Expert Opinion

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Central nervous system delivery of large molecules: challenges and new frontiers for intrathecally administered therapeutics

Ryan G Soderquist & Melissa J Mahoney†
University of Colorado at Boulder, Department of Chemical and Biological Engineering, 424 UCB, Boulder, CO 80309, USA

Importance of the field: Therapeutic proteins and DNA constructs offer promise for the treatment of central nervous system disorders, yet significant biological barriers limit the ability of these molecules to reach the central nervous system from the bloodstream. Direct administrations to the cerebrospinal fluid (intrathecal administration) comprise an emerging field to facilitate the efficient delivery of these biological macromolecules to central nervous system tissues.

Areas covered in this review: Previous reports from 1990 to the present time describing the interactions and turnover of the cerebrospinal fluid within the intrathecal space, characterizations of the effects that therapeutic proteins and DNA have shown after intrathecal delivery through a lumbar route, and reports of emerging technologies to address the limitations of intrathecally administered macromolecules are reviewed.

What the reader will gain: This review provides an overview of the limitations that must be overcome for intrathecally administered biological macromolecules and the recent advances and promising approaches for surmounting these limitations.

Take home message: Emerging approaches that stabilize and sustain the delivery of intrathecally administered biological macromolecules may enhance substantially the clinical relevance of promising therapeutic proteins and DNA constructs for the treatment of various central nervous system disorders.

Keywords: blood–brain barrier, central nervous system, intrathecal, microparticle, PEGylation, PLGA

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1. Introduction

There is a key need to facilitate the clinical relevance of therapeutic treatments for central nervous system disorders. Even after decades of aggressive research in the area, the number of people suffering from debilitating or fatal central nervous system diseases still far outnumbers those dying of all types of systemic cancer or heart disease, and central nervous system disorders remain the world's leading cause of disability and necessitate more hospitalizations and prolonged care than almost all other diseases combined [1]. Debilitating central nervous system disorders include brain tumors [2], epilepsy [3], cerebrovascular diseases [4], neurodegenerative disorders including the widespread Parkinson's [5] and Alzheimer's diseases [6], multiple sclerosis or autoimmune encephalopathy [7], and chronic neuropathic pain [8]. Significant biological barriers that impede the delivery of drugs to the brain and

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Article highlights.

- Biological macromolecules are particularly limited by the formidable obstacles of brain and spinal cord drug delivery. The development of improved delivery methods is required to render these macromolecule-based therapeutic approaches clinically relevant.
- Single administration strategies refer to administrations that can provide a sustained therapeutic effect without the need for implantable infusion pumps and repeated intrathecal administrations.
- Initial studies indicate that protein PEGylation has significant potential for enhancing the CSF stability, spinal cord tissue distribution and therapeutic efficacy over time of intrathecally administered proteins.
- PEI complexation with DNA has also been shown to increase its resultant gene expression in the central nervous system. After an intrathecal administration polymers with improved biocompatibilities are expected to increase the clinical utility of the polymer complexation approach for intrathecal gene delivery.
- The encapsulation of therapeutic proteins and plasmid DNA within biodegradable polymer microparticles offers an approach that can promote therapeutic macromolecule release for prolonged periods of time.
- A single approach is unlikely to be optimal for every therapeutic candidate. Even though all of these delivery approaches still require development and extensive clinical testing before they are accepted for widespread clinical use, polymer-mediated delivery strategies will significantly expand the utility of intrathecally administered therapeutic treatments.

This box summarises key points contained in the article.

spinal cord have dictated the physical properties of potential drug candidates for many of these disorders, and have consequently limited the number of clinically relevant treatment approaches for many of these conditions.

Biological macromolecules, including therapeutic proteins and DNA, are among the list of promising drug candidates that are particularly limited by the formidable obstacles of brain and spinal cord drug delivery. Promising macromolecules for central nervous system delivery include therapeutic neurotrophins [9,10] and anti-inflammatory cytokines [11,12] along with therapeutic plasmid DNA facilitating the endogenous production of neurotrophins [13,14] and therapeutic cytokines [15,16]. The development of improved delivery methods is required to render these macromolecule-based therapeutic approaches clinically relevant.

