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Insider access: pepducin symposium explores a new approach to GPCR modulation

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The inaugural Pepducin Science Symposium convened in Cambridge, Massachusetts on March 8–9, 2009 provided the opportunity for an international group of distinguished scientists to present and discuss research regarding G protein–coupled receptor-related research. G protein–coupled receptors (GPCRs) are, arguably, one of the most important molecular targets in drug discovery and pharmaceutical development today. This superfamily of membrane receptors is central to nearly every signaling pathway in the human body and has been the focus of intense research for decades. However, as scientists discover additional properties of GPCRs, it has become clear that much is yet to be understood about how these receptors function. Everyone agrees, however, that tremendous potential remains if specific GPCR signaling pathways can be modulated to correct pathological states. One exciting new approach to this challenge involves pepducins: novel, synthetic lipopeptide pharmacophores that modulate heptahelical GPCR activity from inside the cell membrane.

Keywords: pepducins; G protein–coupled receptors; transmembrane signaling; meeting report

On March 8–9, 2009, a group of scientists involved in pepducin research presented their latest findings at the Pepducin Science Symposium in Cambridge, Massachusetts. This highly focused two-day event provided timely highlights to some of the most exciting areas in G protein–coupled receptor biology. The symposium was organized by Stephen Hunt III (Ascent Therapeutics), Athan Kuliopulos (Tufts Medical Center), and Thomas Sakmar (The Rockefeller University).

Introduction and history of pepducins

G protein–coupled receptors (GPCRs) are, arguably, the most important molecular target class in drug discovery and pharmaceutical development today. GPCRs are membrane receptors that are central to just about every signaling pathway in the human body: visual, olfactory, and taste sensory systems;

synaptic neurotransmission; targeted cell migration in developmental pathways and inflammation; stem cell homing; hormone signaling systems; and other important mechanisms involving cell–cell communication all involve GPCR activation. Nearly one in three commercialized compounds are designed to modulate GPCR function, and the annual revenue from sales of these drugs makes up a significant fraction of all pharmaceutical revenue. However, as scientists discover additional properties of this receptor superfamily, they also continue to realize how much is not yet understood about receptor function. Everyone agrees, however, that tremendous potential remains if specific GPCR signaling pathways can be modulated to correct pathological states. The Pepducin Science Symposium successfully stimulated discussion concerning pepducins and their potential applications in GPCR research and GPCR-targeted drug discovery.

Table 1. A brief history of GPCR research related to pepducins

Opioid receptor heterodimers characterized	1999	Devi
First GPCR (rhodopsin) crystal structure	2000	Palczewski
GABA receptor heterodimers/allosterism elucidated	2001	Pin
Pepducin agonist/antagonist activity demonstrated <i>in vitro</i>	2002	Kuliopulos
First trials for antagonist pepducins in animals	2002	Kuliopulos
Chemokine receptor pepducins developed	2005	Kaneider
Pepducin inhibition activity in tumor models shown	2005	Agarwal
Heterodimer-targeted pepducins developed	2006	Leger
First trials for agonist pepducins in animals	2007	Kaneider
Pepducin activity used to study sepsis and DIC	2007	Spek
First bioinformatic-based pepducin-like molecules developed	2007	Shields
Membrane flipping of pepducins demonstrated	2009	Hamilton

The Pepducin Science Symposium was jointly chaired by Athan Kuliopulos, Thomas P. Sakmar, and Stephen W. Hunt III. The symposium featured many of the scientists who have contributed to the growing body of knowledge surrounding pepducins and GPCRs over the past dozen years and was assembled to discuss the current state of pepducin research (Table 1). Presenters traveled from as far away as the Netherlands, France, and Ireland to share research on topics ranging from membrane biophysics and biochemistry to bioinformatics and molecular oncology, each with a unique perspective on how pepducins might aid in ongoing efforts to understand and modulate GPCR activity. Presentations highlighted new information around the mechanism of action of pepducins and demonstrated ways in which these molecules are already creating some excitement in GPCR-related drug development. The meeting format encouraged an energetic discussion that touched on many “hot-topic” GPCR questions, including dimerization, allosteric modulation, various strategies to achieve agonist and antagonist properties, and the issue of GPCR deorphanization. According to Dr. Kuliopulos, the scientific founder of Ascent Therapeutics, of the greater than 700 GPCRs encoded in the human genome, over 100 with known or presumed therapeutic potential have not yet been successfully targeted, but these may be amenable to pepducin technology. Dr. Kuliopulos, who has been involved in GPCR research for nearly 15 years, is one of the pioneers involved in the discovery of pepducins. He started the

meeting by summarizing key developments in the field.

