EPITOPES FORMED BY NON-COVALENT ASSOCIATION OF CONJUGATES

The present invention relates to a composition for interacting with a ligand, a method for producing such a composition and a method for producing a molecule based on the composition.

Background of the Invention

Protein receptors are known normally to bind to their target ligands via epitopes, which constitute a small proportion of the total protein molecule. For maximum binding or interaction, the structure of the epitope needs to be maintained in a rigid conformation in order to form a binding site containing all the necessary components of the epitope in close proximity. Attempts to produce an analogous peptide constructed solely of the amino acids comprising the binding site often fail because these peptides do not possess the same biological activity as the protein receptor. This is attributed to the peptide having a different conformation in free solution from that of the entire protein receptor. In addition, where the binding site of a protein is constructed of oligo-peptides from different, non-contiguous parts of a protein chain, mixing isolated oligopeptides in free solution does not result in reconstitution of the active binding site.

Being constrained to use such large proteins to present binding-site epitopes gives rise to several problems in development of new receptor-specific therapeutic strategies. One problem is that such large proteins can readily evoke an

immune response. A second problem is that long peptide chains are susceptible to attack by endopeptidases, such as those in the lumen of the gut. Finally, these large proteins can be costly to manufacture, purify and maintain in stable form.

Summary of the Invention

The present invention aims to overcome the disadvantages of the prior art.

In a first aspect, the invention provides a composition for interacting with a ligand, which composition comprises a non-covalent assembly of a plurality of distinct conjugates, each conjugate comprising a head group and a tail group, wherein the tail groups of the conjugates form a hydrophobic aggregation and the conjugates have freedom of motion with respect to each other within the assembly so that, in the presence of a ligand, at least two of the head groups (which are the same or different) are appropriately positioned to form an epitope capable of interacting with the ligand more strongly than each of head groups individually. The head groups are typically hydrophilic and the tail groups typically hydrophobic, eg lipophilic, composed of hydrocarbon chains, halophilic, constructed of fluorocarbon chains, or silane based.

By constructing conjugates with a head group and a tail group in accordance with the present invention, the tail groups can associate to form a hydrophobic aggregation which is typically a supramolecular assembly such as a micelle, a

lamellar structure, a liposome or other lipid structure, in which the conjugate are oriented whereby the head groups are brought into close proximity when in an aqueous phase. Because the conjugates are movable within the assembly, the head groups are able to adopt a number of different positions within the assembly. The head groups, which are typically non-identical, are therefore free to move within the assembly and, surprisingly, to interact cooperatively to induce biological consequences which the head groups on their own are not capable of eliciting. A further unexpected finding is that assemblies composed of combinations of different headgroups are capable of eliciting biological responses or participating in binding with biological receptors while assemblies composed of single headgroups are not capable of acting in this way.

As indicated above, these supra-molecular assemblies are typically particulate or colloidal in nature, usually comprising many hundreds of sub-units (the conjugates) all oriented with the headgroups directed outwards from the centre of the particle as shown in Figure 1a. Each of the conjugates may change its location within the assembly, being free to exchange places with adjacent conjugates by a process of Brownian motion and, in so doing, may migrate over the whole surface of the assembly. Other manifestations of supra-molecular assemblies are cubic phases and coated surfaces.

Each conjugate in the assembly may have a head group selected from one chemical or biological class or a number of

different classes, such as an amino acid or peptide; a peptide analogue; a mono-, di- or poly-saccharide; a mono-, di- or poly-nucleotide; a sterol; an alkaloid; an isoprenoid; an inositol derivative; a single or fused aromatic nucleus; a water-soluble vitamin; a porphyrin or haem nucleus; a phthalocyanine; a metal ion chelate; a water-soluble drug; a hormone; or an enzyme substrate.

In one preferred embodiment, each head group comprises an amino acid or oligo-peptide, which may be the terminal portion of a peptide chain. It is desirable to keep the length of the peptide to a minimum so as to avoid eliciting an immune response where the composition is to be used *in vivo*. Accordingly, it is preferred that the peptide is no more than six amino acids long.

The amino acids employed can be any of the natural amino acids, substituted derivatives, analogues, and D- forms thereof.