2. Delivery considerations

2.1 Intrathecal administration for delivery to the central nervous system

The blood–brain barrier, which has been reviewed extensively previously [1,17], is the chief obstacle for macromolecular delivery to the brain and spinal cord. Briefly, the blood–brain barrier is a membranous barrier consisting of blood capillaries that are structurally different from blood capillaries in other tissues, as capillaries of the brain and spinal cord lack the small pores that allow the rapid movement of solutes from the circulation into organs. These capillaries are lined with a layer of special endothelial cells that lack fenestrations and are sealed with tight junctions, similar to the barriers formed in the skin, bladder, colon and lung, which render the brain and spinal cord practically inaccessible to water-soluble compounds, such as polar molecules and small ions from the bloodstream. Passage of water-soluble compounds from the blood to the cerebrospinal fluid (CSF) surrounding the spinal cord is also limited by this same barrier, and can be referred to as the blood–CSF barrier. Overall, these tight barriers typically act to protect the brain and spinal cord from systemic microbial and viral infiltration, and often protect brain and spinal cord tissues from harmful toxins in the bloodstream, but have also significantly limited the available oral and parenteral therapeutic treatments for central nervous system disorders to water-insoluble compounds.

New strategies that enable water-soluble molecules to cross the blood–brain barrier have been developed and reviewed previously. These efforts include the use of receptor-mediated transport systems, peptidomimetic monoclonal antibodies and particulate drug carrier systems [17,18]. Although these efforts show some promise, the ability to deliver biological macromolecules directly to the CSF, which bathes the brain and spinal cord, is at present one of the most promising approaches that can surmount existing delivery barriers. The administration of a drug to the CSF surrounding the spinal cord is known as intrathecal administration, as the drug is delivered to the intrathecal space of the spinal cord (**Figure 1A**). The intrathecal space is bordered by the spinal cord pia matter on the inside and the arachnoid membrane on the outside, and as such it is also commonly referred to as the subarachnoid space (**Figure 1B**). Intrathecal delivery offers several advantages, beyond the obvious advantage of bypassing the blood–brain barrier, for the administration of biological macromolecules to the spinal cord. Many active drugs are more potent and safer when injected into the intrathecal space, and owing to the increased proximity to the target tissue smaller dosages can be used, which potentially minimizes systemic toxicity [19]. Also, some drugs encounter decreased enzymatic activity in the CSF relative to drugs in the plasma, and because the CSF exchanges molecules with the interstitial fluid of the brain and spinal cord parenchyma (interior tissue), delivery to the CSF can theoretically increase resultant parenchymal drug concentrations [1].

2.2 Historical basis of intrathecal administration

Intrathecal administrations were primarily developed and have most commonly been used for the delivery of analgesics (pain medications), as other treatment avenues are ineffective and lead to unacceptable side effects for a large percentage of those suffering from acute and chronic pain [20,21]. Even though the

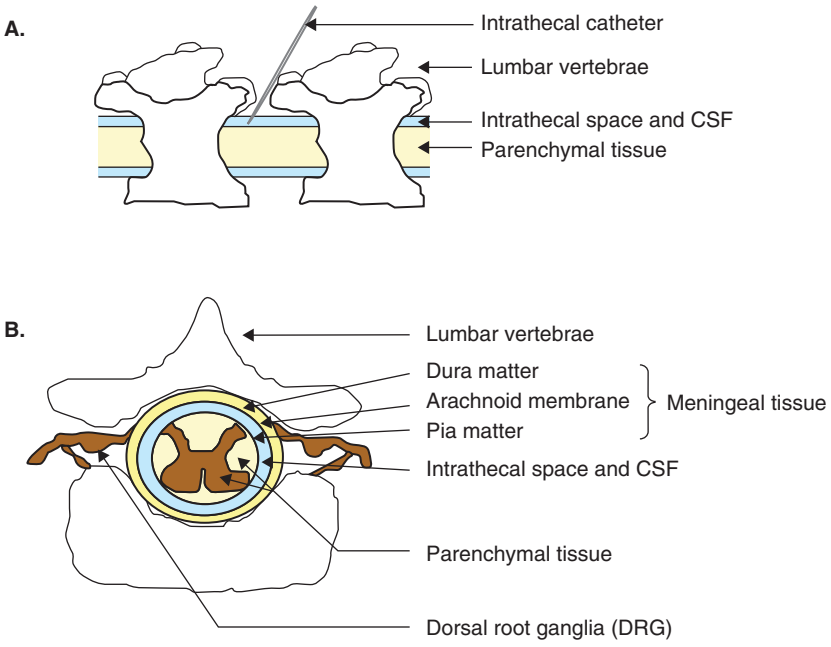

Figure 1. Intrathecal administration and the intrathecal space. A. Schematic diagram of an intrathecal administration. **B.** Schematic diagram of the spinal cord and the intrathecal space.

