Chemical modification of anticancer Parasporins for decreasing of their toxicity

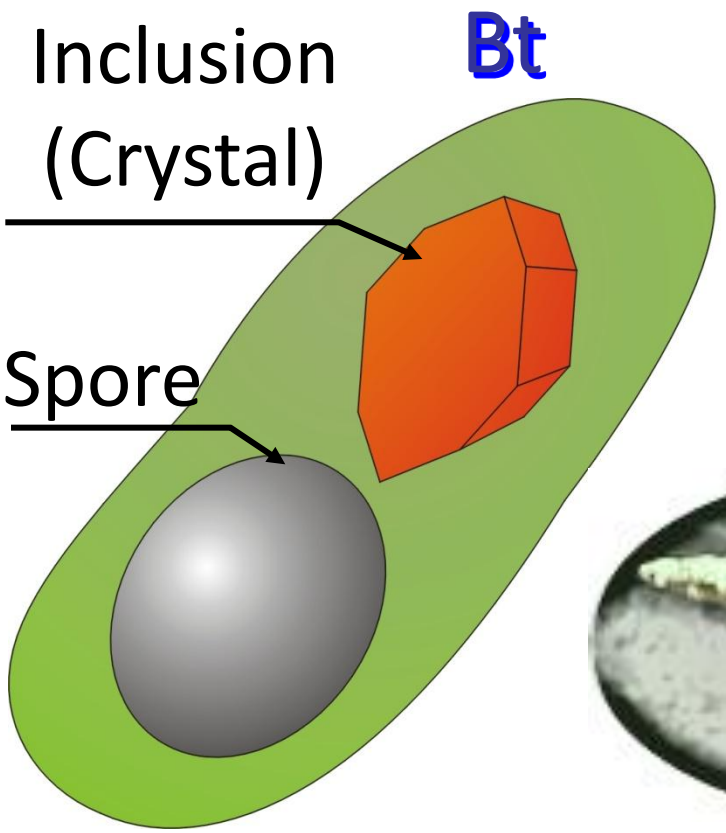
Almas Okassov, Alexander Ilin

Scientific Center for anti-infectious drugs (SCAID), Kazakhstan

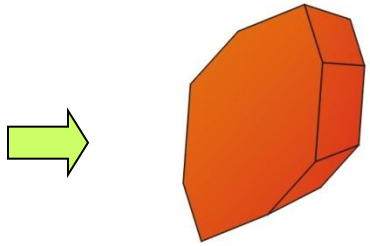
5TH WORLD CONGRESS ON BIOAVAILABILITY & BIOEQUIVALENCE: PHARMACEUTICAL R&D SUMMIT

30th Sep. 2014

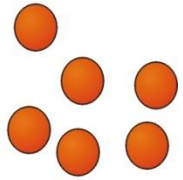
What is *Bacillus thuringiensis* (Bt) and toxins?



