Recherche de nouveaux antiviraux contre les arboviroses
Quelles pistes, quels espoirs et candidats à l'évaluation clinique ?
Enseignements des programmes européens VIZIER & SILVER

Why do we need antivirals ?

1

Fear, and intent to care

Spanish flu
~50 M deaths

2

3

4

1997-200?: avian flu in humans
2003: SARS Coronavirus
2006: Chikungunya spreads to IO islands & India
2011: novel tick-borne phlebovirus in China (NEJM)

5

Bad BUGS, No DRUGS !!!

How to be prepared against emerging viruses ?

- emergence is unpredictable: what will be the next bug ???
- the best preparedness is KNOWLEDGE
- knowledge on different virus families
  - non pathogenic viruses are as important as the bad bugs
- ANTICIPATION is critical

Antivirals available against arboviruses TODAY

- **no treatment for any arbovirus !!!**
  - Flaviviruses → nothing
  - Alphaviruses → nothing
  - Bunyaviruses → nothing

- One old molecule → RIBAVIRIN
  - Multiple mechanisms of actions
  - Claimed to be the worst treatment by many people
  - But because it is the only one so far → tested everytime a crisis situation occurs
    - Either at large level: outbreak, emergence, new virus
    - Or at individual level: Critically ill patient

So what do we do ??? Go fishing !!!

- to conduct distinct strategies in parallel
  - to increase the chances
  - mutual improvements / data exchange / collaborative programs (EU initiatives FP6 & FP7)

- transdisciplinary programs
  - virology
  - chemistry
  - crystallography
  - Industry…

- from hypothesis-driven to technology-driven approaches

Background research activity is pivotal

The wedding of Virologists and Crystallographs
20 European research groups

Background research activity is pivotal

The wedding of Virologists and Crystallographs
20 European research groups

- On one hand,

a long story of genomics of RNA viruses

KU
WNV NS5 99

Barkedji
Chaoyang
LAMV
DENV 3
DENV 1
DENV 2
DENV 4
ZIKV Yap
ZIKV
SPOV
KEDV
AROAV
IGUV
NJLV
BSQV
STRV
KOKV
ITV
BAGV
NTAV
STWV
TMUV
SLEV MSL7
SLEV
ROCV
ILHV
CPCV
USUV SA
USUV Vienna
MVEV
ALFV
JEV
YAOV
KOUV
WNV LEIV
WNV Rabensburg
WNV 99
WNV Hungary06
WNV AY853
WNV India
KUNV
WNV NY385 99

Barkedji
Chaoyang
LAMV
DENV 3
DENV 1
DENV 2
DENV 4
ZIKV Yap
ZIKV
SPOV
KEDV
AROAV
IGUV
NJLV
BSQV
STRV
KOKV
ITV
BAGV
NTAV
STWV
TMUV
SLEV MSL7
SLEV
ROCV
ILHV
CPCV
USUV SA
USUV Vienna
MVEV
ALFV
JEV
YAOV
KOUV
WNV LEIV
WNV Rabensburg
WNV 99
WNV Hungary06
WNV AY853
WNV India
KUNV
WNV NY385 99

- On one hand, a long story of genomics of RNA viruses, including arboviruses
- **On the other hand,**

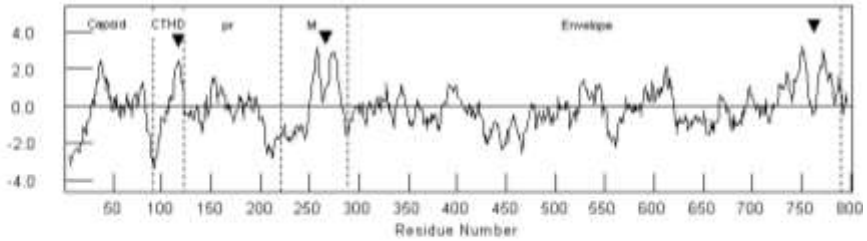
  a strong requirement for medical preparedness against pathogenic emerging RNA viruses

- On one hand, a long story of genomics of RNA viruses, including arboviruses
- **On the other hand,**

a strong requirement for medical preparedness against pathogenic emerging RNA viruses

Since they share similar genome strategies, potential antivirals for individual flaviviruses may display antiviral activity against other viruses within the family (Borowski et al., 2003; Deas et al., 2005).

