Pherecydes Pharma:

Engineered phage banks:

A functional answer to bacteriological threats.

Edinburgh International Phage Conference
July 26th-29th 2008

The starting points.

(at BM-Systems).

- Multi-resistant bacteria are increasingly frequent and widely disseminated in a multitude of environments.

The health threat is very serious

- Progress in molecular biology is such that it has become relatively easy to engineer genetically modified pathogens for which there cannot be any *immediate* counter-measures.

The bio-preparedness challenge is very real.

The questions

(at BM-Systems).

- How to rapidly (less than 30 min) and efficiently ***detect*** the presence of any given LIVE bacterial pathogen?
- How to efficiently ***monitor*** an environment to rapidly (less than 30 min) detect the presence of any LIVE emerging (unknown) bacterial strain?
- How to rapidly and efficiently ***destroy*** any ***unknown*** bacterial pathogen or emerging strain ***without using***

A) Antibiotics: too many resistant strains, and very rapid resistance acquisition.

B) Vaccines: much too slow to act, and small strain variations often lead to inefficacy.

The first apparent answer
(at BM-Systems).

Bacteriophages, the natural predators of bacteria, could present the best potential to act as detectors-killers.

- Many are very host-specific,
- They only replicate in LIVE bacterial cells,
- Many kill the cells in which they replicate,
- As the phage progeny population increases that of the host diminishes (in a « closed » environment, few hosts, if any, should escape), and
- They are extremely numerous and varied (they probably represent the most numerous « life forms » on the planet).

BUT the matter is NOT as simple as it first appears!

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BM Systems Integrative Biology

Negative selection of hypotheses & model building (at BM-Systems).

H1. ***Once isolated, a phage targeting a given host provides a permanent mean of control for that host.***

The stark reality (from model & data).

A) Individuals in the targeted bacterial host population will rapidly and independently mutate to evade attack *(response driven by the increasing levels of bacterial debris and intracellular metabolites in the immediate environment)*.

B) If only ONE individual produces the « right » protective mutation, it will, within 24h, generate a new population resistant to that phage *(the bacterial threat will seem eradicated only to reappear later, but this time, under a form resistant to the phage)*.

Negative selection of hypotheses & model building (at BM-Systems).

H2. ***Should resistance appear in a bacterial population, one only needs to return to a « natural source » of phages to have good chances of finding a new infective phage.***

The stark reality (from model & data).

Initially, this may well be the case.

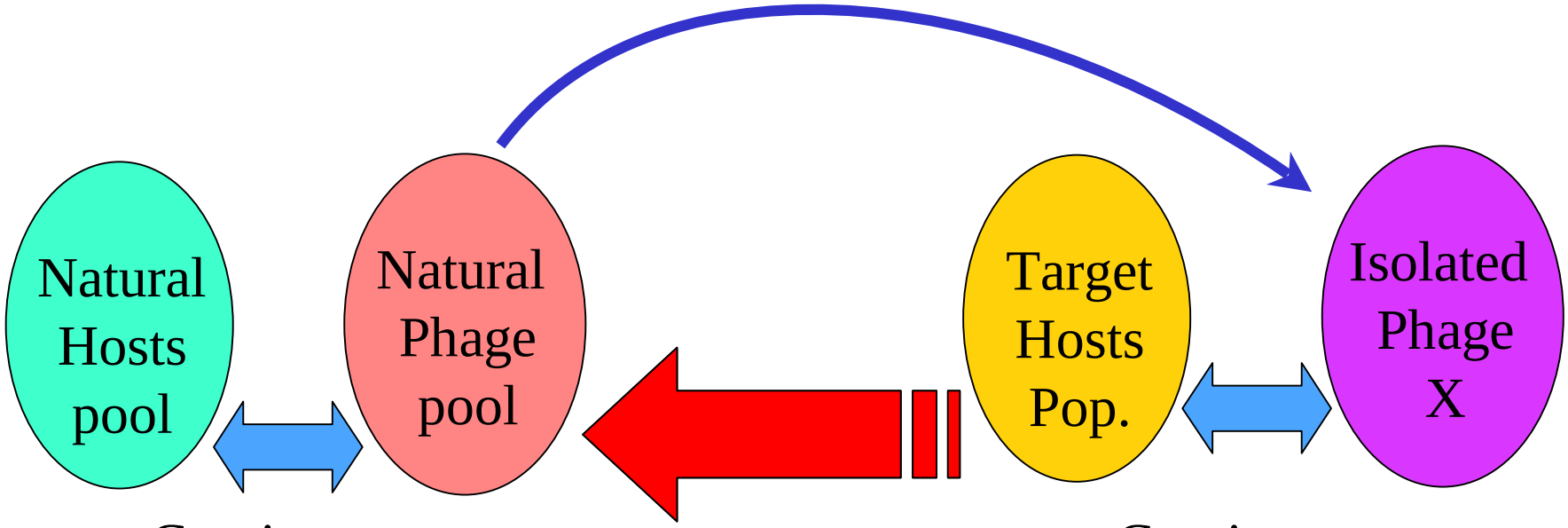
However, for a given host, the more frequent these returns to the « natural source », the greater the chances to fail finding a new appropriate infective phage.

The reasons are: *continuous co-evolution*

versus

***unidirectional predatory pressure*.**

Co-evolution versus unidirectional predatory pressure.



Continuous co-evolution

Ever increasing evolutionary divergence

Continuous co-evolution

+

Ever decreasing chances to find a new efficient lytic phage.

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Negative selection of hypotheses & model building (at BM-Systems).

BM Systems Integrative Biology

H3. ***In that case, one only needs to expose the host to a « cocktail » of phages simultaneously targeting different surface epitopes***.

(any given individual host has practically no chance of simultaneously producing the mutations required to entirely escape attack) .

The stark reality (from model & data).

- Exacerbated predatory pressure inevitably leads to the worst possible situation: mutations of components of host's replicative machinery, hijacked by the phages for their own replication, will now be favoured.
- The phages will be capable of binding to the host, perforate the cell wall, inject their DNA and destroy the host's DNA but will be UTTERLY incapable of replicating, hence entirely defeating the purpose of using phages in the first place!

