

Evaluating the molecular function of zebrafish Anoctamin 1a

Amanda Dimatteo¹, Nicole Stango¹, Adam Rich², Tara Sweet^{3§}

¹Biology, SUNY Geneseo, Geneseo, New York, United States

²Biology, SUNY Brockport, Brockport, New York, United States

³Biology-Psychology, SUNY Geneseo, Geneseo, New York, United States

[§]To whom correspondence should be addressed: tsweet@geneseo.edu

Abstract

Calcium-activated chloride channels (CaCCs) regulate key physiological processes like epithelial secretion, sensory transduction, gastrointestinal pacemaking, and muscle contraction. Zebrafish (*Danio rerio*) are genetically similar to humans, easily manipulated, and are a valuable model organism. We cloned zebrafish Anoctamin 1, a putative CaCC gene, expressed it in mammalian cells, and confirmed its function via halide flux assays. The channel conducted anions and responded to calcium, verifying its role as a CaCC. These findings confirm the functionality of zebrafish CaCCs and lay the groundwork to use the organism to model human physiology and disease.

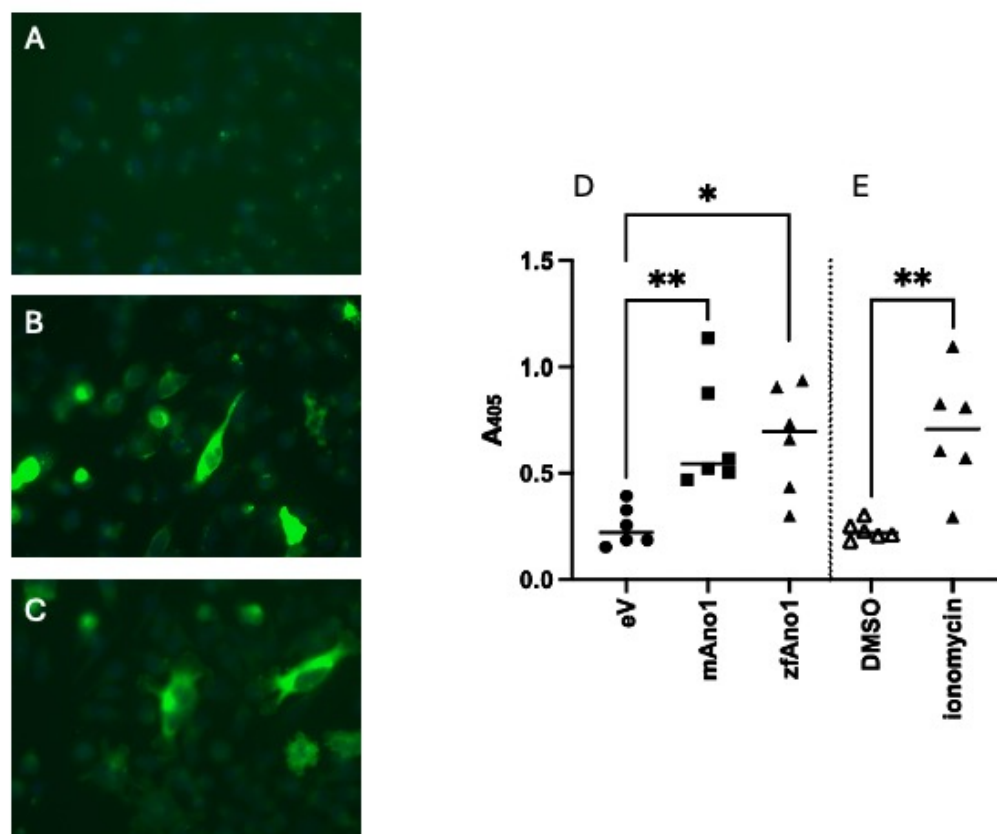


Figure 1. Heterologous Ano1 expression in CHO-K1 cells:

Left: Transfection of plasmids containing (A) vector, (B) mouse Ano1, or (C) zebrafish Ano1. Images were merged and taken at 40X with Ano1 immunoreactivity under the green filter and DAPI staining under the blue filter. Right: (D) Absorbance at 405 nm following a modified Sandell-Kolthoff reaction. Lysates from CHO-K1 cells transfected with empty vector (mean: 0.250, STD: 0.094, n = 6), mouse Ano1 (mean: 0.679, STD: 0.286, n = 6), or zebrafish Ano1 (mean: 0.662, STD: 0.234, n = 6) with the efflux assay run in the presence of 100 μM ionomycin. A Kruskal Wallis test revealed a statistically significant difference in absorbance between groups ($H(3) = 10.15$, $p = 0.002$). Dunn's Post test for multiple comparisons showed significant differences in absorbance between eV and mouse Ano1 ($p = 0.01$), and between eV and zebrafish Ano1 ($p = 0.01$). (E) Absorbance at 405 nm following a modified Sandell-Kolthoff reaction. Lysates from CHO-K1 cells transfected with zebrafish Ano1 with the efflux assay run in the presence of 100 μM of ionomycin, or vehicle. An

independent T-test showed a significant difference in scores between the two groups, $t(10) = 4.16$, $p = .001$. Ionomycin (mean: 0.701, 95% C.I. = 0.414 - 0.988, $n = 6$) had a higher OD405 as compared to the vehicle (mean: 0.230, 95% C.I. = 0.185 - 0.275, $n = 6$), indicating a calcium dependence of the flux.

