Supplementary Material

Structure-based discovery of the novel antiviral properties of naproxen against the nucleoprotein of Influenza A virus

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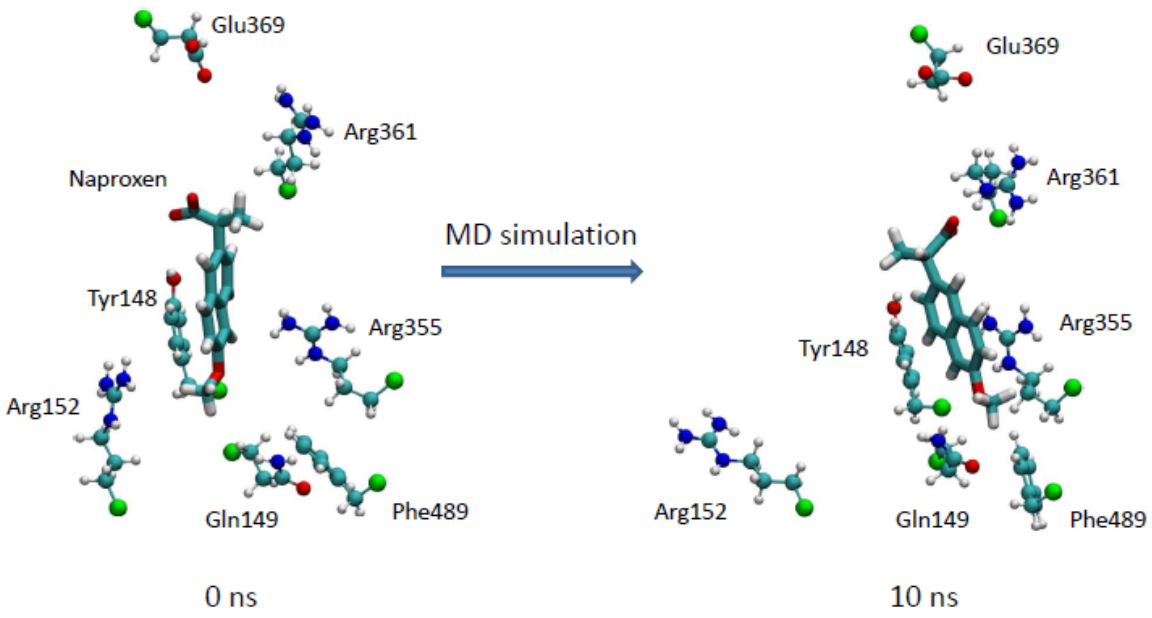
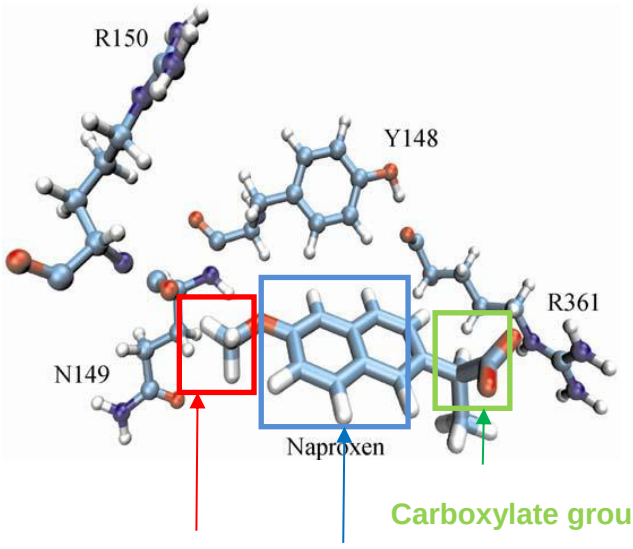
Supplementary Figures 1-6: pages 2-9

Supplementary Movie: naproxen_movie.avi

We visualize the movie with VLC media player www.videolan.org

Supplementary Figure 1:

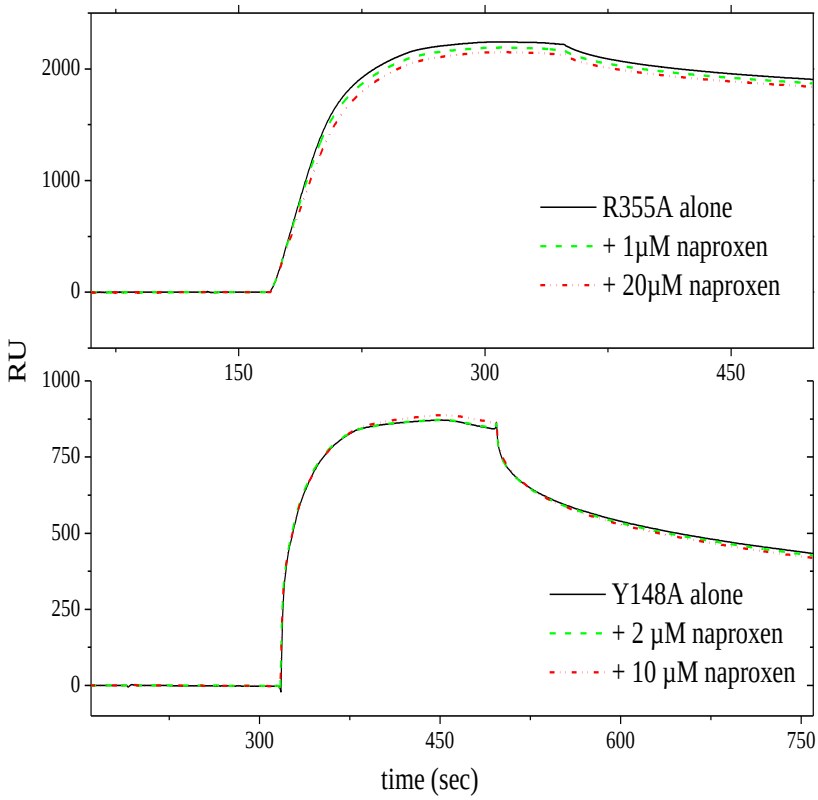




Close-up view of the interactions between naproxen and NP before (left) and after 10 ns (right) molecular dynamics simulations. In the initial step, the naphthalene ring of naproxen stacks on Tyr148, with weak interaction with its OH group while Arg361 forms a salt bridge with Gln369. After 4.5 ns MD simulation, this salt bridge is disrupted, and the rotation of Arg361 enables

electrostatic interactions between the positively charged guanidinium group of Arg361 and the negatively charged carboxylate group of naproxen. This drove a reorientation of the naphthalene core and methoxy group toward Arg355, Phe489 and Gln149 and away from Arg152. A different angle is provided in the bottom view, with the dissection of the different chemical parts of naproxen. A dynamic view of the process can be seen in the attached movie.

