Sensible Sensor Systems are Essential Tools for Humans, Animals, Plants and the Environment: Understanding the Context of *What* to Sense in the Climate of Infectious Diseases, SARS-CoV-2, CoVID-19 and *How* to Prepare to Predict Future Pandemics, Epidemics and Endemics by Implementing Connected Networks of CITCOM

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ABSTRACT

There is little doubt that low-cost "one health" sensor platforms for global surveillance of animals and humans must become a staple for the post-CoVID-19 world. It is essential to implement the paradigm of "**c**anaries **i**n **t**he **co**al **m**ine" (CITCOM) for early detection of infectious agents to proactively practice TETRIS (**te**st, **tr**eat, **is**olate) in every corner of the world. Communities must be cognizant of the catastrophic threat of global transmission which could dessimate key links in the supply chain of essential public goods (food, energy, water, sanitation, healthcare or FEWSH). The diverse spectrum of mortality and morbidity due to unchecked spread of infection by SARS-CoV-2 is a lesson in geopolitical carnage which must not be repeated. Sensible sensor systems and making sense of sensor data must become the frontier in our plight to prevent future pandemics. CITCOM is a simple mnemonic for connectivity and a metaphor similar in concept to the internet of things (IoT). Connected data, non-obvious relationship analysis (NORA) and ability to identify emerging *patterns* of *contextually relevant* clues are the trinity of preparatory pillars for the post-pandemic era. Building trust in data-informed public health services will be a herculean task. Identifying targets which may be used for detection and prevention requires a deep understanding of the biological milieu and molecular processes that underlie disease and dysfunction.This discussion spits out amorphous, non-linear and overlapping "dots" of haphazard realities which must be *connected in context* to evolve a dynamic fabric of connected events to make sense of the data. It may provide guidance for experts and novices or offer fodder for imagination, invention, innovation and implementation.

This article is dedicated to the memory of two mentors

Glenn T. Seaborg

April 19, 1912 - February 25, 1999

Chemist, Scientist, Statesman, Educator & Humanist

Glenn T. Seaborg. 20th April 1998 in Room 70A, LBL (Lawrence Berkeley Laboratory, UC Berkeley). Last birthday (personal photo, Shoumen Datta). www2.lbl.gov/Publications/Seaborg/bio.htm

Clive W. J. Granger

4 September 1934 – 27 May 2009

Mathematician, Economist, Scholar & Humanist

Sir Clive Granger. 9th September 2008 in Sammy's Restaurant on Inch Beach, County Kerry, Ireland. Last birthday (personal photo, Shoumen Datta). www.econlib.org/library/Enc/bios/Granger.html

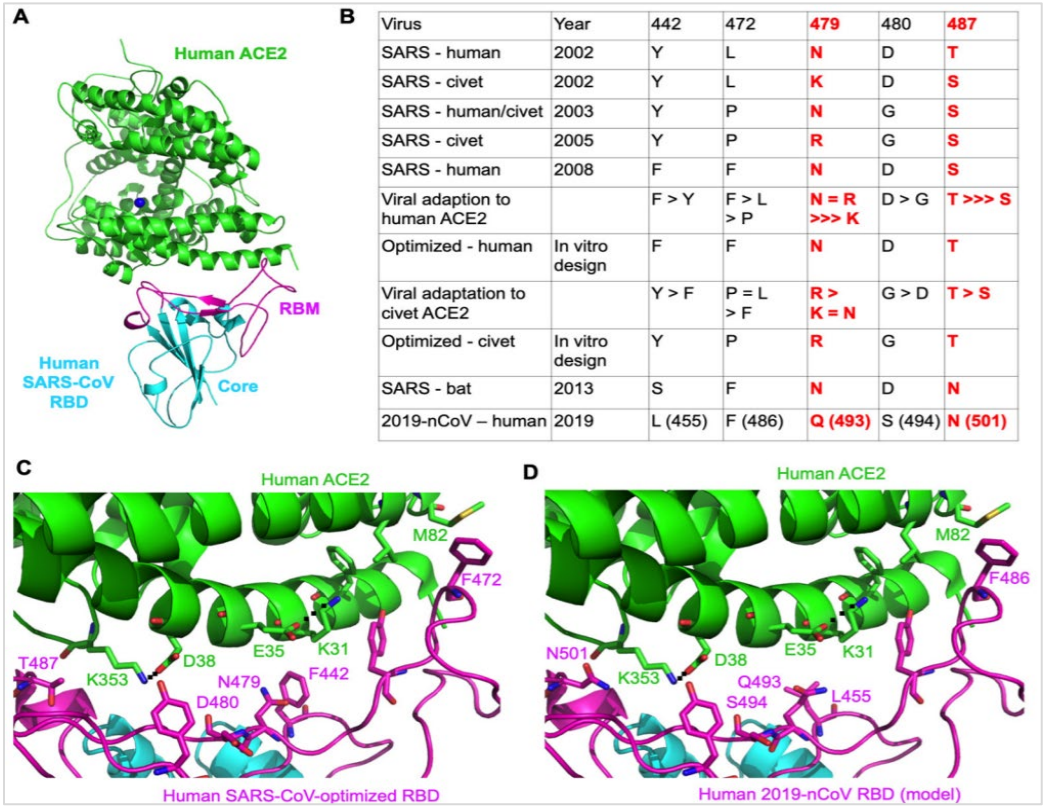
DISCUSSION

IMPORTANCE OF STUPIDITY: DISCUSSING AND DISCLOSING IDEAS WHICH FAILED

Circa 1813 it was documented that iodine[1] and later iodized table salt, could reduce incidences of goiter. Could an ***"almost no-cost"*** solution serve as a first line of defense against the ongoing pandemic which was predicted[2] in 2007? Could *Na-bicarbonate drinks* sufficiently or slightly alter/change the pH of human blood (make it more alkaline), within physiologically acceptable levels, to perturb the binding between the SARS-CoV-2 Spike (S) protein receptor binding domain (RBD in the virus[3]) and its host receptor [4] (ACE2)?

RATIONALE

Could changes in blood/plasma pH[5] trigger amino acid residues (pKa driven) in the S protein RBD to undergo ''microscopic'' conformational change? Could this change alter the affinity of the RBD for ACE2 receptor? Could it be sufficient to disrupt the binding of the virus with its host receptor to prevent entry and infection? Could a solution of sodium bicarbonate (alkaline drinking water) suffice to induce minor changes in blood pH? If the urine turns the litmus paper blue, it may indicate that the internal body pH is sufficiently alkaline. The pH-induced conformational change and/or steric hindrance (Ramachandran[6] Plot) is not impossible due to the bulky phenylalanine residue in position 486 (F486 in h2019-nCoV S1 RBD[7]) and in positions 442 and 472 (Fig 1 section C optimized RBD). Could we expect changes in other polar residues in this active region? Could we determine the minimum change in arterial blood pH which may induce sufficient conformational deformation to reduce viral entry (is metabolic alkalosis[8] better than metabolic acidosis, arterial pH 7.3?).



Virus	Year	442	472	479	480	487
SARS - human	2002	Y	L	N	D	T
SARS - civet	2002	Y	L	K	D	S
SARS - human/civet	2003	Y	P	N	G	S
SARS - civet	2005	Y	P	R	G	S
SARS - human	2008	F	F	N	D	S
Viral adaption to human ACE2		F > Y	F > L > P	N = R >>> K	D > G	T >>> S
Optimized - human	In vitro design	F	F	N	D	T
Viral adaptation to civet ACE2		Y > F	P = L > F	R > K = N	G > D	T > S
Optimized - civet	In vitro design	Y	P	R	G	T
SARS - bat	2013	S	F	N	D	N
2019-nCoV – human	2019	L (455)	F (486)	Q (493)	S (494)	N (501)

Figure 1: Conservation and variation of amino acid residues in the active binding region of SARS-like viruses (ref 7).

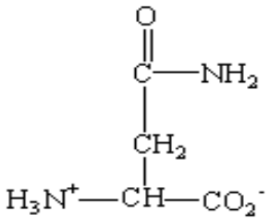
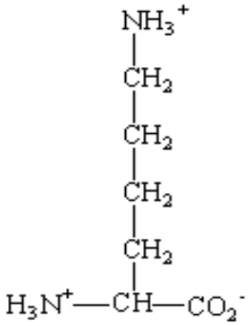
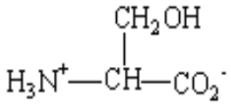
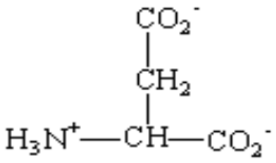
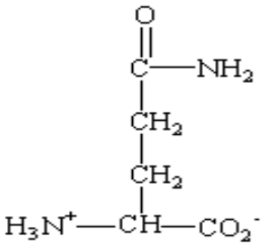
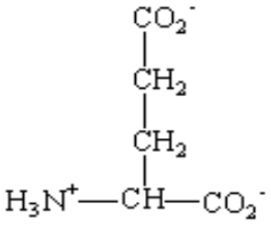
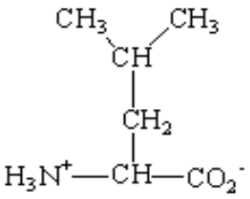
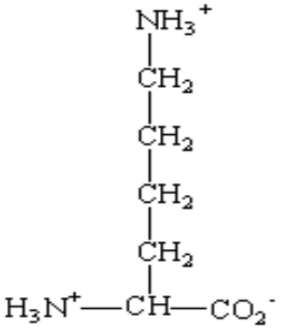
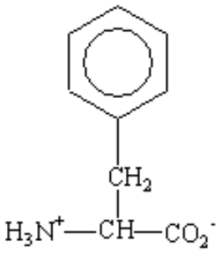
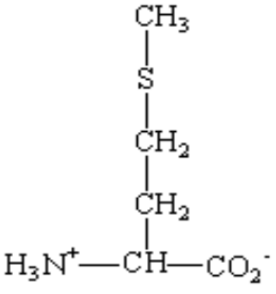
h2019-nCoV (amino acid charges[9] shown at pH 7)		hACE2 (amino acid charges shown at pH 7)	
N501-Asparagine		K353-Lysine	
S494-Serine		D38-Aspartic Acid	
Q493-Glutamine		E35-Glutamic Acid	
L455-Leucine		K31-Lysine	
F486-Phenylalanine		M82-Methionine	

Table 1A: Amino acid charges (ref 9)

Charged (side chains often form salt bridges):
- Arginine - Arg - R
- Lysine - Lys - K
- Aspartic acid - Asp - D
- Glutamic acid - Glu - E

Polar (form hydrogen bonds as proton donors or acceptors):
- Glutamine - Gln - Q
- Asparagine - Asn - N
- Histidine - His - H
- Serine - Ser - S
- Threonine - Thr - T
- Tyrosine - Tyr - Y
- Cysteine - Cys - C

Amphipathic (often found at the surface of proteins or lipid membranes, also classified as polar):
- Tryptophan - Trp - W
- Tyrosine - Tyr - Y
- Methionine - Met - M (may function as a ligand to metal ions)

Hydrophobic (normally buried inside the protein core):
- Alanine - Ala - A
- Isoleucine - Ile - I
- Leucine - Leu - L
- Methionine - Met - M
- Phenylalanine - Phe - F
- Valine - Val - V
- Proline - Pro - P
- Glycine - Gly - G

Table 1B: Just an absurd idea?

The promise or the peril due to the narrow range of normal blood pH (7.35-7.45) is the critical issue in this suggestion. Triggering metabolic alkalosis is the thorn in any attempt to elevate blood pH (Lasix[10]). In vitro cell culture assays with stringent pH control may offer clues about the degree to which the pH must be disrupted in order to reduce the affinity between S-RBD and ACE2. Results from animal models may indicate whether it has any merit or if it is physiologically unsafe due to undesirable side effects. Eating licorice (glycyrrhizic acid is the active component) causes a metabolic syndrome which mimics primary hyperaldosteronism. Chronic intoxication with glycyrrhizic acid may cause hypertension, metabolic alkalosis and hypokalemia with low plasma renin activity. Maybe just enough but not too toxic? Can the reverse work, that is, inducing acidosis[11] with Diamox? The latter is supposedly well tolerated by many individuals.

Name	Abbr.		Molecular Weight	Molecular Formula	Residue Formula	Residue Weight ($-H_2O$)	pKa[1]	pKb[2]	pKx[3]	pI[4]
Alanine	Ala	A	89.10	$C_3H_7NO_2$	C_3H_5NO	71.08	2.34	9.69	–	6.00
Arginine	Arg	R	174.20	$C_6H_{14}N_4O_2$	$C_6H_{12}N_4O$	156.19	2.17	9.04	12.48	10.76
Asparagine	Asn	N	132.12	$C_4H_8N_2O_3$	$C_4H_6N_2O_2$	114.11	2.02	8.80	–	5.41
Aspartic acid	Asp	D	133.11	$C_4H_7NO_4$	$C_4H_5NO_3$	115.09	1.88	9.60	3.65	2.77
Cysteine	Cys	C	121.16	$C_3H_7NO_2S$	C_3H_5NOS	103.15	1.96	10.28	8.18	5.07
Glutamic acid	Glu	E	147.13	$C_5H_9NO_4$	$C_5H_7NO_3$	129.12	2.19	9.67	4.25	3.22
Glutamine	Gln	Q	146.15	$C_5H_{10}N_2O_3$	$C_5H_8N_2O_2$	128.13	2.17	9.13	–	5.65
Glycine	Gly	G	75.07	$C_2H_5NO_2$	C_2H_3NO	57.05	2.34	9.60	–	5.97
Histidine	His	H	155.16	$C_6H_9N_3O_2$	$C_6H_7N_3O$	137.14	1.82	9.17	6.00	7.59
Hydroxyproline	Hyp	O	131.13	$C_5H_9NO_3$	$C_5H_7NO_2$	113.11	1.82	9.65	–	–
Isoleucine	Ile	I	131.18	$C_6H_{13}NO_2$	$C_6H_{11}NO$	113.16	2.36	9.60	–	6.02
Leucine	Leu	L	131.18	$C_6H_{13}NO_2$	$C_6H_{11}NO$	113.16	2.36	9.60	–	5.98
Lysine	Lys	K	146.19	$C_6H_{14}N_2O_2$	$C_6H_{12}N_2O$	128.18	2.18	8.95	10.53	9.74
Methionine	Met	M	149.21	$C_5H_{11}NO_2S$	C_5H_9NOS	131.20	2.28	9.21	–	5.74
Phenylalanine	Phe	F	165.19	$C_9H_{11}NO_2$	C_9H_9NO	147.18	1.83	9.13	–	5.48
Proline	Pro	P	115.13	$C_5H_9NO_2$	C_5H_7NO	97.12	1.99	10.60	–	6.30
Pyroglutamatic	Glp	U	139.11	$C_5H_7NO_3$	$C_5H_5NO_2$	121.09	–	–	–	5.68
Serine	Ser	S	105.09	$C_3H_7NO_3$	$C_3H_5NO_2$	87.08	2.21	9.15	–	5.68
Threonine	Thr	T	119.12	$C_4H_9NO_3$	$C_4H_7NO_2$	101.11	2.09	9.10	–	5.60
Tryptophan	Trp	W	204.23	$C_{11}H_{12}N_2O_2$	$C_{11}H_{10}N_2O$	186.22	2.83	9.39	–	5.89
Tyrosine	Tyr	Y	181.19	$C_9H_{11}NO_3$	$C_9H_9NO_2$	163.18	2.20	9.11	10.07	5.66
Valine	Val	V	117.15	$C_5H_{11}NO_2$	C_5H_9NO	99.13	2.32	9.62	–	5.96

[1] pKa is the negative of the logarithm of the dissociation constant for the -COOH group.
[2] pKb is the negative of the logarithm of the dissociation constant for the $-NH_3$ group.
[3] pKx is the negative of the logarithm of the dissociation constant for any other group in the molecule.
[4] pI is the pH at the isoelectric point.
Reference: D.R. Lide, *Handbook of Chemistry and Physics, 72nd Edition*, CRC Press, Boca Raton, FL, 1991.

Table 1C: Amino acid reference[12] chart. Can CITCOM deploy sensor arrays to detect[13] infectious agents in sewers?

THE HICCUP

Human angiotensin converting enzyme 2 (hACE2) is essential for physiology[14]. Pulmonary hACE2 plays an important role in acute lung failure. ACE2 inhibitors[15] may reduce viral entry but certainly harmful to humans. The suggestion to perturb the ***configuration*** and reduce ***affinity*** or make the binding "wobbly" (think wobble[16] hypothesis) may be problematic. The gulf between the pKa and pKb (Table 1) of amino acids indicates that *slight* change of blood pH may not lead to a sufficient degree of conformational disruption (see Fig 4 in ref 17).

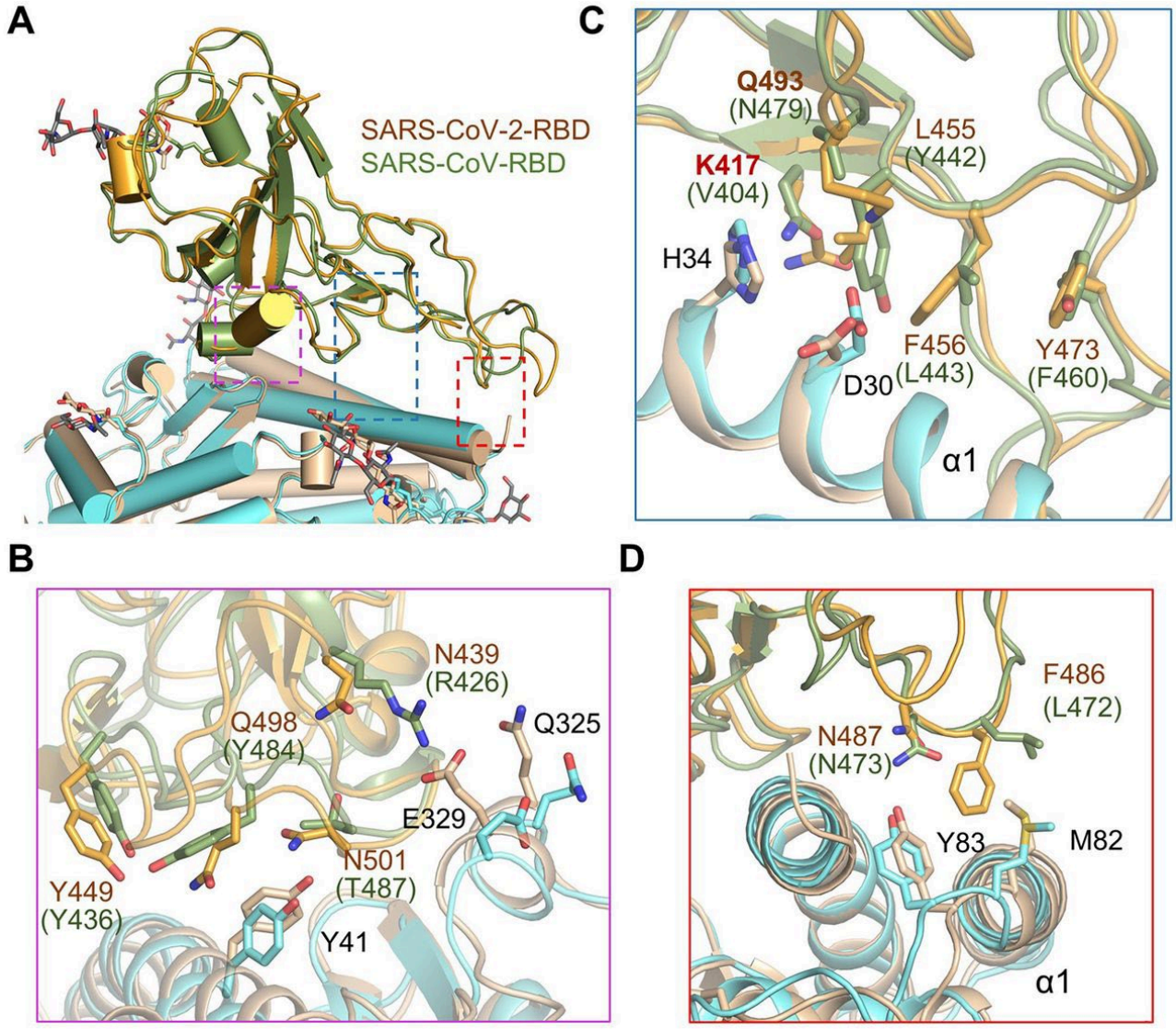


Figure 2: From Yan *et al* - Interface comparison[17] between SARS-CoV-2-S1 RBD (brown) and SARS-CoV-RBD (green) with hACE2 (black) reveals changes which could impact affinity. Change from Val404 to Lys417 may result in tighter association because of the salt bridge formation between Lys317 and Asp30 of ACE2 (see C, above and Fig 4C in ref 17). Change of Leu472 to Phe486 may also make stronger van der Waals contact with Met82 (see D). However, replacement of Arg426 to Asn439 may weaken the interaction by losing one important salt bridge with Asp329 on ACE2 (see B).

