AstraZeneca
IMED Biotech Unit

Establishing strategies to meet the bioanalytical needs of Oligonucleotide Therapeutics in Pre-clinical Models and beyond

Neil Henderson, Nucleotide Bioanalysis Group, AstraZeneca Gothenburg

EBF 2015 Open Meeting, Barcelona

Bioanalytical needs of oligonucleotide therapeutics

Outline of presentation

- Introduction to Nucleotide Bioanalysis Group , AstraZeneca
    - Who are we & why are we here
- Oligonucleotide therapeutics
    - What are they and how do they work
- Overview of bioanalytical approaches
    - Oligonucleotide (PK bioanalysis) — **1 case study**
    - modRNA derived-protein ((PK) PD bioanalysis) — **3 case studies**
    - modRNA (PK bioanalysis) — **1 case study**
- Summary

Bioanalytical needs of oligonucleotide therapeutics

Nucleotide Bioanalysis Group overview

Gothenburg NucBio Team

Group remit – expand the role of the (BA/TK) group beyond small molecules in New Modality area (e.g. RNA, Oligonucleotides and peptides) for toxicology and PK, PK/PD studies

Bioanalytical work arena	Type

Investigational | **GLP** | **UK**

Formulation studies

PK, PK/PD, TK

Rationale for group

Increased demand for pre-clincial bioanalysis from New Modality projects

moderna messenger therapeutics

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Mode of action of New Modality drugs

Regulation of protein synthesis

Oligonucleotides and active metabolites

ASO

anti-miR

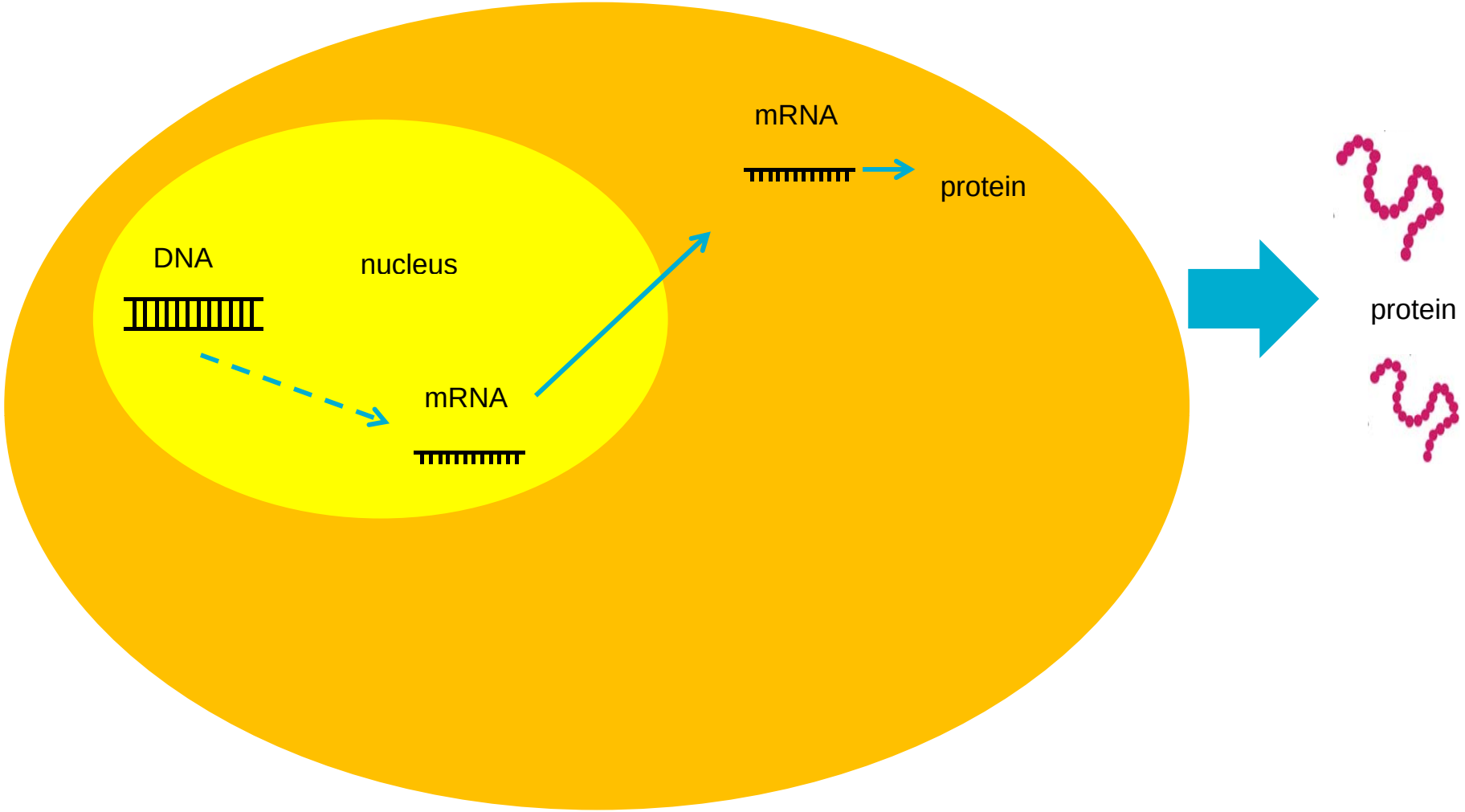
Increased induction of protein synthesis

