« Scientific Writing of Biomedical articles »

Day 1: Friday, March 21

- Comparing various styles in scientific writing
- Readability exercises 1 and 2
- How to write an Abstract and Title
- How to write an Introduction

Homework

1. *Write the Title and Abstract to your research and post it in Moodle*

Day 2 : Friday, April 4

- Which is the best title?
- Comments on Abstracts
- Grammar in scientific writing
- Readability 3
- How to write a poster *(designated participants will bring a poster)*

Homework

2. *Write the Introduction to your research for **day 3** and make them available to your peers (bring copies, post them on Moodle or email it on the day).*
3. *Read the section on Discussion in the booklet and prepare to comment on a discussion.*

Day 3 : Friday, April 11

- Peer review of participant's Introductions
- How to write a paragraph
- The discussion and conclusion

Day 4: Wednesday, May 7

- Presentations

Homework

4. *Send your corrected introduction and abstract to the trainer as agreed in class.*

Day 6: Individual sessions of 30 minutes on Wednesday May 14 (choose a date in Moodle)

What is good style for scientific writing?

Below you will read four different versions of part of a scientific paper. The extract is taken from an introductory section of a published paper which describes an investigation of how the secretion of growth hormone from the anterior pituitary of rats is affected by cyclic AMP.
*Indicate which you find the **best** and **worst** version for communicating science and give reasons for your choice.*

Version O

This study aimed at devising a simple *in vitro* system for further growth hormone secretion control mechanism investigations, based on growth hormone release rate measurements from isolated rat anterior pituitary fragments. Choice of rat pituitary was due to its relatively small size, thus permitting fragment section with minimal tissue damage, and ready availability in fresh state. The University of Wessex recently developed a rapid, sensitive, specific double-antibody radioimmunoassay technique (Black & White), 1990), whose validity has been demonstrated in application to determination of hormone release into media previously utilized for incubation of rat anterior pituitary fragments (Lavender, Greenhedge, and Hawthorne 1992) was used in these investigations. Preliminary findings, including demonstration of a direct effect of diburyryl cyclic AMP on growth hormone secretion stimulation *in vitro*, have been published (Brown & Green, 1993). This paper shows rat pituitary fragment preparation to provide a reproducible and sensitive system for *in vitro* investigation of growth hormone release release regulation, usable to distinguish between hormone leakage (occurring presumably from cell damage during gland fragment isolation) and true secretion. Study of growth hormone secretion and investigation of the metabolic conditions necessary for cyclic AMP- stimulated secretory processes to occur actively in the gland have been performed with this system.

Version G

We set out to produce in this work a simple *in vitro* system which we could use to look further into the ways in which secretion of growth hormone can be controlled. We based our method on measurement of the rate at which growth hormone is released from isolated fragments of anterior pituitary from rats. We chose to use rat pituitary because it is easy to get fresh supplies and it is relatively small. This meant we could obtain fragments with only a little tissue damage.

In our work, we used the rapid, sensitive and specific double antibody technique for radioimmunoassay of growth hormone in rats developed recently in the University of Wessex. (1) We

knew it had been shown to be valid when used to check on the amounts of hormone release into the media in which fragments of rat anterior pituitary had been incubated previously (2).

We have published some of our findings previously (3), including our demonstration of the direct effect dibutyryl cyclic AMP has on the stimulation of growth hormone secretion *in vitro.* In this paper, we present our evidence to support the concept that rat pituitary fragment preparation gives a reproducible and sensitive system for *in vitro* investigation of the regulation of growth hormone release. Also, we describe how, by using it, we have been able to distinguish between hormone leakage, which we presume occurs because of damage to cells during isolation of gland fragments, and true secretion. We have used the system to study growth hormone secretion, and we have also used it to investigate what a pituitary gland's requirements are when it is in an actively secreting state, responding to stimuli from cyclic AMP.

Version J

Our aim was to devise a simple system for further *in vitro* investigation of ways of controlling secretion of growth hormone. The investigation was to be based on measuring the rate at which growth hormone is released from isolated fragments of anterior pituitary from rats. We chose rat pituitary because it is relatively small, so fragments can be obtained with only slight tissue change. Also, fresh pituitary is easy to get. In the investigation, we used the rapid, sensitive and specific technique developed at the University of Wessex [1] for double – antibody radioimmunoassay of growth hormone from rats. This assay has been shown [2] to be valid when used to calculate how much hormone is released into the media in which fragments of anterior pituitary from rats have been incubated.

