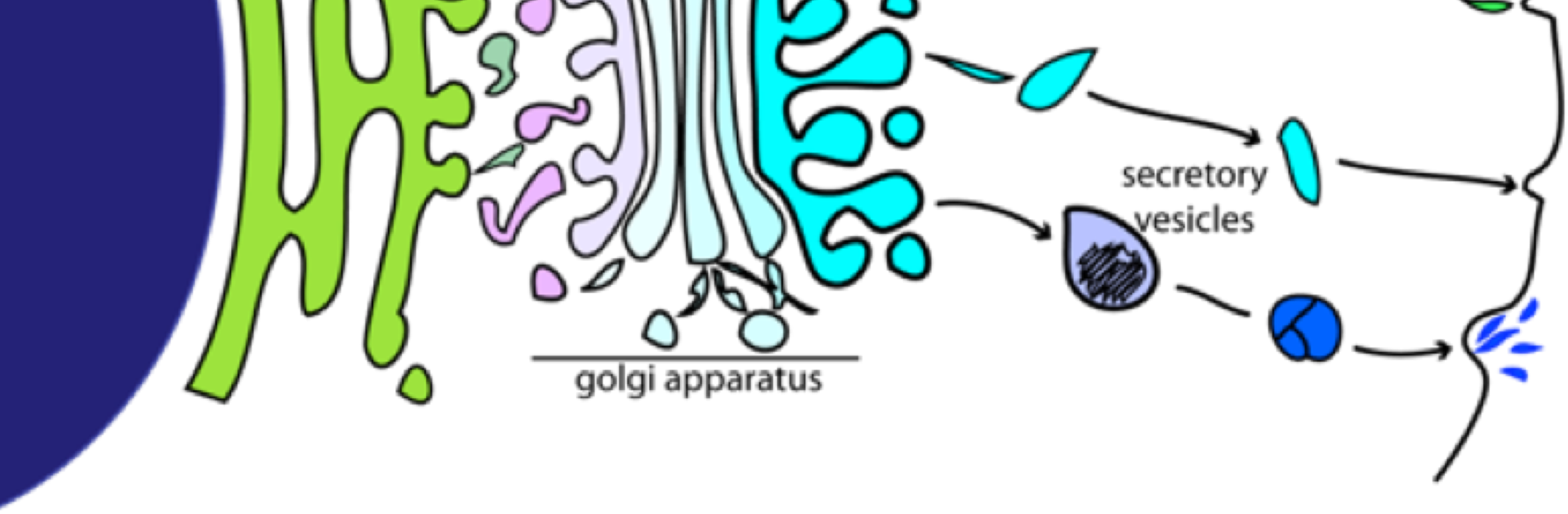


Cell Permeable Miniature Proteins

Delivering Curative Therapies

Alanna Schepartz
Sterling Professor
Department of Chemistry

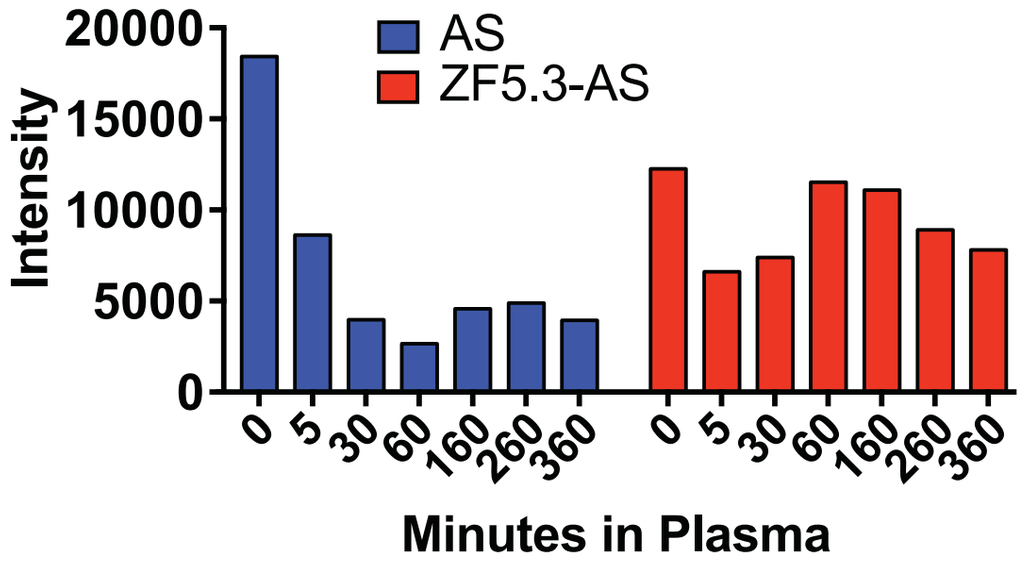
BLAVATNIK FUND
Yale Innovation

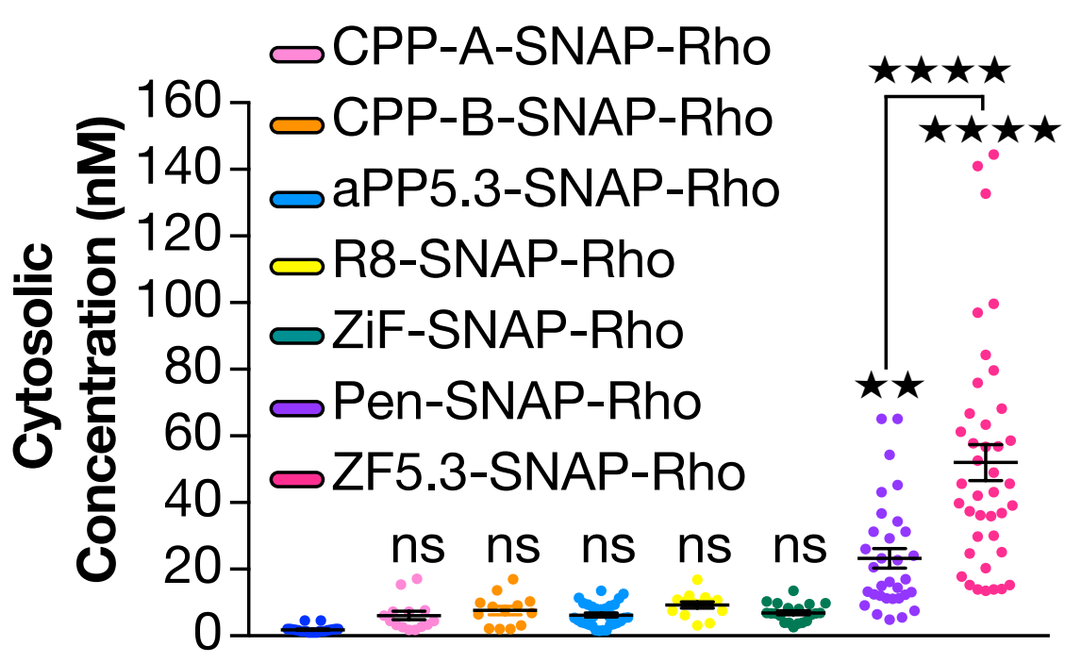
Exolva Therapeutics

Cell Permeable Miniature Proteins

- Reach cytosol and nucleus with efficiencies as high as 75%
- Efficiency higher than all other ‘CPPs’ tested, with or without cargo
- Multiple cell types (SK-Hep1)
- Deliver active payloads 10 – 32 kD
- High and tunable serum stability
- Non-toxic; genetically encodable; easily manufactured
- Key difference: CPMPs utilize well-defined, non-destructive mechanism;
- Key difference: CPMP increases plasma stability of protein cargo
- Fundamental patents and applications covering both scaffold and delivery

CPMP improves plasma stability





CPMP still better even with cargo attached

Using a CPMP to deliver AS and correct an ‘inborn error of metabolism’: CTLN-1

The Problem:

- Type 1 citrullenemia is an **incurable disease**
- Results from deficiency or absence of the urea cycle enzyme **argininosuccinate synthetase** (AS)
- Severe AS deficiency results in **hyper-ammonemia and irreversible neurological damage, coma, or death**

