Abstracts TOC Chemical Biology Symposium 24/8

Bringing the Science of Proteins into the Realm of Organic Chemistry

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Total chemical synthesis of proteins was one of the 'Grand Challenges' of 20th century synthetic organic chemistry, from the time of Emil Fischer. A general solution to this challenge was provided by the chemical ligation concept. In **chemical ligation**, *unprotected* peptide segments are condensed by reaction of *unique, mutually reactive functional group*s, one on each of the reacting segments, with formation of a *non-native bond* at the ligation site.[1] Almost overnight, chemical ligation enabled the synthesis of the long polypeptide chains found in typical protein molecules and the straightforward preparation of synthetic enzymes as homogeneous molecular species of defined chemical structure and full catalytic activity.[2] Within two years, we also introduced the **native chemical ligation** reaction, in which unprotected peptides are reacted via an initial thioester-linked unnatural reaction product that spontaneously rearranges to give a native peptide bond at the ligation site.[3]

Since then, more than a thousand syntheses of protein molecules by native chemical ligation have been reported. A variety of novel ligation reactions has been devised, both non-native and peptide bond-forming. To date, all ligation reactions are based on the original chemical ligation concept: i.e. *chemoselective reaction of unprotected peptide segments enabled by (initial) formation of an unnatural bond joining the two reacting segments.* I will describe the impact of chemical ligation on the total synthesis of protein molecules, and present selected examples of the application of organic chemistry to enzymes and other proteins enabled by modern chemical ligation methods.[4-8]

1. Constructing proteins by dovetailing unprotected synthetic peptides: backbone engineered HIV protease. M. Schnölzer, S. Kent. *Science*, **256**, 221-225 (1992).
2. Synthesis of proteins by chemical ligation of unprotected peptide segments: mirror-image enzyme molecules D- & L-HIV protease analogues. R. deLisle Milton, Saskia Milton, Martina Schnölzer, Stephen B.H. Kent, in "Techniques in Protein Chemistry IV", R. Angeletti, ed., Academic Press, New York, 1993, pp. 257-267.
3. Synthesis of proteins by native chemical ligation. Philip E. Dawson, Tom W. Muir, Ian Clark-Lewis, Stephen B.H. Kent. *Science*, **266**, 776-779 (1994).
4. Bringing the science of proteins into the realm of organic chemistry: total chemical synthesis of SEP (synthetic erythropoiesis protein). Stephen B.H. Kent. *Angew. Chem. Int. Ed.*, **52**, 11988–11996 (2013).
5. Efficient total chemical synthesis of $^{13}C{=}^{18}O$ isotopomers of human insulin for isotope-edited FTIR. B. Dhayalan, A. Fitzpatrick, K. Mandal, J. Whittaker, M. A. Weiss, A. Tokmakoff, S. B. H. Kent. *ChemBioChem,* **17**, 415-420 (2016).
6. Novel protein science enabled by total chemical synthesis. S.B.H. Kent, *Protein Science*, **28**, 313-328 (2019).
7. Chemical synthesis of an enzyme containing an artificial catalytic apparatus. Vladimir Torbeev, Stephen B.H. Kent. *Aust. J. Chem.*, **73**, 321–326 (2020).

8. A designed bivalent D-protein potently inhibits retinal vascularization and tumor growth. Paul S. Marinec, et al., *ACS Chemical Biology,* **16**, 548–556 (2021)

Good vibrations!

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Synopsis

Stimulated Raman scattering (SRS) microscopy is a new imaging technique which can be used to detect specific chemical bonds within small molecules or across whole cells;[1] giving high contrast, label-free imaging and providing intracellular quantification. Recent research in our group has focused on the design of synthetic Raman-active labels which exploit spectroscopically bioorthogonal functional groups;[2,3] the use of both dual-colour SRS and multi-modal imaging to probe intracellular drug distribution and drug resistance mechanisms;[4] and analysis of the quantitative data which SRS imaging provides to allow the kinetics of bioorthogonal reactions to be studied in the intracellular environment itself.[5]

