Enzymatic activity of lipases correlates with polarity-induced conformational changes: a Trp-induced quenching (TrIQ) fluorescence study

***Lid-sequence alignment***

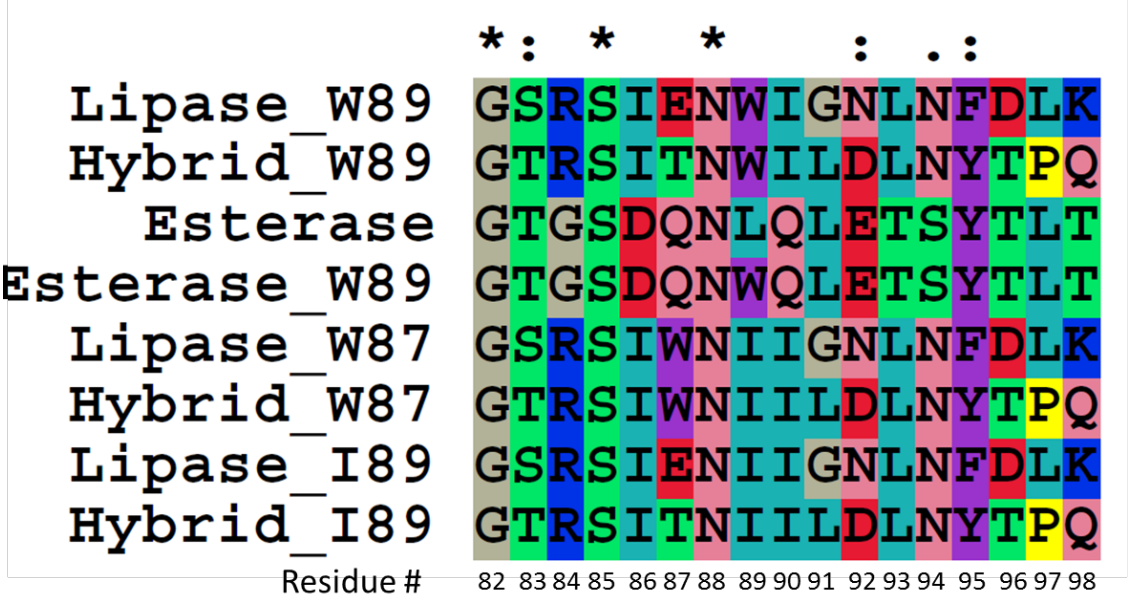


**Figure S1)** Multiple sequence alignment of the lid-region in TL lipase and investigated lid-variants spanning 17 residues (residues 82-98, TL lipase numbering) using ClustalX 1.83 (*1*). Color-code: Grey; Gly, Green; Ser and Thr, Yellow; Pro, Pink; Asn and Gln, Purple; Phe, Tyr Trp, Red; Asp and Glu, Blue; Arg and Lys, Cyan; Iso, Ala and Leu. Asterisks indicate a single fully conserved residue, colons indicate regions of fully conserved residues of high similarity and the period indicates a region of conserved residues with low similarity according to the Gonnet 250 protein weight matrix. The nomenclature used here e.g. Lipase_W89, Hybrid_W89 and Esterase refers to the titles; “TLL”, “3L” and “2L”, respectively, used in Skjold-Jørgensen et al., 2014 (*2*).

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***Mass spectrometry analysis***

**Table S1.**

The intact molecular weight determined for all lipase mutants using liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS). Untreated, reduced and labeled samples were analysed. The specific mutations introduced in each variant are also shown.

| LC-ESI-MS analysis | | Molecular weight Da | | | Molecular weight Da | | | Molecular weight Da | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variant | Mutations | Theoretical[a] | Reduced Sample | Δ | Untreated | Reduced Sample | Δ | Labeled Sample | Reduced Sample | Δ |
| Lipase_W89 | I255C | 29299.2 | 29299.4 | 0.2 | 29419.3 | 29299.4 | 119.9 | 29490.4 | 29299.4 | 191.0 |
| Lipase_W87 | E87W W89I I255C | 29283.3 | 29283.4 | 0.1 | 29402.4 | 29283.4 | 119.0 | 29472.4 | 29283.4 | 189.0 |
| Hybrid_W89 | S83T E87T G91L N92D F95Y D96T L97P K98Q I255C | 29328.3 | 29328.3 | 0.0 | 29447.3 | 29328.3 | 119.0 | 29518.4 | 29328.3 | 190.1 |
| Hybrid_W87 | S83T E87W W89I G91L N92D F95Y D96T L97P K98Q I255C | 29340.3 | 29340.4 | 0.1 | 31814.2[b] | 31695.2[b] | 119.0 | 31886.3[b] | 31695.2[b] | 191.1 |
| Esterase | S83T R84G I86D E87Q W89L I90Q G91L N92E L93T N94S F95Y D96T K98T I255C | 29164.0 | 29163.2 | 0.8 | 29282.2 | 29163.2 | 119.0 | 29353.2 | 29163.2 | 190.0 |
| Esterase_W89 | S83T R84G I86D E87Q I90Q G91L N92E L93T N94S F95Y D96T K98T I255C | 29237.0 | 29236.2 | 0.8 | 29356.2 | 29236.2 | 120.0 | 29427.3 | 29236.2 | 191.1 |
| Lipase_I89 | W89I I255C | 29226.2 | 29225.4 | 0.8 | 29344.4 | 29225.4 | 119.0 | 29415.4 | 29225.4 | 190.0 |
| Hybrid_I89 | S83T E87T W89I G91L N92D F95Y D96T L97P K98Q I255C | 29255.2 | 29255.4 | 0.2 | 31567.1[b] | 31448.1[b] | 119.0 | 31638.2[b] | 31448.1[b] | 190.1 |

[a] theoretical mono-isotopic, non-glycosylated mass of the samples with a single reduced cysteine.

