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Multivalent ACE2 engineering—A promising pathway for advanced coronavirus nanomedicine development

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Abstract

The spread of coronavirus diseases has resulted in a clarion call to develop potent drugs and vaccines even as different strains appear beyond human prediction. An initial step that is integral to the viral entry into host cells results from an active-targeted interaction of the viral spike (S) proteins and the cell surface receptor, called angiotensin-converting enzyme 2 (ACE2). Thus, engineered ACE2 has been an interesting decoy inhibitor against emerging coronavirus infestation. This article discusses promising innovative ACE2 engineering pathways for current and emerging coronavirus therapeutic development. First, we provide a brief discussion of some ACE2-associated human coronaviruses and their cell invasion mechanism. Then, we describe and contrast the individual spike proteins and ACE2 receptor interactions, highlighting crucial hotspots across the ACE2-associated coronaviruses. Lastly, we address the importance of multivalency in ACE2 nanomedicine engineering and discuss novel approaches to develop and achieve multivalent therapeutic outcomes. Beyond coronaviruses, these approaches will serve as a paradigm to develop new and improved treatment technologies against pathogens that use ACE2 receptor for invasion.

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Introduction

Recently, the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its associated deadly COVID-19 disease has been a global hurdle of both health and economic concerns. Before this, the world also experienced the spread of SARS-CoV-1 [1–4] and Middle East respiratory syndrome coronavirus (MERS-CoV) [5–7] at varying degrees. In addition, the seasonal threat of common cold viruses (i.e., HCoV-NL63 and HCoV-HKU1) is still unquenched [8–10]. Unfortunately, the seemingly unending spread of COVID-19 and seasonal flu cases, and the periodic appearance of new strains, continue to cause debilitating impacts on all aspects of human life [11,12]. These global viral insurgencies necessitate a clarion call to develop antidotes such as vaccine [13–17] and therapeutics [18–21] to combat the associated catastrophic consequences. Global vaccination has become a promising prophylactic remedy to subdue the impact of these diseases. However, a significant surge in vaccine hesitancy still exists, which needs to be addressed [22–26]. Also, the efficacy of existing vaccines is being challenged by the unpredictable evolution of new variants [27–30]. For these reasons, the hope of achieving herd immunity through vaccination remains in doubt [31]. The present challenge makes the quest for therapeutic clinical interventions crucial and essential research.

One important approach in viral disease therapy development is the deployment of engineered host receptor decoys that could inhibit cell entry and viral invasion. Accordingly, many receptors have been proposed as targets for several disease treatments [32–35]. The receptor candidate of interest in recent times is angiotensin-converting enzyme 2 (ACE2), which facilitates the invasion of HCoV-NL63, SARS-CoV-1, SARS-CoV-2 and associated variants of concern (VOC) [36–40]. The prospects of ACE2 decoys have sparked several innovative multivalent and multifunctional engineering endeavours towards improving treatment technologies against coronaviruses.

This article highlights and raises interest in promising and innovative ACE2 engineering pathways for SARS-CoV-1, NL63-CoV and SARS-CoV-2 therapeutic development. First, we briefly discuss some human-associated coronaviruses and their cell invasion mechanism. Then, we describe and contrast the individual spike proteins and receptor interactions, highlighting crucial hotspots across the coronaviruses. We also discuss the importance of multivalency and multifunctionality in ACE2 nanomedicine engineering, facilitating the development of innovative treatment technologies against pathogens that use the ACE2 receptor to propagate their invasiveness.

General mechanism of cell invasion of ACE2-associated human coronaviruses

Human-associated coronaviruses (HCoVs) are enveloped positive-sense RNA viruses classified under the Coronaviridae family. Seven pathogenic human-associated coronaviruses have emerged, namely: HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV-1, MERS-CoV, and SARS-CoV-2 [40–42]. Among these viruses, HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1 cause common cold disease, whereas SARS-CoV-1, MERS-CoV and SARS-CoV-2 cause severe pneumonia-like malady [42,43].

One important weapon of HCoVs is their distinct protrusions, called spike glycoproteins (S protein), which initiate viral attachment to corresponding host cell-surface receptors [40,44]. Interestingly, host invasion, spread, and pathogenesis would be improbable without the spike glycoproteins. Recent evidence has revealed receptors such as sialic acid receptor (for HCoV-229E, HCoV-OC43 and HCoV-HKU1) [45–47], dipeptidylpeptidase 4 (DPP4 or CD26; for MERS-CoV) [48–51] and ACE2 (for SARS-CoV-1, NL63-CoV and SARS-CoV-2) [52–57] as important for viral invasion. There is also submission of CD147 as a possible receptor for SARS-CoV-2 in lymphocytes [58]. These receptors have been influential in the elucidation of virus tropism. For context and recency, we here-forward focus on ACE2 and its associated HCoVs.

Generally, cell entry and invasion begin with the engagement of the receptor-binding motif (RBM) of the S protein with the virus-binding motif (VBM) of cell-surface ACE2 (Fig. 1). The RBM-VBM binding activates the cell-surface proteolytic priming machinery (e.g., transmembrane protease serine 2, TMPRSS2) required for membrane fusion and endocytosis. In the case of SARS-CoV-2, pro-protein convertase furin putatively pre-activates the S protein to complement the activity of TMPRSS2 [59]. Subsequently, the fused virus transitions into endosomes and eventually associates with the lysosomal membrane for virus unpacking by proteases such as cathepsins [40,59–61]. Notably, HCoV-NL63 unpacking may not require cathepsins [62]. All the same, these viruses consequently adopt the host cell replication and translation machinery to propagate and invade other cells [56]. It is worth noting that the S protein of SARS and NL63 coronaviruses share overlapping contact points on ACE2 VBM [52]. Also, the genomes of SARS-CoV-1, NL63-CoV and SARS-CoV-2 are equipped with papain-like proteases that actively regulate viral spread [63–65]. Further transmission and invasion of the virus is driven by cell-to-cell fusion [66].

The S protein of NL63-CoV, SARS-CoV-1 and SARS-CoV-2: similarities and differences

The S protein is the main molecular key that mediates cell invasion. Generally, the S protein is a bulbous shaped oligomer composed of three identical protomers (Fig. 2a). Each fully glycosylated S protomer is a 180–200 kDa protein distinctly separable into an N-terminal receptor-binding subunit (S1) and a C-terminal transmembrane subunit (S2) [40,56,67,68].

