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Introduction

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Introduction

This 15th conference in the Bipartitioning and Biopurification series was held at Brunel University 14–19 June, 2009. It brought together a range of scientists from industry, research institutes, and universities united by an interest in addressing fundamentals and practical aspects of bioseparation methods. Over the 5 conference days there were 12 keynote presentations supported by 33 orals and 47 posters. These covered molecular aspects of protein-media interactions, protein purifications, affinity separations of biomolecules, bioindustrial, medical and analytical applications and process validation, bioparticle and complex biostructure purification, new and unconventional partitioning material, emerging technology, and future challenges. The final programme can be viewed at http://www.bpp2009.com/programme.php. This shows the range of separation methods covered, demonstrating that the conference series continued to broaden from separations with Aqueous Two Phase System (ATPS), which had been the basis of the conference series until the Vancouver meeting in 2003. Separations covered a range from small molecules, proteins, viruses, and cells and addressed both fundamental, theoretical issues, and practical, commercial issues.

As is the nature of conferences, some work was in initial stages, not ready yet for publication but stimulating to hear, while other work was in the process of publication. In this special issue we present 11 papers that bring together some of the new work presented with succinct reviews of aspects of the field. The papers fall roughly into two categories. The first: molecular-level experimental and/or theoretical investigations of the basis for the performance of a specific separation process; the long-range goal here is to be able to invert this information to enable the design of optimal processes and separations media for a given target species a priori. The second: the development of novel processes or separations media to address specific or platform bioseparation needs.

The first contribution is from Steven Cramer's group and describes an analysis of the binding behavior of several well-characterized charge variants of cytochrome C to a cation exchange resin. Variations in retention were consistent with the locations of charged residue modifications as assessed by computed electrostatic potential maps; modifications in either of the two binding sites identified for the wild type protein had much greater impact than modifications outside these sites. The Holy Grail here is the use of minimal target protein structural data to predict retention behavior for given chromatographic media.

Maria Elena Lienqueo and co-workers next relate their work on the experimental validation of predicted chromatographic fusion tag performance for protein purification. Wild type cutinase along with two hydrophobically-tagged mutants were studied and good agreement with model yield and purity predictions was found. This holds open the eventuality that an optimal purification tag for a specific target protein might be selected from amongst a library of available tags wth minimal experimentation.

The third contribution from Barbara Andrews and Juan Asenjo reviews their experimental and theoretical work on correlating the partitioning behavior of proteins in polymer/polymer and polymer/salt aqueous two-phase systems with measures or calculations of their surface hydrophobicity. They have found that the average surface hydrophobicity computed from 3D structure data and the inverse of the ammonium sulphate concentration, "$1/m^*$", at which the protein solubility equals a reference concentration, in this case 2 g/L, were satisfyingly predictive of relative target partitioning behavior. While 3D structure data may not be available for a particular target protein, the m^* solubility measurement is facile and can provide predictive ability for aqueous two-phase partitioning behavior for seemingly any target.

Raquel Aires-Barro's group describes the structural response of human IgG to extraction in thermoresponsive aqueous two-phase systems in the fourth paper. While the forward extraction step into a UCON/dextran system was found to be innocuous, the back extraction step via thermo-precipitation of the UCON proved to be potentially perturbing; higher temperatures and lower pHs providing the biggest perturbations. This work points out the importance of understanding the physical state of the target species through the separations process: this approach can be used to define windows of operation where separation performance is good and target structural perturbation is minimal.

Next, the identification of a particularly promising polymer/salt aqueous two-phase system for the purfication of a plant lectin is presented in a paper by the Aires-Barros and Cavada groups. Purification performance for a poly(ethylene glycol)/citrate system was evaluated experimentally as a function of three logical parameters: PEG molecular weight, ionic strength, and pH. Conditions were identified that resulted in both high yield (70%) and high selectivity (98%), potentiating a single-step purification.

The sixth contribution from Pina and co-workers compared a fluorimetric screening technique for selecting affinity ligands from solid-phase combinatorial libraries in both microscope-based and multi-well plate-based formats.

