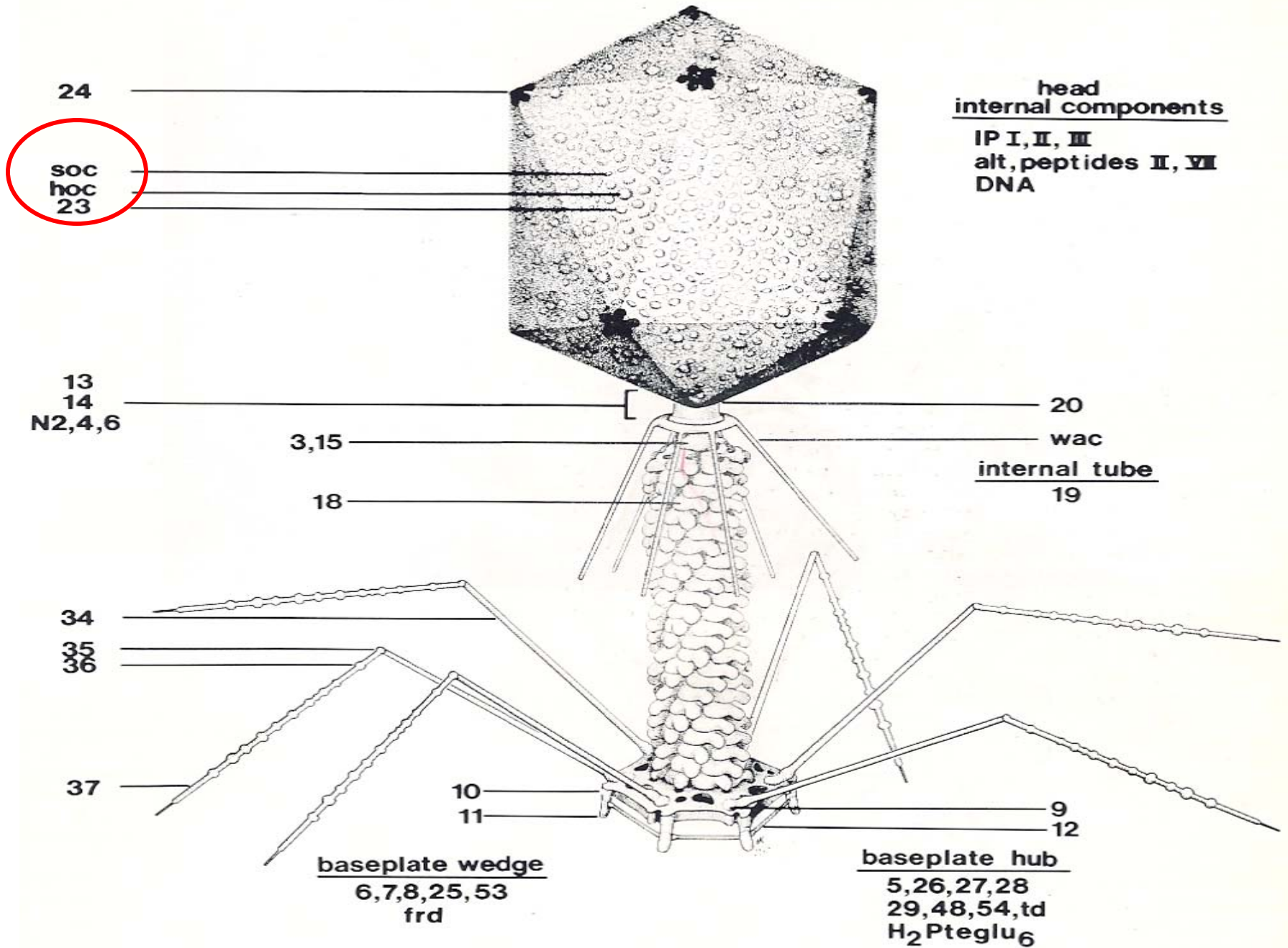
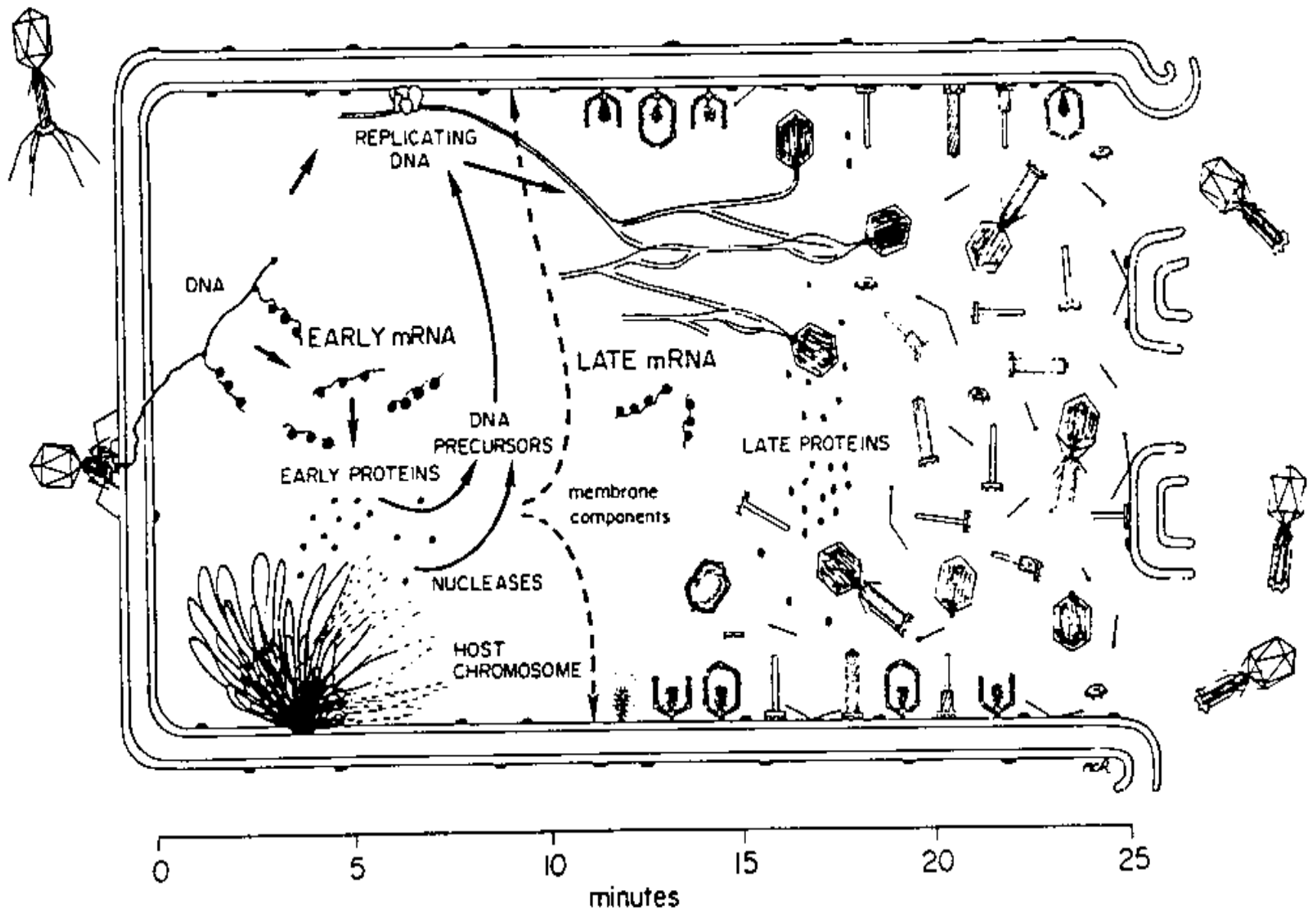
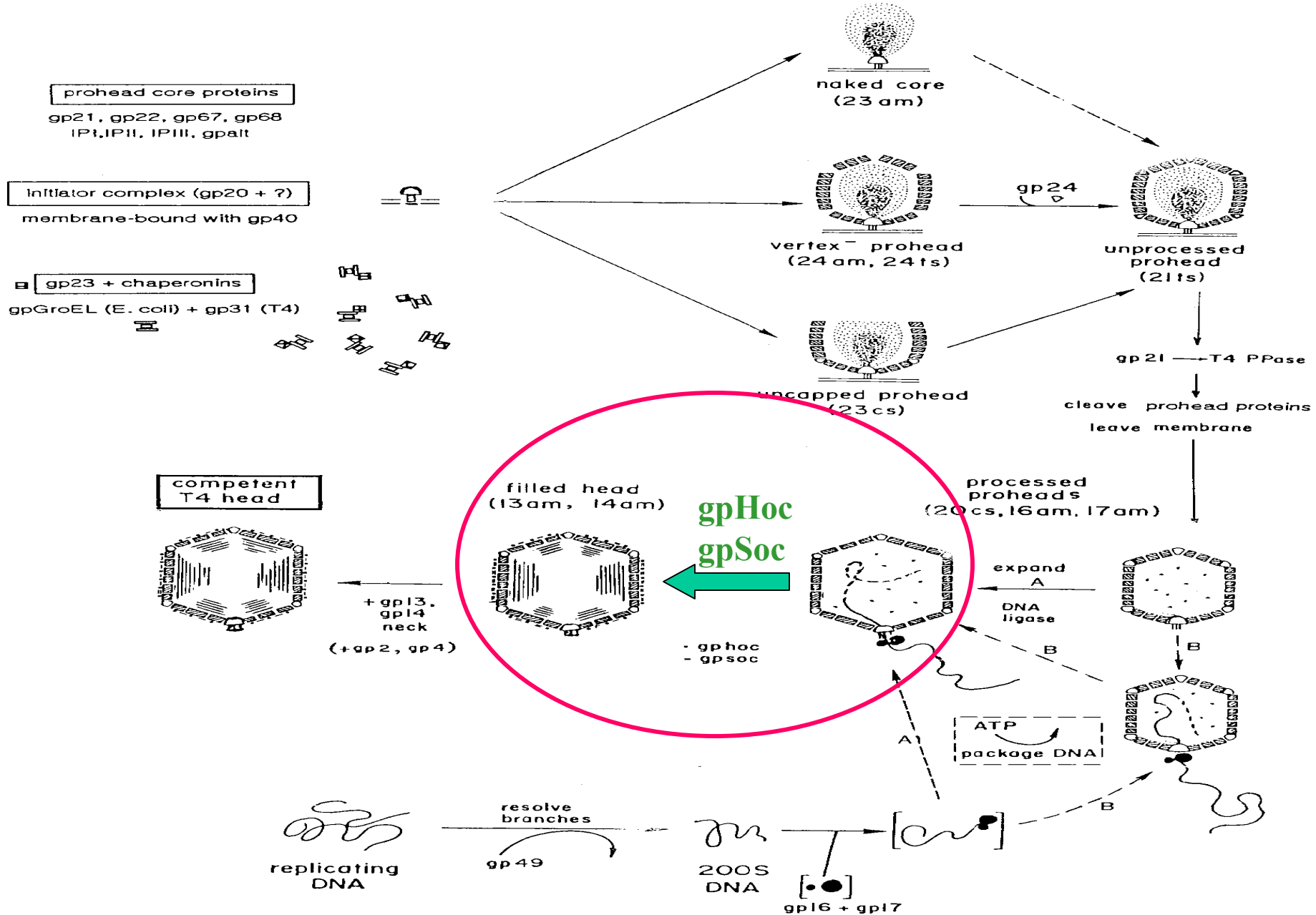


