

Supporting Information for

**Preparation of Viromimetic Rod-Like Nanoparticle Vaccine (RLNVax) and Study  
of Their Humoral Immune Activation Efficacy**

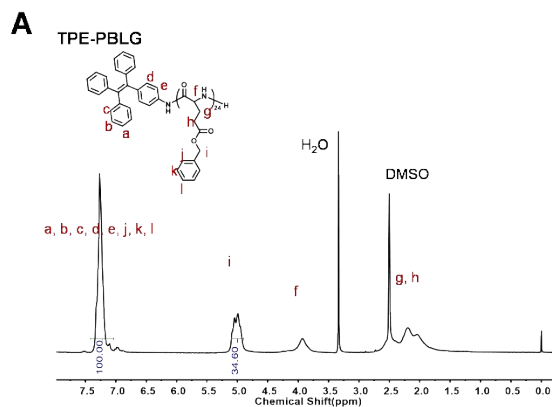
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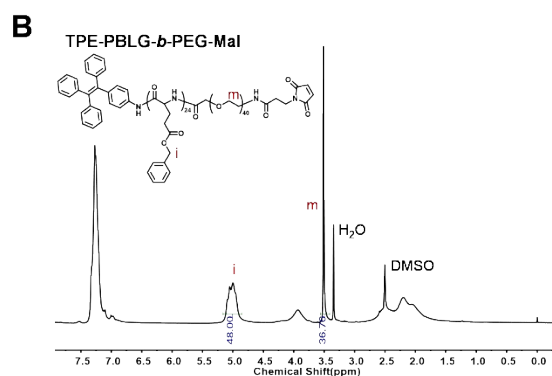
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Assuming the degree of polymerization of PBLG is  $n$ , the following relationship can be established:

$$\frac{A_{abcdeijkl}}{A_i} = \frac{19 + 5n}{2n}$$

Then the degree of polymerization  $n$  can be calculated by quantifying the peak areas in the proton nuclear magnetic resonance (NMR) spectrum.



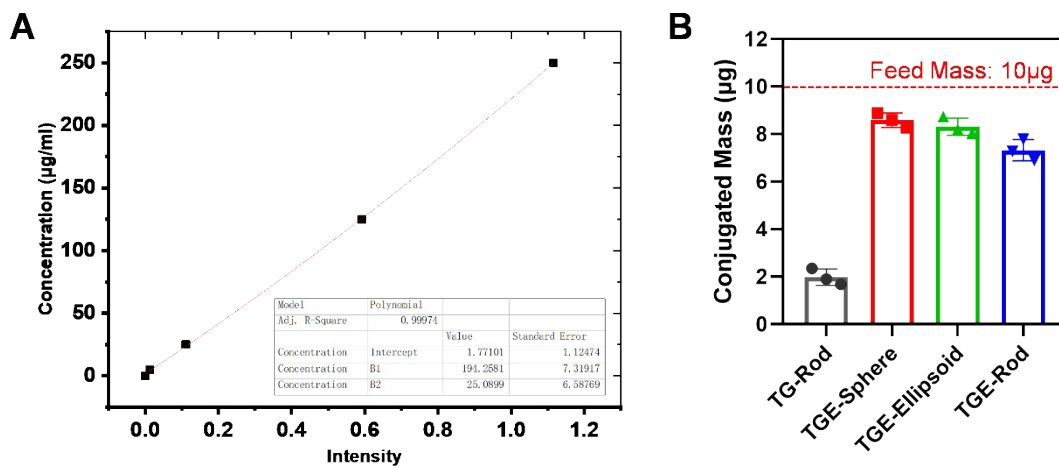
For PBLG with a degree of polymerization of 24 and PEG<sub>2k</sub> with a degree of polymerization of 40, the modification efficiency of PEG can be calculated using the following formula:

$$E\% = \frac{24 \cdot 2 \cdot A_m}{40 \cdot 4 \cdot A_i} \times 100\%$$

**Figure S1. Calculation of the Degree of Polymerization of TPE-PBLG and the Modification Efficiency of PEG in TPE-PBLG-*b*-PEG-Mal.**

(A) The degree of polymerization of PBLG was determined using <sup>1</sup>H NMR spectroscopy. When the integral of the reference peak ( $A_{abcdeijkl}$ ) is normalized to 100, the integral of the peak ( $A_i$ ) is 34.60. Thus, the degree of polymerization of PBLG is calculated to be 24 using the appropriate formula.

