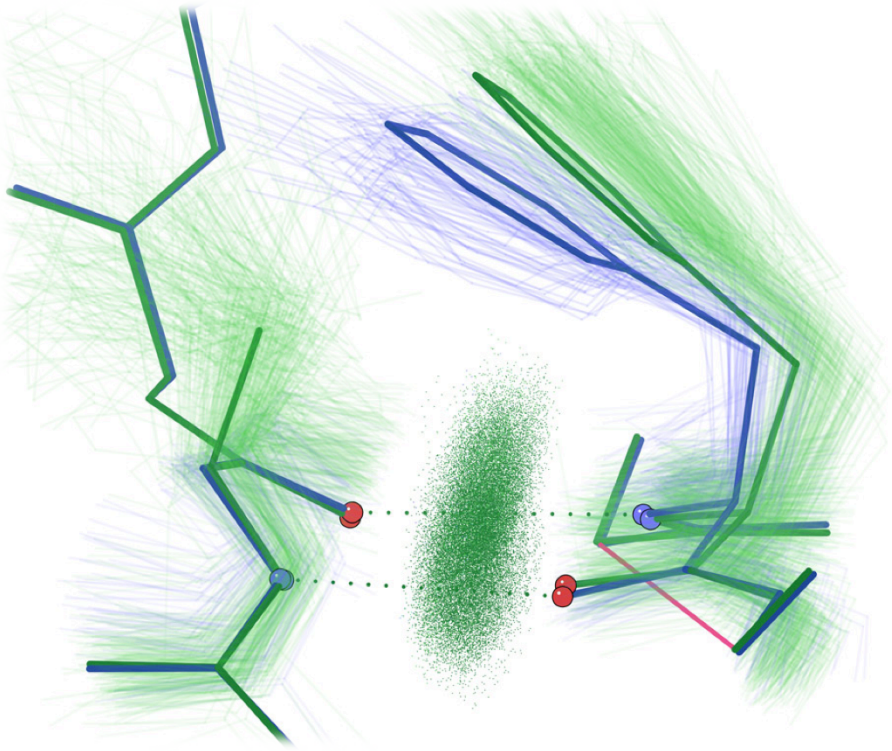
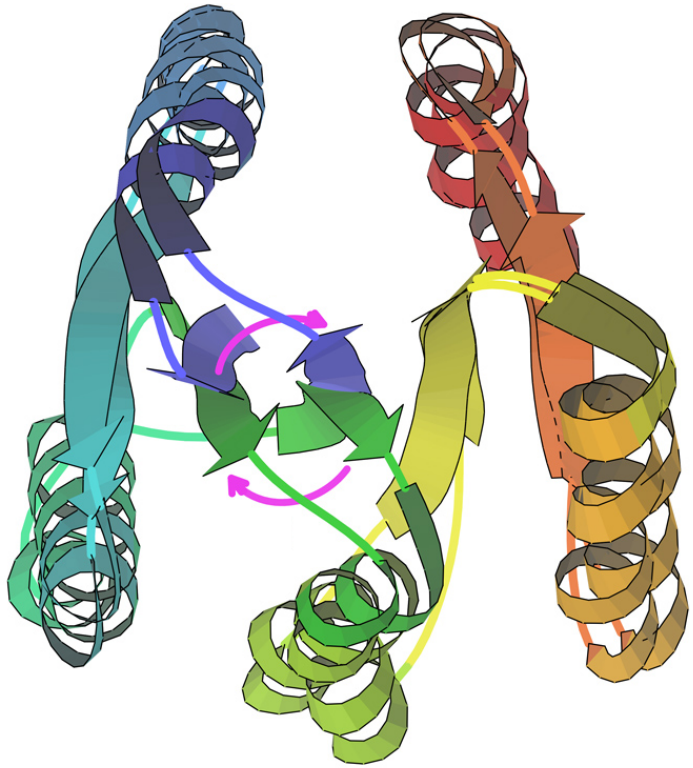


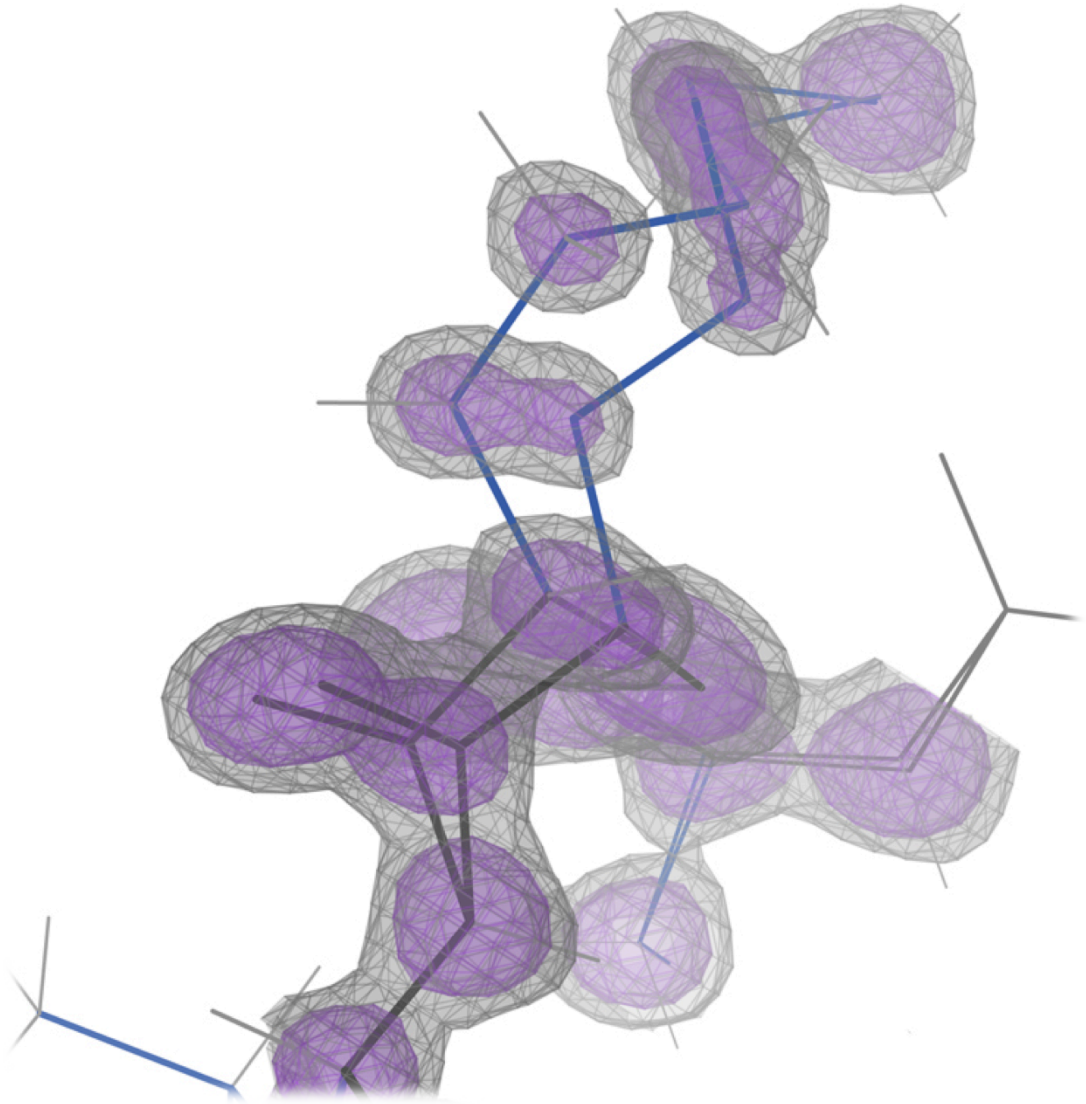


Intended and **Unintended** Backbone Flexibility in Protein Design

Daniel Keedy
Richardson Lab, Duke University
RosettaCon, August 6, 2011
(Last talk!)

