Soluble target quantification:

challenges in method development

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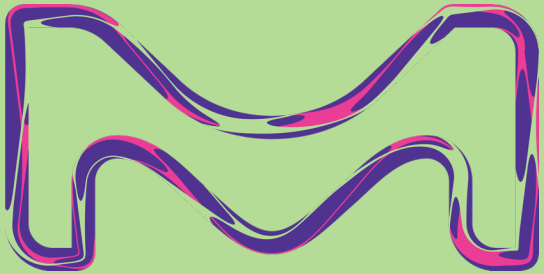
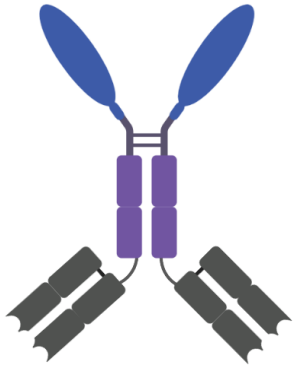


MERCK

Introduction
The Drug and the Target

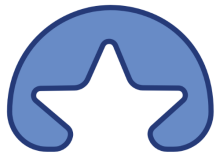


XX-IgG

DRUG: Bispecific fusion protein

anti-TARGET Fabs

TARGET → present in membrane or in soluble form (sTARGET)



TARGET ligand

TARGET expressed on APC membrane (mTARGET)

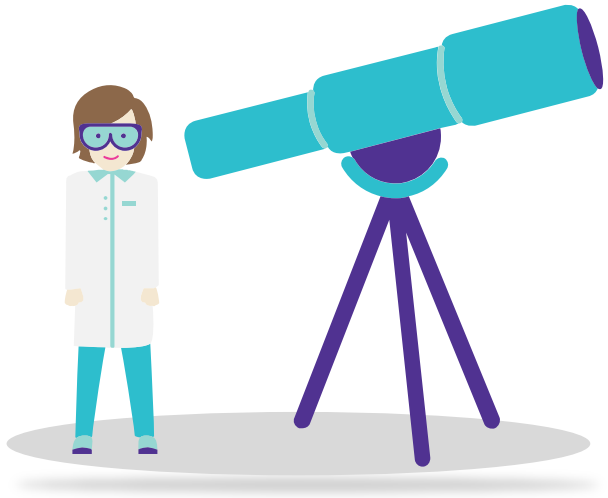
TARGET also present in soluble form (sTARGET)

AIM: quantification of sTARGET

DISCLAIMER: the soluble target (sTARGET) will be called and graphically represented during the presentation as a sTAR not for biological reasons but to help in understanding

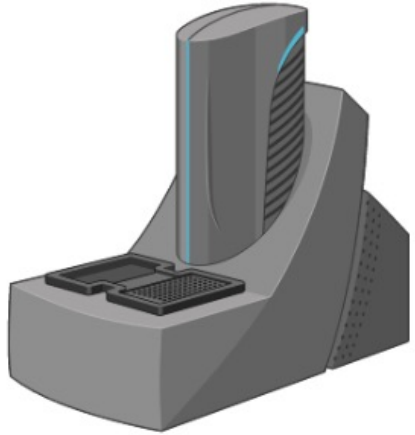
The Challenge
sTAR Quantification

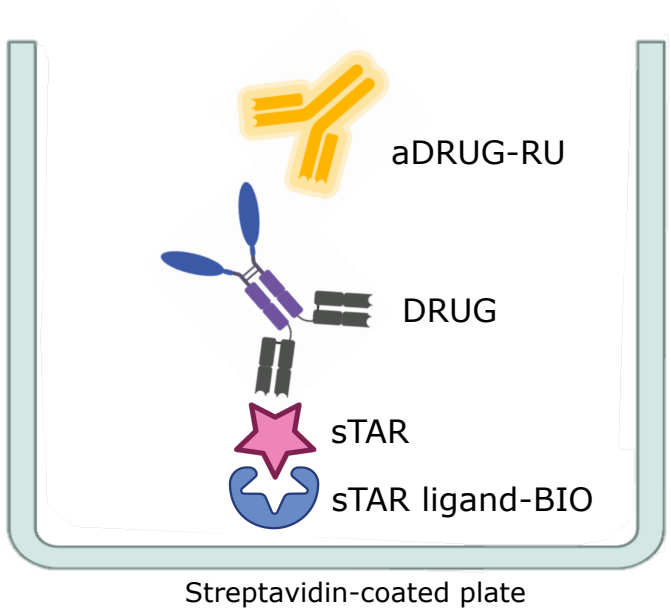
- Quantification of sTAR in the presence of high amount of DRUG
- DRUG present in mg/ml (cMAX 3 mg/ml) vs sTAR present in ng/ml
- sTAR bound to DRUG



