

Assessing Surface Binding Events on Golgi Microsomes Isolated from Cancer Cells by Capillary Electrophoresis

Clara Davoine, Gaël Cobraiville, Lionel Pochet, and Marianne Fillet*



Cite This: *Anal. Chem.* 2026, 98, 19652–19662



Read Online

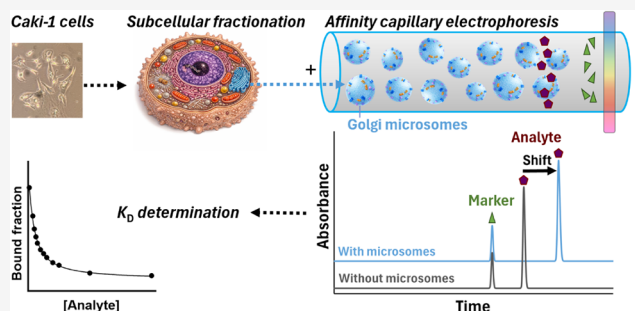
ACCESS |

Metrics & More

Article Recommendations

Supporting Information

ABSTRACT: Intracellular membrane proteins represent a vast and pharmacologically essential class of therapeutic targets, yet their characterization within native pathological environments remains experimentally challenging. Here, we report what is, to the best of our knowledge, the first direct affinity measurements performed at the surface of Golgi microsomes isolated from cancer cells. By integrating affinity capillary electrophoresis (ACE) with a refined mathematical framework, we overcome long-standing obstacles associated with membrane protein analysis, including the need for target modification, intrinsic enzymatic activity, labeled probe, or large protein excess. The microfluidic format further minimizes sample consumption while enabling both direct and indirect detection modalities, thereby expanding applicability to impure compounds and ligands lacking charge or UV absorbance. Applying this strategy to UGCG, a Golgi-resident membrane protein of oncological relevance, we demonstrate quantitative ligand-binding measurements on fairly monodisperse vesicular fractions (~150 nm) that preserve native intracellular architecture. Collectively, this work establishes a robust, broadly applicable platform for target engagement studies in complex biological systems and highlights the potential of ACE to accelerate drug discovery efforts involving intracellular or extracellular membrane-bound targets.



INTRODUCTION

Evaluation of drug–target interactions is a key component in the development of new drugs. The main issue when developing *in vitro* assay is to ensure the integrity of the target. This is particularly true for membrane proteins which play crucial roles in numerous cellular processes, such as signal detection and transduction, transport, enzymatic reactions, and cell–cell communication. Dysfunctional membrane proteins lead to various pathological conditions and serve as targets for many approved drugs.^{1–5} Both mechanistic studies and drug discovery campaigns focusing on a membrane protein require reliable structural and functional information. Although many *in vitro* characterization assays exist, they usually require detergents that remove the native environment. However, membrane proteins rely heavily on the membrane environment.^{4,6} Their interactions with lipids and neighboring membrane proteins determine both their structure and functional behavior, underscoring the need for assays that preserve the native local environment.⁶

Recent studies have highlighted affinity capillary electrophoresis (ACE) in partial-filling mode as a powerful tool for validating target engagement and determining dissociation constants (K_D).^{7–10} ACE detects binding events by monitoring changes in the electrophoretic mobility of an analyte upon interaction with its target. As a microfluidic biophysical method, it requires only minimal amounts of unmodified

proteins, allows quantification over a wide range of affinities under near-physiological conditions and on nonenzymatic targets, and can analyze impure samples thanks to its inherent separation capability.^{11,12} Although ACE has proven effective for measuring affinity with purified soluble proteins, its application to membrane proteins has not yet been explored due to their dependence on a native lipid environment.

The Golgi apparatus serves as the central trafficking nexus of the cell, orchestrating the post-translational modification, sorting, and targeted delivery of secretory and membrane proteins as well as lipids. In mammalian cells, this indispensable membrane-bound organelle is organized as a polarized stack of five to seven flattened cisternae. The *cis* face of the Golgi receives newly synthesized cargo from the endoplasmic reticulum, whereas the *trans* face and the *trans*-Golgi network mediate its sorting and export to distinct intracellular compartments or the extracellular space.¹³ Post-translational processing of proteins and lipids is mediated by a

Received: March 2, 2026

Revised: May 23, 2026

Accepted: May 29, 2026

Published: June 24, 2026



diverse set of Golgi-resident enzymes, including glycosidases, glycosyltransferases, and luminal proteases.¹⁴ Accumulating evidence indicates that disruptions in Golgi architecture and function are implicated in a broad spectrum of diseases. In cancer, aberrant glycosylation patterns are closely associated with disease progression and poor prognosis.^{13,14} Accordingly, quantitative determination of the binding affinities of candidate ligands to Golgi-resident proteins is essential for elucidating mechanisms of cellular regulation and for the discovery of therapeutic agents. A range of analytical approaches has been developed to probe such interactions, including time-resolved fluorescence resonance energy transfer (TR-FRET)-based assays,¹⁵ radioligand binding assay,¹⁶ fluorescence polarization assay,¹⁷ surface plasmon resonance (SPR),¹⁸ or affinity selection mass spectrometry (AS-MS).¹⁹ Each of these methods, however, presents limitations. Displacement assays may fail to detect interactions occurring outside the labeled probe. SPR necessitates immobilization of one binding partner, which can alter binding behavior. AS-MS may show reduced sensitivity for ligands that dissociate on rapid time scales.²⁰

In this context, ACE may reveal itself as a versatile and complementary strategy for Golgi-targeted drug discovery. In this study, UDP-glucose ceramide glucosyltransferase (UGCG, also known as glucosylceramide synthase), the rate-limiting Golgi enzyme for glucosylceramide synthesis, was chosen to benchmark our ACE approach.^{19,21,22} Large-scale production of this intracellular integral membrane protein remains challenging,^{21,22} and its structure and mechanistic details are still incompletely characterized.¹⁹ The D1, D2, D3, (Q/R)XXRW motif defines its active site (Figure 1).²³ UGCG

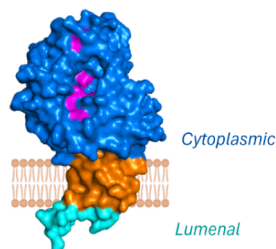


Figure 1. UGCG, a therapeutic target in oncology. Predicted structure from TmAlphaFold database (membrane plane determined by TMDET algorithm).³² In pink, D92, D144, D236, RMIRW272-276 reported active sites.

function is modulated by its environment, forming heterodimers or higher-order oligomers with proteins such as sphingomyelin synthase 1, c-Fos, and Reticulon-1C.^{22,24–26} Oligomerization may influence its localization and activity.^{24,26,27} UGCG is frequently upregulated in multiple cancers, where its inhibition reduces malignancy, mitigates drug resistance, and may enhance immunotherapy by reversing tumor-associated immunosuppression.^{21,28–30} These characteristics establish UGCG as both a biomarker and a promising therapeutic target.^{21,29} Traditional evaluation relies on enzymatic assays with fluorescent²² or deuterated³¹ substrates, and AS-MS has been applied for high-affinity binder identification.^{19,20} However, no method currently enables quantification of fast-equilibrium interactions.

Here, we report the first demonstration of ACE applied to Golgi membrane fractions isolated from cancer cells, preserving endogenous protein and lipid microenvironments. This approach extends the scope of ACE to low-abundance,

membrane-embedded targets and simultaneously broadens drug discovery strategies toward physiologically relevant membrane proteins.

EXPERIMENTAL SECTION

Materials

Cell culture consumables and the Micro BCA Protein Assay Kit were obtained from Thermo Fisher Scientific (Waltham, MA). OptiPrep Density Gradient Medium, bovine serum albumin (heat shock fraction, protease-free, fatty acid-free, essentially globulin-free, pH 7, $\geq 98\%$), 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate (CHAPS), UDP- α -D-glucose disodium salt, 4-methylmorpholine (NMM), and trypsin inhibitor from *Glycine max* (soybean, 1 mg inhibits 1.8 mg of trypsin with activity of approximately 10,000 BAEE units per mg of trypsin) (SBTI) were purchased from Merck (Darmstadt, Germany). Eliglustat hemitartrate and miglustat were supplied by BLD Pharmatech GmbH (Reinbek, Germany), and ceramide C8 was obtained from Avanti Polar Lipids (Alabaster, AL). Sequencing grade modified trypsin (10,868 BAEE units per mg) was acquired from Promega Corporation (Madison, WI). Poly(vinyl alcohol) (PVA) capillaries were provided by Agilent Technologies (Santa Clara, CA).

