PROGRAMME

ORAL SESSIONS

PROGRAMME – ORAL SESSIONS Sunday, 9

18.00 - 19.30 S01 Plenary Lectures

Chair: Patrick Masson (Russia), Kamil Kuca (Czech Republic)

18.00 - 18.45 **S01-1** **Chronic illness from organophosphorus toxicant exposure**
Oksana Lockridge *(USA)*, Lawrence M. Schopfer

18.45 - 19.30 **S01-2** **The catalytic power of phosphotriesterases for the hydrolysis and destruction of organophosphorus nerve agents**
Frank M. Raushel *(USA)*

19.30 - 21.00 Welcome party

Conference venue: **Campus**, Building A, Hradecká 1227, Hradec Králové
Included in the registration fee

PROGRAMME – ORAL SESSIONS — Monday, 10

08.15 - 08.30 — **Opening ceremony**
Chair: Kamil Kuca

08.30 - 11.30 **S02** — **Structure and dynamics of cholinesterases and OP hydrolases**
Chair: Yuan-Ping Pang (USA), Joel Sussman (Israel)

08.30 - 08.50 **S02-1** **Recent breakthroughs in the structure/function studies of acetylcholinesterase**
Joel L. Sussman *(Israel)*, I. Silman

08.50 - 09.10 **S02-2** **Coupling of acetylcholinesterase to the interfacial phase state**
Bernhard Fichtl, Stefan Nuschele, Konrad Kaufmann, **Israel Silman** *(Israel)*, Matthias F Schneider

09.10 - 09.30 **S02-3** **The other side of AChE: Allosteric sites and modulators**
Carlos Roca, Carlos Requena, Víctor Sebastián-Pérez, Sony Malhotra, Chris Radoux, Concepción Pérez, Ana Martinez, Juan Antonio Páez, Tom L. Blundell, **Nuria E. Campillo** *(Spain)*

09.30 - 09.50 **S02-4** **Partial unfolding of insect acetylcholinesterase: Steps toward cysteine-targeting insecticides**
Yuan-Ping Pang *(USA)*

09.50 - 10.10 *COFFEE BREAK*

10.10 - 10.30 **S02-5** **The protonation state of Glu197 and its important role in stabilizing catalytic triad of butyrylcholinesterase**
Junjun Liu *(China)*, Xiao Wan

10.30 - 10.50 **S02-6** **Recipes to design specific ligands of human butyrylcholinesterase**
Florian Nachon *(France)*, Jacques-Philippe Colletier, Nicolas Coquelle, Xavier Brazzolotto

10.50 - 11.10 **S02-7** **Exploring the evolutionary potential of the αE7 carboxylesterase**
Galen J. Correy *(Australia)*, Colin J. Jackson

11.10 - 11.30 **S02-8** **Protein dynamics of phosphotriesterase: Two cations required for enzyme catalysis**
Yuan-Ping Pang *(USA)*

11.30 - 12.30 *LUNCH and POSTERS*

PROGRAMME – ORAL SESSIONS Monday, 10

12.30 - 15.15 S03 Interaction of cholinesterases with substrates, inhibitors and reactivators

Chair: Terrone Rosenberry (USA), Franz Worek (Germany)

12.30 - 12.50 **S03-1** **New Progress in Drug Design, Discovery and Development Involving Cholinesterases**
Fang Zheng, **Chang-Guo Zhan** *(USA)*

12.50 - 13.10 **S03-2** **In searching for the mechanism of butyrylcholinesterase activators**
Jure Stojan *(Slovenia)*

13.10 - 13.30 **S03-3** **Computational analysis of reaction mechanisms for optimization of butyrylcholines-terase-based catalytic bioscavengers against organophosphorus agents**
Sofya Lushchekina *(Russia)*, Bella Grigorenko, Alexander Nemukhin, Sergei Varfolomeev, Patrick Masson

13.30 - 13.50 **S03-4** **Enhancement in pyridinium oxime-assisted reactivation of tabun-inhibited acetylcholinesterase achieved by active site mutations**
Zrinka Kovarik *(Croatia)*, Maja Katalinić, Nikolina Maček Hrvat, Goran Šinko, Tamara Zorbaz, Anita Bosak

13.50 - 14.10 **S03-5** **The search for resistance-breaking and species-selective mosquitocidal inhibitors of *Anopheles gambiae* AChE**
Paul R. Carlier *(USA)*, Jeffrey R. Bloomquist, Jonah Cheung, Jianyong Li, Max Totrov

14.10 - 14.30 **S03-6** **Steric Effects in the Decarbamoylation of Carbamoylated Acetylcholinesterase**
Kunisi S. Venkatasubban, Joseph L. Johnson, Jamie L. Thomas, Abdul Fauq, Bernadette Cusack, **Terrone L. Rosenberry** *(USA)*

14.30 - 14.45 **S03-7** **Assessment of scoring functions for AChE-ligand interactions**
Goran Šinko *(Croatia)*

14.45 - 15.00 **S03-8** **Phenyl valerate esterase activity of human cholinesterases**
Jorge Estévez *(Spain)*, María Romo, Marina Terol, Iris Mangas, Miguel Ángel Sogorb, Eugenio Vilanova

15.00 - 15.15 **S03-9** **Effects of memantine and its metabolite Mrz 2/373 on soman-induced inhibition of bovine erythrocyte acetylcholinesterase *in vitro***
Miloš P. Stojiljković *(Bosnia & Herzegovina)*, Ranko Škrbić, Milan Jokanović

15.15 - 15.40 *COFFEE BREAK*

PROGRAMME – ORAL SESSIONS Monday, 10

15.40 - 18.10 S04 Reactivators of AChE, OP inhibitors – mechanism of toxicity, detection and analytical methods, diagnosis of exposure, detoxification and therapy; counter-terrorism strategies

Chair: Franz Worek (Germany), Terrone Rosenberry (USA)

15.40 - 16.00 **S04-1** **Oximes with ortho-positioned chlorine moiety exhibit improved physical-chemical properties, efficient reactivation of inhibited human acetylcholinesterase and reduced in vivo toxicity**

David Malinak, Tamara Zorbaz, Adam Skarka, Martina Hrabinova, Nikola Marakovic, Jana Janockova, Ondrej Soukup, Jan Misik, Daniel Jun, Kamil Kuca, Zrinka Kovarik, **Kamil Musilek** *(Czech Republic)*

16.00 - 16.20 **S04-2** **Design and synthesis of bifunctional fluoropyridinaldoxime reactivators for nerve agent-inhibited human acetylcholinesterase**

Jagadeesh Yerri *(France)*, José Dias, Florian Nachon, Rachid Baati

16.20 - 16.40 **S04-3** **Combination of oximes with overlapping reactivation spectra: obidoxime and HI-6**

Timo Wille *(Germany)*, Horst Thiermann, Franz Worek

16.40 - 17.00 **S04-4** **Design of broad spectrum antidotes**

Cecilia Lindgren, Nina Forsgren, Christine Akfur, Lotta Berg, David Andersson, Franz Worek, Anna Linusson, **Fredrik Ekström** *(Sweden)*

17.00 - 17.15 **S04-5** **Demonstration of the First Small Molecule Therapeutics for Resurrection of the Aged form of Acetylcholinesterase after Exposure to Organophosphorus Chemical Nerve Agents and Pesticides**

Andrew J. Franjesevic *(USA)*, Qinggeng Zhuang, Ola, Nosseir, William H. Coldren, Christopher S. Callam, Christopher M. Hadad

17.15 - 17.30 **S04-6** **Nanotechnology strategies using oximes-loaded lipid nanoparticles for brain protection against organophosphorus poisoning**

Tatiana N. Pashirova *(Russia)*, Irina V. Zueva, Anissa Braïki, Konstantin A. Petrov, Vasily M. Babaev, Evgenia A. Burilova, Darya A. Samarkina, Ildar Kh. Rizvanov, Eliana B. Souto, Ludovic Jean, Pierre-Yves Renard, Patrick Masson, Lucia Ya. Zakharova, Oleg G. Sinyashin

17.30 - 17.50 **S04-7** **Utilizing Structure-Activity Relationships and Mechanistic Insights to Design Non-Oxime Reactivators**

C. Linn Cadieux *(USA)*, Zachary Canter, Kevin Martin, Keith Morgan, Michael Hepperle

17.50 - 18.10 **S04-8** **New Non-Oxime Reactivators of Organophosphate Inhibited Acetylcholinesterase with Promising Reactivation Potency**

Martijn de Koning *(Netherlands)*, Franz Worek, Gabriele Horn, Marco van Grol

PROGRAMME – ORAL SESSIONS Tuesday, 11

08.00 - 09.40 S05 Enzymes and proteins other than ChEs interacting with OP

Chair: Oksana Lockridge (USA), Patrick Masson (Russia)

08.00 - 08.20 **S05-1** **Identifying axonal transport-related targets for reversing the adverse effects of organophosphate exposure**
Sean X. Naughton, **Alvin V. Terry, Jr** *(USA)*

08.20 - 08.40 **S05-2** **Diagnosis of Poisoning with O-Isobutyl-S-[2-(diethylamino)ethyl] methylphosphonothioate (VR) under Antidotal Therapy with Carboxim**
Nadezhda L. Koryagina *(Russia)*, Elena I. Savelieva, Anton I. Ukolov, Darya S. Prokofieva, Nataliia S. Khlebnikova, Tatiana I. Aliushina, Elena S. Ukolova, Andrey S. Radilov, Nikolay V. Goncharov

08.40 - 09.00 **S05-3** **Copper-dependent hydrolysis of trichloronate by turkey serum and albumin**
Damianys Almenares-López *(México)*, Antonio Monroy-Noyola

09.00 - 09.20 **S05-4** **Mass Spectral Detection of Diethoxyphospho-Tyrosine Adducts on Proteins from HEK293 Cells Using Monoclonal Antibody depY for Enrichment**
Seda Onder *(Turkey, USA)*, Lawrence M. Schopfer, Ozden Tacal, Thomas A. Blake, Rudolph C. Johnson, Oksana Lockridge

09.20 - 09.40 **S05-5** **Innovative Biocatalysts as Tools to Detect and Inactivate Nerve Agents**
Elena Porzio, Francesca Bettazzi, Luigi Mandrich, Immacolata Del Giudice, Odile F. Restaino, Serena Laschi, Ferdinando Febbraio, Valentina De Luca, Maria G. Borzacchiello, Teresa M. Carusone, Franz Worek, Antonio Pisanti, Piero Porcaro, Chiara Schiraldi, Mario De Rosa, Ilaria Palchetti, **Giuseppe Manco** *(Italy)*

09.40 - 10.00 *COFFEE BREAK*

PROGRAMME – ORAL SESSIONS Tuesday, 11

10.00 - 12.10 S06 Stoichiometric bioscavanger, biotechnology and therapeutical aspects

Chair: Palmer Taylor (USA)

10.00 - 10.10
Commemoration of John Casida
Palmer Taylor *(USA)*

10.10 - 10.30 **S06-1**
Ionizable, Zwitterionic Oximes as Countermeasures to Volatile Organophosphate (OP) Exposure
Palmer Taylor *(USA)*, William C. Hou, Jeremiah Momper, Yan-Jye Shyong, Zoran Radic, John McDonough, Zrinka Kovarik, Yvonne Rosenberg, K. Barry Sharpless

10.30 - 10.50 **S06-2**
Development of pre- and post-countermeasures against OP toxins in macaques
Yvonne Rosenberg *(USA)*, James Fink, Lingjun Mao, Xiaoming Jiang, Jonathan Lees, Jerry Wang, Tara Ooms, Narayanan Rajendra, Zoran Radic, Palmer Taylor

10.50 - 11.10 **S06-3**
Human Plasma-Derived Butyrylcholinesterase Is Behaviorally Safe and Effective in Cynomolgus Macaques (Macaca fascicularis) Challenged with Soman
Todd M. Myers *(USA)*

11.10 - 11.30 **S06-4**
Design of a combined aptamer for paraoxon and acetylcholinesterase by in silico approach
Daria A. Belinskaia *(Russia)*, Pavel A. Avdonin, Nikolay V. Goncharov

11.30 - 11.50 **S06-5**
Prokaryotic expression of human butyrylcholinesterase as a tool for catalytic bioscavenger development
Xavier Brazzolotto *(France)*, Alexandre Igert, Virginia Guillon, Gianluca Santoni, Florian Nachon

11.50 - 12.10 **S06-6**
Bioscavengers and the medical management chain
Thomas M. Mann *(England)*, H. Rice

12.10 - 13.40 *LUNCH and POSTERS*

PROGRAMME – ORAL SESSIONS — Tuesday, 11

13.40 - 16.00 S07 Catalytical Bioscavangers – PON and PTE

Chair: Eugenio Vilanova (Spain), Florian Nachon (France)

13.40 - 13.50
Commemoration of Doug Cerasoli
Tamara C. Otto *(USA)*

13.50 - 14.00 **S07-1**
Borderline between catalytic and non-catalytic bio scavengers: the example of albumin and reversible B-esterases
Eugenio Vilanova *(Spain)*, Jorge Estévez, Miguel Ángel Sogorb, Iris Mangas, Antonio Monroy

14.00 - 14.20 **S07-2**
Catalytic Scavengers Provide Broad-Spectrum Protection against Organophosphorus Nerve Agents
Shane A. Kasten, Sandra J. DeBus, Thuy L. Dao, Michael V. Boeri, Zachary A. Canter, Sean M. Hodgins, Robyn. B. Lee, Douglas M. Cerasoli, **Tamara C. Otto** *(USA)*

14.20 - 14.40 **S07-3**
Paraoxonase 1 variant I-F11 gene therapy using adeno-associated virus8 (AAV8) offers long-term protection against G-type chemical warfare nerve agents
Venkaiah Betapudi, Deborah M. Doctor, **Nageswararao Chilukuri** *(USA)*

14.40 - 15.00 **S07-4**
Organophosphate Hydrolase (OPH) Designed as a Tethered Monomer
Jaffet Santiago Garcia, Cetara Baker, Richard Sweeney, **Stephen Kirby** *(USA)*

15.00 - 15.20 **S07-5**
A new animal model to investigate organophosphorus poisoning and enzymatic decontamination
Laetitia Poirier *(France)*, Pauline Jacquet, Laure Plener, Cédric Torre, Eric Ghigo, David Daudé, Eric Chabrière

15.20 - 15.40 **S07-6**
Paraoxonase-2 dependent redox control of platelet physiology
Victoria Petermann *(Germany)*, H.Kleinert, K.Jurk

15.40 - 16.00 **S07-7**
Copper with chicken serum albumin show stereoselective hydrolysis of chiral phosphoramidates
Antonio Monroy-Noyola *(México)*, Miguel Angel Sogorb, Eugenio Vilanova

18.00 ***Walking tour around the city***

The tour will begin and finish in front of the Cathedral of the Holy Spirit.
Free tickets at the registration desk (limited amount!)

20.00 ***Concert in the Cathedral of the Holy Spirit***

PROGRAMME – ORAL SESSIONS Wednesday, 12

08.30 - 11.30 S08 3D section – structure and dynamics of α/β hydrolases and OP hydrolases, in silico methods for designing of modulator

Chair: Zoran Radić (USA), Jonah Cheung (USA)

08.30 - 08.55 **S08-1** **Insights into the Yin and the Yang of Acetylcholinesterase Inhibition by Mechanistic X-Ray Crystallography**
M. Bartolini, M.L. Bolognesi, J. Korábečný, K. Kuca, **Doriano Lamba** *(Italy)*, A. Pesaresi, X. Zha

08.55 - 09.20 **S08-2** **Photo-induced release of an acetylcholinesterase inhibitor**
Eugenio de la Mora *(France)*, Johannes Broichhagen, Peter Mayer, Elisabet Artursson, Fredrik Ekström, Joel Sussman, Israel Silman, Dirk Trauner, Giorgio Schirò, Martin Weik

09.20 - 09.45 **S08-3** **Structural studies of *Anopheles gambiae* acetylcholinesterase provide insight towards improved insecticides for malaria vector control**
Jonah Cheung *(USA)*, Arshad Mahmood, Ravi Kalathur, Lixuan Liu, Max Totrov, Paul Carlier

09.45 - 10.05 *COFFEE BREAK*

10.05 - 10.30 **S08-4** **Room-temperature crystallography and neutron scattering studies of human acetylcholinesterase to inform the design of oxime reactivators**
Oksana Gerlits, Mikolai Fajer, Xiaolin Cheng, Donald Blumenthal, Palmer Taylor, Zoran Radić, **Andrey Kovalevsky** *(USA)*

10.30 - 10.55 **S08-5** **Crystal structures of human cholinesterases in complex with supramolecular ligands**
José Dias *(France)*, Xavier Brazzolotto, Xiao-Yu Cao, Artur Stefankiewicz, Jean-Marie Lehn, Florian Nachon

10.55 - 11.20 **S08-6** **Modifications of Cholinesterase Structure and Function in Covalent Organophosphate Conjugates Visualized in 2D, 3D and VR**
Zihan Zheng, Wanlu Yu, Jacqueline Rohrer, Alexandria Tran, **Zoran Radić** *(USA)*

11.20 - 11.30 **Concluding sentence**
Zoran Radić *(USA)*

11.30 - 13.00 *LUNCH and POSTERS*

13.00 *Trips*

13.00 National Stud Farm at Kladruby nad Labem
13.15 Kuks hospital
13.30 Hrádek u Nechanic chateau

PROGRAMME – ORAL SESSIONS Thursday, 13

08.00 - 11.30 S09 Biological functions, development and non-cholinergic function of cholinesterases

Chair: Shani Shenhar-Tsarfaty (Israel), Israel Silman (Israel)

08.00 - 08.15 **Commemoration of Eric Barnard**
Israel Silman *(Israel)*, **Karl W. K. Tsim** *(China)*

08.15 - 08.35 **S09-1** **Cholinergic mechanisms at the core of skeletal and retinal histogenesis**
Gesine Bachmann, Afrim Bytyqi, Florian Frohns, Matthias Rieke, Gopenath Thangaraj, **Paul G. Layer** *(Germany)*

08.35 - 08.55 **S09-2** **Butyrylcholinesterase as a ghrelin modulator impacting anxiety, stress, obesity, and drug cravings**
Stephen Brimijoin *(USA)*, Y. Gao, L.Y. Geng, V.P. Chen

08.55 - 09.15 **S09-3** **Assembly of PRiMA-linked form of acetylcholinesterase in neurons: the role of enzyme inhibitor acting as chemical chaperon**
Karl W. K. Tsim *(China)*, Etta Y. L. Liu, Miranda L. Xu, Xiang P. Kong, Qiyun Wu, Ran Duan, Tina T. X. Dong

