Department of Chemistry
Cumulative Examinations

March 23, 2019

You may choose to answer any exam from any area covered in the examination booklet. Each exam may contain multiple parts. You may answer more than one exam but each exam is scored separately and is treated as an individual examination result. Thus, answering parts of two exams with a score of 50% would not yield a 100% grade for this cumulative exam. Instead you would receive 50% on each examination attempted.

This booklet contains five examinations.

1) Analytical Cumulative Examination, Pages 1-2
2) Biochemistry Cumulative Examination, Pages 3-18
3) Inorganic Cumulative Examination, Pages 19-21
4) Organic Cumulative Examination, Pages 22-23
5) Physical Cumulative Examination, Page 24

On your examination booklet:

1) Print your student ID number.
2) Print the Exam Booklet number.
3) Print the question number you are answering.
4) Print the Exam Date.

Do not write your name anywhere on the examination booklet. Each exam will be scored anonymously. If you attempt more than one exam, you must use a separate examination booklet for each examination.

When you complete the examination, return the examination and your answer booklet to the proctor. Exam results will be posted on bulletin board #2B on the north side of the hall near BRWN 2124.
1) (10 points) (a) Briefly describe (1-2 sentences) what determines the resolution limit for optical microscopy, using an equation and a sketch.

(b) The Nobel Prize for Chemistry in 2014 was awarded for super-resolution microscopy. Briefly describe (1-2 sentences and a sketch) one of the methods for achieving super-resolution.

2) (20 points) In general, methods for achieving super-resolution utilize fluorescence microscopes.
(a) Sketch a diagram of a fluorescence microscope, labeling the beam path and any important parts.

(b) Calculate the percentage of photons collected by a 1 cm² detector located 2 cm from a point emitter, selecting reasonable values for quantities required.

(c) Sketch a Jablonski diagram for fluorescence and a reasonable graph of absorption and emission spectra for a fluorophore. Label the Stokes shift in both diagrams.

(d) A common fluorescence label in biological imaging is GFP, a protein for which the 2008 Nobel Prize was awarded. If a fluorophore with an excitation maximum in the green exhibits a 20 nm shift in its emission peak, calculate the corresponding difference in energy, making a reasonable choice for a green wavelength.

3) (10 points) The 1991 Nobel Prize and part of the 2002 Prize were given for the development of nuclear magnetic resonance spectroscopy methods for molecular structure determination.
(a) Briefly describe (1 sentence and a labeled diagram) the difference in energy that is important in NMR. Describe what this means in connection with a ‘300 MHz’ NMR instrument.

(b) Briefly describe (1-2 sentences) the chemical differences NMR is able to detect between protons. What units are used to graph the detected difference, and what does the unit mean in the context of part (a)?

4) (10 points) The 2007 Nobel Prize in Chemistry was awarded for studies of chemical processes on solid surfaces.
(a) Briefly explain, using a simple diagram and 2-3 sentences, the photoelectric effect and how it is related to X-Ray Photoelectron Spectroscopy (XPS).

(b) What information is learned about the sample in XPS?
5) (15 points) The 1986 Nobel Prize in Physics was awarded in part for the development of electron optics, which make transmission electron microscopy (TEM) possible.

(a) Briefly explain the difference in electron optics in relation to the optics in, say, a fluorescence microscope, and why the different design is necessary.

(b) Sketch a TEM instrument diagram, labeling relevant parts and the beam path. Briefly describe two key considerations related to the sample structure, in achieving reasonable contrast in a TEM image.

(c) The 2017 Nobel Prize in Chemistry was awarded for cryo-electron microscopy. Briefly describe (1-2 sentences) what types of samples this technique typically applies to, what the ‘cryo’ refers to, and why it is important for that type of sample.

6) (15 points) The other half of the 1986 Nobel prize was awarded for the development of scanning tunneling microscopy (STM).

(a) Sketch an STM tunneling junction, labeling important parts, distances, and potentials. Describe in 1-2 sentences how the feedback loop works.

(b) STM enables real-space imaging of objects such as fullerenes, which have a carbon cage structure. (The discovery of fullerenes was the topic of the 1996 Nobel Prize.) Provide a reasonable estimate of the diameter of C\textsubscript{60}, a common fullerene.

(c) Compare the typical lateral and vertical resolutions of STM and AFM.

7) (10 points) The development of the STM ultimately led to the development of the atomic force microscope (AFM).

(a) Sketch an AFM instrument diagram, labeling relevant parts including the sample and the beam path.

(b) Describe, using labeled sketches, two different imaging modes that are commonly employed.

8) (10 points) Several Nobel Prizes have related to structure determination by X-ray diffraction.

(a) Sketch the condition for constructive interference in Bragg diffraction, labeling important quantities.

(b) Provide an approximate wavelength for an X-ray, and a comparison with the length scale of structural features commonly measured by X-ray diffraction.
Biochemistry Cumulative Exam

Signal Transduction

March 23, 2019

General instructions: Part I is based on general knowledge. Part II is based on the attached manuscript. Your answers should be brief (3-6 sentences) and diagrams are accepted. All parts of a question must be answered for full credit, and any abbreviation used must be written out the first time it is used. Write all answers in the blue exam book.

1. a) Why must signaling be regulated in a temporal and a spatial fashion? (5 points)
   b) How might scaffolding proteins contribute to this process? (5 points)

2. Both heterotrimeric G protein Gα subunits and small GTPases, such as Ras, can be mutated in cancer. Often, these mutant proteins are unable to hydrolyze GTP. Explain how this type of mutation results in cancer. (10 points)

3. Many transmembrane signaling proteins are post-translationally modified by the addition of lipid groups. Loss of this modification can prevent normal signaling from occurring. Briefly describe why this loss of lipidation could change signaling. (10 points)

4. All signaling enzymes must be desensitized. What does this mean? (5 points)

5. ATP and GTP are essential for cell signaling to occur. Explain what ATP and GTP are used for during cell signaling. Assume the cell has sufficient NTPs for all biological processes essential for life. (10 points)

The following questions are based on the article “GPCR-G Protein-β-Arrestin Super-Complex Mediates Sustained G Protein Signaling.” A.R.B. Thompson et al/Cell 166, 907-919 (2016).

