deuterating reagent ultimately becomes detached from the complex, with the net effect of one hydrogen atom being replaced with a deuterium atom. This H/D exchange process may repeat multiple times in the gas phase. Other pathways that are independent of the charge site and involve the interaction of the deuterating reagent directly with the labile hydrogen atom are typically higher in energy and result in slow or no H/D exchange.

Within a non-covalent complex containing protonated amine groups, one might anticipate that a crown ether could remain permanently associated with one specific protonated amine group, thus blocking the possibility of H/D exchange at that site. However, complexes such as $[(18C6)_7{\cdot}Lys_{15}{+}7H]^{7+}$, in which each of the seven protonated amines is coordinated by a crown ether molecule, still undergo virtually complete H/D exchange. Schalley's innovative strategy provides compelling evidence that the extensive exchange of all labile hydrogens for deuteriums within the peptide/crown ether complexes is facilitated by extensive shuttling of the mobile crown ethers. In this way, all amine groups — even those initially coordinated by crown ether molecules — become available for H/D exchange (Fig. 1).

Such a process entails synchronous disruption and formation of multiple hydrogen bonds with net survival of the original non-covalent complexes despite the significant re-positioning of the crown ethers. Each 18C6 molecule migrates with an associated proton between amine groups and does not remain permanently fixed at its initial ammonium binding site or move as a neutral molecule between ammonium sites. Furthermore, comparative experiments using a conventional acid-terminated peptide and an amide-terminated peptide prove that, when possible, the peptide adopts a zwitterionic form — in which a proton has transferred from the C-terminal carboxylic acid to one of the amino groups — affording an additional protonated amine group, which is essential for the relay mechanism.

The impressive ability of H/D exchange reactions to yield insight into structures of gas-phase ions and aid in unravelling the dynamic behaviour of supramolecular assemblies is showcased in elegant fashion by Schalley's team. The implications of these findings are far-reaching. There are opportunities for significant molecular mobility within non-covalent complexes — as illustrated by the migration of crown ether molecules along a peptide — and this example establishes a precedent for potentially more elaborate movement in other types of supramolecular assemblies. Mapping this type of dynamic behaviour in biologically relevant complexes remains an intriguing challenge for the future and could lead to new insight into the mechanisms of biological function. ❐

Jennifer S. Brodbelt is in the Department of Chemistry and Biochemistry, University of Texas, Austin, Texas 78712, USA. e-mail: jbrodbelt@mail.utexas.edu

References

1. Schneider, H.-J. *Angew. Chem. Int. Ed.* **48,** 3924–3977 (2009).
2. Weimann, D. P., Winkler, H. D. F., Falenski, J. A., Koksch, B. & Schalley, C. A. *Nature Chem.* **1,** 573–577 (2009).
3. Loo, J. A. *Int. J. Mass Spectrom.* **200,** 175–186 (2000).
4. McCullough, B. J. & Gaskell, S. J. *Comb. Chem. High T. Scr.* **12,** 203–211 (2009).
5. Bohrer, B. C., Merenbloom, S. I., Koeniger, S. L., Hilderbrand, A. E. & Clemmer, D. E. *Annu. Rev. Anal. Chem.* **1,** 293–327 (2008).
6. Benesch, J. L. P., Ruotolo, B. T., Simmons, D. A. & Robinson, C. V. *Chem. Rev.* **107,** 3544–3567 (2007).
7. Wyttenbach, T. & Bowers, M. T. *J. Am. Soc. Mass Spectrom.* **10,** 9–14 (1999).
8. Campbell, S., Rodgers, M. T., Marzluff, E. M. & Beauchamp, J. L. *J. Am. Chem. Soc.* **117,** 12840–12854 (1995).

ANALYTICAL CHEMISTRY

A dendritic signal amplifier

Exponential signal amplification is achieved when a single molecule of analyte initiates a chain reaction in which a dendrimer releases a coloured 'reporter' molecule, and ultimately four further molecules of the analyte.

Magdalena A. Swiderska and Jean-Louis Reymond

New analytical techniques require the ability to detect trace amounts of a wide variety of analytes with both high selectivity and sensitivity. Immunoassays based on antibodies achieve excellent selectivities when detecting important analytes of biological origin, such as allergens, hormones or antibodies[1]. Such assays are not unusual to us and are found in everyday life — the most common being a pregnancy test, which detects the presence of a diagnostic hormone.