09.15 - 09.35 **S09-4** **Evolution of the first disulfide bond in the cholinesterase-carboxylesterase (COesterase) family: Possible consequences for cholinesterase expression in prokaryotes**
Arnaud Chatonnet *(France)*, Xavier Brazzolotto, Thierry Hotelier, Nicolas Lenfant, Pascale Marchot

09.35 - 09.55 *COFFEE BREAK*

09.55 - 10.15 **S09-5** **Acetylcholinesterase in neuromuscular synaptic clefts of vertebrates**
Edna Blotnick-Rubin, **Lili Anglister** *(Israel)*

10.15 - 10.35 **S09-6** **Respiration during organophosphate and carbamate intoxication when acetylcholinesterase is not anchored at cholinergic synapses**
Eric Krejci (*France*), Aurélie Nervo, Imene Kellout, Anne Sophie Hanak, Guilhem Calas, Florian Nachon

10.35 - 10.55 **S09-7** **Single nucleotide polymorphisms in the genes encoding AChE and its miR-608 regulator co-modulate anxiety and blood pressure**
Alon Simchovitz *(Israel)*, Nimrod Madrer, Rotem Haviv, Geula Hanin, Shani Shenhar-Tsarfaty, Einor Ben Assayag, Shlomo Berliner, Zehava Solomon, Hermona Soreq

10.55 - 11.15 **S09-8** **Dioxin suppresses AChE expression in neuron and muscle**
Heidi Qunhui Xie *(China)*, Yingjie Xia, Tuan Xu, Yangsheng Chen, Yali Luo, Rui Sha, Yiyun Liu, Li Xu, Bin Zhao

11.15 - 11.30 **S09-9** **Wnt3a induces the transcription of acetylcholinesterase: an enzyme playing a role in osteoblastic differentiation**
Miranda L. Xu *(China)*, Etta Y. L. Liu, Qiyun Wu, Duan Ran, Tina T. X. Dong, Karl W. K. Tsim

11.30 - 12.30 *LUNCH and POSTERS*

PROGRAMME - ORAL SESSIONS — Thursday, 13

12.30 - 14.45 S10 Alzheimer's disease and diseases related to cholinesterases

Chair: Ana Martinez (Spain), Maria-Laura Bolognesi (Italy)

12.30 - 12.50 **S10-1** **Restoring mitochondria (dys)function and acetylcholine levels as a prospective therapeutic strategy for Alzheimer's disease**
Fernanda Borges *(Portugal)*

12.50 - 13.10 **S10-2** **From dual binding site AChE inhibitors to chameleon molecules: discovery of potent BuChE inhibitors**
Carlos Roca, Talita P.C. Chierrito, Concepción Perez, Loreto Martinez, Nuria Campillo, **Ana Martinez** *(Spain)*

13.10 - 13.30 **S10-3** **Discovery and development of neuroprotective and disease-modifying anti-AD drug leads from the Chinese medicine**
Marvin Mak, Wei Cui, **Yifan Han** *(China)*

13.30 - 13.50 **S10-4** **Fifty shades of cholinesterase immobilization and their application to drug discovery**
Manuela Bartolini *(Italy)*, Anna Tramarin, Edoardo Fabini, Piotr Drączkowski, Marina Naldi, Daniele Tedesco, Krzysztof Jóźwiak, Vincenza Andrisano

13.50 - 14.10 **S10-5** **Serum cholinesterase activity and Alzheimer disease comorbidities - can bariatric surgery change your sympathetic prone state?**
Shani Shenhar-Tsarfaty *(Israel)*, Shiri Sherf-Dagan, Galia Berman, Shira Zelber-Sagi, Oren Shibolet, Itzhak Shapira , David Zeltser, Shlomo Berliner, Ori Rogowski

14.10 - 14.30 **S10-6** **Indazolylketones: hit to lead optimization of a multitarget drugs**
Pedro González-Naranjo, Natalia Pérez, Concepción Pérez, Carlos Roca, Rocio Girón, Eva Sánchez-Robles, Ángeles Martín Requero, Maria L. de Ceballos, **Nuria E. Campillo** *(Spain)*, Juan Antonio Páez

14.30 - 14.45 **S10-7** **Butyrylcholinesterase genetic polymorphism and neuroimaging biomarkers in Alzheimer's disease**
DeBay Drew R. *(Canada)*, Maxwell Selena, Luke David, Fisk John D., Burrell Steve, Bowen Chris V., Song Xiaowei, Black Sandra E., Darvesh Sultan

14.45 - 15.10 *COFFEE BREAK*

PROGRAMME - ORAL SESSIONS Thursday, 13

15.10 - 17.40 S11 Multi-target-directed ligands in Alzheimer's disease primarily targeting cholinesterases

Chair: Maria-Laura Bolognesi (Italy), Ana Martinez (Spain)

15.10 - 15.30 **S11-1** **Case studies for successful combination of ChE inhibitors and GPCR ligands (cannabinoid 2 and histamine 3 receptors)**
Dominik Dolles, Fouad H. Darras, Antonios Drakopoulos, Andrea Strasser, Hans-Joachim Wittmann, Christoph A. Sotriffer, Steffen Pockes, Bassem Sadek, Tangui Maurice, **Michael Decker** *(Germany)*

15.30 - 15.50 **S11-2** **From acetylcholinesterase inhibitors to multi-target-directed ligands (MTDLs): a step forward in Alzheimer's disease drug discovery**
Maria-Laura Bolognesi *(Italy)*

15.50 - 16.10 **S11-3** **From selective butyrylcholinesterase inhibitors to multi-target-directed ligands as lead compounds for Alzheimer's disease**
Urban Košak, Damijan Knez, Boris Brus, **Stanislav Gobec** *(Slovenija)*

16.10 - 16.30 **S11-4** **Discovery and characterization of tacrine/huprine-tryptophan heterodimers as novel multipotent compounds against Alzheimer's disease**
Jan Korabecny *(Czech Republic)*, Katarina Spilovska, Manuela Bartolini, Barbara Monti, Doriano Lamba, Rosanna Caliandro, Alessandro Pesaresi, Vendula Hepnarova, Daniel Jun, Martina Hrabinova, Rafael Dolezal, Jana Zdarova Karasova, Ondrej Soukup, Eva Mezeiova, Eugenie Nepovimova, Maria Laura Bolognesi, Kamil Kuca

16.30 - 16.50 **S11-5** **Novel conjugates based on γ-carbolines, carbazoles, phenothiazines, and aminoadamantanes as multifunctional agents for Alzheimer's disease treatment**
Galina F. Makhaeva *(Russia)*, N.P. Boltneva, N.V. Kovaleva, S.V. Lushchekina, E.V. Rudakova, R.J. Richardson, S.O. Bachurin

16.50 - 17.10 **S11-6** **Pleiotropic prodrugs: a novel polypharmacology approach to treat neurodegenerative diseases**
Christophe Rochais *(France)*, Patrick Dallemagne

17.10 - 17.25 **S11-7** **Toward an innovative treatment of Alzheimer's disease: Design of multi-target directed ligands (MTDLs) targeting acetylcholinesterase (AChE) and alpha-7 nicotinic receptors (alpha-7 nAChRs)**
Mégane Pons *(France)*, Buron Frédéric, Ludovic Jean, Sylvie Chalon, Sylvain Routier, Pierre-Yves Renard

17.25 - 17.40 **S11-8** **Molecular modeling in search of new, multi-target ligands against Alzheimer's disease. Exploring the biochemical multiverse.**
Jakub Jończyk *(Poland)*, Dawid Panek, Anna Więckowska, Justyna Godyń, Marek Bajda, Tomasz Wichur, Anna Pasieka, Damijan Knez, Anja Pišlar, Jan Korabecny, Ondrej Soukup, Vendula Sepsova, Raimon Sabaté, Janko Kos, Stanislav Gobec, Barbara Malawska

20.00 ***Gala dinner***

Hotel Nové Adalbertinum, Velké náměstí 32, Hradec Králové
Included in the registration fee

PROGRAMME – ORAL SESSIONS Friday, 14

08.00 - 09.20 S12 Varia

Chair: Kamil Kuca (Czech Republic), Ondrej Soukup (Czech Republic)

08.00 - 08.20 **S12-1** **Design of a butyrylcholinesterase mutant for detoxifying cocaine and its toxic metabolites in concurrent use of cocaine and alcohol**

Fang Zheng *(USA)*, Xirong Zheng, Ting Zhang, Xiabin Chen, and Chang-Guo Zhan

08.20 - 08.40 **S12-2** **7-Methoxyderivative of tacrine is a 'foot-in-the-door' blocker of GluN1/GluN2 and GluN1/GluN3 NMDA receptors**

Martina Kaniakova, Lenka Kleteckova, Katarina Lichnerova, Kristina Holubova, Kristyna Skrenkova, Miloslav Korinek, Jan Krusek, Tereza Smejkalova, Jan Korabecny, Karel Vales, Ondrej Soukup, **Martin Horak** *(Czech Republic)*

08.40 - 09.00 **S12-3** **The *Caenorhabditis elegans* pharynx as a model system to investigate and mitigate against the effects of anti-cholinesterase drugs**

Patricia Gonzalez *(United Kingdom)*, Christopher Green, John Tattersall, Lindy Holden-Dye, Vincent O'Connor

09.00 - 09.20 **S12-4** **Pharmacokinetics of bis-pyridinium mono-aldoximes**

Huba Kalász *(Hungary)*, Kamil Kuca, Kamil Musilek, Gellért Karvaly, Syed Nurulain, Kornélia Tekes

09.20 - 09.30 Concluding remarks

10.00 Shuttle bus to Prague Airport

ABSTRACTS

ORAL SESSIONS

S01 Plenary lectures

Chairs: Patrick Masson (Russia), Kamil Kuca (Czech Republic)

S01-1 Chronic illness from organophosphorus toxicant exposure

Oksana Lockridge, Lawrence M. Schopfer

Eppley Institute, University of Nebraska Medical Center, Omaha, NE, 68198-5900 USA, olockrid@unmc.edu

The mechanism of toxicity from acute exposure to organophosphorus toxicants (OP) is understood. Thousands of publications have confirmed that AChE inhibition results in muscle weakness and respiratory failure. AChE activity returns to normal levels within one month, but symptoms can persist for a lifetime. For example, people exposed to sarin in the 1995 Tokyo subway attack still have adverse symptoms 23 years later. Farmers and sheep dippers exposed to OP pesticides have an elevated risk of psychiatric disorders and suicidal behavior. Epidemiology studies show an association between OP exposure and Alzheimer's disease and Parkinson's disease. We propose a mechanism to rationalize these observations independent of cholinesterase inhibition. Mass spectrometry analysis of OP-treated proteins shows that OP make stable adducts on tyrosine and lysine. Furthermore, we have mass spectrometry evidence that OP-lysines promote crosslinks between proteins. The crosslinked proteins are visualized as protein aggregates on SDS gels and Western blots. Mass spectrometry has identified γ-glutamyl-ε-lysine and aspartyl-ε-lysine isopeptide bonds between crosslinked peptides. We propose, but have not yet proven, that isopeptide crosslinked proteins form stable, insoluble aggregates in the brain, similar to the protein aggregates found in Alzheimer's, Parkinson's, and prion diseases. In summary, we propose that chronic neurotoxicity from OP exposure is initiated by OP-lysine formation followed by protein aggregation. Our proposed mechanism could apply to a variety of compounds and lead to an understanding of neurotoxicity induced by many chemicals.

Keywords
crosslinking, protein aggregates, mass spectrometry

S01-2 The Catalytic Power of Phosphotriesterases for the Hydrolysis and Destruction of Organophosphorus Nerve Agents

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Phosphotriesterase (PTE), an enzyme originally isolated from *Pseudomonas diminuta*, is capable of catalyzing the hydrolysis of many organophosphorus nerve agents. The turnover number for the enzymatic hydrolysis of paraoxon (diethyl p-nitrophenyl phosphate) by PTE is ~500,000 min^{-1}. The protein adopts a distorted $(\beta/\alpha)_8$-barrel structural fold and the active site is perched at the C-terminal end of the β-barrel. The water used for nucleophilic attack of the substrate bridges two divalent metal ions in the active site and is further activated by the side chain carboxylate from an aspartate residue that resides at the end of β-strand 8. Upon binding to the active site, substrates are further activated for hydrolysis by a direct interaction of the phosphoryl oxygen with the β-metal ion. The chemical reaction is initiated via the direct attack of the bridging water/hydroxide at the phosphorus center of the substrate and proceeds with an inversion of stereochemistry. Wild-type PTE is stereoselective for the hydrolysis of chiral substrates. However, the catalytic preferences for the hydrolysis of chiral substrates can be enhanced, relaxed, or inverted by selective mutation of key residues in the active site that dictate the size and shape of the substrate-binding cavity. The extreme toxicity and persistence of the G-type (sarin and soman) and V-type (VX and VR) organophosphorus nerve agents makes the detoxification of these compounds of significant interest. A rational and random mutagenesis strategy has been developed and implemented for the evolution of mutant forms of PTE that are more fully optimized for the catalytic destruction and detoxification of the most toxic organophosphorus nerve agents.

S02 Structure and dynamics of cholinesterases and OP hydrolases

Chairs: Yuan-Ping Pang (USA), Joel Sussman (Israel)

S02-1 Recent Breakthroughs in the Structure/Function Studies of Acetylcholinesterase

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The synaptic enzyme acetylcholinesterase (AChE) terminates transmission at cholinergic synapses by rapidly hydrolysing acetylcholine. Examination of the 3D structure of AChE[1] shows that the active site is located at the bottom of a deep and narrow gorge, lined largely by aromatic residues, with its peripheral anionic site located at the top, near the entrance to of the gorge. 3D structures of AChE have been determined for the *Torpedo, Electrophorus*, mouse, *Drosophila* and human enzymes. Overall, more than a hundred crystal structures of AChEs, and of covalent conjugates and reversible complexes with various inhibitors and substrate analogues have been determined. Although the 3D structure of the enzyme itself, and of its molecular dimer, are highly conserved, subtle structural differences are seen to occur upon the binding of certain inhibitors. These changes are well correlated with molecular dynamics data, and appear to be of functional significance.

Unfortunately, upon heterologous overexpression, many proteins misfold or aggregate, thus resulting in low functional yields. Human AChE is a typical case of a human protein that necessitates mammalian systems to obtain functional expression. Using a novel computational strategy, we designed an AChE variant bearing 51 mutations that improved core packing, surface polarity, and backbone rigidity. This variant expressed at ~2,000-fold higher levels in *E. coli* compared to wild-type hAChE, and exhibited 20°C higher thermostability with no change in enzymatic properties or in the active-site configuration as determined by crystallography[2,3].

Keywords
acetylcholinesterase; improved stability; improved expression; Proteopedia; 3D structure

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S02-2 Coupling of Acetylcholinesterase to the Interfacial Phase State

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The state of the lipid interface is known to influence activity of membrane-bound enzymes. Indeed, many enzymes exhibit changes in activity at phase transitions in the membrane to which they are attached. We utilized a Langmuir trough in which detergent-soluble *Torpedo californica* acetylcholinesterase (DS-*Tc*AChE)[1] was anchored to the solvent face of a phospholipid monolayer in order to study this phenomenon. A peak in activity was observed at the compressibility maximum accompanying the transition between the ordered and fluid phases. Neither molecular nor physical alterations affected this correlation qualitatively, as shown by varying lipid type, pH over 2 units, temperature over 20°C, and lateral pressure over 10 mN/m. Thus the only consistent correlation is between the thermodynamic state of the interface and the measured activity. Our data are consistent with a theory in which the interface state and its corresponding fluctuations control catalytic activity[2]. It was earlier demonstrated that pH-pulses initiated by local acidification of the monolayer propagate, in analogy to sound, at velocities up to 1.4 m/s[3]. We have now shown that such a pulse, by transiently modifying compressibility, can concomitantly and reversibly enhance the activity of DS-*Tc*AChE attached to the monolayer. Our data demonstrate a feasible mechanism for signaling between widely separated biological entities that differs fundamentally from the molecular mechanisms currently accepted, and is also very much faster.

Keywords
acetylcholinesterase; Langmuir trough; monolayer; compressibility; acoustic propagation

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S02-3 The other side of AChE: Allosteric sites and modulators

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The best-known function of acetylcholinesterase (AChE) is the hydrolysis of the neurotransmitter acetylcholine, however we are increasingly aware of the multifunctionality of this enzyme [1]. The non-hydrolytic functions of AChE are driven by allosteric sites as the peripheral allosteric site (PAS) responsible for amyloidosis in Alzheimer's disease through interaction with β-amyloid peptide.

We would like to show our work about the identification and characterization of new allosteric sites in AChE, using computational tools. This study has allowed us to identify allosteric inhibitors by virtual screening using our in-house MBC chemical library [2] guided by structure-based and fragment hotspot strategies. The identified compounds were also screened for *in vitro* inhibition of AChE and three of them were observed to be active. Further experimental (kinetic) and computational (molecular dynamics) studies have been performed to verify the allosteric activity. Thus, new compounds have been developed as allosteric modulators that may be valuable pharmacological tools in the study of non-cholinergic functions of AChE.

Keywords
AChE, allosteric sites, Alzheimer's disease, molecular dynamics, allosteric inhibitor

Acknowledgement
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S02-4 Partial unfolding of insect acetylcholinesterase: Steps toward cysteine-targeting insecticides

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To obtain insight into the development of thermally stable insect acetylcholinesterases, 200 distinct, independent, unrestricted, unbiased, isobaric–isothermal, 316-ns molecular dynamics simulations of a substrate-bound mosquito acetylcholinesterase responsible for cholinergic functions (AP-agAChE)[1] were performed using forcefield FF12MC[2] and PMEMD of AMBER 11 with a periodic boundary condition at 1 atm and 340 K. In-depth conformational analysis of these simulations with an aggregated simulation time of 63.2 microseconds revealed partially unfolded regions of AP-agAChE that could be stabilized with mutations for developing thermally stable AP-agAChE variants and thereby enabling rigorous characterization[3] of cysteine-targeting anticholinesterases as potential insecticides that are effective and environmentally safe and also spare beneficial insects[1].

Keywords
insect acetylcholinesterase, protein unfolding, protein engineering, anticholinesterase, and cysteine-targeting insecticide,

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S02-5 The protonation state of Glu197 and its important role in stabilizing catalytic triad of butyrylcholinesterase

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The Glu197 of butyrylcholinesterase (BChE) has been long considered as deprotonated in various studies, e.g. discovering the dynamical characters, interpreting the binding properties of inhibitors, and proposing hypotheses for BChE-catalyzed reaction mechanism. By performing a series of 100 ns molecular dynamics simulations, we accidently discovered that Glu197 needed to be protonated to have the structures simulated appropriately, whereas the deprotonated Glu197 eventually caused the collapse of catalytic triad with long enough simulation time.[1] we found that a highly conserved water molecule required Glu197 to be protonated in order to form an important hydrogen bond network, which supported His438 to be preserved within the catalytic triad. Interestingly, catalytic triad and Glu197 have been long recognized for possibly deviating largely from their crystal structure positions, which could be catalytic deficient and is generally considered as the result from difference between crystal and aqueous

environment. Here, our results suggest that the large deviations of catalytic triad and Glu197 from crystal structure are caused by inappropriate protonation state of Glu197. This finding of the unexpected protonation state of Glu197 shall provide an important clue that has been long missing for the better understanding of BChE related puzzles or even reconsideration of some BChE-catalyzed reaction mechanisms.