intrathecal administration technique must be performed by skilled medical personnel in aseptic conditions [19], current clinical practices in spinal pain intervention by intrathecal administration can be done on an out-patient basis, have a good safety record with a low rate of complications, and have led to considerable improvements in the quality of life for the treatment populations [22,23]. Although intrathecal delivery strategies were not commonly used until after the later 1980s for the treatment of persistent pain [20], at first glance it is rather surprising that more explorations have not been conducted with the delivery of therapeutic molecules for the treatment of more central nervous system disorders beyond small molecule analgesics and chemotherapeutic agents.

2.3 Limitations for macromolecule delivery

Existing limitations for the intrathecal delivery of biological macromolecules may partially explain the lack of extensive development in this area. Although the increased proximity of the CSF to the brain and spinal cord parenchyma is an advantage of intrathecal delivery, inconsistencies can arise between the interstitial fluid of parenchymal tissues and the CSF. In some cases, the concentration of a compound in the CSF can be used as a surrogate for assessing the concentration of a compound within the parenchymal tissue, but it has been shown that the CSF concentration is not necessarily an accurate predictor of unbound drug concentrations in the brain and spinal cord tissue [24]. Both the physical properties of a drug and the physiological state of the tissue, which can be altered by disorders such as epilepsy [25] and conditions such as hyperthermia [26], can increase the discrepancies that are observed between drug concentrations in the CSF and the interstitial fluid. This suggests that there may be a physiologically dependent and relatively uncharacterized CSF–brain barrier that consequently does not guarantee drug penetration into parenchymal tissues on intrathecal administration, and may be an increasing limitation for biological macromolecules of increasing size and hydrophilicity [27-29].

Rapid clearance from the CSF is another significant limitation of intrathecally administered treatments. CSF in the brain is produced in choroid plexus, descends into the lateral, third and fourth ventricles along with the spinal cord and then ascends to the superior sagittal sinus where it is exposed to arachnoid granulations. These granulations are valve-like structures that allow CSF to pass into the lumen of the sinus when the CSF pressure exceeds venous pressure [30]. The CSF essentially passes through these granulations without filtration, which enables the passage of cellular components and macromolecules. CSF can also pass to the periphery through arachnoid villi and spinal nerve roots along the spinal cord, and CSF components that have passed into the interstitial fluid, if left unbound by parenchyma, can drain into lymphatic tissues in smaller quantities [31]. The main absorption of CSF through the central nervous system is ultimately to the blood. This occurs at multiple sites along the length of the brain and spinal cord by the above mechanisms, and is commonly referred to as bulk flow [32].

2.4 Implantable infusion pumps

To overcome the diffusive limitations and clearance of intrathecally administered molecules and promote more sustained and continuous drug concentrations in the spinal cord and its surrounding tissues, first generation approaches have utilized surgically implanted reservoir pumps connected to implanted intrathecal catheters. These surgically implanted pumps have the advantage of overcoming the need for repeated intrathecal injections and also can be designed to allow for the modification of infusion rates. These devices have shown many drawbacks, however, as they are expensive, invasive, have complications resulting from the surgical procedure itself, lead to infection and inflammation and have catheter dysfunctions over time [1,19,20]. A particular concern with observed catheter dysfunctions is the high incidence of intrathecal granuloma formations, which have the potential for spinal cord compression and paralysis [20]. Finally, therapeutic agents must be maintained in an aqueous reservoir, which is not suitable for biological macromolecules that have limited stability in solutions at physiological temperatures [33].