Some of our findings, including the direct stimulation of growth hormone secretion by dibutyryl cyclic AMP *in vitro*, have been published previously [3]. In this paper, we show that a preparation of fragments from pituitaries is a reproducible and sensitive system for investigating *in vitro* how the release of growth hormone can be regulated. Using this preparation, it is possible to distinguish between true secretion and hormone leakage (which presumably occurs because cells are damaged when fragments of gland are isolated).We have used the preparation to study the secretion of growth hormone, and to investigate the metabolic conditions required if the pituitary secrete actively to cyclic AMP.

Version P

The purpose of the study reported here was the establishment of an unsophisticated *in vitro* system, based on measurement of the rate of release of growth hormone from isolated fragments of anterior pituitary from rats, which would provide a suitable technique for further elucidation of the mechanisms by which regulation of the growth hormone secretory process in the cells of the anterior pituitary may be achieved. The choice of rat anterior pituitary for these studies was made by virtue of the ready availability of this gland in the fresh state and its relatively small size which permits fragments being obtained with minimal damage to the tissues involved.

The investigations described in the following pages were carried out utilizing the rapid, sensitive, specific, double-antibody radioimmunoassay technique for rat growth hormone measurement recently developed in the Biochemistry Department of the University of Wessex (Black and White, 1990). That such a process has validity has been demonstrated by its application to the determination of hormone release into media in which incubation of rat anterior pituitary fragments has previously been carried out. (Lavender, Greehedge, Hawthorne, 1992).

A preliminary publication (Brown & Green, 1993) presented an account of some of the experimental findings reported below in this paper, including evidence demonstrating the direct role of dibutyryl cyclic AMP in the stimulation of secretion of growth hormone *in vitro.*

The present paper presents evidence to demonstrate that a sensitive and reproducible system for the *in vitro* investigation of the growth hormone release regulatory mechanisms is provided by the rat pituitary fragment preparation. Utilizing this system, distinction is possible between hormone leakage – occurring, it is presumed, due to cell damage during isolation of gland fragments – and true secretion. Studies of growth hormone secretion have been carried out employing this system, and also investigations of the metabolic requirements exhibited by the gland when stimulation to an actively secreting state bay cyclic AMP is occurring.

Ref: Adapted from “Good style” writing for science and technology
Author: John Kirkman
Publisher: E&FN Spon

Readability exercise 1

Put the action in the verb

1. These models have been subjected to examination by……(ref)

2. In a recent survey it was shown that the spreading of the disease was continuing.

3. We made an investigation on how……..

4. Our results have been submitted to criticism

5. This drug produced an inhibitory effect on the development of the disease.

Rewrite these wordy sentences

1. We are convinced of the fact that articles written in clear English will have more chance of being published.

2. Arteriosclerosis in one of the main causes of death in the Western world. Many risk factors are known, the most important being……

3. At this moment in time our department is investigating the possibility of recruiting another researcher.

4. Protein quantification was performed.

5. Rat pituitary was chosen because of its ready availability in the fresh state

Readability exercise 2

Put these sentences in the active voice.

1. New guidelines were laid down in the institute in the hope that there will be a restriction in the length of the documents to 5 pages by the researchers .

2. Increase funding for cancer research was the final decision of the committee.

3. The pain was reduced considerably by drug X.

4. It was agreed by two scientists that a new method for detecting bacteria in the gastrointestinal tract was necessary.

5. Instructions will often not be followed by the patient.

Untangle these noun clusters

6. Early childhood food disorder misdiagnosis often occurs because of unfamiliarity with recent research literature describing such conditions.

7. There are many elderly over-the-counter drug users in this town.

8. …..can be configured to meet a wide range of user data communication requirements. (1)

9. This stage of the project will be followed by control equipment selection and purchase. (1)

10. The antigen was prepared from whole rat liver homogenate.(2)

Avoid empty word

11. It was observed in the course of the experiment that…….

12. The results would seem to indicate that……..

13. It has been suggested/observed that……

Ref 1 John Kirkman, Good Style, E&FN Spon 1994 **Ref** 2 Mimi Zeiger, Essentials of Writing Biomedical Research Papers, McGraw Hill 1991

The Title

What is the purpose of the title?

