Application of Computational Biology in Veterinary Medicine

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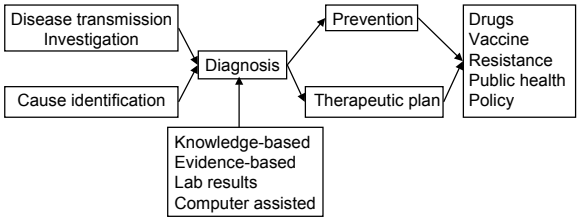
Application of Computational Biology in Veterinary Medicine

Traditional medicine protocol

Application of Computational Biology in Veterinary Medicine

Traditional medicine (and prevention) protocol



Application of Computational Biology in Veterinary Medicine

The NEW ENGLAND JOURNAL of MEDICINE

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Avian Influenza A (H5N1) in 10 Patients in Vietnam

Tran Tinh Hien, M.D., Nguyen Thanh Liem, M.D., Nguyen Thi Dung, M.D., Luong Thi San, M.D., Pham Phuong Mai, M.D., Nguyen van Vinh Chau, M.D., Pham Thi Suu, M.D., Vo Cong Dong, M.D., Le Thi Quynh Mai, M.D., Ph.D., Ngo Thi Thi, M.D., Dao Bach Khoa, M.D., Le Phuc Phat, M.D., Nguyen Thanh Truong, M.D., Hoang Thuy Long, M.D., Ph.D., Cao Viet Tung, M.D., Le Truong Giang, M.D., Ph.D., Nguyen Dac Tho, M.D., Le Hong Nga, M.D., Nguyen Thi Kim Tien, M.D., Ph.D., Le Hoang San, M.D., Le Van Tuan, M.P.H., Christiane Dolecek, M.D., Tran Tan Thanh, B.Sc., Menno de Jong, M.D., Ph.D., Constance Schultsz, M.D., Ph.D., Peter Cheng, M.Sc., Wilina Lim, M.B., B.S., Peter Horby, M.B., B.S., for the World Health Organization International Avian Influenza Investigative Team,* and Jeremy Farrar, F.R.C.P., D.Phil.

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Table 1. Epidemiologic Data.

Patient No.	Location in Vietnam	Occupation	Epidemiologic Information
1	Ha Nam	Student	Family members are farmers who do not keep poultry, but many chickens in neighborhood unexpectedly died in the preceding 2 wk; mother died of influenza A (H5N1) Jan. 9, 2004; father and younger sibling healthy.
2	Nam Dinh	Not available	No information available on exposure to sick poultry; 7-yr-old sister died of acute respiratory illness on Dec. 29, 2003; parents and two other siblings healthy.
3	Bac Ninh	Student	Family members are farmers who kept chickens, which died unexpectedly 5 days before onset of illness; parents and older sibling healthy.
4	Ha Tay	Student	Family members are farmers who kept chickens, which died 2 wk before onset of illness; chickens died in patient's house and neighbors' houses during week before onset of illness; parents and 7 other siblings healthy.
5	Ho Chi Minh City	Student	Patient bought duckling as pet and cared for it in her house for 3 days, duck had diarrhea and died, patient buried it, dug it up a day later and reburied it; both patient and brother handled duck; patient also ate barely cooked eggs (Vietnamese delicacy) 2 days before onset of illness; neighbors kept 40 chickens, but no illness reported in these birds; fever developed in patient 3 days after she bought duck; no other poultry or animals at home; no other household members or relatives sick.
6	Ho Chi Minh City	Student	Frequently attended cockfights; held roosters and chickens; no illness reported in the chickens or in 20 people involved in cockfighting; patient walked through live-poultry market 50 m from house on his way to school.
7	Soc Trang	Student	Extensive exposure, including handling of 10 dead or dying chickens in patient's homestead; father and patient prepared dead chickens for eating (removed feathers, washed, cut meat) 3 days before onset of illness; no other household members or relatives sick; no other poultry or animals at home.
8	Lam Dong	Farmer	Direct handling of 50 chickens, including dead chickens, at home (which was also a restaurant); patient and father prepared chickens for eating; no other household members or relatives sick; no other poultry or animals at home.
9	Lam Dong	Farmer	Direct handling of chickens in patient's homestead 3 days before onset of illness; he prepared dead chickens for eating; no one else in family sick.
10	Lam Dong	Farmer	Direct handling of sick ducks and chickens in patient's home; many sick poultry in the district; no other illness in family.

Figure 1. Map of Vietnam Showing the Reported Cases of Avian Influenza A (H5N1).

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Table 2. Clinical Characteristics of the Patients on Admission.

Variable	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Days between exposure to poultry and onset of illness	—	—	—	—	3	2	3	4	3	3
Days since onset of illness	3	7	7	5	8	6	5	6	5	7
Sex	Female	Male	Male	Female	Female	Male	Female	Male	Male	Male
Age (yr)	12	5	10	8	8	13	16	18	24	23
Cough	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Dyspnea	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Sputum	No	No	No	No	Yes	No	Yes	Yes	Yes	Yes
Diarrhea	Yes	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Rash	No	No	No	No	No	No	No	No	No	No
Myalgia	No	No	No	No	No	No	No	No	No	No
Conjunctivitis	No	No	No	No	No	No	No	No	No	No
Fever	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Temperature (°C)	39.5	38.8	39.0	38.5	38.5	39.6	40.0	40.0	39.5	38.7
Blood pressure (mm Hg)	90/60	112/54	105/80	80/40	104/64	110/70	110/60	100/60	110/60	120/80
Respiratory rate (breaths/min)	65	70	64	60	40	40	40	60	50	28
Crackles	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Wheeze	No	No	No	No	No	Yes	No	No	No	No
Other	Enlarged liver	—	—	Bleeding gums	—	—	—	—	—	—

Table 3. Laboratory Values at Presentation.*

Variable	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Hemoglobin (g/dl)	13.4	12.6	12.4	12.3	11.3	13.4	11.9	14.5	15.8	17.6
Leukocyte count (per mm³)	2,100	3,400	2,800	1,900	1,200	2,700	3,000	1,700	1,900	2,100
Lymphocyte count (per mm³)	1,100	710	860	250	300	900	500	500	800	700
Neutrophil count (per mm³)	850	2,410	1,900	780	700	1,300	2,500	1,100	1,100	1,300
Platelet count (per mm³)	45,000	174,000	135,000	91,000	117,000	81,000	70,000	69,000	62,000	62,000
CD4:CD8 ratio	NA	NA	NA	NA	0.71	NA	0.62	0.75	0.59	1.08
ALT level (U/liter)	53.7	NA	NA	265	354	254	47	NA	NA	89
AST level (U/liter)	278	NA	NA	1,217	320	1,058	20	NA	NA	110
Serum creatinine (μmol/liter)	50	64	NA	27	34	14	71	89	43	121
Serum glucose (mmol/liter)	NA	NA	NA	NA	NA	NA	19.0	13.5	11.7	4.9
Oxygen saturation during receipt of 40% oxygen (%)	50	70	86	50	95	85	67	81	80	90
Day of illness on which PCR for H5N1 performed	5	7	9	6	12	6	5	6	5	7
Viral culture	+	+	NA	NA	Pending	Pending	Pending	Pending	Pending	Pending
Influenza antigens	NA	NA	NA	NA	+	–	–	+	–	–
Blood culture	–	–	–	–	–	–		–	–	–
Outcome	Died (day 6)	Died (day 17)	Died (day 14)	Died (day 7)	Recovered	Died (day 9)	Died (day 14)	Died (day 9)	Died (day 6)	Recovering

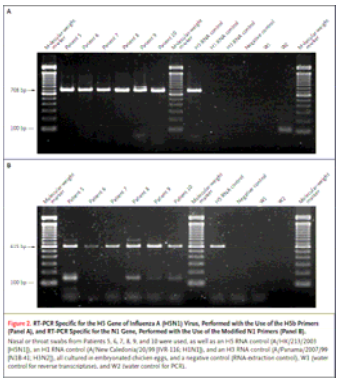


Figure 2. RT-PCR Specific for the H1 Gene of Influenza A (H5N1) Virus, Performed with the Use of the H5b Primers (Panel A), and RT-PCR Specific for the N1 Gene, Performed with the Use of the Modified N1 Primers (Panel B).