Solubilization in midgut

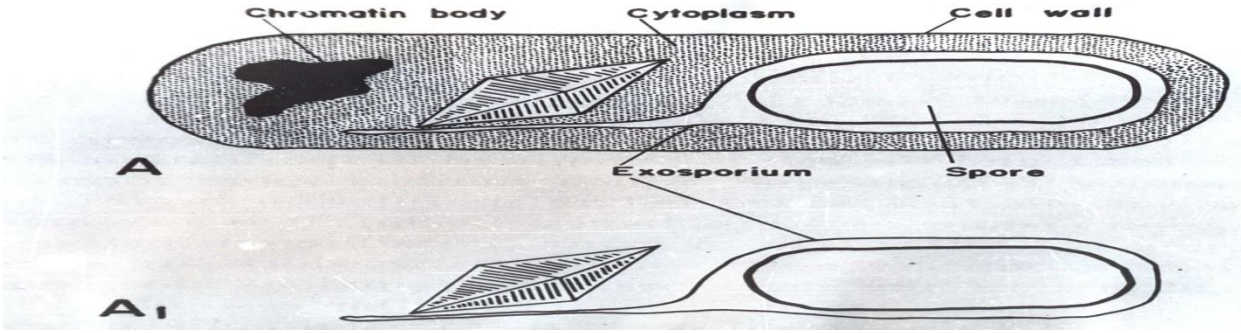


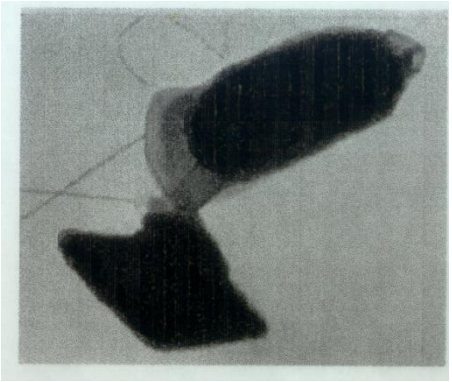
Toxin

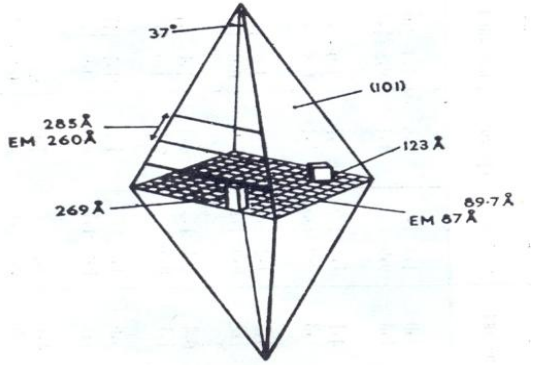


Processing by proteases

B. thuringiensis: formation of crystalline inclusions







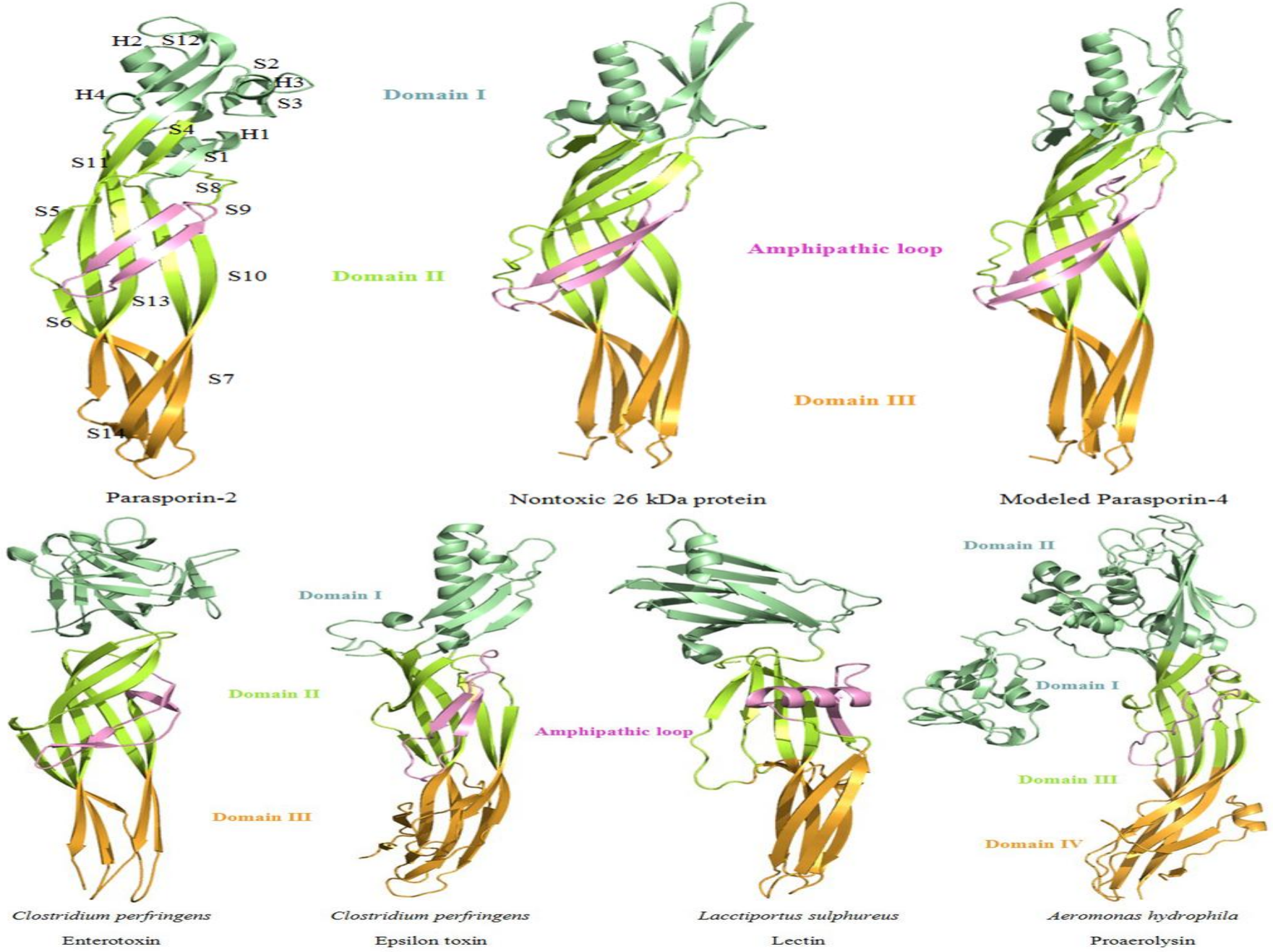
The thin crystallographic methods for study of entomocide crystals of *Bt* ssp. *berliner (thuringiensis)* culture show that width of lines is within 260-285 Å, diameter located on it micromolecules is 87-89 Å (Holmes, Monro, 1965). The differences in size of these macromolecules specific for cultures of 5 subspecies studied of *Bt* - ssp. *entomocidus* 208 Å, ssp. *thuringiensis* – 223 Å, ssp. *dendrolimus* – 227 Å, ssp. *galleriae* 270 Å, ssp. *subtoxicus* – 277 Å have been established (Vankova, Kralik,1966).
Ultrathin section of hardly cultivated entomopathogene - *Bacillus popilliae* during sporulation is shown on the slide.

