

 **me of Journal:** *World Journal of Cardiology*

Manuscript Type: EDITORIAL

Not all arrestins are created equal: Therapeutic implications of the functional diversity of the β arrestins in the heart

Lymperopoulos A, Wertz SL, Pollard CM, Desimine VL, Manning J, McCrink KA.
Functional differences of cardiac β arrestins

Anastasios Lymperopoulos, Shelby L. Wertz, Celina M. Pollard, Victoria L. Desimine, Jennifer Maning¹, Katie A. McCrink^{2,3}.

Laboratory for the Study of Neurohormonal Control of the Circulation, Department of Pharmaceutical Sciences (Pharmacology), College of Pharmacy; Nova Southeastern University, Fort Lauderdale, FL 33328, USA.

¹Present address: Jackson Memorial Hospital, Miami, FL 33136, USA.

²Present address: Massachusetts General Hospital, Boston, MA 02114, USA.

³American Foundation for Pharmaceutical Education (AFPE) "Gateway to Research" Scholar.

Author contributions: All authors performed literature research and contributed to the writing of the manuscript. A Lymperopoulos supervised the project and wrote the paper.

 **upported by** a NSU's President's Faculty Research & Development Grant (PFRDG).

Conflict-of-interest statement: The authors declare no conflict of interest related to this publication.

Correspondence to: Anastasios Lymperopoulos, PhD, FAHA, FESC, Associate Professor of Pharmacology, Department of Pharmaceutical Sciences, Nova Southeastern

University College of Pharmacy, 3200 S. University Dr., HPD (Terry) Bldg/Room 1338,
Fort Lauderdale, FL 33328-2018, USA. al806@nova.edu


Telephone: +1-954-262-1338

Fax: +1-954-262-2278

Abstract

The two ubiquitous, outside the retina, G protein-coupled receptor (GPCR) adapter proteins, β arrestin1 and -2 (also known as Arrestin2 and -3, respectively), have three major functions in cells: GPCR desensitization, i.e. receptor decoupling from G-proteins; GPCR internalization via clathrin-coated pits; and signal transduction independently of or in parallel to G-proteins. Both β arrestins are expressed in the heart and regulate a large number of cardiac GPCRs. The latter constitute the single most commonly targeted receptor class by FDA-approved cardiovascular drugs, with about one-third of all currently used in the clinic medications affecting GPCR function. Since β arrestin1 and -2 play important roles in signaling and function of several GPCRs, in particular of adrenergic receptors (ARs) and angiotensin II type 1 receptors (AT₁Rs), in cardiac myocytes, they have been a major focus of cardiac biology research in recent years. Perhaps the most significant realization coming out of their studies is that these two GPCR adapter proteins, initially thought of as functionally interchangeable, actually exert diametrically opposite effects in the mammalian myocardium. Specifically, the most abundant of the two β arrestin1 exerts overall detrimental effects on the heart, such as negative inotropy and promotion of adverse remodeling post-myocardial infarction (MI). In contrast, β arrestin2 is overall beneficial for the myocardium, as it has anti-apoptotic and anti-inflammatory effects that result in attenuation of post-MI adverse remodeling, while promoting cardiac contractile function. Thus, design of novel cardiac GPCR ligands that preferentially activate β arrestin2 over β arrestin1 has the potential of generating novel cardiovascular therapeutics for heart failure and other heart diseases.

Key words: adverse remodeling; β arrestin; biased signaling; cardiac myocyte; cardiac fibroblast; contractility; functional divergence; G protein-coupled receptor (GPCR); heart failure; hormone; myocardial infarction; signal transducer.

 **re tip:** Presumed functionally similar for a long time, we now know that the two β arrestins display significant functional diversity in several organs and tissues, including in the cardiovascular system. Their functional distinction also in the mammalian heart has been clearly documented over the past few years. β arrestin1, which is far more abundant than β arrestin2 in almost every tissue including the myocardium, opposes the cAMP-dependent pro-contractile signaling of the β_1 AR, and promotes cardiac apoptosis, inflammation, and other adverse remodeling-associated processes post-MI. Conversely, β arrestin2 promotes catecholamine-dependent cardiac contractility directly, via SERCA2a potentiation, and indirectly, by leaving β_1 AR's cAMP-dependent pro-contractile signaling unaffected.

INTRODUCTION

Out of the four mammalian arrestins, only the two ubiquitous (outside the retina) Arrestin-2 and -3, also known as β arrestin1 and -2 respectively, are expressed in the mammalian cardiovascular system. Like in almost every tissue, β arrestin1 protein is ~10-15-fold more abundant than β arrestin2 in the circulatory system, as well^[1]. Both β arrestins regulate all non-opsin G protein-coupled receptors (GPCRs), also known as seven transmembrane-spanning receptors (7TMRs), including those responsible for neurohormonal regulation of cardiovascular physiology^[2,3]. For instance, cardiac function (contractility) is tightly controlled by the activity of β -adrenergic receptors (β ARs) located in the membranes of cardiac myocytes^[4-8]. Cardiac structure and morphology are regulated by angiotensin II (AngII) type 1 receptors (AT₁Rs) present (mainly) in cardiac fibroblast and endothelial cell membranes^[4,7]. Even the production and release of the regulatory hormones per se, whether it be catecholamine and corticosteroid release by the adrenal glands or activation of the renin-angiotensin-aldosterone system (RAAS) by the juxtaglomerular apparatus of the kidneys or release of

neurotransmitters by central and peripheral neurons innervating cardiovascular organs, is under tight regulation by various GPCRs^[1,4,7].

Cardiovascular GPCRs can signal either through G-proteins or β arrestins with the natural, endogenous agonist hormones activating both signal transducers at each receptor fully and equally^[1,9]. Several “biased” GPCR ligands have been discovered that (relatively) selectively activate either G proteins or β arrestins^[1,9]. This “bias” in terms of the activated signal transducer is always relative but the concept of “biased” signaling and its attainability for therapeutic purposes has been challenged recently. Specifically, recent studies have shown that receptors can activate both G-proteins and β arrestins at the same time^[10] or that β arrestins do not even need to bind the agonist-activated receptor to get (“catalytically”) activated^[11]. Additionally, it was very recently clearly demonstrated that G-protein activation is absolutely necessary, at least initially upon agonist activation, for β arrestin activation and signaling to follow^[12,13]. This sequence of activation of the two signal transducers, i.e. G-proteins being activated first followed by activation of β arrestins, is also corroborated well by the majority of structural studies on mechanisms of GPCR activation done to date. Specifically, the receptor seems to require the interaction with the heterotrimeric G-protein in order to become fully activated by the agonist. In other words, in the absence of a G-protein, agonist binding per se is simply insufficient for the receptor to break the huge energy barrier that prevents it from reaching the active state^[2,14]. Taken together, G-protein activation and signaling appears to be a prerequisite for β arrestin signaling by GPCRs and thus, discrimination between these two families of signal transducers for any given GPCR ligand, which represents the foundation of the “biased signaling” concept for GPCRs, is essentially unfeasible. However, whereas the selective stimulation of G-protein vs. β arrestin signaling for therapeutic purposes is most likely impossible, selective stimulation of β arrestin1 vs. β arrestin2 might be feasible, similarly to the selective stimulation (or inhibition) of various G_α subunits, which is pharmacologically achievable and currently exploited therapeutically. The first hint at signaling and functional differences between the two β arrestins came over a decade ago with the realization that β arrestin1, but not β arrestin2

