Targeting the Immunoproteasome

Dustin McMinn

CDD Community Meeting
April 4, 2014

Disclosures

I am an employee of Onyx, an Amgen subsidiary.

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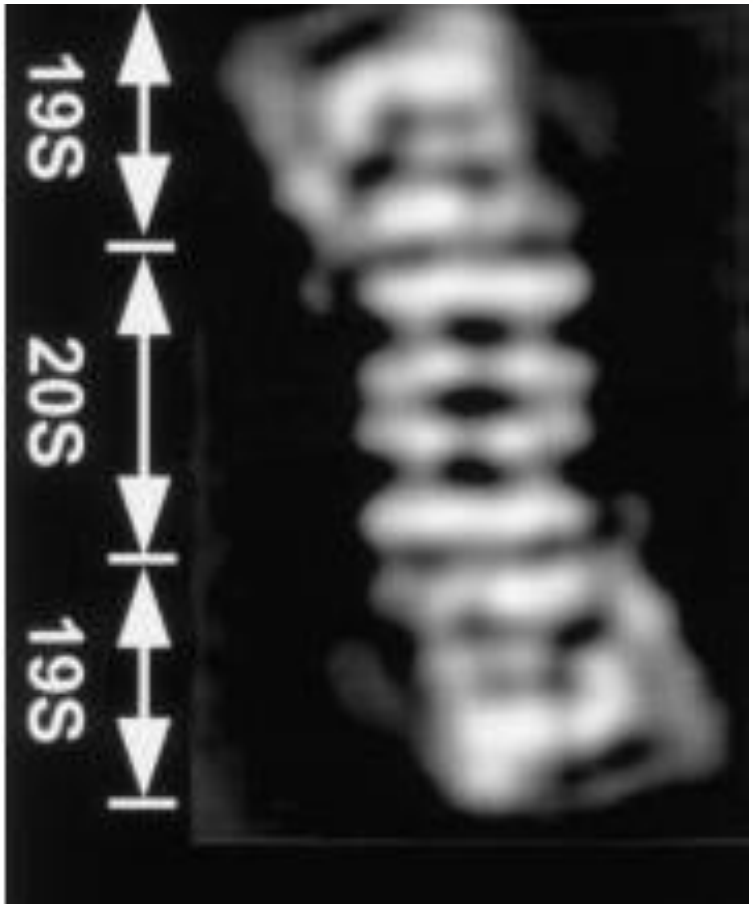
Agenda

- Overview of immunoproteasome as a therapeutic target
- Structural basis for selectivity of ONX 0914
- Structural basis for enhanced selectivity for next generation immunoproteasome inhibitors (iPI's)

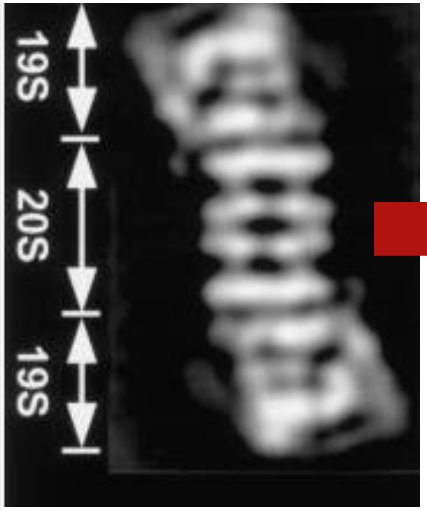
Proteasomes



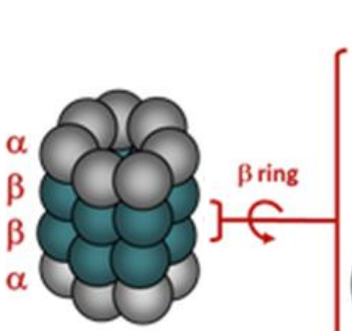
- 2000kDa mega-complexes
- Proteasomes degrade excess/damage "marked" (ubiquitinated) protein
- 19S regulatory particle mediates identification/entrance
- 20S core particle mediates catalytic protein cleavage

J. Struct. Biol. **1998**, *121*, 19.

Catalytic Subunits of the Constitutive- and Immunoproteasomes

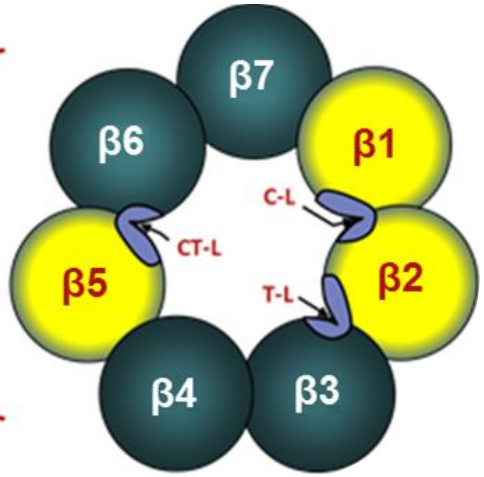


J. Struct. Biol. **1998**, *121*, 19.



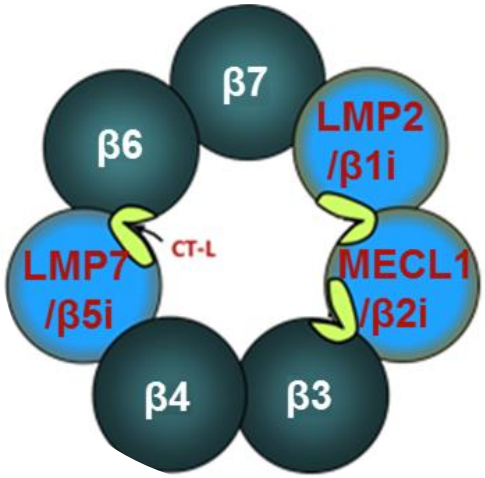
Constitutive Proteasome

Ubiquitous
(e.g. Heart, Kidney, Liver)



Immunoproteasome

Immune System
(e.g. Monocytes, T/B - cells)



Targets of Anti-Cancer Agents

Bortezomib, carlfilzomib target β5 & β5i

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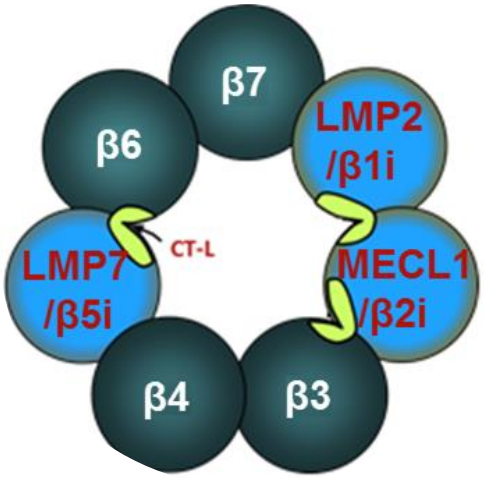
Specialized Roles of Immunoproteasome

Immunoproteasome

Immune System
(e.g. Monocytes, T/B - cells)



- Antigen presentation, T cell proliferation/survival, regulation of cytokine production
- Induced by inflammatory cytokines (e.g. IFN-γ)
- Increased expression in psoriasis, RA, MS, Crohn's , Sjogren's…

Nat. Rev. Immunol. **2010**, *10*, 73.

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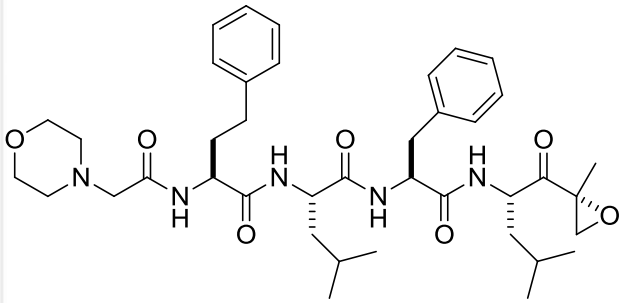
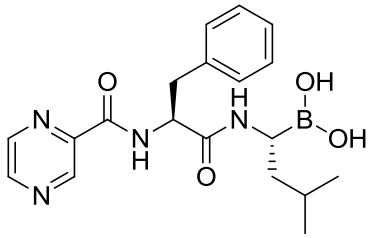
Selective vs. Combined Inhibition of β5 and β5i

- CFZ and BTZ target both β5 and β5i, multiple myeloma
- Leukocytes predominantly express β5i
- Malignant transformed leukocytes express both β5 and β5i



Carfilzomib - β5/β5i non-selective

Selectivity (c20s/i20s) = ~1

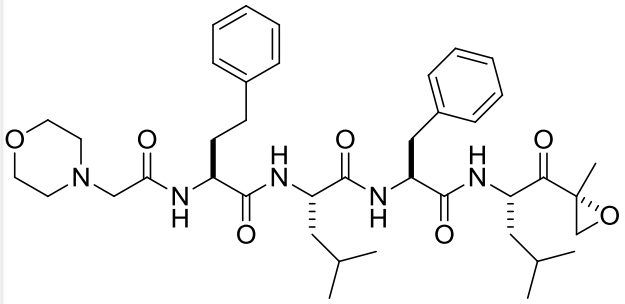


Bortezomib - β5/β5i non-selective

Selectivity (c20s/i20s) = ~1

Selective vs. Combined Inhibition of β5 and β5i

- CFZ and BTZ target both β5 and β5i, multiple myeloma
- Leukocytes predominantly express β5i
- Malignant transformed leukocytes express both β5 and β5i
- Selective inhibitors of β5i and β5 obtained
- Effect on cell viability and caspase 3/7 activation in myeloma cells upon independent and combined selective inhibitor treatment was determined



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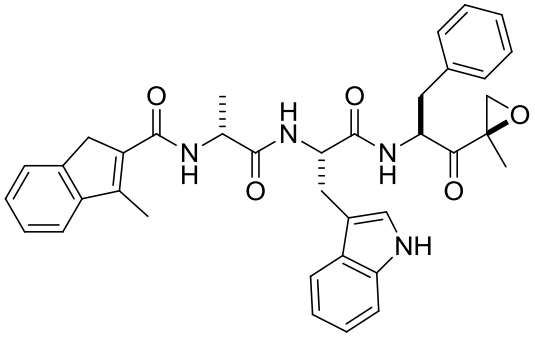
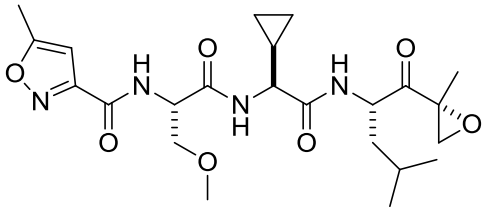


PR-924 - β5i selective

Selectivity (c20s/i20s) = 52

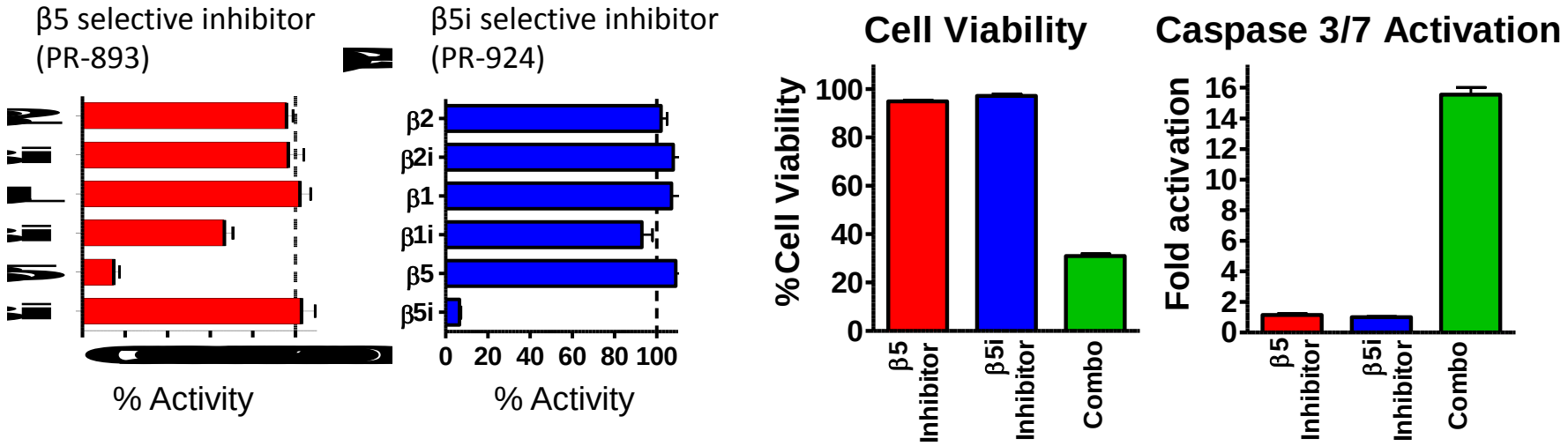


PR-893 - β5 selective

Selectivity (i20s/c20s) = 16

Effect on Myeloma Cell Death and Caspase 3/7 Activation

- Selective inhibition has no effect on MM1.S viability
- Dual inhibition sufficiently drives cell death and caspase 3/7 activation
- Potential therapeutic window for selective inhibition of the immunoproteasome



Blood. **2009**, *114*, 3439.

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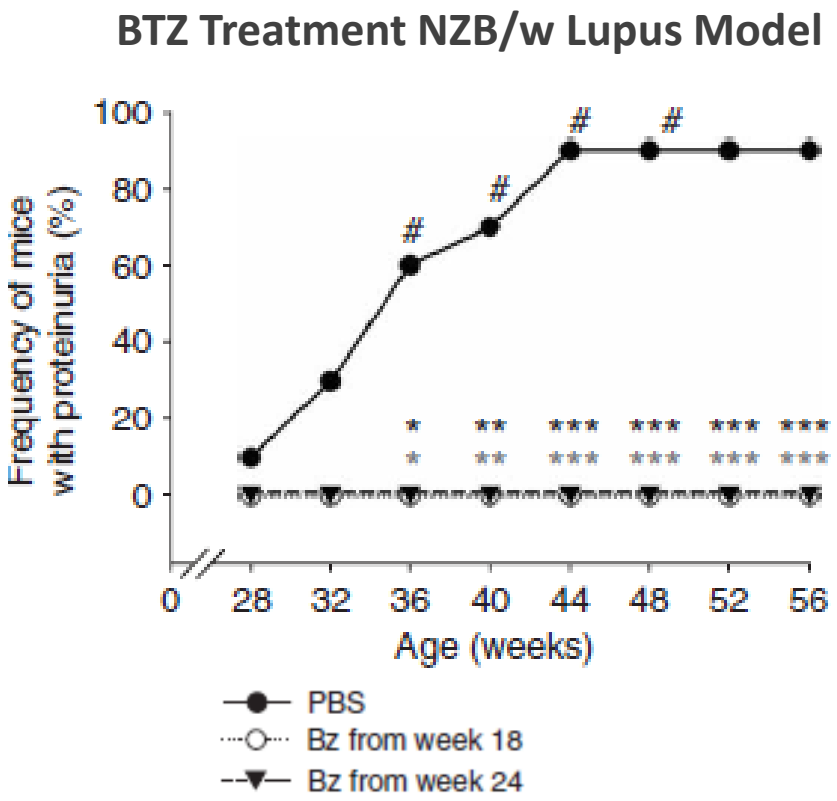
Proteasome Inhibition Toward Autoimmune Disease: A Potential Therapy for Lupus



- Bortezomib blocks disease progression in a clinically relevant mouse model of lupus (SLE)
  - ↓Long lived plasma cells
  - ↓Proteinuria
  - ↓Anti-ds DNA autoantibody

Neubert et al, *Nat Med* 2008

Proteasome Inhibition toward Autoimmune Disease: A Potential Therapy for Lupus

SAT0203

SUCCESSFUL TREATMENT OF REFRACTORY SLE PATIENTS WITH THE PROTEASOME INHIBITOR BORTEZOMIB – A CASE SERIES

R. E. Voll [1, 2,*], T. Alexander [3, 4], R. Peukert [1], A. Rubbert [5], J. Rech [1], T. Braun [1], M. Wiesener [6], K.-U. Eckardt [6], B. Hoyer [3, 4], A. Taddeo [3], A. Reisch [1], G.-R. Burmester [3, 4], A. Radbruch [3], G. Schett [1], F. Hiepe [3, 4]

[1]Rheumatology and Clinical Immunlogy, University Erlangen-Nürnberg, Erlangen, [2]Rheumatology and Clinical Immunlogy, University Medical Center Freiburg, Freiburg, [3]Deutsches Rheumaforschungszentrum, [4]Rheumatology, Charite University Medicine, Berlin, [5]Hemato-oncology, Section Rheumatology, University Cologne, Cologne, [6]Nephrology, University Erlangen-Nürnberg, Erlangen, Germany

Background: Long-lived plasma cells secreting pathogenic autoantibodies may contribute to refractory disease courses of SLE, because long-lived plasma cells are resistant to conventional therapies (1). We showed that the proteasome inhibitor bortezomib, which is approved for the treatment of relapsed multiple myeloma, eliminates plasma cells including long-lived ones and ameliorates lupus nephritis in mouse models of SLE (2).

Objectives: We collected data of all refractory SLE patients treated with bortezomib in our clinics to investigate the efficacy and potential side effects of bortezomib treatment in a case series.

Methods: At 3 university centers we treated 13 patients with bortezomib, who had not sufficiently responded to tr did not tolerate conventional drugs. The patients had given informed consent to off-label treatment before they received bortezomib intravenously at a dose of 1.3 mg/m^2 body surface at day 1, 4 and 8, some additionally at day 11. Most patients also received 20 mg of dexamethasone together with bortezomib. Treatment cycles were repeated up to four times with an time interval of usually 10 to 14 days in-between cycles. The following clinical and laboratory parameters were monitored: SLEDAI, urine sediment, circulating plasma cells, 24 hour-proteinuria, creatinine clearance, complement C3 and C4, antibodies to double-stranded (ds) DNA and ENA, vaccine antibody titers to hepatits B surface antigen and tetanus toxoid.

Results: No serious side effects were observed upon bortezomib treatment. One patient experienced myalgias, fever and headache next day after the first 3 bortezomib applications. Three of seven patients who were treated with 4 bortezomib injections per cycle developed polyneuropathies, which were reversible upon discontinuation of treatment. One patient developed a reversible thrombocytopenia after 4 treatment cycles, no other relevant hematologic toxicities were observed. The disease activity score SLEDAI and anti-dsDNA antibody titers significantly decreased in all patients, in a few patients anti-dsDNA nearly disappeared, whereas ENAs were decreased by up to 50% only. In general, complement levels increased. In all patients with active lupus nephritis, proteinuria declined within 6 weeks of treatment; one patient reached normal protein excretion within 4 months. Protective vaccine antibody titers to hepatitis B surface antigen and tetanus toxoid decreased, but remained within the protective range. Total IgG levels slightly declined in most patients.

Conclusions: The proteasome inhibitor bortezomib may provide an effective new therapy for refractory SLE. Pathogenic antibodies, but also protective antibody titers decline upon bortezomib treatment. Clinical trials should be initiated to explore the use of bortezomib as induction therapy in patients with refractory SLE.

References: (1) Hiepe F. et al. Nat Rev Rheumatol 2011, 7: 170-178.

(2) Neubert K. et al., Nat Med. 2008, 14: 748-755.

