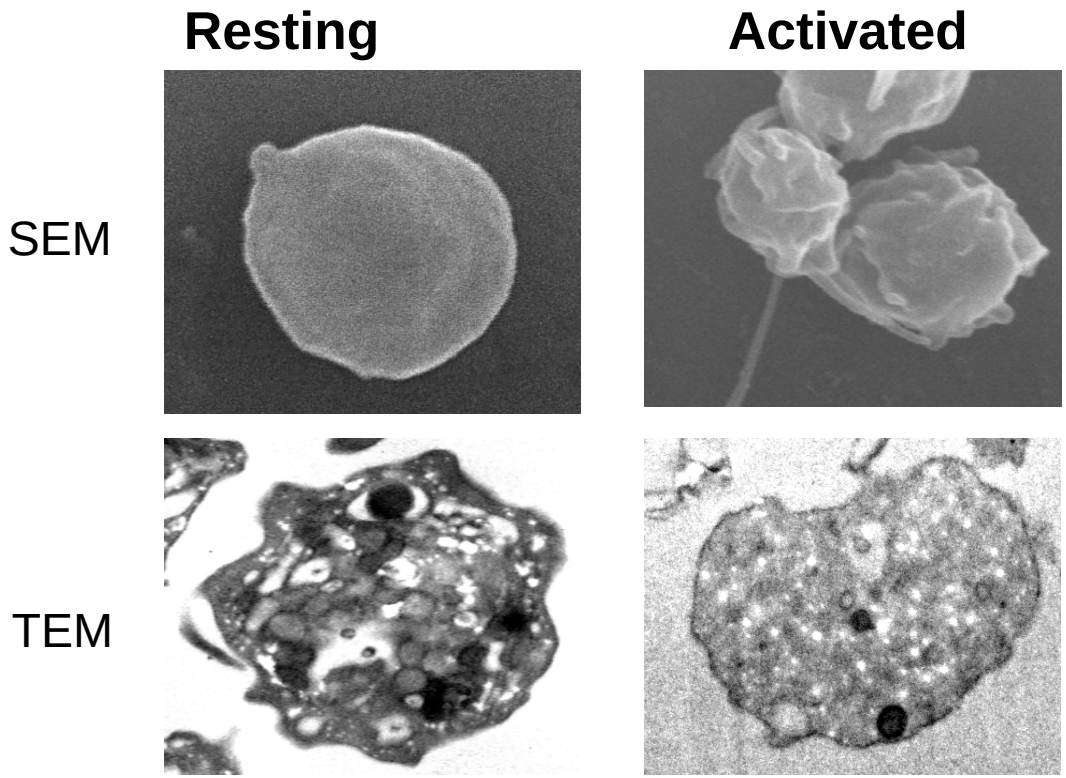
Cellphire

Loading Platelets with Biological Agents for Enhanced Local Delivery

Sponsor:
NIH NIDDK

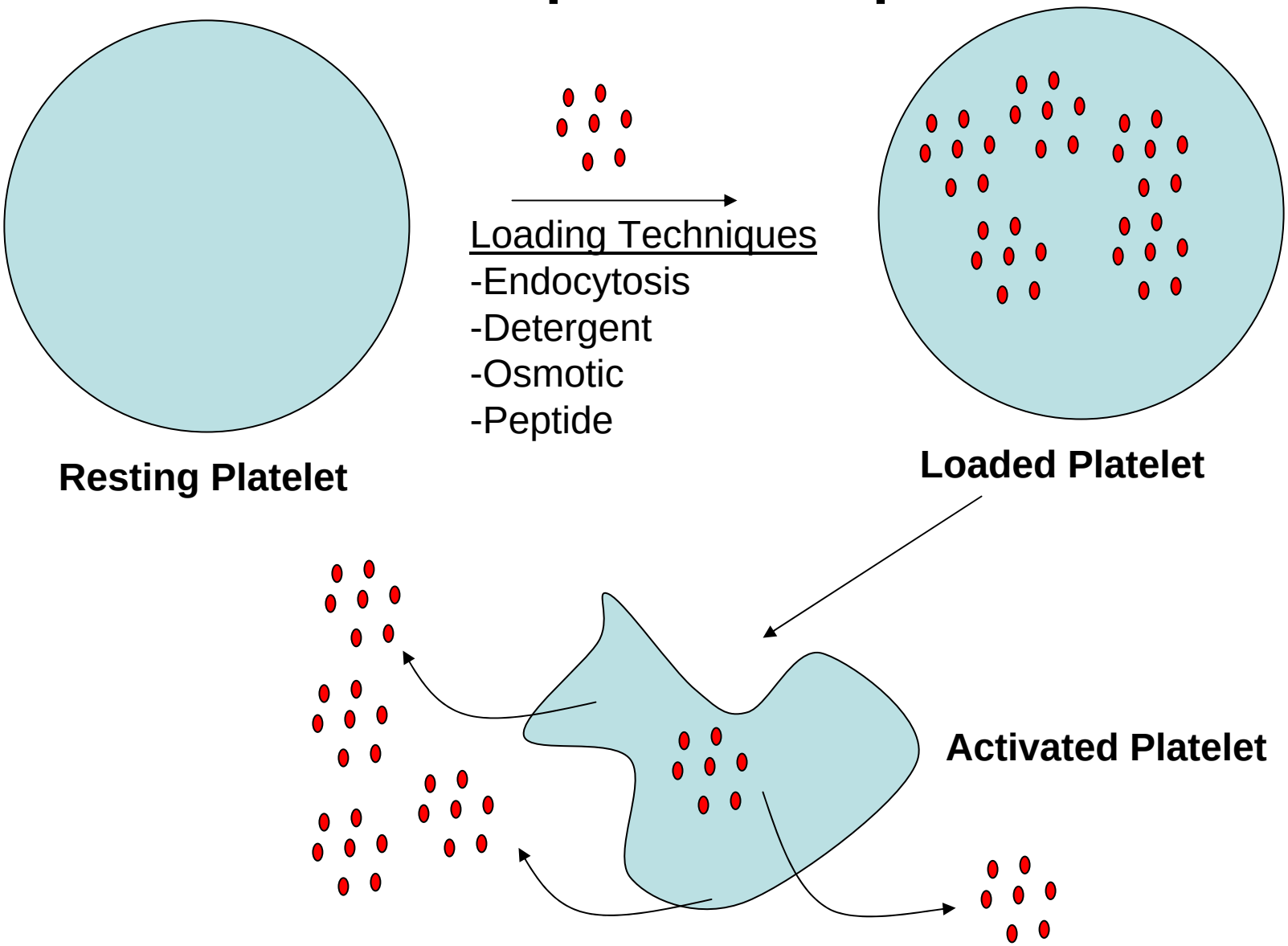
DARPA

Project Overview: Use Platelets to engineer uptake and delivery of therapeutic compounds to specific sites



- **Contain surface markers for specific targets**
- **Activation induced release of internal contents**

