SMALP Copolymers frequently asked questions

aqueous polymer solutions designed for biomedical research

Are the lipids surrounding the protein preserved in the particle?

Yes, and protocols exist for analyzing them using Mass Spectrometry. Teo et al. Sci Rep. 2019 Feb 12;9(1):1813.

At what pH can I use the polymers?

SMA and DIBMA works above pH 7 while SMI works effectively below pH 7.5 (Hall et al. Nanoscale. 2018 Jun 7;10(22):10609-10619.)

At what temperature should I solubilize proteins?

This is a case of balancing the stability of the protein, the time you want to wait for solubilization and the lipids in the membranes. Standard solubilizations are carried out at RT but elevated temperatures (E.g.) 37°C can accelerate the process but could denature your protein. Lower temperatures will make the process much slower and generally don't provide any advantages.

Can I dialyse the polymer to change buffers?

Yes, any dialysis tubing with a molecular weight cut off lower than 10,000 amu. (Lee et al.Nat Protoc. 2016 Jul;11(7):1149-62.)

Can I use DTT and other common additives?

Many common additives used in protein purification (e.g. DTT, TCEP, Glycerol, PEG) are compatible with the polymer systems.

Can I use high NaCl concentrations with the polymer particles?

Yes, in some cases using elevated NaCl (e.g. >150 mM) can also help to prevent particle aggregation.

Can I use polymer solubilized proteins in downstream experiments?

Yes, there is a growing literature on using polymer solublised proteins in a range of downstream studies including Cryo-EM, X-ray Crystallography, Circular Dichroism, Fluorescence etc. The best approach is to look for papers that have developed the method you have chosen.

Can I use other affinity methods to purify my protein?

Yes, the polymer particles have been used successfully in other systems including FLAG, Myc , Biotin and antibody based purifications.

Can I use the polymer to extract proteins from live cells?

Yes, you can add the polymer to live cells and release proteins from the surface. For mammalian cells you need to be careful of DNA release and may need to add DNase. For E. coli and other Gram negatives you may need to add lysozyme to break the cell wall to release the inner membrane. Paulin et al Nanotechnology. 2014 Jul 18;25(28):285101.

Can polymer solubilized proteins be separated using size exclusion chromatography?

Yes, a standard superdex 200 column provides a good separation

Do I need to put the polymer in all my buffers like I do with detergent?

One of the advantages of the polymer system is that you only need to add it at the solubilization stage compared to detergent that must be included in all buffers.

Do polymer solubilized proteins run on Native PAGE?

Yes, Pollock et al. recently published the SMA PAGE method that separates intact polymer-protein particles providing a novel method for determining native molecular mass of membrane proteins. Pollock et al. Biochim Biophys Acta Biomembr. 2019 Aug 1;1861(8):1437-1445

Do polymer solubilized proteins run on SDS PAGE?

Yes, the SDS sample preparation method disrupts the polymer particle leaving the protein to migrate in the PAGE unhindered. However as with all membrane proteins care must be taken in how these samples are prepared. For example some proteins migrate best after boiling while others do not.

Does the polymer work for all membranes?

Yes, all organisms have membranes made from phospholipids and the polymers solubilize these lipids very easily. Remember the method is solubilising lipids around the protein and not the protein directly.

For how long can I store the polymer?

In dry form the polymer is stable for at least 12 months. In liquid form the polymer is stable for at least 6 months at 4°C unopened. Once open the material should be used with one month.

How do divalent cations alter the activity of polymers?

SMA is sensitive to divalent cations (e.g. Mg^{2+} and Ca^{2+}) and will precipitate at concentrations above 5mM. DIBMA is more resistant and stays in solution above 30 mM while SMI is resistant to higher concentrations of divalent cations. (Hall et al. Nanoscale. 2018 Jun 7;10(22):10609-10619.)

How do I change the buffer for the polymer?

You can either add buffer to the polymer to bring it to the correct pH or you can dialyse the sample into the required buffer. (Lee et al.Nat Protoc. 2016 Jul;11(7):1149-62

How do I exchange the protein from the polymer particle back into a membrane?

There is currently no standard method for doing this but there are individual publications showing different methods that have been used successfully. Dörr et al. Proc Natl Acad Sci U S A. 2014 Dec 30;111(52):18607-12.

How do I quantify my proteins when they are encapsulated in polymers?

Both SMA and SMI have styrene side chains which interfere with measurements of concentration using UV spectroscopy. For proteins in SMA and SMI particles you can use dye binding assays (e.g. Bradford) (Lee et al. Nat Protoc. 2016 Jul;11(7):1149-62.)

How do I store proteins when they are in polymer particles?

Once in the disc the protein is relatively stable (certainly more stable than detergent preparations). In general proteins can be stored at either 4°C for periods of up to a week and -20°C for more extended periods. However, each protein is different and so it is important to do stability experiments on your own protein. Interestingly there have been reports of freeze-drying proteins in discs as a storage method. (Wheatley et al. Biochem Soc Trans. 2016 Apr 15;44(2):619-23.)

How do I transfer protein from polymer particles into detergent?

There are several publications in the literature that show methods to achieve this. It is possible that each protein/polymer/lipid/detergent combination may require its own method.