Intended

Backbone Flexibility

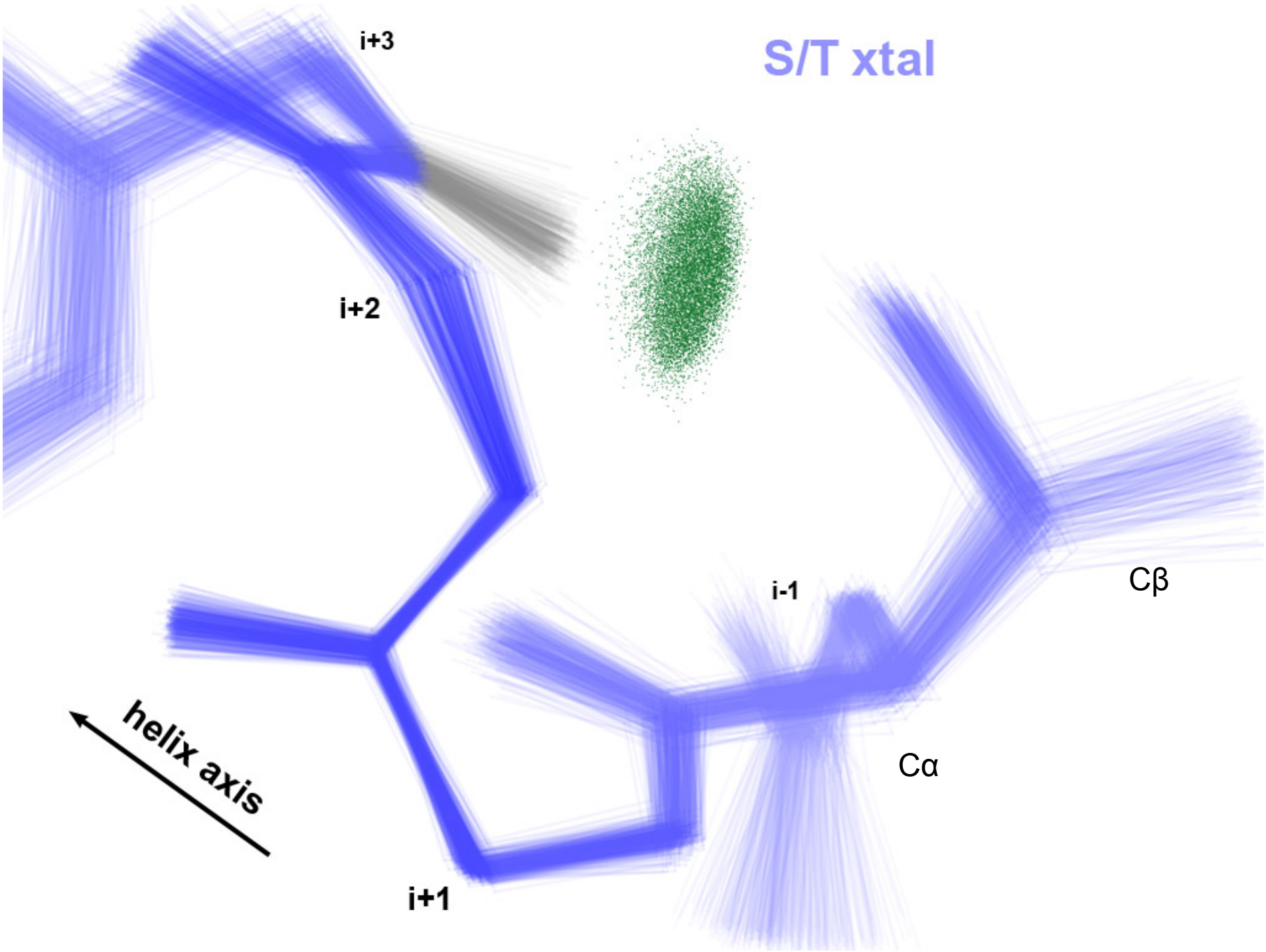


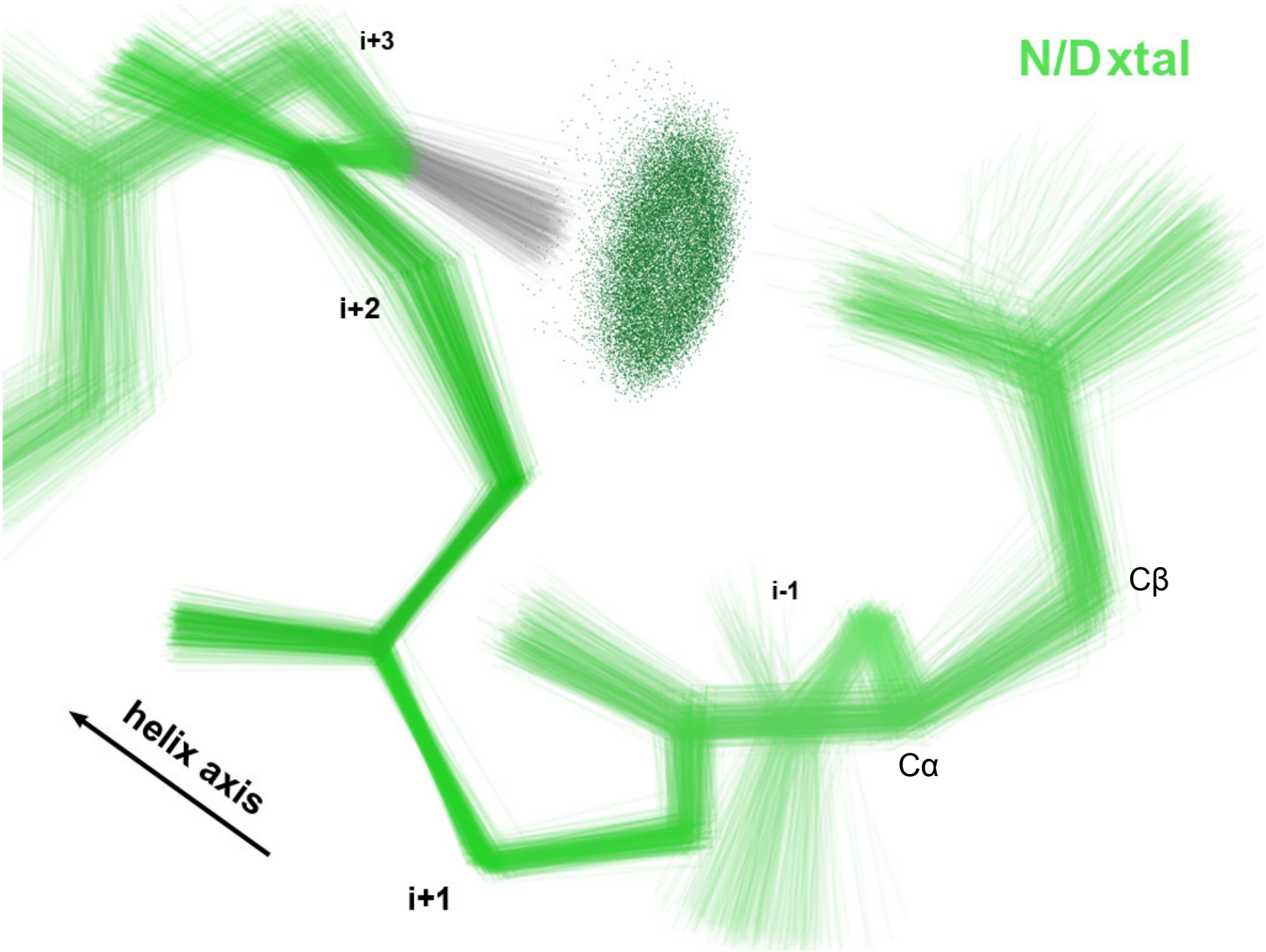
1us0, 0.66 Å,
Lys100

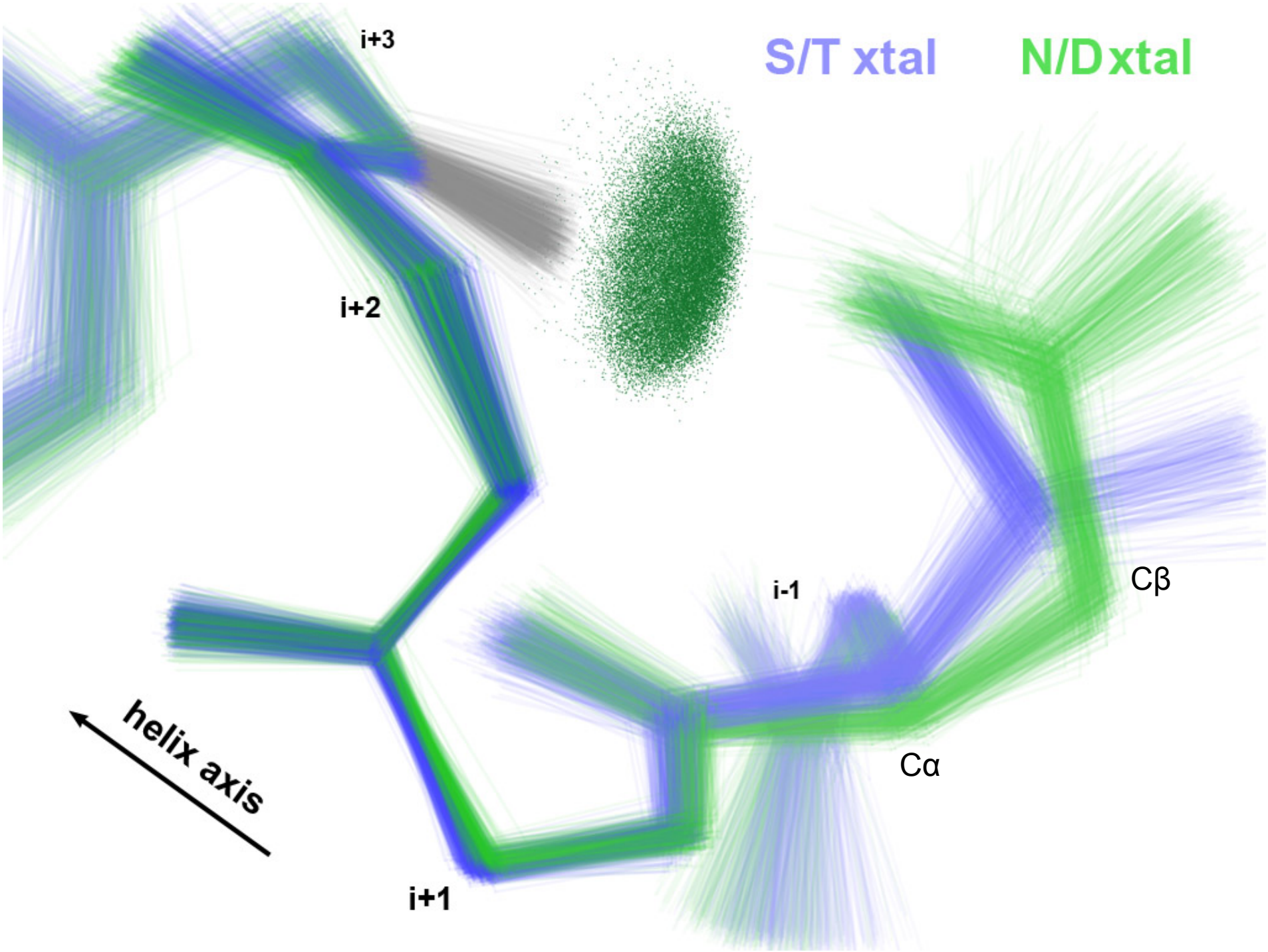
Dynamic backrubs are seen trapped in crystal structures