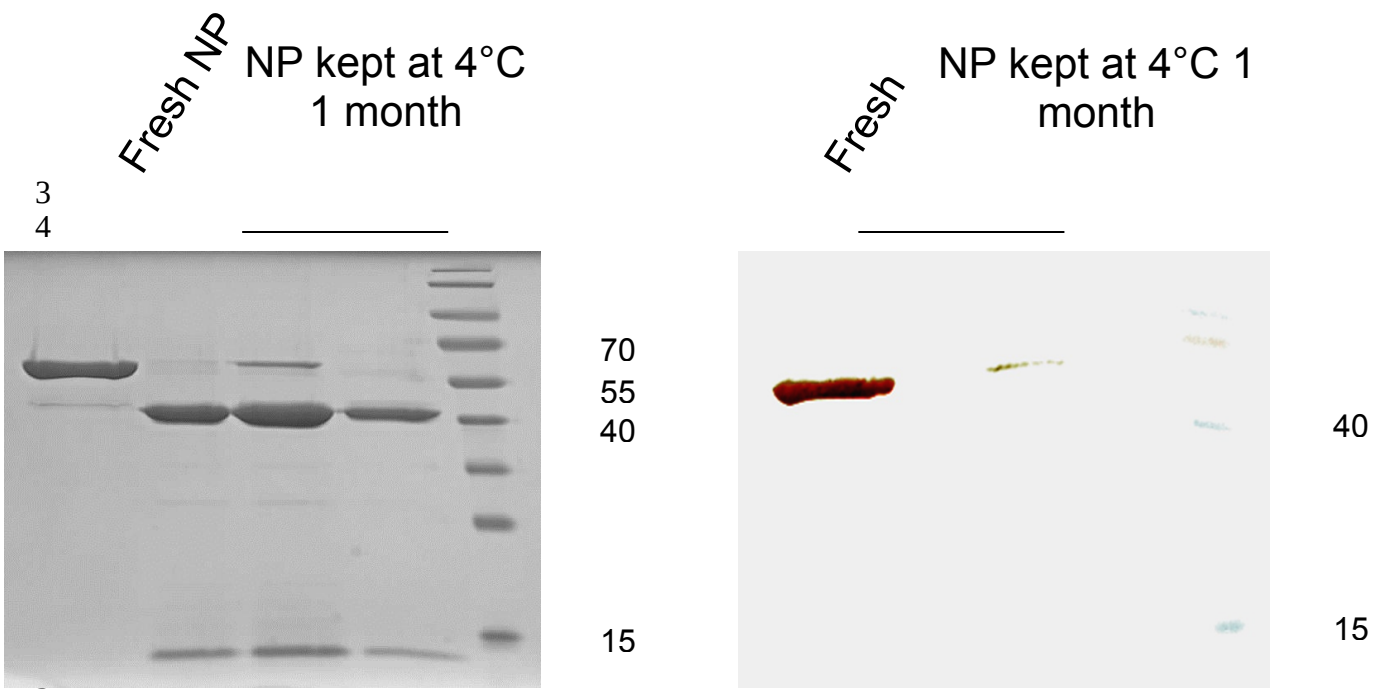
Supplementary Figure 2:



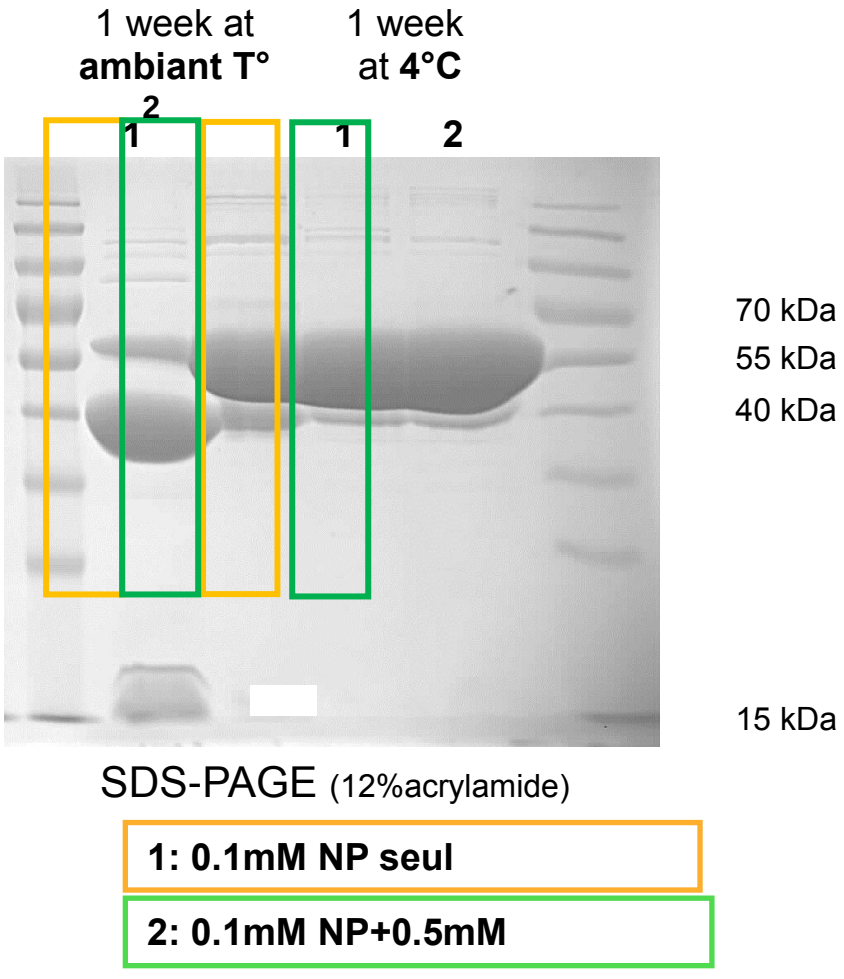
SPR data show the lack of interaction of naproxen with the mutated proteins Y148A and R355A: the signal of the protein- RNA complex (black curves) remains almost unchanged (R355A) or identical (Y148A) with or without naproxen added. This shows that naproxen is unable to compete with RNA binding to these mutated proteins, since the essential residues insuring naproxen binding to NP were mutated. This behavior was also seen with the mutant R361A (Figure 2B), in contrast with the competition observed with the wild-type NP (Figure 2A).

