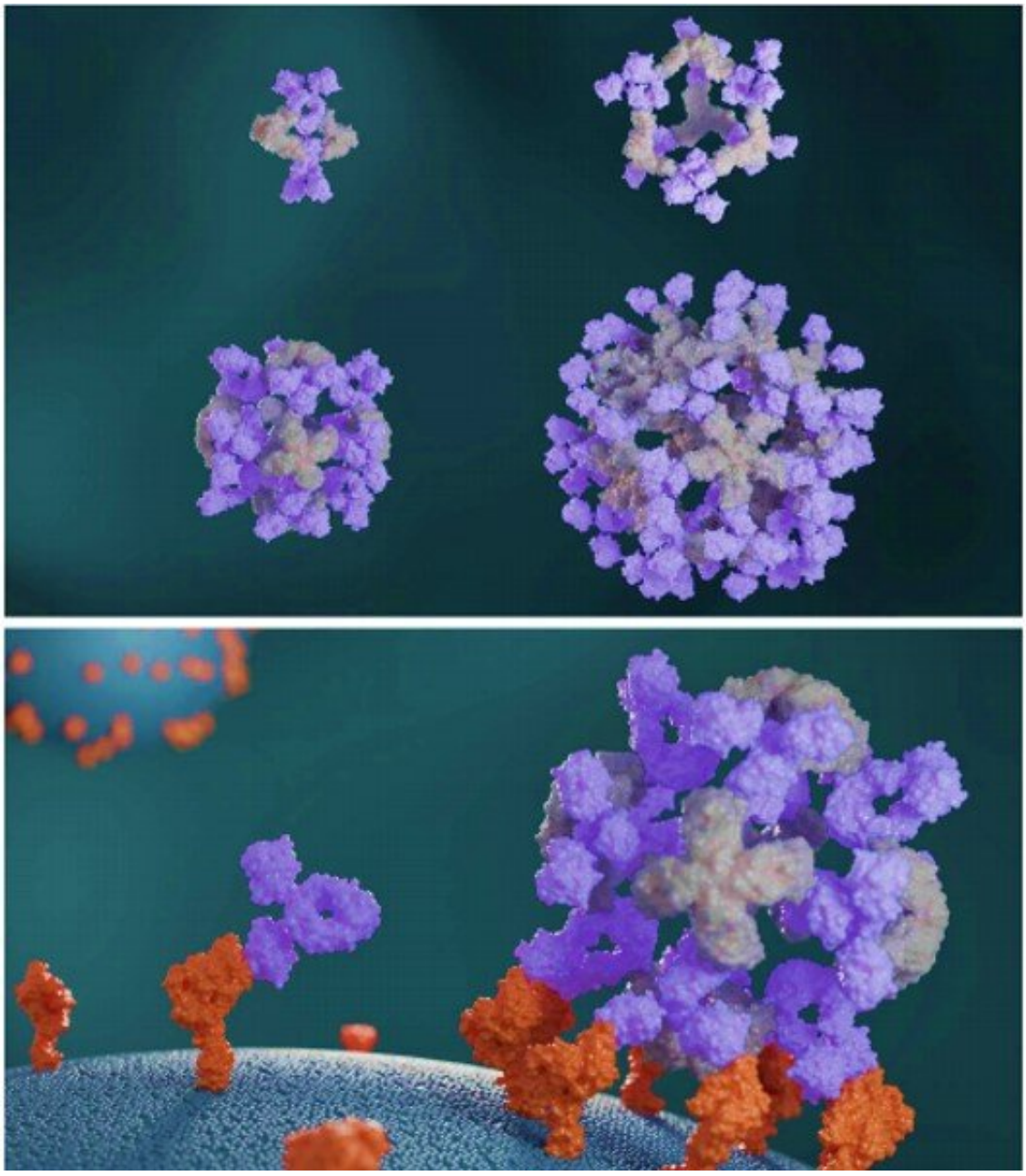
Designed proteins assemble antibodies into modular nanocages