The tail groups of the conjugates may be all the same or may be a mixture of different tail groups, each of which preferably comprises a hydrophobic group selected from a linear, branched, cyclic, polycyclic, saturated or unsaturated construct, with or without hetero-atoms included in the structure which can be substituted or unsubstituted, for example, a lipidic amino acid analogue; a prostaglandin; a leukotriene; a mono- or diglyceride; a sterol; a sphingosine or ceramide derivative; and a silicon or halogen-substituted derivative of such a hydrophobic group. The tail group preferably has from 6 to 24 carbon atoms and more

preferably comprises from 10 to 14 carbon atoms. More than one tail group may be present in each conjugate. For example, one or more lipidic amino acids with hydrocarbon side chains may form part of each conjugate, linked to one or more amino acids in the head group.

Any chemical method may be used to link the head group to the tail group. For example, each conjugate may further comprise a spacer group linking the head group to the tail group so as to facilitate presentation of the head group on the surface of the non-covalent association. Such spacer groups are well known and include, for example, amino acids, hydroxy acids, sugars and polyethylene glycol.

In a further aspect, the present invention provides a composition as defined above, for use as a medicament, a prophylactic or a diagnostic.

An advantage of the invention is that strong specific binding interactions can be achieved with conjugates in which the head groups are small in comparison to conventional biological receptors. If the head group is an oligo-peptide, for example, then the length of the peptide chain would not normally exceed ten amino acids and would preferably be six or less. Accordingly, compositions according to the present invention can be made far less immunogenic than their protein counterparts.

In accordance with this aspect of the invention, not only can the composition of the present invention be formulated to

interact with a ligand *in vitro* but also the composition can be used *in vivo*, optionally formulated with a suitable diluent, excipient or carrier in accordance with a suitable delivery route.

In a further aspect, the present invention provides use of a conjugate comprising a head group and tail group for the preparation of the composition as defined above.

There is further provided a method for producing a composition for interacting with a ligand, which method comprises:
(a) providing a plurality of distinct conjugates, each conjugate comprising a head group and a tail group; and (b) forming from the plurality of conjugates, by noncovalent association thereof, an assembly in which the tail groups aggregate hydrophobically and in which the conjugates exhibit freedom of motion relative to one another so that, in the presence of a ligand, at least two of the head groups are appropriately positioned to form an epitope capable of interacting with the ligand more strongly than each of head groups individually. Each conjugate is preferably as defined above.

The conjugates may be dispersed in aqueous phase by a variety of known methodologies for the preparation of lipid vesicles, including mechanical mixing, exposure to high shear forces, sonication, solvent dispersion or codissolution with detergents. Typically, the non-covalent supra-molecular assemblies formed thereby will be composed of several

different conjugates mixed together. Additional lipidic materials may optionally be added to alter surface properties, to aid in the dispersion of the conjugates, to stabilise the non-covalently associated assembly of conjugates, to aid in the presentation of head groups of the conjugates, or to permit the construction of vehicles which can be targeted by the epitopes formed upon random movement of the conjugates and appropriate positioning of the head groups within the assembly.

An important aspect of the method according to the present invention involves the step of identifying the plurality of conjugates which has the desired biolgical activity. In a preferred aspect, this step comprises

(i) selecting a set of conjugates with an array of head groups;

(ii) forming a non-covalent association therefrom, in which the tail groups aggregate hydrophobically and in which the conjugates exhibit freedom of motion with respect to one another;

(iii) assaying for sufficient interaction between the non-covalent association and the ligand;

(iv) optionally repeating steps (i) to (iii) using a set of conjugates with a modified array of head groups; and

(v) on finding sufficient interaction in step (iii), selecting the set of conjugates as the plurality of conjugates in step (a).

Examples of assays for "sufficient interaction" may include binding assays such as those utilising the ELISA principle for detection of association between antibody and antigen. Other suitable *in vitro* assays include modification of fluorescence of environmentally-sensitive membrane-bound fluorescent probes, precipitation reactions, enhancement or inhibition of enzyme activity etc. Assays relying on the abilty of materials to alter the behaviour of cells cultured *in vitro* may also be appropriate, such as assays for cell death, cell proliferation, apoptosis, inhibition or stimulation of cell-to-cell contact, secretion of cytokines or other soluble products, synthesis of specific m-RNA, intracellular vesicular transport, alteration of cell signalling processes etc. *In vivo* assays in whole animals or humans may also be carried out, for example incorporation of radiolabel into the supramolecular assemblies, followed by investigation of its subsequent distribution after administration by various routes.

