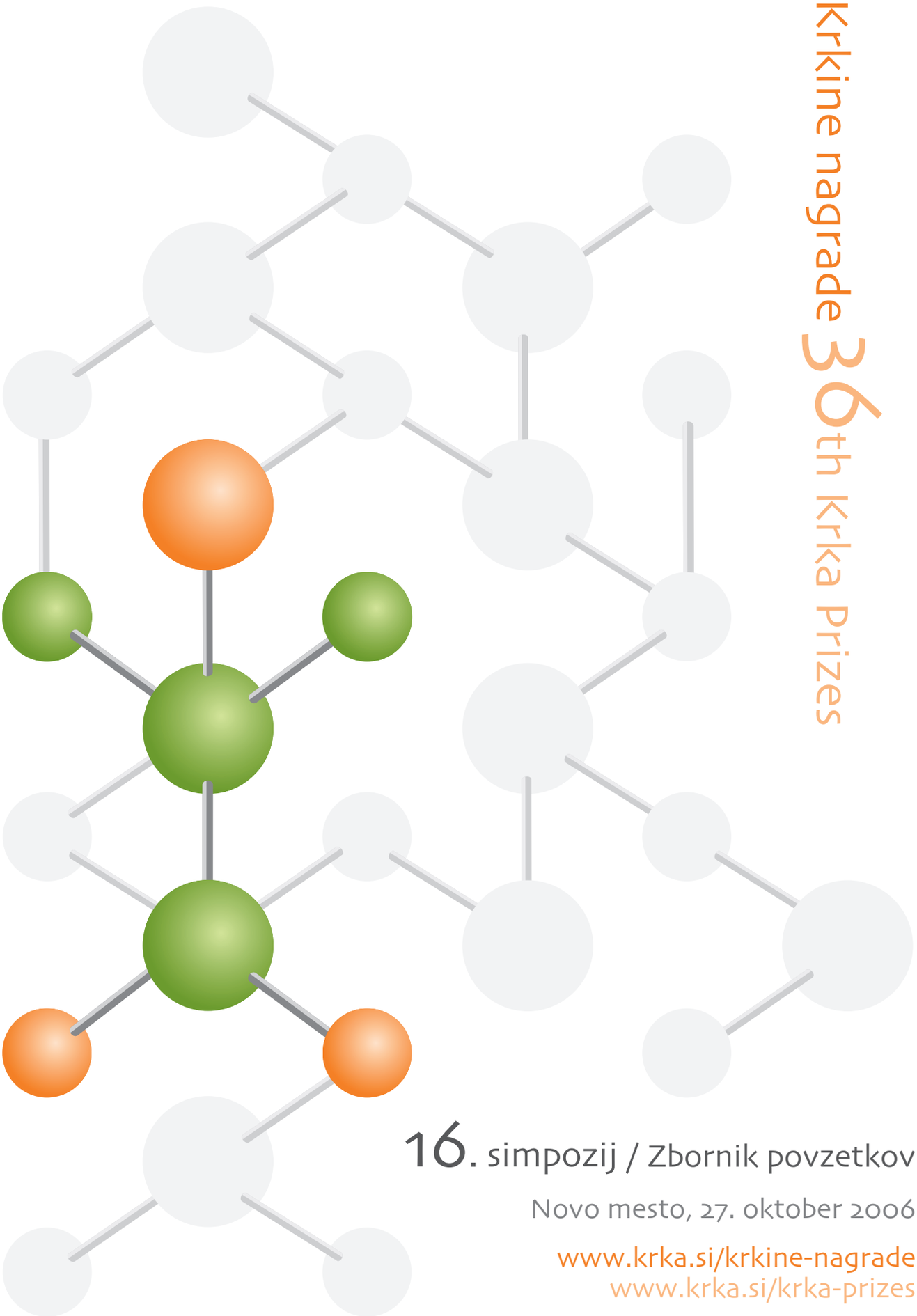
36. Krkine nagrade 36th Krka Prizes



16. simpozij / Zbornik povzetkov

Novo mesto, 27. oktober 2006

www.krka.si/krkine-nagrade
www.krka.si/krka-prizes

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VSEBINA / CONTENTS

36. Krkine nagrade / 36th Krka Prizes

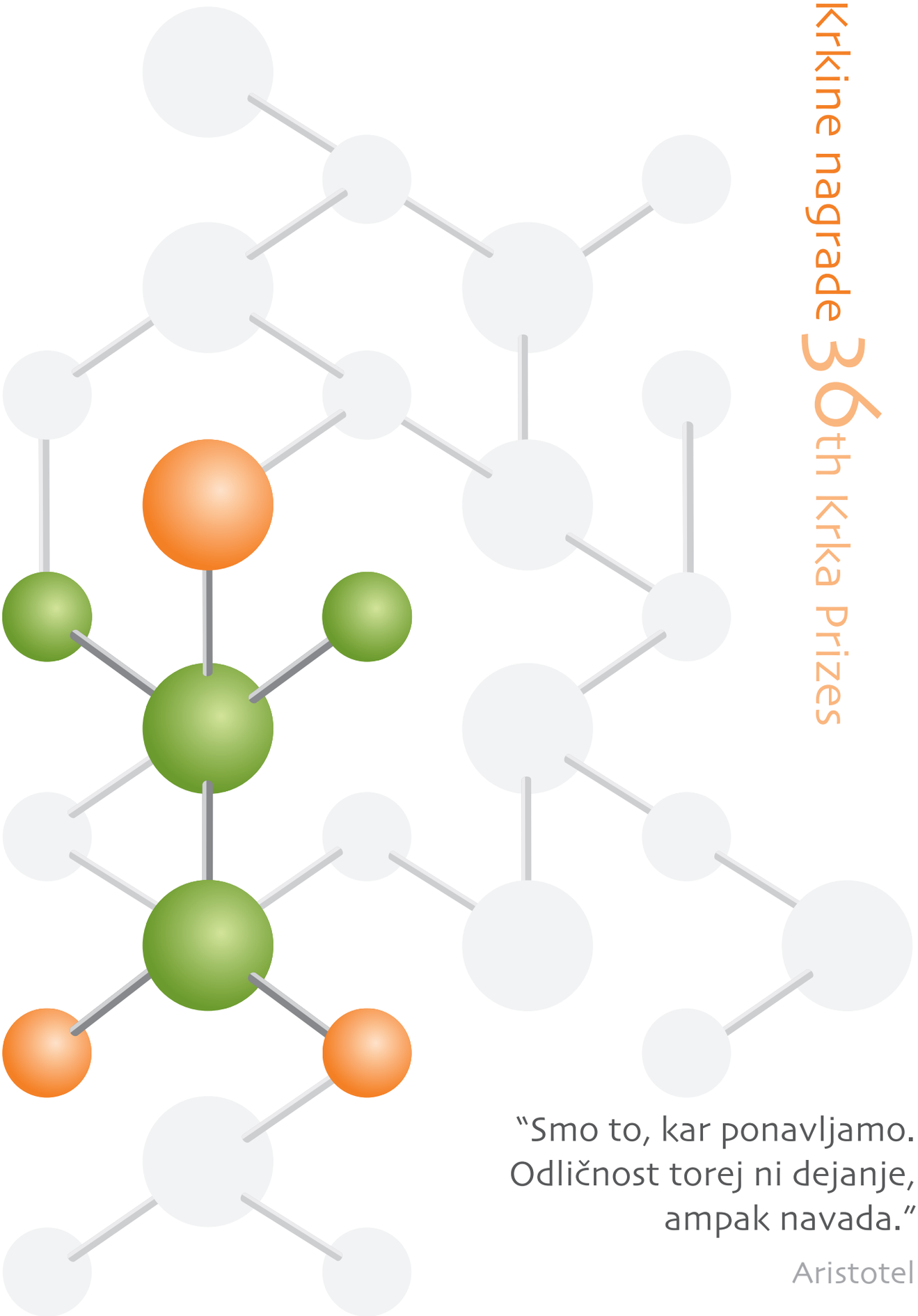
16. simpozij / 16th Symposium
Zbornik povzetkov / Book of Abstracts

Krkine nagrade za posebne dosežke na področju raziskovalnega dela / Krka Prizes for Special Achievements in Research

Krkine nagrade / Krka Prize

E - Krkine nagrade / e - Krka Prize Winners

36. Krkine nagrade 36th Krka Prizes



"Smo to, kar ponavljamo.
Odličnost torej ni dejanje,
ampak navada."

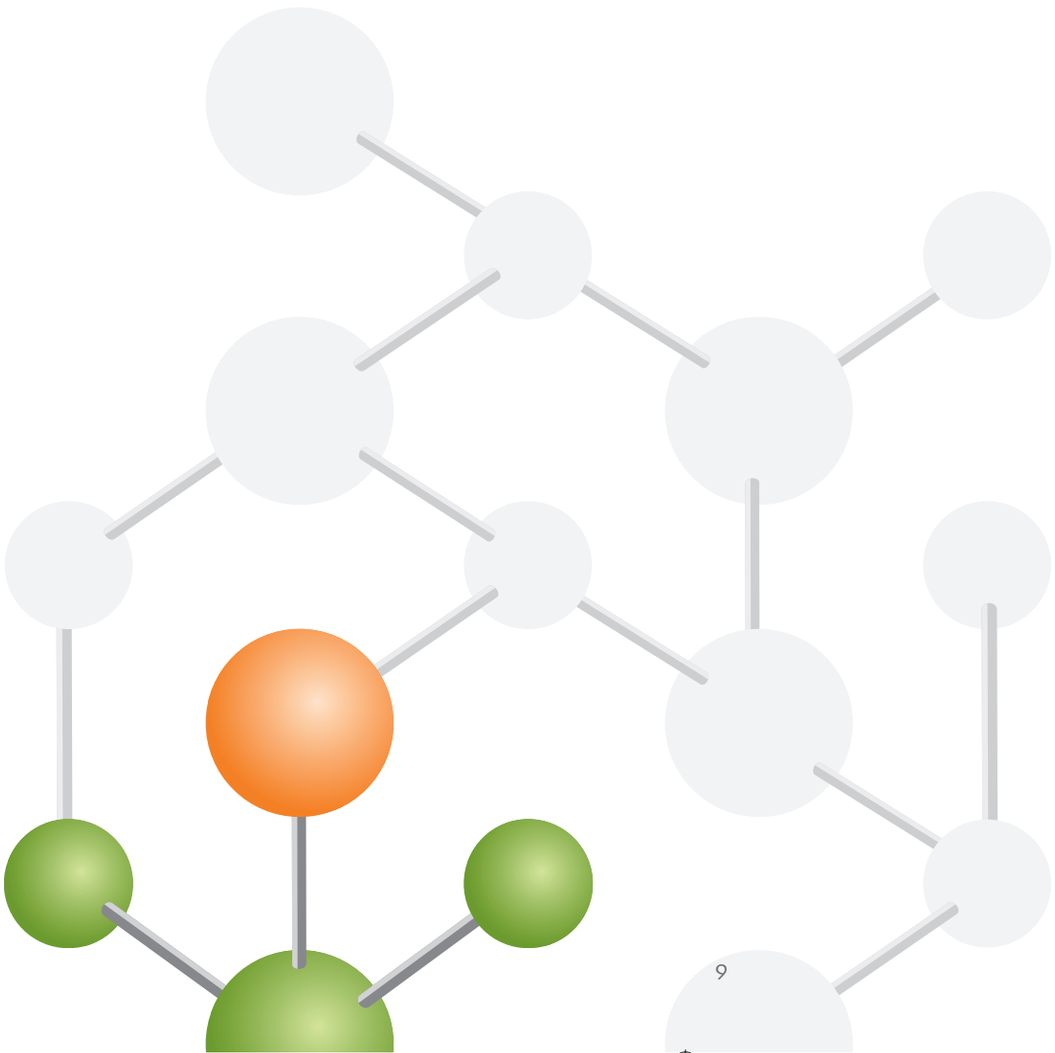
Aristotel

UVODNE BESEDE

Kakor so za pričetek in izdelavo raziskav potrebni preblisk, pronicljivost in vztrajnost, tako so za pravilen izbor potrebne izkušnje, znanje, pa tudi pogled z nekega posebnega zornega kota. Prav vrednotenje razvojnih in raziskovalnih dosežkov je ena od ključnih tem današnjih pogovorov o znanosti. Kadar govorimo o dosežkih posameznih raziskovalcev ali o celotnih raziskovalnih organizacijah, opazujemo in ocenjujemo rezultate glede na naše cilje. Krka je kot razvojno naravnano podjetje življenjsko vezano na raziskovalno delo, tako v hiši kakor pri mnogih naših zunanjih sodelavcih. Te sodelave si želimo še več. Prav tako pa si prizadevamo, da bi nam bila bližje, da bi se vse sposobnosti mladih znanstvenikov čimprej odrazile v naših rezultatih. Zato smo še posebej veseli, da je mnogo nagrajenih nalog vedno bližje našim uporabnikom. Prepričani smo namreč, da neposredno uporaben rezultat ni le dobra novica za podjetje. Še pomembnejše je, da novi izdelki in tehnologije omogočajo kvalitetnejše in zdravo življenje našim uporabnikom: pacientom, zdravnikom, farmacevtom, skratka vsem tistim, ki jim je zdravje prvi in najpomembnejši cilj. Toda najpomembneje je, da energijo in modrost vsi od mladih raziskovalcev do mentorjev usmerimo v pravilen, tvoren razvoj vsakega znanstvenika posebej. Samo rezultati, ki nagrajujejo s svojo uporabnostjo in kakovostjo so tisti, ki nas vse, zlasti vas, ki ste na začetku svoje poti, nezgrešljivo peljejo naprej.

V pričujočem zborniku predstavljamo dela Krkinih nagrajencev, tistih, ki jih je po skrbnem preudarku in recenzijah številnih strokovnjakov izbral Znanstveni odbor.

Dr. Aleš Rotar
predsednik Sveta
Sklada Krkinih nagrad



OPENING WORDS

Just as inspiration, keen intellect, and persistence are required for the initiation and elaboration of a research project, experience, knowledge and a special perspective are necessary to make a proper selection of the most significant research contributions. It is exactly the evaluation of research and investigative achievements that is one of the core topics of the present-day discussions on science. When discussing achievements of individual researchers or research organisations, we observe and evaluate the results of research work in relationship to our goals. In Krka, as a research-oriented company, research work is of essential importance, both that conducted as an inside activity and that contributed by our many external associates the number of which we wish will be further increasing. Another goal our company is striving to achieve is to bring research nearer to the company, to make the creative potential of young researchers soon reflect in the company's results. It is thus a great pleasure for us to see that an increasing number of the awarded research projects are approaching the needs of our customers. We are convinced that research that is directly applicable is much more than good news for the company. What is more important, with new products and technologies we can improve the quality of life and health of our customers: the patients, the physicians, the pharmacists, and of all those to whom health is the first and most important concern; but, above all, it is our primary goal to direct our energy and wisdom, from young researchers to mentors, towards a creative development of each single scientist. Only results rewarding through their applicability and quality provide the right way to further progress to us all and, in particular, to you, young researchers beginning their academic course.

These proceedings present the work of Krka Prize winners chosen by the Scientific Board after careful consideration and review by experts.

dr. Aleš Rotar
President of the Krka
Prizes Foundation

KRKINI NAGRAJENCI 2006 / KRKA PRIZE WINNERS 2006

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2101	Aleksander Berus	dijak	Stanka Florijančič
2102	Alenka Bevk	študentka farmacije	Tea Lanišnik Rižner Stanislav Gobec
2103	David Bevk	doktorand	Branko Stanovnik
2104	Matija Breznik	diplomant kemije	Ksenija Kogej
2105	Valerija Brezovar	dijakinja	Jana Goršin Fabjan
2106	Mateja Cegnar	doktorandka	Julijana Kristl Janko Kos
2107	Jožica Dolenc	doktorandka	Jože Koller
2108	Sana Fajdiga	doktorandka	Romana Marinšek Logar Mojca Narat
2109	Aljaž Godec	študent farmacije	Miran Gaberšček
2110	Ivana Herak	dijakinja	Jana Goršin Fabjan
2111	Darja Herman	doktorandka	Vita Dolžan Katja Breskvar
2112	Marko Kastelic	dijak	Vesna Krošelj Stanislava Florijančič
2113	Andreja Kavčič	diplomantka Biotehniške fakultete	Aljoša Bavec
2114	Nina Kermc	diplomantka kemije	Darinka Brodnjak-Vončina Mitja Kolar Matej Stergar
2115	Matjaž Kirn	diplomant Biotehniške fakultete	Peter Raspor Hrvoje Petković
2116	Mitja Kolenc	dijak	Jana Goršin Fabjan
2117	Simon Kovačič	dijak	Marko Štefanič
2118	Damjan Krajnc	doktorand	Peter Glavič
2119	Jaka Kranjc	dijak	Stanka Florijančič

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	2120	Katarina Kristan	doktorandka	Gregor Anderluh
	2121	Simona Legiša	dijakinja	Vesna Krošelj Stanislava Florijančič
	2122	Barbara Luštek	diplomantka Filozofske fakultete	Marjeta Vrbinc
	2123	Črt Malavašič	magister farmacije	Branko Stanovnik Jurij Svete
	2124	Uroš Maver	študent farmacije	Marjan Bele Stanko Srčič
	2125	Iva Ocvirk	dijakinja	Marinka Kovač
	2126	Ljerka Ožbolt	magistrica	Samo Kreft Vekoslava Stibilj
	2127	Mateja Pate	doktorandka	Branko Krt Matjaž Ocepek
	2128	Nataša Pavšelj	doktorandka	Damijan Miklavčič Véronique Préat
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	2133	Nina Slapar	doktorandka	Kristina Gruden Borut Štrukelj
	2134	Matej Smolič	dijak	Marinka Kovač
	2135	Žiga Sovič	magister farmacije	Metoda Lipnik-Štangelj
	2136	Primož Šket	doktorand	Janez Plavec
	2137	Aleš Špes	diplomant Biotehniške fakultete	Peter Raspor Hrvoje Petković
	2138	Žiga Špiclin	študent elektrotehnike	Boštjan Likar
	2139	Katja Trontelj	magistrica	Damijan Miklavčič Vladka Čurin Šerbec
	2140	Darko Uršič	magister farmacije	Albin Kristl Simon Žakelj

	Št.	Nagrajenci	Status	Mentor / Somentor(ji)
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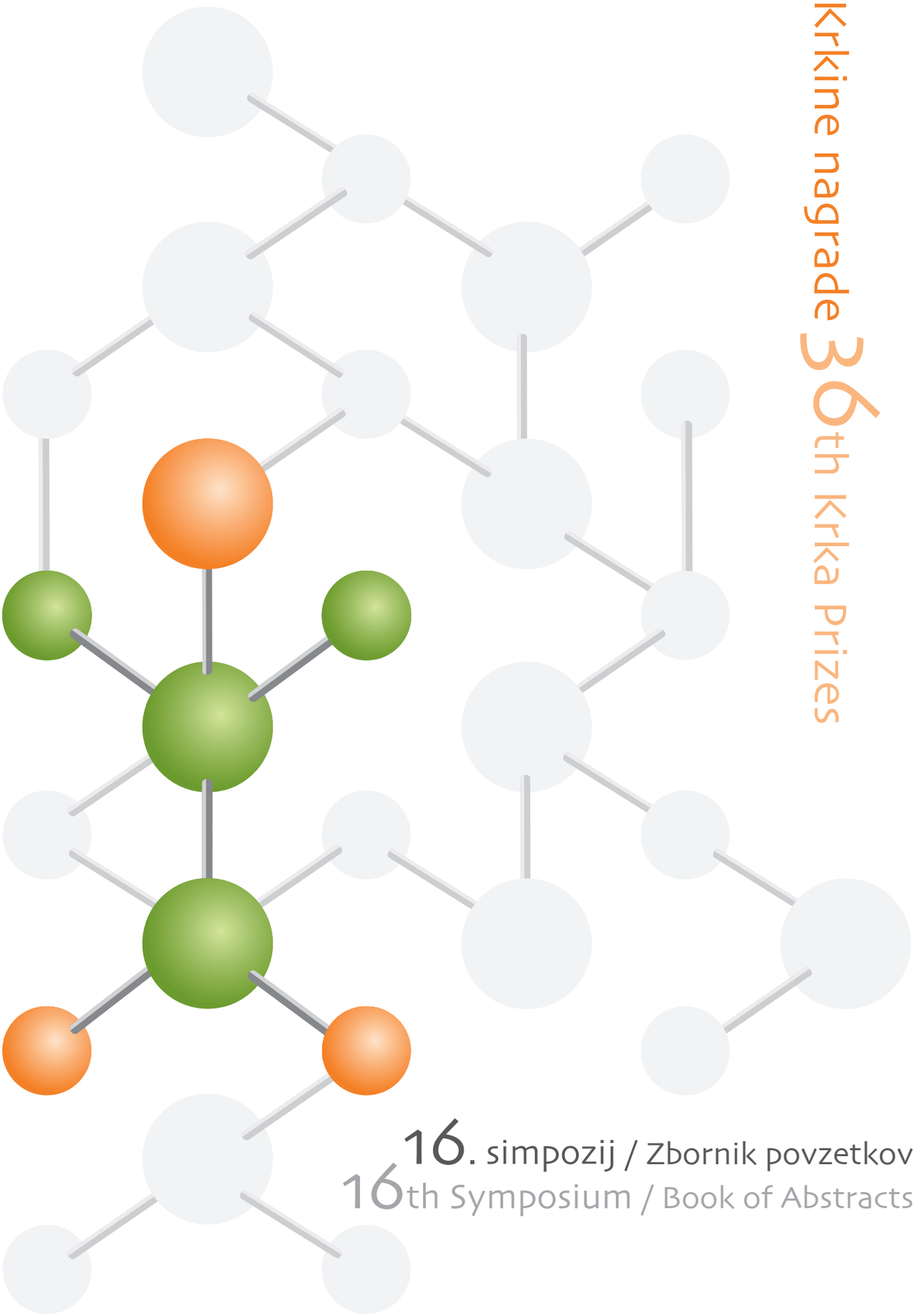
	2142	Matej Vehovc	magister farmacije	Stanislav Gobec Bogdan Štefane
	2143	Andrej Virag	magister	Slovenko Polanc
	2144	Jernej Zadnik	doktorand	Odon Planinšek Franc Vrečer
	2145	Peter Zajc	dijak	Marinka Kovač
	2146	Borut Zupančič	študent kemije	Michael Stephan Barbara Mohar
	2147	Jernej Zupančič	dijak	Stanka Florijančič

36. Krkine nagrade 36th Krka Prizes



16. simpozij / Zbornik povzetkov
16th Symposium / Book of Abstracts

ODLIČNOST, PROFESIONALIZEM, ODGOVORNOST DO BOLNIKOV IN DRUŽBE

Mark Twain je napisal: »Če vedno delate prav, boste nekatere razveselili, preostale pa začudili.«

Kaj se dogaja z veliko farmacevtsko industrijo v svetu? V Ameriki je bistveno izgubila na ugledu. Velike firme kupujejo manjše, se združujejo, jih prevzemajo prijateljsko in sovražno. Za bolnike pa je bistveno, da se je raziskovalna produktivnost zmanjšala. V letih okrog 1960 so lansirali 80-100 pomembnih novih zdravil letno, v letih okrog 1980 50-60 in v letih okrog 1990 30-40. Raziskovanje tržišča je skoraj vedno zgrešilo pri napovedih o velikih novostih (blockbuster) npr. za zaviralce receptorjev beta, antagoniste H2, inhibitorje ACE, zaviralce serotoninskega privzema, omeprazol itd. V farmacevtski industriji je eden od principov razmišljanja, dela in vodenja sledenje odličnosti. Gotovo to vključuje kakovost, zanesljivost in stalno izboljševanje. Kasneje se je prizadevanje po odličnosti razširilo na profesionalizem.

Profesionalizem pomeni lastnosti, standarde, obnašanje in metode v nekaterih profesijah. Ne gre za katerikoli poklic, ampak za profesionalce, ki opravljajo delo ekskluzivno, kompleksno in s posebnimi pooblastili.

