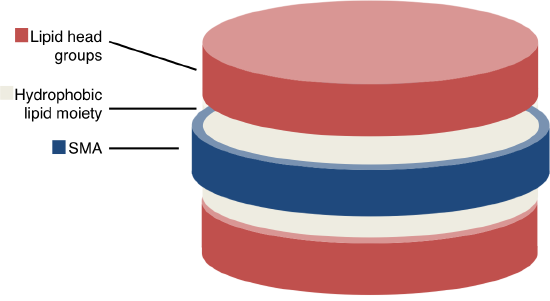
**Freedom to Operate: Styrene Maleic Acid LipoProtein (SMALP)**

1. **Background to the SMALP Technology**
2. **SMALP**

Despite the great progress recently made in resolving their structures, investigation of the structural biology of membrane proteins still presents major challenges. The study of membrane proteins is often hampered by their tendency to misfold when extracted by detergent. A styrene-maleic acid co-polymer offers an interesting alternative to existing detergents, in that it is able to insert into biological membranes, form a nanodisc with the membrane contents in the centre of the disc and leave the membrane as a stable intact nanodisc. The lipid bilayer and resident membrane proteins are held within this disc, as depicted in the figure below. Once in the nanodisc, membrane proteins purified without exposure to further detergents, they show heightened thermal stability and retain full functional activity. This creates a number of commercial opportunities for life science companies.

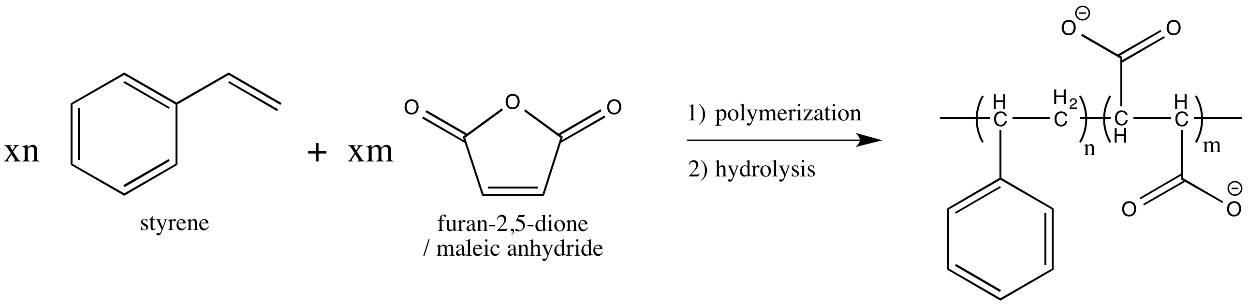
尽管近年来在解决膜蛋白结构方面取得了巨大进展，但对膜蛋白结构生物学的研究仍然面临着重大挑战。膜蛋白的研究经常受到洗涤剂提取时错误折叠倾向的阻碍。苯乙烯-马来酸共聚物为现有洗涤剂提供了一种有趣的替代品，因为它能够插入生物膜中，形成膜内容物位于圆盘中心的纳米圆盘，并使膜成为稳定完整的纳米圆盘。脂质双层和驻留膜蛋白被保持在该盘中，如下图所示。一旦进入纳米盘，即在不暴露于进一步清洁剂的情况下纯化的膜蛋白，它们显示出更高的热稳定性并保持完整的功能活性。这为生命科学公司创造了许多商业机会。



1. **Manufacture of Styrene Maleic Acid (SMA)**

SMA copolymer is formed from polymerization of a mixture of styrene and maleic anhydride in various ratios (3:1 and 2:1 being the most common). The anhydride moieties can subsequently be hydrolyzed to maleic acid. The alternating hydrophobic residues (styrene) and hydrophilic (maleic acid) is thought to be determining for the SMA properties of membrane solubilisation.

