EXAMINATION CHEMICAL BIOLOGY 2020-2021

Tuesday, 06-04-2021

13.00-16.00

Provided with this examination is one research paper on activity-based protein profiling.

https://www.nature.com/articles/s41589-020-00689-z.pdf

As explained during the course, printed manuscripts and hand-written notes are allowed at the examination. Electronic devices or internet connection are not allowed. For some questions, different solutions may apply.
10 points per question. You may answer the questions in any order. The exam counts 90% of the final grade and 10% of the grade is based on the presentations during the course.

A) Explain the design of the activity-based probes (ABPs) developed in this paper. What is the mechanism of these vinyl sulfone ABPs?

B) Which strategies do the authors adopt to improve selectivity of Cy5-QS1-VS (14)? (see extended data Figure 4C, right panel).

C) In Extended Data Figure 4C (left panel), additional bands are observed, which probably belong to endogenous biotinylated proteins. How could you avoit the labeling of such bands? Propose a new ABP design and mechanism, and a workflow of the experiment.

D) Can you provide a mechanism by means of which the SARS Cov-2M inhibitor **16** could be masked and anctivated on demand?

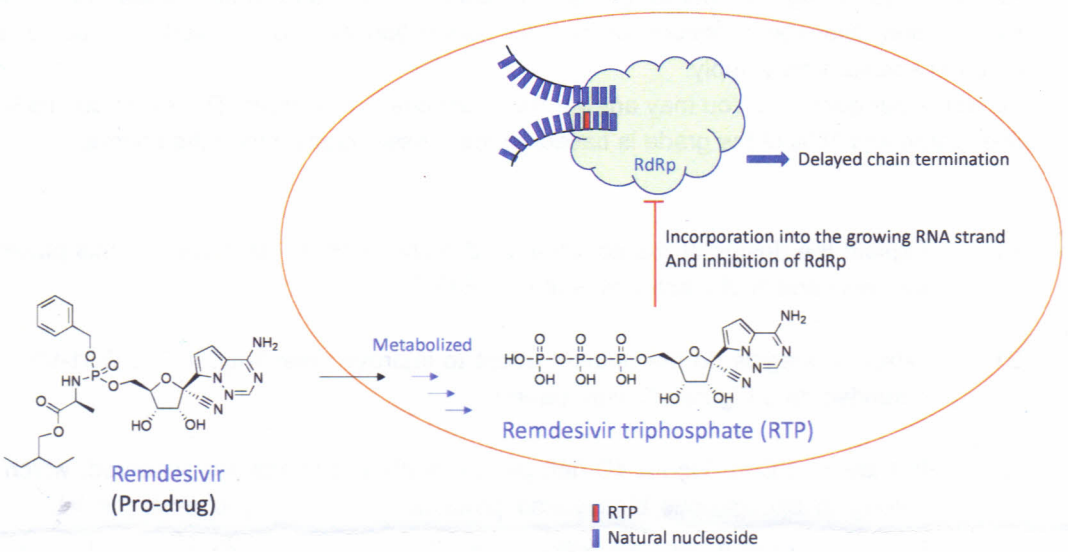
E) One of the disadvantages of activity-based protein profiling is that the methodology reports the number of copies of active enzyme, thus the enzyme gets inactivated. Can you design a reporter substrate that would inform on the activity of M^{pro}?

F) The authours unveil the mechanism of their inhibitors and probes by crystallographic studies, revealing amongst others the amino acid residue within the active site that resacts with the electrophilic warhead of the inhibitors/ABPs. Could you think about a different way to do so?

G) Confocal microscopy with Cy5-QS1-VS probe yielded some background fluorescence in the control sample (healthy patient) and the authors opted to use a Bodipy analogue for these experiments. Can you design a probe, or a strategy, that would allow for the exploration of different fluorofores at will in living cells?

H) Following the previous question, could you think of a different activity-based protein profiling strategy more suitable for living cells or tissues, in case the fluorescent or biotinylated ABPs turn out to be poorly cell permeable?

I) Remdesivir is a phosphoramidate prodrug that is metabolized in cells to yield an active nucleoside triphosphate (NTP) analog that it is refered to as remdesivir triphosphate (RTP). Biochemical studies showed that the RdRp can use RTP as a substrate, leading to the incorporation of remdesivir mono-phosphate (RMP) into the growing RNA product. After RMP incorporation, the RdRp extends RNA by three more nucleotides before it stalls. This stalling mechanism is specific to coronaviruses because the RdRp of Ebola virus can add five RNA nucleotides after RMP incorporation before it stalls.
Provide a chemical proteomics strategy that would allow for labeling of such RNA product.



J) At the time this paper was published there were no vaccines for the treatment of SARS-Cov-2 available yet. In the meantime, several vaccines have (fortunatety) been approved and if all goes well, we will be vaccinated all of us, (in the Netherlands at least, and if we wish so), within the next 12 Months. One aim of the paper is to discover SARS-Cov-2-selective inhibitors. Do you think these are still needed, or not necessary now that we have vaccines?