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Synthesis of pseudocyclic peptide of protein S of SARS-CoV-2 and its interaction with diagnostically significant antibodies of COVID-19 patients

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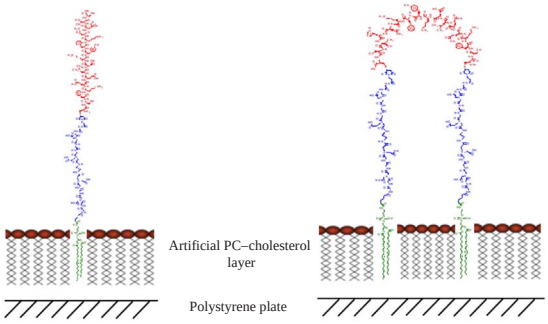
An approach to the design of pseudocyclic peptide epitopes is proposed: lipid tails are attached to both C- and N-termini of the peptide through hydrophilic spacers; thanks to two lipid anchors, the conjugate is inserted into the membrane at two sites simultaneously, and the peptide becomes conformation-constrained. Despite the presence of two long lipid regions, the high hydrophilicity of the spacer makes the entire structure water-soluble, which is crucial for integration into living cells.



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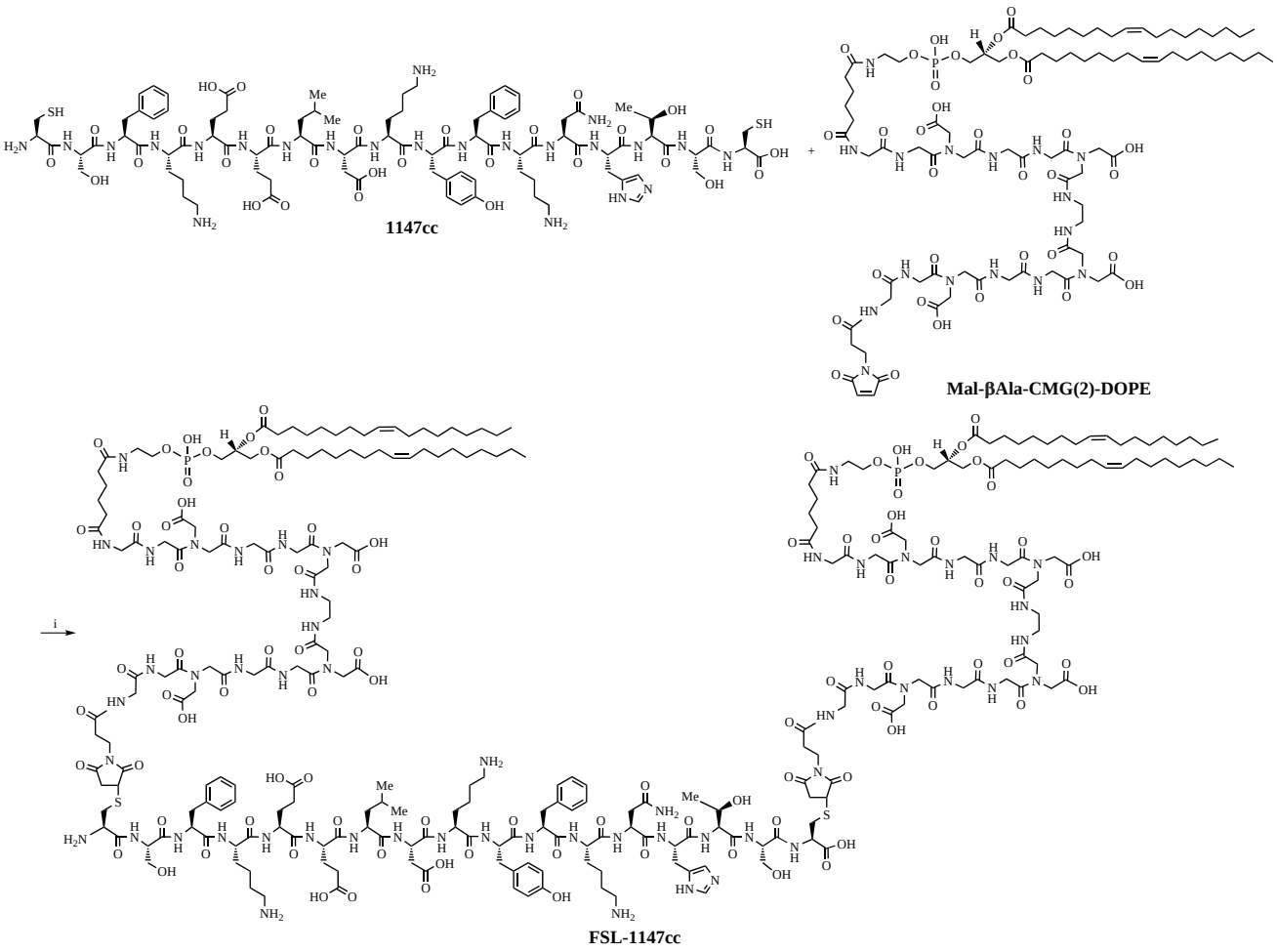
Screening a dozen of peptides, fragments of the theoretically predicted antigenic determinants of SARS-CoV-2 protein S, showed that one of them, the 15 a.a. region 1147, allows for the identification of COVID-19 patients with high specificity and sensitivity.[1] It turned out to be unexpected and paradoxical that region 1147 is located in the protein stalk region, close to the membrane of the viral particle, *i.e.*, is, most likely, spatially shielded from interaction with antibodies. At the same time, this region of the trimeric protein is conserved with respect to mutations, and in similar trimeric hemagglutinins of other viruses this region turned out to be a broadly protective immunogen.[2–5]

