The Secret Life of Mitochondria

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APPENDIX

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Summary

Mitochondrion is an essential organelle that functions both intracellularly and extracellularly. Previous literatures have been demonstrated the crucial role of intracellular mitochondria in energy production, calcium homeostasis, antioxidative stress, and cell death pathways. Moreover, mitochondria are observed to move among cells. However, it reminds unknown whether their functionality after being transported. Therefore, this thesis aims to uncover the innovative biological function of mitochondrial transfer, focusing on three key mechanisms: free mitochondria, extracellular vesicles (EVs), and tubular structures.

Mitochondrial transplantation (MT) is a novel therapeutic method for addressing a variety of disease conditions and preventing cell death. Healthy mitochondria, freshly isolated from cells or tissues, serve as the primary source for MT. These mitochondria are directly incubated with cells or injected into tissues, where they work to repair the damage. We investigated the therapeutic potential of healthy mitochondria in rescuing neuronal cells from ferroptosis. Extracellular mitochondria were observed to incorporate into both healthy and ferroptotic neurons. The incorporated mitochondria in healthy neurons were able to ameliorate mitochondrial respiration in recipient cells. In the ferroptotic neurons, extracellular mitochondria alleviated oxidative stress, decreased cell death, and restored the fragmentation of neuronal networks. The mechanism behind neuroprotective effects of extracellular mitochondria on ferroptosis were attributed to the enzymatic complexes embedded in electron transport chain of mitochondria, with complex I, III, and V identified as the primary contributors. Furthermore, using microfluidic devices, we were able to separate cell bodies and networks of primary cortical neurons (PCNs). Through application of extracellular mitochondria to only the networks of PCNs, we observed their transport to the cell bodies. This finding highlights a potential clinical application aspect: mitochondria could be administered peripherally to repair in central compartments, provided some axonal connections remind intact and undamaged.

Free mitochondria are not the unique format of extracellular mitochondria released from donor cells. Alternatively, they can also encapsuled into lipid membrane structures—EVs. EVs are shown to carry not only functionally intact mitochondria but also mitochondrial components, including proteins and nucleic acid. The alteration of mitochondrial contents in EVs offers insights into cellular health that potentially serves as biomarkers for diagnosing and monitoring disease conditions. We next focused on the proteomic analysis on EVs derived from neural progenitor cells (NPCs) from a patient with *presenilin 1 (PSEN1) ΔE9* mutation, a mutation related to familiar Alzheimer's disease (AD). Through analyzing the protein contents within EVs, we identified several proteins upregulated in AD EVs compared to isogenic EVs, including complement factor (CFB), protein tyrosine phosphatase receptor type C (PTPRC), pregnancy zone protein (PZP), alkaline phosphatase (ALPL), lysyl oxidase-like 4 (LOXL4), inter-alpha-trypsin inhibitor heavy chain 5 (ITIH5), alpha-2-antiplasmin (SERPINF2), periostin (POSTN), apolipoprotein C-III (APOC3), and transferrin (TF). The upregulation of these proteins indicates the involvement of neuroinflammation, extracellular matrix (ECM) remodeling, lipid metabolism, and iron metabolism in AD pathogenesis, underscoring the pivotal role of EVs in the progression of AD.

With a special focus on mitochondrial proteins, we found the upregulated proteins in AD EVs were mainly coming from mitochondrial matrix, while the upregulated proteins in isogenic EVs were mainly from mitochondrial membranes. Moreover, AD NPCs exhibited impaired mitochondrial function compared with isogenic NPCs. The encapsulation of mitochondrial matrix proteins in EVs reflect an attempt to manage mitochondrial dysfunction. These EVs could potentially propagate stress signals, contributing to the spread of mitochondrial dysfunction and cellular damage in recipient cells.

Mitochondria are released regardless of the presence or absence of membranes, yet tubular structures also function as highways for transporting mitochondria between cells. Next, we focused on understanding the biological role of mitochondrial transport through these tubular structures. By co-culturing two cell types, we demonstrated that neuronal mitochondria can be transferred through cytoplasmic extensions to glioblastoma (GBM) cells, the cellular subunits of one of the most malignant brain tumors. The transferred mitochondria of neurons were found to alleviate the proliferation and mitochondrial respiration of the cancer cells. Using transwell inserts to prevent the formation of tunneling nanotubes (TNTs) and tumor microtubes (TMs), we showed that mitochondria transfer from neurons to GBMs primarily depends on tube formation. Our study provides new insights on the tumor survival strategy in the brain: hijacking mitochondria from neurons, the most abundant brain resident cell with intricate tubular networks.

