Binding profiles to different amyloid-beta species for lecanemab, aducanumab and gantenerumab

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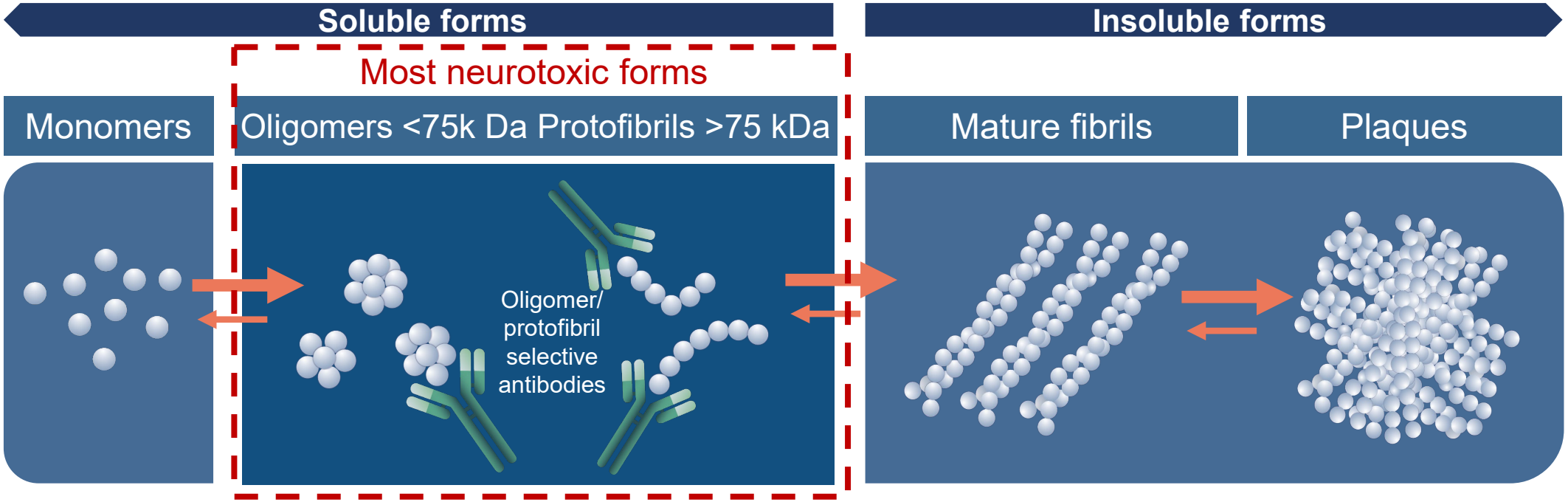
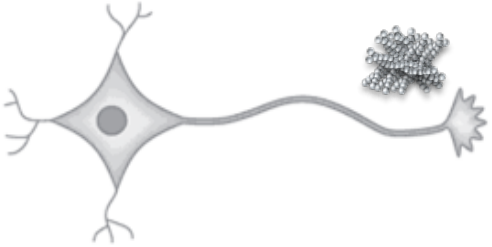
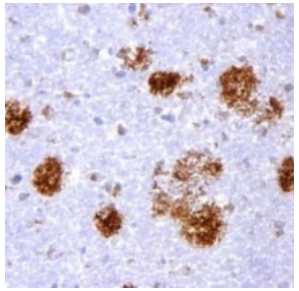
Disclaimer: Co-founder of BioArctic

Targeting most neurotoxic forms of aggregated Aβ is important when designing therapies for Alzheimer's disease