[b] molecular weight of corresponding N-linked glycosylated samples. The peak representing the non-glycosylated species could not be detected for these untreated and labeled samples.

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The untreated samples show an observed molecular weight corresponding to ~120 Da above the theoretical weight. The ~120 Da are ascribed to disulphide formation with free cysteine occurring during protein expression and purification (*3*) hence blocking the sulfhydryl group of the introduced surface exposed cysteine residue in all variants. The difference in molecular weight between reduced (0.5 mM TCEP added to remove cysteine-reacted species) and bimane labeled samples was ~190 Da corresponding to the molecular weight of a single bimane molecule (expected MW: 191 Da). No peaks were observed at higher m/z ratios suggesting successful labeling of each variant with a 1:1 protein:bimane labeling ratio.

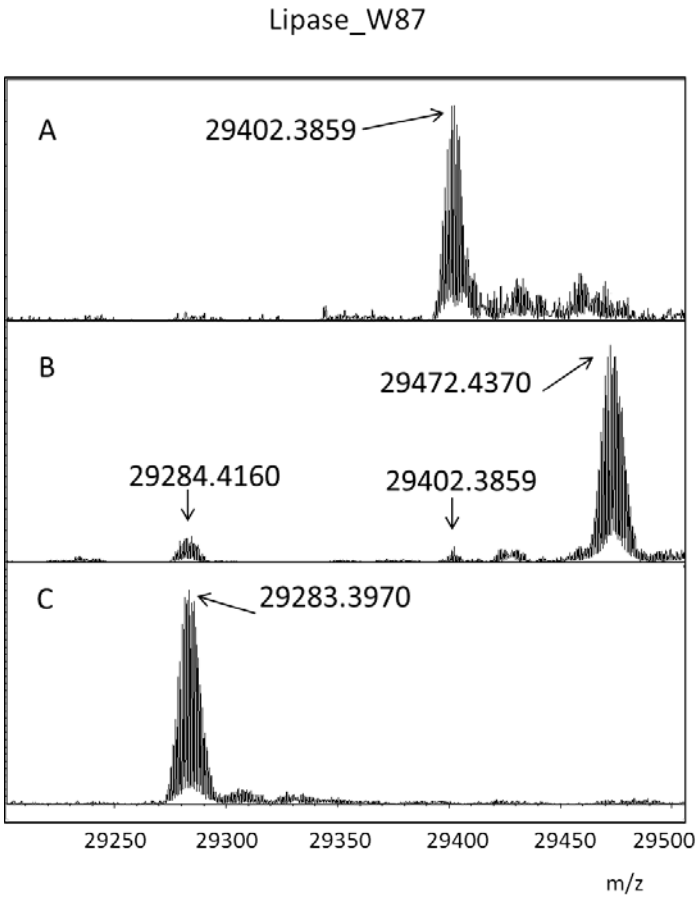


**Figure S2.** Electrospray ionization (ESI) mass spectrum of Lipase_W87. **A)** Untreated sample, **B)** Bimane labeled sample, **C)** Reduced sample (~30 x molar excess of TCEP). The untreated sample has the sulfhydryl group of the introduced cysteine residue (C255) blocked (presumably from disulphide coupling to a free cysteine during protein expression) giving rise to a peak ~120 Da above the observed peak for the reduced sample (which corresponds to the theoretically calculated molecular weight). For the bimane labeled sample, the peaks representing the untreated and reduced protein species are greatly diminished while a large peak is observed ~190 Da above the molecular weight of the reduced species, corresponding to the molecular weight of a single bimane molecule. Protein concentration was ~15 μM. in all experiments.

***Absorbance spectra***

Absorbance spectra were taken for each variant in order to match the amount of bimane in each sample. Also, these spectra were investigated for initial evidence of static complex formation between bimane and Trp. In a ground state complex with its quencher (Trp) it is expected that bimane shows a red-shift in its absorbance maximum (*4*).

