Assessing Enzyme Activities Using Stable Isotope Labeling and Mass Spectrometry

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Running Title:
Assessing enzyme activities using CAPE
Abbreviations

ABPP, activity-based protein profiling; CAPE, catch-and-release activity profiling of enzymes; CAR, catch-and-release; FP, fluorophosphonate; FP-CAR, fluorophosphonate catch-and-release reagent; ICAT, isotope-coded affinity tags; SILAC, stable isotope labeling with amino acids in cell culture; STI, soybean trypsin inhibitor; LYPLA1, Acyl-protein thioesterase 1; FASN, Fatty acid synthase; WBSCR21, Williams-Beuren Syndrome critical region protein 21 form D; APEH, Acylamino-acid-releasing enzyme; PAFAH1B3, Platelet-activating factor acetylhydrolase IB γ subunit; ESD, Esterase D; ACOT2, Peroxisomal acyl-coenzyme A thioester hydrolase 2A; ABHD10, Hypothetical protein FLJ11342; SIAE, Sialate O-acetylersterase precursor.
SUMMARY

Activity-based protein profiling has emerged as a valuable technology for labeling, enriching and assessing protein activities from complex mixtures. This is primarily accomplished via a two step identification and quantification process. Here we show a highly quantitative and streamlined method, termed Catch-and-release Activity Profiling of Enzymes (CAPE), which reduces this procedure to a single step. Furthermore, the CAPE approach has the ability to detect small quantitative changes that may have been missed by alternative mass spectrometry-based techniques.
INTRODUCTION

Mass spectrometry and stable isotopes have recently surfaced as fundamental tools for large-scale proteomics analyses. Among these techniques are isotope-coded affinity tags (ICAT) (1) and stable isotope labeling by amino acids in cell culture (SILAC) (2), which have recently been used to study an array of biological questions (3-7). These technologies utilize differential labeling of proteins with carbon and nitrogen stable isotopes, giving rise to isotopically “light” and “heavy” samples. The major strength of these methods is their direct application to mass spectrometry, which can yield rapid protein identification. Quantification can also be achieved by comparing intensities of light and heavy peptide peaks. While these methods are useful for obtaining quantitative measurements of protein levels, they are strongly biased towards the most abundant proteins.

To go beyond a mere listing of general protein levels, alternative methods must be employed for gaining better sensitivity and readout of protein activity. One of the most elegant among these procedures is activity-based protein profiling (ABPP) (8), which includes the use of functional tags that specifically bind to and covalently modify active enzymes but not their endogenously inhibited or inactive zymogen forms. By only labeling functional proteins within a specific enzyme class, catalytically active proteins can be selectively enriched and subsequently quantified to yield relative activity levels between biological samples. Functional probes for many classes of enzymes have already been described, including those specific for serine hydrolases, metalloproteases, kinases and others (9, 10). To date, however, characterization of proteins by ABPP has been inefficient by requiring two steps. Qualitative information is acquired from mass spectrometry while accurate quantitative data must be obtained from the relative in-gel fluorescent intensities of the labeled enzymes (11). Recent gel-free studies have attempted to simplify this process but were limited by the semi-quantitative nature of the analysis (12) or were still reliant on a two-step procedure using capillary electrophoresis and mass spectrometry (13). A highly quantitative, streamlined approach that takes advantage of stable isotope labeling and mass spectrometry would greatly benefit the field of functional proteomics.
EXPERIMENTAL PROCEDURES

Synthesis of cleavable fluorophosphonate catch-and-release (FP-CAR) probe

\[ X + Y \rightarrow Z \]

Reaction of isotopically “light” intermediate reactant (Y) from Gartner et al. (14) and an alkyl chain-linked fluorophosphonate (X) was carried out in DMF for 4 hr as described (15) to yield final FP-CAR reagent (Z). High resolution mass spectrometry was performed on final product to confirm expected mass (Supplemental Fig. 1).