Pepducins were developed in the late 1990s when scientists at the Tufts Medical Center literally turned the problem of GPCR-targeted drug discovery inside-out—instead of looking at GPCRs from outside the cell, they began looking at ways to target the receptors at the intracellular face, on the flip side of the cell membrane. As elaborated by Dr. Kuliopulos, “pepducins are designed to target recalcitrant GPCRs at the inside surface of the cell. Because pepducins can penetrate the cell and can either agonize or antagonize their targets, they are a potent tool both for research and therapeutic applications. Pepducins can greatly increase both the range of tractable targets and the specificity with which we can modulate their effect.” As originally published in the *Proceedings of the National Academy of Sciences*,¹ pepducin lipopeptides can exhibit agonist or antagonist activity for their cognate receptor (Fig. 1). The initial strategy was to synthesize a series of third intracellular loop (i3) peptides attached to an N-terminal lipid tag that would partition the peptide into and across the lipid bilayer of whole cells. The N-terminal lipid would also serve to anchor the intracellular loop peptide to the lipid bilayer, increasing the effective concentration in the vicinity of the GPCR target. Subsequent studies showed that certain pepducins based on the i1, i2, and i4 loops could also act as either agonists or antagonists of receptors, such as protease-activated receptors (PAR1, PAR2, PAR4) and chemokine receptors (CXCR1, CXCR2, and CXCR4).^{2,3,4}

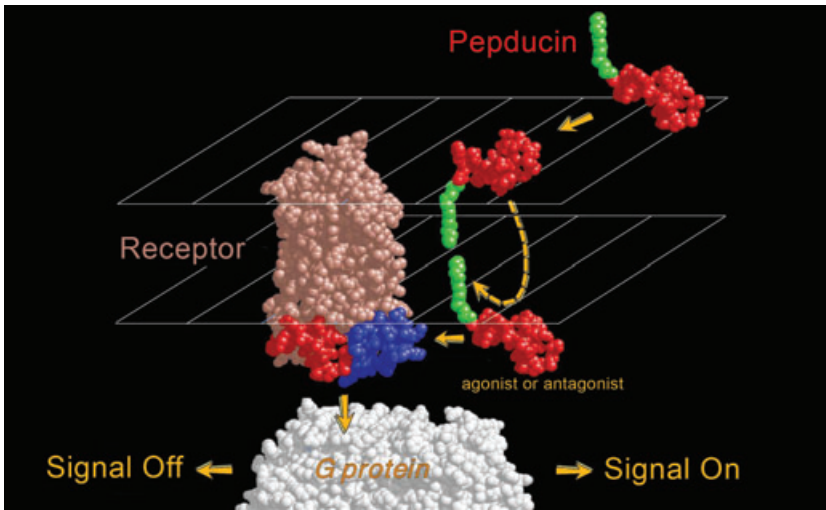


Figure 1. Proposed mechanism of membrane flipping and interaction of pepducins with their cognate receptor on the inner leaflet of the lipid bilayer. Palmitoylated peptides derived from the 3rd (i3) intracellular loop of PAR1 act as either full agonists or antagonists of PAR1, depending on the sequence of the i3-peptide. The palmitate moiety (green) first partitions the lipopeptide into the outside surface of the cell membrane. The lipid tether then facilitates flipping of the pepducin across the lipid bilayer where it can interact with its cognate receptor and either block or stimulate signaling to associated G proteins. (Adapted from Covic *et al.*¹).

In addition to serving as useful tools to study receptor signaling and mechanisms in cell-based systems, pepducins were shown to possess attractive drug-like characteristics that were deduced from pharmacokinetic, pharmacodynamic, biodistribution, and other preclinical studies in mice, guinea pigs, and baboons. The first pepducin studies in mice, published in *Nature Medicine*,⁵ showed that a pepducin based on PAR4 (a thrombin receptor expressed in platelets and other cells), P4pal-10, prevents activation of platelets and extends bleeding time, characteristics that were consistent with the phenotype observed in mice deficient in PAR4; in addition, P4pal-10 inhibited large vessel thrombosis in a ferric chloride (FeCl_3) injury model of mouse carotid arteries.⁶ Single intravenous bolus administration of P4pal-10 (0.28 mg/kg) reduced occlusion of the carotid artery to 30% as compared with untreated mice (90%, $P < 0.001$, $n = 31$). Kuliopulos also described more recent studies where PAR1-based pepducins were effective in blocking metalloprotease-PAR1-dependent arterial thrombosis in guinea pig and in human whole blood,⁷ and were the first uses of pepducin agonists in animals.⁸

A dynamic model for GPCR activation

- A recently elucidated model of GPCR activation shows dramatic conformational changes between active and inactive GPCR states at the intracellular junction.
- Findings help explain the mechanism of action for pepducin agonism or antagonism of GPCR receptors.