In summary, our study investigated three distinct mechanisms of mitochondrial transfer: free mitochondria, EVs, and tubular structures. Our findings demonstrate biological significance of mitochondria transfer in the brain, encompassing roles in neuroprotection, molecular biomarker discovery, as well as tumor facilitation. The functional outcomes of mitochondrial transfer attribute to the source of mitochondria. Healthy donor cells provide mitochondria that can rescue damage cells from cell death or enhance tumor proliferation. Conversely, cells from physiological condition secrete mitochondrial fractions as part of stress-response mechanism, where damaged components are expelled to mitigate intracellular dysfunction.

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Samenvatting

De mitochondriën zijn organellen die zowel intracellulair als extracellulair essentiële functies uitvoeren. Uit eerdere studies is gebleken dat intracellulaire mitochondriën een cruciale rol spelen bij energieproductie, calciumhomeostase, antioxidatieve stress en celdood. Bovendien is waargenomen dat mitochondriën zich tussen cellen verplaatsen. Het is echter nog onduidelijk of hun functionaliteit behouden blijft na transport. Deze scriptie richt zich op het onthullen van innovatieve biologische functies van mitochondriale overdracht, met de nadruk op drie belangrijke transportmechanismen: vrije mitochondriën, membraanblaasjes (EV's) en tubulaire structuren.

Mitochondriale transplantatie (MT) is een nieuwe therapeutische methode om verschillende ziektes te behandelen en celdood te voorkomen. Gezonde mitochondriën, vers geïsoleerd uit cellen of weefsels, dienen als de primaire bron voor MT. Deze mitochondriën worden geïncubeerd met cellen of geïnjecteerd in weefsels, waar ze helpen schade te herstellen. We onderzochten de therapeutische potentie van gezonde mitochondriën om neuronale cellen te redden van ferroptose. Extracellulaire mitochondriën werden opgenomen in zowel gezonde als ferroptotische neuronen. In gezonde neuronen verbeterden de opgenomen mitochondriën de mitochondriale ademhaling in de ontvangende cellen. In ferroptotische neuronen verminderden extracellulaire mitochondriën oxidatieve stress en celdood. Ook droegen ze bij aan het herstel van de fragmentatie van neuronale netwerken. Het neurobeschermende effect van extracellulaire mitochondriën bij ferroptose werd toegeschreven aan de enzymcomplexen in de elektronentransportketen van mitochondriën, waarbij complex I, III en V als de belangrijkste bijdragers werden geïdentificeerd.

Verder hebben we met behulp van microfluidische technieken de cellichamen en netwerken van primaire corticale neuronen (PCN's) gescheiden. Door extracellulaire mitochondriën alleen toe te dienen aan de netwerken van PCN's, observeerden we hun transport naar de cellichamen. Deze bevinding benadrukt een potentiële klinische toepassing: mitochondriën kunnen perifeer worden

toegediend om centrale compartimenten te repareren, mits enkele axonale verbindingen intact en onbeschadigd blijven.

Vrije mitochondriën zijn niet de enige vorm van extracellulaire mitochondriën die door donorcellen worden vrijgegeven. Ze kunnen ook worden ingekapseld in lipidemembraanstructuren, EV's genaamd. EV's bevatten niet alleen functioneel intacte mitochondriën, maar ook mitochondriale componenten, waaronder eiwitten en nucleïnezuren. De detectie van veranderingen in mitochondriale inhoud in EV's biedt inzichten in de cellulaire gezondheid en kan mogelijk dienen als biomarker voor het diagnosticeren en monitoren van ziektes.