Bacillar insecticidal species / subspecies

Preparation base	Toxin	Target insect
B.thuringiensis subsp. thuringiensis	Exotoxin	*Diptera*, etc.
B.thuringiensis (different subspecies)	Crystal	*Lepidoptera*
B.thuringiensis subsp. israelensis	Crystal	Mosquitoes, black flies
B.thuringiensis subsp. sd./tenebrionis	Crystal	*Coleoptera*, Colorado potato beetle
B.popilliae	Crystal	Japanese beetle, Amphimallon
B.sphaericus	Crystal	Mosquitoes

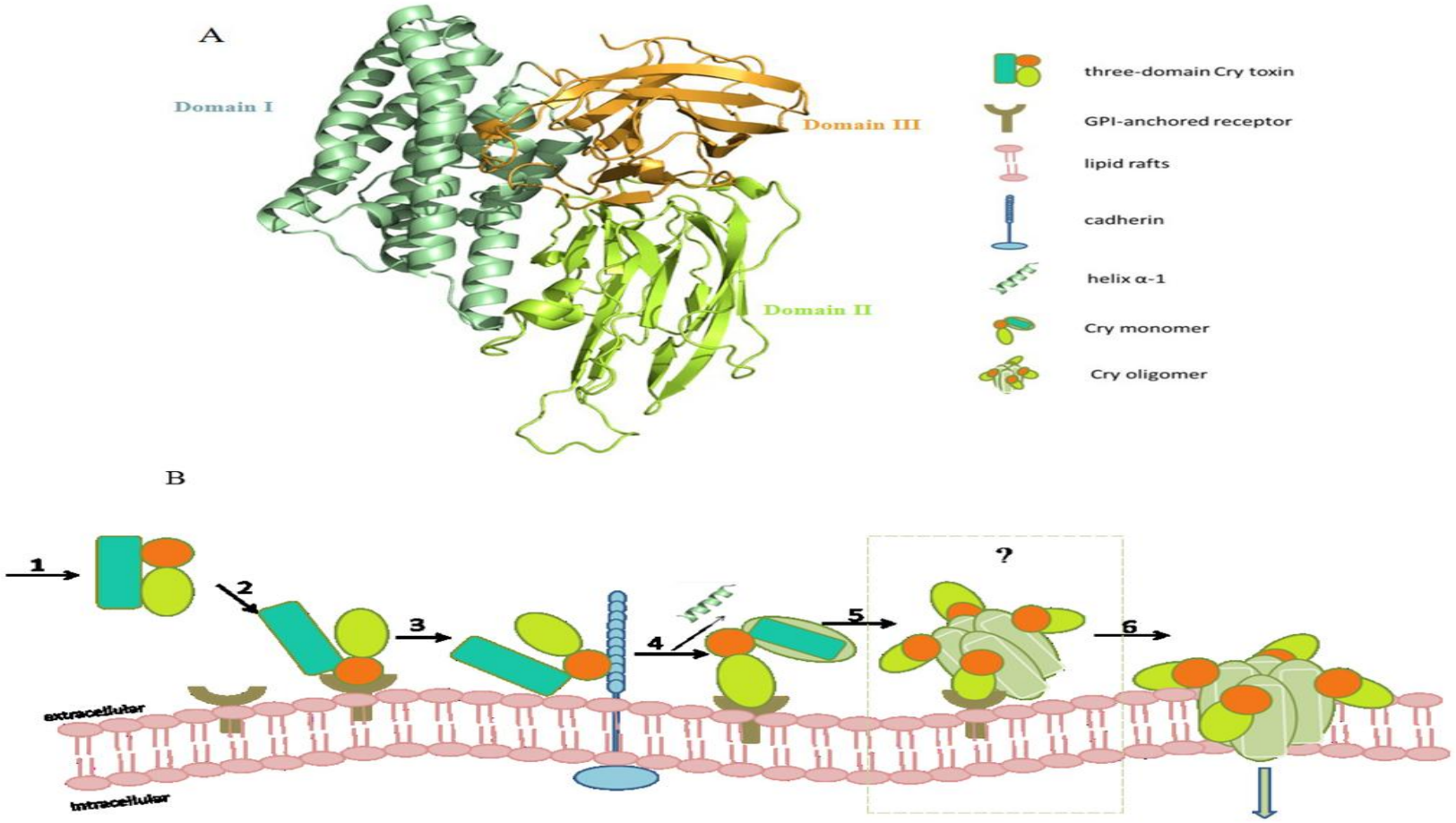
Characteristics of B. thuringiensis crystalline toxins

