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Exploring G protein-coupled receptors and yeast surface display strategies for viral detection in baker's yeast: SARS-CoV-2 as a case study

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1. Abstract

Viral infections pose intense burdens to healthcare systems and global economies. The correct diagnosis of viral diseases represents a crucial step towards effective treatments and control. Biosensors have been successfully implemented as accessible and accurate detection tests for some of the most important viruses. While most biosensors are based on physical or chemical interactions of cell-free components, the complexity of living microorganisms holds a poorly explored potential for viral detection in the face of the advances of synthetic biology. Indeed, cell-based biosensors have been praised for their versatility and economic attractiveness, however, yeast platforms for viral disease diagnostics are still limited to indirect antibody recognition. Here we propose a novel strategy for viral detection in *Saccharomyces cerevisiae*, which combines the transductive properties of G Protein-Coupled Receptors (GPCRs) with the Yeast Surface Display (YSD) of specific enzymes enrolled in the viral recognition process. The GPCR/YSD complex

might allow for active virus detection through a modulated signal activated by a GPCR agonist, whose concentration correlates to the viral titer. Additionally, we explore this methodology in a case study for the detection of highly pathogenic coronaviruses that share the same cell receptor upon infection (*i.e.,* the Angiotensin-Converting Enzyme 2, ACE2), as a conceptual example of the potential of the GPCR/YSD strategy for the diagnosis of COVID-19.

2. Introduction

Biosensors are analytical devices that employ a biological recognition element (e.g., enzymes, antibodies, DNA/RNA, whole cells, etc.) and respond to a target compound by generating a detectable signal that is typically proportional to the concentration of the analyte. Since the development of the first biosensor for glucose detection in the 1960s, biosensors have been extensively studied in multiple areas of research, attaining commercial success in clinical, food, environmental, and biothreat analysis (Bahadır and Sezgintürk 2015).

In this context, developing biosensors for detecting viruses is particularly significant since the diagnosis of viral diseases is notably challenging. As viral infections often lead to generic symptoms, detection relies on time-consuming, expensive, or highly complex processes (Kievits *et al.* 1991). To date, different biosensing strategies have already been proposed for the detection of a wide range of viruses with much success, including Human Immunodeficiency Virus (HIV), Hepatitis virus, Ebola virus, Zika virus, Influenza virus, Dengue virus, West Nile virus, and others (Caygill, Blair and Millner 2010; Saylan *et al.* 2019; Castillo-Henríquez *et al.* 2020; Cesewski and Johnson 2020; Khan *et al.* 2020; Ozer, Geiss and Henry 2020; Qureshi and Niazi 2020; Ribeiro *et al.* 2020). Among these strategies, relevant examples of already commercialized biosensors for the diagnosis of viral diseases in humans are the Influenza A and B virus (Quidel; Corisbio a; Krejcova *et al.* 2012), HIV (OraQuick; Chinamedevice; Haleyur Giri Setty and Hewlett 2014), and Adenovirus tests (Corisbio b), which enable direct quantitative viral detection in respiratory specimens. In fact, biosensor-based diagnostics of viral diseases have proven to be so valuable - for the development of inexpensive, sensitive, rapid, miniaturized, and portable Point-Of-Care (POC) tests - that this technology has been

widely accessed for the detection of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), responsible for the Coronavirus Disease 2019 (COVID-19). Current biosensing approaches and opportunities for COVID-19 testing have been deeply reviewed elsewhere (Bhalla *et al.* 2020; Chauhan *et al.* 2020; Cui and Zhou 2020; Fani *et al.* 2020; Morales-Narváez and Dincer 2020; Torabi *et al.* 2020).

Over the last two decades, enormous advances in nanotechnology, materials science, and genetic engineering tools have turned microbial biosensors into an increasingly hot topic in many areas of research, from environmental monitoring and food control to medical and biotechnological applications (Chang *et al.* 2017). Among the advantages of using microbes as biosensors are their low cost and ease of production, including the possibility of applying genetic and/or evolutionary engineering techniques to improve selectivity and sensitivity towards target compounds (Ault and Broach 2006; Fukuda *et al.* 2011). Additionally, unlike other detection technologies, microbial biosensors open up the possibility of high-throughput screening, enabling testing of multiple cell designs, thereby assisting synthetic biology applications.

The use of yeast in biosensors design offers powerful alternatives, given the ease of their genetic manipulation, high tolerance towards harsh conditions, eukaryotic nature, and the possibility of storage for long periods of time. Particularly, *Saccharomyces cerevisiae* is the most common chassis of choice due to the wealth of information as a model organism, numerous genome-editing tools, and its Generally Recognized As Safe (GRAS) status. In addition, *S. cerevisiae* has been exploited in virus research not only as a model system for understanding basic biological processes triggered by viruses, but also as a screening tool for antiviral drugs, as a production system for recombinant viral antigen, and as a vaccine vehicle (Galao *et al.* 2007).

Most common yeast biosensing strategies involve the expression of a reporter gene under the control of an inducible promoter, which is activated in the presence of the target compound either via direct ligand-receptor interaction or mediated by a signaling pathway coupled to G Protein-Coupled Receptors (GPCRs) (Adeniran, Sherer and Tyo 2015). In the former case, detection of the target compound occurs

within the intracellular space, whereas in the latter, sensing happens through membrane-bound receptors. In addition to GPCRs, another useful extracellular system for biosensing applications is the Yeast Surface Display (YSD) of reporter proteins or enzymes on cell membranes, the expression of which can also be controlled in response to the target compound (Park 2020). Although less explored in synthetic biology, examples of transcription-independent endeavors for yeast biosensing can be found elsewhere (Adeniran, Sherer and Tyo 2015). While these strategies were proven efficient for numerous applications, the complexity of active virus detection remains a challenge for yeast-based viral diagnostics so far. **Table 1**, which congregates some of the most prominent cell-based biosensors for the detection of human-pathogenic viruses, accounts for only one yeast biosensing strategy that targets an antibody response.

In this work, we explore how the integration of both the GPCR and YSD-based endeavors into a single yeast cell could represent a novel strategy for broadening the yeast biosensing opportunities for whole virus detection. Subsequently, we assess how this strategy could potentially be effective for the diagnosis of challenging viral diseases with the design of a conceptual biosensor for SARS-CoV-2 detection that we have named 'CORONAYEAST'. To the best of our knowledge this is the first known attempt in using yeast as a whole-virus biosensing platform.

3. The GPCR-based approach in yeast biosensors

GPCRs constitute a large family of integral proteins with seven-transmembrane α-helical domains present in eukaryotes. These receptors are responsible for sensing a variety of extracellular signals with high selectivity, including nutrients, hormones, neurotransmitters, light, taste compounds, and odorants, thereby regulating important physiological processes. Due to their role in diverse diseases, GPCRs are targets for more than 30% of prescribed pharmaceuticals, which function as GPCR agonists or antagonists (Hauser *et al.* 2017). Upon ligand binding to a specific GPCR, diverse intracellular signaling pathways are activated leading to the modulation of different target effectors, such as adenylate cyclases or Mitogen-Activated Protein Kinases (MAPKs), ultimately

triggering a cellular response (Pierce, Premont and Lefkowitz 2002). This activation is mediated by GPCRs in association with heterotrimeric guanine nucleotide-binding proteins (G proteins) - peripheral proteins composed of Gα, Gβ, and Gγ subunits - highly conserved among eukaryotes (Versele, Lemaire and Thevelein 2001).

The GPCR systems identified in yeast are involved in glucose and pheromone sensing (Versele, Lemaire and Thevelein 2001), the latter being the most relevant for biosensor design. Glucose sensing and signaling in *S. cerevisiae* is mediated by the GPCR/G protein pair Gpr1/Gpa2 (Gα), with no Gβ and Gγ proteins associated (Gancedo 2008). [For more detailed studies on glucose sensing and signaling by Gpr1/Gpa2, please refer to Versele, Lemaire and Thevelein 2001; Rolland, Winderickx and Thevelein 2002; and Gancedo 2008].

The pheromone-mating pathway, in turn, is the mechanism employed by haploid *S. cerevisiae* to detect cells of the opposite mating type in order to fuse their plasma membrane and nuclei to form a diploid cell. While *MAT*a cells express the α-factor receptor (Ste2) to sense α-factor pheromones, *MAT*α cells detect a-factor pheromones by expressing a-factor receptors (Ste3). These two GPCRs (Ste2 and Ste3) are coupled to the same G protein, composed of Gpa1 (Gα), Ste4 (Gβ), and Ste18 (Gγ) subunits, engaging the same downstream components. When binding to its specific pheromone, a conformational change in the receptor results in the dissociation of the Ste4/Ste18 dimer from the complex and the activation of the G protein. Free Ste4/Ste18 can now associate with three different effectors and initiate signal propagation. As a result, the effectors are recruited to the plasma membrane activating a MAPK cascade, that causes the phosphorylation of the Ste12/Dig1/Dig2 transcription factor complex to induce the expression of hundreds of pheromone-responsive promoters (Bardwell 2005).

Many yeast-based biosensors leverage the similarities between yeast and mammalian signaling (Versele, Lemaire and Thevelein 2001) and couple the native yeast pheromone pathway to heterologous GPCRs from higher eukaryotes (Lengger and Jensen 2020). By introducing reporter genes under the control of pheromone-responsive promoters, for instance, FIG1 or FUS1, the presence of an external analyte can be easily accompanied by the expression of the reporter gene via colorimetric (□-galactosidase and

carotenoids), fluorescent (GFP, YFP, RFP), or luminescent readouts (bacterial and firefly luciferase) (Nakamura, Kondo and Ishii 2018). Nevertheless, to guarantee functional coupling of heterologous GPCRs to yeast signaling, some additional engineering is required, as extensively reviewed by Lengger and Jensen (2020). Recently, a model yeast cell for tuning GPCR signaling was constructed harboring a minimal set of signaling components (Shaw ***et al.*** 2019).

Biosensor strategies targeting GPCR systems are of special interest for medical/health research, including pathogen detection and drug discovery (Doijen *et al.* 2019; Lengger and Jensen 2020). Indeed, the role of mating GPCRs on fungal virulence in mammalian and plant hosts (Brown *et al.* 2018) was harnessed by Ostrov and colleagues to detect pathogenic fungi (2017). In this remarkable work, the authors established a highly specific and sensitive *S. cerevisiae*-based biosensor for the detection of pathogen-derived peptides (fungal pheromones) in complex samples coupled to a readout visible to the naked eye.