Description

Anoctamins (TMEM16 proteins) are a diverse family of calcium-activated transmembrane proteins broadly expressed in various tissues. Some members of this family act as ion channels that conduct ions in response to elevated intracellular calcium levels, while others serve as phospholipid scramblases that redistribute lipids across the membrane bilayer. These proteins regulate processes such as membrane potential, cellular excitability, and phospholipid distribution. Specific members like ANO2 (TMEM16B), expressed in photoreceptors and olfactory neurons, are crucial for sensory transduction (Dauner et al., 2013; Stephan et al., 2009). ANO6 (TMEM16F) functions as a scramblase in blood cells and is essential for proper blood coagulation (H. Yang et al., 2012).

Anoctamin 1 (ANO1, also called TMEM16A or DOG1), the founding member of this family, functions as a calcium-activated anion channel that conducts chloride ions across membranes (Caputo et al., 2008; Schroeder et al., 2008; Y. D. Yang et al., 2008). ANO1 is expressed in diverse cell types including epithelial cells, interstitial cells of Cajal (ICCs), smooth muscle, skeletal muscle, and sensory neurons (Dayal et al., 2019; Griffin et al., 1982; Hwang et al., 2009). In epithelial tissues, ANO1 regulates chloride secretion vital for fluid and electrolyte balance in organs such as the lungs, intestines, and salivary glands (Centeio et al., 2021; Ousingsawat et al., 2024; Romanenko et al., 2010). In ICCs, ANO1 contributes to rhythmic electrical oscillations that drive gastrointestinal motility (Hwang et al., 2009). In smooth and skeletal muscle, it influences contractility and membrane excitability, impacting vascular tone and muscle function (Dayal et al., 2019; Heinze et al., 2014). ANO1 also participates in sensory transduction related to pain and itch perception (Huang et al., 2013; Kim et al., 2024). Dysregulation or mutation of ANO1 has been associated with conditions such as gastrointestinal dysmotility, vascular disorders, and various cancers, underlining its importance in health and disease (Al Sharie et al., 2023; Li et al., 2022; Pinard et al., 2023).

The zebrafish (*Danio rerio*) is a valuable vertebrate model for studying human physiology and disease due to its genetic similarity to humans—approximately 70% of human genes have zebrafish orthologs. Zebrafish embryos develop externally, are transparent during early stages, and are amenable to genetic manipulation techniques like CRISPR/Cas9, facilitating the creation of disease models. Their rapid development, high reproductive rate, and suitability for high-throughput screening make them ideal for exploring disease mechanisms and therapeutic interventions across a range of conditions, including cancer, cardiovascular, neurodegenerative, and congenital diseases.

To investigate zebrafish Ano1 function and its relevance to vertebrate physiology, we cloned the putative *ano1* gene from zebrafish larvae, expressed it in a mammalian cell system, and assessed its functional properties.

Cloning and Sequence Analysis of Zebrafish ano1

The zebrafish *ano1a* gene was cloned into the pcDNA3.1+ mammalian expression vector and sequenced via Sanger sequencing. Multiple overlapping reads confirmed high similarity to the predicted *Danio rerio* anoctamin 1 X2 isoform on chromosome 7 (GenBank XM_068222213.1). Sequence comparison revealed 13 differences: 11 silent nucleotide changes and two non-silent substitutions at positions 94 and 222. These resulted in amino acid changes: glutamate replacing aspartate at position 14 and glycine replacing glutamate at position 57.

Protein sequence analysis showed high conservation with human and mouse ANO1 orthologs, especially within the transmembrane regions TM3 to TM10. The critical sites for calcium binding were conserved, indicating potential functional similarity (Le et al., 2021). However, the zebrafish isoform lacked exons previously referred to as b, c, and d which alter the channel's calcium sensitivity, voltage dependence and gating kinetics (Ferrera et al., 2009; Strege et al., 2017; Xiao et al., 2011).

Expression of Zebrafish Ano1 in Mammalian Cells

CHO-K1 cells were transfected with the cloned zebrafish Ano1a construct, mouse Ano1, or empty vector to assess heterologous protein expression. Immunostaining using anti-human ANO1 antibodies revealed plasma membrane and cytoplasmic localization of zebrafish Ano1, comparable to mouse Ano1 expression patterns. Figure 1, panel A shows no signal was detected in empty vector controls, confirming specificity and successful expression of zebrafish Ano1a.