which has a nuclear export signal sequence (NES), can translocate to the nucleus where it regulates gene transcription^[15]. Since then, the experimental evidence supporting functional divergence between the signaling properties of the two β arrestins both in vitro and in several tissues and organs in vivo, including in the heart, has been mounting at an accelerating pace. Thus, β arrestin isoform-selective targeting may have a place in the design and development of novel drugs. Below, we review this evidence known so far for the cardiac β arrestins and discuss what it could signify for heart failure drug development.

FUNCTIONAL DIFFERENCES BETWEEN THE TWO β ARRESTINS IN CARDIAC β AR SIGNALING

The β_1 AR is by far the predominant β AR subtype in human adult cardiac myocytes, representing 75-80% of total β AR density, followed by the β_2 AR, which comprises about 15-18% of total cardiomyocyte β ARs and the remaining 2-3% is β_3 ARs^[4,7,16]. β_1 AR stimulation by catecholamines results in the dissociation of the stimulatory G protein alpha subunit ($G\alpha_s$) from $G\beta\gamma$. $G\alpha_s$ stimulates adenylyl cyclase (AC) to produce cyclic adenosine monophosphate (cAMP), which, in turn, activates protein kinase A (PKA) and regulates different intracellular, sarcolemmal and myofibrillar substrates^[4,5,7]. Thus, cAMP-dependent signaling in cardiomyocytes mediates the cellular effects of β_1 AR activation on stimulation of cardiac chronotropy, inotropy, dromotropy, and lusitropy (**Figure 1**)^[4,5,7]. As co-factors of GPCR-kinases (GRKs) in β AR desensitization/downregulation, β arrestins normally diminish the inotropic and β -adrenergic reserves of the failing heart and their inhibition should theoretically be beneficial in acute decompensated heart failure (ADHF)^[4,7]. Indeed, genetic deletion of β arrestin1 in the heart results in several desirable therapeutic effects in heart failure, such as dramatic improvements in both cardiac β -adrenergic and inotropic reserves, amelioration of adverse remodeling and increased survival post-myocardial infarction (MI) (**Figure 1**)^[17,18]. In contrast however, cardiac β arrestin2 has been shown to be cardio-protective, as it inhibits cardiac apoptosis, inflammation, and significantly attenuates

overall adverse remodeling post-MI (**Figure 1**)^[19]. One of the underlying mechanisms for the anti-inflammatory effects of cardiac β arrestin2 is nuclear factor-kappaB (NF κ B) inhibition in cardiac myocytes, which, again, appears to be mediated only by β arrestin2 and not by β arrestin1 in the heart (**Figure 1**)^[19,20]. Importantly, β_1 AR-stimulated β arrestin2 was also recently documented to increase cardiac contractility both directly and indirectly (**Figure 1**)^[20]. Directly, by interacting with Sarco/Endoplasmic Reticulum Ca^{2+} -ATPase (SERCA)-2a leading to enhanced Small Ubiquitin-related MOdifier (SUMO)-ylation of the latter^[20]. This process, deficient in human heart failure, is known to directly stimulate SERCA2a activity, thereby increasing cardiac contractility^[21]. β arrestin2 also increases cardiac function indirectly by leaving the β_1 AR-stimulated cAMP-dependent pro-contractile signaling intact (i.e. not desensitizing it) in cardiac myocytes in vitro and in post-MI heart failure mice in vivo (**Figure 1**)^[20]. Importantly, these effects are not shared by the vastly more abundant in the human heart β arrestin1^[22].

One of the salient mechanisms for the anti-apoptotic effects of cardiac β arrestin2 is transactivation of the epidermal growth factor receptor (EGFR) by the cardiac β_1 AR (**Figure 1**)^[18,23]. β arrestin1 seems again unable to stimulate this and instead, promotes cardiac apoptosis post-MI (**Figure 1**)^[18]. Older studies had reported that mice expressing a mutant β_1 AR that cannot undergo GRK-dependent desensitization or activate β arrestins in their hearts lack cardiac EGFR transactivation and suffer from massive cardiac apoptosis and left ventricular dilatation compared to wild type controls^[23]. Interestingly, the β -blocker drug carvedilol, an inverse agonist towards G-protein activation^[16], is a weak β arrestin-biased agonist that activates ERKs (Extracellular signal-Regulated Kinases) via EGFR transactivation also^[24,25]. It should be pointed out though that carvedilol's "biased" β arrestin agonism has been demonstrated only in heterologous recombinant cell systems without cardiovascular (or any other physiological) relevance (mostly, transfected HEK293 cells). However, if this holds true in actual cardiomyocytes in vivo, it might be part of the mechanism for this β -blocker's cardio-protective effects. However, several studies do not lend support to this notion; sustained β_1 AR activation

by catecholamines, markedly more potent activators of β arrestin-dependent ERKs in the heart than carvedilol, increases cardiac adverse remodeling even in the absence of cardiac injury^[26]. Moreover, carvedilol is also a β_2 - and α_1 AR antagonist, which may interfere with its β arrestin-activating properties in the heart^[27]. On the other hand, carvedilol's effects in the heart are virtually exclusively mediated by the β_1 AR, due to its relative selectivity for the human β_1 AR over the other human AR subtypes (β_2 AR and α_1 AR) and to the vast preponderance of the β_1 AR over the rest of AR subtypes in the human adult myocardium^[16]. Last but not least, recent studies have been unable to directly detect β arrestin interactions with either β_1 AR or β_2 AR bound to carvedilol, including a study reporting the intriguing finding that carvedilol requires G_{α_i} protein recruitment to the β_1 AR in order to induce EGFR transactivation via β arrestins^[28-31]. Nevertheless, carvedilol has been shown to selectively recruit β arrestin2 to the hyperfunctional human polymorphic Arg389 β_1 AR in cardiomyocytes in vitro^[32]. Therefore, the more robust, compared to its Gly389 counterpart, pro-contractile signaling of this β_1 AR variant^[33] might be, at least partly, due to its unique β arrestin2-interacting tropism. In this vein, very recent data from our lab indicate that carvedilol-bound β_1 AR uniquely stimulates β arrestin2-dependent SERCA2a activity and fractional shortening of cardiomyocytes in vitro^[34].

