SENSORS AND PROBES

A magnetic alternative to FRET

A new approach measures nanoscale distances based on magnetic resonance tuning.

Fluorescence resonance energy transfer, or FRET, is widely applied in biology research. In this phenomenon, energy is transferred from a donor chromophore in an excited state to an acceptor chromophore. The magnitude of the energy transfer depends on the distance between the donor and the acceptor, which enables FRET to be exploited as a 'ruler' to measure nanoscale distances. Because it is an optical technique, however, the application of FRET in living organisms is quite limited. A recently reported sensing mechanism akin to FRET but based on the measurement of a magnetic resonance imaging (MRI) signal may open up multiple novel *in vivo* applications.

This new approach, called magnetic resonance tuning, or MRET, was developed by Jinwoo Cheon of Yonsei University in Korea, along with his colleagues. MRET refers to the interaction that takes place between a paramagnetic enhancer compound (such as gadolinium–DOTA) and a superparamagnetic quencher nanoparticle (such as a zinc–iron oxide nanoparticle). Like FRET, MRET is distance dependent: the strength of the T1 MRI signal depends on the separation between the enhancer and the quencher. Beyond a critical separation distance, the enhancer's electron spin fluctuation accelerates the relaxation of protons in water, thus producing a strong T1 MRI signal. However, as the enhancer and quencher move closer together, the spin fluctuation slows, and the T1 MRI signal weakens, eventually turning off below the critical separation distance. Therefore, MRET, like FRET, can also serve as a nanoscale ruler.

Cheon's group carried out several experiments to showcase potential applications of MRET. They looked at three types of molecular interactions: cleavage, binding and folding. They detected the cleavage of a peptide or sulfonate bond linking enhancer and quencher by a protease or an oxidant, respectively. They monitored the hybridization reaction between complementary DNA strands, as well as the copper-mediated click chemistry reaction between

GENOMICS

SLICE-ING A GENOME'S ARCHITECTURE

Thin slices through nuclei provide an unbiased view of the complex 3D organization of mammalian genomes.

Throughout the centuries, patience has been extolled as a virtue. Take the advice of the Persian poet Saadi, who counseled having patience, for "all things are difficult before they become easy." Fast-forward from 13th-century Iran to present-day Germany, where Ana Pombo from the Max Delbrück Center for Molecular Medicine in Berlin and her colleagues are setting these words in action by devising an orthogonal approach for mapping genome architecture.

The original idea for the work dates back to a discussion between Pombo and Paul Edwards at the University of Cambridge in 2004. They speculated that if one extracted all DNA from thin slices through nuclei at different time points, it should be possible to follow contacts between chromosomes over time. At the time, technical limitations prevented the realization of this concept; most important, high-throughput sequencing was still in its infancy. "We took so long not because we were not ready, but because we had to wait for some technology to become available," recalls Pombo. And the waiting paid off.

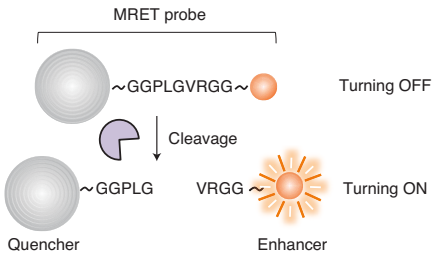
Thirteen years after the idea was conceived, genome architecture mapping (GAM) emerged as a way to discover the 3D genome, side by side with orthogonal biochemical approaches based on chromosome conformation capture (3C) methods and optical methods such as fluorescence *in situ* hybridization (FISH).

Conformation capture methods are based on mild fixation of chromatin, DNA shearing, and ligation of DNA ends in close proximity. This ligation step is prone to artifacts. In addition, 3C techniques rely on isolated cells, which makes some cell types inaccessible to biochemical 3C analysis. GAM does not share these caveats.

Robert Beagrie, a PhD student in the Pombo lab, was the driving force behind the initial GAM data set. He first collected thin slices from a cell pellet, then located and

alkyne-functionalized enhancer and azide-functionalized quencher. And they detected the folding of oligonucleotides upon a change in pH or the addition of a metal.

They also performed studies in cancer cell lines with an MRET probe designed to sense the activity of the cancer biomarker matrix metalloprotease 2 (MMP-2). The activity levels they measured in cell lines correlated well with commercial kit results. Finally, they sensed MMP-2 activity in mice with xenograft tumors and observed a strong T1 MRI signal at the tumor site, and a lack of signal in animals treated with an MMP-2 inhibitor.



Magnetic resonance tuning (MRET) monitors the cleavage of a peptide by MMP-2, a cancer biomarker. Reprinted with permission from Choi *et al.* (2017).

Cheon believes that MRET will be useful for many different sensing applications. "Thanks to the deep penetrating propensity of magnetic fields, MRET can be a valuable tool to explore a wide range of biological events, such as enzymolysis, pH variation, and protein–protein interactions, especially at complex tissue and whole-body levels," he says. Of course, many challenges will have to be overcome before MRET can be broadly used *in vivo*. The probes are rapidly cleared by the mononuclear phagocyte system; Cheon thinks that modifying the nanoparticle surface by applying a 'stealth' coating could help the probe evade the immune system and improve circulation time. Targeting the probes to specific locations *in vivo* is also a challenge; exploring different delivery vehicles may address this issue. "We hope that our research can provide a stepping stone for other researchers who are developing smart MRI nanoprobes for monitoring a wide range of biological events," says Cheon.

Allison Doerr

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Choi, J.-s. *et al.* Distance-dependent magnetic resonance tuning as a versatile MRI sensing platform for biological targets. *Nat. Mater.* http://dx.doi.org/10.1038/nmat4846 (2017).

isolated individual nuclei in each slice by laser microdissection microscopy. Independent amplification and sequencing generated DNA profiles from individual nuclei. The cosegregation of loci in each nuclear profile, the frequency with which loci are seen together compared to a background distribution, allows one to infer interacting regions.

To derive precise and quantitative contact maps from these interaction profiles, the Pombo lab teamed up with Mario Nicodemi from the University of Naples in Italy, and his PhD student Antonio Scialdone. The result was SLICE, a model for the statistical inference of cosegregation.

The researchers applied GAM and SLICE to mouse embryonic stem cells and replicated much of the known genome architecture, from topologically associating domains (TADs) at 1-Mb resolution to chromatin loops within TADs at 30-kb resolution. GAM's resolution is determined by the number of slices and is therefore tunable.

One of the strengths that Pombo sees in GAM is its ability to find specific contacts across long genomic distances of tens of megabases. The team used FISH to validate these contacts, but their function remains to be discovered. Another advantage of GAM is the quantitative mapping of triplet and higher-multiplexed interactions. The researchers could quantify the interactions of three TADs containing known super-enhancers.

They are currently focusing on expanding GAM to dopaminergic neurons in the brain. By staining tissue slices with a marker for certain neurons, they can isolate those neurons during microdissection and subject them to GAM.

And Pombo does not consider the current rendition of GAM as the final word. The long wait to streamline it continues: "if we project into the future," she says, "maybe there will be quantitative, direct sequencing of DNA molecules, and then we can avoid amplification and it becomes super easy with just one incubation."

Nicole Rusk

RESEARCH PAPERS

Beagrie, R.A. *et al.* Complex multi-enhancer contacts captured by genome architecture mapping. *Nature* http://dx.doi.org/10.1038/nature21411 (2017).