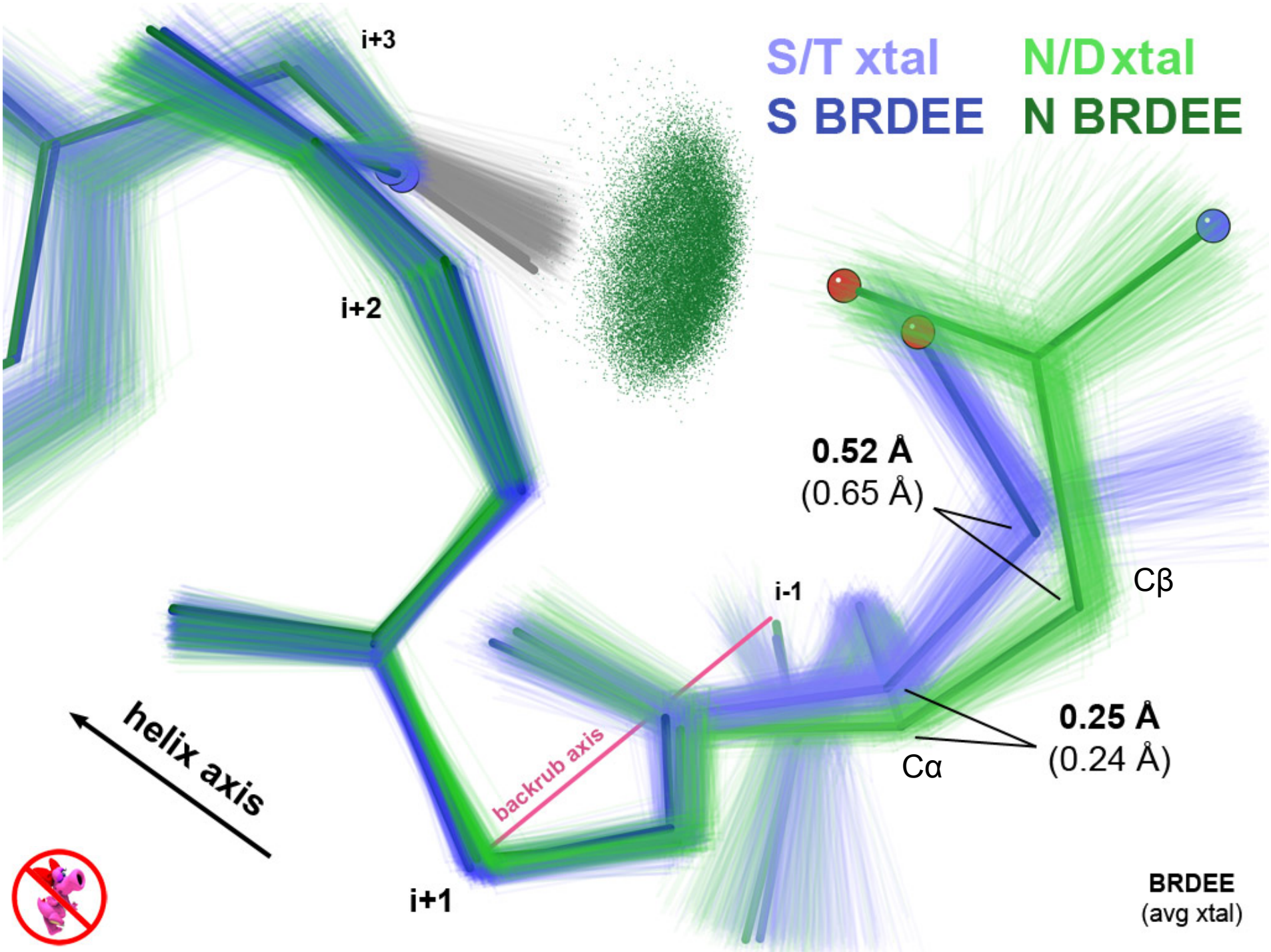
Are backrubs also relevant on longer timescales?

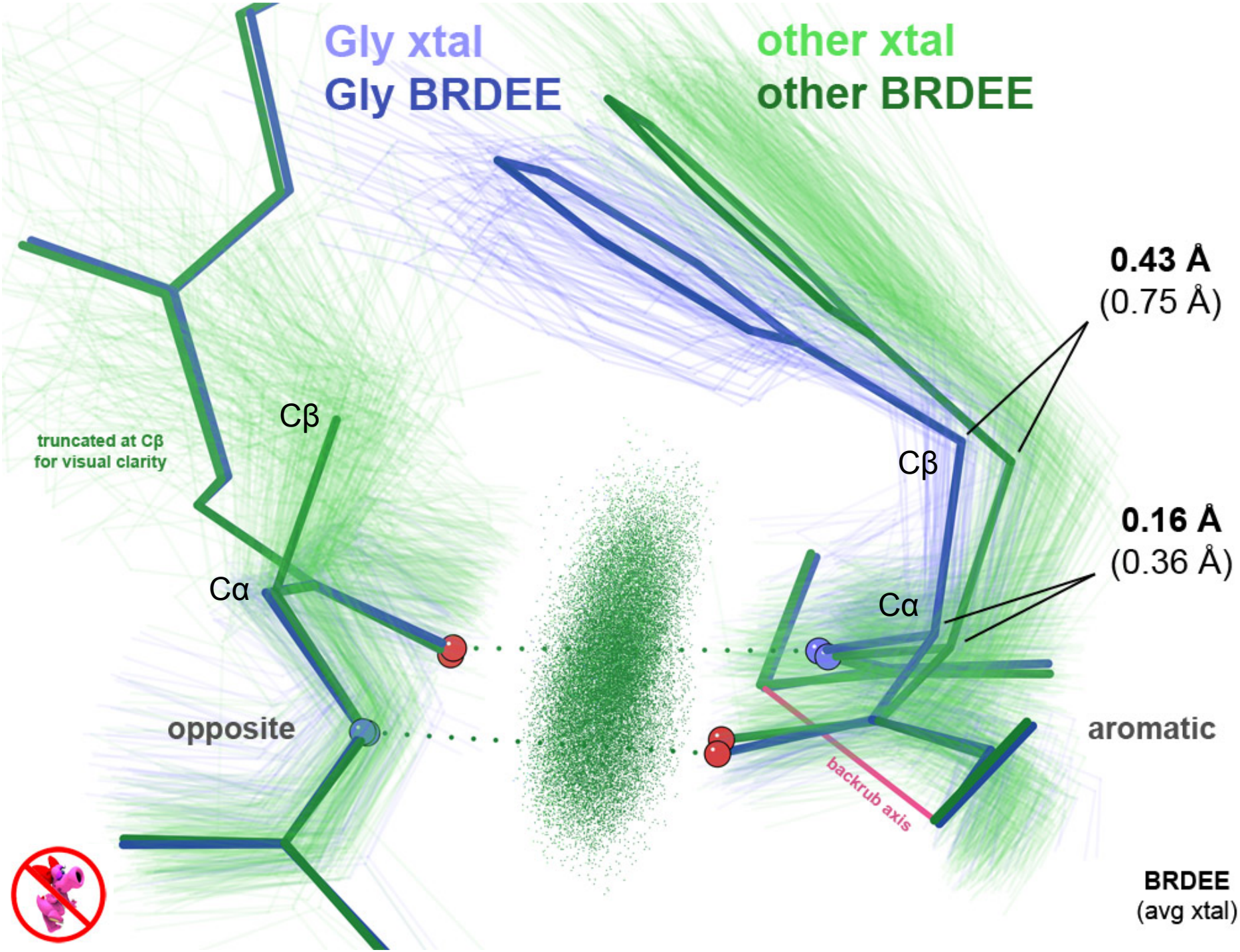
Davis, et al. (2006) *Structure*


S/T xtal
i+3
i+2
i-1
Cβ
Cα
helix axis
i+1


N/D xtal
i+3
i+2
i-1
Cβ
Cα
helix axis
i+1


S/T xtal
N/D xtal
i+3
i+2
i-1
Cβ
Cα
helix axis
i+1


S/T xtal
N/D xtal
S BRDEE
N BRDEE
i+3
i+2
i-1
i+1
0.52 Å
(0.65 Å)
Cβ
0.25 Å
(0.24 Å)
Cα
backrub axis
helix axis
BRDEE
(avg xtal)


Gly xtal
Gly BRDEE
other xtal
other BRDEE
0.43 Å
(0.75 Å)
truncated at Cβ
for visual clarity
Cβ
Cβ
0.16 Å
(0.36 Å)
Cα
Cα
opposite
aromatic
backrub axis
BRDEE
(avg xtal)

