Lawrence Livermore National Laboratory

Nanolipoprotein Particles - Platform for Functional Membrane Proteins and Countermeasures

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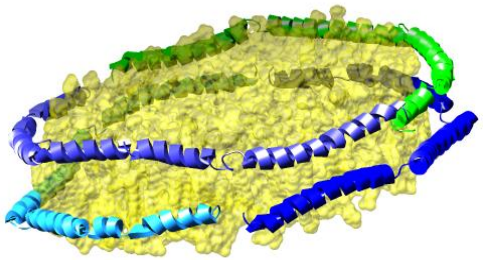
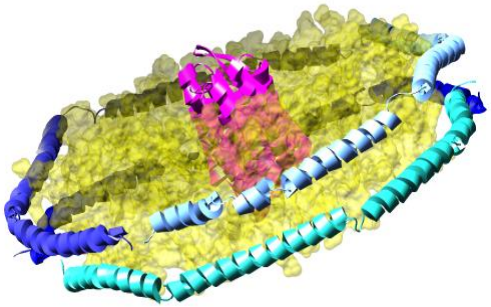
LLNL-PRES-415397

We have created nanoplatform technology broaching new dimensions in Biosecurity, Energy Security & Healthcare





Nanolipoprotein Particle (NLP)

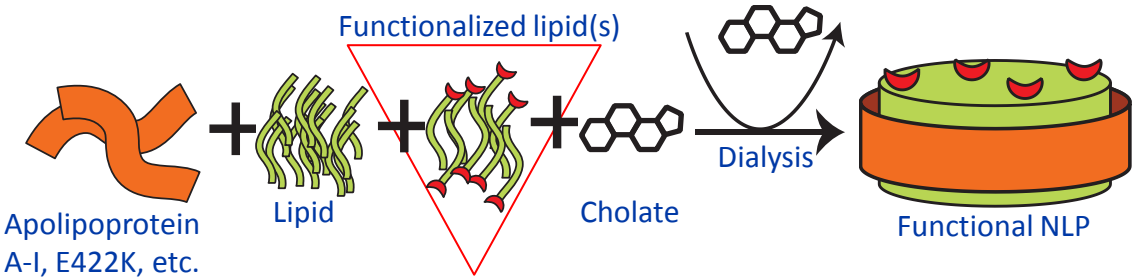
- new platform technology -

- Threat characterization
- Therapeutics & Vaccines
- Environmental Monitoring & Bioenergy
- Risk Assessment
- Clinical Surveillance

(Multi)functionalized NLP are easily prepared by incorporating functional lipids

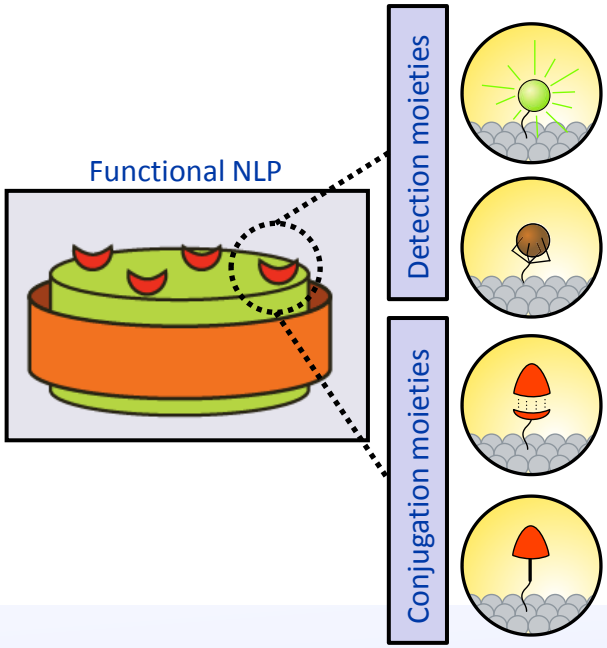
P. Hoeprich(LLNL), N. Fischer (LLNL), C. Blanchette (LLNL)

Incorporating functional lipid(s) into NLP → Functional NLP



- Functional group is located on NLP bilayer surface
- Multifunctional NLPs can be prepared by incorporating multiple functionalized lipids
- Complementary cargo molecules can be conjugated onto a single NLP (*e.g.* targeting peptides and ODNs)
- Multiple copies of each cargo can be immobilized onto a single NLP

