

Abstract collection from the 1st structural biology and biophysics symposium– SBF 2026

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EDITORIAL

Dear readers,

With great pleasure, we present to you a special issue of the European Pharmaceutical Journal, which is represented by an abstract collection from the Structural Biology and Biophysics Symposium that took place on 30th April 2026 in Bratislava at the premises of the Institute of Virology. The conference was organized by the Institute of Neuroimmunology and Institute of Virology of Slovak Academy of Sciences and by the Department of Pharmaceutical Chemistry and Department of Galenic Pharmacy, Faculty of Pharmacy of the Comenius University in Bratislava, in cooperation with the Slovak Biophysical Society. Indeed, we were pleased to host at the symposium in Bratislava approximately 50 participants—structural biologists and macromolecular crystallographers, biophysicists, pharmaceutical chemists, and analytical chemists from Slovakia and the Czech Republic. The symposium covered the work of structural biology groups from Bratislava and Kosice that are jointly measuring protein crystals at synchrotron light sources, mainly at protein crystallography beamlines P13 and P14 operated by European Molecular Biology Laboratory (EMBL) at PETRAIII storage ring in Hamburg. Structural biologists also presented biophysical and functional characterization of their respective protein targets.

During the symposium, nine lectures including the invited lecture delivered by Dr. Tomas Koval from the Institute of Biotechnology in Prague were presented, along with 11 posters that were presented by PhD students.

The symposium was organized as the deliverable of the project PREAGGRMOL (9I03-03-V04-00623) funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia.

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Understanding the versatility of S1–P1 nucleases

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Abstract S1–P1 nucleases are medium-sized enzymes (25–40 kDa) that share a distinctive active site built around a cluster of three zinc ions. They function as phosphoesterases, cleaving the P–O3' bond in nucleic acids and producing 5'-mononucleotides. What makes this enzyme family particularly interesting is its broad substrate range: S1–P1 nucleases can cleave almost any nucleic acid, often without regard to sequence or secondary structure. Members of this family are found in plants, fungi, trypanosomatids, and some bacteria, where they contribute to nutrient scavenging, programmed cell death, and even pathogen–host interactions. Several members have even found applications in biochemistry and biotechnology (e.g., S1 nuclease protection assay) (Koval et al., 2017). In our work, we focus on two representative enzymes: S1 nuclease from *Aspergillus oryzae* and SmNuc1 from the opportunistic pathogen *Stenotrophomonas maltophilia*. Both proteins form crystals that diffract to subatomic resolution and can accommodate any naturally occurring nucleotide—or even nucleotide pairs (Adamkova et al., 2025). These high-resolution structures allow us to investigate subtle structural features and clarify what drives the remarkable substrate promiscuity of the S1–P1 family. At the same time, they help explain why different family members display distinct preferences, such as acidic versus basic pH optima or differing activities toward DNA and RNA, providing new insight into both their biological roles and potential biotechnological applications.

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References

- T. Koval, J. Dohnalek *Biotechnol. Adv.* (2017) 10.1016/j.biotechadv.2017.12.007.
K. Adámková, M. et al. *FEBS J.*, 292, (2025) doi/10.1111/febs.17265

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Opportunities for biophysical and structure characterization of proteins at the Institute of Neuroimmunology, SAS Bratislava

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Abstract The Institute of Neuroimmunology of the Slovak Academy of Sciences (INSAS) is a leading research organization in the fields of neurodegenerative disorders and neuroimmune interactions. In the last 30 years, INSAS has been involved in a few large research projects of basic and applied neuroscience and immunology, including the investigation of neuroinflammation, testing of the first-in-humans active vaccine against pathological tau protein in tauopathies, and development of a passive immunotherapy for coronavirus disease 2019 disease. The Laboratory of Structural Biology of Neurodegeneration at INSAS is actively supporting neuroscience and immunology research of the institute and pursues an own independent line of research of intrinsically disordered proteins. Moreover, during the last 5 years, the laboratory became a nucleating seed of an informal Slovak macromolecular crystallography consortium, not only supporting the collective access to European macromolecular synchrotron beamlines but also collecting a critical amount of biophysical and structural biology research infrastructure and know-how, allowing for an up-to-date investigation of topical biological and biomedical problems. The aim of our effort is to offer the research possibilities on collaborative bases to other research groups in the fields of biophysics, biophysical chemistry, and structural biology. Thanks to a grant from the Recovery and Resilience Plan for Slovakia, INSAS takes part in the Advanced Protein Biotechnology Consortium (APBC), which allowed for a recent upgrade of our infrastructure. The APBC is led by the University of Pavel Jozef Safarik in Kosice, Slovakia and comprises the Institute of Experimental Physics, SAS and four international partners including research institutions from Czechia, Germany, the Netherlands, and the United Kingdom. Two nonacademic partners from Slovakia and Czechia complement the project team. What can we offer? INSAS infrastructure includes a wide portfolio of prokaryotic and eukaryotic expression systems (*Escherichia coli*, insect cells, CHO and HEK cell lines), protein purification protocols, state-of-the-art biophysical methods for protein characterization (dynamic and static light scattering, calorimetry methods, circular dichroism (CD) and Fourier transform infrared (FTIR) spectrometry, high-throughput surface plasmon resonance (SPR) and biolayer interferometry (BLI) instruments, stopped-flow methods), and X-ray crystallography. We will present examples of outputs of various methods in recent research projects.

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Structure of tau aggregation promoting conformational antibody DC11 and of its oligomeric targets

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Abstract A key yet unresolved question of the pathogenesis of Alzheimer's disease (AD) and other tauopathies is the cause and mechanism of transition from the unstructured monomeric tau protein to the insoluble filaments deposited in the brain tissue. In the physiological state, tau protein exists as a conformational ensemble of interconverting structures and on the scale of transition from monomeric through oligomeric and filamentous species, we can observe conformations reacting with specific antibodies, mainly with DC11, which is able to specifically discriminate between tau proteins isolated from healthy brain and tau proteins isolated from the brain of an AD patient. The antibody also recognizes the recombinant truncated tau proteins up to the shortest fragment tau321–391 (Vechterova et al., 2003). It was found that conformational antibodies DC11 and MN423 have catalytic pro-aggregatory effects in tau aggregation assay, whereas the antibody DC8E8 has inhibitory effects on tau filament formation (Kontsekova et al., 2014). This may imply the possible mechanism of induction of pathological tau conformation, in which the antibody prepared against pathological tau imprints the pathological conformation into the physiological tau proteins in solution and therefore speeds up tau aggregation. Therefore, information about the conformational epitopes of these antibodies is of high significance. To further uncover the binding mode of the conformational antibody DC11, we used nuclear magnetic resonance spectroscopy (NMR) for epitope mapping using ¹³C, ¹⁵N labeled tau321–391 (Njemoga et al., 2026) and tau297–391 (dGAE) and recombinantly prepared Fab fragment of DC11 antibody. The overlay of heteronuclear single quantum coherence (HSQC) spectra showed the region of tau between the residues 370 and 390 to be affected by the binding of DC11, that is, its C-terminal region. The results were corroborated using hydrogen deuterium exchange mass spectrometry. We solved the X-ray structure of DC11 Fab fragment to a resolution of 1.33 Å crystallized in a space group P 1 21 1 and deposited it into the protein databank (PDB) with ID 9H8H. We also obtained crystals of DC11 Fab and crystals of tentative complexes between DC11 and either tau321–391 or tau peptide tau371–387 in different space groups. We further measured the synchrotron small angle X-ray scattering (SAXS) data to characterize the conformational ensembles of tau321–391 and tau297–391 in both batch and size exclusion chromatography coupled SAXS modes. We also attempted to characterize the complexes between tau proteins and DC11 Fab fragment. DC11 also binds oligomeric and fibrillar tau forms. Tau oligomers are thought to be the most toxic species in the pathogenesis of AD; however, intermediate fibrils were observed early during the aggregation reaction of truncated tau dGAE (297–391) by cryoelectron microscopy (Lövestam et al., 2024). We performed docking and molecular dynamics simulation of DC11 Fab fragment with tau peptide, tau oligomer (trimer of tau321–391), and tau protofibril to further uncover the binding mode of DC11 antibody and shed light on the tau pre-aggregation conformation.

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References

- Vechterova, L.; Kontsekova, E.; Zilka, N.; Ferencik, M.; Ravid, R.; Novak, M. (2003). *Neuroreport*, 14, 87–91.
Kontsekova, E.; Zilka, N.; Kovacech, B.; Skrabana, R.; Novak, M. (2014). *Alzheimers Res Ther*, 6, 45.
S. Njemoga, P. Majerova, J. Piestansky, Z. Gazova, P. Kaderavek, E.E. Barrera, Z. Bednarikova, O. Cehlar, Gradual N-terminal truncation of tau dGAE (297–391) reveals the importance of 321–325 sequence for early steps of fibril formation, *International Journal of Biological Macromolecules* 362 (2026) 152066.
Lövestam, S., Li, D., Wagstaff, J.L. et al. Disease-specific tau filaments assemble via polymorphic intermediates. *Nature* 625, 119–125 (2024).

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Analysis of the effect of the immune evasion protein UL144 on tumor cells derived from hematologic malignancies

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Abstract Human cytomegalovirus (HCMV), a member of the β -herpesvirus subfamily, has evolved sophisticated strategies to evade host immunity and establish lifelong latent and persistent infection. A key component of this immune evasion repertoire is the viral glycoprotein UL144, which is a structural homolog of the host receptor herpesvirus entry mediator (HVEM), a member of the tumor necrosis factor receptor superfamily. While cellular HVEM interacts with multiple ligands—including tumor necrosis factor ligand superfamily member 14 (TNFSF14, also known as LIGHT), lymphotoxin- α , B and T lymphocyte attenuator (BTLA), CD160, and herpes simplex virus glycoprotein D—UL144 displays a highly restricted binding profile. Specifically, UL144 selectively engages BTLA (B and T lymphocyte attenuator), leading to the suppression of T-cell activation and downstream immune responses. This selective mimicry enables HCMV to fine-tune host immune signaling pathways, thereby promoting viral persistence and immune evasion. Here, we expressed and purified the UL144 protein using a baculovirus expression system in Sf9 cells. The recombinant protein, secreted into conditioned medium, was purified by immobilized metal affinity chromatography [nickel-nitrilotriacetic acid agarose (Ni-NTA agarose)] followed by size exclusion chromatography using a Superdex 75™ 10/300 GL column. The interaction between UL144 and BTLA was investigated in the JVM-2 cell line derived from a patient with B-prolymphocytic leukemia, in which we confirmed the high surface expression of BTLA by flow cytometry. The UL144–BTLA interaction was validated using a pull-down assay. JVM-2 cells were incubated with soluble His-tagged UL144 (225 μ g) for 1 and 4 h, which was followed by washing three times with phosphate-buffered saline, cell lysis, and incubation with His-tag affinity Dynabeads to capture UL144-associated complexes. The bound proteins were eluted, resolved by Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and analyzed by Western blot using an anti-His antibody. A specific UL144 signal was detected in pull-down fractions from treated samples, indicating a specific association of UL144 with cellular BTLA. A modest increase in signal intensity at 4 h suggests time-dependent stabilization of the interaction. To further elucidate the intracellular mechanisms underlying potential UL144-mediated antitumor effects, the CA-46 cell line, derived from Burkitt lymphoma, was treated with UL144 (225 μ g) for 72 h, followed by RNA sequencing analysis. Differential expression profiling revealed 173 significantly deregulated genes (128 upregulated, 45 downregulated). Upregulated genes were associated with transcriptional regulation, apoptosis, and oxidative stress responses, whereas downregulated genes were enriched in pathways related to integrin signaling, glycolysis, and B-cell activation. These findings indicate that UL144 exerts antitumor activity through induction of apoptotic and stress-related pathways while concurrently suppressing B-cell activation, highlighting its potential immunomodulatory role in malignant B cells.

