CLAIMS

1. A multispecific antigen-binding molecule comprising:

a first antigen-binding domain (D1); and

a second antigen-binding domain (D2);

wherein:

D1 specifically binds a target molecule (T);

D1 is derived from an antigen-binding molecule that binds but does not substantially inactivate T on its own;

D2 specifically binds an internalizing effector protein (E);

the simultaneous binding of T and E by the multispecific antigen-binding molecule attenuates the activity of T to a greater extent than the binding of T by D1 alone;

E is a cell surface-expressed molecule that is directly internalized into the cell;

T is a cell surface-expressed target molecule;

the binding affinity of D2 for E is at least 10% weaker than the binding affinity of D1 for T.

2. The multispecific antigen-binding molecule of claim 1, wherein E is selected from the group consisting of CD63, MHC-I, Kremen-1, Kremen-2, LRP5, LRP6, transferrin receptor, LDLr, MAL, V-ATPase, and ASGR.

3. The multispecific antigen-binding molecule of claim 1 or 2, wherein D2 comprises a ligand, or portion of a ligand, that specifically binds E.

4. The multispecific antigen-binding molecule of any one of claims 1 to 3, wherein T is selected from the group consisting of IL-4R, IL-6R, PRLR, Nav1.7, GCGR, and HLA-B27.

5. The multispecific antigen-binding molecule of any one of claims 1 to 4, wherein D1 comprises a ligand, or portion of a ligand, that specifically binds T.

6. The multispecific antigen-binding molecule of claim 1, wherein D1 and/or D2 exhibits pH-dependent binding to its antigen.

7. The multispecific antigen-binding molecule of claim 6, wherein D1 binds T with lower affinity at acidic pH as compared to neutral pH; and/or wherein D2 binds E with lower affinity at acidic pH as compared to neutral pH.

8. The multispecific antigen-binding molecule of claim 1, wherein D1 and/or D2 comprise(s) at least one antibody variable region.

9. The multispecific antigen-binding molecule of claim 8, wherein D1 and/or D2 comprise(s) a heavy chain variable region (HCVR) and a light chain variable region (LCVR).

10. The multispecific antigen-binding molecule of claim 8 or 9, wherein the multispecific antigen-binding molecule is a bispecific antibody.

11. A method for inactivating or attenuating the activity of a target molecule (T), the method comprising contacting T and an internalizing effector protein (E) with a multispecific antigen-binding molecule, wherein the multispecific antigen-binding molecule comprises a first antigen-binding domain (D1) and a second antigen-binding domain (D2), wherein:

D1 specifically binds T;

D1 is derived from an antigen-binding molecule that binds but does not substantially inactivate T on its own;

D2 specifically binds E;

the simultaneous binding of T and E by the multispecific antigen-binding molecule causes inactivation of T to a greater extent than the binding of T by D1 alone;

E is a cell surface-expressed molecule that is directly internalized into the cell;

T is a cell surface-expressed target molecule;

the binding affinity of D2 for E is at least 10% weaker than the binding affinity of D1 for T.

12. The multispecific antigen-binding molecule of claim 1, wherein D2 comprises an antigen-binding portion of an anti-CD63 antibody.

13. The multispecific antigen-binding molecule of claim 12, wherein D1 comprises an antigen-binding portion of an anti-IL-4R antibody.

14. The multispecific antigen-binding molecule of claim 12, wherein D1 comprises an antigen-binding portion of an anti-SOST antibody.

15. The multispecific antigen-binding molecule of claim 1, wherein T is a tumor-associated antigen.

16. The multispecific antigen-binding molecule of claim 15, wherein the multispecific antigen-binding molecule is conjugated to a drug, toxin or radioisotope.

17. The multispecific antigen-binding molecule according to claims 1-10 and 12-16, wherein D2 binds E with low affinity.

18. A use of a multispecific antigen-binding molecule of claim 15 and a second antigen-binding protein that specifically binds T at an epitope that is non-overlapping with the epitope to which D1 binds, in the manufacture of a medicament for targeting a tumor in a subject.

19. The use of claim 18, wherein the second antigen-binding protein is conjugated to a drug, toxin or radioisotope.

20. The multispecific antigen-binding molecule of claim 17, wherein D2 binds E with a K_D of greater than about 10 nM to about 1 μM, as measured in a surface plasmon resonance assay at about 25° C.

CLAIMS

1. A multispecific antigen-binding molecule comprising

a first antigen-binding domain (D1); and

a second antigen-binding domain (D2);

wherein

D1 specifically binds a target molecule (T);

D1 is derived from an antigen-binding molecule that binds but does not substantially inactivate T on its own;

D2 specifically binds an internalizing effector protein (E);

the simultaneous binding of T and E by the multispecific antigen-binding molecule attenuates the activity of T to a greater extent than the binding of T by D1 alone;

E is a cell surface-expressed molecule that is directly internalized into the cell;

T is a cell surface-expressed target molecule;

the binding affinity of D2 for E is at least 10% weaker than the binding affinity of D1 for T.

