

Molecular Evolution and Design of Pegylated CB 2782 as a Complement Factor C3-Inactivating Protease for Dry AMD

ASBMB Symposium on Serine Proteases and Extracellular Proteolysis

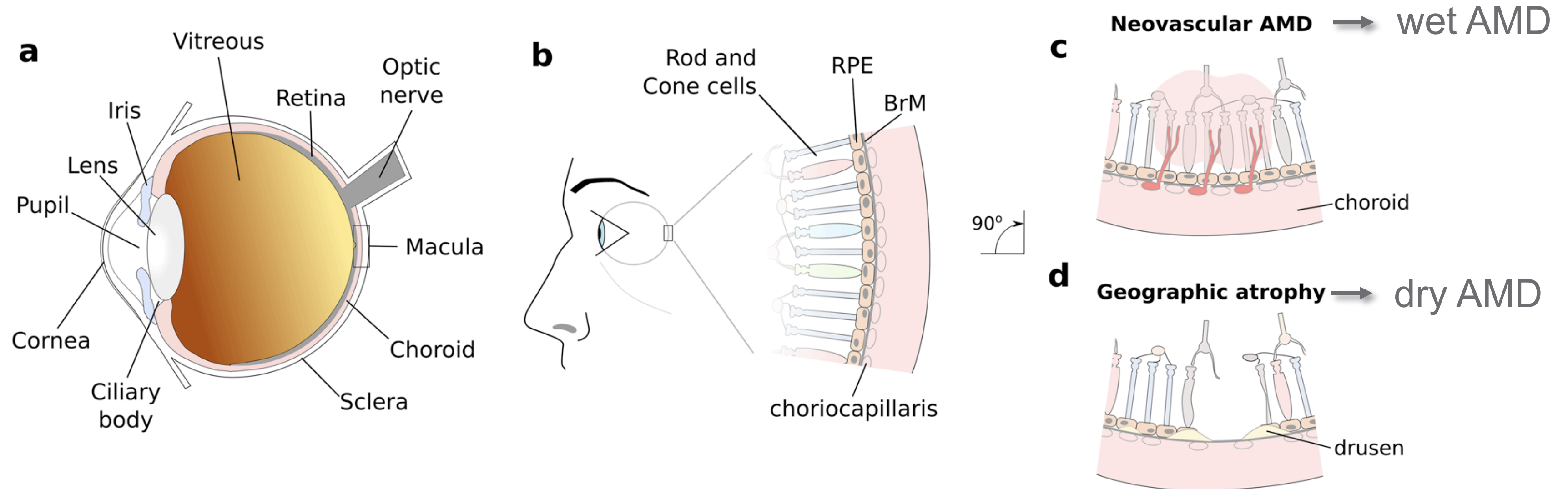
September 15th 2019

Grant E. Blouse, PhD

VP Translational Research



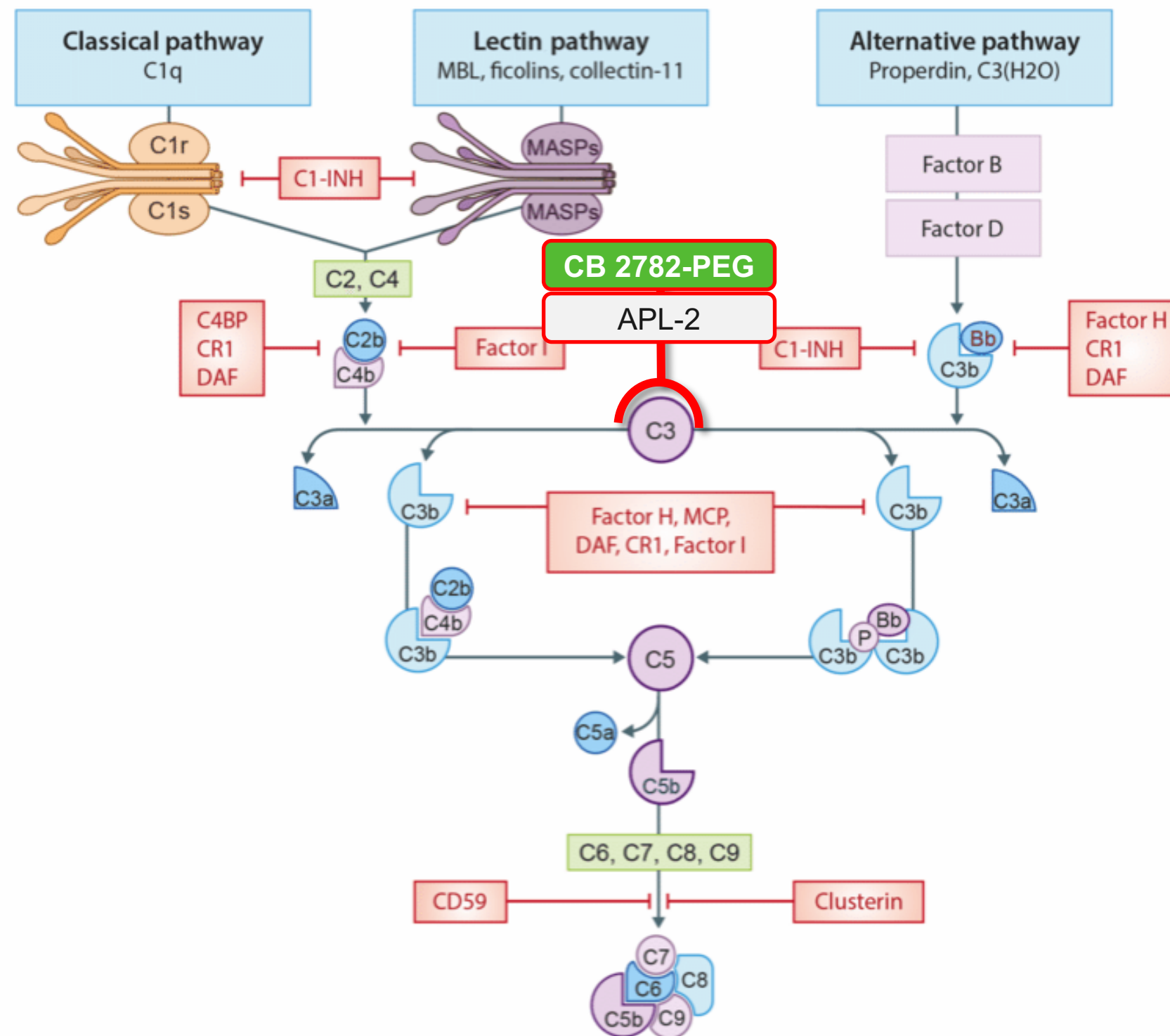
Age-Related Macular Degeneration (AMD)



Clark and Bishop "The eye as a complement dysregulation hotspot" Semin Immunopathol (2018) 40:65–74

- + Wet and dry AMD are distinct diseases of which both **lead to vision loss and blindness**
- + Geographic atrophy (GA) results in progressive loss of photoreceptors and irreversible central vision loss
- + Unlike wet AMD, **no marketed treatment is available for dry AMD**

C3 is the only validated target for GA in dry AMD



Advanced dAMD, or geographic atrophy (GA), has a devastating impact on vision and leads to blindness

+ No currently approved therapies

C3 is the only clinically validated target in GA

- + Apellis APL-2 (anti-C3 PEGylated cyclic peptide) completed P2
- + 15 mg intravitreal injection in randomized P2 (n=246)
 - Qmo - 29% inhibition of GA (p=0.008)
 - Q2mo - 20% inhibition of GA (p=0.067)