FP-CAR reactions with trypsin and trypsinogen. Bovine trypsin (acetylated) and trypsinogen were purchased from Sigma (St. Louis, MO). Trypsin and trypsinogen were incubated at 10 µg/mL (UV-vis) in 50 mM Tris-HCl (pH 8.0) with 5 µM FP-CAR reagent for 1 hr at room temperature. FP-CAR reaction conditions (5 µM for 1 hr at room temperature) were consistent throughout this report. For silver staining, equal amounts of protein (225 ng) were run on a 1 mm 4-12% gradient gel (Invitrogen, Carlsbad, CA) and stained with silver. For avidin blotting, equal molar amounts of protein (100 fmol) were resolved by SDS-PAGE as described above and transferred to PVDF. Membrane was blocked in casein (Vector Laboratories, Burlingame, CA) followed by incubation with avidin-HRP (1:10,000 dilution, Vector Laboratories) for 1 hr at room temperature. After 5 washes in PBS-tween 20 (PBS-T), membrane was treated with Western Lightning Chemiluminescence Reagent Plus (PerkinElmer Life Sciences, Inc., Boston, MA) and exposed to Hyblot CL autoradiography film. Trypsin (10 µg/mL) was treated with a 3-molar excess (26 µg) of soybean trypsin inhibitor (STI) for 2 hr at room temperature (compared to untreated control) followed by incubation with FP-CAR. Reaction was carried out at pH 7.0 due to STI instability under basic conditions. Silver staining and avidin-blotting were carried out as described above. Trypsinogen (1 µM) was treated with enteropeptidase (1 nM, Sigma) for 30 min (compared to untreated control) followed by incubation with FP-CAR. The enteropeptidase reaction was carried out in sodium citrate buffer at pH 5.6 to prevent auto-activation of
trypsinogen (16). Protein was subjected to silver staining and avidin-blotting as described above, with 1 pmol protein used for avidin-blotting.

For mass spectrometry analysis of the trypsin active-site peptide, unmodified Bovine trypsin (Sigma) was incubated at 10 µg/mL in 50 mM Tris-HCl (pH 8.0) with 5 µM FP-CAR reagent for 1 hr at room temperature. Protein was separated by SDS-PAGE and stained with Coomassie blue. After destaining, band was excised and processed for reduction/alkylation. Briefly, gel pieces were reduced with TCEP for 90 min at 50°C followed by alkylation with iodoacetamide. Sample was incubated with trypsin overnight and prepared for tandem mass spectrometry by passage through a C_{18} STAGE tip (17).

**Cell culture and protein preparation.** LNCaP and LNCaP-LN3 cells were plated at 1x10^6 cells/15 cm dish in 8 dishes each and cultured as described (18). Briefly, cells were grown in RPMI-1640 media (Invitrogen) with dialyzed serum (Gibco). LNCaP cells were cultured with ^{12}C^{14}N-lysine and ~arginine (“light” condition), whereas LNCaP-LN3 medium contained ^{13}C_{6}^{15}N_{2}-lysine and ^{13}C_{6}^{15}N_{4}-arginine (Isotec, Sigma)(“heavy” condition). Media was supplemented with 1 nM dihydrotestosterone (DHT) to promote optimal growth of testosterone-sensitive prostate cancer cells (19). Cells were cultured for 6-7 days, changing media every 2-3 days until 70-80% confluency was reached. (Note: cells grown under dialyzed conditions showed similar growth rates and no noticeable differences in morphology as cells cultured with non-dialyzed serum.) For whole-cell lysate (WCL) protein preparation, plates were washed 3X in ice-cold PBS and collected in 50 mM Tris-HCl (pH 8.0) and 1% Triton X-100. Cells were dounce homogenized 20X on ice and ultracentrifuged at 20,000Xg for 5 min at 4°C. Pellets were discarded and supernatant protein concentrations were determined by BCA assay (Pierce Biotechnology, Rockford, IL).