Bacteria have existed for nearly 4 BILLION Years. They have so far resisted to EVERYTHING.
And it is certainly **NOT** for lack of phages!

The model-derived solution.
(at BM-Systems)

- ***What, in essence, is the problem?***

The bacterial host will try anything to escape predation and we have no idea what will be the successful strategy. Furthermore, this strategy is likely to vary between locations (populations) for a same host.

- ***What do we need to achieve?***

We must be capable of always preceding the host's escape strategies, no matter what they could be.

- ***The best-fit solution*** (model-derived):

We have only ONE option: we MUST use a stochastic approach.

It becomes necessary to

- abandon all idea of « natural phage pools » and,
- stochastically engineer phage banks in order to produce particles capable of targeting anything and everything while maintaining their capacity to replicate in the face of host's evasion attempts.

The Pherecydes answers.

Two proprietary technologies (invented at BM-Systems) allowing the production of stochastically engineered phage banks.

TAPE™Ⓟ:

A technology allowing to rapidly & simultaneously introduce defined densities of random mutations in any number of selected regions within a gene while conserving intact any number of defined coding domains in this same gene.

Ab-ACCUS™Ⓟ :

A recombination technology allowing the rapid & efficient production of phage banks in which every individual differs from all others for any number of selected genes or other sequences.

Applicable to any phage and to any known sequence.

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TAPE (Targeted Accelerated Protein Evolution)

A model-derived technology allowing massive targeted diversification of protein-based detection/eradication systems.

(antibodies, phage homing fibrils, etc…)

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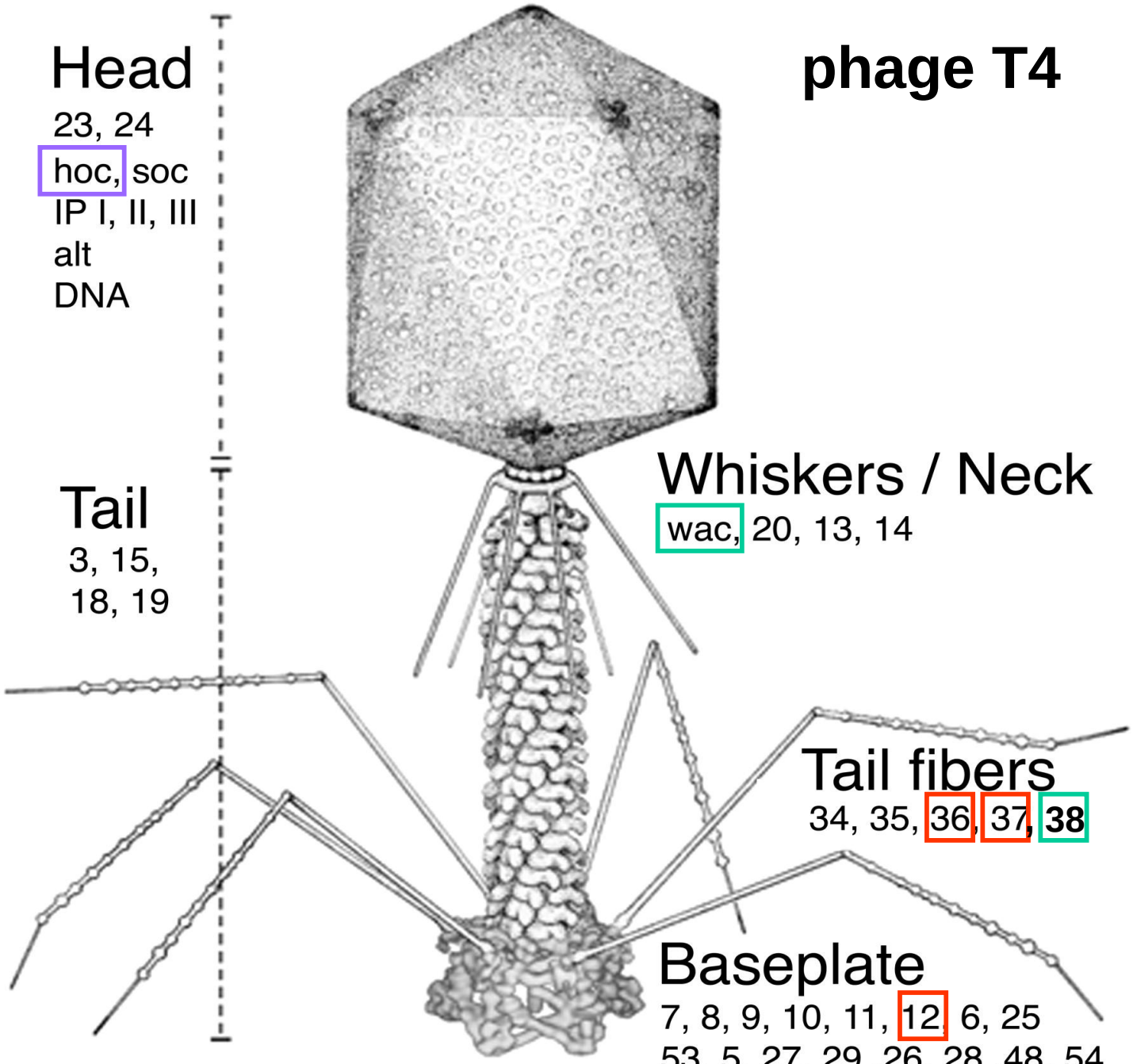
phage T4



