20.109

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Effective Scientific Writing

What's Scientific Writing?

How is it different from literary writing?

Differences Between Scientific Writing & Literary Writing

- **No surprise endings**  in in Scientific Writing
- Scientific documents are designed to **help readers skip some things** to focus on others
- Writing is **transparent** - content is paramount

transparency takes skill

What is "Transparent" Writing?

Transparent writing is like a window - your ideas are the view

Do you want your reader to think: "What a clever sentence."
or
"Wow, this makes perfect sense."

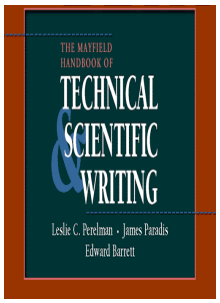
Time to Convert…

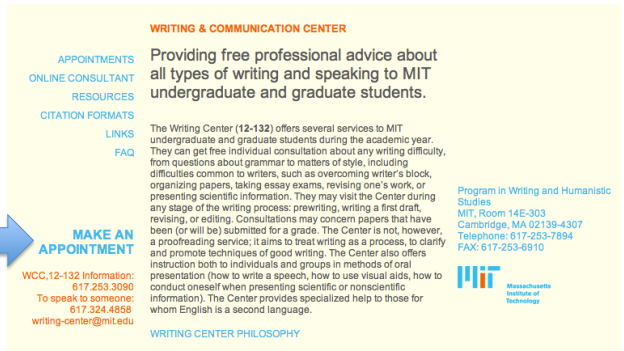
…..ready for professional writing?

Old Habit		New Habit
Begin writing a paper at 2AM that's due at 9AM		Write a first draft **early, get feedback,** and revise it **more than once**
Use language that sounds *scholarly, serious, and smart*		Use language that exactly suits your content
Meet the page count		Write to communicate

Good MIT Resources

http://web.mit.edu/writing/temp2/home.htm





http://writing.mit.edu/wcc

Take advantage of your access to Walter Holland – dedicated writing tutor in BE

Many students think that drafting is the entire writing process

What are some ways to do this?

A Model of the Writing Process

Episode 1: Planning

Goals/Questions
- What do I know about my topic?
- What is my purpose for writing?
- Who are my intended readers and how much do they know about my topic?
- How is this task like others I have had before?
- What structure will work best for my topic?

Strategies
- Clustering
- Freewriting
- Conversation
- Brainstorming
- Reading and research on your topic

Episode 2: Drafting

Goals
- Repeat planning questions
- Strive toward accurately rendering your *intentions*

Strategies
- Any or all of those you used for planning.
- Outlining
- Visual Representations of your topic

Episode 3: Revising

Goals
- Repeat planning/drafting questions
- Address Higher-Order Concerns rather than Later-Order Concerns

Strategies
- Any or all of those you used for planning and drafting
- Seeking feedback
- Glossing your text

Episode 4: Editing/Proofreading:

Strategies
- Editing in several passes with a different focus on each pass
- Reading draft aloud

Gillespie, Paula and Neal Lerner. *The Allyn & Bacon Guide to Peer Tutoring*. Needham Heights, MA: Allyn & Bacon, 2000.

Parts of a Research Report

Guidelines: Paper Structure

- Title
- List of Authors
- Abstract
- Introduction
- Materials and Methods
- Results, including figures and tables
- Discussion
- References

