

# Aptamer-LNP Conjugation Optimization for Targeted Drug Delivery: A Comparative Study

Hiba A.M. Gafar<sup>1</sup>, Alexandra R. Paul<sup>1,3</sup>, Mauro Giacca<sup>2</sup>, Adam A. Walters<sup>1</sup>, Khuloud T. Al-Jamal<sup>1,3</sup>

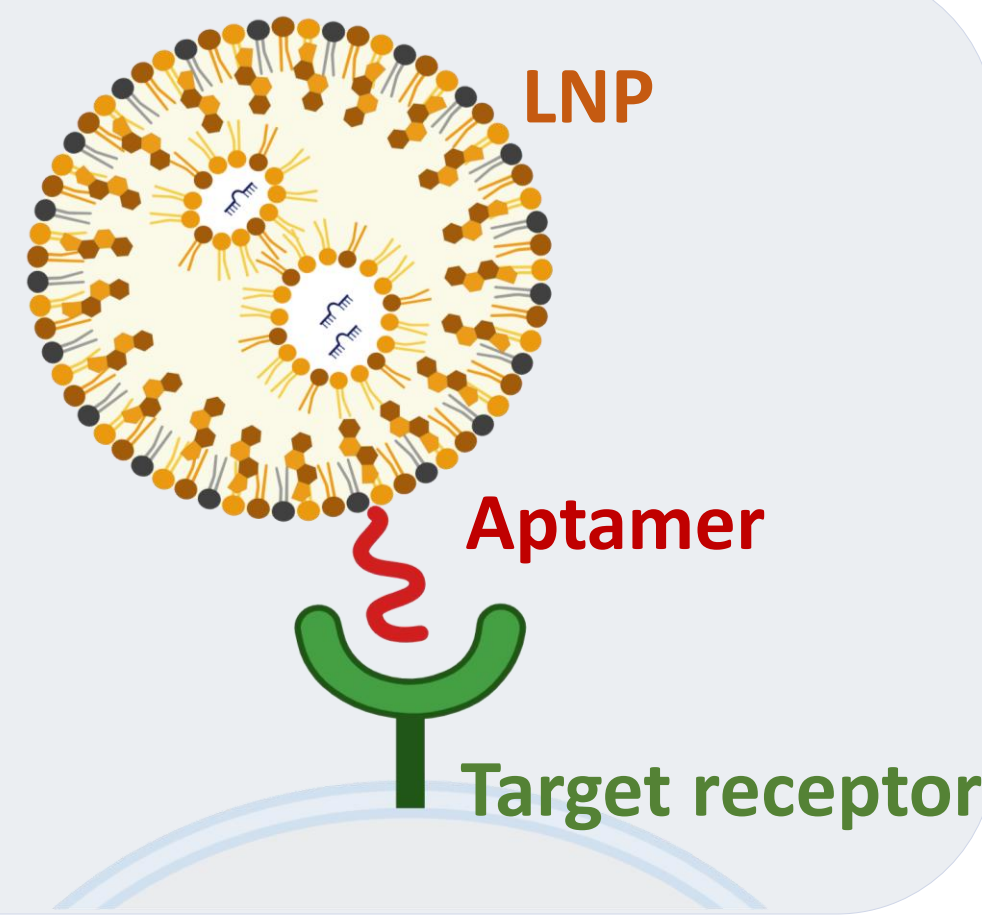
<sup>1</sup>Institute of Pharmaceutical Science, Faculty of Life Sciences & Medicine, King's College London, London SE1 9NH, UK

<sup>2</sup>School of Cardiovascular and Metabolic Medicine & Sciences, King's College London, SE5 9NU

<sup>3</sup>Department of Pharmacology and Pharmacy, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong SAR

## INTRODUCTION

LNP-mediated drug delivery is often limited by non-specific cellular uptake. Aptamers can improve targeting specificity, but their effectiveness depends on how they are conjugated to the nanoparticle surface (1).



## OBJECTIVE



Compare maleimide-thiol (2) and cholesterol-mediated aptamer conjugation (3) strategies for LNP functionalization to identify the optimal approach for stable conjugation and evaluate their impact on cellular association and uptake.