Voll, et al, *EULAR,* 2012

Arastu-Kapur, *Clin. Cancer Res.* 2011

- Clinical trial with BTZ in refractory SLE
  - 13 pts with refractory lupus
  - IV bortezomib at a dose of 1.3 mg/m^2
- Response
  - Significant decreases in disease activity score (SLEDAI) and anti-dsDNA antibody
  - 50% ENA ab decrease
  - Rapid decrease in proteinuria (6 weeks)
- AE
  - 1 patient with thrombocytopenia
  - 3 of 7 patients treated with 4 injections/cycle developed PN
  - **Suggested immunoselective PI's outside the BTZ/boronic acid class may be beneficial for long term treatment.**

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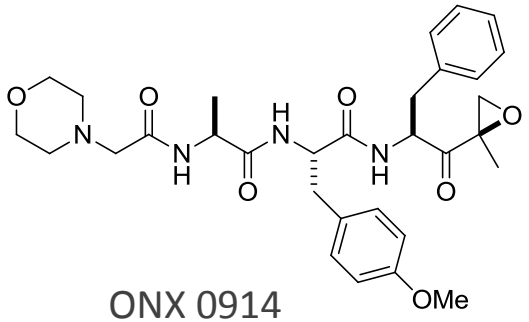
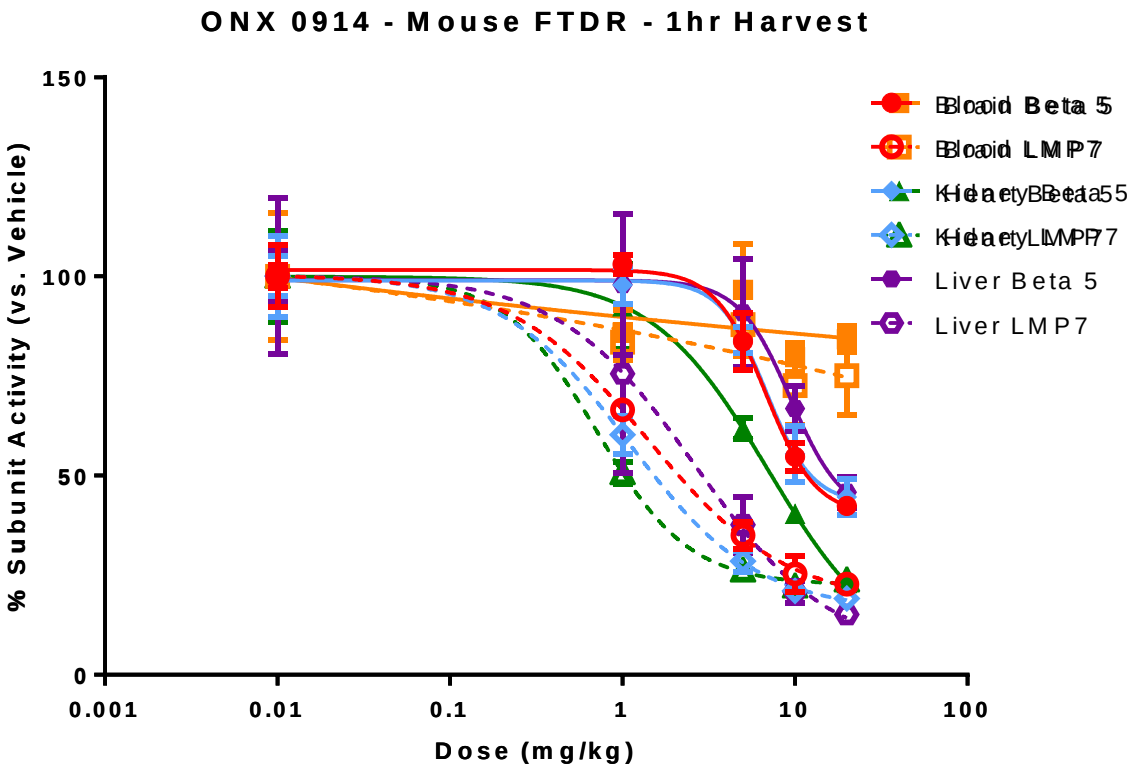
ONX 0914, Potent and Selective Immunoproteasome Inhibitor



ONX 0914
(PR-957)

Species	i20s Potency IC_{50} (nM)	Selectivity (c20s/i20s)
Human	28	12
Mouse	36	7



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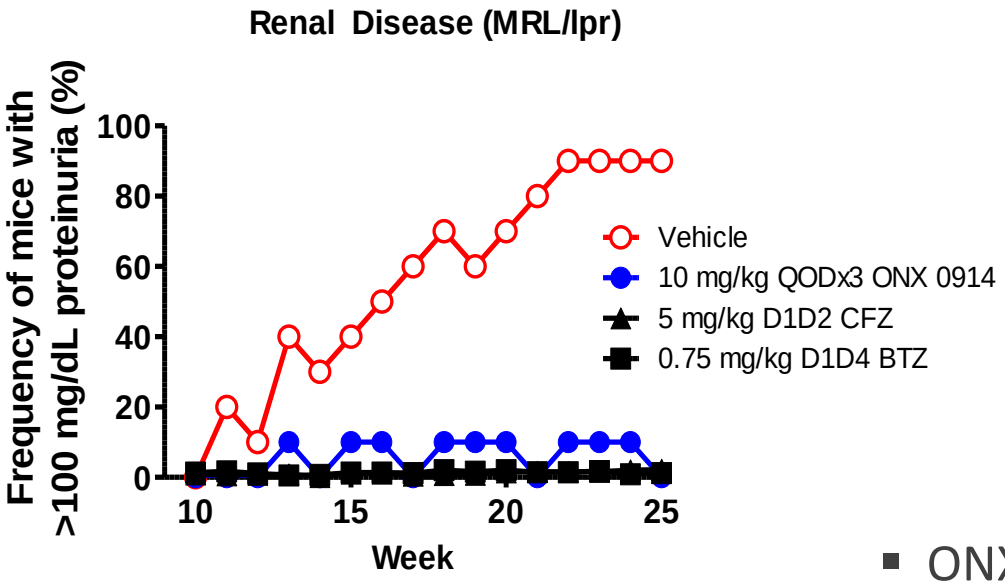
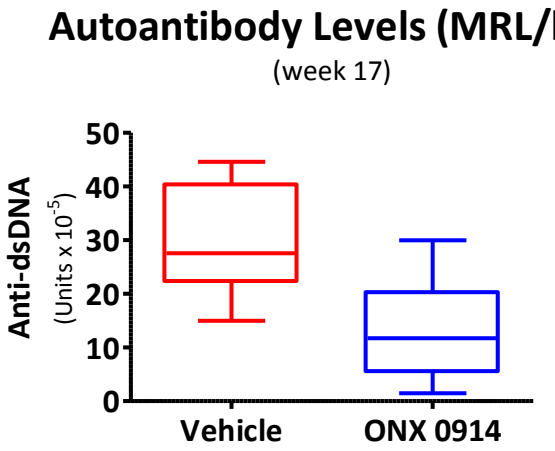
Long Term Treatment with ONX 0914 Prevents Disease Progression in Mouse Models of Lupus Nephritis





SLE Model (MRL/lpr)

- Cytokines (IFN-α, TNF-α, IL-6, IL-1β)
- Autoantibody driven
- T/B-cell Dependent

- ONX 0914 3X/week inhibits development of nephritis in MRL model of lupus
- Similar outcome with ONX 0914 as non-selective inhibitors CFZ and BTZ
- Correlation to decrease in auto antibody production observed

Arthritis Rheum. **2012**, *64*, 493.

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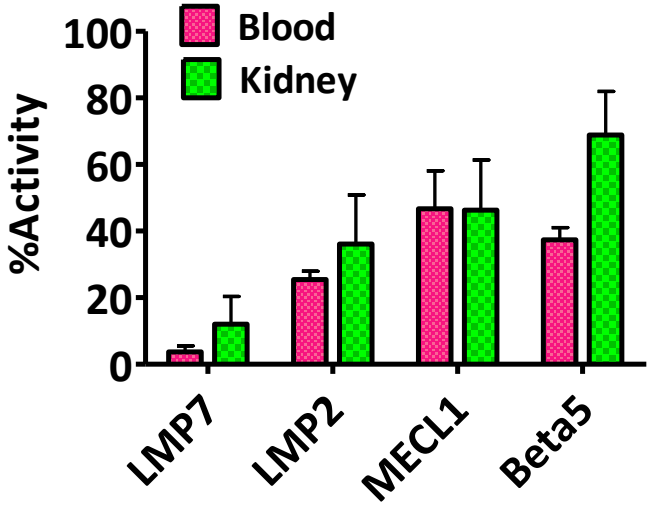
A Single Dose of ONX 0914 Reverses RA in Mice

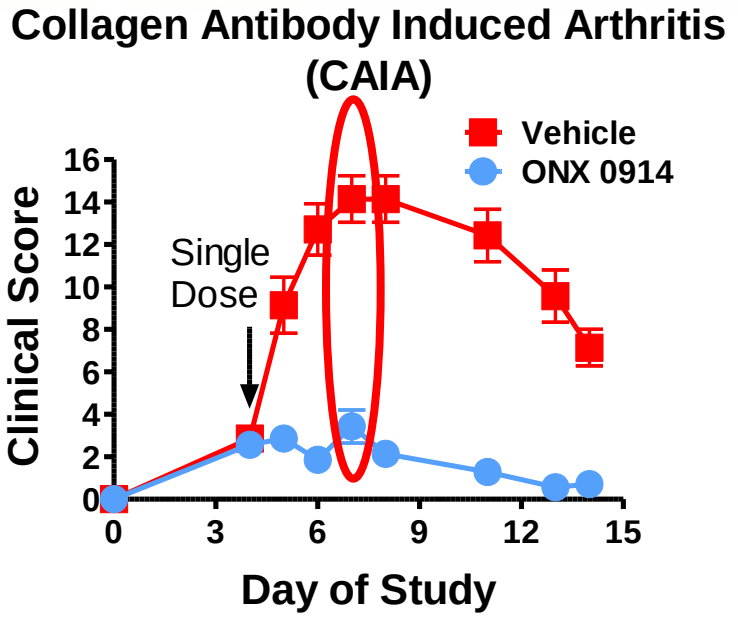
ONX 0914 10 mg/kg (i.v.)

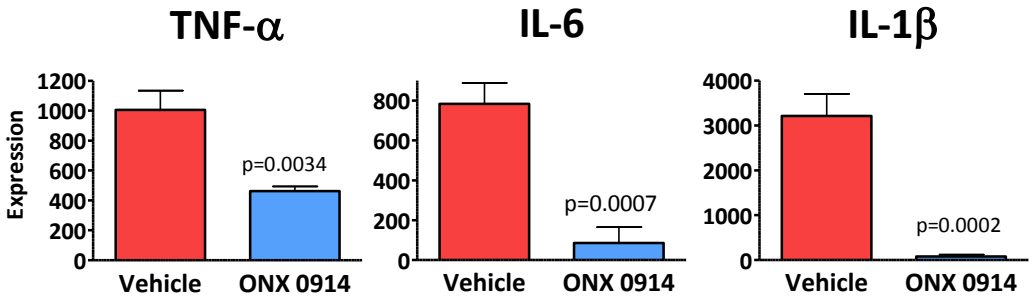
Active site Profile

Disease Model

Paw mRNA







Nat. Med. **2009**, *15*, 781.

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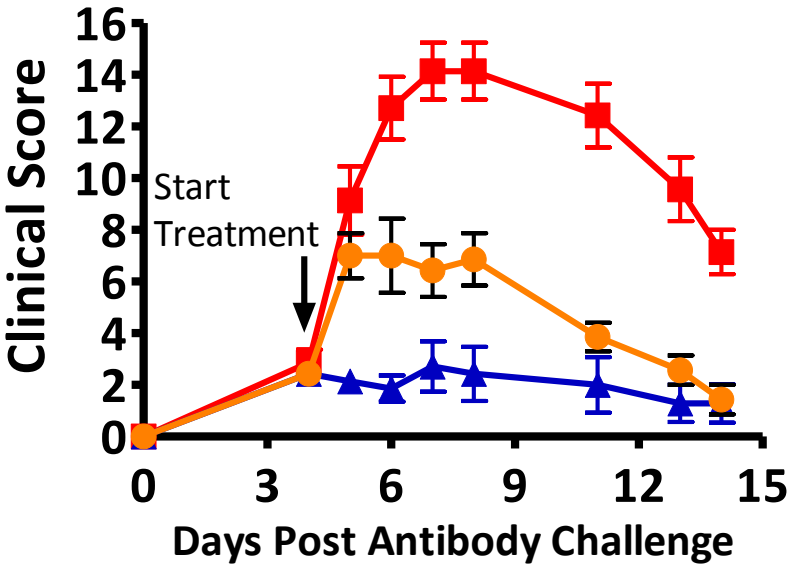
ONX 0914 Compares Favorably to Standard Anti-TNF Treatment (Enbrel®) in Mouse RA Models

- Vehicle
- 10 mg/kg ONX 0914 (QOD)
- 10 mg/kg Enbrel (QOD)

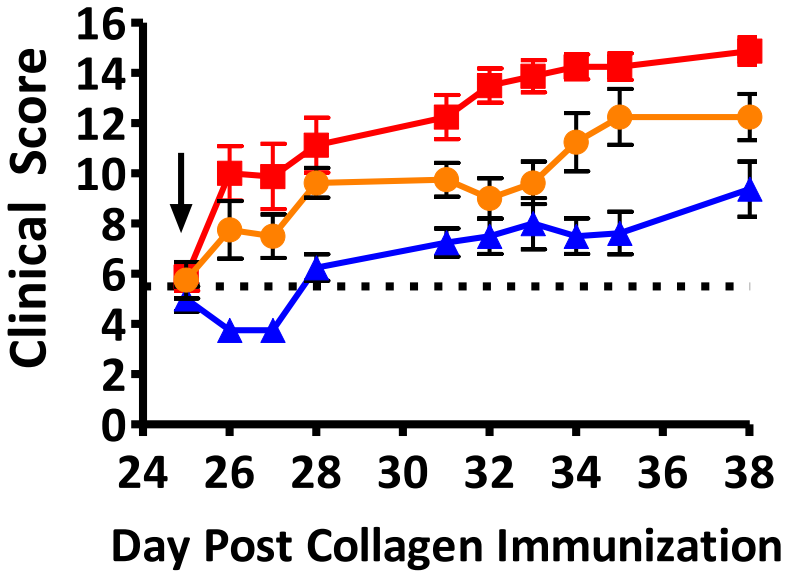
Anti-Collagen Antibody Model



Anti-Collagen Ab Model (CAIA)

- T/B-cell independent

Collagen Immunization Model



Collagen Immunization Model (CIA)

- T/B-cell Dependent

Muchamuel, et al., *Nat. Med.*, 2009

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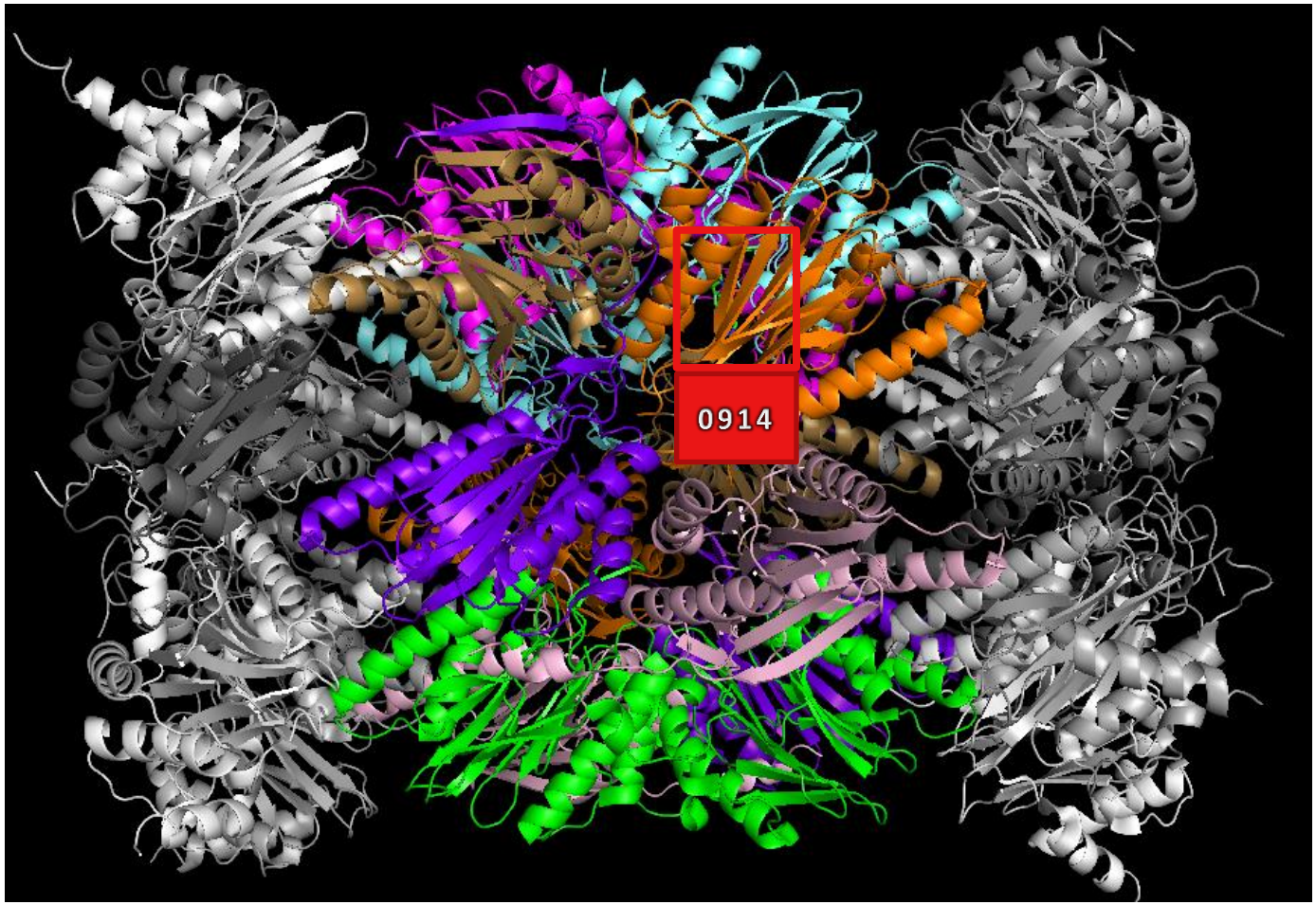
Structural basis for selectivity of ONX 0914



- Structure-based design of selective iCP inhibitors has recently been enabled with the publication of crystal structures of ONX 0914 bound to mouse constitutive (CP) and iCP (Cell, **2012**, *148*, 727)

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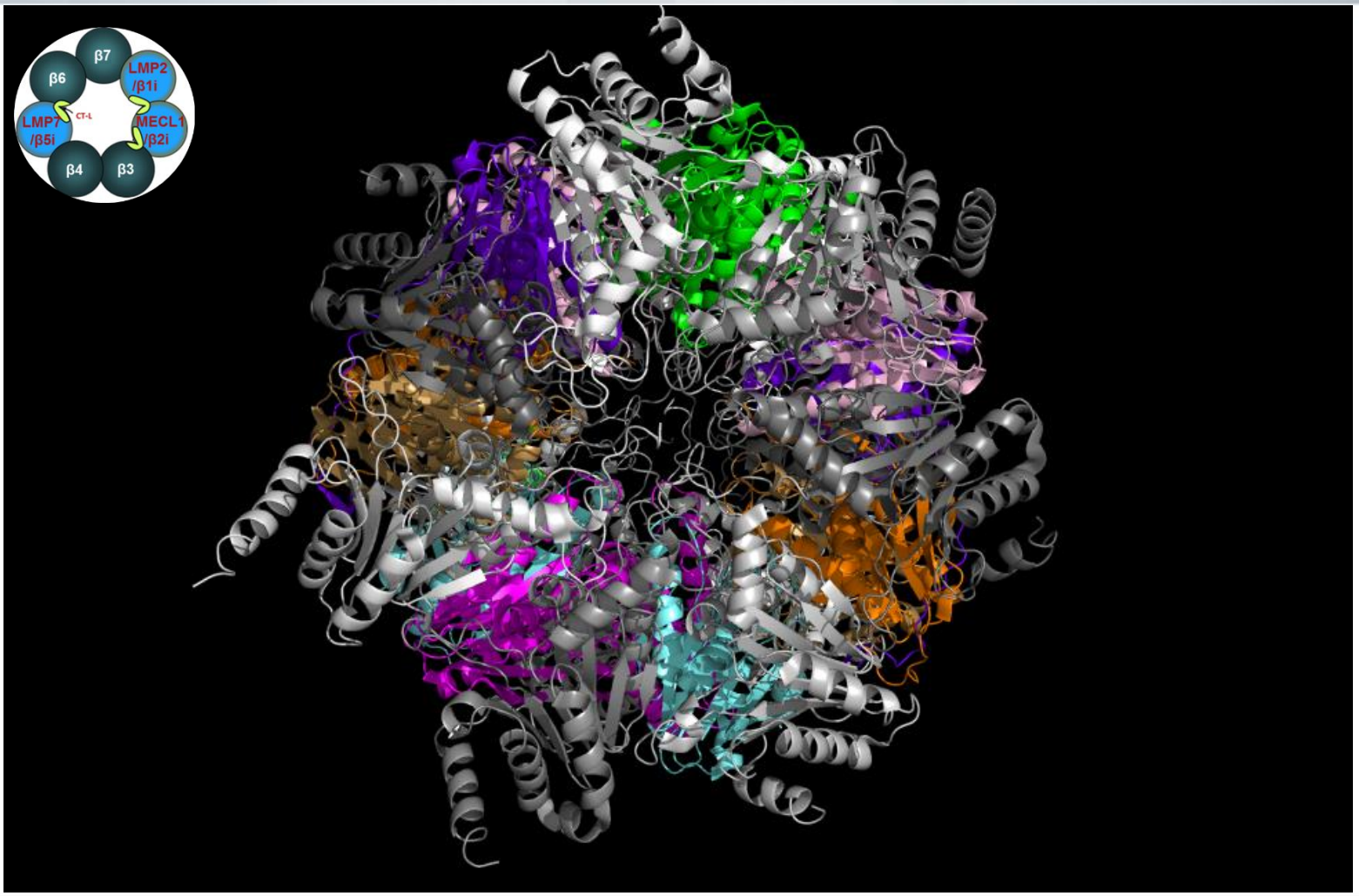
ONX-0914 Selectively Binds to Immunoproteasome at Subunit β5i



