Proteolysis Laboratory – F. Xavier Gomis

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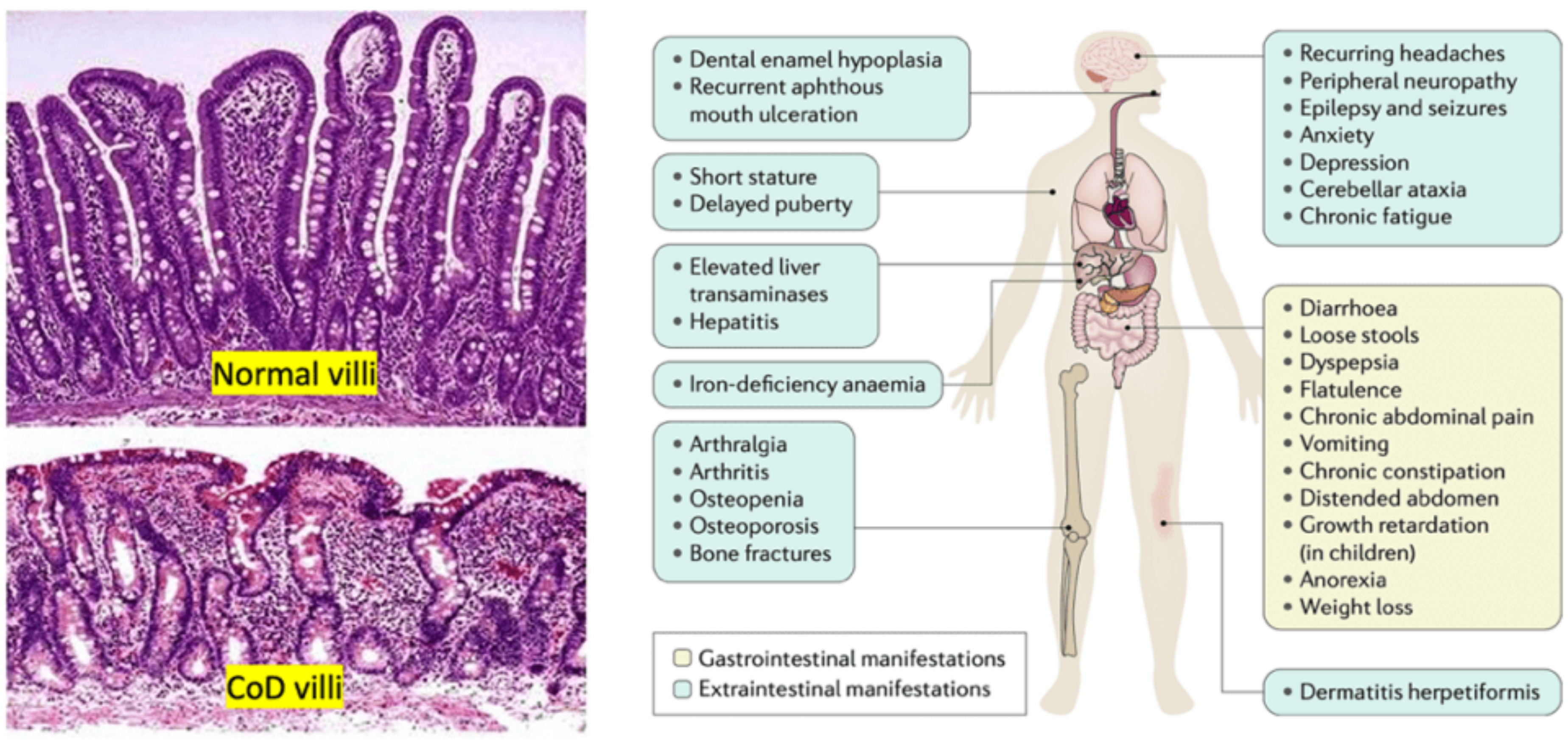
Lab presentation

The work carried out in the ***Proteolysis Lab*** *is centred on the* **analysis of the molecular determinants of function and regulation of proteolysis at the protein level**. *We study* **peptidases** *and their zymogens, mostly from host-microbiome interactions, as well as their complexes with small-molecule and protein inhibitors. Another line of research deals with the molecular analysis of other interaction mediators between animal hosts and their microbiomes.* **Employed techniques include molecular biology, biochemistry, biophysics, and X-ray crystallography**, *among others. Our lab is fully furnished with state-of-the-art equipment, which enables us to carry out cutting-edge science projects.*

One recent project has focused on **coeliac disease** (CoD), a chronic autoimmune enteropathy that affects individuals with genetic and environmental sensitization to dietary gluten, namely a group of cereal prolamin storage proteins rich in proline and glutamine. Prolamins that trigger CoD include gliadin and glutenin in wheat, hordein in barley, and secalin in rye. Intestinal damage is inflicted by as little as ~10 mg of dietary gluten per day, which is <0.1% of the amount found in a typical western diet. CoD is a global health burden across all age ranges, with a worldwide serological prevalence of 1.4% that increases by 7.5% every year.

