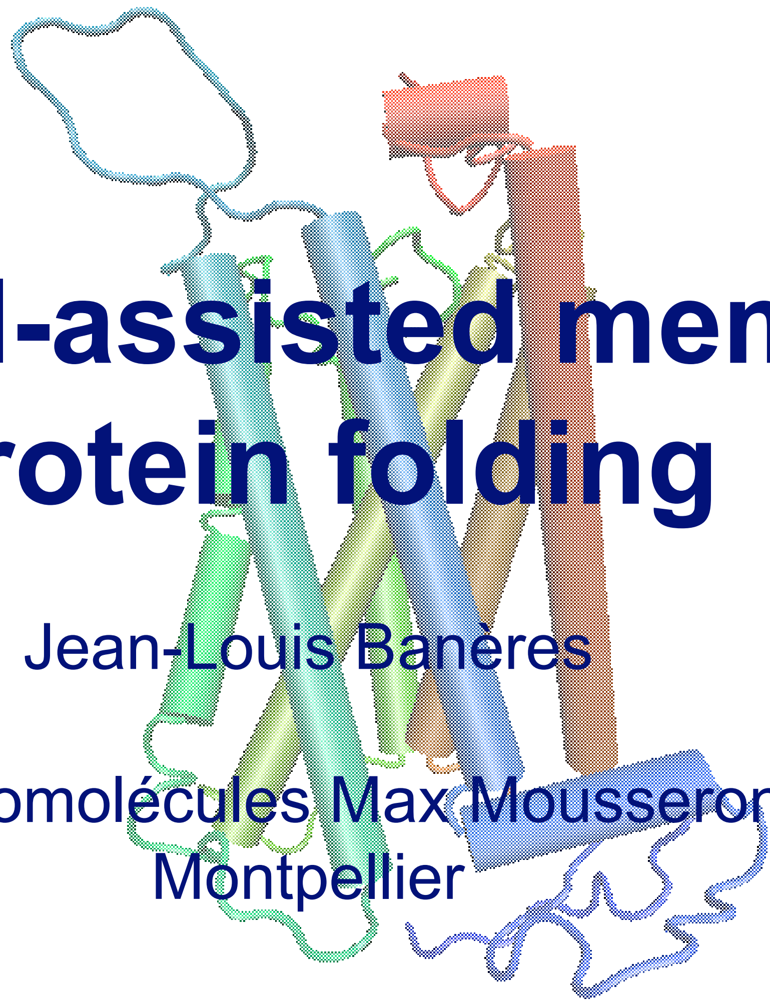
Applications of amphipols to membrane proteins studies, Workshop 2010
IBPC, Paris, march 8 2010



Amphipol-assisted membrane protein folding

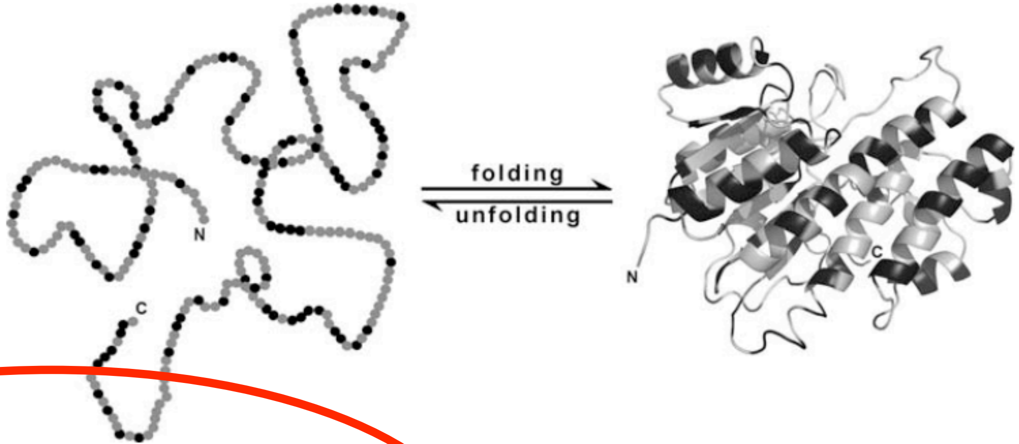
Jean-Louis Banères

Institut des Biomolécules Max Mousseron (IBMM)
Montpellier

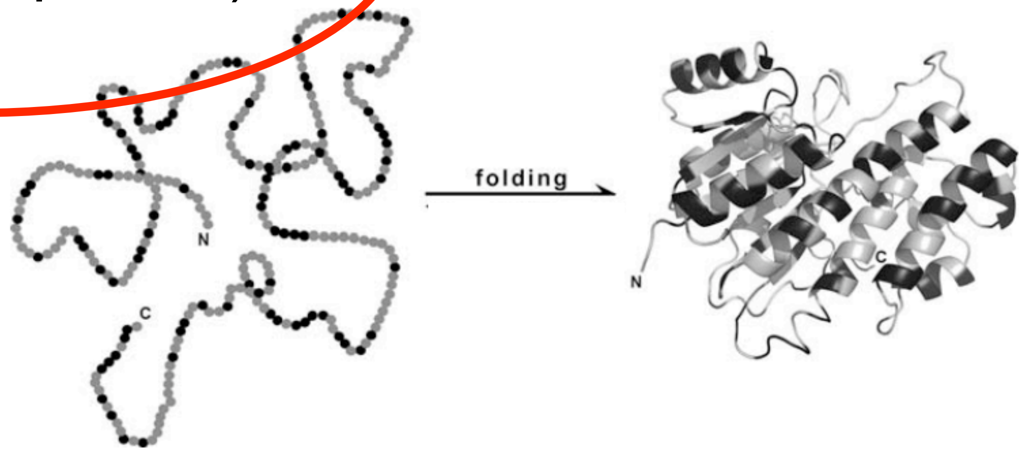
IBMM Institut des Biomolécules Max Mousseron

Refolding membrane proteins : what for ?

"fundamental" studies
(understand how these proteins adopt their native fold)



"practical" studies
(get the folded protein)



Structures of membrane proteins solved by solution NMR

An interesting statistic…

Table 1

Structures of integral β-barrel membrane proteins solved by solution NMR[a].

Protein	Organism	Number of residues	Number of β-strands	Detergent	Deuterated detergent	Refolding
OmpX	*E. coli*	148	8	DHPC	No	Yes
PagP	*E. coli*	164	8	DPC, β-OG	Yes, yes	Yes
OmpA	*E. coli*	177	8	DPC	Yes	Yes
OmpA	*K. pneumoniae*	210	8	DHPC	Yes	Yes
OmpG	*E. coli*	280	14	DPC	Yes	Yes
VDAC-1	*H. sapiens*	283	19	LDAO	Yes	Yes

[a] Abbreviations: DHPC, di-hexanoyl-phosphatidylcholine; DPC, dodecyl-phosphocholine; β-OG, β-octylglucopyranoside; LDAO, mineoxide.

Table 2

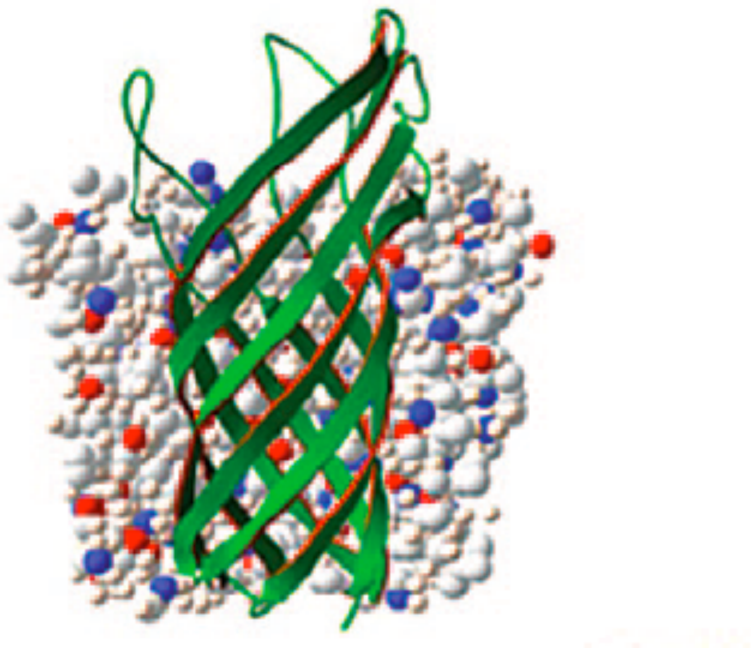
Structures of integral α-helical membrane proteins with more than two transmembrane helices solved by solution NMR[a]

Protein	Organism	Number of residues	Number of TM α-helices	Detergent	Deuterated detergent	Refolding
M2	*Influenza virus*	42	4 × 1	DHPC	Yes	Yes
DsbB	*E. coli*	176	4	DPC	Yes	No
Phospholamban	*H. sapiens*	52	5 × 1	DPC	Yes	Yes

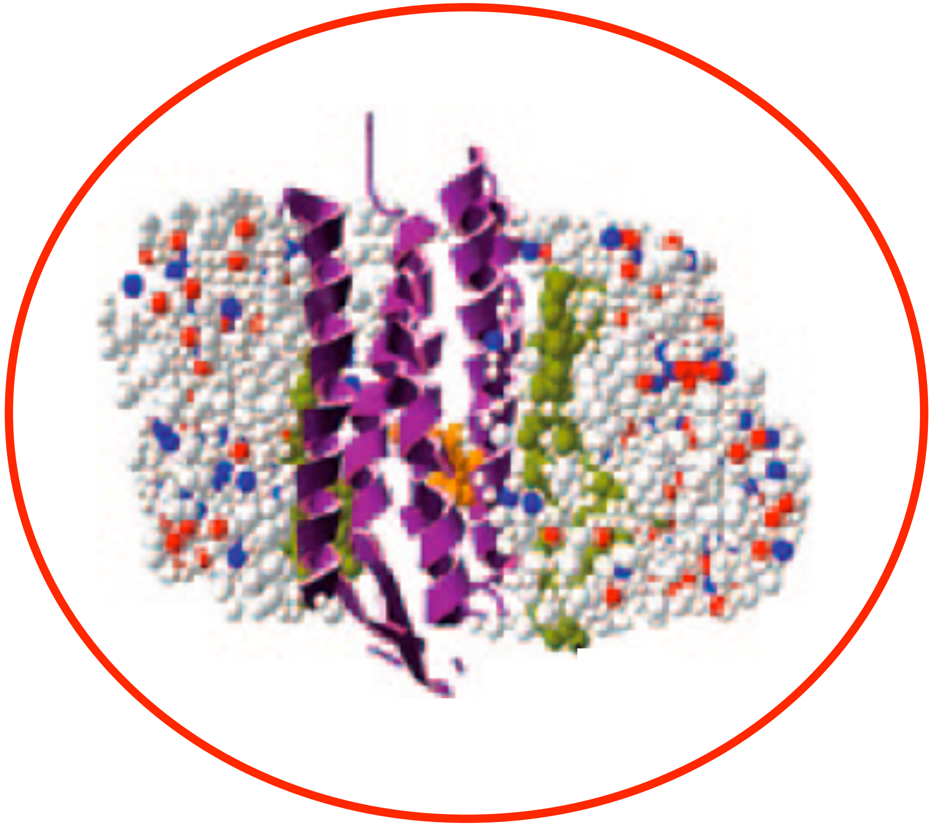
[a] Abbreviations: DHPC, di-hexanoyl-phosphatidylcholine; DPC, dodecyl-phosphocholine; TM, transmembrane.

Hiller & Wagner(2009). *Curr Opin Struct Biol*. **19**:396-401.

Membrane proteins



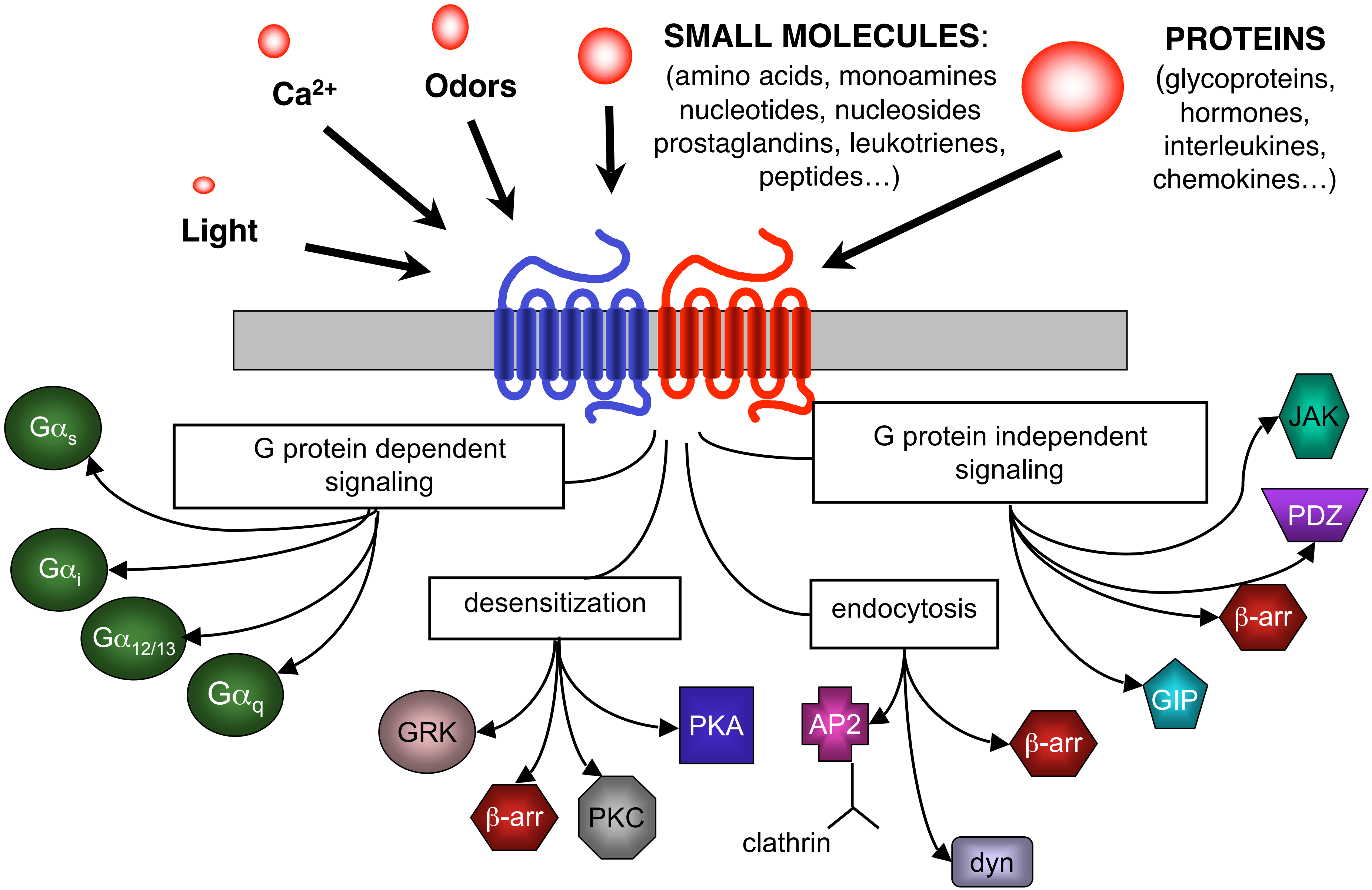
β-barrels



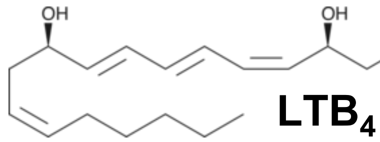
α-helical

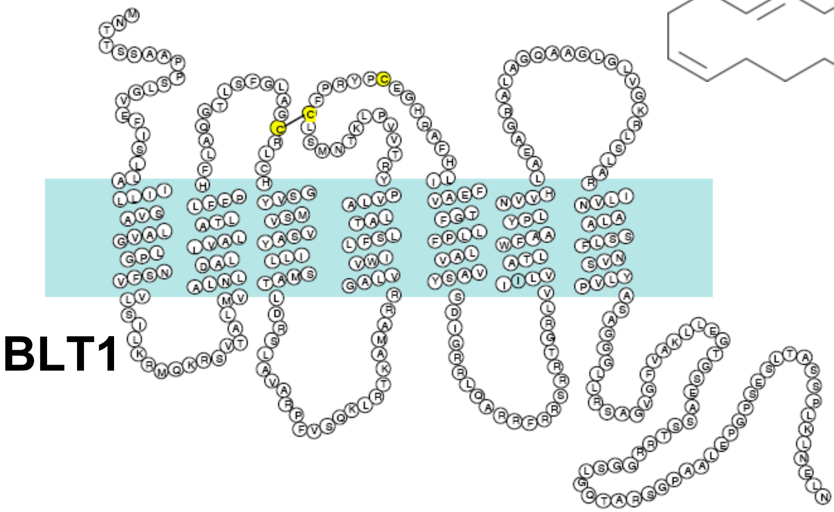
Popot, J.L. (2010). *Ann. Rev. Biochem*. In press

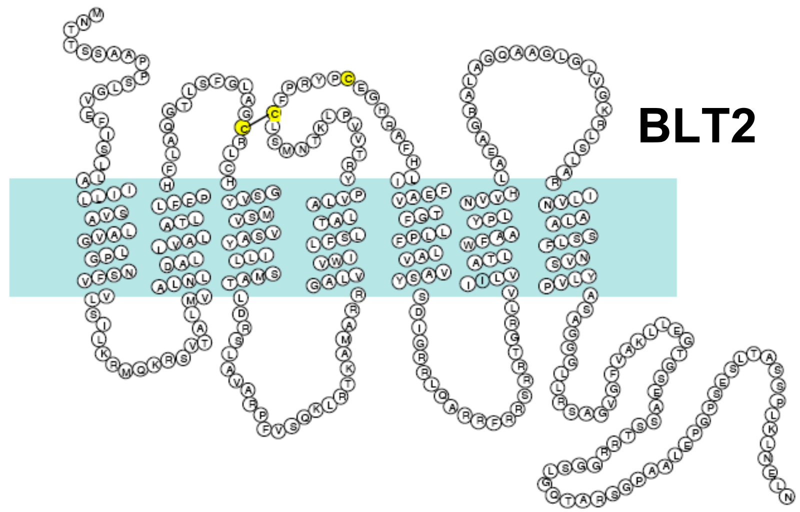
G Protein-Coupled Receptors

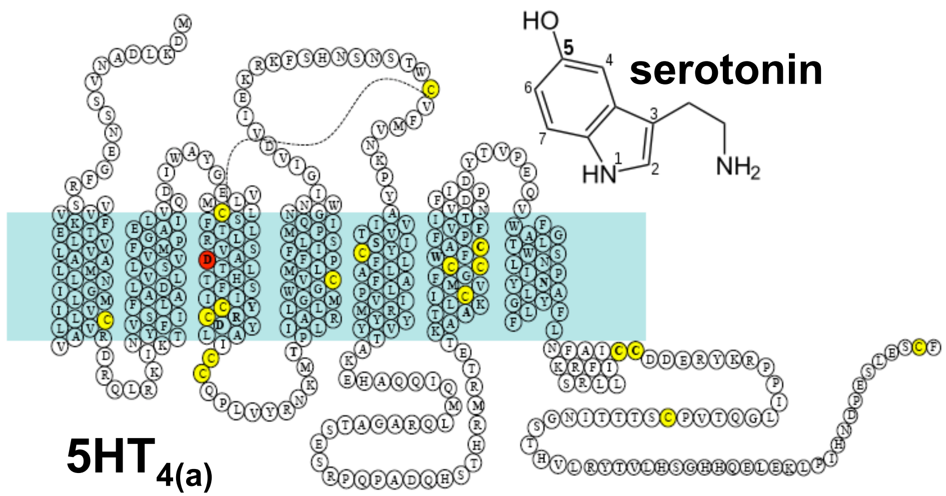


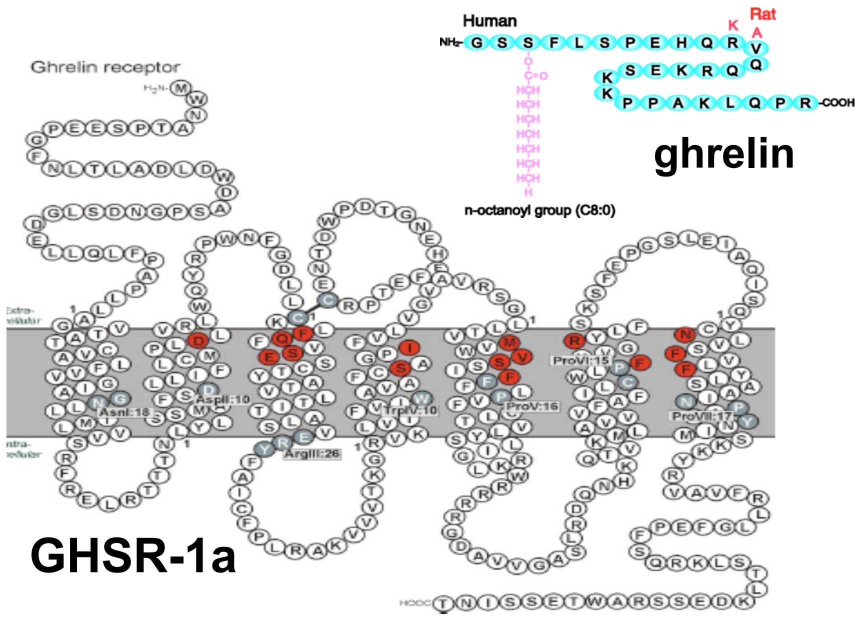
Model systems











Production

Solubilization

Refolding/renaturation

Functional assays

Purification

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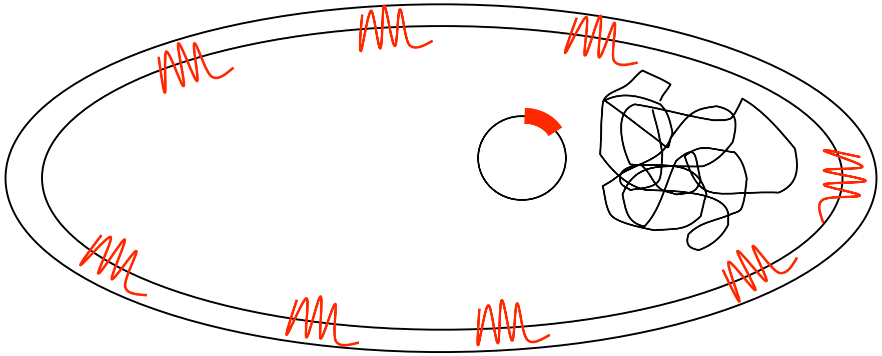
Recombinant GPCRs

