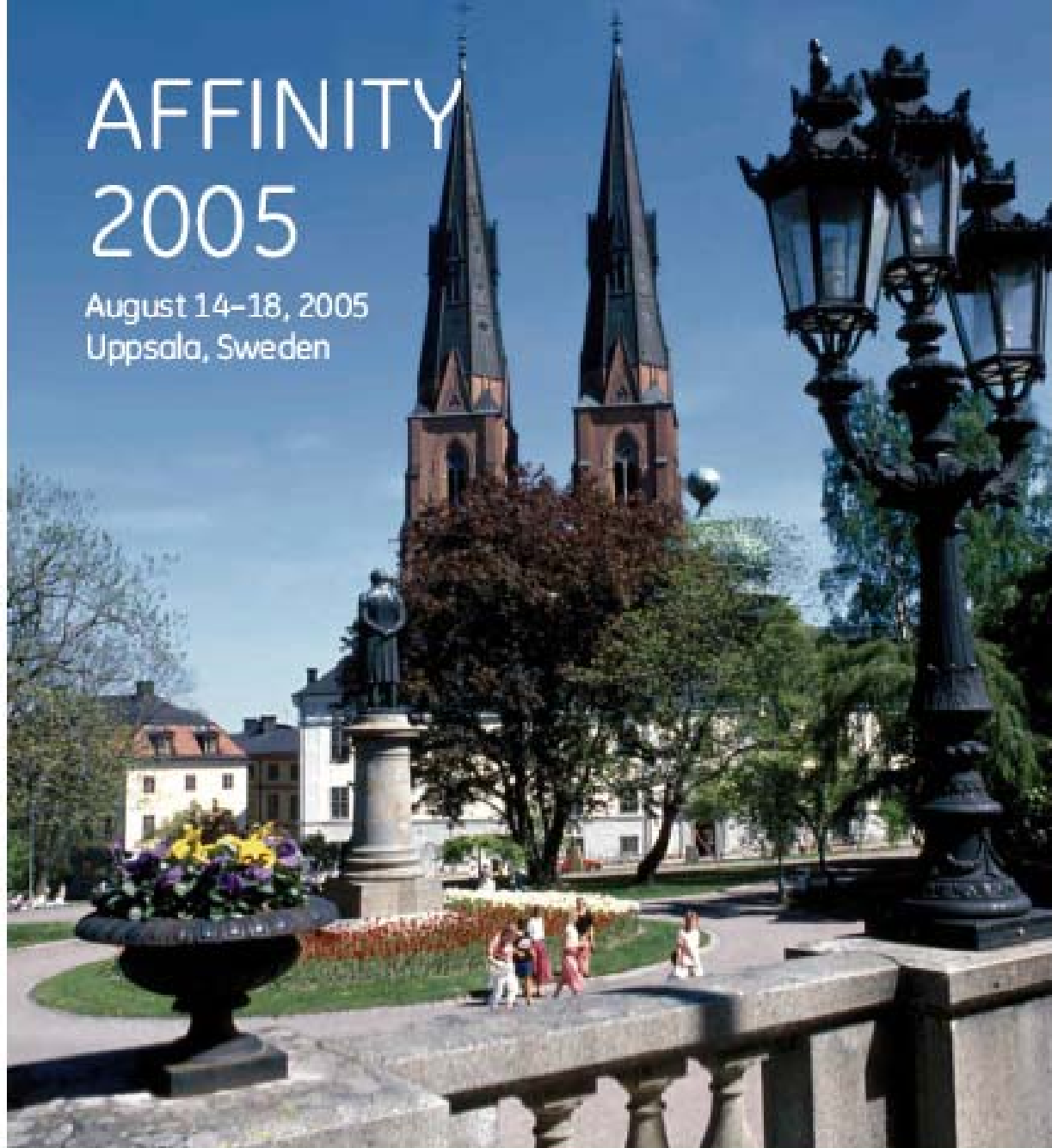


AFFINITY 2005

August 14-18, 2005
Uppsala, Sweden



PROGRAM AND ABSTRACT BOOK

16th Biennial Meeting of
the International Society for
Molecular Recognition (ISMR)



ISMR

GE Healthcare



Welcome to AFFINITY 2005

It is a great pleasure to welcome you to Uppsala and the 16th Affinity Conference. Uppsala hosts northern Europe's oldest university, the workplace for luminaries such as Linnaeus, Celsius, and Arrhenius, and this has been the site of path-breaking developments in protein separation from the early 1920's to the present. Well known are Profs. The Svedberg (the ultracentrifuge), Arne Tiselius (electrophoresis and chromatography), and Jerker Porath (gel filtration, affinity chromatography and IMAC). Thus Uppsala seems to be a most appropriate place to discuss and share new breakthroughs in affinity related interaction phenomena and their applications.

On behalf of the Organizing Committee I would like to welcome you to Uppsala and AFFINITY 2005 with a hope that the structure and program of the meeting will support fruitful discussions with old friends and colleagues, and the opportunity to make new friends, while exploring molecular interaction systems for discovery and purification sciences.

Lars Hagel

Organizing Committee

Assoc. Prof. L. Hagel,
GE Healthcare,
meeting chairman

Prof. K. Caldwell,
Uppsala University

Prof. M. Uhlén, Royal
Institute of Technology

Prof. S. Ohlson,
University of Kalmar

Prof. B. Mattiasson,
University of Lund

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University of Texas at
San Antonio

Prof. C. Lowe, University
of Cambridge

Dr. E. Boschetti,
Ciphergen Inc.

General information

Venue

The welcome reception and the introductory note by Prof. William A. Goddard III will take place at the Uppsala University Hall, close to the centre of Uppsala, on Sunday, August 14, 17.30–20.30.

The meeting is held at the Ultuna campus of SLU, the Swedish University of Agricultural Sciences in Uppsala, located about 7 km south of the centre of Uppsala. The scientific sessions and the exhibition takes place in the Main Lecture Building. The poster sessions takes place in the adjacent building where also the restaurant is situated.

Registration

The registration desk is open as follows:

Sunday, August 14: 17.00–19.00 at Uppsala University, Main Building

Meeting days at the Ultuna campus, Main Lecture Building:

Monday, August 15: 08.00–12.00, 15.00–18.00

Tuesday, August 16: 08.00–11.00, 15.00–18.00

Wednesday, August 17: 08.00–11.00, 15.00–16.00

Thursday, August 18: 09.00–11.00, 15.00–17.00

Transportation

Bus service to Ultuna campus from the hotels is provided as follows:

A complimentary bus marked "AFFINITY 2005" leaves from outside Hotel Gillet/Hotel Muttern/Sunnersta Herrgård at 07.45 on Monday, Tuesday and Wednesday and at 08.45 on Thursday. The bus will return to the hotels 15 minutes after the last session from Monday to Thursday.

It is possible to go by regular city bus 9 and 20 to Ultuna and back to city. The one-way fare is 20 SEK and a schedule is found in the documentation.

Taxi can be ordered by phone at + 46 18 100000. The fare is approximately 150 SEK.

Oral Sessions

All oral sessions will take place in the Main Lecture Hall of the Ultuna campus. Those who will give oral presentations are kindly asked to bring their presentation on a memory stick and meet with the Session Chair at the Registration Desk no later than 30 minutes prior to start of the session for which the oral is scheduled. Speaker names are indicated by bold text in the program.

Poster Sessions

The poster sessions are scheduled for Monday (posters 801–825) and Tuesday (posters 826–858) in the building adjacent to the Main Lecture Building. Posters shall be mounted before 14.00 on Monday and shall be on display until 15.00 on Thursday. Posters are mounted on the

poster board marked with the number of the abstract as given in the Program.

Discussion Forum

Active participation in the Discussion Forum on Monday and Tuesday is encouraged. Please contact the Chair person for each forum if you want to contribute with illustrations, e.g. transparencies.

Coffee and Lunch Break

Coffee will be served in the foyer outside the Main Lecture Hall. Lunch and dinners (except for the Gala Dinner) will be served in the building adjacent to the Main Lecture Building. Coffee and meals are included in the registration fee.

Social Activities

A Welcome Reception with a light buffet dinner is held on Sunday, August 14, from 17.30 to 19.00 in the Faculty Rooms in the main building of Uppsala University (close to the city centre). The formal opening of the meeting and a keynote lecture by William A. Goddard III will follow directly upon the reception.

On Wednesday three parallel excursions are planned. Pre-registration for these are necessary and must be made before Monday 12.00 at the registration desk, unless this was booked together with the registration to the meeting. Excursions are subject to a minimum number of attendees.

Option 1: Guided trip to Skokloster Castle. SEK 400

Option 2: Guided tour of Uppsala. SEK 400

Option 3: Tour to Uppsala Life-Science Industry. SEK 100

Fee includes guide, coach, entrance and coffee.

Buses will leave outside the Main Lecture Building at Uppsala Campus at 15.20 and will return to Hotel Gillet/Hotel Mutterstock/Sunnersta Herrgård at approximately 17.45

The Meeting Gala Dinner will take place at the historic Uppsala Castle on Wednesday evening 19.30. In addition to a fabulous dinner we will enjoy Swedish folk music, typical student mans choir and much more! The fee for the Gala Dinner is included in the registration fee.

Dress Code

There is no dress code for the conference. The Reception and the Gala Dinner are festive events, but no formal dress is required.

Message Center

A Message Center is found at the Registration Desk in the Main Lecture Building of the Ultuna campus.

Sponsors

The fairly moderate fee of the conference had not been possible without cash contributions from our sponsors. Please visit their exhibition outside the Main Lecture Hall of the Ultuna campus.

Program

Sunday August 14, 2005

17.30-19.00 **Welcome Reception**

19.00 **Welcome and Introduction**

Chairman: Lars Hagel, GE Healthcare Uppsala, Sweden

19.15-20.00 **Keynote Lecture**

From system biology to molecular dynamics

William A. Goddard III, California Institute of Technology, Pasadena, USA

Monday August 15, 2005

Session 1: Modelling of surface interactions

Session chair: Karin Caldwell, Uppsala University, Uppsala, Sweden

09.00-09.15 **Chairman's introduction**

09.15-10.00 **Plenary Lecture**

101 Bacterial adhesion under flow: bonds that strengthen under force.

Viola Vogel, Biologisch-Orientierte Materialwissenschaften, ETH, Zurich, Switzerland.

10.00-10.35 **102 A-priori prediction of protein affinity in chromatographic systems using state-of-the-art structure-property multi-scale modeling techniques,**

Asif Ladiwala¹, Qiong Luo², Ting Yang¹, Curt M Breneman² and Steven M. Cramer¹.

¹ Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, USA

² Department of Chemistry, Rensselaer Polytechnic Institute.

10.35-11.05 **Coffee break**

11.05-11.40 **103 Size Distribution of Hydrophobic Patches Allows for the Selection of Hydrophobic Interaction Chromatography (HIC) Media for Protein Purification.**

Markus Laub, Kristin Zurlinden, Herbert P. Jennissen

Institut für Physiologische Chemie, Universität Duisburg-Essen,

Hufelandstr. 55, D-45122 Essen, Germany

11.40-12.15 **104 On the Thermodynamics of Protein Chromatography**
Jørgen M. Møllerup

Department of Chemical Engineering, DTU, Building 229, DK-2800 Lyngby, Denmark

12.15-13.45 **Lunch break**

Session 2: Characterisation of surface interactions

Session chair: Bo Mattiasson, University of Lund, Lund, Sweden

- 13.45–14.00 **Chairman's Introduction**
- 14.00–14.45 **Plenary Lecture**
201 Design and characterization of novel sensing devices: From self-assembled monolayers to micro- and nanostructured hydrogels
Bo Liedberg, Division of Molecular Physics, IFM, Linköping University, SE-581 83 Linköping, Sweden
- 14.45–15.20 **202 Weak affinity chromatography using carbohydrate binding modules**
Reine Johansson¹, Lavinia Cicortas Gunnarsson², Mats Ohlin², Sten Ohlson¹
¹ Department of Chemistry and Biomedical Sciences, University of Kalmar, Kalmar, Sweden,
² Department of Immunotechnology, Lund University, Lund, Sweden
- 15.20–15.50 **Coffee break**
- 15.50–16.25 **203 Structure-Based Antagonism of the HIV-1 Envelope Host Cell Receptor Machine Through Conformational Entrapment by Dual Receptor Antagonists**
Irwin Chaiken, Hosahudya Gopi, Karyn McFadden, Simon Cocklin and Sabine Baxter
Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia PA 19102 USA
- 16.25–17.00 **Pierce Award Lecture**
Large-scale affinity purification of protein complexes: getting functional and structural insights into cellular assemblies
Bertrand Seraphin
Centre de Génétique Moléculaire, CNRS, Gif sur Yvette Cedex, France
- 17.00–18.30 **Poster session for posters 801–825, presenters are requested to stand by their posters**
- 18.30–20.00 **Dinner**
- 20.00–21.00 **Discussion Forum: Weak and strong affinity interactions – pros and cons?**
Chair: M.A. Vijayalakshmi, Université de Technologie de Compiègne, COMPIEGNE, France

Tuesday August 16, 2005

Session 3: Design of affinity ligands

Session chair: Jan-Christer Janson, Uppsala University, Uppsala, Sweden.

08.30–08.45 **Chairman's Introduction**

08.45–09.30 **Plenary Lecture**

301 Why is it so Difficult to Design Ligands that Bind to Proteins?

George M. Whitesides

Department of Chemistry and Chemical Biology, Harvard University Cambridge MA 02138, USA

09.30–10.05 **302 Universal Method for Synthesis of Highly Selective Artificial Gel Antibodies against Proteins, Viruses and Cells; Some Techniques to Study the Selectivity; and Applications.**

S. Hjertén¹, N. Ghasemzadeh¹, M.-C. Hjertén¹, Á. Végvári¹, I. Bacskay², A. Kilár², M. Rezelí², A. Takátsy², F. Kilár², A. Ballagi³, A. Elfving³, H. Cheng⁴, J. Sedzik⁴, T. Aastrup⁵, H. Andersson⁵

¹Dept. of Biochemistry, Uppsala University, Uppsala, Sweden; ²Inst. of Bioanalysis, University of Pécs, Pécs, Hungary;

³Center for Surface Biochemistry, Uppsala University, Uppsala, Sweden; ⁴Dept. of Biosciences at Novum, Karolinska Institutet, Stockholm, Sweden; ⁵Attana AB, Stockholm, Sweden

10.05–10.35 **Coffee break**

10.35–11.10 **303 Nano Imprint Biotechnology**

Klaus Mosbach *, Lei Ye and Huiqi Zhang

Center for Molecular Imprinting Lund University, Sweden

11.10–11.45 **Young Investigator Award Presentations**

11.45–13.15 **Lunch**

Session 4: Combinatorial strategies for ligand discovery

Session chair: Mathias Uhlén, Royal Institute of Technology, Stockholm, Sweden

13.15–13.30 **Chairman's Introduction**

13.30–14.15 **Plenary Lecture**

401 Mimicking natural combinatorial strategies for the creation and selection of molecular diversity

Greg Winter

MRC Laboratory of Molecular Biology, Hills Road, Cambridge

14.15–14.50 **402 Design of small organic molecule affinity ligands**

E. Carredano, A. Axén, H. Baumann, Oguz Ersoy,

GE Healthcare, Uppsala, Sweden

14.50–15.20 **Coffee break**

- 15.20–15.55 **403 An Artificial Protein L (PpL) for the Purification of Immunoglobulins and Fab Fragments by Affinity Chromatography**
Jonathan M. Haigh^a, Xiaoping Yang^a, Erik Gimble^b, **Christopher R. Lowe^a**
^aInstitute of Biotechnology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QT, UK,
^bBioniqs Ltd. Heslington Hall, University of York, Heslington, York, UK. YO10 5DD, UK
- 15.55–16.30 **404 Reducing protein concentration range of biological samples using combinatorial ligand libraries**
E. Boschetti, L. Lomas
CIPHERGEN Biosystems Inc. Fremont CA, USA
- 16.30–18.15 **Poster session, posters 826–858, presenters are requested to stand by their posters**
- 18.15–20.00 **Dinner**
- 20.00–21.00 **Discussion Forum**
Design of affinity ligands – are there any new approaches?
Chair: Mathias Uhlén, Royal Institute of Technology, Stockholm, Sweden

Wednesday August 17, 2005

Session 5: Strategies for industrial affinity purifications

Session chair: Lars Hagel, GE Healthcare, Uppsala, Sweden

- 08.30–08.45 **Chairman's Introduction**
- 08.45–09.30 **Plenary Lecture**
501 Industrial Protein Purification Process Design: Integrating the Steps from Raw Source to Final Product
Conan J. Fee
Department of Materials & Process Engineering, University of Waikato, Private Bag 3105, Hamilton, New Zealand.
- 09.30–10.05 **502 Novel high capacity and high alkaline stable protein A affinity chromatography media: characterization and engineering considerations**
Rainer Hahn, Philipp Bauerhansl and Alois Jungbauer
Department of Biotechnology, University of Natural Resources and Applied Life Sciences, Vienna, Austria,
- 10.05–10.35 **Coffee break**
- 10.35–11.10 **503 Affinity adsorption processes for food industries: need or dream?**
Karin Merck
Agrotechnology and Food Innovations B.V., Bornsesteeg 59, 6708 PD, Wageningen, The Netherlands
- 11.10–11.45 **504 Selective and specific capturing of target molecules**
E. Houtzager, G. de Roo, I. Vijn, K.J. Francoijs, P. Sijmons
CatchMabs BV, Wageningen, the Netherlands
- 11.45–13.15 **Lunch break**