Farmacevtska industrija je industrija, ki najbolj vpliva na obnašanje, počutje človeka. Zato ni nenavadno, da je razen letalske industrije najbolj regulirana industrija. Delo profesionalcev je posebej pomembno za družbo in ga ni mogoče povsem poplačati z denarjem. Profesionalno delo vključuje dva posebna elementa, privrženost znanju in spretnostim ter vzdrževanje zaupnega odnosa s strankami. Profesionalizem in profesionalnost postajata življenjski interes, zagotavljata notranje priznanje in pomenita tudi družbeno vrednoto.

Od leta 1994 do 2004 je Slovenija namenila za raziskave in razvoj okrog 1,5 % družbenega proizvoda, objavila 3365 prispevkov v mednarodno priznanih revijah. Ti so bili 19.691-krat citirani, to znaša 9,9 citatov na 1000 prebivalcev. V celoti stanje ni slabo, mednarodna zastopanost pa je zelo različna po strokah. Zagotovo pa slabša od držav, s katerimi se želimo primerjati.

Etično odločanje temelji na znanju. Znanje pa je treba uporabljati ustvarjalno in družbeno odgovorno. Inovativno mišljenje z odprtostjo za novo ni nujno samo v veliki in bogati ameriški farmacevtski firmi, ampak tudi v generični in vsaki ambulanti družinske medicine. Le domišljeni projekti in naloge bodo zagotavljali učinkovito in varno zdravljenje, dolgo življenjsko dobo zdravilnih učinkovin ter profesionalno integriteto vseh udeleženih. Izsledki kliničnih raziskav z zdravili danes niso samo preprosti medicinski izidi, ampak govorimo tudi o njihovi družbeni ceni in družbeni koristi ter vplivu na kakovost življenja.

Prof. dr. Jože Drinovec
predsednik Znanstvenega odbora

EXCELLENCE, PROFESSIONALISM, AND RESPONSIBILITY TO PATIENTS AND SOCIETY

Mark Twain wrote: "Always do right. That will gratify some of the people, and astonish the rest."

What is happening with the big pharmaceutical industry? In the USA, it has lost much of its reputation. Big companies are buying smaller ones, they are merging, or they are subject to friendly or hostile takeover. And, what is most important for the patients, research productivity has decreased. In the period around 1960, eighty to hundred important new medicinal products were launched annually. In the period around 1980 their number fell to fifty to sixty, and in the period around 1990 it further fell to thirty to forty. Market research almost always failed in predicting blockbusters, such as in the case of beta-receptor inhibitors, H2-antagonists, ACE inhibitors, serotonin reuptake inhibitors, or omeprazole. One principle of thinking, working, and leading in pharmaceutical industry is to follow the path of excellence. Certainly this involves quality, reliability, and ongoing improvements. Striving for excellence gave rise to professionalism.

Professionalism stands for characteristics, standards, behaviour, and methods used in certain professions. It is not implied in every occupation but attributed to professionals, whose carrying out of their profession is exclusive and complex and under special authorisation.

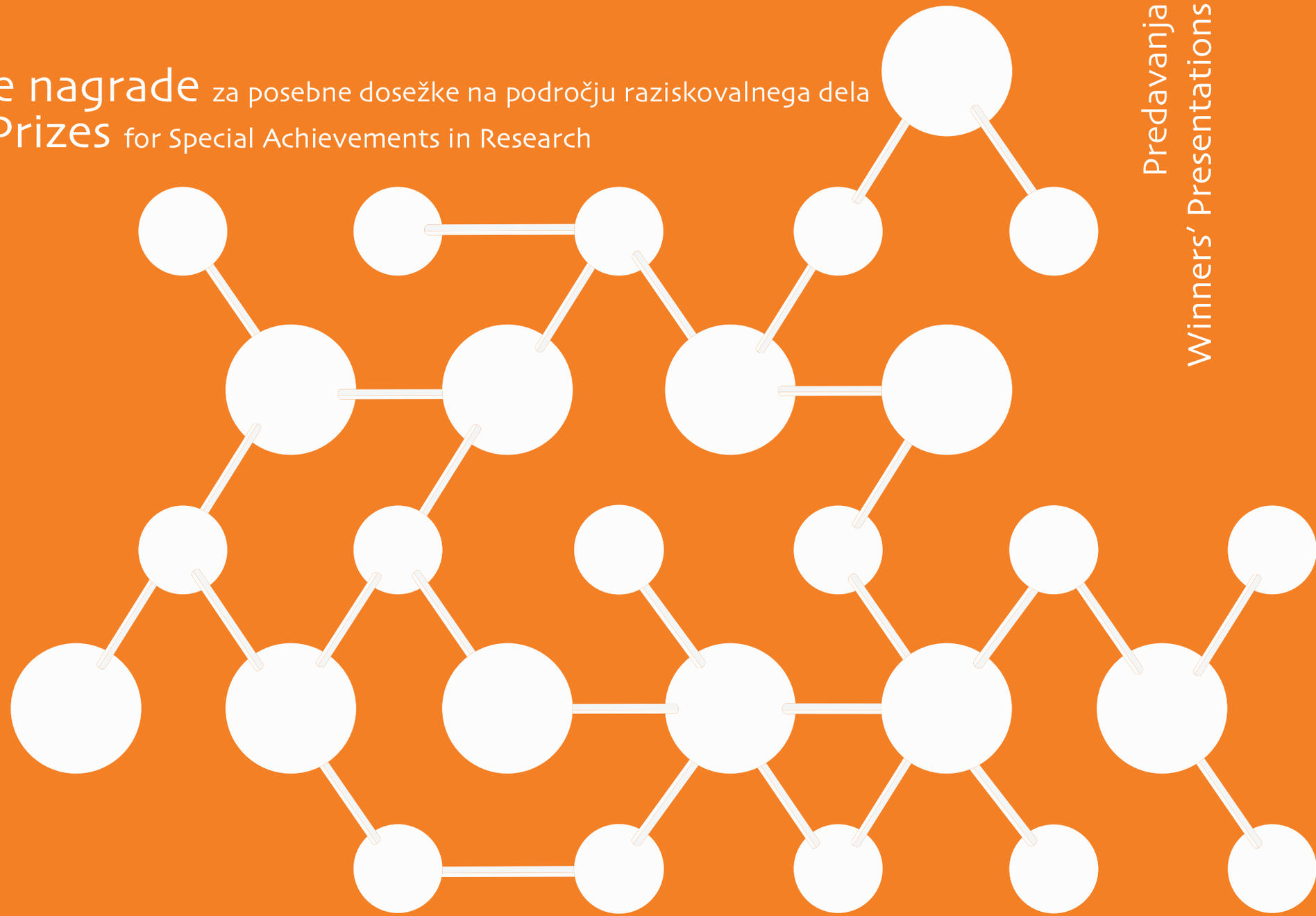
The pharmaceutical industry is an industry that most strongly affects the behaviour and well-being of people. It is therefore not surprising that it is, except for the aircraft industry, the most regulated industry. The work performed by professionals is of special importance for the society and cannot be fully compensated with money. It involves two special elements – dedication to knowledge and skill and maintenance of a relationship of trust with the customers. Professionalism and professionality are becoming a central life interest; they bring an inner recognition of one's worth and are looked upon as a societal value. .

In the period between 1994 and 2004, Slovenia earmarked 1.5 percent of the gross product for research and development and contributed 3365 articles to internationally recognised journals. The number of citations of these papers by other authors was 19,691., i.e. 9.9 citations per 1000 inhabitants. Overall, the situation is not bad. However, international representation is greatly varying between disciplines, and is definitely poorer as in those countries which we wish to compare with.

Ethical decisions are grounded in knowledge. And knowledge should be used creatively and with the responsibility to the society. Innovative thinking and openness to new ideas is of essential importance, not only in a wealthy American pharmaceutical company but also in a generic company and in every family practice. Only with elaborate projects and tasks we shall be able to maintain effective and safe medical treatment, long life of active substances and professional integrity of participants in this process. Today, findings of clinical studies are not only simple treatment outcomes but involve many aspects of societal cost, social benefit and the quality of life.

Prof. Jože Drinovec
President of Scientific Board

Krkine nagrade za posebne dosežke na področju raziskovalnega dela
Krka Prizes for Special Achievements in Research



Predavanja
Winners' Presentations





DIMETILAMINOMETILIDENSKI DERIVATI DIALKIL ACETONDIKARBOKSILATOV V SINTEZI HETEROCIKLIČNIH SISTEMOV

D. Bevk
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani
Mentor: B. Stanovnik
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani

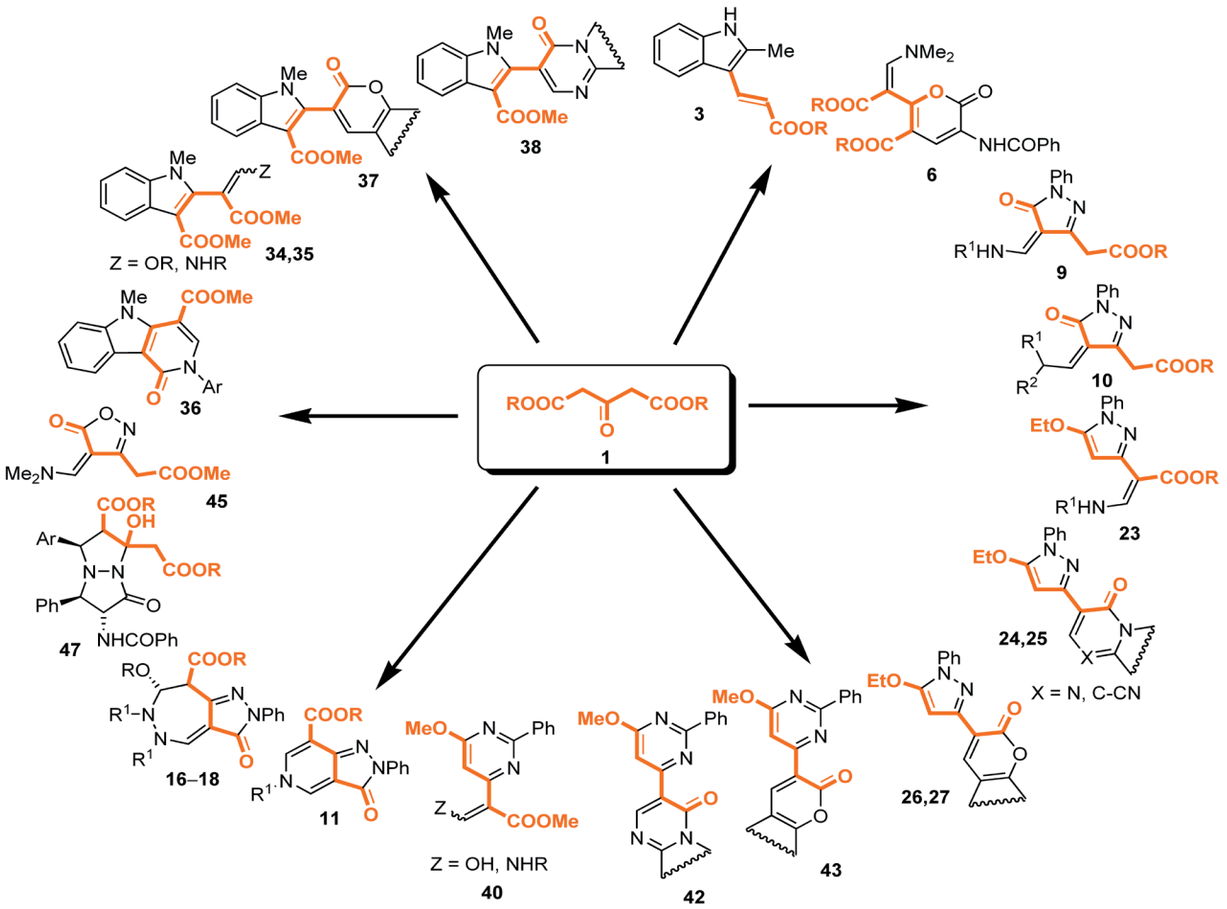
Tekom doktorskega študija smo poskusili uporabiti acetondikarboksilate **1** v sintezi heterocikličnih sistemov s pomočjo enaminonske metodologije. Najprej nas je zanimalo, kako ustrezna mono- ter bis-dimetilaminometilidenska derivata acetondikarboksilata reagirata s *C*-nukleofili. Pri tem je v vseh primerih nastal 2,4,6-tris(metoksikarbonil)fenol (**2**). Izmenjava dimetilaminske skupine je potekla samo z 2-metilindolom, tako da je nastal metil (*E*)-3-(2-metil-1*H*-indol-3-il)propenoat (**3**).
Iz dimetil aceton-1,3-dikarboksilata (**1b**) ter metil 2-benzoilamino-3-dimetilamino-propenoata (**4**) smo sintetizirali ustrezen piranon **5**, ki smo ga z DMFDMA pretvorili v metil (*Z*)-3-benzoilamino-6-[1-(dimetilamino)-3-metoksi-3-oksoprop-1-en-2-il]-2-okso-2*H*-piran-5-karboksilat (**6**). Tudi s tem reagentom nadaljnje pretvorbe z nukleofili niso potekale. Potekla je le odcepitev dimetilaminometilidenskega fragmenta, tako da smo dobili izhoden piranon **5**.
Glavni del študija je potekal na področju pirazolov, ki smo jih pripravili po postopkih iz literature iz dialkil aceton-1,3-dikarboksilata (**1a,b**) ter fenilhidrazina in hidrazina, tako da so nastali etil in metil [(*Z*)-4-(dimetilamino)metiliden-5-okso-1-fenil-4,5-dihidro-1*H*-pirazol-3-il]acetat (**7a** in **7b**), ter etil (*Z*)-2-{4-[(dimet ilamino)metiliden]-5-okso-4,5-dihidro-1*H*-pirazol-3-il}acetat (**8**). Pri sobni temperaturi je potekla reakcija z DMFDMA v toluenu do ustreznih dimetilaminometilidenskih derivatov.
S tako pripravljenimi reagenti smo izvajali izmenjave dimetilaminske skupine z *N*- in *C*-nukleofili do ustreznih produktov **9**, **10**. V določenih primerih smo uspeli amino substituirane produkte **9** z DMFDMA v DMF pretvoriti v 5-substituirane-pirazolo[4,3-*c*]piridin-7-karboksilate **11**.
Zaradi zanimivosti pirazolo[4,3-*c*]piridinskega sistema smo sintetizirali ustrezna reagenta **12**, **13** z dvema dimetilaminometilidenskima skupinama, s katerima smo pripravili 5-substituirane-pirazolo[4,3-*c*]piridin-7-karboksilate **11** direktno v eni stopnji. Reakcije so potekale z alifatskimi amini, aromatskimi, heteroaromatskimi, aminokislinami, hidroksilaminom ter hidrazini. V primeru hidrazinov so nastali ustrezni *N*-amino derivati in ne diazepini.
Za sintezo ustreznih diazepinov smo tako morali uporabiti 1,2-disubstituirane hidrazine, in sicer smo uporabili 1,2-dimetilhidrazin (**14**) ter 1,2-dietilhidrazin (**15**). Pri tem je poleg samega nastanka [1,2]diazepinskega obroča potekla tudi adicija alkohola na dvojno vez, v katerem so reakcije potekale tako, da so nastali etil (7*R**,8*S**)-7-alkoksi-5,6-dimetil in 5,6-dietil-3-okso-2-fenil-2,3,5,6,7,8-heksahidropirazolo[4,3-*d*][1,2]diazepin-8-karboksilati **16**, **17** ter etil (7*S**,8*R**)-7-alkoksi-5,6-dimetil-3-okso-2,3,5,6,7,8-heksahidropirazolo[4,3-*d*][1,2]diazepin-8-karboksilati **18**.
Sinteza oksima **19** je potekala direktno iz pirazolona **8**. Pri poskusu katalitskega hidrogeniranja nastalega produkta je na zraku potekla oksidacija do derivata rubazonske kisline **20**, ob tem pa smo izolirali še etil (5-etoksi-1-fenil-4,5-dihidro-1*H*-pirazol-3-il)acetat (**21**). Tega smo pripravili po drugi poti, s pomočjo *O*-etiliranja pirazolona **8**, ki smo ga z DMFDMA v toluenu pretvorili v etil (2*E*)-(5-etoksi-1-fenil-1*H*-pirazol-3-il)propenoat (**22**). Z amini smo izvajali izmenjave dimetilaminske skupine do etil (2*E*)-2-(5-etoksi-1-fenil-1*H*-pirazol-3-il)-3-(substituirani amino)propenoatov **23**. Poleg samih izmenjav pa so z dinukleofili potekale v ocetni kislini tudi nadaljnje ciklizacije na estrsko skupino do nastanka pirido[1,2-*a*]pirimidin-4-ona **24**, pirazolo[1,5-*a*]pirimidin-7-ona **25** ter kondenziranih piranonov **26** in **27**.
Po postopku iz literature smo poskusili pripraviti etil 3-etoksikarbonilindol-2-acetat (**28**), pri tem pa smo dobili namesto pričakovanega produkta indol s hidrolizirano estrsko skupino na mestu 3 **29** in 5-etoksipirazol **21**.
Sintezo (indol-2-il)acetatnega derivata **30** smo izvedli, prek vmesnega hidrazona **31**, iz 1-fenil-1-metilhidrazina (**32**) ter dimetil aceton-1,3-dikarboksilata (**1b**). Z DMFDMA v toluenu smo na aktivno metilensko skupino uvedli dimetilaminometilidenski fragment, tako da smo dobili metil 2-[(*E*)-2-(dimetilamino)-1-(metoksikarbonil)vinil]-1-metil-1*H*-indol-3-karboksilat (**33**), ki smo ga z amini pretvorili v amino substituirane produkte **34**. Poleg izmenjav dimetilaminske skupine z amini so potekale tudi izmenjave z vodo ter alkoholi. Pri tem so nastali ustrezen hidroksi- **35a** in alkoksi-metilidenski derivati **35**. Iz 3-nitrofenilega- ter 4-nitrofenilnega-aminometilidenskega derivata **34a**,**b** je po obdelavi z natrijevim metoksidom potekla ciklizacija aminske skupine na estrsko skupino

na mestu tri, tako da je nastal piridinski obroč in smo dobili ustrezna γ-karbolina **36**. Pretvorbe reagenta **33** z dinukleofili v ocetni so vodili do nastanka indolov **37** in **38**, ki imata biciklični sistem vezan na mestu 2 glede na indolski sistem.

Reakcija med acetondikarboksilatom ter benzamidinom je potekla do (pirimidin-4-il)acetata **39**, ki smo ga prav tako pretvorili z DMFDMA do dimetilaminometilidenskega derivata, tako da je nastal metil 2-(6-hidroksi-2-fenilpirimidin-4-il)acetat (**40**). S tako pripravljenim reagentom smo izvajali izmenjave dimetilaminske skupine z amini. Nastali so ustrezni produkti **41**. Poleg izmenjav z amini smo izvajali tudi reakcije z dinukleofili, pri katerih poteče najprej izmenjava dimetilaminske skupine, potem pa poteče še ciklizacija na estrsko skupino do ustreznih kondenziranih pirimidinov **42** in kondenziranih piranonov **43**.

S hidroksilaminom (**44**) smo dimetil aceton-1,3-dikarboksilat (**1b**) pretvorili v metil (5-okso-4,5-dihidroizoksazol-3-il)acetat (**45**), ki je reagiral z DMFDMA. Nadaljnje pretvorbe z nukleofili niso potekale.

Nazadnje smo acetondikarboksilat **1b** uporabili še pri reakciji, kjer je potekla cikloadicija le-tega na azometin imin **46**. Tako smo dobili pirazolo[1,2-*a*]pirazol **47**.



DIMETHYLAMINOMETHYLIDENE DERIVATIVES OF DIALKYL ACETONEDICARBOXYLATES IN THE SYNTHESIS OF HETEROCYCLIC SYSTEMS

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During our study, acetonedicarboxylates **1** were being used in the synthesis of heterocyclic systems, using enaminone methodology. At first we were interested in the reactivity of mono- and bis- dimethylaminomethylidene derivatives of acetonedicar-boxylate react with *C*-nucleophiles. In all cases 2,4,6-tris(methoxycarbonyl)-phenol (**2**) was formed. Substitution of dimethylamino group took place only in case of 2-methylindole, to furnish methyl (*E*)-3-(2-methyl-1*H*-indol-3-yl)propenoate (**3**).
From dimethyl acetone-1,3-dicarboxylate (**1b**) and methyl 2-benzoylamino-3-dimethylaminopropenoate (**4**) the corresponding pyranone **5** was synthesized, which was further transformed with DMFDMA into methyl (*Z*)-3-benzoylamino-6-[1-(dimethylamino)-3-methoxy-3-oxoprop-1-en-2-yl]-2-oxo-2*H*-pyran-5-carboxylate (**6**). With this reagent, further transformations with nucleophiles also did not take place. Only removal of dimethylaminomethylidene fragment took place, to give the starting pyranone **5**.
Main part of our study was based on pyrazoles, which were prepared according to the literature procedures from dialkyl acetone-1,3-dicarboxylates (**1a,b**), and phenylhydrazone and hydrazine, to form ethyl and methyl [(*Z*)-4-(dimethyamino)-methylidene-5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl]acetates (**7a** and **7b**), and ethyl (*Z*)-2-{4-[(dimethylamino)methylidene]-5-oxo-4,5-dihydro-1*H*-pyrazol-3-yl}acetate (**8**). Treatment with DMFDMA at room temperature resulted in the corresponding dimethylaminomethylidene derivatives.
In this manner prepared reagents, substitutions of dimethylamino group with *N*- and *C*-nucleophiles were being transformed into products **9**,**10**. In some cases, amino substituted products **9** were transformed with DMFDMA in DMF into 5-substituted-pyrazolo[4,3-*c*]pyridine-7-carboxylates **11**.
Because of interest in pyrazolo[4,3-*c*]pyridine system, suitable reagents **12** and **13** with two dimethylaminomethylidene groups were synthesised, which were used for the synthesis of 5-substituted-pyrazolo[4,3-*c*]pyridine-7-carboxylates **11** in a single step. Reactions were performed with aliphatic amines, aromatic, heteroaromatic, amino acids, hydroxylamine, and hydrazines. In case of hydrazines, the corresponding *N*-amino derivatives were synthesised and not diazepins.
For the synthesis of diazepins, 1,2-disubstituted hydrazines were used, such as 1,2-dimethylhydrazine (**14**) and 1,2-diethylhydrazine (**15**). With the formation of [1,2]diazepin ring, also addition of an alcohol to double bond took place, to give ethyl (7*R**,8*S**)-7-alkoxy-5,6-dimethyl and 5,6-diethyl-3-oxo-2-phenyl-2,3,5,6,7,8-hexahydro-pyrazolo[4,3-*d*][1,2]diazepine-8-carboxylates **16**, **17** and ethyl (7*S**,8*R**)-7-alkoxy-5,6-dimethyl-3-oxo-2,3,5,6,7,8-hexahydropyrazolo[4,3-*d*][1,2]diazepine-8-carboxylates **18**.
Synthesis of oxime **19** was performed directly from pyrazolone **8**. Attempt of catalytic hydrogenation of the product, oxidation by air took place, to give derivative of rubazoic acid **20**, and some ethyl (5-ethoxy-1-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)acetate (**21**) was also formed. Compound **21** was also prepared by *O*-ethylation of pyrazolone **8**, which was further transformed with DMFDMA in toluene into ethyl (2*E*)-(5-ethoxy-1-phenyl-1*H*-pyrazol-3-yl)propenoate (**22**). Substitutions of dimethylamino group with various amines gave ethyl (2*E*)-2-(5-ethoxy-1-phenyl-1*H*-pyrazol-3-yl)-3-(substituted amino)propenoates **23**. Beside substitutions also reactions with dinucleophiles in acetic acid were carried out, where subsequent cyclisations to ester group were taking place to form pyrido[1,2-*a*]pyrimidin-4-one **24**, pyrazolo[1,5-*a*]pirimidin-7-one **25** and condensed pyranones **26** and **27**.
Using the literature procedure, ethyl 3-ethoxycarbonylindol-2-acetate (**28**), was tried to be prepared. Instead of the expected indole **28**, indole with hydrolised ester group at the position 3 **29** and 5-ethoxyparazole **21** were formed.
Synthesis of (indol-2-yl)acetate derivative **30** was carried out from hydrazone **31**, which was prepared from 1-methyl-1-phenylhydraozine (**32**) and dimethyl acetone-1,3-dicarboxylate (**1b**). Treatment with DMFDMA in toluene yielded 2-[(*E*)-2-(dimethylamino)-1-(methoxycarbonyl)vinyl]-1-methyl-1*H*-indol-3-carboxylate (**33**), which was further transformed with amines into amino substituted products **34**. As well as with amines, substitution of dimethylamino group also took place with water and alcohols. In this manner the corresponding hydroxy- **35a** and alkoxy-methylidene derivatives **35** were formed. Treatment of 3-nitrophenyl- and 4-

nitrophenyl-aminomethylidene derivatives **34a**,**b** with sodium methoxide resulted in cyclisation of amino group to ester group at the position 3, with the formation of pyridine ring, to give the corresponding γ-carbolines **36**. Transformations of reagent **33** with dinucleophiles in acetic acid furnished idoles **37** and **38** which contain bicyclic ring attached at position 2 of the indole ring.

