She purifies it.

She discovers it.

She separates it.

She loves it.

She lives it.

She owns it.

Mengling Wong

Earned a BS in biochemistry from U.C. Davis. She is a scientific manager in the Analytical Chemistry & Purification department in Small Molecule Discovery Chemistry at Genentech, Inc. Over her 16 years at Genentech, she has acquired analytical and purification skills in the areas of peptides and small molecules. Her interests are in SFC and HPLC technologies and their applications in separating early discovery chiral and achiral molecules. Prior to Genentech, she was at Keystone Division of Biosource International as a laboratory supervisor in the synthesis and separations of oligonucleotides.

THE Q&A

GENERAL AND WORKFLOW

How long have you worked at Genentech in the purification group and what do you enjoy most about your work?
I have spent the last 16 years in the purification group supporting Discovery Chemistry. I enjoy the challenges that my field faces and working with a wide variety of technologies to isolate the molecules of interest.

> *"For our chiral screen, we utilize 15 different phases."*

Overall, what percentage of your purification work is related to chiral compounds versus achiral compounds?
Eight years ago the majority of the compounds were achiral, but today, with the latest technology to separate chiral compounds in a fast and efficient way, it is now about 70 % achiral and 30 % chiral. The trend is toward more molecules with multiple chiral centers as SFC technology has enabled us to isolate all the individual stereoisomers.

For your achiral work, which chromatography separation techniques/modes do you use (RP, NP, SFC) and why?
We rely on RPLC for achiral separation as it is still the best technique that is universally applicable for a broad range of compounds. We use C18 columns for everything from peptides to small molecules. In our lab, we do not utilize NPLC as we want to avoid using toxic, non-environmentally friendly solvents. If RPLC does not separate well then SFC is used.

What portion/percentage of your purification work is performed by SFC compared to HPLC?
Approximately ~35 % of the purifications in our lab are done by SFC. We typically screen all compounds by RPLC and then decide which compounds are appropriate for HPLC vs SFC. Chiral separations are performed by SFC only.

For achiral separation, which phase do you typically screen and which phase/product do you end up using for purification?
Our workflow requires all crude mixtures be screened on C18 stationary phase. If C18 phase does not resolve well or shows poor selectivity, then we will screen by achiral SFC. The columns we use for SFC screening are typically polar stationary phases such as ethyl pyridine, amino, cyano, and diol, but we have also had success using C18 columns.

Have you tried chiral columns for achiral compound separation/purification and what do you think about this idea?
We have tried chiral columns for achiral purification and have had some success. We have observed differential selectivity for chiral phases that has enabled us to isolate molecules where we did not have good separation using achiral phases. Adding chiral phases to the method screen will increase the likelihood of finding a method that will enable you to isolate all the molecules of interest from a given sample.

In terms of chiral separation, how many phases do you typically screen and which phase/product do you use for purification?
For our chiral screen, we utilize 15 different phases from a variety of manufacturers. The majority of the chiral phases are polysaccharides with the best results from coated ones, followed by immobilized phases. Most often the separation works well with amylose-based columns over cellulose. Interestingly, we have observed that the same chiral selector phase from different manufacturers often has different selectivity. I think this gives the customers an opportunity to choose what they want to be included in their screen. Screening a large number of phases is important since there is no "universal" column for Chiral SFC and there is no simple way to predict which stationary phase will work to purify your molecule.

Overall, what are the biggest challenges and bottlenecks in your purification work?
Today, the biggest challenges and bottlenecks in our purification workflow are during the post purification sample handling. If you asked me this same question 8 years ago, my answer would have been the method screening process. However, analytical technology has improved so much that screening now takes less than 2 minutes per compound. We have invested in equipment that can evaporate aqueous mobile phase quickly and lyophilize the compound to a solid form that is easy to weigh out. The removal of HPLC solvents has always been a challenge as it takes longer than SFC. Our workflow uses two stages of drying. The first stage removes the effluent, and the second stage involves transferring the compound to a pre-tared barcoded vial that is used by our central compound management group. For example, a typical HPLC isolation would have fractions of 20-30 mL, dry that down, and then reformat it to 4 mL pre-tared barcoded vials. The challenge is to perform that in an efficient and automated way. Acetonitrile/Water mixture is used to transfer the samples to the final vial (this mixture leads to a nice dry powder) and it is particularly difficult to automate this dissolution process.

