FTO Report

Party B: CRO Co.

New Antibody Service Using SMALP

2023/11/11

Total word count: 1492

(Without report contents, figures, tables and references)

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Brief Summary

Thanks for the entrust of board director of *CRO Co.* We searched 3 related patents and found that 2 have the valves that close. We provided evaluations on technology aspect and suggest avoiding infringement from a commercial perspective.

Please note that we do not state an opinion regarding the risk of infringement.

Background

1.1 Launch Status Assessment

Object of this Freedom to Operate Report (FTO) is therapeutic antibody discovery services for membrane proteins using the technology of Styrene Maleic Acid Lipo-Protein (SMALP). The "*therapeutic antibody discovery services"* entrust us for this report is hereinafter referred to as ***"TADS"***.

Client in this case title of *CRO Co*., British organization *Contract Research Organization*. Potential market for this discovery service application is the UK market. Therefore, this FTO report will be written based on the principle of Section 60 of the *UK Patent Act.* Regarding the respect of infringement.

1.2 Technical Feature

1.2.1 SMA Synthesis Protocol

Styrene–maleic acid (SMA) is the hydrolysed form of the styrene–maleic anhydride (SMAnh) copolymer in chemistry. When styrene(S): maleic acid (Ma) molar ratio is 1:1, the reaction will generate SMA (see figure 1). Monomer sequence distribution in the polymer becomes more complex when S:Ma higher than 1:1 (Dorr et al. 2016).

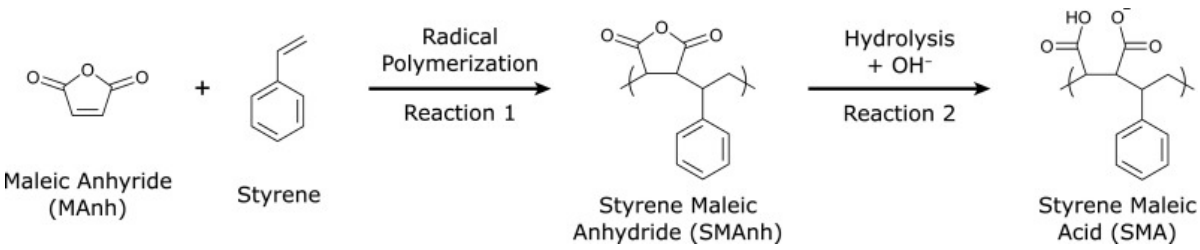


Figure 1. Synthesis of SMA (Dorr et al. 2016).

In scenario of *TADS*, precursor source will be come from commercially available acid anhydride period Netherlands *Inc. Polyscope* with a S: Ma ratio of 3:1 (*Xiran* ®). It should be mentioned that in the reference protocol of TADS, Lee's team use S:Ma ratio 2:1 as SMALP using S:Ma ratio 3:1 precursor contains bilayers, and the synthesis also begin from acid anhydride period (Lee et al.2016a). Then they use basic hydrolysis to dissolve styrene maleic anhydride (SMAnh) copolymer in 1M NaOH and react under solution heating and reflux.

The hydrophilicity lipophilicity balance (HLB) is a wildly used parameter that determines the degree of hydrophilicity or lipophilicity by calculating the molecular weight percentage of the hydrophilic (Mh, molecular mass of the hydrophilic part) and lipophilic parts of the surfactant molecule. The equation is shown below where M means molecular mass of the whole particle (Griffin et al, 1946).

$$HLB = 20 * Mh/M$$

Activation of SMA in *TADS* results in SMA product with HLB of 16.5 and some other by-product (see chart 1). The production can be store as a light white powder (Lee et al. 2016a).

By-product Substance	Proportion by Weight
free monomic styrene	0.25%
free monomer maleic acid plus maleic anhydride	0.3%

Chart 1. By-Product and Proportion in Basic Hydrolysis Synthase of TADS

1.2.2 Protein Solubilization and Formation of SMALPS

SMAs is used in a promising approach to detergent-free solubilize membrane proteins (MP). Most of the other processes to dissolve MB require detergents to extract native MPs from cellular membranes (Dorr et al. 2016). Detergents always cause transient destabilization of MP.

