Session 11: Synthetic and system biology

Lectures

L11.1

Synonymous mutations in the light of translational regulation

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Translational regulation emerges as an important factor modifying protein abundances in eukaryotic cells. It influences protein concentration not only through the efficiency of translation process. What is probably more important is the regulation of mRNA concentration, most likely through degradation of mRNA, induced by quality control mechanisms. Recently we have shown that poly-lysine tracks can have substantial influence on mRNA and protein levels, depending on the codons (AAA vs AAG) encoding these tracks. Here we show the results of systematic screening of mutations within poly-lysine tracks encoded by AAA codons. Mining of the COSMIC database revealed skewed distribution of mutation types towards frameshifts. We argue that gene dosage effect is a plausible interpretation of the effects of certain recurrent synonymous mutations found in cancer tissues.

L11.2

RNA 3D structure prediction for RNA nanodesign

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RNA is a powerful biomaterial for nanotechnology applications due to its structural versatility, property of self-assembly, low free energy of annealing and possibilities for controlling the structure. The interest in RNA nanotechnology for disease therapy increasing and RNAs new roles in nanomedicine applications are expanding. An understanding of how natural RNA molecules fold and assemble is an essential element to allow using RNA for those applications. Similar to understanding protein structure, studies of RNA structure have a decades long history. However, contrary to proteins, methods for RNA 3D structure prediction just recently emerged. These methods are still in the early phrases of development and are not free from problems and limitations. However, understanding the limitations allows their successful utilization.

RNAComposer is a user-friendly and freely available server for 3D structure prediction of RNA. RNAComposer employs fully automated fragment assembly based on RNA secondary structure specified by the user. Predictions of RNA structures characteristic of randomized sequence demonstrating potential of this method to build artificial RNA 3D structures were successful [1].

High accuracy RNA 3D structure prediction is critical if the obtained model will be used for programmed self-assembly. We tested RNAComposer accuracy in RNA 3D structure prediction using the known structure the RNA that assembles into a ring. Subsequently, we applied our method to design a novel RNA motif and functionalize a known nanosquare structure. The workflow for utilizing RNAComposer in RNA design for applications in nanotechnology will be presented and its potential and limitations discussed.

Reference:

1. Popenda M *et al* (2012) *Nucleic Acids Res* **40**: e112.

Oral presentations

O11.1

The emerging role of origami DNA in studying membrane-related biological phenomena

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Most recently, origami DNA approach provided a variety of tools to study biological systems. This technique enables folding of long strands of DNA into nanostructures of defined shape and introduce chemically or structurally functional groups with nanometer precision. We employed origami DNA to construct amphipathic molecules that spontaneously bind to lipid membranes via cholesteryl anchors. We have constructed a set of amphipathic DNA nanostructures of various shapes and functionalities to mimic membrane-related biological events and to determine some features that could be characteristic for such molecules. Our toolbox include DNA origami nanoneedles that enabled us to study their partitioning in phase-separated lipid bilayers and to obtain detailed description of rotational and translational diffusion and isotropic-nematic transition of these molecules on the surface of lipid membranes. We also built membrane-anchored hexagonal DNA origami box that can be opened in a controllable way, which could create signal-driven tethering system potentially useful in spatial organization and regulation of membrane-embedded compartments. Another nanostructures, DNA origami monoliths decorated with sticky oligonucleotides at their lateral sites, appeared to be able to scaffold and deform lipid membrane vesicles. All these features are considered as critical in vesicle biogenesis within living cells, the process which is initiated by membrane deformation events controlled by proteins, such as those belonging to BAR superfamily. Our synthetic biology-inspired approach provide insight into yet unattainable aspects of binding, diffusion and oligomerization of molecules at the surface of biological membranes.

O11.2

Metabolomic scale analysis of the mechanism of C2 ceramide induced cell death

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Ceramide, a bioactive sphingolipid, is known to stimulate the cell death and suppress the cell proliferation. However, there are conflicting reports about the mechanism and the nature of Ceramide induced cell death. In (1) Ceramide has been proven to induce neuronal cells death through the mitogen-activated protein kinases (MAPK) dependent mitochondrial pathway. In the contrary, ultrastructural analysis of Ceramide treated NB16 neuroblastoma cells revealed 75% loss of cell viability mainly due to the development of necrotic cell death (2). Determination of cell death type that prevails in the above mentioned cases, is of particular importance for the selection of adequate bioassays for the measurement of cell viability.
We studied the influence of C2-ceramide, which is the exogenous cell-permeable C2-ceramide, on viability of Neuroblastoma SH-SY5Y cell lines. Transcriptomics data from Affimetrics Human Gene 2.1 ST array was analysed. Using a modified iMat method for transcriptomics data integration with global reconstruction of Human metabolome (Recon2), we have generated a case specific model that allows us an analysis of metabolic bases of ceramide induced death.
We have demonstrated that the inhibition of cell growth is related to the general disregulation of lipids metabolism as well. We also used our neuroblastoma metabolic model to analyse the effects of the inhibition and activation of transcriptomic factors selected using IPA analysis of transcriptomics data in particullar SMARCA4, NUPR1, and KDM5B. The results of our analysis are currently under biological validation.

References:

1. *Fillet M et al* (2003) *Biochem Pharmacol* **65**: 1633-1642.
2. *Ramos B et al* (2003) *Mol Pharmacol* **64**: 502-511.