Reaction of acetonedicarboxylate and benzamidine took place to give (pyrimidin-4-yl)acetate **39**, which was transformed with DMFDMA into dimethylaminomethylidene derivative, and thus methyl 2-(6-hydroxy-2-phenylpyrimidin-4-yl)acetate (**40**) was formed. With this reagent, substitutions of dimethylamino group with amines were performed, what furnished products **41**. Also reactions with dinucleophiles took place, where firstly dimethylamino group was substituted, and secondly cyclisation to ester group took place to yield the corresponding condensed pyrimidines **42** and condensed pyranones **43**.

With hydroxylamine (**44**), dimethyl acetone-1,3-dicarboxylate (**1b**) was transformed into methyl (5-oxo-4,5-dihydroisoxazol-3-yl)acetate (**45**), which reacted with DMFDMA. Further transformations with nucleophile did not take place.

At last, acetonedicarboxylate **1b** was used in the reaction of cycloaddition to azomethin imine **46**. In this manner pyrazolo[1,2-*a*]pyrazol **47** was formed.

RAZVOJ IN VREDNOTENJE POLIMERNIH NANODELCEV ZA TRANSPORT CISTATINA V TUMORSKE CELICE

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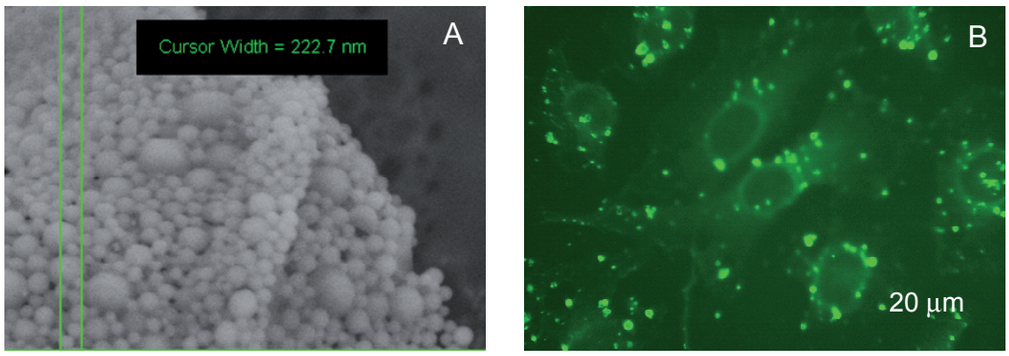
Načrtovanje in oblikovanje nanodelcev kot naprednih dostavnih sistemov za vnos proteinskih učinkovin predstavlja novejše raziskovalno področje oblikovanja zdravil. Tovrstno oblikovanje temelji na inovativnih in empiričnih pristopih, ki posebej obravnavajo specifičnost posameznega proteina in možnosti njegovega vgrajevanja v ustrezni nosilni sistem. Pri našem delu smo izbrali cistatin kot modelno proteinsko učinkovino s potencialnim protitumornim delovanjem in ga vgradili v nanodelce iz kopolimera mlečne in glikolne kisline (PLGA) z dvojno emulzijsko-difuzijsko metodo (slika 1A). Z namenom da ohranimo biološko aktivnost cistatina, smo uveljavljen postopek izdelave nanodelcev ustrezno spremenili in prilagodili, predvsem glede izbire organskega topila in mehanskih obremenitev, potrebnih za nastanek nanodelcev. Z dodatkom ustreznih krio-/lioprotektantov smo aktivnost cistatina v nanodelcih ohranili tudi med postopkom liofilizacije. Nadalje smo vrednotili vpliv različnih derivatov kopolimera PLGA, ki so se razlikovali v molekulski masi in substituciji končnih karboksilnih skupin, na velikost in naboj nanodelcev, vgrajevanje cistatina, njegovo sproščanje in stabilnost.

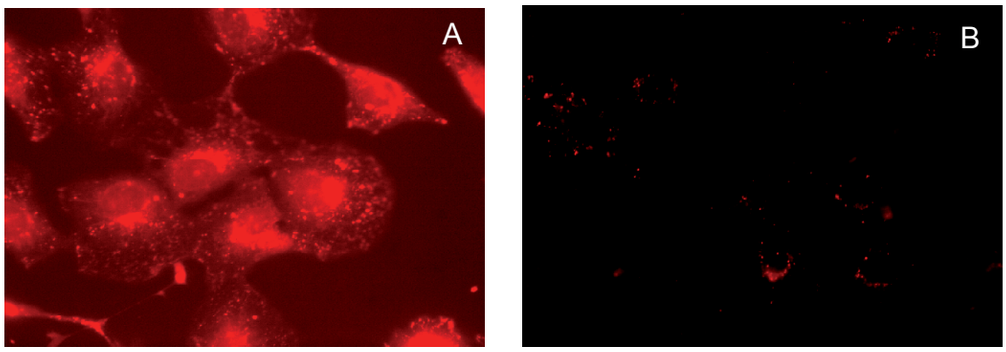
Z uporabo celičnih kultur smo nanodelce vrednotili z biološkega vidika. Glede na lastnosti, ki jih ima cistatin kot inhibitor cisteinskih proteaz, smo izbrali celično linijo transformiranih človeških epitelnih celic raka dojke (MCF-10A neoT), ki omenjene encime izražajo v povečanem obsegu. S proučevanjem citotoksičnosti smo ugotovili, da so PLGA nanodelci pri višjih koncentracijah toksični. Predvidevamo, da je toksičnost nanodelcev najverjetneje posledica adhezije trdnih delcev na celično površino. Pri visokih koncentracijah nanodelcev smo opazili zelo spremenjeno morfologijo celic, ki so se povezovale v mreži podobno strukturo. Slike, posnete s fluorescentnim mikroskopom, so dodatno potrdile toksične učinke nanodelcev, ki so se po vstopu v celice združevali okoli jedra, kar je najverjetneje oviralo izmenjavo snovi, ki jih celica potrebuje za normalno delovanje. Kljub temu, da FDA smernice dovoljujejo terapevtsko uporabo polimerov PLGA, so lahko le-ti glede na naše rezultate v visokih koncentracijah v nekaterih primerih problematični.

Vpogled v celične procese med inkubacijo z nanodelci je omogočila fluorescentna in konfokalna laserska mikroskopija. Za tovrstne poskuse smo uporabili nanodelce s fluorescentno označenim cistatinom. Rezultati so pokazali, da nanodelci lahko vstopajo v celice in da je proces odvisen od koncentracije nanodelcev in časa inkubiranja (slika 1B). V začetku inkubiranja so se nanodelci nahajali na perifernem delu celic in se kasneje pomikali v perinuklearno področje, kjer so se združevali. Možno je, da so ciljali lizosome, ki so polni cisteinskih proteaz. Pri preparatih, kjer smo celice inkubirali s prostim označenih cistatinom v enakih koncentracijah kot v nanodelcih, je bila intenziteta fluorescence bistveno manjša. To kaže, da je difuzija prostega cistatina skozi celično membrano mnogo počasnejša kot endocitoza nanodelcev.



Slika 1. (A) PLGA nanodelci z vgrajenim cistatinom, posneti z vrstično elektronsko mikroskopijo. (B) Vstop PLGA nanodelcev z vgrajenim fluorescentno označenim cistatinom v MCF-10A neoT celice po 30 minutni inkubaciji.

Učinkovitost cistatina, prostega ali dostavljenega z nanodelci, v inhibiciji znotrajcelične cisteinske proteaze katepsina B smo ugotavljali s proteoliznim testom s specifičnim substratom Arg_2-krezil-vijolično (slika 2). Rezultati so pokazali, da je cistatin, ki smo ga dostavili z nanodelci, učinkovito inhibiral proteolizno aktivnost katepsina B, medtem ko prost cistatin takega delovanja ni izkazal.



Slika 2. Proteolizna aktivnost katepsina B v živih MCF-10A neoT celicah, vrednotena z razgradnjo specifičnega substrata Arg_2-krezil-vijolično v rdeče obarvan razgradni produkt. Pred dodatkom substrata smo celice inkubirali z raztopino cistatina (A) in nanodelci z vgrajenim cistatinom (B). Slike smo posneli pri enakih nastavitvah.

Na podlagi teh rezultatov lahko zaključimo, da protein lahko vgradimo v polimerne nanodelce in pri tem ohranimo njegovo aktivnost. Pokazali smo, da metoda izdelave in vrsta polimera bistveno vplivata na lastnosti nosilnega sistema in biološko aktivnost vgrajenega proteina. Z uporabo celičnih kultur smo dokazali, da lahko z nanodelci pospešimo in povečamo vnos proteinske učinkovine v celico v primerjavi z raztopino prostega proteina. In nenazadnje, protein, ki ga na ta način dostavimo v celico, ohrani svojo aktivnost na ciljnem mestu delovanja.

DEVELOPMENT AND EVALUATION OF POLYMERIC NANOPARTICLES FOR TRANSPORT OF CYSTATIN INTO TUMOUR CELLS

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Design and formulation of advanced drug delivery systems with protein drugs, such as nanoparticles, present a new research area in the field of drug formulation. In spite of some successful guidelines development of particular protein formulation still requires innovative and empirical investigation due to specific characteristic of each protein. In our work, cystatin, a potential protein drug in cancer therapy, was incorporated in poly(lactide-co-glycolide) (PLGA) nanoparticles by the double emulsion solvent diffusion technique (figure 1A). In order to preserve the biological activity of cystatin an optimized method was developed, adjusting organic solvents and mechanical forces necessary for nanoparticle production. The results show that appropriate selection of cryo-/lyoprotectants protects nanoparticles-entrapped cystatin during lyophilisation. In addition, several derivates of PLGA polymers differing in molecular weight and containing either free or esterified carboxyl-end groups were investigated systematically to modulate the size and zeta potential of nanoparticles, cystatin loading capacity, the release profile and stability.

For biological evaluation of cystatin-loaded nanoparticles cell culture models were used. According to biological function of cystatin as an inhibitor of cysteine proteases, a transformed human breast epithelial cell line (MCF-10A neoT) was used due to increased expression of these enzymes. Cytotoxic studies of PLGA nanoparticles showed that they are toxic at high concentrations. We propose that toxicity probably arises from adhesion of solid particles to the cell surface. At high concentrations of nanoparticles morphology of cells was changed significantly and reorganization of cell deposit into a mesh like structure was observed. Fluorescent microscopy additionally confirmed possible toxic effect, showing that nanoparticles, after entering the cells, accumulated around the nucleus, which may affect cellular transport of various substances, necessary for normal living function. Although PLGA polymers have been approved by FDA, according to our results the use of high concentrations in some cases might be limited in clinical applications.

The insight into cellular processes during incubation with nanoparticles was enabled using fluorescent and confocal laser microscopy. For these experiments nanoparticles loaded with fluorescently labelled cystatin were prepared. Fluorescent images showed that nanoparticles entered the cells rapidly and that their uptake was time and concentration dependant (figure 1B). At the beginning, nanoparticles were localized in the cell periphery, later they concentrated in the perinuclear region. It is possible that cystatin-loaded nanoparticles targeted lysosomes, which are rich in cysteine proteases. In contrast, images of cells incubated with free labelled cystatin, in amount corresponding to those incorporated in nanoparticles, were stained less extensively, showing that diffusion of free cystatin across the cell membranes is much slower process as compared to endocytosis of nanoparticles.

Figure 1. (A) Scanning electron micrograph of PLGA nanoparticles loaded with cystatin. (B) Internalization of PLGA nanoparticles loaded with fluorescently labelled cystatin into MCF-10A neoT cells after 30 minutes of incubation.

The efficiency of cystatin, either free or delivered by nanoparticles, to inhibit intracellular cysteine protease cathepsin B was evaluated by the proteolytic assay using specific substrate Arg_2-cresyl-violet (figure 2). Results showed that cystatin delivered by nanoparticles effectively inhibited cathepsin B activity whereas free cystatin showed no inhibition of intracellular cathepsin B.

Figure 2. Proteolytic activity of cathepsin B in living MCF 10A-neoT cells imaged by degradation of specific substrate Arg_2-cresyl-violet into the red degradation product. Before the addition of the substrate cells were incubated with free cystatin in solution (A) and nanoparticles loaded with cystatin (B). Instrument settings were kept constant.

In conclusion, our results show that protein can be incorporated into polymeric nanoparticles in the biologically active form. We showed that specific selection of technological procedure and polymer type play a crucial role for the characteristics of the delivery system and preservation of protein biological activity. Furthermore, experiments on cell culture models showed that using nanoparticles as a carrier system we are able to deliver protein drug inside the cell more efficiently compared to the free protein drug in solution. Not least, protein delivered by nanoparticles also maintains its activity at the target site.

VPLIV GENETSKEGA POLIMORFIZMA *CYP2C9* NA PRESNOVO VARFARINA IN NA UČINKOVITOST ANTIKOAGULACIJSKEGA ZDRAVLJENJA

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Varfarin je pri nas najpogosteje uporabljano antikoagulacijsko zdravilo. Zaradi njegove ozke terapevtske širine in velike interindividualne variabilnosti v višini dnevnega odmerka, ki je potreben za dosego terapevtskega učinka, je v času zdravljenja z varfarinom potrebno pogosto spremljanje bolnika in merjenje protrombinskega časa. Varfarin inhibira encim vitamin K epoksid reduktazo (VKOR), kar privede do pomanjkanja reducirane oblike vitamina K. Ta je potreben za delovanje encima γ-glutamil karboksilaza (GGCX), ki postranslacijsko modificira druge od vitamina K odvisne proteine (npr. faktorje strjevanja krvi FII, FVII, FIX in FX ter antikoagulacijske faktorje C, S in Z) in jih na ta način aktivira. Varfarin obstaja v obliki racemata. Najpomembnejši encim, ki presnavlja *S*-varfarin v neaktivno obliko 7-hidroksi varfarin, je citokrom P450 (CYP) 2C9. Gen *CYP2C9* je polimorfen. Poleg alela *CYP2C9*1*, ki ima normalno nukleotidno zaporedje, sta v različnih populacijah pogosto zastopana tudi alela *CYP2C9*2* in *CYP2C9*3*. V pogojih *in vitro* in *in vivo* je bilo pokazano, da imata velik vpliv na metabolno aktivnost encima. Tudi geni za vitamin K epoksid reduktazo, γ-glutamil karboksilazo ter faktorje strjevanja krvi so polimorfni, kar lahko vpliva na farmakodinamiko varfarina.
Z našo raziskavo smo želeli ugotoviti, kakšen je vpliv genetskih dejavnikov, zlasti polimorfizmov v genu *CYP2C9*, na zdravljenje z varfarinom. Najprej smo preučevali vpliv pogostih polimorfizmov *CYP2C9*, ki vodijo v sintezo encimov s spremenjeno sposobnostjo presnove varfarina, nato pa smo analizirali tudi vse eksone ter meje med eksoni in introni gena *CYP2C9*, da bi preverili, če obstajajo še dodatne genetske variante. Zanimal nas je tudi vpliv sočasnega zdravljenja z drugimi zdravili ter vpliv demografskih dejavnikov na višino odmerka varfarina in hitrost njegove presnove. Poleg tega smo hoteli proučiti, ali polimorfizmi v genih *VKORC1*, *GGCX* in *FVII* vplivajo na farmakodinamiko varfarina.
Med slovenskimi bolniki na dolgotrajnem zdravljenju z varfarinom so nosilci polimorfnih alelov *CYP2C9*2* ali *CYP2C9*3* potrebovali statistično značilno nižje odmerke varfarina ter so imeli manjši očistek *S*-enantiomera od bolnikov z genotipom *CYP2C9*1/*1*. Bolniki z enim polimorfnim alelom so potrebovali 44,5% nižje odmerke zdravila in so imeli za 36,9% manjši očistek *S*-varfarina. Bolniki z dvema polimorfnima aleloma pa so potrebovali 66,4% nižje odmerke varfarina in so imeli za 69,2% manjši očistek *S*-varfarina. Izmed negenetskih dejavnikov sta pomembno vplivala na višino odmerka starost in teža bolnika. Pokazali smo, da na presnovo in višino odmerka varfarina vpliva tudi sočasno zdravljenje z drugimi zdravili, zlasti pomembna je bila interakcija med karbamazepinom in varfarinom.
V genu *CYP2C9* smo dokazali novo mutacijo (1060G>A), ki je bila poimenovana alel *CYP2C9*24*. Bolnica je bila heterozigot za to mutacijo in tudi heterozigot za alel *CYP2C9*2*. Primerjali smo presnovo varfarina pri tej bolnici in skupini starejših bolnikov z atrijsko fibrilacijo in genotipom *CYP2C9*1/*2*, ki niso jemali zdravil z vplivom na presnovo varfarina. Opazili smo, da je bolnica prejemala najnižje odmerke zdravila in imela tudi najmanjši očistek *S*-varfarina v primerjavi z izbrano skupino bolnikov. V heterolognem ekspresijskem sistemu kvasovk ter kulturi človeških celic HEK293 smo pokazali, da je encim CYP2C9.24 nepravilno zvit in nima pravilno vezane hemske skupine. Zaradi tega verjetno sledi njegova hitra znotrajcelična razgradnja.
Na farmakodinamiko varfarina sta vplivala polimorfizma v genu *VKORC1*. Polimorfizem v intronu 1 (6484C>T) je bil statistično značilno povezan z nižjimi dnevnimi odmerki zdravila. Polimorfizem v neprevedenem območju na 3'-koncu (9041G>A) istega gena pa je bil povezan z višjimi dnevnimi odmerki.
Z našo raziskavo smo nedvoumno pokazali, da imajo genetski dejavniki velik vpliv na višino odmerka varfarina. Polimorfizmi v genu *CYP2C9* vplivajo na hitrost njegove presnove, polimorfizmi v genu *VKORC1* pa vplivajo na farmakodinamiko zdravila. Z regresijskim modelom smo opisali skupno 60% razlik v višini odmerka varfarina med bolniki. Še vedno pa ostaja vprašanje, ali poznavanje teh dejavnikov lahko kaj doprinese k uspešnosti zdravljenja z varfarinom oziroma, ali je genotipizacija pred začetkom zdravljenja upravičena tudi z ekonomskega vidika. Naši rezultati bi lahko pomagali pri načrtovanju prospektivne klinične študije, ki bi odgovorila na ta vprašanja.

THE INFLUENCE OF *CYP2C9* GENETIC POLYMORPHISM ON WARFARIN METABOLISM AND ANTICOAGULANT TREATMENT

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Warfarin is the most common anticoagulant drug used in Slovenia. Due to its narrow therapeutic index and high interindividual variability in the dose required to achieve the desired therapeutic effect careful monitoring of prothrombin time is necessary. Warfarin inhibits vitamin K epoxide reductase (VKOR), which recycles vitamin K 2,3 epoxide to reduced vitamin K. The reduced vitamin K is a required cofactor for the γ-glutamyl carboxylase (GGCX) that converts precursor forms of blood clotting factors FII, FVII, FIX, FX and endogenous anticoagulant proteins C, S and Z to active zymogens. The predominant enzyme that metabolizes *S*-warfarin to 7-hydroxywarfarin is cytochrome P450 2C9 (CYP2C9). Human *CYP2C9* gene is highly polymorphic. Beside the wild-type *CYP2C9*1* allele the two most common variant alleles in Caucasian populations are *CYP2C9*2* and *CYP2C9*3*. It was demonstrated *in vitro* and *in vivo* that these two allelic variants of *CYP2C9* gene influenced the metabolic activity of the enzyme. Genes for vitamin K-dependent blood coagulation factors, as well as *GGCX* and *VKOR* are also polymorphic, and could influence warfarin pharmacodynamics.

The aim of our study was to analyze the influence of various genetic factors, especially *CYP2C9* gene polymorphisms, on warfarin treatment. First, we focused on the common polymorphisms in *CYP2C9* gene which give rise to proteins with altered catabolic activity and then all exons and intron-exon boundaries of *CYP2C9* gene were screened in order to identify new sequence variations. We were also interested in the effect of concomitant drug treatment and demographic factors on warfarin metabolism and its maintenance dose. The objective of our study was also to determine whether polymorphisms in genes involved in blood coagulation (*VKOR*, *GGCX* and *FVII*) influence interindividual variability in warfarin dose requirement.

Slovene patients with *CYP2C9*2* and/or *CYP2C9*3* polymorphic alleles required statistically significant lower warfarin doses and had lower *S*-warfarin clearance than patients with *CYP2C9*1/*1* genotype. Patients with one polymorphic *CYP2C9* allele required 44.5% lower warfarin dose and had 36.9% lower *S*-warfarin clearance, while patients with two polymorphic *CYP2C9* alleles required 66.4% lower warfarin doses and had 69.2% lower *S*-warfarin clearance as compared to the patients with two normal alleles. Among non-genetic factors patients' age and body-weight influenced warfarin dose. We also demonstrated the influence of concomitant drug treatment on warfarin dose requirement and metabolism. Especially the interaction between carbamazepine and warfarin was shown to be of major clinical importance.

We found a novel *CYP2C9* sequence variant (*1060G>A*), designated *CYP2C9*24* allele. It was present in heterozygous state in one patient who was also a heterozygous carrier of the *CYP2C9*2* allele. The index patient had the lowest daily warfarin dose requirement and the lowest *S*-warfarin clearance in comparison with a group of warfarin patients matched for age, indication, drug co-treatment and *CYP2C9*1/*2* genotype. The results from recombinant yeast expression system and HEK293 cell system indicated that CYP2C9.24 protein was improperly folded resulting in improper heme incorporation and its rapid intracellular degradation.

Polymorphisms of *VKORC1* gene influenced warfarin pharmacodynamics. Polymorphism in intron 1 (6484C>T) of *VKORC1* gene was related to lower dose requirement. In contrast, *9041G>A* polymorphism in 3'-untranslated region of *VKORC1* gene was related to higher warfarin doses.

We showed that genetic factors have significant influence on warfarin dose. Polymorphisms in *CYP2C9* gene influenced warfarin metabolism, while polymorphisms in *VKORC1* gene influenced warfarin pharmacodynamics. With our regression model we could explain 60.0% of variability in warfarin dose. There are still open questions if the knowledge of these parameters is relevant for clinical end points and what is the cost-benefit aspect of pre-prescription genotyping. Therefore, prospective randomized clinical trials are needed and our study could serve as a starting point for designing such studies.

