University of Florida College of Medicine/X6a: Basic and Translational Research Projects

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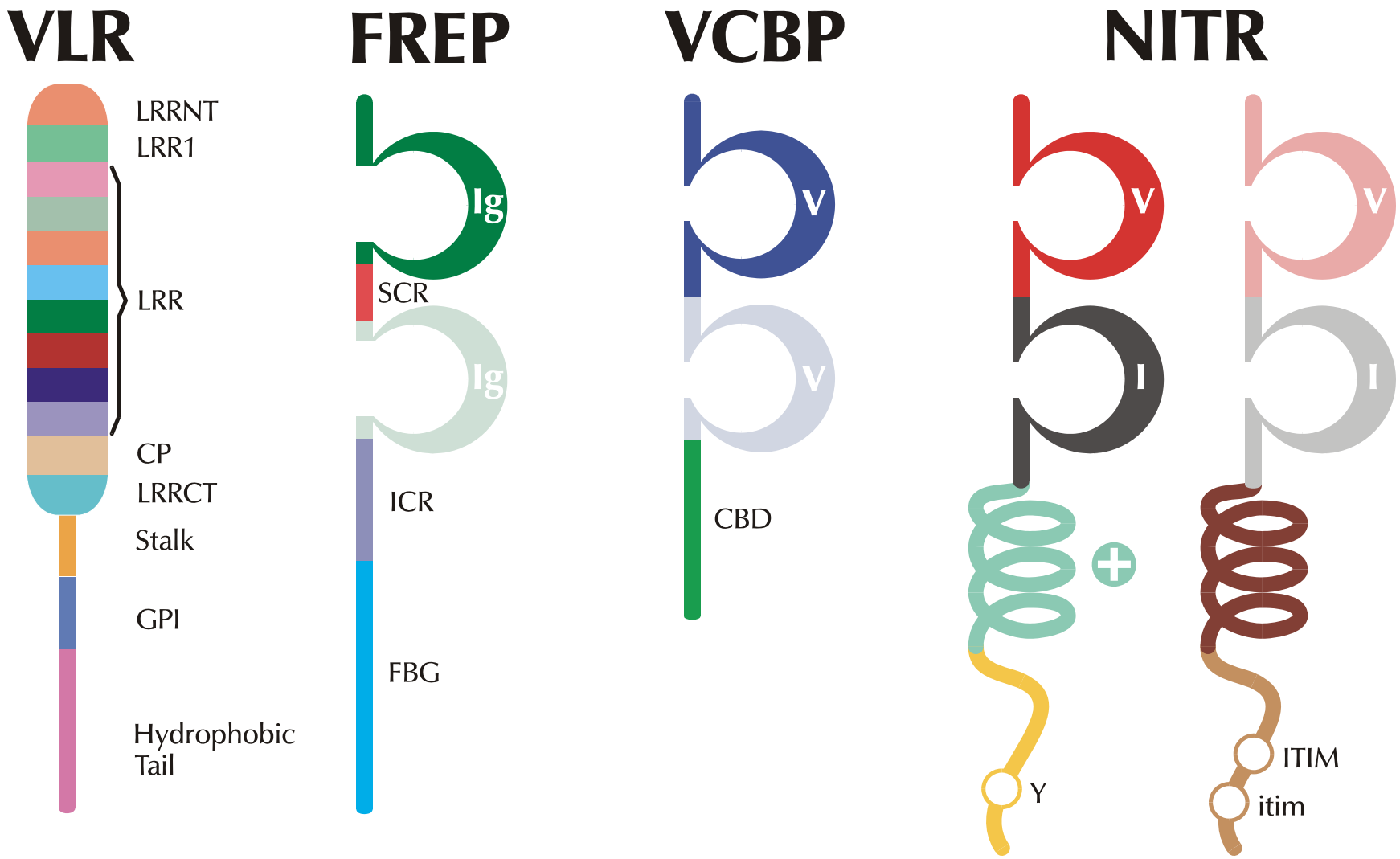
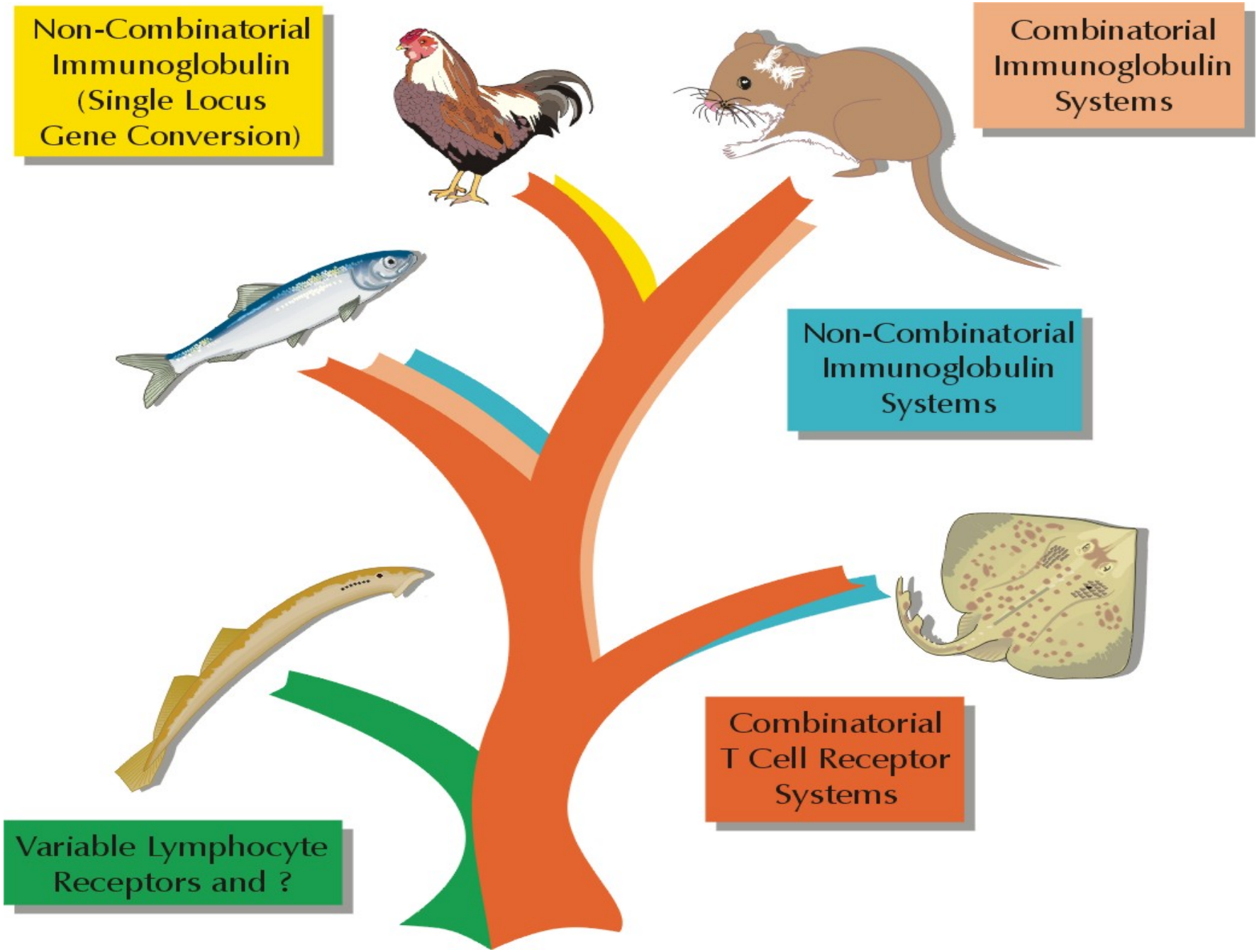
University of Florida College of Medicine/X6a: Basic and Translational Research Projects

- Basic Research
  - Evolution of Adaptive Immunity
    - VCBP3-Variable Chitin-Binding Protein
      - PDB codes-1XT5, 2FBO
    - NITR-Novel Immune-Type Receptors
      - PDB codes-2QHL, 2QJD, 2QQQ, 2QTE, 2B5T, 3BDB
- Translational Research
  - Structure-based Drug Design
    - Cancer target-Focal Adhesion Kinase
      - PDB code-2RA7
    - Type I Diabetes-Insulin:sulfatide complex
      - PDB code-3BRR

	Drosophila (insect)	Sea urchin (echinoderm)	Sea squirts (ascidian)	Lamprey (agnathan)	Shark (chon drichthyes)	Carp (teleost)	Frog (amphibian)	Snake (reptile)	Chicken (bird)	Human (mammal)
Acquired immunity	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Immunoglobulin rearrangement	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Combinatorial T-cell receptor rearrangement	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Polymorphic MHC molecules	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Classical complement pathway	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes
C3 and factor B	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Mannose-binding lectin	No	?	Yes	Inferred	Inferred	Yes	Inferred	Inferred	Yes	Yes
Ficolins	No	?	Yes	Inferred	Inferred	Inferred	Inferred	Inferred	Inferred	Yes
MASPs	No	?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Toll-like receptors	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Antibacterial peptides	Yes	Inferred	Inferred	Inferred	Inferred	Inferred	Yes	Yes	Yes	Yes

Figure 15-10 Immunobiology, 6/e. (© Garland Science 2005)


VLR
LRRNT
LRR1
LRR
CP
LRRCT
Stalk
GPI
Hydrophobic
Tail
FREP
Ig
SCR
Ig
ICR
FBG
VCBP
V
V
CBD
NITR
V
I
Y
V
I
ITIM
itim


Non-Combinatorial Immunoglobulin (Single Locus Gene Conversion)
Combinatorial Immunoglobulin Systems
Non-Combinatorial Immunoglobulin Systems
Combinatorial T Cell Receptor Systems
Variable Lymphocyte Receptors and ?

Identification of diversified genes that contain immunoglobulin-like variable regions in a protochordate

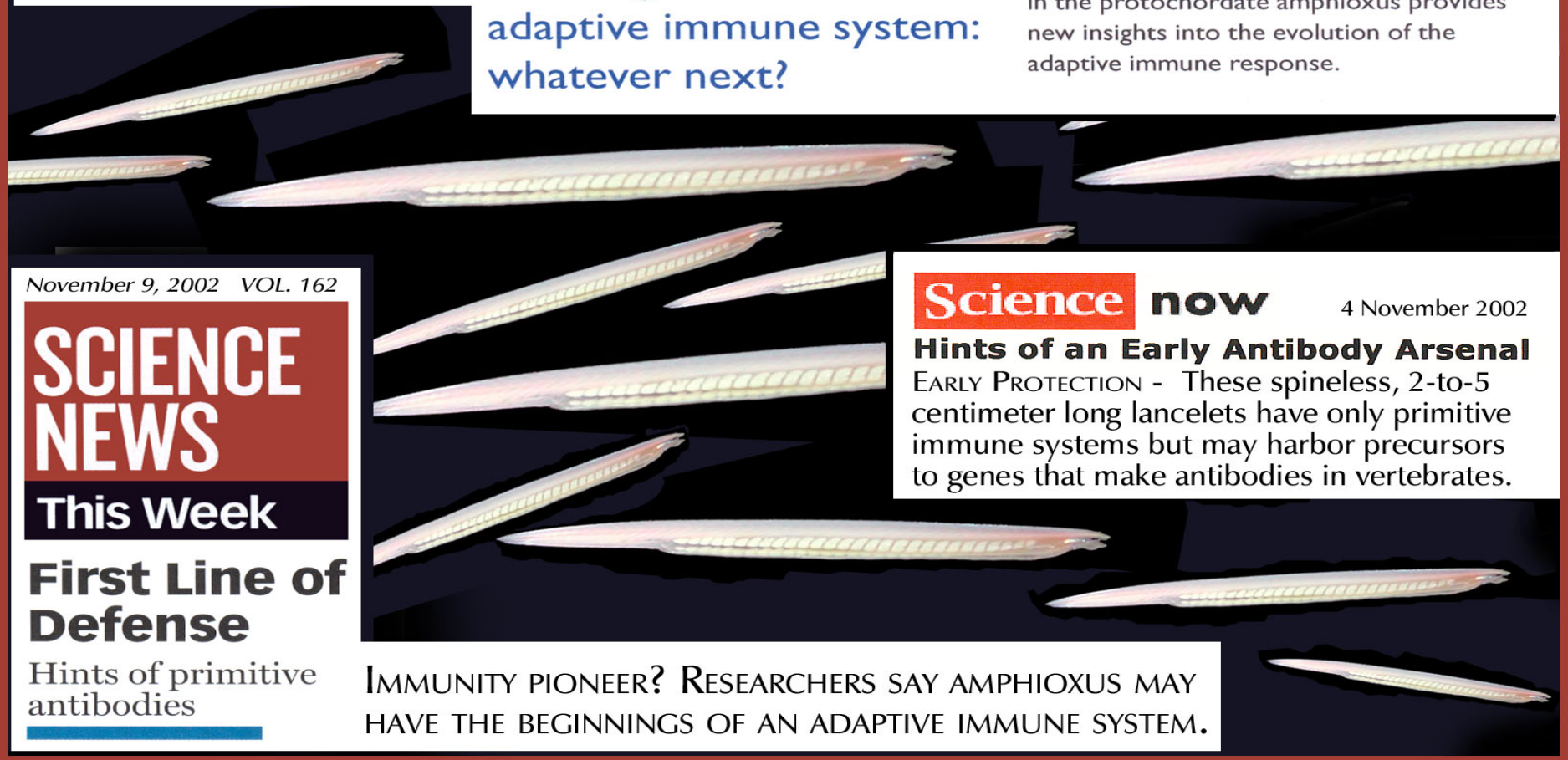
John P. Cannon[1,2], Robert N. Haire[3] and Gary W. Litman[1–3]

Published online 4 November 2002; doi:10.1038/ni849

NEWS & VIEWS

The origins of the adaptive immune system: whatever next?

The discovery of a V-like Ig multigene family in the protochordate amphioxus provides new insights into the evolution of the adaptive immune response.



November 9, 2002 VOL. 162

SCIENCE NEWS

This Week

First Line of Defense

Hints of primitive antibodies

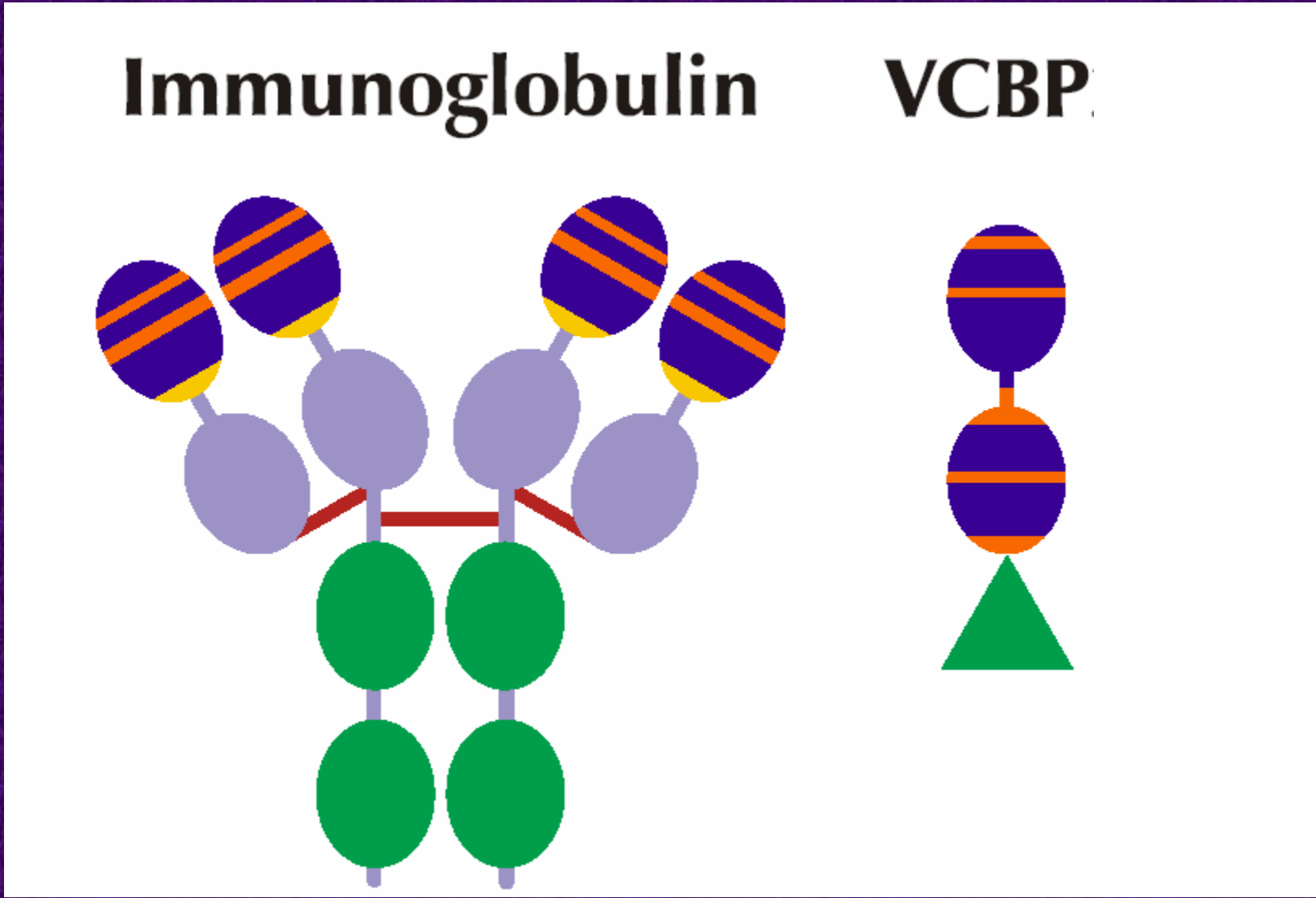
Science now 4 November 2002

Hints of an Early Antibody Arsenal

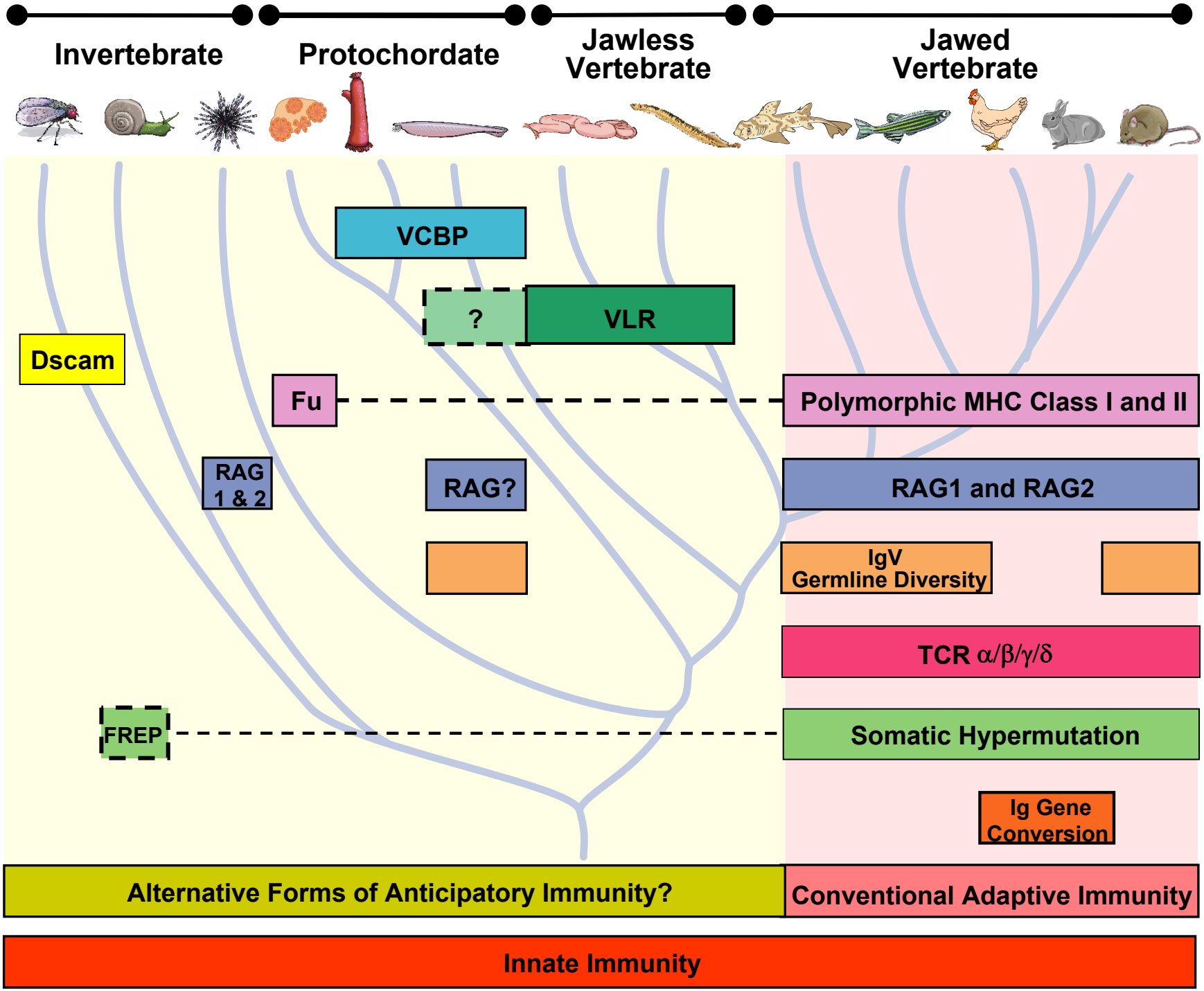
EARLY PROTECTION - These spineless, 2-to-5 centimeter long lancelets have only primitive immune systems but may harbor precursors to genes that make antibodies in vertebrates.

