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Combine and Conquer: Surfactants, Solvents, and Chaotropes for Robust Mass Spectrometry Based Analyses of Membrane Proteins

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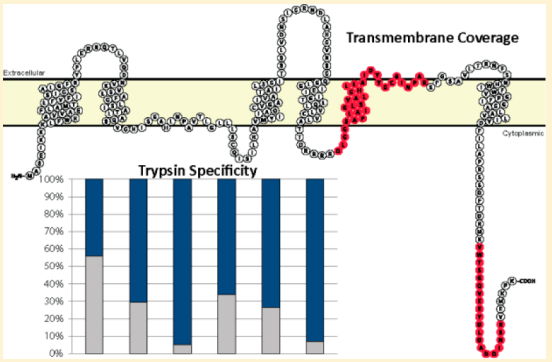
Supporting Information

ABSTRACT: Mass spectrometry (MS) based proteomic technologies enable the identification and quantification of membrane proteins as well as their post-translational modifications. A prerequisite for their quantitative and reliable MS-based bottom-up analysis is the efficient digestion into peptides by proteases, though digestion of membrane proteins is typically challenging due to their inherent properties such as hydrophobicity. Here, we investigated the effect of eight commercially available MS-compatible surfactants, two organic solvents, and two chaotropes on the enzymatic digestion efficiency of membrane protein-enriched complex mixtures in a multiphase study using a gelfree approach. Multiple parameters, including the number of peptides and proteins identified, total protein sequence coverage, and digestion specificity were used to evaluate transmembrane protein digestion performance. A new open-source software tool was developed to allow for the specific assessment of transmembrane domain sequence coverage. Results demonstrate that while Progenta anionic surfactants outperform other surfactants when tested alone, combinations of guanidine and acetonitrile improve performance of all surfactants to near similar levels as well as enhance trypsin specificity to >90%, which has critical implications for future quantitative and qualitative proteomic studies.



Membrane proteins constitute up to 30% of the total human genome and their critical roles in maintaining cellular structure and inter- and intracellular communication have been extensively reviewed.[1−4] Mass spectrometry (MS) based proteomic analyses can offer strategies to identify, quantify, and structurally characterize membrane proteins and their modifications, though these analyses are often challenging because of the inherent properties of this protein class. In particular, integral membrane, or transmembrane (TM), proteins have common structural features that allow for hydrophobic interactions with the lipid bilayer. This characteristic hydrophobicity coupled with the relatively low abundance of TM proteins, when compared to other protein classes,[3−5] results in the underrepresentation of TM proteins in typical global proteomic analyses, which often favor more soluble, abundant proteins and peptides.[3,4,6] Especially in bottom-up proteomic workflows where the enzymatic or chemical digestion of the protein into peptides of suitable m/z for routine analysis by MS is necessary, the efficiency of digestion directly affects the proteome coverage obtained. Particularly for TM proteins, digestion efficiency is hampered by the lack of solubility which results in reduced accessibility of the protein to the protease and sample loss due to precipitation and aggregation.[6]

Ideally, sample preparation conditions for the proteomic analysis of TM proteins should be carefully designed to maximize digestion efficiency without adversely affecting the protease activity or interfering with downstream MS analysis. In general, several classes of MS-compatible reagents are commonly employed to enhance enzymatic digestion of TM proteins including organic solvents, chaotropic agents, and surfactants. These additives aid in the separation of the protein from the lipid content, maintain protein solubility, and assist in protein unfolding to maximize the availability of protease cleavage sites. Organic solvents increase protein solubility by stabilizing the hydrophobic stretches of the proteins, although when used at sufficiently high concentrations, the organic solvent can affect the structure of the proteases in such a way that the apparent K_m value decreases.[4,6] Chaotropes disrupt protein interaction with the lipids in the membrane and

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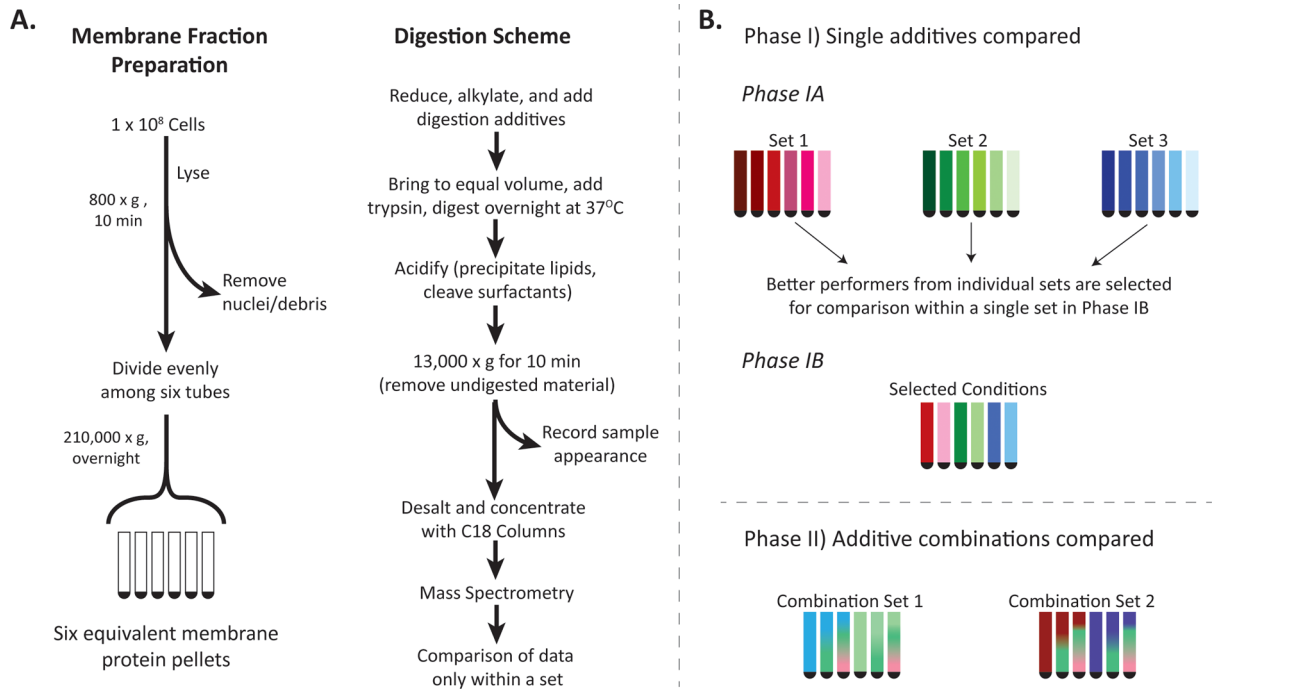