To model the interaction of antibodies with the 1147 epitope, we designed its pseudo-cyclic form. Peptides are made pseudocyclic either due to a specifically organized system of hydrogen bonds, or using a template to which a linear peptide is conjugated by two termini.[6] In the present study, we designed an artificial system based on the embedding of lipidated peptide into phosphatidylcholine (PC)/cholesterol layer: we synthesized derivative of the 1147 peptide with two DOPE (dioleoylphospho-ethanolamine) tails attached at different ends of the peptide. It is believed that cyclic peptides, due to restriction of conformational flexibility, are able to interact better than linear ones with proteins targeted at them.[7] The idea behind our design was that both DOPE residues would be inserted into the membrane, thus forming a conformation-constrained epitope as a pseudo-cycle.

Synthesis and purification of starting peptides [C]SFKEELDKYFKNHTS and [C]SFKEELDKYFKNHTS[C] (1147 cc), as well as FSQILPDPSKPSKRSFIEGGG[C] (peptide-802 as negative control) were carried according to known procedure.[8] According to MS data, all measured masses of peptides were in a good compliance with the calculated ones. Synthesis of mono-DOPE conjugates, generally called FSLs (Function-Spacer-Lipid), has been described earlier.[9] A construct FSL-1147cc with two lipophilic anchors was synthesized similarly by conjugation of the 17-mer peptide (>95% purity) with two moles of spacer-DOPE fragment having a maleimide group, as shown in Scheme 1. Repeating motif of the spacer, designated as CMG(2), is a triglycine, one of the glycine residues of which at the nitrogen atom is modified with a $–CH_2COOH$[9,10] group. This design suggested that, due to the two lipid tails, the FSL-1147cc construct would integrate into the membrane at two points, while the negative charged rigid CMG(2) bridges would ensure that the peptide fragment did not directly contact the artificial membrane, or the plasma membrane in the case of insertion into a living cell.