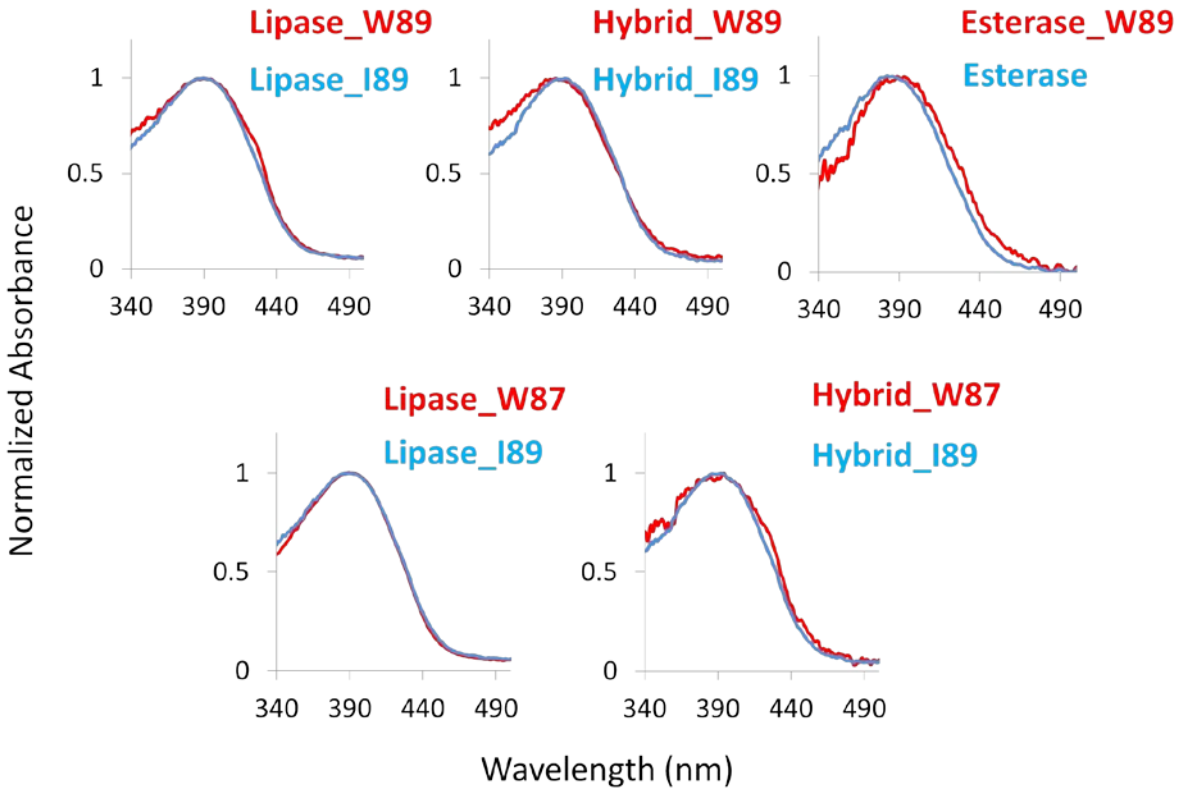


**Figure S3.** Normalized absorbance spectra of bimane labeled at site C255 for each variant with Trp in position 87 or 89 (red line). The spectra were normalized to their Trp-less controls (blue line). Spectral shifts caused by ground-state complex formation between bimane and the quencher (Trp) was investigated and can clearly be seen for Esterase_W89. Absorbance spectra were measured at 22 ºC.

***Determining the amount of free label***

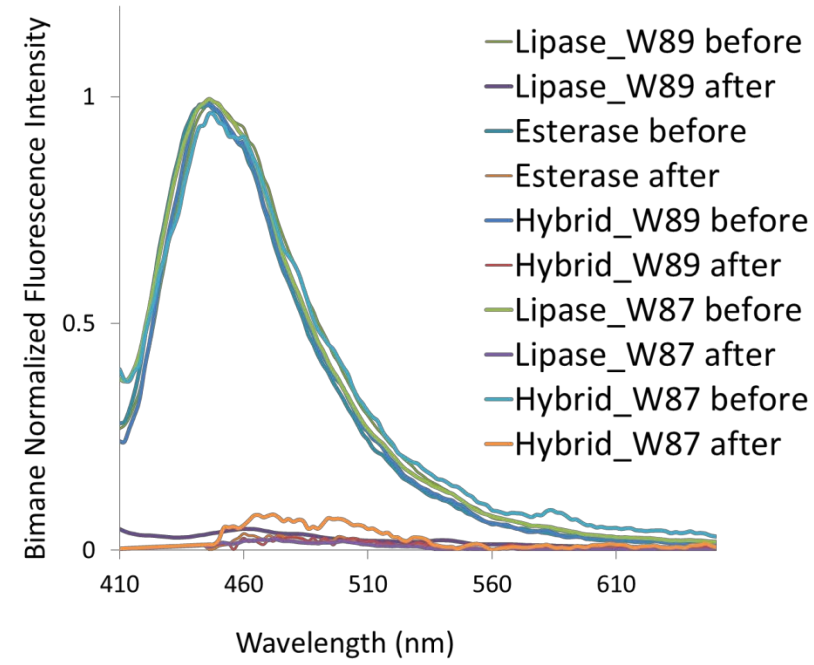


**Figure S4.** Fluorescence emission scans were taken of bimane labeled samples before and after TCA precipitation to elucidate the amount of free, unattached label in the samples. TCA was added and then the samples were incubated on ice for 30 minutes. The fluorescence emission spectrum was measured before and after centrifugation of TCA treated samples at 13.000 rpm at 4 ºC for 20 minutes. Scans represent an average of three consecutive measurements. In all cases, free label represented < 5 %.

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***Calculation of the dielectric constant in solvent mixtures***

**Table S2.**

Volumes, mole-fractions and di-electric constants of the solvent mixtures used in the TrIQ study. Buffer was 50 mM MOPS pH 7.5.

| Volumes | | Mole fractions | | Di-electric constant (ε)[a] |
|---|---|---|---|---|
| Water | Ethanol | Water | Ethanol | Water-Ethanol mixture |
| 100 | 0 | 1 | 0 | 80 |
| 80 | 20 | 0.93 | 0.07 | 69 |
| 60 | 40 | 0.83 | 0.17 | 58 |
| 50 | 50 | 0.76 | 0.24 | 52 |
| 40 | 60 | 0.68 | 0.32 | 46 |
| 10 | 90 | 0.26 | 0.74 | 30 |

[a] calculated using $p_m = \frac{\sum_{i=1}^{n} x_i v_i p_i}{\sum_{i=1}^{n} x_i v_i}$ and $p = \frac{(\varepsilon-1)(2\varepsilon+1)}{9\varepsilon}$ (see also eq. 1 and eq. 2 in Materials and Methods using $v_{H2O}$ = 0,018, $p_{H2O}$ = 17.69, $v_{EtOH}$ = 0.059, $p_{EtOH}$ = 5.33 and assuming that $p$ equals $p_m$. Hence, $p_m$ and $p$ represent the polarization per unit volume. $x$, $v$ and $p$ represent the mole fraction, the molar volume and the polarization of pure component $i$, respectively. ε represents the dielectric constant.