~~**Intended**~~ **Unintended**

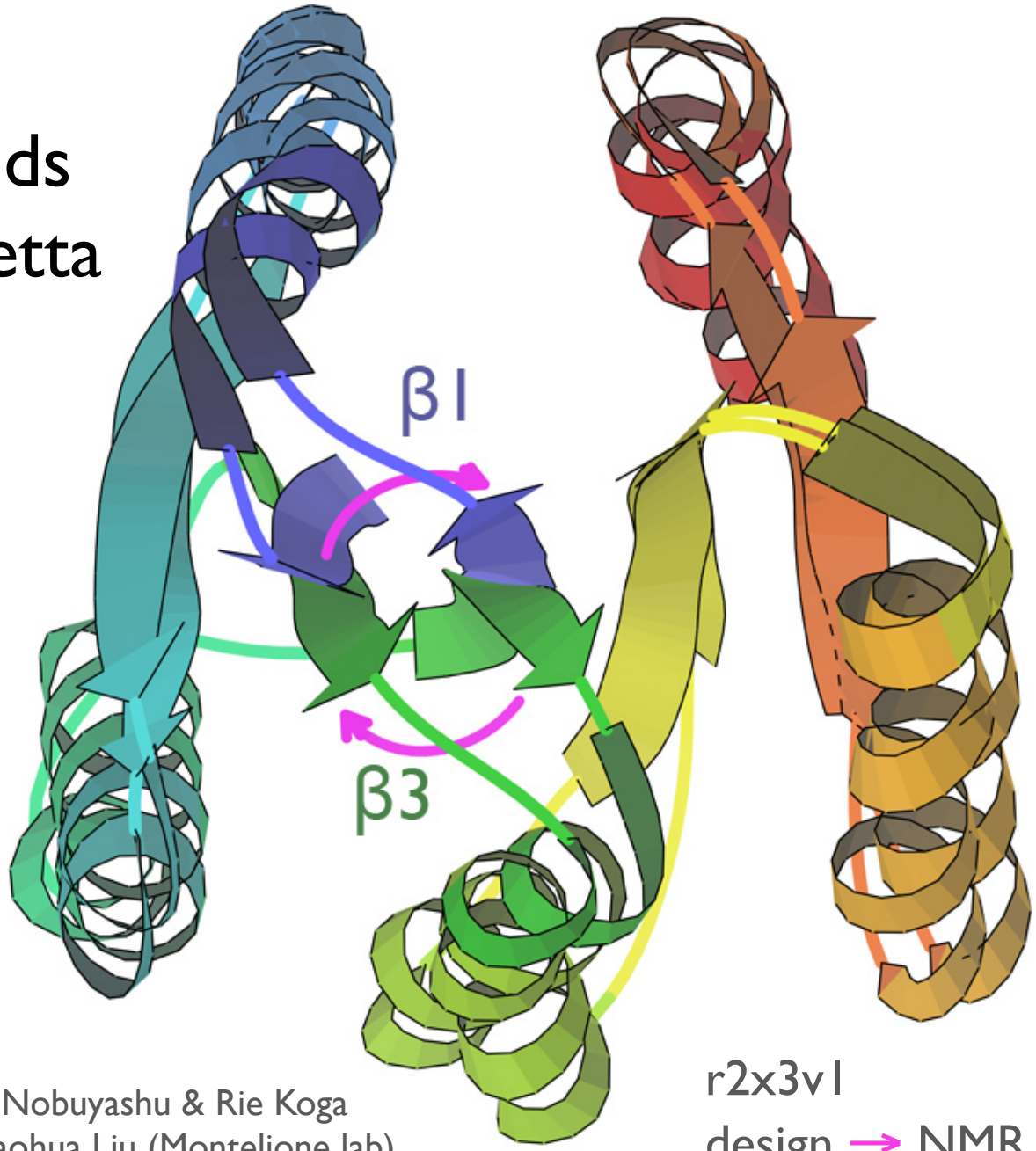
Backbone Flexibility

Strand swaps in Rossmann folds designed by Rosetta

Occurred in 3/4 cases:

- r3x3
- r2x3v1
- r2x3v2
- not r2x2

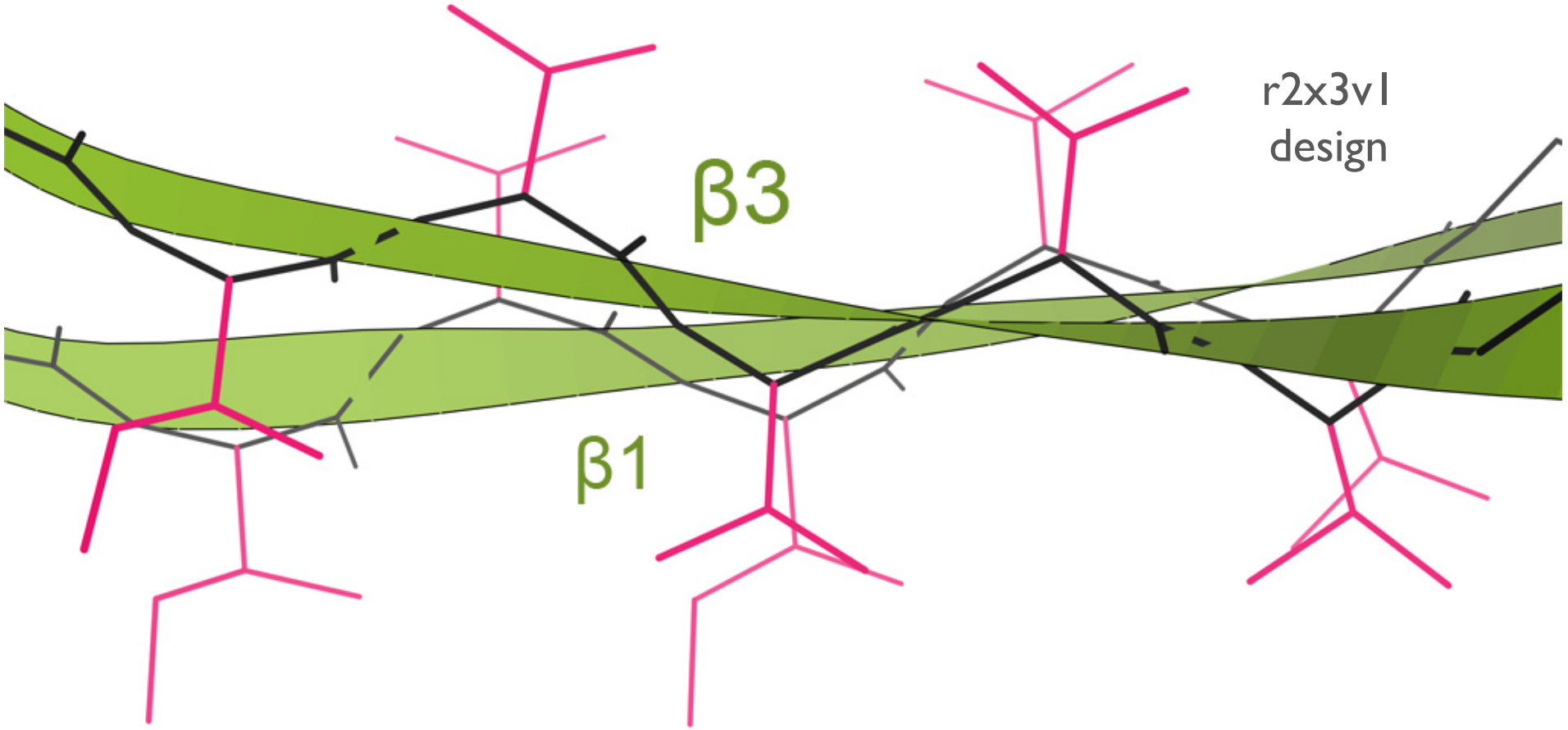
Why?...



Designs: Nobuyashu & Rie Koga
NMR: Gaohua Liu (Montelione lab)

r2x3v1
design → NMR

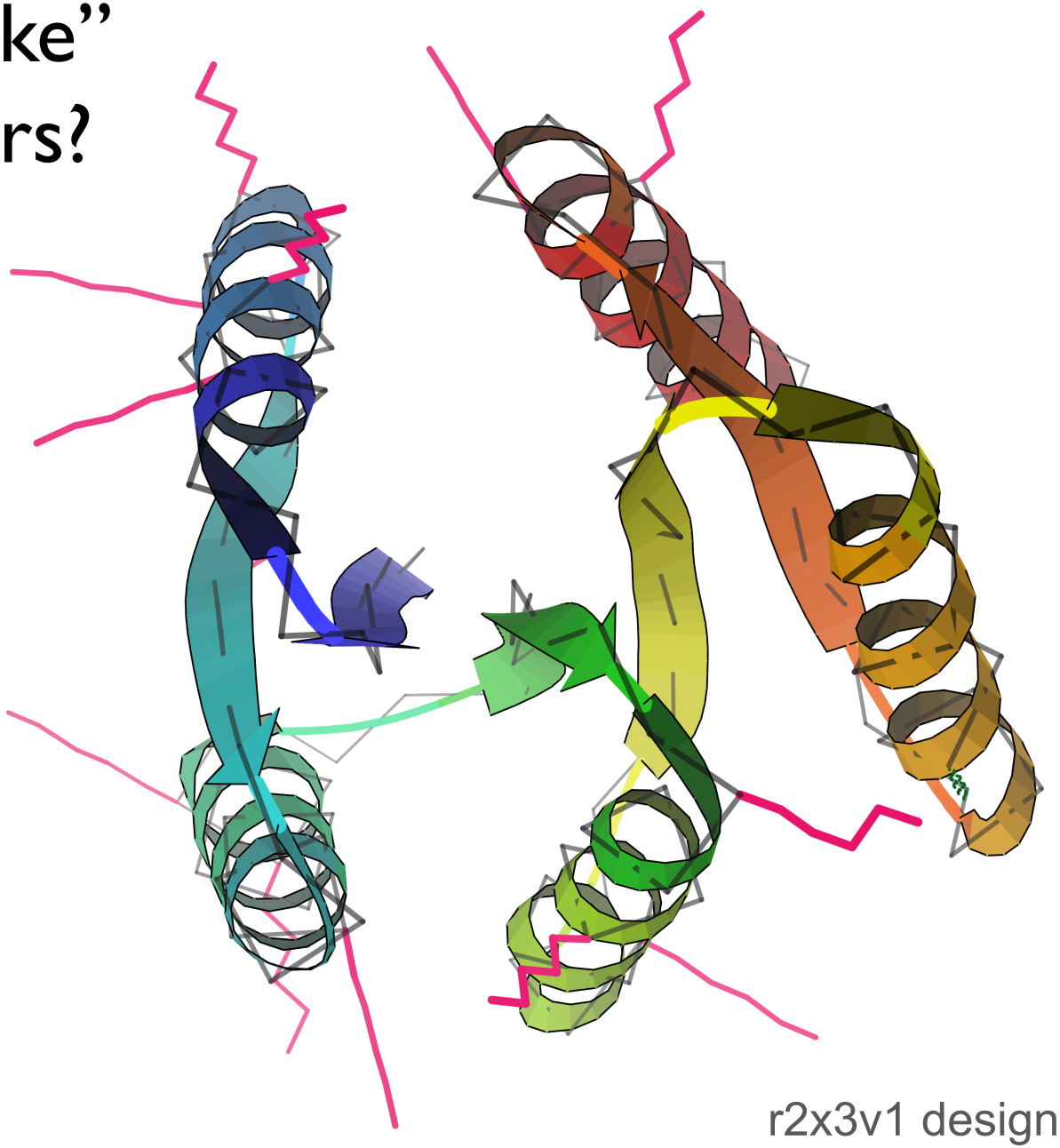
Strands can swap... but why do they?



