CLAIMS

What is claimed is:

1. A method of reducing a hydrolytic enzyme activity rate of a composition obtained from a purification platform, the method comprising subjecting a sample to the purification platform comprising:

    a capture step;

    one or more ion exchange (IEX) chromatography steps; and

    a depth filtration step,

    thereby reducing the hydrolytic enzyme activity rate of the composition as compared to purification of the sample without the depth filtration step.

2. The method of claim 1, wherein each of the one or more IEX chromatography steps is selected from the group consisting of: an anion exchange (AEX) chromatography step, a cation exchange (CEX) chromatography step, and a multimodal ion exchange (MMIEX) chromatography step.

3. The method of claim 2, wherein the MMIEX chromatography step comprises a multimodal cation exchange/ anion exchange (MM-AEX/CEX) chromatography step.

4. The method of any one of claims 1-3, further comprising a virus filtration step.

5. The method of any one of claims 1-4, further comprising an ultrafiltration/diafiltration (UF/DF) step.

6. The method of claim 5, wherein the purification platform comprises, in order:

    the capture step;

    the CEX chromatography step;

    the AEX chromatography step;

    the depth filtration step;

    the virus filtration step; and

    the UF/DF step.

7. The method of any one of claims 1-6, wherein the depth filtration step comprises processing via a depth filter, and wherein the depth filter is a X0SP depth filter, a C0SP depth filter, a D0SP depth filter, a Polisher ST depth filter, or an EMPHAZE™ depth filter.

8. The method of any one of claims 1-6, further comprising a HIC step comprising processing via Sartobind® phenyl.

~~9. A method of reducing a hydrolytic enzyme activity rate of a composition obtained from a purification platform, the method comprising subjecting a sample to the purification platform comprising:~~

~~a capture step;~~

~~a multimodal hydrophobic interaction/ ion exchange (MM-HIC/IEX) chromatography step; and~~

~~a hydrophobic interaction chromatography (HIC) step,~~

~~thereby reducing the hydrolytic enzyme activity rate of the composition as compared to purification of the sample without the HIC step and/or the MM-HIC/IEX chromatography step.~~

~~10. The method of claim 9, wherein the MM-HIC/IEX chromatography step comprises processing via a MM-HIC/IEX chromatography medium and the processing is performed at a pH of about 4.5 to about 9.~~

~~11. The method of claim 9 or 10, wherein the MM-HIC/IEX chromatography step is a multimodal hydrophobic interaction/ anion exchange (MM-HIC/AEX) chromatography step.~~

~~12. The method of claim 11, wherein the MM-HIC/ AEX chromatography step comprises processing via Capto™ Adhere or Capto™ Adhere ImpRes.~~

~~13. The method of claim 9 or 10, wherein the MM-HIC/IEX chromatography step is a multimodal hydrophobic interaction/ cation exchange (MM-HIC/CEX) chromatography step.~~

14. The method of claim 13, wherein the MM-HIC/CEX chromatography step comprises Capto™ MMC or Capto™ MMC ImpRes.

15. The method of any one of claims 9-14, wherein the purification platform comprises, in order:

the capture step;

the MM-HIC/IEX chromatography step; and

the HIC step.

16. The method of any one of claims 9-15, further comprising a virus filtration step.

17. The method of any one of claims 9-16, further comprising an ultrafiltration/diafiltration (UF/DF) step.

18. The method of claim 17, wherein the purification platform comprises, in order:

the capture step;

the MM-HIC/AEX chromatography step;

the HIC step;

the virus filtration step; and

the UF/DF step.

19. The method of any one of claims 9-18, further comprising a depth filtration step.

20. The method of claim 19, wherein the purification platform comprises, in order:

the capture step;

the depth filtration step;

the MM-HIC/AEX chromatography step; and

the HIC step.

21. The method of claim 19 or 20, wherein the depth filtration step comprises processing via a depth filter, and wherein the depth filter is a X0SP depth filter.

22. The method of claim 19 or 20, wherein the depth filtration step comprises processing via a depth filter, and the depth filter is an EMPHAZE™ depth filter or a Polisher ST depth filter.

23. The method of any one of claims 19-22, wherein the depth filter is used as a load filter in conjunction with the MM-HIC/AEX chromatography step.