Nasal or throat swabs from Patients 1, 6, 7, 8, 9, and 10 were used, as well as an H5 RNA control (A/VN/1203/2003 [H5N1]), an H1 RNA control (A/New Caledonia/20/99 [IVR 116; H1N1]), and an H3 RNA control (A/Panama/2007/99 [NIB-41; H3N2]), all cultured in embryonated chicken eggs, and a negative control (RNA-extraction control). W1 (water control for reverse transcriptase), and W2 (water control for PCR).

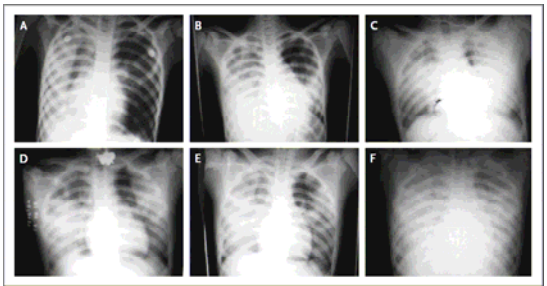


Figure 3. Chest Radiographs.

Radiographs from Patient 5 (Panel A), Patient 7 (Panel B), and Patient 9 (Panel C) show widespread consolidation, collapse, and interstitial shadowing. In Panels D, E, and F, three chest radiographs show the progression in Patient 8 on days 5, 7, and 10 of illness, respectively.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Transmission of Rabies Virus from an Organ Donor to Four Transplant Recipients

Arjun Srinivasan, M.D., Elizabeth C. Burton, M.D., Matthew J. Kuehnert, M.D., Charles Rupprecht, V.M.D., Ph.D., William L. Sutker, M.D., Thomas G. Ksiazek, D.V.M., Ph.D., Christopher D. Paddock, M.D., Jeannette Guarner, M.D., Wun-Ju Shieh, M.D., Ph.D., Cynthia Goldsmith, M.S., Cathleen A. Hanlon, V.M.D., Ph.D., James Zoretic, M.D., Bernard Fischbach, M.D., Michael Niezgoda, M.S., Waleed H. El-Feky, M.D., Lillian Orciari, M.S., Edmund Q. Sanchez, M.D., Anna Likos, M.D., M.P.H., Goran B. Klintmalm, M.D., Denise Cardo, M.D., James LeDuc, Ph.D., Mary E. Chamberland, M.D., M.P.H., Daniel B. Jernigan, M.D., M.P.H., and Sherif R. Zaki, M.D., Ph.D., for the Rabies in Transplant Recipients Investigation Team*

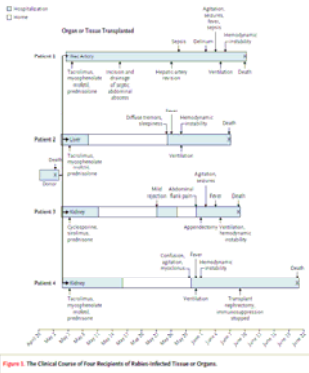
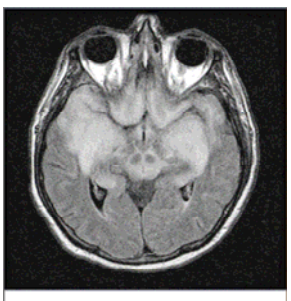


Figure 1. The Clinical Course of Four Recipients of Rabies-Infected Tissue or Organs.



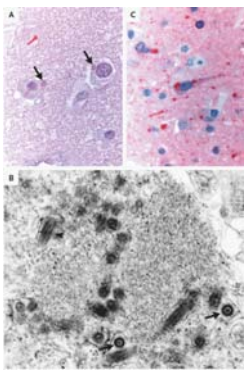
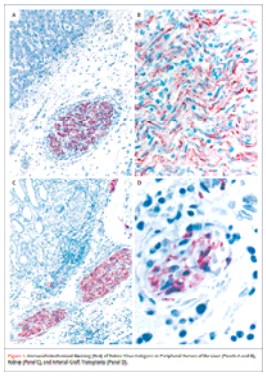


Figure 2. Axial Fluid-Attenuated Inversion Recovery MRI Scan Showing Profound Signal Abnormalities within the Bilateral Frontal and Temporal Lobes, Hippocampi, Basal Ganglia, and Medulla in Patient 2.



The NEW ENGLAND JOURNAL of MEDICINE

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Probable Person-to-Person Transmission of Avian Influenza A (H5N1)

Kumnuan Ungchusak, M.D., M.P.H., Prasert Auewarakul, M.D., Scott F. Dowell, M.D., M.P.H., Rungrueng Kitphati, M.D., Wattana Auwanit, Ph.D., Pilaipan Puthavathana, Ph.D., Mongkol Uiprasertkul, M.D., Kobporn Boonnak, M.Sc., Chakrarat Pittayawonganon, M.D., Nancy J. Cox, Ph.D., Sherif R. Zaki, M.D., Ph.D., Pranee Thawatsupha, M.S., Malinee Chittaganpitch, B.Sc., Rotjana Khontong, M.D., James M. Simmerman, R.N., M.S., and Supamit Chunsutthiwat, M.D., M.P.H.

Table 1. Clinical and Epidemiologic Features of the Family Cluster of Avian Influenza (H5N1).*

Patient	Age	Date of Fever Onset	Date of Pneumonia Diagnosis	Findings on Admission				Antiviral Treatment	Respiratory Isolation	Testing for Hemagglutinin H5	Outcome
				Total White-Cell Count	Absolute Lymphocyte Count	Platelet Count	Chest Radiograph				
	yr			per mm³							
Girl (index patient)	11	Sept. 2	Sept. 7	4500	1350	150,000	Right-lower-lobe consolidation	No	No	Inadequate sample	Died Sept. 8
Mother	26	Sept. 11	Sept. 17	2300	667	90,000	Bilateral lower-lobe consolidation	No	No	Positive (RT-PCR of lung tissue)	Died Sept. 20
Aunt	32	Sept. 16	Sept. 23	5400	1296	230,000	Left-lower-lobe consolidation	Yes	Yes	Positive (RT-PCR of oropharyngeal swab)	Survived; discharged Oct. 7

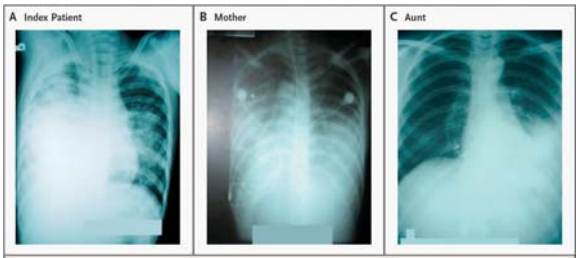

Figure 1. Chest Radiographs from the Three Patients with Avian Influenza A (H5N1).
Panel A shows a chest radiograph from the index patient, an 11-year-old girl, on day 6 of her illness. The image shows right-lower-lobe consolidation and patchy left-lower-lobe infiltrates. Panel B shows a radiograph from the girl's 26-year-old mother on day 9 of her illness. There is bilateral lower-lobe consolidation. Panel C shows a radiograph from the girl's 32-year-old aunt on day 7 of her illness; left-lower-lobe consolidation is visible.

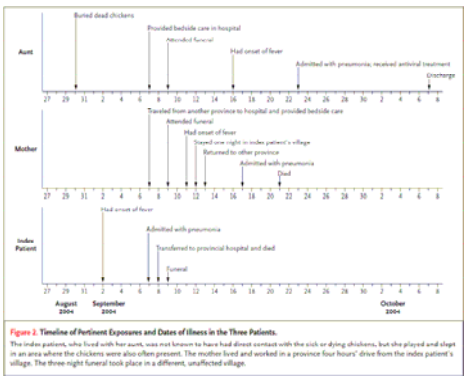

Figure 2. Timeline of Pertinent Exposures and Dates of Illness in the Three Patients.

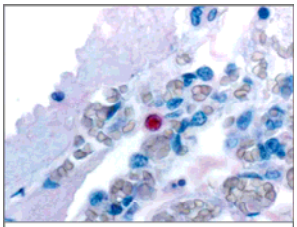

Figure 3. Specimen of Lung Tissue from the Index Patient's Mother.
Immunohistochemical analysis of the specimen shows interstitial pneumonitis and a single epithelial cell containing intranuclear influenza A viral antigens (red) and an antinucleocapsid antibody. Amplification of nucleic acid from this tissue specimen confirmed the presence of influenza A (H5N1) virus.