Head
23, 24
hoc, soc
IP I, II, III
alt
DNA

Tail
3, 15,
18, 19

Whiskers / Neck
wac, 20, 13, 14

Tail fibers
34, 35, 36, 37, **38**

Baseplate
7, 8, 9, 10, 11, 12, 6, 25
53, 5, 27, 29 ,26, 28 ,48, 54

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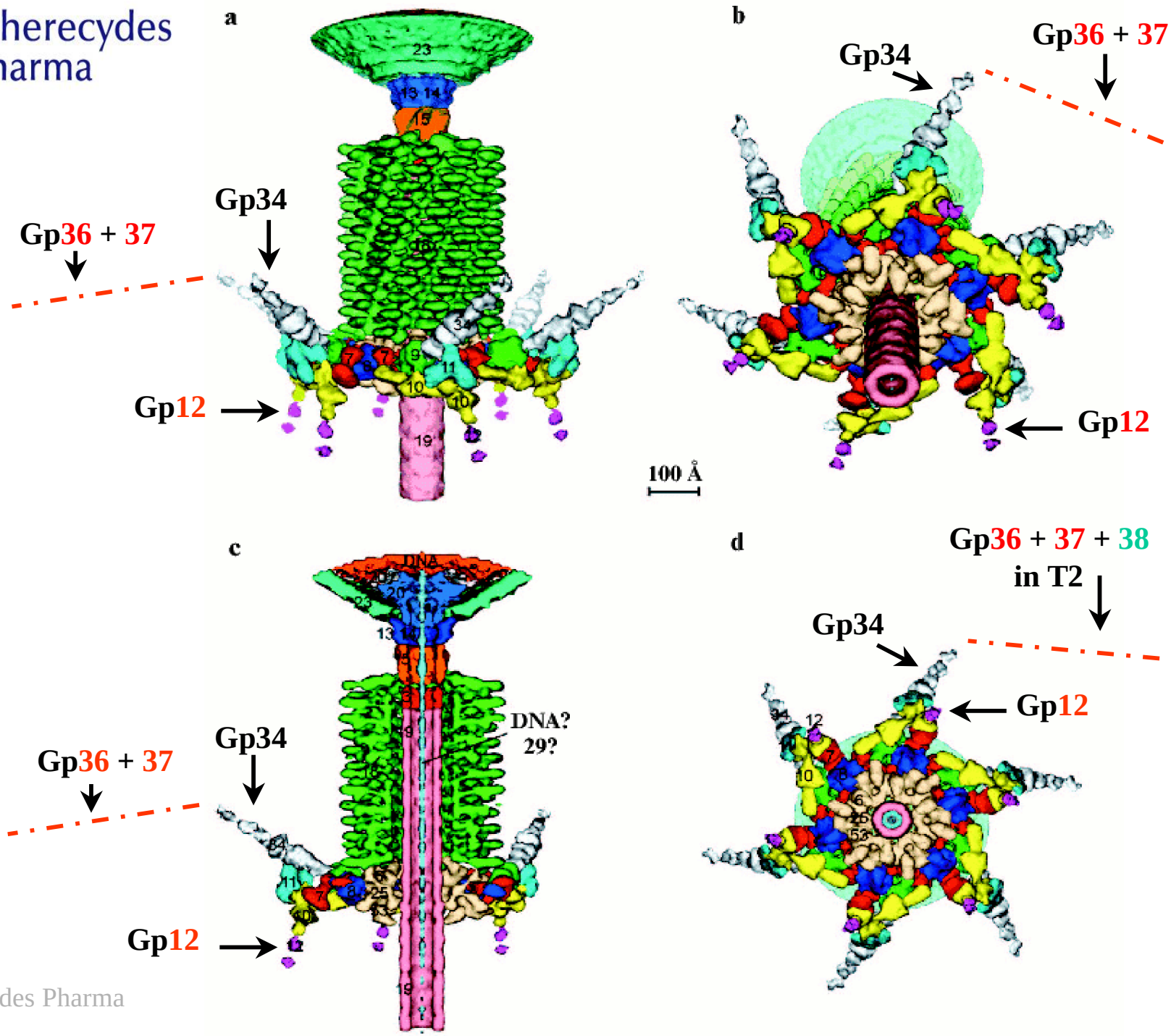