The Opportunity:

- 1:57,000 affected worldwide
- No disease modifying therapy
- Mutations suggests activity >10% would be disease-modifying
- @ $350K/patient/year (based on current ERT costs), 15% treated = $1B/year
- Success validates platform for therapeutic indications where delivery to cytosol is critical

Reminder: Favorable competitive landscape

		Cure disease	Deliver enzyme	Broad scope	High stability	Non-toxic	High delivery efficiency
CTLNI treatments	Buphenyl® or Ravicti®	**x**	**x**	**x**	–	–	–
	Shire, PhaseRx		**x**	**x**	**x**	–	**x**
	SynLogic			**x**	–	–	–
platforms	Aileron, Bicycle	**x**	**x**	**x**			
	TrojanTech, Xigen, Portage, Cellivery, Chondrial				**x**	**x**	**x**
	Cell-permeable miniature proteins						

Recipient of Blavatnik I and 1st Place PITCH Contest: Accomplishments

Objective 1

- Express CPMP-AS fusions/controls
- Evaluate uptake in SK-HEP cells
- Monitor reversal of AS deficiency
- **Milestone:** Overexpression /purification; enhanced uptake; AS deficiency reversal **Q3-4 2017**

Objective 2

- Establish *in vitro* PK and metabolism of CPMP-AS fusions
- Evaluate plasma stability, protein binding
- **Milestone:** acceptable stability ($t_{1/2}$ > 30 min) **Q1 2018**