Functional Characterization Using Iodine Flux Assay

To determine whether zebrafish Ano1a functions as a CaCC similar to its mammalian counterparts (Caputo et al., 2008; Maduke et al., 2000; Y. D. Yang et al., 2008), a colorimetric iodine flux assay was employed (Tang & Wildey, 2004). In this assay, iodine efflux catalyzes the reduction of cerium (Ce), from a yellow Ce^{4+} to colorless Ce^{3+} , allowing quantification via absorbance. Transiently transfected cells loaded with iodine were treated with calcium ionophore ionomycin to activate Ano1 channels, followed by cell lysis and analysis (Liu & Hermann, 1978).

CHO-K1 cells expressing zebrafish or mouse Ano1 showed significantly higher absorbance compared to empty vector controls, indicating successful iodine efflux and functional anion channel activity. This efflux was calcium-dependent, as cells treated with ionomycin exhibited greater absorbance than vehicle-treated cells. These findings confirm that zebrafish Ano1a functions as a calcium-activated halide channel.

The observed 2- to 3-fold increase in absorbance following ionomycin treatment is consistent with activation of a calcium-dependent anion channel, although it may differ in magnitude than changes previously reported for GABA_A receptors by Tang and Wildey (2004). This difference likely reflects distinct channel gating mechanisms: GABA_A is a ligand-gated chloride channel directly activated by GABA, whereas Ano1 is a CaCC that responds to elevated intracellular calcium, which ionomycin induces both directly and indirectly through calcium-induced calcium release. Additionally, greater variability was observed across biological replicates in this study. While the use of transiently transfected CHO-K1 cells may contribute to this variation, other factors such as initial cell density, growth or death rates, calcium handling, expression levels, or cell detachment (potentially due to solution exchange) are also likely contributors. Ongoing work is focused on identifying and minimizing these sources of variability to improve assay consistency and reliability.

This study demonstrates that zebrafish Ano1a is a functional CaCC, capable of conducting anions in a calcium-dependent manner, consistent with its role in other vertebrates such as *Xenopus*, mouse, and human (Caputo et al., 2008; Schroeder et al., 2008; Y. D. Yang et al., 2008). These results provide foundational evidence for the physiological relevance of zebrafish Ano1a and suggest potential roles in processes like osmoregulation, sensory perception, and gastrointestinal function.

For example, zebrafish and other teleost fish must adapt to varying salinity levels during freshwater and seawater transitions (Evans et al., 2005). In euryhaline mummichogs, ano1 expression in gill epithelium increases with salinity exposure, supporting its role in chloride ion transport essential for osmoregulation (Breves et al., 2024). Given the similarity between mummichog and zebrafish Ano1, a similar mechanism may occur in zebrafish.

CaCCs are also important in sensory systems such as olfaction and taste (Dibattista et al., 2024). Zebrafish rely on their lateral line system—a mechanosensory organ detecting water movement and vibration—crucial for navigation, prey detection, and predator avoidance. Although Ano2 is known to be expressed in neuromast hair cells, Ano1 may also contribute to CaCC activity in this sensory network, potentially influencing behavior and environmental response (Lunsford et al., 2023).

In the gastrointestinal system, ICCs generate slow-wave electrical rhythms coordinating gut motility (Huizinga et al., 2014; Sanders, 2019). In mammals, ANO1 is essential for this pacemaker activity, and mutations are linked to dysmotility disorders (Al Sharie et al., 2023). Zebrafish possess ICC-like cells expressing Ano1, suggesting a conserved role in regulating digestive tract function (Uyttebroek et al., 2013). Future research may reveal how Ano1 dysfunction impacts zebrafish gut motility, offering insights applicable to human gastrointestinal disorders.

Recent studies show that Ano1 influences skeletal muscle function in zebrafish by accelerating action potentials. This complements our findings and prior research showing that reducing Ano1 expression via siRNA diminishes CaCC currents in muscle cells (9). These data suggest Ano1 is critical for muscle excitability and contraction, consistent with its role in mammalian muscle physiology.

In summary, we cloned and expressed zebrafish Ano1a, confirming its function as a CaCC through iodine flux assays. Zebrafish Ano1 shares conserved structural and functional characteristics with mammalian counterparts, validating its use in modeling ANO1-associated physiological and pathological processes. This study lays the groundwork for leveraging zebrafish as a model system to explore Ano1's role in sensory biology, muscle function, osmoregulation, and gastrointestinal motility, with implications for understanding human disease mechanisms and potential treatments.

