Supporting Material

accompanying "Phosphorylation Increases Persistence Length and End-to-End Distance of a Segment of Tau Protein". Alexander F. Chin[1], Dmitri Toptygin[1], W. Austin Elam[2], Travis P. Schrank[3], Vincent J. Hilser[1,4]. ([1]Department of Biology, Johns Hopkins University, 3400 North Charles Street, Baltimore, MD 21218. USA; [2]Department of Molecular Biophysics and Biochemistry, Yale University, 260 Whitney Avenue, New Haven, CT, 06511. USA.; [3]Department of Otolaryngology, Head and Neck Surgery, Medical University of South Carolina, 135 Rutledge Avenue, Charleston, SC 29425. USA.; [4]T.C. Jenkins Department of Biophysics, Johns Hopkins University, 3400 North Charles Street, Baltimore, MD 21218. USA.)

Appendix A: Calculation of the freely rotating unit diameter.

The expected steady state anisotropy, r_{ss}, of a fluorophore attached to the model peptides can be estimated by the equation

$$r_{ss} = \frac{r_0}{1+(\tau/\phi)}$$

where r_0 is the fundamental anisotropy, r_{ss} is the measured anisotropy, τ is the fluorescence lifetime, and ϕ is the rotational correlation time. We take $\tau = 2.252$ nanoseconds (1). The rotational correlation time is related to the effective spherical volume of the freely rotating unit, *V*, by the equation

$$\phi = \frac{\eta V}{k_B T}$$

Where η is the dynamic viscosity of the solution, T the temperature, and k_B the Boltzmann constant. We take r_0 = 0.3 at 300nm, η = 1.002×10^{-3} Pa·s, T = 293.15 K, and k_B = 1.381×10^{-23} J/K (2, 3). Rearranging and substituting,

$$V = \frac{k_B T \tau r_{ss}}{\eta(r_0 - r_{ss})}$$

The corresponding diameter, D_{sphere}, of the spherical freely rotating unit is therefore

$$D_{sphere} = 2\left(\frac{3V}{4\pi}\right)^{1/3}$$

A segment of the model peptides of length equal to the persistence length may be simplistically modeled as a rod of length D_{sphere} occupying a spherical volume *V*, flexibly attached to the remainder of the peptide beyond the persistence length. Thus the rough agreement of the rotating unit diameter, D_{sphere}, determined by steady state anisotropy with the persistence lengths determined by time-correlated single photon counting serves as independent validation of the latter, novel technique. The agreement and absolute value of D_{sphere} additionally suggests that the tryptophan fluorophores are unimpeded in their rotation, consistent with monomeric peptides (Supplemental Table 3, Supplemental Table 5).