The wash medium (WM) consisted of 10 mM triethanolamine and 250 mM sucrose, adjusted to pH 7.4 with acetic acid. The homogenization medium (HM) was prepared by supplementing WM with Halt Protease Inhibitor Cocktail and 25 U/mL Pierce Universal Nuclease (Thermo Fisher Scientific, Waltham, MA). A 50% iodixanol working solution was generated by mixing five volumes of OptiPrep with one volume of diluent (60 mM triethanolamine, 1.5 M sucrose, pH 7.4) and subsequently diluted with WM to yield a 15% iodixanol solution. The background electrolytes (BGE) used in capillary electrophoresis consisted of 30 mM sodium phosphate and 250 mM sucrose, pH 7.4. To prepare 100 mL of BGE, 321.1 mg of Na_2HPO_4 (anhydrous), 101.8 mg of NaH_2PO_4 (monohydrate), and 8558 mg of sucrose were added to approximately 80 mL of Milli-Q water. After dissolution, the pH was adjusted to 7.4 with 1 M NaOH. The solution was then quantitatively transferred into a 100 mL volumetric flask and the volume was adjusted with Milli-Q water. Finally, the BGE was filtered through Chromafil Xtra 0.2 μm PVDF syringe filters (Macherey-Nagel, Duren, Germany). Conductivity was measured using a SevenCompact conductimeter (Mettler-Toledo, Columbus, USA). Physicochemical properties of small molecules and the BGE were calculated using MarvinSketch 19.27 (ChemAxon Ltd., Budapest, Hungary) and PeakMaster version 6.0f8,³³ respectively.

Cell Culture

Caki-1 cells (ATCC HTB-46) were obtained from the American Type Culture Collection (Manassas, VA) and maintained at 37 °C in a humidified atmosphere containing 5% CO_2 . Cells were cultured in McCoy's 5A modified medium (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin. Prior to seeding, culture flasks were coated with type I collagen (rat tail, Sigma). The stock collagen solution was diluted 30-fold in PBS, applied to the flask surface, and incubated for 20 min at 37 °C. The coating solution was then aspirated and replaced directly with culture medium without washing.

Golgi Microsomes

Golgi isolation was performed following established protocols (Figure S1).^{34–37} Nineteen T175 flasks, corresponding to $\sim 2 \times 10^8$ Caki-1 cells, were washed three times with 3 mL PBS and twice with 5 mL wash medium (WM) at room temperature. Cells were scraped in 5 mL ice-cold WM, pooled into two 50 mL Falcon tubes, and pelleted by centrifugation at 4 °C (300 g for 20 min followed by 1000 g for 15 min). The supernatant was removed, and cell pellets were combined in 2 mL homogenization medium (HM). Cells were disrupted by 20 passes through a 27-gauge needle. The homogenate was centrifuged at 2000 g for 10 min at 4 °C; the pellet was resuspended in 500 μL HM, centrifuged again, and the resulting

supernatants were pooled. A continuous gradient was prepared in two Ultra-Clear centrifuge tubes (SW40 rotor, Beckman Coulter, Brea, CA) by mixing equal volumes of WM and 15% iodixanol using a Gradient Master (Biocomp Instrument, Tatamagouche, Canada; 135 s, 81.5°, 15 rpm). From the top of each tube, 150 μ L of gradient was removed, and 1.25 mL of low-speed supernatant was layered. Gradients were centrifuged at 200,000 g for 2 h at 4 °C. Fractions (28 $\times$ 500 μ L) were collected by upward displacement, and densities were verified with a refractometer. For ACE experiments, fractions F4, F5, and F6 were pooled and buffer-exchanged into BGE using Amicon Ultra-0.5 centrifugal filter units (10 kDa, regenerated cellulose, Merck KGaA, Darmstadt, Germany). Briefly, 35 μ L of the pooled sample (F_{pool}) was loaded into a filter unit prerinsed three times with 500 μ L BGE (14,000 g , 30 min, 4 °C). Subsequently, 500 μ L of cold BGE was added, and the sample was centrifuged at 4 °C in two steps: 4,000 g for 30 min, followed by 14,000 g for 60 min. The final $\sim$ 30 μ L of ultrafiltrated sample ($F_{\text{pool,UF}}$) was recovered by inverting the filter unit and centrifuging at 2,400 g for 2.5 min.

Western Blot

Samples were separated on a 4–12% Bis-Tris gel using NuPAGE MOPS SDS running buffer (Thermo Fisher Scientific) and electro-blotted onto a 0.2 μ m PVDF membrane (Thermo Fisher Scientific) overnight at 40 mA. Membranes were blocked for 60 min in 10% milk in phosphate-buffered saline with 0.1% Tween 20 (PBST) and incubated overnight with anti-UGCG antibody (clone 1E5, 1:500 in 5% milk in PBST; Cat# SAB1401393, RRID: AB_10608571, Merck). Membranes were then incubated with antimouse IgG HRP-linked secondary antibody (1:4000; Cell Signaling Technology, Danvers, MA) for 120 min. Protein detection was performed using Pierce ECL Western Kit (Thermo Fisher Scientific) and imaged on an Amersham Imager 600 (GE Healthcare, Chicago, IL). For total cell lysate and low-speed supernatant, 2 μ g of protein were loaded per lane, whereas 20 μ L of each ultracentrifuge fraction were applied.

Nanoparticle Tracking Analysis

Particle size distribution and concentration were determined using a NanoSight NS300 (Malvern Panalytical, Worcestershire, UK) equipped with a 532 nm laser and operated with NTA 3.4 Build 3.4.003 software. Samples were diluted with BGE and infused into the microfluidic cell at a flow rate of 40 arbitrary units via a syringe pump. For each sample, five 60 s acquisitions were recorded using a camera level of 13. Particle sizing calculations accounted for the BGE viscosity at 25 °C, measured as 1.222 cP according to a previously reported procedure.³⁸

Activity Assay

Golgi microsomes (3 μ g of total protein) were incubated at 37 °C for 2 h in the BGE supplemented with 200 μ M UDP-glucose, 500 μ M ceramide C8-BSA complex, 5 mM MgCl_2 , 500 μ M maleic acid (internal standard), and either 0 or 100 μ M eliglustat hemitartrate. Reactions were quenched with an equal volume of ethanol, and protein aggregates were removed by centrifugation at 20,000 g for 5 min. Samples were subsequently analyzed by capillary electrophoresis. UGCG-specific UDP-glucose consumption was calculated as the difference between reactions conducted in the absence and presence of 100 μ M eliglustat. A schematic of the protocol is provided in the Supporting Information (Figure S2).

Capillary Electrophoresis

All CE-UV experiments were conducted on an Agilent 7100 capillary electrophoresis system (Agilent OpenLab CDS 2.7) with the cassette thermostatically controlled at 25 °C. Separations were performed in PVA-coated capillaries (75 μ m i.d., 31 cm total length, 8.5 cm effective length) at a constant voltage of -7 kV for anions and $+7$ kV for cations. Each day, capillaries were flushed with BGE for 10 min at 1 bar. Prior to each run, capillaries were conditioned by flushing with BGE for 4 min at 1 bar, except for indirect ACE experiments.

CZE of Golgi Microsomes

The samples were injected by vacuum at -35 mbar for 4 s, followed by a short plug of BGE at -20 mbar for 2 s. The monitored signals

were 205.0 and 280.0 ± 2.0 nm with a reference wavelength at 500.0 ± 50.0 nm. The spectrum between 190 and 400 nm (step, 2 nm) was also stored. The run time was 30 min.

Quantitation of UDP-Glucose

The following injection sequence was used: -20 mbar for 2 s of water, -20 mbar for 4 s of sample, and -5 mbar for 1 s of BGE. The areas under the curve were determined at 205.0 and 260.0 ± 2.0 nm with a reference wavelength at 500.0 ± 50.0 nm for maleic acid and UDP-glucose, respectively. The run time was 5 min.

Direct ACE

$F_{\text{pool,UF}}$ was first injected at -50 mbar for 17 s. Eliglustat solutions at varying concentrations (3.125, 3.75, 5, 6.25, 7.5, 10, 12.5, 15, 20, 25, 50, 100 μ M), in BGE diluted 2 times with water and containing 200 μ g/mL NMM, were subsequently injected at -20 mbar for 4 s, followed by a short BGE plug at -5 mbar for 1 s. UV signals were recorded at 205.0, 210.0, 215.0, 220.0, 225.0, 230.0, 260.0, and 280.0 ± 2.0 nm, with a reference wavelength of 500.0 ± 50.0 nm. Full spectra from 210 to 400 nm (2 nm steps) were also acquired. The run time was 7 min.

Indirect ACE

Prior to each run, the capillary was conditioned by sequentially flushing with BGE for 2 min at 1 bar, followed by BGE containing varying concentrations of miglustat (6.25, 25, 50, 78.125, 100, 156.25, 200, 312.5, 500, 1000, and 2000 μ M) for 2 min at 1 bar. Sample injection consisted of miglustat-spiked $F_{\text{pool,UF}}$ at -50 mbar for 17 s, 5 μ M eliglustat in BGE diluted 2 times with water and containing 200 μ g/mL NMM at -20 mbar for 4 s, and BGE containing different concentrations of miglustat (6.25, 25, 50, 78.125, 100, 156.25, 200, 312.5, 500, 1000, and 2000 μ M) at -5 mbar for 1 s. UV detection parameters were identical to those used for direct ACE experiments. The run time was 7 min.