modified mRNA

mRNA

Bioanalytical needs of oligonucleotide therapeutics

Setting the scene: DNA to mRNA to protein



Bioanalytical needs of oligonucleotide therapeutics

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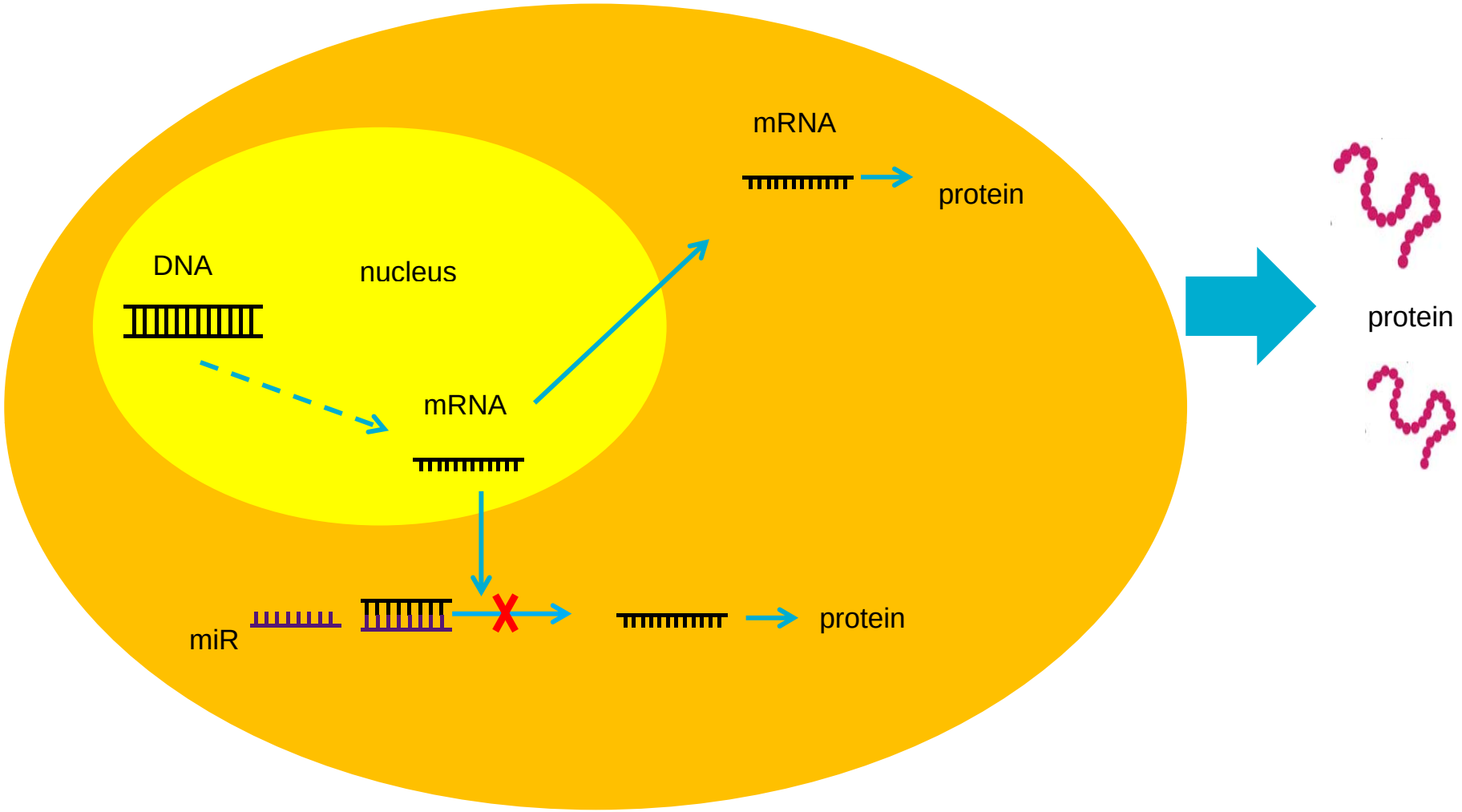
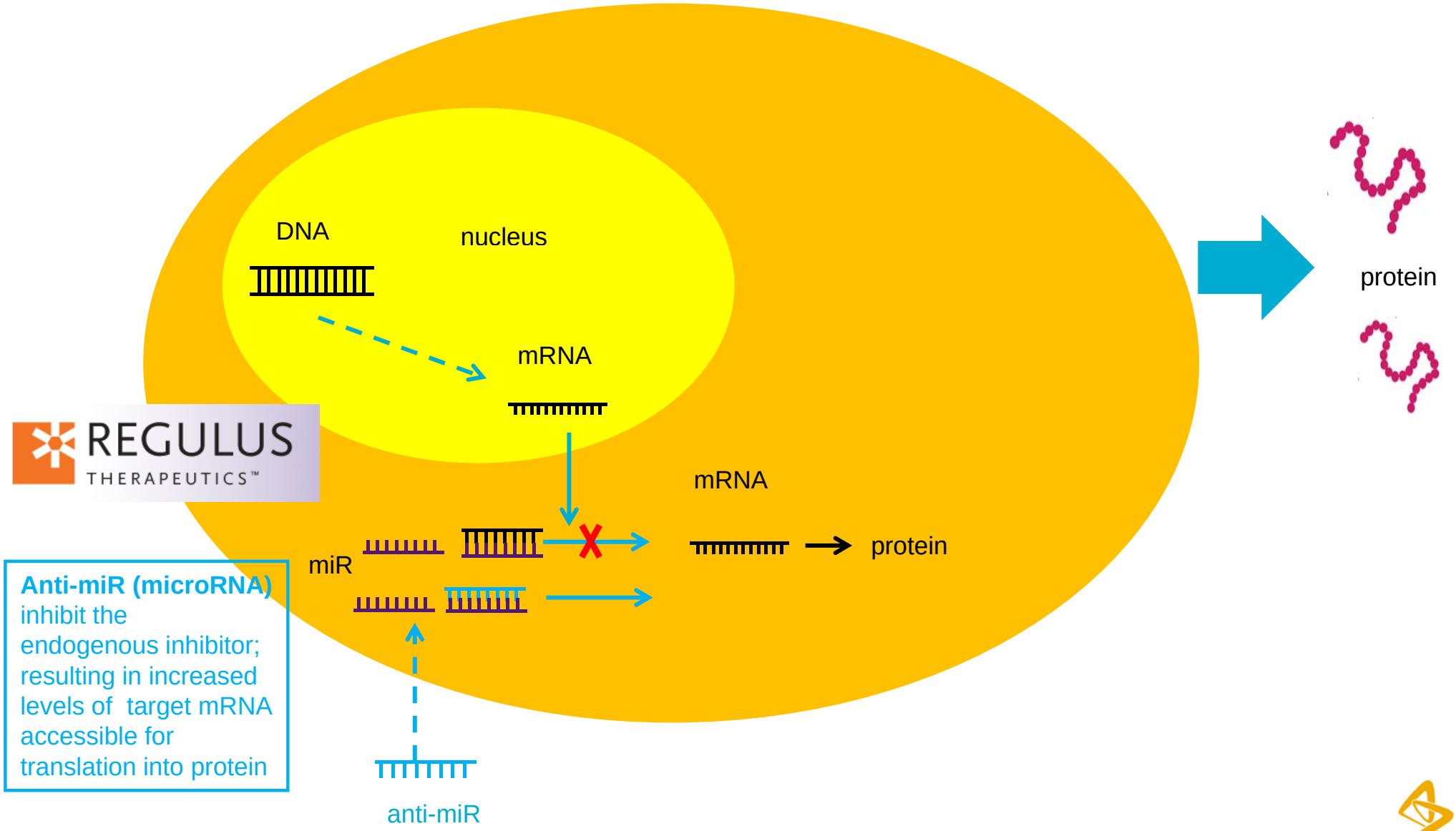


Nucleotide Bioanalysis group (BA/TK)

Setting the scene: biological action of new modalities



Anti-miR (microRNA) inhibit the endogenous inhibitor; resulting in increased levels of target mRNA accessible for translation into protein

Nucleotide Bioanalysis group (BA/TK)