What are the characteristics of a good title?

Can you improve these titles?

1. An analysis of football hooliganism as an expression of social dissatisfaction.

2. Drug X improves pulmonary function in the newborn lambs.

   for a neonatologist ……

3. The effect of calcium antagonist felopide on blood pressure, heart rate, working capacity, plasma rennin activity, urinary catecholamines and aldostone in patients with essential hypertention.

4. Effects of alveolar pressure on lung angiotensin-converting enzyme function in rabbit lungs in vivo. (noun cluster)

What is the difference between these 2 titles?

- Effect of Radio-Frequency Irradiation on Metabolic Rate in Rats
- Reduced Metabolic Rate in Rats During Radio-Frequency Irradiation

And

- Verapamil and Diet Halt the progression of Atherosclerosis in Cholesterol-Fed Rabbits
- Arrested Progression of Atherosclerosis by Verapamil and Diet on Cholesterol-Fed Rabbits

ABSTRACT

Vascular leak is a key driver of organ injury in diseases, and strategies that reduce enhanced permeability and vascular inflammation are promising therapeutic targets. Activation of the Angiopoietin-1 (ANG1)-Tie2 tyrosine kinase signaling pathway is an important regulator of vascular quiescence. Here we describe the design and construction of a new soluble ANG1 mimetic that is a potent activator of endothelial Tie2 *in vitro* and *in vivo*. Using a chimeric fusion strategy, we replaced the extracellular matrix (ECM) binding and oligomerization domain of ANG1 with a heptameric scaffold derived from the C-terminus of serum complement protein C4-binding protein α (C4BP). We refer to this new fusion protein biologic as Hepta-ANG1, which forms a stable heptamer and induces Tie2 phosphorylation in cultured cells, and in the lung following i.v. injection of mice. Injection of Hepta-ANG1 ameliorates VEGF- and lipopolysaccharide-induced vascular leakage, in keeping with the known functions of Angpt1-Tie2 in maintaining quiescent vascular stability. The new Hepta-ANG1 fusion is easy to produce and displays remarkable stability with high multimericity that can potently activate Tie2. It could be a new candidate ANG1 mimetic therapy for treatments of inflammatory vascular leak, such as acute respiratory distress syndrome and sepsis.

The development of small-angle scattering tensor tomography has enabled the study of anisotropic nanostructures in a volume-resolved manner. It is of great value to have reconstruction methods that can handle many different nanostructural symmetries. For such a method to be employed by researchers from a wide range of backgrounds, it is crucial that its reliance on prior knowledge about the system is minimized, and that it is robust under various conditions. Here, we present a method employing band-limited spherical functions to enable the reconstruction of reciprocal space maps of a wide variety of nanostructures. This method has been thoroughly tested and compared to existing methods in its ability to retrieve known reciprocal space maps, as well as its robustness to changes in initial conditions, using both simulations and experimental data. The anchoring of this method in a framework of integral geometry and linear algebra highlights its possibilities and limitations.

Group-Specific Primer and Probe Sets to Detect Methanogenic Communities Using Quantitative Real-Time Polymerase Chain Reaction

Youngseob Yu, Changsoo Lee, Jaai Kim, Seokhwan Hwang

School of Environmental Science and Engineering, Pohang University of Science and Technology, Pohang, Kyungbuk 790-784, South Korea; telephone: +8254-279-2282; fax: +8254-279-8299; e-mail: shwang@postech.ac.kr

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Abstract: Real-time polymerase chain reaction (PCR) is a highly sensitive method that can be used for the detection and quantification of microbial populations without cultivating them in anaerobic processes and environmental samples. This work was conducted to design primer and probe sets for the detection of methanogens using a real-time PCR with the TaqMan system. Six group-specific methanogenic primer and probe sets were designed. These sets separately detect four orders (*Methanococcales*, *Methanobacteriales*, *Methanomicrobiales*, and *Methanosarcinales*) along with two families (*Methanosarcinaceae* and *Methanosaetaceae*) of the order *Methanosarcinales*. We also designed the universal primer and probe sets that specifically detect the 16S rDNA of prokaryotes and of the domain *Bacteria* and *Archaea*, and which are fully compatible with the TaqMan real-time PCR system. Target-group specificity of each primer and probe set was empirically verified by testing DNA isolated from 28 archaeal cultures and by analyzing potential false results. In general, each primer and probe set was very specific to the target group. The primer and probe sets designed in this study can be used to detect and quantify the order-level (family-level in the case of *Methanosarcinales*) methanogenic groups in anaerobic biological processes and various environments.