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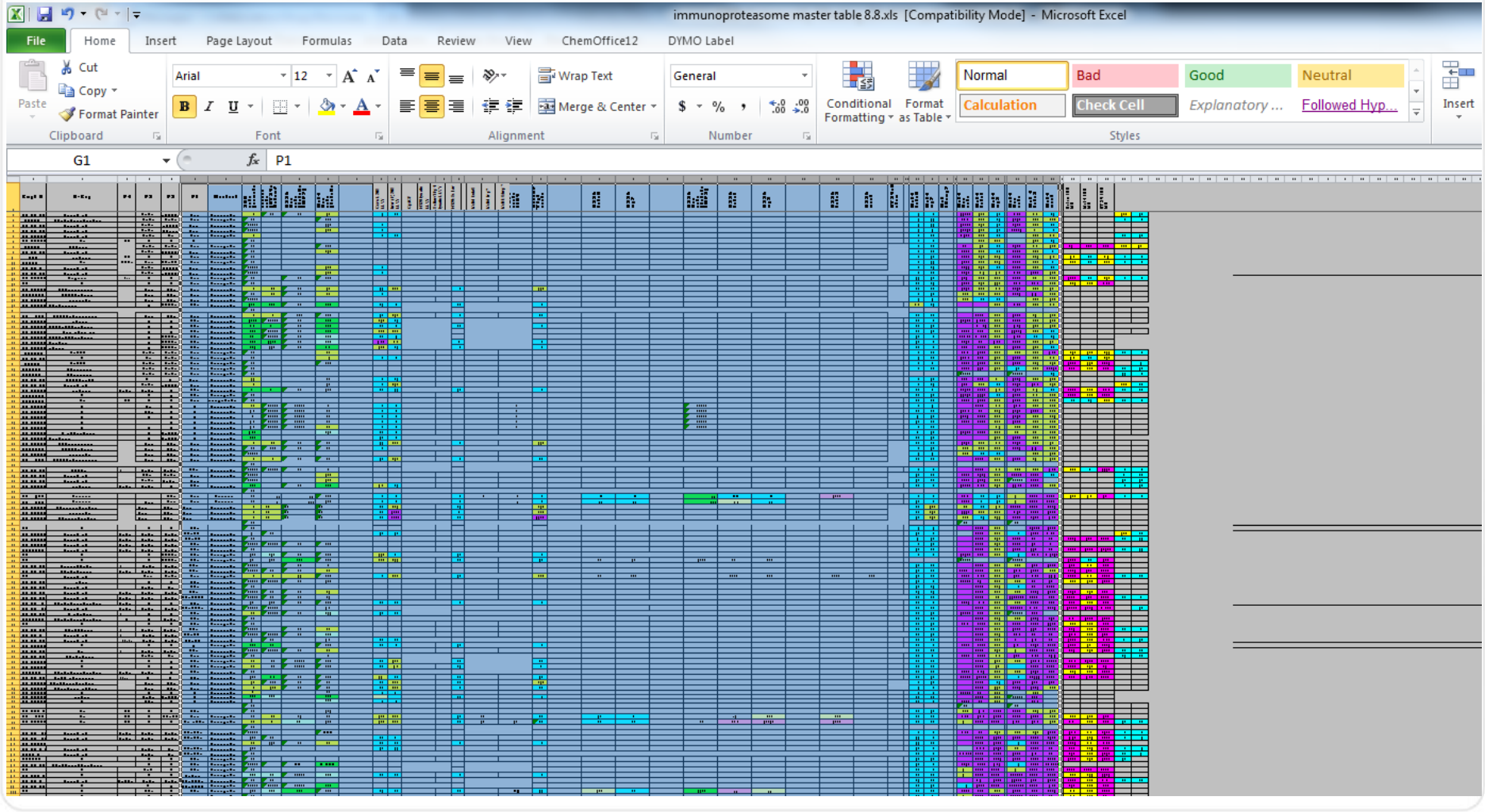
How we started…



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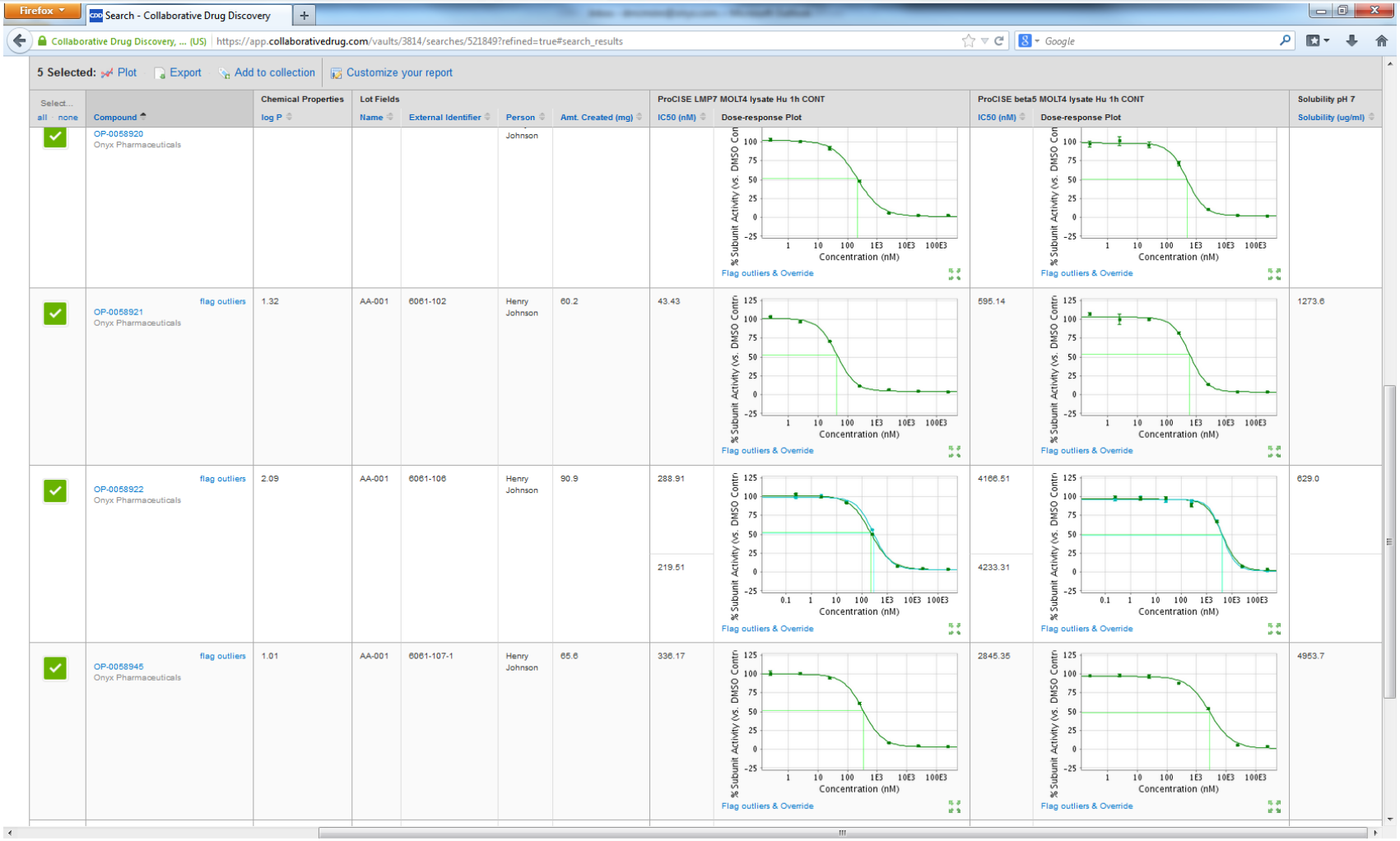
How we got things done…



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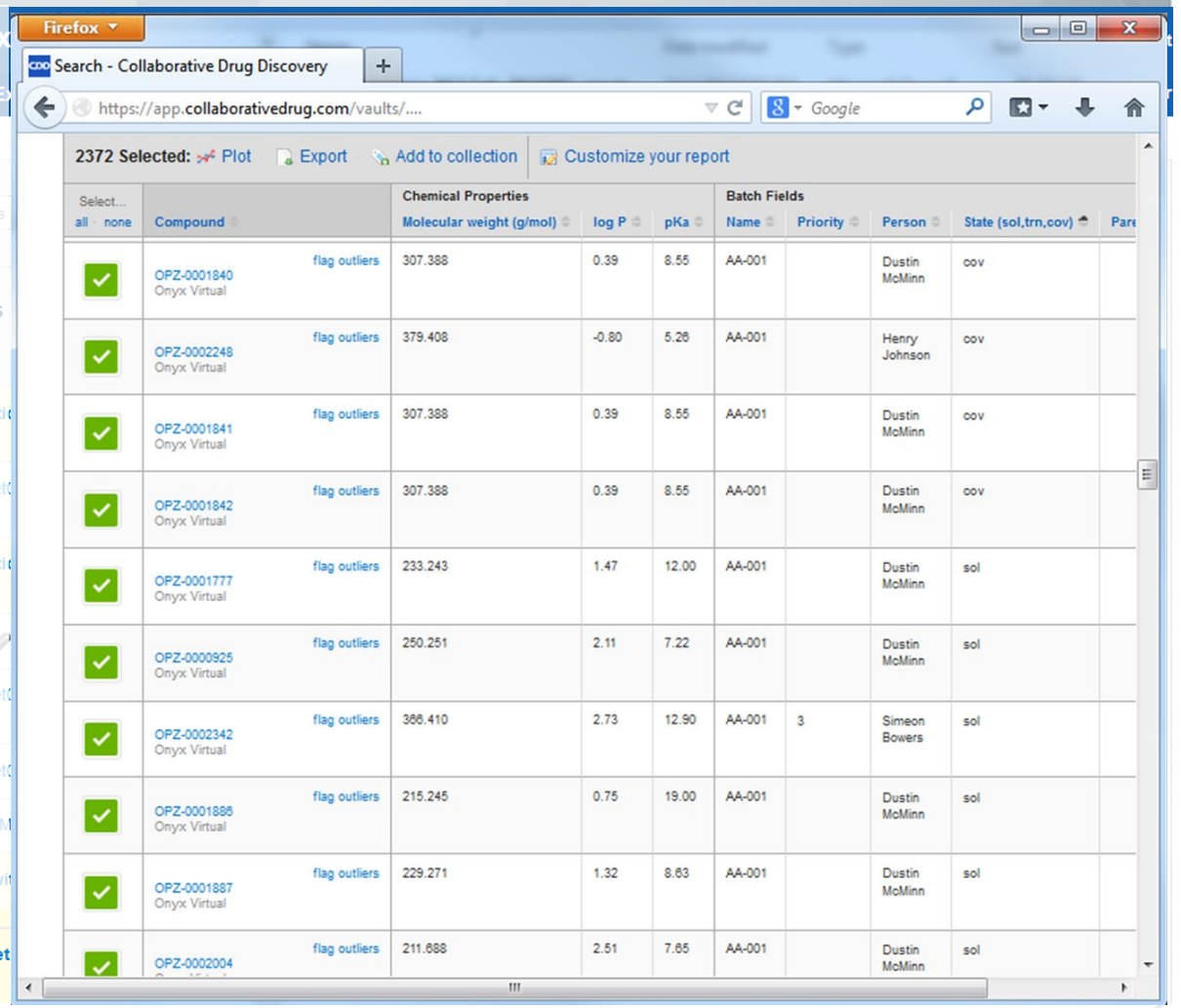
CRO-based SBDD: Meeting the Collaboration Challenge

- Onyx Virtual built upon the CDD web-based platform
  - Import designs, commercial databases
  - Batch fields include transition states, priority, CRO assigned, designer, disclosure descriptors…
  - Rapid, secure information exchange



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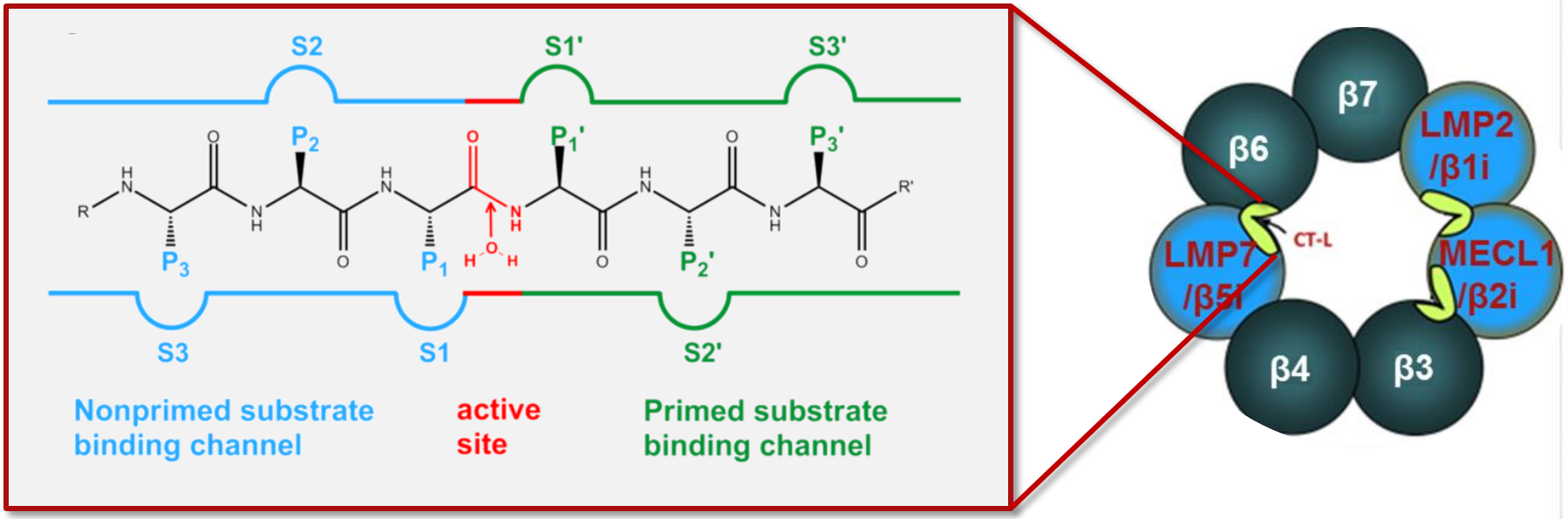
Toward Obtaining an Inhibitor Selective for the Immunoproteasome



- Subunits contain specificity pockets
- Nature of amino acids of peptide being cleaved drives recognition

Cell, **2012**, *148*, 727

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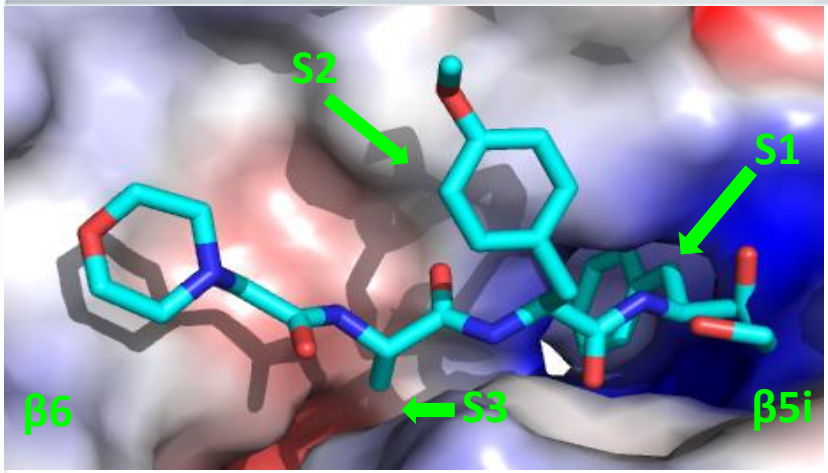
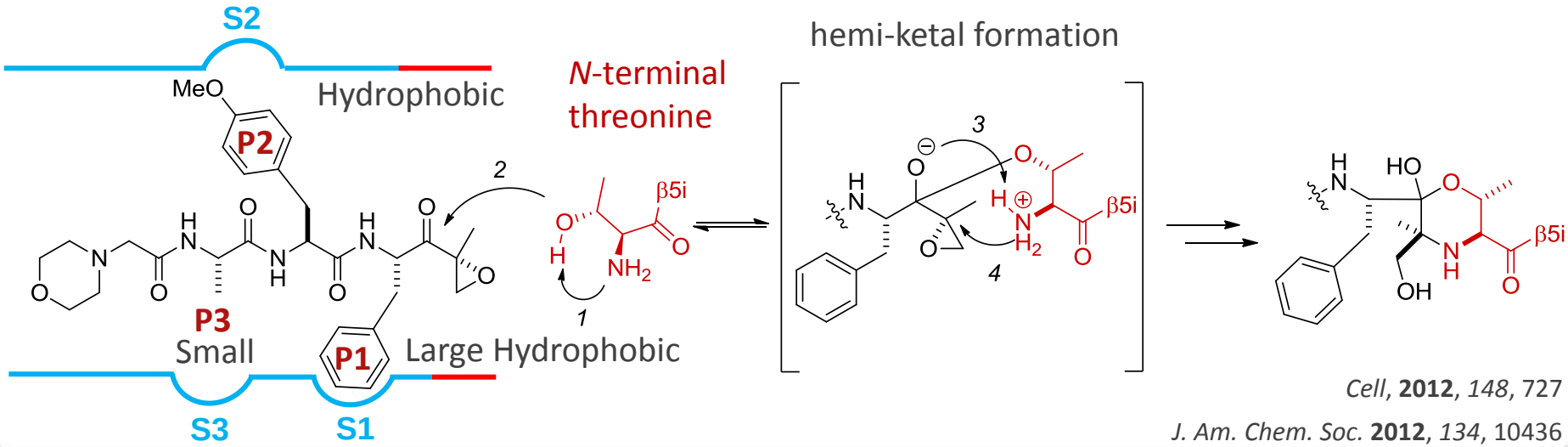
Mechanism for Immunoproteasome Inhibition



- 0914 (shown) binds specific to S-pockets
- Peptide backbone required to differentiate between *N*-terminal threonine binding sites of various subunits (β5i vs. β5, etc.)
- Epoxyketone warhead reacts productively only with *N*-terminal threonines of proteasome