Strategies for incorporating (multi)functionality



Fluorophores
Incorporated into headgroup or tail of lipid

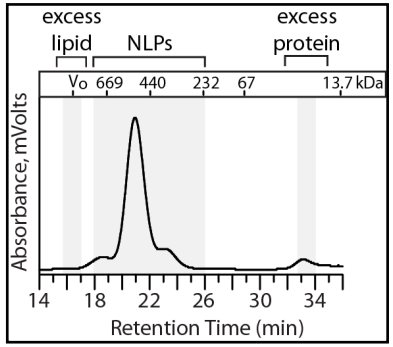
MRI Contrast Agents
e.g. chelated gadolinium

Noncovalent Conjugation of Cargo:
Biotin (Avidin-based proteins)
Chelated nickel (His-tagged proteins)

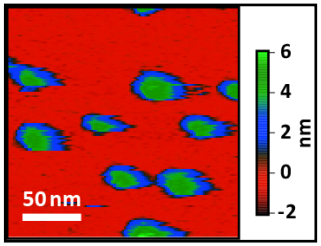
Covalent Conjugation of Cargo:
Azide (Acetylene)
Acetylene (Azide)
"Click" chemistry

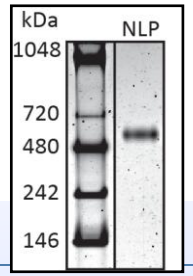
NLP purification/characterization

Size Exclusion Chromatography (SEC)



Atomic Force Microscopy (AFM)





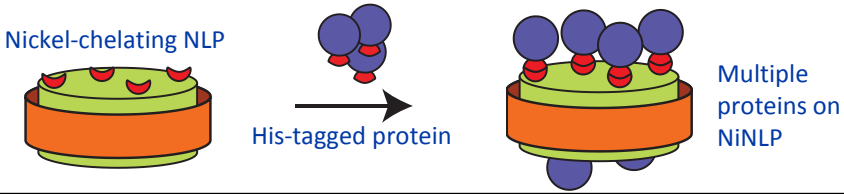
Nondenaturing Gradient Gel Electrophoresis (NDGGE)

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Security, LLC, Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

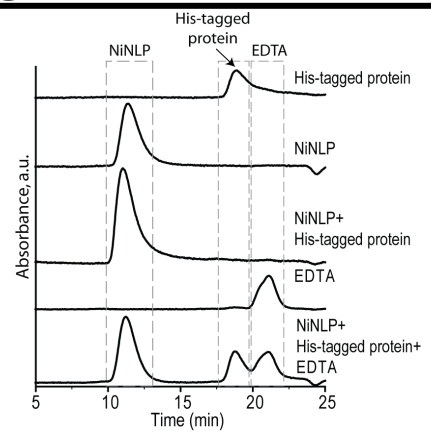
Applications of functional NLPs: Vaccine development and oligonucleotide delivery

P. Hoeprich(LLNL), N. Fischer (LLNL), C. Blanchette (LLNL), P. Mason (UTMB)

His-tagged proteins on nickel-chelating NLPs (NiNLPs): Development of general subunit vaccine platform



- SEC demonstrates reversible His-tagged protein binding to NiNLP
- Fischer N.O. *et al*. (2009) Bioconjugate Chem. 20, 460-465.



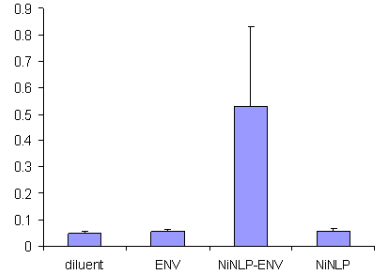
Subunit vaccine platform development

- His-tagged West Nile virus envelope protein (ENV) conjugated to NiNLPs

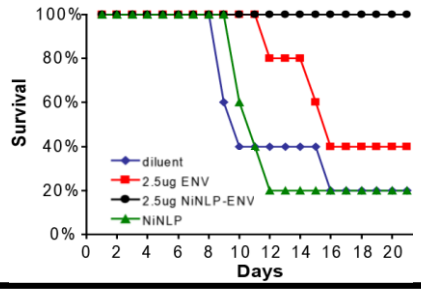
In vivo assessment (single inoculation):

- Enhanced antibody titer against ENV
- Protected against lethal dose of WNV
- NiNLPs themselves are not immunogenic

