# Carrier Proteins for Conjugate Vaccines

*EcoCRM® ($CRM_{197}$), Tetanus toxin and Qß Virus-Like Particles*

## Introduction

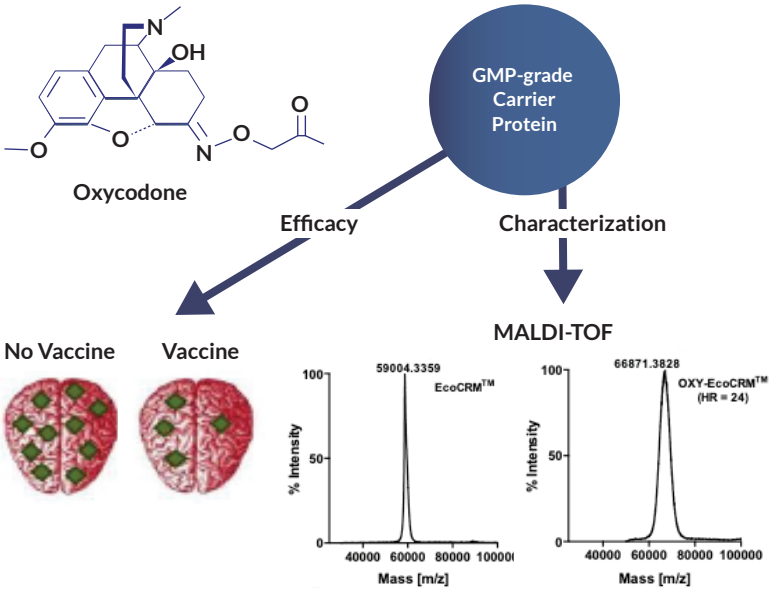
Many antigens are poorly immunogenic unless chemically linked to a carrier protein. Tetanus toxoid, diphtheria toxoid and $CRM_{197}$, a genetically detoxified diphtheria toxin, are among the few carrier proteins licensed for human use. Fina Biosolutions, a conjugate vaccine R&D company, is committed to increasing the availability of carrier proteins and conjugation technology. We have developed an *E. coli* strain with an oxidative cytoplasm that can express disulfide-bonded proteins and have used it to produce carrier proteins in high yield, including two genetically detoxified toxins, EcoCRM® ($CRM_{197}$) and 8MTT (modified tetanus toxin). To expand the range of accessible conjugate carriers, we also present data on Qß, an *E. coli* expressed virus-like particle.

## FINABIO CARRIER PROTEIN PORTFOLIO

| **TTHc**<br>Tetanus toxin fragment | **EcoCRM®**<br>$CRM_{197}$ | **8MTT**<br>Modified tetanus toxin | **Qß**<br>VLP |
|---|---|---|---|
| 50 kDa | 58.4 kDa | 150 kDa | 28 nm-diameter nanoparticle |
| heavy chain fragment C | $CRM_{197}$, a widely used genetically detoxified diphtheria toxin | 8MTT is the first genetically detoxified tetanus toxin | High stability |
| Preclinical | EcoCRM® has been extensively compared to $CRM_{197}$ from other sources[1] | 8 mutations to fully detoxify | Contains T cell epitopes |
| Extensive literature | EcoCRM® available for research & clinical use | $50x10^6$ less toxic than tetanus toxin | High level of symmetry |
| Anti-TTHc neutralizes toxin | | Large size allows for higher hapten: protein ratios | 180 identical subunits |

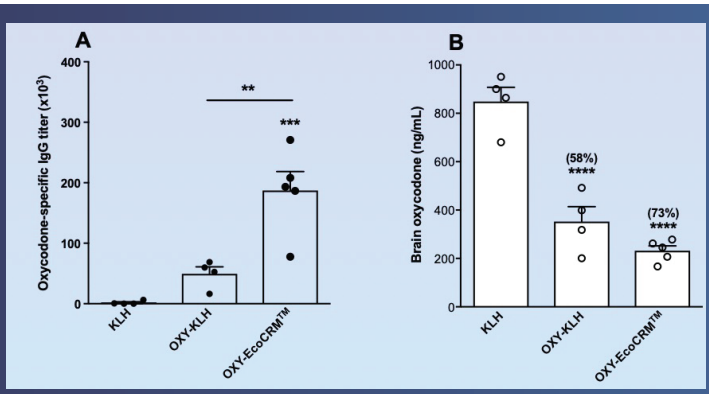
## EcoCRM®($CRM_{197}$) Hapten Conjugate[2]