In their 2008 *Nature* paper, “Crystal structure of the ligand-free G-protein-coupled receptor opsin,” Patrick Sheerer, Jung Hee Park, and colleagues described, for the first time, the crystal structure of the GPCR opsin, the ligand-free version of the visual pigment rhodopsin. Opsin provides the first satisfactory high-resolution structural model for the “on” or active conformation of this class of molecules (Fig. 2).⁹ In his opening remarks symposium co-chair Thomas Sakmar presented an elegant animation depicting the predicted conformational changes between opsin and rhodopsin—a light-activated GPCR commonly accepted as a model for biophysical studies of GPCRs in a ground state conformation. The animation depicted a proposed rotation and outward movement of several

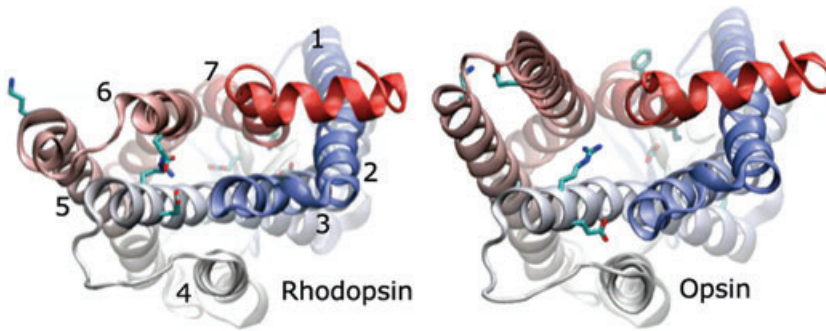


Figure 2. Transition of a GPCR from an inactive to active state. The structure of rhodopsin (pdb:1GZM) is juxtaposed with the structure of opsin (pdb:3CAP). Rhodopsin represents the inactive state of the receptor, and opsin is likely to represent the active-state structure. Transmembrane helices are numbered. The view is from above the cytoplasmic surface. Key differences between the two structures include: (1) an outward movement and rotation of helix 6 in opsin, which affect the orientation of “ionic lock” residues; (2) a general opening of the cytoplasmic surface of opsin; and (3) a dramatic change in the extracellular loop 3 conformation (not shown). In addition, a number of specific amino acid residues known to be important for receptor activation change orientation.

transmembrane helices and significant rearrangement of the loops on the cytoplasmic face of the receptor. Pepducins are believed to interact with the GPCR cytoplasmic loop structures affecting their mobility. Thus, if correct, the model presented helps explain the specificity and mechanism of action of pepducin activity, and may contribute to a broader understanding of the physiological structure of GPCR heterodimers.

It's all relative movement: GPCR activation and regulation through positive allosteric modulation

- Dimerization of GPCRs is thought to aid in fine-tuned allosteric modulation of signaling pathways.
- Positive allosteric modulators are highly desirable therapeutic entities because they can mimic natural timing and localization, achieving fine-tuned specificity with few off-target effects.
- Pepducin candidates that achieve positive allosteric modulation of specific GPCR targets are an important focus of research and development.

Jean-Philippe Pin, head of the Molecular Pharmacology Department within the Institute of Functional Genomics at CNRS, Montpellier, presented his work looking at allosteric modulation of GPCR dimers, suggesting that the purpose of dimers may be to allow very fine-tuning of GPCR signaling. New tools, such as specific labeling of the receptors with fluorescent ligands allowing time resolved—

fluorescence resonance energy transfer (FRET) measurement, can detect dimers and show different ligand binding stoichiometry based on FRET levels in the brain. Understanding how these ligands bind to effect GPCR activation will be important for developing pepducin-based drugs that can turn this knowledge into therapeutics. In particular, positive allosteric modulators (PAMs) are of great interest for drug development efforts because they allow selective enhancement of ligand signaling but have no intrinsic activity by themselves. PAMs can augment the biological activity of a receptor even when very low amounts of natural agonist are present, with very few off-target effects and in a manner that mimics the natural timing and localization of a stimulus response (Fig. 3).

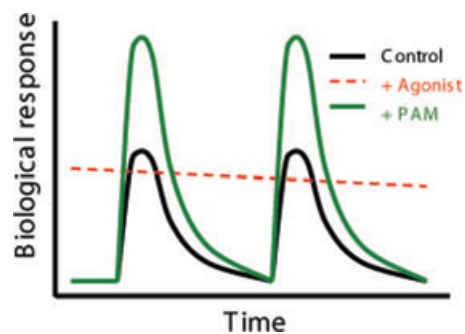


Figure 3. Allosteric modulators maintain the biological activity of the receptor.

In order to explain how PAMs achieve this effect, Dr. Pin's lab looked at the Venus flytrap module (VFTM) of the metabotropic glutamate receptors, which are found naturally in the brain as constitutive dimers¹⁰ and serve as a model for Class C GPCR activation. In this model, a conformational change at one subunit and subsequent relative movement of the VFTMs are both necessary and sufficient for agonist activation of the seven-transmembrane (TM) region.¹¹ In addition, a disulfide bridge was pinpointed as the key regulatory point by which the VFTM controls activation of the heptahelical receptor: breaking this bond allowed a direct activation of the receptor by PAMs. Research has shown that despite the dimeric structure, only one PAM is necessary to enhance regulatory activity and that only one receptor in the dimer pair is activated at a time, suggesting a new level of complexity for GPCR regulation by which each subunit can have an asymmetric effect on the function of the dimer as a whole.^{12,13}

No chaperones! Figuring out flip-flop transport

- An important characteristic of pepducins is their ability to translocate through the cell membrane.
- Special techniques have been developed to monitor transmembrane transport.
- Results suggest that pepducins may be optimized to rapidly access intracellular targets even in the absence of chaperone proteins.

