R01 Countdown: Tools for Writing Concise and Compelling Grants

8 recommended elements for writing a concise and compelling specific aims page

Prioritize crucial paragraphs	• Construct aims page to stand alone. Leave reviewers excited to read more (the 'POW factor') • Take a 30,000' view. Prioritize crucial paragraphs by allocating more room; prune other paragraphs • Within ½ page, include (only) 1-2 sentences about clinical significance, followed by 1-2 paragraphs setting up scientific significance, and then 1 separate paragraph setting up innovation • In the remaining ½ page, state the 3 aims as testable hypotheses. Under each aim, concisely provide essential specific methodological details so reviewers can already begin to evaluate rigor • Provide equal level of detail and space to all aims, especially the (often neglected) third aim • **[Preliminary Data]** Integrate different kinds of available preliminary data into all crucial paragraphs, including scientific significance, innovation, and each of the 3 individual aims paragraphs • Make the 1 page easy on the eye with ample white space between paragraphs. Left-justify text • As a check, color code the specific aims page to ensure all 8 recommended elements are included
Clearly differentiate among clinical significance, scientific significance, and innovation	• For 'outstanding' overall impact scores, 1-2s are needed on both NIH criteria (significance and innovation). Receiving 3, 4, or 5s on either criterion (the 'Land of Mush') will not be sufficient • Clearly differentiate among clinical significance ≈ addresses a clinical problem/disease; scientific significance ≈ paradigm shift advancing scientific knowledge ≈ conceptual innovation; and innovation ≈ innovative methods/techniques ≈ technical innovation • Accentuate scientific significance more than clinical significance, including relative # of sentences • Make it easy for reviewers to assign separate and stellar scores for scientific significance and innovation by constructing separate paragraphs for each criterion that stipulate substantively different, non-overlapping strengths. Consider each criterion 'orthogonal' to one another • R01 Countdown strategy: Distinguish what new, compelling paradigm shift is being proposed (scientific significance) from how it will be done (which innovative methods/techniques to be used)
Briefly address clinical significance	• **[Clinical Significance]** In (only) 1-2 sentences, state clinical significance. Include 2-3 convincing, evidence-based numerical details on prevalence, morbidity, mortality, and/or health care costs • Seek out details and synthesize across reference citations. Avoid vague phrases like 'increased risk'
Use a contrast tactic to underscore scientific significance	• Make it easy for fast-moving reviewers to detect scientific significance (the new compelling paradigm shift) by starting a new paragraph rather than burying it deep within a dense paragraph • **[Paradigm Shift]** In 1 concise, powerful, bolded topic sentence, state the paradigm shift. Reviewers return to this sentence repeatedly for writing their reviews and orally presenting to study section • **[Synthesized Limitations]** In 1-2 concise sentences, synthesize the limitations of any prior paradigm(s), including the rigor (or lack thereof) of prior relevant research • Choose 1 of 2 contrast tactics to explicitly juxtapose the paradigm shift with prior paradigm(s): **[1] [FUNNEL DOWN]** Lead with synthesized limitations (and preliminary data), then paradigm shift **[2] [GO BOLD]** Lead with paradigm shift, then limitations (and prelim data). **[GO BOLD]** is fun to read
Use contrast sentences to highlight innovation	• **[Innovation Topic Sentence]** Make it easy to detect innovation by starting a new paragraph. Lead with 1 brief, bolded topic sentence that includes the key term 'innovative.' State # of innovative methods • **[Innovation]** Then, in 1 sentence, describe each innovative method. Use numerical transition words (e.g., First, Second) to introduce each innovation sentence • **[Innovation Contrast]** Just because it's never been done before doesn't make it innovative. Instead, follow each innovation sentence with 1 succinct contrast sentence that explicitly juxtaposes the strengths of the innovative method with the limitations of existing methods in the field • To underscore the contrast between strengths of the innovative method and limitations of existing methods, use the same dimensions and the same key terms in the same order in both sentences • Use convincing numerical details in the innovation and contrast sentences when possible • To score 1-2s on innovation, integrate 2-3 innovative methods, not just 1. Set aims apart from pack
Identify team expertise	• **[Team Expertise]** In 1 sentence, identify the different expertise areas of the multidisciplinary team • Use the same key terms for disciplines as used for terms in significance and innovation paragraphs
State a hypothesis and method details for each aim	• **[Hypothesis]** In 1 sentence, explicitly state the direction of hypothesis for each aim (e.g., bigger, 'badder,' better). Reviewers are compelled by grants that marshal evidence and take a stand • **[Method Details]** To address rigor, include essential, specific methodological details for each aim • Underline each innovative method from the innovation paragraph earlier if being used for an aim
Write concisely	• Avoid scientific jargon. If it is a multidisciplinary grant, particular reviewers may only have expertise in 1 discipline and little knowledge of even basic vocabulary for the other disciplines • Avoid ice-cream consumption. This is the pervasive academic tendency to use more complicated or highfalutin words than necessary. Instead, use clear simple language…eat more ice cream • Eliminate pink fluff. Delete all repetitive or vague words, phrases, or sentences that do not explicitly add new information—or risk reviewers being distracted by their email or cell phones • Avoid idiosyncratic acronyms. Why make busy reviewers work? Rather, condense in other ways • To systematically condense as concisely as possible, combine Mimi Zeiger's writing techniques (Zeiger M. Essentials of Writing Biomedical Research Papers. 2nd ed. McGraw-Hill; 2000): 'repeat key terms' (p. 58), 'use a consistent point of view' (p. 84), 'put parallel ideas in parallel form' (p. 89), and then condense • To enhance 'continuity' (Zeiger, p. 58) for an easy, seamless read, use another Zeiger combination: 'repeat key terms' (p. 58) and 'use a consistent order' (p. 83), including across all tables and figures