Computational biochemists may help model the interaction and determine the van der Waals radii (distance) necessary to ***diminish*** the ability to form salt bridges, hydrogen bonds (determinants of molecular interaction) between the amino acid side chains in the binding region (Figures 1 and 2). Values of pKa sufficient to keep the molecules (amino acid side chains) apart (increase in van der Waals radii, disrupt salt bridges, polar interactions) may reduce affinity or binding between S1 RBD and hACE2. Armed with this pKa value, a hematologist may be able to deduce the degree of alkalosis (or acidosis) necessary, albeit hypothetical, to deliver that ***micro-pKa change*** necessary to reduce binding. For example, to weaken the F486-M82 interaction (van der Waals) or take advantage of the replacement of Arg426 (CoV) to Asn439 (CoV-2) to disrupt the weak salt bridge with Asp329 of hACE2 (see Figure 2B).

This is an entirely theoretical idea without any supporting evidence. The use of sodium bicarbonate may pose concentration-dependent tolerance issues with respect to sodium/electrolyte management and tissue distribution of pH effect(s). Alteration of blood pH is a challenge to homeostasis and profound metabolic dysfunction. This suggestion is similar to decreasing or increasing body temperature to control an otherwise negative impact on the normal physiology. But, altering body temperature affects enzyme kinetics, cognition and memory. *Would we prefer an infection-free but brain-dead person?*

However, ***the nail on the coffin*** of this idea is the observation that micro-modification of blood (arterial) pH may not have any effect on the binding between SARS-CoV-2 RBD and hACE2 because angiotensin-converting enzyme 2 is localized on, and mediates infection through, the apical plasma membrane[18] of respiratory epithelial cells (not the basolateral). Progeny virus was released into the apical chamber at titers up to 5 logs higher than those recovered from the basolateral chambers[19] of polarized cultures. Therefore, changing the pH of the blood may not[20] help. Do we need to change the pH in alveolar space? The normal pH range of the alveolar fluid is 6.5-6.8 and if we change the pH to 7.5, what can we expect? How will it influence the physiology of the lungs in CoVID-19 patients? Will change in pH affect ACE2 activity[21] and create new problems for patients using ACE inhibitors to control hypertension?

The structural data from Yan *et al* (Figure 2) provides clues with respect to the potential of biomimicry using Spike (S) protein ("ligand decoys"). In general, the "active site" of interaction (Fig 2 on pg. 4 and Fig 4 in ref 17) involves the peptidase domain (PD) of hACE2 and the SARS-CoV-2 Spike (S) protein α1 helix (RBD, Spike subunit 1). The α2 helix and the linker between β3 and β4 pleated sheets of hACE2 also contribute to the "groove" (focus for biomimicry). The idea is to design and create a computationally optimized "groove" to be synthesized as a peptide (polypeptide) which is just sufficient in length to generate the required *functional* secondary structure *in vivo*. The "decoy" active site mimics a segment of the peptidase domain (PD) of ACE2. Can we sufficiently differentiate this peptide from known biological ligands which binds to ACE2? Is it at all plausible to allow individuals to self-administer with this peptide or a cocktail[22] of decoy ligands? Will the peptide trigger immune response? Can immunity to the peptide, reduce the efficacy of this approach for recurring pandemics? Can the design include conformational and/or allosteric[23] switches[24] to act as sensors[25] and/or expose one or more highly antigenic epitopes ***after*** binding?

The *in vitro* success of biomimicry using synthetic peptides[26] to act as decoy ligands for SARS-CoV2 RBD is a hypothesis inspired by crystallographic models. But, bioavailability in the affected organ (apical cells in alveolar space) and efficacy of binding *in vivo* are issues which can halt therapeutic success. Could we explore protein databanks[27] (PDB) to find a "naturally" existing peptide as a decoy ligand? Could we use micelles[28] for delivery as a nasal/bronchial spray?

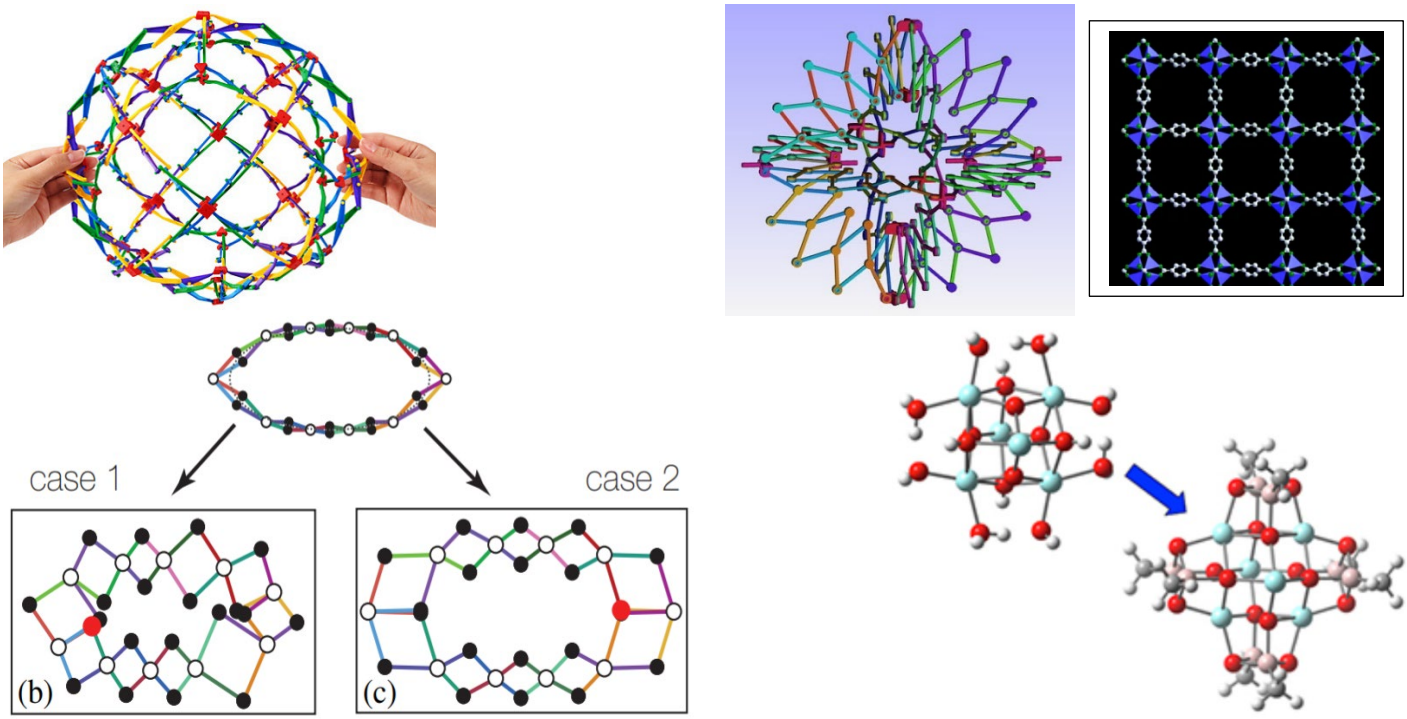


Figure 3: Ideas[29] from Charles Hoberman[30] as a part[31] of the decoy[32] as a "trap" by attaching *Hoberman Sphere-esque* structures to engulf viral particles. Could we configure metal organic frameworks (MOF[33] see right, top and bottom) or COFs (covalent organic frameworks[34]) to act as *nano electro chemo systems* (NECS) which may be "dumb" "mechanical" macrophages triggered (by decoy signal) to capture infectious agents? Is stupidity[35] good for science and innovation?

CITCOM: CANARY IN THE COAL MINE – TRANSFORMING THE CONCEPT INTO A GLOBAL PARADIGM?

Canaries[36] offered their lives (their death was the evidence, the data) to keep humans safe, for almost a century. During 2010's the "nanocanary" was as a biosensor[37] designed[38] to assess toxicity of engineered nanomaterials. The "canary" must be rescued from ignorance[39] and enabled to support the implementation of tools to PREDICT[40] what the future may hold, in terms of public health. Sensors in the sewer[41], a subset of CITCOM (referred to as "SENSEW"), is in need of synergistic integration of basic science research with engineering to transform the vision of CITCOM into a systemic monitoring platform rather than just a knee-jerk[42] "to-do" reaction.

What are we seeking through CITCOM? The oversimplified (but not incorrect) answer is that we are trying to identify clues (for SENSEW, sensors in sewers or wastewater) which may indicate[43] signs of approaching diseases which may become an epidemic or pandemic. The expectation is that by identifying 'ground zero' cases early enough, we may better prepare[44] to prevent spread of the causative agents (humans, animals, bacteria, viruses, prions).

This scenario makes sense in view of the pandemic, but as a paradigm, CITCOM is more than just SENSEW. In a strategic sense, *CITCOM must address the essential pillars of how society functions* because without any one of these pillars, social dysfunction ensues, albeit with variable rates of direct and indirect mortality and morbidity. These pillars are global public goods or FEWSH, an acronym for food, energy, water, sanitation and health, the latter includes human as well as animals and plants (one health).

CITCOM deployments in essential verticals (food, water, sanitation, health) may better inform the FEWSH ecosystem but supply chain agility and optimization will vary with the environment, economy, infrastructure and leadership. What we lack most is our understanding of the synergy necessary for different sources of data with different degrees of dynamic weights to deliver ***actionable information*** of value, *a priori*, to enable planning for emergencies. Peacetime planning for pandemics are error prone with each supply chain analyst claiming their vertical as essential ("*chacun voit midi à sa porte*" or "everyone sees noon at their doorstep"). Post-pandemic planning may remove the "wool over our eyes" and sharpen our sense of urgency by delineating the chasm that exists in supply chain planning.

Figure 4: Transformation of supply chain management is key to pandemic logistics but not central. FEWSH is the future focus to guarantee global public goods access, as equitably as possible. Decline of LVMH[45] *-esque* lifestyle and a surge of health and healthcare services for humans and animals are expected. Education[46] and manufacturing ***aren't*** losers.

CITCOM-FEWSH ENTENTE

End-to-end implementation of the CITCOM metaphor within FEWSH is a gigantic task. It combines basic and applied R&D plus engineering design and deployment, to be executed by PPP (public-private partnerships) and agencies (local, national, global). It calls for keen synergistic integration and oversight by creating a Department of Health Security (HealthSec) with similar urgency which created Department of Homeland Security (DHS) following 9/11. Global security and economy may depend on HealthSec and DHS, equally. FEWSH cannot be viewed as piece-meal. It is analogous to viewing the heart rate monitor (oscilloscope) displaying the PQRST waveform. PQRST represents the inextricably linked FEWSH. PQRST represents heart rhythms which is key to the status of life itself. FEWSH is the pulse which indicates global socio-economic wellness. Network of connectivity between CITCOM nodes is an indicator of this "pulse" and ***the ability to astutely observe the meaning of the fluctuations*** if the data departs from the baseline. It is key to grasp the ***community-specific nature of the baseline*** which will vary depending on the socio-economic milieu.

The complexity of "pulse" may be appreciated by recognizing that the human heart PQRST waveform has a "normal" range and any change in frequency, amplitude or phase underlies a function and/or dysfunction, for example mitral valve stenosis or aortic valve dysfunction (murmur) or arrythmia (atrial flutter). PQRST may be affected irreversibly by factors that exert influence ***on*** the performance of the heart muscle. FEWSH may be affected by factors and fluctuations due to asymmetry in supply chain performance and imbalance of products vs services that converge on global public goods ***delivery***. The state of the heart, PQRST waveforms ***collectively***, influences our general physiology, for example, respiratory rate (RR), end tidal carbon dioxide volume (etCO2), blood oxygen saturation (pO2), arterial blood pH variation (acidosis/alkalosis), mental health (stress, depression) and self-inflicted harm (recreational drug abuse).

However, analysis of FEWSH may be impossible, as a collective entity. The principle of deconstruction and reconstruction must be applied in order to reduce the complexity and then reassemble the key performance indicators (KPI) for each sub-unit and sub-system to synthesize the collective output and value(s). The metaphor of CITCOM is a ***horizontal approach*** because each element of FEWSH influences the outcome (global public goods). Each "vertical" of FEWSH may be examined through the CITCOM lens. The platform of CITCOM with its common forms and functions are FEWSH monitoring tools. The "eyes" of CITCOM are defined as detection, data, diagnoses and decision[47] (4D). The pre-pandemic 4D platform approach is applicable to the current post-pandemic CITCOM strategy with modifications[48] as necessary, to adapt and satisfy the KPIs for each sub-unit and sub-system with an additional focus on essential goods.

Paralysis by analysis may suffocate the preparation which is necessary for implementation of CITCOM in the real-world. The economics of technology will determine adoption, especially in nations with economic constraints[49] with underserved populations. But, a far more complex problem, especially for SENSEW, is the lack of sanitation services for about a billion[50] people in the triple A zone (Asia, Africa, Amazon represents the triple A zone which are "hotspots" for evolution of microbes which may be linked to the etiology of future epidemics and pandemics). Lack of access to water[51] for billions foments poor hygienic practices. The ubiquitous use of low-cost sensors to monitor the environment is essential. Without sensor data, this problem ***will*** explode, periodically.

*Do we know what to sense and how to sense? Millions of papers are published on sensors but how many commercially available sensors are used by the **underserved** communities? What are the top 10 health sensors, globally?*

WHAT IS NEW? NOT MUCH.

We are not unaware of the causes or the scale of these events. Yet our plans and progress are still immersed in haphazard approaches. One reason for this "haphazard" article is to touch a range of interwoven topics, diverse and distant in terms of disciplines. Trans-disciplinarity is not tolerated by magazines, journal articles and scientific papers because traditional publication "houses" are keen on "target" audience, formatting, anti-sharing ethos and pay-walls. This essay and its digressions may appear incoherent or just a sprinkling of too many disorganized "dots" without spelling out 'reasons' or commenting on the connections to illustrate how we can proceed on path to global harmony.

For each of the FEWSH verticals (we will not dare to address energy in this essay) we need to ask a set of semi-standard questions and modify/adapt depending on follow-up questions. Could we ask the questions in Table 2 for each of the domains: food, water, sanitation, health (human health and animal health as in the "one health" approach)?

What may emerge from the information (Table 2) may act as a compass for follow-up questions along specific tracks and serve as a navigator for several (thousand) action items, action plans, strategic plans to fuel many *roadmaps*. From the perspective of CITCOM, a robust end-to-end[52] system complete with mobile data communication and link to near real-time decision system is ***the value*** that the CITCOM ***platform*** should deliver, globally. The challenge is to make the metaphor of CITCOM available and applicable to each and every verified target for each and every vertical. It is a herculean task to make sense of data from a vast range of sensors. Information from sensor data is the foundation.

Are these suitable questions for biological entities (including parasites & prions) as causative agents?	4D ▪ reference 47
01 What are the top five biological agents[53] in terms of mortality?	DETECT
02 What are the top five biological agents[54] in terms of morbidity?	DETECT
03 What are the potential for epidemics or pandemics due to these agents?	DETECT
04 What are the known/anticipated modes of transmission?	DETECT
05 What are the known/anticipated rates of transmission (spread)?	DETECT
06 What are the known/anticipated natural barriers that may prevent natural transmission?	DETECT
07 What are the known/anticipated human-induced barriers that may prevent transmission?	DETECT
08 Are there chemical or biological or engineered targets/tools that can detect these agents *in vivo*?	DETECT
09 Are there chemical or biological or engineered targets/tools that can detect these agents *in vitro*?	DETECT
10 Are there any experimental or commercial sensors available to help detect these agents?	DETECT
11 Are these sensors or sensor systems in a deployable form factor?	DETECT
12 Are these sensors or sensor systems field tested and/or are field deployed at present?	DETECT
13 Are there any available data or database from these sensors?	DATA
14 Are there any available data from sensors which are reliable for use as primary diagnosis?	DIAGNOSIS
15 Are there any available data from sensors which are reliable for use as secondary diagnosis?	DIAGNOSIS
16 Are there any available data from sensors which are reliable for use in epidemiology?	DECISION
17 Are there any available data from sensors which are reliable for use in public health decisions?	DECISION
18 Are there any available data from sensors which are reliable for use in supply chain decisions?	DECISION

Table 2: Could we answer these questions with respect to food, water, sanitation and health (humans and animals)?

CITCOM: OVERSIMPLIFID METAPHOR AND PLATFORM FOR TOOLS BUT THE QUESTIONS ARE INFINITELY COMPLICATED

Sensor data is useless unless the ***target to detect*** is useful for the purpose. If the purpose of CITCOM is to serve as an early warning system for the next global health crisis then we must ***detect the signals*** as close as possible to the origin. PREDICT[55] and other similar landmark alliances[56] are examples of how to ask and probe the correct questions. The deployment of CITCOM ***close to the origin of the signal*** is replete with ambiguity because the origin may not be in the human domain, for example, in cases of epidemics and pandemics (SARS, MERS, H5N1, CoVID-19). This discussion must delve deep into the animal kingdom, as initiated by PREDICT. The human-animal interface may hold the key in our surveillance attempts to detect and define targets for biological signals. The "patient zero" concept may justify when SENSEW (sensors in the sewer) may be a subset of the CITCOM paradigm but unfortunately the triple A zone (Asia, Africa, Amazon) where these interactions may happen may also lack sewer systems. Deploying SENSEW may be cost prohibitive for most of the world. But, affluent nations may develop and deploy ***mobile-lab-on-a-drone*** (mLOAD).

Identifying a potential biological signal (approaching health crisis, if left unchecked) is a complex task. An array of targets will be necessary to improve confidence. Corroborating evidence may strengthen the decisions. It is not a point solution but a fabric of data, information and evidence, curated and verified, that must be made available to decision systems and decision makers, with sufficient time to plan and execute a pre-emptive action at or near the source. Most importantly, a failed outcome, failure to predict, failure of any one mission and failure to be correct must not be the grounds for diminishing the vigor of the program. Failure will teach us what we need to learn, perhaps better, to use the tool with accuracy and precision, as long as the humans in the loop are sufficiently open, knowledgeable and focus on credibility that only uncompromising scientific rigor can offer. Pandemics of the future will not be solved with yesterday's ideas. Recurrence of pandemics/endemics are likely. ***Teams must learn on and from the ground***.

The focus on One Health[57] in ***combination*** with tools from CITCOM, will generate benefits even for events that may not be a global catastrophy (but try using that logic to console the mother of the child who died from eating romaine lettuce). This is where the subset of SENSEW for sanitation and applications in the food security supply chain[58] as well as water and wastewater[59] (re-use in agriculture) will benefit on multiple levels (including energy). The global awareness of the CITCOM paradigm must proselytize the faith in early warning sensor systems and evangelize the power of the tools which are not exclusive to epidemics and pandemics. Cumulative global death toll due to food borne illnesses and water contamination may be reduced by providing open access to these tools[60] for populations in emerging economies. The pursuit of CITCOM paradigm through public support and funding will better prepare the world for health emergencies.

Sensible sensor systems are our allies in WW III. Without its use, the 21st and 22nd centuries may experience the same fate[61] as ancient Rome, an irony which may not be lost on Italians in view of the CoVID-19 massacre in Italy. The end-to-end sensor system is a convergence of many parts that must act seamlessly (discussed in millions of other publications). The frequent digressions in this article are aimed to discuss the science of sensing/identifying targets.

