
PHARMAIN

Enabling and improving human therapeutics

June 2011

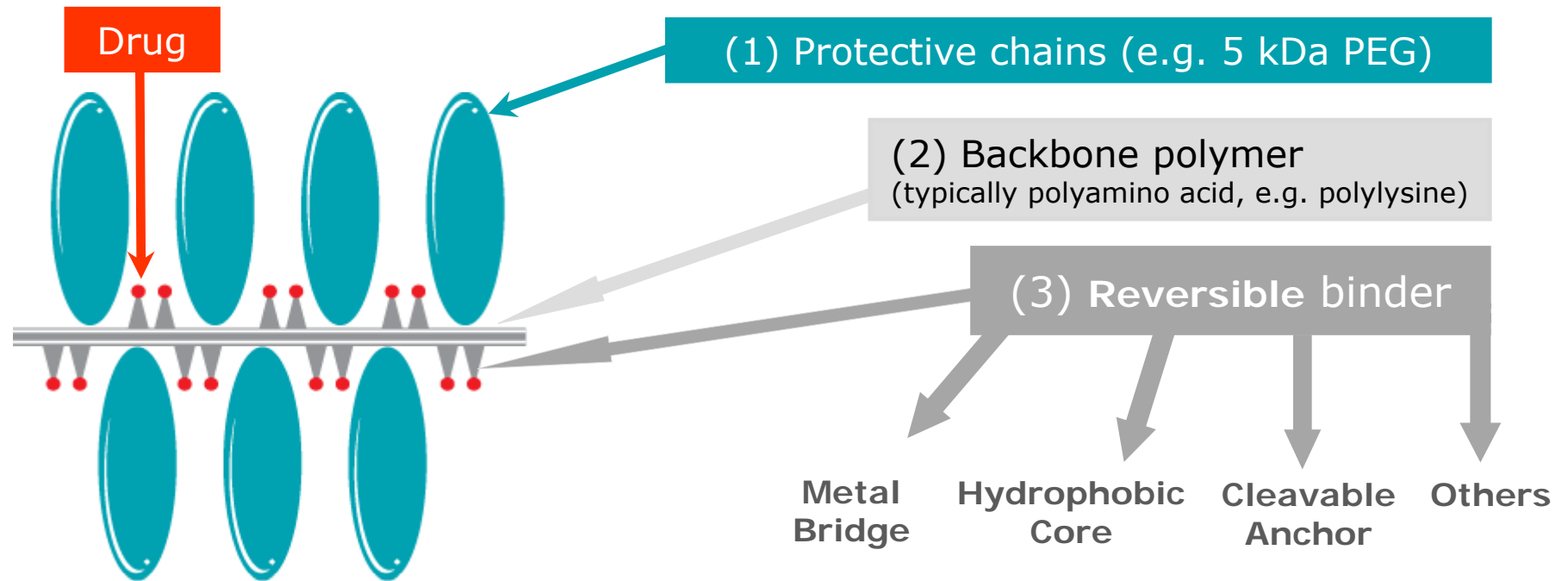
Introduction

- Seattle-based biopharmaceutical company focused on **enabling and improving injectable therapeutics** based on our patented and proprietary drug delivery technology
- Focused on peptides, proteins, and selected small molecule therapeutics
- Experienced and creative team with broad skills and established support infrastructure
- Strong patent portfolio – over 10 issued and pending patent families