R01 Countdown: Tools for Writing Concise and Compelling Grants
Michaela Kiernan PhD, Stanford Medicine

Citation: Kiernan M. R01 Countdown: Tools for Writing Concise and Compelling Grants [Handouts]. https://doi.org/10.25740/yy394qb6954

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Examples of specific aims elements from awarded NIH grants[1]

Briefly address clinical significance	• **[Clinical Significance]** Osteoporosis affects 50% of women and 25% of men over age 50, increasing fracture risk. Hip fractures are particularly devastating as 20% of adults with a hip fracture die within 1 year and another 50% never walk independently again. **[PI Wu R01 AR073773]** • **[Clinical Significance]** Of the 500,000 adults who suffer a stroke each year, 15% die within 30 days and 30% are still not functionally independent 90 days later. **[PI Govindarajan R01 HS026207]**
Use a contrast tactic to underscore scientific significance **[FUNNEL DOWN]**	• **[Synthesized Limitations]** Devastating movement and seizure disorders can be dramatically alleviated via deep brain stimulation and ablation surgeries that target innervation sites of specific fiber pathways. Diffusion magnetic resonance imaging (dMRI) fiber tracking is the only imaging method available to map these fiber pathways, improve targeting accuracy, and identify new targets for these surgical treatments. Unfortunately, clinical application of dMRI for neurosurgical guidance is impeded by the lack of understanding for the influence of histological features on accurate fiber tracking. **[Paradigm Shift] To determine the influence of histological features, we will compare high-resolution postmortem dMRI fiber tracking against direct optical observation of individual neurons using CLARITY in the same intact, fixed human brain tissue specimens.** We propose that for a given voxel-size and degree of diffusion-weighting of the MRI signal, there will be detection limits regarding the histological features (minimum size, myelination, density, and distance from neighboring pathways) for any given fiber pathway to be accurately mapped with dMRI fiber tracking. **[PI McNab R01 NS095985]** • **[Synthesized Limitations]** Prior risk factor models suffered from serious limitations such as limited measurement of core risk factors in only one or two domains; little systematic examination within or between girls and boys or across age or developmental stages; and small samples with low power. **[Paradigm Shift]** We will test **a parsimonious and integrative model comprised of four sets of core risk factors across domains (key clinical symptoms, cognitive factors, genome-wide single nucleotide polymorphisms, and neural factors) and compares psychiatric outcomes across gender, age, and developmental stages.** We will leverage strengths of a large multimodal database (~10,000 8-21-year-old youth, 50% girls). **[PI Singh R56 MH107243]** • **[Synthesized Limitations]** We face two important challenges when improving clinical outcomes in neonatal resuscitation. First, conditions requiring resuscitation such as preterm birth are infrequent. Second, the recommended algorithm is complex, with six decision points made on a second-to-second basis. Healthcare teams lack practice implementing the technical knowledge, behavioral skills, and teamwork required for optimal resuscitation. **[Paradigm Shift] To address these two challenges and advance resuscitation science, we will use in situ simulation to train healthcare teams and improve clinical outcomes as it allows teams to practice infrequent and complex scenarios**. In situ simulation will also account for contextual factors such as equipment, personnel, policies, and hospital-specific factors influencing the complex steps of resuscitation. **[PI Lee R01 HD087425]**
Use contrast sentences to highlight innovation	• **[Innovation Topic Sentence] Two innovative features of our approach reduce the workload and risk compared to prior, *ex vivo* live imaging.** **[Innovation #1]** First, we image the midgut in situ within a living animal, **[Innovation Contrast #1]** which extends viability up to 8 times longer than ex vivo imaging. **[Innovation #2]** Second, we use a 1.0 NA 20X dipping objective, **[Innovation Contrast #2]** which captures 4 times more cells with comparable micron resolution relative to standard 40x objectives. These features yield geometrically more data in fewer imaging sessions, which reduces workload and risk. **[PI O'Brian R01 GM116000]** • **[Innovation Topic Sentence] To deconstruct and target glioblastoma within the human peritumoral astrocyte microniche, we developed three innovative methods that leverage primary human tissue.