IMMUNITY PIONEER? RESEARCHERS SAY AMPHIOXUS MAY HAVE THE BEGINNINGS OF AN ADAPTIVE IMMUNE SYSTEM.

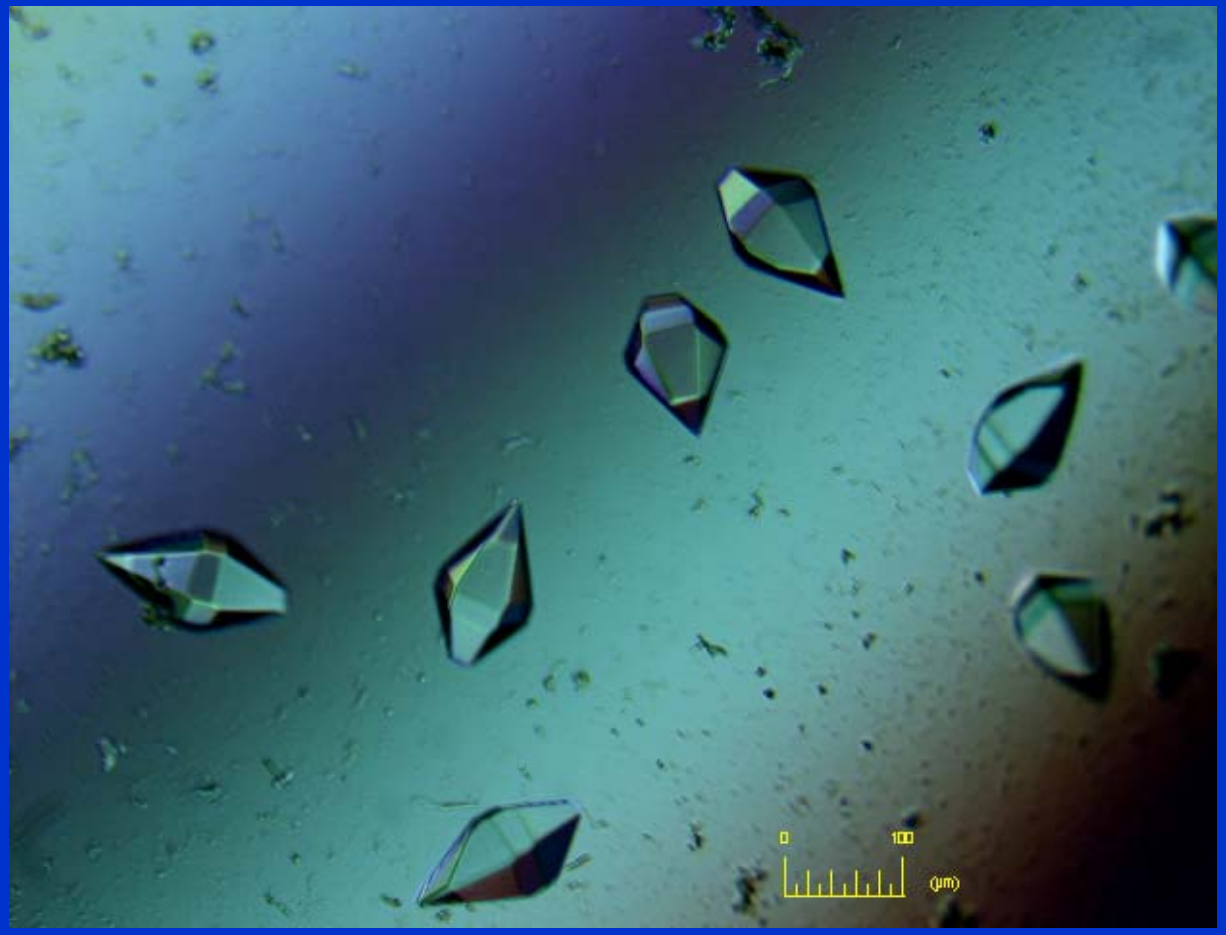
VCBPs have characteristics of a "primordial" antigen receptor



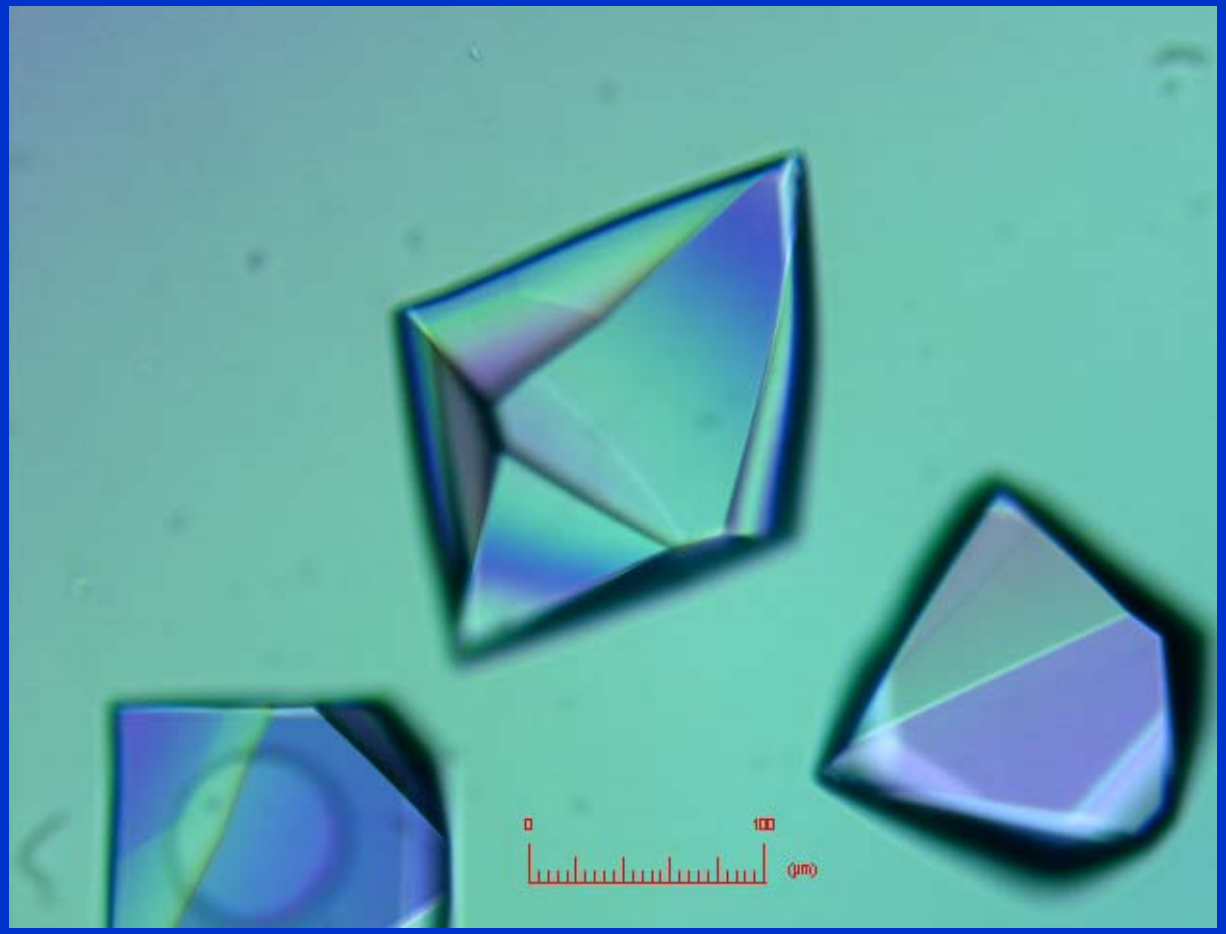
Amphioxus (*Branchiostoma floridae*)


Invertebrate
Protochordate
Jawless Vertebrate
Jawed Vertebrate
VCBP
?
VLR
Dscam
Fu
Polymorphic MHC Class I and II
RAG 1 & 2
RAG?
RAG1 and RAG2
IgV Germline Diversity
TCR α/β/γ/δ
FREP
Somatic Hypermutation
Ig Gene Conversion
Alternative Forms of Anticipatory Immunity?
Conventional Adaptive Immunity
Innate Immunity

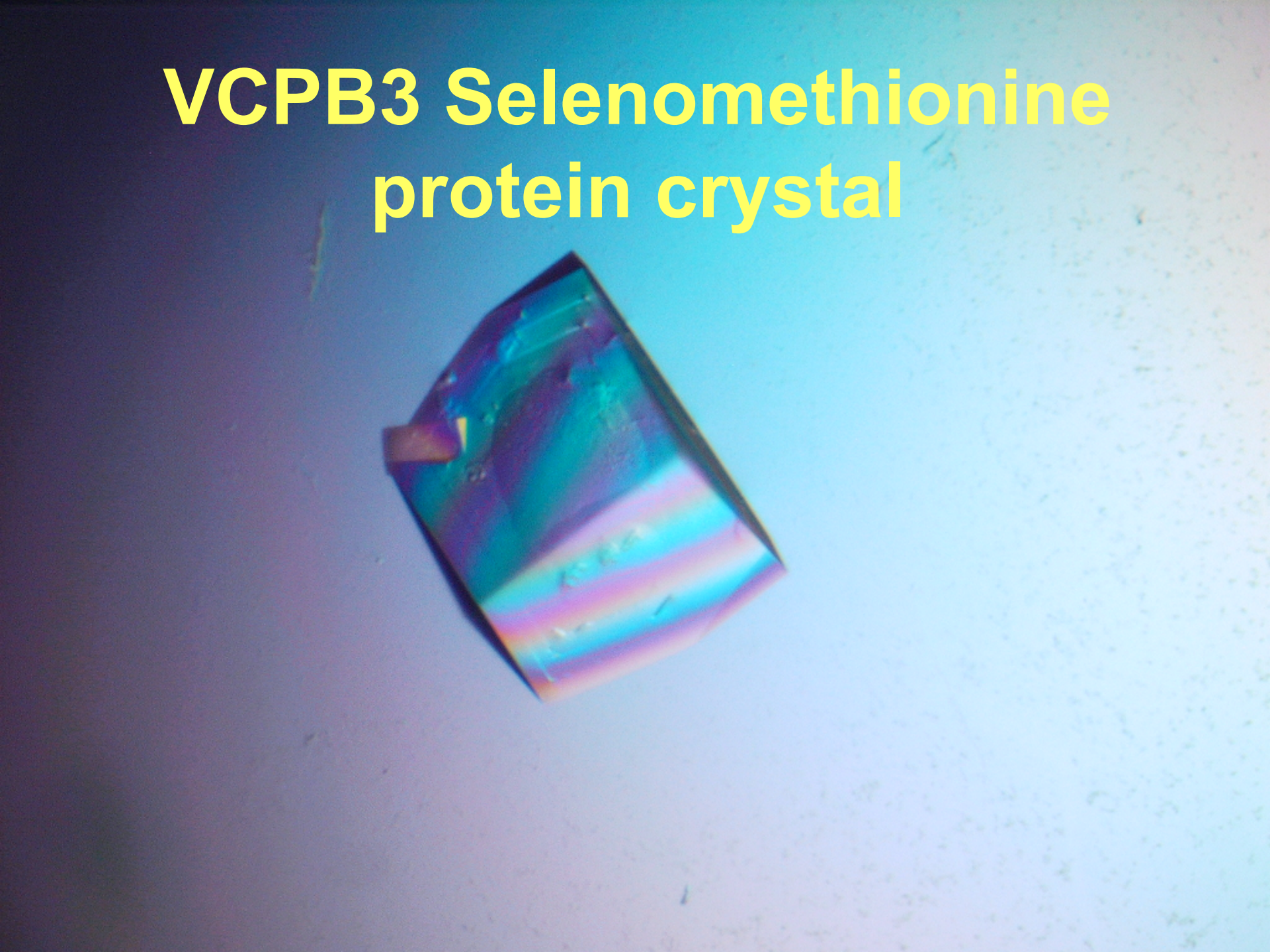
VCBP3-Cryoscreen, PEG 1500, 20 % glycerol



VCBP3-Second round of optimization, Na Acetate, pH 7.5



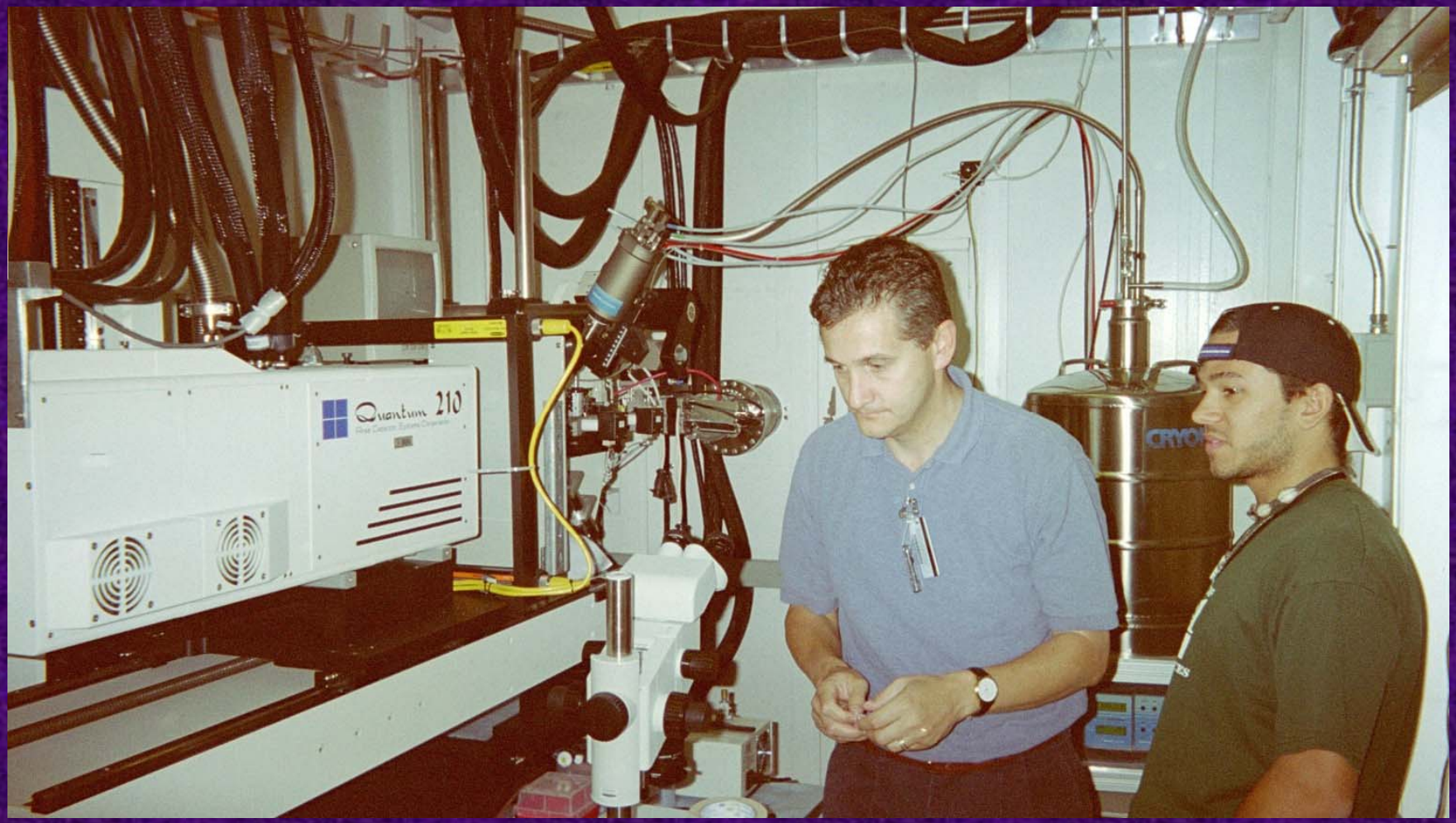
VCPB3 Selenomethionine protein crystal



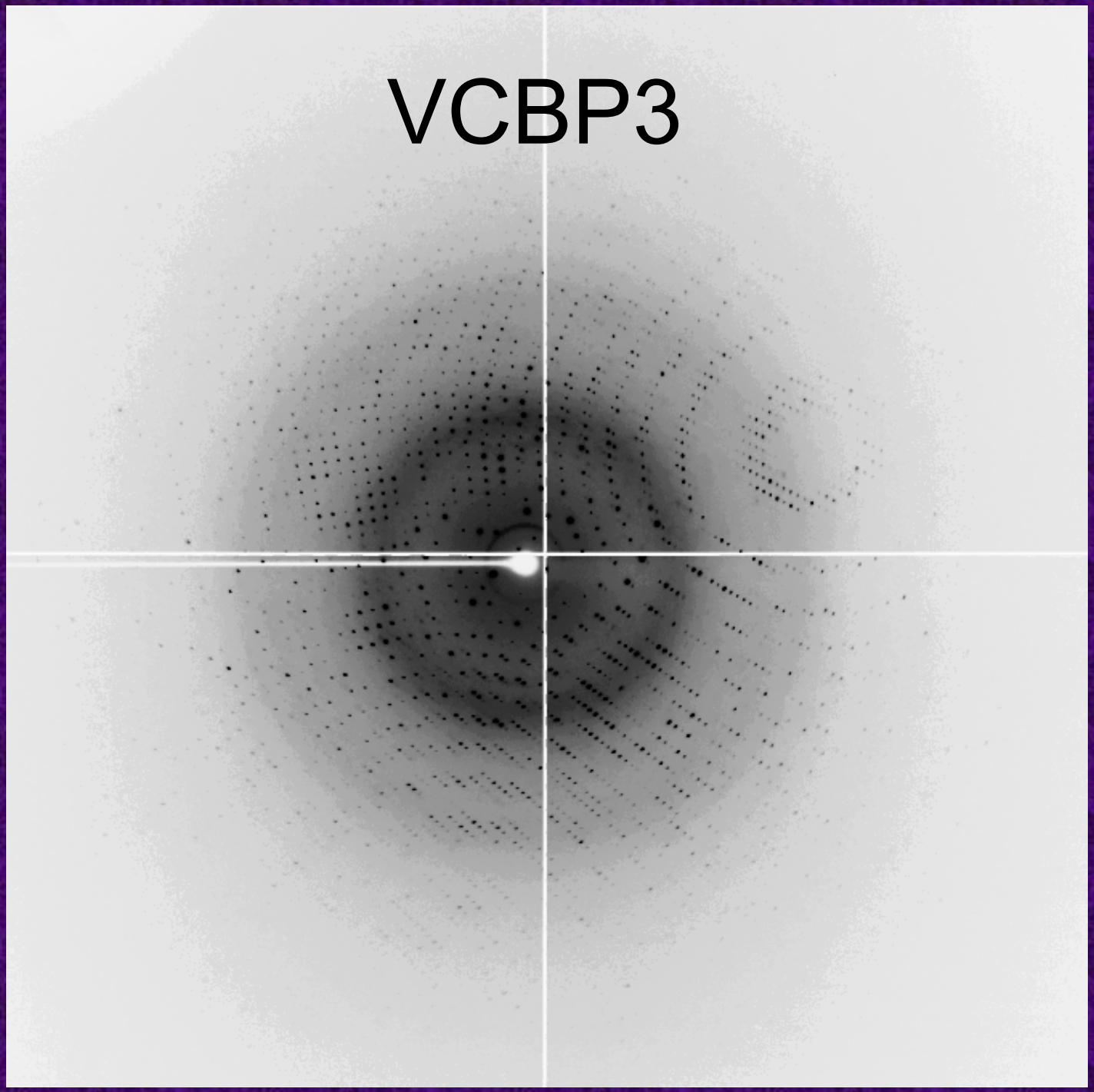
VCBP3 D1 crystal structure

- X-ray data
  - National Synchrotron Light Source, Brookhaven National Laboratory, beamline X6A
  - 3 λ MAD experiments
  - Native x-ray data
- Phase determination
  - SAD and MAD (SOLVE)
  - Solvent flattening
  - Automated chain tracing (81%)
  - Manual building