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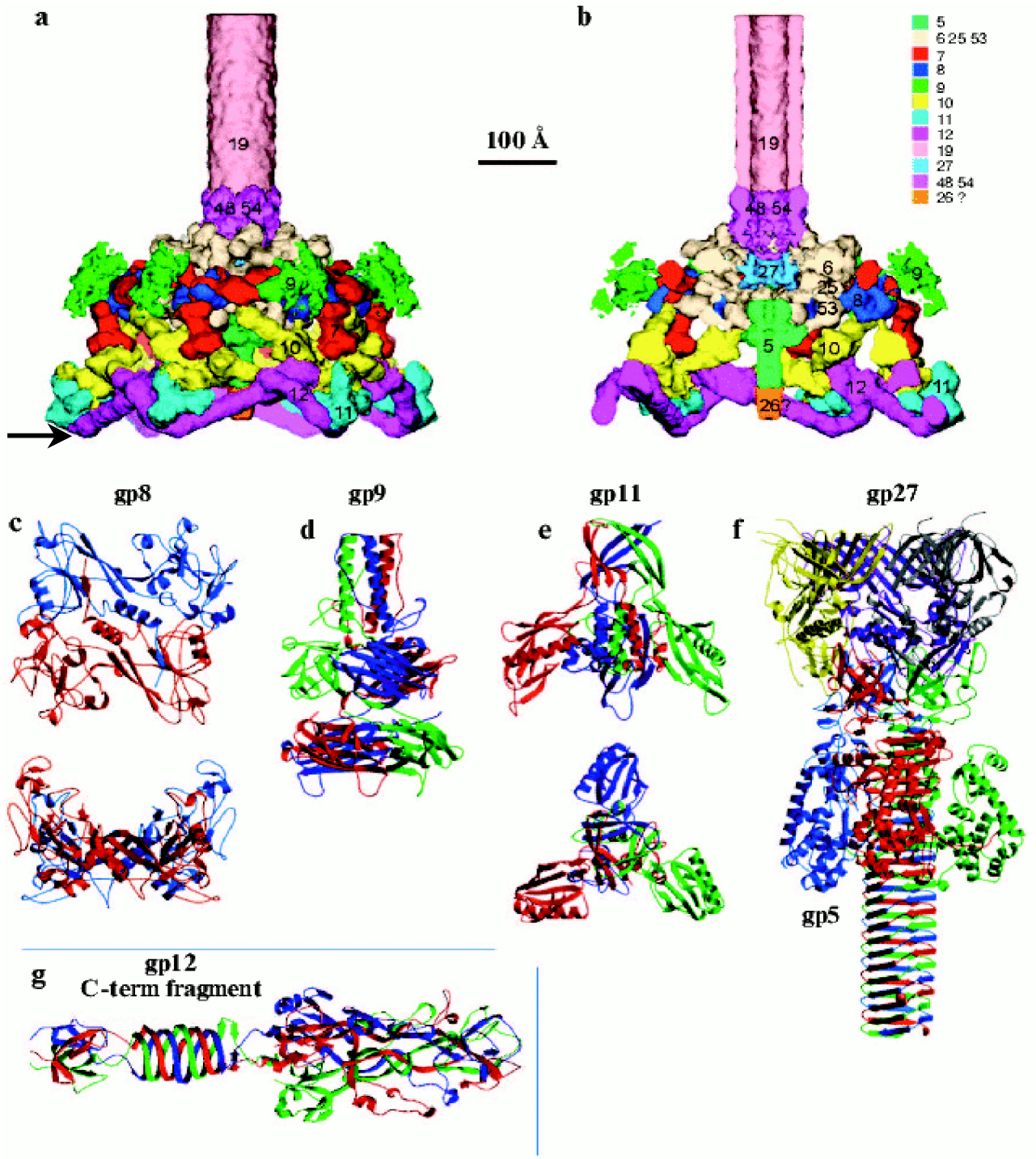
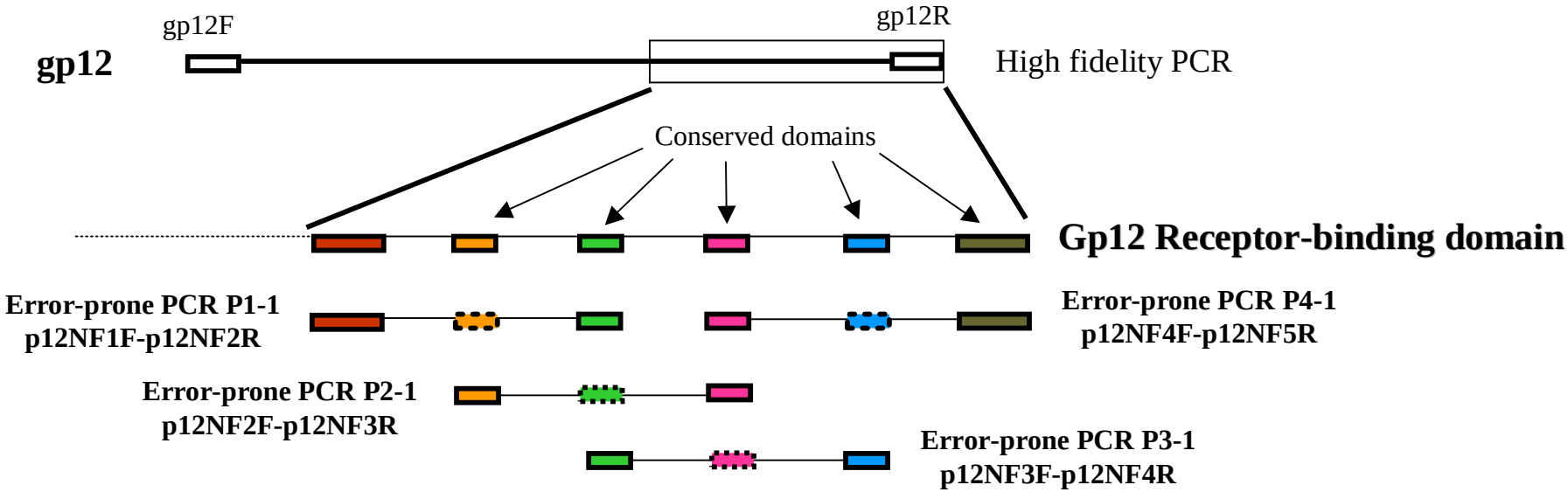
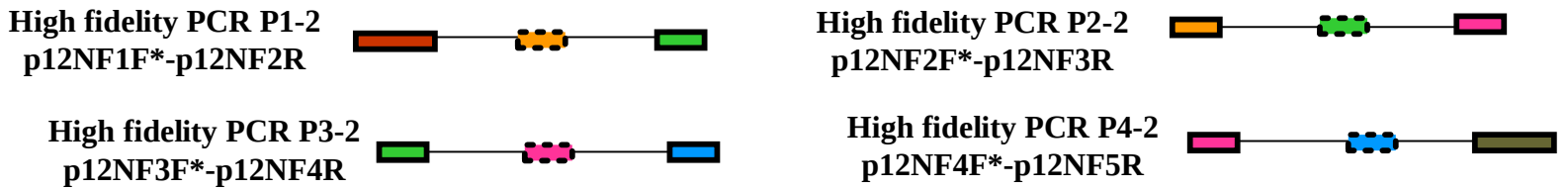