Methods

Tissue was harvested from a pool of fifteen anesthetized unsexed 7 days post fertilization larvae generated from a multi-fish breeding of the ab/ab line housed in the lab of Dr. Rich. Larvae that did not show age-appropriate development were excluded. Previous experiments using this number of larvae produced sufficient RNA to amplify a segment of the ano1 by PCR. Wild-type zebrafish stocks, originally obtained from the Zebrafish International Resource Center, were housed and maintained in our facility at SUNY Brockport. All procedures were conducted following established protocols and in full compliance with the SUNY Brockport IACUC guidelines (Approval Number: Tissue Collection PROTO202400005) (Rich et al., 2007).

Cloning of zebrafish ano1

Tissue was homogenized in Qiazol lysis reagent (Qiagen, 79306). RNA was phase separated from the lysate by addition of chloroform-isoamyl alcohol (Sigma, C0549) and precipitated with isopropanol (Sigma, I9516). From the RNA, cDNA was prepared using a verso cDNA synthesis kit with oligo dT primers per manufacturer's instructions (Thermo Scientific, AB-1453). Primers were designed based on the published zebrafish genomic sequence (GRCz11) to capture the isoforms

overlapping coding regions and incorporate cloning restriction enzyme cut sites. The sequence of the forward primer was cgacacacaggtacCGCTGTACGGTCAACCTGG and the sequence of the reverse primer was cagacttctaGATTACGCTTGTGAACTGGGCCGCGAGCGG. cDNA was amplified with high-fidelity polymerase Pfu[®] Hotstart II (Thermo Scientific, F-549L), cleaned, digested and cloned into expression vectors pcDNA3.1+ (Invitrogen, V79020). The nucleotide sequence the insert was determined by Sanger Sequencing (Genewiz, South Plainfield, NJ), and the full-length sequence has been uploaded to Genbank (PQ62605).

Sequence Analysis of ano1

Using the clustal omega algorithm (available through <https://www.ebi.ac.uk/jdispatcher/>), the cloned zebrafish sequence was aligned with zebrafish predicted sequences (X1: XM_009303471.3, X2: XM_068222213.1, X3: XM_009303473.3, X4: XM_017356841.2). The zebrafish nucleotide sequence was translated to a protein sequence using ExPasy (available at <https://web.expasy.org/translate/>) and aligned with the clustal omega algorithm to human Anoctamin 1a (NP_060513.5) and mouse Anoctamin 1 “a” also referred to as anoctamin-1 isoform 2 (NP_001229278.2). These isoforms were chosen as known sequences and for consistency with previously published literature (Le et al., 2021), but the authors note zebrafish ano1 shows stronger alignment to the hypothetical human isoform ANO1 X6 (XP_054224207.1) largely because there is more available sequence that aligns at the N-termini.

Cell culture

CHOK1 cells (ATCC CAT# CCL-61) were grown in F12-K medium (ATCC 30-2004) containing 10% FBS (Gibco, A52095) and 1X Penicillin-Streptomycin-Glutamine mixture (Gibco, 10378). They were cultured in a tissue culture incubator at 37 °C with 5% CO₂. For imaging, 2 ml cells with a density of ~125,000/ml were dispensed into 12 well-containing D-lysine-coated (Gibco, A38904) glass coverslips. After overnight culture, cells were transfected using lipofectamine 2000 per manufacturer’s instructions (Invitrogen, 11668019). To evaluate expression of ano1, cells were transfected with an empty pcDNA3.1+ plasmid containing no insert, a pCMV6 plasmid containing mouse Ano1 (NM_178642, Cat # MC205263, Origene) or the pcDNA 3.1+ plasmid containing the cloned zfAno1. Materials were diluted at the following ratios, 1.5 µg DNA: 250 µl opti-mem (Gibco, 31985) and 3µl lipofectamine 2000: 250µl opti-mem per well. Three wells were transfected per group. Experiments were performed after the cells were cultured in the dish for a minimum of 20h. The experimenter was not blinded to groups.

For efflux assay, 1 ml of cells with a density of ~125,000 cells/ml were dispensed into a 24-well plate and cultured at 37 °C for four hours. Cells were transfected with lipofectamine 2000 per manufacturer’s instructions. For the flux assay, the cells were transfected with an empty pcDNA3.1+ plasmid containing no insert, a pCMV6 plasmid containing mouse Ano1 (NM_178642, Cat # MC205263, Origene) or the pcDNA 3.1+ plasmid containing the cloned zebrafish ano1. Six wells were transfected per group. Materials were diluted at the following ratios, 1 µg DNA: 50 µl opti-mem and 5µl lipofectamine 2000: 50µl opti-mem per well. Experiments were performed after the cells were in culture for a minimum of 20h. The experimenter was not blinded to groups.