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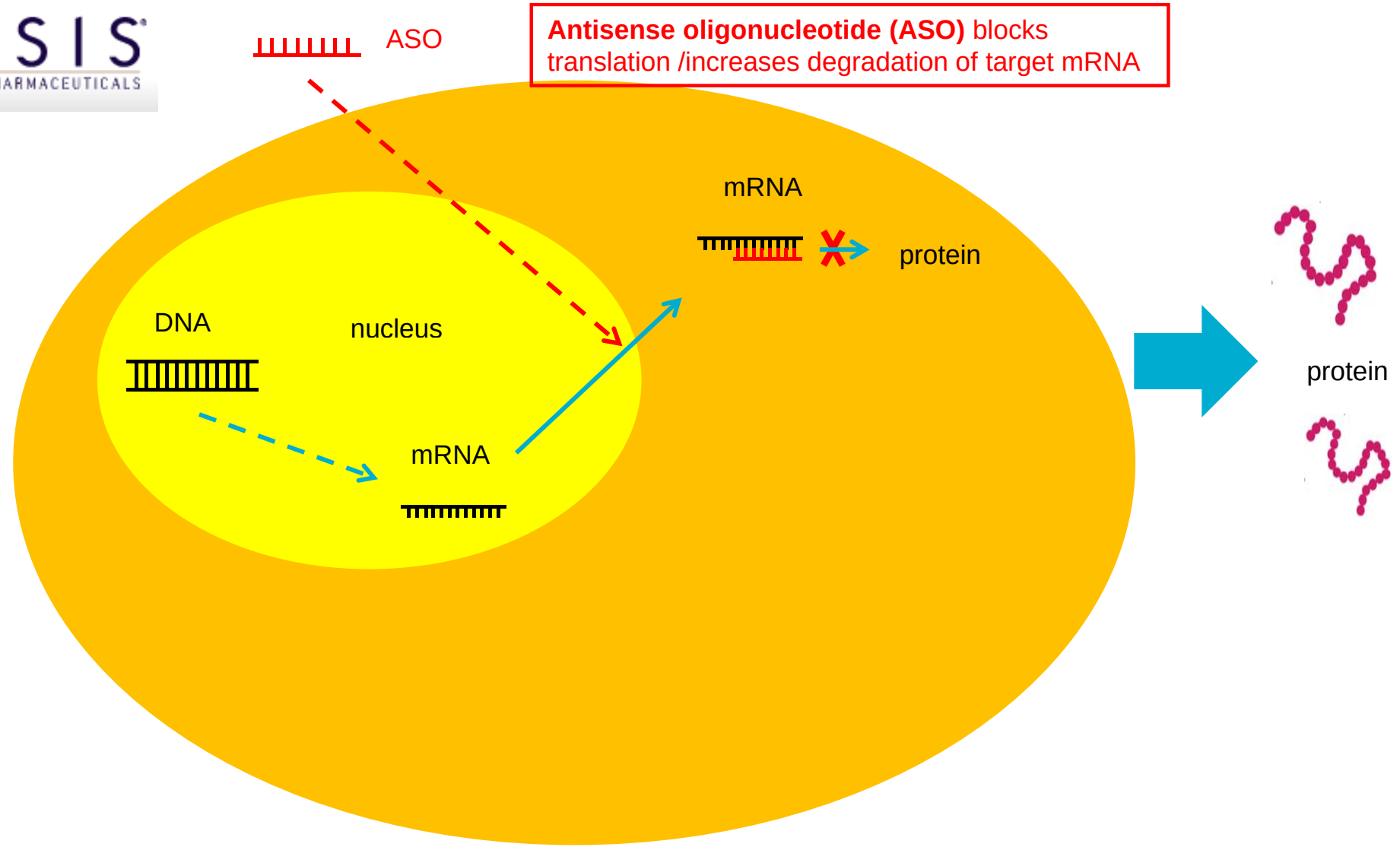

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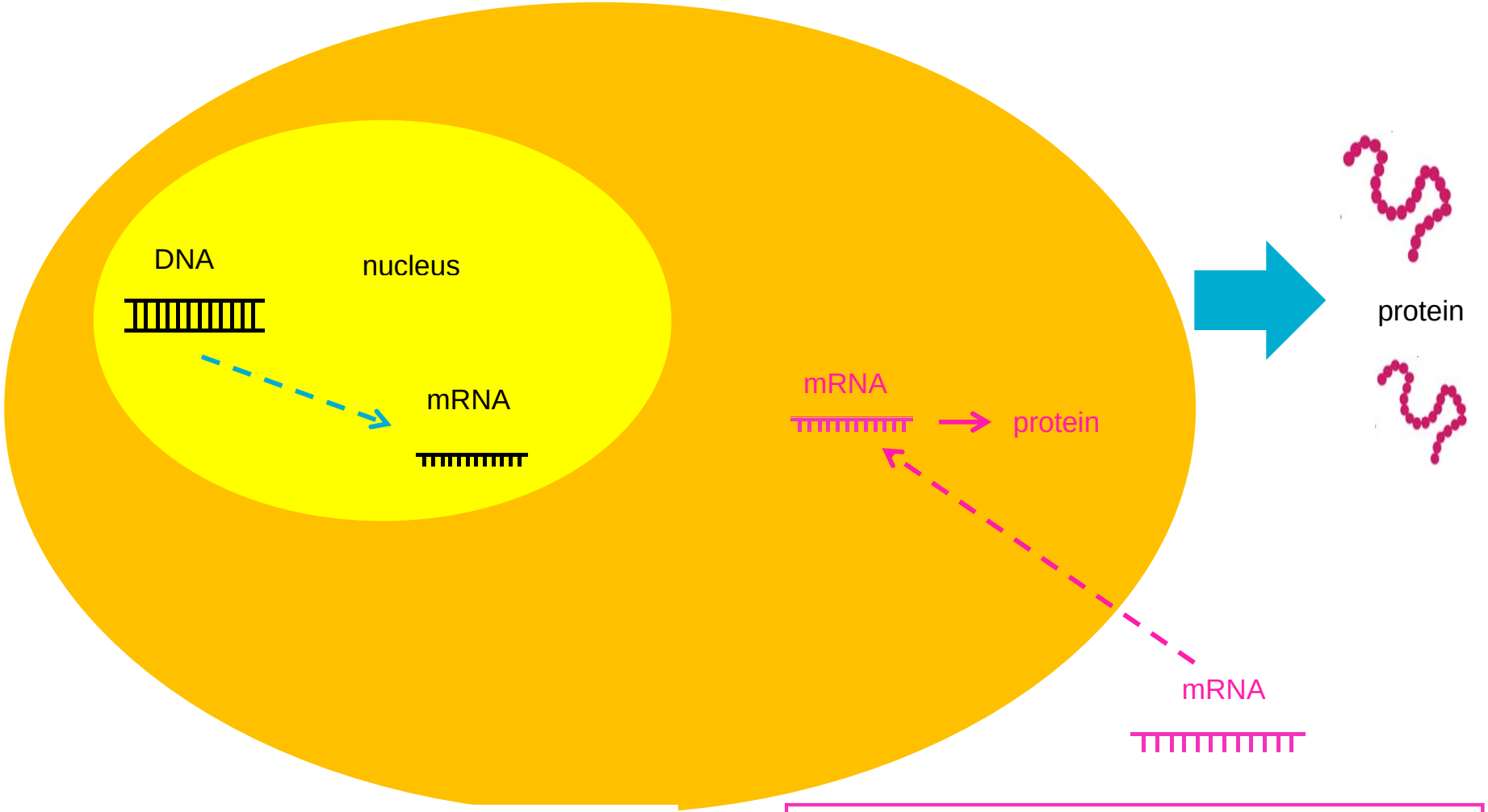


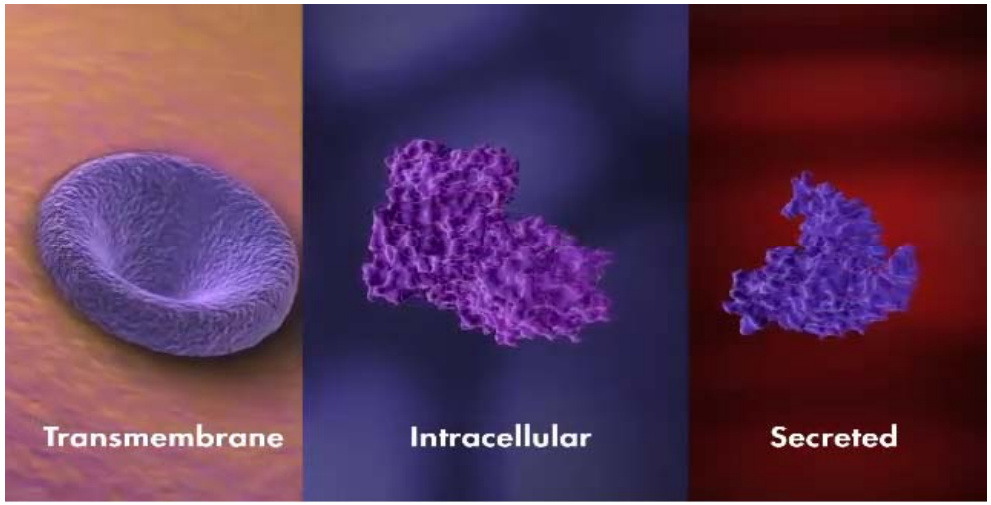
moderna
messenger therapeutics

Chemically **modified mRNA (modRNA)** functions as direct template for protein translation

