Broader subject: Molecular capsules

Title: Hopping protons in supramolecular catalysis

Standfirst: Supramolecular catalysis can emulate many features of enzymatic transformations. Now, a complex proton wire mechanism — enabling the dual activation of a nucleophile and an electrophile through reciprocal proton transfer — has been shown to operate during the β-glycosylation of sugars within a self-assembled capsule.

Authors: Cally J. E. Haynes[1,*] and Larissa K. S. von Krbek[2,*]

1 Department of Chemistry, University College London, 20 Gordon Street, London WC1H 0AJ, UK
2 Kekulé-Institut für Organische Chemie and Biochemie, Rheinische Friedrich-Wilhelms-Universität Bonn, Gerhard-Domagk-Str. 1, 53121 Bonn, Germany

* email: cally.haynes@ucl.ac.uk, larissa.vonkrbek@uni-bonn.de

The supramolecular research community has long been inspired by our knowledge of the structure and function of enzymes, both in their ability to recognise substrates with a high degree of selectivity and in the complex chemical transformations that can be catalysed under mild conditions. In particular, the development of supramolecular catalysts aims to emulate the selectivity and catalytic activity of enzymes using synthetic constructs. Great progress has been made in mimicking key features of enzymes such as selective substrate recognition, reactant nanoconfinement, and the stabilisation of high energy intermediates, leading to novel, substrate-selective and stereochemically controlled reaction processes mediated by robust synthetic constructs[1].

Although nanoconfinement within supramolecular hosts can perturb the reactivity of bound substrates, more complex, long-range substrate activation mechanisms known to operate in enzymes have yet to be replicated within synthetic constructs. One such example is the proton-wire mechanism, in which protons can hop along an interconnected, hydrogen-bonded network (or “wire”) within an enzyme[2]. This can facilitate the dual activation of both a nucleophile and an electrophile (Figure 1a) and enable catalysis in isolated binding pockets. Now, a study published in *Nature Chemistry* from Konrad Tiefenbacher and colleagues has identified such a proton-wire mechanism operating within a supramolecular capsule **I** (Figure 1b)[3]. This mechanism underpins the challenging and synthetically useful β-glycosylation of

saccharides under mild conditions, taking supramolecular catalysis on an additional leap towards enzyme mimicry.