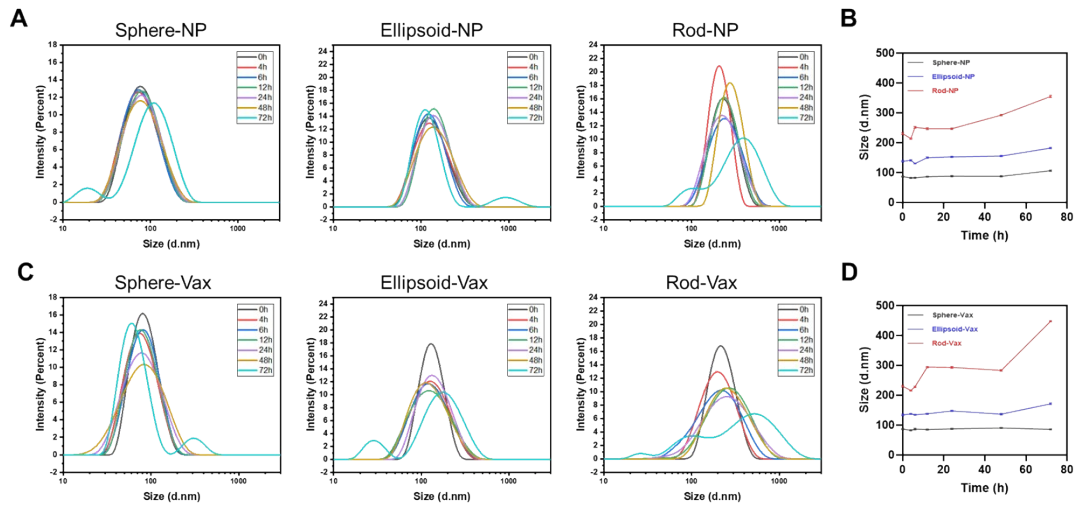
(B) The modification efficiency of PEG in TPE-PBLG-*b*-PEG-Mal was determined using <sup>1</sup>H NMR spectroscopy. When the integral of the reference peak ( $A_m$ ) is normalized to 48, the integral of the peak ( $A_i$ ) is 36.76. Therefore, the modification efficiency of PEG in TPE-PBLG-*b*-PEG-Mal is calculated to be 23% using the appropriate formula.



**Figure S2. Quantification of OVA Protein Modification on the Surface of Nanoparticles of Different Shapes Using the BCA Protein Assay.**

(A) Standard calibration curve of protein concentration versus absorbance intensity in the BCA protein assay (absorption wavelength: 562 nm).

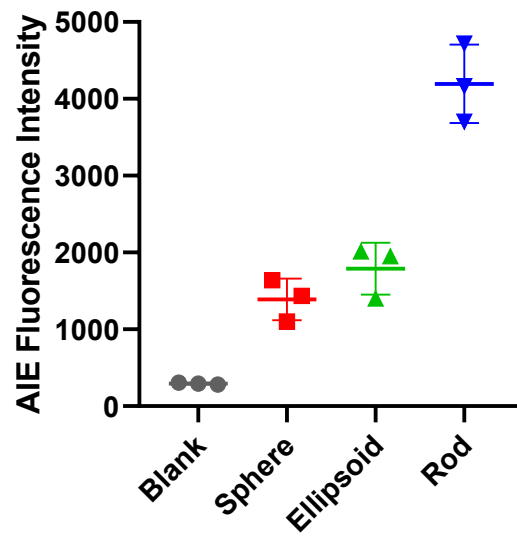
(B) Quantification of OVA antigen protein modified on the surface of nanoparticles of different shapes (with an initial OVA protein input of  $10\mu\text{g}$ ).



