BEST PRACTICE EBOOK

Analytical and Chemical Enhancements to Improve Efficacy and Accuracy of Therapeutic Oligonucleotides

See how top pharma players are addressing impure, unstable, and off-target oligos

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Introduction

Oligonucleotide therapeutics represent a rapidly growing and increasingly powerful class of drugs. By interfering with the production of proteins linked to diseases, for example by blocking the production of harmful proteins, or by increasing the production of helpful proteins, they can go straight to the source of a morbidity.

Oligos' precision and potential to open up the druggable space make them attractive modalities for drug developers, clinicians, and indeed patients. Furthermore, the chemistry behind oligonucleotides is crucial to their success as therapeutics. Oligo developers are therefore working on using chemistry to surmount the key challenges of this modality's development. These include improving stability, delivery, immunogenicity, and off target effects.

We gathered together industry leaders for a half-day digital event, not only showcase the power of this emerging modality, but also unlock its full potential. This means going after the challenges which haunt oligonucleotide development: improving delivery, stability, and uptake. This eBook will see best practice solutions practiced by experts to tackle these difficulties.

First, we'll look at the efforts in analytical and quality control to assess the delivery, stability, and the uptake of oligonucleotide therapeutics:

- **A&M Stabtest**'s Thomas Franz will show of his company's efforts to overcome interference that common agents like triethylamine have on oligo analysis.
- Ulrike Rieder will show how **Novartis** is planning to address the complex impurity profiles of oligonucleotides beyond the standard guidelines.
- Brooke Koshel of **Wave Life Sciences** discusses how stereopure oligos require consideration of additional quality attributes including identity, selectivity, stability, and stereopurity, and the analytical methods essential for this.

Then we'll see how to tackle those same challenges via chemical methods:

- Anna Rydzik of **AstraZeneca** will discuss efforts to enhance the stability, potency, selectivity, and uptake of microRNA therapeutics.
- Isaac Marks from **Avidity Biosciences** will explain how to deal with poor cell uptake and rapid clearance of traditional RNA therapeutics.
- Sasha Ebrahimi from **GSK** will explain how a new form of nanotechnology can fix the false positives often encountered with spherical nucleic acids like nanoflares for intracellular delivery and real-time cellular diagnostics.

We hope you find this eBook informative in finding the best practices for overcoming these challenges.

Tom Cohen
Senior Digital Content Editor, Oxford Global

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Key Speakers Include

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BROOKE KOSHEL,
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Wave Life Sciences

ISAAC MARKS,
Senior Scientist,
Avidity Biosciences

SASHA EBRAHIMI,
Principal Investigator,
GSK

THOMAS FRANZ,
Study Director,
A&M Stabtest

ULRIKE RIEDER,
Scientific Lead,
Novartis

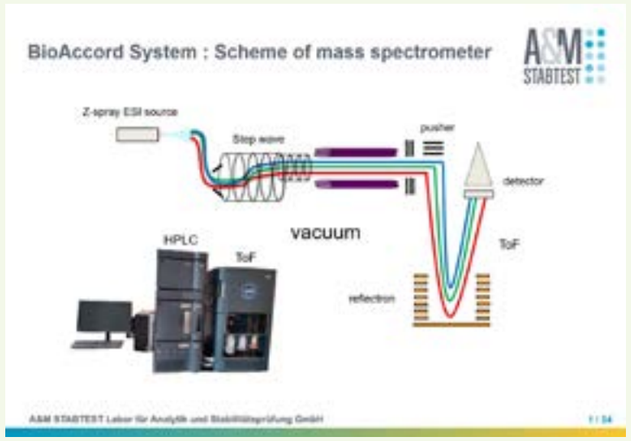
Part 1: Analytical Methods Optimisation

Determine Identity, Purity and Quantity of Your Oligos All in One Platform

Thomas Franz is a Study Director for A&M Stabtest, a GMP-certified and FDA-inspected contract service organization that provides analytical testing services for the pharmaceutical industry. The focus of his presentation was on using liquid chromatography-mass spectrometry (LC-MS) for identity and purity analysis of oligonucleotides.

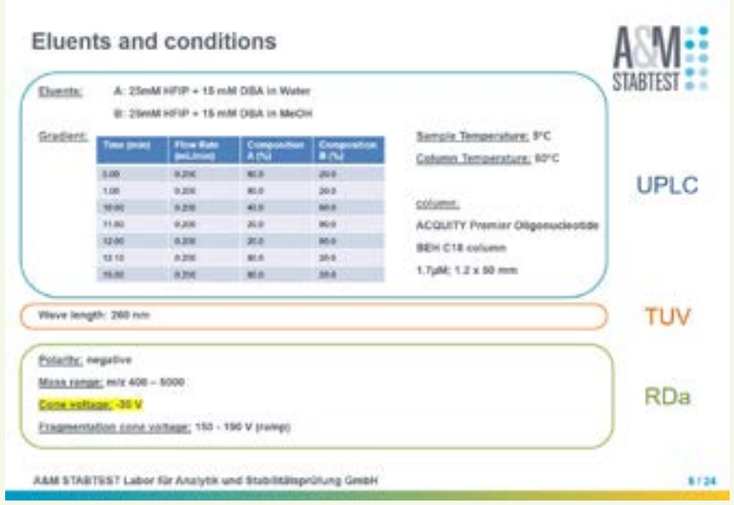
Franz first provided an overview of the organization, which has two sites - one dedicated to biopharmaceutical and ATMP testing, and another focused on small molecule analysis and inhalable drug testing. The goal is to offer clients a full range of analytical services under one roof.

For oligonucleotide and intact protein analysis, the organization uses the Waters BioAccord system with the waters_connect platform. The presenter explained the principles of time-of-flight mass spectrometry used in this system, which allows for analysis of both proteins and oligonucleotides.