SMAs exhibit a significantly different mode of action from detergents. Addition of SMAs to synthetic or biological lipid membranes leads to the spontaneous formation of disc-shaped particles with a diameter of approximately 10 nm (Jamshad et al. 2015). In this novel SMAs bound nano disk, the bilayer structure of the incorporated lipid molecules is stabilized(Orwick et al. 2012). It had different names from different research at early stages, but nowadays most used name right now is SMALPs (see chart 2).

Name	Related Research
SMA–lipid particles (SMALPs)	Knowles et al. 2009
Lipodisq particles	Orwick et al. 2012
native nanodiscs	Dörr et al. 2014

Chart 2. Other names for the particles and related publication

Add powdered SMA polymer to membrane solution at a ratio of 1g:10g (Lee et all. 2016a), MP with up to 36 transmembrane domains will spontaneously form a 9-12nm diameter SMALP structure (Orwick et al. 2012; Knowles et al. 2009). Research proved that protein structure remained stable in SMALP. After that protein can still be purified directly by wildly used methods such as chromatography (Scheidelaar et al.2015; Dorr et al. 2016).

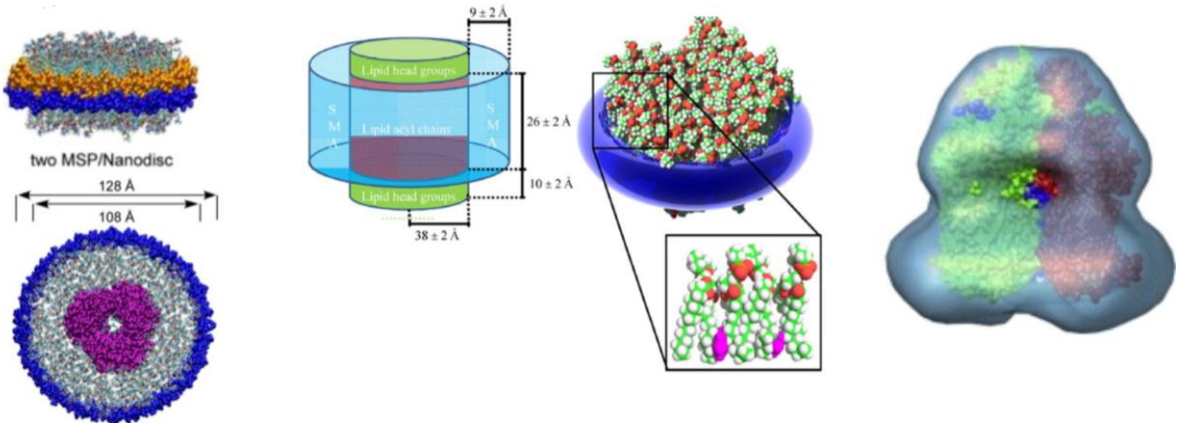


Figure 2. Comparison of membrane proteins in nano-discs. Shows MSP, lipid only SMALP, egative stain 3D reconstruction of SMALP from left to right (Lee et all. 2016b).

Related Patents

The search was conducted by a professional patent search company in PatBase. The most relevant from this comprehensive patent search are 3 patents (assumingly) granted in UK shown below.

2.1 Patent 4

WO2011/004158,

SOLUBILISATION OF MEMBRANE PROTEINS

This patent is published on 13 January 2011. It focusses on method to solubilise a membrane protein. It come from CTB-TTF project (Grant et al. 2005) Malvern group collaborate with Overduin's group from University of Birmingham (UBir).

This method is applied to molecular in cell membrane including proteins and related lipids. It is done by mixing copolymer of 1:2 to 10:1 styrene and maleic acid, with cellular component to form soluble macromolecular assemblies of the copolymer, lipids, and proteins.

What we need to remind Party B is that the invertor of this patent is Timothy Dafforn, Michael Overduin and Timothy Knowles. Tim Dafforn is the corresponding author of the journal article from Nat Protocol "A method for detergent-free isolation of membrane proteins in their local lipid environment" (see Lee et al, 2016a). We will mention later that the synthesis method of Lee's article, which is the method being used by party B in TADS, is indeed include in the patent 4.

