DEWETTING ANTIBODIES AGAINST VIROPORINS MIGHT CONTRIBUTE TO DECREASE 2019-nCoV INFECTION SPREAD

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Dewetting transition is transient phenomenon taking place inside the hydrophobic pores of ion channels that forbids water molecules to cross microscopic receptor cavities and leads to impairment of cellular performance. It has been recently suggested that artificially-provoked dewetting transition in ion channel hydrophobic pores might stand for a molecular candidate to erase viruses, bacteria and cancer cells, and to block autoimmune activities. A novel type of high-affinity monoclonal antibody has been suggested, that is equipped with lipophilic and/or hydrophobic fragments that prevent physiological water flow inside ion channels and targets specific trans-membrane receptor structures of influenza viruses. Here we suggest that these dewetting monoclonal antibodies targeting the 2019-nCoV viroporin channels, sprayed in the nasal cavities, might lead to virion impairment, thus preventing inter-human viral transmission.

KEYWORDS: immunology, immunotherapy, receptor; coronavirus; 2019-nCoV.

In fluid mechanics, dewetting stands for the rupture of the thin, liquid continuous film on the solid–liquid or liquid–liquid interfaces, leading to formation of irregular patterns of droplets (Sharmaa, 1996; Sharmaa and Reiterb, 1996; Sackmann and Bruinsma, 2002; Anishkin and Sukharev, 2004; Rosen 2004; Tanaka et al., 2005; Karapanagiotis and Gerberich, 2005; Leroux et al., 2008; Young et al., 2010; Boreyko et al., 2011; Thompson 2012; Gonzalez-Rodriguez et al. 2012; Douezan and Brochard-Wyart, 2012; Rahe et al., 2012; Lapierre et al., 2013; Thiam et al., 2013; Lee et al., 2014; Vargas et al., 2014; Sándor et al., 2017; Alert and Casademunt 2018; Rego et al., 2019). This process, borrowed by physics, has been recently extended to describe also microscopic biological phenomena. In particular, dewetting transitions may occur inside the hydrophobic pores of cellular ion channels. In the narrowest, more hydrophobic parts of receptor holes, a metastable state of dewetting transition forbids water molecules to get inside the cavities, leading to a decrease in conductance channel closure, and impairment of cellular activity. Tozzi (2020) proposed that this peculiar process can be artificially produced to alter the physiological activity of noxious pathogens, such as viruses, bacteria and tumoral cells. In particular, the manufacture of monoclonal antibodies (against cellular receptors) has been suggested, equipped with lipophilic/hydrophobic caps. In the sequel, we will term these antibodies DEMA (DEwetting Monoclonal Antibodies). Once they link monoclonal targets, their artificial hydrophobic device blocks water flow inside receptors, contributing to malfunction of pathological organisms. The factors influencing dewetting in physical systems, in biological structures and also in nervous cells are described by Tozzi (2020).

In brief, a huge range of physical factors, that can be fine-tuned in experimental settings, may contribute to the attainment and preservation of dewetting regimes in countless systems equipped with solid–liquid or liquid–liquid interfaces. Summarizing, when water and ions are enclosed within the sub-nanometer, narrow cellular confines of a ion channel hydrophobic pore, they exhibit an odd behavior (Aryal et al., 2015): near a critical point, a stochastic liquid-vapor water phase transitions takes place (Anishkin and Sukharev, 2004). These transient vapor states are "dewetted", i.e., devoid of water molecules within all, or part of, the pore. The decreased amount of water molecules inside receptors leads to impaired conductance, energetic barriers to ion transit and closure of the channel, in a process termed "hydrophobic gating". It is noteworthy that the principles underlying the metastable dynamical state of hydrophobic gating require a very small tube radius and interactions with a strongly hydrophobic lining (Lapierre et al., 2013).

In the sequel, the above-mentioned physiological phenomenon of dewetting transition will permit us to build strategies to influence the dynamics of droplet formation inside biological channels of 2019-nCoV viroporin channels.