**FP-CAR reaction with cell lysates.** WCL from LNCaP and LNCaP-LN3 was incubated with 5 µM FP-CAR reagent for 1 hr at room temperature. After addition of protease inhibitors (Roche Applied Science, Indianapolis, IN) and phosphatase inhibitors (25 mM NaF, 10 mM Na_{4}P_{2}O_{7}, 1 mM Na_{3}VO_{4}), protein was mixed 1:1 and dialyzed in buffer containing 10% methanol, 50 mM ammonium bicarbonate, 5 mM EDTA and 0.05% SDS for 4 hr at room temperature to remove excess FP-CAR reagent. Three-hundred microliters of avidin solution (50% slurry of immobilized avidin, Pierce Biotechnology) was added to protein sample and rocked overnight at
4°C. Protein sample was transferred to a column, with non-specific avidin binding washed with 50 mL Wash Buffer 1 (100 mM ammonium bicarbonate, 2 M urea, 0.1% SDS, 150 mM NaCl, 0.5% Triton X-100 and 20% glycerol) followed by 50 mL of Wash Buffer 2 (100 mM ammonium bicarbonate, 10% methanol and 0.5% octyl-glucoside (w/v)). Beads were then transferred to Eppendorf tubes and reduced in TCEP (10 mM in Wash Buffer 2) for 90 min at 50°C to cleave FP-CAR reagent and release proteins bound to avidin. Overlays were removed, saved and avidin resin was washed twice in Wash Buffer 2 for 15 min with shaking. Washes were combined with overlays, followed by TCA precipitation, reduction and alkylation. In-solution trypsin digestion was performed overnight, acidified with acetic acid and evaporated in vacuo. Peptides were purified with C18 STAGE tips before they were subjected to LC-MS/MS analyses.

**LC-MS/MS.** Microcapillary liquid chromatography electrospray ionization tandem mass spectrometry (LC-MS/MS) was performed on a hybrid linear quadrupole ion trap/Orbitrap (LTQ Orbitrap) (20) or a hybrid linear quadrupole ion trap/Fourier transform ion cyclotron resonance (LTQ FT) (21) mass spectrometer (Thermo Electron, San Jose, CA) equipped with an Agilent 1100 Series binary HPLC pump (Agilent Technologies, Palo Alto, CA) and a Famos autosampler (LC Packings, Sunnyvale, CA). Peptides were separated on fused silica microcapillary columns (i.d. 125 µm, approx. 5 µm at the tip) which were in-house packed to a length of 18 cm with a C18 reversed phase resin (Magic C18AQ, 5 µm particle size, 200 Å pore size, Michrom BioResources, Auburn, CA). Separation was achieved by applying a gradient from 5-30% acetonitrile in 0.125% formic acid at a flow rate of 300 nL/min for 60 min (CAPE experiments, LTQ Orbitrap), 90 min (SILAC experiments, LTQ Orbitrap) or 35 min (characterization of trypsin active-site peptide, LTQ FT, gradient from 5-70% acetonitrile). MS and MS/MS data were acquired in a data-dependent fashion: 10 LTQ MS/MS spectra were acquired on the most abundant ions detected in a low-resolution master spectrum while high-resolution MS scans were collected in which 1x10⁶ ions were measured over an m/z range of 375-1800 (resolution settings were 60,000 for the Orbitrap and 100,000 for the FT).

**Database searching of MS/MS data.** MS/MS spectra were searched with a fully tryptic enzyme constraint (two tryptic termini and up to two internal missed cleavages) against a concatenated target (forward orientation) and decoy (reversed orientation) human IPI protein
database (ftp.ebi.ac.uk/pub/databases/IPI/current/) using the SEQUEST algorithm (22) and a mass tolerance of ±200 parts-per-million (ppm). Searches were performed with dynamic modifications on methionine (oxidation), lysine and arginine with masses 15.9949, 8.0142 and 10.0083 Daltons (Da), respectively, along with a static modification of 57.0214 Da on cysteines (alkylation). Using in-house software similar in principle to that developed by Kislinger et al. (23), dCn, XCorr and ppm thresholds were optimized for peptide charge states of 1, 2, 3 and 4 to yield an estimated 1% false positive rate (24) with maximum sensitivity.