Proteases provide superiority to peptides or antibodies

+ Sub-stoichiometric dosing and a catalytic mechanism

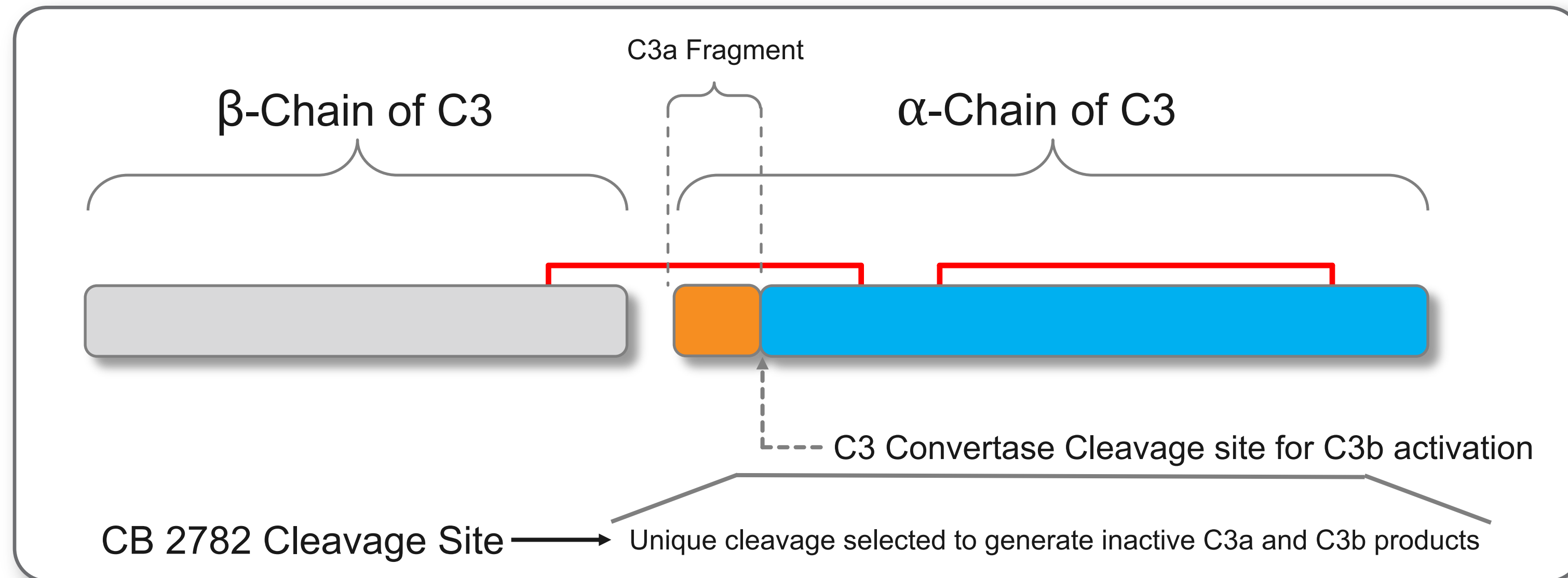
Catalyst's long acting anti-C3 protease is best-in-class

+ Provide superior efficacy and better convenience

+ **Q3mo or Q4mo dosing**

Selection of a specific “inactivating” cleavage site

Schematic of C3 structure and the C3 convertase cleavage site



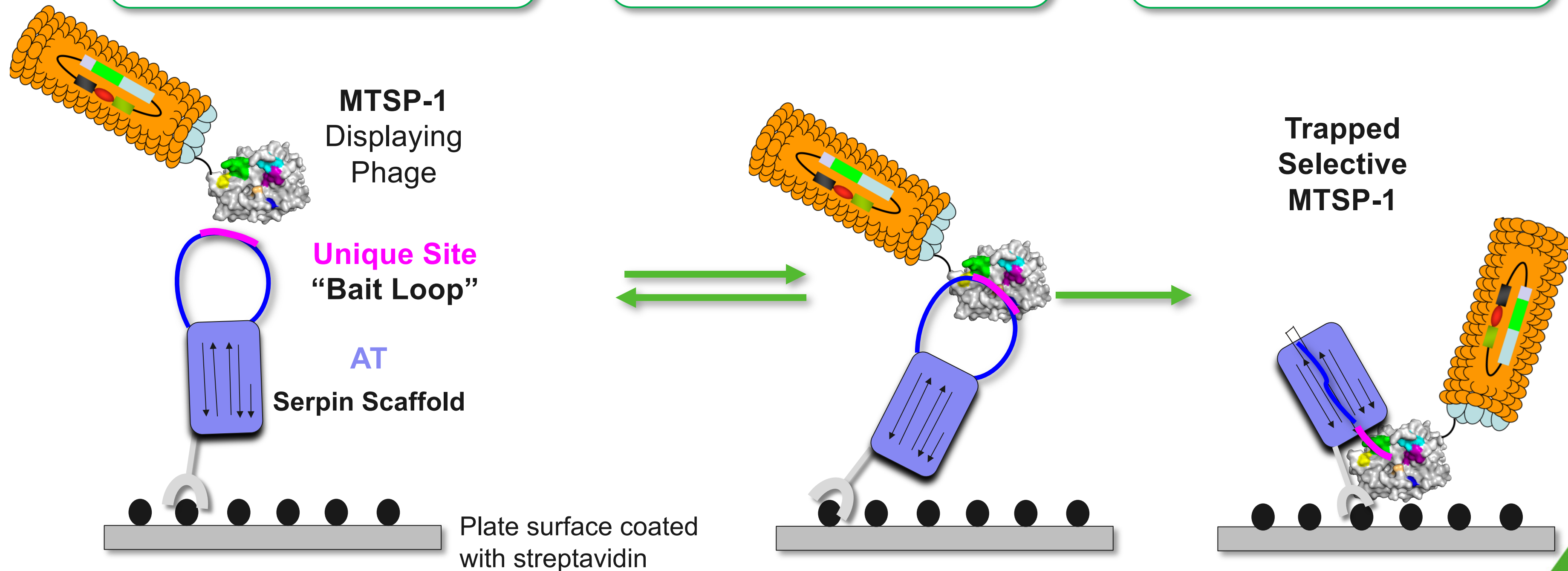
- + CB 2782 was engineered to specifically cleave a single site in C3
 - Divergent from that which is cleaved by the C3 convertases
- + Cleavage of C3 results in an inactive C3a and C3b-related species
 - Cannot be further activated by the C3 convertases

Using SERPINs as a “kinetic” trap to select for catalysis

Phage library presented to a plate coated with biotinylated serpin-AT “Bait”

Catalytic activity of MTSP-1 variant drives cleavage of the unique site in the bait

Serpin-mediated trapping of phage variant that cleaves the unique site

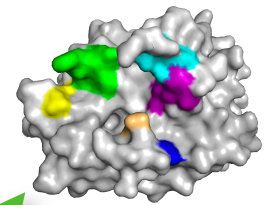


Catalyst Biosciences: Alterase™ Protease Platform

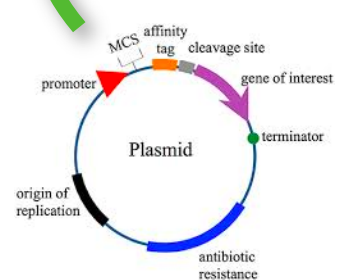
Proprietary Technology for Protease Discovery

- Based on cleavage activity and not on binding by using a serpin-mediated trapping approach
- Allows rapid discovery of new proteases with tailored catalytic activity
- Allows rapid lead optimization by screening for enhanced activity & specificity simultaneously