> *"We use guard columns for all preparative purification."*

STATIONARY PHASES AND COLUMN TECHNOLOGY

Analytical chromatography is moving toward smaller particles with fully porous as well as core-shell types of particles. Do you see this trend moving into preparative chromatography? Have you tried or would you considered using core-shell particle media for purification work?
Yes, I can see this trend moving into mainstream purification realm. We have tested a couple of core-shell particle media and have seen some interesting differences compared to fully porous particles. One example is lower back pressure which leads to shorter run times with higher flow rates. We have not utilized preparative core-shell columns yet as at the time we did not see any benefit. In the world of purification, we strive to attain speed and high loading capacity for the columns we use. The current column we use has met that requirement for a long time. There has not been a column to replace that phase. If there is a core-shell particle that can meet or exceed the loading capacity of our current column then we are willing to try it out.

THE Q&A Mengling Wong

Do you believe that silica based phases will continue to dominate over polymeric phases for small molecule purifications?
Yes, I think silica based phases will continue to dominate the market for small molecule purifications.

It is the phase that a majority of separation chemists are familiar with and works for a wide range of molecules. There is that motto "if it isn't broke, don't fix it."

Are you using hybrid silica based reversed phase media (stable from pH 1-12) for RPLC preparative separation? How do you see the future of hybrid silica based media for separation of small molecules?
Yes, we have been using hybrid silica to purify small molecules. We like the flexibility of utilizing acid and base on one column instead of using multiple columns. It is great to see manufacturers producing hybrid columns as this gives customers more options. I think these hybrid phases will soon be the "go to" phase for small molecule analysis and purification.

> *"I would like to see a universal chiral phase for SFC that eliminates the need to screen 10-15 columns, similar to a C18 column for RPLC."*

What type of PREP product do you use for your purification work in term of particle size, internal diameter and length? Do you see a difference between column hardware and what improvement would you like to see in the future?
Our team is focused on fast, high quality purifications so we need short run times on the order of 15 minutes. The columns we tend to use are short and highly efficient with dimensions of 50 x 30 mm ID, 5 µm. If we need more plate counts, then we will move up the length to 100 mm long columns. The long columns are particularly useful when isolating an impurity that elutes closely to the API in a drug sample.

How important is the use of preparative column protection for both chiral and achiral columns? How much increase in column lifetime do you see when using guard cartridge systems?
We use guard columns for all preparative purification. As a service group that provides purification support in Discovery Chemistry, 95 % of compounds submitted are crude synthetic material. These crude mixtures may contain a wide variety of contaminants including metal catalysts, coupling agents, byproducts, salts, starting material, or even cotton! Most often these contaminants are insoluble and interact with the silica phase strongly whereby preventing the API to bind efficiently causing high pressure and eventually decreasing the column lifetime. Prep columns are high cost investments, especially chiral columns, and we want to extend the lifetime of these columns by using guard columns. Most of the time, guard columns works well, but eventually the prep column will need to be replaced. The lifetime depends on the number of injections and initial purity of the samples. In our experience, some columns last 6 months while others may last a couple of years.

What do you expect from a stationary phase/column manufacturer in terms of availability, choice of phase, delivery time, and financial stability?
I expect column manufacturers to provide the best service possible to their customers. This requires a broad range of silica phases, bonded phases, different dimensions, and ability to take risks and try innovative products that differentiate them from other vendors. Consistent high quality products are a must. Our work requires preparative separation which means that once we try an analytical phase and it works well, we will need a corresponding prep size. The manufacturer should be able to provide preparative columns in any dimensions without sacrificing efficiency. Having columns in inventory that could be quickly delivered to customers would be fantastic.

If you were to write a wish-list to manufacturers regarding the various types of phases used in different modes in preparative chromatography, where do you see the most potential for improvements and which new phases would help you achieve your purification goals?
This is a typical question that gets asked by manufacturers selling columns or hardware technology.

I would like to see a universal chiral phase for SFC that eliminates the need to screen 10-15 columns, similar to a C18 column for RPLC. Maybe develop a hybrid chiral phase that has both cellulose and amylose phase in one column that will have unique selectivity of both types of polysaccharide. I also would like to eliminate the need to add additives to the mobile phase. Perhaps make columns that include the acidic moiety or basic moiety that will suppress the molecule's ionization. Most of our separations require either acid or base additives in the mobile phase to suppress ionization thus obtaining sharper peaks and acceptable retention times. However, these additives often lead to the formation of ammonium salts, formic salts, or TFA salts after purification and lyophilization, which then leads to issues with compound stability and assay compatibility.

