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Towards dewetting monoclonal antibodies for therapeutical purposes

Tozzi Arturo

Center for Nonlinear Science, Department of Physics, University of North Texas, 1155 Union Circle, #311427, Denton, TX, 76203-5017, USA

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ABSTRACT

Dewetting transition - a concept borrowed from fluid mechanics - is a physiological process that takes place inside the hydrophobic pores of ion channels. This transient phenomenon causes a metastable state that forbids water molecules to cross microscopic receptor cavities. This leads to a decreased conductance, a closure of the pore and, subsequently, severe impairment of cellular performance. We suggest that artificially-provoked dewetting transition in ion channel hydrophobic pores might stand for a molecular candidate to erase detrimental organisms, such as viruses, bacteria, and cancer cells. We describe a novel type of high-affinity monoclonal antibody, that: a) targets specific trans-membrane receptor structures of harmful or redundant cells; b) is equipped with lipophilic and/or hydrophobic fragments that prevent physiological water flow inside ion channels. Therefore, we achieve an artificial dewetting transition inside receptor cavities, that causes discontinuity within transmembrane ionic flows, channel blockage, and subsequent damage of morbid cells. As an example, we describe dewetting monoclonal antibodies that target the M2 channel of the Influenza A virus: they might prevent water from entering pores thus leading to virion impairment.

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In fluid mechanics, dewetting is a process occurring at solid–liquid or liquid–liquid interfaces. Dewetting stands for the rupture of the thin, liquid continuous film on the substrate surface, leading to formation of irregular patterns of droplets. The opposite process is called spreading. Four stages can be recognized as dewetting proceeds (Sharmaa and Reiterb (1996)): (a) film rupture; (b) hole expansion and coalescence to form a polygonal "cellular" pattern. The dry patches augment when the material is gathered in the rim surrounding the growing hole; (c) disintegration of polymer ridges into spherical drops, due to Rayleigh instability. The droplets size and spacing may vary over several orders of magnitude, since dewetting process starts from randomly formed holes inside the film. A two-tier surface exhibits a two-stage wetting transition: first impalement at microscale texture, then at nanoscale; (d) fingering instability of hole rims during their expansion (Sharmaa and Reiterb (1996)).

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. We show how this peculiar process can be artificially produced to alter the physiological activity of noxious pathogens, such as

E-mail addresses: tozziarturo@libero.it, Arturo.Tozzi@unt.edu.

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viruses, bacteria and tumoral cells. In particular, we suggest the manufacture of monoclonal antibodies (against cellular receptors) 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.

1. Factors influencing dewetting in physical systems

Various physical processes cause, modify and interphere with dewetting phenomena. One of the most important factors is the **spreading coefficient**. It settles on the spontaneous spreading and dewetting for an oil drop placed on a liquid substrate (i.e., water) with ambient gas. The formula of such coefficient S is (Rosen, 2004; Leroux et al., 2008):

$$S = \gamma_{gw} - \gamma_{go} - \gamma_{ow}$$

where γ_{gw} is the gas-water surface tension, γ_{go} is the gas-oil surface tension and γ_{ow} is the oil water surface tension (measured inside the fluids before they are brought in contact). When $S > 0$, spontaneous spreading occurs; in turn, dewetting occurs for $S < 0$. In most dewetting experiments, a thin polymer film is spun onto a substrate. Even in case of $S < 0$, the film does not dewet immediately when located in a metastable state, e.g., when the temperature is below the polymer glass transition temperature. Annealing this metastable film above its glass transition temperature increases the mobility of the polymer-chain molecules, leading to dewetting (Karapanagiotis and Gerberich, 2005).

Temperature. Solid films are usually metastable or unstable in their deposited state. When heated to higher temperatures, they dewet or agglomerate to form islands (Thompson, 2012). This process, driven by surface energy minimization, occurs via surface diffusion well below a film melting temperature, especially when the film is very thin. Films pre-patterning may contribute to the building of ordered arrays of particles and complex patterns of partially dewetted structures, providing insights into the effects of surface energy anisotropy and facets on shape evolution (Thompson, 2012).