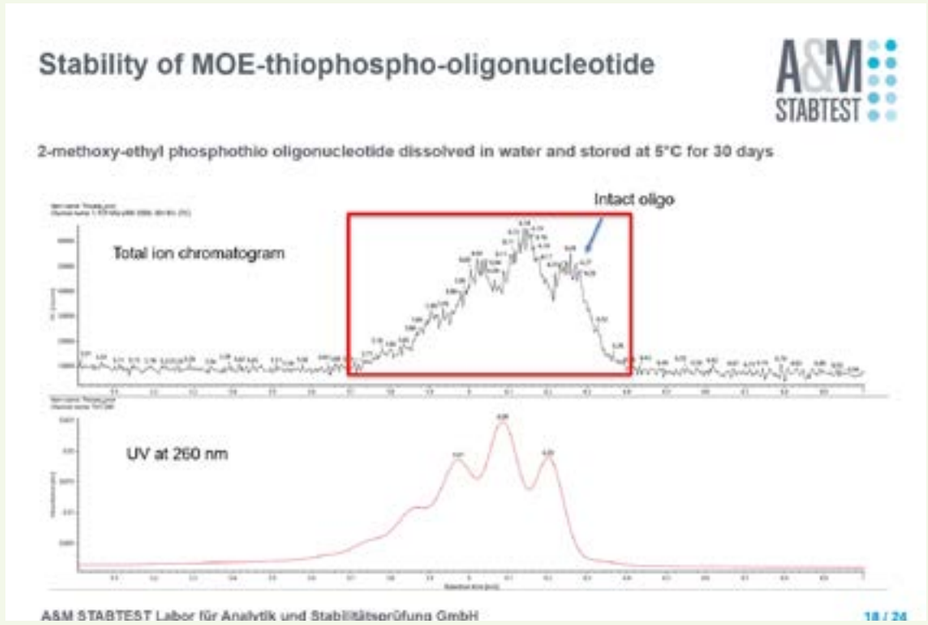


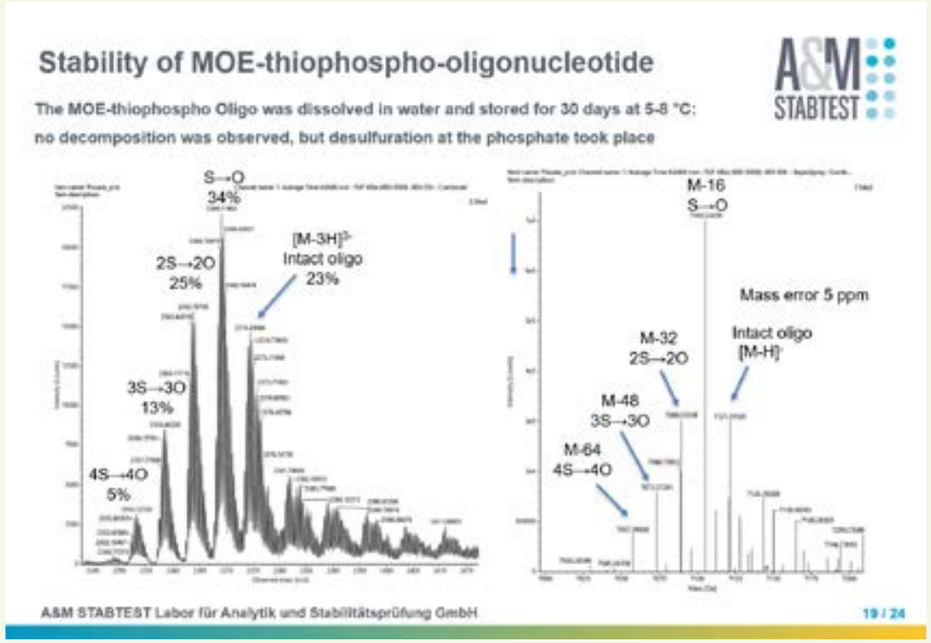
Franz then discussed the method development process for oligonucleotide analysis. After internal discussions, they decided not to use triethylamine, which is commonly used, due to potential negative effects on protein analysis. Instead, they evaluated different alkyl amines and found that dibutylamine (DBA) provided the best sensitivity for oligonucleotide analysis.

Using commercially available oligonucleotide standards, Franz's team optimised the UPLC conditions, including the use of 15 mM DBA and 25 mM hexafluoroisopropanol in the mobile phase. They found that a gradient starting at 85% aqueous and going to 20% aqueous in 9 minutes provided good separation of the oligonucleotides.

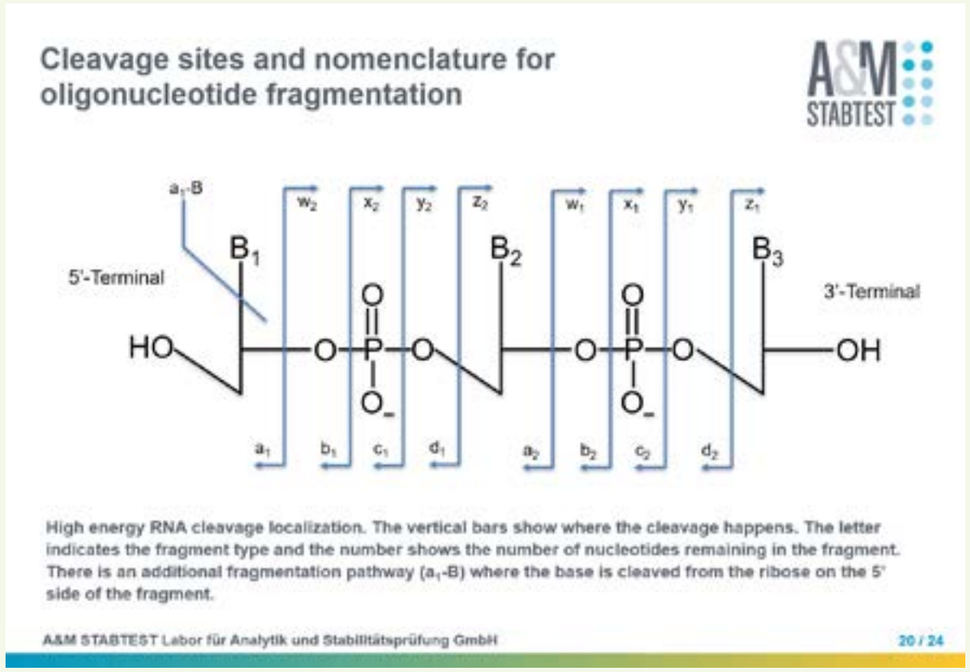


The presentation then covered several case studies, including analysis of a custom 40-mer oligonucleotide and evaluation of the stability of a methoxy ethyl phosphoryl oligonucleotide. For the 40-mer, they were able to detect an impurity present at 0.1% level. For the stability study, they observed stepwise desulfuration of the MOE-thiophospho-oligonucleotide over 30 days of storage.





Finally, Franz discussed the capability of the BioAccord system to perform both intact mass analysis and sequence analysis of oligonucleotides through in-source fragmentation. While manual interpretation of the fragmentation patterns can be challenging, the system has an integrated oligonucleotide sequencing application to facilitate data analysis.