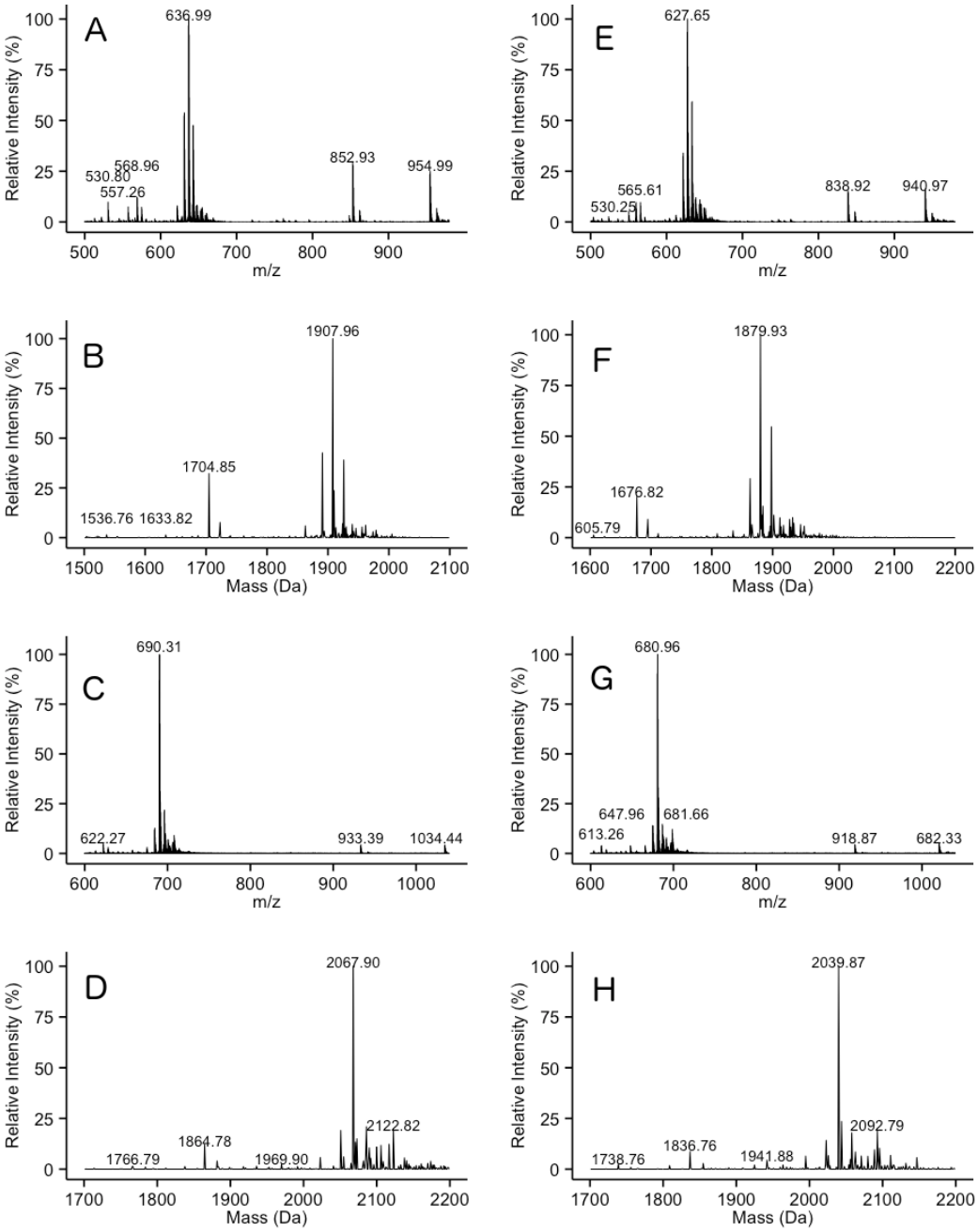

Figure S1. ESI Q-ToF mass spectrometry of CPM-labeled peptides used in this study. Peptide CPM-CAKTPPAPKTPPAW (A) mass spectrum, (B) maximum entropy deconvolution. Peptide CPM-CAK(pT)PPAPK(pT)PPAW (C) mass spectrum, (D) maximum entropy deconvolution. Peptide CPM-CAKSPPAPKSPPAW (E) mass spectrum, (F) maximum entropy deconvolution. Peptide CPM-CAK(pS)PPAPK(pS)PPAW (G) mass spectrum, (H) maximum entropy deconvolution.