April 16 2021, by Thamarasee Jeewandara



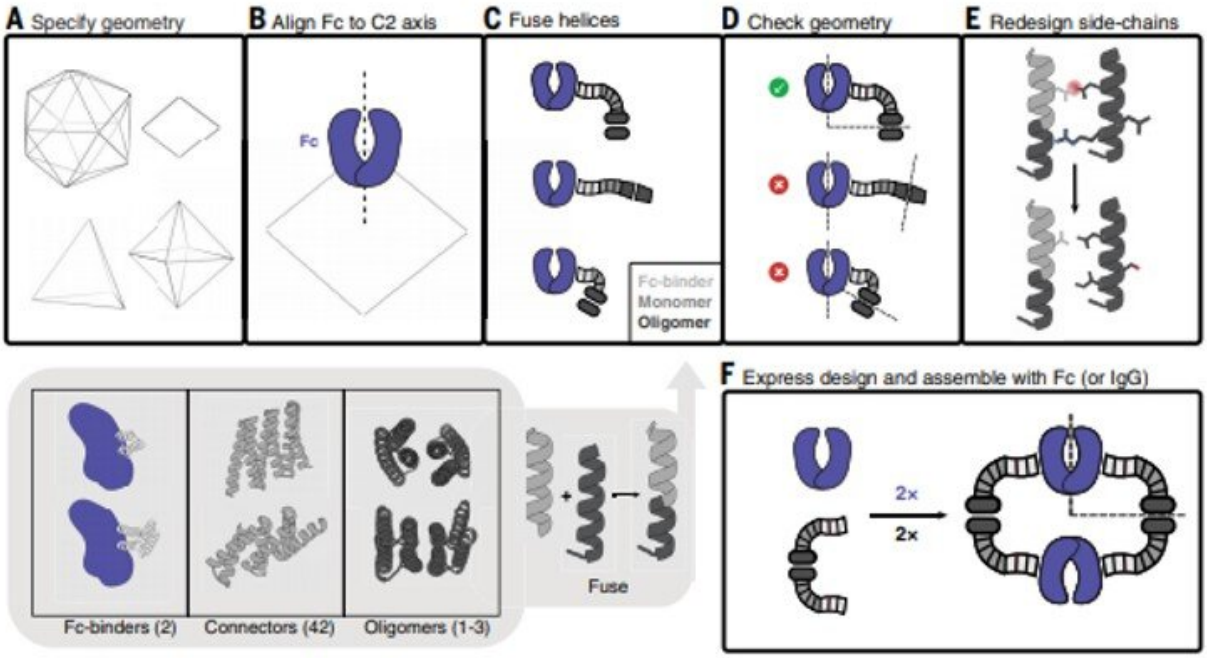
Designed proteins assemble antibodies into large symmetric architectures. Designed antibodyclustering proteins (light gray) assemble antibodies (purple) into diverse nanocage architectures (top). Antibody nanocages enhance cell signaling compared with free antibodies (bottom). Credit: Science, doi:10.1126/science.abd9994

In a new report now published on *Science*, Robby Divine and an interdisciplinary research team at the department of biochemistry, regenerative medicine, and vaccines and infectious disease at the University of Washington U.S. and the School of Medicine, at the Tehran University of Medical Sciences, Iran, conducted computational designs of nanocages to assemble antibodies into precise architectures. During the construction, one structural component formed an antibody or Fc-ligand fusion and the second design formed an antibody-binding homo-oligomer to drive nanocage assembly with different valencies and symmetry. The team hypothesize how this process can also increase the neutralization of a pseudovirus; severe acute respiratory syndrome coronavirus-2 (SARS-COV-2) via α- SARS-COV-2 monoclonal antibodies and Fc-angiotensin-converting enzyme 2 (ACE-2) fusion proteins.

Antibodies in medical research

Antibodies that specifically bind to targets of interest play a central role in biomedical research and medicine. Researchers can generate clusters of antibodies by genetically linking antibody fragments together to improve signaling. It is presently difficult to form antibody assemblies with a variety of precise architectures and valencies. In this work, Divine et al. computationally designed proteins that assembled antibodies into precise architectures with different valencies and symmetries. The team hypothesized that such designs could robustly drive arbitrary antibodies

into homogenous and structurally well-defined nanocages for pronounced effects on cell signaling. The researchers designed proteins to drive the assembly of arbitrary antibodies into symmetric assemblies with well-defined structures. For this, they rigidly fused together three types of "building block" units containing antibody Fc-binding domains, helical repeat connectors and cyclic oligomer-forming modules. In its architecture, the Fc-binding unit positioned itself with the C2 antibody dimer, the cyclic homo-oligomer formed the second cyclic symmetry axis in the nanocage, and the helical repeat connector linked the antibody and cyclic homo-oligomer symmetry axes in the correct orientation to form the antibody nanocages referred to as the AbCs.