Keywords: anaerobic digestion; methanogen; microbial community; primer and probe set; real-time quantitative polymerase chain reaction

INTRODUCTION

Anaerobic processes have provided an efficient means for treating high-strength organic wastewater with production of bioenergy in the form of methane as well as a low generation of sludge. Anaerobiosis of organics involves a consortium of several groups of microorganisms and is based on a series of reactions, the slowest of which determines the overall efficiency of the process (Yu et al., 2002). In anaerobiosis, methanogens play a key role in stabilizing pollution load by participating in the terminal step, methanogenesis. Because methanogenesis is commonly the rate-limiting step in most anaerobiosis, the majority of attention has been given to investigating the most favorable conditions to ensure efficient methanogenesis (Rittmann and McCarty, 2001; Yang et al., 2003).

In almost all engineered anaerobic systems, evaluating the activities of a methanogenic community remains largely process-oriented. Measurable metabolic parameters, like methane formation and substrate biodegradation, are determined as a function of the whole community, which is divided into four different orders: *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, and *Methanosarcinales* (Garrity and Holt, 2001b). The contribution of the individual archaeal groups to these parameters has been nearly impossible to determine, mainly because of extreme difficulties in culturing the archaea in vitro. Furthermore, it has been one of the major limitations of traditional culture techniques that only a small fraction of the methanogens making up a natural community can generally be cultured because of fastidious culture conditions, extremely slow growth rates, and obligate anaerobiosis (Raskin et al., 1994b; Sekiguchi et al., 2001). However, an understanding of methanogenic community structures as well as their dynamics is essential to aid in the prediction and effective control of process operations related to microbial growth (Griffin et al., 1998; Merkel et al., 1999). This is also crucial for development of biological models.

The study of nonculturable organisms has benefited enormously from recent advances in environmental molecular genetics. For example, various hybridization methods, such as fluorescence in situ hybridization (FISH) and dot-blot and whole-cell hybridization, have been valuable for detecting the presence of methanogens in different environments, including laboratory- or full-scale anaerobic bioreactors (Amann et al., 1995; Griffin et al., 1998; Liu et al., 2002; Oude Elferink et al., 1998; Raskin et al., 1994a, 1994b, 1995).

A recently developed quantitative real-time polymerase chain reaction (QPCR) technique (Reischl et al., 2002; Witt-

Correspondence to: S. Hwang

wer et al., 2001) allows rapid, highly sensitive detection of microbial DNA, thus indicating the presence of a target microorganism or group of microorganisms (Nadkarni et al., 2002; Suzuki et al., 2000). Compared with a conventional PCR method employing two primers, a forward and a reverse, an additional fluorescent probe is required in QPCR assays. The fluorescent probe hybridizes with a specific site within the targeted region and each sequence of the primer and probe sets is designed to be specific for a target organism or microbial group. Therefore, a QPCR assay is highly specific and sensitive because three oligonucleotides complementary to the target DNA are simultaneously employed. The QPCR method with a TaqMan (Lie and Petropoulos, 1998; Livak et al., 1995) probe allows reliable detection and quantification of an initial concentration of template DNA in a sample. It also allows a large number of samples to be processed simultaneously. Although QPCR assays have been heavily targeting pathogens for rapid and sensitive detection and quantification (Hein et al., 2001; Martin et al., 2002; Wellinghausen et al., 2001), the use of environmental applications of the method, especially in anaerobic processes, remains in its infancy.

Although many aspects of conducting a QPCR assay, including instrumentation and reagents, have become increasingly simple to perform, one of the most important aspects, designing and selecting primer and probe sets for QPCR application, is still left to the investigators. Failure of amplification reactions or amplification of nonspecific targets is likely attributable to improperly designed primer and probe sets.