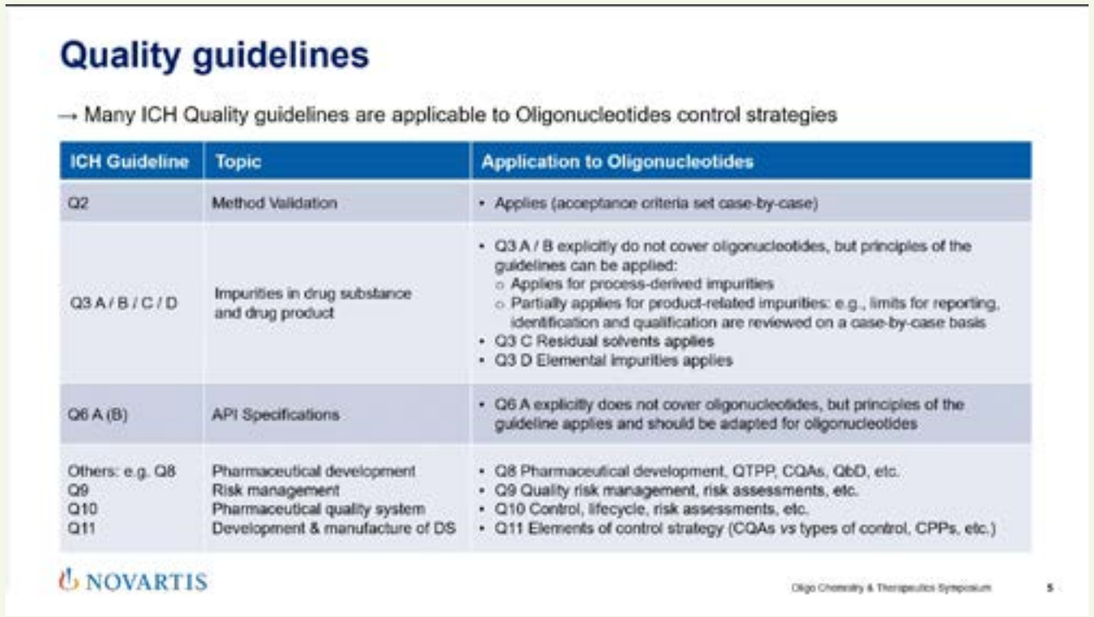


In conclusion, Franz highlighted the key findings, including the advantages of DBA over triethylamine, the optimised UPLC conditions, the mass accuracy and linearity, and the ability to characterise both intact mass and sequence of oligonucleotides using the BioAccord system.

Oligonucleotide Impurity Assessment Strategies Beyond the ICH Guidelines

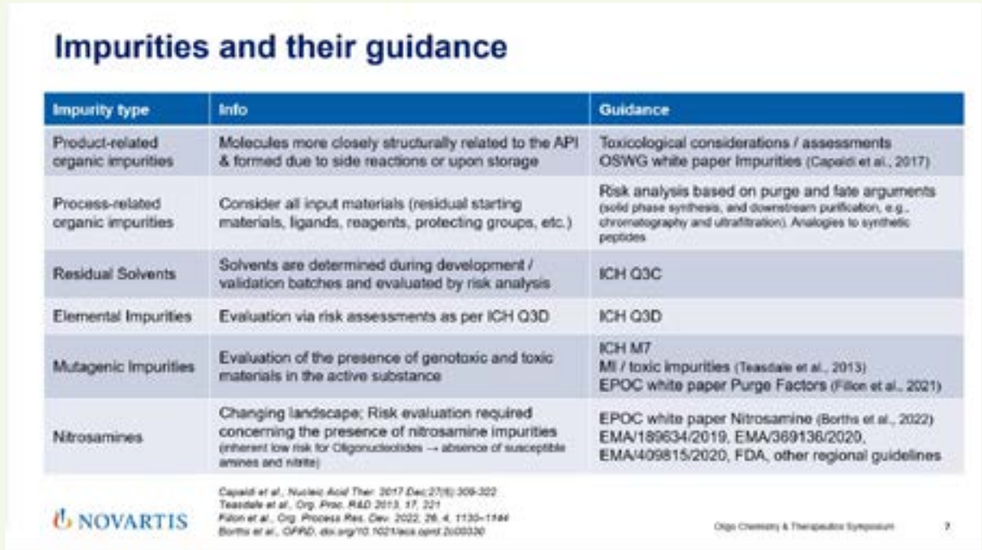
Ulrike Rieder, the Scientific Lead and Project Leader for oligonucleotides and new modalities at Novartis, presented considerations for assessing oligonucleotide impurities. She began by explaining that oligonucleotides are complex molecules composed of nucleotides, with a high molecular weight and a challenging impurity profile.

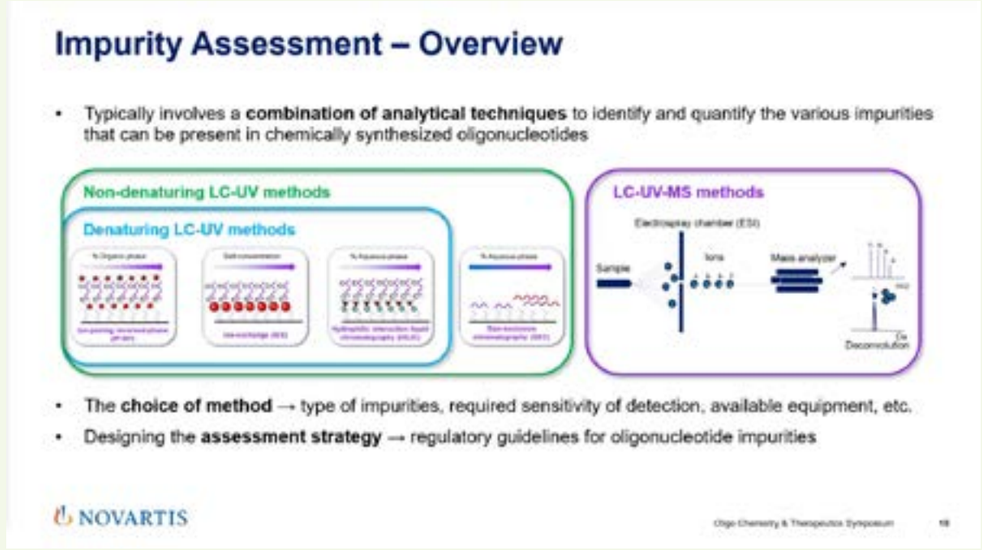
Rieder noted that while most ICH quality guidelines are applicable to oligonucleotides, there are some exceptions. ICH Q3A and Q3B do not explicitly cover oligonucleotides, though the principles can be applied. New guidelines are also emerging, such as the European Medicines Agency (EMA) draft guideline on oligonucleotide development and manufacture, as well as a forthcoming CDE guideline from the Chinese health authority.



Regarding impurities, Rieder outlined the different types and their respective guidance. She focused on product-related impurities, which are structurally similar to the parent compound and can arise from starting materials, incomplete synthesis, side reactions, or degradation. Rieder emphasised the importance of understanding the formation and origin of these impurities to ensure control of the impurity profile.

The presentation then discussed impurity assessment strategies, which typically involve a combination of analytical techniques like LC-UV and LC-UV coupled to MS. Rieder highlighted the strengths and considerations of each approach. Denaturing LC-UV methodologies, such as ion pairing and HILIC, are commonly used, while non-denaturing methods like SEC are applied for double-stranded siRNA. Two-dimensional liquid chromatography was noted as a powerful tool for enhancing separation and hyphenation to MS.





Rieder also addressed the concept of impurity grouping, which can be based on structure, peak profile patterns, or a combination of factors. For siRNA, it is beneficial to use the same denaturing HPLC methods for both the single-strand and double-strand drug substances to enable direct comparison of the impurity profiles.

Overall, Rieder's presentation provided a comprehensive overview of the considerations and challenges in assessing oligonucleotide impurities, highlighting the importance of a thorough understanding of the impurity profile and the strategic use of analytical techniques to ensure quality control.