Over-representation of branched-Cβ sidechains in swapped strands → Different strand pairings approximately isoenergetic

"Un-protein-like" lysine rotamers?

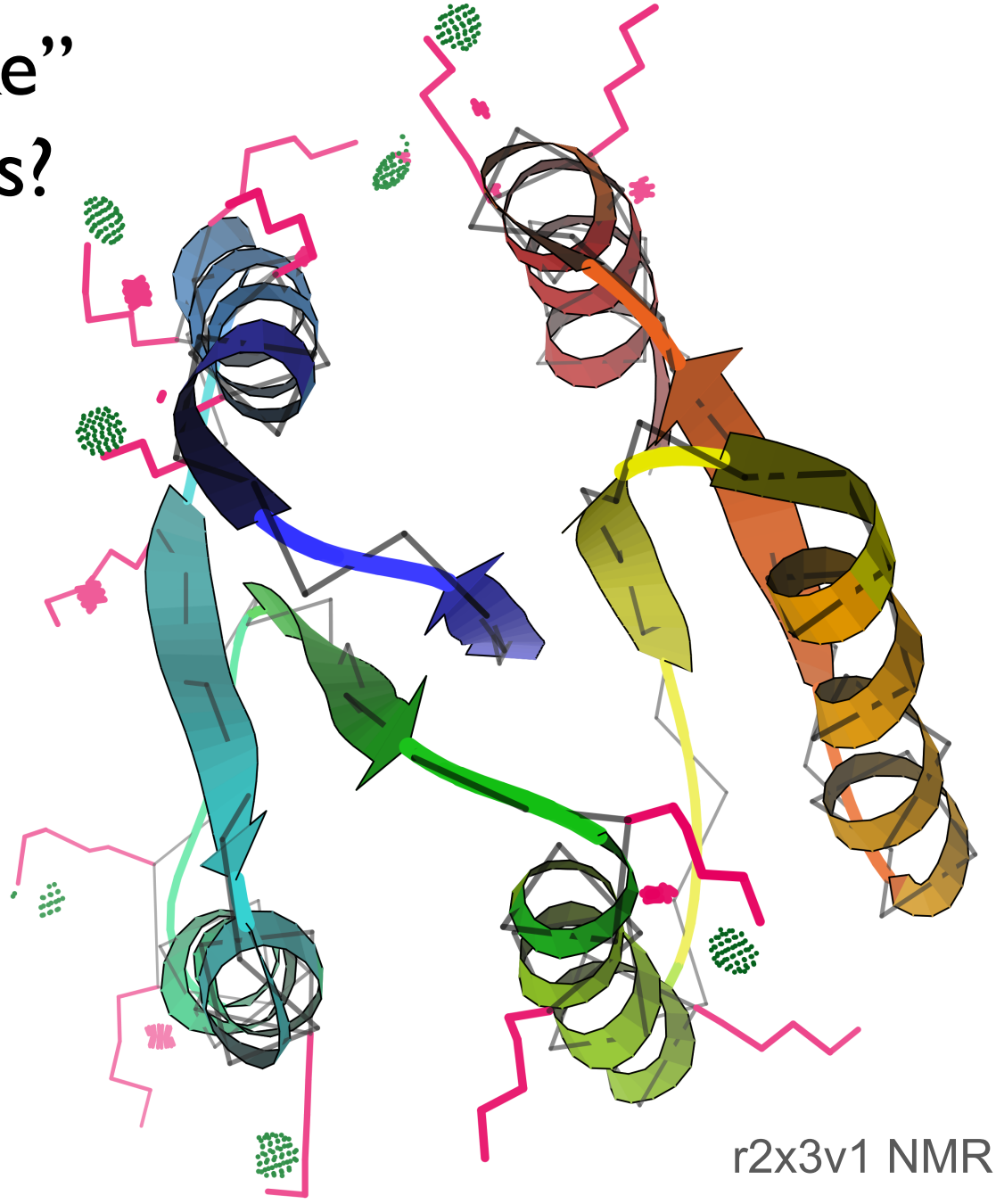
In silico **lysines** point **outward** toward solvent in extended rotamers



r2x3v1 design

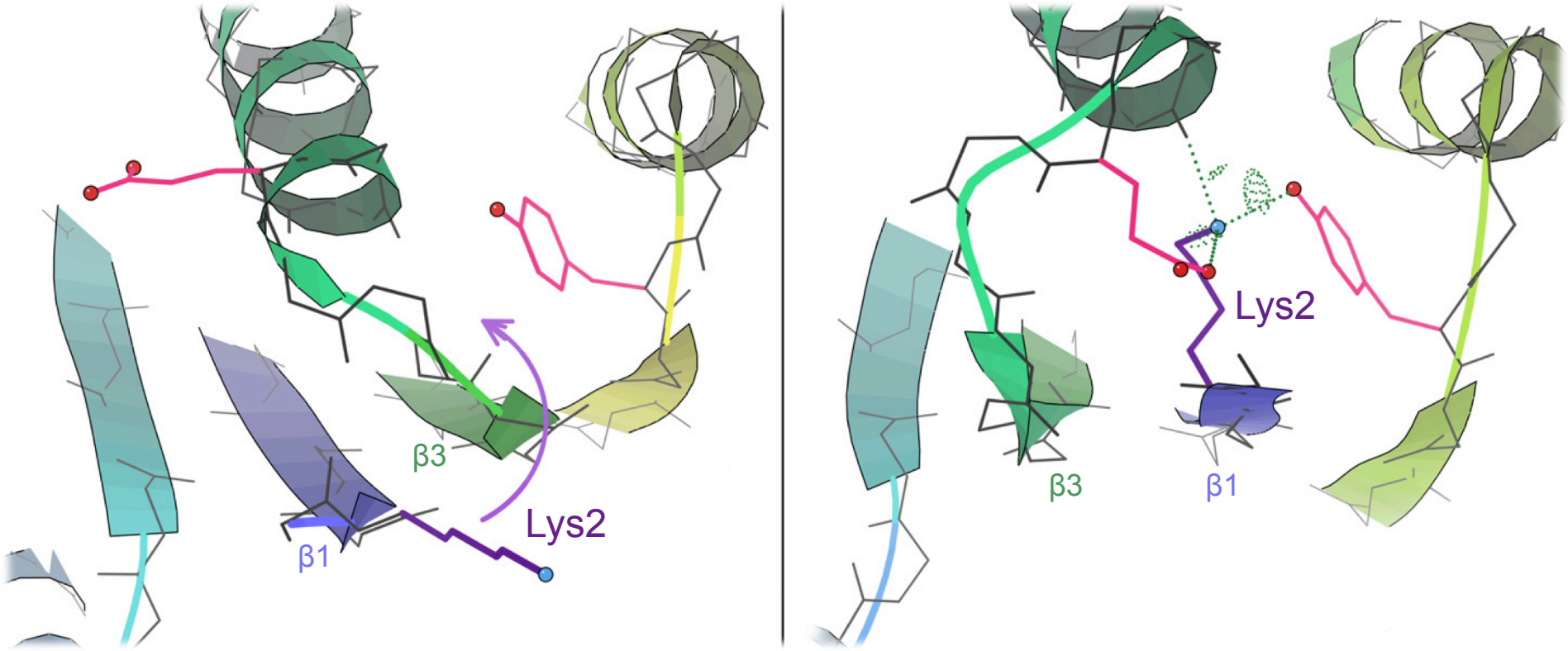
"Un-protein-like" lysine rotamers?

