUE 12 SEPTEMBER 2023

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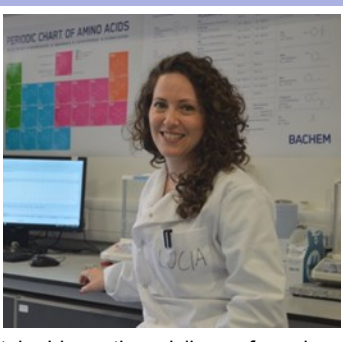
- The new co-editor-in-chief, Lucia Lombardi, *introduces herself* and gives her perspective on the Prosperity Partnership.
- *Highlighted research* from WP3 on the development of digital twins for HPLC methods.
- *Researcher profile:* Maxim Bird (WP4), shares his background and role within the project.
- *A featured publication* furthering the understanding of the hydrophobicity of protected amino acids and protecting groups.
- A reminder about *upcoming events* for PharmaSEL-Prosperity events: the Pharmaceutical Manufacturing Forum and Steering Board meeting.

Co-Editor-in-Chief Perspective: Lucia Lombardi



Greetings, everyone. I am delighted to express my gratitude for the invitation to continue overseeing this newsletter, which shines a spotlight on the remarkable accomplishments of individuals within the PharmaSEL-Prosperity Partnership.

Over the past two-plus years, I have been immersed in an innovative work package (WP4) here at Imperial, working in close collaboration with Lilly. Serving as co-editor-in-chief alongside Hamish for this newsletter fills me with immense joy. I want to acknowledge the dedication and commitment that Othman brought to previous editions, and I aspire to fulfil this role with equal proficiency, ensuring that Othman's absence is felt as minimally as possible. Fortunately, I have the privilege of receiving guidance from and working alongside our many exceptional collaborators on the newsletter!

I am confident that this role will be an invaluable learning experience, enabling me to expand my professional network and delve into the captivating stories behind each research endeavour that the PharmaSEL team wishes to showcase. This journey promises to be a motivating one, deepening my passion for research.

I am enthusiastic about embarking on this new journey, getting to know each of you better, and disseminating the remarkable outcomes, research findings, or any other noteworthy accomplishments that have marked your professional journeys.

Lucia Lombardi
Postdoctoral Researcher
Department of Chemical Engineering, Imperial College London
l.lombardi@imperial.ac.uk

Research Highlight: In-silico HPLC method development (WP3.1)

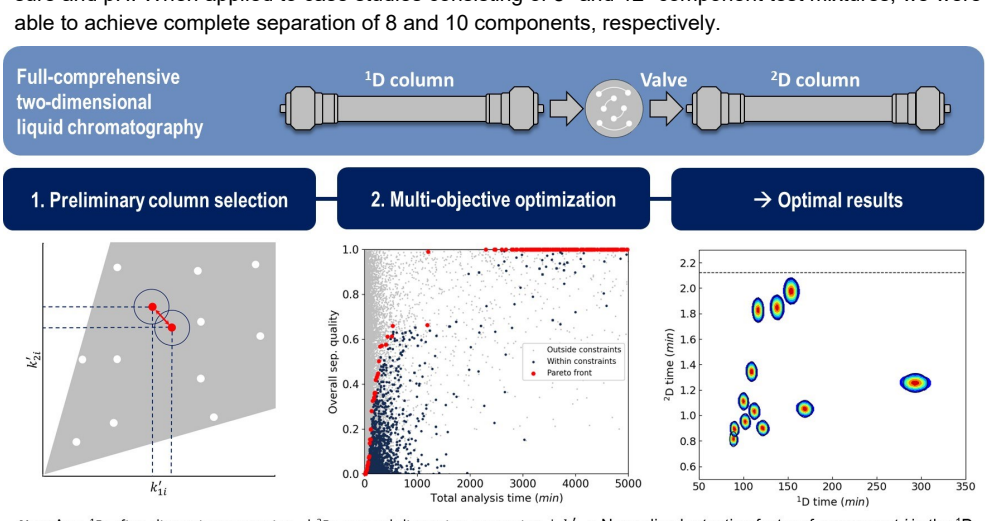
Within work package 3 of the PharmaSEL-Prosperity partnership, my colleagues and I focus on improving purification through high-performance liquid chromatography (HPLC) of active small-molecule pharmaceutical ingredients and therapeutic peptides both at analytical and preparative scales. We will deliver fundamental understanding of HPLC separations, explainable machine learning models for the consideration of novel molecules, digital twins of Lilly's experimental facilities, fundamental insight into peptide hydrophobicity, peptide solubility and injection composition, and new optimization techniques for optimal process design and operations.