Supplementary Figure 3:

A



B



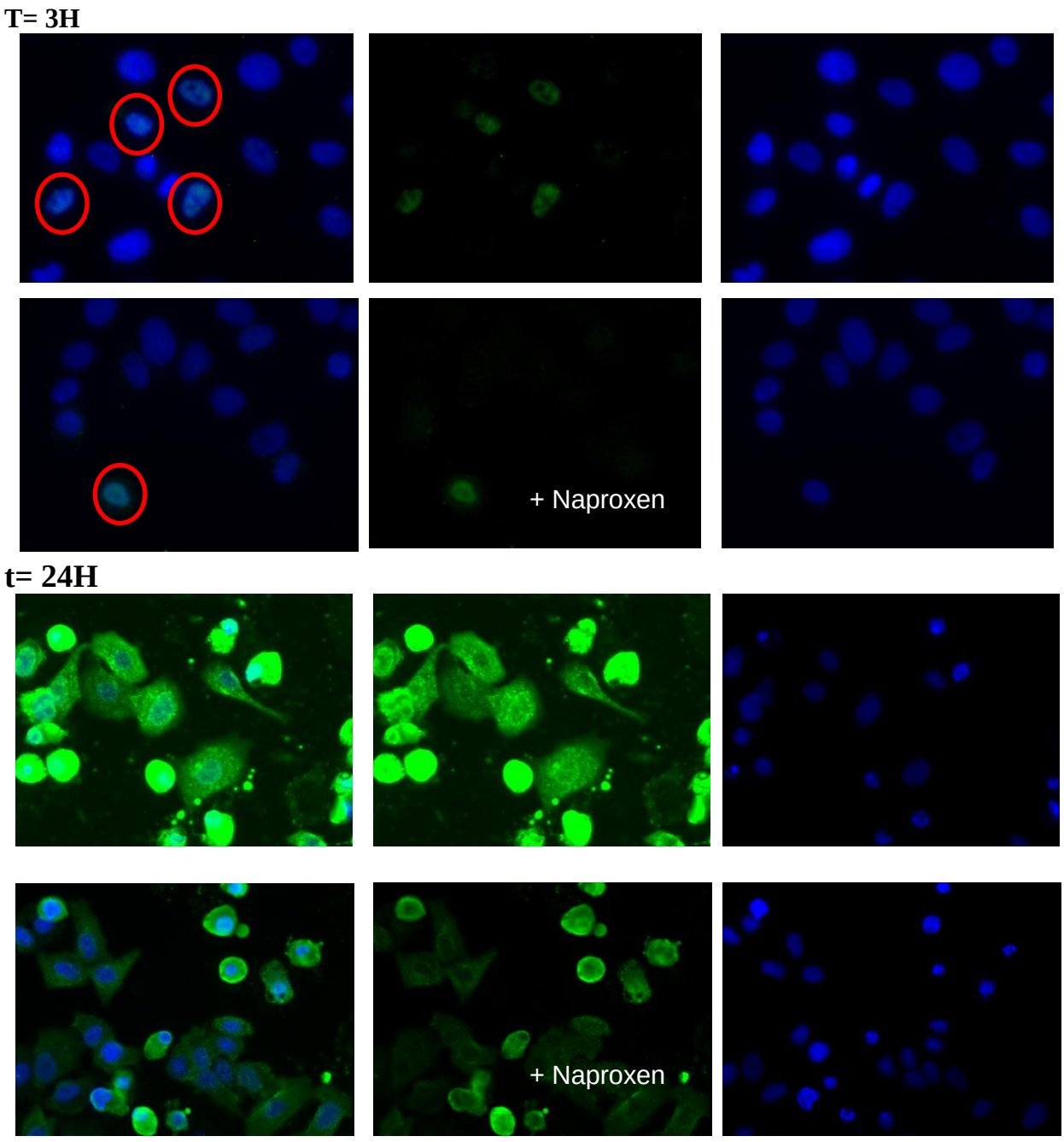
SDS-PAGE (12%acrylamide)