Wij richtten ons vervolgens op de proteomische analyse van EV's afkomstig van neurale progenitorcellen (NPC's) van een patiënt met een *presenilin 1 (PSEN1) ΔE9*-mutatie, een mutatie die verband houdt met de familiaire variant van de ziekte van Alzheimer (AD). Door de eiwitinhoud in EV's te analyseren, identificeerden we verschillende eiwitten die verhoogd waren in AD EV's in vergelijking met isogene EV's, complement factor (CFB), protein tyrosine phosphatase receptor type C (PTPRC), pregnancy zone protein (PZP), alkaline phosphatase (ALPL), lysyl oxidase-like 4 (LOXL4), inter-alpha-trypsin inhibitor heavy chain 5 (ITIH5), alpha-2-antiplasmin (SERPINF2), periostin (POSTN), apolipoprotein C-III (APOC3), en transferrin (TF). De opregulatie van deze eiwitten duidt op de betrokkenheid van neuro-inflammatie, extracellulaire matrix (ECM)-remodellering, lipidenmetabolisme en ijzermetabolisme bij de pathogenese van AD.

Met een speciale focus op mitochondriale eiwitten ontdekten we dat de verhoogde eiwitten in AD EV's voornamelijk afkomstig waren uit de mitochondriale matrix, terwijl de verhoogde eiwitten in isogene EV's voornamelijk afkomstig waren van mitochondriale membranen. Bovendien vertoonden AD NPC's een verminderde mitochondriale functie in vergelijking met isogene NPC's. De inkapseling van mitochondriale matrixeiwitten in EV's weerspiegelt een poging om mitochondriale disfunctie te controleren. Deze EV's kunnen mogelijk stresssignalen verspreiden, wat bijdraagt aan de verspreiding van mitochondriale disfunctie en cellulaire schade in ontvangende cellen.

Mitochondriën worden uitgescheiden ongeacht de aanwezigheid of afwezigheid van membranen, maar tubulaire structuren functioneren wel als snelwegen voor het transport van mitochondriën tussen cellen. We richtten ons vervolgens op het begrijpen van de biologische rol van mitochondriaal transport via deze tubulaire structuren. Door twee celtypen samen te kweken, toonden we aan dat neuronale mitochondriën via cytoplasmatische extensies naar glioblastoma (GBM)-cellen kunnen worden overgedragen, een van de meest kwaadaardige hersentumoren.

De overgedragen mitochondriën van neuronen bleken de proliferatie en mitochondriale ademhaling van kankercellen te verminderen. Met behulp van transwell-inserts om de vorming van tunneling nanotubes (TNT's) en tumor microtubes (TM's) te voorkomen, toonden we aan dat mitochondriaoverdracht van neuronen naar GBM's voornamelijk afhankelijk is van de vorming van tubulaire structuren. Onze studie biedt nieuwe inzichten in de overlevingsstrategie van tumoren in de hersenen: mitochondriën stelen van neuronen, de meest voorkomende hersencellen, aan de hand van complexe tubulaire netwerken.

Samenvattend onderzochten we drie verschillende mechanismen van mitochondriale overdracht: vrije mitochondriën, EV's en tubulaire structuren. Onze bevindingen demonstreren het biologische belang van mitochondriale overdracht in de hersenen, met rollen in neuroprotectie, moleculaire biomarkerontdekking en tumorfacilitatie. De functionele resultaten van mitochondriale overdracht zijn afhankelijk van de bron van de mitochondriën. Gezonde donorcellen leveren mitochondriën die beschadigde cellen kunnen redden van celdood of tumorgroei kunnen bevorderen. Daarentegen scheiden cellen onder fysiologische omstandigheden mitochondriale fracties uit als onderdeel van een stressresponsmechanisme, waarbij beschadigde componenten worden uitgescheiden om intracellulaire disfunctie te verminderen.

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Molecular Pharmacology (MF) family

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羽毛球小分队**鑫哥**和**峰哥**，想念我们一起在 aclo 打球的日子。谢谢你们一直以来的照顾和支持。**鑫哥，**你是一个有追求、有理想的人，你的努力和坚持让我很钦佩。愿你未来的日子里一切顺遂！峰哥，祝你和胥胥师姐幸福美满，小芒果茁壮成长！

陈邓师兄，总是能看到你在实验台前努力的身影，和你的交流中能感受到你对科研的热爱。祝你的科研之路一途坦荡。也祝你和嫂子、蹬蹬美满幸福。**建秋**和**航宇**，和你们一起吃午饭的日子总是那么轻松愉快，每次都让我感到放松和温暖。特别感谢建秋，你总是尽心尽力地帮助我，让我感受到满满的支持和关怀。祝你们毕业顺利，未来前程似锦，一切如愿！