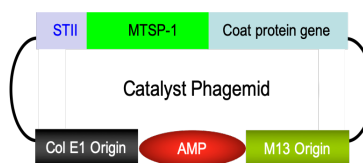
protein production



DNA mutagenesis

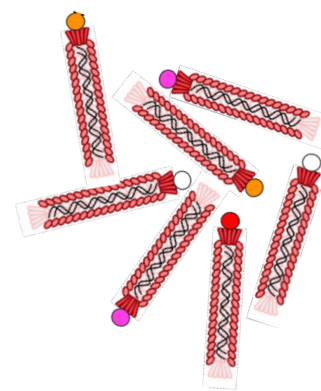


Positive hits for scale up

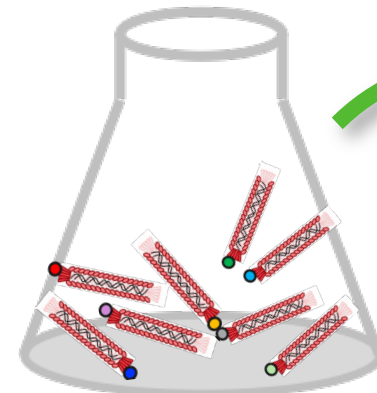


DNA extraction and sequence

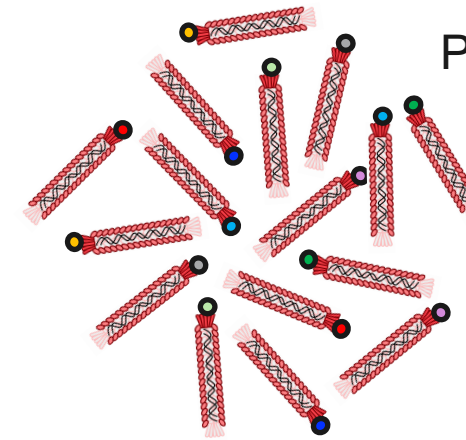
Creation of new MTSP-1 variant libraries



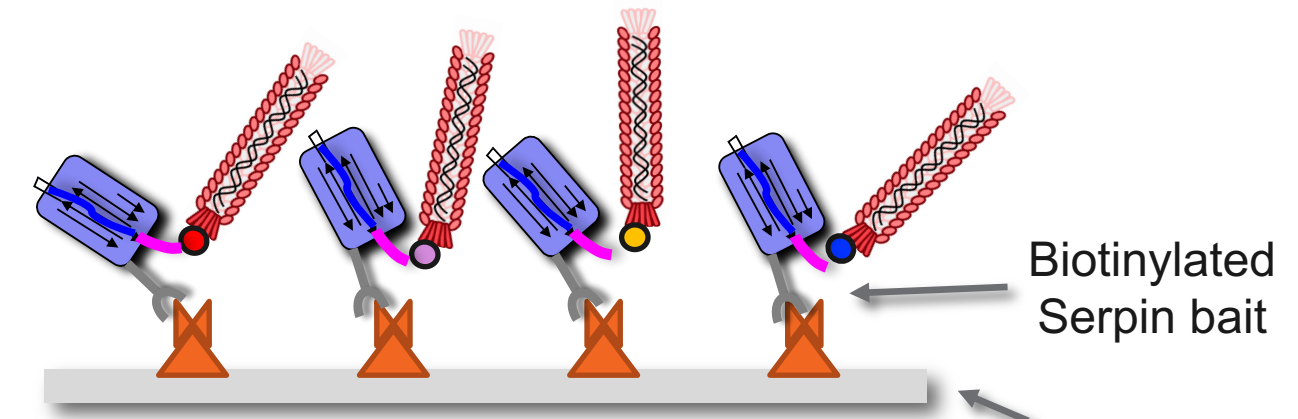
Grow MTSP-1 variant phage library



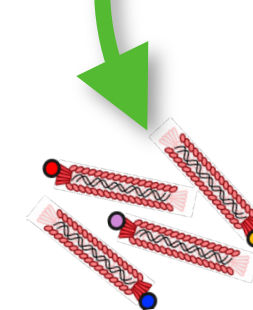
Purify MTSP-1 phage library



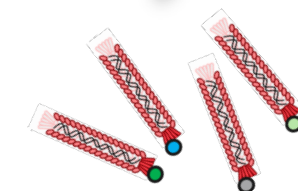
Add MTSP-1 phage library to immobilized serpin bait