The S1 subunit primarily consists of an N-terminal domain (NTD) and a C-terminal domain (CTD) that harbours the RBMs for NL63-CoV, SARS-CoV-1 and SARS-CoV-2 (Fig. 2b). The RBM of NL63-CoV-S comprises three discontinuous loops (RBM 1–3), while that of SARS-CoV-1 and SARS-CoV-2 is a single continuous loop [69]. On the other hand, the S2 subunit houses the fusion peptide, the heptad repeats 1 and 2 (HR1 and HR2), the transmembrane domain and a cytoplasmic domain. These domains together are responsible for fusion with host cells [67,70,71]. It should be noted that the NL63-CoV-S additionally contains the unique domain (a.k.a. Domain 0) and cytoplasmic extension near the N- and C-terminus, respectively. The unique domain is purported not to play a part in receptor interaction [52,72], but the extended cytoplasmic domain plays a role in virus priming towards membrane fusion [73]. Besides the unique and cytoplasmic domains, all other domain arrangements appear similar for these ACE2-associated HCoVs.

The S proteins differ across these viruses in amino acid sequences. For instance, there is about 77% amino acid sequence
similarity in the S proteins of SARS-CoV-1 and SARS-CoV-2 (Wuhan), although they are both β-corona virus. On the contrary, the NL63-CoV (α-corona virus) has only 30% S protein sequence identity to SARS-CoV-1 and SARS-CoV-2 (Wuhan). Furthermore, despite the substantial sequence divergence in the S1 subunit, the S2 subunit of the S protein shows significant sequence conservation across the viruses. Interestingly, the substantial sequence divergence does not change the bulbous S protein trimer shapes of these HCoVs, especially in their closed prefusion state.

Structural alignment of individual S protomers shows a high similarity between SARS-CoV-1-S and SARS-CoV-2-S, with a root mean square deviation (RMSD) of 3.8 Å consistent with the high amino acid sequence similarity [74]. On the contrary, NL63-CoV-S show a substantial deviation from SARS-CoV-1 (RMSD 7.1 Å) and SARS-CoV-2 (RMSD 7.8 Å). Herein, the level of sequence conservation of the S1 subunits is commensurate with the substantial structural deviations (Fig. 2c). For instance, the so-called Domain 0 in NL63-CoV-S NTD has no structural equivalent in SARS-CoV-1 and SARS-CoV-2 (Fig. 2c). Nevertheless, Domain 1 is present in all three coronaviruses, albeit with poor sequence conservation. Also, the RBD core of NL63-CoV deviates from that of SARS-CoV-1 and SARS-CoV-2 in that it comprises two layers of β-sandwich as opposed to a single layer of 5 stranded β-sandwich for SARS-CoV-1 and SARS-CoV-2 [69]. These structural subtleties result in different conformational postures in the open state.

Despite these differences, the RBDs are equally capable of assuming both the close (down) and open (up) conformational states prior to and during receptor engagement, respectively (Fig. 2c-d).

Multivalent and multifunctional ACE2 therapeutics: the rationale and significance

Human-derived soluble ACE2 (sACE2) is a proposed remedy for viral diseases and hypertension [83,84]. Unfortunately, much has not been realized regarding their progress in the clinics, probably due to their rapid elimination half-life (10.4 ± 4.0 h) [85,86]. As an antiviral decoy receptor, sACE2 exhibits a nanomolar affinity for associated viruses, unlike the regular picomolar affinity of clinical-grade antibodies. These limitations have necessitated scientific efforts to improve the efficacy of ACE2 therapeutics, especially in viral disease treatment. To this end, promising face-lifting developments have emerged by exploiting multivalent and multifunctional innovations. The primary rationale for inducing multivalency into therapeutic designs is to promote multiple interaction events en route to disease treatment. Multivalent drug interaction is essential for viral disease
Fig. 2. Structure of S proteins and their interactions with ACE2. a, Surface representation of the S protein trimer of NL63-CoV (top; PDB 7KIP), SARS-CoV-1 (middle; PDB 5XLR) and SARS-CoV-2 (bottom; PDB 6XR8). One S protomer is highlighted in yellow (NL63-CoV), purple blue (SARS-CoV-1) and red (SARS-CoV-2). Colour scheme is used throughout the figure. b, Schematic comparison of the multidomain S protein structure of NL63-CoV, SARS-CoV-1 and SARS-CoV-2. c, Alignment of NL63-CoV, SARS-CoV-1 and SARS-CoV-2 S protein protomers with the RBD in down position. The location of the RBDs, N-terminal domain (NTD), Domain 0 of NL63-CoV in the S1 subunit as well as the S2 subunit are shown. An insertion in the NL63-CoV S2 that deviates from the SARS-CoV-1 and SARS-CoV-2 S2 is highlighted in cyan. d, A cartoon representation of an S protomer from SARS-CoV-2 bound to ACE2 extracted from PDB 7DF4. The dashed lines show the location of the RBD in down position. The arrow shows the direction of movement of the RBD from down to up position to engage ACE2. e, Cartoon showing RBD of NL63-CoV bound to ACE2 based on PDB 3KBH (top panel). Cartoon showing aligned RBDs of SARS-CoV-1 (PDB 6ACK) and SARS-CoV-2 (PDB 7DF4) bound to ACE2. f, Surface representation of ACE2 (PDB 3KBH) showing the location of the virus binding motif (left panel) and the active centre groove (right panel). A zoom-in showing the residues bounds by the HCoVs is shown in the left panel, with the yellow, purple blue and red squares indicating residues that are bound by NL63-CoV, SARS-CoV-1 and SARS-CoV-2 RBDs, respectively. Note residue S19 is bound only by the Omicron variant of SARS-CoV-2. The right panel shows a zoom-in of the substrate recognition and the active site residues. SP: Signal peptide; NTD: N-terminal domain; RBD: receptor-binding domain; RBM: receptor-binding motif; FP: fusion peptide; HR 1&2: heptad repeat 1&2; CD: connecting domain; TM: transmembrane domain; CT: C-terminal domain and CP: cytoplasmic extension/peptide.
therapy in that it could facilitate high-avidity virus binding and neutralization consequences. In theory, it is acknowledged that conferred multivalency increases ligand-receptor interaction (∆G) via combined enthalpic and entropic effects [87,88]. Such level of interaction practically translates into abated off-target outcomes in clinical applications [87,89–91]. Generally, the potency threshold of multivalent therapeutic candidates surpasses monomers and conventional medications [92,93].

On the other hand, incorporating multifunctional attributes into therapies for virus-associated respiratory diseases presents additional armaments for preventing or improving infection- and therapy-associated discomforts such as cytokine release syndrome (CRS) [94,95] and acute respiratory distress syndrome (ARDS) [96,97]. One practical and promising approach of consideration on this account centres on the tandem fusion of potent neutralizers (e.g., ACE2) with cytokine receptors or antioxidative materials of medicinal significance to combat damaging oxidative stress and organ insult. Multifunctional designs incorporating immune and effector function modalities may also expedite recovery from disease.