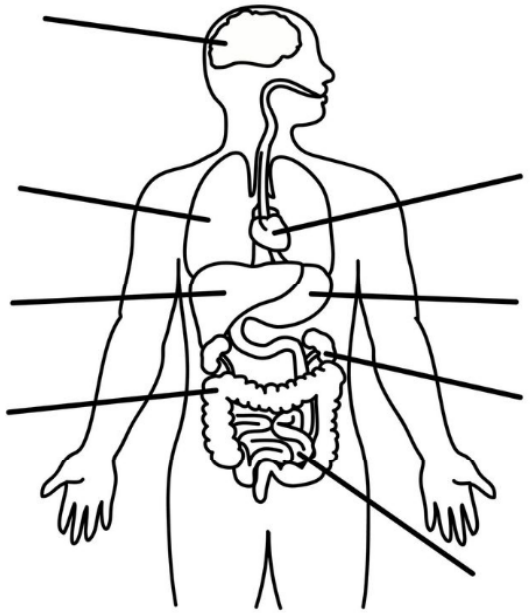
Bioanalytical needs of oligonucleotide therapeutics

Setting the scene: Potential of New Modality drugs?

Potential to target any type of protein



Potential to target any region of body



Potential to target any disease area

Bioanalytical needs of oligonucleotide therapeutics

Setting the scene: what are we measuring?

Drug

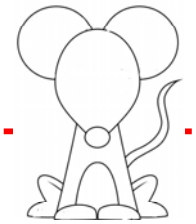
Oligonucleotides and active metabolites

ASO (Antisense Oligos)

anti-miR (microRNA)

human | **modified mRNA**

mRNA (messenger)



Pharmacodynamic (PD) endpoint

Proteins and peptides

Bioactivity (efficacy)

rodent **swine**

non-human primate

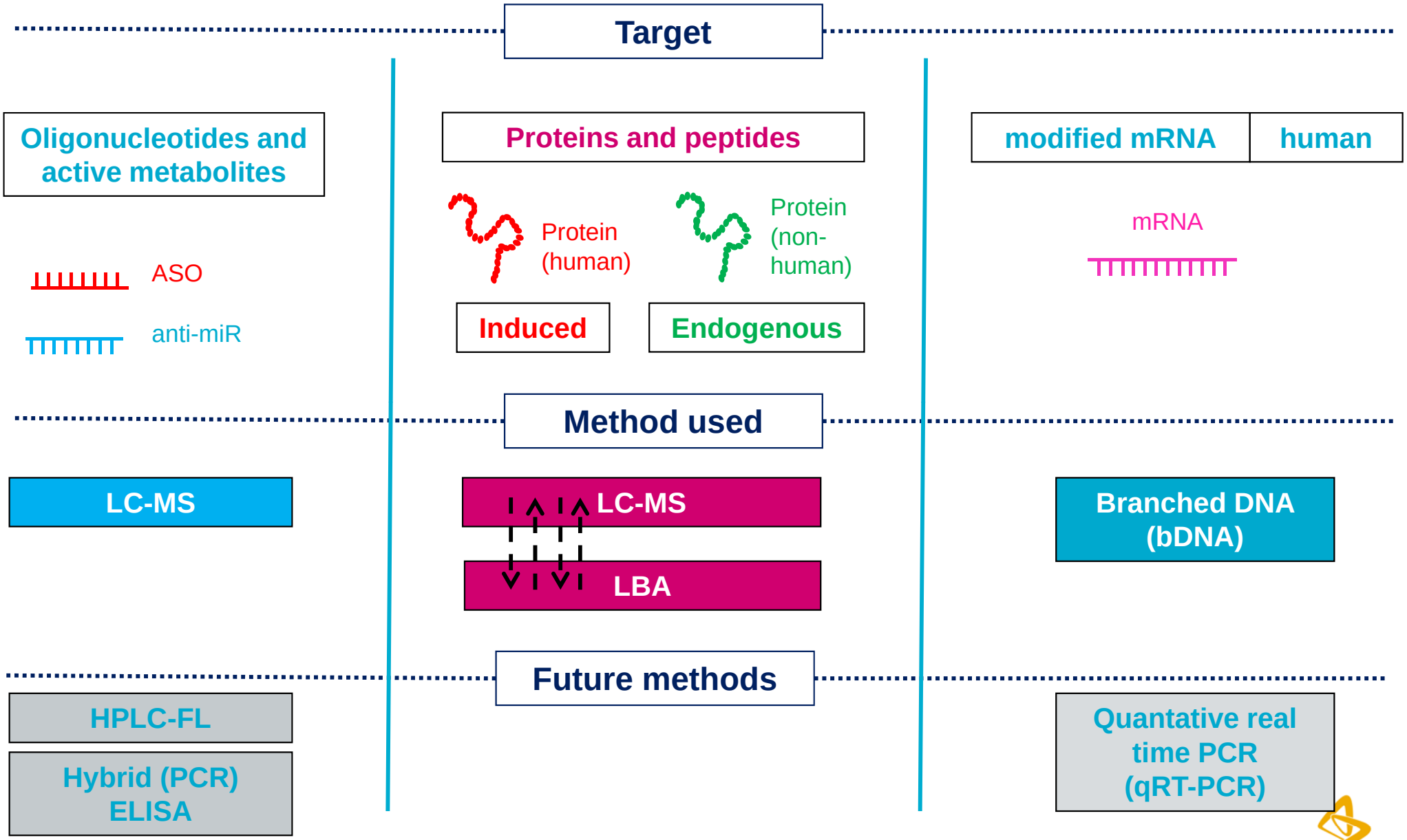
Protein (non-human)

human

Protein (human)

Bioanalytical needs of oligonucleotide therapeutics

Setting the scene: how are we measuring



Bioanalytical needs of oligonucleotide therapeutics

Setting the scene: demands on data generated

Choosing method depends upon

- Preclinical species
- Matrix type

- Matrix volume
- Target

- Hypothesis being tested
- Method approaches available
- Analytical throughput
- What the data will be used for
- Quality standard required

Quality standards depend upon

Investigational		GLP
Wider acceptance criteria	Line of sight	Regulatory guidelines
Meet internal quality demands	Tiered approach	Meet external quality demands

Speed, quality, direct the science, enhance the project understanding

Q1 2016

Bioanalytical needs of oligonucleotide therapeutics

Case studies:

- PK Measurements (oligonucleotides and active metabolites) **Oligonucleotides and active metabolites**
- (PK) PD Measurements (modRNA) **Proteins and peptides**
- PK Measurements (modRNA) **modified mRNA**

Bioanalytical needs of oligonucleotide therapeutics

Case studies:

- PK Measurements (oligonucleotides and active metabolites)

Oligonucleotides and active metabolites

- (PK) PD Measurements (modRNA)

