I. Sojka, D., Franta Z., Horn, M., Mareš, M. and Kopáček, P. Tick gut cells express pattern of cysteine/aspartic proteases similar to schistosomal digestive network (in manuscript).

ABSTRACT
Ticks differ from other hematophagous arthropods in the intracellular localization of hemoglobin proteolysis. Hemoglobin digestion is a critical process for two main reasons: It provides primary nutrition and energy resources and it generates hemoglobin fragments with antimicrobial activity. Digestion in ticks is still poorly understood at the molecular level. We have analyzed the peptidase spectrum in the gut of the hard tick *Ixodes ricinus*, a vector of the Lyme disease and the tick-borne encephalitis. Substrate/inhibitor based profiling demonstrated the presence of endo- and exo-peptidases of cysteine and aspartic class in the tick gut homogenate. Screening of gut-specific cDNA by PCR amplification was performed with primers derived from the conserved regions of the detected peptidases. It resulted in identification of genes coding for (i) cysteine peptidases: cathepsin B and L, and dipeptidyl peptidase I (cathepsin C), and (ii) aspartic peptidase cathepsin D. The gene coding for asparaginyl endopeptidase was described previously (Sojka et al., 2007). The tissue expression analysis by RT PCR revealed that all identified peptidases are expressed in the gut of partially engorged tick females. Taken together, the proteolytic machinery in the tick gut closely resembles the digestive system of *Schistosoma* blood flukes but differs substantially from hematophagous insects relying mainly on serine peptidases.

II. Sojka, D., Hajdušek, O., Dvořák, J., Sajid, M., Franta, Z., Schneider, E.L., Craik, C.S., Vancová, M., Burešová, V., Bogyo, M., Sexton, K.B., McKerrow, J.H., Caffrey, C.R. and Kopáček, P. (2007) IrAE - An asparaginyl endopeptidase (legumain) in the gut of the hard tick *Ixodes ricinus*. Int. J. Parasitol. *37*, 713–724.

ABSTRACT
Ticks are ectoparasitic blood-feeders and important vectors for pathogens including arboviruses, rickettsiae, spirochetes and protozoa.As obligate blood-feeders, one possible strategy to retard disease transmission is disruption of the parasite's ability to digest host proteins.However, the constituent peptidases in the parasite gut and their potential interplay in the digestion of the blood meal are poorly understood.We have characterised a novel asparaginyl endopeptidase (legumain) from the hard tick Ixodes ricinus (termed IrAE), which webelieve is the first such characterisation of a clan CD family C13 cysteine peptidase (protease) in arthropods. By RT-PCR of different tissues,
IrAE mRNA was only expressed in the tick gut. Indirect immunofluorescence and EM localised IrAE in the digestive vesicles of gutcells and within the peritrophic matrix. IrAE was functionally expressed in Pichia pastoris and reacted with a specific peptidyl fluorogenicsubstrate, and acyloxymethyl ketone and aza-asparagine Michael acceptor inhibitors. IrAE activity was unstable at pH P 6.0 and wasshown to have a strict specificity for asparagine at P1 using a positional scanning synthetic combinatorial library. The enzyme hydrolyzedprotein substrates with a pH optimum of 4.5, consistent with the pH of gut cell digestive vesicles. Thus, IrAE cleaved the major protein of

the blood meal, hemoglobin, to a predominant peptide of 4 kDa. Also, IrAE trans-processed and activated the zymogen form of Schistosomamansoni cathepsin B1 – an enzyme contributing to hemoglobin digestion in the gut of that bloodfluke. The possible functions ofIrAE in the gut digestive processes of I. ricinus are compared with those suggested for other hematophagous parasites._ 2007 Australian Society for Parasitology Inc. Published by Elsevier Ltd. All rights reserved.**Keywords:** Asparaginyl endopeptidase; Legumain; Protease; Tick; Midgut; Hemoglobin digestion; Ixodes ricinus

III. Grunclová, L., Horn, M., Vancová, M., **Sojka, D**., Franta, Z., Mareš, M., Kopáček, P.(2006) Two secreted cystatins of the soft tick *Ornithodoros moubata*: Differential expression pattern and inhibitory specificity. Biol Chem. *387*, 1635-1644.

ABSTRACT
Two genes coding for cysteine peptidase inhibitors of the cystatin family (Om-cystatin 1 and 2) were isolated froma gut-specific cDNA library of the soft tick *Ornithodoros moubata*. Both cystatins were clearly down-regulated after a blood meal. Om-cystatin 1 is mainly expressed in the tick gut, while Om-cystatin 2 mRNA was also found in other tick tissues. Authentic Om-cystatin 2 was significantly more abundant than Om-cystatin 1 in the gut contents of fasting ticks and was associated with hemosome-derived residual bodies accumulated in the gut lumen. Om-cystatin 2 was also expressed by type 2 secretory cells in the salivary glands of unfed ticks. The inhibitory specificity of recombinant Om-cystatins 1 and 2 was tested with mammalian cysteine peptidases, as well as endogenous cysteine peptidases present in the tick gut. Both cystatins efficiently inhibited papain-like peptidases, including cathepsin B and H, but differed significantly in their affinity towards cathepsin C and failed to block asparaginyl endopeptidase. Our results suggest that the secreted cystatin isoinhibitors are involved in the regulation of multiple proteolytic targets in the tick digestive system and tick-host interaction. **Keywords:** cystatin; cysteine peptidase inhibitors; midgut; *Ornithodoros moubata*; salivary glands; tick.

IV. Saravanan, T., Weise, C., **Sojka, D**., Kopáček, P. (2003). Molecular cloning, structure and bait region splice variants of alpha2-macroglobulin from the soft tick *Ornithodoros moubata.* Insect Biochem. Mol. Biol. *33*, 841-851.

ABSTRACT
The sequence of a α2-macroglobulin (α2M) from the soft tick *Ornithodoros moubata* (TAM) was determined by cloning and sequencing of overlapping polymerase chain reaction (PCR) and rapid amplification of cDNA ends PCR products. The TAM cDNA sequence is 4944 bp long and contains one open reading frame coding for a protein precursor composed of 1494 amino-acid residues, including a 24-residue signal sequence. The mature protein is cleaved into two subunits similarly to the C3 and C4 components of complement and fish α2Ms. Phylogeny analysis revealed that TAM is closely related to *Limulus* α2M and displaysthe highest similarity to the partial sequence of α2M from hard tick *Ixodes scapularis*. The comparison of conserved cysteine

residues between TAM and human and *Limulus* α2Ms made it possible to predict the pattern of disulfide bridges and explain the atypical molecular arrangement of TAM. Four variants of the TAM bait region differing only in a short central segment were found; our data indicate that TAM exists as a single-copy gene in the tick genome and its bait region variants likely arise by alternative splicing. TAM is produced by tick hemocytes and it is also significantly expressed in salivary glands. TAM mRNA levels were shown to be up-regulated upon blood meal.
 Keywords: *Ornithodoros moubata*; Innate immunity; α2-macroglobulin; Bait region; Thioester