METODOLOŠKI PRISTOPI K MERJENJU IN IZBOLJŠEVANJU TRAJNOSTNEGA RAZVOJA V KEMIJSKI IN PROCESNIH INDUSTRIJAH

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Trajnostni razvoj temelji na tri-dimenzijskem konceptu, ki vključuje okoljsko delovanje, družbeno odgovornost in ekonomski doprinos. Mnoga napredna podjetja kemijske in procesnih industrij so že privzela načela trajnostnega razvoja, vendar so naletela na težave pri merjenju in poročanju o trajnostnem razvoju. Do sedaj je bilo predlaganih le malo metodologij, ki bi omogočale sistematično merjenje in doseganje trajnostnega razvoja. Zaradi tega smo predlagali metodologijo za merjeno doseganje trajnostnega razvoja podjetij kemijske in procesnih industrij, ki temelji na devetih stopnjah. Kot osnovo metodologije smo vzeli niz kazalcev trajnostnega razvoja podjetij, ki je uporaben za večino podjetij v kemijskem in drugih sektorjih. Prednost in posebnost metodologije je v vključitvi matematičnega modela v izračun sestavljenega indeksa trajnostnega razvoja podjetja, ki predstavlja združeno informacijo o delovanju podjetja na vseh treh dimenzijah trajnosti. Uporabnost modela smo preizkusili v več študijah primerov.

Predlagana metodologija vključuje stopnjo, v kateri predlagamo primerjalno ocenitev delovanje podjetja glede na standarde najboljših razpoložljivih tehnik, definiranih v skladu s smernico o celovitem preprečevanju in obvladovanju onesnaževanja. V ta namen smo razvili dva modela: poenostavljeni model in natančnejši, mehko-logični model za primerjanje delovanja podjetij z najvišjimi standardi v sektorju, kot jih definirajo referenčni dokumenti o najboljših razpoložljivih tehnikah. Učinkovitost obeh modelov smo preizkusili v študiji, v kateri smo ocenili vzorčno tovarno glede na porabo energije, vode, drugih surovin in proizvodnjo odpadkov, odpadne vode, stranskih produktov in glavnega proizvoda. Oba modela sta se izkazala kot uporabno orodje za primerjalno ocenjevanje podjetij in tovarn, zlasti zaradi vključitve analize referenčnih dokumentov o najboljših razpoložljivih tehnikah.

Metodologija za merjeno doseganje trajnostnega razvoja podjetij predvideva vključitev procesa nenehnega izboljševanja, saj podjetje na podlagi dobljenih rezultatov zastavi prihodnje naloge in cilje, pri čemer morajo vse tri vidike trajnostnega razvoja nenehno izboljševati. V kemijski in procesnih industrijah bodo izboljšave najpogosteje povezane z rekonstrukcijami obstoječih ali načrtovanjem novih procesov. Običajen pristop k načrtovanju kemijskih procesov obravnava okoljske in socialne vidike kot manj pomembne v primerjavi z ekonomskimi kriteriji. Do sedaj je načrtovanje procesov temeljilo večinoma na mikro-ekonomskih ciljih, kot so vrednost naložbe, obratovalni stroški, vračilni rok in neto sedanja vrednost. Danes začenjajo kemijski inženirji zaradi zavedanja o vplivih odločitev v procesni tehniki na okolje in družbo načrtovati in optimirati kemijske procese s hkratnim upoštevanjem ekonomskih, okoljskih in socialnih dejavnikov. Tovrstno načrtovanje procesov ni lahka naloga. Zaradi nasprotujočih si učinkov ekonomskih kriterijev (npr. obratovalnih stroškov), okoljskih kriterijev (npr. vplivov na globalno segrevanje, zakisanje ozračja itd.) in družbenih kriterijev (npr. varnosti) na procesno delovanje, je zelo težko določiti procesno alternativo, ki hkrati zadostuje vsem tem kriterijem.

V prispevku predlagamo metodologijo za načrtovanje procesov v skladu s trajnostnimi kriteriji, ki omogoča hkratno vključevanje okoljskih, socialnih in ekonomskih kriterijev v računalniško simulacijo procesa. Metodologija vključuje štiri stopnje: začetno, simulacijsko, integracijsko in optimizacijsko stopnjo. Za vzorčno študijo smo uporabili procesne alternative za sintezo amoniaka, ki so bile ocenjene s predlagano metodologijo. Za avtomatsko določitev optimalnega načrta vsake procesne alternative je bil uporabljen procesni optimizer Aspen Plus Optimizer™. Procesno optimiranje z optimizerjem je hkrati upoštevalo okoljske vplive (definirane v skladu z metodo ocenitve življenjskega ciklusa, ki povezuje emisije z okoljskimi vplivi), varnostne vidike (vključene v obliki indeksa varnosti) in ekonomske cilje (dobiček pred obdavčitvijo procesne alternative). Ker nekateri kriteriji (npr. varnost, stroški, okoljski faktorji itd.) ne morejo biti izraženi v istih enotah in sestavljeni v eno namensko funkcijo, metodologija vključuje postopek večkriterijskega odločanja za primerjavo trajnostnega delovanja procesnih alternativ. V ta namen smo uporabili model za integrirano ocenjevanje, ki temelji na analitičnem hierarhičnem procesu. Predlagana metodologija omogoča določitev procesne alternative, ki je v trajnostnem delovanju izboljšana v primerjavi s prvotnim procesnim načrtom.

Glavni cilj načrtovanja trajnostnih procesov postaja doseganje proizvodnje brez odpadkov, ki ga ni mogoče doseči brez pospeševanja sodelovanja med industrijskimi sistemi in koordiniranja povezav med tehnologijami oz. podjetji. Zato smo metodologijo za načrtovanje procesov v skladu s trajnostnimi kriteriji nadgradili z

metodologijo za doseganje proizvodnje brez odpadkov. Metodologija je razširjena na povezave med procesi različnih industrijskih partnerjev, ki jih je mogoče povezati v industrijski grozd. Predlagana metodologija vključuje analizo proizvodnega procesa, procesne izboljšave in optimiranje procesa, inventuro odpadkov in stranskih produktov ter iskanje potencialnih industrijskih partnerjev, s čimer lahko dosežemo koncept industrijskega mutualizma. Metodologijo smo preizkusili v študiji primera.

Metodološki pristopi, ki jih predlagamo v naših raziskavah, so medsebojno povezani, saj je za doseganje trajnosti potrebno hkrati izboljševati delovanje industrijskih procesov in iskati njihove povezave na širšem nivoju eko-industrijskih kompleksov ter vse skupaj na sistematičen način spremljati s primerno metodologijo merjenja trajnostnega razvoja. Z upoštevanjem vseh predlaganih metodologij lahko pripomoremo k izboljšanju in doseganju trajnostnega razvoja v kemijski in procesnih industrijah.

METHODOLOGICAL APPROACHES TO THE ASSESSMENT AND IMPROVEMENT OF SUSTAINABLE DEVELOPMENT IN CHEMICAL AND PROCESS INDUSTRIES

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Sustainable development is based on the three-dimensional concept including environmental performance, social responsibility and economic contribution. Many advanced companies from chemical and process industries have already adopted the concept of sustainable development, but they were faced with difficulties in monitoring and reporting their performance towards sustainability. At present, very few methodologies for systematic assessment and achievement of sustainable development for companies have been proposed. In this work, a methodology for achieving more sustainable business practices in chemical and process industries based on nine steps was proposed. The basis of the methodology was a proposed set of sustainability indicators, which is applicable to most companies in chemical and process industries. The advantage of the methodology was inclusion of a mathematical model for designing a composite sustainable development index that depicted information on performance of companies in all the three dimensions of sustainability – economic, environmental, and societal. The effectiveness of the proposed model was illustrated in three case studies, in which companies were compared regarding their sustainability performance.

The proposed methodology included a step for performance benchmarking in chemical companies, by considering Best Available Techniques, as determined by the Integrated Pollution Prevention and Control Directive. A simple, classic set model and an upgraded, fuzzy logic-based one were proposed for technological assessment of companies. The effectiveness of both models proposed was tested in the performance assessment of the case plant. The performance assessment included raw material extraction, energy consumption, product/by-products generation, water consumption and wastewater release. Based on the results of the case study, both models were recognized as promising ones to support a permitting process required by the Integrated Pollution Prevention and Control Directive. The strength of the model lies in the inclusion of Reference Document on Best Available Techniques in the analysis.

The methodology proposed for achieving sustainable development encourages industry to search for advanced, sustainable business practices. Chemical and process industries increasingly devote attention to the design of environmentally friendly processes, in order to improve their sustainability. The traditional approach to chemical process synthesis has been to treat the environmental and social aspects as secondary to the economic criteria. Process design has been mainly based on micro-economic objectives, such as capital investment, operating cost, payback time and net present value. Nowadays, due to the increased awareness of the impact of process engineering decisions on the environment and society, chemical engineers are starting to design and optimize chemical processes by considering simultaneously economic, environmental and social objectives. However, this is not a trivial task. Conflicting effects of economic (e.g. operational costs, operability), environmental (e.g. global warming, acidification) and social (e.g. safety) criteria on process performance make the determination of process design alternative that can simultaneously satisfy these constraints very difficult.

The traditional approach has been to satisfy the environmental and societal constraints when the technical and economic optimizations of the process have already been performed. The objective of our work was to propose a methodology for sustainable process design which enabled simultaneous integration of environmental, social and economic criteria into computerized simulation of the process. As a case study, several process alternatives of ammonia production were assessed by means of the proposed methodology. Aspen Plus Optimizer™ was used to automatically create optimal design of each process alternative defined by sustainability objectives. Process optimization simultaneously considered environmental objectives (formulated by using Life Cycle Assessment approach that relates emissions to environmental impacts), safety considerations (included in the form of safety index) and economic constraints (income before tax) of each alternative. Since the criteria for total optimum such as safety, cost, and environmental factors cannot be measured by the same metrics and combined in the same objective function, the proposed methodology

considered the procedure for sustainability performance comparison of design alternatives as a multi-criteria decision-making problem. The Integrated assessment model based on analytic hierarchy process was used for solving the multi-criteria decision-making problem. The methodology provided a feasible design alternative that showed improvements toward sustainability when compared to the base case design.

One of the main goals of sustainable development in chemical and process industries is to design processes with zero-waste production. In our work, a methodology for achieving zero-waste production was proposed based on analysis of a production process, the process improvements and optimization, wastes and by-products inventory, and investigation of potential industry partners, in order to achieve a concept of industrial mutualism. The methodology was tested in the case study.

Methodological approaches proposed in our research are interlinked to improve simultaneously the performance of chemical processes and to search for their interconnections on higher level of eco-industrial complexes. This approach needs to be accompanied by a systematic methodology for sustainability assessment. By considering all the proposed methodologies simultaneously, a contribution to improving and achieving sustainable development in chemical and process industries is possible.

VLOGA N-TERMINALNE REGIJE PRI TVORBI TRANSMEMBRANSKE PORE EKVINATOKSINA II, CITOLITIČNEGA PROTINA IZ MORSKE VETRNICE *Actinia equina*

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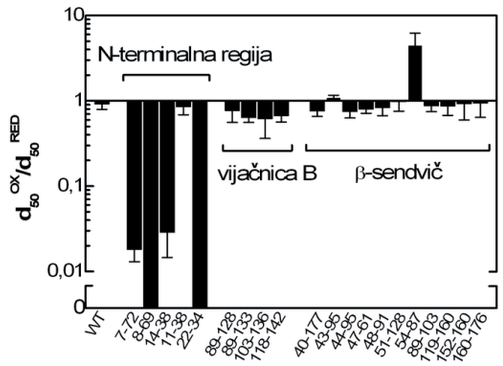
Toksini, ki tvorijo pore, so zelo pomembna skupina naravnih toksinov. Vključeni so v pomembne patološke spremembe in zastrupitve, številni med njimi so virulenčni dejavniki. Poznavanje mehanizmov njihovega delovanja je tako bistvenega pomena za razumevanje bolezenskih znakov, ki jih povzročajo, ter za izdelavo ustreznih zdravil. Hkrati se z razvojem biotehnologije zanje odpirajo nova področja uporabe, npr. v tehnologiji biosenzorjev ali kot insekticidov, zanimivi pa so tudi kot potencialno orodje pri selektivnem ubijanju rakavih celic.

V naši raziskavi smo proučevali molekularni mehanizem tvorbe pore ekvinatoksina II (EqtII). EqtII je 20 kDa velik bazični proteini, ki ne vsebujejo cisteinov in tvori tetramerne, kationsko selektivne pore v umetnih in naravnih lipidnih membranah. Njegova aktivnost je odvisna od prisotnosti sfingomielina (SM) v tarčni membrani. Molekulo toksina tvori kompaktno hidrofobno jedro β-strukture, na katerega sta prislonjeni dve α-vijačnici. Proces tvorbe pore poteka v korakih: toksin se s skupkom izpostavljenih aromatskih aminokislin veže na membrano, nato sledi oligomerizacija in tvorba funkcionalne pore.

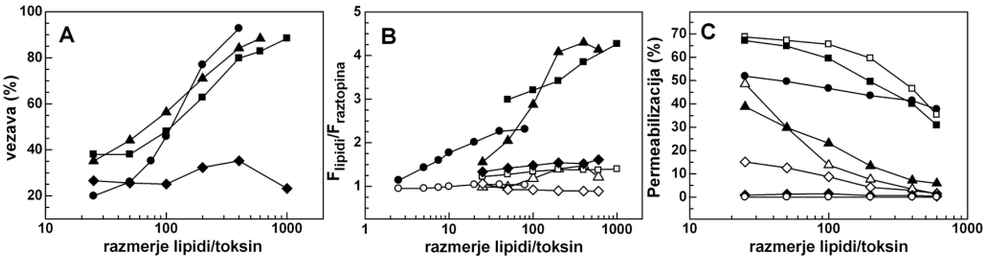
V prvem delu naloge smo ugotavljali, v katerem delu molekule EqtII pride do konformacijskih sprememb, ki omogočajo tvorbo pore. Uporabili smo metodo “dvojne cisteinske mutageneze”. Pripravili smo šestindvajset dvojnih cisteinskih mutantov preko celotne molekule toksina. Med vstavljenima Cys smo z reducirajočimi ali oksidirajočimi pogoji regulirali tvorbo disulfidnih mostičkov in s tem konformacijske spremembe.



Slika 1: Povzetek hemolitične aktivnosti dvojnih cisteinskih mutantov v reducirani in oksidirani obliki. (pri oznakah mutantov so podani le ostanki, ki smo jih zamenjali s Cys; npr. 8-69 pomeni, da smo zamenjali aminokislini na mestih 8 in 69 v cistein).

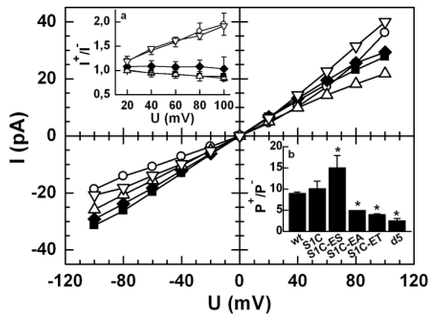
Bistvene razlike v hemolitični aktivnosti med obema oblikama smo opazili le pri mutantih, kjer je bila na jedro molekule vezana N-terminalna regija (slika 1). Pri mutantih iz območja velikih zank na dnu molekule (R31C-K77C, G85C-Y108C, P107C-Y113C) nismo opazili hemolitične aktivnosti. Posebnost je bil mutant S54C-V87C, kjer smo opazili zmanjšano aktivnost v reducirani obliki, vendar normalno aktivnost, v oksidirani obliki (slika 1). Pokazali smo, da je bila slabša aktivnost reducirane oblike mutanta S54C-V87C posledica zmanjšane sposobnosti vezave na membrano, saj se mutacija nahaja v bližini regije, ki je odgovorna za vezavo na membrano. Rezultati so pokazali, da je disociacija N-terminalne amfipatične α-vijačnice od jedra molekule v membrano bistvena za tvorbo pore toksina in da je N-terminalna regija edini del molekule, ki spremeni konformacijo med tvorbo pore. Poleg tega mora biti struktura velikih zank na dnu molekule in POC-vezavnega mesta za učinkovito delovanje toksina ohranjena.

Nadalje smo za proučevanje kinetike posameznih korakov v procesu tvorbe pore uporabili trojnega cisteinskega mutanta (V8C-I18C-K69C). Na ostanek Cys^{18} smo vezali fluorescenčno molekulo IANBD in s tem sledili drugi korak oz. vstavljanje N-konca v membrano. Prvi korak oz. stopnjo vezave molekule na membrano smo določali s testom inhibicije hemolize in celoten proces tvorbe pore opazovali s testom sproščanja kalceina iz lipidnih veziklov.
Pokazali smo, da je vezava EqtII na membrano odvisna od prisotnosti sfingomielina (slika 2A), medtem ko sta vstavljanje N-konca v lipidno okolje in tvorba končne pore odvisna tako od lipidne sestave tarčne membrane kot od koncentracije toksina oz. razmerja med toksinom in lipid (slika 2B in 2C).



Slika 2: Proces tvorbe pore EqtII: (A) Vezava toksina na lipidne vezikle. (B) Vstavljanje N-konca v membrano. (C) Sproščanje kalceina iz lipidnih veziklov. Posamezne korake smo proučevali s pomočjo reducirane (polni simboli) in oksidirane (prazni simboli) oblike trojnega mutanta in lipidnih veziklov različne sestave: (kvadrati) dioleil-fosforilholin (DOPC):SM; (trikotniki) dipalmitoil-PC (DPPC):SM, (diamanti) DOPC in (krogi) eritrocitni duhki.

Nenazadnje smo z metodo planarnih lipidnih membran proučevali topologijo N-konca (1-10 aminokisline) v končni pori ter ugotavljali vpliv tega dela pri učinkovitosti tvorbe pore. Z vezavo nabojev (MTS-reagenti) na ostanek Cys^{1} mutanta S1C smo vplivali na selektivnost por (slika 3, inset b), kar pomeni, da se ostanek nahaja v samem lumnu oz. ustju pore. Razmerje med napetostjo in tokom nam poda informacijo o (pre)razporeditvi nabojev v pori (slika 3 in inset a). Rezultati, dobljeni z dodanim pozitivnim (MTSEA, MTSET) in negativnim nabojem (MTSES) na ostanek 1 ter delecijskim mutantom (Δ5), prvič pokažejo, da se N-konec EqtII razteza na drugo, *trans* stran membrane (slika 3). S pomočjo fuzijskega proteina His_6-EqtII smo rezultate potrdili.



Slika 3: I/V in I^+/I^- (inset a) razmerji mutantov EqtII prikazujeta redistribucijo nabojev skozi poro. (kvadratki) divji tip EqtII, (krogi) mutant Δ5, (dol obrnjeni trikotniki) mutant S1C z vezanim pozitivnim nabojem (S1C-ET), (gor obrnjeni trikotniki) mutant S1C z vezanim negativnim nabojem (S1C-ES), (diamanti) mutant S1C. (inset b) Selektivnost por, ki jih tvorijo divji tip in mutanti EqtII. P^+/P^- opisuje sposobnost prehajanja kationov v primerjavi z anioni.

Poleg tega smo opazili, da odstranitev prvih petih aminokislin (delecijski mutant Δ5) povzroči zmanjšano aktivnost toksina, kljub ohranjeni sposobnosti vezave na membrano, ter pojav prehodnih odpiranj in zapiranj por v začetnem delu procesa tvorbe pore. Po drugi strani pa odstranitev prvih desetih aminokislin (mutant Δ10) vodi v popolno neaktivnost toksina. Rezultati nakazujejo, da N-konec olajša vstavljanje N-terminalne regije v membrano ter pripomore pri stabilizaciji končne pore v membrani.

INVOLVEMENT OF THE N-TERMINAL PART IN THE TRANSMEMBRANE PORE FORMATION OF EQUINATOXIN II, A CYTOLYTIC PROTEIN FROM THE SEA ANEMONE *Actinia equina*

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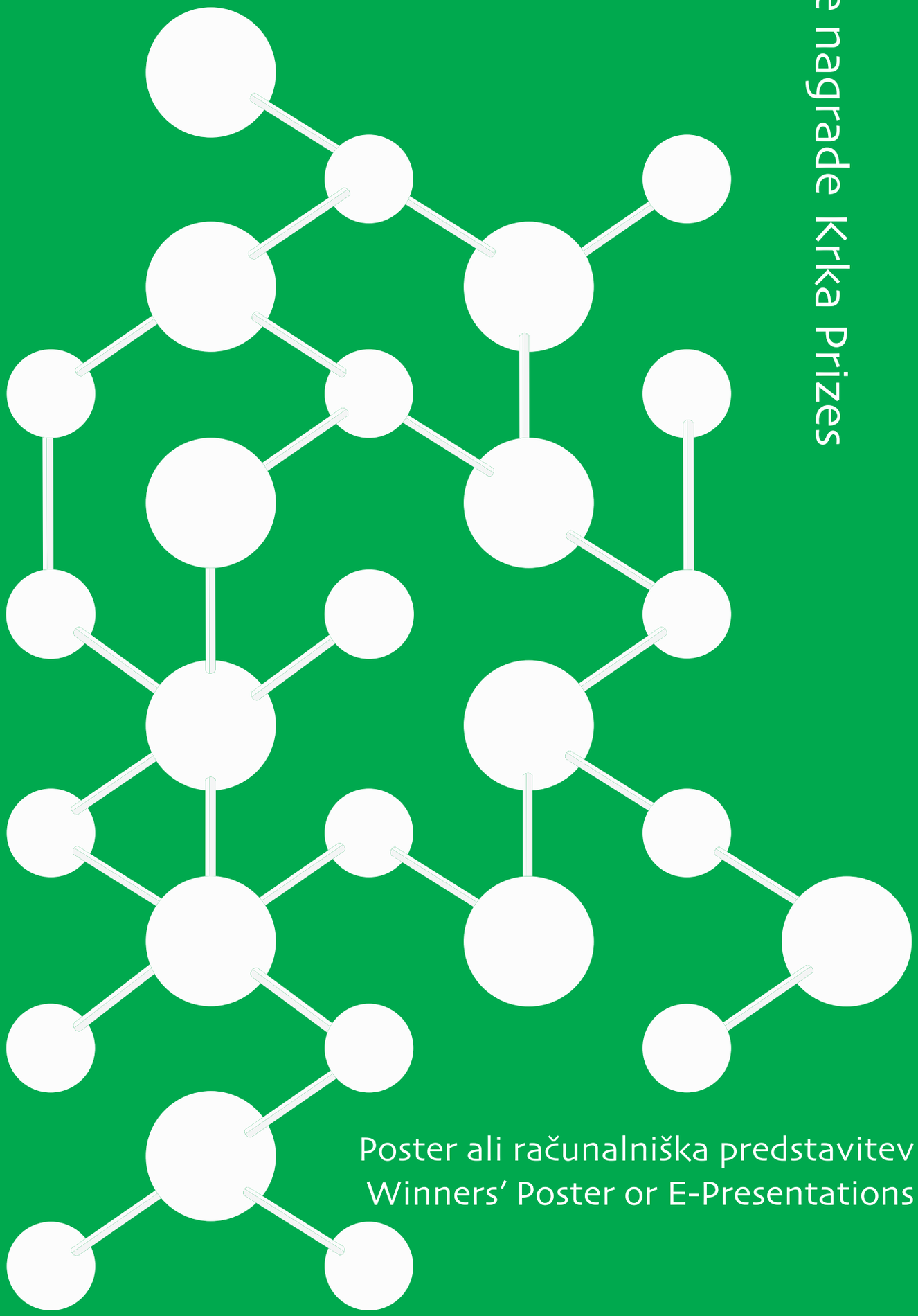
Pore-forming toxins (PFT) are widely distributed proteins, which form lesions in biological membranes, and thereby disrupt the integrity of the cell. Toxin research is therefore aiming to more profound insights into their structure and molecular mechanism in order to understand their role in disease or their potential application to biotechnology or biotherapeutics. In particular, PFT are interesting as potential tools for selective killing of cancer cells, with built-in biological "triggers" that would activate in response to specific biological stimuli. Moreover, they can also be enrolled in the biosensor technology.
The object of our research was molecular mechanism of equinatoxin II (EqtII) pore-formation. EqtII is 20 kDa, cysteineless protein, with sphingomyelin dependent activity. It forms tetrameric, cation selective pores in lipid membranes. EqtII is a single domain protein with a tightly folded hydrophobic β-sandwich core, covered on its two faces by two α-helices. Previous studies proposed at least two steps in the pore formation: weak binding onto the membrane surface by an exposed aromatic cluster, which is followed by oligomerization and pore formation.
Primarily we used double cysteine scanning mutagenesis in order to determine the regions of the molecule that undergo conformational changes during the pore formation. With disulphide bond formation between two chosen amino acids, the conformation of the molecule was trapped in its initial position. If the conformational change is necessary for the pore-formation, the activity would be altered. Therefore, twenty-six double cysteine mutants were prepared and haemolytic activity of mutants in their reduced (S-S not formed) and oxidised (S-S formed) form was measured and compared.
The main differences in haemolytic activity between the two forms were observed only when N-terminal region was attached to the core of the molecule. Besides, no activity of the mutants from the broad loops (R31C-K77C, G85C-Y108C, P107C-Y113C) was detected. Interestingly, the mutant S54C-V87C showed lower haemolytic activity in reduced form, which was a consequence of reduced capability of binding, whereas the binding in the oxidised form was comparable to the wild-type's. All of these mutants have at least one of the amino acids positioned in or close to the region, responsible for binding onto the membrane. The results suggest that the N-terminal region is the only part of the molecule undergoing conformational change during the pore-formation. The dissociation of the helix from the β-sandwich is therefore essential for pore-formation. On the other hand, the structure of the broad loops at the bottom of the molecule and POC-binding pocket need to be preserved in order for the toxin to be able to bind efficiently and continue into the process of pore-formation.
Further, the kinetics of separate steps in the process of pore formation was studied. A triple cysteine mutant (V8C-I18C-K69C) was used. The residue Cys^{18} was labelled by fluorescent probe NBD, with which we could follow the second step, the insertion of the N-terminal α-helix into the lipid bilayer. The first step, the binding, was determined with the inhibition of haemolytic activity. And finally, the whole process was observed with the test of calcein release from lipid vesicles. With this study we could observe three separate steps of the pore formation, which follow each other in an almost instantaneous manner.
Results confirmed that the insertion of N-terminus into the lipid environment and further functional pore formation are dependent on the membrane composition and also on the concentration of the toxin (lipid to toxin ratio). With the observation of kinetics we conclude that the binding, insertion and functional pore-formation follow each other in an almost instantaneous manner.
In the last part the aim was to obtain more information about the topology of the N-terminus (1-10 AA) in the final pore and to study its effect and contribution to pore selectivity and permeability. We used the method of planar lipid membranes (PLM) for functional characterisation of the pore, formed by different toxin mutants.
With S1C mutant, to which additional charges (MTS-agents) were bound and fusion protein His_6-EqtII, the topology of the N-terminus in the final pore was studied. The S1C mutant was able to form pores similar to the wild-type. The addition of charges affected the selectivity, which shows, that the residue Cys^{1} is positioned