元元和**书琦，**很高兴认识你们并和你们成为朋友。你们总是在关心和照顾着我，让我感到无比温暖。谢谢元元，总是为我们准备各种好吃的！还有你和唐天奕每次精心策划的旅行计划，都让我们的每一趟旅程充满欢乐和美好回忆。祝美丽的王瑞阿姨永远开心，笑口常开，生活充满幸福和阳光！书琦，你是我见过最自信的女孩之一。你总是全情投入地对待每一件事情，我相信你一定会取得非凡的成就。自信加上努力，绝对是通往成功的钥匙。祝你们在毕业阶段一切顺利，收获满满的 offer！

孙柳，我们一起经历了几次租房的 drama，格村的风和雨似乎总是在考验我们，但幸好我们相互支持，共同挺了过来。虽然你总说不要美化苦难，但我还是想说，历经风雨后的彩虹，有时候会绽放出别样的美丽风景。感恩有你一路同行，愿未来的日子里，你能沐浴更多的阳光，少些风雨，多些彩虹！小咪的饲料转化率能稍微提高一些。

此外，我还想感谢与我在格村相遇的朋友们：**小丹，皎月，冉哥，强哥，东哥，釜辰，梅姐儿**。

我还想感谢长久以来陪伴着我的朋友们。**昭蓉**，你是我认识最久的朋友。虽然我们现在相隔很远，但每次想起你，总觉得时光未曾拉开距离。我们总能在彼此想起的时候畅聊许久，那种熟悉和默契从未改变。愿你永远自由、美丽，拥有一个充满精彩和幸福的人生！**茜茜**，再次祝贺你成为妈妈！照顾孩子一定很辛苦，但我相信这份辛苦也充满了幸福。作为你的朋友，我真的为你感到由衷的开心。与此同时，我也想提醒你，在悉心照顾天天的同时，一定别忘了多照顾好自己，因为你的健康和快乐同样重要。**曦姐**，祝贺你喜得小棉袄。以后有机会一起旅游。**彭鑫栎**，你总是迁就我，照顾我。我非常感激有你这样一个朋友。但是我也发现，你总是报喜不报忧，似乎不太愿意分享你自己的困扰。希望你以后在伤心难过的时候，也能想到我，我愿意成为你倾诉的对象，做你永远的港湾。无论你遇到什么困难，记得我一直在这里，愿意听你诉说、给你支持。

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在完成论文的同时，我最爱的外婆**邹玉华女士**永远地离开了我们。我这这里写下她的名字是因为她一直以来是别人的妻子、母亲、祖母。但我想这次她也能有自己的名字。邹玉华女士，您一辈子都在为家人默默付出，辛苦操劳，未曾为自己停歇过。我多么希望，您能有更多的时光来为自己活一次，享受生活的美好。可是，遗憾的是，这辈子再也没有机会了。一切都是那么突然。每当想起您，我的心里充满了无尽的思念。还记得去年回家过年时，您把我叫到一边，反复叮嘱我要好好照顾自己，您的眼神里满是关怀与疼爱。虽然您已经离开，但您的爱、您的教诲永远留在我心中。我会带着您的爱，替您感受这个美丽的世界，勇敢地走下去。无论我走得多远，您永远是我心中最温暖的存在。

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最后我要感谢我的**妈妈**。感谢您把我带到这个世界上。还记得小时候，家庭条件并不算宽裕，但你们还是让我去少年宫学了芭蕾、民族舞、拉丁舞和主持。虽然这些并没有成为我后来发展的方向，却让我得以接触到一个丰富多彩的世界。这些经历让我看到了更多的可能性，而我也一直明白，这在当时对你们来说是一份不小的付出，因此我始终心存感激。希望您在未来的日子里始终保持一颗宽容的心，能够从更广阔的角度去看待问题，理解和包容他人的不同观点，这样生活会更加丰富多彩。愿您能享受生活。

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About the author

Tingting Chen (陈婷婷) was born on May 31st, 1995, in Mianyang City, Sichuan Province, China.