Interestingly, ACE2 is a Zn\(^{2+}\) metallopeptidase known to degrade vasoactive molecules (e.g., Ang II, bradykinin metabolites and apelin-13) to control cardiovascular functions and fluid balance [98]. Therapeutic sACE2 has an innate ability to relieve ARDS by degrading angiotensin (Ang) II to Ang 1–7 in the renin-angiotensin system (RAS) [99,100]. During ACE2 and SARS-CoV-2 spike protein interaction, the conversion of Ang II gets impeded, whereas des-Arg 9-bradykinin degradation accelerates, as suggested by biochemical studies [101]. Assuming this biochemical consequence happens in COVID-19 patients, there will be an overflow of undegraded Ang II. Alarmingly, significant levels of undegraded Ang II results in detrimental pro-inflammatory and pro-fibrotic consequences [102,103]. In this regard, therapeutic ACE2 may offer multifunctional support for COVID-19 patients. However, clinical validations are not yet available to substantiate the dual functionality (i.e., antiviral and anti-cardiopathic) of native sACE2 in coronavirus treatment. For instance, it remains unknown whether the catalytic function would disturb the natural balance of endogenous substrates during administration. Accordingly, several scientists have resorted to deactivating the catalytic component to avoid unintended consequences on RAS equilibrium.

**ACE2 catalytic activity deactivation**

The active site of human ACE2 contains a Zn\(^{2+}\) ion coordinated by the HEXXH motif composed of His374, His378 and Glu402 [104]. Also, substrate recognition within the catalytic groove is supported by Glu145, Arg273, His345, Pro346, Asp368, His505 and Tyr510 (Fig. 2F) [104–107]. These structural insights make the zinc-coordination motif and substrate recognition site crucial targets for mutagenesis in scenarios of developing catalytically inactive ACE2 candidates for therapeutic application. Notably, the HEXXH motif is independent of the SARS-CoV-1 and –2 interaction sites [105,108]. A similar argument could be made for HCoV-NL63 since it shares independent interaction sites and the collectrin domain (616–740) versions having the collectrin domain (616–740) out-performed the ACE2(18–740) performance (40–70 pM). This performance was a 10-fold improvement over the wild type ACE2-Ig. Also, all ACE2 (18–740) versions having the collectrin domain (616–740) out-performed their ACE2(18–615) counterparts owing to the dimerization potential of the collectrin domain and the ability to pre-p the ACE2 VBM towards high-affinity virus interaction.

In another study, Tada et al. [127] reported an ACE2 microbody composed of the ACE2 ectodomain fused to the IgG4-Fc domain. This low molecular weight design circumvented the possibility of antibody (Fc) dependent enhancement of viral infection. Interestingly, this innovative design and its H345A mutant did not compromise the binding affinity of the dimeric molecules but afforded up to 10-fold SARS-CoV-2 antiviral enhancement over monomeric sACE2. The design, moreover, inhibited SARS-CoV-2 D613G and other coronaviruses and prevented infection in K18-hACE2 mice. Similar work
was recently reported by Svilenov et al. [128], where their optimized IgG4-Fc engineering accorded ACE2 with a picomolar inhibition prowess against SARS-CoV-2 and its alpha to delta variants. It is worth highlighting that the above developments and optimization of ACE2 dwelled primarily on rational protein engineering skills. However, a lot has also been achieved from a computational optimization perspective. It is appreciable to say that Procko and co. have been the most vibrant group in this area [38,111,129,130]. For instance, their initial work adopted deep mutagenesis scanning techniques to engineer several ACE2 mutants towards high-affinity virus inhibition [111]. The mutagenesis, which sort to enrich the VBM of ACE2, uncovered sACE2 v2 (T27Y, L79T, N330Y, and A386L) and sACE2 v2.4 (T27Y, L79T and N330Y) among several others to bind tightly to SARS-CoV-2-5S. The binding performance of sACE2 v2 and sACE2 v2.4 mutants was 65- and 35-times higher than sACE2 WT. Furthermore, the rational inclusion of the collectrin domain to both sACE2 WT and sACE2 v2.4 produced avid binding kinetics that paralleled the performance of serum IgG from COVID-19 patients. More importantly, the IgG1 Fc fusions of dimerized sACE2 WT, sACE2 v2 and sACE2 v2.4 improved the binding affinities (KD) to 22, 0.2 and 0.6 nM, respectively. Subsequent pharmacokinetics studies of sACE2 v2.4-IgG1 by the same group showed a substantial inhibition of live SARS-CoV-2 and its VOCs in humanized K18-hACE2 mice [129]. The work also reported substantial relief from lung vascular hyperpermeability and oedema formation post-administration of sACE2 v2.4-IgG1.

![Fig. 3. Some protein engineering approaches for achieving multivalent ACE2 therapeutic molecules. a, Monomeric and dimeric native ACE2 formats. b, Immunoglobulin Fc fusions. (c-e) Non-immunoglobulin fusions. (d-e) Challenging but achievable ACE2 multimerization options.](image-url)

Table 1

| Mutations | Substrate | Effect | Ref |
|-----------|-----------|--------|-----|
| T27Y/L79T/N330Y | Mca-APK(Dnp) | Reduced activity | [111] |
| H34V/N90Q/H374N/H378N (CVD118) | Mca-APK(Dnp) | No activity against Mca-APK(Dnp) | [110,112] |
| E145A, R273A, H345A, P346A, D368A, H374A, H378A, E402A, or H505A | Mca-APK(Dnp) | Insignificant activity against AngII | [106] |
| | Ang II | E145A and D368A were active against Mca-APK(Dnp) | |
| | apelin-13 | H345A, P346A and H505A were active against Ang II and apelin-13 | |
| | ND | All mutant showed an inhibited activity | [35,113] |
| H374N/H378N | Mca-APK(Dnp) | No activity | [105] |
| Single mutants of the following: R273Q, R273K, R273L, H245A, H505L, H374N, or H378N | Mca-APK(Dnp) | Nearly eliminated | [114] |
| H374A/H378A | Mca-APK(Dnp) | No activity | [115] |
| T27T/R273Q | Mca-APK(Dnp) | No activity | |
| S128C/V341C | Mca-APK(Dnp) | Nearlly eliminated | |
| | ND | No activity | |

Bold: zinc-coordination mutations; ND: Not determined.
pseudotyped and live SARS-CoV-2 viruses. For instance, the dimerized and Fc-fusion version of the best-enhanced candidate produced an incredible IC_{50} of 28 ng/ml.

