Alkynes in covalent enzyme inhibitors: down the kinetic rabbit hole

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Author Biography

Elma Mons was born on the 7th of December 1989 in Almere, the Netherlands. In 2008, she completed her pre-university VWO Gymnasium education (tracks: Nature&Technology and Nature&Health) at the Baken Park Lyceum (Almere, the Netherlands). From a young age she was exposed to – and interested in – the exact sciences and she decided to study Chemistry at the University of Amsterdam (Amsterdam, the Netherlands). In 2011, she obtained her Bachelors' degree *cum honore*: completing the honours program that allowed her to attend extracurricular courses, which she used for (neuro)pharmacology courses that were part of the biomedical curriculum to learn about drug–target interactions, and how systemic drugs know where to find their target. Next, she obtained her Masters' degree in Chemistry (track: Molecular Design, Synthesis, and Catalysis) at the University of Amsterdam (Amsterdam, the Netherlands) in 2014. The curriculum was tailored towards hands-on lab experience with two 6-month research internships. Her first internship was in the *Synthetic Organic Chemistry* group of Henk Hiemstra at the University of Amsterdam (Amsterdam, the Netherlands), where she worked on methodology for the enantioselective and organocatalytic synthesis of tetrahydroisoquinolines. She then applied for – and was awarded – multiple student travel fellowships to financially support her second internship in the *Organic Synthesis and Chemical Biology* group of Karl Gademann at the University of Basel (Basel, Switzerland). Here, she worked on the semisynthetic introduction of a bioorthogonal alkyne handle onto the natural product withanolide A for mode of action studies using chemical proteomics.

Upon returning to the Netherlands in 2014, Elma started her PhD research in the *Chemical Biology* group of Huib Ovaa at the Netherlands Cancer Institute (Amsterdam, the Netherlands). In this multidisciplinary environment at the interface of chemistry and (medical) biology, chemical tools were used to study biological processes – particularly post-translational protein modification with ubiquitin. In 2016, the Ovaa and Neefjes groups moved to the Leiden University Medical Center (Leiden, the Netherlands) where she continued her PhD research on the recently discovered covalent thiol–alkyne reaction; exploring the versatility of nonactivated alkynes as (latent) electrophiles in covalent CatK inhibitors as well as activity-based probes (ABPs) targeting the catalytic cysteine of deubiquitinating enzymes. Presented with the challenge of evaluating compounds with a covalent binding mode, she focused on the (kinetic) evaluation of (ir)reversible covalent interactions and rational optimization of experimental assay conditions. The primary findings of her research are described in this dissertation.

As of October 2021, Elma is working as a postdoctoral researcher at Leiden University (Leiden, the Netherlands) in the *Biological Chemistry* group of Nathaniel Martin, where she is the lead scientist on the preclinical development of a promising antibiotic candidate for treatment of serious infections with (drug-resistant) Gram-positive pathogens.

List of Publications

Journal Articles

1. Mons, E.; Kim, R.Q.; Mulder, M.P.C. Technologies for Direct Detection of Covalent Protein–Drug Adducts. *Pharmaceuticals* **2023**, *16*, 547. doi:10.3390/ph16040547.

2. Mons, E.; Roet, S.; Kim, R.Q.; Mulder, M.P.C. A Comprehensive Guide for Assessing Covalent Inhibition in Enzymatic Assays Illustrated with Kinetic Simulations. *Curr. Protoc.* **2022**, *2*, e419. doi:10.1002/cpz1.419.

3. Mons, E.; Kim, R.Q.; van Doodewaerd, B.R.; van Veelen, P.A.; Mulder, M.P.C.; Ovaa, H. Exploring the Versatility of the Covalent Thiol–Alkyne Reaction with Substituted Propargyl Warheads: A Deciding Role for the Cysteine Protease. *J. Am. Chem. Soc.* **2021**, *143*, 6423-6433. doi:10.1021/jacs.0c10513.

4. Mons, E.; Jansen, I.D.C.; Loboda, J.; van Doodewaerd, B.R.; Hermans, J.; Verdoes, M.; van Boeckel, C.A.A.; van Veelen, P.A.; Turk, B.; Turk, D.; Ovaa, H. The Alkyne Moiety as a Latent Electrophile in Irreversible Covalent Small Molecule Inhibitors of Cathepsin K. *J. Am. Chem. Soc.* **2019**, *141*, 3507-3514. doi:10.1021/jacs.8b11027.

5. Crane, E.A.; Heydenreuter, W.; Beck, K.R.; Strajhar, P.; Vomacka, J.; Smiesko, M.; Mons, E.; Barth, L.; Neuburger, M.; Vedani, A.; Odermatt, A.; Sieber, S.A.; Gademann, K. Profiling Withanolide A for Therapeutic Targets in Neurodegenerative Diseases. *Bioorg. Med. Chem.* **2019**, *27*, 2508-2520. doi:10.1016/j.bmc.2019.03.022.