According to this method a combinatorial approach is used in which a range of different supra-molecular assemblies (or "probes") is prepared, each containing a different combination of conjugates selected from a pre-synthesised bank. Selection of the appropriate conjugates may be based on known properties of the target ligand or may simply involve the use of a very wide range of head groups to

increase the probability that two or more of the head groups will form an epitope for the ligand. In this way, following the assay for sufficient interaction between the probe and the ligand as described above, the combination of conjugates found to be most effective may be modified by adding further head groups, removing some head groups, or both, and assaying the resultant probes once again for sufficient interaction. Eventually, the most favourable combination of head groups may be identified and selected for use in the composition.

The present invention therefore has a very clear advantage over traditional combinatorial chemistry. In combinatorial chemistry, the identification of the most favourable sequence for binding to a specific receptor must be carried out by synthesis of hundreds of possible combinations of different groups such as amino acids, in different orders, each one having to be tested for efficacy. This process is time-consuming, expensive and is limited by the nature of the chemistry which can be carried out in linking the different components together. In contrast, the present invention simply relies upon proximity of the head groups to provide association-derived epitopes. Once a set of conjugates has been synthesised, no further synthetic chemistry is required, only simple mixing of the conjugates to form the different probes by non-covalent association.

In a preferred simple embodiment, the present method uses conjugates having a single terminal amino acid linked via a spacer to a lipid tail group which can be combined simply by mixing in aqueous medium to form micelles in which different

amino acid side chains would be presented together in a multiplicity of different configurations. Accordingly, the need to present amino acids in a specific order, or with a specific spacing or orientation, is circumvented. On statistical grounds, a proportion of the individual amino acid sub-units will always be associated in an ideal configuration.

In one arrangement, each of the conjugates would have the linear structure: X-spacer-spacer-lipid-lipid, where X represents a single amino acid different for each of the distinct conjugates employed.

When seeking to construct epitopes composed of natural amino acids it is possible to simplify further the number of head groups for selection. One can categorise the amino acid residues found in natural proteinaceous materials into six fundamental classes preferably using in any one class one amino acid rather than all members of that class because of the increased spatial flexibility of amino acids in the terminal position of the head group. This has the effect of reducing considerably the total number of amino acids required for constructing the pre-synthesised bank of conjugates and thereby the total number of head groups used. The main classes of amino acids are set out in Table 1 below.

Table 1

Class	**Representative**
Abbreviation	

Hydrophobic	Leucine	L
Hydroxylic	Serine	S
Acidic	Glutamate	E
Amide	Glutamine	Q
Basic	Histidine	H
Aromatic	Tyrosine	Y

A number of strategies are available for identifying active combinations of amino acid-containing conjugates.

In one embodiment, a restricted number of conjugates is employed to form a range of distinct probes where each probe is an aqueous suspension of supra-molecular assemblies, each assembly consisting of selected conjugates mixed together, and each differing from the other as a result of the inclusion of a different additional conjugate as shown below where each of the letters given represents a conjugate with a different terminal amino acid:

Probe 1 A B C D
Probe 2 A B C E
Probe 3 A B C F
Probe 3 A B C G
.....
.....
Probe x A B C Z

Each of the probes is tested separately in the biological assays for sufficient binding as outlined above.

In a second simple embodiment, an initial probe can be constructed which contains a large number of different conjugates from the bank, and its efficacy compared with probes each lacking a different conjugate in turn, to determine which headgroups in the bank are essential, and which are redundant for the biological interaction being investigated. This approach is illustrated below:

Probe 1 A B C D E . . . Z
Probe 2 A C D E . . . Z
Probe 3 A B D E . . . Z
.....
Probe x A B C D E . . .

Combinations of the alternative approaches as outlined above can be made.

A knowledge of the target ligand may assist in designing a suitable starting array. For example, if the ligand is known to be basic, it would make sense to impart an acidic character to the conjugates by presenting them in the form where a free carboxyl group of the terminal amino acid is exposed. Introducing additional functionality by employing a particular amino acid as a spacer group adjacent to the terminal amino acid may also confer increased specificity. Where the involvement of, say. a short oligo-peptide sequence of known structure has already been implicated in binding to the target ligand, such a sequence may be incorporated into a conjugate to be included in the set of conjugates making up the composition.

