We have identified **three overarching questions** that we invite you all to think about.  
Please use the channel “**Workshop Overarching Questions**” to provide your proposed contributions to these questions.

1. **What do we need to make DNA/RNA nanotech in living cells more predictable and usable?**
2. **In what ways should we be looking to further emulate natural molecular systems? In what ways should we be trying to do things differently?**
3. **What key applications/features can DNA/RNA nanotech in living cells enable?**

## What do we need to make DNA/RNA nanotech in living cells more predictable and usable?

* Wilhelm Huck’s automatic experimental design/parameterisation toolbox. Will this help? Is there a limit to how far you can get with such a setup? Is it always going to struggle against natural stochasticity, or can it be quantitative?
* Should we be looking to use chimeric systems (cyborgs – half living half synthetic) a la Oscar Ces? Will this make them more predictable?
* Should we use simplified environments for prototyping and characterisation? (Richard Murray)
* Should we use Machine Learning OR first-principle modelling OR a combination of both?
* Computational tools for predictive design, verification and realisation of nucleic-acid circuits in living cells.
* Will crowding allow us to make more use of spatial structure, having spatially resolved reaction-diffusion processes?
* Provocative Q: do we know enough (non-eq) physics? Do we know enough (systems) chemistry? Is it (just) an engineering problem now?
* Feedback control vital? Quis Controlet ipsos Controles?
* Erik Winfree: more components = more robust? Redundancy? Cooperativity? How can we systematically design things that work this way. ML works this way? Emergence? Need to have a way of tuning/evolving. Need a structure with this capacity? What’s the timescale? Simulation?
* Evolutionary dynamics will get in the way? Boot up stuff from the ground up (cf Richard Murray’s talk)
* Balance in vivo/in vitro. Difficult to move between the two?
* Look for compatibility first?
* Do you really need to know about the system you’re dealing with in great detail?
* General principles can only get you so far? Fine tuning inevitable?
* Interfacing more systematically with CRISPR?
* Universal fuel for nucleic acid nanotech

## In what ways should we be looking to further emulate natural molecular systems? In what ways should we be trying to do things differently?

* Learn to control crowding and build systems/synthetic cells that take crowding into account (Wilhelm Huck).
* OR, should we do things differently by avoiding crowding, since it makes things more complicated? I think the complexity/functionality tradeoff is an interesting and more general point.
* Can we get synthetic (nucleic acid) systems that interact (specifically?) with their surrounding membrane? Combining chemical information processing with mechanical response/cues.
* Robust, autonomous nucleic-acid systems in living cells? Feedforward vs Feedback vs Buffering systems implemented using nucleic acid circuits.
* Richard Murray’s vision of synthetic cells: spatial organisation, metabolic, sensing and signalling, regulation/computation, actuation. Can we build nucleic-acid systems at the core of each of these?
* We can build dense environments and we can also encapsulate things. Can we encapsulate a dense, structured environment?
* Do we need something like dynamic signal decoding (cf Mustafa Khammash)? Is this just a nice extra, or is it fundamental? If we’re engineering living cells, rather than building synthetic cells, is it forced upon us?
* Should we be building more systems that exploit futile cycles? (Domitilla del Vecchio)
* Should we be looking to implement computational systems like Damien Woods’ in cellular/synthetic cellular contexts. This is very different from nature – is there an advantage we could exploit?
* Do we need something like replication in a synthetic cell? Or can we do without?
* Synthetic machinery for programmed synthesis (of nucleic acid systems) - Andrew Turberfield
* External (thermal) cycling can be very useful. Should we be happy to work in that context, or do we really need to be more self-contained?
* Yannick Rondelez shows how encapsulation is essential for self-contained evolution (without screening). What else does it give you? Concentration of food?
* Coupling DNA nanotech to chromatin, modulate structures of genome, plasmids...

## What key applications/features can DNA/RNA nanotech in or around living cells enable?

* There seems to be industry interest in *synthetic* cells, mainly from a drug delivery perspective (Oscar Ces).
* Single cell –omics applications (molecular resolution, single molecule, genome wide) (Peng Yin).
* Diagnostics is a big part of synbio/nanotech. Would nucleic acids in synbio make for new functionality? Signal processing somehow?
* Are synthetic cells a more promising/engineerable route to applications than engineered biological cells? (Richard Murray)
* Combinatorial exploration of new chemistry (like a ribosome).
* Designing nanotech systems that work around living cells. (cf Oscar Ces).
* Sustainability applications? Why would you give up the billion years of evolution and exponential growth capability?