6. GPCR inactivation at the plasma membrane is proposed to require two steps. What are these steps and what are the proteins that are responsible for them? (5 points)

7. The β2 adrenergic receptor (β2AR), vasopressin-2 receptor (V2R), and a β2V2R receptor were used to study sustained cAMP production in cells. What was the rationale for choosing these receptors? (5 points)

8. What is the difference between an agonist and an antagonist for a receptor? (2 points)
9. The authors propose that the V_2R and the \( \beta_2 V_2R \) display internal signaling, based on the BRET experiments shown in Figure 2. Propose another method to demonstrate that these receptors are signaling from inside the cell. Your answer must include details about what type(s) of experiment(s) is/are being performed, what the data would look like, and how it would be interpreted. Your answer should also include controls. Diagrams are encouraged (20 points)

10. In Figure 3, the authors used fluorescently labeled \( \beta_2 V_2R, G_{\alpha_\delta}, \) and \( \beta Arr-2 \) to demonstrate the proteins are co-localized following receptor activation.

a) Would this result be expected to occur in normal cells? Why or why not? (8 points)

b) Line-scan analysis is often used in experiments to demonstrate where a labeled protein is located across the cell. Briefly describe where the proteins are located in the cell to explain the data shown in Figures 3B and 3C. (10 points)

c) Does this experiment prove the \( \beta_2 V_2R, G_{\alpha_\delta}, \) and \( \beta Arr2 \) directly interact with one another in cells? Why or why not? (10 points)
GPCR-G Protein-β-Arrestin Super-Complex Mediates Sustained G Protein Signaling

Graphical Abstract

Highlights
- Some GPCRs simultaneously interact with both G protein and β-arrestin (βarr)
- In these “megaplexes,” G protein binds to the receptor transmembrane core
- Concurrent with G protein coupling, βarr binds to the receptor C-terminal tail
- G protein activation within megaplexes occurs from internalized compartments

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In Brief
Megaplexes containing a GPCR simultaneously engaged with a G protein and β-arrestin sustain G protein signaling following internalization into endosomes.
G Protein-β-Arrestin Super-Complex Mediates Sustained G Protein Signaling

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SUMMARY

Classically, G protein-coupled receptor (GPCR) stimulation promotes G protein signaling at the plasma membrane, followed by rapid β-arrestin-mediated desensitization and receptor internalization into endosomes. However, it has been demonstrated that some GPCRs activate G proteins from within internalized cellular compartments, resulting in sustained signaling. We have used a variety of biochemical, biophysical, and cell-based methods to demonstrate the existence, functionality, and architecture of internalized receptor complexes composed of a single GPCR, β-arrestin, and G protein. These super-complexes or "megaplexes" more readily form at receptors that interact strongly with β-arrestins via a C-terminal tail containing clusters of serine/threonine phosphorylation sites. Single-particle electron microscopy analysis of negative-stained purified megaplexes reveals that a single receptor simultaneously binds through its core region with G protein and through its phosphorylated C-terminal tail with β-arrestin. The formation of such megaplexes provides a potential physical basis for the newly appreciated sustained G protein signaling from internalized GPCRs.

INTRODUCTION

G protein-coupled receptor (GPCR) signaling ensues when an agonist binds to and stabilizes an active receptor conformation. This agonist-bound GPCR, acting through its transmembrane core, promotes interaction with heterotrimeric G proteins (Gαβγ), thus stimulating guanine nucleotide exchange and separation of the Gα subunit from the Gβγ subunits (Gilman, 1987). G protein subunits then interact with a variety of effectors, such as enzymes and ion channels, to initiate downstream responses (Gilman, 1987; Pierce et al., 2002).

To terminate G protein signaling, cells have devised a specialized desensitization mechanism that includes phosphorylation of receptors by GPCR kinases (GKs) and subsequent recruitment of β-arrestins (βarrs) to the phosphorylated receptor (Moore et al., 2007). βarrs engage both the phosphorylated C-tail and the transmembrane core of the receptor. The latter interaction overlaps with the G protein-binding site and thus sterically hinders further G protein activation (Kang et al., 2015; Shukla et al., 2014; Szczepak et al., 2014). Additionally, βarr binding initiates receptor internalization by interaction with the endocytic machinery (e.g., clathrin, adaptin-2, etc.) (Goodman et al., 1998; Laporte et al., 1999). Depending on the strength of the GPCR-βarr interaction, the receptor may either undergo transient internalization, followed by recycling to the plasma membrane for weak interactions (class A GPCRs), or sustained internalization into endosomes for stronger interactions (class B GPCRs) (Oakley et al., 1999, 2000). Furthermore, βarrs themselves serve as an alternative signaling system by acting as adaptors and scaffolds to interact with numerous signaling molecules (Pierce et al., 2002).

Thus, our current understanding features G protein signaling originating at the cell surface, followed by rapid βarr-mediated quenching of G protein signaling, both by competition with G proteins for receptor interaction and by internalization of the receptors. However, recent findings have begun to challenge these paradigms. A number of GPCRs have been reported to engage in sustained G protein signaling, rather than being desensitized after initial agonist stimulation (Calebiro et al., 2009; Feinstein et al., 2013; Ferrandon et al., 2009; Irannejad et al., 2013; Mullershhausen et al., 2003). Interestingly, this newly appreciated sustained phase of G protein activation appears to be mediated by internalized receptors in endosomes, where they modulate effectors, such as adenylyl cyclase (Calebiro et al., 2009).
an equilibrium between production and degradation of cAMP. These cells were transiently transfected with the β2AR as a prototypical class A GPCR or the V2R as a prototypical class B GPCR. In addition, we used a modified version of the β2AR, the β2V2R, in which the β2AR C-terminal tail has been exchanged with the V2R C-terminal tail. The β2V2R maintains the pharmacological properties of the β2AR, but has significantly higher affinity for β2R than for β2AR, and this increase in affinity manifests predominantly as a change in the receptor internalization pattern from class A to B (Oakley et al., 1999, 2000). In addition, we have successfully purified stable and functional GPCR-β2R complexes using the β2V2R. Therefore, the β2V2R was used to study both the cellular and biophysical basis for sustained G protein signaling by internalized class B GPCR-β2R complexes.

Within the first 5 min of agonist challenge β2AR, β2V2R, and V2R all stimulated cAMP production to a similar extent. Beyond 5 min, the cAMP responses were attenuated to different levels among these receptors and most prominently for the wild-type β2AR (Figure 1A). In addition, the agonist-stimulated cAMP response was diminished slightly more at the β2V2R compared to the V2R (Figure 1A). These results suggest that class BGPCRs promote sustained G protein signaling to a greater degree than does a prototypical class A GPCR.

To investigate whether sustained G protein signaling of class B GPCRs arises from internalized compartments β2AR, β2V2R, or V2R-expressing cells were agonist-stimulated for 10 min to allow for internalization to occur. Then Gs signaling arising from only the cell membrane was inhibited by the addition of 10 μM of a membrane-impermeable antagonist (CGP-12177 for β2AR and β2V2R, or (dCH2)5,D-Tyr(Et)2,Val5,Arg5,des-Gly5)-vasopressin (H-3192) for V2R; Figure 1B) (Jard et al., 1986; Staehelin et al., 1983). To inhibit Gs signaling arising from both the cell membrane and the internalized compartments, 10 μM of a cell-permeable antagonist (ICI-118551 for β2AR and β2V2R or SR121463 for V2R; Figure 1B) was used (Morello et al., 2000; O'Donnell and Warnatt, 1988).

