Controlled membrane translocation:
a mechanism for transmembrane signal transduction and amplification in artificial vesicles

Matthew J. Langton,[a] Flore Keymeulen,[a] Maria Ciaccia,[a] Nicholas H. Williams[b] and Christopher A. Hunter[a]

[a]Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, United Kingdom;
[b]Department of Chemistry, University of Sheffield, Sheffield S3 7HF, UK Email: mjl97@cam.ac.uk

Introduction

The communication of information between the two sides of a lipid bilayer is an essential feature of membrane-compartmentalised systems. In biology, membrane-spanning proteins are responsible for transmission of chemical signals across membranes, and signal transduction is often associated with an amplified signalling cascade.[1] The ability to reproduce such processes in artificial systems has potential applications in sensing, controlled drug delivery and communication in tissue-like constructs of artificial cells. Whilst a number of synthetic systems have been developed to transport chemical signals, such as ions, across lipid membranes via pores or transporters,[2] signal transduction without this physical exchange of matter has proved to be more challenging, and amplification of such a signal has remained uniquely the domain of biology. Here we describe a new mechanism for transmitting chemical signals across membranes based on controlled translocation of a synthetic supramolecular signal transducer from one side of a lipid bilayer membrane to the other (Fig. 1). The molecular motion is reversibly controlled by an extra-vesicle pH change or binding event, and is coupled to catalyst activation on the inside of a vesicle, which leads to a signal amplification process analogous to the biological counterpart.[3,4]

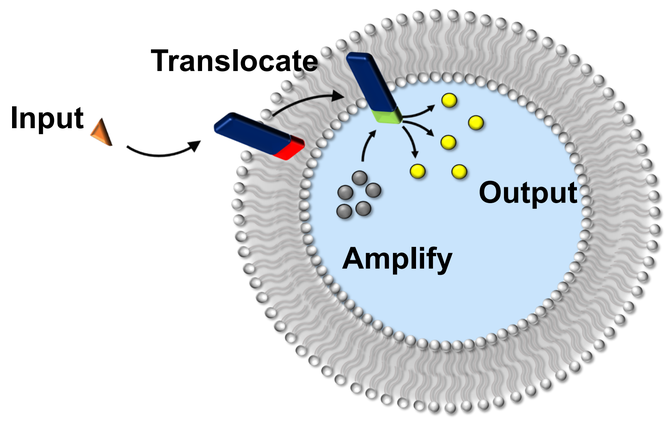

Figure 1: Signal transduction by translocation

pH-controlled signal transduction

The key to controlling membrane translocation of a synthetic signalling molecule is the differing solubility characteristics of polar and apolar head groups at lipid bilayer membrane interfaces (Fig. 2): when the head groups are polar (blue or green), they prefer to sit in the aqueous phase, and when they are non-polar (red or purple), they can enter the membrane. The input signal (hydroxide) switches the external head group from polar to non-polar, and the cofactor switches the internal head group from non-polar to polar. Concerted switching of the head group polarities drives translocation of the transducer across the bilayer. The internal head group is a pro-catalyst that is activated by cofactor binding. In the OFF state, the pro-catalyst is embedded in the membrane and is inactive (red). In the ON state, the activated catalyst is exposed to the internal aqueous phase and turns over an encapsulated substrate to generate the output signal (Fig. 3).[3]