Posters

P11.1

Design and synthesis of substrates and inhibitors of a human kallikrein-related peptidase 7

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Human kallikrein-related peptidase 7 is a serine protease with chymotrypsin-like activity which was originally isolated from stratum corneum tissue – the outermost layer of the epidermis. The hKLK7 is responsible for desquamation process of skin, and the dysfunction in the secretion of this enzyme could provide to many skin disorders [1]. Furthermore increased expression of this enzyme is linked to the prognostic value in many cancers and particularly in ovarian cancer, which has the highest mortality rate among gynecologic malignancies [2].

The first step of studies was investigating the substrate specificity of human kallikrein-related peptidase 7 in both P nonprime and P prime positions based on the Schechter-Berger notation [3]. The peptide libraries were synthetized manually using combinatorial chemistry methods on solid phase. Synthetized substrates were based on FRET (Fluorescence Resonance Energy Transfer) phenomenon – intramolecular transfer of electron excitation energy [4] and they contain in their structure donor (ABZ – 2-aminobenzoic acid) and acceptor of fluorescence (ANB – 5-amino-2-nitrobenzoic acid or Tyr(NO_2)-NH_2 – 3-nitrotyrosine respectively). The first step of our research was to determine the sequence of nonprime positions with the general formula: ABZ-X_3-X_2-X_1-ANB-NH_2. Based on the results of this experiment the next step was to determine the substrate for prime positions using peptide library with general formula: ABZ-Lys-Thr-Leu-Tyr-X_1'-X_2'-X_3'-Tyr(3-NO_2)-NH_2. The next step was synthesis of an activity-based probes based on the structure for non-prime positions with formulas: Biotin-Lys-Thr-Leu-Phe-CMK, Biotin-O2Oc-Lys-Thr-Leu-Phe-CMK and Ac-Lys-Thr-Leu-Phe-CMK, where Phe-CMK was phenylalanine chloromethyl ketone.

The iterative method of deconvolution and RP-HPLC analysis of obtained substrates against hKLK7 allows to determine the sequence for nonprime positions: ABZ-Lys-Thr-Leu-Tyr-ANB-NH_2 and the substrate for prime positions: ABZ-Lys-Thr-Leu-Tyr-Gly-Gln-Val-Tyr(3-NO_2)-NH_2. Kinetic parameters have been designated for both sequences and obtained activity based probes.

References:

1. Rawlings N D *et al* (2013) *Handbook of proteolytic enzymes* **3**: 2788-2792.
2. Tamir A *et al* (2014) *J Ovarian Res* **7**: 109-124.
3. Schechter I *et al* (1967) *Biochem Biophys Res Commun* **27**: 157-162.
4. Foerster T (1948) *Ann Phys* **2**: 55-75.

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P11.2

Characteristics of the human kallikrein-related peptidase 14

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Human tissue kallikrein 14 (KLK14) belongs to the fifteen serine proteases which represent the family of kallikreins. It has been suggested that KLK14 can have a prognostic value in ovarian and breast cancer [1], and it will be used as a biomarker of this kind of malignancy. Furthermore, KLK14 plays an important role as an activator of KLKs proteolytic process in seminal plasma and skin [2], and in pathological processes KLK14 is implicated in skin barrier-related disorders. KLKs are closely related enzymes sharing in part substrate specificity. The aim of this work was to obtain the novel and selective fluorogenic substrate for KLK14, which would able to distinguish substrate specificity of KLK14 from another member of KLK's family. Besides, the sequence of the best hydrolyzed fluorogenic substrate by KLK14 (hydrolyzed also by other enzymes of the KLK's family) was also optimalized. Based on the results, activity-based probes (ABPs) were designed and synthesized. At the end enzymatic studies were conducted.

In order to develop the new substrate for KLK14 two libraries of internally quenched peptides were designed. Their general formulas were as follows: non-prime library ABZ-X_4-X_3-X_2-Arg-ANB-NH_2, prime library ABZ-selected non-prime sequence-X_1-X_2'-X_3'-Tyr(3-NO_2)-NH_2, where in positions X_4 - X_3', the set of all proteinogenic amino acids (except cysteine) were introduced, in the position X_1 Arg was fixed. All libraries were synthesized applying the split-and-mix method and Fmoc chemistry on solid support. Synthesis of activity-based probes were based on the structure of selected non-prime positions for both optimalized substrates for KLK14. Activity-based probes were synthesized also on solid phase using Fmoc/tBu procedure. The general formula of ABPs was: Biotin-P_4-P_3-P_2-Arg-CMK, where Arg-CMK is arginine chloromethyl ketone.

The iterative method of deconvolution performed in solution yield the selective sequence: ABZ-Tyr-Gly-Pro-Arg-Val-Leu-Pro-Tyr(3-NO_2)-NH_2 recognize and hydrolyzed merely by KLK14 and the best hydrolyzed substrate: ABZ-Tyr-Ala-Ala-Arg-Ser-Ala-Arg-Tyr(3-NO_2)-NH_2. Enzymatic studies have shown that ABP with sequence Biotin-Tyr-Gly-Pro-Arg-CMK is the selective and irreversible inhibitor of KLK14 protease.

References:

1. Borgono CA *et al* (2007) *J Biol Chem* **282**: 2405-2422.
2. Emami N *et al* (2008) *J Biol Chem.* **283**: 3031-3041.

Acknowledgements:

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