James Hamilton, professor of physiology and biophysics and research professor of medicine at Boston University School of Medicine, presented his work on novel methods for monitoring transport of fatty acids and related molecules in membranes. Dr. Hamilton's lab has recently partnered with Ascent Therapeutics to look at the interfacial chemistry of how pepducins "flip" through the cell membrane to target GPCRs on the inside of the cell.

A key feature of pepducins is that they are lipidated peptides, a modification in which a fatty acid residue is attached to the peptide to help this molecule bind to a membrane interface; whether this architecture also facilitates movement through a cell membrane is the focus of Hamilton's new collaboration. Dr. Hamilton's presentation touched on a number of techniques that have been developed to visualize the movement of fatty acids in and out of membranes, work that has important implications

for tracking pepducins and understanding their cell-penetrating properties.

One way of tracking this movement is to measure the pH change created when an ionizable molecule translocates from the outside to the inside of the cell membrane (Fig. 4). This technique allows researchers to measure the time between the insertion of a molecule in the extracellular surface and the arrival at the inner surface of the membrane. Additional novel assays can be used to look at the membrane transport characteristics of various fatty acid modifications. By using model phospholipid bilayers that lack membrane proteins, Dr. Hamilton presented evidence supporting a key hypothesis that transmembrane transport can occur in the absence of specialized membrane transport proteins; according to Hamilton, "rapid diffusion makes it unnecessary to postulate that endocytosis is necessary, which means new drugs can be designed that will permit quick access to intracellular targets unchaperoned." Hamilton's team and Ascent Therapeutics aim to apply this membrane transport-tracking method to pepducins. Future research will focus on determining key properties for constructing pepducins with optimal membrane transport properties for use as therapeutics.

Lighten up: new technology illuminates the site of pepducin interactions

- Techniques have been developed that allow scientists to use fluorescence to pinpoint the precise amino acid residue where a pepducin/GPCR interaction takes place.
- This method is being used to propose a biochemical and biophysical model of pepducin/GPCR interaction that will, in turn, be used to design more effective modulators.

Thomas Sakmar, head of the Laboratory of Molecular Biology and Biochemistry at The Rockefeller University in New York, is working to elucidate the biophysical interaction between pepducins and their GPCR targets using novel biochemical and biophysical technologies. The approach relies on an emerging technology for site-specific incorporation of unnatural amino acid residues into GPCRs expressed in mammalian cells in culture.¹⁴ The technology is an adaptation of amber codon suppression methods developed earlier for *E. coli* expression systems. In amber codon suppression, the UAG

