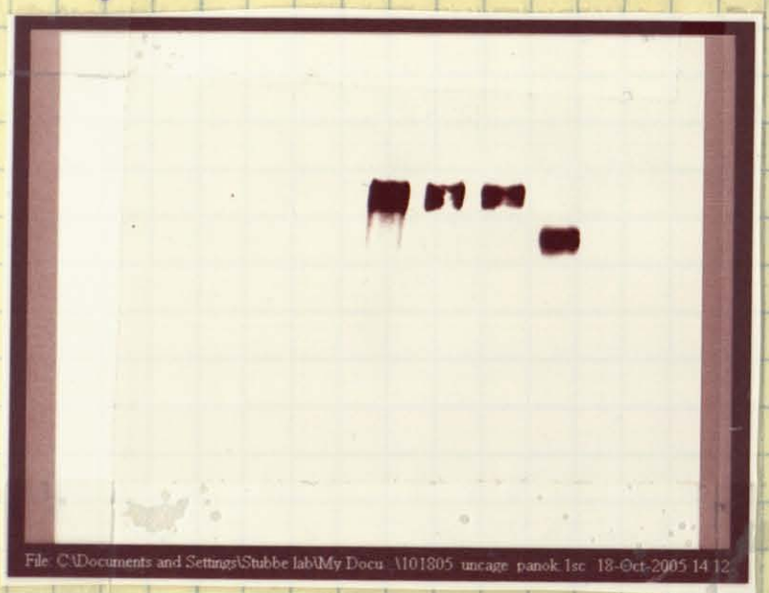
Chemilum. Quantification of cpY31 uncaging

From uncaging on p. 20, a pan and pY31 gel were run. pY31 paxillin (semisynthesized) was used as an internal standard.

1° mouse anti-pan pax (1/1,000)
2° goat anti-mouse HRP (1/10,000)



exposed ≈ 2 min. 4 3 2 1

1° rabbit anti-pY31 pax (1/2,000)
2° goat anti-rabbit HRP (1/20,000)

exposed ≈ 2 min. 4 3 2 1

In 1 6.4 µL benchmark protein ladder
2 6.4 µL semisynthetic cpY31 (see p. 6-19)
3 6.4 µL semisynthetic cpY31 uncaged for 90 s.
4 3 µL of 1/5 semisynthetic pY31

To quantify the density of selected bands:
- zoom in on area w/ bands (zoom icon on top left of screen)
- Under volume select

Calculations of % uncaged based on band density quantification: Oct. 18, 2005

w/ pan 1° ab

		density	st. dev.	density - bckgd	% of pY31 pax (internal standard)
pY31	U3	7711	(362)	5500	1
uncaged	U2	5508	(293)	3297	0.599
caged	U1	5615	(276)	3404	0.619
bckgrnd	B1	2211	(6)	-	-

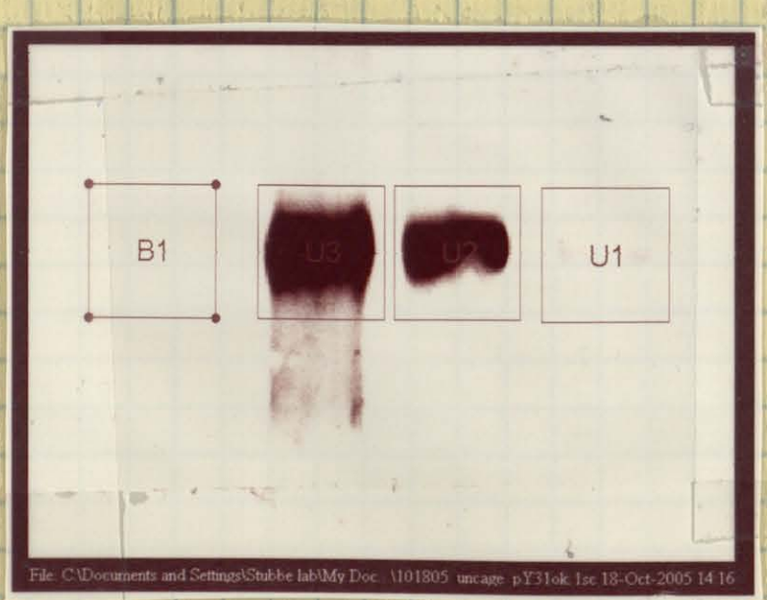
w/ pY31 1° ab

		density	st. dev.	density - bckgd	% of standard
pY31	U3	10650	(637)	8378	1
uncaged	U2	6660	(438)	4388	0.524
caged	U1	2609	(50)	337	0.040
bckgd	B1	2272	(9)		

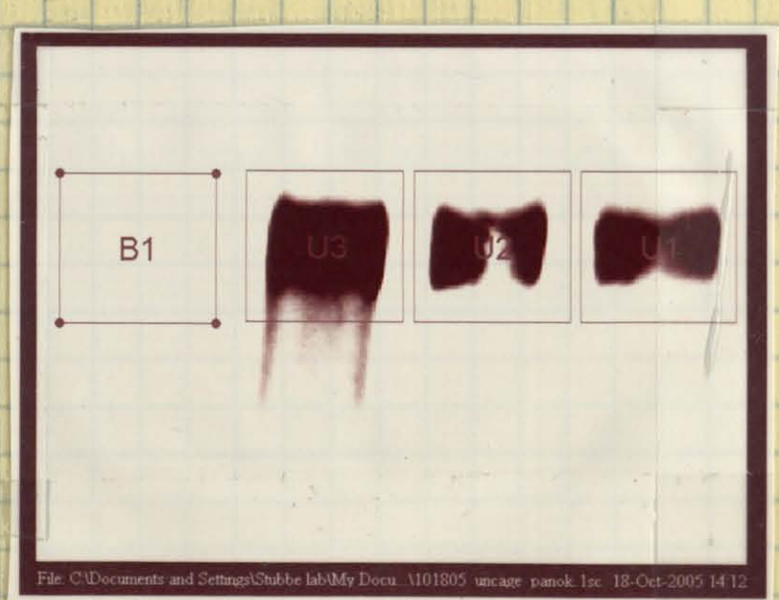
caged: % uncaged = 0.040 / 0.619 = 0.06499 w/ st. dev. ≈ 6.5 ± 0.2 %

90 s. uncaged: % uncaged = 0.524 / 0.599 = 0.8737 w/ st. dev. = 87 ± 4 %

To propagate the standard deviation from each measurement, the % of standard was measured for each band (w/ both pan and pY31 ab) using the density for + and - the st. dev. (see excel spreadsheet for details).



pY31 1° ab



pan pax 1° ab