AMPLE
***A**b Initio* **M**odelling of **P**roteins for Mo**le**cular Replacement

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Outline

- What is AMPLE?
- How it works
- When it works
- Why it works
- How to use it
- What we're doing now

AMPLE

***A**b initio* **M**odelling of **P**roteins for mo**LE**cular replacement

- Joint development by the University of Liverpool and CCP4

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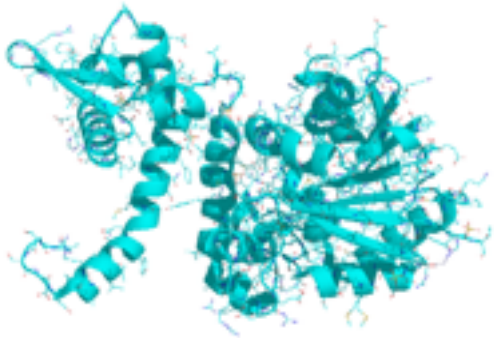
- AMPLE is a comprehensive project to assess the suitability of unconventional models in molecular replacement

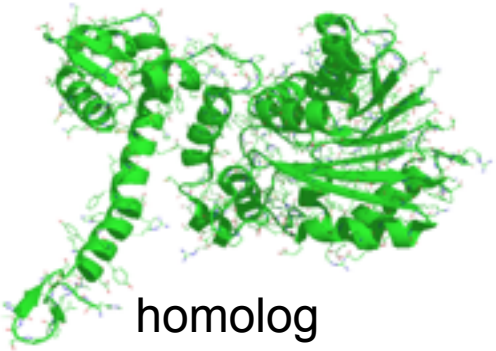
- An additional goal of the project is to make AMPLE into an automated software tool that can be made generally available to potential users through CCP4

Molecular Replacement

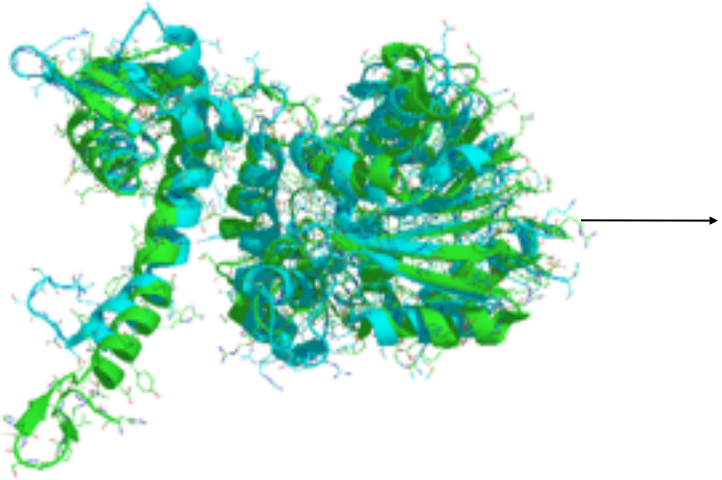
Sequence search for a suitable homolog

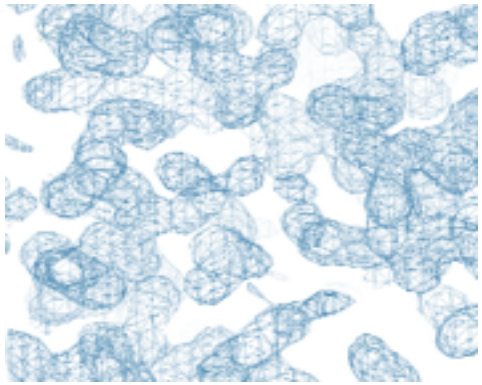


target



homolog





electron density map

- Phases from homolog
- Intensities/positions from target

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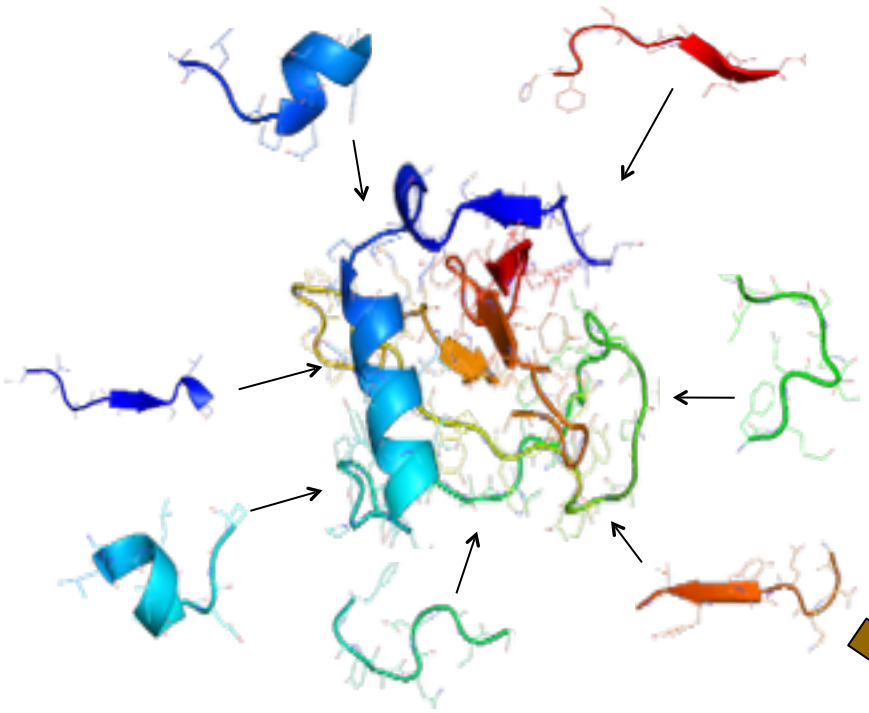
Replacing Molecular Replacement

- Globular proteins with new folds or distant homologies
- Coiled-coils (small changes in sequence or crystallisation conditions can lead to different polymorphs crystallising)
- Helical membrane proteins
- NMR structures as search models
- Not sure exactly what is in the crystal

What to do?

- **AMPLE**
- **ARCIMBOLDO**

Ab Initio Modelling



- Create database of 3- and 9-residue fragments.
- Start from linear chain and splice in fragment geometry.
- Randomly move the fragments under simplified forcefield
- Score the resulting structures.

- 1000's of "decoys" created.
- Decoys are clustered and centroid representatives of largest cluster are considered candidate fold predictions
- Refinement under a more realistic physics-based force field

Decoys vs. final models

- for normal usage > 20,000 decoys are recommended
- refinement stage can take weeks on a supercomputer

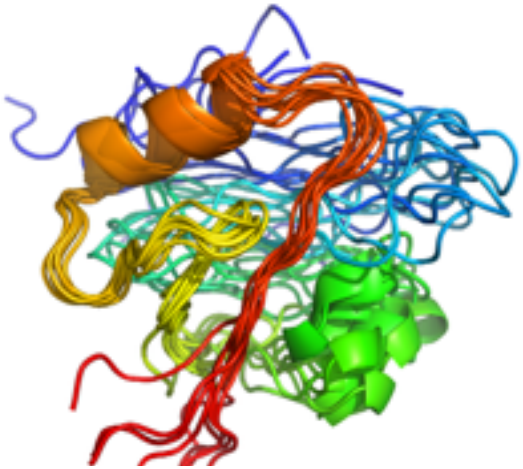
This is beyond the capabilities of most crystallography groups.

But:

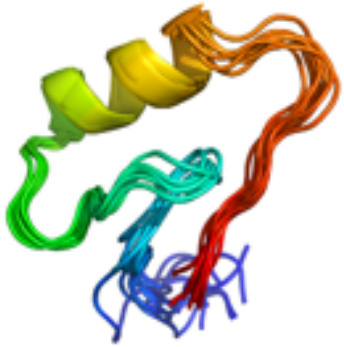
- creating 1000 decoys ~ 1/2 day on a single CPU with ROSETTA
- QUARK modelling is available online and takes ~ 1 day

Ab Initio Models and Molecular Replacement

Simple *ab initio* modelling	Molecular Replacement
Clusters of similar structures	Works well with superposed **ensembles** approximating the target
Within/between clusters, similarity indicates accuracy ∴ trim inaccurate regions leaving more reliable **core**	May only require a **partial** model



trim to accurate core



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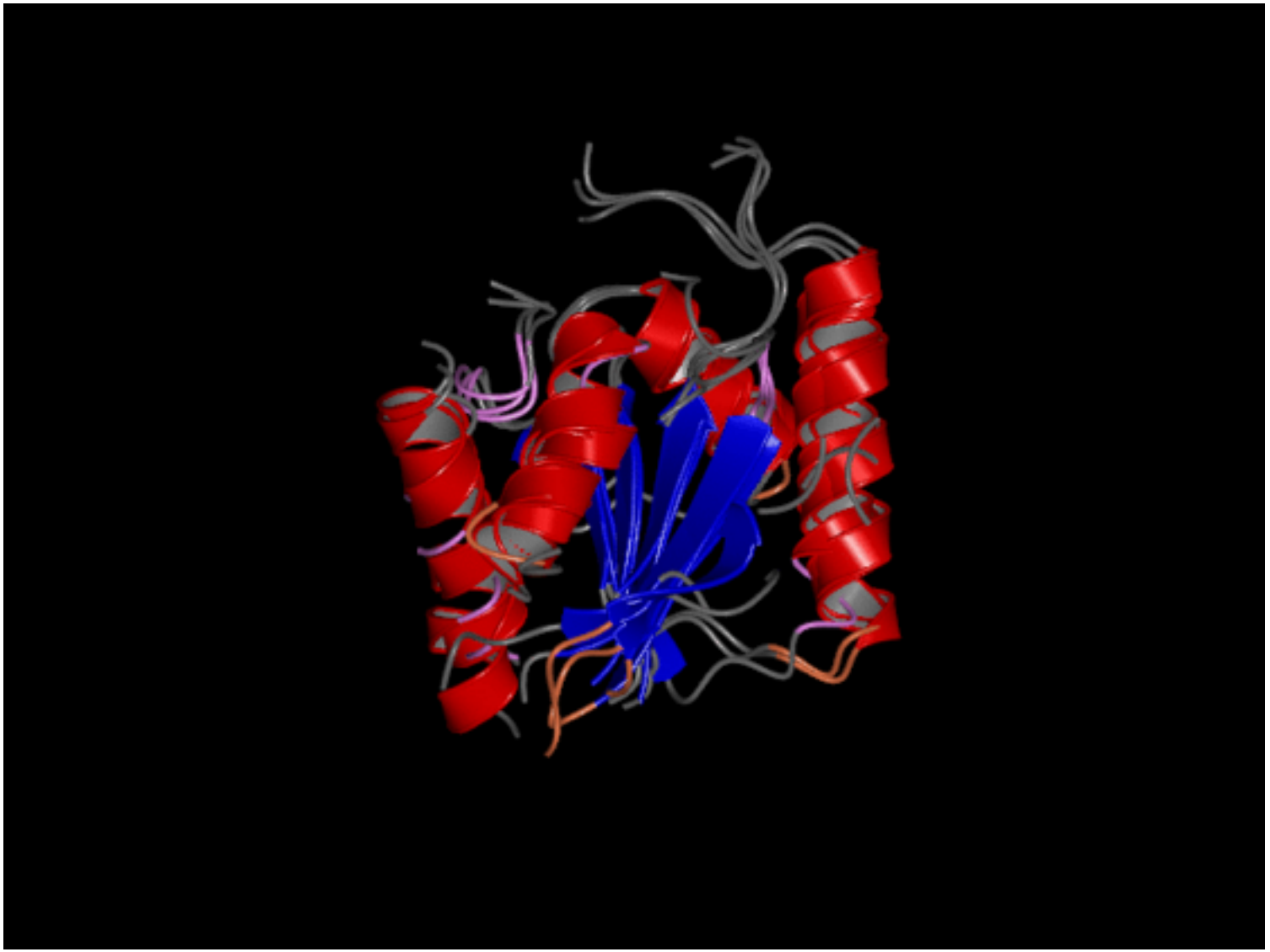
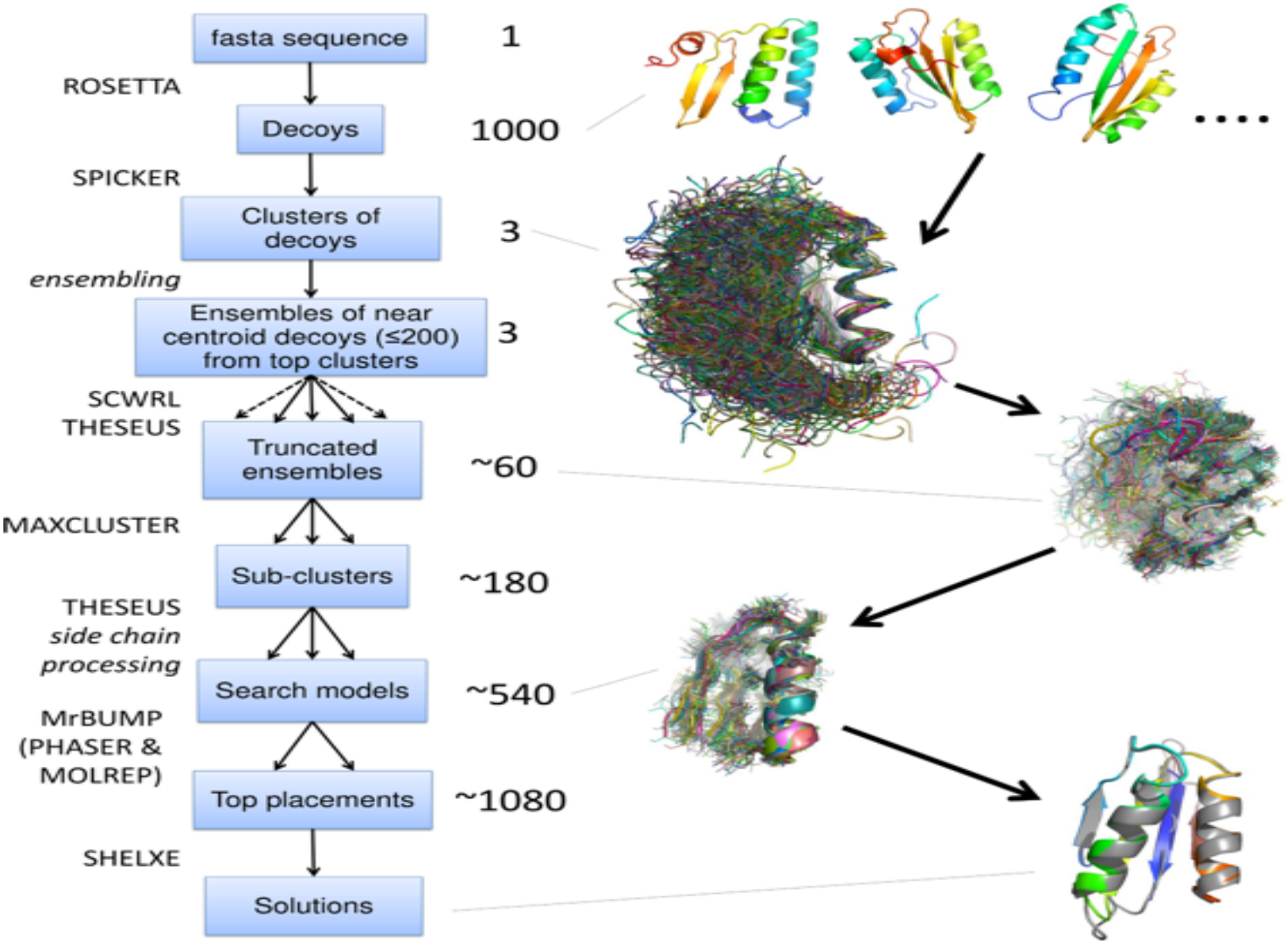
Truncation in action