In a final aspect, the present invention provides a method for producing a molecule for interacting with a ligand. The method comprises producing a composition according to one of the methods defined above; identifying the at least two head groups which form an epitope for the ligand in the composition; and producing a molecule incorporating the functional groups of the at least two head groups optionally spaced apart by one or more linker groups so that the molecule is capable of interacting with the ligand more strongly than each of the head groups individually.

Whilst the compositions of the present invention may themselves be useful in *in vitro* or *in vivo* systems perhaps to induce a biological response in a therapeutic, prophylactic or diagnostic method, in some circumstances a molecule may be produced based on the structure of the above compositions. By identifying the functional groups of the at least two head groups which form the epitope for the ligand a new molecule analogous to the composition may be produced containing the same or a similar epitope. The functional groups may, for example, be incorporated into a .single linear oligo-peptide possibly with one or more linker groups to space the functional groups apart.

Brief Description of the Drawings

The invention will now be described in further detail, by way of example only, with reference to the following Examples and the attached drawings, in which:

FIGURE 1 shows a schematic representation of the surface of a supra-molecular assembly, and how such a composition according to the present invention binds to a target ligand; and

FIGURE 2 shows a schematic representation of the surface of a supra-molecular assembly composed of two non-identical conjugates whose headgroups consist of short-chain linear peptides.

Detailed Description of the Invention

Referring to Figure 1, a section 1 of a composition according to the present invention is shown in the form of a micelle in which the head groups 2 and tail groups 3 together form conjugates 4 (Fig. 1A). A target ligand 5 is presented to the composition 1. Because the conjugates are movable, a rearrangement occurs (Fig. 1B) to allow positioning of the head groups 2 to bind the target ligand 5. Referring to Figure 2, a section of a composition according to the present invention is shown in the form of a supramolecular assembly, in which binding of a ligand to the surface of the assembly is brought about by the creation of an epitope constructed via the non-covalent association of two conjugates composed of short-chain peptides (A), this epitope being able to interact with the ligand more strongly than either of the individual conjugates in isolation (B). The same principle applies for headgroups containing structures other than amino acids.

EXAMPLES

In the examples given below, the standard convention for representation of amino acids by single letters of the alphabet is employed, except that in all cases the letter refers to conjugates as described above in which that particular amino acid occupies the terminal position in the peptide chain. In the examples described here, the lipid comprises two amino acids linked via a peptide bond, in which both of the amino acids are glycine analogues, where in each case the alpha hydrogen has been replaced by a linear hydrocarbon chain containing either 12 or 14 carbons. Linkages between the headgroup and spacer and the spacer and lipid are all via peptide bonds. The headgroup bears a free amino group and the free end of the lipid bears a $CONH_2$ group. The structure of each conjugate is thus: NH_2-headgroup-spacer-amino acid (C_{14} side chain)-amino acid (C_{12} side chain)-$CONH_2$.

Example 1: Stimulation of TNF secretion from macrophages

1. Individual conjugates E, Y, Q, S & H (linked to lipid via a serine-glycine spacer) were prepared as solutions in methanol/dichloromethane 1:1 at a concentration of 5mg/ml.

2. Solutions of the conjugates were dispensed into 7ml glass vials in equal proportions, to give a final volume of 400ul (2mg of solid) in all vials, as shown in the example overleaf. In cases where the volume of organic solution available was insufficient, adjustment was made at a later stage, when the quantity of water added for reconstitution was reduced accordingly, as shown.

3. The contents of all vials were dried down under a stream of nitrogen, then exposed to a vacuum of at least 1mbar overnight in a lyophiliser.

4. On the following day, distilled water was added in volumes as indicated in the table overleaf, to give a final concentration in all vials of 1mg/ml. The vials were capped, warmed to 37 degC and bath-sonicated until clarity was achieved.

5. The samples were then applied to wells of 24-well cluster plates into which cells of the J774A-1 macrophage cell line had been plated (5 x 10^4cells/ml/well). Volumes of 100ul and 10ul of sample were added to individual wells, and the cells were incubated overnight at 37 degC in an atmosphere of 5% CO_2/air.

6. The following day, duplicate volumes of 50ul of supernate were taken from each well and measured for TNF concentration in a capture ELISA assay. Results obtained are shown in the table below.