Keywords
protonation state; Glu197; butyrylcholinesterase; catalytic triad

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S02-6 Recipes to design specific ligands of human butyrylcholinesterase

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Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) hydrolyze the neurotransmitter acetylcholine and function thereby as regulators of cholinergic neurotransmission. Recently, interest has greatly increased in BChE. Firstly, BChE is a good broad spectrum bioscavenger of nerve agent and its efficiency could be significantly increased by the mean of specific reactivators. Secondly, BChE activity in the brain increases with the progression of Alzheimer's disease, thus classifying BChE as a promising drug target in the advanced phase of the disease. AChE and BChE display specificities for substrates and ligands that only partially overlap. This disparity is largely due to differences in the number of aromatic residues lining the active site gorge, which leads to large differences in the shape of the gorge and potentially to distinct interactions with an individual ligand. Considerable structural information is available for the binding of a wide diversity of ligands to AChE. In contrast, structural data on the binding of reversible ligands to BChE was lacking. In the recent years, we solved the X-ray structures of multiple BChE-ligand complexes. Here we will present BChE structures with various ligands, some recently synthesized, to highlight the structural elements leading to their BChE affinity and specificity. These structural data will help to design specific reversible ligands that behave as inhibitors or reactivators.

S02-7 Exploring the evolutionary potential of the αE7 carboxylesterase

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The evolution of insecticide resistance is a model system for studying enzyme evolution. Three insect species have independently evolved catalytic organophosphate (OP) detoxification through a single active-site mutation in the αE7 carboxylesterase. To explore the evolutionary potential of αE7, we subjected αE7 from the sheep blowfly to nine rounds of mutation and screening. The final variant contained 11 mutations which increased the rate of OP-hydrolysis more than 1000-fold. Atomic resolution X-ray crystal structures of the evolutionary intermediates reveal the changes in structure and dynamics at each step in the evolutionary trajectory, and hint at the molecular basis for the increased rate of OP hydrolysis. This work explores the potential for the development of αE7 as an enzyme therapeutic for OP poisoning, and worryingly for insecticide resistance, this work suggests that more efficient OP detoxification could be readily acquired by insect pests.

S02-8 Protein dynamics of phosphotriesterase: Two cations required for enzyme catalysis

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To investigate how protein dynamics facilitates substrate entering and product exiting the phosphotriesterase active site, over 60 distinct, independent, unrestricted, unbiased, isobaric–isothermal, microsecond molecular dynamics simulations of zinc-containing phosphotriesterase in complex with a substrate analog[1] were performed using the second-generation cationic dummy atom model for the zinc divalent cation, forcefield FF12MC[2], and PMEMD of AMBER 16 with a periodic boundary condition at 1 atm and 277 K, 300 K, and 340 K. In-depth conformational analysis of these simulations with an aggregated simulation time of over 76 microseconds revealed atomic and dynamic details on the phosphtriesterase catalysis and its requirement of two cations, which offers insight into re-engineering of phosphotriesterase to develop an improved scavenger against phosphorous-containing inhibitors of acetylcholinesterase.

Keywords
phosphotriesterase, protein dynamics, zinc, scavenger, and protein engineering

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S03 Interaction of cholinesterases with substrates, inhibitors and reactivators

Chairs: Terrone Rosenberry (USA), Franz Worek (Germany)

S03-1 New Progress in Drug Design, Discovery and Development Involving Cholinesterases

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This talk will briefly discuss our newest progress in drug design, discovery and development involving cholinesterases, particularly in three major therapeutic areas.

(1) On the basis of our previous design and discovery of cocaine hydrolases (CocHs) engineered from human butyrylcholinesterase (BChE), we have further developed a novel, long-acting CocH form, and demonstrated the promising clinical potential of CocHs for therapeutic treatment of cocaine overdose and addiction in clinically relevant animal models. One of the long-acting CocHs is currently in the large-scale protein drug manufacturing process development.

(2) It has been demonstrated that a long-acting CocH (enzyme) is capable of both completely blocking cocaine-induced physiological effects and producing the desirable anti-obesity effects. Mice on a high-fat diet gained significantly less body weight when treated weekly with 1 mg/kg enzyme compared to control mice.

(3) Most recently, we have also designed and tested a new therapeutic strategy for heroin detoxification based on a detailed analysis of the cholinesterases-involved chemical transformation and functional change of heroin in the body. It has been demonstrated in our animal models that a carefully selected cholinesterase inhibitor attenuated acute toxicity and physiological effects of heroin, whereas some other cholinesterase inhibitors may actually enhance the acute toxicity and physiological effects of heroin.

S03-2 In searching for the mechanism of butyrylcholinesterase activators

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It is known that cholinesterases show homotropic pseudocooperative effects: their activity at millimolar substrate concentrations is higher than expected by simple saturation kinetics and they are strongly inhibited at the submolar concentrations. However, we have reported that the anionic site directed inhibitors tetramethylammonium and tetraethylamonium too, increase the activity of human butyrylcholinesterase. At that time, the same phenomenon could not be shown for the horse counterpart. Here, it was searched for other putative activators among often used compounds in cholinesterase research. Indeed, imidazole significantly increase the activity of human enzyme, but also its atypical form and the horse enzyme. On the other hand, 2-PAM shows a certain degree of activation with both human enzymes, but inhibits the horse BChE in a classical competitive manner. To avoid substrate activation, the experiments were performed at around 50 micromolar starting substrate concentrations and were followed by its completion in the presence of different modulator(s) concentrations. Subsequently, the effect of 2-PAM on the phosphorylation by DFP was studied, since the bottom of the active site does not differ in these three enzymes. It seems that the distinctive action of activating agents on the wild type, the atypical human and horse BChE is a consequence of differences in the dynamics of the acylation loop at the active site entrance, rather then the composition of the enzyme's peripheral anionic site.

Keywords
reaction mechanism, butyrylcholinesterase activation, kinetics

S03-3 Computational analysis of reaction mechanisms for optimization of butyrylcholinesterase-based catalytic bioscavengers against organophosphorus agents

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Catalytic bioscavengers are second generation bioscavengers. These biopharmaceuticals can be used to degrade toxic organophosphorus agents (OPs) on the skin for decontamination or in the bloodstream for pre-treatment and post-exposure treatment of OP poisoning. Because degradation has to be fast, their catalytic efficiency has be as high as possible (k_{cat}/K_m>10^6 $M^{-1}min^{-1}$). To be of interest, the catalytic activity of certain enzymes, in particular self-reactivating ChEs, has to be increased by several orders of magnitude. This can be reached by computer-redesign, directed evolution of existing enzymes, and combinational strategies.

Rational design of novel ChE-based catalytic bioscavengers requires a better understanding of chemical mechanisms of inhibition, aging of conjugate, and spontaneous reactivation. Kinetic studies, X-ray crystallography and molecular modeling, in particular QM/MM calculations, present valuable insights into specific reaction routes, role of specific amino acids and obstacles against effective reactivation of phosphylated ChEs.

Introducing new functional groups surrounding the phosphylated serine should create a stable H-bonded network susceptible to activate and orient water molecule, stabilize transition states, and intermediates. Direction of nucleophilic attack of water molecule on phosphorus atom may determine whether dephosphylation is favored over aging. Mutations of key residues surrounding human BChE active site, creating new reaction pathways, have been considered. QM/MM calculations suggest that introduction of a histidine, directing attack of water molecule from apical position competes with the aging reaction, while axial direction of water attack does not. Secondary mutations for stabilizing imidazolium upon activation of water molecule lead to lower energy barrier of reactivation reaction [1].

Keywords
catalytic bioscavengers, organophosphorus compound, butyrylcholinesterase, reaction mechanism

Acknowledgement
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S03-4 Enhancement in pyridinium oxime-assisted reactivation of tabun-inhibited acetylcholinesterase achieved by active site mutations

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Tabun represents a phosphoramide class of organophosphophosphates that are covalent inhibitors of acetylcholinesterase (AChE), an essential enzyme in neurotransmission. The currently used therapy in excessive cholinergic stimulation consists of the muscarinic antagonist of acetylcholine stimulation, an anti-seizure drug when indicated and an oxime as the reactivator of inhibited AChE. Since common oximes are particularly ineffective in tabun exposure, we probed the reactivation of phosphoramidate conjugates in more depth by using mutants of AChE and pyridinium oximes to reveal the structural subtleties and yield more information on the architecture of the active centre gorge needed for the reactivation of phosphoramidate agents used in terrorism and as pesticides. Our results indicated that the replacement of aromatic residues with aliphatic ones at the acyl pocket and choline binding site mostly interfered with the stabilization of the oxime's pyridinium ring(s) in the proper orientation of the oxime group toward the phosphorylated active site serine. The peripheral binding site mutation resulted in a 2-5 fold increase in the reactivation rates by bis-pyridinium oximes when compared to the AChE wild type. In the case of mono-pyridinium oximes, we reported a 150-fold enhancement of the maximal reactivation rate for the choline binding site mutation, while the molecular recognition seemed to remain preserved. Therefore, our results emphasized the positive effect of several mutations on oxime embedding and orientation into a position for productive interactions with the tabun-phosphorylated active site serine indicating a future potential for further development of pseudo-catalytic bioscavengers based on AChE mutants.

Keywords
nerve agents, antidotes, 2-PAM, HI-6

Acknowledgment
This work was supported by the Croatian Science Foundation (no. 4307).

S03-5 The search for resistance-breaking and species-selective mosquitocidal inhibitors of *Anopheles gambiae* AChE

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The widespread deployment of insecticide-treated bednets (ITNs) in sub-Saharan Africa has led to a dramatic decline in malaria mortality. However, wide-spread and growing resistance of *Anopheles gambiae* mosquitoes to the pyrethroid class of voltage-gated Na^+ channel modulators used on these nets jeopardizes this achievement, and has prompted the search for suitable insecticidal AChE inhibitors to replace pyrethroids. Such compounds would have three favorable characteristics: excellent contact toxicity towards susceptible adult *An. gambiae*, good contact toxicity to those that bear the G119S resistance mutation of AChE, and very weak inhibition of human AChE.[1] We will review our work on the development of aromatic and heterocyclic core methyl and dimethylcarbamate AChE inhibitors,[2] and including both enzymatic inhibition potencies and mosquito contact toxicities. Finally, the inhibition selectivities of particular compounds will be rationalized in the context of our recently obtained high resolution X-ray structures of G119S *An. gambiae* AChE.[3]

Keywords
mosquito, malaria, carbamate, resistance, G119S

Acknowledgement
We thank the NIH (AI082581) for financial support.

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S03-6 Steric Effects in the Decarbamoylation of Carbamoylated Acetylcholinesterase

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Carbamates are esters of substituted carbamic acids that react with acetylcholinesterase (AChE) in a two-step process, with initial transfer of the carbamoyl acyl group to a serine residue of AChE accompanied by loss of the carbamate leaving group followed by hydrolysis of the carbamoyl enzyme. This hydrolysis, or decarbamoylation, is relatively slow, and half-lives of carbamoylated AChEs range from 4 min to more than 30 days. Since carbamates are poor, slowly reversible AChE substrates, they are effective AChE inhibitors that have been developed as insecticides and therapeutic agents. We show that decarbamoylation rates are independent of the leaving group for a series of carbamates with the same carbamoyl group. For a given leaving group, when the alkyl substituents on the carbamoyl group increased in size from *N*-monomethyl- to *N*,*N*-dimethyl-, *N*-ethyl-*N*-methyl-, or *N*,*N*-diethyl-, the decarbamoylation rates decreased by 4-, 70-, and 1000-fold, respectively. Thus the larger the size of the alkyl groups, the slower the rate of decarbamoylation due to active site distortion. Furthermore, solvent deuterium oxide isotope effects for decarbamoylation decreased from 2.8 for *N*-monomethylcarbamoyl AChE to 1.3 for *N*,*N*-diethylcarbamoyl AChE, indicating a shift in the rate-limiting step from general acid-base catalysis to a likely conformational change.

Keywords
Acetylcholinesterase, Decarbamoylation, *N,N*-diethyl carbamates

S03-7 Assessment of scoring functions for AChE-ligand interactions

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Computer-aided drug design is based on molecular modelling which includes two steps; molecular docking accompanied by scoring docked poses. Molecular docking fits the right molecular "key" to a known receptor "lock" by optimizing the atomic coordinates of a ligand to adapt its 3D structure in such a way to accommodate the binding into the receptor. The second step is the determination of a good fit between the ligand "key" and receptor "lock" using a function that correctly prioritizes the docked ligand poses and predicts their binding affinities by taking into account molecular interactions between the ligand, protein and solvent.

The 68 crystal structures of complexes between acetylcholinesterase (AChE, EC 3.1.1.7) and its ligands, deposited in PDB, were analysed by scoring the functions: LigScore1, LigScore2, PLP1, PLP2, Jain, PMF and PMF04. The scores derived from scoring functions were correlated with an inhibition constant for each ligand (Ki or IC_{50}) in a broad range 10^{-3} – 10^{-12} M. Scores were also correlated with other computational properties as the number of rotational bonds, number of H-bond donor or acceptor atoms, molecular complexity index and topological polar surface area. The linear correlation between the scores derived from the scoring function and matching pKi data resulted in the highest r value for the PLP2 function, r = 0.77, with 10% of the slope error. The LigScore1function resulted in the lowest r value of 0.47 with 23% of the slope error. The PLP2 scoring function is a good candidate in drug discovery related to AChE, although with a higher number of crystal structures of AChE complexes and reliable kinetic data, a better scoring function could be developed.

Keywords
AChE, drug discovery, scoring function, inhibition constant

S03-8 Phenyl valerate esterase activity of human cholinesterases

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The toxicity of organophosphorus compounds (OPs) cannot be explained only by action on acetylcholinesterase or neuropathy target esterase (NTE). A fraction of the membrane bound phenylvalerate esterase activity (PVase) is associated to NTE, the key initiating molecular event in the OP-induced delayed neuropathy (OPIDN). An enzymatic fraction in chicken brain soluble PVase has been reported to be due to a butyrylcholinesterase protein, and we suggested that this enzymatic fraction could be related to the mode of action of the potentiation/promotion phenomenon of the OPIDN. We showed that human butyrylcholinesterase (hBuChE) shows PVase activity. Mipafox, iso-OMPA or PMSF inhibited both activities with similar kinetic constants for both activi-

ties. Moreover, the substrates acethylthiocholine and phenyl valerate showed competition in their activities. The results suggest that both activities are related to the same active center.

This work studies in depth the kinetic interactions between phenyl valerate and acetylthiocholine in human butyrylcholinesterase, showing that the interactions are different to the competitive model of substrates according to the Michaelis-Menten reaction. The approach introduced in this work suggests that other site could be involved in the interaction with phenyl valerate.

In addition, we have observed that human acetylcholinesterase has also phenyl valerate esterase activity, but with lower activity than human butyrylcholinesterase. The level of phenylvalerate esterase activity in cholinesterases depends on the species and the type of cholinesterase. Further evaluation of the molecular interactions is under study.

S03-9 Effects of memantine and its metabolite Mrz 2/373 on soman-induced inhibition of bovine erythrocyte acetylcholinesterase *in vitro*

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Background: Memantine is the non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, used in the treatment of Alzheimer's disease. Memantine pretreatment assured protection of skeletal muscles from poisoning with nerve agents and an interaction between memantine and AChE was proposed [1].

Aim: Memantine and its main metabolite (1-amino-3-hydroxymethyl-5-methyl adamantine, Mrz 2/373) were used to ascertain their interaction with erythrocyte acetylcholinesterase (AChE) *in vitro*. The effect of these two compounds on the kinetics of the soman-induced AChE inhibition and on the aging of the soman-AChE complex was also investigated.

Methods: Bovine AChE activity was measured titrimetrically and the effect on aging of the soman-AChE complex was studied [2].

Results: Memantine and Mrz 2/373 exerted concentration-dependent inhibition of AChE, with Mrz 2/373 being a more potent inhibitor than the parent compound.

Addition of soman $2.5x10^{-8}$ mol/l induced gradual AChE inhibition that became almost 100% after 20 min. Memantine (0.1, 0.5 and 1 mmol/l) and Mrz 2/373 (0.1 and 1 mmol/l) concentration-dependently slowed down the AChE inhibition.

Neither memantine nor Mrz 2/373 prevented the aging of the soman-AChE complex. After 5 min incubation with AChE and soman, AChE activity was 11%, 36% and 30% in control medium and after adding of 1 mmol/l of memantine and Mrz 2/373, respectively.

Conclusion: Since high micromolar and low millimolar concentrations of memantine can be achieved in rats [3], it is quite possible that memantine and Mrz 2/373 can prevent AChE from inhibition by soman, which could, along with known memantine's neuro-protective activity, explain its potent antidotal effect in soman poisoning.

Keywords

acetylcholinesterase, memantine, Mrz 2/373, soman, pretreatment

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S04 Reactivators of AChE, OP inhibitors - mechanism of toxicity, detection and analytical methods, diagnosis of exposure, detoxification and therapy; counter-terrorism strategies

Chairs: Franz Worek (Germany), Terrone Rosenberry (USA)

S04-1 Oximes with ortho-positioned chlorine moiety exhibit improved physical-chemical properties, efficient reactivation of inhibited human acetylcholinesterase and reduced *in vivo* toxicity

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The series of bisquaternary oximes with ortho-positioned chlorine moiety was designed, prepared and evaluated. The novel compounds exhibited valuable pK_a properties [1] with improved *in vitro* reactivation ability of sarin, cyclosarin, VX, paraoxon- and dichlorvos-inhibited human AChE exceeding the standard monoquaternary or bisquaternary reactivators (pralidoxime, methoxime, trimedoxime, obidoxime and asoxime syn. HI-6). Additionally, some chlorinated compounds presented *in vitro* reactivation ability of tabun-inhibited human AChE similar to the efficiency of trimedoxime. The *in vitro* results were further explained by molecular docking study. The *in vitro* non-cytotoxic properties of novel compounds were determined with miscellaneous results. However, assessment of maximum tolerated dose highlighted that the selected chlorinated reactivator is well tolerated by mice on the level similar to the clinically or experimentally used oxime reactivators [2]. The *in vivo* reactivation study is in progress.