Program

Session 6: Molecular interactions for medical applications

Session chair: Sten Ohlson, University of Kalmar, Kalmar, Sweden

- 13.15–13.30 **Chairman's Introduction**
- 13.30–14.15 **Plenary Lecture**
601 Applications of Smart Polymers as protein Conjugates
Allan S. Hoffman and Patrick Stayton (and many coworkers)
Department of Bioengineering, University of Washington, Seattle, WA 98195 USA
- 14.15–14.50 **602 Human Proteome Resource – strategies for protein discovery.**
Sophia Hober
Department of Biotechnology, AlbaNova University Center, Royal Institute of Technology (KTH), Stockholm, Sweden
- 14.50–15.20 **Young Investigator Award Presentations**
- 15.20–17.45 **Excursions (Pre-registration is required)**
- 19.30 **Gala Dinner at Uppsala Castle**

Thursday August 18, 2005

Session 6: Molecular interactions for medical applications, continued

Session chair: Sten Ohlson, University of Kalmar, Kalmar, Sweden

- 09.30–09.45 **Chairmans Introduction**
- 09.45–10.30 **Plenary Lecture**
603 PET, a tracer technology with many applications
B. Långström, G. Antoni, T. Kihlberg, F. Karimi, O. Rahman, S. Estrada, O. Lindhe, H. Engler, G. Blomqvist, O. Itsenko*, J. Barletta*, I. Velikyan*, J. Eriksson
Uppsala Imanet AB, S-751 09 Uppsala, Sweden, *Dep. Org. Chem., University of Uppsala
- 10.30–11.00 **Coffee break**
- 11.00–11.35 **604 Affinity Purification and Characterization of an Anti-PEG IgM**
George K Ehrlich
Hoffmann-La Roche, 340 Kingsland Street, Nutley, NJ 07110
- 11.35–12.10 **Jerker Porath Award Lecture**
- 12.10–13.40 **Lunch**

Session 7: Molecular interactions in the nano-format

Session Chair: Richard Willson, University of Huston, Huston, TX, USA

- 13.40–13.55 **Chairman's Introduction**
- 13.55–14.40 **Plenary Lecture**
701 Molecular assembly platform to direct bio-specific responses in biomaterials, biosensors and targeted drug delivery
Marcus Textor
 BiolInterfaceGroup, Laboratory for Surface Science and Technology, Department of Materials, ETH Zurich, CH-8093 Zurich, Switzerland
- 14.40–15.15 **702 Reversible and Directional Self-Assembly of Bio-Molecular Templates for Nanotechnology Interconnects**
 Lian Wang*, Chelsea Benally*, **Roberto Guzman***, Ye Yang[†], Pierre Deymier[‡], Heather McLaughlin* Ian Jongeward* and James Hoying**.
 *Department of Chemical & Environmental Engineering. [†]Department of Material Science and Engineering. [‡]Department of Pediatrics. **Biomedical Engineering, University of Arizona, Tucson, AZ 85721 USA.
- 15.15–15.40 **Coffee break**
- 15.40–16.15 **703 Multi-Ligand Decorated Nanoparticles as Diagnostic Platforms**
 K. Fromell, A. Sahlholm, M. Andersson and **K.D. Caldwell**
 Department of Surface Biotechnology Uppsala University, 75123 Uppsala, Sweden
- 16.15–16.30 **Concluding remarks**
 Meeting chairman
 Announcement of Affinity 2007

Monday August 15

Session 8: Poster Session

17.00–18.30

- 801 Multiple frequency detection of antibody-antigen interactions via a novel acoustic wave biosensor**
 Kioupritzi E., Araya-Kleinsteuber, B., Roque, A.C.A., Stevenson, A.C., Lowe, C.R.
 Institute of Biotechnology, University of Cambridge, Tennis Court Road, Cambridge, UK CB2 1QT. Tel: +44 (0) 1223 334152
 Fax: +44 (0) 1223 334162, email: ek283@cam.ac.uk
- 802 Critical Interactions for Anisotropic Adsorption of Particles to Polymer Grafted Surfaces**
 Hans-Olof Johansson*¹ and Fernando Barroso da Silva²
¹Department of Biochemistry, Lund University, P.O.Box 124, S-22100 Lund, Sweden. Tel +46-46-2228189; Fax +46-46-2224534; e-mail Hans-Olof.Johansson@biokem.lu.se
²Faculty of Pharmaceutical Sciences at Ribeirao Preto, University of Sao Paulo-USP. Av. Do Café, s/no-FCFRP/Bloco A-sala 133, BR-14040-903, Ribeirao Preto SP, Brazil. Tel +55(16)6024219; Fax +55(16)6332960, e-mail: fernando@fcfrp.usp.br
 * Corresponding author

- 803 Stereoselective recognition of positively or negatively charged chiral and achiral molecules by transferrin**
Ferenc Kilár¹, Petronella Kuti¹, Csenge Sági¹, Nándor Sánta², Vlad Chindea², Béla Tótkés²
¹Institute of Bioanalysis, Faculty of Medicine, University of Pécs Szigeti út 12., H-7624 Pécs, Hungary
²University of Medicine and Pharmacy, Targu Mures, Romania
- 804 Predicting protein retention time in hydrophobic interaction chromatography**
M.E. Lienqueo¹, A. Mahn², J.C. Salgado¹, I Rapaport³ and J.A. Asenjo¹
¹Centre for Biochemical Engineering and Biotechnology, Department of Chemical and Biotechnology Engineering, University of Chile, Beauchef 861, Santiago, Chile, e-mail mlienque@ing.uchile.cl
²Center for Molecular Cell Studies, Institute for Biomedical Sciences, University of Chile.
³Centre for Mathematical Modeling, University of Chile.
- 805 Affinity measurement of carbohydrate binding to recombinant *Aleuria aurantia* lectin using fluorescence spectroscopy**
Johan Olausson¹, Lena Tibell¹, Bengt-Harald Jonsson², Peter Pålsson¹
¹Institute of Biomedicine and Surgery, Division of Cell Biology, Linköping University, SE-581 85 Linköping.
²Molecular Biotechnology/IFM, Linköping University, SE-58183 Linköping, Sweden.
- 806 Phosphatase Inhibitors – A Biacore Approach to Study Small Molecule Inhibitor Interactions with Protein Phosphatases**
Peter Stenlund, Åsa Frostell-Karlsson, Annika Remaeus, Åsa Edström and Olof Karlsson
Systems and Applications, Biacore AB, Uppsala, Sweden.
- 807 Bioanalytical applications of a non contact acoustic sensor**
Araya-Kleinstauber, B., Stevenson A.C., Roque, A.C.A., Kioupritzi, E. & Lowe, C.R.
Institute of Biotechnology, University of Cambridge, Tennis Court Road, Cambridge, UK CB2 1QT.
Tel: +44 (0) 1223 334152 Fax: +44 (0) 1223 334162, email: ba239@cam.ac.uk
- 808 Immobilized Metal Affinity Chromatography of Nucleic Acids**
Ajish Potty¹, Yuchun Fu¹, Tony Cano¹, Sindhu Balan², George E. Fox², and Richard C. Willson^{1,2}
¹Department of Chemistry and Biomedical Sciences, University of Houston, 4800 Calhoun Rd, Houston, TX 77204-4004,
²Department of Biology and Biochemistry, University of Houston, 4800 Calhoun Rd, Houston, TX 77204-5934
- 809 A label-free continuous fluorescence-based immunosensor**
Henrik A. Engström¹, Per Ola Andersson² and Sten Ohlson¹
¹Department of Chemistry and Biomedical Sciences, University of Kalmar, SE-391 82 Kalmar, Sweden.
²Swedish Defence Research Agency, Division of NBC-Defence, SE-901 82 Umeå, Sweden.
- 810 A TiO₂-Binding Protein from Rhodococcus Strain GIN-1 (NCIMB 40340) – Mode of protein-oxide interaction**
Gideon Fleminger, Jenny Komanovsky, Irene Brudo and Golan Gertler
Department of Molecular Microbiology and Biotechnology, George Wise Faculty of Life Sciences, Tel Aviv University,
Tel Aviv 69978, Israel
- 811 Thermodynamic Dissection of Association and Dissociation in Interaction Between Lysozyme and Monoclonal Antibodies**
A. Zhukov¹, T. Nakanishi², K. Tsumoto², I. Kumagai², K. Andersson¹, H. Nordin¹, C Wass¹,
Å Edström¹ and R. Karlsson¹
¹Biacore AB, Uppsala, Sweden, ²Tohoku University, Sendai, Japan.
- 812 Determining the assay consistency for biosensor based direct binding assays**
Annie Näslund, Christina Wass, Annika Remaeus and Åsa Edström
Dept. Systems and application, Biacore AB, Uppsala Sweden.

- 813 An acoustic sensor design based on a non-contact transducer format**
Roque, A.C.A., Araya-Kleinsteuber, B., Kioupritzi, E., Stevenson, A.C., Lowe, C.R.
Institute of Biotechnology, University of Cambridge, Tennis Court Road, Cambridge, UK CB2 1QT.
Tel: +44 (0) 1223 334152 Fax: +44 (0) 1223 334162, email: car38@cam.ac.uk
- 814 Capacitive affinity biosensor as a tool to monitor low concentrations of biomolecules**
Martin Hedström, Igor Galaev and Bo Mattiasson
Department of Biotechnology, Center for Chemistry & Chemical Engineering, Lund University, P.O. Box 124, SE-22100 Lund, Sweden
- 815 Exploitation of the Janus-faced nature of β -cyclodextrins as affinity ligands in CE and CEC**
Ákos Végvári
Dept. Of Biochemistry, Uppsala University, P.O.Box 576, SE-751 23 Uppsala, Sweden
- 816 Affibody® Molecules: Affinity Proteins for Biotechnology Applications**
Martin Nilsson and Thomas Bergman
Affibody AB, Voltavägen 13, 161 02 Bromma, Sweden
- 817 Polymeric IMAC-adsorbents and their use in the adsorption of Arsenic ions from aqueous solutions**
Javier E. Garcia*, Roberto Guzman* and Jerker Porath**
*Chemical and Environmental Engineering Department, University of Arizona, Tucson, AZ.
**Centre for Separation Sciences, Uppsala University, Uppsala, Sweden.
- 818 Enhanced Affinity Gel Permeation Chromatography of Proteins Using a Multiligand-Water Soluble Affinity Carrier**
Javier E. Garcia*, Jerker Porath** and Roberto Guzman*
*Chemical and Environmental Engineering Department, University of Arizona, Tucson, AZ.
**Centre for Separation Sciences, Uppsala University, Uppsala, Sweden.
- 819 Dynamic Binding Capacities of Phosphorylase b on Experimental Butyl-Modified Sepharose™ 4B, and Surface-Extended Sepharose 6 Critical Hydrophobicity HIC Chromatography Media**
Kristin Zurlinden¹, James M. Van Alstine² and Herbert P. Jennissen¹
¹Institut für Physiologische Chemie, Universitätsklinikum Essen, Hufelandstr. 55, D-45122 Essen, Germany;
²GE Healthcare, Protein Separations, Uppsala, 112 54, Sweden
- 820 A New IMAC Medium for Scaling up Purification of Histidine-tagged Proteins**
Jon Lundqvist, Lars C. Andersson, Ann Bergh, Ellinor Ancker, Anna Heijbel, Helena Lindgren, Karin Torstenson and Katarina Öberg
GE Healthcare, Protein Separations, SE-751 84 Uppsala, Sweden
- 821 Automated Multi-step Purification of Histidine-tagged proteins from Crude Cell Lysates**
Anna Sjöberg, Ellinor Ancker, Ann Bergh, Jon Lundqvist, Katarina Öberg, Nina Forsberg
GE Healthcare, Bio-Sciences, Protein Separations, SE-751 84 Uppsala, Sweden
- 822 Hydrophilic Rigid Activated Media by Encapsulation of Inorganic Particles by Dispersion Polymerization**
M.M. Rhemrev-Boom and M.A. Jonker
ResQ lab B.V., Nijverheidsweg 14, 7948 NE Nijveen, the Netherlands

823 Polymer-modulated permeation control for affinity adsorption chromatography. An Strategy to Enhance Peptide and Small Proteins Fractionations

Roberto Guzman*, Lian Wang*, and Jerker Porath**

*Chemical and Environmental Engineering Department, University of Arizona, Tucson, AZ.

**Centre for Separation Sciences, Uppsala University, Uppsala, Sweden.

824 Synthesis and Studies of Chelating Liposomes: Binding of Metal Ions and Proteins and Effect of PEG on Aggregation and Binding Interactions

Shujuan Yan and Roberto Guzmán,

Chemical and Environmental Engineering Department, University of Arizona, Tucson, AZ 85721

825 Selective adsorption of monoclonal antibodies against mutant amidase from *Pseudomonas aeruginosa* on tailor-made immobilized metal chelates

Sónia Martins¹, Jorge Andrade¹, Amin Karmali^{1*} and Maria Luísa Serralheiro²

¹ Centro de Investigação de Engenharia Química e Biotecnologia do Instituto Superior de Engenharia de Lisboa, Rua Conselheiro Emídio Navarro 1900 Lisboa - Portugal, Tel: 351.21.8317052, Fax: 351.21.8317267

² Centro de Química e Bioquímica da Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1760 Lisboa - Portugal.

*To whom correspondence should be addressed. e-mail: akarmali@deq.isel.ipl.pt

Tuesday August 16

Session 8: Poster session continued

16.30–18.15

826 AffilinTM– And Its Application In Affinity Chromatography

E. Fiedler, C. Reimann, M. Fiedler, T. Scheuermann, G. Proetzel, R. Rudolph, and U. Fiedler

Scil Proteins GmbH, Heinrich-Damerow-Str. 1, 06120 Halle/Saale, Germany

Tel.+49 345 27996330, Fax+49 345 27996332, <http://www.scilproteins.com>, e-mail: affilin@scilproteins.com

827 Potential of homocysteine thiolactone scaffold towards libraries of media with new selectivities

Jean-Luc Maloisel, Nicolas Thevenin, Nils Norrman, Johanna Karlqvist, Miranda Varedian

GE Healthcare, Björkgatan 30, SE-751 84 Uppsala, Sweden

828 Affinity ligands for transferrin

Hörður Filippusson

University of Iceland Science Institute, Department of Biochemistry, Dunhaga 3, IS-107 Reykjavik, Iceland

829 Detection of weak antibody fragments using phage display

Eva Åström & Sten Ohlson

Department of Chemistry and Biomedical Sciences, University of Kalmar, SE-391 82 Kalmar, Sweden

830 Adsorption studies of recombinant cutinase onto a combinatorial library of synthetic affinity ligands

Ruiu, L.^{1,2}, Roque, A.C.A.^{1,2}, Lowe, C.R.², Taipa, M.A.¹

¹Centro de Engenharia Biológica e Química, Instituto Superior Técnico, Avenida Rovisco Pais, 1049-001 Lisboa, Portugal.

831 Development and applications of a new Protein A media intended for industrial use

Hans J Johansson

GE Healthcare, SE-751 84 Uppsala

- 832 Affinity cryogel monoliths for cell chromatography and cell surface profiling**
 Maria B. Dainiak^{a,b}, Fatima M. Plieva^{a,b}, Igor Yu. Galaeva and Bo Mattiasson^a
^aDepartment of Biotechnology, Center for Chemistry and Chemical Engineering, Lund University, P-O. Box 124, SE-22100 Lund, Sweden
^bProtista Biotechnology AB, IDEON, SE-22370 Lund, Sweden
- 833 An Optical Biosensor (SPR) Assay For Investigations of Enantioselective Affinities A Comparison with A Validated HPLC Method**
 Robert Arnell, Natalia Ferraz, Peter Sandblad and Torgny Fornstedt
 Dept. of Surface Biotechnology, BMC Box 577, S-751 23 Uppsala, Sweden
- 834 Ion-exchange Macroporous Hydrophilic Gels with Grafted Polymer Brushes**
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Abstract

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Bacterial adhesion under flow: bonds that strengthen under force

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In contrast to common expectations, the adhesion of *E. coli* to surfaces can be enhanced by shear flow. While the adhesive strength of most receptor-ligand interactions is exponentially reduced if pulled apart by force, some receptor-ligand complexes exist that strengthen under force which is the hallmark for catch bonds. Although the existence of catch bonds was theoretically predicted by Dembo et al in 1988, the first experimental demonstration of their existence was given only recently, i.e. for the bacterial adhesin FimH that is located at the tip of type I fimbriae of *E. coli* and then for p-selectin. For mannose-liganded FimH, we will discuss a structural model how a force-induced structural perturbation at a location remote from the binding site can switch FimH from a short-lived to a long-lived state. Force-activation of FimH leads to a complex 'stick-and-roll' bacterial adhesion behavior in which *E. coli* preferentially rolls over mannose-coated surfaces at low shear but increasingly sticks firmly as the shear is increased. These "stick-and-roll" transitions are not due simply to fluid transport or increases in bond number as evidenced by many controls. For example, *E. coli* bacteria when binding to anti-FimH antibody-coated surfaces, thus forming slip-bonds, detach more easily when the shear stress was increased, and flow chamber viscosity studies show that the binding mode depends on shear stress (and therefore force) rather than shear rate (and transport). Physiological implications will be discussed as well as first technical applications.