TOWARDS DEWETTING MONOCLONAL ANTIBODIES

In this Section, we consider the possibility to build high-affinity monoclonal antibodies able to dewet the ion channels of target cells, thus leading to their impairment and, possibly, death. Antibodies with high affinity for receptors of pathological cells could be equipped with lipophilic structures, covalently attached, e.g., to their Fc region. These hydrophobic/lipophilic components must serve two purposes: a) to prevent water to penetrate inside the receptor ionic channels; b) to avoid immune Fc-mediated responses. In plain terms, we could state that, thanks to DEMA, a sort of cork provides a tight seal that prevents water to fill the receptor channels of pathogenic cells, causing collapse of unwanted organisms. DEMA could be used to counteract different types of pathogenic cells, such as viral, bacterial and tumoral ones. In the sequel, we will provide the example of Influenza A M2 proton channel, in an effort to develop a novel drug able to neutralize the virion. In Tozzi (2020), we provided an example, describing how to build D**EMA against influenza A virus M2 receptor** (Pielak and Chou, 2011; Rossman et al., 2010; Cho and Wrammert, 2016; Fiers et al., 2009; Cady et al., 2010; Homeyer et al., 2016).

The following paragraph is from Tozzi (2020), including the Figures. We suggest to use DEMA as a novel drug against all the strains of Influenza A (**Figure 1, top**). The very structure of M2 let us hypothesize that a monoclonal antibody that prevents water to enter the M2 channel might disrupt Influenza A pathogenetic activity. Indeed, water plays a foremost role in M2 functioning (**Figure 2A**). The channel, highly selective for protons, is activated by low pH and has a low conductance. Conduction mechanism involves: a) the exchange of protons between the His37 imidazole moiety, responsible for proton selectivity and pH modulation, and b) the water confined to the M2 bundle interior. Water molecules within the pore form hydrogen-bonded networks or "water wires", from the channel entrance to His37. When a proton gradient occurs, conformational changes facilitate asymmetric diffusion through the channel. Indeed, protons diffusing through the channel need not be localized to a single His37 imidazole, but rather they may be delocalized over the entire His-box and associated water clusters. Furthermore, pore-lining carbonyl groups stabilize hydronium ions through second-shell interactions that involve the bridging of water molecules. A ring of methyl groups from Val27 tightens the N-terminal side of the pore to ~3.1 Å, narrowing the entrance and preventing water molecules from penetrating the channel (Pielak and Chou, 2011). Small motion or "channel breathing" may thus be required for water to enter the pore. It is widely accepted that water molecules are needed inside the channel cavity for supporting proton conduction. Water molecules, provided with a diameter of ~3 Å, start to exhibit liquid–vapor transitions and stochastic switches between wet and dry states within a hydrophobic pore of diameter less than ~14 Å. The most dynamic range for these transitions is between 9 and 12 Å: below this range, the pore will be largely dewetted (Aryal et al., 2015). The pore widens after Ser31 and becomes the widest at Gly34 position, that is equipped with an inner diameter of ~ 6 Å. The channel then narrows towards the C terminus, as the sidechains of His37 and Trp41 constrict the inner diameter to 1.7 and 1.4 Å, respectively.

The presence of a hydrophobic (or lipophilic) part located on the constant chain of DEMA, could, according to our theoretical previsions, stop water flow inside the M2 channels (**Figure 2B**), leading to viral malfunction and, possibly, removal from the infected human body.