Keywords
organophosphate, antidote, oxime, chlorinated oxime, pK_a

Acknowledgment
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S04-2 Design and synthesis of bifunctional fluoropyridinaldoxime reactivators for nerve agent-inhibited *human* acetylcholinesterase

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Acetylcholinesterase (AChE) is a key enzyme of the Central Nervous System (CNS), which hydrolyzes the neurotransmitter acetylcholine.[1] By targeting AChE, organophosphorus nerve agents (OPNA) and organophosphorus pesticides irreversibly inhibit the cholinergic transmission, which is leading to death if untreated.[2] Over several years, our group and colleagues have been concentrating on the development a new class of non-permanently charged bifunctional reactivators, that display higher affinity for AChE and high *in vitro* and *in vivo* efficiencies compared to 2-PAM and Hi6.[3] By analogy, recently, we designed bifunctional reactivators that comprise a peripheral site ligand (PSL) connected to a fluorinated reactivator function using a covalent linker. On the basis of our previous work on the synthesis of central hybrid reactivators bearing 6-alkanyl-3-hydroxy-2-pyridinadoxime moiety, and with the goal to develop reactivator with greater lipophilicity and enhanced blood brain barrier (BBB) permeability, we decided to substitute the 3-hydroxy group, initially designed to decrease the oxime pka, with a more electronegative and electron-withdrawing group such as fluorine. Fluorine is known to modulate the pka of the proximal oxime, the conformational bias and the binding properties via molecular interactions. This structural change, compared to the known 6-substituted 3-hydroxy-2-pyridinadoxime scaffold, appeared valuable for both practical and fundamental reasons, eventually providing reactivators with increased reactivation potency and better pharmacological profiles.

Keywords
Acetylcholinesterase (AChE), Central Nervous System (CNS), Organophosph-orus nerve agents (OPNA), 2-Pyridine Aldoxime Methyl Chloride (2-PAM) and Bifunctional reactivators.

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S04-3 Combination of oximes with overlapping reactivation spectra: obidoxime and HI-6

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Despite extensive oxime research in the last 60 years pralidoxime is still the standard oxime in e.g. United States, British and French forces and obidoxime standard therapy for OP poisoning in several European countries. Oxime research focusses on highly potent oximes with activity against selected nerve agents, broad-spectrum oximes with activity against relevant nerve agents and centrally active (non-)oximes but virtually no compound brought significant improvements compared to the established obidoxime and pralidoxime. In the US MMB-4 is sought to replace pralidoxime and in Germany, France, UK, Canada and other European countries HI-6 is in advanced development for use as nerve agent antidote. Yet, both compounds are not considered as broad-spectrum antidotes and as a mid-term solution combinations of oximes in service with overlapping reactivation potency e.g. obidoxime and HI-6 have been proposed. We here set out to analyze the combination of obidoxime and HI-6 in both a static and dynamic model against poisoning with nerve agents and organophosphorus compound pesticides in vitro. In a cuvette based system the combination of HI-6 and obidoxime both 30 µM for sarin-, cyclosarin-, tabun-, VX- and paraoxon-inhibited human AChE did not result in an impaired reactivation compared to the sole use of both oximes but in a broadened spectrum. Similar results were gained with a dynamic model allowing simulation of nerve agent and pesticide toxicokinetics and oxime pharmacokinetics resembling in vivo conditions. Additional experiments in species closely related to humans e.g. swine are necessary to analyse a potential benefit in vivo.

Keywords
HI-6, obidoxime, reactivation, nerve agents, pesticides

S04-4 Design of broad spectrum antidotes

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The design of reactive molecules such as nerve agent antidotes is inherently challenging due to two intertwined processes imperative for their efficiency: The reversible binding of the initial non-covalent complex in a low energy conformation and the chemical reaction that proceeds *via* a transition state of high(er) energy. Furthermore, a structural and chemical diversity among different nerve agents and their corresponding complex with AChE complicates the design of broad-spectrum antidotes. The development of broad spectrum antidotes has proven challenging and although progress has been made, no new drugs with improved properties have been launched in several decades. Herein, we report a rational, structure-based approach for the development of broad-spectrum antidotes. Based on a hit molecule identified in a high throughput screening targeting the non-inhibited species of AChE, 18 new analogous molecules were designed and synthesized. This resulted in a set of compounds with a diversity in their potency, as desired for subsequent (quantitative) structure-activity relationship ((Q)SAR) modeling. The 18 compounds were investigated for their ability to bind to four different phosphonylated forms of AChE (*i.e.* human AChE inhibited by the nerve agents VX, VR, and tabun, or the substance DFP). The QSAR model was subsequently used to guide the development of a novel set of pyridinium-oxime based broad spectrum antidotes. The mechanism of reactivation of the developed antidotes has been investigated using a combination of X-ray crystallography and molecular modelling.

S04-5 Demonstration of the First Small Molecule Therapeutics for Resurrection of the Aged form of Acetylcholinesterase after Exposure to Organophosphorus Chemical Nerve Agents and Pesticides

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Organophosphorus (OP) compounds are potent acetylcholinesterase (AChE) inhibitors that have found use as both chemical warfare agents (CWAs) and as pesticides. Following inhibition of AChE by OP compounds, a competitive dealkylation reaction of the phosphylated serine residue occurs – a process referred to as aging. Current therapeutic reactivators of OP-inhibited AChE, mainly oximes, are not effective once aging has occurred. For the first time, we have demonstrated *in vitro* conversion of the aged AChE to the native form using small drug-like molecular therapeutics. As part of this effort, a diverse library of small molecule therapeutics have been developed to both recover the activity of aged-AChE, termed resurrection, as well as the activity of inhibited-AChE, referred to as reactivation. The structure of such therapeutics is derived from pyridyl-based quinone methide precursors (QMPs), sharing structural similarities to known therapeutic oximes. A structure-activity relationship study of synthesized QMP therapeutics was conducted to determine the effect electron-donating and electron-withdrawing groups have on the efficiency of both processes and to design optimized small molecule therapeutics for *in vivo* biological efficacy. Our successes will be presented.

S04-6 Nanotechnology strategies using oximes-loaded lipid nanoparticles for brain protection against organophosphorus poisoning

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Nanotechnological „two-in-one" approach using nanoparticles for packaging two oximes in single carriers and nose-to-brain delivery for brain protection against poisoning by organophosphorus agents have been developed. Strategies for designing nanocarriers for drug delivery to the CNS and crossing the BBB showed that nanoparticles based on natural and biodegradable materials are promising. Solid lipid nanoparticles (SLNs) are biocompatible, biodegradable and have very low toxicity, thereby fulfilling the requirements of preclinical safety [1]. 2-PAM and a novel reactivator of VX-, paraoxon-, and tabun-phosphylated AChE [2] a poorly water soluble 6-(5-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)pentyl)-3-hydroxypicolinaldehyde oxime (3-HPA), were loaded in SLNs to offer distinct release profile and half-life for both oximes. To increase the therapeutic time window of both oximes, SLNs with two different compartments were designed. Oxime-loaded SLNs of hydrodynamic diameter 100-160 nm and zeta potential (from -30 to -25 mV) were stable for a period of 10 months at 4ºC. SLNs displayed longer circulation time in the bloodstream compared to free 3-HPA and free 2-PAM. Oxime-loaded SLNs were suitable for intravenous administration. Paraoxon-poisoned rats (0.8×LD50) were treated with 5mg/kg of 3-HPA-loaded SLNs and 2-PAM+3-HPA-loaded SLNs. Brain AChE reactivation up to 30% was slowly achieved in 5 h after administration of 3-HPA-SLNs. Synergistic effect and increased reactivation up to 35% was observed with combination of both oximes.

In addition, new cationic liposomes based on L-α-phosphatidylcholine and cationic surfactant were administered *via* the intranasal route. These liposomes were found to reach directly central AChEs. This last approach provides evidence that reactivation of central AChEs can be achieved by a non-invasive approach that bypasses the BBB.

Keywords
Solid-Lipid Nanoparticles, Blood-brain barrier, Acetylcholinesterase, Oxime, Paraoxon.

The work was supported by Russian Science Foundation, grant No. 14-50-00014.

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S04-7 Utilizing Structure-Activity Relationships and Mechanistic Insights to Design Non-Oxime Reactivators

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Organophosphorus nerve agents are highly toxic compounds which pose a threat worldwide. These compounds induce toxicity by covalently binding to the active site serine of acetylcholinesterase, which results in inhibition of the enzyme. Without functional acetylcholinesterase, the levels of the neurotransmitter acetylcholine in neuromuscular junctions rise quickly, causing overstimulation of the nervous system, which will culminate in death if not treated. Current treatments rely on small molecules to interact with inhibited enzyme to disrupt the covalently bound phosphorus moiety at the active site. The most effective molecules incorporate a pyridinium oxime which acts via direct nucleophilic attack on the phosphorus to achieve reactivation of the enzyme. These compounds have limited effectiveness because the charged portion of the molecule does not allow them to cross into the central nervous system where acetylcholinesterase inhibition is most harmful. The results of studies that characterized a small molecule reactivator (4-amino-2-((diethylamino)methyl)phenol [ADOC]) that does not incorporate an oxime but is capable of reactivating nerve agent-inhibited enzyme as well as or better than current treatments have been used to inform the design of additional novel compounds. This study describes the *in vitro* characterization of these novel compounds as reactivators of phosphonylated human acetylcholinesterase.

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This research was supported by the Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S&T Division.

S04-8 New Non-Oxime Reactivators of Organophosphate Inhibited Acetylcholinesterase with Promising Reactivation Potency

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Organophosphate (OP) compounds inhibit the enzyme acetylcholinesterase (AChE) resulting in severe symptoms and ultimately death. OP intoxications are currently treated by administration of atropine and certain oxime compounds (Obidoxime, HI-6 or 2-PAM). The latter compounds contain nucleophilic oximes that reactivate OP-inhibited AChE by liberating the phosphylated serine. However, these oximes have several drawbacks such as their intrinsic toxicity, their permanent charge which thwarts penetration of brain tissues and their inability to effectively reactivate all types of nerve agent inhibited AChEs. Therefore, the search for new (non-ionic) antidotes of nerve agent poisoning is of great importance. Recently, several papers reported on the discovery of non-oxime compounds as a result of the in vitro or in silico screening of libraries of bioactive compounds and approved drugs. For instance, Katz et al reported[1] a novel class of compounds in which the 4-amino-2-(diethylamino)phenol (ADOC) appeared to be a key motif responsible for reactivation of OP-inhibited AChE.[2] In addition, several structural derivatives of ADOC were synthesized and evaluated for OP-AChE reactivation by Cadieux et al.[3] That study provided valuable information on key structural features of ADOC with respect to reactivation potency and enzyme inhibition, but unfortunately, none of the reported derivatives performed equal or better than the ADOC parent. We here report the design and synthesis of a new series of ADOC derivatives. We report that one of the compounds synthesized so far showed a remarkably improved in vitro performance compared to ADOC towards VX-, sarin-, cyclosarin- and paraoxon-inhibited human AChE.

Keywords
reactivator, non-oxime, acetylcholinesterase

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S05 Enzymes and proteins other than ChEs interacting with OP

Chairs: Oksana Lockridge (USA), Patrick Masson (Russia)

S05-1 Identifying axonal transport-related targets for reversing the adverse effects of organophosphate exposure

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The chemicals known as the organophosphates (OPs) are found in hundreds of useful agricultural, industrial, and commercial products; however, they have also been associated with a variety of adverse health effects in humans and other non-target organisms. The acute toxicity of OPs is attributed to the inhibition of the enzyme acetylcholinesterase; however, this mechanism is inadequate to explain all of the long-term adverse effects of OPs. In both live imaging studies in primary neuronal culture as well as in manganese-enhanced magnetic resonance imaging (MEMRI) studies of the brains of living rats, we have observed impairments in axonal transport (AXT) associated with both the insecticide OP chlorpyrifos and the nerve agent OP diisopropyl-fluorophosphate. These observations may be important since AXT is an essential process that is responsible for the movement of a variety of important macromolecules to and from a neuron's cell body. In this presentation, a brief overview of the results of these neuronal culture (trafficking) and MEMRI experiments will be provided. In addition, the results of experiments conducted to date to identify specific molecular targets of OPs that might negatively influence axonal transport will be summarized. These targets include post-translational modifications of structural proteins that affect AXT through the regulation of microtubule dynamics and stability (e.g., Tau phosphorylation, Tubulin Acetylation), and specific signaling kinases (e.g., ERK GSKIIIβ) that are known to regulate various components of the AXT process. These experiments are expected to help us begin to develop novel therapeutic strategies to improve the neuronal deficits associated with OPs.

Keywords
Pesticide, Nerve Agent, Agriculture, Gulf War Illness

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S05-2 Diagnosis of Poisoning with *O*-Isobutyl-*S*-[2-(diethylamino)ethyl]methylphosphonothioate (VR) under Antidotal Therapy with Carboxim

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The choice of biomarkers for establishment of exposure to organophosphorus compounds (OPs) is made based on the results of assessment of the real situation with account for such factors as the required timeframe for providing the results of expert examination, nature and volume of biosamples, available equipment, and the degree of confidence of information on the influencing factor (substance, dose, way of entry, use of antidote).

We estimated the efficiency of express methods of diagnosis of exposure to OPs, specifically, Ellman's cholinesterase activity assay, as well as GC-MS/MS and HPLC/MS/MS determination of OPs fluoride-regenerated from protein adducts and low-molecular hydrolytic metabolites of OPs, respectively. The objects of study were blood and urine samples of rats exposed to VR in a dose of 2×0.4LD50 under conditions of antidotal therapy with Carboxim {5-[[[2-[benzyl(diemthyl)ammonio]ethyl]amino]carbonyl]-2-[(hydroxyimino)methyl]-1-methylpy-ridinium dichloride}.

Carboxim therapy led to AChE reactivation 3 h after exposure to VR, while in the absence of the therapy the AChE activity recovered within 3 days.

Fluoride regeneration of VR from its blood plasma protein adducts was possible within 7 days after poisoning irrespective of whether the therapy was applied or not.

O-isobutyl methylphosphonate was detected in urine 24 h after exposure in the urine samples of animals both subjected and not subjected to antidotal therapy, whereas after 3 days it was detected exclusively in the urine samples of animals not given the antidote.

It was also found that blood plasma levels of free and esterified fatty acids can serve as an additional toxicodynamic parameter of VR poisoning.

Keywords
nerve agents; VR; markers; Carboxim; antidotal therapy

S05-3 Copper-dependent hydrolysis of trichloronate by turkey serum and albumin

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Trichloronate is a racemic organophosphatioate insecticide. It induced delayed neuropathic in hens and human. The avian are species with greater susceptibility to organophosphorus poisoning due to their low levels of A-esterases. However, a copper-dependent hydrolyzing activity of hexyl dichlorophenyl phosphoramidate (HDCP), known as "antogonistic stereoselectivity" was recently identified in chicken serum. This study shows the activating effect of copper on the hydrolysis of trichloronate enantiomers by turkey serum and albumin (TSA) using chiral chromatography with CHIRALCEL OD column and heptane HPLC as mobile phase. The trichloronate hydrolysis levels (µM remaining concentration of each isomer) quantified at 37 °C, pH 7.4 and 60 minutes of turkey serum (10 µL) incubated with 300 µM of copper were statistically higher $p<0.05$) for (-)-trichloronate (65 %) than (+)-trichloronate (32%). This estereoselective hydrolysis observed in turkey serum was confirmed by the incubation of 200 µg of turkey serum albumin (amount of this protein estimated in the 10 mL of turkey serum) with 400 µM of racemic trichloronate and 300 µM of copper at physiological condition during 60 minutes; hydrolysis values of 90% and 72% were obtained for (-)-trichloronate and (+)-tricholoronate. In conclusion, the present study evidences the hydrolysis of an organophosphatioate racemic for an A-esterase activity in turkey serum and identifies albumin as the cuproprotein responsible of this Cu^{2+}-dependent stereoselective hydrolysis of this chiral insecticide in the turkey serum.

Keywords
trichloronate, chiral organophosphatioate, hydrolysis, turkey, albumin, serum

S05-4 Mass Spectral Detection of Diethoxyphospho-Tyrosine Adducts on Proteins from HEK293 Cells Using Monoclonal Antibody depY for Enrichment

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Chronic illness from exposure to organophosphorus toxicants is hypothesized to involve modification of unknown proteins. Tyrosine readily reacts with organophosphorus toxicants in proteins that have no active site serine. We developed a monoclonal antibody, depY, that specifically recognizes diethoxyphospho-tyrosine in proteins and peptides, independent of the surrounding amino acid sequence[1]. Our goal was to identify diethoxyphosphorylated proteins in human HEK293 cell lysate treated with chlorpyrifos oxon. Cell lysates treated with chlorpyrifos oxon were examined by ELISA and capillary electrophoresis Western blot. Tryptic peptides were analyzed by liquid chromatography-tandem mass spectrometry. The depY antibody recognized diethoxyphospho-tyrosine containing proteins by ELISA and Western blotting. Mass spectrometry identified 40 diethoxyphospho-tyrosine peptides from 24 proteins in immunopurified samples, but found only 9 diethoxyphospho-tyrosine peptides from 6 proteins when the same sample was not immunopurified on depY. The most abundant proteins in the cell lysate, Histone H4, Heat shock 70 kDa protein 1A/1B, Heat shock protein HSP 90 beta, and Alpha-enolase, were represented by several diethoxyphospho-tyrosine peptides. It was concluded that use of immobilized depY improved the number of diethoxyphospho-tyrosine peptides identified in a complex mixture. The mass spectrometry results confirmed the specificity of depY for diethoxyphospho-tyrosine peptides independent of the context of the modified tyrosine, which means depY could be used to analyze modified proteins in any species.

Keywords
chlorpyrifos oxon, diethoxyphospho-tyrosine antibody, mass spectrometry, ELISA, Western blot

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S05-5 Innovative Biocatalysts as Tools to Detect and Inactivate Nerve Agents

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Pesticides and warfare nerve agents are frequently organophosphates (OPs) or related compounds. Their acute toxicity highlighted more than ever the need to explore applicable strategies for the sensing, decontamination and/or detoxification of these compounds. Herein, we report the use of two different thermostable enzyme families capable to detect and inactivate OPs. In particular, mutants of carboxylesterase-2 from *Alicyclobacillus acidocaldarius* and of phosphotriesterase-like lactonases from *Sulfolobus solfataricus* and *Sulfolobus acidocaldarius*, have been selected and assembled in an optimized format for the development of an electrochemical biosensor and a decontamination formulation, respectively. The features of the developed tools have been tested in an *ad-hoc* fabricated chamber, to mimic an alarming situation of exposure to a nerve agent. Choosing ethyl-paraoxon as nerve agent simulant, a limit of detection (LOD) of 0.4 nM, after 5 s of exposure time was obtained. Furthermore, an optimized enzymatic formulation was used for a fast and efficient environmental detoxification (>99%) of the nebulized nerve agent simulants in the air and on surfaces. Crucial, large-scale experiments have been possible thanks to production of grams amounts of pure (>90%) enzymes.