Imaging of Ano1

For examination of Ano1 immunoreactivity, CHO-K1 cells were washed with DPBS with calcium and magnesium (Gibco, 14040) and fixed with acetic acid: ethanol (1:3) for 30 seconds. Cells were blocked in PBS (Kodak) with 10% NGS (Fisher, 10000C) and 0.1% triton-X (Fisher, BP151), stained using a rabbit anti-human Anoctamin 1 antibody (Abcam, ab53212) diluted at 1:100 in PBS with 1% NGS. Antibody staining was visualized using an Alexa 488 conjugated goat anti-rabbit secondary antibody (Fisher, A-11008), diluted at 1:1000. Cells were cover slipped with Vectashield containing DAPI (Vector Laboratories, H200010). For examination of GFP signal, coverslips washed with PBS and inverted on slides.

Chemicals were purchased from Fisher Scientific (Pittsburgh, PA) except when otherwise stated. They were sulfuric acid 96% (CAS 7664-93-9), arsenic (III) trioxide (oakwood chemicals, 1327-53-3), ammonia Ce (IV)-sulfate (oakwood chemicals, CAS 10378-47-9), ammonium hydroxide 25% (CAS 1336-21-6), ammonium chloride (CAS 12125-02-9), sodium iodide (CAS 7681-82-5), calcium chloride dihydrate (CAS 10035-04-8), sodium phosphate monobasic (CAS 10049-21-5), magnesium chloride (CAS 7786-30-3), potassium iodide (7681-11-0), sodium hydroxide (CAS 1310-73-2).

Iodine Flux Assay

An Iodine Flux Assay was conducted as described by Tang and Wildey (Tang & Wildey, 2004).

Solutions

Iodine-loading buffer consisted of the following components: 150 mM NaI, 2 mM CaCl₂, 0.8 mM NaH₂PO₄, 1 mM of MgCl₂, and 5.4 mM of KI; adjust pH to 7.4 with 1 N sodium hydroxide.

Detection buffer I was prepared by dissolving 19.8 g of arsenic (III) acid in a mixture of 300 ml-deionized water and 50 ml of 25% ammonia. Sulfuric acid was added to adjust pH to 8.5. Another 28 ml of sulfuric acid and 25 g of ammonium chloride were added to the solution. The final volume was adjusted to 1000 ml with deionized water. Then to slow

reaction time, the solution was diluted from a concentration of 100 mM, as used by Tang and Wildey, to 15 mM with deionized water.

Detection buffer II was prepared by dissolving 10 g of ammonia Ce (IV)-sulfate in 474 ml of deionized water. Twenty-six milliliters of sulfuric acid were then added, bringing the final volume to 500 ml. Iodine-loading buffer consisted of the following components: 150 mM NaCl, 2 mM CaCl₂, 0.8 mM NaH₂PO₄, 1 mM of MgCl₂, and 5.4 mM of KI; adjust pH to 7.4 with 1 N sodium hydroxide.

Cell lysis buffer consisted of 1% triton X-100 in DPBS.

Assay reaction time and standard curve generation

Sodium iodide was dissolved in water to a final concentration of 1.3 mM. A serial dilution of 1:3 was performed in 96-well plates to final concentrations of 1300, 433, 144, 48, 16, 5.3, 1.8, 0.59, 0.2, 0.07, 0.02, and 0.007 μM. In a 96-well plate, 100 microliters of sample was added to each well (n=3), followed by the modified Sandell-Kolthoff reaction as described below. OD405 was measured after the mixture was incubated at room temperature for 10, 15, 20, 25 and 30 min. We examined the OD405 versus concentration curves and selected 15-minute incubation time for subsequent experiments.

Iodine efflux assay

A modified protocol for measuring iodine efflux rate was used. 20 hours following transfection, medium was removed, and 1 ml of iodine-loading buffer was added, and cells were incubated for 4 hours at 37 °C. Incubation buffer was removed, and cells were placed into 500 μl of DPBS (with calcium and magnesium and 5% DMSO) with or without 100 μM ionomycin (Thermoscientific, CAS # 56092-82-1) for 30 min at 37 °C. Cells were washed two times with 1 ml DPBS containing calcium and magnesium. Cells were lysed with 250 μl of cell lysis buffer and Iodine concentrations were measured with the modified Sandell-Kolthoff (SK) reaction. Typically, 250 μl of lysed sample which contained different concentrations of iodine were mixed with an equal volume of Detection Buffer II and to this was added 250 μl of Detection Buffer I. Solutions were applied in time across, not within groups.

The mixture was incubated at room temperature for 15 min before the OD405 reading with Synergy HTX microplate reader (Biotek). The data presented are raw absorbance readings and have not been normalized or adjusted. The experimenter was not blinded to groups.