24. The method of claim 23, wherein the MM-HIC/-AEX chromatography step comprises processing via Capto™ Adhere or Capto™ Adhere ImpRes.

25. The method of claim 19, wherein the purification platform comprises, in order:

the capture step;

the MM-HIC/AEX chromatography step;

the depth filtration step; and

the HIC step.

26. The method of claim 25, wherein the depth filtration step comprises processing via a depth filter, and wherein the depth filter is a X0SP depth filter, a C0SP depth filter, or a D0SP depth filter.

27. The method of claim 25, wherein the depth filtration step comprises processing via a depth filter, and the depth filter is an EMPHAZE™ depth filter or a Polisher ST depth filter.

28. The method of claim any one of claims 25-27, wherein the MM-HIC/-AEX chromatography step comprises processing via Capto™ Adhere or Capto™ Adhere ImpRes.

29. A method of reducing a hydrolytic enzyme activity rate of a composition obtained from a purification platform, the method comprising subjecting a sample to the purification platform comprising:

a capture step;

one or more ion exchange (IEX) chromatography steps; and

a hydrophobic interaction chromatography (HIC) step,

~~thereby reducing the hydrolytic enzyme activity rate of the composition as compared to purification of the sample without the HIC step.~~

~~30. The method of claim 29, wherein the one or more IEX chromatography steps is a cation exchange (CEX) chromatography step.~~

~~31. The method of claim 29 or 30, further comprising a virus filtration step.~~

~~32. The method of any one of claims 29-31, further comprising an ultrafiltration/diafiltration (UF/DF) step.~~

~~33. The method of claim 32, further comprising a depth filtration step performed at any stage prior to the UF/DF step.~~

~~34. The method of claim 33, wherein the purification platform comprises, in order:~~

~~the capture step;~~

~~the CEX chromatography step;~~

~~the HIC step;~~

~~the virus filtration step; and~~

~~the UF/DF step.~~

~~35~~9. A method of reducing a hydrolytic enzyme activity rate of a composition obtained from a purification platform, the method comprising subjecting a sample to the purification platform comprising:

one or more ion exchange (IEX) chromatography steps;

a hydrophobic interaction chromatography (HIC) step; and

a depth filtration step,

thereby reducing the hydrolytic enzyme activity rate of the composition as compared to purification of the sample without the HIC step or the depth filtration step.

~~36~~10. The method of claim ~~35~~9, wherein the reduction is as compared to purification of the sample without the HIC and the depth filtration step.

~~37~~11. The method of claim ~~35~~ 9 or ~~36~~10, wherein each of the one or more IEX chromatography steps is selected from the group consisting of: an anion exchange (AEX) chromatography step, a cation exchange (CEX) chromatography step, and a multimodal ion exchange (MMIEX) chromatography step.

~~38~~12. The method of claim ~~37~~11, wherein the MMIEX chromatography step comprises a multimodal cation exchange/ anion exchange (MM-AEX/CEX) chromatography step.

~~39~~13. The method of any one of claims ~~35~~9-~~38~~12, further comprising an ultrafiltration/diafiltration (UF/DF) step.

~~40~~14. The method of claim ~~39~~13, wherein the purification platform comprises, in order:

the CEX chromatography step;

the HIC step;

the MMIEX chromatography step;

the AEX chromatography step;

the depth filter step; and

the UF/DF step.

~~41~~15. A method of reducing a hydrolytic enzyme activity rate of a composition obtained from a purification platform, the method comprising subjecting a sample to the purification platform comprising:

a capture step;

one or more ion exchange (IEX) chromatography steps;

a multimodal hydrophobic interaction/ ion exchange (MM-HIC/IEX) chromatography steps; and

one or both of:

a hydrophobic interaction chromatography (HIC) step; and

a depth filtration step,

thereby reducing the hydrolytic enzyme activity rate of the composition as compared to purification of the sample without the HIC step or the depth filtration step.

4216. The method of claim 4115, wherein the reduction is as compared to purification of the sample without the HIC and the depth filtration step.

4317. The method of claim 41-15 or 4216, further comprising a virus filtration step.

4418. The method of any one of claims 4115-4317, further comprising an ultrafiltration/diafiltration (UF/DF) step.

4519. The method of any one of claims 4115-4418, wherein the depth filtration step is performed as a load filter for the MM-HIC/IEX chromatography step, as a load filter for the HIC step, or following the HIC step.