Aggregated Aβ fibrils in amyloid plaques

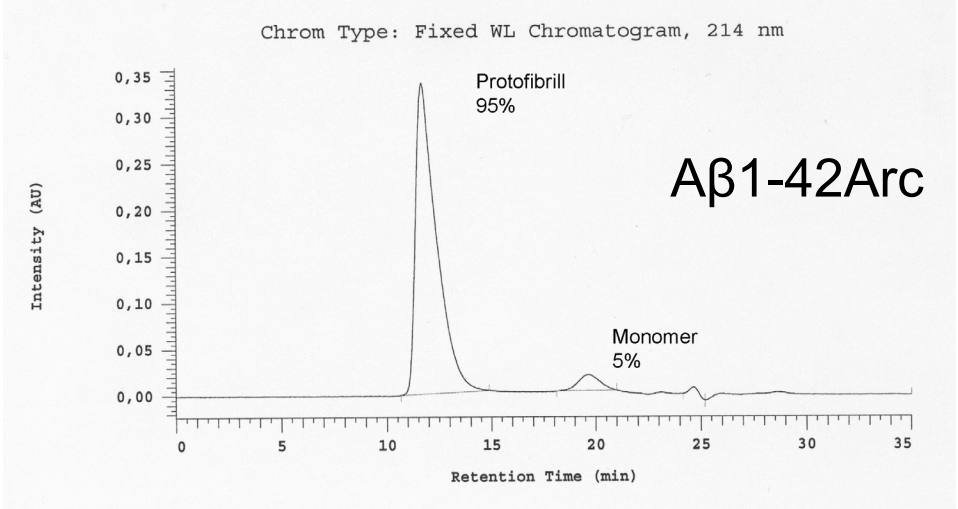


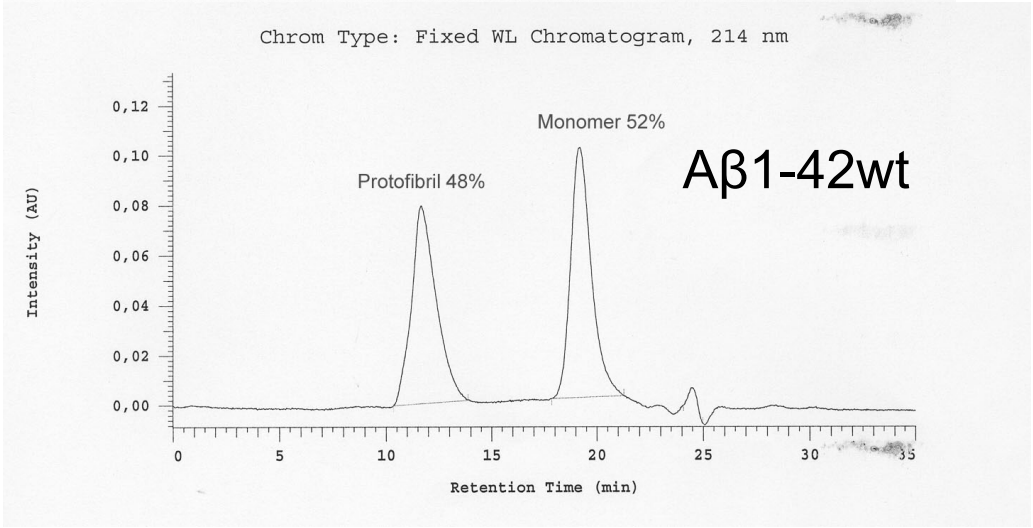


Walsh et al. 1997 J Biol Chem; Harper et al. 1997 Chem Biol; Nilsberth et al. 2001 Nat Genet; O'Nuallain et al. 2010 J Neurosci; Lannfelt et al. 2013 J Intern Med; Lannfelt et al. 2014 Alz Res Ther

Accelerated protofibril formation of Arctic mutation Aβ (Aβ1-42E22G)

Sixe Exclusion Chromatography on a Superdex 75 column





Our definition of protofibrils:
Soluble aggregated Aβ eluting in the void volume of a Superdex 75 column
Protofibrils: >75 kDa
Oligomers: <75 kDa

Protofibrils are found in all AD cases but are more prominent with the Arctic mutation

Nilsberth et al. 2001 Nat Neurosci
Johansson et al. 2006 FEBS J

Investigations of three anti-Aβ antibodies

- Lecanemab, aducanumab and gantenerumab were investigated using inhibition ELISA, immunodepletion and Surface Plasmon Resonance (SPR, Biacore)
- Affinity and selectivity to different *in vitro* generated species of Aβ such as monomers, oligomers, small and large protofibrils and fibrils, were evaluated side-by-side
- Soluble aggregates (oligomers/protofibrils) of Aβ are considered to be the most toxic forms
- Except for lecanemab, the antibody analogues were produced from publicly available sequences

