

Posudek oponenta habilitační práce

Masarykova univerzita

Fakulta Přírodovědecká fakulta MU
Habilitační obor Biomolekulární chemie

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Habilitační práce Nucleic acid interactions and genome stability

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Text posudku (rozsah dle zvážení oponenta)

The research work of Dr. Ctirad Hofr presented in this Habilitation Thesis is devoted to interactions between nucleic acids and other molecules- either drugs or selectively binding proteins.

The first part reports studies of the interaction between the antitumor drugs cisplatin and oxaliplatin, and DNA. The author carried out microcalorimetry measurements to show, firstly, that the thermodynamic profiles of cisplatin-DNA complexes differ substantially from those of the ineffective configuration-isomer transplatin. Secondly, in studies carried out on alternative potential metallodrugs which were synthesized in the groups of N. Farrell, P. J. Sadler and L. Messori, the candidate provided evidence for the different effects that these metal complexes have, upon DNA binding, on DNA melting and DNA recognition by cellular proteins. In the summary of this part, the author concludes that these findings have been used in the design of more effective anticancer drugs. Here the keen reader would wish to learn more about the specific insights from the fundamental research that have found application in the design of new drugs.

The microcalorimetry experiments of C. Hofr constitute an important and indispensable pillar of numerous seminal papers published by the Brabec group (partly in collaboration with the above-mentioned groups). Because of his expertise, Mr. Hofr was invited for a postdoc stay at the Department of Chemistry and Chemical Biology at Rutgers, The State University of New Jersey, USA (2002-2003).

In the study of the chirality-dependent structural effects on DNA displayed by the enantiomers of the oxaliplatin-analogue Pt-dimethylbutane (paper by Malina et al (2000)), the authors concluded that hydrogen bonding is responsible for the observed differences in structure and thermodynamics, and this conclusion is repeated in this Thesis. However, this conclusion was subsequently invalidated by Delalande et al. (Biophys. J. 88, 1-11 (2005)) in the same laboratory. How could Dr. Hofr ignore the work of his own colleagues?

In the second part, Dr. Hofr investigated the interaction between telomeric DNA and telomere-binding proteins. Using the electrophoretic mobility shift assay, isothermal titration calorimetry, fluorescence anisotropy, and surface plasmon resonance measurements for the determination of dissociation constants, he obtained valuable insights into the mechanism of selective protein recognition of telomeric DNA.

The third part is devoted to protein binding to double-stranded RNA. C. Hofr contributed fluorescence anisotropy measurements to three solution structure studies on protein-RNA

systems, which were published in high impact journals.

Overall, the manuscript is well and understandably written, especially for the general reader. On the other hand, the work of the opponents would have been significantly easier if the proper achievements of the author were more clearly distinguished in the text.

Dotazy oponenta k obhajobě habilitační práce (počet dotazů dle zvážení oponenta)

1. Could the candidate specify what is meant by "... was the first study that quantitatively demonstrated the sequence-specific recognition..." (Section 3.3)?

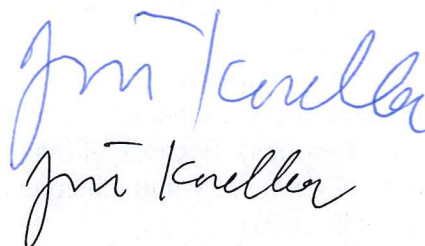
2. The paragraph of the article published in Biochem J. entitled "Binding kinetics" seems to comment only on measurements of the binding affinity, i.e., on thermodynamics. Is the subtitle in error?

In fact, the whole article promises „a quantitative study of telomere-binding specificity and kinetics“, yet the opponent was not able to find any kinetic data in the paper. Could the author point out the truly kinetic experiments in this work, or, eventually, in other studies? SPR does not seem to have been used here to obtain rate constants, why?

Závěr

Habilitační práce Ctirada Hofra „Nucleic acid interactions and genome stability“ *splňuje* požadavky standardně kladené na habilitační práce v oboru Biomolekulární chemie.

V Brně, 17. září 2014



Jiri Kruller