In a similar manner, many viruses exploit human GPCR systems to their own benefit, taking control over the downstream signaling pathway to ultimately ensure their successful propagation (Sodhi, Montaner and Gutkind 2004). A typical example is the chemokine receptor CCR5 - the primary co-receptor of HIV - which is crucial for viral entry and pathogenesis (Berger, Murphy and Farber 1999). Not surprisingly, viruses have also evolved to encode their own GPCRs in order to evade the host's immune response and assist virus dissemination (Sodhi, Montaner and Gutkind 2004).

Despite the role of GPCRs in viral pathogenesis, GPCR-based biosensors have been neglected for viral detection, which might be due to an insufficient understanding of the virus-receptor-ligand relationship or of the signaling pathway that is induced. Furthermore, the dependence on GPCRs (either cellular or viral) to gain entry to cells is currently limited to a group of viruses, while many others employ strategies independent of intracellular signaling (Grove and Marsh 2011).

4. The YSD approach in yeast biosensors

Besides GPCR-based biosensing techniques, surface display of peptides or proteins has allowed a plethora of applications in yeast in the last decade. Regarding the use of such strategy for the sensing of extracellular stimuli, *S. cerevisiae* has presented advantages in comparison to bacterial platforms because of its larger cell size that allows practical microscopic observations or cell sorting for quantitative measurements using flow cytometry (Shibasaki 2019). Also, the post-translational modification machinery of yeasts grants the correct folding and secretion of glycosylated proteins that harbor multiple disulfide bonds, which makes YSD an interesting alternative for the expression of complex eukaryotic proteins (Han *et al.* 2018). These properties have endowed *S. cerevisiae* with biosensing abilities using cell surface engineering.

Overall, most yeast display systems consist of an expression cassette with a strong promoter controlling the coding region of a signal peptide fused to an anchor protein and the protein that should be displayed on the cell membrane surface. Generally, this strategy uses structures known as glycosylphosphatidylinositols (GPIs) - normally covalently linked to the C-terminus of proteins - which provide stable bonds between these proteins and the cell membrane. Yeasts offer multiple options as cell surface anchor proteins, including Aga1p, Aga2p, Cwp1p, Cwp2p, Tip1p, Flo1p, Sed1p, YCR89w, and Tir1p (Cherf and Cochran 2015). Usually, the coding sequence of an epitope is also introduced in the expression cassette to allow the exposure of the displayed protein in immunological assays (Yang *et al.* 2019). The fusion of the protein of interest to the GPI-anchor protein generally results in the exposure of up to 100,000 copies of the fused protein to the cell surface of *S. cerevisiae* (Boder and Wittrup 2000).

Traditionally, YSD technology has enabled the development of biosensors through a signal-transducing element activated by a promoter region of a gene that responds to environmental changes (Shibasaki 2019). This approach allowed the successful application of *S. cerevisiae* as a glucose cell sensor, where the expression of a fluorescent protein-encoding gene inserted between a secretory signal sequence and the α-agglutinin-encoding gene was induced by the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) promoter (for glucose-rich

conditions) or by the *Candida tropicalis* isocitrate lyase (UPR-ICL) promoter (for glucose depletion conditions) (Ye *et al.* 2000). A similar strategy has already been proposed for sensing ammonium and phosphate ions (Shibasaki *et al.* 2001).

Although important advances have been made using the signal transduction methodology, it represents a narrow possibility regarding biosensing activity - especially with respect to virus recognition. Extracellular modifications caused by viral infections are complex and hardly capable of being naturally sensed by yeasts to promote cell surface signaling. It is important to note that a virus receptor is not necessarily a protein that interacts with some ligand for signaling purposes or cell-cell interaction but acts by providing a point of attachment to the target cell and enabling fusion events (Coffin 2013). A yeast-based active virus biosensor would require more features other than the receptor surface display to induce signaling. An alternative could be the use of intracellular artificial transcription factors, as the one described by Matsunaga *et al*. in mammalian cells (Matsunaga *et al*. 2020).

The YSD strategy expands its applicability when dealing with protein-protein interactions, hence posing as a promising alternative for using yeasts as viral detection biosensors. The display of the ZZ domain derived from *Staphylococcus aureus* on *S. cerevisiae* cell surface has been used to adsorb IgG type antibodies and used as an immunoadsorbent for Enzyme-Linked ImmunoSorbent Assay (ELISA) of antibodies or for sandwich ELISA of antigens (e.g. human serum albumin) (Nakamura *et al.* 2001). Yeast cells expressing both single-chain variable fragment (scFv) antibodies and gold-binding peptide (GBP) on its cell surface have been successfully employed to detect *Salmonella* TM43-E10 surface antigen through sandwich format (Venkatesh *et al.* 2015). More recently, a modified yeast was described to display Hepatitis C Virus (HCV) core antigen linked to GBP as a dual-affinity biobrick chimera, able to detect anti-HCV core antibody as an optical or electrochemical immunoassay tactic (Aronoff-Spencer *et al.* 2016). These biobrick approaches pose an interesting alternative for using yeast as POC devices. Following the use of antibodies, nanobodies - camelids small (15 kDa) and stable single-domain IgG fragments - have also been reported as useful tools for *in vitro* application in yeast-derived diagnostic kits (McMahon *et al.* 2018; Uchański *et al.* 2019).

Advances in using *S. cerevisiae* as a genetically renewable whole-cell immunoadsorbent could lead to exciting applications for POC immunoassay diagnostics. However, when dealing with newly discovered diseases, the utilization of antibodies or antigens for diagnostic purposes possesses an important drawback, since extensive knowledge on immunological patterns is required for these applications. When confronted with a pandemic situation, such as the COVID-19 outbreak, whole-virus detection represents a more effective and sensitive tool for disease control. Even so, no yeast-based strategy has yet been described. Next, we assess how a combination of the GPCR and the YSD-based strategies could represent an innovative alternative to harness *S. cerevisiae* as a virus biosensing module.

5. A novel GPCR/YSD combined approach in yeast biosensors

It is well known that virus infections are mediated by their binding to specific surface molecules on target cells, in a highly specific manner. These receptors are usually proteins required by the cell for some normal function and are co-opted by the virus during its entry (Coffin 2013). Given the viral infection mechanism and yeast's biosensing capabilities, a sole GPCR or YSD strategy falls short in detecting the complex and specific changes of this pathogenicity scenario.

Therefore, here we contemplate how the combination of both above-disclosed strategies in a GPCR/YSD complex could broaden the possibilities of using *S. cerevisiae* as a detection platform for whole viruses. By harnessing the signaling sensitivity and specificity of GPCRs with the multiple options of cell surface immobilization, virus detection by engineered yeast cells could be indirectly determined through the sensing of metabolite imbalances caused by the dual activity of a displayed protein - that could act as a viral receptor or as a catalytic enzyme. In other words, an immobilized protein that has a natural catalytic activity and also enrolls in viral binding might have its function altered in the presence of the virus, leading to environmental changes that might be transduced by a GPCR system. A graphic scheme of this strategy is presented in **Figure 1.**

The GPCR/YSD strategy showcases the possibility of magnifying the range of GPCR-based biosensors for pathogen detection, thereby enabling the use of rapid *S. cerevisiae*-based detection tests, similar to the one described by Ostrov *et al*. (2017), for the recognition of pathogens that do not necessarily bear a GPCR system. This is the case for important prokaryotic pathogens (bacteria, archaea, and protozoa) and most viruses, which together account for most of the agents responsible for infectious diseases worldwide (Wolfe, Dunavan and Diamond 2007; Jones *et al.* 2008).

Interestingly, such a GPCR/YSD complex strategy finds a parallel in the traditional concept of a biosensor in which two modules, known as the bioreceptor and the transducer, are employed for functional sensing. Even though the yeast cell performs as both the receiving and transducing elements - as is the case for all cell-based biosensors - it is indisputable that within the cell such modules can be identified. In this correlation, the yeast display of viral-interacting proteins functions in a similar fashion as a receptor module - directly interacting with the virus, while the GPCR acts as the transducer, ultimately leading to the prompt of the output protein through the sensing of a by-product.

In the next section, we envision how such a strategy could be employed for the detection of SARS-CoV-2 and, potentially, other viruses that use the same cell receptor upon infection. The conceptual example, named CORONAYEAST, was based on the recent findings on the host-virus interaction patterns of the highly impacting pandemic pathogen responsible for the COVID-19 outbreak, serving as a theoretical baseline that might be exported for other viral infections. The development of this concept could lead to actual physical diagnostics devices when exposed to the well-known design-build-test-learn cycle.

6. CORONAYEAST: an *S. cerevisiae*-based diagnostic concept for COVID-19

By January, 2021, SARS-CoV-2 has infected more than 90 million people and caused nearly 2 million deaths worldwide, posing a threat not only to healthcare systems but also to the global economy. Confronted with this scenario, we showcase how we could benefit from the GPCR/YSD strategy for the development of a COVID-

19 diagnosis platform, based on an *S. cerevisiae* biosensor that here we call CORONAYEAST. This biosensor concept is built upon the GPCR/YSD strategy for enabling the sensing of a target that is not naturally sensed by conventional - GPCR-only or YSD-only - concepts: in this case, a whole virus. It stands out as a potential application example of how the GPCR/YSD strategy could be decisive for the development of biosensors for highly-important economic and social purposes.

In summary, the biosensor functioning is based upon the dual role played by the Angiotensin-Converting Enzyme 2 (ACE2) in human cells. It is the SARS-CoV-2 sole receptor (Li *et al.* 2003) as well as the effector of Angiotensin II (Ang II) homeostasis within the Renin-Angiotensin System (RAS) (Burrell *et al.* 2004). In this system, Ang II acts as the agonist of the human Ang II GPCR type 1 (AT_1) (Zhang *et al.* 2017), which triggers a cell response through the activation of a signaling cascade. The heterologous expression of ACE2 and AT_1 in the yeast strain would allow the mimicking of the human RAS via the GPCR/YSD strategy. Coupled with the downstream insertion of easy-to-observe and -interpret reporter genes, this strain could represent a promising alternative for viral detection.

The following sections cover in more depth (1) the motivation behind this concept, (2) the mechanism behind SARS-CoV-2 infection, and the RAS imbalance that allowed for its development, and (3) how the GPCR/YSD strategy could be implemented for developing the CORONAYEAST.