Cell, **2012**, *148*, 727

J. Am. Chem. Soc. **2012**, *134*, 10436

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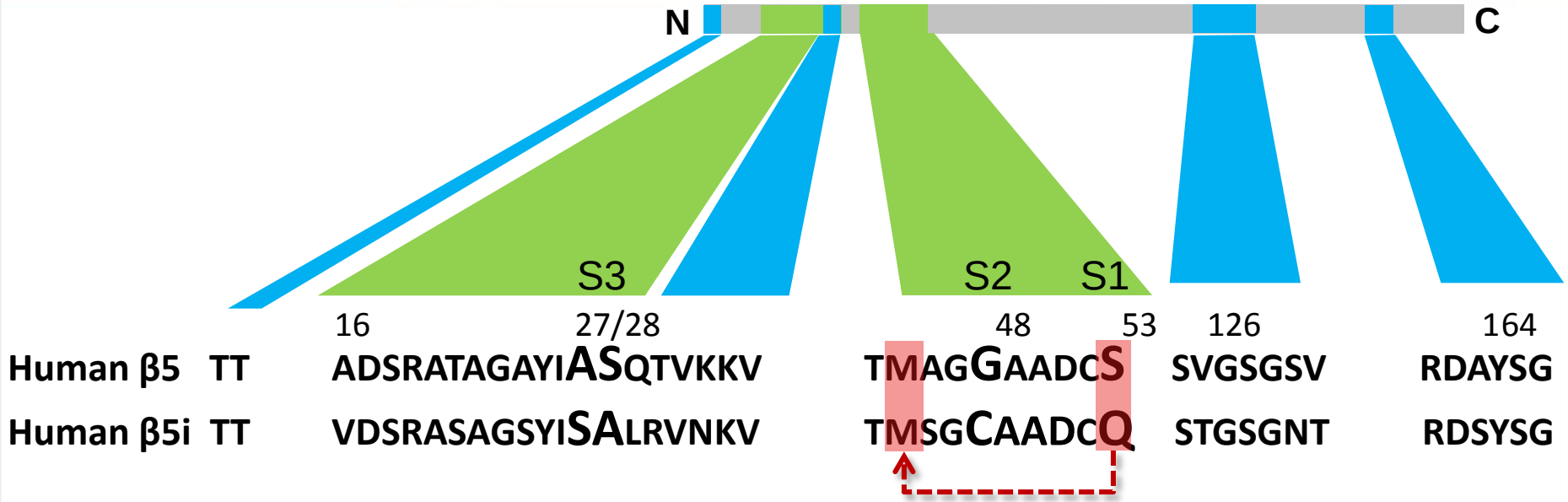
β5 and β5i Homology: S1 pocket



- Mouse, human sequence homology >90%
- Human homology model established at Onyx
- Selectivity of ONX 0914 arises from key residue differences within selectivity pockets of β5 and β5i

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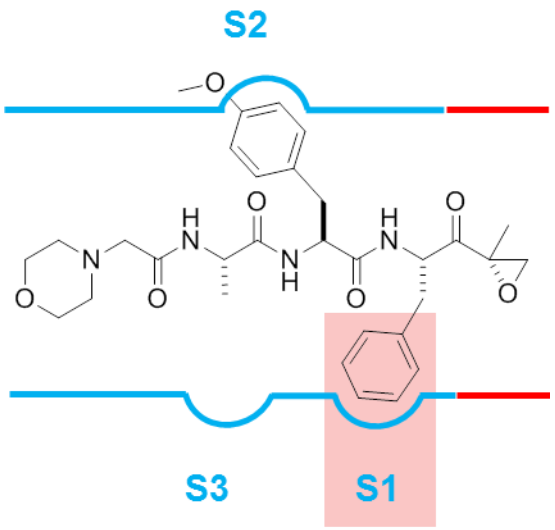
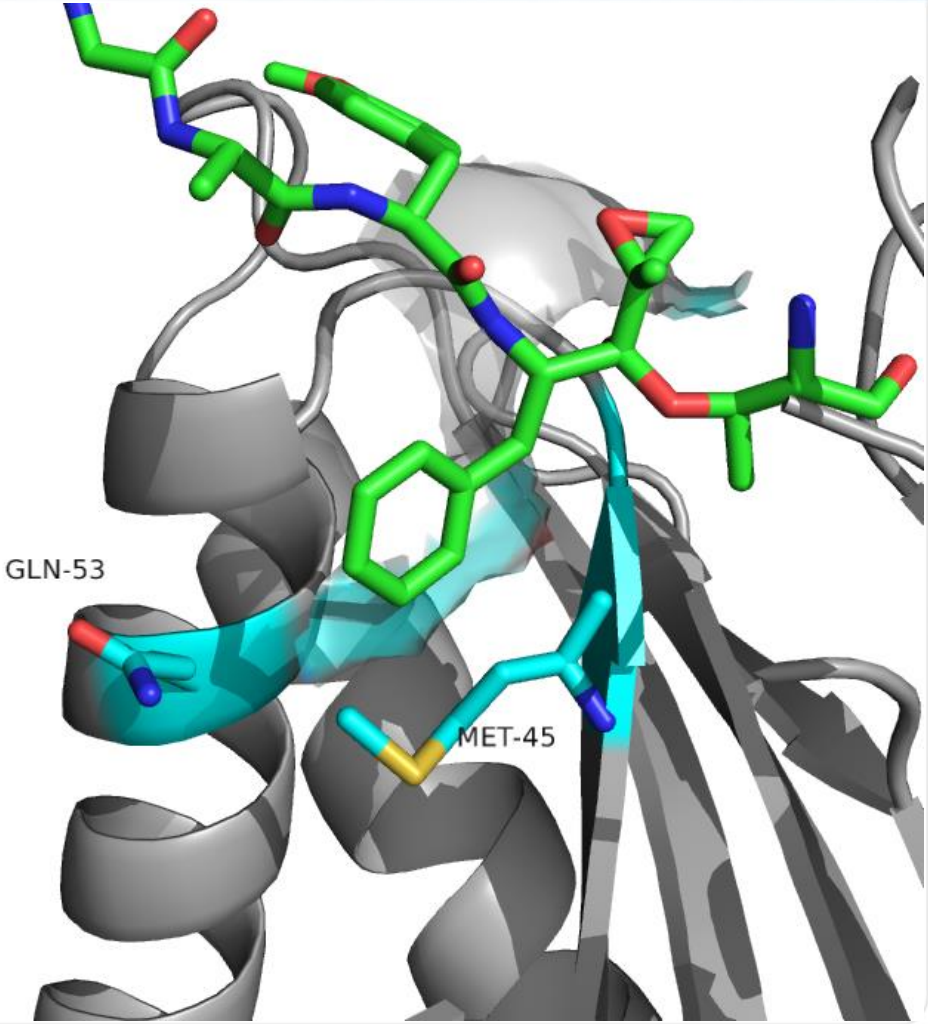
S1 Pocket, β5i Accommodates Larger P1 Residues

- Placement of Met45 side-chain is selectivity driver for ONX 0914 scaffold
- Position of Met45 stabilized by hydrophobic interaction with Gln53
- Results in a large S1 pocket





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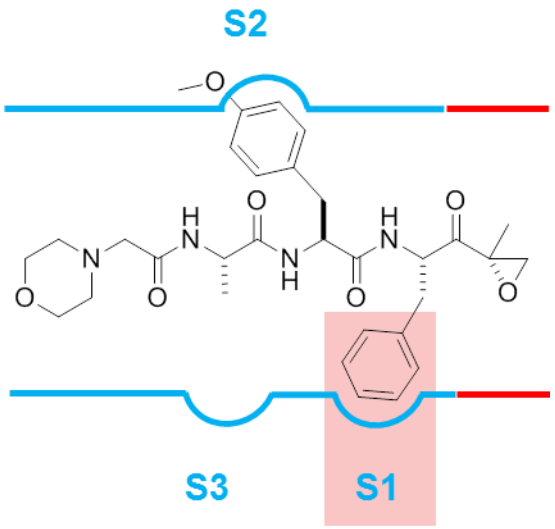
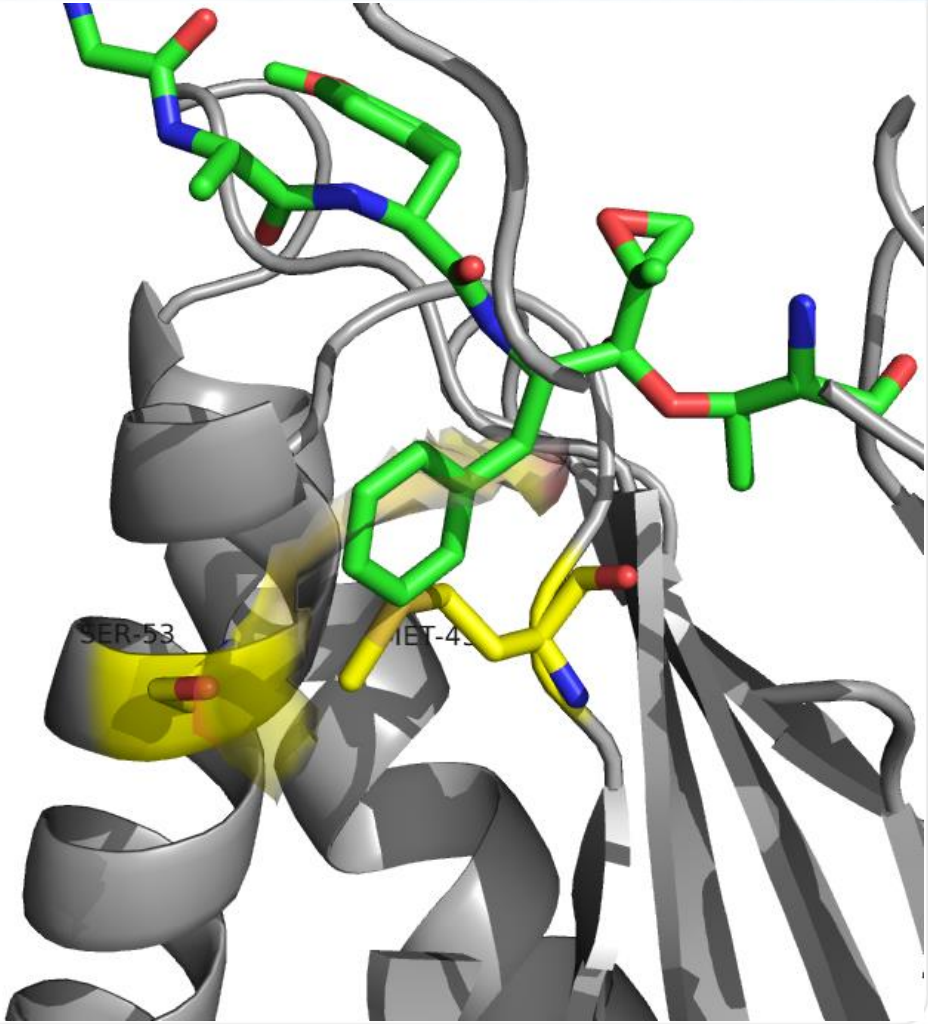
β5 Smaller S1 Pocket

- In β5 Q53 to S53 switch frees M45, shrinks S1 pocket
- Smaller P1 residues allowed
- Sets up M45-P1F clash





ONYX PHARMACEUTICALS
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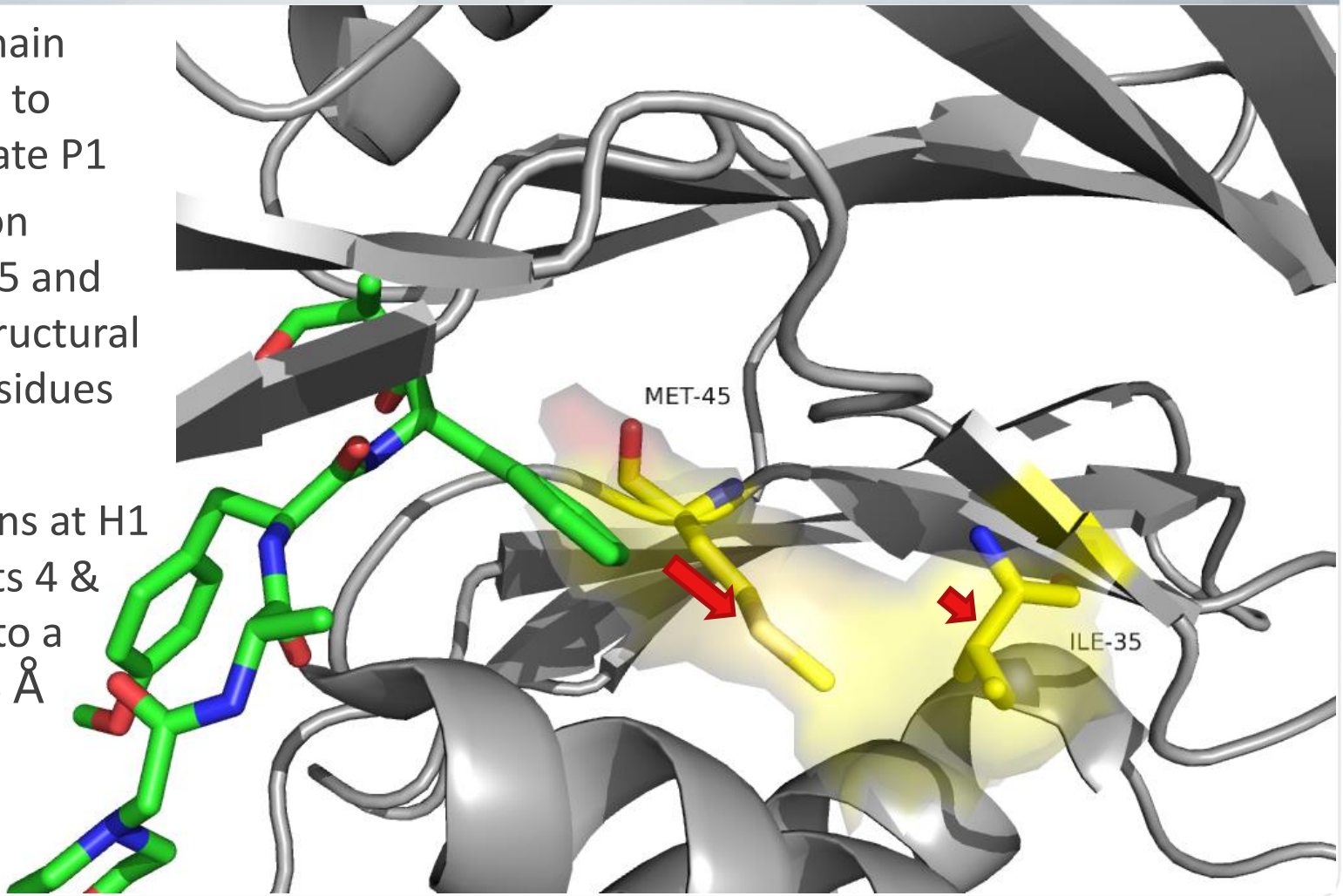
β5 Smaller S1 pocket

- M45 side chain must rotate to accommodate P1
- M45 rotation reorients I35 and cascades structural changes, residues (34 to 76)
- Perturbations at H1 and β-sheets 4 & 5 translate to a rmsd = 0.65 Å



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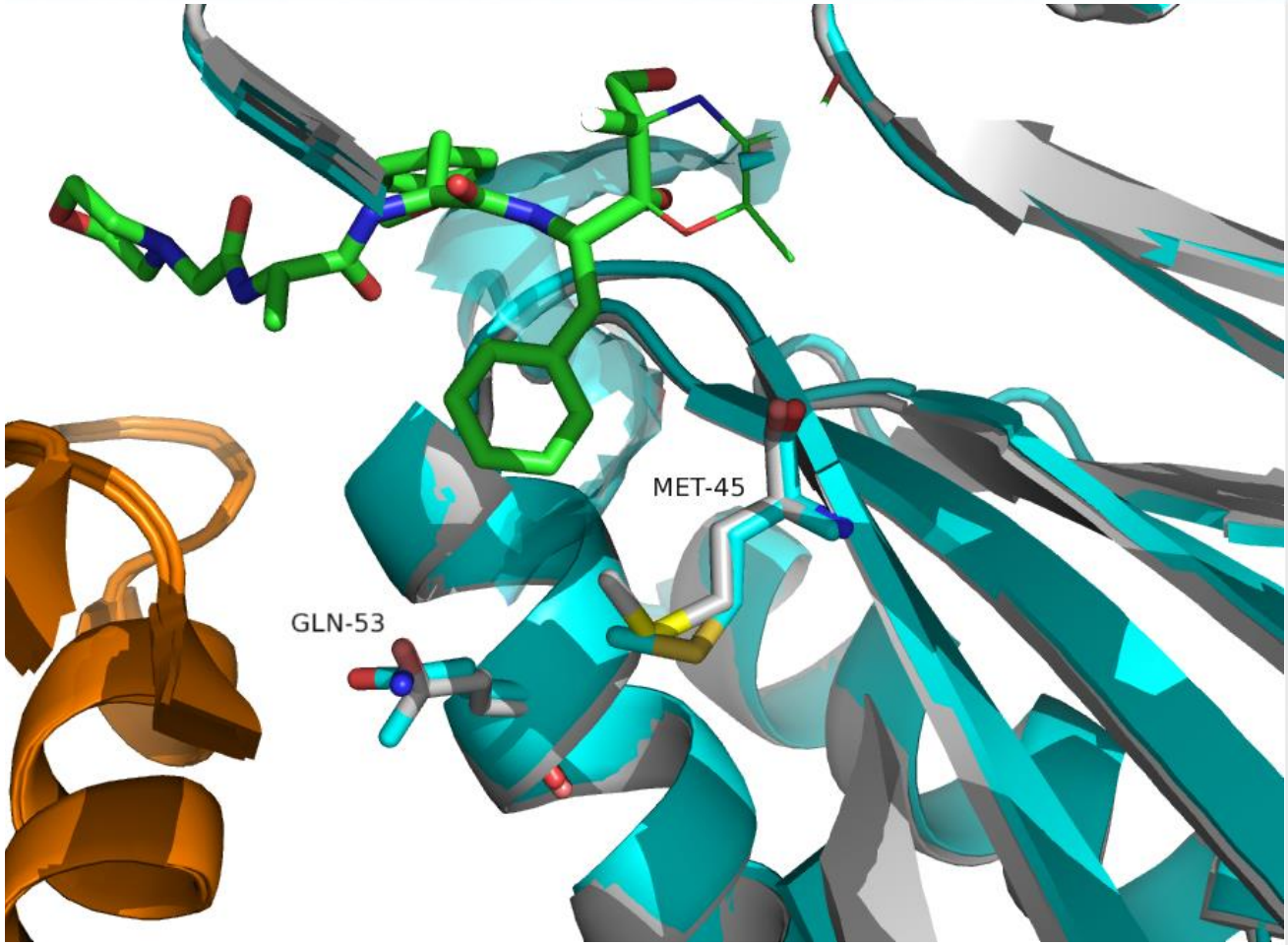
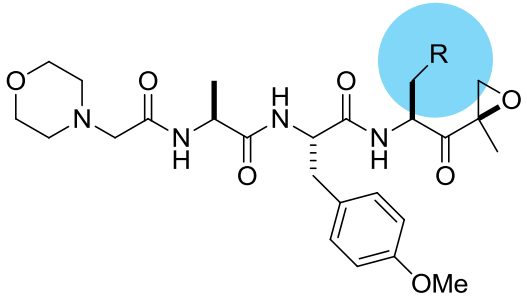
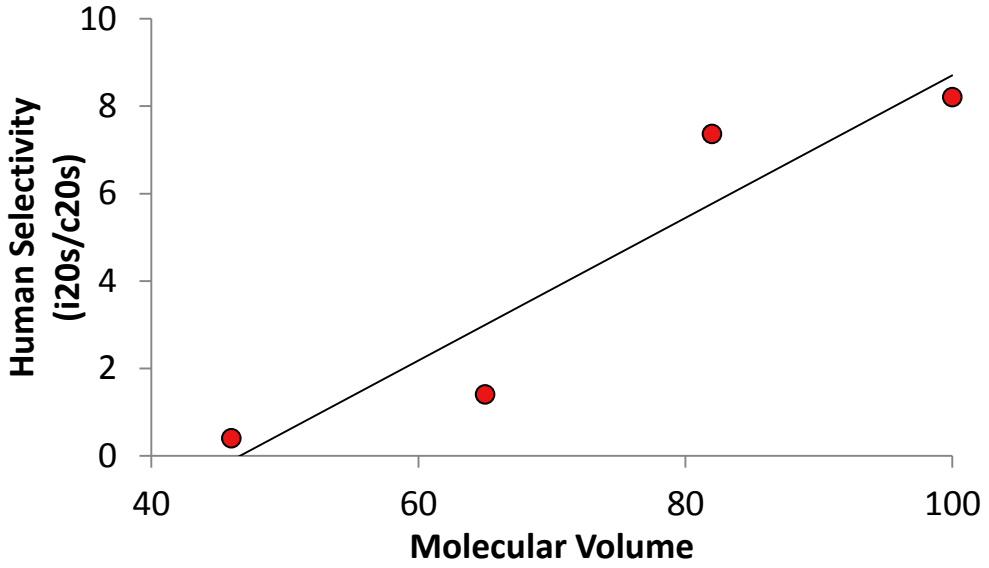
β5i Minimally Perturbed upon Binding to 0914