Data collection

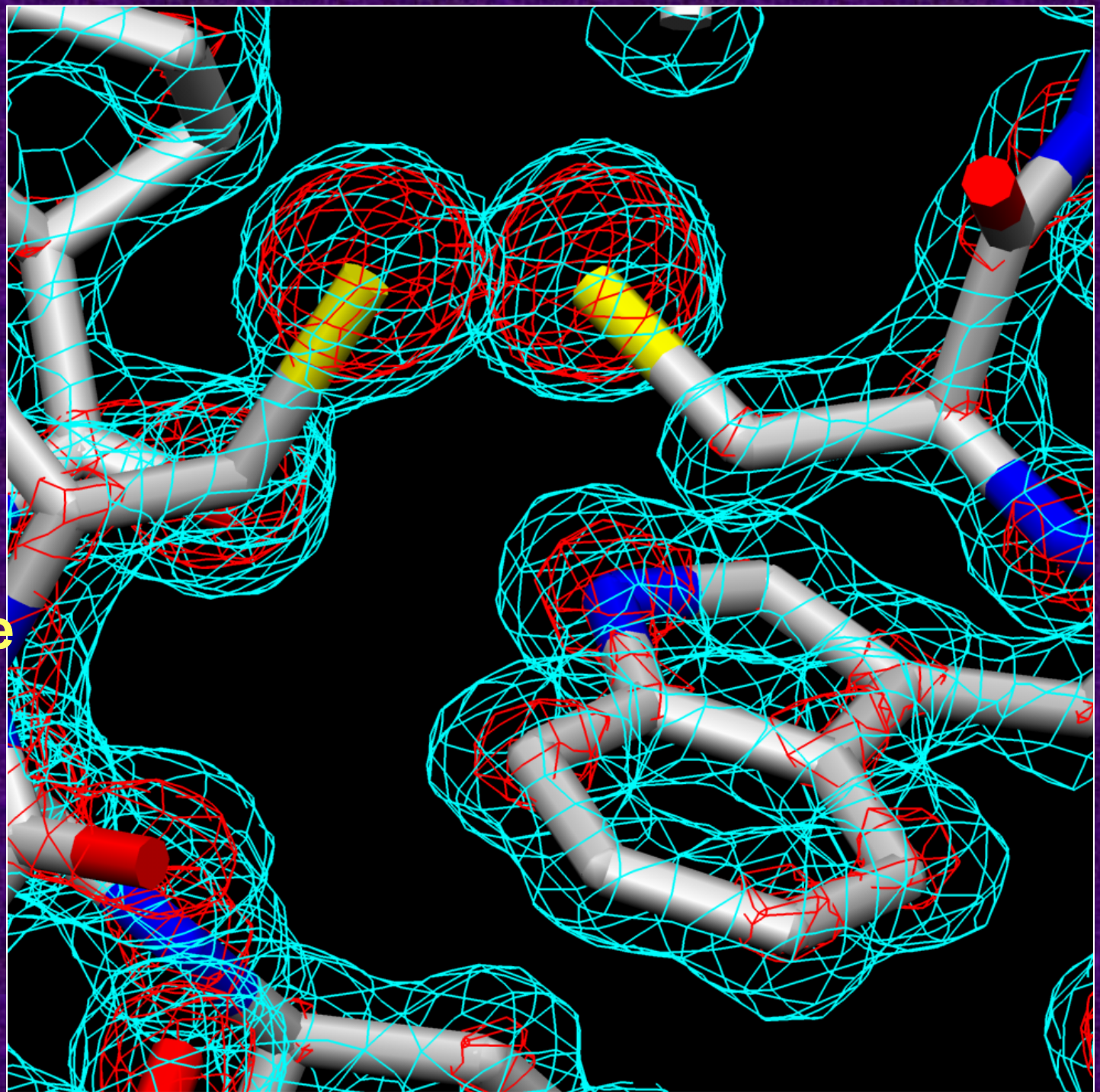


VCBP3



Structure Determination

Trp42 packed against Cys27-Cys110 disulfide bond (BF)



PRELIMINARY DATA: Data collection and reduction statistics

Table 3. Data Collection and Reduction Statistics for VCBP3 crystals.
*Values for the highest resolution shell are in parenthesis.

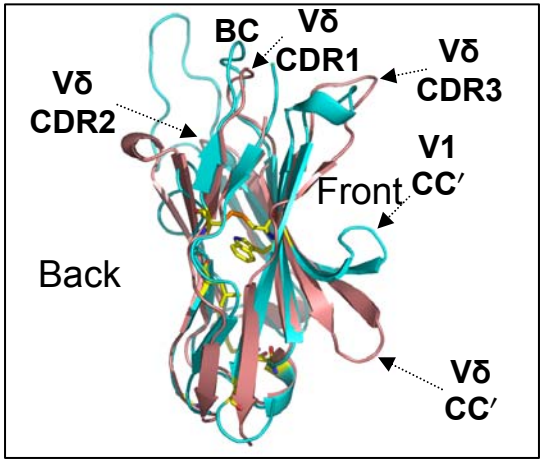
	Selenomethionine Derivative			Native
Wavelength (A)	0.97960 (inflection)	0.97908 (peak)	0.95007 (remote)	0.80000
No. of frames	360	360	360	360
Detector distance (mm)	100	100	100	110
Oscillation step (degrees)	1	1	1	1
Observed reflections	1,732,169	1,788,754	1,767,260	2,010,078
Unique reflections	103,915	103,983	89,107	57,420
Redundancy	8.7	8.7	9.3	14
Resolution range (A)	30-1.17	30-1.17	30-1.25	30-1.15
	(1.18-1.17)	(1.18-1.17)	(1.26-1.25)	(1.16-1.15)
Space group	$P3_121$	$P3_121$	$P3_121$	$P3_121$
Cell parameters	a,b=59.16	a,b=59.13	a,b=59.86	a,b=59.19
	c=79.35	c=79.30	c=80.28	c=79.27
Mosaicity (degrees)	0.54	0.54	0.54	0.70
(I/sigma)average	41.9(2.6)	49.2(3.2)	42.3(4.4)	59.3(6.0)
Reflections (%) >3σ	77.8(27.6)	79.8(35.5)	81.6(50.5)	81.8(56.3)
Completeness (%)	99.1(85.5)	99.3(89.9)	100(100)	99.8(99.7)
Rsym (%)	5.2(34.1)	4.6(31.1)	5.9(37.3)	4.9(37.0)

VCBP3 D1 crystal structure

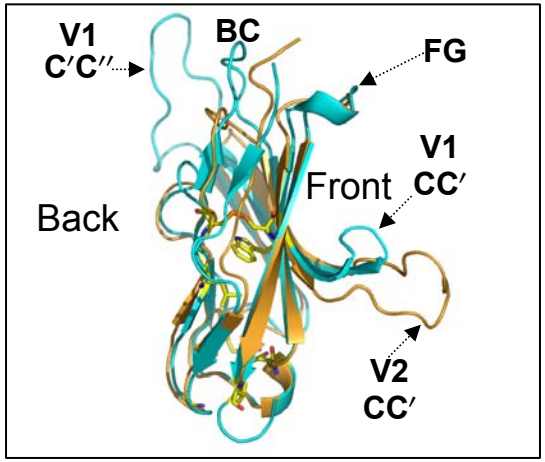
- Refinement
  - Rigid body
  - CNS to 1.5 Å
  - SHELX to 1.15 Å

- Structural Analysis
  - DSSP
  - DALI
  - PARVATI
  - HBPLUS, NCI

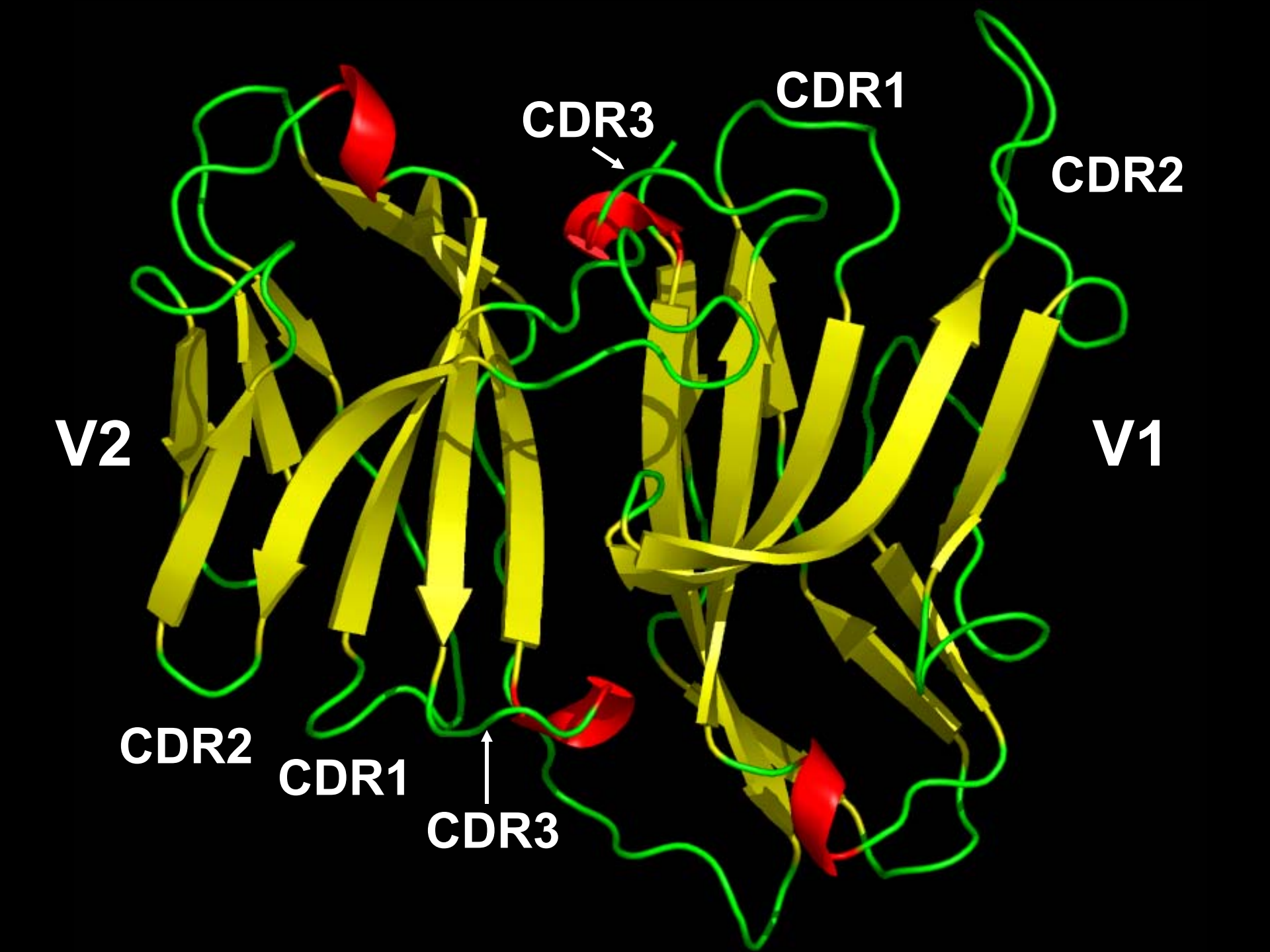
TCR



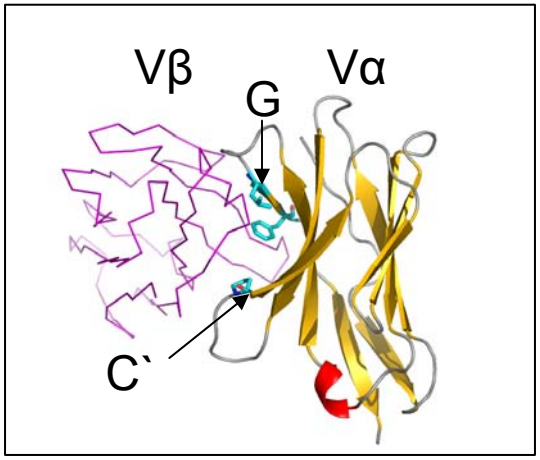
VCBP3



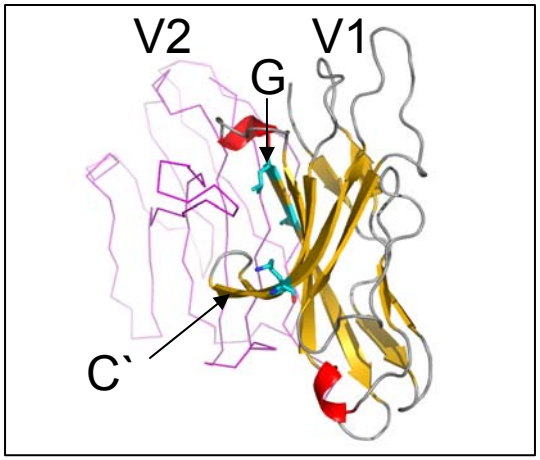
VCBP3, expressed in amphioxus, which lacks an adaptive immune system, is most structurally similar to a TCR Vδ domain.


CDR1
CDR3
CDR2
V2
V1
CDR2
CDR1
CDR3

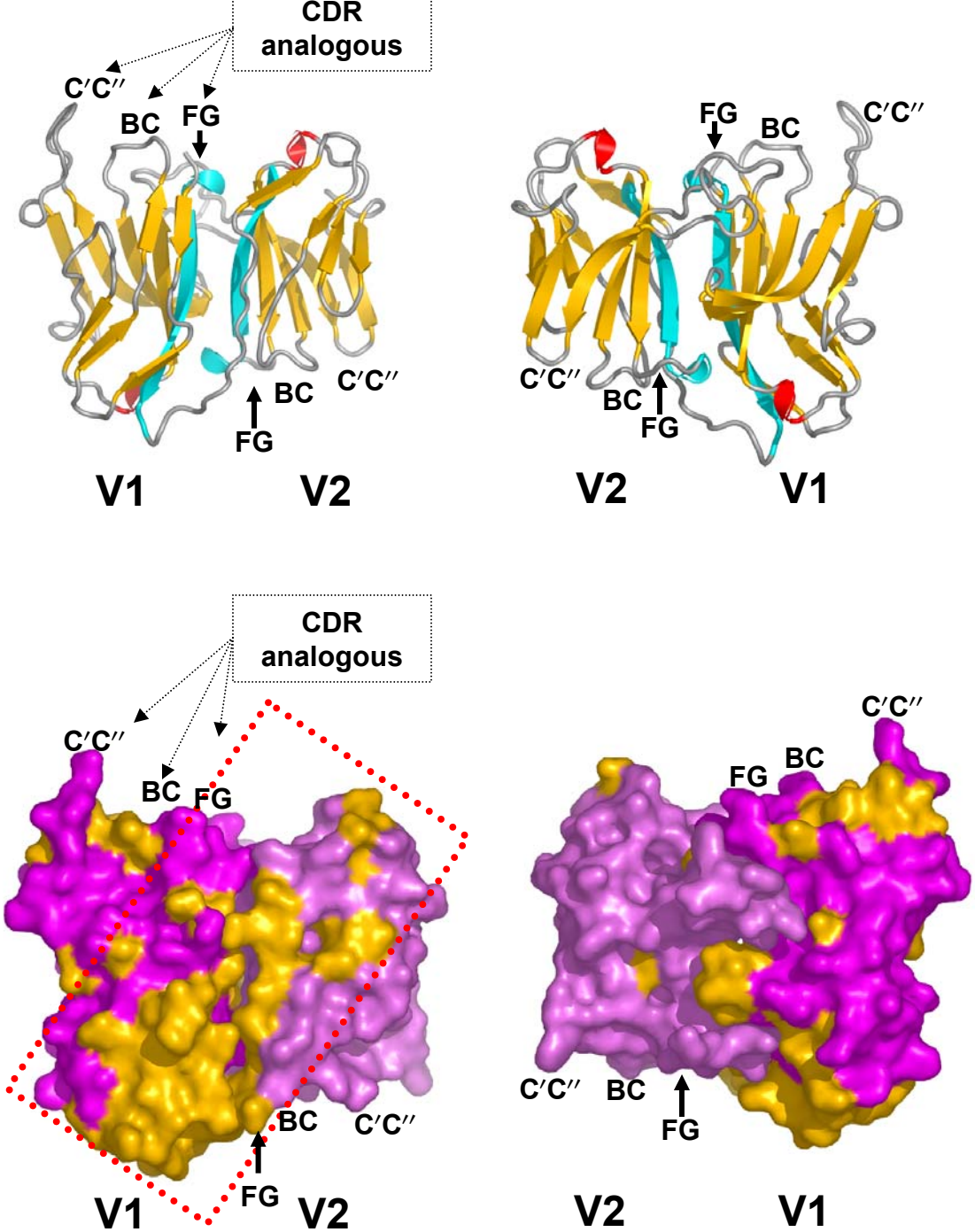
TCR



VCBP3

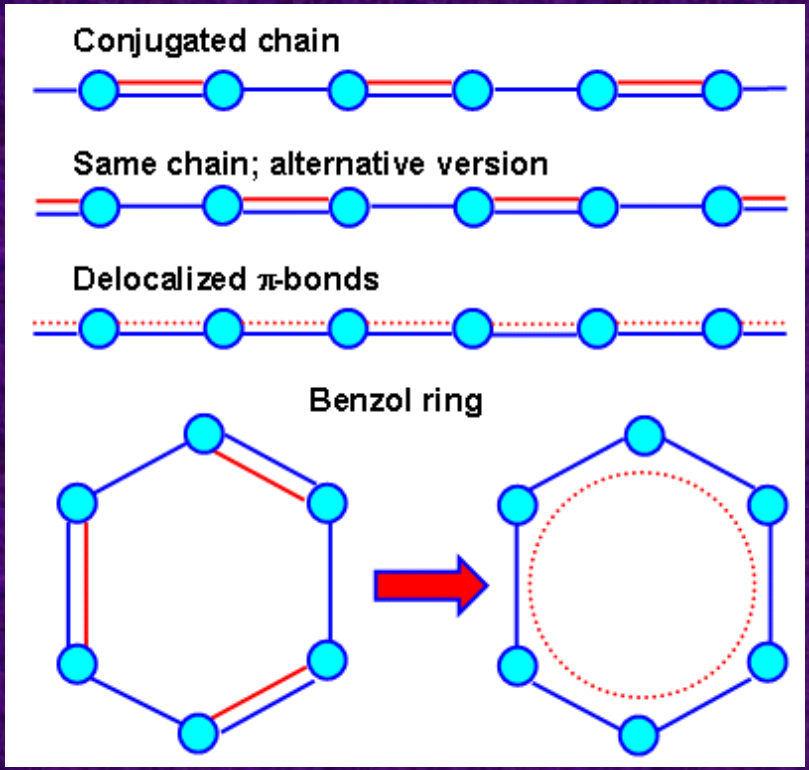


Both exhibit 3-layer packing in which residues in front sheet edge strands fold over the central strands to form conserved interactions.


CDR
analogous
C′C″
BC
FG
BC
C′C″
FG
V1
V2
FG
BC
C′C″
C′C″
BC
FG
V2
V1
CDR
analogous
C′C″
BC
FG
BC
C′C″
FG
V1
V2
C′C″
BC
FG
C′C″
BC
FG
V2
V1

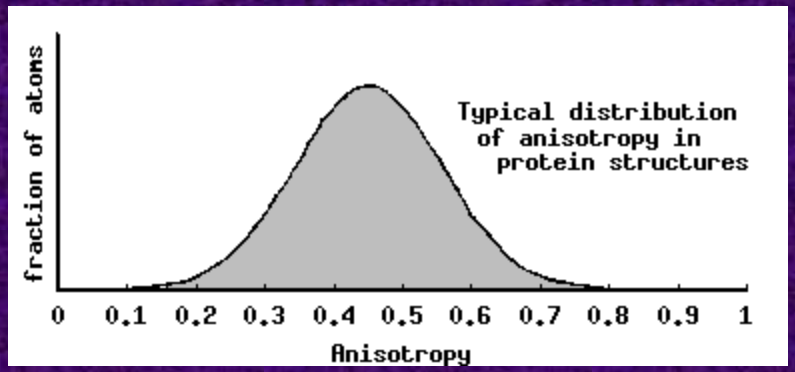
What structural features are apparent at atomic resolution (1.2Å or higher) ?