My own main area of responsibility is developing new frameworks for in-silico HPLC method development, based on either mechanistic (first principles), hybrid, surrogate or shortcut models. Method development is crucial in chromatography to speed up the analysis time, guarantee the purity of the product, and determine the optimal design and operation for the process. As an example, we have designed an in-silico strategy for method development and optimization of full-comprehensive two-dimensional liquid chromatography, a technique used for resolving many components in complex mixtures.

Case study

Full-comprehensive two-dimensional liquid chromatography (LCxLC) has been developed to increase the resolving power of traditional one-dimensional, or single column, chromatographic techniques. LCxLC consists of operating two chromatographic columns in series, having either different separation mechanisms or different stationary phases. At the interface between the two columns, a modulation valve fractionates the effluent from the first column (first dimension) and sends each of these fractions to the second column (second dimension) for further separation, such that the components coeluting together from the first column are then separated in the second column. The introduction of this second column in the process leads to additional challenges when it comes to method development as two separations must be optimized simultaneously and additional constraints are required, such as on modulation time to avoid under-sampling. Due to the lack of simple and systematic ways to develop LCxLC methods, designers usually resort to heuristic approaches such as trial-and-error and one-dimension-at-a-time strategies, which not only are tedious and time-consuming, but also lead to suboptimal and sometimes even wrong designs.

To speed up the development of full-comprehensive LCxLC methods, we have developed an in-silico two-step framework. The first step is to select two columns, in our work reverse phase, having separation mechanisms as orthogonal as possible to increase the separation space and, hence, the resolving power of the method. We propose a new metric for preliminary column selection that intrinsically accounts for both local effects and band broadening. We have shown that this metric is more effective than any other metrics reported in the literature. In addition to identifying the best pairing of columns, the methodology aims to determine, through multi-objective stochastic optimization, the optimal design and best operating conditions for both columns to resolve as many components as possible in the given mixture. The novel contribution of our work is in the underlying optimization algorithm as we have introduced a shortcut, or surrogate, model that only involves algebraic equations. Computational time is therefore no longer a limiting factor and a full stochastic optimization takes only a few minutes, a huge improvement compared with the several hours required by rigorous simulations. Validation against rigorous simulation results reveals that the algebraic shortcut model is highly accurate. With this framework, we can study the impact of column design, stationary phase chemistry, flow rate, pressure and pH. When applied to case studies consisting of 8- and 12- component test mixtures, we were able to achieve complete separation of 8 and 10 components, respectively.



Notation: ^{1}D = first-dimension separation | ^{2}D = second-dimension separation | k'_{1i} = Normalized retention factor of component *i* in the ^{1}D column | k'_{2i} = Normalized retention factor of component *i* in the ^{2}D column

Relevance to Eli Lilly

Pharmaceutical and bio-pharmaceutical industries are interested in technologies and methodologies that allow them to reduce the cost of downstream purification of the API. Purification typically accounts for 50-80% of the overall pharmaceutical manufacturing cost, and normally takes place using reverse-phase liquid chromatography. Our work is therefore clearly of benefit to Eli Lilly. In-silico HPLC method development is faster and cheaper than performing expensive and time-consuming analytic chemistry in the lab, leading to a reduction of manufacturing costs, a reduction of the environmental impact (i.e., less solvent and less energy required), and, most importantly, a faster time to market. Furthermore, in a quality by design (QbD) context, successful models and simulations can help improve decision-making, support scientific and regulatory efforts, foster pharmaceutical manufacturing innovation, and improve product quality and process throughput.

Future work

In the future, we will introduce explainable machine learning models within the in-silico framework for LCxLC method development which would allow us to consider novel molecules given only their molecular structure. This model will be the first of its kind, leading to LCxLC methods without the need for experimentation.

Acknowledgments

This work would have not been possible without the support of our Eli Lilly collaborators, Jinsheng Zhou, Scott Hartzell and Dr Colin Bent, as well as my supervisors, Professor Eva Sorensen, Professor Luca Mazzei and Dr Maximiliam Besenhard; and the collaboration of fellow researchers Dian Ning Chia and Fanyi Duanmu.