Statistical Analysis

We utilized GraphPad Prism software to conduct statistical analyses for assessing differences among experimental groups. Specifically, for the flux study analyzing the effect of plasmid we applied Bartlett's and Forsyth's tests to evaluate the homogeneity of variances across groups, ensuring that assumptions for subsequent analyses were met. Following this, we performed a Kruskal Wallis test for overall significant differences among group means. When significant results were found, we conducted Dunn's post hoc tests to identify specific group differences. For the flux study evaluating the effect of ionomycin, we used the Shapiro-Wilk test to assess normality and, for the normally distributed data, applied an independent samples t-test to compare differences between two groups. These analyses enabled us to accurately assess and interpret variations within and across experimental conditions.

Reagents

STRAIN	GENOTYPE	AVAILABLE FROM
Ab/Ab	Danio Rerio	ZIRC
PLASMID	GENOTYPE	DESCRIPTION
pcDNA3.1+	empty	Invitrogen, V79020
pcDNA3.1+	zfAno1	Genebank PQ62605 insert in between XbaI and KpnI
pCMV6	mAno1	NM_178642, Cat # MC205263, Origene

ANTIBODY	ANIMAL AND CLONALITY	DESCRIPTION
anti-human Anoctamin 1 antibody	Rabbit polyclonal	Abcam, ab53212, diluted 1:100
Anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488	Goat Polyclonal	Fisher, A-11008, diluted at 1:1000.

References

- Al Sharie AH, Abu Mousa BM, Al Zubi YO, Al Qudah MA, Jaradat SA, Barakat A, Altamimi E. 2023. A Novel ANO1 Gene Variant is Associated with Intestinal Dysmotility Syndrome Masquerading as Hirschsprung Disease: A Case Report.. *JPGN reports*. 4: e317. DOI: [10.1097/PG9.0000000000000317](https://doi.org/10.1097/PG9.0000000000000317)
- Breves JP, Posada MA, Tao YT, Shaughnessy CA. 2024. Salinity and prolactin regulate anoctamin 1 in the model teleost, *Fundulus heteroclitus*.. *American journal of physiology. Regulatory, integrative and comparative physiology*. 327: R479. DOI: [10.1152/ajpregu.00188.2024](https://doi.org/10.1152/ajpregu.00188.2024)
- Caputo A, Caci E, Ferrera L, Pedemonte N, Barsanti C, Sondo E, et al., Galiotta LJV. 2008. TMEM16A, a membrane protein associated with calcium-dependent chloride channel activity.. *Science (New York, N.Y.)*. 322: 590. DOI: [10.1126/science.1163518](https://doi.org/10.1126/science.1163518)
- Centeio R, Ousingsawat J, Cabrita I, Schreiber R, Talbi K, Benedetto R, et al., Kunzelmann K. 2021. Mucus Release and Airway Constriction by TMEM16A May Worsen Pathology in Inflammatory Lung Disease.. *International journal of molecular sciences*. 22 DOI: [10.3390/ijms22157852](https://doi.org/10.3390/ijms22157852)
- Dauner K, Mobus C, Frings S, Mohrlen F. 2013. Targeted expression of anoctamin calcium-activated chloride channels in rod photoreceptor terminals of the rodent retina.. *Investigative ophthalmology & visual science*. 54: 3126. DOI: [10.1167/iov.13-11711](https://doi.org/10.1167/iov.13-11711)
- Dayal A, Ng SFJ, Grabner M. 2019. Ca²⁺-activated Cl⁻ channel TMEM16A/ANO1 identified in zebrafish skeletal muscle is crucial for action potential acceleration.. *Nature communications*. 10: 115. DOI: [10.1038/s41467-018-07918-z](https://doi.org/10.1038/s41467-018-07918-z)
- Dibattista M, Pifferi S, Hernandez Clavijo A, Menini A. 2024. The physiological roles of anoctamin2/TMEM16B and anoctamin1/TMEM16A in chemical senses.. *Cell calcium*. 120: 102889. DOI: [10.1016/j.ceca.2024.102889](https://doi.org/10.1016/j.ceca.2024.102889)
- Evans DH, Piermarini PM, Choe KP. 2005. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste.. *Physiological reviews*. 85: 97. DOI: [10.1152/physrev.00050.2003](https://doi.org/10.1152/physrev.00050.2003)
- Ferrera L, Caputo A, Ubby I, Bussani E, Zegarra Moran O, Ravazzolo R, Pagani F, Galiotta LJV. 2009. Regulation of TMEM16A chloride channel properties by alternative splicing.. *The Journal of biological chemistry*. 284: 33360. DOI: [10.1074/jbc.M109.046607](https://doi.org/10.1074/jbc.M109.046607)
- Griffin GE, Fagan EF, Hodgson HJ, Chadwick VS. 1982. Enteral therapy in the management of massive gut resection complicated by chronic fluid and electrolyte depletion.. *Digestive diseases and sciences*. 27: 902. DOI: [10.1007/BF01316574](https://doi.org/10.1007/BF01316574)
- Heinze C, Seniuk A, Sokolov MV, Huebner AK, Klementowicz AE, Szijarto IA, et al., Hubner CA. 2014. Disruption of vascular Ca²⁺-activated chloride currents lowers blood pressure.. *The Journal of clinical investigation*. 124: 675. DOI: [10.1172/JCI70025](https://doi.org/10.1172/JCI70025)
- Huang F, Wang X, Ostertag EM, Nuwal T, Huang B, Jan YN, Basbaum AI, Jan LY. 2013. TMEM16C facilitates Na⁽⁺⁾-activated K⁺ currents in rat sensory neurons and regulates pain processing.. *Nature neuroscience*. 16: 1284. DOI: [10.1038/nn.3468](https://doi.org/10.1038/nn.3468)
- Huizinga JD, Chen JH, Zhu YF, Pawelka A, Mc Ginn RJ, Bardakjian BL, et al., Chen D. 2014. The origin of segmentation motor activity in the intestine.. *Nature communications*. 5: 3326. DOI: [10.1038/ncomms4326](https://doi.org/10.1038/ncomms4326)
- Hwang SJ, Blair PJA, Britton FC, O Driscoll KE, Hennig G, Bayguinov YR, et al., Ward SM. 2009. Expression of anoctamin 1/TMEM16A by interstitial cells of Cajal is fundamental for slow wave activity in gastrointestinal muscles.. *The Journal of physiology*. 587: 4887. DOI: [10.1113/jphysiol.2009.176198](https://doi.org/10.1113/jphysiol.2009.176198)
- Kim H, Shim WS, Oh U. 2024. Anoctamin 1, a multi-modal player in pain and itch.. *Cell calcium*. 123: 102924. DOI: [10.1016/j.ceca.2024.102924](https://doi.org/10.1016/j.ceca.2024.102924)