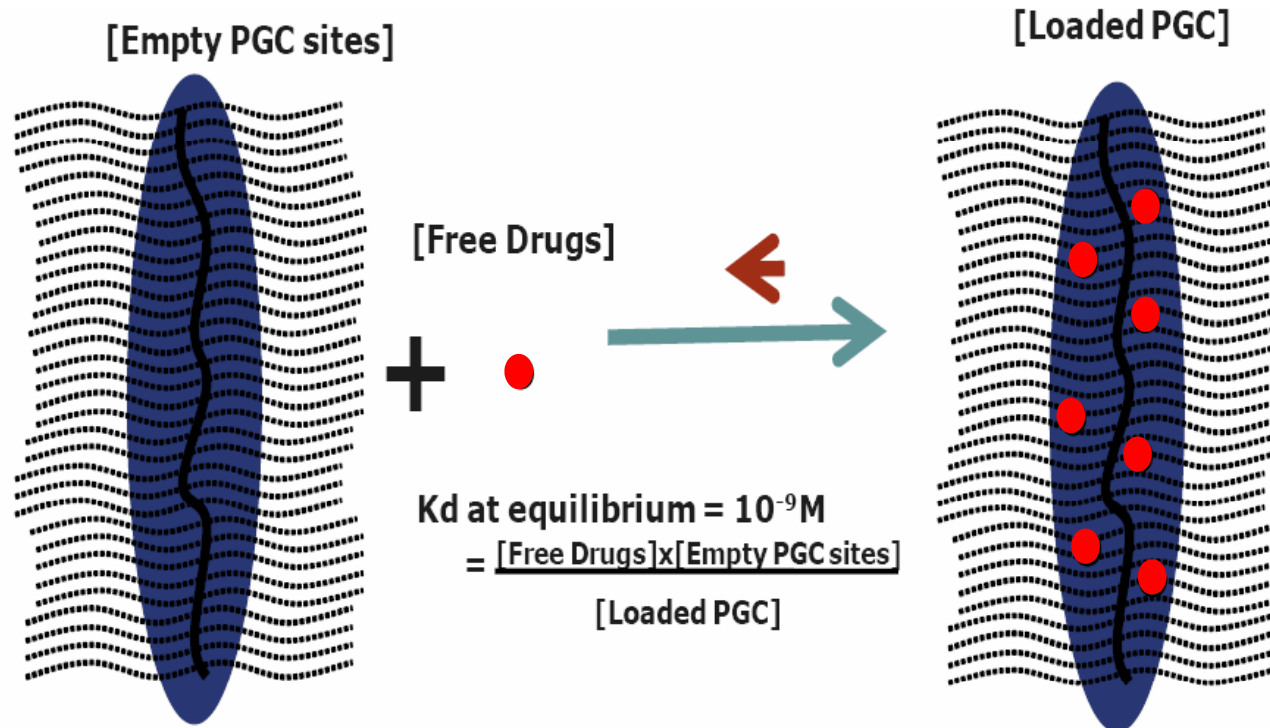
Our Innovative Platform - PGC

Protected Graft Copolymer (PGC) – Very Broad Capability

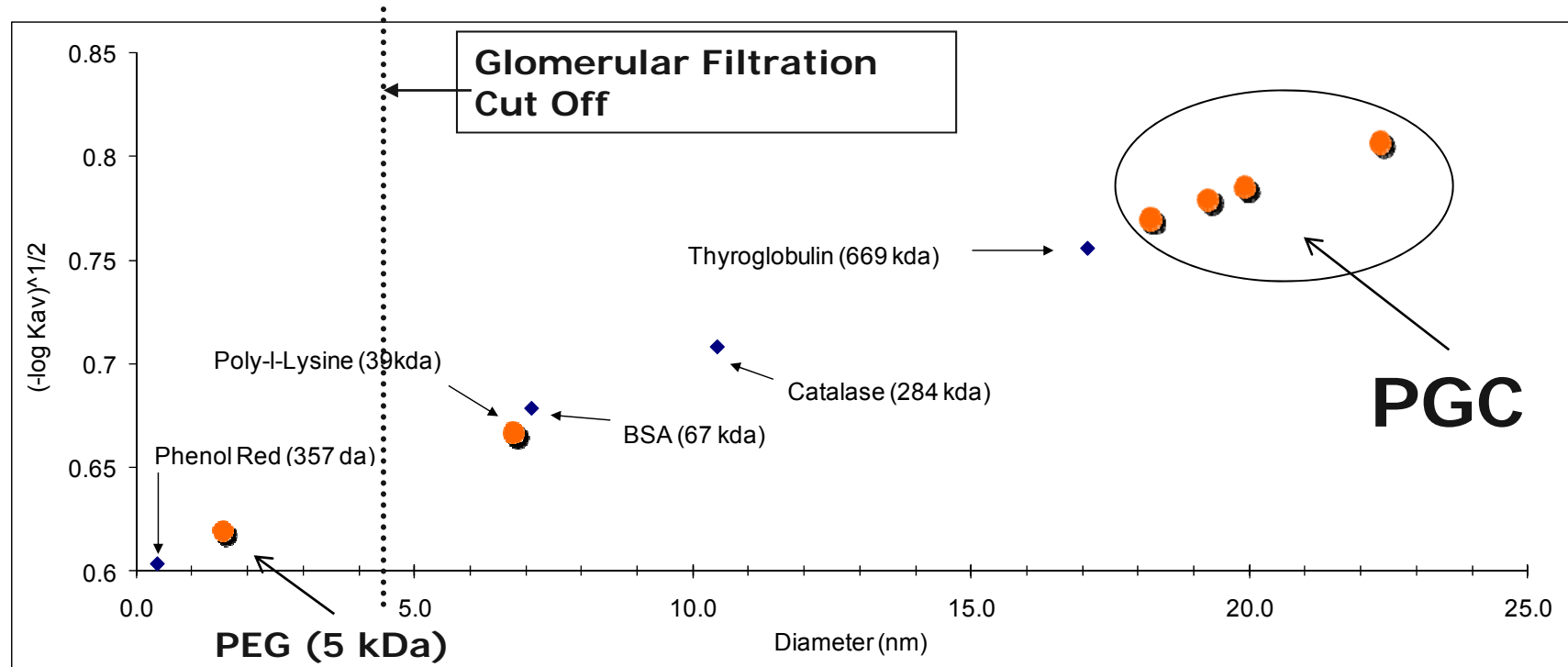


Nanocarrier diameter ~ 20nm

Affinity Binding Mechanism of PGC



Hydrodynamic Diameter of PGC



PGC Application Guide

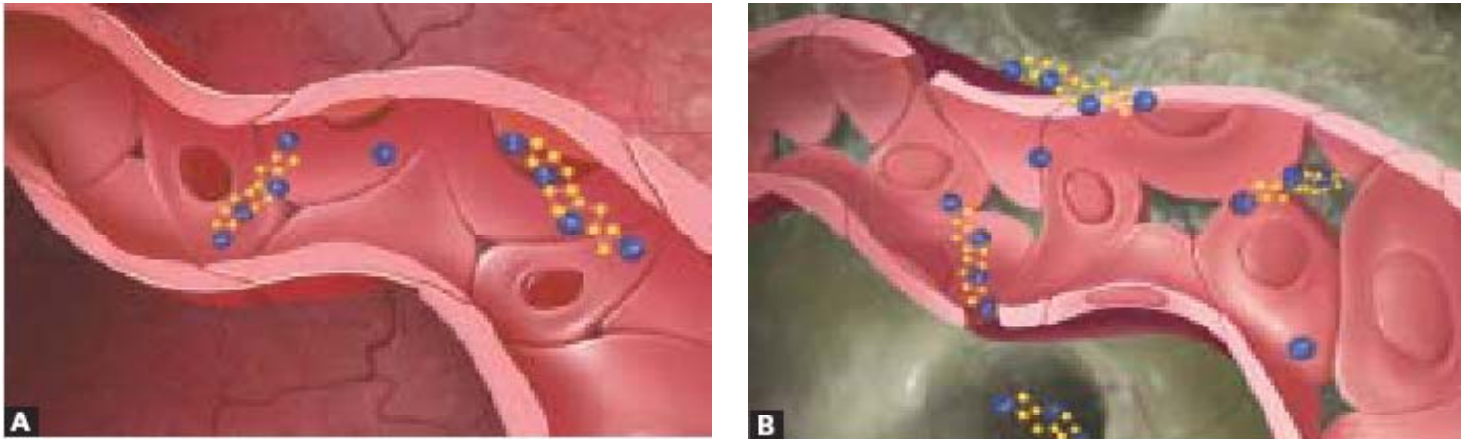
PGC Technology	Peptides/Proteins	Small Molecules
Hydrophobic core	Lipophilic, contains α -helix. Loading from 2-10% [†]	Lipophilic, Loading from 2-5% [†]
Metal Bridge	Binds Zn, Cu; His-tag. Loading from 10-100% [†]	Binds Zn, Cu. Loading not tested and need TBD on a case-by-case basis
Charged Core	Isoelectric point >8.0, multiple formal charges. Loading 20-50% [†]	Must have very high charge density to bind. Loading not tested

[†]Loading is defined as weight of API per weight of carrier, so 100% loading is a 1:1 ratio of API to carrier (mg/mg)