A conjugate vaccine has been developed that elicit opioid-specific IgG antibodies that reduces opioid distribution to the brain. Immunization selectively blocks opioid-induced behaviors, such as opioid self-administration, and prevents opioid-induced respiratory depression and bradycardia in mice and rats. KLH was used as the carrier protein in initial studies. Due to its mollusk origin and poor characterization, native KLH is not an ideal carrier. This study compared oxycodone conjugates using EcoCRM® and KLH as carrier proteins[1].




EcoCRM® was analyzed by MALDI-TOF before and after conjugation to the OXY hapten. A ratio of 24 haptens per $CRM_{197}$ was calculated from the MW difference. KLH conjugates were too large to be characterized by mass spec.

The oxycodone-EcoCRM® conjugate showed superior efficacy to the previously established oxycodone-KLH. In contrast to KLH conjugates, EcoCRM® conjugates are easier to characterize.




Induction of oxycodone-specific IgG antibody prevents oxycodone distribution to the brain in mice.

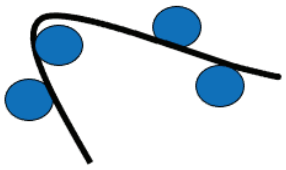
BALB/c mice (n ≥ 4/group) were immunized s.c. with 100 µg unconjugated KLH (control), OXY-KLH, or OXY-EcoCRM®, formulated with 1 mg of alum adjuvant on days 0, 14 and 28. On day 35, mice were challenged with 2.25 mg/kg oxycodone, and brain and serum collected for analysis of vaccine efficacy.

A. Oxycodone-specific serum IgG titers analyzed by ELISA.
B. Oxycodone concentration in the brain 30-min after oxycodone challenge, analyzed by GC/MS. Above bars, percentages (%) indicate decrease in brain oxycodone compared to KLH.

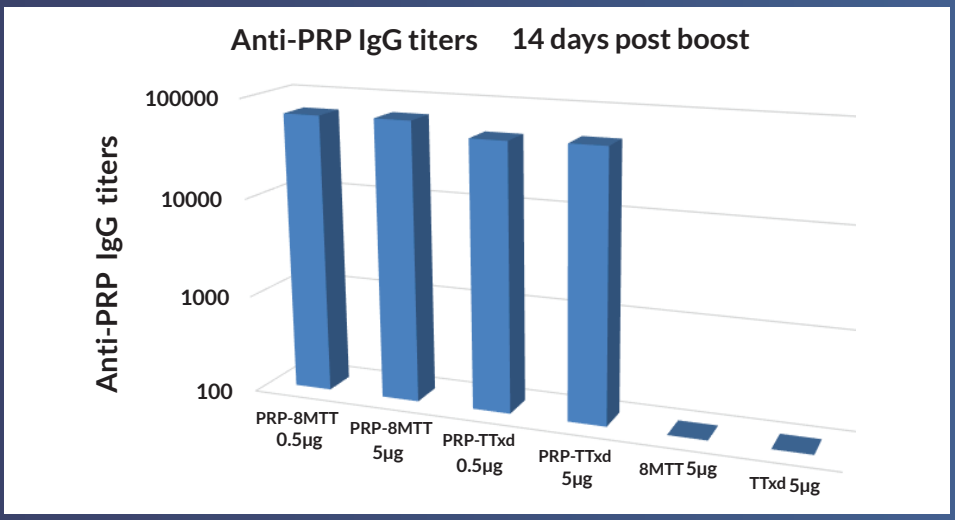

FinaBio
Fina Biosolutions LLC

FinaBio.com 877-346-2246 (877-FinaBio) info@FinaBio.com

## 8MTT vs Tetanus Toxoid



Polysaccharides are poorly immunogenic unless linked to a carrier protein. 8MTT or tetanus toxoid were conjugated to *H. influenzae* b capsular polysaccharide (Hib PRP). Outbred mice were immunized with 0.5 or 5µg of conjugated PRP absorbed to Alhydrogel® on day 0 and 14. Anti-PRP titers were determined from a day 28 bleed.