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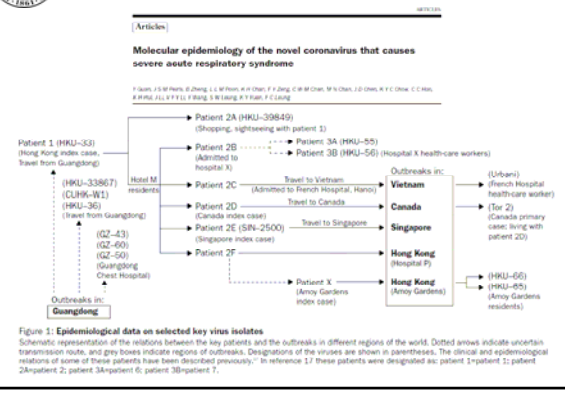

Figure 1: **Epidemiological data on selected key virus isolates**
Schematic representation of the relations between the key patients and the outbreaks in different regions of the world. Dotted arrows indicate uncertain transmission route, and grey boxes indicate regions of outbreaks. Designations of the viruses are shown in parentheses. The clinical and epidemiological relations of some of these patients have been described previously.[7] In reference 1,7 these patients were designated as: patient 1=patient 1; patient 2A=patient 2; patient 3A=patient 6; patient 3B=patient 7.

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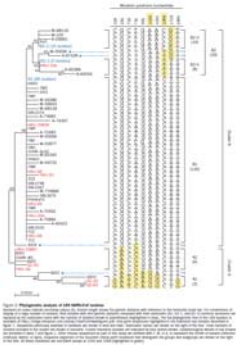

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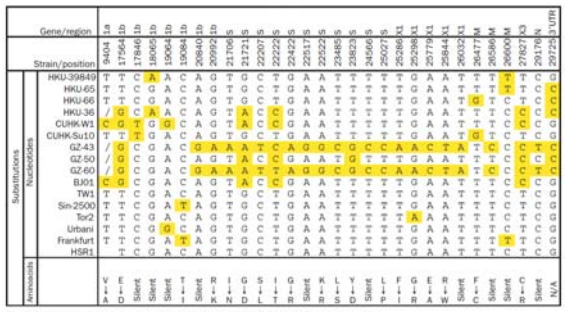

Figure 3: **Nucleotide and aminoacid substitutions of SARS-CoV isolated in Amoy Gardens**
Comparisons were made with other virus isolates by full genome sequence alignment. Except for the Hong Kong and Guangdong samples, the index case or first uploaded sequence from other cities and countries was chosen. Nucleotides were numbered on the basis of the HKU-39849 full sequence.* Slash indicates that the sequence data are not available, and nucleotides highlighted in yellow are those that differ from the majority at the corresponding location. Only substitutions occurring in more than one virus are shown.

Application of Computational Biology in Veterinary Medicine

Now let's get back to the basic

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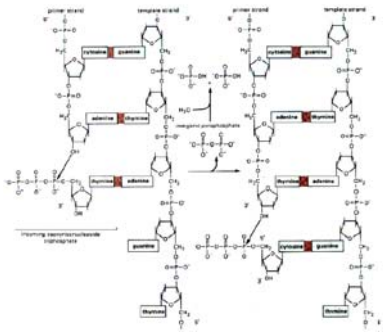

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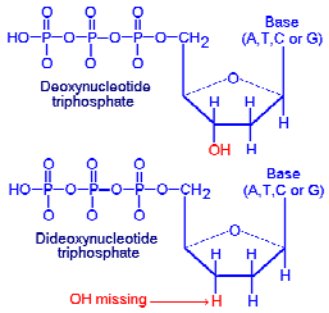

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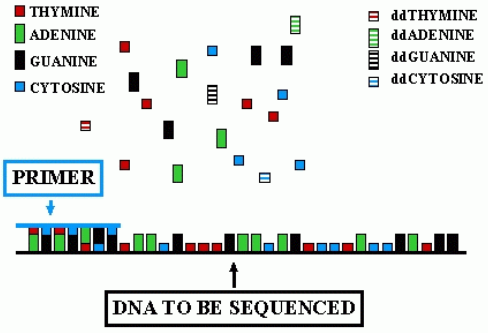

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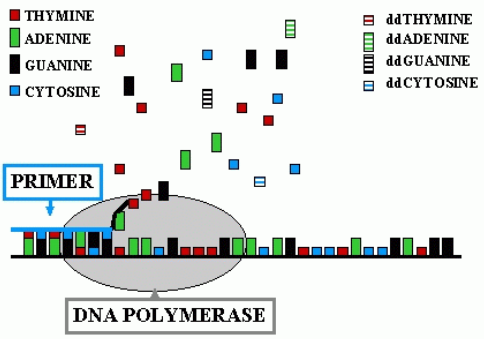

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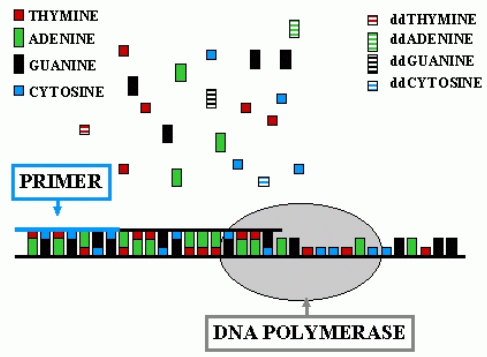

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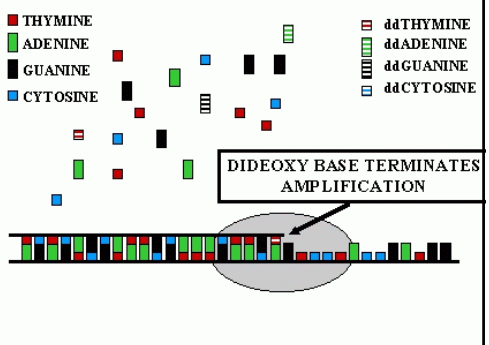

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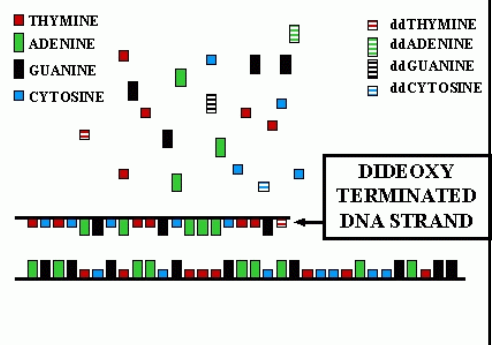

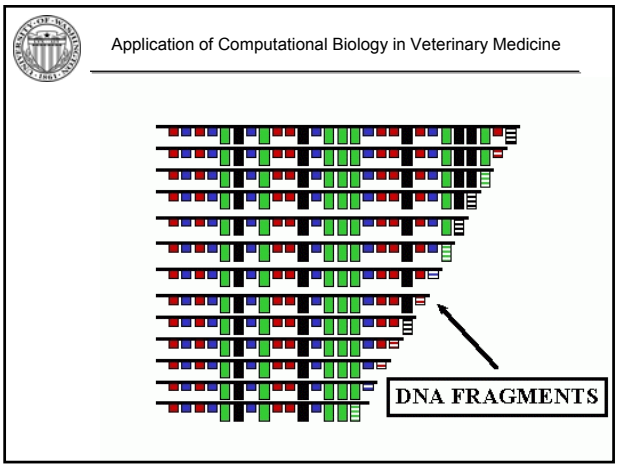
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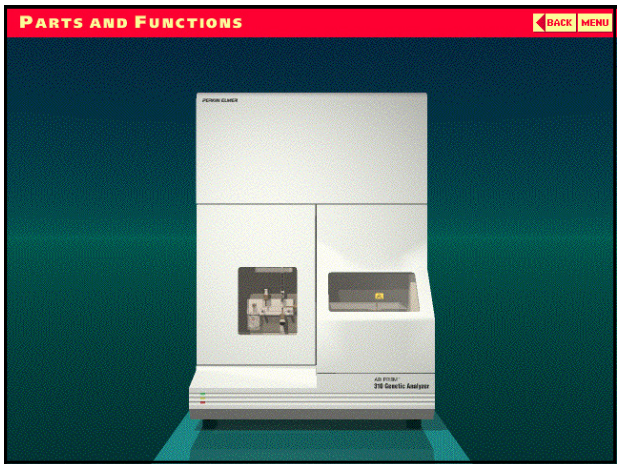
DNA FRAGMENTS