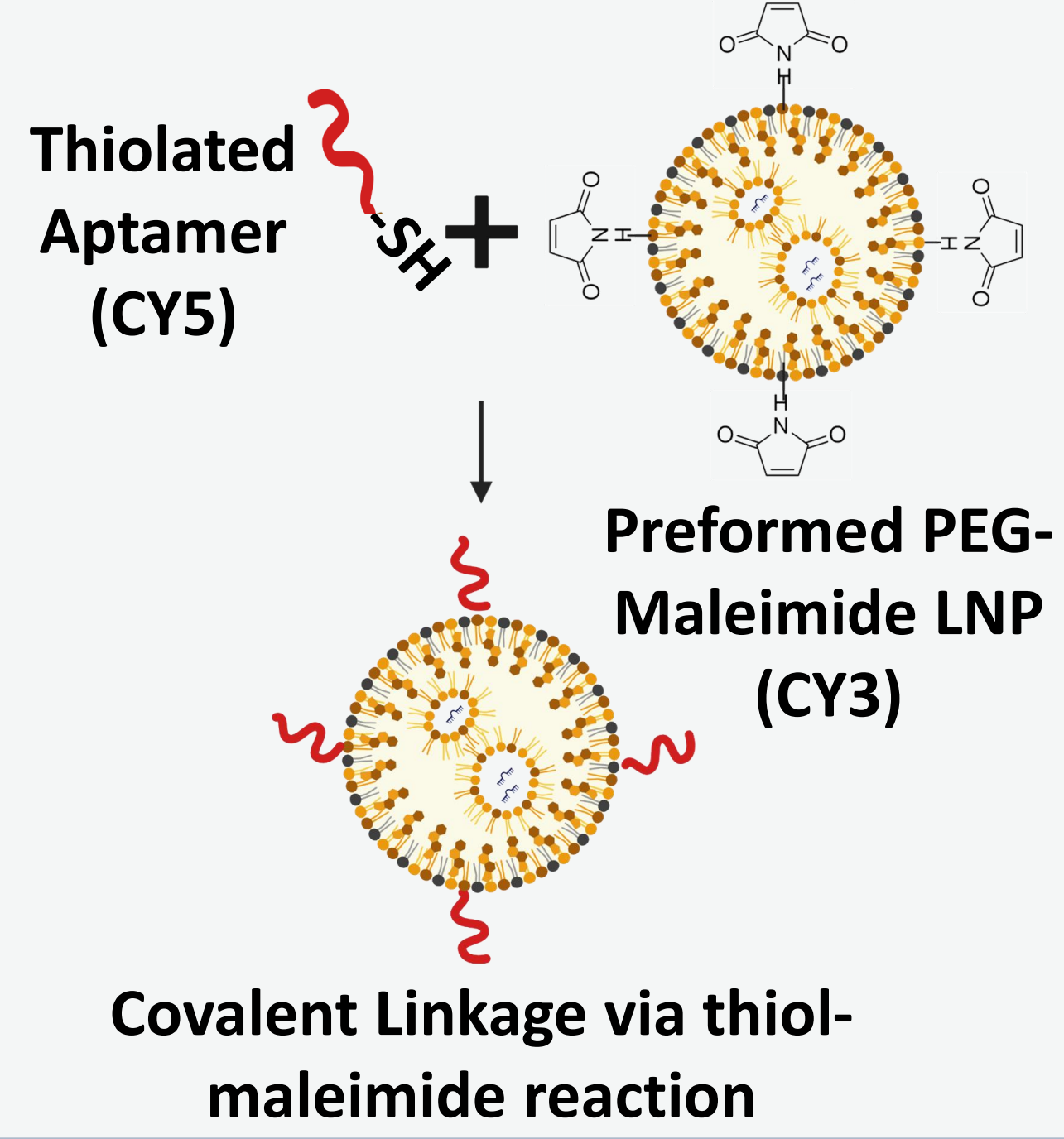
## KEY TAKEAWAY



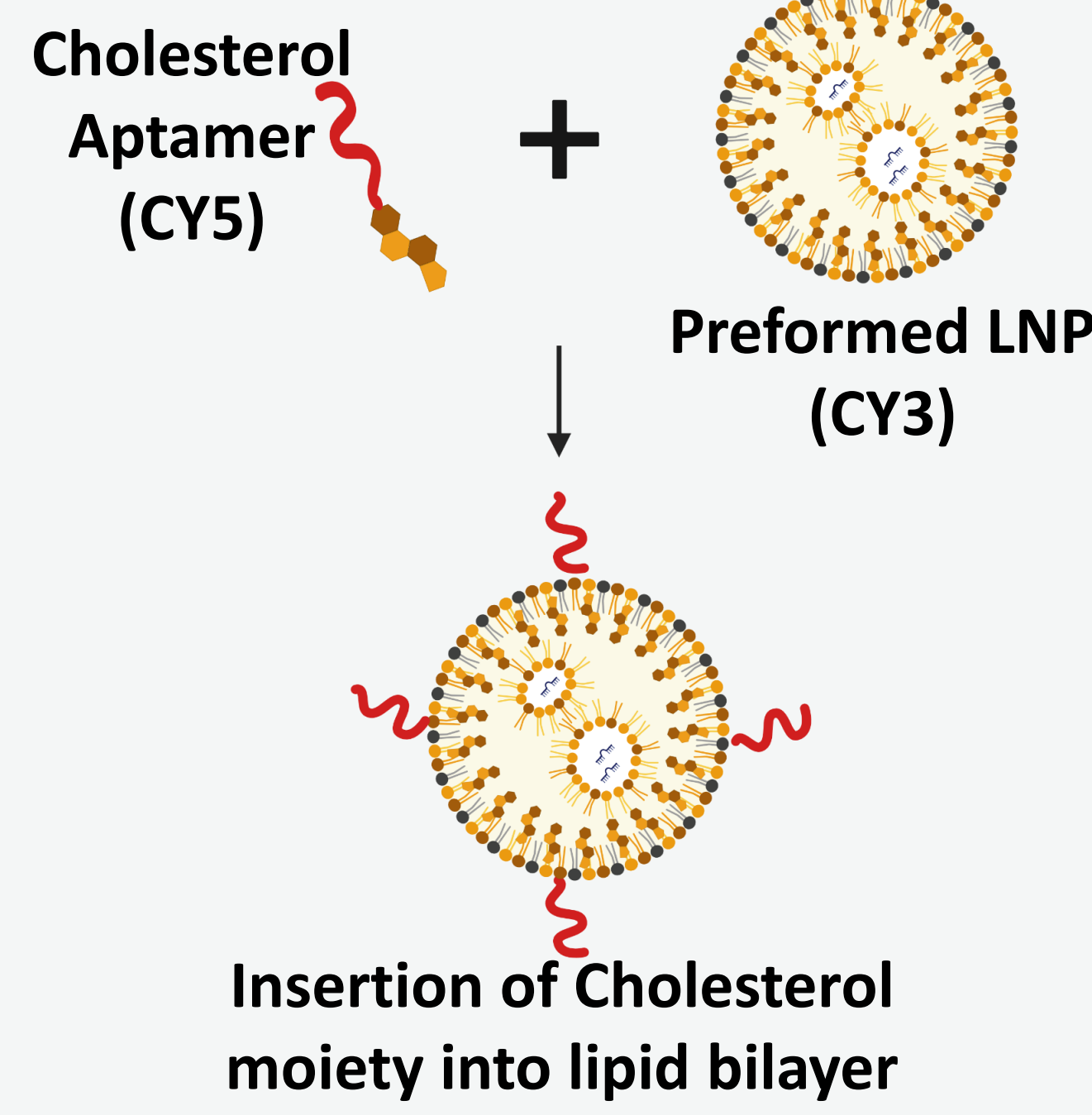
Cholesterol-mediated anchoring provides the most robust aptamer-LNP conjugation; however, cholesterol-driven cell association can mask receptor-specific uptake.