SMA共聚物是由苯乙烯和马来酸酐按不同比例（3:1和2:1是最常见的）的混合物聚合而成。酸酐部分随后可以水解成马来酸。疏水残基（苯乙烯）和亲水残基（马来酸）的交替被认为是决定膜溶解SMA性质的因素。



1. **SMA lipid particles (SMALPs)**

Originally being patented as Lipodisq, (patent 11 below: principally a drug delivery tool for hydrophobic pharmaceuticals), SMALPs can be used to purify membrane proteins (Knowles and co-workers: ref 2). Since that time, a variety of proteins have been purified successfully using the SMALP approach with the following results:

SMALP最初以Lipodisq的名义获得专利（以下专利11：主要是疏水性药物的药物递送工具），可用于纯化膜蛋白（Knowles及其同事：参考文献2）。从那时起，使用SMALP方法成功纯化了多种蛋白质，结果如下：

* + Membrane proteins of 1-36 transmembrane domains spontaneously incorporate in SMALP nanodiscs of 9-12nm diameter (refs 1, 3-5);

1-36个跨膜结构域的膜蛋白自发结合到直径9-12nm的SMALP纳米盘中（参考文献1，3-5）；

* + The proteins in SMALP nanodiscs can be purified by conventional methods (affinity, ion exchange, size exclusion chromatography) without removing them from the nanodisc;

SMALP纳米盘中的蛋白质可以通过常规方法（亲和、离子交换、尺寸排阻色谱）进行纯化，而无需将其从纳米盘中去除；

* + The proteins in SMALP nanodiscs are stable, display native structure and are fully active;

SMALP纳米盘中的蛋白质是稳定的，显示出天然结构并且是完全活性的；

* + The incorporation of membrane proteins in SMALP nanodiscs offers an attractive platform to discover new drugs to individual membrane proteins.

将膜蛋白掺入SMALP纳米盘中为发现单个膜蛋白的新药提供了一个有吸引力的平台。

A number of research articles describing progress with SMALP to date are identified below, along with a comprehensive resource from the SMALP academic community. These are not essential reading, however they are a useful resource to consult as needed.

下面列出了一些描述迄今为止SMALP进展的研究文章，以及来自SMALP学术界的综合资源。这些不是必要的读物，但它们是一种有用的资源，可以根据需要进行咨询。

**Key papers**

1. Orwick, M. C. et al. Detergent-free formation and physicochemical characterization of nanosized lipid-polymer complexes: Lipodisq. Angew. Chemie - Int. Ed. 51, 4653–4657 (2012).
2. Knowles, T. J. et al. Membrane proteins solubilized intact in lipid containing nanoparticles bounded by styrene maleic acid copolymer. J. Am. Chem. Soc. 131, 7484–7485 (2009).
3. Orwick-Rydmark, M. et al. Detergent-free incorporation of a seven-transmembrane receptor protein into nanosized bilayer lipodisq particles for functional and biophysical studies. Nano Lett. 12, 4687–4692 (2012).
4. Swainsbury, D. J. K., Scheidelaar, S., van Grondelle, R., Killian, J. A. & Jones, M. R. Bacterial Reaction Centers Purified with Styrene Maleic Acid Copolymer Retain Native Membrane Functional Properties and Display Enhanced Stability. Angew. Chemie Int. Ed. 53, 11803–11807 (2014).
5. Scheidelaar, S. et al. Molecular Model for the Solubilization of Membranes into Nanodisks by Styrene Maleic Acid Copolymers. Biophys. J. 108, 279–290 (2015).

Further resources: [www.smalp.net](http://www.smalp.net)

1. **Scenario for Assignment**

**CRO Scenario**: A contract research organisation (CRO Co) is evaluating the freedom to operate in the supply of therapeutic antibody discovery services to membrane proteins using SMALPs as a means of preparing target proteins and screening for antibodies with the desired properties (as set out below).