Under these conditions, for the β2AR, both antagonists quickly inhibited almost all Gs signaling (Figure 1C). However, for the β2V2R, only ICI-118551 fully blocked Gs signaling, whereas CGP-12177 only partially inhibited it (Figure 1C). These results indicate that a significant fraction of the β2V2R-stimulated cAMP originates from internalized compartments. In a similar fashion, the V2R-stimulated cAMP response was only partially antagonized when exposed to the cell-membrane-impermeable antagonist, H-3192, but fully antagonized following the addition of the cell-membrane-permeable antagonist, SR121463 (Figure 1C). Therefore, V2R-mediated Gs signaling at 10 min post-agonist-stimulation is, in part, due to internalized receptors. These results demonstrate that both the β2V2R and V2R stimulate Gs signaling from internalized compartments, whereas the β2AR does not seem to exhibit such behavior using this method.

**Monitoring Heterotrimeric Gs Activation at Internalized Compartments by Class A and Class B GPCRs Using Bioluminescence Resonance Energy Transfer Biosensors**

To confirm that the receptor-stimulated cAMP response from internalized compartments results from Gs activation, and
not from other signaling cascades, we directly monitored agonist-stimulated heterotrimeric Gs activation by bioluminescence resonance energy transfer (BRET), which detects the proximity between two proteins within a 10 nm range (Manallan and Bouvier, 2007). Following Gs activation, the Gαs subunit separates from the Gβγ subunits. This separation was detected following agonist challenge of β2AR, β2V2R, or V2R-expressing cells as a decrease in BRET between the functionally validated BRET pair RucII-117-Gαs and GFP10-Gy1 (Figures 2A, 2B, and S1). Such BRET-based assays have been developed as sensors of G protein activation (Galés et al., 2006).

To specifically monitor Gs activation/separation at internalized compartments, we developed an agonist washout protocol. In short, agonists were allowed to stimulate receptors for varying time intervals (0.5 to 14 min), followed by a washout of the agonists. Next, the cells were incubated for 20 min in agonist-free media, and responses were recorded (Figures 1B and 2C). Since washout only removes agonist from the extracellular environment, but not from the intracellular environment, which contains internalized receptors, the receptors insensitive to agonist washout must be activating the Gs from within internalized compartments (Figure 1B).

As shown in Figure 2C, the agonist washout protocol blunted Gs activation in β2AR, β2V2R, and V2R-expressing cells. However, by increasing the duration of stimulation prior to agonist washout, and thereby allowing more receptors to internalize, a substantially diminished signal reduction by agonist washout was observed (Figure 2C). This dampening in signal reduction was most prominent, and statistically significant, for β2V2R and V2R, which still maintained 54% and 64% of their Gs activity, respectively, when cells were pre-stimulated for 14 min followed by agonist washout, as compared to the unwashed control conditions (Figure 2C). For the β2AR, increasing the duration of stimulation prior to agonist washout did not result in a statistically

Figure 1. Sustained Gs Signaling from Internalized Compartments by β2AR, β2V2R, and V2R
(A) Real-time cAMP measurements, using ICUE2-expressing HEK293 cells, in response to agonist stimulation of β2AR (red), β2V2R (blue), and V2R (black). For β2AR and β2V2R, 1 μM ISO was used to stimulate cells. For V2R, 100 nM AVP was used to stimulate cells. Surface expression of all GPCRs was matched. Data represent the mean ± SE of N = 3 experiments and n ≥ 30 cells. Area under the curve (AUC) was used to calculate the total cAMP response for each GPCR, and one-way ANOVA was performed to determine statistical differences relative to β2AR (**p < 0.01; ***p < 0.0001) and β2V2R (#, p < 0.01). Responses.
(B) Schematic representation of the experimental design used to demonstrate sustained Gs activation and signaling from internalized GPCRs.
(C) Real-time cAMP measurements utilized to demonstrate intracellular Gs signaling by GPCRs. Agonist-stimulated cAMP responses (100 nM ISO for β2AR and β2V2R or 100 nM of desmopressin [DESMO] for V2R) was antagonized at 10 min by the addition of 10 μM of cell-membrane-impermeable antagonist (CGP-12217 for β2AR and β2V2R, or H-3192 for V2R; shown in blue). The impact of cell-membrane-impermeable antagonists was measured relative to total antagonism caused by cell-membrane-permeable antagonists (ICI-118551 for β2AR and β2V2R or SR141878 for V2R). Data represent the mean ± SE of N = 3 experiments and n ≥ 87 cells. AUC was used to calculate the total cAMP response for each GPCR after the respective treatments. One-way ANOVA was performed to determine statistical differences among the antagonists compared to DMSO (p < 0.05; ***p < 0.001; ****p < 0.0001) or compared to cell-membrane-impermeable antagonists (#, p < 0.05; ###, p < 0.0001).
See also Figure S1.
significant difference in remaining Gs activity when compared to 0.5 min pulse stimulation (Figure 2C). In this setup, the class B GPCRs, β₂V₂R and V₂R, seem to activate heterotrimeric Gs from internalized compartments, whereas the class A GPCR, β₂AR, does not to any significant degree.

**Co-localization of GPCR, βαrr, and Gαs in Endosomes by Confocal Microscopy**

Receptor internalization from the plasma membrane into endosomes is driven by formation of a GPCR-βαrr complex. Therefore, to investigate whether G protein may interact with GPCR-βαrr complexes in endosomes, we began by tracking the cellular localization of functionally validated N-terminal SNAP-tagged GPCRs (SNAP-β₂AR, SNAP-β₂V₂R, or SNAP-V₂R) pre-labeled with SNAP-Surface 649 fluorescence substrate, mStrawberry-βαrr2, and mEmerald-67-Gαs expressed in HEK293 cells following agonist treatment using confocal microscopy (Figure S1).

Prior to agonist stimulation, SNAP-tagged receptors (β₂AR, β₂V₂R, or V₂R) and mEmerald-67-Gαs were predominantly located at the plasma membrane, whereas mStrawberry-βαrr2 was evenly distributed throughout the cytoplasm (Figures 3A, S2, and S3A). Following agonist stimulation, mEmerald-67-Gαs rapidly translocates from the plasma membrane to
the cytoplasm, and after 5 min of receptor stimulation mEmerald-67-Gαs was predominantly distributed within the cytoplasm (Figures 3A, S2, and S3A). In contrast, following agonist stimulation, mStrawberry-βarr2 was recruited from the cytoplasm to the activated SNAP-tagged receptors (5 min post-stimulation) at the plasma membrane followed by GPCR-βarr complex internalization into endosomes (>20 min post-stimulation) (Figures 3A, S2, and S3A). At longer agonist exposure times (>20 min post-stimulation), increased mEmerald-67-Gαs intensity can be visualized in β2V2R-βarr2 and V2R-βarr2 containing endosomes (Figures 3 and S3), but this was not observed for the β4AR, which is likely because it only forms transient complexes with βarr2 (Figure S2). Line-scan analysis of all three fluorophores within these endosomes demonstrates co-localization of β2V2R/V2R, βarr2, and Gαs, supporting the hypothesis that "megaplexes" of class B GPCRs, βarr, and heterotrimERIC Gs form in endosomes (Figures 3B, 3C, S2B, and S3C).