4620. The method of any one of claims 4115-4519, wherein each of the one or more IEX chromatography steps is selected from the group consisting of: a cation exchange (CEX) chromatography step, an anion exchange (AEX) chromatography step, and a multimodal ion exchange (MMIEX) chromatography step.

4721. The method of any one of claims 4115-4620, wherein the MM-HIC/IEX chromatography step is a multimodal hydrophobic interaction/ anion exchange (MM-HIC/AEX) chromatography step.

4822. The method of claim 4721, wherein the MM-HIC/AEX chromatography step comprises processing via Capto™ Adhere or Capto™ Adhere ImpRes.

4923. The method of any one of claims 1-34-8 and 4115-4822, wherein the capture step comprises processing via affinity chromatography.

5024. The method of any one of claims 1-34-8 and 4115-4822, wherein the capture step is performed in a bind-and-elute mode.

5125. The method of claim 49-23 or 5024, wherein the affinity chromatography is selected from the group consisting of a protein A chromatography, a protein G chromatography, a protein

A/G chromatography, a FcXL chromatography, a protein XL chromatography, a kappa chromatography, and a kappaXL chromatography.

~~52~~26. The method of any one of claims 1-6, ~~19, 20,~~ and ~~35~~9-~~51~~25, wherein the depth filtration step comprises processing via a depth filter.

~~53~~27. The method of claim ~~52~~26, wherein the depth filter is used as a load filter.

~~54~~28. The method of claim ~~52~~ 26 or ~~53~~27, wherein the depth filter comprises a substrate comprising one or more of a diatomaceous earth composition, a silica composition, a cellulose fiber, a polymeric fiber, a cohesive resin, and an ash composition.

~~55~~29. The method of claim ~~54~~28, wherein at least a portion of the substrate of the depth filter comprises a surface modification.

~~56~~30. The method of claim ~~55~~29, wherein the surface modification is one or more of a quaternary amine surface modification, a cationic surface modification, and an anionic surface modification.

~~57~~31. The method of any one of claims ~~52~~26-~~56~~30, wherein the depth filter is selected from the group consisting of: a X0SP depth filter, a D0SP depth filter, a C0SP depth filter, an EMPHAZE™ depth filter, a PDD1 depth filter, a PDE1 depth filter, a PDH5 depth filter, a ZETA PLUS™ 120ZA depth filter, a ZETA PLUS™ 120ZB depth filter, a ZETA PLUS™ DELI depth filter, a ZETA PLUS™ DELP depth filter, and a Polisher ST depth filter.

~~58~~32. The method of claim ~~57~~31, wherein

the depth filter is the X0SP depth filter, the D0SP depth filter, or the C0SP depth filter, and wherein the processing via the depth filter is performed at a pH of about 4.5 to about 8, or

wherein the depth filter is the EMPHAZE™ depth filter, and wherein processing via the depth filter is performed at a pH of about 7 to about 9.5, or

wherein the depth filter is the Polisher ST depth filter, and wherein processing via the depth filter is performed at a pH of about 4.5 to about 9.

~~59. The method of claim 57, wherein the depth filter is the EMPHAZE™ depth filter, and wherein processing via the depth filter is performed at a pH of about 7 to about 9.5.~~

~~60. The method of claim 57, wherein the depth filter is the Polisher ST depth filter, and wherein processing via the depth filter is performed at a pH of about 4.5 to about 9.~~

~~61~~33. The method of any one of claims 9-~~60~~32, wherein the HIC step comprises processing via a HIC membrane or a HIC column.

~~62~~34. The method of claim ~~61~~33, wherein processing via the HIC membrane or the HIC column is performed using low salt concentrations.

~~63~~35. The method of ~~any one of~~ claim~~s 59-61~~33, wherein processing via the HIC membrane or the HIC column is performed in flow-through mode.

~~64~~36. The method of any one of claims ~~61~~33-~~63~~35, wherein the HIC membrane or HIC column comprises a substrate comprising one or more of an ether group, an ethyl group, a propyl group, an isopropyl group, a butyl group, a hexyl group, an octyl group, and a phenyl group.