The AMPLE pipeline



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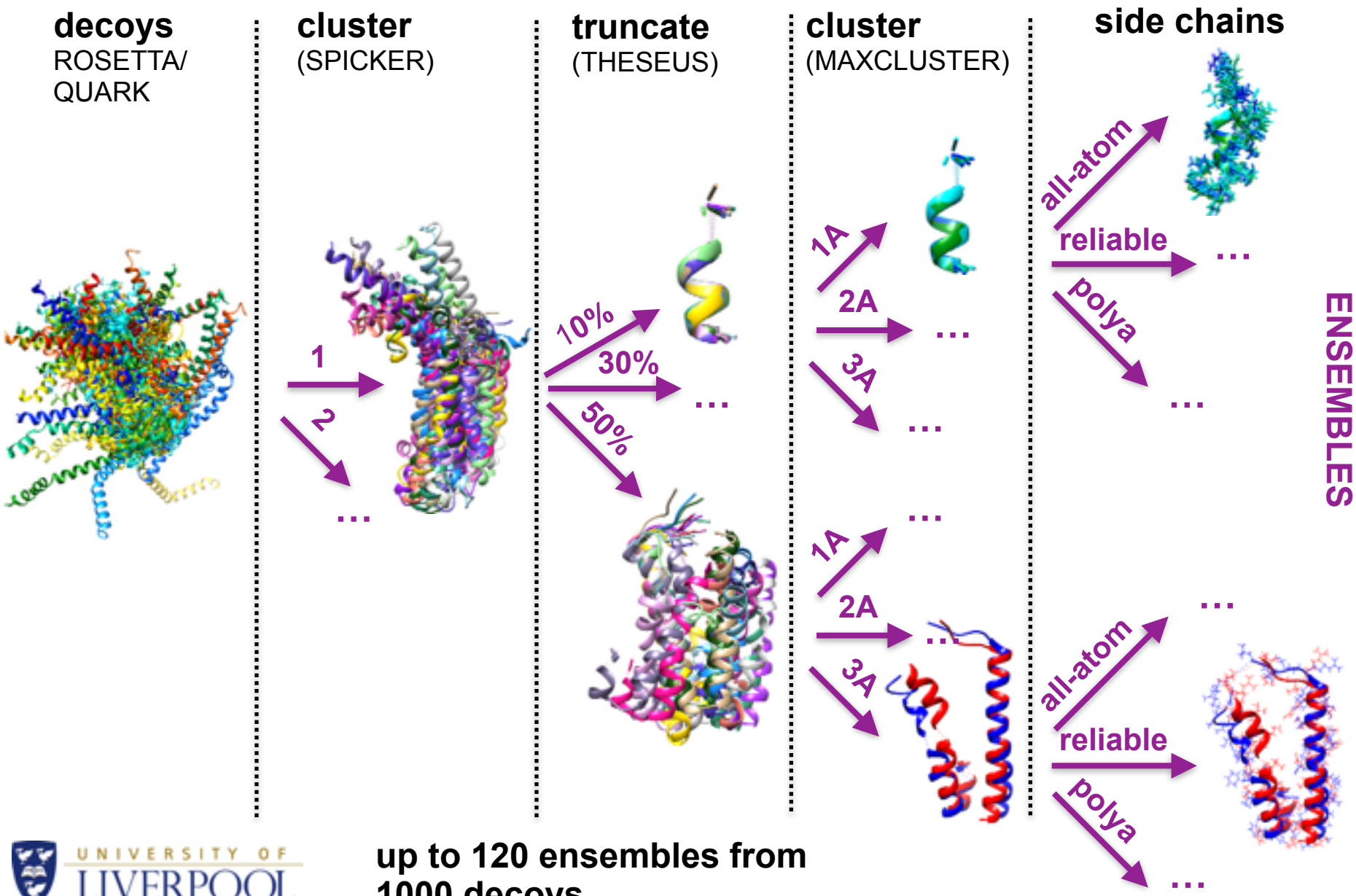
Bibby, J., Keegan, R.M., Mayans, O., Winn, M.D., Rigden, D.J., 2012. Acta Crystallographica Section D Biological Crystallography 68, 1622–1631.

Pathways to ensembles



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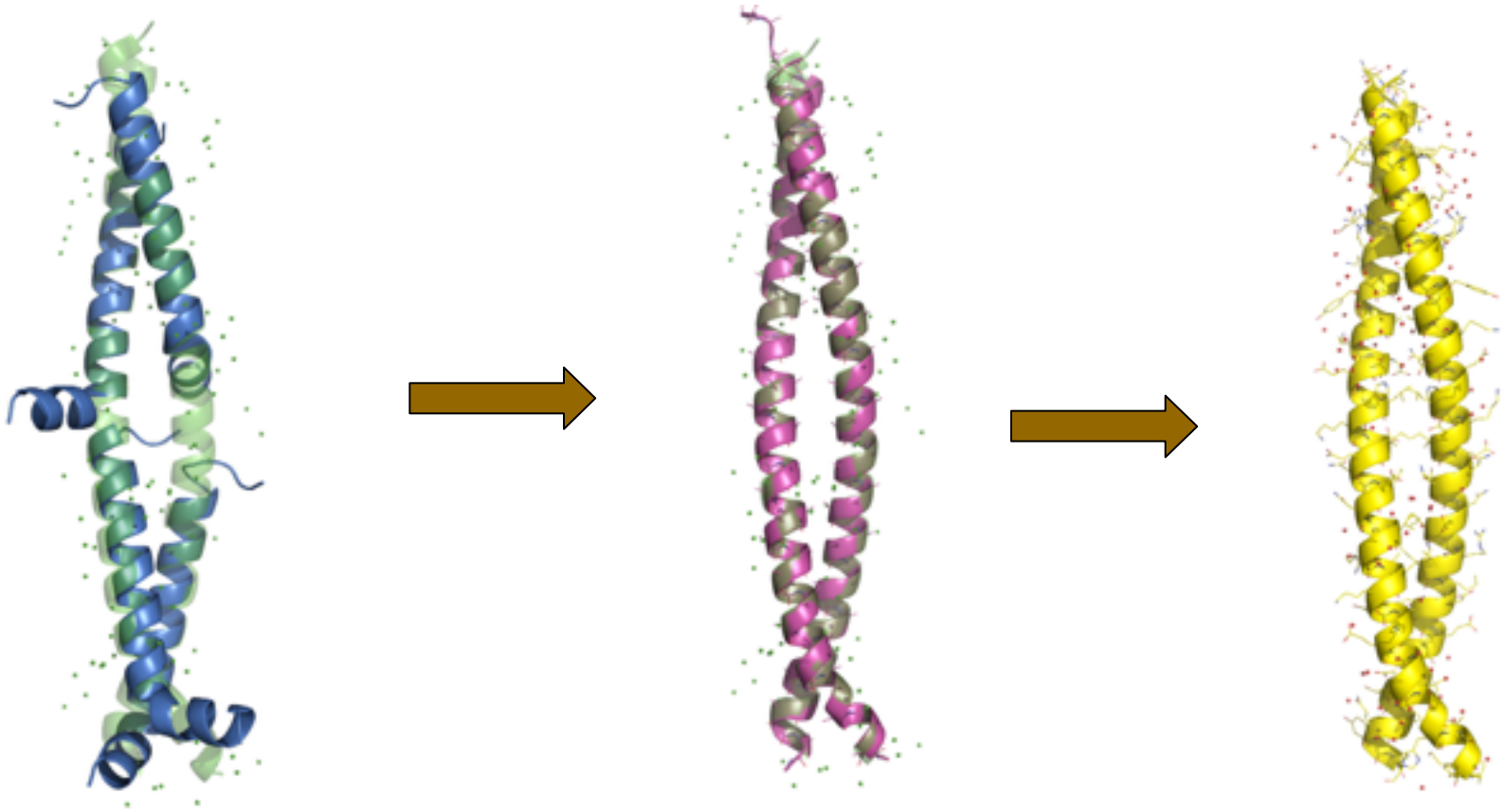
up to 120 ensembles from 1000 decoys