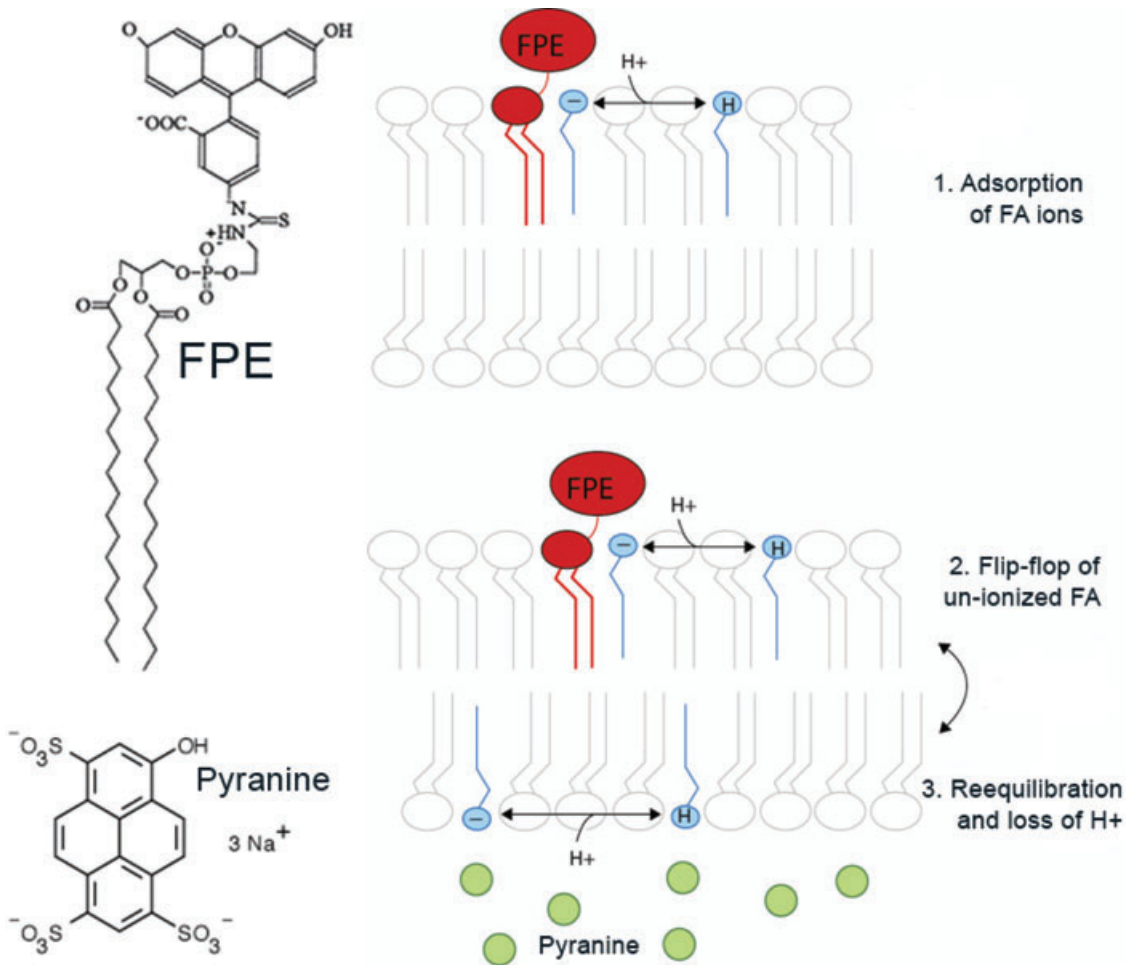


Figure 4. Artificial vesicle system to study pepducin flipping. Schematic showing the structures of the membrane surface potential probe, FPE, in the outer leaflet of the bilayer (top), and the entrapped pH probe, pyranine (bottom), at the opposite side of the bilayer in the aqueous volume. FPE fluorescence reports the number of FA anions that intercalate into the outer leaflet (adsorption). Following flip-flop of un-ionized FA, pyranine detects the release of protons as the FA reach ionization equilibrium in the inner leaflet of the bilayer.

termination codon (*amber codon*) is introduced into a gene of interest by site-directed mutagenesis; in the presence of an engineered suppressor-tRNA/amino acyl-tRNA synthetase pair, read-through occurs during protein synthesis and either natural or unnatural amino acids can be introduced at the amber codon site. In principle, depending on the suppressor-tRNA/amino acyl-tRNA synthetase orthogonal pair, a variety of unnatural amino acids can be introduced. For example, Dr. Sakmar's laboratory to date has reported the introduction of *p*-L-acetyl-phenylalanine, *p*-L-benzoyl-phenylalanine, and *p*-L-

azido-phenylalanine into heterologously expressed rhodopsin and/or chemokine receptors.

The introduction of *p*-L-benzoyl-phenylalanine is particularly useful for binding-site mapping studies since it serves as a photoactivatable crosslinker. The idea would be to introduce *p*-L-benzoyl-phenylalanine adjacent to putative pepducin binding sites on a target receptor, then crosslink in the presence and absence of pepducin and score for crosslinking. The site-directed crosslinking technology is currently being used to test the hypothesis that pepducins interact with

the intracellular cytoplasmic surface of their target GPCR. An alternative hypothesis is that they may interact with the receptor-G protein–ternary complex, or with the G protein itself. Using the cytokine CXCR4, a series of site-directed mutants have been created and optimized for use in the crosslinking reporter structure. Through a progressive screening process this should allow researchers to determine the key sites for pepducin/GPCR interaction.

Initial assays have provided proof of concept for the experimental methods, and the team is currently refining their protocol with the ultimate goal of proposing a biochemical and biophysical model of pepducin/GPCR interaction. “We are encouraged by the initial proof-of-concept experiments and hope that on-going work will add to the growing body of knowledge related to peptide interactions with the GPCR superfamily, which may help researchers design more effective modulators,” concluded Dr. Sakmar.

Mitigating morphine dependence: A two-pronged approach

- Opioid receptors are GPCRs that regulate neuronal pathways, including pain, sedation, appetite, and mood.
- These receptors are thought to form heterodimers that regulate pathways differently than either dimer alone.
- Studies of opioid receptor signaling have elucidated some of the nuances of heterodimer regulation in morphine dependence and are contributing to the development of new and potentially improved therapeutic compounds.

Lakshmi A. Devi, professor of pharmacology and systems therapeutics at the Mount Sinai School of Medicine in New York, presented research from her work studying a new mechanism of modulation of opioid receptors involved in opiate addiction, with the goal of improving the analgesic properties of morphine.

Opioid receptors are an important family of GPCRs that regulate a number of neuronal pathways. The role of dimerization in GPCR function—a key feature of the potential mechanism of action of pepducins—has won broader acceptance recently¹⁵ because of research (some of which was highlighted at this symposium) that supports a model of het-

erodimerization and joint regulation of the mu and delta opioid receptors (MOR and DOR, respectively) in the brain. Morphine is a classic ligand for the mu opioid receptor. This receptor can exist either as a homodimer or as a heterodimer in combination with one of the other two known opioid receptors, delta or kappa. Research presented by Dr. Devi demonstrates that when the two receptors associate with each other, DOR ligands can enhance MOR activity and may inhibit the development of morphine tolerance.¹⁶

A series of publications has suggested that targeting the mu-delta opioid receptor heterodimer can effect a temporal change in the morphine signal in the brain, as evidenced by a switch from rapid activation of the opioid receptor to a slower, more persistent activation. When morphine binds to MOR, it yields an acute effect, but when MOR is in a heterodimer configuration with DOR, morphine binding yields a longer, more persistent effect. This latter type of signaling is due to the recruitment of β -arrestin 2 by the heterodimer, but not the MOR receptor alone (Fig. 5). The signal generated via the β -arrestin 2 pathway is thought to be involved in the development of drug-dependency.^{17,18} That observation prompted Dr. Devi’s group to try a simultaneous two-pronged ligand combination approach that could effectively modulate the MOR-DOR heterodimer activity. These researchers used a combination of ligands to concurrently agonize MOR while antagonizing DOR and were able to increase the analgesic effect of morphine.¹⁹ This is an exciting development and important proof of concept, as this approach yields compounds with greatly improved therapeutic profiles.