Enzymatic activity of lipases correlates with polarity-induced conformational changes: a Trp-induced quenching (TrIQ) fluorescence study

## Table S3.

The amplitude-weighted average lifetime <τ> for bimane attached to C255 in each lipase variant (with Trp in position 89 or 87) in solutions of varying di-electric constants (e.g. 80, 69, 58, 52, 46, 30). The standard deviation from a minimum of two lifetime measurements is shown. Experiments were conducted at 22 ºC.

| **Lipase_W89** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **ε** | **A1** | **τ 1** | **A2** | **τ 2** | **A3** | **τ 3** | **χ2** | **<τ>** | **average** | **stdev** |
| 80 | 3284.9 | 16.20 | 2861.1 | 4.473 | | | 1.001 | 10.74 | 11.04 | 0.42 |
| | 4138.1 | 16.17 | 3049.8 | 4.781 | | | 1 | 11.34 | | |
| 58 | 4056.20 | 16.15 | 2172.00 | 5.65 | 4007.00 | 0.80 | 1.007 | 7.91 | 7.93 | 0.03 |
| | 3842.70 | 16.22 | 2261.80 | 5.64 | 3733.00 | 0.84 | 0.982 | 7.95 | | |
| 46 | 4801.20 | 14.42 | 3348.90 | 5.61 | 3059.00 | 0.88 | 0.988 | 8.09 | 8.06 | 0.17 |
| | 4695.60 | 14.63 | 3669.80 | 5.68 | 2868.00 | 0.95 | 0.989 | 8.21 | | |
| | 4828.20 | 14.37 | 3332.70 | 5.53 | 3342.00 | 0.84 | 0.994 | 7.88 | | |
| 30 | 3773.00 | 16.64 | 3362.00 | 6.61 | 4197.00 | 1.02 | 0.986 | 7.88 | 7.79 | 0.14 |
| | 4200.30 | 15.80 | 3527.30 | 5.41 | 3932.00 | 0.92 | 1.028 | 7.63 | | |
| | 3772.60 | 16.64 | 3362.00 | 6.61 | 4197.00 | 1.02 | 0.989 | 7.87 | | |
| **Hybrid_W89** | | | | | | | | | | |
| **ε** | **A1** | **τ 1** | **A2** | **τ 2** | **A3** | **τ 3** | **χ2** | **<τ>** | **average** | **stdev** |
| 80 | 4471.80 | 15.04 | 2321.60 | 4.19 | | | 1.017 | 11.33 | 10.84 | 0.69 |
| | 3303.90 | 15.79 | 1671.30 | 6.39 | 1309.00 | 1.68 | 0.994 | 10.35 | | |
| 58 | 3630.30 | 15.71 | 2812.00 | 6.08 | 2710.00 | 0.99 | 1.01 | 8.39 | 8.41 | 0.03 |
| | 3815.50 | 15.64 | 2335.40 | 6.13 | 2956.00 | 0.94 | 0.97 | 8.43 | | |
| 46 | 3542.30 | 15.62 | 4544.40 | 6.72 | 2734.00 | 1.75 | 0.998 | 8.38 | 8.42 | 0.05 |
| | 4741.70 | 14.50 | 2782.50 | 5.88 | 2864.00 | 0.94 | 1.002 | 8.45 | | |
| 30 | 3646.60 | 15.86 | 3724.20 | 5.71 | 4029.00 | 0.98 | 0.994 | 7.28 | 7.30 | 0.02 |
| | 3736.60 | 15.85 | 3572.90 | 5.79 | 4164.00 | 0.96 | 1.01 | 7.31 | | |
| **Esterase** | | | | | | | | | | |
| **ε** | **A1** | **τ 1** | **A2** | **τ 2** | **A3** | **τ 3** | **χ2** | **<τ>** | **average** | **stdev** |
| 80 | 5951.2 | 17.62 | 4807.6 | 5.598 | | | 0.975 | 12.25 | 12.23 | 0.03 |
| | 5996.1 | 17.59 | 4868.5 | 5.572 | | | 0.99 | 12.21 | | |
| 58 | 7942.60 | 15.51 | 2576.40 | 5.82 | | | 1.011 | 13.14 | 13.14 | |
| 46 | 7646.30 | 15.16 | 3142.90 | 5.14 | | | 1.059 | 12.24 | 12.24 | |
| 30 | 7765.00 | 15.58 | 3029.70 | 5.18 | | | 0.987 | 12.66 | 12.66 | |

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Table S3 (cont.)