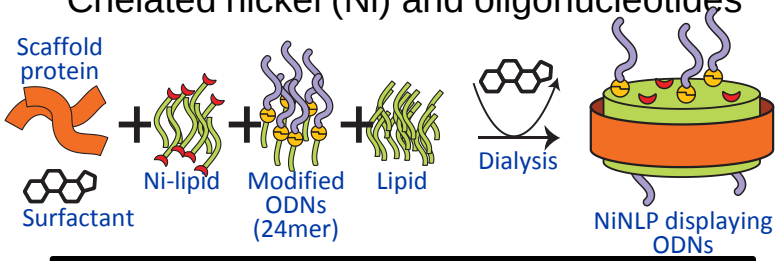
ENV antibody titers (5 weeks)

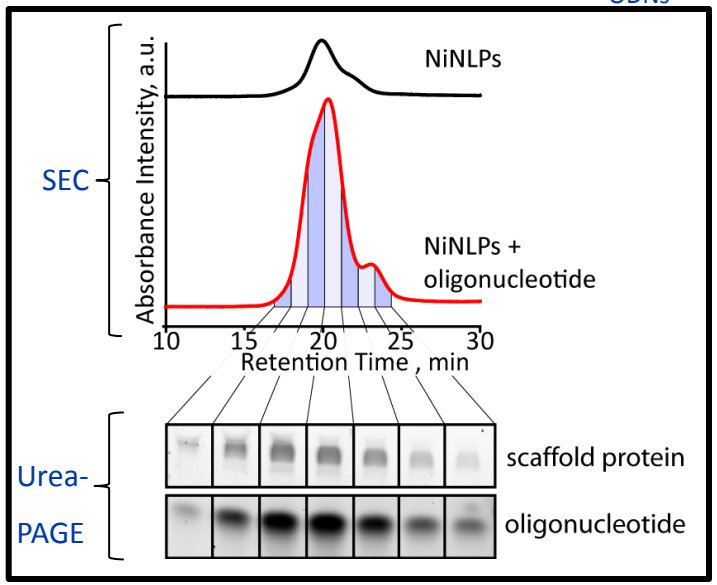


Mouse Survival curve



Multifunctional NLPs: Chelated nickel (Ni) and oligonucleotides



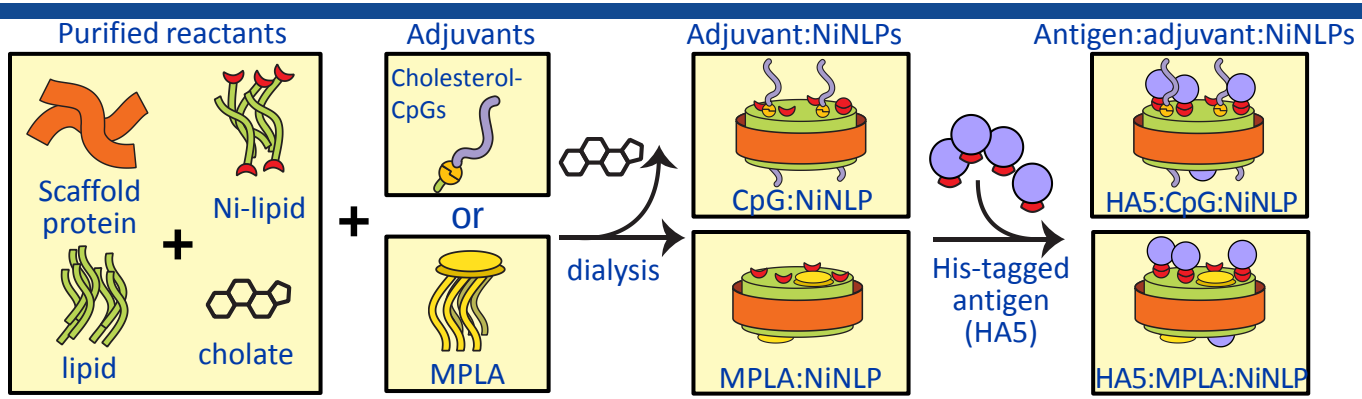


- Oligonucleotides can be readily incorporated into NLPs that feature additional functionality (e.g. chelated Ni)
- His-tagged targeting moiety for cellular targeting and/or delivery can be incorporated via chelated Ni

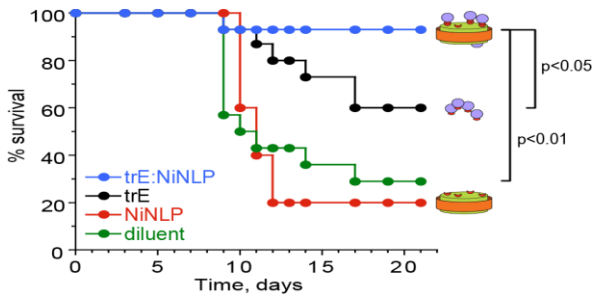
This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Security, LLC, Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

