EcoCRM™

A Recombinant CRM_{197} Carrier Protein

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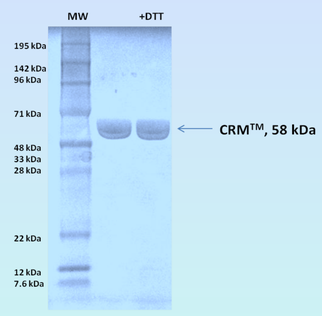
EcoCRM™ Affordable CRM_{197} Carrier Protein

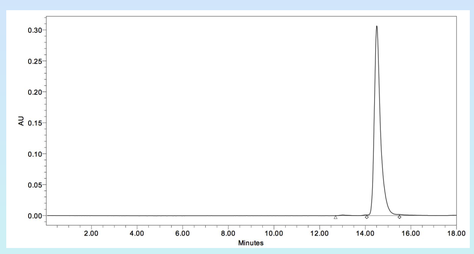
CRM_{197} , a genetically detoxified diphtheria toxin, is widely used as a carrier protein in conjugate vaccines. It was originally expressed as a secreted protein in *Corynebacterium diphtheriae*. Until recently, both "native" and recombinant CRM_{197} has been difficult to obtain and/or expensive.

Fina BioSolutions has developed a new CRM_{197}, EcoCRM™, produced in *E.coli*. EcoCRM™ is expressed as soluble protein in the cytoplasm of a widely-used *E.coli* expression strain. High expression of EcoCRM™ in a BL21 strain and a simple purification method allows low cost production and the promise of significantly reducing the cost of this component of conjugate vaccines.

The data presented show that EcoCRM™ is an excellent carrier protein for a variety of antigens.



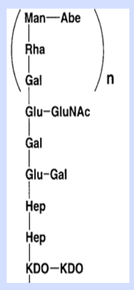
SDS PAGE EcoCRM™
>99% purity. No nicking evident.



SEC HPLC of EcoCRM™
Dimer is <0.6%. Purity >99%.

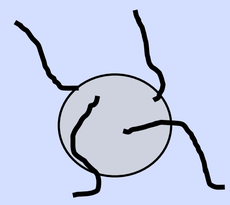
S. Typhimurium Core-OPS-EcoCRM™ Conjugate

Development of a glycoconjugate vaccine to prevent invasive Salmonella Typhimurium infections in sub-Saharan Africa. Baliban et al. PLoS Negl Trop Dis 11(4):e0005493, 2017

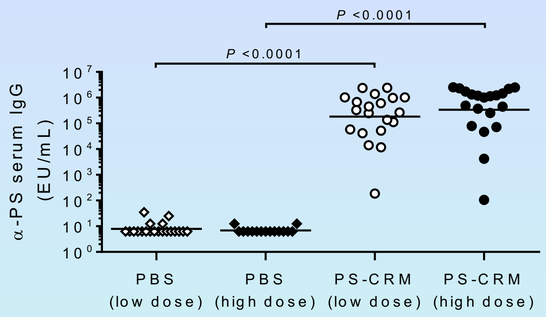


***S.* Typhimurium core O-polysaccharide (STm-COPS)**

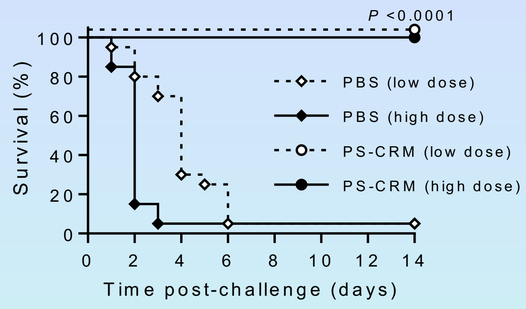
***STm-COPS* was covalently linked to EcoCRM™ via its KDO group**



$STm\text{-}COPS^{KDO}$-EcoCRM™ neoglycoconjugate



Serum IgG titers against STm-COPS from mice (n = 20/group) immunized with 2.5 μg $STm\text{-}COPS^{KDO}$:CRM_{197} or PBS. Solid bars indicate the GMT.

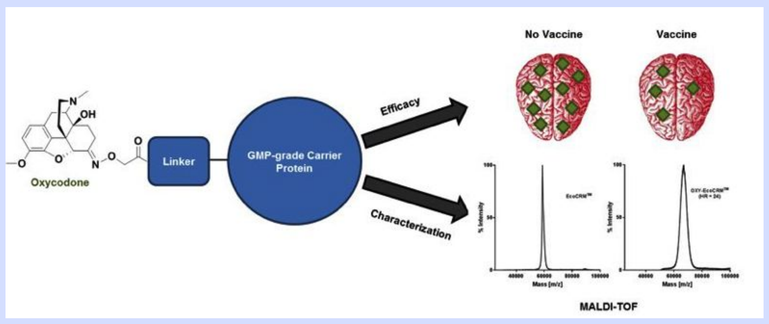


Kaplan-Meier survival curves of mice immunized with $STm\text{-}COPSKDO\text{-}CRM_{197}$ (circle) or PBS (diamond) after challenge (n = 20/group) with 1 or $5x10^6$ CFU of STm D65.