Literature

W. E. Thomas, E. Trintchina, M. Forero, V. Vogel, E. Sokurenko, Bacterial adhesion to target cells enhanced by shear-force, *Cell*, 109 (2002) 913.

W. E. Thomas, L. M. Nilsson, M. Forero, E. V. Sokurenko, V. Vogel, Shear-dependent 'stick-and-roll' adhesion of type 1 fimbriated *Escherichia coli*, *Molecular Microbiology* 53 (2004) 1545.

M. Forero, W. Thomas, C. Bland, L. Nilsson, E. Sokurenko, V. Vogel, A catch-bond based smart nano-adhesive sensitive to shear stress, *Nanoletters*, 4 (2004) 1593.

A-priori prediction of protein affinity in chromatographic systems using state-of-the-art structure-property multi-scale modeling techniques

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Methods development for a given protein separation often entails the initial screening of various classes of stationary phase materials and mobile phase conditions in order to identify chromatographic conditions with sufficient selectivity. In the present work, an approach is presented for the a priori prediction of adsorption isotherm parameters and preparative chromatographic behavior in both ion-exchange and hydrophobic interaction systems. Predictive Quantitative Structure Property Relationship (QSPR) models were generated for the experimentally determined isotherm parameters using a set of novel interpretable molecular descriptors and Support Vector Machine (SVM) learning algorithms. The predictive ability of these models was demonstrated and the important molecular property descriptors identified by the feature selection process were closely examined to provide insight into the nature of selectivity in these different chromatographic systems. These models allow for the direct comparison of the properties of the biomolecules that are responsible for their affinity under different resin/mobile phase combinations. The predicted isotherm parameters were subsequently employed in traditional column chromatography models to successfully predict column performance under a range of operating conditions. In addition, a novel technique for developing an alignment free quantitative comparison technique for protein shape and electron distribution was employed to study distinct patches on the proteins in order to search for patterns associated with chromatographic affinity. Finally, the use of sequence dependent 2D molecular property descriptors alone for building QSPR models for ion exchange systems is presented. These developments represent the state-of-the-art in structure-property modeling as applied to chromatography and can provide significant insight into the nature of affinity in various chromatographic systems and can facilitate downstream process development and optimization.

Size Distribution of Hydrophobic Patches Allows for the Selection of Hydrophobic Interaction Chromatography (HIC) Media for Protein Purification

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Critical hydrophobic interaction chromatography (CHIC) is a recently developed method [1] for the selective and reversible binding of proteins using gel libraries comprising homologous series of n-alkyl agaroses differing in alkyl surface concentration. The experimental determination of the critical surface concentration on butyl- and hexyl-Sepharose revealed that neither α -amylase nor ubiquitin bind to any gel even at the highest degree of substitution (40 $\mu\text{Mol/ml}$ packed gel). In contrast BSA, Ca^{++} -calmodulin, fibrinogen and phosphorylase β adsorb to hexyl-Sepharose in a critical hydrophobicity range of 4–10 $\mu\text{Mol/ml}$ packed gel and to butyl-Sepharose in a range of 20–30 $\mu\text{Mol/ml}$ packed gel. A critical number of hydrophobic patches [2] with a size of more than 400 \AA^2 seems to be a binding prerequisite for protein ligands as both α -amylase and ubiquitin lack patches of this size. The rationale behind this finding might be a critical number of alkyl chains/ hydrophobic surface area which can be calculated for a degree of substitution of 30 $\mu\text{Mol/ml}$ to be ~ 1 alkyl chain/ 200 \AA^2 . We believe that the correlation between alkyl chain length, critical hydrophobicity and the number of hydrophobic patches $> 400 \text{\AA}^2$ provides a rational basis for the prediction of binding behaviour in HIC.

References

1. Jennissen HP (2000) *Int J Bio-Chromatogr* 5, 131–163
2. Lijnzaad P, Berendsen HJ, Argos P (1996) *Proteins* 26, 192–203

On the Thermodynamics of Protein Chromatography

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Proteins are large complex molecules where the three dimensional structure is essential to their diverse biological functions and activity. In spite of their diverse functions, they are a relatively homogeneous class of molecules because all are the same type of linear polymer, build of various combinations of the same 20 amino acids. The large size of the polypeptide chains enables them to fold back on themselves so that many simultaneous interactions take place among different parts of the molecule forming a rather compact molecule. The interaction with water and salts plays an important role in stabilizing the proteins. Therefore, the structure, the stability and the properties of proteins in solution are governed by such factors as pH, the charge and surface charge distribution, the hydrophobicity, the nature and concentration of the salt and the presence of solvents.

A variety of factors that govern the protein properties are utilized in the development of processes for the recovery of biological products including the binding and release of protons, the non-covalent association with non-polar groups (often hydrophobic interactions), the association of small ions (ion exchange). Despite the fact, the proteins are large complex molecules, their compact nature enable us to develop rather simple consistent models of their thermodynamic behaviour in solution that can be used to model ion exchange [1] and hydrophobic interaction [2] chromatography. Besides, measurements on HIC media can be utilised to determine the Cohn salting-out coefficient.

Design and characterization of novel sensing devices: From self-assembled monolayers to micro- and nanostructured hydrogels

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The present contribution deals with the development of novel biochips and microarray platforms for protein analysis and characterization. The first part of the talk focuses on the use of 2D-sensing layers based on alkanethiolate self-assembled monolayers (SAMs) tailored with carbohydrate recognition groups. A generic technology for the optimization of such SAMs are described and illustrated for a well-known model system- streptavidin/biotin. Alkanethiolate SAMs are also combined with modern printing techniques like Micro Contact Printing (mCP) for microarray applications. A few examples of such microarrays are described and characterized using scanning probe microscopy (SPM) optical imaging techniques, in particular, surface plasmon microscopy and imaging ellipsometry. The second part of the talk deals with nano- and microstructured 3D-hydrogels for the production of protein microarrays. A novel concept based on carboxymethylated dextran hydrogels and mCP of cationic surfactants is introduced for the fabrication of reversible hydrophobic barriers. Alternative hydrogel printing strategies based on polyelectrolyte printing are also described and applied for a number of protein model systems.

Weak Affinity Chromatography Using Carbohydrate Binding Modules

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Recently, a combinatorial library was constructed based on a carbohydrate binding module (CBM) from a thermo stable *Rhodothermus marinus* xylanase, and a number of variants were selected by screening the library against a variety of targets like the carbohydrate polymers Avicel[®], birch wood xylan and ivory nut mannan. As these proteins are highly stable they have a potential to be useful in a variety of biotechnological applications.

To assess this matter we investigated the potential of different molecular variants of this CBM and their wild-type counterpart in high performance chromatographic applications. They were immobilised on macroporous silica, and oligomers of glucose (glucose-cellopentaose) and xylose (xylose-xylohexaose) were used as analytes at 25 and 65°C. We found that the various CBM had the ability to separate the glucose- and/or xylose-oligomers, and the affinity between the ligand and the analyte could be controlled by changing the temperature.

In conclusion, the high thermal stability and the ability to be evolved *in vitro* makes the CBM an excellent ligand to use in weak affinity chromatography, and we are certain that CBM can be an alternative to other binding-proteins, such as antibodies and lectins, in various downstream biotechnological and analytical applications.

Structure-Based Antagonism of the HIV-1 Envelope Host Cell Receptor Machine Through Conformational Entrapment by Dual Receptor Antagonists

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Increasing understanding of the molecular and structural nature of the encounter complex of HIV-1 viral envelope protein gp120 with host cell receptors CD4 and co-receptor (CCR5/CXCR4) makes it increasingly feasible to devise antagonist strategies based on the structural mechanism of this molecular machine. We and others have successfully designed structure-based CD4 mimics that compete for CD4 binding, but these also mimic a more deleterious property of CD4 by enhancing binding of Env to co-receptor and hence are not optimal antagonists. As a consequence, we have begun to focus on a fusion inhibitor, the 12-residue peptide, 12p1 [RINNPWSEAMM], which was discovered initially by phage library screening (*Ferrer and Harrison, 1999*). This peptide inhibits the interaction of gp120 with both CD4 and 17b, an antibody that recognizes an epitope overlapping the CCR5 binding site. We have found that this competition appears to occur by an allosteric mechanism that involves binding at a site other than one of the receptor sites and stabilization of a gp120 conformation with reduced affinity for the receptors. More recently, we have employed click chemistry to form triazoles at residue position 6 of 12p1 and found that some of these triazoles lead to peptides that bind to gp120 with several orders of magnitude greater affinity than the parent 12p1 and with retention of the dual antagonist activity.

In coordination with the above work on 12p1, we have initiated efforts to form chimeric fusions of the dual antagonist peptide with cyanovirin-N (CV-N), a small (11 kD) protein that inhibits HIV-1 cell fusion, has been demonstrated to bind to sugar moieties of gp120 with nanomolar affinity and is currently being evaluated as a topical microbicide. We have created a series of chimerae with 12p1 linked to the C-terminal domain of CV-N through a penta-peptide linker of one through five repeats. The proteins were expressed in the BL21 (DE3) strain of *E. coli*, isolated by osmotic shock and purified over a nickel-NTA column. The ability of the CVN-12p1 chimera with five repeats of the linker (L5) to bind to gp120 was confirmed by both SPR (Biacore) and ELISA assays and shows that CV-N can tolerate extensions to its C-terminal domain with no interference to its ability to bind to gp120. Importantly, the L5 chimera detectably inhibits the YU2 -17b interaction more potently than CV-N alone, indicating an affect of the 12p1 domain of the protein. However, the overall increase of antagonist activity of the CVN fusion vs CVN alone is limited due mainly to the much higher gp120 affinity of CVN than 12p1. As a consequence, current work is focusing on generating chimerae of CVN with higher potency partners of 12p1 devised through click chemistry. Conjugates of 12p1 and CVN-12p1 fusions offer a path to improved mechanistic understanding of how diverse HIV-1 entry inhibitors carry out their functions and to the development of enhanced-activity antagonists for therapeutics and microbicides.

Why is it so Difficult to Design Ligands that Bind to Proteins?

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The ability to design drugs (so-called “rational drug design”) has been one of the long-term objectives of chemistry for 50 years. It is an exceptionally difficult problem, and many of its parts lie outside the expertise of chemistry. The much more limited problem-how to design tight-binding ligands-would seem to be one that chemistry could solve, but has also proved remarkable recalcitrant. The question is “Why is it so difficult?” and the answer is “We still don’t entirely know.” This talk will discuss some of the technical issues-potential functions, enthalpy/entropy compensation, protein plasticity, and others-that contribute, and suggest areas where fundamental understanding of protein-ligand interactions falls short of what is needed.

Universal Method for Synthesis of Highly Selective Artificial Gel Antibodies against Proteins, Viruses and Cells; Some Techniques to Study the Selectivity; and Applications

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Highly selective artificial gel antibodies against *different classes* of antigens can be prepared by an imprinting method which, thus, is universal: An aqueous solution of the antigen is supplemented with appropriate monomers. Following polymerization the gel is granulated and the antigen is removed. The degree of selectivity can be determined by various techniques. We will pay special attention to free zone electrophoresis in a rotating capillary and QCM (Quartz Crystal Microbalance). In the former method the mobility of the complex between the gel antibody and the antigen present during the polymerization is determined, as well as the mobilities of complexes between this gel antibody and antigens which are structurally related to the above antigen. The combination of this synthesis of artificial gel antibodies with analysis by free zone electrophoresis permits detection of extremely small differences in the structure of biomolecules and bioparticles, which will be demonstrated by several examples (for instance, we can differentiate between different strains of bacteria, between wild type and a mutant of virus, between proteins from different species, etc.).

The gel antibodies have many advantages compared to those raised in experimental animals (an ethical problem): more stable, probably more selective, more cost-effective to produce, and they can be synthesized not only against proteins, but also against particles, such as viruses and cells, for instance bacteria (in animals these particles are degraded metabolically).

Nano Imprint Biotechnology

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Molecular imprinting of polymers (MIPs) is a technique often employed in the fabrication of biomimetic polymeric recognition matrices that is rapidly attracting increasing industrial interest. By the technology, recognition matrices, possessing high substrate site selectivity and specificity can be prepared. The physical and chemical characteristics of molecularly imprinted materials are highly appealing. These materials exhibit high physical and chemical resistance against external degrading factors. Thus, molecularly imprinted polymers are remarkably stable against mechanical stresses, high temperatures and pressures, resistant against treatment with acid, base or metal ions, and stable in a wide range of solvents. The storage endurance of the polymers is also very high. Furthermore, the polymers can be used repeatedly without loss of their "memory effect".

The application of these robust molecular recognition elements in particular those prepared by non-covalent imprinting as developed in Lund will be briefly discussed:

Biosensor mimics ⁽¹⁾, artificial antibodies ⁽²⁾, drug discovery (screening with plastic receptors ⁽³⁾ and synthesis of new drugs in imprinted nano cavities) ⁽⁴⁾, purification, regio-stereo specific syntheses.....

For further reading, see especially from the Lund Group ^(5 and 6):

References

- 1) Haupt, K. & Mosbach, K., *Chemical Reviews* 100 (7), 2495-2504 (2000)
- 2) Vlatakis, G., Andersson, L.I., Müller, R. & Mosbach, K. Drug assay using antibody mimics made by molecular imprinting. *Nature* 361, 645-647 (1993).
- 3) Ye, L. Yu, Y. and Mosbach, K. Towards the Development of Molecularly Imprinted Artificial Receptors for the Screening of Estrogenic Chemicals. *Analyst* 126, 760-765 (2001).
- 4) Yu, Y., Ye, L., Haupt, K. and Mosbach, K. Formation of a Class of Enzyme Inhibitors (Drugs), Including a Chiral Compound, by Using Imprinted Polymers or Biomolecules as Molecular-Scale Reaction Vessels. *Angew. Chem. Int. Ed.* 41, 4459-4463 (2002).
- 5) Ye, L. and Mosbach, K. *J Inclusion Phenomena and Macrocyclic Chemistry*, 41, 107-113 (2000) ed. Shinkai.
- 6) Ye, L. and Mosbach, K. *Nature Materials*, review, in press

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Mimicking natural combinatorial strategies for the creation and selection of molecular diversity

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Populations of antibodies capable of binding to a myriad antigens are created in nature by random combination of multiple genetic segments. During the early 1990s we were able to mimic this process in vitro by the creation of antibody repertoires by random combination of the same genetic elements. For selection we used phage display and capture on antigen-coated matrices, and several antibodies created by these technologies are in clinical use. More recently we have explored combinatorial strategies to generate novel folded proteins, by shuffling sub-domain gene segments from other proteins, and thereby mimicking "exon-shuffling" in early protein evolution. Not only does this give rise to folded protein domains, but to those with novel domain architectures, as shown by the X-ray crystallography of two of these proteins. One of these structures includes a deep cavity for binding of ligands. We suggest that these results may illuminate early events in protein evolution and protein folding.

Design of small organic molecule affinity ligands

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Natural molecules like proteins and carbohydrates which specifically bind to target proteins are plausible candidates to function as affinity ligands. However, such ligands are less likely to be stable during the extreme acidic and basic conditions which are typical in cleaning-in-place protocols. This concern has triggered the search for stable organic molecules as affinity ligands and a number of publications on this subject have been produced. Different classes of compounds include derivatives and mimetics of natural binders, and other small organic molecules obtained by screening various types of compound libraries. Small-molecule libraries can also be designed and synthesised around a particular type of scaffold. For example the triazine scaffold has been used repeatedly in the development of dye-biomimetics. The process of obtaining ligands can be ordered in a scale that goes from the empiric to the knowledge-based. In practice, all the methods have contributions of both characters.

In this presentation, the different ligand-design and ligand-screening methodologies will be discussed. Specific examples as representatives for the knowledge-based approach based on *in silico* screening in combination with in solution screening will be presented.

An Artificial Protein L (PpL) for the Purification of Immunoglobulins and Fab Fragments by Affinity Chromatography

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A new combinatorial chemistry solid-phase strategy is being developed to generate libraries of synthetic ligands using a novel "peptoidal" chemistry where functional monomers can be sequentially attached onto a solid matrix. It is envisaged that higher order polymers with multi-functional monomers will be immobilised on adsorbent scaffolds to form affinity resins for whole IgG, Fc, Fab and scFv fragments.

Protein L biomimetic ligands will be developed using stable peptoidal chemistry. The design of these second generation PpL mimic ligands will be based on a combination of the knowledge of a characterised PpL- humanVk interface and the previously designed triazine-based 8/7 ligand (Roque *et al.*, 2005). There will be scope to incorporate flexibility within the ligand structures that may allow for the generation of more specific tailor-made affinity ligands for purification of biotherapeutic proteins. Furthermore, this chemistry may offer the ability to incorporate more than two functional groups into the ligand (the triazine-based ligands are limited to R1 and R2) through higher order polymer formation. From the newly synthesised library, putative lead compounds will be identified and structurally refined to improve their affinity and increase purification efficacy with the intent of producing PpL-based ligands that bind antibodies and their fragments from a variety of crude sources.