FUNCTIONAL DIFFERENCES BETWEEN THE TWO β ARRESTINS IN CARDIAC AT_1R SIGNALING

Despite its very low abundance in adult human myocardium (density ratio of AT_1R/β_1AR in the non-failing human heart: $\sim 1:15$)^[16], the AT_1R plays important regulatory roles in the heart, but mainly via actions in cardiac fibroblasts and endothelial cells, rather than in cardiac myocytes^[4,7,35-37]. The AngII peptide analog SII ([Sar¹-Ile⁴-Ile⁸]-AngII) does not elicit G_q protein signaling when bound to the AT_1R ^[9,38]. When it was discovered to induce β arrestin signaling from the AT_1R , the concept of biased signaling was introduced and ushered in a completely new field in cardiovascular drug development with companies designing novel “biased” AT_1R ligands that only activate

β arrestins while retaining G-protein-blocking properties. SII has now been documented to not be completely β arrestin-“biased”, as it can activate some G-protein types (e.g. G_s , G_i) from the AT_1R ^[9]. Nevertheless, studies have shown that AT_1R -elicited β arrestin-dependent signaling in cardiac myocytes leads to cardiomyocyte proliferation without hypertrophy, which is $G_{q/11}$ protein signaling-dependent, and may even result in positive inotropy and lusitropy depending on which GRK isoform is involved (the so-called receptor “bar-coding” by GRKs). Interestingly, which β arrestin is engaged is also crucial^[39,40]. Specifically, GRK2-dependent phosphorylation followed by β arrestin1 binding seems to reduce, whereas GRK6-dependent phosphorylation followed by β arrestin2 interaction seems to promote AT_1R -induced contractility in primary murine adult cardiomyocytes (**Figure 1**)^[39]. However, AT_1R -activated β arrestins have no effect on contractility in isolated Langendorff-perfused cardiac preparations^[41]. Furthermore, a recombinant $AT_{1A}R$ capable of only signaling through β arrestins inhibits myocardial apoptosis and fibrosis, and enhances cardiomyocyte hypertrophy, upon transgenic overexpression in mouse hearts^[42]. Interestingly, a β arrestin2-“biased” ligand at the AT_1R is very beneficial in mice suffering from dilated cardiomyopathy as it prevents maladaptive signaling and improves myofilament Ca^{2+} sensitivity^[43]. Thus, cardiac AT_1R promotes hypertrophy and cardiomyocyte proliferation via the classic G_q protein/phospholipase C- β signaling pathway, which is inhibited by the β arrestins (classic AT_1R desensitization), and, at the same time, β arrestin2 (but not β arrestin1) can increase cardiac function via its cardiac AT_1R -dependent signaling (**Figure 1**).

Based on the above studies, several SII-derivative peptides have been synthesized and tested in animal models of ADHF with promising initial results^[44,45]. Unfortunately, these compounds failed in large phase III clinical trials (BLAST-AHF, ClinicalTrials.gov number, NCT01966601). There are probably several reasons for this. First, findings in animal models do not always translate into humans. Second, the compounds might have not been completely β arrestin-“biased” (i.e. maybe they had some weak, residual activity towards certain G-proteins). One intriguing possibility is that, due to the significantly lower abundance of the cardioprotective β arrestin2, compared to the cardio-toxic

β arrestin1, in human cardiomyocytes^[22], these β arrestin-“biased” compounds stimulated, in reality, β arrestin1 instead of β arrestin2 in the patients` hearts and that`s why their clinical outcomes were not the desired ones. Finally, it is very likely that these compounds stimulated the AT₁R only in cardiac fibroblasts, which would preclude any clinical benefit for ADHF patients. In fact, both β arrestins have been shown to regulate human cardiac fibroblast differentiation and to mediate collagen synthesis in human failing left ventricle-derived fibroblasts, thereby promoting the adverse remodeling process of cardiac fibrosis^[46].

Notably, β arrestins have been reported to mediate signaling by the mechanical stretch-activated (unliganded) cardiac AT₁R^[47], which has been suggested to underlie one of the fundamental laws of cardiac physiology, the Frank-Starling mechanism of cardiac contractility^[48]. Although intriguing, this finding is very difficult to interpret, given that the Frank-Starling mechanism is hemodynamically/biomechanically governed rather than dependent on hormonal receptors/effects. Besides, if it was mediated by a cardiac GPCR, then that receptor would definitely be the β_1 AR, the by far most abundant GPCR (and at least 15-fold more abundant than the AT₁R) in cardiomyocytes^[16].

Finally, β arrestin-mediated signaling by the AT₁R that can regulate cardiac function occurs in the adrenal cortex, as well. Specifically, the AT₁R promotes the adrenocortical production of aldosterone, a cardio-toxic hormone elevated in chronic human heart failure, via both G_{q/11}-proteins and β arrestin1^[35,49-51]. In fact, adrenal β arrestin1 is essential for aldosterone production, since, in mice lacking the β arrestin1 gene, circulating aldosterone levels do not increase even in the presence of MI ^[18]. Interestingly, the prototypic AT₁R antagonist (ARB, angiotensin receptor blocker) losartan is a relatively G protein-“biased” antagonist, which means it cannot suppress β arrestin1-dependent aldosterone production^[51-54]. In contrast, candesartan and valsartan are potent β arrestin-inverse agonists at the adrenal AT₁R and very effective at suppressing aldosterone in vitro and in vivo^[52,53]. These differences among ARBs, which are all orthosteric antagonists, in their potency at blocking AT₁R-activated β arrestin signaling may reflect their differential abilities to suppress β arrestin signaling by the unliganded

(i.e. constitutively active) AT₁R^[47,55]. In other words, the ARBs seem to be inverse agonists not only for G-proteins but also for β arrestins at the AT₁R.

THERAPEUTIC IMPLICATIONS IN HEART FAILURE

It becomes clear from the above that the signaling effects of the two β arrestins in the heart are not just different but actually diametrically opposite. This is true for several other mammalian organ systems and tissue types^[56-58] and is completely corroborated by molecular, biophysical, crystallographic, and proteomic studies^[59-62]. It also makes sense from the evolutionary point of view, as functional redundancy of proteins is usually not favored by natural selection. Only during embryonic development might the two β arrestins be able to compensate for each other, since single β arrestin-knockout mice (either β arrestin1- or β arrestin2-knockouts) are viable and breed normally but the double β arrestin-knockout mouse is embryonic-lethal^[63].