Why build a SARS-CoV-2 biosensor?

SARS-CoV-2 can be transmitted through several different routes (van Doremalen *et al.* 2020; Wang *et al.* 2020b; Xiao *et al.* 2020). On average, each infected person transmits the virus to 1.4 - 6.49 people (Liu *et al.* 2020; Wu, Leung and Leung 2020), while the incubation period ranges from 4.4 to 5.5 days (Jiang, Rayner and Luo 2020). On top of that, advances in the understanding of SARS-CoV-2 immunity as a non-permanent short-termed protection raise concerns regarding the pandemic and post-pandemic transmission dynamics of COVID-19 (Long *et al.* 2020b; Seow *et al.* 2020; Vabret 2020), which may continue affecting communities as late as 2025 (Kissler *et al.* 2020). The inconsistency of symptoms, the sharing of symptoms with other diseases (Udugama *et al.* 2020), and the high rates of asymptomatic cases (Bai *et al.* 2020; Mizumoto *et al.* 2020; Nishiura *et al.* 2020)

account for the importance of the application of accurate diagnostic methods based on the direct or indirect detection of the virus, as a way to contain COVID-19 transmission.

COVID-19 testing is currently performed worldwide using three different methodologies: nucleic acid-based tests, immunoassays, and Computed Tomography (CT) scan.

Nucleic acid-based diagnostics - such as RT-PCR and CRISPR-based tests - target specific RNA molecules. RT-PCR tests present high accuracy rates; however, they depend on RNA extraction and expensive equipment/reagents, pose biological safety hazards, do not test for active viral particles (test solely for the presence of the viral genetic material), and undergo a long waiting time for results, especially due to the accumulation of tests in test centers, bottlenecking the delivery of results (Yang and Rothman 2004; Li *et al.* 2020). Presently, new RT-PCR-based tests are emerging, yet a methodology that overcomes all these caveats is yet to be developed (Lu *et al.* 2020; Vogels *et al.* 2020; Yu *et al.* 2020). On the other hand, CRISPR-based tests usually rely on CRISPR/Cas12 (type V) or CRISPR/Cas13 (type VI) systems (Broughton *et al.* 2020; Ding *et al.* 2020; Hou *et al.* 2020; Xiang *et al.* 2020). These tests are highly accurate and have surpassed many caveats intrinsic to the RT-PCR tests, such as timing and price range. However, they are also dependent on RNA extraction, do not test for active viral particles, and are highly-susceptible to unknown genomic variations that may produce critical negative impacts on the assay efficiency. Immunoassays, such as ELISA and Rapid Detection Tests (RDTs) - lateral flow assays - have an economical and practical appeal; however, are imprecise and ineffective for the diagnosis of early infections and active cases (Lv *et al.* 2020; Tang *et al.* 2020b), since seroconversion is only reached approximately 13 days after symptom onset (Long *et al.* 2020a). Ultimately, COVID-19 diagnostics via the combination of clinical symptoms analysis and chest CT scan is not capable of targeting or identifying specific viruses and is infeasible in asymptomatic cases (Li and Xia 2020).

In this scenario, the development of a diagnostic method that associates high accuracy and sensitivity with cost-effectiveness and handiness has become the target of a new technological race. Therefore, fast and valuable work towards the

development and commercialization of efficient biosensors capable of detecting the whole active SARS-CoV-2 virus, its antigens, or its genetic material are currently being investigated (Jiao *et al.* 2020; Mavrikou *et al.* 2020; Moitra *et al.* 2020; Qiu *et al.* 2020; Seo *et al.* 2020). Even so, microorganism-based approaches for SARS-CoV-2 sensing, such as yeast-based biosensors, are still an unexplored field that could offer high sensitivity, low cost, and equipment independence.

In this context, the full understanding of the physiological outcomes of the SARS-CoV-2 infection, more specifically, its effects over the Ang II levels allow us to contemplate the application of the GPCR/YSD concept for the development of such a system. Following, we will cover this issue as an important foundation for the development of CORONAYEAST.

How does SARS-CoV-2 affect Ang II levels? A brief overview on the infection physiology

Coronaviruses comprise a single-strand positive-sense RNA genome that encompasses four open reading frames encoding structural proteins as well as genes responsible for the expression of non-structural proteins (Su *et al.* 2016). Similarly to other human-pathogenic strains of coronavirus (Forni *et al.* 2017), such as SARS-CoV and HCoV-NL63 (Cui, Li and Shi 2019), SARS-CoV-2 binding to host cells is mediated by the adhesion of the viral surface glycoprotein, Spike, to the ACE2 protein (Li *et al.* 2003; Wu *et al.* 2009; Zhang *et al.* 2020).

The Spike protein is a trimer, composed of three S1 receptor-binding subunits placed over an S2 stalk - which contains the necessary elements for membrane fusion (Li 2015; Shang *et al.* 2020a). SARS-CoV-2 attachment to host cells occurs through a high-affinity association (Lu, Wang and Gao 2015; Shang *et al.* 2020b; Zhang *et al.* 2020) between the SARS-CoV-2 Spike protein S1 unit C-terminal domain (CTD) and the peptidase domain (PD) of ACE2 (Lan *et al.* 2020; Letko, Marzi and Munster 2020; Wang *et al.* 2020a; Zhang *et al.* 2020). Subsequently, SARS-CoV-2 entry into the host cells takes place through membrane fusion, a process mediated by the proteolytic activation of the boundary between S1 and S2 subunits (Hoffmann *et al.* 2020; Ou *et al.* 2020; Wang *et al.* 2020a).

Apart from functioning as a viral receptor during infection, ACE2 essentially functions as a transmembrane type I glycoprotein (mono-carboxypeptidase), sharing a 40% structural identity with the Angiotensin-Converting Enzyme (ACE) (Tipnis *et al.* 2000; Burrell *et al.* 2004). Both enzymes are predominantly involved in the Ang II homeostasis in the RAS, in which they perform antagonistic roles (Santos, Campagnole-Santos and Andrade 2000). While ACE cleaves the physiologically inactive decapeptide Angiotensin (Ang I) into the vasoactive octapeptide Ang II (Skeggs Jr., Kahn and Shumway 1956; Bakhle 1968; Yang, Erdös and Levin 1970), ACE2 cleaves a single residue from Ang II C-terminus to generate Angiotensin$_{(1-7)}$ (Ang 1-7).

Ang II acts as a potent vasoconstrictor and pro-inflammatory particle (Donoghue *et al.* 2000) through the sensing and activation of the AT_1 receptor (Chiu *et al.* 1991; Zhang *et al.* 2017). Ang 1-7 counteracts Ang II effects (Ferrario *et al.* 1997, 1998) through an efficient binding to the GPCR Mas (Mas receptor) (Santos *et al.* 2003). In addition, the Ang II GPCR type 2 (AT_2 receptor) also plays an important role in the RAS homeostasis by counterbalancing the cellular responses triggered by the AT_1 receptor, in a similar fashion to the Mas receptor (Carey, Wang and Siragy 2000; Higuchi *et al.* 2007; Zhang *et al.* 2017). The AT_2 receptor was believed to be activated solely by Ang II. However, recent studies also describe Ang 1-7 as a lower affinity ligand of the AT_2 receptor (Bosnyak *et al.* 2011; Villela *et al.* 2015). A visual scheme illustrating how ACE2 compensates ACE's function by negatively regulating Ang II can be found in **Figure 2.**

SARS-CoV-2 attachment to ACE2 receptor in the host cell leads to its downregulation and consequent increase in Ang II levels and activation of the AT_1 receptor (Imai *et al.* 2005; Kuba *et al.* 2005; Glowacka *et al.* 2010). However, the mechanisms behind the decrease in ACE2 expression are still unclear, as the CTD-PD association does not physically inhibit the catalytic site of ACE2 (Li *et al.* 2005). The most accepted explanations for this phenomenon are (1) the ACE2 internalization upon viral infection mechanism, which induces a loss of the catalytic effect of the receptor at the external site of the membrane (Imai *et al.* 2005; Zhang *et*

al. 2020) or (2) the degradation of the ACE2 receptor following the formation of the receptor-glycoprotein complex, as suggested by Glowacka *et al* (2010).

The Ang II asymmetry on the RAS caused by the reduced ACE2 activity related to SARS-CoV-2 infection is a common outcome of the COVID-19 disease and is discussed in several papers as a potential contributor to the manifestation of the Acute Respiratory Distress Syndrome (ARDS), due to Ang II importance on severe lesions in the respiratory tree (alveolar wall thickening, edema, infiltrates of inflammatory cells, bleeding) (Imai *et al.* 2005; Kuba *et al.* 2005; Lin *et al.* 2018; Gheblawi *et al.* 2020; Tay *et al.* 2020).

On top of its physiological outcomes, the exacerbation of the *CTD-ACE2-Ang II-AT$_1$* axis due to the viral infection stands out not only as a potential therapy target but also as a pivot for the development of novel COVID-19 diagnostics tests, such as CORONAYEAST, which recognizes and marks the viral presence through Ang II sensing.

How could the GPCR/YSD principle be implemented for the COVID-19 diagnosis?

As previously presented, the development of the yeast biosensor concept emerges from understanding the two different roles of ACE2 in the human organism: (1) as an essential component of the RAS, mediating Ang II degradation into Ang 1-7 (Tikellis and Thomas 2012); and (2) as a viral receptor (Hoffmann *et al.* 2020), in an infection scenario. The formation of the ACE2-CTD complex downregulates the enzyme's catalytic function, causing higher concentrations of Ang II to more effectively activate AT_1 (Ciulla 2020; Zhang *et al.* 2020). The *CTD-ACE2-Ang II-AT$_1$* axis was therefore chosen as the starting point for our conceptual yeast-based biosensor for ACE2-dependent coronaviruses presented here.

We predict that the heterologous expression of ACE2 at the yeast membrane surface would allow for its participation in both the viral binding and the catalysis of available Ang II. On the other hand, the replacement of the yeast's pheromone sensing GPCR with the human AT_1 would allow for Ang II detection. In the presence of the virus, the functional hijack of the yeast's ACE2 enzyme by the viral Spike protein would translate into a reduced cleavage of extracellular Ang II, due to ACE2

downregulation. The excess of Ang II would lead to greater activation of the AT_1 biosensory pathway, culminating in the overexpression of a reporter gene triggered by the MAPKs effectors. Diversely, in a viral-absence scenario, the ACE2 enzyme available on the cell membrane surface would actively cleave Ang II into Ang 1-7. The decreased Ang II concentration would consequently limit the activation of the AT_1 receptor and the subsequent promoter.