The sensitivity of an assay, however, depends entirely on how the antibody–antigen complex is detected. The simplest and least-sensitive method is 'immunoprecipitation', whereby a precipitate formed by antibody–antigen aggregation is detected. Enzyme-linked immunosorbent assay (ELISA) achieves a much higher sensitivity by using a microtiter-plate format, in which the antigen is identified on the plate by measuring the activity of a reporter enzyme attached to the antibody[2]. For example, the reporter enzyme may be designed to react with a chromogenic substrate, producing a coloured or fluorescent marker, only when the antibody is bound to the antigen. This allows linear amplification of the signal because each reporter enzyme can produce multiple markers. Later, the immuno-PCR technique[3] improved the sensitivity of ELISA by linking the antibody to a piece of double-stranded DNA, rather than to an enzyme. The antibody is then detected indirectly by amplifying the reporter DNA strand using the polymerase chain reaction (PCR). This exponential process allows detection of the antibody, and hence the corresponding analyte, at much lower concentrations.

In PCR, each DNA strand produces two daughter strands in each cycle of the reaction, resulting in exponential amplification. The technique is very efficient at detecting trace amounts of DNA, and has established itself as the absolute reference in terms of signal amplification for sensitive detection in bioanalytical chemistry. Indeed, PCR has exceeded expectations in analytical efficiency by such a wide margin that no one has dared to replicate its features in any other system, chemical or biochemical. Now, in *Journal of the American Chemical Society*, Sella and Shabat report[4] a new exponential signal amplifier based on a dendritic chain reaction (DCR) (Fig. 1). This achievement is the culmination of a long pursuit of dendrimer-based signal amplification, initiated a few years ago with the concept of 'self-immolation'.

Dendrimers are molecular trees with a regular structure that have unusual and

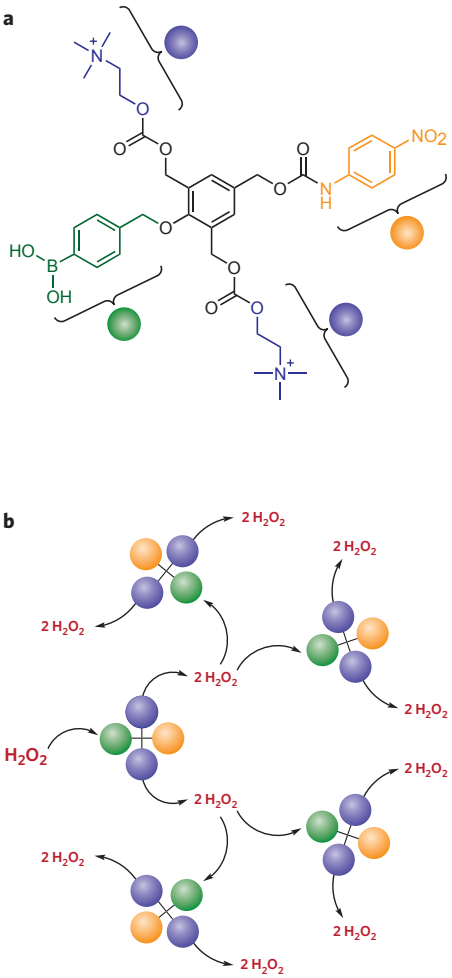

Figure 1 | DCR exponentially amplifies the signal produced in response to an analyte. **a**, Structural formula of the dendritic amplifier. The coloured circles are used to represent the dendrimer branches in **b**. **b**, Amplification cascade triggered by H_2O_2. The released choline (blue) is oxidized by molecular oxygen and the enzyme choline oxidase. This produces two molecules of H_2O_2, which then activates the next boronic acid trigger (green), causing the release of two more choline molecules, as well as a molecule of nitroaniline (yellow), which is detected colorimetrically. In just two cycles of the DCR, a single molecule of analyte has released five molecules of the coloured reporter, and a further twenty molecules of the analyte.

extremely useful macromolecular properties of interest in chemistry and medicine[5]. The concept exploited by Shabat is deceptively simple: activation of a chemical trigger at the dendrimer core starts an elegant disassembly process that leads to the release of multiple reporter molecules. This so-called self-immolative process can amplify the initial signal by a factor of two, four or eight, depending on the number of branches in the dendrimer. It is reminiscent of how a single cycle of PCR doubles the number of copies of DNA strands. Self-immolative dendrimer disassembly was first reported in 2003 by three research groups simultaneously — dendrimers were shown to release a fluorescent marker on photochemical activation[6], a coloured marker on deallylation[7], or the anti-cancer drug Paclitaxel (Taxol) on reduction of a nitro group to aniline[8]. Although these initial systems all suffered from poor aqueous solubility, this was rapidly overcome by Shabat, who both improved aqueous solubility and succeeded in triggering the self-immolative disassembly using an enzyme[9]. Shabat also reported a system that detects microgram amounts of the explosive triacetone triperoxide[10]. In this case, the reaction that triggers the disassembly is based on the oxidation of a phenyl boronic acid at the core of the dendrimer. The oxidant is hydrogen peroxide (H_2O_2), known to be present as an impurity in triacetone triperoxide — the importance of this particular trigger will rapidly become clear.