MRBUMP MR pipeline



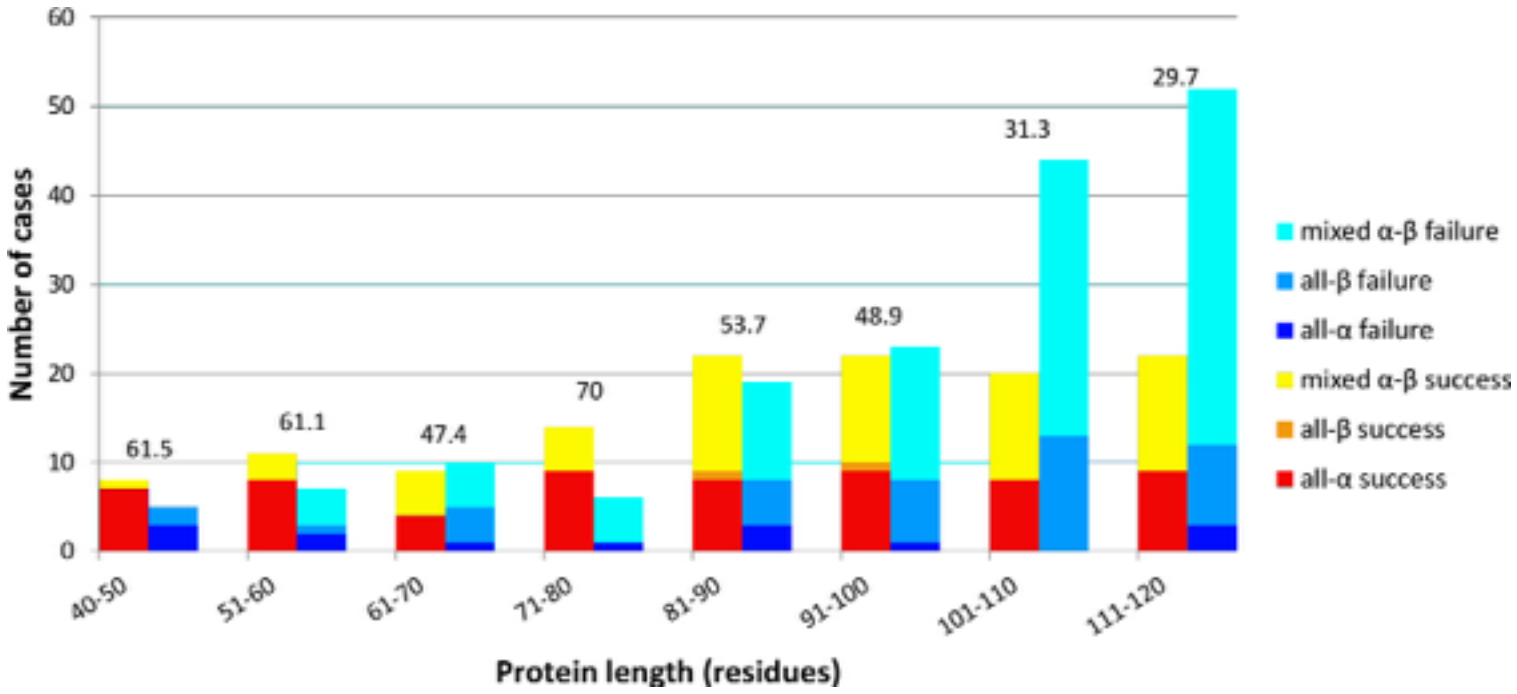
PHASER: places search model

SHELXE: density modification and tracing of the main chain

ArpWARP/BUCCANEER completes the rebuild

Initial AMPLE success

- Test set of 295 small globular proteins (40-120 residues) from the PDB, resolution of 2.2 Å or better
- Single molecule in the asymmetric unit
- Mixture of all-α, all-β and mixed α-β secondary structure



Initial success rate: all-α: 80%, all-β:2%, mixed α-β: 37%

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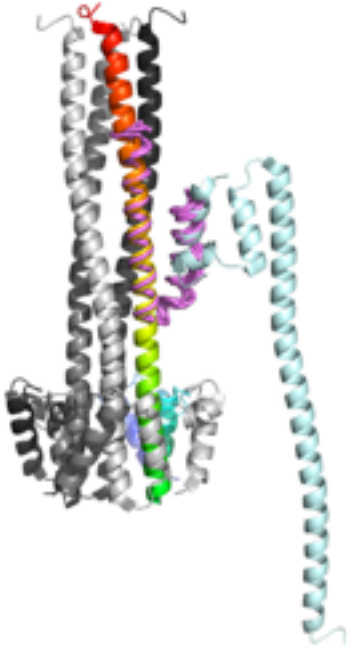
Coiled-coils

- Among the most ubiquitous and functionally important class of proteins
- 2-5 amphipathic helices wound around each other in a supercoil with sequence repeats of 7 or 11 residues
- Notoriously difficult to solve with MR techniques
- *But*, are exclusively α-helices
- Selected 94 cases from the PDB:
  - 12 < residues < 253
  - 0.9 < resolution Å < 2.91



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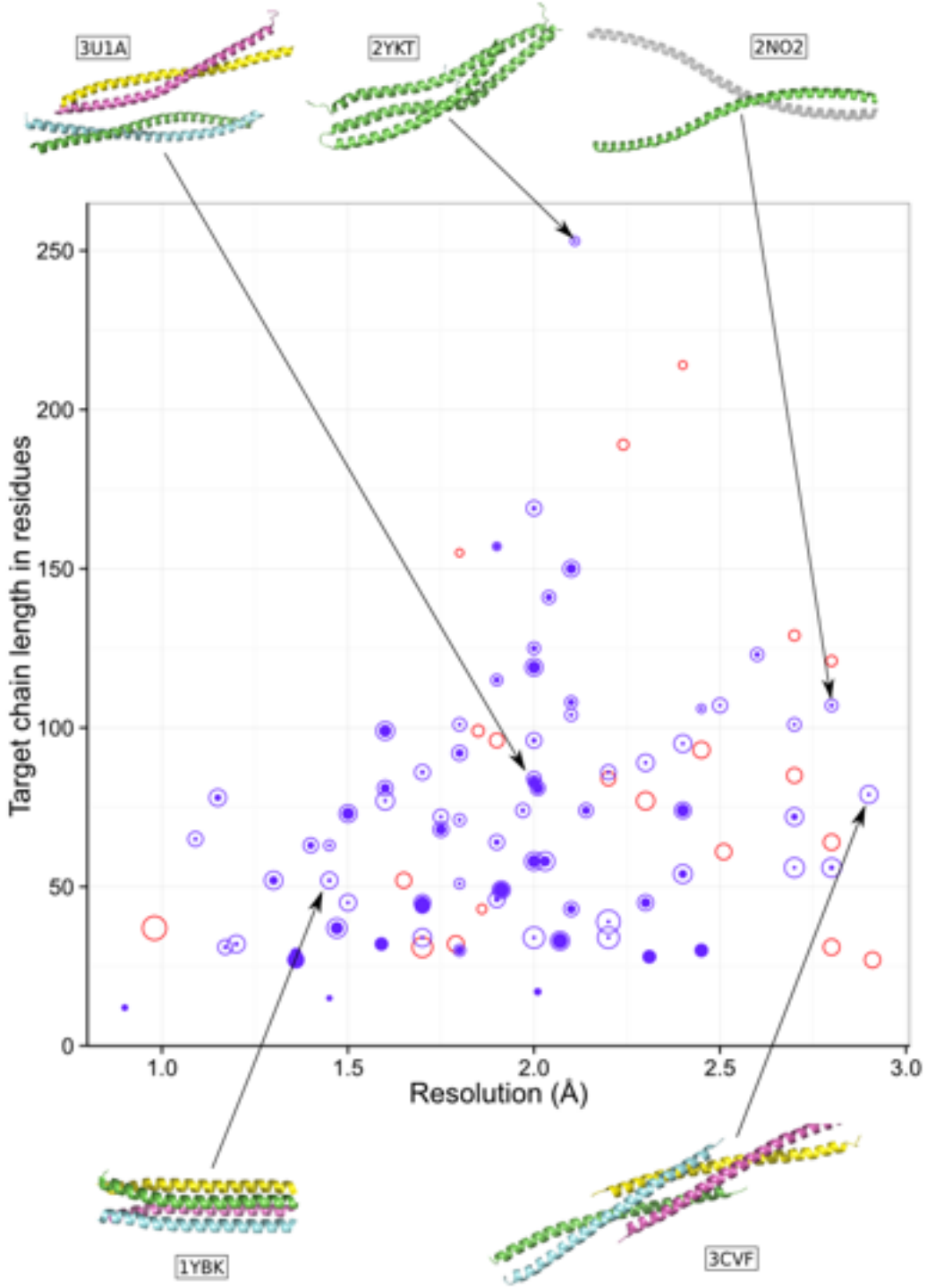
Coiled-coils

Solved more than **78%** with an average CPU time of less than 24 hours.

Successes included:

- **3U1A:** 334 residues
- **3CVF:** resolution of 2.8A
- **2NO2:** a domain of Huntingtin-interacting protein 1, that contains a long, unconventional coiled-coil-like assembly originally phased experimentally using MAD
- **1YBK** right-handed coiled coil



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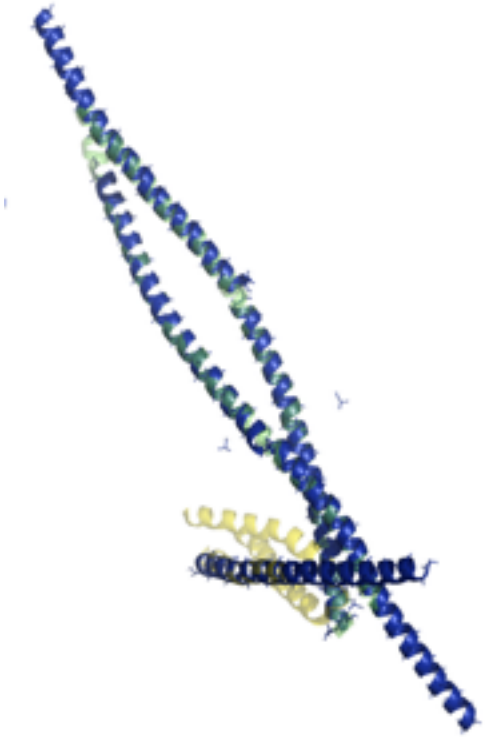
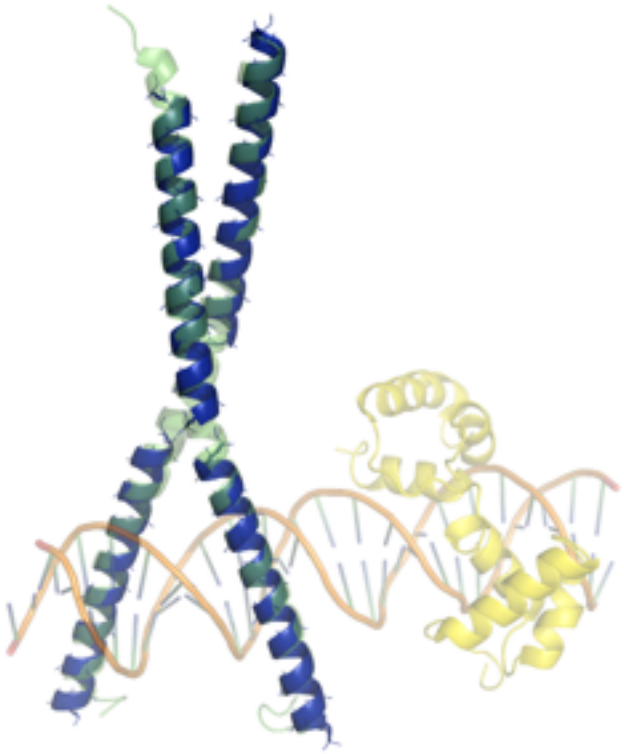
Complex Ambitions

Coiled-coils are often involved in complexes - could AMPLE's ability to solve coiled-coils extend to complexes?





- **1X79**: three chains, 322 residues, ~70% coiled-coil @ 2.41Å
- **1H8A**: MyB/DNA complex of 278 residues ~ 60% coiled-coil @ 2.23Å.

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Transmembrane Proteins

- Experimentally very difficult to work with/crystallise
- Represent ~30% of all proteins
- Make up < 3% of structures in the Protein Data Bank
- Presence in the membrane constrains their shape, so they can be easier to model
- MR with *ab initio* transmembrane models hasn't been tried yet