Therefore, via the application of the GPCR/YSD strategy, the sensing of SARS-CoV-2 - or any ACE2-binding virus such as SARS-CoV and HCoV-NL63 - by the mutant yeast strain would be modulated by Ang II concentration and the consequent expression of a reporter gene. A schematic representation of the biosensing strain molecular mechanisms in the scenarios with and without SARS-CoV-2 can be seen in **Figure 3**.

Engineering *S. cerevisiae* to function as a biosensor for SARS-CoV-2 would require the heterologous expression of (1) hACE2 - the human angiotensin-converting enzyme 2; (2) *AGTR1* - the Ang II AT_1 receptor-encoding gene; (3) Gpa1/$G\alpha_{i3}$ - the yeast and human chimeric G protein to improve downstream signaling; and (4) reporter gene(s) activated by the AT_1 signaling pathway, that would result in a visual outcome in the viral presence. Additionally, the presence of Ang II in the system would be paramount for the correct function of the engineered *S. cerevisiae.* Ang II could be manually added (as a buffer) or even produced and secreted by the yeast itself.

The heterologous expression of hACE2 in microorganisms has already been performed in bacteria of the species *Lactobacillus paracasei* (Verma *et al.* 2019). In this matter, *S. cerevisiae* has been widely used for the production of heterologous mammalian proteins (Buckholz and Gleeson 1991; Baghban *et al.* 2019), requiring only codon optimization for successful functional expression. However, the functional expression of hACE2 in the biosensing strain would require not only the correct folding of the protein but also its display at the membrane surface so that it would be spatially available for binding to ACE2-dependent coronaviruses or reducing Ang II to Ang 1-7. For this reason, GPI-based protein display deems necessary.

On the other hand, the challenge of engineering a recombinant yeast strain that expresses the AT_1 receptor was already described by Nakamura, Ishii and Kondo (2014). The authors succeeded in the functional activation of the human GPCR signal by inserting a single mutation of Ala at Asn295 into the AGTR1 receptor and by using the chimeric Gα protein, G_{i3}tp. The signal transduction activated by the AGTR1(N295A)/G_{i3}tp complex in the presence of Ang II, modulates the *FIG1* promoter, which, fused to the reporter protein ZsGreen, emits fluorescence once activated.

As for the Ang II delivery to the system, a full comprehension via empirical analysis of the interaction dynamics between the SARS-CoV-2 Spike protein and the yeast-displayed ACE2 deems necessary. Uncovering the timing underlying the blockage of the ACE's catalytic function is imperative for understanding when and how Ang II should be delivered to the system. In this matter, Ishii *et al.* (2012) have described a plasmid enabling Ang II secretion which allows autocrine signaling and could be used in the formation of an autonomous biosensing device. However, if considered pertinent, the addition of Ang II to the system as a running buffer is not only feasible but also practical.

As described by Nakamura, Ishii and Kondo (2014), the fluorescent signal emitted by ZsGreen can be effectively modulated by the concentration of Ang II via the AT_1-based transduction. Levels as low as 368 μM of Ang II have been detected by the heterologous AT_1, hence posing as a highly sensitive biosensor. It is postulated that the fluorescence emitted by the biosensing strain would be proportional to the viral titer: the greater the presence of SARS-CoV-2, the greater the ACE2 downregulation and, therefore, the greater the concentration of extracellular Ang II. The opposite should also be true and the time for sensing differential Ang II concentration should be around 4 hours, as previously stated by the group. This strain could potentially be used in laboratory tests as the biosensing component of a quantitative diagnostic, as shown in **Figure 4A**.

In order to establish a multi-mode device that would not rely solely on laboratory apparatus for results interpretation, the pheromone signaling cascade could be coupled to a biosynthetic pathway that ultimately produces a pigment visible to the naked eye when activated. The use of lycopene for this matter has

already been described as highly sensitive and specific (Ostrov *et al.* 2017). In a similar fashion, yeast-based GPCR/YSD biosensing devices such as the CORONAYEAST could be coupled to the lycopene production pathway enabling the qualitative detection of SARS-CoV-2 without the need for additional equipment. The simple addition of the yeast-based biosensor to clinical samples would allow a naked-eye visible color change, which should occur after 8 hours of incubation (Ostrov *et al.* 2017). An illustrative representation of this qualitative device is shown in **Figure 4B**.

Briefly, the biosensing strain in ready-to-test and control conditions could be packed in a paper container, as described by Ostrov *et al.* (2017). A prominent cellulose dipstick would be immersed in the sample and, by capillarity, the specimen would come in contact with the biosensory yeasts, activating or not the reporter gene due to the presence or absence of viral particles, respectively. As it is known that saliva is a large reservoir of SARS-CoV-2 (Azzi *et al.* 2020), the development of a non-invasive test would allow the testing to be personal and confidential. The dissemination of this type of POC test - simple, sensitive, specific, and inexpensive - would allow for effective tracking of the mobility dynamics of viruses. In a hypothetical scenario, users could share their test results through a mobile application at regular intervals. These data could be accessed by appropriate health organizations, allowing the application of effective public policies to control the spread of the disease.

The main advantages of the CORONAYEAST diagnostic compared to current testing techniques would be: (1) To be functional throughout the infection manifestation, once it is responsive to active viral particles capable of binding with ACE2; (2) To be highly specific to ACE2-binding viruses, since it is based on a GPCR signaling pathway that is only activated through the presence of Ang II; (3) To be highly sensitive, because small changes in Ang II concentration can be detected by the AT_1 receptor, leading to significant changes in the reporter genes expression intensity (Nakamura, Ishii and Kondo 2014); (4) To be inexpensive, by reliance on solely yeast propagation (when expressing an Ang II autocrine signaling), with no resistance markers that must be maintained; (5) To be simple to use and interpret,

by not requiring RNA extraction or viral priming of any kind, and acting as a POC test with a visible outcome; and (6) To offer the possibility of continuous home testing due to the previous listed advantages, allowing a precise and comprehensive tracking of virus mobility.

Remarkably, the CORONAYEAST concept detailed here should be able to detect all viruses dependent on ACE2 to initiate infection: SARS-CoV-2, SARS-CoV, and HCoV-NL63. While high ACE2-affinity has been thoroughly described for SARS-CoV in a similar fashion as for SARS-CoV-2, the interaction with HCoV-NL63 is weaker, which is proposed to partly explain the different pathological consequences of infection by HCoV-NL63 when compared to the other two viruses (Mathewson *et al.* 2008; Abdul-Rasool and Fielding 2010). Empirical clinical assays with HCoV-NL63 and the biosensing strain deems necessary to infer whether the interaction between HCoV-NL63 and the CORONAYEAST would be strong enough to lead to detection. In addition, it is important to notice that other human pathogenic coronaviruses - such as HCoV-229E and HCoV-OC43, responsible for common cold, do not use ACE2 for binding to the host cell and, therefore, would not be detected by the CORONAYEAST.

Given the similarities between ACE2-dependent viruses' structural components and infection mechanisms, the CORONAYEAST could stand out as a practical multi-diagnostic alternative. That versatility could introduce cross-reactions among ACE2-binding coronaviruses, but since the surveillance of highly-impacting SARS-like pathogens has not yet overlapped (Peeri *et al.* 2020), it would not be an issue. Furthermore, it might also play an important role in the detection of new human-pathogenic coronavirus strains that may develop over time, as the ACE2 protein is critical for the infection of most of the coronaviruses that have been responsible for pandemics, and mutation rates of the viral genome are very high (Pachetti *et al*. 2020; Tang *et al*. 2020a).

7. Perspectives

As synthetic biology continues to fastly advance towards multitargeted possibilities by harnessing genetic engineering tools, new ideas sprout to fully take advantage of this progress in already traditional applications. Biosensors represent

an area where synthetic biology is mostly fruitful, given the importance of precisely detecting and sorting diverse stimuli using the complexity of a living system. And while yeast, especially *S. cerevisiae*, have traditionally served as biocatalysts for different purposes, their application in the detection of whole-microorganisms that do not bear GPCR systems have not been implemented yet. Enabling these sensing systems could allow for the availability of sensitive, specific, and commercially attractive biosensors for the detection of highly relevant pathogens. In an endeavor to expand the biosensing capabilities of *S. cerevisiae*, here we presented a perspective of how the combination of traditional approaches may endow yeast with virus-sensing abilities.

Although the CORONAYEAST example represents a well-rounded idea of how the interaction between a surface-displayed protein and a target ligand could indirectly modulate signal transduction and be used for the diagnosis of viral diseases in yeast, the application shall not be restricted to SARS-CoV-2. A glimpse of other demands is possible knowing that all viruses require binding to specific molecules on the surface of the target cell prior to infection. Furthermore, the specificity of this ligation would account for virus-specific yeast biosensors.

To substantiate the universality of this approach and provide another application example, an envisioned execution is the detection of the Human T cell Leukemia virus (HTLV), which is associated with leukemia and neurological syndromes. It has been described that HTLV's receptor-binding domain interacts with GLUT-1 - the mammalian glucose transporter -, inhibiting the sugar transport and finally perturbing the glucose metabolism (Manel *et al.* 2003). The expression of GLUT-1 in a "null" hexose transport *S. cerevisiae* background has already been described by Wieczorke *et al.* (2003). We envision that the GPCR/YSD strategy could enable HTLV's sensing through extracellular glucose concentration variation due to the virus interaction with GLUT-1 (acting as the membrane receptor) followed by GPCR-mediated glucose detection coupled, for instance, to the mating pathway. While the understanding of the viral interaction with heterologous receptors expressed in yeast reserves technological challenges, adequate binding for effective signaling could potentially be surpassed via directed evolution efforts focused on the receptor protein affinity with the virus.