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Characterization of phosphoregulation in the human mitochondrial mortalin–GRPE1 chaperone complex

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Abstract Mitochondrial protein quality control (PQC), mediated by proteases and chaperones, is essential for preserving cellular integrity and preventing disease. Central to this network is the mtHSP70/mortalin system and its co-chaperones TID-1, GRPE1, and GRPE2. While GRPE1 is phosphorylated at Ser47, the functional impact of the modification remains unclear. Using biochemical assays and cryoelectron microscopy, we characterized purified human mortalin complexes. We demonstrated that GRPE1 phosphorylation enhances its interaction with mortalin without altering the complex's overall structure or ATPase activity. However, this phosphorylation significantly inhibits refolding of substrates such as luciferase. In addition, we found that the mitochondrial LON protease selectively degrades GRPE2, but not GRPE1. Our findings suggest that GRPE1 phosphorylation modulates PQC by altering mortalin–substrate interaction dynamics, providing new insights into the regulatory mechanisms of mitochondrial chaperones.

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The interdisciplinary approach combining *in vitro* and *in silico* methods to better understand the effect of the cardiac-associated mutations located on the N-terminal domain of human ryanodine receptor 2

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Abstract Ryanodine receptors (RyRs) are the largest ion channels presently known ($M_r \approx 2.2$ MDa). They are located in the sarcoplasmic reticulum (SR) membrane, and their main physiological function is to release Ca^{2+} from SR into the cytoplasm of myocytes, resulting in muscle contraction. RyRs are multidomain homotetrameric proteins where each monomer is composed of ≈ 10 domains. Isoform 2 of this receptor (Peng et al., 2016) is predominantly present in the heart tissue. Its dysfunction, often caused by missense mutations in the *RYR2* gene, triggers several types of cardiac diseases (Catecholaminergic polymorphic ventricular tachycardia (CPVT1), Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia (ARVC/D2), syncope of unknown origin, Sudden infant death syndrome (SIDS)) (<https://www.hgmd.cf.ac.uk/ac/index.php>). These mutations often affect the oligomeric state of individual domains, their biophysical properties and dynamics, or their interactions with neighboring domains or partner molecules, all of which alter the opening and closing of the channel (Bauerová-Hlinková et al., 2020). The object of our study is the N-terminal domain of RyR2 (NTD), which serves as a scaffold and support of the RyR2 central part and is involved in the opening and closing of the channel (Borko et al., 2014). Over 70 arrhythmia-associated mutations have been identified in this domain (<https://www.hgmd.cf.ac.uk/ac/index.php>). In our studies, we focused on characterizing the RyR2 NTD in its native and mutant forms (Borko et al., 2014; Bauer et al., 2020), which are associated with CPVT1 and ARVC/D2. To understand the effect of these mutations on human RyR2, we applied a multidisciplinary approach involving biochemical, biophysical, and structural analyses combined with molecular dynamics. Three of the mutations we have studied, I419F, R420W, and L433P, occur in the central helix of NTD (Borko et al., 2014). Size exclusion chromatography (SEC) and flow induced dispersion (FIDA) analysis revealed that the I419F and L433P mutations affect the oligomeric state of the protein. Circular dichroism spectroscopy suggested that the L433P variant increases its α -helical content by 10%. Differential scanning calorimetry (NanoDSF) showed that the thermal stability of the I419F mutant decreases by $\approx 5^\circ\text{C}$. Molecular dynamics simulations suggested that all mutations uniquely affect the dynamics of NTD, and therefore the function of the whole RyR2 channel.

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References

- Peng, W., Shen, H., Wu, J., Guo, W., Pan, X., Wang, R., Chen, S.R.W., Yan, N. (2016). *Science*. 354(6310): aah5324.
Bauerová-Hlinková, V., Hajdúchová, D., Bauer, J.A. (2020). *Molecules*. 25(18): 4040.
Borko L, Bauerová-Hlinková V, Hostinová E, Gašperík J, Beck K, Lai FA, Zahradníková A, Sevcík J. *Acta Crystallogr D Biol Crystallogr*. 2014 Nov; 70(Pt 11): 2897-912.
Bauer, J.A., Borko, L., Pavlovič, J., Kutejová, E., Bauerová-Hlinková, V. (2020). *J. Biomol. Struct. Dyn.* **38**(4): 1054–1070.

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The effect of S616L, a CPVT1-associated mutation, on the stability and structure of a three-way domain interface in the human cardiac ryanodine receptor

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Abstract Catecholaminergic polymorphic ventricular tachycardia type 1 (CPVT1) is a heritable genetic disease caused by mutations to *hRyR2*, the cardiac isoform of the human ryanodine receptor (Bauerová-Hlinková et al., 2020). One such mutation, S616L (Marjamaa et al., 2009), is found in the α -helical part (NSol) of the *hRyR2* N-terminal domain close to a three-way domain junction with the long α -helical bridging solenoid (BSol) and the shorter junctional solenoid (JSol). In this study, we used molecular dynamics simulation to study the effect of S616L on the residues of this junction. We found that the mutation weakens the interface by increasing the gap between NSol and BSol in the closed form of the channel by 0.4 Å. It also reduces the density of NSol in the vicinity of the mutation site and increases the conformational flexibility of this region. In addition, we determined the impact of this mutation on the biophysical and biochemical properties of a 655-residue fragment containing the entire *hRyR2* N-terminal domain and found that it slightly increases the thermal stability of the fragment.

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References

- Bauerová-Hlinková, V., Hajdúchová, D., Bauer, J.A. (2020). *Molecules*. 25(18): 4040.
Marjamaa, A., Laitinen-Forsblom, P., Lahtinen, A.M., Viitasalo, M., Toivonen, L., Kontula, K., Swan, H. (2009) *BMC Med. Genet.* 10: 12.

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From natural variation to rational design: dissecting stability and function in staphylokinase

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Abstract Staphylokinase (SAK) is a fibrin-selective thrombolytic agent whose clinical use is limited by immunogenicity (Warmerdam et al., 2002). In addition to engineered low-immunogenic variants such as SAK STAR 3A (K74A_E75A_R77A), naturally occurring variants (e.g., SAK 42D) differ in sequence and stability, providing a framework to study sequence–structure–function relationships (Štulajterová et al., 2025). Here, we systematically analyzed two mutational pathways: the engineered 74–77 region and the natural transition between SAK STAR and SAK 42D (positions 34, 36, and 43). Across both systems, fibrinolytic activity was largely preserved, whereas thermal stability was highly residue dependent. R77 was identified as a major stabilizing determinant, while G36R substitution emerged as the dominant destabilizing factor in the 42D variant. *In silico* major histocompatibility complex (MHC) class II prediction indicated that substitutions in the 74–77 region modulate predicted epitope presentation and revealed an inverse relationship with protein stability. Together, these findings highlight shared biophysical constraints governing SAK stability and function.

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References

- P.A.M. Warmerdam, K. Vanderlick, P. Vandervoort, H. De Smedt, S. Plaisance, M. De Maeyer, D. Collen, Staphylokinase-Specific Cell-Mediated Immunity in Humans¹, *The Journal of Immunology* 168 (2002) 155–161.
- M. Štulajterová, L. Ambro, D. Sedláková, M. Nemergut, P. Kohout, S. Mazurenko, R. Varhač, A. Strunga, M. Toul, Z. Prokop, J. Damborský, M. Tomková, E. Sedlák, Assessing the impact of His-tags on activity and stability of staphylokinase variants, *International Journal of Biological Macromolecules* 328 (2025).

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Substrate matters: screening α -synuclein variants to improve RT-QuIC performance in synucleinopathies

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Abstract Parkinson's disease and related synucleinopathies are driven by the misfolding and aggregation of alpha-synuclein (α -Syn), a process highly sensitive to its structural and conformational properties. Even minor sequence modifications, such as affinity tags, may alter aggregation behavior and seeding activity, yet their impact remains insufficiently understood. We systematically compare wild-type (WT) α -Syn and its C-terminally His-tagged variant to assess how the tag influences structural properties and aggregation kinetics. Recombinant proteins were characterized using dynamic light scattering to evaluate size distribution and sample homogeneity. Aggregation behavior and seeding activity were quantified using *in vitro* assays, including real-time quaking-induced conversion. Our results revealed distinct differences between WT and His-tagged α -Syn in both structural properties and kinetic profiles, highlighting the sensitivity of α -Syn aggregation to subtle sequence modifications. These findings underscore the importance of substrate design in aggregation assays and provide insight into how conformational variability translates into functional seeding behavior.

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Preparation and characterization of oligomers formed by truncated tau proteins *in vitro* and *in silico*

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Abstract Soluble oligomers—not relatively inert mature filaments—are thought to be the toxic agents of several neurodegenerative diseases. Oligomers can propagate between neurons and could be isolated by *in vivo* microdialysis from brain interstitial fluid. Tau oligomers can be induced *in vitro* by seeding with amyloid- β (A β) oligomers or using tau with modified cysteines (Lasagna-Reeves et al., 2012; Gerson et al., 2017). However, intermediate filaments with shorter amyloid interface compared to mature filaments were observed early during tau aggregation (Lövestam et al., 2024). Previously, we summarized structural data about oligomers formed by A β , α -synuclein, tau and prion protein, that show substantial lack of structural information about tau protein oligomers (Cehlár et al., 2024). Isolated *in vitro* tau oligomers prepared from truncated tau were characterized by ion mobility mass spectrometry, size exclusion chromatography, dot blot, and Western blot using oligomer-specific antibodies T22, A11, and TOMA-1. Moreover, representative structures of tau dimers and trimers were obtained after clustering of coarse-grained molecular dynamics simulations. A set of 27 previously reported small molecules with the potential to inhibit tau aggregation was docked into the presumed binding pockets identified in these structures. The inhibition was probed by measuring the ThT signal of the aggregation reaction in the presence of small molecules.