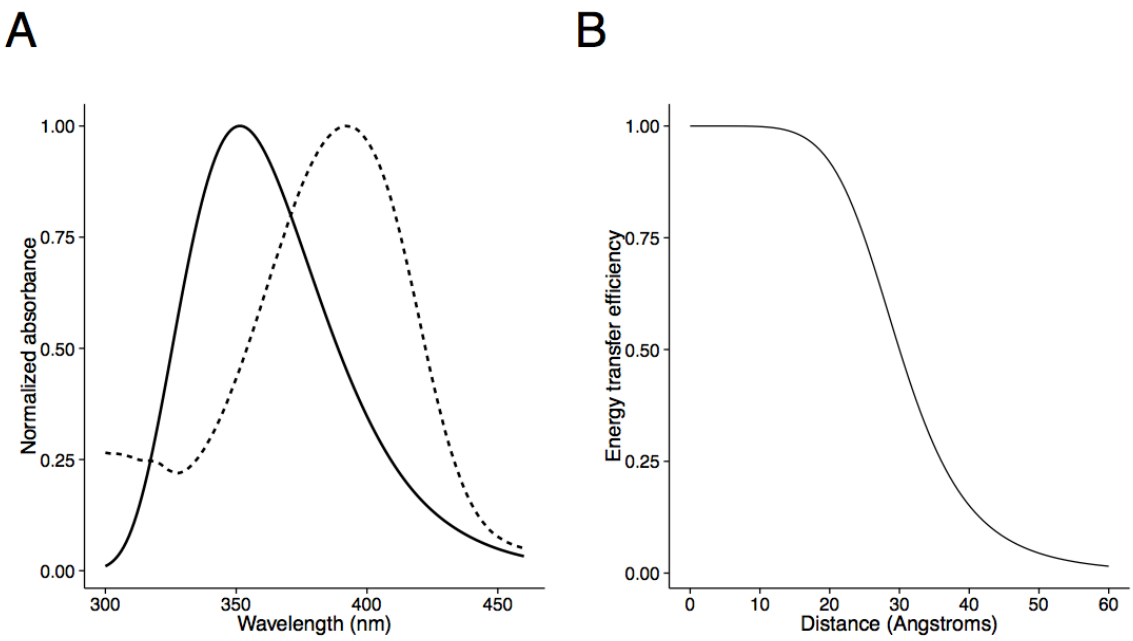

Figure S2. The modified Forster radius of the Trp-CPM FRET pair was calculated to be R_1 = 45.86. Assuming a donor quantum yield of 0.13 and κ^2 = 2/3, the Forster distance R_0 = 30.51 Å was calculated. (A) Normalized emission spectrum of Trp (solid) and absorption spectrum of Arg-(CPM)Cys-Arg (dashed) in peptide buffer, which were used to calculated R_1. (B) Distance dependence of calculated energy transfer efficiency with a Forster distance of 30.66 Å, assuming monoexponential donor decay and no translational diffusion.

