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

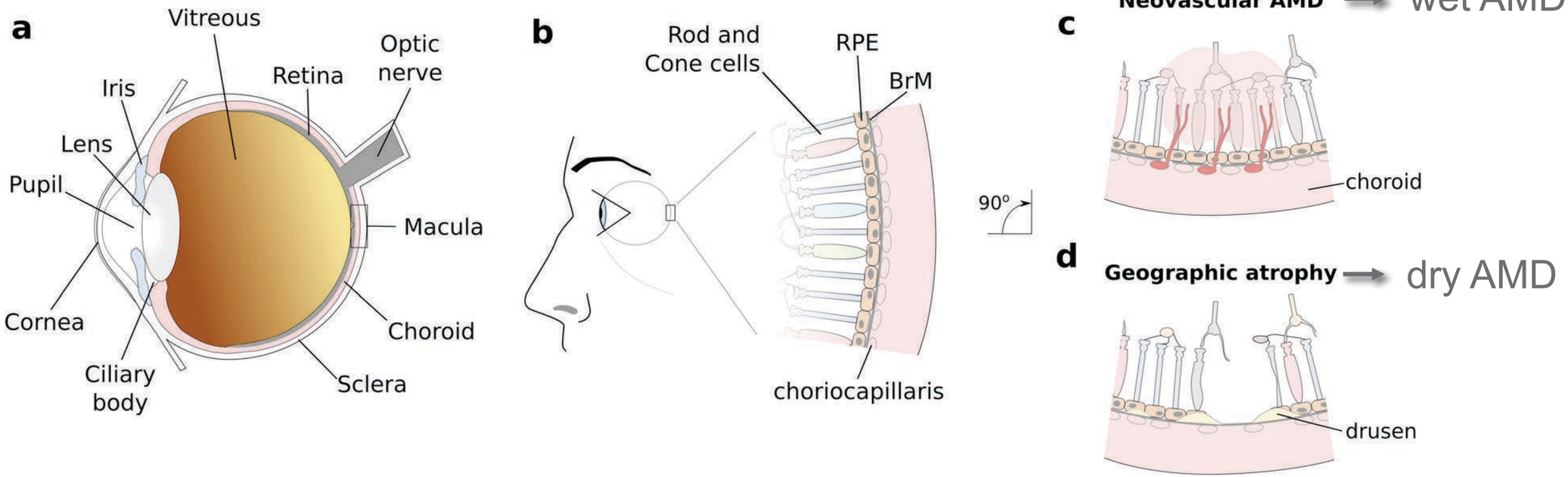
September 15th 2019

Grant E. Blouse, PhD
VP Translational Research

CATALYST BIOSCIENCES

Age-Related Macular Degeneration (AMD)

Nasdaq: CBIO

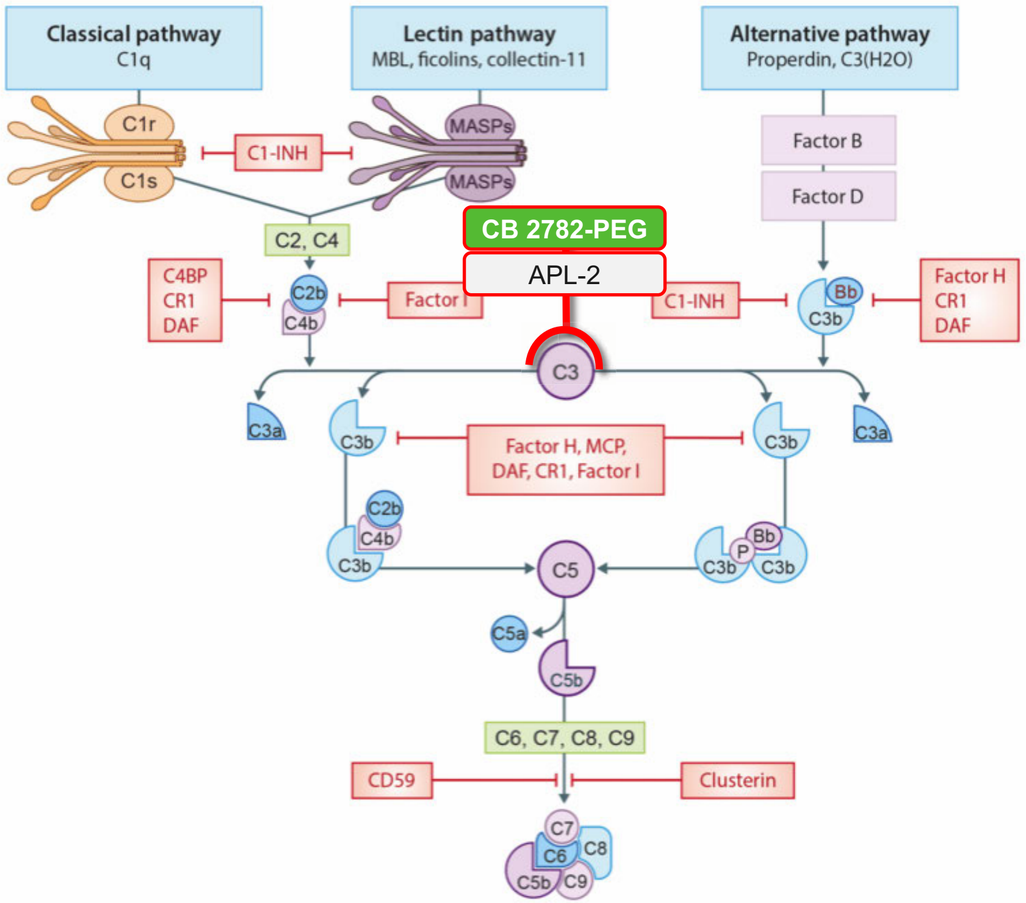


Clark and Bishop "The eye as a complement dysregulation hotspot" Semin Immunopathol (2018) 40:65–74