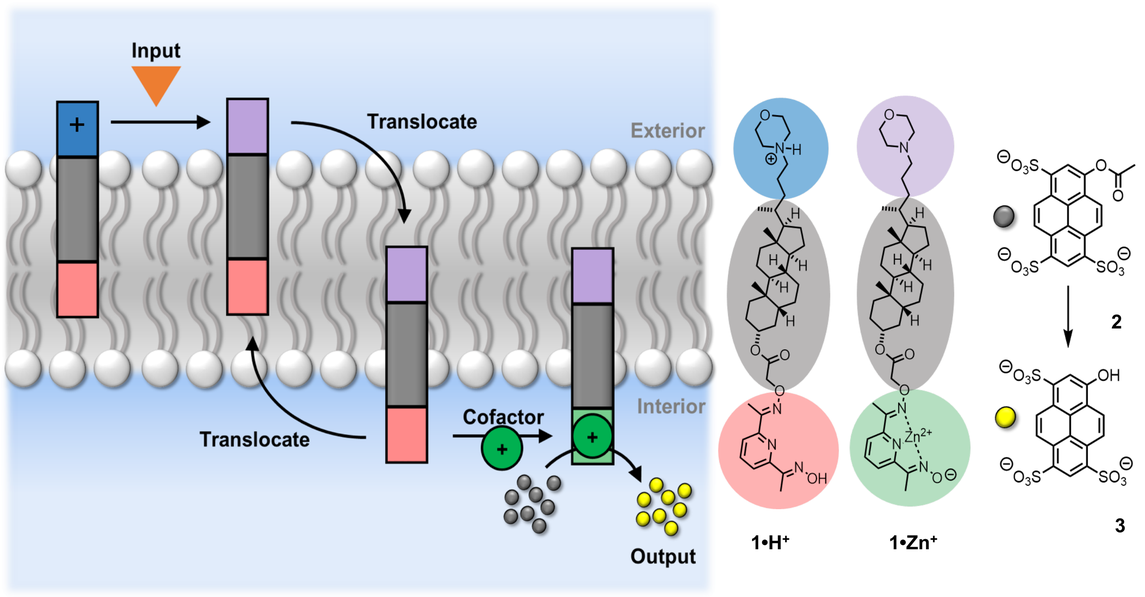

Figure 2: Signal transduction by translocation. The input signal (orange) neutralises the charged external head group (blue) allowing transducer **1** to translocate through the membrane. Subsequent binding of a metal cation cofactor from the internal solution of the vesicle to the catalytic head group (red) activates the catalyst (green), which generates the fluorescent output signal **3** (yellow) by catalysing the hydrolysis of the encapsulated substrate **2**.