Step 1: gp12 High fidelity amplification and mutagenesis



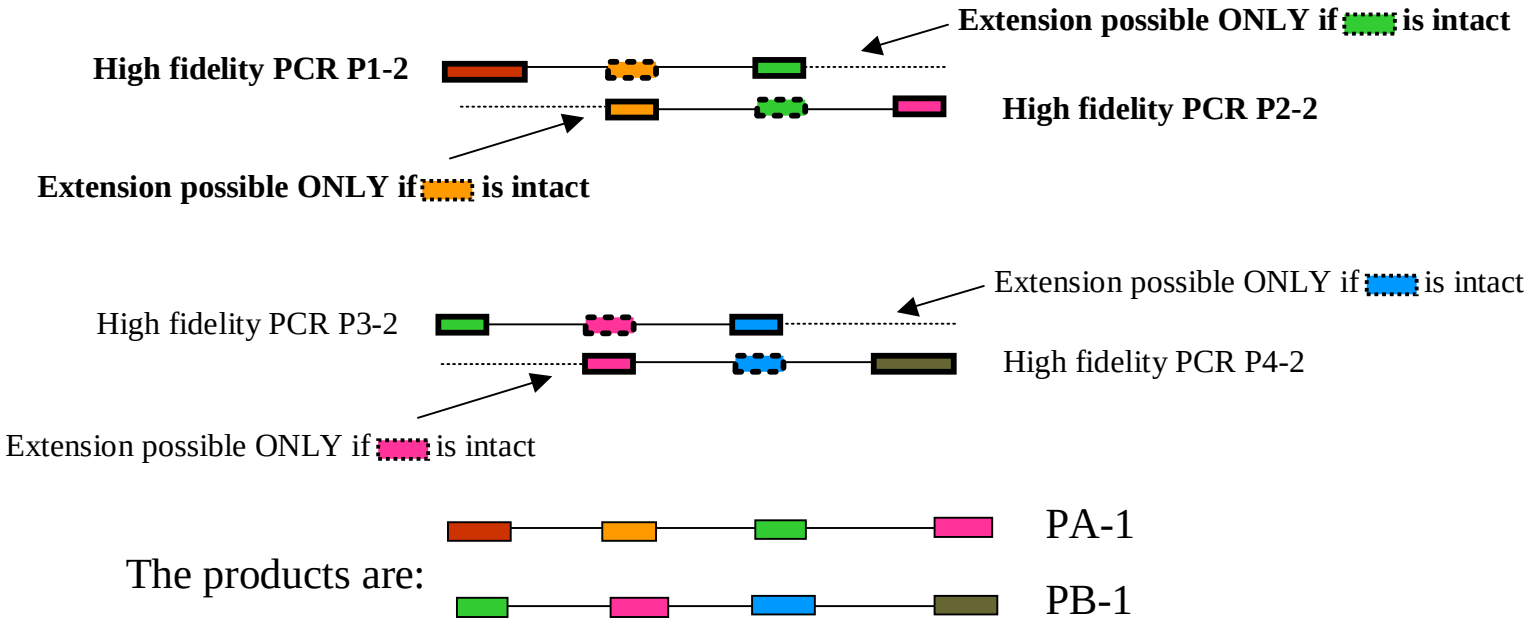
Step 2A: Selective high fidelity amplification of desired fragments

This sub-step selectively amplifies the fragments where the external priming sites , , , and have been preserved (mutation-free).



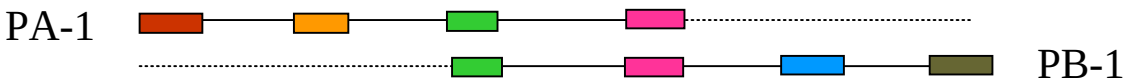
Step 2B: Selective high fidelity reconstruction of desired fragments.

In this sub-step, fragments that terminate with a priming site corresponding to the internal conserved domain found in other fragments serve as primers for the selective amplification of the fragments where the internal conserved domains , , and have been preserved (mutation-free).



Step 3: Reconstruction of pg12 RBD via high fidelity PCR.

In this step, the PA-1 and PB-1 fragments serve as extension primers for each other and result in reconstituted Gp12 Receptor-Binding Domains in which all the « conserved » sequences have been preserved while the variable regions have been extensively mutated.