+ 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

Nasdaq: CBIO



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

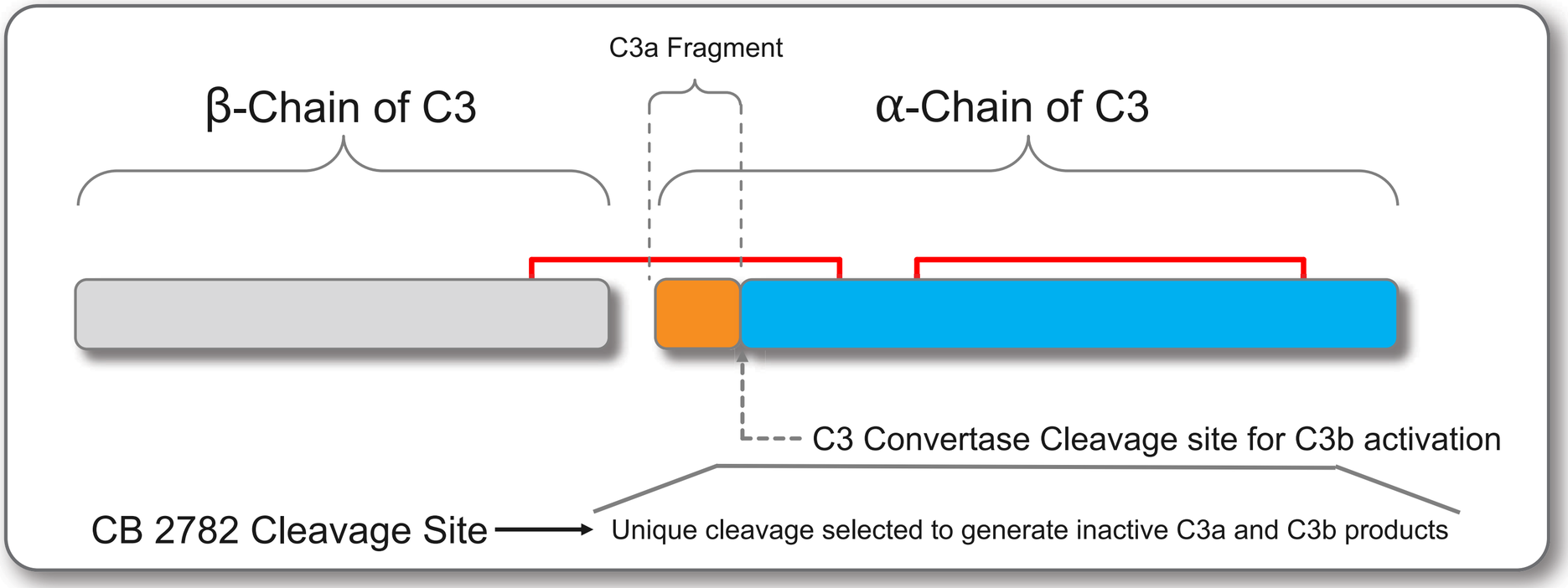
+ Sub-stoichiometric dosing and a catalytic mechanism

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

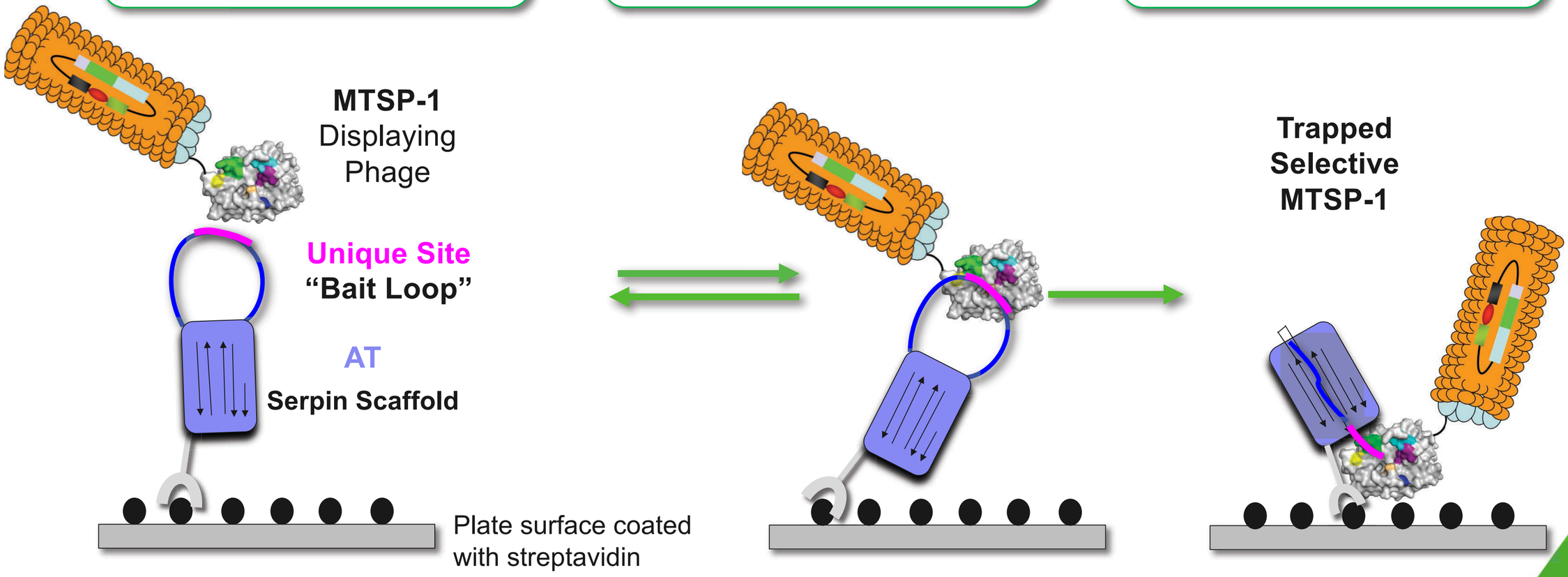
Using SERPINs as a “kinetic” trap to select for catalysis