Phage T4 Display For Vaccine Delivery





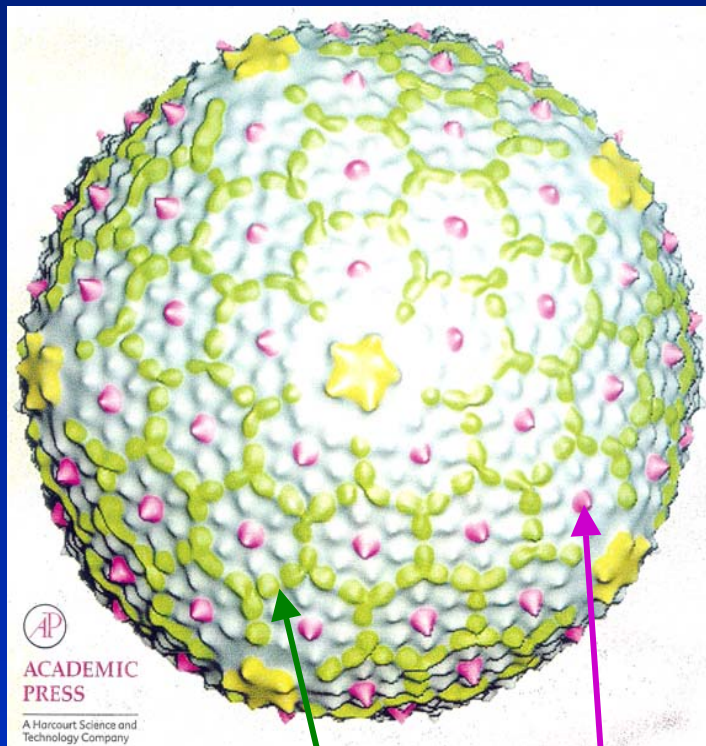
Overview of the T4 developmental program. (Courtesy of Frederick A. Eiserling.)