Figure 1. Overall experimental strategy: (A) Workflow of the membrane protein enrichment strategy and subsequent protein digestion scheme. (B) Summary workflow of phase I, where single additives were compared, and phase II, where additive combinations were compared.

stabilize the unfolded form of the protein allowing for better cleavage.[6] Surfactants, which are typically amphipathic, dissociate the protein from the lipid and solubilize the hydrophobic domains in an aqueous environment.[6]

The incorporation of various surfactants, solvents, and chaotropes for improving enzymatic digestion of complex protein mixtures has been the topic of several recent studies. Delipidation of membrane fractions using surfactants or chloroform extraction has been shown to increase proteome coverage of crude protein mixtures.[7,8] A comparison of the efficiency of digestion of three commercially available surfactants in aqueous and organic solvents on brain homogenate found that these solubilization strategies reduced the amount of starting material required to detect a broad range of proteins and observed a complementarity among the different conditions.[9] The efficiency of organic solvents, surfactants, and chaotropic agents—including combinations of the three—on the digestion of tomato microsomal fractions revealed that the various strategies provided complementary proteome coverage and that the efficiency of surfactants as compared to organic solvents was partially due to higher enzymatic activity in the aqueous environment of the surfactants.[10] The effect of chaotropes, organic solvents, and sequential digestion with multiple proteases on the number of proteins identified from membrane fractions enriched from mammalian cell culture found that each strategy increased the number of proteins identified as well as the percentage of membrane proteins identified, but concluded that a single approach is not applicable to all membrane studies.[6] Finally, the utility of various organic solvents, surfactants, and buffers on the extraction and digestion of the mouse brain proteome found that using a detergent-based protocol allowed for up to 40 times the protein yield as compared to that of organic solvents and acids.[11] However, this study utilized surfactants that were not MS-compatible, and thus required additional processing to prevent the surfactants from interfering with MS analyses. While these previous studies have collectively concluded that additives such as surfactants, organic solvents, and chaotropes can enhance the enzymatic digestion of proteins, the current literature lacks a comprehensive analysis of the effect of these additives specifically for the digestion of TM proteins, which is notably the most difficult class of proteins to access in proteomic studies.

The current study was designed to address this gap by focusing specifically on the effect of additives on membrane-enriched cellular subfractions and extends beyond current literature by including more commercially available surfactants than have been included in previous studies, examining multiple concentrations of the additives, and examining combinations of additives that have complementary properties. This study evaluated eight commercially available MS-compatible surfactants [Invitrosol, PPS Silent Surfactant, Progenta anionic acid labile surfactant (AALS) I, Progenta AALS II, Progenta cationic acid labile surfactant (CALS) I, Progenta CALS II, ProteaseMax, and RapiGest SF], two organic solvents (acetonitrile and methanol), and two chaotropes (urea and guanidine HCl) on the enzymatic digestion efficiency of membrane protein-enriched complex mixtures in a multiphase study using a gelfree MS approach. Although previous studies have investigated methods for digestion after solubilizing membrane fractions in sodium dodecyl sulfate (SDS),[11,12] our study aimed to avoid MS-incompatible surfactants like SDS entirely. First, the effect of various concentrations of individual additives on digestion efficiency was evaluated. This comparison was then followed by an evaluation of various combinations of the best performing individual additives. Multiple parameters, including the number of peptides and proteins identified, total sequence coverage, sequence coverage of TM domain, average peptide hydrophobicity, and digestion specificity (i.e., tryptic termini) were used to evaluate the TM protein digestion performance. Results have implications for future analyses of membrane proteins when maximum

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Table 1. Summary of the Final Concentrations of Additives Tested, Measured Critical Micelle Concentration (CMC), and the Resulting Sample Appearance Postdigestion