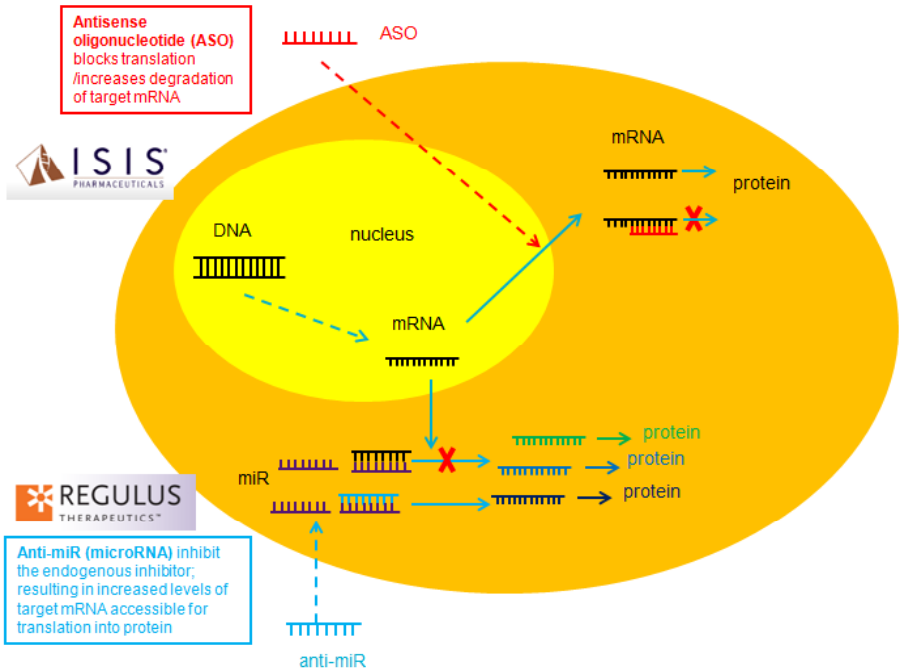
Proteins and peptides

- PK Measurements (modRNA)

modified mRNA

ASO

anti-miR



Bioanalytical needs of oligonucleotide therapeutics

Drug

Oligonucleotides

Case study 1: Oligonucleotide PK analysis

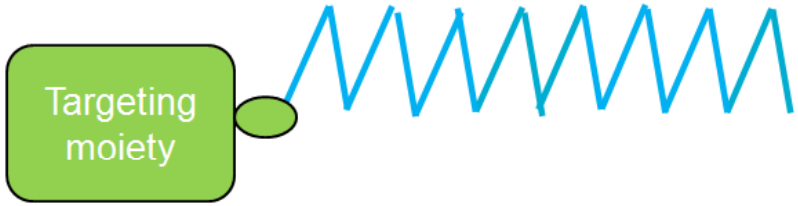
Use of LC-MS

Q3 2015

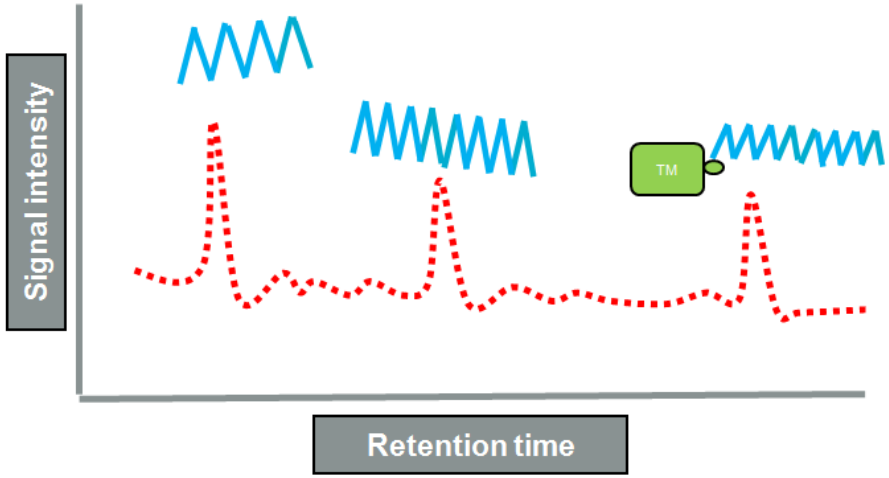
Develop a method to analyse investigational PK dose of Oligo Y

Targeting moiety helps direct oligo

Average oligo size approx. 20 nucleotides



"clean" profile of entire molecule + metabolites



Expect high concentrations of drug to build up in tissues

Current LC-MS methods are Fit for Purpose

ASOs have chemical modifications for increased stability

Focusing on the modifications allows identification of "cleaner" profiles

Challenges with LC-MS methods

Sample preparation steps take time

Rate of Column turnover

Sharing equipment with small molecule bioanalysts

Risk of instrument contamination

Potential for Carryover

Dedicated LC-MS solely for oligo work is desirable

Bioanalytical needs of oligonucleotide therapeutics

Case studies:

- PK Measurements (oligonucleotides and active metabolites)

Oligonucleotides and active metabolites

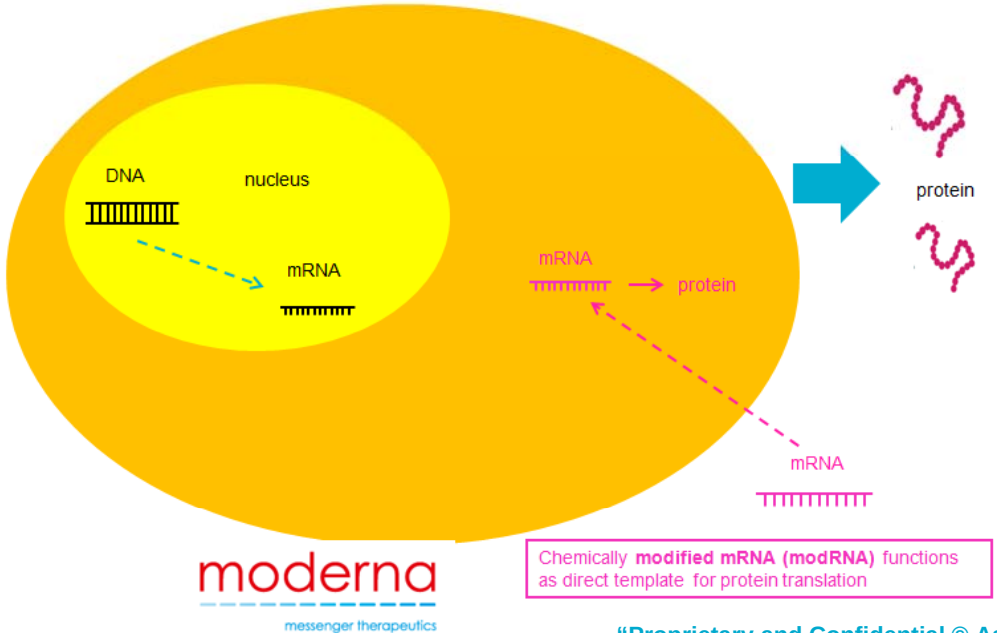
- (PK) PD Measurements (modRNA)

Proteins and peptides

Protein

- PK Measurements (modRNA)

modified mRNA



Bioanalytical needs of oligonucleotide therapeutics

PD endpoint

Case study 2: Establishing suitable assays

modRNA

Proteins and peptides

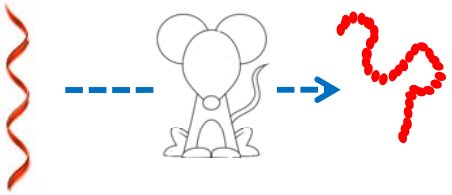
Screening assays

Balancing the right method with the question that requires answering is important