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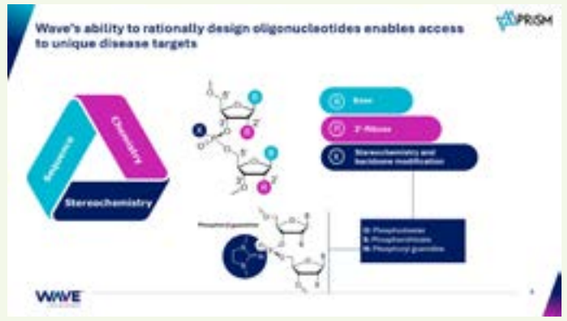
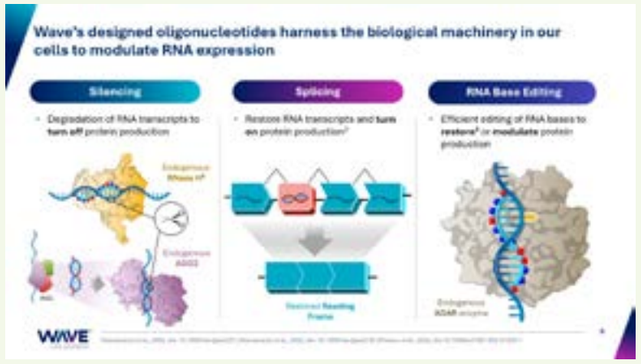
Unlocking Oligonucleotide Stereopurity With The Latest Analytical Methods

Brooke Koshel, the Director of Process Development at Wave Life Sciences, provided an overview of the company's Prism platform and the analytical methods used to characterise their stereopure oligonucleotides.

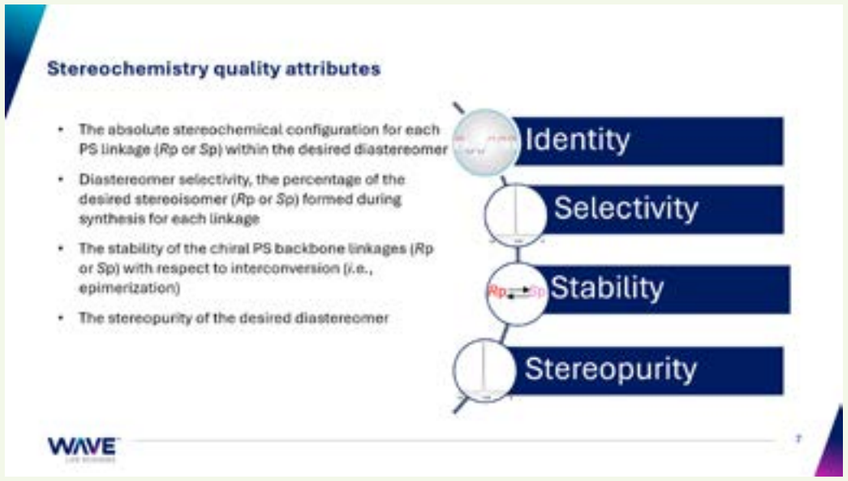
Koshel began by introducing Wave's Prism platform, which highlights the relationship between oligonucleotide sequence, stereochemistry, and chemistry. By controlling the design of oligonucleotide bases, 2' ribose modifications, stereochemistry, and backbone modifications, Wave is able to access unique disease targets in a more innovative way compared to using a predefined toolkit.



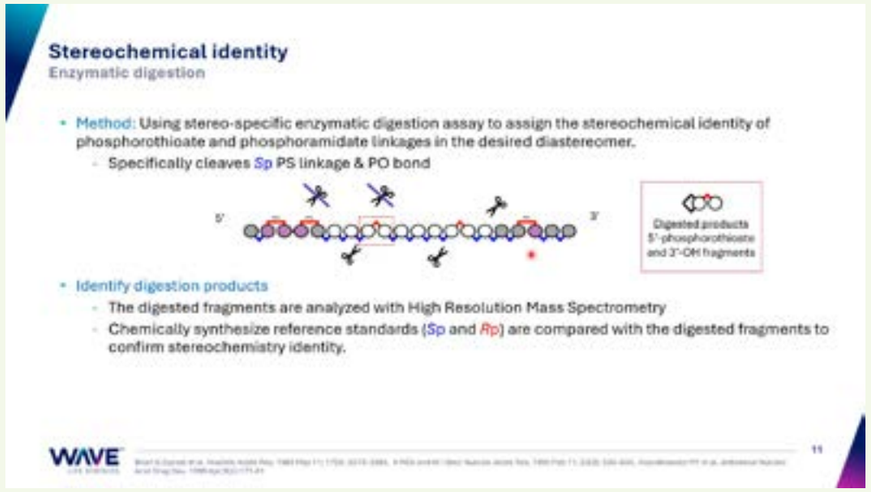
A key focus of Koshel's presentation was Wave's use of PN chemistry, which they introduced in the literature in 2022. PN linkages are neutral, decreasing the net charge of the oligonucleotide, and are being explored to potentially improve pharmacokinetics and efficacy. Wave's oligonucleotides, which span various modalities including RNA interference, antisense silencing, splicing, and editing, have demonstrated biological and therapeutic benefits.



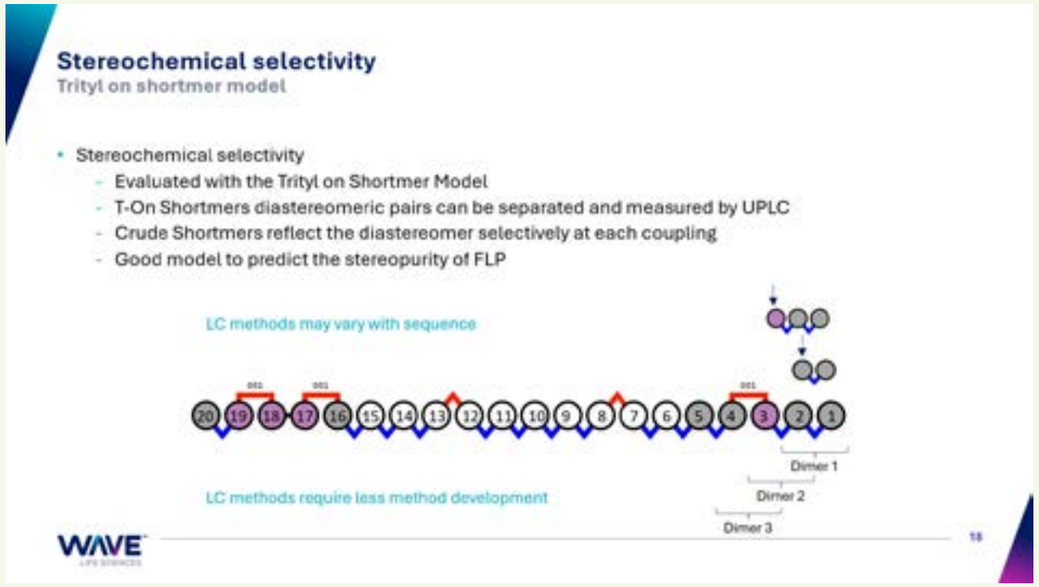
While Wave uses common industry approaches to analyse their oligonucleotides, the stereopure nature of their molecules requires consideration of additional quality attributes. These include identity, selectivity, stability, and stereopurity. Koshel explained that identity confirmation involves LCMS sequencing and UPLC methods to monitor elution time against reference standards. Stereochemical identification is crucial to avoid potential sequence errors during synthesis.