Type of substrate (at room temperature). Rahe et al. (2012) reported the formation of extended molecular layers of C(60) molecules on a dielectric surface at room temperature. In sharp contrast to previous C(60) adsorption studies on prototypical ionic crystal surfaces, a wetting layer was obtained, when calcite (CaCO(3))(10 14) surface is chosen as the substrate. Non-contact atomic force microscopy data confirms an excellent match between hexagonal lattice of the molecular layer and peculiar unit cell dimensions. The difference observed microscopically upon C(60) adsorption on CaCO(3)(10 14) compared to other dielectric surfaces was explained by a macroscopic picture that takes into account surface energies. This example demonstrates that a simple surface-energy based approach provides a valuable estimate for choosing molecule-insulator systems suitable for molecular self-assembly at room temperature (Rahe et al., 2012).

Hole growth, fingering instability, drop diameters and film thickness. Sharmaa and Reiterb (1996) found an excellent match between theoretical predictions and experimental results concerning instabilities and various stages of dewetting of thin (<60 nm) films on coated substrates. Thin polystyrene films (>10 nm), prepared on silicon wafers with three different nano-sized (~1 nm) coatings, dewet spontaneously above the glass transition temperature, leading to the growth of cylindrical holes with wavy rims. In the case of films that are much thicker than the coating, the wettability of the substrate by polystyrene has no influence on the number density of holes. This is explained by the dominating influence of long-range Lifshitz–van der Waals interactions originating from the bulk substrate. In later stages, polygon diameter is rather independent of the coating: this is explained by the insensitivity of the first stages to the coating properties and by the competition between Rayleigh instability (leading to droplets) and drainage from ridges (leading to coalescence). On the contrary, hole growth, fingering instability and drop diameters are highly affected by the surface properties (wettability) of coatings. An increase in film thickness (h) decreases the number density of initial holes ($\alpha h-4$), increases the polygon diameter ($\alpha h2$), augments the drop diameter, and makes the fingering instability stronger.

Wettability of the fluid, vibration, superhydrophobic structures. Several natural superhydrophobic structures display hierarchical two-tier roughness (Boreyko et al., 2011). The wetting and dewetting properties of two-tier roughness is a function of working fluid wettability, where the surface tension of water/ethanol drops is tuned by the mixing ratio. When the ethanol concentration of deposited drops was gradually increased on one-tier control samples, the impalement of the microtier-only surface occurred at a lower ethanol concentration, compared with the nanotier-only surface. The corresponding two-tier surface exhibited two-stage wetting transition, first due to impalement of the microscale texture, then to the nanoscale one. Subsequently, the impaled drops were subjected to vibration-induced dewetting. Both drops impaling one-tier surfaces and two-tier roughness could not be dewetted. However, on the two-tier surface, drops impaling just the microscale roughness exhibited a full dewetting transition upon vibration. Therefore, it has been suggested that two-tier roughness is essential for preventing catastrophic, irreversible wetting of superhydrophobic surfaces (Boreyko et al., 2011).

Electrowetting and detachment of droplets from solid surfaces. Electrowetting on superhydrophobic surfaces (EWOSS) can be generated by an alternating current signal, often leading to droplet impalement (Lapierre et al., 2013). To avoid this unwanted occurrence, either superhydrophobic surfaces must show robustness to high pressure, or an external energy must be used to dewet the droplets. Lapierre et al. (2013) described a novel approach to actuate liquid droplets via a modulated EWOSS signal (MEWOSS). This technique, that allows dewetting caused by periodic vibrations induced through electrowetting actuation, permits the investigation of a huge range of superhydrophobic surfaces. Detachment of droplets from solid surfaces is a a crucial process in practical applications, such as heat transfer and digital microfluidics. Electrowetting actuations with square pulse signals were used to detach droplets from hydrophobic surfaces (Lee et al., 2014). The threshold voltage for droplet detachment turned out to be almost constant for various droplet volumes ranging from 0.4 to 10 μL. Also, droplets can be detached more easily when the width of applied pulse is well-matched to the spreading time. When the droplet was actuated by a double square pulse, the threshold voltage was reduced by ~20% from that for a single square pulse actuation. Finally, droplets can be detached from the solid bottom surface without using a top needle electrode (Lee et al., 2014).

Active matter wetting transition. Sándor et al. (2017) showed that, in the presence of random pinning substrates, sterically interacting self-propelled disks exhibit transitions among different states. In particular, we are in front of an example of active matter wetting transition: starting from a phase of separated cluster state, the disks can spread out and homogeneously cover the substrate. This transition is regulated by disk density, substrate strength, cluster size (that dips at the wetting-dewetting transition) and fraction of sixfold coordinated particles (that drops when dewetting occurs) (Sándor et al., 2017).