## 1. CONJUGATION STRATEGIES COMPARED

### MALEIMIDE-THIOL COVALENT CONJUGATION



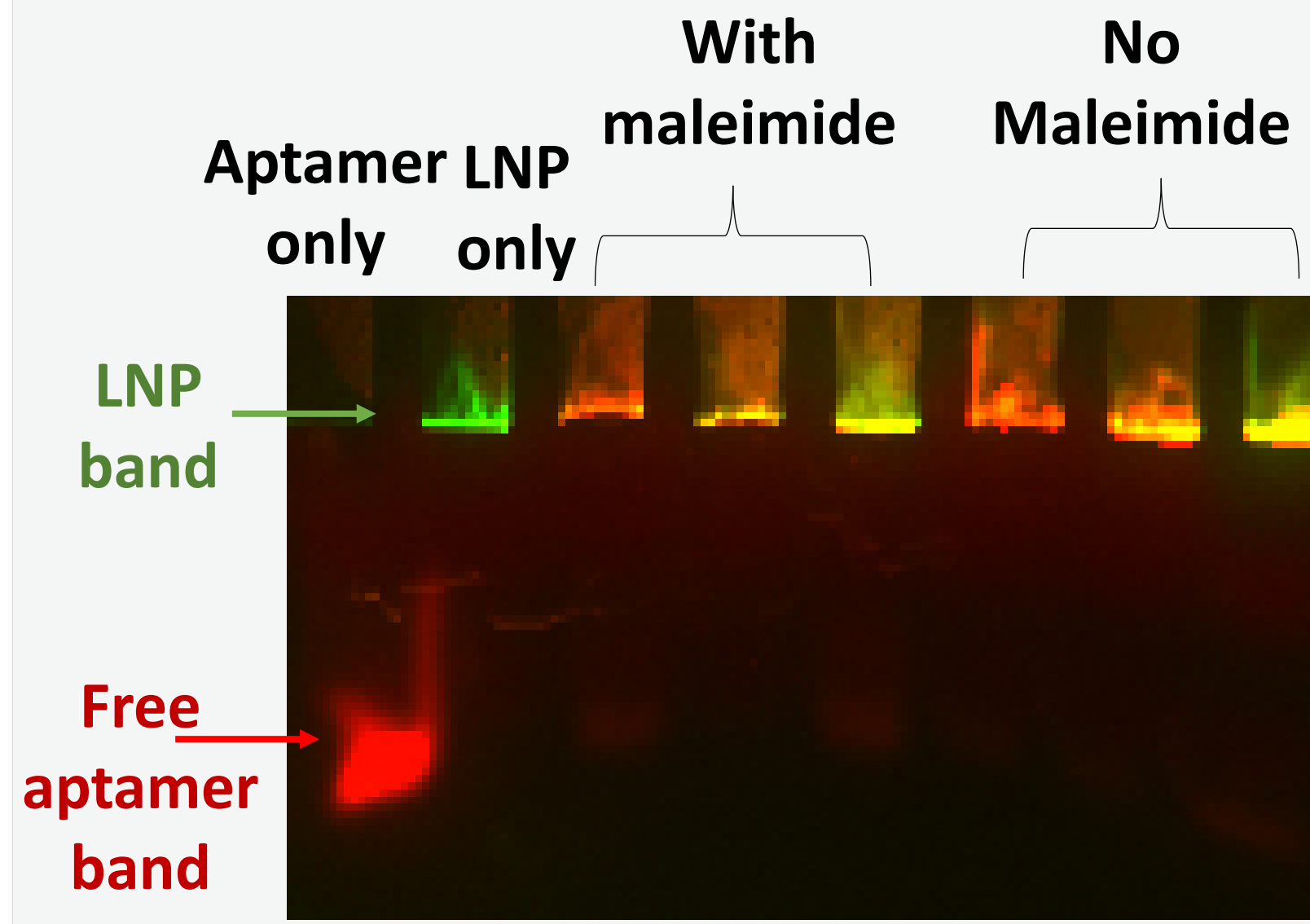
### CHOLESTEROL-MEDIATED ANCHORING