	Volume of conjugate dispensed					Volume of water added
	E	Y	Q	S	H	
E	260ul					1.3ml
Y		400ul				2.0ml
Q			310ul			1.55ml
S				360ul		1.8ml
H					400	2.0ml
EY	200ul	200ul				2.0ml
EQ	200ul		200ul			2.0ml
ES	200ul			200ul		2.0ml
EH	200ul				200ul	2.0ml
YQ		200ul	200ul			2.0ml
YS		200ul		200ul		2.0ml
YH		200ul			200ul	2.0ml
QS			200ul	200ul		2.0ml
QH			200ul		200ul	2.0ml
SH				200ul	200ul	2.0ml
QSH			133ul	133ul	133ul	2.0ml
YSH		133ul		133ul	133ul	2.0ml
YQH		133ul	133ul		133ul	2.0ml
YQS		133ul	133ul	133ul		2.0ml
ESH	133ul			133ul	133ul	2.0ml
EQH	133ul		133ul		133ul	2.0ml
EYH	133ul	133ul			133ul	2.0ml
EYS	133ul	133ul		133ul		2.0ml
EYQ	133ul	133ul	133ul			2.0ml
EQS	133ul		133ul	133ul		2.0ml
EYQS	50ul	50ul	50ul	50ul		1.0ml
EYQH	50ul	50ul	50ul		50ul	1.0ml
EYSH	50ul	50ul		50ul	50ul	1.0ml
EQSH	50ul		50ul	50ul	50ul	1.0ml
YQSH		50ul	50ul	50ul	50ul	1.0ml
EYQSH	40ul	40ul	40ul	40ul	40ul	1.0ml

	OD_{450} in J774 supernates		
	100ug	10ug	0ug
E	0.628	0.098	0.013
Y	0.313	0.053	
Q	0.083	0.015	
S	0.348	0.143	
H	0.632	0.206	
EY	0.198	0.027	
EQ	0.113	0.022	
ES	0.211	0.225	
EH	0.167	0.037	
YQ	0.245	0.034	
YS	0.786	0.363	
YH	0.541	0.133	
QS	0.212	0.025	
QH	0.135	0.027	
SH	0.515	0.177	
QSH	0.253	0.032	
YSH	0.712	0.229	
YQH	0.290	0.020	
YQS	0.519	0.119	
ESH	0.380	0.246	
EQH	0.107	0.026	
EYH	0.254	0.042	
EYS	1.289	0.355	
EYQ	0.191	0.064	
EQS	0.209	0.027	
EYQS	0.777	0.206	
EYQH	0.224	0.067	
EYSH	0.262	0.146	
EQSH	0.149	0.185	
YQSH	0.319	0.045	
EYQSH	0.375	0.073	

It can be seen that some, but not all, of the combinations of different headgroups elicit strong biological responses, indicating that the response is specific to those particular combinations. The example illustrates the way in which the conjugates described can be employed in the combinatorial

approach to identify efficacious combinations for the purpose of eliciting a desired biological response.

Example 2: TNF secretion from macrophages

Comparison of supra-molecular assemblies containing a mixture of conjugates, with a mixture of supra-molecular assemblies each containing a single conjugate

Samples were prepared as described in Example 1, with or without the inclusion of additional lipidic materials as described below. The combination of conjugates Y, S and L was chosen since this combination was a good performer in the experiment described in Example 1.

Probes containing phosphatidyl choline were prepared at a ratio of phospholipid to conjugate of 2:1 wt/wt.

Probes containing octyl glucoside were prepared at a ratio of glycolipid to conjugate of 1:1 wt/wt.

Results shown in the table below are optical densities at 450nm of TNF ELISAs conducted on 18 hour culture supernatants. The concentration of conjugate in the wells was 10ug/ml

	OD_{450} of TNF ELISA
EYS	0.390
E+Y+S	0.059
medium control	0.000
EYS:OG	0.559
(E+Y+S):OG	0.193
OG control	0.228
EYS:PC	0.320
(E+Y+S):PC	0.130
PC control	0.081

This example shows that combinations of the conjugates can elicit biological responses either when presented alone, or when presented in conjunction with other lipids, such as phospholipids or lipid sugars. It also shows that for efficacy to be manifested, it is important for all of the conjugates to be presented in combination on the same supra-molecular assembly, and that activity is not observed if the same conjugates are presented together at the same time, but separated on different supra-molecular assemblies. This suggests that it is important to present the conjugates in close proximity to each other, in order to permit the formation of epitopes formed by non-covalent association of the conjugates, which can participate in specific binding with cell-surface receptors.