In vitro **lysines** tuck **inward** instead to form intramolecular **H-bonds**



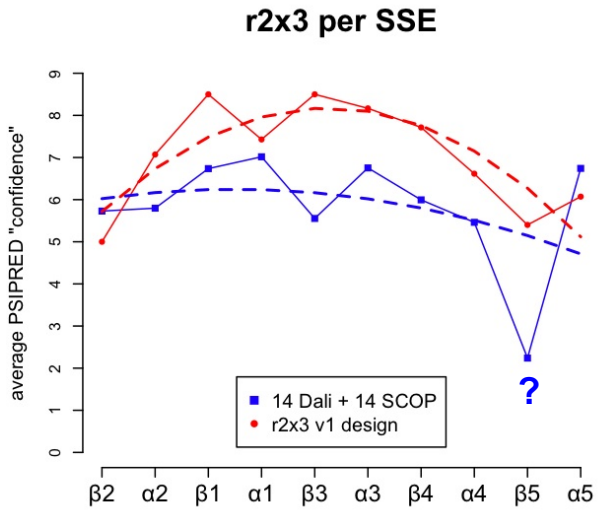
r2x3v1 NMR

“Un-protein-like” lysine rotamers?



r2x3v2 design → NMR

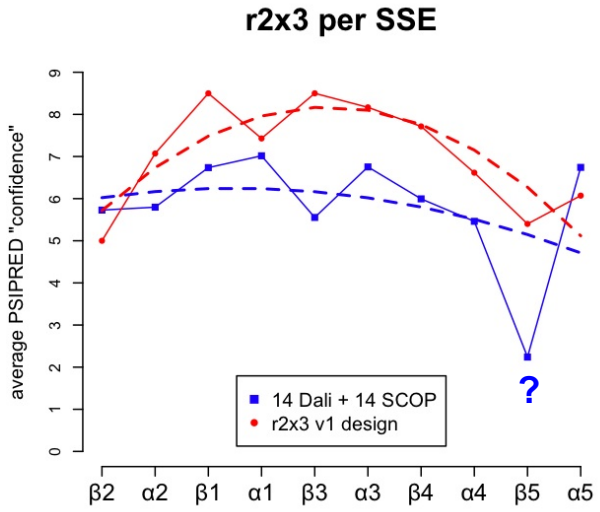
“Un-protein-like” folding pathway?

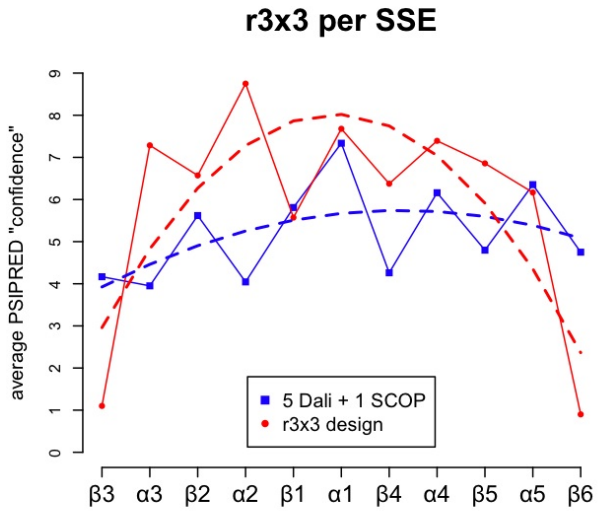


Assuming
secondary structure prediction confidence
≈ rate of secondary structure folding

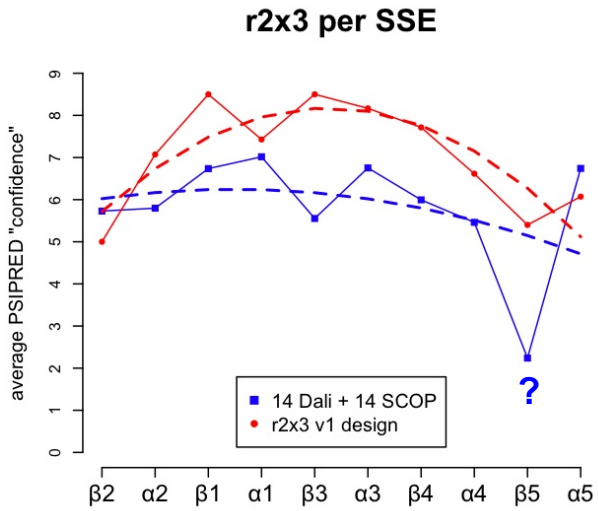
Do natural Rossmann proteins delay folding of their last strand (β5) relative to the designs?