Mass produced reagents serving as common binding surfaces for pathogens are ephemeral in efficacy due to rapid changes in molecular targets. Despite investments in R&D we are without sufficient ammunition against the increasingly terrifying roster of emerging infectious diseases due to HIV, Ebola, Lassa, West Nile, Nipah, SARS, MERS, Zika and Marburg virus. The biological building blocks (proteins, nucleic acids) must be creatively designed to serve as complementary molecules that may bind to targets which can identify and detect biological intruders with specificity and sensitivity. This simple idea of complementarity is profoundly difficult to reproduce reliably in sensor systems. Also, ***sensors must be affordable in AAA nations for home use***, to reduce transmission and global diffusion of infection.

3D printed laser inscribed graphene (LIG) turbostrat sensors are lower in cost to microfluidic systems. But, attaching the complementary molecule on ***LIG must be calibrated to be reproducible***. Testing whether the target binds with specificity and sensitivity, under non-optimum (harsh) conditions (extreme pH, chemical contaminants) is a tall order. Duration of the binding must be sufficient to generate a signal which can be transduced to a receiver or gateway. This is where the metaphor of CITCOM meets with the metaphor of IoT (internet of things) by enabling the signal from the sensor to be connected wirelessly to a smartphone or via the internet to data, information and decision systems. If combined and if functional, then we have some form of a rudimentary sensible sensor system. Ideas relevant to sensible sensor systems are discussed in a follow up (ref 238) to this article ('ADD' https://doi.org/10.26434/chemrxiv.13102877).

The nexus of CITCOM and IoT could be transformative if feasible and pragmatic, globally. Although low-cost LIGs may be single use sensors, the economics of this technology may enable widespread diffusion. The alternative is to pursue the implementation of hysteresis and create a reusable sensor for continuous monitoring. But, at what cost?

The nature of complementarity (mentioned above) is the open question. Answers to question numbers 8 and 9 from Table 2 may provide clues. A synthetic peptide (decoy ligand based on hACE2 binding surface) that binds *in vitro* (ref 33) to the Spike protein subunit 1 of SARS-CoV-2 (S1 RBD) is one candidate. Can it attach to LIG sensors (using linkers to bind to LIG electrodes introduces room for errors) and bind the virus if the Spike protein is made available?

This hypothetical *modus operandi* does not guarantee that the secondary structure of the synthetic peptide will provide stable binding and generate a detectable signa even if it binds to the S1 RBD. The source of S1 RBD for this test is an open question but a synthetic version can be manufactured based on crystallographic structure (ref 17). It remains to be seen if structure-function (in addition to many other issues) may cloud the test due to conformational changes.

The binding of a target to a complementary molecule is almost a standard operating procedure (SOP) for sensor engineering but the biophysical idiosyncrasies of primary, secondary and tertiary structure of peptides, polypeptides and proteins could make this process less dependable due to alteration of binding constants. The latter may induce instability if the test samples have variable pH, chemicals (colloids, surfactants, redox agents) and abundance of other microbes.

Even if we can calibrate, titrate and replicate the results to demonstrate specificity and sensitivity agnostic of the testing solution for single use sensors, we may be far from successful in claiming that this sensor is an effective detection tool. Viral mutations could make the complementary molecule on the sensor, ineffective for detection. What if the binding is influenced by variations in the hACE2 protein due to polymorphisms?

Using a combination of theoretical and protein chemistry tools, it may be possible to predict a number of potential variations of SARS-CoV-2 S1 RBD which may still bind ACE2 and variations of ACE2. Rather than one, we may produce an array of peptides/polypeptides as complementary molecules to which combinations of variants in both proteins. But it is a trial and error approach without any known metrics to predict its potential for success.

If we have an array of complementary molecules, what happens in terms of sensor engineering? If we have 100 different molecules, do we aim to produce and functionally test (calibrate, titrate, replicate) 100 different sensors? If this is adopted as a SOP for all the other variations, then we will have to generate tens of thousands of sensors just to test one virus (due to potential variability in a few hundred coronavirus strains that may evolve). No matter how inexpensive the sensors may be, this may be a fool's errand.

A less foolish approach may be to take advantage of the technological advances in creating nanowire sensors, which are certainly at a higher cost bracket than laser inscribed graphene sensors. Discussed in "4D" (ref 47), nanowires increase the surface area for attachment. Perhaps all 100 variations of the complementary molecule may be attached on one sensor. This ***sensor nanoarray*** is reminiscent of microarrays from the 20th century. However, this approach may stir some "warm" technological debates and what benefits it may deliver. Do we need hundreds of different molecules?

Enveloped viruses display protein molecules on their surface but their genetic material (RNA or DNA) is protected by the envelope. Mild acid/base treatment is sufficient to expose the genetic material. The latter is SOP for lab tests for SARS-CoV-2 . The viral single stranded ribonucleic acid is subjected to quantitative reverse transcription (qRT) using reverse transcriptase (enzyme uses RNA as the template to synthesize DNA, "reversing" of the central dogma of biology). Following qRT, the DNA is subjected to polymerase chain reaction (PCR) using very specific sets[62] of primers[63] to amplify the amount of DNA in order to confirm detection of the virus, if present in the sample. Capturing this lab test on a chip or a sensor is the Holy Grail, for some experts.

Could we use ssDNA (think aptamers) to create a complementary molecule? The assumption is that a lysed sample containing SARS-CoV-2 RNA may bind the complementary ssDNA immobilized on the sensor and generate a signal. Can we create a DNA nanoarray sensor for RNA viruses? Is it impossible? Will it be reliable? Is it affordable?

In the SENSEW subset of the CITCOM paradigm, robotics and automation in sampling and testing is inevitable but then such tools will be out of reach for the geographies that need it the most, the triple A zones. Leaving aside the low-cost factor, robotic sampling of sewer water (wastewater) or swabs from food items (produce, meat) on conveyor belts and sensors embedded in retail packaging are possible. Drones may collect samples from remote areas in triple A zones (soil, vegetation, animal waste, etc). Sensors-on-drones can process samples and upload data to gateway drones, borrowing old ideas from "in-network processing" in mesh networks and MANET (mobile ad hoc networks).

This is conventional thinking, based on what we knew, yesterday. Nothing new was added, today, yet.

If these conventional tools were implemented, it still may be incapable of preventing molecules of mass destruction from unleashing their infectious enthusiasm, occasionally. But, it may suffice to contain smaller and less devastating outbreaks, reduce health impact due to food and water borne illnesses. Infrastructure investing can improve access to water, the practice of hygiene, and accelerate the pace of adopting managed sanitation services in AAA zones. But events that could bring civilization to a halt, remains out of our grasp because we are trying to solve tomorrow's problems with yesterday's ideas (ignore the re-purposed buzz words created by consultants peddling snake oil sales).

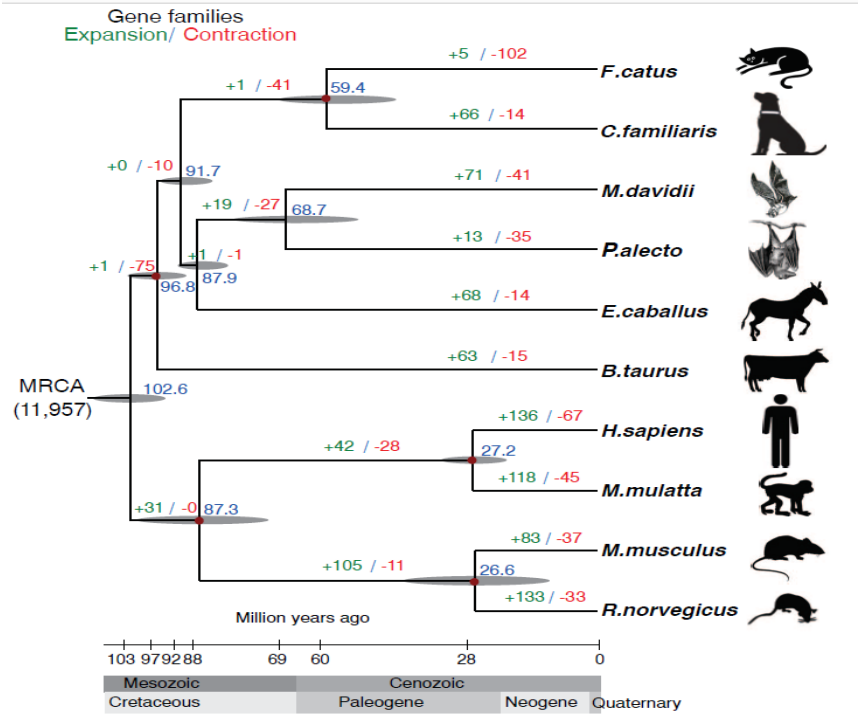
We are searching for needles in the haystack and it is not an "one time" search but a continuous process because evolution is unstoppable. Every potential new version of an organism is a "new needle" we must race to find, before it finds us, unprepared, unhinged, and unprofessional. Is the "new needle" capable of unleashing death and destruction?

Everything in this discussion, thus far, is "old" news. It is handicapped by the dead weight of old technology. Can we afford to wait for imagination to generate new thinking, invention to sow new principles and innovation to lead new practices? Should the old guards carry on with the dogma through this stagnation? Is it possible that we may be approaching an inflection point in ***basic science research?*** Basic science is the only option available for uncovering new clues to solutions. In medicine, the importance of vaccines cannot be overstated (examples from polio, smallpox, rabies). But, medical miracles are few and far between (unless it is in social media). We have not witnessed any breakthroughs or gene therapies or "brain in the box" solutions but just a surfeit of unctuous grins. The "business as usual" muddles on with malaria, lupus erythromatosus, TB, HIV, Ebola, Alzheimer's and antibiotic resistance. Much to the chagrin of Barry Marshall[64] gastritis still kills 14,500 individuals per annum due to eminently controllable *Helicobacter pylori* infections[65].

Detection, data, diagnosis and decision (4D) in the context of CITCOM have exposed grave gaps in our basic knowledge necessary to mount credible resistance to halt epidemics and pandemics. While we increase our investment in basic science, molecular epidemiology and global information arbitrage, to build knowledge bridges to *mind the gap*, it will be inhuman to do less to promote CITCOM's most practical subset, SENSEW. Nearly half a million people die each year from food[66] borne illnesses and 3.4 million people die from water[67] related diseases, of which diarrheal diseases claim about 2 million lives[68] each year, mostly children[69] under the age of five. Even the US[70] is not immune to water[71] related crisis, albeit far less severe than the double AA world (Africa and Asia). It is imperative that we transform the vision of CITCOM through implementation of SENSEW. Target detection for food/water borne illnesses may be less difficult to implement using sensible sensor systems designed to optimize the operation of SENSEW for sanitation.

CITCOM MEETS ONE HEALTH - SKETCHING THE VAST CONTEXT OF HUMAN AND ANIMAL HEALTH (FEWSH)

Deep within the realm of unknown unknowns are thousands or millions of zoonotic viruses with pandemic potential, including coronaviruses (SARS, MERS), paramyxoviruses (Nipah), filoviruses (Ebola) and influenza viruses. Targets to identify and detect these viruses as well as the basic biological understanding of the nature of their virulence was launched by the U.S. Agency for International Development (USAID) since the scare of the 2005 avian flu (H5N1). USAID PREDICT[72] gathered specimens from more than 10,000 bats and 2,000 other mammals in search of dangerous viruses. They detected about 1,200 viruses that could spread from wild animals to humans, signaling pandemic potential. More than 160 were novel coronaviruses, much like SARS-CoV-2, as well as a new Ebola[73] virus. How and when these viruses will infect other species related by phylogenomics[74] is the broader, bigger and bone-chilling form of uncertainty.



Phylogenomic analysis. Maximum-likelihood phylogenomic analysis of 2492 genes from *M. davidii*, *P. alecto*, and eight mammalian species. Divergence time estimates in blue, gene family expansion events in green, and gene family contraction events in red. MRCA, most recent common ancestor.

Figure 5: Phylogenomic analysis (ref 74): The truth about cats and dogs[75] being[76] infected[77] by SARS-CoV-2

Figure 6: The discovery[78] of transposons in maize by Barbara McClintock[79] made it abundantly clear that genes and segments of genes "jump" from one genome to another (displayed as variation in kernel color in the photograph[80]). The phylogenomic ancestry between ten mammalian species shown in Figure 5 (ref 74) and other studies[81] supports the fact that zoonotic viruses are "jumping" (may be still undetected) and can/will "jump" between species.

The timeline of such cross-species jump and the genetics of virulence[82] are still under investigation in various[83] microbes without clarity about ways to control these multigenic events, even after more than half a century of research. The lessons from the infamous 20th century Spanish Flu depends on the interpretation[84] but scientific record[85] of events[86] from a historical perspective reveals that the period was far more extended **(1888-1920)** than discussed[87] or socialized.

"The 1889-1890 pandemic may have originated, among others, in the following countries: China (following the 1888 flooding); Athabasca in Canada (May 1889); Greenland (summer of 1889), Tomsk in Siberia or Bukhara in Uzbekistan (October 1889). We know with certainty that the first cases appeared in St. Petersburg (Russia) on 27th October 1889 and expanded rapidly via railway to whole Europe. In Paris, the first cases were recorded on 17th November; in Berlin and Vienna on 30th November; in London around middle of December and in southern European countries, from Italy to Portugal towards the end of December. The influenza spread overseas to America in January 1890, with the first cases appearing in Boston and New York. During the first months of the year, it spread throughout North and South America, Africa, Asia and Oceania, arriving by August to island of Madagascar, Jamaica and Santa Helena. In Paris, (ref 86) the first cases (17th Nov 1889) were benign and affected, among others, employees of large commercial stores, and of the post and telegraph office services. From 15th December (1889) onwards, the virus became extremely virulent and mortality rose steeply. The period of maximal viral effect took place between 16 December 1889 and 31st January 1890, when over 5,042 deaths were recorded in Paris." (Quoted from reference 85)

Table 3: This excerpt (ref 85, 86) reveals a different timeline of the Spanish Flu (1888-1920). ***It wasn't just 1918-1919.***

The brewing lag phase of viral pandemics was duly cautioned[88] and a hypothesis[89] as to how it may have evolved was perhaps ignored (?). The exclusion of cats, dogs and rodents (ref 89) may be due to the fact that these animals are too "ubiquitous" to be noteworthy. The specific reference to horses (ref 89) brings 6 of the 10 (Fig 5) phylogenetically related mammals (including humans) in close proximity in Étaples, France, during 1916-1917 (quote below, ref 85).

"Étaples (1916-1917): Oxford et al (references 88 and 89) have suggested that the beginnings of the pandemic occurred in the British military base at Étaples, situated in the north of France (Department of Pas-de-Calais). This military base, which was quite important throughout the First World War, was occupied by 100,000 soldiers within a space of 12 square km. The base was situated near sea marshes with abundant migratory birds. Nearby, there were many farms with pigs, ducks and geese reserved as food for the soldiers, and horses which were used as a means of transport. The mixture of crowded soldiers, animals and 24 types of war gasses, which were massively used at the war fronts – many of which were mutagenic – might have been the cause of the appearance of the first outbreak of the epidemic between December 1916 and March 1917. According to Oxford et al, "in the outbreak from December 1916 to March 1917, soldiers were admitted to the base hospitals, suffering from acute respiratory infection, high temperature, and cough at a time when recognised influenza was present. Clinical examination showed, in most cases, signs of bronchopneumonia, and pathology history showed acute purulent bronchitis. This outbreak was further clinically characterised by heliotrope cyanosis described extensively in the ensuing 1918 outbreak, and very high mortality". Hammond et al (1917) described this forgotten epidemic of purulent bronchitis, which Abrahams et al (1919) compared with the great pandemic of the Spanish Influenza: "We emphasize our view that in essential, the influenza pneumococcal purulent bronchitis that we and others described in 1916 and 1917 is fundamentally the same condition as the influenza pneumonia of this present 1918 pandemic". (Reference 85)

Table 4: Quote from reference 85. Did the Spanish Flu begin in France (Étaples, 1916) or China (1888)? Did USAID PREDICT pick up these clues (ref 85, 88, 89 were published prior to 2009)? Lack of leadership and cross-pollination of information are reasons why the caution sounded by Oxford *et al* (ref 88) was left to be archived in the annals of history.

Cartoon 1: Can this[90] be one of the principles of prediction, especially when it comes to epidemics and pandemics?

Going back a couple thousand years brings us to Rome and the observation[91] that "Roman history might be considered the age of pandemic disease." Is this relevant to our current crisis? Could we afford to ignore CITCOM?

"Roman history might be considered the age of pandemic disease. Three times the empire was rocked by mortality events with stunning geographical reach. In AD 165 an event known as the Antonine Plague, probably caused by smallpox, erupted. In AD 249, an uncertain pathogen swept the territories under Roman rule. And in AD 541, the first great pandemic of Yersinia pestis, the agent that causes bubonic plague, arrived and lingered for over two hundred years. To understand how the Romans lived and died, much less the fate of their empire, we must try to reconstruct the specific juncture of human civilization and disease history that the Romans encountered. The pathogens that have regulated human mortality are not an undifferentiated array of enemies. The biological particulars of germs are unruly and decisive facts of history. The history of germs has been dominated by the brilliant model devised in the 1970s and most famously expressed by William McNeill in his classic *Plagues and Peoples*. For McNeill, the connective thread of the story was the rise and then confluence of different Neolithic germ pools. Agriculture brought us into close contact with domesticated animals; cities created the population densities needed for germs to circulate; the expansion of trading networks led to the "convergence of the civilized disease pools," as pathogens that were endemic in one society leapt ravenously into virgin territories. In recent years the shine of the classic model has started to fade. The ground has quietly but decisively shifted around it. The 1970s were the peak of a triumphant moment in western medicine. One by one the scourges of the past fell before the advance of science. There was confident talk of a transition in which infectious disease would become a thing of the past. But the terrifying roster of emerging infectious diseases—HIV, Ebola, Lassa, West Nile, Nipah, SARS, MERS, Zika, to name only a few of several hundred—shows that nature's creative destruction is far from spent. And all of these emerging infectious diseases have something insidious in common: they arose from the wild, not from domesticated species. Pathogen evolution and zoonotic diseases from the wild now loom larger than before in the dynamics of emerging infectious diseases." (Reference 91)

Table 5: Déjà vu or time for SENSEW? Excerpt from "The Fate of Rome" by Kyle Harper (Chapter 1, reference 91).

POSSIBILITIES, PRINCIPLES AND PRACTICE OF POST-PANDEMIC PREDICTION

It is a worn out cliché but prevention is still better than cure[92] unless there is a cure. Polio, smallpox and MMR vaccine highlights our optimism but the lack of HIV or Malaria vaccine indicates that cures may be elusive. In order to prevent we must detect and it appears that our tools for detection are lacking. Are we exploring other possibilities[93] or still asking the wrong question and searching in the wrong places? Can we change[94] our habits? Can we 'sharpen' the tools and create sophisticated sensors[95] and systems to detect some of the targets in the domain of global public goods (FEWSH)? Can we apply the CITCOM metaphor to food and water? Implementation of SENSEW for sanitation may no longer be limited to illicit[96] drug detection using enantiomeric[97] profiling. SENSEW is gaining momentum[98] but could use help from reliable, semi-automated, robotics-assisted, wireless sensor systems for data acquisition and distribution.

The ***key*** to "practice" is still in short supply in the sensor world. New tools for detection[99] of ***nucleic acids*** may be expensive (for example, using CRISPR-Cas12) or inexpensive[100] (based on CRISPR-Cas9[101]). Detection is vital because of potential widespread[102] "silent" infection by virus[103] which continues before and after symptoms[104] appear or disappear. New approaches to ***detect viral RNA*** with lasers[105] are also in progress.

We suggest binding tools which may use nanowire sensors[106] discussed in the pre-COVID version of "4D" (47). Could we converge nanowire sensor conjugated[107] with quantum dots[108] as sensors for traditional[109] saliva test? The corona "family" may use ACE2 as the host cellular receptor. Changing host attachment is an evolutionary process (thousands of years?). Therefore, agnostic of viral evolution and consequent mutations in the RBD, the host point of entry (hACE2) may remain the same for centuries. Could a nanowire quantum dot "dangling" (immobilized) ACE2 as a target serve as a detection tool? If successful, we use a similar approach for Noro, Ebola, Marburg, as well as less life-threatening but high frequency events caused by Salmonella, Listeria, Escherichia, etc. In each case we focus on the host receptor which acts as the primary gateway to humans and animals. Co-receptors may be important too (for example, TMPRSS2 for SARS-CoV-2) but only if it is also a binding target.