- Overlay of unbound β5i and bound β5i
- M45, majority of total structure, minimally perturbed



Selectivity Increases with Size of P1 Substituent





ONYX PHARMACEUTICALS
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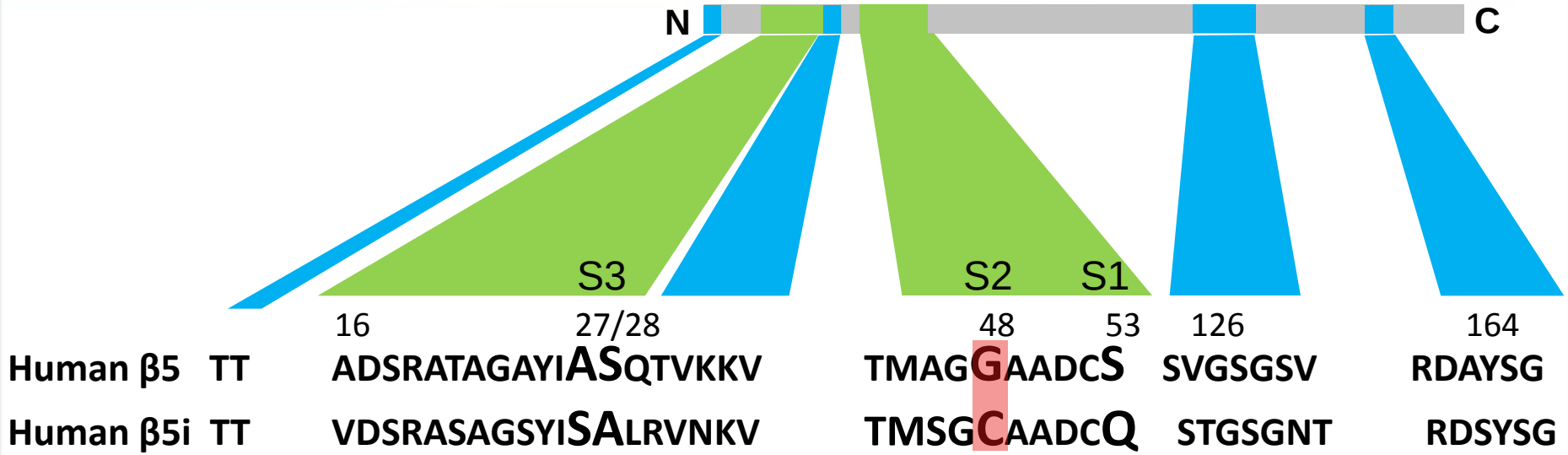
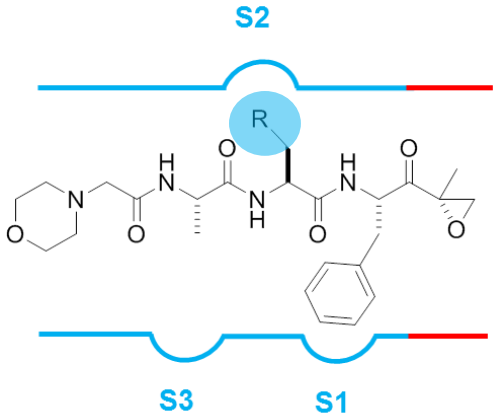
β5 and β5i Homology: S2 pocket

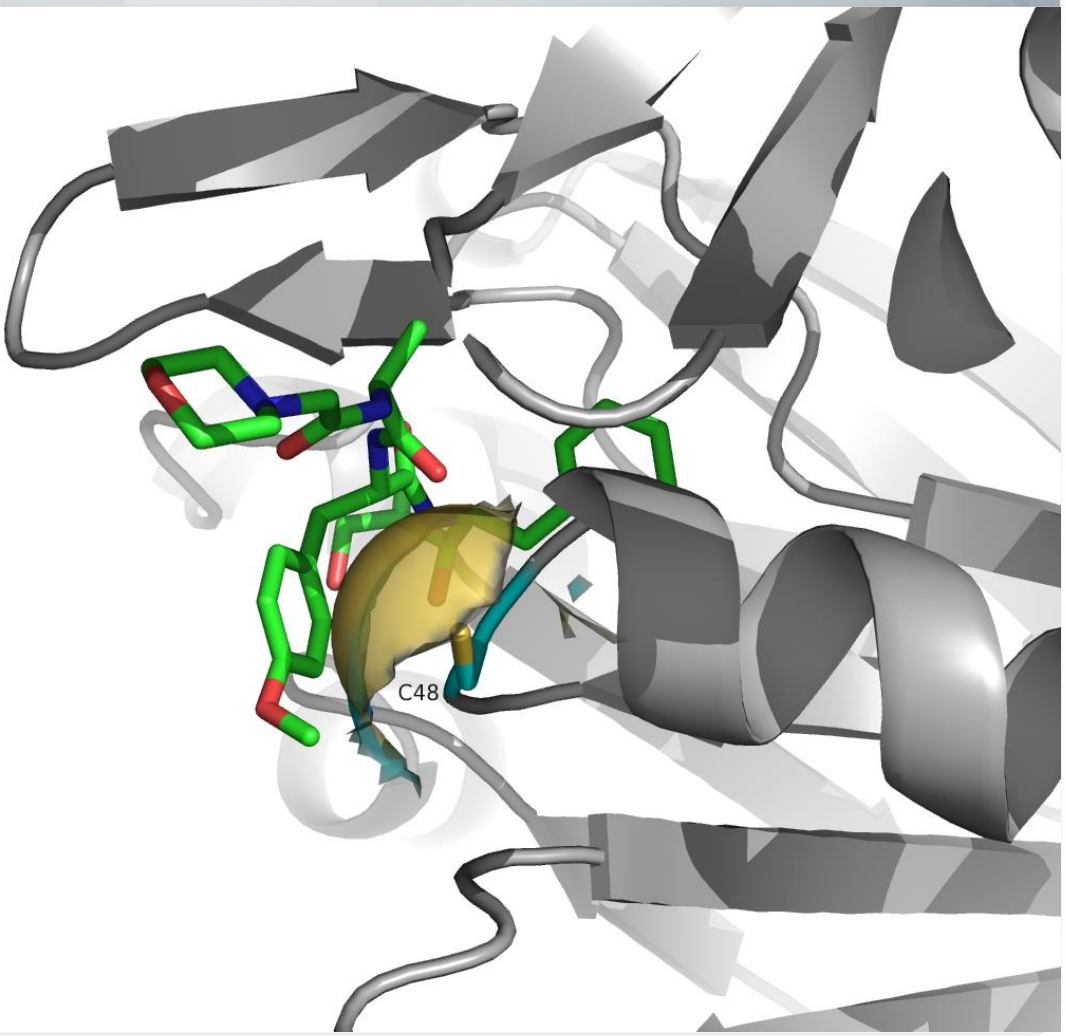


- Mouse, human sequence homology >90%
- Human homology model established
- Selectivity of ONX 0914 arises from key residue differences within selectivity pockets of β5 and β5i

The S2 pocket of β5 *vs* β5i

- Shallow S2 pocket of β5 bears G48
- β5i S2 pocket formed by C48
- Electrostatic sulfur-aromatic interactions drive binding and selectivity





Zauhar, R.J., *Biopolymers*, 2000, *53*, 233

ONYX PHARMACEUTICALS
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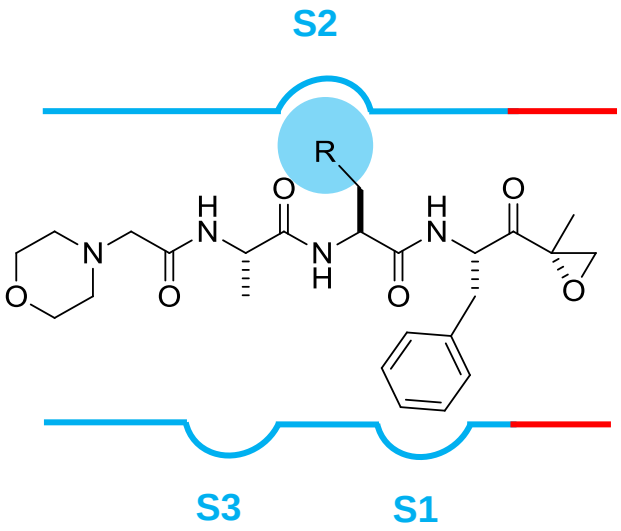
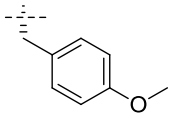
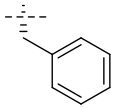
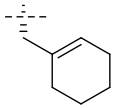
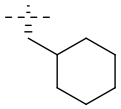
P2 Aromatics Show Superior Selectivity



R	LLVY i20S Hu IC_{50} (nM)	β5i:β5c Selectivity
	21	12
	47	7
	650	2
	390	1
	400	1

ONYX PHARMACEUTICALS
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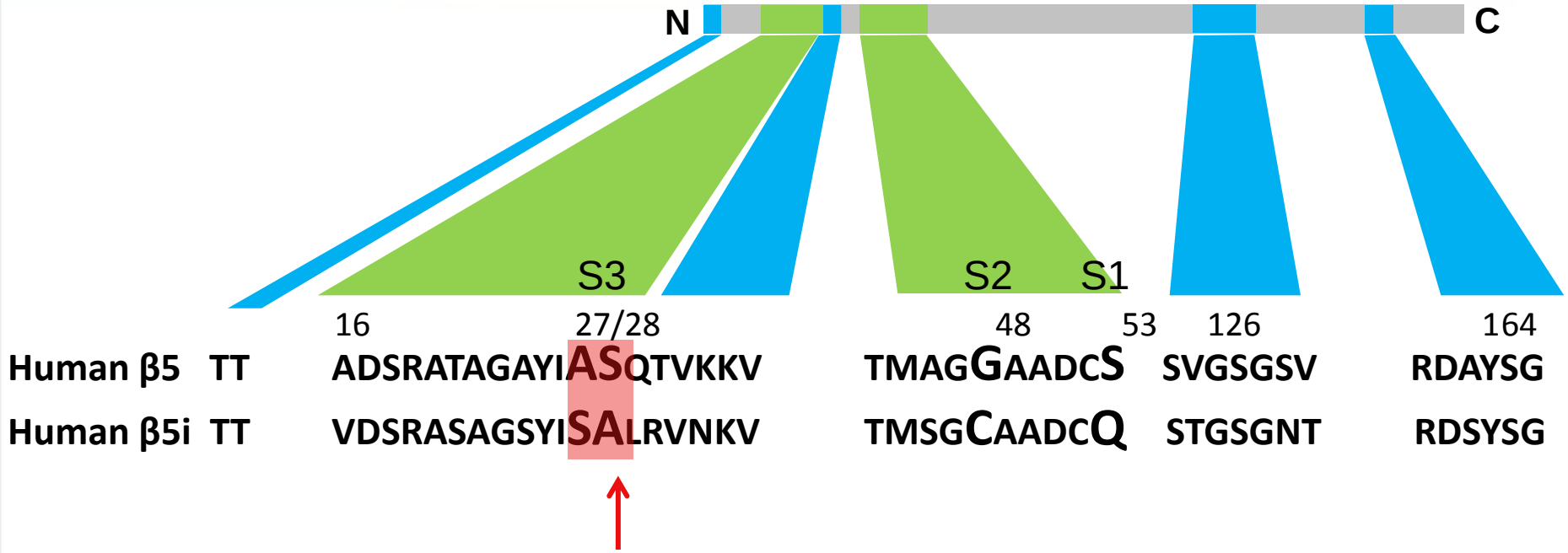
β5 and β5i Homology: S3 pocket



- Ala27 in β5, S27 in β5i, exterior of S3 pocket
- Ser28 in β5, A28 in β5i, deep within S3 pocket

Cell **2012**, *148*, 727.

ONYX PHARMACEUTICALS
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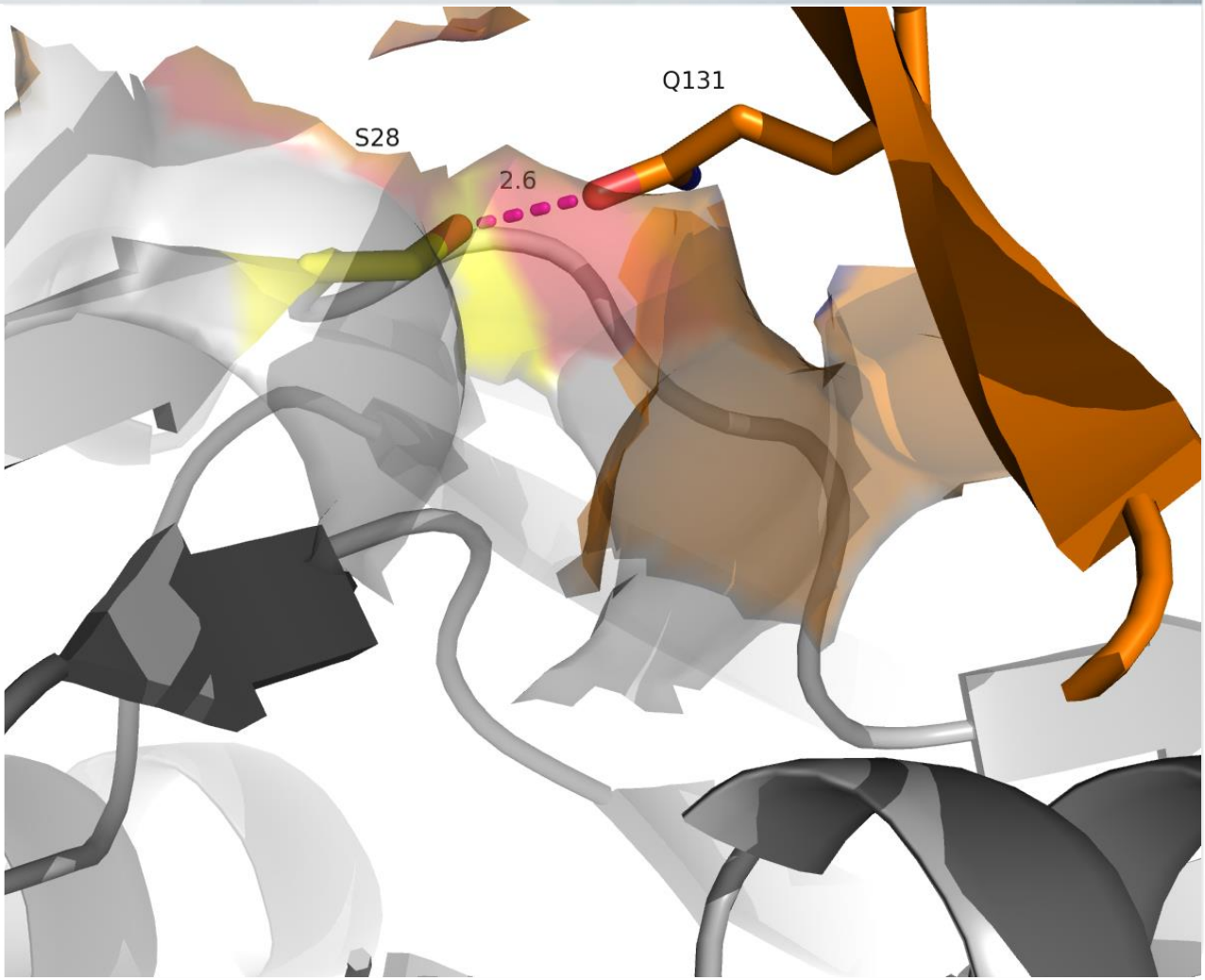
Glu131 of β6 interacts with S28 of β5