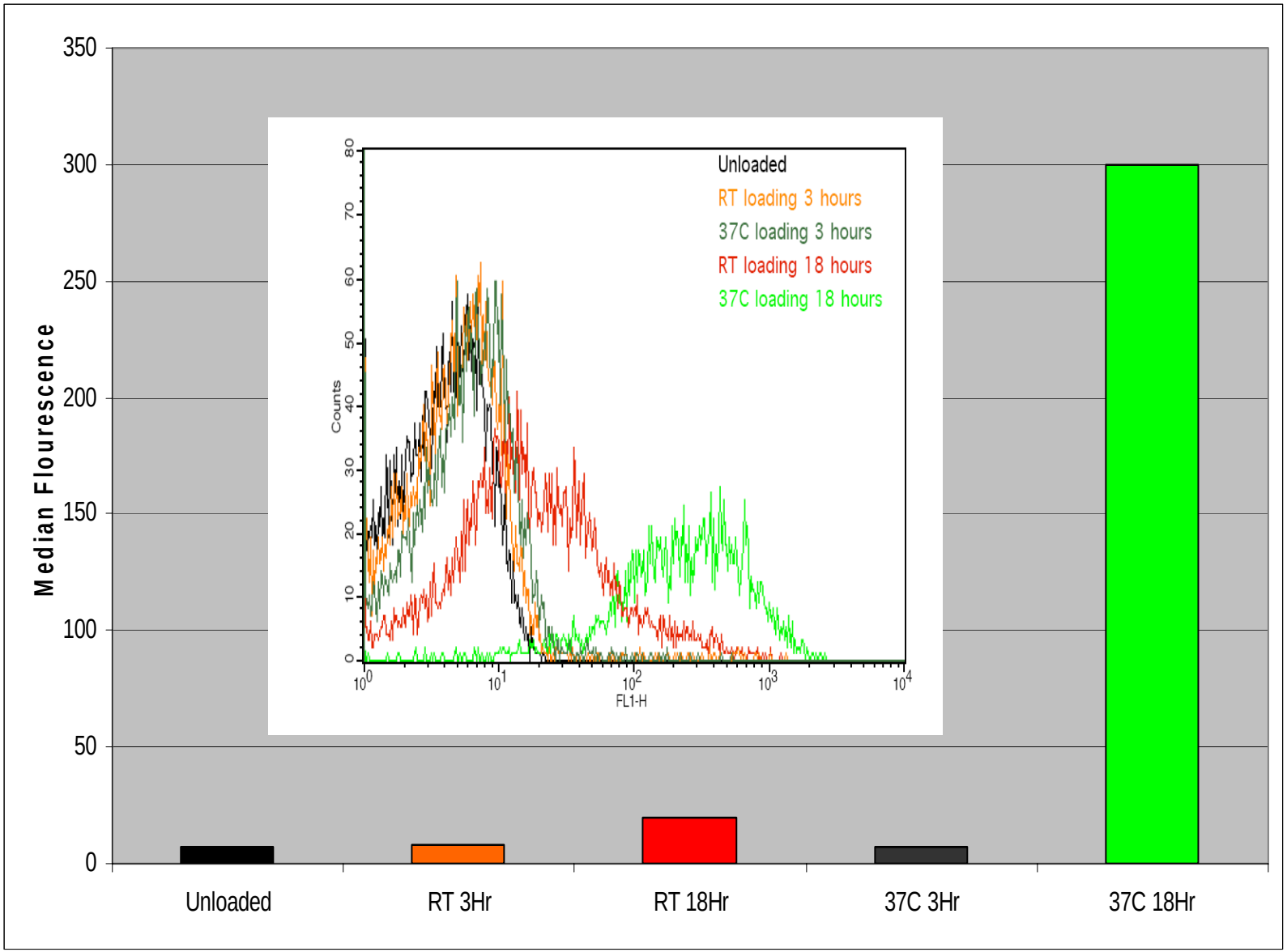
Conceptualization of Platelet Loading and Release of Therapeutic Compounds