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References

- L. Lasagna-Reeves, C., Castillo-Carranza, D., Sengupta, U. et al. Alzheimer brain-derived tau oligomers propagate pathology from endogenous tau. *Sci Rep* 2, 700 (2012).
- Gerson, J.E., Sengupta, U., Kaye, R. (2017). Tau Oligomers as Pathogenic Seeds: Preparation and Propagation *In Vitro* and *In Vivo*. In: Smet-Nocca, C. (eds) *Tau Protein. Methods in Molecular Biology*, vol 1523. Humana Press, New York, NY.
- Lövestam, S., Li, D., Wagstaff, J.L. et al. Disease-specific tau filaments assemble via polymorphic intermediates. *Nature* 625, 119–125 (2024).
- Cehlár, O., Njemoga, S., Horváth, M., Cizmáza, E., Bednarikova, Z., Barrera, E.E. Structures of Oligomeric States of Tau Protein, Amyloid- β , α -Synuclein and Prion Protein Implicated in Alzheimer's Disease, Parkinson's Disease and Prionopathies. *Int. J. Mol. Sci.* 2024, 25, 13049.

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Prediction of ryanodine receptor states by the AlphaFold3 server

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Abstract AlphaFold3 can correctly predict the protein structure ab initio (Abramson et al., 2024), whereas its capability to accurately predict multiple functional states of a single protein has not been comprehensively studied yet. In this study, we assessed AlphaFold3 predictions for three ryanodine receptor paralogs, which differ in the number of experimental structures available for use in the prediction (48 in three states, 17 in three states, and none for RyR1, RyR2, and RyR3, respectively). Experimental structures were compared to 300 AlphaFold3-simulated models of the C-terminal quarter (approximately 5000 residues of the tetrameric channel) of human RyRs (100 models per paralog). All structures were oriented on the central domain, and positions of the other domains were compared with those of the closed and inactivated rRyR1 reference states (Zahradníková et al., 2025). Furthermore, we studied the geometry of the triangle of residues W4716 - F3753 - I4996, which is significant in channel functioning (Murayama et al., 2018). The experimental structures were shown to have three principal conformations that were closed, open, and primed/inactivated (Zahradníková et al., 2025). In contrast, AlphaFold3 predictions were less varied: all RyR1 models were predicted to be in the primed state and all RyR2 models in the closed state. The domain positions of RyR3 models formed a continuum between the closed and primed conformations, with ~16% in the closed state, none in the primed state, and ~84% of the models had no experimental counterpart. These findings indicate that AlphaFold3 is not able to capture the spectrum of functional conformations of RyR channels and can predict nonphysiological conformations, especially with RyR3.

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References

- J. Abramson, J. Adler, J. Dunger et al. Nature (2024) 10.1038/s41586-024-07487-w.
A. Zahradníková, J. Pavelková, M. Sabo et al. PLoS Comput. Biol. (2025) 21:1–40.
T. Murayama, H. Ogawa, N. Kurebayashi et al. Commun. Biol. (2018) 1:98.

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α -Synuclein: from purification to aggregation

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Abstract Synucleinopathies, including Parkinson's disease (PD), dementia with Lewy bodies, and multiple system atrophy, are neurodegenerative disorders characterized by misfolding and aggregation of the presynaptic protein α -synuclein (α -Syn). PD is the most common synucleinopathy, and development of sensitive diagnostic methods remains a major challenge. Real-time quaking-induced conversion (RT-QulC) is a sensitive seeding assay capable of detecting misfolded α -Syn in samples. Here, wild-type α -Syn was expressed and purified using anion exchange and size exclusion chromatography, yielding a predominantly monomeric and homogeneous protein. The purity and structural homogeneity of the preparation were confirmed by chromatographic, electrophoretic, and dynamic light scattering analyses. To assess its aggregation ability and to generate preformed fibrils (PFFs), *in vitro* incubation followed by sedimentation analysis was used. PFFs were subsequently used as seeds in RT-QulC assays and showed robust amplification of ThT fluorescence, whereas negative controls remained largely signal free.

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Expression and isolation of native and mutant Repeat12 domain of human cardiac ryanodine receptor 2

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Abstract Ryanodine receptor (RyR) is the largest known ion channel. Its cardiac isoform, RyR2, is responsible for calcium homeostasis in cardiomyocytes (Otsu et al., 1990). RyR2 is located in the membrane of the sarcoplasmic reticulum, where it mediates the release of calcium ions from intracellular stores into the cytosol, thereby crucially contributing to cardiac contraction (Fabiato, 1983). The channel is a homotetramer and can be divided into several parts, including the pore region, column, handle, and clamp regions (Peng et al., 2016), which are responsible for interacting with the various binding partners that affect the function of this receptor. The clamp region includes the Repeat1-2 domain, in which 14 mutations have been identified to date that are primarily associated with cardiac arrhythmias and cardiomyopathies (Human Gene Mutation Database—RYR2). In our study, we focused on the wild-type and R1051C mutant forms of the Repeat1-2 domain, the latter associated with an increased risk of cyclic vomiting syndrome (Human Gene Mutation Database—RYR2). Both variants were successfully expressed and purified using immobilized metal ion affinity and size exclusion chromatography, which resulted in samples of approximately 95% purity.

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References

- Otsu K., Willard H. F., Khanna V. K., et al. (1990) J. Biol. Chem. 265(23), p. 13472.
Fabiato A. (1983) Am. J. Physiol. 245(1), p. C1.
Peng W., Shen H., Wu J., et al. (2016) Science 354(6310), p. aah5324.
Human Gene Mutation Database – RYR2. Available from: <https://www.hgmd.cf.ac.uk/ac/gene.php?gene=RYR2> (accessed 22 May 2026).

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Specific analytical approaches for detail characterization of the keyhole limpet hemocyanin

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Abstract Novel trends in biologics are associated with the development of antibody drug conjugates, therapeutic protein conjugates, and vaccines which could be effective in situations where the convenient drugs and therapeutic strategies are ineffective. In case of innovative immunotherapeutics, carrier proteins and protein-based adjuvants are gaining great interest. Special attention is paid to the hemocyanins, large metalloglycoproteins present in the hemolymph of mollusks. One of the crucial hemocyanins used in preparation of immunotherapeutics against cancer, Alzheimer's disease, and dementia is keyhole limpet hemocyanin (KLH), which is obtained from the hemolymph of the giant keyhole limpet *Megathura crenulata*. KLH forms a stable supramolecular structure with a molecular weight at the megadalton level. This complex structure may result in some difficulties during the quality assurance process. Generally, the biologics are much more complex than small-molecule drugs, and small variations in the production process may strongly impact the final demanded product. Thus, quality assurance of biologics results in much higher analytical effort compared to small molecules. However, knowledge about the properties of KLH is over 20 years old. Still, there are missing appropriate analytical strategies and modalities for its detail characterization. The present work deals with the implementation of various analytical strategies—based on size exclusion chromatography, amino acid analysis, peptide profiling (mapping), or N-glycan analysis—for the characterization of KLH. Special attention is given to the quantitative approach for KLH determination, which relies on the liquid chromatography-mass spectrometry (LC-MS) analysis of a specific peptide that is unique to the KLH molecule after peptide cleavage using formic acid.

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Surface-exposed HCMV UL141 mimics TIGIT structural motifs to subvert immune surveillance on mature virions

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Abstract Human cytomegalovirus UL141 is a multifunctional immunoevasin that antagonizes DNAM-1/CD96 ligands and Tumor necrosis factor ligand superfamily member 10 (TNFSF10, also known as TRAIL) death receptors. Using quantitative proteomics, we identified UL141 as the predominant viral protein at the plasma membrane of infected cells, with abundances comparable to glycoprotein B throughout lytic progression. Virion-incorporated UL141 exists in a full-length, EndoH-resistant form, signifying transit through the secretory pathway. Surface plasmon resonance assays with intact virions demonstrated that UL141 remains receptor-competent, engaging soluble receptors with particle-specific avidity. Structure-guided interface modeling of the UL141–TRAIL-R2 complex delineated two high-confidence binding surfaces—an exposed N-terminal Ig-like domain and a rear C-terminal domain—both tolerant to glycan occlusion. Isothermal titration calorimetry with a clinical anti-TIGIT antibody confirmed that UL141 mimics T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains (TIGIT) structural motifs for CD226-axis ligand recognition, using a site distinct from its TRAIL-R2 interface. This architectural versatility permits concurrent subversion of disparate immune surveillance mechanisms in cell-tethered and free-virion contexts. Targeted mutagenesis corroborated the functional primacy of these interfaces, as receptor-binding-defective variants retained virion assembly competence. Collectively, these observations establish UL141 as a virion-exposed, receptor-competent evasin, underscoring its therapeutic potential and necessitating systematic interrogation of its interactome on mature virions.

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Interactions of small molecules with tau proteins

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Abstract Since the characterization of intrinsically disordered proteins in 1975, our knowledge of this group of proteins blossomed. Despite examination of these proteins being a Sisyphean task, with modern day's methodology, we are able to understand more about the proteins' appearance, behavior, and relations to other molecules.

An example of this never-ending research is the study of tau protein, its pathology, and potential therapeutical avenues. There are many compounds that are claimed to interact with tau and possibly inhibit its aggregation, leading to diseases that are collectively referred to as tauopathies. Amid these interactive partners are small molecules, molecules with low molecular weight and relatively simple structure. With features such as predictable behavior in the system, stability, cell permeability, and a wide range of methods of administration at their disposal, small molecules represent interesting candidates for future drug development in the treatment of tauopathies.

Based on available studies (Vagrys et al., 2020; Kiss et al., 2018), we focus on three forms of the tau protein (Tau40 (1–441), Tau151–391/4R, and tau dGAE) and three small molecules (methylene blue, rosmarinic acid, and quercetin) that are believed to interact and inhibit the aggregation of tau.

Due to the properties of small molecules and probable interactions with tau, we decided to explore the kinetic behavior of tau–small molecule complexes, alongside contributing to the development of a standardized protocol for future investigations of these complexes.

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References

- Vagrys, D.; Davidson, J.; Chen, I.; Hubbard, R. E.; Davis, B. Exploring IDP–Ligand Interactions: Tau K18 as a Test Case. *Int J Mol Sci* 2020, 21 (15).
R. Kiss, G. Csizmadia, K. Solti, A. Keresztes, M. Zhu, M. Pickhardt, E. Mandelkow, G. Tóth, Structural Basis of Small Molecule Targetability of Monomeric Tau Protein, *ACS Chem. Neurosci.* 2018, (9) 2997–3006.