Additionally, it is important to note that a GPCR/YSD complex strategy for yeast biosensing is not restricted to the detection of viruses or pathogens, as it may find interesting applications in other areas. The surface display of proteins with catalytic activities may allow the detection of molecules that do not directly activate a signaling cascade, but yet could be transformed into a detectable analyte - as a GPCR agonist or a trigger to another signal transduction pathway. Even though the strategy represents an indirect measurement for the target detection, it implements the use of yeasts as biosensors for previously neglected analytes. A simple example would be the use of anchored α-amylases in yeast, as previously described by Selwal *et. al.* (2017), for the construction of a novel *S. cerevisiae* starch biosensor. Again, the amylase would act as the receptor (using the YSD strategy), while signal transduction could be performed by a glucose-inducible promoter or a GPCR system - knowing that this sugar is the product of the enzymatic activity over starch. Starch concentration would be correlated to the hexose presence in the medium, requiring previous standardization protocols for the use as a functional biosensor.

The prospect of expanding the promising, yet underexplored, field of yeast-based viral biosensors via the implementation of the GPCR/YSD strategy presented here might lead to important advances in detection devices in several fields. Ultimately, we hope this review serves as a catalyst for new ideas in the yeast-biosensors research field, addressing current and future detection challenges.

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10. Conflicts of interest

None declared.

11. References

Abdul-Rasool S, Fielding BC. Understanding Human Coronavirus HCoV-NL63. *Open Virol J* 2010;**4**:76–84.

Adeniran A, Sherer M, Tyo KEJ. Yeast-based biosensors: design and applications. *FEMS Yeast Res* 2015;**15**:1–15.

Aronoff-Spencer E, Venkatesh AG, Sun A *et al.* Detection of hepatitis C core antibody by dual-affinity yeast chimera and smartphone-based electrochemical sensing. *Biosens Bioelectron* 2016;**86**:690–6.

Ault AD, Broach JR. Creation of GPCR-based chemical sensors by directed evolution in yeast. *Protein Eng Des Sel* 2006;**19**:1–8.

Azzi L, Carcano G, Gianfagna F *et al.* Saliva is a reliable tool to detect SARS-CoV-2. *J Infect* 2020;**81**:e45–50.

Baghban R, Farajnia S, Rajabibazl M *et al.* Yeast expression systems: overview and recent advances. *Mol Biotechnol* 2019;**61**:365–84.

Bahadır EB, Sezgintürk MK. Applications of commercial biosensors in clinical, food, environmental, and biothreat/biowarfare analyses. *Anal Biochem* 2015;**478**:107–20.

Bai Y, Yao L, Wei T *et al.* Presumed asymptomatic carrier transmission of COVID-19. *JAMA - J Am Med Assoc* 2020;**323**:1406–7.

Bakhle YS. Conversion of angiotensin I to angiotensin II by cell-free extracts of dog lung. *Nature* 1968;**220**:919–921.

Bardwell L. A walk-through of the yeast mating pheromone response pathway. *Peptides* 2005;**26**:339–50.

Berger EA, Murphy PM, Farber JM. Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. *Annu Rev Immunol* 1999;**17**:657–700.

Bhalla N, Pan Y, Yang Z *et al.* Opportunities and challenges for biosensors and nanoscale analytical tools for pandemics: COVID-19. *ACS Nano* 2020;**14**:7783–807.

Boder ET, Wittrup KD. Yeast surface display for directed evolution of protein expression, affinity, and stability. *Methods in Enzymology*. Vol 328. Academic Press, 2000, 430–44.

Bosnyak S, Jones ES, Christopoulos A *et al.* Relative affinity of angiotensin peptides and novel ligands at AT1 and AT2 receptors. *Clin Sci (London, Engl 1979)* 2011;**121**:297–303.

Broughton JP, Deng X, Yu G *et al.* CRISPR–Cas12-based detection of SARS-CoV-2. *Nat Biotechnol* 2020;**38**:870–4.

Brown NA, Schrevens S, van Dijck P *et al.* Fungal G-protein-coupled receptors: mediators of pathogenesis and targets for disease control. *Nat Microbiol* 2018;**3**:402–14.

Buckholz RG, Gleeson MAG. Yeast systems for the commercial production of heterologous proteins. *Bio/Technology* 1991;**9**:1067–72.

Burrell LM, Johnston CI, Tikellis C *et al.* ACE2, a new regulator of the renin–angiotensin system. *Trends Endocrinol Metab* 2004;**15**:166–9.

Carey RM, Wang ZQ, Siragy HM. Role of the angiotensin type 2 receptor in the regulation of blood pressure and renal function. *Hypertension* 2000;**35**:155–63.

Castillo-Henríquez L, Brebes-Acuña M, Castro-Rojas A *et al.* Biosensors for the detection of bacterial and viral clinical pathogens. *Sensors* 2020;**20**:6926.

Caygill RL, Blair GE, Millner PA. A review on viral biosensors to detect human pathogens. *Anal Chim Acta* 2010;**681**:8–15.

Cesewski E, Johnson BN. Electrochemical biosensors for pathogen detection. *Biosens Bioelectron* 2020;**159**:112214.

Chang H-J, Voyvodic PL, Zúñiga A *et al.* Microbially derived biosensors for diagnosis, monitoring and epidemiology. *Microb Biotechnol* 2017;**10**:1031–5.

Chauhan DS, Prasad R, Srivastava R *et al.* Comprehensive review on current interventions, diagnostics, and nanotechnology perspectives against SARS-CoV-2. *Bioconjug Chem* 2020;**31**:2021–45.

Cherf GM, Cochran JR. Applications of yeast surface display for protein engineering. In: Liu B (ed.). *Methods in Molecular Biology (Clifton, N.J.)*. New York, NY: Springer New York, 2015, 155–75.

Chinamedevice. *http//www.chinamedevice.com/ChinaSuppliers/5028/One-step-Anti-HIV-1-2-Antigen-Test-strip-HIV-TEST-KIT-453608.html (last accessed December 11, 2020)*.

Chiu AT, Roscoe WA, McCall DE *et al.* Angiotensin II-1 receptors mediate both vasoconstrictor and hypertrophic responses in rat aortic smooth muscle cells. *Receptor* 1991;**1**:133–40.

Ciulla MM. SARS-CoV-2 downregulation of ACE2 and pleiotropic effects of ACEIs/ARBs. *Hypertens Res* 2020;**43**:985–6.

Coffin JM. Virions at the gates: receptors and the host–virus arms race. *PLoS Biol* 2013;**11**:e1001574.

Corisbio a. *https//www.corisbio.com/Products/Human-Field/Influenza-A-and-B-viruses.php (last accessed December 11, 2020)*.

Corisbio b. *https//www.corisbio.com/Products/Human-Field/Respiratory-*

Adenovirus.php (last accessed December 11, 2020).

Cui F, Zhou HS. Diagnostic methods and potential portable biosensors for coronavirus disease 2019. *Biosens Bioelectron* 2020;**165**:112349.

Cui J, Li F, Shi Z-L. Origin and evolution of pathogenic coronaviruses. *Nat Rev Microbiol* 2019;**17**:181–92.

Ding X, Yin K, Li Z *et al.* Ultrasensitive and visual detection of SARS-CoV-2 using all-in-one dual CRISPR-Cas12a assay. *Nat Commun* 2020;**11**:4711.

Doijen J, Van Loy T, Landuyt B *et al.* Advantages and shortcomings of cell-based electrical impedance measurements as a GPCR drug discovery tool. *Biosens Bioelectron* 2019;**137**:33–44.

Donoghue M, Hsieh F, Baronas E *et al.* A novel angiotensin-converting enzyme–related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ Res* 2000;**87**:E1–9.

van Doremalen N, Bushmaker T, Morris DH *et al.* Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *N Engl J Med* 2020;**382**:1564–7.

Fani M, Zandi M, Soltani S *et al.* Future developments in biosensors for field-ready SARS-CoV-2 virus diagnostics. *Biotechnol Appl Biochem* 2020, DOI: 10.1002/bab.2033.

Ferrario CM, Chappell MC, Dean RH *et al.* Novel angiotensin peptides regulate blood pressure, endothelial function, and natriuresis. *J Am Soc Nephrol* 1998;**9**:1716–22.

Ferrario CM, Chappell MC, Tallant EA *et al.* Counterregulatory actions of Angiotensin-(1-7). *Hypertension* 1997;**30**:535–41.

Forni D, Cagliani R, Clerici M *et al.* Molecular evolution of human coronavirus genomes. *Trends Microbiol* 2017;**25**:35–48.

Fukuda N, Ishii J, Kaishima M *et al.* Amplification of agonist stimulation of human G-protein-coupled receptor signaling in yeast. *Anal Biochem* 2011;**417**:182–7.

Galao RP, Scheller N, Alves-Rodrigues I *et al.* Saccharomyces cerevisiae: a versatile eukaryotic system in virology. *Microb Cell Fact* 2007;**6**:32.

Gancedo JM. The early steps of glucose signalling in yeast. *FEMS Microbiol Rev* 2008;**32**:673–704.

Gheblawi M, Wang K, Viveiros A *et al.* Angiotensin-converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system: celebrating the 20th anniversary of the discovery of ACE2. *Circ Res* 2020;**126**:1456–74.

Glowacka I, Bertram S, Herzog P *et al.* Differential downregulation of ACE2 by the spike proteins of severe acute respiratory syndrome coronavirus and human coronavirus NL63. *J Virol* 2010;**84**:1198–205.

Grove J, Marsh M. The cell biology of receptor-mediated virus entry. *J Cell Biol*

2011;**195**:1071–82.

Guerreiro MR, Freitas DF, Alves PM *et al.* Detection and quantification of label-free infectious adenovirus using a switch-on cell-based fluorescent biosensor. *ACS Sensors* 2019;**4**:1654–61.

Haleyur Giri Setty MK, Hewlett IK. Point of care technologies for HIV. *AIDS Res Treat* 2014;**2014**:497046.

Han L, Zhao Y, Cui S *et al.* Redesigning of microbial cell surface and its application to whole-cell biocatalysis and biosensors. *Appl Biochem Biotechnol* 2018;**185**:396–418.

Hauser AS, Attwood MM, Rask-Andersen M *et al.* Trends in GPCR drug discovery: new agents, targets and indications. *Nat Rev Drug Discov* 2017;**16**:829–42.

Helma J, Schmidthals K, Lux V *et al.* Direct and dynamic detection of HIV-1 in living cells. *PLoS One* 2012;**7**:e50026.

Higuchi S, Ohtsu H, Suzuki H *et al.* Angiotensin II signal transduction through the AT1 receptor: novel insights into mechanisms and pathophysiology. *Clin Sci (London, Engl 1979)* 2007;**112**:417–28.