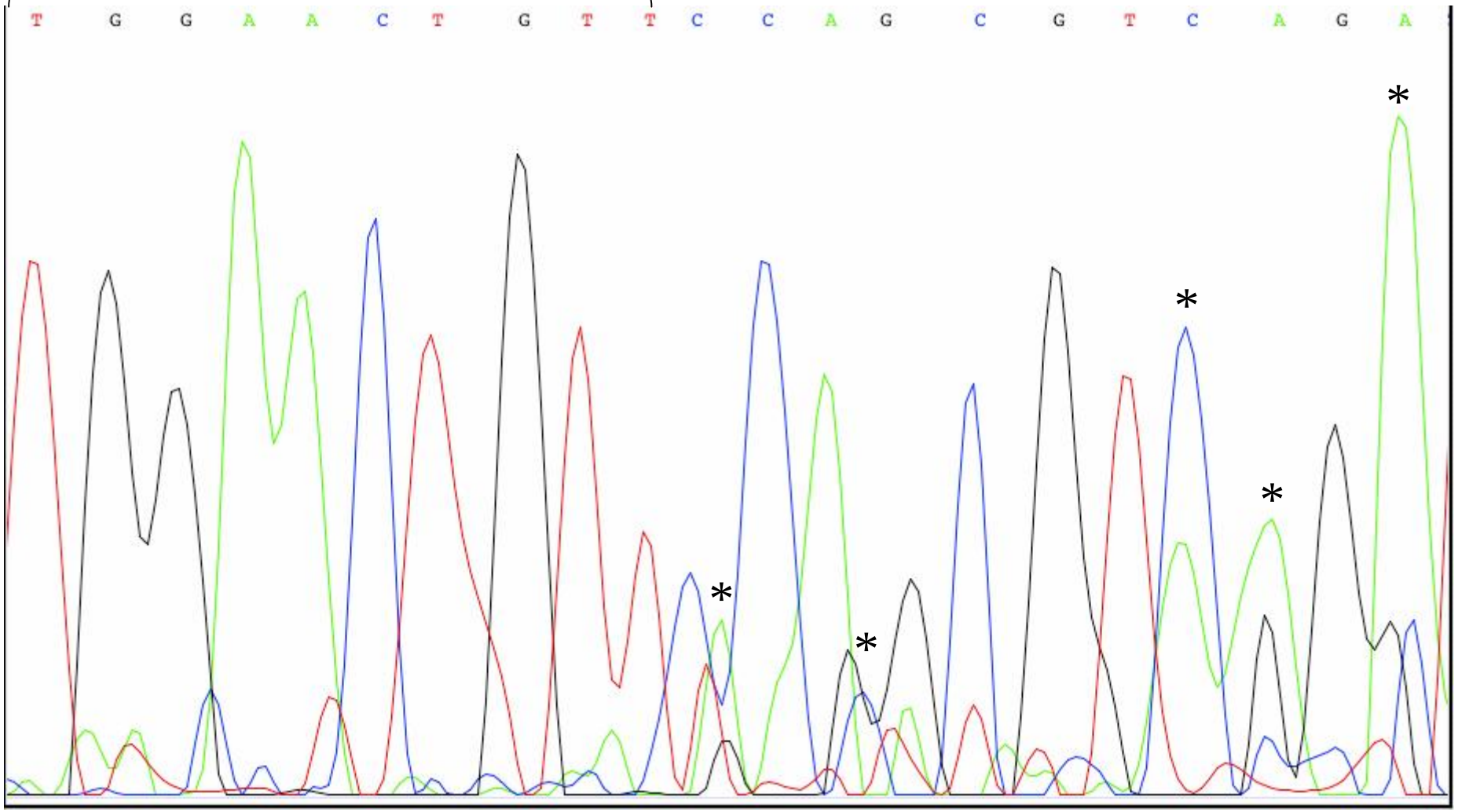


TAPE-mediated targeted mutagenesis

(batch-sequencing)

Conserved Domain

Stochastic mutagenesis : peaks superposition



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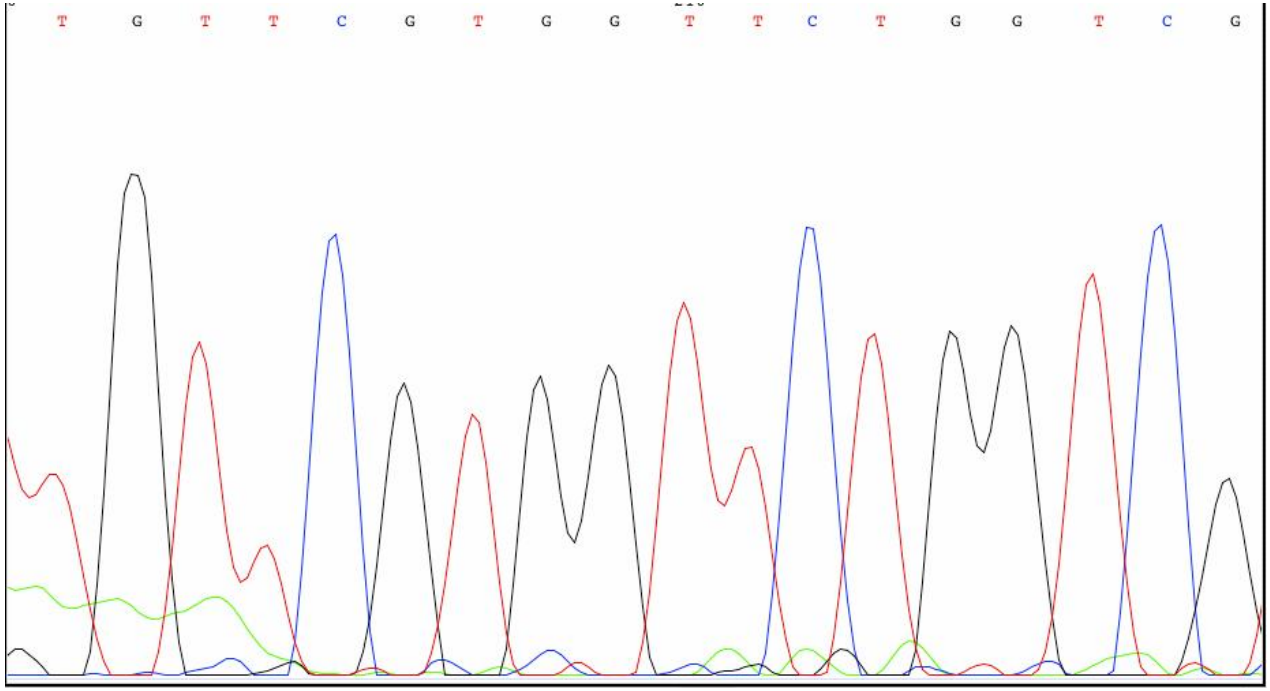
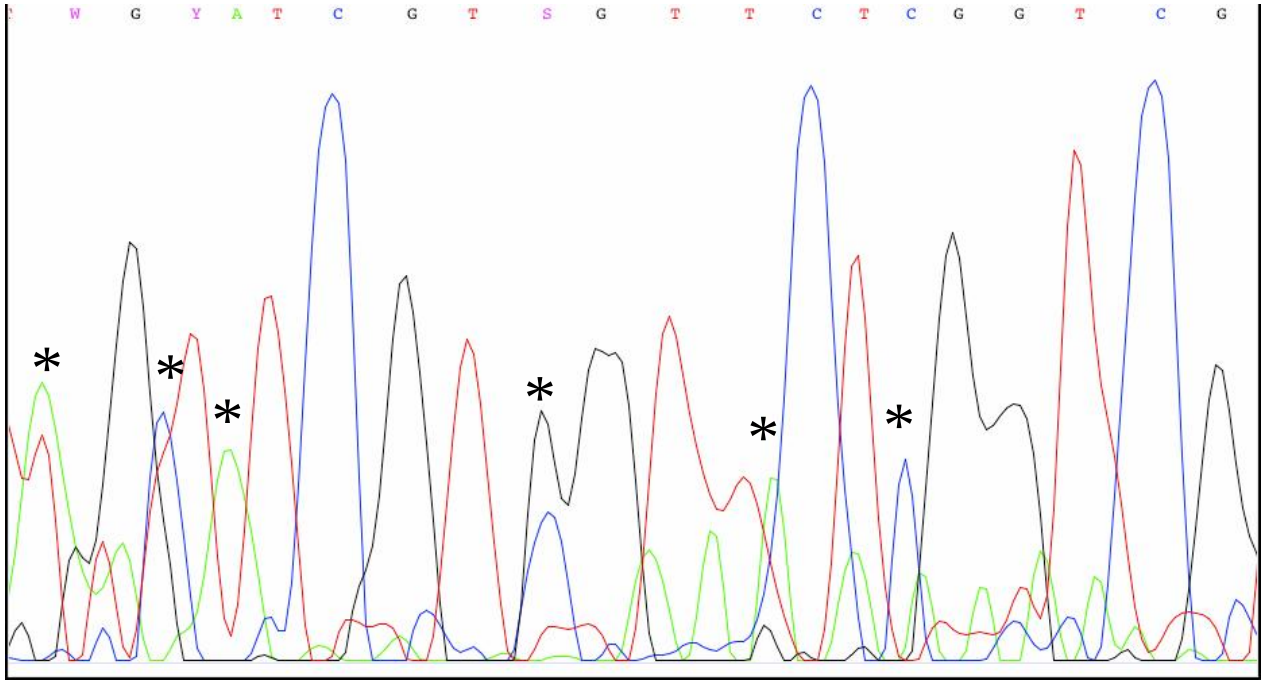
Sequence of origin