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Alternative delivery route of biologics—tocilizumab nebulization study

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Abstract Nebulization represents a noninvasive route of drug delivery that enables the administration of therapeutics both locally within the lungs and systemically through pulmonary absorption. The delivery of biologics via inhalation provides several important advantages. Because the drug is delivered directly to the site of action, lower doses are typically required, which may reduce treatment costs while maintaining or improving therapeutic efficacy. This approach also allows for targeted intervention in the respiratory tract, with the potential to achieve both local and systemic effects. Furthermore, inhalation-based therapies can be suitable for self-administration, eliminating the need for trained healthcare personnel and enhancing patient compliance. Here, we investigated the possibility of tocilizumab to be applied by inhalation delivery route. The experiments were realized with the use of the pharmacopeial *in vitro* human pulmonary system model (Single Stage Glass Impinger) and the commercial drug Tyenne[®], a biosimilar drug containing the monoclonal antibody (mAb) tocilizumab. Analyses of the samples obtained from the nebulization study were performed using the Acquity UPLC H-Class chromatographic instrument equipped with the UV-detector set at a constant wavelength of 280 nm. An in-house developed size exclusion chromatography (SEC) analytical method was applied. The chromatographic separation was carried out using an Acquity UPLC[®] BEH450 SEC column (2.5 μ m, 4.6 $\times$ 150 mm). As a mobile phase, 25 mM phosphate buffer (pH 6.8) with addition of 250 mM NaCl was used. The flow rate of the mobile phase was set at 0.3 mL/min. Throughout the analyses, the column temperature was maintained at room temperature. The injection volume was 10 μ L. The presence of nebulized mAb tocilizumab at a concentration level of 2 mg/mL was investigated in the collected samples from each particular compartment of the *in vitro* model apparatus by the SEC-UV method. The presence of tocilizumab in the upper chamber of the apparatus (representing the upper respiratory tract) and also in the lower chamber of the apparatus (representing the lower respiratory tract, lungs), confirmed effective formation of suitable particles accessible in the respiratory tract.

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Quality control of modern dosage forms for tocilizumab applied by inhalation

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Abstract Biopharmaceuticals (including monoclonal antibodies) are typically delivered into organisms by intravenous, subcutaneous, or intramuscular application. However, recent trends are pushed toward modern and alternative application strategies of monoclonal antibodies, such as inhalation delivery route. Development of such drug formulations also demands appropriate analytical methods for their quality control to ensure safety and efficacy. In our work, the quality control and stability investigation of prepared tocilizumab formulations for inhalation were realized using the size exclusion chromatography (SEC) method. SEC experiments were conducted using the YOUNG IN Chromass (Anyang-si, Korea) chromatography system with UV detector (set at a wavelength 280 nm). A 25 mM phosphate buffer with 300 mM sodium chloride was used as the mobile phase. Separation was performed on the Xtimate SEC-2000 (5 μ m, 4.6 $\times$ 300 mm) column. Moreover, further strategies for quantitative determination of tocilizumab were proposed. The stability study of four tocilizumab formulations (tocilizumab formulated with: a) phosphate-buffered saline, b) histidine and arginine, c) proline, and d) glycine with NaCl) was realized under the following incubation conditions: –80 °C, –20 °C, 4 °C for 24 h, and 60 °C for 2 h. Tocilizumab showed appropriate stability in formulated buffer under such tested conditions. Tocilizumab formulated in the Pro buffer at pH 5.8 was used for pivotal testing of nebulization procedure. No significant formation of aggregates was observed after the nebulization procedure. Furthermore, detailed quantitative information on tocilizumab can be obtained using modern analytical strategies such as liquid chromatography or capillary electrophoresis coupled with mass spectrometry.

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Structural study of tau protein involved in Alzheimer's disease

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Abstract Molecular dynamics (MD) simulations were employed as a main tool to study conformational preferences associated with the pathological functions of protein tau. Previous results suggest sequence-specific aggregation of tau; however, key conformational changes triggering the self-assembly remain unknown. In particular, the importance of tau residues 321–325 was found to be essential to the self-assembly of tau monomers, because we observed a strong aggregation tendency of tau 321–391 and the incapability of tau 326–391 to aggregate. To reveal the structural mechanisms underlying tau aggregation, we performed simulations of tau 321–391 and 326–391 monomers and dimers, as well as their truncated variants. MD simulations revealed increased propensity of 321–325 sequence toward beta-structures in all atom simulations, but rather helical propensity in coarse-grained simulations. Moreover, simulations successfully replicated sequence-specific beta-sheet propensity in agreement with computationally predicted and experimentally established amyloid-nucleating motifs (Njemoga et al., 2026). We also used MD simulations to investigate the behavior of short tau peptides selected from solved structures of tau protein within the protein databank (PDB). First of the simulated peptides spanned from 298 residue to 311 residue of full-length tau and encompassed intramolecular main chain–main chain hydrogen bond formed by Gly302 and Ser305 with the reference structure under pdbID 5MO3. The second peptide spanned from 382 to 394, encompassing intramolecular hydrogen bond formed by the side chain of Asp387 and the main chain of Gly389 with reference structure under pdb ID 2V17. The main goal was to observe whether these hydrogen bond motifs affect the stability of the peptides by simulating both wild-type and mutated variants. After the analysis, we did not observe any major effect on the stability of the conformational ensemble of the peptides. However, for the 382–394 fragment, both WT and mut fragments adopted hairpin conformation stabilized mainly by salt bridges. Interestingly, the additional hydrogen bond motif in the WT variant shifted the hairpin conformation toward the N-terminus when compared to the mutant fragment.

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References

- S. Njemoga, P. Majerova, J. Piastansky, Z. Gazova, P. Kaderavek, E.E. Barrera, Z. Bednarikova, O. Cehlar, Gradual N-terminal truncation of tau dGAE (297–391) reveals the importance of 321–325 sequence for early steps of fibril formation, *International Journal of Biological Macromolecules* 362 (2026) 152066.

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Development and optimization of magnetic bead-based tools for the isolation of *Coxiella burnetii* cells from tissue samples

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Abstract *Coxiella burnetii*, the causative agent of Q fever, is difficult to detect due to its intracellular lifestyle and limitations of current diagnostics. This study developed a magnetic bead-based platform for selective capture of *C. burnetii* using covalently immobilized specific immunoglobulins. Magnetic beads with four different surface chemistries—epoxy, tosyl, amino, and carboxyl—were functionalized and systematically evaluated for their ability to bind *C. burnetii* whole cells. Binding performance was assessed across a range of bacterial concentrations to identify optimal conditions. Following incubation and standardized washing steps, capture efficiency was analyzed using protein quantification assays and *C. burnetii*-specific quantitative polymerase chain reaction. Preliminary experiments were extended to homogenized tissue matrices to assess performance in complex biological environments, which revealed feasibility and also challenges linked to matrix effects and recovery efficiency. Overall, this work establishes a foundation for magnetic bead-based enrichment of *C. burnetii* and supports further development toward diagnostic application in complex samples.

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Riociguat and Terbutaline Reduce Endothelin-1 Production in a Rat Model of Pulmonary Arterial Hypertension

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Abstract Endothelin-1 (ET-1) plays a critical role in pulmonary arterial hypertension (PAH) and is currently targeted by ET-1 receptor antagonists. However, strategies aimed at reducing cellular ET-1 expression have been less thoroughly explored. In preliminary experiments, we targeted multiple signalling pathways to identify those regulating endothelin-1 gene (*Edn1*) expression in A7r5 vascular smooth muscle cells. Based on these findings, we selected the guanylate cyclase stimulator riociguat (RIO) and the β_2 -adrenergic receptor agonist terbutaline (TER) for experimental treatment in a rat monocrotaline (MCT) model of PAH. Rats were sacrificed either after 4 weeks (MCT4w) or prematurely when reaching humane endpoints due to acute health deterioration (ptMCT). Drug treatment commenced 2 weeks after MCT injection. In the PAH model, we observed increased right ventricle and lung weights, along with elevated heart and respiratory rates, accompanied by increased *Edn1* expression in the pulmonary artery, right ventricle and left ventricle. Plasma ET-1 protein levels were elevated in both monocrotaline groups. Although the treatments did not significantly improve biometrical indicators of organ damage, TER reduced heart rate and RIO decreased respiratory rate. At the molecular level, both treatments favourably influenced the expression of the physiological myosin isoform Myh6 and atrial natriuretic peptide in the right ventricle. RIO did not affect elevated *Edn1* expression in the ventricles and only partially reduced *Edn1* overexpression in the pulmonary artery. Interestingly, TER-treated MCT4w rats did not show *Edn1* overexpression in the ventricles, and *Edn1* overexpression was attenuated in terminally ill TER + ptMCT rats. With the exception of TER + ptMCT, neither RIO- nor TER-treated rats showed increased plasma levels of ET-1 protein. In conclusion, riociguat and terbutaline reduced *Edn1* gene expression in vascular smooth muscle cells and in the monocrotaline model of PAH. Therapies aimed at reducing ET-1 levels may offer therapeutic benefits in PAH.

Keywords *endothelin-1 – monocrotaline – pulmonary arterial hypertension – riociguat – terbutaline*

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Potential Involvement of miRNAs in the Protective Effect of Molecular Hydrogen on Radiation-Induced Heart Disease in Older Rats

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Abstract Radiation-induced heart disease (RIHD) is a known harmful side effect of mediastinal radiotherapy applied in oncological diseases. RIHD involves structural and functional abnormalities of the pericardium, coronary vessels, myocardium, valves and conduction system. The underlying pathological mechanisms are complex and mainly related to endothelial cell damage, oxidative stress and inflammation. These can cause cardiomyocyte disorders, tissue fibrosis and ultimately heart failure. To overcome these complications, it is necessary to look for specific therapeutic interventions, which are still lacking. Studies have shown that molecular hydrogen (H₂) possesses radioprotective effects in different experimental models, including RIHD. H₂ could be effective in alleviating RIHD through various mechanisms, for example selective neutralization of hydroxyl radicals, protection against inflammatory and apoptotic damage, antifibrotic and antihypertrophic effects, etc. Available information shows that microRNAs (miRNAs) might be involved in the radioprotective effect of H₂. In this work, our research team investigated the effect of 6-weeks of H₂ administration to 1-year-old Wistar rats irradiated in the mediastinal area with a single dose of 10 Gy (4–5 Gy/min). The results show that the inhalation of H₂ effectively reduced heart damage (lactate dehydrogenase), improved oxidative stress (malondialdehyde, superoxide dismutase activity) and inflammatory (pro-inflammatory cytokines) parameters, as well as had a positive impact on heart function (infarct size, left ventricular end-diastolic pressure) parameters measured 6 weeks after irradiation. This study also reveals that H₂ administration on the RIHD rat model also normalizes dysregulated expression of selected miRNAs (miRNA-21, -30e, -208 and miRNA-142). Based on these results, we conclude that H₂ administration represents a promising treatment strategy for patients undergoing mediastinal irradiation with radiation-induced cardiac damage. However, to clarify other mechanisms of H₂ action and to verify the effectiveness of H₂ therapy in clinical practice, further studies are needed, especially focusing on the still unclear mechanism of its biological effect.

