Nanoscale pH Sensing and Control for Molecular Communication Applications

L. Erbacher, S. Zurmühl, T. tom Dieck, D. Wegner, R. Schober, H. Sticht, and K. Castiglione
Friedrich-Alexander-Universität Erlangen-Nürnberg

***Abstract*—In this work, the concept of a nanodevice (ND) for sensing and controlling the pH in nanoscale systems is introduced. The ND presented herein is based on polymersomes which have been functionalized with light-driven proton pumps, thus enabling transmembrane proton transport. The current setup is outlined, initial experimental results are highlighted, and short α-helical peptides are introduced as novel pH-sensors for the ND.**

I. Introduction

pH is a crucial factor in cellular and organismal function, as it affects biochemical reactions, enzyme activity, membrane potential, and overall homeostasis. Therefore, changes in pH can provide information about the health and disease status of organisms. For example, cancer cells often exhibit an altered pH balance, with a more alkaline intracellular environment and an acidic extracellular environment, aiding in tumor progression [1]. Since the early detection of diseases, especially tumors, is one of the most interesting areas of potential applications of molecular communication (MC), the development of biocompatible nanoscale tools for pH measurements could prove useful in this context. Additionally, various MC systems have been built that leverage the pH value as an information signal. These works include a biological testbed featuring genetically modified light-responsive *Escherichia coli* [2] and a flow-based testbed employing acids and bases as signaling molecules [3]. In both testbeds, the pH changes were measured with macro-scale pH probes, which are unsuitable for many application scenarios of MC on the micro- or nanoscale. To overcome these limitations this project aims to develop novel nanoscale tools for pH sensing and control. Although bacteria with typical cell diameters in the micrometer range are already quite small, their cellular complexity limits the reproducibility and controllability of the system. Using synthetic biology, we want to significantly reduce not only the compartment size but also the biological complexity and therefore use polymersomes with typical diameters of 100 nm as chassis for ND development [4]. These vesicles are functionalized with (macro)molecules that enable pH sensing and control on the nanoscale. Depending on the MC scenario, it might be of interest to influence the intravesicluar pH, e.g., to drive a different transport process via the generated proton motive force [5], to only measure pH, or to combine both aspects. Therefore, we want to establish a set of modular tools for pH sensing and control and investigate them in suitable testbeds.

II. Current Setup

We propose a ND design based on polymer vesicles, i.e., polymersomes, in which molecules can be enclosed in their core or embedded in their polymer membrane [6]. According to the current state of the art, pH-sensitive small-molecule dyes, such as pyranine, are encapsulated in the ND core (Fig. 1). The fluorescence intensity of pyranine shows a linear correlation with pH in the range of pH 6 to 8 [7] as can be seen in Fig. 2a, which allows for the determination of the intravesicular pH. On a macroscopic level, the effect of a pH change on pyranine fluorescence can be demonstrated by adding acid to a pyranine solution, which leads to a shift in fluorescence intensity that can be detected externally with a spectrophotometer (Fig. 2b). To provoke a pH shift on the nanoscale that is restricted to the ND core, we use the light-driven proton pump proteorhodopsin from proteobacteria [8]. These proton pumps are inserted unidirectionally into the membranes and transport protons into the ND upon illumination (Fig. 1), resulting in a pH change (Fig. 2c). However, as pyranine only has a limited pH detection range, other pH-sensitive molecules must be established to make the ND widely applicable. A promising group of molecules for this application are peptides.

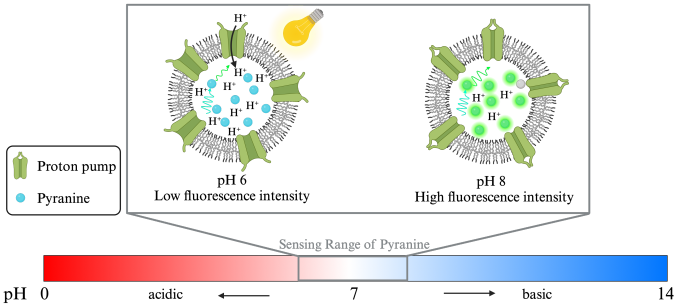
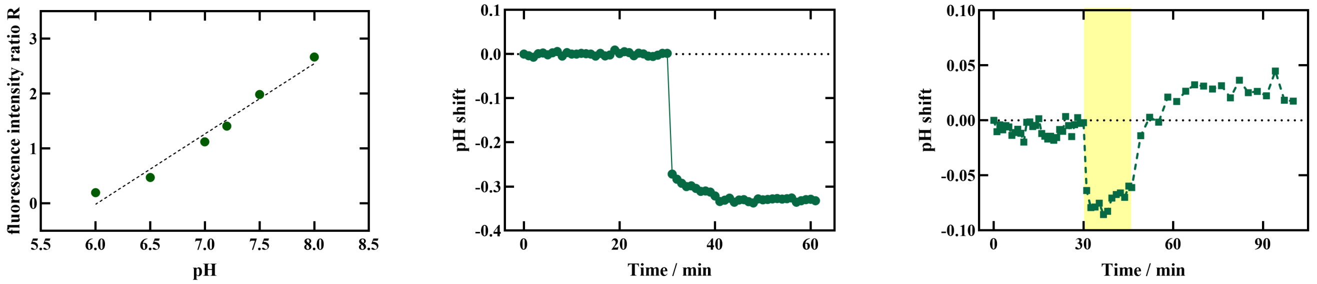


Fig. 1: Schematic overview of polymersomes with proton pumps and pyranine during illumination and darkness. Created with BioRender.com.