Formulation experiments need quick turnaround of data

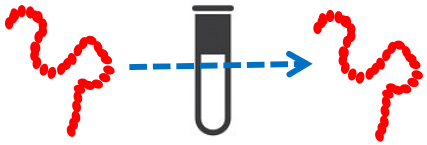
Timecourse experiments

Small *in vivo* sample sizes



Identifying a suitable assay

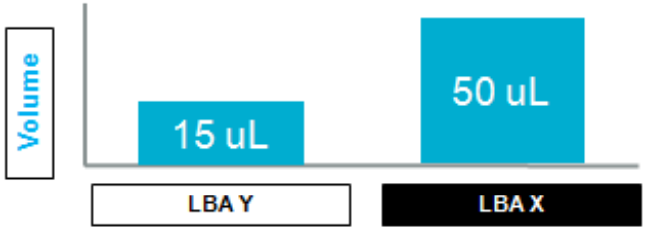
Assay to measure total level of Protein W



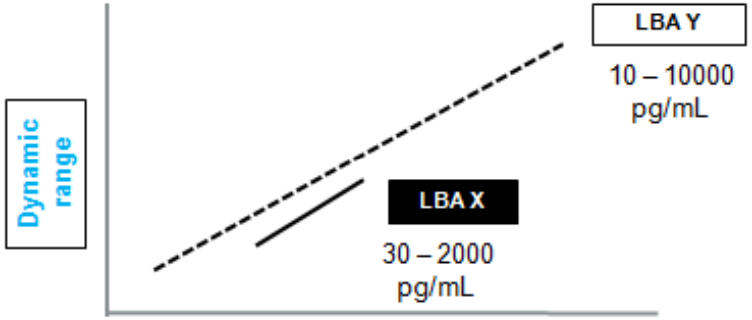
Purified protein spiked into (large volume of pooled) plasma or tissue homogenates (in vitro)

Can we identify assays with suitable qualities including low sample volume and large dynamic ranges?

Low sample volume



Large dynamic range



LBA-Y has desirable assay properties for single (“one shot”) analysis batches

Bioanalytical needs of oligonucleotide therapeutics

PD endpoint

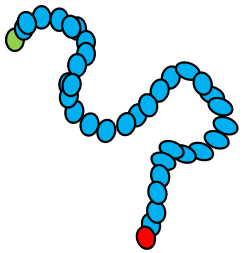
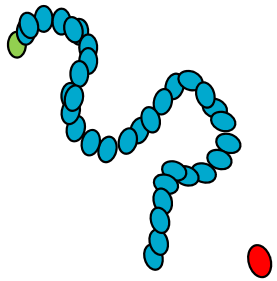
modRNA

Proteins and peptides

Case study 3: Establishing suitable assays

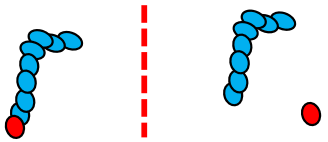
Detecting full length proteins

Degradation of an encoded protein can lead to inactivation of a protein

Full length protein	Protein (n – 5 amino acids)
	
Functional	Non-functional

May need methods that are specific for full length proteins

Digestion of the protein into discrete fragments followed by LC-MS



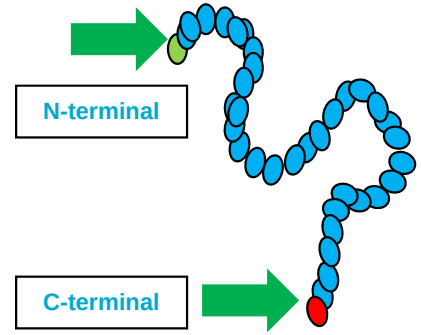
Intact fragment

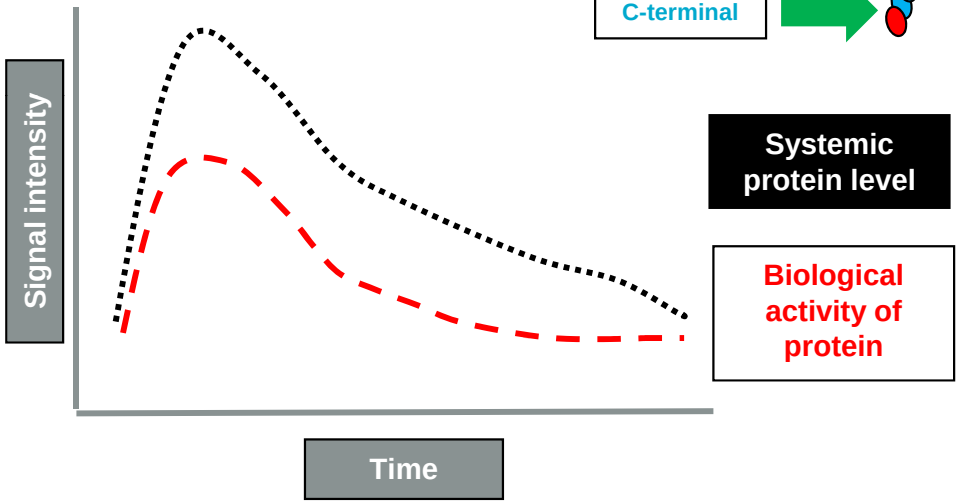
Degraded fragments

LBA approach using an antibody pair that are raised against N-terminus and C-terminus

Commercial reagents/kit available

Generate in-house reagents





Direct correlations between circulating protein level and biological activity over time can then be demonstrated

Bioanalytical needs of oligonucleotide therapeutics

PD endpoint

modRNA

Proteins and peptides

Case study 4: Establishing suitable assays

Adapting to situation & innovating where possible

Q1 2015

Measure secreted protein-V

Only had one anti-protein V polyclonal antibody

Unique target

No commercial reagents

In-house polyclonal antibody

LBA

LC-MS

LC-MS method used

2 day sample prep

An assay with quicker turnaround time was desirable for screening

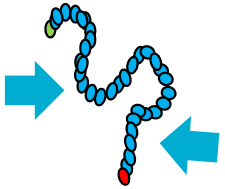
Immunoaffinity column purification with polyclonal antibody had limited success

Q3 2015

New antibody produced in-house

Now have potential to make a “sandwich” LBA

In-house polyclonal antibody



New monoclonal antibody

Gyrolab method used

3 hr turnaround

No sample clean up

Small sample volumes

Multiple assays are used within the same project depending upon the question being asked

Bioanalytical needs of oligonucleotide therapeutics

Case studies:

- PK Measurements (oligonucleotides and active metabolites)

Oligonucleotides and active metabolites

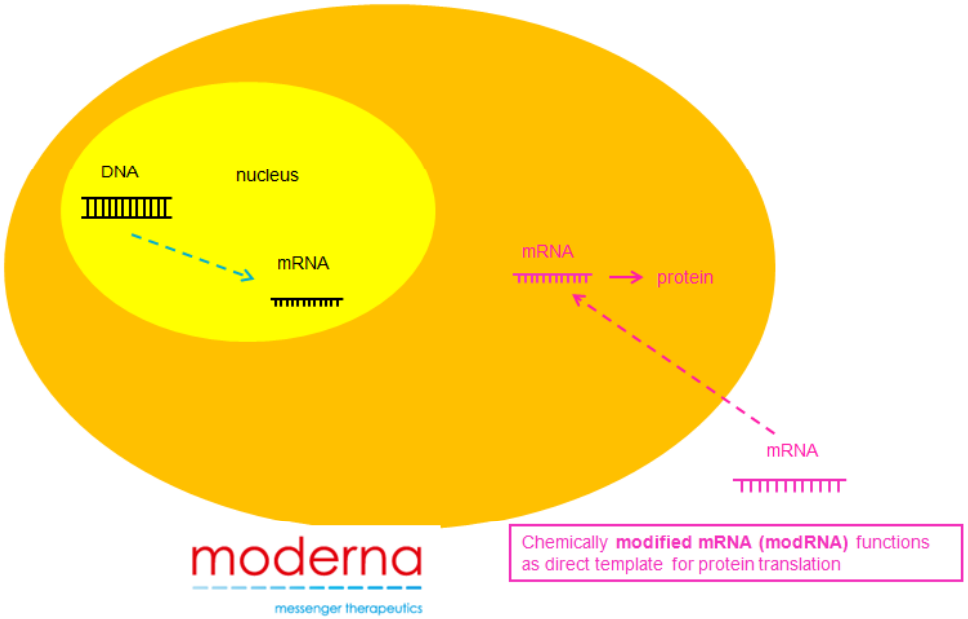
- (PK) PD Measurements (modRNA)