** **[Innovation #1]** First, we developed a single brain cell isolation technique and RNA sequencing (RNA-seq) analysis to define the transcriptomes of individual migrating glioblastoma and their peritumoral neighbors. **[Innovation Contrast #1]** Prior studies in the field relied on bulk glioblastoma and peritumoral samples in which more numerous non-tumor or bystander cells drown out the migrating glioblastoma gene signature. **[Innovation #2]** Second, we developed novel immunopanning separation methods to isolate and culture mature human brain cell subtypes to be able to conduct multiple studies on live, pure, mature, human normal, peritumoral, and glioblastoma astrocytes. **[Innovation Contrast #2]** Previous cell sorting techniques (e.g. FACS) kill the primary brain cells quickly, lead to reactive states, are contaminated with other cell types, or can only be done on fetal rodent cells. **[Innovation #3]** Third, we have adapted primary human glioblastoma models (cell and slice culture, human-in-mouse intracranial xenograft), and CLARITY imaging to validate our genes of interest. **[Innovation Contrast #3]** Prior studies relied upon multiple passaged, murine, or non-infiltrating glioblastoma cell lines, which do not faithfully recapitulate human disease, and thin sectioning of tissue for imaging, which disassembles the glioblastoma-peritumoral astrocyte interactions. **[PI Gephart R01 CA216054]** • **[Innovation Topic Sentence] We will leverage two innovative methods to comprehensively assess the effects of PTH1R and Wnt signaling on bone formation.** **[Innovation #1]** First, we will use mass cytometry to analyze >40 protein parameters, allowing us to distinguish mesenchymal stem cells, osteoprogenitors and osteoblasts, and to simultaneously assess PTH1R and Wnt-activated signaling cascades in each population. **[Innovation Contrast #1]** Past studies relied on fluorescence which is limited to <18 parameters and therefore unable to simultaneously distinguish cell populations and assess signaling cascades. **[Innovation #2]** Second, we will use single-cell RNA-sequencing of osteoprogenitors to assess the individual and combined effects of PTH1R and Wnt signaling on the osteoblast gene network. **[Innovation Contrast #2]** Prior bulk RNA-sequencing methods were unable to examine gene expression in rare osteoprogenitors. **[PI Wu R01 AR073773]**
Identify team expertise	• **[Team Expertise]** Our research team includes experts and inventors of high-resolution postmortem dMRI and CLARITY 3D histology. We have established a strong collaboration across the disparate fields of MRI physics, neuroradiology, neuropathology, neurosurgery, neurology, and bioengineering (tissue cleaning and optical imaging). **[PI McNab R01 NS095985]**
State a hypothesis and method details for each aim	• **Aim 1: To test the association of multi-level risk factors with neonatal intensive care unit (NICU) quality of care. [Hypothesis]** We hypothesize sociodemographic and neighborhood factors are independently and jointly associated with quality of care within NICUs as vulnerable infants may receive worse care (Aim 1a) and across NICUs as vulnerable populations may have to seek care in low-quality hospitals (Aim 1b), over and above standard maternal and infant clinical and hospital factors. **[Method Details]** We will capitalize on the existing infrastructure of the California Perinatal Quality Care Collaborative to study a population-scale sample of >30,000 very low-birth-weight infants (<1500 grams) in 130+ NICUs. NICU quality of care will be assessed using the nationally recommended composite Baby-MONITOR measure (primary outcome). **[PI Profit R01 HD084667]** • **Aim 2: [Hypothesis] Determine if ferrous (Fe^{2+}) iron content is higher in hippocampal specimens with high and low Alzheimer's disease pathology than those with no pathology.** **[Method Details]** Fresh frozen sections will be scanned with X-ray microscopy (via microfluorescence) and electron microscopy (via energy loss spectroscopy) at larger and smaller fields of view, respectively. **[Preliminary Data]** In our pilot data, these methods visualize the same iron seen by MR-histology in human Alzheimer's disease and discern oxidation state (Fe^{2+} vs. Fe^{3+}). **[PI Zeineh R01 AG061120]**