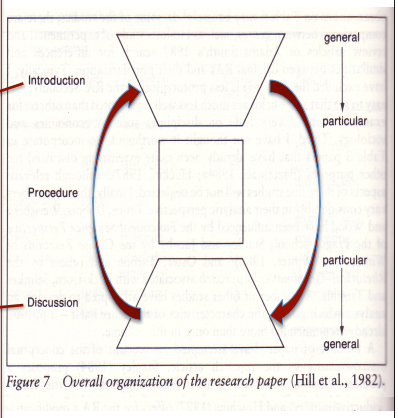
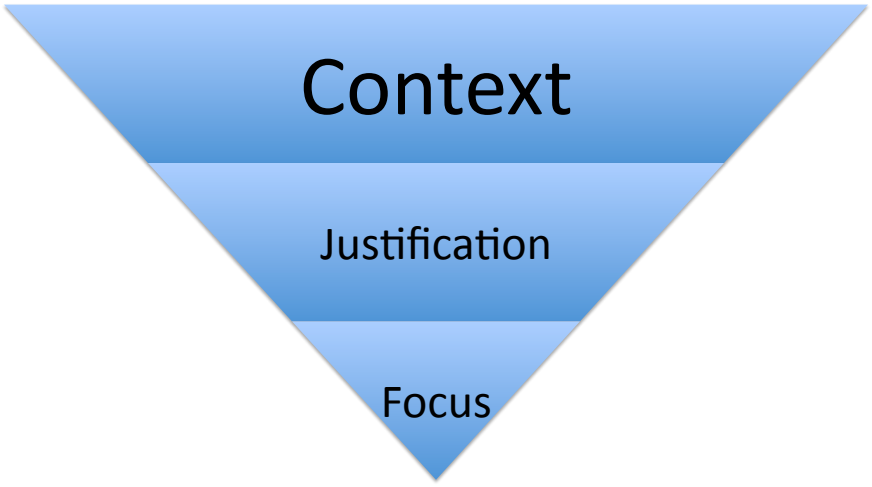


Figure 7 Overall organization of the research paper (Hill et al., 1982).

The body of the paper moves from general (CONTEXT) to specific (YOUR WORK) and then back to general again (INCORPORATE YOUR WORK INTO CONTEXT).

Intro and Disc. Are like bookends around your methods and data – should be closely related to one another – come full circle

Guidelines: Introduction



Context, Focus, Justification

- **Context**: Orient your reader to the published literature related to the study you are presenting
- **Justification**: Show how your work fits into and extends previous work
- **Focus**: What question are you addressing? What is your hypothesis. Define your research space, stake out territory.

Niles & Marletta (2006)

Recent advances in genomics and proteomics are increasing our understanding of transcriptional and post-transcriptional gene regulation and how various gene products integrate into networks (*1–4*). Understanding the functional importance of specific proteins in these contexts has been aided by several methods, including targeted gene knockouts/mutant collections, RNA interference in permissive organisms, and yeast two-hybrid studies, used in combination with microarray transcriptional profiling and mass spectrometry (*3*, *5–10*).

Context, justification, or focus?

Niles & Marletta (2006)

Given the integration of small molecules into these critical circuits, broadly applicable strategies that facilitate the systematic elucidation of their roles in these contexts are required to improve our understanding of cellular physiology.

Context, justification, or focus?

Niles & Marletta (2006)

To evaluate the hypothesis that nuclei acid aptamers can be used to explore the role of small molecules in regulating cellular pathways, we have used Escherichia coli heme biosyhthesis as a model for a product feedback inhibited system.

Context, justification, or focus?

Guidelines: Materials & Methods

- Enough detail to allow **replication**
- **Avoid protocol-like detail e.g.,**
  - Refer to kit name and manufacturer's protocol
  - Use concentrations rather than volumes
- Use **Subheadings** to facilitate skipping around
  - But **avoid the catalogue approach**
- **Use PAST TENSE and FULL SENTENCES**
  - No bullet points – no lists in text

….This may be a much bigger challenge than you realize….

Why?

from a 4% agarose gel, and 150 pmol was used as template for *in vitro* transcription using the Ampliscribe T7 Flash Kit (Epicentre Biotechnologies) spiked with 1 μL of α-^{32}P-ATP (3000 Ci mmol^{-1}, 10 μCi μL^{-1}, PerkinElmer). Reaction times ranged from 4 h to overnight, at the end of which DNase I (1 U) was added at 37 °C for 30–60 min to digest the template DNA. RNA was purified by phenol–chloroform extraction followed by ethanol precipitation at −20 °C.