In sum, 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. In the sequel, the above-mentioned phenomena will permit us to build strategies to influence the dynamics of droplet formation inside biological channels of pathogenic agents.

2. Dewetting phenomena in biological structures

Dewetting processes have been identified in many biological contexts. In case of living agents, dewetting occurs at solid-liquid interface (i.e., cellular structures and the water surrounding them). Dewetting contributes to cellular layer stability and mechanical responses, therefore representing a physiological process that contributes to metabolic patterns and homeostasis. Cellular layer stability was investigated by Douezan and Brochard-Wyart (2012), who showed that cohesive cellular layers deposited on non-adhesive substrates are metastable and dewet with a mechanism of nucleation and growth of dry patches. Two mechanisms contribute to compromise the integrity of cellular layers: indeed, dewetting can be induced either chemically by a non-adhesive surface treatment, or physically by a decrease in the substrate rigidity. The interpretation of cellular opening dynamics in terms of dewetting of viscous films can be used to estimate parameters characterizing the mechanical response of cellular layers.

Cell–cell and cell–tissue contacts between extracellular matrix and cell surface glycocalix. Cell adhesion is controlled by an interplay among membrane elasticity, short-range (lock-and-key) forces mediated by cell surface receptors and short-/long-range nonspecific interactions (Sackmann and Bruinsma, 2002). Cell adhesion, once explored through simplified models that mimic cell and tissue surfaces, enable local measurements of cellular shape changes and adhesion forces. Cell adhesion can be described in terms of first-order dewetting transitions that allow formation of adhesion plaques and permit very low receptor densities. The glycocalix repeller molecules, with their dewetting force, play a key role for the control of adhesion transition and mechanical stability of the adhering cells, by relaxing the strength of the binding forces. Because the stress fibers that stabilize adhesion domains are counteracted by leverage enforced by hydrodynamic shear forces (Sackmann and Bruinsma, 2002), this means that dewetting processes stand for a crucial issue in cell adhesion.

Tanaka et al. (2005) assessed wetting and dewetting of hydrated biopolymer layers mediating cell–cell and cell–tissue contacts, i.e., the extracellular matrix and the cell surface glycocalix. The sum of the net effects of the various interfacial forces, that is referred to as the disjoining pressure, was used as a semi-quantitative measure to describe the thermodynamics of hydrated interlayers. Disjoining pressures were measured through external forces to maintain the equilibrium distance between two parallel surfaces (in case of living cells, the two parallel surfaces stand for two neighboring plasma membranes). Using artificial models of extracellular matrix and glycocalix, Tanaka et al. (2005) described stable cell–cell contacts in terms of the wetting (or spreading) of complex fluids on polymer surfaces. The adjustment of wetting interactions via thin hydrating layers enabled them to transform three-dimensional cell membranes into quasi-two-dimensional films on macroscopically large surfaces. Fine-tuning of local conditions at the interface allowed selective wetting of native cell membranes on microstructured polysaccharide films. In sum, this approach suggests a large potential for individual detection of biological functions in confined geometries.

Substrate stiffness. On a surface, living tissues can either form a droplet-like cell aggregate or spread as a monolayer of migrating cells (Alert and Casademunt, 2018). These processes of tissue wetting and/or spreading depend on the mechanical properties of the substrate, such as stiffness. Taking into account that cells exert larger active traction forces, Alert and Casademunt (2018) showed the occurrence of tissue wetting transition at a critical substrate stiffness that decreases with tissue size. On substrates with stiffness gradient, the tissue spreads faster on the stiffer side. Furthermore, the tissue can wet the substrate on the stiffer side, while dewetting from the softer one. The stiffer-side interface can transiently drag the softer-side interface toward increasing stiffness, against its spreading tendency. These two effects related to stiffness gradients result in directed tissue migration, i.e., a phenomenon that can be produced by both dewetting on the soft side and hydrodynamic interactions between tissue interfaces.