| **Esterase_W89** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **ε** | **A1** | **τ 1** | **A2** | **τ 2** | **A3** | **τ 3** | **χ2** | **<τ>** | **average** | **stdev** |
| 80 | 965.40 | 15.03 | 1990.80 | 4.48 | 2951.00 | 0.77 | 0.997 | 4.35 | 4.24 | 0.16 |
| | 873.10 | 14.29 | 1693.30 | 4.05 | 2541.00 | 0.70 | 0.96 | 4.13 | | |
| 58 | 1870.90 | 13.87 | 2479.10 | 5.46 | 2762.00 | 0.98 | 1.006 | 5.93 | 5.93 | |
| 46 | 3435.20 | 14.37 | 3420.00 | 5.53 | 3013.00 | 1.03 | 1.035 | 7.23 | 7.23 | |
| 30 | 3685.40 | 15.08 | 4172.80 | 5.17 | 2716.00 | 1.25 | 1.049 | 7.62 | 7.62 | |
| **Hybrid_W87** | | | | | | | | | | |
| **ε** | **A1** | **τ 1** | **A2** | **τ 2** | **A3** | **τ 3** | **χ2** | **<τ>** | **average** | **stdev** |
| 80 | 1878.60 | 13.04 | 2181.20 | 4.75 | 2988.00 | 0.68 | 0.96 | 5.23 | 6.17 | 1.13 |
| | 1555.80 | 14.29 | 2316.80 | 5.45 | 2432.00 | 0.82 | 0.997 | 5.85 | | |
| | 1095.10 | 15.59 | 2061.00 | 7.09 | 1513.40 | 1.98 | 0.995 | 7.43 | | |
| 69 | 2603.90 | 13.24 | 2612.20 | 5.24 | 3740.00 | 0.75 | 0.999 | 5.69 | 5.69 | |
| 58 | 3268.60 | 13.38 | 2929.90 | 5.31 | 3721.00 | 0.80 | 0.992 | 6.27 | 5.72 | 0.86 |
| | 3966.40 | 12.60 | 3503.40 | 4.08 | 6520.00 | 0.27 | 1.035 | 4.72 | | |
| | 2475.50 | 13.89 | 2707.40 | 5.62 | 3352.00 | 0.88 | 1.014 | 6.16 | | |
| 52 | 4004.10 | 13.90 | 3321.60 | 5.27 | 4744.00 | 0.66 | 1.016 | 6.32 | 6.32 | |
| 46 | 4118.60 | 14.74 | 3862.40 | 5.98 | 4435.00 | 0.94 | 0.988 | 7.09 | 7.45 | 1.22 |
| | 5165.10 | 14.18 | 4315.90 | 4.81 | 5330.00 | 0.27 | 1.024 | 6.45 | | |
| | 4055.40 | 13.56 | 6919.30 | 6.02 | | | 1.035 | 8.81 | | |
| 30 | 5044.30 | 16.05 | 3262.60 | 6.05 | 4134.00 | 0.97 | 0.98 | 8.41 | 8.64 | 0.20 |
| | 4614.00 | 16.24 | 3007.40 | 5.91 | 3430.00 | 1.03 | 1.046 | 8.71 | | |
| | 3857.00 | 15.51 | 7032.90 | 5.10 | | | 1.057 | 8.79 | | |
| **Hybrid_I89** | | | | | | | | | | |
| **ε** | **A1** | **τ 1** | **A2** | **τ 2** | **A3** | **τ 3** | **χ2** | **<τ>** | **average** | **stdev** |
| 80 | 5300.00 | 18.27 | 3342.20 | 5.84 | | | 1.025 | 13.46 | 13.53 | 0.06 |
| | 5327.30 | 17.51 | 2413.40 | 4.78 | | | 1.025 | 13.54 | | |
| | 4650.20 | 17.60 | 2133.60 | 4.81 | | | 1.023 | 13.58 | | |
| 69 | 6483.60 | 18.02 | 3985.40 | 6.16 | | | 1.04 | 13.51 | 13.51 | |
| 58 | 6674.60 | 17.47 | 3921.60 | 6.06 | | | 1.025 | 13.25 | 12.98 | 0.70 |
| | 6441.00 | 17.38 | 3345.50 | 6.05 | | | 1.073 | 13.51 | | |
| | 5874.00 | 16.79 | 3986.10 | 5.41 | | | 1.073 | 12.19 | | |
| 52 | 7365.60 | 15.99 | 3718.60 | 5.45 | | | 1.048 | 12.45 | 12.45 | |
| 46 | 6870.90 | 15.91 | 3775.80 | 6.17 | | | 1.022 | 12.46 | 11.69 | 0.69 |
| | 6545.30 | 15.62 | 4136.20 | 5.01 | | | 0.986 | 11.51 | | |
| | 5216.60 | 15.96 | 5556.40 | 6.55 | | | 1.076 | 11.11 | | |
| 30 | 7123.60 | 15.63 | 3585.50 | 5.69 | | | 1.01 | 12.31 | 11.96 | 0.49 |
| | 6523.00 | 15.47 | 3661.40 | 4.72 | | | 1.066 | 11.61 | | |

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Table S3 (cont.)

| **Lipase_W87** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **ε** | **A1** | **τ 1** | **A2** | **τ 2** | **A3** | **τ 3** | **χ2** | **<τ>** | **average** | **stdev** |
| 80 | 1494.10 | 13.36 | 2502.90 | 4.61 | 4287.00 | 0.72 | 1.01 | 4.17 | 4.71 | 0.64 |
| | 1677.70 | 13.46 | 2130.10 | 4.38 | 3773.00 | 0.66 | 0.983 | 4.54 | | |
| | 1461.60 | 14.25 | 2053.20 | 4.83 | 2585.00 | 0.88 | 1.002 | 5.42 | | |
| 69 | 2172.20 | 13.31 | 2989.80 | 4.87 | 4768.00 | 0.73 | 0.96 | 4.73 | 4.73 | |
| 58 | 2585.00 | 13.56 | 3262.50 | 5.30 | 4654.00 | 0.83 | 0.982 | 5.35 | 5.87 | 0.46 |
| | 2394.30 | 14.56 | 3370.90 | 5.77 | 3488.00 | 0.93 | 1.04 | 6.22 | | |
| | 2618.10 | 14.00 | 3087.40 | 5.31 | 3648.00 | 0.92 | 1.001 | 6.03 | | |
| 52 | 4138.40 | 13.70 | 3482.80 | 5.43 | 4600.00 | 0.87 | 1.02 | 6.51 | 6.51 | |
| 46 | 3831.30 | 14.54 | 4439.30 | 5.88 | 3191.00 | 1.08 | 1.055 | 7.44 | 7.24 | 0.19 |
| | 4055.60 | 14.26 | 3317.80 | 5.51 | 3630.00 | 0.92 | 0.945 | 7.22 | | |
| | 3024.90 | 14.15 | 5027.00 | 6.54 | 3103.00 | 1.00 | 0.959 | 7.06 | | |
| 30 | 4873.40 | 15.77 | 3003.60 | 6.39 | 3063.00 | 1.06 | 1.076 | 9.08 | 9.66 | 0.80 |
| | 6345.60 | 15.07 | 4629.50 | 4.39 | | | 1.004 | 10.57 | | |
| | 4771.90 | 14.83 | 5921.20 | 4.91 | | | 1.045 | 9.34 | | |
| **Lipase_I89** | | | | | | | | | | |
| **ε** | **A1** | **τ 1** | **A2** | **τ 2** | **A3** | **τ 3** | **χ2** | **<τ>** | **average** | **stdev** |
| 80 | 5916.10 | 17.02 | 3462.50 | 5.13 | | | 1.071 | 12.63 | 13.09 | 0.66 |
| | 5711.30 | 17.61 | 2819.40 | 5.34 | | | 1.054 | 13.56 | | |
| 69 | 6726.30 | 15.49 | 4020.80 | 5.00 | | | 1.015 | 11.57 | 11.57 | |
| 58 | 6709.60 | 16.53 | 4015.40 | 5.49 | | | 1.035 | 12.40 | 12.41 | 0.11 |
| | 6709.70 | 16.66 | 3674.00 | 4.98 | | | 1.071 | 12.53 | | |
| | 6459.90 | 16.43 | 3558.00 | 4.83 | | | 1.033 | 12.31 | | |
| 52 | 7256.70 | 15.94 | 3438.60 | 5.54 | | | 0.992 | 12.60 | 12.60 | |
| 46 | 6980.80 | 15.97 | 3907.20 | 6.00 | | | 0.999 | 12.39 | 11.78 | 0.53 |
| | 6395.90 | 15.79 | 4261.00 | 5.15 | | | 1.05 | 11.54 | | |
| | 5360.80 | 16.26 | 5235.90 | 6.47 | | | 1.029 | 11.42 | | |
| 30 | 7369.60 | 15.44 | 3476.30 | 5.17 | | | 1.064 | 12.15 | 12.11 | 0.05 |
| | 6979.60 | 15.74 | 3760.00 | 5.27 | | | 1.01 | 12.07 | | |