Computational screening for novel GPCR ligands

- Pepducins are synthesized using known amino acid sequences from target areas of interest.
- Novel sequences can be identified through bioinformatics and computational screening methods.
- Computational techniques can be used to aid in the design of novel pepducins that target specific GPCRs.

Denis Shields, professor of clinical bioinformatics at the University College of Dublin, presented his work using bioinformatic computational methods to find targets of interest through whole genome

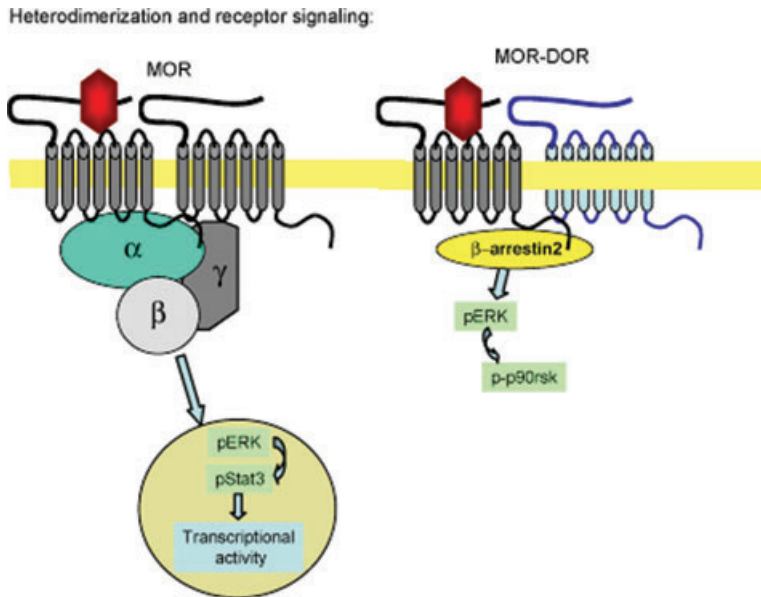


Figure 5. Recruitment of β -arrestin 2 in MOR-DOR heterodimer-mediated ERK phosphorylation compared to MOR-mediated ERK phosphorylation leads to differentiation in signaling pathways. (Adapted from Rozenfeld, R. & L.A. Devi.¹⁹)

screens. Bioinformatic screening can help scientists cost-effectively identify similarities between known protein-protein interactions and screen for novel, bioactive proteins that share these characteristics. For peptiducins, this approach can be used to elucidate new target sequences shared by multiple receptors in a family of interest, allowing researchers to synthesize peptiducins with highly desirable properties.

In the project presented researchers looked for conserved juxtamembrane regions with characteristics of known human platelet activity modulators, with the hope of finding sequences that could lend themselves to anti-clotting applications. High-throughput functional assays were then used to test the activity of more than 50 newly identified peptide sequences with attached N-terminal lipid tags to confer cell-penetrating and tethering properties. First, computational bioinformatic tools were used to find conserved juxtamembrane regions within groups of proteins known to be expressed on the surface of platelets. Subsequent high-throughput *in vitro* platelet functional assays identified many agonists and antagonists based on these lipidated-juxtamembrane sequences. The joint application of bioinformatics and computational methods holds

great promise to aid in the design of novel peptiducins to target specific GPCRs and to modulate discrete downstream signaling pathways. Bioinformatics approaches in development for the more efficient and effective identification of potentially bioactive peptiducins and other oligopeptides include conservation modeling, recurrent motif discovery, and structural modeling.

Inhibiting neutrophil activation in sepsis

- GPCRs play a role in the development of sepsis and are implicated in complications of this condition relating to coagulation factors in the blood.
- Peptiducin antagonists have been shown to inhibit specific receptors and can confer a protective effect in animal models of sepsis.