Producers	Crystal shape	Target insect
B.thuringiensis different subspecies	Rhomboid	*Lepidoptera*
B.thuringiensis subsp. israelensis	Spheric irregular	Mosquitoes, black fly
B.thuringiensis subsp. sd/tenebrionis	Cubic, cylindric,	*Coleoptera*, potato beetle



Chengchen Xu et. al. J. *Toxins,* 2014

Mechanism of action



Chengchen Xu et. al. J. *Toxins,* 2014

Parasporin-2 Actions on Human Cancer Cells

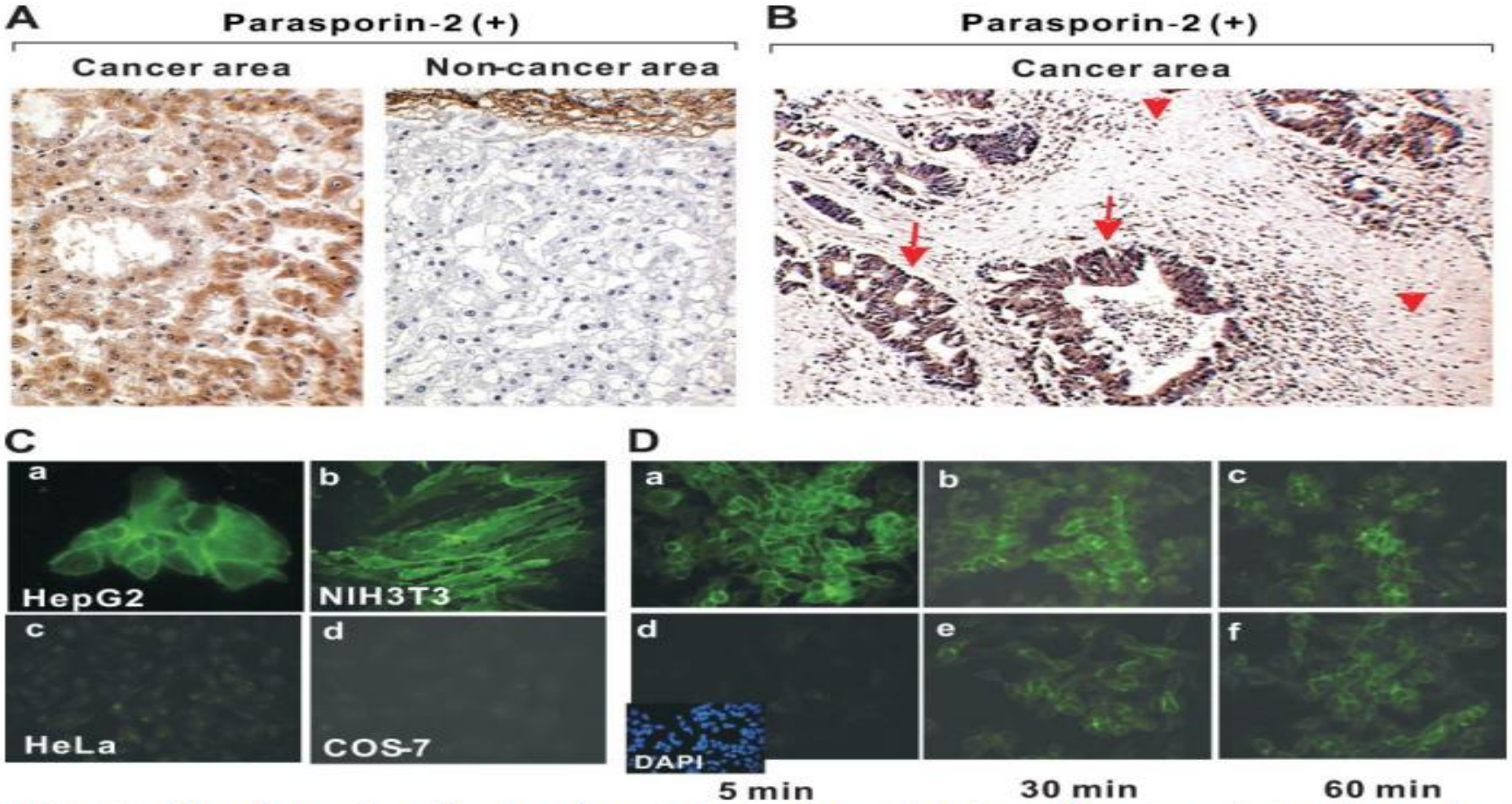
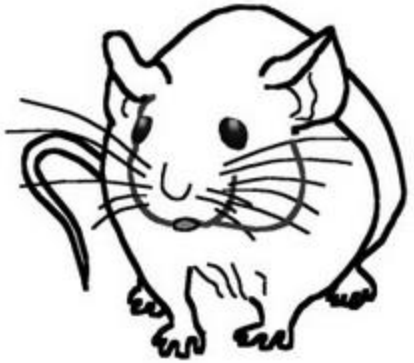