2.2 Patent 11

WO2008/065451,

COMPOSITIONS COMPRISING MACROMOLECULAR ASSEMBLIES OF LIPID AND SURFACTANT

This patent is published on 5 June 2008. It is the originally patent of *SAMLP* although name of it is *Lipodisq* represent in the document.

It provides composition comprising lipids and surfactants. The surfactant in it has an HLB number of less than 20. The lipids and surfactants form the less than 100nm macromolecular assemblies.

2.3 Patent 12

WO2007/115165

STYRENE-MALEIC ANHYDRIDE COPOLYMERS FOR BIOAPPLICATIONS AND THEIR PREPARATION

This patent is published on 11 October 2007. It focusses on the solvent free technology to prepare SMA.

It is mentioned that solvent-free method results in a reduction in the amount of residue, such as unreacted styrene and/or maleic anhydride monomers, making copolymers particularly suitable for biological applications.

Infringement Comparison

3.1 Similar Proportion of Precursor Substances with Patent 4

TADS used precursor of S:Ma=3:1 which infringe the property right of Patent 4.Patent 4 emphasized that all copolymer of styrene and maleic acid used in membrane protein solubilising wherein the styrene : maleic acid ratio is between 1:2 and 10:1 is against their claim 1.

3.2 Similar Surfactant HLB and Size of SMALP of Patent 11

In claim 1 of Patent 11, it is confirmed that all SMALP 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. TADS represent a surfactant HLB of 16.5 and Assembly Size of about 10 nm, which constitute infringement of patent 11.

3.3 No Methodological similarity with Patent 12

The patent 12 use solventless method to reduce the residence. They claim all styrene-maleic anhydride copolymer having less than 0.050% by weight unreacted styrene monomer (see claim 1). In scenario of TADS, it uses the method of traditional basic hydrolysis protocol with 0.25% unreacted styrene which is higher than patent 12. Patent 12 also claim styrene-maleic anhydride copolymer having less than 0.2% by weight unreacted maleic anhydride and maleic acid combined (see claim 2), and the ratio of TADS is 0.3% which is also higher (see chart 1). Therefore, Party B has not infringed Patent 12 from by-product ratio.

3.4 Conclusion of Infringement Comparison

We can see that during the SMA synthesis period Party B did not constitute infringement of patent 12 as it used traditional processes with more residual product. However, it is likely to constitute infringement of patent 4 as its precursor material ratio is analogous. During the protein solubilization period, Party B presumably constitute infringement of patent 11 as their consistent chemical properties of SMAPL. (See chart 3)

	Possible Infringement Aspects								
	SMA Synthesis Method	IN	S: MA Ratio	IN	Surfactant HLB	IN	Assembly Size	IN	Final
TADS	Basic Hydrolysis Protocol	-	3:1	-	16.5	-	~10nm	5	
Patent 4	Basic Hydrolysis Protocol	N	1:2 to 10:1	Y	-	N	-	N	Y
Patent 11	-	N	-	N	>20	Y	>100nm	Y	Y
Patent 12	Solventless Method	N	-	N	-	N	-	N	N

Chart 3. Possible infringement aspects of TADS. IN stands for infringement. Y means there is infringement and N means no infringement. Yellow shading means the focus of the patent.