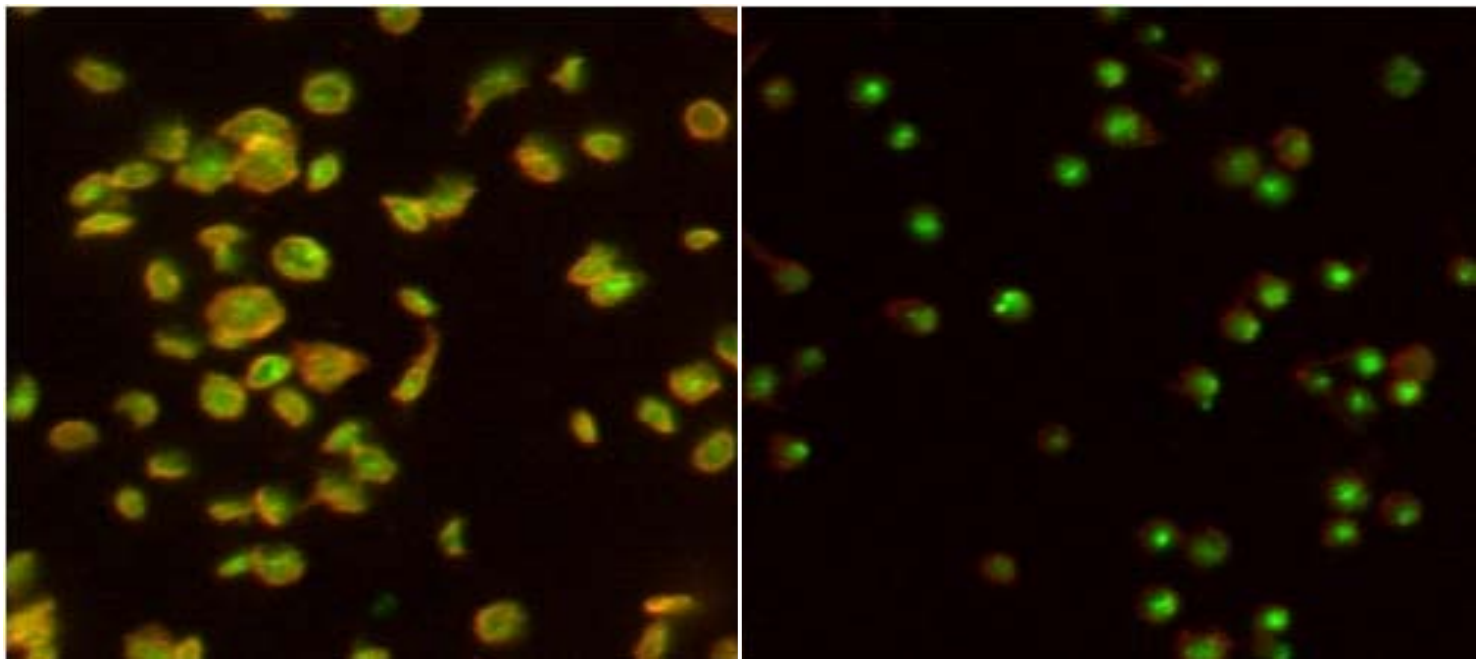
Summary of Loading Techniques

Loading Technique	Methodology
Endocytotic loading	Compounds and Platelets are co-incubated at 37^{o}C
Osmotic hypertonic/hypotonic loading method (Influx cell loading reagent from Molecular Probes (Bothell WA))	Compounds are mixed in a hypertonic medium and added to platelets
Detergent induced permeabilization	Platelets permeabilized with saponin and then compounds are added
Peptide mediated transport (Morris et al., Nat. Biotech 19: 1173 (2001) KETWWETWWTEWSQPKKKRKV	Peptide treated platelets are incubated with compounds