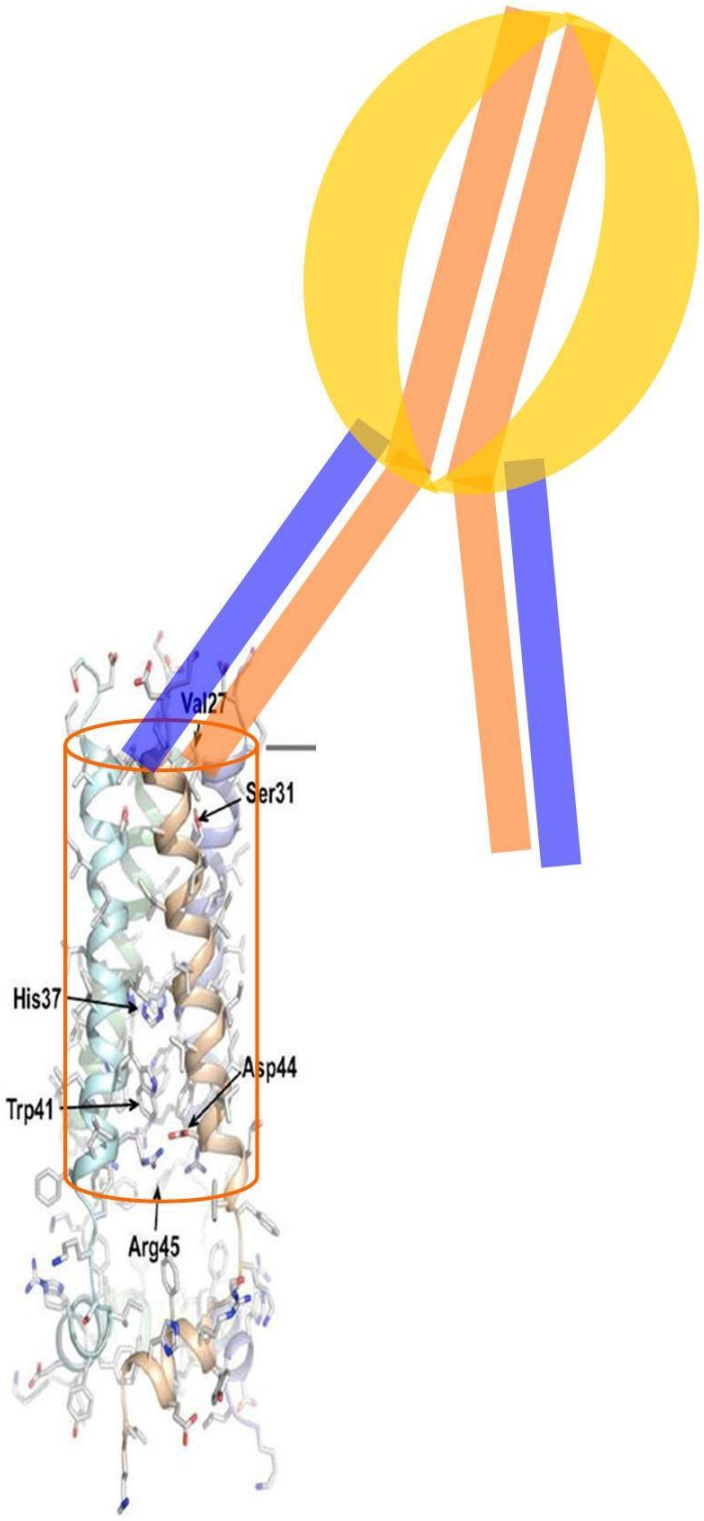

Figure 1. **Bottom**: high resolution structures of the AM2 channel domain. Solution structure of residues 18–60 in 1,2-Dihexanoyl-sn-Glycero-3-Phosphocholine micelles at pH 7.5 (Modified from Pielak and Chou, 2011). **Top**: Dewetting antibody (DEMA) against the Val27 area. The Fc region of this artificial antibody is surrounded by an hydrophobic structure (yellow shape). Channel and antibody are drawn to scale: the transmembrane section (red cylinder) is about 30 Å long. From: Tozzi (2020).