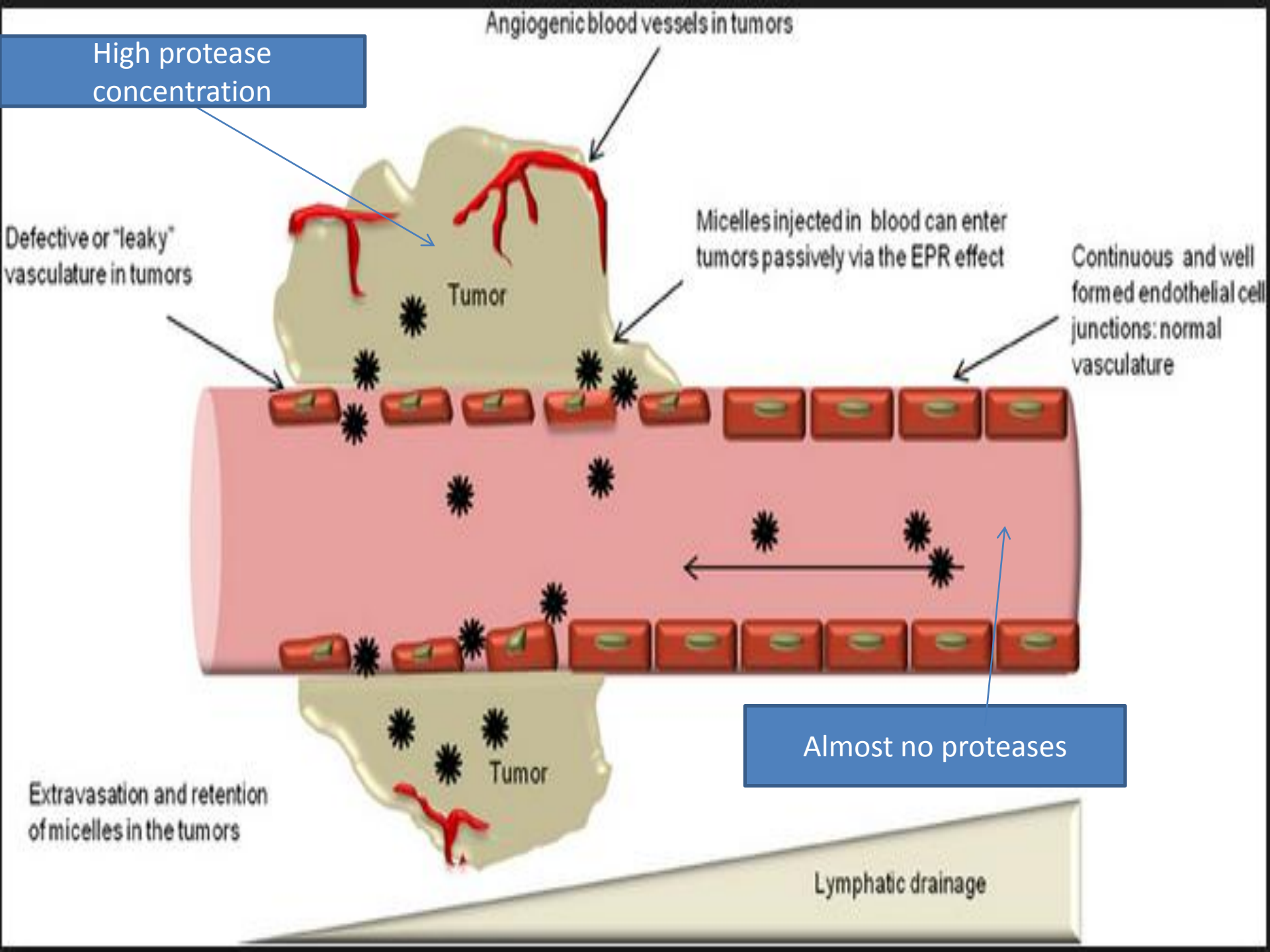
FIGURE 4. **Associations of parasporin-2 with various cell types.** *A*, detection of parasporin-2 in hepatocellular carcinoma. Sections of cancer and non-neoplastic tissues were incubated with parasporin-2, mounted on the same glass slides, and analyzed immunohistochemically using an anti-parasporin-2 antibody. Peroxidase activity was visualized using 3,3′-diaminobenzidine as the chromogen. Note the extensive presence of parasporin-2 in hepatocellular carcinoma cells but not in non-neoplastic liver cells, suggesting that parasporin-2 binds specifically to the cancer cells. *B*, detection of parasporin-2 in colon cancer cells. Sections of colon cancer tissues were treated as described above. The columnar cancer and fibroblastic cells were indicated by *arrows* and *arrowheads*, respectively. The toxins efficiently bound to the cancer cells but not to the peripheral fibroblastic cells. *C*, indirect immunofluorescence observations of cultured mammalian cells treated with parasporin-2. Cultured cells were treated with parasporin-2 (1.0 μg/ml) at 37 °C for 5 min. The intoxicated cells were then washed with PBS, fixed, incubated with an anti-parasporin-2 antibody and detected with a secondary antibody conjugated with a fluorescent dye. *D*, kinetics of parasporin-2 binding to HepG2 cells. HepG2 cells were incubated with 1.0 μg/ml (*panels a–c*) or 0.1 mg/ml (*panels d* and *e*) parasporin-2 at 37 °C for 5 (*panels a* and *d*), 30 (*panels b* and *e*), or 60 min (*panels c* and *f*) and treated as described for *B*. In *panel*) *d*, an image of the cell nuclei stained with 4′,6′-diamidino-2-phenylindole (*DAPI*) in the same visual field is inserted.