Head Assembly of Bacteriophage T4

A NOVEL BACTERIOPHAGE T4 DISPLAY SYSTEM

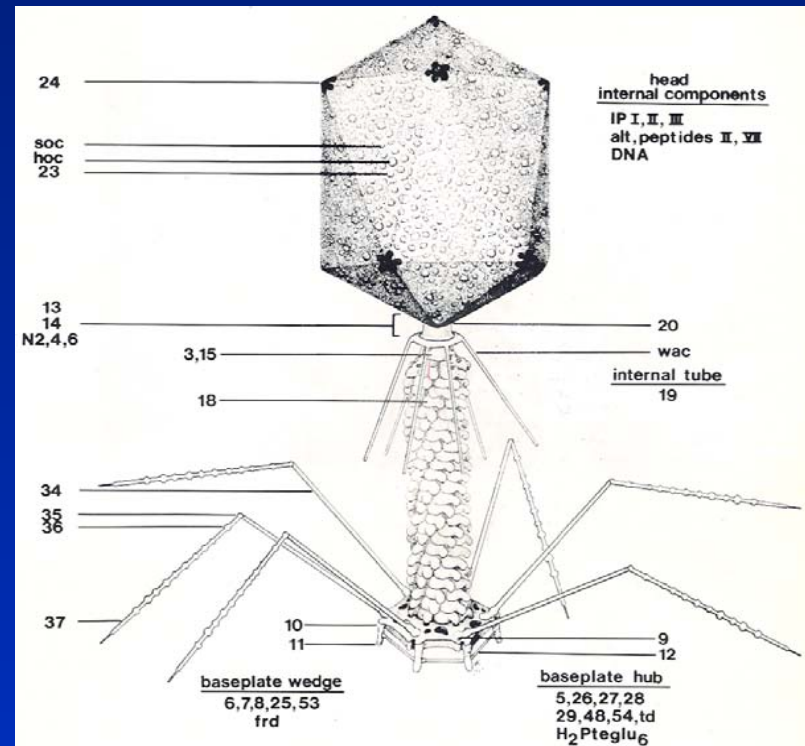
Bacteriophage T4 Capsid: cryo
EM reconstruction at 27Å