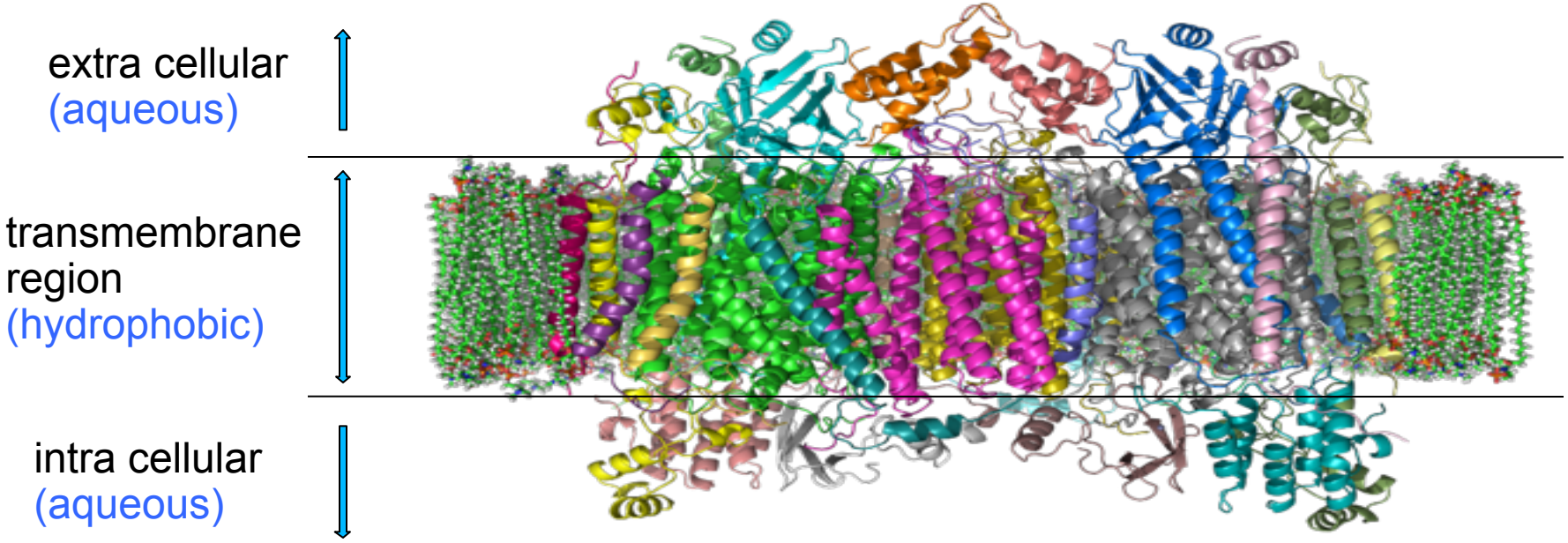

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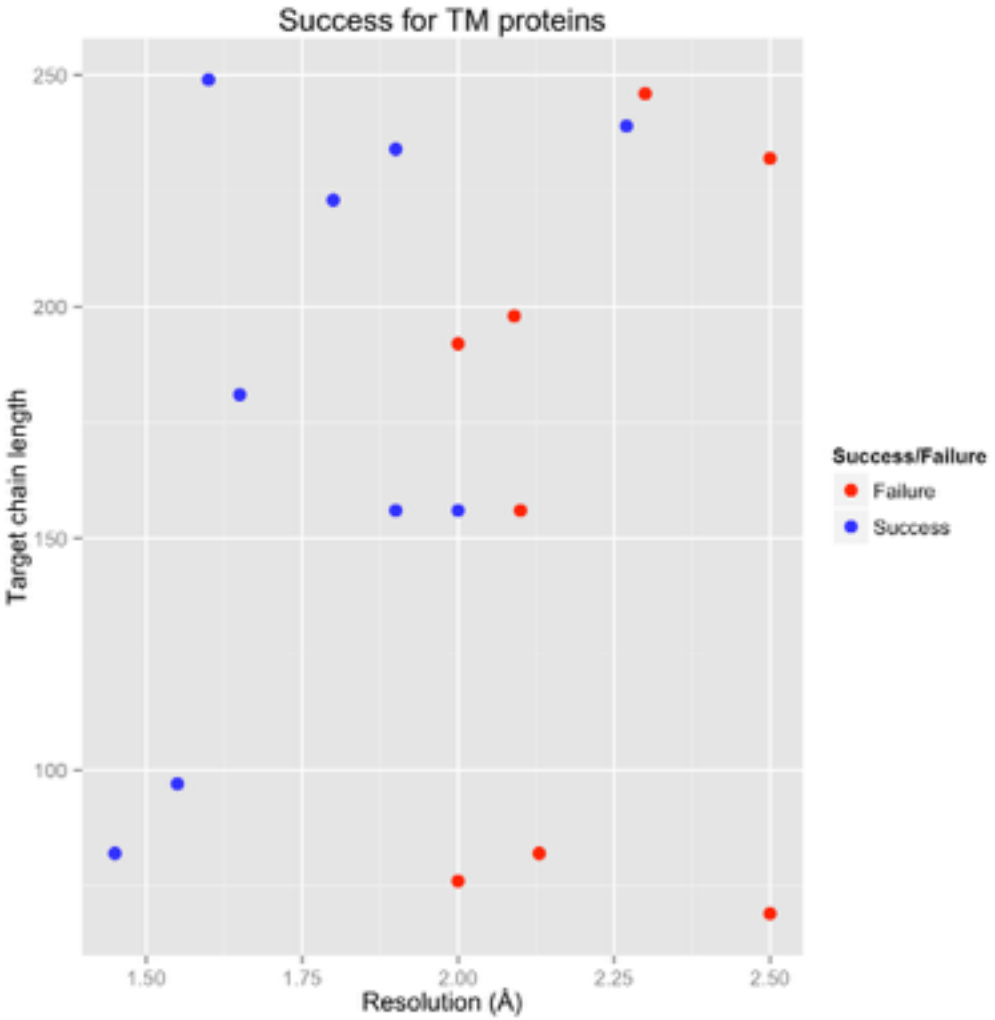
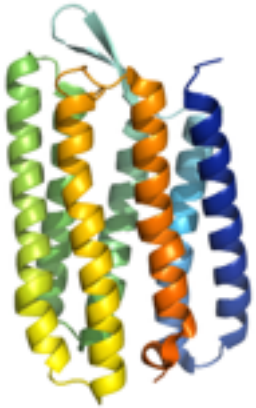
Image: http://en.wikiversity.org/wiki/File:Cytochrome_C_Oxidase_1OCC_in_Membrane_2.png

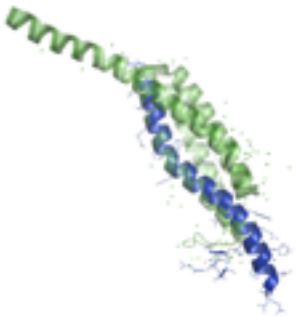
Transmembrane Results

- Selected 18 alpha-helical transmembrane proteins:
  - 23 – 249 residues
  - 1.45 – 2.5A resolution
- 9 clear successes
- Successes extended to 249 residues (3HAP).





3HAP



3PCV

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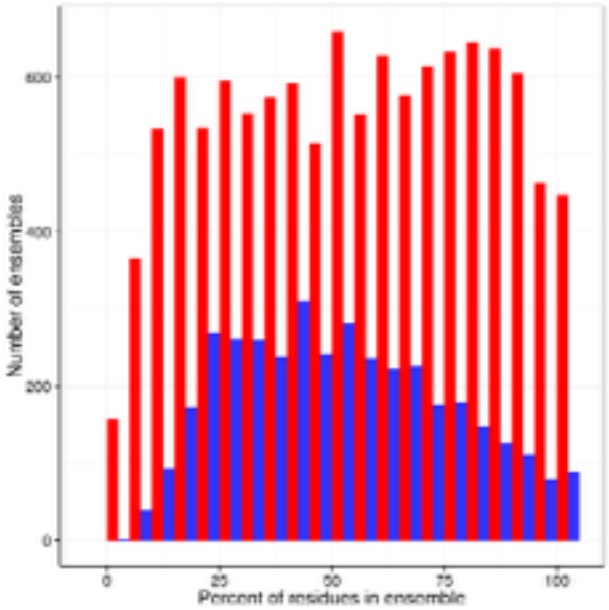
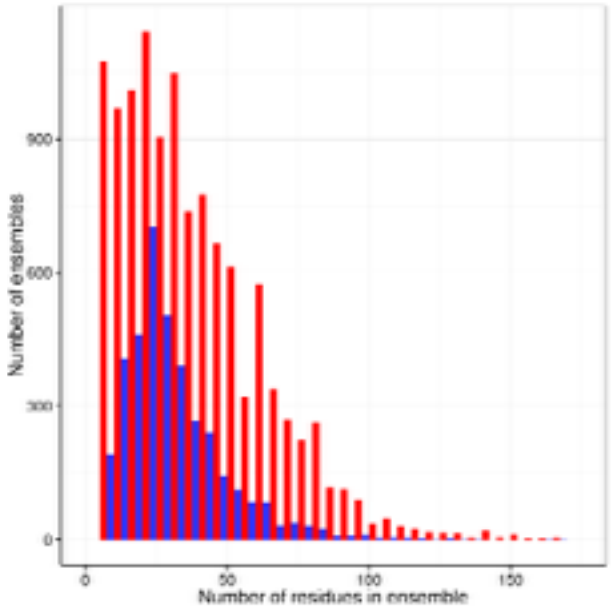
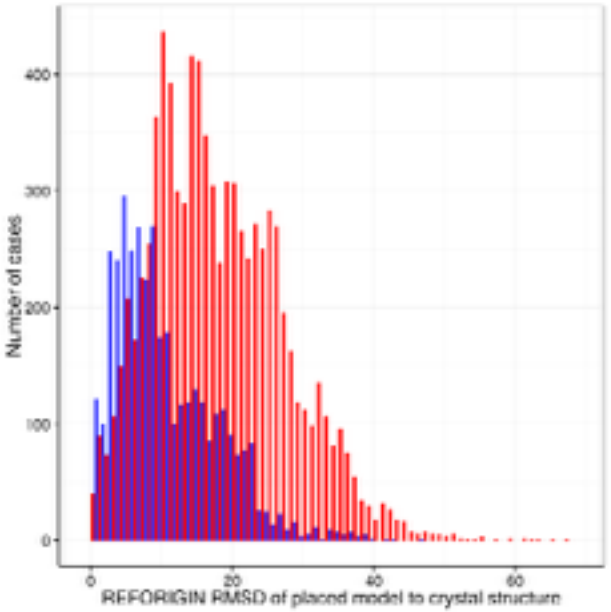
What is solving these structures?

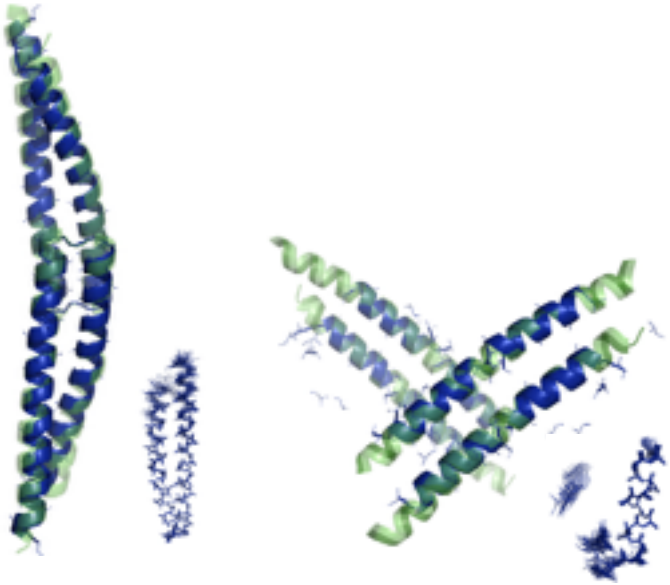


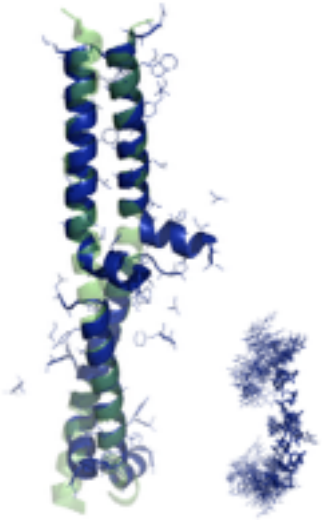


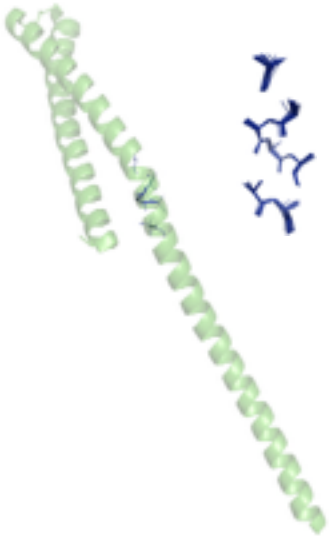


- Sample all proportions of the model but usually less than half
- Short (~25-30 residues) search models
- Search model seems to be in the wrong place!

What is solving these structures?