Lipid bilayers and droplets. The behavior of lipid bilayer is crucial to understand biological processes such as ions trafficking between cells (Vargas et al., 2014). The standard procedures to explore the properties of lipid bilayer and hemifused states, that typically use either supported membranes or vesicles, have several shortcoming in terms of bio-relevance or accessibility for measurements. Vargas et al. (2014) studied the formation of individual free-standing hemifused states between cell membranes, using an optimized microfluidic scheme that allows for simultaneous optical and electrophysiological measurements. At first, two model membranes were formed at a desired location within a microfluidic device, using a variation of the droplet interface bilayer technique. Then, the two model membranes were brought into contact, forming a single hemifused state. For all tested lipids, the hemifused state between free-standing membranes was created within hundreds of milliseconds, i.e. several orders of magnitude faster than previously reported. Formation of hemifused state was a two stage process, whereas the second stage can be explained as a dewetting process in no-slip boundary condition. The formed hemifusion states are long-living and can be triggered by the proper electric fields (Vargas et al., 2014).

Lipid droplets, i.e., the dispersed phase of an oil-in-water emulsion in aqueous cytosol, are almost ubiquitous intracellular organelles with fundamental roles in cellular metabolism. They store oil-based reserves of metabolic energy and components of membrane lipids. Lipid droplets are. Their unique architecture is characterized by an interface between the dispersed oil phase and the aqueous cytosol, that permits dewetting. This mechanism enables cells to use emulsified oil according to their requirements for metabolic energy or membrane synthesis. The regulation of phospholipid surfactants composition at the surface of lipid droplets is crucial both for their own homeostasis and for protein targeting to their surfaces (Thiam et al., 2013). This emphasizes the importance of basic biophysical principles of emulsions for lipid droplet biology.

Opening of transendothelial cell macroapertures (TEMs) in pathogenic bacteria. Pathogenic bacteria can cross from blood vessels to host tissues by opening of TEMs. To induce TEM opening, bacteria intoxicate endothelial cells with proteins that disrupt their contractile cytoskeletal network. Cell membrane tension is no longer resisted by contractile fibers, leading to the opening. Gonzalez-Rodriguez et al. (2012) modeled the opening of TEMs as a new form of dewetting. Their model predicted the minimum radius for hole nucleation, the maximum TEM size, and the dynamics of their opening, in agreement with experimental data. The physical model was then coupled with biological experimental data to reveal that proteins missing in metastasis control the line tension at the rim of the TEM and opposes its opening. More, their studies clearly show that, while liquid dewetting is irreversible, cellular dewetting is transient.

Hydrophobic binding cavity of protein active sites, channels

and receptors. The interactions of proteins and their phase behavior are influenced by their own relationships with the surrounding hydration waters. Rego et al. (2019) found that, akin to extended hydrophobic surfaces, proteins situate their hydration waters at the edge of a dewetting transition, making them susceptible to unfavorable perturbations. The strength of the unfavorable potential needed to trigger dewetting is roughly the same for all the assessed proteins, depending just on the width of the hydration shell being perturbed (Rego et al., 2019). Analyzing the solvation of protein active sites, Young et al. (2010) observed a drying transition in the narrow hydrophobic binding cavity of Cox-2. They identified five proteins that, in molecular dynamics simulations, underwent drying transitions in their active sites. Because these cavities need not to desolvate before binding hydrophobic ligands, they often exhibit very large binding affinities. Furthermore, the Authors demonstrated that drying in protein cavities is not unique to Cox-2. The dynamics of confined water in capillaries and nanotubes suggests that ion cell gating may involve not just modifications in the pore geometry, but also transitions between water-filled and empty states. Webb et al. (2015) studied the molecular mechanisms of GoSlo-SR-5-6, a novel large-conductance Ca2+-activated K+ channel agonist. According to their results, the bulky D ring of GoSlo inserts into a hydrophobic pocket between the S4S5L and S6C and perturbs the interaction between these two regions, resulting in channel opening and voltage sensor activation. A straightforward explanation supporting their suggestion could be a state of dewetting transition.