Objective 3

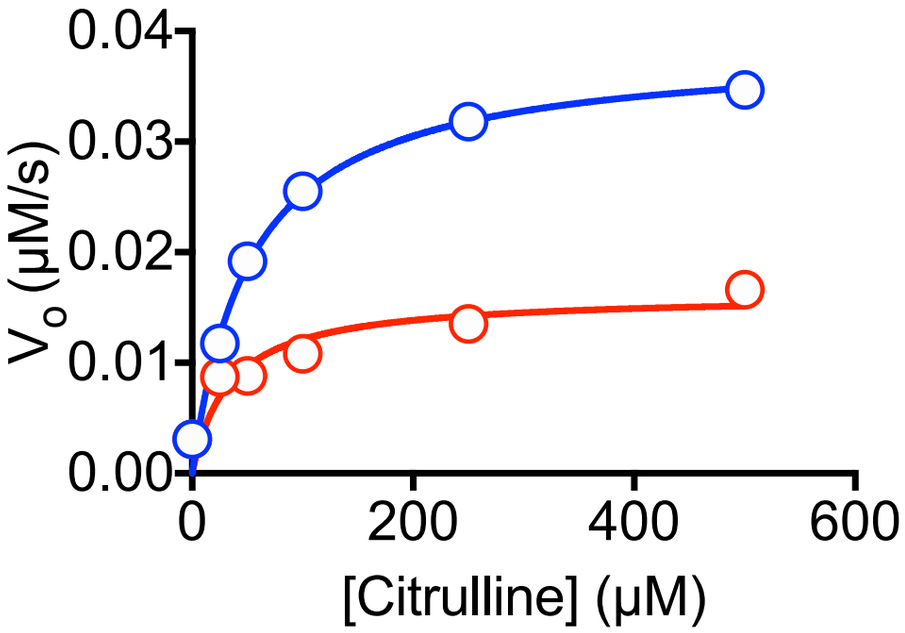
- Establish *in vivo* PK and bio-distribution of C57BL/6 CPMP-AS fusion and control mouse models
- **Milestone:** presence in plasma; acceptable distribution liver **Q3 2018**

Objective 4

- Evaluate CPMP-AS fusion in *fold* mouse model of human CTLN
- Assess lifespan, weight, length, coat density, plasma NH3, citrulline
- **Milestone:** Demonstrate the a CPMP can deliver an active enzyme in an animal to reverse a serious metabolic disease **Q2 2019**

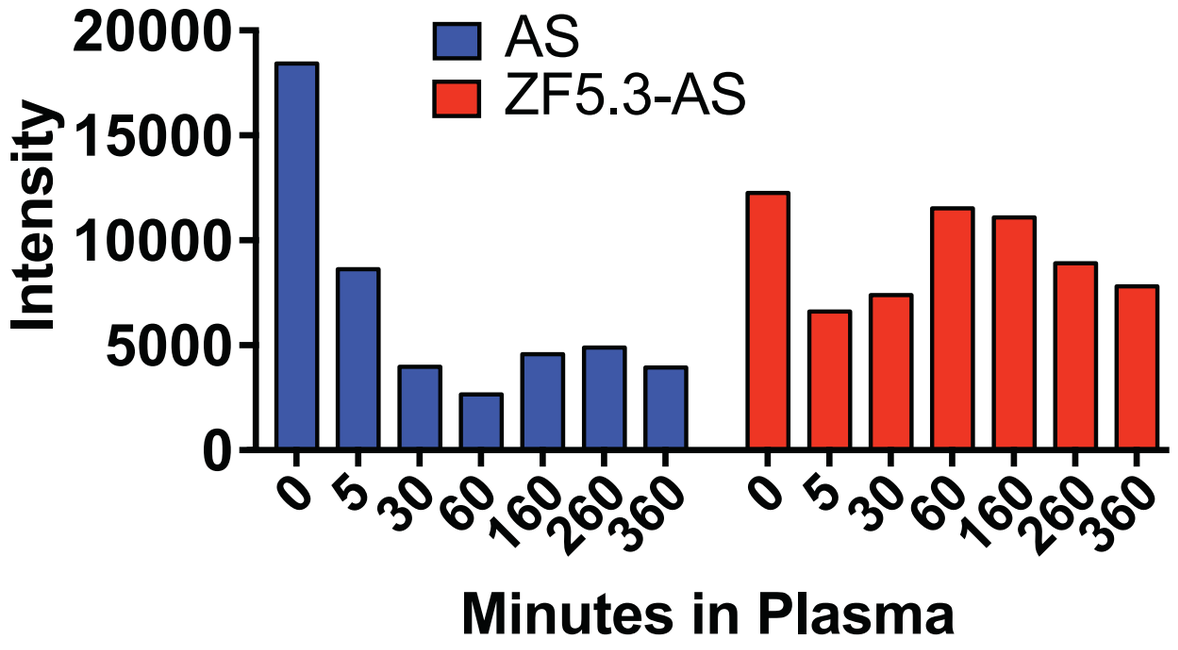
Progress: *in vitro* activity preserved; *in cellulo* stability enhanced

ZF5.3-AS retains catalytic activity



Kinetic Param	AS	ZF5.3-AS
K_M (μM)	52.54 ± 2.674	33.36 ± 13.77
k_{cat} (s^{-1})	0.3336 ± 0.0004	0.1400 ± 0.0001

