

**DOCTORAL CANDIDATE:** Lise Berven

**DEGREE:** Philosophiae Doctor

**FACULTY:** Faculty of Mathematics and Natural Sciences

**DEPARTMENT:** Department of Pharmaceutical Chemistry, School of Pharmacy

**AREA OF EXPERTISE:** Pharmacognosy

**SUPERVISORS:** Anne Berit C. Samuelsen, Harald Thidemann Johansen, Rigmor Solberg, Svein Olav Kolset

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**DISSERTATION TITLE:** *Regulation of the tumor-associated protease legumain by dietary fiber and endogenous carbohydrate polymers*

**Partikulært  $\beta$ -glukan fra gjærsopp hemmer enzymaktiviteten til den kreft-assosierte proteasen legumain i makrofager. Alginat og glykosaminoglykaner akselererer autoaktiveringen av prolegumain via ioniske interaksjoner.**

The lysosomal protease legumain is found to be highly expressed in various types of solid tumors. Legumain has shown to be up-regulated in and secreted from tumor-associated immune cells (macrophages) and has been detected on the surface of cancer cells, cancer-associated endothelial cells and macrophages. We have shown that yeast-derived  $\beta$ -glucan is internalized by macrophages and inhibits the activity of legumain in macrophages. Also, we showed that macrophage legumain activity is inhibited by the mushroom extract AndoSan, and part of this effect may be attributed to  $\beta$ -glucan. Furthermore, alginates which are negatively charged co-polymers of mannuronic acid (M) and guluronic acid (G) were able to increase the activity of legumain in macrophages, but with different potency; Alginates with 75 % M > 60 % M  $\approx$  50 % M > 94 % M  $\approx$  100 % M. Activity was lost upon treatment with alginate lyase, which catalyses the degradation of alginate, showing that molecular size is essential for the activity. Activity was also lost upon chemical reduction of alginate carboxylic acid groups which produces derivatives that are lacking the negative charges, showing that also the polymer charge and conformation are essential for the activity. Additionally, the negatively charged endogenous polysaccharide heparin was able to increase the activity of legumain in these cells. Legumain is synthesized as a proenzyme and undergoes pH-dependent autoactivation of the proform in order to reach an enzymatically active form. We showed that both alginates and glycosaminoglycans accelerate the autocatalytic activation of prolegumain at pH 4.0 and pH 5.0. At pH 5.5 only the alginate with 75 % M was able to promote prolegumain autoactivation. The accelerated autoactivation was due to strong interactions between the negatively charged polysaccharides and the positively charged C-terminal part of prolegumain.