- S28 of β5 forms hydrogen bond to Q131 of β6 subunit



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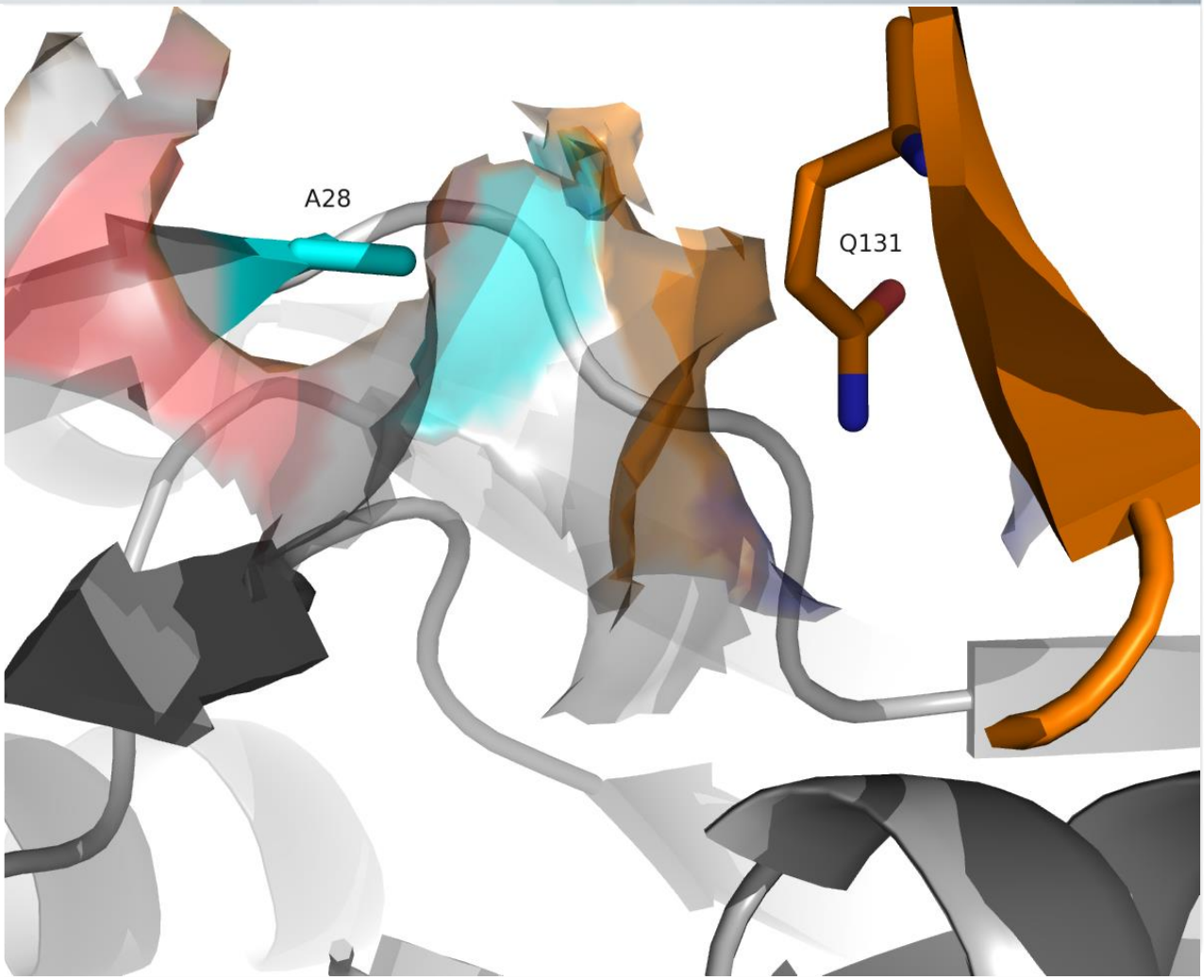
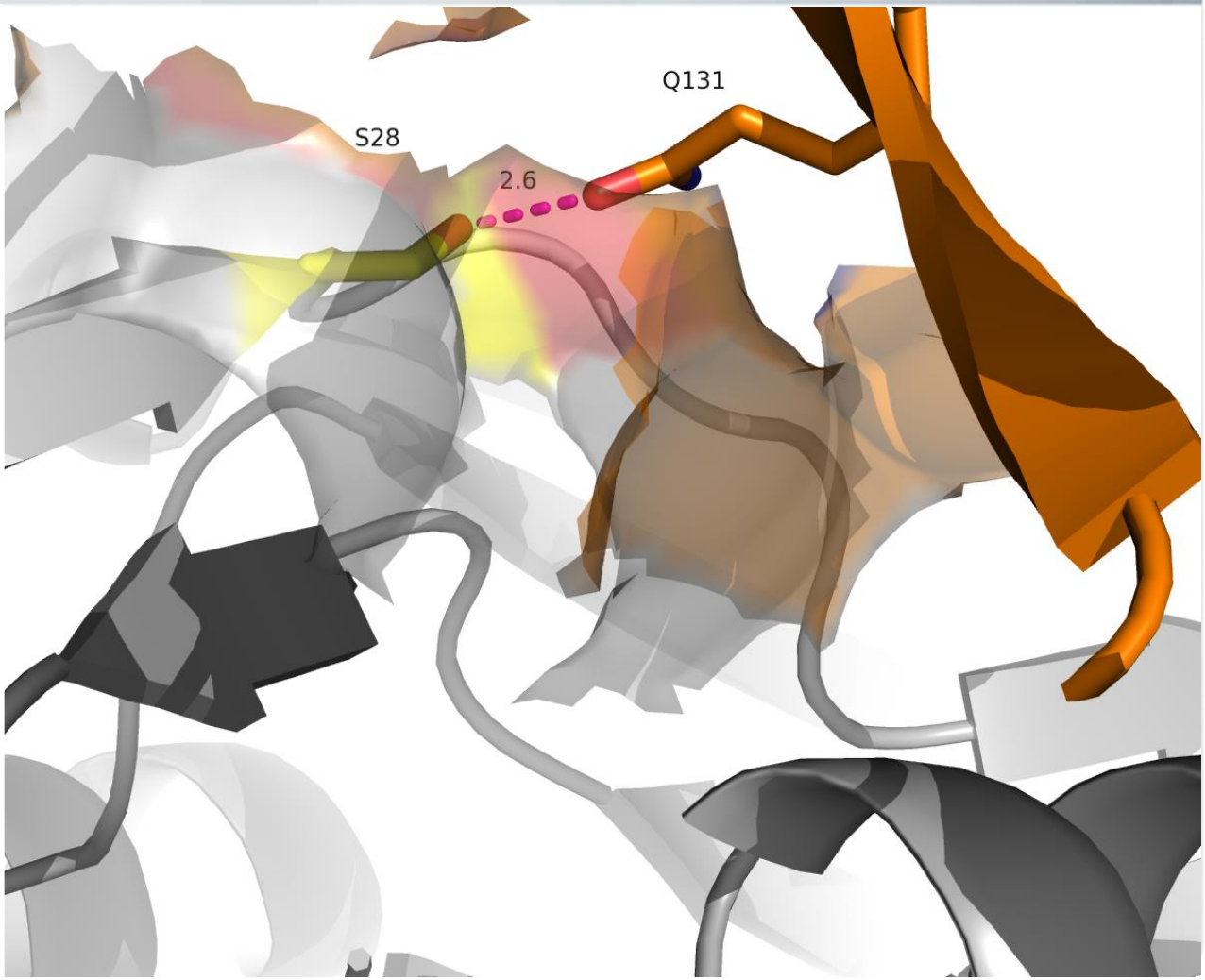
S28 of β5 Switched to A28 of β5i

- S28A switch in β5i liberates Q131 of β6, relaxes pocket floor



Glu131 of β6 interacts with S28 of β5

- Disrupting the β5:β6 protein subunit interaction, a potential selectivity determinant?



ONYX PHARMACEUTICALS
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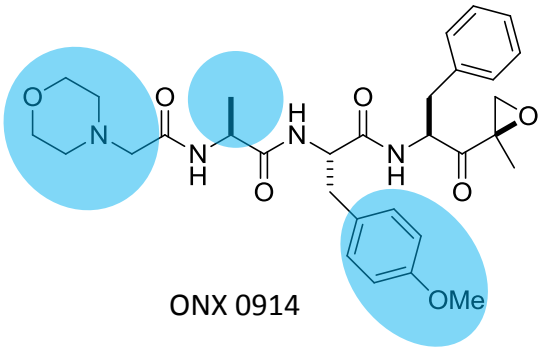
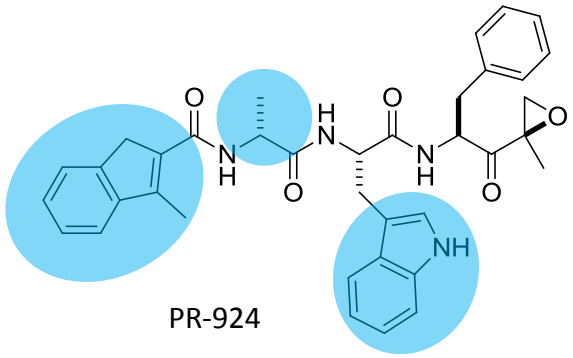
Human β5i Homology Model with PR-924 Reveals Unique Binding Mode



ONX 0914

ProCISE β5i IC_{50} (nM)	39
Selectivity (β5/β5i)	11



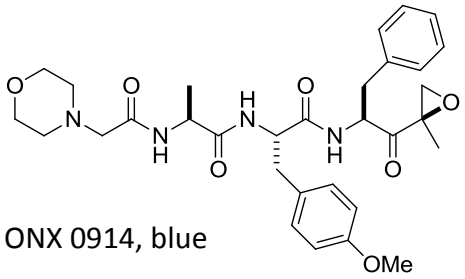
PR-924

ProCISE β5i IC_{50} (nM)	34
Selectivity (β5/β5i)	38

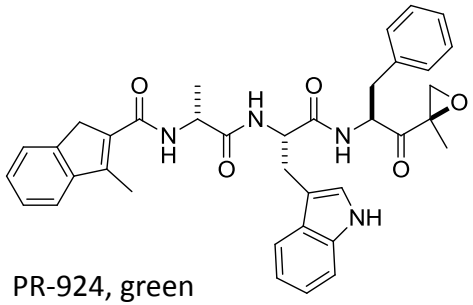
- PR-924, equipotent, ca 4x selective
- Key structural differences in *C*-terminal tail, P2, **and change in stereochemistry at P3**

Human β5i Homology Model with PR-924 Reveals Unique Binding Mode

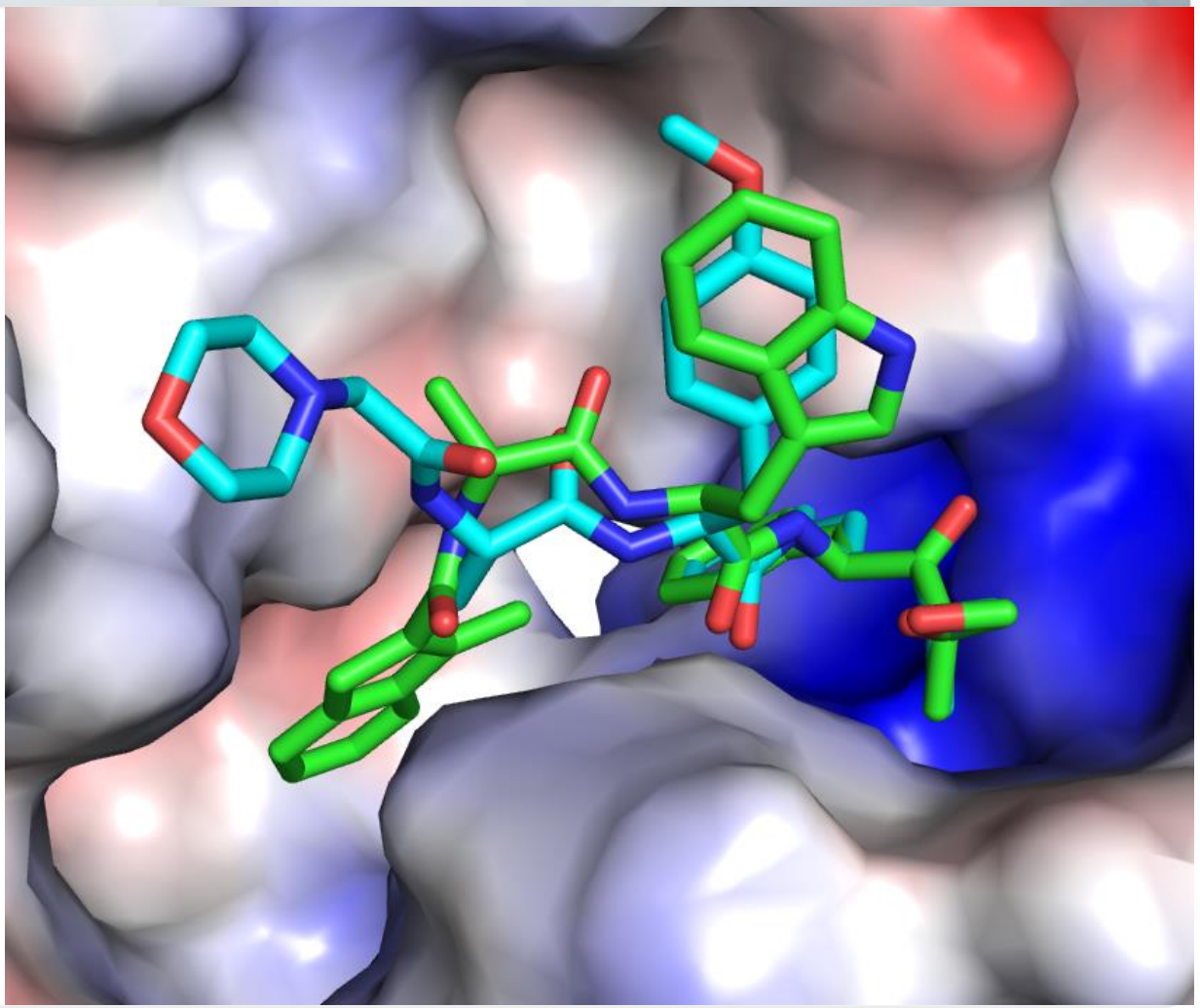
- Human β5i model built from mouse structure
- The hydrophobic tail of PR-924 resides in the S3 pocket.



ONX 0914, blue



PR-924, green



ONYX PHARMACEUTICALS
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Human β5i Homology Model with PR-924 Reveals Unique Binding Mode

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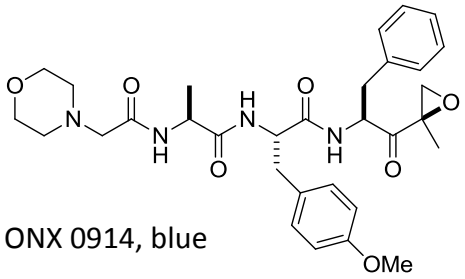
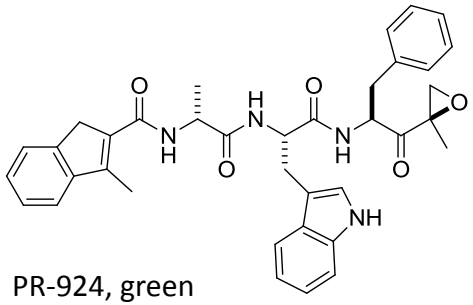
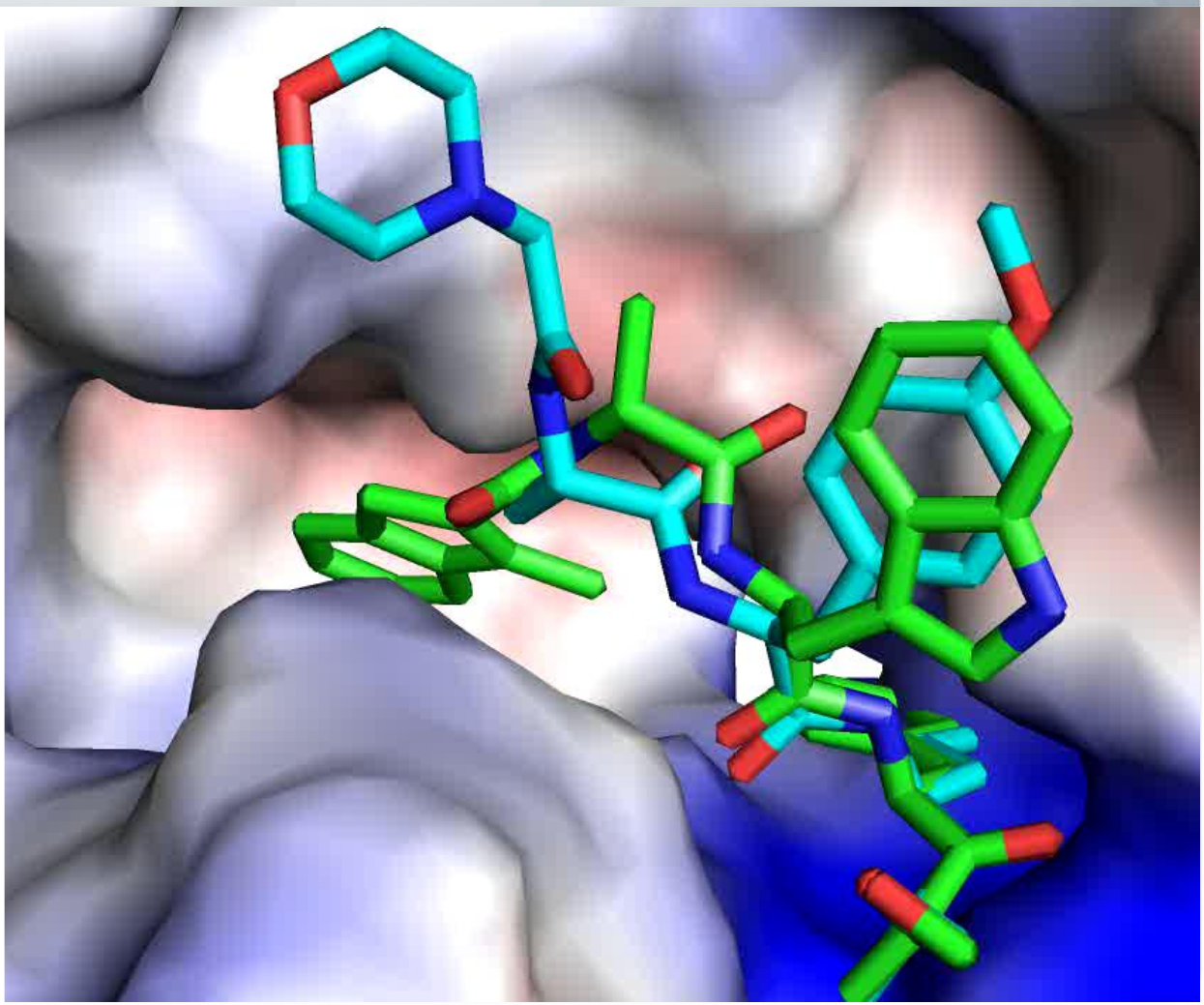


ONX 0914, blue



PR-924, green



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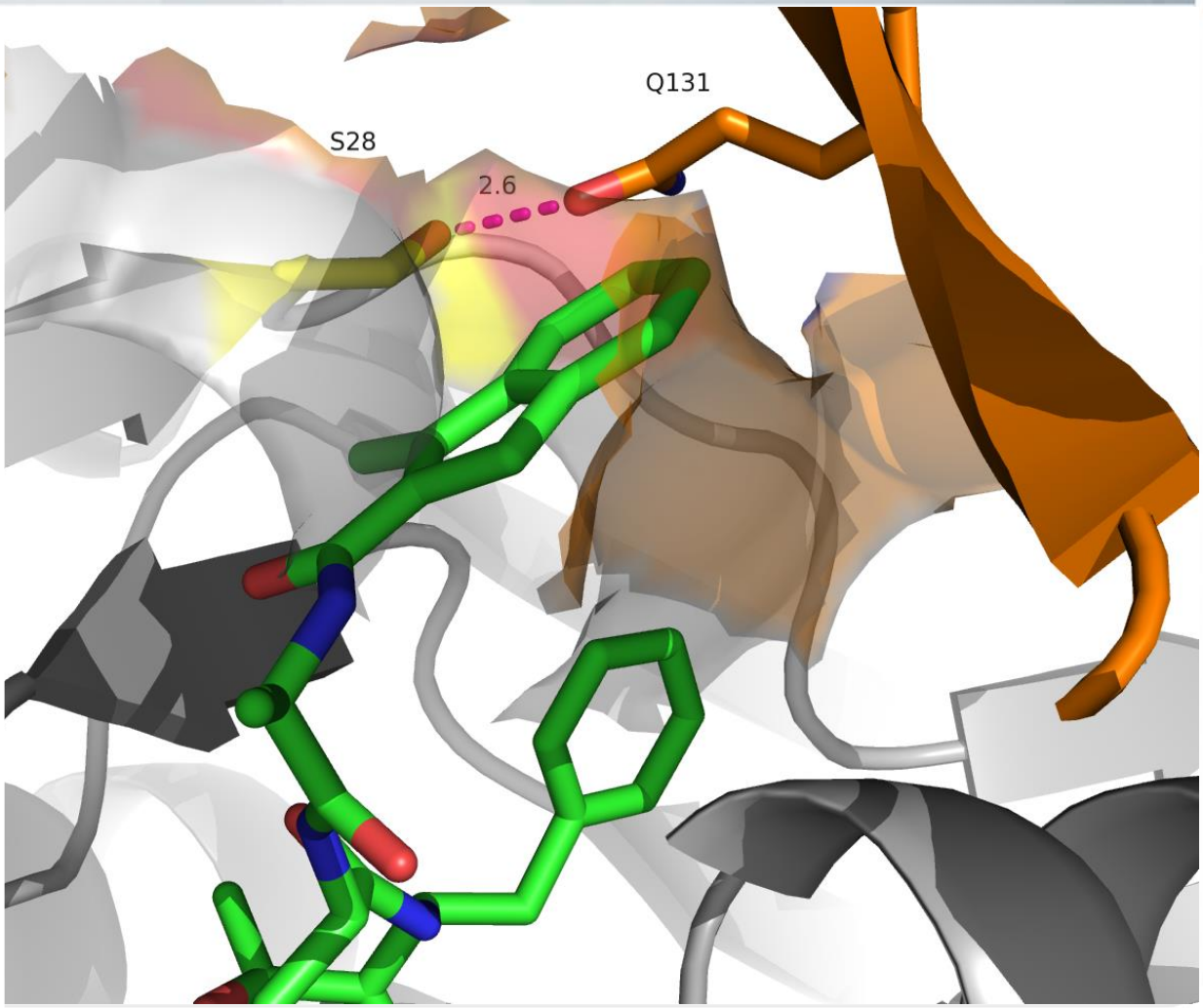
β5/β5i Sequence Differences in Deep S3 Pocket Drive Selectivity of PR-924

- S3 pocket of β5 is restricted by S28:Q131 interaction
- Overlay of PR-924 in β5 disrupts S28:Q131 interaction