Fluorescence Spectra of Lucifer Yellow Endocytotic-loaded Platelets



Fluorescence Microscopic Images of Biotin Endocytotic-loaded Platelets

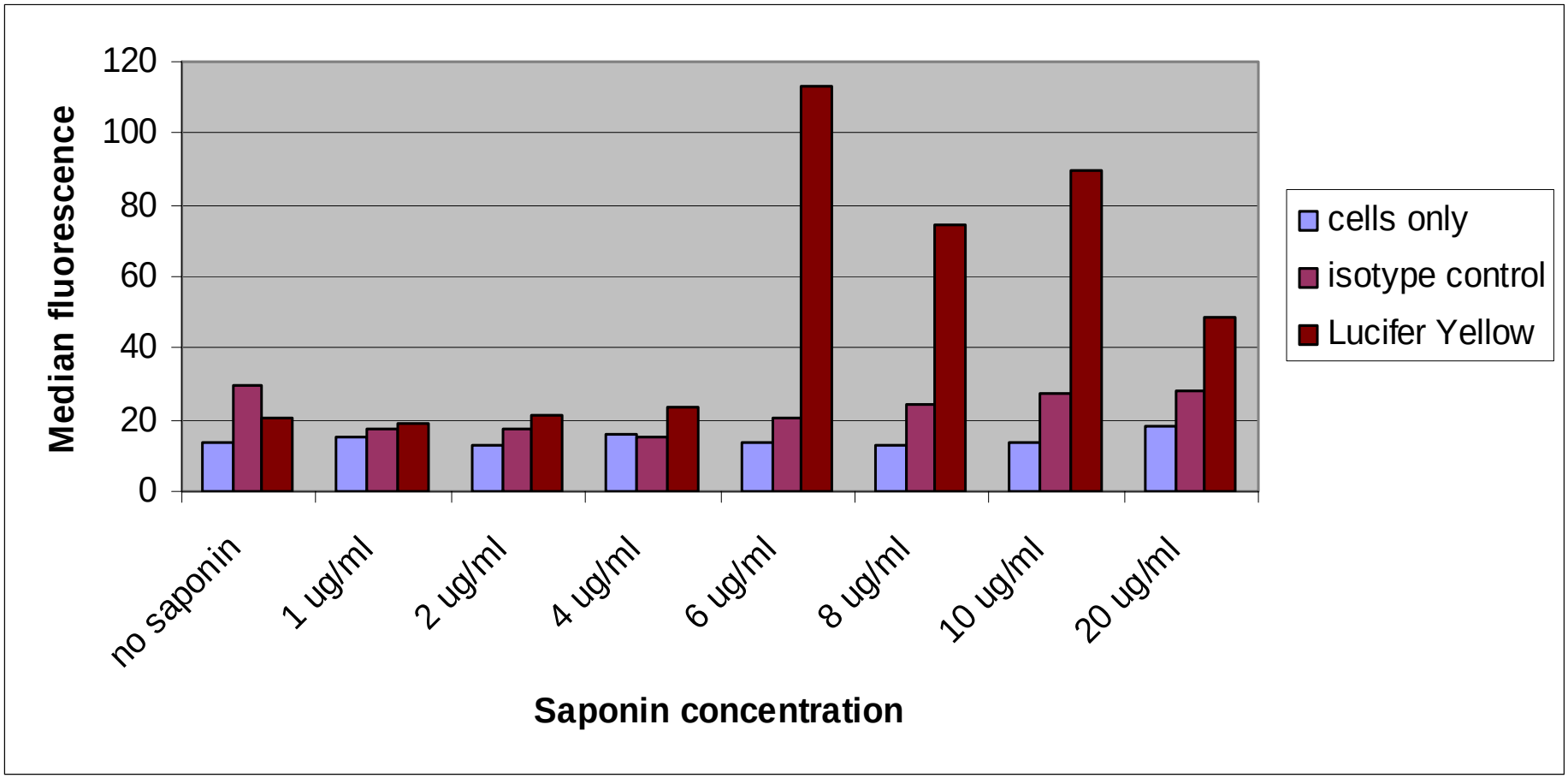


Biotin loading 37^{o}C T=2 hrs

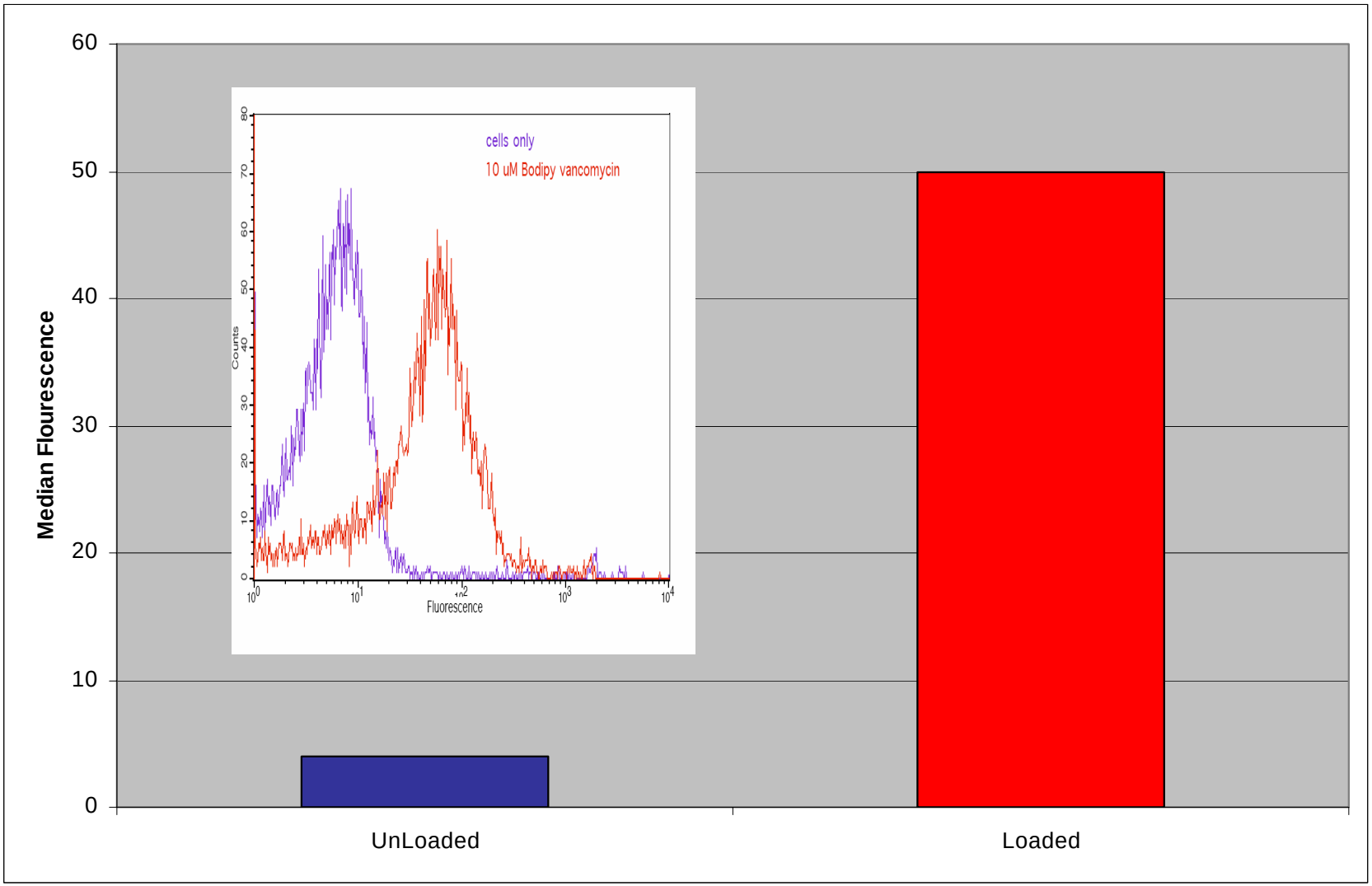