Conserved enzymatic AA patterns



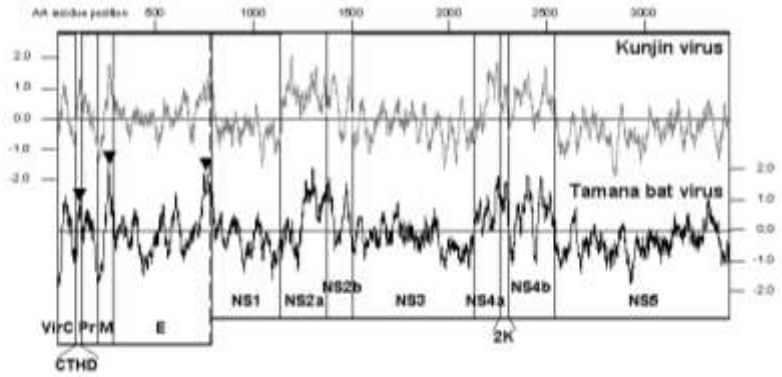


FIGURE 2. Hydropathy plots.

Figure 2a: structural part of the polyprotein of TABV (sliding window = 11 AA, increment = 1 AA).

Figure 2b: Comparison of the hydropathy profiles of the complete polyproteins of TABV and KUNV (sliding window = 25 AA, increment = 1 AA).

Pointers (▼) show the hydrophobic signal sequences that possibly act for the translocation in the lumen of the ER of the pr, E and NS1 proteins.

- On one hand, a long story of genomics of RNA viruses, including arboviruses
- **On the other hand,**

a strong requirement for medical preparedness against pathogenic emerging RNA viruses

Since they share similar genome strategies, potential antivirals for individual flaviviruses may display antiviral activity against other viruses within the family (Borowski et al., 2003; Deas et al., 2005).

- On one hand, a long story of genomics of RNA viruses, including arboviruses
- **On the other hand,**

a strong requirement for medical preparedness against pathogenic emerging RNA viruses

Since they share similar genome strategies, potential antivirals for individual flaviviruses may display antiviral activity against other viruses within the family (Borowski et al., 2003; Deas et al., 2005).

Antivirals

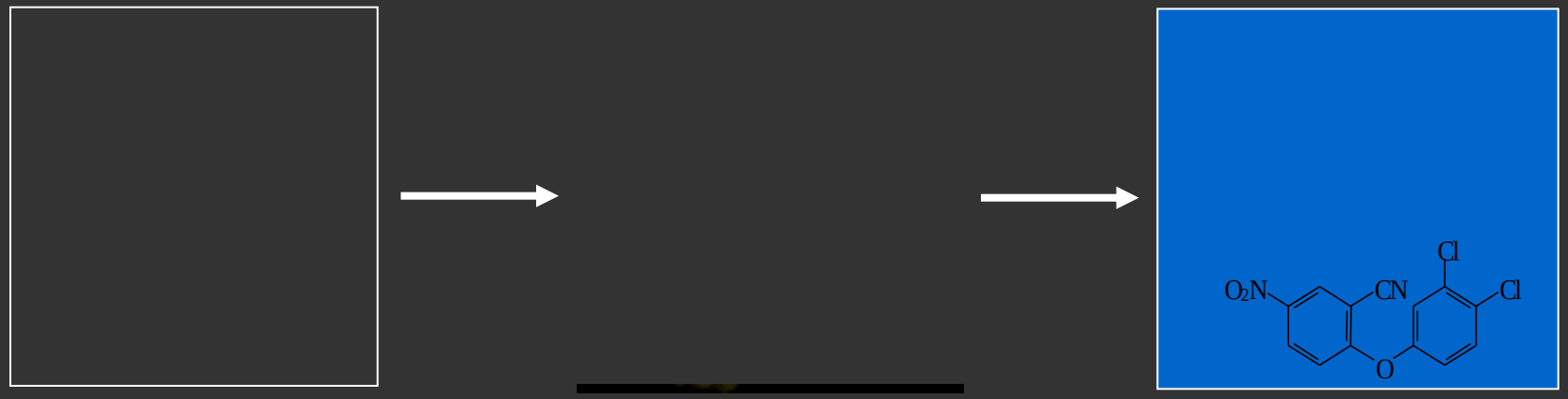
Is it possible to obtain large spectrum drugs ?