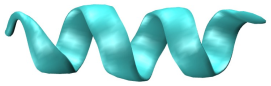
III. Peptides as pH sensing molecules

Peptides are a class of bio-molecules which are increasingly utilized within a variety of fields. Their amino acid sequence determines properties such as their two-dimensional shape (secondary structure) and stability. Such peptides can be rationally designed or optimized to perform a specific task with high accuracy [9]. Our work focuses on peptides with an α-helical secondary structure, with the aim of identifying peptides that undergo a structural change from an α-helix to an unstructured random coil upon pH change (Fig. 3). To avoid the cost- and labor-intensive experimental identification of candidates that might meet these requirements, we employ

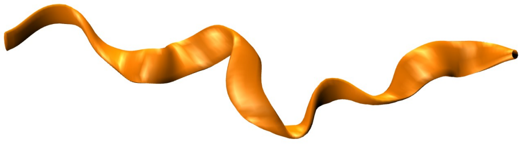


(a) (b) (c)

Fig. 2: a) Ratiometric detection of pH using pyranine. The fluorescent dye was excited at 415 nm and 460 nm, whereas the emission wavelength was set at 514 nm. For calibration, the fluorescence intensity ratio R = F415/F460 is plotted as a function of pH. b) pH shift of a pyranine solution induced by addition of acid after 30 minutes. c) pH shift of pyranine encapsulated in polymersomes with inserted proton pumps. After equilibration, the NDs are illuminated for 15 minutes with light of 530 nm, as indicated by the yellow shaded area. When the light is switched off again, the system returns to the basic state over time. The slight overshoot of the pH can be attributed to photobleaching effects in the system, which affect the ratiometric pH measurement.



(a) Peptide O13 [12] shows a stable α-helical secondary structure at a basic pH.



(b) Peptide O13 [12] in a random coil at an acidic pH.

Fig. 3: Peptide O13 displays a change in secondary structure from structured to unstructured when the pH changes from basic to acidic. At a basic pH, negatively charged glutamate side chains interact with positively charged arginine side chains stabilizing the α-helix.

molecular dynamics simulations to characterize and optimize these peptides *in silico* [10]. As a starting point, we selected known α-helical peptides from the literature that are stabilized in their helical shape by the formation of salt bridges (interactions between oppositely charged side chain functional groups) [11], [12]. We observe that peptides that rely on stabilization by interactions involving these side chain groups, respond to the pH change by losing their helical structure. These results lay the foundation for our future work, where we plan to rationally design and optimize peptides to cover pH ranges specifically customized for different applications of the ND.

IV. CONCLUSION AND FUTURE WORK

Polymersomes form the basis for the development of our ND, since they can be functionalized with various (bio-)molecules, allowing for a wide range of applications. Our first step is the development of a pH-detecting and pH-regulating ND. Thus far, we are able to vary the pH within the polymersomes using external illumination. In order to maximize the versatility of the NDs it is necessary to extend the detectable pH range. Peptides are promising candidates for this application, as they can be rationally designed to undergo structural changes at specific pH ranges, whilst their inherent biocompatibility agrees with the intended biological applications of our polymersome-based NDs.

REFERENCES

[1] M. Damaghi, J. W. Wojtkowiak, and R. J. Gillies, “pH sensing and regulation in cancer,” *Frontiers in Physiology*, vol. 4, p. 370, 2013.

[2] L. Grebenstein *et al.*, “A Molecular Communication Testbed Based on Proton Pumping Bacteria: Methods and Data,” *IEEE Trans. Mol. Biol. Multi-Scale Commun.*, vol. 5, no. 1, pp. 56–62, Oct. 2019.

[3] N. Farsad, D. Pan, and A. Goldsmith, “A novel experimental platform for in-vessel multi-chemical molecular communications,” in *Proc. IEEE Global Commun. Conf.* IEEE, 12 2017, pp. 1–6.

[4] S. T. Poschenrieder, S. G. Wagner, and K. Castiglione, “Efficient production of uniform nanometer–sized polymer vesicles in stirred–tank reactors,” *Journal of Applied Polymer Science*, vol. 133, no. 14, 2016.

[5] T. tom Dieck *et al.*, “Nanoscale transmitters employing cooperative transmembrane transport proteins for molecular communication,” in *11th Annual ACM Intern. Conf. on Nanoscale Computing and Commun.* New York, NY, USA: ACM, 10282024, pp. 14–20.

[6] L. Klermund and K. Castiglione, “Polymersomes as nanoreactors for preparative biocatalytic applications: current challenges and future perspectives,” *Bioprocess and Biosystems Engineering*, vol. 41, no. 9, pp. 1233–1246, 2018.

[7] Y. Avnir and Y. Barenholz, “pH determination by pyranine: Medium-related artifacts and their correction,” *Analytical Biochemistry*, no. 347, pp. 34–41.

[8] R. Goers *et al.*, “Optimized reconstitution of membrane proteins into synthetic membranes,” *Commun. Chem.*, vol. 1, no. 1, pp. 1–10, Jun. 2018.

[9] M. Erak *et al.*, “Peptide chemistry toolbox - Transforming natural peptides into peptide therapeutics,” *Bioorg Med Chem*, vol. 26, no. 10, pp. 2759–2765, Jun. 2018.

[10] D. A. Case *et al.*, “The Amber biomolecular simulation programs,” *J Comput Chem*, vol. 26, no. 16, pp. 1668–1688, Dec. 2005.

[11] B. M. Huyghues-Despointes, J. Martin Scholtz, and R. L. Baldwin, “Helical peptides with three pairs of Asp-Arg and Glu-Arg residues in different orientations and spacings,” *Protein Sci.*, vol. 2, no. 1, pp. 80–85, 1993.

[12] M. Petukhov *et al.*, “Design of stable *A*-helices using global sequence optimization,” *J. Pept. Sci.*, vol. 15, no. 5, pp. 359–365, Feb. 2009.