2.5 Single administration approaches

The examples provided previously require multiple and continuous administrations in order to attain a sustained therapeutic effect. Single administration strategies refer to administrations that can provide a sustained therapeutic effect without the need for implantable infusion pumps and repeated intrathecal administrations. To obtain sustained therapeutic effects from a single injection of biological macromolecules, including therapeutic proteins or DNA constructs, efforts must focus on prolonging the stability and residence of these drugs in the CSF, to the extent permitted by the limitations of bulk flow. Promoting the penetration of biological macromolecules into the parenchymal tissue, and prolonging their residence in the meningeal tissue (dura matter, arachnoid membrane and pia matter; **Figure 1B**) surrounding the intrathecal space and spinal cord, is also needed in order to sustain the therapeutic effect of a single administration. Stabilization of biological macromolecules in the meningeal tissues surrounding the spinal cord is of particular interest for central nervous system disorders in which immune–central nervous system communication is hypothesized to play a key role. These meningeal tissues, which are comprised of highly immunocompetent cell types, have been shown to produce a range of cytokines involved in the development and maintenance of spinally derived neuropathies [34], may play a key role in other central nervous system disorders [35], and are readily exposed to intrathecally administered macromolecules.

2.6 Polymer–protein bioconjugation

Polymer–protein bioconjugation is rapidly becoming a common technique for the enhancement of protein stability in therapeutic applications that rely on oral and parenteral delivery, and may also be a useful approach for enhancing the stability, residence and tissue penetration of intrathecally administered proteins. Previous work with systemically administered proteins has in fact shown that covalently attaching a synthetic polymer to a protein significantly prolongs its half-life in the bloodstream [36-38]. Polyethylene glycol (PEG) is the most common polymer used for the modification of proteins owing to its excellent biocompatibility [39,40] and approval by the US FDA [41]. Although the attachment of PEG to a protein (PEGylation) can sterically hinder the protein's access to receptors and subsequent *in vitro* biological activity, PEGylated proteins typically have an increased systemic circulation time in the bloodstream [41-43], which compensates for marginal activity losses and often results in an overall *in vivo* therapeutic benefit [44,45]. For intrathecal applications, however, where the impact of bulk flow significantly reduces protein residence times in the CSF relative to the bloodstream, minimizing the activity losses after PEGylation is of increasing importance. With a reduced timescale for the onset of efficacy before protein clearance, it is necessary to identify PEGylation strategies that sufficiently preserve protein biological activities while increasing their stability and residence in the intrathecal space as much as possible.

PEGylation can also significantly enhance the diffusion of a molecule in tissues. Even though attaching PEG to a protein increases the overall size of a molecule, and larger molecules would theoretically show a reduced ability to diffuse through tissue, PEGylation creates a hydration layer around a protein that increases its solubility [46,47], reduces its nonspecific electrostatic interactions [48], and shields it from receptor-mediated uptake by surface tissues [40]. These effects therefore increase the potential for PEGylated proteins to penetrate and diffuse into tissue [49], including brain and spinal cord parenchyma. Previous work has indeed shown that PEGylated proteins have enhanced diffusion in *ex vivo* brain tissue slices [48] and *in vivo* penetration into the spinal column and forebrain after prolonged exposure to continuous intrathecal infusions [50].

PEGylation has also been shown to decrease the immunogenicity of proteins and shield them from enzymatic degradation and antigenic determinants of the immune system in the bloodstream [46,51]. These agents are less abundant in the CSF than in the bloodstream, but there is increasing evidence for the presence of serine proteases and antigenic determinants in the CSF [34,52]. These effects can also impart extra protection to PEGylated proteins from proteases and antigenic determinants in brain and spinal cord parenchymal and meningeal tissues [53,54]. Initial studies indicate that protein PEGylation has significant potential for enhancing the CSF stability, spinal cord tissue distribution and therapeutic efficacy over time of intrathecally administered proteins. Intrathecally administered PEGylated brain-derived neurotrophic factor shows an enhanced stability in the CSF and penetration into spinal cord tissue relative to the unmodified protein [55],

and it has more recently been shown that intrathecally administered PEGylated interleukin-10 has an enhanced *in vivo* therapeutic efficacy over time for the treatment of neuropathic pain [56].

2.7 Polymer complexation

Lipids and cationic polymers have been shown to interact with negatively charged DNA in a self-assembly process [57] that results in the formation of liposomes or polyplexes, respectively [58]. The most widely used synthetic polymer for gene delivery by complexation is polyethylenimine (PEI) [59]. PEI complexation has been shown to reduce intrathecal gene delivery requirements by a half to one-tenth [60-62]. PEI complexation with DNA has also been shown to increase its resultant gene expression in the central nervous system 10-fold [63] to 40-fold [60] after an intrathecal administration. Also, it has been shown that intrathecally administered PEI/DNA complexes can show a significant migration to the dorsal root ganglia and significantly enhance the regeneration of transected rat sciatic nerves [64].