in the pore lumen or in its mouth. Besides, addition of charges affected also the ration between voltage and current. The I/V and I^+/I^- ratios give us the information of (re)distribution of charges through the pore. Therefore, this was the first indication, that the N-terminus is transferred to the other, *trans* side of the membrane.
The conductance of a fusion protein His^6-EqtII pores is, in contrast to the wild type EqtII, voltage dependent. With higher voltages applied the conductance grew non-linearly. But with negative voltages applied, the current through the membrane was very small and closures were observed with increasing negative voltages. Histidine-tag, attached to the N-terminus, has a positive charged in experimental conditions used, but the selectivity was not affected. The results confirmed, that the N-terminus of the toxin extends through the pore to the *trans* side of the membrane.
Additionally, the removal of first five N-terminal amino acids (mutant Δ5) results in a reduced activity of the toxin, with retained membrane binding abilities. Besides transient opening and closing of pores in PLM, were also observed. Whereas deletion of first ten amino acids (mutant Δ10), results in the absence of the pore-forming activity. Therefore we conclude that the N-terminus (1-10 AA) enhances the insertion of the whole N-terminal region (1-30 AA) into the membrane and partly helps in stabilisation of the final pore structure.

Krkine nagrade Krka Prizes



Poster ali računalniška predstavitev
Winners' Poster or E-Presentations

TERMODINAMSKA STABILNOST KOMPLEKSOV LIGAND-DNA NA OSNOVI SIMULACIJ MOLEKULSKE DINAMIKE

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Majhne molekule, ki se vežejo v ožji kanal deoksiribonukleinske kisline (DNA), lahko vplivajo na prepis in podvajanje dvojne vijačnice. Poznavanje fizikalnih in kemijskih lastnosti nastanka kompleksov ligand-DNA je zato pomembno pri razvoju zdravil za rakava obolenja. Priznano orodje za raziskavo strukturnih, dinamičnih in termodinamičnih lastnosti kemijskih in biokemijskih sistemov so računalniške simulacije molekulske dinamike. V doktorskem delu so predstavljene simulacije molekulske dinamike dveh ligandov, ki se vežeta v ožji kanal DNA, netropsina in distamicina. Molekulsko dinamiko netropsina in distamicina smo simulirali prosto v raztopini in v kompleksu z DNA. Rezultate simulacij smo uporabili za natančne raziskave interakcij obeh ligandov z dvojno vijačnico DNA in s topilom. Z uporabo metode termodinamske integracije smo izračunali relativno Gibbsovo prosto entalpijo vezave netropsina in distamicina v vezavno mesto AAAAA v ožjem kanalu DNA. Na osnovi kovariančne matrike atomskih nihanj smo izračunali tudi spremembo konfiguracijske entropije, do katere pride ob omenjeni vezavi. Le-ta je bila pri razlagi termodinamskih meritev doslej v celoti zanemarjena, zato rezultati, predstavljeni v pričujočem doktorskem delu, predstavljajo pomemben prispevek k izboljšanju razumevanja tvorbe kompleksov ligand-DNA.

THERMODYNAMIC STABILITY OF DRUG-DNA COMPLEXES FROM MOLECULAR DYNAMICS SIMULATIONS

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Small molecules that bind to the minor groove of DNA are known to interfere with gene expression at the level of transcription and replication and are of considerable interest to the discovery of novel drugs. Molecular dynamics simulations are a well established tool for obtaining structural, dynamical, and thermodynamical properties of a system. In this thesis we have performed molecular dynamics simulations of two DNA minor groove-binding ligands, netropsin and distamycin, both free in solution and in complex with DNA. The interaction of the ligands with the DNA was studied as well as their hydration. Using the thermodynamic integration method the relative free energies of binding of netropsin and distamycin to the DNA minor groove have been calculated. Moreover, the configurational entropy change of both ligands has been estimated using the covariance matrix of atom-positional fluctuations. The results suggest that both netropsin and distamycin lose a significant amount of configurational entropy upon binding to the DNA minor groove. Since this contribution has so far been neglected in the interpretation of their thermodynamic binding profiles the presented results allow for an improved description of the binding of netropsin and distamycin to the DNA minor groove and contribute to a better understanding of the drug-DNA complex formation.

VPLIV SEVOV IZ RODOV *Salmonella* IN *Lactobacillus* NA RAVEN IZRAŽANJA INTERLEUKINA 8 IN PROTEINA TOPLOTNEGA STRESA 70 V CELICAH Caco-2

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Ob okužbi črevesnega epitela s salmonelo *S. enteritidis* pride do izločanja interleukina 8 (IL-8) na mestu vnetja, kar lahko vodi v kronično vnetje črevesja in druge bolezni prebavil. Namen dela je bil preveriti, če laktobacili *L. gasseri* LF221 (LF) in *L. rhamnosus* BGT10 (T10) lahko zaščitijo modelne epitelne črevesne celice Caco-2 pred škodljivimi vplivi *S. enteritidis* 857 (Se857). Izpostavitev celic LF ali T10 in naknadno Se857 ne kaže zaščitnega vpliva. Pri sočasni inkubaciji celic Caco-2 z laktobacili (LF ali T10) in salmonelo pa LF in T10 ovirata invazijo in sintezo IL-8, ki jo spodbudi Se857. LF in T10 ne ovirata adhezije Se857, zato z Se857 verjetno ne tekmujeta za ista vezavna mesta na celicah. Za indukcijo IL-8 v celicah Caco-2 je vsaj delno odgovorna invazija salmonele. Produkti, ki jih LF in T10 izločata v okolje, zmanjšajo sposobnost Se857 za indukcijo sinteze IL-8 v celicah Caco-2. Ti produkti imajo vpliv le pri nizki pH vrednosti in le do določene koncentracije Se857. Se857 zniža transepitelno električno upornost (TEER) celic Caco-2. LF in T10 ne znižata TEER. Pri izpostavitvi celic Caco-2 laktobacilom LF ali T10 pred izpostavitvijo Se857, se zmanjša sposobnost Se857 za znižanje TEER, kar kaže na zaščitno vlogo laktobacilov. Tega vpliva ni pri izpostavitvi celic Se857 in laktobacilom sočasno. Pridobljeni rezultati opravičujejo nadaljnja proučevanja probiotske uporabe *L. gasseri* LF221 in *L. rhamnosus* v prehrani človeka.

INFLUENCE OF STRAINS FROM GENERA *Salmonella* AND *Lactobacillus* ON INTERLEUKIN 8 AND HEAT SHOCK PROTEIN 70 EXPRESSION LEVEL IN Caco-2 CELLS

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Salmonella enteritidis induces expression of interleukin 8 (IL-8) in intestinal epithelium, which may lead to chronic inflammation and gastrointestinal diseases. The aim of this study was to find out if lactobacilli *L. gasseri* LF221 (LF) and *L. rhamnosus* BGT10 (T10) could protect intestinal epithelial model cells Caco-2, against the negative effects of *S. enteritidis* 857 (Se857). Preincubation of Caco-2 cells with LF or T10 prior to Se857 exposure doesn't have protective effect. Simultaneous exposure of Caco-2 cells to Se857 and lactobacilli (LF or T10) inhibits invasion and IL-8 induced by Se857. LF and T10 do not interfere with adhesion of Se857 to Caco-2 cells. Therefore LF and T10 probably don't compete with Se857 for the same adhesion sites on Caco-2 cells. Invasion of Se857 partially accounts for IL-8 induction. Pretreatment of Se857 by LF or T10 products (SCS) inhibits IL-8 secretion in Caco-2 cells. SCS effect develops in acidic conditions and only to limited concentration of Se857. Se857, but not LF and T10, decreases transepithelial electrical resistance (TEER) of Caco-2 cell monolayer. Preincubation of cells with LF or T10 prior to Se857 exposure, reduces ability of Se857 to decrease TEER. This shows the protective role of lactobacilli. Simultaneous exposure of Caco-2 cells to Se857 and lactobacilli doesn't give such effect. Our results suggest further investigations of *L. gasseri* LF221 and *L. rhamnosus* for probiotic use in human nutrition.

MOLEKULARNA TIPIZACIJA SEVOV *Mycobacterium avium*, IZOLIRANIH PRI DOMAČIH ŽIVALIH IN LJUDEH V SLOVENIJI

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V naši raziskavi smo z metodo verižne reakcije s polimerazo (PCR) in metodo analize polimorfizma dolžin restrikcijskih fragmentov (RFLP) genotipizirali izolate bakterije *Mycobacterium avium* iz živali in ljudi. V raziskavo smo zajeli 213 izolatov vrste *M. avium*: 52 podvrste *M. avium* subsp. *avium* iz živali, 118 podvrste *M. avium* subsp. *hominissuis* (58 iz živali in 60 iz ljudi) in 43 podvrste *M. avium* subsp. *paratuberculosis* iz živali. Vse izolate podvrst *M. a. avium* in *M. a. hominissuis* (n = 170) smo tipizirali z metodo PCR. Tipizacija je uspela pri 98 izolatih podvrste *M. a. hominissuis*. Z metodo *Pst*I-*BstE*II-IS*900* RFLP smo pri 10 izolatih podvrste *M. a. paratuberculosis* definirali dva genotipa. Z metodo *Pvu*II-IS*901* RFLP smo pri 52 izolatih podvrste *M. a. avium* definirali 7 genotipov, z metodo *Pst*I-IS*901* RFLP pa pri 41 izolatih prav tako 7 genotipov. Ugotovili smo, da so izbruhe mikobakterioz povzročili sevi istega genotipa. Prenosa okužbe med različnimi živalskimi vrstami nismo potrdili. Z metodo *Pvu*II-IS*1245* RFLP smo uspešno genotipizirali 57 izolatov podvrste *M. a. hominissuis* iz živali in 57 iz ljudi. Enake genotipe smo odkrili pri izolatih iz obeh virov. Naši rezultati podpirajo domnevo, da se bolezen ne prenaša med živalmi, temveč se te okužujejo iz okolja. Pri ljudeh smo posumili na okužbo v bolnišničnem okolju, dokazali prenos okužbe med bolniki, odkrili monoklonalne in poliklonalno okužbo ter reaktivacijo bolezni, posumili pa smo tudi na reinfekcijo.

MOLECULAR TYPING OF *Mycobacterium avium* STRAINS ISOLATED FROM DOMESTIC ANIMALS AND HUMANS IN SLOVENIA

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In our study, genetic diversity of *Mycobacterium avium* isolates from animals and humans was assessed using polymerase chain reaction (PCR) typing and restriction fragment length polymorphism (RFLP) analysis. A total of 213 *M. avium* isolates were investigated: 52 *M. avium* subsp. *avium* isolates from animals, 118 *M. avium* subsp. *hominissuis* isolates (58 from animals and 60 from humans) and 43 *M. avium* subsp. *paratuberculosis* isolates from animals. All *M. a. avium* and *M. a. hominissuis* isolates (n = 170) were PCR typed. Typing succeeded in 98 isolates. *Pst*I- and *BstE*II-IS*900* RFLP resulted in two genotypes among 10 *M. a. paratuberculosis* isolates. Using *Pvu*II- and *Pst*I-IS*901* RFLP, 52 and 41 *M. a. avium* isolates were typed, respectively. Seven *Pvu*II and 7 *Pst*I genotypes were defined. The outbreaks of mycobacteriosis were caused by the strains of the same genotype. Transmission of the infection between different animal species was not confirmed. Using IS*1245*-*Pvu*II RFLP, 57 *M. a. hominissuis* isolates from animals and 57 isolates from humans were typed. Identical genotypes were found among the isolates from both sources. Our findings indicate the environment as the source of infection; the disease is probably not transmitted between the animals. Investigation of human isolates revealed a suspicion of nosocomial infection; transmission of the infection between the patients was also demonstrated. Monoclonal and polyclonal infections and reactivation of the disease were found while re-infection was suspected.

VNOS GENOV V KOŽO Z ELEKTROPORACIJO

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Membrana biološke celice v splošnem ni prepustna za večje molekule, ob prisotnosti zadosti visokega električnega polja pa se permeabilnost celične membrane poveča. Ta pojav imenujemo elektropermeabilizacija in omogoči molekulam, kot so nekatere zdravilne učinkovine ali DNK, vstop v celično notranjost. V raziskavi obravnavamo vpliv električnih parametrov in geometrije elektrod na vnos genov v kožo z elektroporacijo. S pomočjo *in vivo* poskusov smo pokazali, da s kombinacijo kratkega, visokonapetostnega elektroporacijskega pulza, ki mu sledi dolg, nizkonapetostni elektroforetični pulz, dosežemo uspešen vnos genov v kožo, brez poškodb tkiva. Nadalje smo pokazali, da je tak način vnosa genov primeren za aplikacije kot je genska imunizacija kože, kjer potrebujemo kratkotrajno prisotnost genov. Proces elektropermeabilizacije kožnega tkiva je v opisan tudi z numeričnimi modeli. Naredili smo tudi uvodno primerjavo med zunanjimi, ploščatimi elektrodami in različnimi postavitvami polja nebolečih igelnih mikroelektrod. V prihodnosti bomo numerične modele še izboljšali, narejeni pa bodo tudi *in vivo* poskusi z uporabo igelnih mikroelektrod, kar bo vodilo v nadaljnji razvoj postopkov vnosa genov v kožo s pomočjo elektroporacije. Rezultati raziskav bodo vodili v klinične študije, z željo po uveljavitvi te nevirusne metode vnosa genov za gensko terapijo.

GENE TRANSFECTION IN SKIN BY MEANS OF ELECTROPORATION

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A biological cell is, in general, impermeable for larger molecules; however, the application of electric pulses to cells, either in suspension or tissue, permeabilizes the cell membrane. This phenomenon is termed electropermeabilization and makes it possible for larger molecules that otherwise can not cross the membrane, such as drug molecules or DNA, to enter the cell. In this study, the influence of different parameters of electric pulses and electrode geometries on DNA electrotransfer in skin was investigated. *In vivo* experiments showed that using a combination of a short high voltage, permeabilizing pulse, followed by a long low voltage, electrophoretic pulse, yields efficient electrogene transfer in skin and no tissue damage. Further, we showed that DNA electrotransfer in skin is adapted to applications such as immunization or local skin treatment, where a short-term expression in the skin is needed. We described the process of skin electropermeabilization with numerical models that will be further improved in the future. Also, a preliminary comparison between plate electrodes and different settings of painless microneedle electrodes was made. Together with pending *in vivo* experiments using microneedle electrode arrays, further advance of DNA electrotransfer in skin will be achieved. The results of our research will lead to clinical studies with the aim of establishing this non-viral gene delivery method as a successful approach to gene therapy.

MOLEKULARNI VIDIK ADAPTACIJE KOLORADSKEGA HROŠČA (*Leptinotarsa decemlineata* Say) NA OBRAMBNI ODGOVOR RASTLINE

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Koloradski hrošč (KH), najpomembnejši krompirjev škodljivec, zelo hitro razvije odpornost proti kemičnim insekticidom, zato se pojavlja potreba po alternativnih oblikah kontrole. Po napadu KH se v krompirju inducirajo velike količine proteaznih inhibitorjev, ki so del rastlinske obrambe. KH zaobide takšno obrambo s sintezo prebavnih cisteinskih proteaz (intestainov), ki so neobčutljive na krompirjeve inhibitorje. Da bi raziskali obseg in molekulske osnove insektne adaptacije, smo izolirali številne cDNA intestaine, uvrščene v dve novi skupini, D in E. Molekulski modeli intestainov D in E kažejo, da imajo papainsko zvitje in se od sorodnih proteaz razlikujeta v aminokislinah v vezavnem mestu za substrat/inhibitor, ki predvidoma onemogočajo interakcijo s krompirjevimi inhibitorji in vplivajo na substratno specifičnost obeh skupin. Izražanje genov za intestaine D se poveča za dvakrat v dolgotrajni adaptaciji ličink, hranjenih s krompirjevimi listi z visoko vsebnostjo endogenih protezanih inhibitorjev. Na osnovi rekombinantnih intestainov smo pokazali, da različno uravnana encimska aktivnost in različna substratna specifičnost posameznih skupin intestainov pomembno prispevata k izogibanju negativnega vpliva krompirjevih obrambnih molekul na fiziološko stanje žuželke. Naša študija dokazuje, da je adaptacija koloradskega hrošča na obrambni odgovor krompirja zelo kompleksen proces in bo v pomoč nadaljnemu ciljanemu iskanju okolju prijaznejših biotskih načinov zaščite rastlin pred škodljivci.

MOLECULAR ASPECT OF COLORADO POTATO BEETLE ADAPTATION (*Leptinotarsa decemlineata* Say) TO PLANT DEFENSE RESPONSE

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The most important potato pest, Colorado potato beetle (CPB), rapidly acquires resistance to pesticides, so there is a need for alternative means of control. High levels of protease inhibitors are induced in potato as part of a defense response to insect attack. However, the CPB overcomes this defense by inducing digestive cysteine proteases (intestains) insensitive to potato inhibitors. The aim of this study this study was to explore the extent and molecular basis of insect adaptation. Numerous intestain cDNAs, classified in two new groups, D and E, have been isolated from adapted guts. Molecular modelling predicts that intestains D and E possess the papain-like fold. They differ from related proteases in residues in the substrate/inhibitor binding sites that are expected to reduce interaction with the potato inhibitors and influence substrate specificity. Intestain D genes are up-regulated two-fold in the long-term adaptation to the challenge of potato defense protease inhibitors. On the basis of recombinant intestains we have shown that both, differentially regulated enzyme activity and diverse substrate specificities of intestain groups, play an important role in combating negative effects of potato defense molecules on insect physiological condition. Our study enlightens the complex nature of CPB adaptation process to potato defence response. We believe that deeper understanding of the detailed mechanism of insect adaptation should lead to effective means of environmentally friendly plant protection against pests.

NMR ŠTUDIJE VEZAVE KOVINSKIH IONOV ZNOTRAJ G-KVADRUPLEKSNIH STRUKTUR DNK

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Molekule DNK lahko tvorijo tudi številne strukture višjega reda, kot so na primer G-kvadrupleksne strukture. Za tvorbo takšnih struktur je nujno potrebna prisotnost kationov. V zadnjem času se je povečalo zanimanje za G-kvadrupleksne stukture DNK, saj je tvorba takšnih struktur udeležena pri številnih pomembnih bioloških procesih. Pomembno področje raziskav je možnost njihove uporabe v terapevtske namene. Raziskave gredo v smer razvoja majhnih molekul, ki se vežejo in pomagajo zvijati oziroma stabilizirati G-kvadrupleksne strukture, z namenom razvoja novih zdravil proti rakavim in viroznim obolenjem. S pomočjo NMR spektroskopije smo pokazali, da je zvitje G-kvadrupleksnih struktur močno odvisno od samega sekvenčnega zaporedja, kakor tudi od narave kationov prisotnih v raztopini. Že majhna sprememba v sekvenci bogati z gvanini lahko povzroči veliko spremembo v sami topologiji zvitja G-kvadrupleksa. Oligonukleotidno zaporedje $d(G_3T_4G_4)$ v prisotnosti tako K^+ in $^{15}NH_4^+$ kakor tudi Na^+ ionov tvori G-kvadrupleksno strukturo sestavljeno iz treh G-kvartetnih ravnin, dveh dodatnih gvaninov, ki sta na različnih koncih G-kvartetne osnove ter ne sodelujeta pri tvorbi G-kvartetov. Dve timidinski zanki potekata po diagonali oziroma po robu zunanjih G-kvartetov. S pomočjo ^{15}N označenega amonijevega iona smo pokazali, da sta znotraj G-kvadrupleksa $d(G_3T_4G_4)_2$ dve vezavni mesti za katione, ki pa se močno razlikujeta po svojih preferencah za vezavo določenega kationa. Z NMR eksperimenti smo potrdili mešano obliko G-kvadrupleksa $d(G_3T_4G_4)_2$, ki jo stabilizirata en $^{15}NH_4^+$ in en K^+ ion, kar je prvi opisan primer v literaturi in prestavlja vmesno stanje v prehodu med dvo-$^{15}NH_4^+$ v dvo-K^+ obliko G-kvadrupleksa $d(G_3T_4G_4)_2$.

NMR STUDIES OF METAL ION BINDING INSIDE G-QUADRUPLEX DNA STRUCTURES

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DNA molecules can adopt besides several higher-order structures, including G-quadruplex structures. In the past years G-quadruplex DNA structures are a subject of great interest since their formation has been suggested to play a role in variety of important biological processes as well as due to their potential therapeutic applications. The major requirement for the formation of such structures is the presence of cations. Scientists have started with development of small molecules that can bind and help in formation and stabilization of G-quadruplex structures in order to develop anti-cancer and anti-viral drugs. By the use of NMR spectroscopy we have shown that the folding topologies of G-quadruplex structures critically depend on the sequence details as well as nature of cations present in solution. $d(G_3T_4G_4)$ sequence folds into dimeric fold-back G-quadruplex structure, which consists of three G-quartet planes, two overhanging guanine residues and diagonal as well as edge-type loops in the presence of K^+, $^{15}NH_4^+$ or Na^+ ions. By the use of ^{15}N-labeled ammonium ions, we have shown that $d(G_3T_4G_4)_2$ G-quadruplex exhibits two cation binding sites between three of its G-quartets. NMR experiments confirm existence of a mixed mono-K^+-mono-$^{15}NH_4^+$ form that represents intermediate in the conversion of di-$^{15}NH_4^+$ into di-K^+ form of $d(G_3T_4G_4)_2$ G-quadruplex.