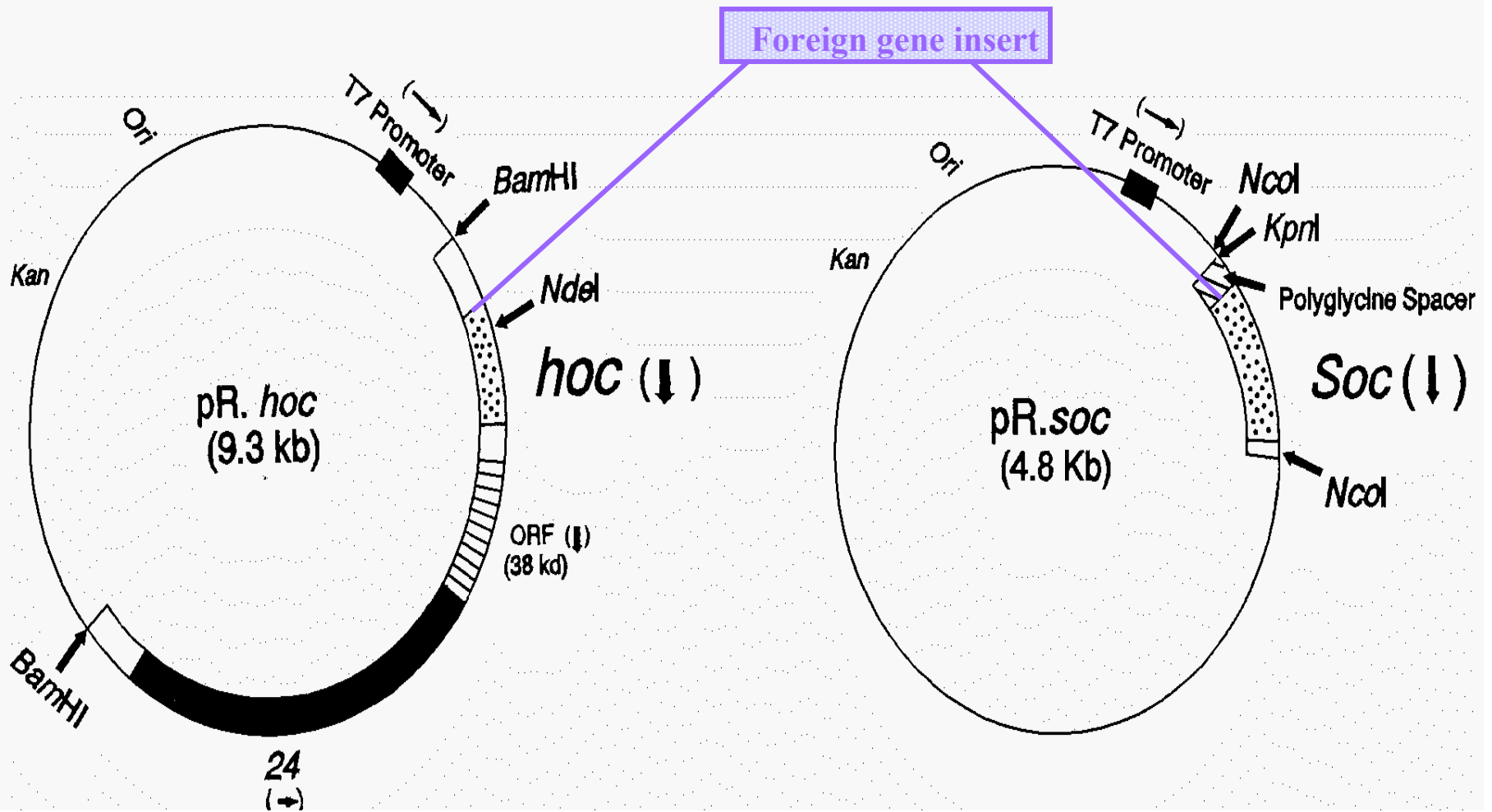


Soc, 840 copies

Hoc, 150 copies

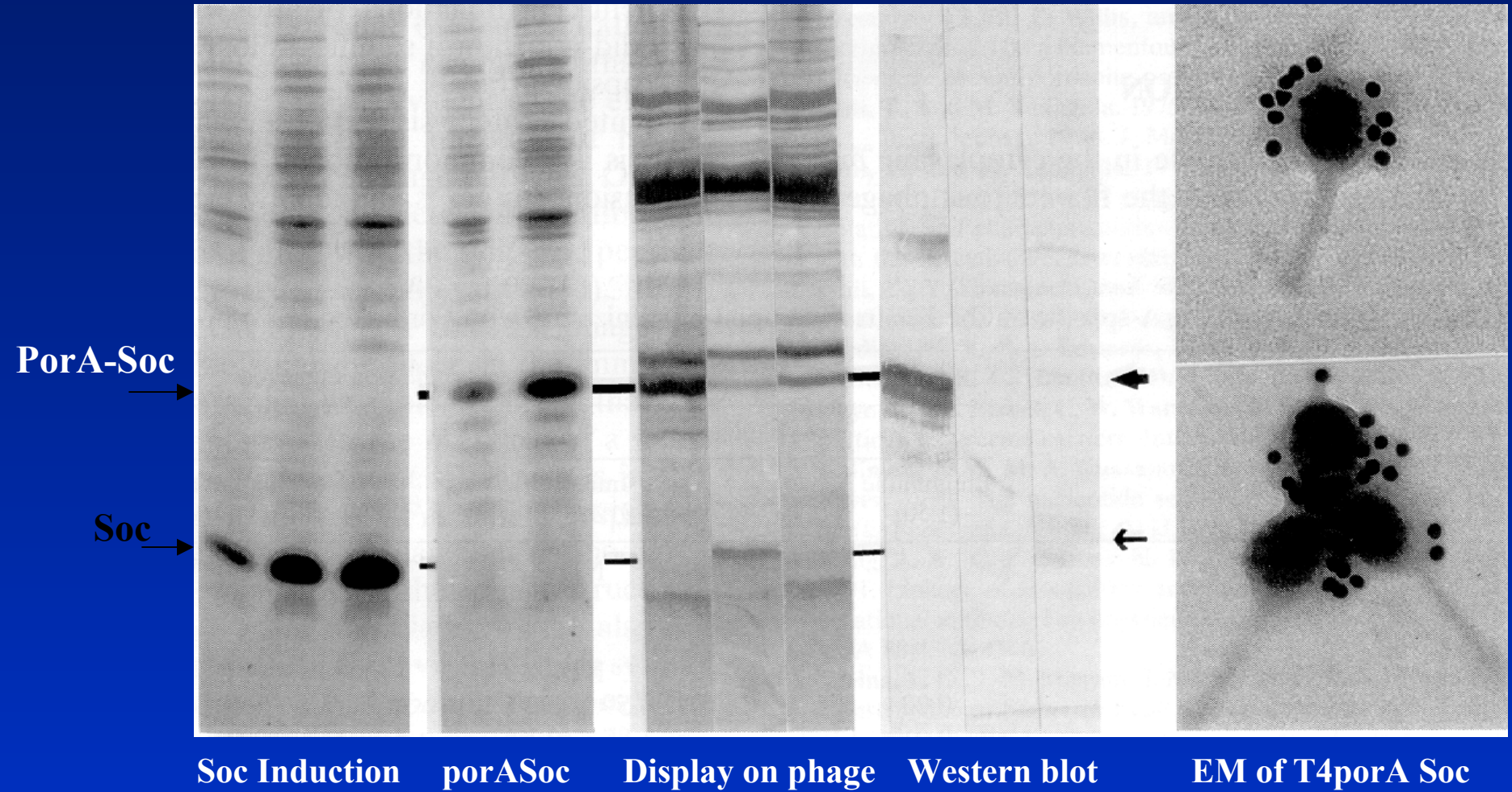
Schematic of Bacteriophage T4





hoc, soc, display vectors

Expression of PorA-Soc fusion protein and its display on T4 phage



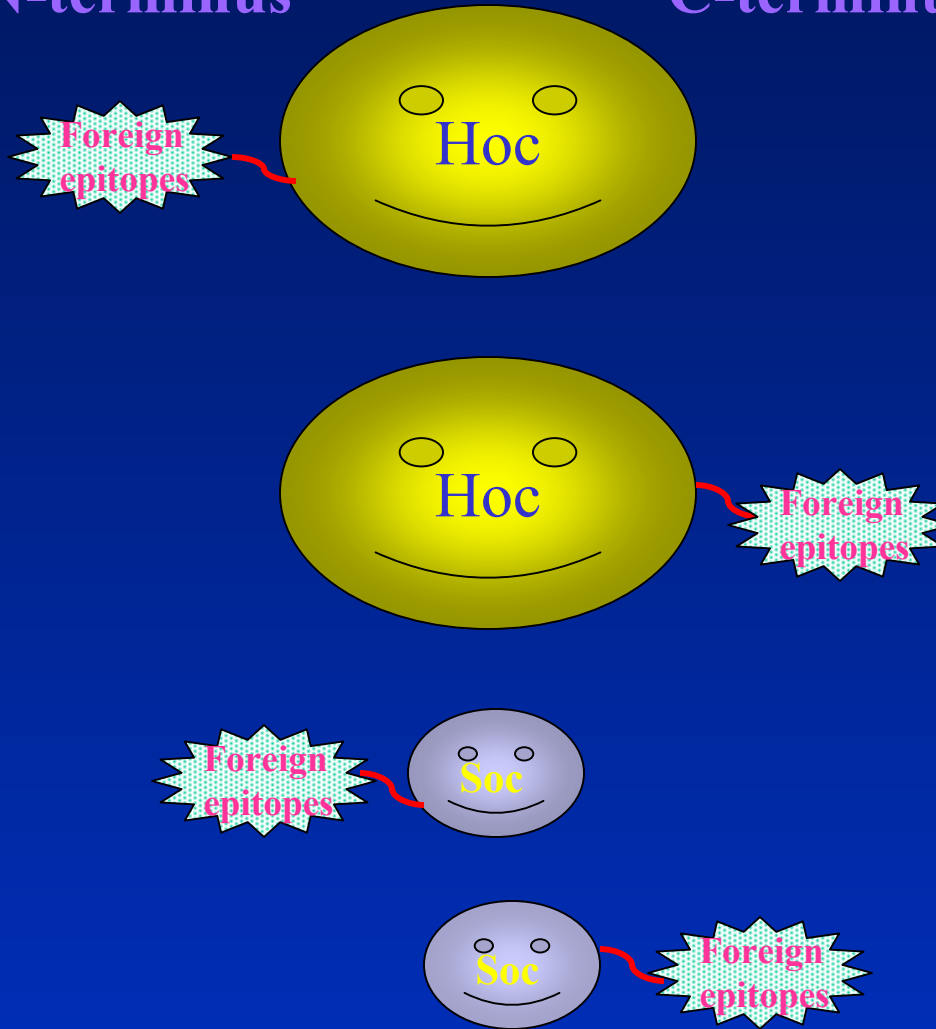
J. Jiang, L.Abu-Shilbayeh, and V.B. Rao *Infect. Immun.* 65:4770-4777 (1997)

TABLE 2. Induction of PorA-specific antibodies in mice upon immunization with PorA-Hoc or PorA-Soc fusion phages^a

