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## Background

**Ancestral Sequence Reconstruction** uses the sequences of extant proteins to predict the sequences of ancestral ones.

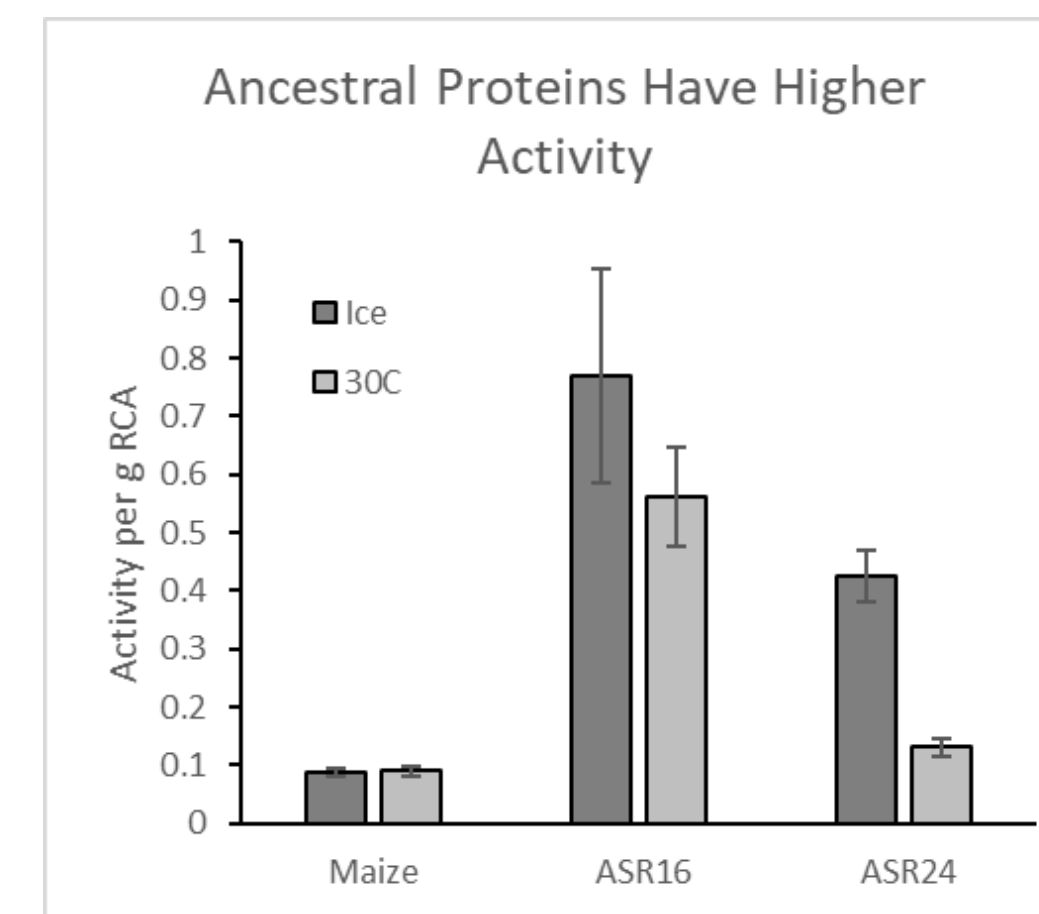
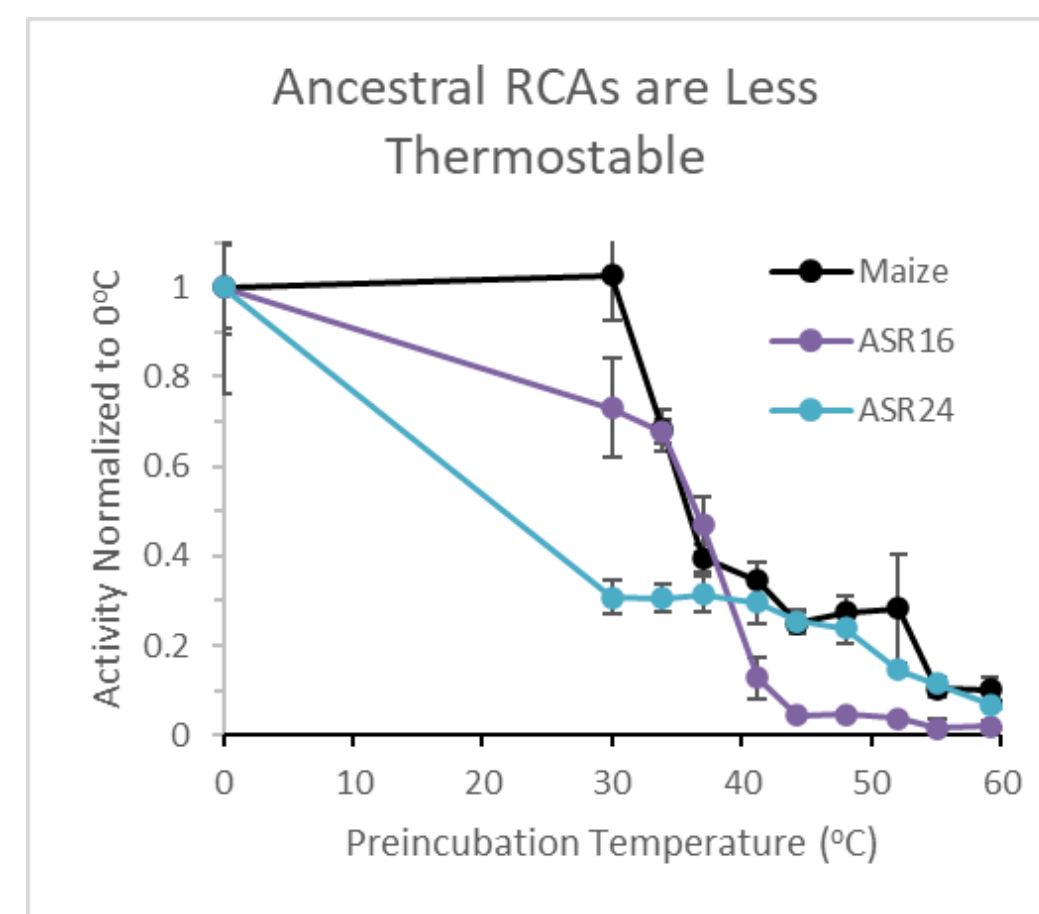
Ancestral proteins are often **more stable**, more promiscuous, and/or more active<sup>1</sup>