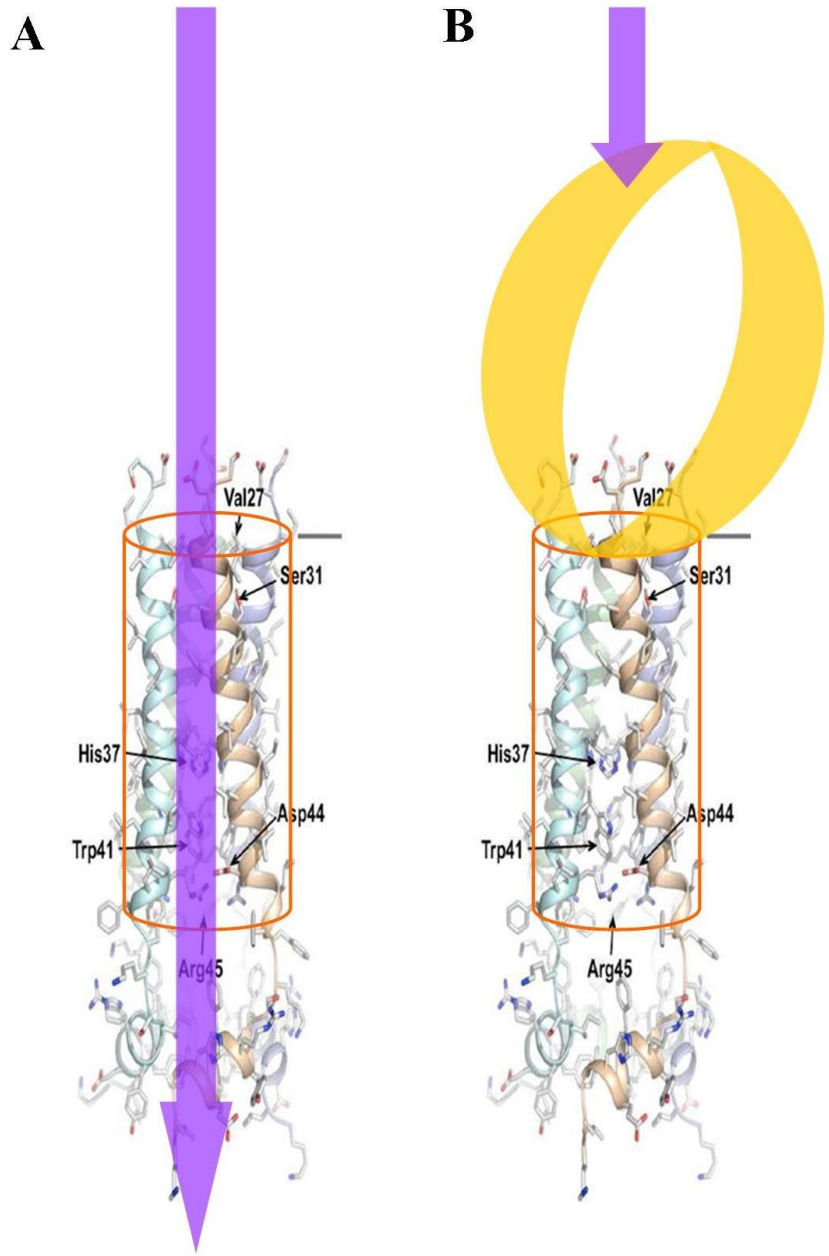

Figure 2. Water flow inside M2 channel. **2A**: In physiological conditions, water is allowed to flow inside the M2 channel, giving rise to proton gradients crucial for pathogenic activity of Influenza A virus. **2B**: When a DEMA targets the upper part of the M2 receptor, its hydrophobic component prevents water to go through the M2 receptor channel (**right side**), leading to impairment of virionic metabolic pathways. From: Tozzi (2020).