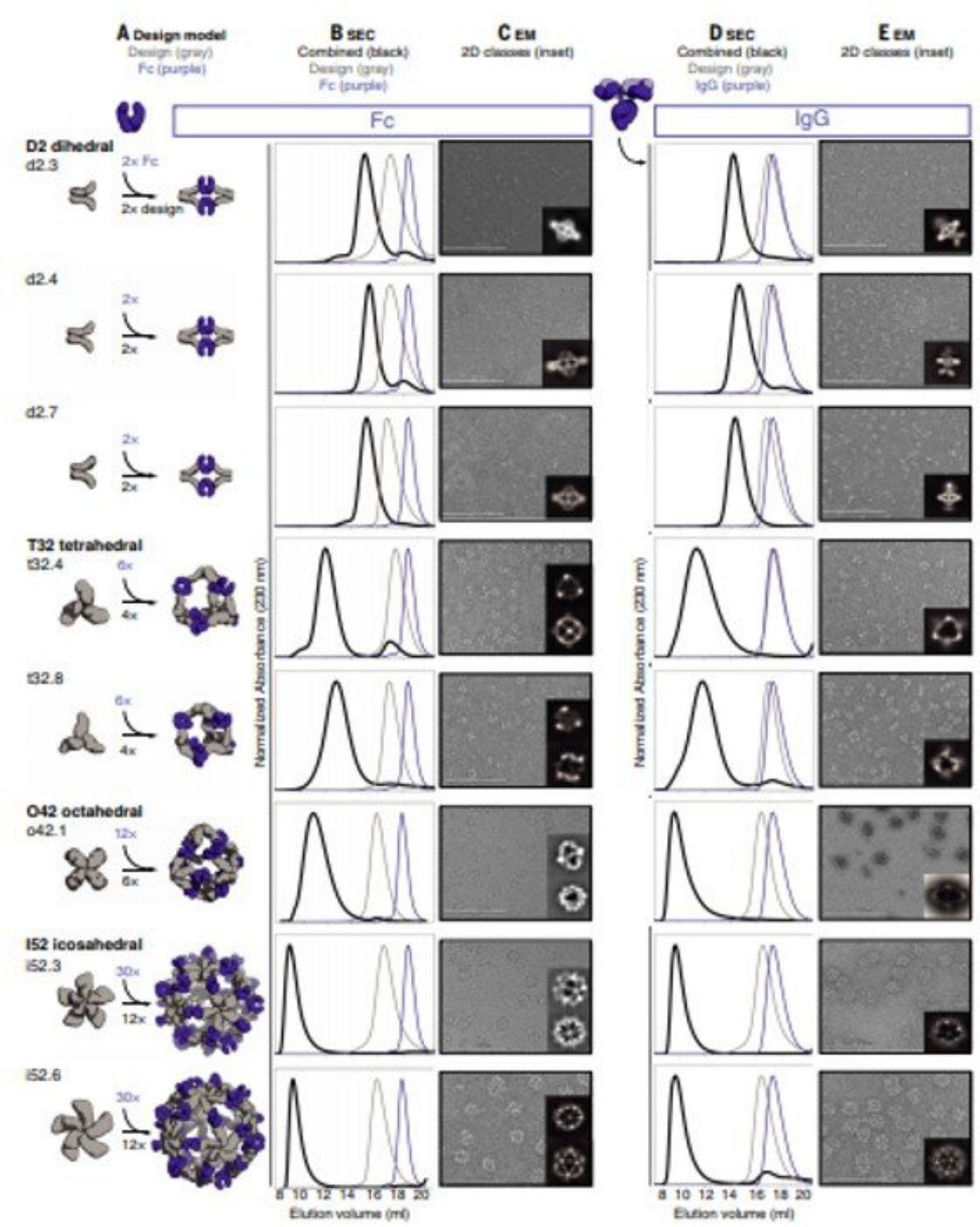
Antibody nanocage (AbC) design. (A) Polyhedral geometry is specified. Clockwise from top left: icosahedral, dihedral, octahedral, and tetrahedral geometries are shown. (B) An antibody Fc model from hIgG1 is aligned to one of the C2 axes (in this case, a D2 dihedron is shown). (C) Antibody Fc-binders are fused to helical repeat connectors that are then fused to the monomeric subunit of helical cyclic oligomers. All combinations of building blocks and

building block junctions are sampled (gray-shaded bottom inset; numbers in parentheses refer to the number of building blocks available). (D) Tripartite fusions are checked to ensure successful alignment of the C2 Fc symmetry axes with that of the polyhedral architecture (in the case of the D2 symmetry shown here, the C2 axes must intersect at a 90° angle). (E) Fusions that pass the geometric criteria move forward with side-chain redesign, where, for example, amino acids are optimized to ensure that core-packing residues are nonpolar and closely packed and that solvent-exposed residues are polar. (F) Designed AbC-forming oligomers are bacterially expressed, purified, and assembled with antibody Fc or IgG. Credit: Science, doi:10.1126/science.abd9994