Table 1. Expression systems for G protein-coupled receptors (GPCRs)

Expression system	GPCR	Advantages	Disadvantages	Refs
Escherichia coli	Adenosine a2a, neurotensin	Fast, easy, safe, scaleable	Lack of post-translational modifications, membrane toxicity, low yields, fusion protein required	[27,28]
	Leukotriene receptor BLT1	High expression in inclusion bodies	Refolding required	[29]
Halobacterium salinarium	Rhodopsin, yeast α-factor, serotonin 5HT2c	Fast, colorimetric assay, scaleable	Cloning, transformation more complicated, fusion protein required	[34]
Lactococcus lactis	Not tested yet	Easy, safe	Lack of post-translational modifications	[35]
Saccharomyces cerevisiae	Yeast α-factor, dopamine D1A	Relatively easy, scaleable	Hyper glycosylation, thick cell wall, clone selection	[10] [11]
Schizosaccharomyces pombe	Dopamine D2	Relatively easy, scaleable	Non-mammalian glycosylation	[12]
Pichia pastoris	β2-adrenergic and other GPCRs	Relatively easy High biomass	Thick cell wall, clone selection	[13]
Baculovirus	Neurokinin-1 β2-adrenergic	Mammalian-like	Slow virus stock production	[14] [36]
Transient mammalian	Several GPCRs e.g. 5HT1E	Mammalian	Transfection methods to be established for each cell line	[37]
Stable mammalian	Rhodopsin Several GPCRs (e.g. α2 adrenergic)	Inducible	Slow, stability problems	[16] [15]
Viral, SFV	Neurokinin-1 >GPCRs	Broad host range Extreme expression Scaleable	Relative expensive Safety issues	[38] [39] [19]
Cell-free translation	β2-adrenergic	Simple, fast	Very low yields	[a]

Lundstrom K. (2005). Structural genomics of GPCRs. *Trends Biotechnol*. **23**, 103-8.

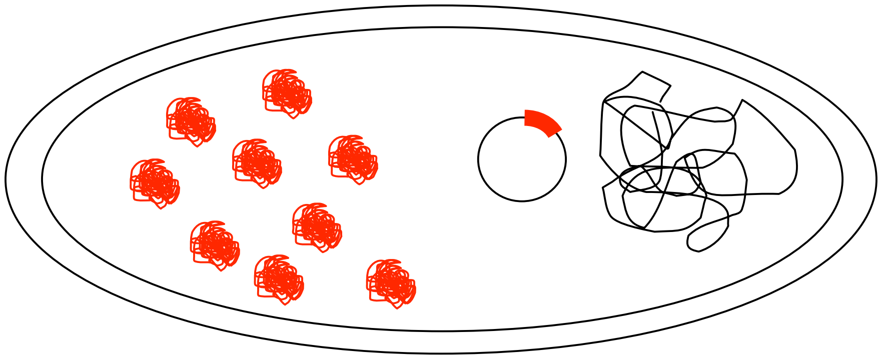
GPCR expression in *E. coli*



Functional expression:

NTS1 (MBP fusion; Grisshammer et al. (1993) Biochem J.)

CB2 (MBP-TRX fusion; Yeliseev et al. (2007) *Prot. Exp. Purif.*)



Expression in inclusion bodies:

OR5 (Kiefer et al. (1996) *Biochemistry*)

BLT1 (Banères et al. (2003). *J. Mol. Biol.)*

5-HT4a (Banères et al. (2005) *J. Biol. Chem.)*

GPCR expression in *E. coli*

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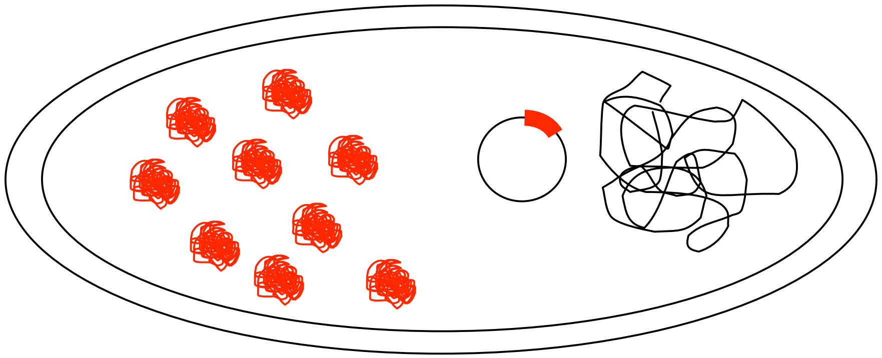

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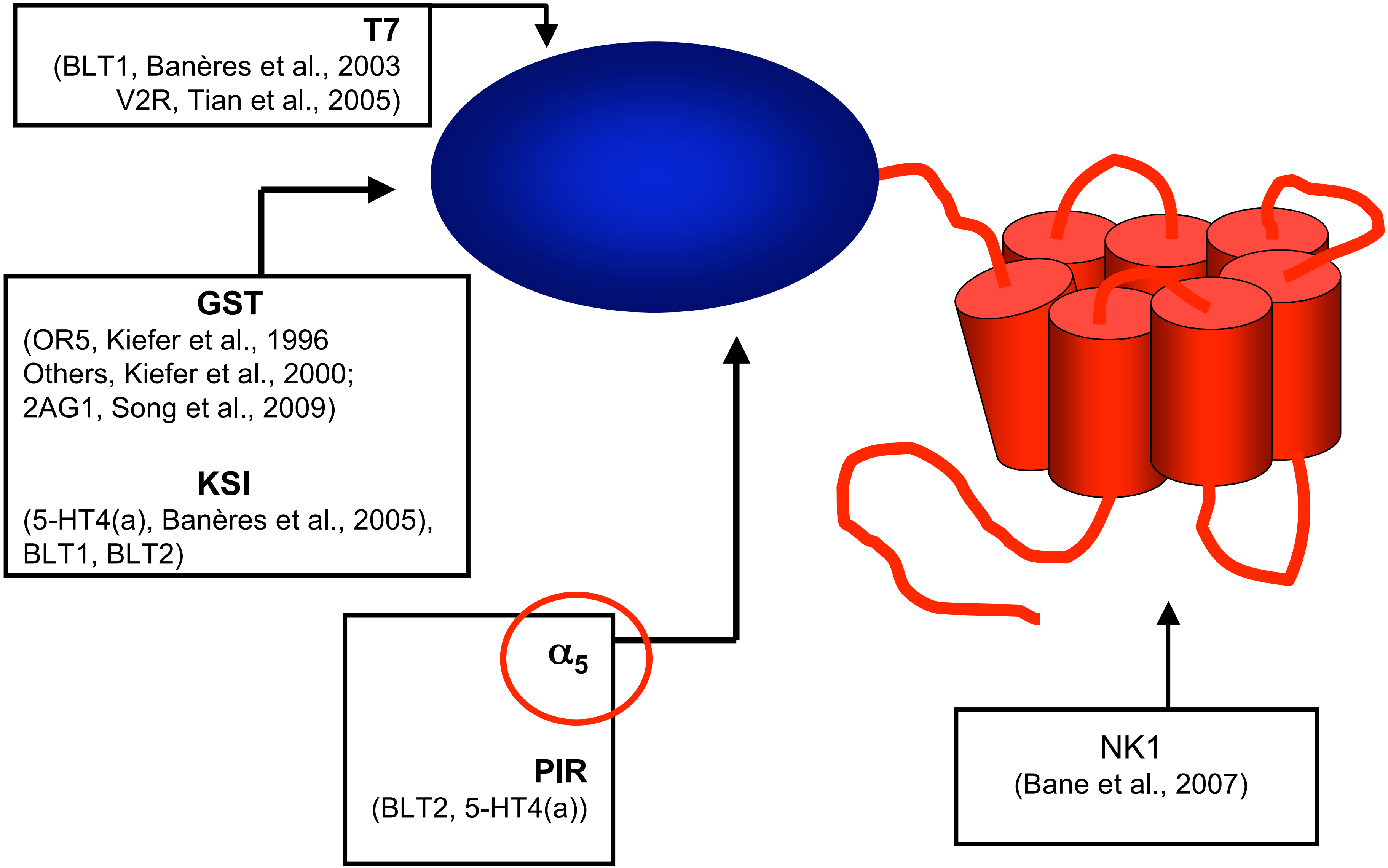
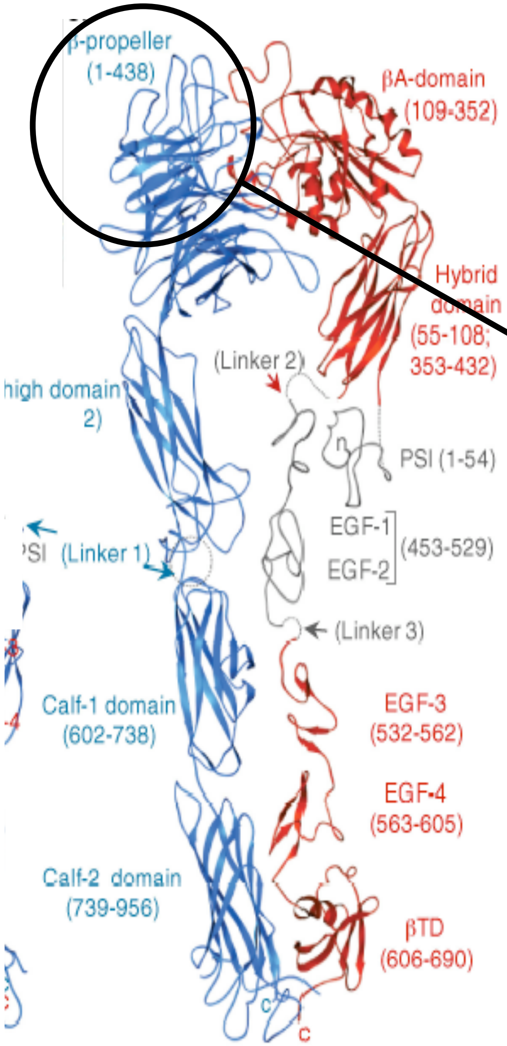
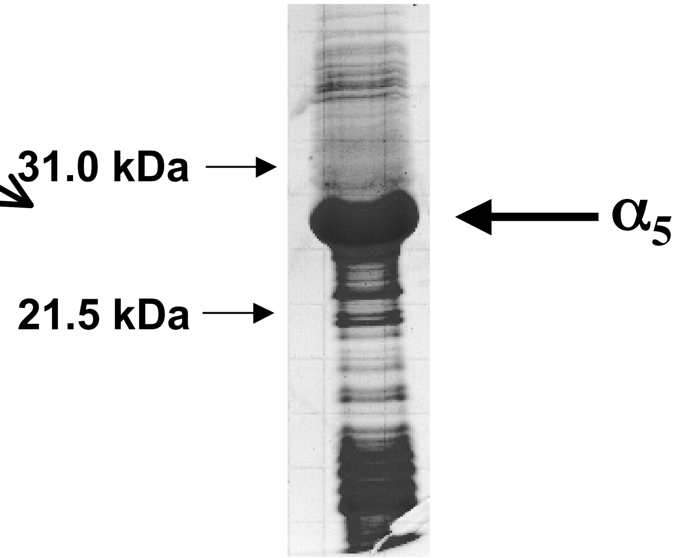
…

Accumulation in inclusion bodies



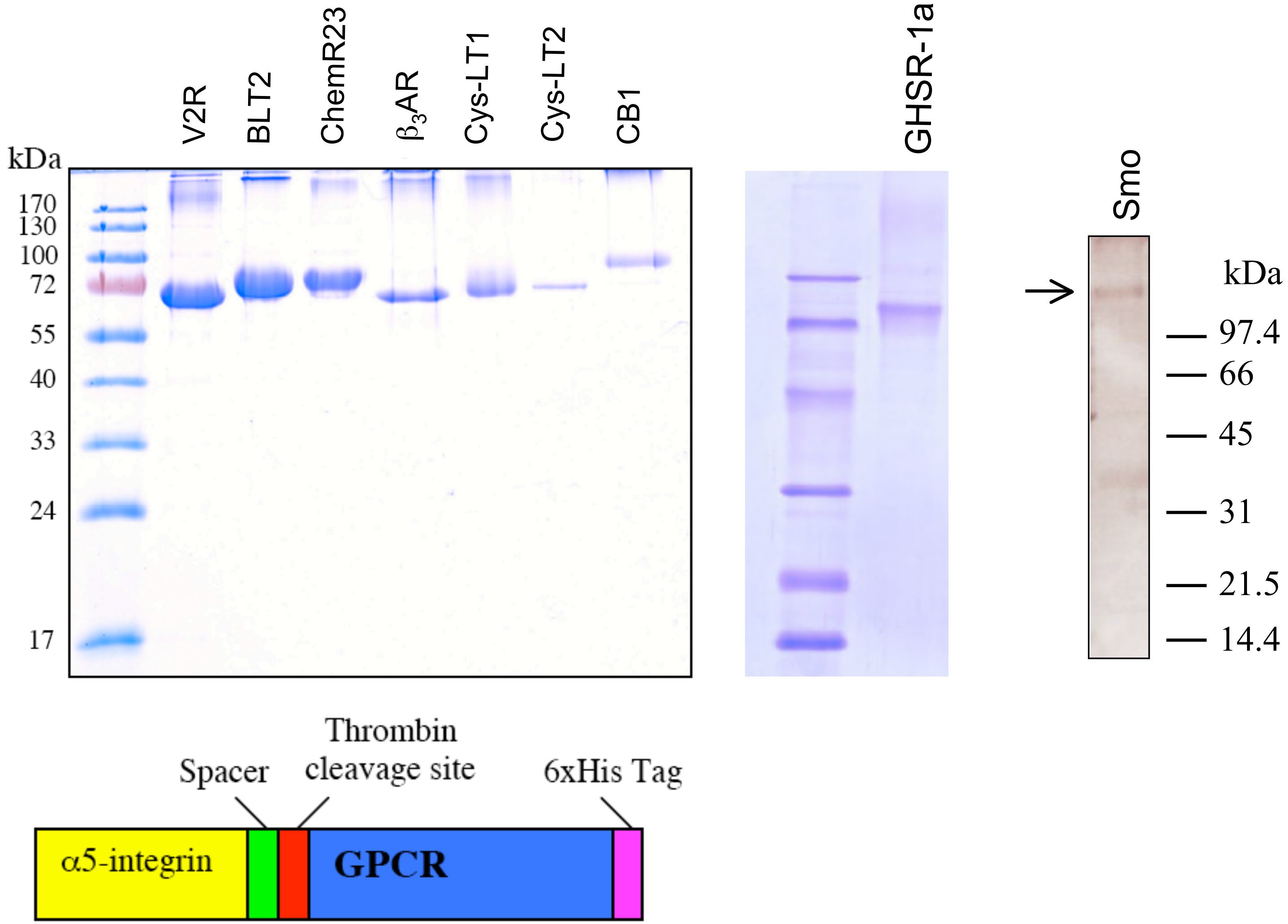
Integrin α_5 fragment





Banères et al. (1998). *J Biol Chem*. **273**:24744-53.

α_5:GPCR fusions



Arcemisbéhère *et al.* (2010) *J. Biol. Chem.* **285**:6337-47

IBMM
Institut des
Biomolécules
Max Mousseron

Production

Solubilization

Refolding/renaturation

Functional assays

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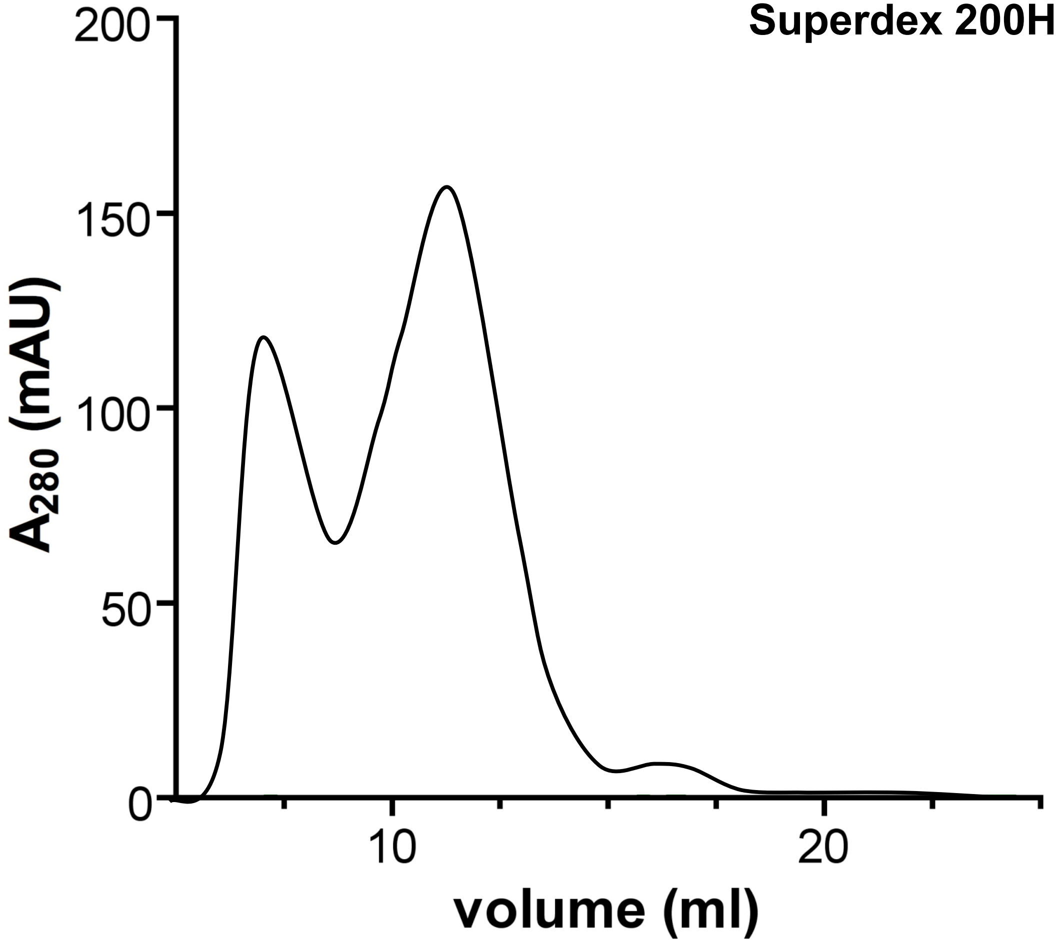
BLT1 solubilization



5-$HT_{4(a)}$ solubilization

Chaotropic agent	detergent	other	Aggregation (Ragg)
urea (8M)	-	-	22
-	SDS 0,2%	-	3,7
-	SDS 0,4%	-	1,3
-	SDS 0,6%	-	1,2
	Sarcosyl 1%	-	4,2
-	DDM 1 mM	-	8,9
urea (6M)	SDS 0,4%	-	1,4
-	DPC	-	6,3
urea (6M)	Sarcosyl 1%	-	6,6
-	SDS 0,6%	Glycerol 10%	1,1
-	-	CHCl3/MeOH	0,5