CORONAVIRUS RECEPTORS

Our account of dewetting transition paves the way for innovative strategies. To make an example, dewetting enables researchers to build synthetic asymmetric model membranes with lipid composition/architecture that mimics the outer membrane of human pathogens, such as Pseudomonas aeruginosa (Maktabi et al., 2019).
Here we take into account the 2019 novel coronavirus 2019-nCoV, recently announced by the World Health Organization. Bioinformatics analysis on a virus genome is available and has been compared with other related coronavirus genomes: overall, the genome of 2019-nCoV has 89% nucleotide identity with bat SARS-like-CoVZXC21 and 82% with that of human SARS-CoV (Chan et al., 2020). Recent studies on coronaviruses have described the presence of a viroporin, i.e., a ion-channel protein (**Figure 3**). The latter is generated by the CoV E protein when it forms homotypic interactions which allows it to oligomerise (Schoeman and Fielding, 2019). Viroporins are viral-encoded membrane pore-forming proteins that can modulate cellular ion channels; they have been suggested to regulate and function in multiple stages of the viral life cycle, from viral entry to assembly and release, and even pathogenesis (Schoeman and Fielding, 2019).

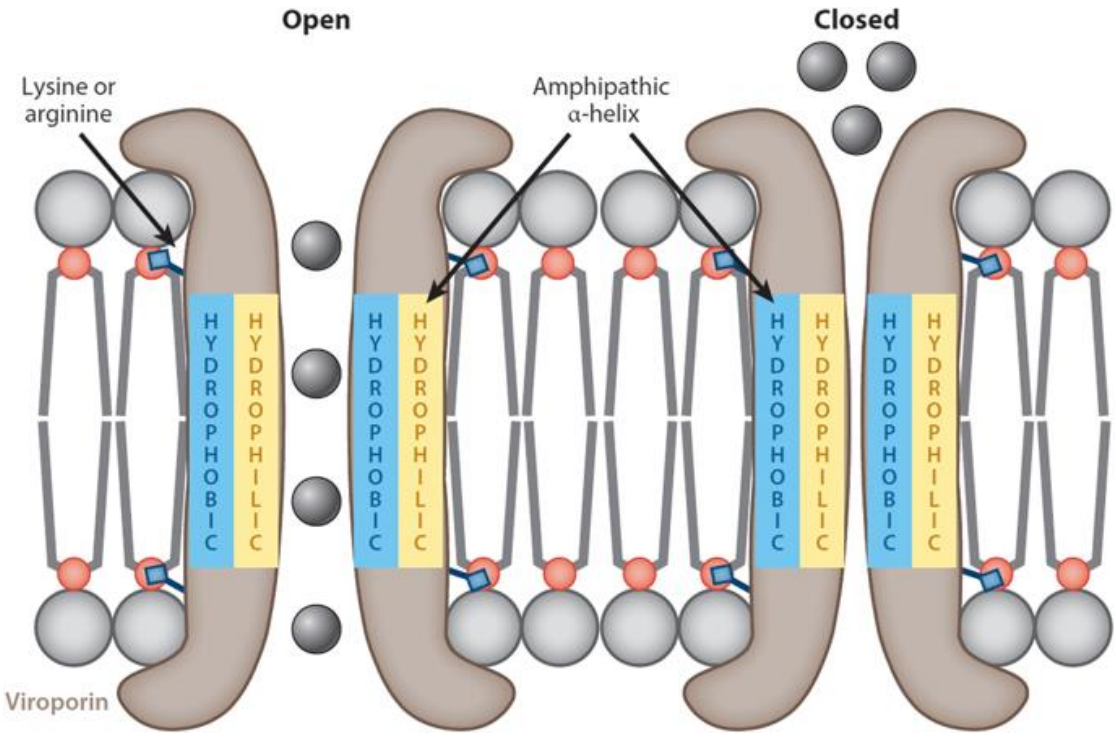

Figure 3. Coronaviruses' viroporin structure and motifs. For further details about this Figure, see Schoeman and Fielding (2019).