Sequence after
TAPE-mutagensis
(batch-sequencing)



Phage engineering.

Host-targeting mechanism.

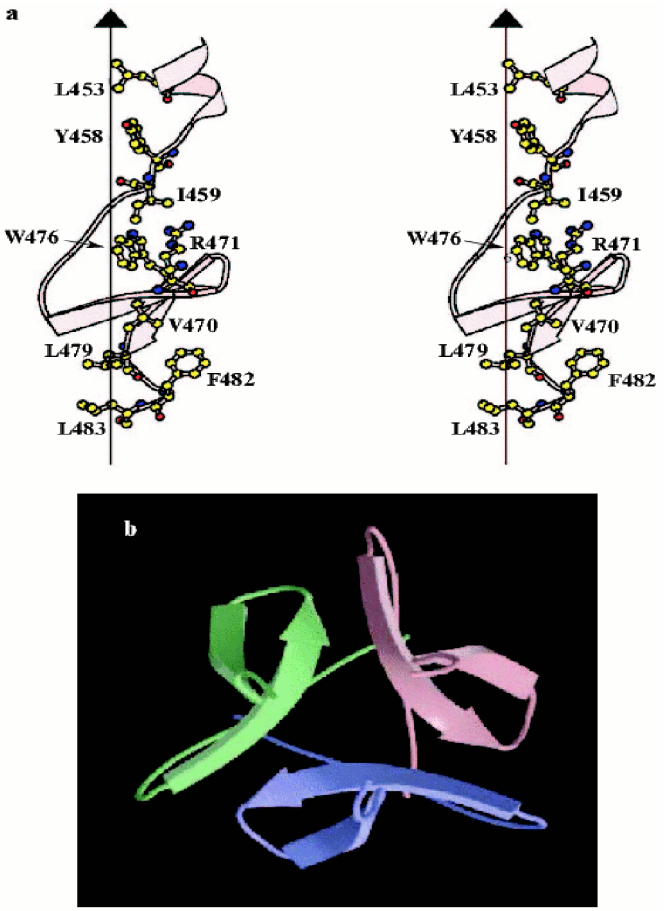
(Using "Tape" + "Ab-Accus")

The phage host-targeting proteins are stochastically engineered to encode hypervariable regions interspersed between constant domains.

The host-range resulting from each engineering manipulation is unknown.

But, each engineering step generates thousands of variants for a given protein, and

When several proteins are being thus manipulated, the number of variants that can be generated nears practical infinity.



Starting from a single phage, this results in the creation of a large bank in which each individual differs from all the others for at least one component of its host-targeting system.

The « Detector-Killer » Principle.

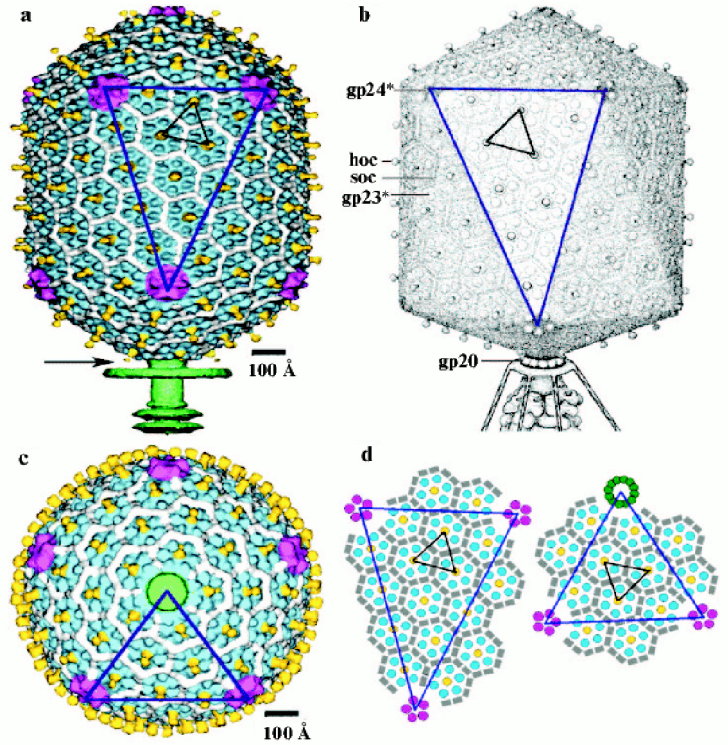
Detection mechanism.