Contrary to the yeast-based random mutagenesis approach reported by Glasgow et al. [112], Higuchi et al. [115] adopted a human cell-based directed evolution approach to engineer ACE2 decoys towards improved binding characteristics against SARS-CoV-2 and its VOCs. Notably, this human cell-based approach surmounts experimental artefacts that could stem from post-translational modification disparities. The work obtained four high-affinity ACE2 variants (denoted 3N39, 3N39v2, 3J113v2 and 3J220v2) whose inhibition efficacy was 100-fold superior to the wildtype for both soluble and Fc-fusion forms. All ACE2 designs were stable and could likewise inhibit SARS-CoV-1, except 3J113v2. Further disulfide bond mutations (S128C/V343C) ensured the closed conformation of ACE2 was rigidified for optimum stability and functionality. Notably, no viral escape emerged in cell studies, and the administration of the ACE2 therapy into SARS-CoV-2 infected hamsters led to a reduced viral load and pathological consequences.

A structure-guided glyco-engineering approach has also received recognition for optimizing ACE2 for efficient virus inhibition [131]. In complementation with molecular dynamics (MD) simulations, the approach revealed some attractive N-glycan hot spots on ACE2 that improves binding affinity and neutralization competency of SARS-CoV-2 and associated viruses.

The Ig-fusion strategy continues to explode with similar outcomes. What remains uncommon in this area is engineered IgA-Fc and IgM-Fc scaffolds that could, respectively, afford a tetravalent and decavalent antiviral arsenal for coronavirus treatment. In this respect, Tanaka et al. [39] reported an ACE2-IgAFc candidate among several ACE2-IgG1Fc variants developed through MD simulation-based optimization. The ACE2-IgAFc demonstrated avid binding characteristics (K_{d} 0.166 nM), potentially neutralizing both wildtype and VOCs (i.e., N501Y, L452R; N501Y and E484K).

From the IgM perspective, the full-length or tailpiece IgM-Fc could be explored to achieve the avid effect demonstrated in other studies. [21,132] In this case, the IgM-Fc fusion may elicit humoral cytolytic reactions and pathogen agglutination (i.e., the formation of aggregated immune complexes) as reported for bacterial pathogens [133]. This agglutination effect results in a superior neutralization activity to IgA-Fc and IgG-Fc. Similarly, there exists a substantial opportunity to use several polymeric Fc-fusions as has been done in other areas [134,135]. The admitted challenge in these scarcely explored areas would be how to obtain reasonably high titres while avoiding aggregations.

### Table 2

Some ACE2 decoys based on immunoglobulin Fc fusions.

| Design          | ACE2 Mutations | SARS-CoV-2-5 | SARS-CoV-2-6 | Viruses (or S protein) tested | Ref. |
|-----------------|----------------|--------------|--------------|-----------------------------|------|
| ACE2-Ig         | WT             | 11.2 nM      | 0.1 μg/ml    | SARS-CoV-1                  | [113]|
| mACE2-Ig        | H274N', H345A' | ND           | 0.03 μg/ml   | SARS-CoV-2                  | [127]|
| ACE2            | ND             | 1.24 μg/ml   | SARS-CoV-1/2 | SARS-CoV-2                  | [127]|
| ACE2 microbody  | WT             | 0.36 μg/ml   | ND           | WIV1, YRDa11                | [38,111]|
| ACE2 microbody (H345A) | H345A' | 0.15 μg/ml   | ND           | Rv2431, Rv6084              |      |
| ACE2 WT         | H34A, T92Q, Q325F, A386L | 140 – 150 nM | ND           | SARS-CoV-1                  | [38,111]|
| sACE2 v1        | T27Y, L197, N330Y | 80 nM       | ND           | SARS-CoV-2                  |      |
| sACE2 v2        | T27Y, L197, N330Y | 2.3 nM      | ND           | HCov-NL63                   |      |
| sACE2 v2        | T27Y, L197, N330Y | 3.8 nM      | ND           | SARS-CoV-2                  | [112]|
| sACE2 WT (dimer)| T27Y, L197, N330Y | ND           | < 1 nM       | SARS-CoV-2                  | [112]|
| sACE2 v2.4 (dimer)| T27Y, L197, N330Y | ND           | ND           | SARS-CoV-2                  | [112]|
| sACE2 WT-Fc     | T27Y, L197, N330Y | 22 nM       | ND           | SARS-CoV-2                  | [112]|
| sACE2 v2-Fc     | H34A, T92Q, Q325F, A386L | 0.2 nM      | ND           | SARS-CoV-2                  | [112]|
| sACE2 v2.4-Fc   | T27Y, L197, N330Y | 0.6 nM      | ND           | SARS-CoV-2                  | [112]|
| CDV012 WT (Fc)  | H34V           | 10.0 ± 0.98 nM | 0.43 ± 0.39 μg/ml | SARS-CoV-1                  | [112]|
| CDV014 (dimer, Fc) | H34V         | 3.2 ± 0.36 nM | 0.35 ± 0.19 μg/ml | SARS-CoV-2                  |      |
| CDV019 (dimer, Fc) | K31F, H34I, E35Q | 0.89 ± 0.11 nM | 0.31 ± 0.16 μg/ml | HCov-NL63                   |      |
| CDV118 (dimer, Fc) | H34V, N90Q, H374N', H378N' | 0.95 ± 0.22 nM | ND           | SARS-CoV-2                  | [112]|
| CDV310 (Yeast display) | A25V, T27Y, H34A, F460D, H454L' | 0.4 ± 0.03 nM | 0.058 ± 0.03 μg/ml | SARS-CoV-2                  | [112]|
| CDV373 (Yeast display) | Q18R, K181, N330D, H34S, E35Q, W69R, Q76R | 0.12 ± 0.01 nM | ~0.028 μg/ml | SARS-CoV-2                  | [112]|
| ACE2-Fc WT      | ND             | 0.13 μg/ml   | ND           | SARS-CoV-1                  | [106]|
| ΔACE2-Fc Arg273Aa | R273A'       | 0.19 μg/ml   | ND           | SARS-CoV-2                  |      |
| ΔACE2-Fc His378Aa | H378A'       | 0.16 μg/ml   | ND           | SARS-CoV-2                  |      |
| ΔACE2-Fc Glu402Aa | E402A'      | 0.24 μg/ml   | ND           | SARS-CoV-1                  | [126]|
| ACE2-Ig-v1      | ND             | ~ 4 μg/ml    | ND           | SARS-CoV-1 Pangolin-CoV-2020 | [106]|
| ACE2-Ig-v3 (tetramer) | D30E         | 0.68 nM      | ND           | nCoV-RaTG13                 |      |
| ACE2-Ig-v4 (hexamer) | D30E         | 0.07 nM      | ND           | SARS-CoV-1 Pangolin-CoV-2020 | [106]|
| WT              | ND             | 17.63 μM     | 24.8 μg/ml   | SARS-CoV-1                  | [115]|
| 3N39            | A25V, K26E, K31N, E35K, N64I, L79F, N90 | 0.29 μM | 0.056 μg/ml | SARS-CoV-1                  |      |
| 3N39v2          | A25V, K31N, E35K, L79 | 0.64 nM | 0.082 μg/ml | SARS-CoV-2                  |      |
| 3J113v2         | K31M, E35K, Q60R, L79F | 1.14 μM | 0.33 μg/ml | SARS-CoV-1 Pangolin-CoV-2020 | [106]|
| 3J220v2         | ND             | 3.98 μM      | 0.068 μg/ml  | SARS-CoV-1 Pangolin-CoV-2020 | [106]|
| hACE2-Fc        | ND             | 2.65 μM      | 10.8 μg/ml   | SARS-CoV-1/2                 | [35]|
| hACE2-Fc mutant | H374N', H378N' | 16.1 nM      | 46.73 μg/ml  | HCov-NL63                   | [39]|
| ACE2-IgG1Fc     | ND             | 21.40 μM     | 1.61 μg/ml   | SARS-CoV-2                  |      |
| ACE2-IgG1Fc (mutant) | H34A         | 4.09 μM      | ND           | SARS-CoV-1 Pangolin-CoV-2020 | [106]|
| ACE2-IgG1Fc (mutant) | T27Y         | 8.01 μM      | ND           | SARS-CoV-1 Pangolin-CoV-2020 | [106]|
| ACE2-IgG1Fc     | T27Y, H34A    | 0.56 μM      | 0.11 μg/ml   | SARS-CoV-1 Pangolin-CoV-2020 | [106]|
| ACE2-IgG1Fc     | ND             | 0.166 μM     | ND           | SARS-CoV-1 Pangolin-CoV-2020 | [106]|