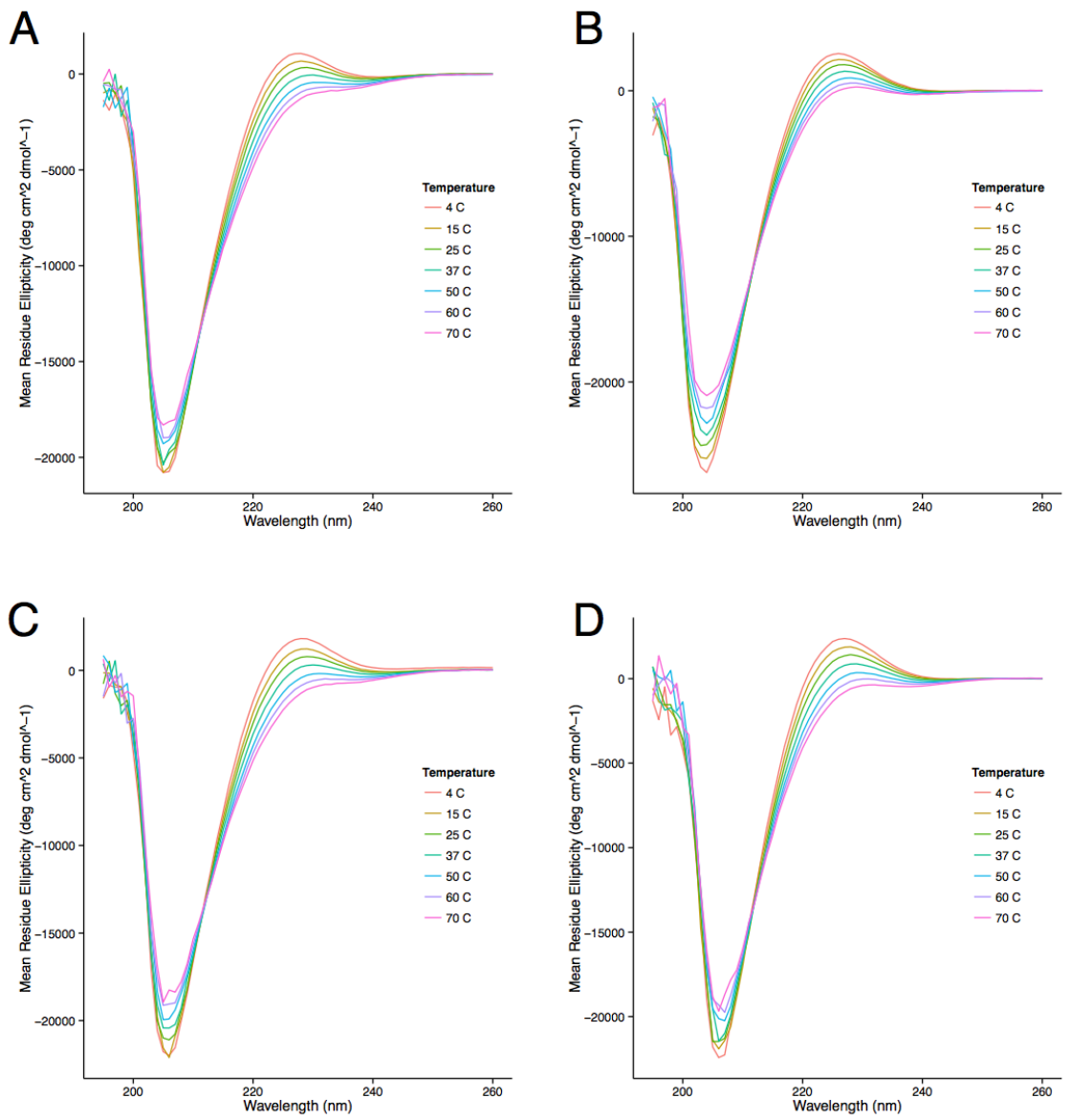

Figure S3. Temperature series circular dichroism spectra of the test peptides containing sequence corresponding to residues tau 173 to 183. (A) Peptide containing AKTPPAPKTPP. (B) Peptide containing AK(pT)PPAPK(pT)PP. (C) Peptide containing AKSPPAPKSPP. (D) Peptide containing AK(pS)PPAPK(pS)PP.

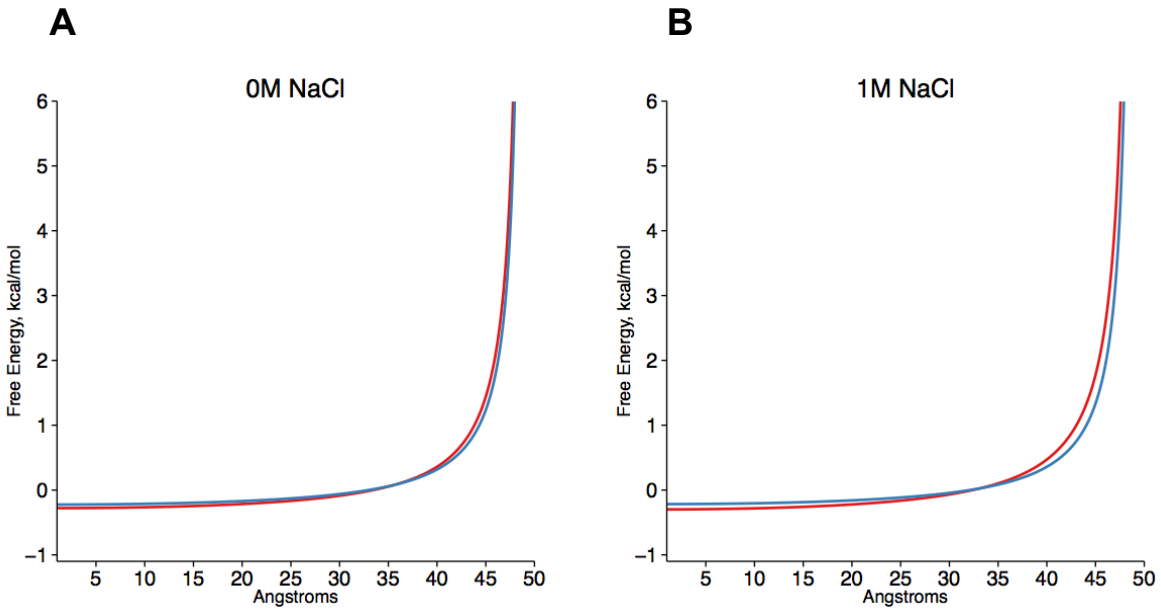

Figure S4. Free energy change upon phosphorylation. Blue = threonine containing peptides. Red = serine containing peptides. (A) Measured in 0M NaCl. (B) Measured in 1M NaCl.