The experiments

To form antibody cage (AbC)-designs, Divine et al. rigidly fused antibody constant domain-binding molecules to cyclic oligomers using helical spacer domains. They facilitated the process through helical spacer domains so that the symmetry axes of the dimeric antibody and cyclic oligomer could be at orientations that generated diverse dihedral or polyhedral architectures. The scientists optimized the junction regions between the connected building blocks to fold to the designed structures. The fusion approach relied on large sets of building blocks with many possible fusion sites per building block as stringent geometric criteria to form the desired symmetric architecture. The team used a computational method for antibody cage design to generate dihedral, tetrahedral, octahedral and icosahedral AbCs and used a naming convention to describe the final nanocage architectures. Divine et al. next expressed synthetic genes encoding the designs in bacterial cultures of Escherichia coli. For instance, the successful designs included D-2 decahedral (three designs), T-32 (one design) and 152 icosahedral (two designs) architectures containing two, six, 12 or 30 antibodies, respectively. They characterized the Fc AbCs using small-angle X-ray scattering and electron microscopy. The reconstructed nanocages were in close

agreement with the computational design models. To assess the stability of nanocages, Divine et al. used dynamic light scattering readings to obtain encouraging stability to allow the characterization of their biological impact next.

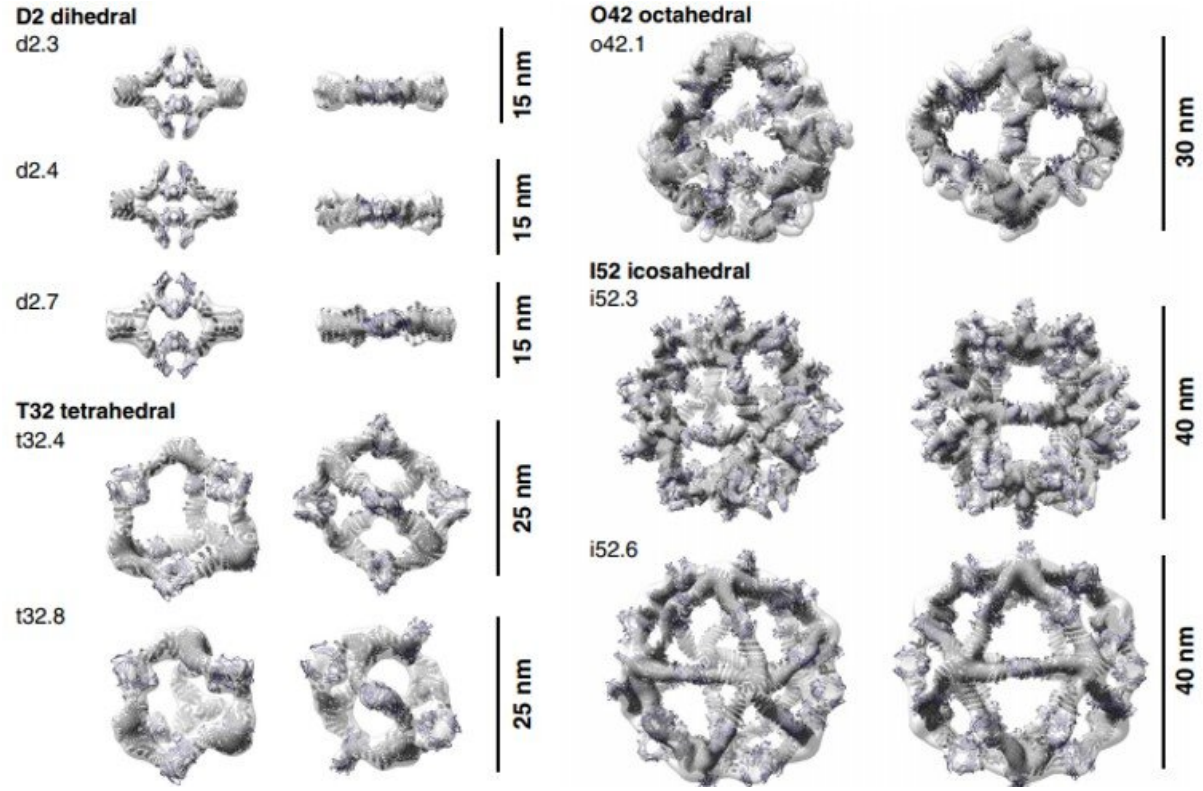


Structural characterization of AbCs. (A) Design models, with antibody Fc (purple) and designed AbC-forming oligomers (gray). (B) Overlay of representative SEC traces of assembly formed by mixing design and Fc (black)