Biotin loading 37^{o}C T=16 hrs

Streptavidin-PE biotin Binding (Red)
Avidin-Alexa Fluor-biotin Binding (Green)

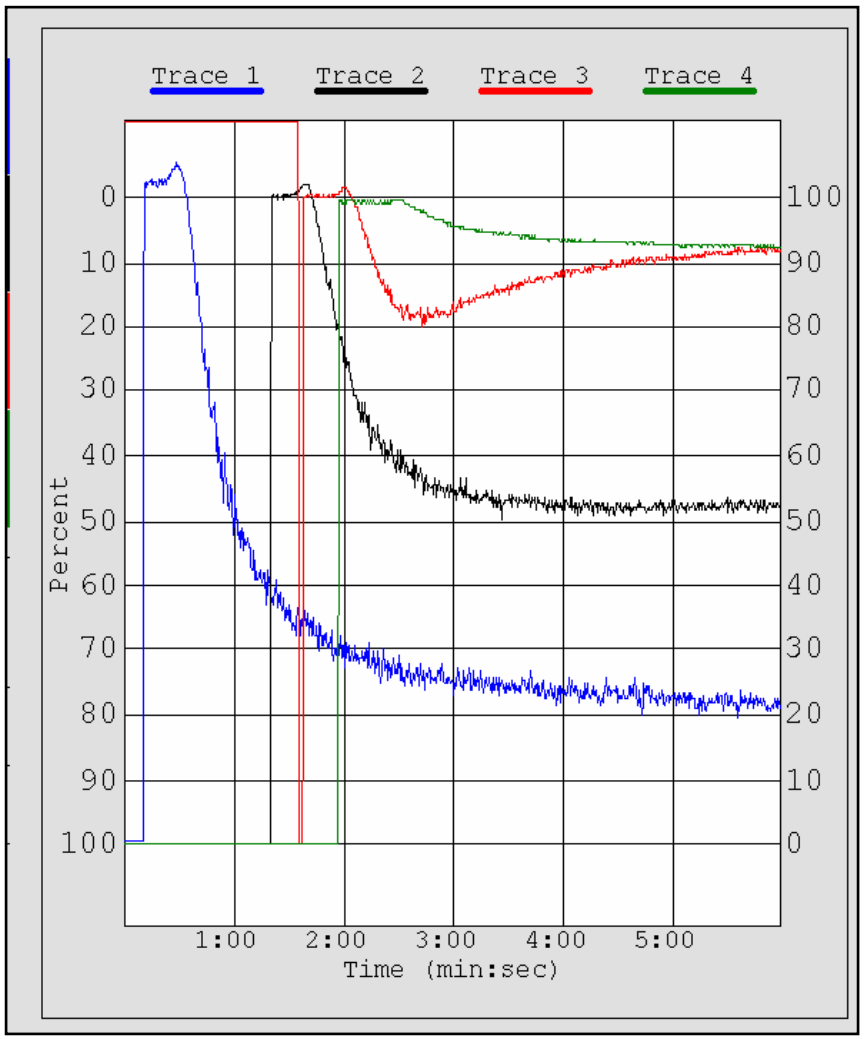
FACS Analysis of Detergent-mediated Loading of Lucifer Yellow and Isotype Control