Rationale to target Aβ protofibrils

Aβ protofibrils

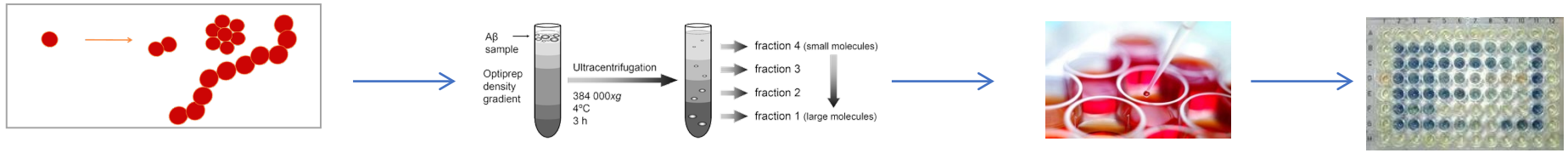
- First observed in 1997, defined by size and structure
- Aβ protofibrils are toxic and detected in the brain
- Increased propensity by the Arctic mutation
- Relevant for all forms of AD

Targeting protofibrils with antibody

- Minimal interference with normally occurring monomeric Aβ
- No sequestering of antibody in the periphery
- Lower binding to Aβ fibrils – lower risk for side-effects, ARIA-E?

Walsh et al. 1997 J Biol Chem; Harper et al. 1997 Chem Biol; Nilsberth et al. 2001 Nat Genet; O'Nuallain et al. 2010 J Neurosci; Lannfelt et al. 2013 J Intern Med; Lannfelt et al. 2014 Alz Res Ther

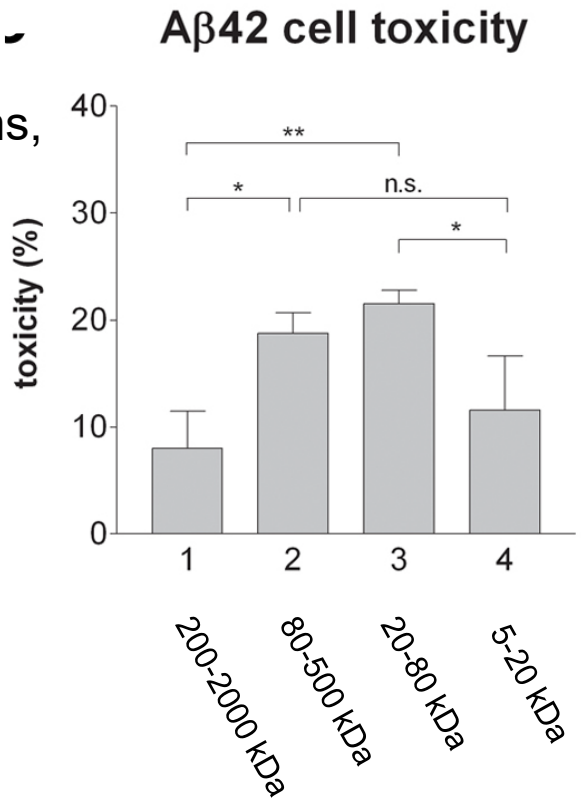
Intermediate sized Aβ42 oligomers/protofibrils: the most toxic species



Ultracentrifugation, of all soluble forms, intermediate sized most toxic

Adjusted for protein (Aβ) and optiprep concentration of each fraction

MTT toxicity assay



Fraction 2 and 3 most toxic

Most soluble Aβ from AD brain:

- In fraction 2
- Size of 80-500 kDa

Sehlin et al. 2012 PLoS ONE

Definition of Aβ protofibrils and oligomers

Our definition of protofibrils:

- Soluble aggregated Aβ eluting in the void volume of a Superdex 75 column, >75 kDa in size
- Do not pellet at 16,000 x g centrifugation
- Protofibrils have a beta-sheet structure and do not migrate as globular standard proteins
- Method: incubation of Aβ1-42 and separation of protofibrils from fibrils and monomers by centrifugation and Size-Exclusion Chromatography (SEC)

Our definition of oligomers:

- Less than 75 kDa in size
- Method: photo-induced cross-linking (Bitan et al. 2001 JBC) followed by separation and collection of oligomer fractions of different sizes using a Superdex 75 column

Methods used in this presentation

Inhibition ELISA

A two-step assay where the antibody to be tested was pre-incubated in solution with Aβ of different species in increasing concentrations. The mix was added to a microtiter plate precoated with Aβ protofibrils. If the antibody binds to Aβ in the pre-incubation step, fewer antibodies will bind to the immobilized Aβ on the microtiter plate.