The convergence of nanowire with quantum dots could be synergistic. The "fly in the ointment" for NQTs (Nanowire Quantum doT) may be the signal strength if the system is devoid of qRT-PCR or any amplification step. If the sputum (saliva) of an asymptomatic/pre- or post-symptomatic human/animal contains only a few viral particles, will that generate sufficient change in optical recognition? Can we find tools to amplify the signal (the optical recognition) using some type of enhancement or resonance that induces a "domino" effect (*in vivo* one could imagine allosteric mechanisms but *in vitro* allosteric manipulation and variant configuration is just a theoretical idea).

The ability to spit directly into a vial with the suspended sensors may be pivotal for accelerating the healthcare supply chain management systems. Capturing the signal (transduction) using a simple holo-lens and an app on a mobile phone may ignite global diffusion and open up market of billions (MOB) who could benefit from "at-home" saliva tests. The sensor and the test must be agnostic of the medium being tested. Instead of saliva, the protocol may use wastewater or sewer waste or install the sensor inside commodes or urinals to implement the "pay-per-pee" payment (PAPPU) for prevention and detection using the platform sensor system as a low-cost surveillance tool for human and animal health.

Also, other signal transduction systems, for example electrochemical impedance spectroscopy (EIS) may be used in connection with mobile devices (ref 52). The mention of mobile devices (for example, smartphone) underscores the importance of making this tool a part of the *sensor-as-a-service* portfolio to serve markets of billions (MOB). The cost of the primary *at-home or anywhere* (AHA) detection service must not pose an insurmountable barrier. AHA may adopt micropayment tools, for example, pay-a-penny-per-use or PAPPU, a creative economic instrument (ref 49) to reduce transaction costs and implement pay-per-use tools to usher the pay-a-price-per-unit (PAPPU) paradigm.

The nexus of CITCOM, IoT and PAPPU could be catalytic. We need brilliance, uncompromising rigor in entrepreneurial invention and the credibility to coalesce the intellectual camaraderie, engineering innovation and novel creativity to unleash economic instruments which will connect billions to access the fruits of science in service of society. An end-to-end operational cohesion will be quintessential for the delivery of nanowire quantum dots or electrochemical impedance spectroscopy as a global ***service*** to better respond to the next epidemic or pandemic or public health crisis. The theoretical pillars of this idea are grounded but *will the orchestration and convergence generate the desired results?*

CAN CONVERGENCE CATALYZE THE CREATION OF SENSOR SYSTEM PLATFORM(S) TO SERVE THE MOB?

The proposal (previous page) is a monumental task of combining transdisciplinary principles with creative tools which may appear to be theoretically logical but unproven in terms of basic science or laboratory experiments. Can we engineer the system to attain the metrics based on key performance indicators? Basic and applied questions remain unanswered and need actual experiments to prove or disprove ideas and hypotheses related to this platform approach.

The encouragement for this host receptor approach is due to the fact that such a sensor system may act as a **"platform"** for a ***family of viruses*** (MERS, SARS and CoV2 are all in the Corona virus family) agnostic of the "bag of tricks" (mutations) to defy and/or evade the host system. Sensors using ACE2 as the binding target may identify MERS, SARS and SARS-CoV2-2019 viruses because the ***virus family*** (irrespective of the variants) ***still*** (in an evolutionary sense) uses ACE2 as the cellular receptor to enter human cells. Interference from glycan shields on viral proteins is expected.

The benefit of the "platform" approach of using host receptor protein, if successful, may be extended to deal with the dangerous family of Filoviruses (Filoviridae) which include Marburg and Ebola viruses. Lessons and techniques from creating ACE2 sensible sensor system can catalyze detection system for Filoviruses by replacing ACE2 with, for example, the human cellular receptor[110] targeted by Filoviruses, the endo/lysosomal cholesterol transporter protein Niemann-Pick C1 (NPC1).

Could we extend the platform approach to human Noroviruses[111] which also infect swine and cattle? HuNoVs are non-enveloped positive sense RNA viruses which infect[112] B cells, facilitated by commensal bacteria expressing an appropriate histo-blood group antigen (HBGAs). Human NoVs bind specific glycans expressed by commensal bacteria in the gut lumen. Viral entry is mediated by transcytosis of the virus-bacteria complex. HBGAs used by HuNoVs are also expressed on host (human) enterocytes and secreted into the gut lumen. It is possible the virus uses both host-derived and bacterial-expressed glycans to stimulate immune cell infection (B cells). In this case, the proposed detection platform sensor system could use HBGAs (with an array of bacterial-expressed glycans) as targets to bind with NoV VP1 or VLPs (NoV genogroups expressing major capsid protein VP1, which can self-assemble into virus like particles, VLPs). It is noteworthy that glycan-based[113] sensors in the context of influenza virus[114] are available and may be informative.

Such a sweeping platform strategy which harbors potential and promise is not without its perils, in terms of performance. So many things can go wrong. We must establish protein-protein *binding kinetics* between Spike (S1) protein RBD (receptor binding domain) and ACE2 or the extracellular enzymatic domain of ACE2 which binds to S1 RBD. A plethora of unknowns exist in the *process*[115] and *materials*[116] necessary to tether ACE2 to the sensor surface. It may not be feasible to attach ACE2 to a nanowire sensor because of the process necessary to generate nanowires may interfere with the stability of ACE2 or truncated versions. Use of ACE2 synthetic peptides[117] may be an alternative.

ACE immobilized on magnetic beads[118] and FLAG-tagged ACE[119] are reasons for optimism that ACE2 may be tethered to a sensor surface (with linker molecules) with favorable binding kinetics between S1 RBD and ACE2 or ACE2 peptides. Potential structure-function issues, conformational resonance/stability, secondary structure (protein, synthetic peptides), zwitterionic charges on linker molecules and unknown unknowns may influence binding (performance, signal to noise ratio) using nanowires[120] or other materials including the low-cost laser inscribed graphene turbostrat sensors.

It may be an ephemeral milestone if we can create a sensor with attached (link with histidine tag? lectins? PANHS?) ACE2 which binds to a target (S1 RBD) *in vitro*. Transducing and capturing the signal is the next phase where conjugation of the sensor with quantum dots[121] (Cadmium? Carbon?) is one proposed optical tool. Using LIG sensors and electrochemical impedance spectroscopy (EIS data as Bode Plots, phase shift) tools are another option.

Creating the nanowire quantum dot (**NQT**) is an unchartered domain. The optical signal is of great value as a simpler detection tool (compared to EIS) which could use smartphone cameras (or HoloLens tools). Perhaps we can create **m**agnetic beads linked with **q**uantum do**t**s (**MQT**) based on prior work with immobilized ACE2 (ref 117) and ACE (ref 118) on magnetic beads for ligand fishing. The strength of the optical signal is key and is a ***key unknown***. Can we ***capture*** the ***net*** optical change (signal above noise) using a mobile device (smartphone camera or HoloLens tools)?

If we can resolve all of the above, only then, we may claim a potential prototype of a platform sensor system. It will be accessible to billions through *convergence* with mobile data using the information platform of the smartphone. Unless it is accessible for the poor, it is neither radical nor revolutionary or smart.

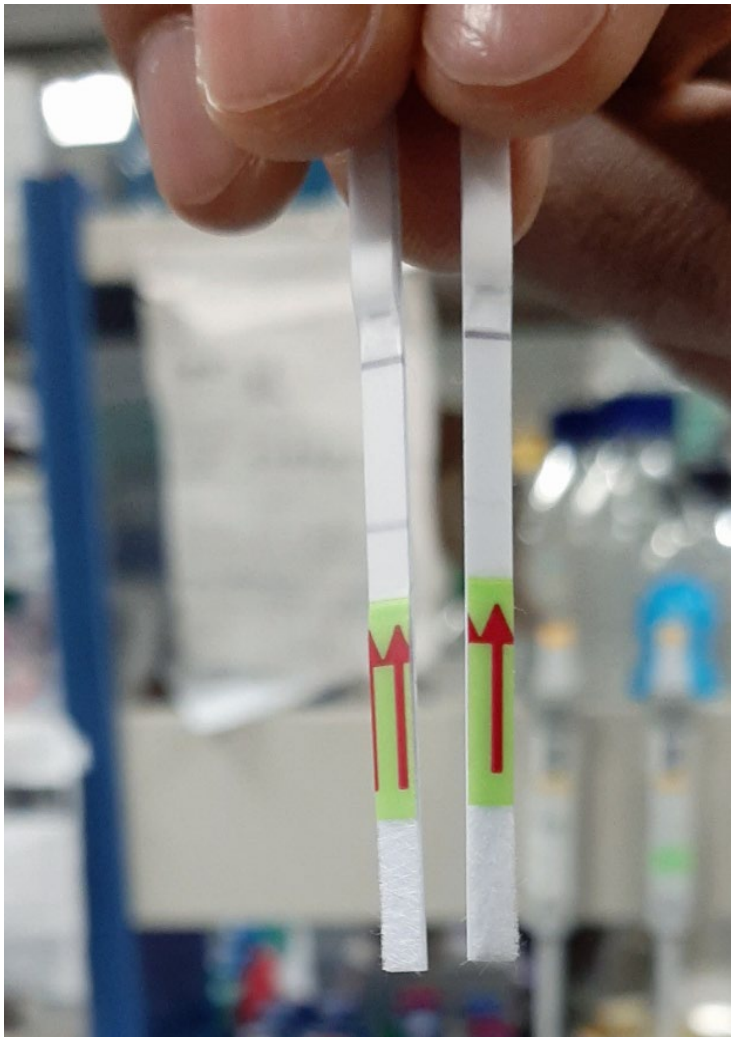


Figure 7: The low-cost (INR500, USD7) paper-strip test for SARS-CoV2 developed by scientists in India (reference 100).

TESTING ANIMALS (FOOD SUPPLY CHAIN) FOR VIRAL INFECTION: BROAD SPECTRUM OF PHYLOGENETIC CONSERVATION

Lessons[122] from LRP6[123] (LDL Receptor Related Protein 6[124]) and CCR5[125] (chemokine receptor type 5 or CD195) resonate with the evolutionary conservation of angiotensin converting enzymes[126] gene in subkingdom Eumetazoa (Figure 8). The presence of ACE/ACE2 gene in almost all organisms in the vast subkingdom Eumetazoa is significant but the architecture of the protein(s), structure and function may vary. In humans, the angiotensin-converting enzyme[127] (ACE2) family of dipeptidyl carboxydipeptidases also exists as an ectoenzyme on endothelial cells and has homology to human angiotensin 1 converting enzyme (ACE). The secreted protein cleaves angiotensin I into angiotensin 1-9, and angiotensin II into the vasodilator angiotensin 1-7. The organ- and cell-specific expression of this gene in humans suggest a role in the regulation of cardiovascular and renal function, as well as fertility. The presence of 1,308 ACE/ACE2 genes from arthropods to humans indicates an age-old structure-function role for this *ancient* gene (ACE/ACE2).

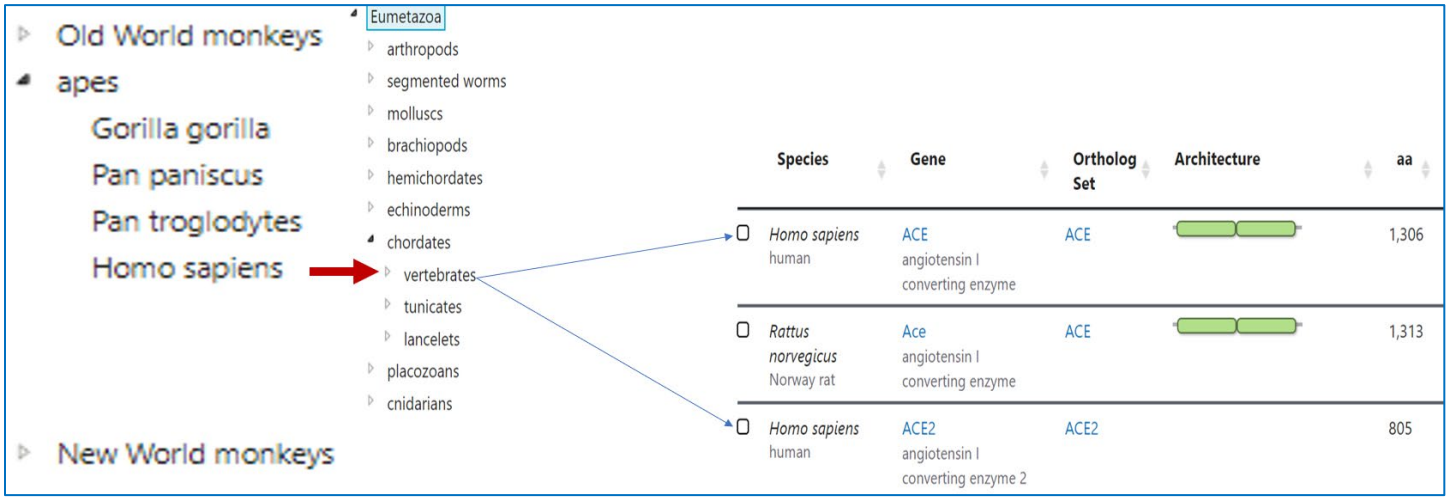


Figure 8: ACE gene and its ortholog (ACE2 gene) – 1,308 genes[128] distributed over the entire sub-kingdom Eumetazoa.

For this discussion, we focus on the fact that the ***encoded ACE2 protein still serves as a receptor for the spike (S) glycoprotein of corona virus family*** (including hCoV-NL63 and human severe acute respiratory syndrome corona viruses, SARS-CoV and SARS-CoV-2). This is the ***basis*** of our broad-spectrum approach to expand our testing to include animal body fluids (saliva) to reliably detect infection by virus in the asymptomatic state using the 'platform' sensor system tethered to the ACE2 protein as the cross-species detector. In this protein-based approach (in contrast to nucleic acid and antibody-based detection) we may detect ***any strain of infecting corona virus which has the S protein.***

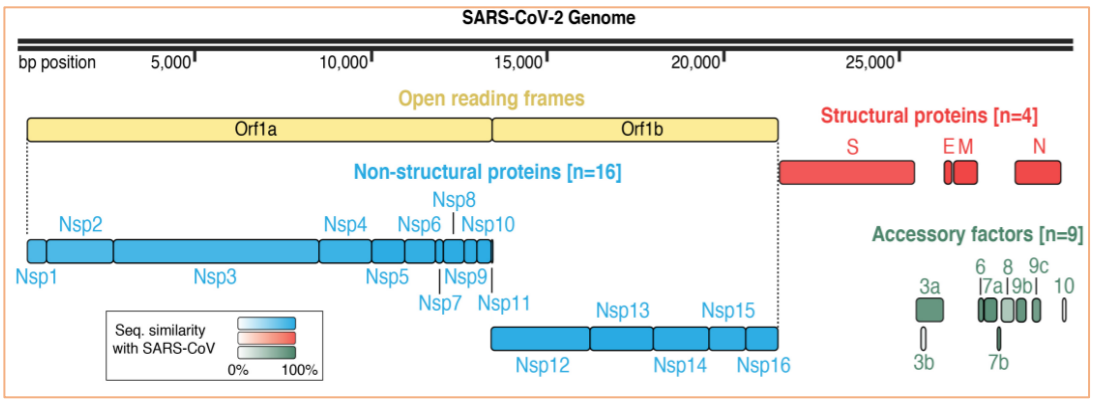


Figure 9: SARS-CoV-2 genome[129] is similar to SARS-CoV but 3' Orfs in SARS-CoV-2 possesses Orf3b and Orf10 and Orf8 is intact (but less virulent SARS-CoV encodes Orf8a and Orf8b).

The core assumptions are [a] Corona virus strains (emerging/mutating) still use the Spike (S1) glycoprotein (***oblivious of new mutations in S1***) to target the ACE2 protein as the host receptor for entry into human cells [b] ACE2 genes and proteins are conserved in the animals (to be included for testing) when compared to humans in terms of structure and function with respect to cellular entry by the virus. Figure 10 suggests that assumption [a] is not without merit because both SARS-CoV and SARS-CoV-2 uses ACE2 as the host receptor despite (mutations) changes in the primary structure of the S proteins between the two strains of the corona virus.

Figure 10: Primary sequence alignment of severe acute respiratory syndrome coronavirus (SARS-CoV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike proteins, with conserved amino acid residues shown in black and non-conserved residues are shown in color. The receptor binding domain (RBD) is a 193 amino acid region (aa 318–510) in the SARS-CoV S protein, which can independently bind to the host ACE2 cellular receptor.[130] The 33 amino acids in the region 460–492 in the SARS-CoV Spike protein RBD (rows 3 and 4, boxed region) that contains the critical residues that contact ACE2, less than half (15/33) are conserved in SARS-CoV-2.

Assumption [b] is supported by the observation that Spike proteins derived from human SARS-CoV or SARS-CoV-like viruses of masked palm civets (pc) and raccoon dogs[131] (rd) were tested for their ***entry efficiency*** into human cell lines. The results (ref 131) reveal rdACE2 is a more efficient receptor for Spike proteins derived from human SARS-CoV, an observation that indicates that the S protein from the ***family*** of corona viruses recognizes (binds to) ACE2 as a cellular receptor for entry into humans as well as raccoon dogs (*Nyctereutes procyonoides*) in the order Carnivora.

It is interesting to note that while rdACE2 is a more efficient receptor for Spike proteins derived from human SARS-CoV, rdACE2 is not ***as efficient*** a receptor for Spike proteins derived from SARS-CoV-like viruses of masked palm civets (pc) and raccoon dogs (rd). It is equally informative to observe that compared to human ACE2 and pcACE2, the rdACE2 is a more efficient receptor for Spike proteins derived from human SARS-CoV. The fact that rdACE2 is more efficient receptor for Spike proteins derived from human SARS-CoV but less efficient for Spike proteins derived from rdSARS-CoV suggests differences in protein-protein interactions and perhaps potential variation in binding constants introduced during the evolutionary journey (Figure 11) of the ACE2 gene from racoon dogs to humans. The gene for the Spike protein of the corona virus family also mutated and evolved over millions of years but it is not surprising that the "clock speed" or rate of evolutionary genomic change between the ACE2 and Spike gene are quite different, generating evolutionary advantages or "fit" (efficiency) but not synchronized, naturally, due to significant biological differences between viral versus animal evolution. Over this time period, the ACE2 protein appears to be well conserved (Fig 12).