Aptamer Selection. RNA (200–1000 pmol) in diethyl pyrocarbonate (DEPC)-treated water was denatured by heating to 70 °C for 5 min, allowed to cool to RT, and refolded in selection buffer (SB), with composition 100 mM Tris–acetate, 200 mM sodium acetate, 25 mM potassium acetate, 10 mM magnesium acetate, 0.05% Triton X-100, and 5% DMSO. For subtraction (rounds 1–3 and 8), RNA in ~200 μL of SB was added to ~100 μL of subtraction resin pre-equilibrated in SB and incubated at ambient temperature with gentle mixing for 30 min. The supernatant and 3 × 50 μL of SB washes of the subtraction resin were recovered and incubated with selection resin (200 μL) for 1 h at ambient temperature with gentle agitation. The selection resin was washed with 11 column volumes of SB and eluted with 2.5 mM hemin (Sigma-Aldrich) in SB (6 × 200 μL aliquots with 10 min between additions). The eluted RNA in each fraction was ethanol precipitated overnight at −20 °C with 20 μg of glycogen as a carrier, and amplified using 8 × 50 μL Ready-To-Go RT-PCR tubes (Amersham) and ~300 pmol each 5′- and 3′-primers. RT was carried out at 42 °C for 40 min, and the reverse transcriptase was inactivated at 95 °C × 5 min, followed by 18 PCR cycles (94 °C × 30 s, 57 °C × 60 s, 72 °C × 60 s) and a 7 min final extension. The RT-PCR products were pooled, concentrated, and purified using 4% agarose gel, and the desired length product was extracted and ethanol precipitated. DNA was resuspended in DEPC-treated H_2O for the next round of *in vitro* transcription. At the sixth and eighth selection rounds, the evolved library was blunt end cloned into pSTBlue-1 vector (Invitrogen) and used to transform competent NovaBlue *E. coli* cells (Invitrogen). Single colonies were used for mini-prep cultures from which plasmid encoding a single aptamer was isolated for sequencing and archiving. Plasmids were sequenced at either Elim Biopharmaceuticals or the UC Berkeley Sequencing Facility using the SP6 primer.

Determining Aptamer Heme-Binding Properties. Binding of rounds two and seven libraries to the protoporphyrin IX scaffold was qualitatively determined by fluorescence spectroscopy using a FluoroMax-2. Refolded library RNA (~2 μM) and PPIX in the more physiologic SHMCK buffer (20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [Hepes], 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7) were incubated at ambient temperature, and the fluorescence emission spectrum was measured after excitation at 400 nm. Binding of selected aptamers from the round 8 library to heme was determined by UV–vis spectroscopic difference titrations using a Cary 300 Bio spectrophotometer equipped with a dual cell Peltier accessory (Varian). Briefly, reference and sample cuvettes containing SHMCK only and refolded RNA (~1–2 μM) in SHMCK were prepared, and heme was added in 0.1–0.2 μM aliquots to each sample and reference cuvette pair at 25 °C while stirring continuously. Difference spectra were recorded every 5 min, and $\Delta A_{405\text{-}390}$ was plotted against log[heme] and fitted to eq 1 to determine aptamer apparent heme-binding dissociation constants.

$$\Delta A_{405\text{nm}} = m_1 + (m_2 - m_1)/(1 + 10^{((m_3 \log[\text{Heme}]) - (\log K_d^{\text{app}}))}) \quad (1)$$

The variables are as follows: m_0 = log[heme]; m_1 = minimum $\Delta A_{405\text{nm}}$; m_2 = maximum $\Delta A_{405\text{nm}}$; m_3 = Hill coefficient; m_4 = apparent K_d.

Cloning of Aptamers into the RNA Expression Vector pGFIB. Plasmid pGFIB was obtained as a gift from Prof. William McClain (University of Wisconsin, Madison). With primers CCG GAA TTC AAT ACG ACT CAC TAT AGG GAG CTC AGA ATA AAC GCT CAA (5′-EcoRI) and GCC CTG CAG GGG CCT CAT GTC GAA (3′-PstI), full-length aptamers were PCR amplified from the archival plasmid, purified by 4% agarose gel, extracted, and resuspended in ddH$_2$O after ethanol precipitation. Aptamers (0.36–0.50 μg of DNA) were double-digested with *Eco*R1 (20 U) and *Pst*I (20 U) for 4 h at 37 °C in 1× *Eco*R1 Unique Buffer (New England Biolabs), then ligated into pGFIB (~4.5 μg digested with 20 U of *Eco*R1 and 20 U of *Pst*I for 5 h at 37 °C, then treated with 0.6 U of calf intestinal phosphatase at 37 °C for an additional 30 min) using the Rapid Ligation Kit (Roche). DH-5α *E. coli* cells were transformed using aliquots of the ligation reactions and grown overnight on Luria-Bertani plates supplemented with 50 μg mL^{-1} carbenicillin. Single colonies were selected for mini-culture and plasmid isolation, and aptamer insertion was verified by sequencing using the M13 forward primer.