Regarding the myocardium, all the functional studies on β arrestins done so far are in relation to either β ARs or the AT₁R. Studies on β arrestin signaling from other cardiac GPCRs in cardiac cells are lacking. Based on the available data for β_1 AR, β_2 AR, or AT₁R signaling through β arrestins in cardiac cells, it can be safely concluded that β arrestin1 is the arrestin responsible for the classic desensitization of the cAMP-dependent pro-contractile signaling of the β_1 AR. This quite simply means that β arrestin1 exerts overall negative inotropy and is responsible (together with the elevated in human heart failure cardiac GRK2) for the diminished inotropic and adrenergic reserves of the failing human heart. In addition, β arrestin1 promotes cardiac apoptosis, inflammation, and accelerates cardiac adverse remodeling post-MI. In direct contrast, β arrestin2 promotes β_1 AR-mediated cardiac contractility both directly and indirectly. Directly, via augmentation of SERCA2a activity, and indirectly, by leaving the β_1 AR's cAMP-dependent pro-contractile signaling intact (i.e. no desensitization). On the other hand, it inhibits apoptosis & inflammation, and overall attenuates cardiac adverse remodeling post-MI, via stimulation of a variety of molecular pathways, such as EGFR transactivation and NF κ B inhibition, which β arrestin1 does not activate. Induction of ERK and of other

mitogenic/proliferative molecular signaling pathways in cardiomyocytes play auxiliary roles in β arrestin2's reverse remodeling effects, as well. Of note, the same functional distinction between the two cardiac β arrestins (i.e. β arrestin1 being detrimental, β arrestin2 being beneficial) applies to cardiac AT₁R signaling, too. Regardless of how small the contribution of this GPCR to the overall contractile function of the cardiac myocyte is, β arrestin2 again appears to promote contractility and cardiomyocyte survival in response to AT₁R activation. Conversely, β arrestin1 (again in conjunction with GRK2) opposes the AT₁R-dependent pro-contractile signaling in cardiac myocytes. However, β arrestin1 might exert an indirect beneficial effect in the hypertrophic heart by desensitizing (reducing) the pro-hypertrophic signaling of the cardiac AT₁R through G_{q/11}-proteins. In conclusion, based on their observed effects on the signaling of both β_1 ARs and AT₁Rs in cardiac myocytes, documented either directly (in β arrestin-knockout mice) or indirectly (with the use of "biased" receptor ligands), cardiac β arrestin2 stimulation and/or β arrestin1 inhibition might be valid therapeutic strategies in human heart failure. By the way, it is interesting to note here that probably the exact opposite is the case in the systemic vasculature. In vascular smooth muscle cells, β arrestin1 appears beneficial in terms of vasodilation and attenuation of hyperplasia and β arrestin2 seems to promote hypertrophy/hyperplasia^[58]. This should not come as a surprise at all, given the different cellular machineries and GPCR types involved in β arrestin signaling between cardiomyocytes (mainly β_1 AR) and vascular smooth muscle cells (mainly AT₁R and, to a lesser extent, β_2 AR). Besides, this is exactly what happens with the major second messenger cAMP: stimulated by the β_1 AR, it is pro-contractile in cardiomyocytes, but stimulated by the β_2 AR, it is pro-dilatory in vascular smooth muscle.

CONCLUSIONS/FUTURE PERSPECTIVES

In the adult myocardium, the actions of β arrestin1 are detrimental, since they result in depletion of the functional and adrenergic reserves of the heart. In contrast, β arrestin2 is beneficial, since it can increase both of these cardiac reserves or at least preserve them in the face of a cardiac insult/damage, such as an MI. Thus, from the therapeutic standpoint,

cardiac β arrestin1 blockade or β arrestin2 stimulation should be pursued for heart disease treatment. Of course, there are at least three very important questions awaiting answer in future studies in order to fully validate these strategies as therapeutic options for human heart failure. First, the effects of the two β arrestins on the signaling of other important cardiac GPCRs in the heart, i.e. beyond the β ARs and the AT_1R , need to be elucidated. The second issue to resolve is what the effects of the two β arrestins in other tissues/components of the cardiovascular system are, e.g. vasculature, platelets, adrenals, etc. This is particularly important if the pharmacological targeting of the β arrestins with systemically administered agents is being explored. For instance, β arrestin2 is beneficial in the heart, in platelets, and in vascular endothelium but might be detrimental in vascular smooth muscle^[58]. Its exact effects in all of these tissues need to be thoroughly investigated and determined, if a drug that stimulates this β arrestin isoform is to be designed and developed. Finally, the third and therapeutically salient unanswered question pertains to the expression levels of the cardiac β arrestins in the failing human heart. Although β arrestin2 protein is significantly under-expressed, compared to β arrestin1, in the non-failing human adult myocardium^[22,64], which makes cardiac-specific β arrestin2 gene transfer an enticing approach for heart failure treatment, its protein levels (and if they change) in human heart failure remain unknown. However, given that it is significantly expressed at the mRNA level, and in fact at levels comparable to those of β arrestin1 mRNA, in the failing human heart^[64], it is quite plausible that it might be upregulated at the protein level, similarly to GRK2, as a homeostatic mechanism of the failing human myocardium to confer cardioprotection. The only study done to date investigating β arrestin expression in failing human hearts is quite old (was published almost 25 years ago) and could not detect any changes in the protein levels of either β arrestin^[64]. Nevertheless, although β arrestin1 protein probably does not change in human heart failure, because it is already highly expressed in normal, healthy human hearts, that study failed to detect any β arrestin2 protein at all, even in normal healthy human hearts, probably due to technical deficiencies of the antibody it used^[64]. Therefore, it is rather inconclusive with regard to cardiac β arrestin2 protein expression in humans

and whether cardiac β arrestin2 protein changes in human heart failure remains an open question. In fact, a much more recent study done in human cardiac fibroblasts isolated from left ventricles of heart failure patients hinted at β arrestin2 protein being upregulated in human failing hearts^[46]. In any case, more studies are certainly warranted, especially in human cardiac specimens, to unravel the full spectrum of physiological (and pathophysiological) actions of the two cardiac β arrestins, beginning with the answering of the three outstanding questions mentioned above. Only then will the true potential of individual cardiac β arrestin isoform targeting for heart failure therapy be revealed, so that the pharmaceutical industry can begin its realization.