First Approach = conserved targets (VIZIER EU FP6 project)

A clear link with genomics studies

VIZIER: VIral enZymes Involved in Replication

Goal : Identification of **new targets** from **RNA viruses** through a **structural characterization** of the replicative system

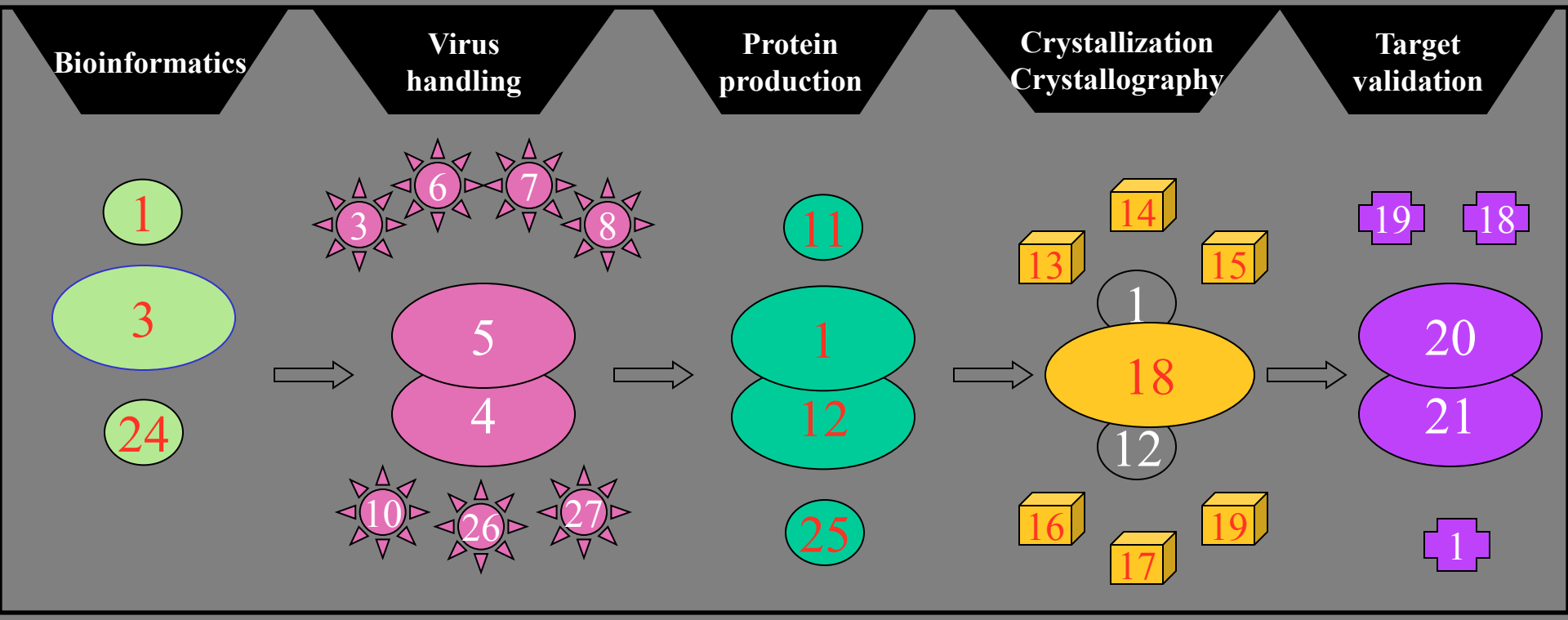


Structural Genomics project supported by the European Union

Frametime: November 2004 - April 2009