PGC Targeting

*- Passive targeting to sites
of enhanced vascular permeability -*

PGC Mechanism of Passive Targeting

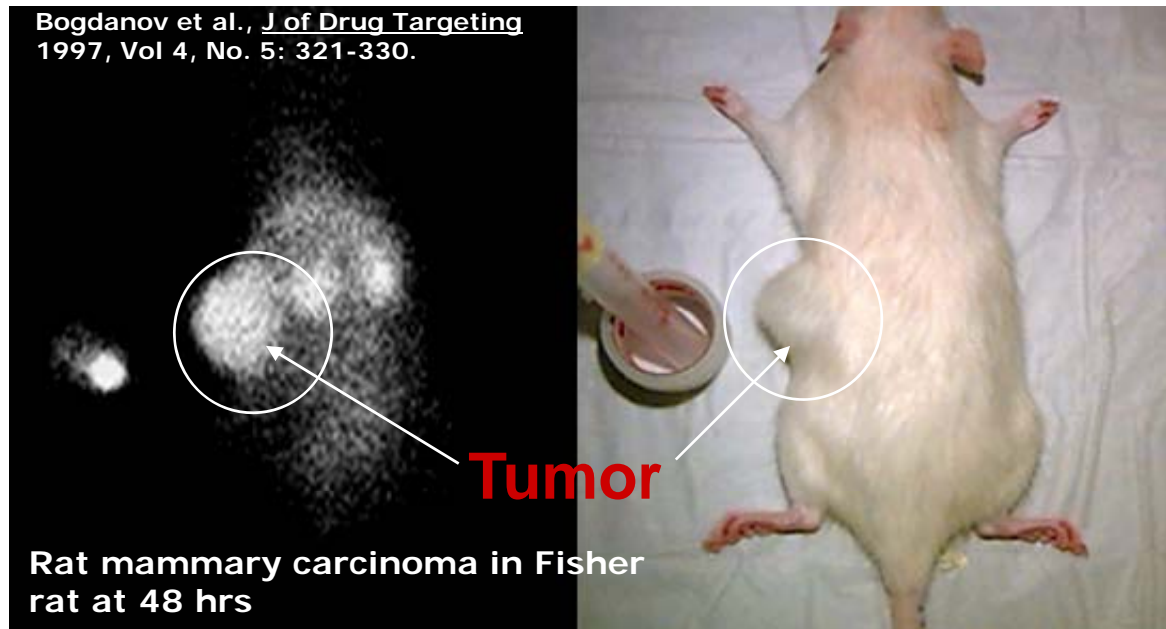


Pore Size in Normal Vasculature	Carrier Size	Pore Size in Abnormal Vasculature
< few nm	20 nm	10s – 100s nm

- PGC carrier flows through healthy blood vessels (A)
- At sites of infection, inflammation, and in tumors, the blood vessels (B) have openings that make them porous to large molecules
- PGC extravasates through the abnormal vessels to interstitial area
- Carrier with drug payload accumulates in sites of enhanced vascular permeability

PGC Tumor Targeting

PGC long circulation and EPR effect results in higher tumor accumulation



Rat carcinoma R3230AC in Fisher rats. Imaged with PGC co-labeled with Gd and ^{111}In .

Active vs. Passive Targeting

Oncology

Specific antibody targeting is more rapid

At 48h copolymer accumulation is comparable to specific antibody

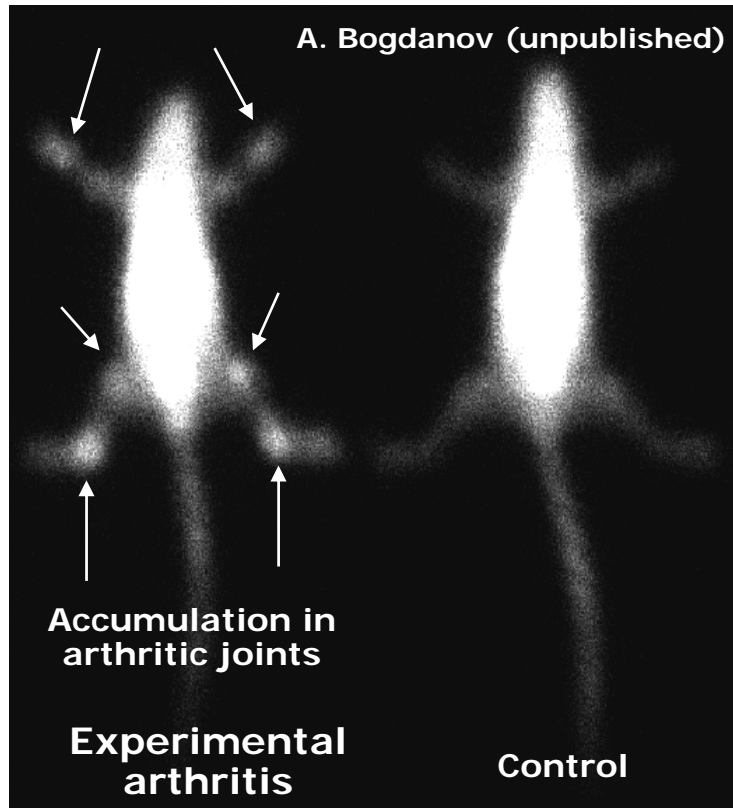
% injected dose/g tissue				
24h		48h		
mAb	PGC	mAb	PGC	hIgG control
6.0 ± 3.2	2.8 ± 1.5	5.8 ± 0.4	5.2 ± 1.7	4.1 ± 1.4

Biodistribution of ¹¹¹In-labeled PGC and BR96-DTPA (mAb) in mice bearing human small cell Lung carcinoma xenografts.

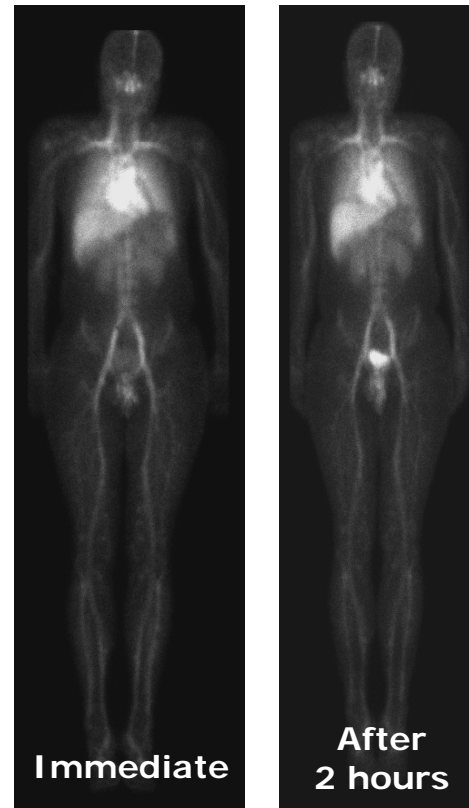
After 48h, accumulation of PGC is comparable to specific mAb, and superior to the non-specific antibody control (hIgG). *Marecos et al., Bioconjugate Chem., Vol. 9, No. 2, 1998*