[1]Examples included with permission of Principal Investigators, see NIH RePORTER for grant details.

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Example of specific aims using 8 recommended elements (color coded and annotated) **[PI ZUCHERO R01 NS119823]**

Myelin is essential in vertebrates for rapid nerve signaling and its loss in diseases like multiple sclerosis causes severe disability in patients. In the central nervous system, oligodendrocytes build myelin by first extending their processes to ensheath axons, then wrapping spirally around the axon while compacting their membranes. In chronic multiple sclerosis lesions, oligodendrocytes ensheath axons but fail to wrap, suggesting that wrapping is a rate-limiting step for remyelination. To ultimately understand why remyelination fails in multiple sclerosis, we first aim to understand the mechanism by which myelin wraps normally.

It was long hypothesized that the assembly of actin filaments provides the force required to drive wrapping, but we and others recently discovered that the dramatic disassembly of the oligodendrocyte actin cytoskeleton is required for wrapping[1,2]. This finding was completely unexpected and suggests two models for wrapping. Cycles of actin disassembly and reassembly could be required to "ratchet" the oligodendrocyte membrane forward[2]. In contrast, based on our strong preliminary data, **we propose that actin disassembly acts as a "trigger" to initiate actin-independent wrapping and that its major role is to allow myelin to compact**.

To break through existing technical barriers that limited previous studies of actin's role in myelin wrapping, we developed **three innovative methods**. First, we created first-in-class genetic tools to experimentally induce actin disassembly (DeActs[3]) or block actin disassembly (StablActs) in oligodendrocytes during wrapping in vivo. Previous studies relied on cell-permeable drugs (which are not cell-type specific) or on conditionally knocking out actin disassembly factors (which results in weak phenotypes due to compensation by related genes[1,2]). Second, we integrated two complementary, advanced microscopy techniques: correlative light electron microscopy (CLEM) using the fluorogenic actin probe SiR-actin to quantify actin filament levels and myelin ultrastructure in the same myelin sheaths; as well as super-resolution microscopy (STORM) to achieve ~20 nm resolution in tissue sections. Previous approaches destroyed myelin ultrastructure or lacked sufficient resolution to localize actin filaments in myelin. Third, we developed a novel reporter to live-image myelin compaction in primary oligodendrocytes, allowing us to determine whether actin disassembly precedes and is required for compaction. Previously, it was only possible to detect compaction by staining fixed cells or tissue, preventing a mechanistic dissection of how compaction is initiated. These innovative methods and our multidisciplinary expertise in the cell biology of myelination uniquely position our team to test three aims:

Aim 1. Determine whether actin disassembly precedes and persists during myelin wrapping in vivo. We will define the developmental time course of myelination (P4-P90) in the dorsal spinal cord using electron microscopy (Aim 1.1), then quantify actin filament levels in myelin in the same region and at the same time points using light microscopy (Aim 1.2). We will use CLEM to measure actin filament levels in the same myelin sheaths that we identify ultrastructurally as "ensheathed" or "wrapping" (Aim 1.3), and STORM to resolve the subcellular localization of actin filaments in myelin (Aim 1.4). Finally, we will quantify actin filament levels in mature myelin that has been experimentally induced to reinitiate and synchronize wrapping by conditionally deleting PTEN from oligodendrocytes (Aim 1.5). In our preliminary studies, actin filament levels in myelin plunged before the onset of wrapping, consistent with the "trigger" model of wrapping.