Example 3: Enhancement of Oral Uptake

1. Individual conjugates L, S, E & Q (conjugated to lipid via a tyrosine-glycine spacer) were prepared as solutions in benzyl alcohol at a concentration of 10mg/ml.

2. 75ul of ^{14}C-cholesterol oleate (3.7MBq/ml in toluene) was dispensed into four 7ml glass screw-capped vials and dried down under a stream of nitrogen.

3. 400ul of each of the solutions in (1) was added to one of the vials in (2) and shaken overnight at room temperature.

4. Solutions of the conjugates were dispensed into 7ml glass vials in equal proportions, to give a final volume of 80ul (0.8mg of solid) in all vials, as shown in the example below.

	L	S	E	Q
L	80ul	-	-	-
S	-	80ul	-	-
E	-	-	80ul	-
Q	-	-	-	80ul
LS	40ul	40ul	-	-
LE	40ul	-	40ul	-
LQ	40ul	-	-	40ul
SE	-	40ul	40ul	-
SQ	-	40ul	-	40ul
EQ	-	-	40ul	40ul
LSE	27ul	27ul	27ul	-
LSQ	27ul	27ul	-	27ul
LEQ	27ul	-	27ul	27ul
SEQ	-	27ul	27ul	27ul
LSEQ	20ul	20ul	20ul	20ul

5. 2ml of distilled water was added to each of the vials with vortexing. The vials were then capped and bath-sonicated for 20 minutes.

6. The samples were then frozen in liquid nitrogen and lyophilised overnight.

7. The following day, each vial was reconstituted with 2ml of distilled water and sonicated again until clear dispersions were achieved.

8. The samples were administered by oral gavage to Balb/c female mice (20-25g weight - four mice per group) at a dose of 0.3ml per animal.

9. 75ul heparinised blood samples were taken by tail venupuncture at 45, 90 and 180 minutes after administration.

10. Each sample was diluted in 0.5ml of PBS, which was then centrifuged, and 0.4ml of the supernate was transferred to a scintillation vial to which 2ml of Optiphase Hisafe 3 (Wallac) was added with mixing.

11. Activity in the samples was measured in a scintillation counter.

Percentage uptake was estimated on the basis of a 2ml blood volume, of which 1ml was assumed to be plasma.

Results are shown in the table below.

	% uptake in bloodstream		
	45mins	90mins	180mins
L	0.90	1.39	0.61
S	1.12	1.14	0.81
E	0.85	1.55	0.79
Q	1.40	3.00	0.81
LS	2.87	2.38	0.66
LE	2.59	2.22	0.49
LQ	5.05	2.15	0.45
SE	4.21	1.66	0.70
SQ	4.67	1.45	0.67
EQ	3.72	2.65	0.59
LSE	1.91	1.20	0.97
LSQ	6.23	1.90	0.80
LEQ	2.77	1.73	0.98
SEQ	3.06	1.52	0.63
LSEQ	2.45	1.74	0.81

It can be seen that some, but not all, of the combinations of different headgroups enhance uptake of label via the oral route, indicating that the response is specific to those particular combinations. The example illustrates the way in which the conjugates described can be employed in the combinatorial approach to identify efficacious combinations capable of acting as targeting ligands.

Example 4: ELISA Fc binding

1. 100ul of goat IgG (1mg/ml) was added to 20ml of PBS and 100ul was placed in each well of a flat-bottomed microtitre plate.

2. The plate was incubated for several days at +4degC.

3. 2mg of each of the conjugates Y, F, W, L, S, E, Q & R (each linked to lipid via a serine-glycine spacer) were weighed into 1ml glass vials and 200ul of benzyl alcohol added to give solutions of each conjugate at a concentration of 10mg/ml.

4. The solutions were dispensed in 7ml glass screw-capped vials as follows:

Vial No.	Y	F	W	L	S	E	Q	R
1	20ul	20ul	20ul	-				
2	20ul	20ul	-	20ul				
3	20ul	-	20ul	20ul				
4	-	20ul	20ul	20ul				
5					20ul	20ul	20ul	-
6					20ul	20ul	-	20ul
7					20ul	-	20ul	20ul

5. The contents of each vial were mixed well by vortexing, then 1.5ml of distilled water was added to each vial.

6. The vials were capped and bath-sonicated for five minutes to give crystal clear dispersions.

7. The plate from step (2) was washed in PBS/0.02% Tween 20 and then blocked by incubating for one hour with 1% BSA in PBS (300ul/well).