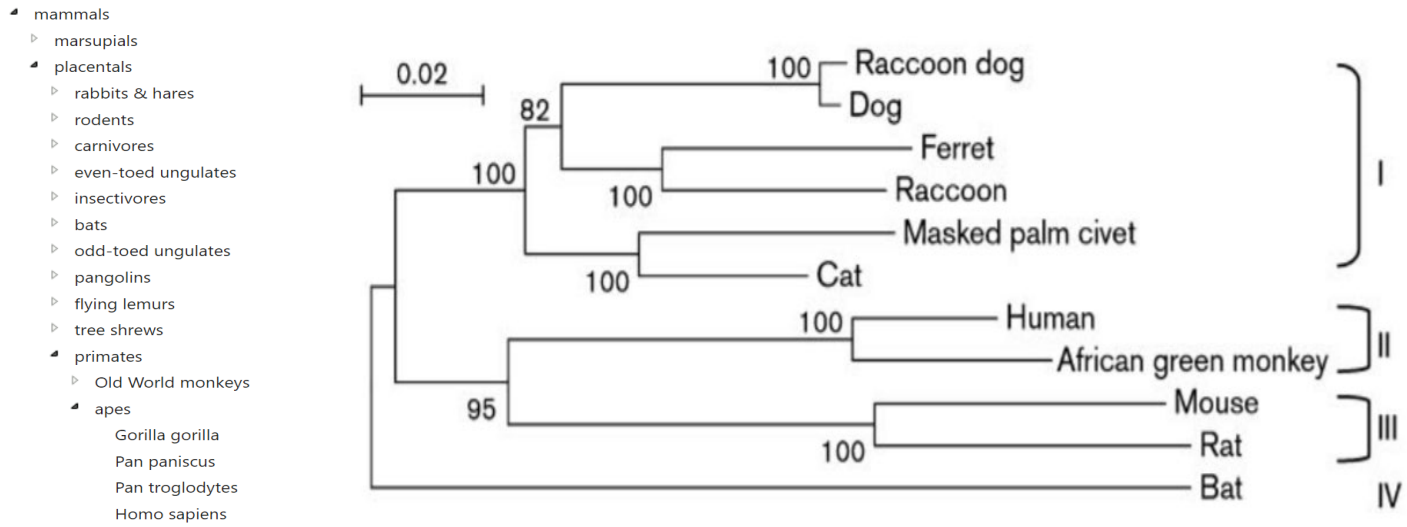
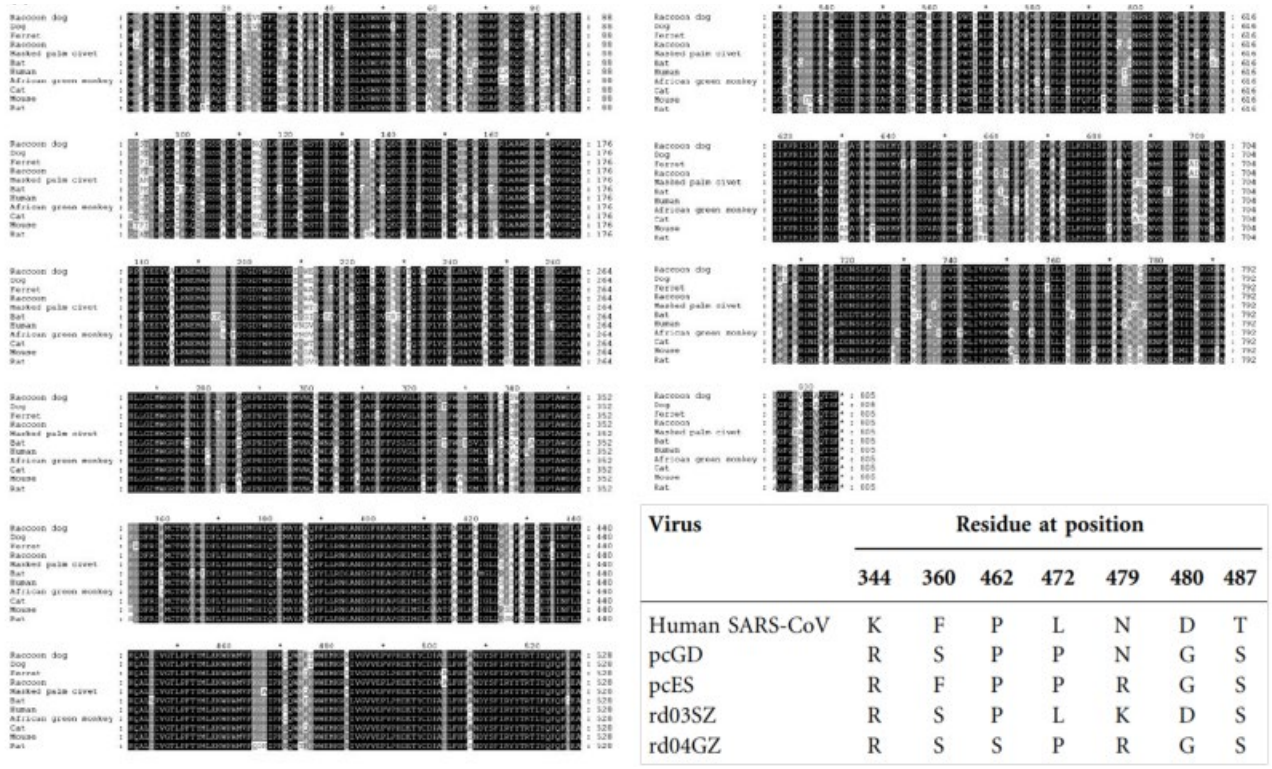


Figure 11: The evolutionary journey of the ACE2 gene from racoon dogs (ref 131), which are closer to dogs (domestic dogs evolved approximately 6 million years ago[132]) to humans (0.2 million years ago, MYA). Over this time period (6MYA – 0.2MYA) the ACE2 protein appears to be well conserved (see Figure 12).



Virus	Residue at position						
	344	360	462	472	479	480	487
Human SARS-CoV	K	F	P	L	N	D	T
pcGD	R	S	P	P	N	G	S
pcES	R	F	P	P	R	G	S
rd03SZ	R	S	P	L	K	D	S
rd04GZ	R	S	S	P	R	G	S

Figure 12: Comparative protein sequence analysis reveals differential genomic evolution between ACE2 protein and viral Spike protein (ref 131). The rdACE2 gene encodes an 804 amino acid protein, which is identical in size to the ACE2 protein of dog, but one aa shorter than those of human (805 aa) and palm civet. Sequence alignment (black indicates identical amino acids): rdACE2 protein is highly conserved in evolution and has an amino acid sequence identity of >80% between cat, raccoon, palm civet (pc), human, mouse, rat, ferret and bat. ACE2 from raccoon dog (rd) is almost identical (>99%) to dog ACE2, with only six amino acid which are different (residues 26, 52, 59, 326, 340, 353). Box on the bottom right shows the amino acid differences in the RBDs of different viral Spike proteins (S glycoproteins).

ACE2 protein	24*	27*	30	31*	32	33	34*	35	36	37*	38*	39	40	41*	42*	45*	79*	82*	83*	90*	91	92	93	325*	329*	330*	353*	354*	355	356	357
Raccoon dog	L	T	E	K	F	N	**Y**	E	A	E	E	L	S	Y	Q	L	L	T	Y	D	**S**	T	**V**	Q	E	N	**R**	G	D	F	R
Dog	L	T	E	K	F	N	**Y**	E	A	E	E	L	S	Y	Q	L	L	T	Y	D	**S**	T	**V**	Q	E	N	K	G	D	F	R
Ferret	L	T	E	K	F	N	**Y**	E	A	E	E	L	S	Y	Q	L	**H**	T	Y	D	P	**I**	I	**E**	**Q**	N	K	**R**	D	F	R
Raccoon	L	T	E	**N**	F	N	**N**	E	**T**	E	E	L	S	Y	Q	L	**Q**	T	Y	D	P	T	**N**	Q	E	N	K	G	D	F	R
Palm civet	L	T	E	**T**	F	N	**Y**	E	A	**Q**	E	L	S	Y	Q	**V**	L	T	Y	D	**A**	**K**	I	Q	E	N	K	G	D	F	R
Bat	L	T	E	K	F	N	**T**	E	A	E	**D**	L	**F**	Y	Q	L	L	T	Y	D	P	**E**	**L**	**E**	E	**K**	K	G	D	F	R
Human	**Q**	T	**D**	K	F	N	**H**	E	A	E	**D**	L	**F**	Y	Q	L	L	**M**	Y	**N**	**L**	T	**V**	Q	E	N	K	G	D	F	R
Mouse	**N**	T	**N**	**N**	F	N	**Q**	E	A	E	**D**	L	S	Y	Q	L	**T**	**S**	**F**	**T**	P	**I**	I	Q	**A**	N	**H**	G	D	F	R
Rat	**K**	**S**	**N**	K	F	N	**Q**	E	A	E	**D**	L	S	Y	Q	L	**I**	**N**	**F**	D	**A**	T	I	**P**	**T**	N	**H**	G	D	F	R
Human SARS-CoV S	N473	Y475		Y475, Y442			Y440, N479			Y491	Y436			Y484, T486, T487	Y436, Y484	Y484	L472	L472	N473, Y475	T402				R426	R426	T486	G488, T487, Y491	Y491, G488			

Figure 13: Contacts between ACE2 and SARS-CoV RBD (receptor binding domain or RBM, receptor binding motif). ACE2 residues in contact with S1 RBD are listed by their position and aa. Non-identical amino acid residues are shown in bold. The residues in the viral S protein from human isolates that contact ACE2 are shown at the bottom of each column. Columns with a star (*) denote 18 residues of ACE2 known to make direct contact with the SARS-CoV Spike protein. A loop and β5 (residues 353–357) had 1 variation in rdACE2 (K353R) and Histidine in mouse and rat ACE2 proteins (K353H). This position is critical for ACE2 binding to SARS-CoV Spike protein (ref 131). Differences in the RBDs of SARS-CoV-2 and SARS-CoV are expected (see Figure 10). In particular, of the 33 amino acids in the region 460–492 in the SARS-CoV S protein that contains the critical residues that contact[133] ACE2, less than half (15/33) are conserved in SARS-CoV-2 (ref 131), which is one reason why cross-neutralizing antibodies may not be as effective.

The ACE2 protein comparative analysis (Figure 13) indicates a non-neutral change (rdACE2 K353R) at the highly conserved region, residue 353, from lysine (K) to arginine (R), in raccoon dogs. Results (ref 131) indicate rdACE2 is a more efficient receptor for Spike proteins derived from human SARS-CoV. Although speculative, replacing lysine with arginine may enable more/stronger salt bridges and the guanidino group of arginine may induce better steric fit as well as the opportunity for hydrogen bonds. Table in Figure 12 indicates that position 487 for the human viral S protein (which makes contact with ACE2 position 353, see Figure 13) is a threonine (T487) but viral S protein from palm civet and raccoon dog contains serine (S487, see table in Figure 12). The threonine to serine change in position 487 may have conformational implications for protein-protein interactions due to evolutionary rate of change[134] in phosphorylation. The convergence or combination of the changes – rdACE2 to arginine (residue 353) and human viral S protein RBD to threonine (residue 487) – may have contributed to the improved efficiency of viral entry in human cell lines (ref 131).

The discussion of the molecular characteristics in the raccoon dog ACE2 protein interaction is particularly significant because it provides a context with respect to cross-species interaction. Raccoon dog (rd), native to East Asia, is one of the suspected intermediate hosts of severe acute respiratory syndrome coronavirus (SARS-CoV). In China, raccoon dogs are skinned alive. Video[135] shows workers cutting the skin and fur from an animal's leg while the free limbs are kicking and writhing. When the fur is finally peeled off over the animals' heads, the bloody bodies are discarded. This fact and the data (ref 131) suggests a potential role of raccoon dog in the SARS outbreak and as a potential contributor to the CoVID-19 pandemic. It is a cautionary tale why vigilance in monitoring animals is necessary and prudent for the global economy to preserve the networks that supply nutrition to far corners of the world.

The one health[136] approach to population public health and precision public health must embrace animal health both for meat industry and the role of animals as pets. The implications for the global meat markets in supporting the food supply chains cannot be overstated. Constructing a ***platform sensor system*** using an evolutionary conserved protein as a primary detector (ACE2) is a strategic scientific perspective. The latter is essential to inform engineers to create tools and technologies which may be necessary to mitigate future risks and meet the emerging challenges in food safety.

	Species	GenBank accession No.	Homology (%)
Wild Boar	Sus scrofa	NM_001123070.1	99.5
Domestic Pig	Sus scrofa domestica	GQ262781.1	99.1
Cow	Bos taurus	NM_001024502.4	88.8
Cat	Felis catus	NM_001039456.1	87.4
Dog	Canis lupus familiaris	NM_001165260.1	86.6
Rat	Rattus norvegicus	NM_001012006.1	82.0
Rhesus Monkey	Macaca mulatta	FJ170098.1	84.8
Goat	Capra hircus	NM_001290107.1	89.2
Zebrafish	Danio rerio	NM_001007297.1	49.7
Humans	Homo sapiens	NM_021804.2	84.9

Figure 14: Comparative Genomic Analysis of the ACE2 gene (from pigs[137]): Nucleotide sequence homology between ACE2 from different animals in the usual meat supply chain (pig, cow, goat) as well as pets (cats and dogs). Homologs of ACE/ACE2 are extensively distributed in animals, including vertebrates (chimpanzee, cow, rabbit, mouse, **chicken**, goldfish and electric eel) and invertebrates (house fly, mosquito, horn fly, silkworm, fruit fly, *Caenorhabditis elegans* and bacteria). The 1,308 genes in the subkingdom Eumetazoa indicates a very early origin[138] in the biological world.

The evolutionary preservation of virus and host in terms of molecular mechanisms of infection is the primary reason for the pursuit of a platform strategy using the host protein as target. Use of neutralizing antibodies[139] (nAbs) as immobile targets on sensors are attractive for detection of specific virus strains. But it is clear from Figures 9 through 13 that viral mutations may alter the molecular nature of nAbs, rendering it less efficient (variation in titre) or useless with emerging viral strains. Use of nAbs across species may be difficult to justify in a platform approach because SARS-CoV-2 antibody is specific for the current pandemic but may be less useful for other Corona virus family members using the evolutionary conserved ACE2 receptor across the animal kingdom. In SARS-CoV, the primary target of neutralizing antibodies is the RBD122, a 193 amino acid region (amino acids 318–510) in the viral S protein, which can bind to the host target ACE2 receptor. Most monoclonal antibodies to SARS-CoV do not bind[140] or neutralize SARS-CoV-2 due to significant differences in the RBDs of SARS-CoV-2 and SARS-CoV shown in Figure 10 (in particular, the 33 amino acids in the region 460–492) and see Figure 16 (magenta spheres). Nevertheless, mouse antiserum raised against SARS-CoV protein can cross-neutralize SARS-CoV-2 pseudo virus, indicating overlapping neutralizing epitopes[141] between the two viruses (ref 131). Shared or overlapping epitopes seed doubt in terms of specificity.

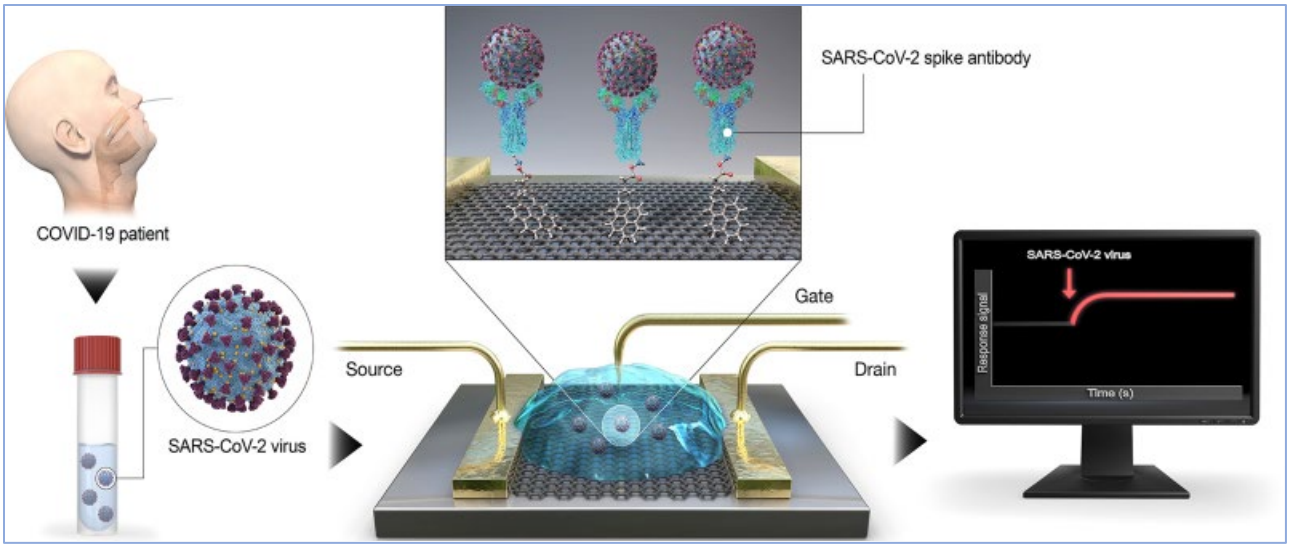


Figure 15: Field-effect transistor (FET)-based biosensor[142] uses SARS-CoV-2 Spike protein antibody conjugated to graphene *via* 1-pyrenebutyric acid *N*-hydroxysuccinimide ester (PANHS[143]), as a linker. FET gate circuit board needed to measure the difference in current flow (free vs bound antibody) may be relatively expensive (ADC, microcontroller). Data visualization (results) on a smartphone may increase the diffusion of the tool for mass consumption at a low cost.

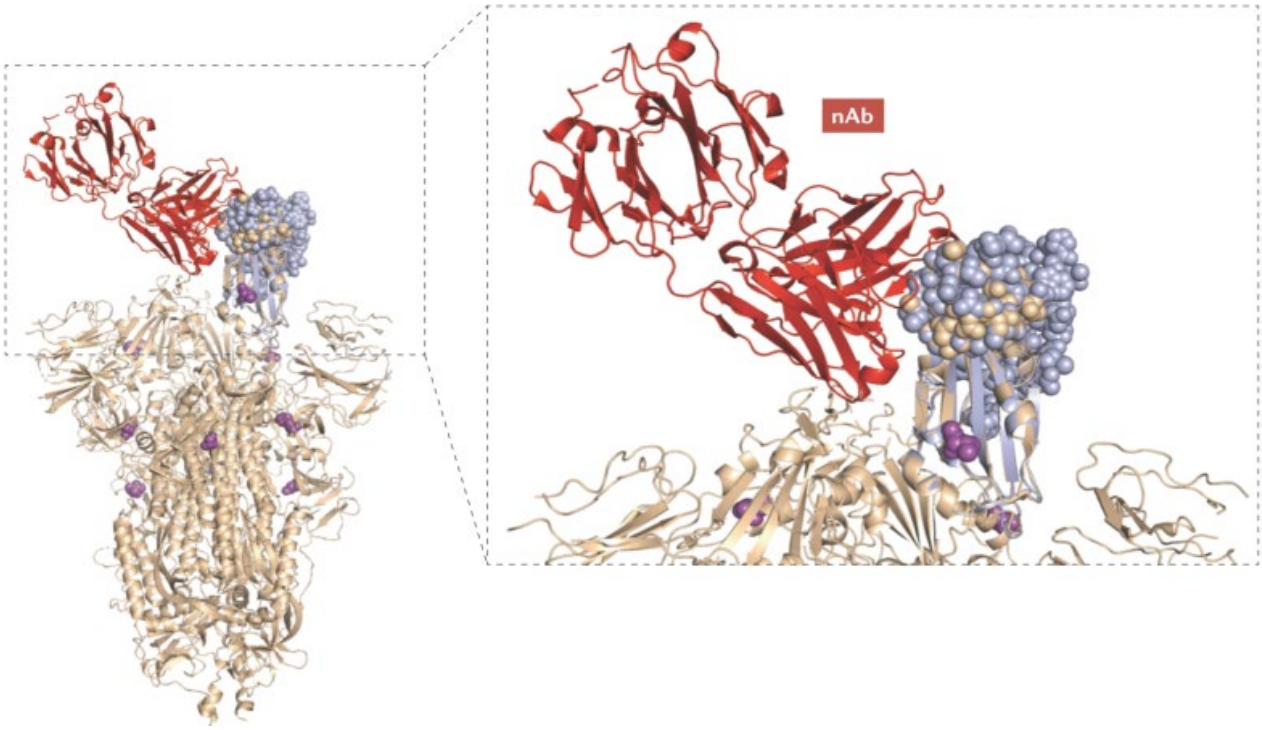


Figure 16: The 3D structure (ref 130) of SARS-CoV-2 (Protein Data Bank ID 6VSB42, peach ribbon) is superimposed on SARS-CoV receptor-binding motif (RBD) complex with the neutralizing antibody (nAb; red ribbon) interfacing with the RBD (Protein Data Bank 2DD8, purple ribbon). Peach and purple spheres denote the RBDs of SARS-CoV-2 and SARS-CoV, respectively. Magenta spheres are **non-synonymous** alterations in the SARS-CoV-2 Spike protein. The latter may modify binding constant (titre) between nAbs and viral Spike proteins, decreasing the value of nAbs as target molecules for a platform approach in creating a sensor system to detect virus families infecting animals and humans.