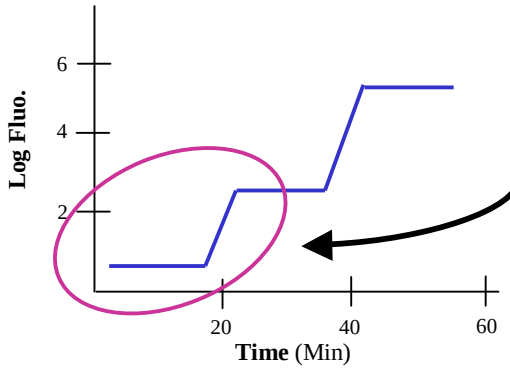
(Using “Tape” + “Ab-Accus”)

The phage capside is engineered to encode a fluorescent protein.

Upon finding its host the phage replicates, giving rise, 20 min later, to a fluorescent progeny (50 to 300 progeny particles per bacterium)

This gives rise to a massive, highly localised, increase in fluorescence.





If this happens,

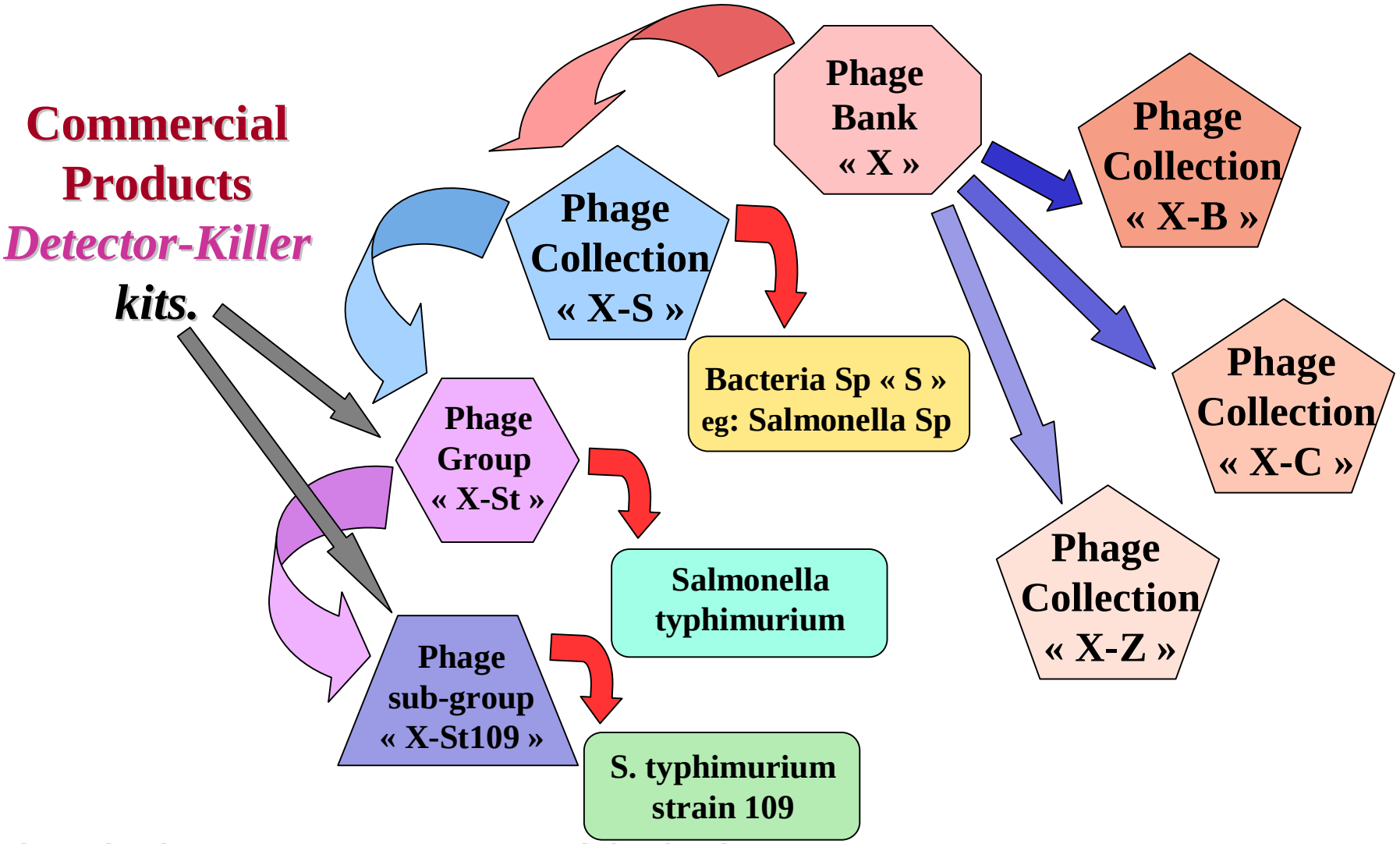
the target bacterium is ***present*** AND ***alive***

and

this population can be successfully eradicated.

The Pherecydes Products.

Commercial Products
Detector-Killer kits.



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IN CONCLUSION.

Bacteriophages are merely

A MEANS

not

AN END

- The company, currently negotiating a collaborative agreement with a diagnostics firm, is opened to collaborations.

BM Systems
Integrative Biology

But for BMSystems, that is merely another successful model.

- **CNS** → Creutzfeld-Jakob**, Alzheimer disease*
- **Systemics** → Breast cancer***, Tamoxifen resistance***, Metastasis*
- **infection/Immunology** → Antibacterial bio-agent**, Hepatitis C, CFS
- **Tissue differentiation** → Adipocytes growth control, Müllerian regression**
- **Metabolism** → Hypercholesterolemia,
- **White biotech** → Program Synthons (Bioproduction processes)**

***: Experimentally validated and published models
** : Experimentally validated models
* : Models under experimental evaluation.

Thank you to:

- **The systems biology teams at BM-Systems.**
  (*Directed by Dr. F. Iris, P-H Lampe & M. Gea*)

- **The R&D teams at Pherecydes Pharma.**
  (*Directed by Dr. F. Pouillot & T. Reynaud*)

- **The validation team at IBT.**
  (*Directed by Dr. V. Genty)*

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