To provide another example from human pathogens, the heptameric structure of the small mechanosensitive channel of Escherichia coli (MscS) displays a relatively wide (7–15 Å), yet highly hydrophobic, transmembrane pore, that exhibits dewetting and low conductance (Anishkin and Sukharev, 2004). Indeed, irrespective of the initial conditions, simulations converged towards the same state of intermittent vapor-liquid transitions. The pore is water-filled in the wider part and empty in the narrow hydrophobic hole. The polar gain-of-function substitution L109S resulted in a stable hydration of the entire pore. Steered passages of Cl(−) ions through the narrow part of the pore consistently produced partial ion dehydration, when the proper force of 200–400 pN was used. This means that the crystal MscS structure does not represent an open state, and that the MscS gate (that is similar to the nicotinic ACh receptor) involves a vapor-lock mechanism. This means that limited changes of geometry or surface polarity can locally switch the regime between water-filled (conducting) and empty channels.

Summarizing this Section, a straightforward conclusion can be drawn. When water and ions are enclosed within the subnanometer, 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 crucial question is: what are the physiological implications of the dewetting transitions taking place inside the ion channels of living cells? 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).

3. Dewetting monoclonal antibodies come into play

The recently-developed concept of “supramolecular chemistry” suggests that complex chemical entities, including the ones correlated with dewetting transition, can be reversibly built, starting from molecular components bound together by labile non-covalent interactions (Lehn, 2007; Tozzi, 2015). Through self-organized, self-assembled and dynamic transitory processes, the storage of information occurs at the molecular level, while its retrieval, transfer and processing at the supramolecular one. Crowding-induced changes in self-assembling macromolecules point towards systems capable of generating well-defined functional supramolecular architectures (Foffi et al., 2013). Sequential self-organization on increasing scale in non-equilibrium systems leads to the emergence of novel features/properties at each level. Incorporating dewetting transitions in this perspective appears particularly relevant for the development of novel drugs. 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.

An example: building DEMA against influenza A virus M2 receptor. The transmural, pH-regulated Matrix-2 (M2) proton channel is a crucial element of influenza A virus envelope (Pielak and Chou, 2011; Rossman et al., 2010). The M2 channel is a homotetramer consisting of helices stabilized by two disulfide bonds. The extracellular N-terminal domain encompasses the residues 1–23, while the transmembrane segment (TMS) (residues 24–46) forms the pore of the ion channel and is one of the targets of amantadine (Fig. 1, **bottom**). In turn, the intracellular C-terminal domain (residues 47–97) plays roles in virus budding and assembly. The crucial residues are the imidazole of His37 (pH sensor) and the indole of Trp41 (gate). Activated by low pH, M2 leads to proton conductance essential for viral replication. Furthermore, M2 regulates the virion core acidity, contributes to receptor-mediated endocytosis, promotes viral replication inside the nucleus of the infected cell, supplies the formation of filamentous strains, membrane scission, stabilization and release of the budding virion (Thomaston et al., 2015; Ichinohe et al., 2010). M2 is the target of several broadly neutralizing antibodies against influenza A, even if clinical results are still lacking and/or under clinical development (Cho and Wrammert, 2016; Fiers et al., 2009). M2 is the target of anti-influenza A drugs too. In particular, aminoadamantanes are specific M2 channel blockers that lead to incomplete viral uncoating an failure in promoting infection (Cady et al., 2010). However, the M2 gene is susceptible to relatively frequent mutations, so that the emergence of resistant strains has led to partial discontinuation of aminoadamantanes (Homeyer et al., 2016).

We suggest to use DEMA as a novel drug against all the strains of Influenza A (Fig. 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 (Fig. 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