References

Roque, A. C. A., Taipa, M. A., Lowe, C. R. (2005), "An artificial protein L for the purification of immunoglobulins and Fab fragments by affinity chromatography." *J. Chrom. A.* **1064**: 157-167

Reducing protein concentration range of biological samples using combinatorial ligand libraries

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The discovery of polypeptides/proteins of diagnostic relevance from a biological liquid is complicated by their vast number and the large concentration range. Depletion methodologies or fractionation have been used but they failed to significantly enrich proteins present in trace amount. Here we report an approach that allows the reduction of protein concentration range of a complex mixture like neat serum through the simultaneous dilution of high abundance proteins and the concentration of low abundance proteins in a single step. This methodology utilizes solid-phase ligand libraries of large diversity, generated *via* standard "split, couple, recombine" combinatorial chemistry. With a controlled sample-to-ligand ratio it is possible to modulate the relative concentration of proteins such that a large number of polypeptides that are normally not detectable by classical analytical methods become easily accessible.

Application of this method to reduce the dynamic range of unfractionated serum is specifically described along with treatment of other biological liquids. Analytical SELDI MS technology and mono- and bi-dimensional electrophoresis demonstrate the reduction in protein concentration range. Specific examples linking this approach with additional fractionation methods demonstrate a further increase of the number of detectable species.

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Industrial Protein Purification Process Design: Integrating the Steps from Raw Source to Final Product

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The success of industrial protein purification processes is determined ultimately by process economics, although other factors, including regulatory, safety and environmental concerns also play a part. The process steps involved are determined by both the product specifications and the characteristics of the input material. Purifications of biopharmaceuticals from traditional cell cultures share a relatively common set of overall constraints: final product values are typically high, volumes are relatively low, and the technologies used, such as affinity chromatography, have matured accordingly. In contrast, production of high-value endogenous proteins from food sources is usually driven by the desire to extract profit from waste streams: values are modest, volumes are high and the technologies used borrow much from the food industry. High-volume, low-cost production of biopharmaceuticals from transgenic animals and plants shares more in common with extraction of proteins from food than with traditional cell culture in the initial stages of downstream processing, though final product specifications may be stringent. In this presentation, various bioseparation strategies will be described in light of the requirements imposed by source materials, product specifications and the need for economic viability in the integrated process.

Novel high capacity and high alkaline stable protein A affinity chromatography media: characterization and engineering considerations

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Staphylococcal Protein A is the most widely generic ligand for purification of monoclonal and recombinant antibodies in laboratory as well as industrial scale. Besides selectivity, dynamic binding capacity and regenerability are the most important features of an industrial Protein A affinity medium. Currently the highest binding capacity of commercially available Protein A medium is in the range of 40 mg/ml and moderate stability towards alkaline conditions are given. An exception is the controlled pore glass based media which are not resistant to alkaline conditions. Two improved Protein A prototype media named Xtra and SuRe and ProSep-vA Ultra have been investigated for static capacity, dynamic capacity and tested for 50 purification cycles using a real feedstock. Equilibrium capacity, dynamic binding capacity and adsorption kinetics for human IgG was determined and the capture of IgG from a crude feedstock was investigated. No significant loss of performance over 50 cycles could be observed for all three tested media. Protein A ligand leakage was in the range of 1–3 ppm for MabSelect SuRe and in the range of 30–40 ppm for MabSelect Xtra and ProSep-vA Ultra respectively. Host cell protein content of eluates from MabSelect SuRe and MabSelect Xtra was about 10 times lower compared to ProSep-vA Ultra. Finally engineering considerations were made to which extent these prototype media would improve the productivity and overall performance of an industrial antibody purification process. Special emphasis was being put on the processing of low titer and high titer feed stocks in the engineering considerations.

Affinity adsorption processes for food industries: need or dream?

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Due to changing demands of consumers (functional foods, nutraceuticals, natural ingredients), changing needs of industries (process intensification) and more stringent legislation (safety, water and energy reduction) the need for new separation techniques in the food industry has increased. Although chromatography is mostly employed in the pharmaceutical industry, now also the food industries, especially the industries producing nutraceuticals, flavours and fragrances tend to consider 'novel and expensive' separation techniques such as chromatography, membrane assisted affinity sorption (MAAS), pervaporation and supercritical processing as processes that could result in new products and in economic production processes. Membrane Assisted Adsorptive Separation is a separation technology in which the specificity of adsorption and the high capacity of microfiltration are combined in one unit operation. In an overview the importance of adsorptive separations for the food industry will be explained and developments in this field especially with respect to cost-effective affinity separations will be summarised. Examples of projects carried out by Agrotechnology and Food Innovations B.V for industrial customers will be given to underline the increasing importance of affinity adsorptive processes in the food industry.

Selective and specific capturing of target molecules

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Selective and specific capturing of target molecules is a mean that have long been on the wish list of industries. Due to the lack of economical attractive and sustainable capture tools mainly traditional chemical modified surfaces have been applied to separate target molecules, often involving a large series of steps and high costs.

The industrial Molecular affinity (iMab) platform offers a first generation economical attractive and sustainable solution in large scale selective separations. Integration of molecular bioinformatics, molecular tools and intelligent selection and screening methods appeared to be a critical decision for the development of iMabs. The development follows an iterative process of modeling, selection, screening, and functional testing. By making use of the potential of the almost unlimited protein space, iMabs can in principle be designed to fit in almost every separation process with optimal performance and beneficial economics. The success of this approach has been shown by the generation of several selective binders like e.g. mouse IgG, lactoperoxidase, lactoferrin, PME and PGE binders.

The capture properties of iMabs enable the use and development of separation means other than columns, among which free flow particle separation (e.g. magnetic beads) and dead-end filter capturing.

Applications of Smart Polymers as Protein Conjugates

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Over the past twenty years, we have been combining “smart” polymer molecules with different biomolecules for many unique applications in medicine and biotechnology. Our early work on these interesting hybrid materials was mostly focused on the random conjugation of a smart polymer to a protein, usually effected through reaction of an activated group on the polymer with a protein lysine amine group. The bioconjugate may be precipitated from solution by stimulating the smart polymer to phase separate. We have used this phenomenon for physical and affinity separations, and immunoassays.

For the past ten years, we have extended these initial studies to the selective conjugation of the smart polymer to a specific site on the protein. The specific site has usually been a cysteine -SH group, which is selectively cloned into the protein at a specific site. By conjugating the polymer near the binding site of the protein, we have been able to control the ligand binding activity of the protein, and we have also effected the release of a bound ligand when the smart polymer is cycled through its phase separation transition. We have extended these studies to thermally-induced, size-controlled binding of biotinylated proteins to streptavidin. Single-stranded oligonucleotides (ODNs) have been conjugated to a smart polymer and also to streptavidin, allowing hybridization of the ODNs to control the distance of the polymer from the active site and also to effect the phase separation of the complex conjugate. We recently cloned a streptavidin mutant having the peptide cell receptor -GRGDS- inserted into its sequence, and complexed a biotinylated temperature-sensitive polymer to the streptavidin to provide for temperature-controlled cell attachment to a polymer surface. We also recently synthesized photo-sensitive polymers and conjugated them to an enzyme for photo-induced “on-off” control of the enzyme-substrate reactions. Most recently we have applied smart polymers and their conjugates in microfluidic devices. This talk will review these smart polymer systems.

Human Proteome Resource – strategies for protein discovery

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The availability of the human genome sequence creates a range of new possibilities for biomedical research and permits a more systematic approach to the analysis of the corresponding proteins. The Human Proteome Resource (HPR) program has been set-up to allow the exploration of the human proteome with Antibody-based Proteomics (www.hpr.se). The basic concept is to generate, in a systematic and high-throughput manner, specific affinity ligands (antibodies) to all human proteins, and subsequently use these for functional analysis of the corresponding proteins in a wide range of assay platforms, including (i) a protein atlas for tissue profiles, (ii) specific probes to evaluate the functional role of individual proteins, and (iii) affinity reagents for purification of the specific proteins and their associated complexes for structural and biochemical analyses. A public protein atlas is being created to describe the tissue distribution and sub-cellular localization of human proteins both in normal and disease tissue.

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PET a tracer technology with many applications

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PET is tracer technology based on the use of short-lived positron emitting radionuclides. It has been applied in basic science for more than 2 decades mainly in neuroscience. It is now moving into general clinical research and clinical applications, especially with the advent of the combined PET/CT-scanners. A very exciting application area for PET is its use as a tool in development of drugs. PET being a valuable technique to transfer knowledge from preclinical to the clinical settings might be expensive but most likely cost effective. Methods to validate hypothesis for the drugs or tracers mode of action, the planning for clinical trials to estimate efficacy early in the development process is important in the drug development process. An important question in early clinical trials is if preclinical information from in vitro studies on gene modified cells or animals are relevant for drug effects in man. The use of PET-tracers offers an attractive alternative to characterize drug distribution and the interaction in target systems in vivo in man.

A key feature is the development of selective tracer molecules for new molecular targets and therefore there is a need of development of high throughput labelling chemistry combined with efficient bioassays. Other interesting perspectives are related the PET-microdosing concept, which includes safety and toxicology assessment adjusted to the small sub-pharmacological doses usually applied when using the PET-technique. That also needs improvements in the chemistry to label potential lead compounds for early pharmacological applications and validations in man.

In the presentation examples on new chemistry and applications in screening for new potential tracers using PET Microdosing technology will be discussed.

Affinity Purification and Characterization of an Anti-PEG IgM

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Anti-PEG IgM was purified by affinity chromatography using variable length PEG chains (5 kDa, 10 kDa, 20 kDa and 30 kDa) as affinity ligands. Maximal binding of anti-PEG IgM was observed using the 30 kDa PEG-derivatized NuGel (single passage). Purified Anti-PEG IgM was characterized for binding to pegylated proteins/peptides by Surface Plasmon Resonance, Western Blotting and ELISA. Anti-PEG IgM, in solution and adsorbed on 20K PEG-derivatized NuGel, was subjected to pepsin digestion followed by affinity chromatography. SDS-PAGE analysis of eluates in both preparations yielded one fragment that was similar in size. However, an additional lower molecular weight band was observed in solution-digested affinity purified material that was not present in the eluate from the material subjected to pepsin digestion on the affinity matrix. The lower MW fragment could be eluted under milder conditions, suggesting loss of binding multiplicity. Sizing of the two fragments by mass spectrometry yielded molecular weights of 133 kDa (both) and 83 kDa (solution). N-terminal sequencing of both fragments resulted in primary sequences (heavy and light chains) that were not only identical to each other but also to those of native IgM. The Anti-PEG IgM fragments were characterized for binding to pegylated interferon alfa-2a by ELISA. The results from these studies suggest that anti-PEG IgM can be used as probes in detection assays for PEG-conjugated biotherapeutics in pre-clinical and clinical studies.

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Molecular assembly platform to direct bio-specific responses in biomaterials, biosensors and targeted drug delivery

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Surface modifications based on biochemical principles are important tools for the fabrication of biosensor chips, biomedical devices such as implants and catheters, and of drug delivery carriers. One possible design approach is eliminating non-specific adsorption rendering surfaces "protein-resistant" and adding to such a silent surface biological functionalities such as DNA/RNA, peptides, proteins/antibodies, growth factors or vesicles. Preservation of active conformation and optimum presentation of surface-immobilized moieties is a particular challenge to the surface engineer in this field.

Novel molecular assembly systems based on alkane phosphates and poly(ethylene glycol)-grafted polyelectrolytes are presented in the context of controlling the interactiveness of oxide and other charged surfaces with biological media. Schemes for the immobilization of bioligands to protein-resistant surfaces cover covalent coupling, biospecific interactions, and metal-organic complexation (NTA-Ni-histag). Bioligands include peptides, proteins and saccharides as well as functionalized vesicles.

It is further shown how inorganic templates produced by lithography can direct the spatially selective organization of molecules to produce biologically adhesive patterns in the micro- and nanometer range in a non-interactive background.

Applications of molecular assembly systems are discussed in the context of bioaffinity sensor chips for DNA and protein analysis, titanium medical implants and polymeric microspheres for targeted delivery of drugs.

Reversible and Directional Self-Assembly of Bio-Molecular Templates for Nanotechnology Interconnects

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In this work we present our research in biorecognition for development of Reversible Bio-Nanointerconnects. Microtubules (MTs) are self-assembled subcellular proteinaceous filaments with nanoscale diameters and micrometer scale lengths. MTs are biopolymers assembled from two, related protein monomers; α and β tubulin. The aspect ratio of MT, the reversibility of their assembly and ability to be metallized make them excellent candidates to serve as templates for the fabrication of nanowires. Our work focuses on developing technology for bottom-up approaches to nano-electronics manufacturing inspired by biological processes. We will present as a first step for the fabrication of nanoscale interconnects in microelectronic devices the molecular assembly for the functionalization with self-assembled monolayers (SAMs) of a gold surface with a gamma-tubulin as a biospecific linker for MTs. Our in situ approach to manufacturing a MT interconnection on a silicon wafer using biomolecular templates consists of (a) a starting electrode functionalized with a derivatized MT nucleating complex (cap) via specific affinity recognition ligands, (b) controlled growth of MTs from the starting electrodes toward a target electrode, (c) binding of the MT plus end to capping agent bound to the target electrode via specific ligands, and (d) disassembly of uncapped MTs and subsequent metallization of interconnecting protein template.

Multi-Ligand Decorated nanoparticles as Diagnostic Platforms

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The rapid cellular response to a molecular stimulus is often due to the existence of highly accessible receptor molecules positioned on the surface of the cell or its organelles, from where a binding event is communicated via a signalling cascade. Such cascades increase in efficiency the shorter the distance between binder and signal generator. The dimensions of most cellular organelles are in the submicron range, and their sizes are well mimicked by many readily available synthetic nanoparticles. We have developed a set of attachment chemistries that allow the simultaneous linking of receptors (IgY molecules) and enzymes (luciferase) to nanoparticles in the 160-250 nm diameter range. In addition, the particles are equipped with groups allowing them to be attached to the surface of an analytical chip where they can be observed, e.g. with a CCD camera.

By means of an example, particles with a diameter of 240 nm have each been decorated with 500 molecules of luciferase and 150 antibody molecules in a platform construct to be used for a point-of-care diagnostic device. When in use, the binding of analyte will lead to a proportionate production of ATP, in turn triggering bioluminescence within the device with an intensity proportional to the concentration of analyte. Clusters of well-characterized particles are firmly positioned on the read-out surface, where wash- and reagent solutions are rapidly provided, and where signal quantification is readily accomplished

Multiple frequency detection of antibody-antigen interactions via a novel acoustic wave biosensor

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A novel acoustic approach known as the Magnetic Acoustic Resonant Sensor (MARS) using a planar spiral coil and an impedance detector has been used to study physico-chemical interactions at the quartz-liquid interface up to 1.1 GHz¹⁻⁵. This particular work presented here considers the response of an acoustic wave immunosensor based on antibodies immobilised via glutaraldehyde and their exposure to their antigen, however instead of conventional single frequency operation, multiple frequencies are used to extract more detailed information from the antibody antigen interaction process. Fluorescence microscopy using FITC labelled antibodies is used to validate the immobilisation procedure and Sauerbrey⁶ and Kanazawa³ response models are used to explore the nature of the multi-frequency acoustic response.

References:

- 1) Stevenson, A. C., & Lowe, C. R. Magentic-acoustic resonator sensors (MARS): A new sensing methodology, *Sensors and Actuators* 72, 32-37, (1999).
- 2) Stevenson, A. C., Araya-Kleinsteuber, B., Sethi, R. S., Mehta, H. M. & Lowe, C. R. Hypersonic evanescent waves generated with a planar spiral coil. *Analyst* 128, 1175-1180 (2003).
- 3) Stevenson, A. C., Araya-Kleinsteuber, B., Sethi, R. S., Mehta, H. M. & Lowe, C. R. The acoustic spectrophonometer: A novel bioanalytical technique based on multifrequency acoustic devices. *Analyst* 128, 1222-1227 (2003).
- 4) Stevenson, A. C., Araya-Kleinsteuber, B., Sethi, R. S., Mehta, H. M. & Lowe, C. R. The application of the acoustic spectrophonometer to biomolecular spectrometry: a step towards acoustic fingerprinting. *Journal of Molecular Recognition* 17(3): 174-179 (2004).
- 5) Stevenson, A. C., Araya-Kleinsteuber, B., Sethi, R. S., Mehta, H. M. & Lowe, C. R. Planar Coil Excitation of a Multifrequency Shear Wave Transducer *Biosensors and Bioelectronics* 20(7): 1298-1304 (2005).

Critical Interactions for Anisotropic Adsorption of Particles to Polymer Grafted Surfaces

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Adsorption of rod-and disc shaped model particles to a surface with end-grafted polymers was studied by Monte Carlo simulations. The partition coefficients for different particles were calculated using statistical mechanical perturbation theory. In this study, the adsorption of particles with different shapes (rods and discs), orientation parallel or perpendicular and different sizes (in terms of polymer segment units) was investigated for different grafting densities of an athermal polymer. The interaction between the model particle and the polymer was also investigated by switching the interaction from attractive to athermal (i.e. zero interaction). At a critical polymer protein interaction particles adsorb to the surface grafted polymers independently of particle orientation, size, shape and grafting density. The critical interaction between polymer segment and particle was determined to lie between close -0.25 kT for particle orientated perpendicular to the surface. The disc shaped particles had a distant dependent critical interaction. In the attractive systems the preferred orientation of the rods is parallel to the surface (at the surface), for disc particle this orientation is perpendicular.

Parts of this work were supported by the Center for Bioseparations (CBioSep) at the University of Lund via collaboration with GE Healthcare.