Nasdaq: CBIO

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

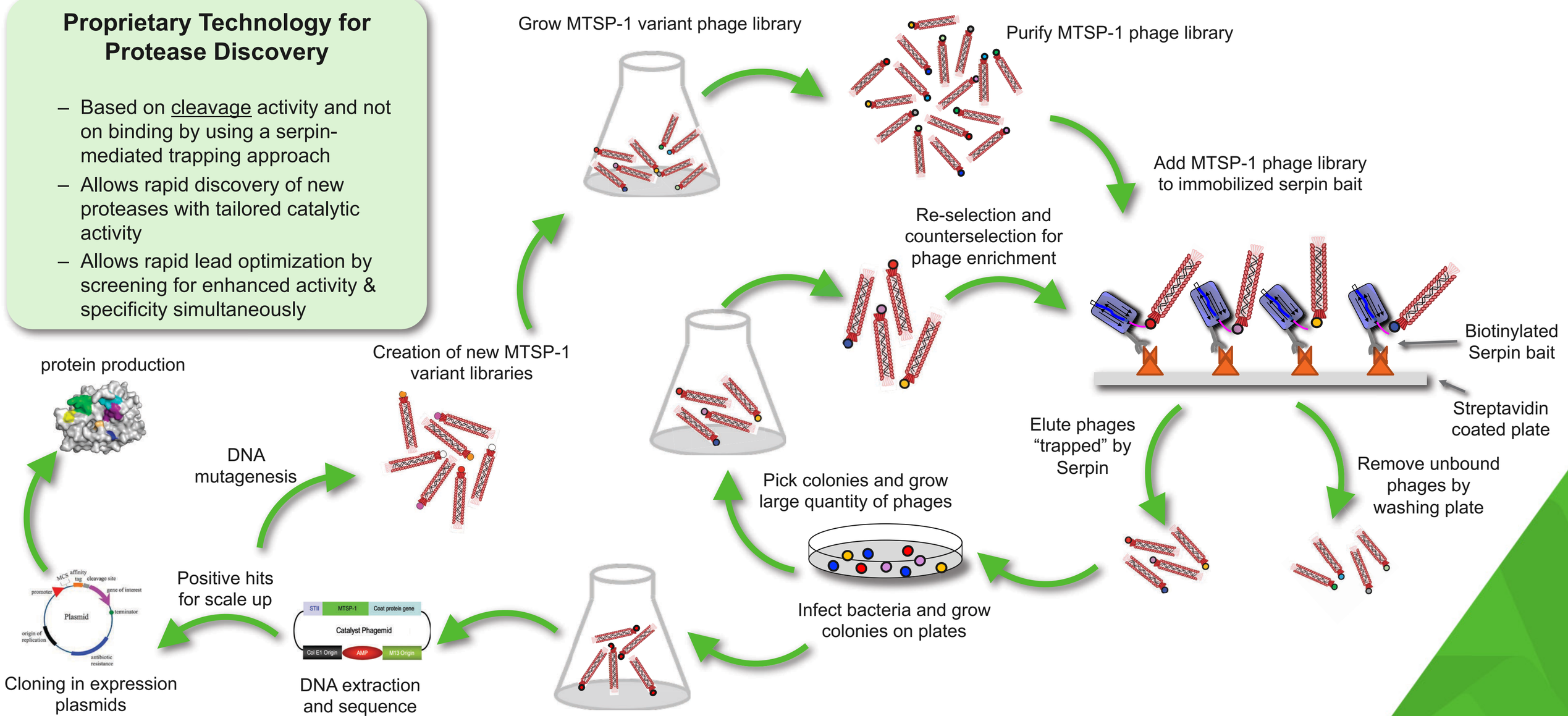


Catalyst Biosciences: Alterase™ Protease Platform

Nasdaq: CBIO

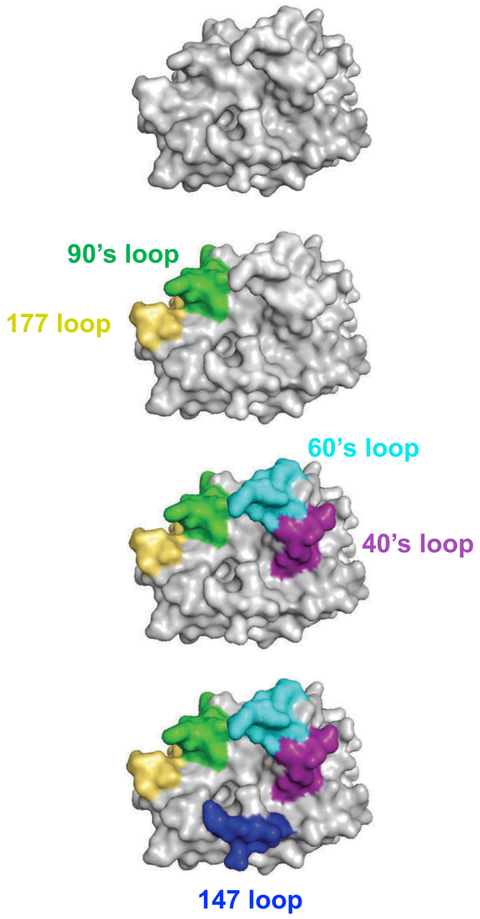
Proprietary Technology for Protease Discovery

- 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



Molecular evolution of CB 2782 for C3-specific cleavage

Nasdaq: CBIO



MTSP-1

CB 2013

CB 2426

CB 2470

CB 2782
(lead candidate)

Select ⇧ Catalysis

Human Plasma
(counter selection)

Antithrombin (AT)
(counterselection)