- Le SC, Liang P, Lowry AJ, Yang H. 2021. Gating and Regulatory Mechanisms of TMEM16 Ion Channels and Scramblases.. *Frontiers in physiology*. 12: 787773. DOI: [10.3389/fphys.2021.787773](https://doi.org/10.3389/fphys.2021.787773)
- Li H, Yu Z, Wang H, Wang N, Sun X, Yang S, Hua X, Liu Z. 2022. Role of ANO1 in tumors and tumor immunity.. *Journal of cancer research and clinical oncology*. 148: 2045. DOI: [10.1007/s00432-022-04004-2](https://doi.org/10.1007/s00432-022-04004-2)
- Liu C, Han T, Stachura DL, Wang H, Vaisman BL, Kim J, et al., Miller YI. 2018. Lipoprotein lipase regulates hematopoietic stem progenitor cell maintenance through DHA supply.. *Nature communications*. 9: 1310. DOI: [10.1038/s41467-018-03775-y](https://doi.org/10.1038/s41467-018-03775-y)
- Liu C, Hermann TE. 1978. Characterization of ionomycin as a calcium ionophore.. *The Journal of biological chemistry*. 253: 5892.
- Lunsford ET, Bobkov YV, Ray BC, Liao JC, Strother JA. 2023. Anion efflux mediates transduction in the hair cells of the zebrafish lateral line.. *Proceedings of the National Academy of Sciences of the United States of America*. 120: e2315515120. DOI: [10.1073/pnas.2315515120](https://doi.org/10.1073/pnas.2315515120)
- Maduke M, Miller C, Mindell JA. 2000. A decade of CLC chloride channels: structure, mechanism, and many unsettled questions.. *Annual review of biophysics and biomolecular structure*. 29: 411. DOI: [10.1146/annurev.biophys.29.1.411](https://doi.org/10.1146/annurev.biophys.29.1.411)
- Ousingsawat J, Centeio R, Reyne N, Mc Carron A, Cmielewski P, Schreiber R, et al., Kunzelmann K. 2024. Inhibition of mucus secretion by niclosamide and benzbromarone in airways and intestine.. *Scientific reports*. 14: 1464. DOI: [10.1038/s41598-024-51397-w](https://doi.org/10.1038/s41598-024-51397-w)
- Pinard A, Ye W, Fraser SM, Rosenfeld JA, Pichurin P, Hickey SE, et al., Milewicz DM. 2023. Rare variants in ANO1, encoding a calcium-activated chloride channel, predispose to moyamoya disease.. *Brain : a journal of neurology*. 146: 3616. DOI: [10.1093/brain/awad172](https://doi.org/10.1093/brain/awad172)
- Rich A, Leddon SA, Hess SL, Gibbons SJ, Miller S, Xu X, Farrugia G. 2007. Kit-like immunoreactivity in the zebrafish gastrointestinal tract reveals putative ICC.. *Developmental dynamics : an official publication of the American Association of Anatomists*. 236: 903. DOI: [10.1002/dvdy.21086](https://doi.org/10.1002/dvdy.21086)
- Romanenko VG, Catalan MA, Brown DA, Putzier I, Hartzell HC, Marmorstein AD, et al., Melvin JE. 2010. Tmem16A encodes the Ca²⁺-activated Cl⁻ channel in mouse submandibular salivary gland acinar cells.. *The Journal of biological chemistry*. 285: 12990. DOI: [10.1074/jbc.M109.068544](https://doi.org/10.1074/jbc.M109.068544)
- Sanders KM. 2019. Spontaneous Electrical Activity and Rhythmicity in Gastrointestinal Smooth Muscles.. *Advances in experimental medicine and biology*. 1124: 3. DOI: [10.1007/978-981-13-5895-1_1](https://doi.org/10.1007/978-981-13-5895-1_1)
- Schroeder BC, Cheng T, Jan YN, Jan LY. 2008. Expression cloning of TMEM16A as a calcium-activated chloride channel subunit.. *Cell*. 134: 1019. DOI: [10.1016/j.cell.2008.09.003](https://doi.org/10.1016/j.cell.2008.09.003)