The Beginning of Method Development
First Assay Format

- Platform: Mesoscale Discovery - MSD GOLD™ 96-well Streptavidin QUICKPLEX® Plate
- Scouting of capture/drug/detection reagent concentrations
- 4-step assay

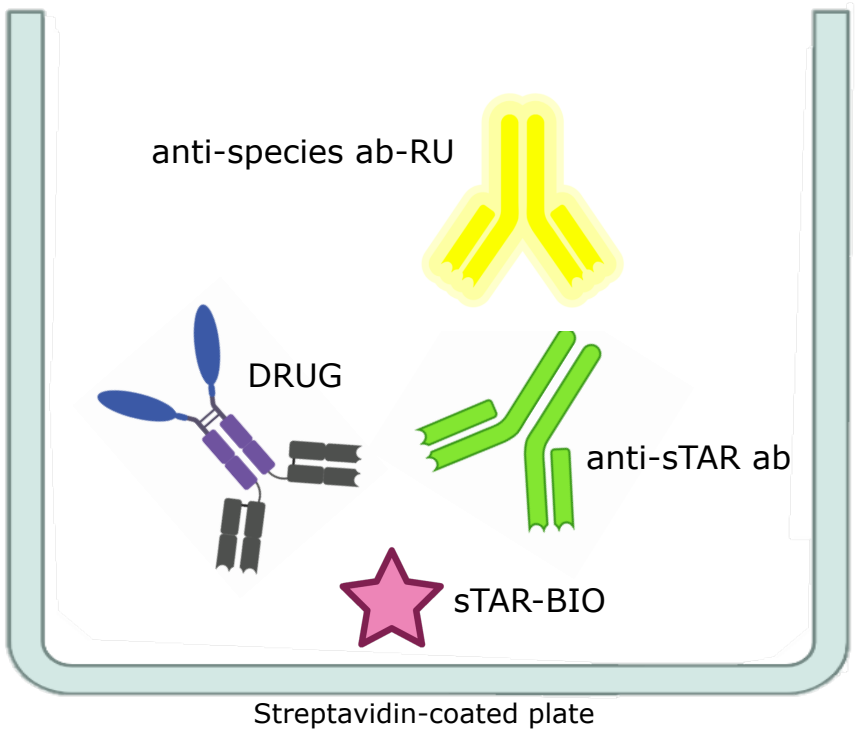




INTERFERENCE BETWEEN DRUG AND sTAR LIGAND

ASSAY FORMAT DISCARDED

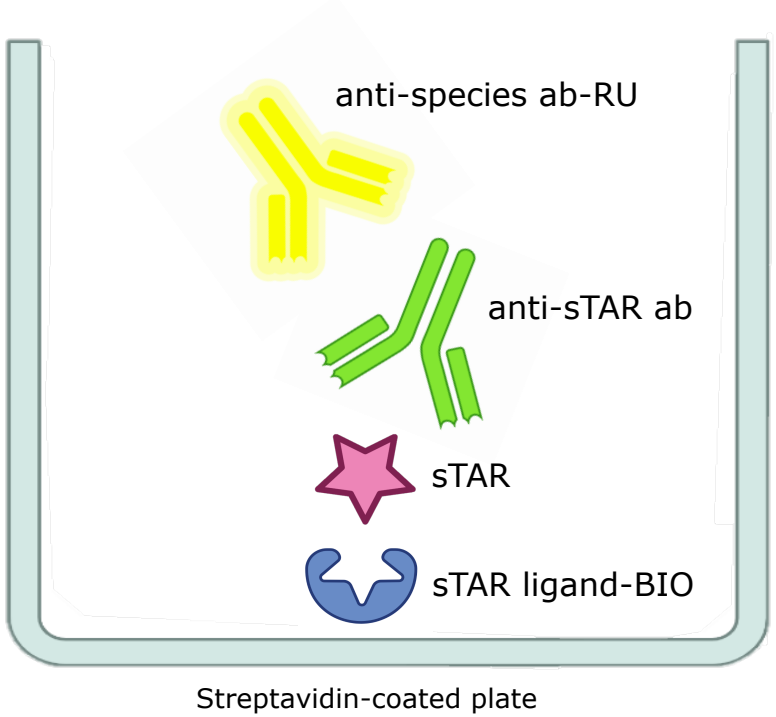
A Different Approach
Finding a Non-Competitive anti-sTAR Antibody with the DRUG