Recombinant phage used for immunization	<i>A</i> ₄₀₅ at the dilution indicated			
	Expt 1		Expt 2	
	Preimmune (1:50)	Immune (1:1,000)	Preimmune (1:50)	Immune (1:100)
Control <i>soc</i> mutant phage with CFA		ND	<0	<0
T4. <i>porA</i> (P1.3).Soc with				
No adjuvant		ND	<0	0.58 ± 0.47
Alhydrogel	0.02	0.59 ± 0.30	<0	0.69 ± 0.52
CFA	0.01	1.85 ± 0.12	<0	0.61 ± 0.40
T4. <i>porA</i> (P1.3). <i>hoc</i> with				
Alhydrogel	0.01	0.39 ± 0.13		ND
CFA	0.02	1.34 ± 0.33		ND

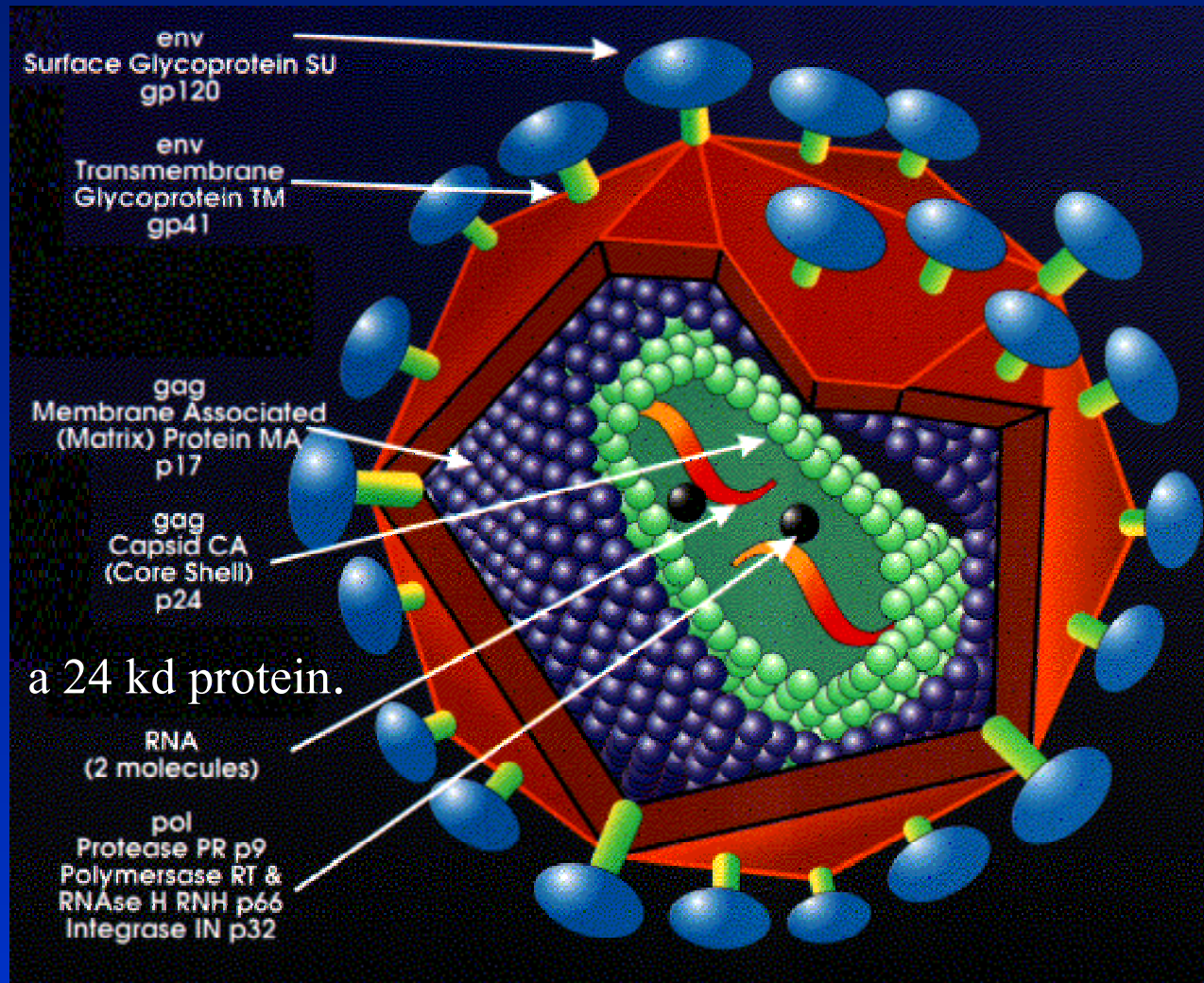
N-terminus

C-terminus



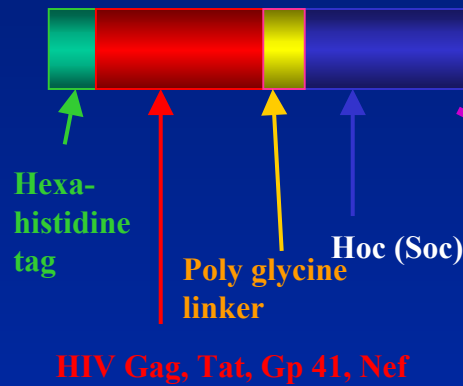
Both N- and C- termini of Hoc and Soc can be used to display foreign epitopes

➤ Can an effective multicomponent vaccine be made against HIV infection?



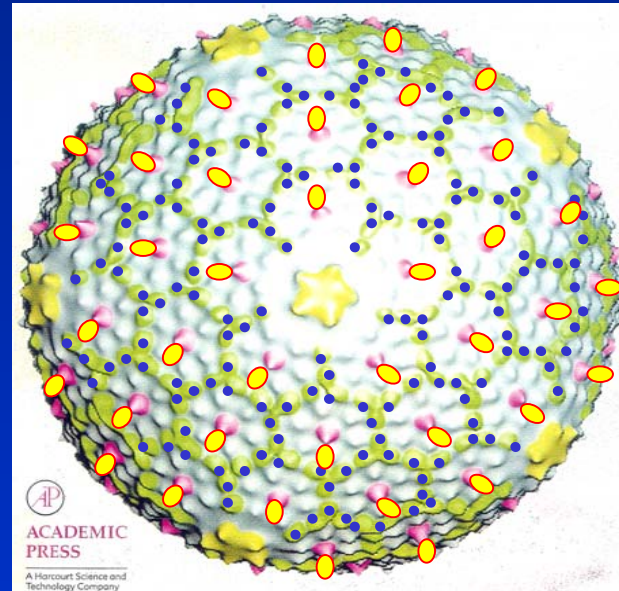
In Vitro Assembly

Engineer the recombinant
into the Hoc/Soc expression
Vector, express the antigen in *E. coli*,
purify the protein by Ni-agarose
chromatography