ND: Not determined.
* Mutation targeted at reducing or inactivating the ACE2 enzymatic function.

Non-immunoglobulin based multimers

Common in this area is the use of trimeric foldon scaffold [136,137], de novo coil-coil systems (e.g., 3HB) [138] and the
rigid) and lengths. Interestingly, the rigid designs (i.e., ACE2-rigid-3HB and ACE2-rigid-foldon) remarkably outperformed their flexible counterparts and monomeric sACE2. Specifically, ACE2-rigid-3HB and ACE2-rigid-foldon showed an extremely tight interaction (K_D < 1 pM) with prefusion stabilized SARS-CoV-2 S protein trimer compared to the monomeric ACE2 (K_D 25 nM). Nonetheless, the flexible candidates, i.e., ACE2-flexible-3HB (4.4 nM) and ACE2-flexible-foldon (0.34 nM), also outperformed the short linker versions. An assessment of virus inhibition with these designs followed a ranking of ACE2-rigid-3HB (IC_{50} 0.40 nM) > ACE2-rigid-foldon (IC_{50} 0.48 nM) > ACE2-flexible-foldon (IC_{50} 1.58 nM) > ACE2-flexible-3HB (IC_{50} 3.46 nM) > monomeric ACE (IC_{50} > 50 nM), which was similar in SARS-CoV-1 neutralization assessment. Interestingly, ACE2-flexible-foldon was ranked the most potent among all the candidates as it showed an ‘avidified’ inhibition towards SARS-CoV-1, WIV1, Rs3667 and the D614G mutant of SARS-CoV-2.

On a similar tangent, Xiao et al. [141] used a structure-based approach to engineer trimERIC ACE2 (18−615)-foldon that showed a binding affinity of ~60 pM for SARS-CoV-2-S. Herein, dimeric (18−740) and monomeric ACE2 (18−615) versions had binding affinities of 12−22 nM and 77 nM, respectively. A subjection of ACE2 (18−615)-foldon to a point mutation (T27W) produced a marked high affinity (1.2 nM).

Lastly, Miller et al. [142] adopted the tetramerization domain of p53 for engineering a super-potent tetramERIC ACE2 decoy against SARS-CoV-2. Compared to the monomeric (K_D 22 ± 3 nM) and dimeric ACE2 counterparts (K_D 21 ± 7 nM), the tetrameric design showed a strong 3.9 ± 0.3 nM affinity. A subsequent study by the same group revealed that the tetrameric format greatly enhanced VOCs neutralization [143].

A question remains whether scientists could go higher in multivalence by exploring other non-immunoglobulin scaffolds such as virus-like particles [144], ferritin [145,146] and other protein cages [147,148]. Like the high-order immunoglobulin scaffolds, the challenge of these multi-affinity designs would be centred on facile expression, protein titres and aggregation. However, overcoming such a hurdle would pave the way for super-targeted ACE2 antiviral engineering.

Particulate nanotechnologies

Secreted extracellular nanovesicles

Extracellular vesicles (EVs) have attracted many biopharmaceutical investments in advanced therapeutics [149]. Before then, EVs were initially known to mediate intercellular communications [150]. However, medicinal advancement has catalyzed applications in bioactive cargo shuttling, immune response regulation, oxidative stress management, and inflammation control over the years [150–152]. These applications essentially depend on the size and outer envelope composition, which, by extension, inform the biodistribution and safety patterns during administration [153–154]. Current developments even make it possible to engineer EVs for disease-specific applications [150,155–157]. Therefore, the knowledge transfer for COVID-19 therapy development was much-expected (Fig. 4).

For instance, Coccozza et al. [158] engineered and compared the potency of ACE2-EVs from natural ACE2-expressing cell lines (i.e., Calu-3, a human lung epithelial cell line; and Caco-2, a human epithelial colorectal cell line) and genetically-modified HEK293T-ACE2. All EVs were extracted from culture supernatant and purified via size-exclusion chromatography. The Calu-3 and Caco-2 associated EVs showed the presence of EV markers such as CD63, CD81 and ADAM10 but with relatively lower levels of membrane-integrated ACE2 than that of the HEK293T-ACE2 cell line. The HEK293T-ACE2 derived EVs were found to contain HSP70 in addition to CD63, CD81 and ADAM10. Interestingly, all EVs showed significant inhibition of SARS-CoV-2 pseudovirus in a magnitude relative to the level of integrated ACE2 (EV- Caco-2 < EV- Calu-3 < EV- HEK293T-ACE2). Further interaction of TMPRSS2 into HEK293T-ACE2-derived EVs resulted in an improved reduction in viral infectivity. The TMPRSS2 associated enhancement may probably be due to the S-protein priming activity TMPRSS2. Nonetheless, about 500–1500 times more soluble recombinant human ACE2 proteins were required to match the level of inhibition achievable by ACE2- EVs or ACE2- TMPRSS2-EVs.