Therefore, this study focused on the design and the characterization of group-specific primer and probe sets using 16S rRNA gene (rDNA) sequences of methanogens that are likely to be found in engineered anaerobic processes. The phylogenetically based classification was used to select the different target groups for the development of primer and probe sets. Six separate primer and probe sets for detection of 16S rDNA of organisms belonging to the orders *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, and *Methanosarcinales*, and the families *Methanosarcinaceae* and *Methanosaetaceae*, were designed for a QPCR assay. We also modified the universal primer and probe sets that could specifically detect the 16S rDNA of prokaryotes, and of the domains *Bacteria* and *Archaea*. The method employs the use of a QPCR system with a dual-labeled oligonucleotide TaqMan probe, which binds to the PCR products internally.

MATERIALS AND METHODS

Design of Primer and Probe Sets

Phylogenetic relationships based on 16S rRNA sequence comparisons from the Ribosomal Database Project (RDP) II (Cole et al., 2003) were used to define the target groups of methanogens outlined in *Bergey's Manual of Systematic Bacteriology* (Garrity and Holt, 2001b). The primer and probe sets for each target group were designed based on regions of identity within 16S rRNA sequences. Among the six primer and probe sets developed in this study, four and two sets were designed to detect order-level methanogenic groups and family-level acetoclastic methanogenic groups, respectively. Each order-level specific set was targeted to detect the orders *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, and *Methanosarcinales*, denoted MBT, MCC, MMB, and MSL, respectively. Each set that was specific to family-level acetoclastic methanogens was designed to detect the families *Methanosarcinaceae* and *Methanosaetaceae*, denoted Msc and Mst, respectively. Three additional primer and probe sets targeting prokaryotes and the domains *Archaea* and *Bacteria*, as reported earlier (Amann et al., 1990; Raskin et al., 1994b; Takai and Horikoshi, 2000), were also modified, and denoted PRK, ARC, and BAC, respectively.

A total of 93 methanogens (data not shown) were selected, and complete or partial rDNA sequences of the type strains in each group were aligned using phylogenetic analysis software, PHYDIT (Chun, 1995). The regions of identity (i.e., signature sequences) within the multiple alignments of 16S rDNA for each microbial group were manually investigated to find the candidate sequences for use as either primers or probes. As suggested in the guidelines set by Applied Biosystems (Foster City, CA) for the design of primers and probes, amplicon size, melting temperature (T_m), percentage of G + C bases, possibility of self-complementarity of the sequence, possibility of forming primer–primer or primer–probe dimers, and the degree of degeneracy were investigated.

The T_m of all TaqMan probes was designed to be about 5° to 10°C higher than the T_m of the two primers in corresponding sets. The percentage of G + C bases was adjusted to be between 40% and 70%. TaqMan probes with a G base on the 5′ end were excluded to avoid a continuous quenching effect. Primers and probes with a strong possibility of either self-complementarity or formation of dimers were excluded. Primers or probes with more than two degenerated bases were also rejected.

The matching efficiency of a candidate sequence is defined as the ratio of the number of organisms that are complementary to the sequence to all organisms in the target group and was analyzed by the PRIMROSE (Ashelford et al., 2002). For each methanogenic group, we tested different combinations of primers and probes that could detect the corresponding groups. Among several possible combination sets that satisfy the criteria from Applied Biosystems, a combination of two primers and a probe with the highest matching efficiency was selected for the target group. Specificity of each primer and probe set was examined online using a PROBEMATCH program from the RDP II. TaqMan probes were labeled with a fluorescent reporter dye (6-carboxyfluorescein) and a fluorescent quencher dye (6-carboxytetramethylrhodamine), which

Readability exercises 3

Parallel ideas

1. Pulsation of the cells or cell masses may be quick and erratic or may occur at fairly regular and leisurely intervals. (1)

What do you expect after "quick and erratic or"?

2. The drug has two functions: calming the patient immediately and to reduce nausea after a few hours.

Are the tenses of the verbs parallel?