**Peptide and protein quantification.** Peptides were quantified with the VISTA program as described (18). While CAPE led to the identification of 25 hydrolase proteins (Supplemental Table 1), only peptides with the highest VISTA confidence scores were used for quantification, and only proteins detected with at least two quantified peptides in each of the four experiments (control and experimental, with biological replicates for each) were considered for analysis. We note that additional non-serine hydrolase proteins were identified in our study predominantly due to the LTQ instrument sensitivity. We also note that the vast majority of these proteins in our raw dataset were single-hit identifications and thus excluded from analysis. Peptide ratios were determined by dividing area under the curve of the extracted chromatograms for the heavy and light peptides, respectively. Ratios were then subjected to log₂ transformation and redundant peptides were excluded from analysis based on confidence measurements including VISTA score, XCorr and S/N values. For purposes of identification and quantification, peptides were considered unique if they had different charges or different states of methionine oxidation. Non-redundant peptide ratios were averaged from each protein to calculate an average protein ratio.

**SILAC analyses of LNCaP and LNCaP-LN3 cells.** For SILAC analyses, WCLs collected from LNCaP and LNCaP-LN3 were mixed at a 1:1 ratio by protein concentration (200 µg from each, 400 µg total), boiled for 3 min in sample buffer and resolved with a NuPAGE 4-12% gradient gel (Invitrogen). Gel was stained with Coomassie blue and processed into 10 samples as described (18). Peptides were clarified with Sep-Pak C₁₈ Cartridges (Waters Corporation, Milford, MA) prior to mass spectrometry analysis. LC-MS/MS was carried out as described above.
**Spectral counting.** All redundant MS/MS spectra from serine hydrolases shown in **Supplemental Table 2** were divided into light and heavy isotopic populations. C-terminal peptides not containing either lysine or arginine were excluded from analysis. Spectra were summed for proteins from each of the two control and two experimental samples and averages were calculated. Protein ratios were determined by dividing the average number of heavy and light spectra for each protein followed by $\log_2$ transformation.
RESULTS AND DISCUSSION

As discussed for cleavable labeling reagents (14), smaller tags are more amenable to mass spectrometry because there are fewer potential sites of fragmentation during collision-induced dissociation, simplifying the analysis of the fragment ion spectrum. Drawing on the advantages of the newly developed catch-and-release (CAR) tags, we sought to apply this cleavable technology to the analysis of enzyme activity and to facilitate simultaneous protein identification and quantification by mass spectrometry. The use of fluorophosphonate (FP) tags to profile serine hydrolases by ABPP has been described in a number of studies and was chosen here as a proof-of-principle application. Thus, our scheme makes use of an FP catch-and-release probe (FP-CAR, Fig. 1A and Supplemental Fig. 1). This reagent consists of (i) an FP group that will react with the active-site nucleophile in serine hydrolases, (ii) a disulfide moiety which is selectively reducible due to steric hindrance, (iii) a biotin tag for the binding and enrichment of active hydrolases and (iv) long linkers on each side of the cleavable site to allow for the binding and recovery of full-length proteins following avidin capture.

While the reactivity properties of our cleavable FP are in accordance with previously published FP tags (Supplemental Fig. 2), the active-site serine residue of trypsin was also identified using our cleavable reagent coupled with tandem mass spectrometry (MS/MS). High mass accuracy MS spectra for the unmodified and FP-modified versions of the trypsin active-site peptide (Fig. 1B) as well as a corroborating MS/MS fragmentation pattern (Fig. 1C) confirm both the binding of our cleavable reagent to the trypsin active-site nucleophile and its compatibility with mass spectrometry.

Having established that the FP-CAR reagent behaves as expected with single proteins, we turned our attention to its use in complex mixtures from a cellular model of tumor metastasis. A general analysis of serine hydrolase activity has been explored in various models of tumorigenicity (11) but has yet to be performed in prostate cancer. The LNCaP prostate cancer line and a highly metastatic variant, LNCaP-LN3 (25), were chosen for this purpose. A genomic profile examination of prostate cancer showed that commercially available LNCaP cells cluster with clinically localized prostate cancer specimens (26), indicating that LNCaP represents a biologically relevant cellular model. LNCaP cells are also androgen-sensitive, suggesting that they represent an early stage of prostate cancer.