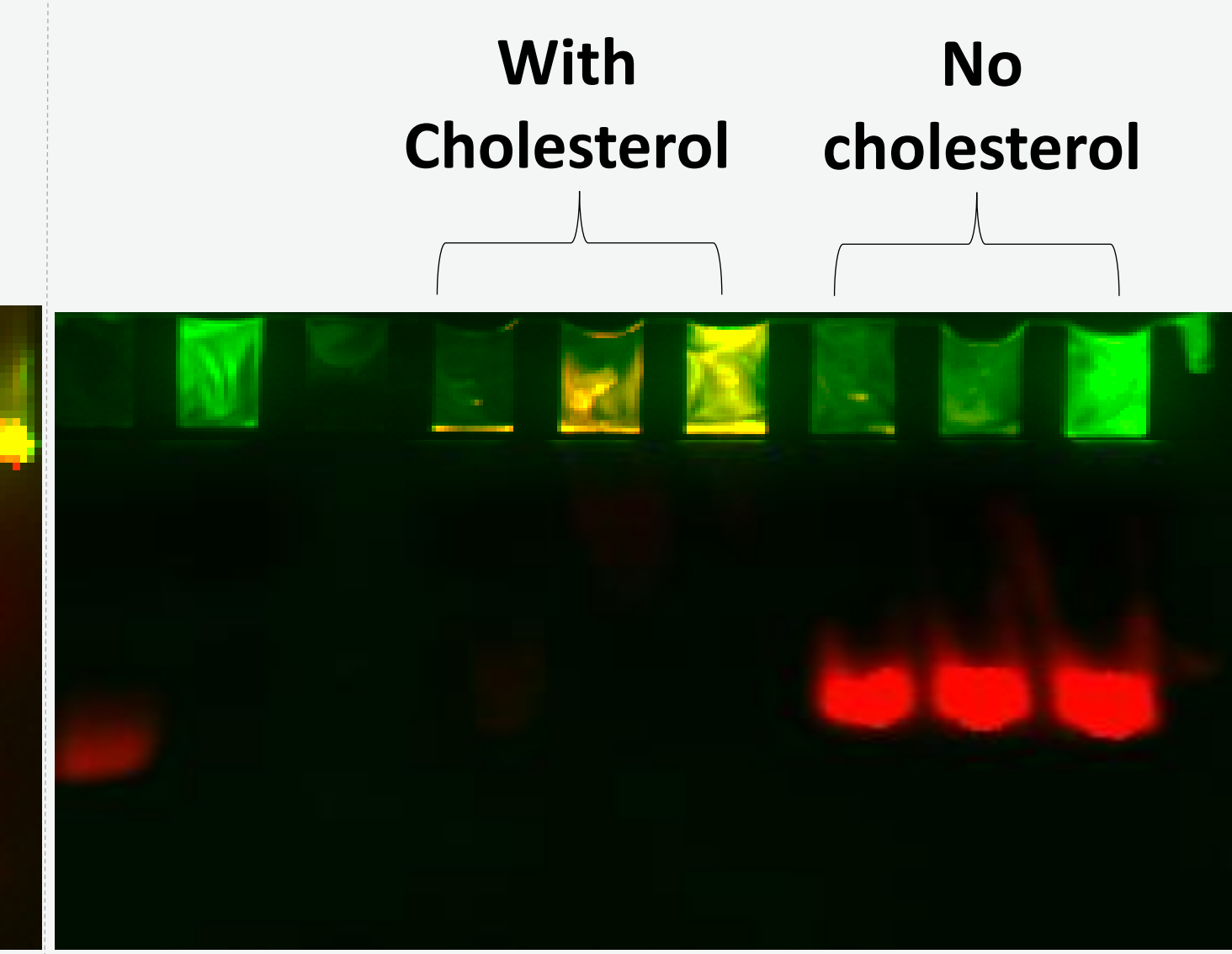
LNPs prepared by lipid self-assembly and labelled with DOPE-CY3. 2.225E-10 moles of CY5-labelled aptamer were incubated overnight with the LNPs.