- Stephan AB, Shum EY, Hirsh S, Cygnar KD, Reiser J, Zhao H. 2009. ANO2 is the ciliary calcium-activated chloride channel that may mediate olfactory amplification.. *Proceedings of the National Academy of Sciences of the United States of America*. 106: 11776. DOI: [10.1073/pnas.0903304106](https://doi.org/10.1073/pnas.0903304106)
- Strege PR, Gibbons SJ, Mazzone A, Bernard CE, Beyder A, Farrugia G. 2017. EAVK segment "c" sequence confers Ca²⁺-dependent changes to the kinetics of full-length human Ano1.. *American journal of physiology. Gastrointestinal and liver physiology*. 312: G572. DOI: [10.1152/ajpgi.00429.2016](https://doi.org/10.1152/ajpgi.00429.2016)
- Tang W, Wildey MJ. 2004. Development of a colorimetric method for functional chloride channel assay.. *Journal of biomolecular screening*. 9: 607. DOI: [10.1177/1087057104266740](https://doi.org/10.1177/1087057104266740)
- Uytendaele L, Shepherd IT, Hubens G, Timmermans JP, Van Nassauw L. 2013. Expression of neuropeptides and anoctamin 1 in the embryonic and adult zebrafish intestine, revealing neuronal subpopulations and ICC-like cells.. *Cell and tissue research*. 354: 355. DOI: [10.1007/s00441-013-1685-8](https://doi.org/10.1007/s00441-013-1685-8)
- Xiao Q, Yu K, Perez Cornejo P, Cui Y, Arreola J, Hartzell HC. 2011. Voltage- and calcium-dependent gating of TMEM16A/Ano1 chloride channels are physically coupled by the first intracellular loop.. *Proceedings of the National Academy of Sciences of the United States of America*. 108: 8891. DOI: [10.1073/pnas.1102147108](https://doi.org/10.1073/pnas.1102147108)
- Yang H, Kim A, David T, Palmer D, Jin T, Tien J, et al., Jan LY. 2012. TMEM16F forms a Ca²⁺-activated cation channel required for lipid scrambling in platelets during blood coagulation.. *Cell*. 151: 111. DOI: [10.1016/j.cell.2012.07.036](https://doi.org/10.1016/j.cell.2012.07.036)
- Yang YD, Cho H, Koo JY, Tak MH, Cho Y, Shim WS, et al., Oh U. 2008. TMEM16A confers receptor-activated calcium-dependent chloride conductance.. *Nature*. 455: 1210. DOI: [10.1038/nature07313](https://doi.org/10.1038/nature07313)

Funding: This work was supported by funds from SUNY Brockport, SUNY Geneseo, NIH Grant R15-DK071588-02, and a SUNY RF Collaboration Award.

7/30/2025 - Open Access

Author Contributions: Amanda Dimatteo: formal analysis, investigation, methodology. Nicole Stango: formal analysis, investigation, methodology. Adam Rich: funding acquisition, project administration, supervision, writing - original draft, writing - review editing, resources. Tara Sweet: conceptualization, data curation, formal analysis, investigation, methodology, project administration, supervision, writing - original draft, writing - review editing.

Reviewed By: Anonymous

Nomenclature Validated By: Anonymous

History: Received June 18, 2025 **Revision Received** July 14, 2025 **Accepted** July 24, 2025 **Published Online** July 30, 2025 **Indexed** August 13, 2025

Copyright: © 2025 by the authors. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Citation: Dimatteo A, Stango N, Rich A, Sweet T. 2025. Evaluating the molecular function of zebrafish Anoctamin 1a. microPublication Biology. [10.17912/micropub.biology.001706](https://doi.org/10.17912/micropub.biology.001706)