***CRO方案：一家合同研究组织（CRO Co）正在评估使用SMALP为膜蛋白提供治疗性抗体发现服务的自由度，以此作为制备靶蛋白和筛选具有所需特性的抗体的手段（如下所述）。***

1. **The proposed service**
2. Membrane proteins will be expressed in relevant mammalian or other cells as a fusion protein (that bears an affinity purification tag);

膜蛋白将作为融合蛋白（带有亲和纯化标签）在相关哺乳动物或其他细胞中表达

1. A target membrane protein will be extracted from the cells by exposure of the cells to a styrene maleic acid co-polymer at a suitable concentration;

目标膜蛋白将通过将细胞暴露于合适浓度的苯乙烯-马来酸共聚物而从细胞中提取

1. Styrene Maleic Acid ("SMA") Copolymer will be prepared from a precursor source: styrene maleic anhydride (Xiran SZ25010: S/MA ratio 3:1; Mw 10000 from Polyscope);

苯乙烯-马来酸（“SMA”）共聚物将由前体来源制备：苯乙烯-马来酸酐（Xiran SZ25010:S/MA比例3:1；Mw 10000来自Polyscope）；

1. The active styrene maleic acid polymer will be prepared using the protocol from Nat Protoc. 2016 Jul;11(7):1149-62 . In the laboratories of CRO Co., activation of SMA using this protocol results in a product (styrene maleic acid) with HLB of 16.5, free monomic styrene content of 0.25% (by weight), and free monomer maleic acid plus maleic anhydride of 0.3% by weight.

活性苯乙烯-马来酸聚合物将使用Nat Protoc的方案制备。2016年7月；11（7）：1149-62。在CRO公司的实验室中，使用该方案活化SMA得到HLB为16.5、游离单体苯乙烯含量为0.25%（重量）、游离单体顺丁烯二酸加顺丁烯二酸酐为0.3%（重量）的产物（苯乙烯-马来酸）。

1. The target protein will be solubilised by SMA (as prepared above), purified using the appropriate affinity chromatography approaches to a suitable purity, and provided is to the client as a suspension in an appropriate buffer. The client will perform the screening assays.

目标蛋白将通过SMA溶解（如上所述制备），使用合适的亲和层析方法纯化至合适的纯度，并作为合适缓冲液中的悬浮液提供给客户。客户将进行筛选化验。

1. **Patent Search**

A patent search was conducted to provide the data to assess the freedom to operate in specific areas. The search was conducted by a professional patent search company in PatBase. You have been provided with the most relevant patents from this comprehensive patent search set and the claims that are most relevant to the report have been highlighted. These are set out below:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Case | Title | **Patent ID** | Abstract | Claim |
| 4 | SOLUBILISATION OF MEMBRANE PROTEINS | **US2012142861B2** | A method is provided for solubilising a membrane protein. The method is applied to cellular material comprising the membrane protein and an associated membrane lipid. A copolymer of styrene and maleic acid, wherein the styrene:maleic acid ratio is between 1:2 and 10:1, is mixed with the cellular material to cause the copolymer, lipid and protein to form soluble macromolecular assemblies. | 1 |
| 提供了一种用于溶解膜蛋白的方法。该方法应用于包括膜蛋白和相关膜脂质的细胞材料。将苯乙烯和马来酸的共聚物与细胞材料混合，使共聚物、脂质和蛋白质形成可溶性大分子组装体，其中苯乙烯与马来酸的比例在1:2和10:1之间 | | | | |
| 11 | COMPOSITIONS COMPRISING MACROMOLECULAR ASSEMBLIES OF LIPID AND SURFACTANT | **WO2008/065451** | A composition comprising lipid and surfactant, characterised in that the surfactant has an HLB number of less than 20 and in that the lipid and surfactant are in the form of macromolecular assemblies of less than 100 nm in diameter. | 1-5 |
| 一种包含脂质和表面活性剂的组合物，其特征在于，所述表面活性剂具有小于20的HLB数，并且所述脂质和表面活化剂为直径小于100nm的大分子组装体的形式。 | | | | |
| 12 | STYRENE-MALEIC ANHYDRIDE COPOLYMERS FOR BIOAPPLICATIONS AND THEIR PREPARATION | **WO2007/115165** | The present invention discloses styrene-maleic anhydride copolymers preparations using solventless techniques. The solventless method resulted in reduced amounts of residues, such as unreacted styrene and/or maleic anhydride monomers, which makes the copolymers particularly suitable for bioapplications. | 1-6 |
| 本发明公开了使用无溶剂技术的苯乙烯-马来酸酐共聚物的制备。无溶剂方法导致残留物的量减少，例如未反应的苯乙烯和/或马来酸酐单体，这使得共聚物特别适合生物应用。 | | | | |

For the purpose of this exercise please assume that the patent applications are granted in the UK in the form that has been supplied to you (the documents are in fact just applications or US patents).