In 2013, she earned her bachelor's degree in veterinary medicine from Southwest University, China.

Driven by a strong passion for science, she pursued her master's degree at the same university under the guidance of Prof. Rendong Fang. She completed her master's degree in 2020, specializing in Preventive Veterinary Medicine. Her master's research primarily focused on the impact of foodborne pathogens on public health, with additional interests in bacterial infections and host defense mechanisms.

In 2021, Tingting began her Ph.D. studies at the University of Groningen, in the Department of Molecular Pharmacology, the Netherlands, under the supervision of Prof. Amalia Dolga and Prof. Ulrich Eisel. Her Ph.D. research focuses on investigating the role of extracellular mitochondria in neuroscience.

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List of publications

During PhD study

1. **Chen, T.**, Majerníková, N., Marmolejo-Garza, A., Trombetta-Lima, M., Sabogal-Guáqueta, A. M., Zhang, Y., Ten Kate, R., Zuidema, M., Mulder, P. P. M. F. A., den Dunnen, W., Gosens, R., Verpoorte, E., Culmsee, C., Eisel, U. L. M., & Dolga, A. M. (2023). Mitochondrial transplantation rescues neuronal cells from ferroptosis. Free radical biology & medicine, 208, 62–72. https://doi.org/10.1016/j.freeradbiomed.2023.07.034

2. Sanghvi, S., Sridharan, D., Evans, P., Dougherty, J., Szteyn, K., Gabrilovich, D., Dyta, M., Weist, J., Pierre, S. V., Gururaja Rao, S., Halm, D. R., **Chen, T**., Athanasopoulos, P. S., Dolga, A. M., Yu, L., Khan, M., & Singh, H. (2025). Functional large-conductance calcium and voltage-gated potassium channels in extracellular vesicles act as gatekeepers of structural and functional integrity. Nature Comm., 2025,01. https://www.nature.com/articles/s41467-024-55379-4#citeas

3. D. Chen, C. Zhao, J. Zhang, C. W. J. Knol, A. Osipyan, N. Majerníková, **T. Chen**, Z. Xiao, J. Adriana, A. J. Griffith, A. S. Gamez, P. E. van der Wouden, R. P. Coppes, A. M. Dolga, H. J. Haisma, F. J. Dekker (2024). Small Molecule MIF Modulation Enhances Ferroptosis by Impairing DNA Repair Mechanisms. *Adv. Sci.*, 11, 2403963. https://doi.org/10.1002/advs.202403963

4. Zhang, Y., Shaabani, S., Vowinkel, K., Trombetta-Lima, M., Sabogal-Guáqueta, A. M., **Chen, T.**, Hoekstra, J., Lembeck, J., Schmidt, M., Decher, N., Dömling, A., & Dolga, A. M. (2024). Novel SK channel positive modulators prevent ferroptosis and excitotoxicity in neuronal cells. Biomedicine & pharmacotherapy. 2024, 171, 116163. https://pubmed.ncbi.nlm.nih.gov/38242037/

5. Sabogal-Guáqueta, A. M., Marmolejo-Garza, A., Trombetta-Lima, M., Oun, A., Hunneman, J., **Chen, T.**, Koistinaho, J., Lehtonen, S., Kortholt, A., Wolters, J. C., Bakker, B. M., Eggen, B. J. L., Boddeke, E., & Dolga, A (2023). Species-specific metabolic reprogramming in human and mouse microglia during inflammatory pathway induction. Nature communications, 14(1), 6454. https://doi.org/10.1038/s41467-023-42096-7

6. Ortí-Casañ, N., Boerema, A. S., Köpke, K., Ebskamp, A., Keijser, J., Zhang, Y., **Chen, T.**, Dolga, A. M., Broersen, K., Fischer, R., Pfizenmaier, K., Kontermann, R. E., & Eisel, U. L. M. (2023). The TNFR1 antagonist Atrosimab reduces neuronal loss, glial activation and memory deficits in an acute mouse model of neurodegeneration. Scientific reports, 13(1), 10622. https://doi.org/10.1038/s41598-023-36846-2