ONYX PHARMACEUTICALS
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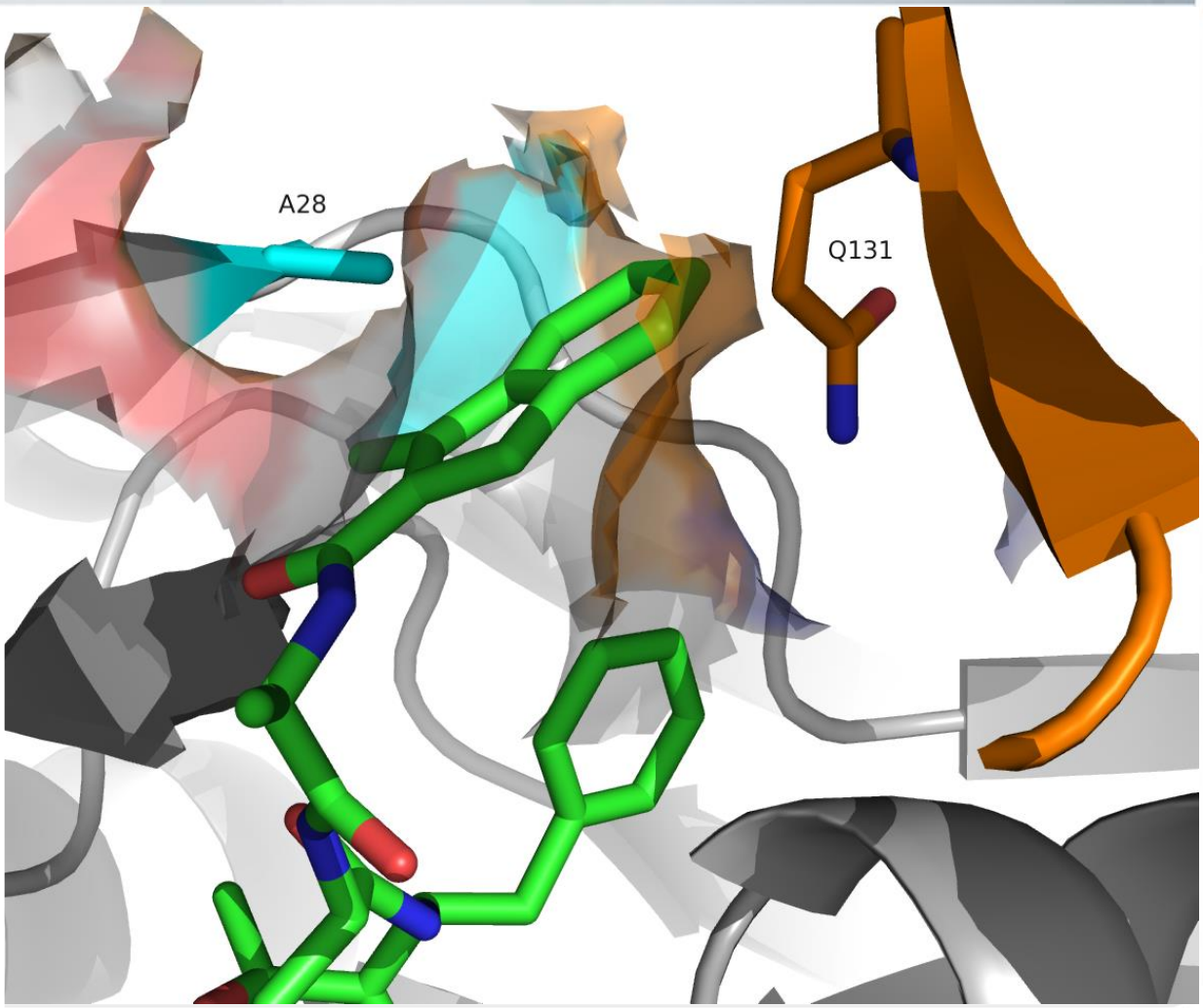
β5/β5i Sequence Differences in Deep S3 Pocket Drive Selectivity of PR-924

- S28A change frees Q131, relaxes the S3 pocket
- S3 pocket of β5i accommodates PR-924



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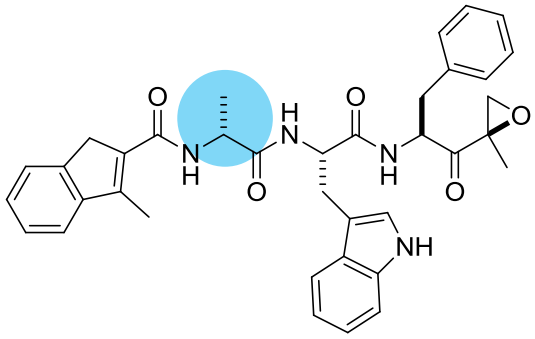
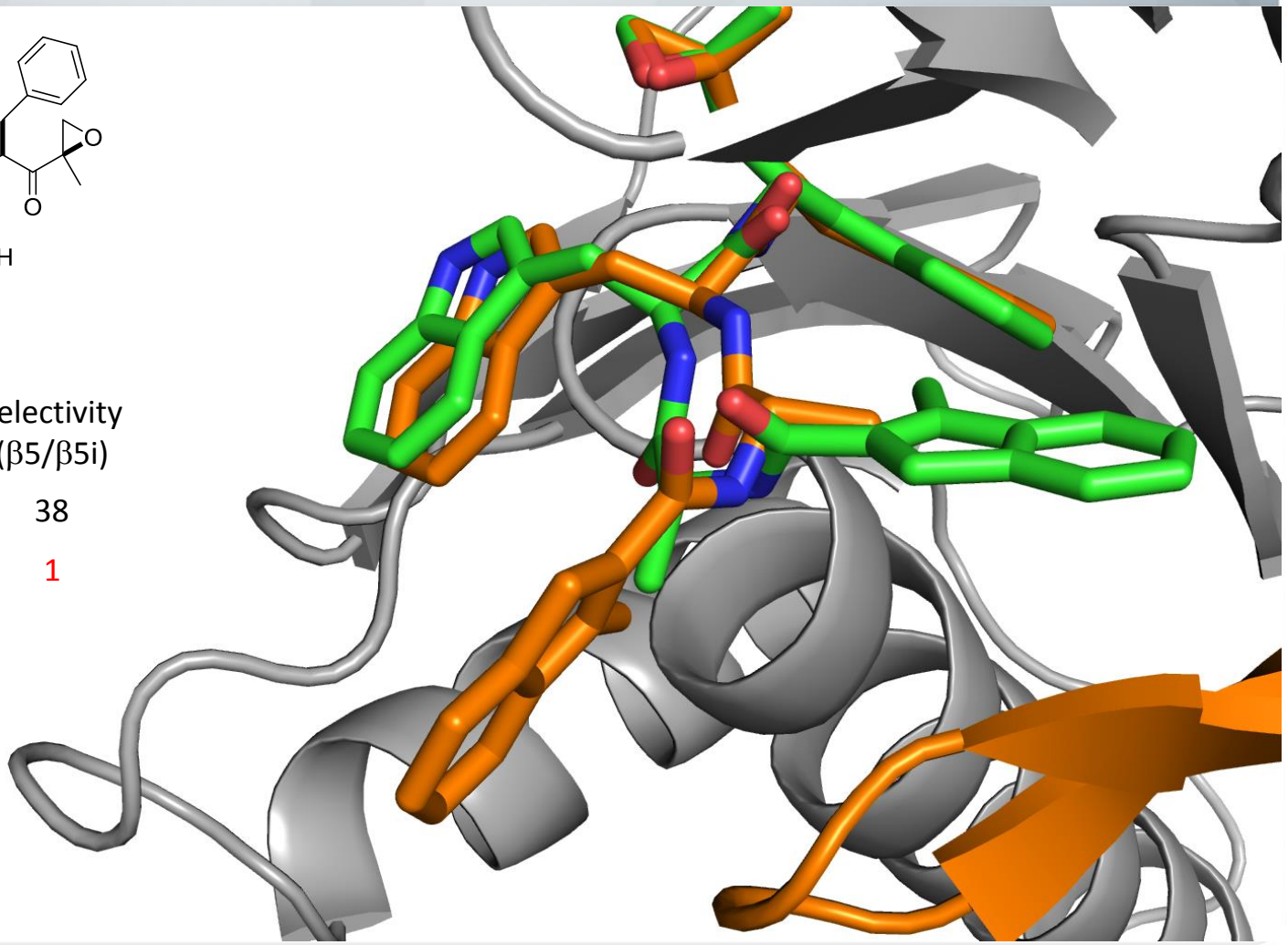
P3 Stereochemistry of PR-924 Dictates Rotation of PR-924 *N*-cap



	Selectivity (β5/β5i)
D-ala (PR-924, green)	38
L-ala (orange)	1



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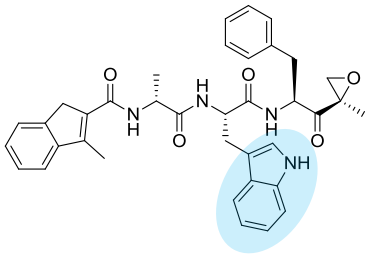
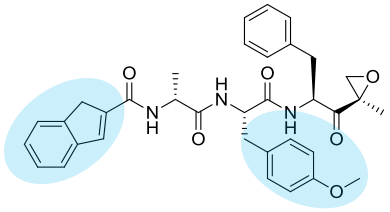
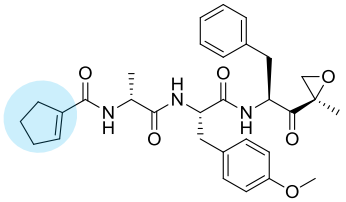
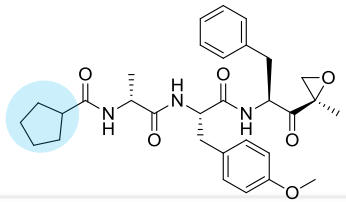
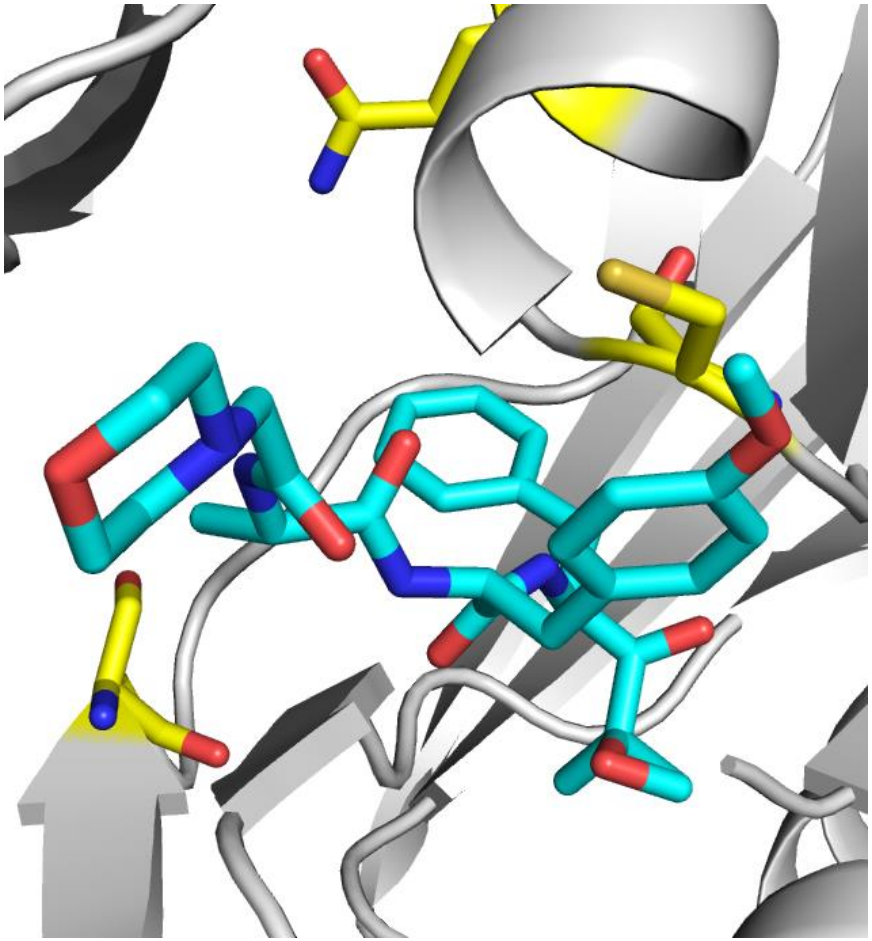
D-Ala *N*-cap SAR

- Hydrophobic burial drives selectivity
- W-P2 offers limited contribution to potency/selectivity
- Selectivity loss with smaller C-terminal tail
- Smaller tails fail to reach the S28:Q131 interaction
- SAR supports a novel binding mode, potentiating the design of highly selective iPI's

Structure	ProCISE LMP7 MOLT4 lysate IC_{50} (nM)	β5i:β5c Selectivity
	34	38
	57	41
	539	8
	59	8

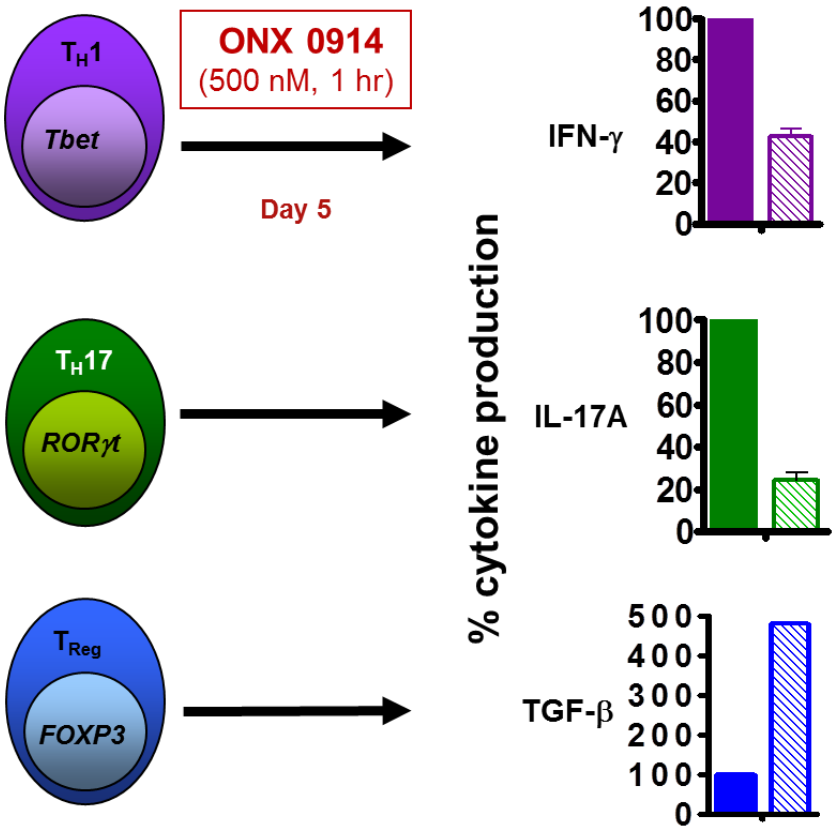
Summary

- Selective immunoproteasome inhibition represents a potentially broad based anti-inflammatory therapy



Summary

- Selective Immunoproteasome inhibition represents a potentially broad based anti-inflammatory therapy
  - β5i selective compounds inhibit cytokine production



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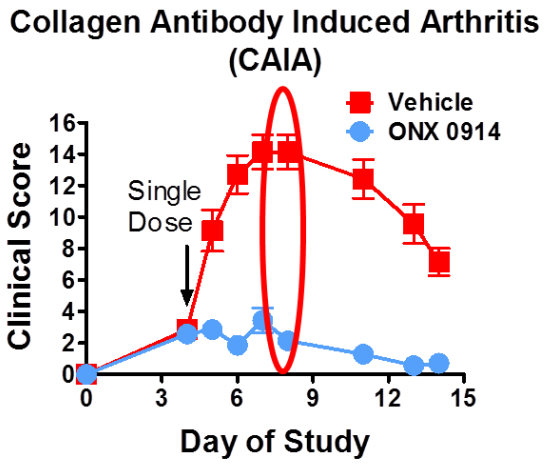
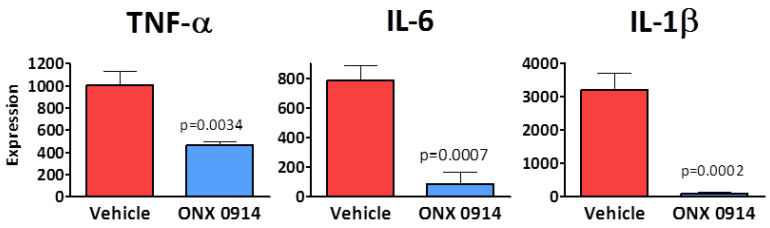
Summary

- Selective Immunoproteasome inhibition represents a potentially broad based anti-inflammatory therapy
  - β5i selective compounds inhibit cytokine production
  - Efficacious in relevant animal models of disease (RA, SLE)





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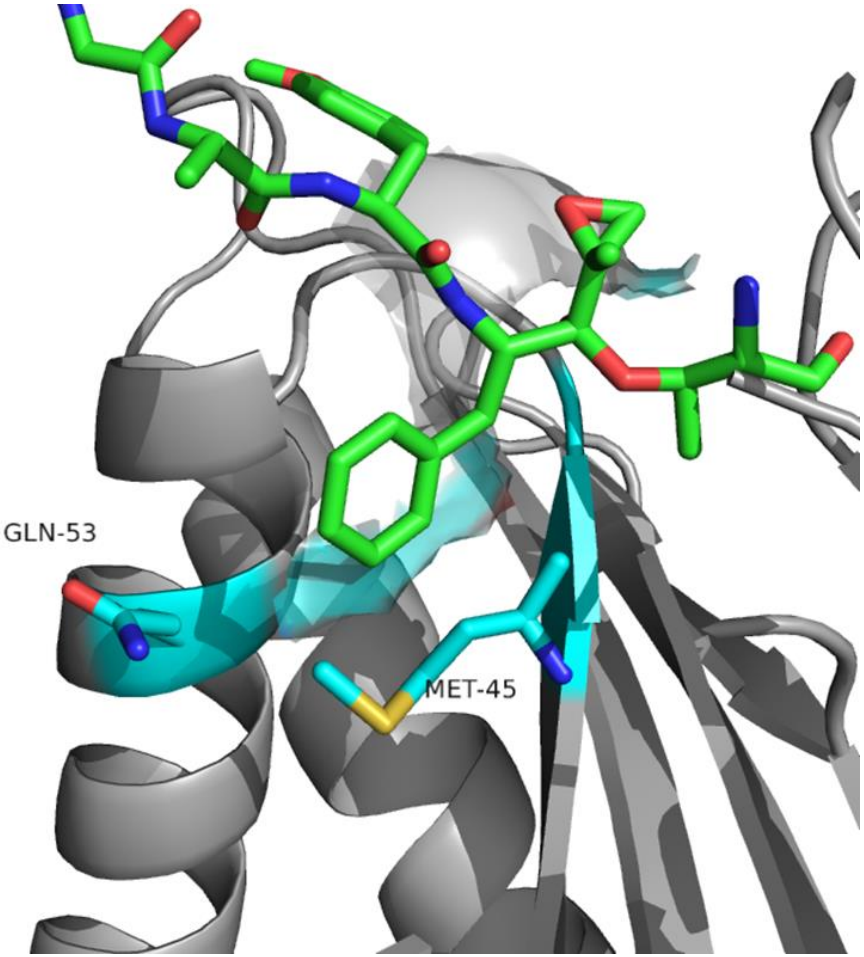
Summary

- Selective Immunoproteasome inhibition represents a potentially broad based anti-inflammatory therapy
  - β5i selective compounds inhibit cytokine production
  - Efficacious in relevant animal models of disease (RA, SLE)
- Constitutive & immunoproteasome /0914 crystal structures clarify a basis for β5/β5i selectivity