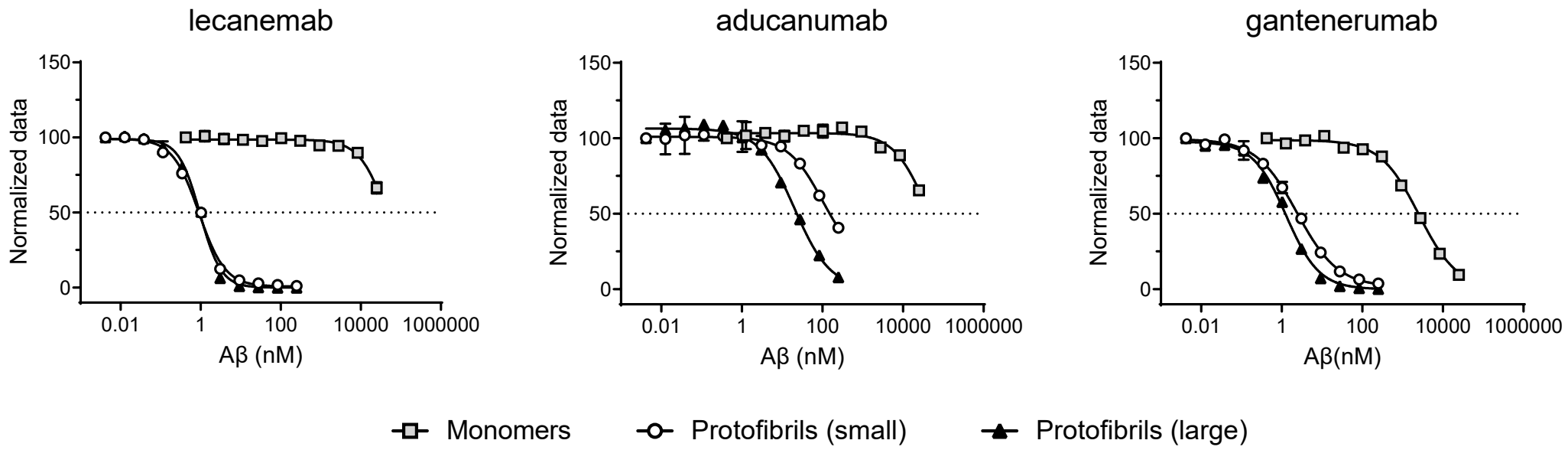
Immunodepletion

Antibodies were incubated with Aβ protofibrils in solution and bound fraction separated from the supernatant by protein A magnetic separation. The supernatant was analyzed for remaining Aβ protofibrils.

Surface Plasmon Resonance (Biacore)

Antibodies were captured with an anti-human antibody immobilized on a CM5 chip and Aβ1-40 monomers were injected over the captured antibodies. Protofibrils or fibrils were immobilized on a CM5 chip and antibodies were injected over the chip.

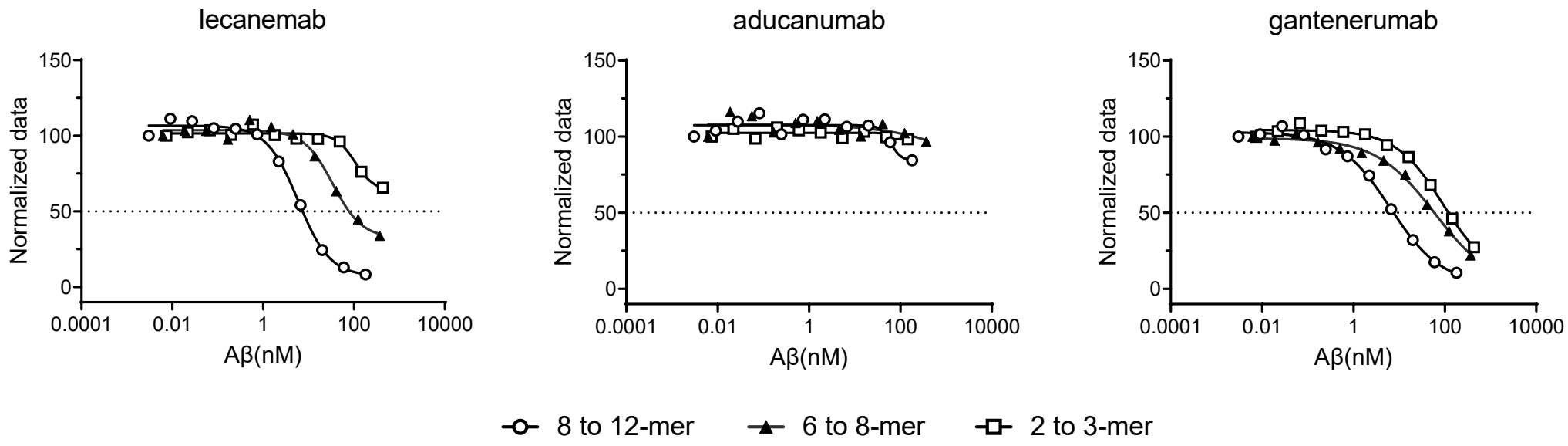
Binding to Aβ protofibrils strongest for lecanemab compared to aducanumab and gantenerumab (inhibition ELISA)