PARTS AND FUNCTIONS
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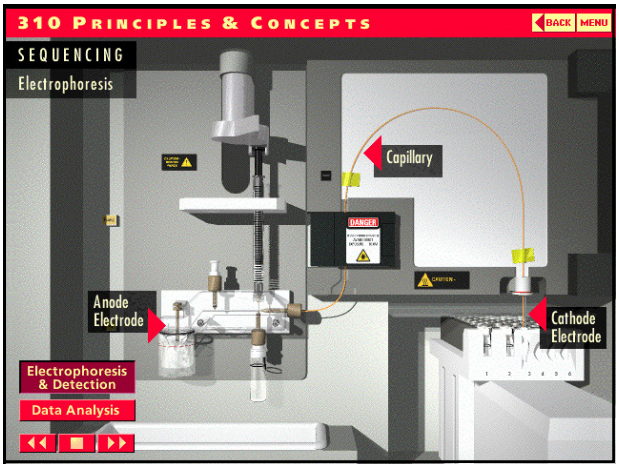
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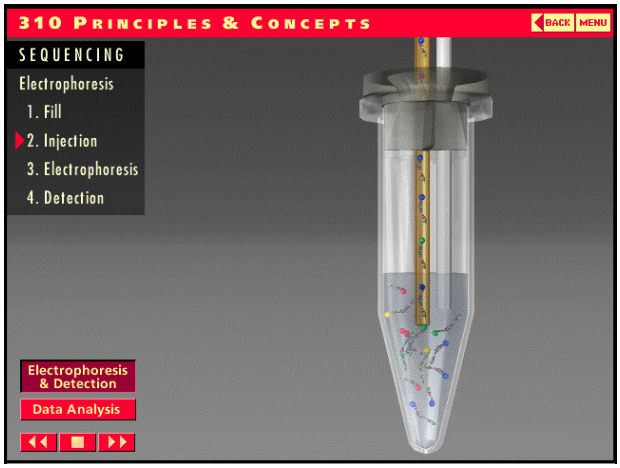
Data Analysis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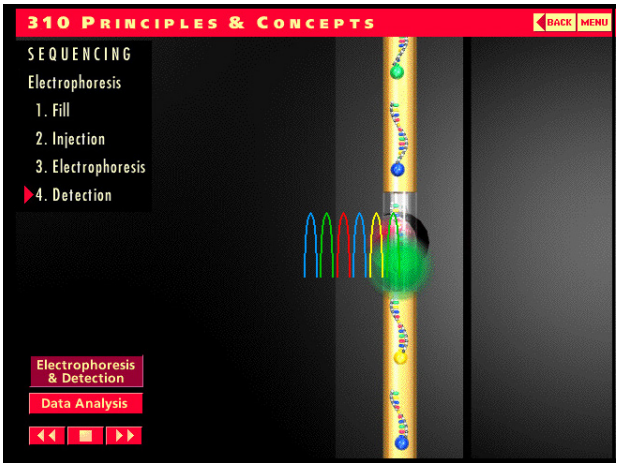
2. Injection
3. Electrophoresis
4. Detection
Electrophoresis & Detection
Data Analysis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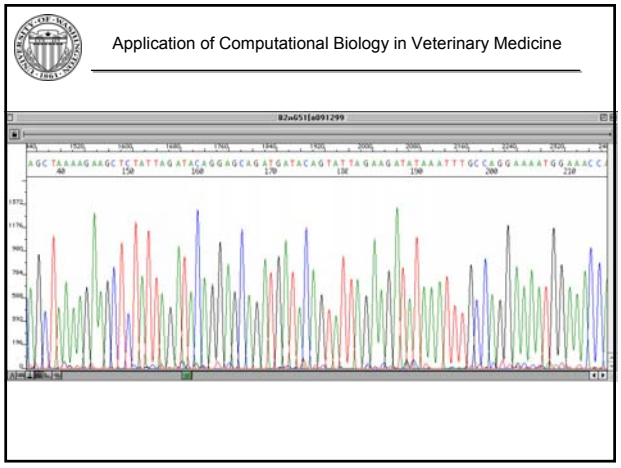
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Maximum parsimony algorithm for finding minimum number of mutations on a tree

Specie A A G G T
Specie B A T G T

Sister taxa: AGGT ATGT

Ancestor: A G/T GT

C G/T G A/C A C/A G A/T

G
CT GA
AC
A

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Sites 819-824 in cytochrome oxidase 1

Chicken ACCCAT
Mouse ATGACA
Rat ATGACA
Human ACCAAA

Rat Mouse Human Chicken

OR

Rat Human Mouse Chicken

Tree A Tree B

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Rat ATGACA
Human ACCAAA
Mouse ATGACA
Chicken ACCCAT

A TG/CC A C/A A

ATGACA

Tree A
Eight mutations

A TGACA
CCCAT

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Rat ATGACA
Mouse ATGACA
Human ACCAAA
Chicken ACCCAT

ATGACA

A TG/CC A C/A A

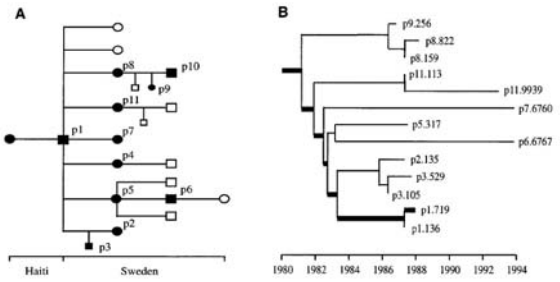
Tree B
Five mutations

ACC A/C A A/T

Application of Computational Biology in Veterinary Medicine

Reliable / Accuracy?

Phylogenetic analysis of HIV transmission case in Sweden



Probable transmission of HIV from orthopedic surgeon to patients in France

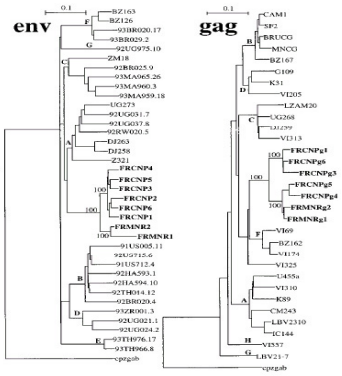
- •In July 1995, an orthopedic surgeon practicing in the hospital of Saint-Germain-en-Laye had been accidentally infected with HIV while performing surgery 12 years earlier.
- •In September 1995, he asked the director to screen all of his former patients and informed the press of his request.
- •In October 1995, the French Ministry of Health offered HIV testing to patients who had been operated on by the surgeon.
- •Letters were mailed to 3004 patients (May 1983 to October 1993)
- •1218 (41%) patients responded, 218 were reported by their families to have died and had no info on sero status, 983 (33%) reported HIV test results, 982 were negative for HIV and 1 was positive for HIV

Probable transmission of HIV from orthopedic surgeon to patients in France

- •A 53-year-old surgeon received a diagnosis of AIDS in March 1994
- •He stopped performing surgery in October 1993
- •He was married, had only heterosexual contacts with his wife, denied using injection drugs, never received a blood tx, no history of STD.
- •In May 1983, he had a needle stick injury while operating on a patient on a patient who had a multiple transfusions. This patient later died
- •30 days after an accident, he developed a symptoms consistent with the HIV seroconversion syndrome
- •The surgeon reported frequent opportunities for blood exposure as well as frequent actual blood exposures
- •He often tightened suture wires with his fingers and sometimes tied sutured with the needle still attached percutaneous injuries with sharp objects previous in contact with blood occurred as frequently as once a week
- •These injuries sometimes (but rarely) involved blood from an HIV infected patient

Probable transmission of HIV from orthopedic surgeon to patients in France

- •A 67-year-old French woman who had undergone an invasive procedure in June 1992 (placement of a total hip prosthesis with bone graft)
- • She was widowed in 1986 and had no sexual intercourse a few years before the death of her husband
- • The donor of bone graft tested negative for HIV in November 1995
- • The donors of the 2 units of packed RBC also tested negative for HIV (one in April 1994 and one in May 1994)



Transmission of HIV to a patient during an invasive dental procedure

CBS' news program "60 Minutes" revisited the case of Jensen Beach, Florida, dentist David Acer a case that came to the nation's attention in 1990 when one of Dr. Acer's patients, 23-year-old college student Kimberly Bergalis, went public with her story. Both died of AIDS: Acer in 1990 and Bergalis in 1991.