Keywords: Monte Carlo simulation; end-grafted polymers; critical adsorption; anisotropy

Stereoselective recognition of positively or negatively charged chiral and achiral molecules by transferrin

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Transferrin can be used for chiral recognition of small molecules, such as drugs. Several approaches have been applied in capillary electrophoresis using biopolymers for separation, but the binding mechanism of enantiomers has not been studied in details. In this study we made an attempt to link model calculations and capillary electrophoresis results. Docking of chiral and achiral compounds was performed by applying the Sybyl software providing numerous alternative conformations characterized by lipophylicity, binding energy, etc. The proper configuration that is in accordance to the real placement of the ligand and receptor was chosen by a systematic comparison of theoretical data to real experiments. Capillary zone electrophoresis of the drugs was performed through a distinct transferrin zone (injected prior to sample injection). Changes in the electrophoretic patterns (*i.e.*, in the migration properties) of the molecules reflected the interactions with the protein. The successful separation of optical isomers of positively charged or negatively charged beta-blockers, as well as other drugs show that the interaction is a complex phenomenon. With the molecular modelling we tried to characterize the binding areas at the iron-binding site of iron-free transferrin. The model-calculations are in excellent agreement with the capillary electrophoresis results, showing that such an approach is suitable for mapping interaction sites on protein surfaces.

Predicting protein retention time in hydrophobic interaction chromatography

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Hydrophobic interaction chromatography (HIC) is an important technique for the purification of proteins. It is based on the reversible interaction between the hydrophobic surface patch on a protein and the hydrophobic surface of a chromatographic medium at moderately high concentrations of antichaotropic salt. In this work, we present three different approaches for predicting protein retention time in HIC.

The first approach correlates the protein retention time in HIC with protein "average surface hydrophobicity". This methodology starts from the protein three-dimensional structure data and considers the hydrophobic contribution of the exposed amino acid residues as a weighted average. This model can be applicable to stable proteins with a relatively homogeneous surface hydrophobicity distribution. The main disadvantage of this methodology is that it does not consider the effect of the distribution of the surface hydrophobicity on protein retention.

The second approach is based on the good correlation level between the average surface hydrophobicity of the interfacial zone (local hydrophobicity, LH) and protein retention time in HIC.

A conformational sampling procedure, in which different protein–ligand conformations are examined to find the correct one, called "molecular docking simulations", was carried out in order to identify the interaction zone of the protein with the hydrophobic ligand. Once the interaction zone was identified, the local hydrophobicity was determined, considering the amino acid residues belonging to that zone and their exposure level.

This methodology could be used to adequately represent the chromatographic behavior in HIC for proteins with a heterogeneous surface hydrophobicity distribution and without a large number of tedious experiments but only using computational simulation.

A third approach carries out a prediction of the average surface hydrophobicity of a protein using only its aminoacidic composition, without knowing its three-dimensional structure. This approach uses "generic" properties of aminoacids existing in databases such as molecular weight, bulkiness, average solvent accessibility, etc. The performance of this model was similar than that observed in the model based on the three dimensional structure of proteins proposed by the first approach.

Using these models it is possible to test different operating conditions for the purification of a target protein and select the best conditions.

This work is supported by Fondecyt project 1030668.

Affinity measurement of carbohydrate binding to recombinant *Aleuria aurantia* lectin using fluorescence spectroscopy

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Lectins are carbohydrate binding proteins that are involved in many recognition events at molecular and cellular levels, Aleuria Aurantia (AAL) is a fucose-binding lectin composed of two identical 312-amino acid subunits. Each subunit contains five binding sites for fucose. The lectin has been extensively used in purification and analysis of fucosylated glycans.

We have expressed a recombinant form of AAL in *E. coli*. The recombinant lectin was purified by his-tag affinity chromatography and fucose-binding activity of the recombinant lectin was confirmed using lectin-ELISA. Tryptophan fluorescence measurement with free fucose was used to estimate K_d values of three of the five possible fucose-binding sites. The strongest binding site showed a K_d value of < 1 μM. Furthermore differences in binding specificity towards defined fucosylated oligosaccharides was examined using the same assay. The direct fluorescence approach provides a convenient method to measure weak affinity interactions and is especially valuable in the study of protein-carbohydrate interactions.

Phosphatase Inhibitors – A Biacore Approach to Study Small Molecule Inhibitor Interactions with Protein Phosphatases

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Reversible protein phosphorylation of serine, threonine and tyrosine residues by protein kinases and phosphatases, is important for the regulation of cellular signal transduction and controls many cellular functions. Disturbances in this regulation have been implicated in a growing number of diseases, making kinases and phosphatases useful targets for therapeutic intervention.

The suitability of surface plasmon resonance (SPR) technology has been widely demonstrated in many drug discovery applications. Here, we present a novel and straightforward methodology for analyzing small molecule binding to the prototypic tyrosine phosphatase, PTP1B, and to two members of the PPP family of protein serine/threonine phosphatases, PP1 and PP2B (calcineurin). Emphasis was placed on investigating the immobilization conditions of the phosphatases by using reducing conditions, inhibitors and metal ions. Kinetic characterizations of inhibitor binding, either to phosphatases alone or in complex with regulatory protein subunits were performed. The methodology was also used to test inhibitor specificity toward different phosphatases. This information, together with detailed kinetic data on inhibitor binding, resolving affinities into kinetic rate constants, may be of great significance for the development of highly specific and high affinity phosphatase inhibitors.

Bioanalytical applications of a non contact acoustic sensor

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This work presents an electrodeless technique termed the Magnetic Acoustic Resonator Sensor (MARS), which uses a planar spiral coil to vibrate a single quartz crystal from 6 MHz to 1.1 GHz, and obtain multifrequency acoustic spectra of molecules. Under these conditions, the biophysical properties of the solid-liquid interface can be investigated in greater detail.

The MARS technique has demonstrated substantial potential for sensing purposes with outstanding results in the measurement of amplitude, frequency and Q factor spectra⁽¹⁻⁴⁾. This is achieved by focussing the acoustic wave onto the chemical recognition layer to provide information on interfacial molecular relaxation. Initial experiments have demonstrated that the multiple frequency measurements can be used to clearly distinguish between polyols and protein systems.

The development of this system promises applications in physics, biology, surface physical chemistry and biosensing in general, which includes acoustic 'fingerprints' for chemical recognition purposes, in vivo monitoring and the study of biomolecular interactions.

References

- 6) Stevenson, A. C., Araya-Kleinsteuber, B., Sethi, R. S., Mehta, H. M. & Lowe, C. R. Hypersonic evanescent waves generated with a planar spiral coil. *Analyst* 128, 1175-1180 (2003).
- 7) Stevenson, A. C., Araya-Kleinsteuber, B., Sethi, R. S., Mehta, H. M. & Lowe, C. R. The acoustic spectrophonometer: A novel bioanalytical technique based on multifrequency acoustic devices. *Analyst* 128, 1222-1227 (2003).
- 8) Stevenson, A. C., Araya-Kleinsteuber, B., Sethi, R. S., Mehta, H. M. & Lowe, C. R. Planar Coil Excitation of a Multifrequency Shear Wave Transducer *Biosensors and Bioelectronics* 20(7): 1298-1304 (2005).
- 9) Stevenson, A. C., Araya-Kleinsteuber, B., Sethi, R. S., Mehta, H. M. & Lowe, C. R. The application of the acoustic spectrophonometer to biomolecular spectrometry: a step towards acoustic fingerprinting. *Journal of Molecular Recognition* 17(3): 174-179 (2004).

Immobilized Metal Affinity Chromatography of Nucleic Acids

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Immobilized metal-chelate affinity chromatography has been widely used in the purification of proteins, and has also been applied to nucleotides. Transition metal ions chelated with metal coordinating ligands interact with aromatic nitrogens through d- π orbital overlap, in a manner analogous to the interaction with histidine. We have recently been investigating the application of metal-chelate affinity to RNA, DNA, and oligonucleotides, which are well adsorbed through interactions involving exposed purines. We have found that the difficult separation of plasmid DNA from chemically-similar genomic DNA contaminants can be efficiently achieved by a combination of IMAC with selective renaturation after partial denaturation, leading to exposure of bases in kinetically-trapped misfolded genomic DNA. We also report that the inclusion of moderate quantities of neutral solutes (ethanol, n-propanol, DMSO, etc.) to the binding buffer substantially enhances the binding affinity of nucleic acids for IMAC adsorbents. Synergy between salts, surface charge and alcohols can allow for very efficient elution, in some cases using pure water as the eluant.

A label-free continuous fluorescence-based immunosensor

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In an earlier study we have demonstrated that fluorescence spectroscopy can be used to investigate weak or transient binding between monoclonal antibodies and carbohydrate antigens. Here we have used Total Internal Reflection Fluorescence (TIRF) flow system for detection of fluorescence changes in antibodies (e.g. monoclonal antibody 39.5) upon binding at low affinity with maltose or panose. These antibodies were coupled on a quartz surface and showed an enhancement in the fluorescence intensity of tryptophan when binding to carbohydrate antigens. When using a structural analog to maltose, cellobiose with a $\beta^{(1-4)}$ bond instead of a $\alpha^{(1-4)}$, no fluorescence intensity change was induced. This direct fluorescence approach to measure the binding of an interacting antigen-antibody pair does not require any fluorophore labeling.

We believe that the introduction of fluorescence techniques will be a valuable complement to current surface based techniques to measure interaction of e.g. antibody with antigen and in particular they will offer solutions for continuous measurement of transiently binding antigens.

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A TiO₂-Binding Protein from *Rhodococcus* Strain GIN-1 (NCIMB 40340) – Mode of protein-oxide interaction.

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A *Rhodococcus* strain (Rh. GIN1), capable of adsorption to TiO₂ particles, has been previously isolated in our Lab. A specific TiO₂-binding protein (TiBP), located on the cell wall of this bacterium, was purified by affinity chromatography on TiO₂, hydrophobic chromatography and gel filtration. Similarly to the intact bacterium, this 52 kDa protein adheres strongly to TiO₂ at high salt concentrations. It binds to TiO₂ (rutile) faster, stronger and at a higher capacity than to the anatase isoform. Comparison of the characteristics of TiBP binding to the two crystalline phases of TiO₂, as well as its behavior in hydrophobic chromatography, suggested a specific adsorption mechanism to the oxide surface. Partial sequencing revealed homology of TiBP with dihydrolipoamide dehydrogenase, a 2×55 kDa homodimeric acidic enzyme, usually found intracellularly in pro- and eukaryotes, but occurs also as surface protein in a number of bacteria. We analyzed the binding of this protein to both anatase and rutile TiO₂. From these analyses we concluded that the main interaction occurs between the carboxylic groups of the proteins and the Ti⁺⁴_{5c} surface atoms. In addition, hydrophobic interactions and hydrogen bonds seem to occur. This adsorption mechanism suggests a unique specific binding, in contrast to other proteins (such as hemoglobin, cytochrome c and albumin), which are known to adsorb to TiO₂ non-specifically, mainly via less specific electrostatic interactions and hydrogen bonds.

Thermodynamic Dissection of Association and Dissociation in Interaction Between Lysozyme and Monoclonal Antibodies

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The thermodynamics of interaction between hen egg lysozyme (HEL) and variable regions of monoclonal anti-HEL (HyHEL-10 Fv) has been studied by ITC and SPR analysis. The goal was to compare equilibrium data provided by the techniques and further characterize association and dissociation using SPR. Wild type and two mutants (LY50F and HY33AY53A) of HyHEL-10 Fv were analyzed. Equilibrium thermodynamic parameters obtained by the two techniques showed very good agreement implying that (i) interaction is enthalpically driven, (ii) mutations decrease affinity (wt>LY50F>HY33AY53A) and make DG less negative by decreasing the negative change in enthalpy.

Kinetic data provided by SPR further demonstrated that the effect of the mutations on DG is entirely attributable to dissociation, while association remains unaffected. However, splitting the activation free energy ΔG^\ddagger into the enthalpic and entropic terms reveals that HY33AY53A has activation pattern different from both wild type and LY50F, possibly implying a different activation mechanism. This demonstrates the additional value of the kinetic data provided by SPR for understanding molecular recognition.

Determining the assay consistency for biosensor based direct binding assays

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The binding kinetic constants (k_a , k_d) and affinity (K_D) were measured for seven low molecular compounds interacting with immobilized carbonic anhydrase II. The assay was run as independent replicates using 3 different preparations of stock solutions, varying immobilization levels and using different sensor chip spots of a Biacore S51 surface plasmon resonance biosensor. The affinity varied between 15 nM (acetazoleamide) and >100 μ M (sulpiride). Generally, the relative standard deviation in kinetic constants and affinity varied between 5 % and 20 %. Larger variations were found for compounds with very rapid association rate constants ($k_a > 10^6 \text{ M}^{-1}\text{s}^{-1}$). The more visible the curvature was in the sensorgrams, the smaller was the spread in the determined association rate constants.

An acoustic sensor design based on a non-contact transducer format

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A unique sensing apparatus, comprising an electromagnetic field detector and an acoustic resonator has been used as a wireless system for remote acoustic sensing within test tubes and other common lab ware vessels. Different inductors were tested as potential generators of an electric flux in order to measure the acoustic response, and to compare it with the sensing characteristics of a known non-contact standard. Preliminary results based on chemical sensing of harmonics in the 6–100 MHz range are presented, and potential applications of the new technology explored. This approach will be of interest to life scientists and biochemists that require high performance assay methodologies that do not use chemical labels.

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Capacitive affinity biosensor as a tool to monitor low concentrations of biomolecules

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Capacitive biosensors have been developed based on the use of a gold surface which is covered by a self-assembled monolayer of alkylthiols. Some of alkylthiols have a terminal reactive group to which proteins might be immobilized. The capacitive biosensors were used to monitor ligand binding to the immobilized protein. When using receptor proteins, it was possible to register concentrations of reactants down to 10–15 moles per liter. The capacitive biosensor can also be used for continuous monitoring, thereby making it suitable for process monitoring and control.

Several examples will be presented.

Exploitation of the Janus-faced nature of β -cyclodextrins as affinity ligands in CE and CEC

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The β -cyclodextrins are cyclic oligosaccharides, built up of seven D-glucopyranosyl units connected by α -(1,4) glycosidic linkages, forming a conical ring-like spatial structure. The cavity of the molecule is hydrophobic whereas the hydroxyl groups (21 per molecule) at the rims of the ring represent a hydrophilic part of the molecule. It is well known that the γ -cyclodextrins are able to form inclusion complexes with organic molecules, possessing an aromatic group (*i.e.*, a hydrophobic moiety). The phenomenon is frequently utilized in separations of drug enantiomers (*e.g.*, β -blockers), where the complexes formed are stabilized by specific interactions between the hydroxyl groups on the rim of the β -cyclodextrin molecules and the hydrophilic moieties of the enantiomers. However, the ability of β -cyclodextrins to form inclusion complexes can be utilized not only as a chiral selector, but also as a universal separation ligand for both aromatic and alkyl compounds.

The mechanisms for the separation of drug enantiomers, peptide conjugates and aromatic organic homologues will be discussed. The β -cyclodextrin ligands were covalently attached to a homogeneous gel support, prepared *in situ* in a piece of fused silica tubing for both electrophoresis and electrochromatography in capillaries and a hybrid microdevice.

Affibody® Molecules: Affinity Proteins for Biotechnology Applications

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Affibody® molecules are novel, small and robust affinity ligands, which can be designed to bind with specificity and selectivity to any protein target. The Affibody® molecules are based on a 58 amino acids, alpha-helical domain of staphylococcal protein A (SPA). All Affibody® molecules have the same basic structure and the unique binding properties of each single molecule are acquired by genetic randomization of the binding surface of the Affibody® scaffold, using combinatorial protein engineering. Specific Affibody® molecules that bind to a desired target protein are isolated from libraries containing billions of different variants. Affibody® molecules with high specificity and high affinity (nanomolar) have been selected against target proteins of various origins and various sizes. The high flexibility and the ease of engineering of Affibody® molecules, enable construction of multimers for enhanced binding capacity, and introduction of sites for oriented immobilization or conjugation. Furthermore, Affibody molecules withstanding a broad range of physical conditions, including extreme pH and elevated temperature have been developed. Affibody® molecules are readily produced in gram amounts in *E. coli* and have also been chemically synthesized. Affibody® molecules are advantageously used as basic tools for protein expression profiling, protein localization and protein purification.

Polymeric IMAC-adsorbents and their use in the adsorption of Arsenic ions from aqueous solutions

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In this work we present adsorption studies of the use of new arsenic adsorbents based on binding to chelating polymers.

The method based on IMAC technology is unique among existing purification methods since it generates very small amounts of waste products in form of non-bulky concentrated arsenicals. The adsorbents can, in principle, be regenerated indefinitely and the small amounts of chemicals needed can be converted to common salt or other harmless chemicals. In this approach polyethyleneimine-grafted-agar gels are used to bind metal ions of strong arsenic affinities. These immobilized metal ions in turn function as adsorption sites for arsenates and arsenites. According to this concept, arsenic in the form of arsenate or arsenite can be removed from the water samples by adsorption on polymer-immobilized metal ions. Arsenite in order to be adsorbed is first oxidized to arsenate and subsequently removed. Arsenic is concentrated 10^4 – 10^6 times (or more) before being displaced from the solid phase. Collected effluent or supernatant is further concentrated. Arsenic can be subsequently eluted as concentrated soluble alkali arsenate and subsequently precipitated e.g., as insoluble copper arsenate and then further processed to a final stage for its safe disposal in compact form.