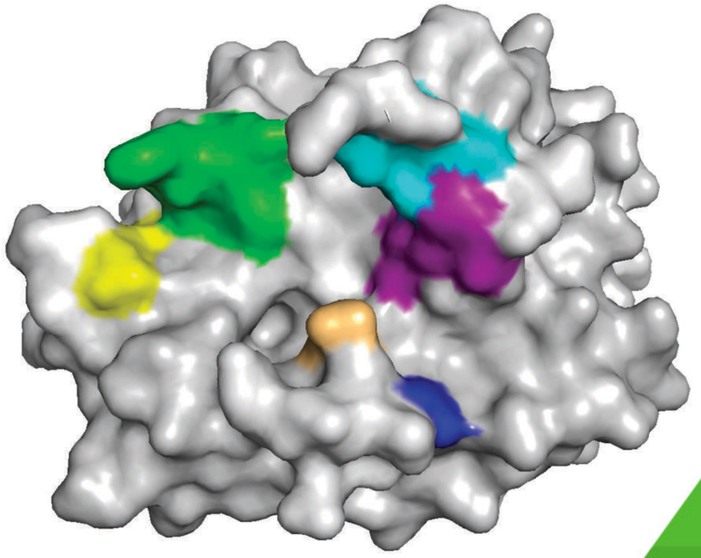
Directed Engineering

α_2-Macroglobulin
(counterselection)

Evolved Candidate

MTSP-1 mature	637	640	661	664	666	705	706		707	708	759	783	802
Chymotrypsin	38	41	60b	60e	60g	96	97	97a	98	99	151	175	192
MTSP-1	**Q**	**I**	**D**	**F**	**Y**	**D**	**F**		**T**	**F**	**G**	**Q**	**Q**
CB2013	Q	I	D	F	Y	D	T	E	G	L	G	L	E
CB2426	H	A	V	R	W	D	T	E	G	L	G	L	D
CB2470	H	A	V	R	W	D	T	E	G	L	N	L	D
CB2782	H	S	T	S	W	K	G	V	P	L	H	L	D

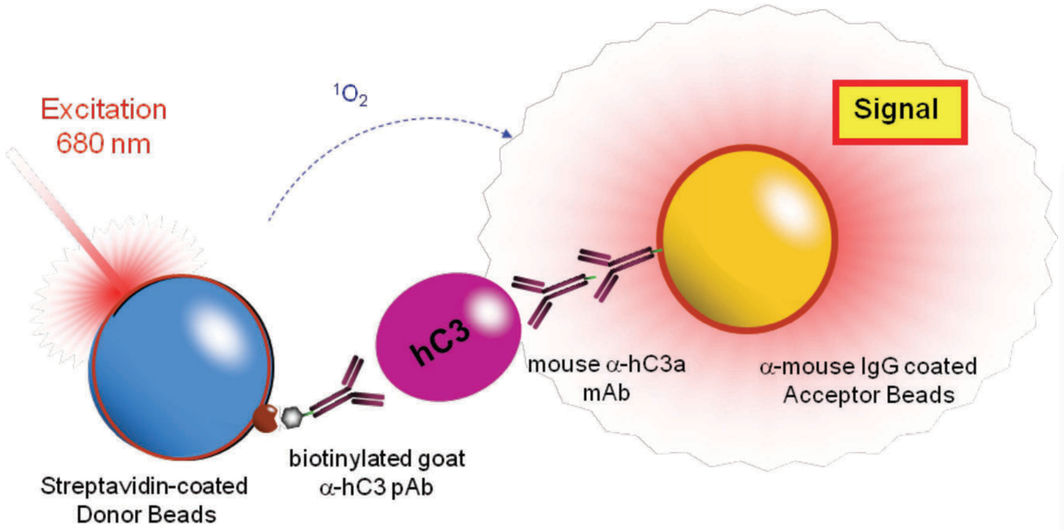
40's loop · 60's loop · 90's loop · 147 loop · 177 loop · S1 pocket