6. Mons, E.; Wanner, M.J.; Ingemann, S.; van Maarseveen, J.H.; Hiemstra, H. Organocatalytic Enantioselective Pictet–Spengler Reactions for the Syntheses of 1-Substituted 1,2,3,4-Tetrahydroisoquinolines. *J. Org. Chem.* **2014**, *79*, 7380-7390. doi:10.1021/jo501099h.

Patents

7. Ovaa, H.; Mons, M. W. E.; van Boeckel, S. Cathepsin Inhibitors. WO2019112426A1, 13 June, 2019.

Acknowledgements

The quest for a PhD cannot be completed without the support of a *fellowship*.

I owe my gratitude to my doctoral supervisor Huib Ovaa, who gave me the scientific freedom to investigate the thiol–alkyne reaction as I saw fit (even if that meant disappearing into a kinetic rabbit hole). Unfortunately, real life did not have a 'happily ever after' but I am sure he would have been proud to see the completion of this fairytale. I am also grateful for Sjaak Neefjes and the *Ovaa trinity* (Monique Mulder, Gerbrand van der Heden van Noort, and Paul Geurink), for stepping up after Huib passed away and keeping his legacy alive.

This dissertation would have had a closer resemblance to an encyclopedia than to a storybook, printed in multiple volumes and never truly reaching completion, if it were not for the guidance of my fairy *PhD support team* Monique Mulder and Robbert Kim. Their guidance stimulated me to focus on completing projects, and stop working on abandoned projects, which helped me defeat the seemingly unconquerable mountain of unfinished projects.

I would like to thank Olaf van Tellingen (NKI) and Hermen Overkleeft (LEI) for their support as members of the *OOA doctoral committee*, and Mario van der Stelt (LEI) for supervising the OTUD1 inhibitor project. Although the majority of their work did not make it into this dissertation, my bachelor students Robin van Veen (HSL) and Jill Hermans (HSL) helped with the chemical synthesis of various building blocks and inhibitors.

Special thanks to the past and current members of the Ovaa and Neefjes research groups for the productive and friendly environment: it was normal to help each other, while also having fun in- and outside of the lab, and I consider many of you to be friends rather than former co-workers. I would like to specifically acknowledge the support of Bjorn van Doodewaerd (LUMC) in the detection of covalent adducts by top-down MS, and the work of the protein facilities for expression and purification of (custom) enzymes: Patrick Celie (NKI), Angeliki Moutsiopoulou (LUMC), and Robbert Kim (LUMC). It is impossible to overestimate the importance of the peptide facility that provided a steady supply of ubiquitin ABP precursors: Henk Hilkmann (NKI), Dris el Atmioui (NKI/LUMC), Cami Talavera (LUMC), Paul Hekking (LUMC), and Duco van Dalen (NKI). After struggling to survive our first months at the LUMC, our knight(ess) in shining armor Pauline Hoftijzer arrived and she rescued us from many stressful bureaucratic situations. Finally, we could always count on Lennert Janssen and Raymond Kooij for magically keeping the lab stocked and organized (and for handling the fall-out when the cleaning gnomes forgot about their duties after certain theme parties).

My journey into the kinetic rabbit hole of irreversible covalent inhibition would have ended prematurely without Sander Roet (NTNU): his kinetic simulation scripts – along with the long nightly discussions to explain the algebraic models in theoretical articles – were a turning point in my understanding of enzyme kinetics, and together we were able to mix the two 'flavors' of covalent inhibitor kinetics literature.[*] Finally, I would like to thank my friends and (extended) family. Many of you may not have contributed to, or even understand, the science in this dissertation but your moral support was crucial in the past decade.

[*] Literature pertaining covalent inhibitor kinetics came in two distinct flavors that were hardly ever mixed: the first flavor was a practical experimental protocol for medicinal scientists with a general equation or two for data analysis but devoid of details on assay condition restrictions or kinetic mechanisms. The second flavor was hardcore (enzyme) kinetics for theoretical scientists, with complex mathematical derivatizations of new (algebraic) models from which it is not always evident how this translates to experimental assay conditions. What was missing was a mix of these flavors: stepwise experimental protocols with the accompanying data analysis protocols, with emphasis on the connection between assay conditions and the algebraic model, and pointers on visual inspection of the (raw) data to identify violations of assumptions embedded in the algebraic models. Experimental data is often 'contaminated' by artefacts, and it consumes too much of precious assay reagents, but we were able to establish this link using kinetic simulation data.

All we have to decide is what to do
with the time that is given to us

J.R.R. Tolkien – The Fellowship of The Ring

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