- Detection of sTARGET despite the high concentration of DRUG in samples
- Co-incubation of 3 antibodies anti-sTAR with the DRUG
- **1 out of 3 anti-sTAR antibody binds the target without competing with the DRUG**

The Definitive Approach
Immunoassay Format

- 4-step assay
- Scouting of best reagent concentration
- Test of this assay format with the addition of DRUG to sTAR to create real-life samples

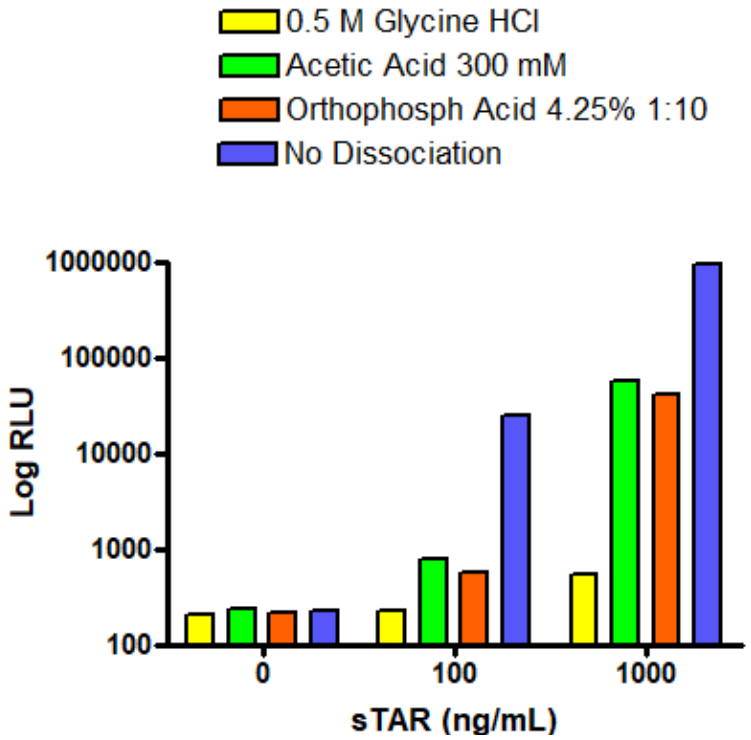


Approaching the Dissociation
Different Acid Screenings

sTAR is bound to the DRUG → need to DISSOCIATE the complex

Test acid-neutralizing buffer setting on sTAR to see the impact on the target

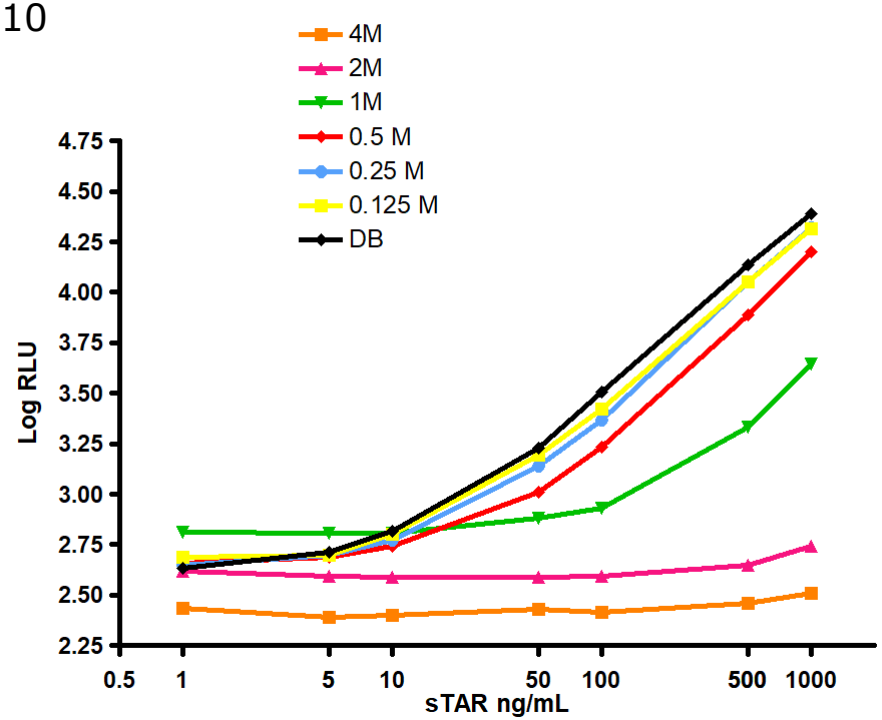
- Orthophosphoric Acid 4.25% 1:10 + Tris-HCl 2M pH 10
- Acetic Acid 300 mM + Tris-HCl 1M pH 10
- 0.5 M Glycine HCl pH 2.6 + Tris HCl 2 M pH 8.0