additive	final concentrations tested	CMC (%) 25 °C	CMC (%) 37 °C	sample appearance postdigestion
Organic Solvent				
acetonitrile	20%, 40%			cloudy, floating particulate, small pellet
methanol	60%			cloudy with particulate, medium pellet
chaotropic agent				
guanidine-HCl	1 M			cloudy with particulate, medium pellet
urea	1.6 M			cloudy, medium pellet
Surfactant				
Invitrosol	1×, 2×	0.064	0.059	clear, medium pellet
PPS Silent Surfactant	0.1%, 0.2%	>0.1	>0.1	cloudy, medium pellet
AALS I	0.1%, 0.2%	>0.1	>0.1	cloudy, medium pellet
AALS II	0.1%, 0.2%	>0.1	>0.1	clear, small pellet
CALS I[a]	0.1%, 0.2%	0.002	0.003	cloudy, large chunks of particulate
CALS II[a]	0.1%, 0.2%	0.023	0.025	cloudy, large chunks of particulate
ProteaseMax	0.05%, 0.1%	>0.1	>0.1	cloudy, small pellet
RapiGest	0.1%, 0.2%	0.064	0.089	clear, small pellet
Combinations				
Invitrosol+G[c]	2× Invitrosol, 1 M guanidine	0.049	0.058	clear, small pellet
Invitrosol+G/A[c]	2× Invitrosol, 1 M guanidine, 20% acetonitrile	0.191	0.188	clear, medium pellet
AALS I+G	0.2% AALS I, 1 M guanidine	*b*	*b*	clear, medium pellet
AALS I+G/A	0.2% AALS I, 1 M guanidine, 20% acetonitrile	*b*	*b*	clear, small pellet
AALS II+G	0.2% AALS II, 1 M guanidine	*b*	*b*	clear, small pellet
AALS II+G/A	0.2% AALS II, 1 M guanidine, 20% acetonitrile	*b*	*b*	clear, small pellet
RapiGest+G	0.2% RapiGest, 1 M guanidine	0.015	0.034	clear, small pellet
RapiGest+G/A	0.2% RapiGest, 1 M guanidine, 20% acetonitrile	0.132	0.162	clear, small pellet

[a]These digestion conditions were not continued to the MS analysis because of their postdigestion appearance. [b]Addition of guanidine resulted in some precipitation, which likely affects fluorescence detection (i.e., CMC value) at these conditions. [c]Abbreviations: G, guanidine; A, acetonitrile.

sequence coverage is required (e.g., mapping protein−protein interactions, identification of isoforms, and post-translational modifications), and moreover, for quantitation of membrane and soluble proteins when reproducible and specific enzymatic digestion is required.

EXPERIMENTAL SECTION

Cell Lysis, Membrane Protein Preparation, and Protein Digestion. The experimental strategy is summarized in Figure 1 and details are provided in the Supporting Information. Surfactants included Invitrosol (Life Technologies), PPS Silent Surfactant (Agilent), Progenta anionic surfactants (AALS I, AALS II), and cationic surfactants (CALS I, CALS II) (Protea Biosciences), ProteaseMax (Promega), and RapiGest SF Surfactant (Waters) (details in Table S1 in the Supporting Information) and samples were brought to the final concentration of surfactant, chaotrope, or organic solvent as listed in Table 1. Mixed membrane pellets were obtained as described in Supporting Information, and 100 mM fresh NH_4HCO_3 was added to the mixed membrane pellet in the ultracentrifuge tube and the volume of NH_4HCO_3 was adjusted such that all digestion conditions were performed in the same total volume. Throughout the remainder of the digestion protocol, the samples were vortexed at 750 rpm in the ultracentrifuge tube using a Thermomixer (Eppendorf). Subsequently, samples were reduced with 5 mM tris(2-carboxyethyl)phosphine (Sigma) for 20 min at 37 °C and then alkylated with 10 mM iodoacetamide (Sigma). Twenty micrograms of sequencing grade modified trypsin (Promega) was added, pH was adjusted to 8.5 when necessary, and samples were allowed to digest overnight at 37 °C. The resulting peptide samples were brought to a concentration of 0.5% trifluoroacetic acid (Thermo), incubated for 30 min at 37 °C to degrade the acid-labile surfactants, and then centrifuged at 13 000 rpm for 10 min to remove lipids, particulates, and undigested material. After centrifugation, the appearance of the resulting solution was observed (i.e., clear, cloudy, presence of particulates, etc.) and recorded in Table 1. Some conditions were not further analyzed by MS due to the large amount of undigested material and aggregation (noted in Table 1). To avoid any of the undigested material and/or precipitated lipids from being carried onto the next step, 400 μL of the supernatant (out of ∼450 μL total) was subsequently desalted and concentrated using C18 Micro spin columns (Harvard Apparatus) according to manufacturer's instructions and dried in vacuo.

Mass Spectrometry and Data Analysis. Two technical replicates of each sample were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) on an LTQ linear ion trap (Thermo) as described in the Supporting Information. For all analyses summarized in Figure 2−4, the results were based on the fully tryptic digest search. A separate database search was conducted as described in Supporting Information, but against a semitryptic peptide database, and was used to assess the total number and percentage of spectra matched to semitryptic peptides (i.e., only a single tryptic terminus), as summarized in Figure 5. Finally, to assess the ability for each digestion condition to access peptides that span