Risk Aversion Suggestions

4.1 patent 4

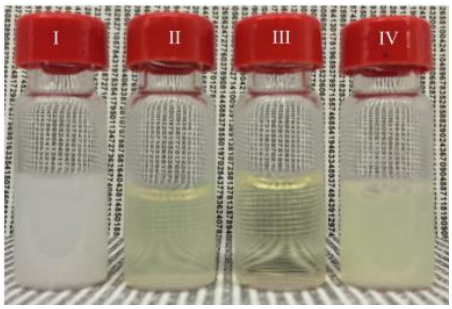
	Technological Option	Commercial Option
Risk Aversion Suggestion	1A. To make S:Ma ratio <1:2 1B. To make S:Ma ratio >10:1.	2A. Exclusive license 2B. Cross licence 2C. Non-exclusive license 2D. Technology shares
Feasibility Analysis	Most research indicated that SMA (3:1) is the best ratio of SMALP solubilization effectiveness and downstream stability (Hall et al. 2018). SMA (3:1) display highest intensity of hydrophobic/ lipophilic balance required for complete dissolution of lipid bilayers. There is no current literature evidence to suggest that <1/2 StMA or >10/1StMa still have solubility or amphipathic. It is analysed that the former is completely hydrophilic while later is hydrophobic (Juan et al. 2019).  **Figure 3**. Vial I is a control of just POPC/POPG vesicles, vial II is 2:1 ratio StMA, vial III is the addition of a 3:1 ratio StMA and vial IV is 4:1 StMA. (Andrew et al. 2016)	Here, business department of the company should provide specific profit models and expected funding for further analysis. If SMALP does not involve key profitability, then 2C and 2D option will be more effective. Negotiation with UBir for exclusive licence is not excluded if bid is good and Party B is adequately capitalised. Our evaluation recognizes the potential commercial value of Patent 4. The Intellectual Property and Commercialisation (IPaC) team of UBir limit grants of IP from professor to funder. It is recommended that the board not only hire Prof. Dafforn et al. as consultant, but also establish an interest binding long-term relationship with the UBir.
Recommendations to the Board	Base on the above analysis, the technological option can be ruled out. There is no need to waste company costs in order to experimentally verify the substrate ratio excluded by patent 4. This report preferent recommendation to the board is **not to avoid patent 4**, but by commercial approach. We support negotiating with university directors and patent holders whether they can hold project shares or grant us non-exclusive license (2C&2D).	

Chart 4. Option to avoid infringement to Patent 4

4.1 patent 11

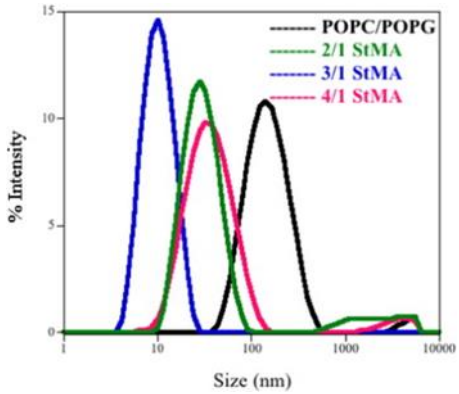
	Technological Option	Commercial Option
Risk Aversion Suggestion	1A. To make HLB>20 1B. To make size of SMALP>100nm.	2A. Acquisition 2B. Non-exclusive license 2C. Technology shares
Feasibility Analysis	The HLB scale ranges from 0 to 20 (Griffin, 1949). Option 1A is excluded. SMALP nanoparticle size depends only on the SMA ratio. It is difficult to obtain particles larger than 100 nm through conventional technical. The yield is too low at small peaks around 7000nm.  **Figure 4**. StMA means styrene-maleic acid. (Andrew et al. 2016)	Malvern Cosmeceutics Ltd, founded in 2005, own Patent 11. Commercial products were launched under the tradename Lipodisq®. Investigation reveals that SMALP related patent are its main valuation source. The current valuation of the company is £80,048.00 (provided by company check.com). Our evaluation reveal that it is worth conducting acquisition negotiation. Consider layout of upstream capital, if the acquisition is not smooth, consider option 2B or 2C.
Recommendations to the Board	This report preferent **not to technically avoid** patent 11. Perhaps due to poor management of the patent owner company, we believe that the current valuation of the company is lower than patent value. Conduction of acquisition negotiation by Party B is suggested(2A).	

Chart 5. Option to avoid infringement to Patent 11

Conclusion

The service conducted by Party B may involve infringement of patent 4 and patent 11. Considering the difficulted to technically avoid these two patents, we suggest requesting authorization from UBri for patent 4 and acquisition of Malvern Cosmeceutics Ltd for patent 11. Regarding informational deficiencies of funds and actual communication situation, other options are also in view.

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