Proteins and peptides

- PK Measurements (modRNA)

modified mRNA

mRNA



Bioanalytical needs of oligonucleotide therapeutics

Drug

modRNA

Case study 5: PK analysis of (modified) mRNA

Branched DNA (bDNA) method

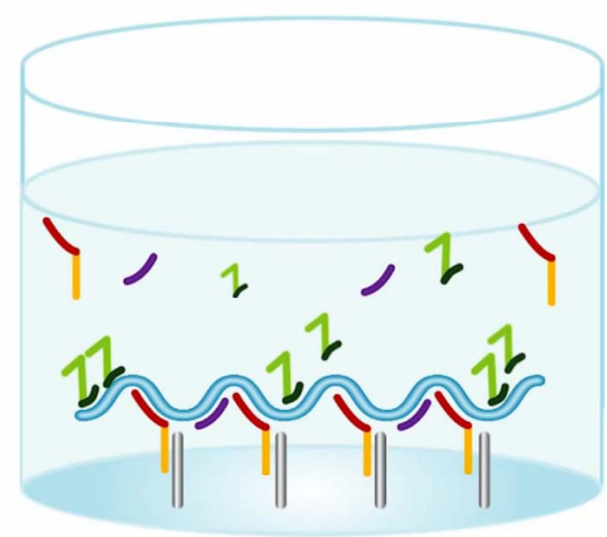
affymetrix
Biology for a better world

ELISA-like workflow

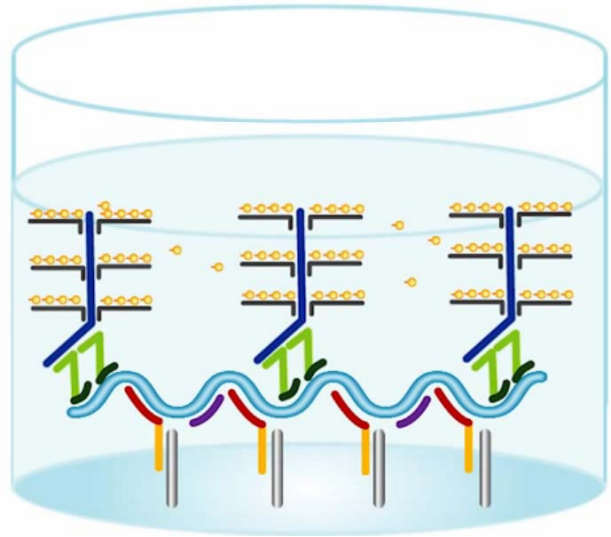
Incubation steps

Wash steps

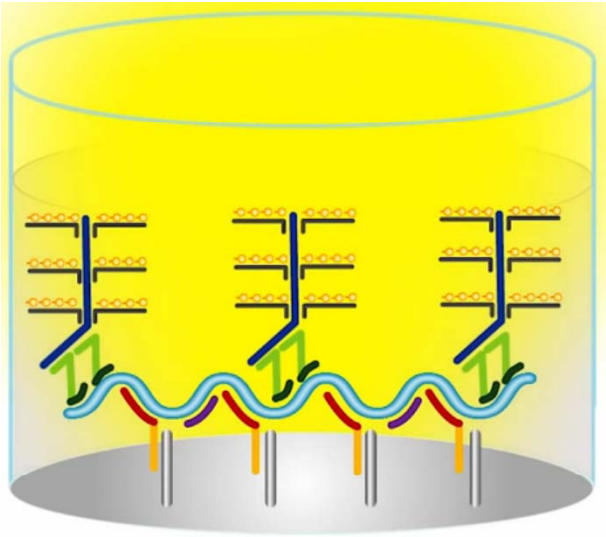
Add a chemiluminescent substrate



Specifically capture mRNA



Amplify signal



Read catalysed substrate

Bioanalytical needs of oligonucleotide therapeutics

Drug

modRNA

Case study 5: PK analysis of (modified) mRNA

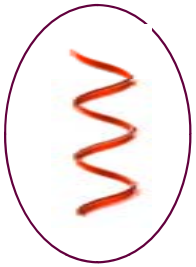
How are we using bDNA method?

