

$\text{Ag}_3^{2+}$  (72.8 kcal mol<sup>-1</sup>), between the  $\text{P}^{\text{tr}}\text{r}^{-1}$  and  $\text{Ag}_3^{2+}$  (95.9 kcal mol<sup>-1</sup>), which means that these complexes are preferably formed in aqueous solutions under acidic and alkaline pH, respectively. The latter complex possesses a long-wave maximum at 541 nm and a major peak at 361 nm in the absorption spectrum. The results obtained can be used for the development of fluorescent and surface-enhanced Raman scattering (SERS)  $\text{P}^{\text{tr}}$  biosensors. The calculations were carried out using the facilities provided by Resource Center «Computer Center of SPbU» (<http://cc.spbu.ru/en>). The work was supported by the Russian Science Foundation grant 20-73-10029.

#### P-05.2-44

#### Encapsulated mutant form of methionine $\gamma$ -lyase: steady-state kinetic and pharmacokinetic parameters of antitumor enzyme

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The methionine dependence is a well-known phenomenon in metabolism of cancer cells. Methionine  $\gamma$ -lyase (EC 4.4.1.11, MGL) catalyzes the  $\gamma$ -elimination reaction of L-methionine and thus effectively inhibits the growth of malignant cells. Therefore, the application of the MGL in enzyme anticancer therapy is relevant. To minimize several problems typical of therapeutic proteins including high plasma clearance, short half-life in the bloodstream, and high immunogenicity, the mutant form V358Y MGL with increased catalytic efficiency in the  $\gamma$ -elimination reaction of L-methionine was encapsulated in polyionic vesicles and steady-state kinetic and pharmacokinetic parameters of encapsulated enzyme were determined. The catalytic efficiencies of the encapsulated and naked enzymes in the  $\gamma$ -elimination reaction of typical substrates were comparable. The inclusion of V358Y MGL in polyionic vesicles allowed us to increase the stability of the enzyme in the blood stream by almost one order of magnitude compared to the naked enzyme ( $\tau_{1/2} = 50.8$  and 7.4 h, correspondingly). Thus, encapsulation of V358Y MGL in polyionic vesicles provides an improvement of the pharmacokinetic characteristics of the enzyme for further study as an antitumor agent for in vivo trials. Acknowledgements The work was supported by the Russian Science Foundation (project No. 20-14-00258).

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#### Detection of tyrosine using metal nanoclusters

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Tyr is involved in the synthesis of neurotransmitters, catecholamines, thyroid hormones, etc. There is a number of pathologies associated with impaired Tyr metabolism: phenylketonuria, hypothyroidism, tyrosinemia, alkaptanonuria, and vitiligo. We have shown the possibility of Ag nanocluster (NC) synthesis on Tyr. Ag NC can be applied for SERS and fluorescent Tyr detection. However, prior to development of Tyr biosensor one should understand the theoretical basics of interactions between Tyr and NCs. We calculated the binding energy ( $E_b$ ) between Tyr and  $\text{Ag}_n^z$  ( $n = 2-8$ ;  $z = 0-1$ ) NCs using DFT-D3 method: PBE functional with 6-31G(d,p) basis set, effective core potential

LANLTZ, and COSMO model for water. Since the growth of Ag NC occurs at pH 12.5 we studied both  $\text{T}^{\text{yr}}\text{r}^{-1}$  and  $\text{T}^{\text{yr}}\text{r}^{-2}$ : protonated and deprotonated through the side-chain ( $\text{p}K_a = 10.5$ ).  $\text{Ag}_5^+$  had the highest  $E_b$  equal to 87.3 kcal mol<sup>-1</sup>. The absorption spectrum of the  $\text{T}^{\text{yr}}\text{r}^{-2}/\text{Ag}_5^+$  complex had a long-wave maximum 525 nm and a major peak at 394 nm. At high pH values, Tyr can reduce  $\text{Ag}^+$  ions directly into Ag NCs or nanoparticles in the absence of additional reducing agents. In this case, the absorption peak at about 400 nm can be used to detect Tyr. The alternative strategy of Tyr detection is to use the already known Tyr RNA-aptamer (5'-GGGCAGUCACUCGUAGAUGGCCUACAGCGGUAAUACGGGGGUCAUCAGAUAGGGAGGCC-3'). We performed a docking of Tyr and the aptamer using AutoDock Vina 4.2 and found  $E_b$  equal to 7.9 kcal mol<sup>-1</sup> ( $K_d = 16 \mu\text{M}$ ), whereas the experimental value is 7.6 kcal mol<sup>-1</sup> ( $K_d = 22 \mu\text{M}$ ). Tyr forms H-bonds with A19, U46, A48, and hydrophobic interactions with U20, G21, and C47. We designed the aptamer-conjugated Ag NCs to develop a sensor for Tyr detection. The research was carried out using the facilities provided by Centre for Chemical Analysis and Materials Research, Optical and Laser Materials Research Centre, and Computer Center of SPbU. The work was supported by the Russian Science Foundation grant 20-73-10029.

#### P-05.2-46

#### Stressed by hydrogel? Physiological response of yeast cells embedded in thermoresponsive Pluronic F127: a prerequisite for bioreactor development

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One class of “smart” materials are thermoresponsive polymers, macromolecules that change their physicochemical properties with temperature. Pluronic F127 is one such biologically-compatible, reversely-gelling polymer, i.e. a material that is liquid near 0°C but hydrogel at room temperature. Such properties make F127 and its functionalised derivatives interesting for 3D bio-printing, as they can be mixed with bacteria, yeast, algae, or mammalian cells to form next-generation bioreactors. However, while initially promising, cells embedded in such hydrogels are impaired quickly, which requires tricky and expensive coupling between hydrogel design and the start of the bioprocess. To investigate the cause of this deterioration, we characterised the physiological state and stress response of yeast cells trapped in the hydrogel. We constructed a set of *Saccharomyces cerevisiae* strains expressing GFP-based ratiometric biosensors that measure cells’ cytoplasmic pH and energetic state. Moreover, we constructed strains expressing fluorescent proteins under the control of newly-designed promoters induced by a specific stress response. Our results provide insights into the interaction between yeast cells and hydrogels, allowing us to use functionalised F127 as a hydrogel matrix to construct yeast biosensors for the detection of common aquatic pollutants.