S06 Stoichiometric bioscavanger, biotechnology and therapeutical aspects

Chairs: Palmer Taylor (USA)

S06-1 Ionizable, Zwitterionic Oximes as Countermeasures to Volatile Organophosphate (OP) Exposure

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Small ionizable, zwitterionic oximes of limited toxicity show successful outcomes in non-human primates upon intramuscular post-treatment of exposures to OP's, that enter via the respiratory tract. Along with their inherent limitations, we consider the bases for success in post-exposure treatment of OP toxicity and reversal of OP-induced sequelae of symptoms[1]. (1) High vapor pressure OPs carry the largest acute exposure risk in mass terrorism. Toxic OPs released from explosive devices or into controlled ventilation environments are governed by partial pressure and Fick's Second Law of Diffusion (inverse square of the distance); (2) Low molecular weight, zwitterionic oximes confer optimal nucleophile orientation and activity within the confines of the OP -impacted, active center gorge of human acetylcholinesterase (AChE). (3) We emphasize features of ionizable neutral oximes of low toxicity that allow facile passage of membranes to peripheral and central AChE targets and optimal attack angles in the AChE active center. Hence, for volatile OP's, antidotes must rapidly enter the circulation, post-exposure, to chase the offending OP. Following entry, antidotes should then hastily equilibrate between tissue compartments and cross the blood-brain barrier. Accordingly, we examine the ionization states of zwitterionic oximes and other cationic and anionic (F^-) nucleophiles in relation to their kinetic parameters of reactivation[2]. Toxicities, both realized and potential, of nucleophilic antidotes in different ionization states, and pharmacokinetics in mice and macaques, under control and exposure conditions, emerge as critical factors for determining *in vivo* antidote efficacy. Data will be presented on multiple OP's and their enzyme conjugates[1-3], comparator oximes and in three animal species/strains.

Keywords
zwitterionic oximes, reactivation, organophosphate conjugates, CNS permeability, antidotes

Acknowledgement
Collaborators at TSRI, USAMR-ICD, IMROH, & PlantVax are gratefully acknowledged.

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S06-2 Development of pre- and post-countermeasures against OP toxins in macaques

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Deliberate sarin releases in Syria with large numbers of fatalities emphasize the need for OP countermeasures for both military and civilian populations. Therapeutic countermeasures involve several strategies: (i) preventing OP poisoning through administering pre-exposure treatments that scavenge OPs before they inhibit their physiological AChE targets in the brain and in the periphery (ii) post-exposure oxime that can rapidly reactivate OP-inhibited AChE or (iii) a combination of both. In terms of a pretreatment, our recent studies have demonstrated that administration of an aerosolized (aer)-rHuBChE employing a user friendly nebulizer, forms a protective pulmonary bioshield in the lungs of macaques which to date remains intact for at least 4 days. Thus 8 mg/kg of aer-rHuBChE deposited in the lung can prevent symptoms and inhibition of RBC-AChE and plasma BChE following a high (55ug/kg) inhaled dose of aer-paraoxon (Px) 4 days later; an amount known to inhibit circulating ChEs by >95% and cause tremors. In terms of oxime efficacy, macaque studies have demonstrated that a single IM post-exposure injection of the zwitterionic, centrally acting oxime RS194B (62-80ug/kg) plus low-dose atropine rapidly reactivates OP-inhibited RBC-AChE and circulating BChE and dramatically reverse both early and advanced clinical OP symptoms following lethal inhalation exposure to both sarin vapor (49.6ug/kg) and lethal aerosolized paraoxon (100ug/kg).

The increased efficacy of nebulizers in humans and the known synergy between aer-rHuBChE pretreatment with IM RS194B post exposure bodes well for a prophylactic or combination treatment which can protect against potent inhaled OP agents for >6 days without multiple injections.

Keywords
aer-human butyrylcholinesterase, sarin, paraoxon, oxime, reactivation, macaques.

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S06-3 Human Plasma-Derived Butyrylcholinesterase Is Behaviorally Safe and Effective in Cynomolgus Macaques (*Macaca fascicularis*) Challenged with Soman

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Organophosphorus compounds (OP) pose a significant threat. Administration of human butyrylcholinesterase (Hu BChE) may reduce or prevent OP toxicity. Thus, we evaluated the safety and efficacy of Hu BChE in monkeys using sensitive neurobehavioral tests while concurrently characterizing absorption and elimination in the presence and absence of high-dose soman exposure to predict time course and degree of protection. Eight young adult male cynomolgus macaques were trained on two distinct automated tests of neurobehavioral functioning. Hu BChE purified under current-Good-Manufacturing Practices (CGMP) was injected intramuscularly at 13.1 mg/kg, producing an average peak plasma value (C_{max}) of 28 Units/ml. The apparent time to maximum concentration (T_{max}) approximated 12 hours and the elimination half-life approximated 80 hours, returning to pre-administration (baseline) levels by 14 days. No behavioral disruptions following Hu BChE administration were observed on either neurobehavioral test, even in monkeys injected 24 hours later with an otherwise lethal dose of soman. Thus, Hu BChE provided complete neurobehavioral protection from soman challenge. These data replicate and extend previous results that used a different route of administration (intravenous), a different species (rhesus macaque), and a different BChE product (non-CGMP material). The addition of two sensitive neurobehavioral tests coupled with the PK/PD results convincingly demonstrates the neurobehavioral safety of plasma-derived Hu BChE at therapeutic levels. Protection against an otherwise-lethal dose of soman by a pre-exposure treatment dose that is devoid of side effects establishes a foundation for additional testing using other exposure routes and treatment times, other challenge agents/routes, or other classes of organophosphate scavengers.

Acknowledgements and disclaimers

Opinions, interpretations, conclusions, and recommendations are those of the author(s) and are not necessarily endorsed by the US Army

This research complied with the Animal Welfare Act and implementing Animal Welfare Regulations and adhered to the principles noted in The Guide for the Care and Use of Laboratory Animals.

This work was funded by the Defense Threat Reduction Agency, Medical S&T Division.

S06-4 Design of a combined aptamer for paraoxon and acetylcholinesterase by in silico approach

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Poisoning by organophosphates (OPs) takes one of the leading places in the total number of exotoxicoses. Detoxication of OPs at the first stage of poisoning could be achieved with the help of aptamers, which are able to bind poisons in the bloodstream [1]. The effectiveness of the aptamers for OPs could be strengthened by their possibility to bind non-covalently with the peripheral anionic site (PAS) of acetylcholinesterase (AChE) defending the active site gorge from OPs molecules. In the present work, we have applied for the first time the *in silico* design of a combined aptamer for paraoxon and PAS of AChE. Based on the published sequence of an aptamer binding organophosphorus pesticides [2], its three-dimensional model was constructed. The most probable binding site for paraoxon was determined by molecular docking and molecular dynamics (MD) methods. Then the nucleotides of the binding site were mutated consequently and the values of free binding energy were calculated using MD trajectories and MM-PBSA approach [3]. On the basis of the energy values, the sequences that bind paraoxon most efficiently have been selected. Molecular docking of sixteen possible nucleotide pairs into PAS of AChE was performed and the pairs that bind with PAS most efficiently have been selected. The 5'-end of the aptamers for paraoxon was modified based on the results of molecular docking. The calculations have shown that the final aptamers interact with paraoxon and PAS of AChE more efficiently than AChE interacts with paraoxon.

Keywords

acetylcholinesterase, aptamer, molecular modeling, paraoxon

Acknowledgement

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S06-5 Prokaryotic expression of human butyrylcholinesterase as a tool for catalytic bioscavenger development

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Currently, the best bioscavenger candidate against nerve agent intoxication is human butyrylcholinesterase (BChE). However, the effective dose cost, estimated to about 200 milligrams of pure enzyme, remains challenging despite the production and purification progresses realized these last years. A strategy for reducing dosage and cost would be to turn this scavenging protein into a nerve agent hydrolyzing enzyme, a catalytic bioscavenger. Up to now, screening of large mutant libraries has been hindered by the restricted eukaryotic expression of active BChE. Here we present the successful prokaryotic expression of an active human BChE variant designed with *PROSS*, a sequence- and structure-based algorithm for the soluble prokaryotic expression of difficult proteins. The protein is easily purified with two simple chromatographic steps. Despite 47 point mutations, the enzyme presents similar enzymatic parameters than the wild-type enzyme and its active site gorge structure is identical to that of the native enzyme produced in eukaryotic systems as determined by X-ray crystallography. These data validate the prokaryotic expression of human BChE which will greatly facilitate the screening of variants with nerve agent hydrolytic properties. We have initiated animal studies to assess the protein potency (immunogenicity, pharmacokinetic and bioscavenger efficiency) and will study the production of the tetramer form. On the other hand, we are currently developing high-throughput protocols for the prokaryotic expression, purification and screening of nerve agent hydrolysis.

S06-6 Bioscavengers and the medical management chain

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Survival and recovery from nerve agent poisoning requires a continuum of medical care, starting with a rapid initial response followed by continued support though the medical chain. In a military context, research into countermeasures to nerve agent poisoning has traditionally focussed on first-aid, pretreatment and prophylaxis; however, there are many opportunities to optimise the management of nerve agent-poisoned casualties.

We have previously demonstrated the efficacy of bioscavenger as a post-exposure, pre-symptomatic therapy in guinea-pigs poisoned by VX via the dermal route. Data will be presented on the efficacy of bioscavenger before, on and after the appearance of signs of poisoning and the influence of introducing a delay between initial treatment and the administration of bioscavenger. Treatment regimens including bioscavenger offered near-complete protection against the VX challenge, in the absence of continuing supportive therapy.

The potential for bioscavenger use within the treatment chain could range from pre-exposure to hospital use. Inclusion of bioscavenger has potential to reduce the level of medical care, monitoring and therapeutic intervention for casualties that have been poisoned percutaneously. The results will be discussed in the context of the UK military medical management chain and the considerations and constraints of the operational environment.

Keywords
Bioscavengers, Medical Management, VX, guinea-pigs

S07 Catalytical Bioscavangers - PON and PTE

Chairs: Eugenio Vilanova (Spain), Florian Nachon (France)

S07-1 Borderline between catalytic and non-catalytic bio scavengers: the example of albumin and reversible B-esterases

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Protective mechanism against organophosphorus compounds (OPs) toxicity are mainly based in molecular processes frequently divided conceptually in (A) catalytic and (B) non-catalytic bio-scavengers. Modified natural proteins and small molecules have been developed for applying in therapy and protection. The catalytic ones are mainly associate to the classical concept of A-esterases (phosphotriesterases, PTEs, i.e. paraoxonase); they hydrolyze carboxylesters and OPs by a divalent cation dependent mechanism. The non-catalytic scavengers are mainly associated to covalent binding to proteins, especially B-esterases with a serine or tyrosine residue, which hydrolyzes carboxylesters. However, if an OP is bound (organophosphorylation), its represents an enzymatic inhibition in some cases considered "the target" of toxicity or initial molecular event (IME) in their mode of action developing toxicity (adverse output pathway, AOP). The binding to proteins also represents a sequestration avoiding the OP interaction to other protein. However, there are protein binding OPs (non-catalytic bioscavengers) which can be slowly dephosphorylated, having a role as catalytic scavenger. A proportion of B-esterase activity in serum and brain shows reversible inhibition and their protective role just in situ in the target tissue of toxicity need to be investigated. Serum albumin is other example of B-esterase mainly thorough a tyrosine residue; its role in detoxication have been demonstrated and adducts applied as biomarker of exposure. Moreover, for a specific phosphoramidate family hydrolysis capacity may be enhanced by copper, probably by a mechanism not related with its B-esterase activity. Therefore, we have examples in the borderline between non-catalytic and catalytic scavengers.

Keywords
A esterase, B esterase, scavengers, albumin, phosphorylation

S07-2 Catalytic Scavengers Provide Broad-Spectrum Protection against Organophosphorus Nerve Agents

Shane A. Kasten, Sandra J. DeBus, Thuy L. Dao, Michael V. Boeri*, Zachary A. Canter*, Sean M. Hodgins*, Robyn. B. Lee, Douglas M. Cerasoli, **Tamara C. Otto**

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Efforts to develop a single enzyme capable of catalyzing the hydrolysis of a broad spectrum of organophosphorus (OP) compounds into non-toxic products have produced multiple candidate enzymes on different structural scaffolds. While protection against multiple OPs from a single enzyme has been obtained, no single enzyme has been identified that can provide protection against all G- and V-type OP nerve agents. The most promising candidate enzyme platform is the bacterially produced recombinant variant of organophosphorus hydrolase (OPH) from *B. diminuta. In vivo* protective efficacy of candidate OPH scavengers as prophylactics was tested in guinea pigs by administering the enzyme via a carotid catheter, followed 20 minutes later by a subcutaneous injection of increasing doses of the OP nerve agents GA, GB, GD, GF, VX, VR, or VM. A stage-wise, adaptive dosing experimental design was used to determine the median lethal dose (LD_{50}) of each OP in the context of enzyme prophylaxis. We report that a combination of two different OPH variants is capable of providing protection against at least 2 x LD_{50}s of all of the OPs tested. The results indicate that broad spectrum prophylactic protection against OP intoxication can be provided with a cocktail of two different catalytic scavengers with appropriate catalytic activity. Formulation of the enzymes to promote circulatory stability will be discussed.

Keywords
catalytic scavenger, prophylaxis, organophosphorus hydrolase

The views expressed in this abstract are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. This work was supported by the NIH CounterACT Center of Excellence grant U54 NS058183 (to D.M.C.) and by the Defense Threat Reduction Agency-Joint Science and Technology Office, Medical S&T Division.

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S07-3 Paraoxonase 1 variant I-F11 gene therapy using adeno-associated virus8 (AAV8) offers long-term protection against G-type chemical warfare nerve agents

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The paraoxonase 1 variant I-F11 affords asymptomatic protection against the lethal effects of G-type chemical warfare nerve agents (CWNA). Here, we tested whether adeno-associated virus8 (AAV8) is able to deliver I-F11 for extended periods of time and at levels affording asymptomatic protection against 2-5LD50 doses of G-type CWNA in mice. I-F11 gene expression levels in mouse blood were assessed under the influence of three different promoters and found to be significantly higher with TBG compared to CMV and CASI. A single tail vein or intramuscular injection of AAV8-TBG-I-F11 resulted in robust production of the enzyme, which reached concentrations of up to 1 to 2 mg/ml in mouse blood for up to 6 months. Mice containing 0.75 mg/ml or higher concentrations of I-F11 in their blood were afforded asymptomatic protection against multiple 5LD50 exposures of GD, GF, GA, and GB, a total of 9 exposures over a seven-week period. We also conducted studies showing that I-F11 is most efficacious in offering protection against GD followed by GF, GB and GA. Analysis of the mouse blood for serum chemistry and hematology parameters, and tissues by H&E staining, indicated no appreciable changes between control mice, mice overexpressing 1-F11 for 6 months, and mice surviving repeated G-agent exposures. These data suggest that AAV8-mediated catalytic bioscavenger gene therapy using 1-F11 is a safe, efficacious, and long-lasting pre-treatment strategy against G-agents.

Keywords

Gene Therapy, AAV8, Chemical Warfare Nerve Agents, Paraoxonase 1 variant I-F11, Safety and Efficacy

Disclaimers/Acknowledgments

The views expressed in this abstract are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. The experimental protocol was approved by the Animal Care and Use Committee of the US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD and all procedures were conducted in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals* (National Research Council), and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. Funding for this work was provided by Joint Science and Technology Office (JSTO), Defense Threat Reduction Agency (DTRA), Department of the Army.

S07-4 Organophosphate Hydrolase (OPH) Designed as a Tethered Monomer

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Organophosphate hydrolase (OPH) mutants have shown potential use as a medical countermeasure against organophosphorus compounds (OPs). OPH is typically expressed in bacteria as a homodimer. Two separate subunits (35 kDa each) self-assemble through non-covalent bonding at the enzyme face close to the putative active site. OPH homodimers do not secrete expediently from mammalian cells. This causes potential problems when trying to express the protein from a heterologous plasmid or viral delivery system. To enhance secretion of OPH from mammalian cells, we sought to increase protein solubility without catastrophic detriment to activity and without addition of fusion proteins. To this end, we designed OPH to be expressed as a tethered monomer by joining two OPH subunits with a poly-glycine linker. We created the single polypeptide OPH with a tether 10 or 35 amino acids in length between the two halves, and named them T10 and T35 respectively. Western blot analysis and paraoxon hydrolysis assays revealed that T10 was being produced and retained some activity against paraoxon. This was a surprise as we expected T10 to have no enzymatic activity. T35 monomer (75 kDa) was also being produced and retained 71% of specific activity against paraoxon compared to untethered OPH. T10 and T35 showed no significant decrement in activity against the nerve agent sarin. Both constructs showed high molecular weight aggregates greater than 250 kDa in dynamic light scattering and native polyacrylamide gels. These tethered constructs are the first attempts known for producing OPH as a single polypeptide.

Keywords

Organophosphate hydrolase, tether, monomer, sarin

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S07-5 A new animal model to investigate organophosphorus poisoning and enzymatic decontamination

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Freshwater planarians from Platyhelminthes, harboring a mammal-like cholinergic nervous system, have emerged as a promising *in vivo* model for investigating neurotoxicity. Moreover a large proportion of stem cells provide planarian an unconventional capacity of regeneration allowing for developmental disruption studies. *Schmidtea mediterranea (Smed)* was used as model for

organophosphorus (OP) poisoning and for evaluating the efficacy of detoxifying enzymes.

Acetylcholinesterase and butyrylcholinesterase from planarian (*Smed*-AChE and *Smed*-BChE) share 35% identity with their human counterpart (*Hs*-AChE and *Hs*-BChE). Structural predictions revealed strong similarities between planarian and human enzymes. Cholinesterase activities were detected in crude planarian homogenates after grinding and were inhibited after organophosphorus exposition. *In situ* Hybridization was further used to localize cholinesterases in planarians and showed two different patterns, *Smed*-AChE being mainly detected in cephalic ganglion and ventral nerve cords while *Smed*-BChE distribution was diffuse.

Survival, behavior and regeneration were analyzed in whole planarian exposed to four OP [1]. The toxicity of OP degradation products generated by enzymatic hydrolysis with the robust phosphotriesterase enzyme *Sso*Pox, from the archea *Sulfolobus solfataricus* [2], was further evaluated. OP were found to be highly toxic to planarians causing severe mortality and behavior disruption at sublethal concentrations as well as growth disruption during regeneration after cutting. Enzymatic decontamination drastically reduced toxicity and enhanced both mobility and development. These results underline that degradation products have a lower impact than initial organophosphorus substrates. A biotechnological application based on a filtration column incorporating detoxifying enzymes was developed to decontaminate wastewater with planarian as biosensor.