PGC Selective Permeability and Long Circulation

Arthritis imaging



Blood pool imaging



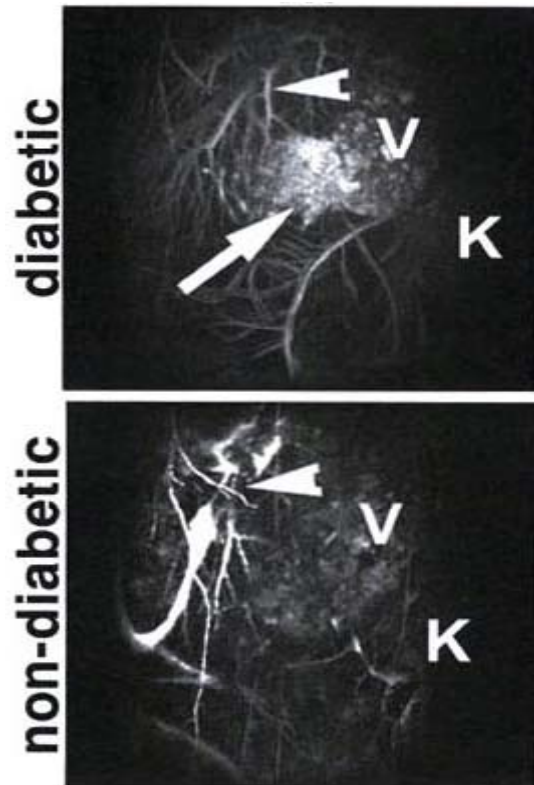
Long blood circulation
 $T_{1/2} = 20.6 \text{ h}$ (1)

Anterior whole
body images

Massachusetts General
Phase I trial

(1) Callahan et al; *AJR*,
171, July 1998

PGC Targeting of Inflamed Pancreas in Type-1 Diabetes Model



Accumulation in pancreas is visually evident in the diabetic animal

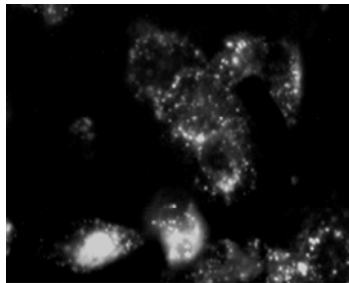
3D MRI 1h after injection of PGC in T1D mouse model and healthy control. The short arrow is the hepatic vasculature, pancreas is shown in the center. (For reference, K is the kidney, V is the stomach).

Peak contrast was seen at 17 h, wash-out at 40 h

Medarova et al., *Diabetes* 56:2677-2682, 2007

PGC Enables Avidity Targeting

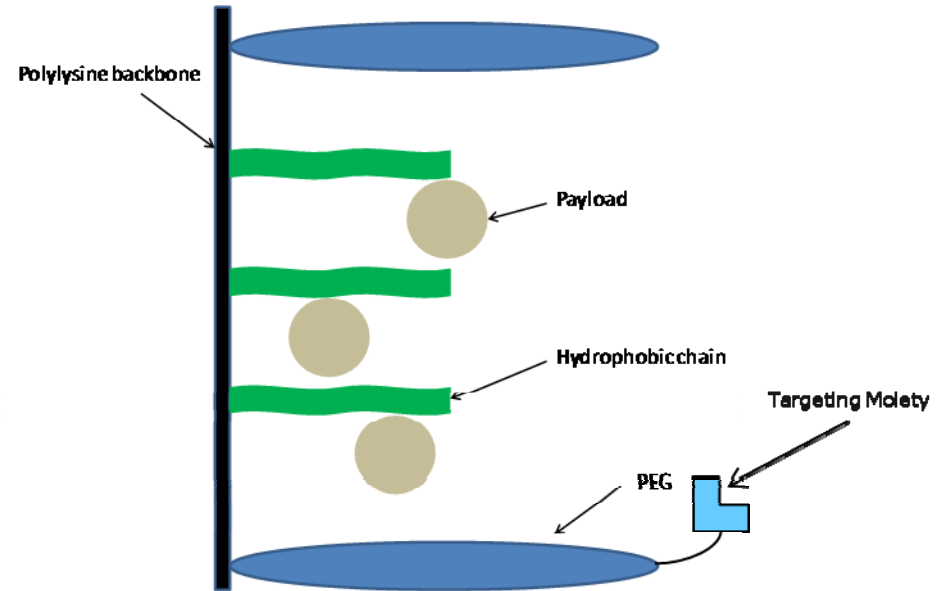
PGC Conjugated to Specific Antibody Targets Presenting Cells in Vitro



Human endothelial cells, treated with IL-1b to express E-selectin



Human endothelial cells, untreated control



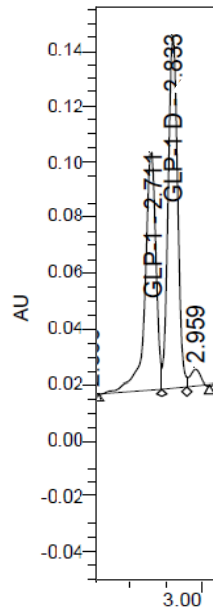
PGC-Ab against E-selectin: Active targeting against E-selectin bearing cells

Shown above is fluorescence microscopy of human endothelial cells (Human EC) after treatment with anti-E-selectin (a targeting antibody) conjugated to PGC carrying indocyanine fluorophores (Cy5.5). IL-1 β is used to express E-selectin (top image).

Pipeline Examples

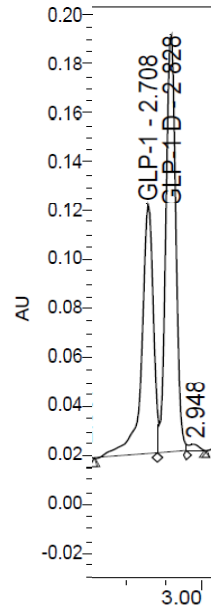
- Glucagon Like Peptide 1 (GLP-1)
- Basal Insulin
- Lysostaphin
- Natriuretic Peptides
- Fatylated Terlipressin
- Vasoactive Intestinal Peptide (VIP)

PGC Protects GLP-1 from DPP-IV Degradation



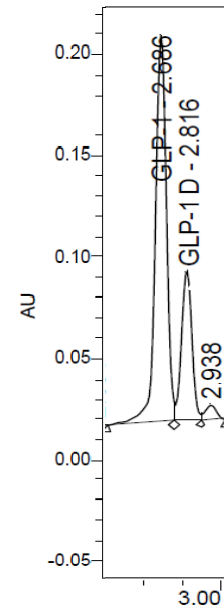
Peak Name	RT	Area
GLP-1	2.711	367344
GLP-1D	2.833	463920