**Rubisco Activase (RCA)** is a chaperone for Rubisco, but **is not thermostable**. It inhibits photosynthesis at high temperature<sup>2</sup>.

We reconstructed RCA and compared its thermostability to the protein from *Zea mays* (maize).

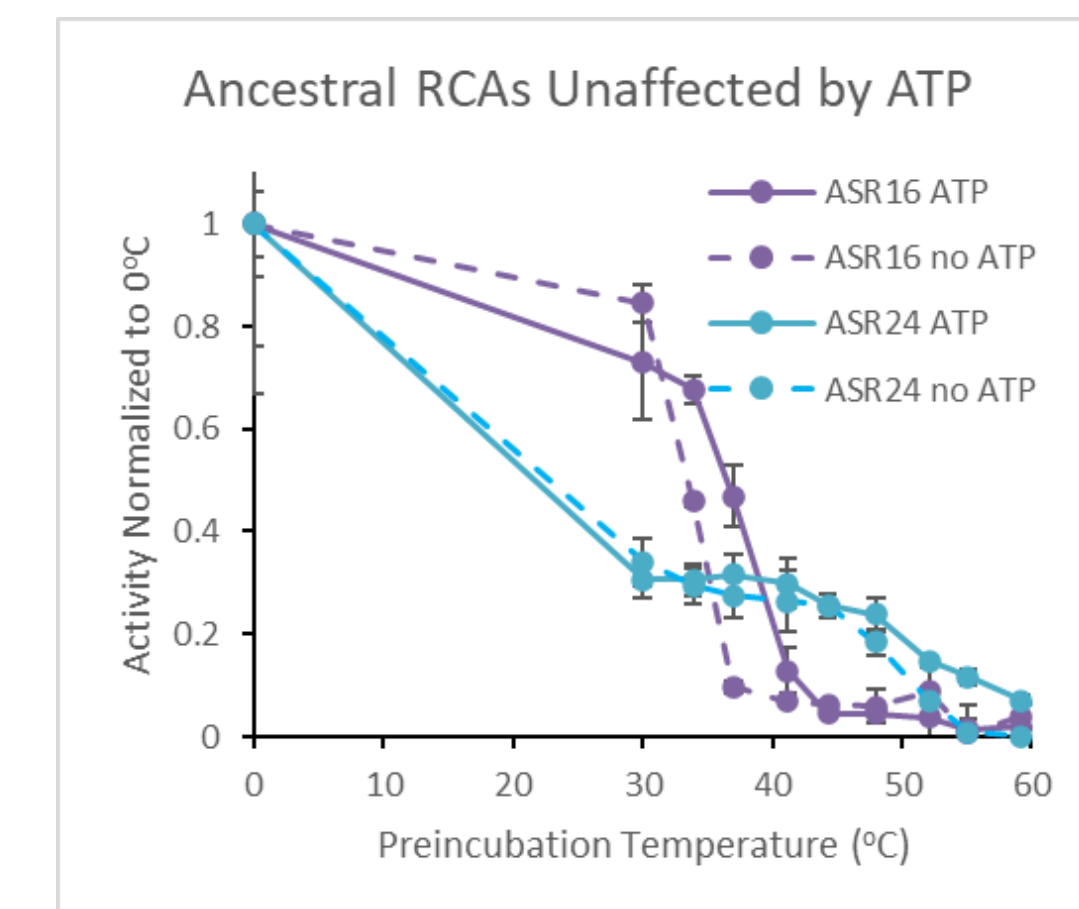
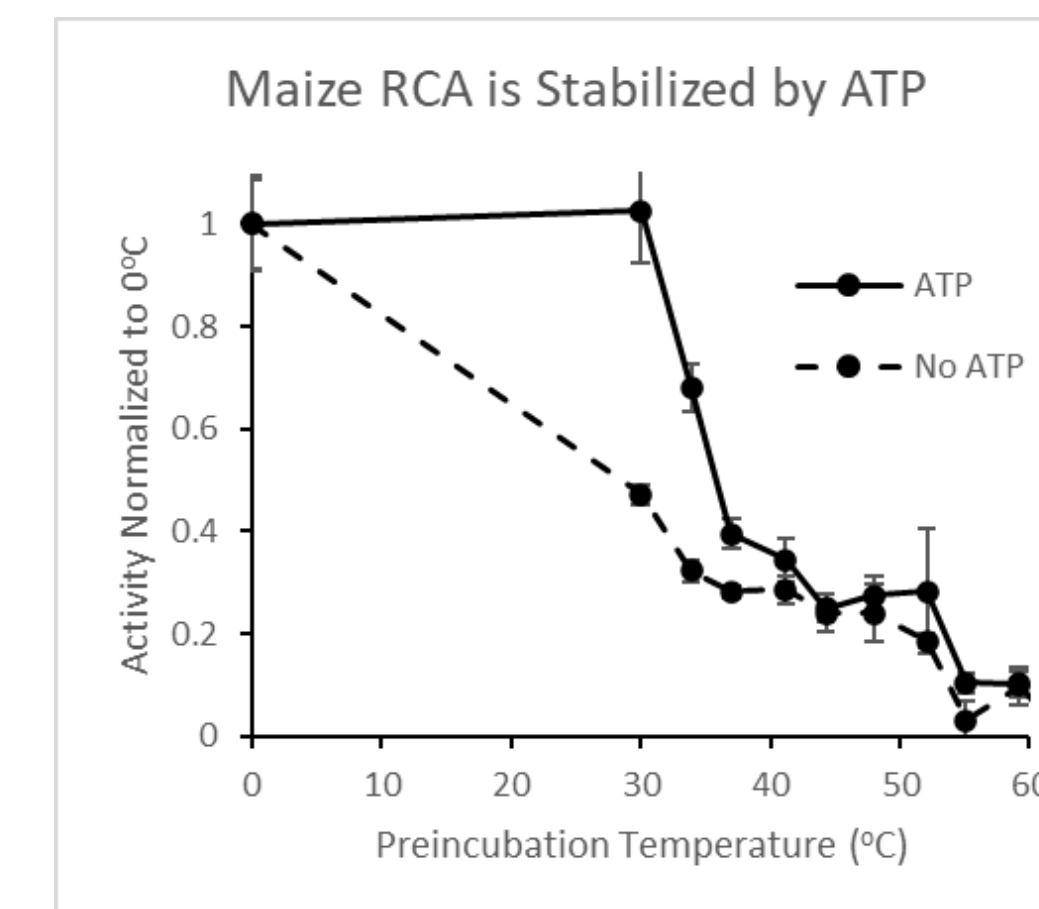
## Thermostability