Keywords
Organophosphorus poisoning, Planarian, Cholinesterase, Pesticides, Bioremediation

Acknowledgement
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S07-6 Paraoxonase-2 dependent redox control of platelet physiology

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Background and Objective: Platelets are not only central players in hemostasis and thrombosis but also important modulators of immune responses, inflammation and cancer. Activated platelets generate reactive oxygen species (ROS) that modulate platelet function through redox signaling and oxidative stress. The anti-oxidative enzyme paraoxonase-2 (PON2) is known to counteract inflammation and atherosclerosis. Recently, we showed that PON2-deficient mice exhibit tissue factor-dependent hypercoagulability[1]. Here, we investigated the role of PON2 in ROS production, phenotype and activation of platelets from PON2-deficient mice.

Methods: Platelet count and mean platelet volume (MPV) were determined by a cell counter. Flow cytometry was used to quantify platelet surface receptors, intracellular ROS and platelet function in diluted citrate-anticoagulated platelet-rich plasma. Platelet aggregation was analyzed by light transmission aggregometry in platelet-rich plasma.

Results: Platelets from PON2-deficient mice displayed increased basal and agonist-induced ROS levels accompanied by decreased platelet count but increased MPV compared to wildtype platelets. PON2-deficient platelets showed increased surface expression of the von Willebrand receptor (vWF) GPIbα, vWF-binding, P-selectin surface expression, but no αIIbβ3 integrin/fibrinogen receptor activation ex vivo. Botrocetin induced enhanced binding of vWF to PON2-deficient platelets in vitro. However, agonist-induced αIIbβ3 integrin activation, P-selectin surface expression and platelet aggregation were impaired compared to wildtype platelets. Interestingly, addition of 0.5 mM Ca^{2+} to platelet-rich plasma normalized platelet hyporeactivity.

Conclusion: Our data demonstrate that PON2 plays a crucial role in platelet ROS production, phenotype and function. Reactivity of platelets from PON2-deficient mice depends on extracellular Ca^{2+}-concentration.

Keywords
Paraoxonase-2; Reactive oxygen species; Platelets; Hemostasis; Ca^{2+} homeostasis

Reference

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S07-7 Copper with chicken serum albumin show stereoselective hydrolysis of chiral phosphoramidates

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Chiral analogous compound of methamidophos insecticide are only poorly hydrolyzed by Ca^{2+}-dependent phosphotriesterases in mammals tissues including the human serum. We reported the hydrolysis of O-hexyl O-2,5-dichlorophenyl phosphoramidate (HDCP) in chicken serum. The hydrolysis of the R-(+)-HDCP isomer is strongly increased in vitro in the presence of 30-250 µM

copper. It is the opposite estereoselectivity of that showed by liver Ca^{2+}-dependent activity. We name it as „antagonistic stereoselectivity“. Diluted chicken serum (10 µL in 1 mL solution of 400 µM HDCP) or the equivalent amount of commercial chicken serum albumin (CSA 216 µg/mL) with 100 µM Cu^{2+}, showed about 50% and 75% of R-(+)-HDCP hydrolysis after 60 and 120 min. In the same conditions other commercial serum metalloproteins with high affinity to Cu^{2+} (cuproproteins) as human serum ceruloplasmin or horse kidney metallothionein did not showed significant Cu^{2+}-dependent hydrolysis. Moreover, other divalent cations (Zn^{2+}, Fe^{2+}, Ca^{2+}, Mn^{2+} and Mg^{2+}) did not showed this activation. The results confirm that the CSA is the protein responsible of „antagonistic stereoselectivity“ that had been observed in the chicken serum. The effect of copper on the hydrolysis of HDCP by other animal albumins is shown in this work.

Keywords
Albumin, copper, hydrolysis, stereospecificity, phosphotriesterases, organophosphorus

S08 3D section - structure and dynamics of α/β hydrolases and OP hydrolases, in silico methods for designing of modulator

Chairs: Zoran Radić (USA), Jonah Cheung (USA)

S08-1 Insights into the Yin and the Yang of Acetylcholinesterase Inhibition by Mechanistic X-Ray Crystallography

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Drug discovery and development is a complex and expensive process. Thanks to the exponential growth of molecular data and advancement in technologies, efforts have been tremendously amplified. Among new approaches multipotent compounds are emerging as the next paradigm in drug discovery [1] and includes: (i) single drug acting on multiple targets of a unique disease pathway, or (ii) single drug acting on multiple targets pertaining to multiple disease pathways. These compounds are thought to have best beneficial effects in the treatment of complex diseases, like Alzheimer's Disease, in which the simultaneous regulation of various pathological aspects may more efficiently interfere with the disease progression. Systematic integration of the data derived from different disciplines including computational modeling, X-ray crystallography, synthetic chemistry, *in vitro / in vivo* pharmacological tests, is mandatory for the selection of best-in-class compounds. In this context, we report on the key contribution of X-ray crystallography in highlighting peculiar mode of interaction of promising multi-target directed ligands, designed by combining the tacrine fragment to distinct pharmacophores i.e. juglone [2], benzofuran [3] and *L*-tryptophan with a linker of a suitable length.

Overall, the structural analysis highlights the molecular determinants responsible for the optimal binding of the multi-target ligands to AChE and pinpoints the utility of hybridization strategies in structure-based drug design programs. It also unveils the validity of X-ray crystallographic structures determination at certain milestones along the development of interacting inhibitory drugs based on molecular modeling studies.

Keywords
Acetylcholinesterase Inhibition, Structure-Based Drug Discovery, X-ray Crystallography, Alzheimer's Disease, Multi-target directed ligands.

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S08-2 Photo-induced release of an acetylcholinesterase inhibitor

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Light–induced isomerization of enzyme ligands allows controlling specific biological processes in time and space. Photoisomerisable azobenzene-based inhibitors allow photo-control of acetylcholine (ACh) signalling by regulating acetylcholinesterase (AChE), the enzyme that catalyses ACh hydrolysis in the central and peripheral nervous system. By regulating AChE, this family of inhibitors would allow spatial and temporal regulation of ACh levels in the synaptic cleft. Adequate regulation of ACh levels is an essential part of Alzheimer's disease (AD) treatment and other common pathologies. Win this work we present the crystal structures of AChE in complex with three different azobenzene derived inhibitors, we confirmed AzoTHA-1 as the only photoactive compound and we determined its structure in its *cis*- and *trans*- isomeric forms bound to AChE. Three-dimensional structures, supported by online UV-Vis spectroscopy and kinetic data, explain why only AzoTHA-1 is an effective photoactive AChE inhibitor and suggest possible ways to improve photoactive drugs. We utilised S/WAXS to follow photo-isomerisation induced-changes in the wide-angle scattering region to demonstrate that photoisomerisation of the inhibitor induces its release from AChE's active site.

Keywords
Photopharmacology, Alzheimer disease, acetylcholinesterase dynamics.

S08-3 Structural studies of *Anopheles gambiae* acetylcholinesterase provide insight towards improved insecticides for malaria vector control

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Malaria is transmitted by the *Anopheles gambiae* mosquito in sub-Saharan Africa and tropical regions where the disease is prevalent. Indoor spraying with anticholinesterase insecticides is a proven method to control populations of the mosquito and to reduce spread of the disease; however, widespread use of insecticides has led to the rise of an insecticide-resistant G119S mutant acetylcholinesterase in the mosquito which threatens ongoing disease-control efforts. We have solved high resolution X-ray structures of the G119S mutant acetylcholinesterase of *An. gambiae* (G119S AgAChE), in the ligand-free state and in complex with a potent difluoromethyl ketone inhibitor, revealing the structural basis of insecticide resistance[2]. Although resistance-breaking inhibitors of G119S AgAChE exist, they also inhibit human acetylcholinesterase and thus lack the necessary species selectivity to be safely used as insecticides. In our structures, we see specific features within the active site gorge, including an open "back door", that are distinct from human acetylcholinesterase. These differences provide a means for improving species-selectivity in the rational design of improved insecticides for malaria vector control.

Keywords
acetylcholinesterase, structure, malaria, insecticide

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S08-4 Room-temperature crystallography and neutron scattering studies of human acetylcholinesterase to inform the design of oxime reactivators

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Human acetylcholinesterase (hAChE) is responsible for degrading neurotransmitter acetylcholine at synapses of the nervous system. Organophosphate (OP) nerve agents and pesticides inactivate hAChE through chemical modifications of the catalytic serine. The current generation of oxime antidotes is not highly efficient. Insights into the molecular structures of AChEs from various species reveal possible limitations in enhancing reactivation rates, but provide only limited information, because the structures have been obtained at cryo-temperatures. Moreover, X-ray crystallography usually cannot resolve positions of hydrogen atoms involved in proton transfer processes during reactivation. Thus, we use room-temperature X-ray and neutron crystallography to obtain structures at physiological conditions and to visualize hydrogen atoms.

Several X-ray structures of native and VX and POX-conjugated hAChE in complex with oxime reactivators, RS2-170B and RS-194B have been obtained. hAChE crystallized in a unit cell (a=124.3, c=129.1 Å; $P3_1$) amenable to neutron crystallography. For the first time we show how RS2-170B binds in the non-modified and OP-conjugated active site gorge at room temperature. RS-194B is observed with its oxime group pointing away from the catalytic Ser203 and the reactivator is pushed out to bind at the peripheral site in the VX-modified structure. Dynamics of hAChE was probed by neutron vibrational spectroscopy to look at harmonic vibrations. POX binding induces significant changes in the acyl pocket loop conformation expelling the weakly binding RS-194B from the active site gorge completely, and the loop becomes more dynamic. We hypothesize that increased dynamics of the acyl pocket loop contributes to the POX-conjugated hAChE resistance to reactivation.

Keywords
room-temperature crystallography, neutron vibrational spectroscopy, oxime reactivator, protonation state, hydrogen bonding, protein dynamics.

Acknowledgement
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S08-5 Crystal structures of human cholinesterases in complex with supramolecular ligands

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Human acetylcholinesterase (hAChE) and butyrylcholinesterase (hBChE) are related enzymes. hAChE plays a key role in neurotransmission and is the target of organophosphorus nerve agents. hBChE is good a natural stoichiometric scavenger of nerve agents, preventing their diffusion to the central and peripheral nervous system where they inhibit hAChE.

hAChE and hBChE display different specificities for substrates and ligands due to differences in the number of aromatic residues lining the active site gorge. These aromatic residues are essential for the binding of quaternary and aromatic ligands.

Some molecules containing quaternary and/or aromatic moieties form supramolecular structures by chelating Zinc. The nature of these molecules suggested that they could have affinity for the aromatic residues in the active site gorge of human cholinesterases. It was confirmed by determining their inhibition properties. A key question was whether these supramolecular ligands bind to human cholinesterases as their Zn-complex or monomeric form? The X-ray structures of two supramolecular complexes binding to the gorge of the hAChE and the hBChE reported herein showed that either cases are possible. These structural data on two new types of ligand can be used to design original cholinesterases inhibitors or reactivators.

Keywords
Acetylcholinesterase, butyrylcholinesterase, inhibitors, metallosupramolecular complexes.

S08-6 Modifications of Cholinesterase Structure and Function in Covalent Organophosphate Conjugates Visualized in 2D, 3D and VR

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Backbone conformations in hundreds of PDB deposited cholinesterase (ChE) X-ray structures show surprising similarity with typical variability of ~1Å or less among native and liganded acetylcholinesterases (AChEs; 3.1.1.7) and as low as ~2 Å between AChEs and butyrylcholinesterases (BChEs; 3.1.1.8). The largest backbone deviations are observed in their covalent conjugates with organophosphate (OP) inhibitors. Those deviations are likely to influence approach, binding and reaction efficacy of nucleophilic oxime reactivators of ChEs the only true antidotes of OP intoxicated individuals and therefore need to be considered in structure based design of improved oxime antidotes.

We developed a novel, reference point based principle for overlay-independent pairwise comparison of liganded and non-liganded Cα conformations from respective PDB structures and encoded it in JAVA based computer algorithm for quick analysis. Comparisons are based on differences in distances between each Cα pair based on differences in the angle between center of mass, reference point and each of Cα in the comparison, revealing a subset of Cα in two structures that maintains their relative positions in the 3D space best and that can be used as tethering points for overlay of compared structures.

Using NanoPro (Nanome Inc.) VR software, we visualized results of pairwise structure analyses creating .pdb format 3D graphs to identify interaction matrices between amino acids revealed upon ligand binding.

Structure comparisons will be paralleled to OP inhibition and oxime reactivation parameters for some of analyzed ChE-OP-oxime systems to emphasize the importance for complete molecular target template characterization in the structure based antidotes design.

Keywords
organophosphates, oxime reactivators, backbone conformation, 3D structure, VR

Acknowledgement
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S09 Biological functions, development and non-cholinergic function of cholinesterases

Chairs: Shani Shenhar-Tsarfaty (Israel), Israel Silman (Israel)

S09-1 Cholinergic mechanisms at the core of skeletal and retinal histogenesis

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Recently we could establish major cholinergic impact on vertebrate *in vivo* and *in vitro* skeletogenesis (1,2). Cholinergic mechanisms are also at the core of formation of the vertebrate retina. Retinal histogenesis of a so-called inner plexiform layer (IPL) was disturbed in an AChE KO mouse (3). Characterized best by their ChAT expression, the only cholinergic cells in all vertebrate retinae are so-called *starburst amacrine cells* (SACs), which send processes into synaptic IPL sublaminae. We documented that SACs are derived from a larger pool of postmitotic AChE$^+$ cells. A developmental comparison of ChAT$^+$ and AChE$^+$ cells revealed a close spatial localization of both proteins first within individual cells (nuclear ChAT, vs. extranuclear AChE), and later between adjacent cells, e.g., ACh-secreting and -degrading cells have the same cell lineage origin, and later remain in close apposition (4). Using our 3D stem cell organoid approach (retinal spheroids), we could show that ChAT$^+$ cells were first to initiate IPL formation by establishing two synaptic sublaminae. Unexpectedly, the earliest ChAT$^+$ cells co-expressed markers of Müller glial precursors (MCPs), indicating that a direct SAC precursor i) gives rise to neurons and glial cells, and ii) that these premature cholinergic cells drive earliest processes of network formation in vertebrate retinae, e.g. could function as *IPL founder cells* (5, cf. also 6,7). These findings could have profound relevance for a basic understanding of neuronal network formation.

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S09-2 Butyrylcholinesterase as a ghrelin modulator impacting anxiety, stress, obesity, and drug cravings

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Our recent studies on butyrylcholinesterase (BChE) have led us to conclude that this enzyme has a major physiological role in regulating levels and impact of ghrelin, the "hunger hormone." A key step toward this realization was finding that, over time, group-housed mice given AAV8-BChE expression vector showed a sharp drop in fighting. Eventually we linked this reaction to a large decrease in plasma ghrelin, which is involved in food-seeking and stress. At first, we assumed that lowered ghrelin was reducing stimulation of growth hormone secretagogue receptors in brain. Instead, treated mice showed *larger* pulses of circulating growth hormone after i.v. ghrelin injection. In other words, high plasma BChE enhanced sensitivity of ghrelin's target, the growth hormone secretagogue receptor, involved in emotional behaviors. That also fits BChE's impact on feeding. BChE knockout mice have high ghrelin levels that drive overeating and obesity. BChE-enhanced mice have low plasma ghrelin, they resist obesity on high-fat diet and show less rebound weight gain after a forced low-calorie diet. These findings suggest that BChE gene transfer could have substantial therapeutic impact on obesity and other conditions that involve ghrelin.

S09-3 Assembly of PRiMA-linked form of acetylcholinesterase in neurons: the role of enzyme inhibitor acting as chemical chaperon

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Acetylcholinesterase (AChE) is anchored onto cell membranes by a transmembrane protein PRiMA (Proline-Rich Membrane Anchor) as a tetrameric globular form that is prominently expressed in vertebrate brain. Several lines of evidence suggest that the dimer formation probably represents an intermediate in the assembly of the tetramer. In addition, the assembly of AChE tetramers with PRiMA requires the presence of a C-terminal "t-peptide" in the AChE catalytic subunit ($AChE_T$). This protein assembly could be affected by chaperons. AChE inhibitors (AChEIs) are the most established treatment strategy for Alzheimer's disease (AD). Many AChEIs are membrane permeable, and thus which could act as chemical chaperons in affecting the protein assembly of PRiMA-linked AChE in the endoplasmic reticulum (ER). In cultured neuroblastoma or cortical neuron, application of AChEIs, including tacrine (Cognex), rivastigmine (Exelon), but not donepezil (Aricept) and galantamine (Razadyne), caused an accumulation of

the unfolded AChE being retained in ER fraction: the AChEI-bound enzyme was not able to transport to Golgi/plasma membrane fraction. As a result, the transcripts encoding AChE and PRiMA were decreased by 50% in the AChEI-treated cultures. In parallel, an increase of ubiquitin-associated enzyme degradation was revealed. The treatment of AChEI in the cultures induced the expression of apoptotic markers, e.g. cleaved caspase 3. In parallel, the apoptotic cell number and mitochondrial membrane potential (MMP) were increased in a dose-dependent manner. The AChEI-bound enzyme retained intracellularly could induce a result of ER stress, as indicated by increased expressions of BiP and CHOP in the treated cultures. The AChEI-induced ER stress resulted with an activation of cAMP signaling, which could regulate the expressions of miR132 and miR212. These findings provide guidance for the drug design and discovery in AD based on inhibition of AChE.

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S09-4 Evolution of the first disulfide bond in the cholinesterase-carboxylesterase (COesterase) family: Possible consequences for cholinesterase expression in prokaryotes

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Within the alpha/beta hydrolase fold superfamily of proteins, the COesterase group (carboxylesterase type B, block C, cholinesterases…) diverged from the other groups through addition of an N-terminal disulfide bond and simultaneous increase in the mean size of the protein (1). This disulfide bond creates a large loop, which is essential for the high catalytic activity of cholinesterases through formation of the upper part of the active center gorge. In some non-catalytic members of the family, the loop may be necessary for heterologous partner recognition. The shuffling of this portion of protein occurred at the time of emergence of the fungi/metazoan lineage. Homologous proteins with this N-terminal disulfide bond are absent in plants but they are found in a limited number of bacterial genomes. In prokaryotes, the genes coding for such homologous enzymes may have been acquired by horizontal transfer. However the cysteines of the first disulfide bond are often lost in bacteria. Natural expression in bacteria of CO-esterases comprising this disulfide bond may have required compensatory mutations or expression of new chaperones. This disulfide bond may also challenge expression of the eukaryote-specific cholinesterases in *E. coli*. Recently , catalytically active human acetylcholinesterase and butyrylcholinesterase were successfully expressed in *E. coli*. The key was the use of a peptidic sequence optimized through the Protein Repair One Stop Shop process, an automated structure- and sequence-based algorithm toward expression of properly folded, soluble eukaryotic proteins with an enhanced stability (2,3). Surprisingly however, the crystal structure of the optimized butyrylcholinesterase variant expressed from bacteria revealed co-existing 'close' and 'open' states of the first disulfide bond. Whether the 'open bond' involves two cysteines (i.e., the bond never formed) or two half-cystines (i.e., the bond properly formed, then broke during the production/analysis process) cannot be inferred from the structural data. Yet, this observation suggests that this first bond is difficult to maintain in *E. coli*-expressed cholinesterases.