In a recent study, El-Shennawy et al. [159] extracted ACE2-EVs via ultracentrifugation from engineered HEK293T-ACE2 and HeLa-ACE2 culture supernatants. The EVs were determined to harbour CD63, CD81, TSG101 and Syndemdin-1 along with the surface ACE2. The HEK293-sourced EV appeared to be more potent than the HeLa-sourced EVs, showing a SARS-CoV-2 pseudovirus neutralization (IC_{50} 8.01 and 13.63 pM, respectively. Overall, the EVs were 60- to 80-fold more potent than sACE2 in preventing pseudotyped virus infection. The EVs also neutralized SARS-CoV-2 variants (α, β, and δ) and protected transgenic human ACE2 mice from lung injury and death. Similarly, Wu et al. [160] extracted ACE2-EVs from culture supernatants of engineered stable HEK293T-ACE2 cells via a differential ultracentrifugation approach. These bioengineered EVs had an average size of 58.5 nm. The EVs were able to efficiently block the infection of SARS-CoV-2 in cell assays and mice models without any observable changes in blood chemistry and tissue injury.

Instead of relying on endogenous EV components, it has become possible these days to fuse molecules of interest to EV-forming subunits for efficient assembling and integration into vesicles. An example is reported by Kim et al. [161], where ACE2 (wildtype, sACE2.v1 and sACE2.v2, reported earlier [111]) was genetically fused to the CD9ΔTM4 (CD9 lacking the transmembrane domain region 4). The ACE2-CD9ΔATM4 construct was expressed in HEK293T cells and purified via tangential flow filtration, showing a size range of 50–200 nm. All the ACE2-EV versions effectively neutralized wildtype SARS-CoV-2 and associated VOCs (ΔD614G; K417N-E484K, N501Y; and L452R-T478K-D614G), with the sACE2.v1 and sACE2.v2 associated EVs performing much better than the wildtype in agreement with earlier reports [111]. Further studies in K18-hACE2 mice led to an abrogation of SARS-CoV-2-induced consequences.

Also, Xie et al. [162] fused the sequences of ACE2 and plasma membrane (PM)-targeting molecule of neuronal growth-associated proteins GAP43 to produce EVs enriched with surface-exposed ACE2. Herein, it was discovered that palmitoylation of the Cys141 and Cys498 residues in the ACE2 potentiates high-level ACE2 integration in the EVs. In other words, de-palmitoylation of the cysteine residues dramatically decreased the level of integrated ACE2 in the EVs. Nevertheless, the ACE2-EVs efficiently neutralize pseudotyped and authentic SARS-CoV-2 in cell assays and transgenic mice with no observable adverse consequences.

Extruded cell membrane nanotrap

Extruded cell membrane (ECM) nanotrap have also been reported. ECMs are plasma membrane particles derived from cells through a series of membrane shredding processes (Fig. 4b). The technology has gained traction in vaccine and prophylaxis development because of the ability to retain and preserve essential native molecules and integral receptors with desirable immune, pharmaco-cytic, and self-adjuvanting functionalities [163,164]. The ECM nanotrap concept is called artificial exosome and has proven to offer impressive biocompatibility and pharmacodynamic advantages in drug delivery applications [164]. This drug development approach became quite established recently, leveraging on processes such as hypotonic lysis, mechanical disruption, and gradient centrifugation.
or dialysis [163,165–167]. It is therefore of no surprise that these developments have opened opportunities for use in ACE2 therapeutics engineering.

For instance, Li et al. [168] synthesized human lung spheroid cells (LSCs)-derived ACE2 vesicles via the serial extrusion of LSC plasma membrane to ascertain their neutralization efficacy on SARS-CoV-2 infectivity. The membrane nanovesicles showed a marked reduction of viral invasion by accelerating phagocytic clearance of SARS-CoV-2 mimics in mice and monkey studies. Furthermore, the work reported no observable toxicity or abnormality in major organs. There was also no significant elevation in cytokine levels among control and tested groups.

Also, Rao et al. [169] reported a nanodecoy derived from the fusion of extruded ACE2-expressing HEK293T and human myeloid mononuclear THP-1 cell membranes for synchronous neutralization of virus and inflammatory cytokines. The vesicle formulation was facilitated by serial sonication. Interestingly, as the ACE2 component targeted virus neutralization, the essential components from the monocyte (specifically, CD130/CD116) ensured the attenuation of inflammatory cytokines such as interleukin 6 (IL-6) and

![Fig. 4. ACE2 extracellular vesicle synthesis and application for virus inhibition. a, Secreted extracellular nanovesicle synthesis approach. b, Extruded cell membrane nanotrapping synthesis. c, Membrane encapsulated nanotherapeutics synthesis. d, Sample cryo-EM images of HEK-derived engineered ACE2 EVs stained for the presence of ACE2 (left panel) and CD81 (right panel) (Source: [159]). Scale bars = 100 nm.](image-url)
granulocyte–macrophage colony-stimulating factor (GM-CSF). Overall, the work reported a significant blocking of SARS-CoV-2 mimics in addition to relieving IL-6 and GM-CSF discomforts in an acute pneumonia mouse model to improve long health.

Similarly, Zhang et al. [170] designed inhalable nanocatchers to neutralize SARS-CoV-2 and its D614G variant. Herein, the nanocatchers were produced by the extrusion of the plasma membrane of HEK293-A CE2 cells. The vesicles harboured a high density of exterior A CE2 molecules that neutralized SARS-CoV-2 wildtype (IC<sub>50</sub> 9.5 μg/ml) and D614G variant (IC<sub>50</sub> 16.3 μg/ml). The nanocatchers showed good stability and biocompatibility with no apparent cytotoxicity. Interestingly, using hyaluronic acid (HA) excipient in the intranasal nanocatcher formulation significantly improved lung retention.

In the same way, Wang et al. [171] prepared an A CE2-nanoparticle from A CE2-rich cells via membrane extrusion and reported dose-dependent trapping of SARS-CoV-1, SARS-CoV-2 and the D614G variant. Strikingly, the A CE2-particle further enhanced mitochondrial Optic atrophy 1 (OPA1) levels to attenuate the virus’s activity towards mitochondrial dysfunction.

Membrane encapsulated nanotherapeutics

In recent times, membrane encapsulated nanotechnologies have been another prevalent advancement in therapeutics engineering (Fig. 4c). In this approach, researchers leverage cell plasma membranes for decorating nanoparticles with desired therapeutic characteristics, including selective antigen targeting and prolonged circulation time [172–175]. Notably, this brilliant plasma membrane coating approach has effectively addressed many biointerfacial challenges of synthetic nanoparticle drug delivery systems in other areas [176]. For instance, the technology has gained enormous attraction in trapping and detaining diverse pathogens over the years [177–180].