**Agonist-Stimulated Interaction between βarrs and Gs Subunits**

To confirm close molecular proximity between different megaplex components following receptor stimulation, we utilized BRET. If megaplexes form, G protein and βarr will simultaneously interact with a single active receptor, and thus, measurement of BRET between functionally validated Gs subunits (Gαs or Gγ2) and βarr1/2 following agonist stimulation can serve to detect these complexes (Figures 4A and S1). In BRET titration experiments, agonist-stimulation for 20 min of either β2V2R or V2R increased the BRET signal between RLucI-67-Gαs and GFP10-βarr1/2 (Figures 4B and 4D). A significant agonist-promoted BRET increase between RLucI-Gγ2 and GFP10-βarr1/2 was also detected, indicating that the agonist-promoted recruitment of βarr1/2 to the β2V2R or V2R brings βarr1/2 into close proximity to both the Gαs and Gγ subunits. In contrast, no change in the BRET signal was detected between GFP10-βarr1/2 and either RLucI-67-Gαs or RLucI-Gγ2 following ISO treatment of β4AR-transfected HEK293 cells (Figures 4B and 4D). Interestingly, in BRET kinetic experiments, which are slightly more sensitive than titration experiments, a weak ISO-promoted BRET signal was observed between GFP10-βarr1/2 and RLucI-67-Gαs but not RLucI-Gγ2 in cells expressing β4AR. (Figures 4C and 4E). In BRET kinetic experiments, for both β2V2R and V2R, the agonist-mediated signals between βarr1/2 and both RLucI-67-Gαs and RLucI-Gγ2 were pronounced and rapidly increased until plateauing ~10 min after agonist treatment.

In the resting state heterotrimeric Gs is initially located at the cell membrane and βarr is cispersed within the cytoplasm, thus, we tested whether the BRET signals detected following agonist stimulation could have arisen from random collisions between the plasma membrane-translocated βarr and any membrane proteins. To control for this possibility, BRET was measured between the membrane protein RLucI-CDB and GFP10-βarr1/2 following agonist-stimulation of β4AR, β2V2R or V2R (Figure 4A). In this setup, no BRET response was observed following agonist stimulation of any of the receptors indicating that the BRET detected between βarr and both Gαs and Gγ2 reflects molecular proximity consistent with the formation of megaplexes (Figures 4B–4E). Taken together, these experiments provide further evidence that megaplexes containing receptor, βarr and Gs form robustly at both the β2V2R and V2R.
Figure 4. Interaction between βarr1/2 and Either Gαs or Gγ2 following Agonist Stimulation of β2AR, β2V2R, or V2R
(A) Schematic representation of the experimental design used to monitor agonist-promoted BRET between RlucII-Gαs (1), RlucII-Gγ2 (2), or RlucII-CD8 (3) and GFP10-βarr1/2.
(B and D) BRET titration curves using a constant amount of RlucII-Gαs, RlucII-Gγ2, or RlucII-CD8 and increasing amounts of GFP10-βarr1 (B) or GFP10-βarr2 (D) monitored 20 min after agonist stimulation. Data are expressed as net BRET absolute values and represent the mean ± SE and are pooled from N = 3–6 experiments. Surface expression of all GPCRs was matched.

(legend continued on next page)
**GPCR-βarr Fusion Proteins Activate Heterotrimeric G Protein following Agonist Stimulation**

The BRET and confocal data shown thus far support the existence of megaplexes and suggest that these complexes occur more readily for the class B GPCRs, β2V2R and V2R, and minimally for the class A β2AR. However, these results do not demonstrate whether GPCR-βarr complexes can directly activate G protein. To investigate whether GPCR-βarr complexes can interact with, and activate, G protein in a cellular environment we generated fusion proteins of GPCR-βarr and investigated their ability to activate Gs in HEK293 cells. We used the β2V2R as our model class B GPCR to be consistent with the biophysical experiments in this study where we assessed the ability of a purified β2V2R-βarr1 complex to interact with, and activate, Gs (see below). To date, we have been unable to purify biochemically functional GPCR-βarr complexes using other class B GPCRs, such as the V2R. The β2V2R has been rigorously characterized both herein and in previous studies; it displays similar biological properties to the V2R, as well as to other class B

![Figure 5](image-url)

**Figure 5. Functionality and Capability of β2V2R-βarr1/2 Fusions to Activate Gs in HEK293 Cells**

(A) Functional assessment of β2V2R-βarr1/2 fusions using radioligand competition binding experiments. Both agonist (ISO) and antagonist (ICI-118551) successfully competed off [32P]-CYP at β2V2R, β2V2R-βarr1 and β2V2R-βarr2. Data represent the mean ± SE of N = 3-4 experiments.

(B) Cellular localization of SNAP-β2V2R and SNAP-β2V2R-βarr1/2 fusions pre-labeled with SNAP-Surface 549 fluorescent substrate (549) using confocal microscopy (100× objective, N = 3 experiments, and n ≥ 16 cells).

(C) Characterization of 1 μM ISO-stimulated ERK1/2 phosphorylation response at 10 min post-stimulation in mock, β2V2R, β2V2R-βarr1, and β2V2R-βarr2-transfectect cells (N = 6 experiments).

(D) ISO-stimulated Gs activation in mock (gray), β2V2R (black), β2V2R-βarr1 (red), and β2V2R-βarr2 (blue) transiently transfected cells determined by BRET titration curves 30 min after stimulation (N = 4 experiments).

(E) Real-time cAMP measurement, utilizing HEK293-IEU2 cells, in response to ISO-stimulation of β2V2R (black), β2V2R-βarr1 (red), and β2V2R-βarr2 (blue). Data represent the mean ± SE of N = 3 experiments and n ≥ 93 cells. Surface expression of GPCRs was matched in all experiments. See also Figures S1 and S4.

Initially, we tested the functionality and stability of the β2V2R-βarr1/2 fusions. Immunoprecipitation (IP) of the FLAG-tagged fusion proteins, expressed in HEK293 cells, confirmed that the fusions were intact and not subjected to cellular cleavage (Figures S4A and S4B). When compared with β2V2R, ISO-promoted displacement of [125I]-cyanopindolol (CYP) binding for both β2V2R-βarr1/2 fusions revealed a bihcsrh curve reflecting a higher affinity state for agonist (Figure 5A): β2V2R-βarr1 dissociation constant $\log K_{D}$ = $-7.11 ± 0.03$ (41%) and $\log K_{D}$ = $6.01 ± 0.03$ (59%); and β2V2R-βarr2 dissociation constant $\log K_{D}$ = $-8.29 ± 0.03$ (49%) and $\log K_{D}$ = $-6.47 ± 0.03$ (51%). These results are characteristic of the allosteric effect of βarr on receptor binding properties, as previously reported (Gurevich et al., 1997), and confirm the functional interaction between the two moieties of the fusions. Fusion to βarr had no effect on the affinity of the antagonist ICI-118551 (Figure 5A). When expressed in HEK293 cells both fusions constitutively internalize resulting in a relatively low amount of β2V2R-βarr1/2 fusions being present at the cell membrane (Figure 5B). This internalization pattern further shows

(C and E) Kinetics of agonist-promoted BRET between GFP10-βarr1 (C) or GFP10-βarr2 (E) and Rlicil-Gxs, Rlicil-Gy2, or Rlicil-CID8 obtained for all three GPCRs. Each kinetic point represents the mean ± SE of AΔBRET between agonist-stimulated and vehicle-treated conditions (N = 3–10 experiments). Two-way ANOVA was performed to determine significant differences between CID8 and Gxs or Gy2 for each time point ($^a$ p < 0.05; $^b$ p < 0.01; $^c$ p < 0.001; $^d$ p < 0.0001). See also Figures S1 and S5.
that βarr, as a fusion partner, retains its functionality to promote βarr-mediated receptor endocytosis. Furthermore, when stimulated with ISO, the β2V2R−βarr1/2 fusions promote ERK1/2 phosphorylation at 10 min post-stimulation (Figures 5C and S4C). These results indicate that both individual proteins, of the β2V2R−βarr1/2 fusions, are functional when expressed in cells.