	Unlabeled peptide molecular weight (Da)	Labeled Peptide expected mol. weight (Da)	Observed molecular weight (Da)
CAKTPPAPKTPPAW	1505.81	1908.26	1907.96
CAK(pT)PPAPK(pT)PPAW	1665.81	2068.26	2067.90
CAKSPPAPKSPPAW	1477.75	1880.20	1879.93
CAK(pS)PPAPK(pS)PPAW	1637.75	2040.20	2039.87

Table S1. The CPM labeling reaction performed on the peptides used in this study was confirmed using electrospray mass spectrometry. All peptides are N-terminal acetylated and C-terminal amidated. Conjugation of the CPM-maleimide label to a peptide is expected to add 402.45 Da. Reported observed molecular weights were obtained by maximum entropy deconvolution of spectra after lock mass correction.

Change	$\langle v^3 \rangle_{fcf}$	$f^2 \cdot n$	Γ	J	n^{-4}	R_1^6	$\Gamma^{1/6} \cdot R_1$
Tau-pT to Tau-pS	-2.98%	0.00%	-2.98%	4.30%	0.00%	4.30%	0.20%
Tau-T to Tau-pT	-1.41%	0.00%	-1.41%	2.12%	0.00%	2.12%	0.11%
Tau-T, 0 to 1M NaCl	0.01%	1.27%	+1.28%	2.10%	-2.65%	-0.61%	0.11%
Tau-pT, 0 to 1M NaCl	-0.04%	1.27%	+1.23%	2.46%	-2.65%	-0.25%	0.16%
Tau-pS, 0 to 1M NaCl	0.14%	1.27%	+1.41%	2.66%	-2.65%	-0.06%	0.22%

Table S2. Theoretically estimated relative changes in Γ, R_1 , and $\Gamma^{1/6} \cdot R_1$ upon phosphorylation, residue substitution, and addition of NaCl to the solvent. The relative systematic error in the persistence length, mean end-to-end distance, and contour length equals the change in the product $\Gamma^{1/6} \cdot R_1$. Γ is directly proportional to $\langle v^3 \rangle_{fcf} \cdot f^2 \cdot n$, where $\langle v^3 \rangle_{fcf}$ is the mean cube of the emission wavenumber averaged over the Franck-Condon factor envelope, $f = (3n^2)/(2n^2+1)$, is the local field correction factor for the empty spherical cavity model, and n is the refractive index of the solvent (4). The factor R_1^6 is directly proportional to $J \cdot n^{-4}$ (1). The refractive index of water at T = 293K, 0M and 1M NaCl, was taken from Aly and Esmail (5).

Peptide	Excitation (nm)	Emission (nm)	Steady State Fl. Anisotropy	Rotating unit diameter (Angstroms)
CAKTPPAPKTPPAW	300	355	0.033	12.863
CAK(pT)PPAPK(pT)PPAW	300	355	0.022	11.120
CAKSPPAPKSPPAW	300	355	0.021	10.962
CAK(pS)PPAPK(pS)PPAW	300	355	0.035	13.233
(CPM)-CAKTPPAPKTPPAW	300	355	0.050	15.145
(CPM)-CAK(pT)PPAPK(pT)PPAW	300	355	0.039	13.790
(CPM)-CAKSPPAPKSPPAW	300	355	0.042	14.100
(CPM)-CAK(pS)PPAPK(pS)PPAW	300	355	0.043	14.238
(CPM)-CAKTPPAPKTPPAW	387	484	0.122	-
(CPM)-CAK(pT)PPAPK(pT)PPAW	387	484	0.122	-
(CPM)-CAKSPPAPKSPPAW	387	484	0.124	-
(CPM)-CAK(pS)PPAPK(pS)PPAW	387	484	0.124	-

Table S3. Fluorescence anisotropy of model peptides used in time correlated single photon counting FRET experiments, containing sequence corresponding to residues numbered tau 173 to 183. Steady state fluorescence anisotropy was measured at both the FRET donor, tryptophan, and FRET acceptor, CPM. Rotating unit diameters for tryptophans were calculated as in Appendix A.