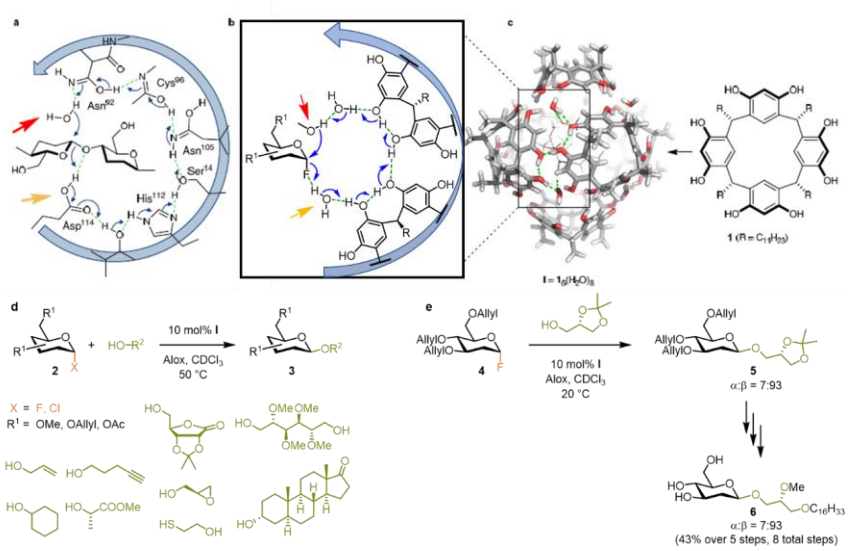

Fig. 1 | Proton wire activation mode and substrate scope. a) The active site of an inverting cellulase enzyme[8]. The nucleophile (water, indicated by a red arrow) is connected to the general acid (Asp114, indicated by a yellow arrow), which activates the electrophile (glycosidic oxygen), through a proton wire. Hydrogen bonds are shown as green dotted lines. Individual proton transfer reactions are indicated by small blue arrows and the overall transfer of protons around the active site is represented by the large blue arrow. b) The proposed, analogous proton wire activation mode within the binding pocket of supramolecular capsule **I**. The catalyst activates the nucleophile (red arrow) and electrophile (yellow arrow) synchronizing both reaction partners through seven hydrogen bonds (green dotted lines). c) Catalyst **I** self-assembles from six resorcin[4]arene units (**1**) and eight water molecules. d) Selected examples demonstrating the substrate scope of the O-glycosylation reactions mediated by catalyst I (alox, aluminium oxide). Structures of nucleophiles are shown in green and the halide displaced from the anomeric carbon is shown in orange. e) The synthesis of known anti-tumour agent 6 in eight instead of the previously reported 15 total steps9. Panels a) and c) reproduced from ref. 3, Springer Nature Ltd.

The supramolecular capsule[6] **I** is a hexamer of resorcin[4]arenes (**I** = $\mathbf{1}_6(H_2O)_8$) held together by a 60-hydrogen-bond network between the phenolic hydroxyl groups of the resorcin[4]arenes and 8 additional water molecules (Figure 1c). The Tiefenbacher and co-workers[7,8] as well as others[9] have previously demonstrated the ability of capsule **I** to catalyse various reactions such as terpene cyclisations, through confinement effects and proton-shuttle mechanisms. In the present study, they make unique use of **I**'s hydrogen bond network for the dual activation of the electrophile and nucleophile inside the capsule

(Figure 1b). In addition to the dual activation, this proton wire mechanism also dictates the diastereoselectivity of the glycosylation reaction favouring the β-glycoside for a range of substrates.

To elucidate the mechanism and understand this remarkable β-selectivity, the team conducted a broad range of control experiments and detailed mechanistic studies. Blocking the cavity of capsule **I** with another guest, disassembling the catalyst in a competitive solvent, and replacing the catalyst with the structural subunit 4-hexylbenzene-1,3-diol under the same reaction conditions prevented product formation in good selectivity or even completely. Replacing the mildly acidic capsule with Bronsted or Lewis acids led to only trace conversion at best and failed to induce diastereoselectivity. Size competition experiments with differently sized nucleophiles and one electrophile or *vice versa* led to the selective glycosylation of the smaller pair, despite their similar reactivity. These control experiments not only indicate that the reaction takes place inside capsule **I**, but also that the capsule was involved in the reaction mechanism through more than just confinement effects.

Kinetic investigations, including the secondary kinetic isotope effect, suggested a loose S_N2 mechanism to be responsible for the observed β-selectivity, resulting in inversion of configuration from the α-substituted substrates. When investigating the reaction between a glycosyl fluoride derivative **2** (Figure 1d) and methanol as the nucleophile, first-order reaction kinetics were observed for both at low concentrations. However, at methanol concentrations > 0.1 M saturation was observed, with higher methanol concentrations having a detrimental effect on conversion, which could stem from the capsule being saturated with methanol. Hence, the bulk methanol concentration ceases to affect the reaction rate inside the capsule at these higher methanol concentrations.

The involvement of a proton-wire mechanism in the reaction was elucidated by detailed molecular dynamics simulations (QM/MM enhanced sampling, quasi-classical trajectories), a proton inventory study, and control experiments with a related capsule, which does not need water molecules to complete its hydrogen-bond network. Because of the lack of water in the hydrogen-bonding rim of the related capsule, it is able to bind the reaction partners, but does not catalyse the reaction. The proton inventory study, which involves measuring the kinetic isotope effect at different deuterium/proton ratios, indicated the involvement of multiple exchangeable protons in the rate determining step which further supports the proton-wire hypothesis. From these studies, Tiefenbacher and co-workersconcluded a synchronized activation to be the most likely mechanism in this catalyst system.

In addition to the remarkable enzyme mimicry, capsule **I** also demonstrated a wide substrate scope for catalysing glycosylation reactions (Figure 1d). A high tolerance for functional groups both at the glycoside donors and nucleophiles was observed with the cavity size

being the only limitation. The reaction of glucosyl and mannosyl fluorides and chlorides was explored, showing the capsule to be a general catalyst for β-selective glycosylation reactions. Remarkably, with the use of capsule **I**, Tiefenbacher and co-workers were able to synthesize a known anti-tumour agent **6** in eight instead of the previously reported 15[5] total steps (Figure 1e), thereby further demonstrating the general applicability of their supramolecular catalysis approach.