- **Ordered H atoms**
- **Assess protonation states**
- **Determine if π electrons in aromatic residues are shared equally**
- **Anisotropic B factor refinement**



Aniosotropy in the VCBP3 D1 crystal structure

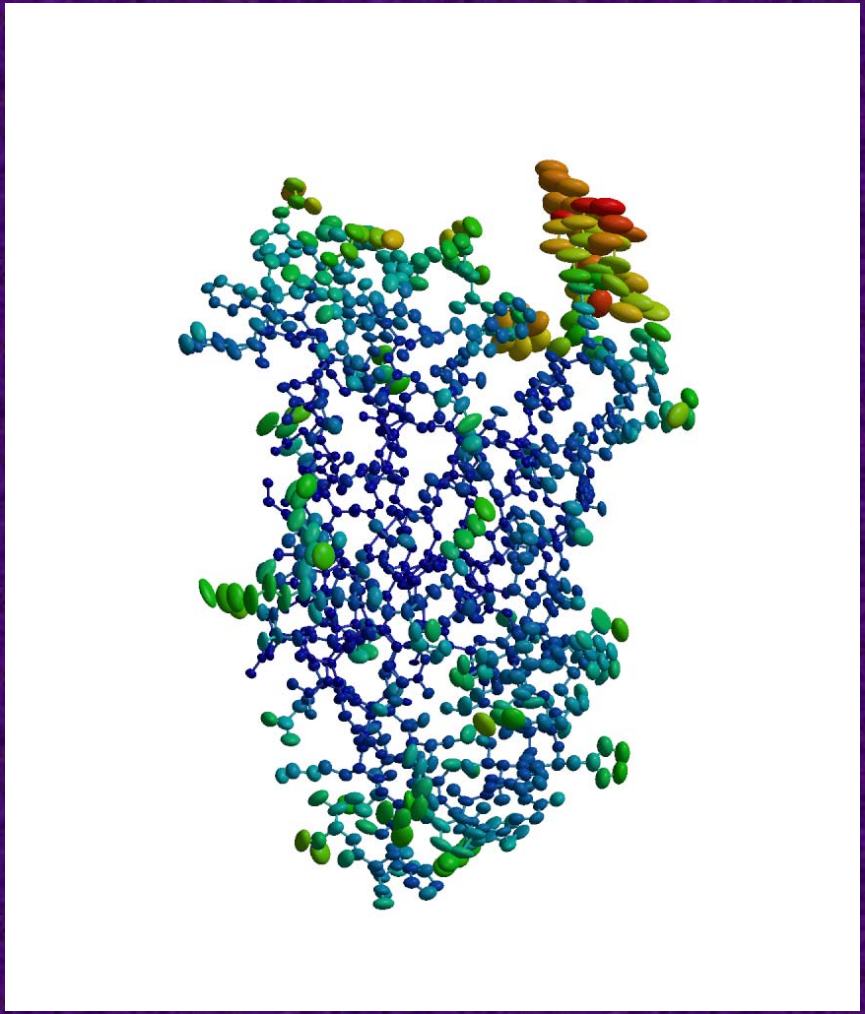
Anisotropy is defined as the square of the axial ratio of an ellipsoid representing the atom. The ratio is 1.0 for a perfectly isotropic (spherical) atom.



Leu^{82}
Extreme anisotropy

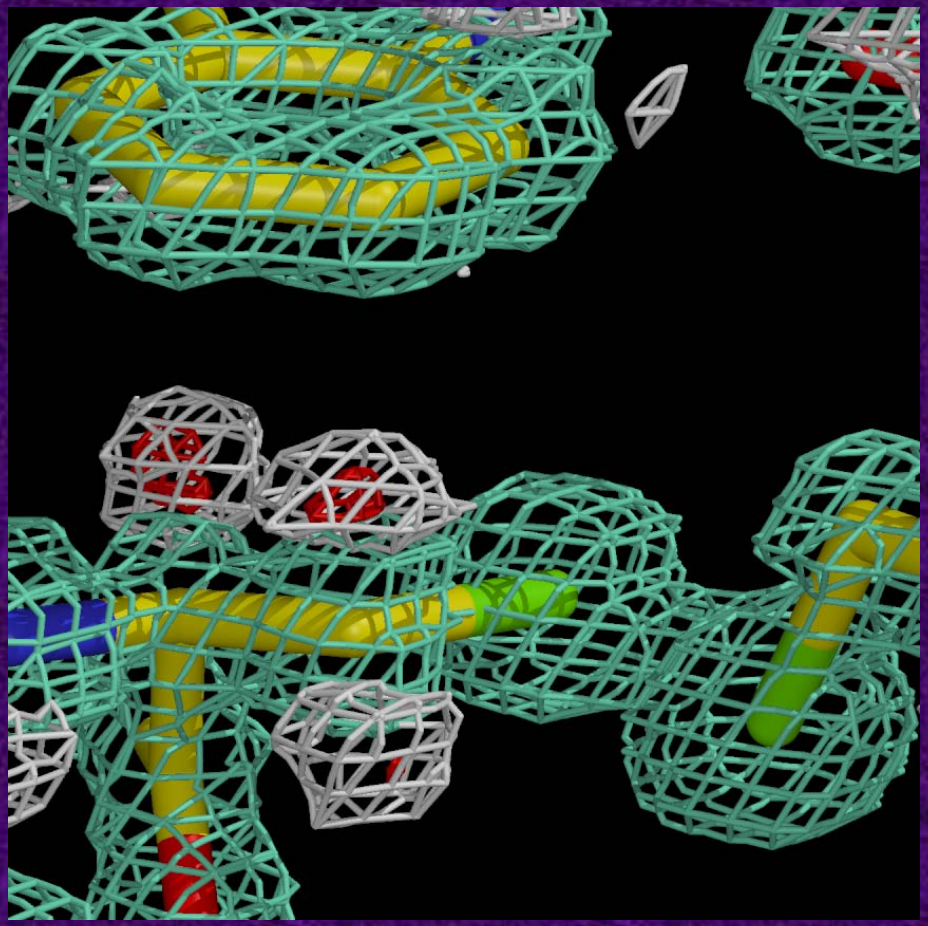
Aniosotropy in the VCBP3 D1 crystal structure

Anisotropy: having a different value when measured in different directions



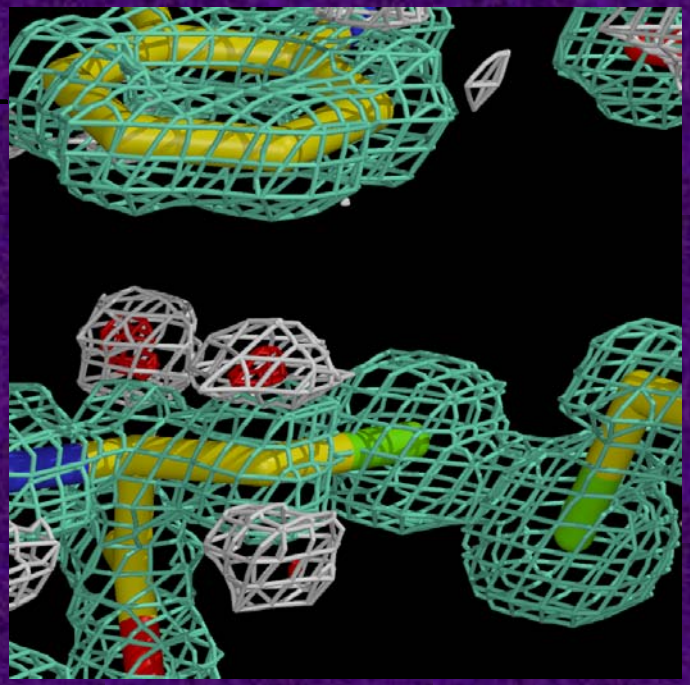
H atoms apparent in omit electron density maps

Non-canonical interaction between evolutionarily conserved Cys110 and Trp42 π electrons



Non-canonical interactions at the core of VCBP3 D1 between Trp^{42} and Cys^{110}.

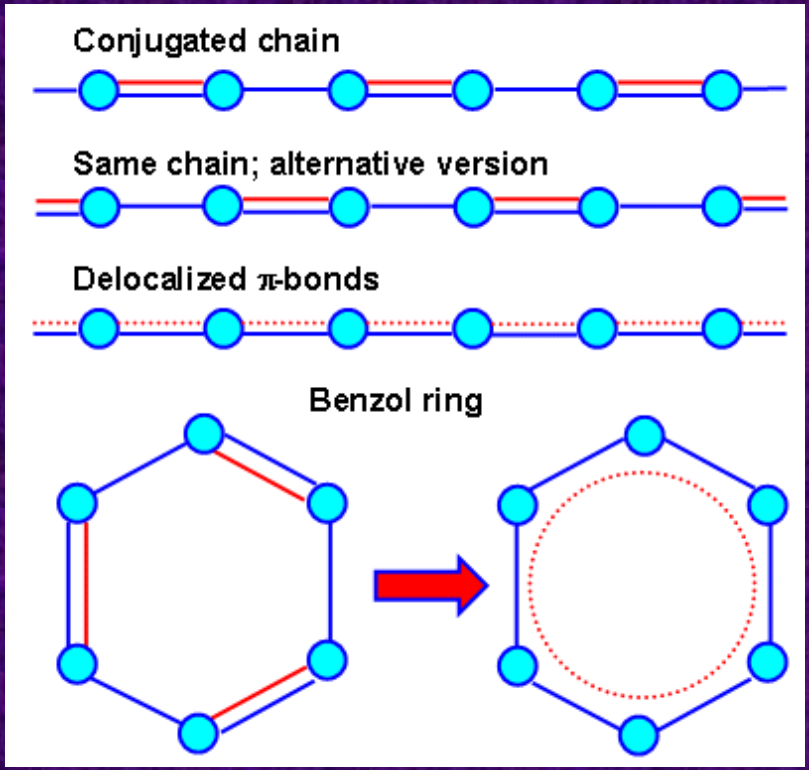
	VCBP3 D1	Chothia (38)	IMGT Ig (39)	NCI (40)	donor	acceptor
Gly	20	16/15	16	0	-	-
Cys	27	23/22	23	CH---OC	0	1
Trp	42	35/36	41	CH---PI CH---OC PI---PI	2	3
Arg	84	61/66	75	CH---OC	0	1
Leu	95	73/80	89	CH---OC	0	1
Asp	104	82/86	98	CH---OC	0	2
Tyr	108	86/90	102	CH---PI CH---OC PI---PI	1	3
Cys	110	88/92	104	CH---OC CH---PI	1	1



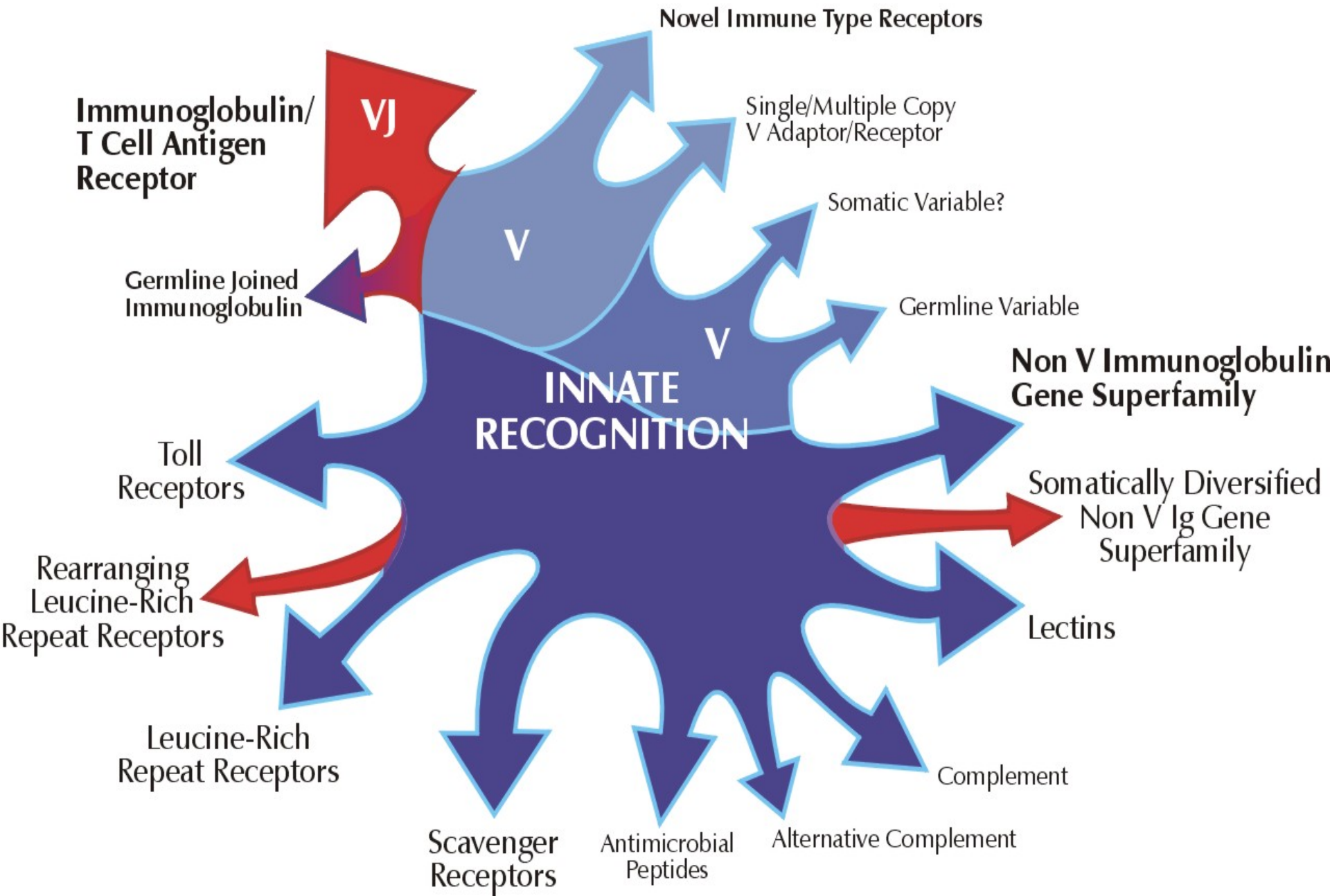
2Fo-Fc electron density calculated from phases to 1.15 Å was contoured at the 2σ level (colored in blue). Non-canonical interactions mediated by π electrons and H atoms apparent in the Fo-Fc electron density are shown at the 3σ level (red), 2σ level (white) and superimposed onto the final model (yellow for carbon, green for sulfur, blue for nitrogen, red for oxygen atoms).

What structural features are apparent at atomic resolution (1.2Å or higher) ?

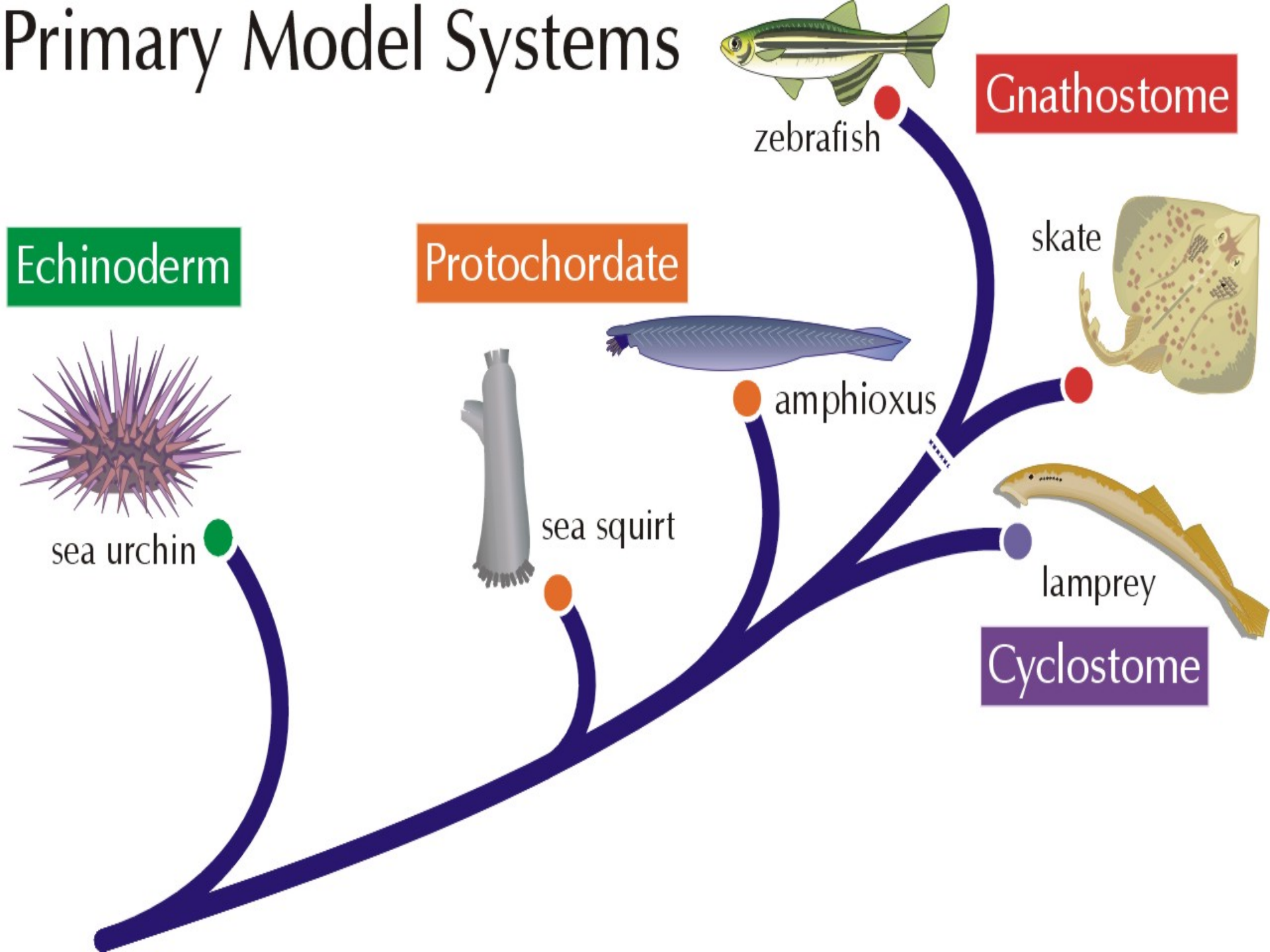
- **Ordered H atoms**
- **Assess protonation states**
- **Determine if π electrons in aromatic residues are shared equally**
- **Anisotropic B factor refinement**



