Referee’s opinion

On the PhD thesis of Mária Soltésová, entitled as „Fast Dynamic Processes in Solution Studied by NMR Spectroscopy“.

The dissertation was accomplished within a joint PhD program between Stockholm University and Charles University in Prague under the supervision of Dr. Jan Lang (Prague) and prof. Jozef Kowalewski (Stockholm). The program belongs to Physics including specialisation in Biophysics, Chemical and Macromolecular Physics.

The topics chosen by the candidate is modern and evergreen at the same time. There is a long tradition of NMR relaxation studies at the Physical Chemistry of SU that is now further strengthened by a thesis connected to a student from Prague and his supervisor. In fact NMR has two faces: the energy levels of spin systems (NMR Hamiltonian) and relaxation that governs the return of spin-states towards equilibrium. Since the latter is dependent on the stochastic behaviour of spins approaching the Avogadro number, interpretation of molecular dynamics from NMR relaxation data is far from trivial. NMR relaxation phenomena are exclusively connected to rotational diffusion however, translational diffusion of dissolved molecules can be also monitored by field gradient NMR. The candidate applied all methods in her work, in order to have detailed information on molecules in solution ranging from simple to complex systems. The thesis is organized as follows: a short introduction (2 pages) is followed by a kind of literature survey, first on the studied systems of host-guest systems, oligo-and polysaccharides and ethanol clusters (7 pages). Then, in the theory and methods part NMR basics, chemical exchange and NMR relaxation is overviewed (27 pages). This part is followed by the discussion of her five papers, concentrating briefly to the results without details. (She has not only the fish but the web?) 109 papers are cited in the list of references. In the end, the five papers are attached: in two of them MS is the first Author (the ethanol paper is „submitted“). In the introduction one motivation of her work is underlined: the choice of relatively simple systems compared to biological entities is a way towards the understanding of weak molecular interactions. This approach is welcome for the elaboration of more complex system studies. In section 2, we get an introduction to the molecular systems studied by the candidate. Though cryptohanes are not very simple hosts, their chosen guests are. Ethanol is a classical target of NMR, and we learn that clustering is present. Oligo and polysaccharides seem less complicated than big proteins or DNA, but this is not necessarily true for their NMR relaxation. Section 3 covers also the basic principles of NMR, that is useful for the newcomers on the field. However, I think the 3.1-3.2 chapter could have been left out or shortened. The simple theoretical description of translational diffusion, two-site exchange and host-guest equilibrium is given. NMR relaxation is dealt with in somewhat more detail, especially for $^{13}$C nuclei, except CSA mechanism, that is less important for carbohydrates. Cross-correlated cross-relaxation is described for dipole-dipole relaxation in AMX spin systems. Different Lipari-Szabo „model-free“ interpretations of relaxation data are described, but the SRLS approach is missing here and deferred to section 4.

In section 4. the cryptophane story is reloaded based on two papers, the second one is a kind of revision of the earlier one on cryptophane-C/CHCl$_3$ or CH$_2$Cl$_2$ host-guest systems. (The two papers (I. and II.) are shared between Z. Takács and M. Soltésová as it is correctly stated in the list of papers.) This is a really
complicated host-guest system, because the host is conformationally mobile, the binding of the guests are weak, and exchange is present everywhere. This is the reason why NMR requires support from computational chemistry, that allows to make qualitative distinctions between the host conformers. The second paper resolved the contradiction (paper I) between the possible orientations of the CHCl₃ guest within the cryptophane cage, suggesting the CH vector points rather towards the cap without OMe groups, now in agreement with NOESY/ROESY NMR data and DFT calculations. The results may have implications concerning the conformational selection model of molecular recognition.

In the toughest paper III, the complicated ¹³C relaxation of oligosaccharides (a trimannoside-6-¹³CH₂ labelled- and a blood group related pentasaccharide LNF-1) is proposed to solve by a diffusive chain model (DCM) that can be thought as an extension of the SRLS model of Freed and Polimeni. The aim is to determine theoretically the conventional ¹³C NMR measurables (T₁, T₂, NOE) and dipole-dipole cross correlated relaxation rates. If the calculated and experimental values agree within the error limits, then the model and the derived parameters are accepted. To this end a fairly sophisticated "integrated" approach is required in several steps: (i) molecular dynamics (MD) simulation (2-300 ns long), (ii) hydrodynamics (HD), (iii) multidimensional diffusive description of global and local dynamics. As shown in the supporting info, the calculated and observed NMR measurables agree quite well, either from anisotropic MF or DCM₂ models. Perhaps theoretical and experimental spectral densities could have been compared directly, in addition to comparison of NMR data (e.g. generated from the Brownian dynamics trajectory and the observed relaxation data). The new "integrated" DCM approach – though demanding – may be useful for the characterisation of the fast global/local dynamics of flexible molecules.

Paper IV is about the dynamics of the E. coli O91 O-antigen polysaccharide with three hydroxymethyl and one methyl groups, all ¹³C labelled. Earlier, the same polysaccharide was thoroughly investigated using the ¹³C-1 (all anomic carbons) labelling (Widmalm 2004). I think the sugar ring labelling is due to historical reasons, but it is more difficult for the reader to follow the paper in the non-sequential EADBC order and seeing no difference between methyl and methylene groups in the tables. The relatively short τₐₕ = 5.4 ns correlation time (compared to globular proteins of the same 11 kDa molecular mass) was borrowed from that work. The insensitivity of the τₐₕ value on the quality of LS fits is strange. Different versions of the Lipari-Szabo methods has been tested, including the extension of Skrynnikov for methyl groups (LS₄). The extended LS model with fast and slow local time scales fit the data most accurately. The difference between A and C&D rings is interpreted as a consequence of the different configuration at C4, having an axial oxygen in the interglycosidic bond in case of A (galacto), in contrast to the equatorial (gluco), close to the rotating CH₂OH groups.

Paper V. (Ethanol paper, submitted)

Here the size of H-bonded ethanol clusters was determined in deuterated hexane solutions as a function of temperature. To this end the spherical TMS was used as a reference in translational diffusion (DOSY) experiments. Since DOSY provides the apparent hydrodynamic radius, this must be converted to cluster size. To this end the HydroNMR program of de la Torre was used. TMS served also as a probe to determine the viscosity of deuterated hexane as a function of temperature (equation 3., not 2.) from the
published viscosities of n-hexane. The connection between the hydrodynamic radius and cluster size is based on DFT calculations. It is stated that ethanol monomers exist above 330K and up to hexa/heptamers are present below 210K.

**Summary:**

Reading the details of the papers I confirm that the experimental NMR work carried out by the Candidate was of excellent quality. The interpretation of the results, and conclusions derived are sound and critical at the same time. I accept those results that are attributed to the Candidate as new scientific results, that were demonstrated in four publications and one submitted paper. Based on this, I recommend to give her the requested PhD degree, supposed that her defense is successful.

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