→ Refolding yield: 0.6% (urea (8M))

→ Refolding yield: 6% (SDS 0,2%)

→ Refolding yield: 16% (SDS 0,6% + Glycerol 10%)

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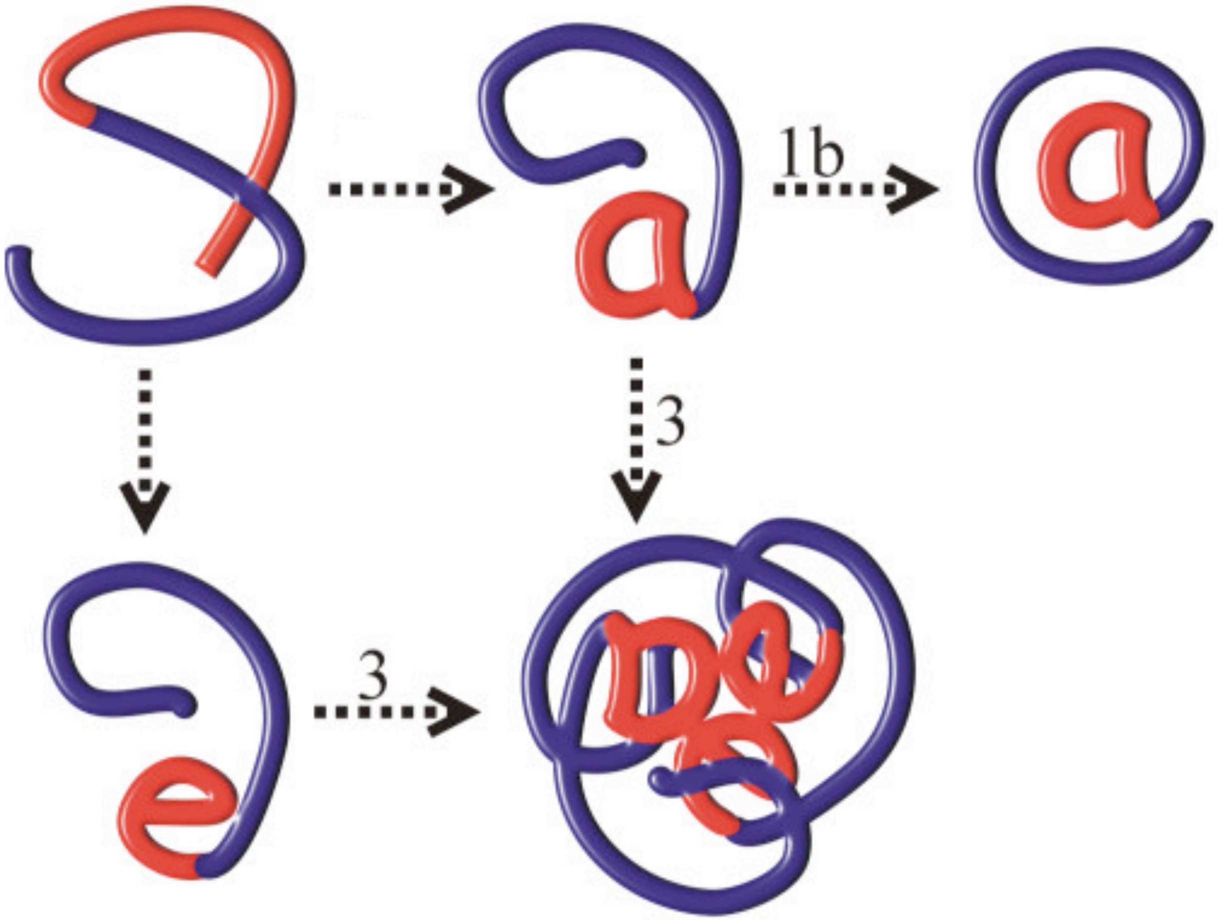
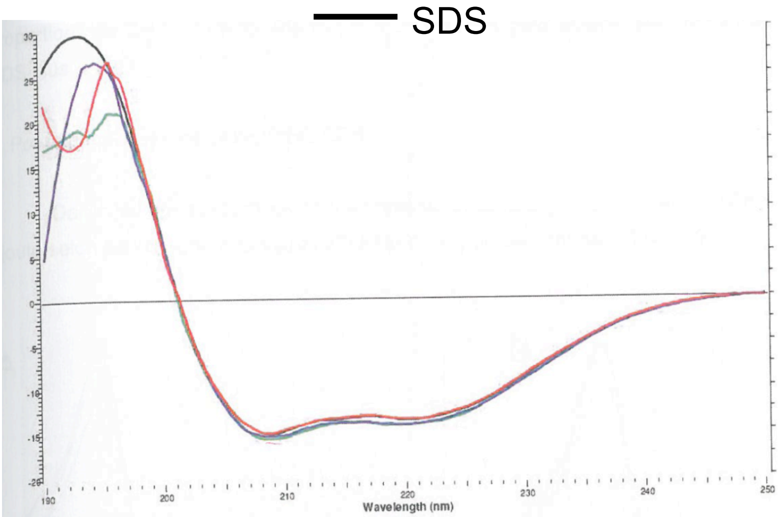
Refolding



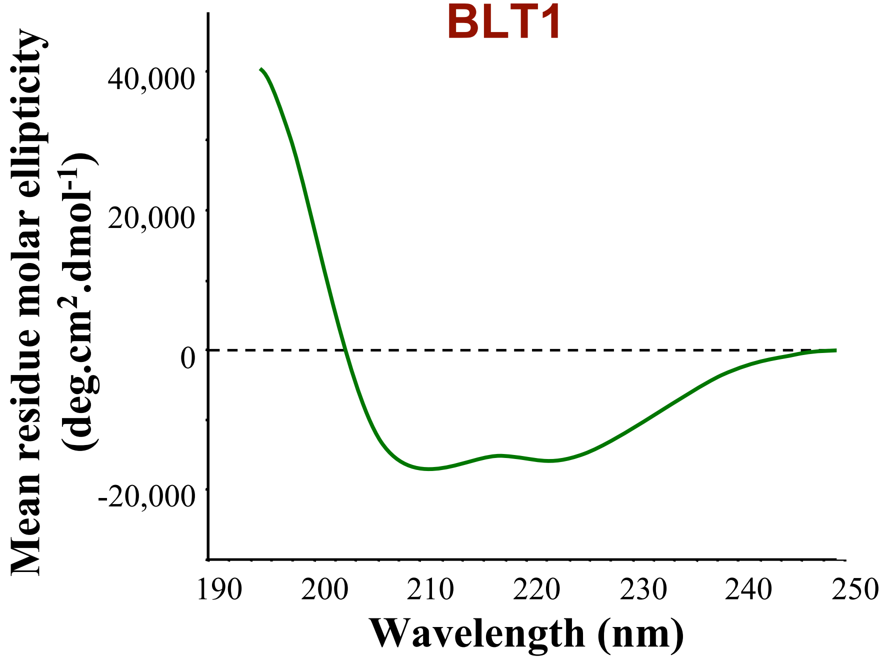
SDS-"unfolded" state of the protein

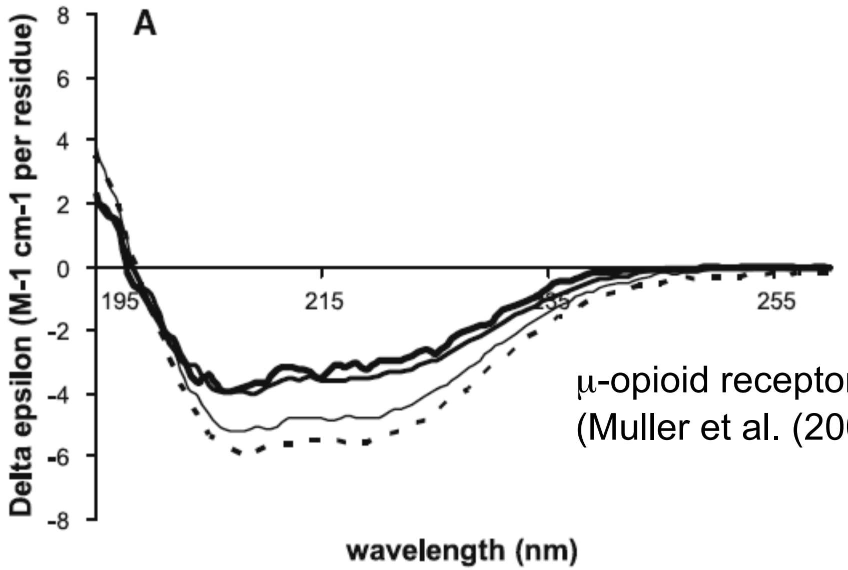
bacteriorhodopsin

Dahmane (2007). Ph. D Thesis.



BLT1

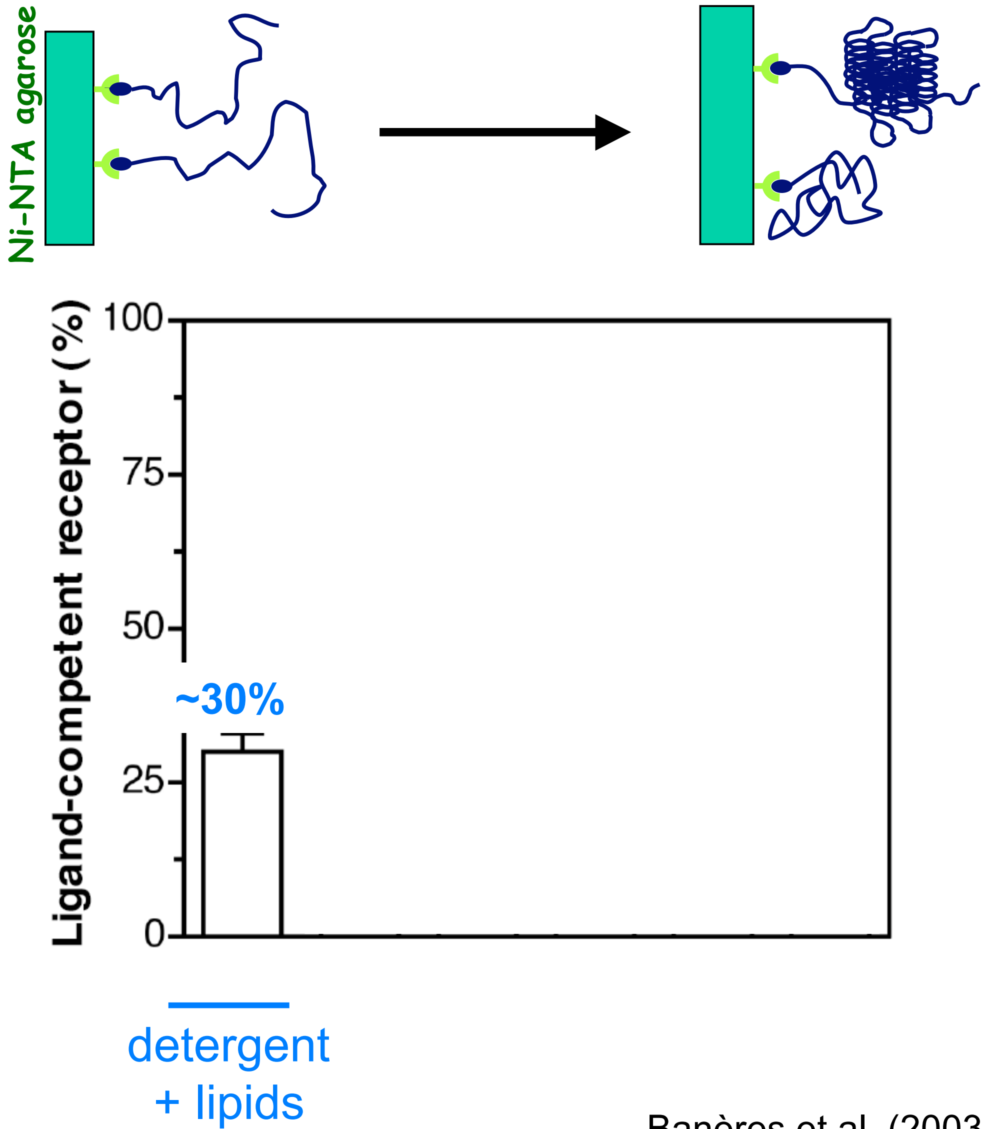




μ-opioid receptor

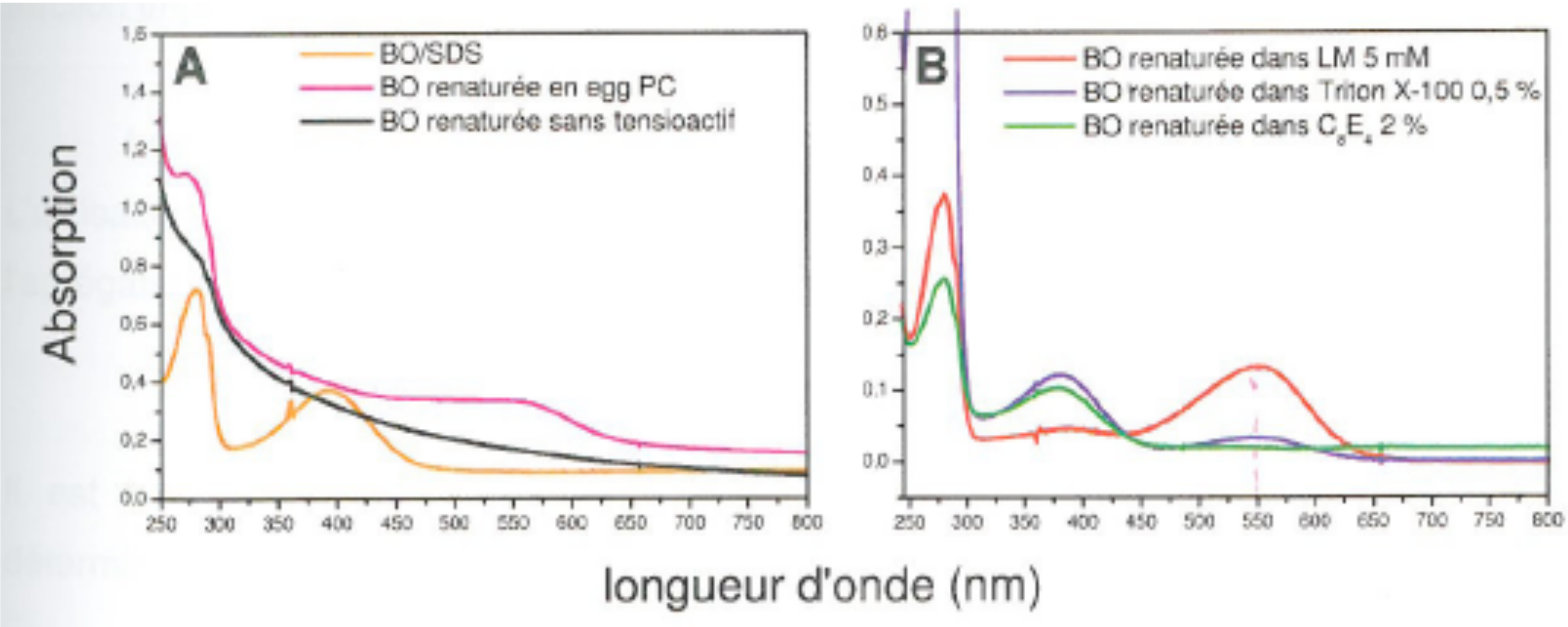
(Muller et al. (2008). *J Membr Biol*. **223**:49-57.)

Detergent-assisted BLT1 refolding



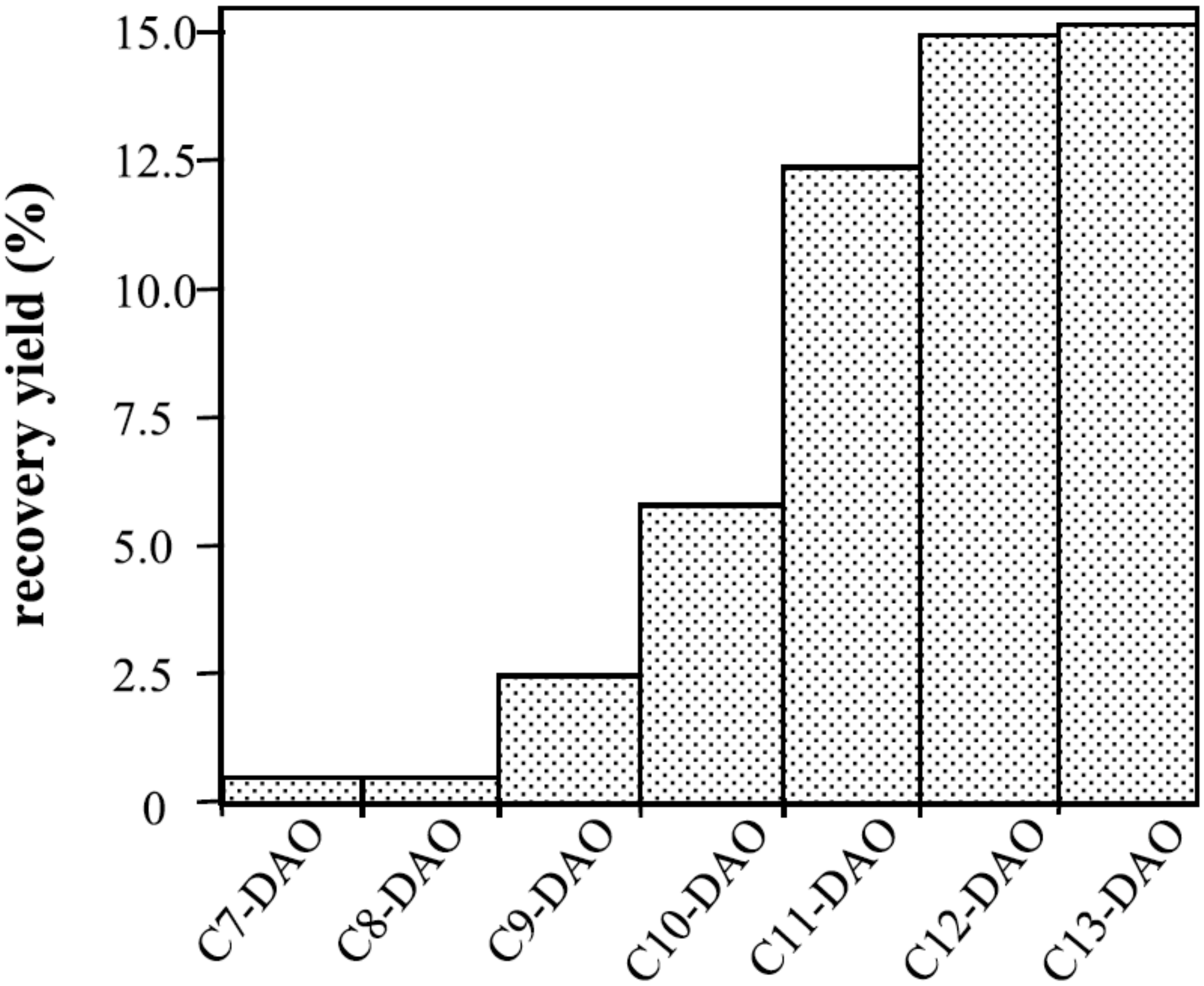
Banères et al. (2003). *J Mol Biol.* **329**:801-14.