Sakae Kitada et. al. J. Biological Chemistry, 2006

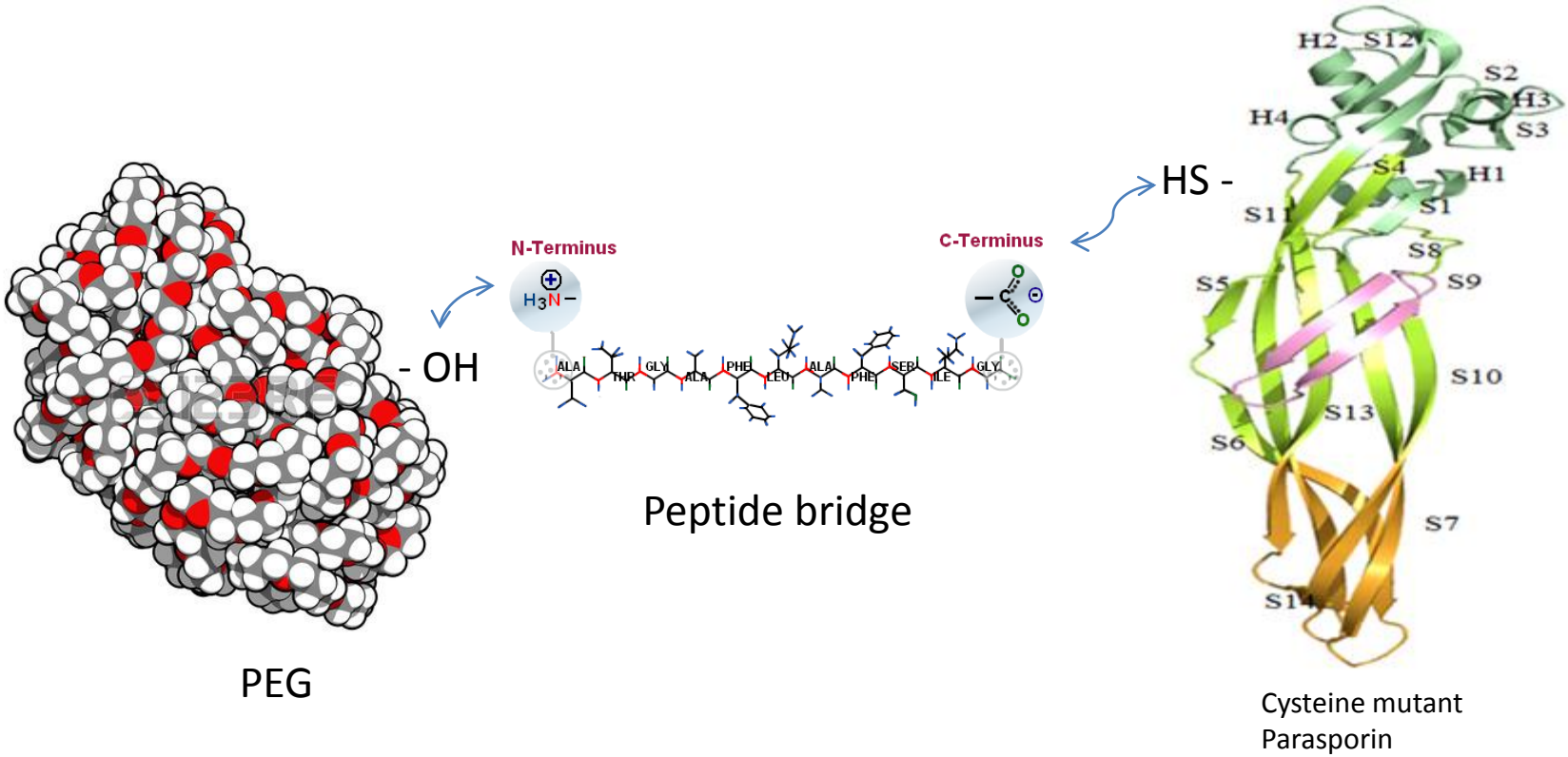
As personal communication, Prof. Sakae Kitada (Kyushu institute of technology, Japan) recently found anti-tumor effect of PS2 (0.2mg/kg) to mice implanted a mouse cancer cells. After injection tumor size was significantly decreased. Under the influence of described dose quarter of mice don’t survive.



The scheme proposed for modification of antitumor Parasporins is based on more high permeability of vessels for high molecular compounds in foci of tumorigenesis with high concentration of proteases. This condition permits to create the construction composed from active Parasporin coupled with a linker particle having diameter of 40-50 nm *via* a peptide bridge. Selective delivery of these conjugates to the foci of tumor formation with further proteolytic activation should decrease therapeutic dose of the active substance and decrease concentration in normal healthy organs and tissues with common vascular permeability and resulting in decreasing of general negative effect for the organism.