Would you consider packing your own column to save cost and have more flexibility?
No, thank you. Packing columns is a challenge and an art.

SFC/EQUIPMENT

Today, SFC is used primarily in drug discovery and smaller scale environmental labs, do you think that this technology will become a production scale technology one day? What is the biggest challenge that SFC needs to overcome to make it a widely-used technique?
Yes, I see SFC evolving to where it is more routinely used for large scale separations. SFC is widely used in drug discovery to rapidly isolate individual stereoisomers for compounds with one or more chiral centers. This has enabled medicinal chemists to focus on designing molecules and not worry about coming up with stereoselective syntheses. SFC is also a green technology, which makes it much more attractive than normal phase separations.

I think the biggest hurdle for SFC in production scale would be carbon dioxide infrastructure and collection. It is a big investment in bringing preparative SFC to a lab. Questions that come to mind is how does one set up carbon dioxide: liquid Dewar, mini-bulk systems, or house CO_2? I think people who are new to SFC will be surprised that it is expensive to get the infrastructure set up before actually using the SFC equipment. When dealing with purification in kilogram scale we are looking at greater than 1000 g/ min of carbon dioxide. The problem I foresee is that these high flow rates will result in high velocity of CO_2 exiting after the BPR into the collection vessel. An efficient phase separator is needed to separate the gas from the liquid polar mobile phase or most of the desired compound will be lost via aerosol formation. Today, CMOs normally working with kilogram scale APIs will most often have large production scale HPLC, SMB, or continuous chromatography.

Which applications/class of compounds benefit the most from the advantages of SFC separations?
Historically the biggest benefit was the rapid isolation of stereoisomers for chiral small molecules. SFC not only enables faster separations, it also enables shorter drying times relative to HPLC. More recently SFC is being applied to library purifications of achiral compounds and we see this area expanding over the next few years. SFC is an orthogonal separation technique to RPLC so compounds that normally don't retain well by RPLC such as polar compounds, or compounds that are unstable in additives, can now be isolated by SFC without additives.

> *"The biggest market for SFC is in the area of development and regulatory filings. If government agencies can approve the use of SFC equipment for drug development then this will have a big impact."*

What is the practical maintenance cost of SFC compared to conventional LC's?
I think the maintenance cost of SFC is a little more than LC. If an experienced SFC user knows the fluidics of SFC, then the maintenance cost can be low and he can troubleshoot himself. However, if a person is new to SFC then he will solely rely on the vendor to repair the system and this can be costly with payments up front for annual service agreements. It also is difficult to source spare parts if the SFC manufacturer is outside the customer's country, obtaining the parts can be challenging as these may not be available with their normal suppliers.

What is the most serious limitation in SFC?
The most serious limitation is the ability to purify all compounds from polar ionic compounds to non-polar ones. We have issues with long peptides greater than 10 amino acids. It would be nice to replace RPLC with SFC, but I do not see this in the near future.

Do you see the SFC market increasing this year… 5 years? Do you see other purification techniques increasing as well or decreasing due to SFC?
I think the SFC market will increase at a slow pace for the next 5 years. The biggest market for SFC is in the area of development and regulatory filings. If government agencies can approve the use of SFC equipment for drug development then this will have a big impact.

Another area is in drug discovery research where SFC provides a strategic advantage as it speeds up the drug discovery cycle by shortening the time from compound synthesis to biological testing. This strategic advantage combined with the latest instrument technology making SFC systems more like HPLC systems, should lead to more widespread adoption. Finally, more companies are putting an emphasis on green technologies and reducing environmental impact, and SFC is certainly a key technology in that area.

What is one change or improvement you hope to see in the SFC separations industry over the next few years?
As an SFC advocate and user, I would like to see SFC manufacturers collaborate on their technology and software development. SFC companies should address customers feedback seriously, especially from experienced SFC users. There are many different designs in preparative systems from very simple to over engineered systems. All SFC systems should include features such as an intuitive user interface, logical commands to collect fractions, ease of method transfer from analytical to prep, and ability to have different injection options. Combining better software solutions with robust hardware is a smart way to do business.

How important is the "Green Chemistry" concept and what impact does it have on the overall chromatography market?

Green Chemistry is very important at Genentech. It is part of our annual company goal to reduce waste, energy and water consumption, as well as to reduce the use of hazardous chemicals in our laboratories. Companies similar to ours that have chemistry departments actively follow the 12 Principles of Green Chemistry. I think being aware of the chemicals we use and how we dispose of them reduces costs, improves efficiency, and increases a safe environment for everyone.