SUGGESTIONS

Dewetting transition, characterized by an unusual behavior of water supramolecular assembly, stands for an increasingly important feature that has been already used to assess countless morphological and/or functional biological structures, such as protein cavities, extracellular matrix and glycocalix, lipid droplets, lipid bilayers, cell adhesion, macroapertures opening in endothelial cells. In the narrowest, more hydrophobic part of cellular channels, a metastable state of dewetting transition of water molecules takes place, leading to decrease of conductance and closure of the pore.

Dewetting mechanisms can be used to achieve novel therapeutic weapons against a wide range of diseases. Further, the possibility to artificially modulate dewetting processes could lead to the development of new drugs (with mechanisms different from DEMA) that might be relevant in development, regeneration, self-immunity, infection and cancer.
Here we suggest to build a dewetting monoclonal antibody against the viroporins of 2019-nCoV (or against other viral channels characterized by dewetting transition). Once achieved a drug, it could be sprayed into the nose. Indeed, administred through the nose, such monoclonal dewetting antibody might contribute to inactivate the virions in the human airways, before 2019-nCoVs are able to link to the host's cellular membranes.
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REFERENCES

1) Alert R, Casademunt J. 2018. Role of Substrate Stiffness in Tissue Spreading: Wetting Transition and Tissue Durotaxis. Langmuir. 2018 Oct 25. doi: 10.1021/acs.langmuir.8b02037. [Epub ahead of print]
2) Anishkin A, Sukharev S. 2004. Water dynamics and dewetting transitions in the small mechanosensitive channel MscS. Biophys J. 2004 May;86(5):2883-95.
3) Aryal P, Sansom MS, Tucker SJ. Hydrophobic gating in ion channels. J Mol Biol. 2015, 427(1):121-30. doi: 10.1016/j.jmb.2014.07.030.
4) Boreyko JB, Baker CH, Poley CR , and Chen CH . Wetting and Dewetting Transitions on Hierarchical Superhydrophobic Surface. Langmuir, 2011, 27 (12), pp 7502–7509 DOI: 10.1021/la201587u
5) Cannon RC, O'Donnell C, Nolan MF. Stochastic ion channel gating in dendritic neurons: morphology dependence and probabilistic synaptic activation of dendritic spikes. PLoS Comput Biol. 2010 Aug 12;6(8). pii: e1000886. doi: 10.1371/journal.pcbi.1000886.
6) Cady SD, Schmidt-Rohr K, Wang J, Soto CS, Degrado WF, Hong M. 2010. Structure of the amantadine binding site of influenza M2 proton channels in lipid bilayers. Nature. 463 (7281): 689–92. doi:10.1038/nature08722
7) Chan JF, Kok KH, Zhu Z, Chu H, To et al. 2020. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microbes Infect. 2020 Dec;9(1):221-236. doi: 10.1080/22221751.2020.1719902.
8) Cho A, Wrammert J. 2016. Implications of broadly neutralizing antibodies in the development of a universal influenza vaccine. Curr Opin Virol. 2016 Apr;17:110-115. doi: 10.1016/j.coviro.2016.03.002. Epub 2016 Mar 28.
9) Douezan S, Brochard-Wyart F. 2012. Dewetting of cellular monolayers. Eur Phys J E Soft Matter. 2012 May;35(5):34. doi: 10.1140/epje/i2012-12034-9.
10) Fiers W, De Filette M, El Bakkouri K, Schepens B, Roose K, et al. 2009. M2e-based universal influenza A vaccine. Vaccine. 2009 Oct 23;27(45):6280-3. doi: 10.1016/j.vaccine.2009.07.007.
11) Gonzalez-Rodriguez D, Maddugoda MP, Stefani C, Janel S, Lafont F, et al. Cellular dewetting: opening of macroapertures in endothelial cells. Phys Rev Lett. 2012 May 25;108(21):218105
12) Homeyer N, Ioannidis H, Kolarov F, Gauglitz G, Zikos C, et al. 2016. Interpreting Thermodynamic Profiles of Aminoadamantane Compounds Inhibiting the M2 Proton Channel of Influenza A by Free Energy Calculations. Journal of Chemical Information and Modeling. 56 (1): 110–26. doi:10.1021/acs.jcim.5b00467
13) Ichinohe T, Pang IK, Iwasaki A. 2010. Influenza virus activates inflammasomes via its intracellular M2 ion channel. Nature Immunology. 11 (5): 404–10. doi:10.1038/ni.1861
14) Foffi G, Pastore A, Piazza F, Temussi PA (2013) Macromolecular crowding: chemistry and physics meet biology (Ascona, Switzerland, 10-14 June 2012). Phys Biol 10(4):040301
15) Haider B, Duque A, Hasenstaub AR, McCormick DA. Neocortical network activity in vivo is generated through a dynamic balance of excitation and inhibition. J Neurosci. 2006 Apr 26;26(17):4535-45.
16) Karapanagiotis, Ioannis; Gerberich, William W. (2005). "Polymer film rupturing in comparison with leveling and dewetting". Surface Science 594 (1–3): 192–202. doi:10.1016/j.susc.2005.07.023.
17) Lapierre F, Coffinier J, Boukherroub R, Thomy V. Electro-(de)wetting on Superhydrophobic surfaces. Langmuir, 2013, 29 (44), pp 13346–13351. DOI: 10.1021/la4026848