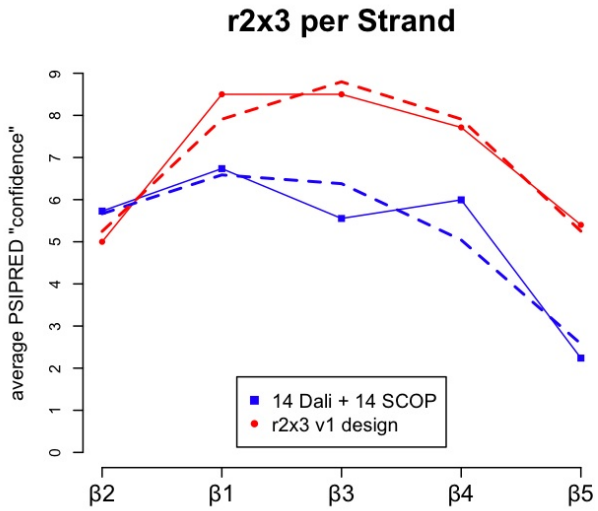
Unlikely – not generally applicable

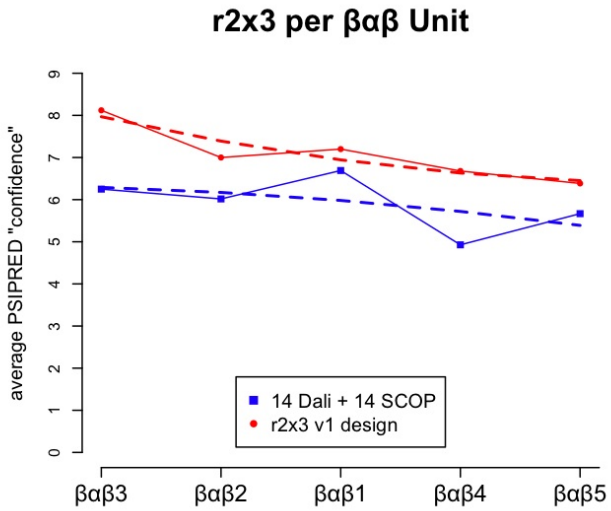


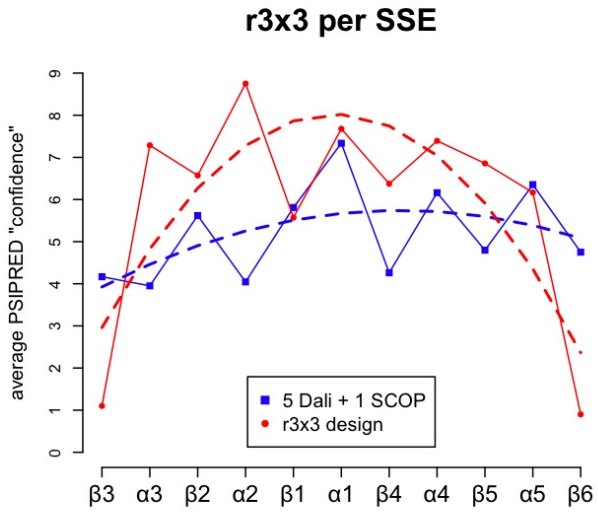


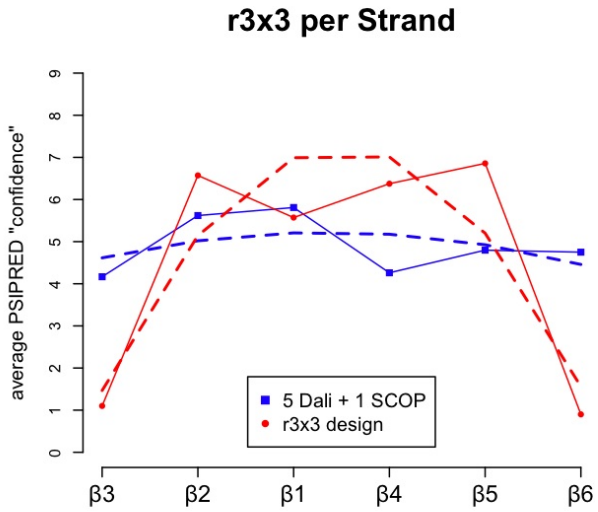
Unlikely – not generally applicable

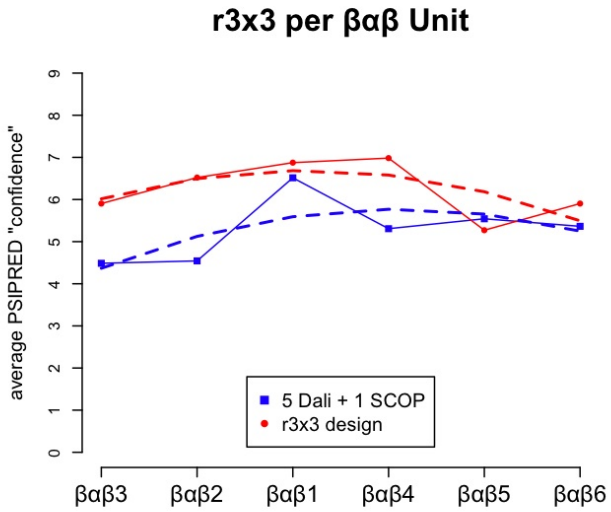


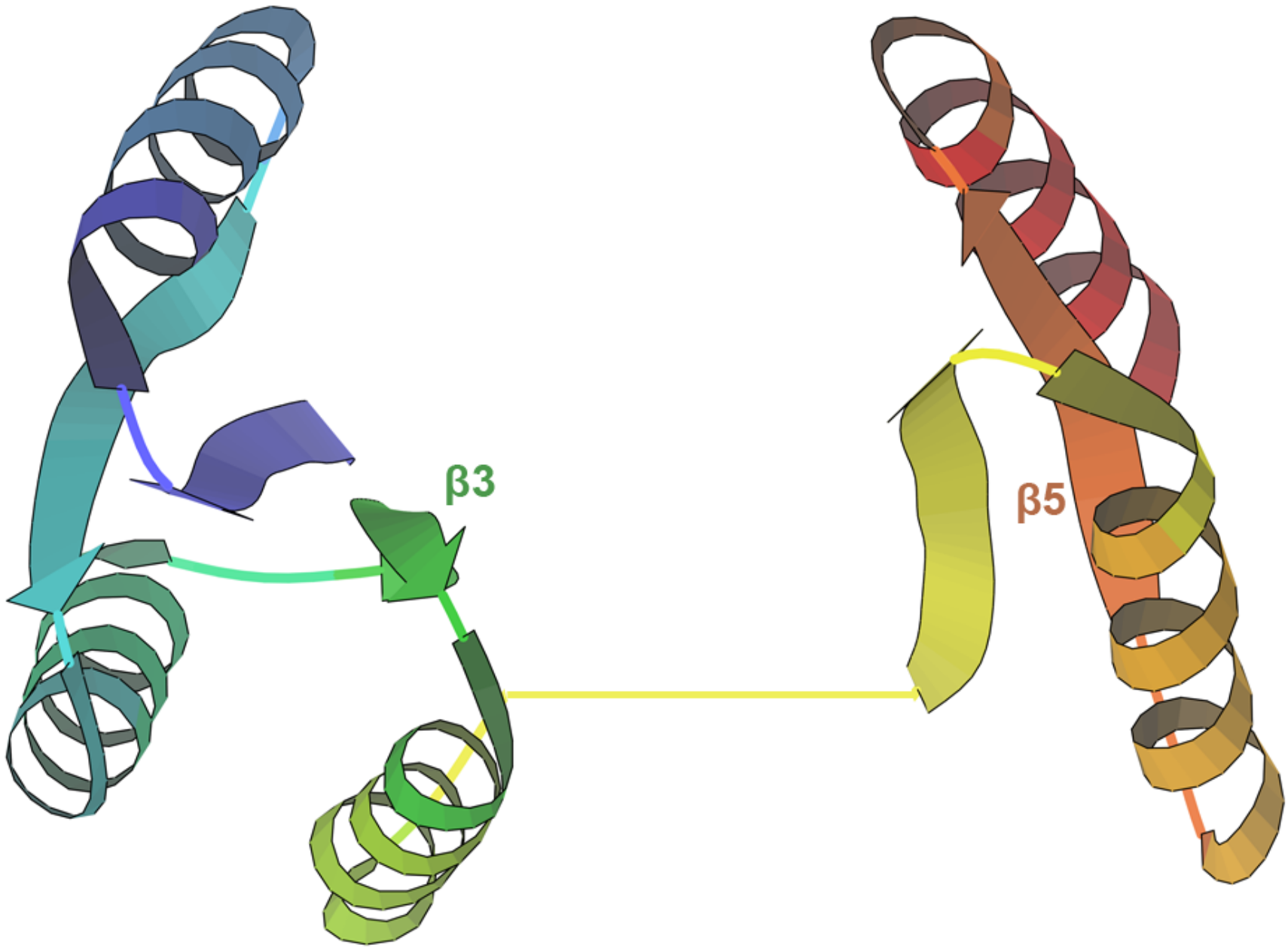


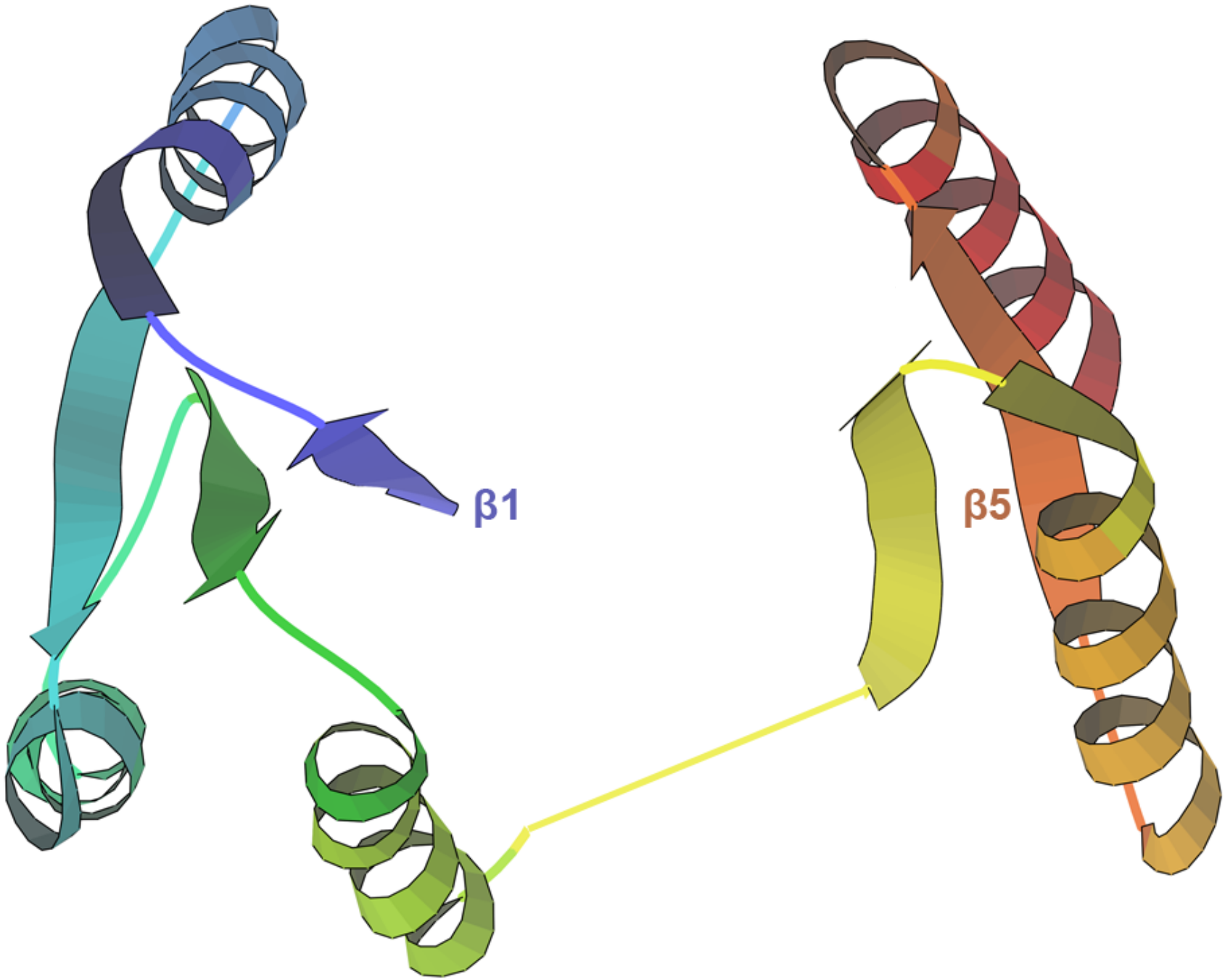






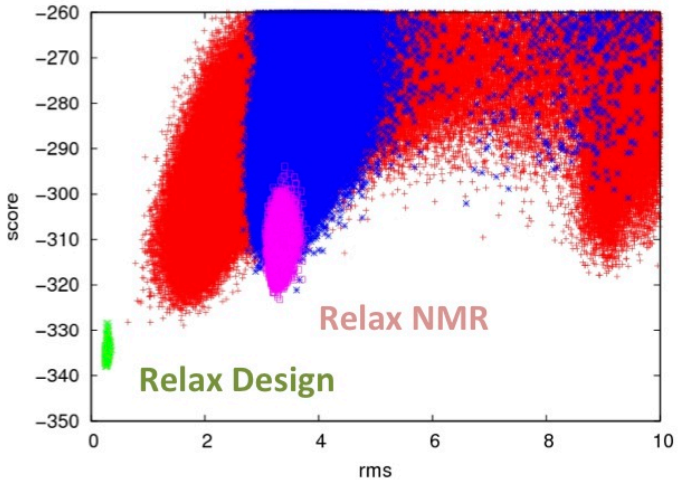



β3
β5

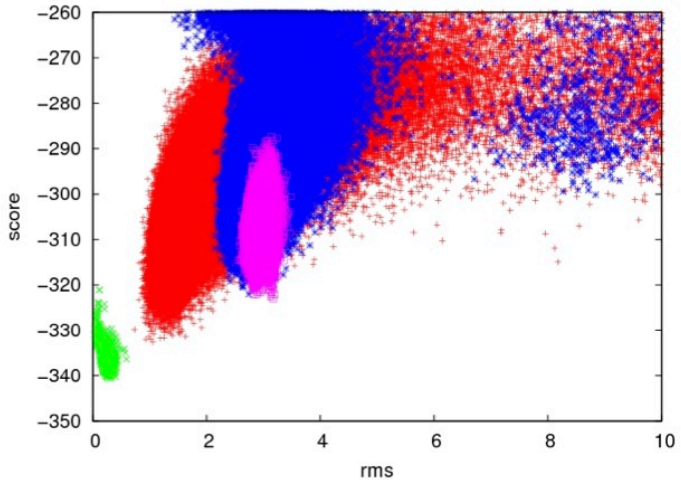

β1
β5

Energy gap between design & NMR

"Flavodoxin-like" (Nobu-version, 2l69)