At least 5 patients involved

Patient A :young college student woman
Patient B :elderly woman, married > 25 yr, her spouse tested -ve for HIV Ab
Patient C :young man, multiple heterosexual partners (all HIV seronegative)
Patient D :a man with AIDS with established risk factors for HIV infection
Patient E :a woman whose epidemiologic and lab tests has not been completed
Patient F
Patient G

All denied sexual contact with the dentist

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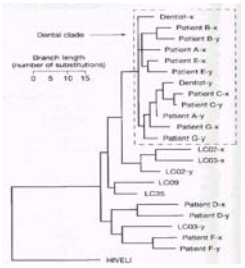


The viruses of the dentist and patients A, B and C are closely related in their V3 sequences with an average difference of 3.4%

The average viral sequence difference for patient D and seven control patients was approximately 13% (range 8% - 15%)

Application of Computational Biology in Veterinary Medicine

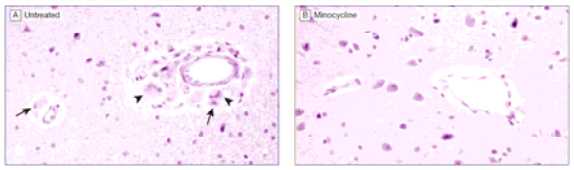
Now we are going to find a magic bullet

Application of Computational Biology in Veterinary Medicine

Neuroprotective and Anti–Human Immunodeficiency Virus Activity of Minocycline

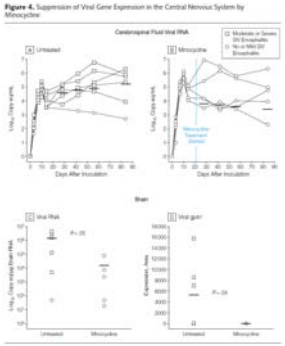
M. Christine Zink, DVM, PhD
Jennifer Uhrlaub, BS
Jesse DeWitt, BS
Tanni Voelker, BS
Brandon Bullock, MS
Joseph Mankowski, DVM, PhD
Patrick Tarwater, PhD
Janice Clements, PhD
Sheila Barber, PhD

Figure 1. Brain Sections of Macaques Infected With Simian Immunodeficiency Virus



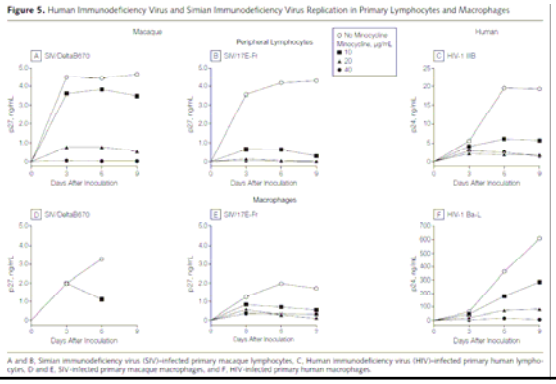
A, Brain section from an SIV-infected untreated macaque demonstrating typical changes of severe SIV encephalitis with numerous epithelioid macrophages (arrows) and multinucleated giant cells (arrowheads) in perivascular spaces. B, Brain section from an SIV-infected macaque treated with minocycline, showing no inflammation. Hematoxylin-eosin, original magnification ×200.

Application of Computational Biology in Veterinary Medicine



Application of Computational Biology in Veterinary Medicine

Figure 5. Human Immunodeficiency Virus and Simian Immunodeficiency Virus Replication in Primary Lymphocytes and Macrophages



A and B, Simian immunodeficiency virus (SIV)–infected primary macaque lymphocytes. C, Human immunodeficiency virus (HIV)–infected primary human lymphocytes. D and E, SIV-infected primary macaque macrophages, and F, HIV-infected primary human macrophages.

Application of Computational Biology in Veterinary Medicine

Virtual screening of HIV-1 protease inhibitors against human cytomegalovirus protease using docking and molecular dynamics

Research question

- Cytomegalovirus retinitis is most common ocular opportunistic infection in immunocompromised or immunosuppressed patients, i.e. patients with AIDS or organ transplant recipients.
- In AIDS patients, it usually seen in patients with CD4+ < 50 per mm^3
- Clinical features: May be asymptomatic, blurry or cloudy vision, floaters, light flashes, loss of central or peripheral vision, vitreous cells with mild anterior chamber inflammatory reaction.
- Management: Intravenous anti-viral agents such as gancyclovir, cidofovir and foscarnet which may be used individually or in combination. Alternative intravitreal gancyclovir implant (the effect lasts in 6-10 months)

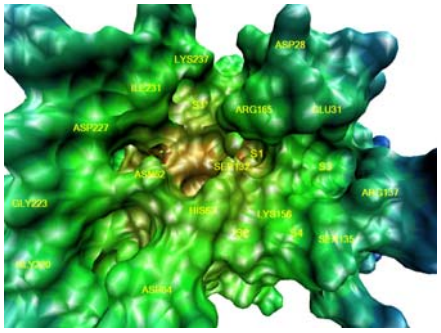
Virtual screening of HIV-1 protease inhibitors against human cytomegalovirus protease using docking and molecular dynamics

Research question

- Deayton J, *et al*. Loss of cytomegalovirus (CMV) viraemia following highly active antiretroviral therapy in the absence of specific anti-CMV therapy. *AIDS* 1999; **13**:1203-1206.
- Casado JL, *et al*. Incidence and risk factors for developing cytomegalovirus retinitis in HIV-infected patients receiving protease inhibitor therapy. Spanish CMV-AIDS Study Group. *AIDS* 1999; **13**: 1497-1502.
- Macdonald JC, *et al*. Highly active antiretroviral therapy related immune recovery in AIDS patients with cytomegalovirus retinitis. *Ophthalmology* 2000; **107**: 877-881.
- Reed JB, *et al*. Highly active antiretroviral therapy-associated regression of cytomegalovirus retinitis: long-term results in a small case series. *Retina* 2001; **21**: 339-343.

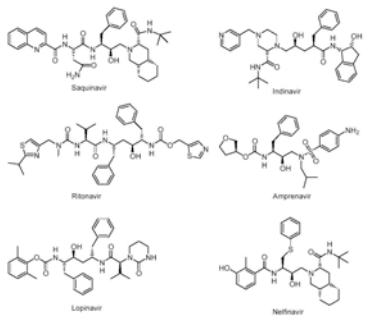
Virtual screening of HIV-1 protease inhibitors against human cytomegalovirus protease using docking and molecular dynamics

Substrate binding site of CMV protease



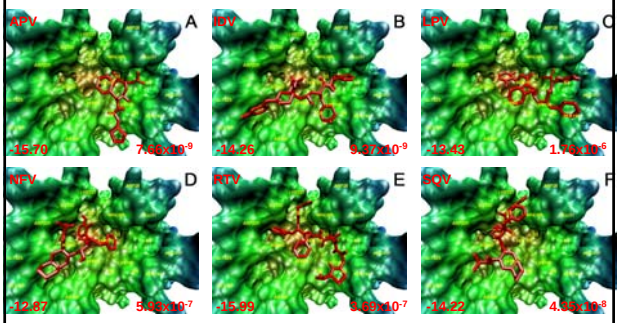
Virtual screening of HIV-1 protease inhibitors against human cytomegalovirus protease using docking and molecular dynamics

List of six HIV-1 protease inhibitors



Virtual screening of HIV-1 protease inhibitors against human cytomegalovirus protease using docking and molecular dynamics

Binding modes/energies of HIV-1 protease inhibitors to the CMV protease

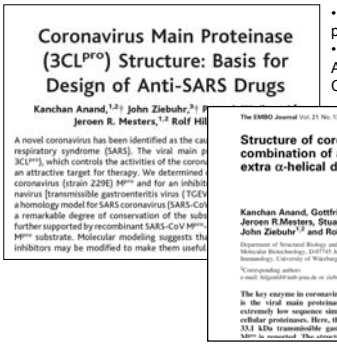


Virtual screening of HIV-1 protease inhibitors against human cytomegalovirus protease using docking and molecular dynamics

Summary

- This study provides evidence for the inhibitory activity of two approved inhibitors, amprenavir and indinavir, against the CMV protease.
- Including either of these two inhibitors in a HAART regimen should help to control the CMV viral load in HIV-1-infected patients.
- The activity of the CMV protease would be inhibited soon after starting HAART, in contrast to inhibition by promoting immune system restoration, which may take several weeks.
- Structural studies of human herpes proteases indicates homology among several subtypes.