7. de Ávila Narciso Gomes, R., Marmolejo-Garza, A., Haan, F. J., García, T. M., Chen, T., Mauthe, M., Moreira Franco Parisotto, Y. E., Murakami, M. M., Jr, Marie, S. K. N., Baptista, M. S., Dolga, A. M., & Trombetta-Lima, M. (2023). Mitochondrial dysfunction mediates neuronal cell response to DMMB photodynamic therapy. Biochimica et biophysica acta. Molecular cell research, 1870(3), 119429. https://doi.org/10.1016/j.bbamcr.2022.119429

During master study

1. **Chen T,** Jiang J, Ye C, Xie J, Chen X, Xu D, Zeng Z, Peng Y, Hu DL, Fang R: Genotypic characterization and antimicrobial resistance profile of Salmonella isolated from chicken, pork and the environment at abattoirs and supermarkets in Chongqing, China. BMC Vet Res. 2019;15(1):456

2. Feng S, **Chen T**, Lei G, Jiang J, Peng Y, Fang R: The research progress in the regulation of intestinal flora by inflammasome - A review. Chinese Journal of Preventive Veterinary Medicine 2018,40(10):971-975 (in Chinese, share first author).

3. Feng S, **Chen T**, Lei G, Hou F, Jiang J, Huang Q, Peng Y, Ye C, Hu DL, Fang R: Absent in melanoma 2 inflammasome is required for host defence against Streptococcus pneumoniae infection. Innate immunity 2019, 25(7):412-419. (share first author)

4. Zhang T, Du H, Feng S, Wu R, **Chen T**, Jiang J, Peng Y, Ye C, Fang R: NLRP3/ASC/Caspase-1 axis and serine protease activity are involved in neutrophil IL-1beta processing during Streptococcus pneumoniae infection. Biochemical and biophysical research communications 2019, 513(3):675-680.

5. Feng S, Huang Q, Ye C, Wu R, Lei G, Jiang J, **Chen T**, Peng Y, Fang R: Syk and JNK signaling pathways are involved in inflammasome activation in macrophages infected with Streptococcus pneumoniae. Biochemical and biophysical research communications 2018, 507(1-4):217-222.

6. Ye C, Huang Q, **Chen T**, Jiang J, Hou F, Xu D, Peng Y, Fang R, Chen J: First detection and genotypic analysis of goat enzootic nasal tumor virus 2 in Chongqing, China. Archives of virology 2019, 164(6):1647-1650.

7. Huang Q, Ye C, **Chen T**, Jiang J, Peng Y, Chen J, Fang R: EvaGreen-based real-time PCR assay for sensitive detection of enzootic nasal tumor virus 2. Mol Cell Probes 2019, 44:51-56.

8. Hou F, Peng L, Jiang J, **Chen T**, Xu D, Huang Q, Ye C, Peng Y, Hu D. L., & Fang R. ATP Facilitates Staphylococcal Enterotoxin O Induced Neutrophil IL-1β Secretion via NLRP3 Inflammasome Dependent Pathways. Frontiers in immunology, 2021, 12, 649235.

9. Xu D, Wu X, Peng L, **Chen T**, Huang Q, Wang Y, Ye C, Peng, Y, Hu, D, & Fang R. The Critical Role of NLRP6 Inflammasome in Streptococcus pneumoniae Infection In Vitro and In Vivo. International journal of molecular sciences, 2021, 22(8), 3876.

10. Peng L, Jiang J, **Chen T**, et al. Toxic Shock Syndrome Toxin 1 Induces Immune Response via the Activation of NLRP3 Inflammasome. Toxins (Basel). 2021;13(1):68.

Awards and conferences

1. 2024 Targeting Mitochondria Best Poster Presentation Awards
2. 2022 BCN Winter Meeting Poster Prize
3. 2024 Second World Congress Targeting Extracellular Vesicles (Oral Presentation)
4. 2023 Dutch Neuroscience meeting (Poster Presentation)
5. 2024 Dutch Pharmacological Society Spring Meeting (Oral Presentation)
6. 2023 The Netherlands Society for Extracellular Vesicles (NLSEV) (Poster Presentation)
7. 2023 Ferroptosis: When metabolism meets cell death (Poster Presentation)
8. 2022 AD/PD meeting (Poster Presentation)
9. 2021 Dutch Medicines Days (Poster Presentation)

THE SECRET LIFE OF MITOCHONDRIA