To test whether β2V2R, as part of the β2V2R−βarr fusions, retains its ability to activate Gs, changes in BRET were measured between RLucII-117-Gαs and GFP10-Gy1 in response to ISO stimulation as compared to vehicle treatment. As shown in Figure 5D, ISO stimulation of β2V2R−βarr1/2 induced a decreased BRET between RLucII-117-Gαs and GFP10-Gy1, reflective of the Gs subunits separation and activation, although to a lesser extent than non-fused β2V2R. These results demonstrate that both β2V2R−βarr1/2 fusions can activate Gs to some degree, following agonist-mediated receptor stimulation, despite their constant coupling to functional βarr1/2.

To further confirm the ability of the β2V2R−βarr1/2 fusions to activate Gs, real-time kinetic studies of ISO-stimulated cAMP production were undertaken. Both β2V2R−βarr1/2 fusions were able to initiate Gs signaling (Figure 5E), providing further support that G proteins are capable of being activated by GPCR−βarr complexes.

**In Vitro Formation and Isolation of Megaplexes**

To further investigate whether GPCRs can form megaplexes by simultaneously interacting with βarr and heterotrimeric G protein, we attempted to form such megaplexes in vitro and to then isolate them by co-immunoprecipitation (coIP). For this study, we used the FLAG-β2V2R expression construct to form stable complexes with βarr1 in st9 insect cells, as previously described (Shukla et al., 2014). Following stimulation with the high-affinity agonist, BI-167107 (BII), β2V2R forms appreciable amounts of complex with βarr1 that can be purified by affinity purification and size-exclusion chromatography (SEC). To obtain highly stable β2V2R−βarr1 complexes, that remain intact throughout the purification, we added the conformationally active antibody binder, Fab30, which binds to and stabilizes active βarr1 conformations (Shukla et al., 2013, 2014). We were unable to obtain monodispersed and functional V2R−βarr1 complexes using this approach (data not shown).

To test whether this Fab30-stabilized BI-occupied β2V2R−βarr1 complex (Fab30 complex) interacts with the heterotrimeric Gs, we added purified Gs in excess to the Fab30 complex and pulled-down the FLAG-β2V2R using M1 anti-FLAG beads. As shown in Figure 6A, βarr1 and all three components of the heterotrimeric Gs (Gαs, Gβ1, and Gγ2) were pulled-down together with FLAG-β2V2R in a stoichiometric fashion. A similar result was observed when using protein A/G agarose beads, which bind Fab30, to pull-down the individual components of the megaplex, confirming that it forms in vitro (Figure 6A).

**Megaplex In Vitro Functionality**

To test the functionality of the receptor in the megaplex we first investigated whether binding of the heterotrimeric Gs to the Fab30 complex is agonist-dependent by FLAG-tag coIP (Figure 6C). The Fab30 complex was able to bind Gs to a similar extent as agonist BI-bound β2V2R (Figures 6C and S5A). In contrast, the agonist carazolol (Cz)-bound β2V2R had almost no ability to bind Gs indicating that the binding of the heterotrimeric Gs to β2V2R in the Fab30 complex is dependent on an agonist-stabilized active receptor conformation (Figures 6C and S5A).

We next investigated whether the β2V2R maintains its guanine nucleotide exchange factor (GEF) functionality with respect to the heterotrimeric Gs when residing in the megaplex. To assess GEF functionality, the megaplex was formed in the presence of either GDP or non-hydrolysable GTPγS. An exchange of GTPγS to GTPγS in the heterotrimeric Gs causes activation of the Gα subunit and separation from the Gβγ subunits (Figure 6B). This separation event was followed by a FLAG-tag coIP. The addition of GDP caused a small decrease in Gs binding to the BI-occupied β2V2R and Fab30 complex (Figures 6D and S5B). However, the addition of GTPγS led to a nearly complete separation of the Gα subunit from both the BI-occupied β2V2R and Fab30 complex (Figures 6D and S5B). This dramatic effect indicates that the receptor retains its GEF functionality while residing in the megaplex and can promote G protein activation and separation. Interestingly, unlike the Gα subunit, the Gβγ subunits remained in complex with both the Fab30 complex and BI-occupied β2V2R after GTPγS treatment (Figure S5B).

**Figure 6. In Vitro Formation and Functional Characterization of the Megaplex**

(A) Coomassie-stained gels of representative coIP experiments of the megaplex by either M1 anti-FLAG beads (to pull-down FLAG-β2V2R; left) or protein A/G agarose beads (to pull-down Fab30; right) (N = 4 experiments).

(B) Schematic presentation of the biochemical steps in G protein activation in the megaplex: (1) heterotrimeric G protein is recruited to the GPCR-βarr "tail" conformation to form the megaplex in an agonist-dependent manner; (2) activated receptor in the megaplex stimulates GDP-GTP exchange in the heterotrimeric G protein, causing activation and separation of the Gα subunit, and (3) activated Gα subunit has intrinsic GTPase activity causing hydrolysis of GTP to GDP and inorganic phosphate (Pi).

(C) M1 anti-FLAG coIP experiment of BI-occupied β2V2R, Fab30 complex, or Cz-occupied β2V2R both with and without heterotrimeric Gs present. Gs binding was determined and quantified by western blot using an anti-Gαs antibody. Data represent the mean ± SE of N = 4 experiments. One-way ANOVA was performed with pairwise comparison to BI-β2V2R (**p < 0.0001).

(D) M1 anti-FLAG coIP experiment with either BI-occupied β2V2R-Gs complex or megaplex in presence of control buffer, 20 μM GDP, or 20 μM GTPγS. Gα subunit separation was determined and quantified by western blot using an anti-Gα subunit antibody. Data represent the mean ± SE of N = 4 experiments. Two-way ANOVA was performed to assess significant differences between control buffer (p < 0.05; *p < 0.01; ***p < 0.0001) and GTPγS. There were no statistical differences between the BI-occupied β2V2R-Gs complex and the megaplex.