Results shown are from fitting the fluorescence lifetime decay using an exponential decay model, $I(t) = \sum_{i-1}^{n} A_i \, e^{\frac{-t}{\tau_i}}$, where I(t), $A_i$ and $\tau_i$ are the fluorescence intensity, the amplitude of the ith component and lifetime of the ith component, respectively. The goodness of fit was evaluated from the reduced chi-square parameter, $\chi^2$.

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***CD spectroscopy***

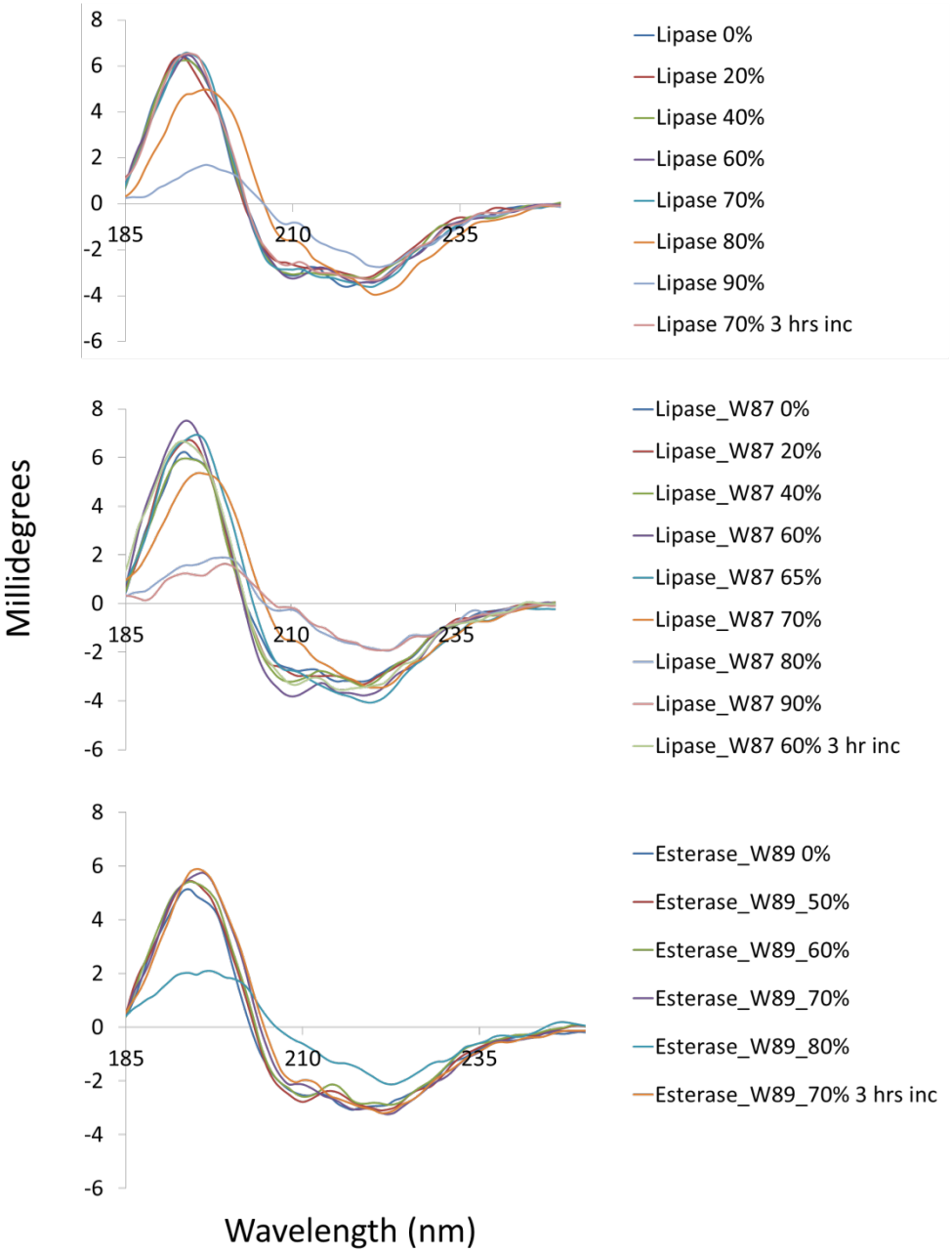


**Figure S5.** Far-UV CD spectra of TL lipase (Lipase wild-type), Lipase_W87 and Esterase_W89 in different water-ethanol mixtures. The measurements were conducted at room temperature. 10 scans were taken with 100 nm/min scan speed. At the highest tolerated