Keywords antioxidant – heart disease – irradiation – molecular hydrogen – oxidative stress

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Targeting Fatty Acid Oxidation to Treat Heart Failure with Preserved Ejection Fraction

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Abstract Alterations in cardiac energy metabolism are an important contributor to heart failure severity. Despite this, there is considerable confusion as to what changes in cardiac energy metabolism occur in heart failure with preserved ejection fraction (HFpEF). To assess this, we measured cardiac fatty acid oxidation and glucose oxidation in a mouse model of HFpEF [which involved administering 13-month-old C57BL/6N female mice a 60% high-fat diet and L-NAME (0.5 g/L/day) for 8 weeks to induce HFpEF]. We observed an increase in fatty acid oxidation in isolated working hearts from HFpEF mice, which was accompanied by a marked decrease in cardiac glucose oxidation. An increased reliance of the heart on fatty acid oxidation as a source of ATP production results in a decrease in cardiac efficiency (cardiac work/O₂ consumed). Our goal was therefore to inhibit fatty acid oxidation in HFpEF mice as an approach to normalize cardiac energy metabolism. These studies were performed in a mouse model of diabetic cardiomyopathy (DbCM), which displays HFpEF symptoms and has high cardiac fatty acid oxidation rates and decreased glucose oxidation rates. A significant contributor to the increased fatty acid oxidation observed in DbCM hearts is the weakening of the regulatory control of mitochondrial fatty acid oxidation by malonyl-CoA. We therefore treated DbCM mice with an inhibitor of malonyl-CoA decarboxylase (MCD), which increases malonyl-CoA in the heart. A 4-week treatment with the MCD inhibitor, CBM-30011 (10 mg/kg/day), resulted in a significant decrease in cardiac fatty acid oxidation and a parallel increase in glucose oxidation in DbCM mice. An improvement in diastolic function was also observed in the DbCM mice (as assessed by echocardiographic E/e'). We conclude that in HFpEF mice the heart switches from glucose oxidation to fatty acid oxidation as the main source of ATP production. However, inhibiting fatty acid oxidation can increase the contribution of glucose oxidation to ATP production and lessen the severity of HFpEF.

Keywords *fatty acid oxidation – glucose oxidation – HFpEF – diabetic cardiomyopathy*

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Mapping Single-Cell Gene Expression in Heart Failure

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Abstract Heart failure is an important cause of mortality and morbidity globally and carries a significant economic burden. The advances in pharmacotherapy have led to improved outcomes for patients with heart failure. However, there remain patients who do not respond to the treatment; their condition deteriorates and ultimately results in untimely death or heart transplantation. Heart failure resulting from varied aetiologies is characterized by heterogeneous patterns of cardiac tissue remodelling that are not adequately reflected in the current therapies. Addressing this heterogeneity will be key for improving outcomes. Single-cell RNA sequencing allows addressing this unmet clinical need by studying transcriptomic phenotypes of individual cells contained in heart tissue in health and in disease at a large scale. Spatial transcriptomic technologies allow the localization of cells in niches, which enables inferences about cell-cell interactions. In patients with advanced heart failure, we have been able to interrogate the cell type abundance shifts and perturbations to cellular gene expression programmes that associate with heart failure in general but also with individual aetiologies and genotypes. With refined understanding of gene activation heterogeneity in heart failure, we will be able to validate putative disease mechanisms that may be amenable to therapeutic targeting.

Keywords *heart failure – RNA – transcriptomics*

Funding This work was supported by The British Society for Heart Failure Research Fellowship, the British Heart Foundation Clinical Research Training Fellowship, the Rosetrees Trust and Alexander Jansons Myocarditis UK.



Interplay Between Stress Sensors and Cardiac Excitation-Contraction Coupling

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Abstract The endoplasmic reticulum (ER) plays a central role in cellular stress responses via mobilization of ER stress coping responses, such as the unfolded protein response (UPR). Activation of UPR is associated with multiple cardiac conditions and has been shown to be either protective or detrimental to the heart. The serine/threonine protein kinase/endoribonuclease (IRE1 α) is the most evolutionarily conserved ER stress sensor and a key UPR stress signal transducer. Impaired calcium homeostasis activates ER stress responses, resulting in severe cardiac fibrosis that is prevented by the inhibition of IRE1 α . There are two pools of IRE1 α in cardiac muscle cells: one is localized to perinuclear ER responding to cellular stresses and the other is at the junctional sarcoplasmic reticulum (SR), a subspecialized membrane system involved in the regulation of excitation-contraction (E-C) coupling. The junctional SR containing ryanodine receptor/calcium channel forms membrane contacts with the T-tubules containing the L-type calcium channel. Deletion of the exons 20 and 21 of the *IRE1a* gene (encoding the RNase domain of IRE1 α) in adult cardiac myocytes caused severe heart dilation and mild fibrosis. We discovered that cardiomyocytes with deleted exons 20 and 21 of the *IRE1a* gene have prolonged calcium transients that can be corrected with L-type calcium channel inhibitors. Our results support an alternative function of IRE1 α associated with L-type calcium channel function. These findings provide new and unexpected insights into the link between E-C coupling and stress coping responses in muscle cells.

Keywords *ER stress – E-C coupling – calcium channel – calcium signalling – dyads – sarcoplasmic reticulum*

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Causes and Consequences of Double-Stranded DNA Breaks in Heart Failure

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Abstract The nuclear genome is exposed to numerous internal and external stressors, which affect the structure and function of DNA. It is estimated that each mammalian cell develops 104–105 lesions in the nuclear genome every day. The stressors cause DNA oxidation, alkylation and cross-linking, among others, which disturb the delicate homeostatic balance that maintains genomic stability. The DNA lesions also affect the replicative function of the DNA and RNA transcription. A consequence of the replicative and transcriptional stress is the generation and accumulation of double-stranded breaks (DSBs) in the DNA, which are the most dangerous form of DNA lesions. The genomic stressors are increased in pathological conditions such as heart failure. Likewise, the DNA repair mechanisms are impaired. Collectively, these changes lead to the accumulation of DNA lesions, including DSBs. The increased DSBs in the nucleus activate the nuclear component of the DDR, including the tumour protein 53 (TP53) and its downstream target cyclin-dependent kinase inhibitor 1A (CDKN1A), leading to cell cycle arrest and cell death. Likewise, a subset of the DSBs are released into the cytosol, where they are sensed by the DNA-sensing proteins, leading to activation of the nuclear factor-kappa B1 (NFkB1) and interferon regulatory factor 3 (IRF3) and consequent expression of the senescence-associated secretory phenotype (SASP). Expression of SASP contributes to the pathogenesis of heart failure by inducing senescence, inflammation, cell cycle arrest and cell death. Genetic blockade of the cytosolic or nuclear DNA imparts salubrious effects in the experimental models of heart failure. The stage is set to test potential pharmacological inhibitions of these pathways, initially in large animals and subsequently in humans with heart failure.

Keywords *DNA damage – double-stranded breaks – heart failure – inflammation*

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Phosphodiesterases in Atrial Fibrillation: From Mechanisms to Therapeutic Approaches

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Experimental Cardiovascular Research, Hamburg, Germany*

Abstract Atrial fibrillation (AF) is the most common sustained arrhythmia in the clinical setting, which is associated with significant morbidity and mortality. AF is also associated with severe cellular calcium (Ca^{2+})-handling abnormalities in atrial myocytes that may contribute to the initiation and maintenance of the arrhythmia. This so-called remodelling is supposed to involve abnormal phosphorylation of key Ca^{2+} -handling proteins. In addition, previous studies suggest that impaired phosphodiesterase (PDE)-dependent regulation of spatiotemporal distribution of cAMP may contribute to Ca^{2+} -handling abnormalities associated with AF. We investigate how PDE8 controls the spatiotemporal response of intracellular cAMP in key relevant Ca^{2+} -handling nanodomains by measuring real-time cAMP, ICa_L , spontaneous Ca^{2+} releases from the sarcoplasmic reticulum, protein expression and localization using highly sensitive and specifically targeted cAMP biosensors, patch-clamp, confocal Ca^{2+} imaging, contractility measurements and biochemical experiments.

Keywords : atrial fibrillation – phosphodiesterases – cAMP – calcium handling

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Regulation of Sarcotubular, Mitochondrial and Nuclear-Envelope Calcium Signalling in Human Stem Cell–Derived Cardiomyocytes

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Abstract While calcium signalling of SR by Ca²⁺ transporters of the surface membrane (ICa and INCX) has been well studied, possible regulation of mitochondrial and nuclear Ca²⁺ signalling by these proteins remains not fully explored. Here we investigate whether there are privileged interactions between Ca²⁺ transporters of surface membrane and mitochondria, SR or nuclear envelope (NE), by comparing their Ca²⁺ activity with cytosolic Ca²⁺, using genetically engineered fluorescent probes GCaMP3/GCaMP6 targeted to mitochondria, nuclear Lamin B, and SR lumen. In whole-cell clamped and TIRF- or confocal-imaged cardiomyocytes, caffeine-triggered SR Ca²⁺ release caused rapid rise in cytosolic calcium ($\Delta F/F \sim 0.5$; fura-2), followed by delayed and slower mitochondrial Ca²⁺ uptake ($\Delta F/F \sim 1-2$; mito-GCaMP6), and much larger NE Ca²⁺ uptake ($\Delta F/F \sim 10$; LaminB-GCaMP6). Calcium-Induced Calcium Release (CICR)-triggered SR Ca²⁺ release induces Ca²⁺ uptake by NE that follows and outlasts cytosolic Ca²⁺ signal significantly. Long-duration application of 5 mM caffeine to empty SR of its calcium content first transiently increases mitochondrial calcium, then triggers mitochondrial pacing despite the absence of SR calcium release. Lowering Na concentration in Ca²⁺-depleted SR increased the baseline calcium concentrations of SR, mitochondria and NE, enhancing the mitochondrial pacing rate. Spontaneous pacing was rapidly suppressed by blocking Ica but not by suppressing SR calcium uptake with CPA. We suggest that while activation of CICR is critical in triggering SR Ca²⁺ release that replenishes NE Ca²⁺ and initiates spontaneous pacing, calcium influx on NCX regulates mitochondrial pacing that sustains autorhythmicity in the absence of SR calcium release.

