**Abstract**

对目前及未来发展方向的review

**Introduction**

固有特性：1）刺激先天免疫应答和适应性免疫应答innate and adaptive immune responses

2）颗粒结构有利于抗原提呈细胞的摄取

3）self-adjuvanting 自调节

**Structurally Diverse VLPs**

Enveloped & Non-enveloped (Capsid-based，含HA &/ NA)

可无细胞合成 大多在高等真核生物细胞中合成（e.g.酵母，昆虫）

**Functionally Versatile VLPs**

Prophylactic VLP immunogen： enveloped VLPs + viral proteins from unrelated viruses / multiple serotypes of the same virus → vaccine design

Novel antigens are placed on the surface of VLPs either by molecular fusion or chemical conjugation → polyvalent antibody response（e.g. HPV

Delivery vessels（Packaged VLPs）→ 基因/药物治疗载体

**Computational VLP-Based Vaccine Design (and Redesign)**

1.数据库：VIPERdb

启发：

结构表位预测

better structural epitope prediction

结构测定技术：X-ray crystallography / cryo-electron microscopy

↓

病毒表面肽表达的表征

Limitation：未考虑生物系统动力学的静态构象；结晶结构可能不能代表蛋白质在其自然溶液条件下的状态

2.计算机：指导实验和辅助数据解释 → 发展至今：bioinformatics and 3D structure prediction

Computer modeling of VLPs has largely focused on the self-assembly kinetics of VLPs, directed at minimizing protein aggregation during processing

建模限制：数据体积庞大 → 解决方法：Multiscale models

利用分子动力学(MD)模拟研究了HPV VLPs亚基与衣壳表面暴露环的构象变化及其对免疫原性的影响

3.生物信息学分析（基于系统发育）被用来优化VLPs的表位

用流感表位在FHV VLPs上表达的三维结构同源建模预测实验免疫学结果（Three-dimensional structure homology modeling of inﬂuenza epitopes for presentation on FHV VLPs was employed to predict and understand experimental immunological results）

对HIV表位进行MD模拟，以预测抗体结合的表位同源构象 → highlights that the length and hydrophobicity of the epitope and the site of epitope insertion are crucial for achieving the greatest structural similarity to the native HIV epitope

VLP结构对预期免疫结果十分重要：与天然表位结构越接近，免疫应答质量越高

计算机建模可以利用一系列计算工具对肽或蛋白序列的未知结构进行有根据的预测，这些

信息适用于VLP设计、重设计或实验数据解释

**Current and Emerging Paradigms for VLP Production**

纯化困难： 颗粒大小

↓ 内部：封装入宿主蛋白/DNA

驱动VLP结构修改 外部：与宿主蛋白结合

常用方法：Density gradient ultracentrifugation 密度梯度超离心，但无经济拓展性 → 改进chromatographic media 色谱介质

细胞内组装原理尚不清楚，比较不可控。故体外拆卸、重组较为重要

基于大肠杆菌系统的无细胞组装在经济效益方面表现更好，高效率无污染。this expression system supports the building of modular VLPs and capsomeres to target highly mutable and variable pathogens, providing for rapid response during epidemic sand pandemics and at low cost for neglected diseases

Polyomavirus(多瘤病毒) capsid protein VP1 to present antigenic modules of pathogens, 模块化平台

**VLP Characterization**

功能性与物理性质：大小、多分散性 评价疫苗的安全性与效力

全面性质：氨基酸组成、分子量和VLP纯度的测定分析，用Mass spectrometry (MS)质谱分析、SDS-PAGE、reversed-phase high performance liquid chromatography (RPHPLC)反相高效液相色谱法、Western Blot

VLP形态和状态通过：超离心法(AUC)、密度梯度超离心法和透射电镜(TEM)获得。使用低温电子显微镜和原子力显微镜(AFM)的其他可视化技术在样品处理过程中由于快速冷冻而导致结构变形的可能性较小，而在溶液中进行分析的可能性较小。Methods such as enzyme-linked immunosorbent assay (ELISA) , dot blot, immune precipitation or immune diffusion assay are often used to analyze antibody binding to VLPs.

**Formulation of VLP Vaccines**

安全运输与存储通过adjuvants and excipients保障，其中铝盐是人类最广泛使用的佐剂，可增强抗原的免疫原性。有些物质enhance antigen-speciﬁc activation of T-cells。配方中还有防腐剂、缓冲剂等。赋形剂，如甘油、壳聚糖谷氨酸盐和甘氨酸，也被报道对VLPs具有显著的稳定作用。

新配方：(i)减少剂量，(ii)提高免疫原性，(iii)取消冷链，(iv)提高长期储存的稳定性

**Innovative VLP-Inspired Vaccines**

the engineering of viral structural proteins to create novel immunogenic structures to tailor immunogenicity of VLPs towards heterologous pathogens

Accumulating evidence demonstrates that effective immunogens can be generated through engineering of the capsid proteins such that assembly into VLPs is precluded. 降低VLP生产的复杂性

Assembly-incompetent capsomeres of papillomavirus and polyomavirus, produced in bacterial expression system, are suitable for heterologous epitope presentation as well as immunization against their cognate virus. 细菌表达系统中无装配能力的病毒适合用作于其同源病毒的异种表位表达和免疫。capsomeres作为VLPs的结构构件，往往比VLPs稳定性更高

负载VLP可包裹特异性抗原

Enveloped VLPs have proved especially useful in that they can easily be targeted to speciﬁc cell types through the insertion of targeting domains as fusions to glycoproteins in a manner similar to pseudo typing. 它们可以很容易地通过插入目标结构域作为糖蛋白的融合物而被定位到特定的细胞类型

**Concluding Remarks**

One signiﬁcant challenge for heterologous epitope presentation on VLPs is producing antigenic epitopes that are structurally similar to those found on the parental pathogens.

Enhanced computer modeling to predict the structure of inserted epitopes on a VLP surface, to guide the design of efﬁcacious VLPs, is urgently needed. 迫切需要增强的计算机建模来预测VLP表面上插入的表位结构，从而指导有效的VLP设计

这些方法将大大减少疫苗开发时间和与当前VLP生产相关的高疫苗成本