2IC9: 2 copies
RIO=125
RIO_in=125

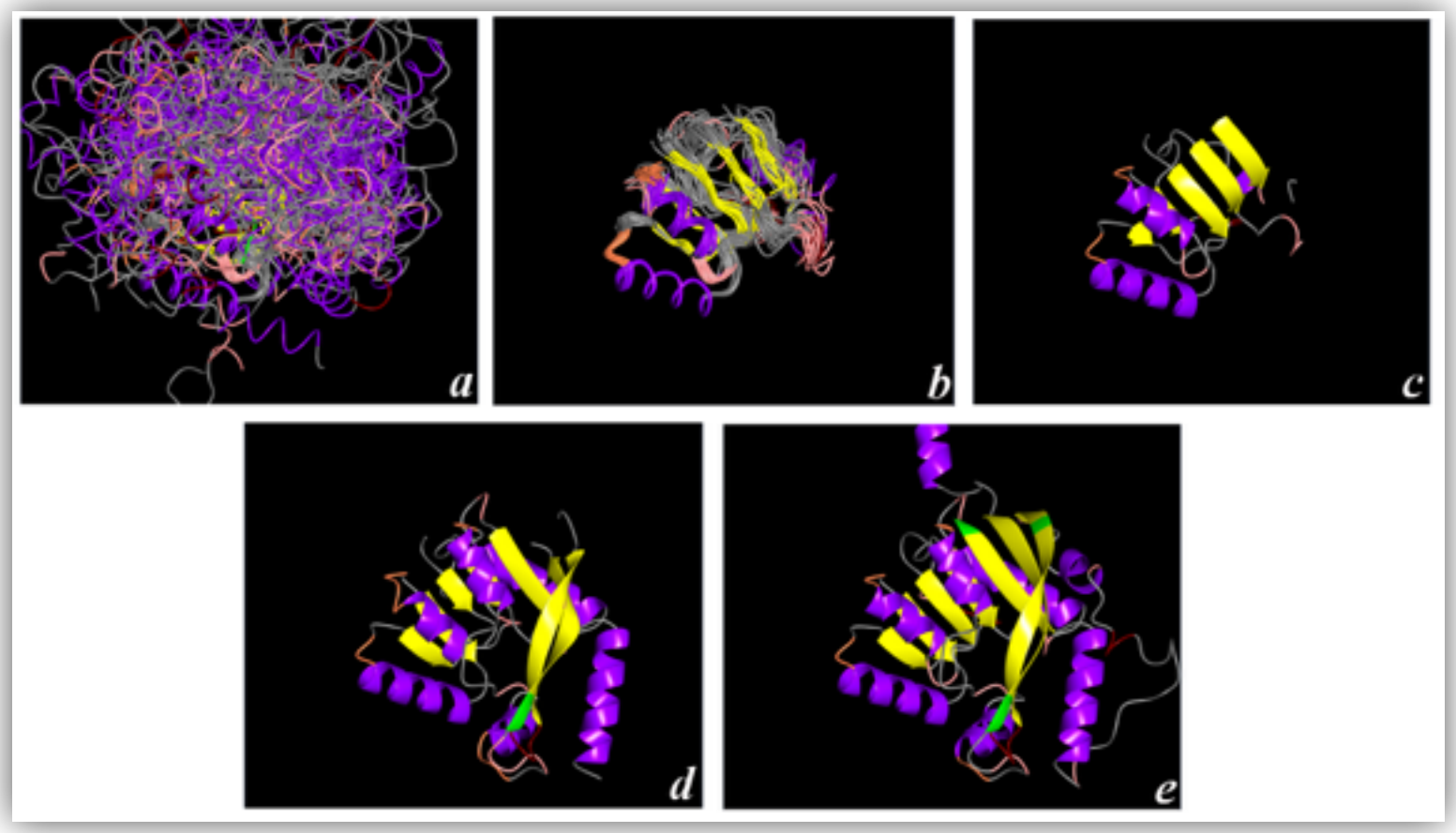
3H00: 8 copies
RIO=110
RIO_out=110

1UII: 4 copies
RIO=91
RIO_in=23
RIO_out=68

3OKQ: 1 copy
RIO=4
RIO_out=4

A mixture of in-register and out-of-register placements

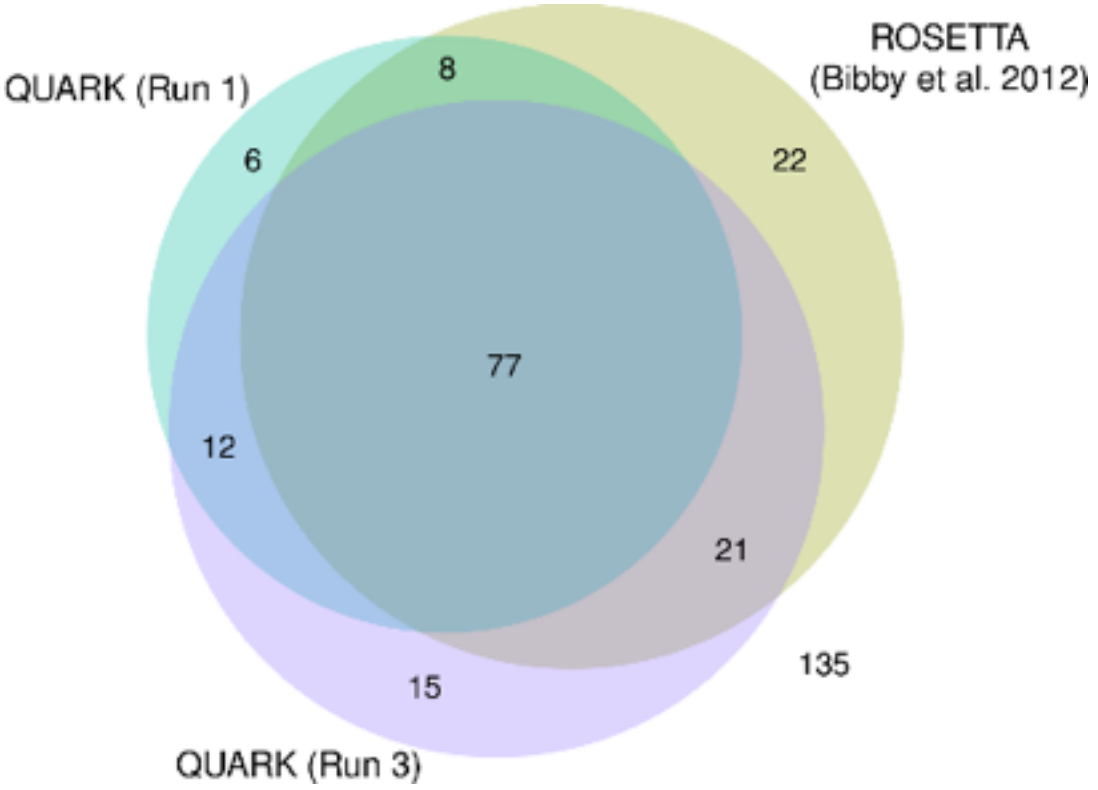
Exploiting distant homologues



a. Clustered decoy models, *b*. Truncated ensemble, *c*. Positioned MR solution, *d*. Shelxe c-alpha trace, *e*. Completed structure

QUARK vs ROSETTA

- Re-did testing using QUARK-generated decoy models
- Results are similar to ROSETTA but there are some targets that could only be solved by one or the other

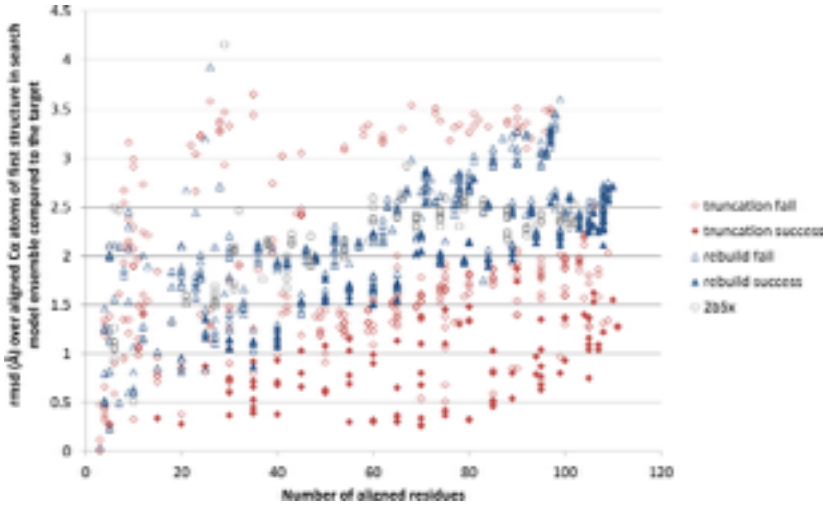


Remodelling related NMR structures or distant homologues

- **Truncation**, where the default AMPLE truncation strategy is applied to the models in the NMR ensemble.
- **Rebuilding**, where the NMR ensemble models are subjected to idealisation, comparative modelling and relaxation with ROSETTA, before being subjected to the standard truncation strategy.

25 test cases (FINDCORE)

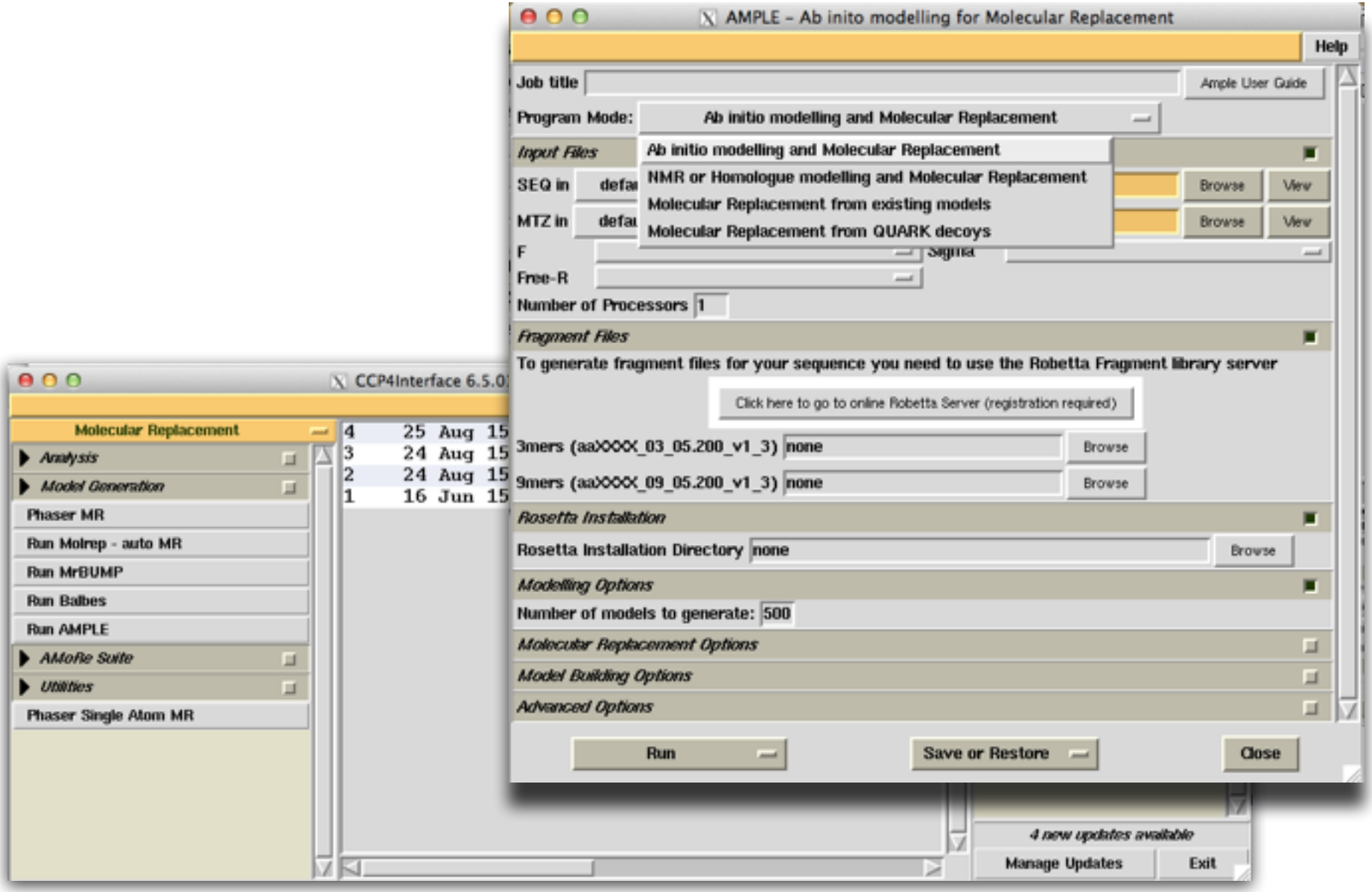
- 19 by truncation alone
- 3 with remodelling