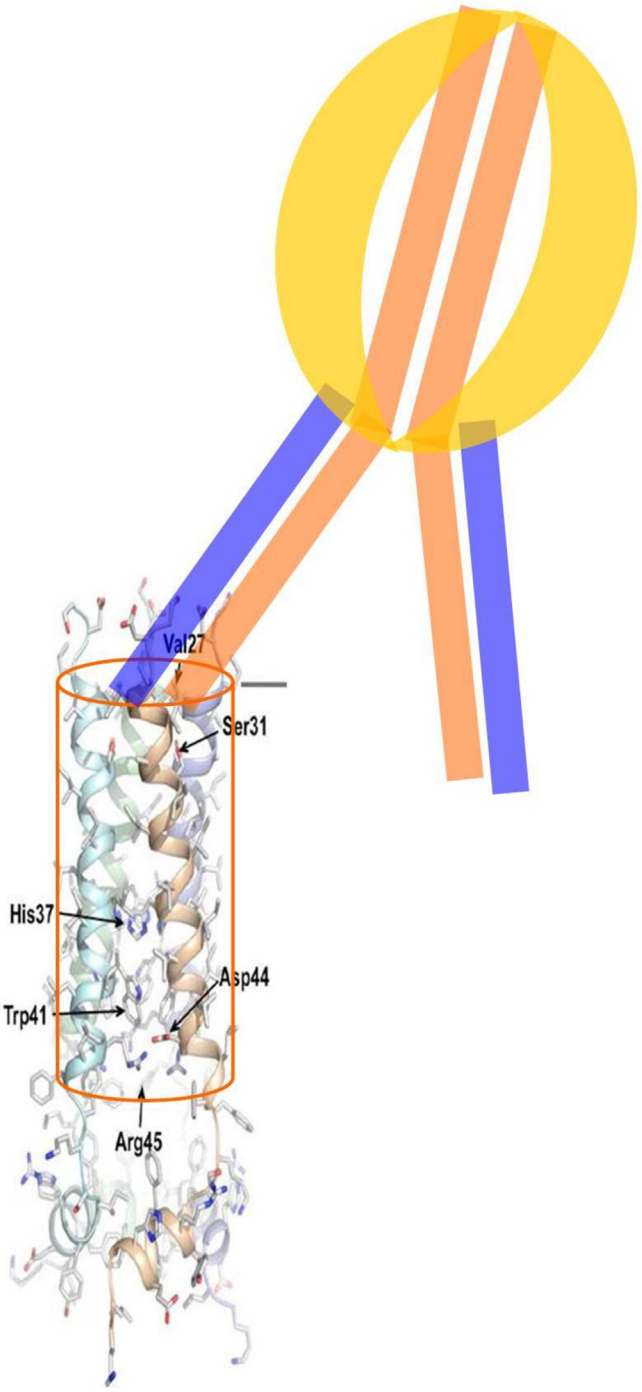

Fig. 1. Bottom: high resolution structures of the AM2 channel domain. Solution structure of residues 18–60 in 1,2-Dihexanoyl-sn-Glycero-3-Phosphocholine micelles at pH 7.5 (Modified from Pielak and Chou, 2011). **Top**: Dewetting antibody (DEMA) against the Val27 area. The Fc region of this artificial 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.

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

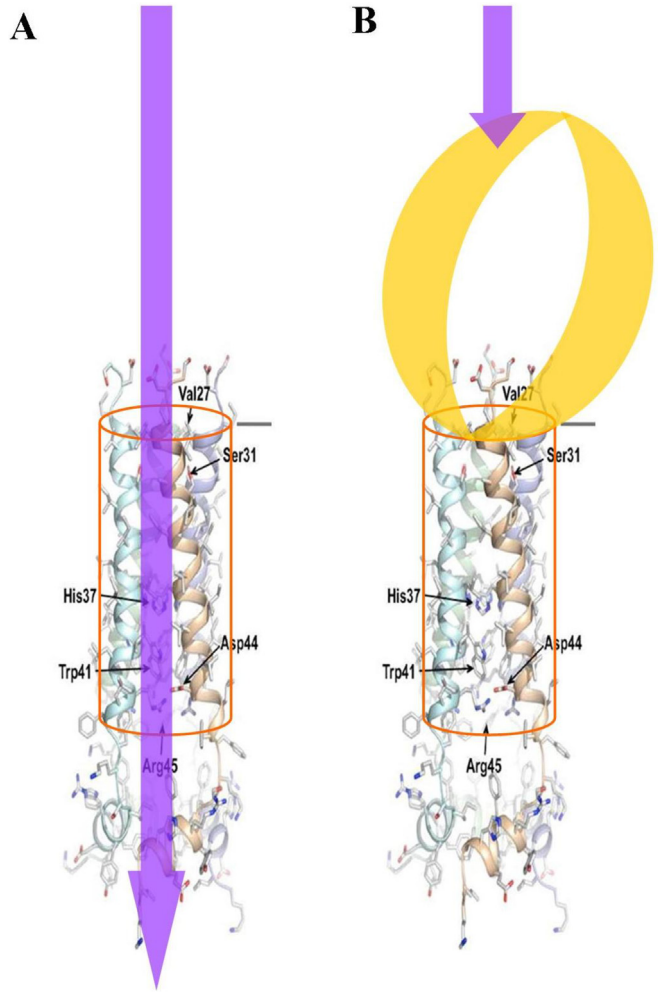

Fig. 2. Water flow inside M2 channel. **2A**: In physiological conditions, water is allowed to flow inside the M2 channel, giving rise to proton gradients crucial for pathogenic activity of Influenza A virus. **2B**: When a DEMA targets the upper part of the M2 receptor, its hydrophobic component prevents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

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 (Fig. 2B), leading to viral malfunction and, possibly, removal from the infected human body.