Means : 25 “partners” (public research centers)
>100 full time researchers involved
13 M€ funding by EEC in the 6th Framework
coordination: Aix-Marseille Université

An original organization: The VIZIER Consortium



Why so many viruses in VIZIER ?

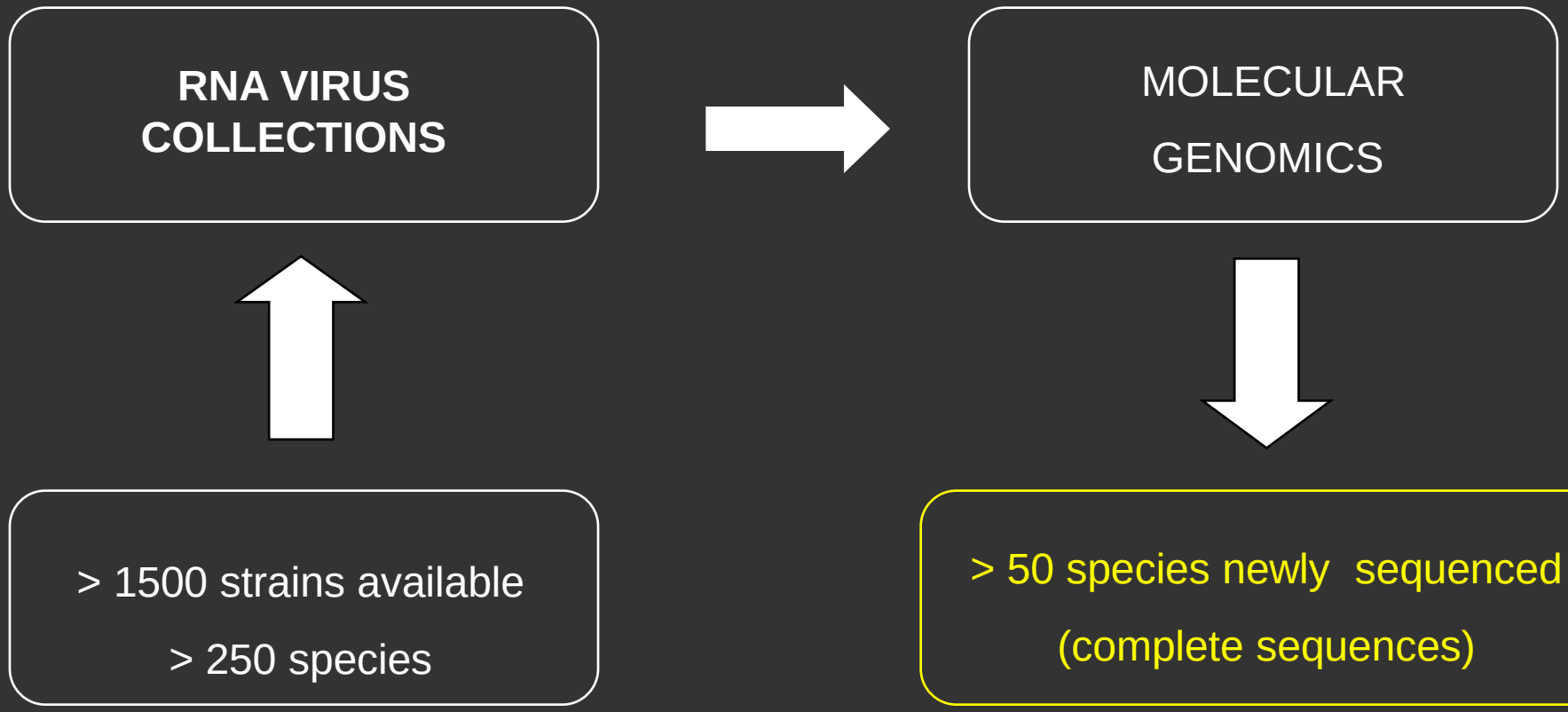
- "circus strains" are not valid models
  - circus strains are multi-passaged collection strains which patterns have been modified overtime