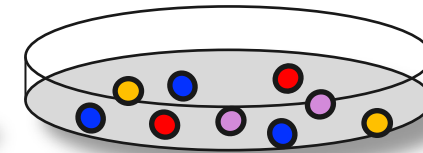
Elute phages "trapped" by Serpin



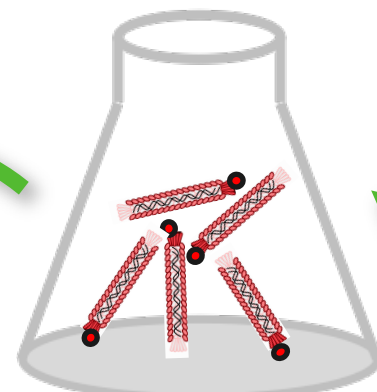
Remove unbound phages by washing plate



Pick colonies and grow large quantity of phages

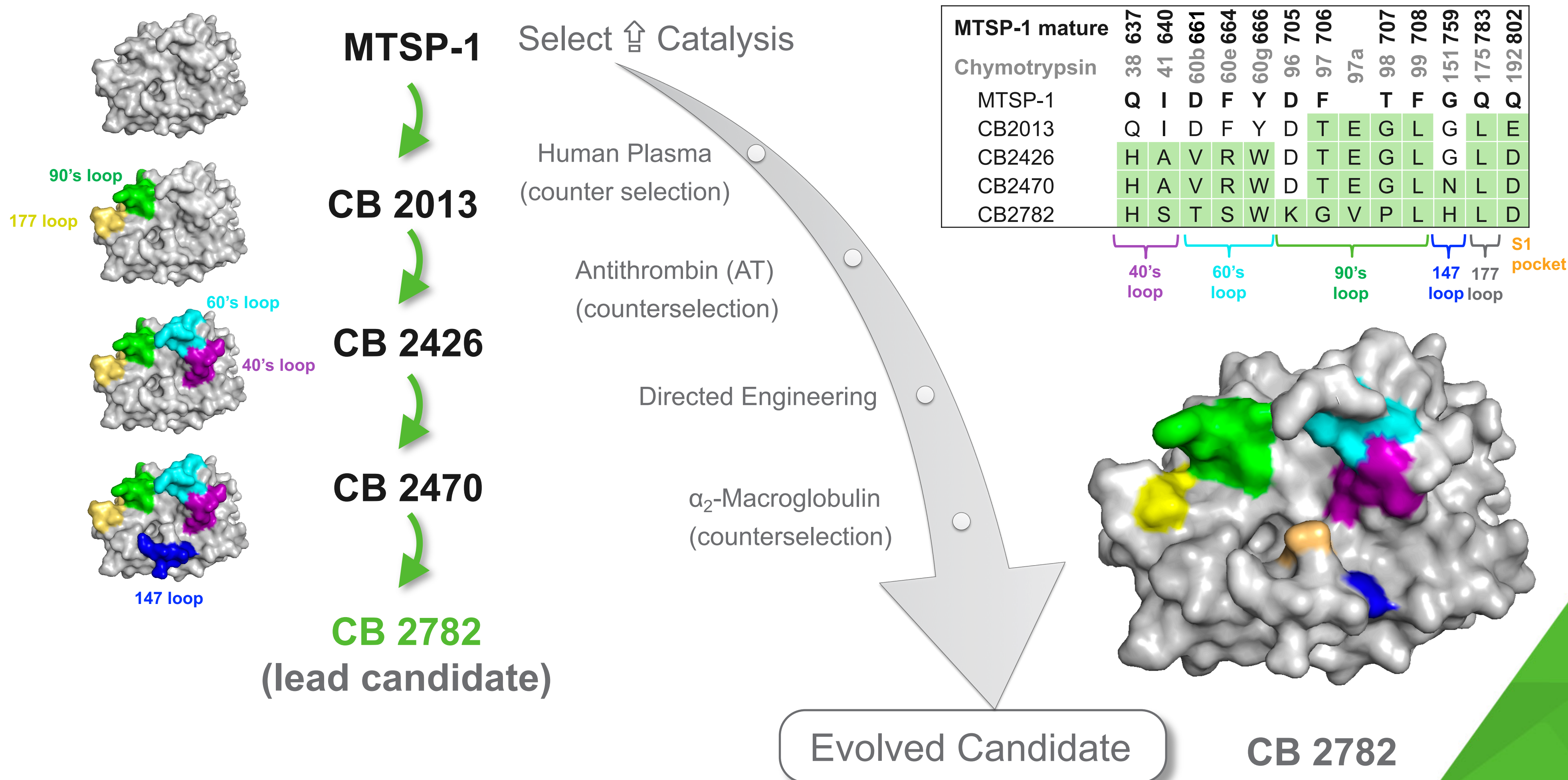


Infect bacteria and grow colonies on plates

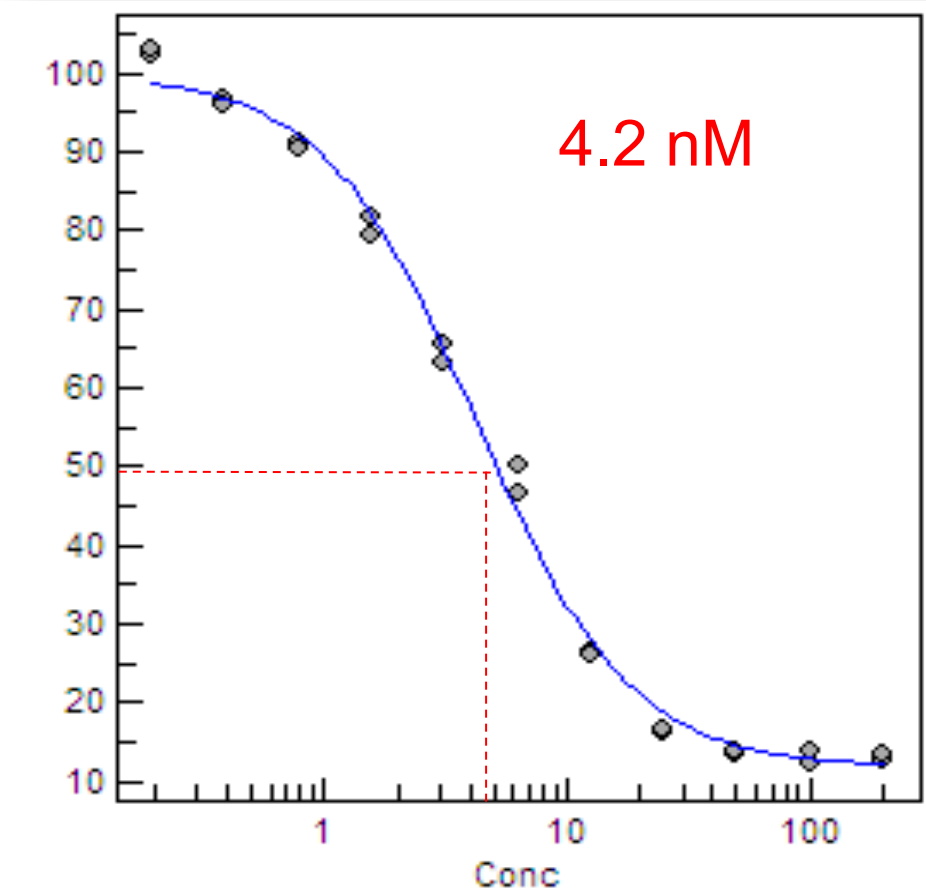
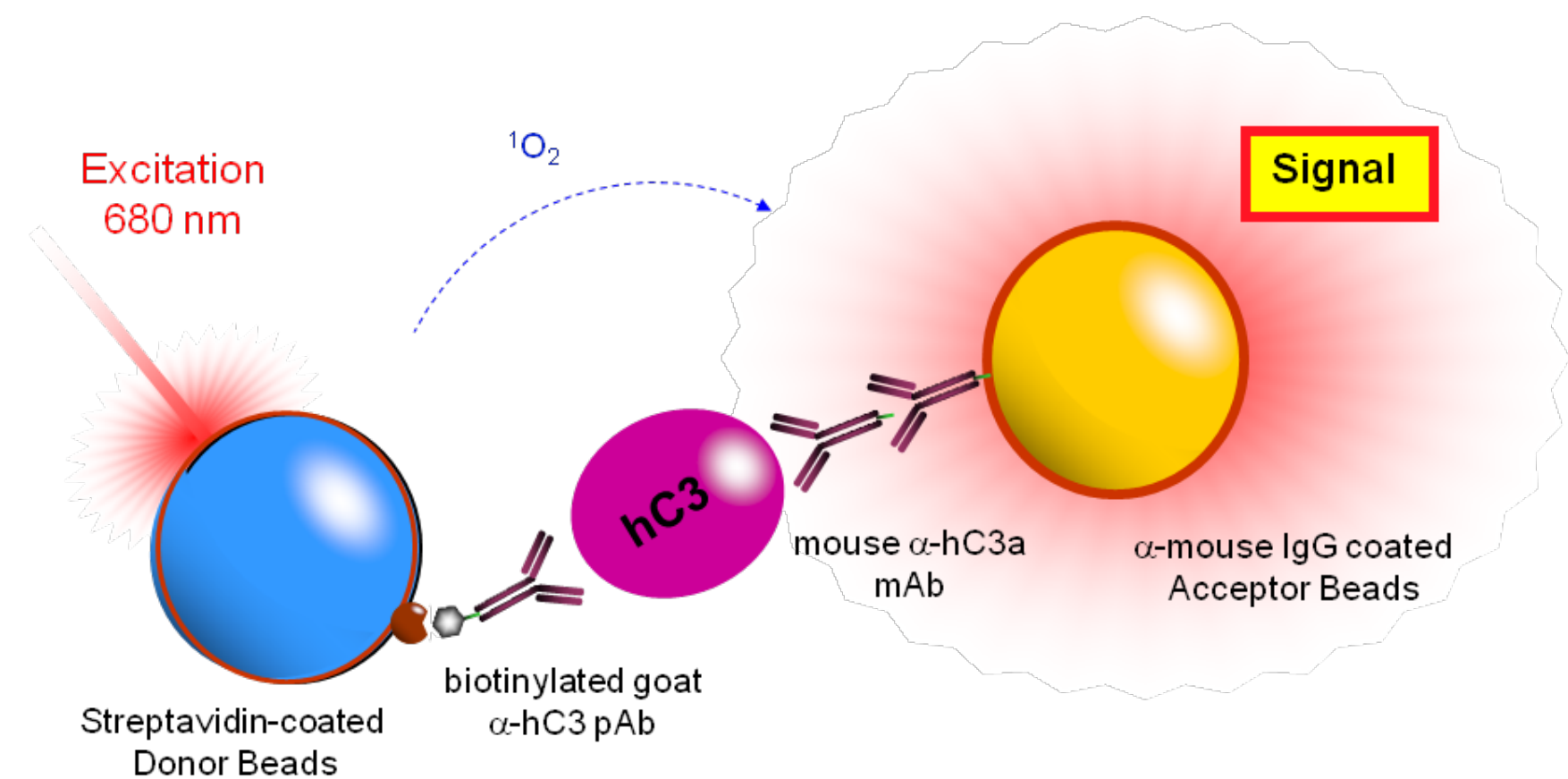