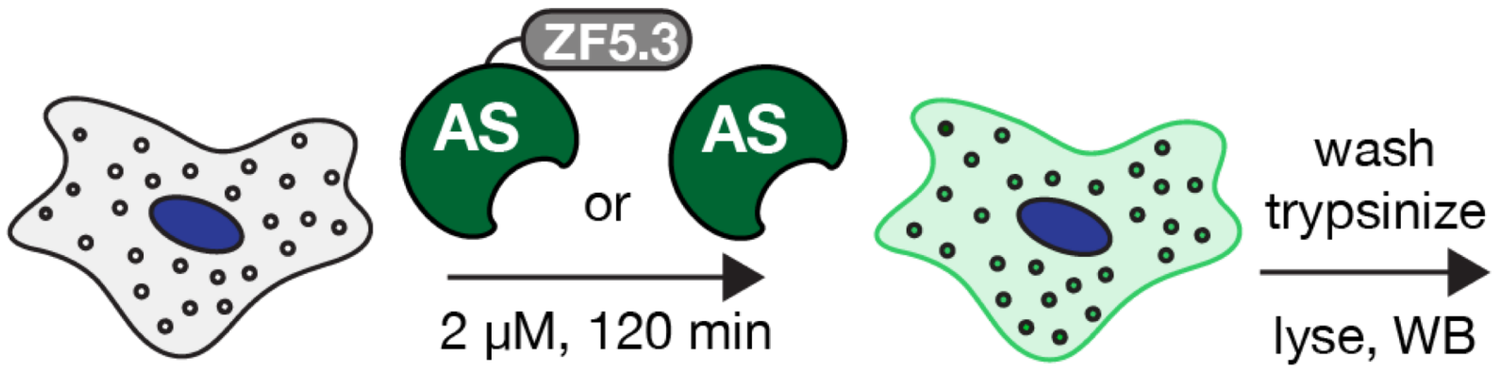
ZF5.3 improves plasma stability of enzyme

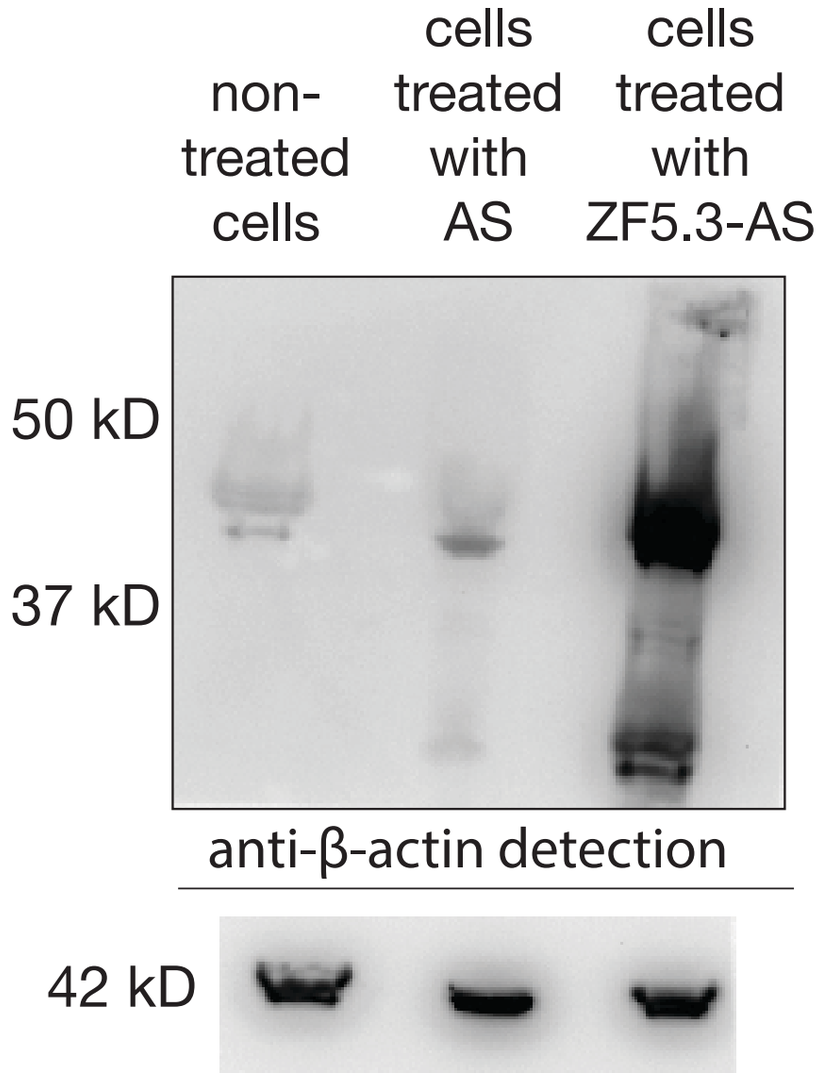


Little or no degradation of ZF5.3-AS after 6 h in plasma

Also: Little or no plasma protein binding detected by SPR

Progress: ZF5.3-AS can be delivered to SK-Hep1 cells





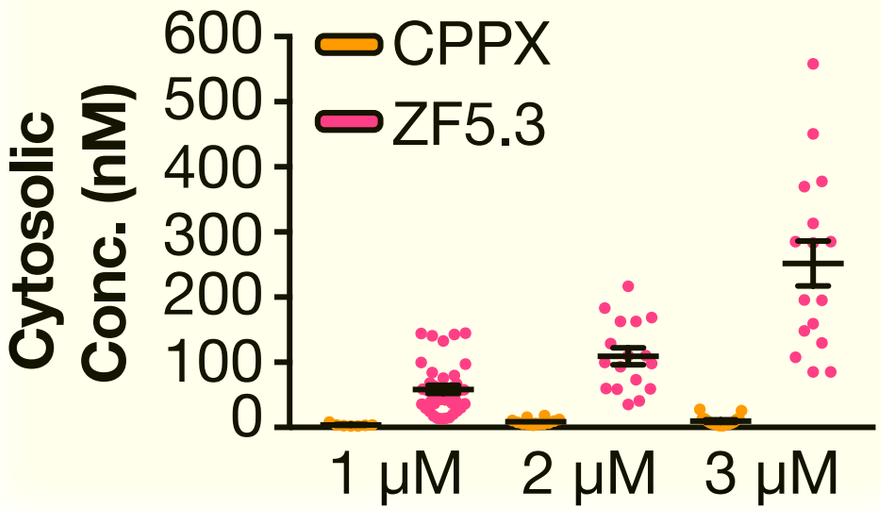
In progress (data by 5/8)