Rewrite these wordy sentences

1. The mice exhibited a 100% mortality

2. There was a significant increase in weight in six patients. (2)

3. Since these data have a rather sensitive nature, there is good reason to handle them with care.

4. It is possible that we shall receive funds for this project before the end of the year.

Ref. 1 : Mimi Zeiger, Essentials of Writing Biomedical Research Papers, McGraw Hill 1991
Ref. 2 Turk & Kirkman. Effective writing. second edition

Tenses exercise

Fill in the correct tense in the space

1. The population based Cancer Registry (collect) …… data of all patients in the South of the Netherlands since 1955.

2. Although numerous articles (write) …… on this disease, little research (done) …… on the psychological effect.

3. A study by Smith revealed that patients who (receive) …… the placebo, recovered less quickly than those who (be give) …… real medication.

4. More recently advances (make)…….using computational hydrodynamics to study……

5. The government (spend) ……a lot of money on AIDS research last year.

6. By 2035 psychologists certainly (develop) …… a model to predict this kind of behaviour.

7. During the last 2 years Smith and Jones (report)……..results which are similar to ours.

8. Every year campaigns to prevent AIDS (set up)…….. in Africa.

9. A month ago Smith and Jones (report) …….. results which are similar to ours.

10. Recently efforts (made)…… to determine the cause of the earthquake.

11. The number of deaths (decrease) ……. since the vaccination became obligatory.

12. Although Smith and Jones (already report)……. these results, we (feel)……. that there should be further investigation.

Punctuation

1. There are many disturbing factors poor eye sight fatigue poor reading ability anxiety or undue caution distractibility and inadequate motivation

2. Unfortunately though incorrect predictions were made about both negative and positive experiments

3. When treating cancer patients preservation and/or improvement of quality of life is an important goal

4. After the incubation of cells for 50 hours at 35° their growth was stopped with XXX.

5. Cell incubation was stopped with XXX after incubation for 50 hours at 35

6. Outcomes result from a complex interaction of medical care and genetics environmental and behavioural factors

7. This consideration is important in any research yet it is overlooked if not denied

8. Frequently adjusted totals need to be scrutinized (1)

9. The orifice through which the exhaust gases leave the chamber is larger than……(1)

10. He eats shoots and leaves (2)

ref.1 Full marks. John Kirkman . Second edition
ref 2 Title of book by Truss, 2006

How to write a good paragraph

A paragraph should be well organized and should flow**.**

Organization
Write a topic sentence, at the beginning, to state the message of the paragraph.
The supporting sentences should be organized logically.

Flow
Do not skip steps
Repeat key words
Use link words or phrases to show the logical link between ideas.

Read the following paragraph and answer the questions below.

Hypothermia is currently used to prevent myocardial damage during surgery requiring circulatory arrest, but the effect of hypothermia per se on the myocardium is not clear. Some studies on dogs have found that hypothermia causes myocardial damage. In those studies subendocardial hemorrhages, calcified necrosis and fatty degeneration were seen in the heart after 1-4 hours at body temperatures between 21 and 30°C (9,10). However, other studies in dogs and rabbits have not found myocardial damage under similar conditions of hypothermia (6,8). Similarly, studies of patients who were hypothermic as a consequence of various medical disorders have found no evidence of permanent myocardial damage. In those patients, although serum activity of total creatine kinas MB isoenzyme was increased, there was no clinical or postmortem evidence of acute myocardial infarction.

- Where is the topic sentence?

- How is the paragraph structured?

- Which expressions move the story forward?

- Is the argumentation clear?

Adaptation from: Essential of Writing Biomedical Research Papers by Mimi Zeiger, McGraw-Hill, ISBN 0-07-072833-X

What is the difference between these 2 paragraphs

Utility costs for argon process are 75% greater than for the proposed hydrogen process. Initial capital cost is 5.4 million euros, roughly 3 times the hydrogen process cost. However, annual income from the sale of argon increased ammonia production, and reduced natural gas requirements elsewhere in the plant iis 160% higher than that generated by the hydrogen process. Present worth analysis shows that the argon process is the better investment. The present worth of the argon process is 10 million euros. The present worth of the hydrogen process is 4.14 million euros.

