A structure-based approach for novel immunodiagnostics targeting *Trypanosoma cruzi* and *Schistosoma spp.*

Stefano De Benedetti[1]*, Flavio Di Pisa*[1]*, Louise Gourlay*[1] *and Martino Bolognesi*[1]

[1]University of Milano, Dip. Bioscienze , Via Celoria 26, Milano, Italy.

INTRODUCTION

Neglected tropical diseases, Chagas Disease and Schistosomiasis are caused by the parasites *Trypanosoma cruzi* and *Schistosoma spp*, respectively and are most commonly found in Latin America and Central Africa, affecting more than 210 million people worldwide (http://www.who.int).

T. cruzi is usually transmitted via the faeces of blood-sucking bugs (Triatominae). T. cruzi enters the human body through microlesions, by breaking through intact mucous membranes, blood transfusions, organ transplantations, congenitally and by ingestion of contaminated food and drink.
The disease is generally characterized by an acute phase, asymptomatic and the later chronic phase, associated with organ damage [1].

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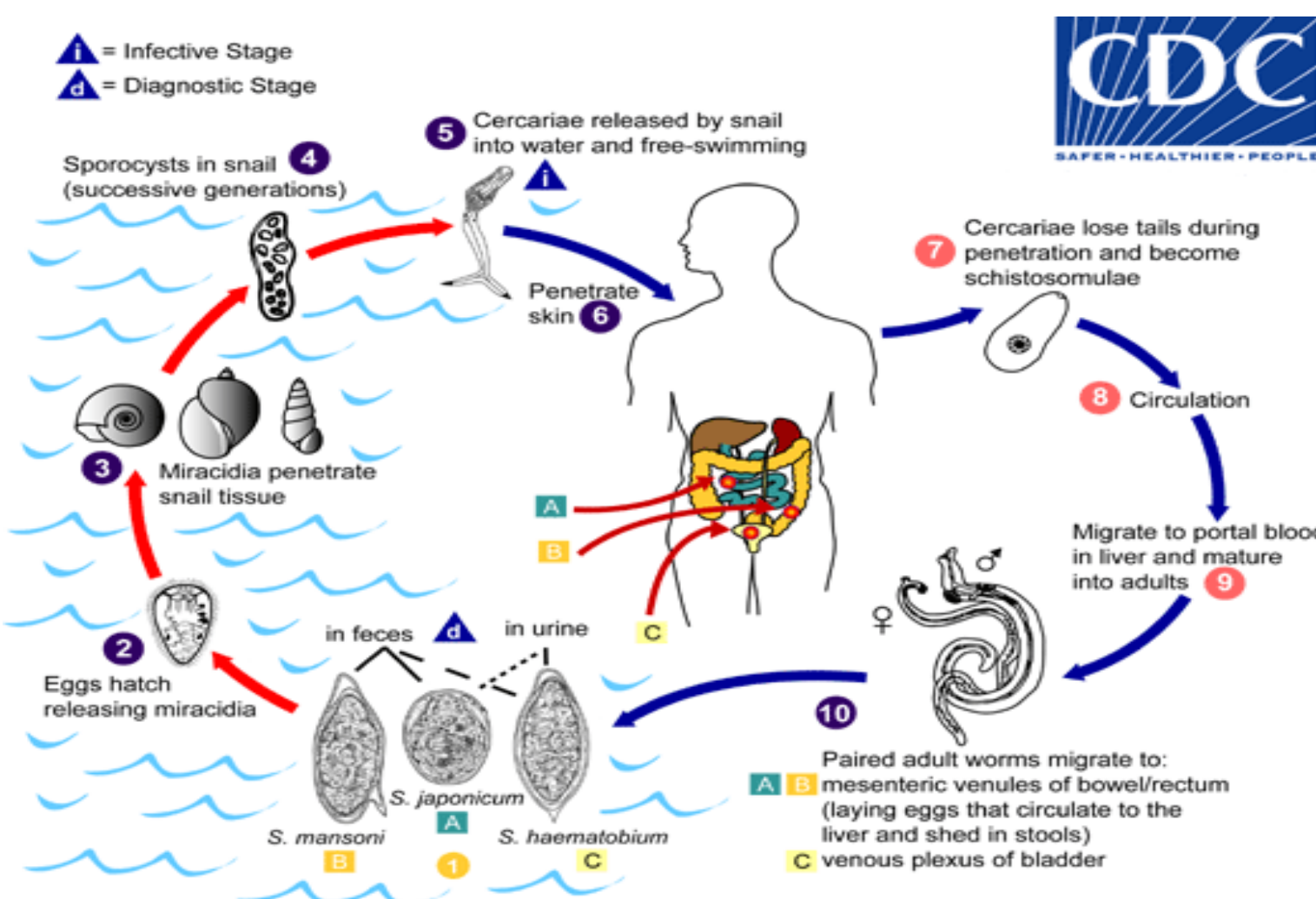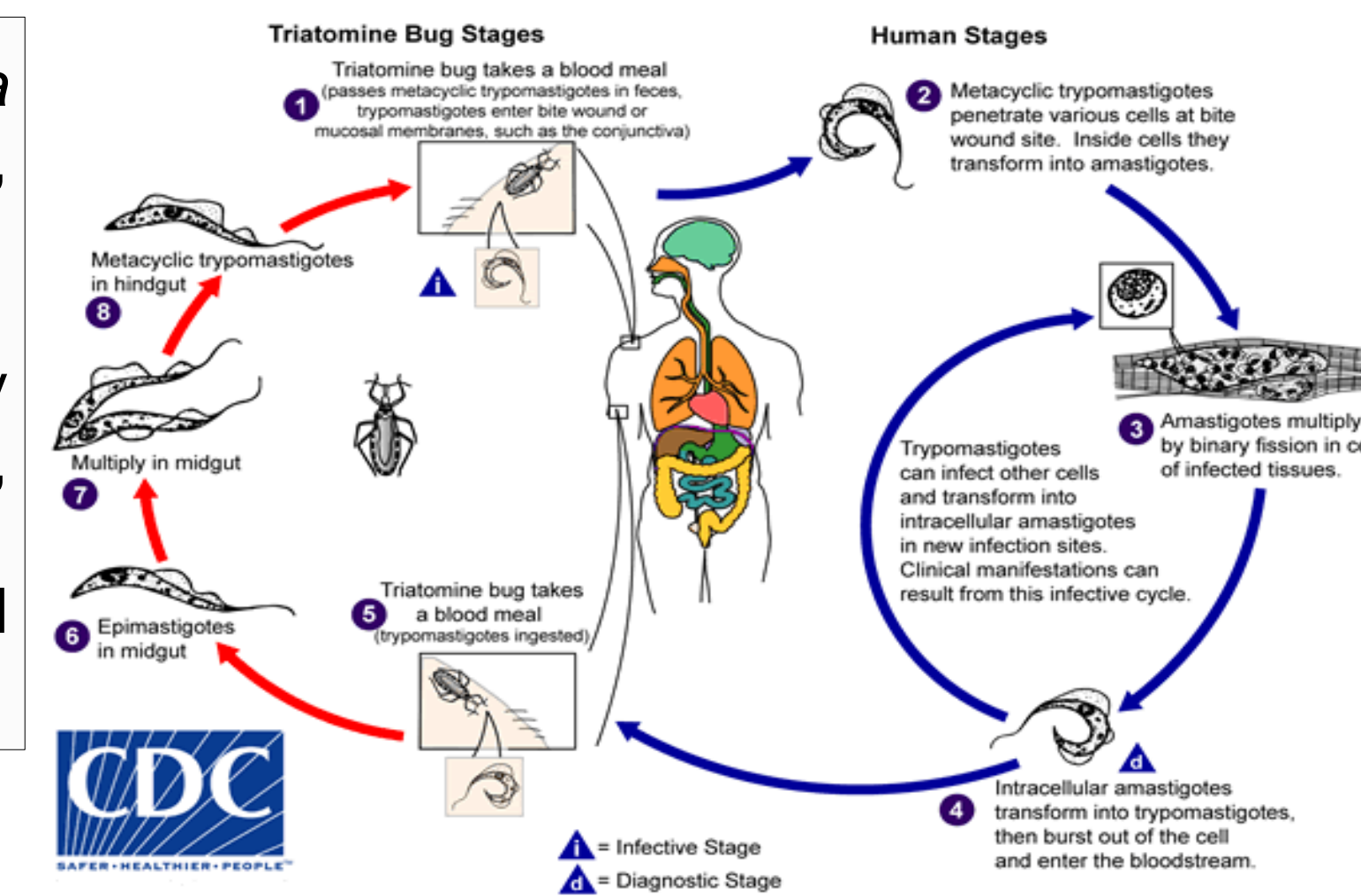
Schistosoma mansoni is usually transmitted by Biomphalaria freshwater releasing infectious larvae (**cercariae**), which burrow into human skin upon contact with contaminated water sources. The parasite transforms into a **schistosomulum** that enters the circulation and migrates to the hepatic portal and mesenteric veins. Here, schistosomula develop into sexually mature **adults** (male and female forms) that can evade immunity and thrive for many years.
Intravascular adult females produce hundreds of **eggs** daily during this time, which either cross the intestinal lumen to continue the lifecycle or circulate to the liver where they induce a robust host immunological response. Chronic inflammation of the liver ultimately results, leading to portal vein hypertension and severe hepatic fibrosis [2].