with those of the single components in gray (design) or purple (Fc). (C) EM images with reference-free 2D class averages in inset; all data are from NS-EM, with the exception of designs o42.1 and i52.3 (cryo-EM). (D and E) SEC (D) and NS-EM representative micrographs with reference-free 2D class averages (E) of the same designed antibody cages assembled with full human IgG1 (with the 2 Fab regions intact). In all EM cases shown in (C) and (E), assemblies were first purified via SEC, and the fractions corresponding to the leftmost peak were pooled and used for imaging to remove any excess design or Ig component. Scale bars, 200 nm. Credit: Science, doi:10.1126/science.abd9994

Effects on cell signaling

The designed AbCs provided a general platform to understand the effect of valency and geometry of receptor engagement on signaling pathway activation. The wide-range of receptor binding antibodies and natural ligands formed with the AbC method developed in this work allowed ready and systematic probing of the effect of geometry and valency of receptor subunit association on cell signaling for almost any pathway. To explore the practicality of this approach, Divine et al. assembled antibodies or Fc-ligand fusions targeting a variety of signaling pathways into Antibody cages (AbCs) and studied their impact on signaling. For instance, the AbCs, formed with a death receptor-targeting antibody induced apoptosis (cell death) of tumor cell lines that had hitherto remained unaffected by the soluble antibody or the native ligand. In addition, the assembly of Fc-fusions or antibodies in AbCs, allowed enhanced angiopoietin pathway signaling, CD-40 signaling and T-cell proliferation. The AbC formation further allowed the neutralization of an in vitro pseudovirus, such as severe acute respiratory syndrome coronavirus 2.