Identifying inhibitors of the SARS Coronavirus protease


Coronavirus Main Proteinase (3CL^{pro}) Structure: Basis for Design of Anti-SARS Drugs

Kanchan Anand, [1,2†] John Ziebuhr, [3†] Jeroen R. Mesters, [1,2] Rolf Hil

A novel coronavirus has been identified as the ca respiratory syndrome (SARS). The viral main p 3CL^{pro}), which controls the activities of the coron an attractive target for therapy. We determined coronavirus (strain 229E) M^{pro} and for an inhibit navirus [transmissible gastroenteritis virus (TGE a homology model for SARS coronavirus (SARS-Co a remarkable degree of conservation of the sub further supported by recombinant SARS-CoV M^{pro} M^{pro} substrate. Molecular modeling suggests th inhibitors may be modified to make them useful


The EMBO Journal Vol. 21 No. 13 pp. 3213–3224, 2002

Structure of coronavirus main proteinase reveals combination of a chymotrypsin fold with an extra α-helical domain

Kanchan Anand, Gottfried J.Palm, Jeroen R.Mesters, Stuart G.Siddell, John Ziebuhr and Rolf Hilgenfeld

Department of Structural Biology and Crystallography, Institute of Molecular Biotechnology, D-07745 Jena and Institute of Virology and Immunology, University of Würzburg, D-97078 Würzburg, Germany

*Corresponding authors

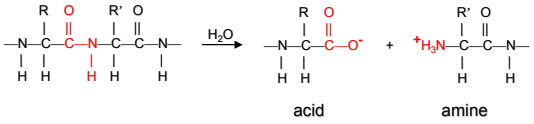
The key enzyme in coronavirus polyprotein processing is the viral main proteinase, M^{pro}, a protein with extremely low sequence similarity to other viral and cellular proteinases. Here, the crystal structure of the 33.1 kDa transmissible gastroenteritis (coronavirus

The central and C-proximal regions o processed by a 33.1 kDa viral cystein called the 'main proteinase' (M^{pro}) '3C-like proteinase' (3CL^{pro}). The na ase' was introduced originally becaus specificities of the coronavirus M^{pro} proteinases (3C^{pro}) and the identificat principal catalytic residue in the co ruses β-barrel fold (Gorbalenya *et al.*, however, several studies have reveal ences in both the active sites an between the coronavirus and picorn and Brown, 1995; Lu and Denison, 1997, 2000; Heggi *et al.*, 2002). Also, reported for a number of picornav

- AG7088 may be a good starting point for drug development.
- *In vitro* evidence showed that AG7088 failed to inhibit SARS CoV.

Identifying inhibitors of the SARS Coronavirus protease

Protease (protein hydrolase) catalyze amide (peptide) bond hydrolysis in protein or peptide substrates:

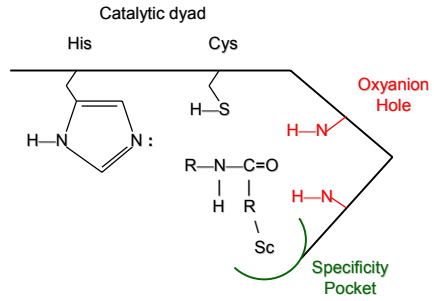


Identifying inhibitors of the SARS Coronavirus protease

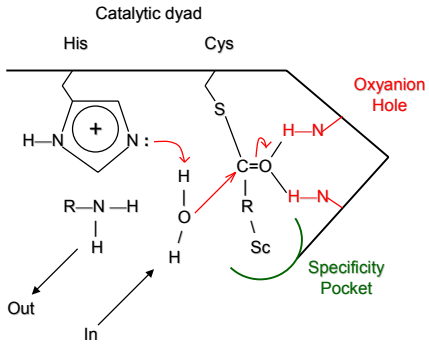
Coronavirus cysteine protease has three important parts in their active site:

1) Specificity pocket	a pocket which interacts with the side chains of specific amino acids
2) Catalytic dyad	two residues, histidine and cysteine, are present in the active site
3) Oxyanion hole	two free amide N—H groups available to donate hydrogen bonds

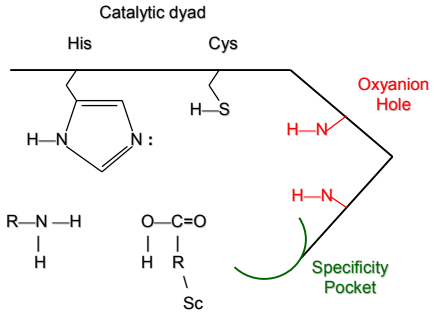
Identifying inhibitors of the SARS Coronavirus protease

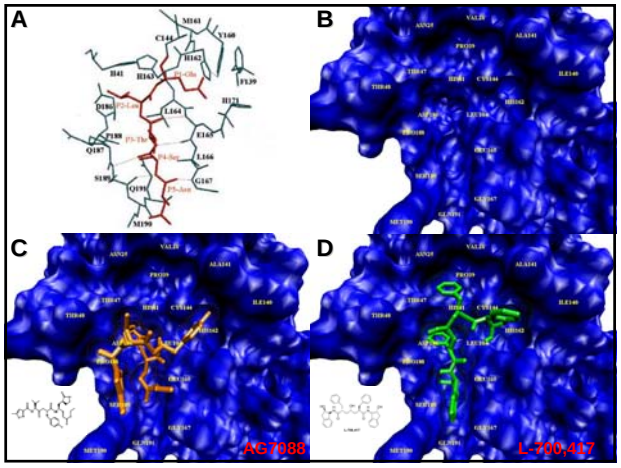


Identifying inhibitors of the SARS Coronavirus protease

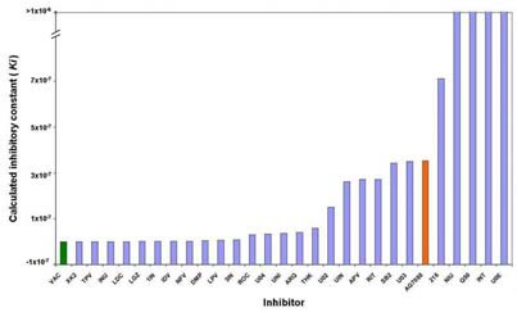


Identifying inhibitors of the SARS Coronavirus protease





Identifying inhibitors of the SARS Coronavirus protease



Identifying inhibitors of the SARS Coronavirus protease

Joseph Sung, MD, PhD

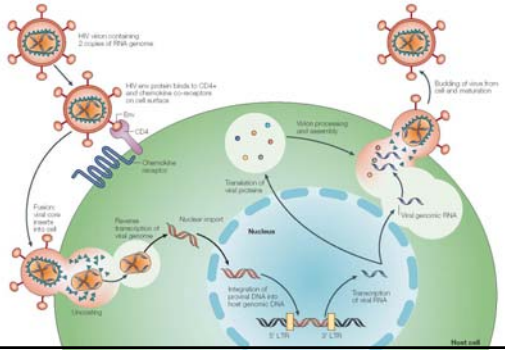
Old Drugs for a New Bug

Influenza, HIV Drugs Enlisted to Fight SARS

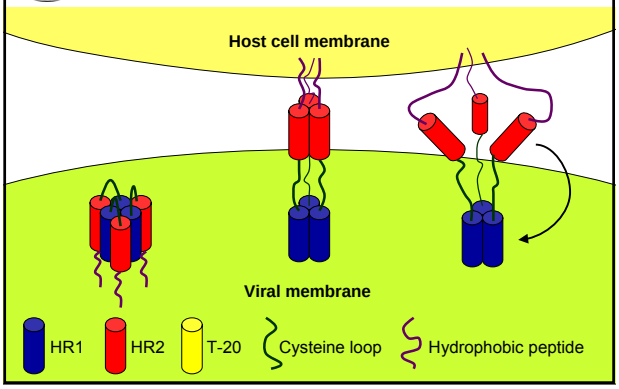
At a June WHO meeting, Joseph Sung, MD, PhD, reported that the drug appeared to cut death rates. At 30 days after onset of symptoms, none of the 34 patients taking Kaletra as an initial treatment (in combination with the antiviral ribavirin) had died; in contrast, 10% of the 690 patients taking only ribavirin had died. The researchers also found that the longer physicians waited to give