By coupling this labeling approach to metabolic stable isotopic labeling and mass spectrometry, we were able to obtain quantitative data for a list of proteins reflecting relative
functional activity. The major advantage of this method is that using differential isotopic labeling coupled with mass spectrometry will result in more accurate quantification of enzyme activity. A schematic for comparing LNCaP and LNCaP-LN3 cells using our procedure, termed catch-and-release activity profiling of enzymes (CAPE), is shown (Fig. 2A).

To assess which proteins exhibited significant quantitative changes, CAPE was repeated using LNCaP cells cultured under both heavy and light conditions as a control. This experiment confirmed that an expected ratio of 1:1 (no change) could be maintained throughout the labeling and purification process.

CAPE experiments led to the confident identification of 25 hydrolase proteins (Supplemental Table 1) from LNCaP and LNCaP-LN3 whole-cell lysates (WCL). Using stringent identification and quantification criteria, nine hydrolases from this list were chosen for further analysis. The hydrolase activities for these proteins displayed accurate reproducibility among biological replicates for both experimental and control conditions (Fig. 2B).

The average control log2 ratio of -0.086 with a standard deviation of 0.18 (13%) shows that the relative activities of most of the serine hydrolases remained unchanged (Supplemental Table 2). A significant change was defined as increasing or decreasing more than three standard deviations from the mean, as determined from the average control protein ratio. Thus, log2-transformed ratios less than -0.63 or greater than 0.46 represent significant experimental changes, as confirmed by Student’s t-test. Unexpectedly, none of the enzymes showed upregulated activity, whereas roughly half exhibited a significant decrease in activity (Fig. 2B). This suggests that a downregulation of specific serine hydrolases may contribute to or result from the progression of metastasis in these cells.

To validate our data, we chose a number of additional control experiments to show that the labeling of enzymatic activity was specific. As with most enzymes, serine hydrolase activity is dependent on three-dimensional structure integrity. Boiling the WCLs prior to labeling with FP-CAR and using the same criteria for peptide identification and quantification led to an overall reduction in activity, with only one out of the nine hydrolases found (fatty acid synthase, FAS). Only a small number of peptides were identified for FAS in this control, indicating that this identification was likely due to minor amounts of protein refolding and reconstituted activity after sample boiling. Moreover, to ensure that hydrolase identification was dependent on FP-CAR labeling and not simply due to non-specific binding to avidin beads, the CAPE procedure
was repeated with no FP-CAR reagent as a background control. None of the nine measured hydrolases were identified in this control experiment.

While CAPE ratios showed clear reproducibility at the protein level, a quantitative analysis at the peptide level was required to determine intraprotein variability. As expected, the peptides used for protein quantification from control samples showed predominantly no change (Supplemental Fig. 3).

Optimized by Mann and colleagues, SILAC has been widely used to quantitatively evaluate protein levels using mass spectrometry (2). To assess the utility of CAPE in comparison to existing methods, the same WCLs from LNCaP and LNCaP-LN3 were mixed at a 1:1 ratio by protein concentration and subjected to SILAC analysis. The consistency of our method is shown in Supplemental Table 2 in which similar quantitative protein levels were observed between SILAC and CAPE. Due to WCL complexity, proteins analyzed by SILAC were first resolved by SDS-PAGE, fractionated 10-fold and subjected to a longer HPLC elution gradient in an attempt to identify and quantify low-abundance proteins. Supplemental Table 2 reveals that this increase in resolution failed to quantify three of the nine serine hydrolases, including two of the most significantly dysregulated enzymes, peroxisomal acyl-coenzyme A thioester hydrolase 2A (ACOT2) and sialate O-acetylesterase precursor (SIAE). While no differences were observed in quantitative levels for serine hydrolases overlapping in both SILAC and CAPE experiments, we note that CAPE directly enriched serine hydrolases and has the potential for characterizing protein activities that may be missed by SILAC alone. Alternatively, because the same metabolically labeled samples can be analyzed by both methods, thereby minimizing experimental variability, we suggest that SILAC and CAPE could be used as complementary techniques for gaining insight into post-translational mechanisms regulating protein activity in a variety of biological models.