Cloning in expression plasmids

Molecular evolution of CB 2782 for C3-specific cleavage

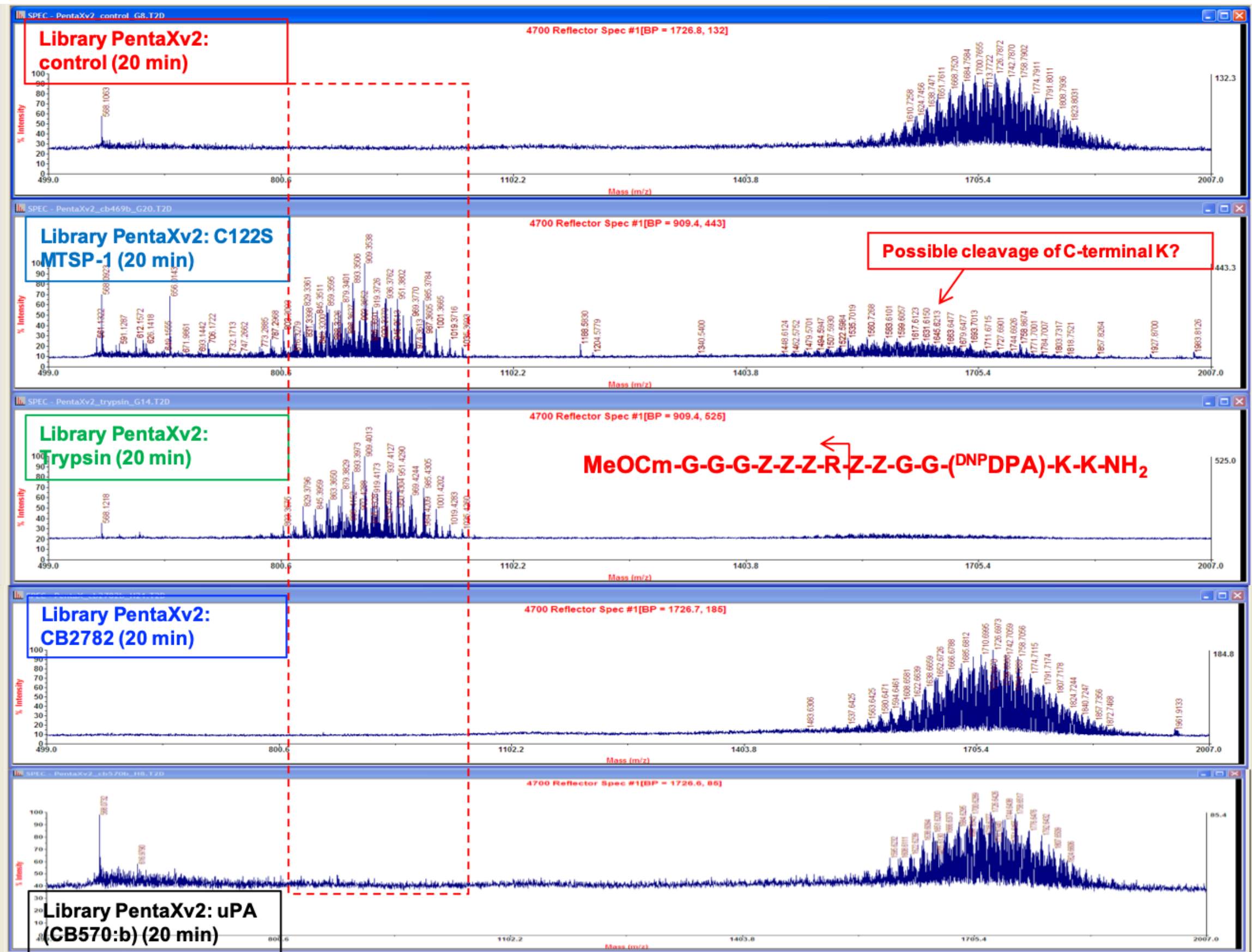


CB 2782 shows significant improvement in cleavage of C3

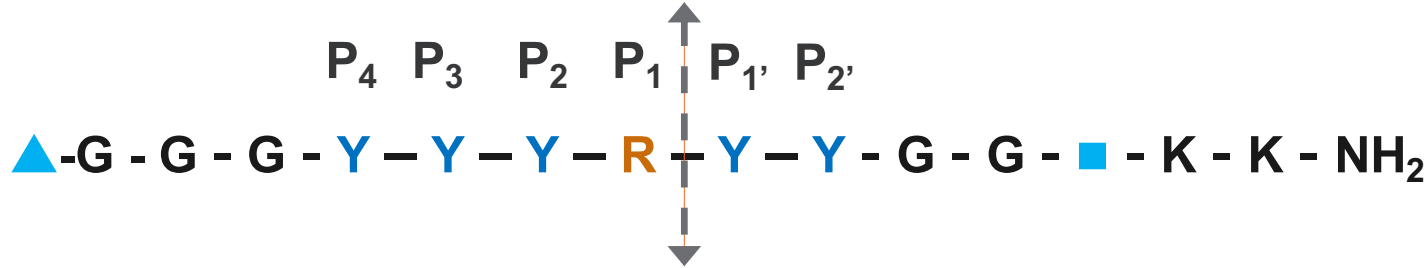


	MTSP-1 (nM)	CB 2782 (nM)	Ratio
Buffer	13.9	6.9	2
Human Plasma	2800	92	30
Cyno Plasma	3500	25	140