(1) Determine cytosolic concentration

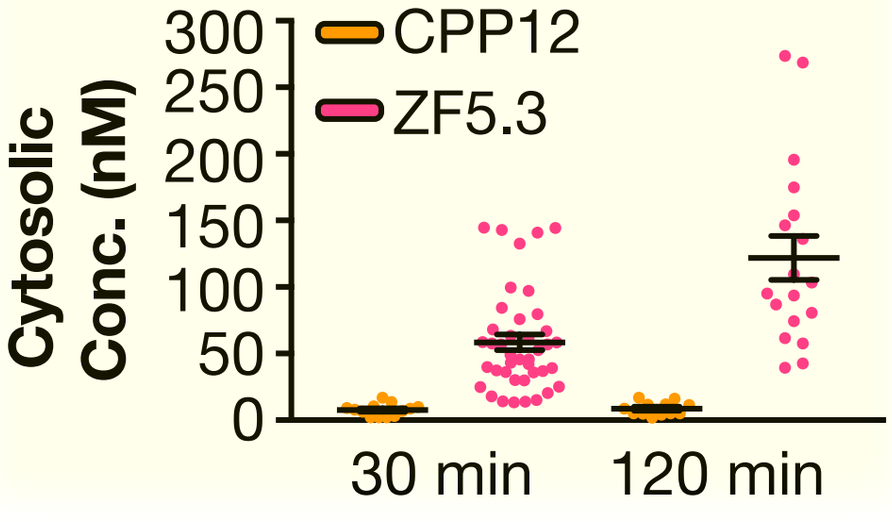
(2) Demonstrate phenotype reversal in SK-Hep1 cells

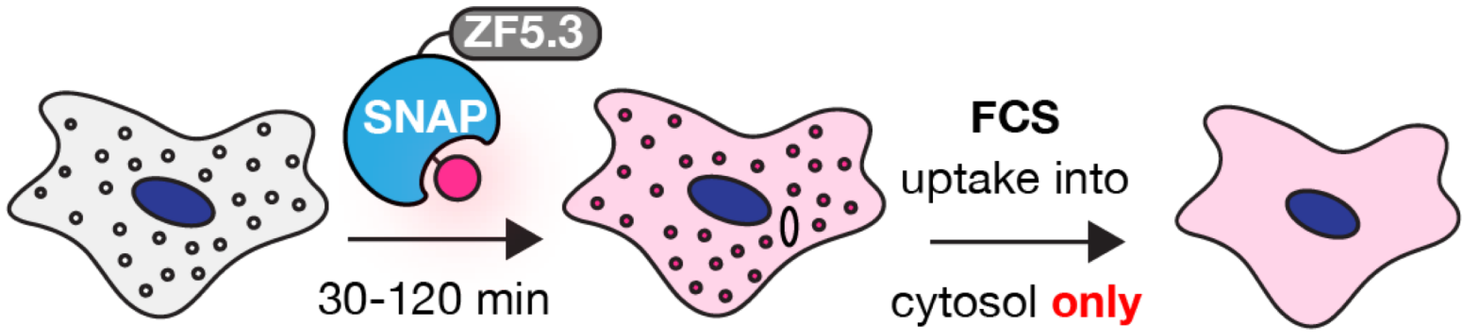
ZF5.3 remains a superior delivery vehicle even when cargo is attached