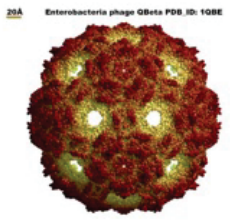

Anti-PRP titers were comparable regardless of whether tetanus toxoid or 8MTT was used as the carrier protein. Anti-tetanus toxin titers were also similar.

8MTT development at FinaBio was supported by NIH SBIR R43AI148018

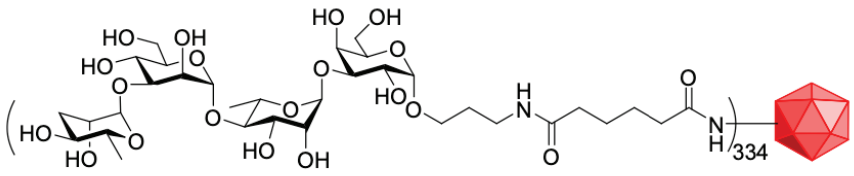
## Qß Virus-Like Particles[3]

Qß VLP are assembled from the Qβ bacteriophage capsid protein. Each subunit is about 14kDa and provides ample amines and carboxyls for conjugation. *E. coli* RNA is packaged during VLP assembly and is a potential TLR7 ligand.

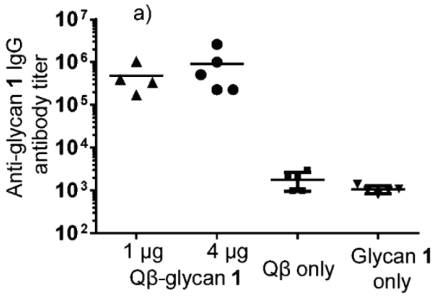
**QßVLP 28 nm particle**

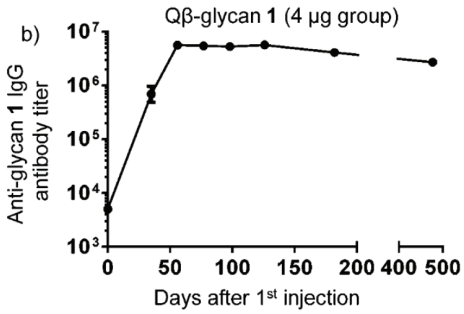


**Synthetic *Salmonella* Enteriditis-derived glycan**




**Qβ-glycan 1**




Robust and long-lasting anti-glycan **1** antibody responses were inducedby Qß–glycan **1** in mice. (a) Anti-glycan **1** IgG titers on day 35 after the first immunization in Qß–glycan **1** (1 and 4 mg of glycan) immunized and controlgroups. Each symbol represents one mouse. Geometric mean titers (GMTs) are indicated by solid bars. (b) Average IgG titers induced by Qß–glycan **1** (4 mg) monitored over 477 days. The persistence trend in the 1 mg group was similar.

## Summary

Conjugate vaccine optimization includes selection of a suitable carrier protein. FinaBio's vaccine portfolio provides access to a variety of conjugate carrier proteins. Along with FinaBio's expertise in conjugation chemistry, this facilitates the development of conjugate vaccines.

### References

[1] Analytical Comparability Assessments of Five Recombinant $CRM_{197}$ Proteins from Different Manufacturers and Expression Systems. J Pharm Sci. 107:1806, 2018.
[2] Pre-clinical efficacy and characterization of candidate vaccines for treatment of opioid use disorders using clinically viable carrier proteins. Mol Pharm 15:4947, 2018.
[3] Synthetic and immunological studies of *Salmonella* Enteritidis O-antigen tetrasaccharides as potential anti-*Salmonella* vaccines. Chem.Commun. 55:4519, 2019.

FinaBio
Fina Biosolutions LLC

FinaBio.com 877-346-2246 (877-FinaBio) info@FinaBio.com