Data Treatment

The areas under the curve were calculated using Agilent OpenLab CDS 2.7 software. The migration times of eliglustat and NMM were determined with CEVal version 0.6i327 using signal A (205 nm) and deconvoluted signal, respectively. The deconvolution script processed as follows: 1) signal A (205 nm) and signal B (210 nm) traces were smoothed by moving average using a window size of 1.25 s, 2) the baseline value of each signal was obtained by averaging the absorbance before the plateau jump with an exclusion of the 1000 first points, 3) the $F_{A/B}$ ratio was determined by eqs 1 and 4) the deconvoluted trace was constructed using eq 2.

$$F_{A/B} = \text{average}_{t \in [t_{\text{peak1}} - 60\text{s}; t_{\text{peak1}} - 30\text{s}]} \left(\frac{Abs_A(t) - \text{baseline}_A}{Abs_B(t) - \text{baseline}_B} \right) \quad (1)$$

where $F_{A/B}$ is the ratio between the signals A (205 nm) and B (210 nm) of the matrix component, t_{peak1} corresponds to the time of the first peak (~ 4 min) that is embedded in the plateau, $Abs(t)$ is the absorbance value of the signal mentioned as a subscript at time t , and baseline is the average of the absorbance value before the plateau of the signal mentioned as a subscript.

$$Abs_{\text{deconvoluted}}(t) = Abs_A(t) - (Abs_B(t) - \text{baseline}_B)F_{A/B} \quad (2)$$

where $Abs_{\text{deconvoluted}}(t)$ is the absorbance value at time t of the deconvoluted signal.

RESULTS AND DISCUSSION

Our previous work established that ACE enables reliable hit validation and quantitative determination of dissociation constants (K_D) using purified soluble proteins.^{7–10} However, extending this approach to membrane proteins presents distinct challenges. These proteins frequently display limited stability in solution and a strong propensity for aggregation due to their hydrophobic domains.⁵ Moreover, correct folding and functional integrity generally depend on the presence of a lipid

bilayer and a defined membrane microenvironment.^{1,3,39,40} Variations in lipid composition, including cholesterol content and other membrane constituents, can profoundly influence membrane organization and protein function.⁴⁰ Collectively, these features complicate membrane protein-targeted drug discovery while simultaneously creating opportunities for selective modulation in specific cellular contexts.³

In this study, we evaluated ACE for membrane protein–ligand binding assays using UGCG, the rate-limiting Golgi enzyme for glucosylceramide synthesis. UGCG has traditionally been isolated from rat liver tissue, as attempts to obtain the recombinant protein in preparative quantities have met with limited success.²² Solubilization of UGCG using detergents is limited and affects its activity.^{27,41,42} Moreover, nine splice variants are reported in the Ensembl database for the *UGCG* gene, which further increases its complexity.⁴³ In order to provide a source that better reflects human malignant physiology and preserves UGCG in its native conformation, we instead used Golgi microsomes from the Caki-1 cell line, a well-established human model of metastatic clear cell renal cell carcinoma.⁴⁴ The Caki-1 cell line was selected based on three criteria: (1) upregulation of UGCG at the protein level,^{45,46} (2) suitability for drug screening due to genetic and phenotypic stability, and (3) its widespread use as a metastatic disease model.

However, the effective target concentration in Golgi microsomes is substantially lower than what can be achieved with purified proteins, meaning that ACE measurements must be operated with high sensitivity under low-concentration conditions while maintaining physiological compatibility. To maximize sensitivity, detection must be performed at low wavelengths. In consequence, minimally UV-absorbing BGE must be used to reduce baseline noise. Phosphate buffer is useful in this respect. Besides its UV transparency, the BGE must be low-conductive and isotonic to avoid the generation of high current during electrophoresis while maintaining the integrity of Golgi microsomes. To achieve the isotonicity without increasing conductivity, sucrose was used as a nonconductive alternative to sodium chloride. The optimized BGE was composed of 30 mM sodium phosphate with 250 mM sucrose (pH 7.4), which provides a low UV cutoff (204 nm), isotonicity, low conductivity ($3274 \pm 18 \mu\text{S}/\text{cm}$, representing a 75% reduction relative to phosphate-buffered saline, $13283 \pm 161 \mu\text{S}/\text{cm}$), and high buffering capacity at physiological pH (13.2 mM).

Isolation and Characterization of Golgi Fractions from Cancer Cells for Interaction Studies

To isolate Golgi membranes from Caki-1 cells, the low-speed supernatant was fractionated using a preformed continuous iodixanol density gradient. Based on the refractive index of the collected fractions (Figure S3) and the reported buoyant density of Golgi membranes (1.04–1.07),³⁷ we examined fractions to identify UGCG. Western blot analysis confirmed the presence of UGCG, with fractions 2 and 5 exhibiting the strongest signal (Figure 2). In both fractions, a prominent band was observed at approximately 45 kDa, consistent with the predicted molecular mass of UGCG derived from its amino acid sequence.

Fractions were then analyzed by capillary zone electrophoresis (CZE) to evaluate the quality of the isolated fractions that contained UGCG. Relative to the matrix control, only negatively charged species were detected (Figure S4, blue

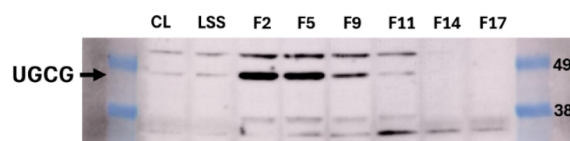


Figure 2. UGCG levels in the total lysate, low-speed supernatant, and selected ultracentrifuge fractions of Caki-1 cells. The most intense ~45 kDa band were fractions 2 and 5. In fraction 11, the ~45 kDa band was weak, while a strong band at ~33 kDa was present, consistent with a splice variant of UGCG lacking amino acids known to be critical for its activity.^{23,43} The blue bands in the two external columns are the molecular weight markers (49 and 38 kDa). CL: total cell lysate, LSS: low-speed supernatant, F: fraction, UGCG: UDP-glucose ceramide glucosyltransferase.

lines). Under negative polarity conditions, the electropherograms of the fractions 2, 4, 5, and 6 included a broad peak (from 4 to 30 min, Figure 3) that is characteristic of

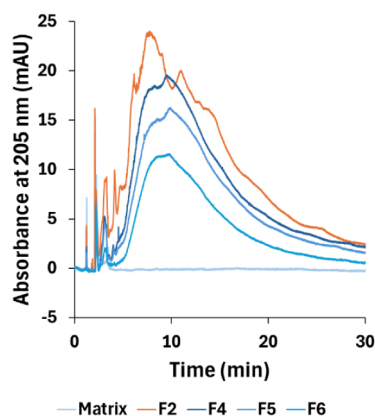


Figure 3. Electropherograms under negative polarity conditions of the selected ultracentrifuge fractions of Caki-1 cells. In all fractions, a broad peak (from 4 to 30 min) was observed and corresponds to nanoparticles of heterogeneous size.⁴⁷ However, this peak was distorted by contaminants in fraction 2 (sample zone). AU: absorbance unit, F: fraction.

heterogeneous nanoparticles.⁴⁷ This is known that the Golgi apparatus of most cultured cells forms vesicles of heterogeneous size after cell disruption (i.e., Golgi microsomes).³⁷ As expected for a fraction of the sample zone (Figure S3), fraction 2 exhibited a peak of Golgi microsomes distorted by contaminants. Because significant distortion of the Golgi microsomes peak was not observed in fractions 4, 5, and 6, these fractions were pooled to obtain sufficient material for subsequent analyses (F_{pool}). The particle concentration and size distribution of the Golgi microsomes were further characterized by NTA (Table S2 and Figure S5), validating the presence of nanometric vesicles in F_{pool} . A span value of 0.66 was measured, indicating a fairly monodispersed size distribution of the Golgi microsomes.⁴⁸

The next step in our workflow was to validate that UGCG remained in an active conformation. UGCG catalyzes the formation of glucosylceramide by transferring glucose from UDP-glucose to ceramide. Traditionally, UGCG activity assays use a radioactive,⁴⁹ fluorescent,²² or deuterated³¹ substrate. Their product is then quantified by scintillation counter, TLC or HPLC with a fluorescence detector, or HPLC-MS, respectively. All these techniques require a labeled substrate that is highly expensive. To monitor the UGCG activity of the

prepared Golgi microsomes in a label-free manner, we developed a CZE method that quantifies the consumption of UDP-glucose. The details of the analytical developments can be found in the [Supporting Information \(Supplemental Data–1\)](#). Briefly, samples were incubated with C8-ceramide, UDP-glucose, Mg^{2+} , an internal standard, and either 0 or 100 μM eliglustat, a specific UGCG inhibitor.⁵⁰ Reactions were quenched with ethanol immediately after mixing or following a 2 h incubation at 37 °C, and residual UDP-glucose was quantified by CZE ([Figure S6](#)). UGCG-specific activity was determined from the difference in UDP-glucose consumption measured in the absence and presence of eliglustat. Since Golgi microsomes may contain other enzymes capable of using UDP-glucose as a substrate (e.g., UGGT1, UGGT2, PLOD3, ALG5),^{45,46,51,52} it was necessary to determine the fraction of the total UDP-glucose consumption specifically attributable to UGCG. As shown in [Figure 4](#), Golgi membrane fractions

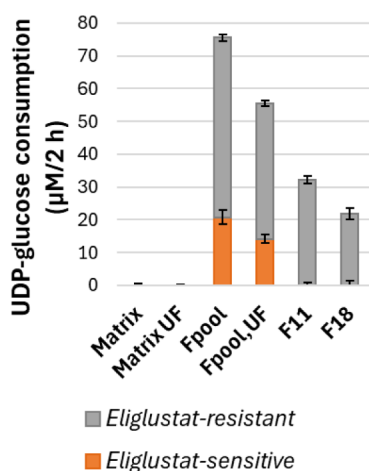


Figure 4. UDP-glucose consumption after 2 hours by membrane fractions isolated from Caki-1 cells. All tested fractions showed UDP-glucose consumption relative to the matrix controls, indicating that the enzymes remain active following cellular fractionation (F_{pool}, F11, and F18) and ultrafiltration-based buffer exchange (F_{pool,UF}). As anticipated from their low 45 kDa UGCG content observed by Western blotting, fraction F11 and F18 displayed no detectable UGCG-related activity. F11: ultracentrifuge fraction 11, F18: ultracentrifuge fraction 18, F_{pool}: pooled ultracentrifuge fractions (4, 5, and 6), F_{pool,UF}: pooled ultracentrifuge fractions (4, 5, and 6) after ultrafiltration, Matrix UF: matrix after ultrafiltration, UGCG: UDP-glucose ceramide glucosyltransferase.

consumed UDP-glucose relative to the matrix controls, confirming that enzymatic activity is preserved following cellular fractionation. At 100 μM , eliglustat inhibited UDP-glucose consumption in F_{pool} by $27 \pm 3\%$, whereas F11 (Golgi) and F18 (smooth endoplasmic reticulum) displayed no inhibitor-dependent change. This was expected, as F11 and F18 serve as negative controls due to their low 45 kDa UGCG abundance observed by Western blot analysis ([Figure 2](#)). These results demonstrate that our label-free activity assay specifically reflects UGCG activity.