Ancestral sequences have higher activity but lower thermostability



Purified protein was incubated at indicated temperatures for 1hr  
 Activity was measured by a spectrophotometric ATPase assay<sup>5</sup>

Ancestral proteins are not stabilized by ATP



Extant RCA proteins are known to have improved thermostability when incubated with ATP<sup>6</sup>  
 This may be an evolutionary innovation rather than an ancestral trait

## Reconstruction

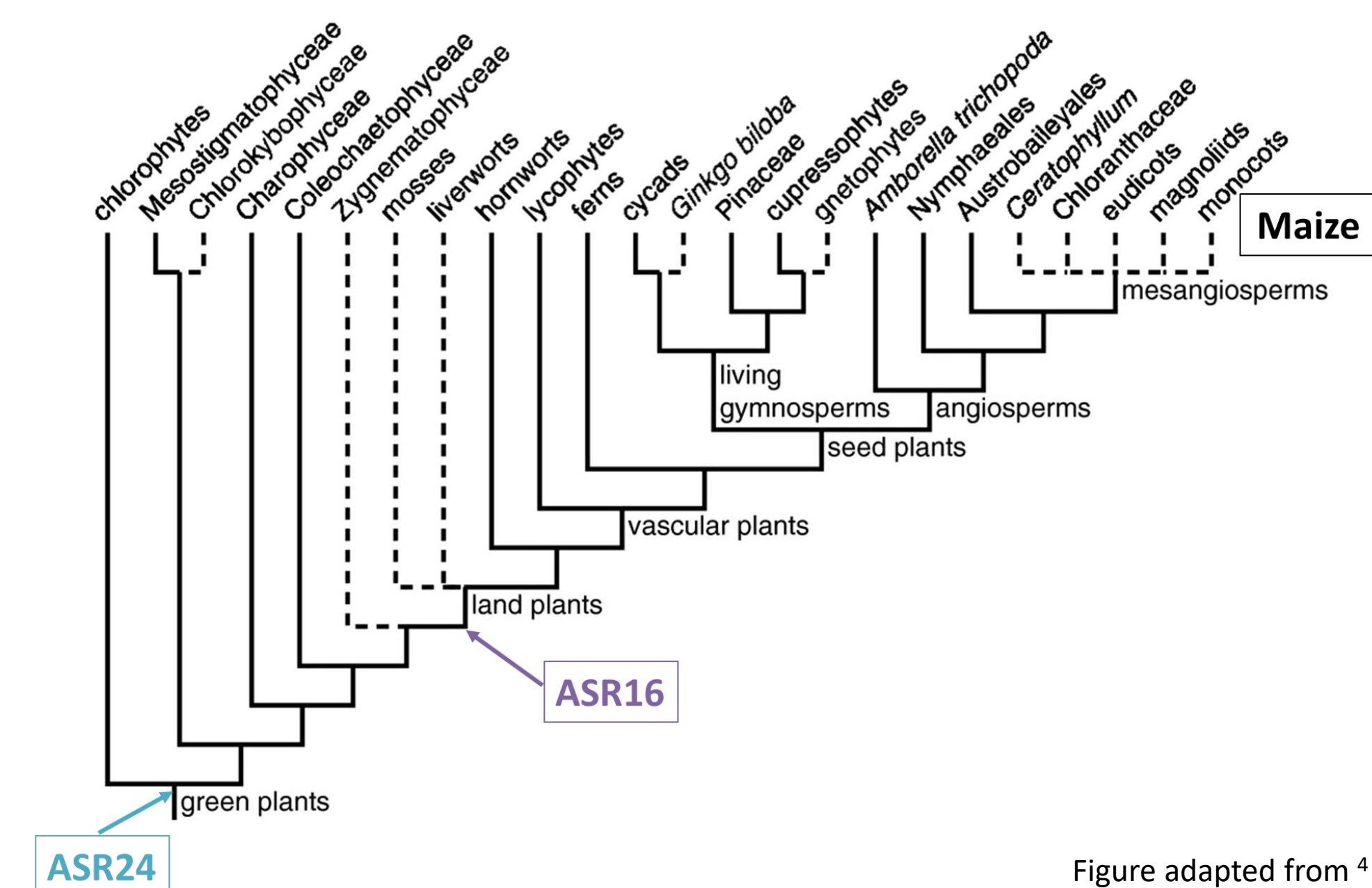


Figure adapted from <sup>4</sup>

	1	78
Zea mays	MAAEVDETKQTDEDKRWGL--AYDISDDQDDITRGGKLVNLFQAPM--GDGTHVAVLSSYDYISQGGKSYNFDNMM	
ASR16	---MEVDETKQTKDRWKGL--GYDSDDDQDDITRGGKLVNLFQAPM--GSGTQNAVMSYBYISQGGKSYNFDNMM	
ASR24	---MAAEVDETKQTKDRWKGL--AYDISDDQDDITRGGKLVNLFQAPM--GDGTHVAVLSSYDYISQGGKSYNFDNMM	
.....		
	79	156
Zea mays	DGFYIAKGFMDKLVVHLSKNFMFLPNIKVPLILGIWGGKGGKSFQCELVFAKMGITPIMMSAGELESGNAGEPAKLI	
ASR16	DGFYIAKGFMDKLVVHLSKNFMFLPNIKVPLILGIWGGKGGKSFQCELVFAKMGITPIMMSAGELESGNAGEPAKLI	
ASR24	DGFYIAKGFMDKLVVHLSKNFMFLPNIKVPLILGIWGGKGGKSFQCELVFAKMGITPIMMSAGELESGNAGEPAKLI	
.....		
	157	234
Zea mays	RQRYREASDLIK-KGKMSCLFINDLDAGAGRMGGTQYTVNNQMVNATLMNIADNPTNVQLPGMYNKEDNPRVPIIVT	
ASR16	RQRYREASDLIK-KGKMSCLFINDLDAGAGRMGGTQYTVNNQMVNATLMNIADNPTNVQLPGMYNKEDNPRVPIIVT	
ASR24	RQRYREASDLIK-KGKMSCLFINDLDAGAGRMGGTQYTVNNQMVNATLMNIADNPTNVQLPGMYNKEDNPRVPIIVT	