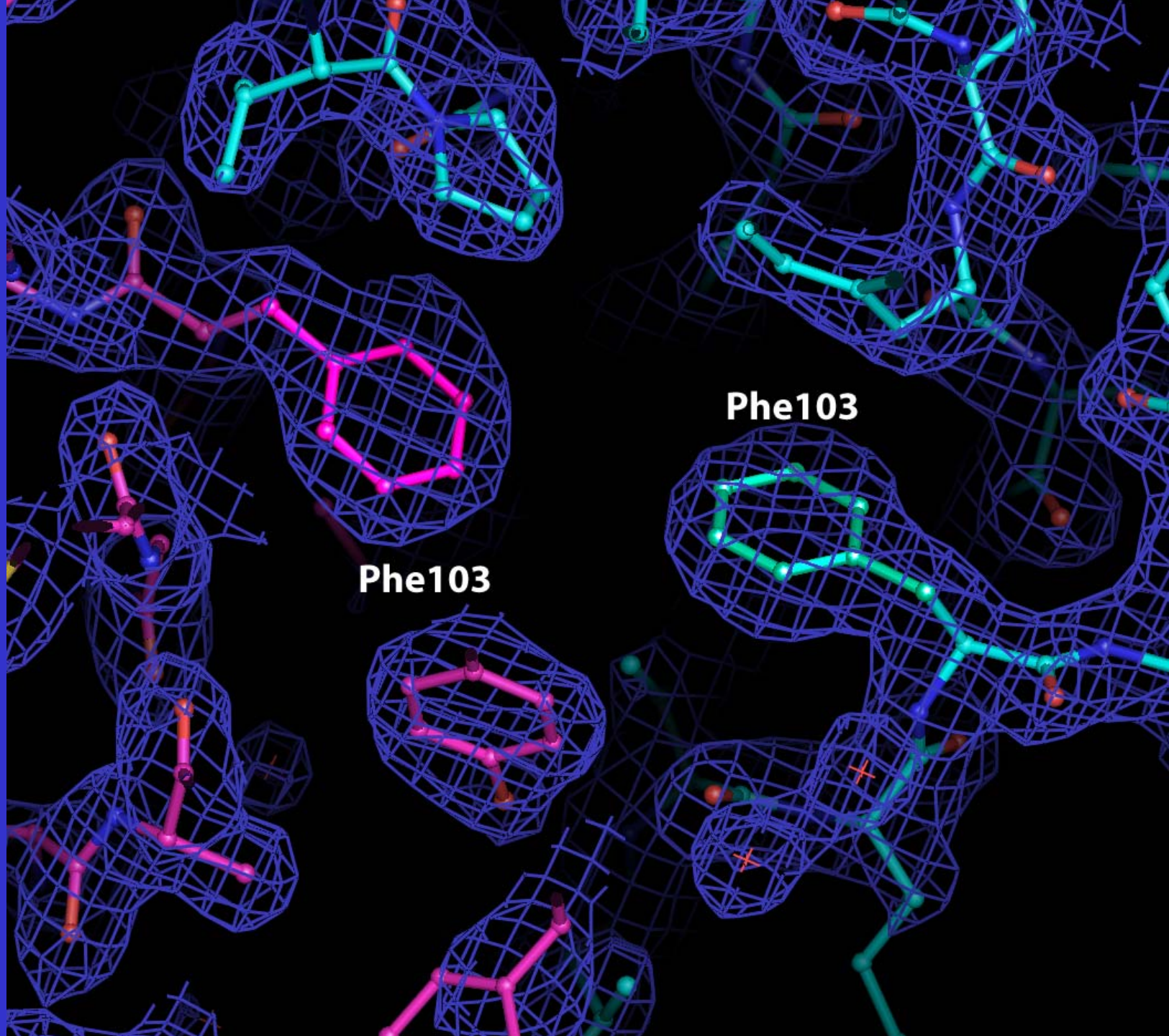
REARRANGEMENT/ RAG-MEDIATED RECOMBINATION

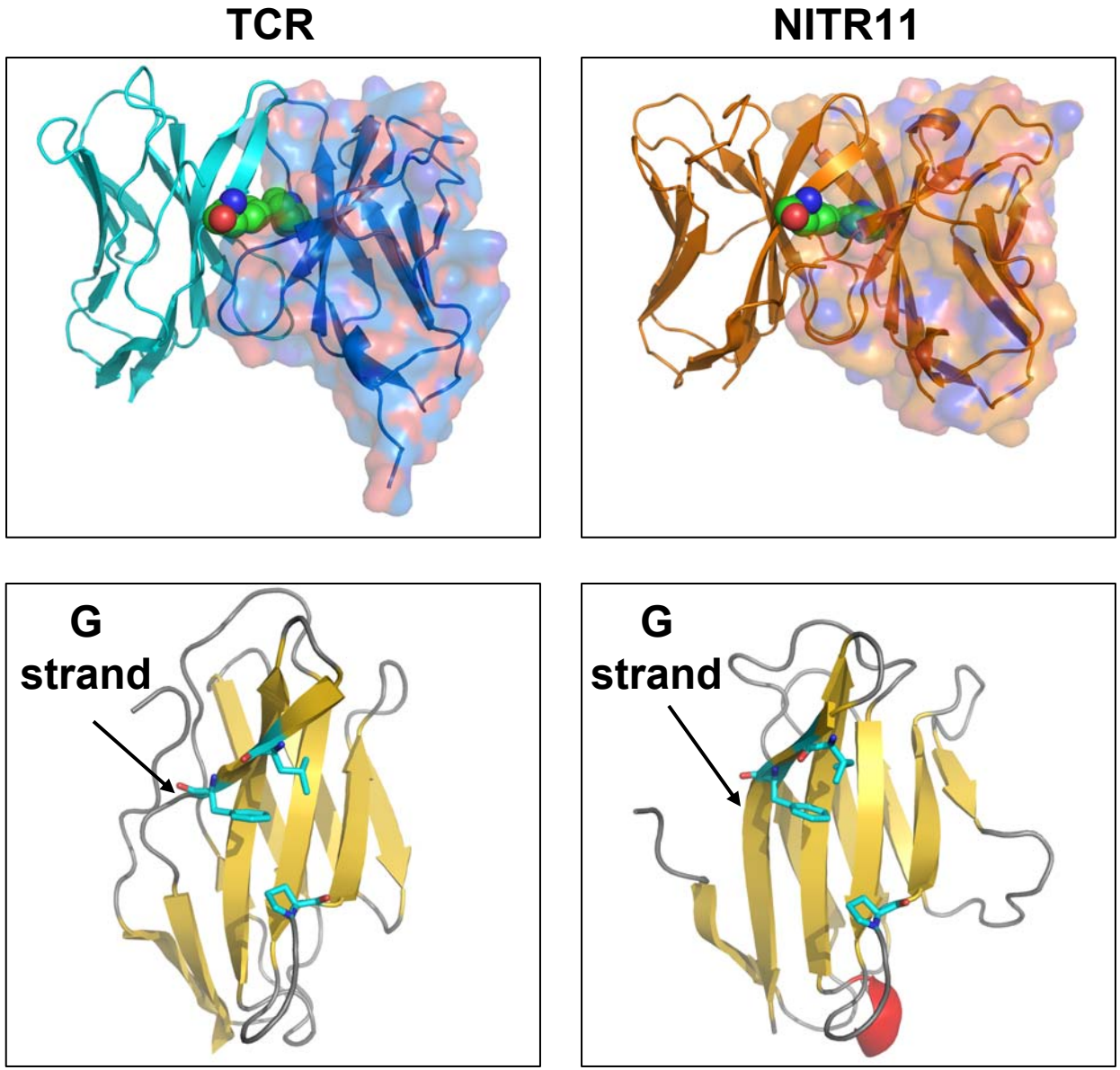


Primary Model Systems



Conserved interactions in the central portion of the homodimer interface of NITR11, solved by SAD to 1.65 Å

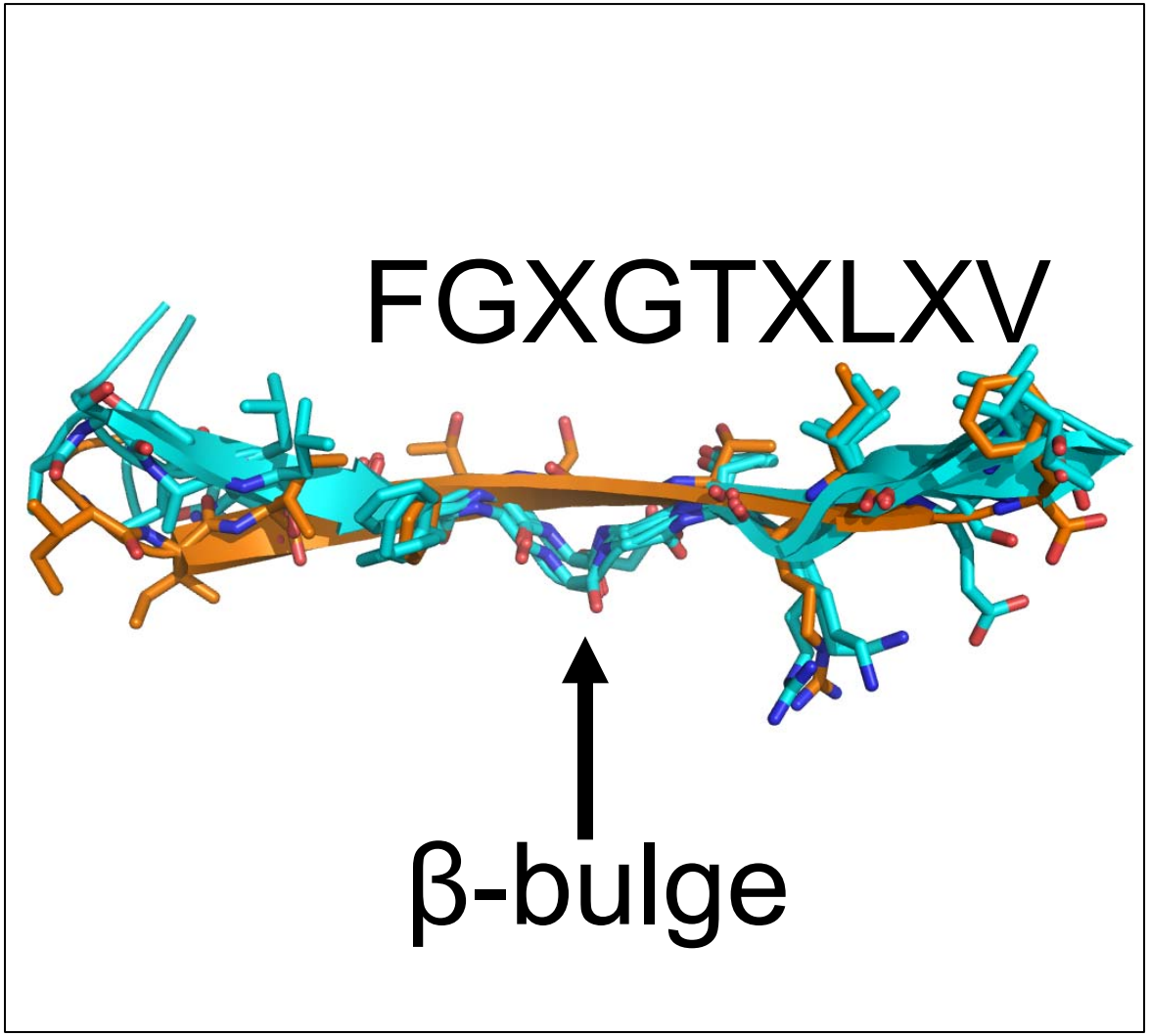




Both exhibit 3-layer packing in which residues in front sheet edge strands fold over the central strands to form conserved interactions.

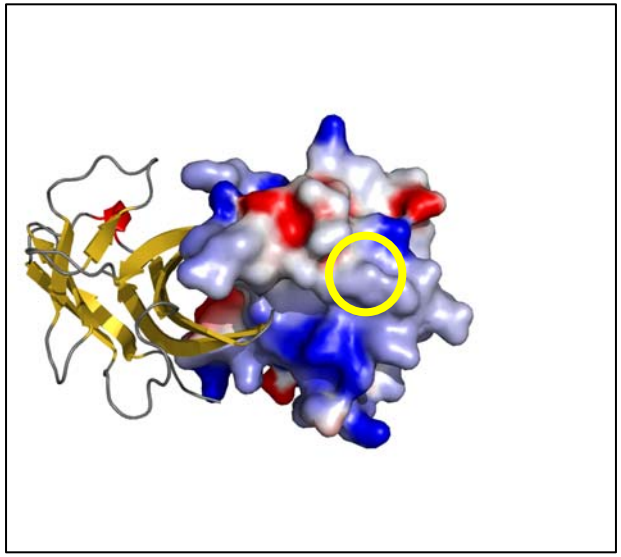
Crystal structure of a disulfide linked dimer of NITR11

NITR11 exhibits the same dimerization mode with and without interchain disulfide bonds

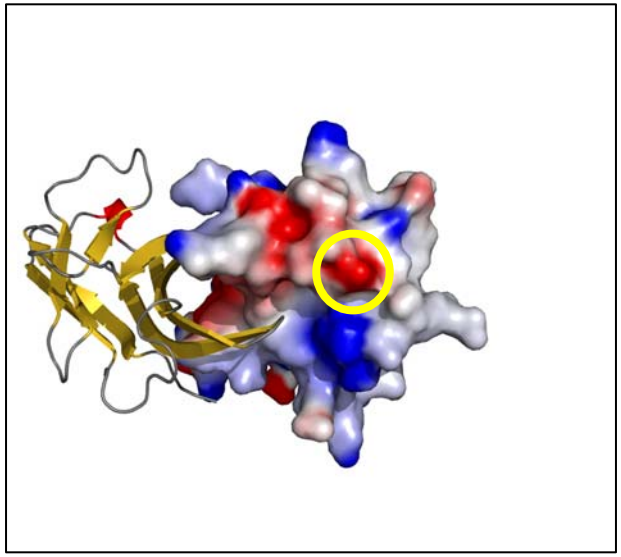


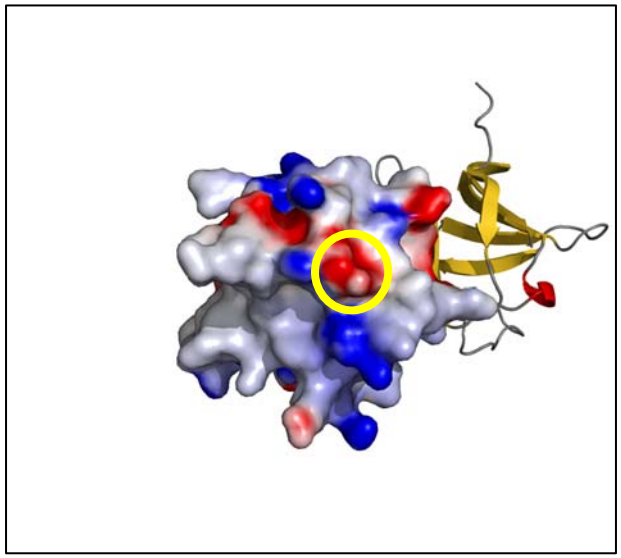
The G strands of NITRs and antigen receptors share structural and functional features

NITR11 +

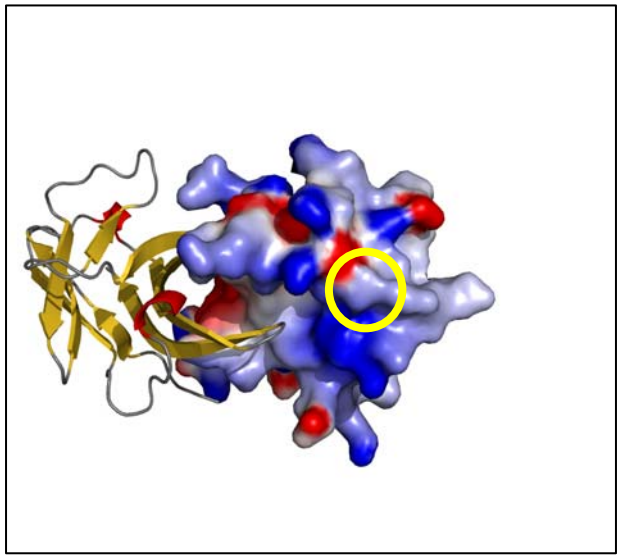


NITR11 N50D -





NITR10 -



NITR10 D50N +

Pinpointing the site of interaction between NITR11 and allogeneic ligand

University of Florida College of Medicine/X6a: Basic and Translational Research Projects

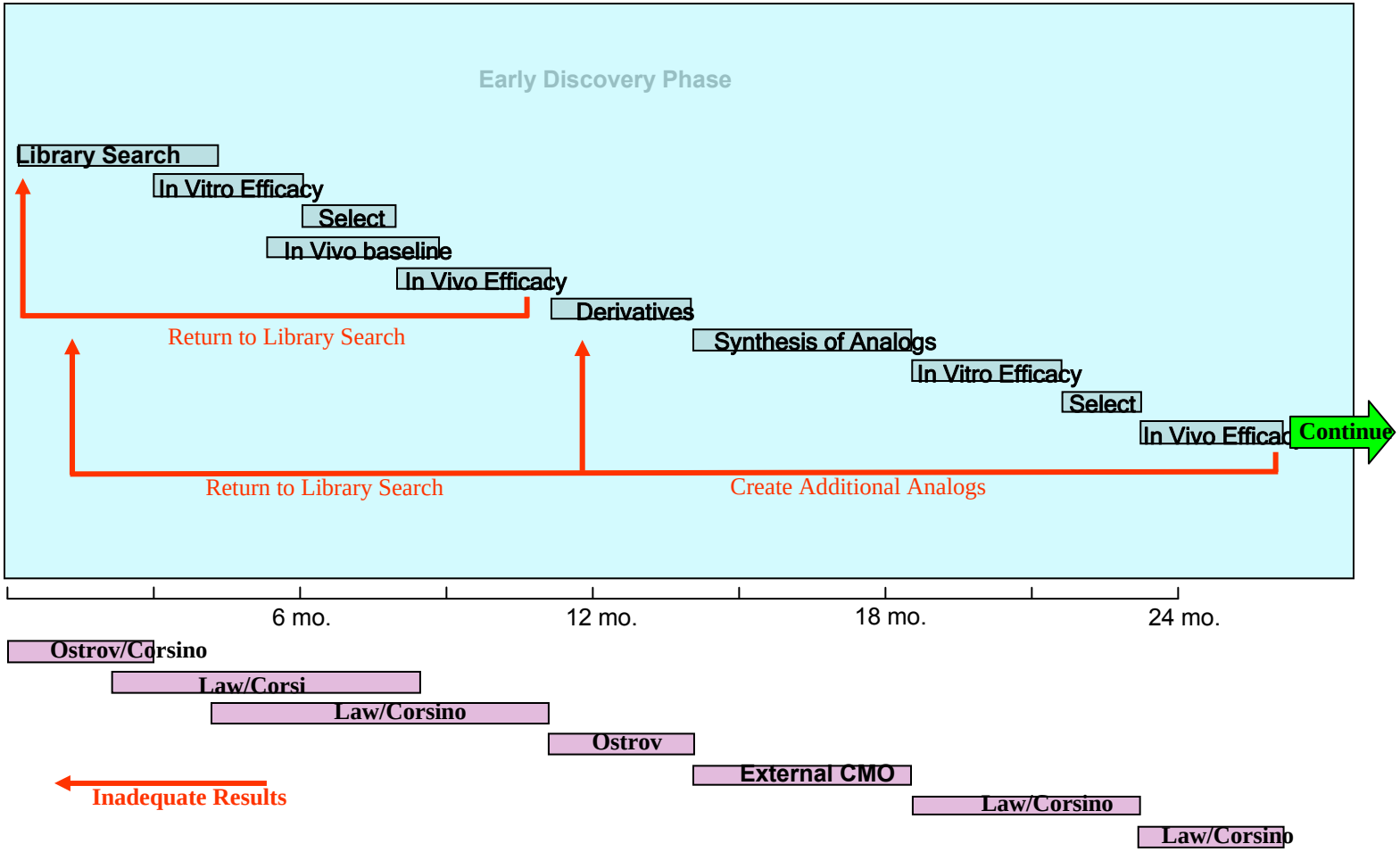
- Translational Research
  - Structure-based Drug Design
    - Cancer target-Focal Adhesion Kinase
      - PDB code-2RA7
    - Type I Diabetes-Insulin:sulfatide complex
      - PDB code-3BRR

Therapeutic goals

Target	Clinical Goal	Structural Basis	Biological Activity
hACE2	SARS	Crystal	Inhibitor
FAK	Breast cancer	Crystal	P-P Inhibitor
hACE2	Hypertension	Crystal	Activator
p27/cdk2	Breast and Prostate cancer	Crystal	Inhibitor
mJAK2	Leukemia	Homology model	Inhibitor
E. coli DNA gyrase	Anti-microbial	Crystal	Inhibitor

