

protomer. However, our knowledge regarding the function of the second site remains unknown. Additionally, the mechanism of structural impact of S635 and T690 phosphorylation on Rad50 structure and global conformation of MRN complex is also elusive. In this study we produced a central 182-aa fragment of human Rad50 with phosphomimetic mutation T690E residue which was subjected to UV-Vis spectroscopy and spectropolarimetric studies with Zn(II) in order to determine the stability of non- and phosphomimetic states. N-terminally fluorescently labeled proteins, were subjected to examine conformational changes of Zn(II) complex under phosphorylated and dephosphorylated states. Our study indicates that phosphorylation of Rad50 at T690 decreases affinity of zinc hook to Zn(II) ion and promotes major conformational change in coiled coil region in the homodimer. This work was supported by the National Science Center of Poland under Opus grant no. 2014/13/B/NZ1/00935.

### P.18-060-Wed Effect of crowding on oligomeric state of sHsps at elevated temperatures

N. Chebotareva, S. Roman, V. Mikhaylova, T. Eronina, B. Kurganov

*Department of Structural Biochemistry of Proteins, Bach Institute of Biochemistry, Federal State Institution "Federal Research Centre "Fundamentals of Biotechnology" of the Russian Academy of Sciences" 33, bld. 2 Leninsky Ave., Moscow 119071, Russia, Moscow, Russia*

Protein aggregation is a universal and unfavorable process for all cells leading to the production of non-native protein structures. It is known that protein aggregates are a hallmark of an increasing number of human diseases including neurodegenerative disorders. Small heat shock proteins (sHsps), as a class of molecular chaperones, form a large family of ubiquitous proteins, which act to prevent protein aggregation. As a rule, sHsps tend to form highly dynamic assemblies of different size and composition, which exchange subunits constantly. It is supposed that the polydispersity and quaternary structure dynamics play an important role in cellular sHsp chaperone function. The detailed mechanism of sHsps chaperone function remains debatable; however, it is often supposed that the large assembly of sHsps undergo reversible dissociation followed by interaction with unfolded proteins and subsequent reassociation to large chaperone-substrate complexes. Unfortunately, there are no data on oligomeric states of sHsps collected either directly in vivo or under conditions realistically mimicking the cell interior. Here, we present a few studies on assembly/disassembly and oligomeric distributions of several sHsps at elevated temperatures in vitro in the presence of agents that mimic crowded conditions. We showed by analytical ultracentrifugation that  $\alpha$ -crystallin and  $\alpha$ B-crystallin dissociated at elevated temperatures (40 and 48°C) in dilute buffer solutions. However, under crowded conditions sHsps tend to form large assemblies at elevated temperatures. For example, sedimentation coefficient,  $s_{20,w}$ , of HspB5 increases from 11 S in dilute solution to 20 S and 40 S in the presence of crowded agents and molecular mass of HspB5 increases from 480 kDa to 2 MDa. This study was funded by the Russian Foundation for Basic Research (grant 16-04-00560-a).

### P.18-061-Mon Physico-chemical properties of the chimeric tobamovirus coated with hordeivirus capsid protein with the deleted C-terminal region

S. Makarova<sup>1</sup>, A. Makhotenko<sup>1</sup>, A. Khromov<sup>1</sup>, V. Makarov<sup>2</sup>, N. Kalinina<sup>2</sup>

<sup>1</sup>*Biological Department, Lomonosov Moscow State University, Leninskie Gory, bld.1/12, Moscow, Russia,* <sup>2</sup>*Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Leninskie Gory, bld.1/40, Moscow, Russia*