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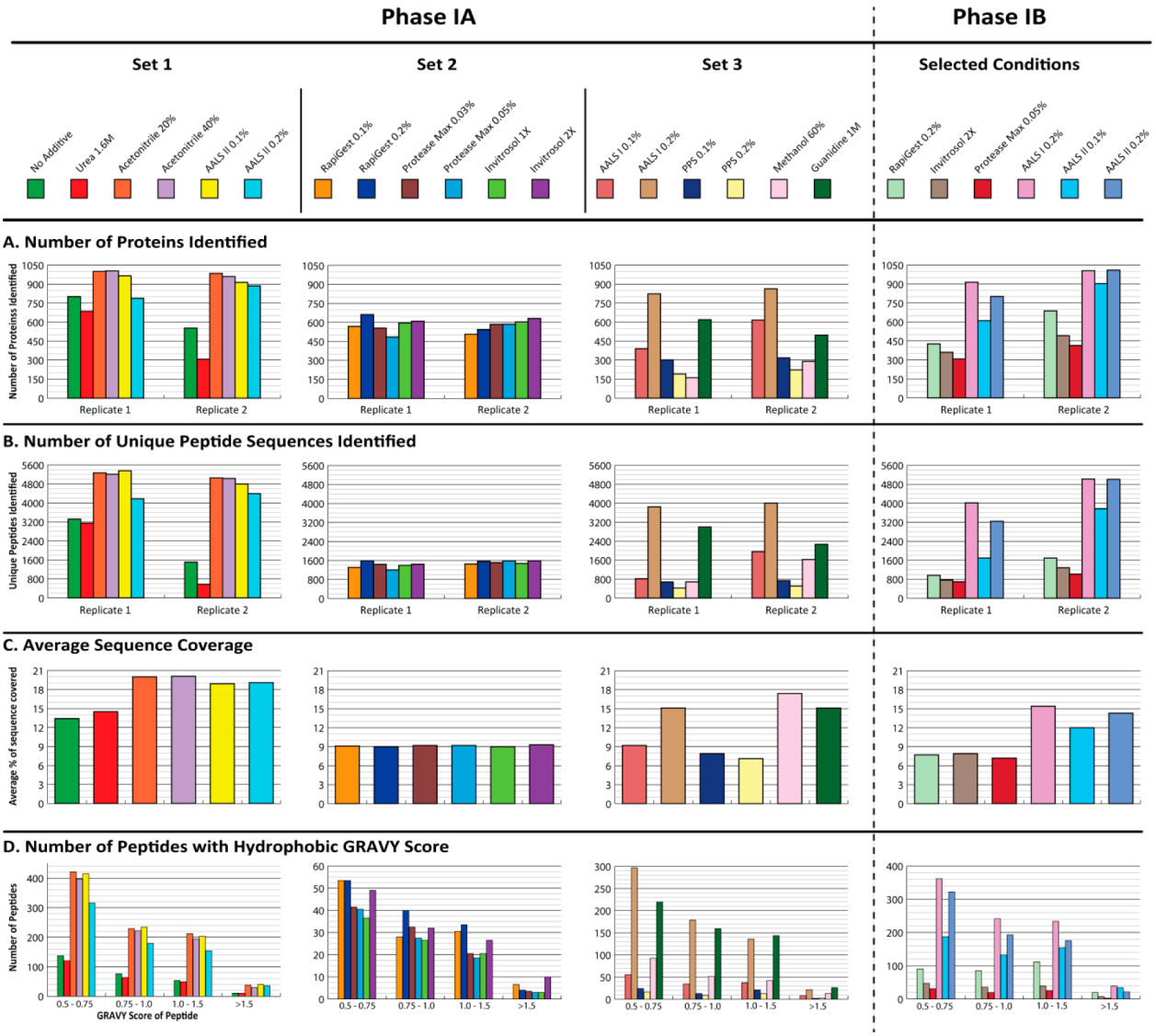

Figure 2. Summary of results from phase I (individual additives). For each tube set, (A) the total number of proteins identified, (B) number of unique peptide sequences identified, (C) average sequence coverage, and (D) number of peptides with hydrophobic GRAVY scores are shown. In (A) and (B), values for each biological replicate are plotted separately to illustrate consistent overall trends.

the predicted TM domains, a custom software tool was developed to map identified peptides onto TM topology information curated in UniProt, which is a combination of experimentally determined information and predictions, utilizing the predictive tools TMHMM, Memsat, Phobius, and hydrophobic moment plot method.[13] The mapping software, PeptideEclipse, is open source and can be accessed at http://ulo.github.io/PeptideEclipse/. Within these studies, a TM peptide is defined as a peptide that contains at least one amino acid from the annotated TM domain from UniProt.

Critical Micelle Concentration (CMC) Measurement. CMC of each surfactant was determined by a fluorometric method as described,[14] using 30 μM 1-anilinonaphthalene-8-sulfonic acid (1,8-ANS) (Sigma 10417-5G-F) for each assay, and measured at 25 and 37 °C in 100 mM NH_4HCO_3, NH_4HCO_3/1.0 M guanidine, and NH_4HCO_3/1.0 M guanidine/20% acetonitrile to accurately mimic each digestion condition tested. The excitation and emission wavelengths were 388 and 480 nm, respectively, and data were acquired using a FlexStation 3 MicroPlate Reader (Molecular Devices).

RESULTS AND DISCUSSION

Phase I: Comparison of Individual Additives Reveals Benefits of Anionic Surfactants. Relevant properties of each additive used are summarized in the Supporting Information and CMC values are included in Table 1. In general, the addition of guanidine or guanidine/acetonitrile affected the CMC; values for Invitrosol and RapiGest in the presence of guanidine are slightly lower than that in NH_4HCO_3 alone, but in the presence of guanidine/acetonitrile the CMCs are approximately 2 times higher than that in NH_4HCO_3 alone. However, for AALS I, AALS II, and ProteaseMax, the addition of guanidine resulted in some precipitate that likely affected the fluorescence measurement used to determine the CMC; thus, the effect of the additives remains unclear for these surfactants. Overall, no clear trend between surfactant performance (described below) and CMC value was revealed, which is consistent with a previous report.[15] As a first step toward assessing differences among digestion conditions, the post-digestion sample appearance, including the clarity of the sample, the size of the pellet, and presence of floating

