CHEMICAL, BIOLOGICAL & MATERIALS ENGINEERING

100 E. Boyd, Sarkeys Energy Center, T-301
405-325-5811
The University of Oklahoma
Norman, Oklahoma

PHILLIPS 66 SEMINAR SERIES, 2017 – 2018

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DR. TODD M. PRZYBYCIEN

PROFESSOR OF BIOMEDICAL ENGINEERING AND CHEMICAL ENGINEERING
CARNEGIE MELLON UNIVERSITY
PITTSBURGH, PENNSYLVANIA

Our seminar

"USING DRUG DELIVERY TECHNOLOGY TO IMPROVE BIOSEPARATION PERFORMANCE IN AFFINITY CHROMATOGRAPHY"

Protein A (ProA) affinity chromatography is a highly selective – and expensive – capture and separation technique in use throughout the biopharmaceutical industry for the purification of monoclonal antibodies (mAbs) – a key class of biopharmaceuticals with global sales of over 90B USD in 2016. While ProA typically removes ≥98% of mAb process impurities, the remaining small, but significant amounts of host cell proteins (HCPs), DNA, product aggregates and fragments and other impurities necessitate multiple subsequent polishing purification steps downstream. In this work, we aim to decrease the post-ProA affinity chromatography separations burden on mAb downstream processes by discouraging impurity interactions with the ProA resin. To accomplish this, we've taken a page from the protein drug delivery playbook and employed "PEGylation", a polymer-based protein engineering technique where poly(ethylene glycol) chains are covalently attached to a protein surface, in this case, the immobilized ProA of the affinity chromatography media. The governing hypothesis is that the attached PEG chains will impede the non-specific binding of contaminant species to the ligand and to bound mAbs via an excluded volume effect. An added benefit should be a shielding of the ProA ligand from proteolytic attack and fouling. This steric exclusion/shielding effect is the same as that used to dramatically extend the half-lives of protein drugs in the bloodstream.

Following extensive characterization of the mass transport implications of ProA media PEGylation, we explored the specificity and robustness of the PEGylated media we generated relative to unmodified, commercial ProA media. In one study, we studied the interactions of a mAb and a model protein contaminant on unmodified and PEGylated ProA resins using confocal laser scanning microscopy (CLSM). Experiments were conducted with both sequential and simultaneous loading of mAb and contaminant on resins modified with both 5 and 20 kDa PEG chains. Through analysis of the CLSM images, we demonstrated that PEGylated ProA is effective at reducing the interaction of the contaminant with mAbs that are bound to the affinity ligand; the magnitude of the reduction was dependent on the size of the PEG chain used. Additionally, we demonstrated that PEGylation reduces the amount of contaminant aggregates that bind to the outer surface of the resin particles. These promising results not only suggest that the PEGylated resins will have anti-fouling behavior, but also result in a lower level of product-associated impurities, a major source of impurity carryover in ProA chromatography. In a follow up study, we tested the selectivity of the PEGylated resins via separation of mAbs from Chinese hamster ovary harvest cell culture fluid (CHO HCCF). Here, we examined the relationships between the size and extent of the PEG modification used on the resin on product recovery and HCP eluate content; we demonstrated up to a 40% increase in selectivity for the PEGylated resins. In a third study, we demonstrated that PEGylated ProA media resist proteolytic attack against a common serine protease, chymotrypsin, by retaining a higher percentage of static binding capacity over the same digestion period compared to unmodified resin.

Ligand PEGylation is a new approach to improving the selectivity and longevity of ProA resins and holds similar promise for affinity chromatography systems with other macromolecular ligands..

TUESDAY, FEBRUARY 20, 2018
COOKIES AND COFFEE -- 2:50 P.M.
SEMINAR -- 3:00 P.M.
SARKEYS ENERGY CENTER, A-235

THIS IS A REQUIRED SEMINAR FOR CHE 5971

Accommodations on the basis of disability are available by contacting the office.