REFERENCES

1. **Desimine VL**, McCrink KA, Parker BM, Wertz SL, Maning J, Lymperopoulos A. Biased Agonism/Antagonism of Cardiovascular GPCRs for Heart Failure Therapy. *Int Rev Cell Mol Biol* 2018; **339**: 41-61 [PMID: 29776604 DOI: 10.1016/bs.ircmb.2018.02.007]
2. **Hilger D**, Masureel M, Kobilka BK. Structure and dynamics of GPCR signaling complexes. *Nat Struct Mol Biol* 2018; **25**: 4-12 [PMID: 29323277 DOI: 10.1038/s41594-017-0011-7]
3. **Peterson YK**, Luttrell LM. The Diverse Roles of Arrestin Scaffolds in G Protein-Coupled Receptor Signaling. *Pharmacol Rev* 2017; **69**: 256-229 [PMID: 28626043 DOI: 10.1124/pr.116.013367]
4. **Lymperopoulos A**, Rengo G, Koch WJ. Adrenergic Nervous System in Heart Failure: Pathophysiology and Therapy. *Circ Res* 2013; **113**: 739-753 [PMID: 23989716 DOI: 10.1161/CIRCRESAHA.113.300308]
5. **Lymperopoulos A**, Garcia D, Walklett K. Pharmacogenetics of cardiac inotropy. *Pharmacogenomics* 2014; **15**: 1807-1821 [PMID: 25493572 DOI: 10.2217/pgs.14.120]
6. **Siryk-Bathgate A**, Dabul S, Lymperopoulos A. Current and future G protein-coupled receptor signaling targets for heart failure therapy. *Drug Des Devel Ther* 2013; **7**: 1209-1222 [PMID: 24143078 DOI: 10.2147/DDDT.S35905]

7. **Capote LA**, Mendez Perez R, Lymperopoulos A. GPCR signaling and cardiac function. *Eur J Pharmacol* 2015; **763**: 143-148 [PMID: 25981298 DOI: 10.1016/j.ejphar.2015.05.019]
8. **Lymperopoulos A**, Bathgate A. Arrestins in the cardiovascular system. *Prog Mol Biol Transl Sci* 2013; **118**: 297-334 [PMID: 23764059 DOI: 10.1016/B978-0-12-394440-5.00012-7]
9. **Whalen EJ**, Rajagopal S, Lefkowitz RJ. Therapeutic potential of β -arrestin- and G protein-biased agonists. *Trends Mol Med* 2011; **17**: 126-139 [PMID: 21183406 DOI: 10.1016/j.molmed.2010.11.004]
10. **Ranjan R**, Dwivedi H, Baidya M, Kumar M, Shukla AK. Novel Structural Insights into GPCR- β -Arrestin Interaction and Signaling. *Trends Cell Biol* 2017; **27**: 851-862 [PMID: 28651823 DOI: 10.1016/j.tcb.2017.05.008]
11. **Eichel K**, Jullié D, Barsi-Rhyne B, Latorraca NR, Masureel M, Sibarita JB, Dror RO, von Zastrow M. Catalytic activation of β -arrestin by GPCRs. *Nature* 2018; **557**: 381-386 [PMID: 29720660 DOI: 10.1038/s41586-018-0079-1]
12. **Grundmann M**, Merten N, Malfacini D, Inoue A, Preis P, Simon K, Rüttiger N, Ziegler N, Benkel T, Schmitt NK, Ishida S, Müller I, Reher R, Kawakami K, Inoue A, Rick U, Köhl T, Imhof D, Aoki J, König GM, Hoffmann C, Gomeza J, Wess J, Kostenis E. Lack of beta-arrestin signaling in the absence of active G proteins. *Nat Commun* 2018; **9**: 341 [PMID: 29362459 DOI: 10.1038/s41467-017-02661-3]
13. **Luttrell LM**, Wang J, Plouffe B, Smith JS, Yamani L, Kaur S, Jean-Charles PY, Gauthier C, Lee MH, Pani B, Kim J, Ahn S, Rajagopal S, Reiter E, Bouvier M, Shenoy SK, Laporte SA, Rockman HA, Lefkowitz RJ. Manifold roles of β -arrestins in GPCR signaling elucidated with siRNA and CRISPR/Cas9. *Sci Signal* 2018; **11**: 549 [PMID: 30254056 DOI: 10.1126/scisignal.aat7650]
14. **Weis WI**, Kobilka BK. The Molecular Basis of G Protein-Coupled Receptor Activation. *Annu Rev Biochem* 2018; **87**: 897-919 [PMID: 29925258 DOI: 10.1146/annurev-biochem-060614-033910]

15. **Ma L**, Pei G. Beta-arrestin signaling and regulation of transcription. *J Cell Sci* 2007; **120**: 213–218 [PMID: 17215450 DOI: 10.1242/jcs.03338]
16. **Yoshikawa T**, Port JD, Asano K, Chidiak P, Bouvier M, Dutcher D, Roden RL, Minobe W, Tremmel KD, Bristow MR. Cardiac adrenergic receptor effects of carvedilol. *Eur Heart J* 1996; **17**: 8-16 [PMID: 8733065 DOI: 10.1093/eurheartj/17.suppl_B.8]
17. **Conner DA**, Mathier MA, Mortensen RM, Christe M, Vatner SF, Seidman CE, Seidman JG. beta-Arrestin1 knockout mice appear normal but demonstrate altered cardiac responses to beta-adrenergic stimulation. *Circ Res* 1997; **81**: 1021-1026 [PMID: 9400383 DOI: 10.1161/res.81.6.1021]
18. **Bathgate-Siryk A**, Dabul S, Pandya K, Walklett K, Rengo G, Cannavo A, De Lucia C, Liccardo D, Gao E, Leosco D, Koch WJ, Lymperopoulos A. Negative impact of β -arrestin-1 on post-myocardial infarction heart failure via cardiac and adrenal-dependent neurohormonal mechanisms. *Hypertension* 2014; **63**: 404-412 [PMID: 24218435 DOI: 10.1161/HYPERTENSIONAHA.113.02043]
19. **Watari K**, Nakaya M, Nishida M, Kim KM, Kurose H. β -arrestin2 in infiltrated macrophages inhibits excessive inflammation after myocardial infarction. *PLoS One* 2013; **8**: e68351 [PMID: 23861891 DOI: 10.1371/journal.pone.0068351]
20. **McCrink KA**, Maning J, Vu A, Jafferjee M, Marrero C, Brill A, Bathgate-Siryk A, Dabul S, Koch WJ, Lymperopoulos A. β -Arrestin2 Improves Post-Myocardial Infarction Heart Failure via Sarco(endo)plasmic Reticulum Ca^{2+} -ATPase-Dependent Positive Inotropy in Cardiomyocytes. *Hypertension* 2017; **70**: 972-981 [PMID: 28874462 DOI: 10.1161/HYPERTENSIONAHA.117.09817]
21. **Kho C**, Lee A, Jeong D, Oh JG, Chaanine AH, Kizana E, Park WJ, Hajjar RJ. SUMO1-dependent modulation of SERCA2a in heart failure. *Nature* 2011; **477**: 601-605 [PMID: 21900893 DOI: 10.1038/nature10407]
22. **McCrink KA**, Maning J, Vu A, Jafferjee M, Marrero C, Brill A, Bathgate-Siryk A, Dabul S, Koch WJ, Lymperopoulos A. Cardiac β arrestin2 Improves Contractility