- The example of anti proteases and HIV must not be forgotten

- clinical / environmental isolates are pivotal: genetic diversity is not useless
- virus genetic diversity and quasispecies must be turned into an advantage

- our experience in VIZIER is that
  - 20 constructs based on genotypes of the same virus may be necessary to obtain one crystal structure
  - 1 AA change can drastically alter or improve the protein properties

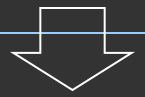
- "secondary aspects" of VIZIER have been considered at the outset
  - Diagnostics tools
  - Tools to discover new viruses
  - evolutionary aspects and mechanistic consequences on emergence


RNA VIRUS COLLECTIONS
MOLECULAR GENOMICS
> 1500 strains available
> 250 species
> 50 species newly sequenced
(complete sequences)

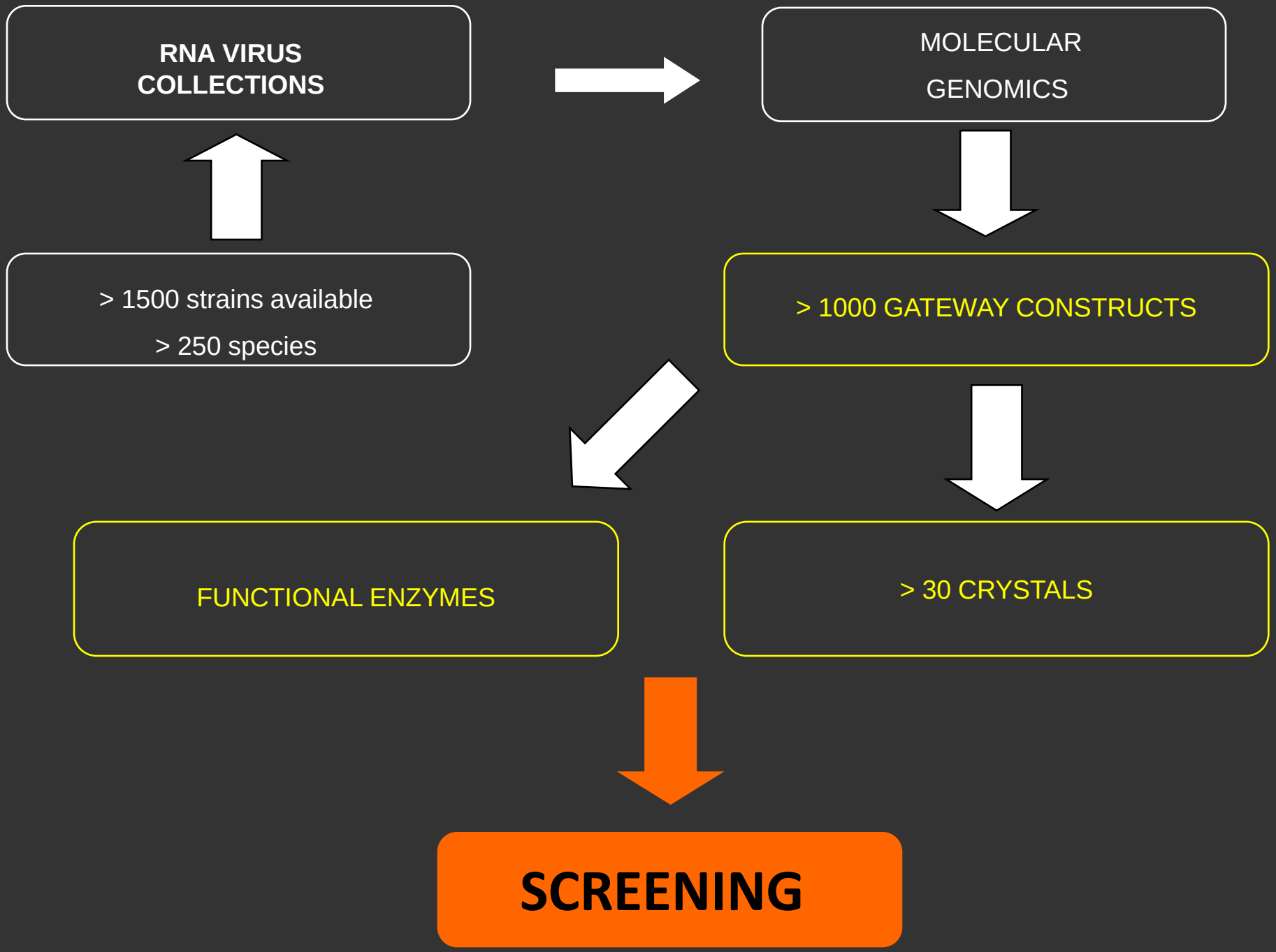
The VIZIER achievements

The invaluable contribution of "exotic viruses" aka "weird viruses

Yokose virus methyl transferase	Wesselsbron virus methyl transferase	Modoc virus methyl transferase	Meaban virus methyl transferase
Chikungunya virus nSP3 macrodomain	Venezuelan Equine Encephalitis virus nSP3 macrodomain		Kunjin virus helicase



PATENT


RNA VIRUS COLLECTIONS
MOLECULAR GENOMICS
> 1500 strains available
> 250 species
> 1000 GATEWAY CONSTRUCTS
FUNCTIONAL ENZYMES
> 30 CRYSTALS
SCREENING

The aims of were virus genomics
& crystal structures of virus enzymes

After

SILVER
(Oct 2010-Sept2014)

(22 partners, €12 million)