Peptide	Salt (mol/L NaCl)	Scattering ratio (2 hours / 0 hours)
CAKTPPAPKTPPAW	0	0.987
CAKTPPAPKTPPAW	1	0.953
CAK(pT)PPAPK(pT)PPAW	0	1.029
CAK(pT)PPAPK(pT)PPAW	1	0.935
CAKSPPAPKSPPAW	0	1.083
CAKSPPAPKSPPAW	1	1.031
CAK(pS)PPAPK(pS)PPAW	0	0.979
CAK(pS)PPAPK(pS)PPAW	1	0.887
(CPM)-CAKTPPAPKTPPAW	0	0.998
(CPM)-CAKTPPAPKTPPAW	1	0.992
(CPM)-CAK(pT)PPAPK(pT)PPAW	0	1.029
(CPM)-CAK(pT)PPAPK(pT)PPAW	1	0.935
(CPM)-CAKSPPAPKSPPAW	0	1.110
(CPM)-CAKSPPAPKSPPAW	1	1.027
(CPM)-CAK(pS)PPAPK(pS)PPAW	0	0.973
(CPM)-CAK(pS)PPAPK(pS)PPAW	1	1.082

Table S4. Steady state fluorescence emission spectra were collected from freshly prepared model peptides both immediately after preparation and two hours later (approximately the duration of a time-correlated single photon counting experiment) after storage at room temperature. The ratio of the scattering peak intensities observed at the excitation wavelength was calculated, expected to be equal to 1 if there was no change in the oligomeric state of the peptide, expected to be equal to 2 if the peptides dimerized. No dimerization process was observed, independent of time or salt concentration.

Peptide	Salt (mol/L NaCl)	p (Å)	p 95% CI (Å)	Mean E2ED (Å)	Mean E2ED 95% CI (Å)	Reduced Chi Square
(CPM)-CAKTPPAPKTPPAW	0	12.487	0.075	29.445	0.065	1.631
(CPM)-CAK(pT)PPAPK(pT)PPAW	0	15.349	0.054	31.689	0.038	1.141
(CPM)-CAKSPPAPKSPPAW	0	12.180	0.053	29.173	0.047	1.439
(CPM)-CAK(pS)PPAPK(pS)PPAW	0	14.401	0.046	31.000	0.035	1.629
(CPM)-CAKTPPAPKTPPAW	1	11.239	0.234	28.293	0.228	1.142
(CPM)-CAK(pT)PPAPK(pT)PPAW	1	14.089	0.523	30.760	0.403	1.394
(CPM)-CAKSPPAPKSPPAW	1	11.297	0.205	28.350	0.198	1.431
(CPM)-CAK(pS)PPAPK(pS)PPAW	1	13.324	0.218	30.154	0.179	1.087

Table S5. Numerical values of the experimentally determined persistence lengths (p), mean end-to-end distance (E2ED), their symmetric 95% confidence intervals (95% CI), and the model fit reduced chi square parameters of model peptides used in time correlated single photon counting FRET experiments, containing sequence corresponding to residues numbered tau 173 to 183.

Supporting References

1. Toptygin, D., A.F. Chin, and V.J. Hilser. 2015. Effect of diffusion on resonance energy transfer rate distributions: Implications for distance measurements. The Journal of Physical Chemistry B. 119: 12603-12622.

2. Valeur, B., and G. Weber. 1977. Resolution of the Fluorescence Excitation Spectrum of Indole Into the 1La and 1Lb Excitation Bands. Photochemistry and Photobiology. 25: 441-444.

3. Kestin, J., Moredechai, S., and W.A. Wakeham. 1978. Viscosity of Liquid Water in the Range -8C to 150C. Journal of Physical and Chemical Reference Data. 7: 941-948.

4. Toptygin, D. 2003. Effects of the Solvent Refractive Index and Its Dispersion on the Radiative Decay Rate and Extinction Coefficient of a Fluorescent Solute. Journal of Fluorescence. 13: 201-219.

5. Aly, K. M., and E. Esmail. 1993. Refractive index of salt water: effect of temperature. Optical Materials. 2: 195-199.