UGCG topology analysis of F_{pool} (adapted from Coste et al.⁵³) revealed that $93 \pm 6\%$ of Golgi microsomes are sealed in the right-side-out orientation, as determined from the differential impact of external proteolysis on enzyme activity. Details can be found in the [Supporting Information \(Table S1 and Supplemental Data–2\)](#).

To obtain reliable results using a partial-filling ACE method, it is essential to maintain comparable conductivity between the target plug and the BGE. Consequently, the buffer composition of F_{pool} must be close to the target-free BGE. To achieve this, an ultrafiltration-based buffer exchange was performed on F_{pool}, yielding F_{pool,UF}. The impact of this step, summarized in [Table S2](#), was assessed through total protein quantification by BCA assay, microsome purity analysis by CZE ([Figure S4](#), orange traces), nanoparticle concentration and size determination by NTA ([Figure S5](#)), and UGCG activity measurement ([Figure 4](#)). The ultrafiltration step efficiently reduced matrix components (iodixanol and triethanolamine) while retaining $78 \pm 3\%$ of total protein, similar size distribution, $66 \pm 3\%$ of particles, and $69 \pm 9\%$ of UGCG activity per particle. The particle recovery is consistent with previous reported data,⁵⁴ and the active conformation of UGCG was preserved throughout the process. Therefore, affinity measurements can be reliably performed using F_{pool,UF}.

Affinity Assessment of Eliglustat Binding to Cancer-Derived Golgi Microsomes

The last stage of our developments was to set up a partial-filling ACE method using cancer-derived Golgi microsomes as target source. In this configuration, a defined segment of the capillary was filled with Golgi microsomes ([Figure 5A](#)), while the remaining length contained the target-free BGE. Upon application of an electric field, the potential ligand, namely analyte A, migrated through the microsome-containing zone and was subsequently detected in the microsome-free electrolyte. Consequently, the observed electrophoretic mobility (μ_{obs}) reflects the contributions from the analyte A mobility within the microsomeal plug ($\mu_{A,eff}$), its mobility in the BGE (μ_A), and the electro-osmotic flow (μ_{EOF}). Within the microsomeal zone, the effective mobility ($\mu_{A,eff}$) represents a population-weighted average of the mobilities of the free analyte (μ_A) and the analyte-microsome complex.^{55,56} Among the well-characterized UGCG inhibitors,⁵⁷ we selected eliglustat as known UGCG ligand to evaluate our ACE method due to its high potency, high selectivity, and established use in clinical cancer research.^{28,29}

Prior to affinity measurements, key ACE parameters, including the Golgi microsome plug length and the electro-osmotic flow (EOF) marker, were optimized. Although the fraction of eliglustat bound is independent of plug length, the migration time shift upon binding increased linearly with plug length. Longer plugs therefore enhanced the magnitude of the migration shift, improving the precision of bound fraction determination. The plug length was set to ~ 3.5 cm, corresponding to the maximum length that allowed complete migration of eliglustat through the target plug before detection. In ACE, preventing interactions between the binding partners (Golgi microsomes and analytes) and the capillary surface is essential. For this reason, we used PVA-coated capillaries, which also nearly suppress EOF and thereby enhance the mobility difference between Golgi microsomes and eliglustat, increasing the magnitude of the migration shift upon binding. However, because PVA coatings are physically adsorbed onto silica, they undergo slight but gradual erosion over repeated injections. Over 91 injections on the PVA-coated capillary, the EOF increased from 0.18 to $0.31 \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$, yet it remained negligible compared with the EOF typically observed with bare fused-silica capillaries (around $4 \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$). However, the EOF cannot be assumed constant, and its

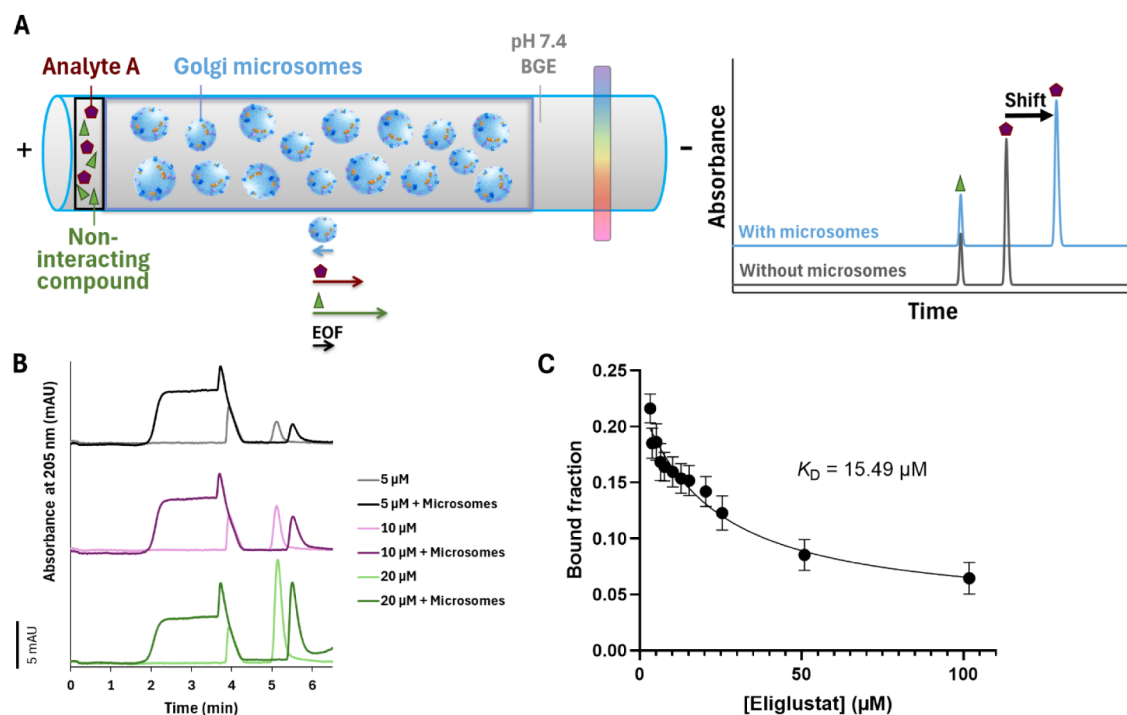


Figure 5. Partial-filling ACE method in direct mode. (A) Principle: First, the capillary is partially filled with the Golgi microsomes. Then, a short plug containing analyte A and the noninteracting compound is injected. After voltage application, the noninteracting compound and the analyte A cross the Golgi microsomes plug and arrive at the detector. Upon binding of the analyte A, a shift is observed in its migration time. (B) Electropherograms: The addition of a 3.5 cm plug of Golgi microsomes shifted the migration time of eliglustat. The shift was reduced with increasing concentration of eliglustat. (C) Affinity determination of eliglustat: The resulting bound fraction calculated from migration times were plotted against the eliglustat concentration to determine the affinity. More details can be found in Figure S8 of the Supporting Information. BGE: background electrolytes, EOF: electro-osmotic flow.

determination at each injection is therefore critical for calculating the bound fraction of eliglustat.⁵⁶ A neutral, noninteracting EOF marker could not be used for two reasons: (1) residual iodixanol in the Golgi microsome plug produces a strong UV background in that region of the electropherogram, and (2) neutral markers substantially extend analysis time. Instead, we employed *N*-methylmorpholine (NMM), a cationic, noninteracting compound that migrates before eliglustat and can be detected at concentrations that do not alter the pH. NMM has already been validated as a buffer component for ACE–MS binding studies⁹ and is structurally related to Good's buffers, which are known to be stable, nonmetabolized, and not act as substrate analogues or enzyme inhibitor.⁵⁸ For each run, the EOF was determined from the observed electrophoretic mobility of NMM. Effective electrophoretic mobility of NMM in the BGE was measured at $1.260 \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$ (RSD = 1.3%, $n = 91$). In addition, the effect of the applied electric field on Golgi microsomes was evaluated using fraction F9, which is comparable to F_{pool} but contains a lower abundance of the 45 kDa UGCG protein (Figure 2). The sample preparation is described in the Supporting Information (Supplemental Data-3). The response of flexible vesicles to an electric field is known to depend on several parameters, including the conductivity ratio between the media inside and outside the vesicle, vesicle size and shape, membrane elasticity, and field strength.⁵⁹ Under the conditions investigated, the electric field did not induce significant alterations in the Golgi microsome integrity. Comparable particle size distributions and concentrations were observed before and after electrophoresis, confirming that the ACE conditions do not cause long-term deformation of the Golgi

microsomes (e.g., rupture) or adsorption on the capillary wall (Figure S7 and Table S3).