- Best performance: Acetic Acid BUT no reproducibility
- Reduction of the signal: acidic environment spoils sTAR?
- **Need a milder dissociation!**

A Milder Dissociation
High Ionic Strength with Magnesium Chloride ($MgCl_2$)

- Incubation time 30 min
- 1° dilution in $MgCl_2$ 1:10
- 2° dilution in buffer 1:10



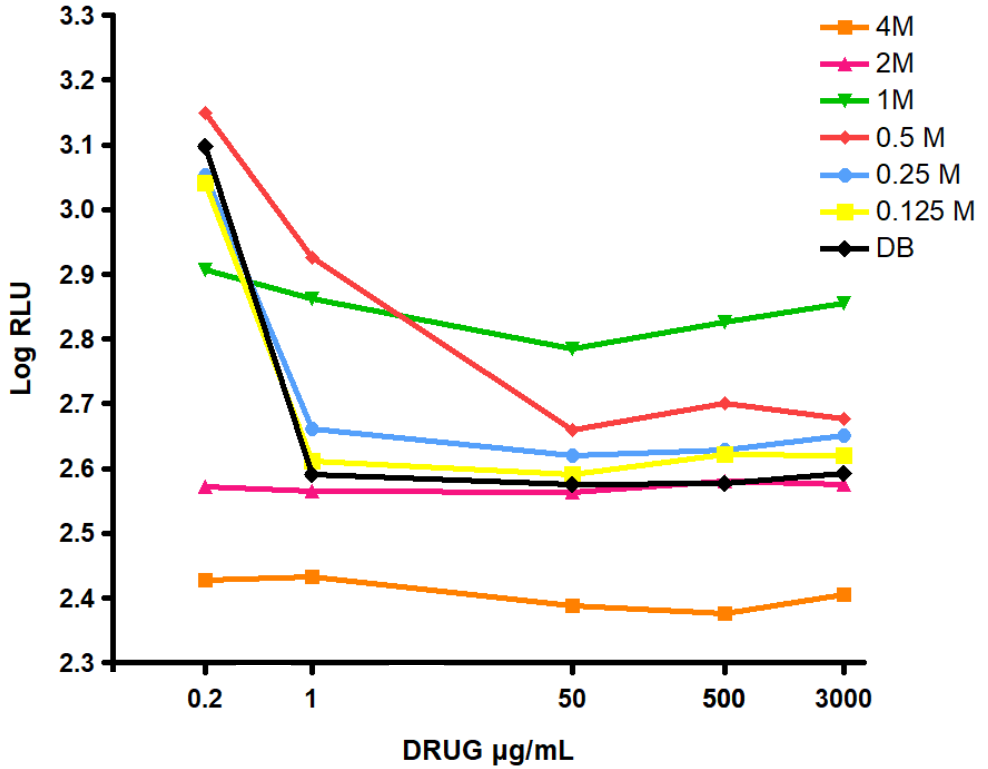
sTAR curve not impacted by treatment with **0.5 M $MgCl_2$** respect acids or $MgCl_2$ higher concentrations

High ionic strength dissociation assay (HISDA) for high drug tolerant immunogenicity testing, *Jordan G, Pohler A, Guilhot F, Zaspel M, Staack R*, Bioanalysis (2020) 12(12)

Dissociate the Complex
Mimic the Real-Life Sample Dissociation (I)

- Incubation time 30 min
- 1° dilution in $MgCl_2$ 1:10
- 2° dilution in buffer 1:10