Hoffmann M, Kleine-Weber H, Schroeder S *et al.* SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 2020;**181**:271–80.

Hou T, Zeng W, Yang M *et al.* Development and evaluation of a rapid CRISPR-based diagnostic for COVID-19. Krammer F (ed.). *PLOS Pathog* 2020;**16**:e1008705.

Imai Y, Kuba K, Rao S *et al.* Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 2005;**436**:112–6.

Ishii J, Yoshimoto N, Tatematsu K *et al.* Cell wall trapping of autocrine peptides for human G-protein-coupled receptors on the yeast cell surface. Kendall D (ed.). *PLoS One* 2012;**7**:e37136.

Jiang X, Rayner S, Luo M-H. Does SARS-CoV-2 has a longer incubation period than SARS and MERS? *J Med Virol* 2020;**92**:476–8.

Jiao J, Duan C, Xue L *et al.* DNA nanoscaffold-based SARS-CoV-2 detection for COVID-19 diagnosis. *Biosens Bioelectron* 2020;**167**:112479.

Jones KE, Patel NG, Levy MA *et al.* Global trends in emerging infectious diseases. *Nature* 2008;**451**:990–3.

Khan MZH, Hasan MR, Hossain SI *et al.* Ultrasensitive detection of pathogenic viruses with electrochemical biosensor: State of the art. *Biosens Bioelectron* 2020;**166**:112431.

Kievits T, van Gemen B, van Strijp D *et al.* NASBA isothermal enzymatic in vitro nucleic acid amplification optimized for the diagnosis of HIV-1 infection. *J Virol Methods* 1991;**35**:273–86.

Kintzios S, Bem F, Mangana O *et al.* Study on the mechanism of Bioelectric Recognition Assay: evidence for immobilized cell membrane interactions with viral fragments. *Biosens Bioelectron* 2004;**20**:907–16.

Kissler SM, Tedijanto C, Goldstein E *et al.* Projecting the transmission dynamics of SARS-CoV-2 through the postpandemic period. *Science* 2020;**368**:860–8.

Krejcova L, Hynek D, Adam V *et al.* Electrochemical sensors and biosensors for influenza detection. *Int J Electrochem Sci* 2012;**7**:10779–801.

Kuba K, Imai Y, Rao S *et al.* A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med* 2005;**11**:875–9.

Lan J, Ge J, Yu J *et al.* Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* 2020;**581**:215–20.

Lengger B, Jensen MK. Engineering G protein-coupled receptor signalling in yeast for biotechnological and medical purposes. *FEMS Yeast Res* 2020;**20**:foz087.

Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. *Nat Microbiol* 2020;**5**:562–9.

Li F. Receptor recognition mechanisms of coronaviruses: a decade of structural studies. *J Virol* 2015;**89**:1954–64.

Li F, Li W, Farzan M *et al.* Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science (80-)* 2005;**309**:1864–8.

Li W, Moore MJ, Vasilieva N *et al.* Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 2003;**426**:450–454.

Li X, Geng M, Peng Y *et al.* Molecular immune pathogenesis and diagnosis of COVID-19. *J Pharm Anal* 2020;**10**:102–8.

Li Y, Xia L. Coronavirus disease 2019 (COVID-19): role of chest CT in diagnosis and management. *Am J Roentgenol* 2020;**214**:1280–6.

Lin C-I, Tsai C-H, Sun Y-L *et al.* Instillation of particulate matter 2.5 induced acute lung injury and attenuated the injury recovery in ACE2 knockout mice. *Int J Biol Sci* 2018;**14**:253–65.

Liu Y, Gayle AA, Wilder-Smith A *et al.* The reproductive number of COVID-19 is higher compared to SARS coronavirus. *J Travel Med* 2020, DOI: 10.1093/jtm/taaa021.

Long Q-X, Liu B-Z, Deng H-J *et al.* Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med* 2020a;**26**:845–8.

Long Q-X, Tang X-J, Shi Q-L *et al.* Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med* 2020b;**26**:1200–4.

Lu G, Wang Q, Gao GF. Bat-to-human: spike features determining 'host jump' of coronaviruses SARS-CoV, MERS-CoV, and beyond. *Trends Microbiol*

2015;**23**:468–78.

Lu R, Wu X, Wan Z *et al.* A novel reverse transcription loop-mediated isothermal amplification method for rapid detection of SARS-CoV-2. *Int J Mol Sci* 2020;**21**:2826.

Lv H, Wu NC, Tsang O-TY *et al.* Cross-reactive antibody response between SARS-CoV-2 and SARS-CoV infections. *Cell Rep* 2020;**31**:107725.

Manel N, Kim FJ, Kinet S *et al.* The ubiquitous glucose transporter GLUT-1 is a receptor for HTLV. *Cell* 2003;**115**:449–59.

Mathewson AC, Bishop A, Yao Y *et al.* Interaction of severe acute respiratory syndrome-coronavirus and NL63 coronavirus spike proteins with angiotensin converting enzyme-2. *J Gen Virol* 2008;**89**:2741–5.

Matsunaga S, Jeremiah SS, Miyakawa K *et al.* Engineering cellular biosensors with customizable antiviral responses targeting hepatitis B virus engineering cellular biosensors with customizable antiviral responses targeting hepatitis B virus. *iScience* 2020;**23**:100867.

Mavrikou S, Moschopoulou G, Tsekouras V *et al.* Development of a portable, ultra-rapid and ultra-sensitive cell-based biosensor for the direct detection of the SARS-COV-2 S1 spike protein antigen. *Sensors* 2020;**20**:3121.

McMahon C, Baier AS, Pascolutti R *et al.* Yeast surface display platform for rapid discovery of conformationally selective nanobodies. *Nat Struct Mol Biol* 2018;**25**:289–96.

Mizumoto K, Kagaya K, Zarebski A *et al.* Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020. *Eurosurveillance* 2020;**25**:2000180.

Moitra P, Alafeef M, Dighe K *et al.* Selective naked-eye detection of SARS-CoV-2 mediated by N gene targeted antisense oligonucleotide capped plasmonic nanoparticles. *ACS Nano* 2020;**14**:7617–27.

Morales-Narváez E, Dincer C. The impact of biosensing in a pandemic outbreak: COVID-19. *Biosens Bioelectron* 2020;**163**:112274.

Nakamura Y, Ishii J, Kondo A. Construction of a yeast-based signaling biosensor for human angiotensin II type 1 receptor via functional coupling between Asn295-mutated receptor and Gpa1/G i3 chimeric Gα. *Biotechnol Bioeng* 2014;**111**:2220–8.

Nakamura Y, Kondo A, Ishii J. Biosensing techniques in yeast: G-protein signaling and protein-protein interaction assays for monitoring ligand stimulation and oligomer formation of heterologous GPCRs. In: Shihori Tanabe (ed.). *Peripheral Membrane Proteins*. IntechOpen, 2018.

- Nakamura Y, Shibasaki S, Ueda M *et al.* Development of novel whole-cell immunoadsorbents by yeast surface display of the IgG-binding domain. *Appl Microbiol Biotechnol* 2001;**57**:500–5.

Nishiura H, Kobayashi T, Miyama T *et al.* Estimation of the asymptomatic ratio of novel coronavirus infections (COVID-19). *Int J Infect Dis* 2020;**94**:154–5.

OraQuick. *https//www.orasure.com/products-infectious/OraQuick-OTC.html (last accessed December 11, 2020)*.

Ostrov N, Jimenez M, Billerbeck S *et al.* A modular yeast biosensor for low-cost point-of-care pathogen detection. *Sci Adv* 2017;**3**:e1603221.

Ou X, Liu Y, Lei X *et al.* Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat Commun* 2020;**11**:1620.

Ozer T, Geiss BJ, Henry CS. Review—Chemical and biological sensors for viral detection. *J Electrochem Soc* 2020;**167**:037523.

Pachetti M, Marini B, Benedetti F *et al.* Emerging SARS-CoV-2 mutation hot spots include a novel RNA-dependent-RNA polymerase variant. *J Transl Med* 2020;**18**:179.

Park M. Surface display technology for biosensor applications: a review. *Sensors* 2020;**20**:2775.

Peeri NC, Shrestha N, Rahman MS *et al.* The SARS, MERS and novel coronavirus (COVID-19) epidemics, the newest and biggest global health threats: what lessons have we learned? *Int J Epidemiol* 2020;**49**:717–26.

Perdikaris A, Alexandropoulos N, Kintzios S. Development of a novel, ultra-rapid biosensor for the qualitative detection of hepatitis B virus-associated antigens and anti-HBV, based on “membrane-engineered” fibroblast cells with virus-specific antibodies and antigens. *Sensors* 2009;**9**:2176–86.

Pierce KL, Premont RT, Lefkowitz RJ. Seven-transmembrane receptors. *Nat Rev Mol Cell Biol* 2002;**3**:639–50.

Qiu G, Gai Z, Tao Y *et al.* Dual-functional plasmonic photothermal biosensors for highly accurate severe acute respiratory syndrome coronavirus 2 detection. *ACS Nano* 2020;**14**:5268–77.

Quidel. *https//www.quidel.com/immunoassays/rapid-influenza-tests (last accessed December 11, 2020)*.

Qureshi A, Niazi JH. Biosensors for detecting viral and bacterial infections using host biomarkers: a review. *Analyst* 2020;**145**:7825–48.

Ribeiro BV, Cordeiro TAR, Oliveira e Freitas GR *et al.* Biosensors for the detection of respiratory viruses: a review. *Talanta Open* 2020;**2**:100007.

Rider TH, Petrovick MS, Nargi FE *et al.* A B cell-based sensor for rapid identification of pathogens. *Science (80-)* 2003;**301**:213–5.

Rolland F, Winderickx J, Thevelein JM. Glucose-sensing and -signalling mechanisms in yeast. *FEMS Yeast Res* 2002;**2**:183–201.

Santos RAS, Campagnole-Santos MJ, Andrade SP. Angiotensin-(1–7): an update. *Regul Pept* 2000;**91**:45–62.

Santos RAS, Simoes e Silva AC, Maric C *et al.* Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc Natl Acad Sci U S A* 2003;**100**:8258–63.

Saylan Y, Erdem Ö, Ünal S *et al.* An alternative medical diagnosis method: biosensors for virus detection. *Biosensors* 2019;**9**:65.