8. The plate was then washed as before, and 100ul of sample from each of the vials in step (6) was added to wells in column (1) of rows (1) to (7). Row (8) was left as a blank control.

9. Doubling dilutions were performed across the plate by transferring 100ul from wells in column (1) to the adjacent well on the same row in column (2) and mixing, then transferring 100ul to the next column as before, etc.

10. The plate was then incubated overnight at +4 degC.

11. The following day, the plate was washed as before and 100ul of commercial horseradish peroxidase-IgG conjugate (diluted 1/1000 in PBS) was added to each well and incubated at room temperature for 40 minutes.

12. The plate was then washed again, and 100ul of OPD substrate for peroxidase was added to each well and incubated at room temperature for 30 minutes.

13. 20ul of 3M sulphuric acid was then added to each well to stop the reaction.

14. The optical density of each of the wells was measured at 450nm on a plate reader, and the results obtained, after adjustment for background, are recorded below.

Sample		1 in 4	1 in 8	1 in 16	1 in 32	1 in 64
1	YFW	0.001	0.039	0.048	0.053	0.083
2	YFL	1.504	1.484	1.325	0.723	0.051
3	YWL	0.803	0.192	0.022	0.023	0.060
4	FWL	1.034	0.778	0.208	0.031	0.034
5	SEQ	0.029	0.041	0.055	0.057	0.091
6	SER	0.013	0.030	0.044	0.062	0.075
7	SQR	0.000	0.045	0.031	0.054	0.065

It can be seen that maximal binding is achieved with samples 2, 3 and 4 (ie combinations YFL, YWL, and FWL).

It can be seen that some, but not all, of the combinations of different headgroups enter into strong binding interactions, indicating that the response is specific to those particular combinations. The example illustrates the way in which the conjugates described can be employed in the combinatorial approach to identify efficacious combinations for the purpose of eliciting a desired binding interaction.

CLAIMS:

1. A composition for interacting with a ligand, which composition comprises a non-covalent association of a plurality of distinct conjugates, each conjugate comprising a head group and a tail group, wherein the tail groups of the conjugates form a hydrophobic aggregation and the conjugates are movable within the association so that, in the presence of a ligand, at least two of the head groups are appropriately positioned to form an epitope capable of interacting with the ligand more strongly than each of head groups individually.

2. A composition according to claim 1, wherein each conjugate has a head group selected from: an amino acid or peptide; a peptide analogue; a mono- or poly-saccharide; a mono- or poly-nucleotide; a sterol, a water-soluble vitamin; a porphyrin or haem nucleus; a metal ion chelate; a water-soluble drug; a hormone; and an enzyme substrate.

3. A composition according to claim 2, wherein each head group comprises an amino acid.

4. A composition according to claim 3, wherein each head group comprises a peptide comprising the amino acid.

5. A composition according to claim 3 or claim 4, wherein the head groups which form the epitope comprise terminal amino acids selected from at least two of the following:

hydrophobic amino acids, hydroxylic amino acids, acidic amino acids, amide amino acids, basic amino acids, and aromatic amino acids.

6. A composition according to any one of the preceding claims, wherein each tail group is the same or different and comprises a lipophilic group selected from a straight or branched-chain fatty acid, alcohol or aldehyde having at least 8 carbon atoms; a lipidic amino acid analogue; a prostaglandin; a leukotriene; a mono-or di-glyceride; a sterol; a sphingosine or ceramide derivative; and a silicon or halogen-substituted derivative of such a lipophilic group.

7. A composition according to claim 6, wherein each lipophilic group comprises a C_{10} to C_{14} fatty acid.

8. A composition according to any one of the preceding claims, wherein each conjugate further comprises a spacer group linking the head group to the tail group.

9. A composition according to claim 8, wherein the spacer group is hydrophilic.

10. A composition according to claim 8 or claim 9, wherein the spacer group comprises an amino acid, a hydroxy acid, a sugar or a polyethylene glycol.

11. A composition according to any one of the preceding claims, wherein the non-covalent association comprises a lamellar structure, a micelle or a liposome.

12. A composition according to any one of the preceding claims, for use as a medicament, a prophylactic or a diagnostic.

13. Use of a conjugate comprising a head group and a tail group, for the preparation of a composition according to any one of the preceding claims.