In ACE, dissociation constants (K_D) are typically derived from expressions based on the free protein concentration, which are valid under the assumption that the protein is in large excess relative to the analyte.^{56,60,61} Under our experimental conditions, this assumption was not satisfied, as evidenced by the electromigration dispersion of the eliglustat peak (consistent with a low target-to-analyte ratio⁷) and by the dependence of the eliglustat bound fraction on the total analyte concentration ($[A]_0$). We therefore applied the binding model described by Swillens.⁶² In this framework, eliglustat binds to a single class of specific targets with 1:1 stoichiometry, as well as to nonspecific sites. Specific binding is defined by K_D and the total accessible protein concentration ($[P]_0$), while nonspecific binding increases linearly with the free eliglustat concentration ($[A]$) and is characterized by the parameter NS, representing the ratio of nonspecifically bound analyte to $[A]$. Under rapid-equilibrium conditions, the bound fraction of eliglustat is described by eq 3 (Supplemental Data-4).

$$\text{Bound fraction} = \left(\frac{-b + \sqrt{b^2 - 4c}}{2K_D(1 + \text{NS}) - b + \sqrt{b^2 - 4c}} + \text{NS} \right) \cdot \left(\frac{1}{1 + \text{NS}} \right) \quad (3)$$

where

$$b = (1 + \text{NS})K_D + [A]_0 - [P]_0$$

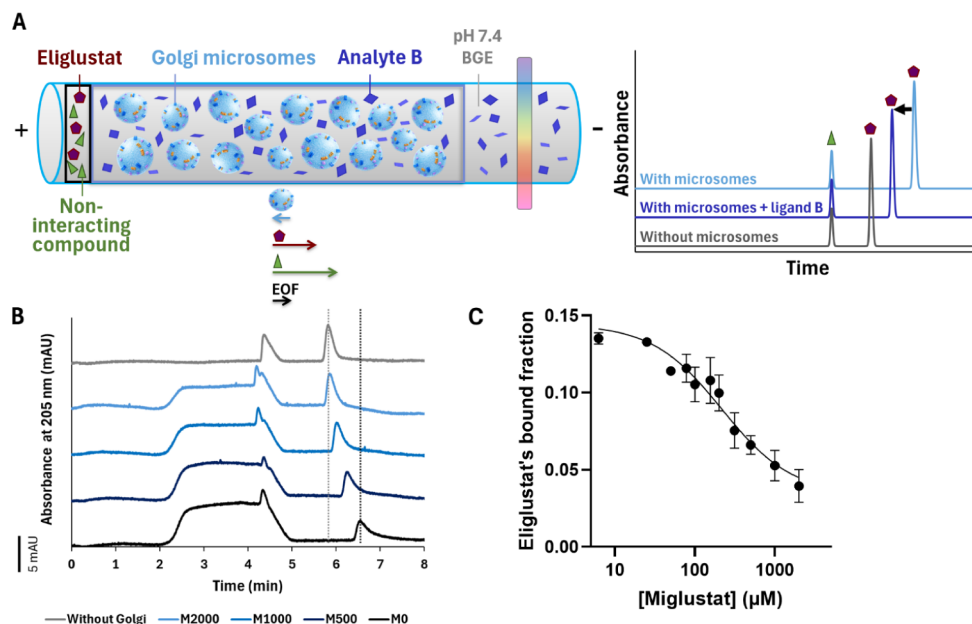


Figure 6. Partial-filling ACE method in indirect mode. (A) Principle: The background electrolytes (BGE) were spiked with analyte B. First, the capillary is partially filled with the Golgi microsomes in the presence of analyte B. Then, a short plug of eliglustat (analyte A) and the noninteracting compound is injected. After voltage application, the noninteracting compound and eliglustat cross the Golgi microsomes plug and arrive at the detector in the spiked BGE. The presence of analyte B reduces the eliglustat binding to the Golgi microsomes. Therefore, the eliglustat migration time shifts back. (B) Electropherograms: The addition of a 3.5 cm plug of Golgi microsomes shifted the migration time of eliglustat. This shift was reduced with the increasing concentration of miglustat in the BGE. (C) Affinity determination of miglustat: The best-fit values of the nonlinear regression were 153.1 [119.1–199.8] μM (K_D) and 2.641 [2.465–2.824] μM ($[P]_0$). For the fitting, the $[A]_0$, K_D , and NS values were fixed at 5 μM , 15.49 μM , and 0.03519, respectively. BGE: background electrolytes, EOF: electro-osmotic flow, Without Golgi: in the BGE with no plug of Golgi microsomes, M0: in the BGE with a 3.5 cm plug of Golgi microsomes, M500: in the BGE containing 500 μM of miglustat with a 3.5 cm plug of Golgi microsomes, M1000: in the BGE containing 1000 μM of miglustat with a 3.5 cm plug of Golgi microsomes, M2000: in the BGE containing 2000 μM of miglustat with a 3.5 cm plug of Golgi microsomes.

$$c = -(1 + NS)K_D[P]_0$$

In our ACE setup, the bound fraction of eliglustat was calculated using eq 4 (Supplemental Data-5):

$$\text{Bound fraction} = \frac{\mu_{\text{obs}} - (\mu_A + \mu_{\text{EOF}})}{(1 - p)\mu_{\text{obs}} - (\mu_A + \mu_{\text{EOF}})} \quad (4)$$

where μ_{obs} is the observed electrophoretic mobility of eliglustat in the presence of the target plug, μ_A is the mobility of the free analyte, μ_{EOF} is the electro-osmotic flow, and p is the fraction of the effective capillary length occupied by the target plug.

The dissociation constant (K_D) of eliglustat was determined by testing different concentrations (Figure 5B), with bound fractions calculated according to eq 4. The resulting data were fitted to eq 3 to obtain K_D , the nonspecific binding parameter (NS), and the total accessible protein concentration ($[P]_0$) (Figure 5C). Measurements were performed independently in duplicate. $[P]_0$ was obtained on the basis of the binding curve, as direct quantitation of UGCG would be unreliable due to its transcript complexity,⁴³ poor intrinsic solubility (CamSol⁶³ intrinsic solubility score at pH 7.4: −3.1), and limited membrane extraction.^{41,42,64} Although $[P]_0$ could vary due to the ultrafiltration step,⁵⁴ K_D and NS were consistent across experiments, as confirmed by an extra sum-of-squares F test ($\alpha = 0.05$). For Caki-1 Golgi microsomes, eliglustat exhibited a K_D of 15.49 [10.08–23.76] μM (Figure S8). Reported inhibition potencies vary with cell type, ranging from 24 nM

in K-562 cells⁵⁰ to 42 μM in JVM-2 cells,⁶⁵ consistent with the lack of a detectable bound fraction in AS-MS experiments.^{19,20}

The binding model used in this study explicitly accounts for nonspecific binding as a function of the free analyte concentration, inherently incorporating the analyte partition into the lipid bilayer as well as all other nonspecific interactions that follow this function. The value of the NS parameter depends on the analyte and the composition of the microsomal membranes. Estimating specific binding by subtracting experimentally determined nonspecific binding from total binding is meaningless when a significant fraction of the analyte is bound. As described by Swillens,⁶² nonspecific binding is no longer a linear function of the total analyte concentration. Consequently, conventional approaches, such as the addition of excess competitor or the use of control microsomes lacking UGCG, lead to an overestimation of nonspecific binding, as they increase the free analyte concentration. To further validate the specificity of the observed binding, four concentrations of eliglustat were tested using F18 under the same experimental conditions as for F_{pool} . F18 is a smooth endoplasmic reticulum fraction that exhibits similar vesicle size distribution and concentration as F_{pool} (Figure S9 and Table S4) but lacks UGCG (45 and 33 kDa; Figure 2). Indeed, at 5 μM eliglustat, a high bound fraction of 0.33 ± 0.02 (superior to F_{pool}) was recorded with F11, suggesting that the inactive 33 kDa UGCG also binds eliglustat. The analysis of multiple eliglustat concentrations enables discrimination between purely nonspecific binding and binding that includes a specific component. In the case of nonspecific binding alone, the bound fraction remains

independent of the total analyte concentration and is equal to the constant $\frac{NS}{(1+NS)}$. As shown in Figure S10, the eliglustat bound fraction measured using F18 remained constant across the tested concentrations, in contrast to the concentration-dependent behavior observed for F_{pool} , thereby supporting the validity of the model.