While the generation of ligand libraries in a variety of formats has become increasingly facile, the rapid and accurate screening of affinity ligands for a given target species remains challenging.

Dürauer and co-workers next describe their development of autocleaving fusion protein tags based on N^{pro}, an autoprotease from classical swine fever virus. These tags lead to the formation of inclusion bodies in *E. coli* expression systems which can be recovered, washed, tag-cleaved, and separated efficiently. Protein engineering of the tag itself was performed to improve solubility behavior and tag cleavage kinetics. Autocleaving tags such as these might be the basis of terrific platform purification processes.

The Rito-Palomares and Glatz groups have collaborated to use aqueous two-phase partitioning in conjunction with 2D gel electrophoresis to characterize the physical property distributions of host cell proteins. An initial partitioning of host proteins, soy proteins in this case, in a polymer/salt system gives a high/low hydrophobicity cut and subsequent analysis of each phase by 2D gel electrophoresis provides the isoelectric points and sizes of the proteins in each phase. While there are some practical hurdles to be overcome, this straightforward characterization protocol may be able to predict which host proteins are most likely to contaminate a given target protein, based on physical property similarities, or may be used to screen different potential hosts for the relative absence of potential contaminants.

The ninth contribution is from Paula Jauregi's group and describes the recovery of bioactive peptides—peptides that can inhibit angiotensin converting enzyme in this case—from whey by combining enzymatic hydrolysis with adsorptive separation. By adsorbing whey proteins from whole whey, primarily β-lactoglobulin and caseinomacropeptides, on an ion exchange resin contained in a membrane retention system, washing and then treating the adsorbed species *in situ* with a protease, they were able to recover bioactive peptides in a single process unit at good yield and purity.

The penultimate contribution from Vennapusa and co-workers examines adhesion forces between cells and expanded bed adsorption (EBA) media in order to get a handle on biomass fouling phenomena. They evaluated the total free interfacial energies of interaction between cells and several EBA matrices using extended DLVO theory with parameter validation via atomic force microscopy. Good correspondence was noted between the tendency to fool and predicted adhesion forces. This information may aid the development of new, lower-fouling EBA media, thereby enhancing the industrial appeal of EBA processes.

The final contribution is David Wood's review of his group's work with self-cleaving intein tags for non-chromatographic protein purification processes. This work moves away from affinity chromatography-based tags to specific, precipitating tags, eliminating the need for costly affinity chromatography media. He describes both phasin-based tags which target intracellular poly(hydroxy butyrate) particles or elastin-like peptide tags which form thermo-reversible precipitates. His results with these systems are discussed in the context of the currently available auto-cleaving tag purification systems. Here, again, the potential of these systems to provide platform purification processes is exciting.

The conference series continues with BPP2011, the 16th in the series, to be held in Puerto Vallarta, Mexico, September 18–22, 2011 under the chairmanship of Marco Rito-Palomares (Tec de Monterey, Mexico), Juan Asenjo (Universidad de Chile), and Todd Przybycien (Carnegie Mellon University, USA) (www.bpp2011.org). BPP2011 will highlight the recent scientific advances in scaleable operations and methodologies to capture, isolate, and purify biologicals. Topics proposed for this conference demonstrate how the series continues to address important aspects of bioseparations: Molecular Aspects of Protein-Media Interactions; Thermodynamics of Phase Formation and Biomolecule Partitioning; Multi-mode Chromatographic Processes; High Titre Processes; Affinity-Enhanced Biomolecule Purification; Purification Unit Operation Modelling and Optimization; Rational/High-throughput Process Design; Integration of Purification Unit Operations; Chromatographic versus non-Chromatographic Processes; High Resolution Protein Purification: Aggregate, Misfolded, and Clipped Form Removal; Particle/Virus-Like Particle Purification; DNA/RNA/PNA Purification; Structure-Processing Relationships for Biomolecules; Bioindustrial, Medical and Analytical Applications; and Emerging Technologies.

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