ethanol concentration for each variant, an additional measurement was made after 3 hours of incubation at 4 ºC. The concentration of protein (typically 1 mg/mL) within a given experiment was the same for all samples.

**Table S4.** The fluorescence emission maxima of the extrinsic 1, 8 – ANS fluorophore added to each variant in the surface exposed hydrophobic surface assay (SEHSA).

| | Trp in position 89 | | | Trp in position 87 | | No Trp |
|---|---|---|---|---|---|---|
| Variant | **Lipase_W89** | **Hybrid_W89** | **Esterase_W89** | **Lipase_W87** | **Hybrid_W87** | **Esterase** |
| $\lambda_M$ (nm)[a] | 478 | 478 | 462 | 478 | 478 | 463 |

**[a]** Fluorescence emission maximum of 1,8 - ANS (in nm). Measurements were conducted in 25 mM MOPS pH 7.5. See full description of experiment under the section “Solvent exposed hydrophobic surface area (SEHSA) assay”, Materials and Methods.

***Effect of bimane labeling on thermal stability of lipase mutants***

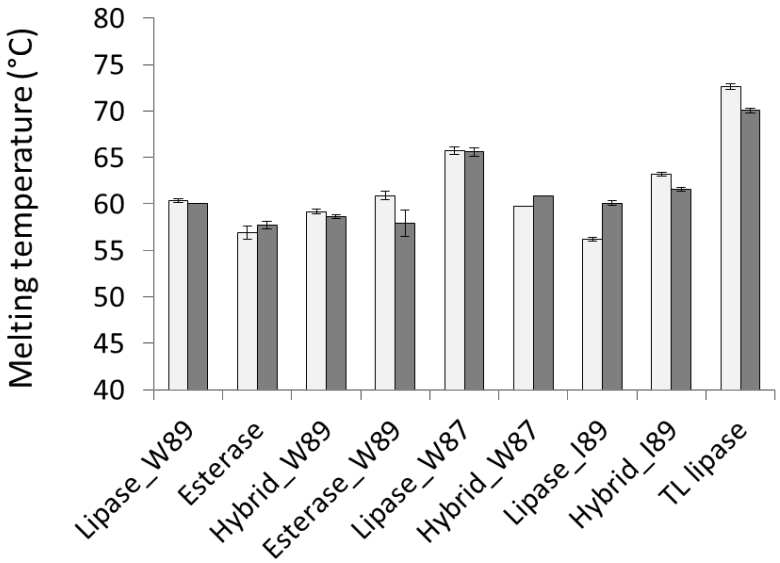


**Figure S6.** Comparison of the thermal stability of each TrIQ variant before (light grey columns) and after (dark grey columns) labeling of bimane at site C255. Values obtained using a thermal melt assay with SyproOrange (SO) as extrinsic fluorophore. The melting temperature ($T_m$) was determined at the maximum slope increment e.g. $\frac{dI}{dT}$. Final lipase concentration was 150 µg/ml. Average spectrum was determined from three independent measurements and subtracted from blank. Error bars denote standard deviation.

***Effect of bimane label on lipase activity***

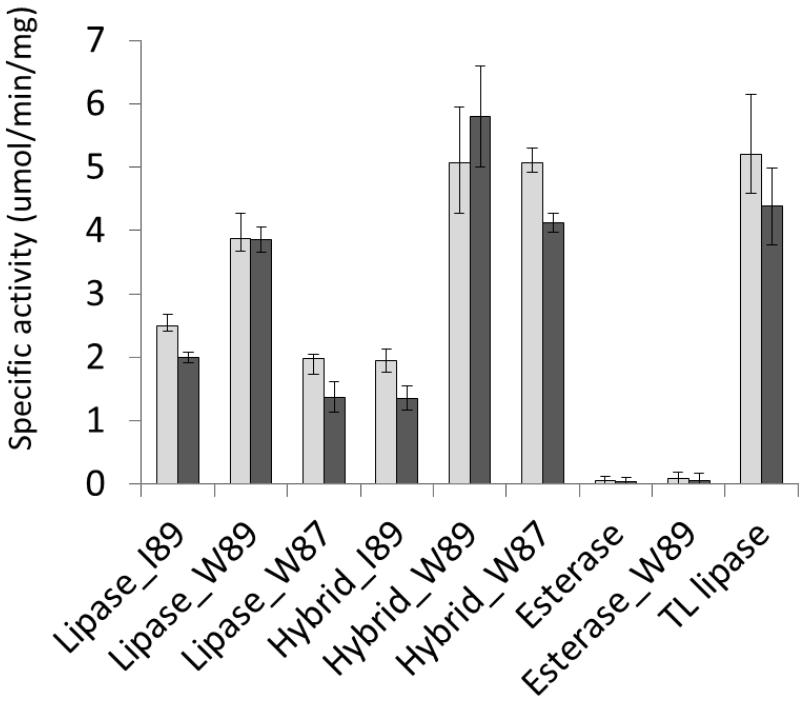


**Figure S7.** Comparison of lipase activity (ability to cleave pNP-palmitate embedded ina lipid layer of lard) for each variant before (light grey columns) and after (dark grey columns) labeling with bimane at site C255. Lipase concentration was 2.5 μg/mL. Assay was run in 100 mM Tris pH 8 + 2 mM $CaCl_2$. Activity was determined from the steepest slope of the absorbance increase (monitored at 405 nm) as a function of time. Error bars denote the standard deviation from triplicate measurements.