- and Adverse Remodeling in Heart Failure, But Is Underexpressed in Humans. *J Am Coll Cardiol* 2017; **70**: 2948-2949 [PMID: 29216991 DOI: 10.1016/j.jacc.2017.10.008]
23. **Noma T**, Lemaire A, Naga Prasad SV, Barki-Harrington L, Tilley DG, Chen J, Le Corvoisier P, Violin JD, Wei H, Lefkowitz RJ, Rockman HA. Beta-arrestin-mediated beta1-adrenergic receptor transactivation of the EGFR confers cardioprotection. *J Clin Invest* 2007; **117**: 2445-2458 [PMID: 17786238 DOI: 10.1172/JCI31901]
 24. **Kim IM**, Tilley DG, Chen J, Salazar NC, Whalen EJ, Violin JD, Rockman HA. Beta-blockers alprenolol and carvedilol stimulate beta-arrestin-mediated EGFR transactivation. *Proc Natl Acad Sci U S A* 2008; **105**: 14555-14560 [PMID: 18787115 DOI: 10.1073/pnas.0804745105]
 25. **Wisler JW**, DeWire SM, Whalen EJ, Violin JD, Drake MT, Ahn S, Shenoy SK, Lefkowitz RJ. A unique mechanism of beta-blocker action: carvedilol stimulates beta-arrestin signaling. *Proc Natl Acad Sci U S A* 2007; **104**: 16657-16662 [PMID: 17925438 DOI: 10.1073/pnas.0707936104]
 26. **Kaur K**, Parra S, Chen R, Charbeneau RA, Wade SM, Jay PY, Neubig RR. Gai2 signaling: friend or foe in cardiac injury and heart failure? *Naunyn Schmiedeberg Arch Pharmacol* 2012; **385**: 443-453 [PMID: 22411356 DOI: 10.1007/s00210-011-0705-z]
 27. **Carr R 3rd**, Schilling J, Song J, Carter RL, Du Y, Yoo SM, Traynham CJ, Koch WJ, Cheung JY, Tilley DG, Benovic JL. β -arrestin-biased signaling through the β 2-adrenergic receptor promotes cardiomyocyte contraction. *Proc Natl Acad Sci U S A* 2016; **113**: E4107-E4116 [PMID: 27354517 DOI: 10.1073/pnas.1606267113]
 28. **Littmann T**, Göttle M, Reinartz MT, Kälble S, Wainer IW, Ozawa T, Seifert R. Recruitment of β -arrestin 1 and 2 to the β 2-adrenoceptor: analysis of 65 ligands. *J Pharmacol Exp Ther* 2015; **355**: 183-190 [PMID: 26306764 DOI: 10.1124/jpet.115.227959]
 29. **Shukla AK**, Westfield GH, Xiao K, Reis RI, Huang LY, Tripathi-Shukla P, Qian J, Li S, Blanc A, Oleskie AN, Dosey AM, Su M, Liang CR, Gu LL, Shan JM, Chen X, Hanna R, Choi M, Yao XJ, Klink BU, Kahsai AW, Sidhu SS, Koide S, Penczek PA, Kossiakoff

- AA, Woods VL Jr, Kobilka BK, Skiniotis G, Lefkowitz RJ. Visualization of arrestin recruitment by a G-protein-coupled receptor. *Nature* 2014; **512**: 218-222 [PMID: 25043026 DOI: 10.1038/nature13430]
30. **O'Hayre M**, Eichel K, Avino S, Zhao X, Steffen DJ, Feng X, Kawakami K, Aoki J, Messer K, Sunahara R, Inoue A, von Zastrow M, Gutkind JS. Genetic evidence that β -arrestins are dispensable for the initiation of β_2 -adrenergic receptor signaling to ERK. *Sci Signal* 2017; **10**: 484 [PMID: 28634209 DOI:10.1126/scisignal.aal3395]
 31. **Wang J**, Hanada K, Staus DP, Makara MA, Dahal GR, Chen Q, Ahles A, Engelhardt S, Rockman HA. Gai is required for carvedilol-induced β_1 adrenergic receptor β -arrestin biased signaling. *Nat Commun* 2017; **8**: 1706 [PMID: 29167435 DOI: 10.1038/s41467-017-01855-z]
 32. **McCrink KA**, Brill A, Jafferjee M, Valero TR, Marrero C, Rodriguez MM, Hale GM, Lymperopoulos A. β_1 -adrenoceptor Arg389Gly polymorphism confers differential β -arrestin-binding tropism in cardiac myocytes. *Pharmacogenomics* 2016; **17**: 1611-1620 [PMID: 27643874 DOI: 10.2217/pgs-2016-0094]
 33. **Liggett SB**, Mialet-Perez J, Thaneemit-Chen S, Weber SA, Greene SM, Hodne D, Nelson B, Morrison J, Domanski MJ, Wagoner LE, Abraham WT, Anderson JL, Carlquist JF, Krause-Steinrauf HJ, Lazzeroni LC, Port JD, Lavori PW, Bristow MR. A polymorphism within a conserved beta(1)-adrenergic receptor motif alters cardiac function and beta-blocker response in human heart failure. *Proc Natl Acad Sci U S A* 2006; **103**: 11288-11293 [PMID: 16844790 DOI: 10.1073/pnas.0509937103]
 34. **Lymperopoulos A**, Desimine VL, McCrink KA, Maning J, Wertz SL, Markan U, Pasupuleti S, Brill A, Parker BM. Positive cardiac inotropy by carvedilol via unique beta-arrestin2-dependent SERCA2a stimulation. *Eur Heart J* 2018; **39**: Suppl_1, 1 [Conference abstract DOI: 10.1093/eurheartj/ehy563.3144]
 35. **Maning J**, Negussie S, Clark MA, Lymperopoulos A. Biased agonism/antagonism at the AngII-AT1 receptor: Implications for adrenal aldosterone production and cardiovascular therapy. *Pharmacol Res* 2017; **125**: 14-20 [PMID: 28511989 DOI: 10.1016/j.phrs.2017.05.009]