Adjuvanted Nanolipoprotein Particles (aNLP) as New Vaccine Countermeasures

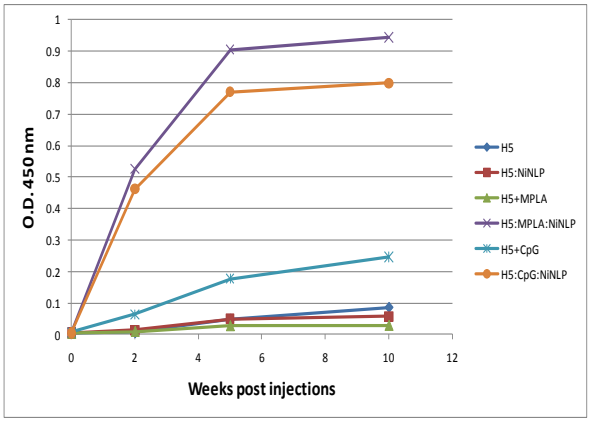
P. Hoeprich(LLNL), N. Fischer (LLNL), C. Blanchette (LLNL), A. Driks (Loyola)



Versatile fabrication approaches permits multiple assemblies for vaccine development



NLP-antigen constructs protect against WNV challenge
- Breast cancer vaccine pre-proposal submitted, DOD
- Dengue vaccine proposal, in progress, E. Harris, UCB



Adjuvanted NLPs show enhanced anti-body production
DTRA grant, approved for funding

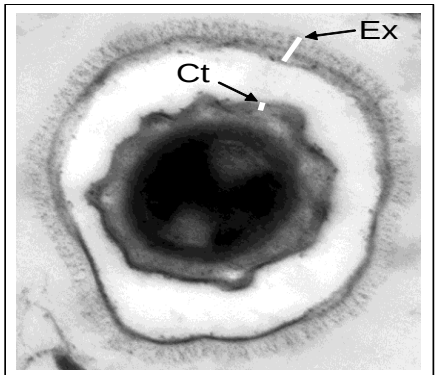


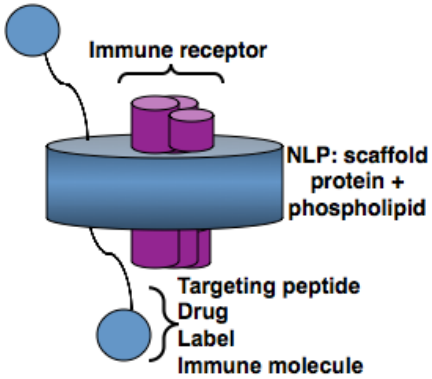
Figure 3. Thin-section electron micrograph of a *B. anthracis* spore. The coat (Ct) and

New Anthrax vaccine
NIH grant 1RO1AI93493-01

Nanolipoprotein particle development for *in vivo* imaging, drug delivery and characterizing receptor function.

Matthew Coleman (LLNL/UCD), Kit Lam (UCD), and William Murphy (UCD)

Multifunction nanolipoprotein particles (NLP)



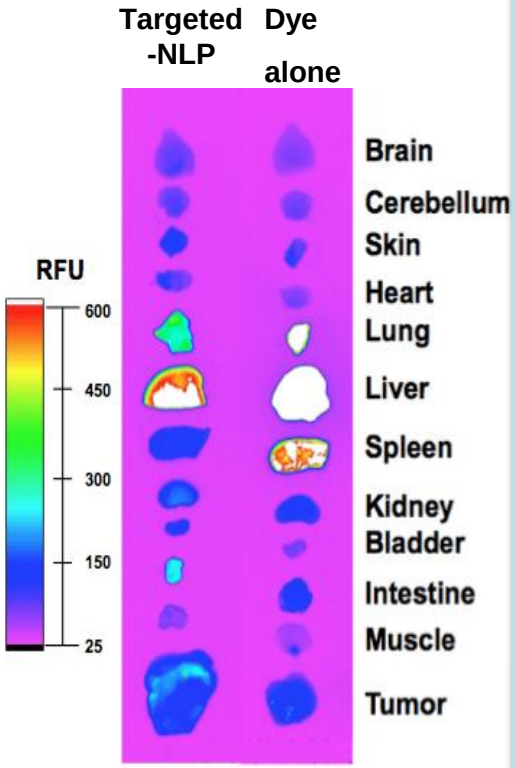
10-20 nm particles that can be generated and purified in a single day.