Detergent-assisted bacteriorhodpsin refolding



Dahmane T. (2007). Ph. D thesis

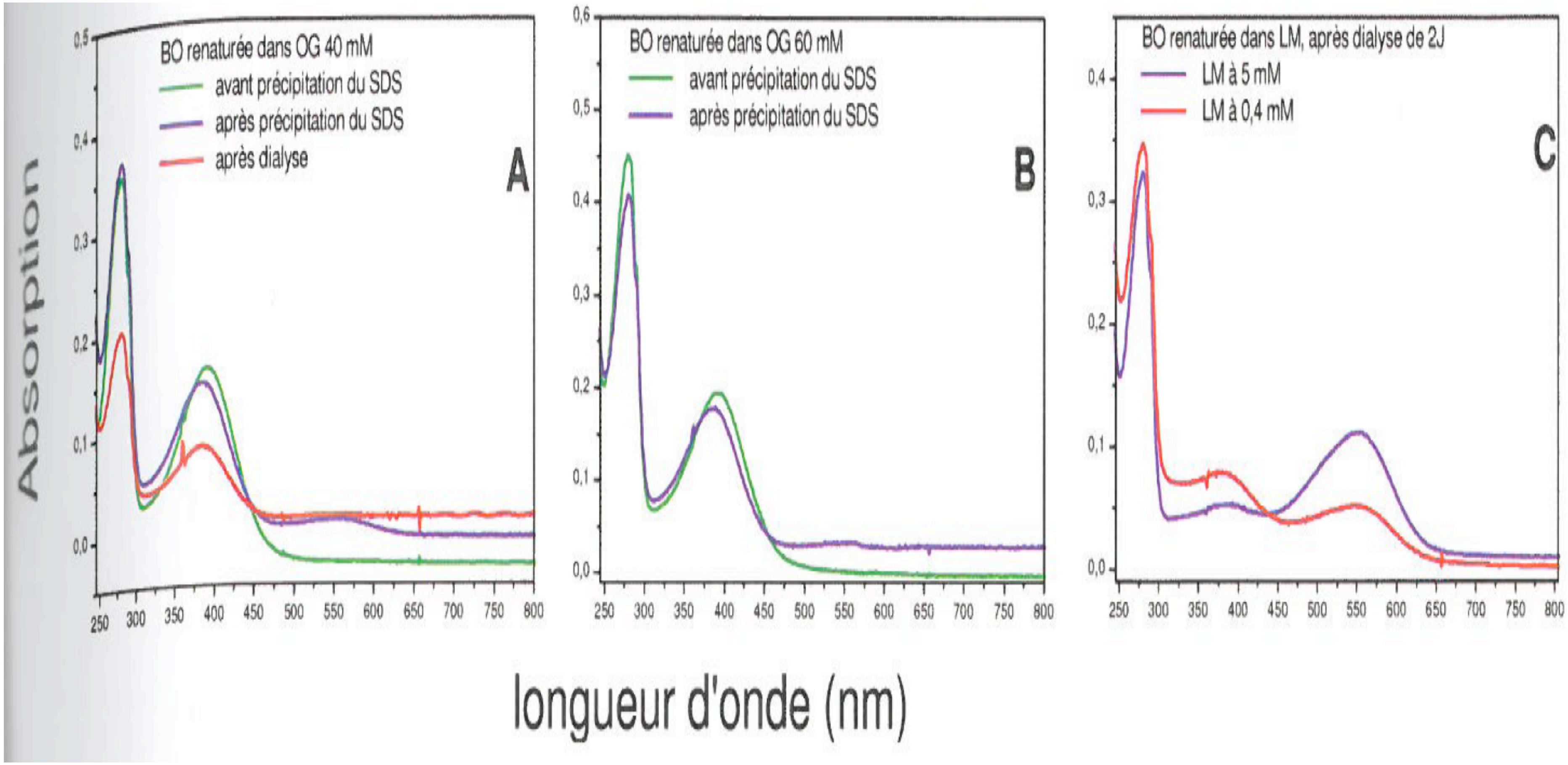
Detergent-assisted BLT1 refolding



Banères et al. (2003). *J Mol Biol.* **329**:801-14.

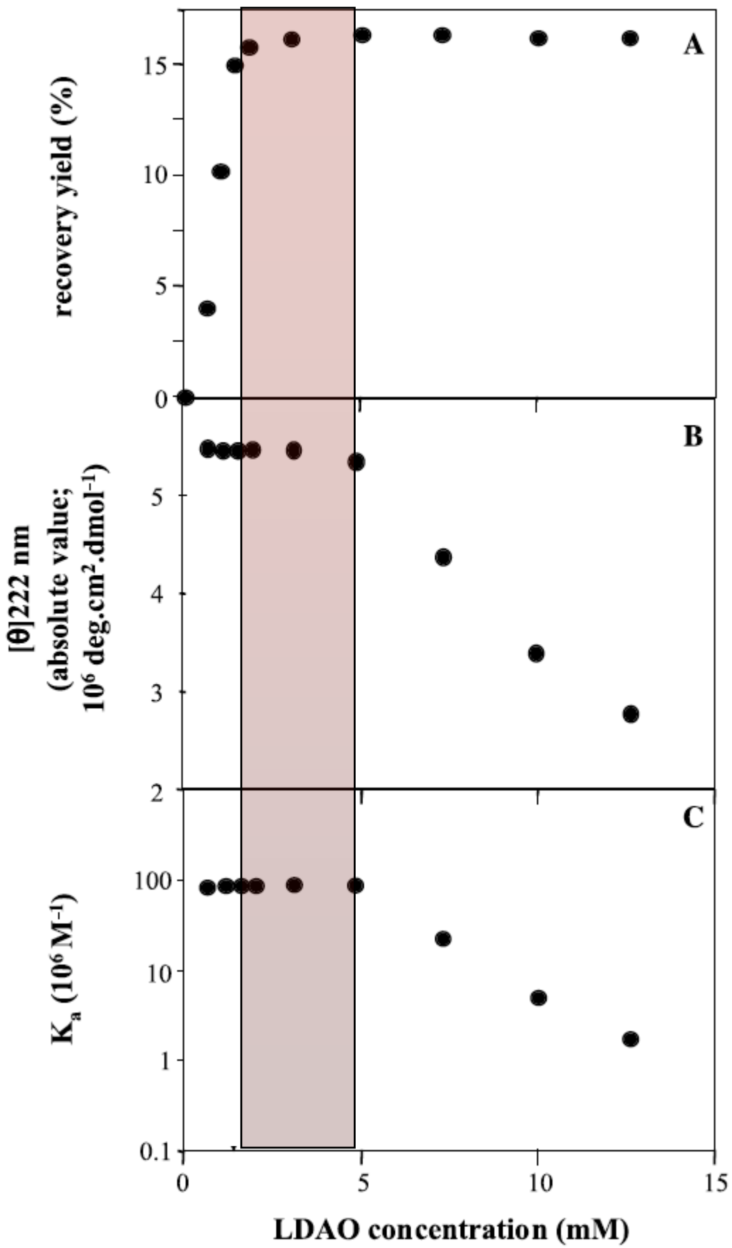
Effect of detergent concentration (1)

Bacteriorhodopsin



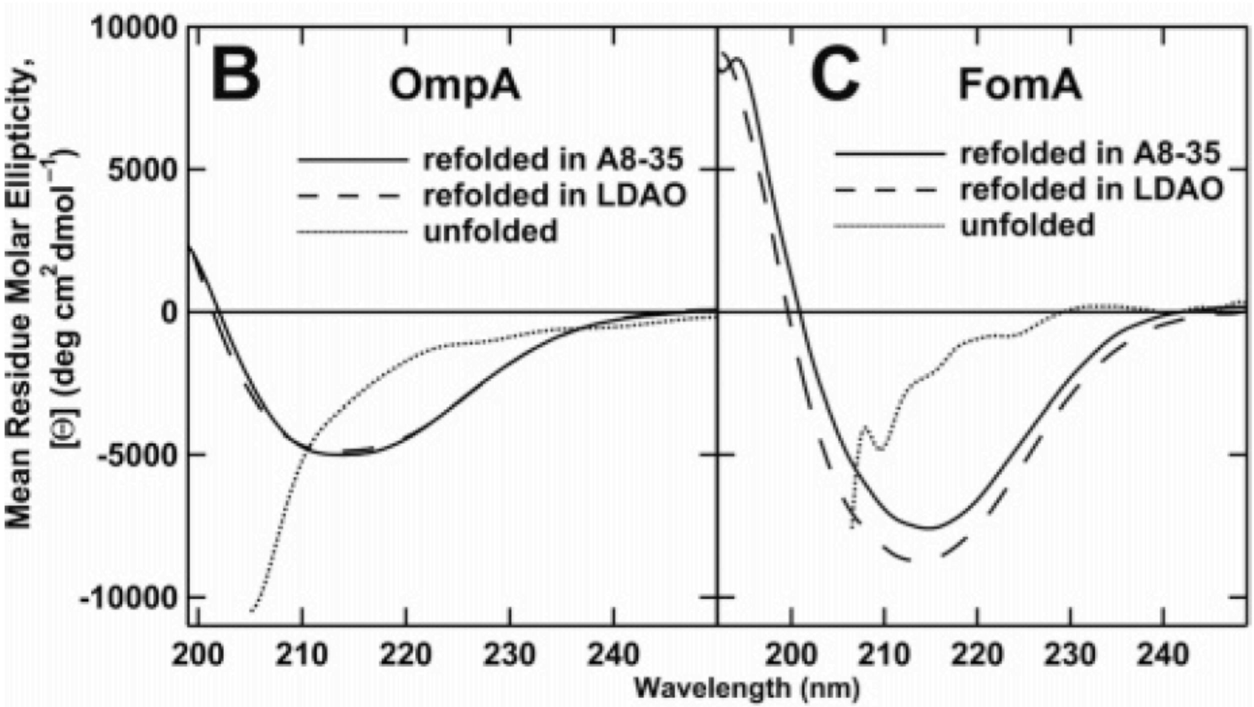
Dahmane T. (2007). Ph. D thesis

Effect of detergent concentration (2)



Banères et al. (2003). *J Mol Biol.* **329**:801-14.

Amphipol-assisted β-barrel protein refolding



Denatured protein in urea

↓

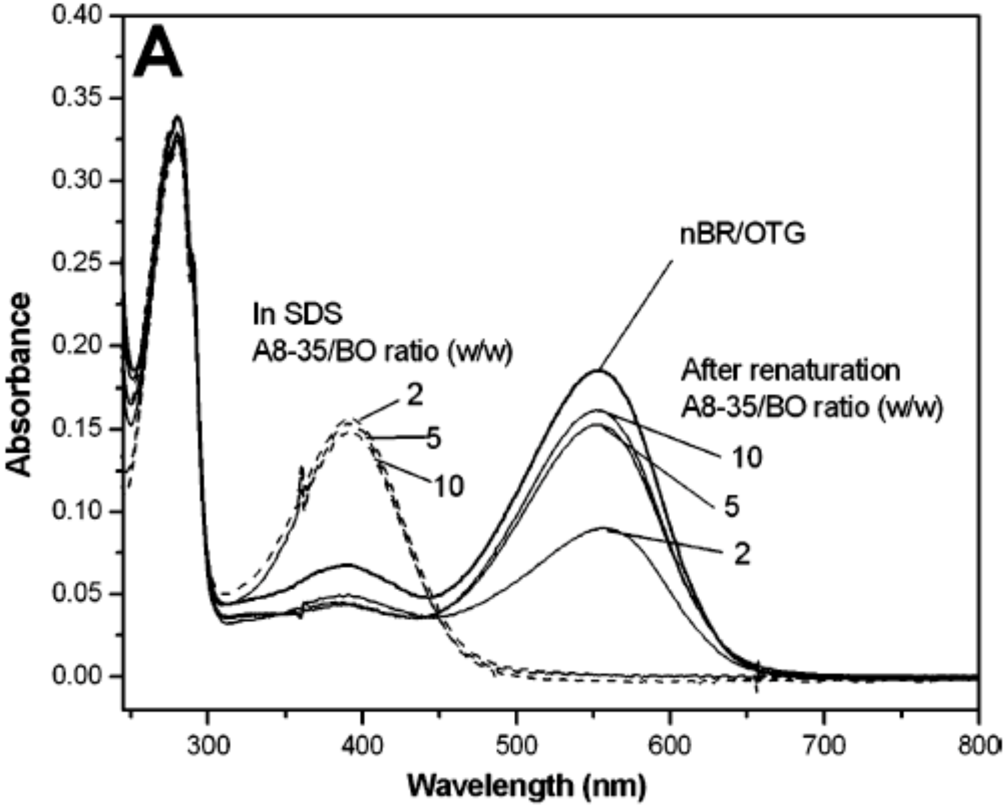
Addition of amphipol A8-35

↓

dilution

Pocanschi et al. (2006). *Biochemistry* **45**:13954-61

Amphipol-assisted bacteriorhodopsin refolding



Denatured protein in SDS

↓

Addition of amphipol A8-35

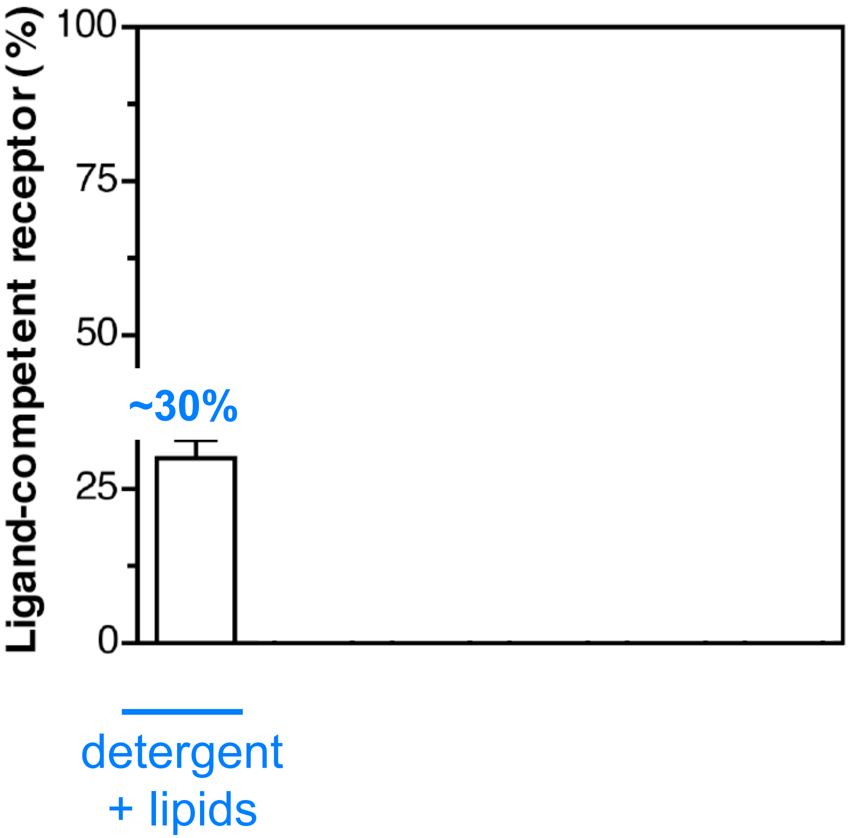
↓

Precipitation of SDS by addition of KCl

Centrifugation – Dialysis of supernatant

Pocanschi et al. (2006). *Biochemistry* **45**:13954-61

Amphipol-assisted BLT1 refolding



Folding of leukotriene BLT1 receptor

Denatured GPCR in SDS

↓

Addition of amphipol A8-35 (± lipids)

↓

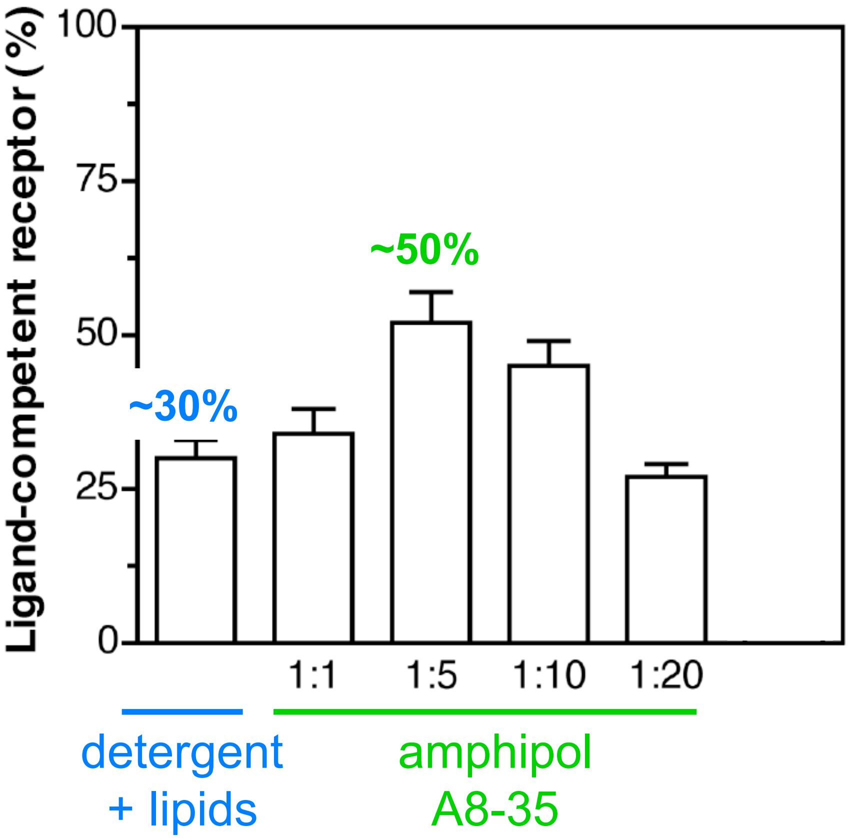
Precipitation of SDS by addition of KCl
Centrifugation – Dialysis of supernatant

↓

Assay for specific ligand binding

Dahmane et al. (2009). *Biochemistry* **48**:6516-21.

Amphipol-assisted BLT1 refolding



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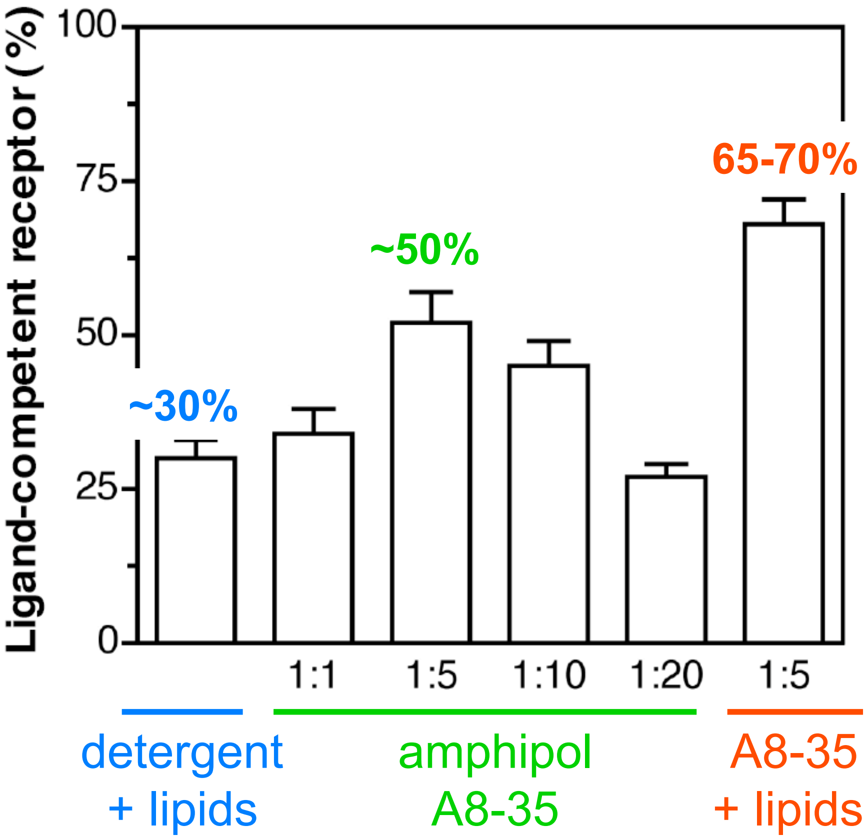

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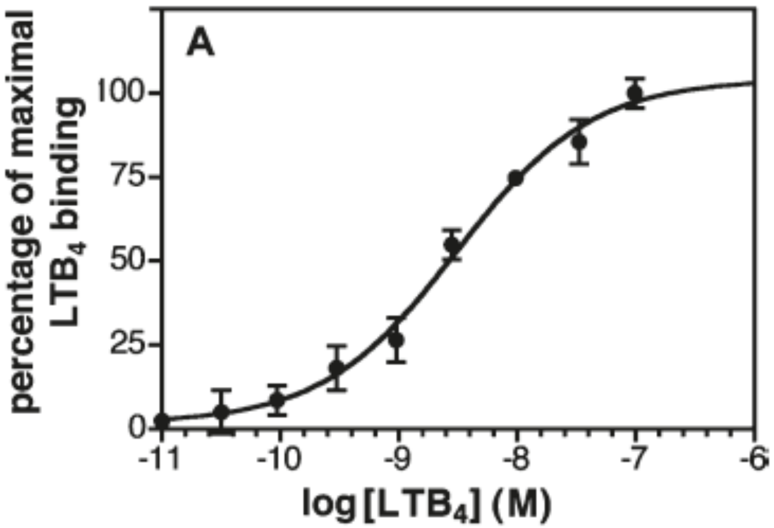