Affinity Assessment of Miglustat by Competitive Displacement Titration

The direct ACE method described above is limited to compounds that are both UV-active and positively charged at physiological pH. To address this limitation, we explored an indirect ACE approach using cancer-derived Golgi microsomes, which also allows detection of weaker interactions. In this mode, a potential competing ligand, analyte B, is added to the BGE, modulating the binding of eliglustat to microsomes, and the resulting shift in the migration time of eliglustat is monitored (Figure 6A). Miglustat was selected as the competing ligand because of its lower potency and selectivity toward UGCG and its chemical structure, which differs markedly from eliglustat.⁵⁷ It is a synthetic *N*-alkylated iminosugar with a pK_a of 7.1.⁶⁶ Given that molecules with distinct physicochemical properties generally exhibit different nonspecific binding behaviors, miglustat ability to displace eliglustat from Golgi microsomes would further confirm the specificity of eliglustat binding.

To account for competition, the binding model was extended to include a second ligand, analyte B, that binds the target with a 1:1 stoichiometry and competes with eliglustat. A ternary complex (target-eliglustat-B) was assumed not to form. Given the expected weak affinity of analyte B, the free concentration $[B]$ was approximated by the total concentration $[B]_0$, as the bound fraction of miglustat is negligible. This simplification avoided the implication of a cubic equation⁶⁷ during mathematical developments. Under rapid-equilibrium conditions, the bound fraction of eliglustat in the presence of the competing ligand B is described by eq 5 (Supplemental Data-6).

$$\begin{aligned} \text{Bound fraction} &= \left(\frac{-b + \sqrt{b^2 - 4ac}}{2K_D(1+NS)([B] + K_B) - b + \sqrt{b^2 - 4ac}} + NS \right) \\ &\times \left(\frac{1}{1+NS} \right) \end{aligned} \quad (5)$$

where

$$a = ([B] + K_B)$$

$$b = (1+NS)K_D([B] + K_B) + K_B([A]_0 - [P]_0)$$

$$c = -(1+NS)K_D[P]_0K_B$$

The dissociation constant of miglustat (K_B) was determined by monitoring the effect of varying miglustat concentrations in the BGE on the bound fraction of eliglustat (Figure 6C). As shown in Figure 6B, increasing miglustat concentrations led to a reduction in the migration time shift of eliglustat. Eliglustat was injected at 5 μM ($[A]_0$), and the resulting bound fractions were plotted against the concentration of miglustat. Fitting these data to eq 5 yielded a K_B of 153.1 [119.1–199.8] μM , consistent with the reported potency range of miglustat (IC_{50} = 22.9–100 μM ⁵⁷).

Collectively, the use of a UGCG-depleted fraction (F18) and a competitive binding assay with a structurally distinct UGCG inhibitor provides converging evidence that the observed mobility shifts of eliglustat arise from specific interactions with UGCG rather than nonspecific effects.

CONCLUSIONS

Membrane proteins constitute 20–30% of the proteome and serve as targets for over half of all approved drugs, yet their study remains hampered by difficulties in production and in preserving therapeutically relevant conformations during drug discovery. There is an urgent need for methods that can interrogate interactions in complex, physiologically relevant environments. Here, we demonstrate that cancer-derived Golgi microsomes provide such a system and show that the ACE methodology overcomes key barriers in membrane protein characterization. ACE requires neither target modification nor intrinsic activity and, in its microfluidic format, dramatically reduces sample consumption. In direct mode, the separative power of capillary electrophoresis is preserved, enabling analysis of impure compounds in a single run, while the indirect mode extends applicability to ligands lacking charge or UV absorbance. Importantly, integration with mathematical modeling eliminates the need for large protein excess and corrects for nonspecific binding, addressing limitations that have long constrained traditional ACE approaches. By enabling the assessment of target engagement in complex, physiologically relevant environments, this approach may support more informed compound selection in the early development process.

Applying this approach to UGCG, an intracellular Golgi-resident membrane protein, we demonstrated that ACE can quantify ligand binding directly at the surface of cancer-derived Golgi fractions. These fractions, composed of fairly monodisperse vesicles (~150 nm), underscore the method ability to interrogate complex, membrane-bound systems. By preserving the native protein composition, including components essential to Golgi architecture, this approach prioritizes physiological relevance. Collectively, this work extends the scope of ACE in drug discovery. This strategy is readily transferable to a broad range of intracellular and extracellular membrane-bound particles of therapeutic relevance, such as liposomes and extracellular vesicles, and thus can probe how vesicle curvature and composition influence binding equilibrium. Together, these advances establish ACE as a robust, versatile platform to study biomolecular interactions in pathologically relevant contexts.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.analchem.6c01494>.

Graphical overviews of the Golgi microsomes isolation and label-free activity assay, detailed descriptions of the analytical development of the label-free activity assay, and the sample preparation of the Golgi microsomes exposed to an electric field; details of the determination of the UGCG topology; full mathematical developments; additional data, including Golgi fraction refractive index, electropherograms, nanoparticle size distribution, nonlinear regression plots for the determi-

nation of the dissociation constants, and total protein content (PDF)

AUTHOR INFORMATION

Corresponding Author

Marianne Fillet – Laboratory for the Analysis of Medicines (LAM), Department of Pharmacy, CIRM, University of Liege, Liège 4000, Belgium; orcid.org/0000-0002-1453-6282; Email: marianne.fillet@uliege.be

Authors

Clara Davoine – Laboratory for the Analysis of Medicines (LAM), Department of Pharmacy, CIRM, University of Liege, Liège 4000, Belgium; orcid.org/0000-0003-1110-8319

Gaël Cobraiville – Laboratory for the Analysis of Medicines (LAM), Department of Pharmacy, CIRM, University of Liege, Liège 4000, Belgium

Lionel Pochet – Namur Medicine & Drug Innovation Center (NAMEDIC – NARILIS), University of Namur, Namur 5000, Belgium; orcid.org/0000-0002-2560-9555

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acs.analchem.6c01494>

Author Contributions

The manuscript was written through contributions of all authors.

Funding

Clara Davoine is a Postdoctoral Researcher of the Fonds de la Recherche Scientifique – FNRS [40017636] and has received funding from the Fondation Léon Frédéricq [2025-259]. Marianne Fillet has received funding from the University of Liège Fonds Spéciaux-Crédits sectoriels [2024-CSRVS].

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We acknowledge Giacomo Baudino and Géraldine Piel for the use of the Gradient Master.