High protease concentration
Angiogenic blood vessels in tumors
Defective or "leaky" vasculature in tumors
Tumor
Micelles injected in blood can enter tumors passively via the EPR effect
Continuous and well formed endothelial cell junctions: normal vasculature
Almost no proteases
Tumor
Extravasation and retention of micelles in the tumors
Lymphatic drainage

Scheme of modification



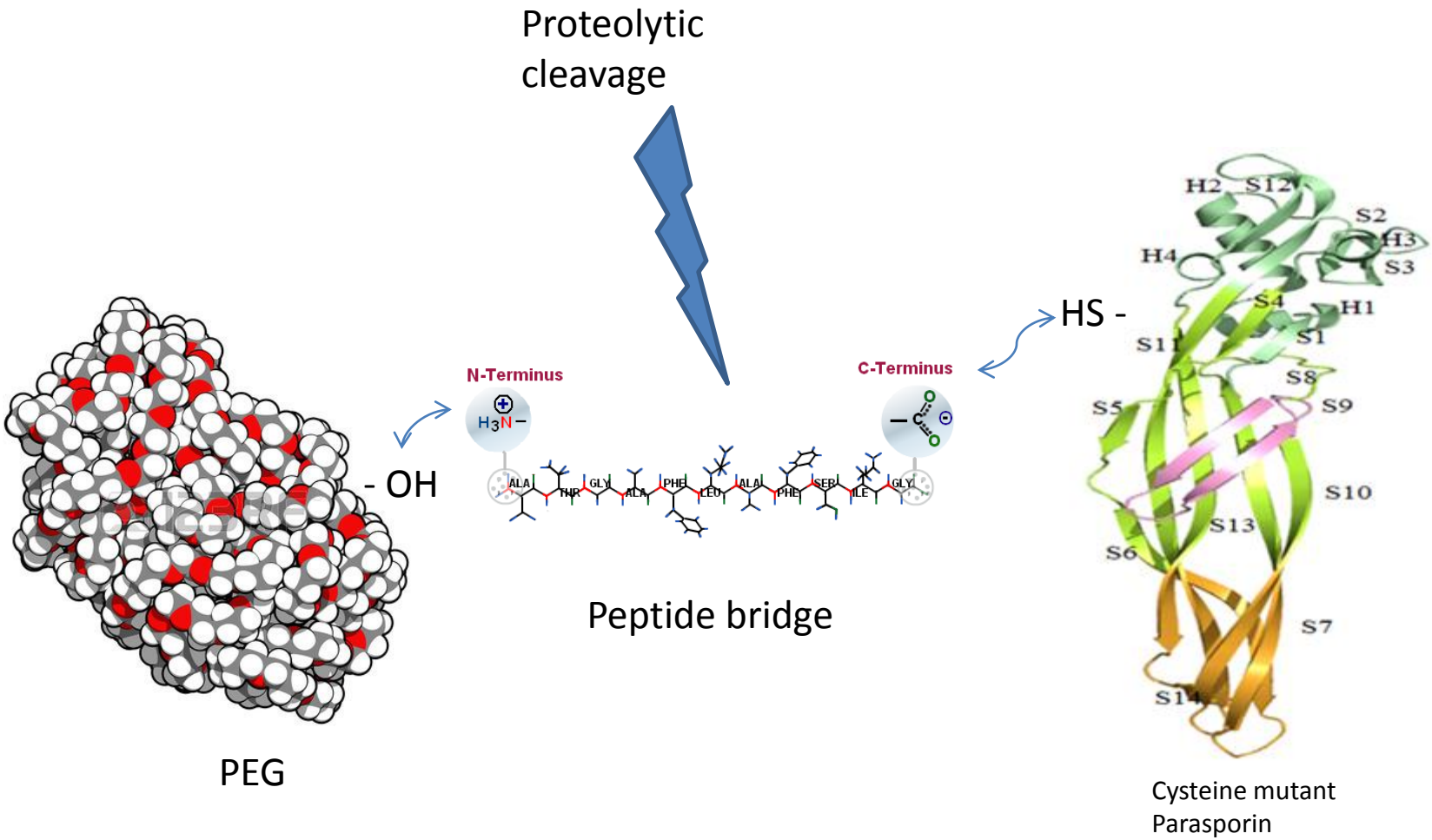
PEG

Peptide bridge

Cysteine mutant
Parasporin

40-50 nm

Scheme of modification



Thank you