出于本练习的目的，请假设专利申请是以提供给您的形式在英国授予的（这些文件实际上只是申请或美国专利）。

1. Review extracts from Section 60 of the UK Patents Act in respect of infringing acts at annexe 1.
2. **Assignment Output**

Prepare a report for the commercial strategy group of CRO Co. that critically evaluates the freedom to operate in areas that fulfil the proposed commercial goal CRO Co. Identify the relevance, or otherwise, of each of the patents provided and provide solutions (technical and/or commercial) to the issues raised [1500 words].

为CRO公司的商业战略小组编写一份报告，对在实现拟议商业目标的领域开展业务的自由度进行批判性评估。确定所提供的每项专利的相关性或其他方面，并为提出的问题提供解决方案（技术和/或商业）[1500字]。

***Schedule 1***

***Section 60 Patents Act 1977***

60.-(1) Subject to the provisions of this section, a person infringes a patent for an invention if, but only if, while the patent is in force, he does any of the following things in the United Kingdom in relation to the invention without the consent of the proprietor of the patent, that is to say –

1. where the invention is a product, he makes, disposes of, offers to dispose of, uses or imports the product or keeps it whether for disposal or otherwise;
2. .where the invention is a process, he uses the process or he offers it for use in the United Kingdom when he knows, or it is obvious to a reasonable person in the circumstances, that its use there without the consent of the proprietor would be an infringement of the patent;
3. where the invention is a process, he disposes of, offers to dispose of, uses or imports any product obtained directly by means of that process or keeps any such product whether for disposal or otherwise.

(2) Subject to the following provisions of this section, a person (other than the proprietor of the patent) also infringes a patent for an invention if, while the patent is in force and without the consent of the proprietor, he supplies or offers to supply in the United Kingdom a person other than a licensee or other person entitled to work the invention with any of the means, relating to an essential element of the invention, for putting the invention into effect when he knows, or it is obvious to a reasonable person in the circumstances, that those means are suitable for putting, and are intended to put, the invention into effect in the United Kingdom.

(3) Subsection (2) above shall not apply to the supply or offer of a staple commercial product unless the supply or the offer is made for the purpose of inducing the person supplied or, as the case may be, the person to whom the offer is made to do an act which constitutes an infringement of the patent by virtue of subsection (1) above.

(5) An act which, apart from this subsection, would constitute an infringement of a patent for an invention shall not do so if –

(a) it is done privately and for purposes which are not commercial;

(b) it is done for experimental purposes relating to the subject-matter of the invention;

(6D) For the purposes of subsection (5)(b), anything done in or for the purposes of a medicinal product assessment which would otherwise constitute an infringement of a patent for an invention is to be regarded as done for experimental purposes relating to the subject matter of the invention.

(6E) In subsection (6D), “medicinal product assessment” means any testing, course of testing or other activity undertaken with a view to providing data for any of the following purposes—

(a) obtaining or varying an authorisation to sell or supply, or offer to sell or supply, a medicinal product (whether in the United Kingdom or elsewhere);

(b) complying with any regulatory requirement imposed (whether in the United Kingdom or elsewhere) in relation to such an authorisation;

(c) enabling a government or public authority (whether in the United Kingdom or elsewhere), or a person (whether in the United Kingdom or elsewhere) with functions of—

(i) providing health care on behalf of such a government or public authority, or

(ii) providing advice to, or on behalf of, such a government or public authority about the

provision of health care, to carry out an assessment of suitability of a medicinal product for human use for the purpose of determining whether to use it, or recommend its use, in the provision of health care.

(6F) In subsection (6E) and this subsection—

“medicinal product” means a medicinal product for human use or a veterinary medicinal product;

“medicinal product for human use” has the meaning given by article 1 of Directive 2001/83/EC(2);

“veterinary medicinal product” has the meaning given by article 1 of Directive 2001/82/EC(3).