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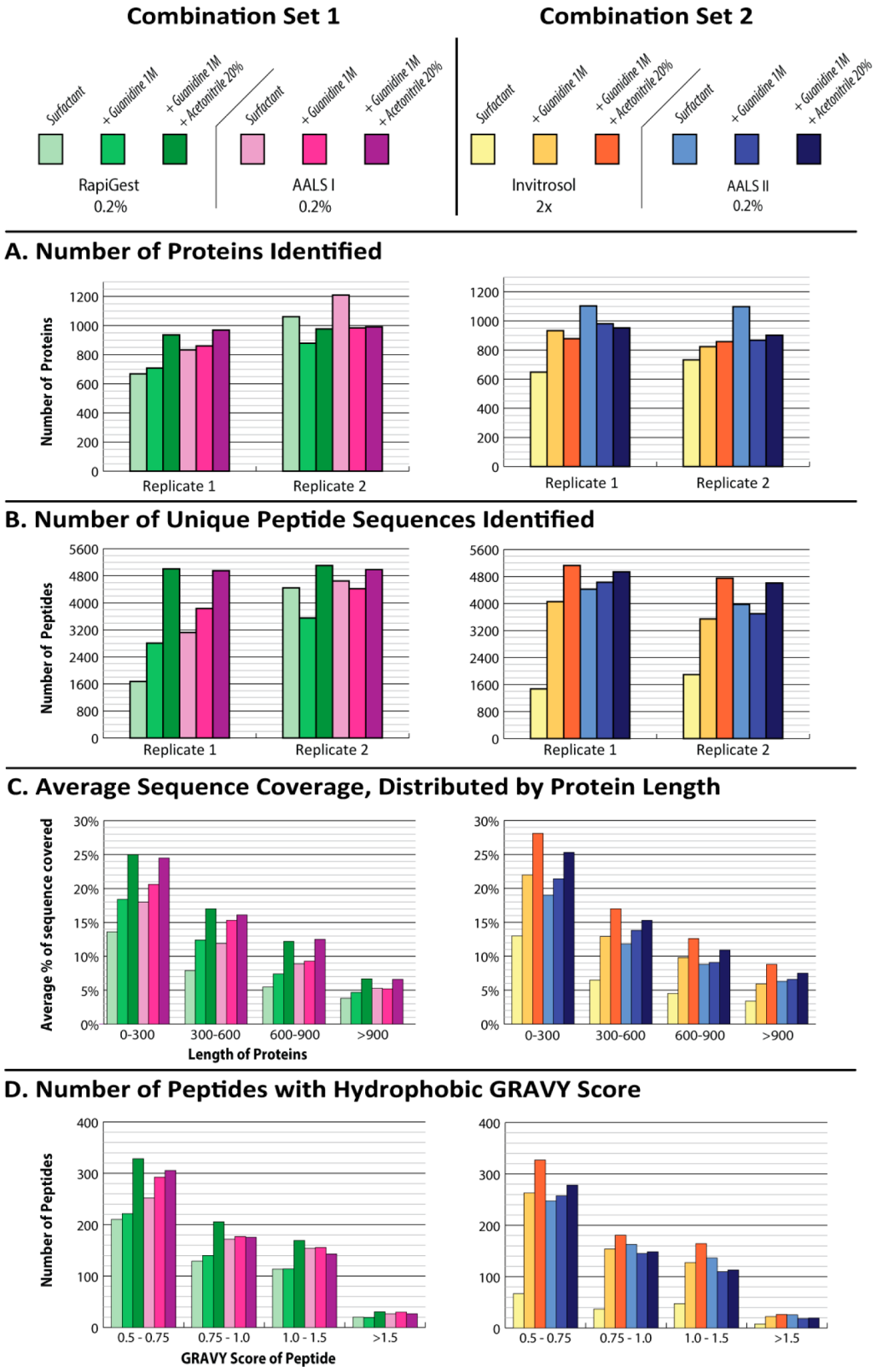

Figure 3. Summary of results from phase II (additive combinations). For each tube set, (A) the total number of proteins identified, (B) number of unique peptide sequences identified, (C) average sequence coverage, and (D) number of peptides with hydrophobic GRAVY scores are shown. In (A) and (B), values for each biological replicate are plotted separately to illustrate consistent overall trends.

particulate are summarized in Table 1. Although these observations are a qualitative and crude measure of the completeness of protein digestion, they are consistent with subsequent quantitative observations where the clear solutions with smaller visible pellets correlate to more complete digestion.