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***Calculating amount of open vs. closed lid conformers***

**Table S5.**

The fraction of closed W89 conformers in solution at a given dielectric constant (ε) was calculated from the relative bimane fluorescence intensities assuming that the fluorescence intensity in Esterase_W89 at ε = 30 represented the fully open lid conformation and the fluorescence intensity for Lipase_W89 and Hybrid_89 at ε = 80 represented the closed conformation. Standard deviation was calculated from two separate experiments.

| | Esterase_W89 | | | |
|---|---|---|---|---|
| | $F/F_0$ | | average | stdev |
| 80 | 0.16 | 0.13 | 0.15 | 0.02 |
| 58 | 0.18 | 0.16 | 0.17 | 0.01 |
| 46 | 0.25 | 0.21 | 0.23 | 0.03 |
| 30 | 0.30 | 0.28 | 0.29 | 0.01 |
| | Lipase_W89 | | | |
| | $F/F_0$ | | average | stdev |
| 80 | 0.68 | 0.57 | 0.63 | 0.08 |
| 58 | 0.56 | 0.53 | 0.54 | 0.02 |
| 46 | 0.36 | 0.36 | 0.36 | 0.00 |
| 30 | 0.38 | 0.34 | 0.36 | 0.03 |
| | Hybrid_W89 | | | |
| | $F/F_0$ | | average | stdev |
| 80 | 0.69 | 0.70 | 0.69 | 0.00 |
| 58 | 0.62 | 0.63 | 0.63 | 0.01 |
| 46 | 0.30 | 0.30 | 0.30 | 0.00 |
| 30 | 0.29 | 0.27 | 0.28 | 0.02 |

$$fraction\ of\ closed\ W89\ conformers = \frac{\left(\left(\frac{F}{F_0}\right)_{sample} - \left(\frac{F}{F_0}\right)_{ref\ \varepsilon 30}\right)}{\left(\left(\frac{F}{F_0}\right)_{sample\ \varepsilon 80} - \left(\frac{F}{F_0}\right)_{ref\ \varepsilon 30}\right)} \qquad Eq.\ 3$$

Where $\left(\frac{F}{F_0}\right)_{sample}$ represents the relative fluorescence emission intensity of a sample compared to its Trp-less control at a given dielectric constant, $\left(\frac{F}{F_0}\right)_{ref\ \varepsilon\ 30}$ is the relative fluorescence intensity of Esterase_W89 at ε = 30.

**Table S6.**

The fraction of open W87 conformers in solution at a given dielectric constant was calculated from the relative bimane fluorescence intensities assuming that the fluorescence intensity equal to unity (no quenching, $\left(\frac{F}{F_0}\right) = 1$) represented the fully open conformation and the fluorescence intensity for Lipase_W87 and Hybrid_87 at ε = 80 represented the closed conformation ( $\left(\frac{F}{F_0}\right) = 0$). Standard deviation was calculated from two separate experiments.

| | Lipase_W87 | | | |
|---|---|---|---|---|
| | $F/F_0$ | | average | stdev |
| 80 | 0.00 | 0.00 | 0.00 | 0.00 |
| 69 | 0.03 | | 0.03 | |
| 58 | 0.04 | 0.11 | 0.08 | 0.05 |
| 52 | 0.25 | | 0.25 | |
| 46 | 0.50 | 0.38 | 0.44 | 0.08 |
| 30 | 0.68 | 1.02 | 0.85 | 0.24 |
| | Hybrid_W87 | | | |
| | $F/F_0$ | | average | stdev |
| 80 | 0.00 | 0.00 | 0.00 | 0.00 |
| 69 | 0.04 | | 0.04 | |
| 58 | 0.05 | 0.23 | 0.14 | 0.13 |
| 52 | 0.17 | | 0.17 | |
| 46 | 0.32 | 0.51 | 0.41 | 0.13 |
| 30 | 0.58 | 0.88 | 0.73 | 0.21 |

$$fraction\ of\ open\ W87\ conformers = \frac{\left(\left(\frac{F}{F_0}\right)_{sample} - \left(\frac{F}{F_0}\right)_{sample\ \varepsilon 80}\right)}{\left(1 - \left(\frac{F}{F_0}\right)_{sample\ \varepsilon 80}\right)} \qquad Eq.\ 4$$

Where $\left(\frac{F}{F_0}\right)_{sample}$ represents the relative fluorescence emission intensity of a sample compared to its Trp-less control at a given dielectric constant.

## References


1. Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., and Higgins, D. G. (2007) Clustal W and Clustal X version 2.0, *Bioinformatics 23*, 2947-2948.

2. Skjold-Joergensen, J., Vind, J., Svendsen, A., and Bjerrum, M. J. (2014) Altering the Activation Mechanism in Thermomyces lanuginosus Lipase, *Biochemistry*.

3. Hedin, E. M., Patkar, S. A., Vind, J., Svendsen, A., Hult, K., and Berglund, P. (2002) Selective reduction and chemical modification of oxidized lipase cysteine mutants, *Canadian journal of chemistry 80*, 529-539.

4. Jones Brunette, A. M. and Farrens, D. L. (2014) Distance Mapping in Proteins Using Fluorescence Spectroscopy: Tyrosine, like Tryptophan, Quenches Bimane Fluorescence in a Distance-Dependent Manner, *Biochemistry 53*, 6290-6301.