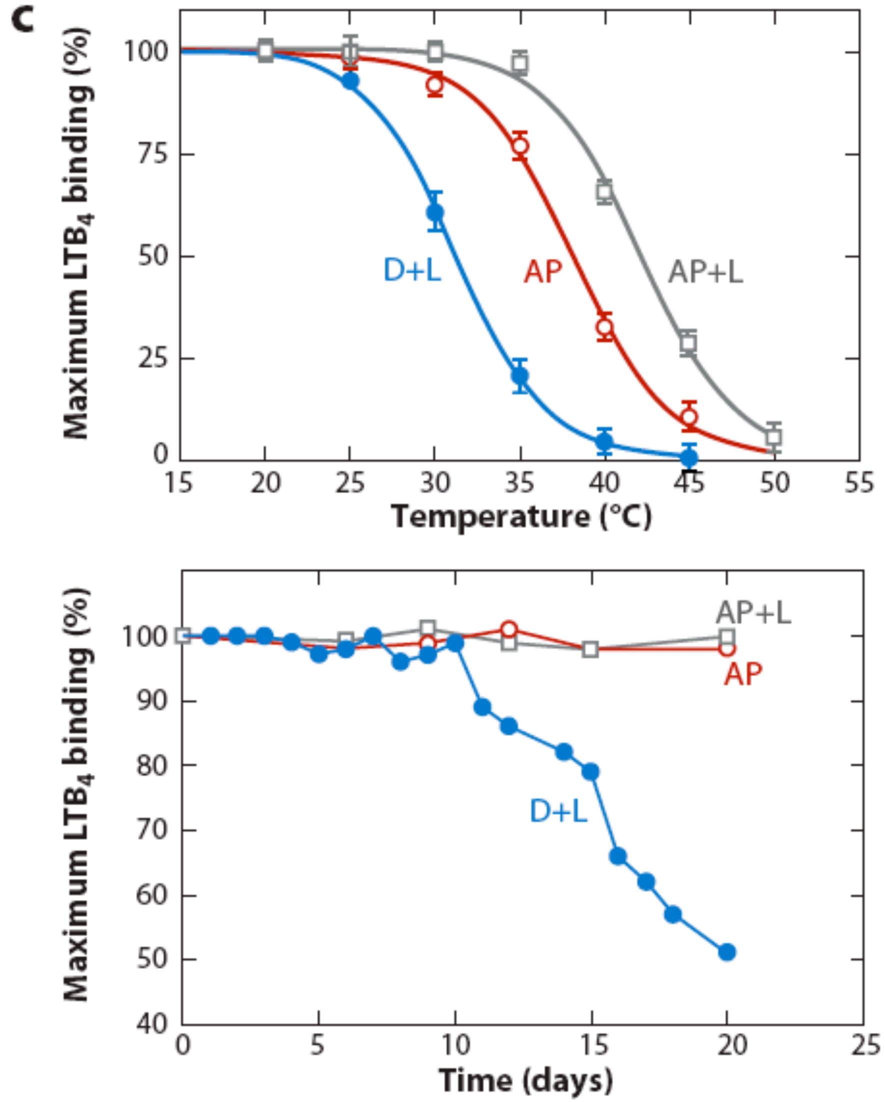

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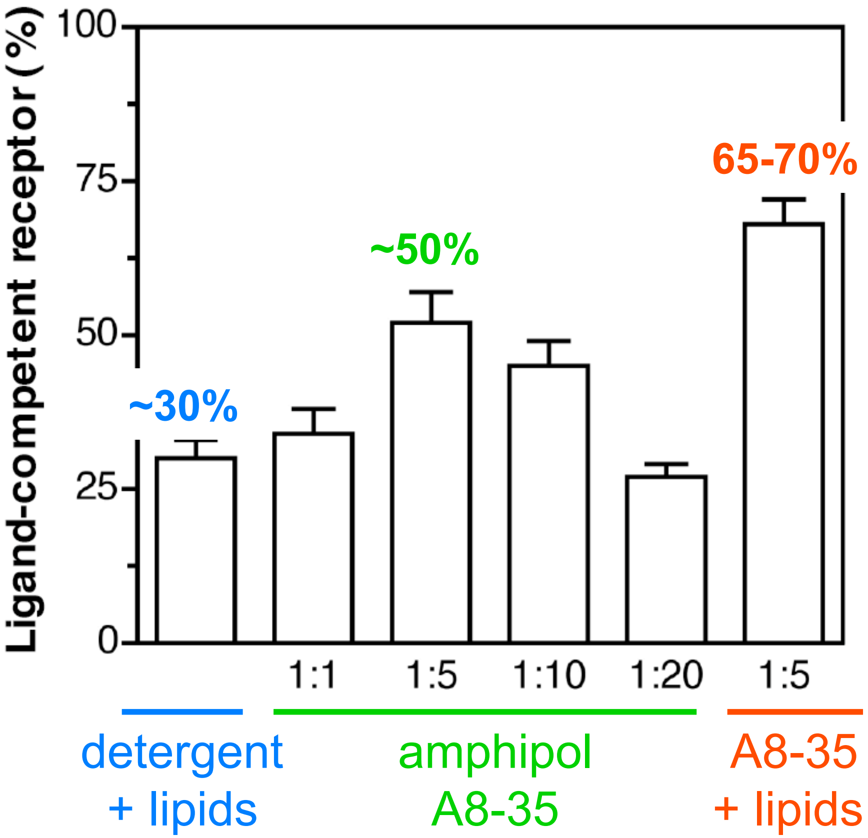
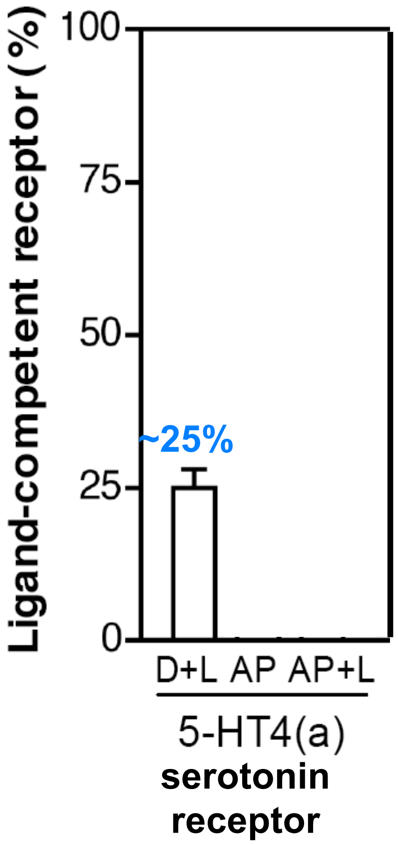


Folding of leukotriene BLT1 receptor



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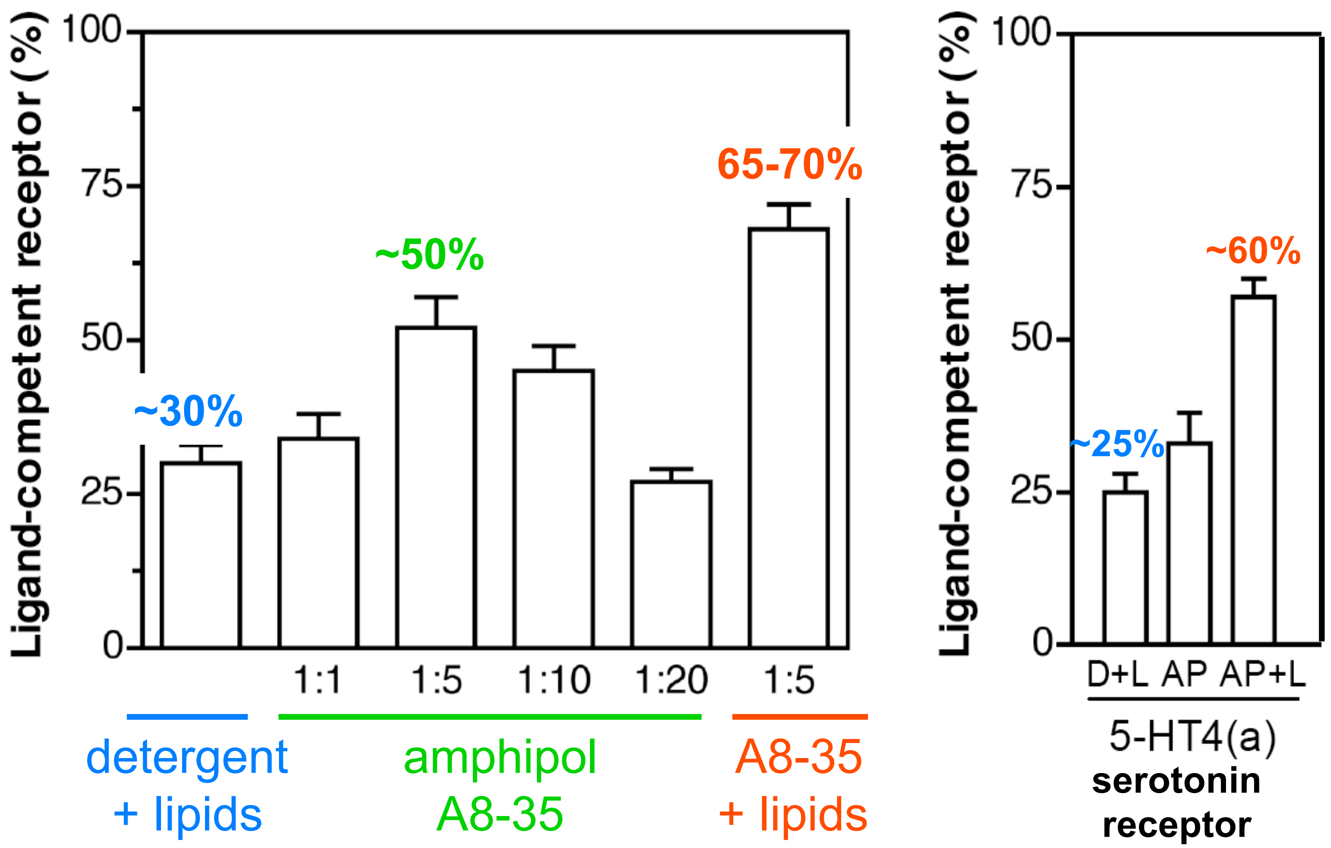

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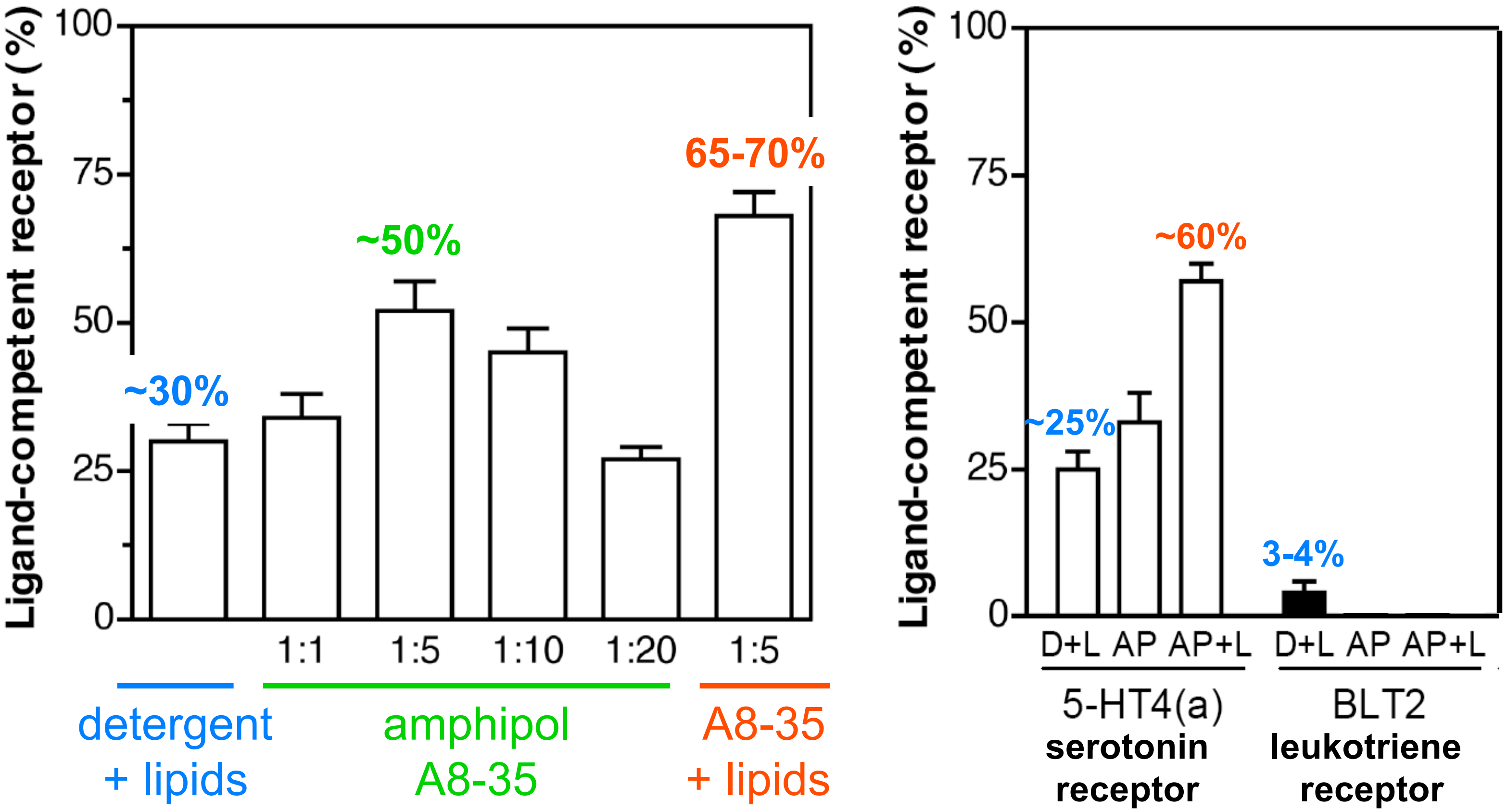

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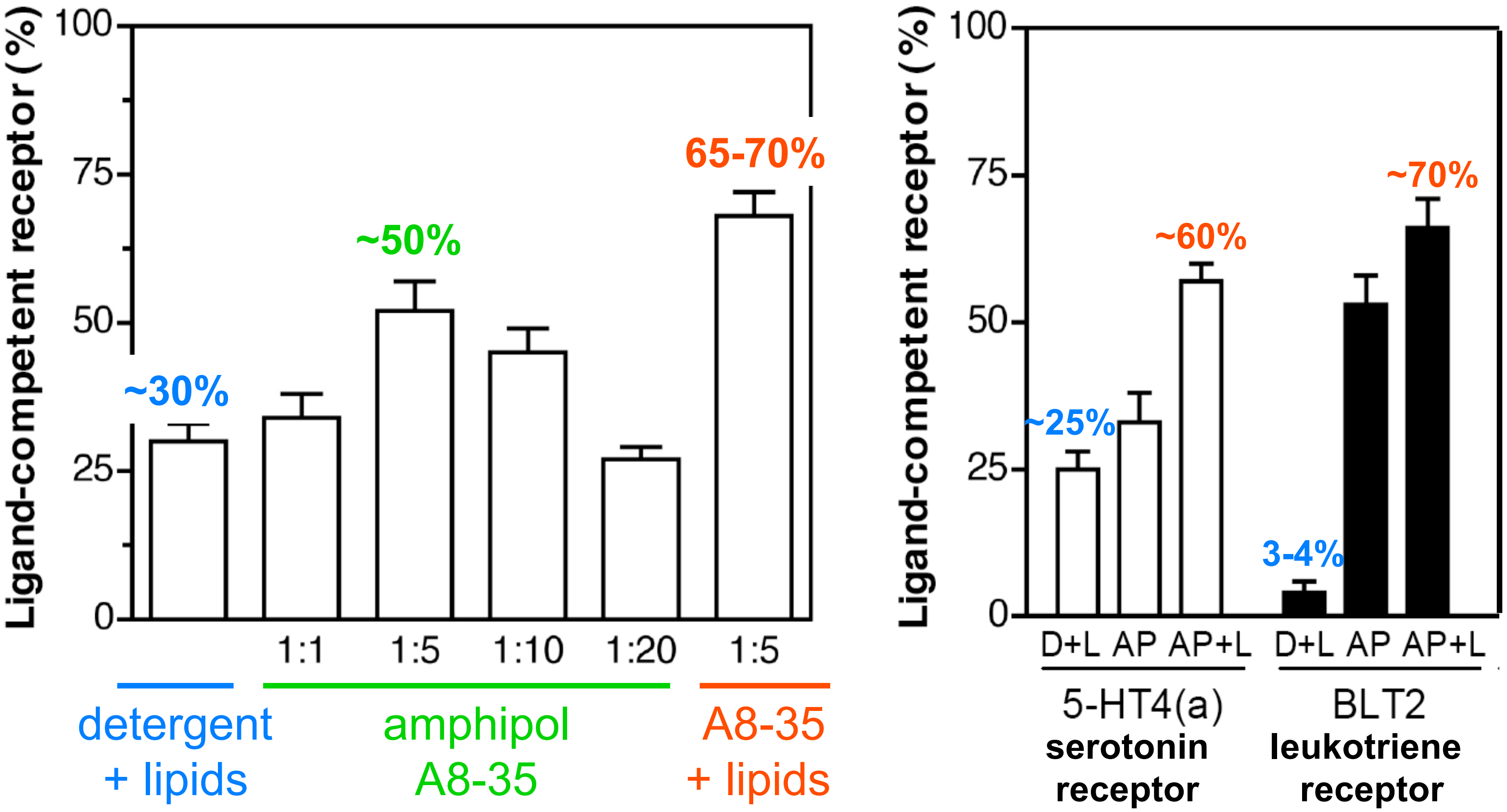

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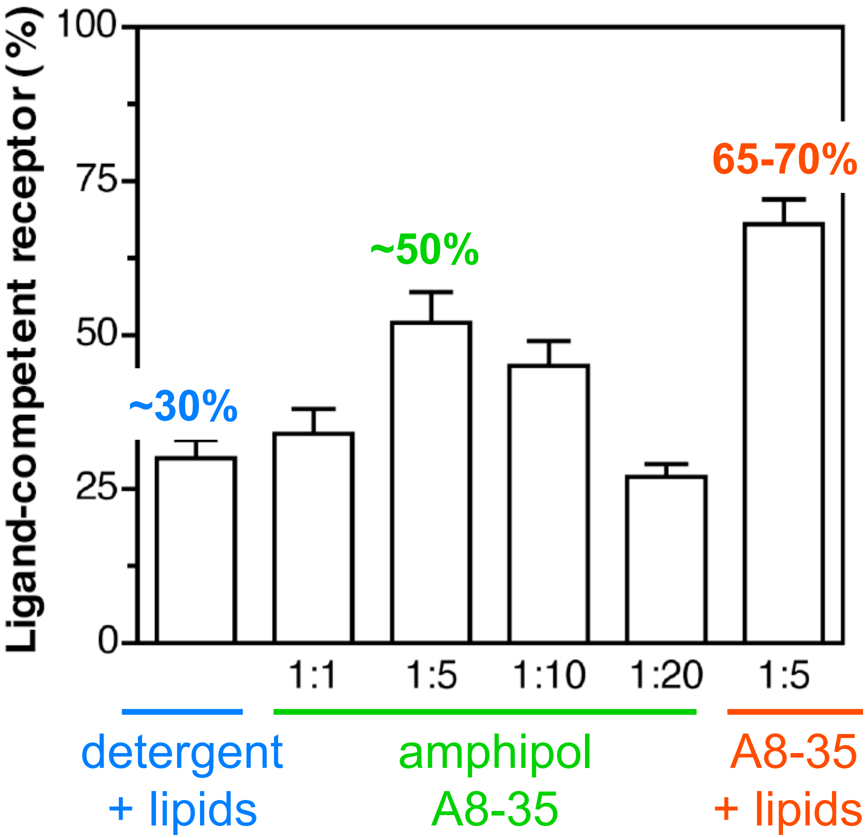