18) Lee SJ, Hong J, Kang KH, Kang IS, Lee SJ. Electrowetting-Induced Droplet Detachment from Hydrophobic Surfaces. Langmuir, 2014, 30 (7), pp 1805–1811. DOI: 10.1021/la404344y
19) Lehn JM (2007) From supramolecular chemistry towards constitutional dynamic chemistry and adaptive chemistry. Chem Soc Rev 36(2):151-160.
20) Leroux, Frédéric; Campagne, Christine; Perwuelz, Anne; Gengembre, Léon (2008). "Polypropylene film chemical and physical modifications by dielectric barrier discharge plasma treatment at atmospheric pressure". Journal of Colloid and Interface Science 328 (2): 412–20.doi:10.1016/j.jcis.2008.09.062. PMID 18930244.
21) Lombardi F, Herrmann HJ, Perrone-Capano C, Plenz D, de Arcangelis L. Balance between excitation and inhibition controls the temporal organization of neuronal avalanches. Phys Rev Lett. 2012 Jun 1;108(22):228703. Epub 2012 May 31.
22) Maktabi S, Schertzer JW , Chiarot PR. 2019. Dewetting-induced formation and mechanical properties of synthetic bacterial outer membrane models (GUVs) with controlled inner-leaflet lipid composition. Biochim Soft Matter. 2019 May 15;15(19):3938-3948. doi: 10.1039/c9sm00223e.
23) Pielak RM, Chou JJ. 2011. Influenza M2 proton channels. Biochim Biophys Acta. 2011 Feb; 1808(2): 522–529. doi: 10.1016/j.bbamem.2010.04.015.
24) Rahe P, Lindner R, Kittelmann M, Nimmrich M, Kühnle A. From dewetting to wetting molecular layers: C60 on CaCO3(10¯14) as a case study. Phys Chem Chem Phys. 2012 May 14;14(18):6544-8. doi: 10.1039/c2cp40172j.
25) Rego NB, Xi E, Patel AJ. 2019. Protein Hydration Waters Are Susceptible to Unfavorable Perturbations. J Am Chem Soc. 2019 Feb 6;141(5):2080-2086. doi: 10.1021/jacs.8b11448. Epub 2019 Jan 22.
26) Rosen MJ. (2004). Surfactants and Interfacial Phenomena (3rd ed.). Hoboken, New Jersey: Wiley-Interscience. p. 244. ISBN 978-0-471-47818-8. OCLC 475305499.
27) Rossman JS, Jing X, Leser GP, Lamb RA. 2010. Influenza virus M2 protein mediates ESCRT-independent membrane scission. Cell. 142 (6): 902–13. doi:10.1016/j.cell.2010.08.029
28) Sackmann E, Bruinsma RF. 2002. Cell adhesion as wetting transition? Chemphyschem. 2002 Mar 12;3(3):262-9.
29) Sándor C, Libál A, Reichhardt C, Olson Reichhardt CJ. 2017. Dewetting and spreading transitions for active matter on random pinning substrates. J Chem Phys. 2017 May 28;146(20):204903. doi: 10.1063/1.4983344.
30) Schnell JR, Chou JJ. 2008. Structure and mechanism of the M2 proton channel of influenza A virus. Nature, 451:591–595.