14. Use according to claim 13, wherein the head group is selected from: an amino acid or peptide, a peptide analogue; a mono- or poly-saccharide; a mono- or polynucleotide; a sterol, a water-soluble vitamin; a porphyrin or haem nucleus; a metal ion chelate; a water-soluble drug; a hormone; and an enzyme substrate.

15. Use according to claim 14, wherein the head group comprises an amino acid.

16. Use according to claim 15, wherein the head group comprises a peptide comprising the amino acid.

17. Use according to claim 15 or claim 16, wherein the amino acid comprises a terminal amino acid selected from hydrophilic amino acids, hydroxylic amino acids, acidic amino acids, amide amino acids, basic amino acids, and aromatic amino acids.

18. Use according to any one of claims 13 to 17, wherein the tail group comprises a lipophilic group selected from a straight or branched-chain fatty acid, alcohol or aldehyde

having at least 8 carbon atoms; a lipidic amino acid analogue; a prostaglandin; a leukotriene; a mono- or di-glyceride; a sterol; a sphingosine or ceramide derivative; and a silicon or halogen-substituted derivative of such a lipophilic group.

19. Use according to claim 18, wherein the lipophilic group comprises a C_{10} to C_{14} fatty acid.

20. Use according to any one of claims 13 to 19, wherein the conjugate further comprises a spacer group linking the head group to the tail group.

21. Use according to claim 20, wherein the spacer group is hydrophilic.

22. Use according to claim 21, wherein the spacer group comprises an amino acid, a hydroxy acid, a sugar or a polyethylene glycol.

23. A method for producing a composition for interacting with a ligand, which method comprises:

(a) providing a plurality of distinct conjugates, each conjugate comprising a head group and a tail group; and

(b) forming from the plurality of conjugates a non-covalent association thereof, in which the tail groups aggregate hydrophobically and in which the conjugates are movable so that, in the presence of a ligand, at least two of the head

groups are appropriately positioned to form an epitope capable of interacting with the ligand more strongly than each of head groups individually.

24. A method according to claim 23, wherein each conjugate is as defined in any one of claims 13 to 22.

25. A method according to claim 23 or claim 24, wherein the non-covalent association comprises a lamellar structure, a micelle or a liposome.

26. A method according to any one of claims 23 to 25, wherein the step of providing the plurality of conjugates comprises

(i) selecting a set of conjugates with an array of head groups;

(ii) forming a non-covalent association therefrom, in which the tail groups aggregate hydrophobically and in which the conjugates are movable;

(iii)assaying for sufficient interaction between the non-covalent association and the ligand;

(iv) optionally repeating steps (i) to (iii) using a set of conjugates with a modified array of head groups; and

(v) on finding sufficient interaction in step (iii) selecting the set of conjugates as the plurality of conjugates in step (a).

27. A method according to claim 26, wherein the array of head groups comprises (i) at least one terminal amino acid from each of the following classes of amino acid:

hydrophobic amino acids, hydroxylic amino acids, acidic amino acids and amide amino acids; and (ii) at least two further terminal amino acids comprising at least one basic amino acid and at least one aromatic amino acid, or at least two basic amino acids or aromatic amino acids.

28. A method according to claim 27, wherein the modified array of head groups used in step (iv) comprises the array of head groups used in steps (i) to (iii) in which the at least two further terminal amino acids are different from those used in steps (i) to (iii).

29. A method according to claim 26, wherein the array of head groups comprises (i) at least one terminal amino acid from each of the following classes of amino acid:
hydrophobic amino acids, hydroxylic amino acids, acidic amino acids, amide amino acids, basic amino acids and aromatic amino acids.

30. A method according to claim 29, wherein the modified array of head groups used in step (iv) comprises the array of head groups used in steps (i) to (iii) in which the at least

one terminal amino acid from one of the classes of amino acid is either absent or replaced by a charged version thereof.

31. A method for producing a molecule for interacting with a ligand, comprising:

(1) producing a composition according to the method of any one of claims 23 to 30;

(2) identifying the at least two head groups which form an epitope for the ligand in the composition; and

(3) producing a molecule incorporating the functional groups of the at least two head groups optionally spaced apart by one or more linker groups so that the molecule is capable of interacting with the ligand more strongly than each of the head groups individually.

32. A molecule for interacting with a ligand, wherein the molecule is obtained by the process as defined in claim 31.