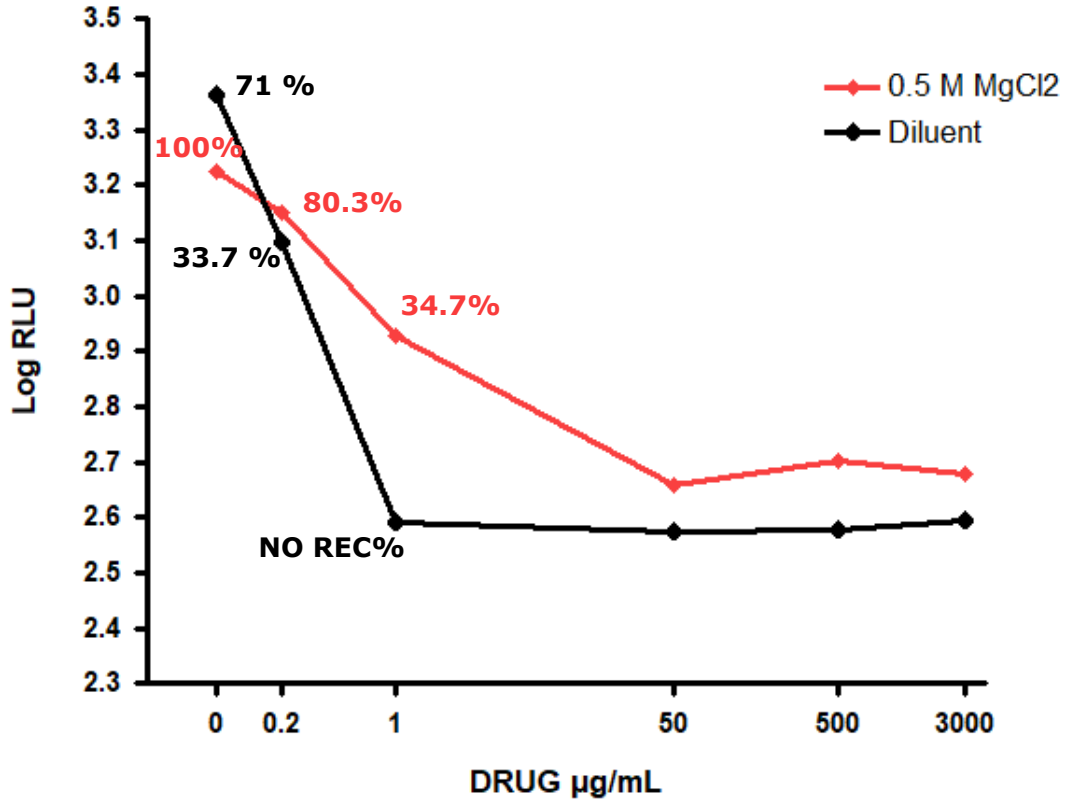
- sTAR samples **100 ng/mL**
- Rapid reduction of the signal with **addition of DRUG** (0.2 – 3000 µg/mL)
- Treatment with **$MgCl_2$ 0.5 M** is the only condition that increases the **drug tolerance**



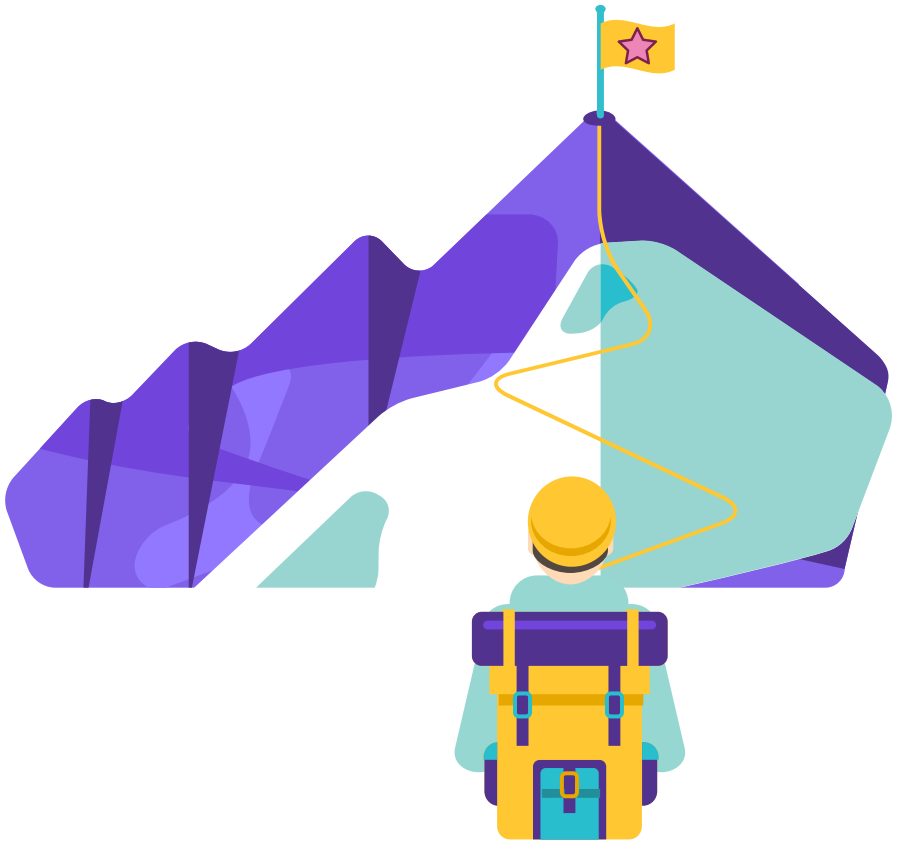
Dissociate the Complex
Mimic the Real-Life Sample Dissociation (II)