Screening Heme-Binding RNA Aptamers for *in Vivo* Function. pGFIB/aptamer constructs were used to transform the *E. coli* heme auxotroph RP523 obtained from the *E. coli* Genetic Stock Center (http://cgsc.biology.yale.edu/top.html). Cells were grown overnight on LB plates supplemented with 50 μg mL^{-1} carbenicillin and 15 μM hemin at 37 °C and stored in the dark at 4 °C. All growth experiments were done in LB containing 50 μg mL^{-1} carbenicillin and supplemented with the appropriate heme concentration as indicated. For high-throughput screening, overnight cultures (1 mL in 15 mL tubes) containing 0.5, 1, 2, and 4 μM hemin were inoculated with RP523 harboring specific pGFIB/aptamer constructs from starter cultures in the early to mid-log phase of growth and incubated at 37 °C and 250 rpm. Optical density measurements (OD_{600}) at ~14–20 h were used to assess the extent of bacterial growth. For growth kinetics studies, 50 mL cultures in 250 mL Erlenmeyer flasks inoculated with mid-log phase starter cultures (~0.5 mL) and supplemented with 1 and 10 μM heme were grown at 37 °C and 250 rpm. OD_{600} readings were taken every 30–60 min to assess growth.

Measurement of δ-ALA Levels in RP523 *E. coli*. Cells were grown in 5 mL of LB containing between 2 and 10 μM heme and harvested by centrifugation after measurement of the OD_{600}. Generally, cells expressing control oligonucleotide **6-5** and aptamers were harvested at similar OD_{600} values, dictated by the maximum OD_{600} attained by aptamer-expressing cells. This ensured that the degree of media heme depletion was similar between control and experimental cultures. Cell pellets were resuspended in 200 μL of 4% heptafluorobutyric acid (HFBA) and lysed by three freeze–thaw cycles followed by sonication for 5 min. Supernatants containing δ-ALA were recovered after centrifugation at 14,000 rpm for 10 min and quantitated using an Agilent 1100 series liquid chromatograph/mass selective detector (LC/MSD) operated in positive ESI mode. A standard curve was constructed using authentic δ-ALA (Sigma-Aldrich) detected by monitoring the m/z = 114 [M − H_2O + H^+]$^+$ and 132 [M + H^+]$^+$ ions in SIMS mode. For the LC, a 150 mm × 3.9 mm, 5 μm Nova-Pak C18 column (Waters) with 25 mM HFBA, 5 mM ammonium acetate (solvent A) and 90:10 methanol/5 mM ammonium acetate (solvent B) as mobile phases were used. The column was eluted at a flow rate of 0.4 mL min^{-1}, according to the following gradient: 5% B for 3 min; 5–25% B over 12 min; 25–100% B over 2 min; an isocratic phase at 100% B for 7 min; 100–5% B over 1 min. For the MSD, the drying gas flow rate and temperature were 12 L min^{-1} and 350 °C, respectively, and the nebulizer gas pressure was 35 psig. The capillary exit and fragmentor voltages were −3000 and 70 V, respectively.