Enzymatic digestion using stereo-specific enzymes described as a key method for confirming sequence identity. The digested fragments are analysed by high-resolution mass spectrometry and compared to chemically synthesised reference standards to verify the correct stereochemistry.



Koshel also discussed the use of dimer modelling and Trityl on modelling to monitor stereoselectivity, the percentage of the desired stereoisomer formed during synthesis. These methods provide a more straightforward way to assess stereoselectivity compared to analysing the full-length product (FLP). Trityl on shortmer modelling is used to confirm the data from the dimer studies, though it requires more method optimization.



Stability and stereopurity assessment involved modifying stereo-defined PN bonds to stereo-random bonds and observing the resulting chromatographic profile. Anion exchange was used as an orthogonal method to analyse chemical impurities, separating them from the collapsing diastereomers.

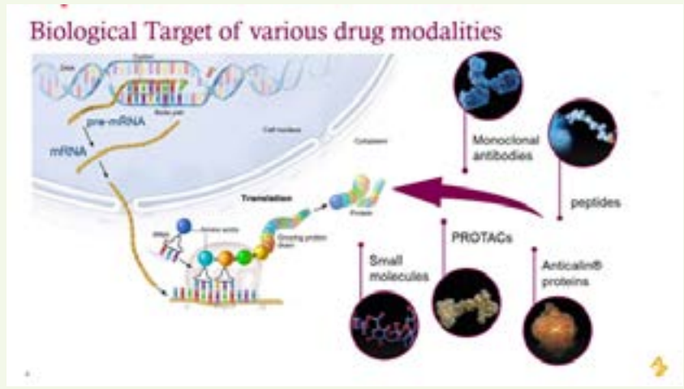
In conclusion, Koshel emphasised that the unique quality attributes of Wave's stereopure oligonucleotides, including identity, selectivity, stability, and stereopurity, are crucial considerations. The analytical methods she described, such as enzymatic digestion, dimer modelling, and shortmer modelling, are essential for characterizing these stereopure molecules.

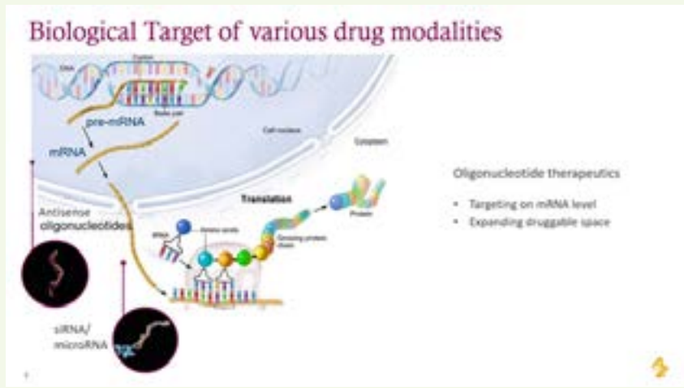
Part 2: Chemical Methods

Navigating the Complexity of microRNA Mimics: Optimising for Stability, Potency, Selectivity, and Uptake

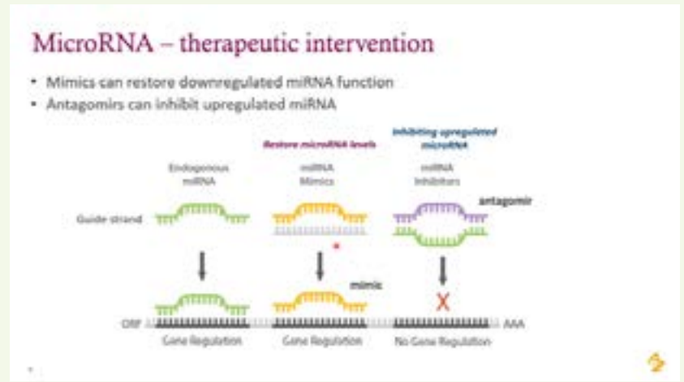
Anna Rydzik, Principal Scientist at AstraZeneca, presented on the development of chemical modification patterns for microRNA mimics, an emerging class of oligonucleotide therapeutics. She began by providing an overview of oligonucleotide therapeutics, highlighting AstraZeneca's work across various drug modalities including antisense oligonucleotides, siRNAs, and oligonucleotide conjugates.

Rydzik explained the classic dogma of molecular biology, where information flows from DNA to mRNA to proteins. While traditional small molecule drugs target proteins, oligonucleotide therapeutics target the mRNA level, allowing for expansion of the druggable space. She then introduced microRNA therapeutics as a relatively new and growing technology compared to the more mature siRNA field.

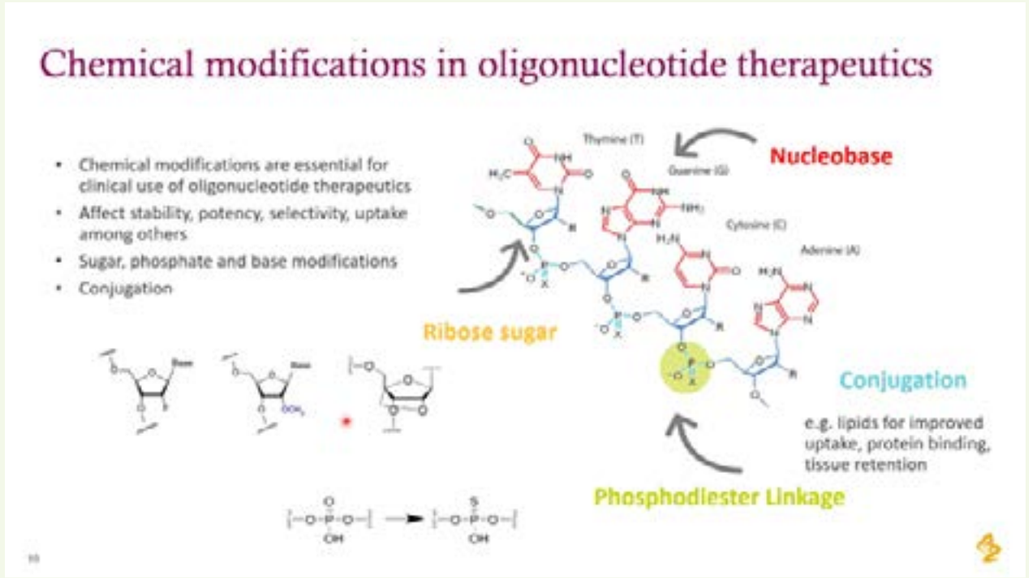




MicroRNAs are intrinsic gene regulation mechanisms that facilitate RNA interference, similar to synthetic siRNAs. However, a key difference is that microRNAs tolerate multiple mismatches and can target numerous genes, whereas siRNAs are designed to be highly specific. Rydzik discussed the therapeutic potential of modulating dysregulated microRNAs, either by blocking elevated levels with antagonists or mimicking downregulated microRNAs with synthetic mimics.



