# Questions for the Selfstudy Material: Genome-wide System Perturbation

## Question 1

As discussed in the handout, off-target effects are a common problem in siRNA experiments. What is the chance that these off-target effects are caused by the siRNA's sequence being present, by pure chance, more than once in the human transcriptome (i.e. the entirety of the sequences transcribed from the genome, which corresponds to ~5% of the whole genome)? Assume random sequences.

## Question 2

Why is it that both the CRISPR/Cas9 and RNAi approach for perturbing systems at the DNA and RNA level respectively were so easily scaled up to the whole genome scale? Do you think that approaches for perturbing the function of proteins in a system can be extended just as easily to the proteome-wide level?

different DNAs and RNAs share the same physical and structural properties. Basically, every gene or transcript has an extremely high similarity to all other possible genes or transcripts, since the fundamental function of all DNA or RNA is the same. The substrate these technologies act on do not possess higher degrees of complexity that would influence the process such as 3D folding, glycosylation, complicated modifications etc.
in proteins it is more difficult, since one would need to specifically target their active site to produce a protein knockdown. since proteins look most of the time structurally different and also have different functions in an organism, it is probably impossible to apply a universal method to knockdown protein due to their high dissimilarity.

## Question 3

Would it be possible to perform an RNAi genome-wide perturbation experiment based on a selection strategy by using chemically synthesized siRNAs that are directly transferred into the cells?

yes. each subcompartment would get another siRNA

## Question 4

Which technology, RNAi or CRISPR/Cas9, would you use, if you want to probe the function of a promoter region in the genome.

CRISPR, since promoter regions do not normally yield mRNA and therefore, there is no target for RNAi

## Question 5

As you have read in the hand-out, the time course of RNAi-mediated gene knock-down experiments is relatively slow. It takes from several hours up to a few days before a reduction in the intra-cellular concentration of the targeted RNAs can be observed.

How does this compare with the time-scale of other processes in the cell/organism (metabolic reactions, transcription, translation etc.)? What does this imply about the changes we will observe as a result of an RNAi-induced perturbation? What else besides the primary effect of turning off the activity of the targeted RNA are we likely to observe?

the other processes are several log scales faster. obviously, there are proteins and metabolites around from before RNAi came into effect, which is why those need to be degraded first (they basically mask the knockdown), until the new phenotype will be visible.