All of these dendritic amplification systems achieved only a single turnover and simply multiplied the signal, but not more. The key to PCR-like exponential signal amplification is to teach the self-immolative dendrimers a new trick: they should respond to a signal from their fellow dendrimers and begin their own disassembly, propagating the signal. In practice, if the dendrimers release not only a reporter molecule, but also more molecules of analyte, then an exponential amplification cascade would be initiated, analogous to a PCR process. Building on the H_2O_2 detector described above, this is precisely what Sella and Shabat now set out to do.

The concept is beautifully simple, but more difficult to realize because building a dendrimer that releases H_2O_2 itself is not possible. The critical enabling factor came with the realization that the dendrimer could be designed to release multiple copies of the aminoalcohol choline. Inclusion of the enzyme choline oxidase, which catalyses the oxidation of the released choline by molecular oxygen, can then produce two new molecules of H_2O_2. The self-immolative dendrimers thus carry the same phenyl boronic acid moiety at their core that was used earlier to detect the explosive, as well as choline and nitroaniline reporter groups in their branches (Fig. 1a). The self-immolation of a single dendrimer molecule triggered by H_2O_2 produces a reporter molecule, and effectively four further molecules of the trigger (Fig. 1b). The process is exponential; PCR has been matched.

From the point of view of dendrimer science, what has been achieved is impressive. The signal transduction between the branches and the core realized by the oxidation of choline to produce H_2O_2 allows the disassembly to formally propagate across successive dendrimer generations, without the need for covalent assembly of the dendrimer. This overcomes the main synthetic drawback of dendrimers as analytical tools, which is their structural complexity. In DCR, only a relatively simple first-generation dendrimer is required, giving hope that the system can be made practical and affordable. Will it be possible to outperform the PCR reaction? To do so, Shabat and his team must now address the weaknesses of their DCR reaction, in particular the spontaneous self-immolation without trigger that seems to be caused by the background hydrolysis of the carbonate linker to choline, which at present limits sensitivity. Furthermore, the DCR concept must be embedded within an analytical system, such as an antibody assay, to demonstrate its utility.

Beyond exponential amplification, another window of opportunity for improving analytical signal-amplification systems resides in autocatalytic systems that mimic biological signal transduction. Each step produces a product that acts not just as a template (as in PCR) or just as a stoichiometric trigger (as in DCR), but rather is a catalyst (or activates a catalyst) to produce more products, a concept promoted as allosteric catalysis for signal amplification[11]. Studies of autocatalysis have been popular in research on the origin of life[12], or in the generation of chiral molecules[13], but are yet to be successfully applied to synthetic signal amplification. Such a strategy could theoretically be even more powerful than PCR or DCR. ❐

Magdalena A. Swiderska and Jean-Louis Reymond are in the Department of Chemistry and Biochemistry, University of Berne, Freiestrasse 3, 3012 Berne, Switzerland.
e-mail: jean-louis.reymond@ioc.unibe.ch

References

1. Hendrickson, E. R., Hatfield Truby, T. M., Joerger, R. D., Majarian, W. R. & Ebersole, R. C. *Nucl. Acids Res.* **23,** 522–529 (1995).
2. Engvall, E., Jonsson, K. & Perlmann, P. *Biochim. Biophys. Acta.* **251,** 427–434 (1971).
3. Sano, T., Smith, C. L. & Cantor, C. R. *Science* **258,** 120–122 (1992).
4. Sella, E. & Shabat, D. *J. Am. Chem. Soc.* **131,** 9934–9936 (2009).
5. Darbre, T. & Reymond, J.-L. *Acc. Chem. Res.* **39,** 925–934 (2006).
6. Amir, R. J., Pessah, N., Shamis, M. & Shabat, D. *Angew. Chem. Int. Ed.* **42,** 4494–4499 (2003).
7. Szalai, M. L., Kevwitch, R. M. & McGrath, D. V. *J. Am. Chem. Soc.* **125,** 15688–15689 (2003).
8. de Groot, F. M. H., Albrecht, C., Koekkoek, R., Beusker, P. H. & Scheeren, H. W. *Angew. Chem. Int. Ed.* **42,** 4490–4494 (2003).
9. Shamis, M., Lode, H. N. & Shabat, D. *J. Am. Chem. Soc.* **126,** 1726–1731 (2004).
10. Sella, E. & Shabat, D. *Chem. Comm.* 5701–5703 (2008).
11. Zhu, L. & Anslyn, E. V. *Angew. Chem. Int. Ed.* **45,** 1190–1196 (2006).
12. Patzke, V. & von Kiedrowski, G. *ARKIVOC* **5,** 293–310 (2007).
13. Soai, K., Shibata, T., Marioka, H. & Choji, K. *Nature* **378,** 767–768 (1995).