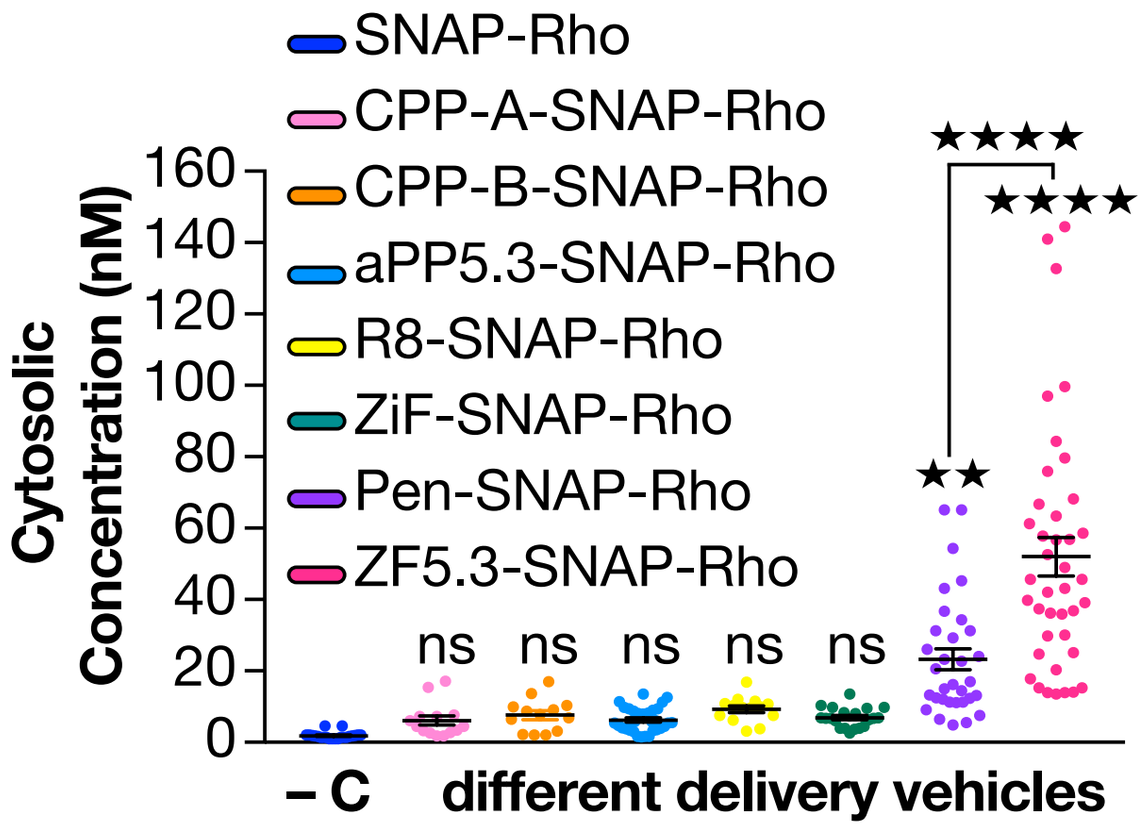
Clear evidence of dose-response



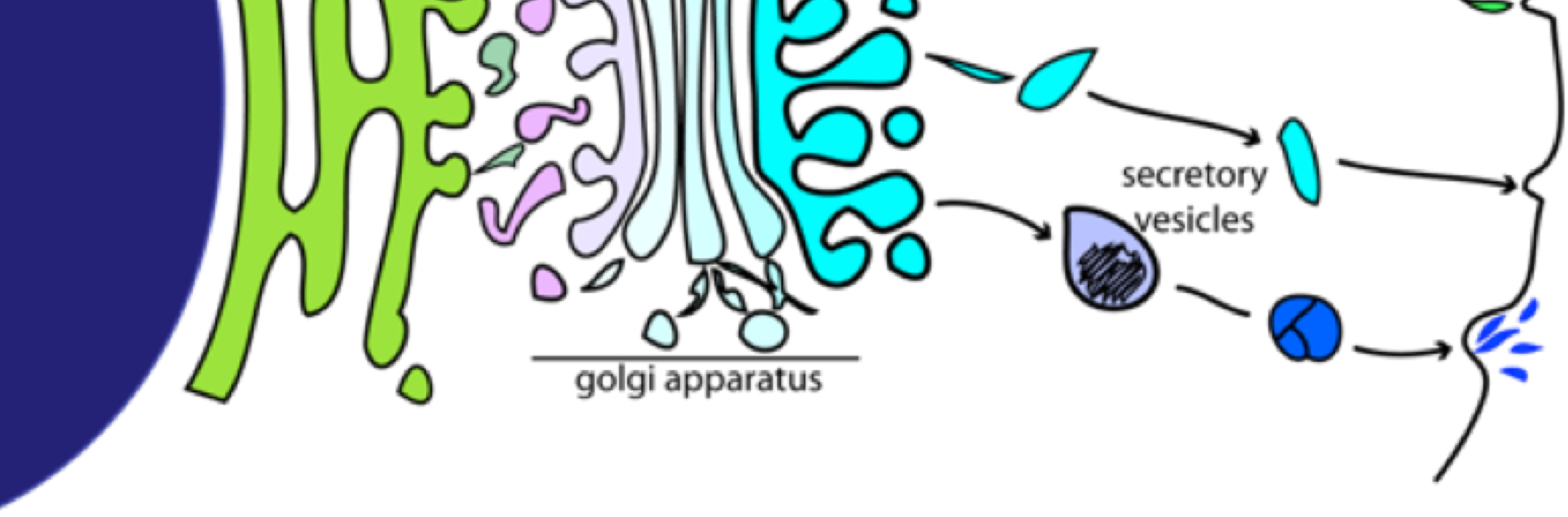
Longer times are better







ZF5.3 still superior even with cargo attached.