4. Conclusions

We showed how dewetting transition, characterized by an unusual behavior of water supramolecular assembly, stands for an increasingly important principle 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.

Several clues point towards the occurrence of dewetting transitions also in nervous cells. Indeed, it is believed that dewetting transition might occur in a wide range of ion channel receptors of the central nervous system (Aryal et al., 2015; Tozzi et al., 2016). Here we provide a few examples:

a) Pentameric ligand-gated ion channels (PLGC), that mediate fast neurotransmission in the nervous system. Nerve signaling in humans and chemical sensing in bacteria both rely on the controlled opening and closing of the ion-conducting pore in PLGC (Zhu and Hummer, 2010).
b) Tetrameric "P-loop" cation channels. This superfamily encompasses various potassium, sodium, and calcium selective channels, the non-selective TRP and cyclic-nucleotide-gated channels. The ability of these channels to select between different cations and to be gated by a diverse range of biochemical and biophysical stimuli enables them to play fundamental roles in the control of nearly all forms of cellular electrical activity.
c) Voltage-gated potassium channels.
d) Small conductance and large conductance Ca2+-activated channels.
e) MthK channels.
f) Two-pore domain channels: this is another subfamily of potassium channels thought to lack a classical bundle-crossing gate.
g) TWIK-1.

Neuronal activity is mediated through changes in the probability of stochastic transitions between open and closed states of ion channels (Cannon et al., 2010). Dewetting transition could lead to increase of inhibition in neurons, therefore influencing the inhibitory/excitatory (E/I) ratio (Xue et al., 2014). Excitation and inhibition are always matched: an optimal E/I ratio across neurons is maintained, despite fluctuating cortical activity levels, through the appropriate strengthening or weakening of inhibitory synapses. E/I ratio is stable, both in vitro and in the intact and spontaneously active cerebral cortex, not only in individual pyramidal neurons (Haider et al., 2006), but also across multiple cortical neurons (Xue et al., 2014) and during neural avalanches (Lombardi et al., 2012). The relationships between the two opposing forces in the mammalian cerebral cortex affect several cortical functions, such as feature selectivity and gain (Xue), memory of past activity. E/I ratio could thus be interpreted as the evidence of a homeostatic mechanism between strengthening and weakening processes in the adaptation of real synapses, both at the single neuron state and at the network excitability level (Lombardi et al., 2012; Tognoli and Kelso, 2014). Therefore, dewetting transition may stand for an important regulatory mechanism, that, influencing neuronal E/I ratio, allows the physiological activities of the brain. The possibility to modulate dewetting transitions through DEMA could be used to counteract neurological diseases through fresh developed drugs.

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) described a microfluidic technique for fabricating monodisperse asymmetric giant unilamellar vesicles, equipped with Gram-negative bacterial outer membrane lipid composition. They generated 50–150 μm diameter water-in-oil-in-water double emulsions that simulate the inner and outer leaflets of the lipid bilayer (Maktabi et al., 2019). Extraction processes by ethanol and micropipette aspiration triggered adhesive interaction between the two lipid monolayers assembled on the water-oil and oil-water interfaces (i.e., dewetting transition), forcing them to contact and to form lipid bilayer membranes that resemble Gram negative bacterial walls.

The last, but not the least, dewetting mechanisms can be used to achieve novel therapeutic weapons against a wide range of diseases. Apart from the above-described DEMA, other totally different approaches might take advantage of dewetting transitions. 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. For example, dewetting can be inhibited or prevented by factors other than DEMA: e.g., by photocrosslinking the thin film prior to annealing, or by incorporating nanoparticle additives into the film, or by modifying temperature, vibration, alternating currents, and so on. Also, surfactants may have a significant effect on the spreading coefficient: as more surfactant molecules are absorbed into the interface, the system free-energy decreases together with surface tension, eventually causing the system to become completely wet. And vice versa.

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