Quantitative RT-PCR. RP523 bacteria expressing **6-5**, **8-1**, **8-7**, **8-12**, **8-13**, and **8-35** were grown in LB containing 2 μM heme. Cells were harvested at OD_{600} ~ 0.3 by adding ice-cold 95:5 ethanol/water-saturated phenol to 11% v/v and centrifuging for 2 min at 4 °C. Supernatants were aspirated, and cell pellets were frozen in liquid nitrogen, then stored at −80 °C until needed. Total RNA was isolated using TRIzol (Invitrogen) according to the supplier's protocol. Approximately 2 μg of RNA was digested with 5 U of RNase-free DNase I (Fermentas) in 10 mM Tris-HCl, pH 7.5, 2.5 mM $MgCl_2$, 0.1 mM $CaCl_2$, at 37 °C for 1.5 h. DNase I was inactivated at 75 °C for

Niles & Marletta (2006)

Guidelines: Results

- **Begin with a short description** of the goal and strategy of the study
- Use **subsections** to describe individual parts
  - Each subsection begins with a bit of **context** –
- Use **Illustrations (Figures & Tables) as a guide** to the structure of the Results Section
- **Do not interpret the data** – just present it in objective terms

Niles & Marletta (2006)

RESULTS AND DISCUSSION

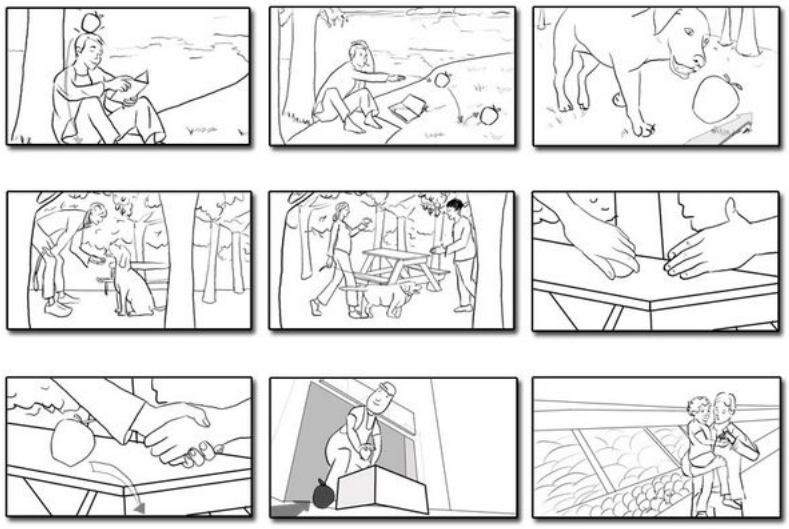
***In Vitro* Aptamer Selection.** Our first objective was to establish high-affinity, expressible heme-binding RNA aptamers for *in vivo* studies.

What's this about?

- **Use subsections to describe individual parts**
- **Each subsection begins with a bit of context**

Guidelines: Tables and Figures

- **Notice the figures in the Niles & Marletta paper**
- Make the illustrations (and their captions) **tell the story graphically – l ike a story board**
- Captions should make data understandable **without reference to the text**
  - **Tables** are used numerical data – often raw
  - **Graphs** show trends
  - **Raw data** e.g., gel, photomicrograph



When to Use Graphs versus Tables

Table 9. Effect of streptomycin, isoniazid, and streptomycin plus isoniazid on *Mycobacterium tuberculosis*[a]

Treatment[b]	Percentage of negative cultures at:			
	2 wk	4 wk	6 wk	8 wk
Streptomycin	5	10	15	20
Isoniazid	8	12	15	15
Streptomycin + isoniazid	30	60	80	100

[a]The patient population, now somewhat less so, was described in a preceding paper (61).
[b]Highest quality available from our supplier (Town Pharmacy, Podunk, IA).

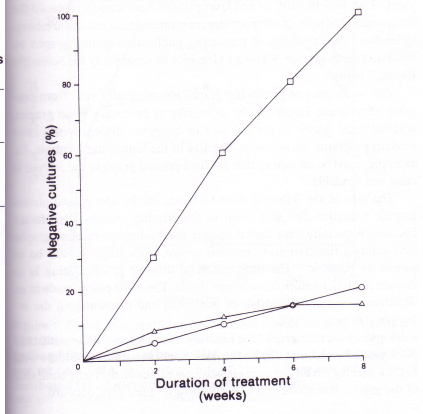


Figure 2. Effect of streptomycin (○), isoniazid (△), and streptomycin plus isoniazid (□) on *Mycobacterium tuberculosis*.
(Courtesy of Erwin F. Lessel.)