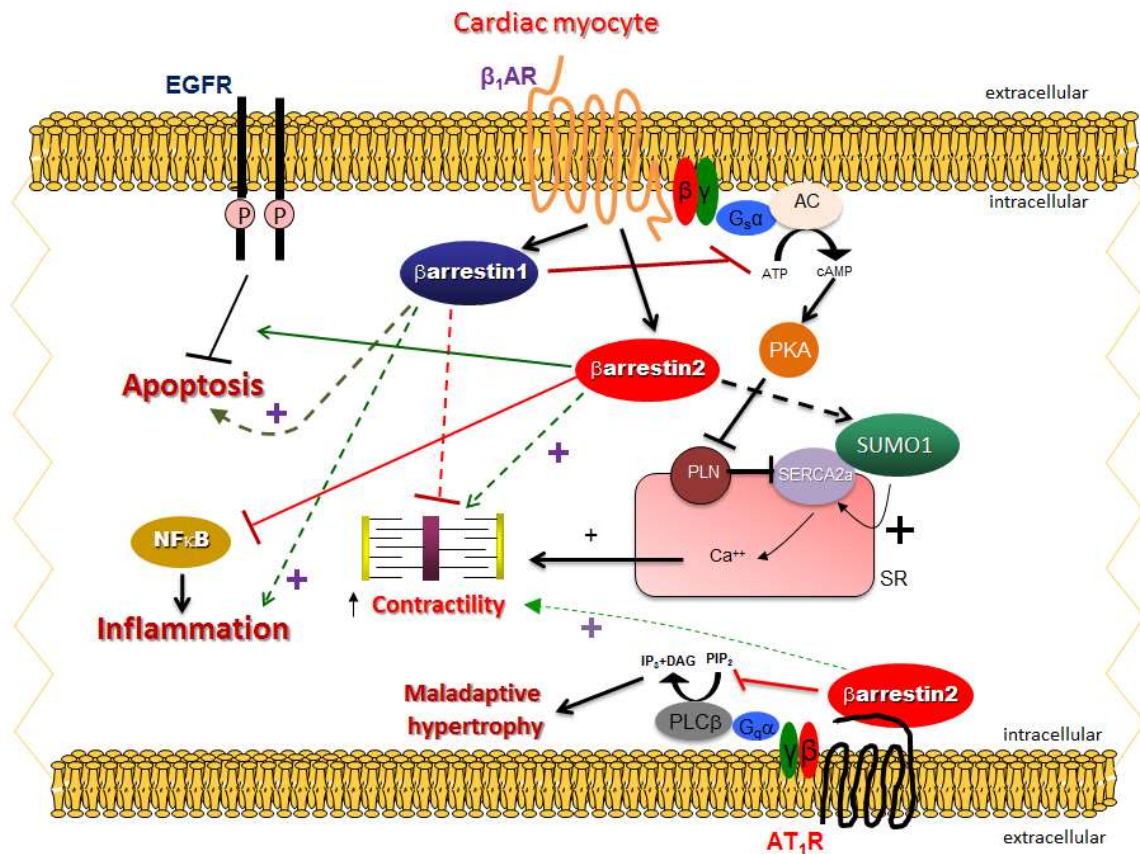
36. **Lymperopoulos A.** Beta-arrestin biased agonism/antagonism at cardiovascular seven transmembrane-spanning receptors. *Curr Pharm Des* 2012; **18**: 192-198 [PMID: 22229558 DOI: 10.2174/138161212799040475]
37. **Thomas WG,** Thekkumkara TJ, Baker KM. Cardiac effects of AII. AT1A receptor signaling, desensitization, and internalization. *Adv Exp Med Biol* 1996; **396**: 59-69 [PMID: 8726686 DOI: 10.1007/978-1-4899-1376-0_7]
38. **Saulière A,** Bellot M, Paris H, Denis C, Finana F, Hansen JT, Altié MF, Seguelas MH, Pathak A, Hansen JL, Sénard JM, Galés C. Saulière A¹, Bellot M, Paris H, Denis C, Finana F, Hansen JT, Altié MF, Seguelas MH, Pathak A, Hansen JL, Sénard JM, Galés C. Deciphering biased-agonism complexity reveals a new active AT1 receptor entity. *Nat Chem Biol* 2012; **8**: 622-630 [PMID: 22634635 DOI: 10.1038/nchembio.961]
39. **Rajagopal K,** Whalen EJ, Violin JD, Stiber JA, Rosenberg PB, Premont RT, Coffman TM, Rockman HA, Lefkowitz RJ. Beta-arrestin2-mediated inotropic effects of the angiotensin II type 1A receptor in isolated cardiac myocytes. *Proc Natl Acad Sci U S A* 2006; **103**: 16284-16289 [PMID: 17060617 DOI: 10.1073/pnas.0607583103]
40. **Zidar DA,** Violin JD, Whalen EJ, Lefkowitz RJ. Selective engagement of G protein coupled receptor kinases (GRKs) encodes distinct functions of biased ligands. *Proc Natl Acad Sci U S A* 2009; **106**: 9649-9654 [PMID: 19497875 DOI: 10.1073/pnas.0904361106]
41. **Aplin M,** Christensen GL, Schneider M, Heydorn A, Gammeltoft S, Kjølbye AL, Sheikh SP, Hansen JL. Differential extracellular signal-regulated kinases 1 and 2 activation by the angiotensin type 1 receptor supports distinct phenotypes of cardiac myocytes. *Basic Clin Pharmacol Toxicol* 2007; **100**: 296-301 [PMID: 17448114 DOI: 10.1111/j.1742-7843.2007.00064.x]
42. **Zhai P,** Yamamoto M, Galeotti J, Liu J, Masurekar M, Thaisz J, Irie K, Holle E, Yu X, Kupersmidt S, Roden DM, Wagner T, Yatani A, Vatner DE, Vatner SF, Sadoshima J. Cardiac-specific overexpression of AT1 receptor mutant lacking G alpha q/G alpha i coupling causes hypertrophy and bradycardia in transgenic mice. *J Clin Invest* 2005; **115**: 3045-3056 [PMID: 16276415 DOI: 10.1172/JCI25330]

43. **Ryba DM**, Li J, Cowan CL, Russell B, Wolska BM, Solaro RJ. Long-Term Biased β -Arrestin Signaling Improves Cardiac Structure and Function in Dilated Cardiomyopathy. *Circulation* 2017; **135**: 1056-1070 [PMID: 28104714 DOI: 10.1161/CIRCULATIONAHA.116.024482]
44. **Violin JD**, DeWire SM, Yamashita D, Rominger DH, Nguyen L, Schiller K, Whalen EJ, Gowen M, Lark MW. Selectively engaging β -arrestins at the angiotensin II type 1 receptor reduces blood pressure and increases cardiac performance. *J Pharmacol Exp Ther* 2010; **335**: 572-579 [PMID: 20801892 DOI: 10.1124/jpet.110.173005]
45. **Boerrigter G**, Soergel DG, Violin JD, Lark MW, Burnett JC. TRV120027, a novel beta-arrestin biased ligand at the angiotensin II type I receptor, unloads the heart and maintains renal function when added to furosemide in experimental heart failure. *Circ Heart Fail* 2012; **5**: 627-634 [PMID: 22891045 DOI: 10.1161/CIRCHEARTFAILURE.112.969220]
46. **Li J**, Philip JL, Xu X, Theccanat T, Abdur Razzaque M, Akhter SA. β -Arrestins regulate human cardiac fibroblast transformation and collagen synthesis in adverse ventricular remodeling. *J Mol Cell Cardiol* 2014; **76**: 73-83 [PMID: 25134464 DOI: 10.1016/j.yjmcc.2014.08.006]
47. **Rakesh K**, Yoo B, Kim IM, Salazar N, Kim KS, Rockman HA. beta-Arrestin-biased agonism of the angiotensin receptor induced by mechanical stress. *Sci Signal* 2010; **3**: ra46 [PMID: 20530803 DOI: 10.1126/scisignal.2000769]
48. **Abraham DM**, Davis RT 3rd, Warren CM, Mao L, Wolska BM, Solaro RJ, Rockman HA. β -Arrestin mediates the Frank-Starling mechanism of cardiac contractility. *Proc Natl Acad Sci U S A* 2016; **113**: 14426-14431 [PMID: 27911784 DOI: 10.1073/pnas.1609308113]
49. **Lymperopoulos A**, Aukszi B. Angiotensin receptor blocker drugs and inhibition of adrenal beta-arrestin-1-dependent aldosterone production: Implications for heart failure therapy. *World J Cardiol* 2017; **9**: 200-206 [PMID: 28400916 DOI: 10.4330/wjc.v9.i3.200]