Gradient-purified
Hoc⁻ Soc⁻
T4 phage

In vitro assembly



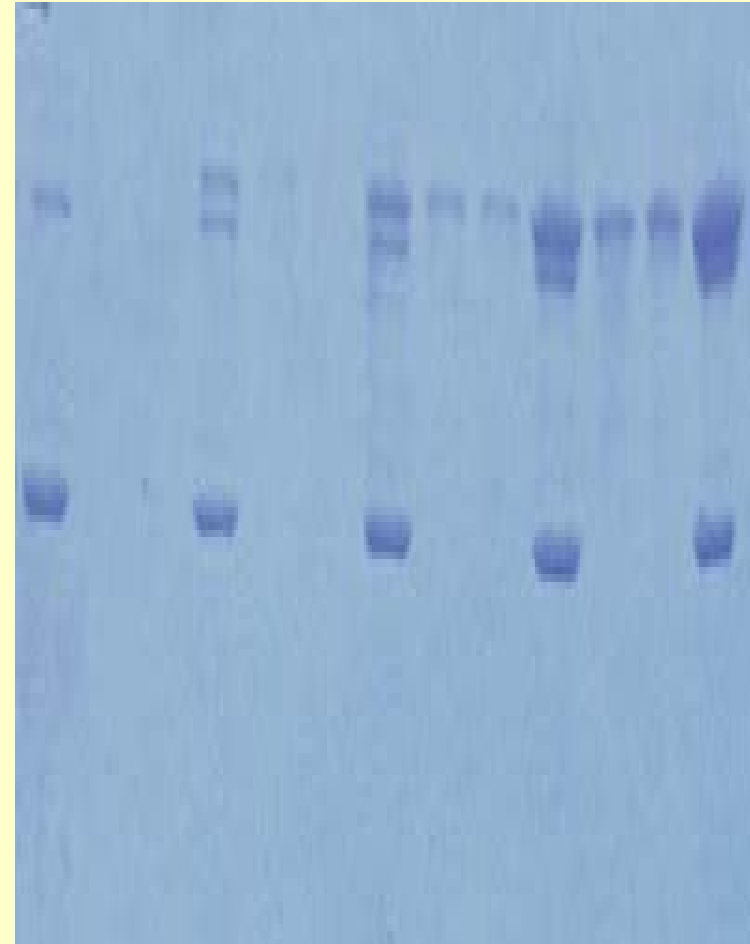
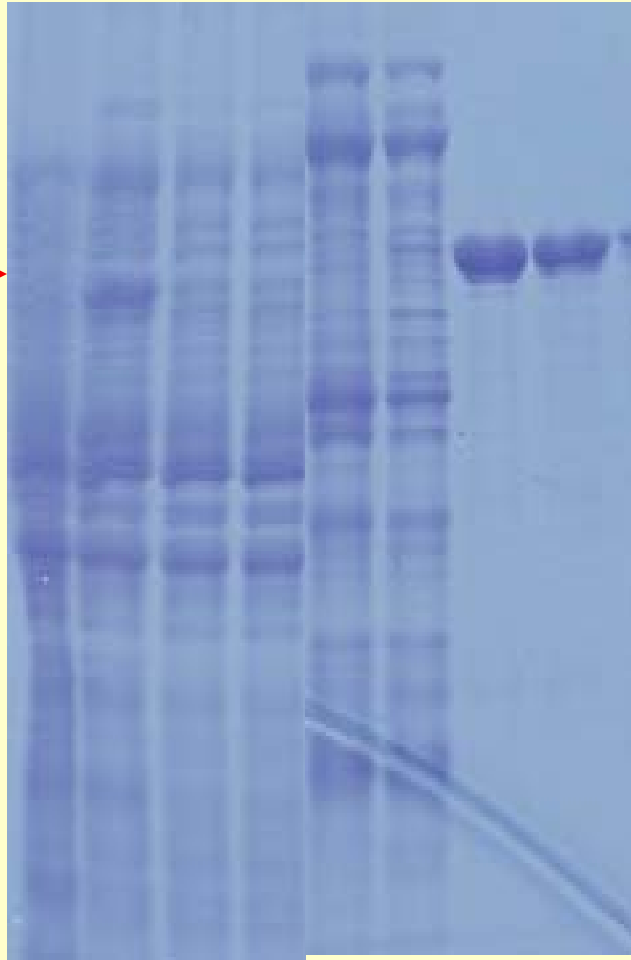
Phage T4 nanoparticles displaying recombinant antigens

III. DISPLAY OF HIV-GAG ON PHAGE T4

- + Ft Ft W W E E

T4 St S P St S P St S P St S P
1:5 1:10 1:25 1:50
T4 phage:Gag-Hoc ratio

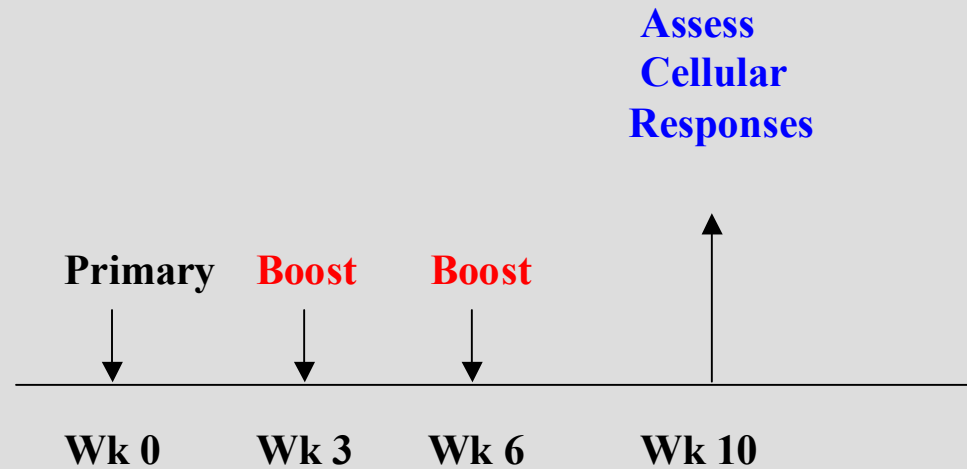
Gag-Hoc
→



- and + IPTG induction
Ft: flow-through
W: 50 mM imidazole wash
E: elution of bound Gag-Hoc

St: starting Gag-hoc
S: unbound Gag-Hoc in supernatant
P: pellet – Gag-Hoc displayed on T4