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S09-5 Acetylcholinesterase in neuromuscular synaptic clefts of vertebrates

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Precise positioning and density of acetylcholinesterase (AChE) in the synaptic cleft is required to correctly control the duration of transmitter action in cholinergic synapses according to the particular functional demands of the synapse. We had previously evaluated the densities of AChE at neuromuscular junctions (NMJs) by EM-autoradiography, using radiolabeled probes. The current study addressed fundamental issues concerning the precise location and distribution of the enzyme in the cleft, i.e., whether it is associated with pre- or postsynaptic membranes, or with synaptic basal lamina (BL), and whether it is present only in the primary cleft (PC) or also in postjunctional folds. Quantitative EM-analysis using nanogold labeled anti-AChE probes demonstrated that AChE sites are almost exclusively located on the BL rather than on pre- or postsynaptic membranes and are distributed in the PC and down the postjunctional folds, with a defined pattern. This localization pattern of AChE is suggested to ensure full hydrolysis of acetylcholine bouncing off receptors, thus eliminating its harmful re-binding. The methodology developed for normal NMJs

provides a benchmark for studying other peripheral and central nervous system synapses under physiological or pathological conditions.

Keywords
nanogold, acetylcholinesterase, basal lamina, synaptic cleft, postjunctional folds

S09-6 Respiration during organophosphate and carbamate intoxication when acetylcholinesterase is not anchored at cholinergic synapses

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Intoxications with organophosphate or carbamate shut down control of breathing in minutes. These central apneas are reversed by atropine the well-known antidote of acetylcholinesterase (AChE) inhibitors. But how the excess of ACh triggers the crisis remains unclear. If the buildup of ACh on the post-synaptic receptors at cholinergic synapses is critical, we expected that mice in which the synaptic transmission is adapted to the deficit of AChE should resist to intoxication with carbamates. AChE is specifically anchored in the synapses by ColQ at the neuromuscular junction (NMJ) and by PRiMA in central nervous system (CNS). We have thus intoxicated mice with paraoxon, physostigmine or pyridostigmine and recorded in great details the modifications of breathing in double chamber plethysmography. Physostigmine triggers very long end inspiration pauses (EIP) in WT whereas pyridostigmine provokes only short EIP. The duration of EIP was changed with physostigmine or pyridostigmine in PRiMA KO mice when the brain was adapted to a huge excess of ACh. Surprisingly, when AChE is absent at the NMJ, EIP were much shorter with physostigmine. If AChE in the respiratory center is a key target, we expected long EIP when AChE is normal in the brain and reduced in muscles. Altogether these observations do not support that the change of the synaptic transmission explains the central shutdown control of breathing when cholinesterases are inhibited. In addition, we observed that methacholine provokes similar alteration of breathing when injected subcutaneously to mice. I will discuss a novel model to reconciliate these observations.

S09-7 Single nucleotide polymorphisms in the genes encoding AChE and its miR-608 regulator co-modulate anxiety and blood pressure

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4 contributed equally to this work

Cholinergic-regulated phenotypes including anxiety, cardiac and immune-related properties show inter-individual variability which might be affected by genomic Single Nucleotide Polymorphisms (SNPs) in the corresponding protein coding genes and their targeting microRNAs (miRs), but the combined impact of such SNP pairs is unknown. We have recently shown that the rs17228616 SNP in the Acetylcholinesterase (AChE) gene reduces the affinity of AChE mRNA to the primate-specific miR-608 and elevates both AChE levels in brain and blood as well as trait anxiety and blood pressure (1) while affecting PTSD-related neural circuits and downregulating numerous brain miR-608 targets (2). Others reported that the rs4919510 SNP in the miR-608 gene reduces miR-608 levels *in vitro* and limits the risk of sepsis following head injury *in vivo* (3). To explore the combined effect of these two SNPs, we tested 444 healthy 30 years old US donors and 101 Israeli ex-prisoners of the 1973 war (EWP), 76 of whom returned with post -traumatic stress disorder(PTSD). Genotyping combined with R-statistics of the corresponding biomedical evidence demonstrated that the rare allele of the AChE SNP was more abundant among non-PTSD EWP donors compared to PTSD patients in this cohort (33 vs 19%, Chi-square 0.03). Moreover, we found in both of these cohorts interaction between the effect of the two SNPs on blood pressure, inflammation and anxiety-related parameters, with the miR-608 SNP stratifying the corresponding impact of the rare allele of the AChE SNP on these parameters. Our findings indicate an interaction between the SNPs in the AChE and miR-608 genes, possibly reflecting modified impact of this primate-specific miR on its numerous downstream targets.

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S09-8 Dioxin suppresses AChE expression in neuron and muscle

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Acetylcholinesterase (AChE, EC3.1.1.7) plays an important role in the cholinergic neurotransmission in central and peripheral nervous systems, which has been widely recognized as a biomarker for monitoring pollution of organophosphate and carbamate pesticides. Recently, a broad spectrum of environmental toxic substances has been found to decrease AChE activity in various species. Dioxin is one of the emerging environmental AChE disruptors, which is a typical persistent organic pollutant with multiple toxic effects on the nervous system. We have reported that dioxin suppresses the expression of neuronal AChE via aryl hydrocarbon receptor (AhR), in which both transcriptional and posttranscriptional regulations could be involved. Moreover,, muscular AChE expression was also disturbed by dioxin exposure. During myogenic differentiation of C2C12 cells, the mRNA expression of AChE T subunit and the enzymatic activity of AChE were significantly suppressed by dioxin exposure in parallel with the disturbances on the myotube formation. However, the addition of AhR antagonist was not able to reverse the suppressive effect of dioxin, suggesting a distinct role of AhR during the myogenic differentiation process. These results further support the notion that dioxin is a novel environmental AChE disruptor which acts on the biosynthesis processes via multiple molecular mechanisms.

Acknowledgments
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S09-9 Wnt3a induces the transcription of acetylcholinesterase: an enzyme playing a role in osteoblastic differentiation

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Acetylcholinesterase (AChE) plays hydrolytic role to terminate cholinergic transmission in vertebrate. AChE is intensively reported to exist in different tissues, and may participate in differentiation process. Here, AChE was demonstrated to participate in osteoblastic differentiation. In rat-derived bone tissues and primary cultured osteoblasts, the expression of AChE was increased in parallel with bone development, as well as osteoblastic differentiation. Transcriptional expression and protein of AChE in differentiating osteoblast could be enhanced by application of Wnt3a. Runx2, a downstream transcription factor in Wnt/β-catenin signaling pathway, played crucial role in Wnt3a-induced AChE expression in osteoblasts. This was confirmed by identification of Runx2-binding site in the *ACHE* gene promoter, over-expression of Runx2 and deletion of the Runx2-binding site in the *ACHE* promoter. Bone defect was observed in *ACHE*-/- mice. The non-enzymatic role of AChE in osteoblast was determined by over-expression system and application of AChE inhibitors. By transcriptomics, AChE was found to influence gene expressions of Wnt/β--catenin signaling components, and may participate in osteoblastic function, e.g. affecting osteoclastogenesis and cell adhesion of osteoblast. A notion of non-cholinergic role of AChE in osteoblast, as well as an insight for elucidating other possible mechanisms in regulation of bone formation was provided.

Acknowledgements
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S10 Alzheimer's disease and diseases related to cholinesterases

Chairs: Ana Martinez (Spain), Maria-Laura Bolognesi (Italy)

S10-1 Restoring mitochondria (dys)function and acetylcholine levels as a prospective therapeutic strategy for Alzheimer's disease

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Alzheimer´s disease (AD) is a progressive and degenerative neurological disorder resulting in memory loss and cognitive decline. The severity of AD dementia was found to correlate with the extent of the cholinergic loss and acetylcholine (ACh) depletion.

In brain synapses ACh can be hydrolyzed by two cholinesterases (ChEs), acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), which were found in neurons and glial cells as well as in AD neuritic plaques and tangles. AChE is the prevalent enzyme in the healthy brain, while BChE is considered to play a minor role in the regulation of synaptic ACh levels. However, in AD advanced stages, AChE activity is decreased while BChE activity is unchanged or even increased, making both ChEs stimulating targets for the treatment of AD. Current AD therapy is based on AChE inhibitors, although they have very modest clinical effects in treating the symptoms of the disease and are unable to halt disease progression.

Oxidative stress (OS) and mitochondrial dysfunction are also considered critical factors in AD pathogenesis. As a result, targeting mitochondrial oxidative stress (OS) in the prodromal phase of AD to slow or prevent the neurodegenerative process and restore neuronal function is thus viewed as a valid therapeutic approach.

As part of our drug discovery program focused in oxidative stress-related diseases, and following a multi-target strategy, new mitochondriotropic antioxidants based on natural scaffolds acting as dual and bifunctional cholinesterase inhibitors have been developed. The results will be reported in this communication.

Keywords

Alzheimer disease, mitochondriotropic antioxidant, cholinesterase inhibitor

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S10-2 From dual binding site AChE inhibitors to chameleon molecules: discovery of potent BuChE inhibitors

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Current pharmacotherapy for Alzheimer's disease (AD) involves compounds aimed at increasing the levels of acetylcholine in the brain through inhibition of AChE. These drugs, known as acetylcholinesterase inhibitors, have been shown to improve cognition and global functions but have little impact on improving the eventual progression of the disease. However, there are evidences that other cholinesterases such as butyrylcholinesterase (BuChE) can play an important role in cholinergic function in the brain, and the long-suspected non-cholinergic actions of acetylcholinesterase, mainly the interference with the beta-amyloid protein cascade, have recently driven a profound revolution in cholinesterase drug research [1-2].

We will present our journey from dual binding site AChE inhibitors as potent beta-amyloid modulators to the more recent serie of indolylpiperidines hybrids with an unexpected and very potent *h*BuChE inhibition. Experimental and computational studies have revealed the chameleon behavior of these molecules able to change their bioactive conformation depending on the cholinesterase binding site. Based on the potent activity of these compounds targeting BuChE, the low cellular toxicity and the *in vivo* target engagement, we can propose these indolylpiperidine derivatives as valuable tools for the study of the role of BuChE in AD and probably as potential drugs candidates for its future pharmacotherapy.

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S10-3 Discovery and development of neuroprotective and disease-modifying anti-AD drug leads from the Chinese medicine

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Alzheimer's disease (AD) represents a chronic and progressive brain disorder, and has now become the most common neurodegenerative disorders among the older population. Although the disease is now seen as major public health problems, the currently available therapeutics only offer temporary symptomatic relieves. Therefore, research and development of more effective and disease-modifying agents for the prevention and/or treatment of AD will have tremendous value from both scientific and economic standpoints.

Over the past few years, our series of studies have identified some highly promising anti-AD drug leads, including those derived from the Chinese medicines, with disease-modifying potential. In this presentation, the multi-neuroprotective effects and the underlying mechanisms of those promising candidates will be comprehensively illustrated and discussed.

Keywords
Neuroprotective effect, Dimers, Alzheimer's disease, Disease modifying, Multiple functions

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S10-4 Fifty shades of cholinesterase immobilization and their application to drug discovery

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New screening methodologies capable of identifying new enzyme inhibitors in a faster, more reproducible and automated way may help early drug discovery. Indeed high throughput screening methodologies for the identification of new cholinesterase inhibitors can reduce screening time and screening costs. In this frame, "immobilized enzymes" [1] can serve as handy and efficient alternatives to conventional in-solution methods. On the other hand, other than massive screening, highly informative approaches may provide decisive information in the selection of best-in-class compounds. Hence, combination of several parameters spanning from inhibition, binding mechanisms and kinetic parameters is important to be considered. In particular, estimation of residence time has recently emerged as critical feature [2]. Therefore, accessing kinetic information on drug binding events at initial stages of the drug discovery process is gaining increasing interest among medicinal chemists.

In the light of these considerations, the talk will present different approaches involving immobilized human cholinesterases (ChEs). Micro-immobilized enzyme reactors (IMERs) can be used in combination with HPLC systems while SPR biosensing technology can be exploited for binding and kinetic investigation. ChE-based IMERs and single or multiple sensing surface(s) can be used in combination as valuable screening tools, which allow to quickly retrieve a set of highly useful information which can assist scientists in the selection of new chemical entities to be further developed.

Keywords
human cholinesterases, automation, bioreactors, sensing surfaces, binding events

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S10-5 Serum cholinesterase activity and Alzheimer disease comorbidities - can bariatric surgery change your sympathetic prone state?

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Alzheimer disease comorbidities, such as hypertension, obesity, metabolic syndrome, diabetes mellitus and inflammation are all associated with impaired sympathetic/parasympathetic response.

Inherited and/or acquired sympathetic prone state, expressed by elevated serum Acetylcholinesterase (AChE) can lead to excessive inflammatory load and cognitive decline.

To evaluate the sympathetic/parasympathetic balance we measured serum cholinesterase activities in stroke, myocardial infarction, diabetes mellitus, morbid obese patients and apparently healthy control. Our findings identify the potential value cholinesterases as possible biomarkers in diseases associated with cerebro-cardiovascular outcome.

Recently we found that serum AChE activity increased with BMI in a dose-dependent manner until it reached a peak level at BMI of 30-35 kg/m², followed by a plateau ($p<0.001$, n=1,450). Similarly, AChE activity increased with waist circumference categories ($p < 0.001$ for men and $P = 0.013$ for women).

The Obesity-related AChE resistance phenotype may be reversed following laparoscopic sleeve gastrectomy (LSG) surgery and correlates with metabolic outcomes (% excess weight loss, %fat, and delta Homeostasis Model Assessment (HOMA)).

Further long-term studies will be needed to validate and evaluate the beneficial effect of AChE reduction post bariatric surgery and its possible relation to cognitive decline.

S10-6 Indazolylketones: hit to lead optimization of a multitarget drugs

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A new family of indazolylketones with a multitarget profile as modulators of cholinergic and BACE-1 enzymes and cannabinoids receptors [1] was designed based on our previous results [2]. We present the synthesis, computational studies and biological evaluation and of a new family of heterocyclic compounds.

Pharmacological evaluation include *in vitro* inhibitory assays in AChE/BuChE enzymes and BACE-1. In addition, functional activity for cannabinoid receptors has been carried out. The results of the pharmacological tests have revealed that some of these derivatives behave as CB2 cannabinoid agonists and simultaneously show BuChE and/or BACE-1 inhibition. Furthermore, studies in human neuroblastoma SH-SY5Y cells and in the lymphoblasts of patients with Alzheimer's disease have shown neuroprotective effects of this family of compounds, as well as their capacity to blunt the abnormal enhanced proliferative activity of AD lymphoblasts. Based on the *in vitro* and functional studies we performed *in vivo* studies of those best compounds employing transgenic mouse (TgAPP) model. The results of the *in vivo* study revealed that some of these compounds could be very promising candidates for the treatment of Alzheimer's disease.

Keywords
Alzheimer's disease, BACE-1 inhibitor, BuChE inhibitor, CB2R agonist, indazolylketone, multitarget drug.

Acknowledgement
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S10-7 Butyrylcholinesterase genetic polymorphism and neuroimaging biomarkers in Alzheimer's disease

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Objective: The influence of butyrylcholinesterase (BChE) genetic polymorphism in Alzheimer's (AD) remains controversial. *BCHE*-K and *BCHE*-A genetic variants cause reduction of BChE, an enzyme implicated in AD. Some studies have reported a protective effect of *BCHE*-K, others suggest increased AD risk, particularly when associated with *APOE4*. We utilized a candidate gene-driven analyses to determine the effects of *BCHE*-K and *BCHE*-A on AD biomarkers using ADNI data (*http://adni.loni.ucla.edu/*).

Methods: Participants were genotyped for *BCHE*-K (615) and *BCHE*-A (785), each stratified into control (C), MCI or AD groups. MRI, ^{18}F-FDG and amyloid-PET were assessed. ANCOVA compared main effects of i)diagnosis, ii)*BCHE*-K, iii)*BCHE*-A and iv)*APOE4* status on each biomarker with age, education and sex as covariates.

Results: The allelic frequency was 20.8%, 4.6% and 26.5% for *BCHE*-K, *BCHE*-A and *APOE4*. For MRI, main effects for diagnosis were significant ($p<0.0001$), with reduction in whole-brain and selected regional volumes (7-27%, $p\leq6x10^{-6}$) in AD vs. C. For ^{18}FDG -PET, the main effect for diagnosis was significant ($p=5x10^{-9}$), with 14% decrease in metabolism in AD vs. C ($p=7x10^{-10}$). For amyloid-PET, the main effects for diagnosis and *APOE4* status were significant ($p=0.034$; $p=3x10^{-6}$), with 12% increase in retention in AD vs. C ($p=0.023$) and 16% increase among carriers of at least one *APOE4* allele vs. non-carriers ($p=8x10^{-6}$). No significant effects of these biomarkers were observed due to *BCHE*-K or *BCHE*-A status ($p\geq0.209$).

Conclusions: These data suggest *BCHE*-K or *BCHE*-A may not significantly effect structural, metabolic or molecular AD biomarkers. Further ROI/voxel-wise analyses are warranted to uncover potential regional changes among AD *BCHE* variants.

Keywords
Alzheimer's disease, butyrylcholinesterase genetic variants, neuroimaging, amyloid-PET, FDG-PET

S11 Multi-target-directed ligands in Alzheimer's disease primarily targeting cholinesterases

Chairs: Maria-Laura Bolognesi (Italy), Ana Martinez (Spain)

S11-1 Case studies for successful combination of ChE inhibitors and GPCR ligands (cannabinoid 2 and histamine 3 receptors)

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The combination of cholinesterase inhibitors with GPCR ligands in hybrid molecules seems highly promising for Alzheimer's disease (AD) therapy, since two very different molecular targets can be addressed at the same time. Nevertheless, significant challenges come with this rationale: a) hybrids might possess too high molecular weights to be orally bioavailable and/or pass the blood-brain-barrier, b) the compounds might act in different concentration ranges, c) and selectivity and affinity has to be optimized for several very distinct targets.