C. Arnold Spek, professor at the Center for Experimental and Molecular Medicine at the University of Amsterdam, addressed sepsis and the physiological complexities that render it an extremely difficult disease to dissect. Dr. Spek presented ongoing research from his laboratory where researchers are using peptiducins to tease out factors that contribute to an “escalating vicious circle,” in which coagulation factors induce inflammation,

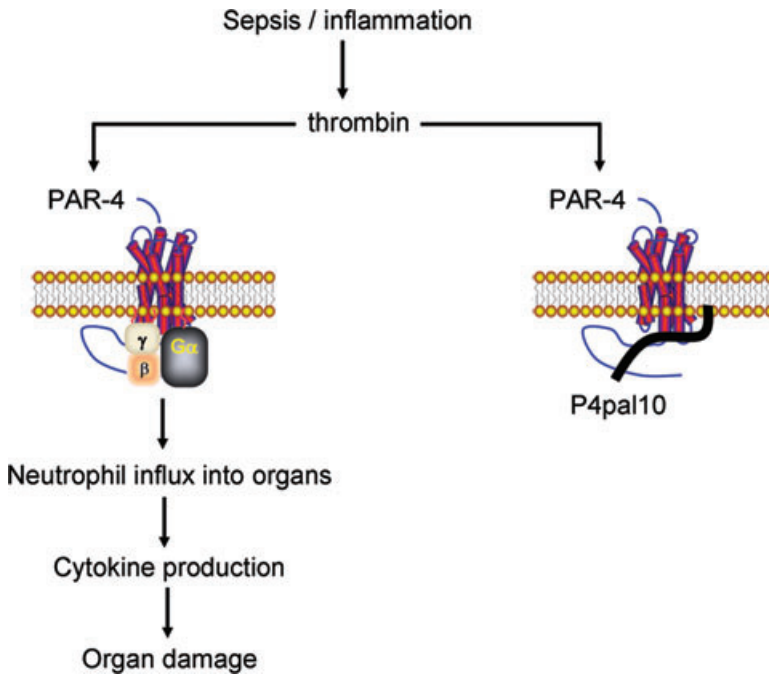


Figure 6. Schematic overview of PAR-4-dependent organ damage in sepsis. Inflammation results in extravascular thrombin generation and PAR-4-dependent neutrophil influx into inflamed organs and subsequent cytokine release, thereby augmenting the inflammatory response leading to organ failure. Treatment with P4-pal10 blocks PAR-4 signaling, thereby preventing neutrophil influx into affected organs and subsequent cytokine-dependent organ damage.

which, in turn, recruits additional coagulation factors and causes systemic inflammation, a cycle that may ultimately lead to multi-organ failure and death.²⁰ Dr. Spek focused on the physiological role of the protease-activated GPCR PAR4 in mouse models of systemic inflammation (Fig. 6). Using a peptidic antagonist against PAR4, Spek and colleagues could reduce neutrophil influx into susceptible organs resulting in a dose-dependent decrease in organ failure and an increase in biomarkers of overall health; the team did not, however, see any correlation to overall survival.²¹ This result implies that while PAR4 inhibits neutrophil inflammation, there is still considerable residual damage due to additional factors.

When the same experiment was performed using neutrophil-depleted animals, the protective effect was not observed, suggesting that the presence of neutrophils is a necessary condition for PAR4 peptidic antagonists to diminish inflammation and organ damage. By contrast, when the experiment

was repeated in platelet-depleted mice, the protective effect of the PAR4 antagonist was observed. Spek's team thus concluded that neutrophils, but not platelets, are necessary for the PAR4 antagonist to confer a protective effect in mice. Additional control experiments confirmed that the effect is specific for PAR4 antagonism.²²

To provide additional support, a migration assay was used to show that the PAR4 peptidic specifically inhibits neutrophil migration toward thrombin but not toward other GPCR ligands. Thrombin-mediated PAR4-neutrophil-specific interaction leads to an increase in local inflammation. Furthermore, Dr. Spek found that administering a PAR4 peptidic antagonist might be protective in systemic inflammatory disease partially due to reduced systemic and local inflammation, as well as to neutrophil responses. Additional planned work will look at PAR4-deficient animals and other models of sepsis to see if the results presented here can be replicated.