Next, we studied the interaction of antibodies isolated from the blood of patients with COVID-19 (affinity isolated) with linear peptide 1147, linear peptide 802 (negative control) and pseudocyclic peptide 1147cc. For this purpose, the constructs were built into a lipid layer formed from PC and cholesterol in the wells of an ELISA plate (polystyrene) using two insertion modes.[9] The first, one-step methodology consists of applying a solution of PC, cholesterol and FSL (the proportion of the latter varies) in ethanol onto the plastic, and the second, two-step methodology involves the formation of a PC–cholesterol layer, and the subsequent insertion of FSL (in varying concentrations) from an aqueous solution. In addition, all three constructs (and also the fourth, namely FSL with PEG instead of peptide – as a second negative control) were analyzed similarly without prior



Scheme 1 *Reagents and conditions*: i, DMSO, room temperature.

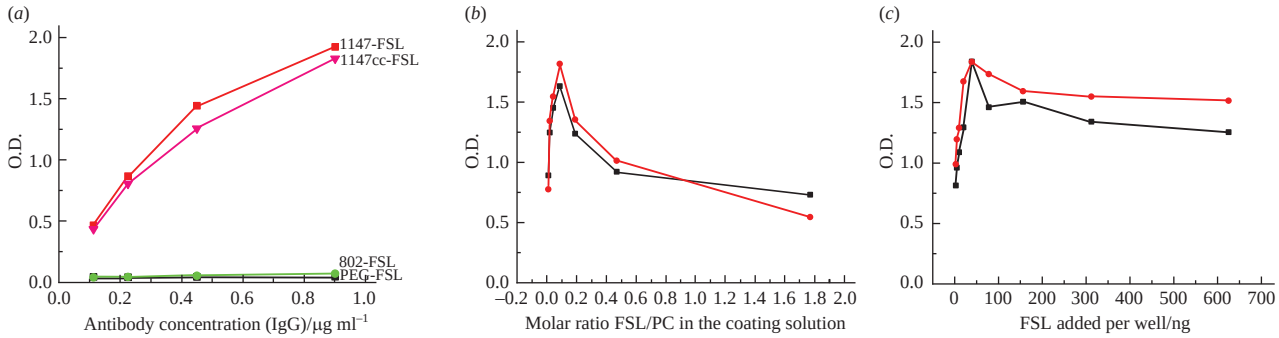


Figure 1 Binding of affinity isolated anti-1147 antibodies to differently presented 1147 peptide, ELISA. (*a*) FSL-1147 and FSL-1147cc were coated directly on plastic surface (PolySorp 96-well plates), FSL-802 and FSL-PEG as negative controls. (*b*) FSL-1147 (black squares) and FSL-1147cc (red circles) were inserted into PC-cholesterol layer using one-stage procedure. (*c*) FSL-1147 (black squares) and FSL-1147cc (red circles) were inserted into the lipid layer using two-stage procedure.

application of PC and cholesterol to polystyrene PolySorp plates, which are used in ELISA for the adsorption of hydrophobic molecules, believing that with this experimental design the molecules are sprawled on the surface of the plastic, when the formation of a peptide pseudocycle is unlikely.

Figure 1(*a*) confirms the specificity of antibodies towards peptide 1147. Figures (*b*) and (*c*) indicate that the 1147cc and 1147 constructs inserted in the lipid layer as antigens are practically indistinguishable, *i.e.*, the pseudocyclic design does not lead to improved interaction with antibodies. This is true both at the initial part of the curves, in other words, where density of the construct is low, and at high density of the inserted construct. Please note that in graph (*c*) antibody binding sharply reaches a plateau, which corresponds to the limit of the degree of insertion of the construct into the pre-existing lipid layer, while in graph (*b*) after a similar sharp rise, the intensity of antibody interaction decreases, which is interpreted as their constrained interaction with high surface density peptide. All observed patterns suggest that antibodies (both IgG, and even more so bulky IgM) will bind poorly or not at all to epitope 1147 of the protein S in composition of the whole virus.

In general, we believe that the proposed approach to the design of conformationally constrained peptide epitopes through double peptide anchoring is significantly simpler than any approach based on covalent cyclization.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2024.06.009.

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