However, to develop a broad-acting countermeasure for COVID-19, Zhang et al. [181] synthesized membrane coated poly(lactic-co-glycolic acid; PLGA) nanoparticles from human lung epithelial cells (LEC) and macrophages (MΦ). The LEC and MΦ membranes were extracted via differential centrifugation techniques. Designated as nanosponges (NS), the LEC-NS and MΦ-NS showed the presence of LEC [A CE2, DPP4 and TMPRSS2] and MΦ [A CE2, CD147 and C-type lectin domain family 10 (CLEC10)] associated surface markers, respectively. Interestingly, LEC-NS and MΦ-NS showed prolonged colloidal stability in buffered solution and neutralized SARS-CoV-2 at IC<sub>50</sub> of 827.1 μg/ml and 882.7 μg/ml, respectively, with no signs of lung injury. Interestingly, CD147 and CLEC10 are also potent coronavirus receptors [58,182] and, thus, their presence in MΦ-NS possibly augmented the neutralization potential. Also, the presence of DPP4 in LEC-NS could render it potent against MERS-CoV. However, these suppositions require further experimental elucidations.

The inclusion of complementary nanoparticles that augment the efficacy of engineered A CE2 holds high promise. A typical example is the A CE2 nanotraps presented by Chen et al. [183] The reported nanotraps is composed of a biodegradable poly lactic acid (PLA) nanoparticle core encapsulated with a liposomal shell-integrating A CE2 and phosphatidylyserines. The engineered A CE2 nanotraps completely inhibited the entry of pseudotyped and authentic SARS-CoV-2 into several cell lines at a magnitude 10-times higher than the blocking efficacy of sA CE2. Additionally, the integration of the phosphatidylyserines in the liposomal membrane provoked the macrophagic clearance of entrapped viruses at the site of infection. Further works in immunocompetent mice and live human lungs affirmed the PLA-based A CE2 nanotraps to abrogate a broad-spectrum SARS-CoV-2 infection with no significant inflammatory consequences.

Similarly, Wang et al. [184] engineered vesicular microspheres from HEK293-A CE2 and MΦ cells treated with lipopolysaccharide (LPS) and interferon-γ (IFN-γ). Herein, the fused A CE2/LPS/IFN-γ vesicles were sheathed around methacrylate hyaluronic acid (HA) hydrogel, which facilitated effective virus neutralization and adsorption of inflammatory factors along the respiratory pathway. Notably, this self-incipient HA-based microsphere may also have the penchant to improve lung retention further, even as the sheath promotes innate and adaptive protection.

Lastly, to address the damaging oxidative stress associated with cytokine upregulation, Ma et al. [185] reported a biocompatible...
Particulate nanochemistry-based therapeutics

Particulate nanochemistry for therapy development has been around for some time now (Fig. 6). In this area, researchers combine unique orthogonal chemistries and nanoparticle scaffolds to develop nanomedicines of clinical relevance. Over the years, techniques such as biotinylation, [186–188] click chemistries, [189–191] \( \pi \)-clamp [192–194], and redox-activated chemical tagging (ReACT) [195,196] have been promising for clinical nanomedicine development.

However, much has not been done directly in multivalent ACE2 therapeutics for coronavirus neutralization.

For example, Satta et al. [197] developed a nano-liposome-human ACE2 (Lipo-hACE2) decoy with SARS-CoV-2 inhibition concentration of 10.68 nM. The Lipo-hACE2 was synthesized from biotinylated liposome, neutravidin and biotinylated ACE2, where the neutravidin served as an anchor connecting the liposome and ACE2. Aside from neutralizing SARS-CoV-2-S and pseudoviruses, the Lipo-hACE2 was also able to neutralize cytokine associated inflammations in murine, mice and human macrophage studies.

The click chemistry technique was also used in another study to engineer algae-based ACE2-microrobots for trapping SARS-CoV-2 in virus infected waterbodies. [198] Herein, the ACE2-microrobots achieved 89% removal of SARS-CoV-2 pseudovirus from infected wastewater. Interestingly, this idea may be easily adopted for human-associated cell therapies against coronaviruses. In this case, cells could be engineered to harbour functional click handles on the plasma membrane for subsequent ‘clicking’ with ACE2. The click functionalization of the ACE2 can be achieved via site-specific

semi-synthetic ACE2-exosome, termed nanobait, for the simultaneous neutralization and prevention of SARS-CoV-2 and cytokine storm, respectively. The researchers invaginated polydopamine (PDA) nanoparticles into ACE2-expressing HEK293T cells and retrieved the nanoparticles as the exocyted product. The PDA adopted an ACE2 sheath from the cell membrane, facilitating high SARS-CoV-2 trapping. In addition to the antiviral effect, the PDA also provided essential antioxidative relief to attenuate cytokine storm challenges in mice studies. All these developments exemplify the advantages of multivalent ACE2-EVs in virus neutralization (Fig. 5).

Fig. 6. Particulate nanochemistry-based therapeutics. a, Some promising orthogonal chemistries. b, Some complementary nanoscaffolds. c, Click chemistry-based synthesis of ACE2 polymersome. d, ACE2 liposome synthesis based on biotin-neutravidin interaction.
ligation techniques such as sortase A transpeptidation [199–202] or genetic code expansion [203–206].

A related example was presented by Ai et al. [207], where azido-expressing THP-1 cells were used to produce heparin-functionalized nanosponges (HP-NS) for SARS-COV-2 neutralization. It should be noted that the researchers did not directly use cells for therapy application. Rather, the azide-functionalized plasma membrane was extracted and coated on PLGA nanoparticles, which were later fused to dibenzocyclooctyne (DBCO)-heparins through click chemistry. The engineered HP-NS achieved an increased neutralization potency (IC_{50} 0.0254 μg/ml) contrary to control particles (IC_{50} 170 μg/ml). The engineered nanosponges also showed the presence of CD130/CD116 receptors in addition to the heparins, which could potentially attenuate IL-6, IFN-γ and IFN-a, respectively.

### Outlook and conclusion

The spread of coronaviruses has resulted in incredible innovations in the development of therapeutics with potentially promising clinical outcomes provided all safety and efficacy requirements are met. So far, there has not been any adverse toxicity and safety concerns around ACE2 decoys from the preliminary studies available. For instance, Huang et al. [208] observed no toxicity effect on epithelial cells. Also, Zhang et al. [209] reported no observable toxicity when sACE2v2.4 was intravenously administered (twice daily at 0.5 mg/kg) into mice for 5 continuous days. Several other research have consistently found no adverse effect with the use of ACE2 therapeutics [83,106,115,160,170,209]. Although higher-level clinical assessments are yet to surface, it is anticipated that the origin of the ACE2 molecule itself may be important for their safe administration.

Predictably, the Fc receptor fusions are the most likely candidates to transition from benchtop to bedside owing to prior clinical history. The only concern with the Fc approach may relate to possible dangers of antibody-dependent enhancement (ADE) of viral infection. In short, ADE results from the interaction of antibody Fc with FcR, which could mediate virus uptake into leucocytes to cause detrimental inflammations and tissue injury [210–213]. Although the ADE immunopathology is not fully understood and standardized in terms of assessment techniques, a careful selection and engineering of Fc candidates that could bypass the dangers of ADE may be critical to expedite clinical acceptance.