Heptad-repeat-2 mutations enhance stability of enfuvirtide-resistant HIV-1 gp41 hairpin structure

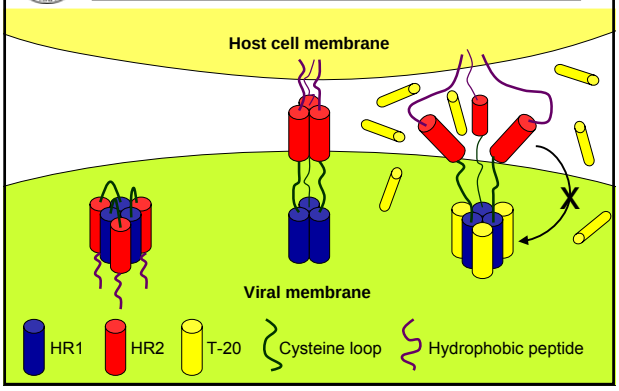
Life cycle of HIV-1



Heptad-repeat-2 mutations enhance stability of enfuvirtide-resistant HIV-1 gp41 hairpin structure



Heptad-repeat-2 mutations enhance stability of enfuvirtide-resistant HIV-1 gp41 hairpin structure



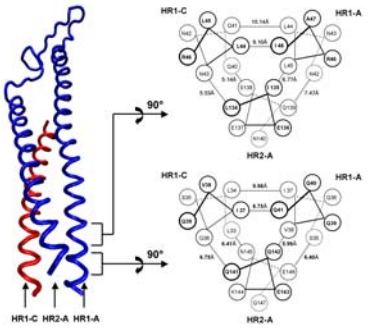
Heptad-repeat-2 mutations enhance stability of enfuvirtide-resistant HIV-1 gp41 hairpin structure

T-20 resistance HR1 mutations

- G36D/S
- I 37V
- V38A/E/M
- Q40H
- N42D/E/S/T
- N43D/K/S
- L45M

Heptad-repeat-2 mutations enhance stability of enfuvirtide-resistant HIV-1 gp41 hairpin structure

Mapping of HR1/HR2 residue-residue interactions



Heptad-repeat-2 mutations enhance stability of enfuvirtide-resistant HIV-1 gp41 hairpin structure

Identification of the corresponding HR2 residues

HR1	Corresponding HR2
G36	Q141 / N145
V38	Q139 / Q142
Q40	S138 / Q141
N42	Q139 / Q142
N43	L134 / S138
L45	S138 / Q139

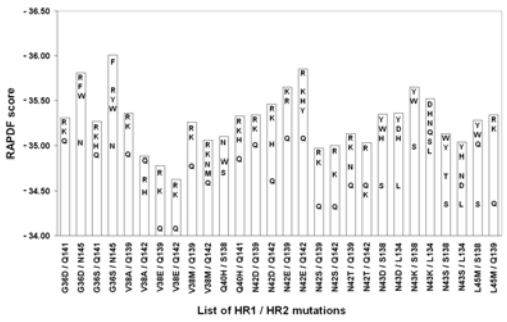
Heptad-repeat-2 mutations enhance stability of enfuvirtide-resistant HIV-1 gp41 hairpin structure

RAPDF reference

- Residue-specific all-atom probability discriminatory function (RAPDF) score
- Samudrala R, Moult J. An all-atom distance dependent conditional probability discriminatory function for protein structure prediction. *J Mol Biol* 1997; **275**: 895-916.
- http://compbio.washington.edu/

Heptad-repeat-2 mutations enhance stability of enfuvirtide-resistant HIV-1 gp41 hairpin structure

Identification of the corresponding HR2 amino acids



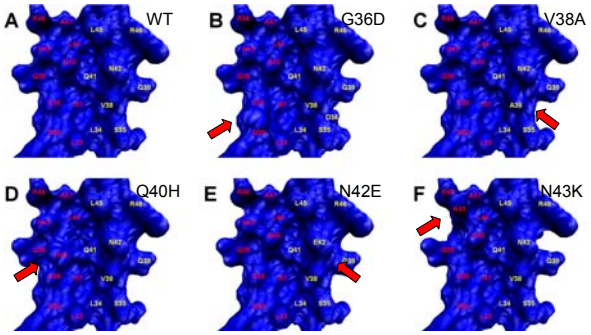
Heptad-repeat-2 mutations enhance stability of enfuvirtide-resistant HIV-1 gp41 hairpin structure

Comparison of the RAPDF scores with EC_{50} and viral fitness levels

HR1 mutation	Viral fitness level	EC_{50} (mg/L)	RAPDF scores HR2 wild-type	RAPDF scores With HR2 mutation
Wild-type	+++++	0.012	-35.12	-
N42T	++++	0.045	-34.66	-35.13 (Q139R)
V38A	+++	0.188	-34.98	-35.36 (Q139R)
N42T+N43K	++	0.388	-34.69	-36.21 (S138Y, Q139R)
N42T+N43S	++	0.727	-34.65	-35.66 (S138Y, Q139R)
V38A+N42D	+	1.685	-34.89	-35.42 (Q139R)
V38A+N42T	+	1.782	-34.49	-35.13 (Q139R)
V38E+N42S	data not available	6.156	-33.73	-35.02 (Q139R)

Heptad-repeat-2 mutations enhance stability of enfuvirtide-resistant HIV-1 gp41 hairpin structure

Comparison of wild-type and mutant surface structures



Heptad-repeat-2 mutations enhance stability of enfuvirtide-resistant HIV-1 gp41 hairpin structure

Designing enfuvirtide derivatives against resistant strains

HR1 mutation	HR2 mutation (residues 134,138,139,141,142,145)	RAPDF score
Wild-type	Wild-type	-35.12
Wild-type	H Y R R R Y	-37.92
Wild-type	- - - - - F	-37.01
Wild-type	- - - - - R	-37.28
G36D	- - - - - F	-37.85
G36S	- - - - - F	-38.01
V38A	- - - - - F	-37.97
V38A+N42D	- - - - - F	-38.44
V38A+N42T	- - - - - F	-37.81
V38E+N42S	- - - - - R	-36.71
V38E	- N - - - R	-37.16
V38M	- - - - - -	-37.86
Q40H	- - - - - -	-37.88
N42D	- - - - - -	-38.50
N42E	- - - - - -	-38.69
N42S	- - - - - -	-37.75
N42T	- - - - - -	-37.76
N42T+N43S	- - - - - -	-37.55
N43D	- - - - - -	-38.11
N43K	- - K - - -	-37.94
N43S	- - - - - -	-37.65
L45M	- - - - - -	-37.92

Prediction of HIV-1 protease inhibitor resistance using a protein-inhibitor flexible docking approach

HIV drug susceptibility testing background

- Monitoring of disease progression: CD4 count, viral load
- Emergence of antiretroviral drug resistant strains
- Monitoring of drug resistance: genotyping, phenotyping
- Phenotyping: expensive, takes time, direct, easy to interpret
- Genotyping: cheap, fast turn around time, indirect, interpretation problems
- Computational methods for genotypic susceptibility interpretation
- Knowledge based: needs large genotype/phenotype data for training
- Physic based: studies interaction between viral enzyme and drugs
- Accuracy of individual and combined methods

Prediction of HIV-1 protease inhibitor resistance using a protein-inhibitor flexible docking approach

Knowledge based machine learning: rule based, decision tree, SVM, ANN

http://hivdb.stanford.edu

http://www.geno2pheno.org

Prediction of HIV-1 protease inhibitor resistance using a protein-inhibitor flexible docking approach

J Acquir Immune Defic Syndr. 2003 May 1;33(1):8-14. Related Articles, Links

LWWonline

HIV-1 protease and reverse transcriptase mutation patterns responsible for discordances between genotypic drug resistance interpretation algorithms.

Ravela J, Betts BJ, Brun-Vezinet F, Vandamme AM, Descamps D, van Laethem K, Smith K, Schapiro JM, Winslow DL, Reid C, Shafer RW.