~~65~~37. The method of any one of claims ~~61~~33-~~64~~36, wherein the HIC membrane or the HIC column is selected from the group consisting of Bakerbond WP HI-Propyl™, Phenyl Sepharose® Fast Flow (Phenyl-SFF), Phenyl Sepharose® Fast Flow Hi-sub (Phenyl-SFF HS), Toyopearl® Hexyl-650C, Toyopearl® Hexyl-650M, Toyopearl® Hexyl-650S, Poros™ Benzyl Ultra, and Sartobind® phenyl.

~~66~~38. The method of claim ~~64~~36 or ~~65~~37, wherein processing via the HIC membrane or the HIC column is performed at a pH of about 4.5 to about 7.

~~67~~39. The method of any one of claims 1-~~8 and 29-66~~38, wherein each of the one or more IEX chromatography steps comprises processing via an IEX chromatography membrane or an IEX chromatography column.

~~68~~40. The method of claim ~~67~~39, wherein the IEX chromatography membrane or the IEX chromatography column is selected from the group consisting of: SPSFF, QSFF, SPXL, Streamline™ SPXL, ABx™, Poros™ XS, Poros™ 50HS, DEAE, DMAE, TMAE, QAE, and MEP-Hypercel™.

~~69~~41. The method of any one of claims 1-~~68~~40, wherein the purification platform is for purification of a target from the sample, and wherein the sample comprises the target and one or more host cell impurities.

~~70~~42. The method of claim ~~69~~41, wherein the target comprises a polypeptide, preferably the target is an antibody moiety.

~~71. The method of any one of claims 1-70, wherein the target is an antibody moiety.~~

~~72~~43. The method of claim ~~71~~42, wherein the antibody moiety is a monoclonal antibody, preferably the antibody moiety is a human, humanized, or chimeric antibody.

~~73. The method of claim 71 or 72, wherein the antibody moiety is a human, humanized, or chimeric antibody.~~

~~74~~44. The method of ~~any one of~~ claim~~s~~ ~~71-73~~42 or 43, wherein the antibody moiety is selected from the group consisting of: an anti-TAU antibody, an anti-TGFβ3 antibody, an anti-VEGF-A antibody, an anti-CD20 antibody, an anti-CD40 antibody, an anti-HER2 antibody, an anti-IL6 antibody, an anti-IgE antibody, an anti-IL13 antibody, an anti-TIGIT antibody, an anti-PD-L1 antibody, an anti-VEGF-A/ANG2 antibody, an anti-CD79b antibody, an anti-ST2 antibody, an anti-factor D antibody, an anti-factor IX antibody, an anti-factor X antibody, an anti-abeta antibody, an anti-CEA antibody, an anti-CEA/CD3 antibody, an anti-CD20/CD3 antibody, an anti-FcRH5/CD3 antibody, an anti-Her2/CD3 antibody, an anti-

FGFR1/KLB antibody, a FAP-4-1 BBL fusion protein, a FAP-IL2v fusion protein, and a TYRP1 TCB antibody.

~~75. The method of any one of claims 71-74, wherein the antibody moiety is selected from the group consisting of: ocrelizumab, pertuzumab, ranibizumab, trastuzumab, tocilizumab, faricimab, polatuzumab, gantenerumab, cibisatamab, crenezumab, mosunetuzumab, tiragolumab, bevacizumab, rituximab, atezolizumab, obinutuzumab, lampalizumab, omalizumab, ranibizumab, emicizumab, selicrelumab, prasinezumab, RO6874281, and RO7122290.~~

~~76~~45. The method of any one of claims ~~69~~41-~~75~~44, wherein the one or more host cell impurities comprises a host cell protein, preferably, the host cell protein is a hydrolytic enzyme, and more preferably, the hydrolytic enzyme is a lipase, an esterase, a thioesterase, a phospholipase, carboxylesterase, hydrolase, cutinase, or a ceramidase.

~~77. The method of claim 76, wherein the host cell protein is a hydrolytic enzyme.~~

~~78. The method of claim 77, wherein the hydrolytic enzyme is a lipase, an esterase, a thioesterase, a phospholipase, carboxylesterase, hydrolase, cutinase, or a ceramidase.~~

~~79~~46. The method of any one of claims 1-~~78~~45, wherein the sample comprises a host cell or components originating therefrom.

~~80~~47. The method of any one of claims 1-~~79~~46, wherein the sample is, or is derived from, a cell culture sample.