**Figure S3. Stability of Particle Size of Nanoparticles with Different Shapes Before and After Protein Modification.**

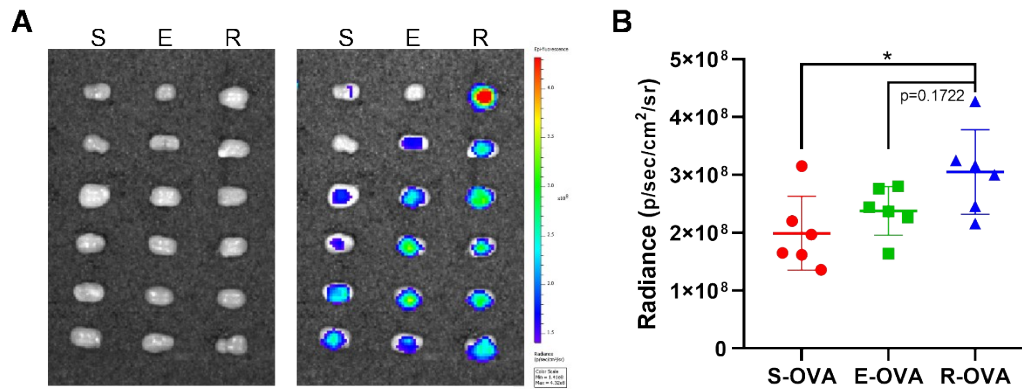
(A-B) Particle size measurements of nanoparticles with different shapes before protein modification at various time points (A) and the corresponding particle size change over time (B).

(C-D) Particle size measurements of nanoparticles with different shapes after protein modification at various time points (C) and the corresponding particle size change over time (D).



**Figure S4. AIE Fluorescence Intensity of 0.5 ml Lymph Node Homogenate Supernatant from Different Groups.**

The "blank" group refers to homogenate of untreated lymph node, which exhibits a background fluorescence intensity of approximately 300.

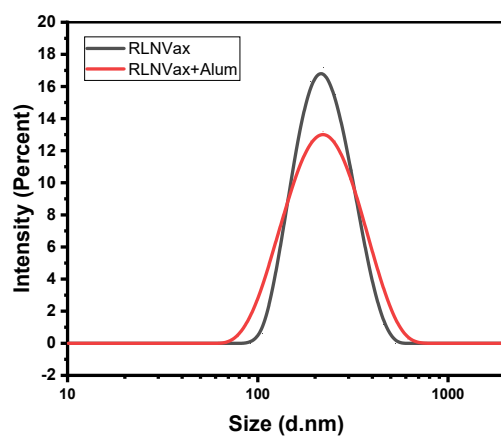


**Figure S5. *Ex Vivo* Fluorescence Imaging Results of Draining Lymph Nodes 72 Hours After Single Injection of OVA/Cy5 Vaccines of Different groups.**

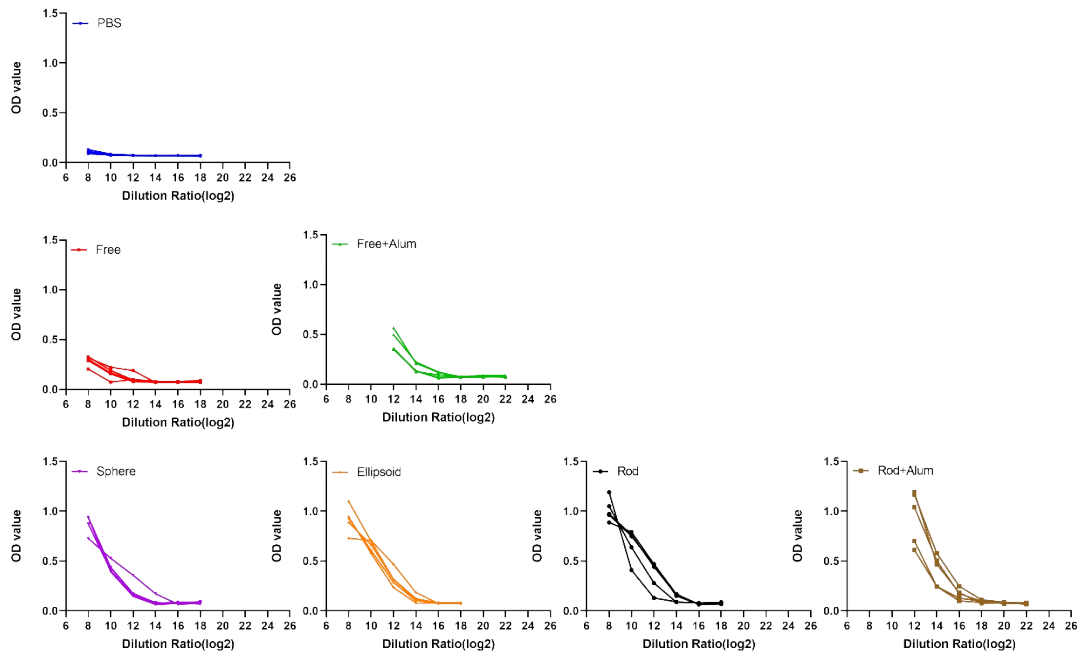
Compared to the 24-hour lymph node imaging results, the overall fluorescence intensity has decreased, but rod-like nanoparticle vaccines still show an advantage over other shapes of nanoparticle vaccines.