(E) Characterizing the ability of BI-occupied β2V2R (top) or Fab30 complex (bottom) to modulate GDP-GTP exchange and Gα activity via GTPase activity. Data represent the mean ± SE of N = 5-6 experiments. Two-way ANOVA was performed to test the effect of Gs presence at each receptor/complex concentrations (**p < 0.01; ***p < 0.0001), and one-way ANOVA tests the effect on Gα modulation by different receptor/complex concentrations (##, p < 0.001). See also Figure S5.
Once activated by the receptor, the Gs subunits display intrinsic GTPase activity. To further characterize the $\beta_2\gamma_2R$ functionality in the megaplex, modulation of Gs, measured as GTPase activity by the Fab30 complex, was investigated. As shown in Figure 6E, the Fab30 complex does indeed positively modulate the GTPase activity of the Gs, and to a similar level as the BI-occupied $\beta_2\gamma_2R$ control.

These in vitro experiments clearly show that the receptor, in the megaplex, retains its full capability to both interact with and to activate heterotrimeric Gs.

**Structural Studies of the Megaplex Using Electron Microscopy**

To investigate the architecture of the megaplex, we formed complexes on a preparative scale and isolated them by SEC (Figures S6A and S6B). To increase the overall stability and homogeneity of the megaplex preparation, we employed two strategies, which were previously utilized to form stable $\beta_2\gamma_2R$-Gs complexes (Rasmussen et al., 2011): (1) we removed GDP from the heterotrimeric Gs by addition of apyrase; and (2) we stabilized the nucleotide-free transition state of the Gs, which is known to interact strongly with the $\beta_2\gamma_2R$ transmembrane core region by adding the conformationally active nanobody binder, Nb35. These two strategies resulted in a stable and monospecific preparation of megaplexes as assessed by SEC and conventional negative-stain EM (Figures 7 and S6) (Peebles and Skiniotis, 2015). The $\beta_2\gamma_2R$ construct used for our in vitro studies was engineered to contain an N-terminal T4-lysosome fusion, (T4L) $\beta_2\gamma_2R$, which can be used as a marker for receptor orientation in EM studies (Shukla et al., 2014; Westfield et al., 2011).

To visualize the structure of the in vitro reconstituted megaplex we applied classification and averaging of the EM particle projections (Figure S6C). The class averages revealed a complex architecture with distinct features that allow domain assignment, with the T4L marking the receptor extracellular face and the heterotrimeric Gs bound diametrically opposite at the intracellular side of $\beta_2\gamma_2R$, in a configuration that appears identical to the previously characterized (T4L) $\beta_2\gamma_2AR$-Gs (Figures 7B and 7C) (Westfield et al., 2011). Additional density attributed to $\beta\gamma\gamma$-Fab30 is observed on the side of the $\beta_2\gamma_2R$-Gs complex. This configuration of $\beta\gamma\gamma$, which interacts with the phosphorylated $\beta_2\gamma_2R$ C-terminal tail, is reminiscent of the "tail" conformation of the (T4L)$\beta_2\gamma_2R$-Fab30 complex that we previously reported by EM (Shukla et al., 2014). In fact, overlaying the averages of the (T4L)$\beta_2\gamma_2AR$-Gs complex and the "tail" configuration of the (T4L)$\beta_2\gamma_2R$-Fab30 complex (using receptor and T4L densities as the common features) results in a projection that appears almost identical to the one of the megaplex (Figures 7B and 7C). In this super-complex, $\beta\gamma\gamma$ is positioned adjacent to the G$\beta\gamma$ subunits and several class averages indicate a direct interface between G$\beta\gamma$ and $\gamma\gamma$ (Figure S6C). This possible G$\beta\gamma$-$\beta\gamma\gamma$ interaction was further explored by glutathione sepharose (GST) pull-down assays, whereby an interaction between the GST-$\beta\gamma\gamma$, in complex with Fab30 and the phosphorylated V$\gamma$R C-terminal peptide

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**Figure 7. Single-Particle EM Analysis of the (T4L) $\beta_2\gamma_2R$-G$\beta\gamma$-$\beta\gamma\gamma$-Fab30 Megaplex**

(A) Representative EM image of negative stained megaplex.

(B) Representative class averages of the megaplex (135 total particle projections).

(C) Class averages of the previously published (T4L) $\beta_2\gamma_2AR$-Gs-Nb35 complex and the (T4L)$\beta_2\gamma_2R$-$\beta\gamma\gamma$-Fab30 complex in the "tail" conformation (images reprinted and modified from Shukla et al., 2014; Westfield et al., 2011). Superimposition of these averages results in a density map identical to the one representing the megaplex. The scale bars in (A–C) correspond to 100, 10, and 10 nm, respectively. See also Figures S6 and S7.
(V$_2$Rpp), and heterotrimeric Gs was observed (Figure S7). Interestingly, when the G$\beta$y subunits were separated from the G$\alpha$s subunit by addition of non-hydrolysable GTP surrogate, GDP-ALF$,^\text{+}$, G$\beta$y binds GST-$\beta$arr1 more prominently whereas G$\alpha$s subunit association with GST-$\beta$arr1 diminishes (Figures S7B and S7C). These results suggest that a direct interaction occurs between $\beta$arr1 and G$\beta$y subunits. Consistent with this finding, we further demonstrated that expression of the G$\beta$y scavenger, T8-$\beta$ARKctk, significantly reduced the $\beta$$_2$V$_2$R-stimulated BRET response between RlucII-Gy2 and GFP10-$\beta$arr1 in HEK293 cells (Figure S7D).

**DISCUSSION**

Sustained G protein signaling by internalized GPCRs has been difficult to incorporate within the classical understanding of GPCR signaling since receptor internalization is thought to be driven by the formation of GPCR-$\beta$arr complexes and because $\beta$arr plays a fundamental role in the desensitization of GPCR-mediated G protein signaling. Thus, we found it surprising that class B GPCRs, including PTHR, $V_3$R, and TSHR, which are known to interact tightly with $\beta$arr, have been shown to promote sustained G protein signaling from internalized compartments (Calebiro et al., 2009; Feinstein et al., 2013; Ferrandon et al., 2009; Wehbi et al., 2013). In the current study we directly demonstrate that by exchanging the C-terminal tail of the $\beta$AR with the V$_2$R C-terminal tail ($\beta$$_2$V$_2$R), the receptor internalization pattern changes from class A to B and that the $\beta$$_2$V$_2$R chimera displays behavior similar to the V$_2$R (Figures 3A and S2) (Oakley et al., 1999, 2000).

Interestingly, as observed with the V$_2$R, this modification significantly enhances the ability of the $\beta$$_2$V$_2$R, relative to $\beta$AR, to promote sustained G protein activation and signaling from internalized compartments (Figures 1 and 2). Previous findings suggest a role for $\beta$arr in sustained G protein signaling by the PTHR and $V_3$R. Co-expression of a constitutively active version of $\beta$arr1 enhances sustained G protein signaling at the PTHR and $V_3$R, and such signaling was diminished by small interfering RNA (siRNA) knockdown of both $\beta$arr1/2 (Feinstein et al., 2011, 2013; Wehbi et al., 2013). In contrast, for the class A GPCR, $\beta$AR, desensitization of Gs signaling is enhanced by a constitutively active $\beta$arr1, but diminished by siRNA knockdown of $\beta$arr1/2 (Violin et al., 2008; Wehbi et al., 2013).