UPORABA INVERZNE PLINSKE KROMATOGRAFIJE ZA DOLOČANJE POVRŠINSKIH LASTNOSTI UČINKOVIN IN POMOŽNIH SNOVI ZDRAVIL

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Vhodne snovi, ki jih uporabljamo v razvoju in proizvodnji zdravil, morajo biti za razumevanje procesov dobro definirane. Za analizo in vrednotenje trdnih snovi uporabljamo različne metode, ki jih lahko delimo na tiste, kjer merimo in opazujemo celotno maso vzorca ali samo njegovo površino. Med slednjimi postaja vse pomembnejša inverzna plinska kromatografija (IGC – Inverse Gas Chromatography).
Namen doktorskega dela je bil razviti, ovrednotiti in v uporabo vpeljati metodo inverzne plinske kromatografije. Običajni plinski kromatograf smo predelali v inverzni plinski.
Z IGC smo proučevali kako metoda priprave trdnih snovi in mletje vplivata na njene površinske lastnosti. Poleg proučevanja lastnosti posameznih snovi smo z IGC meritvami napovedovali adhezivnost visoko-koncentriranih zmesi, ki se uporabljajo v obliki suhih praškov za inhaliranje. Metodo smo uspešno uporabili tudi za vrednotenje termičnih lastnosti trdnih snovi. Polimerom smo določali temperaturo steklastega prehoda.
Rezultate IGC smo potrdili s komplementarnimi analitskimi metodami.
Ugotovili smo, da je inverzna plinska kromatografija široko uporabna komplementarna metoda za določevanje fizikalno-kemijskih lastnosti različnih materialov.

USE OF INVERSE GAS CHROMATOGRAPHY FOR THE DETERMINATION OF SURFACE PROPERTIES OF ACTIVE PHARMACEUTICAL INGREDIENTS AND EXCIPIENTS

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In order to understand the pharmaceutical processes involved in the development and production of pharmaceuticals, the raw materials that are used have to be well characterized. Different methods can be used to characterize and evaluate dry powders. They can be divided into those where the bulk sample is analysed and those where only the surface of the sample is studied. Among the latter, inverse gas chromatography (IGC) is becoming more and more important.
The purpose of the project described in this thesis was to develop, evaluate and introduce into use the method of inverse gas chromatography. A gas chromatograph was modified to enable IGC measurements. IGC was used to study the influence of an isolation procedure and milling on the surface properties of a solid.
Besides characterizing the properties of single powders, the results from IGC analysis were used to predict the adhesion capacity of highly loaded interactive mixtures for use in dry powder inhalers. IGC was successfully used for studying the thermal properties of solid samples. The glass transition temperatures of various polymers were determined. The results were confirmed with other, complementary, analytical methods.
It has been shown in this thesis that the IGC is a valuable complementary technique for physico-chemical characterization of various materials.

UGOTAVLJANJE SELENOVIH SPOJIN V AJDI, GOJENI PRI IZBRANIH RAZMERAH

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Namen dela je bil raziskati sposobnost privzemanja selena pri navadni ajdi (Fagopyrum esculentum) sorte Darja z metodo HG-AFS in identificirati selenove spojine v njenih ekstraktih z metodo HPLC-UV-HG-AFS. Semena ajde smo namakali v deionizirani vodi ali v raztopinah, ki so vsebovale 5, 10 ali 20 mg Se/L v obliki SeVI ali SeIV ali selenometionina (10 mg Se/L). Kalice so največ selena privzele v obliki SeVI, manj v obliki selenometionina in najmanj v obliki SeIV. Selen v selenovih spojinah smo ugotavljali v ekstraktih HCl in po encimski hidrolizi s proteazo XIV. V kislinskih supernatantih kalic iz semen, namakanih v SeVI, smo kot glavno spojino ugotovili SeVI, sledove SeIV in pri koncentraciji 10 mg SeVI/L ter 20 mg SeVI/L tudi prisotnost neznane selenove spojine. V kislinskih ekstraktih kalic iz semen, namakanih v raztopini SeIV ali selenometionina, smo našli neidentificirano selenovo spojino. V encimskem ekstraktu kalic iz semen, namakanih v raztopini SeVI, smo ugotovili prisotnost selenometionina, Se-metilselenocisteina, SeIV in dveh neznanih selenovih spojin. Pri koncentracijah 10 mg L in 20 mg SeVI/L smo opazili tudi vrh za SeVI. V encimskem ekstraktu kalic iz semen, namakanih v raztopini SeIV, smo ugotovili prisotnost selenometionina, Se-metilselenocisteina, sledove SeVI in neznano selenovo spojino. Pri koncentracijah 10 mg SeIV/L in 20 mg SeIV/L smo ugotovili še prisotnost sledov SeIV. V primeru namakanja semen v raztopini selenometionina smo zasledili prisotnost sledov selenometionina in dveh neznanih selenovih spojin. Na vsebnost selenovih spojin vpliva tudi UV-B sevanje.

DETERMINATION OF SELENIUM COMPOUNDS IN BUCKWHEAT GROWN AT SELECTED CONDITIONS

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The aim of our work was to check the ability of common buckwheat (Fagopyrum esculentum), cv. Darja to accumulate selenium. HG-AFS and HPLC-UV-HG-AFS were used to identify selenium compounds. Seeds were soaked in water or in solutions containing 5, 10 or 20 mg Se/L in the form of SeVI or SeIV or selenomethionine (10 mg Se/L). Selenium was identified in acidic supernatants and after enzymatic hydrolysis with protease XIV. In acidic supernatants of sprouts from the seeds, soaked in solutions of SeVI, the main compound was Se(VI). Traces of SeIV were also found and at concentrations 10 mg and 20 mg SeVI/L un unknown selenium compound was detected. In HCl extracts of sprouts from seeds soaked in solutions of SeIV or selenomethionine un unknown selenium compound was detected. In supernatants of sprouts from seeds soaked in solutions of SeVI selenomethionine, Se-methylselenocystein, SeIV and two unknown compounds were detected. A peak coresponding to SeVI was found at 10 and 20 mg SeVI/L. In sprout supernatants from seeds soaked in solutions of SeIV, selenomethionine, Se-methylselenocysteine, SeVI and unknown selenium compound were detected. At the concentrations of 10 and 20 mg SeIV /L SeIV was also found. In enzymatic extract of sprouts from seeds soaked in a solution of selenomethionine, traces of selenomethionine and two unknown selenium compounds were detected. The content of selenium compounds is also affected by UV-B radiation.

TEST RISANJA URE

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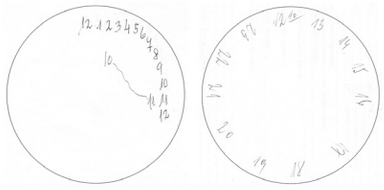
Z našo raziskavo smo želeli poenotiti in poenostaviti sistem ocenjevanja testa risanja ure (TRU) do te mere, da bi bil primerljivo senzitiven in specifičen z bolj uporabljanim Kratkim preizkusom spoznavnih sposobnosti (KPSS). Na podlagi klinične ocene psihiatra smo 158 preiskovancev razdelili na zdrave in dementne (n=119). Vsi so opravili TRU in KPSS (maks. 30 točk). Rezultat TRU pri slovenskih bolnikih z demenco je primerljiv z rezultati, dobljenimi z zahtevnejšimi sistemi ocenjevanja. TRU dobro korelira z diagnozo demenca (-0,64, p<0,001), najbolje z Alzheimerjevo boleznijo (-0,78; p<0,001). Mejo med zdravimi (A) in bolniki z demenco (B, C) smo postavili na 3 od 4 točk (SE 87%; SP 90%). Rezultata obeh testov korelirata dobro (0,632; p<0,001). Z uporabo obeh testov lahko izboljšamo iskanje bolnikov z demenco (SE 93%, SP 79%).

A

B

C



CLOCK DRAWING TEST

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The goal of our study was to simplify and unify scoring system of Clock drawing test (CDT), as to make it comparable in sensitivity (SE) and specificity (SP) to more widely used Slovenian adaptation of Mini - Mental State Examination (KPSS) On the basis of clinical evaluation of a psychiatrist, 158 volunteers were grouped either as »healthy« or »demented« (n=119). Both groups were subjected to CDT and KPSS (maximal score=30). The results suggest that our system is comparable to more elaborate scoring systems in Slovenian dementia patients. CDT corelates well with the diagnosis of dementia (-0,64; p<0,001) and is strongest in Alzheimer's disease (-0,78; p<0,001). Cutt-off score between healthy (A) and demented (B, C) was set to 3 out of 4 points (SE 87%; SP 90%). Results of both test show good corellation (0,632; p<0,001). With the use of both tests, we could improve recognition of patients with dementia (SE 93%, SP 79%).

OPTIMIZACIJA ELEKTROFUZIJE CELIC SESALCEV V POGOJIH *in vitro*

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Elektrofuzija oziroma zlivanje celic z električnimi pulzi je pojav, pri katerem se celice, ki so med seboj v tesnem stiku, zaradi prisotnosti kratkotrajnih visokonapetostnih električnih pulzov zlijejo med seboj. Če celice niso v tesnem stiku, električni pulzi povzročijo samo permeabilizacijo celic, to je začasno neselektivno povečanje prepustnosti membrane. Na oba pojava vplivajo različne lastnosti električnega polja. V pričujočem delu smo proučevali vpliv amplitude in števila pulzov ter smeri električnega polja na zlivanje in na permeabilizacijo celic. Preizkusili smo metodo zlivanja celic z električnimi pulzi na dveh celičnih linijah v pogojih *in vitro*. Primerjali smo celice mišjega melanoma B16F1 in ovarijske celice kitajske hrčice CHOK1. Stopnjo permeabilizacije celic smo določali s fluorescenco vnesenega propidijevega jodida, zlivanje celic pa smo določali mikroskopsko, pri čemer smo si pomagali z barvanjem Giemsa. Zlivanje celic je metoda, ki jo lahko koristno uporabimo v biotehnologiji in biomedicini. V našem delu smo uporabili proučevano metodo zlivanja celic z električnimi pulzi za pridobivanje hibridomov iz mišjih mielomskih celic in človeških limfocitov z modificirano Koehler Milsteinovo hibridomno tehnologijo. Rezultati naše metode so primerljivi s tistimi, ki so jih pred nami pridobili z zlivanjem enakih celic s polietilenglikolom.

OPTIMIZATION OF ELECTROFUSION OF MAMMALIAN CELLS *in vitro*

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Cell electrofusion is a phenomenon that occurs, when cells that are in close contact are fused by means of short, high-voltage electric pulses. When cells are not in close contact, the consequence of the pulses is transient and nonselective increase of permeabilization of cell membranes without fusion. Both phenomena are dependent on the choice of electric field parameters. In this work we studied the influence of amplitude and number of electric pulses and direction of electric field on the degree of permeabilization and fusion yield. We tested the method of electrofusion on two cell lines *in vitro*. We compared mouse melanoma cells B16F1 and chinese hamster ovary cells CHOK1. Degree of cell permeabilization was determined microscopically by fluorescence of propidium iodide. Fusion was detected microscopically with Giemsa staining. Fusion of cells can be useful in biotechnology and biomedicine. In present work electrofusion was used to produce hibridomas from mouse mieloma cells NS1 and human lymfocytes with modified Koehler Milstein hibridoma technology. We obtained viabile human – mouse heterohybridoma in amounts that are comparable to those previously obtained by fusing the same cells with the use of polyethylene glycol.

SINTEZA IN PRETVORBE NEKATERIH *N*-ACILTRIAZENOV IN TRIAZENIDOV

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Triazeni so zelo uporabna in raznolika skupina spojin. Študirali so jih kot substance za zdravljenje raka in so bili uporabljeni za različne namene v organski kemiji. Delo obravnava sintezo, lastnosti in nove aplikacije 1,3-diariltriazenov in njihovih derivatov. Sintetizirali smo tri 1,3-diariltriazene, ki imajo v fenilnem obroču na mestu 2 vezan brom, fluor ali trifluorometilno skupino, na mestu 4 pa nitro skupino. Nadalje smo 1,3-bis(2-kloro-4-nitrofenil)triazen in 2-bromo-4-nitro ter 2-fluoro-4-nitro analoga obdelali s trimetilaminom, trietilaminom ali piperidinom v acetonu, pri čemer smo dobili trialkilamonijeve soli. Na nekatere triazene smo uvedli acetilno, 3-metoksi-3-oksopropanoilno in različne (2*E*)-3-(dialkoksifenil)akriloilne skupine. Z reakcijo trietilamonijevega 2-kloro-4-nitrofenil- in 2-bromo-4-nitrofeniltriazenida z metil propiolatom in etil propiolatom smo pokazali, da nastanejo alkoksikarbonilviniltrietilamonijevi triazenidi s *trans* konfiguracijo. Iste spojine smo dobili tudi z direktno reakcijo iz triazenov, trietilamina in propiolatov. Ugotovili smo, da se estri zlahka preestrijo v različnih nasičenih alkoholih, alilnem alkoholu in 3-metilbut-2-en-1-olu. Reakcije so reverzibilne in potekajo najhitreje v metanolu. Preestrenja z nasičenimi alkoholi smo izvajali tudi pod pogoji obsevanja z mikrovalovi, s čimer smo zelo povečali hitrost reakcije. Izkazalo se je, da pri preestrenjih triazenidni anion igra vlogo katalizatorja. Različne triazene, acilirane triazene, triazenil acetate in amonijeve triazenide so testirali kot inhibitorje celic raka materničnega vratu (HeLa) in D-alanil-D-alanin ligaze, pri čemer so v obeh primerih testirane spojine pokazale obetavno učinkovitost.

SYNTHESIS AND TRANSFORMATIONS OF SOME *N*-ACYLTRIAZENES AND TRIAZENIDES

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Triazenes are very useful and versatile group of compounds. They were studied as anticancer drugs and used in different areas of organic chemistry. This work describes the synthesis, properties and new applications of 1,3-diaryltriazenes and their derivatives. 1,3-diaryltriazenes containing a nitro group and bromine, fluorine or trifluoromethyl group in the phenyl ring were synthesized. In reaction of 1,3-bis(2-chloro-4-nitrophenyl)triazene and 2-bromo-4-nitro or 2-fluoro-4-nitro analogues with trimethylamine, triethylamine or piperidine in acetone, trialkylammonium salts were formed. Acylated triazenes possesing acetyl group, 3-methoxy-3-oxopropanoil group or various (2*E*)-3-(3,5-dimethoxyphenyl)acryloyl groups were also prepared. In the reaction of triethylammonium 2-chloro-4-nitrophenyl and 2-bromo-4-nitrophenyltriazenide with ethyl propiolate in acetonitrile alcoxycarbonylvinyltriethylammonium triazenides with *trans* configuration were obtained. The same compounds were also synthesised with a direct reaction from triazene, triethylamine and alkyl propiolate. These esters are very prone to transesterifications in saturated alcohols, allylic alcohol and 3-methylbut-2-en-1-ol. Reactions are reversible and are particularly fast in methanol. Transesterifications were also performed in saturated primary alcohols at elevated temperatures under microwaves. From transesterifications studies it was obvious that the triazenide anion catalyzes the reactions. Different triazenes, acylated triazenes, triazenyl acetates and ammonium triazenides were tested as inhibitors of cervical carcinoma cells (HeLa) and D-alanyl-D-alanine ligase and in both testings they showed promising activity.

NOVI INHIBITORJI ČLOVEŠKE HIDROKSISTEROID-DEHIDROGENAZE AKR1C1

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AKR1C1 je 20α-hidroksisteroid-dehidrogenaza, ki katalizira od NADPH odvisno redukcijo progesterona v 20α-hidroksiprogesteron, kakor tudi redukcijo alosteričnega modulatorja $GABA_A$ receptorjev 3α,5α-tetrahidroprogesterona v manj aktivno obliko 5α-pregnan-3α,20α-diol. Na ta način je AKR1C1 povezana z nastankom hormonsko odvisnih oblik rakavih obolenj in z bolezenskimi stanji kot so epilepsija, depresija in predmenstrualni sindrom. Človeški rekombinantni protein AKR1C1 smo izolirali s pomočjo bakterije *Escherichia coli* z vstavljenim plazmidom pGEX-AKR1C1, ki nosi zapis za fuzijski protein glutation-S-transferaza-AKR1C1. Encim smo očistili z afinitetno kromatografijo na glutation sefarozi in izolirali s cepitvijo s trombinom. Kot potencialne inhibitorje smo testirali strukturno različne spojine: derivate pirimidina, derivate ftalimida, derivate piridazina ter derivate ciklopentana. Inhibicijo oksidacije splošnega substrata aldo-keto reduktaz, 1-acetonaftola ob hkratni redukciji koencima NAD^+, smo določali spektrofotometrično. Merili smo porast absorbance pri 340 nm. Za vse spojine smo določili odstotek inhibicije encimsko katalizirane reakcije, najboljšim inhibitorjem smo določili tudi IC_{50} vrednosti pri 500 µM koncentraciji substrata. Kot najboljši inhibitorji so se izkazali derivati pirimidina, ki predstavljajo dobre spojine vodnice za iskanje novih inhibitorjev encima AKR1C1.

NEW INHIBITORS OF HUMAN HYDROXYSTEROID DEHYDROGENASE AKR1C1

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AKR1C1 is a 20α-hydroxysteroid dehydrogenase that catalyzes NADPH dependent reduction of progesterone to less potent 20α-hydroxyprogesterone, and reduction of 3α,5α-tetrahydroprogesterone, an allosteric modulator of $GABA_A$ receptor, to less active 5α-pregnane-3α,20α-diol. AKR1C1 is an emerging therapeutic target in the treatment of hormone-dependent forms of cancer, as well as in the conditions such as epilepsy, depressive disorders and premenstrual syndrome. We isolated recombinant human hydroxysteroid dehydrogenase AKR1C1 from the bacteria *Escherichia coli* transformed with plasmid pGEX-AKR1C1 that codes for fusion protein glutation-S-transferase-AKR1C1. AKR1C1 was purified with affinity chromatography by binding to glutathione-sepharose followed by cleavage with thrombin. We examined inhibitory activities of structurally different compounds: derivates of pyrimidine, phtalimide, pyridazine and cyclopentan. The inhibitory potency of the selected compounds towards the recombinant AKR1C1 was measured spectrophotometrically following the oxidation of a common AKR substrate 1-acenaphthenol in the presence of NAD^+ as a coenzyme. Percentage of inhibition was determined for all compounds and IC_{50} values were determined only for compounds that showed more than 30% of inhibition. Derivates of pyrimidin proved to be good inhibitors of AKR1C1 and represent promising lead compound for further modifications in the search for more potent inhibitors of AKR1C1.

ŠTUDIJ KONFORMACIJSKEGA PREHODA IN INTERMOLEKULARNE ASOCIACIJE POD VPLIVOM STRIŽNIH SIL V RAZTOPINAH ATAKTIČNE IN IZOTAKTIČNE POLIMETAKRILNE KISLINE

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V pričujočem delu predstavljamo rezultate študije ataktične (a-PMA) in izotaktične polimetakrilne kisline (i-PMA) v vodnih raztopinah z dodatkom NaCl in $NaClO_4$. Izvedli smo potenciometrične titracije, UV spektroskopijo in meritve sipanja svetlobe pod kotom 90º pri 25 ºC. Izračunali smo spremembo standardne proste energije prehoda. Vrednosti spremembe standardne proste energije prehoda so večje za i-PMA kot za a-PMA. Iz UV spektrov smo določili valovne dolžine, ki ustrezajo maksimumu neionizirane in ionizirane karboksilne skupine ter izozbestično točko. Oba stereoizomera se razlikujeta po valovni dolžini izozbestične točke. Meritve sipanja so pokazale, da v raztopinah a-PMA pride do medmolekulske asociacije pod vplivom strižnih sil. Tega pojava pa nismo zasledili v raztopinah i-PMA.

STUDY OF THE CONFORMATIONAL TRANSITION AND OF SHEAR-INDUCED INTERMOLECULAR ASSOCIATION IN AQUEOUS SOLUTIONS OF ATACTIC AND ISOTACTIC POLYMETHACRYLIC ACID

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In this work results of the study of atactic (a-PMA) and isotactic polymethacrylic acid (i-PMA) in aqueous solutions with added NaCl and $NaClO_4$ are presented. Potentiometric titrations, UV spectroscopy and light scattering measurements were performed at 25 ºC. We calculated Gibbs free energy of the conformational change. Values of Gibbs free energy of conformational change were greater for i-PMA than for a-PMA. From the UV spectra, wavelengths of the maxima that correspond to the unionized and to the ionized carboxyl groups and the isosbestic point were determined. Both stereoisomers differ in the wavelength of the isosbestic point. Light scattering measurements in solutions of a-PMA point to shear-induced intermolecular association. This phenomenon was not observed in solutions of i-PMA.

IZRAŽANJE FUZIJSKE BELJAKOVINE MED ZELENO FLUORESCENČNO BELJAKOVINO IN RECEPTORJEM ZA GLUKAGONU PODOBNI PEPTID-1 V CELICAH OVARIJEV KITAJSKEGA HRČKA

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V zadnjih letih je postala zelena fluorescenčna beljakovina (GFP) pomembno orodje za vizualizacijo celičnih beljakovin in struktur v živih celicah ter za opazovanje celičnih procesov. Z našo nalogo smo želeli s pomočjo fuzijske beljakovine med GFP in receptorjem za glukagonu podobni peptid-1 (hRGP-1) (GFP-hRGP-1) ugotoviti mesto izražanja RGP-1 v celici in preveriti, ali fuzijska beljakovina ohrani funkcionalnost RGP-1. Kot model za proučevanje RGP-1 smo uporabili celice ovarijev kitajskega hrčka (CHO), v katere smo vnesli gen za hRGP-1 ali gen za GFP-hRGP-1. Rezultati so pokazali, da se v plazemski membrani izraža malo GFP-hRGP-1, velika koncentracija pa je v neposredni okolici jedra. Fuzijske beljakovine s specifičnimi poliklonskimi protitelesi proti GFP nismo mogli dokazati, medtem ko smo z uporabo protiteles proti RGP-1 dobili v membranskih vzorcih celic z genom za hRGP-1 proteinsko liso, ki ustreza hRGP-1. Dodatek hormona, glukagonu podobnega peptida-1 (GP-1) poviša aktivnost hRGP-1 in GFP-hRGP-1 v membranskih vzorcih CHO celic, iz česar lahko zaključimo, da GFP-hRGP-1 ohrani funkcionalnost hRGP-1, ki za polno biološko aktivnost potrebuje izražanje v plazemski membrani. Ugotovitev, da je GFP-hRGP-1 indikator mesta izražanja hRGP-1, ki ohrani funkcionalnost hRGP-1, bi lahko imela pomen pri nadaljnjih raziskavah hRGP-1 za potrebe zdravljenja diabetesa mellitusa tipa 2.

EXPRESSION OF A FUSION PROTEIN BETWEEN THE GREEN FLUORESCENT PROTEIN AND THE GLUCAGON-LIKE PEPTIDE-1 RECEPTOR IN CHINESE HAMSTER OVARY CELLS

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In recent years the green fluorescent protein (GFP) has become an important tool for visualization of cell proteins and structures in living cells and for monitoring of cell processes. In our study we used a fusion protein between GFP and glucagon-like peptide-1 receptor (hGLP-1R) (GFP-hGLP-1R) to establish the site of expression of hGLP-1R in the cell. We also tried to test if GFP-hGLP-1R retains biological activities of native hGLP-1R. As a model to study hGLP-1R we used Chinese hamster ovary cells (CHO) in which we transfected gene encoding for hGLP-1R or for GFP-hGLP-1R. The results have shown that minimal amount of GFP-hGLP-1R is expressed in plasma membrane but high concentration is seen in close vicinity of nucleus. We were not able to detect fusion protein using anti-GFP, whereas application of anti-hGLP-1R has resulted in a protein band in membrane preparations of cells transfected with hGLP-1R, corresponding hGLP-1R. Adding glucagon-like peptide-1 (GLP-1) increases activity of GLP-1R and GFP-hGLP-1R in membrane preparations of transfected cells. From this we can conclude that GFP-hGLP-1R retains biological activity of hGLP-1R, which needs to be expressed in plasma membranes to be fully functional. Considering GFP-hGLP-1R as an indicator of hGLP-1R expression which preserves biological activity of native hGLP-1R could be important in further research of hGLP-1R for the need of treatment of diabetes mellitus type 2.