**(A)** *Ex vivo* fluorescence imaging results of the draining lymph nodes. The left panel shows the bright-field image, and the right panel shows the Cy5 fluorescence intensity image. Fluorescence intensity is expressed as Radiance (p/sec/cm<sup>2</sup>/sr), with Min=1.41e8 and Max=4.32e8. **S** represents spheres, **E** represents ellipsoids, and **R** represents rods.

**(B)** Quantitative analysis of Cy5 fluorescence intensity from panel A. **S** represents spheres, **E** represents ellipsoids, and **R** represents rods.

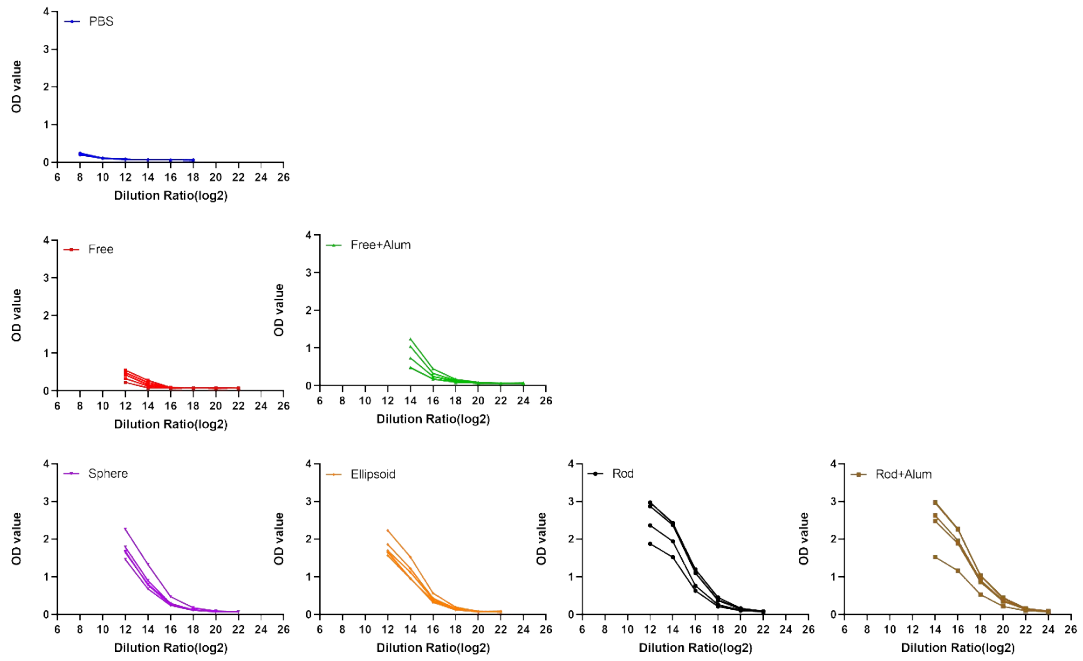


**Figure S6. Comparison of Particle Size of Rod-like Nanoparticle Vaccine Before and After Mixing with Aluminum Adjuvant**



**Figure S7. Changing Curves of Absorption Intensity (OD Value) vs. Dilution Ratio (log<sub>2</sub>) of Serums from Individual Samples in Different Treatment Groups on Day 10 in the Specific Antibody Titer Assay.**





**Figure S8. Changing Curves of Absorption Intensity (OD Value) vs. Dilution Ratio (log2) of Serums from Individual Samples in Different Treatment Groups on Day 20 in the Specific Antibody Titer Assay.**