

**Increased heat stability of  $\alpha$ -chymotrypsin through its confinement in liposomes**M. Yoshimoto<sup>1</sup>, J. Yamada<sup>1</sup>, K. Mizoguchi<sup>1</sup>, P. Walde<sup>2</sup><sup>1</sup>Department of Applied Chemistry, Yamaguchi University, Japan, <sup>2</sup>Polymer Chemistry, Department of Materials, ETH Zurich, Switzerland

For applications of enzymes in confined space, for example inside liposomes (lipid vesicles), the enzyme stability is a critical issue [1]. During the course of our investigations on the entrapment of enzymes inside submicrometer-sized liposomes, we found that the confinement of  $\alpha$ -chymotrypsin in liposomes formed from POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) leads to a significantly increased thermostability of the enzyme. Since through the method used – dispersing a dried POPC layer with an aqueous enzyme solution, followed by polycarbonate membrane extrusion – the enzyme entrapment in the liposomes occurs during liposome formation, a stochastic enzyme distribution among the liposomes is obtained. Heat stability experiments showed that a considerable fraction of liposomal  $\alpha$ -chymotrypsin is still active after being treated at 80 °C for 30 min, whereas the free enzyme is completely deactivated. For liposome-confined  $\alpha$ -chymotrypsin, the heat stability increases as the average number of enzyme molecules per liposome decreases. This high heat tolerance can be explained by a decrease in interactions between partially unfolded enzyme molecules as a result of a decrease in the number of enzyme molecules per liposome compartment. In the extreme case, there is no opportunity for the irreversible formation of enzyme aggregates – which leads to enzyme deactivation – in the case of single enzyme molecule confinement. Whether this finding also holds for other monomeric enzymes is currently under investigation.

*Acknowledgment:* This work was supported by JSPS KAKENHI grant number 15KK0241.

*References:* [1] Kuchler, A., Yoshimoto, M., Luginbühl, S., Mavelli, F., Walde, P. *Nature Nanotechnol.*, **2016**, *11*, 409. [2] Yoshimoto, M., Yamada, J., Baba, M., Walde, P. *ChemBioChem*, **2016**, *17*, 1221.