Selwal KK, Li Y-F, Yu Z. Functional display of amylase on yeast surface from Rhizopus oryzae as a novel enzyme delivery method. *Food Biotechnol* 2017;**31**:233–44.

Seo G, Lee G, Kim MJ *et al.* Rapid detection of COVID-19 causative virus (SARS-CoV-2) in human nasopharyngeal swab specimens using field-effect transistor-based biosensor. *ACS Nano* 2020;**14**:5135–42.

Seow J, Graham C, Merrick B *et al.* Longitudinal evaluation and decline of antibody responses in SARS-CoV-2 infection. *medRxiv* 2020, DOI: 10.1101/2020.07.09.20148429.

Shang J, Wan Y, Luo C *et al.* Cell entry mechanisms of SARS-CoV-2. *Proc Natl Acad Sci U S A* 2020a;**117**:11727–34.

Shang J, Ye G, Shi K *et al.* Structural basis of receptor recognition by SARS-CoV-2. *Nature* 2020b;**581**:221–4.

Shaw WM, Yamauchi H, Mead J *et al.* Engineering a model cell for rational tuning of GPCR signaling. *Cell* 2019;**177**:782–96.

Shibasaki S. Bio-sensing using cell surface display: principles and variations of a cell sensor. In: Ueda M (ed.). *Yeast Cell Surface Engineering*. Singapore: Springer Singapore, 2019, 93–106.

Shibasaki S, Ninomiya Y, Ueda M *et al.* Intelligent yeast strains with the ability to self-monitor the concentrations of intra- and extracellular phosphate or ammonium ion by emission of fluorescence from the cell surface. *Appl Microbiol Biotechnol* 2001;**57**:702–7.

Skeggs Jr. LT, Kahn JR, Shumway NP. The preparation and function of the hypertensin-converting enzyme. *J Exp Med* 1956;**103**:295–9.

Sodhi A, Montaner S, Gutkind JS. Viral hijacking of G-protein-coupled-receptor signalling networks. *Nat Rev Mol Cell Biol* 2004;**5**:998–1012.

Su S, Wong G, Shi W *et al.* Epidemiology, genetic recombination, and pathogenesis of coronaviruses. *Trends Microbiol* 2016;**24**:490–502.

Tang X, Wu C, Li X *et al.* On the origin and continuing evolution of SARS-CoV-2. *Natl Sci Rev* 2020a;**7**:1012–23.

Tang Y-W, Schmitz JE, Persing DH *et al.* Laboratory diagnosis of COVID-19: current issues and challenges. *J Clin Microbiol* 2020b;**58**:e00512-20.

Tay MZ, Poh CM, Rénia L *et al.* The trinity of COVID-19: immunity, inflammation and intervention. *Nat Rev Immunol* 2020;**20**:363–74.

Tikellis C, Thomas MC. Angiotensin-converting enzyme 2 (ACE2) is a key modulator of the renin angiotensin system in health and disease. *Int J Pept* 2012;**2012**:256294.

Tipnis SR, Hooper NM, Hyde R *et al.* A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J Biol Chem* 2000;**275**:33238–43.

Torabi R, Ranjbar R, Halaji M *et al.* Aptamers, the bivalent agents as probes and therapies for coronavirus infections: a systematic review. *Mol Cell Probes* 2020;**53**:101636.

Uchański T, Zögg T, Yin J *et al.* An improved yeast surface display platform for the screening of nanobody immune libraries. *Sci Rep* 2019;**9**:382.

Udugama B, Kadhiresan P, Kozlowski HN *et al.* Diagnosing COVID-19: the disease and tools for detection. *ACS Nano* 2020;**14**:3822–35.

Vabret N. Antibody responses to SARS-CoV-2 short-lived. *Nat Rev Immunol* 2020;**20**:519.

Venkatesh AG, Sun A, Brickner H *et al.* Yeast dual-affinity biobricks: progress towards renewable whole-cell biosensors. *Biosens Bioelectron* 2015;**70**:462–8.

Verma A, Xu K, Du T *et al.* Expression of human ACE2 in Lactobacillus and beneficial effects in diabetic retinopathy in mice. *Mol Ther - Methods Clin Dev* 2019;**14**:161–70.

Versele M, Lemaire K, Thevelein JM. Sex and sugar in yeast: two distinct GPCR systems. *EMBO Rep* 2001;**2**:574–9.

Villela D, Leonhardt J, Patel N *et al.* Angiotensin type 2 receptor (AT2R) and receptor Mas: a complex liaison. *Clin Sci (London, Engl 1979)* 2015;**128**:227–34.

Vogels CBF, Brackney D, Wang J *et al.* SalivaDirect: simple and sensitive molecular diagnostic test for SARS-CoV-2 surveillance. *medRxiv* 2020, DOI: 10.1101/2020.08.03.20167791.

Wang Q, Zhang Y, Wu L *et al.* Structural and functional basis of SARS-CoV-2 entry by using human ACE2. *Cell* 2020a;**181**:894-904.e9.

Wang W, Xu Y, Gao R *et al.* Detection of SARS-CoV-2 in different types of clinical specimens. *J Am Med Assoc* 2020b;**323**:1843–4.

Wieczorke R, Dlugai S, Krampe S *et al.* Characterisation of mammalian GLUT glucose transporters in a heterologous yeast expression system. *Cell Physiol Biochem* 2003;**13**:123–34.

Wolfe ND, Dunavan CP, Diamond J. Origins of major human infectious diseases. *Nature* 2007;**447**:279–83.

Wu JT, Leung K, Leung GM. Nowcasting and forecasting the potential domestic and international spread of the 2019-nCoV outbreak originating in Wuhan, China: a modelling study. *Lancet* 2020;**395**:689–97.

Wu K, Li W, Peng G *et al.* Crystal structure of NL63 respiratory coronavirus receptor-binding domain complexed with its human receptor. *Proc Natl Acad Sci U S A* 2009;**106**:19970–4.

Xiang X, Qian K, Zhang Z *et al.* CRISPR-cas systems based molecular diagnostic tool for infectious diseases and emerging 2019 novel coronavirus (COVID-19) pneumonia. *J Drug Target* 2020;**28**:727–31.

Xiao F, Tang M, Zheng X *et al.* Evidence for gastrointestinal infection of SARS-CoV-2. *Gastroenterology* 2020;**158**:1831-1833.e3.

Yang HY, Erdös EG, Levin Y. A dipeptidyl carboxypeptidase that converts angiotensin I and inactivates bradykinin. *Biochim Biophys Acta* 1970;**214**:374–6.

Yang S, Rothman RE. PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. *Lancet Infect Dis* 2004;**4**:337–48.

Yang X, Tang H, Song M *et al.* Development of novel surface display platforms for anchoring heterologous proteins in Saccharomyces cerevisiae. *Microb Cell Fact* 2019;**18**:85.

Ye K, Shibasaki S, Ueda M *et al.* Construction of an engineered yeast with glucose-inducible emission of green fluorescence from the cell surface. *Appl Microbiol Biotechnol* 2000;**54**:90–6.

Yu L, Wu S, Hao X *et al.* Rapid detection of COVID-19 coronavirus using a reverse transcriptional loop-mediated isothermal amplification (RT-LAMP) diagnostic platform. *Clin Chem* 2020;**66**:975–7.

Zhang H, Han GW, Batyuk A *et al.* Structural basis for selectivity and diversity in angiotensin II receptors. *Nature* 2017;**544**:327–32.

Zhang H, Penninger JM, Li *Y et al.* Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med* 2020;**46**:586–90.

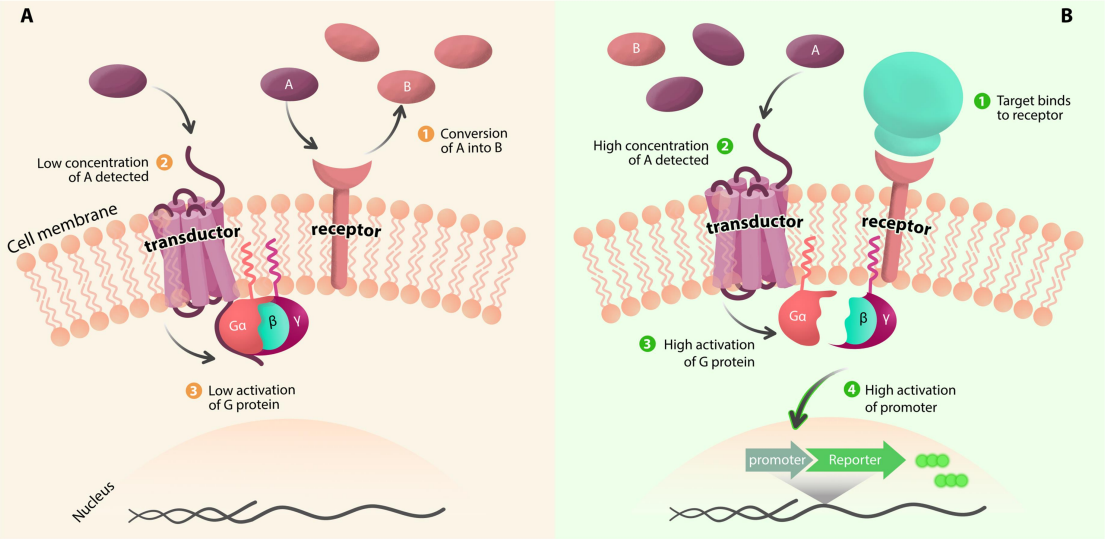

Figure 1. Molecular mechanism of the GPCR/YSD biosensing strategy in yeast. **(A)** Scenario with **no** virus; the receptor - a protein displayed on the yeast membrane surface - exerts its role as a catalytic enzyme, converting molecule A (externally supplemented) into B. The low concentration of A, in a closed environment where no further A is added, poorly activates the transductor - represented by a GPCR system. The biosensor emits an output relative to the *viral absence*. **(B)** Scenario **with** virus; the virus binds to the receptor, causing a decrease in its natural catalytic activity due to its downregulation. Molecule A now accumulates in the extracellular environment, while a lower amount of B is produced. The high concentration of A activates the transducer and, consequently, a signaling cascade that ultimately produces a visual outcome. The biosensor emits an output relative to *viral presence*.