No PGC
After 24 hr digestion



Peak Name	RT	Area
GLP-1	2.708	454154
GLP-1D	2.828	634194

With PGC
After 24 hr digestion

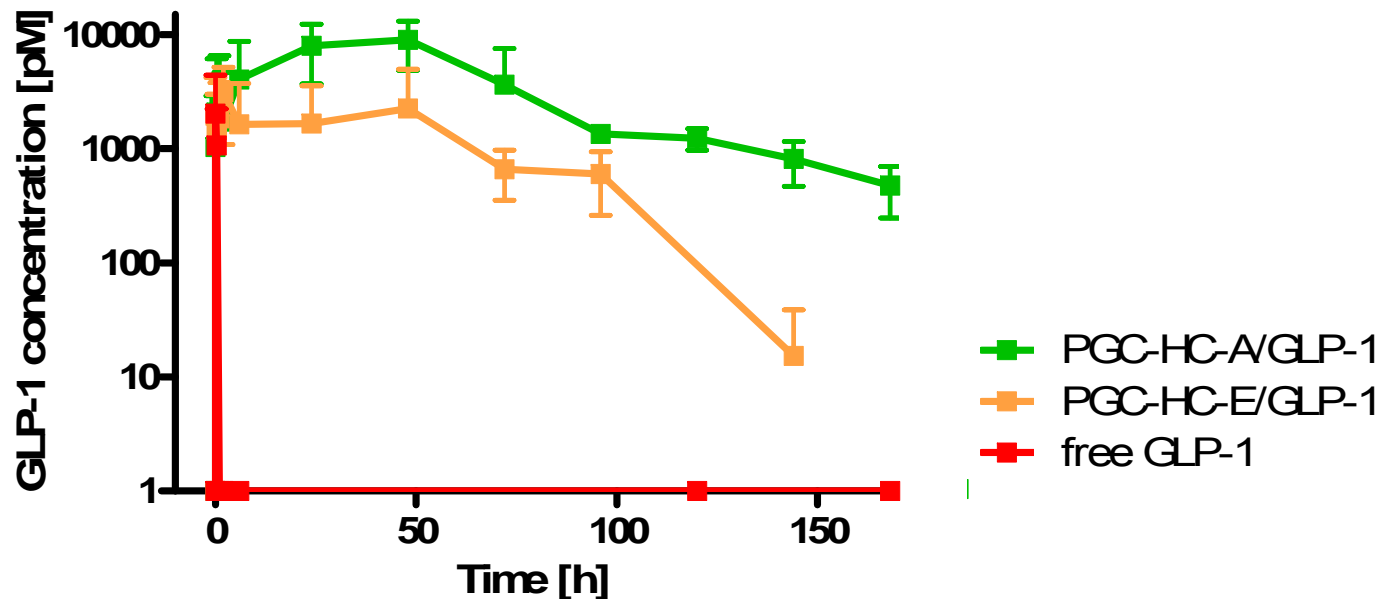


Peak Name	RT	Area
GLP-1	2.686	756783
GLP-1D	2.816	272506

With PGC-C18
After 24 hr digestion

GLP-1 Pharmacokinetics

PGC Hydrophobic Core Carrier

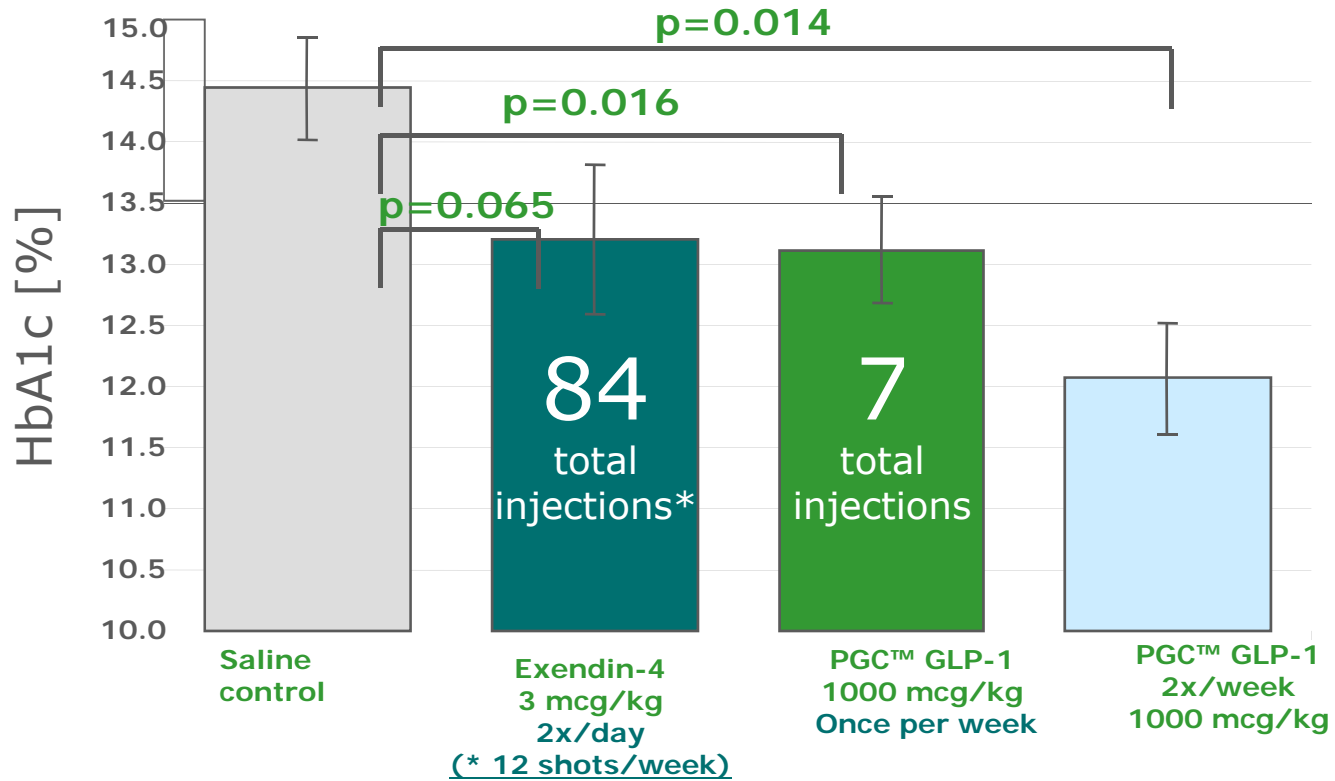


This figure describes two nanocarrier designs. The PGC HC-A has an amide linkage between the PEG and poly-Lysine backbone, the PGC HC-E has an ester linkage in its place. Dosage was 1 mg/kg GLP-1 in Sprague Dawley rats.