CB 2782 shows high specificity



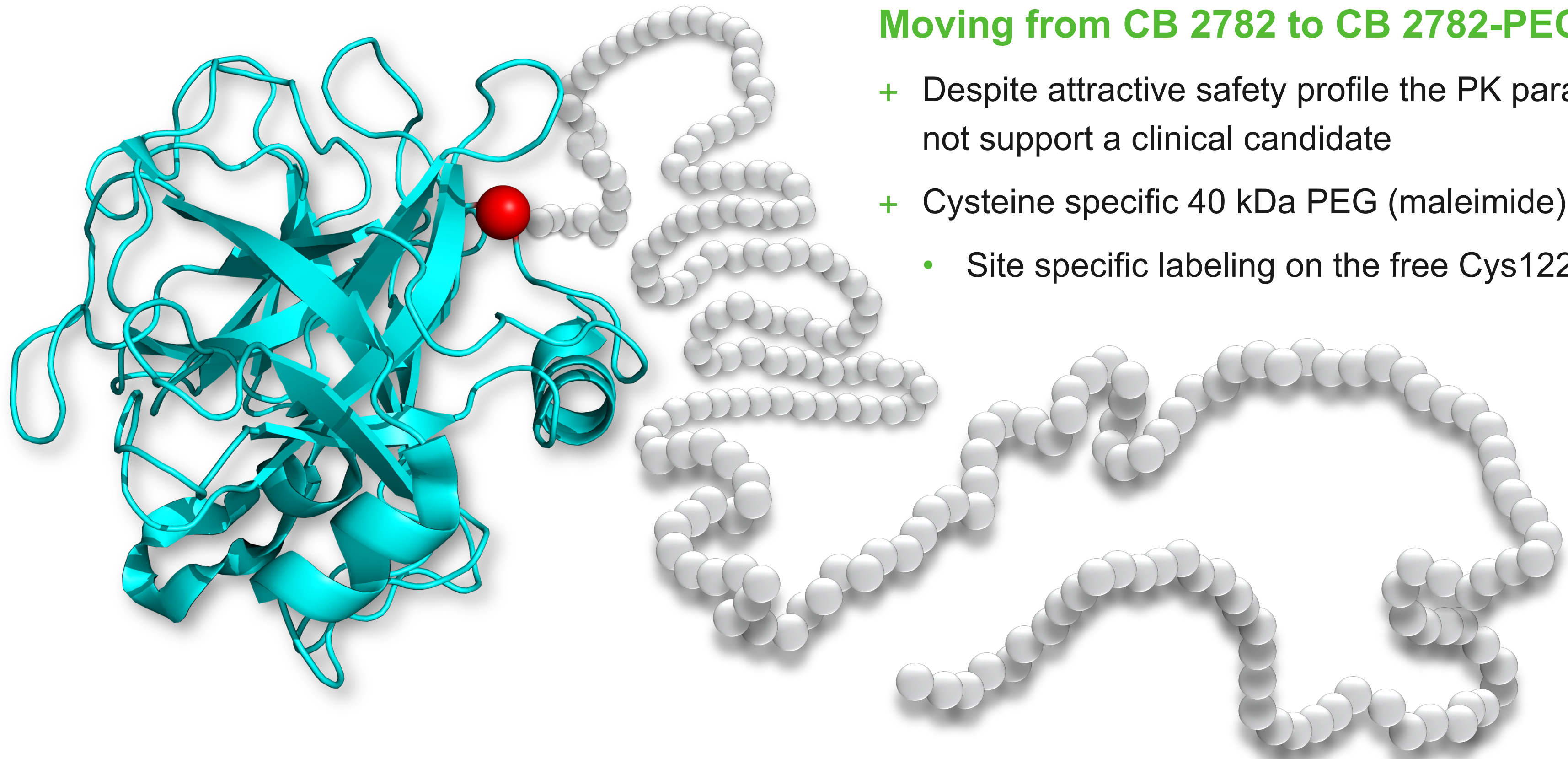
Cleavage of PentaXv2 Library



- Y Any of 18 AAs (excluding R, C)
 - \triangle N-terminal 7-methoxycoumarin-4-acetyl
 - \blacksquare dinitrophenyl-diaminopropyl
- # Peptides
1,889,568 (18⁵)

- + Essentially no detectable cleavage of the PentaXv2 library by CB 2782
- + Near complete cleavage by MTSP-1
- + Complete cleavage by trypsin
- + Very little cleavage by uPA