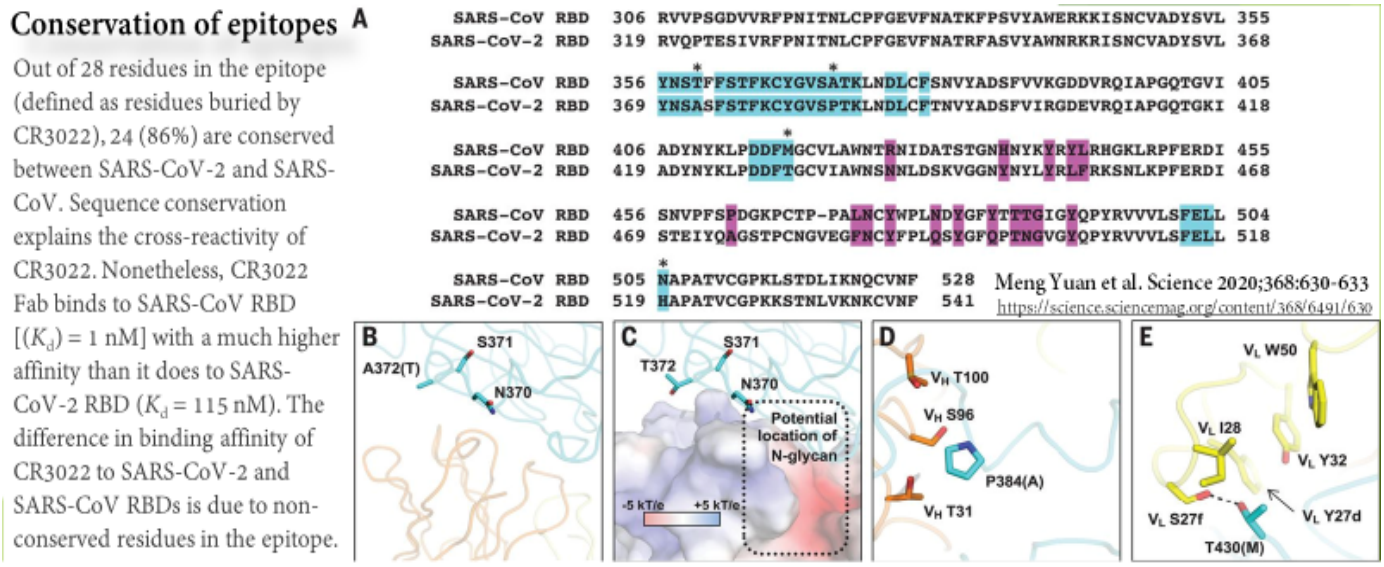


Figure 17: The use of antibodies (nAbs) as detectors are excellent for specific strains of a virus but may not perform adequately in a platform sensor system. Accumulating evidence[144] indicates significant disparity between dissociation constants {CR3022 Fab binds to SARS-CoV RBD [dissociation constant (K_d) = 1 nM] with a much higher affinity than it does to SARS-CoV-2 RBD (K_d = 115 nM)} likely due to non-conserved epitopes and post-translational modifications (N-glycosylation site at amino acid residue 370 on SARS-CoV). Taken together with the change in human SARS-CoV at amino acid residue 487 (see Figure 12) from Serine to Threonine, the data suggests a potential regulatory role for post-translational modifications as a mechanism to modify binding constants in protein-protein interactions. Recombinant proteins produced in bacteria (E. coli) and yeast (S. cerevisiae) may lack necessary post-translation modifications and behave erroneously in *in vitro* assays for protein-protein interactions. The use of insect (Baculovirus) or mammalian cell lines may be helpful but it remains to be determined if the proteins will be post-translationally modified in the absence of specific environments or factors (such as, viral infection *in vivo*).

ALL ADVANTAGES ARE TEMPORARY BUT THE IMPACT MAY BE LONG TERM

The rationale to create an ACE2 sensor for animals and humans using the same target (ACE2) appears to have sufficient phylogenetic, evolutionary and molecular justification. The bridge from science to engineering is under construction. To bring to market fool-proof technology as "AHA" service is vital for surveillance[145]. The ability to detect and perhaps to prevent infections in animals and humans must start with surveillance using a platform strategy or some thematic variation which can detect biological (viral) activity in hot spots. It is foolhardy to sequence all usual suspects. Reinventing this wheel, each time we have a public health crisis, is wasteful. It is devastating if inaction allows the crisis to affect food, water and sanitation networks and disrupt global supply chains. The urgency to explore this or any other cross-species sensor platform strategy for surveillance is due to risk from an estimated 1.67 million[146] unidentified viruses which may infect humans and exist in mammals and water birds. In addition to the human tragedy due to Ebola and other viruses, yet unknown virus strains may emerge from any of the 1.67 million viruses to be more disruptive and lethal compared to SARS-CoV-2. But, sequencing 1.67 million viruses is ***not conclusive for prediction.*** Genomics is a confirmatory tool when surveillance reveals potential "hot spots" that must be tested for viral activity. Even rudimentary programs to deal with this gargantuan multifactorial challenge may cost billions of dollars annually but the cost of a pandemic every century may be trillions[147]. In comparison, surveillance may provide an incredibly good return on investment (ROI) as long as we ***deploy surveillance first***, before we start sequencing all viruses.

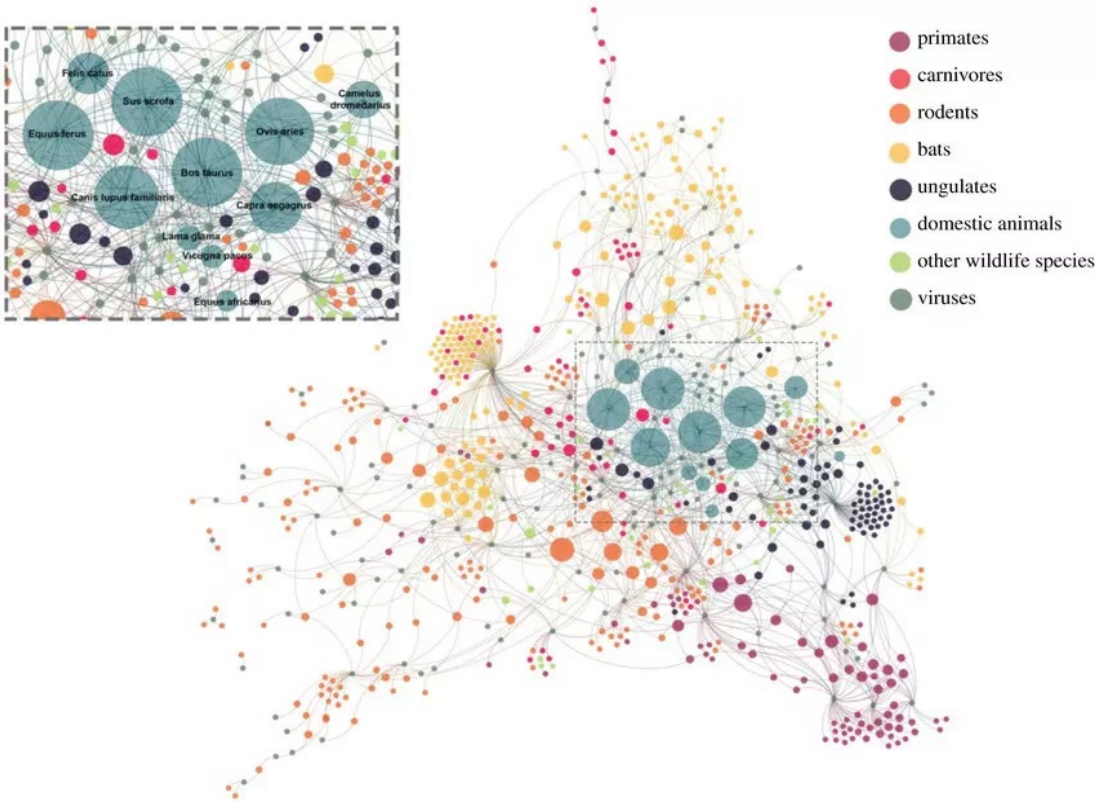


Figure 18: Bipartite network showing wild and domesticated mammalian species and their zoonotic virus associations. Host species harboring the same zoonotic virus are linked by shared zoonotic viruses (grey nodes). Mammalian species nodes are colored by domestication status and taxonomic order for non-domesticated terrestrial wildlife. Species node size is relative to the zoonotic virus richness calculated in that species. Humans are host to all viruses, but not shown.[148]

DETECTION IS NOT ENOUGH: PEPTIDES ON THE VACCINE PLATFORM?

The fundamental scientific step is to reproducibly and quantitatively validate the strength of the *in vitro* protein-protein interaction between the viral S1 RBD and an extra-cellular N-terminal segment of ACE2 (see Figure 19). The degree of success in this step could pave a parallel path but in a different direction. Can we test in human cell culture (*in vitro*) whether the target protein (an extra-cellular N-terminal segment of ACE2, see Figure 19) can neutralize or block or reduce the entry of CoV? In the *in vitro* cell culture scenario, the target protein (ACE2 derived peptide) is expected to bind to the viral S protein and may prevent CoV from binding to the cell surface ACE2. In other words, the target protein (extra-cellular N-terminal segment of ACE2) behaves as if it is a neutralizing antibody. Synthetic (see ref 33) versions[149] of ACE2-based peptides in Figure 19 may be useful. If cells can survive the *in vitro* challenge and if creating point mutations in the target protein (extra-cellular N-terminal segment of ACE2) can eliminate the "neutralizing" ability of the target peptide/protein then the experiment may move *in vivo* for testing in animal models. Usual barriers may include degree of bioavailability, toxicity and ligand sequestration (physiologically relevant ligands may bind to the extra-cellular N-terminal peptide sequence of ACE2 but may not deliver the expected physiological outcome). Creative delivery tools may be necessary in addition to existing protocols, for example, using micelles as delivery vehicle via nasal[150] sprays. Suggestions here are nothing new but rational thought, albeit limited by knowledge and imagination. Can intravenous or subcutaneous administration of peptide(s) influence the binding between SARS-CoV-2 and ACE2 localized on the apical plasma membrane of respiratory epithelial cells in humans? Is this a difficult strategy to mimic?

Inhibitor 1	residue 21 to 55 **I EEQA KTFLD KFNHE AEDLF YQSSL ASWNY NTNIT**
Inhibitor 2	residues 21 to 88 and 349 to 357 **(1) I EEQA KTFLD KFNHE AEDLF YQSSL ASWNY NTNIT EENVQ NMNNA GDKWS AFLKE QSTLA QMYPL QEI** **(2) WD LGKGD FR**
Inhibitor 3	residues 21 to 105 and 323 to 362 **(1) I EEQA KTFLD KFNHE AEDLF YQSSL ASWNY NTNIT EENVQ NMNNA GDKWS AFLKE QSTLA QMYPL QEIQA LTVKL QLQAL QQNGS** **(2) MTQ GFWEN SMLTD PGNVQ KAVCH PTAWD LGKGD FRILM CT**
Inhibitor 4	residues 21 to 95 and 335 to 400 **(1) I EEQA KTFLD KFNHE AEDLF YQSSL ASWNY NTNIT EENVQ NMNNA GDKWS AFLKE QSTLA QMYPL QEIQA LTVKL** **(2) D PGNVQ KAVCH PTAWD LGKGD FRILM CTKVT MDDFL TAHHE MGHIQ YDMAY AAQPF LLRNG ANEGF**

Figure 19: ACE2-based peptide inhibitors of SARS-CoV2 may be used as targets for sensors as well as (?) molecules to prevent virus from binding to cell surface (eg: lung epithelial cells[151] *in vitro*). The 35 amino acid inhibitor 1 (1st row) extends the 23-residue amino acid (ACE2 α1 helix sequence) peptide IEEQAKTFLDKFNHEAEDLFYQS (ref 33). Comparing inhibitors 1 and 3 (ref 117) suggests essential points of contact (included in inhibitor 2) may need a β-pleated sheet (β5) and the loop region for conformational stability – which is the inhibitor #3. Data on average interaction energies (ref 117) suggests that choosing inhibitor #1 versus inhibitor #3 may not make a dramatic difference (unless results indicate otherwise). The shorter length of the peptide sequence of inhibitor #1 (35 amino acids) may be better in terms of its use in an ACE2-dependent platform sensor system as the detector target.

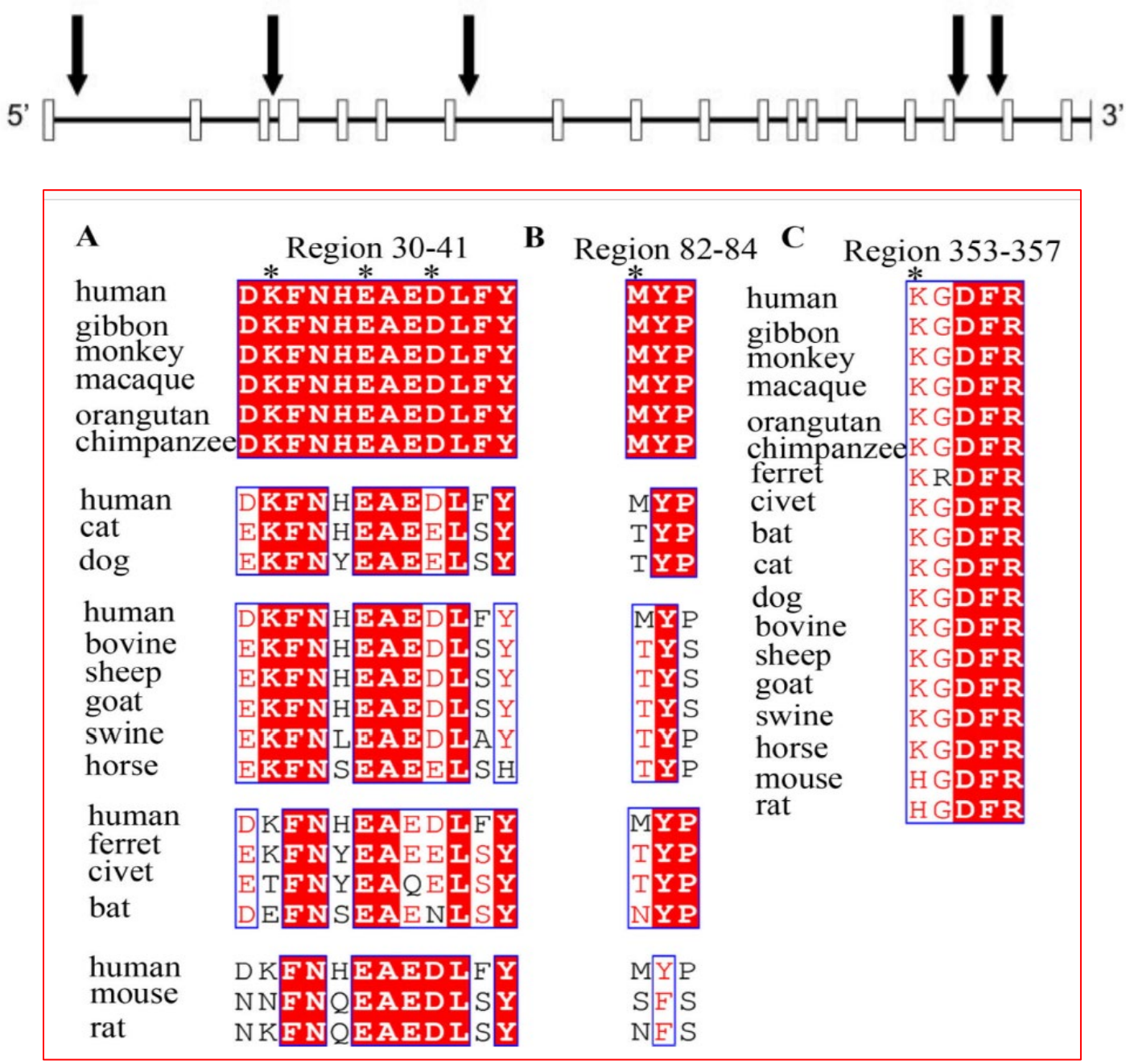


Figure 20: (TOP) Genomic organization of ACE2 (arrows mark SNP, single nucleotide polymorphisms, ref 215). hACE2 contains 18 exons and codes for 805 amino acid protein. ACE2 is a homolog of ACE (may be a duplication of the ACE gene and then fused with another gene). (BOTTOM PANEL) Compare ACE2 based peptides (Figure 19) with sequence alignment of ACE2 from human[152] (UniProt entry Q9BYF1) vs Northern white-cheeked gibbon (UniProt G1RE79), green monkey (UniProt A0A0D9RQZ0), crab-eating macaque (UniProt A0A2K5X283), Sumatran orangutan (UniProt Q5RFN1), chimpanzee (UniProt A0A2J8KU96), cat (UniProt Q56H28), dog (UniProt J9P7Y2), bovine (UniProt Q58DD0), sheep (UniProt W5PSB6), goat (UniProt A0A452EVJ5), swine (UniProt K7GLM4), horse (UniProt F6V9L3), ferret (UniProt Q2WG88), civet (UniProt Q56NL1), Chinese horseshoe bat (UniProt E2DHI7), mouse (UniProt Q8R0I0) and rat (UniProt Q5EGZ1). (A) Region 30–41. (B) Region 82–84. (C) Region 353–357. The conserved residues in the regions are colored in red and the critical residues are marked by asterisks.

UNCONVENTIONAL CONSIDERATIONS FOR THE VACCINE PLATFORM?

The departure from conventional wisdom in enabling non-canonical concepts to ferment on the vaccine platform[153] may be essential to address the potential for infectivity that lurks within millions of viruses. Of particular interest is bidirectional cross-species[154] and cross-kingdom RNA interference[155] tools. Pathogens and pests deliver small RNAs (sRNAs) into host cells to modify host immunity and hosts transfer sRNAs into pathogens and pests to modify their virulence[156] in host-induced gene silencing (HIGS) that includes plants, insects, worms[157] and humans.

Wolbachia pipientis, an intracellular endosymbiont bacteria confers host resistance[158] against RNA viruses in insects. In mosquitoes[159] the presence of *Wolbachia* can inhibit the transmission of certain viruses, such as Dengue, Chikungunya, Yellow Fever, West Nile, as well as the infectivity of the malaria-causing protozoan, *Plasmodium* and filarial nematodes which causes filariasis[160] in humans. Fecal microRNA (miRNA) present in extracellular vesicles (EV) mediate inter-species gene regulation[161] by entering *Fusobacterium nucleatum* (an oral bacterium, indigenous to the human oral cavity, that plays a role in periodontal disease*)* and *Escherichia coli*, specifically to regulate bacterial gene transcripts, as a potential strategy for manipulating the human microbiome. Indeed, small non-coding clustered, regularly interspaced short palindromic repeat (CRISPR) RNAs (crRNAs) have gained great attention as a tool[162] for targeted genome editing[163] and crRNAs are almost ubiquitous as tools for cross-species immune regulation. However, CRISPR tools for adaptive immunity are not invincible. Anti-CRISPR genes[164] which can resist bacteriophage infection were identified in *Pseudomonas aeruginosa* and may be useful as "off-switches" for CRISPR gene editing.

Pseudomonas aeruginosa, a gram-negative opportunistic pathogen that primarily infects immunocompromised hosts, uses outer membrane vesicles[165] (OMVs) to transfer sRNAs to human airway epithelial cells[166] (*in vitro*) with the intent to target host mRNA function and/or stability which may reduce host immune response. It is this particular result which begs the question if ***engineered RNAi as a "vaccine"*** is an option. Is it possible to induce a medically controlled *Pseudomonas aeruginosa* infection which could *effectively* deliver OMVs containing engineered RNAi targeted to inhibit viral (any virus) reproduction (RNA rt, transcription, translation) in human airway epithelial cells, preferred by CoV?