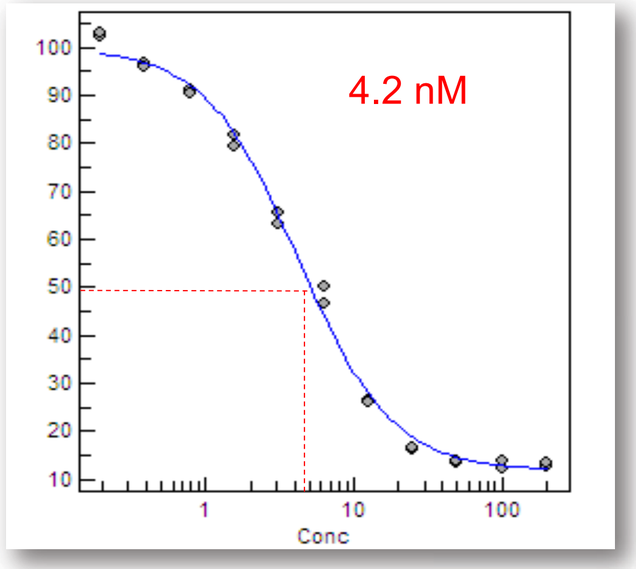


CB 2782

CB 2782 shows significant improvement in cleavage of C3

Nasdaq: CBIO

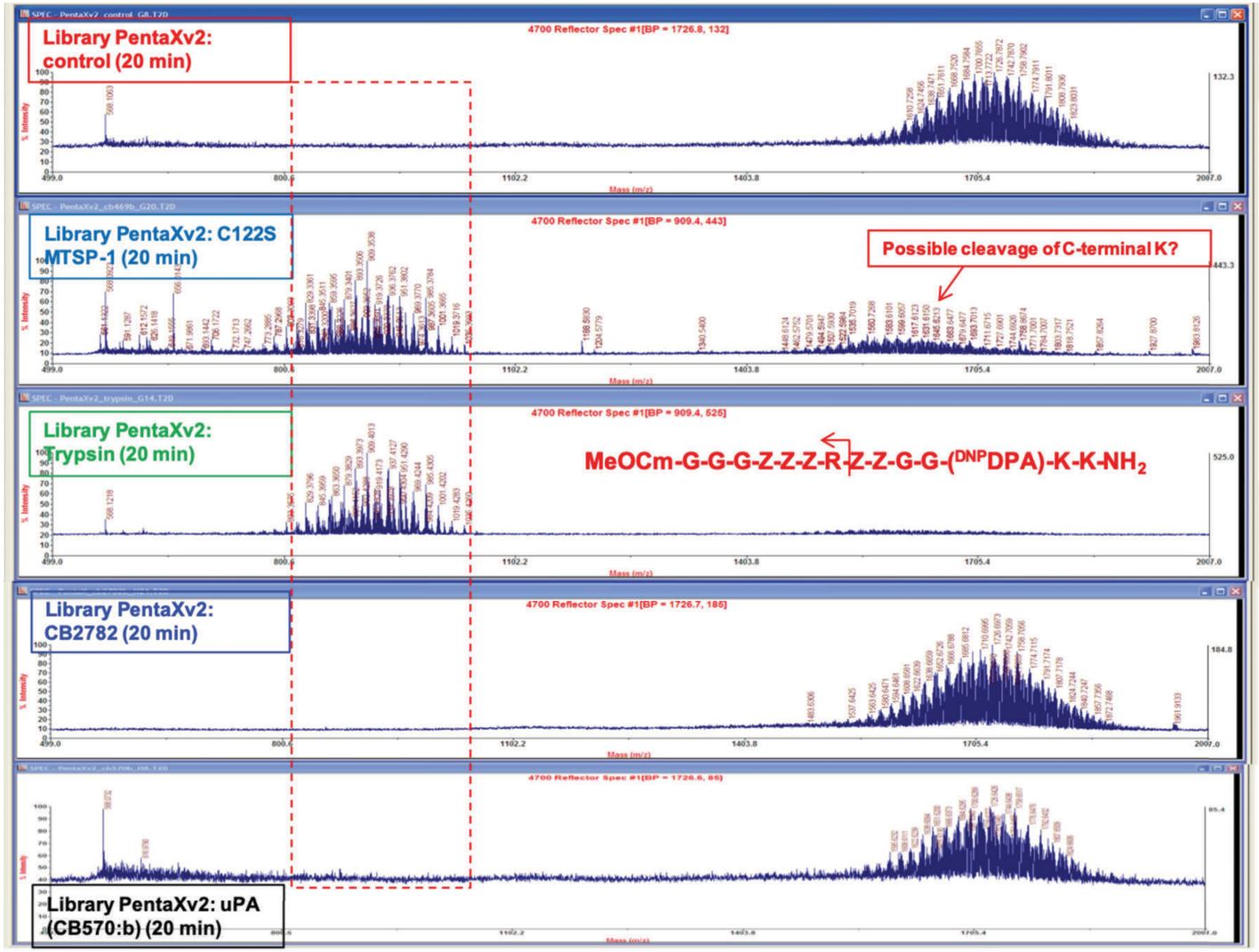




	MTSP-1 (nM)	CB 2782 (nM)	Ratio
Buffer	13.9	6.9	2
Human Plasma	2800	92	30
Cyno Plasma	3500	25	140

CB 2782 shows high specificity

Nasdaq: CBIO



Cleavage of PentaXv2 Library

P_4 P_3 P_2 P_1 $P_{1'}$ $P_{2'}$

▲-G - G - G - Y – Y – Y – R – Y – Y - G - G - ■ - K - K - NH_2

Y Any of 18 AAs (excluding R, C)