Chemical modifications are essential for the clinical use of oligonucleotide therapeutics, affecting stability, potency, selectivity, and uptake. Rydzik explained the complexity of designing microRNA mimics, requiring optimization of both the guide and passenger strands, as well as the overall duplex architecture. While inspiration can be drawn from siRNA modification patterns, direct translation may not be possible as the two systems use the same enzymatic machinery but have distinct requirements.



To illustrate their approach, Rydzik presented the team's work on developing microRNA200c (miR200c) mimics. They selected miR200c as a model system due to its involvement in cancer and fibrosis pathways and used the A549 cell line with low intrinsic miR200c expression to screen for representative target genes.

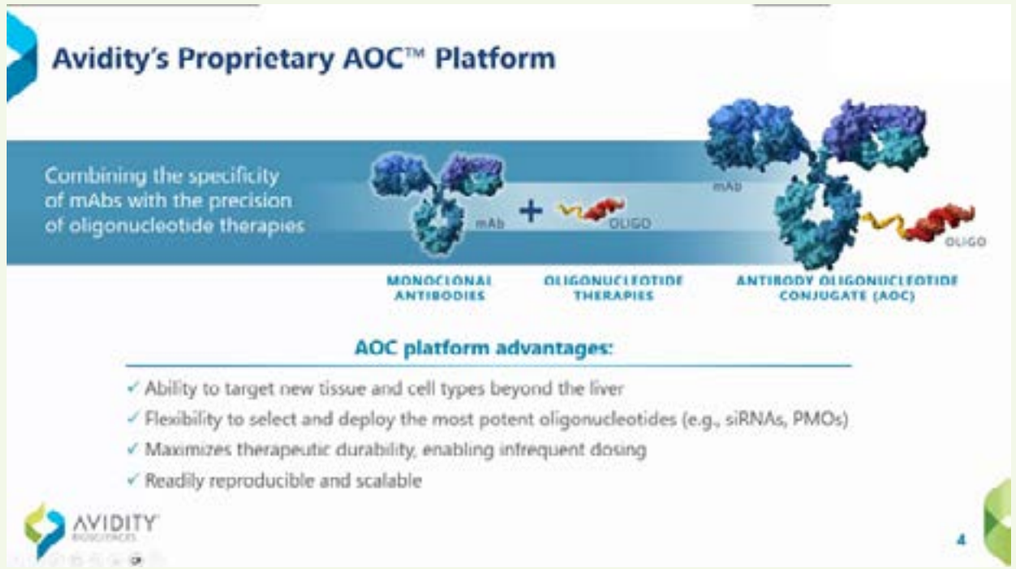
The researchers explored various modification patterns, including those commonly used for siRNAs as well as those reported for microRNA mimics in the literature. They also investigated the impact of duplex architecture, such as shortening the passenger strand. Screening a total of 38 miR200c mimics, they shortlisted 10 designs for further evaluation using next-generation sequencing to assess the whole genome-level effects.

Rydzik concluded by emphasizing the importance of chemical modifications in microRNA therapeutics and the need for continued research to develop effective tools for in vitro target validation and future therapeutic design.

Antibody Oligonucleotide Conjugates: Targeted Delivery of Oligonucleotides for Rare Musculoskeletal Disorders

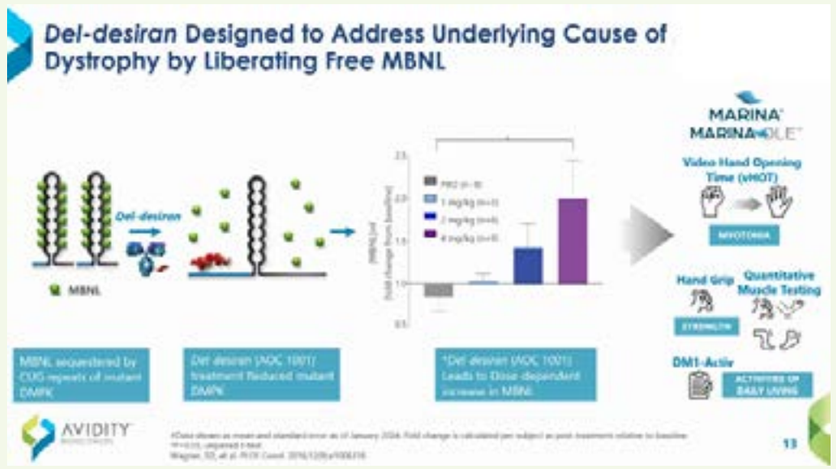
Isaac Marks, a scientist in the chemistry group at Avidity Biosciences, delivered a presentation outlining the company's pioneering work in a new class of antibody drug conjugates called Antibody Oligonucleotide Conjugates (AOCs). Avidity is leveraging this platform technology to improve the delivery of RNA therapeutics beyond just targeting the liver, opening up a host of new possible indications.

At the core of Avidity's AOC approach is the combination of the exquisite specificity and high affinity of monoclonal antibodies with an oligonucleotide cargo that can precisely target the genetic source of disease. By using the antibody as the targeting moiety, Avidity aims to enable delivery of the oligonucleotide payload to new tissue types beyond what has traditionally been possible with unmodified oligonucleotides. The company has selected the transferrin receptor on muscle cells as the target to achieve receptor-mediated uptake of the oligonucleotide cargo.



Marks explained that oligonucleotide therapeutics have faced challenges with limited cell uptake and rapid renal clearance when administered without targeting. Avidity's AOC platform seeks to overcome these limitations by leveraging the antibody component. The presentation highlighted the flexibility in AOC design, including the ability to attach multiple oligonucleotide drugs per antibody while maintaining favourable pharmacokinetics.

Avidity currently has three AOC programs in clinical development – Del-desiran for myotonic dystrophy type 1, Del-brax for facioscapulohumeral muscular dystrophy, and Del-zota for Duchenne muscular dystrophy. The focus of the presentation was on the Del-desiran program, where the company has demonstrated dose-dependent increases in functional MBNL protein levels and improvements in hand opening time, a measure of myotonia, in myotonic dystrophy patients.

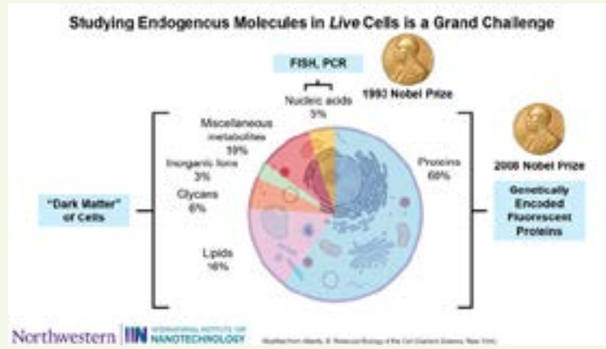


In conclusion, Marks expressed his belief that Avidity is at the forefront of enabling oligonucleotide therapeutics to reach new target tissues beyond the liver. This, he argued, opens up a wealth of new possible indications in areas like cardiology and immunology - a testament to the potential of Avidity's innovative AOC platform. The presentation provided a comprehensive overview of the company's progress in advancing this novel class of RNA therapeutics.