## 2. CONJUGATION VALIDATION (GEL RETARDATION)

### MALEIMIDE-THIOL CHEMISTRY



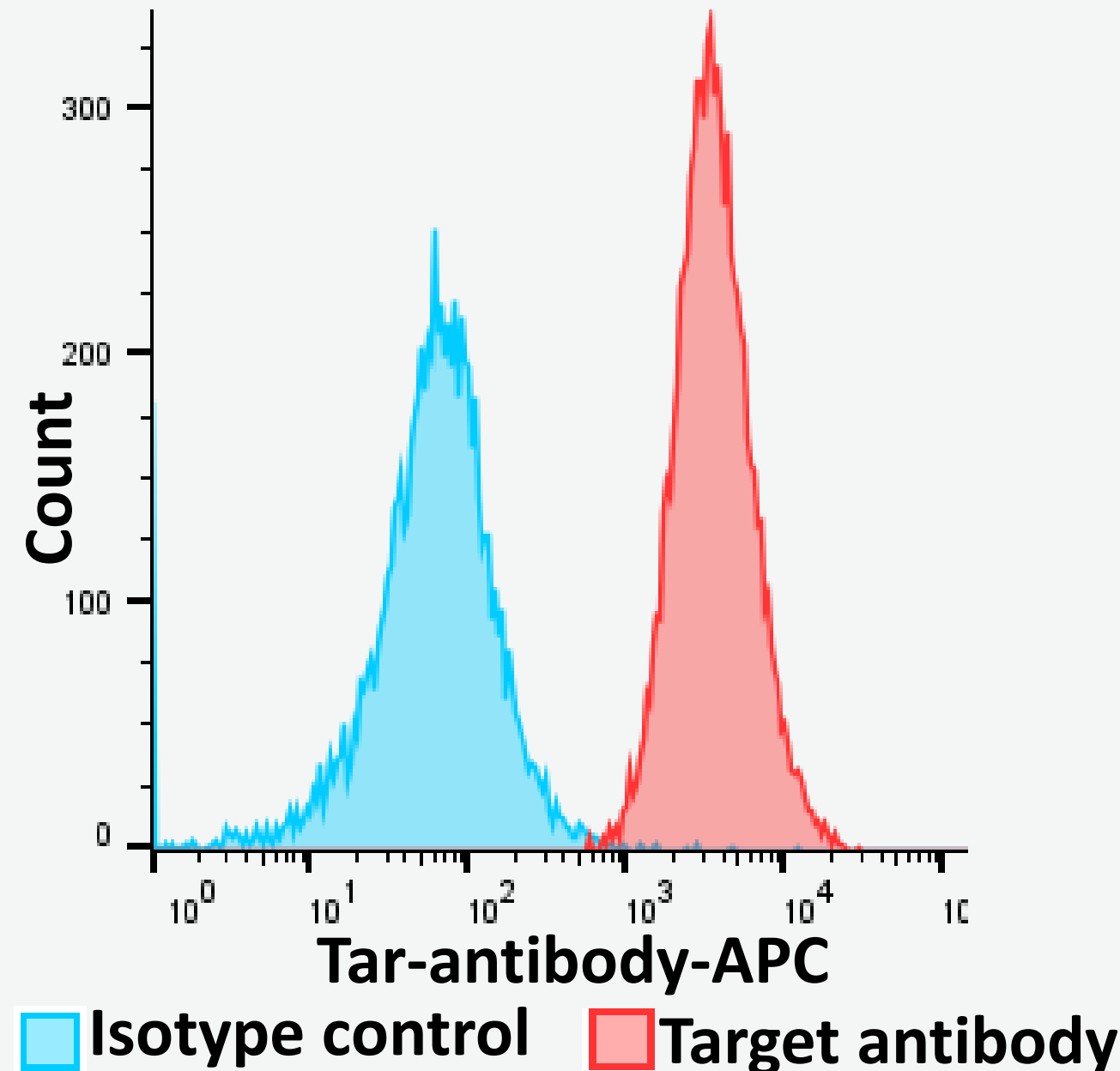
### CHOLESTEROL ANCHORING