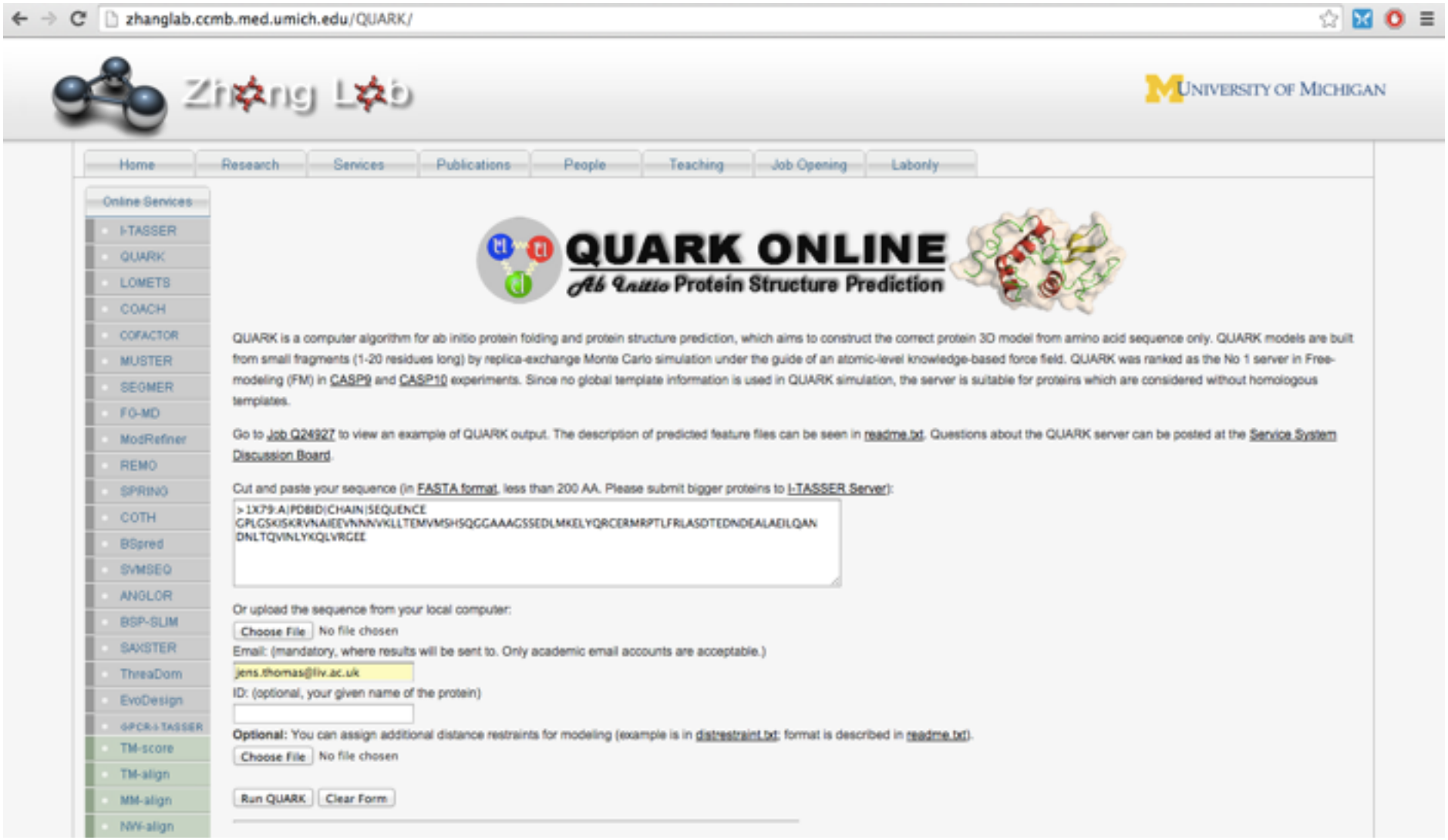
Practicalities

- Available since CCP4 6.3.0.
- Requires installation of several non-CCP4 packages (all freely available to academics):
  - **SHELXE** (now in CCP4 7.0)
  - **Rosetta** (optional)
  - **SCWRL** (optional)
- QUARK models now available so can do modelling online.
- Models can come from anywhere – just need a directory of PDB files
- Can exploit multiple processors on a local desktop, but also runs out-of-the-box on a cluster/supercomputer (SGE/LSF).

Available in CCP4i



Create models with Quark



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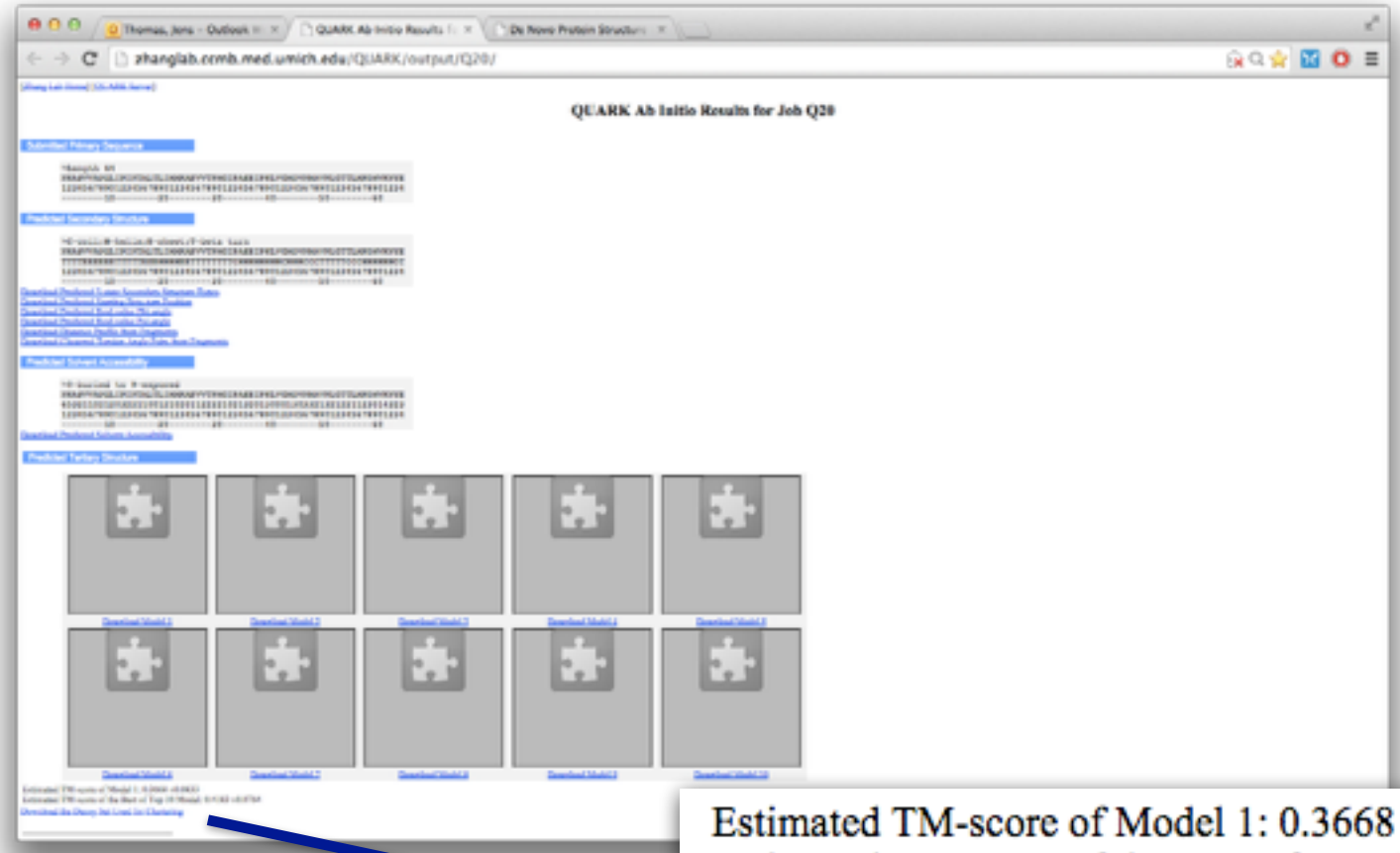
Quark Decoys



Estimated TM-score of Model 1: 0.3668 ±0.0833
Estimated TM-score of the Best of Top 10 Model: 0.4183 ±0.0764
Download the Decoy Set Used for Clustering

decoys.tar.gz

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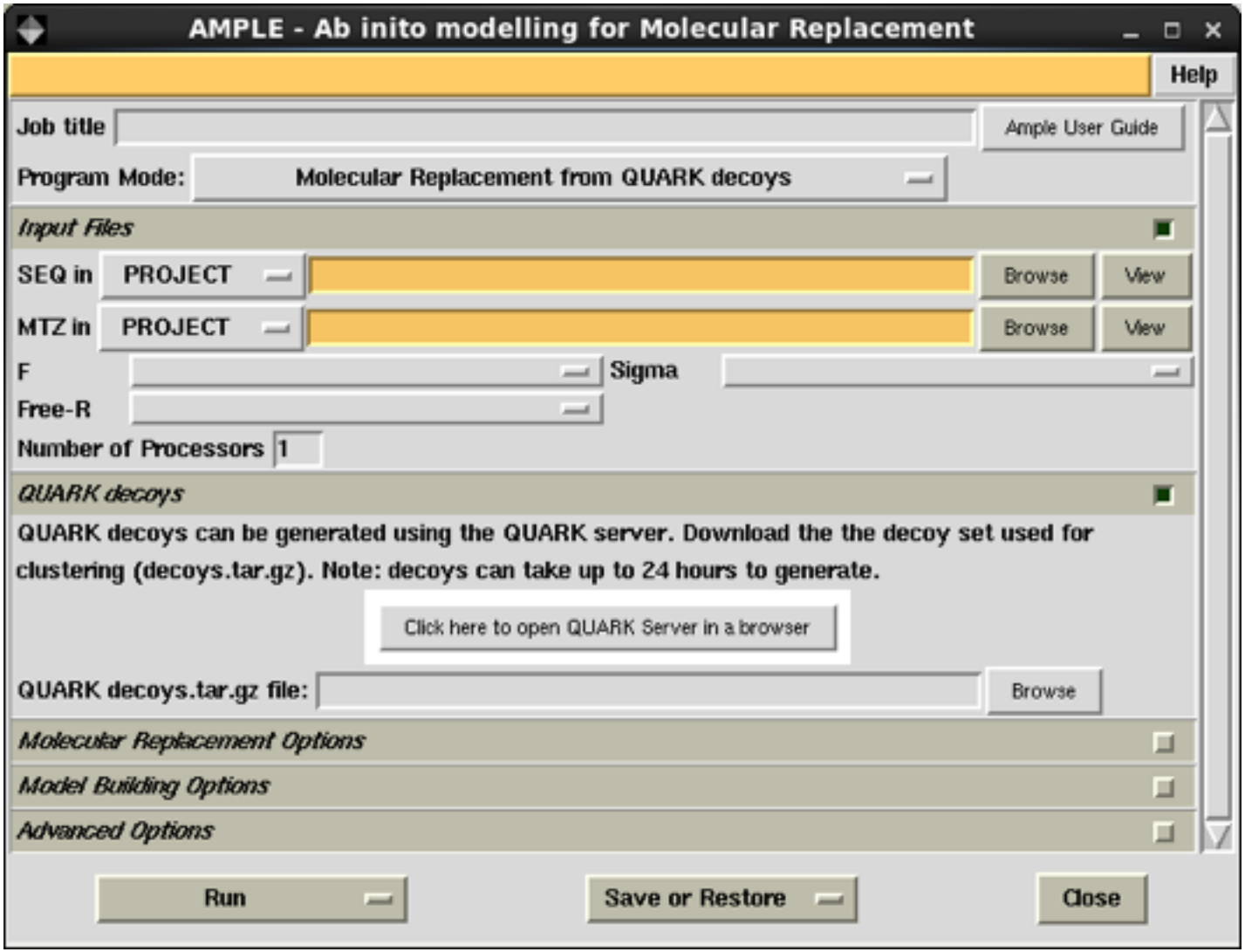
Quark Decoys