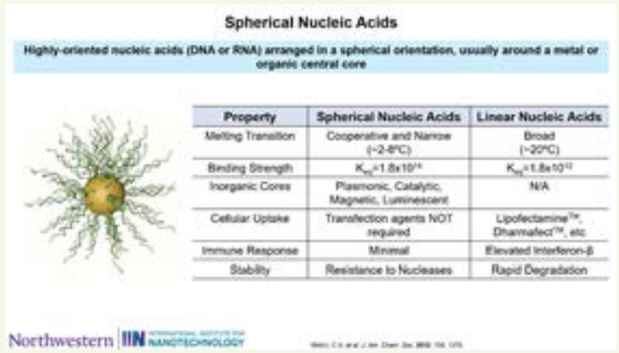
Studying Cells' 'Dark Matter' With the Next Generation of Oligo-Based Probes

Sasha Ebrahimi is a Principal Investigator at GSK within the emerging drug delivery platforms team. The main focus of his presentation was on developing tools to study the "dark matter" of cells – the molecules and processes inside live cells that are difficult to observe using current techniques.

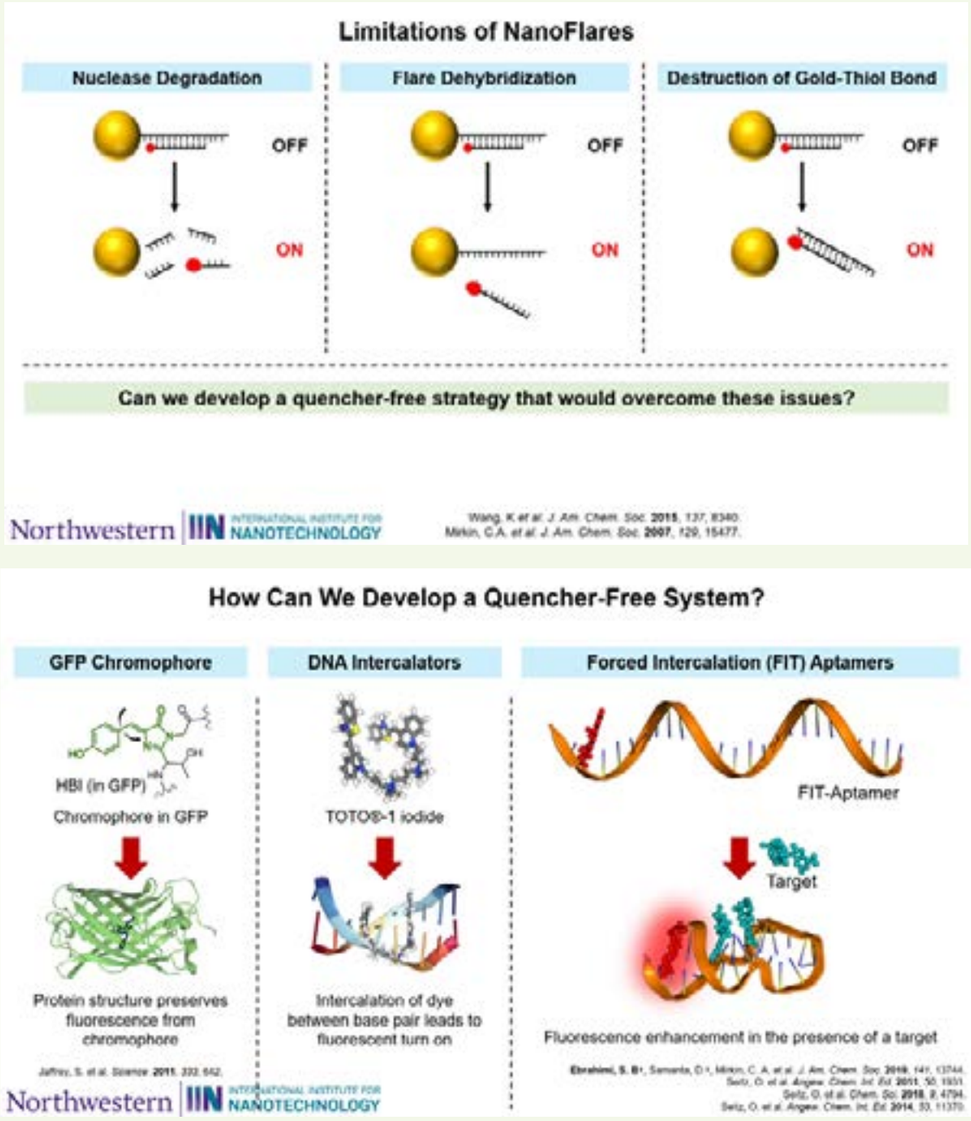
Ebrahimi began by explaining the importance of being able to study the dynamic changes of molecules inside live cells, as these changes are closely tied to disease. However, he noted that while there are powerful techniques to study proteins and nucleic acids, there is a lack of tools to study the rest of the "chemical composition" of cells.



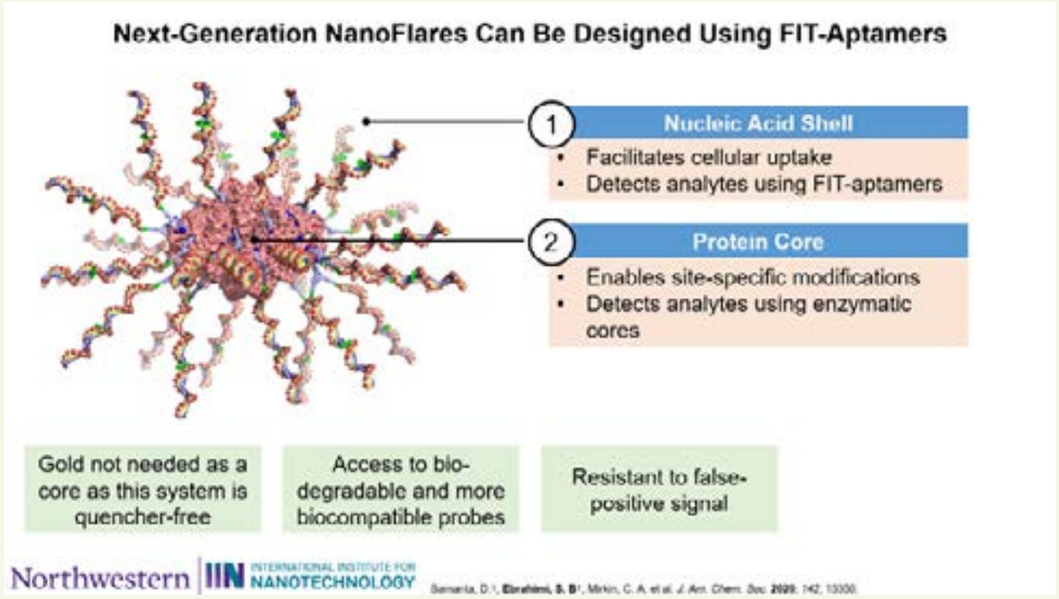
To address this, Ebrahimi's team has been working on a platform based on spherical nucleic acids (SNAs) - DNA densely functionalised around a nanoparticle core. These structures can actively enter cells and are more resistant to degradation than linear DNA. Ebrahimi described how they used SNAs to create "nanoflare" probes that can detect the presence of target mRNAs in live cells by monitoring fluorescence changes.