PGC GLP-1 Efficacy in ZDF Rat Model

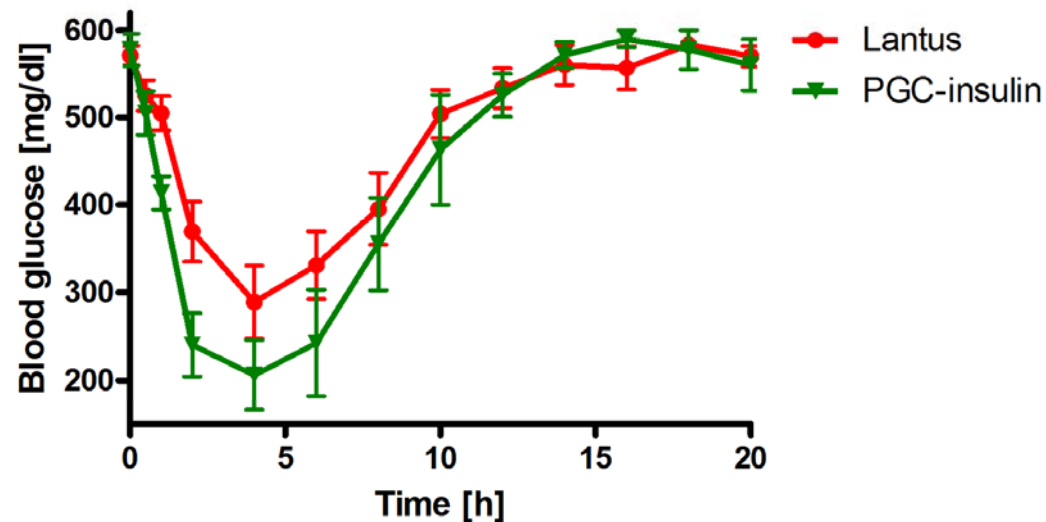
Impact on HbA1c Comparable to Byetta

After 7 weeks of treatment in ZDF rat model



PGC Insulin: Unmodified Basal Form

PGC Insulin vs. Lantus® in diabetic rat model



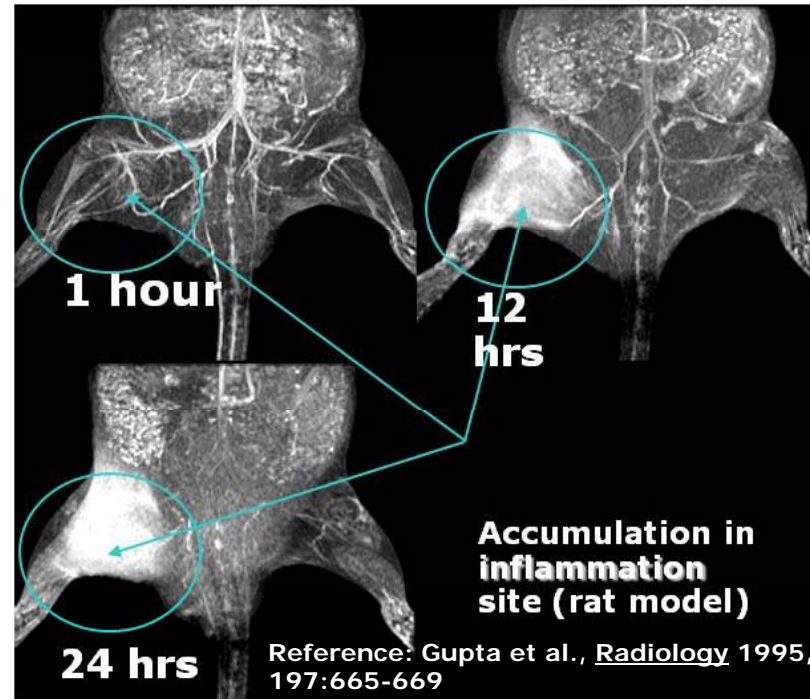
PGC-Insulin (10% loading) was injected subcutaneously into 6 STZ-diabetic Sprague Dawley rats at a dose of 1 mg/kg. Lantus® (Insulin-Glargine, Sanofi-Aventis) was also dosed at 1 mg/kg into the same animals after a 72 h washout period.

PGC Infection and Inflammation Targeting

PGC accumulates in inflamed muscle of rat infection model

INFECTION MODEL

Male Sprague-Dawley rats infected with previously frozen *E coli* in the posterior portions of the muscle in the left thigh. PGC was labeled with Gd for MR imaging (right), and radiolabeled for quantitation (below).



Radiolabeled PGC compared to IgG; Accumulation of PGC is comparable to IgG in inflammation site

Targeting Agent	% of ID at inflammation site	% of ID in healthy muscle tissue	Target/background ratio
Labeled PGC	0.65%	0.09%	7.8
Labeled IgG	0.75%	0.15%	5.3

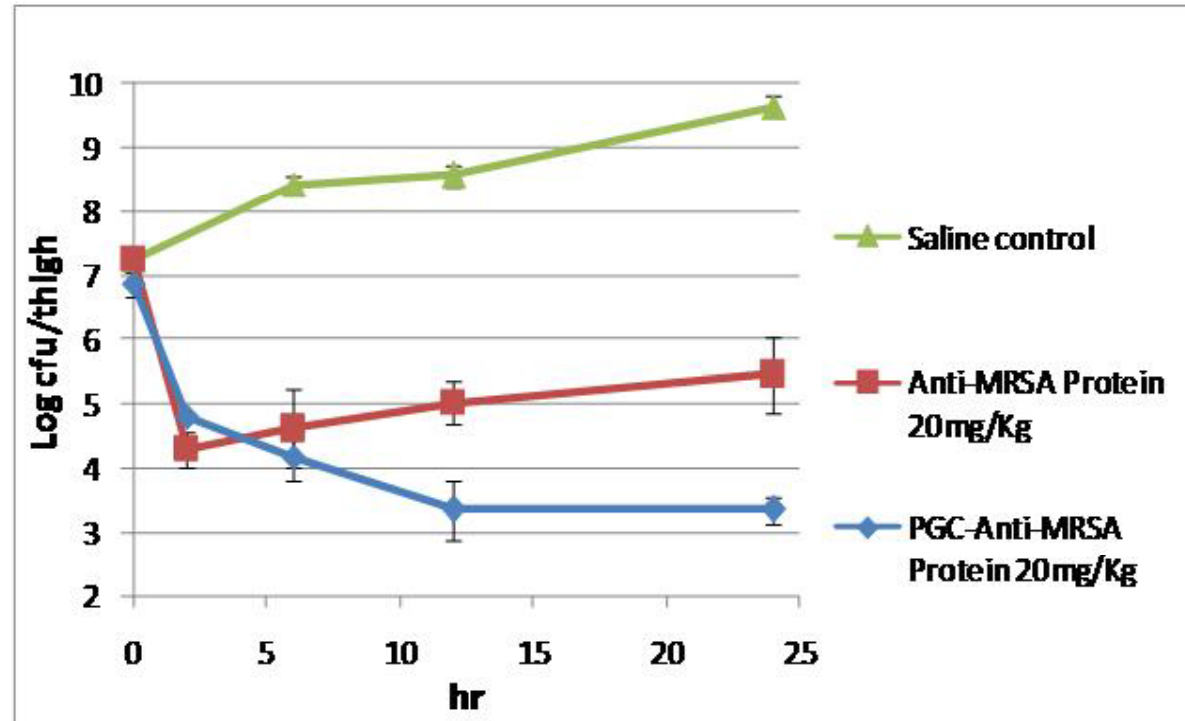
PGC Lysostaphin Efficacy

PGC Lysostaphin reduced MRSA Burden 100-fold relative to unformulated Lysostaphin

Lysostaphin is a 27 kDa metalloendopeptidase.