The argon process is clearly a better investment that the hydrogen process. Although it has higher utility costs (by 75%) and a higher initial capital cost (by 300%), it generates annual income –from the sale of argon, from increased ammonia production, and from reduced natural gas requirements elsewhere in the plant – that is 160% greater than that generated by the hydrogen process. Present worth analysis shows that the argon process is valued at 10.25 million dollars whereas the hydrogen process is valued at only 4.14 million dollars.

Ref: English for Science and Technology. T. Huckin & L.Olson, Mc Graw-Hill Int. Ediitons.

How to end the Discussion and make a point

Which of the 3 conclusion gives the answer **a.** plus implication ,**b**. plus speculation and **c.** plus recommendation?

In summary, our results indicate that expansions of plasma volume by 400ml in untrained men increases stroke volume during exercise by 11% but that further expansion of plasma volume has no apparent hymodynamic benefit. These findings imply that in untrained men the measurements of stroke volume during upright exercise, when blood volume is normal, may not provide an adequate measure of intrinsic myocardial function. It appears that about half of the difference in stroke volume normally observed between untrained and highly endurance-trained men during upright exercise is due to suboptimal blood volume in untrained men.

In conclusion, in this study of patients with retroperitoneal sarcoma, we found that presentation status, high histologic grade, unresectable primary tumor and positive gross margins are strongly associated with death from tumor. Patients with primary disease or a first local recurrence approached with curative intent should undergo aggressive attempts at complete surgery resection. These attempts should include a liberal *en bloc* resection policy to obtain negative margins. Incomplete resection should be undertaken only for symptom relief.

In summary, we have shown that the transforming activity of mutated *ras* is associated with two vertebrate cellular systems thought to be regulated by G proteins, namely phospholipases A_2/C and adenylate cyclase. In both cases the enzyme activity was reduced in cells expressing mutated *ras* at high levels. Since phospholipase and adenylaste cyclase activities were also reduced in cells expressing c- *ras* at high levels, we believe that c-*ras* may normally help modulate systems that are regulated by G proteins and that *ras* transformation may result from a concerted aberration or guanine-nucleotide-regulated systems.

Essentials of Writing Biomedical Research Papers. Mimi Zeiger. Second edition. McGraw-Hill

6. Conclusion

SAXS tensor tomography aims at reconstructing the local three-dimensional reciprocal-space map for each volume element within a three-dimensional sample. This can be achieved through gradient-based optimization. An adequate numerical representation of the three-dimensional reciprocal-space map, for which only a few quantities or coefficients have to be recovered for each voxel, can be critical towards developing an approach that is efficient both in computational and measurement time. Liebi *et al.* (2015) introduced this reconstruction approach using spherical harmonics as a base to represent the reciprocal-space map and demonstrated it with a millimetre-sized sample of trabecular bone. The three-dimensional reciprocal-space map comprises information on the main orientation of the nanostructure for different q ranges and also its degree of orientation. The reciprocal-space map could further be used as input for fitting the underlying nanostructure, similarly to what has been done on two-dimensional SAXS data, for example to retrieve size parameters of the mineralized platelets in bone (Fratzl *et al.*, 2005; Turunen *et al.*, 2016).

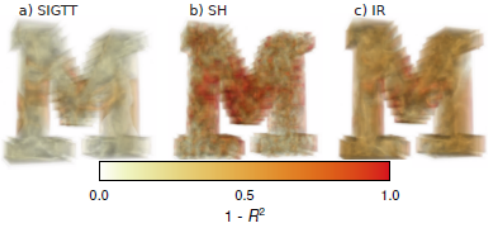

FIG. 3. **Volume renders of errors for reconstructions of "M". a)** Errors for SIGTT. **b)** Errors for SH. **c)** Errors for IR. The error is defined as $1 - R^2$, where R^2 is given by equation (15). Larger errors are rendered with greater opacity and are thus visible even if they are in the interior.