▲ N-terminal 7-methoxycoumarin-4-acetyl

■ dinitrophenyl-diaminopropyl

 # Peptides

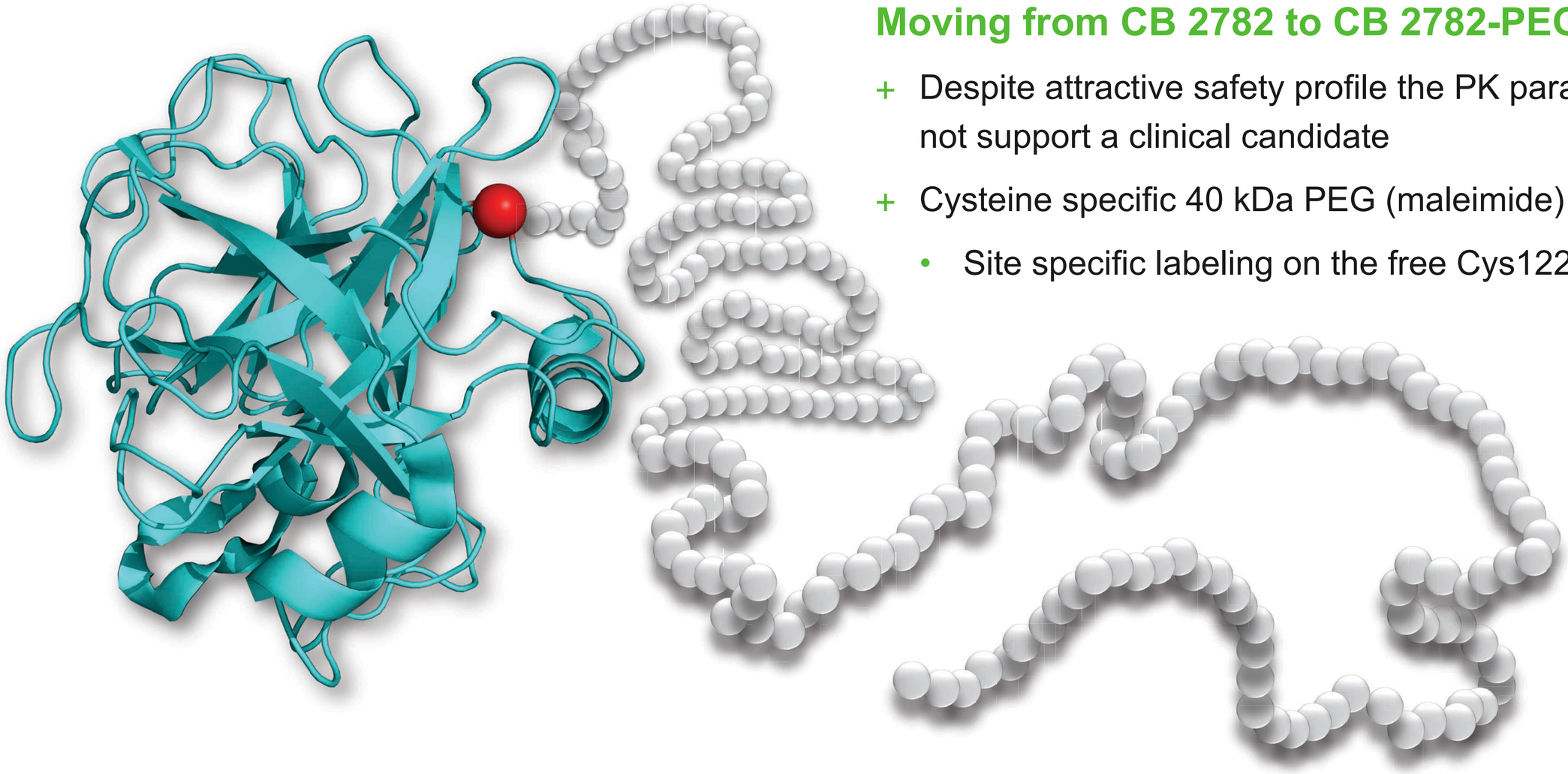
1,889,568 (18^5)

+ 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

Nasdaq: CBIO

Mosaic Biosciences



Moving from CB 2782 to CB 2782-PEG

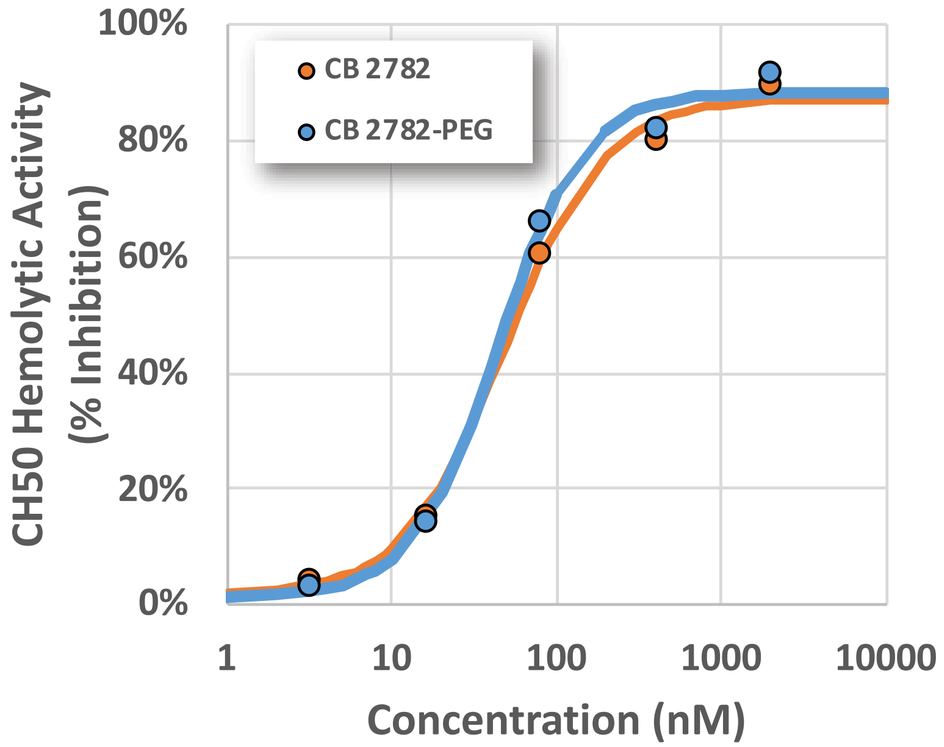
+ Despite attractive safety profile the PK parameters did not support a clinical candidate
+ Cysteine specific 40 kDa PEG (maleimide) conjugated
  - Site specific labeling on the free Cys122