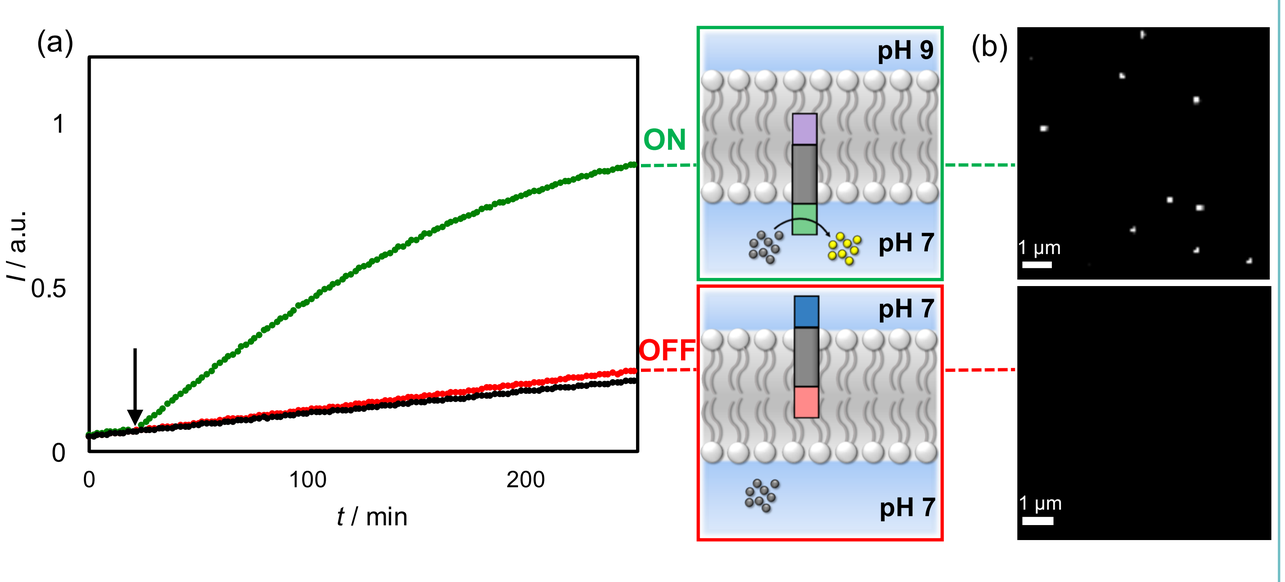

Figure 3. (a) Time dependence of the fluorescence emission intensity from **3** inside vesicles at 510 nm (exciting at 415 nm). All experiments were conducted in 200 nm DOPC/DOPE vesicles containing 2.5 mol% **1**, 250 μM **2**, 250 μM $ZnCl_2$ and 100 mM HEPES buffer at pH 7. Red data: pH 7, green data: initially pH 7, raised to external pH of 9 (arrow). Black data: control in absence of **1**. (b) Total internal reflection fluorescence microscopy (TIRFM) images of vesicles composed of lipids with 2.5 mol% **1** under the same conditions.

Amplification and reversibility

Signal amplification in biology is achieved by coupling the signal recognition process by the transmembrane receptor to an enzyme catalysed reaction. Here, the external signal turns on catalysts on the inside of the vesicle, which can turns over multiple substrate molecules and provides a mechanism for amplification (Fig. 4a). The signaling process is fully reversible and can be switched on and off by raising and lowering the pH of the external solution, which generates reciprocating translocation of the transducer across the lipid bilayer. Fig. 4b shows two cycles of this process.

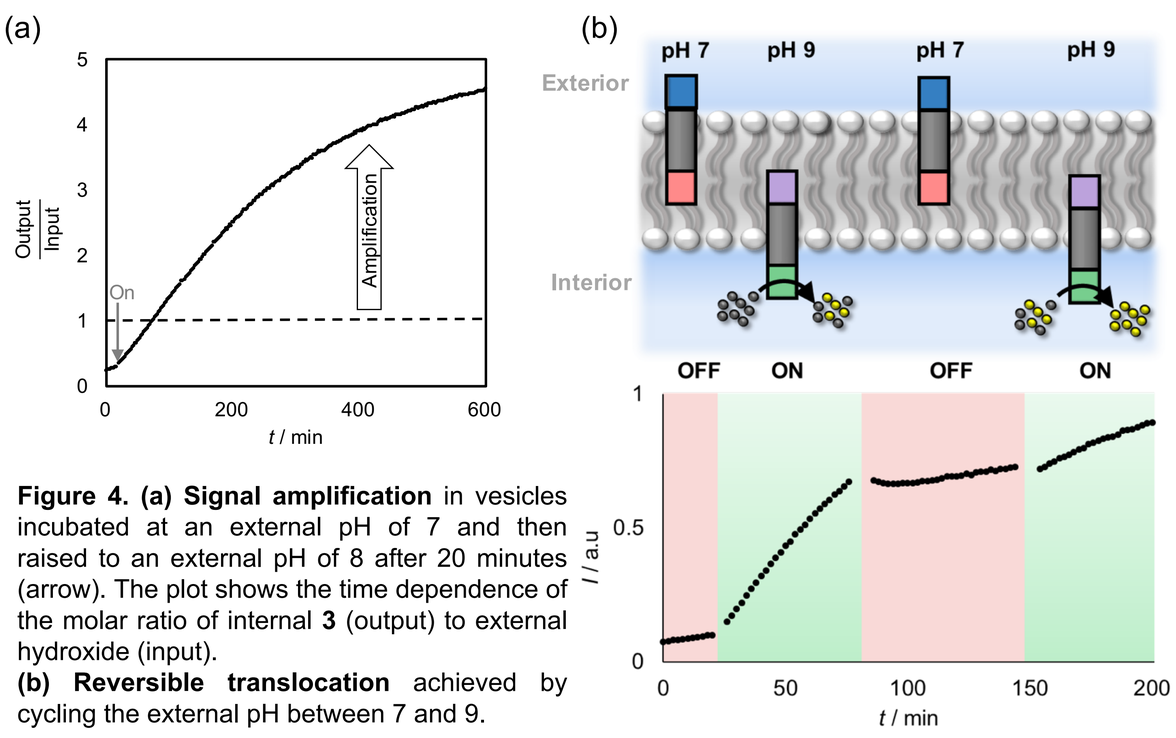

Figure 4. (a) Signal amplification in vesicles incubated at an external pH of 7 and then raised to an external pH of 8 after 20 minutes (arrow). The plot shows the time dependence of the molar ratio of internal **3** (output) to external hydroxide (input).
(b) Reversible translocation achieved by cycling the external pH between 7 and 9.