Development Candidate CB 2782-PEG

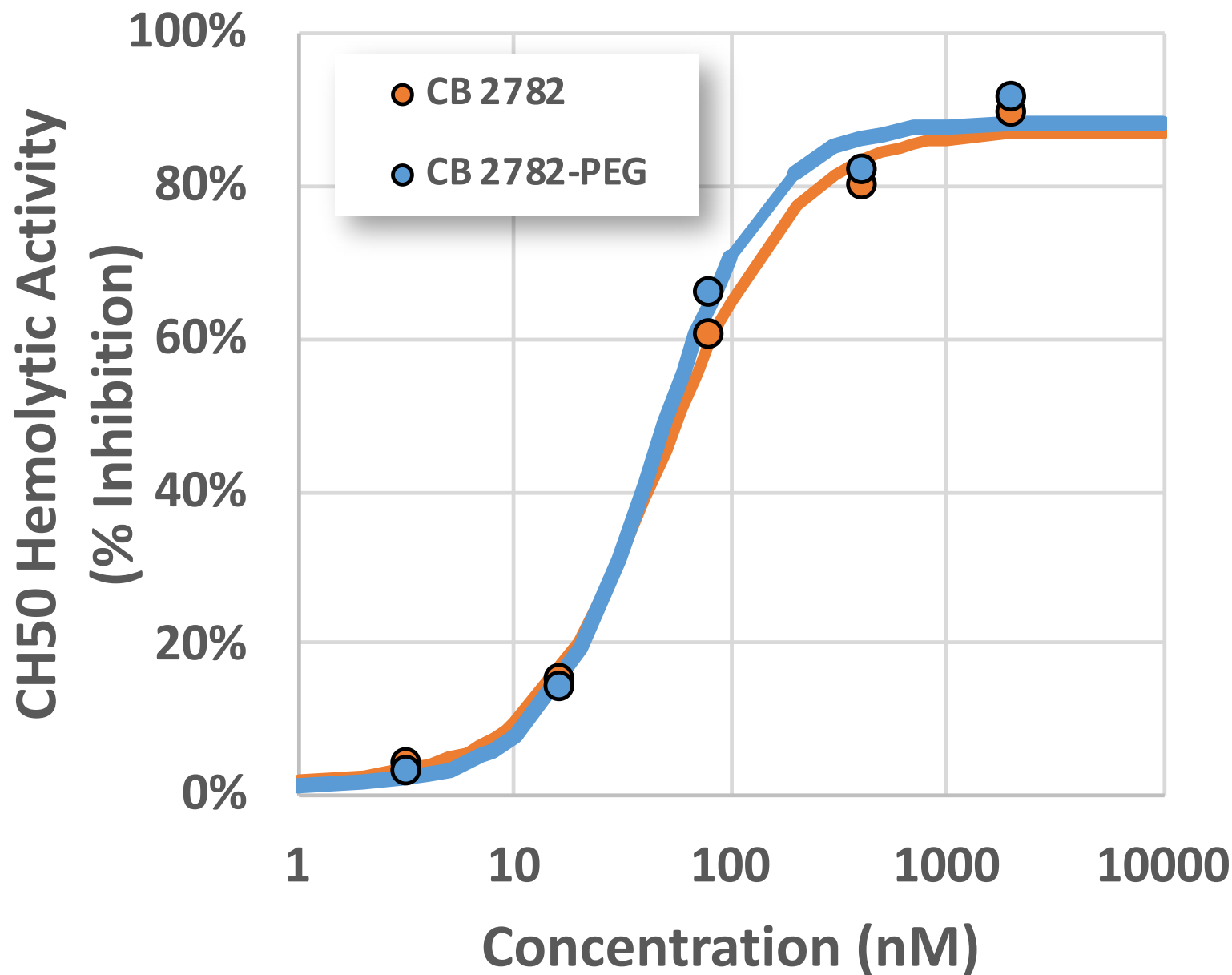


Moving from CB 2782 to CB 2782-PEG

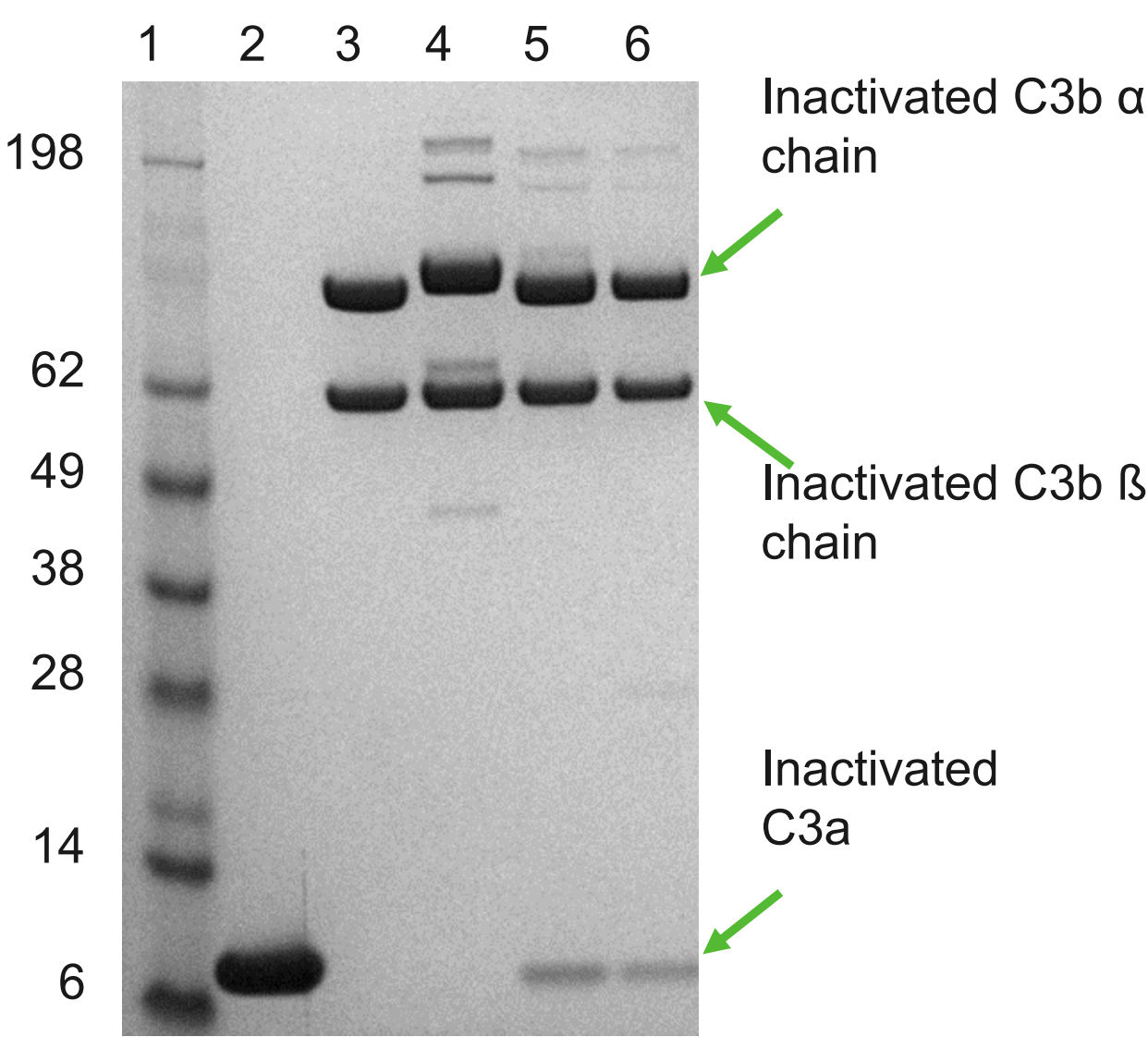
- + Despite attractive safety profile the PK parameters did not support a clinical candidate
- + Cysteine specific 40 kDa PEG (maleimide) conjugated
 - Site specific labeling on the free Cys122

CB 2782-PEG has indistinguishable activity vs CB 2782

CB 2782 and CB 2782-PEG inhibit complement-mediated hemolysis *in vitro*



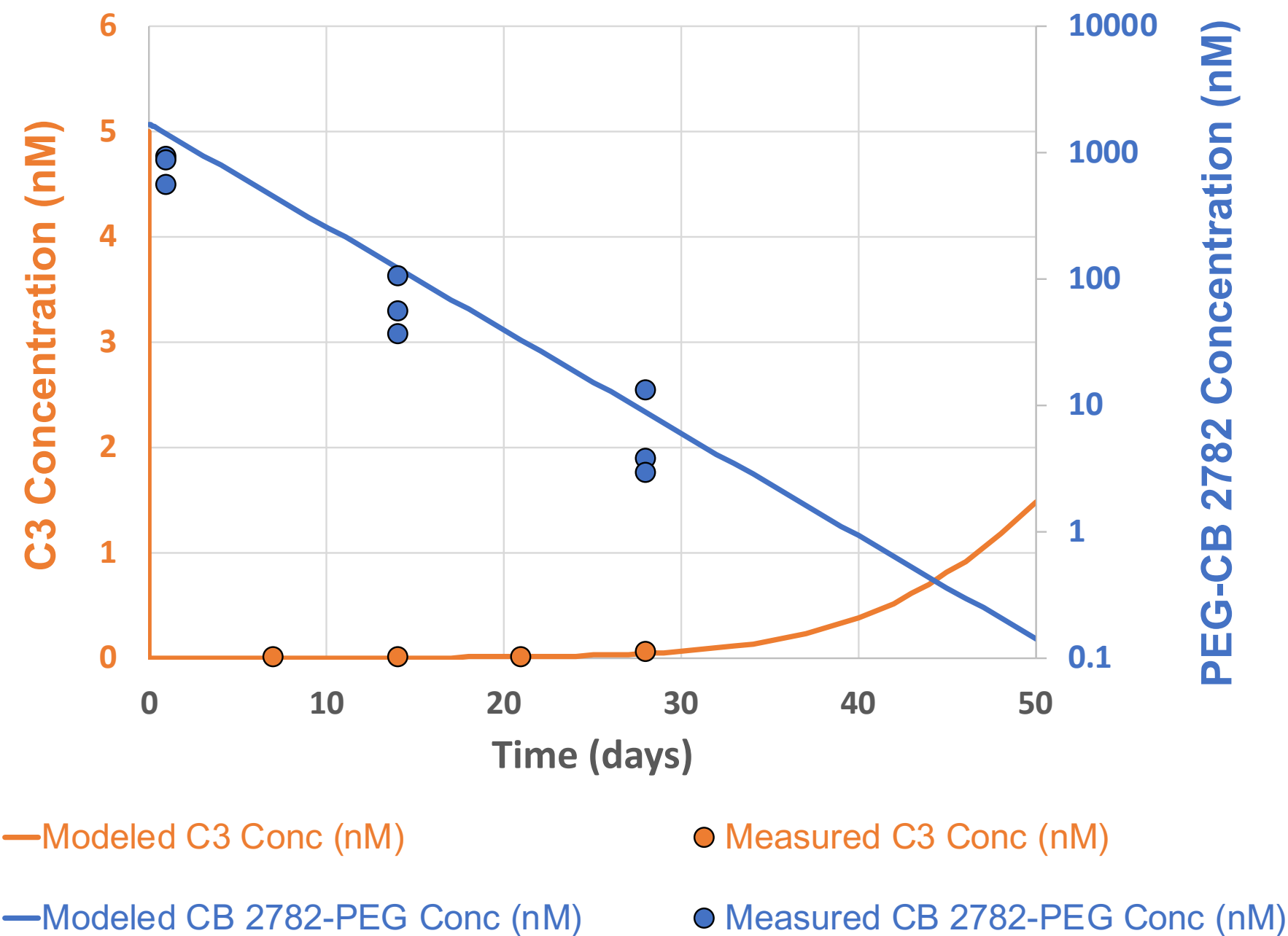
Sub-stoichiometric CB 2782 and CB 2782-PEG specifically cleave C3 at a single site into inactive fragments