Enhanced Affinity Gel Permeation Chromatography of Proteins Using a Multiligand-Water Soluble Affinity Carrier

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A multi-PEG derivative modified with iminodiacetic acid (IDA) was prepared as an affinity macroligand PEG-(IDA Cu²⁺)₆₄, a 700,000 molecular weight multi-armed chelator. This derivative was used as an affinity macroligand to investigate its interaction with proteins and its behavior in gel permeation chromatography. The multi-arm PEG chelator loaded with metal ions (e.g., Cu) is effective in interacting in batch mode with protein mixtures consisting of histidine containing proteins such as bovine hemoglobin, horse heart cytochrome C and ovalbumin. Due to the larger number of histidine residues on the hemoglobin surface the metal affinity binding of the multiligand was more selective towards this biomolecule. After binding, the radius of giration of the molecule-complex is apparently increased due to the large molecular size of the multi-PEG molecule. Next, the mixture of proteins along with the complex hemoglobin-multiPEG ligand was introduced into a gel permeation chromatography column. Due to the "enhanced" molecular size of the bound hemoglobin its retention time in the column was effectively reduced leaving the column considerably faster than the rest of the unbound proteins, allowing with this an efficient protein fractionation. The preparation of such multiligand chelating-derivatives as well as binding, separation and elution studies will be presented.

Dynamic Binding Capacities of Phosphorylase b on Experimental Butyl-Modified Sepharose™ 4B, and Surface-Extended Sepharose 6 Critical Hydrophobicity HIC Chromatography Media

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The Critical Hydrophobicity (CH) approach is one method to enhance the selectivity of hydrophobic interaction chromatography (HIC) for proteins [1, 2]. In essence it involves (i) selection of specific CH media from a homologous library of gels differing in n alkyl surface grafting density, (ii) adsorbing the target protein to the CH-support at high salt concentration and (iii) eluting by a negative salt gradient. The chosen CH gel type thus reflects an application variable balance between media and target protein hydrophobicity.

Dynamic binding capacities have never been reported for media at the CH point. In the present study such measurements were made for butyl-modified experimental CH media based on two very different matrices - Sepharose™ 4B (non-crosslinked 4 % agarose) and Sepharose™ 6 Fast Flow (cross-linked 6 % agarose [3]). The latter media was surface modified with dextran (Dx) polymer chains prior to butyl-grafting. In the first case the alkyl residues are directly coupled to the paracrystalline rigid agarose backbone, in the second case largely to the flexible long dextran chains. Libraries of ten butyl-Sepharose 4B (Seph-C4) gels ranging in alkyl substitution from 1 to 40 $\mu\text{mol/ml}$ packed gel and six butyl-Sepharose 6 gels (SephDx-C4) ranging from 14 to 105 $\mu\text{mol/ml}$ packed gel. The critical hydrophobicity of Seph-C4 was 22 $\mu\text{mol/ml}$ packed gel and that of SephDx-C4 was 70 $\mu\text{mol/ml}$ packed gel. The dynamic binding capacity (10 % breakthrough, retention time 22 min) at 1.5 M NaCl for phosphorylase b was found to be 3.9 mg/ml packed gel for CH-Seph-C4 and 24.3 mg/ml packed gel for CH-SephDx-C4. To our surprise SephDx-C4 could not be readily saturated with protein, suggesting a novel mechanism of adsorption of phosphorylase b on the experimental SephDx-C4 media.

References

- [1] Jennissen, H. P. (2000) *International Journal of Bio-Chromatography*, **5**, 131-163
- [2] Jennissen, H. P. (2002) *Nature Encyclopedia of Life Sciences*, **9**, 353-361
- [3] Amersham Bioscience Product Info (2004) No. 18-1123-82 AC

A New IMAC Medium for Scaling up Purification of Histidine-tagged Proteins

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A new Immobilized Metal Ion Affinity Chromatography medium, Ni Sepharose™ 6 Fast Flow, has been developed and optimized for scaling up purification of Histidine-tagged proteins. The medium is compatible with a wide range of buffers and additives, including denaturants, detergents and reducing agents.

In this study, a number of Histidine-tagged proteins expressed in *E. coli* and *P. pastoris*, with different molecular weight, length of Histidine-tag (Histidine₆ or Histidine₁₀) and expression levels were purified using 1-, 5- and 20-ml pre-packed columns.

Purity and yield of the eluted target proteins were high. The large bead size of the Ni Sepharose Fast Flow medium makes it possible to scale up the purification with preserved high target protein concentration in eluted peaks. A purification on a 20-ml pre-packed HisPrep™ FF 16/10 column resulted in 0.5 g of pure protein (with a concentration of 2.7 mg/ml). Reproducibility in purity and recovery was shown after repeated purification runs without need of stripping, cleaning and/or recharging of the medium. Notably, the leakage of Ni²⁺ was very low during protein purification. This minimizes potential problems such as Ni²⁺-induced oligomerization, precipitation of target protein and loss of binding capacity.

Automated Multi-step Purification of Histidine-tagged proteins from Crude Cell Lysates

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Sample preparation, including centrifugation and filtration of cell lysates, is a time-consuming step in the protein purification workflow. Using the new HisTrap™ FF crude column, histidine-tagged proteins can be purified from sonicated, unclarified cell lysates. HisTrap FF crude is a ready-to-use column, intended for immobilized metal affinity chromatography (IMAC).

ÄKTExpress™, is designed for high-throughput purification of histidine- and GST-tagged proteins. The system enables automated, parallel purification with the possibility to run a number of different multistep protocols. A method wizard supplied with the control software, UNICORN™, makes it easy to create methods for different purification protocols.

In this study, histidine-tagged proteins were purified from crude cell lysates using ÄKTExpress. HisTrap FF crude was used in the first chromatographic step, followed by up to three additional steps such as desalting, ion exchange chromatography, and gel filtration. The results show that the use of HisTrap FF crude together with ÄKTExpress facilitates and enables significant time saving in the purification of histidine-tagged proteins without compromising purity.

Hydrophilic Rigid Activated Media by Encapsulation of Inorganic Particles by Dispersion Polymerization

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Undoubtedly, (immuno)affinity chromatography is one of the most powerful techniques to selectively isolate minor components from a complex mixture. The selectivity of these supports is mainly determined by its coupled ligand (e.g. antibody, enzyme, protein, etc.). However, due to undesirable nonselective adsorption, the advantages of this technique can not be fully utilized. In order to improve the nonselectivity of rigid inorganic particles, a diversity of protocols have been developed and evaluated. These protocols include the covalent coupling of a diversity of hydrophilic polymers onto silicagel particles by applying different methods of activation of the silicagel or hydrophilic polymers prior to coupling. Good results were obtained when silicagel was coated with a modified linear polysaccharide through dispersion polymerization. By adjusting the temperature and time during polymerization, the thickness of the hydrophilic coating could be varied and optimized. To examine its applicability for (immuno)affinity based separations, several ligands have successfully been coupled. The (immuno)affinity supports thus obtained demonstrated a good capacity and stability during daily practice. The nonselectivity of the (immuno)affinity supports were demonstrated by the extraction of real samples (serum, beverages, process waters, etc.).

Polymer-modulated permeation control for affinity adsorption chromatography. An Strategy to Enhance Peptide and Small Proteins Fractionations

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Despite the many efforts to develop efficient protein purification techniques, the isolation of peptides and small proteins on a larger than analytical scale remains a significant challenge. In this work we present an approach that has the potential to obtain peptide separations of higher efficiency by combining molecular size effects and specific chemical affinities in rationally applied adsorption procedures.

On the average, there are, fewer interaction sites per molecule in peptides than in proteins. If, therefore, ligand densities are increased for making peptide adsorption effective, protein adsorption is even more favored. This can be avoided by preventing proteins from entering gel phase regions where affinity ligands are located.

Here we present studies of polymer-modulated permeation control (PMPC) where specific ligands and an inert polymer are covalently bound to a solid-phase adsorbent. The inert polymer serves mainly as obstacle for relatively large proteins. Thus only molecules of a size below a certain defined limit are able to penetrate onto the surface of the gel matrix. If these permeable molecules have strong chemical affinities for the gel-bound ligands, they will be retained. Other permeable solutes with no affinity will pass the bed with a weak retention due to the molecular sieving effects.

Synthesis and Studies of Chelating Liposomes: Binding of Metal Ions and Proteins and Effect of PEG on Aggregation and Binding Interactions

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In this work we studied and characterized the properties of self-assembled ligand-modified liposomes (large unilamellar vesicles) upon binding to metal ions and proteins in solution. The surface of liposomes was modified by coupling a tridentate chelating agent, iminodiacetic acid (IDA), and a flexible polymer, polyethylene glycol (PEG), by different methods. The modified so-called chelating liposomes (liposome-IDA) were able to bind metal ions from solutions, specifically copper (Cu(II)). The liposome-IDA-Cu(II) complex was used to bind histidine-containing proteins such as myoglobin, lysozyme, and the mixture of these. PEG modified liposomes (PEGylated liposomes) were used to increase the stability of liposomes and to prevent aggregation that was experienced when chelating liposomes bound proteins or even metal ions.

Selective Adsorption of Monoclonal Antibodies against Mutant Amidase from *Pseudomonas aeruginosa* on Tailor-made Immobilized Metal Chelates

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The chromatographic behavior of monoclonal antibodies (Mabs) of IgM class against mutant (T103I) amidase from *Pseudomonas aeruginosa* was investigated on immobilized metal chelates. The effect of ligand concentration, the length of spacer arm and the nature of metal ion were investigated on immobilized metal affinity chromatography (IMAC). Mabs against mutant amidase adsorbed to Cu(II), Ni(II), Zn(II), Co(II) and Ca(II)-IDA agarose columns. The adsorption of Mabs onto immobilized metal chelates was pH dependent because an increase in the binding of Mabs was observed as the pH was varied from 6.0 to 8.0. The adsorption of Mabs to metal chelates was due to coordination of histidine residues to metal chelates which are available in the 3rd constant domain of heavy chain (CH3) of immunoglobulins since the presence of 5mM imidazole in the equilibration buffer abolished the adsorption of Mabs to the column. The combination of tailor-made stationary phases for IMAC and a correct design of the adsorption parameters permitted to devise a one-step purification procedure for Mabs of IgM class. Culture supernatants containing Mabs of IgM class against mutant amidase (T103I) were purified by IMAC Co(II) column at pH 8.0. The results presented in this work strongly suggests that one-step purification of Mabs of IgM class by IMAC is a cost-effective and process-compatible alternative to other purification schemes.

Affilin™- And Its Application In Affinity Chromatography

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The generation of novel binding molecules based on protein frameworks (so-called 'scaffolds') via combinatorial engineering is a concept which allows the generation of tailor-made binding proteins with affinities to any chosen target. Recently published strategies apply loops or helices for the creation of the binding site. In contrast, the Affilin™ technology utilizes the rigid β -sheet structures of stable human proteins to construct novel binding sites.

We have chosen the small and highly stable eye-lens protein, γ -crystallin, as well as the human ubiquitin as scaffold for the *de novo* generation of an artificial binding site on the surface of the antiparallel β -sheet. An Affilin™ molecule directed against the recombinant growth factor proNGF has been isolated from complex libraries by *in vitro* selection. This proNGF Affilin™ was characterized with regard to its affinity and selectivity to proNGF. Further, binding activity was tested after immobilization to dextran and sepharose matrices using the C-terminal cysteine or random NHS-coupling. The coupled Affilin™ does not loose affinity or specificity even after several regeneration cycles. We will show data that Affilin™ can be used to purify recombinant proteins from CHO cell supernatant or *E. coli* refolding solutions. The Affilin™ technology is suitable for chromatography, is robust and cost-effective.

Potential of homocysteine thiolactone scaffold towards libraries of media with new selectivities

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The fast expansion of the research in the proteomics area should generate an important increase of potential target proteins. In the field of chromatography, the answer to this challenge will be either to generate products with more general applicability or develop a battery of specific products.

The combinatorial approach used in pharmaceutical industry could be applied in the chromatographic area to synthesise diverse libraries of media. These can be designed based on a multimodal approach combining several kinds of interactions on the same media. The rapid synthesis of libraries of multi-modal media has been performed using the versatile three-functional scaffold, homocysteine thiolactone.

Screening the chromatographic characteristics of the collection of prototypes led to the discovery of new selectivities.

Applications of the library approach towards new IEx, HIC, and affinity media will be presented.

Affinity ligands for transferrin

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Affinity chromatography is increasingly important as a capture or polishing step in the purification or production of proteins. The use of rational computer-aided design of ligands coupled with limited combinatorial synthesis based on triazine chemistry has proven successful in affording stable and specific affinity ligands. This presentation reports attempts to design and synthesize ligands for capturing transferrin, both the apo-form and the holo-form.

Transferrin is a glycoprotein of some 80.000 kDa. One molecule of transferrin binds two molecules of ferric iron. An unusual feature is that the release of iron from holo-transferrin results in a structural change which opens up binding targets which are only accessible in the apo-form. Attempts have been made to exploit this feature in the design of specific affinity ligands for apo-transferrin.

The propagation of animal cells in culture is important in biotechnology, both on a small scale and also on a larger scale, e.g. in the production of proteins by recombinant cells and by hybridoma cells in the production of monoclonal antibodies. Transferrin is a necessary component of media for growing many mammalian and other cells. It is presently produced from bovine plasma. An affinity method for its purification could be of economic importance.

Detection of weak antibody fragments using phage display

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Using surface plasmon resonance (SPR) technology it has been shown that weak antibodies with K_d values in the mM- μ M range have the potential to be used for continuous measurement of weak antigen interactions. For the production, selection and identification of such transiently binding antibodies the phage display technique is attractive, since phage display libraries allow selection of antibodies for almost any target. In this study the interaction between the single chain fragments (scFv) of a weak antibody expressed on the phage surface and its antigen was studied using SPR biosensor technology.

The scFv of the weak murine monoclonal antibody, named 39.5, was cloned and expressed as fusion proteins to the coat protein III on the phage surface. The monoclonal antibody, originally produced against the oligosaccharide Glc α 1-6Glc α 1-4Glc α 1-4Glc (Glc) $_4$, recognises carbohydrate structures containing the Glc α 1-4Glc motif. Phages, expressing 39.5 scFv, were slowly injected over a sensor chip surface immobilised with BSA-(Glc) $_4$ molecules at 10°C. A difference in the interaction between the 39.5 and the control phages to (Glc) $_4$ -BSA was observed, with the 39.5 phages showing a higher association response and a slower dissociation. These results show that SPR biosensor technology can be used to detect interactions between weak scFv expressed on phages and their antigens.

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Adsorption studies of recombinant cutinase onto a combinatorial library of synthetic affinity ligands

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Protein stabilization by immobilization has been proposed to be most effective if the protein is attached to the carrier at that region where unfolding is initiated. A new approach for assessing and extrinsically enhancing protein stability, by integrating the concepts of molecular modelling and solid phase combinatorial chemistry, is addressed. The localization of structural regions connected with early unfolding events is the basis for the proposed strategy. A 64-member combinatorial library of ligands was synthesised on a triazine-substituted agarose matrix using a modified "mix and split" procedure. The triazine scaffold used as the spatial framework for the display of attached functional groups has been shown to deliver effective protein binding ligands. The combinatorial library was assessed for binding to recombinant cutinase from *Fusarium solani pisi* by standard affinity chromatography, and the percentage of protein bound and eluted calculated. A total of 13 ligands were selected for further studies by presenting more than 40 % cutinase bound and eluted.

Development and applications of a new Protein A media intended for industrial use

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The use of affinity media for large scale purification of proteins intended for therapeutic use puts special demands on the design of the media. To be economically viable the medium should ideally show long functional life time, high capacity, allow rigorous cleaning and sanitization in place, low ligand toxicity and easy removal of leached ligand.

This paper will describe the design and development of a new Protein A media with significantly improved alkaline stability. Studies on functional life time, purification performance, leakage removal, sanitization, CIP and comparability to regular rProtein A media will also be presented.

Affinity cryogel monoliths for cell chromatography and cell surface profiling

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Polyacrylamide cryogel monoliths are elastic drainage-protected matrices with large (10–100 μm) interconnected pores. Supermacroporous continuous structure of the cryogel adsorbents ensures free passage of cells through plain cryogels containing no ligands. Cryogel columns with immobilized affinity ligands can be efficiently used for the separation of cells using essentially the same traditional chromatographic set-up and applying the concept of affinity interactions.

Application of drainage-protected cryogel monoliths with various affinity ligands in 96-well format, i.e. when the monoliths are inserted into wells of a standard microplate with open-ended wells, is very suitable for screening for best ligands and best conditions for efficient chromatographic separation of cells from clinical and food samples and for analysis of cell surface properties. The possibility to carry out such screening and to separate different types of microbial cells on affinity cryogel columns was demonstrated by surface profiling and subsequent separation of recombinant *E. coli* cells displaying poly-His peptides, wild type *E. coli* and *Bacillus halodurans* cells using Me(II)-IDA- and Phenyl-cryogel monoliths in 96-well format and using Cu(II)-IDA cryogel monolithic columns.

An Optical Biosensor (SPR) Assay For Investigations of Enantioselective Affinities. A Comparison with A Validated HPLC Method

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Recently, the surface plasmon resonance (SPR) technology, in the form of an optical biosensor, has been suggested for screening affinities of small ligands for different proteins^[1]. Using this optical approach, the adsorption process is followed in real-time by measuring the refractive index at the sensor surface.