Recovery% on sTAR samples + DRUG after $MgCl_2$ dissociation

- sTAR samples **100 ng/mL**
- Recovery % on the CTRL
- With the $MgCl_2$ the recovery with **1 µg/mL** of **DRUG is 34.7%**



New Challenges
When the going gets tough, let the tough get going



The anti-sTAR antibody production was discontinued with no substitute available!

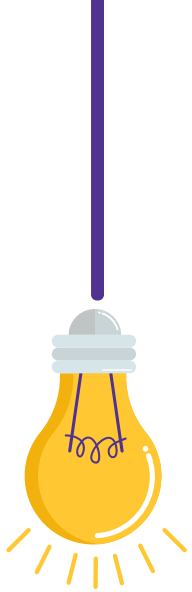
New antibodies research and selection: only the ones where full sTAR sequence was used were selected

Two new antibodies tested → no effective binding both in 3 step or bridging assay format

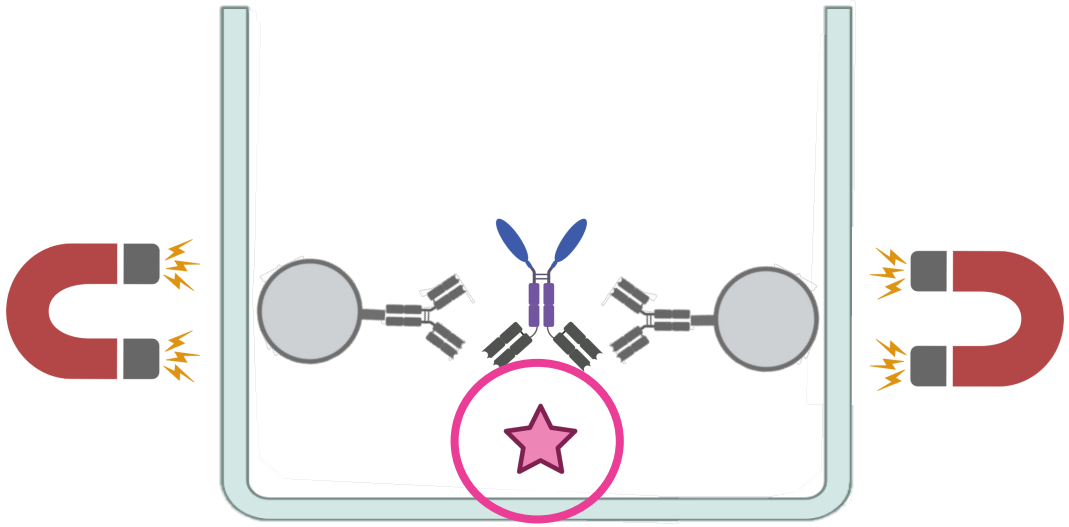
IDEA!

Removing the DRUG
Combination of Dissociation + Drug Removal with Beads

Dynabeads conjugation with antibody (anti-Target idiotype) to remove DRUG from samples