_S._ Typhimurium COPS-EcoCRM™ conjugate provided 100% protection in this animal model

Therapeutic Vaccines for Treatment of Opioid Abuse and Overdose

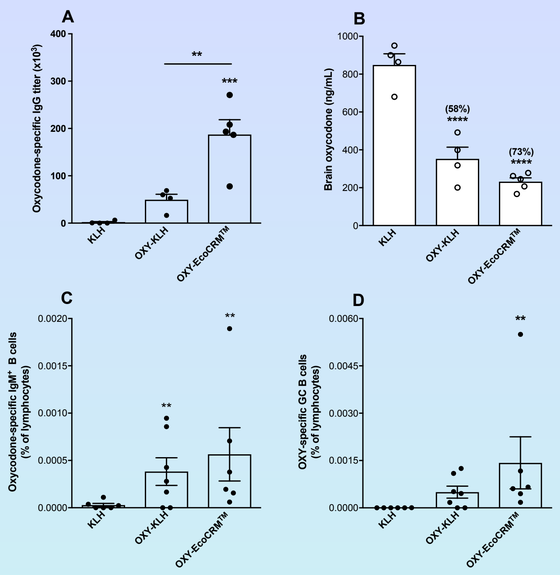
The Pravetoni lab has developed a series of conjugate vaccines that elicit opioid-specific IgG antibodies that reduce opioid distribution to the brain. Immunization selectively blocks opioid-induced behaviors, such as opioid self-administration, and prevents opioid-induced respiratory depression and bradycardia in mice and rats. Current efforts focus on readying for FDA approval and Phase I clinical trial a vaccine consisting of oxycodone (OXY) conjugated to a GMP monomer KLH carrier protein. Due to its mullosk origin and poor characterization, native KLH is not an ideal carrier. This study compared oxycodone conjugates using EcoCRM™ and KLH.



The haptenization ratio of oxycodone-EcoCRM™ can be characterized by MALDI-TOF.

EcoCRM™ was analyzed before and after conjugation to the OXY hapten. A ratio of 24 haptens per CRM was calculated from the MW difference. We were unable to characterize the conjugates containing native KLH.

***IN VIVO* VACCINE EFFICACY**



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 then 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.

Induction of oxycodone-specific B cells

BALB/c mice (n ≥ 4/group) were immunized i.m. with 100 μg KLH, OXY-KLH, or OXY-EcoCRM™, formulated with 200 μg of alum. At 7 days post-immunization, OXY-specific B cell populations from spleen and lymph nodes were isolated by antigen-based magnetic enrichment paired with multi-parameter flow cytometry.

C) Oxycodone-specific IgM^+ B cells.
D) Oxycodone-specific germinal center (GC) B cells.

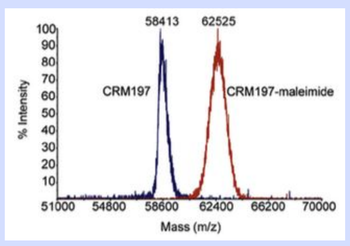
The oxycodone-EcoCRM™ conjugate showed superior efficacy than the previously established oxycodone-KLH. In contrast to KLH conjugates, EcoCRM™ conjugates are easier to characterize.

The Pravetoni group is currently developing vaccines and monoclonal antibodies against heroin, oxycodone, and fentanyl abuse and overdose (Pubmed, "Pravetoni M"). For collaborations or partnership, please contact Dr. Marco Pravetoni at prave001@umn.edu.

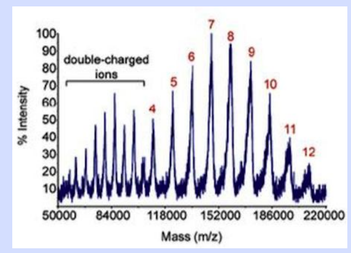
HIV Glycopeptide-EcoCRM™ Conjugate

Synthesis of multivalent glycopeptide conjugates that mimic an HIV epitope. Bailey et al. Tetrahedron. 72:6091, 2016.

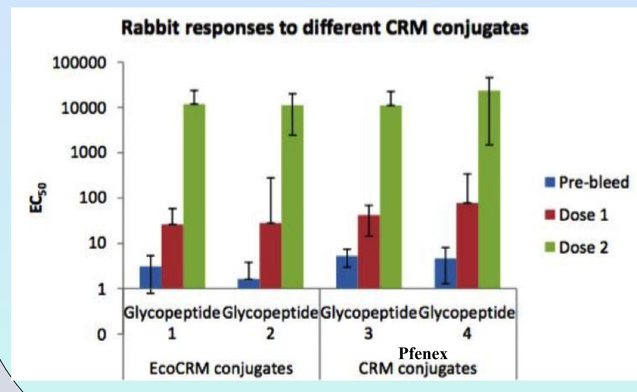
Thio-ether conjugation is frequently used for linking peptides, oligosaccharides and other antigens to a carrier protein such as CRM_{197}.



EcoCRM™ was labeled with an excess of the NHS maleimide reagent sulfo-EMCS. The MW of EcoCRM™ increased from 58,413 to 62,525, (delta=4100). Each linker adds a mass of 208, indicating about 20 of CRM's 39 lysines have been labeled.



Thiol-glycopeptide was conjugated to the EMCS-labeled CRM_{197}. Mass spec of the conjugates indicated ~7 glycopeptides/CRM.



Glycopeptide conjugates of EcoCRM™ and CRM from *P. fluorescens ("Pfenex")* were prepared. Rabbits were immunized twice. After the final bleed,IgG anti-glycopeptide titers were measured using glycopeptide-BSA as the ELISA antigen.

Glycopeptide conjugates with EcoCRM™ & Pfenex CRM induced comparable anti-glycopeptide titers in rabbits.

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EcoCRM™ is an effective carrier protein for conjugate vaccines and is an alternative to CRM_{197} from *Corynebacterium* and *Pseudomonas*. A simple expression system, high yields and the efficient purification of EcoCRM™ can help reduce the cost of conjugate vaccines.