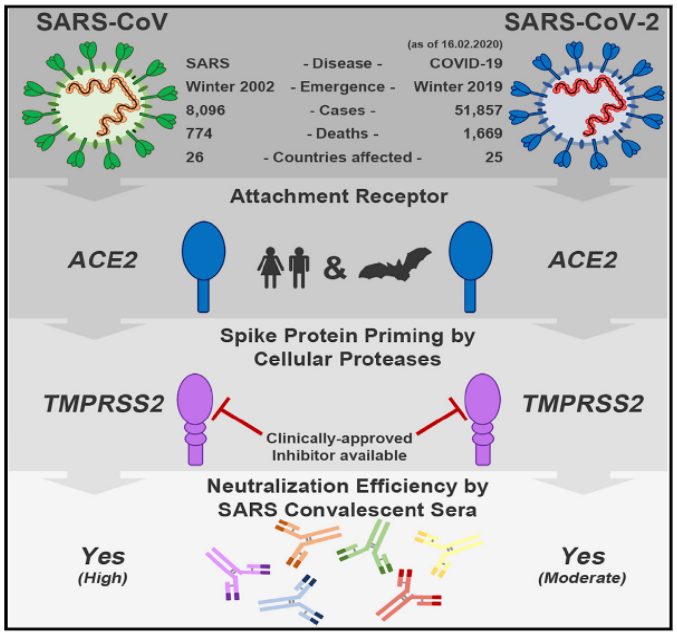
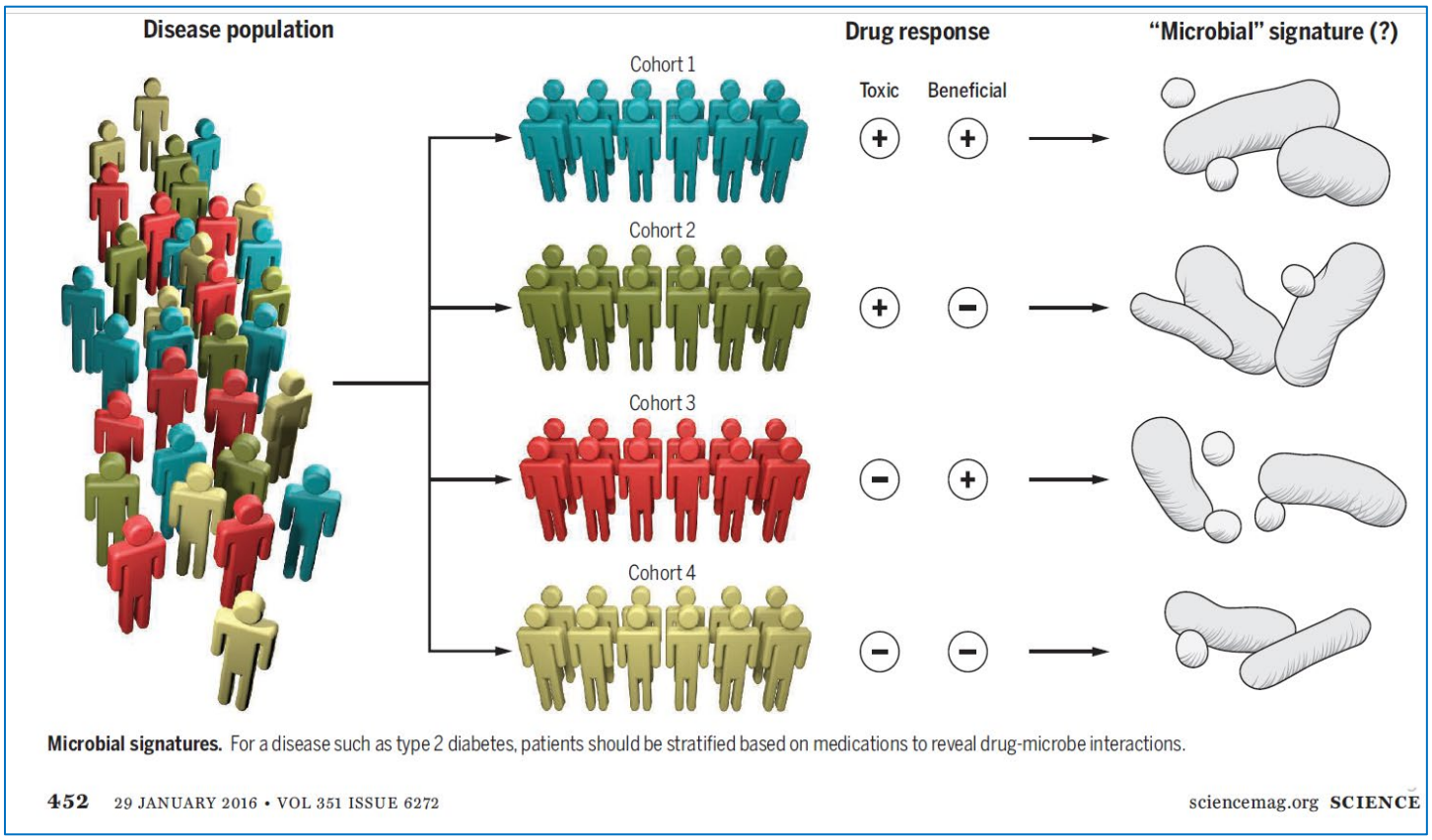


Figure 21: Co-factors may be catalytic for viral entry and TMPRSS2, a known[167] serine protease, may be involved in priming[168] the SARS-CoV family of S (spike) proteins. The amino acid differences in the RBDs of different viral S (Spike) proteins (see Table in Figure 12) reveal that residue 487 is Threonine in Human SARS-CoV but Serine (487) in palm civets (pc) and racoon dogs (rd). In Human SARS-CoV-2 residue 487 is Asparagine (see cryo-EM structure in Figure 2 D). Hence, an interesting question about the substrate and the role of the serine protease TMPRSS2 for S (Spike) protein priming in human SARS-CoV and SARS-CoV-2. It appears to be a target for anti-viral intervention due to inhibition of viral entry when host TMPRSS2 was inhibited by the serine protease inhibitor camostat mesylate.[169]

The non-linearity[170] in COVID-19 infection with respect to age[171], gender (ACE2 gene is on the X chromosome), variable gene expression and pre-existing levels of inflammation (immune status) may also encounter challenges due to the potential for interaction between the virus, pre-existing co-morbidities and the host microbiome. Perhaps, lessons for humans and animals may be extracted (cryptic?) from Jin *et al* (ref 155) on cross-kingdom and cross-species immunity which is a strategy used for protection of food crops. Examples from amyotrophic lateral sclerosis[172] (ALS), type 2 diabetes[173] and Alzheimer's disease[174] reveals complexities due to microbiomes and may partially explain variations[175] in bioavailability in cases of diabetes and Alzheimer's. These clues, in combination, may help to analyze CoV infection and other threats with a wide-angle "panoramic" lens which catalyzes convergence (chemistry, genomics, microbiology) and views non-linear efficacy of treatment (drugs, vaccines) using a ***stratified*** perspective not only based on geography, genomics, metabolomics (proteomics, lipidomics) but also, must include microbiomics.

For those seeking antecedence (precedence) may note that the identification of norovirus[176] in 1968 was followed[177] by traditional[178] observations[179] before the breakthrough by Stephanie Karst (reference 112) demonstrating that norovirus infection is facilitated by commensal bacteria found in the gut microbiome. Norovirus is an example of how infection may be regulated[180] by host organisms. The microbiology[181] and virology[182] of respiratory tract infections[183] presents a range of partners as well as a variety of drugs[184] as potential elements in fomenting "microbiome conspiracies" to enable the organisms to consult and then implement their collective wrath to insult and injure humans and animals.



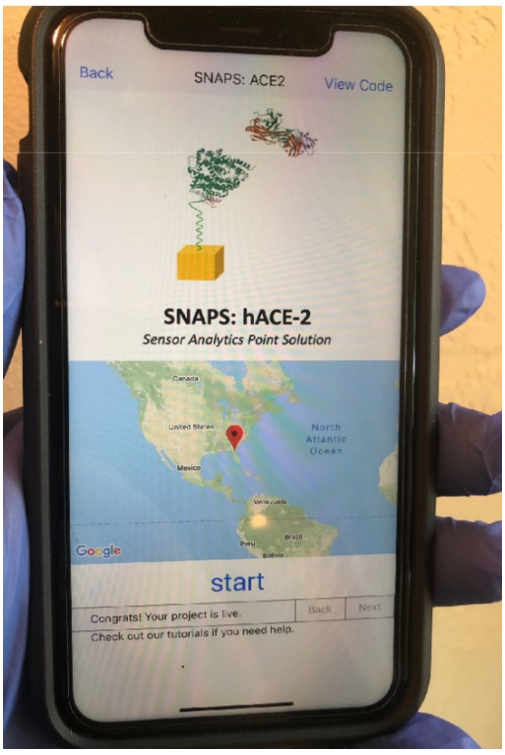
Microbial signatures. For a disease such as type 2 diabetes, patients should be stratified based on medications to reveal drug-microbe interactions.

Figure 22: Stratification based on microbiomics[185] may hold clues to improving our understanding of the non-linear behavior and efficacies of treatments, drugs and vaccines. Stratification by immunogenic[186] profile (HLA[187] and MHC[188]) and amino acid variations in proteins (for example, ACE2, TMPRSS2, viral S protein) may be revealing.

DIGRESSING TO DISCUSS A CONTRARIAN PERSPECTIVE

Is it written in the stars[189] often comes to mind when the scientific complexity is as overwhelming as CoVID-19. Are we hovering over the obvious[190] and ignoring[191] the medium where the "canary" thrives - in the air? What if analysis of particulate matter in the air may hold clues to distant hot spots or indicate signs of emerging events? What if data from testing sea water when ***combined*** with data from sewer water and wastewater is a better predictor than SENSEW SNAPS (48) without data fusion? Elements from food, air, sand, dust[192], soil, water and sanitation sludge may contain useful indicators. The expectation that surveillance will find that "needle in the haystack" may be a mirage. If we find the "needle" how will we know what we found? What if we have the data[193] but analysts may lack the knowledge to grasp the significance of the "needle" hiding in plain sight in front of them?

What if the cycle of pandemics occur at some periodicity similar to variations of the Kondratieff waves[194] and signifies a natural passage of organismal evolution? Because our window on evolutionary and geological events are extraordinarily limited and our understanding is even less, we view this evolutionary cycle as a cleansing routine for the civilization and rush to prevent it from repeating itself. We ought to proactively pursue our plight for prevention, cure (vaccines) and critical care life-saving measures but shouldn't we also entertain the (non-zero) probability that our endeavors may be insufficient to combat natural laws, cryptic to human perception? Should we ignore the philosophies of the West and the East as to how fate[195] may guide our destiny?



FATE AND FREEDOM IN GREEK TRAGEDY

By Walter R. Agard
University of Wisconsin

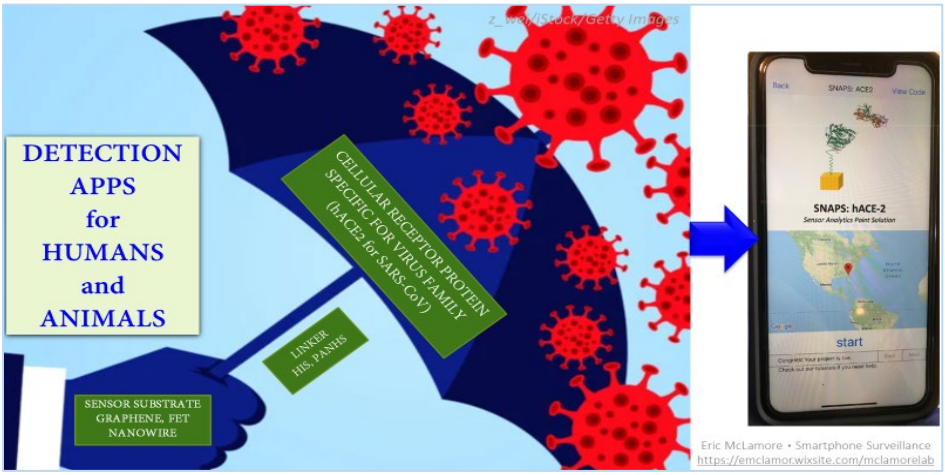
"The strength of Necessity cannot be resisted" (*Prometheus* 105). "From ills given by the gods there is no escape" (*Seven against Thebes* 719). Such is the philosophy of life commonly accepted by critics as the guiding principle of Greek tragedy. The judgment is a natural one to make. Do we not see the characters struggling vainly against fate or heavenly powers that contrive for them disaster?

Figure 23: "AHA" – the surveillance smartphone app[196] (left) representing the sensor platform strategy to detect a virus (any virus) using the host cellular receptor (***virus family-specific receptor*** in humans and/or animals) immobilized on the sensor as a "target" for the virus (protein) to bind (when the sensor is in contact with a sample, for example, saliva from humans and/or animals). (Right) Excerpt from Walter R. Agard's (1933) "Fate and Freedom in Greek Tragedy." (195).

CALL FOR A PLATFORM SENSOR STRATEGY – BUT WE DON'T KNOW ENOUGH TO BE DEFINITIVE

It is impossible to ignore the urgency with which we must strive to predict emerging *hot spots*, globally. Sensors as low-cost mobile services for "citizen surveillance" may be supported by the economics of technology for diffusion, globally. Real-time distributed sensor data (acquired by smartphones) and ***actuation of information arbitrage*** with nodes of analysis (CDC, HHS, WHO) holds the key to identification of *hot spots,* detection of infectious agents and estimating the spread of infectious agents in human populations, animal farms and entities in the food supply chain.

Platform consists of a target **detector molecule** affixed to the **material of the sensor.** When analyte (**pathogen**) **binds** to the detector, it induces a change that is captured as a **signal** by a **mobile** device. The **data** is analyzed, displayed in an **app** and transmitted or stored per admin/user preferences. The signal could be optical (captured by **smartphone**) or electro-chemical (EIS, plasmon resonance, FET).



VARIABLE Virus family (may be unknown)	VARIABLE Host receptor protein DETECTOR (S)	Sensor Engineering materials (graphene) linkers & form factor	Signal Transduction (transmission, storage and analysis)	Smartphone App, Data & Information Arbitrage

Figure 24: Deploying an array of sensors as detectors coupled with surveillance provide public health professionals with information about "hot spots" of activity in humans/animals with respect to virus families. Together with other data (body temperature) it may signal the need for specific testing followed by isolation, to stem the spread of the pathogen. The platform approach is highlighted in the table (bottom) where the biology of the detector protein for the virus in question (first two boxes, left) converges with the engineering elements in a combinatorial *plug and play* approach.

The sensor platform strategy is ***useful for detecting virus families which are known*** and where we know how the virus enters the host cell (humans, animals) using which cellular protein as its receptor. The cellular "receptor" serves as the detector protein for the virus/virus family. This strategy may be applicable to ***preventing recurrence.***

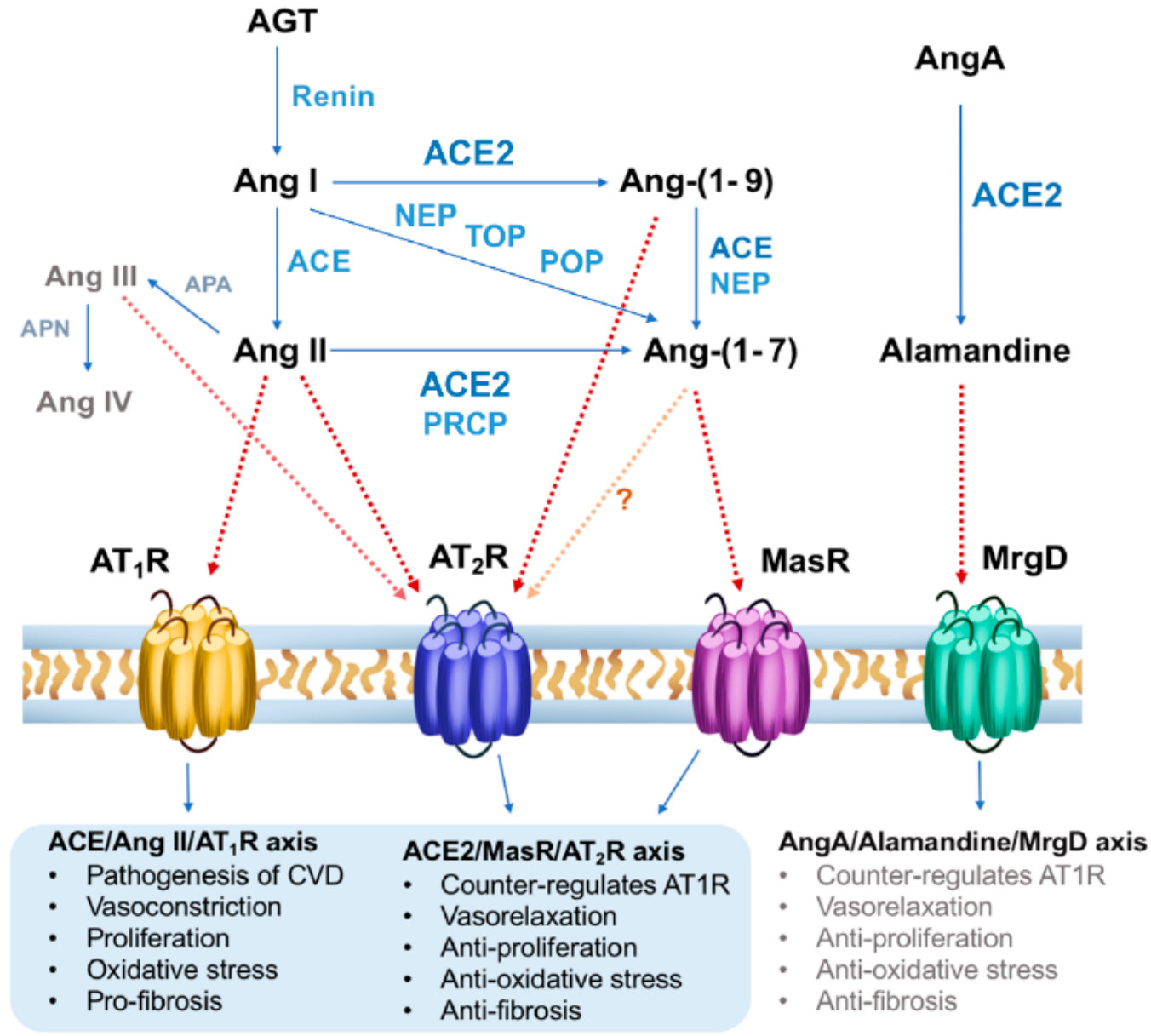
What happens if a completely new virus emerges from the potential group of 1.67 million viruses due to inter-species host switching? The knee-jerk reaction starts with sequencing and genomics, the bread and butter of biology in our quest to understand the virus. Unfortunately, neither the genome of the virus nor its predicted proteins (from open reading frames, orfs) may contain any "clear signature" with respect to its virulence or potential to whip up a pandemic.

Results[197] describing the phylogeny of 1200+ novel RNA-dependent RNA polymerase (RdRp[198]) sequences of bat corona viruses from China could not (?) (did not?) predict the pandemic due to SARS-CoV-2 (CcVID-2019). Genomics is quintessential but sequencing 1.67 million viruses may not sufficiently illuminate our ability to predict any pandemic potential. We may not have "silver bullets" for pandemic prediction within the current realm of knowledge. Sequencing combined with sensor-based surveillance improves the odds. But, local knowledge of "unusual" biological activity in humans and animals may be crucial in these uncertain scenarios. Transparency of data and near real-time information arbitrage are critical factors to drive professional focus, without delay, to explore suspected *hot spot* activities. Neither surveillance nor sequencing holds any magic, but are required, with regards to reducing the risk of the next pandemic.

More effective and less onerous is the deployment of educational tools[199] to reach every nook and corner of the civilization. Let us educate[200] the masses with respect to genetics, phylogenomics, microbiology, virology and physiology in the context of common medical symptoms (fever, cough, asphyxia, diarrhea) and with respect to their daily lives in terms of health, water, food (animal protein in nutrition) and hygiene. Vaccines are emerging after 30 years[201] of research but its distribution may not reach the underserved, soon enough. Scientists must serve society by roughshodding over public opprobrium in the pursuit of purpose, public health[202] and primary care[203]. Education begins by teaching the teachers, who will teach the students. Students, in turn, will teach their parents, families and communities about viruses.

The genetic relationship and similarities between SARS-CoV-1 and SARS-CoV-2 is insufficient to explain how SARS of 2002-2003 was controlled in less than a year after SARS-CoV-1 infected 8437 individuals (813 deaths) versus the aggressive SARS-CoV-2 which has infected >100 million people[204] and global death toll is approaching 3 million. High level of SARS-CoV-2 shedding[205] in the upper respiratory tract, among presymptomatic patients, distinguishes it from SARS-CoV-1, where replication occurs mainly in the lower respiratory tract. The vastly different trajectories cannot be accounted for by what we know.