DRUG 0.2 - 3000 µg/mL
sTAR 500 ng/mL



1 Complex Dissociation

0.5 M MgCl2 – 30 min – 1:5 Dilution

2 Dynabeads addition

1 hour incubation – 1:10 Dilution

3 Magnetic separation and supernatant isolation

sTAR isolated and incubated on a MSD plate

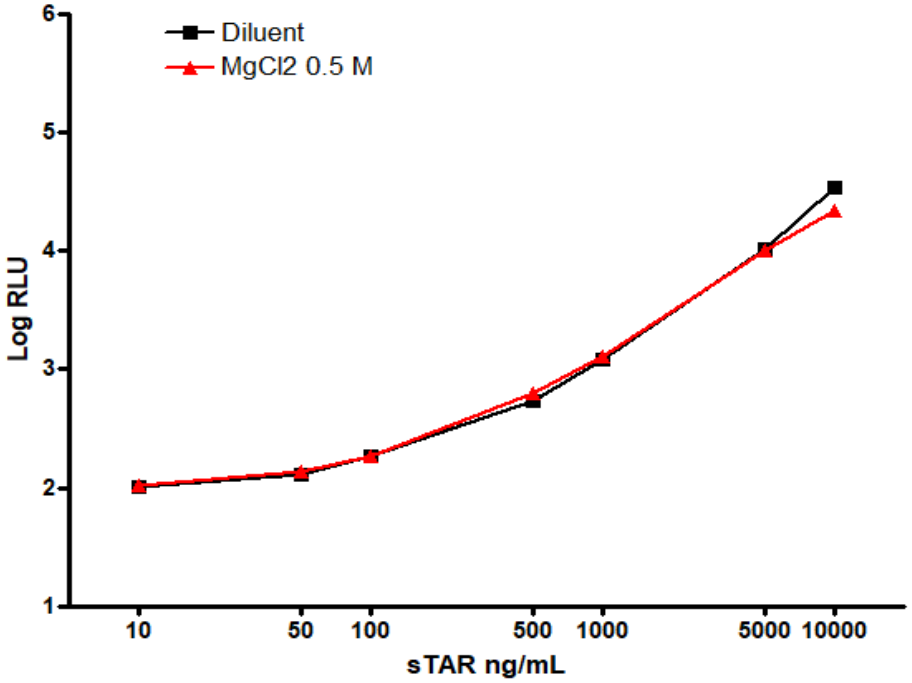
4 3-step assay for sTAR quantification

sTAR ligand-BIO as capture – sTAR – DRUG-RU as detection

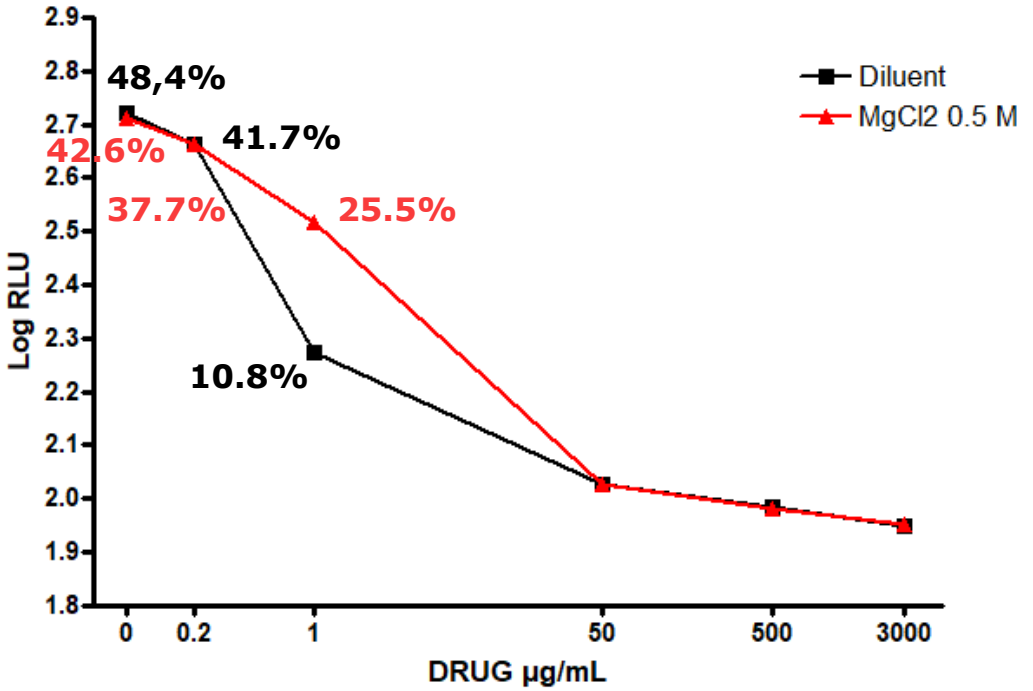
Removing the DRUG
Results

DRUG 0.2 - 3000 µg/mL
sTAR 500 ng/mL

- Treatments do not affect sTAR standard curves



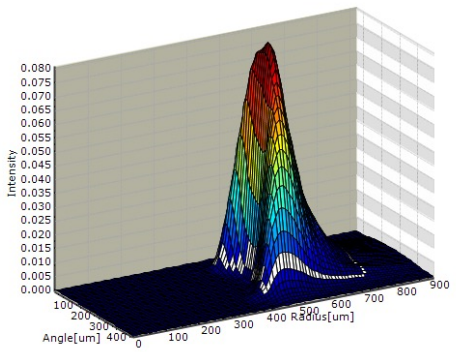
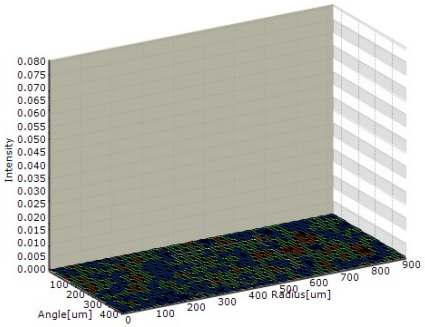
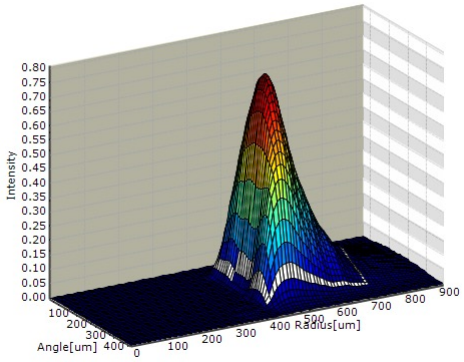
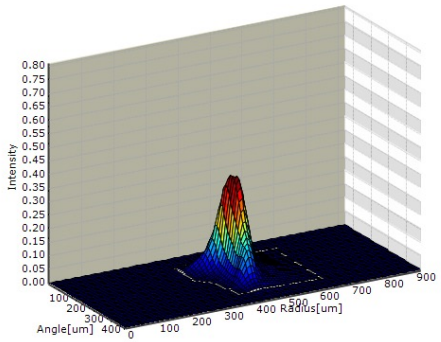
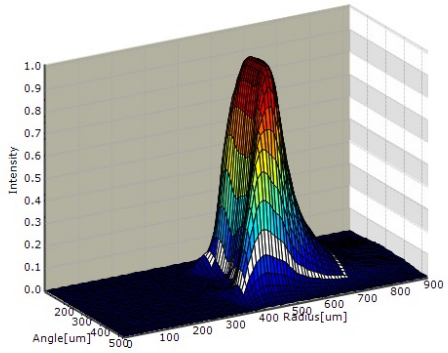
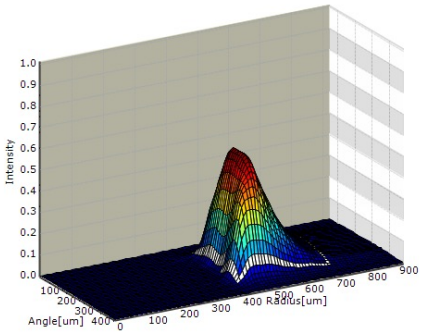
- Similar performances compared to previous experiments without beads
- Low recovery% also in the sTAR controls (<50%)
- **Best recovery with 1 µg/mL (25.5% vs 10.8%)**



Removing the DRUG **Troubleshooting**

Gyrolab analysis with the PK method for DRUG quantification:

- After MgCl2 treatment (**PRE**)
- After Dynabeads incubation (**POST**)

	PRE	POST
500 ng/mL sTAR + **50 µg/mL DRUG**		
500 ng/mL sTAR + **500 µg/mL DRUG**		
500 ng/mL sTAR + **3000 µg/mL DRUG**		

Don't ever stop reaching for the stars...

To Sum Up

- Aim: quantification of a small soluble target present in ng/mL when mg/mL of DRUG is present
- Classic acid dissociation methods tested but sTAR is degraded
- $MgCl_2$ High Ionic Strength dissociation tested with better results: recovery up to 34.7% with 1 µg/ml DRUG
- Bead-based DRUG removal tested – target recovery not improved but troubleshooting highlight a DRUG removal up to 50 µg of DRUG.

Next Steps and Open Points

- Is sTAR still bound to the drug when it is removed?
- Have we reached the platform sensitivity limit?
- Dissociation: do we need to dissociate more? Stronger or longer incubation time?
- Improve sensitivity and sample dilution: test on a different platform?

Acknowledgements

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THANK YOU!

Images created with BioRender