Cell permeable miniature proteins

Blavatnik 2: Establishing a Platform Technology With Broad Scientific Application

Orphan diseases: inborn errors of metabolism

Gene editing tools ✓

Nuclear transcription factors

Protein-protein interaction inhibitors ✓

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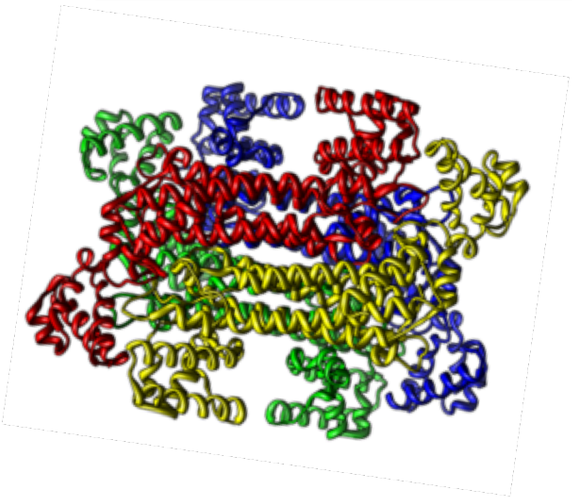
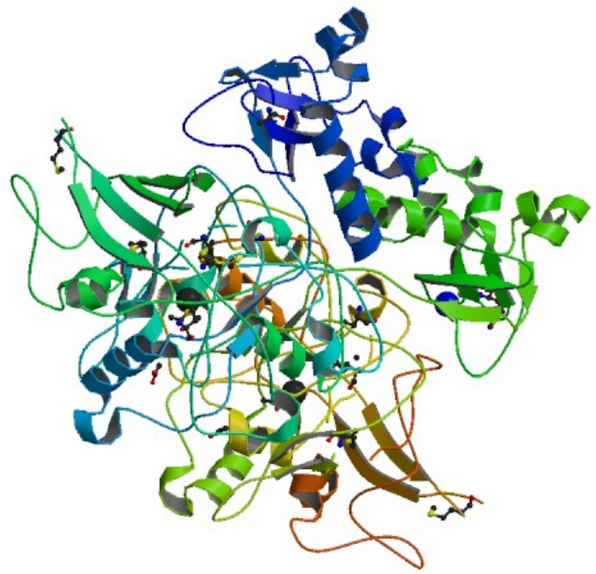
Treating Inborn Errors of Metabolism:
Disease Targets for CPMP Platform



Enzyme: Arginino-succinate lyase (ASL)
Disease: Arginino-succinic Aciduria (urea cycle disorder)

- Tetramer of 49 kD subunits
- Expressed in *E. coli* with N-terminal tag
- Active in the liver cytosol
- Incidence: 1 in 70,000 live births
- No disease-modifying therapy
- No current clinical trials for a DMT
- Primary pharmacotherapy is NaPhenylbutyrate sales 20.8M in 2017
- ASL hypomorphic mouse model exists
- Workflow benefits from experience during Blavatnik I

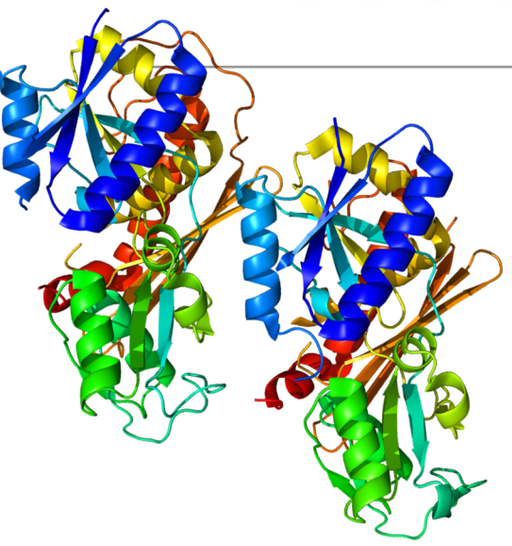


Enzyme: Fumaryl-acetoacetate hydrolase (FAH)
Disease: Tyrosenemia type I (liver disease)

- Lack of FAH leads to toxic elevated levels of fumaryl-acetoacetate, tyrosine and byproducts
- Homodimer of 46 kD; neutral surface
- Expressed in *E. coli* with N-terminal tag.
- Active in liver and kidney cytosol
- Incidence: 1 in 12,000 - 100,000 live births
- One treatment available: nitisinone (sales 2017 $100M); **many bad side effects**
- Two mouse models exist; good in vitro assay
- Cargo is among the smallest and carries no cofactors other than M(II)

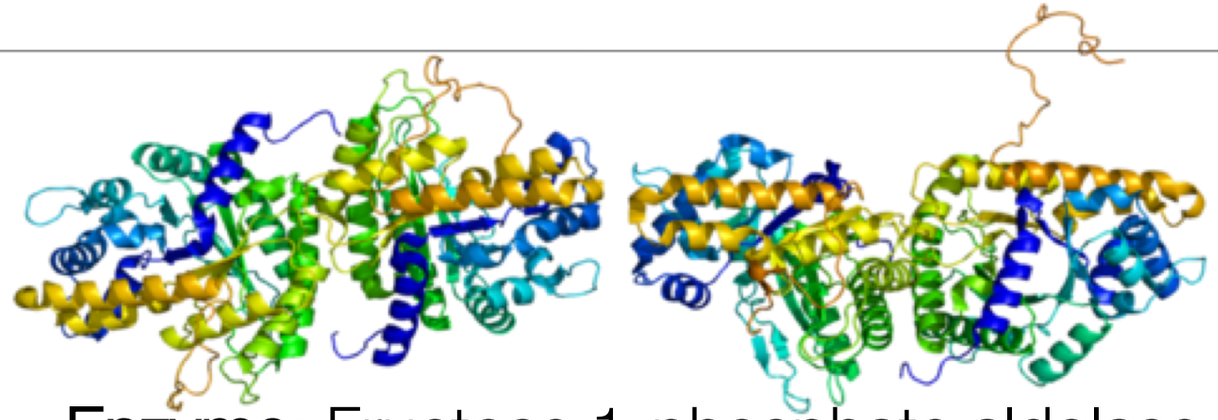
Treating Inborn Errors of Metabolism:

Disease Targets for CPMP Platform



Enzyme: Porpho-bilinogen deaminase
Disease: acute intermittent porphyria (acute abdominal pain)

- Dimer of 42 kD subunits
- Active in liver cytosol
- Incidence: 1 in 10-20,000
- Mouse model available
- Only treatment is Panhematin® (lyophilized heme)
- Delivery of recombinant protein (Porphozy) decreased porphobilinogen for 2 hours
- RNAi approach in clinical trials (Alnylam)
- Carries bound cofactor but can be expressed in bacteria



Enzyme: Fructose 1-phosphate aldolase, isozyme B (ALDOB) **Disease**: hereditary fructose intolerance (HFI) (liver and renal disease)

- Catalyzes cleavage of fructose-1-phosphate to form dihydroxyacetone phosphate and D-glyceraldehyde
- No disease-modifying therapy available; no clinical trials identified
- Incidence: 1 in 10-20,000
- Active in the liver and kidney cytosol.
- Homotetramer of 36 kDa subunits, neutral charge
- Good in vitro assay available
- A mouse model homozygous null for the ALDOB orthologue, Aldo2, recapitulates symptoms of HF.

Plans for Blavatnik 2: Four shots on goal

Objective 1: Q3-4 2018

- Overexpress/purify all four CPMP-enzyme conjugates
- Evaluate activity in vitro
- Evaluate/compare extent of cytosolic trafficking
- Move forward all target effectively

Yale costs < 50K

Objective 2: Q1-2 2019

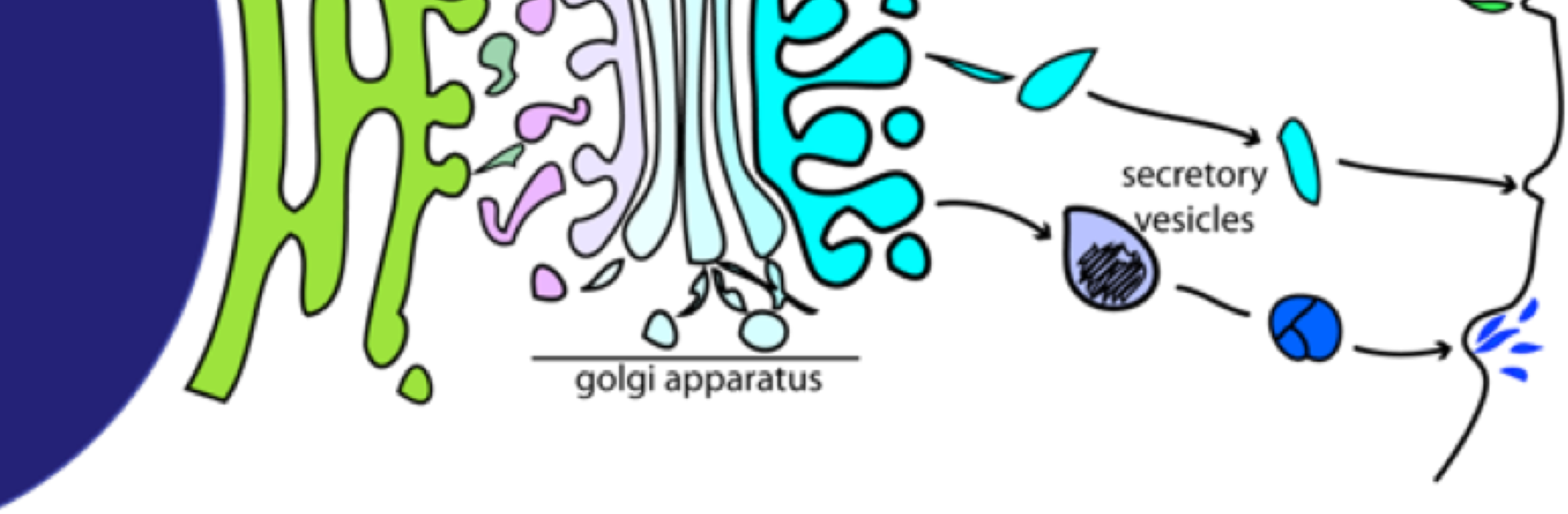
- Establish *in vitro* PK and metabolism of CPMP-enzyme fusions
- Evaluate plasma stability
- Move forward 2-3 targets with high stability/low plasma protein binding

Objective 3: Q3-4 2019

- Establish *in vivo* PK and bio-distribution of CPMP-enzyme fusions in appropriate mouse models
- Move one target forward to establish efficacy in animal

Objective 4 : Q1-2 2020

- Demonstrate CPMPs can deliver an active enzyme to the cytosol of appropriate mouse model to reverse the effects of a serious metabolic disease
- Perform initial non-GLP clinical tox assessment



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