CAPE was also evaluated against spectral counting as a means for relative quantification between low and high metastatic cells. As others have suggested, spectral counting is ideal for detecting large quantitative changes (27) and better suited to the analysis of more abundant proteins (12). However, LNCaP and LNCaP-LN3 exhibited small differences in serine hydrolase activity as assessed by CAPE. Many of these proteins have low spectral count numbers, as well, suggesting they are present in comparatively low concentrations (Fig. 3). The three most dysregulated enzymes showed relatively no change as measured by spectral counting,
highlighting the utility of CAPE and stable isotope labeling for detecting small quantitative changes that may have otherwise been missed by alternative MS-based methods.

In summary, we have described a streamlined approach for identification and quantification of serine hydrolase activities using mass spectrometry, with our results pointing to some potentially interesting metastatic biomarkers. While others have suggested a tumorigenic role for decreased SIAE activity (28), ACOT2 and hypothetical protein FLJ11342 (ABHD10) exhibited markedly decreased expression in the present study but have been poorly characterized in other cancer analyses. Additional experiments are underway to determine the role these enzymes play in metastasis.

Recent work has shown the utility of combined chemical and computational approaches for identifying novel serine hydrolases (29). The use of a highly quantitative method such as CAPE in conjunction with a computational technique that pinpoints specific hydrolases could be valuable, as such an approach could generate additional enzymes whose function may be important in the progression of disease.

Furthermore, the CAR technology could be applied to other activity-based probes to explore functions with high specificity and sensitivity of additional enzyme classes. Considering the milieu of endogenous enzyme inhibitors and post-translational mechanisms that affect protein function, CAPE has the potential for measuring differences in protein activity in a single step that alternative mass spectrometry-based approaches such as SILAC and ICAT could not.

ACKNOWLEDGMENTS
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Addendum – After submission of this work, two studies describing cleavable probes for functional proteomics were published (30, 31).
REFERENCES


accuracy on an Orbitrap mass spectrometer via lock mass injection into a C-trap. 

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FIGURE LEGENDS

Figure 1: Structural features and MS analysis of FP-CAR. (A) FP-CAR reagent noting key structural and functional components, such as fluorophosphonate (i), phosphine-cleavable disulfide linker with sterically hindering dimethyl and isopropyl groups (ii), biotin tag (iii) and long linkers (iv) to allow entry and flexibility during initial capture and subsequent isolation/release of full-length serine hydrolases. (B) High mass accuracy MS scans of peptide DSCQGDSGPVVCDSGK from trypsin containing active-site serine residue (denoted by * in right panel). The doubly charged unlabeled peptide has a monoisotopic $m/z$ ratio of 805.33 (left panel), while the peptide labeled with the cleaved reagent portion has a monoisotopic $m/z$ ratio shifted to 1023.44 (right panel). (C) Assignment of $b$ (red) and $y$ (blue) ion series in MS/MS scan from 1023.44 precursor ion confirms peptide identification and modified-site localization. T denotes the mass of the cleaved FP-CAR reagent (436.2 Da) plus a water molecule (18.0 Da). Large $m/z$ shift between diagnostic ions $y_9$ and $y_{10}$ due to FP-CAR derivatization shows correct labeling at active-site serine residue. Doubly charged species from $y_{13}$ and $y_{14}$ were observed at 842.5 and 922.6, respectively. All peak intensities under x2 umbrella were doubled for visual clarity.