Analysis of Antibiotic (Vancomycin) Osmotic-induced Loading Process

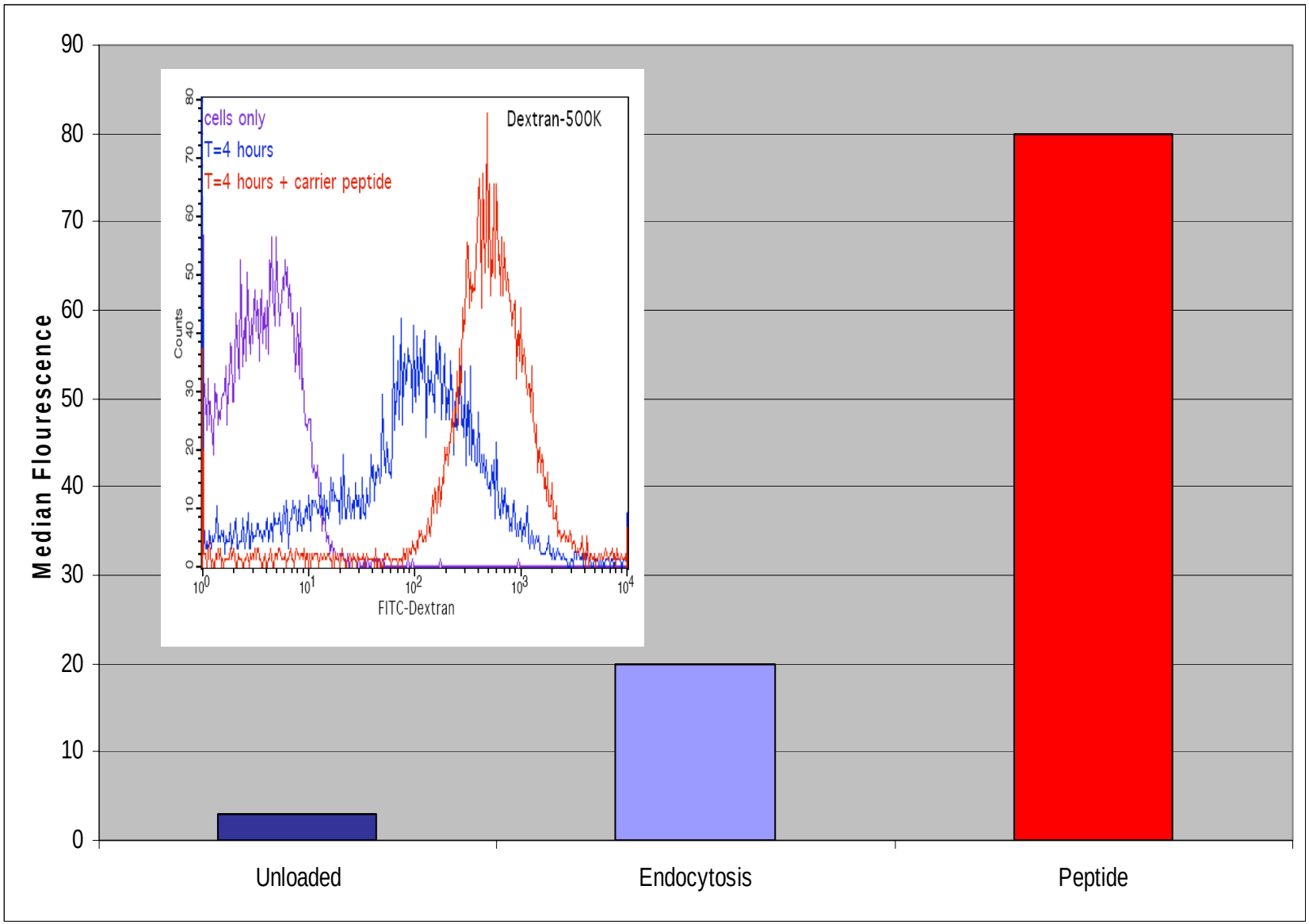


Aggregometry Demonstrating the Effect of Various Concentrations of Carrier Peptide on Platelet Function in Plasma



Carrier peptide (uM)
BLUE: 0 uM
BLACK: 50 uM
RED: 100uM
GREEN: 200uM
Agonist: Collagen