1: 0.1mM NP seul

2: 0.1mM NP+0.5mM

Monomeric NP labeled with a His-tag at its C terminal was purified as detailed in Materials and Methods. A: A freshly purified NP (10µM) sample or kept for a month at 4°C was run on a SDS-PAGE. The corresponding western blot using an anti-His antibody is shown on the right side. B: A similar experiment was performed with 100 µM NP alone (lanes 1) or 100 µM NP + 500 µM naproxen (lanes 2). When kept at room temperature, the cleavage of NP C-terminal is impeded by the presence of naproxen.

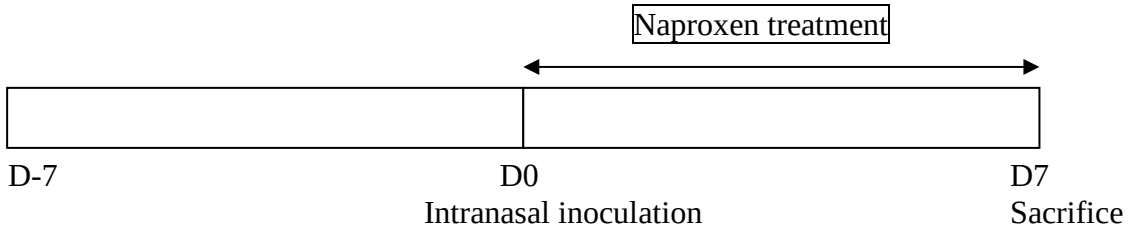
Supplementary Figure 4:



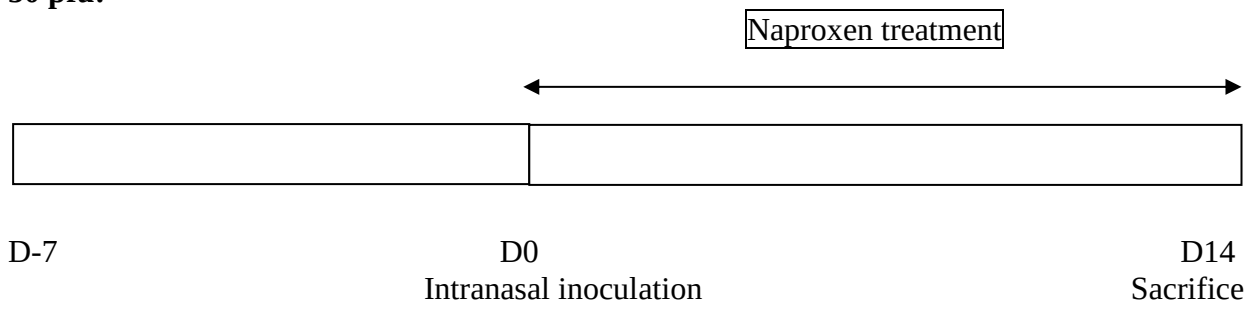
Immuno-fluorescence (IF) of A549 cells infected with Influenza A/WSN/3 stained with DAPI (nucleus, blue) and with a monoclonal anti-NP antibody (green) at t = 3H post-infection. The data show no change in the nuclear localization of NP upon addition of naproxen (50 µM).

Supplementary Figure 5: In vivo protocols

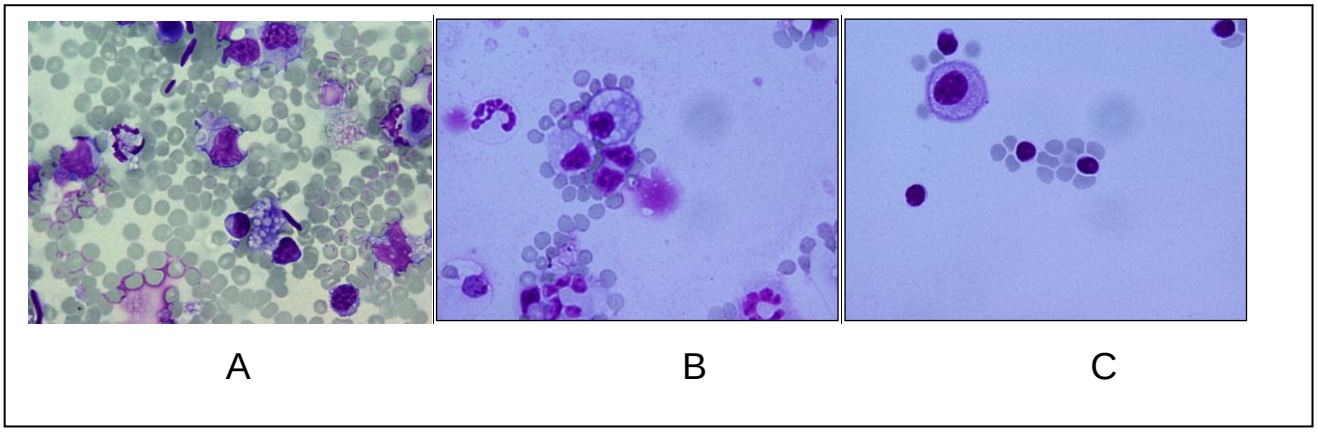
2000pfu:



50 pfu:



Supplementary Figure 6: Comparison of the broncho-alveolar fluid of infected (A), infected and treated with 3 mg naproxen (B) and non infected mice (C)



To collect broncho-alveolar lavage fluids (BAL), the mouse trachea was surgically exposed, cannulated with a syringe and the remaining lobes of the lungs were flushed four times in and out using 1.5 mL D-PBS (Gibco) supplemented with 1mM EDTA (Gibco). After centrifugation (4 min, 500g), viable BAL cells were resuspended at appropriate concentration for cytocentrifugation. BAL supernatants were stored frozen at −20°C. Microscopic examination of representative May-Grünwald and Giemsa stained cytocentrifuge slides are shown below**.**

In A, many red blood cells and a reduced number of macrophages in infected and untreated mice attested at bleeding and inflammation of the lungs; in the healthy control C, only macrophage and monocytes are observed; in infected mice with naproxen treatment (B, 3mg/ day), only a few red blood cells are seen and the macrophages are activated and overall the number of cells is reduced as compared to A.