2. The multispecific antigen-binding molecule of claim 1, wherein E is selected from the group consisting of CD63, MHC-I, Kremen-1, Kremen-2, LRP5, LRP6, transferrin receptor, LDLr, MAL, V-ATPase, and ASGR.

3. The multispecific antigen-binding molecule of claim 1 or 2, wherein D2 comprises a ligand, or portion of a ligand, that specifically binds E.

4. The multispecific antigen-binding molecule of any one of claims 1 to 3, wherein T is selected from the group consisting of IL-4R, IL-6R, PRLR, Nav1.7, GCGR, and HLA-B27.

5. The multispecific antigen-binding molecule of any one of claims 1 to 4, wherein D1 comprises a ligand, or portion of a ligand, that specifically binds T.

6. The multispecific antigen-binding molecule of claim 1, wherein D1 and/or D2 exhibits pH-dependent binding to its antigen.

7. The multispecific antigen-binding molecule of claim 6, wherein D1 binds T with lower affinity at acidic pH as compared to neutral pH; and/or wherein D2 binds E with lower affinity at acidic pH as compared to neutral pH.

8. The multispecific antigen-binding molecule of claim 1, wherein D1 and/or D2 comprise(s) at least one antibody variable region.

9. The multispecific antigen-binding molecule of claim 8, wherein D1 and/or D2 comprise(s) a heavy chain variable region (HCVR) and a light chain variable region (LCVR).

10. The multispecific antigen-binding molecule of claim 8 or 9, wherein the multispecific antigen-binding molecule is a bispecific antibody.

11. A method for inactivating or attenuating the activity of a target molecule (T) in a non-human animal, the method comprising contacting T and an internalizing effector protein (E) with a multispecific antigen-binding molecule, wherein the multispecific antigen-binding molecule comprises a first antigen-binding domain (D1) and a second antigen-binding domain (D2), wherein

D1 specifically binds T,

D1 is derived from an antigen-binding molecule that binds but does not substantially inactivate T on its own;

D2 specifically binds E;

the simultaneous binding of T and E by the multispecific antigen-binding molecule causes I nactivation of T to a greater extent than the binding of T by D1 alone;

E is a cell surface-expressed molecule that is directly internalized into the cell;

T is a cell surface-expressed target molecule;

the binding affinity of D2 for E is at least 10% weaker than the binding affinity of D1 for T.

12. The multispecific antigen-binding molecule of claim 1, wherein D2 comprises an antigen-binding portion of an anti-CD63 antibody.

13. The multispecific antigen-binding molecule of claim 12, wherein D1 comprises an antigen-binding portion of an anti-IL-4R antibody.

14. The multispecific antigen-binding molecule of claim 12, wherein D1 comprises an antigen-binding portion of an anti-SOST antibody.

15. The multispecific antigen-binding molecule of claim 1 wherein T is a tumor-associated antigen.

16. The multispecific antigen-binding molecule of claim 15, wherein the multispecific antigen-binding molecule is conjugated to a drug, toxin or radioisotope.

17. The multispecific antigen-binding molecule according to claims 1-10 and 12-16, wherein D2 binds E with low affinity.

18. A use of a multispecific antigen-binding molecule of claim 15 and a second antigen-binding protein that specifically binds T at an epitope that is non-overlapping with the epitope to which D1 binds, in the manufacture of a medicament for targeting a tumor in a subject.

19. The use of claim 18, wherein the second antigen-binding protein is conjugated to a drug, toxin or radioisotope.

20. The multispecific antigen-binding molecule of claim 17, wherein D2 binds E with a K_D of greater than 10 nM to 1 μM, as measured in a surface plasmon resonance assay at about 25°C.

21. An in vitro method for inactivating or attenuating the activity of a target molecule (T), the method comprising contacting T and an internalizing effector protein (E) with a multispecific antigen-binding molecule, wherein the multispecific antigen-binding molecule comprises a first antigen-binding domain (D1) and a second antigen-binding domain (D2), wherein:

D1 specifically binds T,

D1 is derived from an antigen-binding molecule that binds but does not substantially inactivate T on its own;

D2 specifically binds E;

the simultaneous binding of T and E by the multispecific antigen-binding molecule causes inactivation of T to a greater extent than the binding of T by D1 alone;

E is a cell surface-expressed molecule that is directly internalized into the cell;

T is a cell surface-expressed target molecule;

the binding affinity of D2 for E is at least 10% weaker than the binding affinity of D1 for T.