Division of Infectious Diseases, Department of Medicine, Stanford University, Stanford, California 94301, USA.

HIV Med. 2003 Jan;4(1):72-8. Related Articles, Links

Full text

Discrepant results in the interpretation of HIV-1 drug-resistance genotypic data among widely used algorithms.

Kijak GH, Rubio AE, Pampuro SE, Zala C, Cahn P, Galli R, Montaner JS, Salomon H.

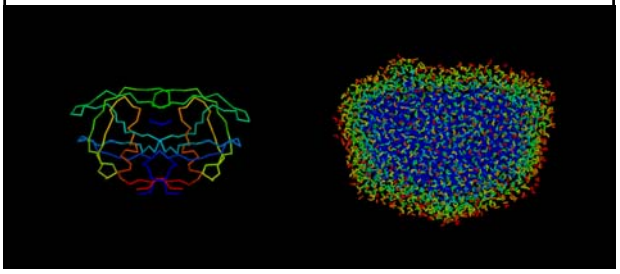
National Reference Center for AIDS, Department of Microbiology, School of Medicine, University of Buenos Aires, Argentina.

Prediction of HIV-1 protease inhibitor resistance using a protein-inhibitor flexible docking approach

Molecular dynamics simulations protocol

Downloaded 25 HIV-1 protease-inhibitor complexes that their experimental binding energies are available. Missing atoms or residues were checked and fixed.

Water molecules and sodium-chloride ions were added until the shell was 10 Å thick.



Prediction of HIV-1 protease inhibitor resistance using a protein-inhibitor flexible docking approach

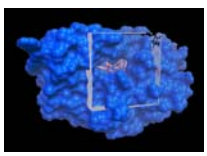
Protein-inhibitor docking protocol

- Protein-inhibitor binding energy calculation: AutoDock 3.0.5

$$\Delta G_{binding} = \Delta G_{vdW} + \Delta G_{elec} + \Delta G_{hbond}$$

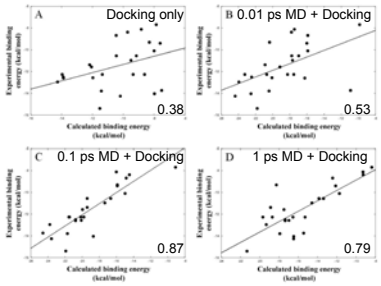
ΔG_{vdW}	12-6 Lennard-Jones potential, dispersion/repulsion
ΔG_{elec}	Coulombic electrostatic potential
ΔG_{hbond}	Directional 12-10 potential

- Set up the ligand & macromolecule.
  - *Ligand:*
    - –Add all hydrogens, compute charges
    - –Distinguish aliphatic and aromatic carbons
    - –Choose root & rotatable bonds
  - *Macromolecule:*
    - –Add polar H, assign charges
    - –Assign solvation parameters
- Pre-compute AutoGrid Maps for all atom types.
- Perform dockings of ligand to target.



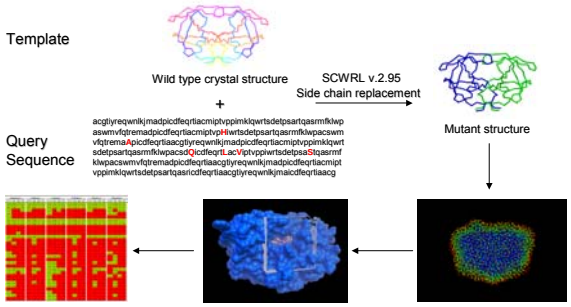
Prediction of HIV-1 protease inhibitor resistance using a protein-inhibitor flexible docking approach

Correlation between experimentally-determined and calculated binding energy



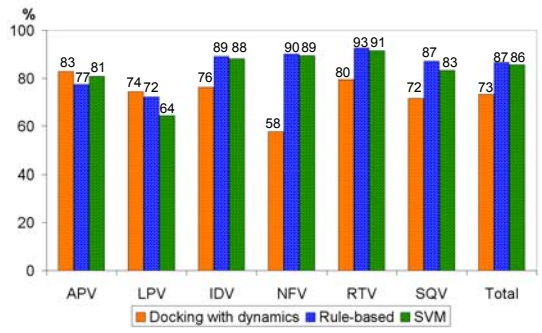
Prediction of HIV-1 protease inhibitor resistance using a protein-inhibitor flexible docking approach

Docking with dynamics protocol



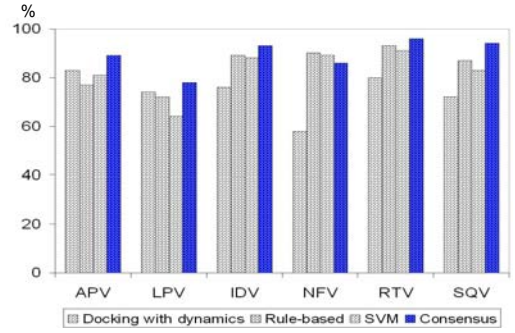
Prediction of HIV-1 protease inhibitor resistance using a protein-inhibitor flexible docking approach

Accuracy of each method

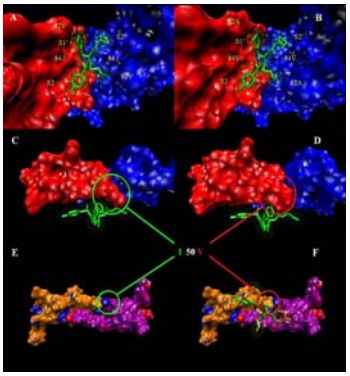


Prediction of HIV-1 protease inhibitor resistance using a protein-inhibitor flexible docking approach

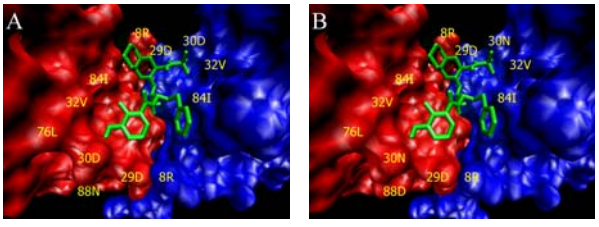
Accuracy of the consensus

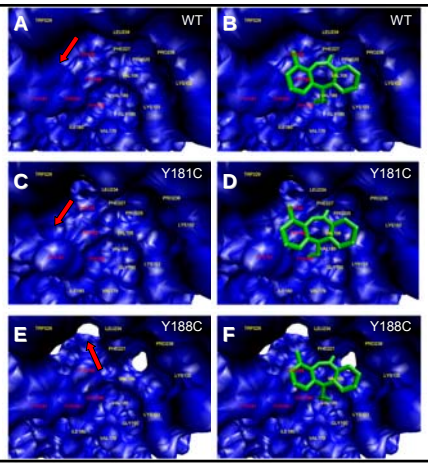


Prediction of HIV-1 protease inhibitor resistance using a protein-inhibitor flexible docking approach

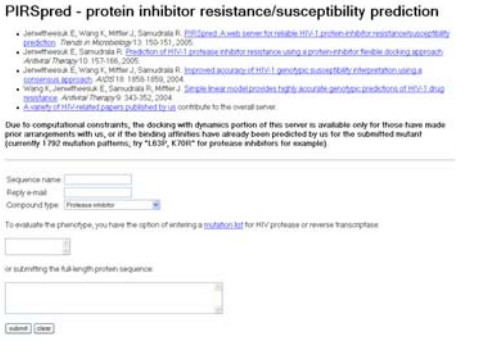


Prediction of HIV-1 protease inhibitor resistance using a protein-inhibitor flexible docking approach





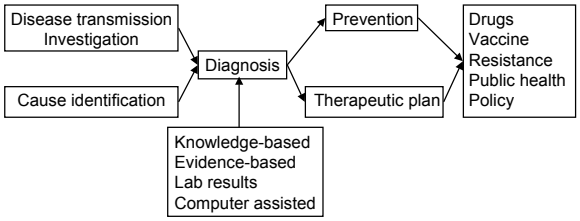
Prediction of HIV-1 protease inhibitor resistance using a protein-inhibitor flexible docking approach



http://protinfo.compbio.washington.edu/pirspred/

Application of Computational Biology in Veterinary Medicine

Traditional medicine (and prevention) protocol



Prediction of HIV-1 protease inhibitor resistance using a protein-inhibitor flexible docking approach

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