Applicable to drug, nucleic acid and protein delivery.

Can carry both stimulatory receptors and drugs.

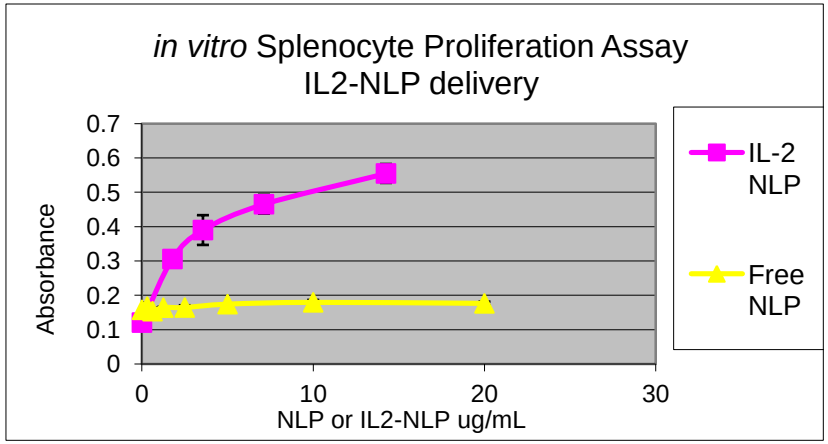
Process is readily automated.

Biodistribution and tumor targeting using LXY3 peptide ($\alpha 3\beta 1$ integrin targeting ligand)



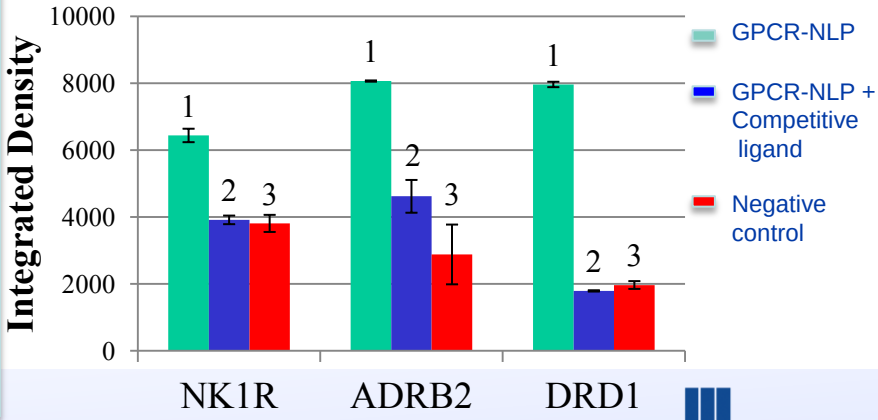
DiD-labeled NLPs injected in to mice (tail vein) and imaged 24 hrs later.

Protein Drug Delivery



GPCR Receptor Characterization

Ligand binding assay ($p < 0.05$)