Reduced SDS-PAGE	
Lane	Sample
1	Ladder
2	C3a
3	C3b
4	C3
5	2 μM C3 treated with 0.2 μM CB 2782-PEG
6	2 μM C3 treated with 0.2 μM CB 2782

CB 2782-PEG eliminates vitreous C3 for at least 28 Days

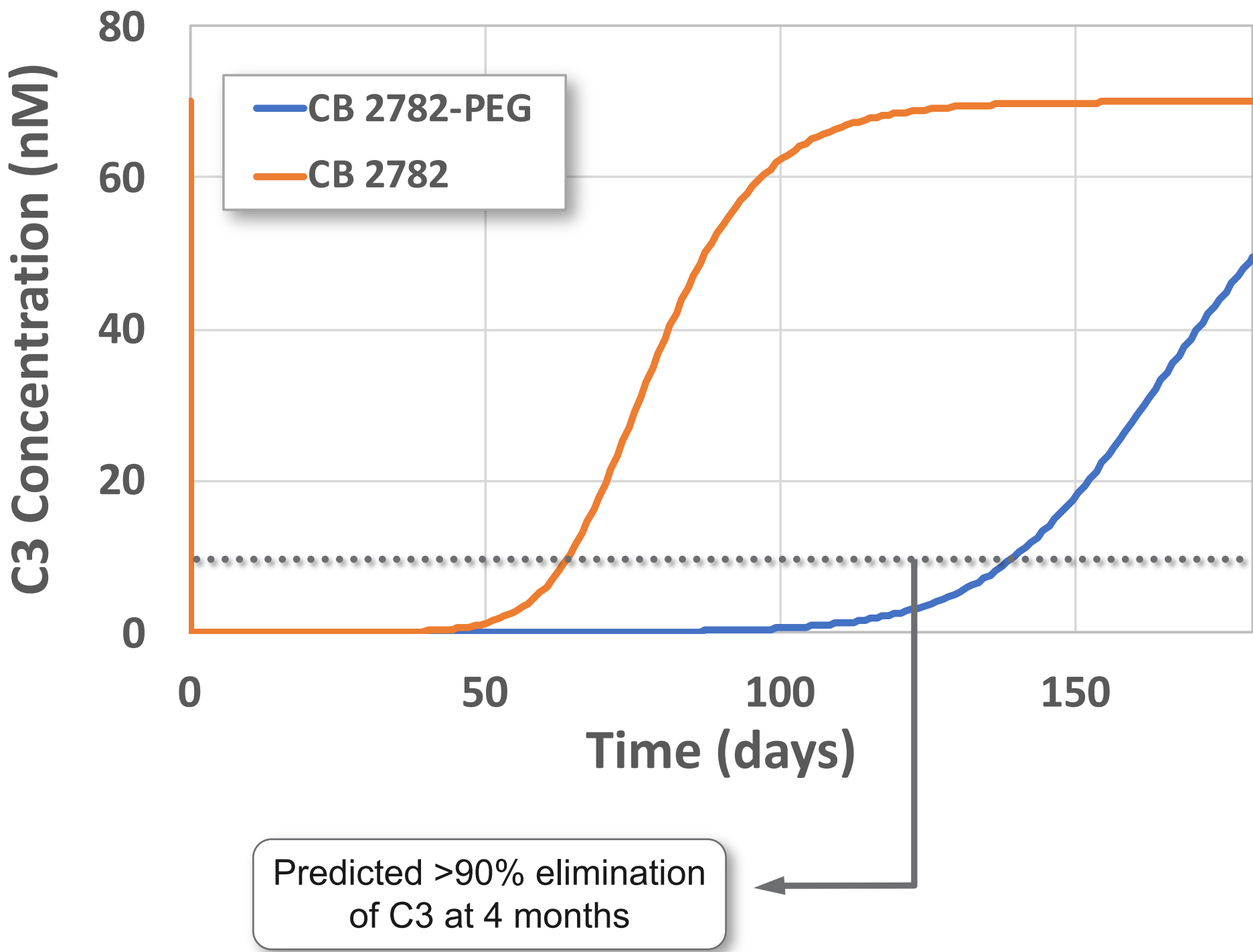
Intravitreal CB 2782-PEG has a half-life of 3.7 days and eliminates at least 99% of C3 in vitreous humor of African green monkeys for at least 28 days



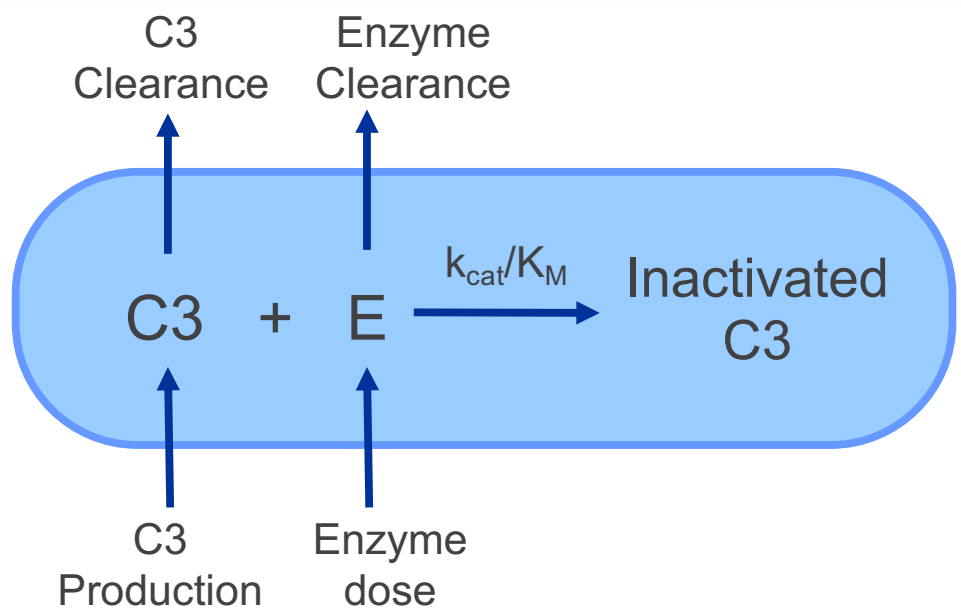
Parameter	CB 2782-PEG
t-half-terminal (d)	3.7
Mean residence time (d)	3.37
Cmax (μM)	0.90
Tmax (d)	1
AUC 0-inf (μM-d)	6.94
AUC 0-t (μM-d)	6.92

Predicted 2.0 mg human dose three to four times a year

Enzyme Model: Fit to observed primate PK/PD data and scaled to the human condition



Model Parameter	African Green Monkey		Human	
	Value	Source	Value	Source
Vitreous Volume (mL)	3.0	Measured	4.4	Literature
C3 Steady State Conc (nM)	5.0	Measured	70	Literature
C3 Vitreous Half-Life (d)	4.4	Literature	8.2	Literature
Enzyme Dose (mg)	0.125	Known	2.0	Known
Enzyme Half-Life (d)	3.7	Measured	8.5	2.3X scaling from AGM to human
Enzyme k_{cat}/K_M (nM ⁻¹ d ⁻¹)	1.88	Fit	1.88	AGM Model



Summary & conclusions

Engineered novel specificity through molecular evolution of MTSP-1

Significantly improved catalysis and stability in a biological milieu

Intravitreal injection resulted in at least 99% elimination of C3 for at least 28 days

CB 2782-PEG has potential for best-in-class efficacy and convenience in dry AMD

Acknowledgements



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