Small protofibrils, approx. 75-300 kDa, large protofibrils, approx. 300-5000 kDa

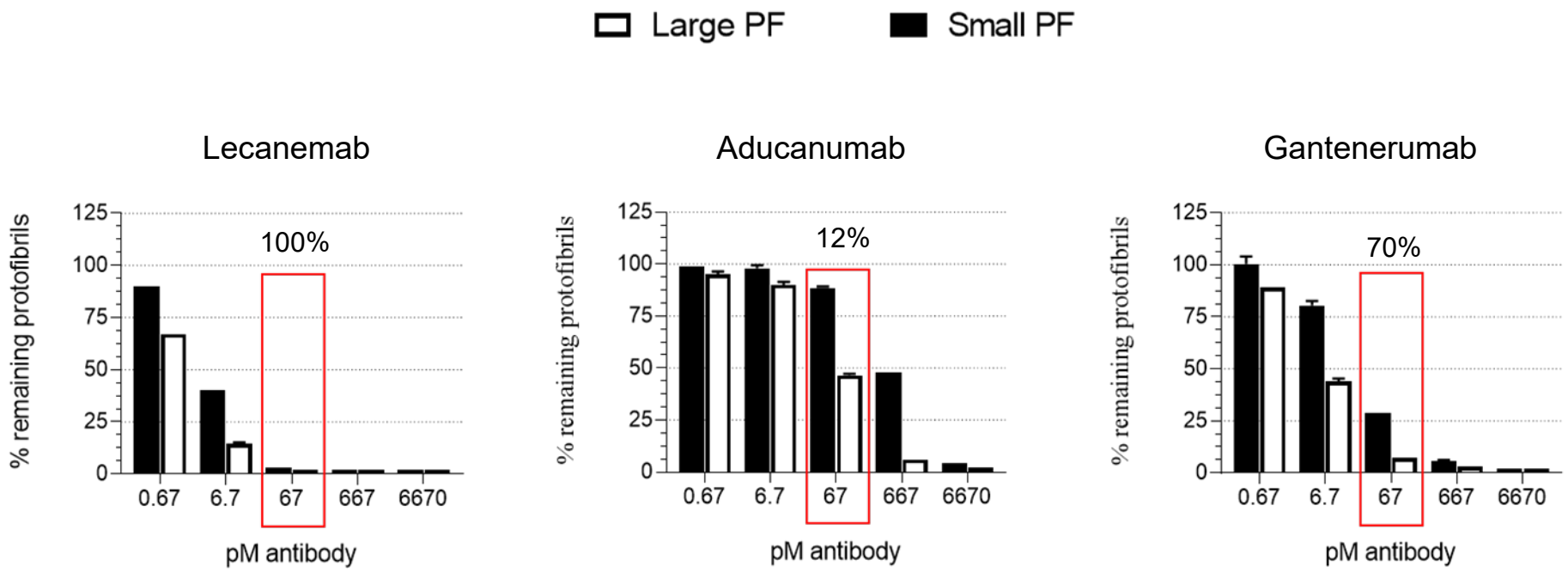
- Lecanemab binds small protofibrils 100x and large protofibrils 25x stronger than aducanumab
- Gantenerumab is less selective and binds monomers with somewhat higher affinity compared to lecanemab and aducanumab