Although these delivery improvements are very promising, major disadvantages from the use of synthetic and natural polycations, including PEI, for complexation are toxicity [65,66], lack of biodegradability and poor biocompatibility overall [67]. Although these considerations have previously limited the broad applicability of this technology, more recent efforts have shown that potential toxicity can be reduced by covalently linking PEI to carrier molecules such as PEG [63] or cyclodextrin [68] and that the biodegradability of PEI can be increased by the incorporation of ester and caprolactone groups [69]. The further identification and development of polymers with improved biocompatibilities is therefore expected to increase the clinical utility of the polymer complexation approach for intrathecal gene delivery.

2.8 Polymer encapsulation

The encapsulation of therapeutic proteins and plasmid DNA within biodegradable polymer microparticles offers an approach that can promote therapeutic macromolecule release for prolonged periods of time with minimized toxicity concerns [70]. The term 'microparticle' refers specifically to a particle with a diameter of 1 – 1000 μm [71], but as intrathecal injections are typically conducted with an 18- to 22-gauge needle, microparticles < 100 μm in diameter are preferred [19]. Although there are variations in the nature of a microparticle, it is usually assumed that a microparticle formulation is a mixture of a polymer and a biological macromolecule that is released over time as the polymer degrades [71].

The most common polymers for biological macromolecule encapsulation and delivery are poly(lactic acid) (PLA) [72] and poly(lactic-*co*-glycolic acid) (PLGA) [72-75] owing to their high biocompatibility and established approval by the US FDA [71]. PLGA degrades into the natural products of lactic acid and glycolic acid that are eventually eliminated from the body without side effects [71,76] and it has been shown that PLGA is biocompatible with and has no evidence of toxicity to neural tissues [33]. Initial work has demonstrated that the encapsulation of intrathecally administered small molecule analgesics into PLGA microparticles can significantly prolong the potency of analgesia with few side effects for pain control [77]. Studies with the intrathecally administered antispasticity drug baclofen have also shown that PLGA encapsulation can enable its therapeutic presence in the CSF for > 1 month while reducing its toxicity relative to bolus dosages [78,79].

2.9 Microparticle-mediated intrathecal protein delivery

The short (2 – 3 h) half-life of most therapeutic proteins in the CSF necessitates multiple injections to obtain the desired therapeutic effects [80,81]. Microparticle preparations can release protein products at a controlled rate in a sustained dosage form, and can stabilize them further from degradative enzymes and activity loss that occurs in an unprotected environment [70,75]. Many applications for protein delivery have utilized microparticle encapsulation to improve the stability and delivery of therapeutic proteins in the bloodstream and peripheral tissue over time [70,82,83]. Previous work demonstrating the merits of microparticle-mediated intrathecal protein delivery is more limited in scope, but shows promise for increasing the clinical relevance of intrathecally administered protein formulations [33].

2.10 Microparticle-mediated intrathecal gene delivery

Owing to the ability of therapeutic gene delivery to promote long-term therapeutic effects *in vivo*, there has been an increased degree of interest in the use of DNA delivery vectors in the central nervous system [84]. Owing to concerns over the safety of viral-mediated DNA delivery systems [85], most efforts for gene delivery to the spinal cord have focused on the delivery of non-viral plasmid DNA (pDNA), which has a good safety profile and is easy to manufacture [86]. Non-viral pDNA delivery, however, is often hampered by inefficient pDNA uptake and expression [85] and can require multiple high-dose intrathecal injections for enduring therapeutic efficacy in various central nervous system disorders [87].

High dosages of pDNA, which are often necessary for successful *in vivo* gene therapy in the central nervous system [15,80], are unfavorable from both a clinical and a process economics standpoint [88]. pDNA encapsulation with biodegradable PLGA microparticles can help meet the increasing need to induce human responses with lower and fewer doses of pDNA and also protects pDNA from nuclease degradation and rapid clearance [89]. Microparticle encapsulation can be used with large DNA plasmids, has simple preparations with flexibility in use and can offer cell-type specificity after

chemical conjugation with a targeting ligand [90,91]. Microparticle encapsulation also increases the persistence of pDNA released to the local environment, which is critical for prolonged *in vivo* effects [92]. Overall, pDNA encapsulation within biodegradable microparticles can therefore enhance gene transfer by increasing the number of cells expressing the transgene, the extent of transgene expression, or by shielding the vector from clearance and the host's immune response [33]. Even though there is only one reported use of the approach so far [62], improved pDNA delivery over time as a result of microparticle encapsulation offers potential for significantly enhancing and prolonging the ultimate expression of therapeutic proteins in the central nervous system after intrathecal administrations.