affymetrix
Biology for a better world

Purified modRNA for use as Reference Standard & QCs

Non encapsulated

Encapsulated



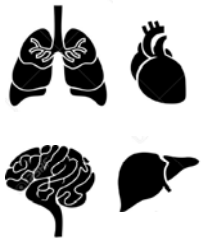
Non protected

Protected

Nuclease-free buffer only

Matrix matched vehicle

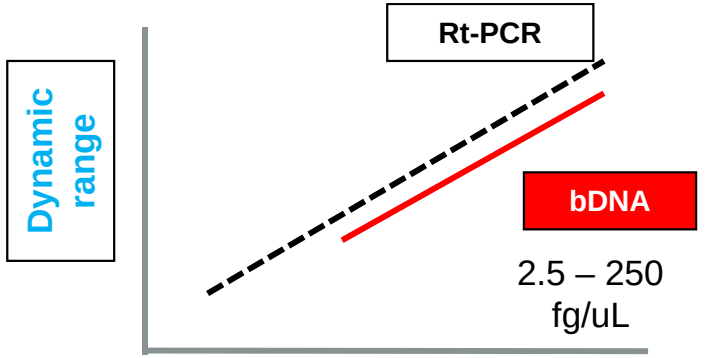
Plasma / Tissue



Instrumentation

Luminometer

Not a dedicated RT-PCR platform



Sensitivity vs. Throughput

Evaluation criteria

Accuracy/ precision

Specificity

Selectivity

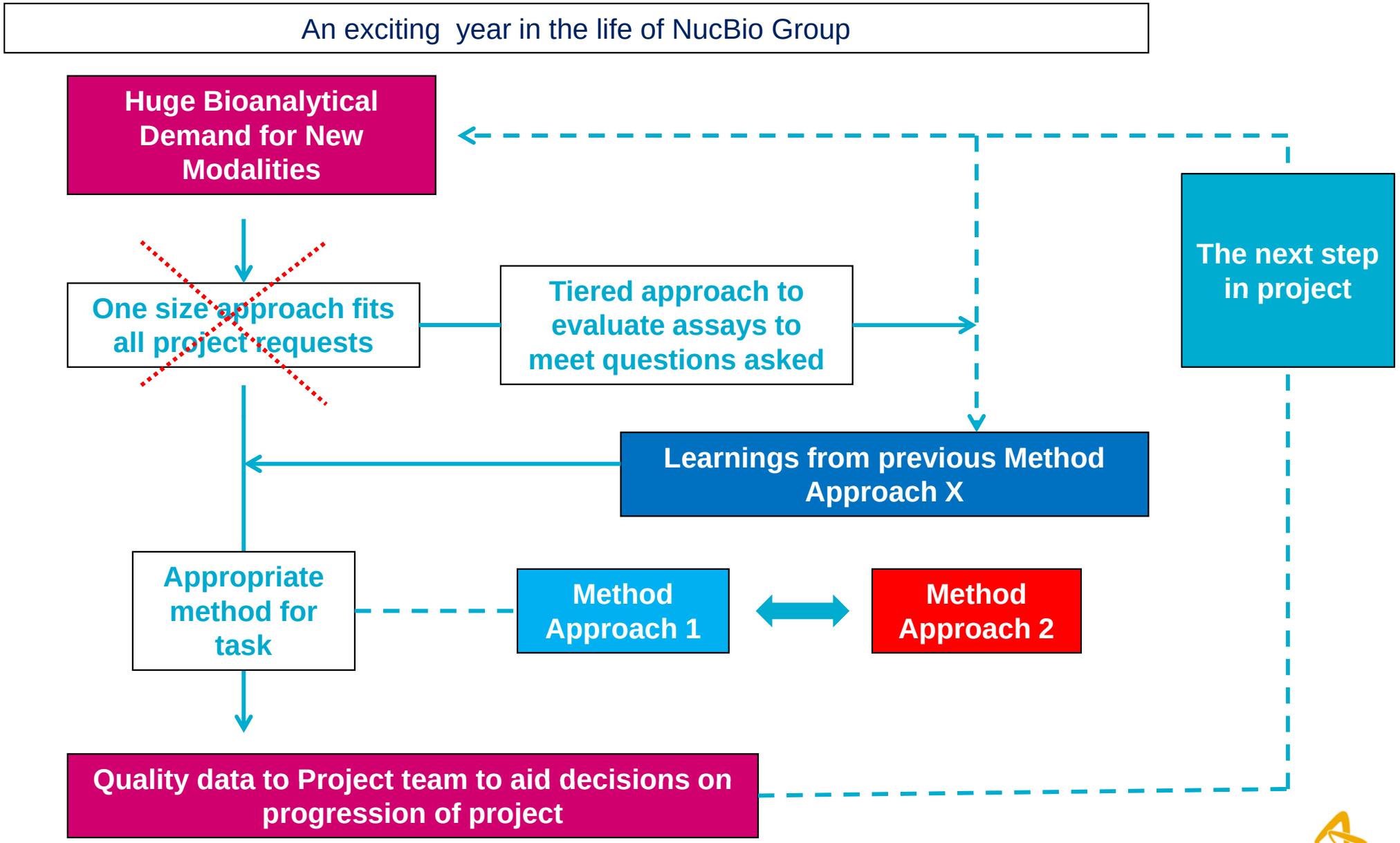
Matrix effects

Prozone

Dilutional linearity

Bioanalytical needs of oligonucleotide therapeutics

Take home message



Bioanalytical needs of oligonucleotide therapeutics

Summary

- New Modalities are an exciting area of drug discovery/development with potential to treat any disease
- Bioanalytical approaches can be simple or complex depending upon the needs of the project
- A combination of bespoke methods and commercially available kits are used
- Applying a “fit for purpose” tiered approach strategy generates confidence in assay performance and data quality for Investigational studies and GLP studies

Bioanalytical needs of oligonucleotide therapeutics

Acknowledgements

- NucBio group (AZ Gothenburg)
  - Åsa Wåhlander
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  - Petra Thulin
  - Daniel Linden
- Collaborators

moderna messenger therapeutics

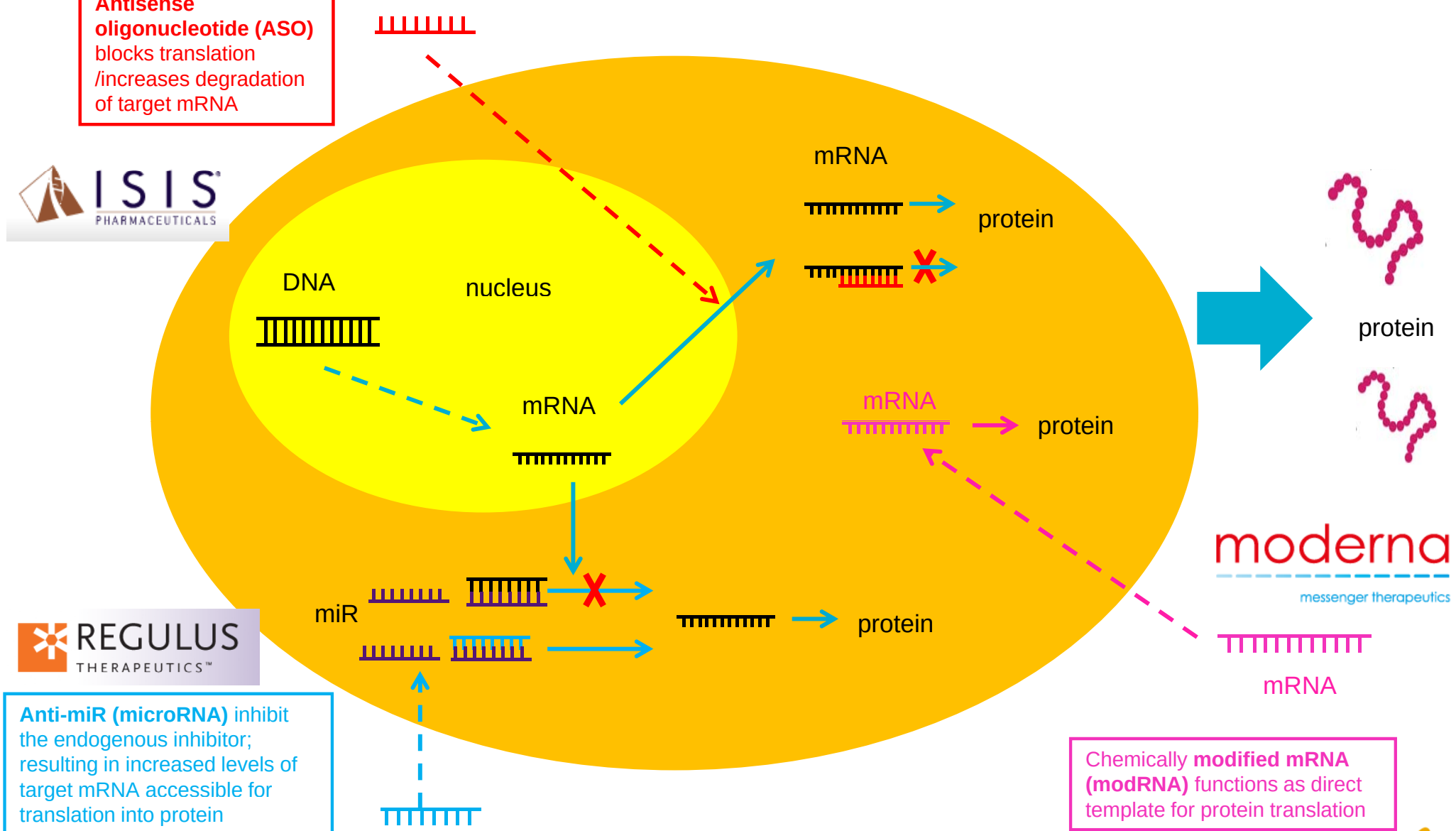
ISIS PHARMACEUTICALS

REGULUS THERAPEUTICS™

Bioanalytical needs of oligonucleotide therapeutics

Setting the scene: biological action of new modalities

Antisense oligonucleotide (ASO) blocks translation /increases degradation of target mRNA



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