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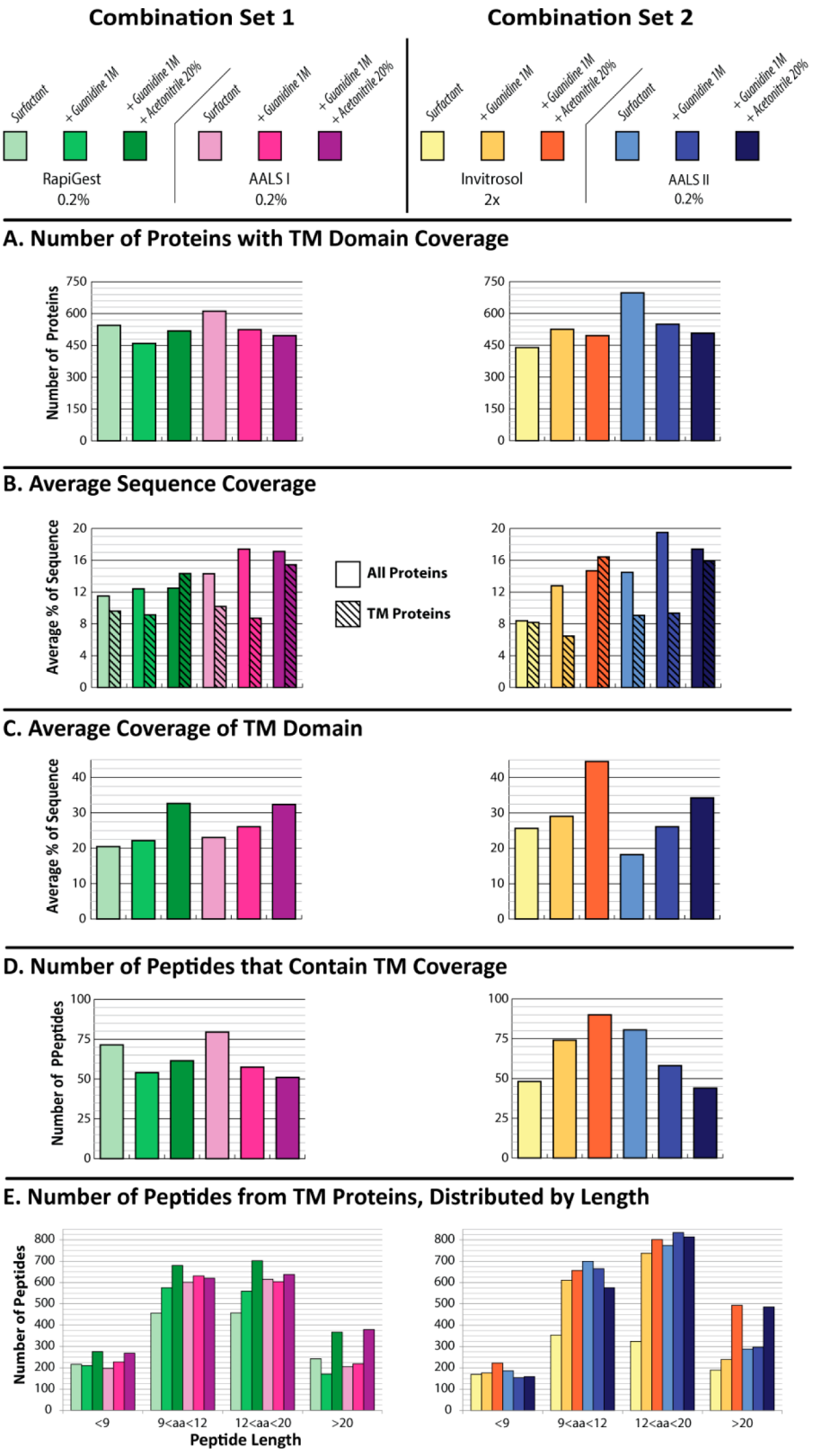

Figure 4. Assessment of TM domain mapping from additive combinations examined in phase II. Data include (A) the number of proteins identified by peptides containing at least one amino acid from the TM domain, (B) the average percent sequence coverage of all proteins and TM proteins, (C) average percent sequence coverage of the TM domain for those proteins identified by at least one peptide with TM coverage, (D) the number of peptides with TM domain coverage, and (E) the number of peptides from TM proteins, as a distribution of total peptide length.

Quantitative comparisons of the digestion conditions are summarized in Figures 2−5 and were carried out within biological replicates of each tube set such that the total protein amount for each comparison set was equivalent. When appropriate, data are grouped according to biological replicates to illustrate that although total protein among replicates/tube

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sets may vary slightly, trends in surfactant performance were consistent among replicates. Overall, among the surfactants, the anionic Progenta surfactants (AALS I, AALS II) performed most favorably across all parameters examined (Figure 2). Of the solvents, acetonitrile performed more favorably, especially for increasing the number of total proteins and TM proteins identified (Figure 2A, Figure 1A in the Supporting Information), and number of hydrophobic peptides (Figure 2D). Of the chaotropes, guanidine was preferable, especially for increasing the number of hydrophobic peptides and average sequence coverage of smaller proteins (Figure 2D, Figure 1B in the Supporting Information). In phase IA, it is possible that the total amount of protein varied among tube sets, and thus assessments regarding the best performing conditions were only made within each set. Thus, to further evaluate the most favorable conditions, the top six performing surfactants from phase IA (referred to as "Selected Conditions") were directly compared within a single tube set (in two biological replicates) in phase IB and included RapiGest 0.2%, Invitrosol 2×, ProteaseMax 0.05%, Progenta AALS I 0.2%, Progenta AALS II 0.1%, and Progenta AALS II 0.2%. Results from phase IB were consistent with those from phase IA, where AALS I and AALS II consistently outperformed the other surfactants in terms of overall proteome coverage, and more specifically, the number of transmembrane proteins and hydrophobic peptides (Figure 2, Figure 1 in the Supporting Information).

Phase II: Comparison of Additive Combinations Reveals Benefits of Guanidine and Acetonitrile. On the basis of previous studies,[9,10] and the data from phase I indicating that some additive classes may assist digestion in several but not all parameters, phase II was conducted to determine whether combining the surfactants with acetonitrile (best solvent) and guanidine (best chaotrope) could further enhance the digestion. These additives were tested in combination with the three top performing surfactants identified from phase IB (AALS I 0.2%, AALS II 0.2%, and RapiGest 0.2%) as well as Invitrosol 2×. Invitrosol, though not a top performer on its own, was selected because of its low cost compared to those of the other surfactants (Table S1 in the Supporting Information) and to investigate whether the vast differences among individual surfactant performances could be mitigated simply by the inclusion of inexpensive additives like acetonitrile and guanidine.