Aim 2. Determine whether inducing actin disassembly in oligodendrocytes increases myelin wrapping. We will adapt DeActs to allow viral-mediated expression in oligodendrocytes in vivo, specifically during wrapping, using the oligodendrocyte-specific promoter pMBP (Aim 2.1). We will determine the expression time course, specificity for oligodendrocytes, and ability to induce actin disassembly in myelin by virally expressing pMBP-DeActs (or membrane-GFP control) in the dorsal spinal cord of neonatal mice (Aim 2.2). We will then quantify ensheathment and wrapping using electron microscopy, and oligodendrocyte morphology using light microscopy, in P8/P16 mice expressing pMBP-DeActs or membrane-GFP control (Aim 2.3). In our preliminary studies, neonatal injection of pMBP-DeActs into the dorsal spinal cord caused loss of actin filaments in myelin but increased wrapping compared to control-injected mice, consistent with the "trigger" model of wrapping.

Aim 3. Determine whether actin disassembly is necessary for myelin compaction. Mechanistically, actin disassembly may be required to allow myelin to compact. We will test this hypothesis by live-imaging compaction and actin filaments in cultured oligodendrocytes and using actin-perturbing drugs to block (jasplakinolide) or induce (latrunculin) actin disassembly (Aim 3.1). In our preliminary studies, blocking actin disassembly prevented compaction. To extend these studies into myelin in vivo, we will further develop StablActs, genetic tools we created to stabilize actin filaments and prevent their disassembly (Aim 3.2). We will determine whether actin disassembly is necessary for myelin compaction (and wrapping) in vivo by virally expressing pMBP-StablActs (or fluorescent protein control) in the dorsal spinal cord of neonatal mice, and quantifying myelin compaction and wrapping at P8 and P16 using electron microscopy (Aim 3.3).

By defining actin's role in wrapping, this project will open up new research directions to understand the cellular mechanisms driving myelination and whether these pathways are perturbed in demyelinating disease.

Commented [MK1]: 30,000' view of page: Note ½ the page includes scientific significance and innovation; the other ½ includes 3 bolded Aims, including testable hypotheses & specific method details. All 3 Aims are equally weighted in detail.

Paragraphs are left-justified with white space around them. Reviewers can easily absorb new info (for the actual aims, the font size for the white space in between paragraphs was 6 point; for the handouts, the font size was decreased to 3 point).

Commented [MK2]: Specific Aims included with permission of the Principal Investigator [PI Zuchero R01 NS119823]. Reference: Kantarci H, Cooper MH, Munch A, Ambiel N, Garcia MA, Iyer M, & Zuchero JB. In preparation, 2021

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Commented [MK3]: [Clinical Significance]: 1 concise sentence provided about clinical significance. Specific evidence-based numerical details were not provided to save space for 3 explanatory sentences about myelination. Explanatory sentences ensure reviewers outside immediate niche understand essential concepts from the start.

Commented [MK4]: [Scientific Significance/Paradigm Shift]: Paragraph proposes a provocative paradigm shift by specifying a new, specific overarching model, & then pits it against an existing model. Scientific significance/paradigm shift presented in 1 succinct bolded sentence. Powerfully hooks reviewers on 1st page.

Commented [MK5]: [Preliminary Data]: Key prelim (& published) data integrated, sets up FUNNEL DOWN tactic.

Commented [MK6]: [Innovation Topic Sentence]: Starts w/ short, topic sentence including key term 'innovative' & enumerates number & type of innovative aspects (e.g., three, methods). Use numerical transition words (e.g., First, Second) to clearly introduce each innovation sentence.