Small-molecule Inhibitor Leads
Versus Emerging
and neglected RNA viruses

SILVER Partners

SILVER Objectives

SILVER Objectives

Do not forget alternative options

Chikungunya virus as an example

- massive outbreak started in 2005 and affected Indian Ocean area and SE Asia > 3 millions people
- unpredicted, perfect paradigm of emergence
- scientific and medical community unprepared
- race to look for countermeasures: vaccines, antivirals

Chikungunya: too late for a new compound !!! what about an old one ???

- chloroquine: active *in vitro,* useless *in vivo* (*de Lamballerie et al 2008)*
  - IFN-alpha / ribavirin: active *in vitro*, not tested *in vivo* *(Briolant et al 2004, de Lamballerie et al 2009)*

•Arbidol (*1-méthyl-2-phényl-thiométhyl-3-carbotoxy-4-diméthyl -aminométhyl-5-hydroxy-6-bromoindolehydrochloride monohydrate*)

• developed at the Russian Research Chemical and Pharmaceutical Institute 20 years ago (Panisheva et al 1988)

• since used in Russia for prophylaxis and curative in acute respiratory infections including influenza

• ARB has shown activity against a number of RNA and DNA viruses (Boriskin et al 2008) suggesting targeting critical steps in virus-cell interactions:

  • incorporates into cellular membranes causing inhibition of virus-mediated fusion (Villalain 2010)

Chikungunya virus as an example

- en culture cellulaire: IC50 ~10µg/ml, avec CC50/IC50 ~30
  - Dose cytotoxique 30 fois supérieure à la dose efficace
  - pas d'effet virucide direct