31) Schoeman D, Fielding BC. 2019. Coronavirus envelope protein: current knowledge. Virology Journal volume 16, Article number: 69.
32) Sharmaa A, Reiterb G. 1996. Instability of Thin Polymer Films on Coated Substrates: Rupture, Dewetting, and Drop Formation. Journal of Colloid and Interface Science. Volume 178, Issue 2, 25 March 1996, Pages 383–399. doi:10.1006/jcis.1996.0133
33) Tanaka M, Rehfeldt F, Schneider MF, Mathe G, Albersdörfer A, et al. Wetting and dewetting of extracellular matrix and glycocalix models. 2005 Journal of Physics: Condensed Matter Volume 17 Number 9. doi:10.1088/0953-8984/17/9/022
34) Thiam AR, Farese Jr RV, Walther TC. The biophysics and cell biology of lipid droplets. Nature Reviews Molecular Cell Biology 14, 775–786 (2013) doi:10.1038/nrm3699
35) Thomaston JL, Alfonso-Prieto M, Woldeyes RA, Fraser JS, Klein ML, Fiorin G, DeGrado WF. 2015. High-resolution structures of the M2 channel from influenza A virus reveal dynamic pathways for proton stabilization and transduction. Proceedings of the National Academy of Sciences of the United States of America. 112 (46): 14260–5.
36) Thompson CV. Solid-State Dewetting of Thin Films (2012). Annual Review of Materials Research. Vol. 42: 399-434. DOI: 10.1146/annurev-matsci-070511-155048
37) Tozzi A. 2015. Information Processing in the CNS: A Supramolecular Chemistry? Cognitive Neurodynamics 9 (5): 463–477.
38) Tozzi A, Fla Tor, Peters JF. 2016. Building a minimum frustration framework for brain functions in long timescales. J Neurosci Res, 94(8): 702–716.
39) Tozzi A. 2020. Towards Dewetting Monoclonal Antibodies For Therapeutical Purposes. Progress in Biophysics and Molecular Biology, 150:153-159. https://doi.org/10.1016/j.pbiomolbio.2019.09.001.
40) Vargas JN, Seemannab R, Fleury JB. Fast membrane hemifusion via dewetting between lipid bilayers. Soft Matter, 2014,10, 9293-9299
41) Young T, Hua L, Huang X, Abel R, Friesner R, Berne BJ. Dewetting transitions in protein cavities. Proteins [2010, 78(8):1856-1869]. DOI: 10.1002/prot.22699
42) Webb TI, Kshatri AS, Large RJ, Akande AM, Roy S, et al. Molecular mechanisms underlying the effect of the novel BK channel opener GoSlo: Involvement of the S4/S5 linker and the S6 segment. Proc Natl Acad Sci U S A. 2015 Feb 17;112(7):2064-9. doi: 10.1073/pnas.1400555112.
43) Zhu F, Hummer G. 2010. Pore opening and closing of a pentameric ligand-gated ion channel. Proc Natl Acad Sci U S A. 2010 Nov 16;107(46):19814-9. doi: 10.1073/pnas.1009313107. Epub 2010 Nov 1.