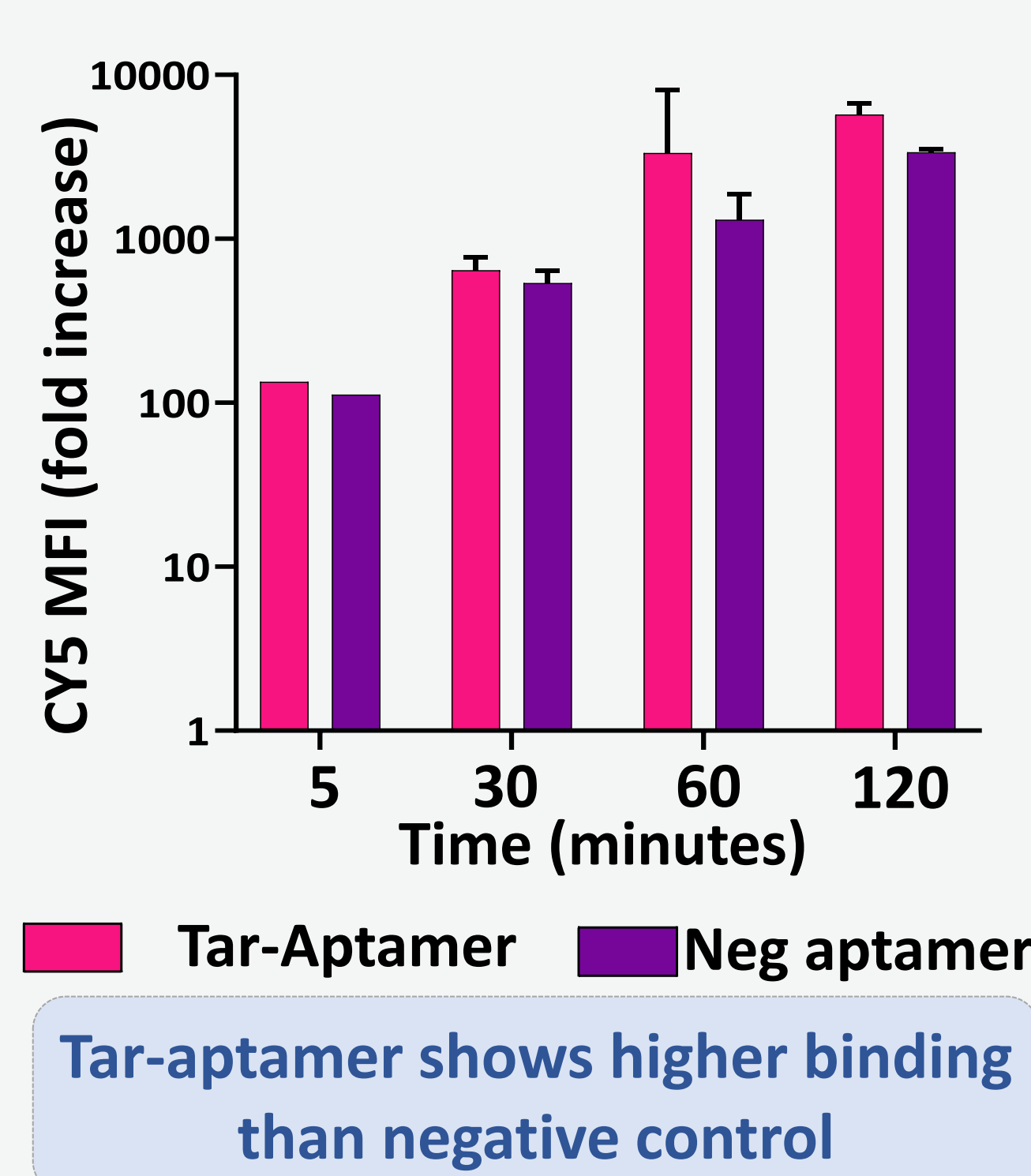
Cholesterol conjugation shows strong and specific association with LNPs. Selected for further biological evaluation