3. Conclusions

Biological macromolecules such as proteins and pDNA encounter significant obstacles after an intrathecal administration, yet they can still show transient therapeutic effects. To overcome the clinical limitations of intrathecally administered biological macromolecules, advanced technologies including polymer conjugation, polymer complexation and polymer encapsulation of biological macromolecules have recently been explored. The findings of these foundational reports indicate that polymer-mediated delivery strategies may significantly enhance the clinical relevance of intrathecally administered biological macromolecules.

4. Expert opinion

Although the administration of therapeutic proteins and pDNA to the bloodstream is the easiest and most traditional route of administration, the obstacle presented by the blood–brain barrier renders this delivery route infeasible for many therapeutic proteins and pDNA constructs. Owing to a previous lack of more advanced administration and stabilization approaches, therapeutic treatments for many central nervous system disorders have been restricted to molecules that can easily traverse the blood–brain barrier. The number of potentially therapeutic molecules that have not been adequately explored because of this obstacle is unknown, but there is a continued and unacceptable cost to individuals and society as a whole owing to a lack of adequate treatments for many central nervous system disorders. This therefore necessitates an adequate exploration of both small and large molecule therapeutic candidates, even if they are unable to cross the blood–brain barrier by means of systemic administration.

Efforts to develop delivery approaches that enable a therapeutic molecule to traverse the blood–brain barrier directly cannot be neglected and show promise [17,18]. The focus of this review, however, is on the potential of intrathecal delivery, which may ultimately be more widely applicable for a range of therapeutics when they are coupled with delivery vehicles that overcome the limitations inherent to the intrathecal space. Over the past two decades the emergence of a clinical infrastructure and trained personnel for intrathecal administration has made this route of delivery much more feasible [20], although the current clinical usage of the approach does not extend beyond the realm of small molecule analgesics. The inherent biological limitations of CSF flow and turnover in the intrathecal space at present require that intrathecally administered therapeutic proteins [80] and pDNA [87] occur with repeated and high dosages in order to promote a sustained therapeutic effect. Repeated and continuous intrathecal administration paradigms can lead to deleterious side effects and patient non-compliance over time [93-95]; hence, significant improvements in the delivery of intrathecally administered macromolecules are still required to render these approaches clinically applicable.

Specific research directions for intrathecally administered macromolecules will probably focus on several different areas, as a single approach is unlikely to be optimal for every therapeutic candidate. The only approach that has toxicity concerns is the use of positively charged polymers in a complexation approach. As concerns over vector toxicity and degradability therefore limit the use of liposomes and polyplexes, efforts with this technology will probably focus on the identification and development of materials that address these concerns adequately. Efforts with therapeutic protein PEGylation will probably focus on PEGylation approaches that minimize biological activity losses while stabilizing and sustaining the presence of therapeutic proteins in the intrathecal space as much as possible. Efforts with the encapsulation of therapeutic proteins and pDNA within degradable microparticles will probably focus on promoting the interaction of these vectors with targeted cell types, while sustaining therapeutic molecule release for as long as possible. Even though all of these delivery approaches still require development and extensive clinical testing before they are accepted for widespread clinical use, it is anticipated that the utilization of polymer-mediated delivery strategies will significantly expand the utility of intrathecally administered therapeutic treatments for a range of central nervous system disorders.

Declaration of interest

The authors have no affiliations with any organizations that have a direct or indirect financial interest in the contents of this report.

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Affiliation

Ryan G Soderquist & Melissa J Mahoney[†]
[†]Author for correspondence
University of Colorado at Boulder,
Department of Chemical
and Biological Engineering,
424 UCB,
Boulder, CO 80309, USA
Tel: +1 303 492 3573; Fax: +1 303 492 4341;
E-mail: melissa.mahoney@colorado.edu