Lysostaphin is a well studied protein with specific activity vs. staph infection and known affinity to Zn.

The PGC-Metal Bridge carrier binds Lysostaphin with high affinity.



A neutropenic mouse infection model was infected with *S. aureus* ATCC 33591, an MRSA isolate. The thighs of the mice (N=12) were infected and treated with a single IV injection after 2 hours. Animals were euthanized and the microbial burden quantified at five time points (4 thighs/2 mice/time point) over a 24hr period.

Natriuretic Peptides

- Family of peptide hormones
 - BNP: Scios (nesiritide, Natrecor) (2001 US)
 - Acute heart failure
 - ANP: Daiichi Sankyo (Carperitide) (1995 Japan)
 - Acute and chronic heart failure
 - Others: not marketed
- Current Administration
 - Continuous IV infusion ($t_{1/2} < 1\text{hr}$)

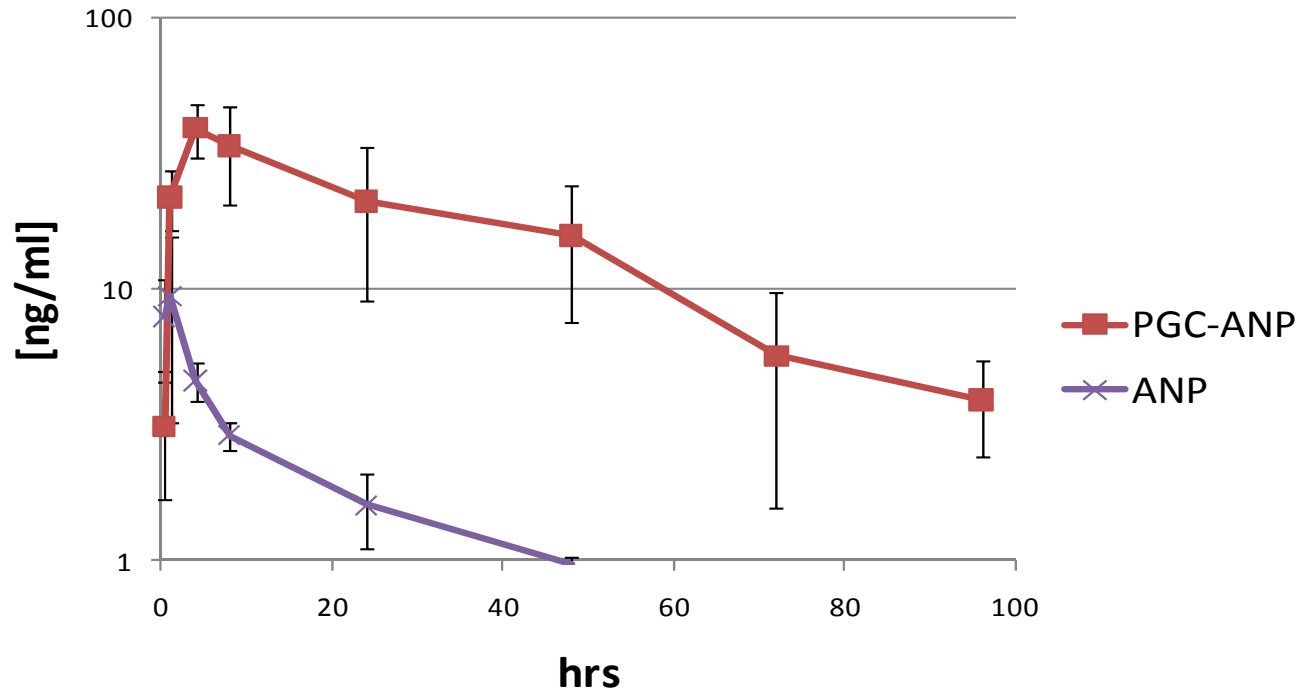
Natriuretic Peptide Profiles

ANP (Atrial Natriuretic Peptide)	BNP (Brain Natriuretic Peptide)
28 aa peptide (3.1 kDa)	32 aa peptide (3.4 kDa)
IV infusion (0.1-0.2 ug/kg/min)	IV infusion (2 ug/kg bolus + 0.01 ug/kg/min)
pI = 10.9 (basic)	pI = 11.6 (basic)
1 disulfide bridge	1 disulfide bridge
Excellent binding with PGC	Excellent binding with PGC

- Other peptides in family can also be formulated

ANP Pharmacokinetics

Subcutaneous Administration



Subcutaneous ANP PK (n=3, female BALB/c mice). PGC was loaded 1% w/w with ANP, mice were dosed at 2 mg/kg.

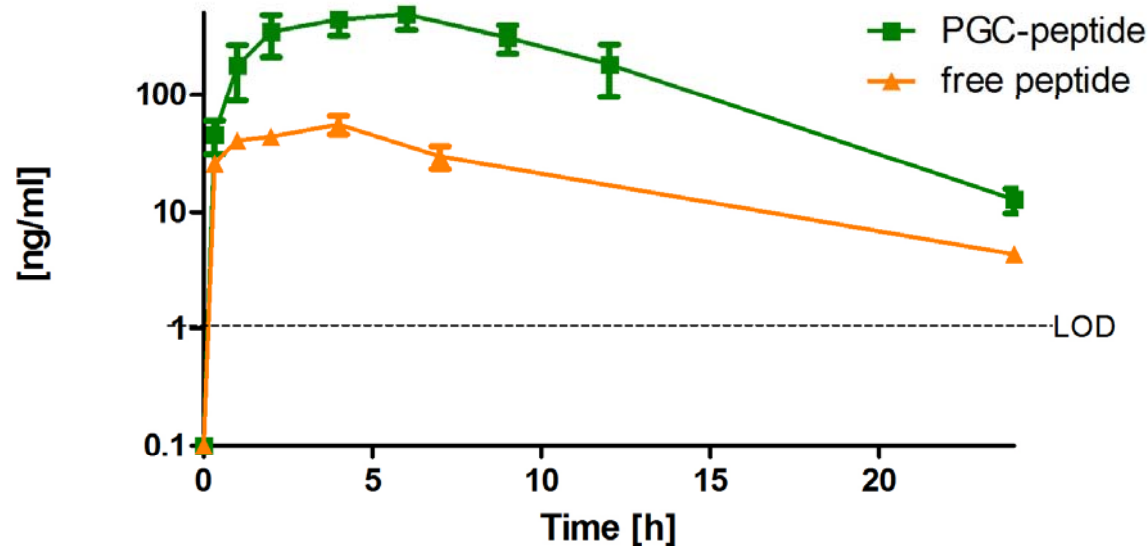
PGC-Fatylated Terlipressin PK

PGC improved AUC of a fatylated peptide

The fatylated (conjugated to a fatty acid chain) peptide has Mw~1.5 kDa.