Keywords mitochondria – hiPSCs – Nuclear envelope – Ca²⁺ signalling – sarcoplasmic reticulum

Vitamin D Decreases Oxidative Stress and MAO Expression in Arterial, Venous and Adipose Tissue Samples from Obese Patients

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Abstract Overweight and obesity represent serious health concerns whose prevalence and severity have been constantly rising worldwide. The global threat of the obesity pandemic is related to the high risk of cardiometabolic comorbidities/complications. Increased body fat leads to vascular and adipose tissue dysfunction mediated via increased oxidative stress and chronic systemic inflammation. Overwhelming experimental and clinical studies have reported the association of vitamin D deficiency with metabolic and cardiovascular pathologies, including chronic venous disease. Monoamine oxidases (MAOs) with two isoforms, A and B, are mitochondrial enzymes that catalyse the deamination of biogenic amines and neurotransmitters with the constant generation of hydrogen peroxide in both vessels and adipose tissue. We here present evidence that (i) suboptimal vitamin D status is associated with increased reactive oxygen species in visceral adipose tissue and vascular samples (mesenteric artery branches and varicose vein explants), (ii) serum levels of vitamin D are inversely correlated with the magnitude of local oxidative stress, (iii) *in vitro* administration of the active vitamin D (1,25(OH)₂-D₃) alleviated both oxidative stress and vascular dysfunction and (iv) the protective effects of vitamin D are related, at least partly, to a decrease in MAO expression. In both types of vascular samples, acute incubation with 1,25(OH)₂-D₃ was responsible for the upregulation of eNOS and downregulation of iNOS in both obese and non-obese patients. Further research is required to elucidate the signal transduction pathways responsible for the antioxidant effects of vitamin D in human samples.

Keywords vitamin D – antioxidant – monoamine oxidase – vascular and adipose tissues

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Assessment of Cardiac Alternans in a Canine Model of Athlete's Heart

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Abstract Regular physical exercise has well-known beneficial effects on health; however, increasing evidence indicates that extreme sports activities – although very rare – can induce various cardiac arrhythmias, such as atrial fibrillation or even sudden cardiac death. A significant number of sudden cardiac deaths among athletes have been attributed to ischaemic origins or other deviations revealed by autopsy. However, the underlying cause of the remaining cases remains unclear. Cardiac alternans indicate a periodic, regular oscillation of the action potential (short-long pattern) and parallel intracellular Ca²⁺ release (large-small pattern). Alternans have been clearly associated with the development of ventricular fibrillation and sudden cardiac death. However, the potential role of cardiac alternans in the 'athlete heart' is unknown. In this study, the potential role of alternans was assessed in arrhythmia development in trained dogs. Beagle dogs underwent a vigorous 16-week long training program and were compared to a sedentary group. Cardiac action potentials were measured by the conventional microelectrode technique from right ventricular tissue samples. Ionic currents were recorded by the whole-cell configuration of the patch-clamp technique. Ca²⁺ transients were monitored by employing fluo-4AM fluorescent dye. All experiments were performed at 37°C. Trained dogs exerted increased susceptibility to ventricular fibrillation as a response to burst pacing. At the cellular level, the I_{to} was downregulated in trained animals. Action potential alternans in endocardial and midmyocardial tissue layers were increased in trained animals. Purkinje-ventricle dispersion during alternans was found to be larger in trained dogs. Ca²⁺ transient alternans were enhanced in trained animals. Ca²⁺ content of the sarcoplasmic reticulum was decreased in exercised animals, while cell shortening was identical between groups. Alternans may contribute to the development of ventricular fibrillation in intensive training-associated arrhythmias. The underlying mechanism of alternans could be SERCA downregulation in trained animals.

Keywords exercise – arrhythmia – remodelling – alternans – fibrillation

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Hydrogen Medicine from Basic Science to Clinical Trials

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Abstract Molecular hydrogen (H_2) has emerged as a promising therapeutic and preventive medical gas. In 2007, we overturned the conventional wisdom that H_2 has no effect on mammalian cells, leading to birth to the field of 'hydrogen medical biology'. H_2 has many advantages, including high efficacy, lack of toxic effects, and diverse functions such as antioxidant, anti-inflammatory, anti-cell death, and energy metabolism stimulation. In addition, H_2 is beneficial not only for severe chronic and acute diseases but also for healthy people. The target molecule of H_2 was identified as the oxidized form of porphyrin, which acts as a catalyst to stimulate the selective reaction of H_2 with hydroxyl radicals. Various types of porphyrins are widely and abundantly distributed throughout the body. Therefore, the repair of oxidized porphyrins by H_2 may bring multiple benefits to various cells. H_2 suppresses free radical chain reactions and modifies signalling mediators involved in lipid peroxidation. We present the findings from animal experiments to clinical trials. The best example of H_2 's effectiveness is recovery after cardiac arrest. Inhalation of H_2 gas has reduced the mortality rate by more than half and more than doubled the number of patients discharged without sequelae. The molecular mechanics will be discussed. The future mission of H_2 medicine is to apply H_2 for treatment, prevention, and quality of life across a wide range of fields. Furthermore, the use of H_2 in agriculture has the potential to produce safe, productive and high-quality foods. H_2 medicine could be practiced with simple and low-cost treatments without adverse effects and contribute to many unsolved serious problems facing today's society. H_2 has the potential to address a wide range of issues, including cardiac arrest, cerebral infarction, metabolic syndrome, advanced cancer, inflammatory cytokine storm, Alzheimer's dementia, healthcare, beauty and agriculture.

Keywords *cardiac arrest – ischaemia-reperfusion – molecular hydrogen – multiple functions – no adverse effects*



Extracorporeal Membrane Oxygenation in Cardiogenic Shock

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Abstract Cardiogenic shock is a critical circulatory condition in which a severely failing cardiac pump is unable to generate sufficient cardiac output, leading to tissue hypoperfusion in vital organs. Despite advances in intensive cardiology care, mortality from cardiogenic shock remains high. During the past years, mechanical circulatory support, including extracorporeal membrane oxygenation (ECMO), has become a standard therapy for cardiogenic shock with the aim of bridging the most critical conditions. Current evidence supporting the use of ECMO (and other mechanical circulatory support systems) in cardiogenic shock remains insufficient. The initiation of ECMO leads to rapid restoration of total circulatory output, blood pressure and tissue perfusion. On the other hand, it may be associated with a possible negative effect on the already severely damaged left ventricle as well as several specific complications. Furthermore, the haemodynamic effect of ECMO also depends on the cause of cardiogenic shock and modulating factors, such as the presence of valvular heart disease (e.g. aortic stenosis or mitral regurgitation) or mechanical complications (e.g. ventricular septal defect). The negative effect of ECMO could be at least partially prevented by synchronized pulsatile extracorporeal flow. Randomized clinical trials have reported a neutral effect of the use of ECMO on clinical outcomes in cardiogenic shock. However, these trials had significant limitations, particularly in the inclusion criteria. Several recently published analyses indicate that detailed haemodynamic examination allows for the identification of subjects who may benefit from mechanical support. Currently, numerous clinical questions remain unanswered, and further research in this field is fundamentally needed.

Keywords : *cardiogenic shock – mechanical circulatory support – extracorporeal membrane oxygenation*

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Sex Differences in Cardiac Tolerance to Oxygen Deprivation: 40 Years of Cardiovascular Research

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Abstract Experimental and clinical studies have clearly demonstrated significant sex differences in myocardial structure and function, both under physiological and pathological conditions. The best example is significant sex differences in cardiac tolerance to ischaemia/reperfusion injury: pre-menopausal adult female hearts are more resistant than male myocardium. The importance of these findings is supported by the fact that the number of studies dealing with this issue has increased significantly in recent years. Detailed molecular and cellular mechanisms responsible for sex differences are yet to be elucidated; however, it has been stressed that the differences cannot be explained only by the effect of oestrogens. In recent years, a promising new hypothesis has been developed, suggesting that mitochondria may play a significant role in the sex differences in cardiac tolerance to oxygen deprivation. However, one thing is clear already today: sex differences are so important that they should be taken into consideration in clinical practice for the selection of the optimal diagnostic and therapeutic strategy in the treatment of ischaemic heart disease. The present review attempts to summarize the progress in cardiovascular research on sex-related differences in cardiac tolerance to oxygen deprivation during the last 40 years, that is from the first experimental observation. Particular attention was paid to the sex-related differences of the normal heart, sex-dependent tolerance to ischaemia-reperfusion injury, the role of hormones and, finally, to the possible role of cardiac mitochondria in the mechanism of sex-dependent differences in cardiac tolerance to ischaemia/reperfusion injury.

Keywords *female heart – cardiac hypoxic tolerance – ischaemia-reperfusion injury – sex difference*

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Myosin Inhibitors Evoke Comparable Contractile Effects in Cardiomyocytes on Different Genetic Backgrounds

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Abstract Mutations in genes encoding certain myocardial proteins result in hypercontractility of the left ventricle (LV) and are thought to drive pro-hypertrophic signalling in hypertrophic obstructive cardiomyopathy (HOCM). Accordingly, targeting the myosin heavy chain- β by small molecules appears to be an attractive approach to prevent the development of HOCM. Indeed, clinical evidence now supports that modulation of the myosin motor can be an effective treatment option for well-defined HOCM patient groups. Moreover, the potential benefit of myosin inhibitors has also been implicated for patients with heart failure with preserved ejection fraction (HFpEF) in the absence of known genetic alterations. However, it is unclear if cardiomyocytes on different genetic backgrounds respond identically to myosin inhibitors or not. Here, we tested the contractile effects of afcamten, a second-generation myosin inhibitor agent, in cardiac preparations of healthy rodents, dogs and humans. Moreover, these effects were also contrasted to those obtained in permeabilized cardiomyocytes isolated from HOCM patients following myectomy. Afcamten reduced maximal Ca^{2+} -activated tensions (F_{max}) and evoked robust rightward shifts in the Ca^{2+} sensitivities of isometric force production of permeabilized cardiomyocytes of all sources. Nevertheless, afcamten-induced Ca^{2+} desensitizations were higher in HOCM cardiomyocytes than those in cardiomyocytes of healthy animals or humans. Our results illustrate largely comparable contractile effects upon myosin inhibitor applications, irrespective of the myosin isoform composition or the presence or absence of mutated myocardial proteins in cardiomyocytes of different species. These data also implicate that pro-hypertrophic signalling can also be limited to similar degrees by afcamten in cardiomyocytes on different genetic backgrounds.