REFERENCES

- (1) Lo, C. H.; Zeng, J. Application of Polymersomes in Membrane Protein Study and Drug Discovery: Progress, Strategies, and Perspectives. *Bioeng. Transl. Med.* **2023**, *8*, No. e10350.
- (2) Yin, H.; Flynn, A. D. Drugging Membrane Protein Interactions. *Annu. Rev. Biomed. Eng.* **2016**, *18* (1), 51–76.
- (3) Gulezian, E.; Crivello, C.; Bednenko, J.; Zafra, C.; Zhang, Y.; Colussi, P.; Hussain, S. Membrane Protein Production and Formulation for Drug Discovery. *Trends Pharmacol. Sci.* **2021**, *42* (8), 657–674.
- (4) Jelokhani-Niaraki, M. Membrane Proteins: Structure, Function and Motion. *Int. J. Mol. Sci.* **2023**, *24* (1), 468.
- (5) Li, H.; Sun, X.; Cui, W.; Xu, M.; Dong, J.; Ekundayo, B. E.; Ni, D.; Rao, Z.; Guo, L.; Stahlberg, H.; Yuan, S.; Vogel, H. Computational Drug Development for Membrane Protein Targets. *Nat. Biotechnol.* **2024**, *42* (2), 229–242.
- (6) Qiu, W.; Guo, Y. Advances in Native Cell Membrane Nanoparticles System. *Curr. Opin. Struct. Biol.* **2025**, *94*, 103130.
- (7) Davoine, C.; Fillet, M.; Pochet, L. Capillary Electrophoresis as a Fragment Screening Tool to Cross-Validate Hits from Chromogenic Assay: Application to FXIIa. *Talanta* **2021**, *226*, 122163.
- (8) Davoine, C.; Pardo, A.; Pochet, L.; Fillet, M. Fragment Hit Discovery and Binding Site Characterization by Indirect Affinity Capillary Electrophoresis: Application to Factor XIIa. *Anal. Chem.* **2021**, *93* (44), 14802–14809.
- (9) Davoine, C.; Fillet, M. Hyphenation of Affinity Capillary Electrophoresis with Mass Spectrometry for the Study of Ligand–Protein Interactions: N -Methylmorpholine Acetate Buffer and Polydopamine-Based Coating as Key Assets. *Anal. Chem.* **2025**, *97* (7), 3988–3995.
- (10) Farcaș, E.; Bouckaert, C.; Servais, A. C.; Hanson, J.; Pochet, L.; Fillet, M. Partial Filling Affinity Capillary Electrophoresis as a Useful Tool for Fragment-Based Drug Discovery: A Proof of Concept on Thrombin. *Anal. Chim. Acta* **2017**, *984*, 211–222.
- (11) Davoine, C.; Pochet, L.; Fillet, M. Advances in Analytical Technologies Detecting and Characterizing Noncovalent Interactions for Fragment-Based Drug Discovery. *TrAC, Trends Anal. Chem.* **2023**, *166*, 117161.
- (12) Olabi, M.; Stein, M.; Wätzig, H. Affinity Capillary Electrophoresis for Studying Interactions in Life Sciences. *Methods* **2018**, *146* (May), 76–92.
- (13) Li, J.; Ahat, E.; Wang, Y. Golgi Structure and Function in Health, Stress, and Diseases. *The Golgi Apparatus and Centriole*. Springer 2019; 7441–485.
- (14) Cui, C.; Sun, C.; Yuan, P.; Tian, S.; Xie, H.; Xu, F.; Li, H. The Golgi Apparatus as a Strategic Target in Cancer: Mechanisms, Diagnosis and Therapeutic Opportunities. *J. Drug Target* **2025**, *33*, 1773–1787.
- (15) Shah, B.; Hunkeler, M.; Bratt, A.; Yue, H.; Jaen Maisonet, I.; Fischer, E. S.; Buhrlage, S. J. Structural Basis of VCP-VCPIP1-P47 Ternary Complex in Golgi Maintenance. *Nat. Commun.* **2025**, *16* (1), 8025.
- (16) Lin, P.; Le-Niculescu, H.; Hofmeister, R.; Michael McCaffery, J.; Jin, M.; Hennemann, H.; McQuistan, T.; De Vries, L.; Farquhar, M. G. The Mammalian Calcium-Binding Protein, Nucleobindin (CALNOC), Is a Golgi Resident Protein. *J. Cell Biol.* **1998**, *141* (7), 1515–1527.
- (17) de Paz, J. L.; Nieto, P. M. Fluorescence Polarization Assays to Study Carbohydrate–Protein Interactions. *Org. Biomol. Chem.* **2025**, *23* (9), 2041–2058.
- (18) Cho, W.; Stahelin, R. Membrane Binding and Subcellular Targeting of C2 Domains. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids* **2006**, *1761* (8), 838–849.
- (19) Fujii, T.; Tanaka, Y.; Oki, H.; Sato, S.; Shibata, S.; Maru, T.; Tanaka, Y.; Tanaka, M.; Onishi, T. A New Brain-penetrant Glucosylceramide Synthase Inhibitor as Potential Therapeutics for Gaucher Disease. *J. Neurochem.* **2021**, *159* (3), 543–553.
- (20) Renaud, J. P.; Chung, C. W.; Danielson, U. H.; Egner, U.; Hennig, M.; Hubbard, R. E.; Nar, H. Biophysics in Drug Discovery: Impact, Challenges and Opportunities. *Nat. Rev. Drug Discovery* **2016**, *15* (10), 679–698.
- (21) Zhao, X.; Pandey, M. K. Central Roles of Glucosylceramide in Driving Cancer Pathogenesis. *Int. J. Mol. Sci.* **2025**, *26* (20), 9879.
- (22) Hirabayashi, Y.; Ishibashi, Y. UDP-Glucose: Ceramide Glucosyltransferase (UGCG). In *Handbook of Glycosyltransferases and Related Genes*; Springer: Japan, Tokyo, 2014; pp. 3–13. DOI: .
- (23) Marks, D. L.; Dominguez, M.; Wu, K.; Pagano, R. E. Identification of Active Site Residues in Glucosylceramide Synthase. *J. Biol. Chem.* **2001**, *276* (28), 26492–26498.
- (24) Wegner, M.-S.; Gruber, L.; Mattjus, P.; Geisslinger, G.; Grösch, S. The UDP-Glucose Ceramide Glycosyltransferase (UGCG) and the Link to Multidrug Resistance Protein 1 (MDR1). *BMC Cancer* **2018**, *18* (1), 153.
- (25) Di Sano, F.; Piacentini, M. Reticulon Protein-1C: A New Hope in the Treatment of Different Neuronal Diseases. *Int. J. Cell Biol.* **2012**, *2012*, 1–9.
- (26) Hayashi, Y.; Nemoto-Sasaki, Y.; Matsumoto, N.; Hama, K.; Tanikawa, T.; Oka, S.; Saeki, T.; Kumasaka, T.; Koizumi, T.; Arai, S.; Wada, I.; Yokoyama, K.; Sugiura, T.; Yamashita, A. Complex Formation of Sphingomyelin Synthase 1 with Glucosylceramide

Synthase Increases Sphingomyelin and Decreases Glucosylceramide Levels. *J. Biol. Chem.* **2018**, 293 (45), 17505–17522.

(27) Marks, D. L.; Wu, K.; Paul, P.; Kamisaka, Y.; Watanabe, R.; Pagano, R. E. Oligomerization and Topology of the Golgi Membrane Protein Glucosylceramide Synthase. *J. Biol. Chem.* **1999**, 274 (1), 451–456.

(28) Dong, L.; Cao, Z.; Chen, M.; Liu, Y.; Ma, X.; Lu, Y.; Zhang, Y.; Feng, K.; Zhang, Y.; Meng, Z.; Yang, Q.; Wang, Y.; Wu, Z.; Han, W. Inhibition of Glycosphingolipid Synthesis with Eliglustat in Combination with Immune Checkpoint Inhibitors in Advanced Cancers: Preclinical Evidence and Phase I Clinical Trial. *Nat. Commun.* **2024**, 15 (1), 6970.

(29) Dong, L.; Cao, Z.; Han, W.; Wu, Z. Synthesis, Function, and Therapeutic Potential of Glycosphingolipids. *Front. Immunol.* **2025**, 16, 16.

(30) Chen, S.; Wang, X.; Jounaidi, Y. UGCG and the Glycosphingolipid Rheostat: A Metabolic Checkpoint Governing Immune Activation and Tumor Immune Evasion. *Signal Transduction Targeted Ther.* **2025**, 10 (1), 298.

(31) Cas, M. D.; Casati, S.; Roda, G.; Pablo Sardi, S.; Paroni, R.; di Fonzo, A.; Trinchera, M. A Sensitive Method for Determining UDP-Glucose: Ceramide Glucosyltransferase (UGCG) Activity in Biological Samples Using Deuterated Glucosylceramide as Acceptor Substrate. *Glycobiology* **2023**, 33 (2), 88–94.

(32) Dobson, L.; Szekeres, L. I.; Gerdán, C.; Langó, T.; Zeke, A.; Tusnády, G. E. TmAlphaFold Database: Membrane Localization and Evaluation of AlphaFold2 Predicted Alpha-Helical Transmembrane Protein Structures. *Nucleic Acids Res.* **2023**, 51 (D1), D517–D522.

(33) Dovhunová, M.; Malý, M.; Dubský, P.; Gerlero, G. S.; Kler, P. A. Generalized Model of the Linear Theory of Electromigration and Its Application to Electrokinetic Chromatography: Capillary Zone Electrophoretic Systems with Complex-Forming Equilibria. *J. Chromatogr. A* **2020**, 1610, 460595.

(34) Graham, J. Homogenization of Mammalian Cultured Cells. *Sci. World J.* **2002**, 2, 1630–1633.

(35) Graham, J. Fractionation of Golgi, Endoplasmic Reticulum, and Plasma Membrane from Cultured Cells in a Preformed Continuous Iodixanol Gradient. *Sci. World J.* **2002**, 2, 1435–1439.

(36) Graham, J. OptiPrep™ Density Gradient Solutions for Mammalian Organelles. *Sci. World J.* **2002**, 2, 1440–1443.

(37) Graham, J. M. Isolation of Golgi Membranes from Tissues and Cells by Differential and Density Gradient Centrifugation. *Curr. Protoc. Cell Biol.* **2001**, 10 (1), 3–9.

(38) Bello, M. S.; Rezzonico, R.; Righetti, P. G. Capillary Electrophoresis Instrumentation as a Bench-Top Viscometer. *J. Chromatogr. A* **1994**, 659 (1), 199–204.

(39) Früh, V.; Ijzerman, A. P.; Siegel, G. How to Catch a Membrane Protein in Action: A Review of Functional Membrane Protein Immobilization Strategies and Their Applications. *Chem. Rev.* **2011**, 111 (2), 640–656.

(40) Levental, I.; Lyman, E. Regulation of Membrane Protein Structure and Function by Their Lipid Nano-Environment. *Nat. Rev. Mol. Cell Biol.* **2023**, 24 (2), 107–122.

(41) Durieux, I.; Martel, M. B.; Got, R. Solubilization of UDPglucose-Ceramide Glucosyltransferase from the Golgi Apparatus. *Biochim. Biophys. Acta, Biomembr.* **1990**, 1024 (2), 263–266.

(42) Paul, P.; Kamisaka, Y.; Marks, D. L.; Pagano, R. E. Purification and Characterization of UDP-Glucose: Ceramide Glucosyltransferase from Rat Liver Golgi Membranes. *J. Biol. Chem.* **1996**, 271 (4), 2287–2293.

(43) Dyer, S. C.; Austine-Orimoloye, O.; Azov, A. G.; Barba, M.; Barnes, I.; Barrera-Enriquez, V. P.; Becker, A.; Bennett, R.; Beracochea, M.; Berry, A.; et al. Ensembl 2025. *Nucleic Acids Res.* **2025**, 53 (D1), D948–D957.

(44) Brodaczewska, K. K.; Szczylik, C.; Fiedorowicz, M.; Porta, C.; Czarnecka, A. M. Choosing the Right Cell Line for Renal Cell Cancer Research. *Mol. Cancer* **2016**, 15 (1), 83.

(45) Nusinow, D. P.; Szpyt, J.; Ghandi, M.; Rose, C. M.; McDonald, E. R., III; Kalocsay, M.; Jané-Valbuena, J.; Gelfand, E.; Schweppe, D.