How long should it take to solubilize a protein using the polymer?

The rate of solubilisation is influenced by the type of lipids in the membrane, the temperature and the protein content. This means that some samples will solubilize in <10 Minutes while others require an overnight incubation. If solubilisation is slow try incubating at 37°C.

How much polymer do I need to solubilize membranes?

In general, you need at least 2.5% w/v in your membrane/cell suspension. The solubilisation works well above this percentage as well and you might want to increase the amount of polymer if you are not getting 100% solubilisation of your protein.

How should I prepare my membranes?

There are methods in the literature for preparing membranes from cells the only thing to be aware of is to keep any divalent cation concentrations below 5mM when using SMA.

I cannot find SMALP 30010P, 25010P, 40005P and 1100I, are they still available?

Yes, these products are still available, but under different names. You can use the table below to find the product you need:

SMALP 40005P	→	**SMALP 140**
SMALP 30010P	→	**SMALP 200**
SMALP 45010P	→	**SMALP 300**
SMALP 1100I	→	**SMALP 140-I**

I do not get a clear peak on my size exclusion column when I use polymer compared to when I use detergent. Is this a problem?

This is quite normal for a polymer stabilised protein. The particle is more heterogenous than a soluble protein so tends to elute as a wider peak. Running the fractions on an SDS PAGE and SMA-PAGE will help you decide which fractions you need to pool.

I have lower yields of protein compared to my detergent purifications.

The polymer system only solublizes protein that is in the membrane and therefore most likely to be fully folded. Detergents can solubilize but protein in the membrane and also partially folded or aggregated material. This can mean that a gross assessment of yield can make detergent solubilization look more efficient than polymer. However, the true test is whether the activity is higher in the detergent, often it isn't. If the activity is higher in the detergent then it might indicate that you need to trial another polymer type that better matches your proteins needs.

My protein ends up in the pellet after solubilization with the polymer. What should I do?

Keep calm, if some protein is solubilized it is possible that the protein in the pellet is an aggregate that is formed when proteins are over expressed. If this is the case, then proceed with the soluble sample. If you think that actually the protein is not an aggregate, try increasing the time and temperature of the solubilisation.

My protein precipitates after size exclusion chromatography?

Make sure that your salt concentration in the running buffer of the column is >100 mM as this inhibits ionic binding to the column and aggregation.

My protein in not active in the polymer particle, what should I do?

Don't Panic, first test whether the polymer is inhibiting your assay. You can also make a lipid only disc by adding lipid to the polymer and see if this is inhibiting the assay. If the assay is not inhibited but the protein is not active then try one of the other polymers that we supply.

What buffer is the polymer in?

The polymers are supplied in water and some residual K^+ from the production process. There is no Buffer so the polymer needs to be buffered to the required pH for use.

What is the difference between Aurorium's SMALP solutions and the Cube Biotech SMALP powders?

Both we and Cube Biotech sell the same polymeric SMA backbone for SMALP. Cube-Biotech sells SMALP polymers as powders, while we sell a 20 percent solid content SMALP solution in water. To our Aurorium SMALP products HEPES and NaCl are added and freeze dried by Cube-Biotech to obtain a easy to use powder that guarantees a stable pH at 7.5 when used. These additional steps results in a sodium counter ion of the Cube-Biotech powders in contrast to our potassium counter ion.

What is the largest protein that can fit in a polymer particle?

There is evidence in the literature that the SMALP discs can solubilize proteins with up to 51 transmembrane helices. It is possible that larger proteins (particularly with domains outside the membrane) can be solubilized. *let us know if you successfully solubilize a larger one

When I add polymer to my membrane solution, I cannot see the solution clarify. What should I do?

Don't panic, for some samples clarification is not complete as non-membrane aggregates remain. These can be removed by centrifugation.

When I stain my PAGE with Coomassie I see a low molecular weight "smear". What is it and how do I get rid of it?

This is free polymer. The smear can be reduced by removing free polymer by size exclusion. Alternatively try another gel visualisation method that is known to be less influenced by the presence of the polymer e.g. Instant Blue Coomassie

Which polymer should I use?

There a few rules for choosing polymers. If divalent cations are important for your work (>5mM) use SMI or DIBMA. If you need to work at low pH use SMI. If you are looking at ligand binding experiments you may also want to choose a polymer that has the same charge as the ligand to reduce non-specific binding. Otherwise if it is the first time you have used the polymer you may want to trial all 3 polymers to see which works best.

Why doesn't my protein bind to NiNTA columns when it is in a SMA particle?

The charges on the SMA polymer can interact with Histidine Tags reducing their affinity for $Ni2^{+}$ and $Co2^{+}$ based affinity resins. It is therefore essential that SMA solubilized proteins are loaded onto resins without any imidazole. The material should be loaded using a batch method ideally overnight and will then elute at lower imidazole (e.g. 20 mM) that you would expect for a soluble protein. Another option is to try extending the Histidine tag to 10 or 12 histidine as this can also increase affinity.

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201 North Illinois Street, Suite 1800
Indianapolis, IN 46204 USA
ask@aurorium.com
www.aurorium.com