How is $\beta$arr involved in receptor-mediated G protein activation at internalized GPCRs? Several studies with the PTHR, $V_3$R, TSHR, $\delta$ opioid, and CCR1 (Audet et al., 2012; Calebiro et al., 2009; Feinstein et al., 2013; Ferrandon et al., 2009; Gilliland et al., 2013; Wehbi et al., 2013) indirectly suggest our megaplex hypothesis for GPCRs. In the present study, however, we provide direct evidence for formation of megaplexes; super-complexes in which the heterotrimeric Gs subunits come into close proximity with $\beta$arr1/2 following stimulation of the $\beta$$_2$V$_2$R or V$_2$R (Figure 4). This event seems to occur at internalized receptors (Figure 3). Furthermore, following agonist addition, $\beta$$_2$V$_2$R-$\beta$arr1/2 fusions retain the ability to activate Gs and promote signaling (Figures 5 and S4). We demonstrated in vitro that the receptor in a purified agonist-occupied $\beta$$_2$V$_2$R-$\beta$arr1-Fab30 complex interacts strongly with heterotrimeric Gs through its transmembrane core, while it couples simultaneously with $\beta$arr1 through the receptor C-terminal tail (Figures 6 and 7). This megaplex architecture allows the receptor to promote GDP-GTP exchange and to activate Gs (Figures 6, S5, and S6) and thus explains how $\beta$arr can drive receptor internalization without interfering with G protein coupling to the receptor.

Why does $\beta$arr partake in sustained internalized G protein activation at class B GPCRs, while desensitizing it at others, such as the prototypical class A GPCR, $\beta$AR? The current study suggests that a strong interaction between the GPCR C-terminal tail and $\beta$arr is required to robustly form a megaplex (Figures 4, 6, and 7). To obtain a highly stable $\beta$$_2$V$_2$R-$\beta$arr1 complex, used for the aforementioned structural studies, this complex was engineered to have a strong interaction between the receptor C-terminal tail and $\beta$arr1, which was accomplished by exchanging the $\beta$AR C-terminal tail with the V$_2$R C-terminal tail and stabilizing the active conformation of $\beta$arr1 with Fab30 (Shukla et al., 2014). $\beta$$_2$V$_2$R and $\beta$arr1 only interact through the V$_2$R C-terminal tail region in the "tail" conformation, which is the arrangement that allows the receptor to interact with Gs. Thus, in order to form megaplexes, it might be a requirement for GPCRs to have a C-terminal tail that promotes a strong interaction with $\beta$arr following phosphorylation. Class B GPCRs have been reported to contain highly conserved serine/threonine phosphorylation site clusters in their C-terminal tails, which are critical for the formation of highly stable GPCR-$\beta$arr complexes (Oakley et al., 2001; Vilarcaga et al., 2002). Therefore, a dependency on a strong C-terminal tail interaction, that promotes the "tail" conformation, could explain why class B GPCRs form megaplexes that lead to G protein signaling from internalized compartments. On the other hand, class A GPCRs lack serine/threonine clusters at their C-terminal tails and, thus, promote a transient interaction between the GPCR C-terminal tail and $\beta$arr (Oakley et al., 2001). Therefore, formation of class A GPCR-$\beta$arr complexes, might be dependent on the interaction between the receptor transmembrane core and $\beta$arr, which would explain why class A GPCRs only form megaplexes to a limited degree.

Overall, our results indicate that megaplexes of a single class B GPCR, $\beta$arr, and heterotrimeric G protein exist and may explain the recently appreciated phenomenon of sustained G protein signaling from endosomes.

**EXPERIMENTAL PROCEDURES**

**Real-Time Measurement of cAMP Production**

HEK293-IOUE2 cell lines transiently transfected with GPCRs were imaged in the dark, on a 37°C temperature-controlled stage, and for the entire stimulation experiment by using a DeltaVision Deconvolution microscope (GE Healthcare) with a CoolSnap HQ2 CCD camera (Photometrics) controlled by SoftWoRx 6.1 (GE Healthcare). Dual-emission ratio imaging used a CFP/YFP dichroic mirror and 470 ± 24 nm and 535 ± 25 nm emission filters for CFP and YFP, respectively.

**BRET Assay**

Following agonist stimulation, transfected HEK293 cells were incubated in 37°C and luciferase substrate co-terminerazine 400a was added 5 min prior to reading BRET in a Synergy Neo microplate reader (BioTek) equipped with an acceptor filter (515 ± 30 nm) and donor filter (410 ± 80 nm). The BRET signal was determined as the ratio of light emitted by GFP10-tagged biosensors (energy acceptors) and light emitted by RlucII-tagged biosensors (energy donors).
Confocal Microscopy
HKE293 cells transfected with fluorescence protein were fixed with ice-cold 6% formaldehyde diluted in DPBS prior to, or at different time points, during stimulation of the GPCRs. Confocal images were obtained on a Zeiss LSM510 laser-scanning microscope using multi-track sequential excitation (488, 568, and 633 nm) and emission (515-540, 585-615, and 650 nm) filter sets.

Co-immunoprecipitation of In Vitro Complexes
Fab30 complex, BI-β2V2R, or Cz-β2V2R were mixed with Gs in a molar ratio of 1:1.5 in presence of control buffer, 100 mM Bi, 100 mM Cz, 20 μM GDP, or 20 μM GTPγS. Next, FLAG-β2V2R was immobilized on M1 anti-FLAG agarose beads followed by extensive wash. Finally, FLAG-β2V2R and associated proteins were eluted by elution buffer containing 1 mg/ml FLAG peptide.

Megaplex Preparation for Structural Studies
To form stable megaplexes, Fab30 complex was incubated with Gs and Nb35 in a molar ratio of 1:1.5:3 for 1 hr at room temperature. Megaplex was treated with 25 μM/ml of apyrase for 1 hr, and the CaCp2 concentration was adjusted to 4 mM. Finally, the megaplex was purified on an SEC column (Superdex 200, 16/600, GE Healthcare).

Electron Microscopy
Megaplexes were prepared for EM using conventional uranyl formate negative staining. The negative-stained sample was imaged at room temperature with a Tecnai T12 electron microscope operated at 120 kV using low-dose procedures. Images were recorded at a magnification of 71,138x and a defocus value of ~1.5 μm on a Gatan US4000 CCD camera. All images were binned (2 x 2 pixels) to obtain a pixel size of 4.16 Å on the specimen level.

See the Supplemental Experimental Procedures for a detailed description of all experimental procedures.

SUPPLEMENTAL INFORMATION
Supplemental Information includes Supplemental Experimental Procedures and seven figures and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2016.07.004.

AUTHOR CONTRIBUTIONS

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Inorganic Chemistry Cumulative Exam
Purdue University
*Solid State Chemistry – Electronic Structures of Solids*
March 23, 2019

This is a 2-hour exam. It contains 6 problems testing your knowledge of the theoretical principles underlying the electronic structure of solids. The maximum possible score is 55 points. Good luck!