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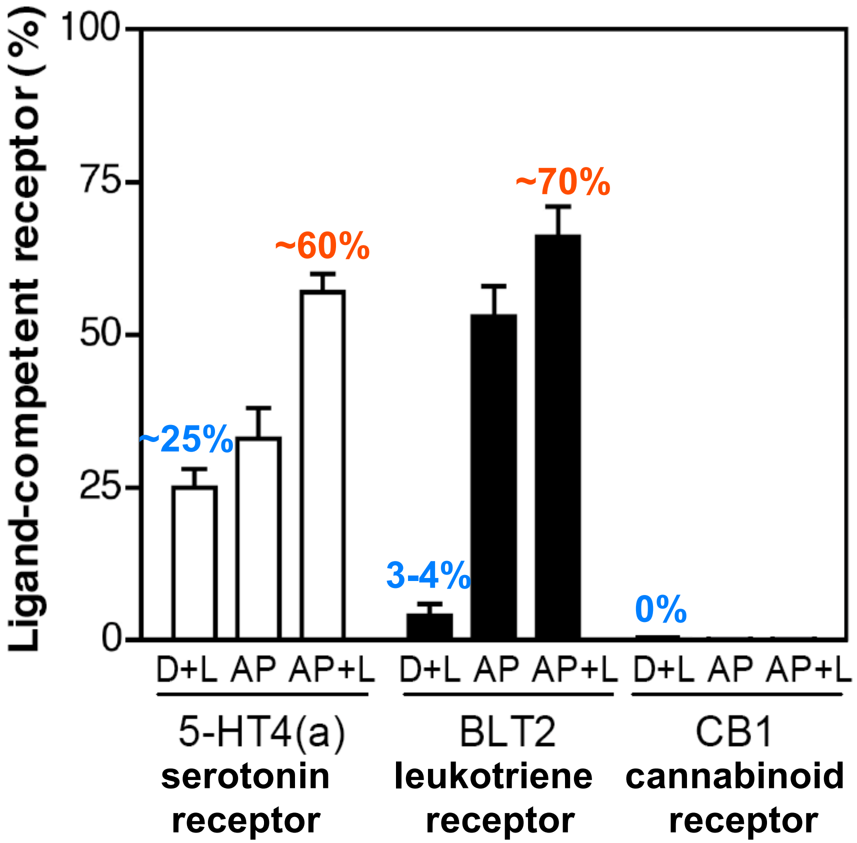

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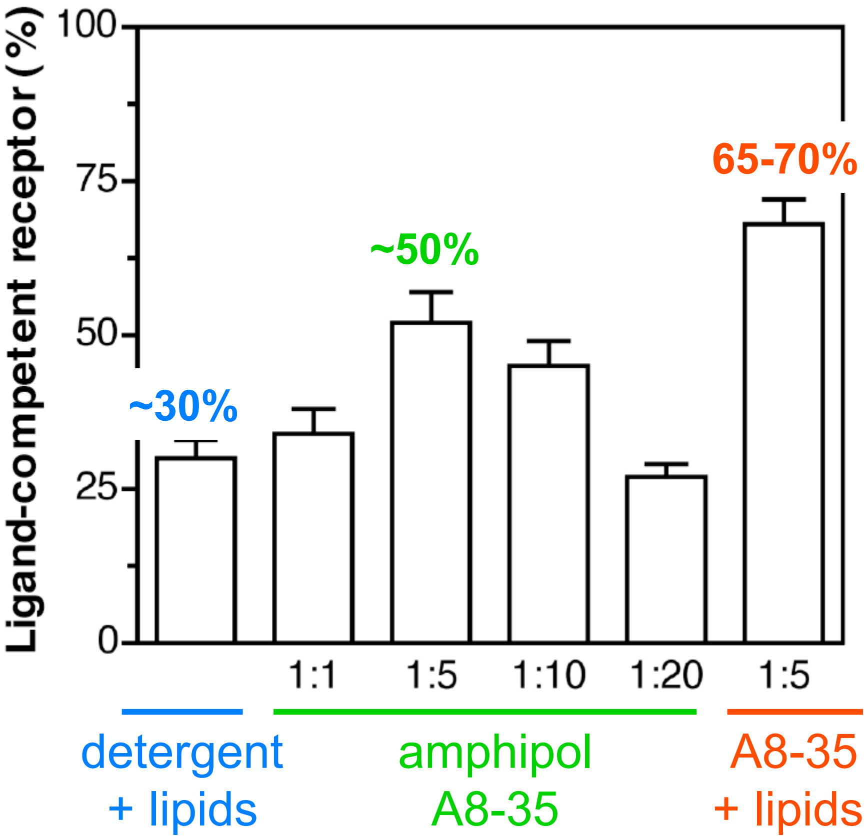

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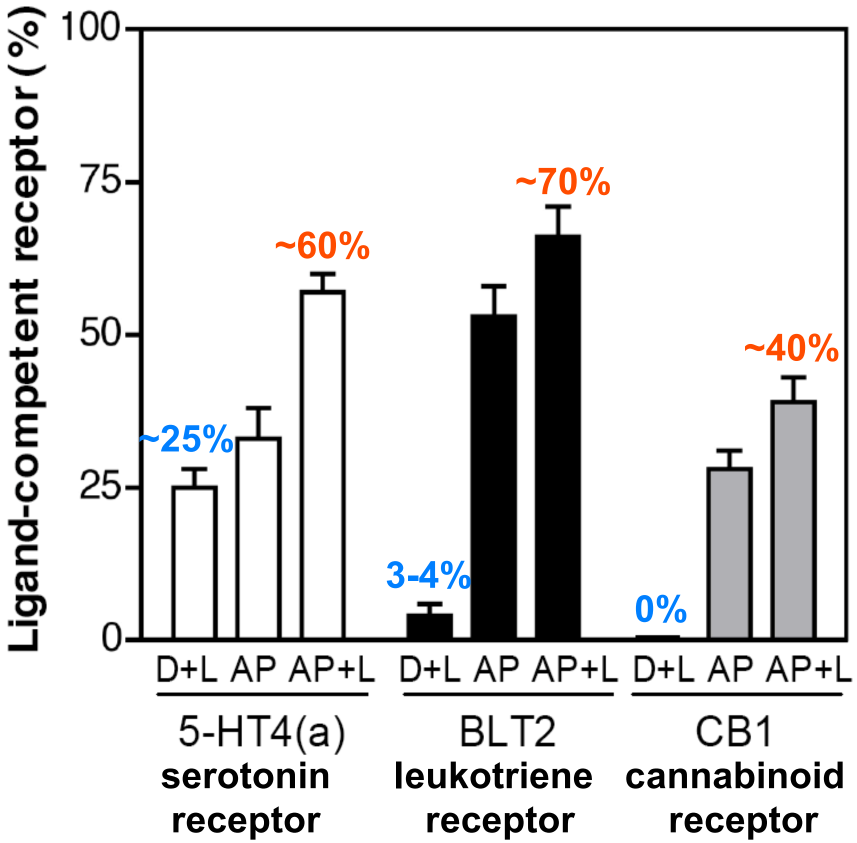
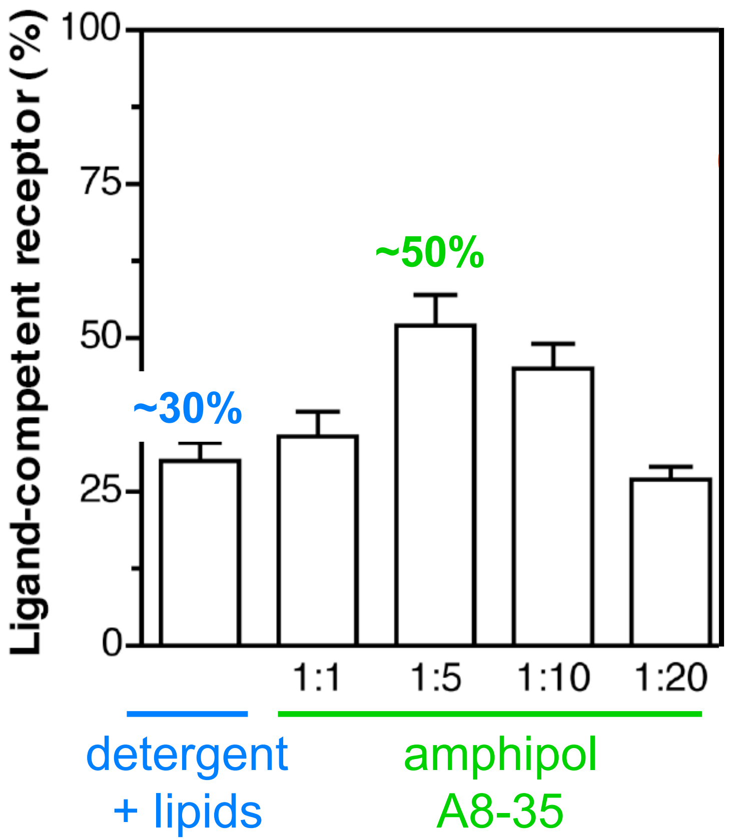
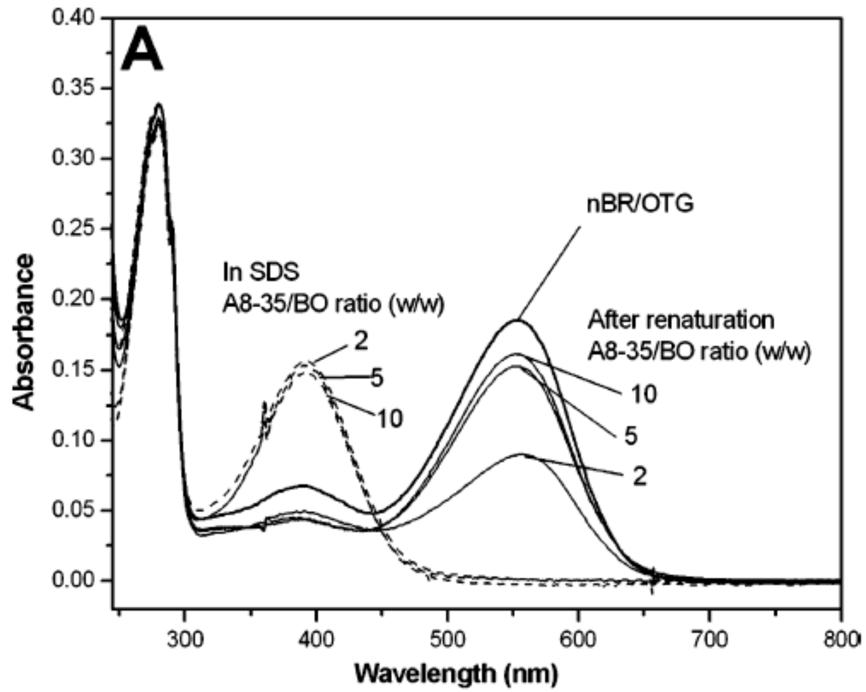


Dahmane et al. (2009). *Biochemistry* **48**:6516-21.