Table 9 and Figure 2 present the same data, which one is better?

Form for Tables and Figures

title

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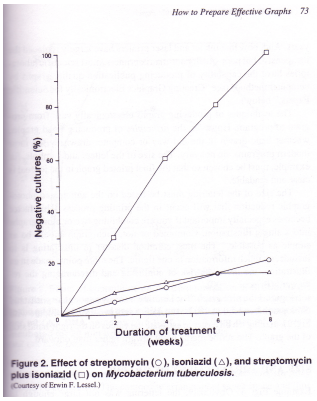

title

Figure 2. Effect of streptomycin (○), isoniazid (△), and streptomycin plus isoniazid (□) on *Mycobacterium tuberculosis*.
(Courtesy of Erwin P. Lessel.)

- Both tables and figures are numbered, and both have brief, informative titles that aren't sentences
- The title appears above a table and under a figure - NOT IN THE FIGURE ITSELF or BELOW THE TABLE
- Refer only to "tables" and "figures," not "graphs, charts, or illustrations"

Guidelines: Discussion

Interpret and contextualize the data

- **Reiterate purpose** (justification)
- **Briefly summarize major findings** (related to focus)
- Relate your work to the **literature** (related to context)
- Identify **shortcomings** and sources of error
- What are the **implications** of the work
- Suggest **future work**

Guidelines: Title & Abstract

Section	Goal	Evaluation		
Title	To give content information to reader	▪ Engaging	▪ Appropriate	▪ Not enough content information or too much
Abstract	To concisely summarize the experimental question, general methods, major findings, and implications of the experiments in relation to what is known or expected.	▪ Key information is presented completely and in a clear, concise way ▪ All information is correct ▪ Organization is logical ▪ Captures any reader's interest	▪ Sufficient information is presented in proper format ▪ Would benefit from some reorganization ▪ Understandable with some prior knowledge of experiment	▪ Some key information is omitted or tangential information is included ▪ Some information is misrepresented ▪ Some implications are omitted ▪ Incorrect format is used

Utilizing RNA Aptmaers to Probe a Physiologically Important Heme-Regulated Cellular Network

Jacquin C. Niles & Michael A. Marletta

ABSTRACT Broadly applicable strategies facilitating direct and selective modulation of the intracellular levels of physiologically important small molecules are essential for dissecting their integral and multiple roles in cellular processes. Therefore, we have been exploring the suitability of RNA aptamers for this purpose. Using the *Escherichia coli* heme biosynthetic pathway as a simple model of a negative feedback regulated process, we show that heme-binding RNA aptamers, developed *in vitro* and expressed intracellularly, induce a heme-dependent growth defect in an *E. coli* heme auxotroph defective in converting δ-aminolevulinic (δ-ALA) acid into downstream products. Relative to a control oligonucleotide, the aptamers also induce δ-ALA accumulation in cells grown under heme-limiting conditions. Increasing the concentration of heme in the media completely reverses both the growth defect and δ-ALA accumulation, except for two aptamers for which reversal is partial. Thus, these aptamers specifically target their cognate ligand *in vivo* and functionally modulate its intracellular concentration, demonstrating that RNA aptamers are useful tools for elucidating the role of heme and possibly other small molecules in regulating cellular networks.

PURPOSE

APPROACH

METHODS

RESULTS

INTERPRETATION

Vigorous Writing is Concise

Advice from the Authorities

The Elements of Style
by William Strunk, Jr., and E. B. White

Omit needless words. Vigorous writing is concise. A sentence should contain no unnecessary words, a paragraph no unnecessary sentences, for the same reason that a drawing should have no unnecessary lines and a machine no unnecessary parts. This requires not that the writer make all his sentences short, or that he avoid all detail and treat his subjects only in outline, but that every word tell....

Avoid fancy words. Avoid the elaborate, the pretentious, the coy, and the cute. Do not be tempted by a twenty-dollar word when there is a ten-center handy, ready, and able.... All [words] are good, but some are better than others.