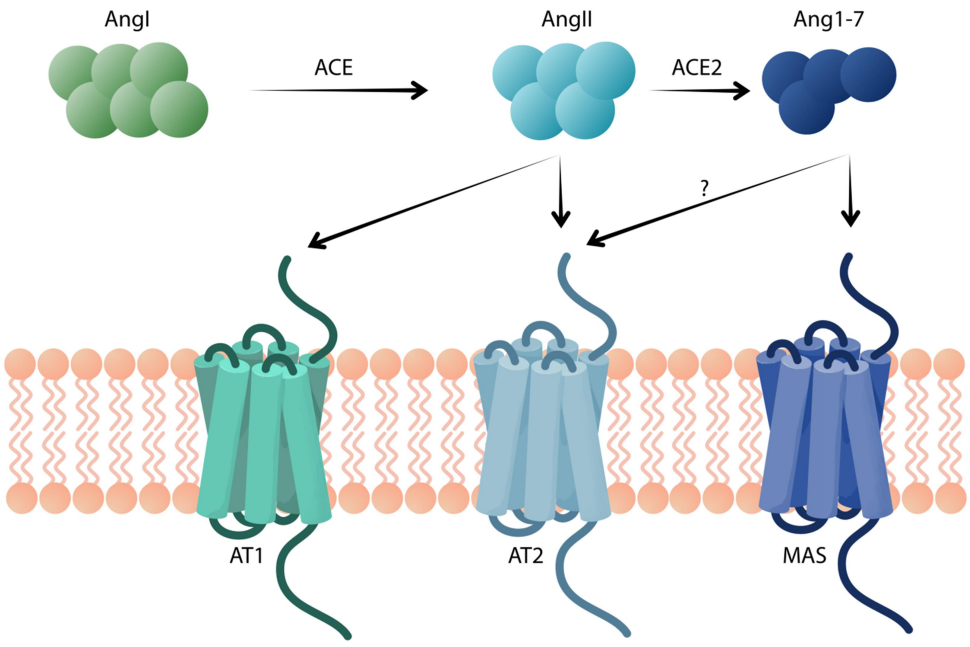

Figure 2. Simplified schematic representation of the RAS with the key components for the biosensing strain concept. Ang II is the product of Ang I metabolization through ACE, and acts as the main agonist for the AT1 receptor. Ang 1-7 is the product of ACE2 activity over Ang II, and binds to the Mas receptor. Both Ang II and Ang 1-7 may act as agonists for AT2. Ang II: angiotensin II; ACE: angiotensin-converting enzyme; AT1: Ang II type 1 receptor; Ang 1-7: angiotensin$_{(1-7)}$; ACE2: angiotensin-converting enzyme 2; AT2: Ang II type 2 receptor; MAS: Mas receptor.

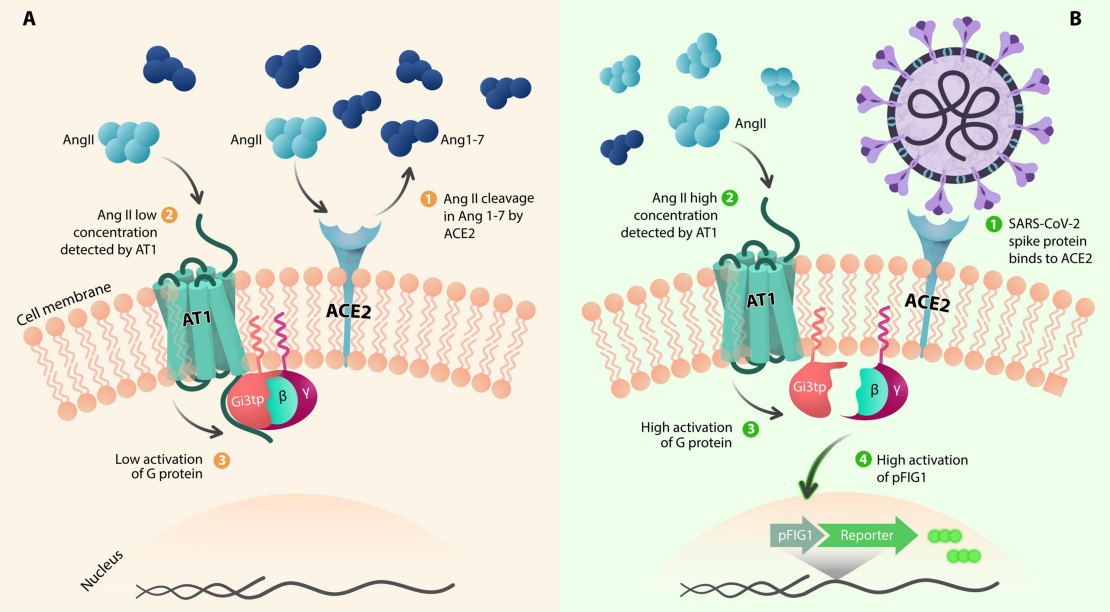

Figure 3. Molecular mechanism of the biosensing strain functioning in a scenario (**A**) without viral particles: as ACE2 is enzymatically active, it metabolizes the extracellular Ang II into Ang 1-7, inducing low activation of the AT1 receptor and, consequently, low expression of the reporter gene; and (**B**) in the presence of SARS-CoV-2: ACE2 is downregulated due to the interaction with the virus' Spike protein, eliciting the accumulation of the extracellular Ang II. The greater the Ang II concentration, the greater the reporter gene activation, allowing visual indication of the SARS-CoV-2 presence. ACE2: angiotensin-converting enzyme 2; Ang II: angiotensin II; Ang 1-7: angiotensin$_{(1-7)}$; AT1: Ang II type 1 receptor; G_{i3}tp: yeast-human chimeric Gpa1/Gα_{i3} Gα subunit; β: yeast Gβ subunit (Ste4); γ: yeast Gγ subunit (Ste18); pFIG1: *FIG1* promoter.

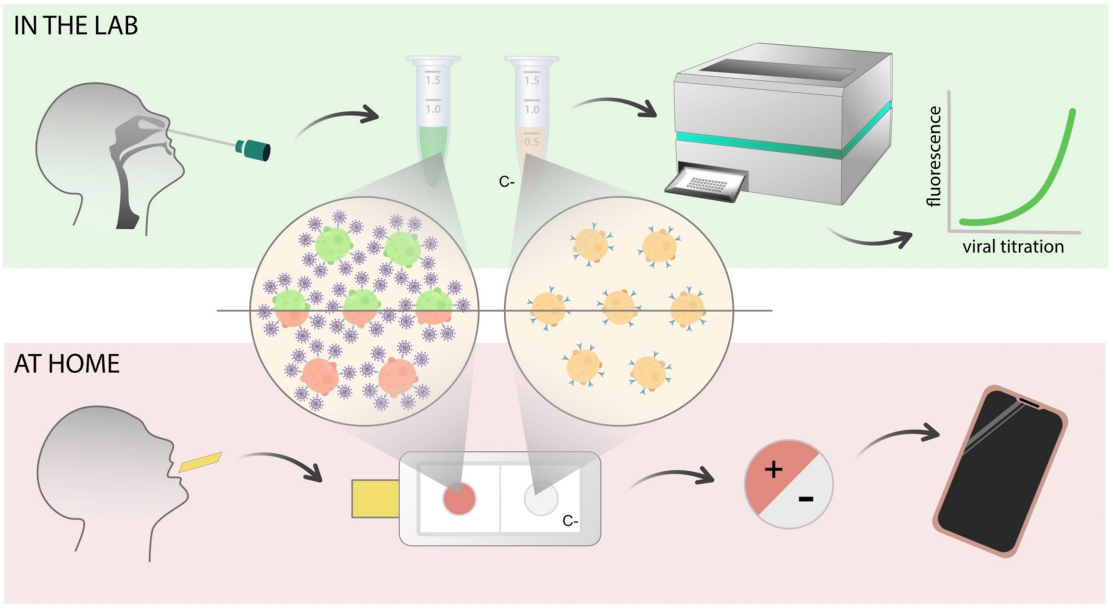

Figure 4. The CORONAYEAST diagnosis possibilities. **IN THE LAB**: Specialized health centers or diagnosis facilities could make use of fluorescent plate readers to correlate the yeast signal intensity with the viral titration in samples. The biosensor fluorescent output is expected to occur after 4 hours of incubation with the patient's saliva, while the result processing depends on a single seconds-lasting point read in the equipment - extinguishing the test accumulation problem and allowing for large amounts of diagnosis a day. **AT HOME**: CORONAYEAST works as a point-of-care testing alternative. The contact of the patient's saliva with the device's cellulose dipstick would allow the activation of the biosensor; a visual result - a color change - would be observable after 8 hours in case of the viral presence. Additionally, a mobile application could be applied to communicate the test results to competent health organizations.

Table 1. Cell-based biosensing strategies for the detection of human-pathogenic viruses*.

Cell-type	Target/Virus	Recognition element	Output	Reference
Vero cell	SARS-CoV-2	Human chimeric spike S1 antibody	Bioelectric	(Mavrikou *et al.* 2020)
Vero cell	Hepatitis B Virus (HBV)	Specific antibodies (anti-HBVs, anti-HBVe) or antigens (HBVsAg)	Bioelectric	(Perdikaris, Alexandropoulos and Kintzios 2009)
Vero cell	Herpes Simplex Virus (HSV-1)	Anti-HSV-1 antibodies	Bioelectric or fluorescent	(Kintzios *et al.* 2004)
Vero cell	Varicella Zoster Virus (VZV)	Anti-VZV antibodies	Bioelectric or fluorescent	(Kintzios *et al.* 2004)
B lymphocyte	Vaccinia virus	Anti- Vaccinia virus antibodies	Bioluminescent	(Rider 2003)
HEK-293 cell	Adenovirus	Adenoviral protease cleavable site	Fluorescent	(Guerreiro *et al.* 2019)
Jurkat cell	HBV	scFv against HBV surface antigen	Luminescent or fluorescent	(Matsunaga *et al.* 2020)

HeLa-Kyoto cell	Human Immunodeficiency Virus (HIV)	Camelid single-domain antibody against HIV capsid protein	Fluorescent	(Helma *et al.* 2012)
EBY100 yeast cell	Hepatitis C Virus (HCV)	HCV core antigen	Fluorescent or electrochemical	(Aronoff-Spencer *et al.* 2016)

*only those with specific recognition elements towards viral-derived components are listed. scFv: single-chain variable fragment.