Protein:amphipol ratio and refolding efficiency

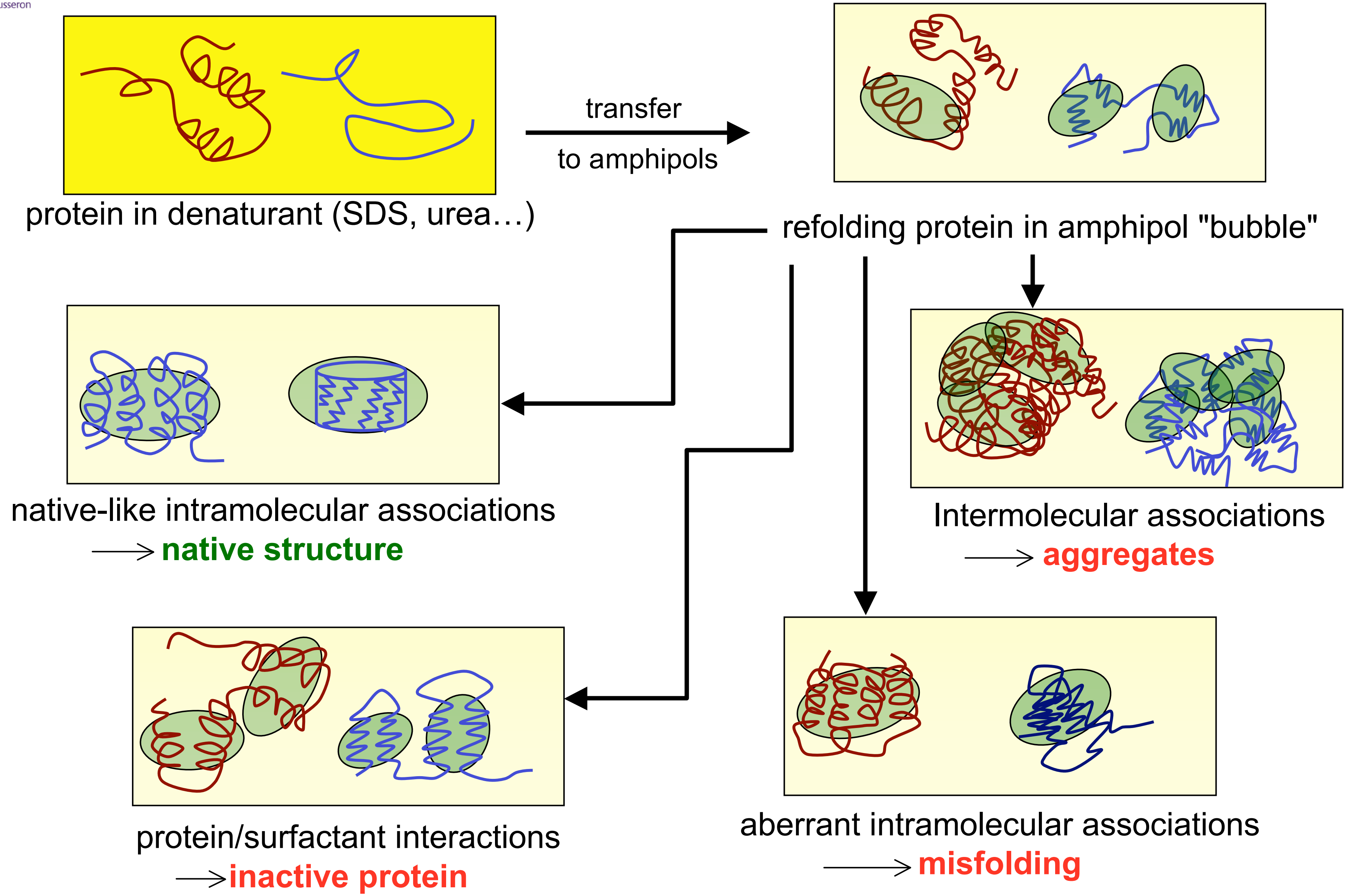


Folding of leukotriene BLT1 receptor



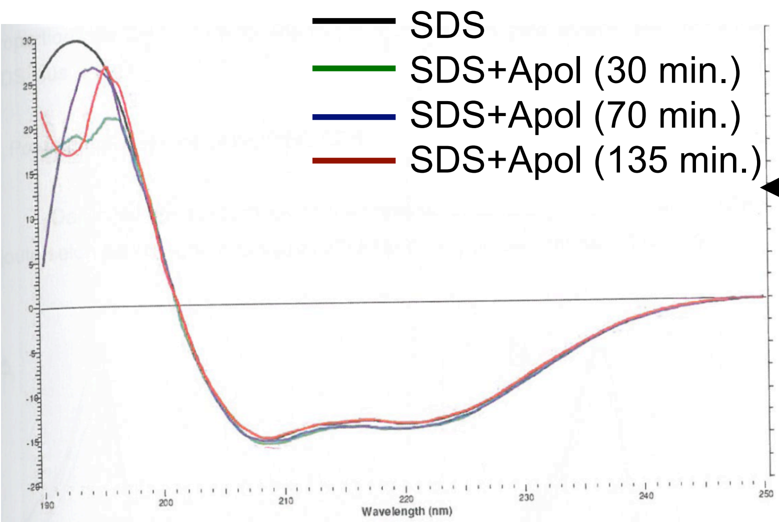
Folding of bacteriorhodopsin

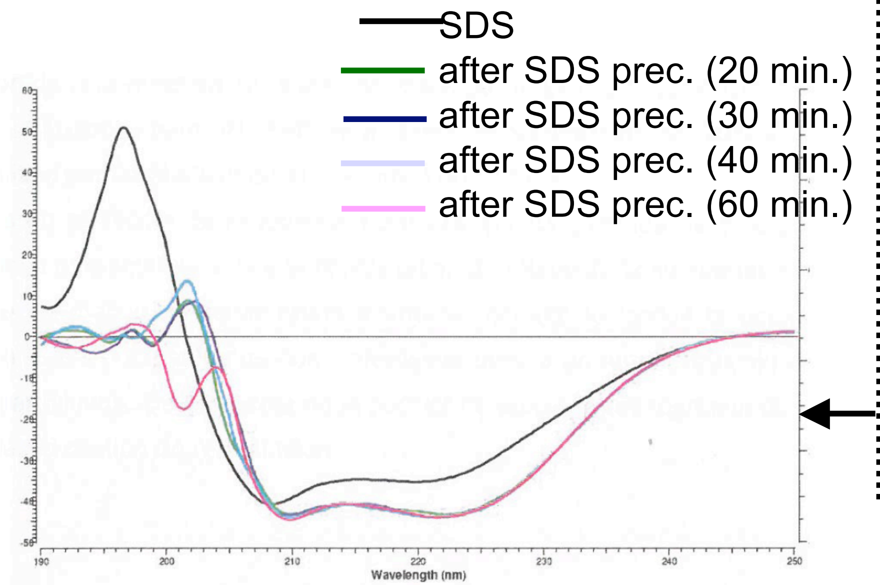
What happens during refolding

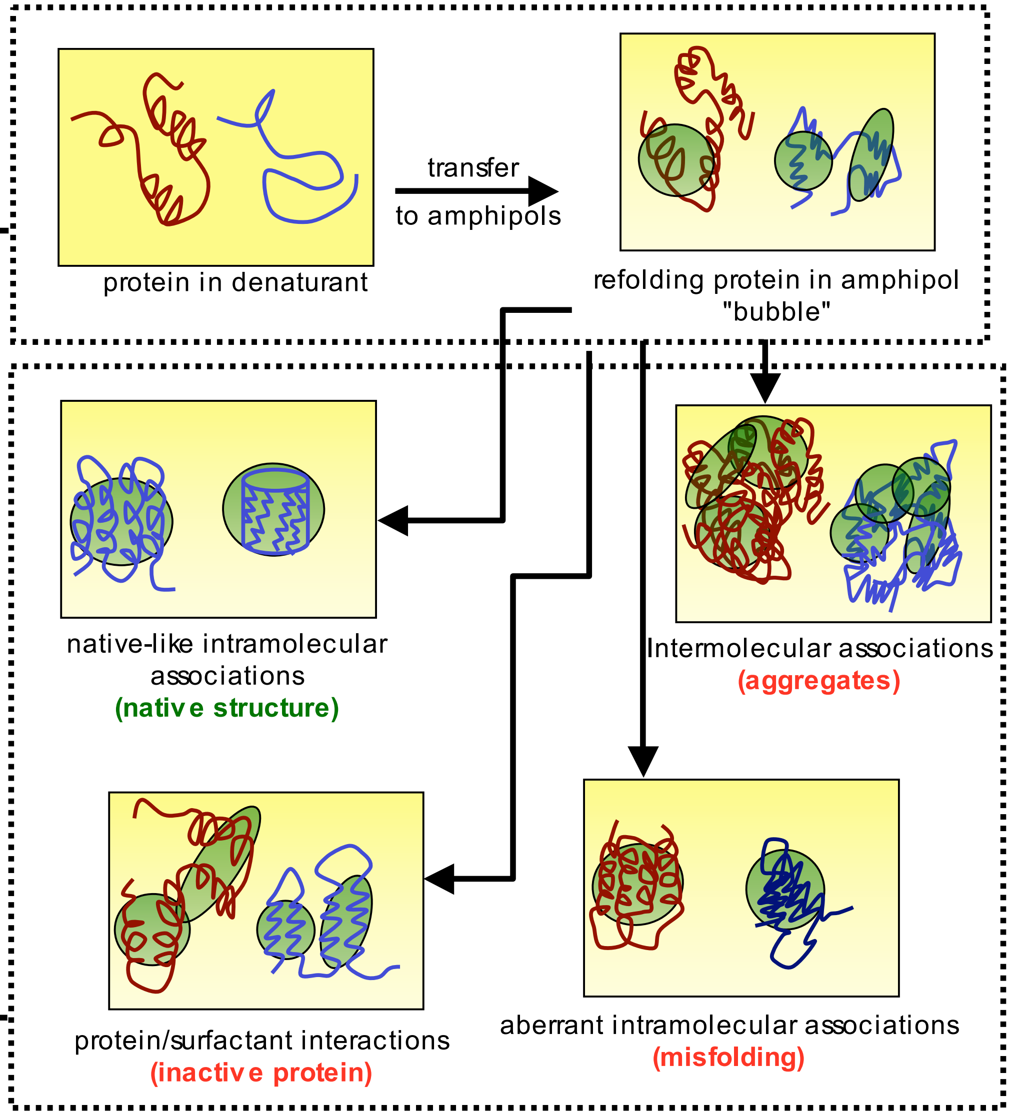


Pocanschi et al. (2006). *Biochemistry* **45**:13954-61

What happens during refolding: bacteriorhodopsin







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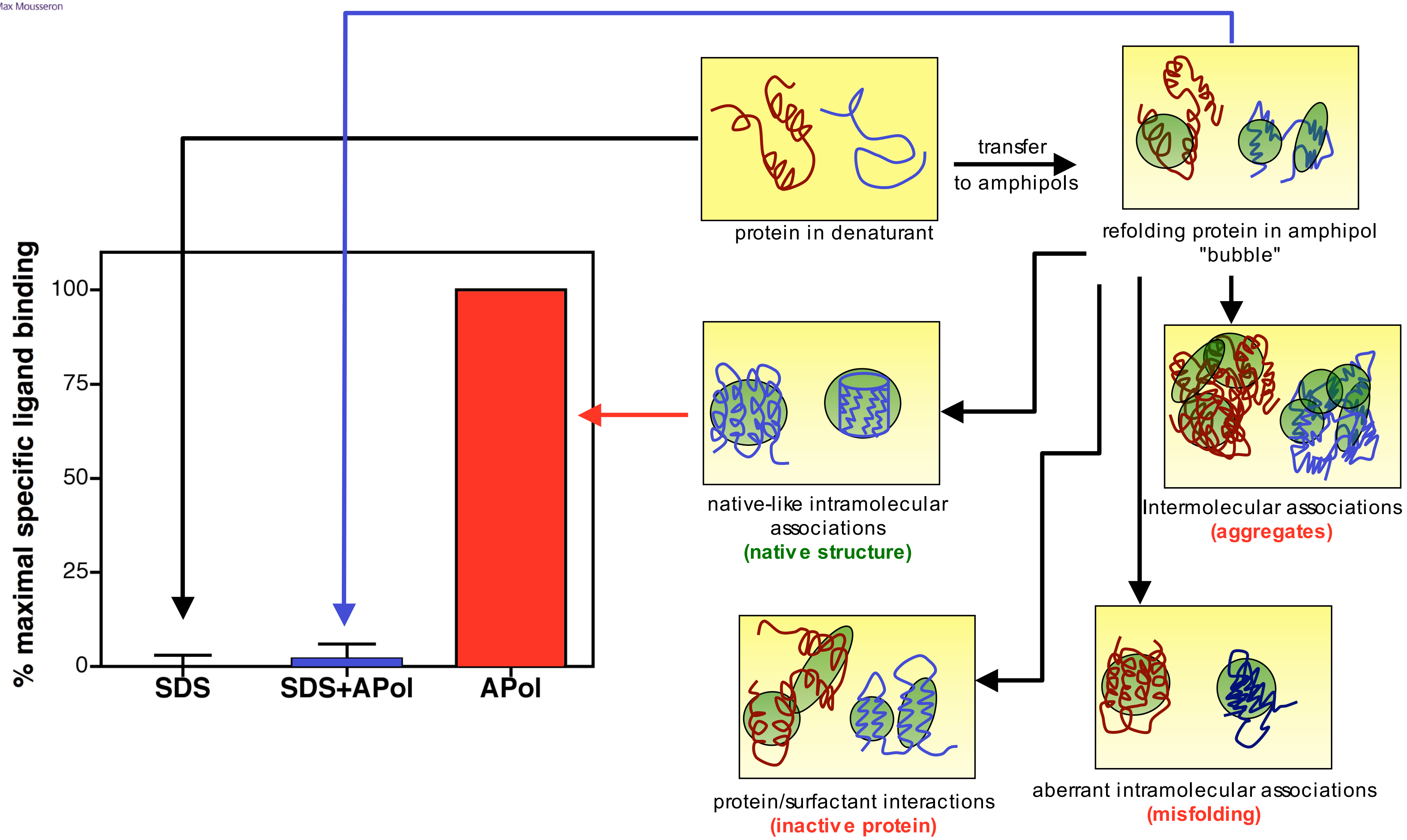
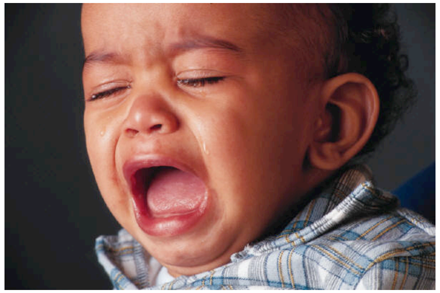
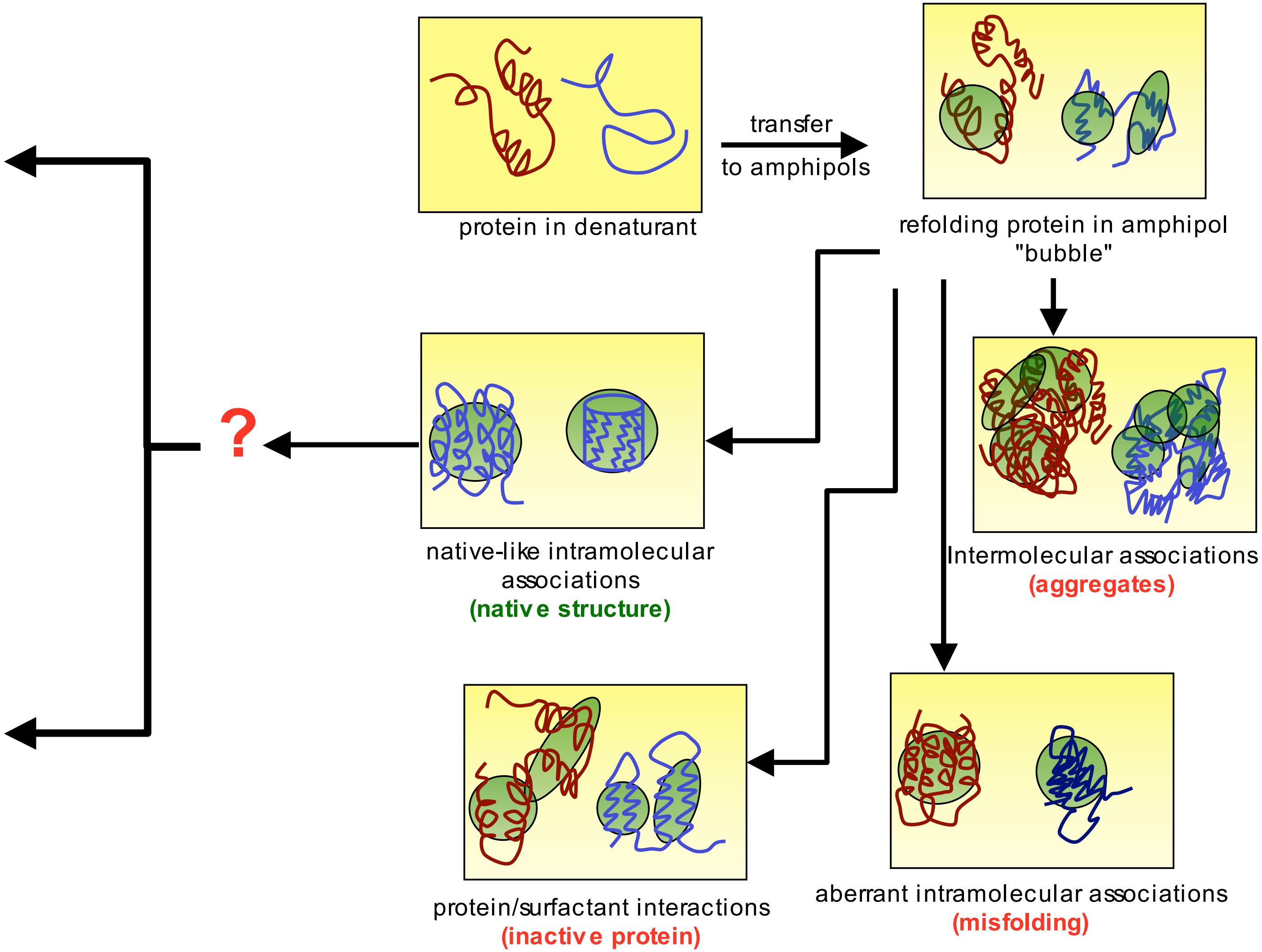
What happens during refolding: GHSR1a