Binding to small Aβ oligomers using inhibition ELISA



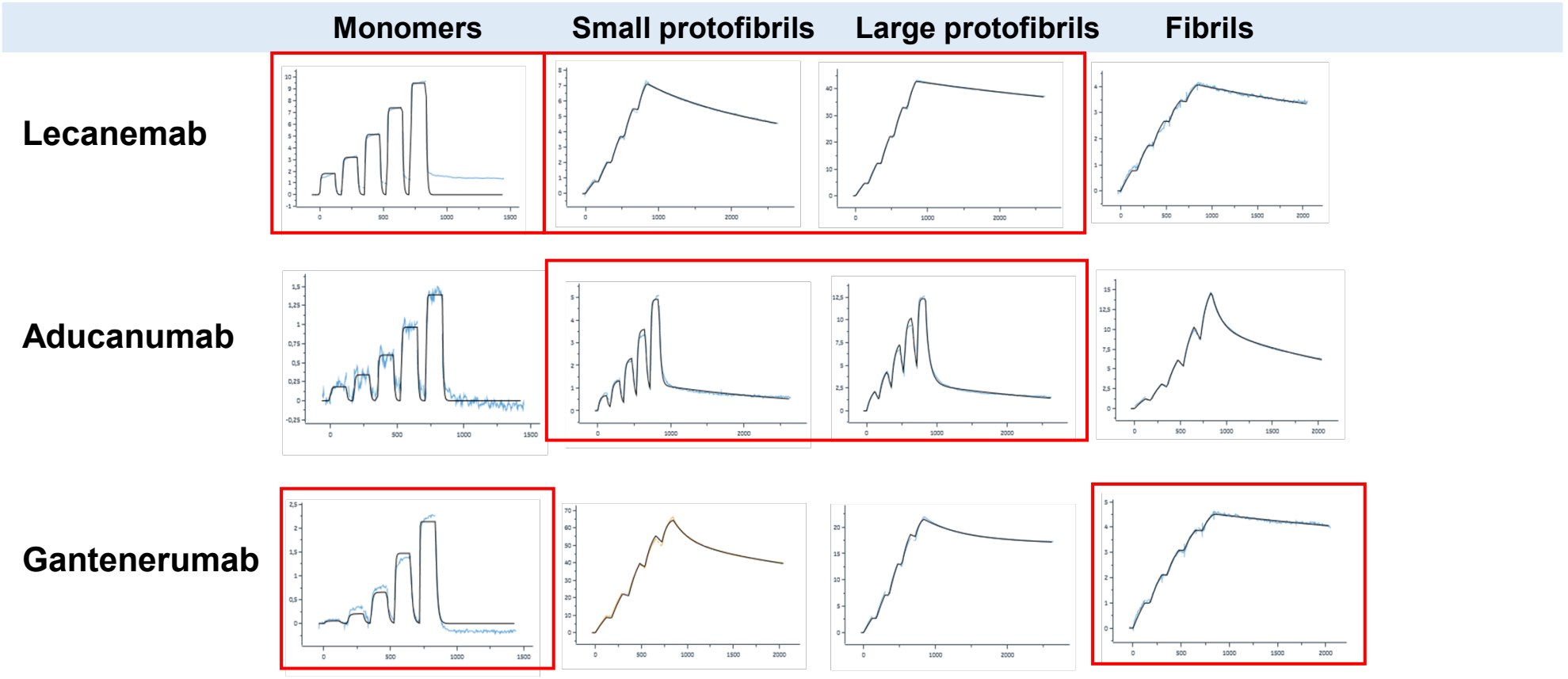
- Lecanemab binds 8 to 12-mer oligomers but had reduced binding to smaller species (2 to 3-mer)
- Aducanumab showed very weak binding to small oligomers
- Gantenerumab demonstrated binding to small oligomers

Immunodepletion of protofibrils: lecanemab more potent than aducanumab and gantenerumab



- Lecanemab was most potent in immunodepleting protofibrils in solution
- Full depletion was achieved with 67 pM of lecanemab, with 667 pM of gantenerumab and with 6670 pM aducanumab
- Results in line with inhibition ELISA

Selectivity for Aβ protofibrils strongest for lecanemab (SPR, Biacore)