.....		
	235	312
Zea mays	GNDFSTLYAPLIRDGRMEKFWAPTREDRIGVCKGIFRTDGVDEEHVQLVDTFPGQSIDFFGALRARVYDDEVRWV	
ASR16	GNDFSTLYAPLIRDGRMEKFWAPTREDRIGVCKGIFRTDGVDEEHVQLVDTFPGQSIDFFGALRARVYDDEVRWV	
ASR24	GNDFSTLYAPLIRDGRMEKFWAPTREDRIGVCKGIFRTDGVDEEHVQLVDTFPGQSIDFFGALRARVYDDEVRWV	
.....		
	313	388
Zea mays	SETGVENIARKLVNSKEGPPTEFPKPTIEKLLYGHMLVAEQENVKRVQLADKYLNAAALGEANEDAMKTGSFFK	
ASR16	SETGVENIARKLVNSKEGPPTEFPKPTIEKLLYGHMLVAEQENVKRVQLADKYLNAAALGEANEDAMKTGSFFK	
ASR24	SETGVENIARKLVNSKEGPPTEFPKPTIEKLLYGHMLVAEQENVKRVQLADKYLNAAALGEANEDAMKTGSFFK	

## Conclusions

- Reconstructed ancestral proteins were highly active but not thermostable compared to maize  
 This breaks the trend that ancestral sequences are usually more stable
- Maize RCA is thermostable and may not be an informative comparison  
 Future work should compare thermostability to RCA from other species such as spinach or Arabidopsis
- Stabilization of RCA by ATP is not an ancestral trait but begins to appear in land plants.

## References

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- The common ancestors of land plants and green plants were reconstructed using the FireProt webserver<sup>3</sup>
- Alignment shows the sequence is significantly conserved
- Proteins were expressed in *Escherichia coli* and purified by Ni-NTA affinity chromatography