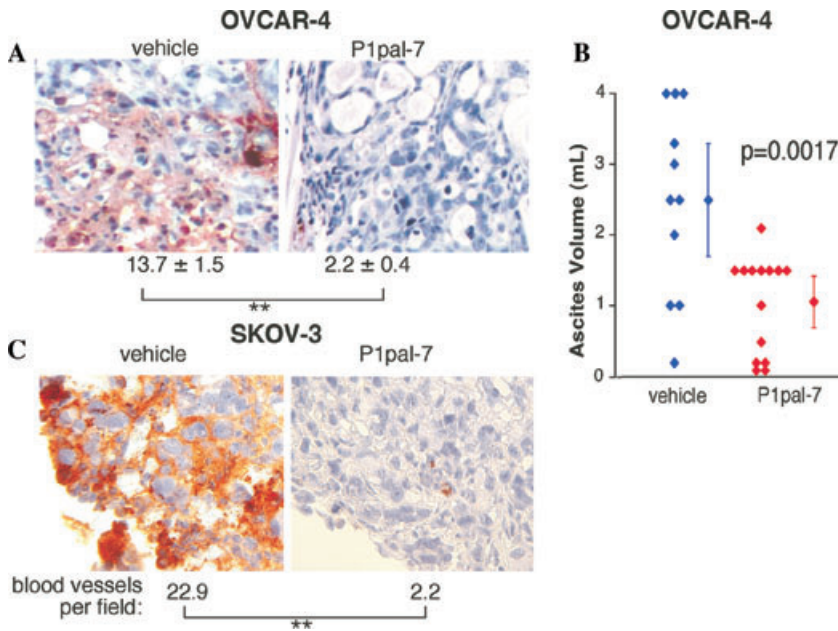


Figure 7. PAR1 antagonist pepducin P1pal7 inhibits angiogenesis and ascites in mouse models of peritoneal ovarian cancer. (A) and (C), PAR1 pepducin, P1pal-7, used as monotherapy inhibits angiogenesis of peritoneal OVCAR-4 and SKOV-3 cancer in mice. Female NCR Nu/Nu mice were injected intraperitoneally (i.p.) with 1.5 million OVCAR-4/SKOV-3 cells and then divided into treatment groups of vehicle (10% DMSO alternate day (A/D), starting on day 2) or P1pal-7 (3.2 mg/kg i.p. A/D, starting on day 2). Intense staining of Weibel-Palade bodies within individual endothelial cells using a rabbit polyclonal anti-von Willebrand factor was scored as a blood vessel in a blinded manner from paraffin-embedded tissue sections from mice with peritoneal OVCAR-4/SKOV-3 cancer. Representative sections and blood vessels (arrow heads) from tumor centers or tumor edge are shown. Vascular density was determined by counting 5–9 fields (160 X magnification) from at least 5 tumors in each group. Mean blood vessel density per field is shown at the bottom. * $P < 0.05$, ** $P < 0.005$. (B), Female NCR nu/nu mice were injected with 1.5 million OVCAR-4 cells and treated with either i.p. vehicle alone (20% DMSO A/D) or the PAR1 pepducin antagonist P1pal-7 (10 mg/kg, A/D) for 40 days; ascites fluid was collected and volume determined. (Adapted from Figures 4 and 5, Agarwal *et al.*²⁴)

Targeting signal pathways in ovarian cancer

- The matrix metalloprotease (MMP) cascade is a key signaling pathway involved in the development of ovarian cancer.
- RNAi knock-down experiments have previously shown that blocking transcription of the PAR1 oncogene can inhibit cancer cell migration and invasion.²³
- Pepducin antagonists against PAR1 have been shown to have a similar therapeutic effect as RNAi knock-down and may be a viable means to target the previously intractable MMP-PAR1 expression pathway.^{22,24,25}

Anika Agarwal, from the Tufts Medical Center, presented her work on targeted therapies for ovarian cancer. There is a great need for new methods to detect and treat this disease, explained Dr. Agarwal; by the time ovarian cancer is detected it has usually progressed to a late stage, and with current treatments, most patients experience relapses due to drug resistance, “as a result, there has been a real upsurge in interest in developing targeted therapies that block key signaling pathways,” she said.

One of these key signaling pathways involves matrix metalloproteases (MMPs), which have emerged as important biomarkers and prognostic factors for a number of cancers. Dr. Agarwal explained how her

lab identified an MMP cascade that culminates in the activation of a protease-activated GPCR, PAR1, which is an oncogene thought to have a role in many cancers. Initial experiments using RNA interference (RNAi) to “knock-down” the expression of the PAR1 gene in ovarian tumors demonstrated a correlation between migration and invasion of malignant ovarian cells and PAR1 expression; this activity was confirmed using a pepducin against PAR1. The PAR1 antagonist pepducin was administered through interperitoneal injection in mice to create a pharmacological barrier blocking PAR1-dependent proliferation of ovarian carcinoma cells. Dr. Agarwal reported that pepducin inhibition of PAR1 significantly decreased ascites formation and angiogenesis, thereby inhibiting tumor growth, invasion, and metastasis (Fig. 7). “These results have generated a lot of excitement because previous efforts to target MMPs have fared very poorly in clinical trials,” concluded Dr. Agarwal, and “we were encouraged to find that inhibition of PAR1 in mice is a new mechanism to inhibit this cancer-proliferation cascade and look forward to exploring pepducins in additional studies.”

In follow-up discussion, many of the audience members were intrigued by the observation that PAR1 is expressed when tumors metastasize, and questioned whether PAR1 could possibly be used as a biomarker to detect the presence of malignant cells. While Dr. Agarwal had not explored that possibility yet, she and the group were optimistic that understanding MMP1-PAR1 expression and interaction may lead to additional breakthroughs for the treatment of ovarian cancer.

Conclusion

The sponsors and chairs of the Pepducin Science Symposium were honored to have more than 30 scientific presenters, GPCR thought leaders, and life science venture capitalists in attendance and are planning a second meeting for 2010 where they will share the results of collaborations forged at the inaugural symposium. They welcome new contributions to the growing field of pepducin science and therapeutics.

Conflicts of interest

Jacquelyn Miller is an employee of MacDougall Biomedical Communications, a service provider

for Ascent Therapeutics Inc. Lakshmi A. Devi receives research funding from Johnson and Johnson. Athan Kuliopulos is a co-founder of Ascent Therapeutics Inc. Athan Kuliopulos and Thomas P. Sakmar serve on the Scientific Advisory Board and receive research funding from Ascent Therapeutics Inc. Stephen W. Hunt III is Senior Vice President at Ascent Therapeutics Inc.

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