Chikungunya & Arbidol

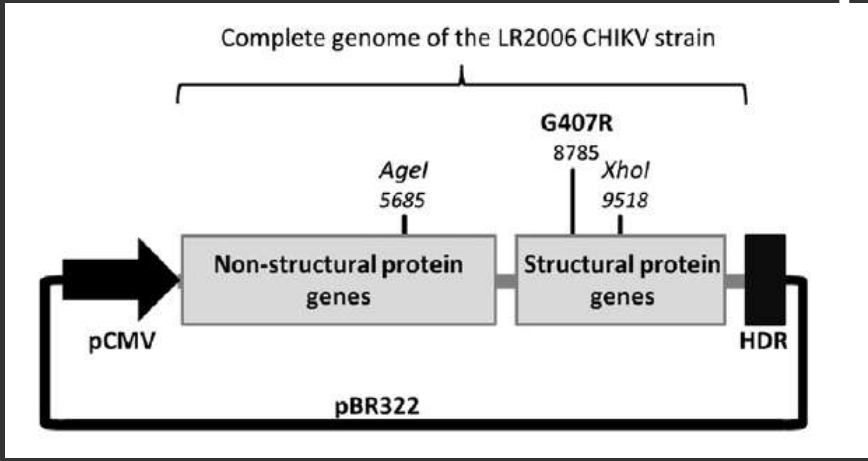
- ARB interferes with the earliest stages of the viral replication
  - Virus attachment and entry in the cell

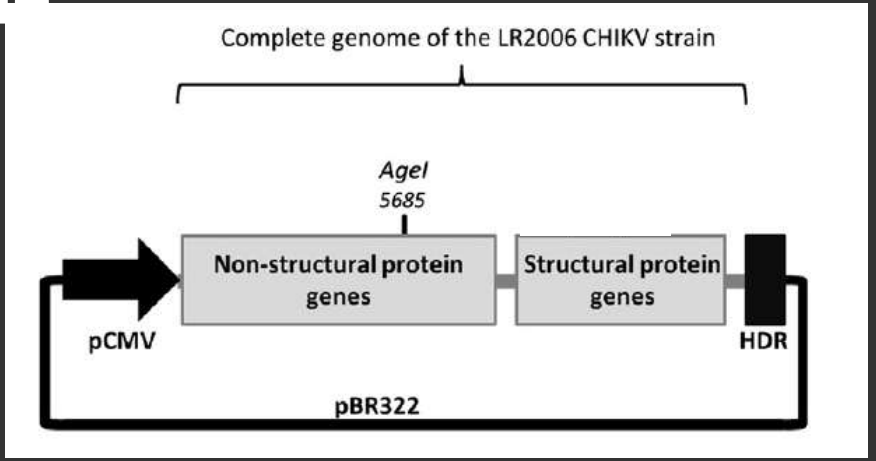
Selection of ARB-resistant mutant

- sucessive passages in cell culture with increasing doses of the drug
  - 4 to 30 µg/ml (IC50 = 10 µg/ml)
  - 17 passages (4 months)
  - ARB-R strain was end point purified in 30 µg/ml
  - complete sequence was determined

    A unique mutation G407R in the E2 viral protein

ARB-R infectious clone / ARB-S infectious clone





ARB-R infectious clone / ARB-S infectious clone

Arbidol and Chikungunya: and NOW !!

- further evaluation in
  - animal models
  - pre-clinical and clinical studies
- to decipher the mechanism of action on CHIKV

- to test Arbidol on other viruses
  - RSV
  - Filoviruses (Ebola, Marburg)
  - Phleboviruses (Toscana & Rift valley fever)

In conclusion

1. Select virus models within each family of interest

2. High throughput compounds / FDA-AMM approved drugs for a variety of viruses representing a panel of virus families
   - using live virus
   - using different protocols to test for various mechanisms: attachement, release, replication

3. **Test a DRUGSTORE** !!! ➔ blind testing of licensed drugs for virus panel

4. Clinical virus isolates are of **MAJOR IMPORTANCE**
   - Never forget anti-protease story for HIV (1986 / 1994)

Thank you for your attention