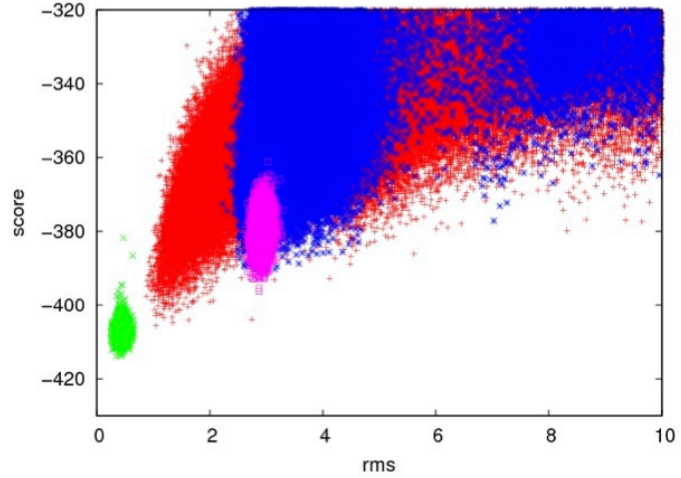
"Flavodoxin-like" (Rie-version, 2lci)



Abrelax w/ CS-fragments

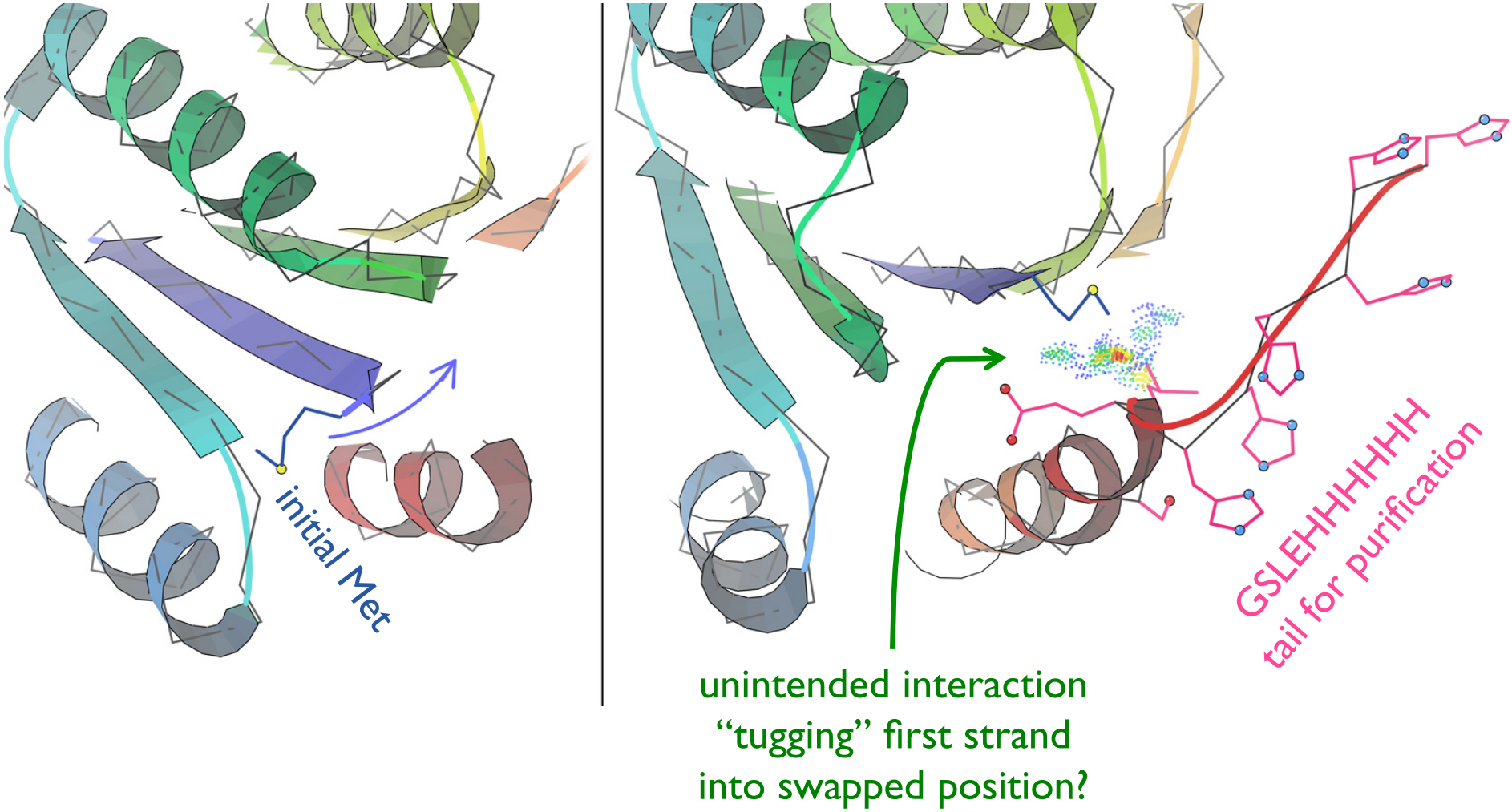
Abrelax w/ CS-fragments + NOE constraints

"R3x3, 2l82"



N- & C-termini may unexpectedly interact

r2x3v2 design → NMR



Thanks to...

"Brought to you in part by Dave the Mage!"

Richardson Lab

- Ian Davis
- Ed Triplett
- Dave & Jane

Donald Lab

- Ivelin Georgiev
- Bruce Donald

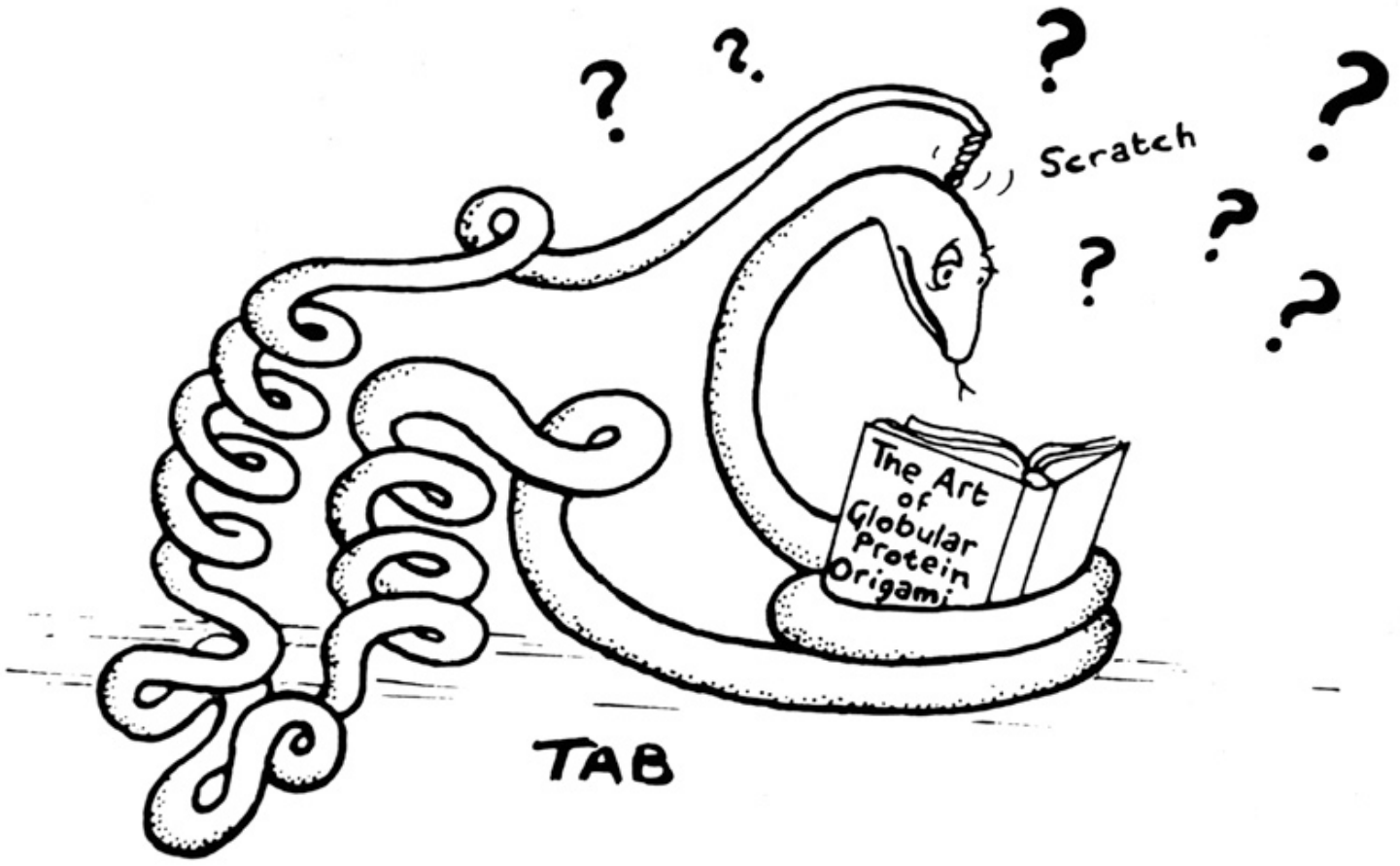
Baker Lab

- Nobuyasu Koga
- Rie Koga
- David Baker

Montelione Lab

- Gaohua Liu
- Guy Montelione

- NOW COLLAPSE DOWN HYDROPHOBIC CORE, AND FOLD OVER HELIX 'A' TO DOTTED LINE, BRINGING CHARGED RESIDUES OF 'A' INTO CLOSE PROXIMITY TO IONIC GROUPS ON OUTER SURFACE OF HELIX 'B' ---



TAB