The conjugated peptide is then bound with high affinity to the PGC-Hydrophobic Core carrier.

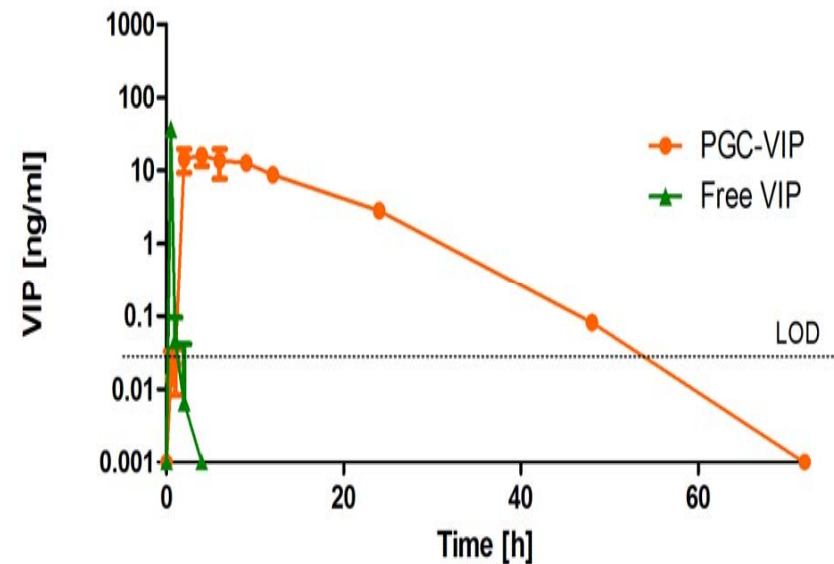
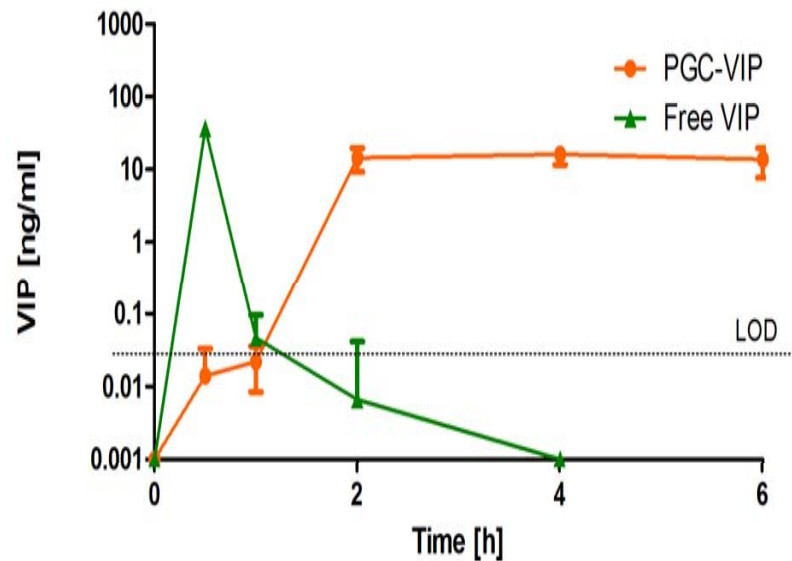
As the fatty acid is removed from the peptide in vivo, this is an example of the PGC-Cleavable Anchor platform.



Sprague-Dawley rats were dosed subcutaneously with a proprietary peptide. A second arm was the PGC formulated peptide. These two arms were both a single dose of 0.5 mg/kg.

VIP Pharmacokinetics

Subcutaneous Administration



Subcutaneous VIP PK (n=5, BALB/c mice). PGC was loaded 0.5% w/w with VIP, mice were dosed at 0.1 mg/kg VIP. The first 6 hours are shown on the left, and the entire 72 h result is shown on the right

PharmaIN Pipeline Examples

Compound	Description	Indications	PharmaIN Improvement	Status
Large (>100 kDa) undisclosed protein	Marketed IV protein by major Pharma	Not disclosed	Increased IV T _{1/2} significantly	Partner funded
Lysostaphin Mid-size MW protein	27 kDa enzyme with anti Staph and MRSA activity	MRSA IV administration	100 fold increase in killing power at 24h in thigh infection model	Available (NIH funded POC)
Natriuretic Peptides	Peptides: ANP and BNP marketed as IV infusion for CHF Very short T _{1/2}	1) Chronic SC admin for CHF patients 2) Disruption of fibrin in cirrhosis 3) Oncology	Significant improvement of PK, IV and SC	Available (NIH funded POC)
hbEGF	Epidermal Growth Factor peptide	Ischemic indications	Significant extension in PK via SC administration	Available (NIH funded POC)
PHIN-837	Peptide	Type 1 and 2 diabetes	Demonstrated exciting HbA1C data in Type 1 mouse model	Available (NIH funded POC)

PharmaIN Pipeline Examples

Compound	Description	Indications	PharmaIN Improvement	Status
Undisclosed peptide	Marketed peptide for CVS disease (IV, hospital use)	Daily SC use for and undisclosed chronic condition	PGC and fatylated peptide yields significant improvement in PK profile and enables SC admin, improved safety	Licensed (partner and NIH funded)
Vasoactive Intestinal Peptide (VIP)	Naturally occurring peptide with CVS and anti-inflammatory properties Very short T _{1/2}	1) Arthritis 2) CVR indications 3) Lung disease	Formulated into long acting SC administration, improved safety	Available (NIH funded POC)
Native GLP-1 peptide	Naturally occurring form of GLP-1 extremely short T _{1/2} Favorable CVS profile	Diabetes (Type 1 and 2)	Formulated as 1/day (possibly 1/week) SC dose	Licensed (NIH funded POC)
Basal Insulin	1/day native human basal insulin	Diabetes (Type 1 and 2)	1/day basal insulin, profile comparable to Lantus	Available (NIH funded POC)

PGC Advantages

- Affinity based binding of drug to carrier
 - No irreversible chemical modification of drugs
 - Improves PK, solubility, and safety
 - Useful for peptides, proteins, and potent small molecules
 - Ease of formulation and manufacturing
- Targeted delivery
 - Passive targeting to areas of enhanced vascular permeability (inflammation, infection, and tumors)
 - Active targeting capability
- Strong IP
- SC, IV, and IM

Thank you!

有難うございます

(Japanese)

תודה רבה

(Hebrew)

Спасибо

(Russian)

感謝

(Chinese)

Vielen Dank

(German)

Salamat Po

(Tagalog)

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