Reaction mediation by supramolecular entities takes its inspiration from natural catalysts such as enzymes and catalytic antibodies showing great promise in unique substrate reactivities and selectivities, yet achieving conversion is a common drawback. In this work, Tiefenbacher and co-workers not only achieve supramolecular catalysis but also report a novel and complex mechanism beyond what had been demonstrated in synthetic catalysts so far with an impressive level of biomimicry.

On top of the conceptually intriguing biomimetic aspects of this work, Tiefenbacher and colleagues showed a new general way towards selective β-glycosylation reactions with a wide substrate scope which is only limited by substrate size and the apolar aprotic solvent system required by the capsule. Future investigations might feature a wider variety of protection groups on the saccharide substrates or higher boiling solvent systems for potential larger scale applications.

Given the prevalence of hydrogen bond-mediated self-assembly in supramolecular chemistry, this work can motivate further investigation and design of proton-wire mechanisms within hydrogen-bonded networks to catalyse new classes of reactions and open up new chemical space. Supramolecular chemistry has taken another leap towards biomimicry, raising the inspiring question — which other complex, nature-inspired strategies and mechanisms could be incorporated into supramolecular systems design?

References

[1] Olivo, G., Capocasa, G., Del Giudice, D., Lanzalunga, O. and Di Stefano, S. *Chem. Soc. Rev*. **50**, 7681–7724 (2021).
[2] Cukierman, S. *Biochim. Biophys. Acta Bioenerg*. **1757**, 876-885, (2006).
[3] Li, TR. *et al. Nat. Chem.* (2022). https://doi.org/10.1038/s41557-022-00981-6
[4] Nakamura, A. *et al. Sci. Adv.* **1**, e1500263, (2015).
[5] Marino-Albernas, J. R., Bittman, R., Peters, A. & Mayhew, E. *J. Med. Chem.* **39**, 3241-3247 (1996).
[6] MacGillivray, L. R. & Atwood, J. L.. *Nature* **389**, 469-472 (1997).
[7] Zhang, Q., Catti, L. & Tiefenbacher, K. *Acc. Chem. Res.* **51**, 2107-2114 (2018).
[8] Merget, S., Catti, L., Piccini, G. & Tiefenbacher, K. *J. Am. Chem. Soc.* **142**, 4400-4410 (2020).
[9] Gaeta, C. et al. *Chem. Eur. J.* **25**, 4899-4913 (2019).

The authors declare no competing interests.

Figure 1. Proton wire activation mode and substrate scope. (a) The active site of an inverting cellulase enzyme[4]. The nucleophile (water, indicated by a red arrow) is connected to the general acid (Asp114, indicated by a yellow arrow), which activates the electrophile (glycosidic oxygen), through a proton wire. Hydrogen bonds are shown as green dotted lines. Individual proton transfer reactions are indicated by small blue arrows and the overall transfer of protons around the active site is represented by the large blue arrow; (b) The proposed, analogous proton wire activation mode within the binding pocket of supramolecular capsule **I**. The catalyst activates the nucleophile (red arrow) and electrophile (yellow arrow) synchronizing both reaction partners through seven hydrogen bonds (green dotted lines). (c) Catalyst **I** self-assembles from six resorcin[4]arene units (**1**) and eight water molecules. (d) Selected examples demonstrating the substrate scope of the O-glycosylation reactions mediated by catalyst **I**. Structures of nucleophiles are shown in green and the halide displaced from the anomeric carbon is shown in orange; (e) The synthesis of known anti-tumour agent **6** in eight instead of the previously reported 15 total steps[5]. Panels a and c reproduced from ref. [3], Springer Nature Ltd.

Commented [KA1]: I've re-worded these sentences so the phrasing is more distinct from that used in the original paper.

Commented [KA2]: OK? Added this short explanation so the significance of the coloured structures is clear.

Commented [L3R2]: Yes, much better. Thank you!