The disease is caused by partially degraded gluten peptides, which are immunogenic and include a 33-residue fragment of wheat α-gliadin (33-mer) that is kind of a standard in the field. These peptides resist further cleavage by digestive peptidases owing to their high proline content. In coeliacs, they cross the mucosal epithelium of the small intestine, where glutamine residues are deamidated by tissue transglutaminase. This enhances the affinity of the peptides for particular alleles of the human leukocyte antigen receptor, which are a requisite for the development of CoD. Receptor binding triggers a severe pro-inflammatory autoimmune response mediated by T cells, with resulting intestinal phenotypes including intraepithelial lymphocytosis, crypt hyperplasia, atrophy of small-intestine villi and mucosal inflammation. These lead, in turn, to the chronic malabsorption of nutrients, diarrhoea, vomiting, bloating, abdominal pain and intestinal lymphomas. Moreover, there are extra-intestinal manifestations, which include delayed puberty, osteoporosis, axonal neuropathy and cerebellar ataxia. Overall, CoD patients face a reduced life expectancy.



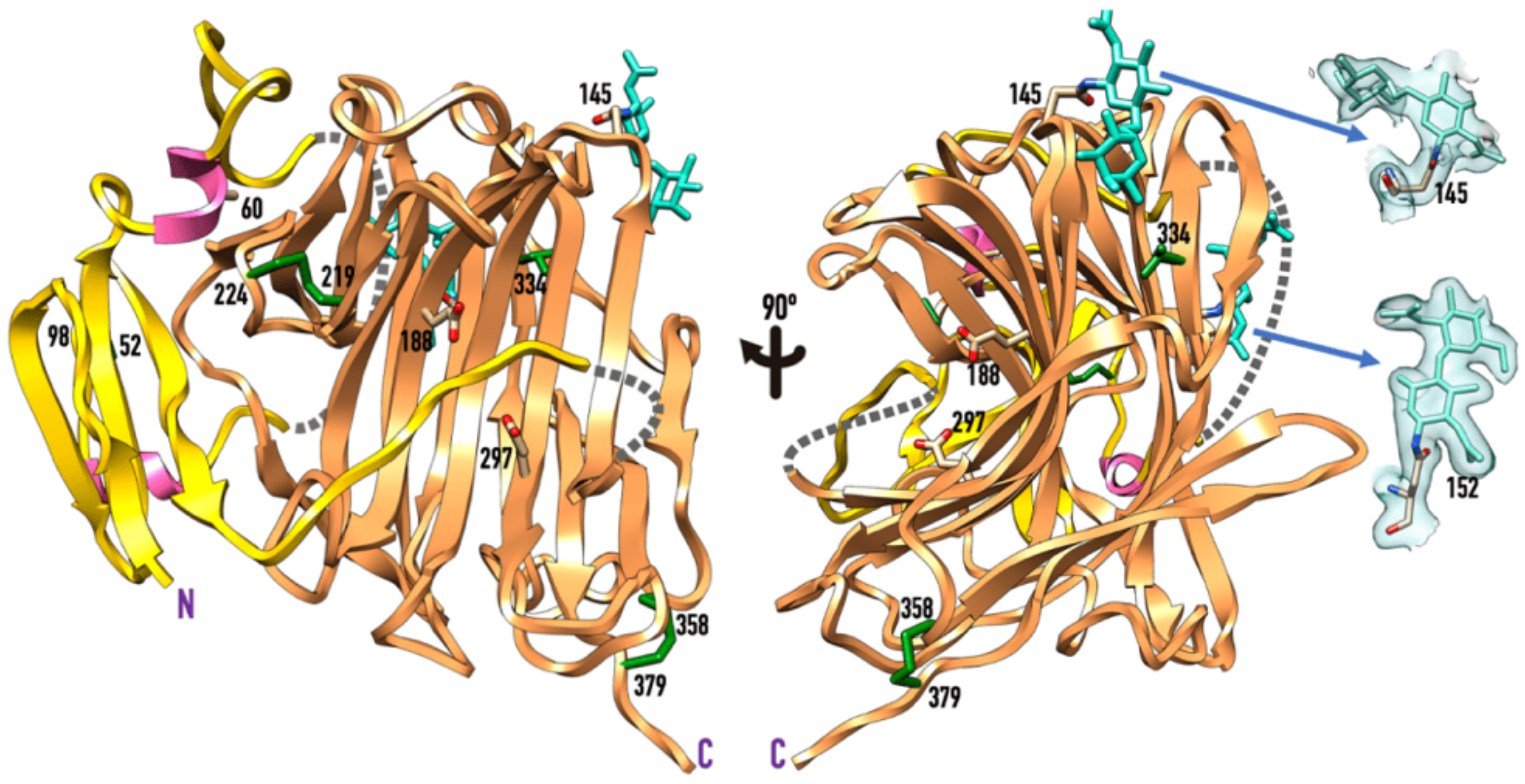
https://celiac.org/about-the-foundation/featured-news/2015/03/biopsy-samples-diagnosis-celiac-disease/