Results from phase II are summarized in Figure 3 and Figure 2 in the Supporting Information, and for the remainder of the study, digestion conditions are abbreviated as follows: NH_4HCO_3 with 1.0 M guanidine is referred to as "+G" and NH_4HCO_3 with 1.0 M guanidine/20% acetonitrile is referred to as "+G/A". The effects of guanidine and acetonitrile on the surfactants varied slightly among the four surfactants compared in this phase. The +G/A condition generally increased the total number of proteins and peptides identified for each surfactant, with the exception of AALS II that showed an increase in total number of peptides, but a decrease in total proteins. For all surfactants, average sequence coverage was improved with +G/A, but only for RapiGest, AALS I, and Invitrosol did the number of hydrophobic peptides increase. Notably, although AALS I outperformed RapiGest when surfactants alone were compared, and AALS II outperformed Invitrosol (phases I and II), RapiGest+G/A was similar to AALS I+G/A, and Invitrosol +G/A was similar to AALS II+G/A in all categories (Figure 3).

Custom Software Reveals Additive Combinations Enhance Transmembrane Domain Accessibility. The number of peptides with hydrophobic GRAVY scores (>0.5) and the number of identified TM proteins are summarized in Figures 2−4 and indicate that AALS I and AALS II provide optimum accessibility of TM proteins among surfactants alone. To more directly assess the ability of each condition to specifically access the TM domain of the protein, the custom software program PeptideEclipse was developed to map the peptide sequences observed onto the annotated TM topology provided in the public database UniProt. For these analyses, the average TM coverage considered only the proteins for which some of the TM was observed (i.e., the proteins included in Figure 4A) and was calculated by dividing the number of observed amino acids from all TM regions by the total number of amino acids predicted to be from all the TM regions. Overall, the addition of guanidine and acetonitrile consistently improved the ability of the surfactants to access the TM domain. First, while the +G/A condition decreased the total number of proteins that were identified by a peptide from the TM domain (Figure 4A), it increased the average coverage of all proteins, and of TM proteins, independent of the number of predicted TM domains (Figure 4B and Figure 2B in the Supporting Information). Second, the +G/A condition led to an increase in TM domain coverage (Figure 4C), despite a decrease in the number of peptides that contained amino acids from within the TM domain for all surfactants except Invitrosol (Figure 4D). Effectively, although the addition of guanidine and acetonitrile decreased the number of peptides from the TM domain, when a peptide from TM domain was observed, higher coverage of the TM domain was achieved. A detailed analysis of the characteristics of the peptides from the TM proteins reveals an explanation for this observation; namely, that the addition of guanidine and acetonitrile resulted in longer peptides being identified (Figure 4E), thus explaining how fewer numbers of unique peptide sequences could give rise to higher TM protein sequence coverage. This trend is consistent with a previous observation that TM domains are represented by longer tryptic peptides than soluble regions.[16] A closer look at the peptides from the TM spanning regions reveals that enhanced digestion specificity (described below) may be one cause of this increase in peptide length because as the fidelity of digestion is increased in the +G/A condition, longer (i.e., fully tryptic) peptides result. Moreover, digestion efficiency of the TM peptides was assessed by combining data from conditions in phase II and considering only peptides with ≥20 amino acids (length of a TM domain) that span the annotated TM domain. For these peptides, surfactant alone yielded 13 peptides with one missed cleavage out of 236 peptide observations whereas surfactant +G/A had 1 peptide with one missed cleavage out of 284 peptide observations. Thus, while it is possible that additional aspects of the analytical technique may affect which peptides are observed more readily, such as the reduced ability to fragment large peptides, preferential ionization of hydrophobic TM peptides vs hydrophilic non-TM peptides, and/or less than optimal handling of higher charge state peptides by the bioinformatics, these trends suggest that the addition of guanidine and acetonitrile improve sequence coverage of TM proteins and specifically the TM domain by improving digestion efficiency and specificity.

Trypsin Specificity Is Enhanced by Acetonitrile—Implications for Quantitation. The specificity of digestion (i.e., percentage of fully tryptic vs semitryptic peptides) was evaluated because of its importance in protein quantitation, which requires predictable and reproducible digestion.[17]

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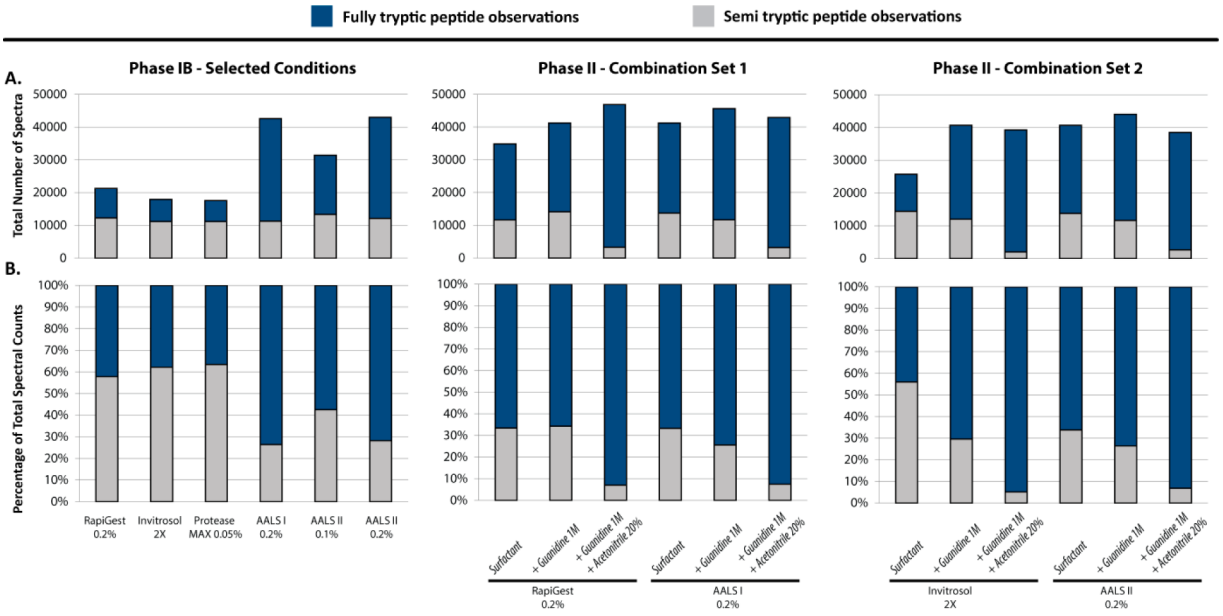