K.; Jedrychowski, M.; et al. Quantitative Proteomics of the Cancer Cell Line Encyclopedia. *Cell* **2020**, 180 (2), 387–402.E16.

(46) DepMap portal. *DepMap data*. <https://depmap.org/portal> (accessed 2026–05–22).

(47) Steć, A.; Heinz, A.; Dziomba, S. Characterization of Extracellular Vesicles by Capillary Zone Electrophoresis: A Novel Concept for Characterization of a next-Generation Drug Delivery Platform. *J. Pharm. Anal.* **2024**, 14 (12), 101004.

(48) Ribeiro, L. N. D. M.; Couto, V. M.; Fraceto, L. F.; de Paula, E. Use of Nanoparticle Concentration as a Tool to Understand the Structural Properties of Colloids. *Sci. Rep.* **2018**, 8 (1), 982.

(49) Matsuo, N.; Nomura, T.; Imokawa, G. A Rapid and Simple Assay Method for UDP-Glucose: Ceramide Glucosyltransferase. *Biochim. Biophys. Acta - Gen. Subj.* **1992**, 1116 (2), 97–103.

(50) McEachern, K. A.; Fung, J.; Komarnitsky, S.; Siegel, C. S.; Chuang, W.-L.; Hutto, E.; Shayman, J. A.; Grabowski, G. A.; Aerts, J. M. F. G.; Cheng, S. H.; Copeland, D. P.; Marshall, J. A Specific and Potent Inhibitor of Glucosylceramide Synthase for Substrate Inhibition Therapy of Gaucher Disease. *Mol. Genet. Metab.* **2007**, 91 (3), 259–267.

(51) *Handbook of Glycosyltransferases and Related Genes* Taniguchi, N.; Honke, K.; Fukuda, M.; Narimatsu, H.; Yamaguchi, Y.; Angata, T. Eds.; Springer: Japan, Tokyo, 2014. DOI: .

(52) Ahmad, S.; Jose da Costa Gonzales, L.; Bowler-Barnett, E. H.; Rice, D. L.; Kim, M.; Wijerathne, S.; Luciani, A.; Kandasamy, S.; Luo, J.; Watkins, X.; Turner, E.; Martin, M. J.; Bateman, A.; Martin, M.-J.; Orchard, S.; Magrane, M.; Ye, S.; Adesina, A.; Ahmad, S.; Bowler-Barnett, E. H.; Carpentier, D.; Denny, P.; Fan, J.; da Costa Gonzales, L. J.; Hussein, A.; Ignatchenko, A.; Insana, G.; Ishtiaq, R.; Joshi, V.; Jyothi, D.; Kandasamy, S.; Lock, A.; Luciani, A.; Luo, J.; Lussi, Y.; Marin, J. S. M.; Raposo, P.; Rice, D. L.; Stephenson, J.; Tototo, P.; Urakova, N.; Vasudev, P.; Wijerathne, S.; Ibrahim, K. T.; Kim, M.; Yu, C. W.-H.; Bridge, A. J.; Aimol, L.; Argoud-Puy, G.; Auchincloss, A. H.; Axelsen, K. B.; Bansal, P.; Baratin, D.; Batista Neto, T. M.; Blatter, M.-C.; Bolleman, J. T.; Boutet, E.; Breuza, L.; Casals-Casas, C.; Echioukh, K. C.; Coudert, E.; Cucho, B.; de Castro, E.; Estreicher, A.; Famiglietti, M. L.; Feuermann, M.; Gasteiger, E.; Gaudet, P.; Gehant, S.; Gerritsen, V.; Gos, A.; Gruaz, N.; Hulo, C.; Hyka-Nouspikel, N.; Jungo, F.; Kerhornou, A.; Mercier, P. L.; Lieberherr, D.; Masson, P.; Morgat, A.; Paesano, S.; Pedruzzi, I.; Pilbout, S.; Pourcel, L.; Poux, S.; Pozzato, M.; Pruess, M.; Redaschi, N.; Rivoire, C.; Sigrist, C. J. A.; Sonesson, K.; Sundaram, S.; Sveshnikova, A.; Wu, C. H.; Chen, C.; Huang, H.; Laiho, K.; Lehvaslahti, M.; McGarvey, P.; Natale, D. A.; Ross, K.; Vinayaka, C. R.; Wang, Y.; Mazumber, R.; Shanker, V.; Zhang, J. The UniProt Website API: Facilitating Programmatic Access to Protein Knowledge. *Nucleic Acids Res.* **2025**, 53 (W1), W547–W553.

(53) Coste, H.; Martel, M. B.; Got, R. Topology of Glucosylceramide Synthesis in Golgi Membranes from Porcine Submaxillary Glands. *Biochim. Biophys. Acta, Biomembr.* **1986**, 858 (1), 6–12.

(54) Lane, R. E.; Korbie, D.; Trau, M.; Hill, M. M. Purification Protocols for Extracellular Vesicles. In *Extracellular Vesicles, Methods in Molecular Biology*, Kuo, W. P.; Jia, S., Eds.; Humana Press: New York, NY, 2017, Vol. 1660, pp. 111–130. DOI: .

(55) Galievsky, V. A.; Stasheuski, A. S.; Krylov, S. N. Capillary Electrophoresis for Quantitative Studies of Biomolecular Interactions. *Anal. Chem.* **2015**, 87 (1), 157–171.

(56) Ansorge, M.; Dubský, P.; Ušelová, K. Into the Theory of the Partial-Filling Affinity Capillary Electrophoresis and the Determination of Apparent Stability Constants of Analyte-Ligand Complexes. *Electrophoresis* **2018**, 39 (5–6), 742–751.

(57) Shayman, J. A.; Hinkovska-Galcheva, V.; Shu, L. Inhibitors of Glucosylceramide Synthase In *Glycolipids: Methods and Protocols*, Kabayama, K.; Inokuchi, J.-I. Eds.; Humana: New York, NY, 2023, pp. 271–288. DOI: .

(58) Ferreira, C. M. H.; Pinto, I. S. S.; Soares, E. V.; Soares, H. M. V. M. (Un)Suitability of the Use of PH Buffers in Biological, Biochemical and Environmental Studies and Their Interaction with Metal Ions – a Review. *RSC Adv.* **2015**, 5 (39), 30989–31003.

(59) Morshed, A.; Dutta, P.; Hossan, M. R.; Dillon, R. Electrodeformation of Vesicles Suspended in a Liquid Medium. *Phys. Rev. Fluids* **2018**, 3 (10), 103702.

(60) Colton, I. J.; Carbeck, J. D.; Rao, J.; Whitesides, G. M. Affinity Capillary Electrophoresis: A Physical-Organic Tool for Studying Interactions in Biomolecular Recognition. *Electrophoresis* **1998**, 19 (3), 367–382.

(61) Nilsson, M.; Harang, V.; Bergström, M.; Ohlson, S.; Isaksson, R.; Johansson, G. Determination of Protein-Ligand Affinity Constants from Direct Migration Time in Capillary Electrophoresis. *Electrophoresis* **2004**, 25 (12), 1829–1836.

(62) Swillens, S. Interpretation of Binding Curves Obtained with High Receptor Concentrations: Practical Aid for Computer Analysis. *Mol. Pharmacol.* **1995**, 47 (6), 1197–1203.

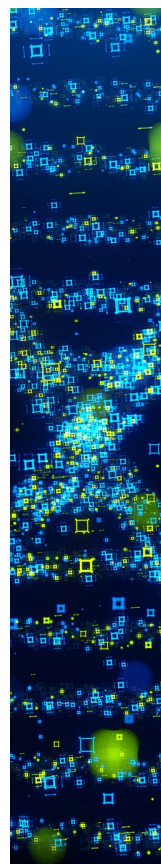
(63) Sormanni, P.; Vendruscolo, M. Protein Solubility Predictions Using the CamSol Method in the Study of Protein Homeostasis. *Cold Spring Harbor Perspect. Biol.* **2019**, 11 (12), a033845.

(64) Cube Biotech. *Solubilization Database of Membrane Proteins*; 2024. <https://cube-biotech.com/solubilization-database>.

(65) Nguyen Van Long, F.; Le, T.; Caron, P.; Valcourt-Gendron, D.; Sergerie, R.; Laverdière, I.; Vanura, K.; Guillemette, C. Targeting Sphingolipid Metabolism in Chronic Lymphocytic Leukemia. *Clin. Exp. Med.* **2024**, 24 (1), 174.

(66) Brumshtein, B.; Greenblatt, H. M.; Butters, T. D.; Shaaltiel, Y.; Aviezer, D.; Silman, I.; Futerman, A. H.; Sussman, J. L. Crystal Structures of Complexes of N-Butyl- and N-Nonyl-Deoxynojirimycin Bound to Acid β -Glucosidase. *J. Biol. Chem.* **2007**, 282 (39), 29052–29058.

(67) Wang, Z.-X. An Exact Mathematical Expression for Describing Competitive Binding of Two Different Ligands to a Protein Molecule. *FEBS Lett.* **1995**, 360 (2), 111–114.



CAS BIOFINDER DISCOVERY PLATFORM™

STOP DIGGING THROUGH DATA —START MAKING DISCOVERIES

CAS BioFinder helps you find the
right biological insights in seconds

Start your search



A Division of the
American Chemical Society