- Lecanemab had its strongest binding to protofibrils, very low binding to monomers
- Aducanumab was a weaker Aβ binder with a very fast on- and off-rates for protofibrils, very low binding to monomers and prefers fibrils
- Gantenerumab had somewhat higher binding to monomers and prefers fibrils

Relative selectivity to different Aβ species for lecanemab, aducanumab and gantenerumab: summarized data from three methods

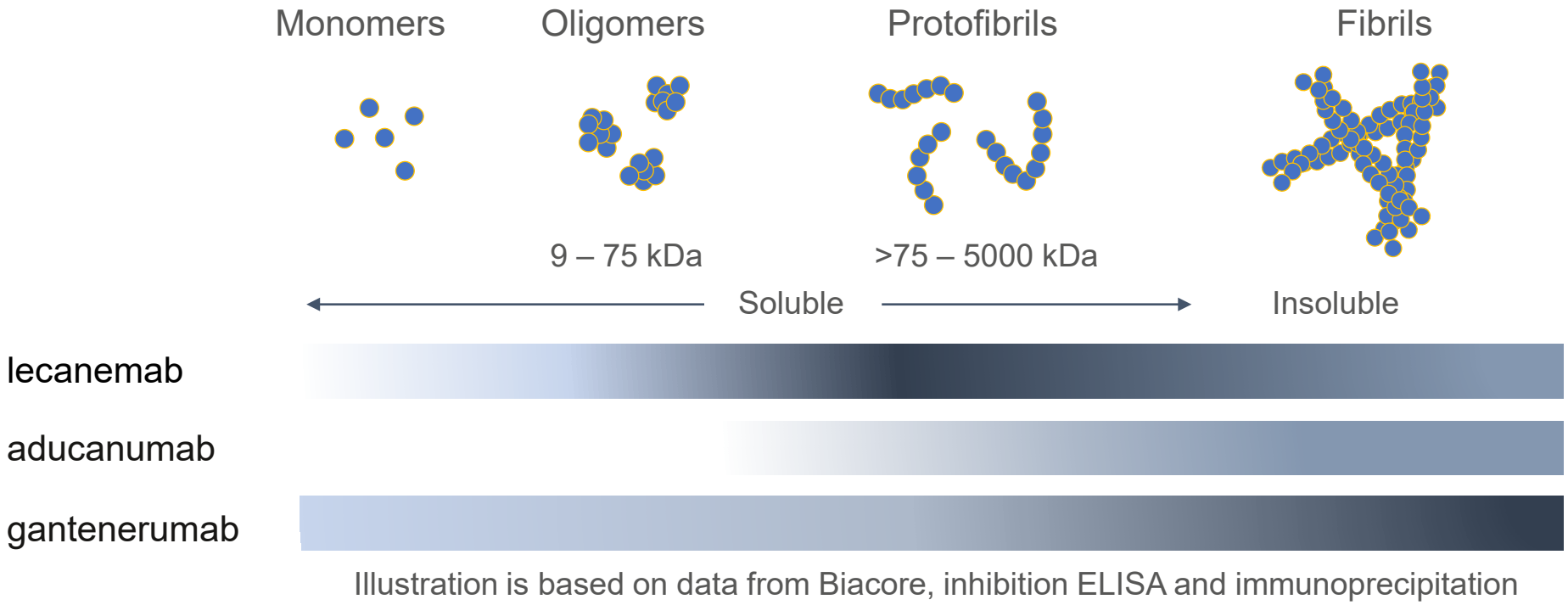


Illustration is based on data from Biacore, inhibition ELISA and immunoprecipitation

- Lecanemab had the highest preference for soluble protofibrils/oligomers versus monomeric and fibrillar forms of Aβ
- Aducanumab and gantenerumab had a preferences for the insoluble fibrils
- Aducanumab showed a lower binding to all Aβ species
- Gantenerumab had somewhat higher binding to monomers and prefers fibrils

Summary

- All three investigated antibodies mainly bind aggregated forms of Aβ
- All three antibodies have low binding strength to monomers
- All three antibodies work through avidity
- The three antibodies have different binding profiles to Aβ
- All three antibodies bind fibrils, but with different selectivity
- Lecanemab was the strongest Aβ binder and had the highest selectivity for protofibrils
- Lecanemab prefers protofibrils and aducanumab and gantenerumab prefer fibrils
- These binding profiles might reflect differences in clinical outcome and also side-effects

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