We replaced the CP gene of turnip vein cleaning tobamovirus (TVCV) by the mutant CP gene of Barley stripe mosaic hordeivirus (BSMV) with deletion of 22 C-terminal amino acid residues (TVCV  $\Delta$ C-CP BSMV). Previously we demonstrated that the infectious cDNA clone of the mutant chimeric virus agroinfiltrated into *N. benthamiana* plants efficiently accumulated in infected and systemic leaves. Here we studied the physico-chemical characteristics of TVCV ( $\Delta$ C-CP BSMV) isolated from the systemic symptomatic leaves. The virus preparation was characterized by transmission electron microscopy (TEM) and atomic force microscopy (AFM). The TEM data demonstrated that the preparation contained a heterogeneous set of filamentous structures with width of 5–7 nm, variable in length forming network clusters of a weak electron density. Similar images were obtained by AFM. Measurement of the hydrodynamic diameter of TVCV ( $\Delta$ C-CP BSMV) particles by dynamic laser light scattering showed the presence of two peaks with sizes of about 100 nm and 800 nm indicating that the virus particles also form aggregates in the solution. However the removal of the C-terminal fragment of the CP BSMV does not significantly affect either the protein structure or its surface properties according to the data of circular dichroism spectroscopy in the near ultraviolet range and the measurement of the surface zeta potential. It is known that the C-terminal disordered fragment of the CP is not involved in the formation of intersubunit interactions in the mature virion but we can not rule out that it participates in the initial stages of the virion assembly. Our data indicate the importance of the integrity of the CP C-terminal region for the correct assembly of virions, and possibility of effective systemic transport of the tobamovirus genome in form of chimeric atypical virions (assumed ribonucleoprotein complexes). This work was supported by the Russian Science Foundation project No 14-24-00007.

### P.18-062-Tue Structural characterization of transferrin-bound ruthenium(III) terpyridine complexes

M. Matijevic<sup>1</sup>, M. Cindric<sup>2</sup>, M. Petkovic<sup>1</sup>, M. Nisavic<sup>1</sup>

<sup>1</sup>*Vinca Institute of Nuclear Sciences, Belgrade, Serbia,* <sup>2</sup>*Ruder Boskovic Institute, Zagreb, Croatia*

Human serum transferrin (Tf) is 80 kDa protein that readily binds and transports  $\text{Fe}^{3+}$  throughout bloodstream and tissues. Transferrin contains two  $\text{Fe}^{3+}$  binding sites where two Tyr, one Asp and one His residues are included in  $\text{Fe}^{3+}$  binding. Also, Tf is believed to transport various metals and metal-based drugs, including Ru anticancer drugs. Since majority of tumor cells overexpress Tf receptor, delivery of Ru drugs via Tf cycle increases drug selectivity. Although some data on Ru(III) drug binding to Tf exists, data on Ru(II) drugs binding to this protein is scarce. In this work, binding of two Ru(II) drugs of general formula  $\text{mer-}[\text{Ru}(\text{L}3)(\text{N-N})\text{Cl}][\text{Cl}]$  (where L3 = 4'-chloro-2,2':6',2"-terpyridine (Cl-tpy); N-N = 1,2-diaminoethane (en) or 1,2-diaminocyclohexane (dach)) to Tf has been confirmed using liquid chromatography (LC) and matrix-assisted laser desorption

and ionization mass spectrometry (MALDI MS). For the purpose of determining exact binding sites, Tf was incubated with 10 fold molar excess of each complex for 24 h. Unbound portion of the complexes was removed by ultrafiltration and the obtained adducts were subjected to trypsin digestion. The resulting peptides were separated using LC, and Ru-containing fractions were collected for MALDI MS analysis. The obtained spectra revealed presence of five ruthenated peptides. Binding amino acids have been determined by MS/MS analysis of target sequences. According to the obtained results, both Ru(II) complexes bind five histidine residues, namely: His642, His300, His585, His289 and His273. Only His585 is included in  $Fe^{3+}$  binding site, while other His residues are mainly located on the protein surface. It can be concluded that both complexes show high affinity towards His residues and since the binding of the complexes does not cause changes in Tf structure, it can be suggested that these compounds can use Tf cycle to be actively transported into tumor cells.