Both Chagas Disease and Schistosomiasis diseases are often misdiagnosed and, if untreated, can be fatal. Diagnosis represents a challenge, due to difficulties in recognizing clinical symptoms and the lack of specific and reliable diagnostic tools [1,3]. Furthermore, they are diffusing also in non-endemic regions, including Lombardia, as a consequence of the massive human migration from endemic areas and the increasing travelling to tropical regions.
For all these reasons, there is an increasing need of rapid diagnostic tools and prevention strategies.

The READy project is based on the identification, design and production of bioreagents (antibodies and synthethic epitopes), kits and diagnostic tools which may expand the perspectives in the diagnostic and vaccine design fields against Chagas Disease and Schistosomiasis.

AIM OF THE READy PROJECT

- Establishment of a regional, scientific network of excellence for the rapid response to bio-emergencies

- Development of a rapid, peptide-based microarrays that present multiple immunoreactive epitopes predicted from 3D protein structures of *Schistosoma* and *Trypanosoma* antigens

GENERAL WORKFLOW

The workflow: from 3D structure to microarray diagnostic tools

Antigen 3D structure determination — Epitope prediction — Epitope extraction and design of peptides: synthesis of easy-to-manage, small sets of *ad hoc* designed peptides — Microarray — Test response

Secondary mAb
Spacer
Signal intensity
Ctrl Peptide1 Peptide2 Peptide3

UniMi

UniMi activities

ANTIGEN SELECTION
- Antigen selection based on literature and public databases
- Criteria for antigen selection: immune sera reactivity, antigenicity, abundance and sequence conservation among different strains, exposure to the host immune system during the acute stage of infection