Monica Tirapelle
Postdoctoral Researcher
Department of Chemical Engineering, University College London
m.tirapelle@ucl.ac.uk

Researcher Spotlight: Maxim Bird, WP4

Maxim completed a MEng in Chemical Engineering at Imperial College London in 2020, including a year at the University of California, Santa Barbara (UCSB). During his undergraduate studies, he worked as an undergraduate research assistant in the Matar Fluids Group mapping CFD data from a visualisation programme onto a virtual reality and haptic interface for application in undergraduate teaching of non-Newtonian flows. Whilst in California, he also worked on a project with the O'Malley group analysing the genome of a Butyrivibrio bacterium species from the microbiome of a San Clemente Island goat to establish its function in the degradation of lignocellulose.

Maxim is currently a PhD research student at Imperial College London in the Surfaces and Particle Engineering Laboratory (SPEL), headed by Prof Daryl Williams, having joined the group in October 2020. His work is focused on determining the chemical stability and aggregation propensity of peptide formulations in GI tract conditions via chromatographic methods for WP4. The primary goal is to establish the effectiveness of immobilised enzyme reactors (IMERs) and Self-interaction Chromatography (SIC) as alternatives to batch reactions and Dynamic light scattering (DLS) experiments, respectively. These strategies both reduce the required amount of sample, whilst also providing a consistent platform upon which varying conditions and formulations may be applied. Maxim is currently assessing the necessity of a linker chain for the immobilisation of peptides in SIC, by correlating SIC results with different resins against Size Exclusion Chromatography data using Semaglutide as an analogue in various simulated GI tract conditions. In future, he plans to apply the method for various formulations to establish its adaptability.

Maxim Bird
PhD Student
Department of Chemical Engineering, Imperial College London
maxim.bird16@imperial.ac.uk

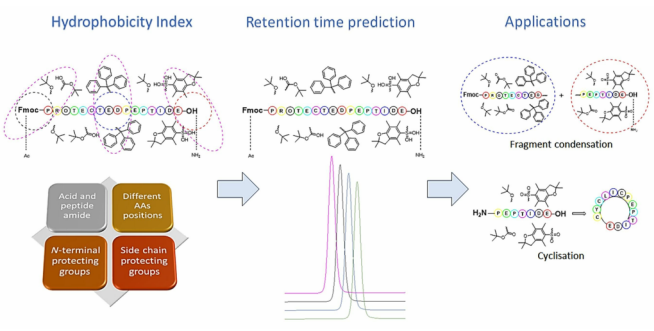
Featured Publication

Determining the Hydrophobicity Index of Protected Amino Acids and Common Protecting Groups

Varshitha Gavva, Othman Al Musaimi, Colin Bent, and Daryl R. Williams

DOI: 10.3390/separations10080456

Abstract



Peptides are in great demand in the pharmaceutical arena and a majority of these peptides contain 20 or more amino acids. They are infrequently synthesised using the fragment condensation approach. A key limitation in adopting this approach more commonly is that protected peptide fragments with high purity are often required prior to the final condensation steps. It is hypothesized that understanding the hydrophobic nature of the protected amino acids will assist with designing optimal fragment purification processes when needed. Whilst a myriad of hydrophobicity indices are reported in the literature for unprotected amino acids, the literature lacks any data regarding the protected amino acids which form the key precursor for the fragment condensation task. In this current study, hydrophobicity indices for protected amino acids with common α-amino and sidechain protecting groups were experimentally determined. Different positions for each amino acid within the peptide chain were considered, namely at the C-terminal and N-terminal as well as internal positions. These data give deep insights on the hydrophobicity of each amino acid with respect to its position in the peptide chain. The data acquired in this research facilitated the prediction of the retention time of protected peptide fragments with an uncertainty of less than ±1.5%.

Upcoming Events

Finally, a reminder that there are two upcoming PharmaSEL-Prosperity events:

- The **Pharmaceutical Manufacturing Forum** is being held on the 4th of October, and is a unique one-day event organized as part of the PharmaSEL-Prosperity Partnership. The event is open to non-PharmaSEL-Prosperity researchers, and includes invited talks and discussion from academia, industry, and regulators, as well as a poster session. Registration is **free** for PharmaSEL-Prosperity members and poster presenters, and all members are encouraged to attend.
- The **Steering Board meeting** is being held on the 5th of October, which will include overviews and updates from each of the Work Packages in the Prosperity Partnership, along with feedback and discussion from the Steering Board. There is also a session at 11:20am (BST) for an open discussion between PhD students/postdocs and the Steering Board members. Attendance and involvement, especially within this session, is very important, and all PhDs/postdocs are expected to attend.

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

Co-Editor-in-Chief
Lucia Lombardi
Postdoctoral Researcher
Imperial College London
l.lombardi@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 Imperi-