Analysis of Peptide Mediated Loading Process compared with Endocytosis



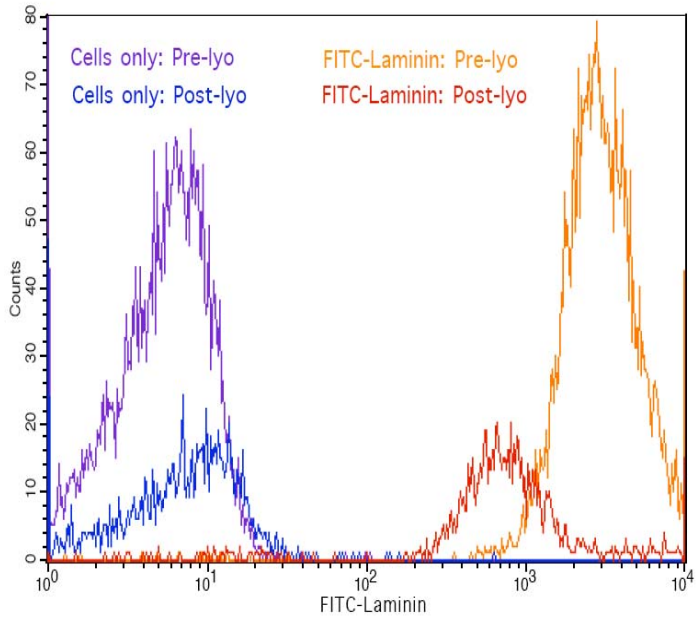
Summary Compounds Tested Under Various Loading Conditions

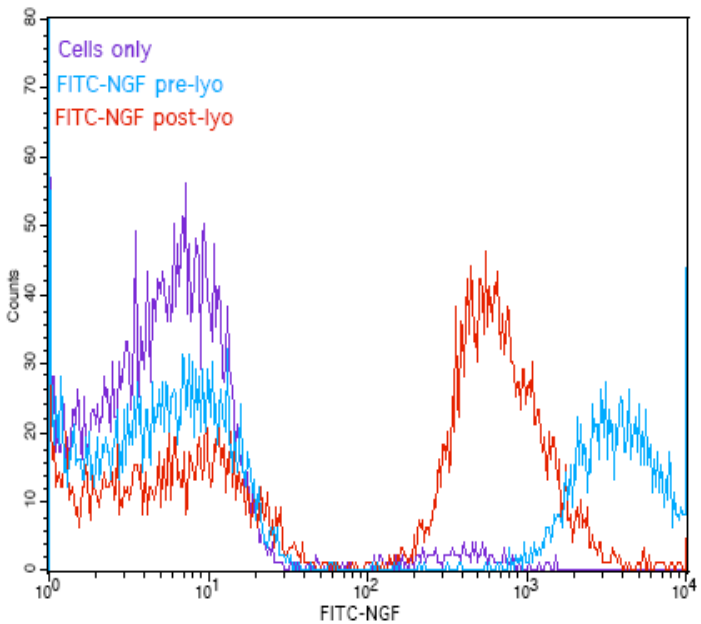
Loading Method	Peptide and Hormone	Anti-microbial	Polymers	Proteins
Endocytotic	FITC-NGF	BODIPY-vancomycin	FITC Dextran 3K, 10K, 500K (0.5mM)	-FITC-albumin -FITC-F(ab)'2 -Histone H1 -Soybean Trypsin Inhibitor -FITC-collagen -FITC-laminin -FITC-bovine IgG -FITC-rabbit IgG
Osmotic	FMLP (200mM)	BODIPY-vancomycin	FITC Dextran 3K, 10K (0.5mM)	-FITC-albumin -FITC-F(ab)'2 -Histone H1 -Soybean Trypsin Inhibitor -FITC-bovine IgG -FITC-rabbit IgG
Detergent				Lucifer yellow (1mM)
Peptide	-FMLP (200mM) -FITC-NGF	BODIPY-vancomycin	FITC Dextran 3K, 10K, 500K (0.5mM)	-FITC-albumin -FITC-bovine IgG -FITC-rabbit IgG -FITC-F(ab)'2 -FITC-collagen -FITC-laminin -Histone H1

Green= Positive Response (Concentration Loaded)
Red= Negative Response

Post-lyophilization Retention of FITC-compounds

1. Peptide mediated loaded platelets with fluorescence compounds were stabilized with Trehalose
2. Pre freeze-dried samples for uptake were analyzed for fluorescence using FACS
3. Samples were freeze-dried using Cellphire's proprietary lyophilization protocol
4. After lyophilization, the freeze-dried samples were reconstituted and their fluorescence measured using FACS





Summary

- Established proof of concept that therapeutic compounds can be loaded into platelets
- Selected uptake of compounds based on different loading conditions
- Upon reconstitution, platelets retained the loaded compounds
- Loaded platelets can be used as vehicle for local delivery and release of therapeutic compounds

Acknowledgments

Dr. Cindy Orser
Dr. Keith Moskowitz
Richard Cliff
Dr. Alan Rudolph
Josh Dee
Nannette Mittereder