In this study we will demonstrate how this technology can be utilized also for quantification of enantioselective drug-protein interactions.

Another good method to obtain the same type of data is the HPLC perturbation peak (PP) method. The PP method involves quantification of the adsorption from the retention times of perturbation peaks (system peaks) at different mobile phase plateau levels. We recently validated a new injection strategy that allows for the determination of adsorption of the single enantiomers directly from racemic mixtures^[2].

The SPR approach to measure enantioselective affinities was compared in detail with the validated HPLC method. The results of both methods were in generally good agreement but we found important differences which will be outlined in this contribution.

1 D.G Myszka, *Anal. Biochem.*, (2004), 329, 316-323.

2 P. Forssén, J. Lindholm, T. Fornstedt, *Anal. Chem.*, (2004), 76, 4856-4865

Ion-exchange Macroporous Hydrophilic Gels with Grafted Polymer Brushes

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Monoliths or continuous bed chromatographic columns with large interconnected pores represent an attractive stationary phase for chromatography of proteins, plasmids, viruses and cells. One of the drawbacks of monolithic materials with large pores is a limited surface area of pore walls resulting in a limited amount of functional groups available on the pore wall surfaces. The grafting of functional polymers to the polymer surface is a versatile approach that enables the preparation of materials with both controlled extent of functional group incorporated and with tailored surface chemistries. Polymer chains anion- and cation-exchange polymers have been grafted onto supermacroporous hydrophilic polyacrylamide gels. The sorption of low-molecular-weight (metal ion, dye) and high-molecular-weight (protein) substances on the grafted monolithic column has been studied. The results indicate that a "tentacle"-type binding of protein to grafted polymer takes place after a certain degree of grafting has been reached.

Capture of Bacterial Endotoxins using a Supermacroporous Monolithic Matrix with Immobilized Polyethyleneimine, Lysozyme or Polymyxin B

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Bacterial endotoxins (BEs) are integrated part of *E. coli*, a microorganism widely used for the production of recombinant proteins. Selective removal of BEs from protein solution is welcomed. Supermacroporous monolith (continuous bed) columns, so called cryogel columns, with immobilized affinity ligands, polyethyleneimine (PEI), Polymyxin B (PMB) and lysozyme were employed for BEs capture. Due to the large interconnected pores it was possible to use cryogel columns at flow rates as high as 10 ml/min. The columns packed with Sepharose CL-4B with immobilized PEI, PMB and lysozyme were impossible to use at these high flow rates due to the collapse of the bed. The dynamic capacities of the cryogel columns were nearly independent of the flow rate. In the presence of EDTA, BEs were quantitatively captured from mixtures with a model protein, bovine serum albumin (BSA) at pH 7.2 with practically no protein losses. Decontamination of exhausted non-clarified *E. coli* cell lysate was successfully achieved with more than 104 BEs clearance at pH 3.6.

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Ion Exchange Media for Separation Large Biomolecules

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Media for chromatography and related separations must offer good selectivity (separation power) in combination with high binding capacity. Such media must offer rapid intra-pore mass transfer and a pore size distribution which balances effective mass transfer versus adsorptive area. Such a balance is particularly important in regard to ion exchange media used to separate large biomolecules (> 150 KDa) such as nucleic acid polymers and some proteins.

Recently ion exchange media has been developed which combines unusually rapid mass transfer and well balanced pore size distribution. The media was tested using proteins whose hydrodynamic radii were varied by grafting with neutral poly(ethylene glycol) polymers.

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CBioSep – Swedish Centre for BioSeparation

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Nine Swedish companies and Lund University co-operate with the support of VINNOVA in a centre dedicated to the separation of biomolecules. We are studying fundamental aspects of bioseparation as well as developing new and improved separation techniques.

The member companies are either producers or users of separation technology. Lund University is represented by four departments, mainly in the bio-area, and with important participation by engineering and theoretical chemistry. We are about 30 scientists working in the centre – PhD students and senior scientists. In addition, a number of company personnel participate in centre projects located partially at the company in question.

Glimpses from our present research projects will be presented:

1. Nanoparticle separation
2. Molecular imprinting
3. Molecular modelling
4. Analysis of contaminants and quality control
5. Separation of membrane proteins for proteomics
6. Stimuli-responsive polymers in biochemical analysis
7. Protein libraries for development of new chromatography materials
8. Production of Amelogenin
9. Process design and process operation in protein purification

Supermacroporous composite monolithic cryogels bearing immobilized metal affinity ligand

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Supermacroporous monolithic cryogels are produced through gelation at subzero temperatures when most of the solvent (water) is frozen while the dissolved substances are concentrated in small non-frozen liquid microphase. The gel formation occurs in this liquid microphase and the crystals of frozen solvents perform like porogen. After melting the ice crystals, a system of large interconnected pores with pore size of 1–100 μm is formed. Due to the large pore size in such cryogels, a crude cell homogenate can be processed directly without clarification.

Composite supermacroporous polyacrylamide- and agarose-based monoliths bearing immobilized metal affinity ligand iminodiacetic acid (IDA) were prepared through free-radical polymerization of low molecular weight precursors (acrylamide and N,N'-methylenebis(acrylamide) or gelation of high molecular weight precursor, agarose, respectively) in the presence of the filler, IDA-Sepharose-6B. Up to 30 % of the filler was possible to incorporate into the cryogel network without losing interconnected porous structure. Environmental scanning electron microscopy (ESEM) revealed uniform distribution of the filler in the cryogel network. The composite monolith columns had much lower back pressure in comparison to the column packed with the filler (IDA-Sepharose-6B).

Cryogels – supermacroporous gels for separation of particulate matter

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Supermolecular aggregates, subcellular particles and cells are representing challenges to bioseparation today. The lack of high resolving technologies to handle this type of matter has become obvious in recent years.

The supermacroporous cryogels offer an interesting opportunity to deal with these separation problems. The gels are produced by carrying out gel formation under freezing conditions. Thus, when making an polyacrylamide gel, the monomers and the catalyst system is mixed, the mixture is frozen and polymerization is allowed to happen between the ice crystals. Such gels are supermacroporous, the pore size can be controlled to some extent by the temperature regimes used and by using additives.

Cancer cells as well as subclasses of lymphocytes have been separated with good resolution using these chromatographic gels. Recently it was also demonstrated that inclusion bodies could be captured as well as quantified using an antibody based procedure.

When screening for e.g. proteins from fermentation broths, it is advantageous to apply the whole mixture on the gel, let the cells and cell debris pass through and capture the protein of interest. This is done via a multi-column microtiter plate chromatography. Monolithic cryogels are placed in the wholes of a microtiter plate with no bottom. The gels are retained in the holder since they are made approx. 0.1 mm wider than the whole where they shall fit. The gels furthermore retain liquid such that the gels are not running the risk of getting dry since the liquid is retained due to capillary forces.

Affinity purification of proteins using quartz crystal microbalance sensor chip

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Affinity purification of proteins using chip-based technology, capable of on-line detection of binding proteins and subsequent elution for identification, is a goal in many proteome applications, centring on finding receptors, transporters, ligands and interacting proteins, by attachment of selected polypeptides to a reporter chip. Here we introduce a biosensor instrument based on quartz crystal microbalance (QCM) technology for affinity purification of proteins. This biosensor instrument has novel advantages and we demonstrate one of these advantages. Proinsulin C-peptide was electro-immobilized to a QCM sensor chip, localizing this low-pI peptide for covalent attachment to activated surface carboxyl groups. The resulting chip was used in a continuous flow biosensor instrument to capture anti-C-peptide antibodies, which could subsequently be eluted in 5 % formic acid between air bubbles for efficient recovery and mass spectrometric identification. The method is reproducible through repeated cycles, providing affinity purification of proteins under real-time monitoring of the binding and elution processes.

***Escherichia coli* K88 Interaction with Serum Porcine IgA Oligosaccharides**

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Diarrhea from enterotoxigenic *Escherichia coli* expressing the K88 fimbrial adhesin causes high mortality and morbidity among newborn and weaned piglets. K88 fimbrial adhesins are surface filaments with lectin activity that allow bacteria to attach to specific glycoconjugates on the surface of intestinal cells. Serum preparations containing high amounts of immunoglobulins (Igs) improve porcine survival, presumably by interfering with adhesin interaction with gut tissues. Reliable commercial sources of porcine Igs are limited. We isolated Igs from serum and test their activity in limiting adhesin interactions in piglets. Serum porcine Igs were isolated in a single step using highly acetylated agarose (HA) hydrophobic interaction chromatography. Only Igs were adsorbed to HA gel in the presence of 0.5 M Na₂SO₄ and were recovered by removing the salt from the buffer. SDS-PAGE, quantitative radial immunodiffusion and immunodetection documented Igs homogeneity. HA capacity (3.9 mg/mL) is similar to that of commercial thiophilic gels, but recovery of IgA and IgM is greater for the HA gel. HA purified Igs prevented *E. coli* K88 interaction with piglet intestinal tissues. K88 bound with specific, high affinity to IgA oligosaccharides, but not to those IgG or IgM. IgA binding inhibited the adherence of K88 strains to porcine intestinal mucins.

Membrane Recognition by the Cholesterol-Dependent Cytolysin Anthrolysin O from *Bacillus Anthracis*

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Cholesterol-dependent cytolysins (CDCs) are a class of membrane-damaging proteins secreted by pathogenic and non-pathogenic gram-positive bacteria. Recently, a toxin of this class was identified in the genome of the etiological agent of anthrax, *Bacillus anthracis*. This toxin, designated anthrolysin O (ALO), was found to be the major secreted cytolysin of *B. anthracis*. In this work, we initiated a study of this potential anthrax virulence factor in an effort to understand the molecular and structural mechanisms of its action, in particular those governing the initial membrane-docking event. Recombinant anthrolysin O (rALO) (residues 35–512) and an N-terminally truncated version of ALO (residues 390–512) from *B. anthracis* were overproduced in *Escherichia coli* and purified to homogeneity. The role of cholesterol in the cytolytic activity of ALO was probed in cellular cholesterol depletion assays. Challenging cells with rALO₃₅₋₅₁₂, but not rALO₃₉₀₋₅₁₂, resulted in cell death by lysis. However, cytolysis could be abolished by depleting the cellular cholesterol using methyl- β -cyclodextrin. Furthermore, the interaction of rALO with model membranes comprised of POPC alone, or with a variety of structurally similar sterols, was probed using Biacore. Both rALO₃₅₋₅₁₂ and rALO₃₉₀₋₅₁₂ demonstrated binding to model membranes composed of POPC and cholesterol, but not at all to other sterols tested. This was also reflected in the inability of rALO₃₅₋₅₁₂ to lyse *Drosophila* cells, which have ergosterol as their major membrane sterol. The rALO₃₉₀₋₅₁₂-membrane interaction exhibited first order kinetics with an equilibrium dissociation constant (K_D) in the low nanomolar range, whereas rALO₃₅₋₅₁₂ exhibited complex kinetics probably due to the multiple events involved in pore formation. Moreover, examination of the differential effect of temperature on the dissociation phase of rALO₃₅₋₅₁₂ and rALO₃₉₀₋₅₁₂ suggested the occurrence of these additional events at the sensor surface and in the time-frame of the assays. The results obtained clarify the pivotal role of cholesterol in the action of rALO and also open the way to use surface plasmon resonance-based methods to characterize the mechanism of membrane disruption caused by this CDC. The biosensor method presented could be used to dissect the kinetics of the individual steps of pore formation by this class of proteins, and other membrane-binding proteins/peptides, and also provides a platform for the screening of inhibitors of such processes.

Metallizable, Site-Specific Coiled Coil Interfaces for Carbon Nanotube Arrays

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Our objective is to interface electrically active proteins, including glucose oxidase and photosynthetic reaction centers, with nanoelectrodes and nanotubes via self-assembling bio-inspired molecular linkers. The coiled-coil motif represents one of the simplest tertiary structures in proteins, containing alpha-helices that are supercoiled with strong affinity and structural integrity. Based on our previously conceived modular anchor-probe approach, we designed a dimeric system in which hydrophobic residues (V,L) form the major interface between the helical components. Charged border residues (E,R) help stabilize the structure, increase peptide solubility and provide specificity to the complex. Histidine residues oriented on the exposed hydrophilic exterior were included as chelating sites for metal ions such as cobalt. A terminal cysteine was incorporated for oriented, thiol-mediated reactions in order to couple to the biosensor chip surface, gold nanoparticles and carbon nanotube (CNT) substrate. Two peptides were produced by solid phase peptide synthesis using Fmoc chemistries, namely the acidic 42 residue peptide MSE4,

CGGWGGEVRALEEEVRALEEEVHALEHEVRALEEEVRALEEK,

and its dimer counterpart, the basic 39 residue peptide MSK4,

WRVRALRERVRALRERVHALRHRVRALRERVRALREGGC.

Purification of the peptides was carried out via high performance liquid chromatography with a C18 column, followed by MALDI TOF mass spectrometry analysis to ensure that the correct mass was achieved. Kinetic surface plasmon resonance biosensor analysis of MSK4-MSE4 assembly revealed a k_d of 0.12 nM in PBS buffer and a slightly lower affinity ($k_d = 0.26$ nM) in buffer containing 1 mM CoCl_2 . ESI-MS confirmed chelation of a single cobalt ion to both the MSK4 and MSE4 peptides in the metallized buffer. For the device-oriented CNT immobilization, tip-selective functionalization was achieved by oxidizing the substrate, adding PDEA/NHS/EDC and reacting with MSK4 in H_2O . MSE4 was reacted with 15 nm gold nanoparticles and added to the MSK4-CNT surface. SEM was used to visualize the site-specific attachment of the MSK4/MSE4-Au on the tips of the CNT array. Control surfaces indicated minimal non-specific adsorption. Sidewall immobilization mediated through a pyrene moiety (P130) is also being explored. We are currently developing cassette-based approaches to produce MSE4-protein fusions via a recombinant approach, and in parallel are developing a novel methodology for a direct electronic to photonic conversion platform by co-assembling photosynthetic reaction centers and coiled-coil fusions.

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Real-time protein interaction analysis in the development of an anti-IgE based immunotherapeutic treatment for allergy and asthma

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METHODS: Animals were administered with species-specific recombinant IgE-derived proteins using several immunization regimes, and serum samples were taken at multiple time-points for characterization of anti-IgE responses. Protein interaction assays included anti-IgE response patterns and qualitative kinetic analysis of antibody-antigen binding stability. Results were compared to a standard ELISA.

RESULTS: Anti-IgE responses were readily detected in both animal models. Several distinctive response patterns were seen in relation to time and dosing regimes, and in most animals, there was a clear correlation between increasing anti-IgE response and decreased circulating IgE. Response patterns correlated well with ELISA, but protein interaction assays were significantly more reproducible (intra- and inter-assay CVs 0.35 % and 3.7 % compared to 22 % and 38 % in ELISA) and better able to detect low/medium affinity antibodies. Qualitative kinetic analysis of primate samples revealed maturation of anti-IgE, manifested by increased stability of antibody-antigen binding over a specific time window of the immunization period.

CONCLUSIONS: The recombinant IgE-derived immunotherapeutic proteins show excellent immunogenic properties in animal tests, providing the basis for an effective new treatment for allergy and asthma. Protein interaction analysis provided significant advantages over a traditional ELISA in these studies (higher information content and better reproducibility) and offers a powerful approach for assessing immunotherapeutic proteins and optimizing treatment regimes.

Design and Use of Affibody Molecules for Tumor Targeting

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Affibody[®] molecules represent a novel class of high affinity, very small, single domain proteins. They are designed by randomization of 13 solvent-accessible surface residues of a 58-residue alpha-helical receptor protein. Their small size holds promise for good penetration properties for therapeutic and diagnostic *in vivo* delivery. Monomeric Affibody[®] molecules can be made by conventional peptide synthesis. Alternatively, highly avid multivalent molecules containing two or more domains may be produced at high levels in *E. coli*. Furthermore, they can be made as fusion protein with an albumin-binding moiety, extending the *in vivo* half-life to days. The properties and facile production make Affibody[®] molecules interesting alternatives to traditional monoclonal antibodies.

Here, we describe the design and use of an Affibody[®] molecule with picomolar affinity for HER2, a receptor protein over-expressed in breast and ovary cancer. Rapid and specific tumor delivery of labeled tracers in HER2-positive tumor xenografts was obtained within one hour post injection. Tumor to blood ratios up to 200 were obtained. Specificity of tumor targeting was proven by competition with unlabeled Affibody[®] molecules or modulation of HER2 expression. Due to the very low background, tumors could be visualized by SPECT imaging as early as one hour post injection.