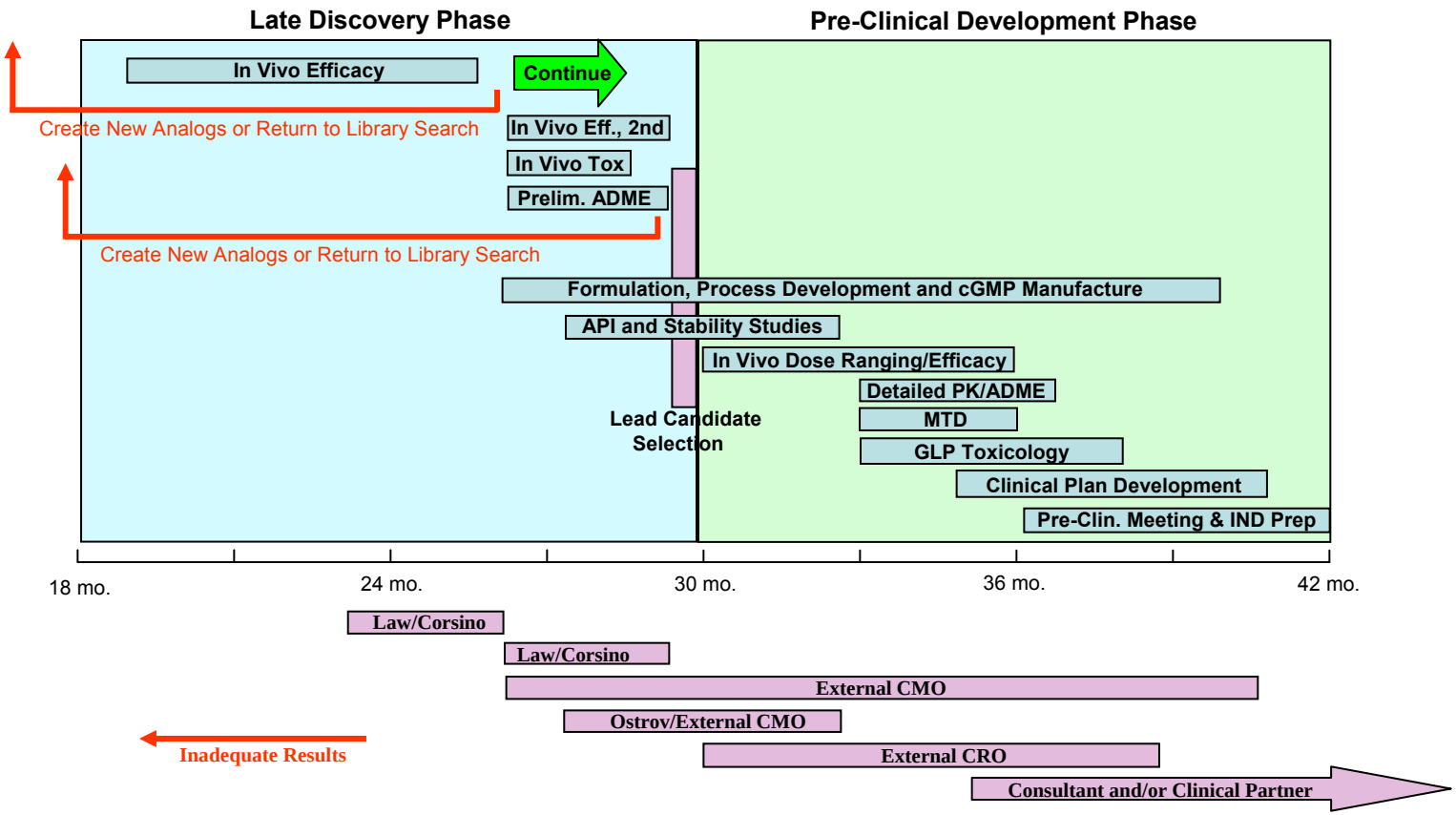
Drug Development Strategy

Early Discovery Phase Activities and Timeline



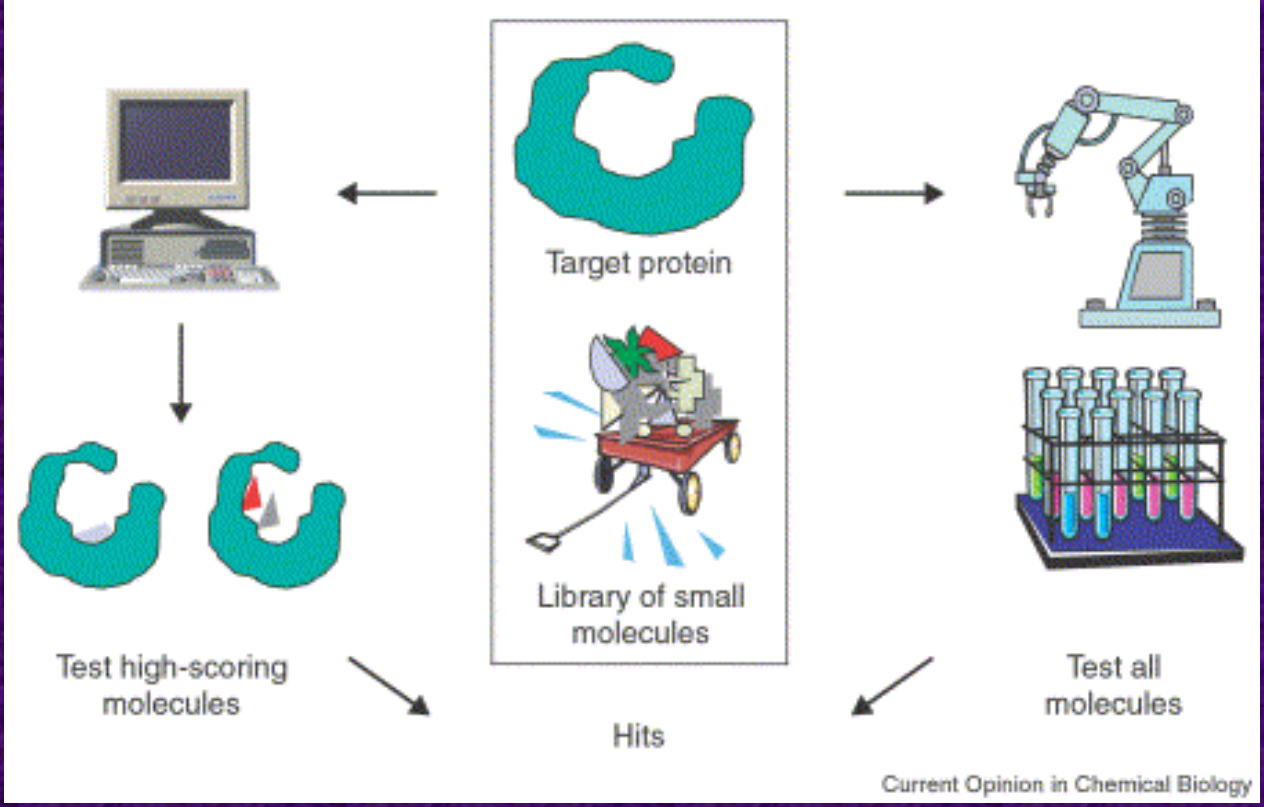
Drug Development Strategy

Late Discovery Phase Activities and Timeline



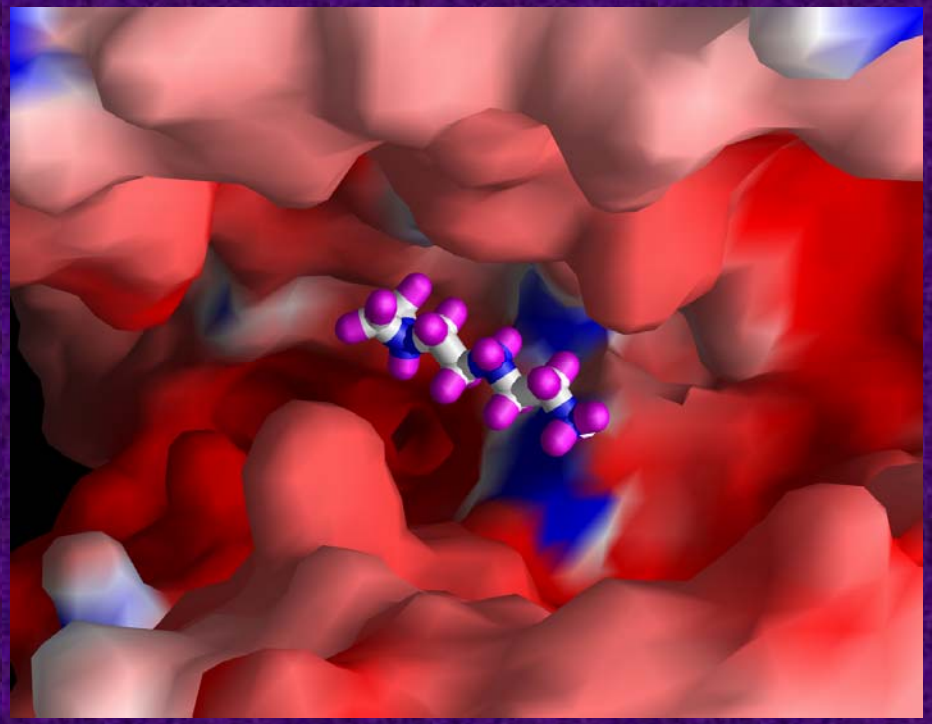
Molecular Docking

High-Throughput Screening



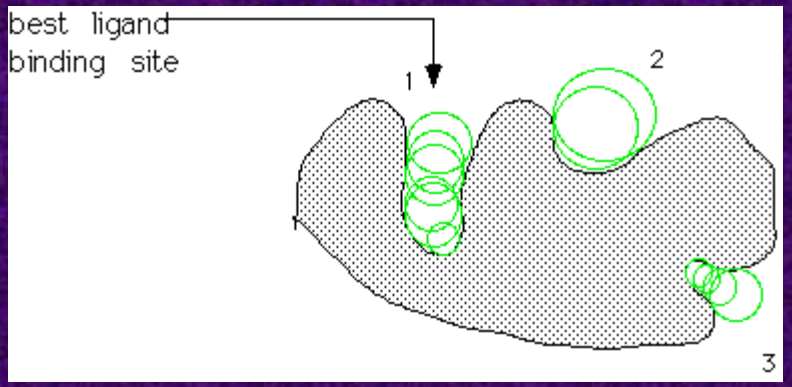
Method:
DOCK6 (UCSF)+ National Cancer Institute/Developmental Therapeutics Program Repository

DTP maintains a repository of synthetic and natural products that have been evaluated as potential anticancer and anti-HIV agents.



This repository has an historic inventory of more than 300,000 non-discreet compounds that have been submitted to DTP from a variety of sources worldwide.

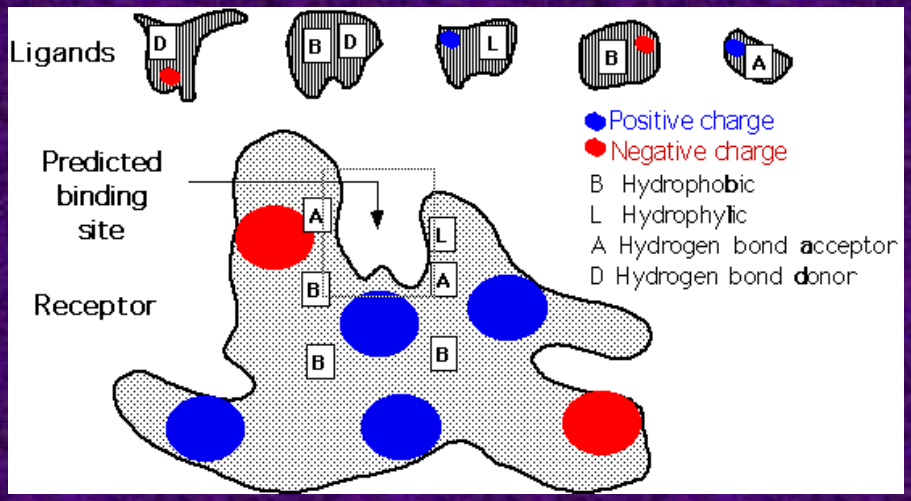
1. Identification of candidate binding sites clusters of overlapping spheres that touch the receptor surface at two points



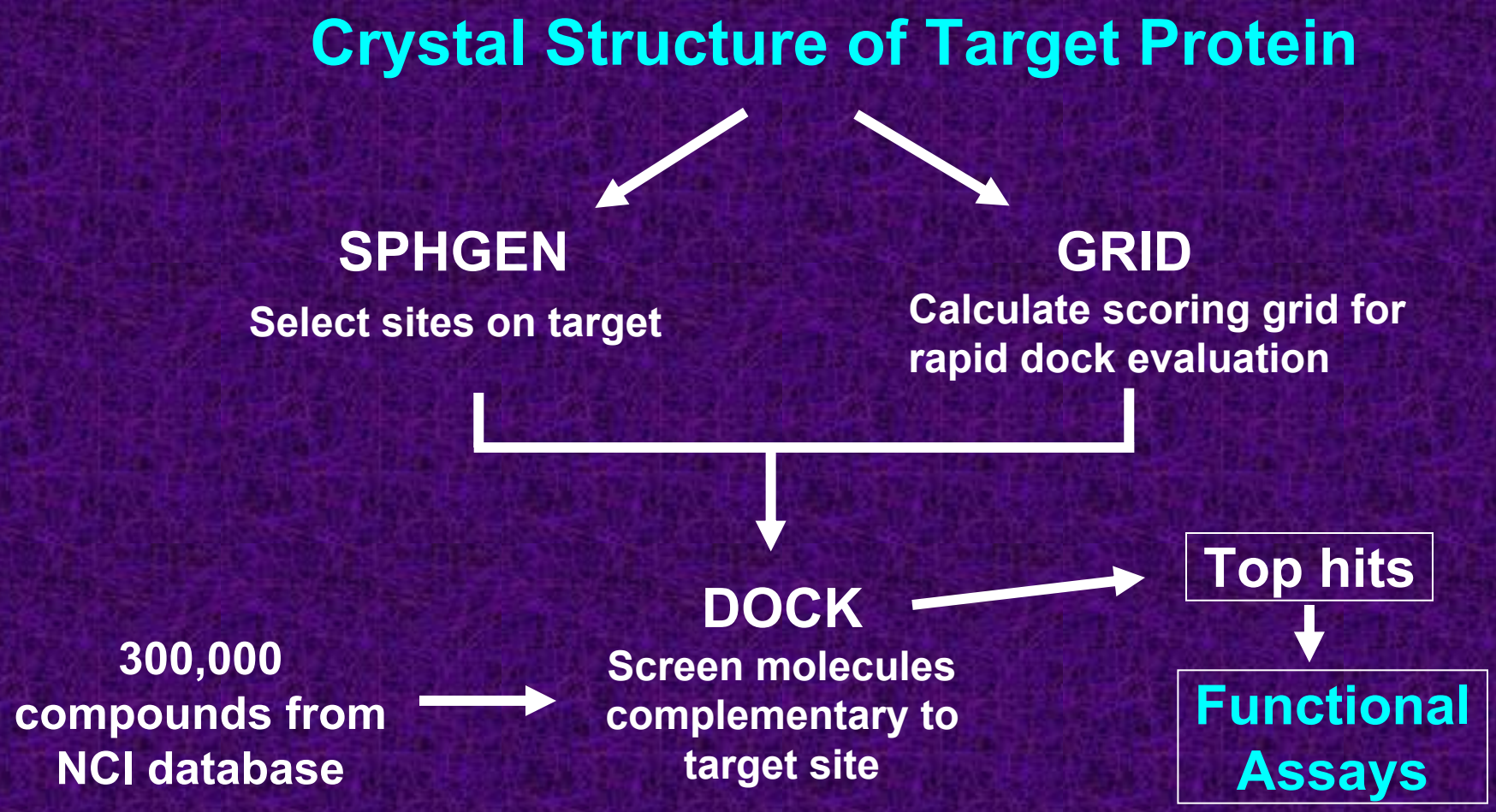
2. Measurement of the geometrical complementarity contact area

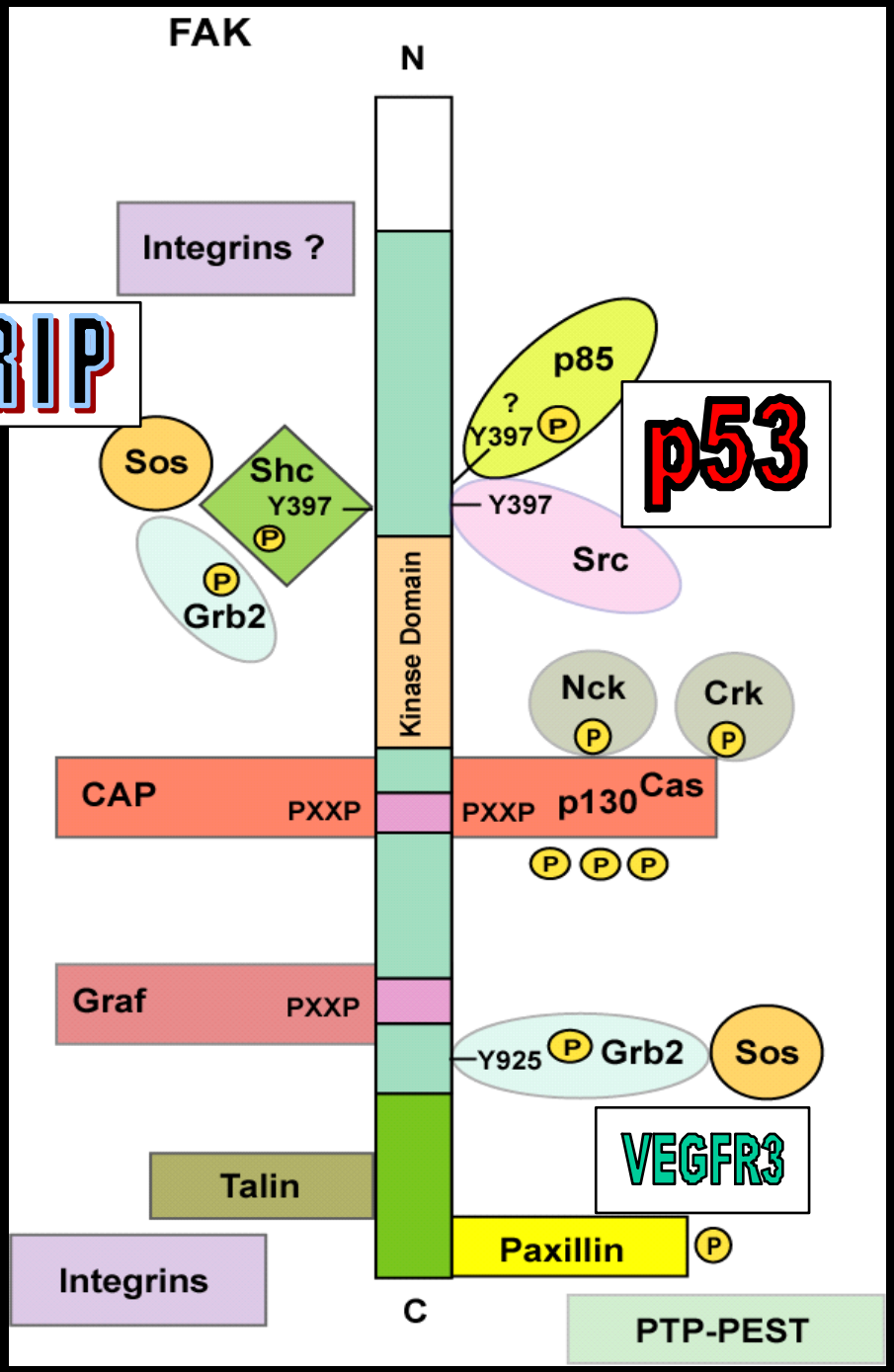
$$E = \sum_{i=1}^{lig} \sum_{j=1}^{rec} \left[\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}} + 332.0 \cdot \frac{q_1 \cdot q_2}{D \cdot r_{ij}} \right]$$