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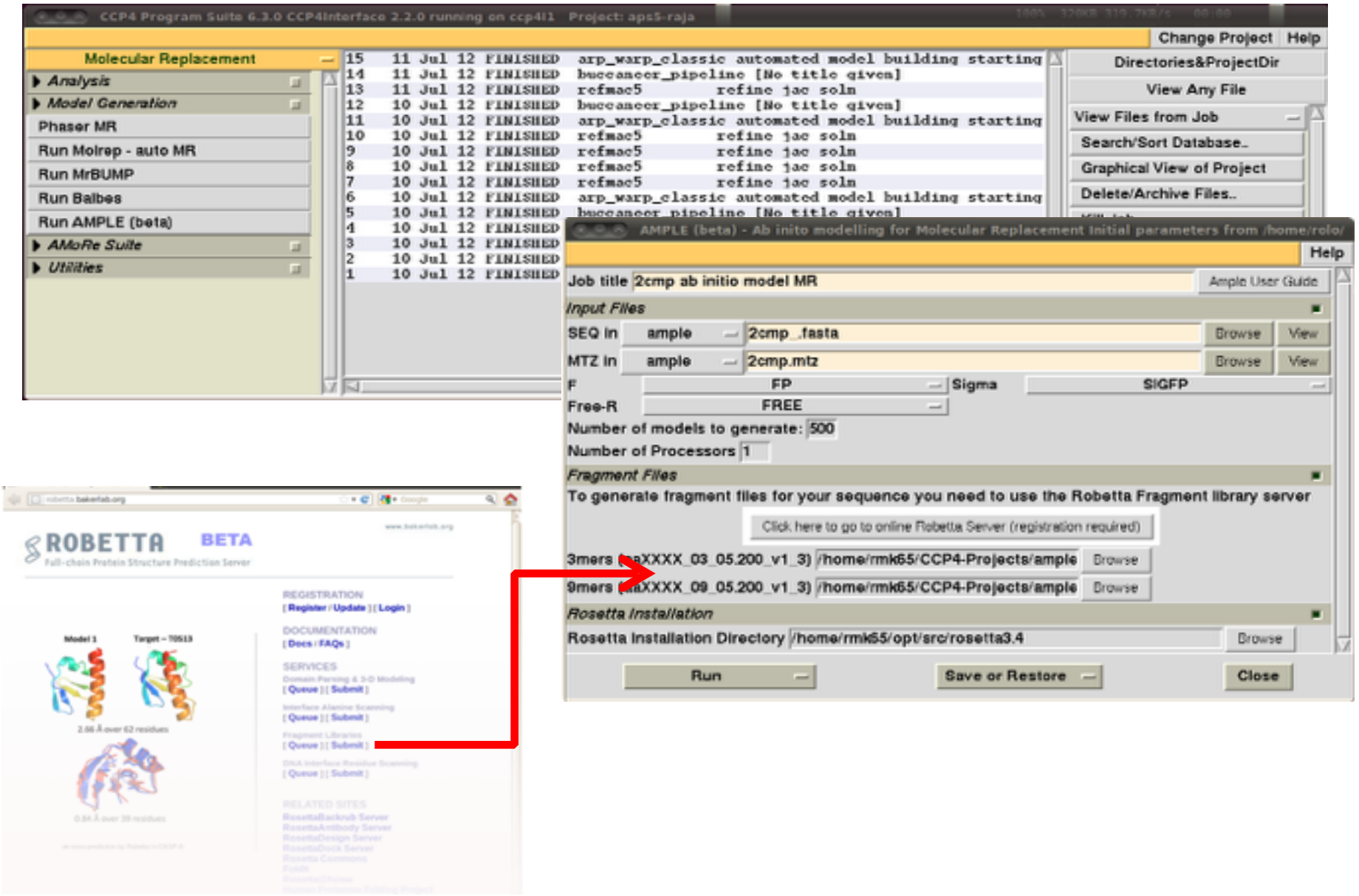
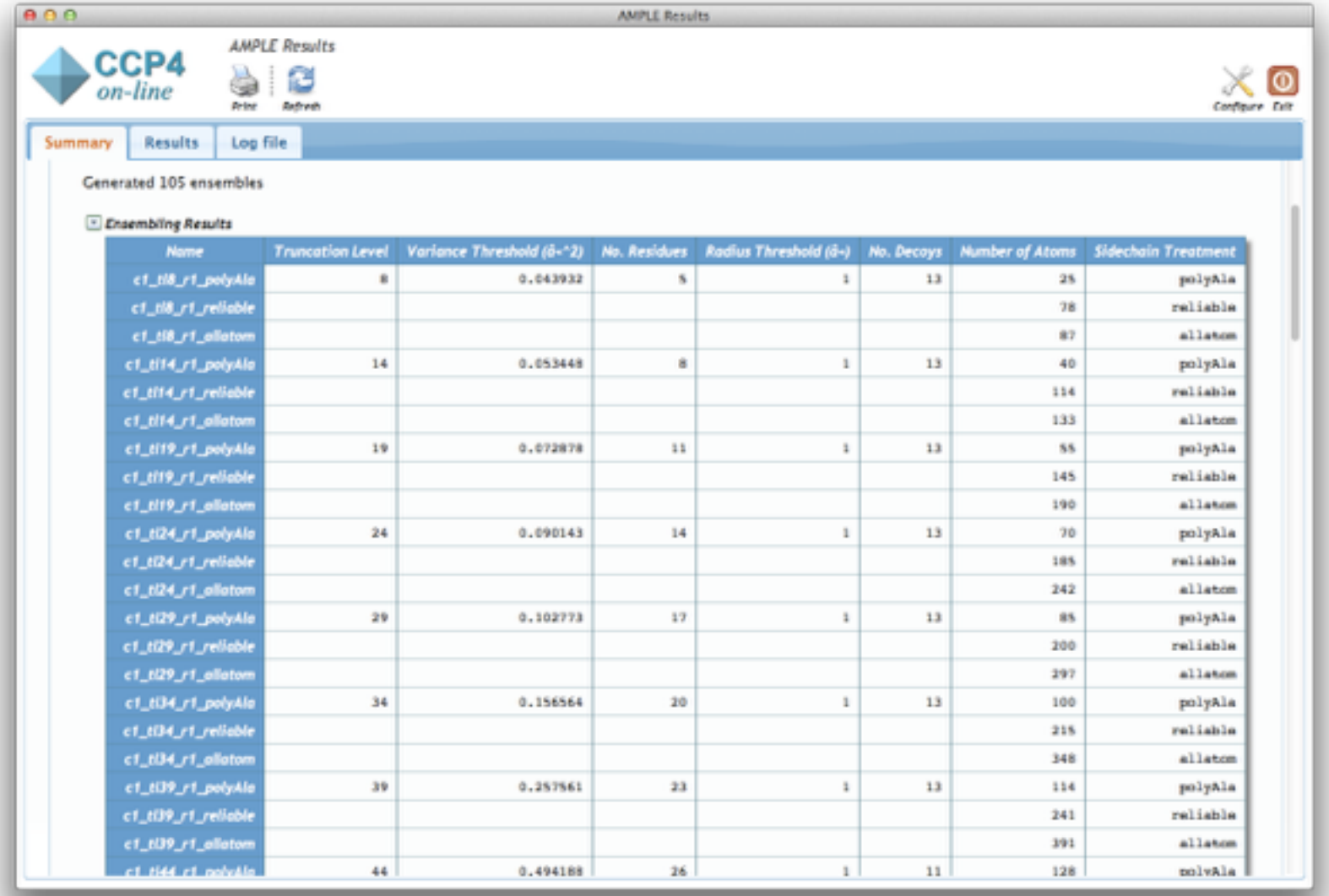
Running Rosetta



Results: Ensembles



Name	Truncation Level	Variance Threshold (Å^2)	No. Residues	Radius Threshold (Å)	No. Decoys	Number of Atoms	Sidechain Treatment
c1_t18_r1_polyAla	8	0.043932	5	1	13	25	polyAla
c1_t18_r1_reliable						78	reliable
c1_t18_r1_allatom						87	allatom
c1_t14_r1_polyAla	14	0.053448	8	1	13	40	polyAla
c1_t14_r1_reliable						114	reliable
c1_t14_r1_allatom						133	allatom
c1_t19_r1_polyAla	19	0.072878	11	1	13	55	polyAla
c1_t19_r1_reliable						145	reliable
c1_t19_r1_allatom						190	allatom
c1_t24_r1_polyAla	24	0.090143	14	1	13	70	polyAla
c1_t24_r1_reliable						185	reliable
c1_t24_r1_allatom						242	allatom
c1_t29_r1_polyAla	29	0.102773	17	1	13	85	polyAla
c1_t29_r1_reliable						200	reliable
c1_t29_r1_allatom						297	allatom
c1_t34_r1_polyAla	34	0.156564	20	1	13	100	polyAla
c1_t34_r1_reliable						215	reliable
c1_t34_r1_allatom						348	allatom
c1_t39_r1_polyAla	39	0.257561	23	1	13	114	polyAla
c1_t39_r1_reliable						241	reliable
c1_t39_r1_allatom						391	allatom
c1_t44_r1_polyAla	44	0.494188	26	1	11	128	polyAla

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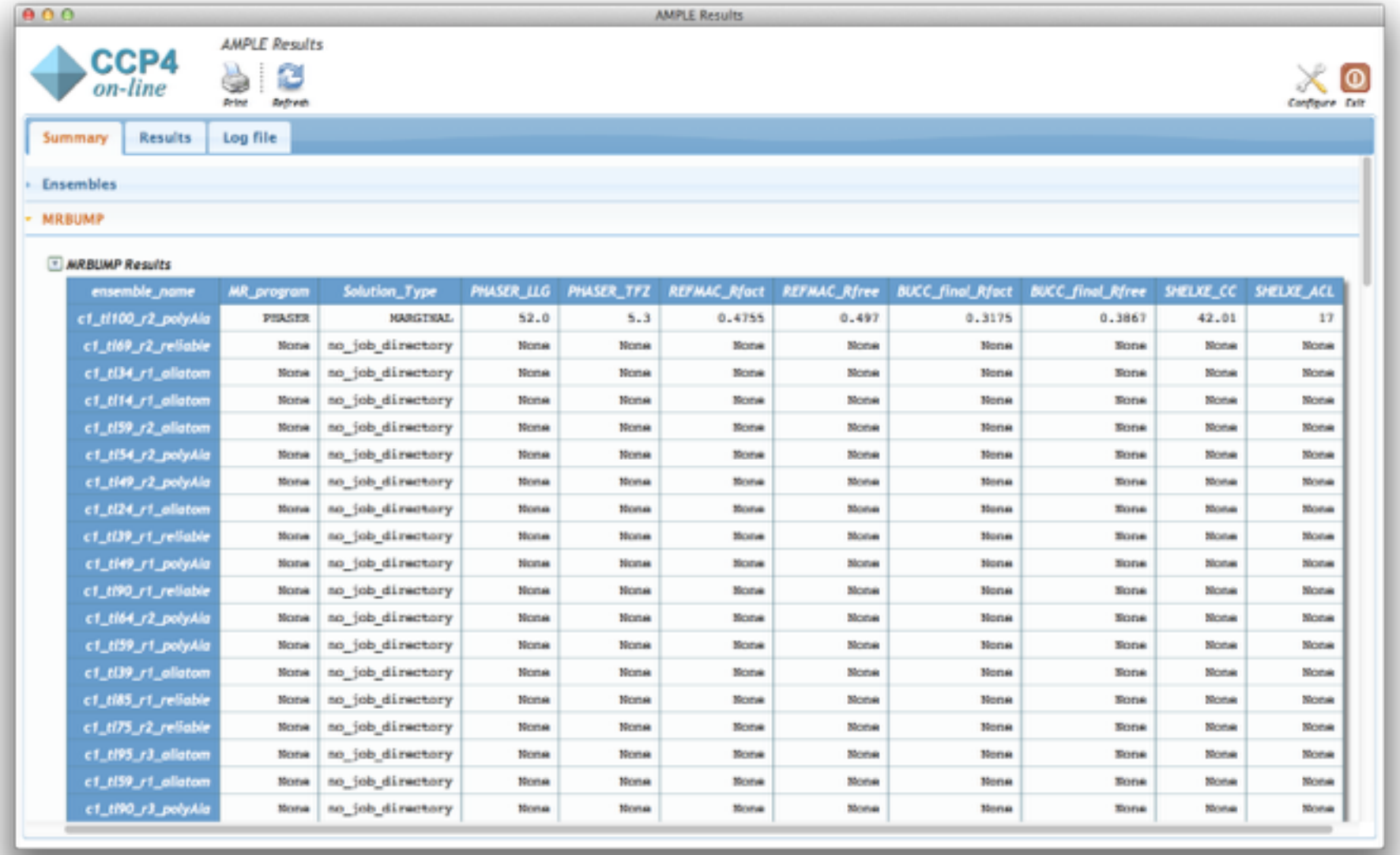
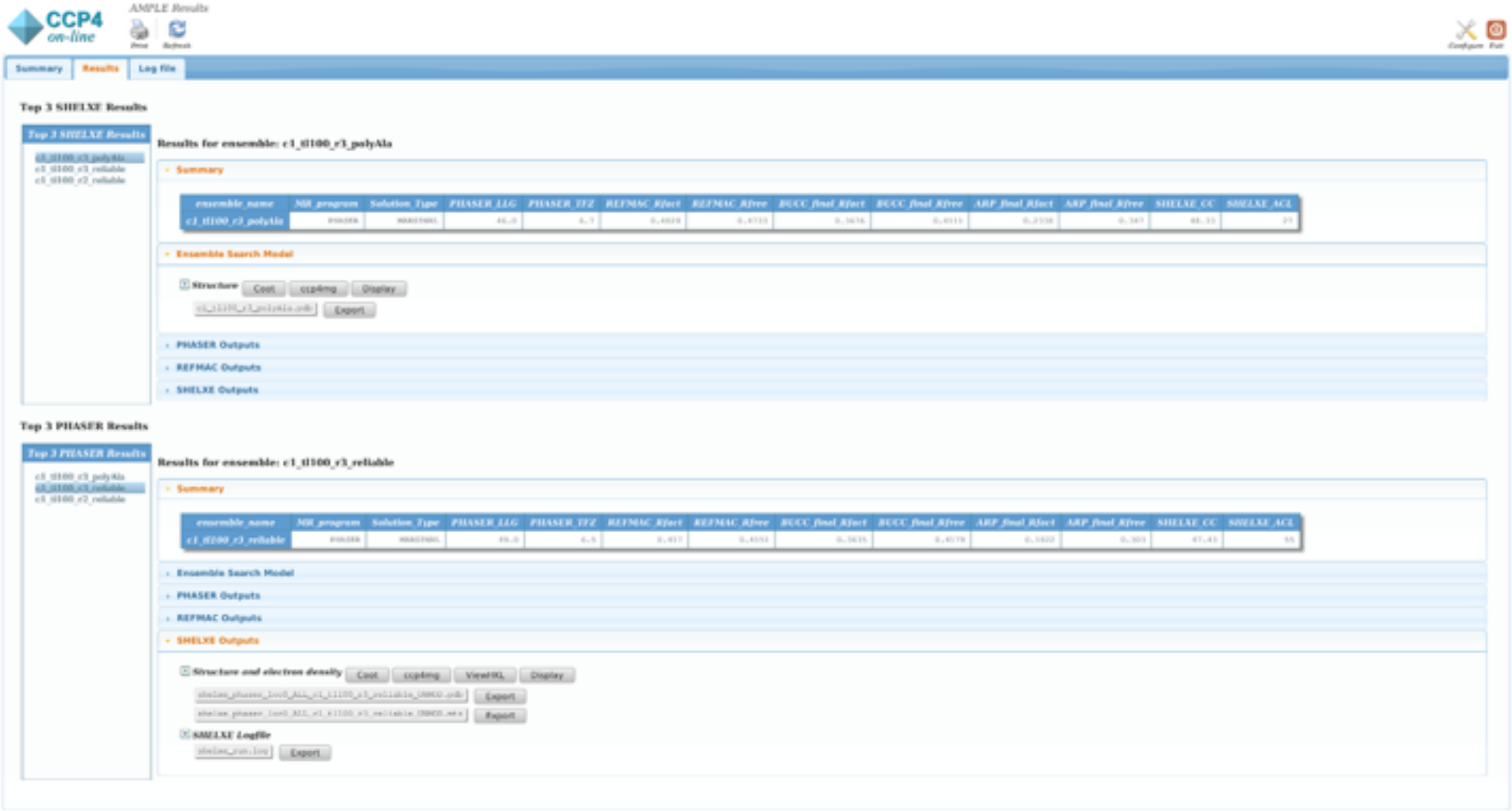
Results: MrBUMP