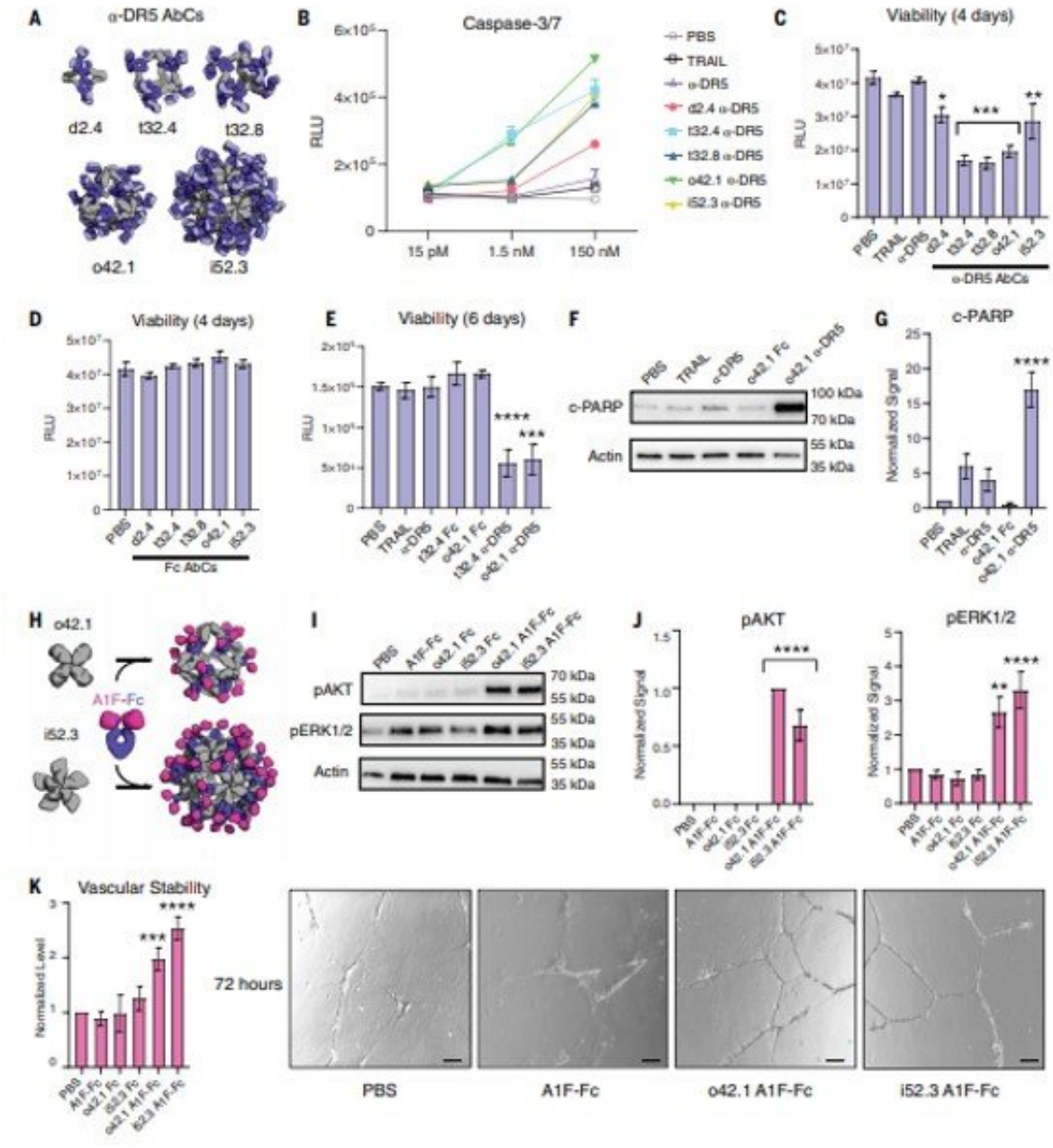


3D reconstructions of AbCs formed with Fc. Computational design models (cartoon representation) of each AbC are fit into the experimentally determined 3D density from EM. Each nanocage is viewed along an unoccupied symmetry axis (left) and rotated to look down one of the C2 axes of symmetry occupied by the Fc (right). Three-dimensional reconstructions from o42.1 and i52.3 are from cryo-EM analysis, all others are from NS-EM. Credit: Science, doi:10.1126/science.abd9994

Outlook

The method detailed in this work, went beyond previous computational design efforts to create protein nanomaterials that integrate form and function. The AbCs therefore used antibodies as both structural and functional components to achieve a wide-range of geometries and orientations. This strategy is applicable to design vaccines with

nanocages assembled with viral glycoproteins using components terminating in glycoprotein binding domains to maximize the proximity of active sites. In this way, Robby Divine and colleagues designed multiple antibody cage forming proteins to accurately cluster any protein-binding antibody into nanocages through controlled valency and geometry. The team used two, six and 12 or 30 antibodies within the AbCs by simply mixing the antibody with the corresponding designed protein without additional covalent modifications. The scientists added receptor-binding or virus-neutralizing antibodies into ABCs to enhance their biological activity across varied cell systems. The team expect this outcome for rapid antibody assembly within ordered nanocages without covalent modifications to have broad applications across research and medicine.



AbCs activate apoptosis and angiogenesis signaling pathways. (A and B) Caspase-3/7 is activated by AbCs formed with a-DR5 antibody (A), but not the free antibody, in RCC4 renal cancer cells (B). (C and D) a-DR5 AbCs (C), but not Fc AbC controls (D), reduce cell viability 4 days after treatment. (E) a-DR5 AbCs reduce viability 6 days after treatment. (F and G) o42.1 a-DR5 AbCs enhance PARP cleavage, a marker of apoptotic signaling; (G) is a quantification of (F) relative to PBS control. (H) The F-domain from angiopoietin-1 was fused

to Fc (A1F-Fc) and assembled into octahedral (o42.1) and icosahedral (i52.3) AbCs. (I) Representative Western blots show that A1F-Fc AbCs, but not controls, increase pAKT and pERK1/2 signals. (J) Quantification of (I): pAKT quantification is normalized to o42.1 A1F-Fc signaling (no pAKT signal in the PBS control); pERK1/2 is normalized to PBS. (K) A1F-Fc AbCs increase vascular stability after 72 hours. (Left) Quantification of vascular stability compared with PBS. (Right) Representative images; scale bars, 100 mm. All error bars represent means ± SEM; means were compared using analysis of variance and Dunnett post-hoc tests (tables S8 and S9). *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001 Credit: Science, doi:10.1126/science.abd9994

More information: Divine R. et al. Designed proteins assemble antibodies into modular nanocages, *Science*, 10.1126/science.abd9994

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