Figure 2: CAPE procedure applied to complex mixtures using a model of tumor metastasis. CAPE was used to assess differential serine hydrolase activity between low (LNCaP) and high (LNCaP-LN3) metastatic prostate cancer cells. (A) CAPE combines multiple existing proteomics methods. Many steps in this schematic are common to SILAC (red), ABPP (blue) and/or CAR (green). Low and high metastatic prostate cancer cells were cultured with light and heavy amino acids, respectively. Whole cell lysates (WCL) were collected, labeled with FP-CAR reagent and mixed at a 1:1 ratio according to measured protein concentration. Serine hydrolases were captured by incubation with avidin and non-specific binding was reduced by washing. Protein was trypsin-digested in-solution and analyzed by tandem mass spectrometry. MS and MS/MS scans provided quantitative and qualitative data, respectively. (B) Red and blue bars correspond to the average serine hydrolase activity levels from control (LNCaP cells cultured under both light and heavy amino acid conditions to confirm an expected 1:1 ratio) and experimental (comparing light LNCaP and heavy LNCaP-LN3 cells to determine differential serine hydrolase activity in PCa metastasis) conditions, respectively, with error bars representing high/low values between biological replicates. Ratios greater than 0 represent
upregulated activity in the high metastatic cells while values less than 0 correspond to decreased activity. Black dashed line shows the mean protein ratio from the control samples, with red dashed lines corresponding to three standard deviations from the mean. Student’s t-test was performed on all peptides used for quantification by combining replicate experiments and comparing intraprotein variability between experimental and control peptide ratios, with * representing p-values <0.00001. Protein abbreviations were taken from Swiss-Prot Gene Names (“Abbreviations” section and Supplemental Table 2). Note: Only proteins detected with at least two quantified peptides in each of the four experiments (control and experimental, with biological replicates for each) were considered for analysis.

Figure 3: Comparison of CAPE and spectral counting for quantification. All redundant spectra for each serine hydrolase were divided into light and heavy isotopic populations. (A) Bars represent the average number of light (solid colors) and heavy (striped colors) spectral counts obtained from the two control (red) and two experimental (blue) samples for each protein. Note: a nearly equal number of light and heavy spectra (236 and 248, respectively) were obtained from the control samples, indicating no bias toward the correct identification of light or heavy spectra. (B) Spectral count ratios from panel A were calculated and log₂-transformed. Displayed are the average ratios from spectral counting from the control (open red squares) and experimental (open blue squares) samples, as well as CAPE ratios for control (solid red squares) and experimental (solid blue squares) samples, as shown in Fig. 2B. Ratios greater than 0 represent upregulated activity in the high metastatic cells while values less than 0 correspond to decreased activity. (C) Representative peptide from ABHD10 shows relatively no quantitative change in control sample (left) as determined by CAPE while the same peptide identified in experimental sample (right) exhibits an approximate 2.5-fold decrease in activity. Control (red) and experimental (blue) monoisotopic [M+2H]^{2+} peaks from light and heavy species are highlighted.
**Figure 1**

**A**

Formula = C\(_{40}\)H\(_{75}\)FN\(_5\)O\(_{10}\)PS\(_3\)
MW = 931.4398

**B**

\[ \text{DSCQGDS}^+ \text{GGPVVC}^+ \text{CSGK} \]

**C**

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\(^*\) Doubly charged species were detected
Figure 2

A

12C14N-Lysine
12C14N-Arginine

Culture low metastatic cells & collect WCL

Label with FP-CAR

Avidin enrichment

Wash/cleave proteins off avidin

Trypsin digestion

13C15N-Lysine
13C15N-Arginine

Culture high metastatic cells & collect WCL

Label with FP-CAR

B

Identification by MS/MS

Control

Experimental

Mean (Control)

3 SD

LYPLA1
FASN
WBSCR21
APEH
PAFAH1B3
ESD
ACOT2
ABHD10
S1CE

Log2Ratio

0

0.5

1

1.5

2

-2

-1.5

-1

-0.5

0

21

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Figure 3

A

Spectral Counts

LYPLA1 FASN WBSCR21 APE1 PAFAH1S1 ESD ACOT2 ABHD10 SAE

Log2Ratio

LYPLA1 FASN WBSCR21 APE1 PAFAH1S1 ESD ACOT2 ABHD10 SAE

B

SC Ctrl CAPE Ctrl SC Exp CAPE Exp

C

ABHD10: FDYSGVGSSDGNSEESTLGK

CAPE Ctrl

Relative Abundance

1010 1014 1018 1022 1026 1030 m/z

CAPE Exp

Relative Abundance

1010 1014 1018 1022 1026 1030 m/z