[1] 'Recent advances in the use of stimulated Raman scattering in histopathology': M. Lee, C. S. Herrington, M. Ravindra, K. Sepp, A. Davies, A. N. Hulme, V. G. Brunton, *Analyst*, **2021**, *146*, 789-802.
[2] 'Design, Synthesis, and Analytical Evaluation of Fsp^3-Inspired Raman Probes for Cellular Imaging': C. F. Steven, M. Lee, G. S. Nichol, P. R. J. Davey, E. Chiarparin, V. G. Brunton, A. N. Hulme, *Eur. J. Org. Chem.*, **2022**, e202200393.
[3] ''Stretching the bisalkyne Raman spectral palette reveals a new electrophilic covalent motif': M. Punaha Ravindra, M. Lee, S. Dimova, C. F. Steven, M. T. J. Bluntzer, V. G. Brunton, A. N. Hulme, *Chem. Eur. J.*, **2023**, *29*, e202300953.
[4] 'Utilizing stimulated Raman scattering microscopy to study intracellular distribution of label-free ponatinib in live cells': K. Sepp, M. Lee, M. T. J. Bluntzer, G. V. Helgason, A. N. Hulme, V. G. Brunton, *J. Med. Chem.*, **2020**, *63*, 2028-2034.
[5] 'Kinetic analysis of bioorthogonal reaction mechanisms using Raman microscopy': W. J. Tipping, M. Lee, V. G. Brunton, G. C. Lloyd-Jones, A. N. Hulme, *Faraday Discussions*, **2019**, *220*, 71-85

Next-generation bioconjugates for intra- and extracellular targeting

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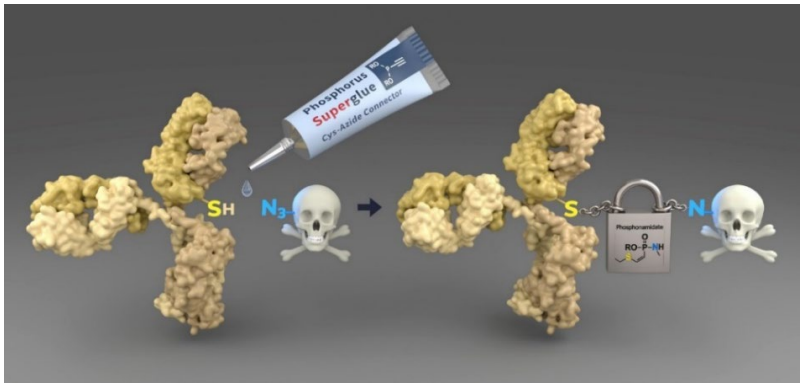
Our lab aims to identify highly selective chemical reactions for the synthesis and modification of functional peptides and proteins.[1] We apply these chemoselective or bioorthogonal reactions to study functional consequences of naturally occurring posttranslational protein modifications (PTMs), in particular phosphorylated Lys- and Cystein-peptides and proteins,[2] as well as to generate novel protein- and antibody-conjugates for pharmaceutical and medicinal applications.[1a,3]
In this presentation, I will focus on our most recent chemical development of Cys-selective P(V)-reagents including unsaturated phosphonamidates (see Scheme),[4] phosphonothiolates[5] and phosphinates.[6] We applied these reactions in a so-called **P5-labeling protocol** for the generation of new antibody-drug conjugates (ADCs)[3] and for the development of cell-permeable proteins.[7] With these next-generation drug conjugates, we show an improved efficacy compared to clinically approved cancer therapeutics on the market for the targeted delivery of pharmaceuticals.



Keywords: Bioconjugation, Bioorthogonal Reactions, Antibody-Drug Conjugates, Cell-permeable Antibodies, Drug Delivery, Staudinger Reactions

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Role of host fucose in cholera intoxication