We have designed – applying computational methods - and synthesized dual-acting ChE-inhibitors that act with high potency and selectivity also at the histamine 3 receptor (hH$_3$R) [1], and the same could be achieved for cannabinoid 2 receptors (hCB$_2$R) [2, 3], both GPCRs represent important AD targets. Regarding dual-acting ChE inhibitors and hCB$_2$R agonists both covalently connected hybrids using the unselective ChE inhibitor tacrine as well as merged small molecules with high butyrylcholinesterase (BChE) selectivity have been obtained and pharmacologically characterized *in vitro*. Representative examples from all sets of compounds have been investigated *in vivo* in different AD mice models [3].

The case studies demonstrate that it is possible to obtain dual-acting compounds that a) act highly selectively and with high affinity at the respective targets, b) work in the same concentration range ("balanced affinity"), c) exhibit pronounced *in vivo* activity.

Keywords
GPCR, cannabinoid, histamine, merged ligands, hybrid molecules

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S11-2 From acetylcholinesterase inhibitors to multi-target-directed ligands (MTDLs): a step forward in Alzheimer's disease drug discovery

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Notwithstanding clinical effectiveness evidences continue to suggest benefit from the acetylcholinesterase inhibitors (AChEIs) in alleviating Alzheimer's disease (AD) symptoms, these drugs do not appear to delay or prevent the underlying neurodegeneration. In this context, novel prospects are offered by the strategy of developing single chemical entities able to modulate multiple targets, i.e. the multi-target-directed ligands (MTDLs). On this basis, several multifunctional AChEIs have been rationally designed with the deliberate aim of enlarging their biological profiles, beyond the ability to inhibit cholinesterases. This is because it has been recognized that a balanced simultaneous modulation of multiple targets critically intertwined in AD pathological cascade can provide a superior therapeutic and toxicological profile compared to the action of a selective AChEI.[1]

Building on this founding principle, we and others have developed several series of anti-AD MTDL compounds that combine cholinesterase inhibition with anti-aggregating, anti-oxidant, and anti-neuroinflammatory properties.[2] As a further step, to explore the possibility to discover new MTDLs based on inexpensive resources, we have developed a series of MTDLs obtained by

properly modifying constituents from the cashew nut shell liquid (CNSL), a waste from cashew nut processing factories.[3] Such hybrid compounds, obtained from renewable and inexpensive material, might be promising bio-based, sustainable MTDLs for AD drug discovery.

Working in the field for almost 20 years, we should draw lessons from the past and try our best to chart innovative directions and hopefully address the scientific and societal challenges of neurodegenerative diseases.

Keywords
Alzheimer's disease, amyloid, acetylcholinesterase, multitarget compounds, neuroinflammation.

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S11-3 From selective butyrylcholinesterase inhibitors to multi-target-directed ligands as lead compounds for Alzheimer's disease

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Alzheimer's disease (AD) is characterized by severe basal forebrain cholinergic deficit, which results in progressive and chronic deterioration of memory and cognitive functions. Similar to acetylcholinesterase, butyrylcholinesterase (BChE) contributes to the termination of cholinergic neurotransmission. Its enzymatic activity increases with the disease progression, thus classifying BChE as a viable therapeutic target in advanced AD. Potent, selective and reversible human BChE inhibitors were developed. First, a hierarchical virtual screening was performed followed by biochemical evaluation of highest scoring hit compounds. Three compounds showed significant inhibitory activities against BChE and the best inhibitor was selected for further SAR studies. More than 100 different analogues were synthesized and among them, two compounds were found to be promising lead compounds as they were not cytotoxic, they crossed the blood-brain barrier and improved memory, cognitive functions and learning abilities of mice in a model of the cholinergic deficit that characterizes AD, without producing acute cholinergic adverse effects. The solved crystal structures of human BChE in complex with the most potent inhibitors revealed their binding modes and provided the structural basis for their further development into multi-target-directed ligands, which in addition to good inhibition of BChE possess good antioxidant, metal chelating, neuroprotective and other properties beneficial for AD.

Keywords
Alzheimer's disease, butyrylcholinesterase, multi-target-directed ligands.

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S11-4 Discovery and characterization of tacrine/huprine-tryptophan heterodimers as novel multipotent compounds against Alzheimer's disease

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Combination of tacrine/huprine, connected through a different linker tether length, with tryptophan led to the generation of a novel, highly-potent family of multi-target directed ligands targeting key molecular mechanisms of Alzheimer's disease. Based on *in vitro* biological profile, the 6-chloro-tacrine-$(CH_2)_6$-*L*-tryptophan heterodimer *S*-K1035 was found to be the most potent inhibitor of human acetylcholinesterase (*h*AChE) and human butyrylcholinesterase (*h*BChE) within the series, with nanomolar IC_{50} values (6.31 and 9.07 nM, respectively). Moreover, *S*-K1035 showed good ability to inhibit $A\beta_{42}$ self-aggregation and *h*AChE-induced $A\beta_{40}$ aggregation. The X-ray crystallographic analysis of *Tc*AChE in complex with *S*-K1035 highlighted the utility of the hybridiza-

tion approach used in the structure based drug design. *S*-K1035 also exerted moderate inhibition against neuronal nitric oxide synthase (nNOS). *In vivo* studies displayed low toxicity profile compared to parent tacrine. *S*-K1035 also significantly ameliorated performances of scopolamine-treated animals.

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S11-5 Novel conjugates based on γ-carbolines, carbazoles, phenothiazines, and aminoadamantanes as multifunctional agents for Alzheimer's disease treatment

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Neurodegenerative diseases are multifactorial. Therefore, their treatment requires drugs that can act simultaneously on multiple pathogenic targets. We synthesized several series of hybrid structures combining certain pharmacophores essential for neurodegenerative disease treatment: γ-carbolines, carbazoles, phenothiazines, and aminoadamantanes [1-3]. Inhibitory activity of these conjugates against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and carboxylesterase (CaE) was studied along with their ability to competitively displace propidium iodide from the peripheral anionic site of electric eel AChE to assess their potential effect on AChE-induced aggregation of β-amyloid. Antioxidant properties were examined computationally with density functional theory and measured experimentally using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and oxygen radical absorbance capacity (ORAC-FL) assays. Binding modes of conjugates to AChE and BChE were studied using quantum mechanical-assisted molecular docking. Results revealed structures that were selective inhibitors of BChE [1,2] or that combined high potency and selectivity toward BChE with high radical-scavenging activity, e.g., conjugates of γ-carbolines and tetrahydrocarbazoles [3]. Conjugates of γ-carbolines and cycloalcaneindoles with the phenothiazine derivative Methylene Blue demonstrated high potency against AChE and BChE combined with effective displacement of propidium from the peripheral anionic site of AChE. Additionally, the conjugates were extremely active in both antioxidant tests. All conjugates were poor CaE inhibitors and therefore expected to lack drug-drug interactions by this pathway. Good agreement was found between experimental and computational results. Lead compounds were identified for future optimization and development of new multi-target drugs against neurodegenerative diseases that combined cognition enhancement with neuroprotective potential.

Keywords
Alzheimer's disease, multifunctional agents, γ-carboline, phenothiazine, aminoadamantane

Acknowledgement
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S11-6 Pleiotropic prodrugs: a novel polypharmacology approach to treat neurodegenerative diseases

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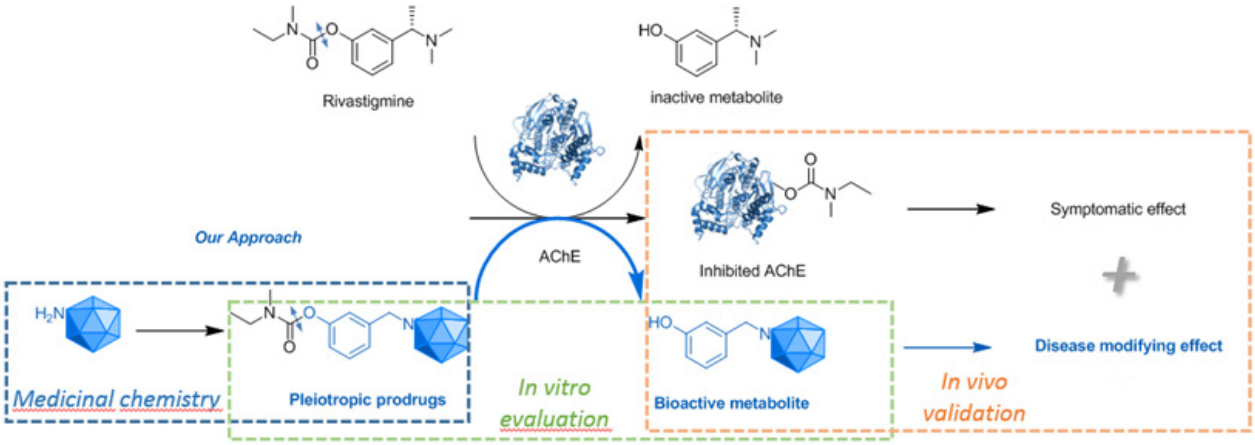
Today, treatment of Alzheimer's Disease (AD) mainly involves acetylcholinesterase inhibitors (AChEIs). AChEIs display solely a symptomatic benefit, alleviating the cognitive disorders associated to AD through a temporary restoration of the cholinergic neurotransmission impaired by the neurodegeneration. The gradual loss of efficiency for AChEIs led to associate them to drugs exhibiting potential disease-modifying properties.

The "Multi-Target-Directed Ligands" (MTDLs) was used in the recent years with a great potential benefit towards multiple targets implicated in the complex AD,[1] as well as other neurodegenerative syndromes, which involve multiple pathogenic factors.

Our contribution to the field led recently to the discovery of Donecopride, the first 5-HT_4R partial agonist, which possesses important acetylcholinesterase (AChE) inhibition properties currently under preclinical development.[2,3] Based on this experience, we have recently developed a novel pleiotropic prodrugs approach to generate promising in vivo active compounds. Based on the structure of rivastigmine, novel MTDLs were designed, acting as prodrugs, able to temporarily covalently bind and inhibit AChE (for a symptomatic effect). and to secondarily release a drug able to selectively reach another AD target (for a potential disease-modifying effect)



This concept was applied to several secondary targets, including different 5-HT receptors of interest[4] for the treatment of AD. The concept, the synthetic development, *in vitro* and *in vivo* evaluation of these candidates and our undisclosed results will be presented for the first time in this communication.

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S11-7 Toward an innovative treatment of Alzheimer's disease: Design of multi-target directed ligands (MTDLs) targeting acetylcholinesterase (AChE) and alpha-7 nicotinic receptors (alpha-7 nAChRs)

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Alzheimer's disease (AD) is a complex and progressive neurodegenerative disorder. The available therapy is limited to the symptomatic treatment and its efficacy remains unsatisfactory [1]. In view of the prevalence and expected increase in the incidence of AD, the development of an effective therapy is crucial for public health. Due to the multifactorial etiology of this disease, the multi-target-directed ligand (MTDL) approach is a promising method in search for new drugs for AD. Aiming at developing new MTDLs, this project consists on the development of new multifunctional agents, which will act simultaneously on the different players in AD pathology. The project aims at developing MTDLs by combining an AChE inhibitory activity with an alpha-7 nAChR activation [2].

Keywords
Alzheimer's disease, MTDLs, AChE, nAChR

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S11-8 Molecular modeling in search of new, multi-target ligands against Alzheimer's disease. Exploring the biochemical multiverse.

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In response to the complex and still not fully understood pathomechanism of Alzheimer's disease, many researchers have turned towards the promising paradigm of designing ligands with a multi-target nature[1]. One of the possible benefits of this approach in

Alzheimer's disease is an opportunity to merge activity against cholinesterases, which are used in the current symptomatic therapies, with disease-modifying targets associated with β-amyloid and tau protein pathways[2]. Optimization of ligand with respect to several biological targets while maintaining good physicochemical parameters is not an easy task. Computer modeling can be a huge help in this task. Computer modeling in the design of biologically active substances can be used to effectively search through the huge, available chemical space, or provide support for drawing conclusions of results obtained during the study[3]. In the work presented here, we would like to describe how the molecular modeling methods were used to design and obtain new series of 1-benzylamino-2-hydroxyalkyl derivatives that are effective against both acetyl- and butyrylcholinesterase as valid, symptomatic targets with an anti-aggregating properties against Tau protein, β-amyloid and inhibition properties against β-secretase (BACE-1) as disease-modified targets[4].

Acknowledgments

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S12 Varia

Chairs: Kamil Kuca (Czech Republic), Ondrej Soukup (Czech Republic)

S12-1 Design of a butyrylcholinesterase mutant for detoxifying cocaine and its toxic metabolites in concurrent use of cocaine and alcohol

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Cocaine abuse is a major medical and health problem. There is no FDA-approved medication for treatment of cocaine overdose and addiction. Statistical data show that 92% of cocaine users also consume alcohol. The risk of immediate death is 18 - 25 times greater for cocaine co-ingested with alcohol than for cocaine alone. Alcohol can react with cocaine to get a series of toxious compounds in body including cocaine, cocaethylene, norcocaine, norcocaethylene and benzoylecgonine.

In combination of our "virtual screening of transition states" computational protocol and artificial intelligence, a novel approach was used to design BChE mutants as multiple functional cocaine hydrolases (mfCocHs) for treatment of toxicity caused by concurrent use of cocaine and alcohol. Comparing the kinetic parameters of *native human BChE* and mf*CocH* against cocaine as well as its four toxic/harmful metabolites (i.e. norcocaine, cocaethylene, norcocaethylene and benzoylecgonine) determined by us, the most effective mfCocH has at least a ~1000-fold improved catalytic efficiency against three of the substrates (cocaine, norcocaine, and cocaethylene), ~100-fold and ~10-fold improved catalytic efficiency against norcocaethylene and benzoylecgonine, respectively.

In vivo studies have revealed that the mfCocH can effectively hydrolyze cocaine and its four metabolites in rats produced from the concurrent abuse of cocaine and alcohol in both addiction and overdose models. The mfCocHs was powerful antidote to treat cocaine (w/ or w/o alcohol) induced toxicity, even from the lethal toxicity after co-administrated 1 g/kg alcohol (IP) and 180 mg/kg cocaine (IP), at any time point as long as the subject is alive before treatment.

S12-2 7-Methoxyderivative of tacrine is a 'foot-in-the-door' blocker of GluN1/GluN2 and GluN1/GluN3 NMDA receptors

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N-methyl-D-aspartate receptors (NMDARs) are glutamate-gated ion channels that mediate excitatory neurotransmission in the mammalian central nervous system (CNS), but their dysregulation results in the aetiology of many human CNS disorders. Several NMDAR modulators including memantine have been used successfully in clinical trials. Indeed, 1,2,3,4-tetrahydro-9-aminoacridine (tacrine; THA) was the first approved drug for Alzheimer's disease (AD) treatment. 7-methoxyderivative of THA (7-MEOTA) is less toxic and showed promising results in patients with tardive dyskinesia. Here, we employed electrophysiological recordings in HEK293 cells and rat neurones to examine the mechanism of action of THA and 7-MEOTA at the NMDAR. We showed that both THA and 7-MEOTA are "foot-in-the-door" open-channel blockers of GluN1/GluN2 and GluN1/GluN3 NMDARs and that 7-MEOTA is a more potent but slower blocker than THA. Furthermore, the inhibitory potency of 7-MEOTA at synaptic and extrasynaptic hippocampal NMDARs was similar, and 7-MEOTA exhibited better neuroprotective activity in rats exposed NMDA-induced lesions in hippocampus when compared with THA and memantine. Finally, intraperitoneal administration of 7-MEOTA attenuated MK-801-induced hyperlocomotion in rats. We conclude that 7-MEOTA is a promising candidate for the treatment of diseases associated with the dysfunction of NMDARs.

Keywords
glutamate receptor; patch-clamp technique; inhibitor; excitotoxicity.

Acknowledgement
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S12-3 The *Caenorhabditis elegans* pharynx as a model system to investigate and mitigate against the effects of anti-cholinesterase drugs

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C. elegans is a free-living worm widely used as model to study neurotoxicology. Despite its simplicity, *C. elegans* has a high level of genetic and molecular conservation with vertebrates. Similar to mammals, intoxication with anti-cholinestereses triggers the accumulation of synaptic acetylcholine causing continuous stimulation of both nicotinic and muscarinic receptors, hypercontracting the muscles of the worm[1]. The pharynx, the nematode feeding organ, depends on cholinergic function. Pharyngeal movements, readily observed in whole organism, are disrupted by impairments in cholinergic transmission. Therefore, quantitative analysis of pharyngeal structure and function has excellent potential to probe anti-cholinesterase mode of action that may translate to human toxicology.

We establish the IC_{50} values for the carbamate aldicarb and the organophosphates paraoxon-ethyl, paraoxon-methyl and DFP, highlighting a distinct dose-time dependence inhibition of pharyngeal activity. In recovery experiments, aldicarb and paraoxon-ethyl but not paraoxon-methyl or DFP intoxicated worms recover the pharyngeal function onto empty and oxime plates. A cycle of aldicarb intoxication-recovery-intoxication revealed aldicarb-induced plasticity as a reduced sensitivity of pre-conditioned worms to a subsequent drug exposure. We investigated molecular determinants of this plasticity by using uncoordinated locomotion and reduced pharyngeal movement mutant worms due to impairments in cholinergic transmission. Interestingly, preconditioned mutant worms exhibits a switch in the aldicarb-induced plasticity observed in wild type, becoming more sensitive to post-exposure of aldicarb. Defining the molecular identity of this mutant will reveal pathways that mediate cholinesterase induced structural reorganization at the pharyngeal NMJ. Thus, the drug and genetic tractability of *C. elegans* offers a new route to anti-cholinesterase poisoning antidotes.

Keywords
anti-cholinesterase intoxication, cholinergic plasticity, *C. elegans*

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S12-4 Pharmacokinetics of bis-pyridinium mono-aldoximes

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Bis-pyridinium mono-aldoxime (BPMA) compounds are potential antidotes against organophosphorus inhibitors of either acetylcholinesterase or these of butyrylcholinesterase. From the points of drug distribution and pharmacokinetics essential characteristics were determined (concentration versus time curves).

Experimental results of pharmacokinetics of BPMA will be detailed with special focus on drug distribution and HPLC analysis of oxime K117.

The concentration of BPMAs decreases fast in the body of rats, and thus they fulfil the basic requirement for antidotes: elimination should be as fast as possible. Their elimination curve should be characterized by the term „tenth-life" rather than half-life.

BPMA compounds penetrate into the brain in considerable amounts of their concentration in the serum. As blood-brain penetration can have vital importance, time of the maximum extent of blood-brain barrier should also be conceived as a novel pharmacokinetic parameter.

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