Experimental data

An ensemble of 10 reconstructions each with some initial conditions randomized were performed using SIGTT, SH and IR on a sample of trabecular bone. For experimental details, see Liebi *et al.* 2015, sample B [15]. The chosen q-range for this reconstruction does not contain the collagen peak, and therefore its reciprocal space map has fiber symmetry. A comparison of a virtual section from each of the three methods can be seen in Fig. 6. Because there is no ground truth to compare to for experimental data, the ensemble of reconstructions was instead analyzed to investigate the robustness of each method against perturbations in the initial conditions. The spherical harmonic representations of the 10 reconstructions were averaged over, voxel-by-voxel, and Fig. 6 shows the results of the averaged reconstruction. The colors of the orientation glyphs indicate the degree to which the anisotropy of the reciprocal space map changes across every reconstruction; the quantity Q is defined in equation (16). The glyphs are scaled according to the square root of the mean anisotropic power of each RSM (the anisotropic power is defined in equation (11)) across the ensemble. The results indicate that SIGTT and IR are robust to perturbations of the initial conditions, but that SH is not. However, the orientations of the averaged SH reconstruction agree well with those of SIGTT and IR. A plausible reason for this difference is that SH is the only method out of the three which depends on Euler angles. Depending on the initial conditions of the angles, the solution may be confined to local minima, as the symmetries of its reciprocal space can only vary across a limited subspace of the total spherical harmonic coefficient space. While IR performed similarly to SIGTT in the chosen q-range, a rank-2 tensor can not contain more than one local maximum per hemisphere. This poses a problem for the method in the case of the RSM of the collagen peak q-range of bone, which contains two distinct maxima - one lies along a great circle, and one lies on the poles orthogonal to that great circle. This symmetry, which requires at least a rank-4 tensor, can be represented by both SIGTT and SH [21].

DISCUSSION

This work has demonstrated the SIGTT method for SAXS tensor tomographic reconstruction of the reciprocal space map in samples using a band-limited spherical function expressed in spherical harmonics (see Methods for details). In three case studies using simulated data with approximately zonally symmetric reciprocal space maps, rank-2 tensors, and complicated-textured higher-order reciprocal space maps, the method produces results superior to the approaches of Liebi *et al.* (2018) and Gao *et al.* (2019)[16, 17]. SIGTT has also been shown to be robust to perturbations in the initial conditions when reconstructing experimental data. The reconstruction of the reciprocal space map up to higher spherical harmonic orders will enable the use of more specific methods of characterization that reveal information about the nature of the sample beyond the main orientation or adherence to a specific, predetermined symmetry. Samples frequently contain multiple domains of various orientations, and the SIGTT method makes it possible to study not just individual domains, but also the boundaries and transitions between them, including voxels with more than one orientation. SIGTT can be applied to more complicated reciprocal space maps, such as those that occur in SAXS measurements of samples with hexagonal symmetries, or wide-angle X-ray scattering measurements. This includes reciprocal space maps which are not necessarily well approximated by a rank-2 tensor. Similarly, SIGTT should make it easier to reconstruct samples with smaller or less well-organized domains. In this way, the reconstruction of the reciprocal space map using band-limited spherical functions makes full use of the data obtained from the collection of scanning SAXS data at multiple angles, and opens up many new venues of analysis. Finally, the anchoring of SIGTT in a framework of integral geometry and linear algebra highlights the potential for algorithms employing alternative schemes both for data acquisition and representation of the reciprocal space map.

METHODS

Discretized formalism

The inverse problem to the forward model of equation (5) is to obtain the distributions $a_m^\ell(\mathbf{r})$ for a set of measured data.

In practical terms, the solution to the inverse problem is best discussed using a discrete formalism. Prior to reconstruction, measurements at a particular value of $\mathbf{q}$ are reduced by binning the measured pixel intensities into azimuthal segments, which corresponds to an integral over a line segment on the sphere. Consequently, for the bin

The Discussion check list

Questions to answer while reading a Discussion

Structure

- ☐ Has the question posed in the **Introduction** been answered?
- ☐ Does each **paragraph** start with a **topic sentence**?
- ☐ Does the rest of the paragraph **support** the topic sentence?
- ☐ Have you clearly mentioned the **similarities** and **differences** to other research?
- ☐ Have the **Results** been transferred smoothly to the **Discussion**?
- ☐ Have you pointed out the **unexpected** findings, if any?
- ☐ Are the **limitations** clear?
- ☐ Does the **importance** of the study stand out?
- ☐ What is the **message** of the **conclusion**?

Style

- ☐ Have you used good **linking words**?
- ☐ Have you avoided "**empty**" words
- ☐ Have you watched out for **sentence length**?
- ☐ Have you used a **combination** of active and passive voice?