12 antigens selected

ANTIGEN PRODUCTION AND PURIFICATION

At this stage:
- **11 genes cloned in pTXB1 and pGEX4T-1 vectors**
- **Expression trials ongoing**
- **Sm SERPIN successfully purified with IMPACT system**
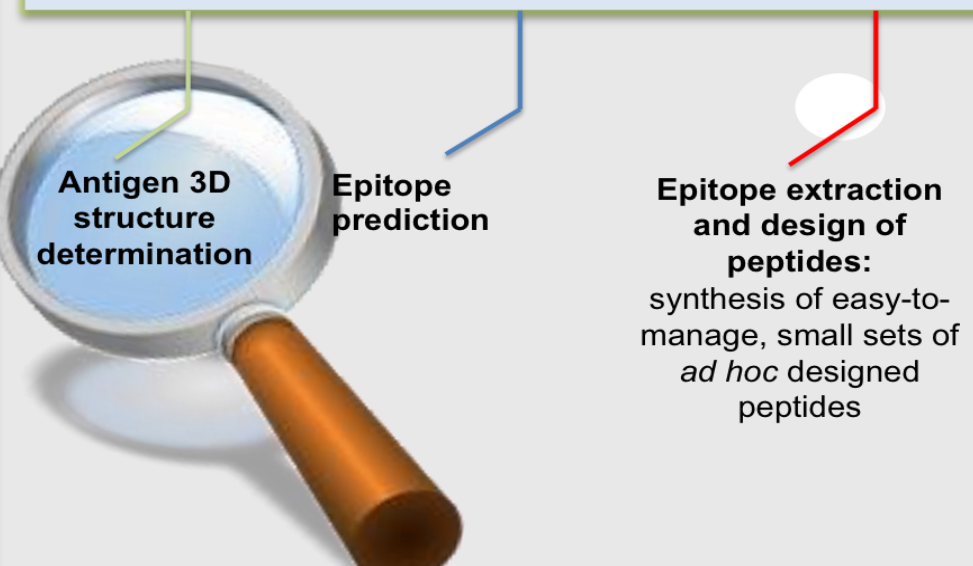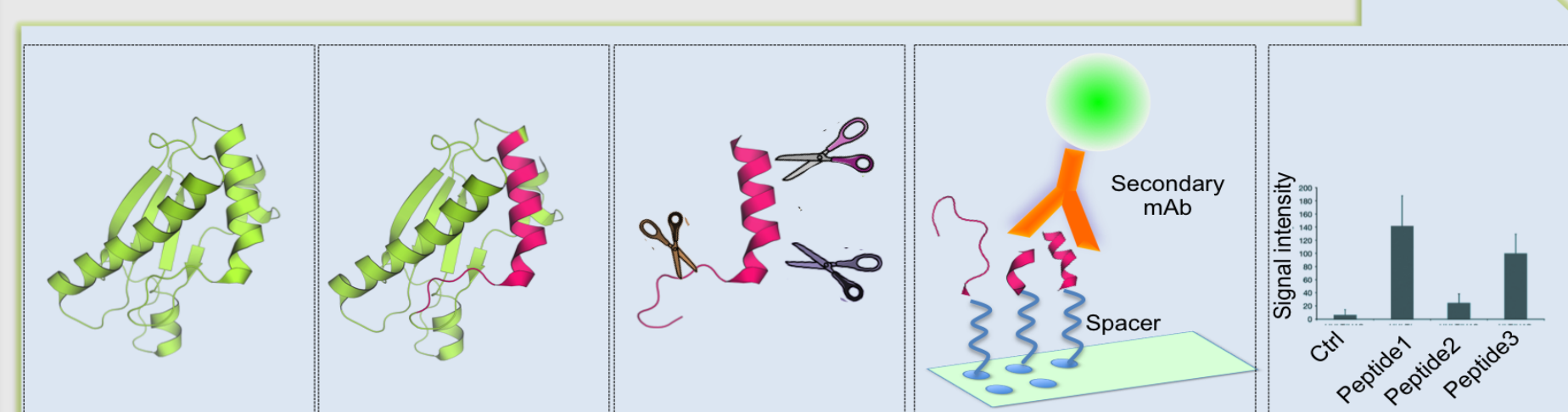
ANTIGEN 3D STRUCTURE DETERMINATION
- Antigen structure prediction using online tools
- Structure modelling on templates (sequence and structural analogues)
- Crystallization screens
- X ray diffraction experiments
  At this stage:
  Sm SERPIN structure refinement

3D

References:
1) Rassi A Jr et al., (2010). Lancet 375: 1388–1402.
2) Norseth et al., (2014). Plos Negl Trop Dis 20;8(11):e3229
3) Gray DJ et al., (2011). BMJ 342.

Contact: stefano.debenedetti@unimi.it