Commented [MK7]: [Innovation and Innovation Contrast]: Each innovation is described in 1 sentence followed by 1 succinct contrast statement explicitly juxtaposing innovation's strengths relative to existing methods' limitations. Use same dimensions & same key terms in same order in both sentences.

Commented [MK8]: Yellow: Pairs of key terms deliberately underscore important contrasts (~20 nm resolution vs lacked sufficient resolution; live-image vs staining fixed cells or tissue).

Commented [MK9]: [Team Expertise]: In 1 sentence, innovation paragraph is wrapped up (ha) & explicitly linked to team's multidisciplinary expertise. Key term 'myelination' repeated from above enhancing continuity.

Commented [MK10]: [Hypothesis]: Each bolded Aim states a specific, testable hypothesis. Under each Aim, hypothesis is not stated again; no wasted words with null.

Commented [MK11]: [Method Details]: Every sentence concisely conveys new, essential, specific details. No pink fluff (no excess words).

Commented [MK12]: [Innovation]: Each Aim explicitly references an innovative method described above in the innovation paragraph; key terms are repeated exactly and underlined to enhance continuity.

Commented [MK13]: [Preliminary Data]: Each Aim includes preliminary data specific to that Aim. Consistent point of view used each time (In our preliminary studies, X affected Y).

Commented [MK14]: [Paradigm Shift]: Phrase (consistent with "trigger"…) elegantly & explicitly links prelim data back to team's provocative model, phrase also underlined & last.

Commented [MK15]: [PI Zuchero R01 NS119823]. Reference: Kantarci H, Cooper MH, Munch A, Ambiel N, Garcia MA, Iyer M, & Zuchero JB. In preparation, 2021

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Aim 3. Determine whether actin disassembly is necessary for myelin compaction. Mechanistically, actin disassembly may be required to allow myelin to compact. We will test this hypothesis by live-imaging compaction and actin filaments in cultured oligodendrocytes and using actin-perturbing drugs to block (jasplakinolide) or induce (latrunculin) actin disassembly (Aim 3.1). In our preliminary studies, blocking actin disassembly prevented compaction. To extend these studies into myelin in vivo, we will further develop StablActs, genetic tools we created to stabilize actin filaments and prevent their disassembly (Aim 3.2). We will determine whether actin disassembly is necessary for myelin compaction (and wrapping) in vivo by virally expressing pMBP-StablActs (or fluorescent protein control) in the dorsal spinal cord of neonatal mice, and quantifying myelin compaction and wrapping at P8 and P16 using electron microscopy (Aim 3.3).

By defining actin's role in wrapping, this project will open up new research directions to understand the cellular mechanisms driving myelination and whether these pathways are perturbed in demyelinating disease.

Commented [MK16]: A powerful writing technique to enhance clarity is to: 'repeat key terms exactly' (p. 58) [Zeiger M. Essentials of Writing Biomedical Research Papers. 2nd ed. McGraw-Hill; 2000].

Repeating key terms makes it substantially easier for reviewers outside the immediate discipline to follow the scientific logic and not get confused or waylaid by insignificant changes in key terms. This is crucially important if a grant is multidisciplinary as reviewers may only have expertise in one discipline and little expertise in other disciplines.

Here, there are numerous sets of key terms that are repeated exactly. This is in contrast to changing up key terms in a myriad of different ways to refer to the same concept.

Especially helpful is repeated use of simple, easy-to-understand key terms for the most important concepts of the grant (assembly, disassembly, reassembly; and compaction).

Also helpful is repeated use of simple, easy-to-understand key terms for basic building blocks of the grant (oligodendrocytes, actin filament, electron microscopy), and verbs (precedes, ensheath, initiate; never mind trigger and wrapping).

Keep the key terms as simple as possible. Don't confuse reviewers with 'ice cream consumption.' Rather, simply 'eat more ice cream.'

There are at least 3 more sets of key terms that are repeated exactly in these Aims, but not highlighted. Can you find them?

Commented [MK17]: [PI Zuchero R01 NS119823]. Reference: Kantarci H, Cooper MH, Munch A, Ambiel N, Garcia MA, Iyer M, & Zuchero JB. In preparation, 2021