3. Measurement of the energy of interaction Van der Waals and electrostatic interactions



Virtual Screening: Structure-based Drug Design Strategy using DOCK6

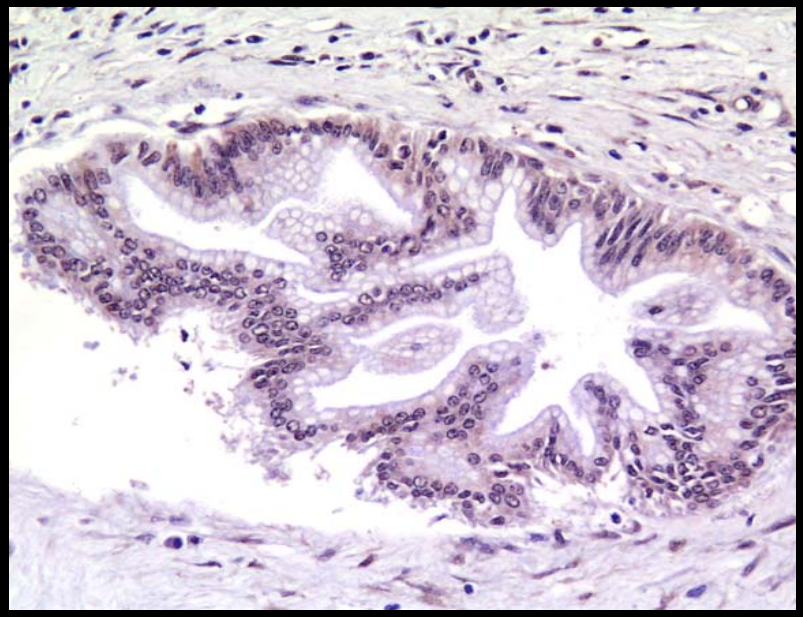




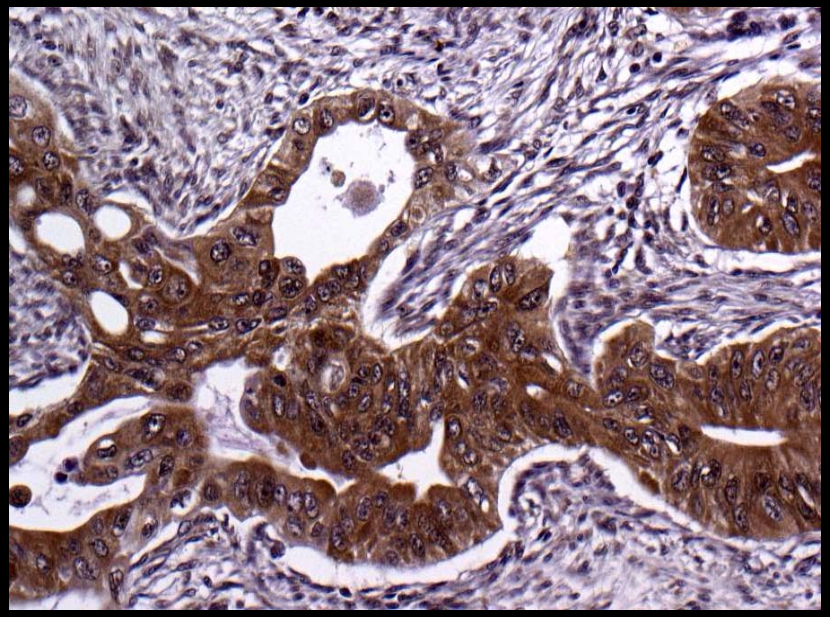
Inhibiting Protein-Protein Interactions with small molecules selected by molecular docking

Target: Focal Adhesion Kinase

FAK is overexpressed in human tumors

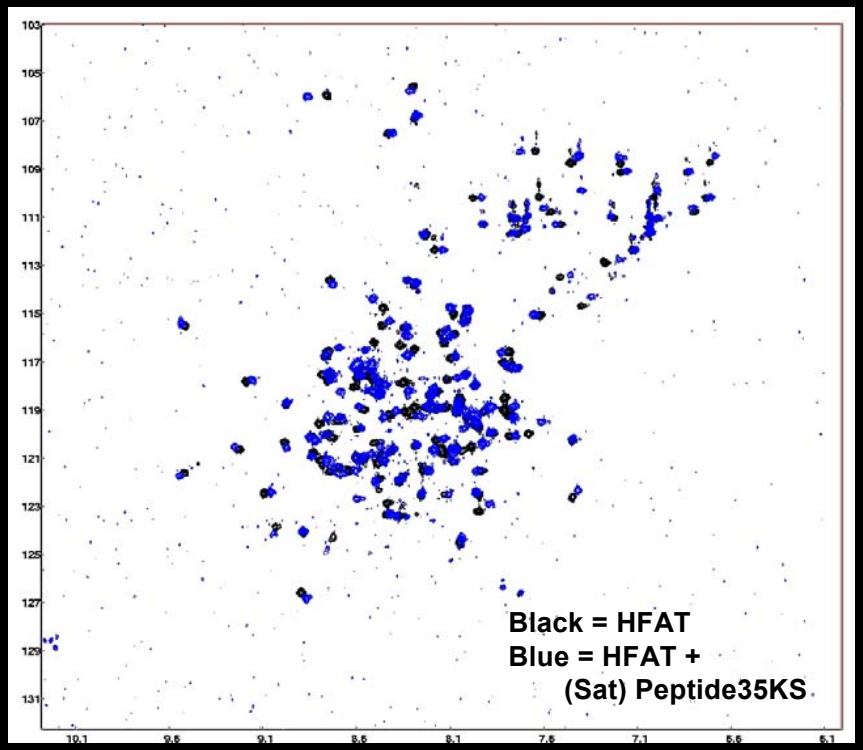


Normal Pancreas

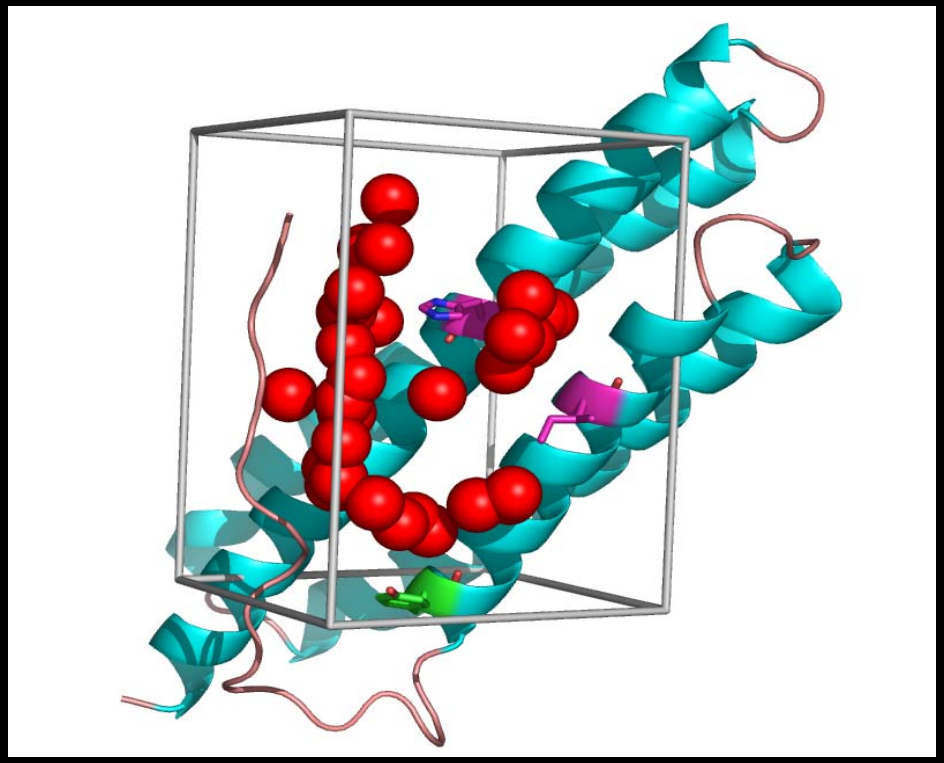


Cancer Pancreas

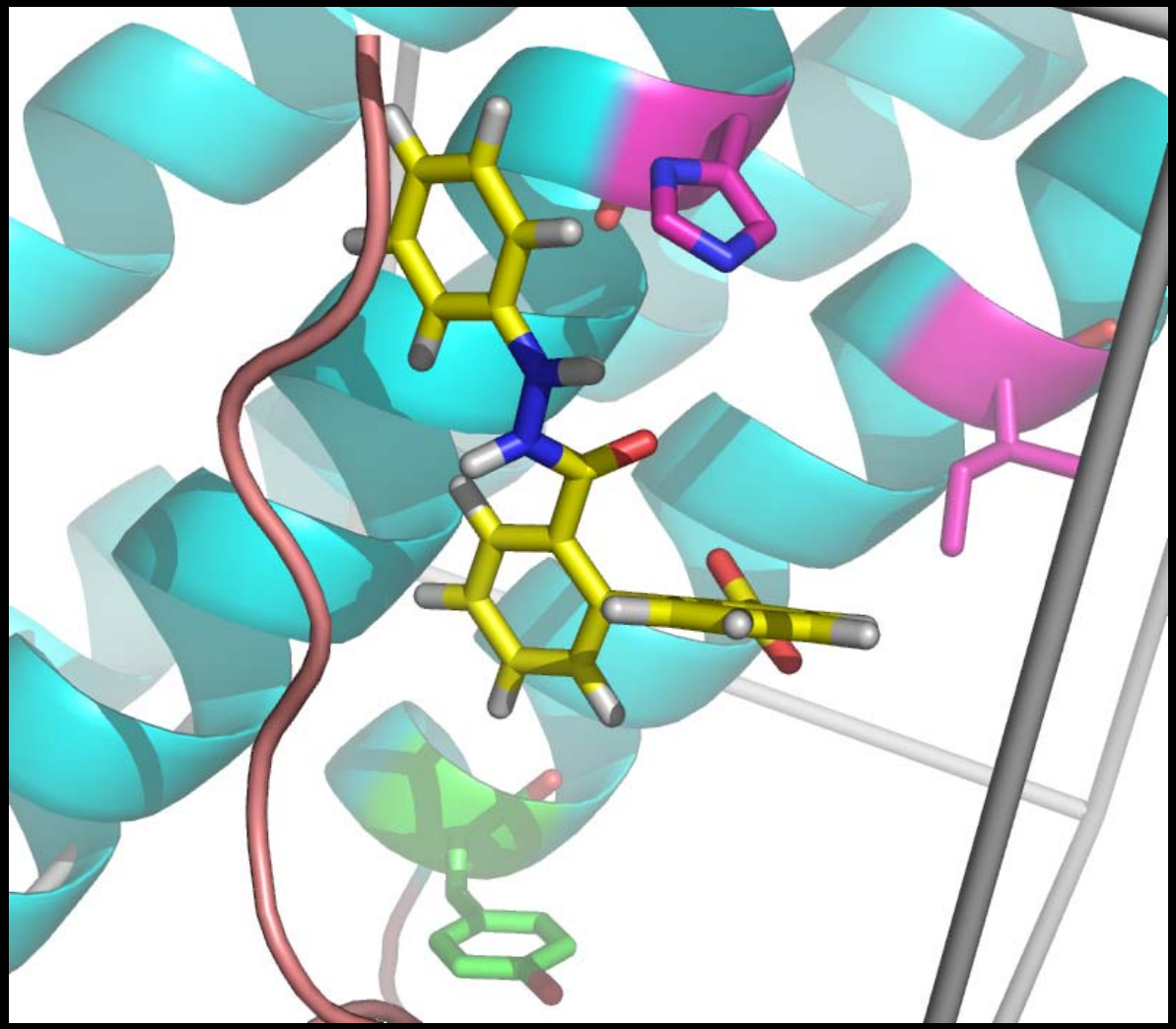
Marked up-regulation of FAK expression in pancreatic cancer. Slides are stained with FAK antibody (brown).



NMR used to map VEGFR receptor binding site on FAK



Site selected for molecular docking

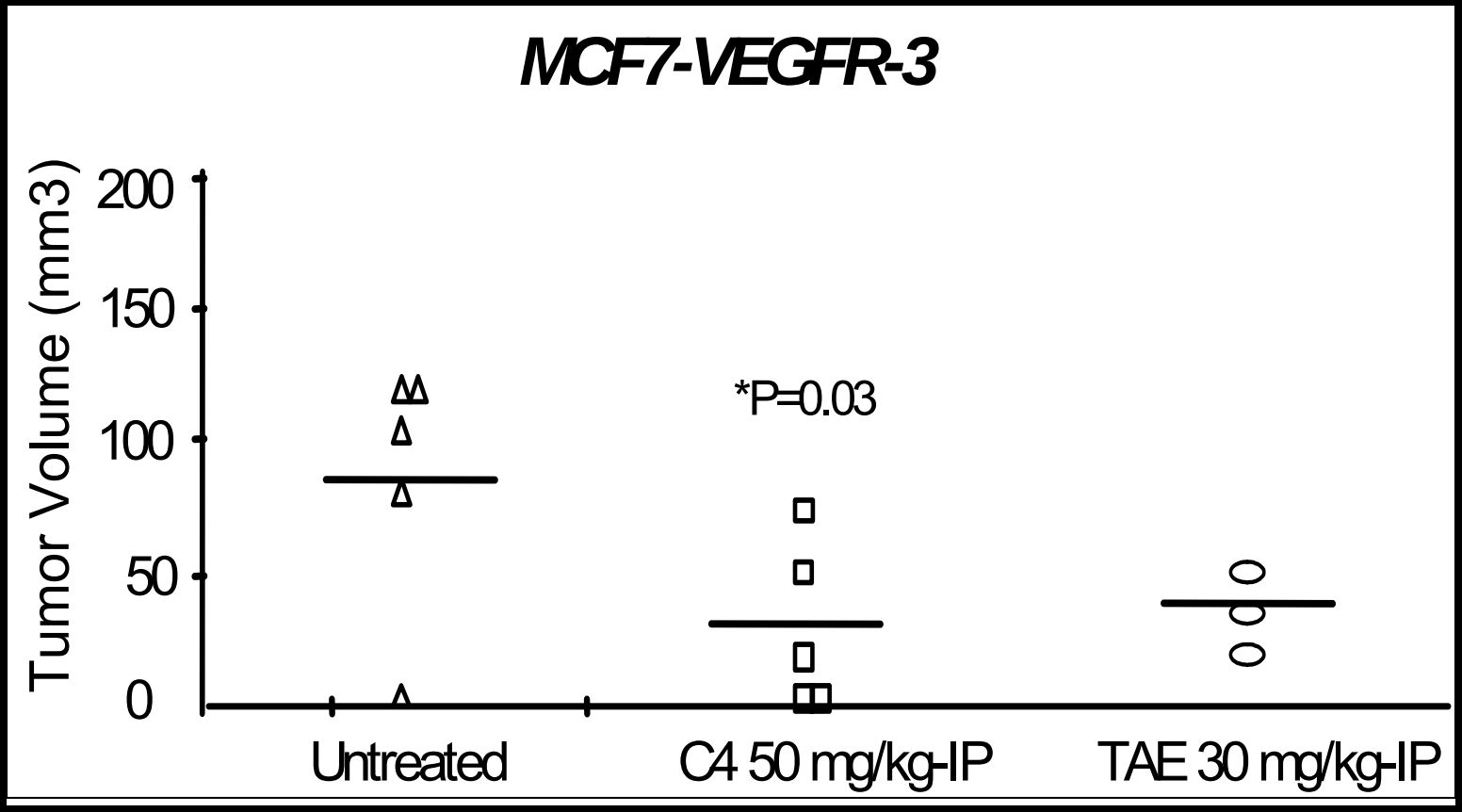


20,000 small molecule compounds with drug-like characteristics (following the Lipinski rules) were docked into the FAT domain of the human FAK crystal structure in 100 different orientations using DOCK5.1. As an example, one such DOCKED ligand, representing the highest scoring compound, 2-[2-(anilinocarbamoyl)phenyl] benzoic acid, is shown

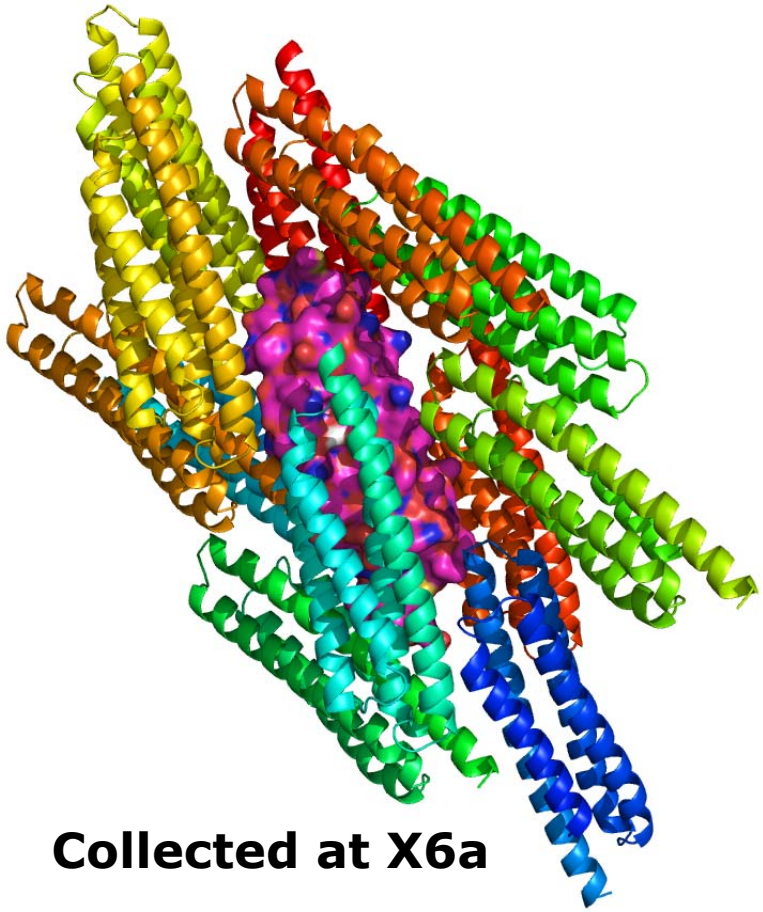
Evidence for Compound C4: biochemical, cellular, *in vivo*, **x-ray crystallography**

In vivo validation of antitumor activity of compound C4

Female nude mice were subcutaneously inoculated with MCF7 breast cancer cells stably overexpressing VEGFR-3. Next day after inoculation, peritoneous injections (IP) with compound C4 (50 mg/kg) or TAE226 (30 mg/kg) (Novartis FAK kinase inhibitor as a control) were started as daily treatment. Tumor size was measured with caliper and tumor volume was calculated. Tumor volume in three groups (5 mice each) after 21 days of treatment was compared.

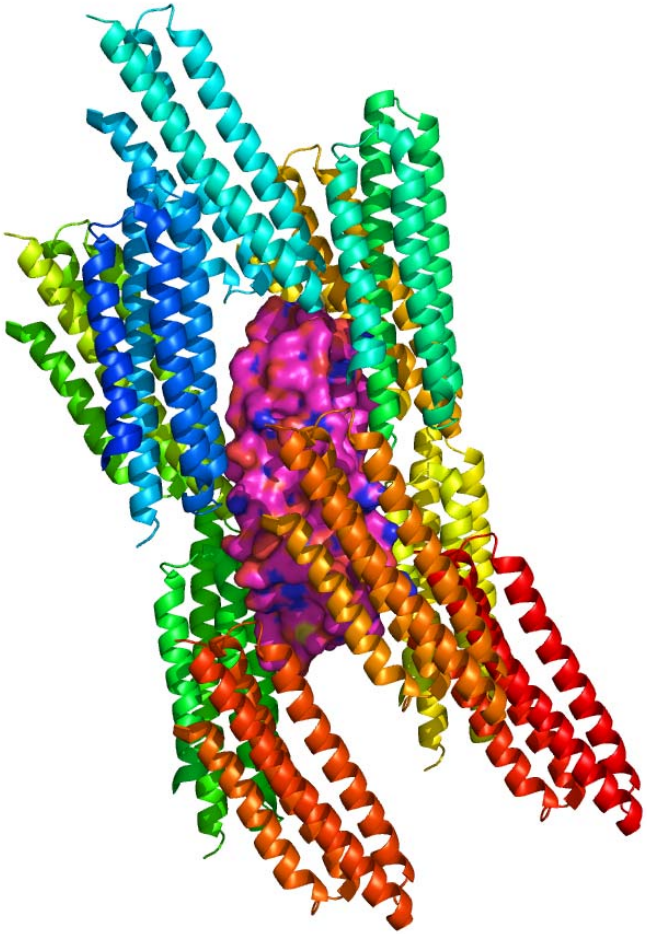


Crystal packing of FAK FAT domain in primitive orthorhombic space group (left), and C-centered orthorhombic space group (right).