ensemble_name	MR_program	Solution_Type	PHASER_LLG	PHASER_TFZ	REFMAC_Rfact	REFMAC_Rfree	BUCC_final_Rfact	BUCC_final_Rfree	SHELXE_CC	SHELXE_ACL
c1_tl100_r2_polyAla	PHASER	MARGINAL	52.0	5.3	0.4755	0.497	0.3175	0.3867	42.01	17
c1_tl69_r2_reliable	None	no_job_directory	None	None	None	None	None	None	None	None
c1_tl34_r1_allatom	None	no_job_directory	None	None	None	None	None	None	None	None
c1_tl14_r1_allatom	None	no_job_directory	None	None	None	None	None	None	None	None
c1_tl59_r2_allatom	None	no_job_directory	None	None	None	None	None	None	None	None
c1_tl54_r2_polyAla	None	no_job_directory	None	None	None	None	None	None	None	None
c1_tl49_r2_polyAla	None	no_job_directory	None	None	None	None	None	None	None	None
c1_tl24_r1_allatom	None	no_job_directory	None	None	None	None	None	None	None	None
c1_tl39_r1_reliable	None	no_job_directory	None	None	None	None	None	None	None	None
c1_tl49_r1_polyAla	None	no_job_directory	None	None	None	None	None	None	None	None
c1_tl90_r1_reliable	None	no_job_directory	None	None	None	None	None	None	None	None
c1_tl64_r2_polyAla	None	no_job_directory	None	None	None	None	None	None	None	None
c1_tl59_r1_polyAla	None	no_job_directory	None	None	None	None	None	None	None	None
c1_tl39_r1_allatom	None	no_job_directory	None	None	None	None	None	None	None	None
c1_tl85_r1_reliable	None	no_job_directory	None	None	None	None	None	None	None	None
c1_tl75_r2_reliable	None	no_job_directory	None	None	None	None	None	None	None	None
c1_tl95_r3_allatom	None	no_job_directory	None	None	None	None	None	None	None	None
c1_tl59_r1_allatom	None	no_job_directory	None	None	None	None	None	None	None	None
c1_tl90_r3_polyAla	None	no_job_directory	None	None	None	None	None	None	None	None

Results: Files



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AMPLE server - coming soon!

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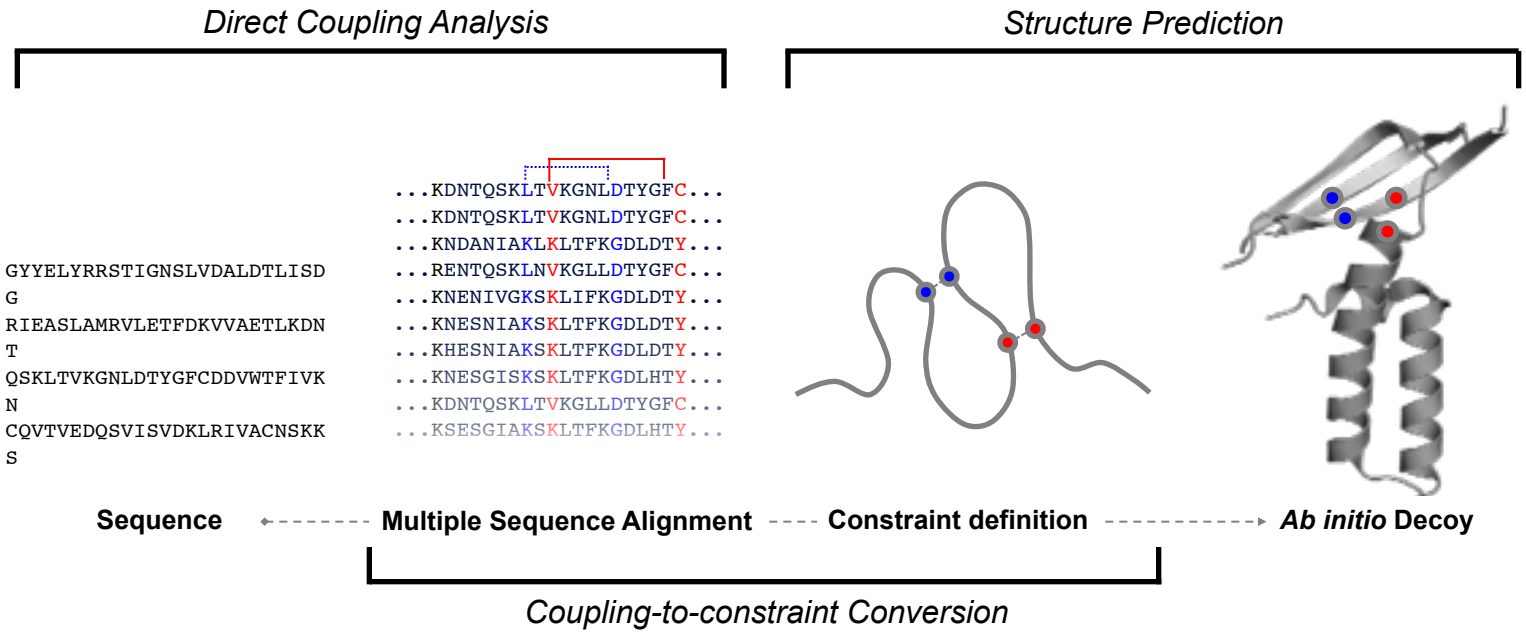
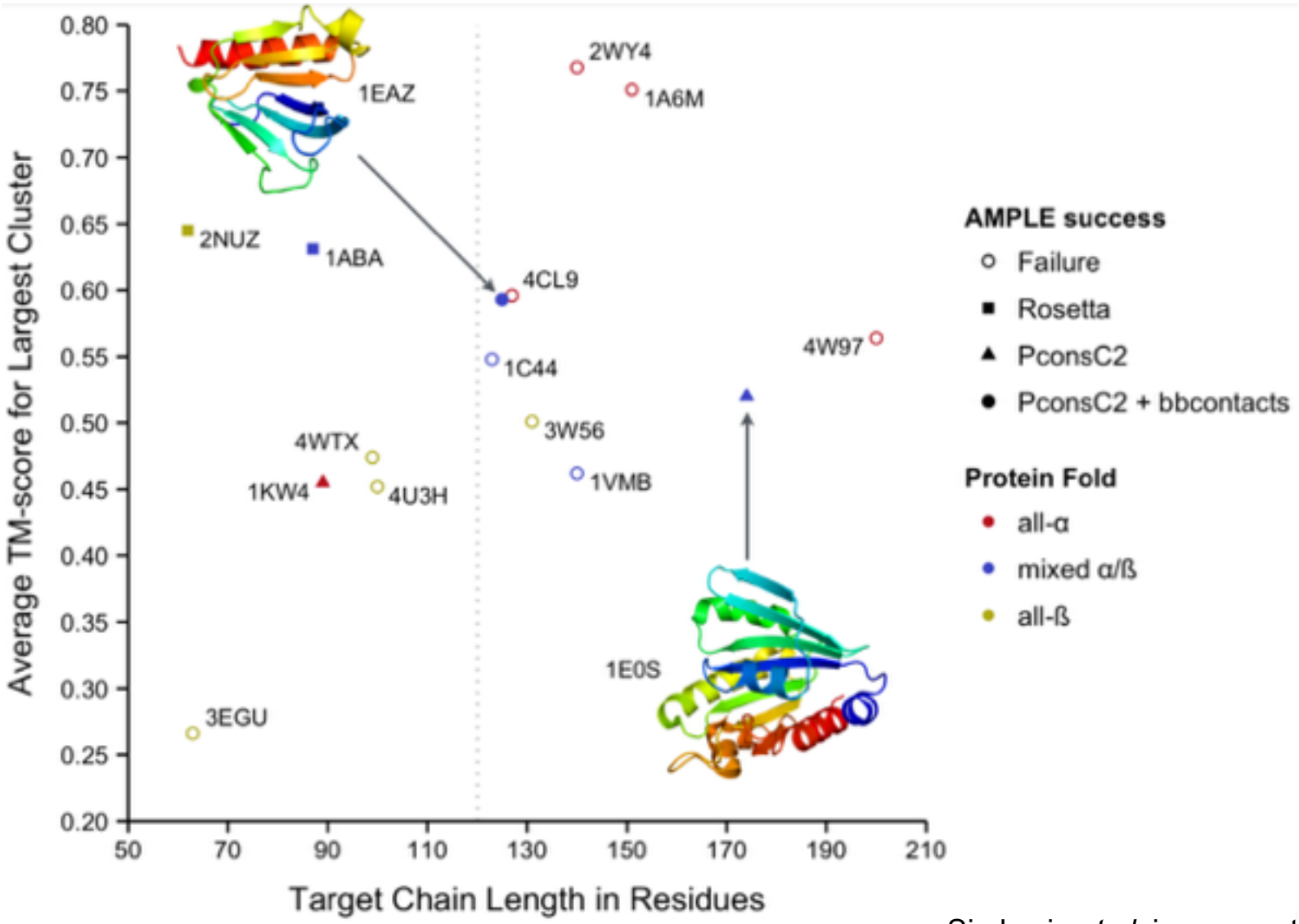
Ongoing developments

Residue-residue contact prediction



With contact predictions AMPLE succeeds with larger and more β-rich proteins



Simkovic *et al.* in preparation

Summary

- AMPLE – a pipeline for Molecular Replacement with unconventional models.
- works particularly well with α–helical proteins.
- solves structures with fragments or more complete models.
- usually best for small proteins with reasonable resolution.
- favourable cases can work > 330 residues and > 2.8Å resolution.
- very easy to use.

Credits

- Dan Rigden, Olga Mayans – IIB, Liverpool University
- Felix Simkovic, Adam Simpkin and Jaclyn Bibby – Liverpool University
- Ronan Keegan, Martin Wynn – CCP4 (STFC)
- BBSRC
- Hartree Center
- **Phaser:** Randy Read, Airlie McCoy & Gabor Bunkozci
- **SHELX:** Andrea Thorn, Tim Gruene & George Sheldrick
- **Refmac:** Garib Mushudov, LMB-MRC Cambridge
- Developers of **Rosetta** and **Quark**
- **Molrep:** Alexei Vagin & Andrey Lebedev
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