CB 2782-PEG has indistinguishable activity *vs* CB 2782

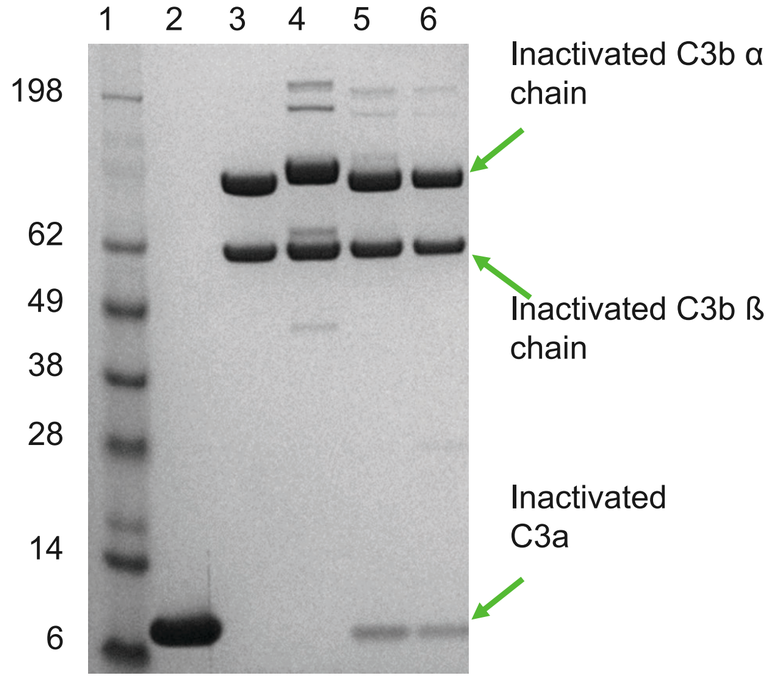
Nasdaq: CBIO

Mosaic Biosciences

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



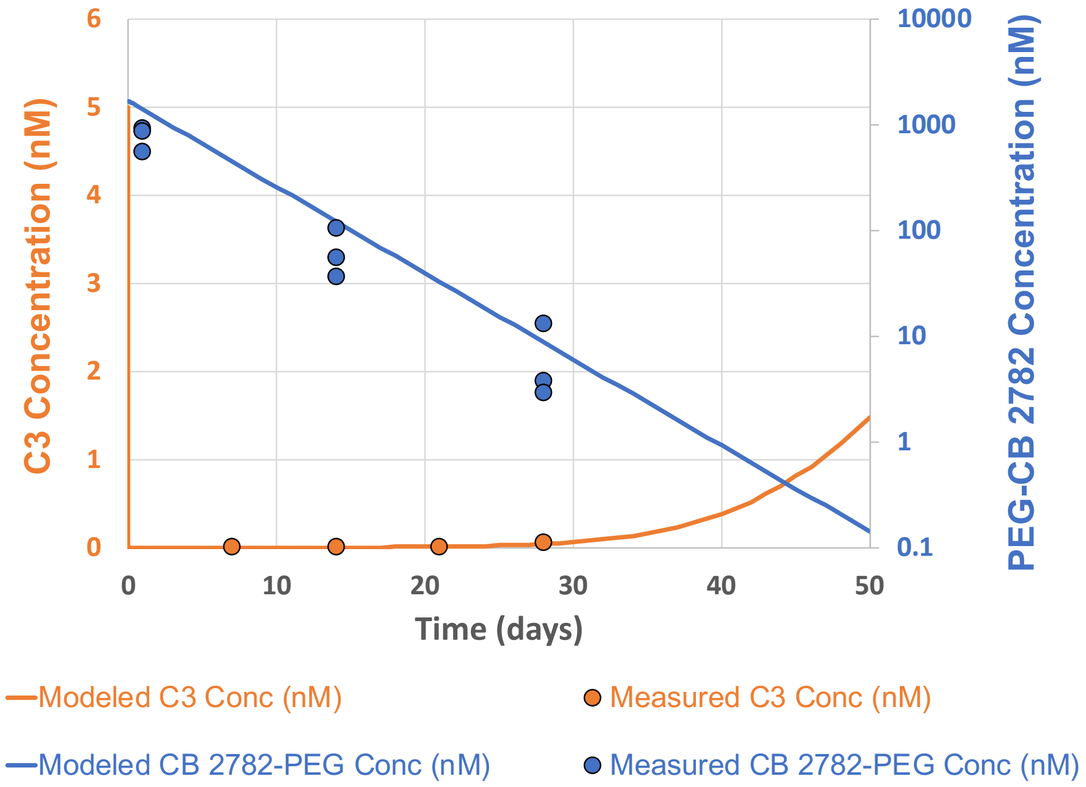
Reduced SDS-PAGE	
Lane	Sample
1	Ladder
2	C3a
3	C3b
4	C3
5	2 µM C3 treated with 0.2 µM CB 2782-PEG
6	2 µM C3 treated with 0.2 µM CB 2782