Both[206] ACE2 (physiologically important in the renin-angiotensinogen system, a carboxypeptidase transmembrane protein that cleaves angiotensin I and II, controls vasoconstriction, blood pressure and hypertension) and the transmembrane protease, serine 2 (TMPRSS2) are crucial for SARS-CoV-2 entry (see Figure 21). ACE2 is the "main" receptor for the spike (S) protein of both SARS-CoV and SARS-CoV-2. TMPRSS2 cleaves the viral S protein at the S1/S2 and the S2' sites, allowing fusion[207] of viral and cellular membranes followed by endocytosis of the virus necessary for replication. TMPRSSS2 serves as a plasma membrane-anchored serine protease that participates in proteolytic cascades of relevance for the normal physiologic function of the prostate (and plays a key role in prostate cancer, see below).



Schematic overview of the renin/ACE/Ang-II/AT_1R axis, ACE2/MasR/AT_2R axis and AngA/Alamandine-MrgD axis, modified from [22]. ACE, Angiotensin converting enzyme; AGT, angiotensinogen; Ang, angiotensin; APA, aminopeptidase A; APN, aminopeptidase N; AT_1R, angiotensin II type 1 receptor; AT_2R, angiotensin II type 2 receptor; MasR, Mas receptor; MrgD, Mas-related G-protein coupled receptor type D; NEP, neutral endopeptidase, POP, prolyloligopeptidase; PRCP, prolylcarboxypeptidase; TOP, thimet oligopeptidase.

Figure 25: It may be both premature and audacious to speculate that one or more ***second messenger systems*** may be contributing and/or manifesting the aggressive nature of CoVID-19 infections and deaths. The mode of entry and tissue targeting by SARS-CoV-2 may hide an insidious mechanism not apparent from relationships[208] due to ACE2. It may involve MAS[209] (through ACE2-ANG(I)-MAS[210] axis) and complex[211] interactions[212] with TMPRSS2. The latter is a target[213] for intervention[214] but may be difficult to treat with specificity and acceptable level of physiological toxicity.

The second question concerns tissue-specific expression[215] and organ targeting by SARS-CoV-2. The high level of virus shedding in the respiratory tract (upper for CoV-2 and lower for CoV-1) suggests that the virus targets the respiratory tract, consistent with the droplet/aerosol mode of transmission (wearing a mask to reduce the spread of infection). Previous work indicated *ACE2* (gene, in italics) is abundant[216] in human epithelia of the lung and small intestine. But, tissue specific expression to facilitate viral infection requires ***co-expression*** of *ACE2* and *TMPRSS2*. In fibrotic human lung tissue, only 1.4% of type II pneumocytes express *ACE2*, and 0.8% of type II pneumocytes expressed both *ACE2* and *TMPRSS2*. In human upper respiratory epithelia, 1.3% of all secretory cells expressed *ACE2* while 0.3% expressed both *ACE2* and *TMPRSS2*.[217] It is incredible that SARS-CoV-2 has infected >100 million people and 3 million deaths by targeting 0.3% of the cells in the human upper respiratory tract epithelia co-expressing ACE2 and TMPRSS2.

Both genes can influence the extent of SARS-CoV infection and sex-related effects because ACE2 is located on the X chromosome[218] while TMPRSS2 (chromosome 21[219]) expression is responsive to androgen/estrogen. Differences in ACE2 and TMPRSS2 levels[220] and genetic variants[221] do not result in significant severity of disease or X-linked bias, in Italy. In other studies[222] the relative risk for death in males was 1.62 after adjustment for age (the coincidence of the golden ratio[223] [1.6, *phi*] is intriguing). In general, men infected with CoVID-19 are worse off [224] and the risk for bald men[225] may be highest. Also, note that [a] females with two X chromosomes (two alleles of *ACE2*) are at a lower risk than mono-allelic males [b] TMPRSS2 is linked[226] to prostate[227] cancer[228] metastasis[229] as well as infection by influenza[230] and parainfluenza[231] viruses [c] the impact from amino acid changes in ACE2 (viral S1 binding region) identified from ~200,000 exomes of unrelated people[232] (see 2004 data on SNPs in ref 215) remains unexplored [d] infection is often asymptomatic and [e] symptoms vary in pathological severity and immune response (see references in Figure 22).

The 332 proteins identified by Gordon *et al* (ref 129) as potential points of interaction between humans and SARS-CoV-2 adds to the already vast landscape of second messenger systems and their inter- and intra-relationships (Fig 25). It is noteworthy that the 3' open reading frame (ORF) in SARS-CoV-2 could generate Orf3b and Orf10, unique to SARS-CoV-2 (Fig 9). The protein from Orf3b was identified to interact with stomatin-like protein 2[233] (STOML2), one human protein, a novel mitochondrial inter-membrane space/inner membrane-localized protein that forms a large hetero-oligomeric complex with Mfn2 (mammalian mitofusins Mfn1 and Mfn2 are large GTPases of the mitochondrial outer membrane that mediate mitochondrial fusion). In the context of proteins, less virulent SARS-CoV-1 encodes[234] Orf8a and Orf8b but Orf8[235] is intact in SAR-CoV-2. Understanding these interactions are crucial for CoVID-19.

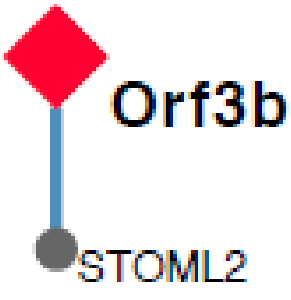


Figure 26: From the 332 proteins identified by Gordon *et al* (ref 129), only human STOML2 may interact with viral Orf3b protein.

STOML2 and the repertoire of 332 human proteins which may interact with SARS-CoV-2 suggests something deeply systemic that may be hijacked by the virus. Is this interaction (Of3b-STOML2) one of the contributing reasons for the incredible mortality and morbidity due to "systemic" functions which goes awry in CoVID-19?

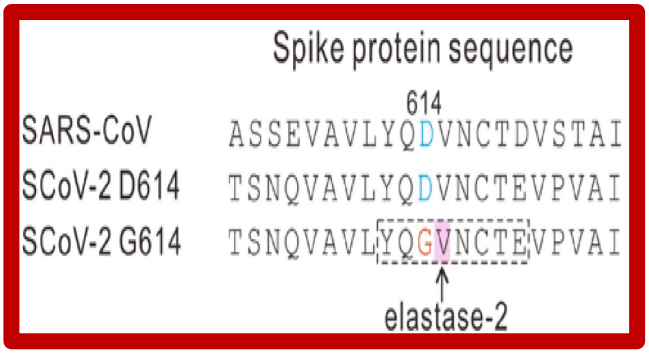
To mitigate the risk of infection we can still think about "anti-viral" strategies for prevention and spread, but once infected (if confirmed positive) the treatment (even for asymptomatic individuals) may have nothing to do with any anti-viral approach. The "domino effect" set in motion by the initial infection by the virus is now transformed to a physiological aberration which may be unstoppable (till death) unless intervention is successful.

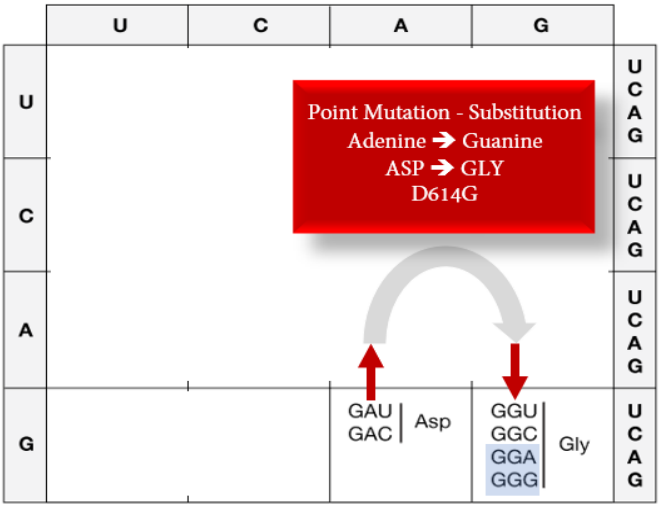
Thus, CoVID-19 may no longer be a viral disease but an accelerated human disease based on physiological functions turned pathological. It may not be at any one point in a specific tissue but may coerce ubiquitous elements (proteins, messengers, pathways) which are central to normal physiology to act abnormally, against "self" perhaps in a manner analogous to auto-immune dysfunctions. Evidence from lung autopsies of seven patients[236] who died from CoVID-19 infection indicates pulmonary vascular endothelialitis, thrombosis, and angiogenesis as the cause of death.

The medical literature is indicating a vast range of anomalies in patients with SARS-CoV-2 infection. In terms of treatment, are we barking up the wrong tree? What if the anti-viral strategy is rationally necessary but insufficient and/or incorrect? The variants[237] of SARS-CoV-2 presents challenges and one non-synonymous mutant dominates. Why?

Mutations in Spike Protein	No. of mutation	No. of wildtype	Total No. of sequences	Mutation (%)
D614G	2995	1637	4632	64.659
A829T	37	4602	4639	0.798
L5F	33	4614	4647	0.710
H146Y	26	4594	4620	0.563
P1263L	15	4631	4646	0.323
V483A	12	4387	4399	0.273
S939F	12	4626	4638	0.259
R78M	10	4623	4633	0.216
E583D	9	4639	4648	0.194
A845S	9	4632	4641	0.194

Figure 27: How can we explain the dominance of D614G mutation (ref 237) in the Spike protein of SARS-CoV-2?





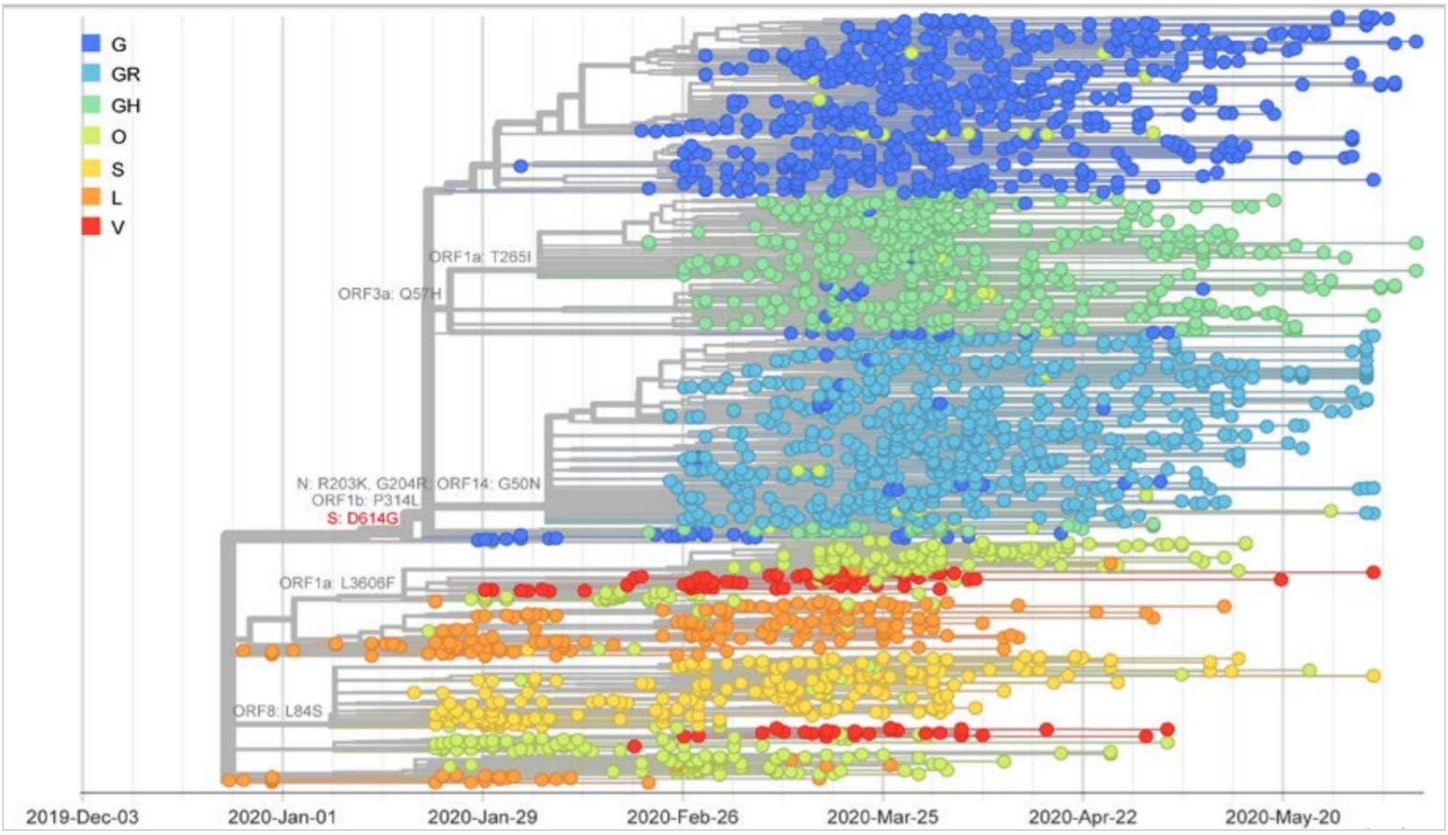


Figure 28: SARS-CoV-2 mutant D614G creates a new serine protease (neutrophil elastase) site which facilitates entry (2X in pseudovirus assays) into host (human) cells and reduces (3-5 fold) neutralization using individual convalescent sera (ref 237). The Aspartate to Glycine change (top left panel) may be an Adenine to Guanine point substitution (top right). The prevalence of D614G (~65%, Fig 27) evident from clade G (blue circles) in the phylogenetic analysis (bottom panel) of SARS-CoV-2 genomes (n = 2,834 from 1/2020 to 5/2020) must ask whether this mutation can be explained by [a] the selective forces of natural selection or [b] the low fidelity (not typical of SARS-CoV-2 coronaviruses) of RNA replication in RNA viruses using the RNA-dependent RNA polymerase or [c] if there is room for any reasonable doubt whether this striking dominance may be an artefact. With >100 million individuals infected by SARS-CoV-2 and ~3million deaths due to this pandemic, it may be usual to expect the unexpected, yet rigorous use of scientific tools, credible data and reproducible verification is necessary to arrive at potential conclusions.

In exploring the range of COVID-19 infections (asymptomatic, paucisymptomatic) and clinical severity, it is unclear how two "similar" viruses can lead to infection of 8437 individuals (813 deaths, ref 215) by CoV-1 versus more than 10 million individuals by SARS-CoV-2 (half million deaths). Any attempt to explain the catastrophic outcome suggests that ***we may not know what we don't know*** about virulence factors and the mechanism of action of SARS-CoV-2. One hypothesis is the potential for involvement of one or more second messenger systems to modulate the outcome, and/or induce systemic physiological failure (for example, vascular disease and/or hormonal dysfunction). Is it time to think radically different? This discussion continues in ADD[238] which explores aptamers.

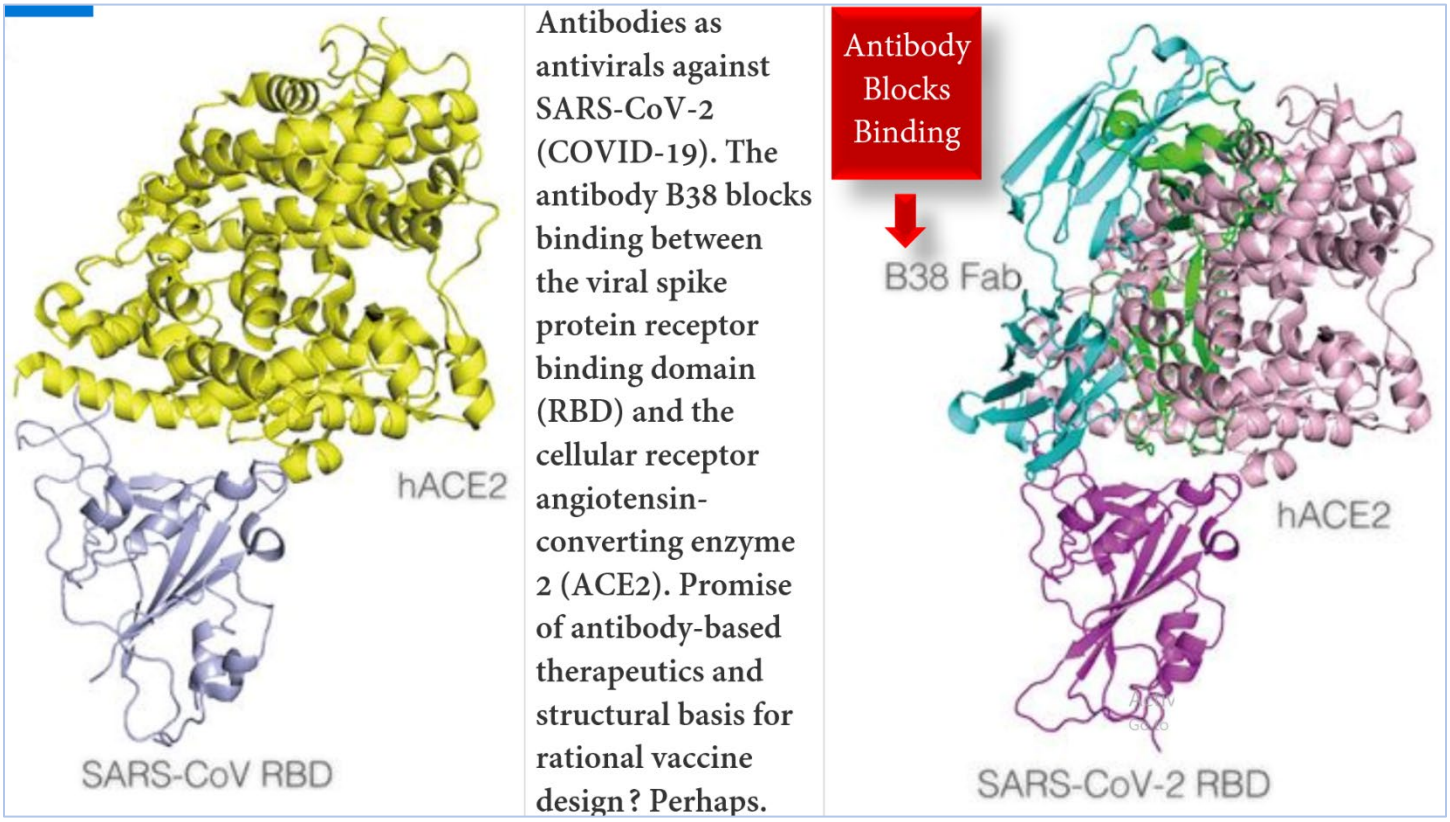


Figure 29: Therapeutic success from anti-viral approaches, alone, may be limited. The promise of 4A8[239] or B38 and H4 (B38 is shown here) pair of non-competing human neutralizing antibodies[240] blocking COVID-19 binding to ACE2, is an example. Using this and other[241] evidence[242] of mutation dynamics[243] as a structural basis to create cocktails of short (?) synthetic polypeptides may be better (?) than reliance on our immune system or other sources of (large) antibodies.

TEMPORARY CONCLUSION

One aim of this essay is to convey the urgent[244] need for a platform approach for surveillance of pets[245] and animals[246] as well as humans. Data from sensor systems may help to prevent recurrence of epidemics and pandemics[247], due to known viruses. Conflicting[248] evidence[249] is also adding to our uncertainty. Sensor-based smartphone detection tools *at home or anywhere* (AHA), using a low cost *pay-a-penny-per-use* (PAPPU) economic instrument, may be affordable for communities under economic constraints in triple A regions (Asia, Africa, Amazonia). Due to the pandemic in progress, the example of choice for the sensor platform is the corona virus family. Therefore, the sensor suggests the use of ACE2 which binds to viral Spike protein, as a detection tool to identify *corona virus members*. By replacing the detector with *other cellular receptor molecules*, we may identify members of *other virus families*. This approach uses pre-identified detector molecules (proteins), hence, surveillance is limited to ***known viruses***.

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The opinions in this essay are entirely due to the author and does not represent the views of the institutions with which the author may be affiliated. This essay wanders but not aimlessly. It aims to bring together observations, in an attempt to seek ***non-obvious*** relationships (NORA).