Figure 5. Specificity of enzymatic digestion: (A) Total number of spectra and (B) percentage of spectra observed that contain two tryptic termini (fully tryptic) or one tryptic terminus (semitryptic) for the analyses in phase IB and phase II.

Especially in the case of selected reaction monitoring (SRM) assays, the ability to reliably obtain fully tryptic peptides is a requirement for accurate quantitation.[17,18] Digestion specificity was determined by counting the total number of spectra matched to peptides with either one or two tryptic termini and because the occurrence of peptides with zero tryptic termini was negligible (<1%), these data were excluded. Examining surfactants alone, the number of semitryptic peptides is relatively constant among all surfactants compared in phase IB, and these analyses reveal that the increase in the total number of spectra observed in the AALS I and II conditions may be due to more fully tryptic peptides (Figure 5A). Thus, in addition to identifying more total peptides and proteins (as shown in Figure 2), inclusion of surfactants should be beneficial for quantitative studies. Examination of the additive combinations from phase II reveals an unexpected yet striking trend, where the addition of guanidine and acetonitrile increases the number of fully tryptic spectra for each surfactant (Figure 5A) while also reducing the number of semitryptic spectra, such that the overall specificity of digestion increases from ∼50% (surfactant alone) to greater than 90% fully tryptic with the inclusion of guanidine and acetonitrile (Figure 5B). Although the analysis of tryptic termini could be better assessed with high mass accuracy data, the trend is consistent across all investigated surfactants and the implications are critical for protein quantitation studies, which rely on the ability to reproducibly generate fully tryptic peptides.

Opportunities for Further Analyses. This study provides an extensive comparison of commercially available MS-compatible surfactants for TM protein digestion; however, opportunities for further analyses remain and may provide additional insights. Alternative digestion enzymes (e.g., Lys-C and Asp-N) may respond differently to the additives used in this study, especially considering the recently described effect that trypsin source (i.e., bovine vs porcine) has on digestion specificity.[19] Additional surfactants (e.g., β-octylglucoside and Progenta zwitterionic surfactants) and solvents (e.g., isopropanol) could also be examined. Moreover, it is expected that when surfactants are included during the cell lysis stage, heat denaturation of proteins prior to digestion, deglycosylation (e.g., PNGaseF), and the use of high mass accuracy instrumentation (which allows for charge state screening to ignore singly charged lipids in precursor selection for MS/MS) may affect the overall TM protein coverage for all conditions tested here. An investigation of the structures and physical properties of the surfactants can suggest which surfactant moieties are most critical in aiding TM protein digestion. On the basis of the results from this study, the anionic surfactants with a sulfate group and a long acyl chain perform the best across parameters measured here, but the relationship between CMC and surfactant performance is unclear. Finally, protein solubilization depends heavily on cosolubilization of lipids,[20] and surfactants not only displace the lipid from the protein but they also maintain the lipids in solution (i.e., out of the way of the protein). Thus, while fibroblasts were analyzed here because they are commonly used for in vitro assays and the results are expected to be generally applicable to any cell type, especially other adherent cell types, it is possible that optimum TM protein digestion conditions will be cell type/tissue specific and depend largely on the lipid composition, which can vary greatly among cell types.[21] Thus, the combinations of solubilizing agents should be tailored to the specific needs of the study and should consider whether maximum digestion specificity is required (e.g., protein quantitation) and/or maximum transmembrane protein coverage is desired (e.g., structural biology and protein−protein interactions).

■ CONCLUSIONS

Using a gelfree approach, the efficiency and specificity of membrane protein digestion was evaluated for a wide range of commercially available MS-compatible surfactants, solvents, and chaotropes. The detailed analyses made possible by the new software program PeptideEclispe allow for novel insights into TM domain accessibility and is thus expected to be beneficial to the broader community for future proteomic analyses. These data demonstrate that the inclusion of guanidine and

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acetonitrile in addition to surfactants maximizes overall digestion specificity, total number of peptides, average sequence coverage among all proteins, and the sequence coverage of the TM domain. A major benefit of the additives examined here, when compared to other detergents (e.g., SDS, Triton-X, NP-40, and CHAPS), is they do not require complicated or extensive methods for removal prior to MS analysis, which can result in protein loss. In addition to membrane-enriched fractions obtained by differential centrifugation, as described here, these digestion strategies should be more generally applicable to other strategies that include protein precipitation (e.g., TCA/acetone, methanol/chloroform) where the subsequent resolubilization is notoriously difficult. Moreover, the unique observations regarding the impact of acetonitrile and guanidine on enzyme specificity are expected to be significant for any quantitative proteomic study, including both soluble and membrane proteins. In conclusion, MS-compatible surfactants, solvents, and chaotropes are easy additions to membrane protein digestion schemes and their benefits for more complete and predictable digestion outweigh their financial cost.

■ ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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