Bioengineered EVs are undoubtedly a mainstay and could be an important game-changer for coronavirus treatment if potential side effects are assessed and ruled out. Currently, the bioengineered EV field is vast expanding with several innovative possibilities to improve therapeutic responses. For instance, ACE2 can be genetically fused to EV membrane protein Lamp2b [214–216], glycosylphosphatidylinositol (GPI) anchor peptides [217,218], platelet-derived growth factor (PDGF) receptor [219–221], and arrestin domain containing protein 1 (ARRDC1) [222–226] for efficient recruitment and integration in vesicles. These developments can bring on board the desirable attributes of EVs in association with bioactive cargo shuttling, immune response regulation, oxidative stress management and inflammation control.

Progress in applications that combine particulate nanochemistry and complementary nanoscaffolds for multivalent and multifunctional ACE2 nanomedicines are also anticipated. Herein, the adoption of site-specific chemistries in these applications may offer a facile approach to controllably orient ACE2 molecules for maximum virus capturing. Additionally, the large available surface area of complementary and novel nanoscaffolds can be adopted to extend the scope of options for multivalent ACE2 nanomedicine engineering.

One key challenge that may possibly associate with nanoparticle-based ACE2 therapeutics is the formation of “protein corona.” Briefly, protein corona occurs via spontaneous adsorption of intracellular fluid (e.g., plasma) proteins onto nanoparticles [227–230]. The phenomenon has been critical for both pristine and protein-coated nanoparticle therapeutics due to its complex influence on nanoparticle’s biological activity, pharmacodynamics, safety and ultimate fate in living systems [231–236]. In part, the complexity depends on the size, morphology, surface charge and chemistry of the nanoparticle [231–236]. Similarly, the extent of protein adsorption also depends on the route of nanoparticle delivery (e.g., intravenous or intranasal) [237–240]. These factors, by extension, also modulate the colloidal stability (or agglomeration propensity) of nanomaterials in biological media [241–244]. At the moment, it remains unexamined how ACE2-coated nanoparticle may be impacted by the occurrence of protein corona. Fortunately, experimental cues in other nanomedicine studies are available that highlight how the phenomenon

### Table 3

Some ACE2 decoys based on nanotechnology.

| ACE2 decoy | Composition | Size (Zeta potential) | IC_{50} | Additional function | References |
|------------|-------------|-----------------------|---------|---------------------|------------|
| ACE2-EV    | ACE2(CD63/CD81) | 160 – 170 nm | ND     | ND                  | [158]      |
| evACE2     | ACE2(CD63/CD81/TSG101/Syntenin-1) | 180 – 200 nm | 41.92 – 93.63 μM | Reduced lung injury | [159]      |
| ACE2 EVs   | ACE2(CD9/CD63/CD81) | 50 – 200 nm | < 40 μg/ml | ND                  | [161]      |
| Palmitoylated ACE2 EVs | ACE2(CD9/CD63/CD81) | -200 nm | 12 ± 0.7 ng/ml | Lung inflammation protection | [162]      |
| ACE2-nanodecoy | ACE2(CD130/CD116 receptors) | -100 nm | - 24 μV | Cytokine neutralization | [169]      |
| Nanodecoy | ACE2(CD130/CD116 receptors) | -100 nm | - 24 μV | Cytokine neutralization | [169]      |
| Nanocatcher | ACE2 with HA incipient | 200 nm | 9.5 μg/ml (WT virus) | ND | [170]      |
| ACE2-nanoparticle | ACE2 | 169 nm | 431.2 μg/ml | OPAI elevation | [171]      |
| Nanosponge (NS) | LEC membrane on PLGA | -100 nm each | 827.1 μg/ml (LEC NS) | Cytokeine neutralization | [181]      |
| ACE2 microsphere | Mε membrane on PGLA | 35 μm | 8827.1 μg/ml (Mε NS) | Cytokeine neutralization | [181]      |
| ACE2 Evan | ACE2/IFN-γ on HA hydrogel | 5–35 μm | 0.2 – 0.8 mg/ml | Cytokeine neutralization | [184]      |
| Exosome-based nanobait | ACE2/PLEX/IFN-γ | 341 nm | 0.2 – 0.8 mg/ml | Cytokeine neutralization | [185]      |
| Lipo-hACE2 decoy | Lipid/neutavidin/ACE2 | 100 nm | 10.68 nM | Cytokeine neutralization | [197]      |

† Different ranges of particle size gave different results:
ND: Not determined.
could obstruct active-targeting, uptake and the ultimate fate of protein-coated nanomaterials [236,238–240,245,246]. The resulting impact may be complementary or deleterious [247–249] and thus worth examining for appropriate interventions. We anticipate that existing nanoparticle dispersion and stabilization strategies that leverage a careful control of the biophysical properties of phase formations and have been previously demonstrated in other protein-nanoparticle formulations [250–253] will be useful in circumventing protein corona and colloidal stability challenges in ACE2-based nano-pharmaceuticals. All the same, clinical safety validations of these approaches would determine their approval into clinical use.

Lastly, we foresee avenues of adopting programmable encapsulation systems, where ACE2 fragments and peptides are controllably expressed on the surfaces of engineered microbes by inductions for medicinal use. The technology has recently been reported for the treatment of cancer, where living bacteria were engineered to controllably evade immune recognition and deliver tumour suppression benefits even at distal site [254]. Although the area looks embryonic, its adoption for ACE2 therapeutics would guarantee the delivery of high doses at diseased site if concerns on safety are addressed.

In conclusion, multivalent ACE2 engineering remains a promising therapeutic approach for current and future coronavirus diseases. Considering the availability of several innovative approaches to confer multivalency and multifunctionality to engineered ACE2, scientists are better equipped to face future related diseases. The transition of multivalent ACE2 therapeutics from benchtop to bedside may not seem a distant dream, should uncompromised safety standards be met (Table 3).

CRediT authorship contribution statement

Eugene M. Obeng: Conceptualization, Writing – original draft (85%), Writing – review & editing (85%), Validation, Visualization (80%). Isaac Fianu: Writing – original draft (15%), Visualization (20%). Michael K. Danquah: Writing – review & editing (15%), Supervision.

Data availability

No data was used for the research described in the article.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Eugene M. Obeng has patent #Engineered ACE2 and uses thereof (Provisional) pending to Monash University.

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Declaration of competing interest

EMO is a co-inventor of engineered ACE2-IgM-Fc molecules for therapeutic use. The issued provisional patent, entitled: 'Engineered ACE2 and uses thereof', is held by Monash University on behalf of the inventors. LF and M.K.D declare no competing interest.

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