Molecular recognition controlled signal transduction

The system can be diversified to respond to molecular recognition processes on the exterior of vesicles. As a proof-of-concept, this is demonstrated by Cu(II)-phenanthroline derivative, **4**. Displacement of the charged copper cation neutralises the transducer and facilitates membrane translocation, and activation of the catalytic head group inside the vesicle (Fig. 5).[4]

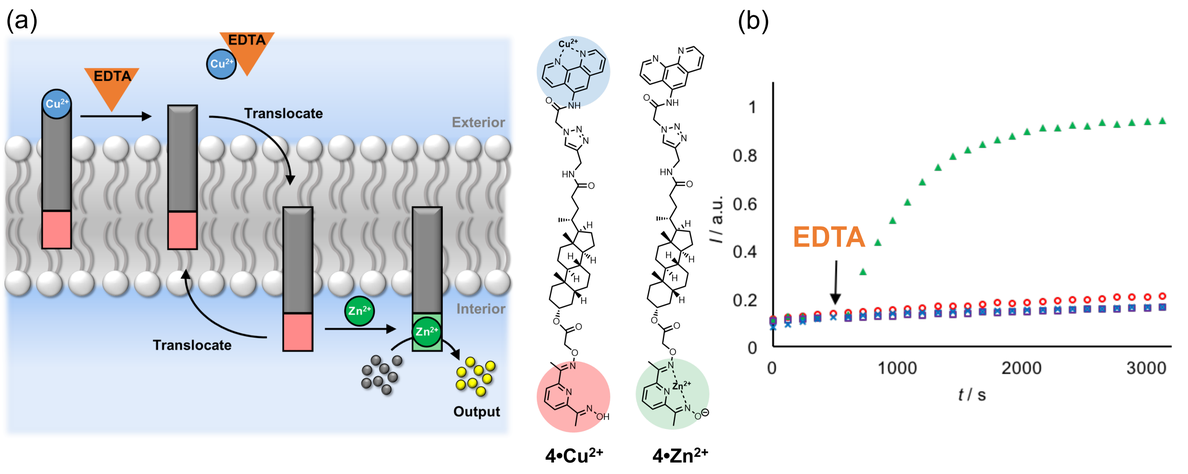

Figure 5. Recognition-controlled signal transduction. (a) Dipslacement of Cu(II) from transducer **4** by EDTA initiates signal transduction and turnover of substrate **2** inside the vesicles. (b) Time dependence of the relative fluorescence emission intensity from **3** inside vesicles containing **4**·Cu^{2+} (red) and following external EDTA addition (green). Blue data: control in absence of **4**·Cu^{2+}.

Conclusions

These results demonstrate that membrane translocation is an effective mechanism for transmembrane signal transduction and amplification. The simplicity of this concept suggests that it will be broadly applicable to diverse stimuli, opening up a wide range of possible applications. The ability to change the internal chemistry of membrane-bound capsules will be crucial for the development of bio-inspired nanotechnologies, capable of interfacing and exchanging information with biological systems.

MJL thanks the University of Cambridge for an Oppenheimer Early Career Research Fellowship. We thank Franziska Kundel and David Klenerman for TIRFM imaging experiments.

References

1. Krauss, G. *Biochemistry of Signal Transduction and Regulation*, 3rd ed.; Wiley: New York, 2006.
2. Matile, S., Jentzsch, A. V., Montenegro, J. and Fin, A., ***Chem. Soc. Rev.,*** 2011, 40, 2453–2474.
3. Langton, M. J., Keymeulen, F., Ciaccia, M., Williams, N. H., and Hunter, C. A., ***Nat. Chem.,*** 2017, **9**, 426-430
4. Langton, M. J., Williams, N. H., and Hunter, C. A, ***J. Am. Chem. Soc.,*** 2017, 139, 6461–6466