## 3. APTAMER BINDING TO GL261

### TARGET PROTEIN EXPRESSION

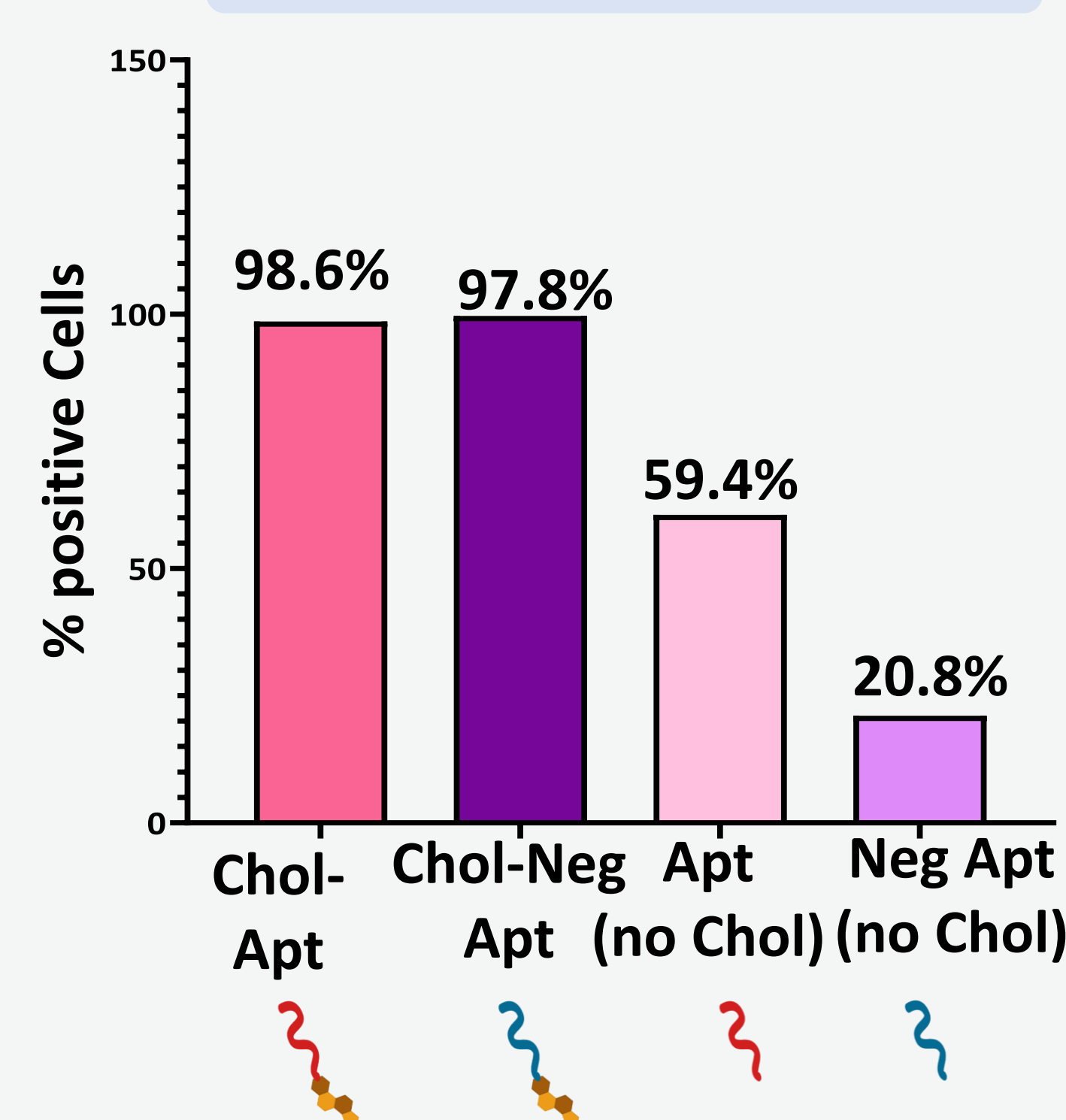


### APTAMER BINDING (CY5 MFI)



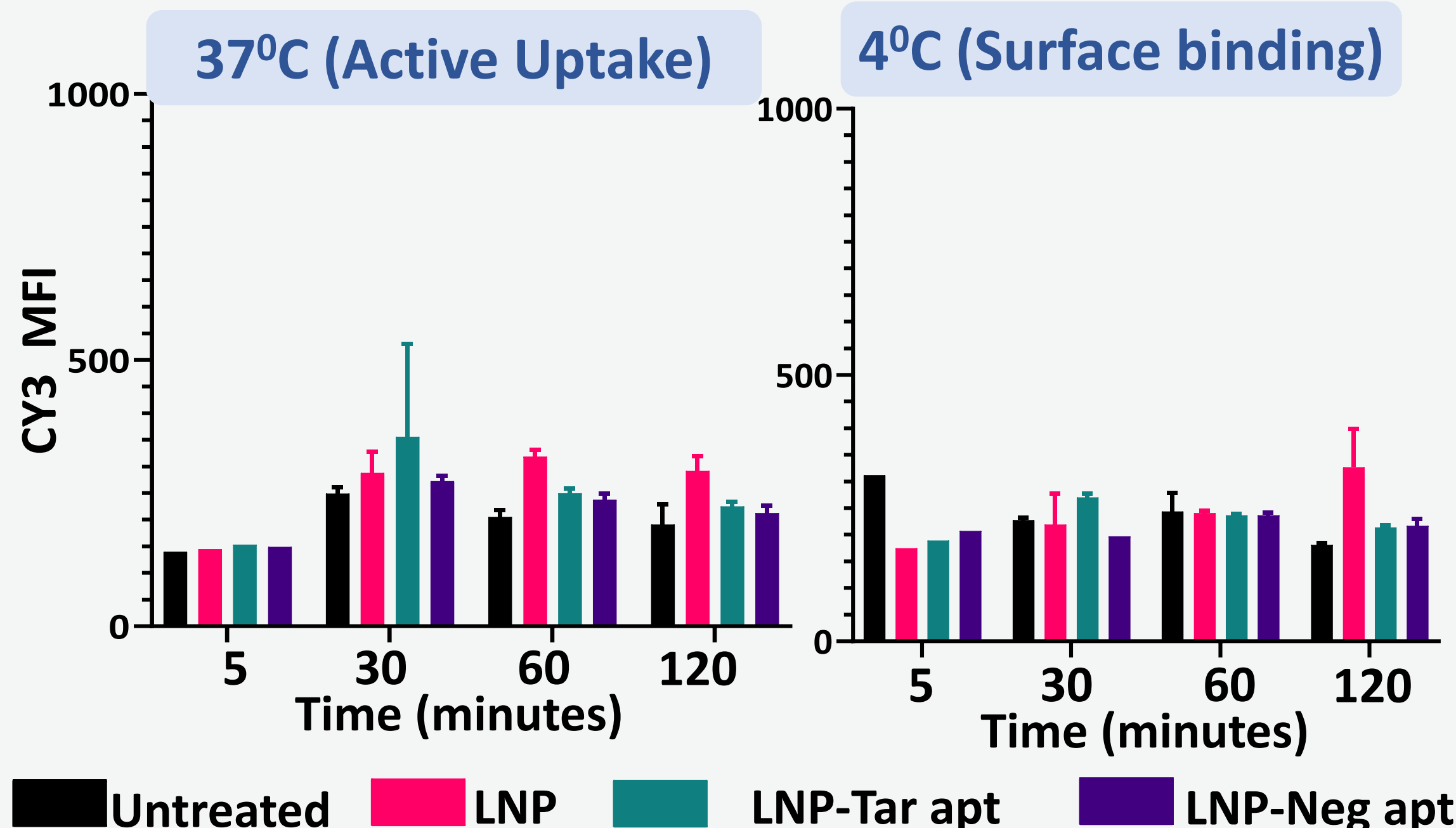
## 4. CHOLESTEROL-DRIVEN CELL ASSOCIATION

### CY5 POSITIVE CELLS (%)



✓Cholesterol is a major driver of cell association. Targeting signals can be masked by cholesterol-mediated interactions.

## 5. NANOPARTICLE UPTAKE SUMMARY (CY3 MFI)



## 6. COMPARATIVE SUMMARY

Evaluation Criterion	Maleimide-Thiol (Covalent)	Cholesterol (Anchoring)
Stable LNP association	★★★★☆	★★★★★
Specificity of conjugation	★★★☆☆	★★★★★
Scalability reproducibility	★★★☆☆	★★★★★
Ease of preparation	★★★★☆	★★★★★

★★★★★Excellent ★★★☆☆ Good ★★☆☆☆ Moderate

## 7. CONCLUSION

- Cholesterol-mediated anchoring provides the most stable aptamer-LNP conjugation.
- Tar-aptamer retains binding activity following conjugation.
- Cholesterol substantially enhances cell association independent of aptamer sequence.
- Increased nanoparticle association does not necessarily translate into receptor-specific uptake.
- Optimal conjugation requires balancing conjugation stability with true targeting specificity

## 8. WHAT'S NEXT?

- ✓ Evaluate nucleic acid cargo delivery and gene silencing.
- ✓ Assess receptor-blocking and competition studies.
- ✓ Further optimise aptamer density to reduce non-specific binding.
- ✓ Validate biodistribution in glioblastoma models.

## CONTACT

Hiba Gafar  
[Hiba.gafar@kcl.ac.uk](mailto:Hiba.gafar@kcl.ac.uk)  
 Institute of Pharmaceutical Science, Faculty of Life Sciences & Medicine, King's College London  
[www.linkedin.com/in/hiba-gafar-663a87123](http://www.linkedin.com/in/hiba-gafar-663a87123)

## REFERENCES

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