Interaction of boronate-containing copolymers with saccharides, glycoproteins, carbohydrate particles and cells

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Water-soluble synthetic polymers carrying pendant phenylboronate functions are capable of reversible binding to carbohydrates, polyols and glycoproteins. We have synthesized and characterized a series of the boronate-containing copolymers (BCC) of acrylamide, N,N-dimethylacrylamide, N-isopropylacrylamide (NIPAM) and studied their interaction with the above compounds, agarose particles and cells. The binding of saccharides to BCC of NIPAM resulted in a shift of its phase transition temperature (ΔT_p), which provided a quantitative measure for the complex formation. The copolymer interaction with adenosine was simultaneously characterized by the ΔT_p and equilibrium dialysis, providing correlation between the ΔT_p and the copolymer saturation with the nucleoside, as well as the association constant. The strongest effects on the ΔT_p were produced by fructose, glucose and lactulose. Among the sugars typical of non-reducing ends of glycoproteins the ΔT_p decreased in the order: N-acetylneuraminic acid > xylose \approx galactose > mannose \approx fucose \gg N-acetylglucosamin. The effect of glycoproteins on the ΔT_p decreased in the order: mucin from porcine stomach (80 % carbohydrates) > horseradish peroxidase (21 %) > human γ -globulin (2 %). All the above BCC were readily adsorbed by agarose gels (up to ca. 20 mg/ml gel) due to boronate-carbohydrate interactions and could be eluted with 10 mM fructose. The adsorption capacity increased with pH in the range from 7.3 to 10. The multiple boronate-carbohydrate interactions allowed specific adhesion of agarose particles and yeast cells on the plane glass surfaces grafted with BCC. The adhered particles and cells could be detached by 20 mM fructose or glucose.

On the Double Nature of Chromatographic Peaks and Fronts: The Result of the Propagation of a Mass and/or a Concentration Wave

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Imagine a LC system equilibrated with an eluent containing a solute, until a concentration plateau has been established. If a small excess of the solute is injected into this equilibrated column, one single peak will appear on the chromatogram. This peak consists of displaced plateau molecules while the injected molecules elutes later and together with a deficiency of plateau molecules and are therefore not visualized by the detector. By selectively label the injected molecules (tracer-molecules) we can provide a deeper insight in the following typical cases:

- Small injections are made on an equilibrated single-component system. It was found that a total of three zones are migrating in the column [1].
- Large injections on the system above. The zones are now deformed although the sum is always a normal overloaded peak.
- Frontal analysis on the system above. Also in this case there are three zones.
- The theory and methodology was extended to the multi-component case: We show how multi-component isotherm surfaces easily can be calculated from the retention times of the injected solutes.

1) J. Samuelsson, P. Forssén, M. Stefansson and T. Fornstedt, *Anal. Chem.* 2004, 76, 953

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Fast discovery of small molecule affinity ligands

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The presentation describes a new invention for fast development of affinity ligands, where up to 20.000 ligands can be screened on-bead and identified in a few hours.

Combinatorial synthesis by the split and mix procedure is a powerful technique for generating vast numbers of diverse chemical compounds on polymer beads with relatively little effort. Traditionally, the technique is hampered by the laborious spectroscopic and chemical analysis, needed to determine the exact structures of the ligand on selected beads. In this way, 6–12 month analysis time could easily be spent just to analyze a tiny fraction of the library.

In the Versaffin™ Technology each bead is encoded, individually tracked, and identified during the synthesis and screening. This decreases the whole ligand development time from months to weeks and increases the amount of information significantly. The bead code further enables evaluation of the ligand-protein binding under varying binding and elution conditions.

The instrument for reading the encoded beads and for quantifying the amount of bound protein is presented. The encoded beads we use are based on functional cross-linked polyethyleneglycol (PEG), which is compatible with water as well as most organic solvents. Thus, the combinatorial synthesis can be carried out in organic solvents and the resulting compounds can be evaluated, still bound to the parent beads, under aqueous conditions. A further advantage of using PEG based beads for on-bead screening is the fact that PEG is biologically inert and therefore does not interfere in a bio-assay.

CD microlaboratories – a fully automated solution for fast and flexible IgG quantification in biopharmaceutical process development

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Efficient process development and optimization, and quality control are crucial for successful production of protein-based drugs, such as recombinant antibodies. Protein quantification is performed during several stages of biopharmaceutical process development and the establishment of quality control procedures. Current technologies deliver good quality results, however, assay times are slow and considerable amount of 'hands-on' time is required.

To overcome these challenges we have developed a miniaturized and integrated assay format for protein quantification within a CD microlaboratory. The CD contains microstructures where multiple samples are analyzed in parallel. Each microstructure contains a pre-packed column of streptavidin-coated particles. Analyte specificity of each column in the CD is determined by addition of biotinylated capture molecules. Samples are passed through the columns followed by complementary, fluorescently-labelled detection molecules. The amount of specifically-bound protein is measured by scanning each column using a laser-induced fluorescence detector integrated into the workstation processing the CD microlaboratories.

Case studies for quantification of human IgG in CD microlaboratories with comparative data from ELISA and HPLC will be presented.

Selective Separation of Cells: supermacroporous monolithic cryogel provide a new tool

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In the areas of biomedical research and diagnostic medicine, it is vital to have specific separation of a discrete population of cells from a mixture. The isolation of specific cell sub-populations is a key factor to the advancement of cell-based therapies of cancer, auto-immune diseases and genetic disorders. Magnetic separation and flow cytometry represent the most powerful tools for cell separations but are limited to analytical applications. Due to low cost and simple operation, cell affinity chromatography can be the preferred approach when the intended application is preparative scale separation. However, for this application the choice of a suitable matrix material is important because, as separation objects, cells are relatively large, rather fragile and sensitive to shear stress. Their diffusivity is negligible and only convective transport can be used. Thus, for cell affinity chromatography the key element is the design of the matrix.

A new type of continuous, supermacroporous, monolithic, cryogel affinity adsorbent, has been developed, which allows selective separation of mammalian cells in a chromatographic format. The affinity adsorbent was used to design a novel cell separation strategy, which was based on the interaction of protein A with cells bearing IgG antibodies on the surface. Protein A was covalently coupled to epoxy activated dimethylacrylamide (DMAA) cryogel matrix. After treating cells with monoclonal antibodies against the surface receptors of specific cells, the labelled cells were selectively bound on the protein A-cryogel column whereas unlabelled cells passed through the column. Human blood lymphocytes were specifically fractionated when the cells were treated with goat anti-human IgG. The IgG-positive B-lymphocytes were efficiently separated from T-lymphocytes when the protein A cryogel column specifically bound IgG-bearing B-lymphocytes, while non-bound T-lymphocytes passed through the column. More than 90% of the B-lymphocytes were retained in the column while the cells in the breakthrough fraction were enriched in T-lymphocytes (81%). Similarly, the capture of human acute myeloid leukemia KG-1 cells expressing the CD34⁺ surface antigen was evaluated when anti-CD34⁺ treated cells were retained on the protein A-cryogel column. The bound cells were released by human or dog IgG without impairing the cell viability. The results show differences.

A novel affinity based controlled release system involving derivatives of dextran with enhanced osmotic activity for potential application in surgery

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Dextran is a highly bio-compatible molecule with osmotic activity. We synthesized histidine derivatives of dextran, DexH (dextran histidine) to test the feasibility of an IMAC based controlled release system. DexH was synthesized by the periodate oxidation method. Effect of periodate oxidation and histidine conjugation on osmotic activity was tested. The oxidized intermediate itself exhibited higher osmotic activity than native dextran. Conjugation with histidine further increased the osmotic activity and the resulting DexH exhibited nine times more osmotic activity than native dextran.. A positive correlation was observed between the extent of coupling of histidine and osmotic activity. Association of DexH was tested on Cu-IDA- Novarose® and Zn-IDA-Novarose®. DexH bound to both these matrices and only partial elution was achieved with step wise lowering of pH and complete elution was possible only with EDTA. As expected, it was found that, DexH in its bound state (DexH-Cu-IDA-Novarose and DexH-Zn-IDA-Novarose) exhibited lesser osmotic activity than the eluted soluble form. The IDA-Cu and IDA-Zn based solid supports bound DexH in a species dependent manner, as IDA-Zn matrix selectively bound DexH with clustered histidine. Further, this DexH with clustered histidine shows higher osmotic activity. A controlled release system is proposed based on this difference in the osmotic activity between the bound and eluted forms of DexH with EDTA as the external trigger to induce this transition in osmotic activity.

This increased osmotic activity due to controlled release translated into mechanical force by which the membrane chamber containing the system swells. This is potentially useful in vascular surgical procedures.

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MAAS enables cost-effective affinity adsorption

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Membrane Assisted Adsorptive Separation (MAAS) is a separation technology in which the specificity of adsorption and the high capacity of microfiltration are combined in one unit operation.

In the current project the usefulness of MAAS for the recovery of valuable components from complex feed-stocks is studied on the basis of an existing industrial process from the food industry: the recovery of lactoperoxidase (LPO) from acid whey.

It was shown that the binding of LPO to a cation exchange adsorbent is highly selective.

Adsorbent beads were cycled through a dedicated pumping system showing that agarose beads are more stable than polystyrene beads.

Based on experiments and some safe assumptions the cost price for the production of LPO in a MAAS system was estimated to be €5–€10/kg (largely depending on the adsorbent price). This implies that MAAS is an economic process for relatively low to high added value ingredients (€20–200/kg, ROI after 14–258 days).

Finally, it was shown that MAAS is a much more efficient recovery system than packed bed chromatography in terms of adsorbent inventory (10–20 times less) and required floor space (7–10 times less). This is because the adsorbent is used much more efficiently in a MAAS system (10 times/hr) than in a packed bed system (1 time/2–12 hr).

Conformational Entrapment of gp120: High Affinity Dual Antagonists through Click Chemistry

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A promising lead fusion inhibitor, the 12-residue peptide, 12p1 [RINNPWSEAMM] was discovered initially by phage library screening. This peptide inhibits the interaction of gp120 with both CD4 and 17b, an antibody that recognizes an epitope overlapping the CCR5 binding site, with micromolar affinity. In this work, we developed a novel methodology for conjugation at gamma-position of proline 6 of 12p1 through click chemistry. The modification of proline 6 with 4-phenyl, 1, 4 disubstituted 1,2,3 triazole, fabricated through [3+2] cycloaddition reaction, leads to a peptide [HNG-105] that binds to gp120 with a K_D of about 12.7 nM and inhibits the binding of gp120 to CD4 as well as CCR5 epitope ligands with IC_{50} values of 22 and 29 nanomolar, respectively. HNG-105 also inhibits the binding of these ligands to trimeric envelope glycoproteins, blocks the binding of gp120 to the native co-receptor CCR5, and inhibits HIV-1 infection of target cells *in vitro*. CD4 saturation analysis of monomeric gp120 experiments on Biacore at a fixed concentration of HNG-105 suggests that suppression of CD4 binding to gp120 is due to allosteric inhibition rather than competitive inhibition of CD4. These results confirmed that HNG-105 preferentially binds to the gp120 prior to the engagement of CD4. Binding of HNG-105 to gp120 showed complete inhibition of interaction with ligands (CD4 and CCR5) that are crucial for viral entry. Because gp120 in its unliganded form is relatively flexible, it is likely that it exists in a range of conformations between its unliganded and activated states. A current working hypothesis is that HNG-105 can stabilize a nonproductive conformation of gp120 and prevent the transition to an active conformation. Direct binding and competition studies of different clades of gp120 with the HNG-105 and its analogue peptides are currently under investigation.

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Cyanovirin-N-12p1, a Novel Chimeric Protein Inhibitor of HIV-1 Envelope Interactions

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The first, critical stage of HIV-1 infection is the fusion of the viral and host cellular membranes mediated by a viral envelope glycoprotein termed gp120. This process is currently being targeted for novel inhibitors of HIV infection. These include directly targeting the CD4 or co-receptors binding site through protein mimetics or by conformationally locking gp120 in a non-productive state thereby preventing it from binding either CD4 or the co-receptor. An example of the latter is 12p1, a linear peptide which allosterically blocks gp120 binding to CD4 and co-receptor. Another unique inhibitor is cyanovirin-N (CV-N), a small (11 kD) protein that has been demonstrated to bind to sugar moieties of gp120 with nanomolar affinity. We have created a series of chimerae with 12p1 linked to the C-terminal domain of cyanovirin through a linker of one through five penta-peptide repeats. The proteins were expressed in the BL21 (DE3) strain of *E.coli*, isolated by osmotic shock and purified over a nickel-NTA column. The ability of a chimera to bind to gp120 was analyzed by both SPR (Biacore) and ELISA assays. The CVN-12p1 chimera with five repeats of the linker (L5) binds to gp120 from a variety of clades and tropisms with identical nanomolar affinity as the unlinked CVN. The ability of L5 to compete with the sCD4 or 17b interaction was analyzed using a Biacore biosensor. Either sCD4 or 17b was immobilized onto a CM-dextran chip and YU2-gp120 mixed with increasing concentrations of the chimera was passed over it. The chimera maintains its ability to inhibit sCD4 in a manner similar to CVN alone arguing for retention of function. The L5 chimera inhibits the YU2 -17b interaction more potently than CVN alone, indicating an affect of both the 12p1 and CVN domains of the protein. These results demonstrate that cyanovirin-N can tolerate a long attachment to its C-terminal domain with no interference to its ability to bind to gp120. Currently, work is focusing on generating chimerae of CVN with higher potency partners containing both natural and non-natural amino acids. We will explore these in order to correlate linker length with site topology for envelope and envelope variants and hope to illuminate cooperative effect of the chimera on envelope binding and conformation. We will further explore the chimerae as possible tools for envelope structure determination in alternative conformational states.

Use of Single Domain Heavy Chain Antibody Fragments as Ligands in Affinity Chromatography

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The burgeoning fields of healthcare and recombinant biotherapeutics require new purification methods to address the technical and economic limitations of protein A, dye mimetics, and immunochromatographic methods. We have developed a technology for the generation of ligands, using the variable domains of heavy chain-only antibodies from the species *camelidae*. These single domain antibody fragments possess beneficial features over existing methods comprising "tuneable" specificity, high affinity, base stability, short development times, flexibility in choice of matrix and the ease of non-animal derived production at any scale in *Saccharomyces cerevisiae*. The specific ligands are generated from antibody fragment libraries. Specific requirements for a ligand in a particular application are incorporated in the library screening procedure. These requirements can then be used in chromatography processes, for instance: pH of elution, contamination in the source material or stability of the ligand/target at a certain pH value. We describe the development of specific ligands for several application areas including the purification of human IgG, Fab fragments and recombinant biotherapeutics. The fields of application can range from industrial process chromatography and healthcare applications such as plasmapheresis, to novel R&D and diagnostic tools.

The design and Synthesis of Carbohydrate Receptors

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The design and synthesis of a series of phenyl boronic acid derivatives containing a polymerisable acrylamido moiety is reported. The coupling of the acrylyl component was achieved in a basic aqueous solvent system giving good to moderate yields of the purified product. The latter were co-polymerised with acrylamide and *N,N'*-methylene-bis-acrylamide to cast hydrogels, within which holograms could be recorded. The hologram acts as a Bragg grating and reflects a narrow band of wavelengths when illuminated with white light. The binding of an analyte, in this case glucose, to its complimentary ligand within the polymer causes a change in the swelling state of the hydrogel, resulting in a change in the reflected wavelengths or colour of the hologram. This change is correlated to glucose concentration and thus forms the basis for a holographic glucose sensor.

Receptor Epitope Usage by Interleukin 5 and its Antagonist Peptide

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The cyclic peptide AF17121 is a library-derived antagonist for human interleukin-5 receptor α (IL5Ra), and inhibits interleukin-5 (IL5) activity. Our previous results have demonstrated that the sixth arginine residue of the peptide is crucial for the inhibitory effect, and several acidic residues in the N- and C- terminal regions also make a contribution though to a lesser extent. However, the recognition mechanism of the receptor has remained unresolved. In the present study, AF17121 was fused to thioredoxin by DNA recombinant techniques and examined for IL5Ra interaction by using a surface plasmon resonance biosensor method. Kinetic analysis reveals that the dissociation rate of the peptide-receptor complex is comparable to that of the cytokine-receptor complex. The fusion peptide competed with IL5 for both biological function and interaction with IL5Ra, indicating that the binding sites on the receptor are shared by AF17121 and IL5. In order to define the epitope residues for AF17121, we investigated its binding footprint on IL5Ra using alanine-substitution of Asp55, Asp56, Glu58, Lys186, Arg188 and Arg297 of the receptor. Marked effects on the interaction were observed in all three fibronectin-type III domains of IL5Ra, in particular residues Asp55 in D1, Arg188 in D2 and Arg297 in D3 domains. This footprint represents a significant subset of that for IL5 binding. The fact that AF17121 mimics the receptor-binding capability of IL5 but antagonizes biological function evokes several models for how IL5 induces activation of the multi-subunit receptor system.

Immobilisation of Ribonuclease I via Epoxy Groups or CDI Groups of CIM Methacrylate Monoliths

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Regularly, the RNA impurities in DNA samples are removed using soluble enzyme ribonuclease A (RNase). In order to avoid the addition of RNase into the analyzing sample, the use of immobilized RNase on solid support is recommended. Very few supports enable efficient interaction between immobilized enzyme and large macromolecules such as DNA having size above 200 nm. Glycidyl methacrylate-co-ethylene dimethacrylate monoliths with inherently present epoxy groups or carboxydiimidazole groups, shortly named CIM epoxy discs, structured as a network of channels around 6000 nm in diameter were used as a support for RNase immobilization. The apparent K_m and turnover number k_3 for immobilized RNase determined by on-line frontal analysis method were respectively 0.52 mM cytidine-2',3'-cyclic monophosphate and 55 s⁻¹ of immobilized RNase. The former is seven times lower than that of soluble enzyme, but the latter is six times higher to that observed for the soluble enzyme. This is an order of magnitude better in comprising to other enzymes immobilized on CIM monoliths. The CIM RNase column was used in real samples for removal of RNA contaminants in DNA samples.

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