### P.18-063-Wed

#### The effect of arginine and ionic strength on aggregation of UV-irradiated glycogen phosphorylase *b*

V. Mikhaylova, T. Eronina, N. Chebotareva, B. Kurganov  
*Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia*

Arginine (Arg) is widely used not only because it stabilizes proteins and accelerates their folding, but also because of its ability to suppress protein aggregation. However, Arg is a charged molecule, and therefore, when studying the action of Arg on protein aggregation, the effects of ionic strength should be taken into account. In this work the effect of ionic strength and Arg on aggregation of UV-irradiated glycogen phosphorylase *b* (UV-Phb) was studied. With this test system we can obtain information on the effects of different agents on aggregation of denatured protein molecules. The process of UV-Phb aggregation includes the rate-limiting monomolecular stage of structural reorganization of the Phb molecule, containing concealed damages, and a relatively fast aggregation stage. The process of structural reorganization of UV-Phb is characterized by a first-order rate constant ( $k_1$ ). The kinetics of UV-Phb aggregation was studied using dynamic light scattering (DLS) at 37°C (0.03 M Hepes buffer, pH 6.8) at various ionic strength values (0.02–0.7 M NaCl or Arg). It was shown that an increase in NaCl concentration caused a decrease in the  $k_1$  value, suggesting a slowdown of the UV-Phb structural reorganization process. Circular dichroism data confirmed this conclusion. Analytical ultracentrifugation and DLS data have shown that an increase in ionic strength leads to the formation of smaller aggregates, testifying that the change in the aggregation pathway occurs. To evaluate the effect of Arg, we conducted experiments at fixed values of ionic strength (0.15 M and 0.5 M NaCl or Arg). It was shown that at a low ionic strength Arg accelerated the process of protein aggregation and induced changes in the aggregation pathway. At high concentrations Arg acts as a charged molecule, and its effect on protein aggregation is due solely to a change in ionic strength of the solution. The study was funded by the Russian Science Foundation (grant 16-14-10055).

### P.18-064-Mon

#### Relationship of growth rate and muscle protein turnover in Atlantic salmon *Salmo salar* L. under natural and artificial photoperiods

N. Kantserova, L. Lysenko, N. Nemova  
*IB KarRC RAS, Petrozavodsk, Russia*

It is known that protein degradation in fish muscles depends on three proteolytic systems such as cathepsins, calcium-dependent proteases (or calpains), and the ubiquitin-proteasome system (UPS). Calcium-dependent proteolysis is a major pathway regulating muscle turnover in fish, while cathepsins and ubiquitin-targeted protein digestion by the proteasome are primarily responsible for bulk protein degradation. Calpain activity is considered as a marker of fish growth and health state at different life stages along with myofibrillar protein expression levels, key digestive and metabolic enzyme activities, lipid contents, etc. This study was conducted to evaluate the effects of natural and artificial (24:0 or 18:6 light:dark, L:D) photoperiods on the growth rate and calpain activity in the skeletal muscles in Atlantic salmon. Maximal growth rate was attained at 24L:0D, followed by 18L:6D, and a minimal was induced by a natural photoperiod. The results suggested positive correlation between length-weight growth dynamics in individuals and calcium-dependent proteolysis level in their skeletal muscles as well as their orchestrated regulation by photoperiod variations. Stimulating effect of a continuous (24L:0D) light photoperiod on growth performance and muscle protein turnover in salmon was concluded. The work was supported by the Russian Science Foundation, project no. 14-24-00102.

### P.18-065-Tue

#### Inhibition of the actin N-terminal acetyltransferase NAA80

M. Baumann<sup>1</sup>, L.M. Myklebust<sup>1</sup>, M. Goris<sup>1</sup>, R. Magin<sup>2</sup>, H. Foyen<sup>1</sup>, S.I. Støve<sup>1</sup>, R. Marmorstein<sup>3</sup>, B. E. Haug<sup>1</sup>, T. Arnesen<sup>1</sup>

<sup>1</sup>University of Bergen, Bergen, Norway, <sup>2</sup>University of Pennsylvania, Philadelphia, United States of America, <sup>3</sup>Perelman School of Medicine, University of Pennsylvania, Philadelphia, United States of America

A common modification of proteins is the acetylation of their N-termini by N-terminal acetyltransferases (NATs). As a member of the NAT-family, NAA80 transfers an acetyl group from acetyl coenzyme (Ac-CoA) to the acidic N-termini of processed animal actins. These actins are integral parts of the cytoskeleton that is responsible for cell shape and motility, thus making NAA80 a viable target for the regulation of cytoskeletal functions. In this work, peptidic bisubstrate inhibitors based on Ac-CoA and the natural actin substrates of NAA80 were developed and for their synthesis we employed Fmoc-based solid phase peptide synthesis (SPPS) to prepare tetrapeptides, which were subsequently attached to coenzyme A through an acetamide linker. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy were used for structure confirmation of the inhibitors, whereas the potency and selectivity of the inhibitors was evaluated through acetylation assays. Furthermore, in complex with the most potent inhibitor, the crystal structure of NAA80 was determined. This work marks the basis for development of more potent and selective inhibitors of NAA80 thereby increasing the number of tools available for regulation of actin and the cytoskeleton.