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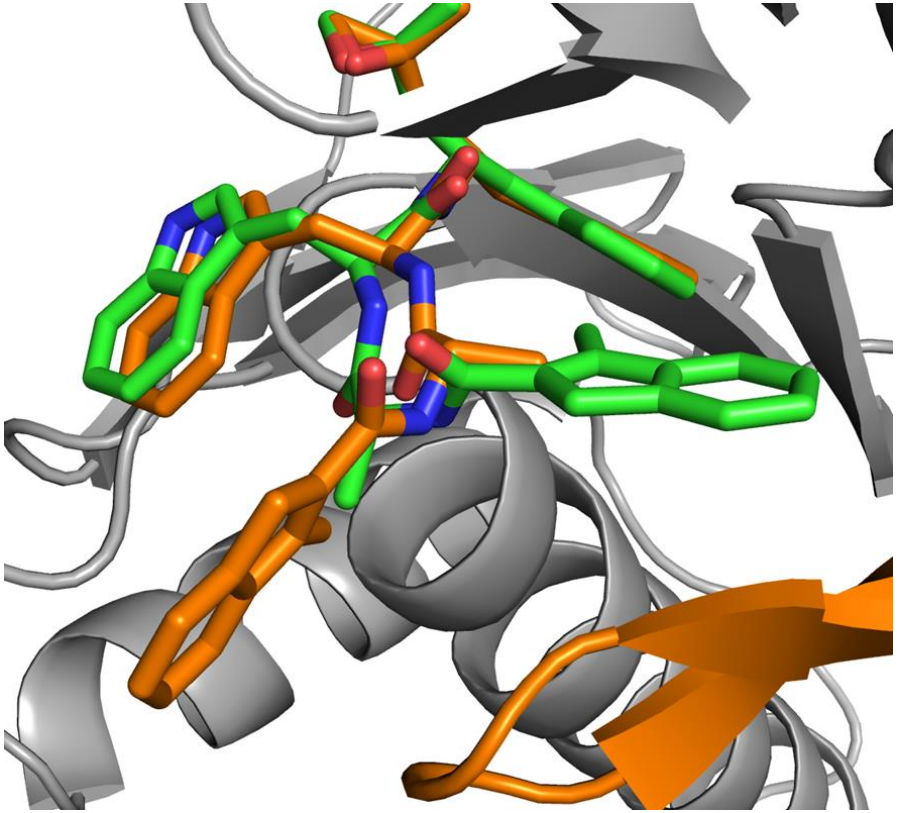
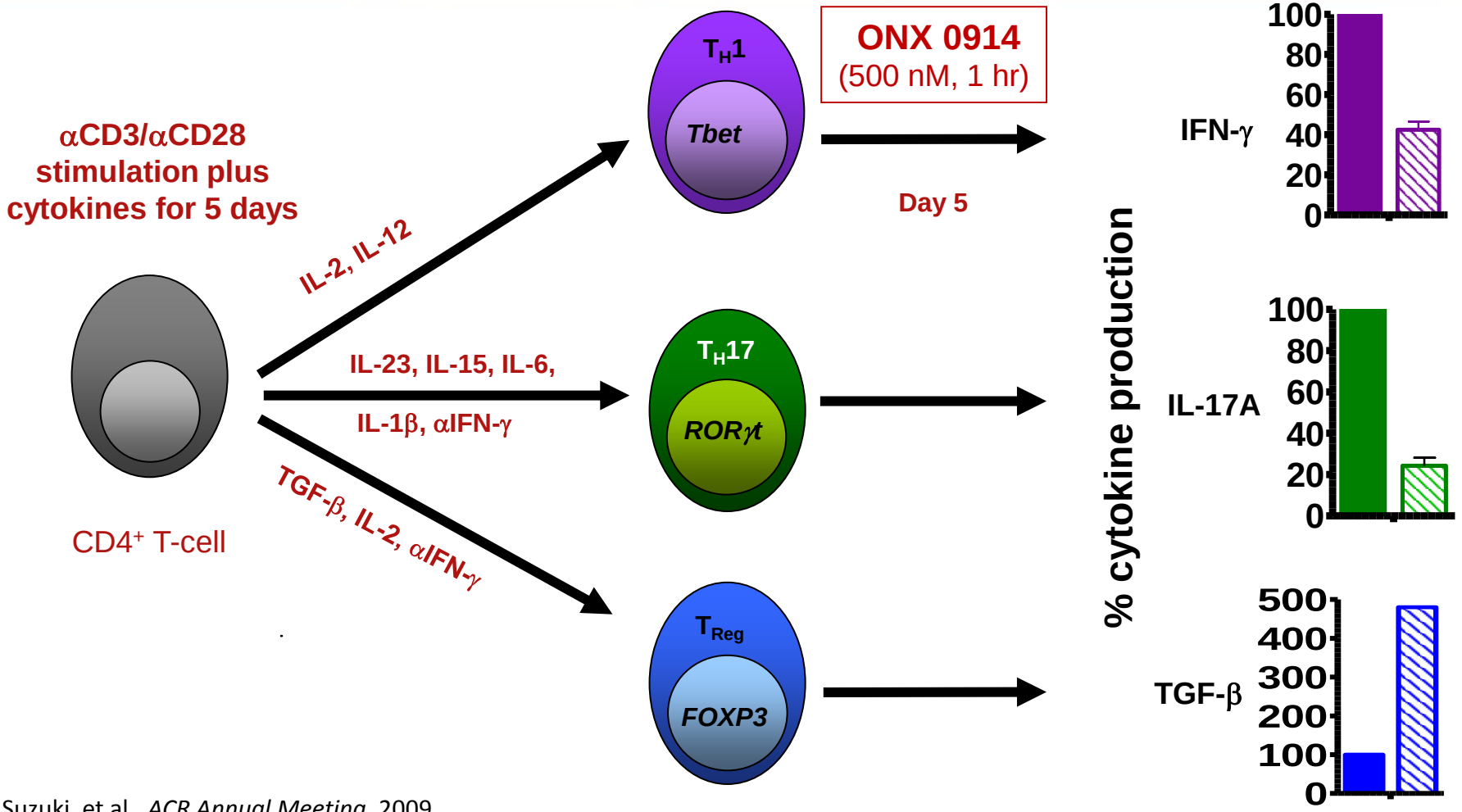
Summary

- Selective Immunoproteasome inhibition represents a potentially broad based anti-inflammatory therapy
  - β5i selective compounds inhibit cytokine production
  - Efficacious in relevant animal models of disease (RA, SLE)
- Constitutive & immunoproteasome /0914 crystal structures clarify a basis for β5/β5i selectivity
- Exploiting residue differences in human homology model may potentiate design of highly selective immunoproteasome inhibitors with novel binding mode



Supplemental

Immunoproteasome Inhibition Impacts Cytokine Production in Differentiated T-cells



Suzuki, et al., *ACR Annual Meeting,* 2009

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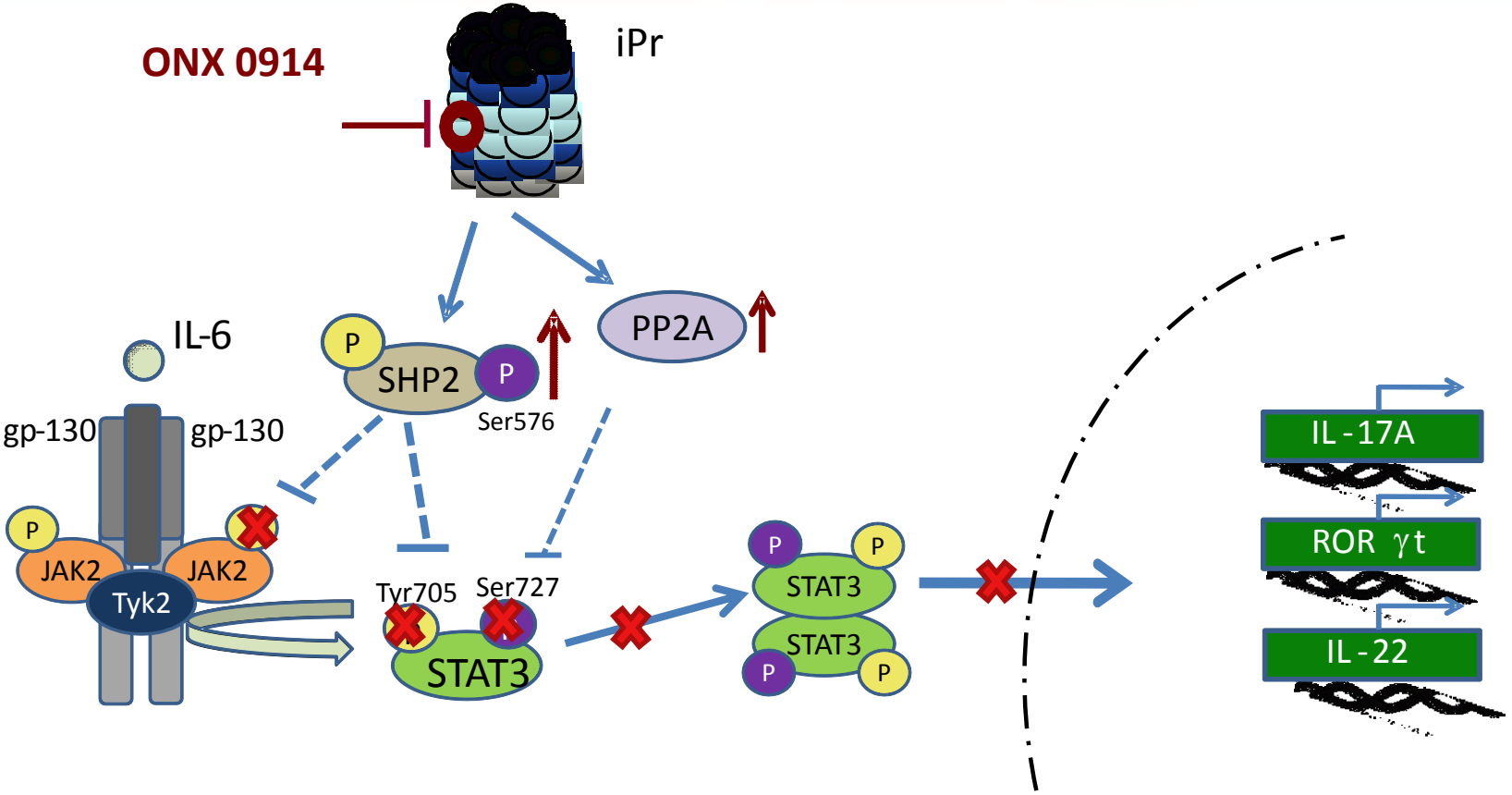
Role of Immunoproteasome Inhibition in Th17 Signaling



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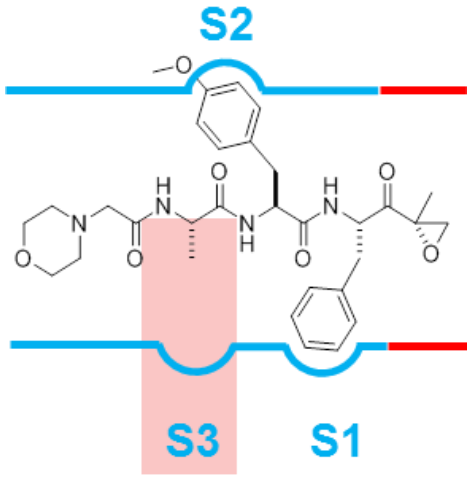
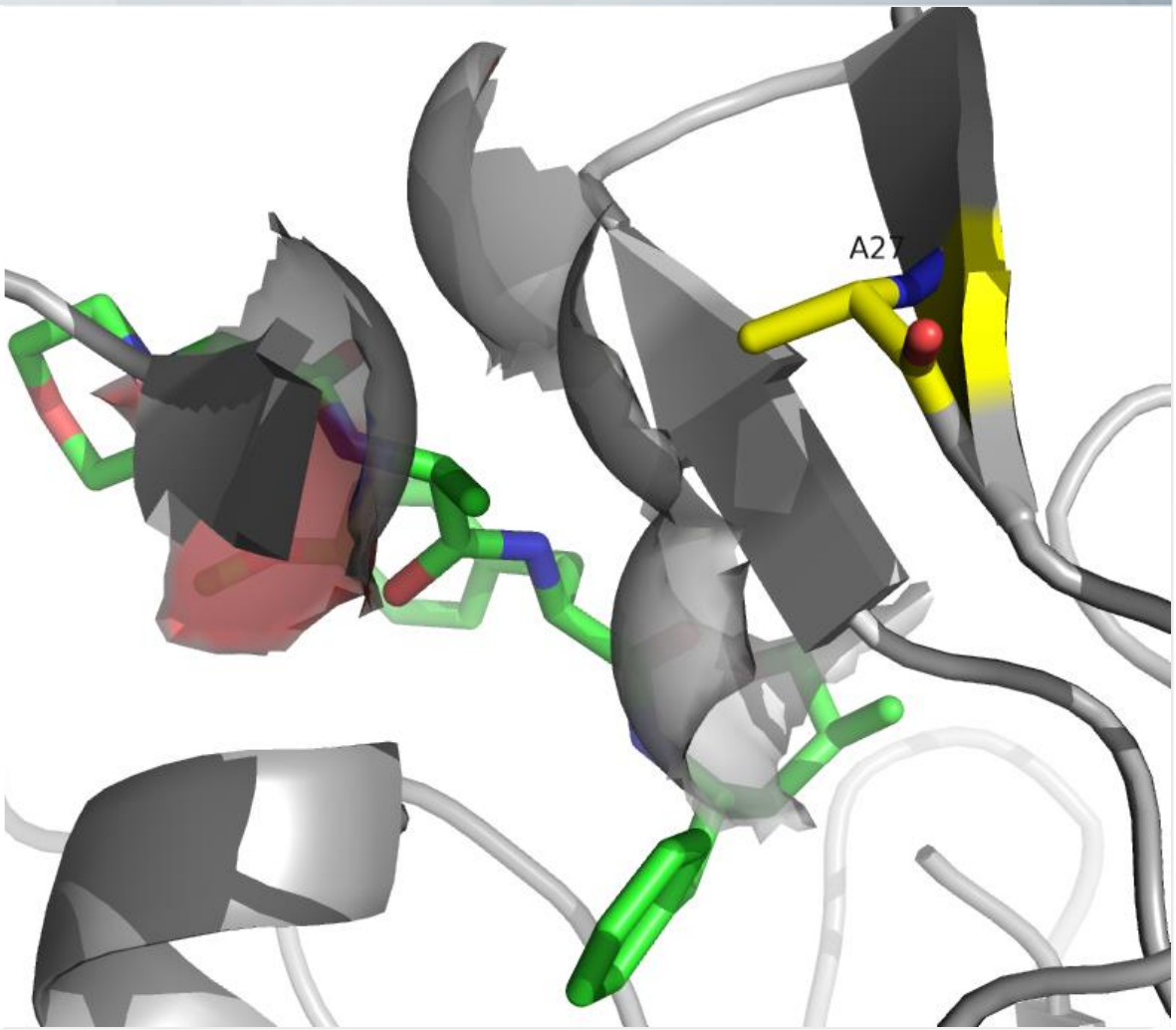
S3 pocket of β5 Subunit

- Ala at 27 position





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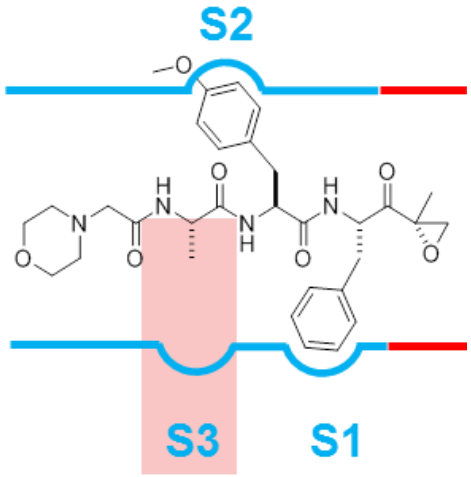
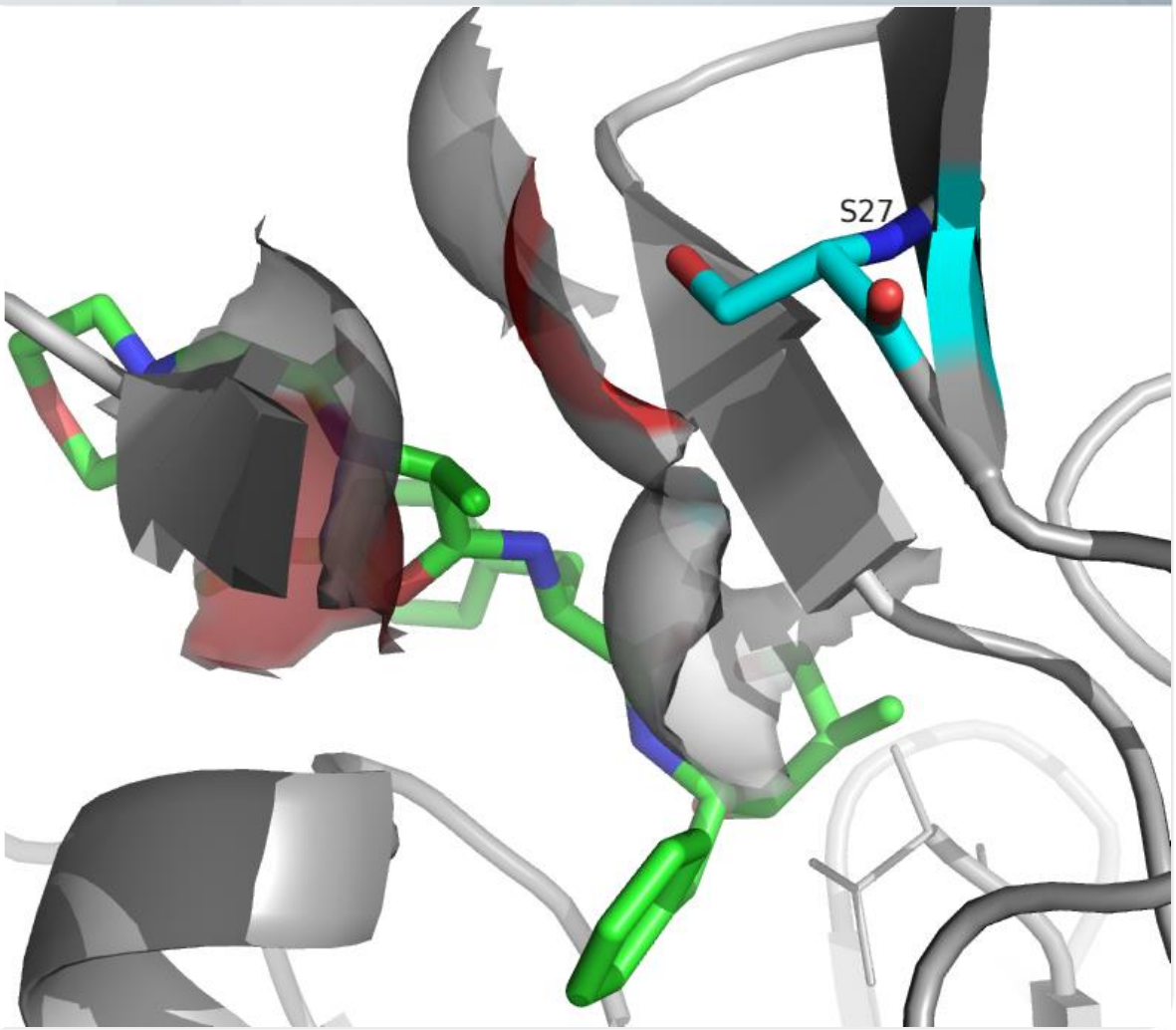
S3 pocket of β5i *vs.* β5

- S27 in β5i "restricts" size of S3 pocket, invokes hydrophilic character
- Underutilized selectivity determinant?





ONYX PHARMACEUTICALS
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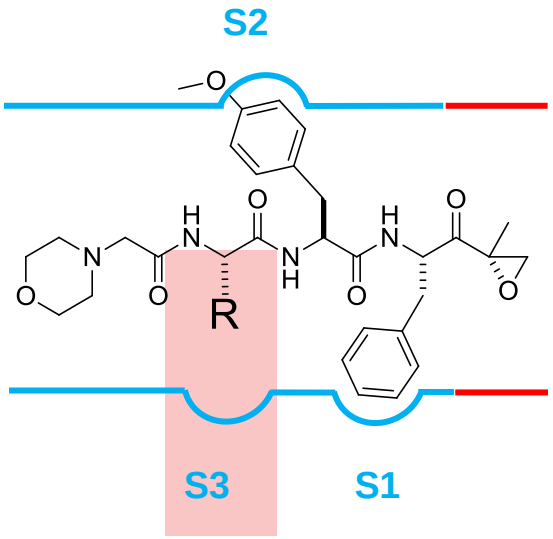
P3 SAR



R	ProCISE LMP7 MOLT4 lysate IC_{50} (nM)	β5i:β5 Selectivity
	21	12
	209	20

- Substitution overcomes entropy
- P3S, comparable potency and selectivity, solubility handle
- Attempts to further direct polar functionality erodes selectivity