



**8th Bulgarian Peptide Symposium is organized by the  
Medical Faculty of the Trakia University, Stara Zagora;  
National Science Fund of Bulgaria and Bulgarian Peptide Society**



# **8<sup>TH</sup> BULGARIAN PEPTIDE SYMPOSIUM with international participation**

## **PROGRAMME**

**Stara Zagora Mineral Baths  
June 08 – June 10, 2018**



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## P20. CONFORMATIONAL AND THERMAL STABILITY OF A CONJUGATED WITH VITAMIN B9 HEMOCYANIN FROM *RAPANA THOMASIANA*

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Hemocyanins (Hcs) are oxygen-transporting proteins that present in the hemolymph of various mollusks and arthropods [1]. They exhibit numerous physiological activities including non-specific stimulation of the immune system, anticancer, antibacterial, antiviral, radioprotective activities, and some other [1]. Chemical modification of the Hcs may result in changes in their structure and can alter or enhance their biological activities. This is the **first report** on the conjugation of a hemocyanin from *Rapana thomasiana* (RtH) with vitamin B9 (RtH-vit B9). The reaction was conducted in two steps. At first, N-hydroxysuccinimide ester of vitamin B9 was obtained, then the activated ester was reacted with the RtH molecules. The target RtH-vit B9 was purified using PD-10 desalting columns. The degree of the modification was estimated spectrophotometrically by the changes in the absorbance at 363 nm and the protein concentration was determined using Bradford's method.

The FTIR spectra of the native and the modified RtH were collected by direct deposition of the samples on attenuated total reflectance (ATR) element in frequency region 4000–600 cm<sup>-1</sup> (ATR) with 128 scanning and at resolution of 1 cm<sup>-1</sup>. Then the ATR-FTIR spectra were Fourier deconvoluted by Opus software version 5.5 using band width of 14 cm<sup>-1</sup>, 2.9 resolution enhancement factor, and Lorentzian lineshape in order to describe quantitatively the changes in the Hc secondary structure that are due to the modification with vitamin B9. Second derivative spectra were obtained using the Savitzky–Golay algorithm based on 25 smoothing points. Then, the relative contribution of each band component of the Amide I band was determined by curve fitting following the procedure of OPUS program. Differential scanning calorimetry was used to monitor the changes in the thermal stability of RtH upon modification with vitamin B9. Correlation between the degrees of modification, changes in the RtH secondary structure and their thermal stability were observed.

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### References:

1. C. J. Coates, J. Nairn, Dev. Comp. Immunol. 45 (2014) 43–55.