1. Sketch approximate $E$ vs. $k$ diagrams for:
   (a) a chain of interacting $s$-orbitals
   (b) a chain of $p_x$-orbitals propagating and interacting along the $x$ direction
   (c) a chain of $p_z$-orbitals propagating and interacting along the $x$ direction
   Explain the change in energy with the variation of the $k$-vector. [10 points]

2. Shown below is the band structure of TiO$_2$, with partial contributions of each element shaded in black. [5 points]
   (a) Which of these partial DOS plots represents the titanium contribution and which one belongs to oxygen?
   (b) Can you distinguish the contribution from the $d$ orbitals of Ti? Can this contribution be divided into contributions from $t_{2g}$ and $e_g$ orbitals?

![Diagram of band structure of TiO$_2$](image)

3. Shown below is a computed band structure of an eclipsed [PtH$_4$]$^{2-}$ stack. [15 points]
   (a) Assign Pt and Pt–H orbitals that generate each of the depicted bands.
   (b) Sketch an approximate DOS diagram for this structure.
4. Shown below are the DOS and COOP diagrams for a mixed-metal nitride, Ce$_2$MnN$_3$. [10 points]
Use these diagrams to answer the following questions:

(a) Classify the material as a metal, semiconductor, or insulator.
(b) Two diagrams on the left show the contribution of cerium 4f and 5d electrons to the total density of states (DOS). What can you conclude about the participation of these electrons in bonding?
(c) How will the Ce–N and Ce–Ce bond lengths change upon oxidation or reduction of the material. Assume that the band structure is not affected by these processes.

5. The computed band structure of vanadium sulfide, VS, is shown on the left. The band structures calculated for the V sublattice (in the absence of S) and the S sublattice (in the absence of V) are also shown. Using these diagrams and your knowledge of the orbital interactions in solids, answer the following questions [5 points]:

(a) Are there metal-metal bonds in the structure?
(b) Why do the band diagrams of the V and S sublattices not add up to the total band diagram of VS?

6. Sketch roughly how the appearance and filling of the 3d-band changes as one go through the first series of transition metals from Ti to Ni. Explain your drawing. [10 points]
Organic Cumulative Exam
Catalytic Reactions in the March 6, 2019 Issue of JACS (Vol. 141, Issue 9)

Provide a plausible mechanism for 4 of the 7 catalytic reactions below.

Your mechanism should include a clear depiction of each intermediate in the catalytic cycle. For transition metal-catalyzed reactions, you may abbreviate the supporting ligands as appropriate. However, the oxidation state of the metal and the inner coordination sphere should be clear from your drawings.

You do not need to account for the stereochemical outcomes of the reactions.

1. Liming Zhang, 3787–3791.

2. Chidambaram Gunanathan, 3822–3827.


\[
\text{Ph}^\text{H} + \text{Ph}^\text{O} \xrightarrow{\text{Catalyst (10 mol\%), } \text{Cs}_2\text{CO}_3 (20 \text{ mol\%})} \text{Ph}^\text{Me} \text{Me}
\]


\[
\text{C=C} + \text{Ph}^\text{O} \xrightarrow{\text{Cu(OAc)}_2 (5 \text{ mol\%}), \text{Ligand (6 \text{ mol\%)}, } (\text{EtO})_2\text{MeSiH} (4.0 \text{ equiv})} \text{Ph}^\text{Me} \text{Me} \text{N}\text{Me}
\]

\[\text{Ar} = 3,5-\text{t-Bu-4-MeO-C}_6\text{H}_2\]

91\% ee

Ligand


\[
\text{Ph}^\text{N} + \text{Me} \text{B} \text{O} \text{Me} \text{Me} \xrightarrow{[\text{Cp}^*\text{RhCl}_2]_2 (5 \text{ mol\%}), \text{AgNTf}_2 (20 \text{ mol\%)}, \text{AgF (2.2 equiv)}} \text{Ph}^\text{N} \text{Me}
\]


\[
\text{Ph}^\text{I} + \text{H}^\text{N}^\text{Boc} \xrightarrow{\text{Catalyst (5 mol\%), } \text{CySH (5 mol\%)}} \text{Ph}^\text{C} \text{H} \text{N}^\text{Boc}
\]

+ CO\text{2} + NaI

Catalyst
1. Consider the diatomic molecule HD (composed of a hydrogen atom whose mass is approximately equal to $m_p$ and a deuterium whose mass is twice as large) which has a bond length $r$ of approximately 0.074 nm. HD has a vibrational frequency of 3600 cm$^{-1}$.

(a) What is the value of the quantum number $J$, and the degeneracy, of the rotational quantum state of HD whose energy is $\frac{3h^2}{\mu r^2}$? (15 points)

(b) What is the root-mean-squared magnitude of the angular momentum of HD in the same rotational state as in part a? What are the possible projections on the z axis? (15 points)

(c) What is the uncertainty (in nm units) in determining the position of the center of the mass of the HD molecule in the ground vibration state? (20 points)

2. A hydrogen anion $H^-$(which is also known as the hydride ion) has two electrons bound to one proton. In answering the following questions you may assume that $H^-$ may be approximated by a system consisting of two non-interacting electrons whose wavefunctions are identical, except for their spin. In other words, each electron can either be in a spin up $\alpha = |+\rangle$ or spin down $\beta = |-\rangle$ state, so $\alpha_1$ and $\beta_1$ represent the two possible spin states of one electron, while $\alpha_2$ and $\beta_2$ represent the two possible spin states of the other electron.

(a) Express the ground state wave function of $H^-$ in terms of the one-electron states $\alpha_1$, $\beta_1$, $\alpha_2$, and $\beta_2$ in a way that is consistent with the fact that electrons are Fermi particles. (15 points)

(b) Use an expression similar to that you obtained in (a) to show that the two ground state electrons cannot have the same spin. (15 points)

3. Consider an electron confined in a one-dimensional box extends from $-L/2$ to $L/2$. Please write down the transition dipole moment integral for transition from $n=1$ to $n=2$ eigenstate and determine whether or not it is optically allowed. (Hint: you may use symmetry rules). (20 points)

$$E_\ell = \frac{\langle \ell^2 \rangle}{2\ell} = \frac{\hbar^2}{2\mu r^2} \ell (\ell + 1) \quad \text{or} \quad E_J = \hbar c B J(J+1)$$

$$E_v = h\nu \left( v + \frac{1}{2} \right) \quad v = 0, 1, 2, 3 \ldots$$

$$\Psi_0 = N_0 e^{-z^2/2\sigma^2} \quad \sigma = \sqrt{\frac{\hbar}{\mu \omega}}$$

$$\Psi_1 = \sqrt{\frac{2}{L}} \cos \left( \frac{\pi}{L} x \right) \quad \Psi_2 = \sqrt{\frac{2}{L}} \sin \left( \frac{2\pi}{L} x \right)$$

$$\mu_{fi} = \langle f | \hat{\mu} | i \rangle = \langle f | e\hat{z} | i \rangle$$