Keywords *myosin inhibitors – afcamten – cardiomyocyte – hypertrophic obstructive cardiomyopathy*

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Adverse Effects of Shift Work on Cardiovascular Health During Pregnancy and Foetal Development

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Abstract Pregnancy is a dynamic process associated with significant physiological changes in the cardiovascular system. During pregnancy, there is an increased metabolic demand on the mother and foetus to ensure adequate uteroplacental circulation for foetal growth and development. Insufficient haemodynamic changes can result in maternal and foetal changes to the cardiovascular system. Shift work during pregnancy results in significant changes to the circadian rhythm of both mother and foetus. Furthermore, shift work is linked to increased risk for developing cardiac disease. Therefore, engaging in shift work during pregnancy may adversely affect the cardiovascular health of the mother and have negative implications for the offspring, including increased risk for cardiovascular disease, obesity, and diabetes. While the link between shift work and negative pregnancy outcomes, such as miscarriage, preterm birth and reduced foetal growth, has been previously established, the outcomes of pregnancy on cardiovascular health in shift working mothers are not well understood. Herein, we utilize a night shift work model in mice and provide new evidence that shift work during pregnancy results in changes in genes controlling endothelial function and vascular regulation, coincident with increased cardiac haemodynamic demand. Furthermore, in silico analysis revealed that genes responsible for hemodynamic and endothelial functions are regulated by the circadian genes Clock and BMAL1. The downregulation of these genes following shift work leads to failed vascular response and insufficient metabolic supply to the maternal-foetal system, resulting in placental insufficiency and restricted foetal growth. Our findings provide the first evidence for a direct link between haemodynamic function and circadian regulation during pregnancy. Understanding the key genes and networks that are associated with circadian rhythms and cardiac haemodynamics during pregnancy may contribute to developing new therapeutic strategies to mitigate the adverse effects of shift work during pregnancy.

Keywords women's heart health – pregnancy – circadian rhythm – endothelial function

Pro-arrhythmic Role of Fibrofatty Infiltration in Human Atrial Appendages

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Abstract Cardiac adipose tissue plays a crucial role in maintaining healthy cardiac function. It communicates with the myocardium through paracrine signalling and provides free fatty acids for beta-oxidation. However, an increase in cardiac adipose tissue has been associated with a higher risk of arrhythmias. This risk is linked to the release of adipokines, which signal fibroblasts to increase fibrosis and alter ion channel profiles in cardiomyocytes. These changes can lead to prolonged action potentials and re-entry circuits. Additionally, intramyocardial adipocytes can act as conduction blocks, slowing down conduction velocity and further increasing the risk of arrhythmias. In this study, we examine the adipose tissue of human atrial appendages in terms of fibrosis, adipose tissue volume and adipocyte size. The left and right atrial appendages were collected from 40 patients undergoing cardiac surgery at Rigshospitalet, Copenhagen (Denmark). Formalin-fixed and paraffin-embedded tissue was sectioned at 4 μm and stained with Picro Sirius Red. The sections were imaged using the AxioScanZ1 and subjected to automated intelligent image segmentation using the QuPath software in order to quantify fibrosis, adipose tissue and adipocyte size. We found that the content of fibrosis was significantly higher in the right atrial appendage (54.3%) compared to the left (35.9%) ($P < 0.0001$). In opposition, we found that the left appendage had a significantly higher amount of epicardial fat (35.0%) compared to the right (11.5%) ($P < 0.0001$). Infiltration of intramyocardial fat was also found to be higher in the left appendage (0.68%) compared to the right (0.21%) ($P = 0.02$). Furthermore, we also observed that the intramyocardial adipocytes are smaller compared to epicardial adipocytes in the left atrial appendage (990.6 vs $1791.9 \mu\text{m}^2$, respectively, $P < 0.0001$) with a trend in the right appendage (901.5 vs $1214.9 \mu\text{m}^2$, $P = 0.09$). Surprisingly, adipocytes in the left epicardial adipose tissue are larger compared to those in the right epicardial adipose tissue (1791.9 vs $1214.9 \mu\text{m}^2$, $P < 0.0001$). A reason for larger adipocytes observed in the left could be that we see a strong correlation between body mass index and adipocyte size in the left ($P = 0.0007$) that is not found in the right appendage ($P = 0.65$). We show structural differences between the left and right human atrial appendages in terms of fibrosis, epicardial adipose tissue and infiltrating adipocytes. Furthermore, adipocyte size also varies drastically between the atrial appendages, which could be partly explained by the increase in left atrial adipose cell size with body mass index, whereas body mass index has no effect on the right appendage. Future work will leverage the unique opportunity of having both left and right atrial appendages for comparative analysis in our patient cohort. We will first investigate if the structural characteristics have a predictive value for post-operative atrial fibrillations.

Keywords atrial fibrillation – cardiac adipose tissue

Trials and Tribulations of Translational Research to Mend a Broken Heart: The Story of Propofol Cardioplegia

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Abstract Patients with occlusive coronary artery disease can be treated by coronary artery bypass surgery (CABG) using cardiopulmonary bypass (CPB) and cardioplegic arrest. However, cardioplegic arrest renders the heart ischaemic and susceptible to reperfusion injury. This injury is augmented by the effects of CPB, which can be largely avoided by performing surgery on a beating heart. However, despite extensive clinical research, adopting beating heart surgery remains controversial. Therefore, an approach to reduce ischaemia reperfusion (I/R) injury associated with cardioplegic arrest remains a major target. A significant number of additives to cardioplegia have thus far failed to translate to clinical practice. Several early studies reported cardioprotection by the general anaesthetic propofol against I/R. Our research aimed to investigate whether propofol cardioplegia is cardioprotective when used during CPB. Work in our unit started in 1996 and showed that the addition of propofol to cardioplegia is protective during cold or warm arrest and in hearts from different species and with different pathologies. Furthermore, CPB surgery on a pig model showed cardioprotection with propofol. Inhibition of the mitochondrial permeability transition pore seems to be the mechanism. Preparations for clinical research started in 2007/8. This involved approval by several regulatory and health agencies/committees. A detailed description of the trial has been published (1). Patient recruitment started in 2010 and finished in 2012. The primary analysis showed that cardiac injury using propofol (6 µg/mL) cardioplegia was not statistically different from that of the intralipid vehicle (2). This inconclusive outcome ($P < 0.051$) promoted an ongoing second trial (3) that involves comparing control cardioplegia (not intralipid) to propofol cardioplegia. Interestingly, the secondary analysis of data provided extensive and seminal information relating to metabolic stress, cardiac injury, pathology and associated molecular changes (e.g. 4).

Keywords : *propofol – cardioplegia – cardiac surgery – ischaemia*

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Cholinergic Mechanisms and Isoprenaline-Induced Heart Failure in Rats

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Abstract Autonomic imbalance is one of the key components in the pathophysiology of heart failure (HF), with an increase in the sympathetic tone and a decrease in parasympathetic activity. Acetylcholinesterase (AChE) inhibitors constitute a large and chemically diverse group of compounds that inhibit AChE. Some of them are used to treat myasthenia gravis, such as pyridostigmine (PYR) or Alzheimer's disease (such as rivastigmine and donepezil), and they have been shown to exhibit some favourable cardiovascular effects. By using the isoprenaline (ISO) model of chronic HF (two doses of 85 mg/kg sc, 24 h apart), we aimed to investigate the potential protective effects of PYR, a peripheral AChE inhibitor. It was administered to Wistar rats in drinking water for 2 weeks at a daily dose of 20 mg/kg, while the control rats drank plain tap water instead. It was shown that PYR caused the attenuation of maladaptive cardiac remodelling, which was noted in histopathological preparations of the myocardium. PYR treatment also led to the preservation of ejection fraction (EF). In the untreated group, EF decreased significantly (from 68.71% to 53.20%), while in the PYR-treated group, EF remained stable (68.76% vs 72.10% after treatment). Slowing down of progressive left ventricle dilatation was also noted, with a significant difference in the left ventricular internal diameter end-systole (LVIDs) being increased in the untreated group (from 4.43 mm to 6.8 mm). In contrast, in the PYR-treated group, there was no significant difference between the starting and end values of LVIDs. Similar changes were found in end-systolic volume (ESV), which showed a 259.37% increase in the untreated group. Thus, by attenuating the decline in systolic function of the left ventricle and slowing the maladaptive cardiac tissue remodelling, PYR represents a promising agent in the treatment of HF.

Keywords heart failure – isoprenaline – pyridostigmine – rats – cardiac remodelling

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Molecular Hydrogen Enhances Platelet Mitochondrial Bioenergetics and Endogenous Coenzyme Q10 Levels in Patients with Metabolic Dysfunction-Associated Steatotic Liver Disease

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Abstract Metabolic dysfunction-associated steatotic liver disease (MASLD) is characterized by increased fat accumulation in liver tissue and is recognized as a hepatic manifestation of metabolic syndrome. Mitochondrial dysfunction and oxidative stress play significant roles in the progression of MASLD. Molecular hydrogen (H₂) has been recognized as a medical gas with antioxidant, anti-inflammatory and antiapoptotic effects, and it can also stimulate energy metabolism. This study aimed to evaluate the effect of adjuvant therapy with hydrogen-rich water (HRW) on platelet mitochondrial bioenergetics and endogenous coenzyme Q10 (CoQ10) levels in patients with MASLD. The study included 30 patients with MASLD and 15 healthy volunteers. Over 8 weeks, 17 patients (H2 group) drank water (3 330 mL/day) enriched with HRW tablets (>4 mg/L H2), while 13 patients (P group) drank water with placebo tablets producing CO2. Mitochondrial bioenergetics in platelets isolated from whole blood was assessed using high-resolution respirometry. The concentration of CoQ10-TOTAL in platelets and plasma was determined by HPLC, and the oxidative stress parameter TBARS in plasma was measured spectrophotometrically. Patients with MASLD exhibited relatively higher CI-linked LEAK respiration, lower CI-linked OXPHOS, and reduced CII-linked ET capacity compared to the control group, along with lower CoQ10 concentrations. Plasma TBARS concentration was higher in the H2 group. After 8 weeks of HRW adjuvant therapy, CoQ10 concentration in platelets increased, plasma TBARS decreased, and the impaired parameters of platelet mitochondrial respiration improved. Our results support the evidence that mitochondria are the primary target of H2 therapy. The application of H2 appears to be a promising new strategy for the targeted treatment of mitochondrial disorders. Additional and longer-term studies are needed.

Keywords MASLD – hydrogen-rich water – platelets – mitochondrial bioenergetics – CoQ10

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Pathophysiology of Cardiovascular Abnormalities During Obesity in Metabolic Syndrome

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Abstract It is now well established that obesity is a global epidemic and a major health hazard. Obesity is known to contribute to an increase in the risk of cardiovascular disease. Unhealthy dietary practices affect human health, and excessive fructose intake has been linked to metabolic syndrome and the occurrence of obesity and concomitant cardiac dysfunction. This study was undertaken to test the hypothesis that long-term consumption of a high fructose (HF) diet induces cardiac abnormalities during obesity in metabolic syndrome. Accordingly, our investigations, conducted in male SD rats fed an HF (60% w/w) diet plus a moderate level of saturated fat (10% w/w lard) for up to 20 weeks, revealed that systolic blood pressure and serum levels of insulin, triglycerides and total cholesterol were significantly increased in the HF fed group. Furthermore, an increase in visceral fat mass and reduced levels of the anti-inflammatory and anti-atherosclerotic cytokine, IL-10, were observed. Others have demonstrated an increase in cardiomyocytes size, left ventricular wall thickness and reduced ejection fraction and fractional shortening subsequent to high-fructose feeding of mice for 12 weeks. These data suggest that long-term consumption of HF can lead to cardiovascular dysfunction. Thus, an effective intervention to reduce HF intake may attenuate the risk for obesity-related morbidities.