DOLOČEVANJE CELOKUPNEGA DUŠIKA V TEKOČIH VZORCIH Z VISOKO TEMPERATURNO KATALITIČNO OKSIDACIJO

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Diplomsko delo predstavlja metodo za določevanje celokupnega dušika (TN) v tekočih vzorcih z visoko temperaturno katalitično oksidacijo. Razvito analizno metodo smo validirali in določili parametre (natančnost, točnost, selektivnost, delovno območje, linearnost metode, mejo zaznave in mejo kvantifikacije). Pri ovrednotenju smo si pomagali s statističnimi testi tako, da smo poiskali morebitne ubežnike z Dixonovim Q testom in Grubbs – Beckovim testom. Točnost metode smo določali s t-testom. Določili smo vsebnost totalnega dušika v standardnih raztopinah kalijevega nitrata (V), natrijevega nitrata (III), amonijevega nitrata (V) in nikotinske kisline ter v učinkovinah losartan kalijeve soli, paracetamola, natrijevega sulfamonometoksina in kloramfenikola. Naš namen je bil ugotoviti, ali je metoda uporabna za vzorce z zelo nizko koncentracijo totalnega dušika. S pomočjo validacije metode z raztopinami KNO_3 smo ugotovili, da je metoda uporabna za vzorce z nizko koncentracijo totalnega dušika, saj smo določili mejo zaznave pri 0,003 mg/L TN, mejo kvantifikacije pa pri 0,01 mg/L TN. Dokazali smo tudi, da je metoda ustrezna glede točnosti, natančnosti in linearnosti za KNO_3. Z razvito metodo lahko določamo zelo nizke koncentracije celokupnega dušika v tekočih vzorcih, vendar smo ugotovili, da metoda ni primerna za določevanje celokupnega dušika v tekočih vzorcih, ki vsebujejo amoniak ali organsko obliko dušika, kar smo tudi potrdili z izbranimi učinkovinami.

DETERMINATION OF TOTAL NITROGEN IN LIQUID SAMPLES WITH HIGH TEMPERATURE CATALYTIC OXIDATION

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Diploma thesis presents method for determination of total nitrogen (TN) in liquid samples with high temperature catalytic oxidation. Analytical method was evaluated by validation (precision, accuracy, selectivity, working range, method linearity, limit of detection and limit of quantitation). We helped with statistics tests like Dixon's test and Grubbs-Beck's test for outliers and t – test for accuracy. We determined total nitrogen in standard solution of potassium nitrate (V), sodium nitrate (III), ammonium nitrate (V) and nicotinic acid and in active substance like losartan, paracetamol, sulfamonomethoxine sodium and chloramfenicol. Our goal was to find out if the method is useful for samples with very low concentration of total nitrogen. While validating the method with standard solutions of potassium nitrate (V) we found out that method is useful for samples with low concentration of TN, our limit of detection is 0,003 mg/L TN, limit of quantitation is 0,01 mg/L TN. We proved precision, accuracy and method linearity for KNO_3. With this method very low concentration of total nitrogen in liquid samples can be determined, but it is not useful for determination of total nitrogen in liquid samples where nitrogen is in form of ammoniac or other organic conformation, which we confirmed with active substances.

PRIMERJAVA ANGLEŠKIH, NEMŠKIH IN SLOVENSKIH NAVODIL ZA UPORABO – LINGVISTIČNA ANALIZA

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Analiza navodil za uporabo v angleškem, nemškem in slovenskem jeziku vodi do ugotovitve, da so kljub približno enakim pravnim določbam, kako naj navodila izgledajo, še vedno opazna številna odstopanja, ki negativno vplivajo na berljivost navodil.
V zvezi z makrostrukturo lahko pri nemških in angleških besedilih opazimo bolj ali manj formalno obliko, ki je povezana tako s pravno funkcijo besedil kot tudi s stremljenjem k berljivosti le-teh. Večini slovenskih navodil je tuje uvodno besedilo, ki informira bolnika o pomembnosti navodila. Medtem ko so naslovi poglavij v nemških in angleških navodilih v obliki vprašanj, se pri slovenskih navodilih še vedno najpogosteje pojavljajo enobesedni termini. Kljub temu da so bolniki v vseh treh jezikih večinoma nagovorjeni osebno, se nagovarjanje v slovenskih navodilih odraža predvsem s pomočjo glagola v 1. osebi množine, kar lahko vzbudi vtis, da stoji prejemnik zunaj skupine strokovnjakov, katerim so navodila dejansko namenjena. Analiza uporabe strokovnih izrazov pa prikazuje, da je večji del terminov dodatno razložen tako v nemškem kot tudi v angleškem jeziku, medtem ko strokovni izrazi ostajajo v slovenskih besedilih nepojasnjeni.
Interpretacija izsledkov vodi k neizogibni ugotovitvi, da je oblika angleških in nemških navodil v primerjavi s slovenskimi bolniku bistveno prijaznejša. Težko pa je oceniti, ali so bolniku prijaznejša angleška ali nemška navodila. Medtem ko so prva preprosta in vsebujejo le tiste izraze, ki so bolniku razumljivi, nudijo nemška navodila dodatne informacije, kar pogosto ustvari vtis zgoščenosti, vendar pa istočasno omogočijo bolniku, da obogati svoje znanje na področju medicine.

A COMPARISON OF ENGLISH, GERMAN AND SLOVENIAN PATIENT INFORMATION LEAFLETS – A LINGUISTIC ANALYSIS

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Analysis of English, German and Slovenian patient information leaflets (PILs) leads to the conclusion that despite approximately the same legal provisions for the formation of PILs being in force, numerous deviations which influence the readability of leaflets can still be observed.
With regard to macrostructure, in the English as well as in the German PILs, an increasingly more formalised format can be observed, which is connected with the legal function of the texts and with the effort to improve readability. The majority of the Slovenian PILs are unfamiliar with the introductory text, which informs the patient about the importance of the leaflet. Moreover, the English and German headings are formatted in the form of questions, while in Slovenian technical terms as headings are still preferred. In spite of the fact that the patient is mainly addressed personally in all three languages, the personal reference is expressed in Slovenian mostly by using verbs in the 1st person plural, which leaves the impression that the recipient is located outside the group of experts for whom the instructions were actually written. Furthermore, the use of technical terms shows that while the majority of medical expressions are explained in English and German, almost all such expressions are left uncommented in Slovenian.
According to the analysis results, patient information leaflets prove to be more patient-oriented in English and German than in Slovenian. However, it is difficult to evaluate whether the German or the English PILs are patient-friendlier. While the latter might be plain and simple, containing only phrases understandable to the patient, comprise the German PILs more information, which creates the effect of density, but enables that the patients enrich their knowledge in the field of medicine.

SINTEZA PEPTIDOMIMETIKOV NA OSNOVI (3*S*,5*S*)-3-AMINOPIROGLUTAMINSKE KISLINE

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Piroglutaminska kislina je uporaben reagent v sintezi enantiomerno čistih spojin. 1,5-Di-*t*-butil (*S*)-3-hidroksimino-2-oksopirolidin-1,5-dikarboksilat monohidrat, ki je bil v štirih stopnjah pripravljen iz (*S*)-piroglutaminske kisline, smo s stereoselektivnim katalitskim hidrogeniranjem pretvorili v 1,5-di-*t*-butil (3*S*,5*S*)-3-amino-2-oksopirolidin-1,5-dikarboksilat, nato pa s HCl v dietil etru tvorili hidroklorid omenjene spojine. Le-tega smo acilirali z različnimi kislinskimi kloridi in na ta način sintetizirali 1,5-di-*t*-butil (3*S*,5*S*)-3-acilamino-2-oksopirolidin-1,5-dikarboksilate, ki smo jih z reakcijo s trifluoroocetno kislino v brezvodnem diklorometanu pretvorili v ustrezne (3S,5S)-3-acilamino-2-oksopirolidin-5-karboksilne kisline. Nato smo na paralelnem sintetizatorju izvedli pripajanje dveh primarnih in dveh sekundarnih aminov na tri izmed štirih sintetiziranih (3*S*,5*S*)-3-acilamino-2-oksopirolidin-5-karboksilnih kislin z EEDQ kot aktivacijskim reagentom in s tem sintetizirali potencialne peptidomimetike. Reakcijo pripajanja vseh štirih pri reakcijah po EEDQ-metodi uporabljenih aminov na (3*S*,5*S*)-3-fenilacetilamino-2-oksopirolidin-5-karboksilno kislino smo izvedli še z uporabo TBTU kot aktivacijskega reagenta.

SYNTHESIS OF (3*S*,5*S*)-3-AMINOPYROGLUTAMIC ACID BASED PEPTIDOMIMETICS

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Pyroglutamic acid is a useful building block in the synthesis of enantiomery pure components. 1,5-Di-*t*-butyl (*S*)-3-hydroxyimino-2-oxopyrrolidine-1,5-dicarboxylate monohydrate, prepared from (*S*)-pyroglutamic acid in four steps, was catalytically hydrogenated to form 1,5-di-*t*-butyl (3*S*,5*S*)-3-amino-2-oxopyrrolidine-1,5-dicarboxylate. A hydrochloride of this compound was formed by adding the solution of HCl in diethyl ether. The hydrochloride was then acylated with various acid chlorides to form 1,5-di-*t*-butyl (3*S*,5*S*)-3-acylamino-2-oxopyrrolidine-1,5-dicarboxylates, which were transformed into (3*S*,5*S*)-3-acylamino-2-oxopyrrolidine-5-carboxylic acids, respectively, by treatment with the solution of trifluoroacetic acid in anhydrous dichloromethane. The reactions between three of the four synthesised (3*S*,5*S*)-3-acylamino-2-oxopyrrolidine-5-carboxylic acids and two primary and two secondary amines were carried out by parallel solution phase approach, using EEDQ as a coupling reagent, to form potential peptidomimetics. Reactions between (3*S*,5*S*)-3-phenylacetylamino-2-oxopyrrolidine-5-carboxylic acid and all four amines used in the parallel solution phase synthesis by the EEDQ method were then also carried out using TBTU as a coupling reagent.

KVANTITATIVNA ANALIZA ZDRUŽBE MAKROINVERTEBRATOV V REKI TEMENICI

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Na podlagi kvantitativne analize združbe makroinvertebratov v reki Temenici smo ocenili ekološko stanje prvega dela te ponikalnice in raziskali vpliv nekaterih okoljskih dejavnikov na sestavo združbe. Vzorčenje s Surberjevim vzorčevalnikom smo izvajali vsake tri mesece od oktobra 2003 do julija 2004 na štirih vzorčnih mestih, obenem smo izvedli tudi meritve nekaterih okoljskih dejavnikov. Analizo združbe makroinvertebratov smo razdelili na analizo mikrorazporeditve in analizo longitudinalne razporeditve. V obeh kategorijah smo naredili primerjavo raznolikosti taksonov s pomočjo diverzitetnih indeksov, analizo prehranskih skupin ter kanonično analizo z odstranjenim trendom (DCA) in kanonično korespondenčno analizo (CCA). Pri vseh analizah se je pokazalo, da so v združbi makroinvertebratov razlike med vzorčnimi mesti večje kot razlike med mikrohabitati. Posebej izstopa četrto vzorčno mesto z izrazitim zmanjšanjem raznolikosti taksonov, kar kaže na močan negativen vpliv mesta Trebnje na ekološko stanje Temenice. Od izbranih okoljskih spremenljivk vzorčnih mest imata največji vpliv na sestavo združbe red vodotoka in saprobni indeks, med spremenljivkami mikrohabitatov pa smo največji delež variabilnosti združbe pojasnili z globino struge.

QUANTITATIVE ANALYSIS OF MACROINVERTEBRATE COMMUNITY IN THE TEMENICA RIVER

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Quantitative analysis of macroinvertebrate community in Temenica river was made to asses the ecological quality of the first part of this disappearing stream and to investigate the influence of some environmental variables on the structure of the community. Samples were colleted with Surber sampler net at four sampling sites along the river every three months from October 2003 until July 2004. Along with sampling we also measured environmental variables. Analysis of the macroinvertebrate community was divided to analysis of microdistribution and analysis of longitudinal distribution. In both categories we performed analysis of taxonomic diversity, analysis of feeding categories structure, detrended canonical analysis (DCA) and canonical correspondence analysis (CCA). All of the performed analysis showed that differences in community structure were much more pronounced among sampling sites than among different microhabitats. The fourth sampling site is especially outstanding with its decrease in taxonomic diversity and thus indicates a strong negative influence of the town Trebnje on the ecological quality of Temenica river. Stream order and index of saprobity have the greatest influence on community structure among the environmental variables of sampling sites, while stream depth is the variable that effects the most the community structure on the level of microdistribution.

RAZVOJ KROMATOGRAFSKE METODE ZA DOLOČANJE OSTANKOV TOPIL V ZDRAVILIH

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Namen raziskave je bil razviti čimbolj enostavno in univerzalno metodo s pomočjo plinske kromatografije, ki bo ločevala čim več ostankov topil, ki jih Krka uporablja v praksi in bo aplikativna na širokem spektru substanc.
V nalogi smo razvili dve metodi za določanje 26 hlapnih organskih topil. Vsa topila smo določali z direktnim vbrizgavanjem topil v raztopini dimetilformamida in z vbrizgavanjem parne faze nad raztopino topila v DMF in vodi. Pokazalo se je, da se v koloni CP-Volamine ločijo metanol, etanol, acetonitril, aceton, propan-1-ol, metilacetat, metilenklorid, propan-1-ol, tert-butil metil eter, butan-2-on, butan-2-ol, n-heksan, kloroform, tetrahidrofuran, ocetna kislina, benzen, cikloheksan, trietilamin, 1,4-dioksan, n-heptan, n-butilacetat, ksilen, diizopropileter, etilacetat, izopropilacetat, toluen. Za ločevanje etilacetata, diizopropiletera, toluena, izopropilacetata smo uporabili kolono AT-624.
Določili smo tudi linearno območje metode posameznega topila in najnižjo mejo, pri kateri lahko topilo kvantitativno ovrednotimo (LOQ, meja določanja) ter mejo, pri kateri topilo še zaznamo (LOD, meja zaznavnosti). Metodo z uporabo kolone CP-Volamin pri direktnem vbrizgavanju in metodo z uporabo kolone CP-Volamin pri vbrizgavanju parne faze, smo primerjali glede na ponovljivost in mejo kvantifikacije.

CHROMATOGRAPHIC METHOD DEVELOPMENT AND ITS APPLICATION FOR DETERMINATION OF RESIDUAL SOLVENTS IN DRUG PRODUCTS

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Objective of the research is to develop a simple and universal gas chromatographic method for separation and determination of residual solvents used in manufacturing process in Krka that will be applicable for wide range of substances.
Two methods for determining 26 volatile organic solvents were developed. All solvents were determined by direct injection of solvents in solution of dimethylformamide and by injection of steam phase over solution of solvent in DMF and water. It turned out that column CP-Volamine separated methanol, ethanol, acetonitrile, acetone, propan-1-ol, methyl acetate, dichloromethane, propan-1-ol, tert-buthylmethyl ether, butan-2-one, butan-2-ol, n-hexane, chloroform, terahydrofuran, acetic acid, benzene, cyclohexane, triethylamine, 1,4-dioxane, n-heptane, n-butyl acetate, xylene, diisopropyl ether, ethyl acetate, isopropyl acetate and toluene. For separating ethyl acetate, diisopropyl ether, toluene and isopropyl acetate column AT-624 was used.
Linear area of the method for individual solvent was assigned as well as the lowest limit where determination of residual solvent is still possible (LOQ - Limit of Quantitation) and the limit where the solvent is still detected (LOD – Limit of Detection). Both methods were compared regarding repetability and the limit of quantitation.

NAJPOGOSTEJŠE POMANJKLJIVOSTI V FARMAKOLOŠKO-TOKSIKOLOŠKEM DELU DOKUMENTACIJE ZDRAVIL PO SPREJETJU EVROPSKE ZAKONODAJE

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Zdravilo se sme tržiti, ko gre skozi postopek registracije na Agenciji Republike Slovenije za zdravila in medicinske pripomočke (v nadaljevanju Agencija), v katerem si prosilec (farmacevtsko podjetje ali predstavništvo s sedežem v Republiki Sloveniji) pridobi dovoljenje za promet z zdravilom. Za pridobitev dovoljenja za promet mora prosilec z registracijsko dokumentacijo dokazati, da je zdravilo kakovostno, varno in učinkovito. Kakovost, varnost in učinkovitost zdravila prosilec dovoljenja za promet dokazuje z registracijsko dokumentacijo, v kateri je akumulirano vse prosilčevo znanje o zdravilu. Oblika in vsebina dokumentacije sta zakonsko določeni.
V nalogi so predstavljeni zakoni, ki predpisujejo obliko in vsebino farmakološko-toksikološkega dela dokumentacije, tj. dela z nekliničnimi podatki, s katerimi prosilec dokazuje varnost zdravila. Predstavljena so vsa obvezna poglavja tega dela dokumentacije in tudi vsebina, ki mora biti v določenem poglavju opredeljena.
Nadalje so predstavljeni kriteriji, po katerih smo ocenjevali formalno in vsebinsko strukturo dokumentacije. Opisana je ugotovljena formalna nepopolnost dokumentacije, vložene na Agencijo od leta 2000 dalje, in najpogostejše vsebinske pomanjkljivosti dokumentacije, ki je bila v postopku evalvacije na Agenciji v prvi četrtini leta 2006.

THE MOST FREQUENT DEFICIENCIES IN PHARMACO-TOXICOLOGICAL PART OF THE APPLICATION DOSSIER OF MEDICINAL PRODUCTS AFTER IMPLEMENTATION OF EUROPEAN UNION LEGISLATION

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A medicinal product may only be placed on the market when a marketing authorisation has been issued by the Agency for the Medicinal Products and Medical Devices of the Republic of Slovenia (further on shortened to *Agency*). In order to obtain marketing authorization, an applicant (pharmaceutical company or representative offices with headquarters in the Republic of Slovenia) must submit application dossier, where the quality, safety and efficacy of the product are approved.
The requirements for the structure and the content of the application dossier are well defined in the legislation, governing medicinal products. In the present study we listed the rules, defining the structure and the content of the pharmaco-toxicological part of the application dossier, where the safety of the medicinal product should be approved. We also presented the requirements for the content of all the mandatory chapters of this part of the dossier.
Furthermore, we selected some basic criteria for the evaluation of the pharmaco-toxicological part of the application dossier, according to the requirements for the structure and the content of the application dossier, defined in the legislation. Afterwards we analyzed faults in the structure of dossiers, which Agency has been receiving from the year 2000 on. Using selected criteria, we also showed the most frequent deficiencies in the content of dossiers being in the marketing authorization procedure at the Agency in the first quarter of year 2006.

DOLOČANJE ZNAČILNIH PODROČIJ NA SLIKAH TABLET

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Zanesljivost in natančnost odkrivanja napak na tabletah s sistemom za vizualno kontrolo kakovosti tablet lahko izboljšamo z ločeno analizo slik tablet po posameznih značilnih področjih. Tipična značilna področja tablet so robovi, napisi in vtiski ter gladke površine tablet. Razvoj in testiranje delovanja postopkov za določanje značilnih področij smo izvedli s pomočjo zbirke slik, ki je vsebovala slike tablet različnih tipov z različnimi značilnimi področji. Kritični del razgradnje slik tablet na značilna področja je bilo samodejno določanje področja vtiska, ki smo ga izvedli s postopki poravnave modela vtiska na slike tablet z vidnim vtiskom. Razvili smo postopek s poravnavo vektorjev glavnih osi, postopek 2D poravnave slik in postopek 1D poravnave krožnih profilov. Kriterija za vrednotenje postopkov poravnave modela vtiska na slike tablet sta bila natančnost poravnave in časovna zahtevnost. S poskusi na dani zbirki slik smo ugotovili, da je postopek 1D poravnave krožnih profilov v splošnem najbolj robusten in hiter. Zaradi velike variabilnosti proizvedenih tablet pa ne moremo predlagati univerzalnega postopka poravnave modela, zato menimo, da je smiselno razviti več različnih postopkov poravnave modela ter z ustrezno kombinacijo teh postopkov optimizirati delovanje sistema za različne proizvode.

SEGMENTATION OF DISTINCTIVE REGIONS ON TABLET IMAGES

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We assume that the performance of visual quality inspection of tablets can be improved by segmenting the images of tablets into distinctive regions, such as engravings, imprints, edges and surfaces. We focused on the segmentation of tablet images with complex imprints, representing various symbols or characters. For this purpose a large image database of different tablets with various imprints and surface properties was acquired. Model based image segmentation was carried out by matching an imprint model to the tablet images. Three different algorithms were proposed. The assessment of the proposed algorithms was based on their matching accuracy and time consumption. The matching algorithm that maximises the cross-correlation between the circular profiles of the imprint model and tablet image was most accurate. This algorithm also outperformed the others in terms of robustness and speed. Due to the large variability of the manufactured tablets, no general approach for segmentation of tablet images can be proposed. A feasible solution is to combine various algorithms in order to best optimise the overall performance of the system for automated visual quality inspection of tablets.

VPLIV IZBRANIH DEJAVNIKOV NA TRANSPORT S-(2,4-DINITROFENIL)GLUTATIONA S SEKRETORNIMI PRENAŠALCI SKOZI TANKO ČREVO PODGANE *in vitro*

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S-(2,4-dinitrofenil)glutation se uporablja kot označevalec aktivnosti MRP (ang. multidrug resistance associated protein) prenašalcev. Povečano permeabilnost označevalca skozi izoliran segment tankega črevesa podgane v določeni smeri (s serozne na mukozno stran ali obratno) pripisujemo povečani aktivnosti MRP prenašalcev, ki transportirajo substrate v omenjeni smeri. Aktivnost MRP2 prenašalca, ki poveča permeabilnost označevalca v smeri s serozne na mukozno stran, je v hepatocitih nadzorovana s posttranslacijsko vezikularno regulacijo. Nanjo vplivajo različni dejavniki kot sta na primer osmolarnost inkubacijskega medija ali prisotnost tauroholne kisline. Naše raziskovalno delo nakazuje, da regulacija MRP2 aktivnosti obstaja tudi v tankem črevesu. Zdi se, da je le-ta podobna regulaciji v jetrih, saj se odziva na enake dražljaje. Poleg tega se aktivnost MRP2 poveča tudi ob prisotnosti glukoze na mukozni strani tkiva. Najverjetnejša vzroka sta kotransport natrija in glukoze z SGLT prenašalci in vezava glukoze na SGLT3 senzor.

INFLUENCE OF SELECTED FACTORS ON TRANSPORT OF S-(2,4-DINITROPHENYL)GLUTATHION BY EFFLUX TRANSPORTERS ACROSS RAT SMALL INTESTINE *in vitro*

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S-(2,4-dinitrophenyl)glutathion is used as a marker of MRP (multidrug resistance associated protein) activity. Increased permeability across isolated segment of rat small intestine in a certain direction (from serosal to mucosal side or vice versa) is assigned to increased activity of MRP transporters that transport their substrates in mentioned direction. Activity of MRP2 transporter, which increases permeability of marker in serosal to mucosal direction, is in hepatocytes regulated with post-translational vesicular regulation. Regulation is induced by different factors like anisoosmolarity of incubation medium and taurocholic acid. Our research is showing that regulation of MRP2 activity is also present in small intestine. It appears that intestinal regulation is similar to hepatic, because it is responding to the same stimuli. Moreover, MRP2 activity is also increased by presence of glucose on mucosal side of the tissue. Most likely reasons are cotransport of sodium and glucose by SGLT transporters and binding of glucose on SGLT3 sensor.