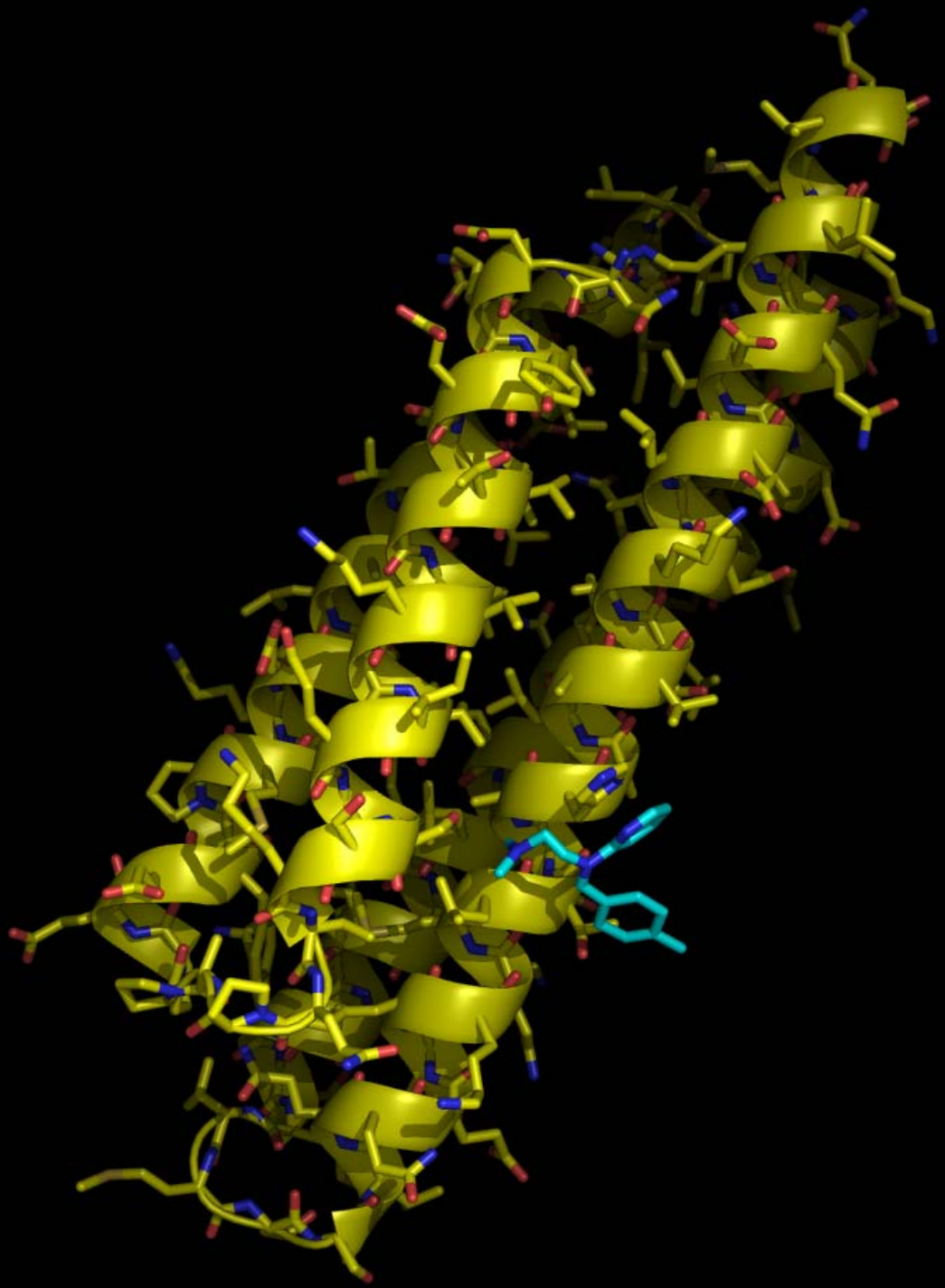


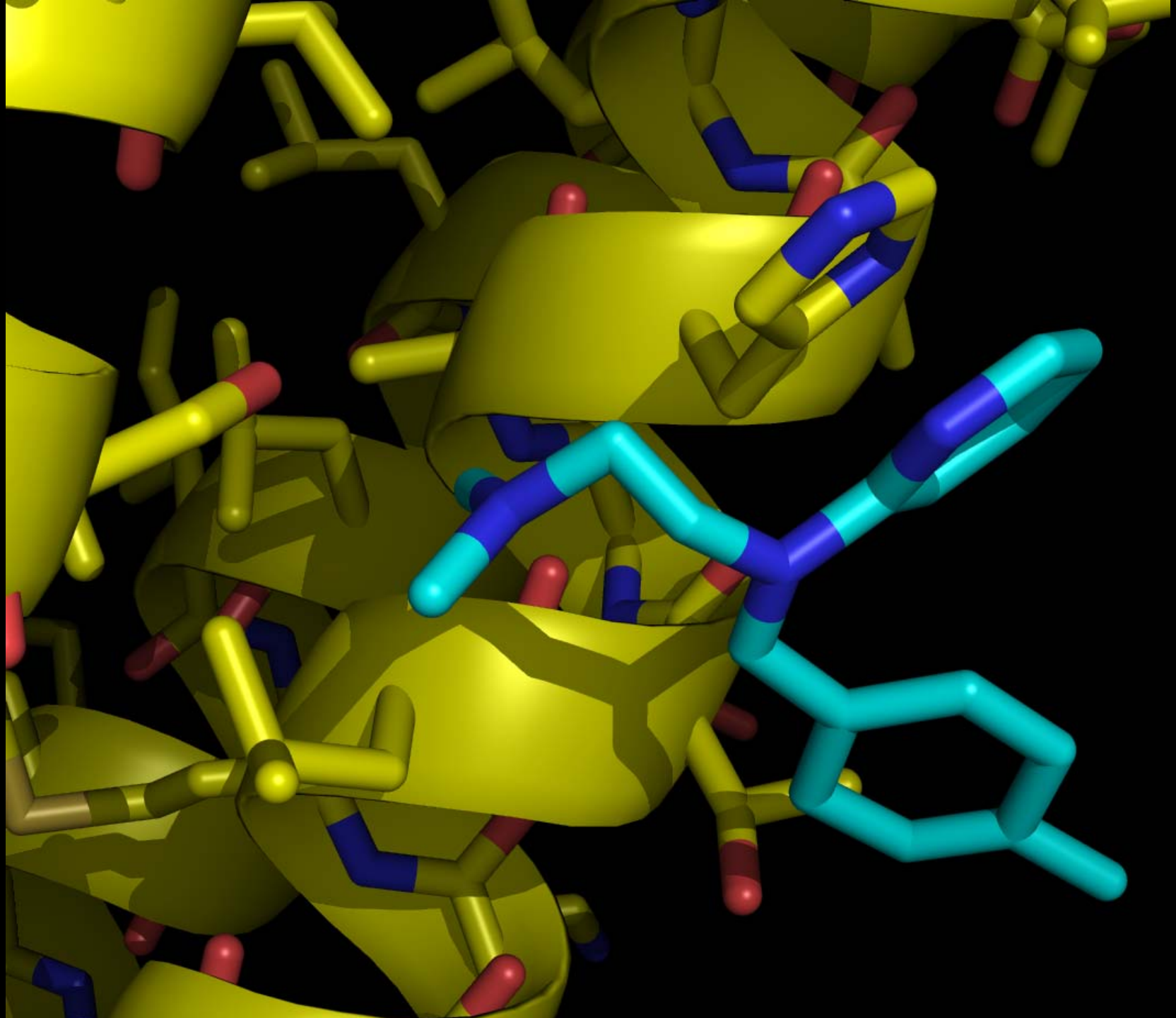
Collected at X6a

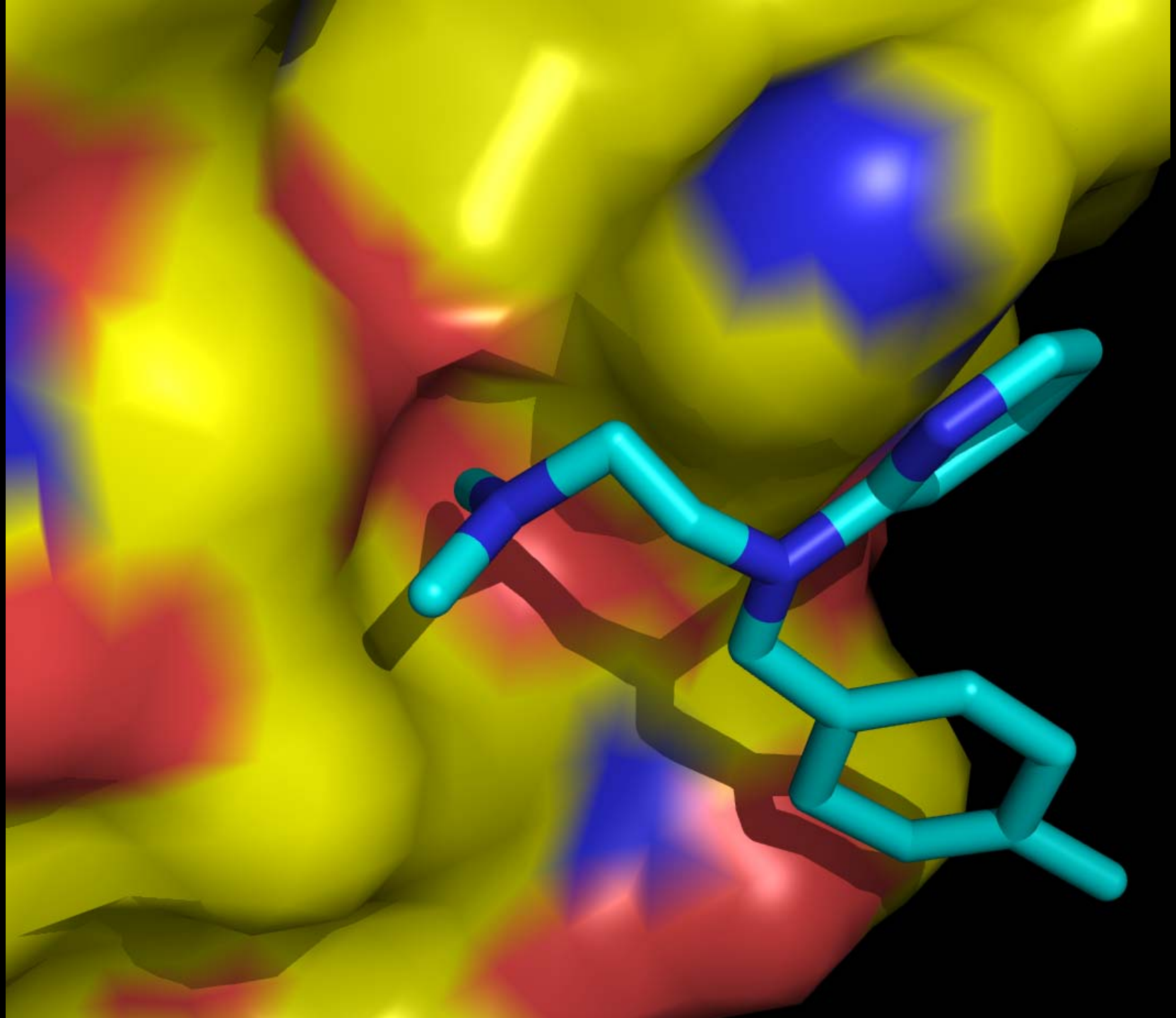
$P2_12_12_1$

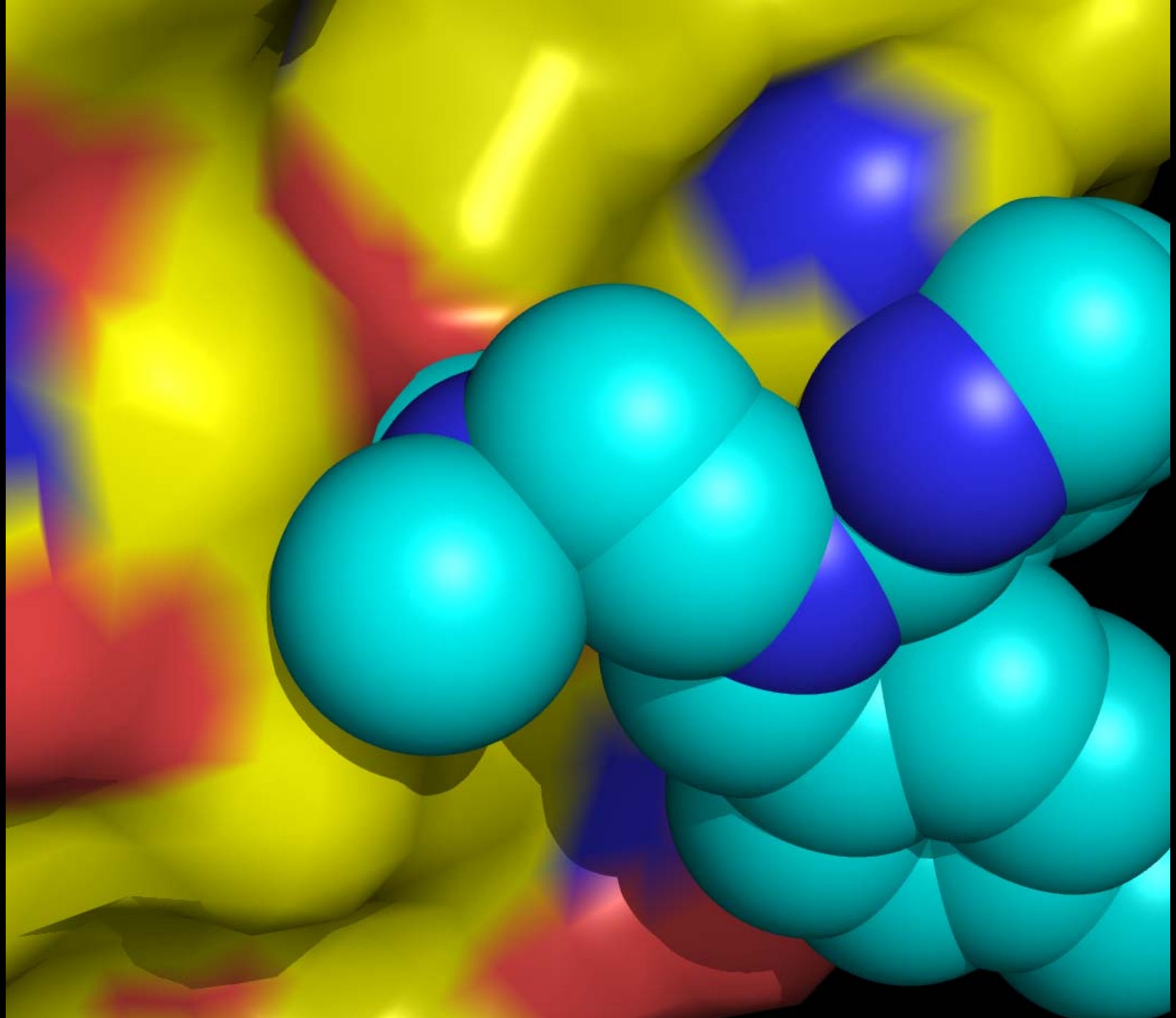


$C222_1$

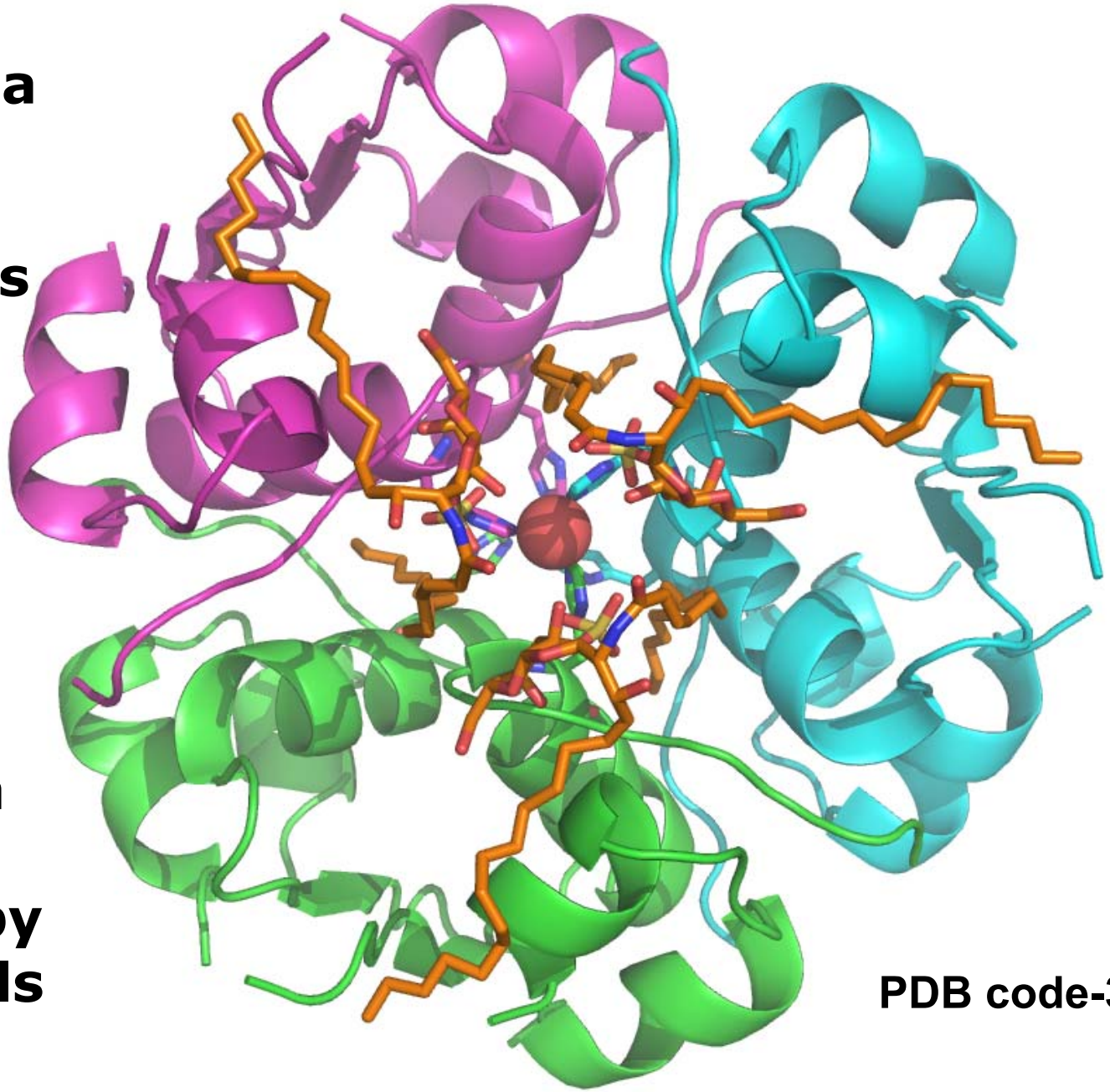








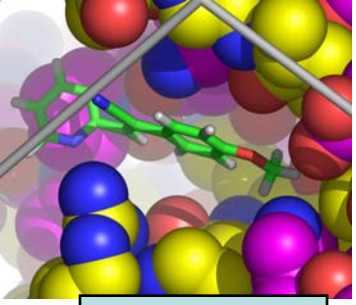
The crystal structure of a complex between 2 autoantigens in Type I Diabetes:



Insulin and sulfatide (a glycolipid recognized by B cells, T cells and MHC molecules)

PDB code-3BRR

FAK Inhibitor Lead Development Scheme

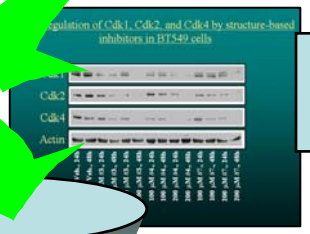


START

3 Existing Protein Kinase inhibitors

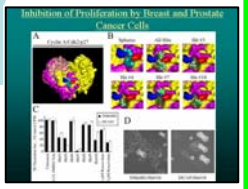
Screen 140,000 compounds from NCI/DTP

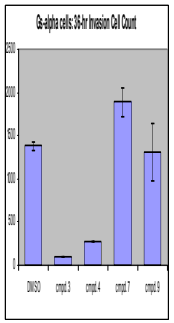
Obtain top 40 scoring molecules



20 - 30 Derivatives from RACHEL

In Vitro Screen



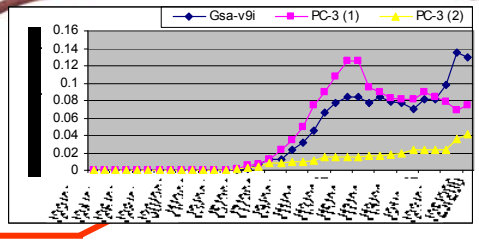


Fails Selection Criteria

2 – 3 Best Candidates

Meets Selection Criteria

In Vivo Efficacy (Family)



Worst Case

Fails Selection Criteria

In Vitro Screen

2 – 3 Best Candidates

In Vivo Efficacy (Lead)

Meets Selection Criteria

In Vivo Efficacy, 2nd Species and Prelim. ADMET

Lead Candidate Selection

FINISH

Conclusions

- Basic questions regarding novel mechanism of immune recognition can be answered by x-ray crystallographic studies conducted at UF/X6a.

- Translational research projects are conducted using a novel high-throughput virtual screening method employing up to 6 million compounds, *in vitro* and *in vivo* testing.

- Drug discovery and development is rapid and economical due to rapid access/data collection capabilities at X6A beamline at the Brookhaven National Lab.

Acknowledgements

NCI Challenge Goal 2015
Eliminating the Suffering and Death Due to Cancer

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