Keywords *high fructose diet – metabolic syndrome – obesity – cardiovascular abnormalities*

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An Investigation on Whether Specific Antidiabetics with Dual Receptor Action Incretin Analogues Have On-Target, Off-Target or Both Effects on Metabolic Heart Function

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Abstract Novel cardioprotective antidiabetic medications include treating patients with dual or multiple receptor agonists to have better cardiovascular benefits with consistently reduced major adverse cardiac events, improvement in heart failure, and diminished cardiovascular and all-cause mortality. Especially in recent years in this field, intensive studies have suggested that active incretin hormone glucagon-like peptide-1 amide (GLP-1) and its analogs have cardioprotective effects by modulating cardiomyocyte metabolism. In addition, a glucose-dependent insulintropic polypeptide (GIP) is a hormone produced in the upper gut and supports the physiological and pharmacological relevance of GIP in metabolic disorders. Tirzepatide (TZPD), as a new generation dual agonist of GIP and GLP-1 receptors, has been shown to have metabolic effects in type 2 diabetic (T2DM) patients, as well as acting as an unbalanced and disproportionate agonist of GIP and GLP-1 receptors, and thereby showing a biased effect on GLP-1 receptors both in experimental animal studies and in cultured cells. In addition to the fact that TZPD administration affects the expression levels of these 2 receptors. It has been suggested that TZPD has a “biased pharmacologic” effect on GLP-1 receptors, and thus TZPD administration with a biased pharmacologic effect has the potential to affect other targets while activating some specific target proteins. Moreover, it has been proposed that GLP-1R activation in pancreatic beta-cells stimulates insulin secretion and activates adenylate cyclase (AC)-mediated cAMP production, cellular Ca²⁺-regulation, and modulates Gas protein activation. Although there are no clear studies to demonstrate a direct target effect of TZPD administration on heart function, the current data on pancreatic beta-cells strongly suggest a similar pathway is also possible in cardiomyocytes associated with its action. However, it is well accepted that off-target activity is the biological activity of a drug that is different from and not at that of its intended receptor. In some cases, off-target activity can be taken advantage of for therapeutic purposes. Therefore, we aimed to investigate both on-target effects and possible target effects of this drug in cardiomyocytes by considering its action in cAMP signaling. We first demonstrated its two receptors (GIP and GLP-1 receptors) localizations in cardiomyoblast (H9c2 cell line) and responses to TZPD application (40 nM). There was a significantly high response to the TZPD application in GIP-R under hyperglycemia and hyperinsulinemia mimicking cells compared to the GLP-1R. Furthermore, this application also induced a significant increase in the depressed cAMP level parallel to the increases in the expression levels of both depressed β_1 - and β_2 -adrenergic receptors. Moreover, the TZPD application significantly decreased the overexpressed β_3 -adrenergic receptor level in hyperglycemia and hyperinsulinemia mimicking cells parallel to a decrease in increased cGMP level. Our study overall demonstrated that a dual-action receptor agonist antidiabetic drug TZPD can provide both on-target receptor action and an off-target action via the cAMP signaling pathway in cardiomyoblasts under pathological stimuli such as in hyperglycemia and hyperinsulinemia, while both types of actions can include important therapeutic approaches for the heart dysfunction under pathological conditions.

Keywords *metabolic heart – antidiabetic drugs – beta-adrenergic receptors – glucose metabolism – mitochondria*

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High-Fat Dysbiosis Diet Induces Epigenetic 1-Carbon Metabolism and HFpEF

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Abstract Although the double and triple hits (i.e., hypertension, diabetes/diet/obesity, ageing) contribute to HFpEF, the contribution of a single-hit high-fat dysbiotic diet (HFD) to HFpEF is unclear. To test whether the HFD causes HFpEF, we administered HFD to normal wild-type (WT, C57) mice and measured HFpEF. Previous studies from our laboratory have observed gut dysbiosis instigating disruption in epigenetic memory (i.e. rhythmic methylation/de-methylation cycle on H3K4, K9, H4K20) via folate 1-carbon metabolism (FOCM) pathways by gene writer (DNMT), eraser (TET/FTO) and homocysteine (Hcy). We observed a link between mitochondrial TCA cycle and gene writer, eraser, and Hcy. A disruption in recycling Hcy back to methionine and impaired clearance by transsulfuration—specifically, mitochondrial sulfur metabolic pathways involving 3MST, CBS, and CSE—leads to a reduction in mitochondrial bioenergetics, ATP, OCR, H₂S, PGC1 α , and TFAM, along with an increase in the fission/fusion ratio, ultimately instigating cardiac dysfunction. The hypothesis is that the HFD causes epigenetic dysregulation and contributes to cardiac dysbiosis by HHcy and a decrease in H₂S promoting HFpEF. To determine whether the lactobacillus lowers homocysteine and increases H₂S, bidirectionally producing folate and lactone/ketone, we created a chronic dysbiotic environment by utilizing WT and DNMT1KO mice, treated w/o HFD and w/o lactobacillus. The *in vivo* data with lactobacillus show that lactobacillus (a folate and lactone/ketone body producer) mitigated HFD-induced HFpEF and cardiac remodelling. The results indicate that HFD-led HHcy negatively impacts cardiac function. The alteration in 1-carbon metabolism leads to cardiac pathological etiologies. The probiotic treatment, a folic acid and Hcy lowering agent producing probiotic mitigates epigenetic defects and HFpEF. The treatment with *Lactobacillus rhamnosus* significantly reduced the levels of cardiac MMP-2 and MMP-9, fibrosis and hypertrophy in the hearts of HFD mice, thus mitigating the HFpEF.

Keywords HFpEF – microbiota – probiotics – homocysteine – epigenetics – 1-carbon metabolism



The Role of Immune Checkpoint Signalling in Heart Failure

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Abstract Immune checkpoint molecules are physiological regulators of the adaptive immune response. Immune checkpoint inhibitors (ICIs), such as anti-PD-1 or anti-CTLA-4 monoclonal antibodies, have revolutionized the treatment of cancer, and their clinical application is rapidly increasing, as reflected by thousands of ongoing clinical trials. However, ICIs cause various immune-related side effects, including acute and chronic cardiovascular toxicities. Among these, ICI-induced acute fulminant myocarditis is the most studied direct cardiotoxicity, although emerging clinical and preclinical data suggest that other ICI-related chronic cardiac toxicities are important, such as accelerated atherosclerosis, heart failure, arrhythmias and additional forms of cardiac dysfunction. These chronic cardiovascular toxicities are often hidden, as they may only occur in the presence of preexisting injuries (ischaemic necrosis, hypertrophy) and comorbidities (metabolic disease). It is therefore of high clinical importance to identify the risk factors and comorbidities that may precipitate ICI-induced cardiotoxicity. The mechanisms of ICI-induced myocarditis and cardiac dysfunction appear to be distinct: myocarditis develops as a specific autoimmune response against cardiac myocytes involving expansion of autoreactive CD8 T cells to cardiac self-antigens (e.g. against alpha myosin heavy chain), whereas cardiac dysfunction/heart failure following ICI therapy is associated with disturbances in cardiac transcriptomic and metabolic effects, likely due to increased expression of pro-inflammatory cytokines. The occurrence of these profound effects on the heart suggests a possible role for immune checkpoint molecules in the maintenance of cardiovascular homeostasis, and therefore, the disruption of physiological immune checkpoint signalling may lead to cardiac diseases, including heart failure.

Keywords heart failure – inflammation – immune checkpoint – T cell – cardiotoxicity



Development of a Novel Class of Ino-Lusitropic Agents

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Abstract Ino-lusitropic therapy would be highly beneficial in heart failure (HF), even at its early stages, but it is underexploited because of the undesirable effects (mainly proarrhythmia) of currently available agents. In the early 2000, we came across a Na^+/K^+ pump blocker, named PST2744, in which we detected an ancillary SERCA2a stimulatory effect. After a thorough bench characterization, this compound, now known as istaroxime, has been tested in large animal models and patients with advanced heart failure, showing ino-lusitropic effects and an excellent safety profile (no proarrhythmia). Since then, we have synthesized and validated a series of derivatives that overcome istaroxime limitations: 1) lack of selectivity for SERCA and 2) pharmacokinetics suitable for acute use only. The outcome of this endeavour is a toolbox of 'pure' SERCA2a stimulators suitable for chronic HF treatment. My talk will go through the development process, what we have learned from it and where it is going.

Keywords *SERCA2a stimulators – heart failure – lusitropy – inotropy – drug development*

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Disruption of Circadian Control of the Heart by Exposure to Light at Night

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Abstract Periods of biological rhythms in the cardiovascular system (CVS) range from milliseconds to days. Disruption of rhythmicity can have fatal consequences, and understanding physiological and molecular mechanisms is necessary to prevent and treat chronodisruption. Circadian rhythms (about 24 h) are important in CVS functioning. The disrupted daily blood pressure (BP) profile (non-dipping) can increase cardiovascular risk, but underlying mechanisms are not understood. We showed that hypertensive patients with the non-dipping BP profile had lower nighttime melatonin levels than dippers. However, the melatonin profile was not suppressed in transgenic rats with the upregulated renin-angiotensin-aldosterone system [TGR(mRen-2)27], which is accepted as the animal model of non-dipping. Aldosterone concentrations were upregulated, the rhythmicity of sympathetic activity was phase-shifted and expression of clock genes in the area postrema, a sensory transducer between blood and brain, was in antiphase in TGR compared to controls. Artificial light at night (ALAN) has increased dramatically, and epidemiological studies suggest its adverse effects on health, which can be mediated by disruption of circadian rhythms. We explored the consequences of ALAN on cardiovascular parameters in normotensive and spontaneously hypertensive rats. Control rats were kept in a light:dark cycle of 12:12, and ALAN groups were exposed to dim light (~2 lux) during the night. Heart rate, BP and locomotor activity were measured by telemetry, and gene expression in the heart was quantified by qPCR. The power of circadian oscillations in BP and heart rate decreased, and the rhythmic pattern of clock and clock-controlled genes and selected metabolic genes was disturbed by light at night. ALAN eliminated rhythmic expression of *Nos3*, *Pkc1ε* and glucocorticoid receptors and suppressed amplitude in *Pdk4* and *Pparγ* daily rhythms. Results suggest that ALAN attenuates circadian rhythms in CVS, disrupts the expression of genes controlling heart metabolism and interferes with the ability of the heart to anticipate and compensate for altered workload.

Keywords *circadian – blood pressure – ALAN – heart – metabolism*

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