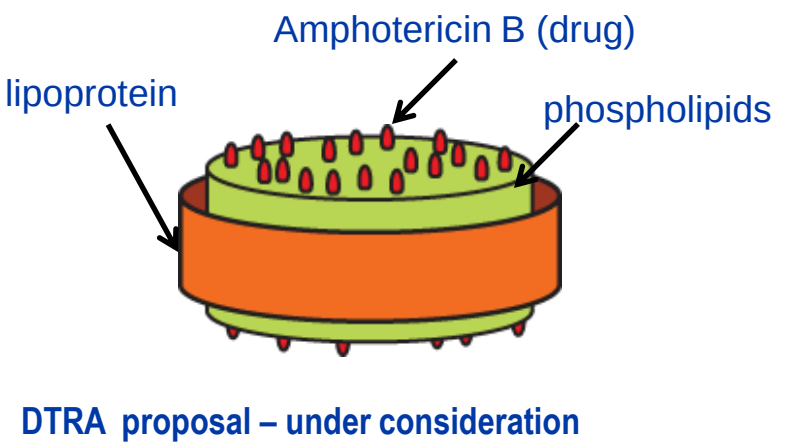


Applications of NLPs: drug delivery and diagnostic *in vivo* imaging

P. Hoeprich(LLNL), N. Fischer (LLNL), C. Blanchette (LLNL), M Oda (CHORI)

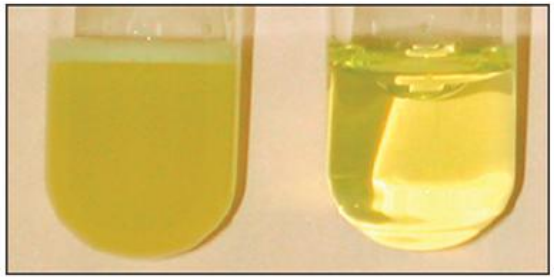
NLPs as drug delivery vehicles

Solubilization of hydrophobic drugs: *no targeting*



DTRA proposal – under consideration

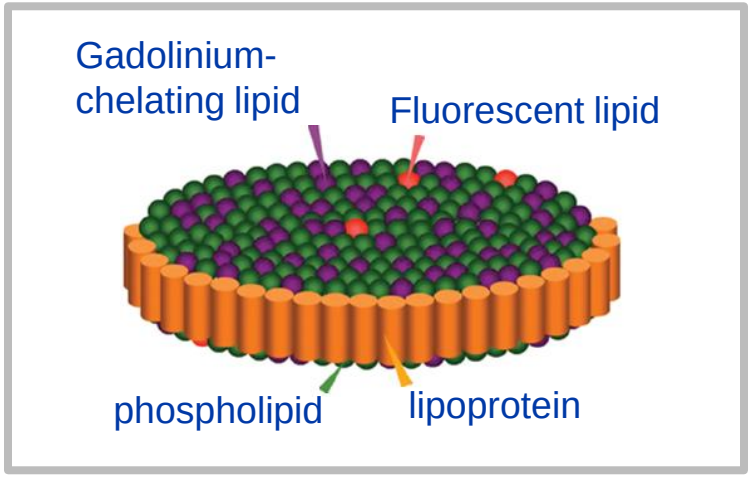
Amphotericin B alone | Amphotericin B in NLPs



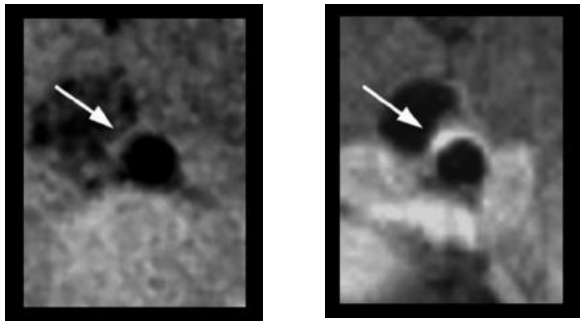
Oda M.N. *et al*. (2006) J. Lipid Res., *47*, 260-267.

NLPs as imaging agent vehicles

Passive targeting to atherosclerotic plaques



Pre-injection | Post-injection

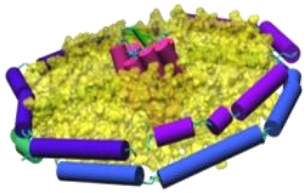


Cormode D.P. *et al*. (2009) Bioconjugate Chem., *20*, 937-943.

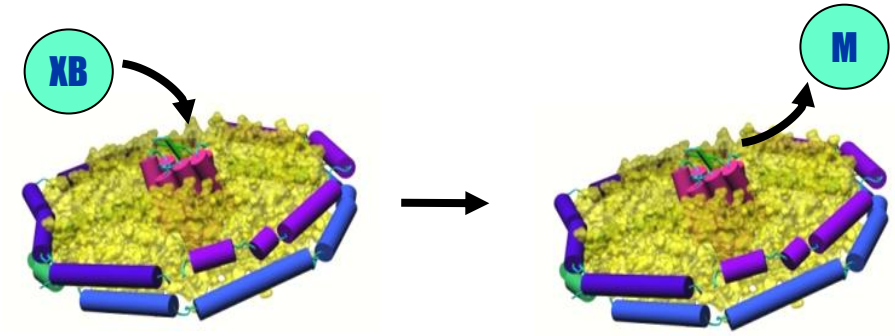
Stable & Soluble NLP-based CYP450 Constructs Facilitate Drug Metabolism

Mike Malfatti (LLNL), Edward Kuhn (LLNL) & Ken Turteltaub (LLNL)

CYP450-NLP



Self-assembly *de novo* of CYP450-NLP
in cell-free reaction



Xenobiotic (XB) binding to soluble cytochrome P450 contained in a Nanolipoprotein particle (NLP) and undergoing metabolic processing to (M)