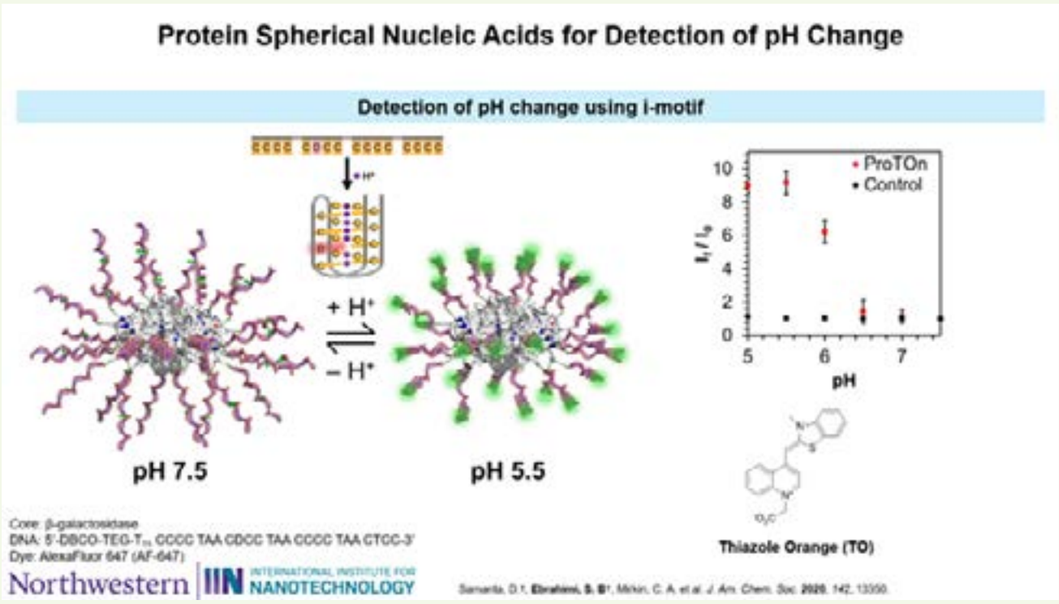


However, nanoflares have limitations, including the potential for false positive signals. To overcome this, Ebrahimi's team developed "FIT aptamers" - aptamers with a dye covalently attached. When the aptamer binds its target, the dye becomes restricted in its rotation, causing a fluorescence change. This approach was shown to have higher signal-to-noise and faster kinetics compared to nanoflares.

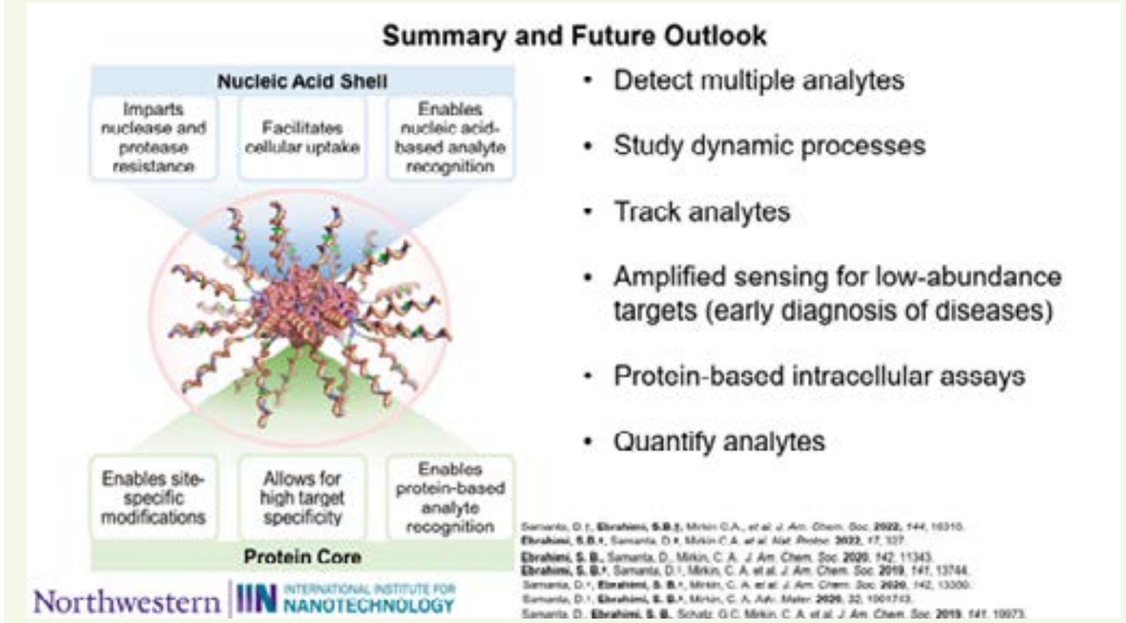


Building on this, the team then combined FIT aptamers with SNAs, using protein nanoparticle cores instead of gold. This allowed them to create pH-sensitive probes that could detect changes in intracellular pH. They further developed glucose-sensing probes by using glucose oxidase as the protein core.





In live cell experiments, these glucose-sensing probes were able to detect changes in intracellular glucose levels, demonstrating their ability to function inside cells. Ebrahimi highlighted the advantages of this platform, including the potential for multiplexing, gaining spatial and temporal information, detecting low abundance targets, and moving towards absolute quantification.



Overall, the presentation showcased the team's innovative work in developing versatile, sensitive, and specific tools to study the complex molecular environment inside live cells. This has important implications for understanding disease mechanisms and enabling new diagnostic and therapeutic approaches.

Report Summary

The challenges facing the development of vaccines and immunotherapies are complex, but companies are actively finding innovative solutions. Throughout this eBook, we've seen how advancements in mRNA technology, nanoparticle delivery, and cancer immunotherapy are paving the way for safer, more scalable, and more precise treatments. However, hurdles like ensuring scalability, addressing reactogenicity, and enhancing the precision of therapeutic targeting remain prominent.

To tackle these issues, companies are focusing on optimizing manufacturing processes, improving delivery mechanisms such as biodegradable lipid nanoparticles, and leveraging data-driven platforms to streamline production. For example, efforts to enhance mRNA vaccine efficacy, as demonstrated by Sanofi, are complemented by breakthroughs in cancer immunotherapy, where neoantigen-targeting technologies like those from NEOGAP Therapeutics are pushing the boundaries of personalized medicine.

Looking ahead, the future of vaccines and immunotherapies appears promising, with continued innovations that could revolutionize the fight against both infectious diseases and cancer. These developments will be further discussed at our upcoming event, NextGen Biomed 2025 from 12 - 14 March 2025, which will feature focused sessions on vaccines and immunotherapies, among other cutting-edge biomed topics. It will provide a platform for in-depth conversations on the latest breakthroughs and how the industry can continue to evolve.

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