INHIBITOR ESTERAZE IZ *Streptomyces* sp.

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Esteraze so v naravi zelo razširjeni encimi in sodelujejo pri številnih reakcijah v organizmu, kjer hidrolizirajo estre na ustrezne kisline in alkohole. Poleg pomembne celične vloge esteraze sodelujejo pri razgradnji ksenobiotikov, vplivajo na transport proteinov ter sodelujejo pri aktivaciji neaktivnih oblik zdravil in neželeni razgradnji zdravil. Zato inhibitorji esteraz predstavljajo zelo pomembno področje pri raziskavah zdravil. V predstavljenih rezultatih smo poskušali izolirati inhibitorja esteraz mikrobnega izvora, delo pa je bilo opravljeno v okviru treh diplomskih nalog. Raziskavo smo začeli z razvojem encimskega testa in presejalne metode za ovrednotenje aktivnosti inhibitorjev esteraz. Številne aktinomicete smo gojili na izbranih gojiščih in v njihovih ekstraktih preverili prisotnost metabolitov z aktivnostjo inhibitorjev esteraz.
Pri dveh sevih aktinomicet smo zaznali značilno višjo inhibitorno aktivnost. V nadaljevanju smo poskušali dvigniti donose še z optimizacijo produkcijskega gojišča in kultivacijskih pogojev. Preizkušali smo vpliv različnih virov ogljika, dušika, izbranih soli in mineralov v produkcijskem gojišču ter uspeli značilno izboljšati donose želenega metabolita. Za nadaljnje povečanje donosov proizvodnih sevov smo izvedli selekcijo v manjšem obsegu, ki je temeljila na izbranih morfoloških in fizioloških lastnostih produkcijskega seva.

AN ESTERASE INHIBITOR FROM *Streptomyces* sp.

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Esterases are ubiquitous enzymes that catalyze hydroxylation of esters into the corresponding acids and alcohols. They take an active role in many reactions in the cell. Apart from cellular functions they also take part in detoxification of xenobiotics, they influence transport of proteins and are involved in activation of pro-drugs including undesired degradation of drugs. Esterase inhibitors thus represent an interesting area in drug discovery research. Here we present results of an attempt to isolate an inhibitor of an esterase of microbial origin, which has been carried out by three diploma students. The project was initiated by development of a sensitive enzymatic assay and a screening method for the evaluation of inhibitory activity of esterase inhibitors. We have tested a number of actinomycetes by cultivation on selected media. The extracts were than tested for the presence of metabolites with esterase inhibitory activity.
Two actinomycetes strains with significant esterase inhibitory activity were identified. In the second step, the production medium and cultivation conditions optimization were carried out in order to improve the yield (activity) of the desired metabolites. The influence of carbon and nitrogen source and the influence of selected salts and minerals were tested. This led to creating a production medium with significantly improved yield (activity) of the target metabolite. To improve the yield of a target metabolite further, the producing strains were subjected to a small scale selection based on morphological and physiological properties of the producing strains, which enhanced the yield even further.

SINTEZA CIKLOPENTANSKIH DERIVATOV KOT POTENCIALNIH ENCIMSKIH INHIBITORJEV

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Aldo-ketoreduktaza AKR1C3 deluje kot hidroksisteroidna dehidrogenaza pri biosintezi in inaktivaciji steroidnih hormonov, zato ima lahko pomembno vlogo pri nastajanju hormonsko odvisnih oblik raka. Sodeluje še pri sintezi prostaglandinov, zaradi česar jo tudi preko tega mehanizma povezujejo z malignimi transformacijami. Da bi določili nove smernice v razvoju inhibitorjev AKR1C3 in pridobili več informacij o odnosu med strukturo in delovanjem, smo sintetizirali različne ciklopentanske derivate. Njihov vpliv na reduktivno sposobnost encima smo določili v prisotnosti substrata 9,10-fenantrenkinona. Ugotovili smo, da ima metilni eter ciklopentanola močno inhibicijsko aktivnost, še večjo pa 3-substituiran ciklopentanski derivat. Oba derivata sta potencialni spojini vodnici novim, močnejšim inhibitorjem AKR1C3, ki bi lahko služili kot nova zdravila različnih oblik raka, kjer je aktiven omenjeni encim (levkemija, rak prostate, dojk, gastrointestinalnega trakta).

SYNTHESIS OF CYCLOPENTANE DERIVATIVES AS POTENTIAL ENZYME INHIBITORS

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Aldo-keto reductase AKR1C3 is involved in the biosynthesis and inactivation of steroid hormones as a hydroxysteroid dehydrogenase and can therefore play an important role in the development of hormone-dependent forms of cancer. It also acts as a prostaglandin synthesis and is often linked to malignant transformations by this mechanism as well. In order to obtain further information on the structure-activity relationship and determine new leads, a series of cyclopentane derivatives were synthesized. Their effect on the reductive activity of AKR1C3 was evaluated using 9,10-phenantrenequinone as a substrate. Methyl ethers of cyclopentanol were found to possess a strong inhibitory activity, and 3-substituted cyclopentane derivatives were even more potent. These compounds represent a good starting point in the search for new, superior AKR1C3 inhibitors that could be important in treatment of various malignant diseases affected by this enzyme (leukemia, breast, prostate and gastrointestinal cancer).

NOVI KATALIZATORJI ZA ASIMETRIČNO HIDROGENIRANJE

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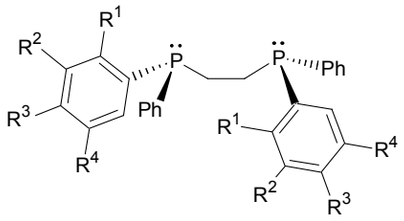
Pripravili smo nove optično čiste derivate DiPAMP liganda, pri katerih smo modificirali sterične in elektronske efekte *o*-anizilne skupine z različnimi substituenti. Novi ligandi so bili zelo aktivni in so dali izboljšane enantiomerne presežke pri Rh-kataliziranem hidrogeniranju dehidro-α-amino kislin in α-substituirane akrilne kisline. Npr., z (*R,R*)-1,2-bis[(fenil)(2,3,4-trimetoksifenil)fosfino]etanom (**5c**) smo hidrogenirali 2-acetamidoakrilno in 2-acetamidocimetno kislino z do 99% ee v 4 min, medtem ko je poteklo hidrogeniranje z DiPAMP ligandom z do 92-95% ee v 15-20 min.



Ligand	DiPAMP	5a	5b	5c	5d
R^1	OMe	OMe	OMe	OMe	OMe
R^2	H	OMe	H	OMe	-(CH)$_4$-
R^3	H	H	H	OMe	
R^4	H	H	OMe	H	H

NEW CATALYSTS FOR ASYMMETRIC HYDROGENATION

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New modified optically pure DiPAMP-like ligands were prepared where the *o*-anisyl group was sterically and electronically modified by the presence of various groups. The new ligands were very active and led to improved high ees in Rh-catalysed hydrogenation of dehydro-α-amino acids and α-substituted acrylic acid. For example, (*R,R*)-1,2-bis[(phenyl)(2,3,4-trimethoxyphenyl) phosphino]ethane (**5c**) gave up to 99% ee in 4 min in hydrogenation of 2-acetamidoacrylic acid and 2-acetamidocinnamic acid, while DiPAMP led to 92-95% ee in 15-20 min.

RAZLIKOVANJE NARAVNEGA VITAMINA C IN ASKORBINSKE KISLINE

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Vitamina C človek ne sintetizira, zato ga mora pridobiti s hrano, ker ga nujno potrebuje.
V raziskovalni nalogi smo primerjali reaktivnost askorbinske kisline in šipkovega čaja. Zbirali smo informacije iz literature in iz spletnih strani.
Izdelali smo navodilo za vajo kot izbirno vsebino za spoznavanje kvantitativne analize s pomočjo jodometrične titracije za določanje sintetičnega vitamina C in reducentov v šipkovem čaju. Kvalitativno smo dokazali, da vitamin C reducira železove(III) ione, vendar je razlika v hitrosti reakcije in obarvanosti raztopin med sintetičnim vitaminom C in šipkovim čajem. S pomočjo računalnika smo merili spremembo pH vrednosti v dveh urah.
Izvedli smo tudi anketo o poznavanju in uporabi šipkovega čaja.

DISTINGUISHING OF NATURAL VITAMIN C AND ASCORBIC ACID

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The human body cannot synthesize the vitamin C that is why we must get it with food because it is vitally important.
In our research we compared the reactivity of the ascorbic acid and rosehip tea.
We gathered information from literature and web sites.
We also compiled the instructions for realizing quantity analysis as an exercise for electives with the help of iodometric titration for the determination of the synthetic vitamin C and reducing agents in rosehip tea.
We proved qualitatively that the vitamin C reduces the ferric iron (Fe^{3+}) to the ferrous iron (Fe^{2+}), however the difference is in the speed of reaction and the colour of solutions between the synthetic vitamin C and rosehip tea. By means of the computer we measured the change of pH value in two hours.
We also carried out survey of how people know and use rosehip tea.

DELOVANJE RAZKUŽILA VIRKON S NA MIKOORGANIZME PRI RAZLIČNIH POGOJIH

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V raziskovalni nalogi smo proučevali vpliv koncentracije razkužila Virkon S v času delovanja ter vpliv temperature na delovanje. Učinek razkužila smo raziskovali na Gram-pozitivni bakteriji *Staphylococcus species* in Gram-negativni bakteriji *Escherichia coli*, za dokaz dezinfekcijskega učinka Virkona S na vse ostale mikroorganizme, na katere deluje, pa smo razkuževali opremo in površine v laboratoriju.
V prvem delu smo razkuževali različne površine v in izven laboratorija z različnima koncentracijama Virkona S, vzemali brise ter jih nanašali na gojišča. Hkrati smo preverjali delovanje na znane mikroorganizme.
V drugem delu smo ugotavljali delovanje razkužila Virkon S v odvisnosti od časa in različnih koncentracij.
Nato pa smo naredili še poskus delovanja razkužila Virkon S pri različnih temperaturah.
Delovanje na znane mikroorganizme smo ugotavljali tako, da smo okužili gojišče in nato razkuževali. Bolj smo se osredotočili na delovanje različnih koncentracij razkužila, čas delovanja, temperaturo vode za pripravo raztopine in starost raztopin na predmete v laboratoriju.

THE ACTIVITY OF THE DISINFECTANT VIRKON S ON MICROORGANISMS AT THE DIFFERENT CONDITIONS

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In our research we have studied the impact of Virkon S disinfectant concentration in its activity time and the temperature influence on the activity.
The disinfectant effect was researched on Gram–positive bacterium *Staphylococcus species* and Gram–negative bacterium *Escherichia coli*, but to prove the disinfectant effect of Virkon S on all other micro-organisms on which it has an impact, the equipment and surfaces in a laboratory were disinfected.
In the first part of our research, we disinfected different surfaces in and outside of the laboratory with different concentrations of Virkon S. We took smears and then we applied them on culture media. We were checking the activity on known micro-organisms at the same time.
In the second part we tried to find out how the activity of disinfectant Virkon S depends on time and different concentrations. After that we did one more experiment of the disinfectant Virkon S activity at different temperatures.
We infected culture medium and then we disinfected it in order to find out its activity on known micro-organisms.
Most of our attention was given to the activity of different concentrations of disinfectant, time of activity, temperature of water for preparing the solution and the age of the solution on objects in the laboratory.

ALI PRAVILNO UPORABLJAMO ZDRAVILA?

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Z izdelavo raziskovalne naloge smo se hoteli seznaniti, kako kot uporabniki lahko vplivamo na varnost in učinkovitost zdravil, s katerimi se vsakodnevno srečujemo. V prvem, teoretičnem delu smo analizirali procese, ki potekajo v telesu, ko zdravilo zaužijemo in ugotovili, da lahko s hrano in/ali pijačo, ki jo zaužijemo z/pred zdravilom, vplivamo predvsem na sproščanje učinkovine iz končnega pripravka, deloma pa tudi na absorpcijo in metabolizem. Poleg tega, da upoštevamo nasvete o pravilni in varni uporabi zdravila, ki jih ob vročitvi da farmacevt, moramo biti uporabniki pozorni še na rok uporabe zdravila ter na proizvajalčeva navodila o shranjevanju.
V drugem delu naloge smo izdelali anketni vprašalnik in izvedli anketo, s katero smo želeli ugotoviti, koliko so ljudje seznanjeni s pravilno uporabo zdravil, s kakšnimi pijačami jih najpogosteje jemljejo in kako ravnajo z zdravili, ki jih kupujejo na zalogo in shranjujejo v domači lekarni.
Rezultate ankete smo uporabili za načrtovanje eksperimentalnega dela, v katerem smo v šolskem laboratoriju poskušali ponazoriti, kako različni napitki vplivajo na razpadnost tablet v želodčnem soku. Kot modelna končna pripravka smo izbrali neobložene tablete Daleron in gastrorezistentne filmsko obložene tablete Naklofen. Da bi dokazali pomen pravilnega shranjevanja zdravila, smo poskuse ponovili tudi s tabletami Daleron, ki jim je pretekel rok uporabe, poleg tega pa so bile nepravilno shranjene.

ARE WE USING MEDICINES CORRECTLY?

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The aim of this study was to review how users can influence the safety and efficacy of medicines they encounter on a daily basis.
Our research paper is divided into three parts. In the theoretical part we analyzed the processes that happen in the body following intake of a medicinal product. We found that consuming food or drink before or during medicinal intake can influence primarily liberation, but also absorption and metabolism of active ingredient. In addition to considering the correct and safe administration of the medicinal product the patient should also be aware of the expiry date and conditions of storage.
In the second part we designed and carried out a questionnaire, with which we wanted to find out, how people use medicines, what kinds of drinks they prefer, when administering tablets and how they store medicines in their home pharmacy.
The results were used to design the experimental part. We used simple laboratory equipment to find out how different drinks affect the disintegration of model tablets: Daleron tablets and Naklofen gastroresistant film-coated tablets. We also repeated the experiments with Daleron tablets that were past their expiry date and had furthermore been stored in inadequate storage conditions.

KEMIČNO ČIŠČENJE

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Kemično čiščenje - je to »pranje« v posebnih topilih ali obdelava s hlapi topila, je to mogoče nanašanje kemikalij na tkanino in ščetkanje s krtačo? Spoznali smo postopek, katera čistila oz. topila uporabljamo pri tem, varnostne ukrepe pri čiščenju, izvedli nekaj trikov kako ohraniti tkanino celo, spoznali smo zahteve inšpekcij in izvedeli, da se po 31. oktobru 2007 obetajo velike spremembe. V bolnišnici Novo mesto uporabljajo posebno kemično čiščenje, pri katerem se poleg standardiziranega postopka in pralnih sredstev uporabljajo še specialna pralna sredstva, kot na primer detergent z encimi. Veliko smo izvedeli o zgodovini čiščenja. Lastnik čistilnice nas je popeljal v svet tkanin in madežev. Naučili smo se prepoznavati različne madeže in jih odstranjevati s pomočjo detaširanih sredstev. Z Beilsteinovo reakcijo smo preverili količino klora v oblekah takoj po pranju in nekaj časa za tem. Topilo perkloroetilen je nevarno, vendar je njegova poraba zelo majhna, saj v večini čistilnic čistijo v zaprtih sistemih. Topilo, porabljeno pri čiščenju, gre najprej v destilator, potem pa sledi ločevanje topila in kontaktne vode v separatorju, kjer dobimo topilo, ki je pripravljeno na ponovno uporabo. Pri čiščenju dobimo tudi majhno količino odpadka, ki se posuši in zbere (odpeljejo ga na sežig). Da smo to preverili, smo v šolskem laboratoriju izvedli destilacijo in nadaljnje ločevanje. Anketa nam je pokazala, da ljudje o čiščenju, čeprav ga pogosto uporabljajo, zelo malo vedo. Mi nismo več med njimi.

DRY CLEANING

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Dry cleaning- is this washing in special solvents or a treatment with vapours of a solvent, is this possibly putting special chemicals on materials and brushing? We saw the procedure, found out which detergents or solvents are used, which safety precautions are taken and what tricks to use to protect a material. We were also told that after 31. October 2007 there will be some enormous changes made by inspection. In hospital Novo mesto is used special chemical cleaning with standard procedure and special chemical agent such as detergent with enzyme. We learned a lot about the history of dry cleaning. We also learned how to remove stains with special chemicals. With Beilstein reaction we examined the amount of chlorine in clothes as soon as they have been washed and a little afterwards. The tetrachloride is dangerous, but its consumption is very small, because the majority of dry cleaners have closed systems. The used solvent first goes into a distiller and then it is followed by the separation of contact water and tetrachloride in separator, where we get a pure solvent which can be reused. Cleaning produces a small amount of waste, which is dried and eventually burnt. To make sure that this is true, we carried out the same procedure in our school laboratory. Survey revealed that people know very little about dry cleaning even though they use it quite often. We, on the other hand, are no longer among them.






Krkine nagrade

VPLIV PROSTORSKE RESTRIKCIJE NA NASTANEK IN STABILNOST POLIMORFNIH OBLIK

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V delu je teoretično predstavljen vpliv prostorske restrikcije na nastanek in stabilnost polimorfnih oblik. Teoretični model je osnovan na termodinamiki homogene nukleacije. Pokazano je, da lahko s prostorsko restrikcijo selektivno pridobimo določeno polimorfno obliko, lahko pa tudi stabiliziramo metastabilne polimorfne oblike. Kriterij, ki odloča o tem, ali bo določen polimorf lahko kristaliziral v porah nosilca ali ne, je zadostna velikost por. Če velja neenakost

$$r^{por} < \frac{2\gamma_i V_{m,i}}{RT \ln S},$$

polimorf *I* ne bo nastal. Metastabilni polimorf *A2* bo stabiliziran pred prehodom v stabilnega *A1*, če je velikost por taka, da velja neenakost

$$r^{por} < \frac{-2\gamma^{A1/A2} V_m}{RT \ln\left(\frac{x_{A1}}{x_{A2}}\right)}.$$

Vpliv na selekcijo je računsko prikazan na primeru sulfatiazola.

THE INFLUENCE OF SPACE RESTRICTION ON THE FORMATION AND STABILITY OF POLYMORPHS

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In this paper we theoretically addressed the influence of space restriction on the formation and stability of polymorphs. The model is based on the homogenous nucleation theory. It was shown that space restriction could be used for polymorph selection or stabilization of metastable polymorphic forms. The criteria that determines whether a polymorphic form can crystallize or not, is sufficient pore size, which also determines the stability of metastable forms. The influence on polymorph selection is presented in the case of sulfathiazole.

PREHAJANJE FARMACEVTSKIH OBLIK SKOZI GASTROINTESTINALNI TRAKT

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Absorpcijo zdravil lahko definiramo kot gibanje aktivne učinkovine od mesta aplikacije, skozi biološke membrane, do mesta, kjer vstopi v krvni obtok. Sistemska absorpcija zdravil je odvisna od mnogih faktorjev, kot npr. fizikalno-kemijskih lastnosti zdravila, narave zdravila in anatomije ter fiziologije mesta absorpcije. Cilj eksperimentalnega dela je bil preveriti topnost gastrorezistentnih pelet v medijih z različno sestavo in pH. V poskuse je bilo vnesenih veliko spremenljivk, kot npr.: sestava gastrorezistentne obloge, njena debelina, vsebnost pelet, pH medija,… Raztapljanje zdravila je bilo testirano na USP (United States Pharmacopoeia) aparaturah I. (košarice) in III. (BioDis). Medija za teste raztapljanja z USP aparaturo I. sta bila 0,1M klorovodikova kislina (za kislo fazo) ter fosfatni pufer (pH 6.8). Pelete so bile najprej dve uri izpostavljene kisli fazi, nato pa še uro v fosfatnem pufru. Mediji za teste raztapljanja z USP aparaturo III. so bili acetatni pufri (pH 5.0 ter 5.4 ali 5.6) in fosfatni pufer (pH 6.8). Vzorci so bili vrednoteni glede na rezultate, dobljene pri UV-VIS-spektrofotometriji. Pelete, zaščitene z etilcelulozno oblogo so pokazale slabo gastrorezistenco. V literaturi sem zasledil, da se ta obloga v kislem mediju pretrga in tako se zmanjša gastrorezistenca zdravila. Pelete so pri testiranju na USP aparaturi III. pokazale celo boljše rezultate od originatorja, saj so se začele raztapljati že pri pH 5.0. Kot najprimernejši plastifikator za gastrorezistenco se je izkazal trietilcitrat.

TRANSIT OF PHARMACEUTICAL DOSAGE FORMS THROUGH GASTROINTESTINAL TRACT

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Drug absorption may be defined as the movement of active drugs from the administration site, across biological barriers, into a site where it is measured in the blood. The systemic drug absorption depends on the physiochemical properties of the drug, the nature of the drug product, and the anatomy and physiology of the drug absorption site. The main goal of the experimental part of this research paper was to determine the solubility of gastro-resistant pellets in mediums with different structure and pH value. Many factors, such as composition of gastro-resistant pellets, thickness of its coating, mass of its containment, pH of the medium,…, were observed. Drug dissolution was tested using USP (United States Pharmacopoeia) apparatus I. (baskets) and III. (BioDis). Mediums for testing with USP apparatus I. were 0,1M HCl (for acid phase) and phosphate buffer (pH 6.8). At first the pellets were exposed to the acid phase for two hours and then an hour in phosphate buffer. Mediums for dissolution testing with USP apparatus III. were acetate buffers (pH 5.0 and 5.4 or 5.6) and phosphate buffer (pH 6.8). Samples were valued regarding results, gained with UV-VIS spectrophotometry. Pellets protected with ethyl-cellulose polymer showed low gastro-resistance. It was confirmed by literature data that the coating was damaged causing a resultant loss in the sustained-release properties. Dissolution testing with USP apparatus III. showed even better results than with the originator tablets. The pellets started dissolving at pH 5.0. The best plasticizer for gastro-resistance turned out to be triethyl citrate.

STRESALNA NAPRAVA Z MODIFICIRANIMI POLIMERNIMI SITI ZA LOČEVANJE IN ANALIZO VELIKOSTI DELCEV

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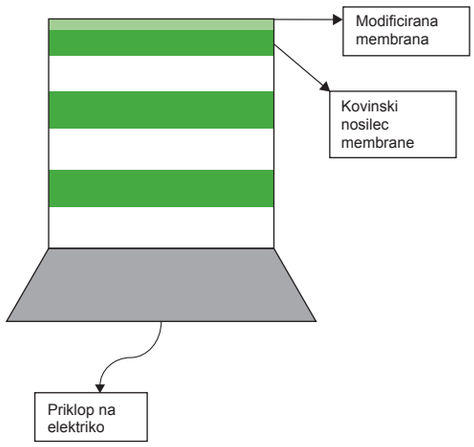
V delu je predstavljena naprava za analizo velikosti delcev, ki v eni stopnji omogoča izolacijo komponente iz praškaste zmesi in analizo porazdelitve velikosti delcev te komponente. Predstavljene so prednosti pred znanimi analiznimi tehnikami ter potencialna uporaba naprave.



NEW SIEVING DEVICE WITH MODIFIED POLYMERIC SIEVES FOR SEPARATION AND PARTICLE SIZE ANALYSIS

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In the present work we presented a new device for particle size analysis, which enables the isolation of a given component from a mixture of powders and the analysis of particle size distribution of this component in a single step process. We presented advantages to other techniques of analysis and the potential use of the device.

Kazalo avtorjev / Author Index

Kazalo mentorjev / Supervisor Index

Uredniški odbor

Natalija Vitezić, glavna urednica
Prof. dr. Jože Drinovec
Mateja Vukšinič Kozoglav

Oblikovanje

2AM|FCB, Ljubljana

Produkcija

Modan informatika d. o. o., Maribor

Tisk

Tiskarna Dalmatin d. o. o., Ljubljana

Izdajatelj

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