~~81~~48. The method of claim ~~80~~47, wherein the cell culture sample comprises a host cell, and wherein the host cell is a Chinese hamster ovary (CHO) cell or an *E. coli* cell.

~~82~~49. The method of any one of claims 1-~~81~~48, further comprising a sample processing step.

~~83~~50. The method of any one of claims 1-~~82~~49, wherein the reduction in the hydrolytic enzyme activity rate is at least about 20%.

8451. The method of any one of claims 1-8350, further comprising determining the hydrolytic enzyme activity rate of the composition and/or determining the level of one or more hydrolytic enzymes in the composition.

~~85. The method of any one of claims 1-84, further comprising determining the level of one or more hydrolytic enzymes in the composition.~~

8652. The method of any one of claims 1-8551, wherein the composition comprises a polysorbate, preferably the polysorbate is selected from the group consisting of polysorbate 20, polysorbate 40, polysorbate 60, and polysorbate 80.

~~87. The method of claim 86, wherein the polysorbate is selected from the group consisting of polysorbate 20, polysorbate 40, polysorbate 60, and polysorbate 80.~~

8853. A pharmaceutical composition obtained from the method of any one of claims 1-8752.

~~89. A formulated antibody moiety composition comprising an antibody moiety and a polysorbate, wherein the composition has a reduced rate of polysorbate hydrolysis, wherein the shelf-life of the composition is more than 12 months.~~

~~90. A formulated antibody moiety composition comprising an antibody moiety and a polysorbate, wherein the composition has a reduced rate of polysorbate hydrolysis activity, wherein the shelf-life of the composition is extended compared to the shelf-life indicated in documents filed with a health authority related to the formulated antibody moiety composition, wherein the shelf-life is extended by at least 3 months compared to the shelf-life indicated in said documents.~~

~~91. The formulated antibody moiety composition of claim 89 or 90, wherein the rate of polysorbate hydrolysis is reduced by at least about 20%.~~

~~92. A formulated antibody moiety composition comprising an antibody moiety, wherein the formulated antibody moiety composition has a reduced degradation of polysorbate, wherein the degradation is reduced by at least about 20% compared to the degradation indicated in~~

documents filed with a health authority related to the formulated antibody moiety composition.

93. A formulated antibody moiety composition comprising an antibody moiety and a polysorbate, wherein the polysorbate is degraded during storage of the liquid composition by 50% or less per year.

94. The formulated antibody moiety composition of any one of claims 89-93, wherein the antibody moiety is a monoclonal antibody.

95. The formulated antibody moiety composition of any one of claims 89-94, wherein the antibody moiety is a human, humanized, or chimeric antibody.

96. The formulated antibody moiety composition of any one of claims 89-95, wherein the antibody is selected from the group consisting of an anti-TAU antibody, an anti-TGFβ3 antibody, an anti-VEGF-A antibody, an anti-CD20 antibody, an anti-CD40 antibody, an anti-HER2 antibody, an anti-IL6 antibody, an anti-IgE antibody, an anti-IL13 antibody, an anti-TIGIT antibody, an anti-PD-L1 antibody, an anti-VEGF-A/ANG2 antibody, an anti-CD79b antibody, an anti-ST2 antibody, an anti-factor D antibody, an anti-factor IX antibody, an anti-factor X antibody, an anti-abeta antibody, an anti-CEA antibody, an anti-CEA/CD3 antibody, an anti-CD20/CD3 antibody, an anti-FcRH5/CD3 antibody, an anti-Her2/CD3 antibody, an anti-FGFR1/KLB antibody, a FAP-4-1 BBL fusion protein, a FAP-IL2v fusion protein, and a-TYRP1-TCB antibody.

97. The formulated antibody moiety composition of any one of claims 89-96, wherein the antibody moiety is selected from the group consisting of ocrelizumab, pertuzumab, ranibizumab, trastuzumab, tocilizumab, faricimab, polatuzumab, gantenerumab, cibisatamab, crenezumab, mosunetuzumab, tiragolumab, bevacizumab, rituximab, atezolizumab, obinutuzumab, lampalizumab, omalizumab ranibizumab, emicizumab, selicrelumab, prasinezumab, RO6874281, and RO7122290.

~~98. The formulated antibody moiety composition of any one of claims 89-97, wherein the polysorbate is selected from the group consisting of polysorbate 20, polysorbate 40, polysorbate 60, and polysorbate 80.~~