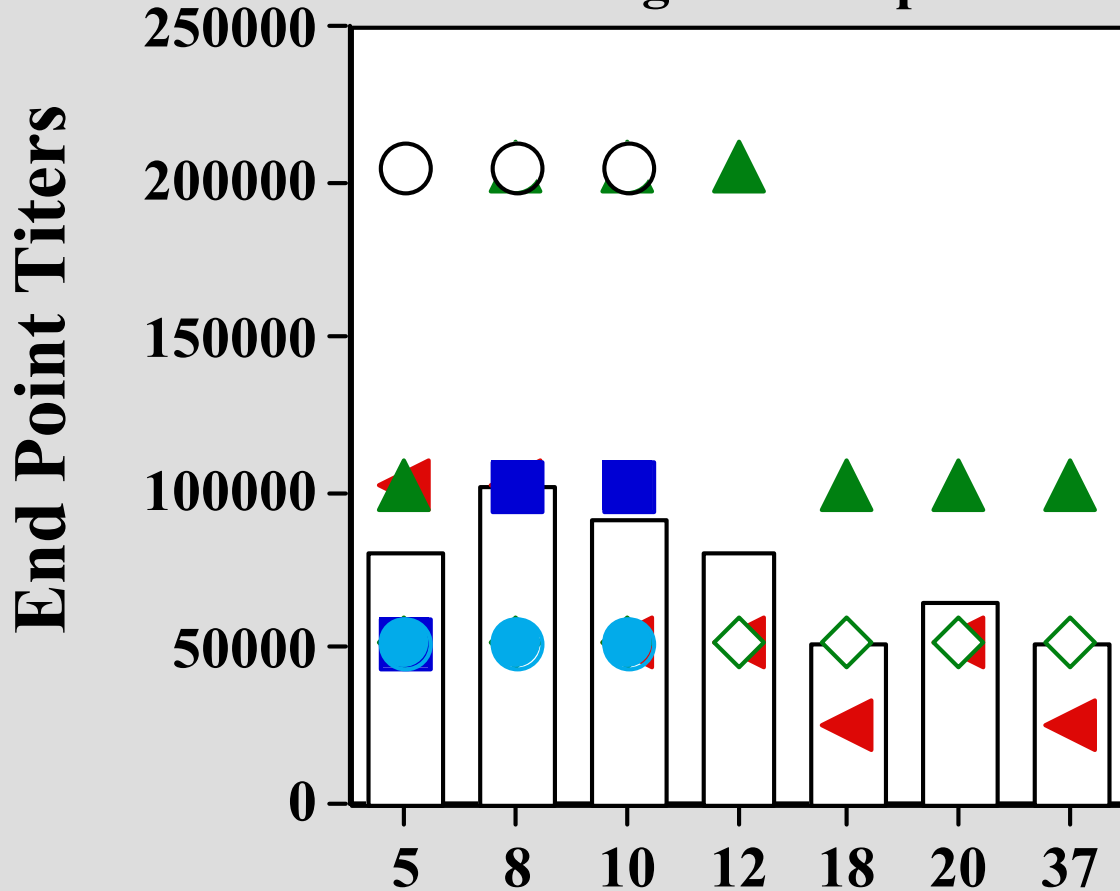
EXPERIMENTAL DESIGN FOR IMMUNIZATION WITH HIV-p24 DISPLAYED ON T4 NANOPARTICLES



IMMUNOGEN: Hoc-p24-T4
Route: Intramuscular
Adjuvant: None

p24-Specific IgG Antibodies

Immunogen: Hoc-p24-T4

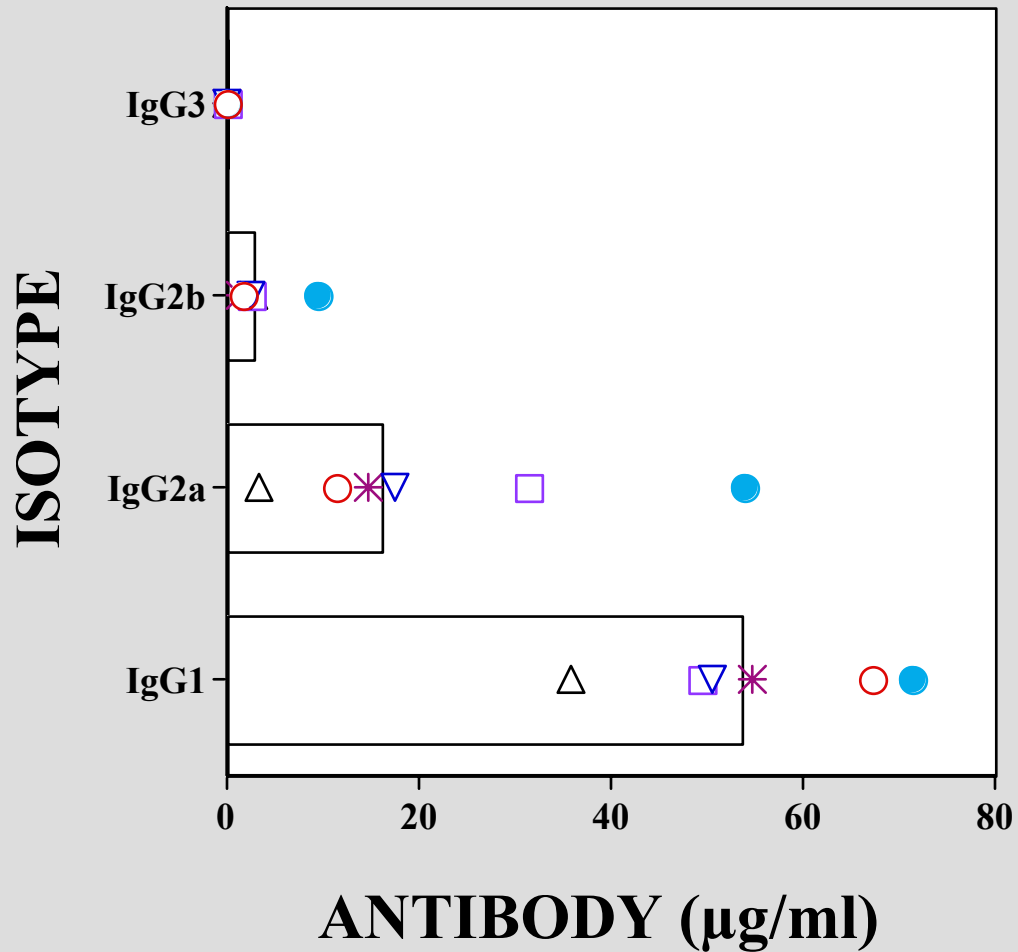


Total protein injected: 10 μ g/mouse
Amt. of p24 in the total protein: 50-100 ng

Long-lasting high titer antibodies are induced with ng amounts of p24

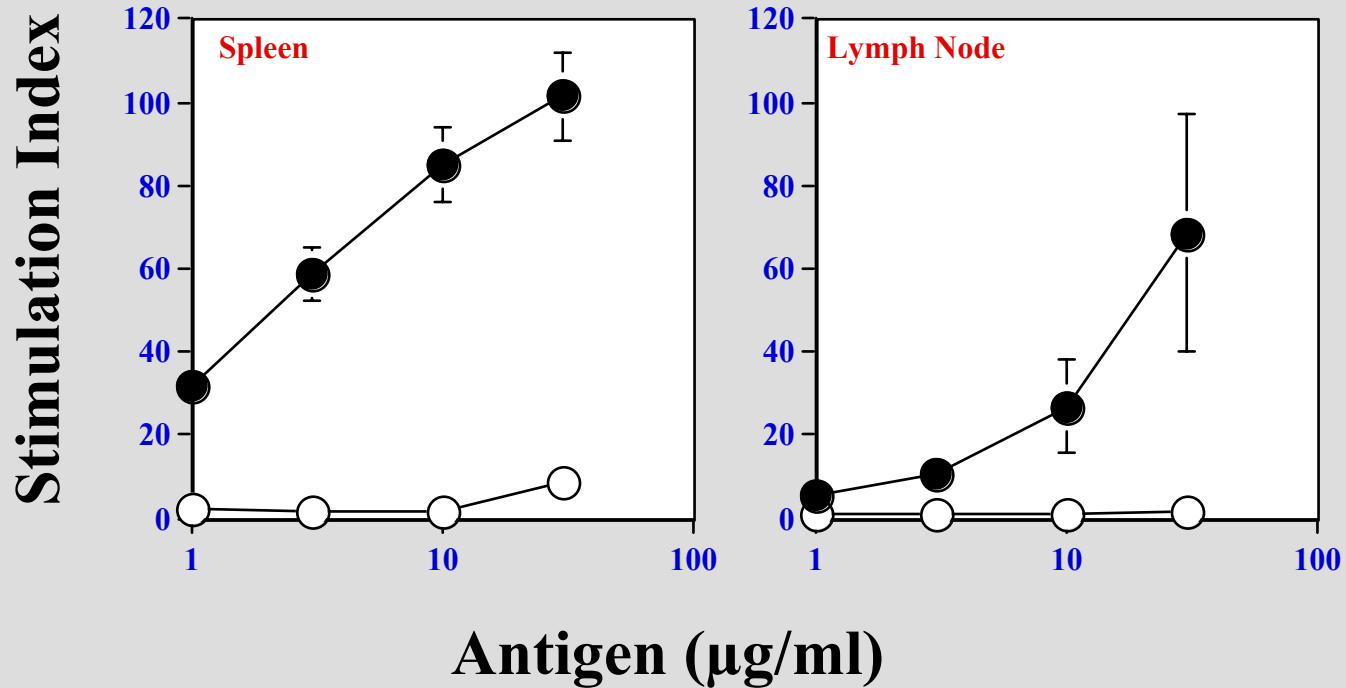
Weeks Post-Immunization

SUBCLASS SPECIFICITY



Conclusion: A mixed Th1/Th2 response is obtained

T Cell Proliferative Responses to p24



Conclusion: Robust T cell proliferation is obtained in the spleen and lymph nodes

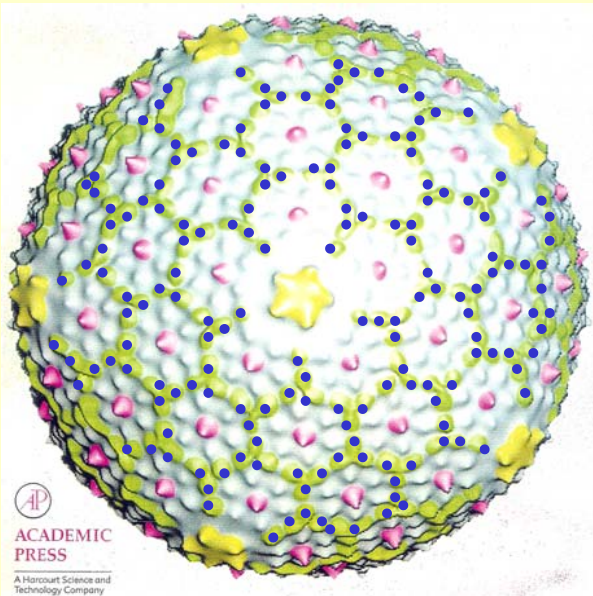
Important Features of the T4 Display System

1. Multicomponent vaccines can be constructed:

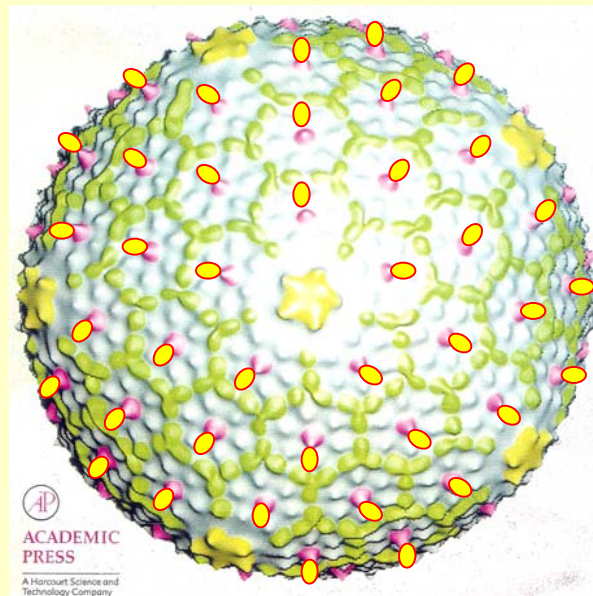
Multiple antigens can be displayed on the same capsid particle.

Soc – 840 copies per each capsid particle

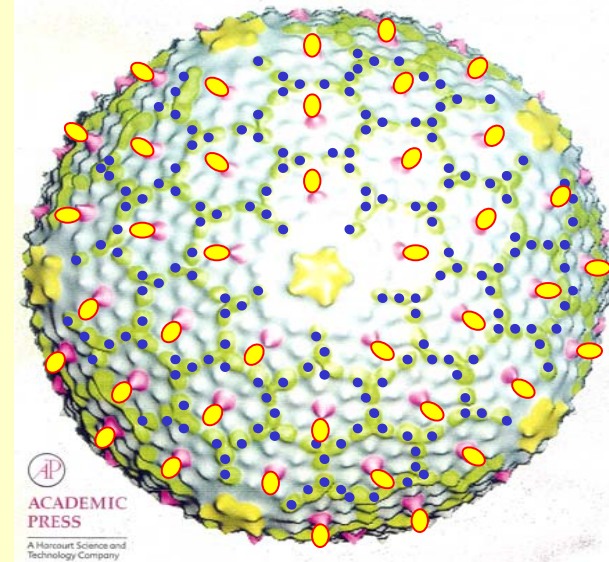
Hoc – 150 copies on each capsid particle



Soc-fusion



Hoc-fusion



Hoc and Soc
fusions

3. The vaccines can be prepared in large quantities safely, cheaply, using standard lab equipment.

T4 life cycle: 30 min

Burst size: several hundreds/infected cell

Approx. titers/one liter of infected *E. coli*: $>10^{13}$

4. Customized vaccine formulations can be developed:

Load different vaccine epitopes onto the T4 capsid surface by *in vitro* binding.

5. Phage T4 is a highly stable particle:

T4 vaccines are expected to be stable and would not necessarily require costly storage equipment.

ACKNOWLEDGEMENTS

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