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The surface of all human cells is covered with a dense coating of glycosylated molecules that dictates the interactions of the cells with their environment. Nowhere is this more true than within the gastrointestinal tract, where the epithelial surface is coated with a mucus layer that is a critical mediator of communication with the diverse community of microorganisms that form the gut microbiome. The intestinal epithelial mucus layer is composed primarily of heavily O-glycosylated glycoproteins, called mucins, along with N-linked glycoproteins and glycolipids. A defining characteristic of the mucus layer is the presence of the fucose monosaccharide. Fucosylation of the intestinal epithelial occurs in a interleukin 22 (IL-22)-dependent manner in response to colonization with commensal bacteria, and fucose serves as nutrient for many gut microbes. However, pathogens also take advantage of intestinal fucosylation and use recognition of fucosylated epitopes as a means to attack host cells. In my seminar, I will discuss how cholera toxin exploits cell surface fucosylation to enter and intoxicate host cells. In a colonic epithelial cell line, knockout of B3GNT5, an enzyme required for synthesis of fucosylated type 1 LacNAc structures on glycosphingolipids (GSLs), reduces cholera toxin binding but sensitizes cells to intoxication. Overexpressing B3GNT5 to generate more fucosylated type 1 GSLs confers significant protection against intoxication, indicating that fucosylated type 1 GSLs act as decoy receptors for cholera toxin. Knockout of B3GALT5, which results in increased production of fucosylated glycoproteins, significantly increases cholera toxin binding and intoxication. Knockout of B3GNT5 in B3GALT5 KO cells eliminates production of fucosylated LacNAc on GSLs but does not reduce intoxication, identifying fucosylated glycoproteins as functional receptors for cholera toxin. These findings provide insight into the molecular determinants regulating sensitivity of host cells to cholera toxin.

The prebiotic origin of the RNA nucleosides and translation

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Keywords: Prebiotic chemistry, purine & pyrimidine bases, wet-and-dry-cycles, Origin of the ribosome

Abstract: The widely accepted RNA world hypothesis suggests that life first emerged from RNA, which is able to (self)-replicate and evolve. Replication of RNA requires formation of the complementary pyrimidine-purine Watson-Crick base pairs A:U and G:C, which are a prerequisite for accurate genetic information transfer. Although prebiotic pathways to RNA building blocks have been reported, no pathway has been able to generate all four constituents of RNA simultaneously.[1, 2] We recently reported a prebiotically plausible new pathway (FaPy-pathway) that is able to generate purine nucleosides.[3] The chemistry is driven exclusively by fluctuations of physicochemical parameters such as pH, temperature and concentration. These conditions allowed in addition the parallel formation of a variety of non-canonical purine nucleosides as living molecular fossil of an early abiotic world.[4] Many of the formed non-canonical RNA building blocks are today assumed to have been part of the genetic system of the last universal common ancestor (LUCA).[5] In order to find a prebiotically plausible scenario for the parallel formation of purine and pyrimidine bases to create the fundamental Watson-Crick base pairing system, we developed new prebiotically plausible chemistry to pyrimidines. The chemistry is compatible with the purine procedures which allowed to generate all four RNA building blocks in the same geochemical environment.[6]
Next to the formation of nucleosides, the emergence of life also required amino acids and the process of translation in which RNA information encodes the formation of proteins. We were able to show that certain RNAs have the property to self-decorate with amino acids and that these amino acids can react directly attached to RNA to peptides. This so far unknown property of RNA forces us to extend the RNA world theory.[7] We postulate that an RNA-peptide world is a good candidate to explain the emergence of life.[8]

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Biophysical proteomics

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Abstract:
In order to understand cellular phenotypes, it is not sufficient to only look at RNA and protein concentration levels since many molecular processes are regulated post-translationally. Mass-spectrometry based proteomics has been instrumental in mapping thousands of post-translational modifications, (PTMs), revealing the extent and complexity of post-translational regulation. A major challenge that needs to be tackled now is the understanding of which of the hundreds of thousands of PTMs are functionally relevant and dissecting the mechanisms. Our lab has developed biophysical proteomics methodologies for systematically assessing protein thermal stability and solubility in order to map on a proteome-wide scale how these biophysical parameters change upon perturbation or due to addition of post-translational modifications. The has enabled us to detect changes in protein activities which are not accessible to standard protein concentration measurements and also to understand which PTMs have an effect on biophysical properties of proteins and are thus likely to be functionally relevant. I will present the most recent results on this topic.

Posters:

Poster 1: Thilde Andersson
Poster 2: Rajeshwari Rajeshwari
Poster 3: Valentin Duvauchelle
Poster 4: Ana Sofia Grosso
Poster 5: Elmeliani M'HAMMED
Poster 6: Josy ter Beek
Poster 7: Thomas Kieselbach
Poster 8: Norman Hoster
Poster 9: Sandra Behren
Poster 10: Thomas Norberg