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

Nasdaq: CBIO

Mosaic Biosciences

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



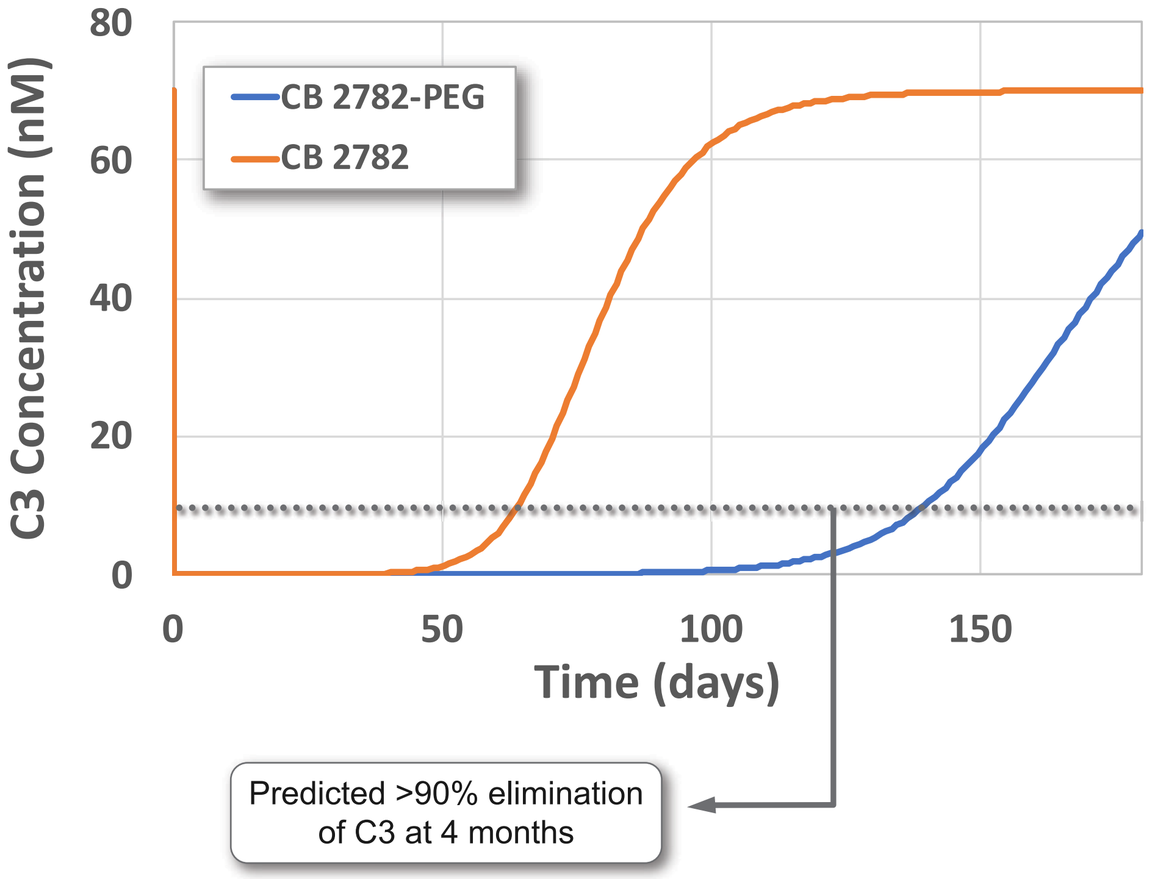
Parameter	CB 2782-PEG
t-half-terminal (d)	3.7
Mean residence time (d)	3.37
Cmax (µM)	0.90
Tmax (d)	1
AUC 0-inf (µM-d)	6.94
AUC 0-t (µM-d)	6.92

Predicted 2.0 mg human dose three to four times a year

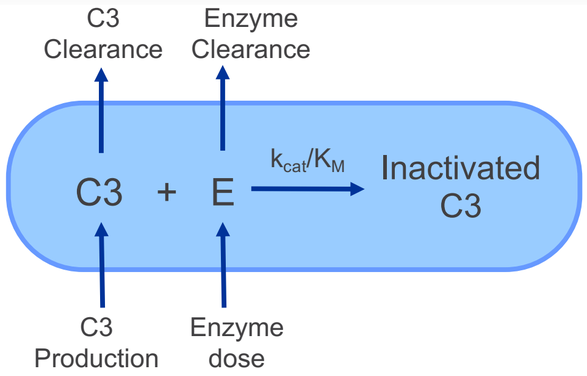
Nasdaq: CBIO

Mosaic Biosciences

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

The Catalyst Biosciences Team
Ed Madison
Vanessa Soros
Mikhail Popkov
Chris Thanos
Hoa Ly
Grant Blouse
Natacha LeMoan
And
Many Others

The Mosaic Biosciences Team
Eric Furfine
Marty Stanton
Matt Traylor