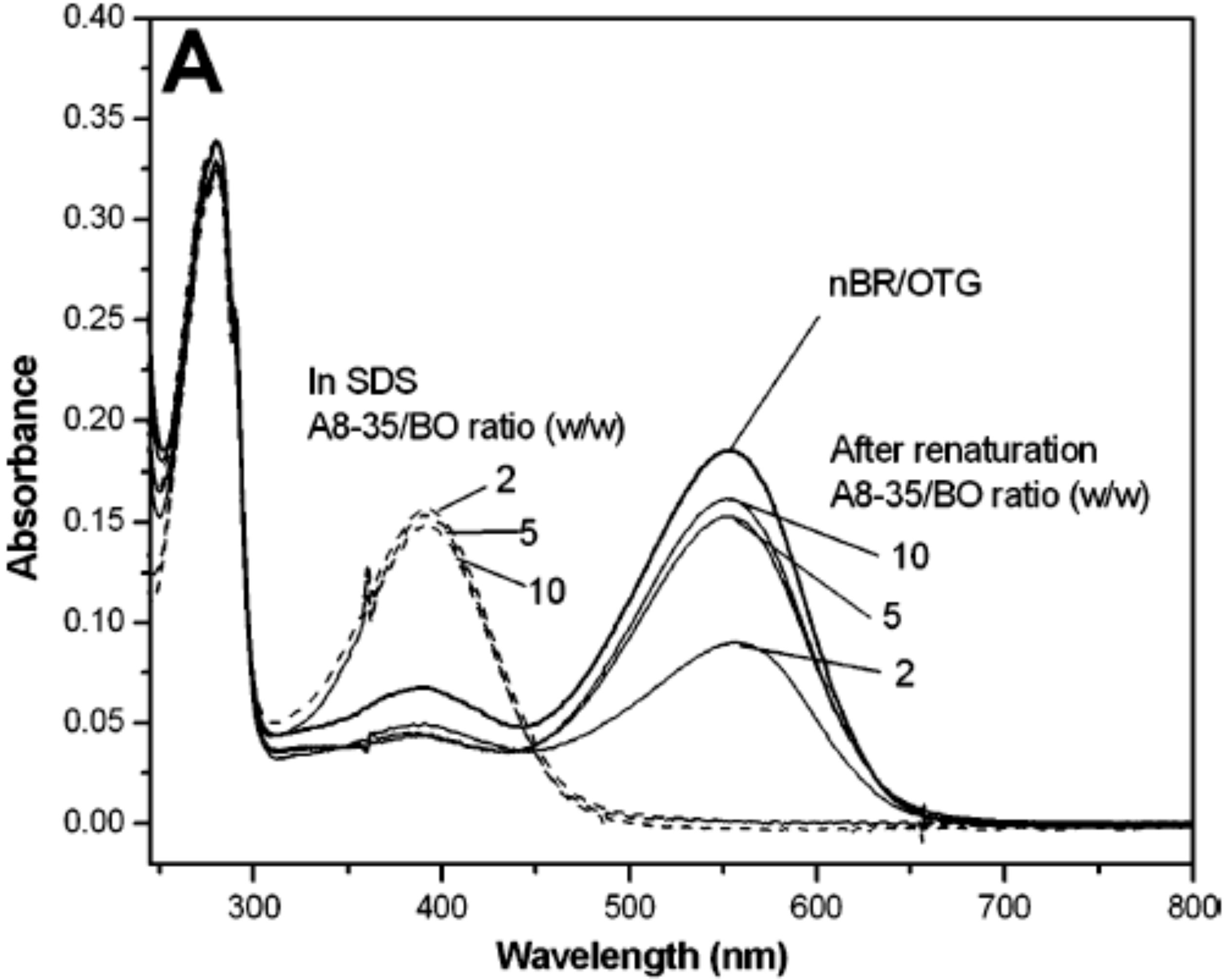


Assessing refolding efficiency

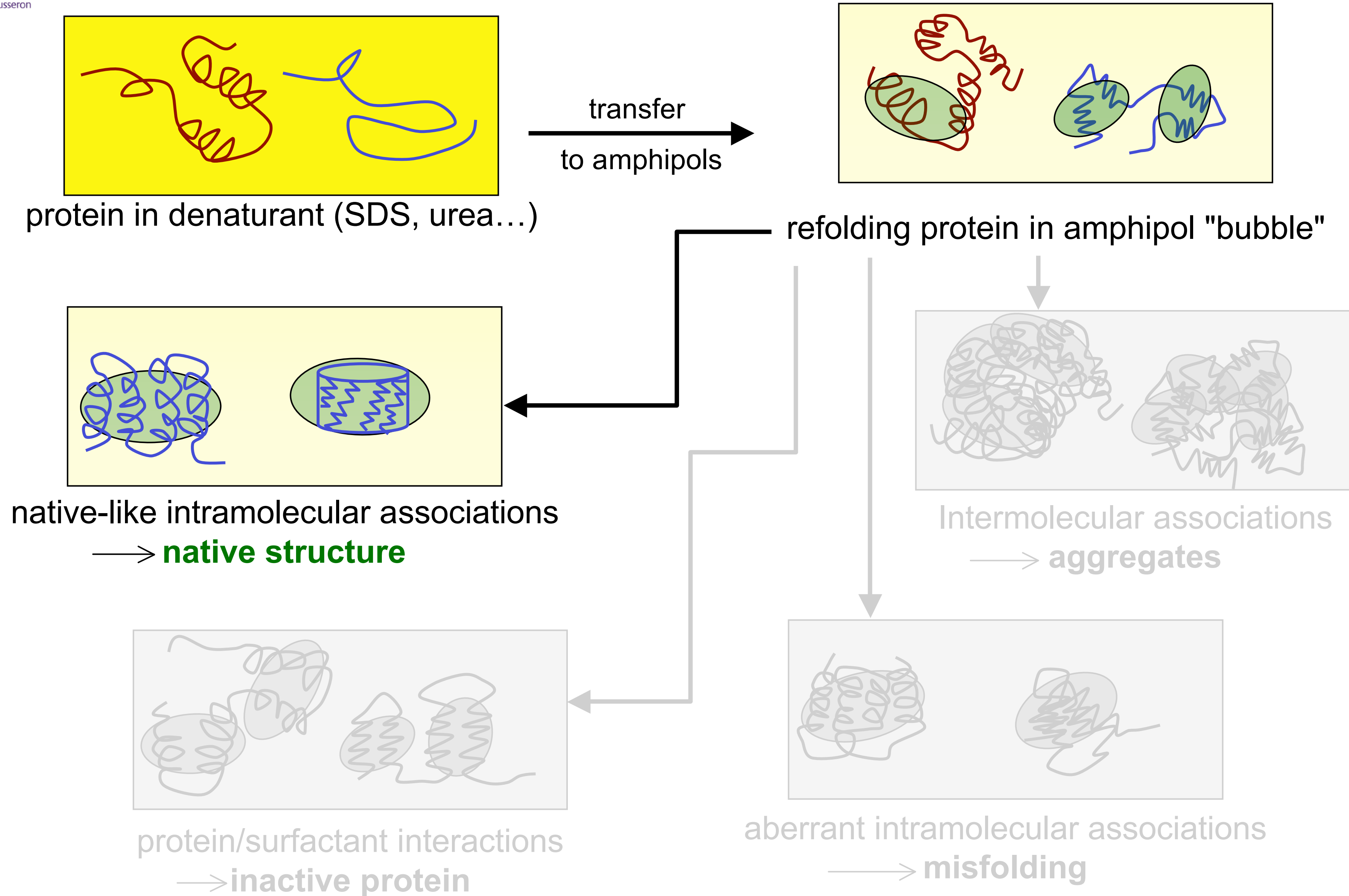




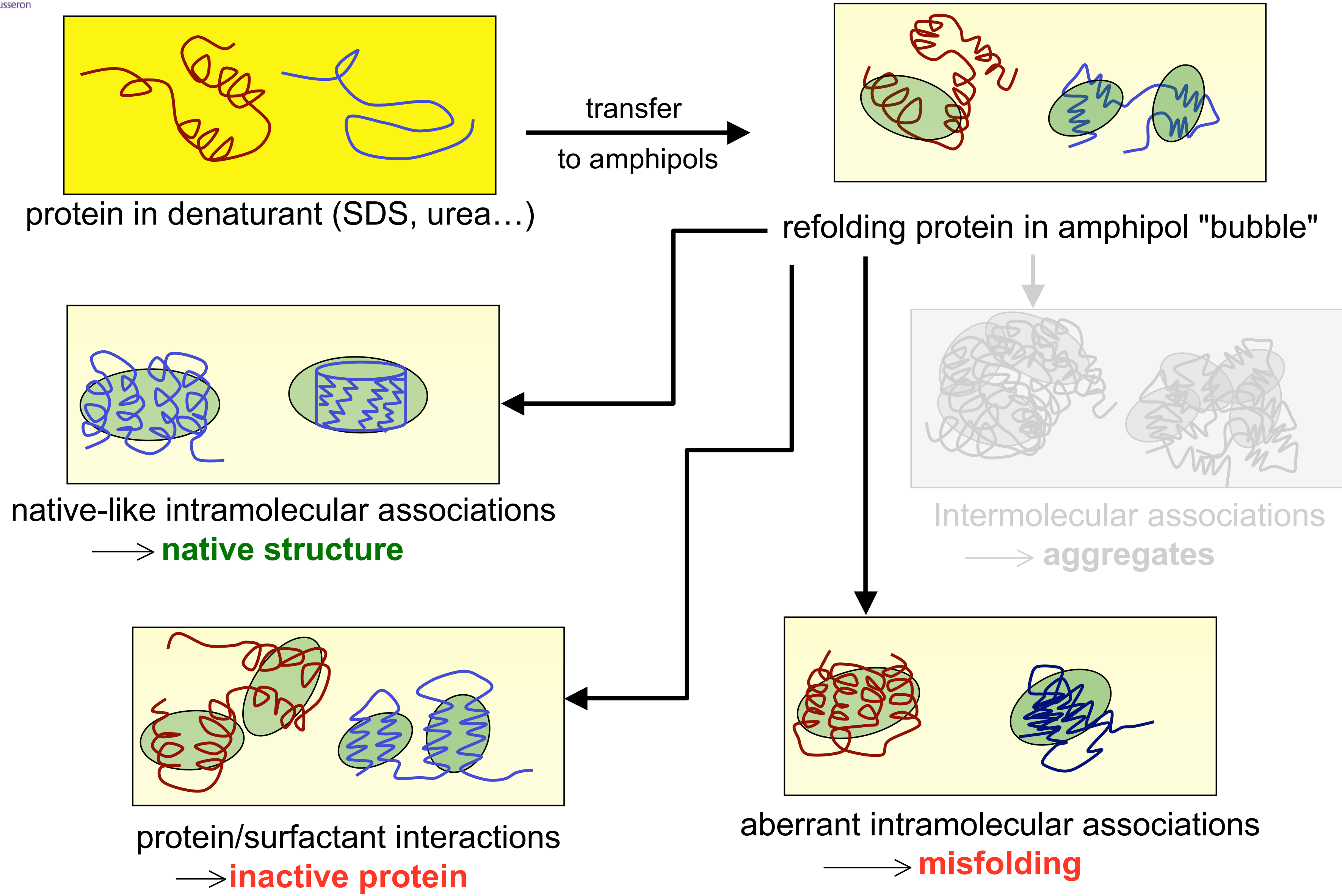
Assessing refolding efficiency



Solubility as an indicator of refolding



Solubility as an indicator of refolding



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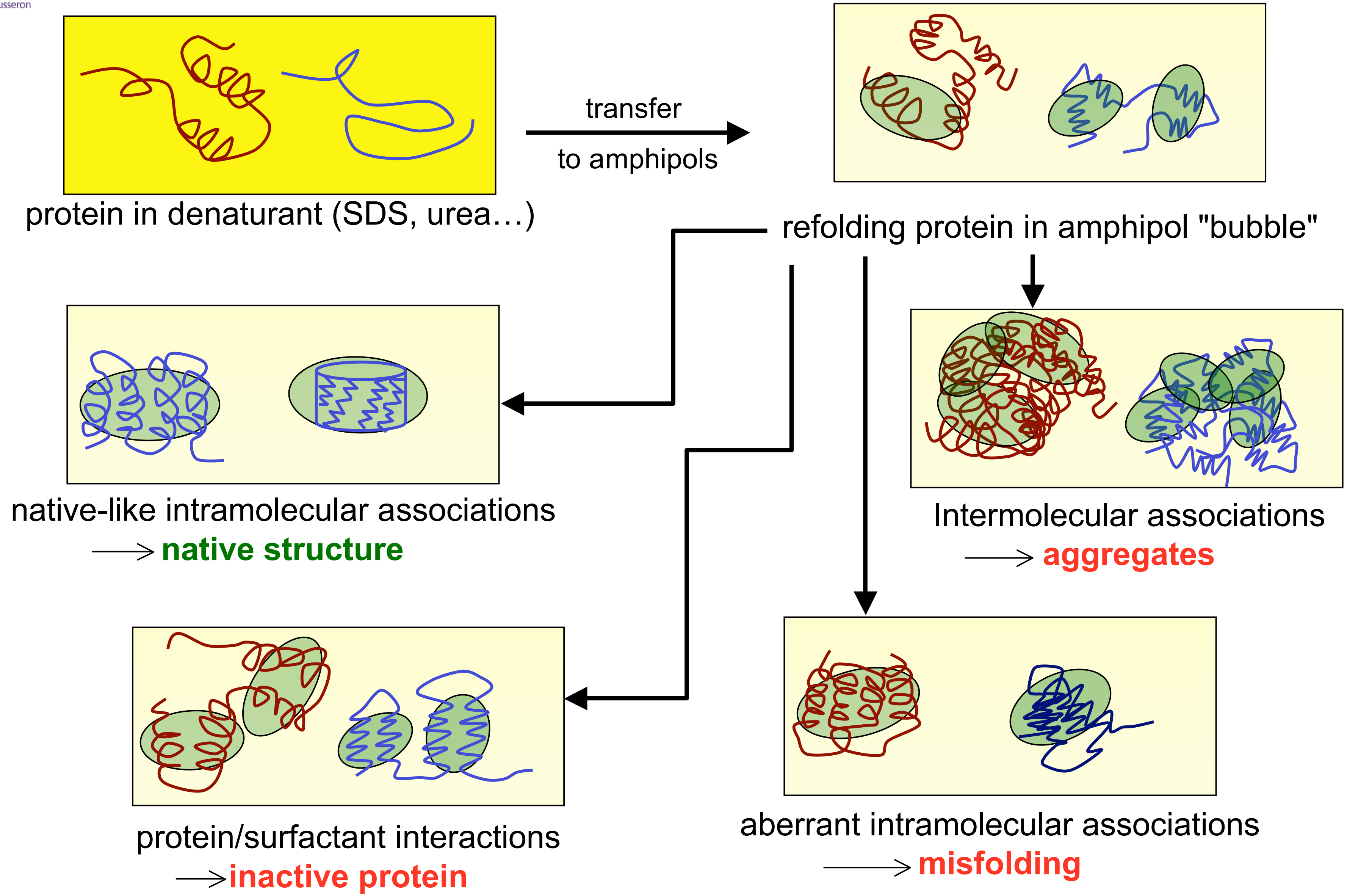
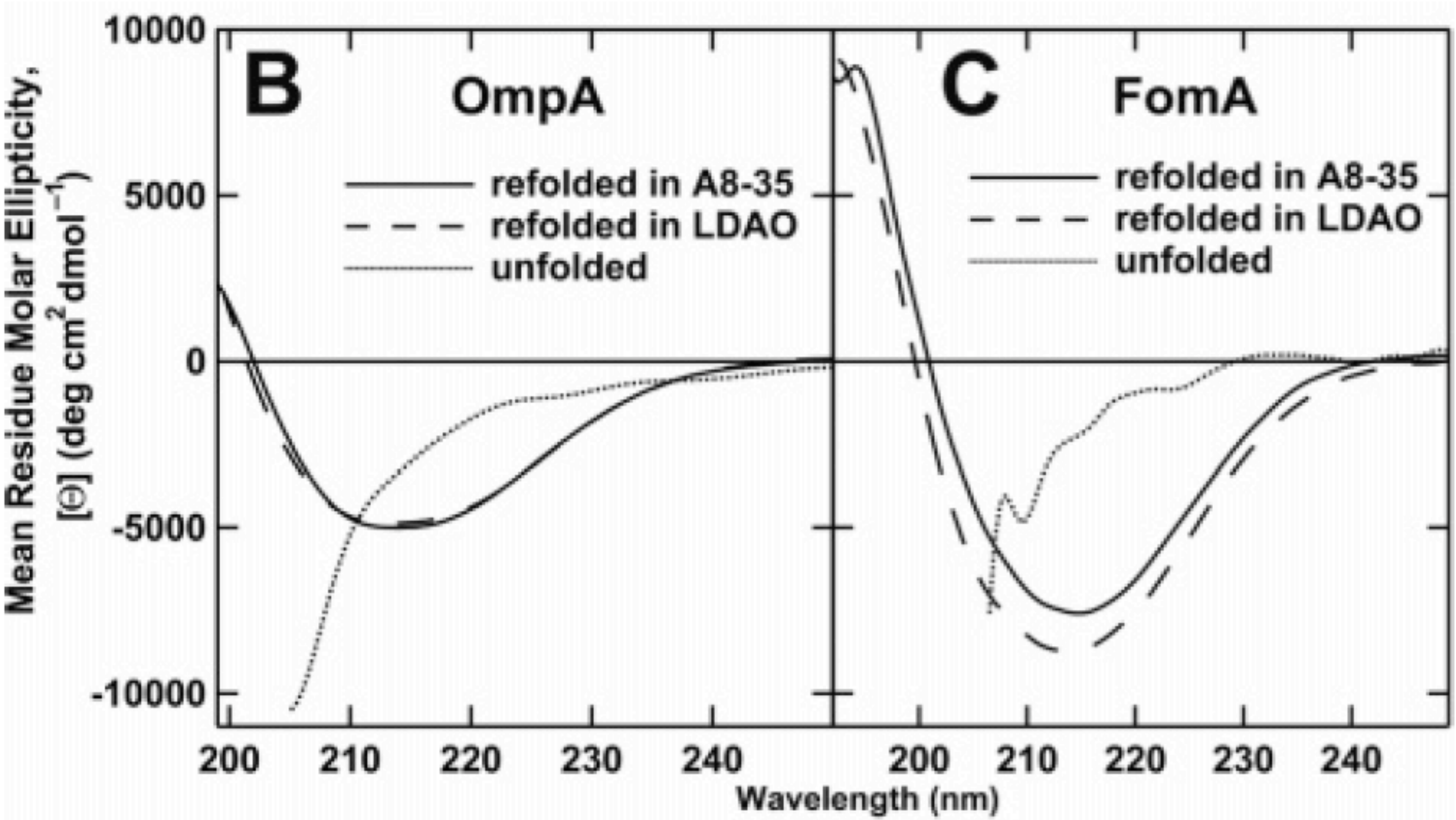
Secondary structure as an indicator of refolding



Amphipol-assisted β-barrel protein refolding



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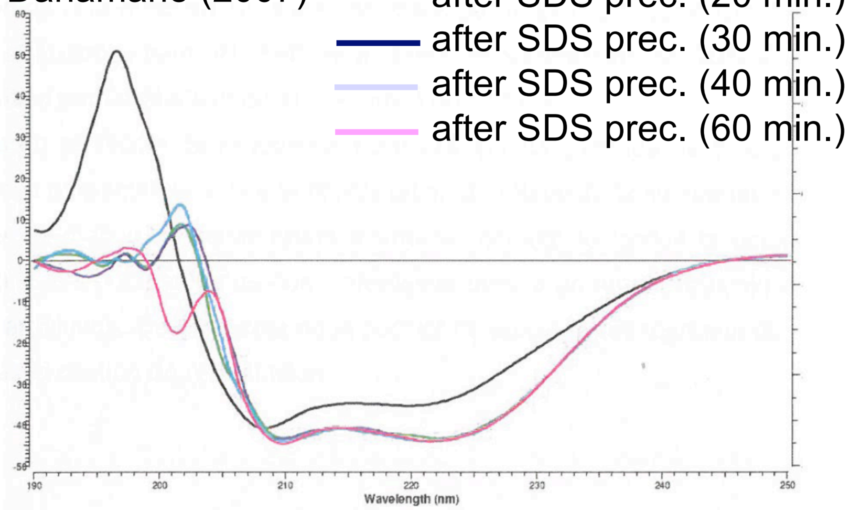
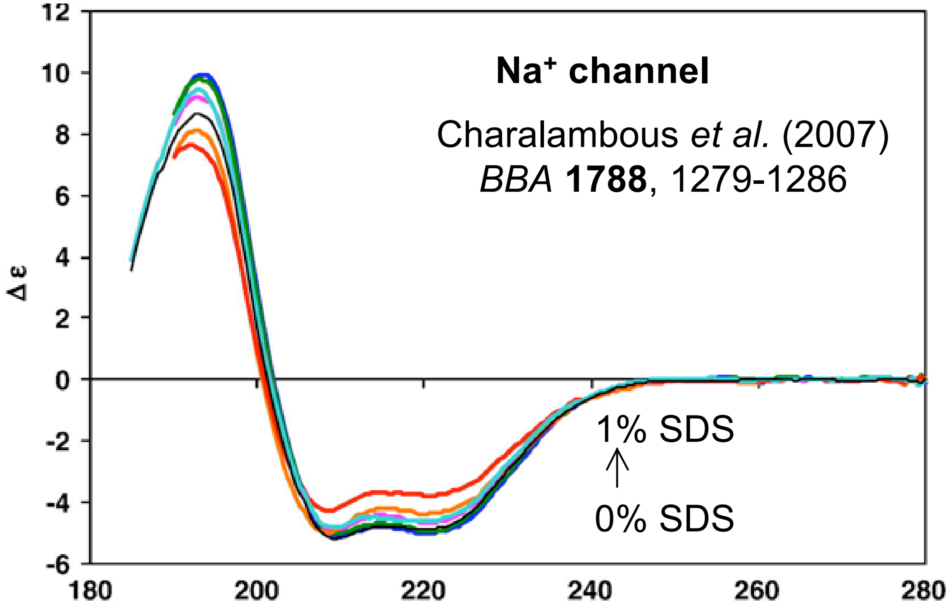
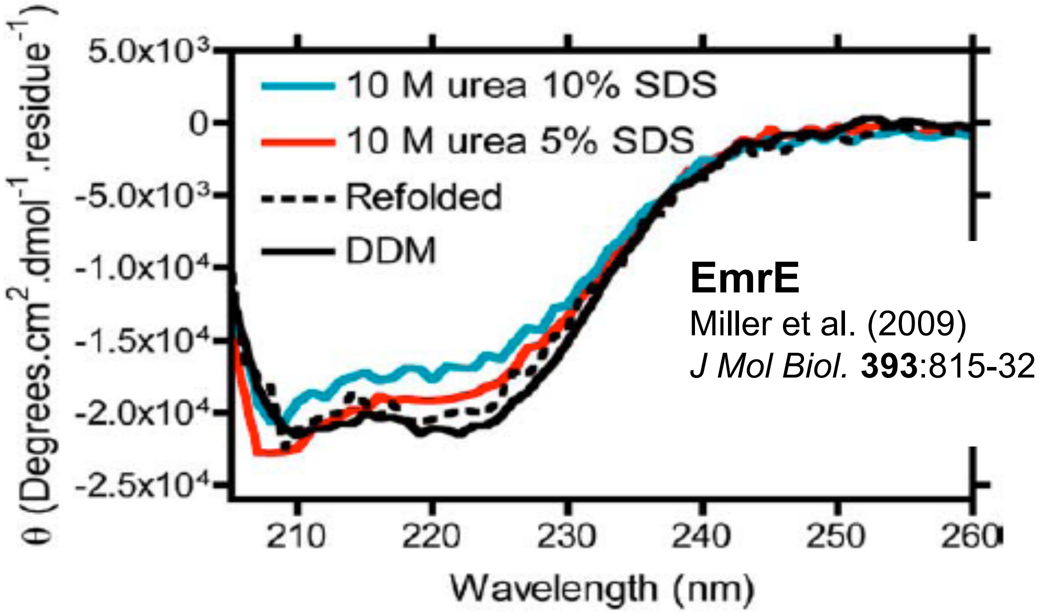
Circular dichroism of folded and "unfolded" membrane proteins

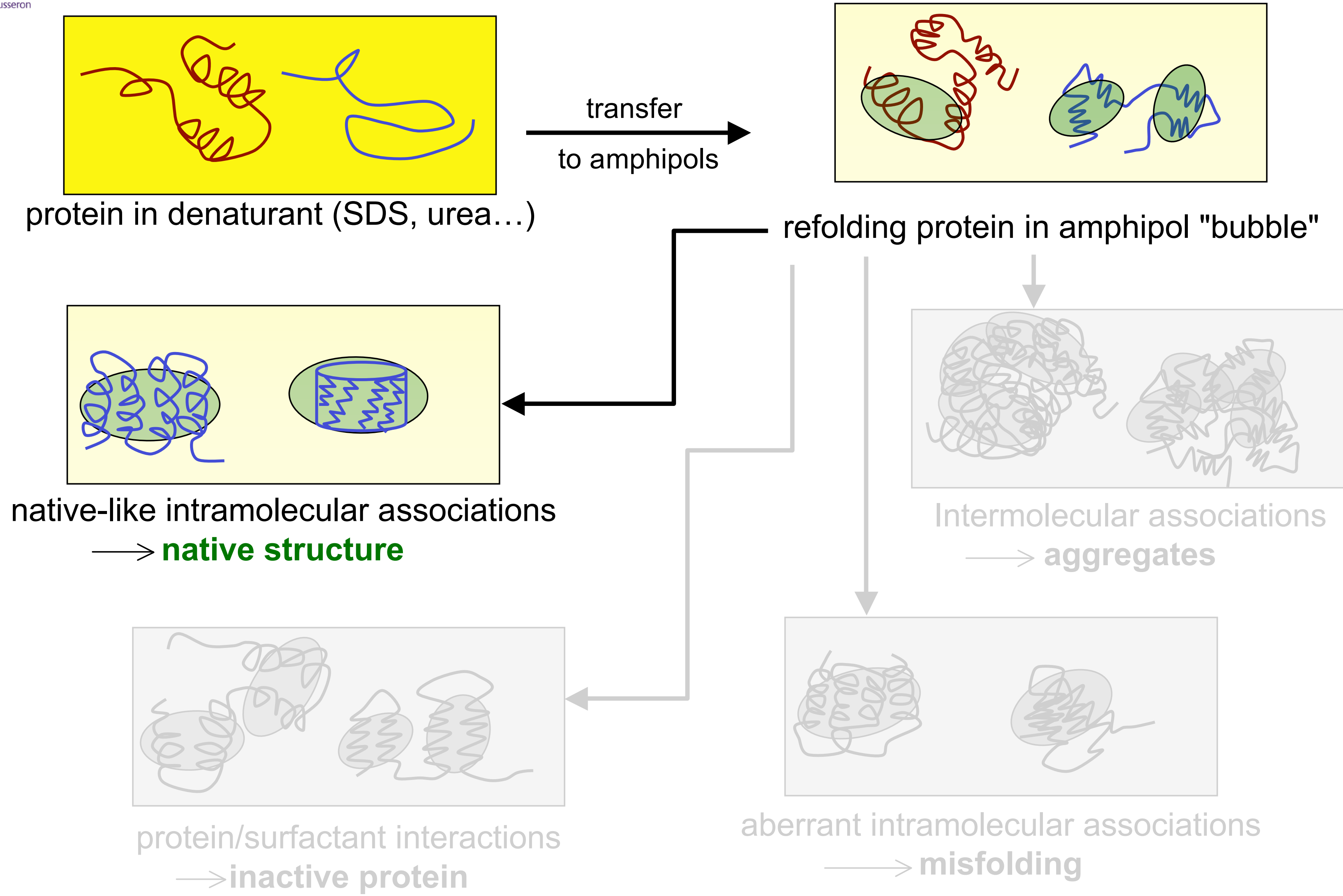
Bacteriorhodopsin
Dahamane (2007)



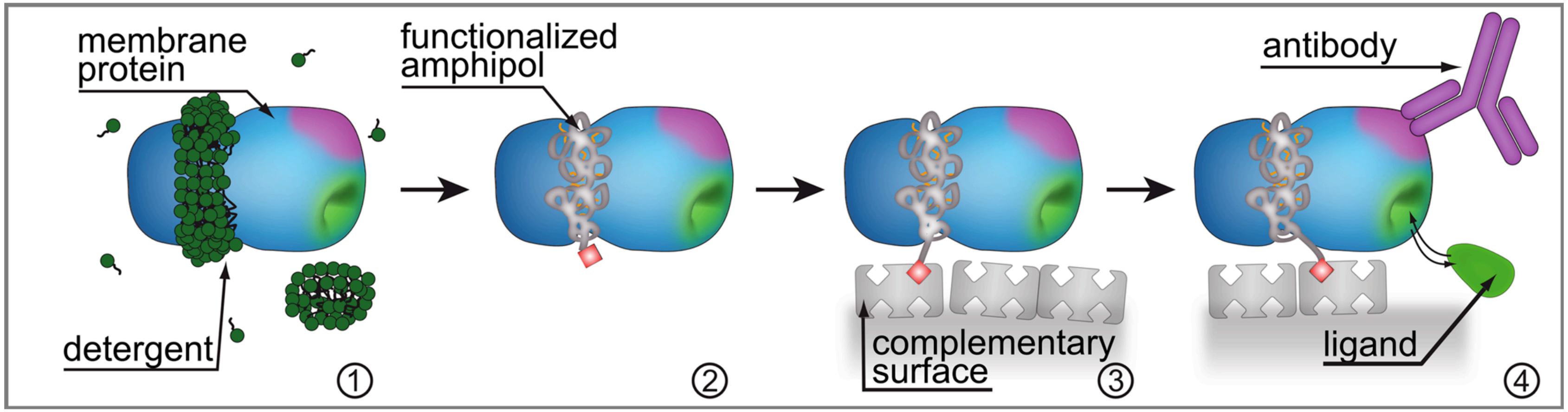




Function as an indicator of refolding



SPR-based functional assays



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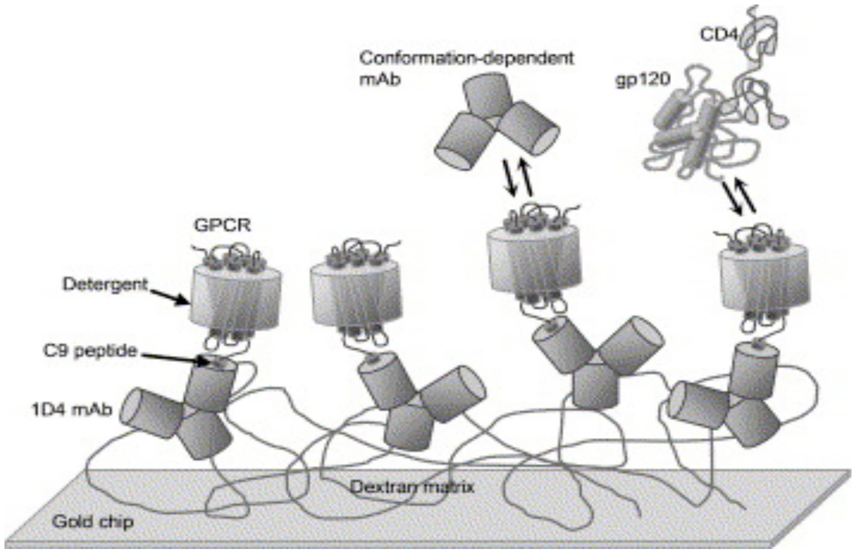

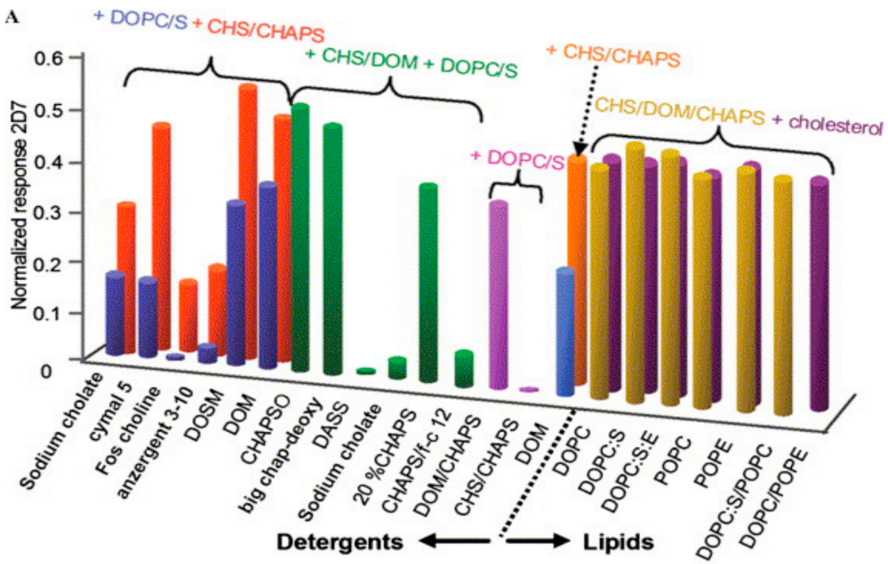

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IBMM (Montpellier)

Marjorie DAMIAN
Jacky MARIE
Sophie MARY
Aimée MARTIN
Jean-Philippe LEYRIS
Jean-Alain FEHRENTZ
Pascal VERDIE

IGF (Montpellier)

Bernard MOUILLAC

IBPC (Paris)

Jean-Luc POPOT
Tassadite DAHMANE
Fabrice GIUSTI
Laurent CATOIRE

Université d'Avignon

Bernard PUCCI
Ange POLIDORI