50. **Lymperopoulos A**, Rengo G, Zincarelli C, Kim J, Soltys S, Koch WJ. An adrenal beta-arrestin 1-mediated signaling pathway underlies angiotensin II-induced aldosterone production in vitro and in vivo. *Proc Natl Acad Sci U S A* 2009; **106**: 5825-5830 [PMID: 19289825 DOI: 10.1073/pnas.0811706106]
51. **Lymperopoulos A**, Rengo G, Zincarelli C, Kim J, Koch WJ. Adrenal beta-arrestin 1 inhibition in vivo attenuates post-myocardial infarction progression to heart failure and adverse remodeling via reduction of circulating aldosterone levels. *J Am Coll Cardiol* 2011; **57**: 356-365 [PMID: 21232674 DOI: 10.1016/j.jacc.2010.08.635]
52. **Lymperopoulos A**, Sturchler E, Bathgate-Siryk A, Dabul S, Garcia D, Walklett K, Rengo G, McDonald P, Koch WJ. Different potencies of angiotensin receptor blockers at suppressing adrenal β -Arrestin1-dependent post-myocardial infarction hyperaldosteronism. *J Am Coll Cardiol* 2014; **64**: 2805-2806 [PMID: 25541135 DOI: 10.1016/j.jacc.2014.09.070]
53. **Dabul S**, Bathgate-Siryk A, Valero TR, Jafferjee M, Sturchler E, McDonald P, Koch WJ, Lymperopoulos A. Suppression of adrenal β arrestin1-dependent aldosterone production by ARBs: head-to-head comparison. *Sci Rep* 2015; **5**: 8116 [PMID: 25631300 DOI: 10.1038/srep08116]
54. **Valero TR**, Sturchler E, Jafferjee M, Rengo G, Magafa V, Cordopatis P, McDonald P, Koch WJ, Lymperopoulos A. Structure-activity relationship study of angiotensin II analogs in terms of β -arrestin-dependent signaling to aldosterone production. *Pharmacol Res Perspect* 2016; **4**: e00226 [PMID: 27069636 DOI: 10.1002/prp2.226]
55. **Tóth AD**, Prokop S, Gyombolai P, Várnai P, Balla A, Gurevich VV, Hunyady L, Turu G. Heterologous phosphorylation-induced formation of a stability lock permits regulation of inactive receptors by β -arrestins. *J Biol Chem* 2018; **293**: 876-892 [PMID: 29146594 DOI: 10.1074/jbc.M117.813139]
56. **Srivastava A**, Gupta B, Gupta C, Shukla AK. Emerging Functional Divergence of β -Arrestin Isoforms in GPCR Function. *Trends Endocrinol Metab* 2015; **26**: 628-642 [PMID: 26471844 DOI: 10.1016/j.tem.2015.09.001]

57. **Lymperopoulos A**, Negussie S. β Arrestins in cardiac G protein-coupled receptor signaling and function: partners in crime or "good cop, bad cop"? *Int J Mol Sci* 2013; **14**: 24726-24741 [PMID: 24351844 DOI: 10.3390/ijms141224726]
58. **Lymperopoulos A**. Arrestins in the Cardiovascular System: An Update *Prog Mol Biol Transl Sci* 2018; *in press* [DOI: 10.1016/bs.pmbts.2018.07.003]
59. **Zhan X**, Gimenez LE, Gurevich VV, Spiller BW. Crystal structure of arrestin-3 reveals the basis of the difference in receptor binding between two non-visual subtypes. *J Mol Biol* 2011; **406**: 467-478 [PMID: 21215759 DOI: 10.1016/j.jmb.2010.12.034]
60. **Zhuo Y**, Vishnivetskiy SA, Zhan X, Gurevich VV, Klug CS. Identification of Receptor Binding-induced Conformational Changes in Non-visual Arrestins. *J Biol Chem* 2014; **289**: 20991-21002 [PMID: 24867953 DOI: 10.1074/jbc.M114.560680]
61. **Gurevich VV**, Gurevich EV. Extensive shape shifting underlies functional versatility of arrestins. *Curr Opin Cell Biol* 2014; **27**: 1-9 [PMID: 24680424 DOI: 10.1016/j.ceb.2013.10.007]
62. **Xiao K**, McClatchy DB, Shukla AK, Zhao Y, Chen M, Shenoy SK, Yates JR 3rd, Lefkowitz RJ. Functional specialization of beta-arrestin interactions revealed by proteomic analysis. *Proc Natl Acad Sci U S A* 2007; **104**: 12011-12016 [PMID: 17620599 DOI: 10.1073/pnas.0704849104]
63. **Luttrell LM**, Gesty-Palmer D. Beyond desensitization: physiological relevance of arrestin-dependent signaling. *Pharmacol Rev* 2010; **62**: 305-330 [PMID: 20427692 DOI: 10.1124/pr.109.002436]
64. **Ungerer M**, Parruti G, Böhm M, Puzicha M, DeBlasi A, Erdmann E, Lohse MJ. Expression of beta-arrestins and beta-adrenergic receptor kinases in the failing human heart. *Circ Res* 1994; **74**: 206-213 [PMID: 8293560 DOI: abs/10.1161/res.74.2.8293560]

Figure 1. The functional distinction between β arrestin1 and β arrestin2 in cardiac myocytes.



ATP: Adenosine triphosphate; P: Phosphorylation; SR: Sarcoplasmic reticulum; SUMO1: Small ubiquitin-like modifier protein-1; PLN: Phospholamban; PIP₂: Phosphatidylinositol 4,5-bisphosphate; IP₃: Inositol 1,4,5-trisphosphate; DAG: 1,2-Diacylglycerol. See text for details and for all other molecular acronym descriptions.