Lindfors et al., Nat Rev Dis Prim, 5:3 (2019)

Currently, there is no treatment for CoD, so patients must adhere to a lifelong strict gluten-free diet, which restores the normal architecture of the intestinal villi. However, gluten-free diets do not provide balanced nutrition, and many coeliacs suffer intestinal symptoms even with adherence to such dietary restrictions. Moreover, gluten is found in most processed foods and medicines, making dietary compliance challenging in western societies. This has created a demand for effective CoD therapies.

One promising approach is the development of endopeptidases that cleave the immunogenic gluten peptides and would thus act as bona fide glutenases for oral enzyme therapy, reminiscent of lactase tablets for lactose intolerance. Such an approach would also benefit patients suffering from non-coeliac gluten sensitivity, which has a worldwide prevalence of up to 13%, and irritable bowel syndrome, with a prevalence of ~0.5%. A candidate glutenase must fulfil stringent criteria for clinical application. First, it should work in the stomach during digestion, before the gastric bolus passes into the duodenum and initiates the autoimmune response, and thus must remain stable and active in the acidic gastric environment (pH ~2.5) as well as resisting gastric pepsin. Second, a reasonable dose should efficiently digest gliadin and the immunogenic gluten peptides when combined with pepsin under gastric conditions, which requires the processing of large quantities of dietary protein. Third, it should not harm intestinal structures or inhibit nutrient absorption, and thus ideally should be inactive at the slightly acidic postprandial pH of the duodenum.

Recently, in a **collaboration** with the **Synthetic Structural Biology** group headed by **Ulrich Eckhard** within the institute and the group of **Francisco José Pérez Cano** from the Faculty of Pharmacy and Food Science of the **University of Barcelona**, we studied neprosin, a peptidase from a carnivorous pitcher plant, as a potential glutenase for therapy in CoD based on previous pioneering work by the group of **David Schriemer** from the **University of Calgary** in Canada (https://profiles.ucalgary.ca/david-schriemer). He hypothesized neprosin might have a function in protein metabolism during prey digestion and/or defence. In combination with other peptidases from the digestive fluid, neprosin was further identified as part of a potential glutenase preparation. Finally, purified neprosin was also considered a useful reagent for proteomics.



Two orthogonal views of proneprosin, with the pro-domain in yellow and the catalytic domain in salmon. The two glycosylation sites are pinpointed.

We established an efficient human recombinant production system to produce high yields of pure neprosin in human cells. We determined that neprosin is a new member of the glutamate peptidase class, with two unique glutamate residues acting as a catalytic dyad, which is distantly reminiscent of the two aspartates of acid peptidases. Moreover, we reported the crystal structure of the neprosin zymogen and its mature form in product-mimicking complexes. In this way, we further unveiled the molecular determinants of latency, its mechanism of activation, as well as its thermal stability, pH profile, general proteolytic and peptidolytic activities, and susceptibility to a panel of peptidase inhibitors. We also tested cleavage of gliadin and the 33-mer *in vitro* to evaluate the ability of neprosin to act as a *solo* glutenase. Moreover, we evaluated the effect in mice and found that co-administration of gliadin and the neprosin zymogen at the ratio 500:1 reduced the abundance of the immunogenic gluten peptides in the small intestine by up to 90%. Neprosin therefore founds a family of eukaryotic glutamate endopeptidases that *a priori* fulfils requisites for a therapeutic glutenase.

Read the whole story freely under https://www.nature.com/articles/s41467-022-32215-1.

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